

CONTRIBUTIONS CONCERNING THE CURRENT STATUS OF AMPHIBIAN SPECIES IN ROMAN TOWN AND ITS SURROUNDINGS

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INTRODUCTION

Amphibians are vertebrate ectothermic tetrapods, with naked tegument rich in glands and strongly vascularized, allowing gas transfers with water and air both as larvae and adults. Lung breathing in adults and branchial breathing in larvae add to the the system of respiration through skin. Real amphibians belong to subclass *Lissamphibia* divided into three *Salientia* groups (frogs and toads), *Caudata* (salamanders and tritons/newts) and *Gymnophiona* (a group of apod amphibians) [38,39].

At present, there are 7.530 species of amphibians in the world according to <http://amphibiaweb.org/lists/index.shtml> website, of which 29 species described this year – information updated on 25.04.2016 (Table 1).

Table 1 Number of species at international level [32]

<i>Lissamphibia</i>	<i>Salientia</i> sau <i>Anura</i>	<i>Caudata</i>	<i>Gymnophiona</i>	Total
FamiLy	55	10	10	75
Genus	445	68	33	546
Species	6642	683	205	7530

In Romanian fauna, only 20 species of amphibians are part of the first two groups (*Salientia/Anura* and *Caudata*), 7 species of amphibian species and 13 urodele. They are found everywhere from the Black Sea to an altitude of 2000 m [38]. The red list of endangered species IUCN identified the group of amphibians as the most threatened group of vertebrates until now, with an extinction risk of 41%. Extinction and reduction in the number of individuals is due to fires, climatic changes, diseases and over-exploitation, which imposes urgent measures being taken to protect of populations of amphibians worldwide. [36].

Importance of amphibians in natural ecosystems. Amphibians play an important role in nature and their sensitivity to environmental conditions can help us determine the health of an ecosystem, this making them important bio-indicators of environmental quality. In addition to being a substantial part of the food chain they feed, in

their turn, on insects carrying the vectors that produce diseases dangerous for humans [36], for example, vectors that cause the disease known as malaria are transmitted by the bite of *Anopheles* mosquitoes; the infection with West Nile virus can be transmitted by the bite of various species of the genus *Culex mosquito*; *Lyme borreliosis* is a disease which is transmitted by tick bite [45] and all these insects and arachnids constitute daily food for amphibians. A number of important chemical compounds are found in the skin of amphibians, which turns them into "hopping pharmacies." However, special attention should be paid to over-exploitation of amphibians while looking for the next miracle cure [36].

Amphibians are extremely sensitive to environmental changes, so they need special habitats both in terrestrial and aquatic environments, as they have permeating skin which easily absorbs chemical toxins. Amphibian larvae keep waters clean by feeding on algae and detritus, so amphibians are considered valuable indicators of environmental stress. Their health is considered to be an indicator of the health of the natural ecosystem as a whole [37, 24, 20]. Amphibians produce a wide range of skin secretions, many of which have significant potential to improve human health through their use as pharmaceuticals. A group of Russian researchers have found more than 76 different antimicrobial peptides secreted by the skin of the red mountain frog (*Rana temporaria* Linnaeus, 1758). These peptides may be useful for the prevention of antibiotic resistant pathogenic strains [37].

The decline of amphibian species. Amphibians have populated earth for over 300 million years, but during the last two decades there has been an alarming number of extinctions; it is believed that nearly 168 species have disappeared and at least 2469 will soon disappear, given that species are in decline (Table 2, Fig. 1). This indicates that the species endangered and threatened will continue to grow (Stuart et al., 2004) [1, 2, 27] unless urgent action is taken to protect them [1, 14].

The most important factor leading to the decline of amphibian populations is habitat destruction [15, 28]. For instance, species of amphibians that live in forests undergoing deforestation will soon disappear because they will

not have the conditions necessary to live in that environment. Worrying is that in the world there are many cases where the habitat is still protected amphibians disappear. There are several reasons for the decline of amphibians, but a disease called "chytridiomycosis" and global climate change are the greatest current threats to the populations of amphibians in combination with other factors [4, 28].

Chytridiomycosis is an infectious disease caused by a fungus and is associated with the global decline of hundreds of amphibian species; it is an important cause of the decline of biodiversity in the animal world. Some researchers have concluded that this is the most serious cause of declining populations of amphibians in recorded history until today [18, 27]. From an ecological point of view, the factors pertain to the crisis in biodiversity at world level (habitat destruction, modification and fragmentation, new species being introduced over-exploitation) and yet, many populations of amphibians suffer a sharp decline even in protected areas or so-called "clean environments" because some depend on more complex mechanisms (climate change, infectious diseases and malformations during embryonal development) [1, 27].

The confirmed factors causing the decline in species of amphibians after Young et al. 2001 are:

Habitat destruction, habitat modification and fragmentation – amphibian habitat is often perceived as being confined to wetlands and other aquatic environments, but they are found in a wide variety of ecosystems (Photo 1 and 2), from rainforest to arid deserts. The destruction of the habitats preferred by amphibians, after Dodd and Smith 2003, has probably the most topical causes of decline and the best can be seen by the action of urbanization [1, 29, 41]. Other causes are deforestation, grazing, pollution with household waste which drastically alter habitats (Photo 3 and 4), and lead to fragmentation and isolation of populations as well as loss of genetic diversity. Due to these causes, millions of amphibians disappear every year [15, 16, 24].

Invasive species – when we say introduced species we do not mean only species introduced from other species of amphibians, referring to other

species of animals, eg fish species introduced into breeding ponds for reproduction which feed on tadpoles that no longer reach the metamorphosis. High mobility, dietary habits and the huge reproductive capacity of other species is a real threat to amphibians [1, 3, 30].

Over-exploitation – amphibians are collected for their meat used as food. Because of their beauty, they are traded as pets being collected directly from nature.

Other reasons for over-exploitation are research, education and medicine, as amphibians are collected for these purposes; in recent years, there have been found research methods that do not involve the death of the animals studied [21, 22, 42].

Climatic changes – amphibians can be more sensitive to climate change than other species because of their permeable skin, their life cycle (oviposition, growth and development of embryos, metamorphosis of larvae) makes them extremely sensitive to small changes in temperature and humidity (Carey Alexander 2003). Due to global warming, some amphibians begin to reproduce earlier (*Lissotriton vulgaris vulgaris* Linnaeus 1758, *Triturus cristatus cristatus* Laurentus 1768) and others later (*Bufo bufo bufo* Linnaeus 1758, *Rana temporaria temporaria* Linnaeus 1758), which can decrease the immunity leading to outbreaks of pathogens and increased mortality. But a more detailed study of the effects of climate change should be performed on a long term basis [3, 5, 10, 20, 26].

UV radiations – for the past decades, the level of UV radiation in the atmosphere has increased significantly; the researchers have discovered that radiation can kill amphibians, having direct and indirect effects. Although it is known that amphibians have defense mechanisms to combat the harmful effects of UV radiation, the consequences vary according to species and populations of the same species, according to the stages of development. UV radiation directly causes sublethal effects. For example, after Langhelle et al. 1999, the effects of UV radiation on the species *Triturus cristatus cristatus* Laurentus 1768 are skin lesions and erratic swimming behaviour.

Table 2. Regional distribution of critically endangered and vulnerable species until 2014

Region	Afro-tropical	Australian	Madagascanian	Nearctic	Neotropical	Oceania
Crx*	26	19	7	41	127	1
Ev**	162	35	60	107	406	20
Total***	760	229	245	440	2231	411
Region	Oriental	Palaearctic	Panamanian	Saharo-Arabian	Sino-Japanese	
Crx*	41	6	231	4	10	
Ev**	266	33	350	5	66	
Total***	1021	198	1098	73	356	

Legend: *CRX- Critically Endangered; **Ev – Vulnerable; ***Total

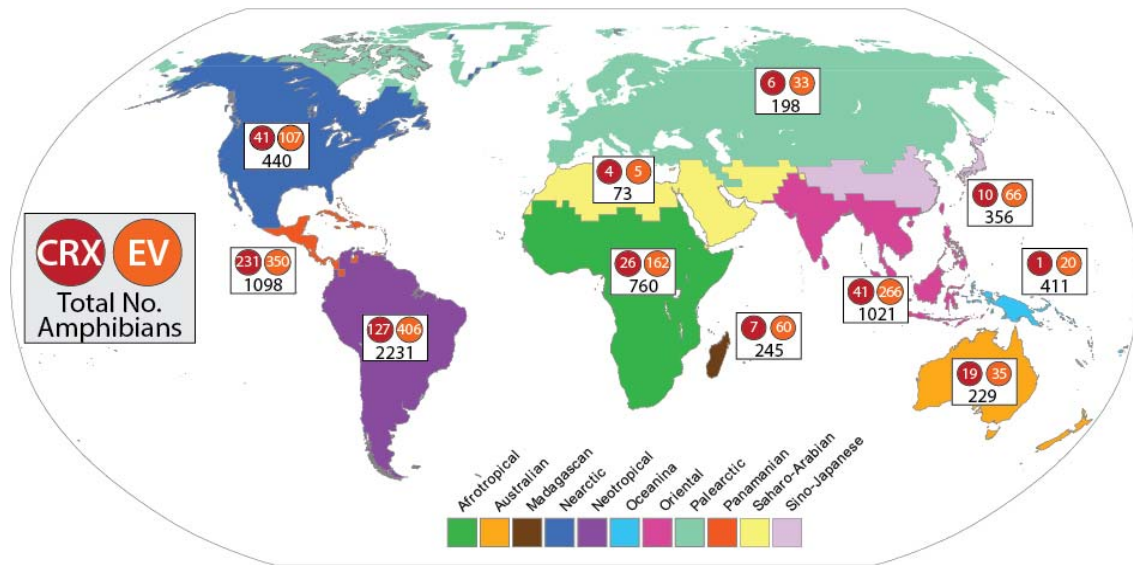


Fig. 1. Distribution of vulnerable and endangered species (updated on 12 May 2014)
(<http://amphibiaweb.org>)

According to Nagl and Hofer 1997, the same effect is manifested on the species *Ichthyosaura alpestris alpestris* Laurentus 1768 and *Rana arvalis arvalis* Nilsson 1842, when exposed to UV radiation: an increase in embryo mortality (Häkkinen et al 2001), whereas for *Bufo bufo bufo* Linnaeus 1758, there is increased embryo death hazard and reduction of the survival rate of larvae (Lizana and Pedraza 1998; Häkkinen et al. 2001). In *Hyla arborea arborea* Linnaeus 1758, skin color (Langhelle et al. 1999) darkens. Long-term studies on UV radiation will guarantee a competent understanding of the effects on populations of amphibians [7, 8, 9, 31].

Chemical contaminants – the major chemical factors of stress on amphibians are pesticides, heavy metals, acid pH and nitrogen pollution. The consequences of stress factors in amphibians are lethal, sublethal, direct and indirect. After Ponduri 1997, 2000 sublethal effects can cause developmental and behavioral abnormalities. They also weaken the immune system, so amphibians become more sensitive to parasites, diseases and UV radiation (Blaustein et al. 2003 Christin et al. 2003 Daszak et al., 2003, Gendron et al. 2003). Certain pesticides may disrupt the endocrine system, leading to sexual defects, such as hermaphroditism (Hayes et al. 2002b, Hayes et al. 2003) [3, 25].

Diseases – are decline factors in populations of amphibians worldwide. Diseases caused by fungi, for example, leading to the death of larvae and adults (Photos 5 and 6). *Batrachochytrium salamandrivorans* fungus is a pathogen that was described in 2013 based on a strain collected from skin tissue of fire salamander *Salamandra salamandra* Linnaeus 1758. This pathogen agent infects salamanders and newts and devastated populations of salamanders in the Netherlands [4, 23,

28, 46]. Viruses belonging to the family *Iridoviridae* are associated with mass mortality in *Rana temporaria temporaria* Linnaeus 1758. Given that it is unclear whether these pathogens have recently appeared in amphibians or coexisted and have recently increased pathogenicity or if amphibian immunity decreased in recent years [6, 10, 17], more detailed studies are required.

Malformations – the increased rate of malformations in natural populations of amphibians is a recently signaled problem recently and can be due to increased ultraviolet radiation, chemical contamination and parasitic infections [40].

Synergism – multiple factors act together to cause lethal and sublethal harm in populations of amphibians such as habitat destruction, introduced species, climate change, UV radiation and diseases with a higher negative effect higher on amphibians than a single factor acting in isolation [43].

Amphibians in Romania

Of the 20 species of amphibians that are found in Romania according to the Red Book of vertebrates (Table 3), 3 species are endangered, 9 species vulnerable, 5 species almost endangered and 3 species unevaluated [11, 12, 13, 19, 38, 44, 47].

MATERIAL AND METHOD

It consisted of a total of ten species of amphibians collected across Roman town, from the municipal park, on neighbouring farmland where there are irrigation ditches, in temporary and permanent pools, in the area of the bridge over Moldova River (joining Roman town with Cotu Vameş village), before its flowing into Siret River, in the Siret river in the vicinity of Roman town and the

bridge over Siret, joining Roman and Gadinti, as well as in Gadinti forest (Table 4).

The species of amphibians were observed during breeding and rainy days for 10 years in March 2006 - April 2016 and were determined by the field guide written by Prof. dr. Dan Cogălniceanu, *Amphibians of Romania* 2002 and Cogălniceanu D., Aioanei F., M. 2000. *Amfibieni din Romania, Determinator* [Amphibians of Romania Determiner], Ed. Ars Docendi, Bucharest, 1-99 and using descriptions and comparisons of images taken personally or retrieved online.

The specimens of adult amphibians were collected by hand from the ground or by spoon net when they were captured in ponds during breeding periods; the larvae were caught by spoon net. Juvenile and adult amphibians were determined in the field or were transported in the laboratory in plastic containers provided with openings for good ventilation; inside the container were placed pieces of sponge or damp moss. The larvae were transported in plastic containers (plastic bottles of 500 ml) filled halfway with water, avoiding shaking during transportation.

All the species were observed in the laboratory: the larvae were analyzed with the naked eye, a magnifying glass or a microscope (photo 7 and 8), making comparisons with descriptions and images used to determine the species.

RESULTS AND DISCUSSIONS

In Roman town, located at the confluence of the rivers Siret and Moldova, located in the central part of Moldova Plateau, Neamt County, we have identified three species of amphibians: *Hyla arborea arborea* Linnaeus in 1758 (Photo 11), *Bufo viridis* Laurentus 1768 (Photo 14) in Abator, Favorit and Muncitoresc districts and *Pelophylax kl. esculentus* Linnaeus in 1758 (Photo 17) in the neighborhood called Colonie sepaevaluated by railways from the rest of the town. From March 2006 – April 2016, in the municipal park of Roman, we identified five species of amphibians, *Lissotriton vulgaris vulgaris* Linnaeus in 1758 (photo 9), *Triturus cristatus cristatus* Laurentus 1768 (photo 10), *Bufo viridis* Laurentus 1768 (photo 14), *Hyla arborea arborea* Linnaeus in 1758 (photo 11) and *Pelophylax kl. esculentus* Linnaeus in 1758 (photo 17).

Table 3. Status of amphibian species in Romania [33]

Crt. No.	Scientific name	Common name	International staus	Status in Romania
1	<i>Salamandra salamandra</i> Linnaeus, 1758	fire salamander	LC	VU
2	<i>Ichthyosaura alpestris alpestris</i> Laurentus, 1758	alpine newt	LC	VU
3	<i>Lissotriton montandoni</i> Boulenger, 1860	Carpathian newt	LC	VU
4	<i>Triturus cristatus cristatus</i> Laurentus, 1768	Northern crested newt	LC	VU
5	<i>Triturus dobrogicus dobrogicus</i> Kiritzescu, 1903	Danube crested newt	NT	EN
6	<i>Lissotriton vulgaris vulgaris</i> Linnaeus, 1758	smooth newt	LC	NT
7	<i>Lissotriton vulgaris ampelensis</i> Linnaeus, 1758	Transylvanian smooth newt	LC	VU
8	<i>Bombina bombina</i> Linnaeus, 1761	European fire-bellied toad	LC	NT
9	<i>Bombina variegata variegata</i> Linnaeus, 1758	Yellow-bellied toad	LC	NT
10	<i>Pelobates fuscus fuscus</i> Laurentus, 1768	spadefoot toad	LC	VU
11	<i>Pelobates syriacus balcanicus</i> Boettger, 1889	Eastern or Syrian spadefoot	LC	EN
12	<i>Bufo bufo bufo</i> Linnaeus, 1758	common toad	LC	NT
13	<i>Bufo viridis</i> Laurentus, 1768	European fire-bellied toad	LC	NT
14	<i>Hyla arborea arborea</i> Linnaeus, 1758	European tree frog	LC	VU
15	<i>Pelophylax ridibundus ridibundus</i> Pallas, 1771	marsh frog	LC	NE
16	<i>Pelophylax kl. esculentus</i> Linnaeus, 1758	edible frog	LC	NE
17	<i>Pelophylax lessonae</i> Camerano, 1882	Italian pool frog	LC	NE
18	<i>Rana dalmatina</i> Fitzinger, 1839	agile frog	LC	VU
19	<i>Rana temporaria temporaria</i> Linnaeus, 1758	terrestrial common frog	LC	VU
20	<i>Rana arvalis arvalis</i> Nilsson, 1842	Moor frog	LC	EN

Legend: EN (endangered); VU (vulnerable); NT (near threatened); LC (least concern); NE (not evaluated).

Table 4. Amphibian species identified in the area of Roman town and its surroundings

Crt. No.	Scientific name	Common name	International staus	Status in Romania
1	<i>Triturus cristatus cristatus</i> Laurentus, 1768	northern crested newt	LC	VU
2	<i>Lissotriton vulgaris vulgaris</i> Linnaeus, 1758	smooth newt	LC	NT
3	<i>Bombina bombina</i> Linnaeus, 1761	European fire-bellied toad	LC	NT
4	<i>Bufo bufo bufo</i> Linnaeus, 1758	common toad	LC	NT
5	<i>Bufo viridis</i> Laurentus, 1768	European green toad	LC	NT
6	<i>Hyla arborea arborea</i> Linnaeus, 1758	European tree frog	LC	VU
7	<i>Pelophylax ridibundus ridibundus</i> Pallas, 1771	marsh frog	LC	NE
8	<i>Pelophylax kl. esculentus</i> Linnaeus, 1758	edible frog	LC	NE
9	<i>Pelophylax lessonae</i> Camerano, 1882	Italian pool frog	LC	NE
10	<i>Rana dalmatina</i> Fitzinger, 1839	agile frog	LC	VU

In recent years, these populations are threatened with extinction due to fish introduced in the recreational lake, which was in the past a proper breeding ground because there were no predators and larvae needed no vegetation to hide. The lack of aquatic vegetation in the lake in combination with fish species lead to increased vulnerability of tadpoles, which are actively hunted and no longer reach metamorphosis. *Lissotriton vulgaris vulgaris* Linnaeus 1758 may have already grown extinct from the lake, the larger fish feeding on mature specimens, which do not exceed 11 cm in length at maturity from head to tail.

On the Siret River and Moldova we have identified three species of the genus *Pelophylax* namely *Pelophylax kl. esculentus* Linnaeus 1758 (foto 17), *Pelophylax ridibundus ridibundus* Pallas, 1771 (foto 18) and *Pelophylax lessonae* Camerano, 1882 (foto 16).

On farmland from Roman area where there are irrigation canals and temporary or permanent pools, we identified a total of seven species of amphibians: *Lissotriton vulgaris vulgaris* Linnaeus in 1758 (photo 9), *Triturus cristatus cristatus* Laurentus 1768 (photo 10), *Bombina bombina* Linnaeus in 1761 (photo 12), *Bufo viridis* Laurentus 1768 (photo 14), *Hyla arborea arborea* Linnaeus in 1758 (photo 11), *Pelophylax kl. esculentus* Linnaeus in 1758 (photo 17) and *Pelophylax lessonae* Camerano 1882 (photo 16). These populations of amphibians are threatened due to chemical contaminants (pesticides, herbicides) used to treat crops, chemical compounds get into the soil then in the water from irrigation canals and in the vicinity of agricultural land. Moreover, the waste dumped by farmers and residents on the outskirts transformed the irrigation canals and ponds in the area in real garbage dumps (photo 3 and 4). Recent climate changes and UV radiation also contribute to decreasing the number of amphibians near Roman town.

In the Siret and Moldova Rivers, we identified three species of the genus *Pelophylax* namely *Pelophylax kl. esculentus* Linnaeus 1758 (photo 17), *Pelophylax ridibundus ridibundus* Pallas 1771 (photo 18) and *Pelophylax lessonae* Camerano, 1882 (photo 16). Specimens do not seem to be endangered.

Gadinti Forest, located in the SE of Neamt County, in the vicinity of Roman municipality, [34] extends over 2,284 hectares [35], in which there are several temporary and permanent pools, both within the forest and on the margin. It is used by amphibians especially during breeding. From March 2006 - April 2016 we identified a number of new species of amphibians in the forest: the urodele *Lissotriton vulgaris vulgaris* Linnaeus in 1758 (photo 9), *Triturus cristatus cristatus* Laurentus 1768 (photo 10), and one amphibian *Bufo viridis* Laurentus 1768 (photo 14), *Bufo bufo bufo* Linnaeus in 1758 (photo 13), *Bombina bombina* Linnaeus in 1761

(photo 12), *Hyla arborea arborea* Linnaeus 1758 (photo 11), *Rana dalmatina* Fitzinger 1839 (photo 15), *Pelophylax kl. esculentus* Linnaeus 1758 (photo 17) and *Pelophylax lessonae* Camerano 1882 (photo 16).

Although over the years a decrease in the number of individuals has not noticed, the species of amphibians in this area may be threatened by habitat destruction, climate change, UV radiation and pollution with waste and household waste that is stored at the forest edge.

CONCLUSIONS

In the area investigated in March 2006 - April 2016, we identified three species of amphibians whose status in the Red Book of Vertebrates of species is vulnerable (VU), four species near threatened with extinction (NT) and three species not yet evaluated (NE).

It is worth noting that traditional species are present in the area, but there are also species which are vulnerable, threatened and not evaluated yet.

There is a risk of decline in the populations of amphibians in Roman town and the surrounding areas due to habitat destruction, pollution by sewage, deforestation, grazing, climate change and UV radiation. This imposes urgent amphibian conservation measures, otherwise, we will face the extinction of this population in the coming years, a situation with irreversible consequences on ecosystems and human beings.

Amphibians represent an important link in the food chain has the feeding of other animals, but, in turn, feed large quantities of insects, which are vectors for the transmission of diseases in man and therefore require more attention from by the authorities.

Amphibian larvae clean the waters in which they develop by feeding on algae, their skin is very sensitive to environmental conditions and pollution thus becoming an important bioindicator for a healthy environment.

ABSTRACT

In the period March 2006 - April 2016 were identified a total of ten species of amphibians in Roman town, in the municipal park, on neighbouring farmland where there are irrigation ditches, in temporary and permanent pools, in the area of the bridge over Moldova River (joining Roman town with Cotu Vameş village), before its flowing into Siret River, in the Siret river in the vicinity of Roman town and the bridge over Siret, joining Roman and Gadinti, as well as in Gadinti forest from Neamt County. The study contributes to a better knowledge of the species of amphibians from the area identifying three vulnerable species, four nearly

endangered species and three species which are still unevaluated.

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Photo 1. Temporary pool used during the breeding of amphibians in the forest Gadinti
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Photo 2. Channel used for irrigation in the agricultural area of Roman town
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Photo 3. Domestic waste pollution in Gadinti forest area
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Photo 4. Domestic waste pollution, channels used for irrigation in the agricultural area of Roman town
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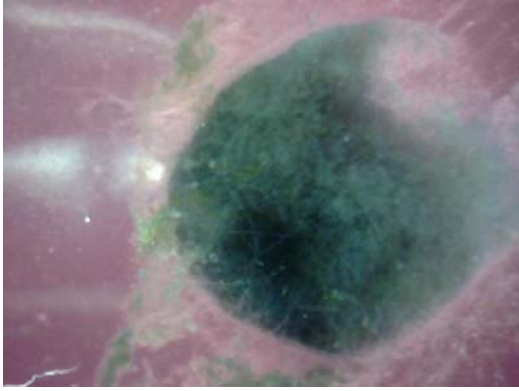


Photo 5. Ou de de *Lissotriton vulgaris vulgaris* Linnaeus 1758 egg affected by fungi seen under microscope
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Photo 6. *Rana dalmatina* Fitzinger 1839 larva affected by bacterias seen under microscope
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Photo 7. *Lissotriton vulgaris vulgaris* Linnaeus 1758 larva seen under microscope – external gills and forelimbs
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Photo 8. *Rana dalmatina* Fitzinger 1839 tadpole seen under microscope – head detail
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Photo 9. *Lissotriton vulgaris vulgaris* Linnaeus 1758 male and female during reproduction period
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Photo 10. *Triturus cristatus cristatus* Laurentus 1768 male specimen during
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Photo 11. *Hyla arborea arborea* Linnaeus 1758
perched on a tree branch Gâdinți forest
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Photo 12. *Bombina bombina* Linnaeus 1761 on a piece
of floating wood
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Photo 13. *Bufo bufo bufo* Linnaeus 1758 juvenile
specimen in Gâdinți forest
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Photo 14. *Bufotes viridis* Laurentus 1768 in Roman
town
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Photo 15. *Rana dalmatina* Fitzinger 1839 in Gâdinți
forest
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Photo 16. *Pelophylax lessonae* Camerano 1882 during
mating season
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Photo 17. *Pelophylax kl. esculentus* Linnaeus 1758 on the bank of Moldova River before flowing into Siret River
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Photo 18. *Pelophylax ridibundus ridibundus* Pallas 1771 on Siret River bank close to the bridge connecting Roman town with Gâdinți village
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