



Infectious diseases in amphibians

Handbook of best practices in
educational discovery activities



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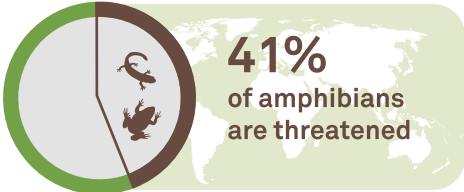
We would like to thank the companies and environmental education organisations in the Montseny area, naturalists and conservation organisations, and schools for their participation in the two working groups on “Emerging diseases in amphibians: biosafety guidelines for educational activities in the aquatic environment of the Montseny”. Their contributions and insights have enriched the content of this handbook of best environmental practices.

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Presentation

In recent years there has been a significant decline in amphibian populations worldwide and 41% of known species are threatened (International Union for Conservation of Nature, 2020). One of the main



factors causing this decline are infectious diseases caused by fungi, viruses or other pathogens, which can lead to mass deaths of specimens. This fact has alerted many groups of scholars and naturalists. Therefore, in order to reduce the

transmission of these diseases, numerous disinfection protocols for field work have been developed and published.



Calotriton arnoldi

The Montseny Natural Park and Biosphere Reserve is home to a unique amphibian that is listed as endangered: **the Montseny newt** (*Calotriton arnoldi*). The entry of pathogens causing these deadly diseases is clearly a serious threat to the wild populations of this unique species.

The discovery of the environment through environmental education activities is a very valuable and attractive educational resource for children to get closer to nature, get to know it and respect it. When exploring a river, a pond or a stream, it is very common to get in the water to look for the organisms that live there and, if necessary, to catch them with a hand net, study them and then release them back into their environment. Aware of the consequences of infectious diseases on amphibians, it is necessary to review these activities and include, in protocols, criteria to avoid their transmission, as is done in amphibian monitoring studies. Thus was born the idea of this handbook of best environmental practices for the discovery of amphibians and riparian habitats, aimed at environmental education institutions, nature schools and regular schools. This is a collection of best practices aimed at disseminating the problems associated with these diseases and raising awareness among the population so that these activities can be carried out without introducing pathogens into the environment.

So what you have here is a document with recommendations that we hope will be useful both in the Montseny and throughout the territory.



The equipment is disinfected very carefully during field work (IR)

What is biosecurity?

This concept refers to all health, management and training measures aimed at preventing or reducing the risk of infectious diseases entering a given environment. In this handbook, when we mention biosecurity, we refer to the disinfection and management guidelines that we recommend applying in discovery activities of riparian habitats to prevent the transmission of emerging diseases to amphibians.

Chytridiomycosis and ranavirus infection are infectious diseases that seriously affect amphibians

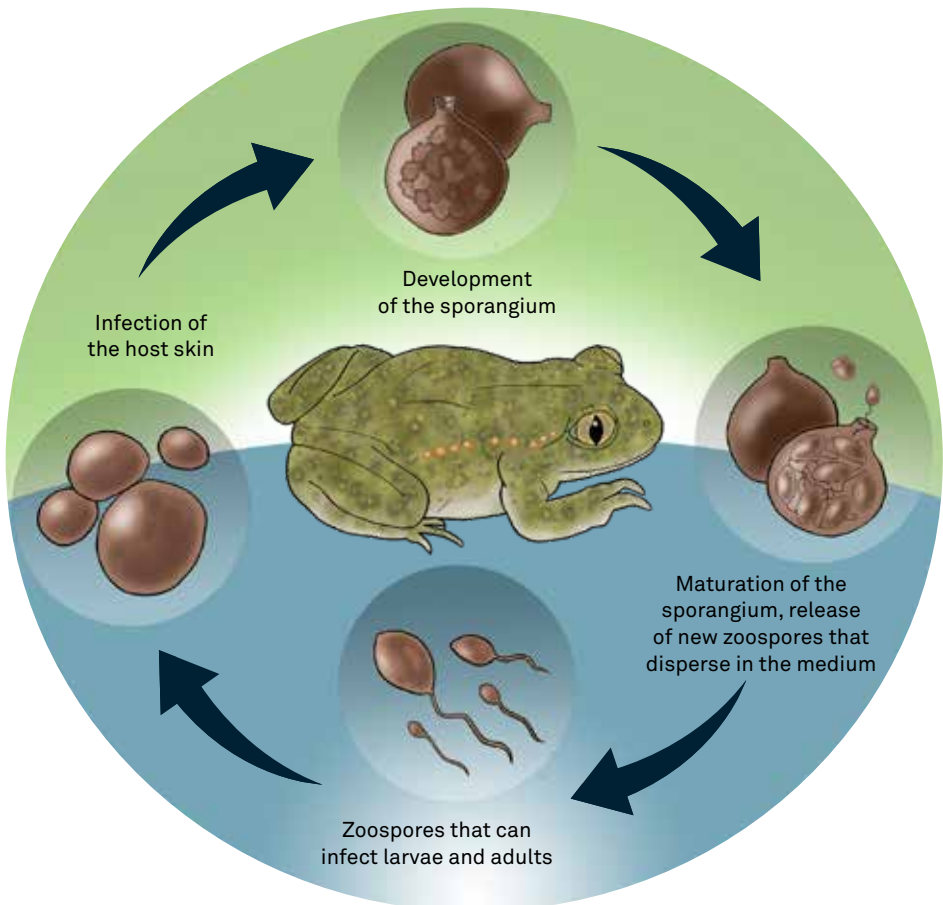
What are emerging diseases?

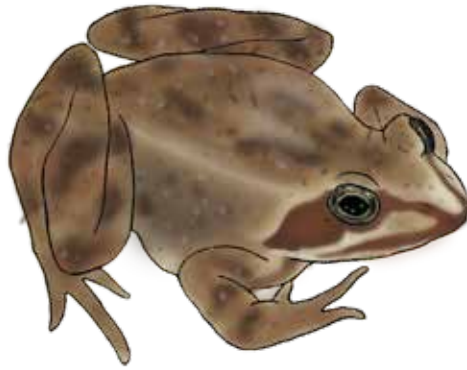
Emerging diseases are diseases caused worldwide by the infection of fungi, bacteria or viruses that colonise new territories, especially as a result of trade and trafficking in exotic species that carry these pathogens. The arrival of these diseases was described for the first time in Europe in 1997, when a significant mortality of midwife toads was detected (*Alytes obstetricans*) on the mountain of Peñalara (Sierra de Guadarrama National Park).

The emerging diseases that mainly affect amphibians are **chytridiomycosis** and infection by **ranavirus**.

Chytridiomycosis

Chytridiomycosis is a disease caused by microscopic fungi of the genus *Batrachochytrium*: *B. dendrobatidis* (*Bd*), which especially affects anurans (frogs and toads), and *B. salamandrivorans* (*Bsal*), which mainly affects salamanders and newts. This fungus colonises the keratinized areas of the skin: in the case of larvae, keratin is found only in the mouth area; in adults, it spreads throughout the skin. Once the chytrid has infected the host, it forms a kind of sac (the sporangia) containing zoospores, mobile reproductive cells that are released into the water and can infect other amphibians or the individual producing them. The skin of infected amphibians loses keratin and has many micro-wounds that prevent the proper exchange of water and ions through the skin, resulting in the infected amphibians dying from metabolic imbalance produced by the loss of regulation.





A red frog (*Rana temporaria*), which is found in the Pyrenees and in the Transversal - Montseny mountain range. In the United Kingdom, red frog populations have declined significantly due to this virus.

Ranavirus

Ranavirus is a virus of the Iridovirus family that infects amphibians, reptiles and fish. Ranavirus infections in amphibians have been shown to contribute to the decline of amphibian populations worldwide. In the Iberian Peninsula, massive mortalities have been reported in Galicia and northern Portugal. Transmission occurs through multiple routes, including contaminated soil, direct contact, exposure through water, and ingestion of infected tissue during predation. These viruses enter the host cell, where virus replication and subsequent cell death occur. Lesions associated with ranavirus infection include generalised inflammation, inflammation of the extremities of the liver, and haemorrhage as the most common symptoms.

Transmission
of this virus
occurs
through
multiple
routes

Can we be vectors of these diseases?

A large number of microorganisms live on our skin, some of which can cause diseases in amphibians if we touch them directly.

If we have visited an area where these pathogens are present, we can carry their spores from one place to another through clothing (especially if it is damp or wet), in the mud on shoes or hand nets, or even with our hands if we have touched an infected individual. Given the ease of transmission of these diseases, we must act very carefully in the discovery activities of continental aquatic environments.



Footwear disinfection by immersing it in a tray with the disinfectant product (DF)

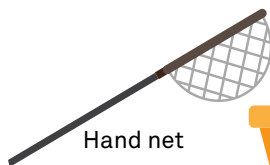
What can we do to prevent the spread of these diseases?

To prevent the transmission of these diseases, we must act very carefully in the discovery activities

By following biosafety rules properly, we can avoid being the vectors of these pathogens. All equipment, both footwear and field tools, must be disinfected at the beginning and end of the activity. For added safety, it is advisable to expose boots, hand nets, trays and containers to the sun for a few days in order to achieve total drying. This way we ensure that the zoospores die.

It is also advisable to carry out the activity in a single location. If despite this recommendation we have to visit different water points or streams, we must have as many sets of equipment (hand nets, trays and containers) as the sites we have to visit, and we must disinfect our footwear before and after each activity.

The equipment must be exposed to the sun to ensure that zoospores die.



Hand net



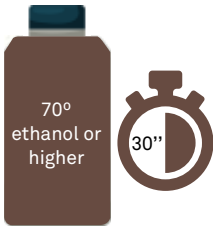
Tray



Footwear

How should we disinfect ourselves before and after the activity?

Of the various disinfectant products that can be used, we suggest those with the lowest impact on the environment:



RECOMMENDATIONS FOR THE APPLICATION OF VIRKON S®



Virkon S® is a product used for the disinfection of livestock facilities that is highly effective in eliminating viruses, fungi and bacteria. This disinfectant is one of the most recommended because it degrades in ten days. It should be prepared the day before or on the same day of use, as it loses effectiveness over the following days. For the preparation, we must follow all the recommendations specified by the manufacturer in the product safety data sheet and apply all necessary personal protective measures, as this product is marketed in powder and can be irritating.

Guidelines for disinfection

NECESSARY EQUIPMENT







FOOTWEAR DISINFECTION

1

Brush the footwear thoroughly to remove traces of mud and organic matter.



SMALL GROUPS

It is more effective to disinfect the footwear by applying the sprayed product.



LARGE GROUPS

The product is best optimised if the footwear is disinfected by immersing it in a tray with the disinfectant product.

2



3



The resulting product should be collected in a closed container.



Disinfection should never be carried out right next to the body of water. Preferably, it should be done in an area with little vegetation.

DISINFECTION OF FIELD EQUIPMENT

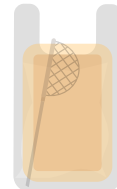
START OF THE ACTIVITY

The equipment must be cleaned and disinfected before arriving at the activity site.



END OF THE ACTIVITY

The equipment must be disinfected at the end of the activity and stored in bags. When we get to the storage area, we should clean it again.



Exposing footwear, clothing and equipment to 40 °C for 30 minutes is also an effective and simple method. So, after disinfecting everything in the storage area, letting it dry in the sun is a good option.



We should never handle amphibians directly with our hands (DF, left and GP, right)

Why is it not advisable to handle amphibians in educational activities?

The handling of amphibians can lead to various effects on the specimens. In addition to the risk of transmission of pathogens by contact, we must keep in mind that their bare skin is covered with mucus that helps them regulate the exchange of water and electrolytes.

It is not advisable to hold an amphibian directly with our hands, as this could cause skin irritation, which could lead to moulting, an increase in body temperature and accelerated desiccation. We must be aware that, by holding an amphibian, we are causing unnecessary stress.

Observation is a viable alternative to manipulation for the study and understanding of amphibians, as it is in other faunal groups, such as birds. Learning how to observe and listen, and teaching non-manipulation aims at a paradigm shift, the achievement of values that give relevance to the way we interact with nature and with these species, and it helps us understand why these species are so sensitive and delicate.

Observation is a viable alternative to manipulation for the study and understanding of amphibians





Handling of a spadefoot toad (*Pelobates cultripes*) (GP)

When manipulation is essential, we must consider it as a one-off and short-term activity and explain it to the people involved.

TIPS FOR PROPER AND RESPONSIBLE HANDLING

The animal must always be released at the exact same place where it was captured

1



One or two people on the educational team should be responsible for handling.



2

It is essential to wear powder-free gloves. Latex gloves are not suitable, as they can cause alterations in the skin of amphibians.

3



The specimen must be held gently, without excessive pressure, to avoid stress or injury.

4

We should hydrate its skin often with water from the same place where the specimen was caught.



In the case of aquatic urodeles, they must be kept trapped inside a tray during observation.

5



We should change our gloves every time we handle a different specimen.



Population monitoring of the Montseny brook newt (*Calotriton arnoldi*). (IR)

Do we need a specific permit for handling amphibians?



For the handling of amphibians or any protected wildlife species we need a specific authorisation that is regulated by the Animal Protection Act¹ and the Natural Heritage and Biodiversity Act². Authorisation to capture wildlife specimens for scientific, management or educational purposes must be requested from the Servei de Fauna i Flora de la Generalitat de Catalunya (Fauna and Flora Service of the Government of Catalonia). More information can be found on the website of this service, where the procedure can be accessed directly³.

Authorisation to capture wildlife specimens must be requested

We should not release amphibians from elsewhere!

Years ago, the capture of tadpoles at water points to study the metamorphosis of amphibians was a very common practice. These larvae

1. Legislative Decree 2/2008, of 15 April, approving the revised text of the Animal Protection Act.

2. Law 42/2007, of 13 December, on natural heritage and biodiversity (Spanish Official State Gazette no. 299, of 14 December 2007).

3. <http://territori.gencat.cat/ca/tramits/tramits-temes/Autoritzacio-per-a-la-captura-dexemplars-de-la-fauna-salvatge-per-motius-cientifics-de-gestio-o-educatiu>



Capture of tadpoles and adult specimens is not allowed (GP)

**Almost all
amphibian
species
present in
Catalunya are
protected**

were kept in an aquarium until they became adult specimens and were then released back into the aquatic environment, either in their place of origin or elsewhere. Current legislation on the protection of animals, the natural environment and biodiversity prohibits moving animals from one place to another, i.e., translocation without specific authorisation, both in the adult, larval and egg-laying stages. Almost all amphibian species present in Catalunya are protected; therefore, their capture requires explicit consent and is only authorised for the conservation of the species. Apart from protection purposes, releasing an animal elsewhere, whether the same species is present or outside its range, increases the risk of disease transmission, can cause severe alterations in recipient populations and abort microevolutionary processes through genetic contamination.

In the event that we find an amphibian trapped in a pond or water point from which it cannot get out, we must assess the situation. If the point is accessible and our safety is guaranteed, we can pick it up applying the biosecurity measures described in this handbook and leave it in the vicinity of the water point. The animal should never be transported to another location, as it could be a carrier of diseases. If we cannot guarantee our safety, we should contact the rural agents to inform them of the animal's situation so that they can assess whether it is necessary to proceed with the rescue.



A specimen of Anatolian crested newt (*Triturus anatolius*), an exotic species detected in Parc del Montnegre i el Corredor. This species is an asymptomatic carrier of infectious diseases and also hybridises with the marbled newt (*Triturus marmoratus*) (WYD).

What should we do if we find a dead amphibian or an introduced species?

When we find a dead amphibian (unless it has been run over) or an introduced species, we must notify the Cos d'Agents Rurals (Corps of Rural Agents) or the Servei de Fauna i Flora de la Generalitat de Catalunya (Fauna and Flora Service of the Government of Catalonia) and inform them of its location. It is very important to warn of the presence of an exotic amphibian because it can be a carrier of pathogens of emerging diseases. Whether it is a dead specimen or an exotic species, we should not touch it directly; handling should always be done with gloves. If it is captured, it must be kept in a sterile container until it is collected.

It is very important to warn of the presence of an exotic amphibian



Cos d'Agents Rurals
+34 93 561 70 00

Servei de Fauna i Flora
+34 93 495 80 00

Useful links and information

AMAT, Fèlix. *Guia d'identificació d'amfibis: Reserva de la Biosfera del Montseny (Amphibian Identification Guide: Montseny Biosphere Reserve)*. Barcelona: Diputació de Barcelona, 2017.

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Catalan Health surveillance programme. 2018. Servei de Fauna i Flora Generalitat, 2017

SOCIETAT CATALANA D'HERPETOLOGIA. *Fitxes d'amfibis i rèptils aquàtics (Sheets of amphibians and aquatic reptiles) [online].* <<https://soccatherp.files.wordpress.com/2020/01/fitxes-herpetofauna-aquatica-de-catalunya.pdf>>



Decalogue to prevent the transmission of infectious diseases in amphibians

- 1 Prevention and disinfection recommendations (biosecurity measures) must be scrupulously followed during activities for the discovery of inland aquatic habitats.
- 2 The persons responsible for the activity must know and apply biosecurity criteria and standards.
- 3 All equipment and footwear should be cleaned and disinfected before and after the activity with the recommended products.
- 4 It is not allowed to capture adult specimens, eggs or tadpoles from the natural environment to take them home, to schools or other places even for educational purposes.⁴
- 5 A specific authorisation from the Servei de Fauna i Flora de la Generalitat de Catalunya (Fauna and Flora Service of the Government of Catalonia) is required for the capture of wildlife specimens for scientific, management or educational purposes.
- 6 Activities that promote the observation of amphibians should be encouraged in order to avoid manipulating them.
- 7 If the handling of specimens is essential, it should always be done with disposable nitrile gloves and avoid using previously used gloves. Moreover, it should only be done by members of the educational team.
- 8 It is advisable to do the activity in a single pond or stream, but in the event that more than one water point is visited on the same day, a different set of clean and disinfected equipment must be used for each location.
- 9 Moving animals from one place to another is prohibited. The translocation of individuals, even if they are a native species, can cause severe alterations to ecosystems.
- 10 It is forbidden to leave specimens of non-native species in the environment, as they can have a catastrophic effect on native flora and fauna.

⁴ Animal Protection Act and Natural Heritage and Biodiversity Act.



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