

# MAKE AN INVENTORY. THE AFRICAN CLAWED FROG

#### TECHNICAL SHEET

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This technical sheet was produced within the framework of the European LIFE CROAA program "Control strategies Of Alien invasive Amphibians - 2016-2022" (LIFE15 NAT/FR/000864) whose main objective is to contribute to the improvement of the state conservation of native amphibian species, in particular by preserving them from the impact of invasive alien species.

This program is coordinated by the Société Herpétologique de France (SHF), and carried out in collaboration with seven partner structures.

Find our more: www.life-croaa.eu

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#### LIFE CROAA project partners

















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### AFRICAN CLAWED FROG, AN INVASIVE EXOTIC SPECIES IN FRANCE

An <u>invasive alien species</u> is defined as a species introduced by man outside its natural range (voluntarily or fortuitously) and whose establishment and spread threaten ecosystems, habitats or native species with ecological consequences. and/or economic and/or negative health (<u>IAS Resource Centre</u>).

Originally from southern Africa and introduced to France several decades ago, the African clawed frog (Xenopus laevis) is now one of France's invasive alien species. The species is listed in <u>annex 1 of the ministerial decree of February 14, 2018</u>, which means that its introduction is prohibited on national territory.

Widely used in research laboratories since the 1950s, this species was released in Deux-Sèvres following the closure of a breeding centre for animal experimentation.

It thus colonized several departments of metropolitan France, such as Deux-Sèvres, Maine-et-Loire, Vienne and Loire-Atlantique. Three new populations were discovered in Gironde (2015), in the North (2018) and in Haute-Garonne (2019).

#### Learn more about invasive alien species regulations

Several legal texts address the issue of invasive alien species at national, European and international levels. In France, the National Invasive Alien Species Strategy was drafted in 2016. It aims to protect marine, freshwater and terrestrial ecosystems, as well as the animal and plant species they host, from the risks and effects associated with biological invasions. Its general objective is to strengthen and structure collective action concerning prevention and awareness, the establishment of surveillance and rapid reaction systems, long-term management means, including the restoration of ecosystems, and the improvement of knowledge.

Guided by these European and national strategies, study and control actions against the clawed frog have been tested by professionals from local authorities and environmental associations (<u>LIFE CROAA project</u>), in order to identify the species, limit its dispersion and if possible to reduce its impact on the natural environment.

The purpose of this sheet is to present the identification criteria for African clawed frog (all stages) and the methods for detecting this species, at the juvenile and adult stages.

→ Learn more about Species targeted by LIFE CROAA



## KNOW HOW TO RECOGNIZE THE AFRICAN CLAWED FROG

The African clawed frog has physical characteristics that differentiate it quite easily from other species of amphibians present in France..

See also these attached photos 1.

#### Laying and eggs

- Laying consists of 300 to 2,500 free-living eggs, isolated or grouped together.
- The eggs are light brown and bicolour (lighter below than above).
- Size: about 1mm.





#### **Tadpole**

- Maximum size: 8 cm.
- Shape similar to that of a small fish with
- the end of the tail very tapered.
- Transparent head.
- Double and side breathing ports.
- Anal opening opening in the axis of the body.
- Eyes in lateral position.
- Wide mouth with two side barbels similar to catfish.
- Moves in a reclined position, head down.



#### Juvenile

- Small individual without tail, up to about 5 cm.
- Moves mainly at the bottom of the pond by moving the front legs.





#### Adult

- Adult size included in 7 and 12 cm.
- Flattened profile.
- Smooth skin, very viscous and slippery.
- Skin mottled with dark spots on a light brown
- background. Lateral sutures, on the flank,
- the throat and the belly. Short, rounded muzzle.
- Absence of eardrum and tongue.
- Circular pupils with golden irises,
- placed above the head.
- Highly developed thighs of hind legs.
- Black claws on the first three toes (hind legs).
- Very developed webbing of the toes.

reduced front legs - smooth and slippery skin - highly developed hind legs - eyes placed on top of the head - lateral sutures - black claws on the first three toes.





(c) Matthieu Berroneau

#### Adult female

- enerally larger than the male.
- Has a cloaca (duct-like organ) protruding between the hind legs.

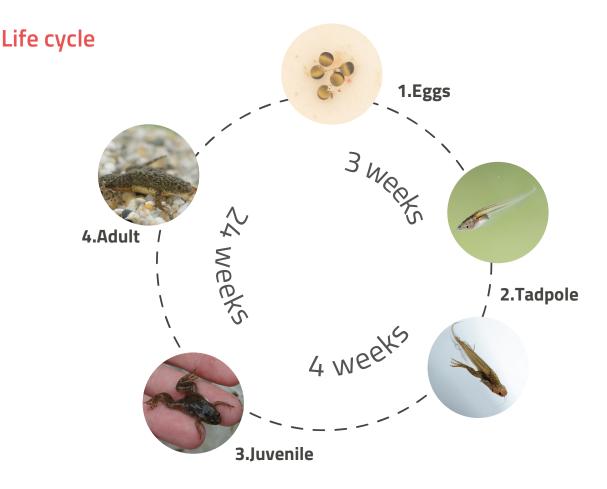


#### Adult male

- Cloaca is less conspicuous than the female.
- During the breeding season, shows black nuptial calluses on the inside of the forelegs.









- 1. The spawning process is triggered as soon as the water temperature reaches 15°C, but spawning is most often found when the water temperature approaches 19-20°C. With eggs usually scattered and isolated, they have no specific shape.
- 2. Females ovulate throughout the year, allowing multiple generations of tadpoles to be observed at the same time. With their 2 barbels, tadpoles look like catfish. They are transparent and very fragile. They move in small groups, head down and waving tail, usually between 2 waters. After a few weeks, the hind and forelegs appear, the tail and barbels begin to disappear. During this stage, their low speed of movement in the water makes them easy prey.
- 3. Juveniles spend most of their time moving around the bottom of the pond. To feed, they move their front legs in the mud to bring prey (molluscs, eggs, small fish, etc.) up to their mouth.
- 4. Sexual maturity can be reached as early as six months after metamorphosis.
- 5. Adult reproduction takes place from May to August. Their ability to move is impressive, from 1 to 5 km per year if the conditions are favourable (depending on the quality of the ecological corridor). African clawed frog has a wide range of prey (mainly other amphibians, insects, crustaceans). The species can live up to 10 years.

(c) photos tadpole with adult legs: Mathieu Berroneau



### ARE THERE AFRICAN CLAWED FROGS IN MY BODY OF WATER?

The African clawed frog is a species that is difficult to detect, which lives mainly underwater, at the bottom of the aquatic areas it occupies. In order to ensure its absence or to confirm its presence, it is advisable to carry out regular inventories of your water features (pond, pond, lake, etc.). The earlier the detection of individuals, the faster the implementation of control actions can be programmed and the local biodiversity preserved.

#### 1. Prerequisites for an inventory

#### 1.1 Obtaining Waivers

Whatever the stage of development, your actions to capture African clawed frogs are likely to result in the accidental capture of other amphibian species. The latter are all protected in France according to the Order of January 8, 2021 fixing the list of amphibians and reptiles represented on the metropolitan territory protected throughout the national territory and the methods of their protection.



Any capture and manipulation, even temporary, of protected species is therefore subject to obtaining a derogation which is the subject of a prefectural order. This derogation request must be submitted to the Regional Environment and Housing Development Department (DREAL) in your region. The lead times can be quite long, think about doing it in advance.

As head of the national network, the SHF coordinates control actions for this species, so we invite you to contact us before any inventory or trapping operation in order to:

- Respecter la réglementation;
- Connaître les modalités de prise en charge des individus;
- Remonter vos données d'observations et de capture.

#### >> contact@lashf.org

#### 1.2 Protecting ourselves and the environment

#### **Hygiene protocol**

Before and after any handling of amphibians, a hygiene protocol must be followed to prevent the transmission of diseases specific to these species, such as ranavirose or chytridiomycosis, responsible for the death of many species of amphibians, reptiles or even of fish (see access to the protocol on page 9).

#### Field clothing

Clothing **covering at least the legs and thighs**, with boots or hiking shoes is recommended in the field. **Over-trousers** such as a fishing raincoat can be used and will be easy to disinfect with a disinfectant such as Virkon®.



The outfits of the agents carrying out the capture actions should ideally be washed every week to avoid storing any pathogens on the clothes. This disinfection step must be carried out at a certain distance from water points to avoid any release of the product into the aquatic environment.

**Rubber gloves** are essential for handling individuals in order to avoid contact with their mucus, but also contact with water contaminated by the possible presence of nutria (leptospirosis). Wearing gloves for trapping in a wastewater treatment plant (STEP) is also compulsory in order to avoid biological risks such as the presence of viruses in the water (model of gloves recommended in the equipment and cost section of each technique of trapping).

We recommend that you wear a light life jacket during field operations near deep water bodies. As a safety measure, it is also advisable to work in pairs on most actions.

Find the protocol for disinfection and use of Virkon® on the SHF website: <a href="mailto:lashf.org/fiches-techniques/">lashf.org/fiches-techniques/</a> > Section "Our other technical sheets" > "Hygiene protocol for amphibian disease control in the field".





#### 2. Equipment and inventory costs

The cost of your inventory may change depending on the surface of the area to be studied. The recommended equipment for carrying out the inventory is as follows:

- Landing net: small professional landing net with 200 mm frame and net with mesh from 1 mm to 5 mm maximum. Around €100 per unit depending on the model.
- Trap with net with double entrances, foldable, round or square. Catfish trap type. Fine stitches from 1 to 10 mm maximum. Average dimensions of 30 x 60 cm. From 10 to 20€ per unit depending on the model. These traps adapt perfectly to shallow water bodies. NB: The trap must be equipped with a surface holding device (empty plastic bottle or pool rope float). This device allows captured species to breathe on the surface and avoid drowning while waiting for the traps to be changed (every 24 hours).

#### Choose a trap adapted to the biodiversity in the field

Two types of foldable traps with double entrances can be used in the field:

- Trap with 10 mm mesh.
- Trap with finer mesh of 1 to 5 mm maximum.

The use of one or the other will depend on the biodiversity found in your body of water and more particularly on the presence or absence of newts.

Indeed, the use of fine-mesh traps quickly became the rule for all water points that could accommodate newts. Indeed, a high risk of mortality by drowning has been observed for these species with traps with meshes that are too wide (the heads of individuals remaining stuck in the meshes of the trap).

Traps with a mesh of less than 5 mm are therefore very strongly recommended for sites rich in amphibians, in particular as soon as the presence of newts is possible.

Traps with 10 mm mesh are recommended for plans of water occupied by fish, or purification lagoons, where no newts are likely to be present. These traps have the advantage of being stronger and less expensive.





• String: To attach the trap to a fixed element placed on the bank (tree, fence post or rebar to be installed yourself), in order to facilitate its recovery during surveys. Ref: Corderie Mesnard, 200 m spool, standard halyard, Ø 2 mm. €10.50 per spool.



- Bait: Dog food (about €6.5/kilo) is to be used to bait your traps and try to improve your catch rate. Place these baits in a small sausage of about 5 cm, in a small resealable net (usually sold with the trap). Be careful, however, they are likely to attract other exotic species potentially present in your bodies of water (crayfish, catfish, etc.). These invasive species must never be returned to the water (for more information, consult the <a href="Invasive Alien Species Resource Center">Invasive Alien Species Resource Center</a>). With regard to the other catches (native species of fish and amphibians), it will be necessary to check the traps every 24 hours to release the individuals not concerned by your capture plan.
- Buckets: Buckets with hermetic lid of 30 L. For the conditioning of the individuals sampled. €10 to €15 per unit depending on the model (example of supplier: Rolléco €7.28 per unit). NB: Pour some water into your buckets before removing the individuals.
- Environmental DNA sampling kit: Includes a sterile filtration capsule, a syringe, a 100 ml sampling ladle, two pairs of sterile gloves, a 2L bag and a buffer solution to store the eDNA once filtered. A kit allows the sampling of a water point with a maximum surface of 1 ha. About 150€/kit for the purchase. 10€ for sending the kit to the laboratory for analysis.
- Gloves: Waterproof dishwashing gloves with sleeves. Choose a model that is neither too wide nor too tight to put them on and take them off easily and maintain a flexible grip. They can be disinfected, washed and used several times; however, be sure to choose a resistant model. From 5 to 7 € per pair depending on the model.
- Fishing Boots: All-terrain rubber boots. They will have to be cleaned systematically from one water point to another (see hygiene measures). From 40 to 100 € per pair depending on the model.
- Waders [optional]: Neoprene material for fishing (entering the water). They will have to be cleaned systematically from one water point to another (see hygiene measures).
   From 65 to 140 € per unit depending on the model.
- Jumelles [optionnel]: For occasional observation of individuals from dawn to dusk.

  From €300 per pair to benefit from professional equipment and/or Polarized glasses
  [optional]: reduces reverberation and increases contrast to better spot tadpoles in the water. From €100 per unit depending on the model.
- Headlamp: 100 lumens max. Minimum €50 per unit depending on the model.
- Rain gauge: To indicate the level of precipitation on your field sheet during your
  inventory sessions (see "rainfall" in appendix 2). From 20 to 80 € per unit depending on
  the model.

If your equipment is used on several sites, consider systematically disinfecting it, as well as your clothing between each site, so as not to spread pathogens harmful to native amphibians. Find the disinfection and use protocol for Virkon® on the SHF website:

lashf.org/fiches-techniques/ > Section "Our other technical sheets" > "Hygiene protocol for the control of amphibian diseases on the ground".



#### 3. Inventory process

The objective is to detect the presence of African clawed frogs in a defined area (the body of water) during several visits spread over the breeding period of this species. It does not necessarily require significant human resources (1 to 3 people depending on the surface of the study area). According to field observations and scientific literature, African clawed frogs are active as soon as the water temperature reaches 14-15°C, with optimum activity around 19 to 22°C (see study by Casterlin, ME and Reynolds, 1980. Hydrobiologia). Remember to regularly check this data using your thermometer in order to best optimize your capture plan.

#### Step 1: The laying if the trap

 Periodicity: 3 visits minimum are to be made in the evening, during the breeding period (from the beginning of May to the beginning of September) to increase the chances of detection of the species.

#### Implementation

For an optimal capture rate, count 2 to 3 traps for 100 m<sup>2</sup>. If the surface area of your water point is greater, plan a number of traps proportional to the aforementioned indication for 100 m<sup>2</sup>. For bodies of water from 800 m<sup>2</sup>, it will be necessary to cap the number of traps at 10 maximum, in order to optimize your capture sessions (logistics and time spent).

**Position your traps** in the evening (previously baited with dog food placed in a sausage of about 5 cm per trap) close to the banks (1 or 2 m) and at the edge of the seagrass so that they touch the bottom a little. If the pond is very shallow (< 30 - 40 cm), they can also be placed in the middle of the pond.

The set time for the traps must be a full night to ensure the capture of the individuals (with a reading after 24 hours maximum).

As a reminder, the traps must imperatively float and be placed horizontally so that the captured individuals can come up to breathe (place a float or an empty plastic bottle inside the trap to create buoyancy). They must also be firmly attached using a string to a fixed element (post, tree) positioned on the bank so that they do not drift.

All the traps are cleared the next morning. African clawed frogs captured at each pass must be counted (if possible, indicate the number of individuals captured by stage and sex - see sheet proposed in appendix 2) and placed in the buckets provided for this purpose (pour a background of water in the receptacles before placing African clawed frogs there to limit their stress).







#### Step 2: Dip net fishing

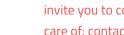
We recommend using a landing net about ten times if visual detectability is not satisfactory (for example if the water is turbid or if more than half of the site is vegetated). Take care not to damage the aquatic vegetation or the bottom of the body of water when using your landing net: make slow movements, passing close to the bottom without removing the substrate or the sediment.

The trapped clawed frogs are counted and placed in buckets (with water bottom). They must be quantified and separated by date of capture and by basin (see appendix 2). Perform this step at least twice on different days.





Reminder: in order to proceed with steps 1 & 2, a derogation for the capture of protected species is required (cf. 1. Prerequisites for an inventory).



As head of the national network, the SHF coordinates control actions for this species, so we invite you to contact us before any trapping operation to find out how individuals are taken care of: contact@lashf.org

#### Step 3: Prospect

During the day or preferably in the early evening, prospect the aquatic site by visual identification (eggs, larvae, adults) using a flashlight if necessary (a recommended power of around 100 lumens), for 10 15 mins. For large bodies of water, several observation points can be made while respecting a distance of 100 m between two points.

African clawed frogs live underwater but juveniles and adults frequently come to the surface to breathe and can thus be observed. Perform this step at least twice on different days.

#### Step 4: Environmental DNA testing

If the absence of the species is proven following the first three steps, this hypothesis should be verified using environmental DNA (eDNA) tests, especially for large bodies of water. This technique aims to trace the specific DNA molecules left in the environment by organisms by taking water samples, which will make it possible to detect the presence of the species at low densities.

Sampling should be avoided in the event of heavy rain or thunderstorms and can be carried out day or night. A kit allows the sampling of a water point with a maximum surface of 1 ha. If the prospected water point is of a larger area, several kits will be used.

- For each sampling point: stir the water column using the ladle to collect homogenized water and maximize the probability of detecting the species.
- In order to avoid any contamination of the samples, it is essential to wear sterile gloves and to take the samples from the bank without stepping into the water. For 1 ha, 20 samples of 100 ml, evenly distributed over the body of water to be sampled, are taken (approximately one sample every 20m) and stored in the 2L bag.



- After collecting the samples, the bag containing all the water samples is in turn homogenized and then injected into the filtration capsule using the syringe. After having filtered all the water in the sachet and expelled the excess water using the syringe filled with air, the filtration capsule is filled, in the direction of the flow, with the buffer solution provided in the kit, then stirred for almost 1 minute.
- The capsule(s) are referenced (date, mesh number, sample number, contributor) and stored vertically, always in the direction of the flow, in their packaging at a stable temperature.

The samples must then be sent to a specialized laboratory for analysis.











## ACCOMPANIMENT AND FOLLOW-UP

As head of the french national network, the SHF coordinates control actions for this species, so we invite you to contact us before any trapping operation in order to:

- Be accompanied for the implementation of a procedure respecting the regulations;
- Know the methods of taking care of individuals;
- Upload your observation and capture data
  - For each capture technique and during your trap readings, record your observations of African clawed frogs in a field sheet (see an example in appendix 2). Any observation of other amphibian species (by visual, auditory observation, or capture) must also be mentioned in your field sheet..
  - Enter all your data respecting the elementary exchange data of the SINP (DEE). If you
    do not have a suitable tool, the SHF makes its own available to you to enter your
    amphibian and reptile data by creating a dataset adapted to your structure and your
    program (metadata):

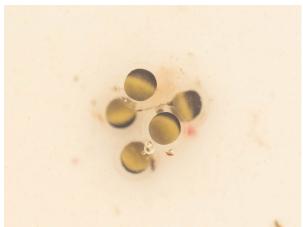
>> Contact us: contact@lashf.org



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#### **ANNEX 1**



(c) Axel Martin



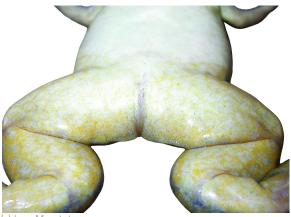
(c) Claude Miaud



(c) Axel Martin



(c) Matthieu Berroneau

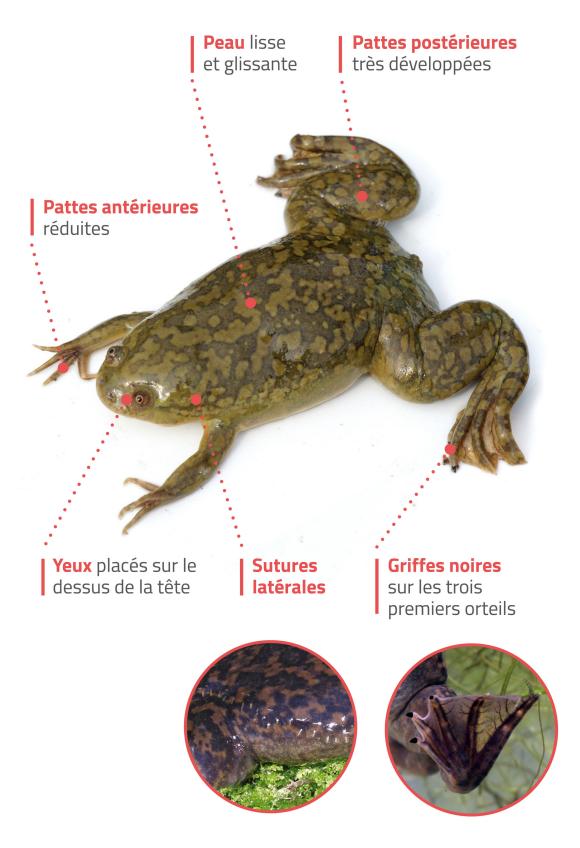






(c) Jean Muratet





(c) Matthieu Berroneau



LAND SHEET Observation of African clawed frog - Passage n°		
Observer name:	Date:	
Geographical coordinates, name and description of	the environment:	
Air (°C) and water (°C) temperature	**************************************	3
Weather report	Rainy / cloudy / sunny	
Rains	**************************************	
African clawed frog	Observation method	Quantity
Spawning	Visual observation / landing net	
Tadpoles	Visual observation / landing net / trap	NB 8:
luvenile.s	Visual observation / landing net / trap	SE SU RA AS
Adult.s Female.s	Visual observation / landing net / trap	22
Adults Males	Visual observation / landing net / trap	30
TOTAL		3
Other species (specify species, stage and sex):	Observation method	Quantity
	Visual observation / landing net / trap	
	Visual observation / landing net / trap	87 33
	Visual observation / landing net / trap	60 3
	Visual observation / landing net / trap	
	Visual observation / landing net / trap	
TOTAL		8 8





#### LIFE15 NAT/FR/000864





