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LIFE CROAA

Control stRategies Of Alien invasive Amphibians

Final report

LIFE15 NAT/FR/000864

Société Herpétologique de France

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LIFE project number

LIFE15 NAT/FR/000864

Final Report

Covering project activities from 01/09/2016 to 31/08/2022

Reporting date

30/11/2022

LIFE project name or acronym

LIFE CROAA – LIFE Control stRategies Of Alien invasive Amphibians

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Project location	France
Project Start Date	01/09/2016
Project end date	31/08/2022
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EU contribution	€1,878,040.34
% of eligible costs	60 %

Data beneficiary	
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Completeness and accuracy of the file

Mandatory elements	**✓ or N/A**
Technical report	
The last template for the type of project concerned (e.g. traditional) was used and all sections were completed, in English. *Only in electronic version*	✓
Index of deliverables with a brief description in the appendix, in English. *Only in electronic version*	✓
Final report: Deliverables not already submitted with the mid-term report attached, including the extension report and the post-LIFE plan. Deliverables written in one or more languages other than English include an English summary. *Only in electronic version*	✓
Financial Report	
The reference period in the financial report (consolidated financial statement and financial statement of each individual beneficiary) is the same as in the technical report, except for any terminated beneficiary for which the end period must be the termination date.	✓
Consolidated financial statements with 5 forms duly completed, signed and dated Signed electronically or if original paper submission dated and signed* and in electronic version (pdfs of signed sheets + complete Excel file)	✓
Financial statements of the coordinating recipient, each associate beneficiary and each affiliate (if applicable), with all completed forms (signed and dated). The financial statements of beneficiaries with subsidiary(s) include the total cost of each subsidiary on 1 line per cost category. *In electronic version (pdfs of signed sheets + complete Excel files) + in the case of the final report the overall summary sheets of each beneficiary electronically signed or if paper submission, signed and dated originals**	✓
The amounts, names and other data (e.g. bank account) are correct and consistent with the grant agreement / in the different forms (e.g. the figures in the individual statements are the same as those indicated in the consolidated financial statement)	✓
Beneficiary certificate for non-expendable property included (if required, i.e. beneficiaries claiming 100% of the cost of non-expendable property) *Electronic signature or if paper submission original dated and signed* and in electronic version (pdfs of signed sheets)*	✓
Attestation on financial statements (if necessary, i.e. for beneficiaries with an EU contribution ≥ € 750,000 in the budget) *Electronic signature or if original signed paper submission and electronic version (pdf)*	N/A
Other audits	
Additional information/clarifications and supporting documents requested in previous Agency letters (unless already submitted or not yet due) *Electronic version only*	✓
This table, page 2 of the final report, completed - each box is checked *Electronic version only*	✓

**signature by a legal or statutory representative of the beneficiary/affiliate concerned*

NOTE

This report has taken into account, to the extent possible, the recommendations provided by the Agency regarding the framework of the report. The number of pages for the technical part (25 pages max.) was not respected, although the information was synthesized. It is important to note that LIFE CROAA is structured around 17 actions, the vast majority of which are broken down into sub-actions. On the other hand, eight beneficiaries worked on this project, so there is a lot of information to convey in the final report. In fact, starting the description of each action on a new page, it was impossible to limit yourself to 25 pages.

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2 LIST OF KEYWORDS AND ABBREVIATIONS

CCT: Community of Communes of Thouarsais
CDPNE: Loir-et-Cher Departmental Committee for the Protection of Nature and the Environment
CEN: Conservatory of Natural Areas
CINEA: European Climate, Environment and Infrastructure Executive Agency
CN: Cistude Nature
CROAA: Control stRtagies Of Alien invasive Amphibians
DDT: Departmental Directorate of Territories
DREAL: Regional Directorate for the Environment, Planning and Housing
EASME: Executive Agency for the Small and Medium-sized Enterprises
eDNA: DNA of the species present left in their environment
FTEs: Full-Time Equivalent
IAS: Invasive Alien Species
ISSG: Invasive species specialist group
IUCN: International Union for Conservation of Nature
MNHN: Muséum National d'Histoire Naturelle
OFB: French Office for Biodiversity
PNRLAT: Loire-Anjou-Touraine Regional Natural Park
PNRLG: Landes de Gascogne Regional Natural Park
PNRPL: Périgord-Limousin Regional Natural Park
SEBB: Syndicat d'Entretien du Bassin du Beuvron
SHF: Société Herpétologique de France
UA: University of Angers
UCO: Catholic University of the West
WWTP: Wastewater treatment plant

3 SUMMARY

Since 2016, the European project LIFE CROAA (Control stRategies Of Alien invasive Amphibians – LIFE15 NAT/FR/000864) aimed to protect indigenous amphibians from the introduction and spread of invasive alien species (IAS), in particular the American Bullfrog (*Lithobates catesbeianus*) and the African clawed frog (*Xenopus laevis*).

This project was born from the alarming observation made in the face of the arrival of invasive amphibians on the territory of metropolitan France. Indeed, IAS are recognized as one of the leading causes of global biodiversity erosion (UNEP, 2005; IPBES, 2019). In particular, they represent a danger to all indigenous species, in particular amphibians, of which about 40% of the species are threatened worldwide (IUCN World Red List). In addition to the threat posed by IAS to local amphibians, habitats are destroyed and fragmented, air, water and soil pollution, the removal of individuals from the natural environment and global warming.

Specifically, IAS are considered to be the third largest pressure on threatened species at European level, after habitat destruction and overexploitation of species (Genovesi et al., 2015). The France is no exception to this phenomenon, with 1,379 species of exotic plants and 708 exotic species of fauna recorded on its metropolitan territory in 2021, of which 120 are considered invasive (Ministry of Ecological Transition (MTE), Action Plan to Prevent the Introduction and Spread of Invasive Alien Species, 2022).

Wishing to react to the threat of invasive amphibians, the Société Herpétologique de France (SHF) coordinated the LIFE CROAA. This project, which is national in scope, is based on the guidelines of this national strategy. It combines improved knowledge, field control actions and awareness campaigns around the American Bullfrog (*Lithobates catesbeianus*) and the African clawed frog (*Xenopus laevis*), in order to:

- Eradicate small American Bullfrog population nuclei (Sologne, Dordogne, Bassin d'Arcachon) in France by improving capture methods;
- Develop a strategy for the control of invasive amphibians when the size of the colonized area limits the possibilities of eradication and apply it to weaken populations and avoid the spread of individuals (African clawed frog in Maine-et-Loire/Deux-Sèvres, American Bullfrog in Gironde);
- Prevent the risk of new introductions by identifying major pathways of introduction of exotic amphibians;
- Create a system for the early detection and assessment of amphibian introductions based on the networking of local actors, the development of a mapping tool and the expertise of IAS managers and scientists;
- Inform, raise awareness and train local actors;
- Ensure the transferability of project results.

Articulated around 17 actions, it has made it possible to:

- Updating the distribution of the two species;
- Improving knowledge of the biology and ecology of both species, including their movements;
- The development of connectivity maps to estimate the risk of spread of the two species according to land cover;
- The identification of a global strategy for the management of the largest outbreaks, for which eradication is not an option;
- The virtual eradication of American Bullfrog kernels from Sologne, Dordogne and Bassin d'Arcachon;

- The implementation of targeted trapping actions within large nuclei with a view to preserving sites presenting a particular challenge for local biodiversity;
- Assessment of the impact of two species on local wildlife;
- Communication and awareness of the general public, school audiences, amateur amphibian and reptile owners and pet stores.

Many deliverables have been produced. In particular, the Technical Guide for the Management of American Bullfrog and African clawed frog is a compendium of the learnings and experiences gained during the project. It details the actions to be implemented in the event of the discovery of the American Bullfrog or the African clawed frog, in terms of surveys, but also in terms of control actions.

The implementation of this ambitious project was possible thanks to the involvement of seven partner structures working locally in the areas concerned by the presence of these two invasive amphibians: the Community of Communes of Thouarsais (Deux-Sèvres, 79), the association Cistude Nature (Gironde, 33), the Departmental Committee for the Protection of Nature and the Environment (Loir-et-Cher, 41), the Landes de Gascogne Regional Natural Park (Gironde, 33), the Loire-Anjou-Touraine Regional Natural Park (Maine-et-Loire, 49), the Périgord-Limousin Regional Natural Park (Dordogne, 24) and the University of Angers (Maine-et-Loire, 49).

4 INTRODUCTION

Invasive alien species (IAS) are present in a large number of continental and marine ecosystems and are now considered a major threat to biodiversity and ecosystem services. Some ecosystems may appear more sensitive because they are already subject to significant anthropogenic stresses. This is the case for continental wetlands. Their continuous degradation and destruction in recent decades has led to a very severe loss of habitats for many species that depend on them. Today, climate change is adding to these adverse conditions. The introduction of IAS appears in this already difficult context as a major new threat likely to contribute to the decline and extinction of populations of already fragile and often highly specialised species. In addition, IAS can sustainably change the functioning of wetland ecosystems and the services they provide. These very diverse areas (ponds, ponds, marshes, flooded areas ...) and the species that occur naturally provide major hydrological, biogeochemical and biological functions. They are home to often high biodiversity and iconic faunal groups that depend strictly on them. Increasingly, these environments are considered by the public to have a high heritage, landscape, recreational or educational value.

Amphibians are a specific component of wetlands, on which they depend for their reproduction and which ensure their survival during an often-important period of their life cycle. They are active organisms within the aquatic biocenosis and the networks of exchange and transformation of energy and matter. Generally herbivorous in the larval stage and prey of many invertebrates and fish for example, they are then predators of many vertebrates and invertebrates in the adult stage and often an important prey for terrestrial predators such as birds or reptiles. Amphibians therefore have a significant functional role in ecosystems and their disappearance constitutes a degradation of these. But conversely, they can also create significant disruption when introduced into a stabilized ecosystem. This is especially the case when an exotic species arrives and proliferates there.

Among invasive amphibians, two species, the American Bullfrog (*Lithobates catesbeianus*) and the African clawed frog (*Xenopus laevis*), are recognized as posing a high ecological risk due to the impacts observed following their introduction on several continents. The former is native to North America and has been introduced to Italy, Germany, France, Spain, Belgium, the Netherlands and Greece. It has been assessed as one of the 100 most harmful invasive species by the *Invasive species specialist group* (ISSG) of the International Union for Conservation of Nature (IUCN). The second is native to southern Africa and has been introduced to France, Italy, Portugal and the United Kingdom and its introduction is also considered a major threat. Both are on the list of priority species for the Union under Regulation No 1143/2014 on the prevention and management of the introduction and spread of invasive alien species.

Both species have a generalist diet like native amphibians. However, due to their large size and very high fecundity, X-American Bullfrogs are first-class competitors and predators for most wetland wildlife stands. The adult American Bullfrog consumes many amphibians and strongly impacts local populations. It can also consume reptiles or small mammals.
The diet of the African clawed frog ranges from copepods to amphibians and fish. Preferred prey varies according to local availability but selective sampling from different groups (molluscs, diptera, odonates, amphibians including larvae) has been observed. In addition to its size, its impact is particularly linked to its presence all year round in the aquatic environment, which is unparalleled in France, and to densities that can be particularly high.

Finally, both species pose a significant health risk to native amphibian populations. They are healthy carriers of the fungus *Batrachochytrium dendrobatidis*. Some strains of this pathogen are responsible for mass

mortalities of amphibians on several continents, making it a major global threat to these vertebrates. The African clawed frog and the American Bullfrog are also carriers of ranavirus, which are also the cause of epizootics causing episodes of mass mortality of amphibians. Both species are therefore reservoirs of major pathogens whose risk of transmission to local amphibian populations increases with the expansion of colonized areas. Recombinations of their exotic viral or fungal strains with local viral or fungal strains pose a significant additional threat.

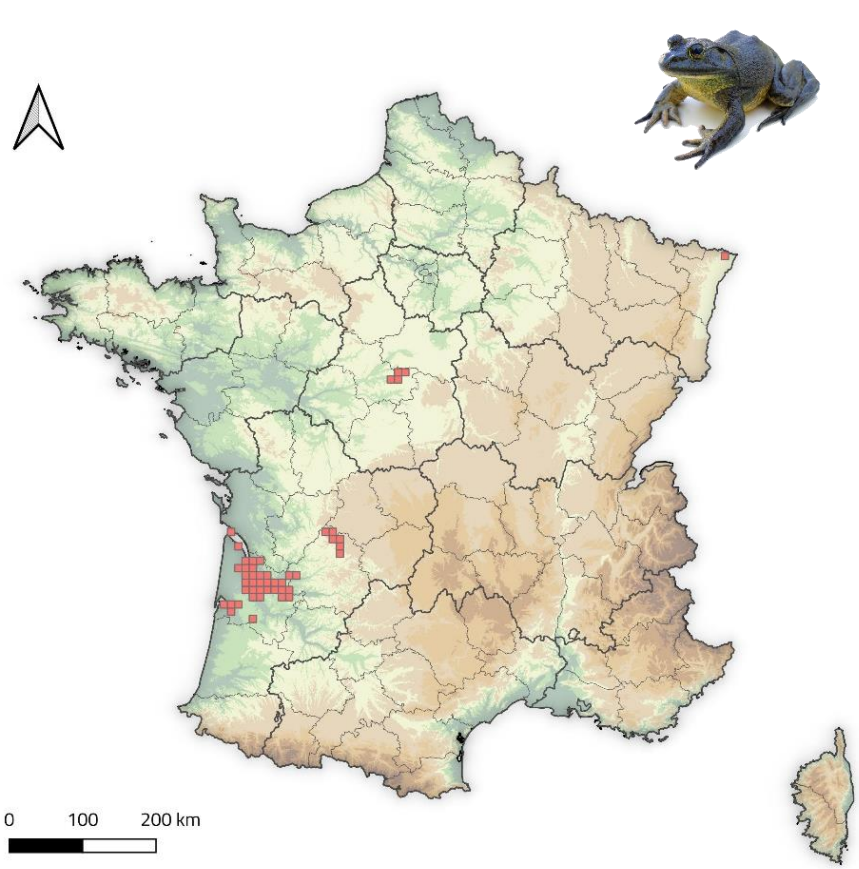
Both species were introduced in France and are naturalized there. The American Bullfrog was imported to Gironde in 1968. From this main core, two secondary nuclei probably resulting from human movements appeared in Dordogne and the Bassin d'Arcachon. An independent introduction took place in Sologne. The Sologne and Dordogne nuclei are circumscribed and have been the subject of control plans for several decades, unlike the other two. These different sectors have been the subject of specific actions under LIFE CROAA. It should be noted that a new population was discovered in Alsace in 2021. An individual was also observed that same year in Saône-et-Loire (Figure 1).
The African clawed frog was introduced in the 1980s in Bouillé-Saint-Paul (Deux-Sèvres) following the closure of a breeding centre providing scientific laboratories. It was detected in the wild in 1998, and its distribution studied gradually from the early 2000s, with the first sampling tests in 2003-2005. The main nucleus, studied within the framework of LIFE CROAA, extends over four departments: Deux-Sèvres, Maine-et-Loire, Vienne and Loire-Atlantique. Three additional and independent populations have been discovered in recent years in Bordeaux (2015), Lille (2018) and Toulouse (2019) (Figure 1).

In 2013, the idea of setting up a synergy between actors involved in the management of these species emerged and the development of a LIFE programme was decided. The LIFE CROAA program (*Control stRategies Of Alien invasive Amphibians*; LIFE15 NAT/FR/000864) was finally launched in September 2016, for a period of six years.

The presence of "small" kernels, which have been managed for several years, and "large" nuclei that are widely extended requires different management strategies. For small nuclei, eradication can be considered and must then be carried to completion. For larger nuclei, it is unreasonable to consider this solution alone because the costs involved can be high and the effectiveness of control over large spaces possibly lower, therefore the risk of failure high. Figure 2 below identifies the nuclei considered "small" and those considered "large" managed by the project. In view of these considerations, the main objective of the LIFE CROAA programme is to establish a general methodology for defining optimal control strategies against exotic amphibians in metropolitan France.

It is oriented around six areas of work:

- Develop a control strategy when the size of the colonized area limits the possibilities of eradication;
- Eradicate or significantly reduce small population nuclei;
- Prevent the risk of new introductions of exotic amphibians;
- Inform, raise awareness and train the general public and local actors;
- Create a system for the early detection and assessment of introductions of exotic amphibians;
- Disseminate the generic approach developed in the project.



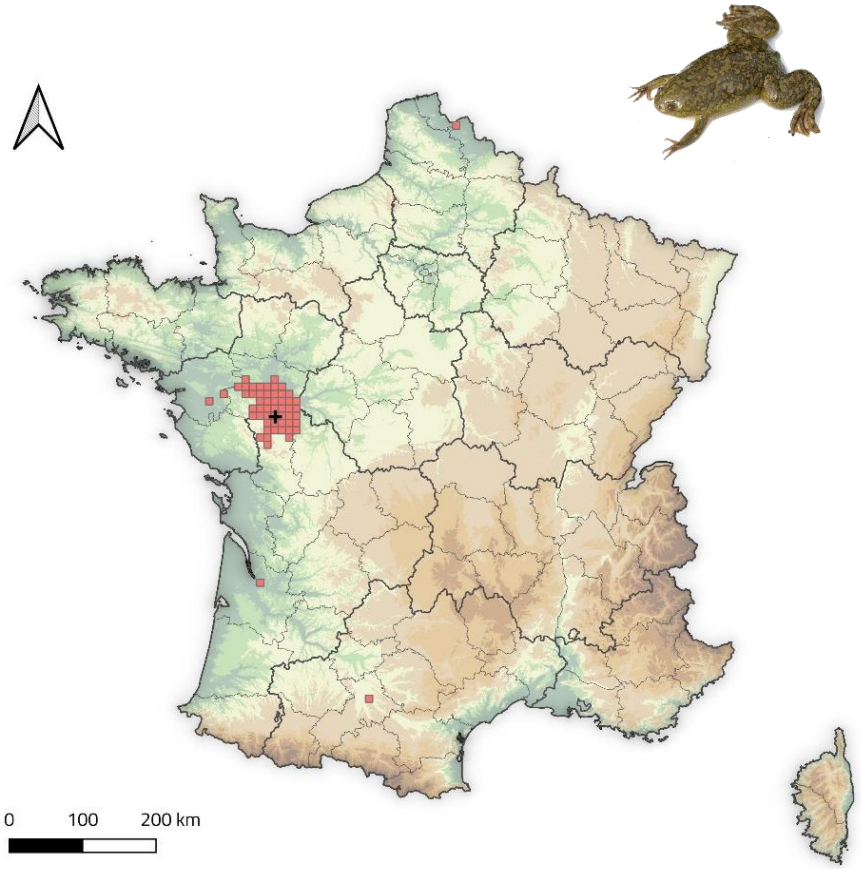


FIGURE 1. Distribution of American Bullfrog (left) and African clawed frog (right) in France in 2022

LIFE15 NAT/EN/000864 - LIFE CROAA

Repo

XL-1 nucleus

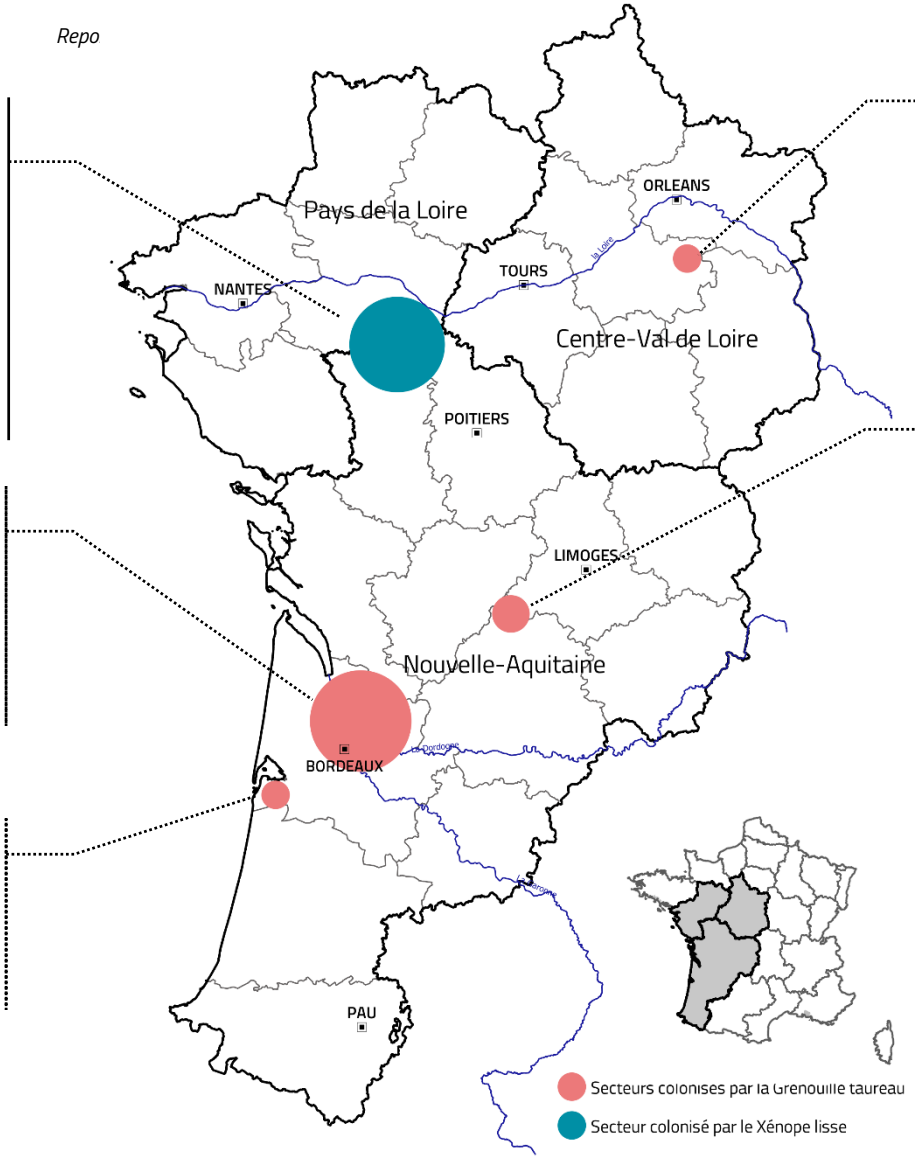
Target species: African clawed frog
Category: "Large" nucleus
Managing beneficiaries: CCT, PNRLAT, with the support of the UA

GT-4 nucleus

Target species: American Bullfrog
Category: "Large" nucleus
Managing beneficiary: CN, with support from the UA

GT-3 nucleus

Target species: American Bullfrog
Category: "Small" nucleus
Managing beneficiary: PNRLG



GT-1 nucleus

Target species: American Bullfrog
Category: "Small" nucleus
Managing beneficiary: CDPNE

GT-2 nucleus

Target species: American Bullfrog
Category: "Small" nucleus
Managing beneficiary: PNRPL

To make the report easier to read, each kernel has been assigned a code:

- GT-1 nucleus: "small" Sologne American Bullfrog population core
- GT-2 nucleus: "small" Dordogne American Bullfrog population core
- GT-3 nucleus: "small" population nucleus of the Bassin d'Arcachon American Bullfrog
- GT-4 nucleus: "large" population core of Gironde American Bullfrog
- XL-1 nucleus: "large" population nucleus of African clawed frog in Deux-Sèvres, Vienne and Maine-et-Loire

FIGURE2. Location of American Bullfrog and African clawed frog population nuclei managed under LIFE CROAA

5 ADMINISTRATIVE PART

5.1 Project management

The team was composed of the SHF, beneficiary coordinator, and seven associated beneficiaries: the Community of Communes of Thouarsais (CCT), the Departmental Committee for the Protection of Nature and the Environment of Loir-et-Cher (CDPNE), Cistude Nature (CN), the Regional Natural Park of Landes de Gascogne (PNRLG), the Regional Natural Park Loire-Anjou-Touraine (PNRLAT), the Regional Natural Park Périgord-Limousin (PNRPL) and the University of Angers (UA). This initial partnership has not been changed.

Partnership agreements have been drafted and signed between the SHF and each of the associated beneficiaries. Amendments have been signed for the PNRLAT, the UA and the CCT to adjust certain amounts (see Mid-Term Report for more details, page 20).

The SHF relied on a monitoring committee, a steering committee and a scientific committee. Further information is given in the review of the F1 action. The minutes of these various committees are available in **Deliverable n°1**.

Apart from these various committees, the SHF regularly exchanged, by email or telephone, and sometimes in physical meetings with the associated beneficiaries, in order to ensure the proper conduct of the actions or to resolve any difficulties. Videoconferences were also organized when a topic concerned several beneficiaries. These essential exchanges have made it possible to ensure rigorous technical, administrative and financial monitoring.

5.2 Communication with the Agency and the external monitoring team

At Agency level, three project managers followed LIFE CROAA: Ms Blanca SAEZ-LACAVE, Ms Rosemarie HINGSAMER and Ms Ana KLENOVSEK. Ms HINGSAMER, accompanied by Mr Spyridon FLEVARIS, Policy Officer at the European Commission, participated in the 2019 external team visit. This was an opportunity to present the progress of the project but also to demonstrate the actions carried out on the ground.

In parallel, following each visit by the external monitoring team, the Agency sent us evaluation letters, to which we responded in the mid-term report for the visits carried out until 2018, and to the NEEMO monitor thereafter. The replies to the last letter of 16/05/2022 are formulated in this report on pages 34 and 90.

LIFE CROAA was followed by two instructors from the NEEMO external team: Frédéric BROCHIER, from 2016 to 2018, then Quirin RENARD, from 2019 to 2022. The SHF had regular exchanges throughout the project with M. BROCHIER and RENARD, mainly by email and telephone.

5.3 Amendments to the grant agreement

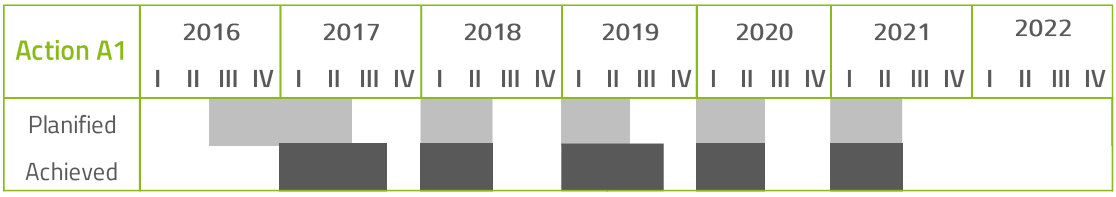
Two amendments were signed during the project (see **Annex 1**). The first, dated 27/09/2018, amended the definition of the conditions applicable to natural persons, the presentation of the VAT certificate and the threshold for presenting the certificate in the financial statements. The second, dated 14/11/2021, concerned the modification of the SHF's bank details. No amendments relating to substantial modifications to the project were made.

6 TECHNICAL PART

6.1 Technical progress by action

6.1.1 Actions A - Preparatory actions, preparation of management and/or action plans

6.1.1.1 A1. Preparation prior to field actions



Responsible beneficiary of the action: SHF

Other beneficiaries involved: CCT, CDPNE, CN, PNRLAT, PNRLG, PNRPL, UA

DESCRIPTION OF TECHNICAL PROGRESS AND DIFFICULTIES ENCOUNTERED BY SUB-ACTION

Progress of the action between 01/09/2016 and 31/08/2022 and results

- ***A1.1. Drafting of scientific and technical protocols***

Three types of protocols have been drafted by the SHF and the UA as part of this sub-action:

- Sampling and inventory protocols for the implementation of Action A2. A different protocol has been defined for each American Bullfrog and African clawed frog population nucleus to take into account the context and local specificities.
- Monitoring protocols for native species for the implementation of Action D2. Two faunal groups were monitored as part of this action: amphibians and aquatic invertebrates.
- Protocol for the application of techniques to combat American Bullfrogs and African clawed frogs under actions C2 and C3. The protocols drafted at the beginning of the project and provided at the time of the mid-term report have evolved in line with Action A3. They have been integrated and improved in the technical guide (action E2).

- ***A1.2. Prior administrative procedures***

All the required authorizations (for the incidental capture of protected species, for the capture and destruction of American Bullfrogs and African clawed frogs, for penetration into private property) have been obtained, on an annual or multi-year basis depending on the department.

On the other hand, in order to facilitate the implementation of actions to combat the American Bullfrog in Sologne, the CDPNE has signed agreements with 9 owners. This allowed the CDPNE to be able to engage in control operations on 19 ponds. In return, the owners benefited from a refurbishment of the emptying system of their pond (4 bodies of water) or maintenance work such as logging and brushing (15 ponds).

- ***A1.3. Training of the teams responsible for implementing the control actions during the duration of the project***

All team members benefited from annual training aimed at strengthening their knowledge and skills for the implementation of field actions.

A total of 13 training courses were conducted:

- 7 training courses on amphibian identification, mainly provided by CN for other associated beneficiaries;
- 5 training courses provided by the French Biodiversity Office of Dordogne (OFB 24) with the aim of training CN, PNRPL and PNRLG agents in the use of a weapon as part of actions to combat the American Bullfrog.

Comparison with projected schedule and expected results

The drafting of the D2 action protocols was slightly delayed at the start of the project, which also delayed the launch of action D2. However, this did not have any substantial consequences on the course of the action.
Regarding administrative authorizations, prefectural orders were issued late in Gironde in 2018 and 2019, which delayed the implementation of control actions (C2 and C3).
The training on amphibians and shooting was able to take place in the first half of each year, allowing the various beneficiaries and trainees to be operational before the implementation of field actions, except for the year 2020 during which amphibian training could not take place, due to the health context.

Difficulties encountered and measures put in place where necessary

The drafting of the protocols was planned at the very beginning of the project. However, the launch of the project, the implementation of administrative and financial monitoring tools and the late recruitment of the administrative and financial coordinator, proved time-consuming and delayed the development of protocols.
As some administrative authorizations were issued late at the beginning of the project, the SHF was responsible for developing a common file for all associated beneficiaries located in Nouvelle-Aquitaine. After analysis by the Regional Scientific Committee for Nature Protection (CSRPN), multi-year authorizations were obtained, valid until the end of the project (except for the PNRPL which had to renew its application for the year 2021). The file is available in **Annex n°2**.

The CDPNE had to face the refusal of some owners to implement the planned operations, despite the signing of an agreement. This is largely due to hunting activities: the owners fearing that the actions to combat the American Bullfrog will disturb the game. The non-signature of conventions or the non-respect of a signed convention are particularly problematic because it prevents a complete management of colonized sites and can lead to the dispersion of individuals to previously uncolonized sites. To counter this difficulty, the CDPNE organized consultation meetings involving State services (DREAL, OFB, DDT). Emphasis was also placed on communication with owners. For example, they were informed live of the actions carried out on their water bodies.

Complementary actions

-

Prospects

-

DELIVERABLES

TABLE1. Deliverables of Action A1

Entitled	Estimated deadline	Status
Catch authorizations for 2017	31/03/2017	**Finalized** Provided with the mid-term report (Annex II.5)
Catch authorizations for 2018	31/03/2018	**Finalized** Provided by email to Quirin Renard in July 2019
Catch authorizations for 2019	31/03/2019	**Finalized** Provided by email to Quirin Renard in July 2019
Catch authorizations for 2020	31/03/2020	**Finalized** Provided during the external team's visit on 25/01/2021
Catch authorisations for 2021	31/03/2021	**Finalized** Provided during the external team's visit on 29/03/2022

6.1.1.2 A2. Updating the distribution and/or colonization front of the African clawed frog and the American Bullfrog

Action A2	2016				2017				2018				2019				2020				2021				2022			
	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
Planified																												
Achieved																												

Responsible beneficiary of the action: SHF

Other beneficiaries involved: CCT, CDPNE, CN, PNRLAT, PNRLG, PNRPL, UA

DESCRIPTION OF TECHNICAL ADVANCES AND DIFFICULTIES ENCOUNTERED

Progress of the action between 01/09/2016 and 31/08/2022 and results

This action was planned at the beginning of the project, with the aim of updating the distribution of the two target species before the concrete implementation of the management actions.

The techniques and protocols adopted for each of the nuclei, as well as the distribution maps produced, are available in Annex II.6 of the mid-term report.

In summary, the 2017/18 inventory showed:

- A strong increase in the African clawed frog since the inventories conducted before the project. The species is now present in Loire-Atlantique and north of the Loire in the Maine-et-Loire department. The natural barrier offered by the river does not seem effective in slowing its expansion.
- An expansion of the Gironde American Bullfrog core compared to the last inventory dating from 2005, especially towards the northwest along the Gironde estuary towards the Médoc, which was expected, but also towards the Dordogne in the east, which was less so.
- A low presence of the American Bullfrog on the Bassin d'Arcachon: only 2 sites were positive out of nearly 200 sites inventoried in 2017. A third site was discovered in early 2018.
- No data of concern on the cores of American Bullfrog of Sologne and Dordogne.

On the other hand, the field and monitoring actions carried out throughout the project made it possible to update the distribution maps annually. The maps produced at the end of the project are available in **Annex n°3**.

In conclusion:

- The project has made it possible to define a total colonization area of nearly 4,700 km² for the American Bullfrog (including more than 4,600 km² on the Gironde core alone) and more than 4,800 km² for the African clawed frog.
- During the project (2016-2022), the ranges of the two large nuclei expanded, the rate of spread being 2.5 times higher in the African clawed frog over the entire area it colonizes than in the American Bullfrog on its Gironde core, yet considered a densely colonized area.
- The area of distribution of the African clawed frog in France is currently very large and a detailed update of the presence of this species would require a considerable human and financial effort. Therefore, it is highly likely that the distribution map produced as part of the project slightly underestimates the true distribution of the species.
- Regarding small nuclei:
    - In Sologne, the American Bullfrog is present on 7 aquatic sites.

- In Dordogne, the presence of the American Bullfrog is proven on a single pond. Monitoring actions must be maintained on 6 other ponds where the presence of the species is suspected.
- On the Bassin d'Arcachon, only one American Bullfrog individual was contacted in 2022 on a single site. Monitoring actions must also continue.

Comparison with projected schedule and expected results

This action has started according to the provisional timetable. As not all of the 900 eDNA kits acquired in 2017 were used in the first year, we decided to continue the inventory in 2018 with the remaining 166 kits.

Difficulties encountered and measures put in place where necessary

This action was particularly time-consuming for large nuclei. In Gironde, the PNRLG came to reinforce CN to carry out the inventory. On the other hand, weather conditions were not optimal in 2017, which had the effect of limiting the ability to sample all pre-identified environments.
Regarding small nuclei, the detection of the American Bullfrog is becoming more and more difficult, the numbers being very small and the individuals smaller and smaller. Inventories based on conventional techniques (eavesdropping, trapping) and molecular techniques (eDNA) therefore appear to be too time-consuming or too costly. New methods were tested as part of the project to overcome this difficulty (see below).

Complementary actions

Thanks to the communication and awareness-raising carried out during the project, an informal network of observers was formed, especially for the African clawed frog.

Prospects

Regarding the African clawed frog, the range is very extensive, but requires regular monitoring to monitor the progress of the species. The use of environmental DNA on this scale seems difficult because of the costs involved. It is therefore proposed to continue to develop the network of observers (professionals**,** naturalists, individuals) capable of transmitting any new observations.

In order to improve the detectability of the American Bullfrog within small nuclei, new techniques have been tested (see action A3): dissemination of American Bullfrog songs in order to trigger a response and thus locate individuals. These methods can be used in the coming years, with the aim of achieving the complete eradication of these populations.

DELIVERABLES

TABLE2. Deliverables of Action A2

Entitled	Estimated deadline	Status
Updated distribution map of the core population of American Bullfrog in Sologne	28/02/2018	**Finalized** Provided with the mid-term report (Annex II.6)
Updated distribution map of the core population of American Bullfrog in Dordogne	28/02/2018	**Finalized** Provided with the mid-term report (Annex II.6)

Updated distribution map of the American Bullfrog population core on the Bassin d'Arcachon	28/02/2018	**Finalized** Provided with the mid-term report (Annex II.6)
Updated distribution map of the population core of American Bullfrog in Gironde (large nucleus)	28/02/2018	**Finalized** Provided with the mid-term report (Annex II.6)
Updated distribution map of the African clawed frog (large nucleus)	28/02/2018	**Finalized** Provided with the mid-term report (Annex II.6)

6.1.1.3 A3. Improved capture techniques

Action A3	2016				2017				2018				2019				2020				2021				2022			
	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
Planified																												
Achieved																												

Responsible beneficiary of the action: SHF

Other beneficiaries involved: CCT, PNRLAT, CN, UA

DESCRIPTION OF TECHNICAL ADVANCES AND DIFFICULTIES ENCOUNTERED

Progress of the action between 01/09/2016 and 30/08/2022 and results

- ***A3.1. Capture of larvae***

For African clawed frog:

According to the literature, tadpoles tend to aggregate under shaded surfaces. Based on this assumption, a capture device was developed and tested. It was an inverted trap (entrance below), equipped with a wooden plate on the top, in order to create a floating and shaded surface (Fig. 3). Unfortunately, this trap proved ineffective since no tadpoles were caught.

FIGURE 3. Trap created and tested to improve the catch rate of African clawed frog tadpoles

For the American Bullfrog:

The best technique for removing tadpoles is pond emptying coupled with seine fishing. However, this method cannot be used systematically, because certain parameters must be taken into account: context, size of the body of water, presence of a drain system, etc.

Tests were therefore carried out throughout the programme to try to increase the number of tadpoles captured per trapping session.

In 2017 and 2018, tests on bait were first carried out, with baited traps (cat pie with vegetables, chicken, beef or sardines) or not, deployed in a standardized way (same number of traps, same number of days). Overall, baited traps seem to show greater effectiveness.

Various trapping devices were also tested in 2018 and 2019: traps, fyke nets, tiles, seines.

The so-called "passive" means (traps and worms) logically produce the best results since they can be left in place without human presence, but are not exhaustive means of capture.
Tests were also carried out to determine the preferential habitats of the larvae: traps were set up under similar conditions (number and period) on bare banks, on seagrass beds or in open water. But no trend could be identified during this experiment.
It should still be noted that large concentrations of larvae have been observed at the edge of the bank at the beginning of the season (spring), when ambient temperatures become favourable again and the first centimetres of the water slide are much warmer than at depth. Trap catches seemed to be more effective at this time, but a large number of tadpoles were still observed in the lower levels, not allowing for exhaustive trapping of this stage in this season.

- ***A3.2. Capture of adults***

For African clawed frog:

As early as 2018, the UA, the PNRLAT and the CCT conducted various experiments to identify the type of trap and the type of bait most effective for catching adults.

Regarding the traps, the PNRLAT created two prototypes, which were then also tested by the CCT:

- Bottom trap type trap (with double entrances) connected to a fishing squall (Fig. 4.a);
- "Bourriche" type trap, comprising a trap with a bottom-facing entrance, fixed in a floating fishing slough (Fig. 4.b).

A third type of trap, the sparrowhawk net (Fig. 4.c), commercially available, has also been tested.

FIGURE 4. Types of traps experimented in order to improve the catch rate of African clawed frog adults. (a) Bottom trap, (b) Bourriche, (c) Sparrowhawk net

Several types of bait have also been studied: fishing baits such as "carp boilies" (different flavours) and "fish pellets", butcher's offal, canned sardines and mackerel, bread, live African clawed frog females, bouillon cubes, cat pate, Nuoc nam sauce, crayfish noquettes.

In 2021, in order to obtain robust results on the most effective baits and traps, the SHF drafted a protocol, applied by the CCT and the PNRLAT on 6 different sites. The following were tested:

- The trap (usually used method), the bourriche and the sparrowhawk net for traps;
- Dog kibble (usually used bait), crayfish and live female clawed frog for bait.

It appears that the bourriche can capture more African clawed frogs (adults and subadults) than other types of trap, when the sparrowhawk net is more effective on juvenile individuals. On the bait side, it is the dog food that allows the maximum capture, all stages combined except tadpoles.

For the American Bullfrog

In order to improve the capture rate of American Bullfrog adults, the experiments conducted were based on the following hypothesis: they are attracted by the song of males. A floating trap, equipped with a trap placed vertically and coupled with a device for broadcasting songs, was therefore developed and tested (Fig. 5). The sounds used were recorded by the UA in partnership with Thierry Lengagne, bio-acoustic researcher at the University of Lyon.

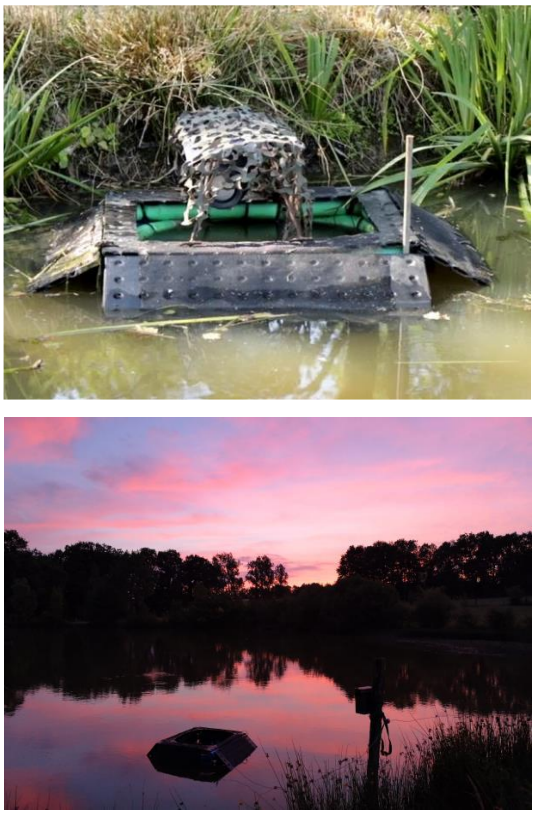


FIGURE 5. Experienced trap to promote the capture of American Bullfrog adults

The experiments carried out in Sologne and Dordogne (small nuclei) and the main Gironde nucleus, did not allow an improvement in the catch rate and the device was therefore abandoned. However, experiments have highlighted the interest of ironing. Indeed, the males answer each other. Thus, it becomes easier to verify the presence of individuals and identify their location for the implementation of control actions.

Based on this observation, the sound equipment for the use of the trap was still used throughout the project.

In addition, for small nuclei, where the numbers are increasingly small, the probability of contacting an individual decreases. A passive detection system was therefore considered. In 2021, the CDPNE tested the use of SM4 sound recorders to automatically and autonomously record sounds in known frequency ranges for a given time. The species present can thus be determined according to the songs, through the Kaleidoscope software coupled with a human verification, for a lower time cost.

This experiment, carried out on several ponds in Sologne, in partnership with Beauval Nature, made it possible to identify the most optimal period of activity of the American Bullfrog: from 7pm to 4am. This information will make it possible to better organize interventions in the field.

- ***A3.3. Creation of refuges without African clawed frog***

The lagoon stations present on the territory colonized by the African clawed frog constitute real "nurseries". Juveniles and adults leave these stations to colonize other nearby waterholes and lagoon stations. The objective of this sub-action was to avoid the colonization of these anthropogenic environments in order to limit the number of "nurseries". The system created (see below) is effective both for plants free of clawed frogs (avoiding any introduction) and for already colonized plants (by avoiding a dispersion of individuals, likely to lead to the colonization of environments and treatment plants (WWTPs) located nearby).

In 2017, the CCT recruited an intern to diagnose four lagoon stations and propose a containment system compatible with the operation of the sites and resistant to the weather as well as the coypu that frequent these stations.

In 2018, the selected device was installed on a first lagoon station: the WWTP of Saint-Martin-de-Sanzay (79). The installation steps were detailed and illustrated in the mid-term report. A monitoring of the number of clawed frogs trying to enter and leave the station was set up until 2021. A total of 8,894 individuals were captured. This monitoring made it possible to analyse the movements of the species, with:

- A clear trend of dispersal around spring between the months of April and June, which correspond to the breeding season.
- An important role of rainfall in dispersion.

Details are given in **Annex n°4**, pages 13 to 15.

As the system has shown great effectiveness, it has been integrated into the actions implemented to combat the species (action C4). Two new stations have been equipped on the territory of the CCT (see action C4).

Comparison with projected schedule and expected results

Due to the disappointing results of the first devices tested, the team wanted to continue the experiments. In its letter of 04/11/2019, the Agency granted an extension until the end of the project for the implementation of this action.

Difficulties encountered and measures put in place where necessary

Unfortunately, the initial assumptions have not been verified in the field and the traps tested have not proved effective. Thanks to the additional time granted by the European Commission new experiments based on the knowledge acquired during the project could be carried out. This is how the standardized study of the effectiveness of baits for African clawed frogs could be conducted or passive detection systems could be tested in order to better identify the bodies of water still colonized in Sologne.

Complementary actions

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Prospects

It would be interesting to continue experimenting with passive detection systems, especially in small populations.

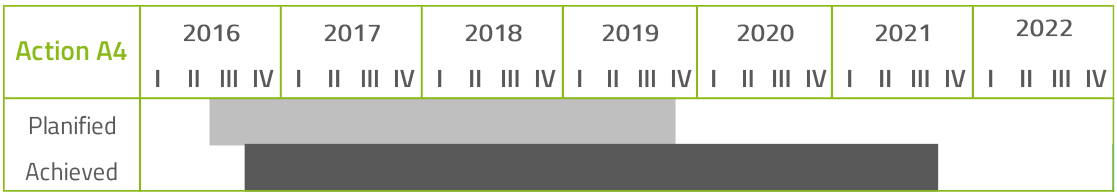
DELIVERABLES

TABLE3. Deliverables of Action A3

Entitled	Estimated deadline	Status
Technical sheet for the trap for catching the larvae of American Bullfrogs	30/09/2018	**Modified and finalized deliverables** The new techniques initially foreseen in the nomination file did not bear fruit. The associated

Technical sheet for the trap for catching larvae of African clawed frogs	30/09/2018	deliverables have therefore been replaced by other data sheets: - Make an inventory - The African clawed frog - Make an inventory - The American Bullfrog - Listening to amphibians: autonomous recording units - The bourriche: trapping system for catching the African clawed frog - Techniques for capturing the African clawed frog - American Bullfrog capture techniques In order to facilitate their dissemination, it was decided to translate them into English.
Data sheet for the sound trap for the African clawed frog	30/09/2018	
Technical data sheet for the sound system attractive to the American Bullfrog	30/08/2018	
Technical sheet for the lagoon preservation system	31/12/2018	**Deliverable finalized** Provided with the mid-term report (Annex II.6)

6.1.1.4 *A4. Definition of optimal control strategies for large population nuclei of invasive amphibians*



Responsible beneficiary of the action: UA
Other beneficiaries involved: CN, SHF

DESCRIPTION OF TECHNICAL ADVANCES AND DIFFICULTIES ENCOUNTERED

Progress of the action between 01/09/2016 and 31/08/2022 and results

- ***A4.1. Survival and dispersal in African clawed frogs and American Bullfrogs***

The objective of this sub-action was to fill knowledge gaps regarding the demographic parameters of American Bullfrogs and African clawed frogs in their home range. For this, several studies have been conducted:

- *For African clawed frog:*
  - Demographic study
  - Estimation of travel costs, on different substrates. The acquired data were used for the calculation of the connectivity maps (action A4.3).
- *For the American Bullfrog:*
  - Tadpole survival study
  - Study on the survival and movement of juveniles
  - Estimation of travel costs, on different substrates. The acquired data were used for the calculation of the connectivity maps (action A4.3).

All the activities carried out in this context are detailed in the activity report of action A4.1, provided to the NEEMO monitor, Quirin RENARD, during his annual visit in January 2021.

In summary, these different studies have highlighted that:

- The numbers of African clawed frog are much larger on the periphery of the range than at its centre. This assumes that the core population is aging.
- The African clawed frog has adaptive abilities for the colonization of new environments. Indeed, the biometric data collected during the demographic study highlighted morphological differences between "colonizing" individuals and individuals in the heart of the range.
- The African clawed frog moves slower on grass surfaces, which could give a concrete indication to reduce pond access to this species. However, it should also be taken into account that the latter dehydrates less quickly on this substrate than on a smooth substrate. There could therefore be a trade-off between travel speed and risk of dehydration.
- Like the African clawed frog, the American Bullfrog moves slower on grass surfaces.

Annex n°5 presents the various scientific articles published.

- ***A4.2. Eco-evolutionary responses of invasive populations and local settlements***

The objective of this sub-action was to determine whether the growth of individuals and reproductive parameters show signs of attenuation of invasiveness (laying size, morphology). For this, several studies have been conducted:

- For African clawed frog:
  - Estimation of reproductive effort
  - Larval development analysis
  - Assessment of local stand responses

- For the American Bullfrog:
  - Measuring the size-growth relationship in American Bullfrogs

All the activities carried out in this context are detailed in the activity report of action A4.2, provided to the NEEMO monitor, Quirin RENARD, during his annual visit in January 2021.

In summary, these different studies have highlighted in African clawed frogs that:

- The tadpole stage could be considered the most sensitive stage. However, the studies conducted seem to show that the attenuation of invasiveness that could have been expected for this stage does not seem to be occurring. Conversely, tadpoles seem to have adapted, they have become more resistant to colder climates and seem to be able to identify new predators.
- Studies conducted on prey species show that they would be able to develop anti-predator responses, which could contribute to the long-term resilience of food webs.

The research that was planned for the American Bullfrog was not conducted because a study, published in December 2016, carried out (independently of LIFE CROAA) the analyses we planned. There was therefore no point in repeating the same work.

- ***A4.3. Cost-effectiveness modelling of control strategies***

The objective of this sub-action was to identify an optimal strategy for the management of large nuclei of American Bullfrog and African clawed frog. To this end, an assessment of the costs of control operations in the areas of action was carried out and connectivity maps were modelled. This modelling work, integrating the data collected under the two previous actions, was finalised in April 2019.

The experience of LIFE CROAA demonstrates that the personnel and transport costs associated with the use of current trapping techniques are incompatible with large-scale and long-term deployment.

In addition, modelling showed very strong connectivity for each species on and around the colonized territory. Given this result and the cost of continuous active control by trapping, it seems impossible to act on the entire front to limit expansion or fragment colonized areas to weaken invasive populations. In contrast, the eastern part of the plain clawed frog is bordered by a naturally drier area where colonization is slowed down. For the American Bullfrog, the map identifies areas of surveillance and priority action between the main core and the small endangered population of the Dordogne. Strong vigilance is required in this area to avoid secondary recolonization in the Dordogne after 20 years of eradication efforts.

Faced with this observation and in order to conduct a collective reflection, beyond the actors of LIFE CROAA, a working seminar was organized within the framework of action E2.5, combining scientific exchanges and strategic reflections. More details are given in the description of action E2.5.

These times of exchange and work led to the drafting of a strategy to control the two large nuclei of African clawed frog and American Bullfrog. In order to make this document accessible to as many people as possible, it was written in English and posted on the project website. This deliverable was given to our NEEMO instructor, Quirin RENARD, during his March 2022 visit.

The strategy thus defined proposes the following lines of work:

- Reduce pressure and maintain control actions: create ecological corridors for native species, eliminate high-productivity sites (wastewater treatment plants for example);
- Reduce pressure through the implementation of trapping actions at the scale of stakes or priority sites;
- Reduce the impact through the implementation of environmental restoration measures;
- Continue communication and awareness-raising actions for the local population.

Regarding the implementation of restoration actions, a complementary study, not initially planned, was carried out in order to evaluate the interest of the restoration of environments and nature-based solutions to reduce the ecological impacts of African clawed frog. The study conducted in 2021 on 43 ponds in the centre of the colonized area, showed that the complexity of the structure of aquatic vegetation in a pond was positively related to the diversity of aquatic invertebrates. The main reason is that the vegetation naturally offers refuges from predation by the African clawed frog which is a large species. Thus, promoting the development of aquatic vegetation through proven ecological restoration methods would mitigate the effects of the invasive over several years while being beneficial in other areas, including water quality. These "ecosystemic" solutions have the advantage of being beneficial for several species and thus make it possible to mobilize existing types of financing.

Comparison with the provisional timetable

Initially, action A4 was to take place over the first three years of the project (2017 to 2019). However, the timetable had to be adapted:

- At sub-action level A4.1:
    - ***Radio-tracking of American Bullfrogs:***
      The first year of the radio-tracking follow-up required an extremely important preparation phase (identification of the owners of the plots of "interest", presentation of the program and its challenges, written access authorizations, test phase for the anaesthesia protocol of the individuals to be equipped, etc.). As a result, the first catching and radio-tracking campaigns of 2017 did not really start until late compared to the beginning of the species' active season in July.

    - ***Dispersal of American Bullfrog juveniles:***
      The captures, the installation of transmitters and refuge plates were carried out on schedule. To maximize the chances of recapture of juveniles during their dispersal, which can be very brief, plate control passages have been significantly increased and extended until 2018.

    - ***American Bullfrog larval survival rate:***

The estimation of the survival rate of larvae was also envisaged over 2 years from 2017, based on the marking of 2000 individuals (1000 individuals per year) on 2 sites using coloured elastomers. Since the larval development of the American Bullfrog generally extends over2 years, it was decided to carry out all catches in the same year, on the same site and to concentrate on the tadpoles "of the year", in order to estimate the survival rate of the larvae over their entire 2-year development.

In 2017, only one spawning was observed throughout the field season and it was not possible to find tadpoles in their first year of development. we have therefore decided to postpone this sub-action to 2018 assuming that the egg-laying, and therefore the tadpoles of the year are more numerous.

- At sub-action level A4.3:

The activities under this sub-action have been within the planned timetable. However, the drafting of the overall strategy has been delayed. Indeed, the working seminar that allowed the identification of the management strategy of the most extensive nuclei took place at the end of 2020 by videoconference, due to the Covid-19 epidemic.

In addition, in view of the results obtained, the additional study on the evaluation of the interest of restoration actions took place in 2021.

The A4 action has therefore been extended until 2021.

Difficulties encountered and measures put in place where necessary

The results of the various field studies on the American Bullfrog have been disappointing:

- No tagged tadpoles could be recaptured. Therefore, the survival rate could not be estimated.
- No juveniles were found under the refuge plates, despite the extension of follow-up for an additional year.
- Movement of tagged adults was restricted, likely due to very dry weather conditions in 2017.

In order to obtain the necessary information for the development of connectivity maps, the UA carried out the same experiment as on the African clawed frog to identify the travel costs of individuals and the resistance to different substrates.

Complementary actions

Thesis funding was obtained through co-funding from the Embassy of France in South Africa and Stellenbosch University. The recruited PhD student (Natasha Kruger) carried out, in South Africa and with funds from the University of South Africa, the larval development experiments on African clawed frog that were planned in the project. John Measey, an internationally renowned specialist in African clawed frog and member of the scientific committee of LIFE CROAA, co-supervised the thesis at Stellenbosch.

In addition, the connectivity maps currently being developed may underestimate the real, already strong connectivity. Indeed, the dispersal capacity of the African clawed frog increases as the expansion progresses. However, this property is not integrated into connectivity models. New modelling is therefore currently underway in collaboration with Dennis Rödder (Leibniz Institute, Bonn) to incorporate the dynamic nature of the expansion. The advantage of this approach is to identify areas that might seem unfavourable to colonization and that would be so when variation in dispersal is taken into account.

Prospects

Further studies could be conducted on nature-based solutions. Indeed, these environmental restoration actions could make it possible to set up local and regional networks favourable to local species. For this, research projects have been submitted by the UA.

DELIVERABLES

TABLE4. Deliverables of Action A4

Entitled	Estimated deadline	Status
Progress report on work under sub-action A4.1	31/12/2019	**Finalized** Provided during the external team's visit on 25/01/2021
Progress report on work under sub-action A4.2	31/12/2019	**Finalized** Provided during the external team's visit on 25/01/2021
2 scientific publications in international peer-reviewed journals (sub-actions A4.1 and A4.2)	31/12/2019	**Finalized** Provided during the external team's visit on 25/01/2021
Large population nucleus connectivity maps for African clawed frogs and American Bullfrogs (sub-action A4.3)	31/12/2019	**Finalized** Provided during the external team's visit on 03/03/2020
Strategy for the control of the American Bullfrog and the African clawed frog	31/12/2019	**Finalized** Provided during the external team's visit on 29/03/2022

6.1.2 Actions C - Concrete conservation actions

6.1.2.1 C1. Establishment of an early detection and assessment system

Action C1	2016				2017				2018				2019				2020				2021				2022			
	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
Planned																												
Achieved																												

Responsible beneficiary of the action: SHF
Other beneficiaries involved: All

DESCRIPTION OF TECHNICAL PROGRESS AND DIFFICULTIES ENCOUNTERED BY SUB-ACTION

Progress of the action between 01/09/2016 and 31/08/2022 and results

- ***C1.1. Development of an early detection system***

- *Tool development:*
  - Smartphone app:

It was initially planned to create a smartphone application. However, due to the growing number of naturalistic applications, this development was ultimately not considered relevant. After discussion with the Agency, the latter accepted the abandonment of this deliverable in its letter of 27/11/2018. In return, the LIFE CROAA team took on the developers of European applications such as "IASTracker" and "Invasive Alien Species in Europe".

Response to the letter of 16/05/2022 – Problem n°1 (Action C1)

Reminder: In reiteration of number 3 of the Agency's letter dated February 22, 2021, you are cordially invited to get in touch with the JRC development team and ensure that *Xenopus laevis* is included in their smartphone app on invasive amphibians.

I remind you that working with the JRC on this action was granted as a substitute for not developing your own application. Therefore, evidence of constructive collaboration with the RCC must be presented to the Agency, which has not been the case in the last two years.

Answer: As early as 2018, the SHF contacted the team in charge of developing the "IAS Tracker" and "Invasive Alien Species in Europe" applications. We have not received any feedback from the developers of the "IAS Tracker" application.

Regarding the "Invasive Alien Species in Europe" application, the SHF proposed from the first exchanges to translate the application into French in order to facilitate its use in France (see **Annex n°6.a**).

In 2021, we got back in touch with the development team again. Data entry for African clawed frog was not possible. Ana Cristina Cardoso, chief scientist at the European Commission's *Joint Research Centre*, said only species listed on the list of species of EU concern were included in the app. As the African clawed frog is about to be included in this list, it was decided to wait. In parallel and in anticipation, the SHF has translated into French the species sheets for *Xenopus laevis* and *Lampropeltis getula*, King Snake, an invasive exotic reptile whose management has also benefited from a LIFE program. Details of the exchanges are available in **Annex n°6.b.**

- Online input tools:

In order to promote the transmission of observations of exotic amphibians and to reduce the time taken to report information to conservation stakeholders, the SHF developed at the beginning of the project an online input form accessible to all (see Annex II.9 of the mid-term report).

However, this tool has shown shortcomings. It was therefore decided in 2021 to migrate to another type of data entry tool, designed for the general public: GeoNature-Citizen. This device has the advantage of being compatible with the SHF database, managed under the GeoNature device. Thus, the data entered are directly entered into the SHF database. Two surveys have been created:

- One on the American Bullfrog: https://enquetes.lashf.org/fr/programs/5/observations
- The second on the African clawed frog: https://enquetes.lashf.org/fr/programs/4/observations

Both surveys are available on the project website: https://www.life-croaa.eu/signaler-amphibien-exotique/

The indexing of the Life CROAA site by Google is such that it appears on the first page during searches such as "report American Bullfrog" or "report frog" or "American Bullfrog France" etc.

Finally, in 2021, in partnership with the National Inventory of Natural Heritage (INPN), we launched two "INPN Species" quests (American Bullfrog and African clawed frog) with the aim of collecting additional observation data. Unfortunately, little data was captured through this channel.

- *Creating a network*

Thanks to LIFE CROAA and their involvement in different scientific and technical groups, local or national, on invasive species, the SHF and the associated beneficiaries are now recognized as reference structures for the African clawed frog and the American Bullfrog.

Thus, the team's expertise was sought when discovering new populations of African clawed frog and American Bullfrog in France – see action E2.4. These different examples demonstrate the good functioning of the local and national network around invasive amphibians.

- ***C1.2. Development of an early assessment system***

The SHF works closely with several national and international experts on biological invasions: members of the French Office for Biodiversity, the French IUCN Committee, scientists... It is also part of the Network of Scientific and Technical Expertise of the Invasive Alien Species Resource Centre.

In addition to requests and requests for expertise in the context of the discovery of new populations, the SHF also provided support for the management of the fire-bellied bell population located in Lorraine and involved its national partners likely to provide additional expertise (see action E2.4).

Comparison with the provisional timetable

In accordance with the provisional timetable

Difficulties encountered and measures put in place where necessary

The development of a new application for smartphones no longer seemed relevant to us given the existence of other applications with the same functionality (see previous paragraph "Progress of the action and results"). In return, we have contributed to the improvement of already existing applications.

Complementary actions

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Prospects

The SHF, as the national association specializing in herpetology, is the head of the national network for all issues related to amphibians and reptiles. It must be the first structure, with the services of the State, to be informed of the observation of American Bullfrog, African clawed frog or any other species of amphibian or invasive reptile on the national territory.

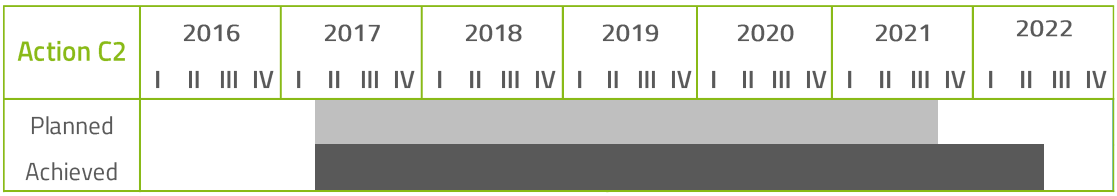
Overall, all the beneficiaries of the project will remain in the years to come the reference structures in terms of management of the African clawed frog and the American Bullfrog and will be able to bring their expertise.

DELIVERABLE

TABLE5. Deliverables of Action C1

Entitled	Estimated deadline	Status
Cartographic tool (IAS portal)	31/07/2017	**Finalized** The first developed form was presented in the mid-term report (Annex II.7). The new forms developed under GeoNature-Citizen are presented in **Annex n°7.**
Evaluation Committee Participation Agreement	30/09/2017	Abandoned
App for smartphones	31/12/2017	Abandoned

6.1.2.2 C2. Eradication of small American Bullfrog population nuclei



Responsible beneficiary of the action: SHF
Other beneficiaries involved: CDPNE, PNRPL, PNRLG

DESCRIPTION OF TECHNICAL PROGRESS AND DIFFICULTIES ENCOUNTERED BY SUB-ACTION

Progress of the action between 01/09/2016 and 31/08/2022 and results

The actions carried out under Action C2 and the results obtained are detailed in **Deliverable n°2**. A summary of the results is given below.

- ***C2.1. Eradication of the American Bullfrog population nucleus in Sologne (GT-1)***

The CDPNE has renewed each year the following actions to target the different stages of development of the species:

- For eggs: prospecting of clutches;
- For tadpoles: installation of traps, emptying of ponds and seine fishing;
- For juveniles and adults: shooting with rifles, installation of trapping barriers.

Brush clearing and logging actions have also taken place on some ponds to facilitate access and implementation of shooting actions, limiting the number of refuges for American Bullfrogs.

The following table (Table 6) shows the number of individuals captured by stage and year.

TABLE 6. Number of American Bullfrog individuals caught in Sologne by year and stage of development

	2017	**2018**	**2019**	**2020**	**2021**
Pontes	7	0	2	0	0
Tadpoles	8 491	14 185	1 388	8	0
Juveniles	1 348	745	7	14	74
Adults	32	23	16	10	10

During the first surveys conducted in 2002 at the time of the discovery of the presence of the species in Sologne, 119 bodies of water had been identified as colonized. At the end of 2021, fewer than 7 sites were considered still colonized. Eradication has not yet been achieved, but the colonized area has drastically decreased. The abundance of individuals continues to fall. This result is very positive, eradication has never been so close. However, it also stresses the need to continue prospecting and control efforts to eliminate the last individuals.

In addition to **Deliverable n°2**, the slideshow in **Annex n°8**, presented during the symposium of restitution of the project, takes stock of the last 20 years of fight against the American Bullfrog in Sologne.

- ***C2.2. Eradication of the American Bullfrog population nucleus in Dordogne (GT-2)***

The PNRPL implemented several control techniques during the project. As for Sologne, the objective is to target all stages of development:

- For eggs: search and prospection of lays;
- For tadpoles and juveniles: trapping at the trap;
- For adults: night shooting accompanied by special game wardens.

The following table (Table 7) shows the number of individuals captured by stage and year.

TABLE 7. Number of American Bullfrog individuals caught in Dordogne by year and stage of development

	2017	**2018**	**2019**	**2020**	**2021**
Pontes	2	1	0	2	0
Tadpoles	655	6 902	41	6 295	16
Juveniles		702	406	166	21
Adults	10	400	304	12	3

It should be noted that the American Bullfrog population in Dordogne was initially established in three sectors:

- Saint-Saud-Lacoussière sector;
- Sector of Piégut-Pluviers;
- Sector of Thiviers.

The surveys and actions carried out as part of the project have highlighted the absence of activity of the species on the first two sectors. The species still seems to be present only in the Thiviers sector. At the end of 2021, the presence of the species (at all stages) was proven on a single body of water.
As in Sologne, eradication has not yet been achieved. However, LIFE CROAA has had a significant impact on the Perigord population thanks to the significant resources that have been deployed. Eradication is almost achieved.

- ***C2.3. Eradication of the American Bullfrog population nucleus in the Bassin d'Arcachon (GT-3)***

The situation in the Bassin d'Arcachon is complex. Unlike Sologne and Dordogne, the level of colonization of the American Bullfrog was poorly known at the beginning of the project, the last inventories dating from 2005. During the first two years of the project, efforts were focused on updating the distribution of the species (see Action A2). Inventories revealed the presence of the species at only 6 sites in 2017. In 2018, a new colonized site was discovered.

The control actions really began in 2019. Due to the context and the difficulties of access to the various colonized sites, their implementation proved to be complete. In total, only 9 individuals were eliminated. In 2021, despite 44 days of prospecting, the species was not contacted. This suggests that the workforce is extremely small and highly localized.

Comparison with the provisional timetable

Control actions were planned from 2017 to 2021. They started well in 2017 in Sologne and Dordogne. In the Bassin d'Arcachon, eradication operations began in 2019, the first two years of the project having been devoted to updating the distribution of the species.
As the entire budget for Action C2 has not been used and the actions must be maintained in order to maximise the chances of achieving eradication, it was decided to continue the actions until June 2022 (the months of July and August having been devoted to the balance sheets and the drafting of the final report). This opportunity made it possible to initiate the 2022 field campaign. Additional funding was sought locally to finish the season.

Difficulties encountered and measures put in place where necessary

The difficulties are twofold:

- *Access to properties:*

Whether in Sologne, Dordogne, or the Bassin d'Arcachon, access to property, private or public, has sometimes proved difficult. In Sologne, a territory with a large hunting vocation, the implementation of eradication actions can come up against this activity during the opening period of hunting. Access to properties is sometimes refused in order to avoid disturbing the game. On the Bassin d'Arcachon, these difficulties of access – for other reasons – were found on a private site belonging to a company, or on a public leisure base managed by the Gironde Departmental Council.

To overcome this, permanent and very regular contacts are ensured with the owners in order to guarantee dialogue and seek compromises on a case-by-case basis.

The regulations also provide for the possibility of calling on the support of the Environmental Police (OFB agents) in order to oblige the owner to accept the implementation of the actions. But none of the associated beneficiaries resorted to this solution, which could have been counterproductive.

- *Detection of individuals:*

On small populations, such as those of Sologne, Dordogne and Bassin d'Arcachon, the numbers and size of individuals are reduced. As a result, they are increasingly difficult to detect. The time of prospecting in the field therefore increases, while the probability of finding an individual decreases. In order to counter this problem, the LIFE CROAA teams have devised a passive listening solution, based on automatic recordings (see Action A3). If a American Bullfrog song is identified, then control operations can be organized without wasting time. This is a real added value. The CDPNE, in partnership with Beauval Nature, has conducted initial experiments on this subject. In view of the very promising results, the CDPNE, the PNRPL and the PNRLG have equipped themselves with recorders, which will also make it possible to continue monitoring actions after the project, an action identified as a priority in the Post-LIFE Plan, presented in Action F3.

Complementary actions

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Prospects

Monitoring and control actions will have to continue after the project in order to achieve the definitive eradication of the three populations (see action F3 – Post-LIFE plan).

DELIVERABLES

TABLE 8. Deliverables of Action C2

Entitled	Estimated deadline	Status
Annual review of eradication operations carried out in 2017	15/01/2018	**Finalized** Provided in the mid-term report (Annex II.8).
Annual review of eradication operations carried out in 2018	15/01/2019	**Finalized** Provided by email to the external monitoring team (Quirin Renard) in July 2019
Annual review of eradication operations carried out in 2019	15/01/2020	**Finalized** Previously provided
Annual review of eradication operations carried out in 2020	15/01/2021	**Finalized** Provided during the visit of the external monitoring team on 29/03/2022
Annual review of eradication operations carried out in 2021	15/01/2022	**Finalized** Provided during the visit of the external monitoring team on 29/03/2022
Final assessment of eradication operations	31/01/2022	**Finalized** Deliverable n°2

6.1.2.3 *C3. Protection of stake sites in large population nuclei of African clawed frog and American Bullfrog*

Action C3	2016				2017				2018				2019				2020				2021				2022			
	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
Planned																												
Achieved																												

Responsible beneficiary of the action: SHF
Other beneficiaries involved: CCT, CN, PNRLAT

DESCRIPTION OF TECHNICAL PROGRESS AND DIFFICULTIES ENCOUNTERED BY SUB-ACTION

Progress of the action between 01/09/2016 and 31/08/2022 and results

- ***C3.1. Protection of stake sites on the population core of the African clawed frog (XL-1 core)***
  - *Actions implemented in the Deux-Sèvres by the CCT*

Two areas at stake for native amphibians were identified in *the* Thouarsais at the beginning of the program:

- The Gouraudière slag heap, located between the Gouraudière quarry and the Sensitive Natural Area of the Vallée du Pressoir, is designed to accommodate a dozen species of amphibians, one of the richest sites in the department.
- The second designated area was first the vicinity of the Joyette stream, the probable dispersal axis of the African clawed frog. The objective was to prevent the progression to the Saint-Varentais. However, following the discovery of the species in the Saint-Varentais in 2018, the initially defined stakes area was replaced by a network of bocage ponds located at a place called La Butte, known to shelter beautiful populations of large newts (Fig. 6).

The objectives were to:

1. Protect the Gouraudière *slag heap* from the invasion of the African clawed frog;
2. Limit the development of the species on the network of ponds of La Butte;
3. Create ecological corridors for native species, in which African clawed frog numbers would be reduced, to facilitate the formation of functional metapopulation systems.

In total, nearly fifty water points were trapped with classic traps baited with dog croquettes. 28,518 individuals (adults and juveniles) were captured and eliminated between 2018 and 2021.

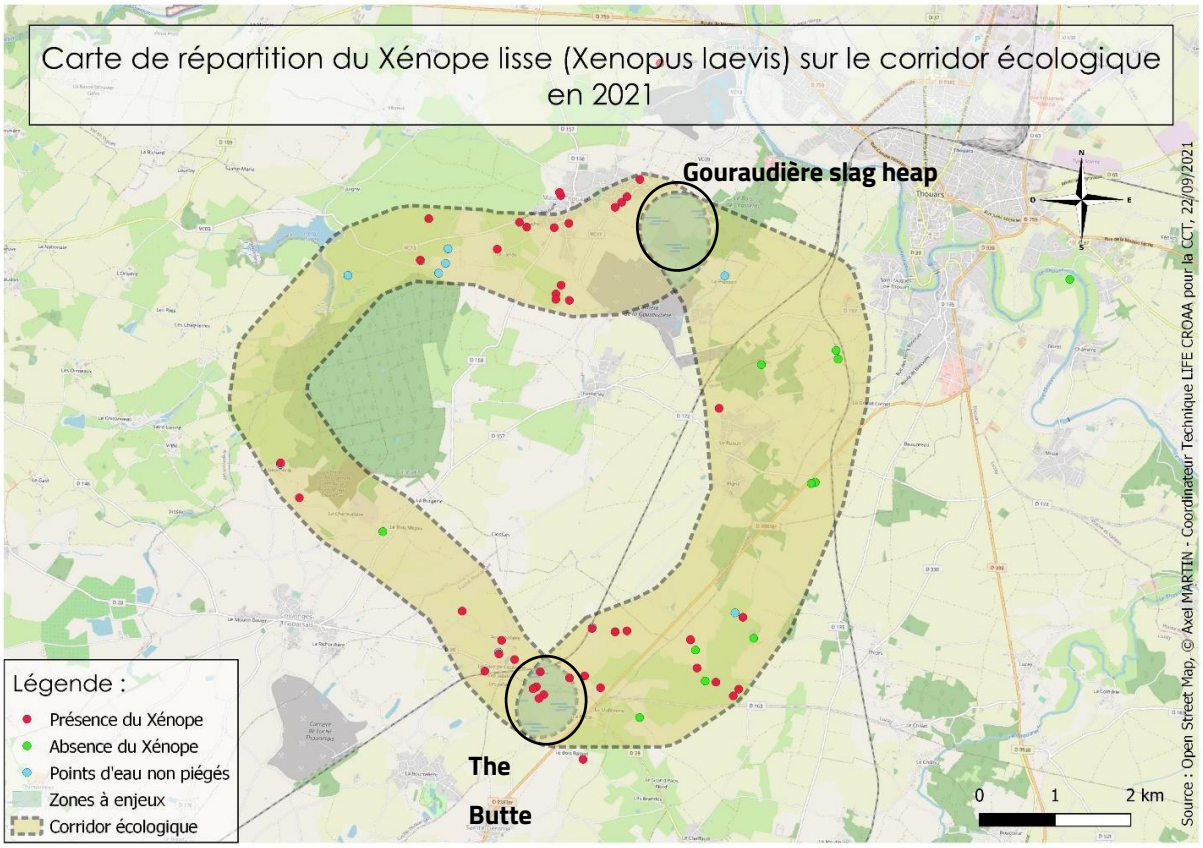

FIGURE 6. Distribution of areas with managed issues in Deux-Sèvres

- *Actions implemented in Maine-et-Loire by the PNRLAT*

A study commissioned in 2018 by the PNRLAT to a group of students from the Catholic University of the West (UCO) made it possible to identify priority sites on which to act (see Mid-term report). Two sectors were selected by the PNRLAT (Fig. 7):

- The Fontevraud/Montsoreau sector, which presents a major challenge for indigenous amphibians;
- The Gennes/RNR sector "Étang de Joreau et boisements de Joreau", which presents a very strong challenge for odonates.

These sites concentrated all the trapping efforts of the African clawed frog between 2018 and 2019.

In parallel, larger areas have been trapped in order to refine knowledge on the foci of presence of African clawed frog, in particular on:

- The municipalities around the forest massif of Fontevraud, and in particular the treatment plants;
- The Mazé sector (presence of an outbreak detected in 2018 on a pond), north of the Loire.

As in Deux-Sèvres, the trapping of the African clawed frog was implemented using traps baited with dog food.

A total of 5,721 individuals were captured between 2018 and 2019.

Note: The PNRLAT continued the control actions on these sectors in 2020 and 2021, by directing time to action C4.

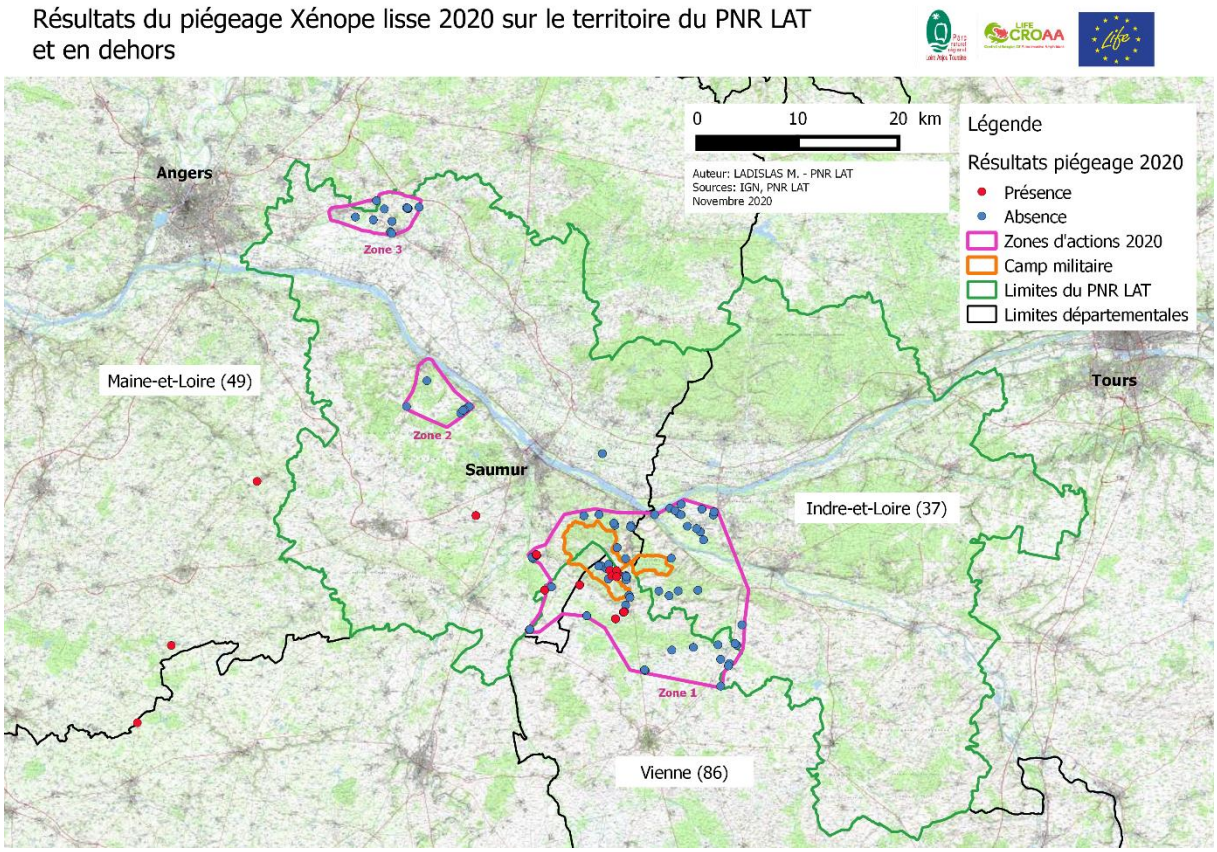

FIGURE 7. Location of areas that have benefited from trapping actions in Maine-et-Loire and Vienne. The map shows the results of the 2020 campaign, but these are the same areas that were managed from 2018 to 2021.

- ***C3.2. Protection of sites at stake in the core population of American Bullfrogs in Gironde (GT-4)***

Control actions were directed towards sites of presence with high stakes for biodiversity. Manual capture, landing net, trapping/jacket trapping and night rifle shooting are the main methods that have been used in regulatory actions.

As all the targeted sites are wetlands, specific ammunition not containing lead was used for firing actions, avoiding any pollution of the environment.

The table below details the number of individuals eliminated per year.

TABLE 9. Number of American Bullfrog individuals eliminated from the main core of Gironde, by stage of development and year

	2017	**2018**	**2019**
Clutches	1	0	1
Tadpoles	2 000	1 520	3 508
Juveniles	0	0	632
Subadults	389	48	187
Adults	66	112	121

Note: CN continued American Bullfrog control actions in 2020 and 2021, marking time to action C4.

Comparison with the provisional timetable

Nucleus XL-1 – Deux-Sèvres: Concerning the Gouraudière slag heap, the temporary ponds could not be trapped. To date, no clawed has been observed. However, the surrounding ponds have been trapped and show a very high population density, especially on some degraded ponds. In addition, the African clawed frog is now present within the ENS of the Vallée du Pressoir.
Regarding the network of ponds of La Butte, despite systematic trapping since 2018, the population of African clawed frog has continued to grow and it is now well established on the site. Only a few ponds, less favourable, are spared.

Nucleus XL-1 – Maine-et-Loire/Vienne: It was initially planned to set up trapping on water bodies near two main rivers (the Layon and the Thouet) identified as a natural expansion route for the African clawed frog and thus avoid an expansion of the species, particularly towards the Loire. However, following the results of action A2 and the numerous records of the presence of eDNA positive African clawed frog on water points in Nord-Loire (Varennes, Villebernier, Champtocé, Varades...), the action was considered outdated.
Thus, the PNRL AT, in consultation with the SHF and the AU, has chosen to orient its strategy from 2018 on the second objective of action C3, aimed at preserving sites with remarkable biological stakes and has relied for this on the mapping work carried out by UCO students.

Nucleus GT-4: Regulatory authorizations, conditional on obtaining a prefectural order, have not always been obtained in a timely manner.
Thus, the control sessions, particularly by shooting, suffered from these delays, and sometimes had to be initiated well after the beginning of the period of activity of the species. This was particularly the case in 2017 (obtained on 31 August) and 2018 (obtained on 19 July).
In addition, action C3 for the protection of sites at stake should, from 2019, evolve into a control strategy (C4) defined on the basis of the data collected as part of action A4, necessary for the realization of predictive modelling carried out by the UA. As explained in A4, the updating of the distribution of the species on the main core of Gironde was initially planned for the end of 2017 but was staggered over two years because of the size of the area to be prospected. Similarly, some results concerning the ecological characteristics of the species, resulting from the follow-ups put in place were obtained one year later than expected. This information, essential to the production by the University of Angers of connectivity models, delayed the definition of the control strategy. Until a real control strategy can be defined on the basis of predictive models, the C3 action was therefore maintained in 2019.

Difficulties encountered and measures put in place where necessary

Nucleus-1 core – Deux-Sèvres: The presence of the African clawed frog in the Saint-Varentin sector, proven by the 2017-2018 inventory, led to the adaptation of the initial strategy, replacing this site with a second site at stake (La Butte) and setting up a systematic trapping of ponds constituting an ecological corridor between the Gouraudière slag heap. and La Butte. This pilot action is innovative: it is the first time that this type of action has been tested to build a network of functional indigenous settlements.

Nucleus XL-1 – Maine-et-Loire/Vienne: The implementation of the action had to be reviewed following the results of action A2 demonstrating a colonization front much more extensive than expected. As a result, it was necessary to identify new areas of action by identifying priority sectors in which to intervene. This work was done in collaboration with students.

Nucleus GT-4: In 2017, control shares were revised downwards in favour of A2 and A4 shares, which are particularly time-consuming. The significant delay in obtaining the prefectural decree also limited eradication

actions. In order to compensate for the delays in obtaining authorizations, mainly due to the fact that part of the action requires the use of a firearm, it has nevertheless been possible to launch numerous campaigns of manual capture, by landing net or by trap. The regulation of larvae and juveniles is also not achieved by shooting and could therefore be started, and although subadults and adults remain more difficult to capture with a net, this could also be feasible in some cases. Harvesting of adults by manual trapping during the season was satisfactory in targeting areas of presence with low riparian vegetation.
No treated sites could be completely eradicated: American Bullfrog numbers, at least for adults and subadults, seemed to decrease significantly during our intervention periods, but many individuals recolonized them the following season. Because of the strong interconnections of water bodies and wetlands, the surrounding individuals systematically took advantage of the decrease in densities to settle their new territories, even if the regulatory actions obviously reduced the densities and the pressure on the treated environments.

Complementary actions

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Prospects

In accordance with the Post-LIFE Plan, control actions must continue on the sites at stake.

DELIVERABLE

TABLE10. Deliverables of Action C3

Entitled	Estimated deadline	Status
Annual review of eradication operations carried out at stake sites on the XL-1 nucleus in 2017	01/01/2018	**Finalized** Provided in the mid-term report (Annex II.9)
Annual review of eradication operations carried out at stake sites on the GT-4 nucleus in 2017	01/01/2018	**Finalized** Provided in the mid-term report (Annex II.9)
Annual review of eradication operations carried out at stake sites on the XL-1 nucleus in 2018	01/01/2019	**Finalized** Provided by email to the external monitoring team (Quirin Renard) in July 2019
Annual review of eradication operations carried out on sites at stake on the GT-4 nucleus in 2018	01/01/2019	**Finalized** Provided during the visit of the external monitoring team on 29/03/2022
Annual review of eradication operations carried out on the wet depressions of the Gouraudière and the Joreau pond (XL-1 nucleus) in 2019	01/01/2020	**Finalized** Previously provided
Annual review of eradication operations carried out on the wet depressions of the Gouraudière and the Joreau pond (nucleus XL-1 nucleus) in 2020	01/01/2021	**Finalized** Provided during the visit of the external monitoring team on 29/03/2022

Annual review of eradication operations carried out on the wet depressions of the Gouraudière and the Etang de Joreau pond (nucleus XL-1) in 2021	01/01/2022	**Finalized** Provided during the visit of the external monitoring team on 29/03/2022

6.1.2.4 *C4. Application of control strategies in large population nuclei of African clawed frog and American Bullfrog*

Action C4	2016				2017				2018				2019				2020				2021				2022			
	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
Planned																												
Achieved																												

Responsible beneficiary of the action: SHF
Other beneficiaries involved: CCT, CN, PNRLAT

DESCRIPTION OF TECHNICAL PROGRESS AND DIFFICULTIES ENCOUNTERED BY SUB-ACTION

Progress of the action between 01/09/2016 and 31/08/2022 and results

This action was dependent on the results of action A4. Indeed, its objective was to apply the strategy developed at the conclusion of action A4 on 110 sites colonized by the African clawed frog and 20 sites colonized by the American Bullfrog. As a reminder, the chosen strategy was built thanks to the involvement of scientific and technical experts, gathered for a working seminar in December 2020 (see action E2.5). The report of the scientific seminar highlights the need to act in a more global context and stresses the lack of knowledge which, to date, and despite the studies carried out in Actions A, prevents the definition of an appropriate strategy on a large scale.

Given the short time remaining until the end of the project and the fact that the budget for Action C4 had not been dimensioned for the actions advocated in the strategy, Action C4, as originally planned, could not be implemented. After discussion with Quirin Renard, NEEMO instructor, and all the beneficiaries of the project, it was decided not to submit an application for an amendment to the grant agreement.

As a result, the beneficiaries involved in this action have worked at two levels:

1. Continue the ongoing field actions undertaken by the PNRLAT and CN at the beginning of the project on action C3, the latter being consistent with the proposals of the strategy identified in action A4.
2. Strengthen communication and lobbying actions with national and local public authorities on the situation of large claws of African clawed frogs and American Bullfrogs. In this context, the SHF exchanged with the OFB to assess the possibility of developing a National Plan for the Control of Invasive Alien Amphibians, which would take into account the recommendations of the strategy resulting from Action A4. In addition, the SHF sent the associated beneficiaries frames of mail to be sent to local institutions in order to alert them to the situation. The PNRLAT has sent a letter to the presidencies of the departments (Maine-et-Loire and Indre-et-Loire) and the regions concerned (Pays de la Loire and Centre-Val de Loire). They are available in **Annex n°9**.

Comparison with the provisional timetable

The action, as originally planned, has been delayed due to the late development of the strategy developed under Action A4. However, the field actions initiated under action C3 were implemented in 2019/2020 by the PNRLAT and CN.

Difficulties encountered and measures put in place where necessary

See above

Complementary actions

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Prospects

Knowledge gaps were highlighted at the December 2020 working seminar. Multi-partner research projects have been submitted in this direction.

DELIVERABLES

TABLE11. Deliverables of Action C4

Entitled	Estimated deadline	Status
Annual review of the implementation of control strategies on the XL-1 nucleus in 2019	01/01/2020	**Abandoned**
Annual review of the implementation of the strategies to combat the GT-4 nucleus in 2019	01/01/2020	**Abandoned**
Annual review of the implementation of control strategies on the XL-1 nucleus in 2020	01/01/2021	**Abandoned**
Annual review of the implementation of the GT-4 nucleus control strategies in 2020	01/01/2021	**Abandoned**
Annual review of the implementation of control strategies on the XL-1 nucleus in 2021	01/01/2022	**Abandoned**
Annual review of the implementation of the strategies to combat the GT-4 nucleus in 2021	01/01/2022	**Abandoned**

6.1.3 Actions D - Monitoring the impact of project actions

6.1.3.1 D1. Assessment of the impact and effectiveness of concrete conservation actions

Action D1	2016				2017				2018				2019				2020				2021				2022			
	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
Planned																												
Achieved																												

Responsible beneficiary of the action: SHF
Other beneficiaries involved: All

DESCRIPTION OF TECHNICAL PROGRESS AND DIFFICULTIES ENCOUNTERED BY SUB-ACTION

To carry out this action, indicators have been defined. They made it possible to analyse the effectiveness:

- The early detection and assessment system (sub-action D1.1),
- American Bullfrog eradication actions implemented on small population nuclei (sub-action D1.2)
- Measures taken to preserve sites at stake on large population centres (sub-action D1.3)
- The control strategy identified in Action A4 and applied in Action C4 (Sub-Action D1.4).

The effectiveness of communication actions was also evaluated.

The table of indicators defined for this purpose is presented in **Annex n°10**.

Progress of the action between 01/09/2016 and 31/08/2022 and results

- ***D1.1. Early detection and assessment system***

The indicators defined were reported annually by the SHF.

- ***D1.2. Eradication of small population nuclei***

The indicators defined were reported annually (2017-2021) by the CDPNE, the PNRLG and the PNRPL. These indicators revealed that the management actions implemented in the small population nuclei had allowed the capture of more than 56,000 individuals, thus tending towards an eradication of the American Bullfrog in these areas (see **Deliverable n°3**). The 85 sites on which the American Bullfrog has been eradicated have also been double-checked using environmental DNA, confirming the absence of the species in these territories.

- ***D1.3. Protection of Issue Sites***

The indicators defined were reported annually (2017-2021) by the CCT, CN and the PNRLAT. These indicators revealed that the management actions implemented in the large population nuclei resulted in the capture of more than 18,000 individuals (see **Deliverable n°3**). Despite a certain investment of the associated beneficiaries involved, these figures have unfortunately not slowed down or reversed the colonization of the American Bullfrog and the African clawed frog on their respective territories. Moreover, faced with the vast territories colonized by the two target species, control actions could only take place on part of the colonized surfaces.

- ***D1.4. Application of control strategies***

As Action C4 has been revised, this sub-action is no longer relevant.

Comparison with projected schedule and expected results

It was expected that the indicators would be identified in early 2017. However, due to the volume of work at the beginning of the project, a slight delay was taken, without this having any impact on the action.

Difficulties encountered and measures put in place to address them

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Complementary actions

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Prospects

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DELIVERABLES

TABLE12. Deliverables of action D1

Entitled	Estimated deadline	Status
Report on the analysis of the effectiveness of eradication/control and communication operations	30/09/2019	**Finalized** Provided to the external monitoring team, during its visit on 29/03/2022
Final report analysing the effectiveness of eradication/control and communication operations	30/06/2022	**Finalized** Deliverable n°3

6.1.3.2 D2. Evaluation of the restoration of ecosystem functions

Action D2	2016				2017				2018				2019				2020				2021				2022			
	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
Planned																												
Achieved																												

Responsible beneficiary of the action: SHF
Other beneficiaries involved: All

DESCRIPTION OF TECHNICAL PROGRESS AND DIFFICULTIES ENCOUNTERED BY SUB-ACTION

Progress of the action between 01/09/2016 and 31/08/2022 and results

The objective of this action was to study the impact of American Bullfrogs and African clawed frogs on the state and resilience of colonized wetlands, by assessing the biodiversity present in these environments through the sampling of amphibians and aquatic invertebrates. The amphibian monitoring protocol is based on the POP Amphibian protocol developed by the SHF. The aquatic invertebrate monitoring protocol is based on the IBEM protocol (Biological Index of Ponds and Ponds) developed by a team of Swiss researchers. These two protocols have been adapted to the objectives of LIFE CROAA.

We expected colonized sites to have a lower overall state, with a lower specific richness in aquatic invertebrates and amphibians than non-colonized sites. The control actions implemented in colonized environments were also intended to counteract the negative effects caused by invasive alien species, by limiting their impacts on the specific richness of the environments, ensuring that colonized environments managed have a better capacity for resilience to the arrival of invasive alien species. Based on sampling of nearly 100 different sites (in 2017/2018 and again in 2021), the results of this assessment showed that sites not colonized by American Bullfrogs or African clawed frogs appeared to host more local amphibians and aquatic invertebrates than colonized sites. Managed colonized sites also tend to have a better state of conservation than unmanaged colonized sites, highlighting the benefit of management actions for the preservation of local amphibians.

The results are detailed in **Deliverables n°4 and n°5**.

Comparison with the provisional timetable

For the initial follow-ups, the schedule was respected for the GT-1, GT-2 and XL-1 cores.
Follow-up was delayed by one year for GT-3 and GT-4 nuclei, due to lack of knowledge on the distribution of the American Bullfrog. The results of Action A2 were indeed necessary to identify the sites to be monitored. Sampling therefore began in early 2018.
Regarding the monitoring at the end of the project, the schedule was respected.

Difficulties encountered and measures put in place where necessary

The method developed at the beginning of the project proved over time not to be the most relevant and gave rise to many difficulties in setting up the action (compliance with the timetable, time-consuming follow-ups, difficulties in identifying the sites to be monitored). A more documented monitoring, before and after the control actions deployed, would potentially have made it possible to detect more robustly the resilience capacity of the sites.

Complementary actions

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Prospects

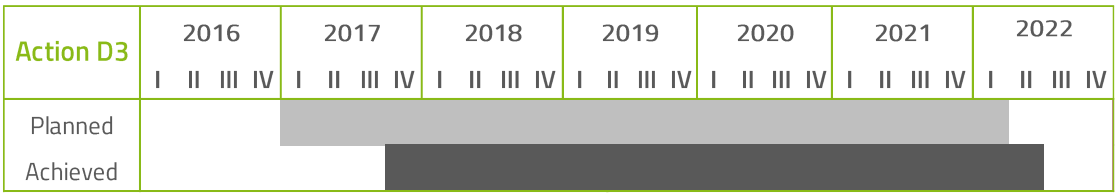
However, the preliminary results of this assessment are consistent with the conclusions of the literature: the presence of the two target species in aquatic sites, through predation and competition for resources, leads to a decrease in specific richness, particularly in amphibians and aquatic invertebrates. Further studies are needed to confirm the early trends observed here. Similarly, monitoring the colonization of sites, newly cleared of invasive amphibians, by local amphibians would be very relevant, in order to estimate the resilience capacity of colonized wetlands.

DELIVERABLES

TABLE13. Deliverables of Action D2

Entitled	Estimated deadline	Status
Report on changes in ecosystem structure for each species	01/03/2022	**Finalized** Deliverable n°4
Report on the assessment of the restoration of ecosystems and their services (in relation to the EU MAES policy)	01/03/2022	**Finalized** Deliverable n°5

6.1.3.3 D3. Assessment of the socio-economic impact of the project



Responsible beneficiary of the action: SHF

Other beneficiaries involved: All

DESCRIPTION OF TECHNICAL PROGRESS AND DIFFICULTIES ENCOUNTERED BY SUB-ACTION

Progress of the action between 01/09/2016 and 31/08/2022 and results

The indicators set out in **Deliverable n°6** were defined and informed each year (2017-2022) by all associated beneficiaries. Based on the method deployed by Nelly Felter (LIFE GypHelp), the evaluation of the socio-economic impact of the project showed that it had made it possible to employ several dozen people on the national territory (57.86 FTE in total). Beyond this direct impact on employment, the indicators also made it possible to identify 867 economic service providers as well as many actors and partners (193 in total), making the LIFE CROAA project more than visible in terms of economic and territorial influence.

In addition, the evaluation of the project also focused on the sociological impact of the project on beneficiaries, schoolchildren and the general public. The SHF called on a contractor to carry out this study. The various interviewees all highlighted that the project was essential for the preservation of ecosystems and that the awareness-raising and communication tools developed were perfectly adapted. The latter were also disseminated through numerous events in which the associated beneficiaries participated. The analysis report is available in the appendix (**Deliverable n°7**).

Comparison with the provisional timetable

There was a slight delay in identifying the indicators, but this had no impact on the progress of the action and the achievement of the objectives set.

Difficulties encountered and measures put in place where necessary

The definition of socio-economic indicators arrived in the middle of the project. Most of them could be informed retroactively, not causing any problems for the realization of the action. Only interviews and surveys could not be launched at the very beginning of the project.

Complementary actions

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Prospects

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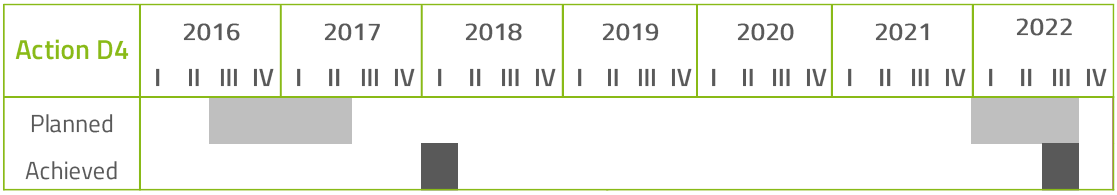
DELIVERABLES

TABLE14. Deliverables of Action D3

Entitled	Estimated deadline	Status
Socio-economic impact report prepared by the external service provider	15/07/2022	**Finalized** Deliverable n°4
Final scoreboard (indicators)	15/07/2022	**Finalized** Deliverable n°5

6.1.3.4 D4. Evaluation of indicators for LIFE projects



Responsible beneficiary of the action: SHF

Other beneficiaries involved: -

DESCRIPTION OF TECHNICAL PROGRESS AND DIFFICULTIES ENCOUNTERED BY SUB-ACTION

Progress of the action between 01/09/2016 and 31/08/2022 and results

The objective of this action was to select and inform the indicators set up by the European Commission to evaluate the LIFE programme.

In January 2018, the SHF therefore identified the indicators concerning the LIFE CROAA project and filled in those for which data were available. Some could not be informed for lack of information at that time.

In August 2022, all data (at the beginning of the project, at the end of the project, five after the end of the project) was completed. Particular attention was paid to the justification of the data entered. The values were validated by the Agency in October 2022.

The analysis of the results is detailed in Part 7 of this report.

The extraction from the KPI platform is available in **Annex n°11**.

Comparison with the provisional timetable

We had initially planned to fill in the indicators in March 2017, but the platform developed by the European Commission was not yet available.

Difficulties encountered and measures put in place where necessary

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Complementary actions

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Prospects

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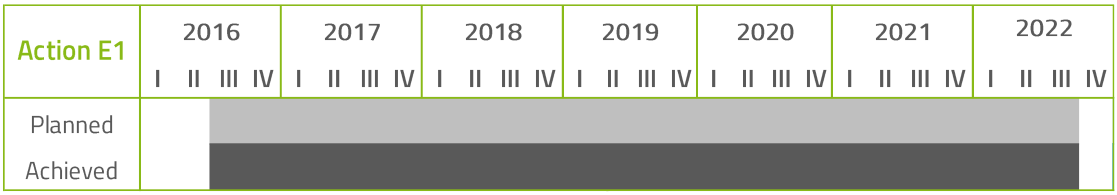
DELIVERABLES

No deliverables are planned for this action.

6.1.4 Actions E - Public awareness and dissemination of results

6.1.4.1 E1. Communication planning and implementation



Responsible beneficiary of the action: SHF
Other beneficiaries involved: All

DESCRIPTION OF TECHNICAL PROGRESS AND DIFFICULTIES ENCOUNTERED BY SUB-ACTION

Progress of the action between 01/09/2016 and 31/08/2022 and results

- ***E1.1. Dissemination plan and communication and awareness-raising tools***

The SHF had to recruit an intern to write the communication plan. However, this was not possible for operational reasons (teleworking staff). The communication plan was therefore drafted internally by the salaried team (see Annex II.12 of the mid-term report).

The table in **Annex n°12** presents the various communication tools that have been created within the framework of this action. For some, the SHF used external service providers. Of all the planned media, two have not been developed:

- *Newsletters*. In return, we regularly posted on the project's Facebook page.
- The presentation brochure of the cartographic tool. Rather than creating a dedicated support for this tool, we have included a presentation in the brochure dedicated to LIFE CROAA.

- ***E1.2. Networking with other European projects***

Linking with other LIFEs dealing with similar issues

Contacts have been made with the following LIFE projects:

- LIFE LAMPROPELTIS (LIFE10 NAT/ES/000565)
- LIFE ASAP (LIFE15 GIE/IT/001039)
- LIFE RAPID (LIFE16 NAT/UK/000582)
- LIFE 3n-American Bullfrog (LIFE18 NAT/BE/001016)

With regard to LIFE LAMPROPELTIS and ASAP, contact did not allow for regular exchanges. The exchanges were richer with the LIFE RAPID. We participated in a seminar organised by the project coordinating team in November 2018, then we exchanged on the actions carried out against the American Bullfrog and the African clawed frog in England.

On the other hand, we have created a close relationship with LIFE 3n-American Bullfrog. Alain De Vocht, member of PXL University, coordinating structure of the project, is part of the scientific committee of LIFE CROAA. Jean Secondi, associate beneficiary (UA) of the latter is also a member of the scientific committee of LIFE 3n-American Bullfrog. Finally, Alain de Vocht presented the project at the LIFE CROAA restitution conference. The technique developed by LIFE 3n-American Bullfrog (sterilization of individuals) could be an avenue to explore for the management of French American Bullfrog populations.

The SHF and the UA also participated in the "Platform Meeting" on Invasive Alien Species organized by the Lombardy Region, coordinator of LIFE GESTIRE 2020 "Nature Integrated Management to 2020" (LIFE 14-IPE 000018), in Milan on 29 and 30 November 2017. It was an opportunity to meet other LIFE project leaders dealing with IAS themes and to present LIFE CROAA.

Linking with other LIFEs in France

The SHF participated in the following interLIFE meetings:

- 2016 – Auzat (09), organised by LIFE+ Desman (LIFE13NAT/FR/000092), from 7 to 8/11/2016
- 2017 – Reunion (974), organised by LIFE+ Petrels (LIFE13 BIO/FR/000075), Dry Forest (LIFE13 BIO/FR/000259) and CapDOM (LIFE09 NAT/FR/000582), from 4 to 8/12/2017
- 2018 – Prémanon (39), organised by the LIFE Tourbières du Jura (LIFE13 NAT/FR/762), from 19 to 21/09/2018
- 2019 – Marseille (13), organised by LIFE Habitats Calanques (LIFE16 NAT/FR/000593), from 30/09 to 02/10/2019
- 2021 – Châtelaillon-Plage (17), organised by LIFE Vison (LIFE16 NAT/FR/000872), Biodiv'OM (LIFE17 NAT/FR/000604) and Gypconnect (LIFE14 NAT/FR/000050), from 20 to 23/09/2021

These meetings have always been rich in learning and sharing experiences.

International Restitution Symposium

The LIFE CROAA restitution conference was held on 18 and 19 May 2022, in Bordeaux. Organized face-to-face and broadcast online, it brought together nearly 90 people. The programme and slideshows of the various interventions are available in **Annex n°13**.

Comparison with the provisional timetable

The launch of the project and the workload in 2017 did not allow for the development of all the communication materials and tools planned for the first year of the project. In addition, it was not relevant to write certain materials before having the results of the first actions (updating of maps, first committee meetings, photo library, etc.), nor before having thought in depth about a communication plan. The communication on IAS is indeed special and can be caricatured, divisive and counterproductive.

Difficulties encountered and measures put in place where necessary

Although there was a slight delay in the production of the various communication media planned, this had no impact on the achievement of the objectives set.

Complementary actions

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Prospects

The SHF and the associated beneficiaries will continue to communicate on the American Bullfrog and the African clawed frog after the end of the project. The tools developed, such as the website, the exhibition, the videos, are excellent communication media that will be used in the coming years. The SHF will continue to update the website regularly.

DELIVERABLES

TABLE 15. Deliverables of Action E1

Entitled	Estimated deadline	Status
Graphic	15/01/2017	**Finalized** Provided in the mid-term report (Annex II.13).
Communication plan	31/03/2017	**Finalized** Provided in the mid-term report (Annex II.12).
Documentary film and thematic videos	21/12/2021	**Finalized** Supplied during visit n°6 of Quirin Renard, NEEMO instructor
Layman's report	15/06/2022	**Finalized** See **Deliverable n°8**

6.1.4.2 E2. Raising awareness of the problem of invasive alien amphibians and disseminating the results of the project

Action E2	2016				2017				2018				2019				2020				2021				2022			
	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
Planned																												
Achieved																												

Responsible beneficiary of the action: SHF
Other beneficiaries involved: All

DESCRIPTION OF TECHNICAL PROGRESS AND DIFFICULTIES ENCOUNTERED BY SUB-ACTION

Progress of the action between 01/09/2016 and 31/08/2022 and results

- ***E2.1. Prevention against intentional or unintentional introductions of exotic amphibians***

  - *Raising awareness among the general public*

Several actions have been carried out for the general public and young audiences:

- **Creation of an educational kit**
  The development of this tool took a long time. Its objective is to communicate widely about the taxonomic group of amphibians, including the risks to these endangered species, in order to allow children to acquire knowledge about their biology and ecology. At the end of the various games offered (6 in total), the children must have acquired knowledge that allows them to discuss and formulate their ideas about the introduction of exotic species.
  The briefcase was published in seven copies. This generated an additional cost, not initially foreseen, but allowed each beneficiary of the project (except the UA) to have its own copies and to provide entertainment to schools. A dematerialized version has been made available free of charge on the LIFE CROAA and SHF websites. An information campaign was launched at the end of 2021 on a large scale to publicize the existence of this tool; More than 1300 downloads were recorded.

- **Animation for young audiences**
  Many animations were carried out during the project.

- **Participation in public events**
  In total, the project team participated in 20 events for the general public. In particular, the SHF has held a stand dedicated to LIFE CROAA every year since 2016 at the Ménigoute International Ornithological Film Festival (79), this festival welcomes about 30,000 people each year.

- **Awareness-raising through the press**
  Finally, many articles were published in the local press.

- *Awareness of amateur amphibian and reptile owners, pet stores and breeding centres*

In 2020 and 2021, the SHF formed a working group, including several members of its Captivity Commission, to identify the best communication strategy to adopt to raise awareness among the general public likely to acquire exotic animals. Several tools have been developed for this:

- Video n°5 was built with the aim of raising awareness of the risks posed by the release of exotic species into the wild. It was widely disseminated on social networks.
- A collection of 4 posters was developed. Three of them highlight three exotic species frequently found in pet stores (a snake, a turtle, a pogona). A fourth poster, highlighting the African clawed frog, has been specially designed for research laboratories using the species as a biological model. These four posters are available for free download in two formats (A3 and A2) in the toolbox of the project *website*. They have also been widely disseminated through targeted awareness-raising campaigns. A specific emailing was created and sent to several hundred recipients (animal store unions, terrariophily unions, zoos, research centres, etc.).

- ***E2.2. Promoting and facilitating the implementation of actions to combat invasive alien amphibians***

Three types of actions were carried out:

- ***Raising awareness among elected officials and local institutions***
  The municipalities in which actions have been implemented have been informed in advance. In addition to facilitating the implementation of actions in the field, it was also an opportunity to raise awareness on the issue of invasive amphibians. For example, in 2017, the PNRLAT sent a letter to 66 municipalities in the Maine-et-Loire department impacted or probably soon impacted by the arrival of the African clawed frog. The purpose of this letter was to inform the municipalities of the involvement of the PNRLAT in LIFE CROAA and the steps in progress.
- ***Owner awareness***
  Information and awareness of owners (mainly private) is an essential step for the implementation of control actions. To avoid any conflict and promote the acceptance of actions on the ground, several means were used during the project: sending information letters, meetings at the beginning of the season, regular exchanges throughout the action period, transmission of summaries of the operations carried out on the site, etc.
- ***Involvement of volunteers***
  For several years, the CCT has been running a network of volunteer trappers on its territory, owners of water points colonized by the African clawed frog. An agreement is signed between the CCT and each trapper. *Ad hoc* equipment is made available to owners; the CCT is responsible for recovering individuals captured for disposal. This resulted in the capture of several thousand African clawed frogs during the project. At the beginning of the project, the PNRLAT envisaged the establishment of a similar network on the Maine-et-Loire side, but this did not succeed because of the time required for the implementation and monitoring of this type of action. The CDPNE also called on more than 200 volunteers throughout the project for the implementation of actions to combat the American Bullfrog in Sologne.

- ***E2.3. Proposals for improvements or adjustments to IAS public policies and regulations***

In order to contribute to better regulation, the LIFE CROAA team:

- Provided its expertise and reviewed the risk analysis on the African clawed frog with a view to its inclusion on the list of invasive alien species of concern for the European Union;
- Drafted proposals for the Ministry in charge of the environment, as part of the draft amendment of the decree of 14 February 2018 on the prevention of the introduction and spread of invasive alien species on the metropolitan territory;
- Made recommendations on a draft order laying down the rules for the keeping of non-domestic animals. This project aimed to make changes to the regulations resulting from the Biodiversity Act and simplification compared to the decrees of 10 August 2004 setting the conditions for authorizing the keeping of animals of certain non-domestic species in establishments for breeding, selling, hiring, transiting or presenting to the public animals of non-domestic species;
- Responded to and disseminated public consultations on IAS.

The list of species of concern for the European Union was amended in July 2022: the African clawed frog is now included. The ministerial decree on the prevention of the introduction and spread of invasive alien animal species on the metropolitan territory should be updated accordingly in the coming weeks.

- ***E2.4. Replicability and transferability of developed methods and techniques***

As part of this sub-action, we have provided our expertise on the populations of African clawed frog and American Bullfrog recently discovered in metropolitan France:

- 2016 – Discovery of the African clawed frog, near Bordeaux (33). CN, also an associate beneficiary of LIFE CROAA, is in charge of the management of this population. It benefits directly from the knowledge and experience gained during the project.
- 2018 – Discovery of the African clawed frog in La Chapelle-d'Armentières, near Lille (59). The Groupe Ornithologique et Naturaliste du Nord-Pas-de-Calais (GON), a local association for the protection of nature, is in charge of managing this population. Many exchanges and meetings (by videoconference) took place in order to guide the GON on the prospections to be carried out and the actions to be implemented. In particular, the SHF was asked to give an opinion on the proposed action plan.
- 2019 – Discovery of the African clawed frog in Toulouse (31). The association Nature En Occitanie (NEO) is in charge of the management of this population. Exchanges were also planned to pass on our knowledge to the NEO team. This led to the realization of surveys and a partial emptying of the colonized basin.
- 2021 - Discovery of the American Bullfrog in Riedseltz (67), near the German border. The SHF was asked to give its opinion on the identification of frog individuals observed in Germany. After several exchanges and recommendations, additional surveys were conducted and validated the presence of the species. An action plan led by the CEN Alsace and implemented by the BUFO association was adopted in 2022.
- 2021 – Discovery of the American Bullfrog with Écuisses (71). The observation was entered into a European input tool and detected by the SHF. On the advice of the SHF, a field check was organized in 2021 by the Natural History Society of Autun, without results. Additional surveys have been scheduled for 2022. At the time of writing, no individuals have been identified.

The SHF was also asked by CEN Lorraine about the population of Fire-bellied *Ringer (Bombina bombina*) introduced in Moselle, in order to issue an opinion on the strategy to be adopted. A meeting with other national experts was planned, but it was not scheduled during the project.

The SHF was also in contact with the LIFE BNIP (Belgian Nature Integrated Project) which wanted to carry out surveys for the African clawed frog, after the discovery of the species at La Chapelle-d'Armentières, located

near the Belgian border. In 2021, a working group was formed between French actors (SHF, GON, CEN Hauts-de-France, DREAL Hauts-de-France) and Belgian actors (DEMNA-SPW, Natagora, INBO) to reflect on a common strategy for monitoring and managing the African clawed frog.

Finally, in order to facilitate the appropriation of the techniques developed and used in the framework of LIFE by other European actors, the *Technical Guide for the Management of American Bullfrogs and African clawed frogs* has been translated into English (see Action E2.5).

- ***E2.5. Dissemination of project results and sharing of experiences and knowledge gained during the project***

The dissemination of results and the sharing of experiences and knowledge gained during the project were ensured through the following activities:

- ***Interventions at local, national or international symposia, congresses, seminars.*** The project was presented at a seminar in South Africa in October 2019. He was also the subject of two papers (2018 and 2021) at the National Congress of Herpetology. Many other presentations were made during the project.
- ***Technical training of OFB agents.*** For two consecutive years (2018 and 2019), the SHF intervened during the training of OFB agents on exotic species, in order to discuss the issue of invasive amphibians and reptiles.
- ***Publication of several scientific articles (see Annex n °5).***
- ***Organization of a working seminar.*** The objective was to identify a management strategy for the most extensive American Bullfrog and African clawed frog nuclei, based on the knowledge gained during the project (see Action A4).
- ***Writing of a technical guide for the management of American Bullfrogs and African clawed frogs.*** This substantial document (130 pages) is structured around 9 chapters. The reader can find general information about IAS, American Bullfrog and African clawed frog. A chapter is also dedicated to regulatory aspects. The technical aspects are treated in the form of a decision tree, so that the manager identifies the actions to be implemented, depending on the situation in which he finds himself. Finally, the last part of the document is devoted to awareness raising and complementary measures to be applied for the preservation of native amphibians. This document is available in two versions, French and English, which are widely distributed. Twenty copies were printed for project beneficiaries, reviewers and project funding partners.

Comparison with the provisional timetable

The creation of the teaching kit has been delayed. The time required for design work has been greatly underestimated.

Difficulties encountered and measures put in place where necessary

Although the pedagogical kit has been delayed, activities with school children have been carried out since the beginning of the project in order to raise awareness among as many students as possible. Other supports were used pending the finalization of the briefcase.

In addition, we have changed our strategy regarding the awareness of amateur amphibian and reptile owners and pet stores. Indeed, the exchanges with the working group showed that the target identified at the outset (the amateur amphibian and reptile owners) was not the right one. Passionate amateur amphibian and reptile

owners are usually aware of the regulations and are careful of their animals. The risk of introduction into the wild comes more from the general public, who are able to give in to a favourite purchase, without having seriously thought about the commitment that this represents. The tools originally planned have therefore been replaced by awareness posters.

Complementary actions

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Prospects

Awareness and communication are essential to avoid the introduction of new species and to implement control actions. The SHF and the associated beneficiaries of the project will continue these actions in general. The tools created will be used in the coming years (pedagogical kit in particular).

DELIVERABLES

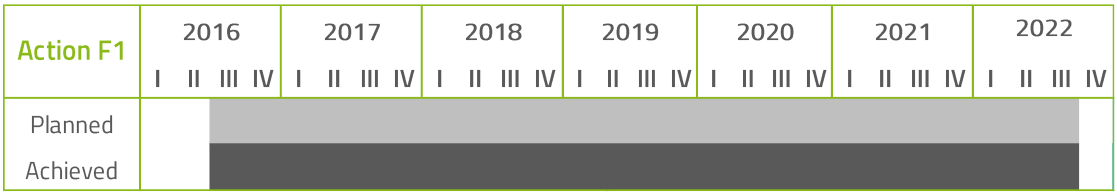
TABLE16. Deliverables of Action E2

Entitled	Estimated deadline	Status
Flyer on the risks of introduction into the natural environment	31/05/2019	**Altered** After internal discussion, it was decided not to make a flyer. Indeed, experience shows that those carried out to present the project and the two target species (action E1) are difficult to sell. We therefore opted for two other types of media: three awareness posters and a video (episode 5). The three awareness posters are intended to be simple and well-illustrated; The species highlighted (a turtle, a snake, a lizard) are species that are easily found in pet stores. Episode 5 was designed to be accessible and impactful. Made in a "Raw" format, the objective is to transmit information, clear and precise, in order to empower anyone wishing to adopt an exotic animal.
Teaching kits	31/05/2019	**Finalized** Presented to Quirin Renard, NEEMO instructor during the visit of 29/03/2022
Information leaflet with CD/DVD	31/07/2019	**Amended** A working group was formed in 2020 with experts in the captivity of alien species, in order to identify a suitable communication strategy. It was decided to abandon the information leaflet in favour of more suitable media. An awareness poster on the African

		clawed frog has been developed for research laboratories using the species as a laboratory animal. A communication campaign was also carried out for pet shops and pet shops' unions, zoos and zoological reserves to allow the wide distribution of public awareness posters and episode n°5.
Guide for the Management of Invasive Alien Amphibians	30/06/2022	**Finalized** See **Deliverable n°9**

6.1.5 Actions F - Project management

6.1.5.1 F1. General coordination of the project and animation of the committees



Responsible beneficiary of the action: SHF

Other beneficiaries involved: All

DESCRIPTION OF TECHNICAL PROGRESS AND DIFFICULTIES ENCOUNTERED BY SUB-ACTION

Progress of the action between 01/09/2016 and 31/08/2022 and results

Within the SHF, the project was coordinated by Myriam LABADESSE for the technical part and Isabelle CHAUVIN for the administrative and financial part.

The F1 action has made it possible to:

- **Ensure the technical, administrative and financial coordination of the project**
  At the end of 2016, the partnership agreements between the SHF and each of the beneficiaries were drafted and signed by both parties. These conventions were annexed to the first progress report. On the other hand, monitoring tools were provided by the SHF to the associated beneficiaries: framework of annual technical reports, timesheets, financial monitoring files, etc. in order to ensure rigorous management and monitoring of the project.
- **Make annual or biennial funding requests to co-funders**
  In addition to the European Commission, five financial partners supported the project: DREAL Nouvelle-Aquitaine, Agence de l'Eau Adour-Garonne, Centre-Val de Loire Regional Council, Nouvelle-Aquitaine Regional Council and Beauval Nature. It should be noted that Beauval Nature withdrew in 2020. Additional requests for funding were then submitted to the DREAL and the Nouvelle-Aquitaine Regional Council, and accepted
- **To convene the various committees of the project:**
  - The monitoring committee met 13 times during the project
  - The steering committee met 4 times during the project
  - The scientific committee met 3 times during the project
- **To welcome Frédéric Brochier, then Quirin Renard, monitors of the external monitoring team**
  Six visits were organized during the project, face-to-face (except in 2020, due to the health context). Field trips were proposed in order to present in a concrete way to the monitors the actions implemented in the field.
- **To draw up the various reports for the European Commission**
  The first progress report were sent on 28/06/2017. The mid-term report, initially scheduled for 30/11/2019, has been brought forward to 30/09/2018, following an official request on 17/02/2018, specifying that we had used 100% of the previous pre-financing instalment, and that this change would allows us to overcome any cash flow problems. This change has slightly disrupted the timing of subsequent progress reports. The three reports were therefore transmitted on 01/06/2017, 30/11/2019, 31/05/2021.

Comparison with the provisional timetable

It was initially planned that the administrative and financial coordinator would be recruited at the launch of the project, i.e. from 1 September 2016. The position was taken 4 months late.

Difficulties encountered and measures put in place where necessary

The late recruitment of the Administrative and Financial Coordinator forced the Technical Coordinator to take on some of the tasks of this post in addition to her own. This had an effect on the launch of some actions leading to a slight delay that has since been made up.

Following failures of the PNRLG and the UA observed by the SHF on administrative and financial feedback, registered letters reminding them of their obligations were sent to them in February 2018, with a copy to Mr. Frédéric BROCHIER. Their respective directorates have been receptive and we have since noted an improvement and compliance of their commitment. These letters have been provided in Annex III.12 of the mid-term report.

Complementary actions

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Prospects

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DELIVERABLES

TABLE17. Deliverables of action F1

Entitled	Estimated deadline	Status
Partnership agreements between the coordinating beneficiary and each associated beneficiary	07/10/2016	**Finalized** Provided with the first progress report
Minutes of each committee: 18 monitoring committees, 6 steering committees, 6 scientific committees	07/07/2022	**Finalized** See **Deliverable n°1**

6.1.5.2 F2. External audit of the project

Action F2	2016				2017				2018				2019				2020				2021				2022			
	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
Planned																												
Achieved																												

Responsible beneficiary of the action: SHF
Other beneficiaries involved: -

DESCRIPTION OF TECHNICAL PROGRESS AND DIFFICULTIES ENCOUNTERED BY SUB-ACTION

Progress of the action between 01/09/2016 and 31/08/2022 and results

As indicated in amendment No 1 of 27 September 2018, an attestation of the financial statements and underlying accounts is required only for beneficiaries for whom the total contribution in the form of reimbursement of the actual costs referred to in Annex III is at least €750,000. Therefore, no beneficiary is concerned. This action therefore did not take place.

Comparison with the provisional timetable

-

Difficulties encountered and measures put in place where necessary

-

Complementary actions

-

Prospects

-

DELIVERABLES

Not applicable.

6.1.5.3 F3. Implementation of a post-LIFE conservation and communication plan

Action F3	2016				2017				2018				2019				2020				2021				2022			
	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
Planned																												
Achieved																												

Responsible beneficiary of the action: SHF
Other beneficiaries involved: All

DESCRIPTION OF TECHNICAL PROGRESS AND DIFFICULTIES ENCOUNTERED BY SUB-ACTION

Progress of the action between 01/09/2016 and 31/08/2022 and results

In order to identify the actions to be implemented at the end of the project, several working meetings were organized between the SHF and the associated beneficiaries from the end of 2021. This led to the drafting of the post-LIFE conservation and communication plan – see **Deliverable n°10**. The document was written in French and English.

It takes stock of the project, the results obtained, its strengths and weaknesses. It also highlights (1) the need to continue management actions for American Bullfrog and African clawed frog populations in the coming years, and (2) the appearance of new outbreaks of colonization, by the American Bullfrog, the African clawed frog or other invasive amphibian species, on the metropolitan territory.

The proposed strategy is divided into two phases:

- Firstly, the immediate continuation of certain concrete actions of the project. This concerns in particular the monitoring and control actions against the GT-1, GT-2 and GT-3 nuclei in order to achieve the total eradication of the American Bullfrog on these small nuclei.

- Second, the drafting of a national plan to combat invasive alien amphibians. This document will propose a global approach (including other problematic species than the American Bullfrog and the African clawed frog) and will provide a framework for the actions to be implemented. It will be a strategic document, the drafting of which will require the creation of a committee of experts, and which will have to be validated at the national level by the National Council for Nature Protection.
  In order to achieve this objective, contacts have been made with the OFB and the Ministry of Ecological Transition and Territorial Cohesion.

Comparison with the provisional timetable

In accordance with the provisional timetable

Difficulties encountered and measures put in place where necessary

-

Complementary actions

-

Prospects

Not applicable

DELIVERABLES

TABLE 18. Deliverable of action F3

Entitled	Estimated deadline	Status
Post-LIFE Conservation and Communication Plan	30/08/2022	**Finalized** See **Deliverable n°10**

6.2 Main deviations or problems encountered and actions taken to remedy them

The difficulties encountered during the project are of two kinds:

1. **Administrative difficulties**

The first difficulty encountered concerns obtaining prefectural orders for the capture and destruction of the American Bullfrog, mainly in Gironde. Indeed, although the requests were transmitted sufficiently in advance, the prefectural orders were received late in 2017 (in September) and 2018 (in July), considerably delaying the implementation of the control actions. In order to facilitate these administrative procedures, the SHF developed in 2020 a file at the scale of the Nouvelle-Aquitaine region, in order to obtain prefectural orders valid until the end of the project for all beneficiaries located in the region (PNRPL, PNRLG, CCT, CN) for the capture and destruction of the American Bullfrog and the African clawed frog. Orders with a period of validity until the end of the project.

The implementation of control actions on private property has also sometimes proved complex. In Sologne, the vast majority of water bodies are private and used for hunting activities. Agreements were signed between the CDPNE and private owners, as soon as the American Bullfrog was identified on site. However, despite the signing of this agreement, an owner refused to empty his pond and implement a seine fishery.
Other beneficiaries of the project have also faced rejections from owners, private or public.
The regulations provide for the possibility of using sworn officers in order to compel the implementation of the action. However, this approach was deemed too aggressive and counterproductive by the LIFE CROAA team. We have strengthened communication and awareness among owners. Thus, very regular contacts are ensured in Sologne throughout the activity phase of the American Bullfrog.

2. **Technical difficulties**

The main technical difficulties concerned the following five actions:

- ***Action A3:*** This action aimed to develop new control techniques to improve the capture rate of American Bullfrogs and African clawed frogs, based on hypotheses from the scientific literature. Unfortunately, the experiments conducted proved inconclusive:
  - The trap to improve the catch rate of African clawed frog tadpoles, based on the assumption that tadpoles tend to aggregate under floating surfaces, did not result in any catches.
  - The trap to improve the capture rate of American Bullfrog adults, based on the use of songs to attract females, did not result in any catches.

  Due to the results obtained, and in order to continue the investigations, the Agency granted additional time for the completion of this action, which was initially scheduled to end in 2019. In this way, we were able to conduct additional experiments (standardized study of the most effective trap and bait for the African clawed frog, passive detection system of the American Bullfrog...).

- ***Action A4:*** Two main difficulties were encountered in this action:
  - Field studies on American Bullfrogs (adult radio-tracking, juvenile dispersal study, tadpole survival study) have not been conclusive, since adults have moved little, no juveniles and tagged tadpoles have been found. In order to obtain all the information necessary for the development of connectivity maps, a laboratory experiment had to be conducted.

- We initially thought that the connectivity maps produced would make it possible to identify fairly clearly sectors on which to act in priority and thus define a strategy for managing the most extensive kernels. However, this has not been the case. We organized a working seminar in 2020 to conduct a global and collegial reflection with scientists and experts of the two targeted species. The results are detailed above, in the description of action A4. The need for a more comprehensive approach thus emerged from the reflections. The lack of knowledge was also highlighted. This had consequences for the C4 action, whose objective was to apply the strategy on many sites colonized by the American Bullfrog and the African clawed frog. Action C4 was therefore adapted: the field actions initiated at the beginning of the project (and which were consistent with the conclusions of the working seminar) continued. In addition, communication and lobbying actions have been strengthened. No amendment to the grant agreement has been drafted and signed for this.

- ***Action C1: From the beginning of the project, the development of the*** initially planned smartphone application appeared irrelevant, due to the growing number of naturalistic applications currently being developed. On the advice of the Agency, and in return for the abandonment of this deliverable, we have contributed to the updating of European applications.

- ***Action C3:*** Following the results of action A2 carried out in 2017 and 2018, the strategies for combating African clawed frog in Deux-Sèvres, Maine-et-Loire and Vienne had to be adapted. Indeed, in the Deux-Sèvres, the initial objective was to avoid the invasion of the species in the Saint-Varentin, but the inventories of 2017/2018 have attested to its presence. As a result, the CCT modified its action plan and focused its efforts on other colonized areas. Similarly, the PNRLAT has adapted its action plan in view of the results of action A2; New priority sectors for action have been identified.

Finally, it is important to stress that LIFE CROAA has also been hit hard by the health crisis linked to the Covid-19 pandemic. Thus, field actions, but also communication, were disrupted in 2020. Field operations have been delayed in some areas due to the lockdown. Some events have been cancelled (national congress of herpetology, festival of Ménigoute...). We were also forced to postpone the holding of the working seminar (actions A4 & E2.5) and to organize it by videoconference. In general, these setbacks did not have a substantial impact on the progress of the project and the achievement of objectives.

6.3 Evaluation of project implementation

Applied methodologies

In general, the methods used in the context of the project were adapted to the objectives sought. Innovative techniques have been used, such as eDNA. This method has the advantage of limiting the time spent in the field. However, it is a complementary technique, which should not completely eclipse conventional field surveys. Some aberrant results were noted in particular in Sologne, with negative results when individuals had been observed.
On the other hand, the protocol put in place within the framework of the D2 action revealed some weaknesses. This type of study would be interesting to renew with a more robust protocol.

Comparison of the results achieved with the expected results specified in the proposal

As a reminder, the expected results as specified in the grant agreement are as follows:

- *The development of a model-based methodology to select the optimal control strategy against an invasive amphibian population.*
  ➤ **Result!** The modelling maps were produced in 2019 (see Action A4). The organization of the working seminar made it possible to identify a strategy for the management of large nuclei.
- *A gain in efficiency of capture methods (20%).*
  ➤ **Result obtained for the African clawed frog and in part for the American Bullfrog!** For African clawed frog, the various experiments conducted have made it possible to identify the most effective type of trap and bait (see Action A3). For the American Bullfrog, the only trap created and experienced did not bear fruit; However, it has highlighted the interest of ironing to improve the detection of individuals and thus improve the catch rate.
- *The eradication or significant reduction of small nuclei.*
  ➤ **Result!** At the end of 2021, the number of sites still colonized by the American Bullfrog in Sologne was estimated at less than 7. In the Dordogne, the species still seemed to be active on a single body of water. On the Bassin d'Arcachon, there was no contact that same year.
- *The preservation of at least two sites of ecological interest within the area of distribution of the African clawed frog and to limit its expansion along known dispersal axes.*
  ➤ **Result obtained for sites of ecological interest!** The pond of Joreau, Regional Nature Reserve located in Maine-et-Loire has benefited from increased monitoring and trapping actions in its surroundings. Today, it is not colonized by the African clawed frog. The second site, the Gouraudière slag heap, very rich in native amphibians, is located in Deux-Sèvres and has also benefited from monitoring and preservation actions. Like the Joreau pond, it is not colonized by the African clawed frog.
  Regarding the dispersal axes, the inventory conducted in 2017/2018 showed that the species was already beyond the known dispersal axes. It was therefore decided to focus on the sites at stake.
- *The eradication of eight sites colonized by the American Bullfrog in Gironde.*
  ➤ **Result not achieved.** The level of colonization of the American Bullfrog in Gironde is such that it is utopian to consider achieving the total eradication of certain sites, located in the range of the species.
- *Raising awareness among pet store brands, laboratories, amateur amphibian and reptile owners and the main naturalist associations.*

➤ **Result!** Specific tools have been produced for this purpose (posters, video – see action E2). Information campaigns were sent by email to several hundred recipients.

- The creation of a network of trappers on the area of the African clawed frog.
  ➤**Result obtained in part!** The CCT strengthened the network of volunteer trappers over the course of the project. However, no network has been set up in Maine-et-Loire; the PNRLAT considered the investment too important (management of administrative authorizations, recovery of individuals captured for rendering, etc.).
- *The creation of networks of operational observers to structure and feed the early detection and assessment system.*
  ➤ **Result obtained in part!** No observer network has been established. However, the SHF relies on its network of regional coordinators, who are the relays at the regional level. On the other hand, the SHF is well identified as the head of the network on the subject of invasive amphibians and alien reptiles. Thus, in case of new observation, it is one of the actors to contact in priority.
- *The publication of at least 4 scientific articles in international journals and the holding of a European seminar.*
  ➤ **Result!** Many scientific articles were written during the project, and an European seminar was held by videoconference on the 15 and 16/12/2020.
- *Advertising of the project on the website.*
  ➤ **Result!** The project website went online in 2018. Since then, regular publications on the project have been posted there.

The table below lists the objectives set, the expected results and indicates the intermediate results already achieved (Table 19).

Results of replication actions

The knowledge and methods developed within the framework of the project have been used for the management of populations of African clawed frog and American Bullfrog discovered in recent years in France (see action E2.4). On the other hand, the major deliverables have been translated into English, in order to facilitate their influence at European and global levels: technical guide, management strategy for the most extensive cores, etc.

Effectiveness of dissemination actions

Many communication actions have been implemented as part of the project, for each target audience (general public, young people, amateur amphibian and reptile owners, pet shops, breeding centers, professionals, managers of natural areas, etc.). Their effectiveness was assessed as part of action D1 (see Deliverable 3). Overall, these actions had a positive effect and made it possible to raise the awareness of a large number of people. They also highlighted the need to conduct a major information and awareness campaign to fight against the abandonment of pets.

Political impacts

LIFE CROAA has contributed to the improvement of regulations and public policies relating to invasive alien species. Details of the actions carried out are available in the description of sub-action E2.3.

In addition to the various requests for expertise on captive fauna, the main achievement of the project consisted in the rereading of the risk analysis of the African clawed frog with a view to its inclusion on the list of species of concern for the European Union. A technical note has also been written for the attention of the OFB and the Ministry of the Environment, with the aim of strengthening French regulations concerning African clawed frogs.

Since July 2022, the African clawed frog is listed as a species of concern for the European Union. French regulations should therefore change in the coming months.

Concerning the elements detailed in the B3 form of the proposal:

- The eradication of small nuclei, in particular that of Sologne, is about to be achieved. The large wetlands of major interest are therefore preserved;
- The Ministry in charge of the environment was involved, particularly with regard to regulatory aspects, through the expertise of Véronique Barthélémy, project manager at DREAL Nouvelle-Aquitaine and member of the steering committee of the project.
- The strategy developed for the management of the most extensive nuclei has been translated into English, in order to facilitate its appropriation by other countries concerned by one or other of the two species. The technical guide, a major deliverable of the project, is also available in English, which contributes to its influence at European and global levels.
- Concerning the early detection system, the SHF and the associated beneficiaries are identified at the national level as reference structures on invasive amphibians. In this way, they are recognized as a reference structure when new populations of bullfrog or clawed frog are discovered.
- Regarding environmentally friendly purchases, particular attention has been paid to eco-labels and methods with a minimum impact on the environment.

TABLE 19. Evaluation of the implementation of the project by sub-action

Action	As planned in the proposition	Completion	Evaluation
A1.1	***- Goal:*** Apply suitable, pertinent methods which are consistent with the scale of the project, in actions A2, C2, C3 and D2 ***- Expected outcomes:*** 3 protocols drafted for actions A2, C2/C3, D2	The three protocols have been drawn up.	The protocol for action D2 was presented late, but this did not have an impact on the implementation of the action.
A1.2	***- Goals:*** Implement field actions under the required administrative permits ***- Expected outcomes:*** Obtain permits for accidental capture of protected specimens and destruction of IAS	Administrative permits were obtained on time by all beneficiaries responsible for implementing field actions, except for CN who obtained their permit to destroy Bullfrogs late, in 2017. In 2020, a common file was drawn up by the SHF in order to obtain authorizations for all beneficiaries located in Nouvelle-Aquitaine.	Action implemented in accordance with expectations, except for CN, without impact on the goals set. The common dossier has made it possible to obtain multi-year authorizations for the capture and destruction of the American Bullfrog and the African clawed frog.
A1.3	***- Goal:*** Train people involved in implementing field actions in identifying amphibians and in capture techniques ***- Expected outcomes:*** 2 training sessions per year, one for amphibian identification, the other for capture techniques	Training sessions have been organized every year, except in 2020 due to the health context.	Action implemented in accordance with expectations, except for 2020, but this had no impact on the implementation of actions, as the staff did not change between 2019 and 2020.
A2	***- Goal:*** Update knowledge of the distribution of Bullfrogs and African clawed frogs in France ***- Expected outcomes:*** Up-to-date distribution maps for the two species	The distribution maps were updated following the inventory carried out in 2017 and 2018. The annual field surveys as part of the control actions also enabled the maps to be updated each year.	Action implemented in accordance with expectations.
A3.1	***- Goal:*** Improve capture rates for Bullfrog and African clawed frog larvae ***-Expected outcomes:*** Create new traps specifically for tadpoles of the two targeted species	Several devices have been tested, based on the data available in the bibliography (see details of action A3.1)	The new traps tested did not show better results than the traps usually used.

Action	As planned in the proposition	Completion	Evaluation
A3.2	***- Goal:*** Improve capture rates for Bullfrog and African clawed frog adults ***-Expected outcomes:*** Create new traps specifically for adults of the two targeted species	Several devices have been tested, based on the data available in the bibliography (see details of action A3.2). For American Bullfrog, the trap did not allow the capture of individuals but it highlighted the effectiveness of the replay to improve the detection of individuals. For African clawed frog, a new trap was created ("bourriche") and showed better results than the trap usually used	For the bullfrog, the results were disappointing but nevertheless allowed to identify an effective method for the detection of individuals. For the African clawed frog, the results are encouraging.
A3.3	***- Goal:*** Weaken the African clawed frog population present in lagoon treatment plants, allowing native amphibian species to recolonise. ***- Expected outcomes:*** Propose an enclosure system for settling pools of lagoon treatment plants, limiting the dispersal of the African clawed frog to the outside	System created in 2017 and implemented in the field in 2018. Monitoring of the number of captured individuals has been in place for 4 years on the first equipped station. Two other stations were equipped in 2021. A total of 8,894 individuals were captured	The confinement system has been very effective.
A4.1	***- Goal:*** Gain a better understanding of the dispersal of adult and juvenile Bullfrogs and African clawed frogs ***- Expected outcomes:*** Obtain biological parameters for dispersion to include in the connectivity model	Various studies have been carried out by CN to assess the movements of adult and juvenile Bullfrogs, and the survival of tadpoles. Individuals have been branded. For the smooth clawed frog, the UA conducted laboratory experiments to assess the movements of the species according to different substrates.	No bullfrog juveniles or tadpoles were found. The experiments carried out on African clawed frogs in the laboratory were therefore carried out for the bullfrog.
A4.2	- ***Goal:*** Gain a better understanding of the survival of African clawed frog larvae - ***Expected outcomes:*** Obtain biological parameters for larval survival to include in the connectivity model	Several studies have been conducted to estimate the reproductive effort, larval development and prey response of African clawed frog.	Action implemented in accordance with expectations
A4.3	- ***Goal:*** Identify an optimal strategy for combating major nuclei of the African clawed frog and Bullfrog populations - ***Expected outcomes:*** Create a connectivity map and analyse the cost-effectiveness of the different strategies possible	Thanks to the data collected in the first two sub-actions, connectivity maps were produced, in order to assess the possible displacement capacities.	The action was carried out as planned. However, the results of the connectivity maps are not

Action	As planned in the proposition	Completion	Evaluation
			encouraging and did not allow to identify a strategy for the management of large nuclei. The scientific seminar (action E2) brought together experts and partners in order to collectively propose a strategy.
C1.1	- ***Goal:*** Implement a functional and effective early detection system - ***Expected outcomes:*** Assemble observations of invasive alien amphibians	The observation entry form created at the start of the project has been replaced by new, more efficient technology. Following the abandonment of the development of an application for smartphone, we have been in contact with the JRC team, for the improvement of the European application. The rapid detection system is in place, the SHF is identified as the reference structure for invasive alien amphibians and is automatically contacted in the event of new observations.	Action implemented in accordance with expectations, except for the smartphone application.
C1.2	- ***Goal:*** Implement an assessment system in the event of the introduction of IAS into the natural environment - ***Expected outcomes:*** Create an expert committee	The SHF works closely with several national and international experts on biological invasions: members of the French Office for Biodiversity, the French IUCN Committee, scientists... It is also part of the Network of Scientific and Technical Expertise of the Invasive Alien Species Resource Centre.	Action implemented in accordance with expectations
C2.1	- ***Goal:*** Eradicate the GT-1 nucleus - ***Expected outcomes:*** Eliminate all specimens of the Bullfrog in Sologne	Eradication actions were carried out as planned each year in Sologne.	At the end of 2021, less than 7 bodies of water were still colonized. These results are very encouraging. Eradication should be achieved in the next few years.

Action	As planned in the proposition	Completion	Evaluation
C2.2	- ***Goal:*** Significantly reduce the GT-2 nucleus - ***Expected outcomes:*** Eliminate all specimens of the Bullfrog in Dordogne	Eradication actions were carried out as planned each year in Dordogne.	At the end of 2021, the Bullfrog was active on a single body of water in the Dordogne. Eradication should be achieved in the next few years.
C2.3	**- *Goal:*** Eradicate the GT-3 nucleus **- *Expected outcomes:*** Eliminate all specimens of the Bullfrog in Bay of Arcachon	Efforts have focused on finding individuals. Eradication actions were carried out as soon as an individual was detected.	Bullfrog numbers are very low in the Bassin d'Arcachon. Eradication is in progress.
C3.1	- ***Goal:*** Preserve key sites at risk from the African clawed frog - ***Expected outcomes:*** Eliminate the African clawed frog from sites identified as key sites	Trapping actions have been implemented around sites presenting a particular challenge in Deux-Sèvres, Maine-et-Loire and Vienne. The selected sites are of major interest for native amphibians and invertebrates.	Action implemented in accordance with expectations
C3.2	- ***Goal:*** Preserve key sites at risk from the Bullfrog - ***Expected outcomes:*** Eliminate the Bullfrog from sites identified as key sites	Actions against the American Bullfrog have been implemented each year.	Action implemented in accordance with expectations
C4	- ***Goal:*** Apply the strategies developed in action A4 - ***Expected outcomes:*** Optimal control populations of the African clawed frog and Bullfrog in major nuclei of the population	The action could not be implemented as initially planned, due to the conclusions of the working seminar (see action A4).	This action did not go as planned. In place, communication and lobbying actions have been carried out.
D1	- ***Goal:*** Assess the effectiveness of concrete conservation and communication actions - ***Expected outcomes:*** Indicators identified and analysed	Several indicators were monitored each year to assess the impact of the project. The eDNA technique was also used each year in SOlogne, as planned.	Action implemented in accordance with expectations
D2	- ***Goal:*** Assess the restoration of ecosystem functions - ***Expected outcomes:*** Monitor native species	Monitoring of native amphibians and invertebrates was set up at the start and end of the project.	Initial monitoring was conducted in 2018 for some populations, instead of 2017. The analysis of the results showed shortcomings in the protocol applied. It would be interesting to renew this monitoring.

Action	As planned in the proposition	Completion	Evaluation
D3	- ***Goal:*** Assess the project's socio-economic impact - ***Expected outcomes:*** Indicators identified and analysed	The socio-economic assessment of the project was entrusted to an external service provider. In addition, indicators were monitored internally.	Action implemented in accordance with expectations
D4	- ***Goal:*** Contribute to assessing specific LIFE indicators - ***Expected outcomes:*** Indicators identified on the European Commission platform	The KPIs were entered on the dedicated platform. They were validated in October 2022.	Action implemented in accordance with expectations
E1.1	- ***Goal:*** Communicate about the LIFE CROAA project - ***Expected outcomes:*** Creation of various communication tools for the project	The various communication media have been created.	Action implemented in accordance with expectations
E1.2	- ***Goal:*** Promote awareness of the project and join the network of other European projects ***- Expected outcomes:*** Connection with other European projects	We contacted several LIFE projects dealing with similar issues and we participated in all inter-LIFE meetings.	Action implemented in accordance with expectations
E2.1	***- Goals:*** Prevent the introduction of alien amphibians into the natural environment ***- Expected outcomes:*** Raise awareness among all people involved (general public, terrarium owners, pet shop owners, natural space managers, etc.)	Appropriate communication tools have been created in order to raise awareness among the various target audiences: educational kit, posters, video, etc. Activities have been organized with schoolchildren and we have taken part in various general public events.	There was a delay in the development of the educational kit, but this had no consequences on the organization of activities with schoolchildren. We have also modified the type of tools initially planned for raising awareness among amateur amphibian et reptile owners. This made it possible to have a better impact.
E2.2	***- Goals:*** Facilitate the implementation of control actions ***- Expected outcomes:*** Increased awareness and training for institutions, local representatives and land owners	Contacts have been made with public institutions and owners of sites that have benefited from control actions. The CCT continued to coordinate the network of volunteer trappers.	Action implemented in accordance with expectations. The PNRLAT did not set up a network of volunteer trappers like the CCT, as was initially planned.
E2.3	***- Goals:*** Improve public policy and regulations surrounding IAS ***- Expected outcomes:*** Adjustments and recommendations to national and/or European authorities	The LIFE CROAA contributed to the impact analysis which led to the inclusion of the African clawed frog on the list of species of concern for the EU. The SHF	Action implemented in accordance with expectations

Action	As planned in the proposition	Completion	Evaluation
		has also drafted a technical note for the FrenchMinistry in charge of the Environment, in order to place the African clawed frog in Appendix 2 of the decree of February 14, 2018, prohibiting any introduction and any use of the species.	
E2.4	***- Goals:*** Replicate and transfer techniques and methods developed during the project ***- Expected outcomes:*** Discussions and visits with French and European partners working on similar subjects	Exchanges took place with several French partners, in charge of the management of new populations of Xenopus smooth and bullfrog recently discovered. We also maintain close relationships with the LIFE 3nBullfrog team. Finally, our expertise was requested concerning the population of Fire-bellied toad introduced in Lorraine.	We did not carry out field visits as initially planned, but were able to disseminate and share the knowledge acquired during the project.
E2.5	***- Goals:*** Disseminate knowledge acquired during the project ***- Expected outcomes:*** Organisation of technical training courses, organisation of a scientific symposium, and a management guide to be drafted	The scientific symposium was organized in November/December 2020. The technical guide for the management of the Bullfrog and African clawed frog has been written, both in French and in English. Training and technical days have also been organised.	Action implemented in accordance with expectations
F1	- ***Goal:*** Coordinate the project - ***Expected outcomes:*** Rigorous project management	The technical, administrative and financial monitoring of the project was ensured throughout the project. 13 monitoring committees, 4 steering committees and 3 scientific committees have been organised. 6 visits by the external team also took place.	Action implemented in accordance with expectations
F2	- ***Goal:*** Ensure proper financial management of the project - ***Expected outcomes:*** An audit conducted by an external auditor	Not applicable	Not applicable

Action	As planned in the proposition	Completion	Evaluation
F3	- ***Goals:*** Ensure conservation and communication actions are continued through to the end of the project - ***Expected outcomes:*** Post-LIFE conservation and communication plan drawn up	The after-LIFE conservation and communication plan has been drafted. It proposes the continuation of actions deemed to be priorities and the drafting of a national plan for the management of all invasive amphibians present in France.	Action implemented in accordance with expectations

6.4 Benefits analysis

6.4.1 Environmental benefits

The environmental benefits of the project are multiple. Initially, concerning the small nuclei of populations colonized by the American Bullfrog, it should be noted that all the management actions of these sites have made it possible to move significantly towards eradication of this species (decrease in numbers year after year). The American Bullfrog has been eradicated from dozens of aquatic sites, and local amphibian populations are likely to trend upward in the coming years. In parallel with the impact that this native American species can have on local amphibians, it is important to note that the presence of this species also has major consequences on the biomass, structure and composition of aquatic algae and invertebrate communities, and on nutrient cycling and primary production of aquatic ecosystems. It is therefore expected that the American Bullfrog-free sites in the small population nuclei will regain some balance in the coming years.

The objective of eradicating the American Bullfrog within small population nuclei has used standard methods (field monitoring, capture with a net, seine, pond emptying, lamp research, rifle shooting) and controlled by the use of environmental DNA. This tool for detecting the presence of DNA from a target species in an environment (a pond for example) made it possible to verify that the species had indeed disappeared from the sites where it had been caught. The combination of all these methods, coupled with monitoring and frequent presence on the ground by the associated beneficiaries of LIFE CROAA, has made it possible to move towards this goal of eradication.

One of the project's flagship actions was to limit (or even stop) the appearance of new outbreaks of populations, both by raising awareness among holders of exotic species about release into the wild, and by developing tools to alert early on the discovery of new colonized sites. Management actions can thus be implemented quickly to prevent animals from dispersing and colonizing other nearby sites. Through the development of early detection tools, the LIFE CROAA project makes it possible to limit the future impacts of colonized outbreaks on local wildlife, and therefore actively participates in the preservation of ecosystems.

In a second step, the project focused on management actions within the large nuclei of populations colonized by the American Bullfrog and the African clawed frog. Despite the more than consequent efforts made by the associated beneficiaries in these territories, it turned out that the control actions had not slowed down or reduced these exotic species in the targeted territories. Indeed, the eradication of an exotic species in an aquatic site located near another site colonized by the same species is ineffective. The species recolonizes very quickly (by terrestrial movement, which makes it difficult to detect) the eradicated site.

The problem of large population nuclei has proved to be more complex than expected. The conclusions of the LIFE CROAA project now tend to focus on actions related to nature-based solutions in large population centres. In other words, in these specific cases, it is a question of restoring habitats in order to offer local amphibians new environments less colonized by exotic species. Ecosystem restoration is a measure that should also limit access to these habitats by alien species (amphibians or others). Indeed, it has been shown that most sites colonized by alien species are degraded sites, impacted by other environmental threats, such as pollution, or habitat fragmentation. These unbalanced sites sometimes still host native species, but are quickly colonized by exotic species that rush into free ecological niches. The restoration of these environments should therefore limit the space available for alien species, and strengthen the populations of native species. In addition, the restoration of ecological corridors could also help to restore connections between aquatic sites, allowing for some ecological functionality. Through these corridors, the arrival of natural predators of amphibians (such as the Gray Heron or the European Otter) could also be a lever to naturally manage exotic amphibian populations.

These ecological restoration actions could not be carried out within the framework of the LIFE CROAA project, but are part of the overall conclusions of the project.

6.4.2 Economic benefits

Since the beginning of the LIFE CROAA project, several dozen people have been deployed on the national territory, with a total of 57.86 FTEs. Beyond this direct impact on employment, the LIFE CROAA project has also generated economic benefits by working with more than 867 identified economic service providers, all beneficiaries combined, over the period 2016-2022. Most of the economic providers were selected close to the associated beneficiaries, promoting a local economic approach. For reasons of particular skills most of the time, some providers were also selected outside the France (Europe). Many actors and partners (193 in total) also collaborated with the associated beneficiaries of the project (in the framework of the steering committees, the scientific committee, data exchange on alien species, etc.). making the LIFE CROAA project more than visible in terms of territorial influence.

6.4.3 Social benefits

The social impacts of the LIFE CROAA project were assessed with beneficiaries, school children and the general public. The results of this evaluation showed a strong interest in the project among the general public and schoolchildren. Both targets were particularly aware of amphibian conservation and threats to these species, and highly appreciated the information disseminated at events. As communication and awareness-raising are a flagship action of the LIFE CROAA project, exchanges with the general public were numerous and frequent throughout the project.

In addition, the LIFE CROAA project has been the source of numerous meetings between partners and has led to the development of many communication media, very well received by the general public and schools, whether through our website or via social networks, but also through the dozens of events in which the beneficiaries participated. Some tools developed as part of the LIFE CROAA project are still disseminated and used in awareness-raising events, especially among young people.

6.4.4 Replicability, transferability, cooperation

The associated beneficiaries of the LIFE CROAA project have always wanted to surround themselves with experts and partners previously confronted with this problem of invasive alien amphibians. This desire led us to choose Belgian, Swiss and South African scientists to form the scientific committee of our project. Work meetings and exchanges with European partners were also held, in order to exchange on control methods and techniques developed during the project, but also to learn from their experience.

In addition, the management tools and measures developed as part of the LIFE CROAA project also aim to be used to combat exotic amphibians (American Bullfrogs, African clawed frogs and other exotic amphibians) in territories other than metropolitan France. Indeed, the project is of wider interest because the two target species have been introduced in several countries of the European Union (Belgium, Netherlands, Germany, etc.). where control programmes have been put in place more or less systematically. There is therefore a real potential for transferability of the results of the LIFE CROAA project. The capture methods used, as well as the control systems developed in this context, have been the subject of technical sheets, available in French and English, which we are already distributing to certain European partners (including Belgium, which has just identified a cluster of African clawed frog population on its territory). These tools allow us to have basic elements in order to exchange and support these partners in the fight against invasive alien amphibians.

Discussions are also underway with the Spanish on a tool for the early detection of colonized sites based on passive listening to amphibian songs. Some exchanges also took place with Switzerland, faced with the arrival of the Southern Newt, the Shepherd's Frog, or the Executioner's Newt (this species was also the subject of a workshop during the 2022 congress of the SHF organized in the Auvergne-Rhône-Alpes region). In France, the approach can also be tested and adapted on other species that would show an invasive nature and whose impact on local species would be recognized, to ensure its transferability to other invasive amphibian species.

The integration of replicability and transferability actions into the project as well as the establishment of substantial networks are intended to allow to experiment with the implementation of the control techniques developed during the project in other territories, and to benefit from our experience the territories newly confronted with the arrival of these invasive species, with the common goal of protecting ecosystems.

6.4.5 Lessons from good practice

Not applicable.

6.4.6 Demonstration

LIFE CROAA is a demonstration project for several reasons:

- It is part of the implementation of the European regulation on IAS (EU Regulation No 1143/2014 of 22 October 2014), and in particular in its relevance to French law.
- It aims to identify a strategy to combat IAS that meets the expectations of the European Commission and to demonstrate its effectiveness.
- It seeks to identify, implement and evaluate a generic strategy on invasive alien diseases, providing the necessary knowledge to ensure proper management of problem populations.
- It targets two species also found in other European countries.

The demonstrative nature of the project is revealed in the various technical sheets written in French and English, detailing the capture and control methods to be implemented in the face of the arrival of the American Bullfrog and/or the African clawed frog in a territory. The LIFE CROAA project has also enabled the development of early detection tools (tools that can easily be used in other territories) and communication and awareness-raising media for the general public and schools.

6.4.7 Policy effects

Important work has been carried out under Action E2.3 to contribute to the improvement of national or European legislation in terms of IAS management.

As a reminder, the American Bullfrog is listed in the list of invasive alien species of concern for the European Union (Implementing Regulation (EU) 2016/1141) and is, in fact, prohibited from introduction, movement, breeding, sale and possession. This was not the case for the African clawed frog which, although introduced in several European countries and causing impacts on local ecosystems, was still not considered a priority IAS at European level. In France, the species was still traded and often bred in terrariums, which considerably increased the risk of introduction (even unintentional) into the natural environment. Including it on the European list would make it possible to control its marketing and stop trade between breeders.

Following the update, at European level, of the list of alien species prohibited on EU territory, the SHF has provided a set of documents so that the French situation is taken into account, and that the African clawed frog appears, in the same title as the American Bullfrog, on the list of invasive alien species of concern for the

European Union. The new list, including the African clawed frog, is currently being drafted and is expected to be released in the coming months. The French regulations should therefore also evolve very soon, and make it possible to strengthen the measures against the species, currently only prohibited from introduction into the natural environment, pending its inclusion on the European list. The inclusion of the African clawed frog on these regulatory documents will be a victory for the members of the LIFE CROAA project, hoping that the resulting regulatory constraints will greatly limit the presence of this species on the national territory.

7 KEY PROJECT-LEVEL INDICATORS

Following the letter sent by the European Commission on 11/12/2017, the SHF was responsible for identifying the indicators specific to the LIFE CROAA project. This work was validated by Mr. Frédéric BROCHIER and Mrs. Blanca SAEZ-LACAVE. The indicator "invasive species" could not be filled in, the results of action A2 (inventory of American Bullfrog and African clawed frog nuclei) were not yet known. We therefore had no indication of the initial state at the beginning of the project.

The final indicators were completed in August 2022; the data entered are available in **Annex n°11**. After verification by the Agency, they were validated in October 2022.

A total of 28 indicators were selected and documented.

Regarding the indicators relating to the management of the American Bullfrog, the data entered correspond to the number of individuals captured within the small nuclei. The objective set was the eradication or significant reduction of these populations. The number of individuals captured at the beginning of the project was 16,352 individuals, compared to 156 at the end. It is estimated that eradication can be achieved within the next five years, so the data entered five years after the project is zero.

With regard to the African clawed frog, since the population managed under the project covers an extremely large area, it is not possible to implement control actions on such a scale. The data entered correspond to field data collected on the Saint-Martin-de-Sanzay WWTP, which benefits from a containment device to capture African clawed frog individuals seeking to enter or leave water bodies. The results are positive, since the number of individuals captured dropped drastically between the beginning and the end of the project. This clearly suggests that targeted measures at priority sites can be effective.

A calculation was also carried out to assess the evolution of the "number of populations/hectare". However, this unity does not seem to us to be relevant in the context of LIFE CROAA. Indeed, the term "population" retains a genetic concept that it was not realistic to follow in the context of the project, for lack of human, material and financial resources.

A large number of selected indicators focus on communication and awareness among the various audiences concerned by the problem of invasive amphibians. Again, the results are positive. The number of tools developed and the number of people sensitized (schoolchildren, students, general public, professionals, etc.) are high; The objectives we had set ourselves have been achieved.

To conclude, the results of LIFE CROAA are encouraging and show the interest of the LIFE programme in being able to act on invasive alien species. Without funding from the European Commission, actions to combat American Bullfrogs and African clawed frogs in France would have had much less scope and impact.

8 COMMENTS ON THE FINANCIAL REPORT

In addition to the table "Control of the completeness and accuracy of the file" presented on page 2, the reference period in the financial report (consolidated financial statement and financial statement of each individual beneficiary) is the same as in the technical report, from 1/09/2016 to 31/08/2022. However, it should be noted that:

- The financial and technical reports of the associated beneficiaries were stopped on June 30, 2022 for 2 reasons: (1) to take into account the summer period of paid leave (and therefore absence) of employees, (2) to allow the coordinating beneficiary to have all the necessary elements upstream in order to compile the data for the final report. However, the individual financial statements of the associated beneficiaries shall indicate an end date of 31/08/2022 in accordance with the grant agreement.
- Regarding the beneficiary coordinator, 3 employees (Isabelle Chauvin, Myriam Labadesse and Audrey Trochet) justified working time for the months of September and October 2022 (for a total of 253 hours or 36 days) in order to write the final report. However, the individual financial statement of the coordinating beneficiary shall mention an end date of 31/08/2022 in accordance with the grant agreement.

The final financial report presents a commitment of expenditure of € 3,130,067.24, for an initial estimated budget of **€ 3,430,179** (completion rate: 91%). The 7 associated beneficiaries did not individually reach their estimated budget, the coordinating beneficiary exceeded his own by 5%.

The following items can be found in the appendix:

- **Annex n°14:** File "LIFE CROAA financial reporting consolidated" in Excel and Pdf formats.

Comments:

- The mid-term report as of 30/09/2018 presented the change in the distribution of the European grant, due to an input error in the application file (see part 10.2 of the mid-term report). The amounts of the European grant, indicated by each associated beneficiary, have been calculated on the basis of this modification, with aid rates of 55.53% for the CCT and 60.48% for the other associated beneficiaries. As these rates have been rounded to 2 decimal places, the amount of the European subsidy for the coordinating beneficiary (SHF) has been calculated on a slightly higher rate (60.58%) in order to fall back on the exact overall rate of 60.00%.
- The bank details entered in the payment request correspond to those mentioned in Amendment No. 2 of 14/11/2021 (in **Annex n°1**), modification made following our official request of 24/09/2021.

- **Annex n°15:** "Personnel costs calculation" files of the 8 beneficiaries in Excel and Pdf formats.

Comment: These files are not required but allow you to understand how we calculated personnel costs, per person and per calendar year, as was already the case during the mid-term report. The dates and types of contract are also mentioned. We then reported personnel costs in the corresponding tabs of the Financial Individual Statement per person per calendar year.

- **Annex n°16:** "Signed financial individual statement" files of the 8 beneficiaries in Excel and Pdf formats.

Comments:

- The PDF versions include the tabs "Individual cost statement" and "Certificate for Nature projects" signed and scanned for each beneficiary. The originals shall be kept by the coordinating beneficiary.
- Considering that an intern, whether or not receiving a gratuity, has student status and is under the responsibility of the institution where he or she studies, the associated beneficiaries who have hosted paid interns have integrated them into the "non-employed staff" tab, as presented in the mid-term report.

- **Annex n°17:** Analysis of expenditure levels by cost category

Comment: This analysis is broken down by category of expenditure and presents (1) the evolution of total expenditure throughout the project in comparison with the forecast and (2) the evolution of each category of expenditure with analysis.

- **Annex n°18:** Analysis of financial gaps by beneficiary

Comment: This analysis is broken down by beneficiary and presents (1) the evolution of expenses throughout the project in comparison with the forecast and (2) the justification of extraordinary cases such as necessary costs not provided for in the budget, or possible changes in the staff mobilized.

8.1 Summary of costs incurred

The table below presents the costs incurred by expenditure category (Table 20).

TABLE 20. Costs incurred by expenditure category

Budget breakdown categories	Budgeted costs	Costs incurred	%
1. Personnel	€2,213,626	2 176 134.73 €	98 %
2. Travel and subsistence	€270,778	191 118.98 €	71 %
3. External assistance	469 895 €	368 745.11 €	78 %
4. Sustainable goods	€97,681	85 257.97 €	87 %
Infrastructure			
Equipment	€97,681	85 257.97 €	87 %
Prototype			
5. Land purchase / long-term lease			
6. Consumables	€63,248	55 984.63 €	89 %
7. Other Costs	90 550 €	48 055.06 €	53 %
8. Overheads	€224,401	204 770.75 €	91 %
TOTAL	**€3,430,179**	**3 130 067.24 €**	**91 %**

The table below shows the costs incurred by type of action (Table 21).

TABLE 21. Costs incurred by type of action

Typical action	Budgeted costs	Costs incurred	%
A - Preparatory actions	€709,878	794 536.07 €	112 %
C - Concrete conservation actions	€1,414,751	923 620.57 €	65 %
D - Impact monitoring and evaluation	€182,090	182 384.29 €	100 %
E - Communication and dissemination of results	€491,663	539 541.69 €	110 %
F - Project management	€631,797	689 984.62 €	109 %
TOTAL	**€3,430,179**	**3 130 067.24 €**	**91 %**

8.2 Accounting system

The mid-term report as of 30/09/2018 presented a detailed statement of the accounting system, including (for each beneficiary): the accounting system used, the cost approval procedure, the time recording system with the control procedure and the integration of the LIFE mention on invoices (see part 10.4 of the mid-term report).

8.3 Partnerships (if applicable)

The mid-term report as of 30/09/2018 presented a detailed state of the functioning of the partnerships, including: financial transactions and financial reporting (see section 10.5 of the mid-term report).

8.4 Financial Statement Certificate

Article II-23-2 of the grant agreement was amended by Amendment No 1 of 27 September 2018: an attestation of the underlying financial statements and accounts is required only for beneficiaries for whom the total contribution in the form of reimbursement of the actual costs referred to in Annex III is at least €750,000. Therefore, no beneficiary is concerned.

8.5 Estimated number of days per person per action

The table below shows the number of days completed by type of action (Table 22).

TABLE 22. Number of days completed by type of action

Typical action	Days planned	Days realised	%
A - Preparatory actions	3 260	4 351	133 %
C - Concrete conservation actions	5 887	4 561	77 %
D - Impact monitoring and evaluation	698	458	66 %
E - Communication and dissemination of results	1 730	1 949	113 %
F - Project management	2 545	2 746	108 %
TOTAL	**14 120**	**14 065**	**100 %**

8.6 Replies to EASME and CINEA letters

Following each annual visit of the NEEMO external monitoring team monitor (Mr. Frédéric Brochier from 2017 to 2018 and Mr. Quirin Renard from 2019 until the end of the program), EASME (until March 2021) and CINEA (from April 2021) sent us successive letters containing recommendations and questions.

We systematically answered questions raised with the NEEMO monitor on his next visit.

Following its 6th and last visit on 29/03/2022, we received a letter from CINEA dated 16/05/2022 and respond below to problem n°2 raised for actions A3, C1 and C2:

Reminder: At the March 29 meeting in Sologne, you highlighted the strong potential of bioacoustics sensors in the early detection and monitoring of invasive amphibians. Your partners have made a convincing case for supporting the high value for money of these devices, which turn out to be relatively inexpensive (€1,500 per item) compared to the human resources required by a regular presence of staff in the field. Although the sensors appear to be a functional solution for detecting and monitoring the species, their purchase cannot be considered eligible as they could only be used for a very limited period of time during the project's duration. Nevertheless, we encourage you to try to establish an equally effective monitoring system in the post-LIFE period.

Answer: Taking into account the evaluation received from CINEA, each beneficiary concerned analysed this point within its structure and with the coordinating beneficiary (SHF). The advantage of this equipment is undeniable in terms of the human resources to be mobilized and the cost corresponding to the regular presence of staff in the field. In addition, the co-financers concerned have validated these investments even if they are committed in the last year of the programme, estimating the added value they represent, and specifying that they will be used after the end of the programme like the majority of equipment in the continuity of the actions to be implemented. This is in line with the certificate signed by each beneficiary, which attests that "durable goods purchased for the project, during the duration of the project and with a longer life than the project, will in future be used exclusively for nature conservation activities".

Finally, the post-LIFE plan (see Action F3 and Deliverable n°11) includes, among the priority actions to be implemented at the end of the project, the monitoring the small American Bullfrog nuclei, in particular through

the use of passive sound recording devices. The acquisition of this equipment will therefore ensure the continuation of this action.

The 3 associated beneficiaries concerned have therefore purchased this equipment and included the expenditure in the financial report, being aware that it may be deemed ineligible at European level. This represents a total amount of €4,155: €1,504 for the CDPNE, €1,704 for the PNRLG and €947 for the PNRPL.