

MAKE AN INVENTORY. THE AMERICAN BULLFROG

TECHNICAL SHEET

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

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

Find out more: www.life-croaa.eu

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THE BULLFROG, AN INVASIVE EXOTIC SPECIES IN FRANCE

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

Originally from United States and introduced into France in the wild several decades ago, the American bullfrog (*Lithobates catesbeianus*) is listed in [appendix 2 of the ministerial decree of February 14, 2018](#), prohibiting its introduction into the national territory and into the natural environment, its transport, peddling, use, exchange, its sale and sale or the purchase of live specimens. Used for ornamental purposes in the 1980s, this species has since colonized several territories in mainland France, such as Loir-et-Cher, Gironde and Dordogne and Bas-Rhin.

→ [Learn more about invasive alien species regulations](#)

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

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

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

→ [Learn more about Species targeted by LIFE CROAA](#)



KNOW HOW TO RECOGNIZE THE BULLFROG

The bullfrog, especially in the juvenile stage, can be confused with other species of the Ranidae family such as species of the genus *Pelophylax* (known as "green frogs"). It is therefore essential to know how to identify it in order to declare an exact presence and to avoid any confusion.

See also these photos in appendix 1.

Laying and eggs

- The clutches consist of 1,000 to 25,000 eggs, spread out in gelatinous layers 50 to 80 cm in diameter in the plants.
- The eggs are dark brown in colour and bicoloured (lighter below than above).
- Dimensions: 1.5 to 2.5 mm.



(c) Matthieu Berroneau

Tadpoles

- Size from 7 to 17 cm (maximum).
- Greenish-brown with pinpoint black dots on head and tail.
- Dorsal fin low, starting at the back of the back and wider than the head.
- Eyes in dorsal position.
- Thick tail end (little tapered).



(c) Jean Muratet

Juveniles

- Small individual without tail, up to about 10 cm (from snout to vent).
- Iris of orange colour.
- Dorsal side usually olive green punctuated with well-defined black dots.



(c) PNR Périgord-Limousin



Adults

- Size: between 12 and 18 cm from the muzzle to the vent.
- Dorsal side olive to brownish in colour dotted with darker spots.
- Long and powerful hind legs.
- Webbing of the toes very developed and not very indented up to the end of the 4th toe.
- Short, rounded muzzle.
- Large eardrum with a diameter greater than or equal to the size of the eye.
- Skin fold surrounding the eardrum but absence of dorso-lateral folds.
- Iris punctuated with black and yellow-orange.



Fold surrounding the eardrum (absence of dorso-lateral folds)
Dorsal side olive to brownish in colour dotted with darker spots
Ventral side yellow in the male and whitish in the female
Large eardrum greater than or equal to the diameter of the eye

Adult female

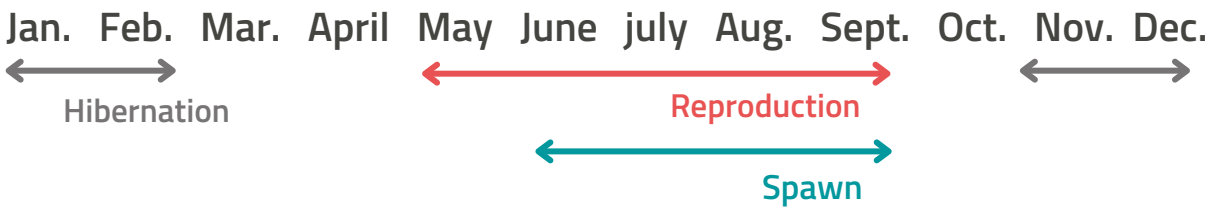
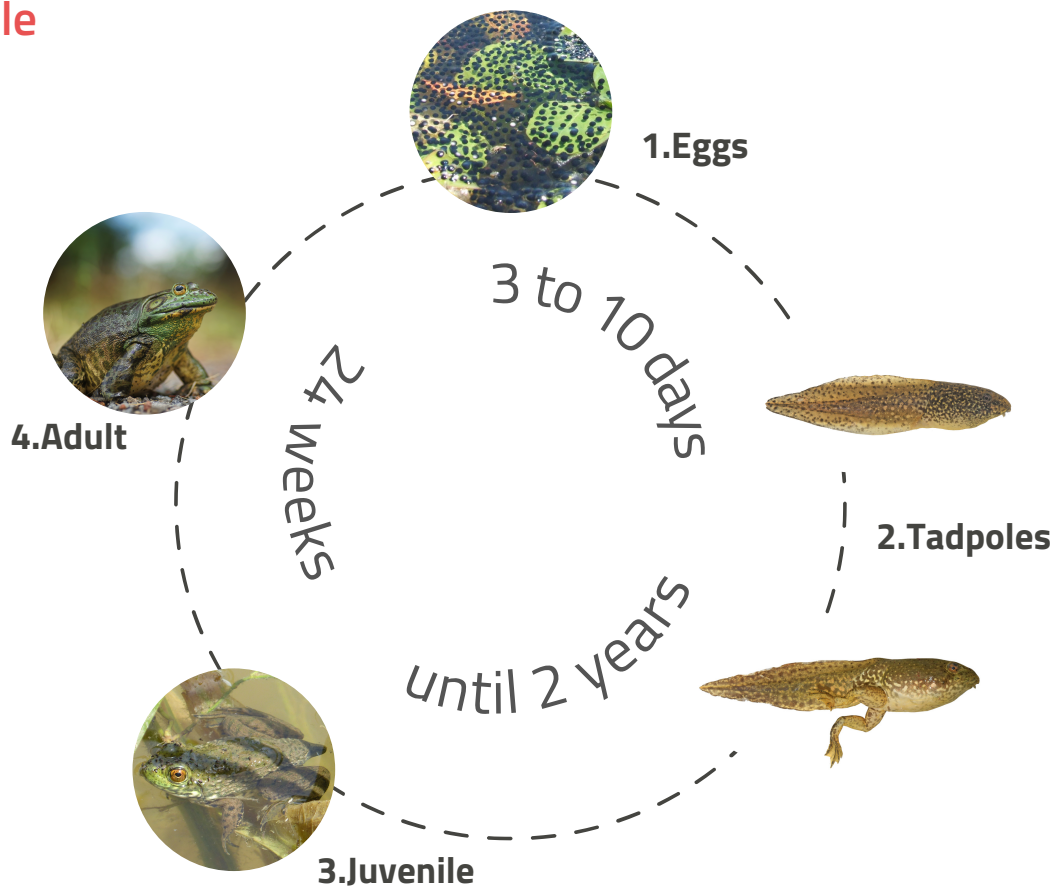
- Ventral face and whitish throat.
- Rounded eardrum equivalent in size to the diameter of the eye.



Adult male

- Ventral face and throat yellowish.
- Rounded eardrum twice the size of the diameter of the eye.
- Vocal sac under the throat.
- Black nuptial calluses on the fingers.





1. Eggs are laid when the water temperature approaches 20°C (ideal temperature: 26°C). The eggs are visible on the surface, attached to plants in sunny areas. They take the form of gelatinous layers with clumped eggs.
2. Tadpole development is slow and temperature dependent. If the metamorphosis only lasts a few days, the lifespan in tadpole form can last up to 2 years! In the event of strong predation, this duration may be shortened. During this metamorphosis the cutaneous respiration becomes pulmonary, legs appear and the tail is gradually reduced. Small tadpoles graze on algae and filter water, while large tadpoles are able to eat small aquatic prey.
3. Juveniles are able to disperse out of the pond in all directions and represent the main stage responsible for dispersal, for the colonization of points of proximity (when the distances between water bodies are small).
4. Sexual maturity is reached 2 to 4 years after metamorphosis, when the individual measures about 10 cm.
5. Adult reproduction takes place from May to September with peak reproduction in June and July. Their displacement capacity is about 300 to 500 m each year. Less active in winter (hibernation), they can also go into aestivation during heat waves in summer. The Bullfrog has a wide spectrum of predation (mainly other amphibians, insects, crustaceans). The species can live up to 16 years.



ARE THERE BULLFROGS IN MY BODY OF WATER?

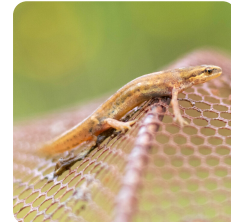
In order to ensure the absence or presence of the bull frog, it is advisable to carry out regular inventories of your bodies of water (pond, pond, lake, etc)

The earlier the detection of individuals, the faster the implementation of control actions can be programmed and the local biodiversity preserved.

1. Prerequisites for an inventory

1.1 Obtaining waivers

Regardless of the stage of development, your actions to capture bull frogs are likely to result in the accidental capture of other amphibian species. The latter are all protected in France according to the [Order of January 8, 2021 setting the list of amphibians and reptiles represented on metropolitan territory, protected throughout the national territory and the terms of their protection.](#)



(c) Axel Martin

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

- As head of the national network, the SHF coordinates control actions for this species, so we invite you to contact us before any trapping operation in order to:
- Be accompanied for the implementation of a procedure respecting the regulations;
- Know the methods of taking care of individuals;
- Upload your observation and capture data.
- >> contact@lashf.org

1.2 Protecting ourselves and the environment

Hygiene protocol

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

Field clothing

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



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

Rubber gloves are essential for handling individuals in order to avoid contact with their mucus, but also contact with water contaminated by the possible presence of nutria (leptospirosis). In the context of eDNA sampling, prefer the use of sterile laboratory-type rubber gloves.

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

Find the protocol for disinfection and use of Virkon® on the SHF website:

lashf.org/fiches-techniques/ > Section "Our other technical sheets"

> "Hygiene protocol for amphibian disease control in the field".



2. Equipment and inventory costs

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

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

Choose a trap adapted to the biodiversity in the field

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

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

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

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

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

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



(c) Axel Martin



(c) Bastien Martin

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



- **Bait:** Dog food (about €6.5/kilo) is to be used to bait your traps and try to improve your catch rate. Place these baits in a small sausage of about 5 cm, in a small resealable net (usually sold with the trap). Be careful, however, they are likely to attract other exotic species potentially present in your bodies of water (crayfish, catfish, etc.). These invasive species must never be returned to the water (for more information, consult the [Invasive Alien Species Resource Center](#)). With regard to the other catches (native species of fish and amphibians), it will be necessary to check the traps every 24 hours to release the individuals not concerned by your capture plan.
- **Bins or plastic buckets:** For packaging captured individuals. Fishing bucket of 20 L minimum with handle. From €10-15 per unit depending on the model. NB: Pour some water into your buckets before removing the individuals.
- **Environmental DNA sampling kit:** For the detection of individuals. Includes a sterile filtration capsule, a syringe, a 100 ml sampling ladle, two pairs of sterile gloves, a 2 L capacity bag and a buffer solution to store the eDNA once filtered. 1 kit allows the sampling of a body of water with a maximum surface of 1 ha. Around €150/kit for purchase and €10 for sending the kit for analysis.
- **Fishing Boots:** All-terrain rubber boots. They will have to be cleaned systematically from one water point to another (see hygiene measures). From 40 to 100 € per pair depending on the model.
- **Waders [optional]:** Neoprene material for fishing (entering the water). They will have to be cleaned systematically from one water point to another (see hygiene measures). From 65 to 140 € per unit depending on the model.
- **Jumelles [optionnel]:** For occasional observation of individuals from dawn to dusk. From €300 per pair to benefit from professional equipment and/or Polarized glasses [optional]: reduces reverberation and increases contrast to better spot tadpoles in the water. From €100 per unit depending on the model.
- **Headlamp [for night search]:** 100 lumens max. Minimum €50 per unit depending on the model.
- **Flashlight [for night search]:** 1000-1200 lumens max. Minimum €100-150 per unit depending on the model.
- **Rain gauge:** To indicate the level of precipitation on your field sheet during your inventory sessions (see "rainfall" in appendix 2). From 20 to 80 € per unit depending on the model.



3. Inventory process

The objective is to detect the presence or to pronounce the absence of the species in a defined area of water, during several passages distributed over the reproduction period of this species. It does not necessarily require significant human resources (1 to 2 people depending on the surface of the study area).

- **Periodicity**

From the beginning of June to the end of September, 3 passages per stage (1 & 2) are to be carried out during the reproduction period of the species to increase the chances of detection. It is recommended as far as possible to carry out these plays when the temperature is mild (20°C minimum), without wind or storm.

- **Implementation**

Step 1: Visual prospecting

This step must be carried out during the day in clear weather.

- Définissez une dizaine de points d'observation tout au long de la zone d'eau prospectée avec idéalement un point tous les 100 m (à adapter selon la dimension de votre point d'eau). These points must be as far away as possible from any noise disturbance (vehicle passing) or visual disturbance (plant screen). Take care not to circulate in the immediate vicinity of the body of water between two observations and to arrive from afar (from outside in relation to the body of water) on the observation points in order to avoid disturbing individuals.
- For adults and juveniles, the search can be carried out either using a pair of binoculars if the surroundings of the body of water are difficult to access or on foot if the site allows it. The observation lasts about ten minutes during which the observers will take care to be particularly discreet.
- The search for the larval stage can be done using a fine-mesh landing net where several fishing strokes will be made all around the water point, preferably near vegetated areas (about ten depending on the size of your point). water). Take care not to damage the aquatic vegetation or the bottom of the body of water when using your landing net: make slow movements, passing close to the bottom without removing the substrate or the sediment.



(c) CDPNE



- Traps can also be set to capture the species at this stage. The set time for the traps must be a full night to ensure the capture of the individuals (change the next morning - set for 24 hours maximum). The traps with or without bait (crayfish traps or dog food) must be floating or with an emerged part so that any captured individuals can breathe (place a float or an empty plastic bottle in the trap to ensure its buoyancy) . These traps must also be firmly attached to a fixed element (stake, tree) so that they do not drift.



(c) PNRLAT



(c) CDPNE

➔ **As a reminder: in order to proceed with these two steps, a derogation from the capture of protected species is required (cf. 2.1 Prerequisites).**

Step 2: Auditory prospecting, passive listening to singing males

During the breeding season, the adult male of the bullfrog produces a characteristic, low-pitched sound that travels several hundred meters. These plays are to be carried out from June to August, between 10 p.m. and 1:30 a.m. The most favourable periods for listening are warm nights when the temperature is between 16 and 25°C, with a light wind (less than 14 km/h).

The 3 listening sessions are to be carried out with 10 days of minimum intervals.

- Define around ten sampling points throughout the area of water surveyed (to be adapted according to the size of the body of water). Given the scope of the song, listening close to the site is not mandatory at the risk of scaring the species away. These points must be as far away as possible from any noise disturbance (vehicle passing) or visual disturbance (plant screen). The site must be in total darkness to start tapping (no flashlight on), it is therefore recommended to carry out these tappings in pairs for safety reasons. It is necessary to remain silent throughout the listening period.
- Listen for 15 minutes at each point in all directions (rotations of the observer to scan the area around him). To improve the perception of the songs, place the hands behind the ears, slightly bent in order to increase the zone of reception of the sound. If during a visit to a listening point, noises disturb the smooth running of the protocol, the observer must wait if possible for the end of the noise disturbance then carry out the 15-minute listening and resume the rest of the prospecting.

NB: the absence of song during a single listening session does not mean with certainty that the bullfrog is not present. Moreover, these techniques work well in the presence of a population. The presence of low numbers or isolated individuals may be more difficult to detect. It is therefore necessary to carry out 3 listening sessions on your site, extending the last two listening sessions by 5 min in order to validate the data and reduce the biases linked to meteorological factors in particular.



Step 3: Environmental DNA testing

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

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

- For each sampling point: stir the water column using the ladle to collect homogenized water and maximize the probability of detecting the species.
- In order to avoid any contamination of the samples, it is essential to wear sterile gloves and to take the samples from the bank without putting your feet in the water as far as possible. For 1 ha, 20 samples of 100 ml, evenly distributed over the body of water to be sampled, are taken (approximately one sample every 20 m) and stored in the 2L bag.
- After collecting the samples, the bag containing all the water samples is in turn homogenized and then injected into the filtration capsule using the syringe. After having filtered all the water in the sachet and expelled the excess water using the syringe filled with air, the filtration capsule is filled, in the direction of the flow, with the buffer solution provided in the kit, then agitated for almost a minute.
- The capsule(s) are referenced (date, mesh number, sample number, contributor) and stored vertically, always in the direction of the flow, in their packaging at a stable temperature. The samples must then be sent to a specialized laboratory for analysis.



(c) Luc Clément, Cistude Nature



(c) CDPNE



ACCOMPANIMENT AND FOLLOW-UP

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

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

>> Contact us: contact@lashf.org



(c) M. Berroneau



ANNEX 1**Bullfrog egg laying - (c) Matthieu Berroneau****Bullfrog tadpole - (c) Jean Muratet****Bullfrog juvenile - (c) PNR Périgord-Limousin**

ANNEX 1

Adult female of bullfrog - (c) Jean Muratet



Adult male of bullfrog - (c) Jean Muratet



Physical characteristics of the bullfrog -

(c) Matthieu Berroneau

Fold surrounding the eardrum (absence of dorso-lateral folds)

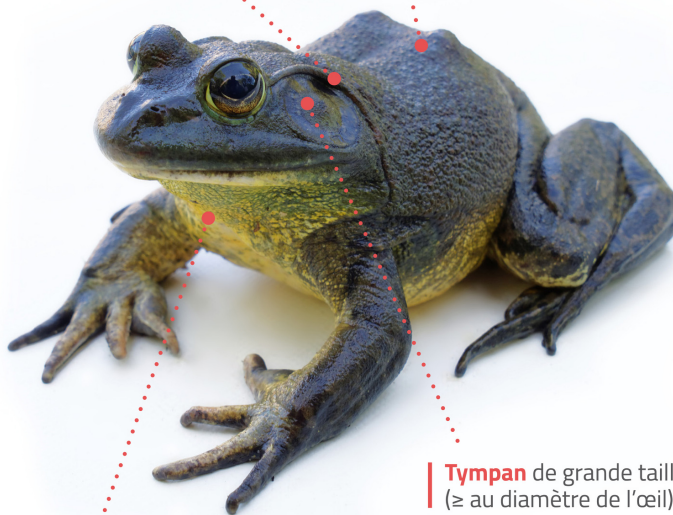
Dorsal side olive to brownish in colour dotted with darker spots

Ventral side yellow in the male and whitish in the female

Large eardrum greater than or equal to the diameter of the eye

Repli entourant le tympan (absence de replis dorso-latéraux)

Face dorsale de couleur olivâtre à brunâtre parsemé de tâches plus foncées



Tympan de grande taille (\geq au diamètre de l'œil)

Face ventrale de couleur jaune chez le mâle et blanchâtre chez la femelle



LAND SHEET		
Observation of bullfrog - Passage n° ...		
Observer name:	Date:	
Geographical coordinates, name and description of the environment:		
Air (°C) and water (°C) temperature	
Weather report	<i>Rainy / cloudy / sunny</i>	
Rains	
Bull frog	Observation method	Quantity
Spawning	<i>Visual observation / landing net</i>	
Tadpoles	<i>Visual observation / landing net / trap</i>	
Juvenile s	<i>Visual observation / landing net / trap</i>	
Adult s Female s	<i>Visual observation / landing net / trap</i>	
Adult s Male s	<i>Visual observation / landing net / trap</i>	
TOTAL		
Other species (specify species, stage and sex):	Observation method	Quantity
	<i>Visual observation / landing net / trap</i>	
	<i>Visual observation / landing net / trap</i>	
	<i>Visual observation / landing net / trap</i>	
	<i>Visual observation / landing net / trap</i>	
	<i>Visual observation / landing net / trap</i>	
TOTAL		





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