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# Proposal and application of a method of identification of terrestrial spotted salamanders by photographic analysis software to estimate population parameters by Capture-Mark-Recapture method.

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*The content of this dissertation is the sole responsibility of the candidate and the host organization and does not engage the scientific responsibility of the tutor and the university*

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# I. Introduction

## 1) General

On a planetary scale, the class of amphibians is judged by the International Union for the Conservation of Nature and these resources (IUCN) to be one of the most threatened class. It is estimated that 41% of known amphibian species are currently at risk of extinction compared to 14% for birds and 25% for mammals (IUCN, 2020). These numbers have been growing for many decades. This difference between the groups is mainly due to the fact that most amphibian species have a bi-phasic life cycle. They are sensitive not only to changes in terrestrial habitats but also to aquatic habitats. The destruction and alteration of these two habitats are the main causes of their decline. This internship is part of a project to improve the knowledge and conservation of the terrestrial spotted Salamander. Amphibians are tetrapods, the oldest of them is dated around 380 million years old (Duguet and Melki, 2003). The current representatives are named the Lissamphibians. They are vertebrates considered small in size, anamniotes, ectotherms and having mostly two pairs of locomotor limbs and lungs. Their skin lacks scales but is called glandular (Duguet and Melki, 2003) which allows them to produce, absorb and exchange substances with the outside environment. Their bare and thin skin is thus the support of respiratory and water exchanges.

## 2) The Terrestrial spotted Salamander (*Salamandra salamandra terrestris*)

The Spotted Salamander (*Salamandra salamandra*) is the most widespread salamander species in France. The subspecies *Salamandra salamandra terrestris* represented in Franche-Comté is present in most of the French territory. Among the class of amphibians, the Salamanders belong to the order of the urodae and to the family of the Salamandridae which contains 16 genera and about 61 species distributed in North America and also in Eurasia, of which 4 species are present in France (Duguet and Melki, 2003). Many of the biological and ecological characteristics of Spotted Salamanders remain to be discovered. The Spotted Salamander is present in almost all of France outside some southern areas and Corsica and reaches 2350 m of altitude in the Pyrenees. The terrestrial habitat is mainly represented by the grove, the deciduous and mixed afforestation of plain and altitude. The species prefers to avoid filtering and acidic basements; hence its rarity in coniferous forests. The aquatic habitat of the larvae is mainly represented by the well oxygenated waters, thermally stable to the very limited stocking of certain torrents, streams, springs or fountains, washhouses or even some pools (Duguet and Melki, 2003). In France and Franche-Comté, Spotted Salamander populations are considered to be of "Minor Concern" (LC) according to the Red Lists of Amphibians of France and Franche-Comté (UICN Comité Français and MNHN, 2015). This species is covered by Annex III to the Berne Convention (Council of

European union, 1979; INPN, 2018) and is also protected in France by Article 3 of the Decree of 19 November 2007 laying down the lists of amphibians and protected reptiles and their protection arrangements (Ministère français, 2007). In Franche-Comté, the Spotted Salamander subspecies represented is easily recognizable by its body having two more or less discontinuous yellow lines with sometimes some yellow or orange punctuations. The dorsal pattern is individual, which allows them to be recognized and differentiated. However, it is necessary to wait for sexual maturity because the shape and length of these color lines change throughout the juvenile and subadult period. Sexual dimorphism is not very marked but it is possible to note that males, unlike females, have a very domed cloaca. Adult salamanders range in size from 11 to 21 cm and this is one of the most reliable criteria for determining whether the individual is an adult or juvenile. The second criterion for determining the age category of a Terrestrial Spotted Salamander is the shape of the dorsal yellow lines; if they are connected on a large part of the back, the individual is still young (Muratet, 2008). However, this criterion is far from reliable because some large salamanders may have connected lines on a part of their body.

Terrestrial spotted salamanders are viviparous lecithotrophs; the embryos will develop in the womb of the female, which, at the time of giving birth, joins the aquatic environment. Ovulation and reproduction takes place in terrestrial environments in summer with a gestation period of a few months (Duguet and Melki, 2003). Whelping depends on the region and may occur in winter in some areas. For example, in northern France the whelping period runs from January to May. It can take place over several nights and the female then returns to her summer site during the day. Migration and reproduction of the salamanders under hormonal control is closely dependent on weather conditions as this species is very sensitive to variations in humidity, temperature, etc. Knowing exactly when migrations and reproductions take place is very complicated because it depends on many factors like the outside temperature at night, if there was rain in the day, etc. The temperature favouring the outputs is estimated between 8°C and 14°C but this can very vary if the previous days were rather dry (Duguet and Melki, 2003). Some salamanders may give birth in winter if optimal climatic conditions are met. Several dozen larvae with their four legs and gills are deposited. During the metamorphosis, the young salamanders lose their gills and acquire lungs to enter into terrestrial environments. The salamanders are very bad swimmers and she only goes back to the water to give birth. It is not uncommon to see dead salamanders drowned during whelping. Males, on the other hand, are not dependent on the aquatic environment. The dispersal perimeter around the aquatic site is generally less than 100 metres for salamanders.

As mentioned above, there are many threats to amphibians. In addition to the destruction of habitats, there are various types of pollution which adversely and rapidly affect amphibians with permeable skin (Serre Collet, 2019). One of the main threats to salamanders remains habitat fragmentation. Every year, the road network kills 25 to 50 million amphibians. Where other amphibian species try to cross the roads as quickly as possible, the salamander will be in the middle of the road to observe what is happening. Reproductive behaviour encourages Salamander males to find the best point of view to locate a female from afar. Unfortunately, the roads become perfect observation point (Serre Collet, 2019). Moreover, it seems that the heat that spreads from macadam attracts them (Perrot, 2018). Emerging amphibian diseases are also emerging threats that are severely affecting global populations. This is why it is essential to organize follow-up, rescue, restoration and creation of aquatic and terrestrial habitats favourable to amphibians. Human activities, such as ATV forest rides in streams or new recreational sports, such as ruisseling (Serre Collet, 2019), alter and destroy nesting sites. To combat this crushing road problem, amphibians are incorporated into the green and blue frames “trames vertes et bleues” (Morand and Carsignol, 2019; Siblet et al., 2011).

### 3) The association “Ligue de protection des oiseaux” (LPO)

The « Ligue de Protection des Oiseaux » or LPO is a non-profit association for the protection of species and their natural environments. The Franche-Comté LPO was created on June 1, 2007 and now has more than 1,000 members and many more volunteers. Like all associations, it is governed by a board of directors and has 12 employees in various sectors of activity. In Franche-Comté, eight local LPO groups are distributed throughout the territory and are supported by the LPO Franche-Comté. These local groups are encouraged by the LPO Franche-Comté to implement projects, actions related to their sector for volunteers. Thus, each person interested in environmental protection can participate. The LPO projects focus on the study and the protection of wildlife (birds, reptiles and amphibians, mammals) and their habitats. Educating and mobilizing people about protection of nature are also important activities of the LPO employees (Ligue de Protection des Oiseaux, 2020). The most important strength of this association is the large number of volunteers who are attached to their cause. The data collected by these individuals is the main pillar of environmental data that they collect through various activities. Communication with the public, informed or not, is also one of the daily actions of LPO employees to prevent and inform about the environment, biodiversity, species and their habitats, etc.

At the crossroads of the improvement of the knowledge of amphibians and their conservation, the voluntary system of road rescue for amphibians of the departmental road 104 between Boussières and Vorges-Les-Pins illustrates very well the problems dealt by the association and its network of volunteers.

### 4) Objectives of the internship

The volunteer amphibian roadside rescue system mentioned earlier was first implemented in February 2019. Since then, the identification of the individuals has been done manually by the device managers, based on photos of the dorsal pattern of each salamander. This manual identification leads to a significant loss of time. In addition, the database created by the volunteers regarding the number of salamanders identified does not reflect the total number of salamanders actually present in the Vorges-Les-Pins area. But this information could make it possible to study the evolution of this population over several years and eventually, authorize the LPO Franche-Comté to implement a rescue plan for the salamander subspecies.

To remedy the problem of time loss encountered by volunteers, the use of photo-identification software should save time on the identification of each individual. One of the objectives of this internship will be to test the photo-identification software name I3S to determine if it can help volunteers. The photos used for the identifications will be sorted and then incorporated into a database. The given photo loss will likely be large. For the volunteers, a standardized protocol for the photographic shooting of salamanders will be developed and tested in order to obtain later photographs always usable by the software. To monitor the evolution of the population over the years, it is necessary to estimate each year its total population. To be able to do this the Capture-Mark-Recapture (CMR) method based on the data collected for 2019 will be use to estimate the population. At the end of this expertise, it will be possible to know whether a scientific study can be carried out based on voluntary data or not.

## II. Material and Method

### 1) Vorges-Les-Pins Amphibian Rescue System

- *The site:*

Since 2019, an amphibian rescue device has been set up on the RD104 road linking Vorges-Les-Pins and Boussières, as shown in figure 1. The "Moulin Caillet" stream and the pond near Highway RD104 are breeding grounds for the various amphibian species in the area. The surrounding forest represents wintering areas for these amphibians present in this sector. The 500 metres of roads separating these two areas, designated by the two yellow and red lines in figure 1, represent a space of strong amphibian migration. This device aims to reduce the crash of amphibians crossing the road especially during their spring and fall migrations. This is a temporary device called a "trap barrier", which was first installed on February 2, 2019 and can be quickly assembled and dismantled. Using a device like this is not intended to harm the rest of the wildlife.

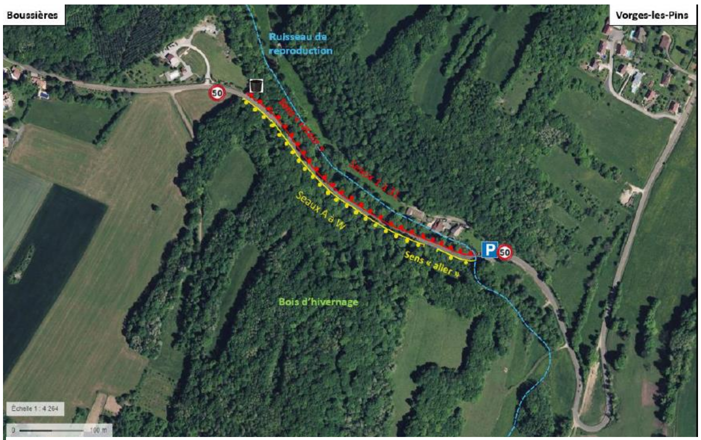



*Figure 1: Location map of the amphibian device of Vorges-Les-Pins on the road RD 104 at the place called "Le moulin Caillet" with schematization of the location of the buckets. Image from the LPO action presentation slideshow.*

- *Assembly and disassembly of the device*

The principle of this device is to place on each side of the road tarpaulins blocking amphibians in their crossing, as shown in figure 2, which forces them to follow the tarpaulin. The tarpaulins can be equipped with flaps to make it easier to block salamanders that are very good climbers. Seals, about 10 metres apart, are also placed on either of the tarpaulins forest-side, in order to recover the amphibians that fall inside. It is important that the buckets touch the tarpaulin so that the animals do not pass between the tarpaulin and the bucket without ever falling into it. The buckets are filled with a mixture of soil and humus that allows individuals to spend the day or night away from predators. Each bucket is equipped with a numbered stick to identify each of them. This stick has a double use because it is placed in the bucket so that if a small mammal like a rodent falls into it, the animal can come out.


*Figure 2: Schematic drawing of an amphibian rescue device from the "Guide de détermination des amphibiens d'Alsace, Conseil département Haut-Rhin, BUFO Alsace".*

For more details on the device installed in Vorges-les-Pins, it is possible to read the PDF associated with the installation and disassembly of the tarpaulins (Michon and et al, 2018) as well as the progress of each evening of rescue is explained in (Michon and Montaz, 2018).

| Session 1 | | | Session 2 | | |
|---|---|---|---|---|---|
| Beginning | End | Time (by night) | Beginning | End | Time (by night) |
| 07-févr-19 | 06-avr-19 | 58 | 27-sept-19 | 03-nov-19 | 38 |

*Table 1:Date of beginning and end of capture session and duration of session (in number of catch nights).*

Table 1 summarizes the dates on which the rescue device was put in place. This corresponds to the capture nights used to estimate the population by Capture-Mark-Recapture methods.

## 2) Capture-Mark-Recapture Method (CMR)

Capture-mark-recapture (CMR) is a method used to estimate the number of individuals in a population or to calculate certain demographic parameters such as survival. This method is based on the capture and recapture of individuals from the same population in a given area. Capture sessions take place over several days and can be referred to as “occasions”. When an animal is captured, it is then marked so that it can be identified during other capture sessions. The methods of marking are diverse and varied, using more or less invasive techniques for animals (Netchaieff, 2014). For this study on salamanders, a photograph of the dorsal pattern of adults is the best marking because these kind of photo-data respect two conditions necessary for a CMR study:

- The marking must be unique and definitive on each individual
- Marking must not affect the survival of the individual

This photo data is also used in other CMR studies involving other animal species such as giraffes (T. Bolder et al., 2012) or yellow-bellied doormen (Vacher, 2017)and (Bonnaire and Baudran, 2016).

Each new individual is referenced in an EXCEL and this allows to obtain a life history of each individual with an annotation 1 during a capture or recapture and 0 when the individual is not found during the occasion. With the help of software such as MARK the population can be estimated if the recapture number is sufficient as it is the most important factor in this method. The higher the recapture number is, the more accurate the estimate will be. Other prerequisites must be respected for the CMR method.

- Sampling should be random so that everyone has the same probability of being captured.
- Released individuals must be able to mingle with the rest of the population
- Capture or recapture must be random

These requirements are always necessary to estimate a population but different modelling can be used. For example, the Lincoln-Peterson principle is based on the above-mentioned requirements and only on a population considered closed. In other cases, population estimation can be done on a population considered open but for this, the Jolly-Sober model must be applied. This model makes it possible to estimate the total population but also other demographic parameters such as the estimation of population survival or abundance. Thus the model takes into account births, deaths, immigration and emigration. For this model a new prerequisite is necessary and say that each individual present at the i n capture until the i+ n capture, have the same probability of survival. To be able to carry out these calculations, the statistical power required to be must higher than that of «Jolly-Sober» calculations where the population is considered closed. For this, many years of monitoring are necessary to estimate the population and survival rate.

In this study, the sampling takes place only on the part of the road where the rescue device is located, so it cannot be considered as truly random. In addition, because the study takes place over a whole year, the population is not closed because there are births and deaths. However, since the database is probably too weak to obtain sufficient statistical power to use the Jolly-Sober model, this study will be based on the Lincoln-Peterson principle. The biases related to the dataset are recognized and will be integrated for the best in the different tests. However, if the statistical power is sufficient with the data collected, tests on an open population may be considered.

### 3) I3S (Interactive Individual Identification System) Photo-Identification software.

During the rescue evenings carried out by the volunteers, photos are taken of each individual to allow a manual identification of the exact number of individuals captured. It is on this basis that the estimate of the total population is made. However, this so-called manual identification is very expensive in time for the two volunteers in charge of identification. This is why, in order to save valuable time for these people, a photo-identification software called I3S (Interactive Individual Identification System) is tested to determine if the photos taken during the year 2019 can be incorporated each year into a reusable database. This is a so-called semi-automatic method already used in many CMR studies (Rey and Timmermans, 2017) (Gardner et al., 2019). It allows when individuals are not identified, to quickly know how many individuals are captured or recaptured. So it’s an additional tool to get a faster estimate of the population. Other photo-identification software exists, such as the WILD-ID software, which has been used many times by researcher Douglas T. Bolder, including his study entitled “A computer-assisted system for photographic mark-recapture analysis.” (T. Bolder et al., 2012). Recently the software I3S (Interactive

Individual Identification System) was used on populations of Pelagic Vipers in the Pas-de-Calais (Rey and Timmermans, 2017) and on a yellow-bellied Sonneur in Alsace (Vacher, 2017).

For this study, it is better to use the I3S software because it is free and also very easy of manipulation. It is also used in several studies on salamanders and the results of these experiments are very convincing. In addition, the software is not the one that decides to accept a match between two individuals of different photo, but leaves the possibility to the user to do so. Unfortunately, the comparison with the WILD-ID software was not possible because the download could not be done properly and its use seems more complex than that of I3S. The software chosen for the study has several versions like I3S Spot, I3S Straighten, I3S Pattern, I3S Classic, etc. Each of these different versions is adapted to a type of particular species. Although the Spot version has already been used on studies with newts whose ventral pattern may be similar to dorsal patterns of some salamanders, it will not be chosen. I3S Pattern seems more suitable for our salamander species because the circles chosen as «key point» do not only correspond to the color marks of the individuals but can also represent the distance between two points of color. Since I3S Pattern also uses ellipses as a «key point» this version can also be used on species such as the spotted newt. I3S Pattern automatically selects the key points after the user has defined the search area. The I3S Straighten version is also used when twisted individuals need to be put back straight. If the salamander is too curved I3S Straighten cannot properly put the individuals straight without changing the spots.

Before being able to test the software it is first necessary to create the database by creating a special folder where the software can classify each new individual. These individuals will have their own specific folder where the software will add any new photo of the individual if a match between two different photos is accepted.

When the database is created, a specific metadata must then be assigned to that database. This metadata must correspond to the characteristics that can help to differentiate each individual. In addition, it is important to choose three reference points that will allow the software to calculate the distances of each drawings pattern for each individual. The reference points must be referenced first then in the metadata as many elements as desired can be added. In

*figure 3: Representation of the three key points by blue circles, chosen to study the salamander with the software I3S.*

the case of Salamanders, the two intersections between the eyes and the parotoid glands and the dorsal intersection between the cloaca and the tail are chosen as you can see one the figure 3. As part of the metadata, the sex and length of the individuals are added.

**Step 1: Tested the software to create a photographic shooting protocol for salamanders.**

In order not to waste time by including in the database photos that cannot be exploited by the software, a sort is carried out. To find out what kind of photos are not usable by the software, it is important to read the recommendations found on the I3S website ("i3s – Reijns - Photo-identification," n.d.). A sort is done on all the photos of the salamander 2019 folder and each photo is renamed as follows: N° du sceau_Sexe_Taille_Date de capture_Identifiant attribué à la Salamandre. Thereafter, this re-naming of each photo allows to know if the unknown individual tested in I3S already had a reference file with a photo at this time. If that were the case, a certain match had to be played. If the match between the two photos was not possible this means that the quality of at least one of the two photos is not sufficient.

In order to confirm the recommendations concerning the photograph of a salamander found on the official site of I3S, some tests will be carried out on a secondary database. These tests will make it possible to create a standardized and popularized protocol for photographing the salamander, adapted to the volunteers. If it is properly applied during the nights of rescues, this protocol will eventually help to stop losing photo-data and it can be find on the appendix 2. In this secondary test database, photos of the same salamander will be incorporated and each of the salamanders present on these photos will presents a problem: Flash, twisted, biases, etc. It is also interesting to see how the original photos and those that were handed right by I3S Straighten will match.

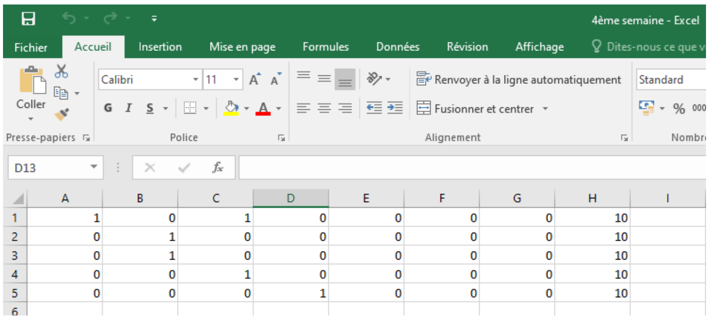
**Step 2: Creation of the database.**

This step consists in creating the database from the photos sorted in step 1. As the tests have shown that the match between two picture of salamander processed by I3S Straighten are better than the starting images, this database will be based only on the photos of straighten individuals. It is therefore necessary that each individual selected be treated with I3S Straighten, even very little crooked salamanders.

### 4) MARK software

To estimate the population size of Spotted Salamanders, the MARK software is chosen for this task. It has already been used in several capture-mark-recapture studies.

First of all, it is important to create EXCEL files usable by the software. Columns should represent a capture occasion and rows should represent a single individual. Each time a new individual is captured, it is noted present in the corresponding occasion. A presence is annotated 1 and an absence 0 at each occasion. Of course an individual present on several occasions is marked as present (1) for each of them. This corresponds to recaptures. An example of an EXCEL file is shown in picture 2. In this table only 5 individuals are present but others can be added. To use the MARK software, it is important to add a last column that categorizes each individual. This column is represented in Picture 2 by column H. This column does not correspond to an occasion but to a category. The choice of categories depends on what we want to highlight. For example, it is possible to separate the population according to sex and therefore 3 categories can be created. Female: 10; Male: 01; Juvenile: 11. The objective of achieving several categories is to be able to estimate different size of categories in the population and to test the differences of capturability for each of them. Although it is necessary to always add this last column, it is not mandatory to always have a minimum of two categories. Only one can be e and each individual can be enough and categorized as "belonging to the same population": 10, as in the example in figure 4.



| | A | B | C | D | E | F | G | H | I |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 10 | |
| 2 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 10 | |
| 3 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 10 | |
| 4 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 10 | |
| 5 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 10 | |

*Figure 4: Example of EXCEL table used for population estimation using MARK software.*

Before that the Salamander population can be estimated it is necessary to determine whether it is closed or not. Over a week of capture, the question does not necessarily arise because the probability for the population to be considered closed is high. This is less feasible on a test carried out over a whole year where mortality and birth rate are not zero. To know the answer, the CloseTest software will be used on each of the tests to find out if the closure of the populations. This CloseTest software is based on two closure test, the first on the *Stanley and Bruhnam* test and the second is call *Otis et al*. test (White, 2008). In addition to announcing whether populations are open or not, these tests tell if the number of data is sufficient. CloseTest files are similar to MARK software but the category column should not be included. The hypothesis H0: the population is closed, will always be the starting hypothesis of the tests carried out on CloseTests.

Concerning the problem of population closure, two phases of tests are carried out. In the population, to reduce birth bias it would be interesting to remove juveniles from the list. However, it is first necessary to know if the statistical power is sufficient with juvenile before being tested on a population restricted to adults. This step is necessary because the collected data probably does not have enough capture or recapture to be carried out. If the statistical power is sufficient or at least strong enough to estimate a population with juveniles, the tests will be conducted on a population restricted to adults. Due to lack of time, it will not be possible to carry out test with juvenile and tests without juvenile in Phase 2. If Phase 1 allows to estimate populations when juveniles are not taken into account in the tests then, Phase 2 will be performed only on the adult population. The phase 2 correspond to test without night of capture with no data (capture and recapture). this means that all nights of capture that does not have at least one recapture or capture are no longer taken into account in the tests. The grouping changes slightly from phase 1.

Typically, the MARK software and the CMR method consider an occasion to be a single night of capture. However, given the low number of catches or recaptures in some capture nights, groupings will be performed between several nights to increase their power. For the first phase, the groupings were done on a week-scale (7 occasion corresponding of 7 consecutive nights of capture over a week), on a monthly scale (4 occasion and an occasion correspond of 7 consecutive nights of capture), on a seasonal scale (4 occasion for each season, each season divided by 4) and on an annual scale (4 occasion for the full year, each season divided by 2). For the second phase, the grouping was done on a week-scale (7 occasion corresponding 7 night of capture), on a seasonal scale (first with 2 occasion for each season, each season. In Phase 1 Test 1, the fourth month differs from the first months. only 6 weeks of capture are found in Season 2. The fourth month therefore corresponds to the 3 month with 5 occasions instead of 4 (the 6th week is not taken into account

| | Phase 1: Each test below is performed on the total population or on the juvenile-free populationpopulation totale ou sur la population sans juvéniles |
|---|---|
| Test 1 bis (weekly) | 1 occasion = 1 night of capture |
| | the tes was carried out with 7 occasions |
| Test 1 (monthly) | 1 occasion = 7 night of capture |
| | the test was carried out with 4 occasions |
| Test 2 (season) | 1 occasion = 1/4 of the nights of the season (14 or 15 night for season 1 and 9 or 10 for season 2) |
| | the test was carried out with 4 occasions |
| Test 3 (annual) | 1 occasion = half of the season ( first season = 29 night, seconde season = 19 night) |
| | the test was carried out with 4 occasions |

*Table 2: Explanation of the tests carried out for phase 1 with or without juveniles.*

because it contains no capture or recapture). divided by 2, and secondly with 4 occasion, each season divided by 4), on an annual scale (4 occasion, each season divided by 2).

| | | Phase 2: juveniles and nights without capture were not taken into account |
|---|---|---|
| Test 1 bis (weekly) | | 1 occasion = one night of capture |
| | | the test was carried out with 7 occasions |
| Test 1 (season) | 1 | 1 occasion = half of the season |
| | | the test was carried out with 2 occasions |
| | 2 | 1 occasion = a quarter of the season |
| | | the test was carried out with 4 occasions |
| Test 2 (annual) | | 1 opportunity = half a season |
| | | The test was carried out with 4 occasions |

*Table 3: Explanation of the tests carried out for phase 2 where juveniles and nights without catch or recapture are not taken into account.*

It is important to test the population in different ways to determine which form of test is the best to properly estimate the total population from the number of individuals introduced into the test. In addition to estimating the population, the CAPTURE program of the MARK software that will be used for the tests also indicates the appropriate model for the population tested. These models are M(T): variation of capture over time, M(B): trape depending, M(h): heterogeneity of capture between individuals. The software also estimates the population size without any of these effects and the model concerned is then M(O). The parameter chosen by the software corresponds to the one that most influences the population tested. When the parameter is determined, the software will perform the calculations taking into account this bias. Sometimes effects can be combined, such as the M(tb) model. The best model is selected on the basis of a maximum value ranging from 0 to 1 based on a specific CAPTURE criteria.

# III. Results

In the appendix 1 you can find completive information about the structure community of amphibians the sector.

## a. Results of database creation

Thanks to the software, a manual identification error was discovered. The individual called Anastasia and the individual called Galia are finally the same individual. Scores between matches of the same individual always remained above 12 but it is still possible to accept it. With the I3S software, matches between two individuals should be below 10. The number of individuals identified by the software is 125, compared to 306 manually identified by volunteers. Problems encountered during database creation mean that some photos are not of good quality. On 141 picture used, 125 were identified and this means that 16 pictures was recapture data.

Approximately ¾ of the photos could not be used by the I3S software analysis because of the poor quality of the picture for

b. Results of estimates population

The red crosses (X) represented in the tables below mean that it is not possible for the MARK software to estimate the population but also the estimate of H0 is rejected by the corresponding test from the CloseTest software. The validated green symbols (V) represented in the tables below mean that the associated closed population test and H0 is therefore accepted.

*Result of the phase 1:*

On the table 4, 18 tests carried out, 6 tests could not to an estimation. Most of the time, estimates with or without juveniles for the same test vary widely. In addition, Test 1 does not show a homogeneity in the estimates relative to each month. Confidence intervals are very wide. In about 3/9 of the cases, the CloseTest software tests disagree.

| | | Test 1 bis | | Test 1 | | | |
|---|---|---|---|---|---|---|---|
| | | première semaine | quatrième semaine | premier mois | deuxième mois | troisième mois | troisième mois, 5 occasions |
| **Avec juvénile** | estimation de la population | 38 | X | 175 | 136 | 338 | 391 |
| | intervalle de confiance | 24 to 67 | X | 127 to 282 | 129 to 175 | 225 to 555 | 272 to 603 |
| | standard error | 10,65 | X | 37,3 | 9,4 | 80,9 | 81 |
| **sans juvénile** | estimation de la population | 11 | X | X | 122 | 206 | 260 |
| | intervalle de confiance | 6 to 49 | X | X | 108 to 213 | 137 to 350 | 175 to 426 |
| | standard error | 8,1 | X | X | 20,47 | 20,5 | 61,5 |

| | | Test 2 | | Test 3 |
|---|---|---|---|---|
| | | première saison | deuxième saison | |
| **Avec juvénile** | estimation de la population | 533 | X | 521 |
| | intervalle de confiance | 413 to 719 | X | 344 to 1464 |
| | standard error | 76,6 | X | 225,7 |
| **sans juvénile** | estimation de la population | X | X | 867 |
| | intervalle de confiance | X | X | 628 to 1248 |
| | standard error | X | X | 154,8 |

*Table 4: Summary of the results obtained for each of the different estimation tests on the total population and on the population with only adults.*

The results of the table 5 show that the models adapted to the different tests vary greatly even within a similar test. For example, test 1 shows differences between the first three months. However, the third month with 4 and 5 occasions has the same test. There may also be differences between juvenile and juvenile results for the same test.

| | Test 1 bis | | | | Test 1 | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | première semaine | | quatrième semaine | | premier mois | | deuxième mois | | troisième mois | | troisième mois, 5occasions | |
| | Stanley & Burnham | Otis et al. | Stanley & Burnham | Otis et al. | Stanley & Burnham | Otis et al. | Stanley & Burnham | Otis et al. | Stanley & Burnham | Otis et al. | Stanley & Burnham | Otis et al. |
| Modèle choisi par le logiciel | M(h) | | X | | M(t) | | M(tb) | | M(O) | | M(O) | |
| Réponse de CloseTest | V | V | V | V | V | V | V | V | V | V | V | V |
| Modèle choisi par le logiciel | M(O) | | X | | M(tbh) | | M(tb) | | M(O) | | M(O) | |
| Réponse de CloseTest | V | V | X | V | V | V | V | V | X | V | X | V |

| | Test 2 | | | | Test 3 | |
|---|---|---|---|---|---|---|
| | première saison | | deuxième saison | | | |
| | Stanley & Burnham | Otis et al. | Stanley & Burnham | Otis et al. | Stanley & Burnham | Otis et al. |
| Modèle choisi par le logiciel | M(t) | | M(tbh) | | M(tb) | |
| Réponse de CloseTest | X | V | V | V | X | V |
| Modèle choisi par le logiciel | M(tbh) | | X | | M(t) | |
| Réponse de CloseTest | V | X | V | V | X | V |

*Table 5: Summary of the results obtained by the CloseTest software on closure tests. Summary of the results of the MARK software regarding the appropriate model choice for each test performed.*

*Result of the phase 2:*

The two tables presenting the results of the tests of phase 2 show us that in the 6 tests carried out, 3 could not give an estimate. As in the first phase, population estimates vary widely and confidence intervals are very wide. In addition, most tests have different models. Regarding closed or open population results, the two tests of the CloseTest software do not always agree. In two out of three cases, they disagree.

| | | Pour une semaine, test 1 bis | Pour la première saison, test 1 | |
|---|---|---|---|---|
| | | | pour 2 occasions | pour 4 occasions |
| **Sans juvénils** | estimation de la population | 2521 | X | X |
| | intervalle de confiance | 131 to 81391 | X | X |
| | standard error | 118473 | X | X |

| | | Pour la deuxième saison, test 1 | | Pour l'année, test 2 |
|---|---|---|---|---|
| | | pour 2 occasions | pour 4 occasions | |
| **Sans juvénils** | estimation de la population | X | 101 | 721 |
| | intervalle de confiance | X | 86 to 219 | 556 to 970 |
| | standard error | X | 25 | 104,27 |

*Table 6: Summary of results obtained for each of the different population estimate tests conducted on the adult salamander population with evening with only capture or recapture.*

The table 7 show a lot of differences between models associate to the test. The model M(tbh) did not lead to an estimation. There is a lot of contradiction for the two tests of closure from CloseTest.

| | | Pour une semaine, test 1 bis | | Pour la première saison, test 1 | | | |
|---|---|---|---|---|---|---|---|
| | | | | avec 2 occasions | | avec 4 occasions | |
| | | Stanley & Burnham | Otis et al. | Stanley & Burnham | Otis et al. | Stanley & Burnham | Otis et al. |
| Sans juvénils | Modèle choisi par le logiciel | M(tb) | | X | | M(tbh) | |
| | Réponse de CloseTest | V | V | X | V | V | X |
| | | Pour la deuxième saison, test 1 | | | | Pour l'année, test 2 | |
| | | avec 2 occasions | | avec 4 occasions | | | |
| | | Stanley & Burnham | Otis et al. | Stanley & Burnham | Otis et al. | Stanley & Burnham | Otis et al. |
| Sans juvénils | Modèle choisi par le logiciel | X | | M(tb) | | M(t) | |
| | Réponse de CloseTest | X | V | V | V | X | V |

*Table 7: Summary of the results obtained for the CloseTest software concerning the result of closure population. Summary of the models chosen by the MARK software adapted to each of the populations.*

A test was conducted by taking into account two categories of population: Male and Female. This test was performed on the juvenile-free population. Since none of the previous tests showed a good estimate with a low confidence interval, it was decided to do this test by dividing the population into 4 occasions where each occasion represents one week. The MARK software has not been able to estimate the population of each group and has also chosen only one model which is M(tbh). The latter model was never able to estimate a single population in this study. CloseTest indicates that the data are insufficient but concludes these two tests with a P-value greater than 0.05 which indicates that we can accept H0, the population is considered closed.

In each tests, the CloseTest software showed that the data are not sufficient despite the fact that it could still calculate the P-value for the *Stanley and Burnham* test and *Otis et al*. This means that make test with open-population is not possible. There is not enough statistical power.

# IV. <u>Discussion</u>

Apart from the error on the Anastasia/Galia salamander explained in part 3) “database result”, each salamander was correctly identified by the volunteers. In addition, the results of all rescue night were carefully annotated in an EXEL file. Biases in the starting database are therefore very low. However, it is possible that errors are present in the sexing and measurement of the size of the individual. The most important bias explaining the difference between the results obtained by the volunteers and those obtained by the I3S software concerns the photographs of salamander dorsal patterns. The rate of photos not usable by the software is much too high. In addition, as volunteers change every night, the technique for taking photos also changes. Although each volunteer is competent, their large number brings bias. The scientific rigour expected for an CMR study is not sufficient in this case. To radically reduce this bias, the tracking method would have to be changed by reducing the number of people tacking the photo-data. But we are once again moving away from the heart of this study. The solution regarding the use of a standardized and popularized protocol remains the best solution. The positive feedback from the volunteers regarding the protocol is encouraging and suggests that the photo-data collected soon will be of better quality and all usable by the software. This will make it possible to expand the database created during this internship. At the moment, the number of salamanders identified by I3S is much lower than the number of salamanders identified manually. In addition, the quality of some photos included in the database is not sufficient. The scores of the matches are still too high but the protocol will reduce this bias because each photo will be normalized.

- The analyses of the estimates obtained in "Results" are based on the result of the work done by volunteers on the manual identification of individuals. The number of individuals identified does not correspond to a population estimate but to the minimum threshold of individuals present in the area. If the total population is taken into account then the estimate must be higher than 306 individuals minimum, corresponding to all individuals identified in 2019. If the tests include only adults, then the estimate must be greater than a minimum of 224 individuals.

As can be seen from the four summary tables, there are many differences in results. First of all, the results for each 1 bis test in phase 1 are not sufficient. Population estimates are always below the minimum number of salamanders identified. Moreover, these estimates do not always come to an end as shown in table 4. It seems that with or without juveniles, the number of recaptures is insufficient. For example, between the spring and fall period, the number of recaptures was only 11 individuals. For phase 2, the 1 bis test does not give a good estimate. Although the estimated number of salamanders exceeds the minimum number of salamanders in the population, the confidence interval is too high. This estimate cannot therefore be considered accurate because this confidence interval includes a possibility of a total population that can be between 131 and 224 which is therefore impossible. Whether for the first or the second phase, the confidence intervals are all extended and some more than others. Regarding the Test 1 series of Phase 1, the estimates vary a lot especially between the two seasons. The first two months have estimates below the acceptable minimum (306 or 224 individuals). Although the confidence interval of the second month is very interesting, it cannot be accepted. These variations are found for both the total population and adult population tests. Some variations over the last two months may be acceptable if their confidence interval was not as wide. The same problem is repeated on each of the tests and it does not seem to change. Tests 1 in the second phase are no better than those in Phase 1. Only one test out of four was able to arrive at an estimate and this one is not acceptable. Looking at the table 7 it is possible to find an explanation for this lack of result. First, two of the four tests did not even have the opportunity to propose a suitable model, which probably means that the data are far too insufficient. By insufficient data, we can imagine a sufficient lack of recapture and/or not enough individuals. This happened several times during this study where the software was not able to estimate a population but give at least the corresponding model. The model in question is the M(tbh) which corresponds to a model where time, heterogeneity of capture and trape depending influence the population. As it can be seen in Tables 7 and 5, whenever these models are chosen by the software, the estimation of the population is impossible to be calculate. It can be assumed that the influence of these three parameters on the population is much too strong to be attenuated by the software. This was also the case for the Test 2 series of the first phase where only one test in four was completed. And so, this way of testing the population by considering an occasion as two weeks of rescue night is not appropriate. Concerning the tests carried out over the full year (Test 3 for the 1st phase and Test 2 for the 2nd phase) we can note a systematic disagreement between the two closure tests, as shown in tables 5 and 7. At least one of the two closures tests assumes that the population is open and therefore rejects H0. In table 4, estimates of tests with or without juveniles differ and the highest estimate is reached when only adults are considered. However, these confidence intervals remain far too wide to be able to consider the estimate as reliable. Looking at tables 5 and 7, we see that time is the parameter that always influences this series of tests. This seems logical as these tests have taken into account all the data contained in a whole year.

Facing these disappointing results, some assumptions concerning explanations for the wrong estimates can be made. It would appear that using the data recovered during the rescue evenings would not be appropriate for a Capture-Mark-Recapture population estimation method. It has already been determined that the lack of recapture is a major problem for our estimates as it decreases the statistical power of the model. The CMR method is based on these recaptures and the data recovery mode greatly influences this parameter. This conclusion is supported by the numerous messages from the CloseTest software indicating a lack of data to perform all the tests. The results of these tests to determine whether the population is closed or not are therefore not necessarily reliable. However, it was necessary to test each population to be sure that this estimation model did not work. In addition, the choice to accept the population as closed even if one of the two tests (*Stanley and Bruhnam* or *Otis et al.*) indicates the opposite, reduces the probability of actually being closed. Over a whole year, it makes sense that this is not the case even if only adults are taken into account. deaths are inevitable as evidenced by road crashes. It would therefore have been interesting to test this population by considering it as open. However, it will probably take several years to obtain sufficient statistical power. As the models chosen by the software for each test differ greatly, even within the same set of tests; this indicates that the population is subjected to many disruptive elements. Concerning this problem, the solution may concern the rescue device because initially it was not intended for a CMR study. So there are obviously several biases that can be found at the protocol level. As explained in the "Explanation of the Capture-Mark-Recapture Method" section, a CMR study requires prerequisites that are assumed to be true in this study. However, this is not entirely accurate. For example, the recapture number is very important to estimate the population by CMR method or there is very little recapture in the dataset used. In (Bonnaire and Baudran, 2016) "Synthesis of the population monitoring method by C.M.R. " it is explained that to improve this recapture problem, several solutions can be considered such as adapting the study surface. In this study, the data collected come only from the D104 section of road between Vorges-Les-Pins and Boussières and therefore it does not really respect the required meadows that assume a heterogeneity of capture on the whole study area. Since each seal remains at the same locations for the duration of the catches, the notions of heterogeneity and random catches are not necessarily respected. To estimate that these required meadows were validated, we based ourselves on the fact that the road is an axis heavily frequented by salamanders. However, as it does not cover the entire study area, several locations are ignored. To achieve true CMR monitoring it would therefore be necessary to extend the capture area to the surrounding forests. However, this adaptation of the CMR protocol no longer corresponds to the use of data from a voluntary rescue device. It would be necessary to find others people to be able to make these catches. For the species studied, the «catches» are adapted but we know that the climatic conditions influence their migrations enormously. Therefore, it is possible not to capture any salamanders on consecutive nights. Considering this, the Tests 1 bis always having several «null» evenings and so are not adapted to estimate the total population of this species. By increasing the number of days contained in an occasion helps to reduce this bias related to climatic conditions. In every occasion there will always be at least one captured individual. At the same time, it increases the likelihood of having an open population. In Table 5, we can see that disagreements between the two CloseTest tests increase as the catch period increases. In relation to this problem there is really no possible solution if we decide to keep the same format of CMR method. The choice of taking into account only the evenings with catches in the second phase also reduces the bias related to the weather. Taking into account only adults, the bias regarding the opening of the population is supposed to be reduced too. However, the results are disappointing. Thus, on all the tests carried out no method really allows us to estimate the population correctly. And so, it will take several years to obtain the statistical power needed for this study.

# V. Conclusion

Given the results obtained with the Closetest and MARK software, it is not possible to estimate by CMR a population of amphibians based on data from one year from a voluntary roadside rescue system. Sampling biases are far too numerous and the solutions that can be provided are beyond the scope of volunteering. While the current I3S database does not currently include all identified individuals, it will quickly facilitate the identification of all individuals. This is a significant saving of time for volunteers in charge of identification. In addition, it will be interesting to try the experiment again when the database will be more important.

Although it is outside the scope of voluntary action, estimating the population of this sector remains an important study to reduce gaps in the biology and ecology of the Spotted Salamander. It might be possible to combine with the voluntary system an internship aimed at collecting random data on the entire air distribution of this population. For example, a distance sampling method may be considered if it is adapted to our species (Besnard and Salles, 2010; Hoffman et al., 2010). New techniques are also being developed, such as POP-Amphibians (Barrioz and Miaud, 2016).

## SWOT analysis:

| | **HELPFUL** | **HARMFUL** |
|---|---|---|
| **Internal origin** | • Handling Salamanders rather facilitated by the box created<br>• Use of three new software and improve handling on Zotero<br>• Improving knowledge of herpetology | • Manipulation of the original database complicated<br>• Insufficient statistical power of the database<br>• R software too complicated for the internship goal |
| **External origin** | • New approach to the Capture-Mark-Recapture method<br>• Very good integration within the association structure LPO Franche-Comté and discovery of this professional sector.<br>• Interaction and communication with volunteers despite containment | • Low reliability of results achieved<br>• difficult to travel in the field due to COVID-19<br>• Disappointing results because it is impossible to estimate the population |

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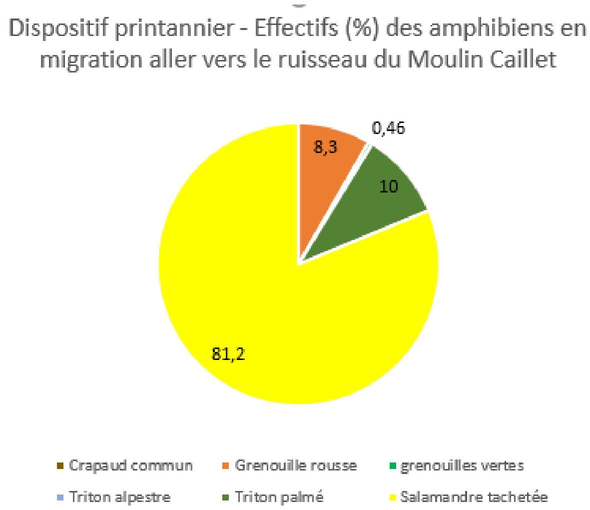
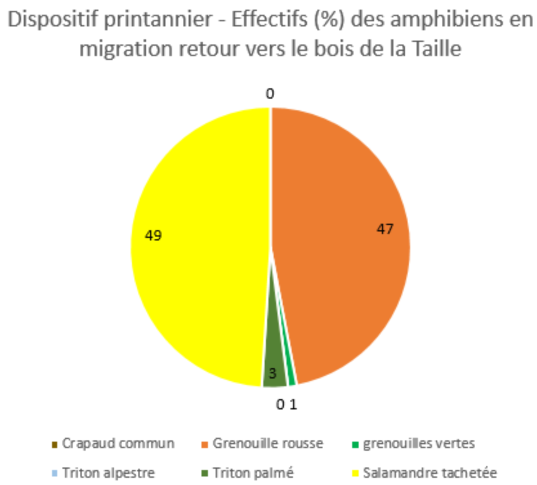
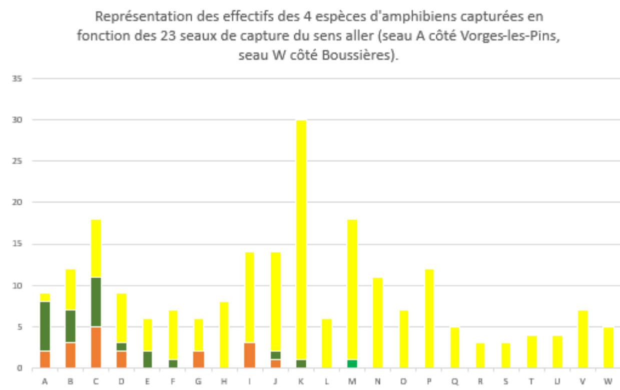
# Appendix 1:

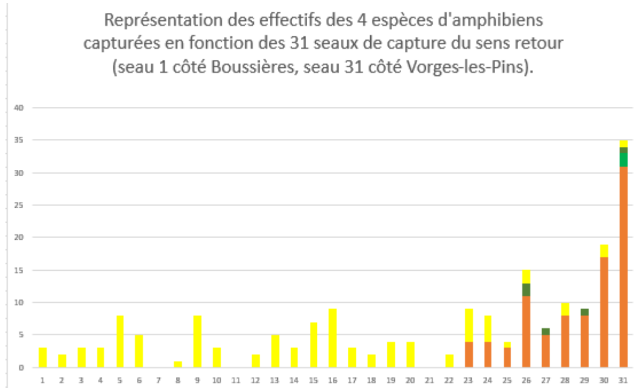
| | Aller | | Retour | | Aller + Retour | |
|---|---|---|---|---|---|---|
| | Printemps | Automne | Printemps | Automne | Printemps | Automne |
| **Salamandre tachetée** | 177 | 242 | 94 | 214 | 271 | 456 |
| **Crapaud commun** | 0 | 5 | 0 | 2 | 0 | 7 |
| **Grenouilles brunes** (Grenouille rousse + Grenouille agile non vérifiée) | 18 | 192 | 91 | 130 | 109 | 322 |
| **grenouilles vertes sp.** | 1 | 2 | 2 | 6 | 3 | 8 |
| **Triton palmé** | 22 | 12 | 5 | 7 | 27 | 19 |
| **Triton alpestre** | 0 | 2 | 0 | 1 | 0 | 3 |
| **tritons sp.** | 0 | 1 | 0 | 0 | 0 | 0 |
| **TOTAL** | 218 | 456 | 192 | 360 | 410 | 815 |

*Table appendix 1: Summary of the number of individuals from each species saved in both size of the device in Vorges-Les-Pins and Boussières*



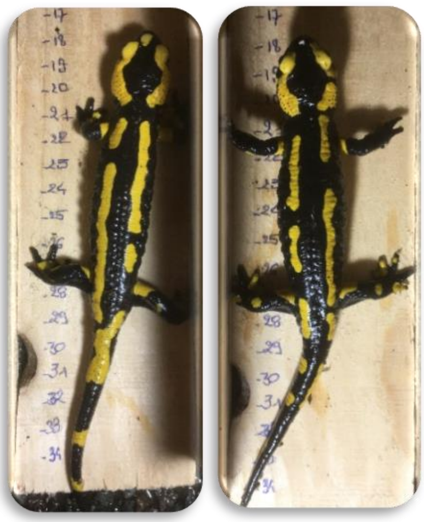



Appendix 2:

# PROTOCOLE DE PRISE DE VUE PHOTOGRAPHIQUE des Salamandres tachetées (*Salamandra salamandra*)

Le patron dorsal (nombre, disposition et forme des taches sur le dos) des salamandres tachetées évolue chez les juvéniles pour « se fixer » à l'âge adulte. Le patron dorsal des salamandres tachetées adultes, propre à chaque individu permet ainsi de les reconnaître individuellement et de les suivre dans le temps.

*Photographie 1:individu juvénile de salamandre tachetée terrestre. La longueur totale est comprise entre 5 et 10 cm et les lignes dorsales sont souvent fusionnées sur le dos.*



*Photographie 2 :Deux individus femelles de salamandres tachetées terrestres dont les patrons dorsaux diffèrent. Les deux femelles mesures environs chacune 18 cm.*

Ce protocole peut être appliqué pour tous les sujets scientifiques concernant les salamandres tachetées. Par rapport à une comparaison « manuelle » des photographies, ce protocole permet de gagner un temps considérable pour l'identification de chaque individu adulte par le biais d'un logiciel de photo-identification de type I3S (Interactive Individual Identification System). Bien que le logiciel analyse des photographies uniquement d'adultes, il est important de photographier également les juvéniles afin de suivre le pourcentage d'immatures de la population chaque saison.

# Méthode :

Pour chaque session de suivi/capture, les opérateurs doivent se munir de la gouttière rectangulaire graduée et du fond de gouttière mise à disposition par la LPO pour le suivi. Cette gouttière est indispensable car elle permettra de mesurer l'individus tout en le confinant dans un endroit où ces mouvements seront restreints. Il sera donc plus facile de prendre des photos nettes, proportionnées et standardisées. Les photos peuvent se faire à l'aide d'un appareil photo ou d'un téléphone portable. L'important est de réussir à avoir des photos nettes de l'individus.

*Photographie 3: boitier en bois permettant de mesurer et photographier les individus.*

Lorsqu'une salamandre est retrouvée dans un seau, commencez par la prendre en photo. Il vous sera plus facile de procéder à deux. Par exemple la personne en charge de la prise de note est celle qui photographie l'animal et la personne en charge de la capture des animaux place les individus dans la gouttière.

1. Placer la gouttière sur un sol plat
2. Déposer l'animal en bout de gouttière où l'extrémité de la queue arrive à l'extrémité ouverte de la gouttière :

D'ordinaire les salamandres vont préférer marcher jusqu'au bout de la gouttière mais il est possible qu'elles essayent de s'échapper. Dans ce cas, il suffit de réitérer l'étape 2.

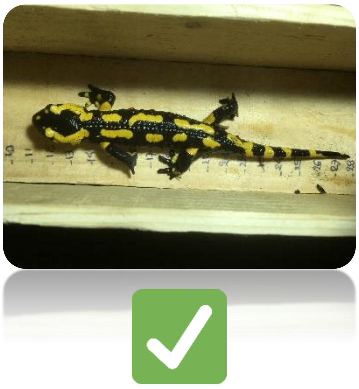
3. L'appareil photo doit être placé à quelques centimètres au-dessus de la gouttière et doit être parallèle à celle-ci.
4. Photographier l'animal : la photo doit être nette et sans reflet. Pour ce faire il faut privilégier l'éclairage de l'animal avec une frontale en laissant le flash de votre appareil photo désactivé. Si l'animal avance dans la gouttière vous pouvez le suivre, mais toujours parallèlement à la gouttière et en prenant votre photo au moment où l'animal est le plus droit possible. N'hésitez pas à réitérer vos prises de vues jusqu'à obtenir une photo nette (voir ci-dessous)

*Photographie 4: exemple de photo pouvant être utilisé par le logiciel I3S.*

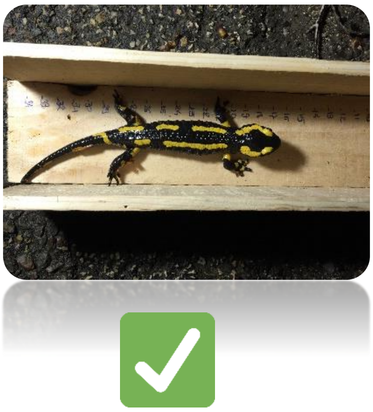
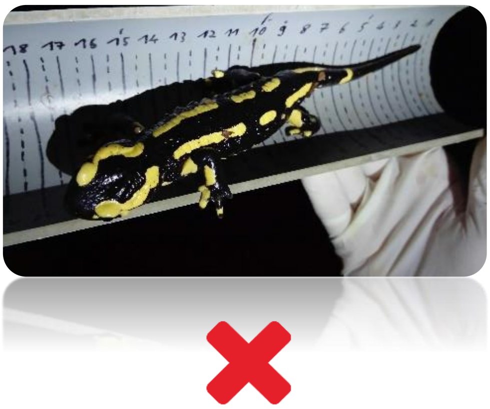
Avant de relâcher l'animal, penser à compiler sur votre fiche de relevé de terrain l'ensemble des informations demandées (déterminer son sexe, étudier attentivement son comportement et s'il présente des « anomalies cutanées » pour le suivi des maladies émergentes, etc.).

# Pour vous aider voici les règles les plus importantes pour obtenir les meilleures photos :

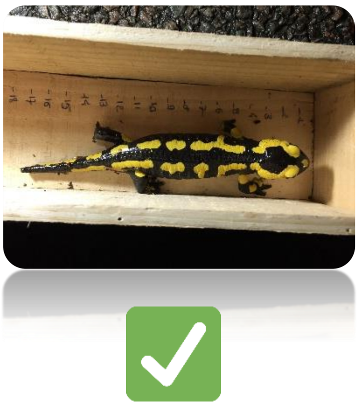
1) **Placer l'individu dans la gouttière**. Cela vous permettra de les mesurer en même temps. Les individus placer sur fond clair sont mieux identifié par le logiciel. Les photos avec des individus sur le sol ou en mains ne sont pas exploitables.



2) **Prendre des photos parallèles** à l'individu avec l'appareil photo placé parallèlement au-dessus de la gouttière.


3) **La photo doit être la plus nette possible**.



4) Dans la mesure du possible essayer de **ne pas utiliser de flash ni de lumière direct**. Les éclairages indirects sont autorisés avec une lampe à proximité de l'individu par exemple.






5) **Les individus droits** sont difficiles à avoir mais sont mieux identifier par le logiciel. Si l'individu est légèrement tordu, la photo peut généralement être utilisée.

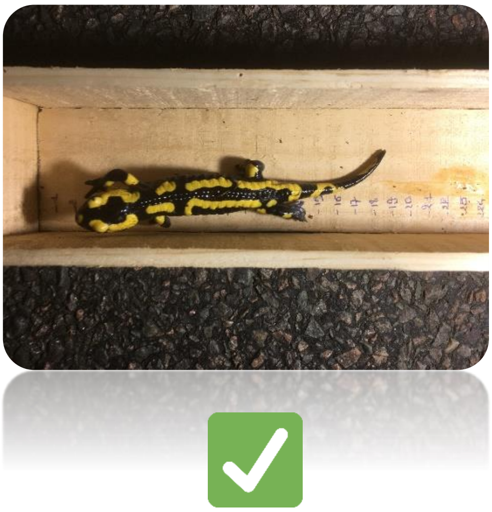





6) Une **distance de maximum 40 cm** entre l'appareil photographique et la salamandre doit être respectée.




7) Ce n’est **pas nécessaire que l’individu soit placé en butée contre le fond de la gouttière.**



# A vous de jouer !

Et comme dirait Monsieur Michael Aguilar :

« *Le désir de bien faire est un puissant moteur. Celui de faire du bien est plus puissant encore*. »

Réalisation :

VERNEREY Fiona et MICHON Alix

Bibliographie :

Site web du logiciel I3S « [https://reijns.com/i3s/faq/](https://reijns.com/i3s/faq/) »

## Abstracts :

For several centuries, the world has seen its biodiversity decline very clearly, as evidenced by the numerous studies on this subject. The amphibian group is one of the most affected with approximately 41% of the species classified as threatened by the IUCN (IUCN, 2020). Roads are particularly deadly for these populations and to remedy this problem, many solutions are being implemented near roads (Morand and Carsignol, 2019). Although the Terrestrial Spotted Salamander (*Salamandra salamandra terrestris*) is considered as "minor concern" in France and Franche-Comté, it is a good example of a species dependent on voluntary lifesaving devices. During the installation of rescue device, many photographic data are collected allowing to manually identify each individual but also to know their movements on the road. The main objective of this course is to estimate the salamander population present in the Vorges-Les-Pins sector by using the volunteer data collected through the amphibian rescue device set up in 2019. The estimates are based on the Capture-Mark-Recapture method. This internship will allow the implementation of a photo-identification software which will help the volunteer to save considerable time on the identification of each individual. Although the results of this study were not conclusive, the use of voluntary data for scientific purposes remains a very interesting idea.

## Résumé :

Depuis plusieurs décennies, le monde voit sa biodiversité déclinée très nettement comme le témoignent les nombreuses études à ce sujet. Le groupe des amphibiens est l'un des plus touchés puisqu'environ 41% des espèces sont classées menacées par l'IUCN (IUCN, 2020). Les routes sont particulièrement meurtrières pour ces populations et pour remédier à ce problème, de nombreuses solutions sont mises en œuvre à proximité des celles-ci (Morand and Carsignol, 2019). Bien que la Salamandre tachetée terrestre (*Salamandra salamandra terrestris*) soit considérée comme « préoccupation mineure » en France et en Franche-Comté, elle représente un exemple d'espèce dépendant des dispositifs de sauvetage bénévole. Lors de la mise en place de dispositif de sauvetage, de nombreuses données-photographiques sont récoltées permettant d'identifier manuellement chaque individu mais également de connaitre leurs déplacements sur la route. L'objectif principale de ce stage consiste à estimer la population de salamandre présente sur le secteur de Vorges-Les-Pins en utilisant les données bénévoles récoltées grâce au dispositif de sauvetage amphibiens mis en place en 2019. Les estimations se basent sur le principe de la méthode de Capture-Marquage-Recapture. Ce stage permettra la mise en place d'un logiciel de photo-identification permettra aux bénévoles référents de gagner un gain de temps considérable sur l'identification de chaque individu. La création d'un protocole de prise de vue photographique

destiné à tous les bénévoles est également indispensable.  Bien que les résultats de cette étude ne se soit pas avérés probant, l'utilisation de données bénévoles à des fins scientifiques reste cependant une idée très intéressante