CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

FACULTY OF THE ENVIRONMENT

DEPARTMENT OF ECOLOGY

DIPLOMA THESIS

Monitoring the most abundant population of the fire salamander (*Salamandra salamandra*) in Prague

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CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

Faculty of Environmental Sciences

DIPLOMA THESIS ASSIGNMENT

B.Sc. Guido Fernández Koch

Nature Conservation

Thesis title

Monitoring the most abundant population of the fire salamander (Salamandra salamandra) in Prague

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Objectives of thesis

Amphibians are currently the most endangered group of vertebrates. This is especially true in urbanised environments, including Prague, the capital city of the Czech Republic. On the other hand, thanks to its unique geomorphology and intensive nature conservation, Prague is home to many amphibian species, including the fire salamander. The largest population of this species in the capital is in Bohnice. The size of the local population was estimated at 1060 individuals on the basis of monitoring in 2015-2020. However, the salamanders are currently threatened by a number of factors (habitat destruction, disturbance) at the site.

The aim of thesis is to estimate the recent (2022-2023) population size and to compare it with the previous status by means of identification of repeatedly recorded individuals (CMR). Further aim is to compare the activity of salamanders during the year, including the winter period. The aim of the review section is to provide an overview of the biology, ecology, habitat requirements and distribution of the study species.

Methodology

The monitoring will use repeated visits (at least 15) during 2022-2023. Salamanders will be photographed for subsequent identification, and the occurrence of individuals will be located by GPS device. Subsequently, recent local population size will be estimated using Mark software and compared with previous data, including potential changes in age structure and sex ratio of the population.

The proposed extent of the thesis

30–40 pages

Keywords

amphibians, Prague, salamanders, Capture Mark Recapture

Recommended information sources

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Author's declaration

I hereby declare that I have independently elaborated the diploma thesis with the topic of "Monitoring the most abundant population of the fire salamander (*Salamandra salamandra*) in Prague" and that I have cited all the information sources that I have used in the thesis and thar are also listed at the end of the document in the list of bibliography. I am aware that my diploma thesis is subject to Act No. 121/2000 Coll. on copyright, on rights related to copyright, and on amendments of some acts, as amended by later regulations, particularly the provisions of Section 35(3) of the Act on the use of the thesis.

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With my signature, I declare that the electronic version of the diploma thesis is the same as the printed version.

Guido Fernández Koch

In Prague on 27.03.2023

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Abstract.

Salamandra salamandra is a well-known salamander species in Europe, extending its range all the way from the Iberian Peninsula to Poland and the Balkan Peninsula. In recent years, its conservation status is being negatively affected by human activities e.g. habitat fragmentation and global warming, plus facilitated spread of infectious diseases. The situation in the Czech Republic is not dissimilar. By national legislation it is classified as an endangered species, due to recent declines that risk the presence of this animal in this country. The aim of this project is to determine the population estimate of the fire salamander in the Black Valley (Černa rokle), Prague, for the year 2023.

To obtain population estimates, firstly, a CMR with18 nighttime visual encounter surveys and four additional datasets was compiled and analysed with the Jolly - Sebber model. The population size of past research was compared with the newly generated population estimate. Other data, such as sex or stage of the individuals, date, rain, and wind were also added. The Jolly – Sebber model gave out a population estimate of 582 fire salamanders for the Black Valley site, with a 95% interval of confidence in between 414 and 819 individuals. This represents a 45% decrease of the reported population for the 2020 census of the Black Valley, respectively. As for the VES (Visual Encounter Surveys), it reported that the population in Black Valley has at least 190 fire salamanders. It was also noted that fire salamanders in the Black Valley still have a natural pattern to the climatic changes according to the seasons of the year.

It was concluded that there is enough evidence to say that the *Salamandra salamandra* population is facing an almost 50% decrease. The seasonal behaviour barely shows any change, though, but it has to be monitored to understand the effects of the climatic changes on the largest fire salamander population of Prague.

Keywords : amphibians, Prague, salamanders, Capture Mark Recapture

Abstrakt

Mlok skvrnitý je velice známý druh žijící v Evropě, jehož výskyt sahá od Pyrenejského ostrova až po Polsko a Balkánský ostrov. V posledních letech je ale jeho ochrana negativně ovlivněna lidskou činností, jako například fragmentací biotopů a globálním oteplováním, které usnadňuje šíření infekčních chorob. Situace v České Republice není rozdílná. Podle legislativy České Republiky je tento druh řazen mezi ohrožené druhy, a to z důvodu nedávného poklesu početnosti, díky kterému je výskyt tohoto živočicha ohrožen. Cílem této bakalářské práce je stanovit odhad početnosti mloka skvrnitého, a to na pražském území Černé rokle pro rok 2023.

Pro získání odhadu velikosti populace bylo nejprve sestaveno CMR s 18ti vizuálními záznamy o nočních setkání zmíněného druhu, a čtyřmi dalšími soubory dat, které byly následovně analyzovány pomocí modelu Jolly-Sebber. Dále byla porovnána velikost populace z předešlých studií s nově vytvořeným odhadem populace. Doplněny byly i další údaje, jako například pohlaví nebo vývojové stadium jedinců, datum, nebo klimatické podmínky jako déšť nebo vítr. Jolly-Sebberův model poskytl odhad populace na 582 mloků skvrnitých pro lokalitu Černé rokle, a to s 95% intervalem spolehlivosti mezi 414 a 819 jedinci. To představuje 45 % pokles v rámci nahlášené populace druhu pro rok 2020 v Černé rokli. VES (vizuální průzkum setkání) uvádí, že populace v Černé rokli čítá nejméně 290 mloků skvrnitých. Bylo také zjištěno, že mloci skvrnití vyskytující se v Černé rokle stále přirozeně reagují na klimatické změny v závislosti na roční období.

Díky dostatku důkazů lze závěrem tvrdit, že populace mloka skvrnitého čelí téměř 50 % poklesu. Sezonní chování tohoto druhu téměř nevykazuje žádné změny, přesto je ale třeba tyto změny sledovat, aby mohly být dopady klimatických změn na nejvíce vyskytovaného mloka v Praze – mloka skvrnitého, postřehnuty a názorně zaznamenány. a dále analyzovány

Klíčová slova: obojživelníci, Praha, mloci, metoda opakovaných odchytů

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List of Abbreviations

1. IUCN: International Union for Conservation of Nature
2. VES: Visual encounter survey
3. AOPK ČR: Agentura ochrany přírody a krajiny Česke Republice (Nature and Landscape Protection Agency of the Czech Republic)
4. Bsal: *Batrachochytrium salamandrivorans*
5. Bd: *Batrachochytrium dendrobatidis*

1. Introduction

Historically, amphibians have been looked after closely due to the rapid extinction rate of its species. They are currently the most endangered group of vertebrates in the world. (Stuart et al., 2004). Many factors are influencing this global event, and the main contributor is the humans. Habitat fragmentation and global warming are the most pressing matters, caused by human activities. There are also natural factors affecting their abundance, e.g. infectious diseases like chytridiomycosis, that are facilitated by humans through commercial trade in amphibians (Picco & Collins, 2008). Focusing on the salamander group, habitat fragmentation and presence of new diseases are particularly pressing matters as of today. Even tough Salamanders are widely spread, their populations tend to be isolated, which makes them rather vulnerable before habitat changes and upcoming threats (Wake & Vredenburg, 2008).

The fire salamander (*Salamandra salamandra*) is the most well-known salamander of Europe, and one of the most common caudate species in this continent (Balogová & Uhrin, 2015). Its range extends all the way from North Africa via the Iberian Peninsula, across Central and Eastern Europe, all the way into the Near and Middle East (Seidel & Gerhardt, 2016). Furthermore, the range of elevation that the fire salamander can withstand is between 0 and 2500 MSL, allowing them to be present in the Pyrenees and the Alps. This is a very interesting feature for this species, and in such a large area it could be difficult to believe that the species is in a constant reduction. However, according to the latest assessment of the IUCN (2023), the populations of fire salamanders are decreasing, and their conservation status has deteriorated to vulnerable. The main causes for this status are human related activities, such as housing and industrial development, wood harvesting, and introduction of invasive species/diseases.

In the Czech Republic specifically, the fire salamander is widely spread. The central, east, and north areas are the ones with the most abundant fire salamander populations (IUCN, 2023) (Kulihová, 2016). Nevertheless, the fire salamander has suffered important declines across time, which has ultimately led it to be catalogued as a endangered species and it is currently protected by national legislation (Brejcha, 2018). This categorization and the creation of laws for the protection and conservation of the fire salamander goes to show the importance of this species in the Czech country and why it is important to study its ecology and their population dynamics, especially in urban areas with high anthropogenic pressure.

In recent years, the Prague's population has increased about 7% since its last census back in 2012 and the forecast is that Prague's population will continuously grow from now on (IPR, 2021). This increase is likely causing more destruction for the habitat, due to people needing new spaces for living and leisure. Fire salamander populations are threatened by these kinds of activities because they are extremely sensitive to changes in their habitat. That is why the main objective of this study is to estimate the abundance of the fire salamander population in Bohnice, Prague during 2023.

1.1. Objectives

As it was stated above, the main goal of the thesis is to estimate population abundance in Bohnice's fire salamander population. Previous studies record this as the largest population of fire salamander in Prague, so the past and current abundances of the fire salamander will also be compared, and it will be determined how the population is being affected by humans and whether there have been any population decreases or increases.

Some secondary aims were also set for this research. Firstly, the climatic data was collected during the field trips. This will determine not only the behaviour of the fire salamanders at specific temperatures, but more precisely understand the winter activity of this population. Secondly, the sizes of the male, female, sub-adult, and juvenile fire salamanders were also compared, in order to set standard sizes for the individuals in this population. Thirdly, the recapture probability was also calculated, in order to obtain a more precise population estimate.

2. Literature review

2.1. General description of the species

2.1.1. Taxonomy

The family Salamandridae, which consists of 21 genera and approximately 139 recognized species, is the second most diverse groups of extant salamanders. As of today, it is divided into three subfamilies: Pleurodelinae, Salamandrininae, and Salamandrinae (AmphibiaWeb, 2024). Salamandridae owes its name to a specific taxon of animals, namely the species under the *Salamandra* genus, an animal that most people are familiar with due to its worldwide recognition and study.

Genus *Salamandra* is large group of terrestrial salamanders and amphibians, it contains six species and approximately 25 subspecies (Burgon et al., 2021). They are a well-known group due to their distinctive colorations. On the one hand, some species are completely black coloured; on the other hand, others are most recognized for their vibrant, highly contrasted black and yellow – and sometimes even red – coloration (Preißler et al., 2019). By their common names, these salamanders are separated in two groups: the alpine salamanders and the fire salamanders, respectively (Lüddecke et al., 2018). The first group comprises the alpine salamander (*Salamandra atra*) and Lanza's alpine salamander (*S. lanzai*), while the other group includes the North African fire salamander (*S. algira*), Corsican fire salamander (*S. corsica*), Near Eastern fire salamander (*S. infraimmaculata*) and the fire salamander (*S. salamandra*) (Seidel & Gerhardt, 2016) (Figure 1). This last one is the species that is most abundant in Central-East and Eastern Europe and, therefore, it inhabits the Czech Republic.

Species of both groups, especially the common fire salamander, include a vast number of subspecies. As it can be inferred by their common names, many species inhabit very specific areas, making them exclusive to a determined land or endemic. To differentiate these unique species is important to determine the site where they were found, as well as focusing on the coloration. For example, there is a salamander species (*S. atra aurorae*) that is almost fully black coloured and that it is also endemic to the dei Seite community in Italy. Another example is an alpine salamander species (*S. atra* *pasubiensis*), which has some yellow elements and is exclusive Passubio massif in Italy too (Beukema et al., 2016) (Bonato & Steinfartz, 2005).

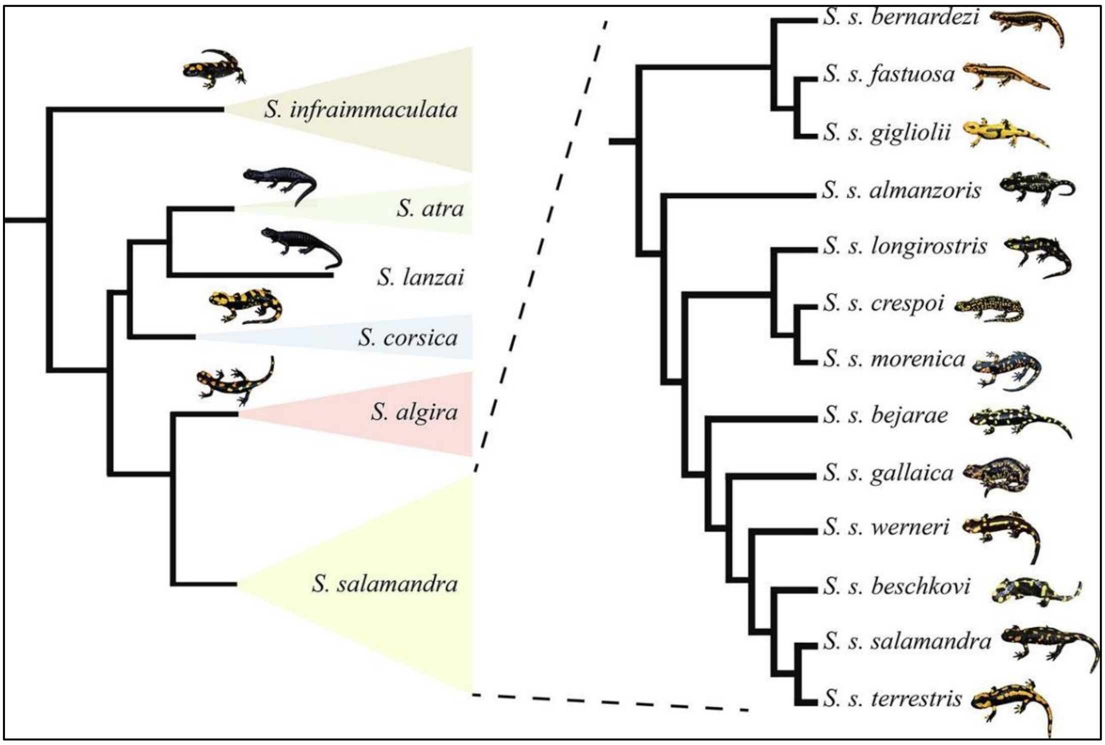

Figure 1. Phylogenetic tree of the Salamandra *genus, with special emphasis on the subspecies of the* Salamanadra salamandra *species* (Burgon et al., 2021).

2.1.2. Morphology

Focusing now on the fire salamander (*Salamandra salamandra*), their coloration is mostly black, yellow, red, and brown. Depending on the subspecies, the display of the colours is also different (Seidel & Gerhardt, 2016). To summarize, the fire salamander can be easily recognized because of its deep-black skin with vibrant different patterns across the back, limbs, and head. Added to these patterns or dots, yellow parotids on the head and two dorsal gland lines on both sides of the backbone can also be identified. It is important to point out that the pattern is generally genetically inherited (Sanchez et al., 2019).

This coloration is an aposematic signal of the fire salamander, indicating to the predator toxicity, noxiousness, or unpalatability. The venom is not supposed to kill larger predators, just traumatizing them, so they remember the consequences of attacking an aposematic prey (Lüddecke et al., 2018). This venom is excreted through the dorsal gland lines, and it consists of three neurotoxin components: Samandarin, Samandaridin and Samanderon. This kind of defence fits perfectly for such a small and vulnerable animal like *S. Salamandra* (Meikl et al., 2010).

However, before becoming this vibrantly coloured animals, larvae of the fire salamander have to go through metamorphosis. At first, larvae have a cryptic coloration, primarily grey, and their pigmentation is regulated by physiological colour changes to match the substrate (Himmer in Sanchez et al., 2019)

S. salamandra is currently Europe's largest salamander species, reaching a total of 20 cm of length (Burgstaller et al., 2021). There is data that supports that this animal can grow as much as 25 or 30 cm during his life. The body has a cylindrical shape, they possess a robust, long, and cylindrical tail, and their size and distribution of the dots or stripes depends on the individual (AmphibiaWeb, 2024; Diputación de Málaga, 2024).

2.1.3. Life cycle

The first step in the life of the fire salamander is the mating season of the adult individuals, during the nights of April, until the end of May (Steinfartz et al., 2006). However, in some populations in Central Europe, the mating season can last until early September (Seidel & Gerhardt, 2016). During the mating ritual and if the female individual responds positively, the male individual deposits a spermatophore – a jelly like substance with sperm on the top – that the female dips her rear body into. Then the sperm can stay in a sperm pocket for up to 2 years (Bayliss, 1939).

After three months, the larvae have developed enough to be released. The larvae are released into a shallow river, and the birthing event can take many days. At "birth", larvae are maximum 3 cm long and weight between 0.1–0.2 g. The first stage of the larval stage is related to many risks, e.g. displacement in their rivers, drying up of pools and predation. However, 40 to 120 days after, these larvae reach the metamorphosis phase, gills and fins are reduced and the yellow spots become visible. The once tiny larvae are now freshly metamorphosed salamanders, measuring 45 – 65 mm and reaching (in some cases) 2 g of weight (Seidel & Gerhardt, 2016).

However, the larvae size can also be influenced by different diets. The development of the head structure can give a better understating of this phenomenon. Through ontogeny, some species are more likely to compete for scarce resources, which models their behaviour to be more aggressive (Manenti et al., 2018). This gives a better development of the mandibular bone structure, and basically the body is afterwards built around the head structure (Bon et al., 2020).

After going through the whole process of metamorphosis and becoming paedomorphy terrestrial fire salamanders, they can live up to 20 years, based in records in central Europe (Burgstaller et al., 2021). Nevertheless, some records state that fire salamanders can reach 30 years of age, and there was even one individual, which currently holds the record for oldest fire salamander, that reached 50 years of age (Citizen Conservation, 2023).

2.1.4. Biology and ecology

Continuing with last section's topic, reproduction is a very distinctive feature of the fire salamander. Actually, its larval development is the most variable and adapted to local environmental conditions: it is the only species of tailed amphibians capable of giving birth to fully developed larvae (Seidel & Gerhardt, 2016). Normally, larvae can begin their life in a variety of habitats, like pools, streams, springs, and epigean and subterranean water bodies (Weitere et al., 2004) until they can undergo metamorphosis around 3- 4 months after being born (Cogliati et al., 2022).

Once the fire salamander larvae are growing up, competition for resources is up and running. Poor growth, delay in the metamorphosis (Warburg, 2009) and less activity (Manenti et al., 2013) are the consequences of a deficient diet in early stages of life. Ergo, as larvae, fire salamander tend to feast on minuscule invertebrates and crustaceans that are in the surface of small ponds or nearby them (Jefferson et al., 2014). Nevertheless, during this phase, many larvae can share the same water body without being related, which ends up in bite attempts between conspecific individuals (Berkowic & Markman, 2019). There is evidence that this aggression can escalate to full-on cannibalism between larvae. This practice may help the survival chances of some individuals in poor habitats during starvation periods (Manenti et al., 2018).

Once the fire salamanders get through the rough path of becoming a fully developed individual, they can start their live as predators. The fire salamander is a carnivorous species throughout their whole life cycle, which means that they are skilful predators (Seidel & Gerhardt, 2016). They prefer to eat larger insects near to the water bodies, or they can change completely to a terrestrial hunt and feast on larger invertebrates such earthworms, slugs, spiders, millipedes, and beetles (Sánchez-Hernández, 2020) (Thiesmeier, 2004).

Unfortunately, fire salamanders are still small and vulnerable animals, causing them to sometimes become the prey, and the presence of toxins in the salamanders' bodies is evidence of it. As mentioned earlier, the coloration of the fire salamander stands out due to the shiny yellow patterns that contrast with the black body. Even though it may not be recognized fully as aposematism in *Salamandra salamandra*, it surely serves to deter predators and these toxins could also have antimicrobial effects. (Lüddecke et al., 2018). The toxins are developed in the later stages of the life cycle of the fire salamanders, and that is why the risk of being predated is bigger in the early stages of life (larval or metamorphosis stages). However, predation is almost inexistent in adult fire salamanders (Ibáñez et al., 2014).

Furthermore, fire salamanders also have to protect themselves from changes in temperature, to which they are known to be quite sensible. During dry conditions their underground activity increases, because they are looking for lower temperature and a more humid environment. This is especially important for their latency period, which comprises from October to March (Balogová & Uhrin, 2014). This behaviour is mostly displayed to protect the fire salamanders from dying by freezing or desiccation (Wells, 2007).

Normally, winter dormancy is practiced in groups of tens of individuals in the same shelter. The reason why the salamanders are found in large groups in these dens is because of their fidelity to the site (Kováč et al., 2014) (Manenti, Ficetola, et al., 2009). They are most usually found during their overwintering under rock outputs, hollow trees, inside of clay deposit or gravel piles or guarded by vegetation (Caldwell in Balogová et al., 2017).

2.1.5. Habitat requirements.

Many amphibians are semi-aquatic and require different habitats occupied at different stages of their biological cycles (Manenti, Francesco Ficetola, et al., 2009). The fire salamander is no exception, in most subspecies search for a habitat which include damp upland forests, usually deciduous, and by clean water sources, such as stream or ponds needed for breeding. Also, habitats that have leaves litter are specially an important factor for the salamanders because they provide a hideout for this species (Gorman, 2008). Nevertheless, these hideouts are not useful only as protection are they are not always made out of leaf litter either. The fire salamanders also need fitting habitats for their overwintering season. Winter dormancy is a behavioural response to changing seasons and withdraws the salamanders from adverse low temperatures and probable death. The habitats usually have rock outcrops, hollow trees, cavities in clay deposits and gravel piles; natural hideouts where salamanders can spend the winter and survive for months (Balogová et al., 2017) (Appendix 2, 3 & 4).

Other than a hideout, the habitat also requires certain characteristics for the deposit and development of the larvae. As mentioned earlier, the female individuals deposit the larvae in first-order streams, mainly in small pools or ponds. This mode of reproduction -stream reproductive mode- is one of the most beneficial for the survival of larvae. The drift of these bodies of water is used as a strategy to regulate population densities, because if certain bodies of water became overcrowded the larval mortality would be higher (Reinhardt et al., 2013).

Following the results of Manenti's research (Manenti, Ficetola, et al., 2009) stream morphology is also an important factor to consider in the habitat of the fire salamander. The importance relays on how it explains the distribution of said species. In this research, the salamanders were associated to shallow streams and with high heterogeneity. This reflects how, in natural watercourses, there is a complex network of riffles, pools and seeps, mainly due to geomorphological causes. Altogether, they represent a measure of quality of the watercourse and diverse freshwater communities. Heterogeneous streams with presence of different elements are extremely important for the larval development of the salamanders. For once, it offers shelter and prey items to younger individuals, while more mature individuals can take advantage and exploit areas with slightly faster current velocity.

All this previous information is important, because it relates to a very specific phenomenon observed in many *S. salamandra* populations around Europe: site-fidelity (Schulte et al., 2007). It is related mostly to terrestrial salamanders with an aquatic stage in the life cycle, and the fire salamander has been a textbook example of site-fidelity. The study performed by Schulte showed how mostly male salamanders have a strong site-fidelity, even more than double as females do. However, it also suggests that these animals have a great tendency to disperse, staying always inside the home range, but only returning to the site mostly during mating season.

Now that the site-fidelity is mentioned, it brings out the question on what the ideal site for the fire salamander looks like. Across these last paragraphs, the water-dependant lifestyle has been covered thoroughly, but very few has been said about the flora. Normally, in old forests with healthy salamander populations, the European beech (*Fagus sylvestris*) and the sessile oak (*Quercus petraea*) are the predominant trees. The presence of these plants, alongside other species of the same genus, represent the ideal terrestrial habitat for the fire salamander. Furthermore, the soil of said forest has to be stagnosolic (poorly drained), which allows the formation of various water bodies alongside these plant species (Reinhardt et al., 2013).

2.2. Distribution across Europe and in the Czech Republic

The fire salamander is distributed across the whole European continent, excluding northern-Europe. It can be found all the way from the Iberian Peninsula, Portugal, Spain, France, Belgium, and Germany, across the Czech Republic and Slovakia, Italy, and Hungary, even until Greece in Balkan Peninsula and Ukraine (Rees, 2019). In Poland, the fire salamander populations can be found in the south Sudetes and Carpathians Mountains (Najbar et al., 2020). So far it has not been found Great Britain, Ireland, the Scandinavian countries, Belarus, or Russia. Some fire salamander subspecies are known for being related to the Alps, and that is because they are widely distributed at altitudes between 200 and 700 m and found at up to 1300 m in some spaces of Austria and Switzerland (Meikl et al., 2010).

Specifically in the Czech Republic, there has been records of sightings of fire salamanders since 1898 (Pražák in Brejcha, 2018) all the way to 2024. Across time, this species has occurred in most parts of the country, especially in the North, East, West and Central Czech Republic. The southern-central section of the country has data for only inaccurate encounters with the fire salamander. They also appear to be isolated populations, which could drive these groups of salamanders to extinction (Kulihová, 2016) (Figure 2).

As for Prague, its presence has been more noticeable in recent years, especially in Prague 7 and 8, in the Podhoří Natural Reserve area and along the river at the northern and southern part of the Czech capital city (Schreib, 2017). Recently, a study has confirmed new locations for the fire salamander in Prague (Figure 3), however Suchdol and Podhoří / Černá rokle are still the locations with the most abundant fire salamander populations: 160 & 1050 individuals respectively (Vojar et al., 2020).

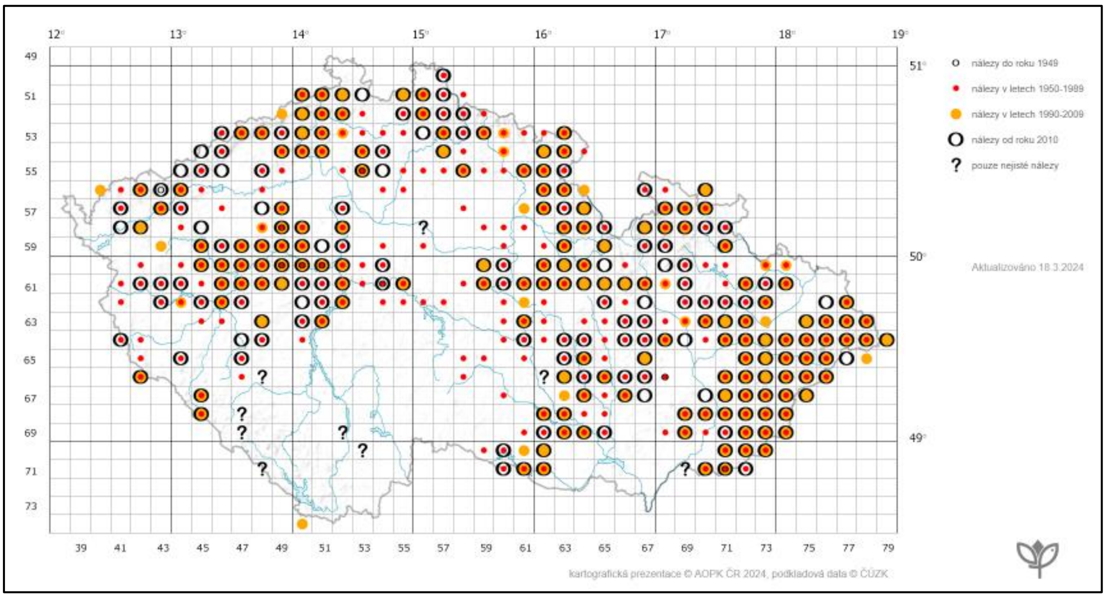

Figure 2. Fire salamander distribution map in the Czech Republic as of March 2024.
Small black circles represent encounters in 1949, red circles represent encounters between 1950 – 1989, orange circles represent encounters between 1990 – 2009 and the large black circle represent the encounters since 2010. The "?" represent inaccurate encounters (© AOPK ČR 2024).

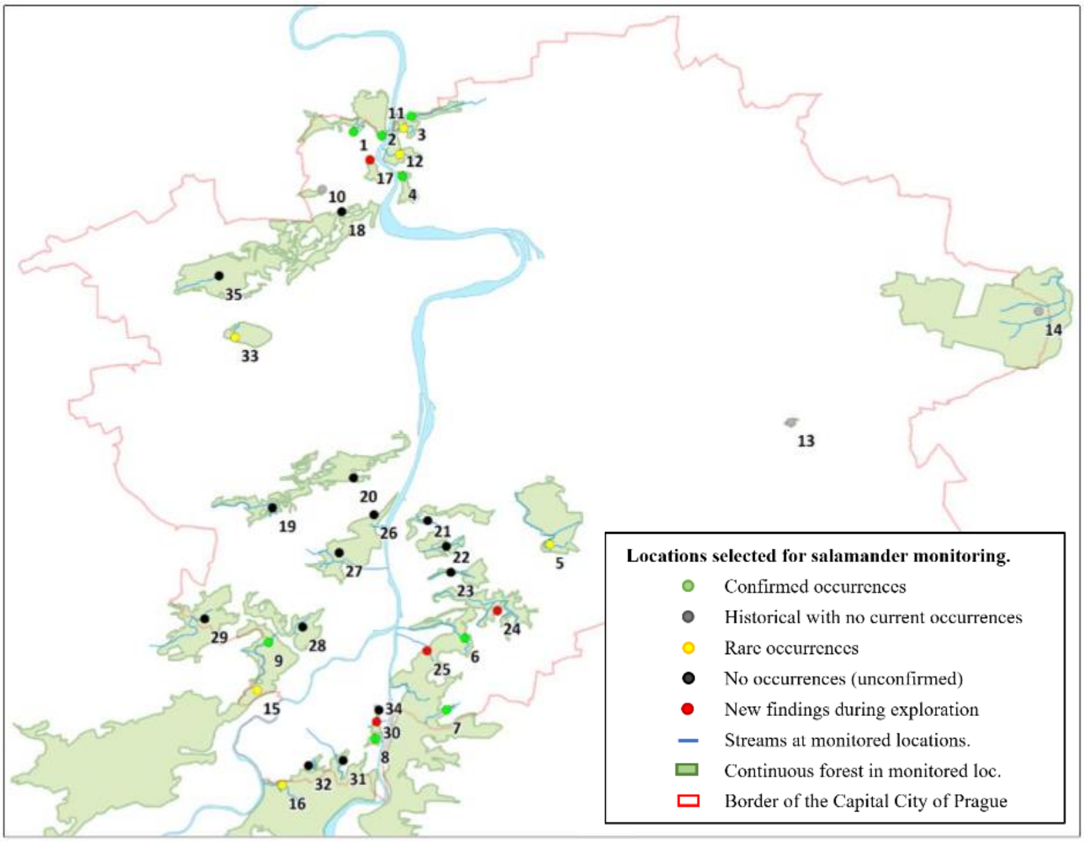

Figure 3. Designated localities for the fire salamander monitoring in Prague (Vojar et al., 2020).

2.3. Threats and conservation

2.3.1. Conservation status

Amphibians have historically survived mass extinctions events, but today's history is very different. The rate at which amphibians are disappearing is an unprecedent event in the history of this group of animals (Catenazzi, 2015). As of 2008 (Stuart et al., 2008), a third of known amphibian species were classified as endangered with extinction by the IUCN. It can only be expected that 15 years after this census, the number has increased.

The status of the fire salamander has been assessed periodically by the IUCN Red List. For almost 20 years, this species has been classified under the "Least Concern" category, but the most recent assessment categorized it under the "Vulnerable" category, with a decreasing population of mature individuals and a continuing decline in area, extent, and quality of the area (IUCN, 2023). In the Czech Republic, the fire salamander status is also very worrying. According to the Czech legislation, the Legal

Protection Category classifies it as a highly endangered species, whereas the Czech Republic Red List considers this species to be vulnerable (AOPK ČR, 2024).

This salamander is usually referred to as a common species, but in plenty of areas the decline is quite severe and they are becoming rare, not only because of its reduced population but also because of the habitat loss (Manenti, Ficetola, et al., 2009). Habitat loss is a topic well related to the fire salamander because it plays a very important role in their ecosystem. As well as other amphibians, they are considered environmental bioindicators, due to their sensitivity to changes in the habitat or presence of environmental contaminants (Arens, 2023). The presence, disappearance, or absence of this animal across time is also an indication of a deteriorated environment.

2.3.2. Causes of the threats

One of the main causes for the decline of biodiversity in general all around the world is the negative effects of human populations on animal species and their habitats. A number of factors contribute to the extinction of amphibians, such as habitat loss, exotic predators, infectious diseases, and road kills (Sinai et al., 2020).

A recent growing issue is the injuring and killing of salamanders due to traffic and roads construction. These factors can not only affect the individuals directly, but also the habitat they inhabit. The building of roads isolates populations and creates vulnerable subpopulations deemed to disappear. It also deteriorates the quality of the habitat, by contaminating and making some food sources or breeding sites inaccessible. It is also known that salamanders, mainly male, are very active on the roads, they cross them relatively slow because they stop for conspicuous posture display. Furthermore, male individuals then to be on or close to roads in search of female individuals, but the only thing they are able to find is deadly traffic (Sinai et al., 2020).

Due to land use change, the habitat loss and degradation has produced reduction in population size. Similarly to the creation of roads, smaller populations become more vulnerable and prone to extinction because of genetic drift, inbreeding and falling into the extinction vortex. This isolation prevents any sort of genetic exchange between subpopulation, which also make them vulnerable to upcoming diseases (which will be covered in the next section). Many conservation and restoration projects are put into action to try and restore the health of the environment, but it is, for the most part, unviable (Bani et al., 2015).

After going through the human caused threats, it is important to address one of the most recent and worrying matter in our hands. Approximately a decade ago the fungi *Batrachochytrium salamandrivorans* (Bsal) was first identified by scientists, which is the driver for the chytridiomycosis disease. Ever since then, it has spread all over Europe affecting populations of salamanders and newts, interestingly enough in both wild and kept individuals (More et al., 2018). This fungus has shown to have great impact on the fire salamander's populations, and the most impressive example of the impact of this outbreak can be observed in the Netherlands, where in the span of 7 years a population of *S. salamandra* decreased by 99.9% (Martel et al., 2014; Spitzen-van der Sluijs et al., 2016).

Since 2010, dead fire salamanders have been spotted across the Netherlands. In this country two major infectious drivers have been historically present: *Batrachochytrium dendrobatidis* and ranavirus; but none showed to affect the populations severely and there was no need to take strong action against them. However, a phenomenon was observed, a steep and sudden decline in the fire salamander populations. Even though the populations of the fire salamander in the Netherlands were relatively small, they were not considered at risk, but this unexpected and quick decline has deemed the dutch fire salamander populations to extinction (Spitzen-Van Der Sluijs et al., 2013).

In addition, the same group of researchers tried to expand their area of work to get more information regarding the presence and movement of this fungus across Europe. 55 sites in three countries were surveyed, and 14 of them presented Bsal infected amphibians, and not only fire salamanders, but also a couple of newts species (Spitzen-van der Sluijs et al., 2016).

Even though the situation in Netherlands and Germany are critical, the bohemian country has had a fight of its own against the presence of this fungus, ever since Bsal was confirmed in Germany (Sabino-Pinto et al., 2015). The Czech Republic has a relatively high Caudata species diversity, therefore the presence of such a deadly illness is worrying for the local populations (Sillero et al., 2014).

The first research to confirm the presence of *Bsal* in Czech Republic gave the fire salamander populations a little rest, as no caught individual yielded positive results for the presence of *Bsal*. Nevertheless, the authors highlighted the importance to avoid the entry of this pathogen to the wild amphibian populations, due to the lack of methods to reduce the impact of a new infectious disease. Also, some actions were suggested to reinforce the prevention of the *Bsal* presence (Baláž et al., 2018).

The accelerated pace at which this fungus *Bsal* was dispersing across Europe seemed to slow down when entering the Czech Republic. A recent study tried to use environmental DNA to detect the presence of *Bsal* and *Bd* (*Batrachochytrium dendrobatidis*) in amphibian populations in the Czech Republic and Spain. This innovative research had as a main goal to identify any occurrence of both fungi in caught individuals of both countries. The horizon in Spain is not as nice as expected, because it showed that the fire salamanders in this location host both pathogens. It was also the first detection of both chytrid fungi in a single location by using eDNA. On the other hand, no *Bsal* was present in any of the assessed salamanders for the Czech Republic. Even though some traces of the fungus *Bd* were found, these results also showed to be optimistic about the health status of the fire salamander population (Lastra et al., 2021).

Bsal is not the only fungi to worry about, though. Chytridiomycosis is a lethal fungal disease caused by *Batrachochytrium dendrobatidis* (Bd), which is causing massive amphibian population declines and extinctions worldwide (Cevallos et al., 2022). Ever since the fungi arrived in Europe 24 years ago (Mutschmann et al., 2000) hundreds of amphibian species have seen a decline in their number and now chytridiomycosis is viewed as the most devastating disease impacting vertebrate biodiversity (Berger et al., 2016). Even though the chytridiomycosis by *Bsal* is showing to be more severre than the one from *Bd*, both have shown their impact and devastation they can create alongside Europe, like they did in Belgium, Germany, and the Netherlands (Lastra et al., 2019).

2.4. *Methods for field work and abundance estimates*

When studying any species, including salamanders, the most important is to monitor the size of its populations and the trends. Normally, it is easier to study the abundance of fire salamander in larval stages in comparison to adult salamander (Wagner et al., 2020), hence many of the abundance estimate focus especially in the younger individuals. This does not mean that the same methods can be used for the study of adult individuals.

The most common method for estimating the abundance of any species is the *in situ* simple count methods, or visual encounter surveys (VES). This methodology can give information about species density (expressed in number of individuals), species composition and an approximate size structure (Freon, 1996). When working with herpetofauna, it has been shown that VES is more effective for identifying species and individuals in comparison to quadrants, for example (Doan, 2003).

Nighttime VES are relatively effective for salamanders, if the climatic conditions favour their activity (Grover, 2006). Therefore, when working with the fire salamanders, researchers have to wait until nighttime and pay close attention to the humidity of the area, because most fire salamander prefer to come out of their hideouts during or after a rainy period. Since adult fire salamanders have exclusive yellow patterns on their back, this can be used in favour of the researchers and use it to measure how many times the same individuals were caught and how many individuals there actually are in the area (Wagner et al., 2020).

The capability to identify different individuals in a population is fundamental for the Capture-Mark-Recapture method, which provides basic information regarding population ecology (Šukalo et al., 2013). Many methods are implemented to recognize individuals over time, such as toe clipping or tattoos, which are quite intrusive approaches and can affect locomotion or survival of the individual negatively (Le Galliard et al., 2011). Furthermore, it has been shown that this technique is not as efficient as other CMR methods. For example, it was recorded that when comparing toe-clipped animals and fluorescent-maked animals, the animals with the fluorescent mark had a 20% higher recapture rate (Davis & Ovaska, 2001).

As a result, photo-identification is the most used non-invasive method in population studies of amphibians nowadays, and it has been utilised to identify salamanders in relatively small databases (Gamble et al., 2008). Although this method means no harm to the species, in identification of salamanders it has only been reliable for short-time studies due to changes in the patterns of spots overtime. Also, it has been stated that this method suggests a lot of time only for the analysis of the photographs (Šukalo et al., 2013).

3. Methods

3.1. Study area.

The field work was conducted in the site Black Valley (Černa rokle), inside of the Natural Reserve Podhoří (Přírodní Rezervace Podhoří), 50.129852, 14.404544. This site is located in the right bank of the Vltava River, in Bohnice, Prague 8, Czech Republic, and the approximate area of the study site is 3.4 ha (Figure 1). In the middle of it runs a stream that is 325 metres long.

This area hosts many species of flora, such as *Quercus* sp., *Acer* sp., some herb species and even protected plant species, such as *Auriana saxatilis*, *Festuca pallens* and *Allium senescens*. This diversity in species composition is thanks to the presence of shale that dates back to the Upper Proterozoic period, and diorite porphyritic rocks all along the valley. Its location is also advantageous for being next to the Vltava riverbed and having a tributary stream and several pools inside of the Black Valley. The actual aim of protection of the area is the preservation of the geomorphic groupings, thermophilus plant communities and the rocky steppes. The protection of these biotic and abiotic factors ultimately helps the conservation of important plant species, thermophilic insects, birds, and the largest population of *Salamandra salamandra* in Prague (Pražská příroda, 2019).

Figure 4. Map of the field study site: Black Valley in the Natural Reserve Podhoří (own elaboration).

3.2. *Field work.*

The field work was conducted from January 2023 until November 2023, with a special emphasis in the autumn season from September to November. This period of time is characterized by a drop in temperature and more frequent precipitation, which are perfect drivers for the activity of fire salamanders. Also, as mentioned before, some of the field trips matched the range of mating season of the fire salamander, as well as their birthing events period.

Normally, the study site was visited one hour after the sunset, to have more chances to see salamanders. The visits lasted from around one hour up to two hours and a half. A total of 18 visits were performed, during which other factors such as the temperature, the rain intensity, the wind strength, and the starting time of the visit were recorded (Appendix 1). It is important to point out, that an external researcher shared 4 datasets of visits that were added to the project's CMR, giving out a total of 22 visits. The summarized information of the visits can be found in the Appendix section (Appendix 2).

The methodology chosen for the finding of salamanders was through visual surveys in night walks after or during a period of rain. It is known that salamanders are mostly active during the night and rain makes them leave their hideouts, which facilitates the detection and collection of data. Visual surveys were chosen because they are a very profitable method for estimating and monitoring wildlife abundances; it offers an inexpensive and unintrusive approach to population surveys (Skalski et al., 2005). The transects for searching fire salamanders along the stream were approximately 200 – 250 metres long. To avoid any damage to the individuals, only the individuals that were on the main path were captured. No search was done in rocky slopes and no rocks were moved to avoid crushing the salamanders.

Every single captured salamander was measured using graph paper, photographed using a mobile phone camera and identified as Adult, Male, Female, Sub-adult, Juvenile or Larvae. When photographing, the salamanders were assigned a number according to the order in which they were captured and the initial of the sex or stage, e.g. 21M was the 21st individual captured and was male. In order to differentiate male and female fire salamanders, the cloacal region was observed. If it looked like as if it was inflated and round, then the individual was a male. On the other hand, if it looked flat and straight, it was a female (Seidel & Gerhardt, 2016). Based on experience, subadults were identified as such because they had a total length smaller than 15 cm and no clear sex signs. Lastly, juveniles were relatively simple to identify; they are the smallest individuals (usually < 10 cm). Individuals identified as Larvae could not be measured. All individuals observed during these visits were photographed using a mobile phone camera. Meanwhile, their location was also being recorded using a GARMIN MapGPS 64s.

Figures 5, 6 & 7. GPS Garmin MapGPS 64s used for the marking of the location of the individuals next to a a female S. Salamandra. Figures 3 & 4 show the same individual (B024) captured in different visits, almost 8 months apart.

3.3. Population estimate based on the VES.

The pictures of the fire salamanders where firstly organized according to the date of capture and then individually compared in between each other, analysing the pattern of the dots or stains along the salamander. Firstly, the pattern of the head, specifically the parotid gland and the dot found sometimes behind the eye. Secondly, the pattern of the back, starting from the upper back going all the way into the lower back. Finally, the pattern of the dots on the tail where also analysed to determine whether it was a new individual or not. If the pattern was unique, the individual was given an identification name using the location and the number B001 (“B” stands for Bohnice and “001” for the first individual in the list), but if it was a recapture, it was marked as such in the CMR list. The total number of individuals caught via VES was obtained after counting all the unique individuals identified in the pictures’ comparison. The patterns in the dorsal part of the individuals serve as their unique ID, therefore the recapture percentage could also be calculated, along with the female to male ratio of the population.

Larvae were not captured for analysing the patterns on their bodies, but they were counted *in situ*. The estimated number of this individuals are also described in the next chapter.

Because this method is non-invasive, the fire salamanders were not marked on the field. The capture and identification method of individuals has shown to be helpful for achieving a more precise capture-recapture index result, which ultimately provides a better population estimation (Joseph & Knapp, 2021). Therefore, the number of individuals obtained through the VES should not be overlooked.

3.4. Population estimate with Statistical Analysis

For analysing the population size, the probability of survival, the probability of new individuals entering the population and the probability of detection, the POPAN model was used, which can be utilized in the software R by using the RMARK package.

The dataset is composed of 22 tables of information of the salamander searches in the Black Valley. The Jolly – Sebber model was used in order to obtain the population estimate. This estimation was obtained through the model: {Phi(~time)p(~time)pent(~time)N(~1)}. It is adjusted so that the probabilities of survival (Phi), of detection (p), of a new individual entering the population through birth or migration (pent) and total number of the superpopulation are dependent on the time in between visits. Otherwise, they would be treated as constants, and the estimation would be different, but not very accurate.

For identifying if the population of the Black Valley follows a 50:50 ratio between male and female individuals, the Chi-square goodness of fit test was also performed. This test confirms if the data obtained follows the expected probabilities or not.

4. Results

4.1. Abundance estimation based on Jolly– Seber model.

In the Natural Reserve Podhoří, a total of 393 salamanders, 271 after-metamorphosis individuals and 122 larvae were observed across 18 own and 4 field trips. After comparing the dotted and stained patterns of the fully developed salamanders, 191 unique individuals could be quantified, divided like this: 77 males, 53 females, 41 sub-adults, 19 juveniles and one dead adult individual. Due to the presence of this deceased individual, we can say that the population of fire salamanders for the Black Valley is at least 190 living individuals.

When working with the Jolly – Seber model, it can be said that the population estimate for the Black Valley fire salamander population stands at 582 individuals (Figure 8). With a 95% confidence interval, the lower class limit is set at 414 individuals and the upper class limit is set at 819 individuals. The complete range of population estimates can be found in the Appendix section (Appendix 6).

When comparing the data obtained to 2020's research (Vojar et al., 2020), the number of the population estimates are totally different. The previous research reported a population estimate of 1050 fire salamanders for the Black Valley site. This entails a 45% decrease in the population estimate of *S. salamandra* in the most abundant wild population of this amphibian in Prague.

The months that registered the highest salamander counts were October, with 101 encounters in total and November, with 92 encounters in total. Figure 9 represents the occurrence of fire salamanders in Black Valley during the time of the study. The highest recorded temperature for this study is 17 °C and the lowest temperature was 5°C.

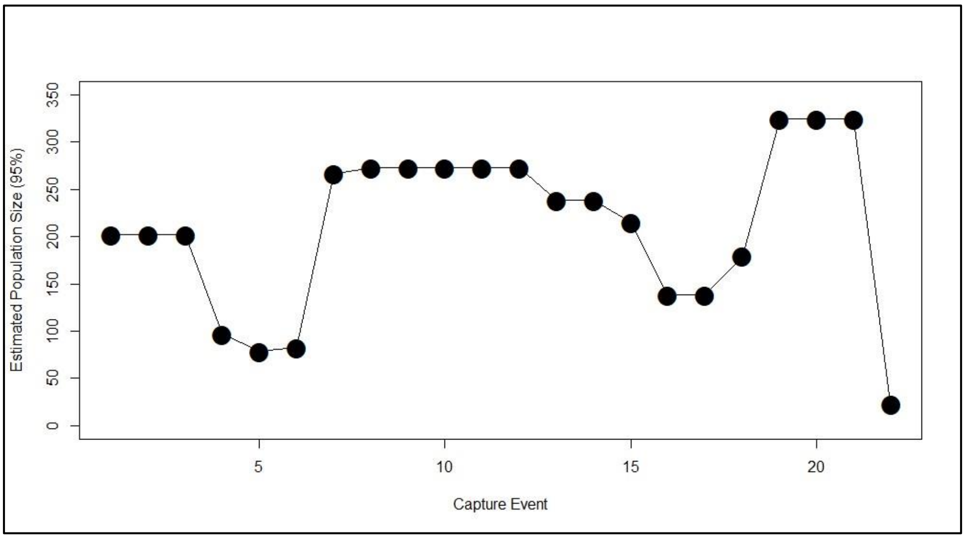

Figure 8. Population estimate from the open-population Jolly-Seber model, with a 95% confidence interval.

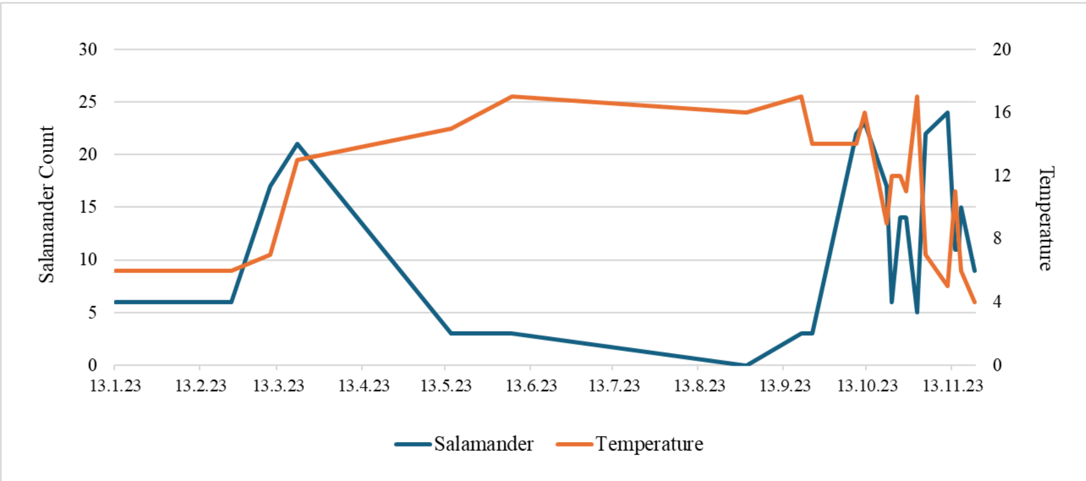

Figure 9. Graphical representation of the abundance of fire salamanders in the Natural Reserve Podhoří, from January to November 2023, with the temperature information (own ellaboration).

4.2. Recapture of individuals

With the CMR methodology, it was possible to compare the 190 caught fire salamanders' pictures and obtain a number of unique individuals. As mentioned before, the yellow patterns are their unique ID, so the number of recaptures could also be calculated and the percentage of recapture too.

For the 2023 population estimate of the Black Valley, the percentage of recapture was of 22.631%. This percentage was obtained by doing a simple Rule of Three, comparing the total number of unique individuals with the number of recaptured individuals.

In comparison to the 2015-2018 research period, it can be seen that both total capture and recapture numbers increase, and therefore the percentage of recapture is also larger (28.428%).

Period	***Visits***	***Total captures***	***Total recaptures***	***% recapture***
2023	22	190	43	22.631
2015 - 2018	12	299	85	28.428

Table 1. Table of information of the recapture incidences recorded for the Black Valley fire salamander during the 2015 – 2018 and 2023 period.

Individuals B053 & B061 (males) (Figure 10a & 10b) were recaptured the most, both of them appeared on six of the 22 datasets. B033, B107 (males) and B039 (female) (Figure 10c, 10d & 10e) follow as the second most frequent individuals, appearing on four of the 22 datasets each.

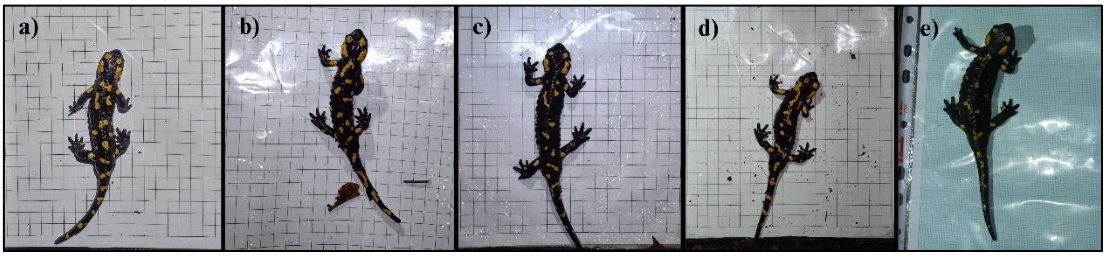

Figure 10. Most recaptured individuals during the research period. Individuals B053 (a) & B061 (b) were recaptured six times. Individuals B033 (c) & B107 (d) and B039 (e) were recaptured 4 times.

With a total of 77 male individuals caught, it was the most prevalent sex in the Black Valley fire salamander population, in contrast to 53 females. However, in order to confirm this prevalence, the chi-squared goodness of fit test was performed. The null hypothesis stated that there would be no difference between male and female proportion (50:50 ratio), whereas the alternative hypothesis stated that there is a significant difference between the observed and expected values.

With a p-value of 0.03 ($p < 0.05$) the null hypothesis was rejected, meaning that the population of fire salamanders in the Black Valley do not have an equal proportion in between both sexes. The results of the chi-squared are described below (Figure 11).

```
        Chi-squared test for given probabilities

data:  fs
X-squared = 4.4308, df = 1, p-value = 0.0353
```

Figure 11. Result of the chi-squared goodness of fit test. X-squared is the value of the chi-square test statistic, df stands for degrees of freedom (1 in this case) and the p-value is the significance level alpha = 0.05

4.3. Size comparison.

15 randomly chosen individuals of each sex / stage were chosen to be measured using the graph paper. The SVL was not chosen, because the salamanders were photographed in a prostrated position, so the cloaca was not visible, and the calculation would not be as precise as the size including the tail. In average, the females are the largest individuals, with a mean size of 18.4 cm, surpassing the mean male length by half a centimetre (17.9 cm), the sub-adults have a small range of size difference, and most are very similar in size; their mean size is 13 cm. Lastly, juveniles are the smallest individuals caught, with a mean length of 8.6 cm. The complete dataset for the results in Table 1 can be found in the Appendix section (Appendix 7).

Given that the mean body length of the male and female individuals is so close to each other, based on the lengths of 15 randomly chosen individuals, a T-test was also performed, in order to see if there were any significant difference in between mean body lengths of female and male fire salamanders.

In order to do so, first all the assumptions were covered, both datasets follow a normal distribution, both datasets have a similar variance, and they are independent from each other. All these calculations made in R-Software are in the Appendix section (Appendix 8). Taking all of this into consideration, it was concluded that the mean body lengths differences between male and female fire salamanders for the Black Valley population are not significantly different (Figure 11).

Sex / Stage	*Mean length (cm)*	*SE*	*UCL*	*LCL*
Male	17.9	0.4662	20	16
Female	18.4	0.3297	20.5	16
Sub-adult	13.0	0.4662	14.5	12
Juvenile	8.6	0.4662	11	6

Table 2. Mean body length measurements of the different sexes and stages of the captured fire salamanders, including the standard error, upper class limit, and lower class limit. SE stands for Standard Error, UCL stands for Upper Class Limit and LCL stands for Lower class Limit.

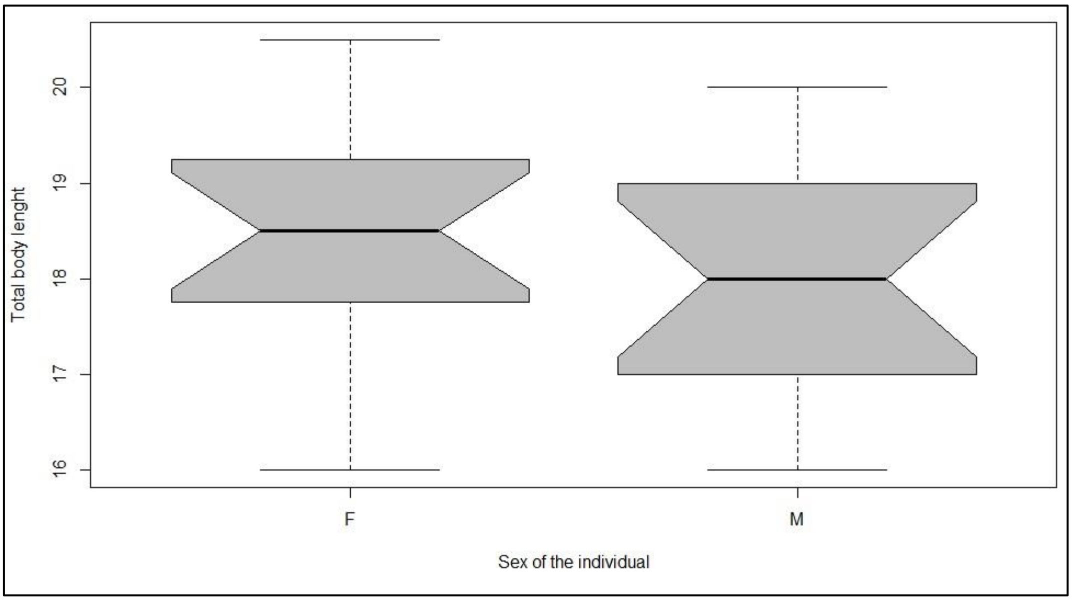

Figure 12. Boxplots of the body lengths of 15 female and 15 male fire salamander individuals with a 95% confidence interval of the median.

5. Discussion

5.1. Past and recent population abundance

As it has been stated, the population estimate for the Black Valley is as today of 582 individuals, which is 468 individuals less than the study conducted back in 2020. During the years 2015 to 2018 only 12 visits were performed to the site, which could be a possible answer as in why the population estimate is higher in previous year. The main reason would be that the confidence interval is quite extensive, the lower class and upper class limits are too far away from each other, so the estimate is also a high number. Since this year the number of visits was 22, the confidence level for this year's study is of 95%, which gives a more precise range of fire salamanders that can be found in comparison to the past study.

Nevertheless, this difference in population estimate should not be overlooked, it is almost a 45% decline in the wild *S. salamandra* population. The reasons for this decline could be explained mostly by human factors. The Black Valley study site is no further than 200 metres from urbanization, and lately new constructions have been made in the area, which involves damage to the environment and the natural habitat of the fire salamander. Also, the study site is connected to a pathway for cyclist, runners, or walkers, so it is customary for people to visit this place. In the case of cyclist, they practice downhill racing or mountain biking, which could potentially harm the salamanders that are crossing the path, or they can cycle over rocky terrain and kill some individuals. As for the runners and visitors, normally they take their dogs to the pools or ponds, that are very important for the breeding of the fire salamander. This can cause the waterbody to degrade, as well as killing a lot of larvae, younger and adult individuals that are nearby the water source.

Now focusing on the CMR results, the recapture percentage showed to be lower in comparison to the last fire salamander population estimate. Based on the results of the CMR, the recapture probability is of 22.63%, which, if we compare to the previous study result (28.43%), it has stayed almost the same. This 6% difference suggests that there is a higher probability to find unique individuals when the total VES repetition is higher.

5.2. Comparison with similar studies

The studies of population estimate of fire salamanders, and animals in general, are important to understand their ecology and the effects of different threats to the individuals. Like in this thesis, Oviedo has an isolated population of *Salamandra salamandra* due to habitat fragmentation and they performed a population estimate using the MARK Software too. After 4 years of data gathering, they obtained a population estimation consisting of 113 fire salamanders (95% CI 100 - 142), and a recapture percentage of 70.4 %. Interestingly enough, the population is not as genetically poor as it might seems, because the fire salamanders have developed genetic compensation techniques to battle genetic depression (Álvarez et al., 2015).

This suggests good news for the fire salamander population in the Black Valley. Both studies focus on a population that is trapped due to anthropocentric causes, being the case in Spain relatively worse than in the Czech Republic. However, in a population estimation of 113 fire salamanders, these animals were already developing genetic compensation techniques in order to prevent the genetic depression. It is known that a population with less genetic diversity is very vulnerable against upcoming diseases, such as chytridiomycosis; so, finding evidence that such a behavioural response is possible, it is safe to say that the population in the Black Valley, with a higher population estimate, can withstand and battle this genetic depression.

Following the results of a 2018 research by Spitzen – van der Slujs, it coincidently determines the abundance estimation of the fire salamander with the Jolly - Seber model too. The results are practically the same as they are in the site of Prague 8: richest encounters in the later months of the year (September - November), whereas the months of late Winter and early Spring also show the lowest presence of *S. salamandra* (Spitzen - van der Sluijs et al., 2018) (Figure 13).

For the Black Valley population, the pattern that is set by the graph generated by the VES results and confirmed by the Jolly – Seber model shows dependency on climate, a shared feature with Spitzen – van der Slujs' work. Even though the model can differentiate capture events, it is important to join methodologies in order to obtain more accurate information about the conditions during the encounter events in which the fire salamanders were found.

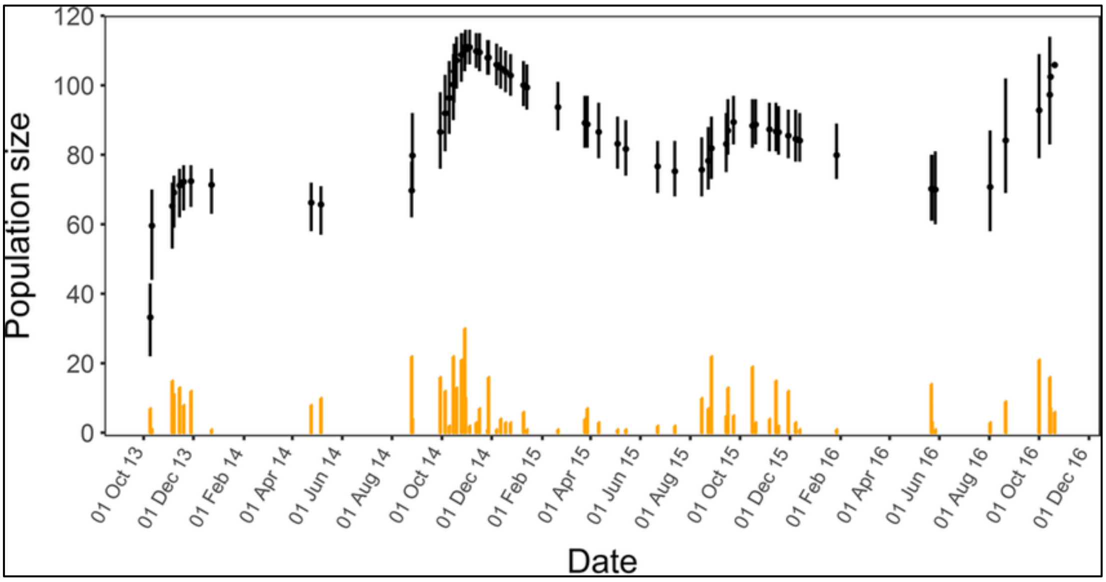

Figure 13. Population size of Fire salamanders at the new site, estimated from open-population Jolly-Seber model (Spitzen - van der Sluijs et al., 2018).

Talking about the conditions during the survey, weather conditions are probably the most important to take into consideration, since the operating and active range of temperatures of salamanders runs from 2°C up to 27°C (Stebbins & Cohen, 2021). However, in the case of fire salamander, the range is even more constrained. A 26 yearlong study revealed that this species is behaviourally active at 1 °C, but the activity suddenly decreases as the temperature rises to 16 °C; these animals become aestivate (Catenazzi, 2016).

Comparing these findings with the results obtained, it is clear to see how the decrease in temperature directly affects the fire salamander count. It can be seen that, as soon as the temperature goes under 16 °C, the population becomes more active, and the amount of fire salamanders exponentially grows. It is also important to note that the highest record of fire salamanders coincides with the second lowest temperature of the research period.

5.3. Potential causes of population decline

The fire salamander in the Black Valley faces enough natural threats as it is, such as chytridiomycosis by *Bsal*. However, there are several human risks that contribute to the decrease and harm to the population.. Due to habitat loss and fragmentation, urbanization has affected on the number of amphibians, and specially salamanders (Riley et al., 2005). In North Carolina, United States, for example, the change from in the land from forest to urbanization caused a decline of 44% in the southern two-lined salamander population. Problems associated with water velocity and loss of the forest were associated with a decrease in survival, primarily in larvae, and colonization probabilities. In these studies, it was also found that exotic species were introduced, making the rates of survival even lower (Price et al., 2006, 2011; Riley et al., 2005).

Following the idea of the last paragraph, it is important to report the presence of certain exotic species. Firstly, five feral cats were observed during the field trips, lurking around the water bodies, or moving outside of the Natural Park. Secondly, several rats were observed running around the rocks that were almost flooded; an area where salamanders were found more than once.

To begin with, the way in which the feral cats influence wildlife is through predation. Several researchers have focused on bird populations when addressing the feral cats as invasive species. Some studies in birds' population in Canada has led to the cats being described as the "largest human-related source of bird mortality in Canada" (Blancher, 2013). Moreover, a study in United Kingdom recorded what cats brought home after "playing outside". The list included small mammals, birds, insects, reptiles, and amphibians (Woods et al., 2003). That being said, feral cats not only damage the fauna itself, but also complete populations of species, and therefore, complete ecosystems. It is believed that diseases transmitted by feral cats, competition, disturbances, and predation can lead species to extinction; but more research is needed to completely understand the magnitude of feral cats in the field (Trouwborst et al., 2020).

As for the fire salamander specifically, only one case related to predation by domestic animals has been reported, and it was surprisingly not by a cat. Due to the playful and aggressive nature of some hunting dogs, many of them tend to play with salamanders. A recent record about the intoxication of a dog by alkaloids of the fire salamander shows how the introduction into nature of domesticated animals can be harmful for this species. Also, according to the authors, cats usually avoid contact with the fire salamander, due to a specific smell that the cats avoid (Erjavec et al., 2017; Habermehl, 1995). It should still be studied whether feral cats have developed a mechanism to ignore that smell that house cats can scent and prevent. Whereas not a single dog was seen during the field trips, it does not rule out the possibility that they can also affect individuals or entire populations of fire salamanders.

Dogs and - not so much - cats have always been described as perfects companions for the household; but there is another animal that is in every household, but most people want out of their house; and so do fire salamanders: rats. As mentioned before, several rats could be observed in the study site, sharing space near the stream that the fire salamanders frequented. The presence of this particular animal does not mean anything good for the fire salamander, because, despite their small bodies, rats can become efficient predators.

When discussing about the dog and cat as predators, this is highly improbable because of their nocturnal habits and their effective chemical defence mechanism (Cupp, 1994). Nevertheless, this anti-predation techniques showed ineffective in Spain, where an individual of *Salamandra infraimmaculata* was reportedly eaten by rats. This animal is an opportunistic omnivore, and their predation on amphibians is known, even in species that carry possibly lethal toxins (Breed & Ford, 2007).

Even though the species is not the same as the one in the Czech Republic (*S. infraimmaculata & S. salamandra*), the alkaloids are similar in composition and action, which does not rule out the possibility that a specialized predation and feeding behaviour could be spread to the fire salamander population in Czechia. Focusing on the Black Valley population, it represents a real danger, considering that it can be devastating for small and isolated populations (Pezaro et al., 2018).

5.4. Sex ratio and young individuals

Lastly, it was also determined that most recaptured individuals were male. The reason for this could be related to the pregnancy of several female individuals found in the latter stages of the research. Normally, the mating season of fire salamanders takes up April and May (Steinfartz et al., 2006), which means that most female fire salamanders were looking up for water bodies to lay their larvae. Both male and female salamanders are dependent on water, so it is difficult to detect any strong displacement from them. Nevertheless, females migrate more often to the water bodies in order to give birth to the aquatic larvae, while male individuals rarely return after metamorphosis (Ficetola et al., 2012). This lack of recapture of female individuals could be attributed to the guard of female salamanders when giving birth, because they stay hidden and safe during these process for several nights (Manenti et al., 2017), while male fire salamanders have more active nights and, ergo, are easier to visualize.

Focusing now on the juvenile salamanders, their total number is relatively low. Even though the amount of found larvae is sizeable, there is a huge probability that they do not reach maturity. This can be due to the unknown distribution, abundance, and quality of the new terrestrial habitat and all the external threats that they will encounter once they leave the pond they hatched in. Predation and desiccation are their most common adversities as they embark in this new stage of life (Rohr & Madison, 2003). Records of the juvenile individuals of the spotted salamander (*Ambystoma maculatum*) showed that only a 17% of these individuals are able to survive one year after metamorphosis (Rothermel & Semlitsch, 2006).

6. Conclusion

Salamandra salamandra populations in the Czech Republic are battling against many anthropocentric and natural threats, causing them to be threatened with extinction. This thesis monitored the largest population in Prague, the Black Valley, Prague 8, and aimed to analyse what the population estimate was for this site. By using the Capture Mark Recapture methodology, Visual Encounter Survey, and the MARK Software, it was found that the population estimate stands at 582 individuals (CI 95%, 414-819), with a recapture percentage of 22.631.

This research provides important evidence about the decline of the *Salamandra salamandra* population in Prague, suggesting a loss of almost 50% of the most abundant fire salamander site. Although education programmes, eradication or removal of invasive species and sustainable developmental plans for the urbanization can help the conservation of this species, the horizon is quite pessimistic in a city that keeps on growing and expanding.

On the other hand, seasonal behaviour appears not to be perturbed by climate or external factors, showing great and quick adaptability to the challenging environment. Mating, larvae laying and moving patterns are still normal. Nevertheless, climate change is a pressing matter that cannot be ignored, and its effects should start to be studied now to prepare a better outcome for the fire salamanders.

As for the replicability of this study, a stronger VES effort is advised. Extending the time of the field surveys in order to see if the patterns of abundance stay the same, or if there is any incoherence. Also, duplicate or triplicate the number of researchers in the field in order to manage all salamander more quickly and easily. As for the data analysis, the more data obtained via VES, the more precise the data is and better conclussions about the real state of the population can be made.

To follow up this research, it is suggested that more information about predation needs to be recollected, to analyse how vulnerable the Czech population of fire salamander is against dogs, cats, and rats. Also, joining the Amphibian & Reptile Wildbook initiative would largely attract more people to investigate and protect salamanders. So far, this project has no information on Czech fire salamanders, so it offers a great opportunity to raise awareness about this sympathetic amphibian and create more projects for its conservation.

In addition, and on a very personal note, the author believes that hunters should be more respectful and comprehensive of the work done in the field by scientists. During this research, in two occasions a hunter was dangerously close to a hotspot and in one occasion, he decided to shoot a wild boar, even though the presence of the researchers was made obvious. Even though it is a legal activity, it shows the lack of commitment of this group of people in helping the preservation of the wild fauna of this country, by not allowing the respective parts to do their jobs. Some agreements should be established, e.g. control of hunting in Natural Reserves during high seasons of salamanders.

Lastly, *Bsal* appears to be a great menace for salamanders in Czech Republic, since it can be as lethal as it is being in the Netherlands and Germany. It is important to address this pathogen as a severe risk for the fire salamander populations of Czechia.

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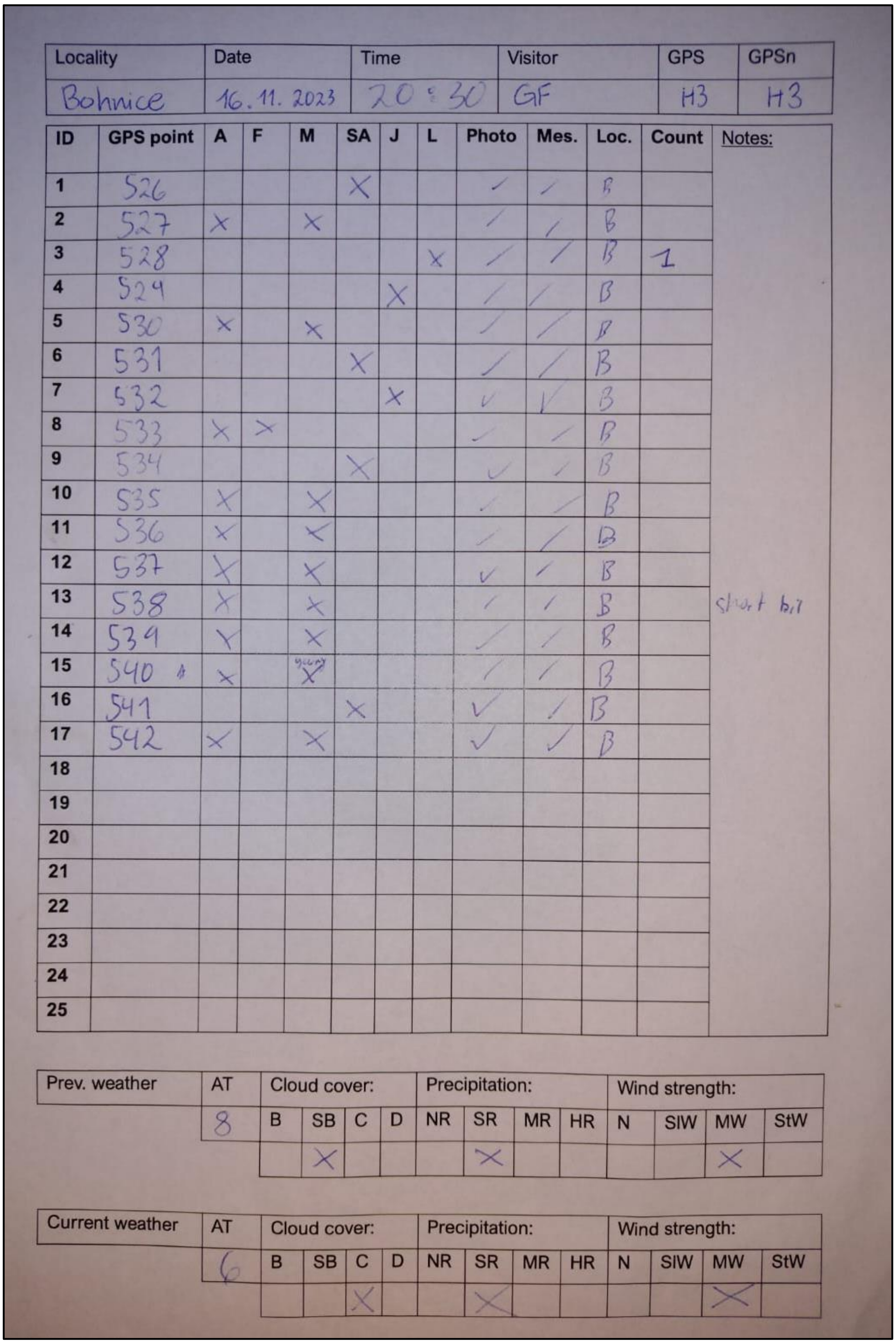
8. Appendix



Locality	Date	Time	Visitor	GPS	GPSn
Bohnice	16. 11. 2023	20 : 30	GF	H3	H3

ID	GPS point	A	F	M	SA	J	L	Photo	Mes.	Loc.	Count	Notes:
1	526				X			✓	✓	B		
2	527	X		X				✓	✓	B		
3	528						X	✓	✓	B	1	
4	529					X		✓	✓	B		
5	530	X		X				✓	✓	B		
6	531				X			✓	✓	B		
7	532					X		✓	✓	B		
8	533	X	X					✓	✓	B		
9	534				X			✓	✓	B		
10	535	X		X				✓	✓	B		
11	536	X		X				✓	✓	B		
12	537	X		X				✓	✓	B		
13	538	X		X				✓	✓	B		short bit
14	539	X		X				✓	✓	B		
15	540	X		X				✓	✓	B		
16	541				X			✓	✓	B		
17	542	X		X				✓	✓	B		
18												
19												
20												
21												
22												
23												
24												
25												

Prev. weather	AT	Cloud cover:				Precipitation:				Wind strength:			
	8	B	SB	C	D	NR	SR	MR	HR	N	SlW	MW	StW
			X				X					X	

Current weather	AT	Cloud cover:				Precipitation:				Wind strength:			
	6	B	SB	C	D	NR	SR	MR	HR	N	SlW	MW	StW
				X			X					X	

Appendix 1. Information table for the VES (Visual encounter survey).
Information regarding location, date, time of start, name of visitor and number of GPS used is on the top part. The second part consists of 20 rows to write down the assigned GPS for every salamander encountered, their stage and sex (A – Adult, F- Female, M -Male, SA – Subadult, J – Juvenile and L - Larvae). At the bottom, the weather conditions during and before the visit are found.

Visit	*Date*	***N***	*A*	*F*	*M*	*SA*	*J*	*L*	*AT (°C)*	*C*	*R*	*W*
1	13.01.23	**6**	6	5	1	0	0	4				
2	24.02.23	**6**	6	3	3	0	0	10				
3	10.03.23	**21**	12	5	6	5	1	26				
4	20.03.23	**21**	18	10	8	2	1	110	13	C	NR	SlW
5	15.05.23	**3**	2	2	0	0	1	0	15	SB	NR	N
6	06.06.23	**6**	4	2	2	2	0	0	17	D	MR	SlW
7	30.08.23	**0**	0	0	0	0	0	0	16	SB	NR	SlW
8	19.09.23	**3**	3	0	3	0	0	0	17	B	NR	N
9	23.09.23	**3**	2	0	2	0	1	0	14	B	NR	N
10	09.10.23	**22**	19	7	12	2	1	0	14	C	MR	MW
11	12.10.23	**23**	19	6	13	4	0	0	16	C	SR	SlW
12	20.10.23	**18**	12	4	8	2	3	1	9	C	NR	SlW
13	22.10.23	**6**	3	1	2	1	2	6	12	C	NR	SlW
14	25.10.23	**14**	11	4	7	3	0	0	12	D	MR	SlW
15	27.10.23	**14**	11	6	5	0	3	0	11	C	NR	MW
16	31.10.23	**5**	3	1	2	1	1	0				
17	03.11.23	**22**	15	6	9	6	1	0	7	D	MR	MV
18	11.11.23	**24**	17	2	15	5	2	5	5	C	SR	MV
19	14.11.23	**14**	6	0	6	4	1	3	11	C	NR	SlW
20	15.11.23	**13**	8	5	3	2	1	2	8	SB	SR	MW
21	16.11.23	**17**	10	1	9	4	2	1	6	C	SR	MW
22	21.11.23	**9**	7	3	4	2	0	0	4	SB	SR	MW

Appendix 2. Summarized information table for the VES. This table contains comprised information of the 18 visits and 4 datasets that were used for this study.

N – total number of salamander caught, A – Adult, F – Female, M – Male, SA – Subadult, J – Juvenile, L – Larvae, AT – Air Temperature at the start of the field trips, C – Cloudiness (B – Bright, SB – Semi bright, C – Cloudy & D - Dull), R – Rain (NR – No Rain, SR – Sleight Rain, MR – Medium Rain) & W – Wind (N – No Wind, SlW – Sleight Wind, MW – Medium Wind).

Appendix 3, 4 and 5. Individuals of fire salamanders in different hideouts. The first picture shows an individual hiding in an easily accessible rock formation. The second shows an individual hiding in a narrower space, with less chances to be captured or preparing for the winter latency period. The last picture shows an individual digging under a rock to hide its entire body, most possibly preparing for the winter hibernation.

Grp.	*Occ.*	***N-hat***	*Standard Error*	*Lower*	*Upper*
1	1	201.94876	82.461827	93.534909	436.02225
1	2	201.94876	82.461827	93.534909	436.02225
1	3	201.94855	82.461871	93.534711	436.02228
1	4	96.912719	49.023929	38.024911	246.99795
1	5	78.313597	39.215057	30.988637	197.91188
1	6	82.355950	69.355254	19.610418	345.86220
1	7	266.58329	4203.1336	2.6666087	26650.573
1	8	273.00249	50.899206	19.02797	392.20733
1	9	273.00228	50.486986	190.57684	391.07713
1	10	273.00228	50.486986	190.57684	391.07713
1	11	273.00228	50.486986	190.57684	391.07713
1	12	272.99109	50.521227	190.52059	391.16053
1	13	238.50712	94.902283	112.50378	505.63321
1	14	238.50509	94.898648	112.50516	505.61838
1	15	215.30597	111.52925	82.803777	559.83750
1	16	138.35049	48.555355	70.936602	269.83048
1	17	138.35049	48.555355	70.936602	269.83048
1	18	179.10448	64.142406	90.658891	353.83640
1	19	324.28882	94.940435	184.85483	568.89634
1	20	324.28882	94.940435	184.85483	568.89634
1	21	324.28882	94.940435	184.85483	568.89634
1	22	22.320061	2281.4575	0.0574411	8672.9727

Appendix 6. Population Estimates of the fire salamander according to every visit to the site with a 95% confidence interval. The model used to obtain these vales is [Phi(~time) p(~time) pent(~time) N(~1)], where Phi represents the probability of survival, p represents the probability of detection, pent represents the probability of new individual entering the population (birth or migration) and N represents the size of the super-population.

No.	***M** (length in cm)*	***F** (length in cm)*	***SA** (length in cm)*	***J** (length in cm)*
1	16	18	12	6.5
2	17	16	12.5	6.5
3	16.5	19	14	10
4	17	18.5	12.5	11
5	19	17	12	10.5
6	17.5	17.5	13.5	11
7	19	18.5	14	10.5
8	18	19.5	12	8.5
9	17	19.5	13	6
10	18	18.5	13	9
11	19	20.5	13	9
12	20	17.5	14.5	9.5
13	17.5	18	14	6.5
14	18	18.5	13	7
15	19	19.5	12	7.5

Appendix 7. Body lengths of 15 random individuals of every sex and stage of the fire salamander population in the Black Valley. M stands for male individuals, F stands for female individuals, SA stands for sub-adults and J stands for juveniles.

```
> shapiro.test(male_size)

        Shapiro-Wilk normality test

data:  male_size
W = 0.95404, p-value = 0.5902

> shapiro.test(female_size)

        Shapiro-Wilk normality test

data:  female_size
W = 0.97361, p-value = 0.9074

> # 2) Equality of variances
> var.test(male_size,female_size)

        F test to compare two variances

data:  male_size and female_size
F = 0.97238, num df = 14, denom df = 14, p-value = 0.9589
alternative hypothesis: true ratio of variances is not equal to 1
95 percent confidence interval:
 0.3264553 2.8963061
sample estimates:
ratio of variances
         0.9723757

> # 3) Independence #met, because the size of females does not depend on males
> t.test(x=male_size, y=female_size, var.equal=T) #Two sample t-test

        Two Sample t-test

data:  male_size and female_size
t = -1.2127, df = 28, p-value = 0.2354
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -1.3445799  0.3445799
sample estimates:
mean of x mean of y
     17.9      18.4
```

Appendix 8. Two sample T-test to identify any significant difference between male and female total body length. Shapiro tests show that both datasets follow a normal distribution, the F – test compare variances and states that both datasets have almost the same variance, and both datasets are independent from each other. That means that all the assumptions are met for the T-test to be performed.