ESTABLISHMENT OF THE REGIONAL MONITORING PROGRAM FOR THE DINARIC POPULATIONS OF ALPINE SALAMANDERS

Rufford Conference: "Explore and protect the natural beauty of Balkans". 27-28.9.2018, Silver Lake (Srebrno jezero), Serbia

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INTRODUCTION

The Alpine salamander (*Salamandra atra*) is a fully terrestrial, viviparous tailed salamander (Fig. 1). Four subspecies are recognized within the species: the nominotypical (*S. a. atra*) inhabitnig the Alps, two in the Italian Prealps (*S. a. pasubiensis* and *S. a. aurorae*) and the one inhabiting the Dinarides (*S. a. prenjensis*, Fig.2).
In the Alps, it is continuously present along a wide altitudinal range (from 433 up to 2.800 m a.s.l.), thus occupying various ecosystems such as humid forests, meadows, pastures and rocky habitats above the tree line. Dinaric populations, on the other hand, are severely fragmented and restricted to isolated, high-altitude areas with a narrow vertical distribution (Fig. 2). The number of occurrence areas along the Dinarides are still questionable. **Dinaric populations are the focus of this study**.

Fig. 1. The alpine salamander, Salamandra atra Laurenti 1768

This salamander is a cold adapted amphibian with the highest activity observed between 5 - 12ºC and when humidity values are above 70%. **The peculiar biology and „slow" lifestyle of these vulnerable animals question their ability to cope with climate change as the speed of adaptation to (warmer) environments seems to be outstripped by global warming**. It is possible that some populations are already extinct such as the one from mt. Treskavica (in Bosnia and Herzegovina – B&H) which presence on this mountian has not been (re)confirmed since the early 1900, despite extensive fieldwork research in the past three years on this mountain (through the support of previous Rufford grants). **Additional threats to the survivorship of these populations are the spreading amphibian pathogens**; In several individuals of alpine salamanders that were kept in captivity (from the year 2016. till present) we registered the dangerous Ranavirus, responsible for amphibian declines worldwide.

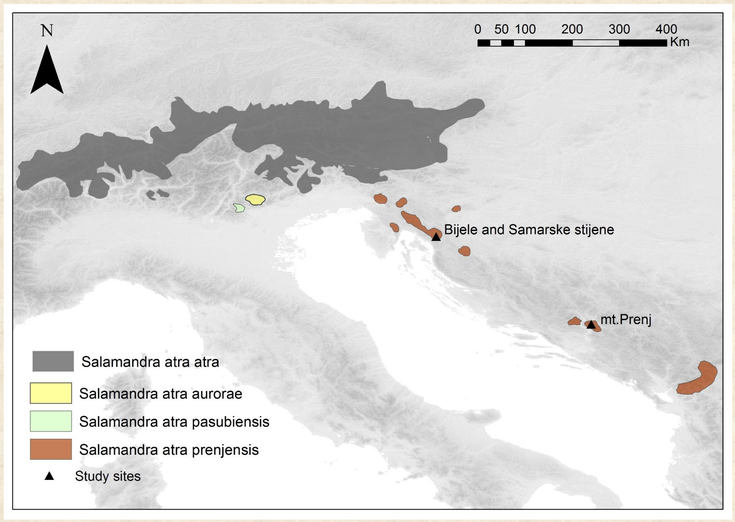

Fig. 2. Distribution areal of the four described subspecies within S. atra showing the study areas encompassed by this project

Through this project we are **setting up a standardized monitoring program that will serve as the basis for a long-term demographic assessment of salamander populations** in Croatia (Gorski kotar, area of Bijele and Samarske stijene) and Bosnia and Herzegovina (mt. Prenj, Fig. 2). During this project we will also **screen captured animals for Rana virus** using molecular techniques.

METHODOLOGY

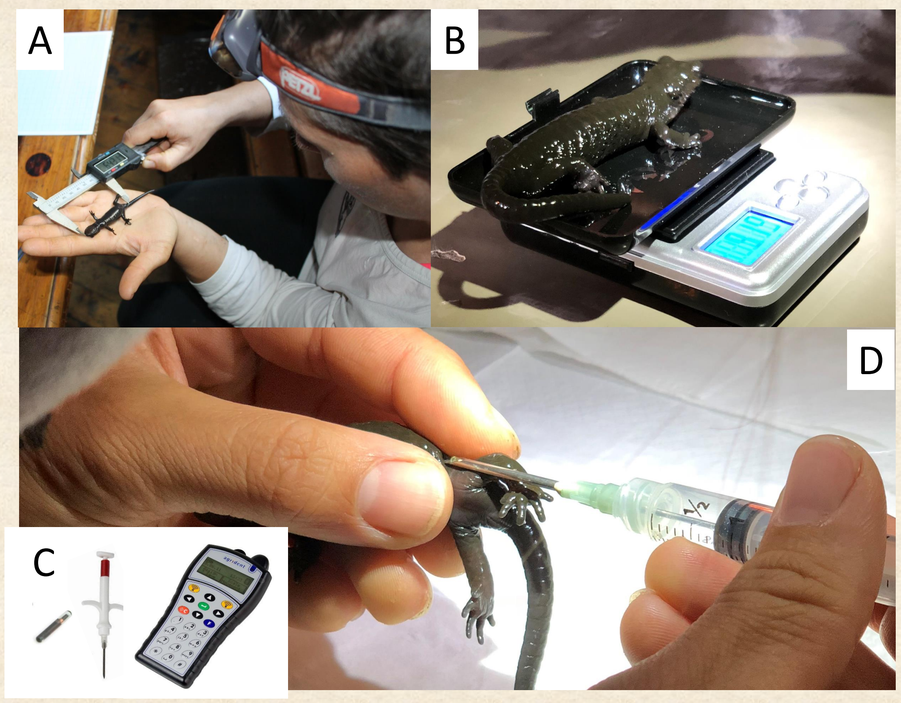

Fig. 3. (A, B) Each animal is measured and weigthed to asses its body condition index. (c) Equipment neccessary fot Pit tagging: PIT tag, a needele and a reader (as in order of appereance). (D) Insertion of PIT tag (present in the needele) in the abdominal cavity of the animal.

The standardized monitoring program we are setting up is based on a **Capture Mark Recapture (CMR) study** using PIT tags (biochips with unique identifying code) that we insert permanently in the animals. This survey method consists in visiting the same site(s) on several occasions, during which, individuals are captured. The individuals that are captured for the first time are **marked by inserting a PIT tag** in them and individuals that have been marked during previous visits are recorded (with a reader, see Fig. 3C). Upon marking/recording, animals are released on their exact place of finding.

The PIT tags (Fig. 3C) are electronic microchips that are 12 mm long and 2 mm wide encased in biocompatible glass which protects the electronic components and prevents tissue irritation. **Each PIT tag has a unique code and is injected with a 12-gauge needle under the animal's skin in the abdominal cavity** (Fig. 3D). Prior to PIT tag insertion we anesthetized the salamanders using MS-222 (amphibian anesthetic). Upon insertion of the PIT tag, we apply surgical glue on the insertion site to prevent the peritoneum or internal organs to exit from the hole made by the needle. The animal recovers fast and its presence during subsequent visits of the project site(s) - recapture sessions - is annotated by **activating the PIT tag using a handheld reader** (Fig. 3C) with which the researcher scans the animals (holding it cca 5 cm above them). If a PIT tag is present inside the animal, the reader generates a close-range, electromagnetic field that activates the tag, which transmits its number. The unique Pit tag code permits a marked individual to be distinguished from every other one in a population.

After the marking procedure we also **measure** the standard length of each animal (snout to vent length) and its weight (on a digital scale, Fig. 3A and 3B) and **picture** it (ventral and dorsal side) to be able to asses the body condition index of each marked individual and register eventual morphology changes. We also **swab** each individual for further Rana virus analysis.

Thanks to the repeated visits on the same location(s), at the end of the seasonal survey, we obtain **a capture history for each marked individual** which is analyzed in the **software: MARK**. Depending on the duration of the survey, several parameters can be estimated; The recapture history after the first year can yield data on **population size** and **capture and recapture probability**. Collected data during the subsequent study years can give **the survival probability, emigration and immigration probabilities, recruitment rate** and **population growth**. In this way the CMR study will **help us to understand species response to changing environments and demographic changes** through the years.

So far we **tagged 74 individuals on mt. Prenj and 41 in Gorski Kotar** (Fig. 4A, 4B). From each population we have **50 body swabs** that will be sent to the **Laboratory of Dr. Frank Pasmans –** the world leading expert of amphibian diseases (Faculty of Veterinary Medicine of Ghent University, Belgium) for **Rana virus screening**.

OTHER ACTIVITIES AND PROJECT PROGRESS

Besides the scientific background, other project ctivities are **educational** and **promotional, such** as:
Raising aweraness: In april 2018, we **depicted a mural** of the alpine salamander on a buildig at the entrance of the city of Jablanica (B&H, underneath mt. Čvrsnica – home of alpine salamaders). The mural slogan is: „**Protect mts. Prenj and Čvrsnica – Protect me**" (Fig. 4C) . This attracted the attention of many people and is functioning as a permanent instrument of **rasing aweraness on the need to protect our natural beauties.** We also started the filming of the **educational movie: „The black salamander from mountain Prenj"** in cooperation with the staff of the Academy of Performing Arts in Sarajevo (release time - summer 2019).
Education and reporting: In June 2018, we held a two day **workshop** (led by expert Julien Courant, PhD in Zagreb) during which staff from HIB and ATRA (see affiliation of authors) were educated for conducting CMR surveys and analysing data in MARK. Julien also gave two **educational presentations** on the same topic for the students of both: University of Zagreb and University of Sarajevo (Fig. 4D).

The data gathered through this project will be **reported to IUCN** and in several **scientific articles**.

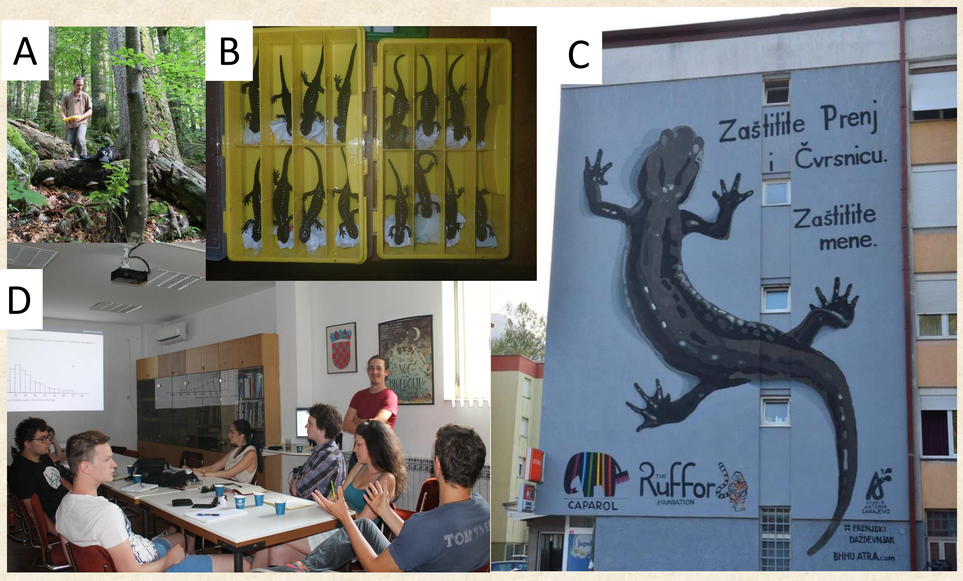

Fig. 4. (A, B) Field reaserches and recovery of tagged individuals. (C) The salamander mural on the building in Jablacnica. D Julien Courant giving an educational lecture at Zagreb University.

For more information please contact the project holder (see first author affilation) or visit Rufford and BHH UATRA webpage (rufford.org , bhhuatra.com