Project Update: October 2013

After the completion of educational lectures throughout Bosnia and Herzegovina (first project activity), we started with the second activity planed within the project, field analyses. During June – September 2013, field analyses were conducted on four different mountains in BiH: Prenj, Čvrsnica, Treskavica and Maglić.

Collection of biological samples was performed on Prenj and Čvrsnica, where also distribution analyses were conducted. On Maglić and Treskavica, just distribution analyses were performed.

Mountain Prenj was visited seven times during the period June – September 2013 at five different sub-locations, five different subpopulations were researched. In total, on mountain Prenj:

- 145 individuals were measured and pictured (61 males, 20 juveniles and 64 females).
- 97 faeces were collected.
- 34 stomach flushes were collected.
- Four traps for insects were set and their contents collected at four different sub-locations.
- Three data loggers were set at three different sub-locations.
- 109 individuals of *Salamandra atra prenjensis* and other amphibian (such as *Bufo bufo* and *Bombina variegata*) individuals were sampled for chytridiomycosis.
- 41 toxin samples were collected from males, females and juveniles.
- 52 DNA samples of *Salamandra atra prenjensis* were collected
- Seven pregnant females and one male were taken from the field to conduct reproduction analyses.
- We managed to register *Salamandra atra prenjensis* at a new sub-location on Mt. Prenj (Osobac).

Mountain Čvrsnica was visited five times during the period August – September 2013 at five different sub-locations. Out of five analysed sublocations, population of *Salamandra atra prenjensis* were registered only at two, which confirms that populations are present on Mt. Čvrsnica but they are not as abundant as on Mt. Prenj. We primary think that the reason of this is influenced by the disappearance of habitat due to the movement of forest range towards higher altitudes. It seems that populations have adapted to the conditions on Mt. Čvrsnica, as we have found them on very different habitats compared to the habitats of Mt. Prenj. Populations of alpine salamanders on Čvrsnica are not as dense as on Mt. Prenj. In total, on Mt. Čvrsnica:

- 73 individuals were measured and pictured (42 males, 4 juveniles and 27 females).
- 10 feces were collected.
- 16 stomach flushes were collected.
- One trap for insects was set and its content collected at one sub-location.
- Two data loggers are set at two different sub-locations.
- 40 individuals of *Salamandra atra prenjensis* were sampled for chytridiomycosis.
- 23 toxin samples were collected from males, females and juveniles.

- 71 DNA samples of *Salamandra atra prenjensis* were collected.
- We managed to register *Salamandra atra prenjensis* at a new sub-location on Mt. Čvrsnica (Pločno).

The collected samples are currently being analysed. For the next 3 months I am hosted by the University of Salzburg (see previous report) where we are going to perform feeding ecology studied of *Salamandra atra* using molecular biology techniques (analyses of feces and stomach flushes through PCR). The insects collected in the field using traps are also an important instrument through which feeding ecology will be defined. We will compare feeding habits - ecology of Austrian population (*Salamandra atra atra*) vs Dinaric population (*Salamandra atra prenjensis*).

Morphological comparison of Austrian and Dinaric individuals will also be performed. For each individuals 10 morphological measurements were taken and each individual was photographed four times (dorsal picture, lateral picture, picture of cloaca and picture of head). Toxins collected on the field from *Salamandra atra prenjensis* individuals will be also compared with the collected toxins of *Salamandra atra* (Salzburg samples) in order to estimate if there is any difference in the amount of active substances of the toxins in those two populations. We expect to register differences since individuals inhabit different habitats and this might have affected the quality of the toxins.

DNA analyses collected on the field will also be used for a population genetics study which can have a huge impact in defining conservation units. This study will be also performed at the University of Salzburg.

This comparison between Austrian and Dinaric population (through all the listed studies) will be a very important step through which we will try to confirm the subspecies status of *Salamandra atra prenjensis* which is still controversial as it is not fully accepted in the scientific community. Additionally, the outcome of this research will have a huge impact for conservation policies in Bosnia and Herzegovina (BiH). BiH is preparing for entering the European Union. One of the first steps will be to prepare the Natura2000 sites for species listed in Habitat Directive, appendix IV, which is the case of *Salamandra atra*. However, considering that *Salamandra atra aurorae* is listed on Appendix II, *Salamandra atra prenjensis* could have a similar signal function for the local government in the designation of critical Natura2000 habitats. In this regard, the research performed through this mobility exchange is crucial to help solving and defining the taxonomic and conservation status of *S. a. prenjensis* in BiH.

During January and February 2014 collected samples will be analysed for chytridiomycosis (a fatal fungal disease attacking amphibians all over the world) at the Veterinary Faculty of the University of Sarajevo. During field analyses, other found amphibians were also sampled for this disease in order to have a clear idea if the disease has reached BiH. These will be the first data for Bosnia and Herzegovina regarding chytridiomycosis analyses. During field work we also found several dead and sick individuals which we collected. We set up a collaboration with Prof. Frank Pasmans from the Laboratory of Veterinary Bacteriology and Mycology of the Clinic for Exotic Animals by the Faculty of Veterinary Medicine of Ghent University (Belgium). We sent him the samples and he and his team will try to identify the cause of death that could be a potential threat for the whole population. The team is

currently working on the case.

In the field we also collected seven pregnant females and one male for reproductive study analyses. Those individuals are currently at the "in-situ amphibian and reptile conservation centre" by the Zoo of Zagreb. This study is conducted in the collaboration with the Croatian Herpetological Association: "Hyla". The females are healthy and are monitored until their deliver, after which we will release them back in the location from which we collected them. Until now the females didn't give birth so we expect this next year (summer 2014).

Five data loggers are set up in the field to monitor and follow abiotic factors and their variation in the habitat of *Salamandra atra prenjensis*. Abiotic factors that are registered each hour (until next year) are: temperature, humidity and dew point. The data from the data loggers will be collected next year in order to have a clear picture of the situation in the habitat according which we can further and more detailed estimate the activity of *Salamandra atra prenjensis* during the whole year as well as to get an insight of eventual occurring climate changes and the impact on *Salamandra atra prenjensis*.

In the next period we will also define conservation units (subpopulations which must be monitored) and the way to monitor them. It is important to notice that during whole of August 2012, Mt. Prenj was burning and the fire reached some of the salamander's habitats that we also analysed. We registered a smaller density of populations in these areas but further research must be conducted to confirm the true level of damage and impact on populations. On Mt. Čvrsnica we registered a loss of salamander habitat due to the movement of the forest range towards higher altitudes. This movement of forest is certainly affected by the fact that no cattle breeding is performed in hot spot areas for salamanders since 1985. *Pinus mugo* is expanding uncontrollably inside the habitat. Further research must be conducted. Distribution analyses on Mt. Treskavica and Mt. Maglić were not successful in terms of registering *Salamandra atra* individuals but we strongly believe that they inhabit these mountains and further researches must be also conducted to prove this. For Mt. Treskavica just very old literature data exist (Radovanović, 1951) indicating that *S. atra* is found there, but no specific locations are given. Maxent data showed Mt. Maglić as a potential habitat for *S.atra* which is possible according to habitat characteristics.

Mountain Prenj: June – September 2013		
Date	Sublocation analysed	Performed work
29. - 31.06.2013	• Podotiš	• Measurement of collected individuals and Photographing • Population analyses (sex ratio and density) • Sampling for chytridiomycosis, • Feces collection, • Setting the traps for insects • Setting the data logger
06. - 10.07.2013	• Kopilice • Osobac	• Measurement of collected individuals and Photographing • Population analyses (sex ratio and density) • Sampling for chytridiomycosis, • Feces collection, • Setting the traps for insects • Setting the data logger and collection of DNA samples from Osobac individuals
19. – 23.07.2013	• Zakantar • Soplje	• Measurement of collected individuals and Photographing • Population analyses (sex ratio and density) • Sampling for chytridiomycosis, • Feces collection, • Setting the traps for insects • Setting the data logger
27 – 28.07.2013	• Podotiš	• Sampling individuals for additional feces collection • Collecting the set traps for insects on all sublocations
31.07.2013	• Podotiš	• Measurement of individuals collected on the previous field and Photographing • Population analyses (sex ratio and density) • Feces collection • Toxin sampling
21. – 23.2013	• Kopilice • Podotiš	• Stomach flushing of individuals and • Collecting the pregnant females for reproduction studies
07. – 09.2013	• Kopilice	• Toxin sampling • DNA sampling

06. - 08.08.2013	• Pod zelenom glavicom • Sjeverne padine Vilinca	• Measurement of collected individuals and Photographing • Population analyses (sex ratio and density) • Sampling for chytridiomycosis, • Feces collection, • Setting the traps for insects • Setting the data logger
10. - 11.08.2013	• Vilinac • Sjeverne padine Vilinca	• Measurement of collected individuals and Photographing • Population analyses (sex ratio and density) • Sampling for chytridiomycosis, • Feces collection, • Setting the traps for insects • Setting the data logger and collection of DNA samples from Osobac individuals
24. – 26.08.2013	• Ledeno jezero • Peharovi stanovi	• Measurement of collected individuals and Photographing • Population analyses (sex ratio and density) • Sampling for chytridiomycosis, • Feces collection, • Setting the traps for insects • Setting the data logger
28 – 30.08.2013	• Peharovi stanovi • Ledeno jezero • Veliki kuk	• Sampling individuals for additional feces collection • Collecting the set traps for insects on all sublocations
16. – 17.08.2013	• Pločno • Peharovi stanovi • Vilinac	• Many individuals registered
Mountain Treskavica: August – September 2013		
Date	Sublocation analysed	Performed work
18. - 20.08.2013	• Konjska vrela • Bijelo jezero • Pašina planina	• Distribution analyses didn't confirm the presence of the alpine salamander on the analysed locations of Mt. Treskavica. Several other amphibians, such as: *Ichtyosaura alpestris*, *Bufo bufo* and *Rana dalmatina* were registered. The found data are very important for conservation purposes of BiH amphibians!
Mountain Maglić: September 2013		
Date	Sublocation analysed	Performed work
05. - 06.09.2013	• Konj	• Distribution analyses didn't confirm the presence of the alpine salamander on the analysed locations of Mt. Treskavica.

Each individual found on the field was collected in a separate box. All the boxes were kept in a plastic bag (on the field) for 4 days waiting to get feces samples needed for feeding ecology study.

Stomach flushing for feeding ecology study

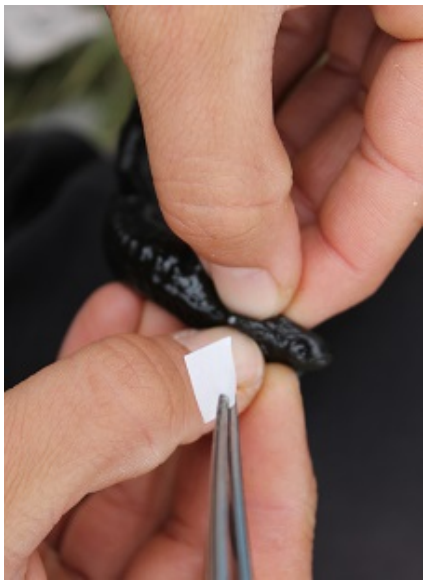
Weighing individual

Chytridiomycosis sampling

Collected feces

Pregnant females were collected for reproductive analyses studies

Habitat of *Salamandra atra prenjensis*



Toxins were collected during field work