Final Evaluation Report

Your Details	
Full Name	Ana Ćurić
Project Title	Conservation of complex aquatic and terrestrial habitats preffered by extreme ontogenetic shapechifter, European common spadefoot toad (*Pelobates fuscus*)
Application ID	21349-2
Grant Amount	4996
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Date of this Report	20th October, 2018

1. Indicate the level of achievement of the project's original objectives and include any relevant comments on factors affecting this.

Objective	Not achieved	Partially achieved	Fully achieved	Comments
Age structure				Since we established the focus locality of *Pelobates fuscus* research in Bosnia and Herzegovina, one of our goals was to determine the great population age structure (Bosnian spadefoot toad biggest population with adequate habitat, least antropogenic impacted). It is interesting that the biggest explosion in breeding was in 2014 (big flooding in Bosnia) and by now the breading seasons were average, with not many juveniles noticed by the end of summer. That is one of our reasons to study the Čardak population age structure. Bigger sample results could gives us an insight of population viability and future predictions of it stability depending on antropogenic pressure, existing habitats and climate changes. The samples were taken from April to September 2018 and in total we sampled 30 phalanges for the skeletochronology procedure. Each caught individual was measured by the standard protocol (standardized protocol made in 2014), morphological and meristic description was taken and from each toad the last two distal phalanges of the fourth toe from the left forelimb was clipped, set in 10 % formalin and stored until the process for skeletochronological analysis . Toads were released into their original capture site right after taking all mentioned parameters and samples. From April to September 2018, phalanges were proceeded in laboratory of Veterinarian Institute „Vaso Butozan", Banja Luka. Procedure takes 5 days and each week 10 samples were proceeded. The phalanges, from 10 % formalin, were washed in running water and decalcified for 15 minutes in 3 % nitric acid (since the samples are small and fragile) and washed again. Phalanges were then dehydrated through a series of

				increasing concentrations of ethanol solution (70 %, 96 %, 100 %), cleared with xylene and then embedded in paraffin (56 – 58 ºC). Frozen phalanges (on - 20 °C) were cut on rotary microtome with a cross section 8-12 m thickness, stained in total for 1 hour and 30 minutes i (H&E staining). Finished samples were examined and photographed on light microscope LEICA DM750 with digital camera EC3 at the two magnification, allowing for simultaneous comparison and facilitating the analysis of the bone growth pattern together with histology professor Smiljana Paraš, Faculty of science and mathematics, University of Banja Luka. By preliminary results, the oldest spadefoot toad in Čardak population is around 10 years old.
Tadpole survey – ethology, ecology, morphometry, meristic and morphology				In April 2017 two egg clutches were noted and published for the first time in Posavina region. The egg clutches were collected and moved in 90 l buckets where we adapted conditions as similar as possible to the natural ones concerning water quality and presence of flora and fauna from natural habitat. Buckets were held outside with enough sunlight that is important for the eggs and tadpoles survival and development. Larvae and tadpoles were fed with aquarium fish food and fish pellets in later stages, combining with plants and algae collected from natural water habitat. After 3 months collecting and photographing the individuals we recorded important stages from egg development to the latest stage before the process of metamorphosis. In total 249 Tadpoles were measured. In total, five parameters were observed considering tadpole morphology. For the morphometry 28 parameters were measured and three for meristic. All data has been statistically proceeded (min, max, standard deviation, average value, PCA). Also, development stages (by Gosner, 1960) were determined for all measured tadpoles. Observed stages were categorized in groups: 1. Egg 2. Larvae

				3. Tadpole The lowest observed larval stage was 11, and the highest tadpole stage was 41. All morphological, morphometry and meristic data were compared through developmental stages by Gosner (1960). The smallest tadpole measured was 5,48 mm in stage 23. The biggest tadpole was 99,04 mm in stage 40. From all measured tadpoles, the highest number were in stages 26 and 27. All detailed results are in preparation to be published. Ethology has been observed on aquarium tadpoles and filming the tadpoles in natural habitat. Giant tadpoles can be easily observed during evening and night in the shallow parts of water body. During the day they are in deeper parts and can be seen on the surface when approaching to take a breath. Smaller tadpoles are concentrated around egg clutches and after they start active phase. One of the great results of tadpole research was a rollup made for European common spadefoot toad tadpole ontogenetic developmental stages. Project rollup can be seen: https://www.rufford.org/files/21349-2%20Promotional%20Materials.pdf
Oral disc morphometry and morphology				For each stage by Gosner (1960) (from stage 23 to 41) oral disc morphometry and morphology was taken and described. Photographs were taken with stereo microscope LEICA EZ4 HD for each stage of several individuals, drawings were made and labial formula determined. These results have been presented at 13th Croatian Biological Congress / 2nd Balkan Herpetological Symposium as a poster and the paper is in preparation (Ćurić, A. (2018): Looking into the mouth of giant tadpoles – morphological and meristic description of *Pelobates fuscus* (Laurenti, 1768) oral disc. 13th Croatian Biological Congress with International Participation, 2nd Balkan Herpetological Symposium, 19-23.09.2018, Poreč, Croatia. Abstract book, pp. 160). Poster results are available at: https://www.researchgate.net/publication/32

				8314756_Looking_into_the_mouth_of_giant_tadpoles_-_morphological_and_meristic_description_of_Pelobates_fuscus_Laurenti_1768_oral_disc
Reserch for new sites and cheking for unreliable literature sites for spadefoot toad presence				During the period from February to July 2018, several localities along rivers Una, Vrbas and Bosna were observed for presence of adults and tadpoles of spadefoot toads. On western part of Posavina region no new localities were found. In central part of Posavina region (Lijevče polje) we also did not found any new localities. This region has a potential for finding spadefoot toads, since the whole area is converted in agricultural land with drainage canals. *Pelobates fuscus* has adapted for this type of antropogenic habitats and there is a need for more intensively research of Lijevče polje. In central part tadpoles were found on Bardača lake, Brajinci, which confirms the first literature data for Bosnia and Herzegovina – the spadefoot toad found in digestive system of night heron (*Nycticorax nycticorax*) (Obratil, 1981). Literure data for Podrašnica locality, Mrkonjić Grad, has been negated. The researched area is too far from the nearest spadefoot toad populations in Posavina region and has no refugial importance. The area of Podrašnica is on 800 m a.s.l. and represent the highest elevation border of European common spadefoot toad distribution, which in combination with found flora and fauna is not representative habitat for the species. Water habitat represent the cold stream with the water temperature measured in April 2018, 12 °C. The flow is medium and fast which is not appropriate for the species breeding and tadpole development. There are no appropriate hydrophytes which spadefoot toad use for egg deposit and whose larvae and tadpoles use it as hiding and resting support. Also, local herpetofauna is not typical for spadefoot toad coexistence. These found species prefer higher elevation, colder water, lower air temperatures. At the site we found only two amphibian speces: *Bombina variegata, Rana dalmatina.*

				Ichtyofauna on breeding site (Čardak locality, Bardača chanals) was not found, which means that locals did not introduce fish in water habitat. These water habitat represent stagnant water bodies which dries up in summer and it is not suitable for fish natural maintenance.
Adult morphometery, morphology, meristics and skeletochronolo hy				Morphometric, meristic and morphological data was collected for all found adult individuals from Čardak locality as previous years by the standardised protocol. For all those individuals finger sample was taken for further procedure of skeletochronology. Samples were held in 10 % formaldehyde. Skeletochronology method was done in collaboration with Veterinary Institute of Republic of Srpska "Dr. Vaso Butozan", Banja Luka and studied at Faculty for science and mathematics, University of Banja Luka. By now we have data collected for 193 individuals, which is around 48 active hours of just taking morphometric measures, morphological description and meristic data.
Choosing new sites for data loggers for the future data comparisson				Data loggers from Čardak locality were burned in an accident (locals did not reported planed burning of dried water vegetation – Typha, *Phragmites*). These data loggers were replaced with new ones since the Čardak locality is of great importance for monitoring physical parameters that has been done for last four years.
Education and project promotion				Preparing and printing all promo material was finished in autumn 2017. Previous mentioned rollup was used during our educational project part. It is consisted of original illustrated photographs of ontogenetic stages from the egg clutch to the adult toad. Rollup was kept 7 days before and after the lecture in high schools and faculties for additional student education. All promotional material can be found on Rufford Small Grant web page (https://www.rufford.org/files/21349-2%20Promotional%20Materials.pdf), consisting of leaflets (in Serbian and English language), eco bags, t-shirts, pencils, stickers and rollup (Serbian language). Main lectures have been held in four towns: Banja Luka, Sarajevo, Mostar, Gradiška.

				Lectures were held in highscools among biology and ecology students and at Faculty of Science, University of Banja Luka with biology and ecology students. One lecture was held with students from The United World College in Mostar and presented in English language. Also, we had a meeting with locals and president of the local community in Čardak, Modriča municipality, where the main research site of spadefoot toad in Posavina region is. By the end of the year 2018 we will install the educational boards for European common spadefoot toad, herpetofauna and natural habitat.
Promo video				During this project we captured important videos and photographs of *Pelobates fuscus* tadpoles from egg stage to the process of metamorphose. Also, we manage to capture videos of spadefoot toads breeding which are the first videos uploaded online and can be found at YouTube site: https://www.youtube.com/watch?v=P0XwclSMLYw&t=4s For the video we used photographs and videos from previous years, and made an educative video where everyone can see the spadefoot toads in their developmental stages, breeding process, their natural and seminatural habitat, main threats that we recorded during these four years of research. The video is made in local language and it is planed to add English narration.

2. Please explain any unforeseen difficulties that arose during the project and how these were tackled.

During this project we had to change the usage of new data loggers that were intended to be used on new locality for data comparison. Instead of new locality we had to install them on the Čardak locality since the previous ones were destroyed in accident.

In Federation of Bosnia and Herzegovina we planned to have lectures in several high schools and were prevented due to Ministry slow work and complicated procedure. License was issued after 8 months and the time was overlapping with other projects activities.

Skeletochronology method took longer than we expected with method needed to proceed the samples for rotary microtome cuttings. With the combine work with other amphibian samples, limited common spadefoot toad samples collected during the field work, availability of the Institute laboratory and faculty microscope room and private issues, we finished sample procedure later than planned. Also, it is very hard to get the smallest medullar cavity cut at mid-diaphyseal level with the small bone samples and cross section over 8 µm, so just several samples ended up perfect. I will need extra time to set up the correlation and standardize the counts for all *P. fuscus* samples to get the best results. All results will be published.

During the summer, after breeding period, from June to September, we did not find any adult individuals which mainly effected skeletochronology study with smaller sample number than we had in plan.

3. Briefly describe the three most important outcomes of your project.

1. Skeletochronology – results are of great importance for population viability insights and future predictions of their maintenance.

2. Tadpole research – many data has been collected during tadpole research: morphology, morphometry, meristic, oral disc morphlogy and oral disc formula compared within stages by Gosner (1960). This data fullfiled so far known data for *Pelobates fuscus* tadpoles and it is of great importance for further biology researches in Bosnia and Herzegovina and neighbouring countries.

3. Local government meeting – recognition of our four year work and supporting nature protection (water habitat, herpetofauna and ornitofauna species).

4. Briefly describe the involvement of local communities and how they have benefitted from the project.

During the project our team has made variety promotional material that has been distributed within the local people, children, students, professors and colleges during meetings and workshops, conferences, field trips and meeting in Čardak. Through this project, promotional material was made in English and local language, focusing on tadpole biology and ecology, spadefoot toad life cycle, vulnerability, preferable terrestrial and water habitat.

Students were interested with the project goals and during this project we had students from Banja Luka and Sarajevo joined our field trips. This project had a big impact in herpetofauna research promotion and education, since this year we have three new active members joined BHHU ATRA, herpetological association in Bosnia and Herzegovina.

Our focus site is Čardak locality, where we found the biggest population of European common spadefoot toads in Bosnia and Herzegovina. One of the main

reasons is that the water (breeding) habitat is the least degraded, and concerning terrestrial habitat, spadefoot toads have adapted to agricultural lands. This project goal was to negotiate for habitat protection, water habitat restoration and to promote the species and its habitat through educational boards. Planed boards will be set up by the end of year 2018. Local community in cooperation with local government will organize the venue concerning educational lecture, project lecture, historical lecture and biodiversity lecture of the focus area followed with ceremonial opening. This venue will assure gathering all local people from Čardak that are the key audience for further conservation steps.

5. Are there any plans to continue this work?

Next step in mentioned protection on focus species and habitat is habitat restauration and potential proclamation of protected area, which we are aware it is a big and complicated step that in Republic of Srpska, Bosnia and Herzegovina will take time. Other main plan is to focus our research on tadpole biology, to study its behaviour and potential overwintering on physiology level.

6. How do you plan to share the results of your work with others?

Our work is promoted during this project through educational lectures within students, conferences, meetings and workshops. Main focus is publishing our work and sharing the information with Republic Institute for protection of cultural, historical and nature heritage of Republic of Srpska that will be joined in regulation on protected and strictly protected species. This is important for future steps of Natura2000 habitats and protected areas proclamations.

We are presenting our references where first and second Rufford project grants had a great impact:

1. Ćurić, A. (2018): Looking into the mouth of giant tadpoles – morphological and meristic description of Pelobates fuscus (Laurenti, 1768) oral disc. 13th Croatian Biological Congress with International Participation, 2nd Balkan Herpetological Symposium, 19-23.09.2018, Poreč, Croatia. Abstract book, pp. 160.
2. Ćurić, A. (2018): Conservation of Complex Aquatic and Terrestrial Habitats Preferred by Extreme Ontogenetic Shapeshifter, European Common Spadefoot Toad – Pelobates fuscus (Laurenti, 1768). 27th Rufford Small Grants Conference „From Mountains to Deep Seas“, 03-06.02.2018, Bar, Montenegro. Abstract book, pp. 21.
3. Ćurić, A., Zimić, A., Bogdanović, T., Jelić, D. (2018): New data and distribution of common spadefoot toad *Pelobates fuscus* (Laurenti, 1768) (Anura: Pelobatidae) in Western Balkans. North-Western Journal of Zoology, 14 (1): 50-59.
4. Zimić, A., Ćurić, A., Šunje, E., Vesnić, A., Lelo, S., Jelić, D. (2018): Sinecology of European common spadefoot tad, *Pelobates fuscus* (Laurenti, 1768) (Amphibia: Anura: Pelobatidae), in the agroecosystems of Bosnia and Herzegovina. 13th Croatian Biological Congress with International

Participation, 2nd Balkan Herpetological Symposium, 19-23.09.2018, Poreč, Croatia. Abstract book, pp. 8.

5. Zimić, A., Čengić, M., Ćurić, A., Šunje, E., Jusić, B., Lelo, S., Jelić, D. (2018): The checklist of reptile fauna (Chordata: Vertebrata: Reptilia) in Bosnia and Herzegovina. 13th Croatian Biological Congress with International Participation, 2nd Balkan Herpetological Symposium, 19-23.09.2018, Poreč, Croatia. Abstract book, pp. 157.

7. Timescale: Over what period was the grant used? How does this compare to the anticipated or actual length of the project?

Duradion of this project has been from May to October 2018 as planed.

The research depended on the species activity, which in total gives about 4 months of intensive field research and total of 14 months of fulfilling our project goals. The grant was approved on 24th July 2017, and by autumn we finished preparation and printing promotional material. Before the project approval we started our field research in May 2017. Education was preformed during the winter period and field research in 2018 started in March.

8. Budget: Provide a breakdown of budgeted versus actual expenditure and the reasons for any differences. All figures should be in £ sterling, indicating the local exchange rate used. It is important that you retain the management accounts and all paid invoices relating to the project for at least 2 years as these may be required for inspection at our discretion.

Item	Budgeted Amount	Actual Amount	Difference	Comments
Field trip – Podrašnica locality	78	69	+9	During the two field research trips we managed to fulfill the planed task and refute the allegations of european spadefoot toad presence at this questionable location.
Shallow and neoprene boots	55	68	-13	
Labaratory	60	29	+31	We bought just needed chemicals that were missing at the Veterinarian Institute for skeletochronology method (microscope slides – glass and cover slides, xylene, 96 % alcohol, 3 % HCl).
Sample preparation	250	250	0	

Printing cost/Promo material	225	446	-221	We used extra money from the gas budget since the design and printing costs were higher that expected.
Handnets (two)	26	30	-4	
Datalogger DT-171 (LOG32) (two)	134	162	-28	
HOBO Software Pro	90	106	-16	Instead of HOBO Software we bought OneSet HOBO Optic USB Base Station and downloaded the software separately for free.
Datalogger PRO-V2 U23-003 (two)	536	461	+75	
GoPro accessorise	86	45	+41	
GoPro Hero Black 5	446	494	-48	
Batteries	30	-	+30	The price is included in" Shallow and neoprene boots".
Measuring scale 0.01	45	-	+45	The price is included in" Shallow and neoprene boots".
Lectures – gas bills	400	470	-70	
Field trip – June to September 2018	360	383	-23	
Field trip – May 2018	300	148	+152	
Field trip – March to April 2018	510	249	+261	
Field trip – July to September 2017	345	279	+66	
Field trip – June 2017	510	468	+42	In May and June fieldtrips were conducted from Zagreb, Croatia. Travel expenses were higher due to 200 km longer trips, higher gas prices and road tolls. Four field trips were done in June.
Field trip – May 2017	510	400	+110	In May and June fieldtrips were conducted from Zagreb, Croatia. Travel expenses were higher due to 200 km longer trips, higher gas prices and road tolls. Five field trips were done in May.
TOTAL	4996	4556	+440	Extra money will be spent for educational boards (design, wooden stand, glass, printing) and covering one part of venue expenses in Čardak, Modriča municipality.

Notes to budget

- *The medium of exchange rate for conversion to Pounds Sterling used in this project was used from NLB Razvojna Banka, Banja Luka on date 23.03.2017. was (http://www.nlbrazvojnabanka.com/Kursnalista.aspx):* ***1 GBP = 2.253520 BAM***
- *The medium of exchange rate for converstion to Pound Sterling used for final calculation is used from from NLB Razvojna Banka, Banja Luka on date 16.03.2018. (https://www.nlb-rs.ba/stanovnistvo/alati/kursne-liste/):* ***1 BAM = 0.45 GBP*** *(1 GBP = 2.221398 BAM)*
- *Budget was rearanged and adjusted due to small differences in eqipment and promo material prices. Also slight differences were caused by currency conversion.*

9. Looking ahead, what do you feel are the important next steps?

The next important step is to save the Bosnian common spadefoot toad population - Čardak population, to restore its water habitat since we notice there is a big chance of habitat degradation and water eutrophication, which is fatal for this toad survival. If we get the good results from the habitat restoration, potential protected area declamation and positive reactions from the locals, I've planed to expand the positive local impact to other localities from eastern to western part of Posavina.

10. Did you use The Rufford Foundation logo in any materials produced in relation to this project? Did the Foundation receive any publicity during the course of your work?

The Rufford Foundation logo was used with printed leaflets, brochures, roll-ups, other promo material, on the web-page (www.bhhuatra.com), facebook page (BHHU ATRA), power point presentations, promo and educational video (https://www.youtube.com/watch?v=P0XwcISMLYw&t=4s) and posters.

The Pelobates project and Rufford fondation was once again promoted through media:
http://www.nocistrazivaca.ba/2018/09/21/ana-curic/

https://www.bhhuatra.com/news/zastita-slozenih-vodenih-kompleksa-i-terestricnih-stanista-preferirana-od-strane-ekstremnog-ontogenetskog-metamorfa-zabe-cesnjarke-pelobates-fuscus

Logo was used according to the instructions received from the RSGF.

11. Please provide a full list of all the members of your team and briefly what was their role in the project.

Ana Ćurić – Project leader. Have been participating in every field trip, taking morphological, morphometry and meristic measurements of adult individuals and tadpoles, taking physical parameters, coordinates. Ana was working on the skeletochronology, taking separate field trips and collecting samples. She has been processing all the data collected during the project writing the results as papers for publishing. Also, she was the lecturer in Banja Luka and Mostar, presenting the

project on conferences in Bar, Montenegro (*Rufford conference "From mountains to deep sea*) in February 2018 and Poreč, Croatia (*2nd Balkan Herpetological Symposium within 13th Croatian Biological Conference*) in September 2018. She was the main negotiator with locals and local govenment in Čardak locality.

Bojana Milinković – Conducting the field trips collecting spadefoot toads for morphometry study and determine herpetofuna specimens and gathering all the field data in digitalized form.

Bojana Vukašinović - Lecturer in Gradiška.

Adnan Zimić – Conducting the field trips collecting spadefoot toads for biological reserach and determine herpetofauna species. Adnan had a big impact on project promotion helping with design and also papers writing and publishing.

Berina Vrhovac – Conducting the field trips, working on data gathering on field and lecturer in Sarajevo.

Tamara Trifković – First year bachelor student joining the field trips for training, learning and helping with spadefoot toad research.

Jovana Herceg - First year bachelor student joining the field trips for training, learning and helping with spadefoot toad research.

Gabijela Jeftić - First year bachelor student joining the field trips for training, learning and helping with spadefoot toad research.

12. Any other comments?

By the end of 2018 all results (skeletochronology and adult morphometry, physical data) will be proceeded and prepared for publishing. Educational boards will be placed in Čardak locality.

We are very satisfied with the project outcome. We managed to achieve even more than we expected. Gas prices varied during the whole researched period so there is a variation in spent money. Then we combined months with less spent money and fulfill other months having more field trips.

I would like to thank RF once again for recognising our enthusiasm and work in protection of European common spadefoot toad and for helping to achieve this project idea and goals, and to continue promotion of herpetology in Bosnia and Herzegovina. This project helped us connect with Faculty of science and mathematics, University of Banja Luka, Department of Biology, Faculty of Science, University of Zagreb, Croatia and Veterinarian Institute "Vaso Butozan", Banja Luka, Bosnia and Herzegovina.

Thank You once again for the opportunity that helped me and my team to realise our project idea!