The Rufford Foundation

Final Report

Congratulations on the completion of your project that was supported by The Rufford Foundation.

We ask all grant recipients to complete a Final Report Form that helps us to gauge the success of our grant giving. The Final Report must be sent in **word format** and not PDF format or any other format. We understand that projects often do not follow the predicted course but knowledge of your experiences is valuable to us and others who may be undertaking similar work. Please be as honest as you can in answering the questions – remember that negative experiences are just as valuable as positive ones if they help others to learn from them.

Please complete the form in English and be as clear and concise as you can. Please note that the information may be edited for clarity. We will ask for further information if required. If you have any other materials produced by the project, particularly a few relevant photographs, please send these to us separately.

Please submit your final report to jane@rufford.org.

Thank you for your help.

Josh Cole, Grants Director

Grant Recipient Details	
Your name	David Brankovits
Project title	Integrating Research and Education to Protect Anchialine Cave Ecosystems in Quintana Roo, Yucatan Peninsula in Mexico
RSG reference	13195-1
Reporting period	May 2014
Amount of grant	£5490
Your email address	brankovd@tamug.edu or david.brankovits@gmail.com
Date of this report	10 May 2014

1. Please 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
1) Stable isotope analysis: investigate organic matter flow within and among anchialine cave systems.			√	This goal was achieved by successfully obtaining water, cave fauna, sediment, soil and plant samples for stable carbon and nitrogen isotope analyses. This allowed, first, to learn about spatial variations in terrestrial organic matter inputs along the cave system (i.e., qualify and quantify the organic material entered the subterranean waterways from different surface environments), and second, to determine the importance of different organic matter sources for the endemic cave-adapted food web community. In addition to the original plans, I was able to utilise further analytical techniques to study carbon and energy inputs in more details within the underground ecosystem (e.g., bio-markers and compound-specific stable isotope analysis). Two scientific publications are in preparation.
2) Water quality analyses: investigate contaminants and pollutants within the underground waterways.			√	Water samples were successfully collected from the underwater caves. These samples were, first, analysed for carbon sources (Dissolved Organic Carbon, Dissolved Organic Carbon and methane) and, second, for water quality measures (nitrates, nitrites, ammonia, total phosphorus, pH, dissolved oxygen, suspended solids, faecal bacteria, and heavy metals). Carbon sources were analysed to compliment objective 1. Water quality measurements were conducted to learn about the contaminants transported within the subterranean waterways. One scientific publication is in preparation.
4) Making an educational movie.		√ in progress		Footages from the field and laboratory were taken to make an educational movie about the subterranean habitats and increase awareness of the biological, ecological and economic importance of anchialine ecosystems. As it was

				originally planned in the project proposal, cutting and finishing up the educational movie will be done late summer or early fall this year (2014).
5) Dissemination of research and ensuring project continuity.		√ in progress		The educational movie and the publications are in preparation; the preliminary results were published in scientific symposiums and were successfully used to apply for further funding to ensure the continuation of this project.

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

Starting collaboration with some of the local conservation groups and scientists was more difficult than I originally anticipated. Nevertheless, the project has overcome this issue because field work allowed making new connections with Mexican cave diver groups and scientists from UNAM (Universidad Nacional Autónoma de México).

Weather has affected the groundwater conditions (e.g., water level and visibility) at sampling sites during the second field trip (December 2013) making sampling and filming more difficult. As a result, some of the objectives had to be postponed to another field expedition (June 2014). All objectives have been achieved eventually (e.g., sample collection) or being fulfilled currently (e.g., part of the filming).

Transporting research equipment to Mexico provided difficulties during our last field trip when, while entering the country, at customs we had to show additional permits to what we had used in the past. This resulted in a week long delay in starting the sampling. Necessary paperwork has been obtained with the help of local collaborators at UNAM.

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

(1) This project has successfully obtained samples to provide the first detailed information on the organic matter flow and contaminants within the Ox Bel Ha, the longest underwater cave system of the world. First, it has been determined what the most important food sources are for endemic cave-adapted fauna living in the subterranean waterways. Second, we collected novel scientific information about the water quality and water chemistry of the stratified water layers. Samples have been collected; part of the laboratory work is done; other part of the lab work and the data analysis are still in progress.

(2) This study has provided unequivocal evidence of the importance of overlying natural vegetation for the anchialine cave ecosystems. This information has crucial importance in management and decision making. We have proof that compounds originated from biogeochemical processes (e.g., fermentation and methanogenesis) in the subsurface soils of surface vegetation (e.g., tropical forest and mangroves) are washed into the cave systems and are utilised by naturally occurring bacteria. These bacteria are then consumed by endemic cave adapted crustaceans. Consequently, we have tantalising evidence that the

surface-derived natural compounds provide crucial energy source for the food web in this cave environment where nutrition is sparse and limited. These surface-derived compounds contain degraded organic matter and reduced compounds, e.g., methane. For example, methane, converted into a package accessible for invertebrates by bacteria, seems especially important in fuelling the studied ecosystem. The preliminary results were recently presented in a scientific symposium with great success. In addition, it will be published in a conservation oriented scientific journal.

(3) During the course of this project, we have successfully developed a new water sampling protocol which allows maximising the information we gather on scuba in a cave environment with complex hydrology. This technique involves two important steps on the field. First, it consists of simplified water sample collection on scuba from the stratified water layers of an anchialine cave system. Second, it involves a protocol for dividing up each water sample into several sub-samples which will be preserved and analysed separately. As a result, we have been able to obtain novel environmental information on water quality and water chemistry in the stratified water layers (i.e., fresh, brackish and salt water). Furthermore, we maximised the information gained during a single cave dive the safest possible way. This is especially important in a potentially hazardous overhead environment where monitoring, sample collection and other surveys are limited by multiple environmental factors. As a result of our success, this technique was shared with Mexican scientists to facilitate wider application. We plan to publish the protocol as a novel method in a scientific journal and make a 3 minute-long method film to share the technique with other scientists or interested parties.

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

Establishing collaboration with Mexican scientists from UNAM was extremely successful. Besides fulfilling the proposed goals on the field, we managed to obtain environmental samples for two UNAM research groups and two Mexican graduate students working in different scientific fields. Helping Mexican scientists and facilitating the research of local students was beneficial for several reasons. First, raising their interest about anchialine cave research could have far reaching impact on this scientific field. Second, their research allowed us to broaden the objectives of this project and answer more questions in terms of the ecology and current environmental condition of these subterranean natural resources.

We have established connections with cave conservation focused local groups and individuals in Quintana Roo. Scientific information on the ecology and biology of the vast cave systems were shared with these parties to aid their fight against developments that, if put through, would destroy some of these subterranean natural resources and would negatively affect the groundwater. Some of the developments are threatening habitats of endemic and endangered anchialine cave invertebrates (mainly crustaceans) that can only be found here.

5. Are there any plans to continue this work?

Yes, there is. (1) It is planned to develop a modified version of our water sampling protocol. This modified version would allow non-scientists to collect water samples independently from us. Creating a network among local cave divers will allow monitoring water quality and chemistry on a

larger spatial and temporal scale. (2) Since our results suggest that bacteria living in these caves have a profound and direct effect on the cave adapted invertebrate community, we plan to thoroughly examine the bacterial diversity in natural versus human impacted (e.g., anthropogenic contamination) cave systems. With the help of preliminary results of the current Rufford project, further funding have been obtained from several organizations (TAMU-CONACYT Research Funds for Collaboration between USA and Mexico, National Speleological Society, Explorer's Club, and Cave Research Foundation) to continue this work.

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

These findings will be published in scientific journals. Three manuscripts will be submitted to different journals. The first one is planned to be submitted by December 2014:

Brankovits, D., Pohlman, J., Iliffe, T., Niemann, H., Alvarez, F. (in preparation). The importance of natural methane inputs for an anchialine cave ecosystem in the Yucatan Peninsula, Mexico.

Two short videos are in preparation. The first movie will be an educational video explaining the biological, ecological and economical values of these subterranean ecosystems and presenting our scientific findings (5-10 min). The second one will be a short method movie about the water sampling protocol that was developed as part of this project (3-5 min).

Project findings and details will be shared via my advisor's website (www.cavebiology.com).

These results have been and will be shared with local nature conservation groups.

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

According to the original proposal, two field trips were planned (June 2013 and January 2014). However, due to unforeseen difficulties (please see point 2.) some of the objectives of the 2nd field trip had to be postponed to June 2014.

Rufford grant was used for the following field activities:

- July - August 2013: First month long expedition to determine the exact location of sampling sites and start collecting samples;
- December 2013: Second expedition to the same sampling sites. Objectives involved sample collections and filming. Difficulties had arisen because of water conditions due to weather and an unexpected permit requirement when we were transporting research equipment into the country. As a result, some of the proposed objectives were postponed.
- June 2014: Third field trip. This field trip was not planned in the original proposal. Objectives involved sample collections and video recording with an emphasis on achieving the postponed objectives of the December 2013 field trip.

8. Budget: Please 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.

Exchange rate: 1.6:1=USD:GBP and 21:1=MXN:GBP

Item	Budgeted Amount	Actual Amount	Difference	Comments
Airfare (Houston-Cancun)	350	350	0	
Extra baggage fee	65	131	-66	Additional baggage fee was paid for the extra field trip in (June 2014) to make up the unaccomplished objectives of the December 2013 field trip.
Rental car and fuel	920	969	-49	
Accommodation	175	195	-20	
Food	420	406	14	
Local field assistants	320	250	70	
Tank rentals and gas fill	440	438	2	
Field GPS unit and accessories	350	0	350	This amount was spent on a different item due to unexpected equipment failure. A GPS was not purchased but we borrowed one for each field trip.
Cave diving equipment	0	350	-350	Cave diving equipment was purchased due to an unexpected equipment failure.
Safety underwater lights	310	344	-34	
Field equipment for research diving	250	250	0	
Sample collecting equipment I. (falcon tubes, vials, and coolers)	400	294	106	Some of the equipment was borrowed instead of purchasing new gear.
Sample collecting equipment II. Glassware and filter for water sampling	500	450	50	Some of the equipment was borrowed instead of purchasing new gear.
Safety backup hard drives for video and data	120	125	-5	
Compact underwater HD video camera	320	313	7	
Other general	200	269	-69	Shipping to and dry ice in Mexico was more costly than originally anticipated.
Unexpected field expenses (~4% of total field budget)	350	350	0	This was used to purchase flight ticket for an extra field trip (June 2014) to make up the unaccomplished objectives of the December 2013 field trip
Total	**5490**	**5484**	**6**	

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

First of all, I intend to finish up the educational movie and the method film (please see point 1.). The first manuscript will be submitted to a scientific journal by the end of this year. As a continuation of

this project I plan to unfold the research in two complementary directions: (1) start a long-term water quality monitoring with the help of local cave divers and (2) examine differences in bacterial diversity between natural and human impacted anchialine cave systems (please see under point 5.).

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

Yes, the logo was used in poster presentations and scientific talks.

11. Any other comments?

I will use scientific findings gained from this project for my PhD dissertation.