

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	Juan Scheun
Project title	Towards the conservation of declining amphibians: a new non-invasive hormone monitoring technique for assessing adrenocortical and reproductive activity
RSG reference	21464-1
Reporting period	February 2017 – February 2018
Amount of grant	£5000
Your email address	Juan@sanbi.org.za
Date of this report	10 April 2018

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
Validate dermal secretion as a matrix for monitoring stress hormone patterns in amphibians				We successfully reached all three of our original objectives with regard to the validation of the stress hormone technique, the matrix, and the most appropriate body location for collecting and monitoring the stress hormone pattern in amphibians.
Validate dermal secretion as a matrix for monitoring reproductive hormone patterns in amphibians				The process of validating this technique required additional months and additional funding. As this was a secondary objective, the attention was kept on the stress analysis. We hope to conduct this research in the near future however.

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

(i) In South Africa all research conducted needs the clearance from the Department of Environmental Affairs (DEA). Unfortunately, this clearance can take months to receive and resulted in the study starting a bit later than planned. As the study grew and the validity test increased in its robustness, new clearance certificates were required. Although this was a timely process in the beginning, it did allow us to develop a clear line of communication with the DEA and resulted in additional amendments being issued in a relatively short period of time.

(ii) To ensure the technique was validated to the best of our ability, we increased the number of individuals used during the study. Furthermore, to further ensure the technique was robust, we included a control group in the study as well. Although both these factors increase the validity of the study many folds over, this in itself made the study logistics complicated. To handle the increase in group numbers and workload, we optimised housing conditions to ensure sampling could be done quickly and efficiently, while giving individuals an enclosure which would ensure minimum housing stress. To minimise the workload, we trained students, interns and volunteers to assist in sample kit preparation, frog handling, enclosure design and cleaning, sample storage and extraction protocols.

(iii) Following the swabbing event, and the beginning of the extraction process, our multitube vortex machine unfortunately broke. We were able to borrow a replacement from colleagues of the University of Pretoria, but this had to be

returned, halting further extractions. The Rufford Foundation was kind enough to allow us to purchase a vortex which could be used for the study specifically. This alone allowed us to finish the extractions and start the analysis of samples at the University of Pretoria.

(iv) As mentioned in the previous section, as a result of the expansion of the stress hormone monitoring study, and the important results acquired, the funding for the secondary, reproductive section was limited. Although we collected a large amount of samples prior to, and during the 2017/18 period which could be used for the reproductive hormone monitoring validation, the financial implication of analysing these samples would have been far in excess of what was available. As such we chose to focus our attention on the ability to monitor stress hormone patterns in amphibians, as this is one, if not the, most important factors which can assist in amphibian conservation practices.

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

1. The project successfully validated the method for monitoring glucocorticoid concentrations in dermal secretions in amphibians. Comparing the different enzyme immunoassays at our disposal for this, meant that we validated the most robust technique in this regard.

2. Additionally, the study confirmed that both the dorsal and ventral body regions could be used to monitoring glucocorticoid secretions. Although the findings point to the ventral region producing dermal secretion at a higher rate than the dorsal region, the use of the ventral region in wild, free-ranging amphibians may be hampered by contamination.

3. The urine glucocorticoid metabolite patterns observed in the frogs supported the notion that the ACTH injection activated the HPA axis, which was observed in the dermal secretions of the study animals. However, with the creatinine analysis failing, a Specific Gravity analysis is now required to confirm the validity of the results.

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

As the project was the first step in developing and implementing a new technique, we could not involve local communities. However, we did train volunteers, interns and students in animal ecology and physiology, handling, sample collection and storage, as well as extraction protocols. Future research will include these and other community members in the field.

5. Are there any plans to continue this work?

Should further funding be acquired we will aim to: (1) validate the method for monitoring reproductive hormones; and (2) collect samples from free-ranging populations in pristine and heavily degraded habitats within South Africa.

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

The results from the study will be published in an internationally recognised, peer-review journal. Additionally, the results will be presented at national and international conferences in 2018/19.

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

Rufford funding was obtained in February 2017.

The date of completion for the study was March 2018.

As a result of the loss of equipment and the delay in sample analysis by the University of Pretoria, the study only concluded in May 2018.

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.

The 5000 **£ sterling** received from the Rufford Foundation resulted in 81 881 South African rands, at R16.3762 for 1 **£ sterling**.

Item	Budgeted Amount	Actual Amount	Difference	Comments
Enzyme immunoassay analyses	4600	4301	299	1. Dermal secretion: glucocorticoid monitoring 2. Urine glucocorticoid confirmation and creatinine analysis
Animal Upkeep, Cage optimization, Ambient condition monitoring, Sampling materials	400	482	82	1. Cage optimization was required to enhance sampling procedures of all individuals 2. Animal upkeep 3. Digital temperature and humidity reader to ensure optimal environmental conditions remained constant
Vortex machine	0	2164	2164	1. Vortex purchase for sample extraction

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

It is of great importance that the technique be employed in the natural environment to ensure it is used for assessing population stress and vulnerability to anthropogenic activities. For this reason, the technique should be a staple of all environmental impact assessment procedures in South Africa and globally.

We hope to use this technique in a number of habitats within South Africa in 2018/19 to determine the effect of human-driven activities and disease (mining, agricultural and urbanisation) on amphibian population stability and survival-likelihood.

Furthermore, we hope to confirm the use of this technique for monitoring reproductive hormone patterns within amphibians to assist with conservation practices of endangered amphibian species. Although this will require further validation and time, the process will be less labour intensive than the validation of stress hormone monitoring.

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

The Rufford Foundation logo was used in all presentations and discussions regarding this study. Specifically, the logo was used during a presentation at the 11th International Conference on Behaviour, Physiology and Genetics of Wildlife held in Berlin, Germany from the 4th-7th of October 2017. Further presentations in South Africa, and globally, will display the Rufford logo when the project is presented.

The manuscript which is in preparation will also ensure that the Rufford Foundation is acknowledged.

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

Dr Juan Scheun – Principle investigator

- Project logistics
- Sample collection
- Sample extractions
- Data analyses
- Manuscript preparation

Prof Andre Ganswindt

- Project logistics
- Sample analyses
- Manuscript preparation

12. Any other comments?

Our thanks go out to the Rufford Foundation for funding this research. We believe this is the first step in enhancing amphibian conservation on a global scale. There are still a number of step to conduct and questions to answer, but we believe this to be a positive, rather than seeing the technique as incomplete.