

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

Your name	Shivan Parusnath
Project title	Conservation genetics and population ecology of the Sungazer (<i>Smaug giganteus</i>), a Threatened endemic lizard
RSG reference	13956-2
Reporting period	1 March 2014 – 1 March 2015
Amount of grant	6000 GBP
Your email address	Shivan.parusnath@gmail.com
Date of this report	12/09/2015

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
Distribution-wide tissue collection			X	Over 500 tissue samples have been collected from Sungazers through non-invasive sampling techniques.
DNA extraction from Sungazer scales			X	Various extraction techniques were tested in order to develop the most promising protocol. We are able to get up to 20 ng/ul DNA from highly degraded tissue samples.
Microsatellite development			X	Microsatellite markers have been selected and tested to develop a final panel of ~20 markers.
Sequencing of Sungazer samples		X		The sequencing of these samples is a time consuming process and is only part way completed. Sequencing is anticipated to complete in late October.
Population study	X			This component of the study did not end up being carried out during this project, and is being planned for inclusion in a more comprehensive population ecology study as a continuation of Sungazer research.

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

Due to this being the second Sungazer conservation project funded by an RSG, field problems experienced in the first leg of research were anticipated throughout the second field season and well prepared for (eg. farm access, rough terrain). Contacts made during the first project made accessing Sungazer populations much easier due to the trust gained from landowners over many years of working with them. Vehicular problems experienced previously (getting stuck in muddy areas, not being able to access certain farms) has necessitated the use of offroad vehicles, which allow greater access to Sungazer populations throughout the distribution.

The primary challenge during this project was developing a technique to extract high quality DNA from Sungazer scales. Due to Sungazers being a threatened species, and seasonal in activity, a non-invasive sampling strategy was adopted early in the project design. This allowed the collection of five times the number of tissue samples than would be possible through the time-consuming and potentially stressful exercise of collecting tissue from

animals. Therefore we amassed an impressive ~500 sample tissue collection, although most of these were degraded and low in DNA yield. We underwent rigorous testing of different extraction techniques and PCR enzymes, to ultimately develop a technique that would work for 95% of these samples. This is a result that itself will be published in a peer reviewed journal.

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

- A. Collection of an extensive sample of tissue, from over 500 Sungazers spread widely across the distribution of the species. This resolution of tissue collection will make this study the most comprehensive research of any African reptile. Furthermore these samples were all collected non-invasively – a technique that is not widely used in reptile biology. Non-invasive sampling allows tissue to be collected without stressing out or disturbing animals, while bolstering the potential sample size.
- B. Developing techniques to extract DNA from degraded tissue samples. Tissue samples that have been collected as shed skin/scales may have been lying in soil, exposed to sunlight and rain for weeks or months before being collected. This results in degradation of the DNA and forensic techniques are necessary to extract DNA that is workable. Through careful testing of various protocols we have been able to develop a protocol that yields DNA in concentrations that are useable in downstream applications.
- C. Development of the first species specific microsatellite markers. Besides the use of these markers in investigating the relatedness of Sungazers within and between populations, there is also the potential of using these markers to assess parentage for animals that are to be traded internationally. Sunagzers are listed as a CITES II species, and require proof of an F2 generation before permits are granted. As there has been no means of proving this with testing prior to these markers being developed, it is believed that many Sungazers poached from the wild have been traded as captive bred. These markers are now freely available for use by national facilities to conduct these tests. It is our hope that this becomes a major obstacle to Sungazer poaching in South Africa.

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

My fieldwork involves travelling across the Free State and Mpumalanga provinces of South Africa and engaging with local landowners in order to facilitate sampling of the Sungazers on their property. As a result of my previous leg of conservation work which was conducted in my previous RSG period, and working closely with the Endangered Wildlife Trust (EWT), we have started facilitating a stewardship programme with landowners who have healthy Sungazer populations on their land. Landowners benefit from tax reductions, while declaring habitat containing Sungazers as protected area. Landowners can also partake in regular surveys of their Sungazer populations on a voluntary basis. This has been identified as a

critical step in monitoring populations in the long term. Furthermore to providing landowners with the opportunity to take part in stewardship programme, this project has allowed several biology undergrad students to partake in field research, with the result that one student has joined the Sungazer research team and is interested in pursuing a Masters in Sungazer biology.

5. Are there any plans to continue this work?

Yes, definitely. This body of research represents the first conservation efforts directed towards this species in the country in over thirty years. As our research progresses, we identify an increasing number of gaps in knowledge that are necessary to successfully conserve the species and its habitat, which is also highly threatened. The first research project aimed to assess the status of the species through intensive surveying and mapping. The second project aimed to investigate how the widespread fragmentation discovered across the distribution is affecting genetic structure, and provide an insight into how Sungazers disperse within and between populations. This will allow us to understand how movement corridors should be planned into the current stewardship programme, as well as paint a more complete picture of the conservation status of the species, from a novel genetic standpoint. The third leg of research stemming from this project will aim to elucidate the population ecology on a fine scale, and in conjunction with the genetic project, provide a complete picture of how Sungazer populations function.

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

The primary findings from this study will be disseminated through the publication of scientific papers in international peer-reviewed journals, as well as international conferences. Two conference presentations have already been delivered within the reporting period - first was the 12th Conference of the Herpetological Association of Africa, and the second was the first 2015 Southern African Rufford Small Grants Conference. While only preliminary results are available for this research at this point, these, along with results from my previous research project funded by my first RSG were presented.

The report from this conference can be found here: http://www.rufford.org/grant_recipients_conference_south_africa_2015. A highlight from the RSG report about my research: "Another project that showed major catalytic results from the funding was the Sungazer Project run by Shivan Parusnath. This project has led to a broader understanding of the conservation requirements and current status of this previously poorly understood species. The results are guiding the development of a Biodiversity Management Plan, update of the red-list status, development of a formal Non-detriment Finding in line with CITES II requirements and are guiding the geographic focus of a broader Biodiversity Stewardship project aimed at protected area expansion in the region. Of course we must never forget the individual development, Shivan is now working towards his PhD in genetics focussed on this same species and is certainly the world expert on the species today."

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

The budget was used roughly within the time period planned (October 2014 – Jan 2015). As with most reptiles, Sungazers are only active throughout the warmer months of the year, and therefore field research has to be done within this time period. The extensive tissue collection was highly efficient, and afforded the collection of ~500 tissue samples – roughly ten times more than could be collected in the same time period if animals had to be captured for this purpose. A part of this research project that has taken significantly more time than anticipated was the design of microsatellites, and the optimisation of the techniques to extract DNA from the degraded tissue samples. In the initial stages of project design we were unsure as to whether the use of microsatellites would be necessary to give us the resolution needed for the study, or if nuclear and mitochondrial DNA would be suitable enough. Eventually we did decide to use microsatellites, supplemented with the other markers to achieve as complete a picture as possible. This did differ slightly from the initial proposal, however the component of the project that the RSG funded was used for the same purpose.

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.

Item	Budgeted Amount	Actual Amount	Difference	Comments
Accommodation	5283	4680	603	Tissue collection took about 20 days less than initially anticipated, due to the success of the non-invasive sampling technique.
Travel costs	2932	3840	908	Fuel costs escalated over the time period more than was anticipated, driving up the total fuel costs for the project. This was confounded with slightly more driving per day to reach our field sites than anticipated.
Total	8215	8520	305	Out of the total £8215 projected for this study, only £6115 was requested from The Rufford Foundation. The remaining costs were recovered from various other small grants from my host institutes, and a small portion of a Mohamed bin Zayed Species Conservation Fund grant received for a broader Sungazer project.

An exchange rate of 1 GBP = 15.9 ZAR was used in the application process and used again here for the purposes of comparing the budgeted amounts to the final amounts.

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

With each stage of conservation research on Sungazers, further gaps in our knowledge of the species' life history become obvious. During the course of this project, a small component relating to population ecology was planned, but became unfeasible due to the non-invasive sampling technique used. Rather than seeing this as a setback, this component has been fleshed out and a more rigorous, long term population ecology study is now planned for the species, by our research team. While in the field, it became clear that a genetics approach alone, while giving us information that would not be possible to collect without many years of fieldwork, would not paint a complete picture of Sungazer population ecology, movement patterns, reproductive systems etc.

The project we have conceptualised involves a population study that looks at long-term patterns through mark-recapture studies of Sungazers marked several years ago with PIT tags, and the seasonal monitoring of Sungazers that will be fitted with radio transmitters. This will also serve as the initiation of a long-term monitoring of several different colonies of Sungazers that are all on private property, but with different land management strategies. Some sites are left completely natural, whereas others are grazed, and at least one is near to a mining area. This will allow us to assess how these different management strategies affect populations over long and short term periods. Eventually, this will lead to more informed decisions about how to manage Sungazer populations and their habitat to ensure prosperity in the future.

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 Sungazer research funded by this RSGF, as well as the first RSGF have been presented at several national and international conferences (two within the reporting period of this grant). All these presentations have prominently feature the RF logo, and the foundation mentioned as primary funder to Sungazer conservation research. There are several publications planned from the results of this project for peer-reviewed journals, which will all acknowledge the RSGF as a primary funder.

11. Any other comments?

As with the first RSG used on Sungazer conservation research, this grant provided a much needed lifeline to the project, without which, this research may have failed to get off its feet. South Africa is a country with an impressive level of biodiversity, but a lack of funding available to conduct research on its vast array of flora and fauna. The RSGF has developed the reputation in South Africa for providing funding to conservation projects that otherwise may not have ever been undertaken, as well as providing early career conservationists an opportunity to engage in field research on a level otherwise impossible.