The Rufford Small Grants Foundation

Final Report

Congratulations on the completion of your project that was supported by The Rufford Small Grants 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	Jonathan Kolby
Project title	Forest canopies as safe havens from Amphibian Chytrid Fungus, phase II: Enigmatic dispersal mechanisms and environmental distribution.
RSG reference	7859-2
Reporting period	Nov 9 2010 – July 8 2011
Amount of grant	£5985
Your email address	J_kolby@hotmail.com
Date of this report	July 7 2011

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
Sample arboreal bromeliad water for chytrid; deploy sampling equipment to collect wind-blown particles.			X	Canopy access rope climbing techniques employed to scale trees and conduct arboreal surveys. Extremely challenging and rewarding. Arboreal research is fantastic and something I hope to promote and help more people explore.
Deploy weather stations to monitor environmental conditions at chytrid sampling sites			X	No complications in deployment, but one of the data loggers malfunctioned and stopped recording information for a period of time during the season.
Radiotrack endangered amphibians to determine dispersal patterns of chytrid infected individuals			X	The presence of much fewer adult frogs than previously encountered at the particular study site hampered the volume of data collection desired. On a few occasions, the waistband irritated the frog's skin and was removed immediately to protect the frog's health. This never occurred during 2009, so it is uncertain why this happened following the same protocol with the same materials in 2010.
Conduct chytrid surveys in endangered amphibian species, arboreal and terrestrial environments (bromeliad and river water), rain, on the exoskeletons of arthropods, and on the soles of footwear.			X	No complications; all components went exceptionally well.

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

The greatest unforeseen difficulty arose during the data analysis portion of my project regarding the detection of chytrid in rainwater. During the project, I did not foresee that the nature of my methodology would not allow me to refute the possibility of amphibian contamination in my buckets of rainwater. Since the filter samples require a high volume of water to produce accurate results,

the rainwater buckets were left to collect water over the span of several rain events to accumulate enough volume to be processed. The buckets were left for approximately two weeks at a time between sampling, and although this worked and enough water had been captured, I cannot visually confirm that a frog did not drip chytrid-laden water drops into my buckets. As a result, I just completed a follow-up field survey for this aspect of my project. I returned to my study site in June 2011 and repeated this study, but with close attention to the previous flaw. I constructed a simple rain collector out of a 4x4m sheet of plastic suspended in a location where no trees were present. A drain was affixed in the center and guided water into a single bucket below. Water was either filtered immediately or sealed and filtered the next day, as time allowed. In this way, I was able to collect a significant volume of rain in a single rain event and closely monitor my equipment to ensure that amphibian contamination was not possible. Although a relatively simple project, the results will be very robust and provide significant support to the preliminary findings produced in 2010.

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

1. My chytrid survey results from 2010 now show that the prevalence of infection in the endangered amphibians I am studying is still quite dynamic. From my first survey in 2007 to the most recent in 2010, infection rates have bounced up and down by nearly 30% in some species between years. This is extremely worrisome and such instability within these species represents a high potential for population declines. Although I have proven that chytrid has been present at my study site in Honduras since 1996, for unknown reasons chytrid has not yet reached stable levels of endemic infection in these amphibians, as would have been expected. It is a widely accepted belief in my field that amphibian mass mortality and extinctions driven by chytridiomycosis occur soon after the initial arrival of chytrid to a particular region, as is currently publicised by the urgent situation in Panama where scientists have been rushing to stay ahead of the "wave" of chytrid to rescue amphibians prior to severe declines and possible extinctions. In contrast, my work in Honduras strongly suggests otherwise; that even after 15+ years of exposure, populations can still be at risk of significant declines and deserve urgent attention as well. To the best of my knowledge, no one else has conducted repeat infection survey within the same amphibian populations over more than one season and it is assumed that infection levels do not change significantly. Although I cannot yet explain why the infection prevalence levels observed in the species in Cusuco have fluctuated so highly, it may be due to the effect of additional stressors which may contribute towards host immunosupression. Just a few weeks ago, a significant portion of the forest was illegally logged at my study site, causing worry that this sudden change in environmental conditions may unfavourably affect individuals already carrying infections and tip the scales towards mortality and decline.
2. I have demonstrated the presence of chytrid in rainwater, providing the first data suggesting a rain-based aerial dispersal pathway of amphibian chytrid fungus. This is arguably the most exciting discovery of my project both in terms of its implications for understanding patterns of regional and global chytrid dispersal and for illuminating the threat of chytrid to arboreal and terrestrial amphibian species and a possible mechanism of exposure. Chytrid is widely accepted to be an aquatic pathogen associated with permanent terrestrial water bodies, and hence arboreal and terrestrial amphibian species have not received as much attention for conservation activities, especially those which undergo direct metamorphosis. I will continue to dedicate a great deal of effort to further investigate rain-driven dispersal of chytrid fungus and consider dispersal ranges, viability of zoopores, and the role it may play in global dispersal.

3. While I fully support and follow responsible research activities and decontaminating footwear and equipment before moving between study sites, the aspect of my project considering a human-footwear based chytrid dispersal pathway demonstrated that this is nearly impossible to occur at any scale of ecological significance. Despite standing in a river of known chytrid presence, it was not possible to detect chytrid on boots which had been submerged after walking only 15m from the water's edge even after many attempts. Water samples from the rivers were simultaneously sampled to confirm the presence of chytrid and all sampled produced positive results, although showed very low concentrations of spores (i.e. 1 chytrid zoospore/L). Accordingly, I conclude that in Cusuco National Park, the movement of people wearing footwear exposed to chytrid-positive water bodies does not affect the patterns of dispersal and distribution of chytrid in this environment, although this may differ in other regions where the concentration of chytrid in river water might be higher. Still, in terms of Cusuco, this is quite significant. Scientists are frequently targeted as an avenue for chytrid to hitch a ride and infiltrate pristine remote locations. In Cusuco, this appears to be untrue, despite the frequent presence of scientists of all taxa, most of whom are neither familiar with chytrid fungus nor informed to decontaminate before and after entering the forest. As such, this brings everything back to the original question; how did chytrid arrive in Cusuco and what drove its subsequent widespread dispersal throughout the forest and across dry land? The movement of people would have been a simple explanation, but now excluding this factor and making note of the absence of any amphibian trade in the region, what is the driving force? This is also something I will heavily focus on during my upcoming PhD.

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

5. Are there any plans to continue this work?

Yes, applying the knowledge developed by this project, I have designed an investigation to tackle the questions surrounding global amphibian chytrid dispersal in an attempt to explain and address the current extinction crisis. With this project, I applied for a PhD at James Cook University, Australia, and I was recently offered candidature. The goal of my project is to use all the data I have collected in Honduras (natural dispersal pathways) and combine it with data I intend to collect from within the international wildlife trade (anthropogenic dispersal pathways) to evaluate global pathways of dispersal and determine whether there are possible mechanisms to control future spread of this and other wildlife diseases. In addition, I intend to re-evaluate countries which thus far have tested negative for chytrid (i.e. Madagascar) and conduct surveys based on my results from Honduras to determine the most likely places and substrates to detect chytrid in the environment and whether the conclusions of previous studies were affected by a sampling bias (i.e. solely amphibian sampling vs. rainwater, bromeliads, and river water). In August 2011, I will begin this PhD. My overarching goal is to determine to what extent the global amphibian extinction crisis is being driven by man's activities versus natural pathogen dispersal dynamics.

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

I am working to publish the results of my research in peer-reviewed journals and intend to submit no less than three major written works this year. One will be about the five-year prevalence of chytrid within a community of critically endangered amphibians, another will be about chytrid dispersal and

distribution within the natural environment, and a third will focus on aerial-based chytrid dispersal via wind-blown rain.

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

The RSG was used between 8/7/10- 10/3/11. The length of this project has remained on schedule, with the exception that one of the laboratories provided results several months later than what was initially agreed upon, due to mechanical failure in the laboratory.

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
Canopy Access (Tree rigging and climbing equipment)	525	525		
Environmental Monitoring Equipment (Vantage Vue Weather Stations and Data Loggers)	289	289		
Radio Telemetry (Miniature radio transmitters x 5)	600	600		
Radio Telemetry (Radio transmitter refurbishment)	170	170		
Radio Telemetry (Headphones, antenna, batteries, harness materials, GPS, etc.)	407	407		
Bd Amphibian Infection Collection Supplies (Sample tubes, swabs, Nitrile gloves, ethanol, weatherproof journals, etc.)	217	217		
Bd Water Filtering Supplies (0.22 uM Sterivex water filters, Disposable Sterile 60 mL plastic syringes, 1 mL syringes, ATL buffer, Lysis solution, etc.)	0	0		
Bd Airborne Particle Collecting Supplies (Windsocks x 15, Supplies for airborne particle collectors, filter paper, window screen mesh, etc.)	767	767		
Bd Molecular Analysis (Chytrid swabs)	1280	1280		
Bd Molecular Analysis (Water Filters)	1312	1312		
Bd Molecular Analysis (Insect samples)	0	0		
Miscellaneous (Int'l Transport of ~200lbs scientific and climbing equipment)	420	420		
Total	5987	5987		

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

The important next step is to pursue my investigation into global chytrid dispersal pathways even further, most notably the possibility of rain-driven chytrid dispersal, which I intend to tackle via the PhD I am about to begin. Although my preliminary evidence suggests the dispersal of chytrid may occur via rain, the presence of chytrid genetic material does not imply viability of chytrid zoospores. Therefore, after I have first confirmed that chytrid does indeed disperse via rain events, I will then see if exposure to chytrid in rain samples is able to transmit infection to tadpoles, inferring chytrid viability.

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

I have not yet used the RSG logo, but included references to the funding provided by RSGF both in presentations I have given and in manuscripts I have submitted for publication.

11. Any other comments?

I am extremely grateful for the support Rufford Small Grants has provided to make this project possible and allow me to pursue my endeavour to address the global amphibian extinction crisis. I am extremely dedicated to my research and am very excited to start my PhD to continue this investigation. Thank you so very much.