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	Daniella Biffi Olivas
Project title	Developing a simple census method for endangered marine otters *Lontra felina*
RSG reference	11689-1
Reporting period	19 months
Amount of grant	£5910
Your email address	d.biffi@tcu.edu
Date of this report	December 2013

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
Collect and map faecal samples in Ica, Moquegua and Tacna			✓	We collected samples in Lima instead of Ica, because we did not find scat in Ica.
Extract otter DNA from the samples			✓	
Use genetic markers to genotype the DNA samples and identify individual otters			✓	
Use these data to construct an estimator using the incidence of faeces to predict the number of individual otters at a site		✓		(See point 9. Next steps)

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

Amplifying nuclear loci from DNA extracted from faeces can be very difficult; it took us more time than we expected to finish the genotyping.

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

The most important outcome of this project was finding that non-invasive genetic typing will ultimately provide more accurate estimates of marine otter abundance than censuring based on sightings. Our results also suggest that with the appropriate sampling design, non-invasive genetic typing could increase our knowledge of otter home range use and movement patterns. The following are our two most important results:

1. *Otter abundance estimated from faecal genotyping is higher than estimates based on visual sightings.*

In 2005, Apaza (Apaza & Romero 2012) estimated a total of 756 ± 86 individuals with a mean density of 1.48 ind/km along 510 km of potential suitable habitat. Valqui (2012), using research conducted between 2008 and 2011, estimated from 789 to 2,131 individuals in Peru along 789 km of suitable habitat with a density of 1 – 2.7 ind/km. Densities between 1 – 2.7 ind/km are the most frequently reported densities in older studies (Valqui 2012). Censuring elusive animals like otters is challenging. Behavioral studies of marine otters, based on direct observation during eight hours of daylight, conclude that they spend 80% of their time out of view (Medina-Vogel et al. 2006).

We estimated otter abundance as a minimum estimate from the number of unique genotypes and by using the programme CAPWIRE, which is based on the classic mark-recapture method and takes into account the fact that we likely did not sample all individuals in an area when we collected faeces. Both unique genotypes and CAPWIRE gave us greater estimates of marine otter abundance (Table 1) and density (3.9 ind/km and 5.5 ind/km, respectively), compared to those made in previous studies in Peru and Chile, by visual censuring. These data suggest that otter

populations may be up to 2X higher than current estimates. In fact, we think our estimates may still be underestimates of the actual population size for three reasons: 1) we conservatively considered genotypes that differed at one locus to belong to the same individual (to account for some genotyping error) and in doing so we may have called two separate individuals the same in some instances, 2) our genetic markers were variable enough to recognise two separate unrelated individuals with high statistical certainty, however; they did not have enough power to reliably discern siblings or parents from their offspring, and 3) we found new individuals during every visit at many of the sites which indicates we probably underestimated the number of otters at some sites (Figure 1). We provide recommendations to address these issues in section 9 below.

Table 1. Comparison of marine otter abundance at seven sites in Peru done by visual censuring and by noninvasive genotyping techniques. Valqui and Apaza estimates obtained from Valqui 2012 and Apaza & Romero 2012, respectively. ND indicates no data.

	Visual censuring		Noninvasive genotyping	
Site	Valqui	Apaza	Genotypes	CAPWIRE
IS	ND	7	17	38
PC	5	2	13	26
PG	9	8	6	12
PP	3	3	12	30
PU	5	3	5	13
QB	5	2	13	29
VV	8	6	4	5

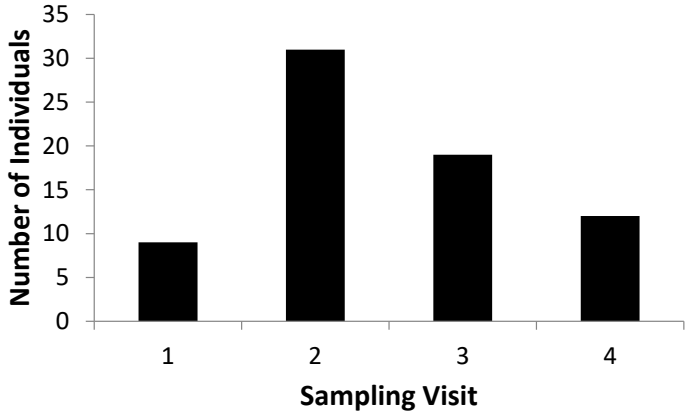

Figure 1. Number of individuals identified for the first time in each sampling visit

2. *The method used in river otters to correlate faecal abundance with otter abundance is not appropriate for marine otters.*

Mowry *et al.* (2011) in their study of river otters removed all faeces from a site then returned 7 days later to collect faeces for genotyping. There is a strong correlation between the number of genotypes and the number of faeces deposited over this 7-day period. In our study, we waited 3 days between the 1st and 2nd visit and the 3rd and 4th visit (due to time and travel constraints) and then 7 days between the 2nd and 3rd visit. We found there was no relationship between the number

of scat for each site and the number of otters estimated from the genetic data for visits 1, 2, 4 and overall visits. However, in our third visit we found a strong correlation between the number of scat and the number of otters identified by using unique genotypes (Figure 2). This sampling period corresponded most closely to the methodology in the Mowry *et al.* (2011) study on river otters. However because we found new individuals during each visit and many individuals were not found across all visits if we simply used the relationship found for visit 3 to estimate otter abundance we would have underestimated abundance by almost 50%.

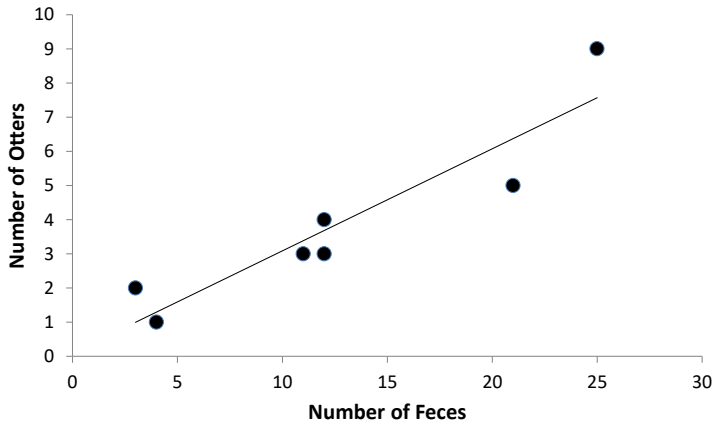

Figure 2. The relationship between number of fresh faeces and number of otters per site determined from genetic typing in the 3rd visit ($y = 0.30x + 0.10$, $R^2 = 0.86$, $P = 0.002$).

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

In collaboration with the non-profit ProDelphinus we gave some workshops to students of the "Miguel Grau" school, in Pucusana, Lima. In the field, I worked with a fisherman and collaborator of the non-profit ProDelphinus.

5. Are there any plans to continue this work?

Although we have reached our proposed goals we are still working in the lab in order to publish the results of this study. Specifically we are:

1. Determining the sex of the individuals identified. We are going to use the genetic markers ZFXY and SRY2 developed for mammals. This is going to potentially help us discern between similar genotypes of related individuals, like siblings or parents and offspring.
2. Amplifying mitochondrial DNA. By obtaining sequences of the mtDNA control region we are going to further study the relatedness and structure of the marine otter populations that we sampled.

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

I presented preliminary results of my research in the Student Research Symposium at TCU and at the 2nd Meeting of Environmental Researchers in Arequipa, Peru. Also, I gave a talk on marine otters at the Universidad Nacional Mayor San Marcos (UNMSM) in Lima, Peru. Finally, I gave a conservation genetics and marine otter talk at the Universidad Científica del Sur (UCSUR) in Lima, Peru.

In the future, we plan to present our results in the Congress of Marine Science in Peru (CONCIMAR) and to develop a scientific publication for Conservation Genetics or Animal Conservation.

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

The grant was used through the whole project, from the fieldwork to the genotyping. It took us about 3 more months to finish the lab work than we anticipated.

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
TRAVEL				
Dallas – Lima – Dallas	887	--	--	Covered by other sources
Lima – Tacna – Lima	443.5	--	--	Covered by other sources
Car rental	1197	100	--	Covered by other sources
Gas	445	282	163	
Toll	32	26	6	
ACCOMODATIONS				
Hotel Tacna x 16 days	1014	596	422	We stayed in total 21 days
Food Tacna x 16 days	507	880	- 373	
Hotel Marcona x 16 days	710	105	605	We change the location to Lima
Food Marcona x 16 days	507	300	169	
PERMITS				
Collection permits	76	--	--	Covered by other sources
Export permits	32	--	--	Covered by other sources
FIELD MATERIALS				
Gloves	12	12	0	
Vials	38	38	0	
Ethanol	19	19	0	
Buffer	7	7	0	
LAB MATERIALS				
DNA extraction kit x 6	1330	1495	- 165	We bought 7 extraction kits
Genotyping	0	1095	- 1095	
Supplies (primers)	0	230	- 230	
EDUCATIONAL MATERIALS				
Workshop expenses	92	92	0	
Printed materials & others	96	96	0	
OTHERS				
Field assistant	--	235	- 235	
Taxis (Fieldwork)	--	50	- 50	
Bus (Moquegua & Tacna)	--	130	- 130	
Postal service (samples)	--	122	- 122	
TOTAL	5910	5910		

1 Peruvian Nuevo Sol = 0.22 British Pound Sterling

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

In order to obtain better estimates of the number of otters from the number of faeces it will be necessary to first develop some marine-otter-specific loci to increase our power to discriminate between related individuals. We would also recommend collecting all faeces at a site, rather than just fresh faeces. We collected only fresh faeces at a site because most previous studies have found that fresh faeces are easier to genotype. The Mowry *et al.* (2011) study found however, that fresh otter scats yielded the lowest amplification rate compared to old scats (1-6 days old). Collecting all faeces at a site may result in more thorough sampling of individuals utilising that site.

The appropriate spatial scale and number of visits to a site that result in the detection of all individuals utilizing a site will also need to be determined. Medina-Vogel *et al.* (2007) determined the home ranges of marine otters were from 1.4 to 4.1 km of seashore and so we considered localities separated by 5 km to be separate sites. We found faeces, however, from the same individuals more than 5 km apart. These data combined with the fact that we detected new individuals over time at a site suggests that otter home ranges may in some instances be larger than previously thought and that home ranges are not very exclusive. In the future, it would be useful to intensively sample several of these same sites on a daily basis for 2-3 weeks in order to determine how many sampling trips it takes to detect all otters at a site. Increasing the sampling distance around these sites by a few km would also help reveal the spatial scale that consistently detects the same otters over time. Once the appropriate spatial scale has been determined then it may be possible to construct a better estimator of otter abundance from faeces counts.

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?

We printed 1000 marine otter cut-offs that were distributed during the educational workshops. During all the talks and presentations, the Rufford Small Grants Foundation was mentioned as one of the collaborators of the project and the logo was shown.

11. Any other comments?

I have attached my master's thesis that goes into more detail about data analyses and estimating population size.