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	Rabin Kadariya
Project title	Genetic Variation, Ecology and Conservation of Asiatic Black Bears (*Ursus thibetanus*) in Mountain Landscape of Nepal
RSG reference	Ref no 18343-1
Reporting period	October, 2015-March, 2017 (18 months)
Amount of grant	£5000
Your email address	rkadariya@yahoo.com
Date of this report	May 16, 2017

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
Assess status and distribution of bears in Annapurna conservation area				We visited eight sites of Annapurna Conservation Area on ground walk of 27 grids of 5X5 km^2. Bear signs such as claw marks on trees, broken branches for fruit, tree and ground platform, faeces, food eating signs on ground, walking tracks , raided bee nests, ground digging, grass bites, broken log for insects, footprints, hairs on trees and fence were recorded in all sites where as fresh fecal sample were collected in seven sites. Survey was carried out from 1422 to 3647 m elevation whereas the maximum elevation of bear sign was recorded at 3582 m.
Assess human bear conflict and local people perception				Crop damage, especially maize depredation, was highly reported in six sites. People had also fair of bear mauling. Local people showed their negative attitudes towards bears as they were facing human causalities and repeated depredation of maize. Maize is the major crop in the mountain farming system of Nepal. Only rare cases of livestock killing were reported by villagers. We also observed snares in the tracks of bears to cropland and poison kept in maize bite of bears which were kept by angry farmers. Beside we heard three gunshots and bear killed during our field survey.
Explore seasonal diet selection and habitat use by Asiatic black bears				More than 250 faecal samples were collected for diet analysis during the period of July to December 2016. The most of

				fecal samples were collected from agricultural land (maize, paddy, and apple) in rainy season (July-September) whereas the autumn (October-December) seasons faeces was collected from forest habitat. Signs of Asiatic black bear were only recorded in mixed forest, mostly in bamboo forest and forest having high abundance of nuts. No any sign was recorded in pine dominated forest.
Study genetic diversity and phylogeny of Asiatic black bears using non-invasive method				A total of 127 putative fresh bear faecal samples were collected for the genetic analysis, of which 46 were collected from agricultural land in July-September period and 81 were collected from forest in October-December period. Similarly, 22 hair samples were collected from fences and broken branches. DNA was extracted from the faecal/hair sample by using extraction kit and stored at -20^{0}C.

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

Hair sample collection: We could not find any bear rubbing trees which was supposed to be entangled by barbed wire for the collection of hair sample. Later, hair trap consisted of a single barbed wired strand approximately 20 m long wrapped around at least four trees, 40-50 cm above the ground in seven sites during the period of November-December, 2015. Honey was baited in the centre of trap at 3 m above the ground level to attract bears. It was hung using string from two trees. It assumed that bait attracted bears, crawled underneath (or over) the barbed wire that snagged their hair in the barbs. Traps were visited every 10 days for 2 months trapping season. Unfortunately, we could not get any bears signs in the trap located in seven locations. In the second visit we set up same hair traps during the July-August, 2016 with carrion bait, which were located near the site of damaged maize field and monitored by automated camera. We were not able to collect bear hairs from the second attempt too. 22 hair samples were found in broken branches and barbed wire fence around agriculture land that were collected in small paper envelopes having silica gel with details of GPS location and characteristics of hairs

Faecal sample collection: It was very difficult to find fresh faecal samples when we organised field survey by selecting random grid from map. To maximise the

collection of fresh faecal samples, grids were selected after confirmation of bear existence in the particular site. So we organised a series of meetings with project staff, community leaders and livestock herders. We set parameters like bear related conflict events, status of fruiting trees and observation of bear fresh faeces or broken trees by livestock herders for the selection of grid. Identification of abundant fruiting tree's site was the first indicator for high chances of getting of fresh faecal sample by investing minimum time, energy and money. We also targeted to collect fresh faecal samples from the agricultural land for easy access.

Quality of DNA: The DNA extracted from the faecal sample, which age was more than 4 days and sample collected in rainy season, was not good so we collected more sample during October-December period. The putative fresh faecal samples were collected to ensure the success of DNA extraction. We could not see any difference for the extraction of DNA and amplification of loci whether it collected in ethanol or silica gel so we choose ethanol and cotton swab which felt easier by field assistants.

Identification of item on faeces: The food items found in the faeces were tried to identify on site by comparing nearby seeds of fruit. The probable food of the bears such as fruit/berries, grasses and leaves of plants were collected for the reference during diet analysis in lab as we could not find any books covered all the seeds of different fruits of Annapurna Conservation Area.

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

Success of Non-invasive samples for DNA extraction and selection of appropriate primers: DNA was successfully extracted from 99 (66%) out of 149 samples of which 75% success within faecal sample (95 in 127) and only 18% in hair samples (4 in 22). DNA could not extract from most of hair samples which were very old and collected from fence and branches. Regarding faecal samples most of the samples collected during rainy season were not succeed for the extraction of DNA. If the fresh faecal samples were collected in autumn season, sufficient amount of DNA can be extracted for the population monitoring and genetic study. 11 microsatellite primers were selected for the individual identification and study of genetic diversity after getting the amplification success in PCR. The bear specific primers were also selected for the sequencing of Control Region of mitochondrial DNA.

Diet of bears in autumn season: We analysed 68 faecal samples collected from five units of Annapurna Conservation Area in autumn season. We identified 10 species of wild fruits, three species of insects, one type of agricultural crop. The grasses and mammals hairs recognised in faeces could not be identified to species level due to the lack of reference and sufficient hair sample in some extent. The species of two distinct seed also could not indentify. The percent frequency of occurrence of wild fruits was 85.29% followed by grasses (42.65 %). The results were same in percent of volume i.e. wild fruits (74.22%) and grasses (21.28%). The contribution of grasses was lower in volume than frequency of occurrence. The grasses were found in 29 samples of which two faecal samples contained only bamboo shoots, four samples only *Arisaema* spp. and five unknown grasses were in trace amount. The *Arisaema*

spp. was one of the major food items during dry period. The whole part of *Arisaema* spp. (fruit, stem and rhizomes) was recorded in faeces. Although, seven faecal samples recorded mammals hairs volumetric contribution was only 1.94% where three samples contained only trace amount. During the survey period, we could not see any agriculture crop in the field; accordingly, we found buckwheat in two faecal samples from trans-Himalayan region. We also observed only one food item in 50% faeces. Similarly, 31%, 13%, 6% faeces contain two, three and four food items respectively. Wild fruits/nuts are major food of bears followed grasses whereas small amount of mammal hairs, insects and crop are also recorded in bear faeces. Although food habit of bears were omnivores, fruits were the most preferred food items in the autumn season. Asiatic black bears consumed any available food source but they gave more preference to larger fruits than smaller berries and grasses. Local herders observed the carrion of ox ate by bears until it was decayed. We also found hairs of ox in the old defecation site of bear.

Increasing human bear conflict: More than 60% maize was damaged in six sites so farmers were more worried for the protection of maize although most of famers guard maize using their local traditional knowledge. In one site more than 2 km long barbed wire fence was constructed with the aim to protect maize from bear damage. Unfortunately both traditional measures and barbed wire fence were not effective. It is urgent need to develop and implement community based crop compensation scheme for the long term survival of Himalayan black bears by minimising retaliatory killing as snares, poison and gunshot were noticed during our study.

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

Development of citizen scientists for wildlife research: Six local youths from ethnic communities were trained for the field sample collection, habitat survey and human bear conflict assessment. They can collect samples, fill designated form and handle GPS. They are acting as citizen scientist for wildlife research in Annapurna conservation area

Capacity enhancement on bear conservation: 62 community leaders participated in bear conservation workshop. The workshop was mainly focused on human-bear conflict assessment, appropriate mitigation measures and bear population monitoring in the local site by using non-invasive method.

5. Are there any plans to continue this work?

Yes, I am very interested to know the present status of bear population, sex ratio, genetic relatedness and diversity within different sites of Annapurna Conservation Area and taxonomic confirmation of Asiatic black bears of Nepal from the successfully collected non-invasive sample during this study and by collecting additional at least 100 fresh faecal samples. There is no any other appropriate method to know the population of bears from the remote mountain landscape

except non-invasive sample. The genetic database will act as reference for the forensic identification of bear killing and control of trading.

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

a. Already shared:

- Flash talk, poster presentation and oral presentation at year end presentation of Graduate School of Veterinary medicine in 2016 and 2017.
- Research progress was presented in the research seminar of Laboratory of Wildlife Biology and Medicine.
- The research progress was presented in the leading progress seminar organised by program for leading graduate school.

b. Future planning:

- An oral presentation will be made on the 5th Sapporo Summer Seminar for One Health (SaSSOH) in September 2017 (confirmed).
- A poster will be presented on bear conservation conference organize by International Bear Association in November 2017 (waiting for result).
- At least two papers will be published in international scientific journals by the mid of 2018. (Start for write up)
- The final technical report will be published in the website of Hokkaido University and Rufford Foundation.

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

Although project period was mentioned from January to December 2016 in application proposal, the first field visit was organised, immediately after approval of project proposal by Rufford Foundation, from 18th October 2015 to 12th January, 2016 for the testing of questionnaire, habitat assessment methods and evaluation of fecal and hair collection methodology. The second field was organized from 27th June 2016 to 2nd January 2017 for the intensive non-invasive sample collection, habitat survey and human bear conflict assessment. It was extend additional 6 months to cover the seasonal data collection by considering field accessibility in highly terrain mountain landscape. Beside field sample collection, it took time for the diet analysis, DNA extraction and selection of appropriate primer during lab work. The field visit and activities was regularly informed to Rufford Foundation with project update.

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
Purchase of map	80	20	60	Only those maps, which were not available at National Trust for Nature Conservation, were purchased
Intensive field survey (Non-invasive sample collection, habitat survey, questionnaire survey)	3190	5639	(2449)	It covered the cost of field survey of principal investigator (local transportation/accommodation) and two assistants during the period of 18 October 2015 to 12 January 2016 and 27 June 2016 to 2 January 2017.
Field accessories and materials	400	662	(262)	Sample collection tubes, gloves, stationeries, field reagents, preservatives, Barbed wire/accessories and bait were purchased.
Faecal analysis for diet	400	459	(59)	Cost of reagents, equipments and remuneration of undergraduate students
Genetic analysis	930	1478	(548)	Cost of DNA extraction kits, reagents, primers and other accessories for the extraction of DNA
Transportation	0	1732	(1732)	Two round airfares from Sapporo, Japan to Kathmandu, Nepal
Total	5000	9990	(4990)	£3950 was granted by Columbus Zoo and Aquarium Conservation Fund and £1040 was paid by Program for Leading Graduate Schools. The in kind contribution of NTNC and additional accessories provided by Laboratory of Wildlife Biology and Medicine are not included

Conversion factor: £1= NPR127

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

This study showed that DNA can be extracted from non-invasive sample especially from fresh faeces of Asiatic black bears. We also tested the amplification success of 25 microsatellite primers, sex primers and bear-specific primers for the sequencing of control region mitochondrial DNA. So the next major steps are to assess the present population of bears and sex ratio by using non-invasive samples. Non-invasive DNA genotyping has become a popular method for studying wild animals. It is especially useful for bears whose direct observation, capturing and tagging are difficult in dense mountain forest. Furthermore, this approach is favoured because non-invasive sampling reduces the risk of injury to endangered wildlife and reduces costs and burden on field works. The genetic relatedness and diversity of bear populations in

conservation area will help us to assess the impact of community based biodiversity conservation in Annapurna Conservation Area as a model. The taxonomic status of Nepal's Asiatic black bears is unknown so sequencing of mitochondrial DNA will be very helpful to identify whether the bear found in Nepal is same sub species recorded in other countries (*Ursus thibetanus thibetanus*) or Himalayan sub species (*Ursus thibetaus laninger*). Beside it is very essential to engage local community by developing community based crop compensation scheme and awareness program for the long term conservation of bears to minimizing retaliatory killing.

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?

Yes, I used the logo of Rufford foundation in posters, flash talk and oral presentation. It was also used in the banner of two stakeholder workshops which were organised for the human-bear conflict assessment. The financial support of Rufford Foundation will be highly acknowledged in the upcoming conferences/seminars and paper publication.

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

The research activities were implemented under my leadership with the close supervision of Professor Toshio Tsubota and Associate professor Michito Shimozuru from Hokkaido University. Numbers of people and organisation supported for the successful organization of research work including field data collection and lab processing. The UInit Conservation Officers and rangers who deployed in different units of Annapurna Conservation Area Project had supported for the coordination with local communities and selection of appropriate site for the bear study. Besides, they also supported for the logistic arrangements of field work. The chairperson and community leaders of Conservation Area Management Committees also provided valuable information and support for the selection of probable candidate for the citizen scientist. The citizen scientist played key role for the collection of fresh faecal sample, habitat survey, onsite crop depredation assessment. The Laboratory of GENETUP was used for the DNA analysis whereas diet was analysed in the zoological lab of Institute of forestry by mobilizing undergraduate students.

Photos:

Objective 1: Assess status and distribution of bears in Annapurna conservation area

Bear food print

Bear food remain on the ground

Caw mark on tree

Ground digging

Bear faeces

Location of bear sign with GPS

Field team in one site

Landscape of study area

Plants highly preferred by bears

Highest location of bear sign found

Objective 2: Assess human bear conflict and local people perception

Human bear conflict workshop

Questionnaire survey

On site conflict assessment

Maize damaged by bears

Damage on paddy land

Quantity of maize damage assessment

Poisson used in maize by angry farmer

Snare set up in the bear track to maize land

Interaction with injured old person

Human Causalities

Scarecrow to avoid bears damage

Early harvesting of maize to escape additional damage by bears

Objective 3: Explore seasonal diet selection and habitat use by Asiatic black bears

Habitat assessment

Mixed forest preferred by bears

Participatory identification of food items in bear faeces

Collection of bear faeces for diet identification

Collection of probable bear food

Preparation of references of all seeds

Washing of bear faeces using sieve

Identification of food items by matching with references

Nut remains in faeces

Insect(larva) found in faeces

Objective 4: Study genetic diversity and phylogeny of Asiatic black bears using non-invasive method

Collection of bear faeces by using sterile cotton swab

Set up of bear hair trap

Trap monitored by automatic camera

Hair sample collected from fences

Lab work for genetic study

Amplification success of extracted DNA from faeces and hairs