EVALUATION OF POPULATION ESTIMATION SAMPLING TECHNIQUES AND ASSESSMENT OF GENETIC DIVERSITY OF GREATER ONE-HORNED RHINOCEROS (*Rhinoceros unicornis*) POPULATION IN DUDHWA NATIONAL PARK, UTTAR PRADESH, INDIA

DISSERTATION SUBMITTED TO SAURASHTRA UNIVERSITY, RAJKOT, IN PARTIAL FULFILLMENT OF THE MASTER'S DEGREE IN WILDLIFE SCIENCE

By

Vibhav Srivastava

Under the Supervision of

Dr. S.P. Goyal

(Supervisor)

Shri Qamar Qureshi

(Co-Supervisor)

Shri Udayan Borthakur

(External Co-Supervisor)

भारतीय वन्यजीव संस्थान
Wildlife Institute of India

JUNE 2013

भारतीय वन्यजीव संस्थान
Wildlife Institute of India

CERTIFICATE

This is to certify that **Mr. Vibhav Srivastava** has carried out an original research titled **"Evaluation of population estimation sampling techniques and assessment of genetic diversity of Greater One-Horned Rhinoceros (*Rhinoceros unicornis*) population in Dudhwa National Park, Uttar Pradesh, India"**, in partial fulfilment of **Master's Degree in Wildlife Science from Saurashtra University, Rajkot**. The study was carried out under our supervision from December 2012 to June 2013. We hereby certify that this work has not been submitted for any other degree to any other university.

Dr. S.P. Goyal	**Shri Qamar Qureshi**	**Shri Udayan Borthakur**
Scientist G	Scientist F	Scientist
Supervisor	Co-supervisor	External Co-supervisor

Date: 28.06.2013
Place: Dehradun

पत्रपेटी सं0 18, चन्द्रबनी, देहरादून – 248 001, उत्तराखण्ड, भारत
Post Box No. 18, Chandrabani, Dehradun – 248 001, Uttarakhand, INDIA
ई.पी.ए.बी.एक्स : +91-135-2640111 से 2640115 फैक्स : 0135-2640117, तार : WILDLIFE
EPABX : +91-135-2640111 to 2640115; **Fax :** 0135-2640117; **GRAM :** WILDLIFE
ई–मेल / **E-mail:** wii@wii.gov.in

CONTENTS

ACKNOWLEDGEMENTS

I would like to convey a sincere acknowledgement to all those persons who have contributed significantly and without whom my dissertation would have been incomplete, especially my parents and my family who are always there with me as support in every condition.

At the foremost, I would like to thank Shri P. R. Sinha, Director and Dr. V. B. Mathur, Dean, Wildlife Institute of India (WII), Dehradun for granting me the permission to carry out my dissertation work.

I am thankful to my supervisor Dr. S.P. Goyal, co-supervisor Shri Qamar Qureshi and external co-supervisor Shri Udayan Borthakur for their guidance, help, encouragement and moral support at the crucial stages of my dissertation. Special acknowledgement goes to Udayan Sir for providing me with the optimised rhino specific microsatellite markers.

Thanks to all the faculties at Wildlife Institute of India for their critical evaluation of the proposal and expert comments which improved my study from time to time.

I sincerely acknowledge Uttar Pradesh Forest Department for granting the permission to carry out field work in Dudhwa Tiger Reserve. My special thanks go to Mr. Rupak De (PCCF, U.P.), Mr. Shailesh (Field Director, Dudhwa Tiger Reserve), Mr. Ganesh Bhat (Deputy Field Director, Dudhwa Tiger Reserve) and Mr. C.K.P. Chaudhary (Ranger) for facilitating field data collection without much hassle. I would also like to thank Director, Vetenerian and staff of Kanpur Zoological Park for letting me to observe rhinoceros there.

I would like to express my gratitude towards Dr. B. Pandav, Dr. Christy Williams and Shri A.S. Negi for their support during the process of fund raising for the project.

I am grateful to Ms. S. Sharma, whose immense support, from proposal building to thesis writing, made it possible to bring this project to completion. I thank her for being tolerant and understanding during the ups and downs in my behaviour during various stages of this project.

I express my sincere gratitude to Dr. S.P. Sinha whose experience and help made the journey of my first independent field work easy, Devendra Sir for helping me in critical times in field, Krishnendu, Pranav, Rekha, Ashish for being jolly company in field.

I heartily thank Puneet Sir, Sujeet Sir, elder brother Rahul, Ved Sir and Bheem Sir for incredulous help during laboratory work. I owe everything I learned in the genetics laboratory to them. The vital inputs from Mukesh Sir, Sudhanshu Sir, Ajit Sir, Nipun Sir and Vinita ma'am can never go unacknowledged. Thank you all for clearing my clumsy thoughts.

Heartfelt thanks goes to Naresh Sir, Sutirtha da, Anant, Bidyut da, Indro da, Swati di and Riddhima di for the helping hand that they extended towards me when I needed the most.

Special thank goes to Deepak, Debabrata, Nitya and Aditya for sharing their wealth of knowledge during 'happy' hours. Thanks to all the WildCubs for making my 2 years of M.Sc. awesome and forever memorable.

I keenly acknowledge Dudhwa frontline staff Chotelal ji, Sanjay ji, Shri Ram ji, Ram Chander ji, Rakesh ji, Deepak ji and Hari ji and mahaouts Lallan ji, Umar ji , Susheel ji and Irshad ji, also chara-cutters Qayamat-ud-din and Ejaaz Ali for being excellent support in field and for keeping the melancholic atmosphere at the Base Camp in Dudhwa lively.

I would also like to express my gratitude towards everyone at Wildlife Forensic Cell especially Thapa ji and Mahavir. Thanks to all the staff at WII Library. GIS Cell, Academic Cell and Accounts Cell for much needed help in respective aspects during various stages of project.

I am grateful to all the inmates of Old Hostel who make this place homely and colourful. I thank Eminem for lovely songs which always helped me overcome my bad mood. Thanks to wildlife, hope it prospers for next several hundred years in India. Last but not the least, I thank the rhinos for sparing me unhurt even though I came in their way so that I can write this thesis.

-Vibhav Srivastava

SUMMARY

The ideology of wildlife conservation emerged with the realization that the wildlife numbers are on a decline in the natural habitats. Since, due to humane limitations we cannot ascertain the exact numbers of a individuals very accurately, therefore, the basic requirement for population estimation arises. Greater one-horned rhinoceros (*Rhinoceros unicornis*), already being declared a globally threatened species, demands much attention towards their surviving numbers in wild. Moreover, with the constant rise in the unethical and illegal human activities, the need to regularly monitor their population is realized. To suffice this requirement, population estimation is largely done in a crude way i.e. by labor intensive block count method in which the probability of missing individuals in dense vegetation is high. Advanced population estimation techniques such as capture-recapture using photographic or DNA fingerprint based individual identification, show promising results within the framework of resources in comparison to use of footprint and dung count methods.

The current study was conducted in Rhino Reintroduction Area (RRA) of 27 km^2 located in Dudhwa National Park. The first objective was to evaluate four population estimation techniques – non-invasive faecal DNA based capture mark recapture (CMR), photographic CMR, dung count and footprint analysis, for their validity in estimation of rhinoceros population with respect to accuracy and precision. The following techniques have been selected out of the others because of their reasonable accuracy and precision obtained when applied to other mega-herbivore (including other species of rhinoceros or elephant) population estimation. I selected Dudhwa National Park (DNP) where the reintroduced rhinoceros population is surviving since 1984-85, with a known population size (32 individuals) so as to compare our estimates.

For non-invasive faecal DNA CMR technique 140 fresh dung samples were collected and out of them 27 unique genotypes were identified by microsatellite analysis. The capture history of these unique genotypes was then analyzed in MARK to arrive at a population estimate. In photographic CMR, 4 remotely triggered camera trap units were deployed in 6 sessions having 7 occasions in each session. For dung count, random elephant transects of length varying from 1 km to 3.2 km were run and dung piles were counted on either sides. The data on dung density was analyzed in DISTANCE. For dung decay rate estimation 20 fresh dung piles were marked in each of the four habitat types and monitored for decay. The defecation rate was estimated by observing captive rhinoceros. In case of footprint technique, a foot ruler was kept besides each rhinoceros footprint before capturing its photograph. Twenty four (length, angle and area) parameters were extracted from the images using Sigma SCANPRO. The resulting variables

were subjected to principle component analysis (PCA) to check for the corresponding variance values in differentiating individual footprints.

It was found that the non-invasive faecal DNA based population estimation and photographic capture mark recapture were the better ones as compared to the other two. However, the data analysis for the dung count and footprint analysis techniques is still under consideration and does not form part of this thesis. These two techniques require further logical modification in study design and statistical analysis to achieve at a reliable estimate. Between the former two, non-invasive faecal DNA based population estimation technique estimated population size (35.10 ± 5.01) close to the known population size of 32. Photographic capture recapture estimated the population size as (25.98 ± 4.91) which was comparatively less accurate than non-invasive faecal DNA CMR.

Knowledge of the genetic status of a confined and isolated population is always beneficial to evaluate their well-being and to avoid any future threat such as that of inbreeding depression. Therefore, second objective of the study was to describe genetic structure of this isolated and reintroduced population. With 27 identified unique genotypes and 10 rhinoceros specific microsatellite markers the genetic variability in this population was examined. It was found that the mean observed heterozygosity level was 0.353 while mean expected heterozygosity level was 0.483. The effective number of alleles per loci was 2.069. When compared to the genetic diversity of the ancestral population in India and Nepal, evaluated in previously published studies, the following results indicated that this population carries lower genetic variability than ancestral populations. The inbreeding test revealed that the population show signs of inbreeding (F_{IS} = 0.39) and which are likely to exaggerate in future as it is more or less closed and non-randomly interbreeding.

Focusing on the conservation needs from management viewpoint we suggest that it is necessary to bring variability in the genetic structure to avoid future dire consequences of inbreeding depression. This can be achieved either by translocating new individuals, preferably males, from other Indian sub-populations of Assam or West Bengal since they have better genetic diversity than the rhinoceros in Nepal.

CHAPTER 1

1.1. GENERAL INTRODUCTION

Quantification of species abundance and genetic diversity significantly influence our understanding of the ecological processes ranging from geographic to molecular scales such as habitat use, patterns of dispersal and colonisation and maintaining genetic viability (Mondol *et al.* 2009, Ramakrishnan *et al.* 1991). Both population size and genetic structure are considered as state variables that impetuously determine the dynamic system of species-niche relationships highlighting the crucial research and management gaps to scrutinise conservation efforts (Mondol *et al.* 2009). However, measurements in field with absolute accuracy is merely speculative owing to challenges such as species behaviour, difficult terrain, visibility constrains in dense habitat, resource limitations and subjective humane errors (Ramakrishnan *et al.* 1991; Savage *et al.* 2010). Therefore, achieving robustness in the study design for field data collection empowers scientists and conservationists to take decisive actions for developing and implementing conservation strategies aimed at effective management and conservation (Ramakrishnan *et al.* 1991, Silveira *et al.* 2003). The question of robustness in abundance estimation and quantification of population genetic structure becomes more acute for remaining wild populations of a narrow ranging species that serve as management units or isolated potential evolutionary significant units (Moritz 1994; Legge *et al.* 1996).

In many instances, due to lack of adequate resources traditional inefficient methodologies for population density estimation, such as block counts in case of elephants (Ramakrishnan *et al.* 1991; Dawson and Dekker 1992) and rhinoceros, are chosen over modern practices in various parts of the world (see Bist 2003; Lahan & Sonowal 1973) leading to discrepancies in long term management plans that hampers the conservation efforts. Consequently, there arises a need for calibration of techniques which can parsimoniously achieve the predefined target with precision and accuracy. Specifically, it is of prime significance as far as management of the second largest terrestrial species *i.e.* rhinoceros is concerned.

Of the extant rhinoceros species, the Greater One-Horned Rhinoceros or Indian rhinoceros *Rhinoceros unicornis* Linnaeus (1758) (taxonomy mentioned in table 1) is a generalist-feeder, mega-herbivore that is notified as Schedule-I species under Wildlife (Protection) Act, 1972 apart from being enlisted amongst the vulnerable species in IUCN Red list (Talukdar *et al.* 2012). Ranging in the grasslands of *terai* landscape, it seconds the white rhinoceros *Ceratotherium simum* Burchell (1817) in size and weight proportions (Penny 1987; Dutta 1991). With a dwindling population of around 2,835 individuals (Sinha *et al.* 2010) greater one-horned rhinoceros are found in 11 protected areas in India and Nepal (Sinha *et al.* 2010). In India, a major population (around 2500 rhinos) is found in the state of Assam but scattered populations are also present in northern West Bengal. A diminutive population was reintroduced into Dudhwa National Park (NP), Uttar Pradesh in 1984-85 as a satellite to the larger ones (Sinha *et al.* 2010; Sale and Singh 1987). This population is kept within an enclosure where, from seven founders in 1985, currently 32 rhinoceros are present (U.P. Forest Department).

Table 1: Taxonomic classification of Greater One-Horned Rhinoceros

Kingdom	Animalia
Phylum:	Chordata
Class:	Mammalia
Order:	Perissodactyla
Family:	Rhinocerotidae
Genus:	*Rhinoceros*
Species:	*unicornis*

Population estimation sampling techniques have been widely used in the wildlife surveys, population census and monitoring spatio-temporal changes that are critical for setting their management and conservation priorities. Efficient and reliable methods are necessary for rapid assessments as the threat to many susceptible wild populations stands high and is on an ever rise (Silveira *et al.* 2003). Footprints, dung, scat and other signs have been widely used as precursor of their presence and abundance in the area

(MacKenzie and Nichols, 2004). Techniques such as counting dung, nests, footprint trails, calls and direct sightings on line or strip transects, deploying remotely triggered camera traps and using faecal DNA for population estimation are comparatively recent and more popular probably because they are robust in their estimates (Silveira *et al.* 2003). The most well known is the camera trapping method which is based on individual identification of animal following with capture mark recapture scheme and have been extensively used in India for tigers by Karanth and Nichols (1998). Hariyadi *et al.* (2011) have used this method to estimate the abundance of critically endangered Javan rhinoceros *Rhinoceros sondaicus* Desmarest (1822) in Ujung Kulon National Park, Indonesia with reasonably low standard error (32 ± 4.32) while Brodie *et al.* (2010) used multi-strata mark recapture models to monitor the population dynamics of African black rhinoceros *Diceros bicornis* Linnaeus (1758) in Namibia. For the conservation of the threatened species the precise information of its population structure and dynamics in wild is essential and camera trapping technique is obviously effective for this. Individual identification of greater one-horned rhinoceros is possible since they differ from one another in their morphological features such as skin folds near neck, horn shape and size, tubercles and ear nicks (Laurie 1981; Dinerstein 2003; Kandel and Jhala 2008). Camera trapping has never been tried in this case perhaps because the existing method of total sweep count is supposed to be conservatively reliable.

If the animal is present or using the area it is certain to leave behind some signs. Convincingly, these indirect sign such as faeces, urine, scrape, scratch and footprints are efficient to predict the animal abundance in the area (MacKenzie and Nichols 2004). It was presumed earlier that individual identification of rhinoceros based on their footprint is a hard task as, being a perissodactyle, it leaves only three hoof marks on the substratum, therefore the use of footprint for the purpose of monitoring was not so popular, however, Santiapillai (1990) did the same satisfactorily for Javan rhinoceros. In 2001 Jewell *et al.* came up with a paper in which they elaborated the robust procedure of individual black rhinoceros identification and subsequently estimated their population with a decent level of precision. Using discriminant functional analysis of objective footprint measurements Alibhai *et al.* (2008) were successful in individual identification of white rhinoceros in Namibia that was utilised further for population estimation. Dung count surveys were rigorously used for population estimation of

elephants both in Asia (Varma 2006) and Africa (Barnes *et al.* 1997) but have never been tried in case of rhinoceros owing to the difficulty arising due to community latrine site or middens (Dutta 1991). However, if the number of animals defecating at a site could be monitored and determined, this problem could be solved to some extent.

Non-invasive genetic sampling and using faecal DNA for population estimation is a comparatively novel method that has been utilised in population estimation of most cryptic species such as Bengal tiger *Panthera tigris tigris* (Gopalaswamy *et al.* 2012) and snow leopard *Panthera uncia* (Janecka *et al.* 2011). Further, this method is most advantageous in case of animals inhabiting difficult terrain such as argali *Ovis ammon* in Afghan Pamir (Harris *et al.* 2010). Their use in context of rhinoceros is still lacking grounds but would be easy and reliable to accomplish. Cunningham *et al.* (2001) estimated the abundance of black rhinoceros in Tanzania using the DNA extracted from the dung piles. Validating this technique on all the currently existing rhinoceros species would be an important nuisance resolving step as there are many rhinoceros habitats in tropical Africa and South-East Asia which are dense enough to generate bias in population estimates by most other methods.

Greater one-horned rhinoceros is counted in wild by block count method where individuals are sought from camp-elephant backs and most probable total number is achieved (Lahan & Sonowal 1973). Forest staff's personal skill of individual identification accounts for the naive estimates of recorded population (Lahan & Sonowal 1973). The chance of missing an individual remains high due to dense vegetation and tall grasslands in the habitat hence, statistically sound sampling based techniques are required for population estimation (Lahan & Sonowal 1973). Sampling methodologies such as photographic capture-mark-recapture, faecal DNA based population estimation, dung counts and footprint (spoor) analysis are relatively modern techniques that have been developed and applied to the abundance estimation of the wildlife in many parts of the world and most of them have been tested for mega-herbivores such as rhinoceros (Jewell *et al.* 2001; Alibhai *et al.* 2008; Brodie *et al.* 2010; Hariyadi *et al.* 2011) and elephant (Goswami *et al.* 1997; Varma 2006; Lukacs *et al.* 2007). Therefore, these techniques have been chosen in this study as they are either implemented or have been attempted in case of other species of rhinoceros throughout the world. Most of the studies using capture-mark-recapture models have been carried

out in African black rhinoceros (Brodie *et al.* 2010) and Javan rhinoceros (Hariyadi *et al.* 2011) but instance of their implementation in greater one-horned rhinoceros is lacking. Further, since some of these techniques are comparatively costlier than traditional methods of total count, therefore they are not being followed actively in greater one-horned rhinoceros population estimation in India and Nepal. With a known population in a given area (32 rhinoceros in 25 km^2 area) of Dudhwa NP, U.P. would essentially provide with an opportunity to assess accuracy and precision of these techniques. Therefore, the first objective of our study is:

Objective 1: **To compare and validate the techniques used in determining rhino abundance estimation with special reference to accuracy and precision.**

Wherein, we tested Non-invasive faecal DNA based CMR. Photographic CMR, Dung count and Footprint analysis methods for their applicability in rhinoceros abundance estimation with respect to accuracy and precision.

Research question: Among the given sampling techniques, which one would provide most accurate and precise results when applied to rhino abundance estimation in Dudhwa NP?

Over the period of 27 years, closed greater one-horned rhinoceros population in Dudhwa NP have been constantly interbreeding producing 4 generations of adult individuals. Prior studies suggest that isolated wildlife populations have been prone to threats at the genetic scale and thus, prior quantification becomes quintessential to predict any upcoming issues (Frankham *et al.* 2002). This raises a question on this population's genetic status owing to the fewer parent individuals (in 1985) and social dominance behaviour of male greater one-horned rhinoceros (Dinerstein 2003; Dutta 1991). It is apparent that with no new alleles emerging and instances of few animals dying without reproducing, a state of loss of allelic diversity, heterozygosity and polymorphism occur attributing to genetic drift and non-random mating (Frankham *et al.* 2002). Thus, lowers the overall reproductive fitness of the individuals and affects the long term sustainability of population at large (Dinerstein and McCracken 1990; Frankham *et al.* 2002). In previous studies, it has been observed that greater one-horned rhinoceros populations have suffered a bottle neck event in past (Zschokke and Baur 2002; Zschokke *et al.* 2011) and carry low microsatellite diversity as compared to

black rhinoceros (Scott 2008). Under this purview, we tested the genetic diversity parameters such as heterozygosity, allelic diversity and individual relatedness to predict the probable inbreeding conditions in this small population.

Despite of all the conservation endeavours there were instances in both free ranging and captive wild animals where the population declined due to intrinsic factors such as their genetic diversity (Frankham *et al.*, 2002). Genetic variation imparts elasticity to the population to make it resilient to any environmental or pathogenic attacks and is crucial in securing its long term survivorship. Greater one-horned rhinoceros has always been very pliant towards these factors that has been proved on the level of their genes by Dinerstein and McCracken (1990) where they estimated that these rhinoceros in Nepal carry high level of heterozygosity despite of passing through a bottleneck in past. Similarly, Harley *et al.* (2005) showed that the past known bottlenecks had hardly affected the black rhinoceros subspecies in Africa. The two major populations of greater one-horned rhinoceros thrive in the Nepalese *terai* and north-east India. They are now separated for many centuries with no report of interbreeding at all. Zschokke *et al.* (2011) carried out the task of comparing the genetic variability in these two larger populations and found that both allelic richness and heterozygosity was higher in Assam sub-population (north-east India) than in the Nepal. They further predicted that the Nepal sub-population have more recently colonised suggesting that Assam sub-population was the founder population from where they dispersed. As indicated from their analysis they suggested that the two populations should not be intermixed as both the populations behave as two separate units. However, the present population in Dudhwa NP is a hybrid between the two (along with the founders) and is doing well in terms of number and breeding capacities, it would be interesting to investigate the detrimental consequences of this reintroduction and cross-breeding on a genetic scale.

Threat of inbreeding depression is more potent in a small, isolated or captive population of wild animals as the same alleles circulate in the system (Frankham *et al.* 2002). Following this fact, Al-Otaibi and Fahmi (2011) investigated the genetic diversity in endangered and captive bred Arabian Oryx (*Oryx leucoryx*) population in Saudi Arabia and found out that although the population has existed for over 15 years, it showed little genetic loss. The study done on the captive Baird's tapirs (*Tapirus bairdii*)

of North and Central American zoos indicated that inbreeding and genetic variation loss has started in the tapirs in Central American zoos (Norton and Ashley 2004). As a remedy the exchange of animals between the North and Central American zoos was suggested for their genetic rescue (Norton and Ashley 2004). Small isolated population of any animal is as good as a captive population in the context of its genetic variability. Since, greater one-horned rhinoceros population in Dudhwa NP are inside an enclosure from the time of their reintroduction a cumulative study encapsulating both the assumed effects of hybridisation between Assam and Nepal rhinoceros and probable inbreeding would be crucial if further reintroduction or translocation of Dudhwa rhinoceros to other areas is seen as a necessary effort towards their conservation.

In order to ensure effective conservation at landscape level, the forest authorities are planning to rehabilitate some individuals in the other areas of Dudhwa NP, such as the proposed *Bhadi taal* area (Sinha *et al.* 2010). Considering this issue, it becomes essential to understand the population genetic structure and aid in dispersal of the species in its historic home range.

The second objective of this study was concerning the genetic variability in this reintroduced population.

Objective 2: To determine the genetic diversity in the present population of rhinos.

Research question: What is the level of heterozygosity and allelic diversity in the present population?

1.2. STUDY AREA

1.2.1. Dudhwa National Park

Dudhwa National Park is situated in the Nighasan Tehsil of district Lakhimpur Kheri, Uttar Pradesh, India and shares its northern borders with Nepal (Tiwari & Joshi 1997). Geographically, it extends between 28°18'N and 28°42'N latitudes and between 80°28'E and 80°57'E longitudes (Aziz 1990) in the *Terai* Arc Landscape. The area enclosed within park boundaries is 680.34 km^2 (core-490.30 km^2 and buffer-190.04 km^2). This landscape is assigned under Biogeographic Zone 7A i.e. The Upper Indo-Gangetic Plain (Rodgers and Panwar 1988). It is one of the highly productive biodiversity rich areas in the *Terai* Arc Landscape (Kumar 2009) and hence, was

notified as Dudhwa Wildlife Sanctuary in 1958 which was later upgraded to the status of National Park in 1977 (Govt. of U.P., Forest Department Notification no. 6991/14-3-1/74, dated 21.1.1977). The park has been declared as Dudhwa Tiger Reserve in 1987 which includes Dudhwa National Park, Kishanpur Wildlife Sanctuary and Katarniaghat Wildlife Sanctuary. The buffer zone is located north of the core region and it is inhabited by the *'Tharu'* tribe (Aziz 1990). Several of their villages are still present within the vicinity of the park in buffer area. The entire are is subdivided into six ranges *viz.* Bankati, Dudhwa, North Sonaripur, South Sonaripur, Sathiana and Bellraien.

Himalayan perennial rivers Mohana and Suheli confines the two boundaries of the protected area. Mohana flows in north adjacent to Indo-Nepal border and river Suheli runs along the southern limits of the park (Aziz 1990). Other than the two rivers, three *nalas i.e.* Nagrol, Neora and Jauraha form the perennial water source that notably influences the wildlife distribution in the park (Singh 2002). A number of lakes and swamplands are interspersed inside the park with Kakraha, Bhadi, Banke, Ranwas, Puraina, Tiger *taals* being the prominant ones (Singh 2002).

Dudhwa National Park lies in the plains of *Terai* region and hence, the terrain is more or less flat with fertile alluvial soil bed while other soil types include sand, loam and clay varying according to the local topography (Singh 2002).

Three distinct seasons are recognised *viz.* Summer (March to June), Monsoon (July to October) and Winter (November to February). The rainfall occurs between June and September reaching an annual average of 150 cm (Singh 2002). The park remains flooded for most parts of monsoon with water-logged conditions in low lying areas in post-monsoon months (Singh 2002).

1.2.1.1. Flora

Dense forest of Sal *Shorea robusta* covers the landscape which is interspersed by marshes and grasslands. The forests of Dudhwa NP can be broadly classified into two categories *viz.* Tropical Semi-Evergreen Forest and Tropical Moist Deciduous Forest (Champion and Seth, 1968). The major trees are Asna *Terminalia allata*, Shisham *Dalbergia sissoo*, Semal *Bombax malabaricum*, Ganjhar *Ficus rumphii*, Kusum *Schleichera oleosa*, Saghun *Tectona grandis*, Dhak *Butea monosperma*, Khair *Acacia katechu*, Haldu *Adina cordifolia*, Lasora *Cordia dichotoma* and Gutel *Trivia nudiflora*. Occurring along the

streams and *nalas* is a riparian zone dominated by Jamun *Syzygium cumini* followed by Putijiya *Drypetes roxburghii* and Kath neem *Murraya koenigii.* The third important ecosystem of the Dudhwa landscape mosaic include large grasslands called *'Phantas'* dominated by Nari *Arundo donax,* Kans *Saccharum spontaneum,* Munj *Saccharum munja,* Narkul *Phragmites karka* and Pater *Typha angustata* (Singh 2002).

1.2.1.2. Fauna

The park harbours about 38 species of mammals, 16 species of reptiles, 400 species of birds and 90 species of fishes. Apart from Greater one-horned rhinoceros , the endangered fauna found here consist of swamp deer *Rucervus duvaucelii duvaucelii,* Bengal tiger *Panthera tigris tigris,* Asian elephant *Elephas maximus,* hispid hare *Caprolagus hispidus,* hog deer *Axis porcinus,* Bengal florican *Haubaropsis bengalensis,* white rumped vulture *Gyps benghalensis* and griffon vulture *Gyps fulvus* (Singh, 2002). Other notable animals are spotted deer *Axis axis,* barking deer *Muntiacus muntjak,* golden jackal *Canis aureus,* fishing cat *Prionailurus viverrinus,* leopard *Panthera pardus,* wild pig *Sus scrofa,* sloth bear *Melursus ursinus,* crocodile *Crocodylus palustris,* Asiatic rock python *Python molurus,* king cobra *Ophiophagus hannah,* Indian roof turtle *Pangshura tecta,* spotted pond turtle *Geoclemys hamiltonii,* great hornbill *Buceros bicornis* and great slaty woodpecker *Mulleripicus pulverulentus* (Singh 2002).

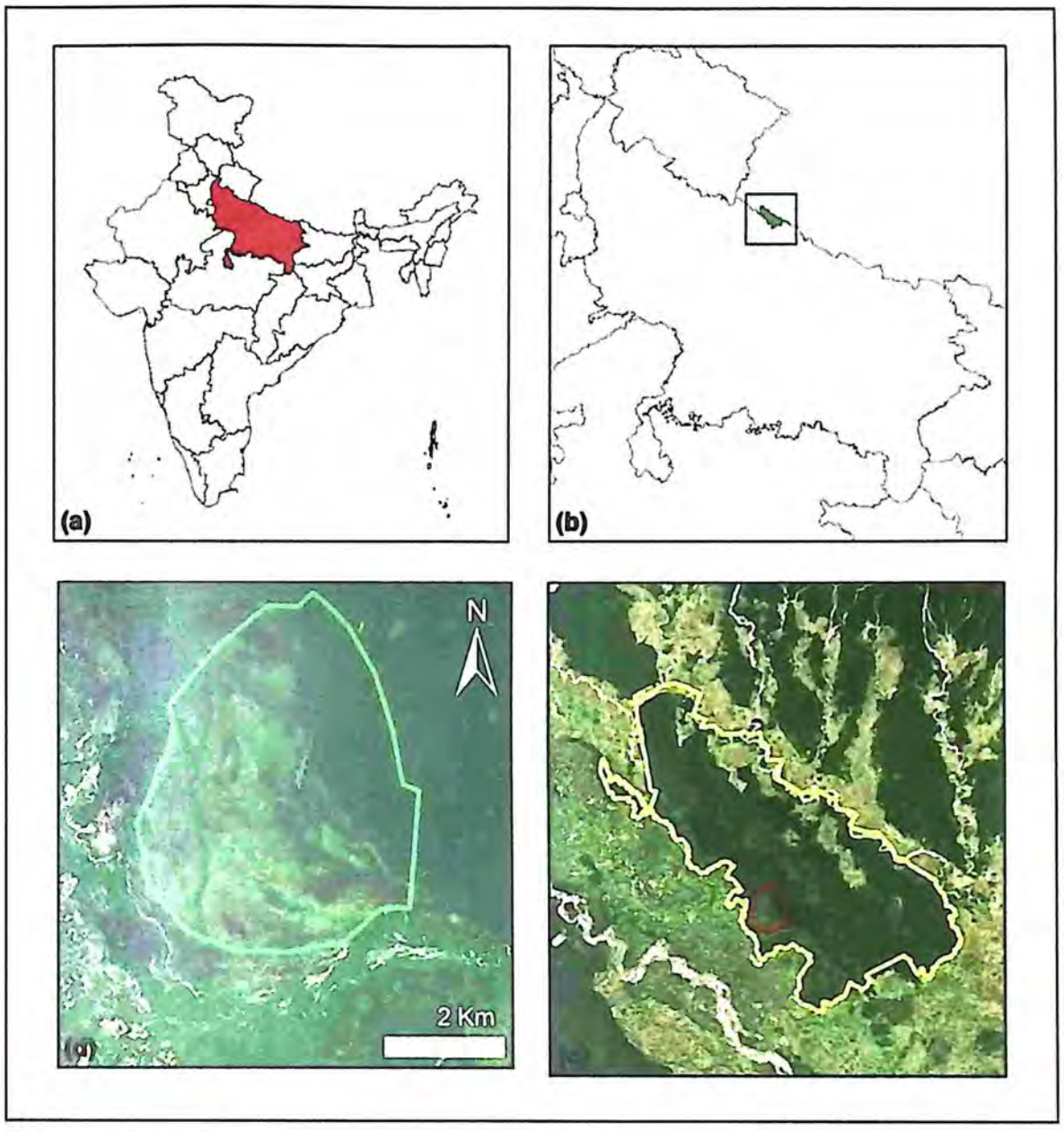

Fig. 1 Map showing:
(a) Uttar Pradesh in India
(b) Location of Dudhwa National Park in Uttar
(c) Location of RRA in Dudhwa National Park
(d) Boundary of RRA

1.2.2. Intensive Study Area: The Rhino Reintroduction Area (RRA)

The Rhino Reintroduction Area or the intensive study area (Fig. 1 and 2) under this study is located in the South Sonaripur range of Dudhwa National Park. The area of about 25 km² has been encircled by 4-wired electric fences which constitute the Kakraha block and a part of Chotapalia block (Aziz 1990; Sinha *et al.* 2010). The entire

18.19 km of the fence is regularly monitored for any damage to ensure that no rhinoceros strays out of the enclosure.

About 80% of the enclosed area has grasslands while remaining is moist deciduous Sal forest with nine permanent lakes in the grassland-woodland ecotone region (Aziz 1990). Few strips of riparian zone exist along the streams and *nalas* intercepting the grasslands. The major vegetation types identified in the RRA are:

1. The North Indian Moist Deciduous Sal Forest
2. Western Light Alluvial Sal Forest
3. Alluvial Savannah Woodland
4. Moist Sal Savannah Forest
5. Tropical Seasonal Swamp Forest
6. Plantations

The moist Sal *Shorea robusta* savannah forests or the grasslands form two types i.e. Narenga Savannah or Upland *phantas* that are dominated by *Narenga porphyrocoma* and Wet Savannah or Low-lying *phantas*. The whole area gets flooded in monsoon with excessive siltation that regulates the ecological succession cycles.

The power fence prevents the rhinoceros from moving out however, other animals usually cross the fence to come in and use the area. Some of the fauna found inside RRA are hog deer *Axis porcinus*, swamp deer *Rucervus duvaucelii duvaucelii*, Bengal tiger *Panthera tigris tigris* and spotted deer *Axis axis*. Asiatic elephants *Elephas maximus* also frequently intrude the RRA by damaging the fence or negotiating the electric wires (Sinha *et al.* 2010). Other faunal species, also found elsewhere in the park, such as Asiatic rock python *Python molurus*, jungle cat *Felis chaus*, porcupine *Hystrix indica*, blue bull *Boselaphus tragocamelus*, Indian soft-shell turtle *Nilssonia gangeticus*, streaked kukri snake *Oligodon taeniolatus* and white rumped vulture *Gyps benghalensis* are present in the RRA boundary (Singh 2002).

1.3. A BRIEF HISTORY OF GREATER ONE-HORNED RHINOCEROS REHABILITATION IN DUDHWA NATIONAL PARK

Historically, the range of greater one-horned rhinoceros once extended all along the flood plains of the river Indus, Ganges and Brahmaputra over the *Terai* and *Dwars* along the foothills of Himalayas (Rookmaaker 1984) but around a century ago, habitat fragmentation, hunting and heavy poaching for its invaluable horn, considerably reduced its distribution ranges (Dinerstein and Price 1991; Dinerstein 2003) (Fig. 3 depicts the historic and current range of greater one-horned rhinoceros in Indian Sub-Continent). The last rhinoceros of Uttar Pradesh was shot in Pilibhit district adjacent to the Dudhwa National Park in 1878 (Sinha *et al.* 2010). The populations declined rapidly and suffered a bottle neck in mid 19th century (Dinerstein and McCracken 1990). The only surviving populations remained in pockets in the North-East India and in some areas falling in the Nepalese Terai (Dutta 1991). They were seen to be highly vulnerable to extinction risks due to poaching, habitat loss and epidemic disease outbreak (Sale and Singh 1987).

The declining population of then endangered greater one-horned rhinoceros in the last remaining refuges of North Eastern India instigated the need for conservation, and repopulating their former ranges was adopted as an alternative strategy to raise their numbers in wild (Sinha *et al.* 2010; Schenkel 1981). Dudhwa National Park was selected, as it met the habitat, space and safety requirements (Sinha *et al.* 2010; Schenkel 1981; Sale and Singh 1987; Singh and Rao 1984). Following final negotiations from the state governments first phase of reintroduction happened in 1984 (Sinha *et al.* 2010; Sale and Singh 1987). Six individuals (2 males and 4 females) from Pobitora Wildlife Sanctuary, Assam were chosen and brought to Guwahati zoo where one of the females died due to capture myopathy (Sinha *et al.* 2010). The remaining five were air lifted to New Delhi and from there they were translocated in special vehicles to Dudhwa NP (Sinha *et al.* 2010; Sale and Singh, 1987; Singh and Rao 1984).

The predefined area in South Sonaripur range was fenced to receive the new members (Singh and Rao 1984). Two more females died within a span of few months after relocation owing to travel stress. In order to revive the population and improve genetic variability in filial generations four female rhinoceros were brought to the park

from Royal Chitwan NP, Nepal in lieu of sixteen camp elephants during the second phase of reintroduction in 1985 (Sinha *et al.* 2010; Sale and Singh 1987; Singh and Rao 1984). Thus, in all seven individuals contributed to the seed population in Dudhwa NP where, at present, 32 individuals are surviving within the RRA.

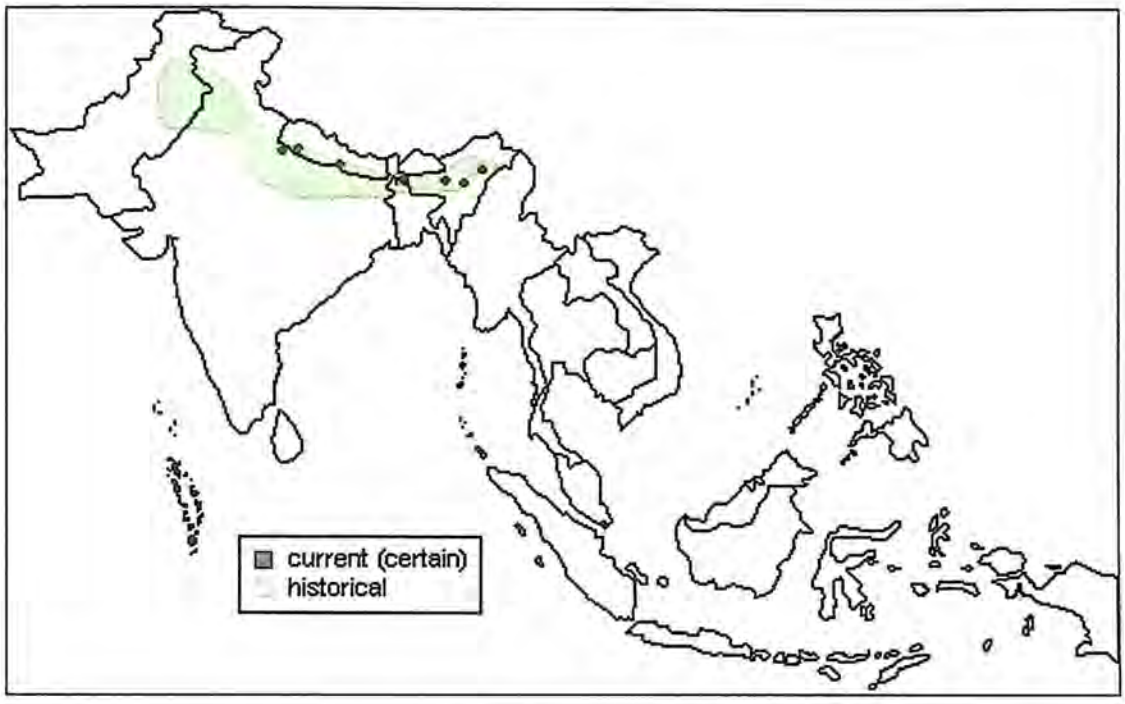

Fig. 3: Historic and current range of greater one-horned rhinoceros in Indian Sub-Continent

CHAPTER 2

GREATER ONE-HORNED RHINOCEROS ABUNDANCE ESTIMATION

2.1. NON-INVASIVE FAECAL DNA BASED POPULATION ESTIMATION

2.1.1. Introduction

Wildlife population size is an important factor for conservationists because, being a state variable, it significantly influences ecological and behavioural attributes of a species, thus, provides vital insights into the protection and management of threatened taxa (Williams *et al.* 2002; Mondol *et al.* 2009; Jewell 2013). Precise and accurate population estimates for rare, elusive and wide ranging species is a tedious task to accomplish (Thompson 2004; Gopalaswamy *et al.* 2012). Precise counts are generally considered preferable over incorrect estimates for small, isolated and rare species, especially when they are of interest to hunters and poachers (Harris *et al.* 2010). In recent times, non-invasive tools, such as faecal DNA capture-mark-recapture (CMR) (Waits 2004; Mondol *et al.* 2009) or footprint analysis (Jewell *et al.* 2001) for sampling population of rare and endangered species are emerging out as promising techniques when supported with well-established statistical models to deal with imperfections in detectability.

Non-invasive faecal DNA CMR works on the principle of marking individuals in first capture session followed by recapture of a proportion of marked individuals in subsequent sampling sessions through DNA fingerprinting, where the DNA is obtained from the species faecal matter (Williams *et al.* 2001; Petit and Valiere 2006). Analogous to camera trap based CMR, where individuals are marked using individually identifiable natural markings such as stripes in tiger *Panthera tigris* (Karanth and Nichols 1998), rosettes in leopard *Panthera pardus* (Henschel and Ray 2003) and jaguar *Panthera onca* (Silver et al. 2004), in this method, individuals are distinguished through their unique genotypic identity.

In last two decades, this technique has been widely applied to population estimation of broad spectrum of species (summarised in Lukacs and Burnham 2005) such as bears *Ursus spp.* (Woods *et al.* 1999), African elephants *Loxodonta cyclotis* (Eggert *et al.* 2003), coyotes *Canis latrans* (Kohn *et al.* 1999), humpback whales

Megaptera novaeangliae (Palsboll *et al.* 1997) and painted turtles *Chrysemys picta* (Pearse *et al.* 2001).

The presents study is an attempt to test this technique on a small and confined population of greater one-horned rhinoceros in Rhino Reintroduction Area so as to determine the precision and accuracy associated with it in population estimation of this species.

2.1.2. Material and Methods

2.1.2.1. Collection of Faecal Samples

Non-invasive faecal (or dung) samples were used as source of DNA as they are effective and widely accepted (Kohn and Wayne 1997). Apart from least interference to animal and ease of collection, faecal samples were selected as their collection permissions are easier to obtain than for invasive blood and tissue samples in case of Schedule I species such as greater one-horned rhinoceros. Moreover, rhinoceros dung is easily identifiable in the habitat based on its texture, odour and colour. The only sympatric species here is elephant whose old dung can be mistaken for rhinoceros dung. But this mis-identification was avoided through the collection of fresh rhinoceros dung with cent percent certainty. Fresh dung (within 1-8 hr post defecation identified based on moisture content or direct observation) was collected either during transect walk (see Sec. 2.3.2.3.) or by random search wherever spotted in the species habitat. Samples were collected from solitary dung piles as well as community defecation sites or middens. Their geographic coordinates were recorded using hand held Global Positioning System (GPS) unit (Garmin eTrex Vista HCx). We used disposable toothpicks to scrape and collect in plastic vials, the outermost transparent mucous layer containing the epithelial cells that slough off from the alimentary canal and come out along with the dung every time the animal defecates. We collected 140 dung samples (ca. 100-150 g) which were sundried and stored in plastic vials over silica gel till they were brought to laboratory for DNA extraction.

2.1.2.2. DNA Extraction

DNA extraction from the dung samples was achieved using commercially available QIAamp DNA Stool mini kit (QIAGEN Inc.) following the manufacturer's

protocols. Precautions were taken at every step so as to check any contamination. DNA was eluted in buffer AE in two aliquots—first in 40 µl and second in 30 µl; and stored at -20 °C.

2.1.2.3. Polymerase Chain Reaction (PCR) Amplification and Genotyping

We used 12 fluorescently labelled rhinoceros specific microsatellite forward and reverse primer pairs *viz.* Rh1, Rh3, Rh4, Rh5, Rh6, Rh7, Rh9, RH10, RH11 (Zschokke *et al.* 2003), SR63, SRIIIA and SR281 (Scott *et al.* 2004) and followed the same multiplex amplification conditions optimised in Borthakur *et al.* (2012). Standardised 10 µl reaction volume containing 5 µl Qiagen multiplex PCR buffer mix (QIAGEN Inc.), 0.125 µM forward and reverse primers each, 1 µl Q-solution (QIAGEN Inc.), 1.5 µl of dung DNA template and rest nuclease free water was amplified in ABI thermal cycler GeneAmp® PCR System 2700 (Applied Biosystems, Singapore). The temperature regime in the PCR started with initial denaturation (95 °C for 15 min) followed by 40 cycles of denaturation (95 °C for 40 s), annealing (T_a for 1 min) and extension (72 °C for 1.5 min). Final extension conditions were 72 °C for 30 min. The PCR products were checked for amplification by agarose gel electrophoresis in 2% agarose. Positive and negative controls were taken to check any chances of contamination.

2.1.2.4. Genotyping and Quality Assessment

Fragment analyses were performed in ABI PRISM 3130 Genetic Analyser (Applied Biosystems, Singapore). The amplified microsatellite alleles were scored using GENEMAPPER ver. 3.7 (Applied Biosystems, Singapore). Electropherograms with low peak heights in Relative Fluorescence Unit (RFU) might incorporate large allele dropout and/or inclusion of false alleles (Buckleton *et al.* 2005). To eliminate this risk, we categorized the electropherograms, generated during analysis of data, into 4 quality categories based on their peak (Scandura *et al.* 2006), Q1 being the best and Q4 being the worst quality. Q1 comprised of samples with peak height of more than 1000 RFU, Q2 comprised of 301 to 1000 RFU, Q3 comprised of 101 to 300 RFU and Q4 contained samples with less than 100 RFU peak height. Data of below 100 RFU were dropped from any further analysis as Applied Biosystems, the manufacturer of ABI PRISM, recommend caution in using data below 150 RFU in GENEMAPPER user manual since it may lead to flawed score generated by amplification discrepancy.

2.1.2.5. Data Analysis

After allele calling the data was screened manually to avoid any mis-score. We used GENECAP software (Wilberg and Dreher 2004) for estimating the number of unique genotypes and calculating $P_{(ID)}$ (Probability of identity) values (Waits *et al.* 2001).

2.1.2.6. Faecal DNA Capture Mark Recapture Analysis

For estimating the population size using faecal DNA a sampling strategy was needed through which we can generate capture histories of genetically identified individuals (unique genotypes). Dung samples were collected from the RRA on a daily basis through the survey of whole area every day, therefore each day was considered as a sampling occasion and the entire study period was taken as one sampling session. Fig. 1 represents the spatial distribution of dung sample collection points inside RRA. Thus, there were 84 occasions in a single sampling session (from 6th January to 30th March, 2013) and the capture history of individuals identified through their dung DNA was generated. We used the program MARK 7.1 (White and Burnham 1999) to analyse the data and estimate animal population size which was later compared with the actual value to ascertain accuracy of this technique.

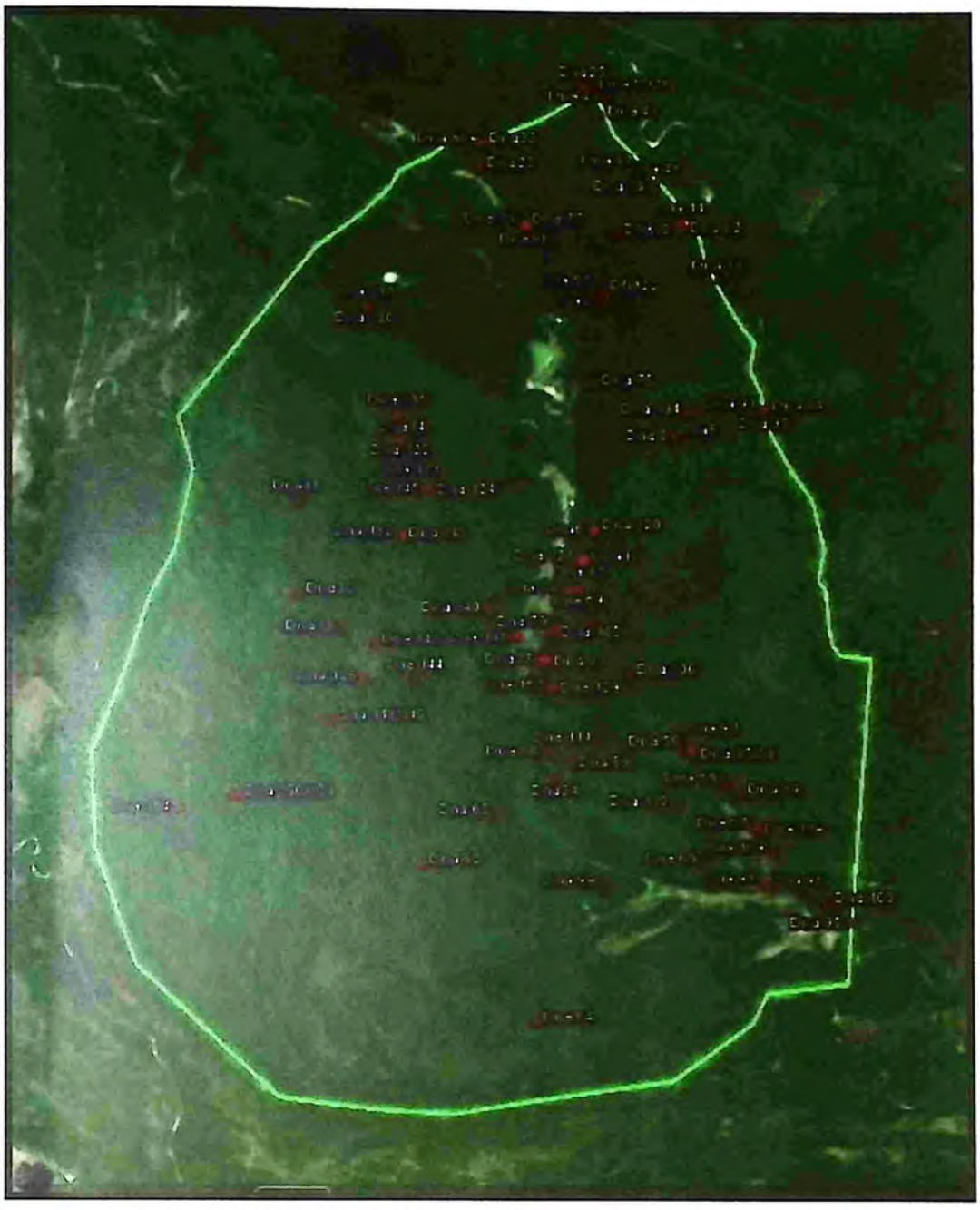

Fig. 1: Distribution of dung sample collection points inside RRA

2.1.3. Results

2.1.3.1. Amplification success

All the 140 dung DNA samples were subjected to analysis and mean PCR amplification success rate across 10 loci was 54.57 % (S.E. ± 7.72%). The amplification success rate per locus has been represented in the Fig. 2. We found that locus Rh7 (success rate 87.9%), Rh11 (success rate 80%) and SRIIIA (success rate 79.8%) were

the best. Locus Rh5 and Rh10 were dropped on account of minimal amplification success rate and poor Q scores.

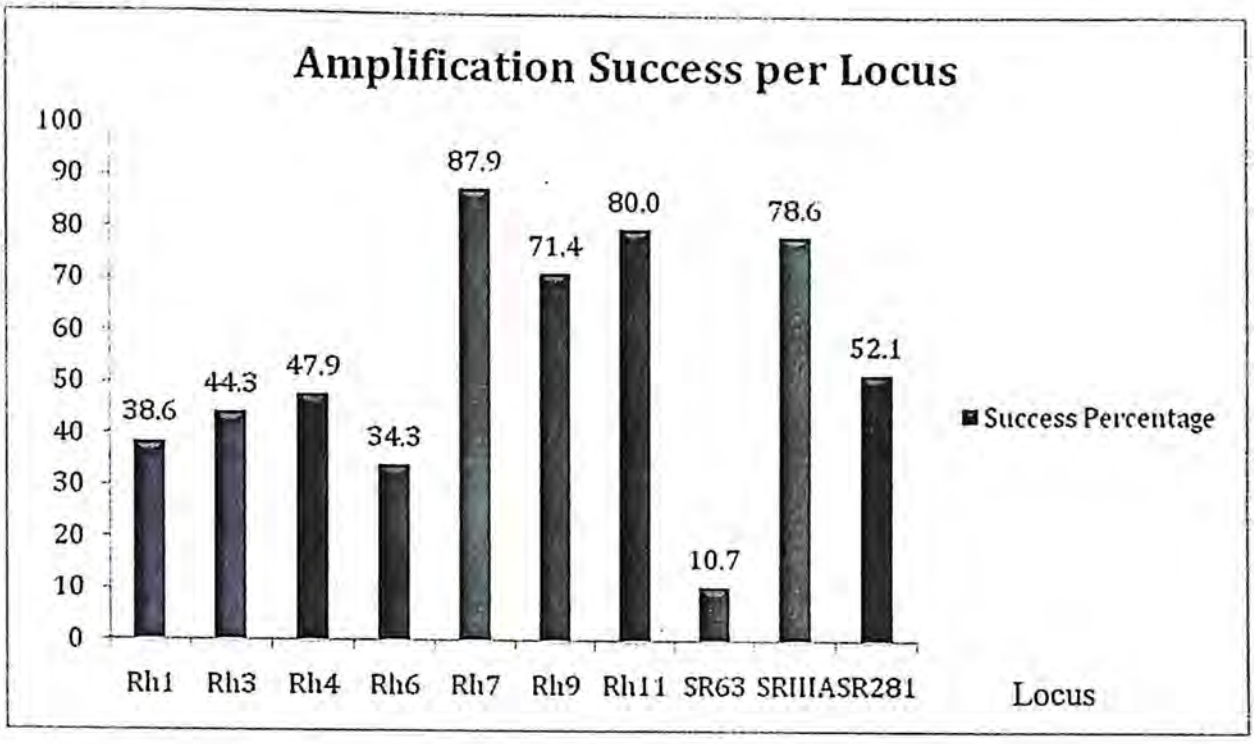

Fig. 2: Success rate of amplification per locus

2.1.3.2. Individual Identification

Out of the 140 samples giving differential amplification for various loci, we selected 79 samples, all of which were amplified in 3 loci *viz.* Rh7, Rh9 and SRIIIA for estimating minimum number of individuals in the population with no mismatch genotype. Fig. 3 shows the estimation of unique genotypes with sequential addition of the three loci Rh7, Rh9 and SRIIIA. We analyzed the data using R software package ALLELEMATCH ver. 2.0 on the basis of unique genotypes. We identified a total of 27 individuals amongst the 79 samples analyzed. Hardy-Weinberg probability of identity (PID_{HW}) was calculated to be 2.8E-02 and sibling probability of identity (PID_{sib}) was found to be 1.7E-01. Fig. 4 depicts the decline in PID values with sequential addition of loci data.

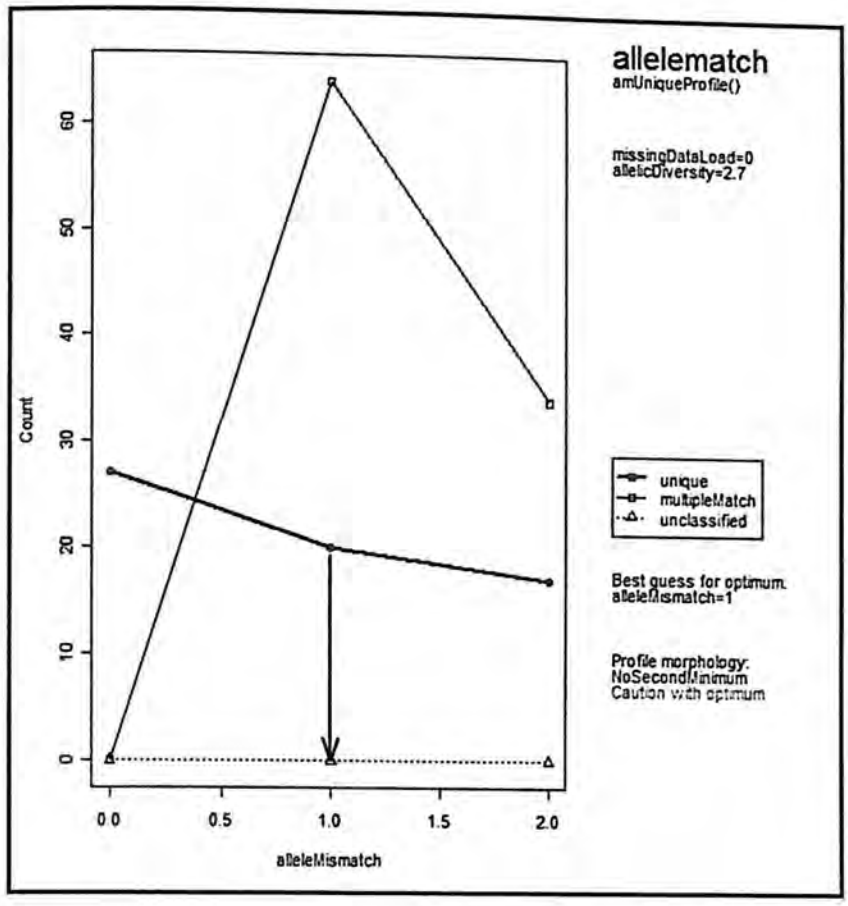

Fig. 3: Unique genotypes with sequential addition of the 3 loci

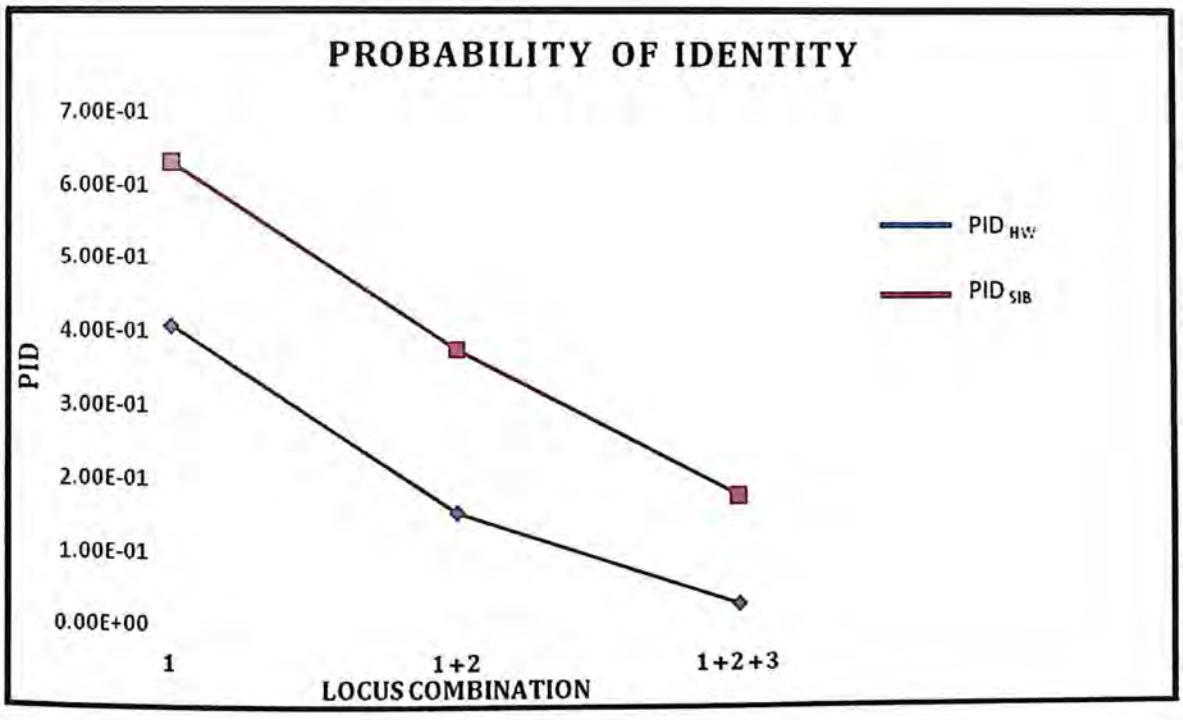

Fig. 4: PID values with sequential addition of loci data

2.1.3.3. Population estimation using Faecal DNA data

The analysis of capture histories revealed that with a total of 84 trap days of sampling efforts, extended over a period of two and a half months, we captured 27 unique individuals (M_{t+1}). Program MARK selected the habitat heterogeneity model (M_h) with minimum AIC value 484.81. The estimated population size (N) was 35.10 (± 5.01). The detection probability (p) was calculated to be 0.096 (± 0.021) and capture probability ($\hat{c}$) was 0.196. Total unique individuals captured were 27 out of which 13 individuals had single captures.

The calculated population size estimate overlapped with the reported total number of individuals (*i.e.* 32 individuals).

2.1.4. Discussion

Counting threatened and elusive wild fauna through their faecal matter, using unique DNA fingerprint of each individual, is a much recent technique in conservation biology (Lukacs and Burnham 2005). Species specific effective field protocols, tools and technology for reliable genotyping of sheared faecal DNA and the models of capture-recapture exist to allow easy implementation of this technique for wide range of species (Lukacs and Burnham 2005).

It was found that success rate of DNA extraction and amplification is moderate (54.57 %) in this study. Lukacs and Burnham (2005) contrive amplification issues to excessively sheared dung DNA or presence of PCR inhibitors, like plant alkaloids in herbivore dung. However, the dung of greater one-horned rhinoceros can be easily identified and procured in rhinoceros habitat due to its prominence unlike scats of elusive carnivores and therefore, the loss in the data can be compensated by collecting large number of dung samples.

Efforts have been made to limit genotyping error as much as possible. Lukacs and Burnham (2005) identified three major problems associated with the non-invasive individual identification. First being the amplification failure, which arises either due to the presence of PCR inhibitors or by vague humane errors. Using standardised protocols and multiplexing schemes we avoided them in our procedures. Moreover, amplification error, if present, gets absorbed into the capture probability later in the analysis of

capture history. Therefore, assuming the capture probability to be less than one (Mondol *et al.* 2010) this error is neglected. The second being genotyping error such as allele dropout or null alleles which cause allele mis-calling. Again, using optimised amplification protocols and refining the data in MICROCHECKER we significantly reduced the error rate. The third is the mutation during amplification which is of extremely rare occurrence which we presumed not to have happened in our case.

Interestingly, the program MARK selected the heterogeneity model to best fit suggesting a significant role of individual behavioural heterogeneity. This may be attributed to the differential detectibility of dung in varied habitats. The detection rate would be higher in more open habitat such as tall grasslands or deciduous forests with thin understory than in the tall grasslands or dense understory forests. In case of greater one-horned rhinoceros habitat, where most of the surveys were done on elephant backs the observer's bias in dung detection may also arise due to dense ground cover. Heterogeneity model also suggests social spacing mechanism and differential use of midden by the species. Dinerstein (2003) reported that a single midden is collectively used by many individuals and are not territorial markings. Individual heterogeneity may vary when few individuals use a midden for longer time and hence, were captured and recaptured several times at the same site.

Although, the fresh dung samples collected gave fair results pertaining to DNA quality, we would suggest use of cotton swabs to acquire only the mucous layer from the fresh dung boli as it is very easily available and identifiable in fresh mega-herbivore's dung. This would further reduce the inevitable side venture of plant alkaloids or other PCR inhibitors in the collected samples.

The non-invasive dung DNA capture recapture is promising in the scenario where the need for wildlife census argues with the disturbance to the natural ecology of the animals. Although, this technique does not seem much economical at first but in the long run becomes reasonable when compared to the disturbance to the species and manpower and other efforts involved, it is more cost-effective and can be used for population estimation of mega-herbivore species such as greater one-horned rhinoceros in its wild habitats.

2.2. PHOTOGRAPHIC CAPTURE MARK RECAPTURE

2.2.1. Introduction

Greater one-horned rhinoceros is a flagship species of *terai* grassland ecosystem (Dinerstein 2003). It is threatened in wild due to habitat fragmentation and illegal killing (Rookmaaker 1984). Functioning as the umbrella species in a fragile yet biodiversity rich *terai*, conservation of this species is decisive for protection of whole range of species and biodiversity in general. Reliable estimates of populations are critical for the conservation of large terrestrial mammals as they play an important role in evaluating the effectiveness of conservation efforts and also provide benchmark data for future management decisions. While the prevalent method for greater one-horned rhinoceros census in India is block count, it fails to address the issues related to key nuisance parameter of detectibility in rigorous manner. Non-invasive technique, such as using remotely triggered cameras (Karanth 1995), are required to regularly monitor their population, especially in such a threat prone scenario.

Capture mark recapture (CMR) technique was initially developed to estimate the abundance of fishes, birds and small mammals where other methods of counting were found ineffective (Otis *et al.*, 1978; Karanth, 1995). The foundation of capture-mark-recapture (CMR) in the wildlife surveys lies in the ability to uniquely identify individuals in population (Karanth and Nichols 1998). In most non-invasive way, unique natural markings, which are less likely to change during the sampling period, have been improvised to estimate population abundance. Karanth (1995) has demonstrated individual tigers *Panthera tigris tigris* can be distinguished on the basis of stripe patterns. Similarly, leopards *Panthera pardus* were identified through rosette patterns (Henschel and Ray 2003), Asian elephants *Elephas maximus* by tusk morphometry (Goswami *et al.* 2007) and blotch structure and arrangement for Indian python *Python molurus* (Bhupathy 1991). Under this scheme, random individuals from a homogenously dispersed population of animals are captured, marked with special, unique identity, released to mingle into the population and recaptured several times in repeated sampling (Pradel, 1996). The photographic CMR is more robust in wildlife inventories under various environmental conditions as compared to line transect and track surveys (Silveira *et al.* 2003). Greater one-horned rhinoceros are always susceptible to illegal hunting and poaching for its valuable horn (Rookmaaker 1984).

In a previous study by Morley and Aarde (2006), it has been reported that population size or density estimation techniques for the large mammals are still poorly developed and demands rigorous efforts. An attempt was made, through this study, to arrive at population estimation of large herbivore through camera trapping technique answering some key questions regarding its applicability in grassland habitat and associated accuracy and precision for arriving at a reliable estimate. Recent expansion of this species population size in Assam and Nepal following the efforts of conservation community, has led to management and ecological questions regarding their abundance, resources and space utilization. Thus, it is important to have a reliable population estimate so as to deal with the scientific, management and conservation issues concerning the species.

2.2.2. Material and Methods

2.2.2.1. Equipments and Survey Design

We used four digital Moultrie (Model: D-40 (MOULTRIE GAME FEEDERS, Alabaster, USA)) camera traps for capturing the greater one-horned rhinoceros images. A reconnaissance survey of the entire Rhino Reintroduction Area (RRA) was carried out to identify the camera stations. The cameras were deployed on road sides and rhinoceros trails where the signs of greater one-horned rhinoceros activity, such as fresh footprints, dung piles and direct sightings, were abundant. Inter-station interval ranged from 1-4 km. Fig. 1 represents the spatial distribution of camera trap locations inside RRA. The digital camera trap units were fixed on tree trunk at a height of 2.5 feet and 3-6 meter away from road, with one unit at a station. Since, the camera trap units were less we divided the area into four sampling blocks based on the observed temporal variation in the species ranging patterns (see sec 2.4.4.) and periodically shifted among the sites. With total trapping nights of 48, we had six sampling sessions with seven trapping nights in each session (extending from 03.02.2013 to 28.03.2013). Due to the seasonal burning of grasslands all sampling occasions were not equal having first two sampling session of 10 days each. All the camera traps were monitored twice in a week to download data and to check for loss by animals or theft.

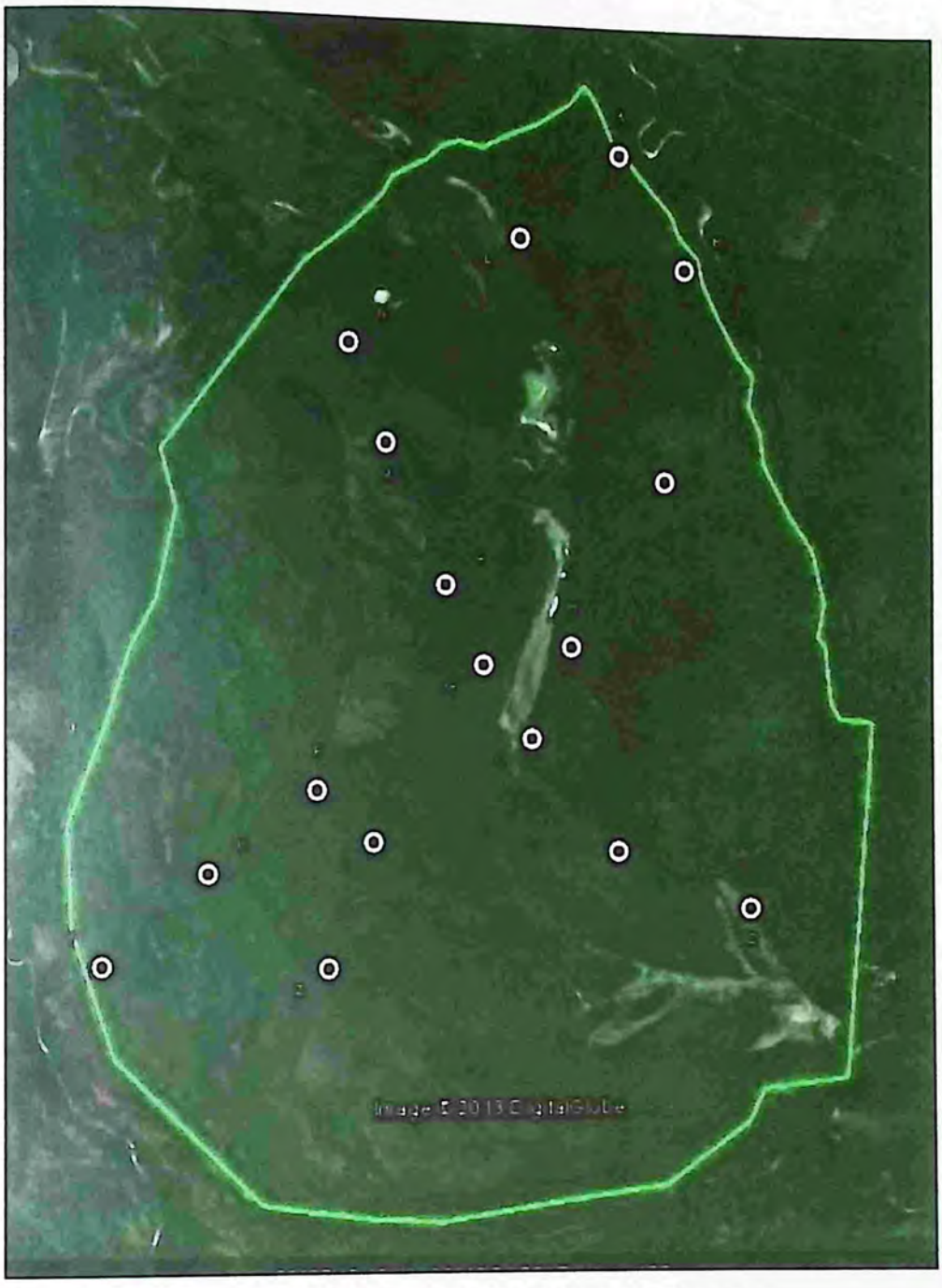

Fig. 1: Location of Camera traps inside RRA

2.2.2.2. Individual Identification

Individuals were identified by close examination of the photographs to discriminate individuals through their physical features such as horn length and thickness, scars on skin, ear nicks and cuts, tuft of hair at the edge of ear pinna and albino spots (Laurie 1981; Dinerstein 2003; Kandel and Jhala 2008). Fig. 2 demonstrates the use of horn shape and size and ear morphology in individual identification of greater one horned rhinoceros. To remove discrepancy in individual

identification, the pictures were matched with the photographic record of Forest Department (prepared by Worldwide Fund for Nature and Natural Resources (WWF) - India). In two cases only the young calves were photographed and identified but the mother did not appear in the picture. They were taken as proxies for their mothers since the calf always remains in close vicinity of the mother (Dinerstein 2003). Hence, assuming that the mother is also present, we have included them into the analysis.

Fig. 2: Example of unambiguous individual identification of greater one-horned rhinoceros using camera-trap photos of head features like horn length and permanent ear bend (Top and Bottom images of same individual (Banke)).

2.2.2.3. Data Analysis

The assumptions of geographic and demographic closure (Otis *et al.* 1978; Karanth 1995; Karanth and Nichols 1998, Karanth *et al.* 2004) hold valid in this population due to confined area and no reported adult mortality during the sampling period. The individuals first captured were considered as marked and their subsequent captures altogether generated the capture histories which were analysed in program MARK 7.1 (White and Burnham 1999). This program uses well established models to deal with the difference in capture probabilities under the influence of factors such as time, trap–response and individual heterogeneity, when assessed as opposed to the null model which assumes that these factors do not affect capture probabilities. We used closed capture models to compare our results as our population always followed the closure assumption during entire sampling period. Individual capture histories for the identified rhinoceros were constructed using a standard 'X-matrix format' (Otis *et al.* 1978; Nichols 1992), in which '1' indicates capture of a particular animal during a particular sampling occasion, while '0' indicates that the same animal was not captured during that occasion. The best-fit model selection was done by the program on the basis of minimum Akaike Information Criterion (AIC).

Further, we subjected the data for appropriate model selection in program CAPTURE (Rexstad and Burnham 1991) to compare the accuracy in the population estimate. Results from both the analysis were compared with the actual value of 32 individuals in Dudhwa National Park to check for accuracy and precision.

2.2.3. Results

With a total of 48 trap nights sampling efforts, extended over a period of two months, we captured 22 unique individuals (M_{t+1}). Program MARK selected the behavioural model (M_b) with minimum AIC value 321.30. The estimated population size (N) was 25.98 (± 4.91). The detection probability (p) was calculated to be 0.036 (± 0.016) while recapture probability ($\hat{c}$) was 0.145. Seven individuals caught only once in the camera traps during the entire sampling period while rest had more than one captures.

CAPTURE program selects the appropriate model using Discriminant Functional Analysis (DFA). Heterogeneity model (M_h) got maximum selection criterion (1.0) followed by behaviour model (M_b) with value 0.96. The program estimated population as 26 (± 3.97) with the detection probability (p = 0.04).

2.2.4. Discussion

Photographic capture mark recapture results showed that this method can be implemented for rhinoceros population estimation in grassland ecosystem. The estimated population of ca. 26 individuals was less than the known population size (32 individuals). However, adding the upper limit of standard error to the estimate (i.e. ca. 31 individuals) brings the population close to the known value. The precision level was high (S.E. ± 3.97) as compared to other similar studies, such as on elephants (N = 134; S.E. ± 14.20) (Goswami *et al.* 2007). The accuracy and precision of this method depends primarily on sampling design besides other factors influencing detection probability (Karanth 1995). Although our sampling efforts were not very well distributed due to field constrains, such as grassland burning, we got a reliable output.

The selection of behaviour (M_b) as significant model in MARK and second most significant in CAPTURE indicated towards the alteration in the behaviour of this species. The major habitat of the species (*i.e.* tall grassland) was burnt during March and April every year as a regular procedure to manage grassland ecosystem (Javed et al. 1999). After burning the habitat becomes more open with increased visibility of camera traps. Perhaps, this significantly influenced their behavioural response towards the camera traps. A behavioural alteration reflecting in the recapture probabilities have been reported in tiger (Wegge *et al.* 2004) and kinkajou *Potos flavus* (Schipper 2007) and is attributed to the animal's sudden encounters with the camera trap units.

We attempted to resolve the issue of individual identification of this species based on camera trap images. Although, the population in Dudhwa National Park was known with individuals already identified we recommend that future camera trap based population estimation of greater one-horned rhinoceros should be done using a pair of camera trap units to capture both the flanks so as to reduce misidentification bias.

Another associated problem encountered during the study is concerned with the habitat that this species occupy. The tall grasslands and swamplands are often non-navigable and unfit for setting up camera traps. In the present case, we placed them only on roadsides and sometimes in open patches within tall grasslands directing towards the opening of an actively used animal trail. Yet the estimated population size was comparable with the known value. Roads are present in all the protected areas where rhinoceros are found. They are utilised for navigation and monitoring as well as for tourism purpose. Therefore, as evident from this study, the photographic CMR method using remotely triggered camera traps can be well used in such landscapes.

Population size is a parameter of paramount importance in both fundamental and applied population biology. The ability to reliably estimate population size from photographs of greater one-horned rhinoceros taken during multi-session sampling experiments is thus a promising step toward increased knowledge of both the technique and the species and consequently better management policies for threatened species.

2.3. DUNG COUNT METHOD FOR POPULATION ESTIMATION

2.3.1. Introduction

The effective management strategies and ecological monitoring of a species is closely linked to aspects related to its abundance and habitat variables. In case of some mammals, it is difficult to detect individuals of a population due to closed habitats or cryptic behaviour (Laing *et al.* 2003). In such instances, indirect surveys of signs are easier to quantify usage of the area by species over a period of time. In addition to this, it also addresses concerns associated with sample error which may arise due to over or underestimate of animal density at the time of direct survey (Jachmann 1991; Laing *et al.* 2003). Convincingly, these indirect sign, such as dung piles, scats, otter spraints, scratch, nests and footprints, are efficient to predict the animal abundance in the area (MacKenzie and Nichols 2004) and hence, used as surrogates to their abundance in the area.

In case of mega-herbivores, such as elephants, it has been reported that dung method yield estimates of abundance comparable with estimates using direct methods and are found to yield more precise estimates of elephant abundance than aerial surveys (Barnes 2002; Laing *et al.* 2003). Despite the broad spectrum of field survey techniques which can be employed for terrestrial mammalian populations, not all can be efficiently implemented with respect to every landscape or species. Considering obstacles pertaining to topography, resource limitations and species behaviour, many effective survey methods fail to produce reliable outputs (Silveira *et al.* 2003). In addition to this, direct animal counts along line transect and its dependency on favourable field conditions, requirement of skilled manpower and bias towards large diurnal species limits the precision and accuracy of population estimate (Barnes 2001; Silveira *et al.* 2003). Thus, it arise the need for ecological monitoring as a quintessential component of any conservation project so that effects of management can be assessed (Kremen *et al.* 1994; Plumptre 2000).

The precise and accurate techniques for assessment of species richness and abundance are important to determine conservation objectives and prioritize necessities (Jewell 2013). Fay (1991) reported the use of dung count methods in several dense forests of Africa to calculate elephant density, thus, emphasising applicability of dung count method for estimating mega herbivore abundance. Varma (2006)

mentioned the use of dung count for estimating Asian elephant abundance in the North-East India. Similar studies to census elephants were carried out using this technique (Dawson and Dekker 1992; Ramakrishnan *et al.* 1991). One of the major drawbacks of direct surveys of animals over indirect surveys of dung is that the direct survey provides only an estimate of average abundance spreading over a period of months. On the other hand, the former gives a precise estimate of abundance for the day of the survey (Marques *et al.* 2001). Thus, it may provide misleading information on habitat use by animal under consideration (Marques *et al.* 2001).

The three central factors governing the dung count technique are defecation rate, dung decomposition rate and dung density (Barnes and Barnes 1992). A steady state is assumed while implementing this technique which means that the dung deposition rate and the dung decomposition rate in a particular habitat is in equilibrium. Dung piles need to be monitored from the day of their deposition to the day they completely vanish to build a survivorship model. The reciprocal of survivorship will provide with the estimated decay rate (Barnes *et al.* 1997; Laing *et al.* 2003).

Dung surveys were rigorously used for population estimation of elephants both in Asia (Varma 2006) and Africa (Barnes *et al.* 1997) but have never been tried in case of rhinoceros owing to the difficulty in estimating dung density arising due to community latrine site or middens. Through the presents study, we addressed the question of validity and precision of dung count survey technique in rhinoceros abundance estimation, by testing it on the isolated population of greater one-horned rhinoceros present in Rhino Reintroduction Area of Dudhwa National Park.

2.3.2. Material and Methods

2.3.2.1. Defecation Rate

Defecation rate or the number of times an individual greater one-horned rhinoceros defecates per day was quantified by monitoring three individual in the Kanpur Zoological Park. They were monitored during the entire length of day for 5 days and number of defecations was counted. Monitoring in night was difficult due to permission issues therefore, number of dung piles gathered during the night was counted the next morning.

2.3.2.2. Decay Rates

To estimate the dung decay rates we marked 20 fresh dung boli in each of the four habitat types *viz.* tall grassland, short grassland, forest and riparian forest and monitored for decay stages on an interval of 7 days. Care was taken while placing these boli, such as keeping them in obscure places so that probability of rhinoceros defecation on it is reduced. We followed Barnes and Jensen (1987) who had described this method for measuring dung decay rate for elephant where they classified the dung decomposition status into six stages:

Stage A- All boli intact, fresh, moist, with odour

Stage B- All boli intact, dry, no odour

Stage C1- More than 50% (but <100%) of all boli intact, whether moist or dry

Stage C2- Less than 50% of all boli intact, whether moist or dry

Stage D- All boli broken up and/or flat mass, whether moist or dry

Stage E- No dung visible *i.e.* fully decayed

2.3.2.3. Transects

Owing to the difficulty in carrying out line transect on foot in a rhinoceros country, elephant transects were carried out for estimating the dung abundance. Transects were randomly laid across the vegetation types which ensured spatial coverage of the entire Rhino Reintroduction Area (RRA). In all 15 transects with lengths ranging from 1 km to 3.2 km were run and the greater one-horned rhinoceros dung piles were counted on either side. The perpendicular distance between the transect centre line and dung pile was measured either with a steel measuring tape or by ocular estimation. Geographic coordinates of each pile was recorded using a hand held GPS unit (Garmin eTrex Vista HCx) along with the habitat type where it was spotted.

2.3.2.4. Data Analysis and Population Estimates

The program DISTANCE 5.0 (Thomas *et al.* 2006) was used to analyse the data collected from the elephant transect survey in conventional distance sampling framework. This program assumes that all dung piles located on transect are detected with certainty and calculates a probability of detection as the perpendicular distance from the transect increases. Therefore, the dung density is calculated in the same way as

animal density is ascertained. The animal abundance from the dung abundance is calculated by the equation:

$N = (Y \times R)/D$ (Dawson and Dekker 1992) ----------1

where,

N=Density of animal

Y= Density of dung piles

R= Daily rate of decomposition

D = Number of times of defecation by an individual per day.

2.3.3. Results

2.3.3.1. Defecation Rate

The defecation rate thus, calculated after monitoring the wild caught individual was found to be 5.6 ± 2.24. We used the rounded off value of 6 defecations per day in our subsequent analysis.

2.3.3.2. Decay rate

None of the dung boli completely decayed during our study period. However, owing to varying environmental and ecological conditions in different habitat, the rate of change of bolus features from one stage to another was significantly different among them. Therefore we assumed the decay rate was 0 as the survivorship was 100 percent during the study period.

2.3.3.3. Dung Density

The DISTANCE program selected half normal uniform model to fit our data based on minimum Akaike Information Criterion score (AIC=479.15). A total of 126 dung piles (N=126) were sighted on 15 transects (K=15) which formed 29 km (L=29) of elephant transect length suggesting our efforts. The encounter rate was 4.32 dung pile/km (C.I. 3.03-6.14) on the entire length of transect walk. Effective strip width (ESW) was calculated to be 4.21 m (C.I. 3.71-4.97 m) with expected cluster size of 2.6 (C.V. 8.61). The detection probability (p) was 0.5 (C.V. 6.48). The variation in detection probability with relation to the perpendicular distance from the transect midpoint in represented in Fig. 1. The dung density calculated by the model was 1352.4/km^2 (C.V. 19.96/km^2).

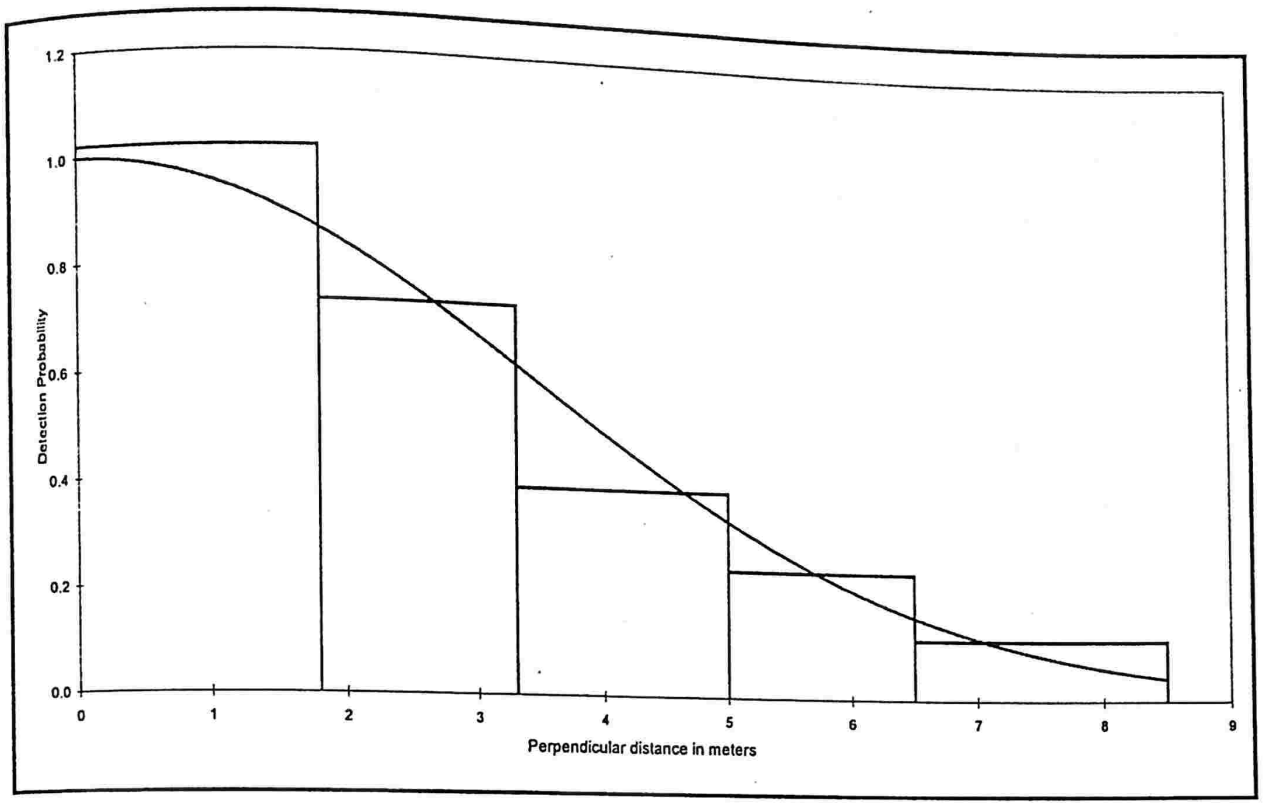

Fig.1: Variation in detection probability from the midpoint of transect

2.3.3.4. Abundance Estimation

The analysis of data is still in progress and the animal abundance would be estimated using the equation 1. Since none of the dung piles, monitored for decay, completely disintegrated during the short study period of 3 months, we took the decay rate to be 1 (*i.e.* all the dung surviving). To correctly estimate the rhinoceros abundance we modified the equation 1. Since, the data on the percentage proportion of fresh dung (Stage A) is available, we will multiply the dung density to arrive at an estimate that nullified the count of all the other decay stage dung piles encountered while walking the transects.

2.3.4. Discussion

Dung count method for population estimation of mega-herbivores, such as species of elephant, is a widely used and effective method but its reliability for greater one-horned rhinoceros abundance estimation was found to be low. Since walking transects in a rhinoceros country is dangerous and ineffective due to tall dense grasslands, elephant transect serve as an alternative. But the subjective problem arises since the camp elephant never walks on a perfectly straight line. The errors associated

with it can be reduced by truncating the entire transect at small intervals and negotiating the bends. The greater one-horned rhinoceros habitat which comprises of predominately tall grasslands and swamplands (Subedi *et al. in press*) makes the dung detection hard but the detection probability can be incorporated using MCDS (Multiple Covariate Distance Sampling) models in DISTANCE software.

The crucial issue that surrounds the entire technique is to calculate the dung decay rate and deposition rate (Plumptre 2000). Although during the short window of our study period none of the marked dung piles completely deteriorated, we recommend that a separate study should be conducted to measure the rate of dung disappearance in each habitat type for every season.

In addition, following individuals over several days to measure the average defecation, as done in case of elephants (Wing and Buss 1970; Tchamba 1991), is difficult in tall grasslands. However, decay rate is critical in this method and therefore, we suggest confining an individual in its natural habitat in a small area (< 1 km^2) by an enclosure and monitor defecation rate. We could not ascertain the defecation rate of the free ranging greater one-horned rhinoceros due to logistic constrains and therefore, had to adhere to the defecation rate enumerated from the captive individual only. Although, it is obvious that there would be certain variations in the defecation rates of free ranging and captive animals due to difference in forage quality, quantity, feeding time and activity pattern of the two and the need to quantify these factors is important for improved design of this study.

Question may arise regarding the observer's bias in approximating the number of dung piles at a latrine site. Greater one-horned rhinoceros has a tendency to defecate on the sight of another dung pile, which Dinerstein (2003) reasoned to be the dung odour that instigate excretion. The animals were seen either defecating over the existing pile or on its sides or in close vicinity of the nucleus pile. Estimating the number of dung piles when a latrine site where animals had defecated on top of existing ones becomes cumbersome but one can approximate the numbers through close observations to general bolus volume and average boli number dropped by the animal.

Although this technique is logistically and economically sound, we have not estimated abundance of rhinoceros from this method. Lack of appropriate defecation and decay rate data hindered the analysis but work is still in progress to adequately come out with a logical estimate, and does not form the part of this thesis.

2.4. FOOTPRINT TECHNIQUE FOR GREATER ONE-HORNED RHINOCEROS POPULATION ESTIMATION

2.4.1. Introduction

With the increasing numbers of globally threatened wildlife species the significance of population estimation and monitoring techniques is realised so as to evaluate the effectiveness of conservation measures (Ramakrishnan *et al.* 1991; Singh *et al.* 2002; Nowell and Jackson 1996; Karanth 2003; Sharma *et al.* 2005). Besides, reliable population estimates also provide baseline information regarding various life history parameters of the species that assist in making future management decisions. Human intervention in the wildlife habitat adversely affects the biology of faunal species (Harihar *et al.* 2009) while most monitoring techniques fail to address this disturbance factor, thus, jeopardise their natural behaviour. Widely accepted sweep count and block count as well as aerial surveys conceived for rhinoceros population estimation alters the natural behaviour of the species due to frequent animal-human encounters and necessitates the use of least disturbing methods.

Investigation of indirect evidences such as faeces, scratch, scrape, sprays, nests and footprint contribute much to the species survey without any direct hindrance to animal or its habitat. Individual identification of animals using their footprint is one of the techniques which succumbs reliable estimates when combined with appropriate sampling design and field craft. Individuals can be distinguished by their footprints in the same way as humans are identified through their fingerprints (Van Strien 1985). This technique is cost effective and least interfering. Nevertheless, decoding animal tracks and footprints have always been a part of field studies for conservation researchers and managers during faunal surveys. It provides with opportunity to record the presence-absence and sometimes abundance of elusive, nocturnal species or species with very low abundance.

Footprints of an animal is defined as the imprint of one foot left in the soil while tracks is a series of footprints of one animal left in the soil when the animal moved over an entire length (Van Strien 1985). Footprint tracking is an age old technique traditionally practiced by indigenous tribal community for hunting and interpreting peculiar animal behaviour (Jewell *et al.* 2001). Footprint (Pugmark for carnivores)

census technique was used for population estimation of tiger *Panthera tigris* based on expert identification of individual animals through their pugmark (Choudhary 1970, 1971, 1972; Sawarkar 1987; Sharma *et al.* 2005; Singh *et al.* 2002). In recent times, it has been deployed to distinguish individual mountain lions *Felis concolor* (Currier *et al.* 1977; Kutilek *et al.* 1983; Fitzhugh and Gorenzel 1985; Van Dyke *et al.* 1986; Grigione and Burman 2000; Sharma *et al.* 2005), population estimation of mountain tapir *Tapirus pinchaque* (Lizcano and Cavelier, 2000), pine marten *Martes martes* (Zalewski 1999) and snow leopard *Panthera uncial* (Riordan 1998). Single footprint of an animal can reveal information such as age-sex, individual identity and physical condition of the animal (Corbett 1944; Abramov 1961; Choudhury 1970, 1971, 1972; Sankhla 1978; Panwar 1979a,b; Jayarajan 1983a,b; Sawarkar 1987; Basappanavar 1988; Gogate *et al.* 1989; Rishi 1997; Singh 1999; Sharma *et al.* 2005).

Tracks of Sumatran rhinoceros *Dicerorhinus sumatrensis* (Van Strien 1985; Borner 1970; Flynn and Abdullah 1983) and Javan rhinoceros *Rhinoceros sondiacus* (Schenkel and Schenkel-Hulliger 1969) were used as a surrogate to animal abundance for these low density cryptic Asian rhinoceros species. But due to insufficient information regarding the true numbers in wild the credibility of this technique was questioned. Jewell *et al.* (2001) used objective footprint together with robust statistical design to validate footprint survey method and classified individual footprints of black rhinoceros *Diceros bicornis* to obtain significant accuracy (87% - 95%). Their detailed study was conducted on *a priori* known population (of 15 individuals) and therefore, reduced the probability of biased estimation due to misidentification. Similar analysis was conducted on individual identification of white rhinoceros *Ceratotherium simum* which effectively estimated their population size as well (Alibhai *et al.* 2008).

Despite simplicity and reasonable accuracy, footprint method has its own drawbacks. Its reliability was debated over the issue of subjectivity that is always involved in discriminating the footprints (Schaller 1967; Singh 1972, 1984; Sankhla 1978; Karanth 1987, 1993, 1999; Karanth & Nichols 2000). The variability in footprint classification, which arises due to changes in shape and size over different substratum, soil texture, soil moisture and soil depth, can never be negated with this technique. Therefore, the technique loses reproducibility and the generalisation becomes invalid.

Scientific literature testing the reliability of this technique for greater one-horned rhinoceros individual discrimination is lacking to the best of our knowledge. Greater one-horned rhinoceros, being the third largest terrestrial mammal (adults weigh ca. 1500—2000 kg), is likely to leave a conspicuous footprint in the substratum. The current study attempts to differentiate between individuals of this species and evaluates this technique for their population estimation.

2.4.2. Materials and Method

The reconnaissance survey was carried out in the entire Rhino Reintroduction Area (RRA) to locate the places where probability of getting fresh footprints was high. Following Jewell *et al.* (2001), we selected only left hind foot impression which was identified using the field expertise of forest staff. Images of footprints were taken from continuous tracks formed on roads that traverse the forest and grassland habitats on accounts of restricted visibility in dense tall grasslands and dangers pertaining to rhinoceros country.

Only foot impression with clear features was photographed by holding the SONY Cyber-shot digital camera (Model: DSC-W320) vertically above it. A 30 cm ruler was placed besides the footprint before photographing it for scalar referencing of morphometry. Geographic coordinates were recorded using hand held Global Positioning System (GPS) unit (Garmin eTrex Vista HCx) for geo-spatial reference. The footprint was obliterated after photographing it to avoid its re-evaluation.

2.4.2.1. Image Measurements

We selected best 60 footprint images for further image analysis. Each digital photograph was calibrated with respect to the 30 cm ruler and analysed in Sigma ScanPro 4 (SPSS Inc.) software. In all 24 geometric measurements (including 13 lengths, 7 angles and 4 area (Fig. 1 a, b, c, d)) were taken for every image which are enlisted in Table 1. The measurements were selected to extract the crucial information regarding the morphometry of the footprint. Some of these parameters have earlier shown to give significant power in discriminating individuals in different species such as Bengal tiger (Sharma *et al.* 2005) and black rhinoceros (Jewell *et al.* 2001).

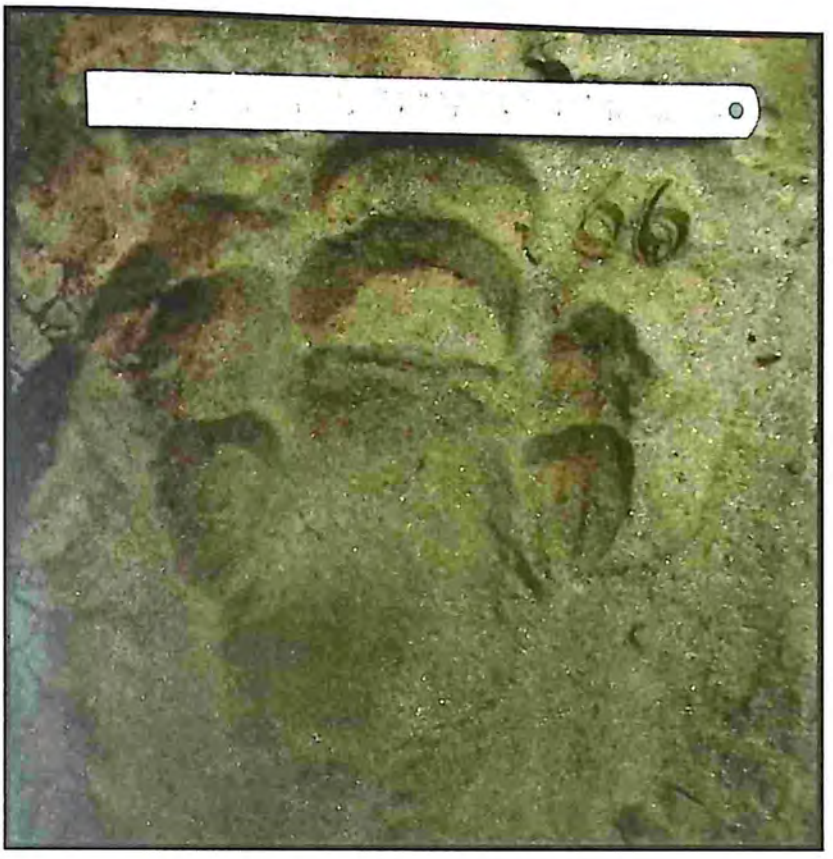

Fig. 1 (a): Footprint image

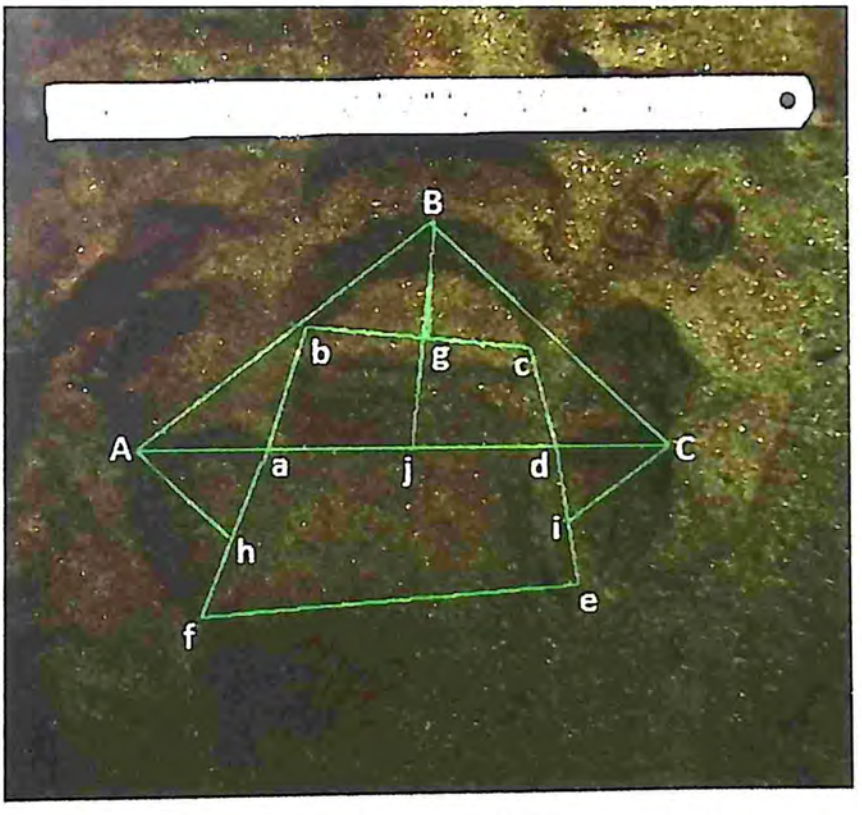

Fig. 1 (b): Footprint showing vertices of measurements

Table 1: Description of parameters taken for analysis

S. No.	Variable	Type	Description
1	Ab	Length	Distance between uppermost point of toe and left lowermost point of second toe.
2	Bc	Length	Distance between left lowermost point and right lowermost point of second toe.
3	Cd	Length	Distance between right lowermost point of second toe and uppermost point of third toe.
4	De	Length	Distance between uppermost point and lowermost point of third toe.
5	Ef	Length	Distance between lowermost point of third toe and lowermost point of first toe.
6	Fa	Length	Distance between lowermost point and uppermost point of first toe.
7	Ah	Length	Distance between lateral most point of first toe and middle point of line intersecting uppermost and lowermost point of first toe.
8	Ci	Length	Distance between Lateral most point of third toe and middle point of line intersecting uppermost and lowermost point of third toe.
9	Bg	Length	Distance between upper most point of first toe and middle point of line intersecting left lowermost and right lowermost point of second toe.
10	AB	Length	Distance between lateral most point of first toe and uppermost point of second toe.
11	BC	Length	Distance between uppermost point of second toe and lateral most point of third toe.
12	CA	Length	Distance between lateral most point of first and third toe.
13	Bj	Length	Distance between uppermost point of second toe and middle point of line intersecting the lateral most points of first and third toe.
14	∠abc	Angle	Angle formed by intersecting uppermost point of first toe, left lowermost point and right lowermost point of second toe.
15	∠bcd	Angle	Angle formed by intersecting left lowermost point, right lowermost point of second toe and uppermost point of third toe.
16	∠cde	Angle	Angle formed by intersecting right lowermost point of second toe and uppermost and lowermost point of third toe.
17	∠def	Angle	Angle formed by intersecting uppermost and lowermost point of third toe with lowermost point of first toe.
18	∠efa	Angle	Angle formed by intersecting lowermost point of third toe with lowermost point and uppermost point of first toe.

19	∠ABC	Angle	Angle formed by intersecting lateral most point of first toe with uppermost point of second toe and lateral most point of third toe.
20	∠BCA	Angle	Angle formed by intersecting uppermost point of second toe with lateral most point of third toe and lateral most point of first toe.
21	bBc	Area	Area covered by second toe depression
22	Dce	Area	Area covered by third toe depression.
23	afA	Area	Area covered by first toe depression
24	fabcde	Area	Area enclosed between the three toes ahead of the planar cushion.

2.4.2.2. Statistical Analysis

The generated geometric measurements were then subjected to Principle Component Analysis (PCA) in SPSS 16.0 (SPSS Inc.) software. This statistical test was selected for preliminary examination of the parameters that has maximum factor weight with successive factoring until no meaningful variance remains (Zar 1984).

2.4.3. Results

The soil condition and habitat structure in greater one-horned rhinoceros habitat was found to be unsuitable for procuring entire sole impression. The alluvial substratum thickens up when wet either by dew or sporadic rains, thus, making it hard enough to form complete impression capturing all necessary features such as hoofs and planer cushion imprints. Consequently, due to above stated field constrains only the hoof marks were photographed and subjected to analysis.

The results of Principle Component Analysis (PCA) signify 6 maximum factor weights which are tabulated in Table 2. Further, we projected these extracted variables on a scatter plot (Fig. 2 a, b, c, d, e) which showed non-significant clusters indicating that none of these variables were statistically powerful enough to discriminate among individual footprint, thereby, this technique for individual identification of greater one-horned rhinoceros in Dudhwa National Park was not applicable.

Table 2: List of factors and their weights

Component	Initial Eigen values			Extraction Sums of Squared Loadings		
	Total	Variance %	Cumulative %	Total	Variance %	Cumulative %
1	6.421	26.753	26.753	6.421	26.753	26.753
2	3.968	16.533	43.285	3.968	16.533	43.285
3	3.441	14.337	57.622	3.441	14.337	57.622
4	3.117	12.987	70.61	3.117	12.987	70.61
5	1.878	7.826	78.436	1.878	7.826	78.436
6	1.134	4.724	83.16	1.134	4.724	83.16
7	0.976	4.066	87.226			
8	0.908	3.783	91.009			
9	0.574	2.393	93.403			
10	0.474	1.976	95.379			
11	0.36	1.499	96.878			
12	0.233	0.97	97.849			
13	0.148	0.619	98.467			
14	0.101	0.422	98.89			
15	0.081	0.336	99.226			
16	0.053	0.22	99.446			
17	0.049	0.204	99.649			
18	0.037	0.156	99.806			
19	0.017	0.071	99.877			
20	0.016	0.065	99.942			
21	0.007	0.028	99.97			
22	0.004	0.017	99.986			
23	0.002	0.01	99.996			
24	0.001	0.004	100			

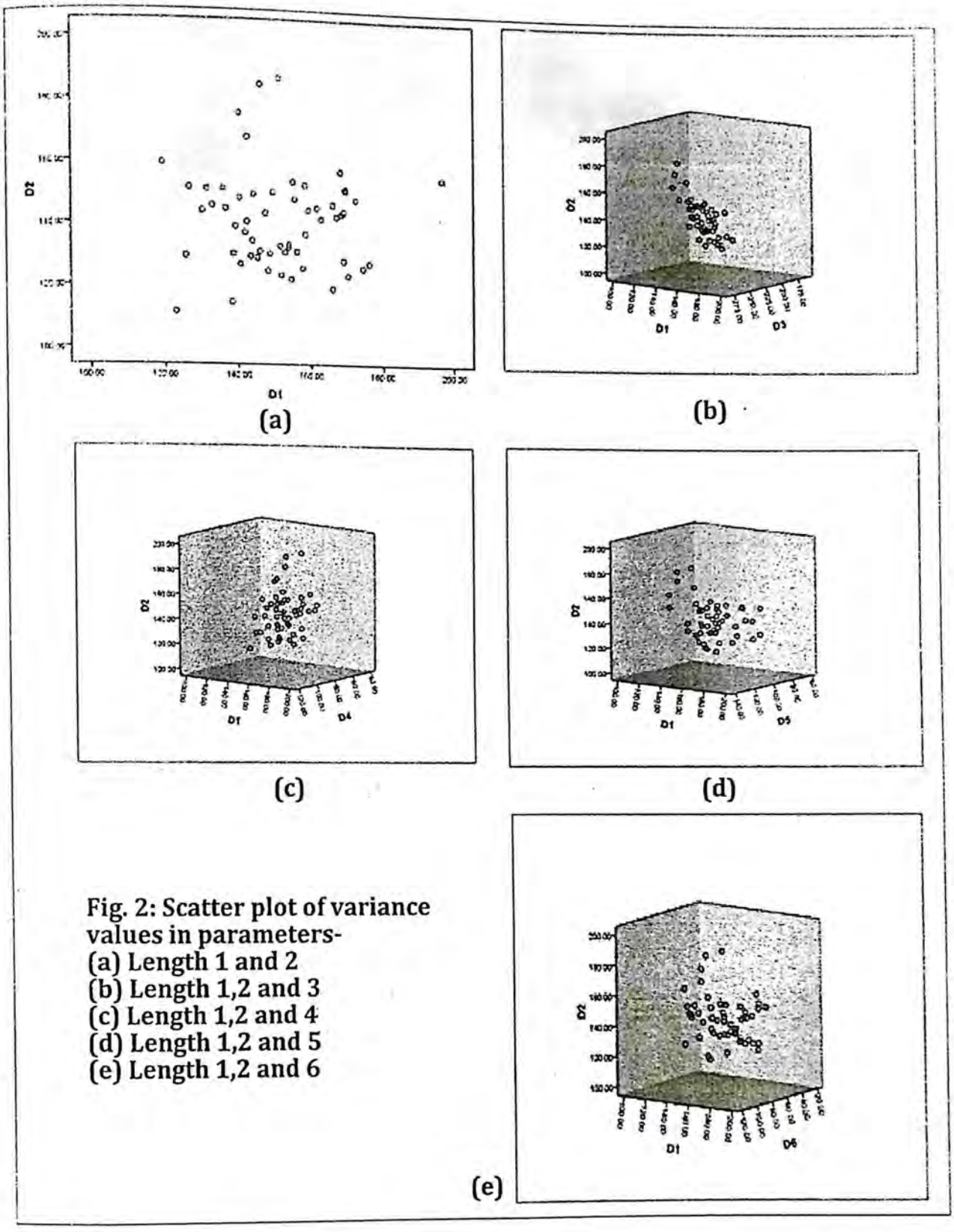

Fig. 2: Scatter plot of variance values in parameters-
(a) Length 1 and 2
(b) Length 1,2 and 3
(c) Length 1,2 and 4
(d) Length 1,2 and 5
(e) Length 1,2 and 6

2.4.4. Discussion

Although, the footprint analysis method was found effective for black rhinoceros and white rhinoceros (Jewell *et al.* 2001; Alibhai *et al.* 2008), the applicability of footprint technique for population estimation of greater one-horned rhinoceros in their natural habitat possess some site specific constrains such as the substratum and rainfall conditions. However, a more robust sampling strategy needs to be developed incorporating the environmental and habitat limitation to get a better understanding of the subject. Since, it was difficult (and dangerous) to walk in this species habitat which comprises of either thick tall grassland or swampland, good quality footprints can majorly be encountered on the roads.

Other major factor that influenced our study was stochastic rainfall which occurred thrice during the survey. The issue associated with it here was flat terrain with alluvial or loamy-clay soil which retains moisture for longer periods. This made the study area non-navigable for a few weeks and ground unfit for good footprint formation. These conditions were in contrast with the habitat conditions of African landscape where the soil is sandy; therefore, the results are non-comparable with the findings of Jewell *et al.* (2001) and Alibhai *et al.* (2008).

An interesting phenomenon pertaining to the behaviour of greater one-horned rhinoceros was observed during the peak winter season when the environmental temperature was very low (< 10 °C). Although, this species is considered as grassland dweller with less instances of their moving into forests (Dutta 1991), we found that major proportion of their population was residing in the woodlands that comprised only 33% of the entire RRA. The suspected reason why they occupied the forest during winter seems thermal-cover that was absent in the open grasslands. This proposition was further supported by the fact that these animals are sensitive to temperature despite of having a very thick skin (Dutta 1991; Dinerstein 2003). Occasionally, they were also spotted foraging in the wallow sites during this period suggesting their preference for aquatic vegetation when the browse became scarce in the forests. Obtaining their foot impressions during this period was difficult as there were less number of roads specifically in forest within RRA (total road length was ca. 3 km) and

the forest floor was covered with thick layer of leaf litter which was unsuitable for footprint formation.

We suggest that future studies regarding footprint technique validation should be undertaken in dry seasons or after the routine grassland burning so as to get better habitat conditions for footprints. Following the identified individuals to acquire footprints with known identity would produce better output as implemented in Jewell *et al.* (2001) and Alibhai *et al.* (2008). Since, it is hard to get planer cushion imprints, given the soil conditions, the track plots should be made from very fine soil grains and a layer of lime can be used for enhancing the contrast in photograph. In addition, automated image analysis software, such as NiSAS software (Jewell *et al.* 2001) should be used to reduce subjective bias and achieve results with precision and accuracy.

2.5. COMPARISON OF POPULATION ESTIMATION TECHNIQUES

One of the main objectives of this study was to compare the different population estimation techniques likely to be applicable in case of rhinoceros population estimation. Since, in Dudhwa National Park, the number of rhinoceros is known *a priori* (*i.e.* 32 individuals) we tested the validity of widely used techniques such as non-invasive faecal DNA based capture mark recapture (CMR), photographic CMR, dung counts on line transects and footprint analysis, to check their precision and accuracy. Reasons to choose these techniques are mentioned in Chapter 1.

We found that both the non-invasive dung DNA based population estimation using CMR framework and photographic CMR were good enough to be used in population estimation of greater one-horned rhinoceros. The population size estimated by non-invasive faecal DNA CMR (of 35 individuals) slightly overestimated the actual population size (of 32 individuals) but the range of standard error in the estimate (*i.e.* ± 5 individuals) overlapped the known population. The population estimated by photographic CMR was 26 individuals which is an underestimate as known population is 32. The error in the estimate (± 5 individuals) also did not precisely overlap the actual value but found close to it with upper limit of estimate as 31 individuals. Fig. 1 represents the population estimates by the two techniques and is compared with the actual numbers obtained from forest department officials.

Moreover, it is observed that in both the techniques the precision is similar (with both showing the S.E. ± 5) but, as compared to the photographic CMR, non-invasive faecal DNA CMR is more accurate with the estimated population closer to the actual value, in this study.

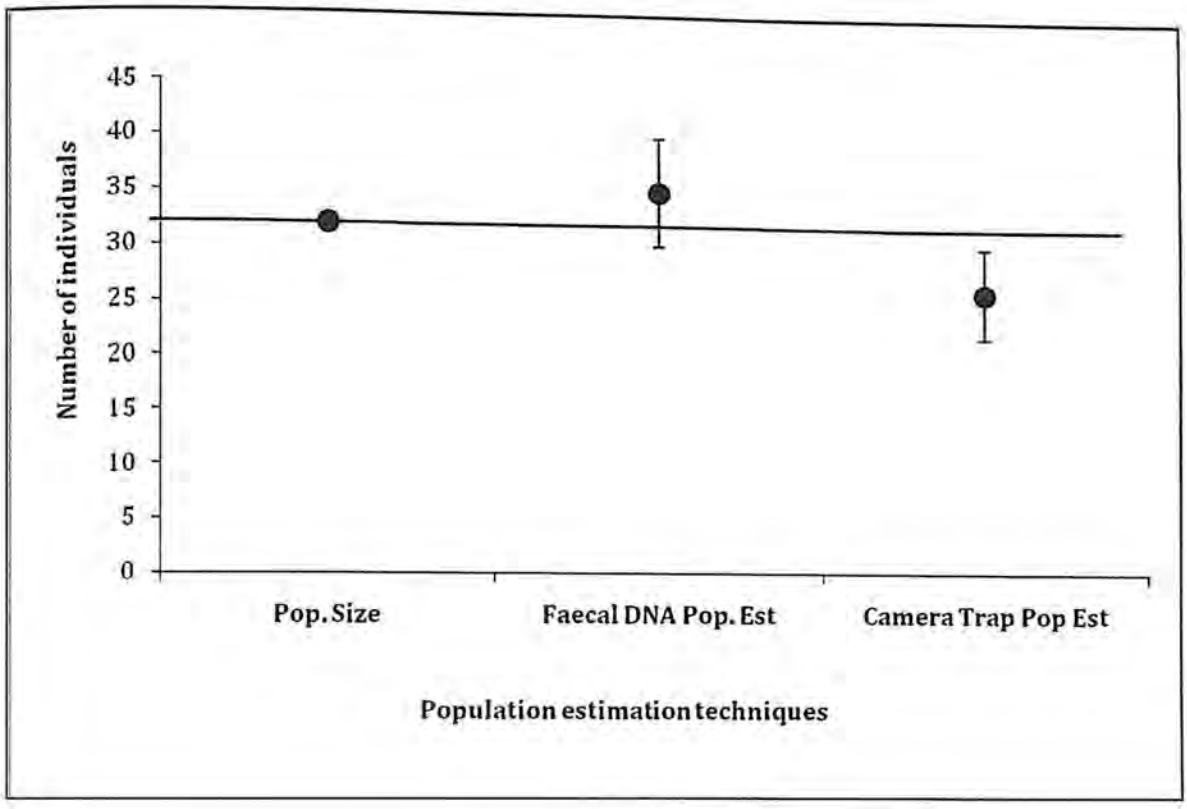

Fig. 1: Graph comparing the results of two techniques with known population size

The dung count method, although applicable for elephant abundance estimation, was not found suitable for rhinoceros population estimation in this study for reasons already discussed in section 2.3.4. It is recommended that before improvising this technique one should resolve the critical issue of rhinoceros dung decay rate. However, preliminary examination of the modified equation, discussed in section 2.3.3.4., has given promising results but needs more scientific scrutiny to find its validity which could not be achieved within the given timeframe. The footprint analysis method had a major drawback as the substratum and weather conditions in *terai* occupied by these animals limit proper footprint acquisition. Therefore, comparison of these two techniques does not form the part of present thesis.

Evaluating the logistic and field constrains associated with the two valid techniques *i.e.* non-invasive faecal DNA CMR and photographic CMR, we found that camera trapping is less effective in tall grassland habitats where deploying camera trap unit is the biggest hurdle. Clearing the roadside grasses for deployment or solitary trees exposes the camera trap to damage inflicted by animals or theft. In addition, in places

where the rhinoceros density is low the roads are not enough to capture adequate number of individuals in camera traps. In non-invasive dung DNA CMR this problem is avoided as rhinoceros dung piles appear prominently in the habitat and can easily be detected from elephant back. The chance of misidentifying an elephant dung pile from rhinoceros dung pile is low and can be further lowered by field experience. The major problems associated with the non-invasive dung DNA CMR lies in fresh sample collection at middens and sampling design. In a small sampling area such as RRA, it was possible but in a larger area, such as that in Assam, block-wise sampling would be more effective than so as to have a spatial coverage each day while collecting fresh dung.

In some of the studies involving camera trapping, the status of the population demographics and social structure of a species have also been evaluated (*e.g.* Asian elephant (Goswami *et al.* 2007)) which shows the multimodal utility of this technique which is hard to quantify with other technique such as non-invasive faecal DNA CMR or track surveys. With camera traps, monitoring the population trend over a length temporal scale is easier as compared to faecal DNA CMR exclusively due to logistic reasons. Although, the comparison of effort and resources involved in the techniques were not included in this thesis but, it would be interesting dimension to predict the utility of the techniques for greater one-horned rhinoceros abundance estimation.

Modelling the misidentification probability in genetic and photographic CMR based population estimation technique is in progress and will provide better insight for error rates. These two techniques may appear less economic and tedious to execute but are reliable and least disturbing to the park's natural ecosystem as well. They are much productive when rapid estimates and long term monitoring of a species is required. For threatened species with narrow home ranges and for the species that occupy dense habitats such as greater one-horned rhinoceros, these techniques should be adapted to keep a track on the conservation demands of this species and its habitat.

Owing to its massive size, it is not difficult to count greater one-horned rhinoceros through direct ground based observations. However, count tallies are fraught with issues while interpreting surveys. Apart from the well-explored problem with detection in dense habitat, individual identification is difficult unless the unique natural markings are prominent enough to be detected during the survey. Mis-

identification will lead the observer into inappropriate estimation, who must therefore, deal with the possibility of tallying the same individuals multiple times. In such situation, robust sampling techniques offer potential solutions to these problems.

On the other hand, concerns regarding the relation between ethics and conservation science is rarely addressed (Jewell 2013). It is important to explore how is the quality of data collected and conclusions drawn are affected, if any technique which interferes the natural behaviour of the individual or population? Non-invasive techniques, such as the ones discussed in this thesis, are rated higher than invasive techniques since they cause minimum interference into the animal behaviour or habitat. Invasive techniques, such as immobilisation not only causes stress and compromises fertility (Alibhai *et al.* 2001) but is also rarely logistically sound. Greater one-horned rhinoceros occupies habitats in *terai-duar* ecoregions which are rich in biodiversity. Extensive monitoring is a necessity especially for such species which are of interest to illegal hunters and poachers (Harris *et al.* 2010) and therefore, it is suggested that they should be preferably utilised as and when needed for rapid or long term monitoring and management of the species.

CHAPTER 3

3.1. GENETIC DIVERSITY IN GREATER ONE-HORNED RHINOCEROS POPULATION OF DUDHWA NATIONAL PARK

3.1.1. Introduction

Scale matters when the priorities for conservation have to be scrutinised and a multi-scale outlook is necessary to achieve the overall conservation of a species (Levin 1992). Moreover, for a mammalian species that has suffered massive range contraction and severe population decline, such as greater one-horned rhinoceros (Dinerstein and Price 1991), the concern of scale becomes all the more critical, as it demands attention from the landscape to molecular level. For an isolated small population the conservation issues arising at the genetic scale needs to be addressed, failing same increases the probability of extinction (Goodman 1987; Lande 1988; Pimm *et al.* 1989; Dinerstein and McCracken 1990). The reasons being random genetic drift due to loss of heterozygosity, allelic diversity and polymorphism which reduces the adaptive fitness and evolutionary potential of the species (Franklin 1980; Allendorf 1986; Charlesworth and Charlesworth 1987; O'Brien *et al.* 1987; Dinerstein and McCracken 1990; Westemeier *et al.* 1998; Harley *et al.* 2005) and progressively renders it vulnerable to environmental, demographic and genetic stochasticity (Gilpin and Soule 1986; Harley *et al.* 2005; Vidya *et al.* 2007).

Low levels of genetic diversity is found in numerous threatened species such as elephant seal *Mirounga sp.* (Bonnell and Selander 1974), British fallow deer *Dama dama* (Pemberton and Smith 1985), cheetah *Acinonyx jubatus* (O'Brien *et al.* 1987) and Baird's tapir *Tapirus bairdii* (Norton and Ashley 2004). Contrastingly, large mammals show higher extinction rates than their smaller counterpart owing to long gestation time, low recruitment rate and large range requirements (Cardillo *et al.* 2005; Garner *et al.* 2005; Scott 2008). The present five species of rhinoceros *i.e.* two—namely black rhinoceros *Diceros bicornis* and white rhinoceros *Ceratotherium simum* in Africa and three *viz.* greater one-horned rhinoceros *Rhinoceros unicornis,* Sumatran rhinoceros *Dicerorhinus sumatrensis* and Javan rhinoceros *Rhinoceros sondiacus* in Asia have been affected by

anthropogenic pressure, habitat destruction, hunting and poaching labelling rhinoceros as an emblem to threatened fauna due to human intervention (Scott 2008).

The extant populations of greater one-horned rhinoceros have survived bottleneck events in the recent past where the population size reached as low as 60-80 individuals in the two major populations in India and Nepal in 18th century (Dinerstein and McCracken 1990; McCracken and Brennan 1993, Dinerstein and Price 1991; Laurie 1978) and has recovered to ca. 2800 individuals in wild by 2010 (Sinha *et al.* 2010). Despite the significant recovery in individual numbers, the information on genetic diversity and population genetic structure is crucial for ensuring better management of the species in fragmented habitat patches.

Genetic diversity in all the populations of rhinoceros species is low but Harley *et al.* (2005) and Scott (2008) showed that the *Diceros bicornis* carried high level of microsatellite diversity as compared to other species of rhinoceros. Similar to *R. unicornis* the *Ceratotherium simum simum* too has revived rapidly from recent bottleneck and therefore, carries low genetic variability (Scott 2008). Although, the number of *C. simum simum* is far more than that of *C. simum cottoni* in wild (ca. 20 individuals), the latter carries slightly higher mean heterozygosity and allelic richness questioning the corroboration between the genetic diversity and population size (Scott 2008) which also evokes necessity to include the evolutionary temporal scale into the correlation as well. The mitochondrial analysis of D-loop region of the elusive *Rhinoceros sondiacus* revealed that the haplotype variability was very low; consequently, Fernando *et al.* (2006) concluded that this species has suffered severe genetic drift owing to founder events, bottlenecks and persistent small population size. Scott (2008), while comparing the microsatellite variability of four rhinoceros species also found that the mean heterozygosity and allelic richness in *Dicerorhinus sumatrensis* was significantly high in contrast to their numbers in wild and tallied with the results obtained by Scott *et al.* (2004). Morales et al. (1997) did mitochondrial D-loop analysis of the *D. sumatrensis* and found 4 haplotypes that suggest that this species carries comparatively higher genetic diversity.

Despite of a drastic population decline in 18th century, protein electrophoresis study of Nepalese sub-population of greater one-horned rhinoceros exhibited high

heterozygosity as compared to other mammals such as *Acinonyx jubatus* and *Dama dama* and therefore, Dinerstein and McCracken (1990) concluded that the bottleneck events in conservation genetics may be overemphasised. The results of the study by Scott (2008) concerning the genetic diversity of greater one-horned rhinoceros was in agreement with the same carried out by Zschokke *et al.* (2011) stating that this species has better genetic health when compared to other rhinoceros species. Further, Zschokke *et al.* (2011) compared the genetic diversity of both the Assam and Nepal greater one-horned rhinoceros populations and found that the Assam sub-population carried slightly higher genetic diversity than Nepal one. Both the mitochondrial and microsatellite analysis of these two sub-populations showed significant genetic differentiation owing to a lengthy period of absolute isolation and separate bottleneck events (Zschokke *et al.* 2011). The findings of existing literature concerning the population genetics of the extant rhinoceros species using nuclear genome related techniques have been summarised in Table 1.

Molecular examination of small sub-populations of a mammalian species whose numbers are regressing due to fragmentation and habitat contraction is important as these populations are most likely susceptible to genetic erosion that exacerbates the problem of population decline (Harley *et al.* 2005). Greater one-horned rhinoceros population in Dudhwa is confined and interbreeding since past 28 years with very few founder members (for details, see Section 1.1), we evaluated the genetic diversity status of this population to assist in management and conservation decisions for *in situ* conservation of this rehabilitated species.

Table 1: Report on genetic diversity of rhinoceros species using nuclear genome

mmon Name	Scientific Name	Sub - species	Method	H_o	H_e	H_{hat}	N_a	Data Source
ite rhino	*Ceratotherium simum*	*Simum*	Microsatellite Analysis	0.597 ± 0.050	0.578 ± 0.028		2.8	Floresceu *et al.* 2003
ite rhino	*Ceratotherium simum*	*Simum*	Protein Electrophrosis			0.013		Merenlender *et al.* 1989
ite rhino	*Ceratotherium simum*	*Cottoni*	Protein Electrophrosis			0.019		Merenlender *et al.* 1989
ite rhino	*Ceratotherium simum*	*Simum*	Microsatellite Analysis	0.342 ± 0.003	0.388 ± 0.005		2.2	Scott 2008
ite rhino	*Ceratotherium simum*	*Cottoni*	Microsatellite Analysis	0.368 ± 0.031	0.338 ± 0.007		2.3	Scott 2008
ack rhino	*Diceros bicornis*	*Minor*	Microsatellite Analysis	0.436	0.459		5.44	Harley *et al.* 2005
ack rhino	*Diceros bicornis*	*Bicornis*	Microsatellite Analysis	0.523	0.505		4	Harley *et al.* 2005
ack rhino	*Diceros bicornis*	*Michaeli*	Microsatellite Analysis	0.731	0.675		5.6	Harley *et al.* 2005
ack rhino	*Diceros bicornis*	*Minor*	Protein Electrophrosis			0.013		Merenlender *et al.* 1989
ack rhino	*Diceros bicornis*	*Bicornis*	Microsatellite Analysis	0.401 ± 0.018	0.455 ± 0.016		2.7	Scott 2008
ack rhino	*Diceros bicornis*	*Minor*	Microsatellite Analysis	0.477 ± 0.014	0.507 ± 0.010		3.1	Scott 2008
ack rhino	*Diceros bicornis*	*Michaeli*	Microsatellite Analysis	0.573 ± 0.005	0.635 ± 0.012		3.9	Scott 2008
dian rhino	*Rhinoceros unicornis*	Nepal	Protein Electrophrosis	0.099 ± 0.045				Dinerstein and McCracken 1990
ndian rhino	*Rhinoceros unicornis*	Assam	Protein Electrophrosis			0		Merenlender *et al.* 1989
ndian rhino	*Rhinoceros unicornis*	Assam	Microsatellite Analysis	0.57 ± 0.23	0.6 ± 0.20		3.75 ± 1.49	Zschokke *et al.* 2011
ndian rhino	*Rhinoceros unicornis*	Nepal	Microsatellite Analysis	0.43 ± 0.29	0.45 ± 0.30		2.95 ± 1.42	Zschokke *et al.* 2011
ndian rhino	*Rhinoceros unicornis*	West Bengal	Microsatellite Analysis	0.36	0.35			Borthakur *et al.* 2012
ndian rhino	*Rhinoceros unicornis*		Microsatellite Analysis	0.338 ± 0.023	0.4280 ± 0.013		2.9	Scott 2008
Sumatran rhino	*Dicerorhinus sumatrensis*		Microsatellite Analysis	0.380 ± 0.021	0.605 ± 0.090		3.8	Scott 2008

3.1.2. Materials and Methods

For details on Sample collection, DNA extraction, PCR Amplification and Genotyping see sec. 2.1.2. We used the genetic data of 27 unique genotypes (individuals) to infer genetic insights of reintroduced rhinoceros population of Dudhwa National Park.

3.1.2.1. Data Analysis

Microsatellite data was analyzed for potential genotyping errors such as allele dropout, false alleles, scoring errors, large allele dropout and null alleles using MICRO-CHECKER v.2.2.3 (Van Oosterhout *et al.*, 2004). The deviation from Hardy Weinberg Equilibrium (HWE) and Linkage Disequilibrium (LD) was assessed using GENEPOP. Basic genetic diversity parameters were assessed using software such as GENALEX 6.5 (for H_o, H_e, N_a, N_e, F_{IS}) (Peakall and Smouse 2006) and CERVUS ver. 3.0.3 (for PIC, PID, PID_{sib}) (Kalinowski *et al.*, 2007). Besides, we also calculated Nei's standard genetic distance (DS), (Nei, 1987) between the individuals from the 27 unique genotypes, developed a similarity matrix based on pair wise values of DS (Nei, 1987) and constructed a Neighbour Joining (NJ) dendrogram using POPULATIONS 1.2.32 software (Langella 2002). Analysis of parameters governing genetic diversity and other related characteristics such as observed and expected heterozygosity and allele diversity per locus, thus found, were compared with the existing reports from the Nepal and Assam sub-populations.

3.1.3. Results

Out of the 12 microsatellites attempted for PCR amplification, Rh5 and Rh10 were excluded from further analysis due to poor amplification performance. The remaining 10 loci were found polymorphic showing no signs of potential genotyping errors like allele dropout, false allele, large allele dropout etc. MICRO-CHECKER detected presence of null alleles in two microsatellites i.e. Rh7 (0.16) and SR281 (0.19). Four out of ten microsatellites have shown deviation from Hardy-Weinberg Equilibrium (Rh1, Rh4, Rh7 and SR281). None of the markers showed linkage disequilibrium proving that their selection for this analysis is valid.

3.1.3.1. Genetic diversity of reintroduced *Rhinoceros unicornis* population of Dudhwa National Park

The observed heterozygosity (H_o) in this population was found to be 0.354 ± 0.064 whereas, expected heterozygosity (H_e) was 0.464 ± 0.061. Genetic diversity statistics have been summarized in Table 2. Polymorphic Information Content (PIC) value indicates markers information yielding capacity and it has been suggested that markers with PIC value > 0.5 were considered good for population genetic analysis (Bostein *et al.* 1980). Four makers (Rh1, Rh4, Rh7 and SR281) have shown PIC value greater than 0.5 suggesting their better prospect for population genetic study on rhinoceros. Fixation index (F_{IS}) value, if positive represents inbred population while if negative shows no inbreeding in the population. The mean F_{IS} value was found 0.39 suggesting the highly inbred nature of this population.

The NJ tree, constructed using Nei standard genetic distance, is based on the allele frequency in the population (Nei, 1987). It showed three major clusters—cluster 1, cluster 2 and cluster 3. Out of them, cluster 2 accounted for more than two third of the population (20 out of 27 individuals) indicating towards the high relatedness between individuals.

3.1.3.2. Comparison of Genetic Diversity

We compared the genetic diversity found in this population with the same found in existing literature concerning different rhinoceros species implying microsatellite analysis. Fig. 3 graphically represents H_o found in different populations of four rhinoceros species. We have included the population reference only for greater one-horned rhinoceros for exact comparison. It was found that H_o level in this population (0.353) was comparable to that of white rhinoceros (0.342) (Scott 2008), slightly less than Sumatran rhinoceros (0.380) (Scott 2008) and much less than the least found H_o in black rhinoceros (0.401) (Scott 2008). Among the greater one-horned rhinoceros populations H_o in Assam sub-population was maximum (0.570) followed by Nepal sub-population (0.430) (Zschokke *et al.* 2011). In this perspective, H_o of this population fell close to the West Bengal sub-population (0.36) (Borthakur *et al.* 2012) and a pool of Indian and Nepalese sub-population (0.338) (Scott 2008). Similar trend was observed when N_a found in different rhinoceros species was compared. Fig. 4 graphically represents N_a found in different populations of four rhinoceros species. We have

included the population reference only for greater one-horned rhinoceros for exact comparison. Black rhinoceros carried maximum genetic diversity among all the extant rhinoceros species. Since, H_o is not affected by the sample size (Lu-Na and De-Xing 2004); therefore, we concluded that the genetic diversity in this population is low and similar to other rhinoceros species and same species populations.

Table 2: Summary of Generic Diversity Parameters

LOCUS	N_a	N_e	H_o	H_e	PIC	F_{IS}	LD
Rh1 *	3	1.733	0.231	0.440	0.377	0.455	No
Rh3	2	1.257	0.077	0.212	0.183	0.623	No
Rh4*	3	1.823	0.294	0.465	0.401	0.349	No
Rh6	2	1.087	0.083	0.083	0.077	-0.043	No
Rh7 *#	4	2.934	0.444	0.672	0.595	0.326	No
Rh9	2	1.800	0.444	0.453	0.346	0.000	No
Rh11	6	2.765	0.760	0.651	0.595	-0.190	No
SR63	3	2.667	0.500	0.714	0.555	0.200	No
SRIIIA	2	2.000	0.333	0.509	0.375	0.333	No
SR281 *#	4	2.625	0.368	0.636	0.550	0.405	No
Mean	3.1	2.069	0.353	0.483	0.405	0.390	

* locus show divergence from Hardy-Weinberg Equilibrium; # locus having null alleles; N_a = No. of Different Alleles; N_e = No. of Effective Alleles; H_o = Observed Heterozygosity; H_e = Expected Heterozygosity; PIC = Polymorphic Information Content; F_{IS} = Fixation Index, LD= Linkage Disequilibrium

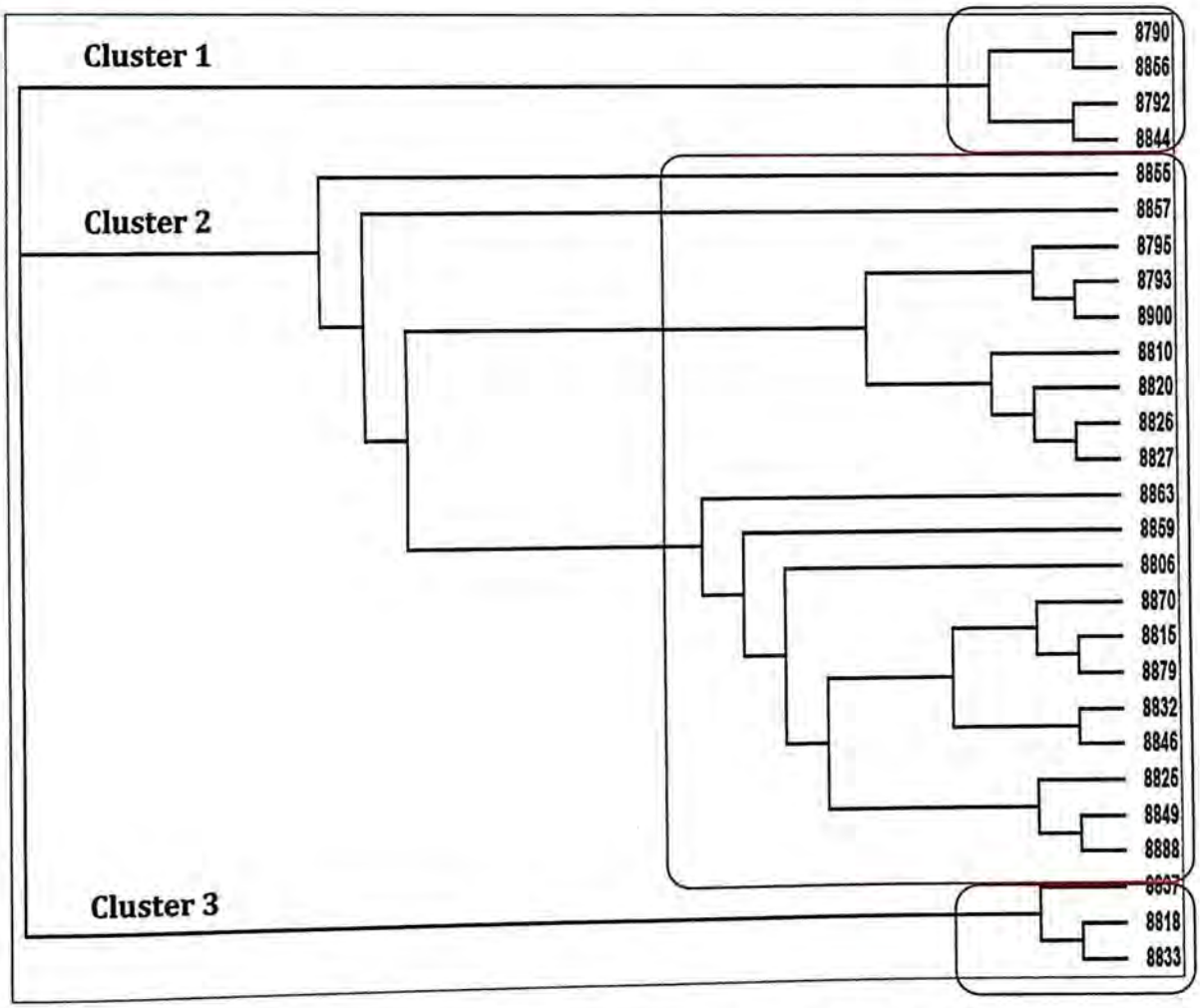

Fig. 2: Neighbour Joining (NJ) dendogram showing 3 major clusters

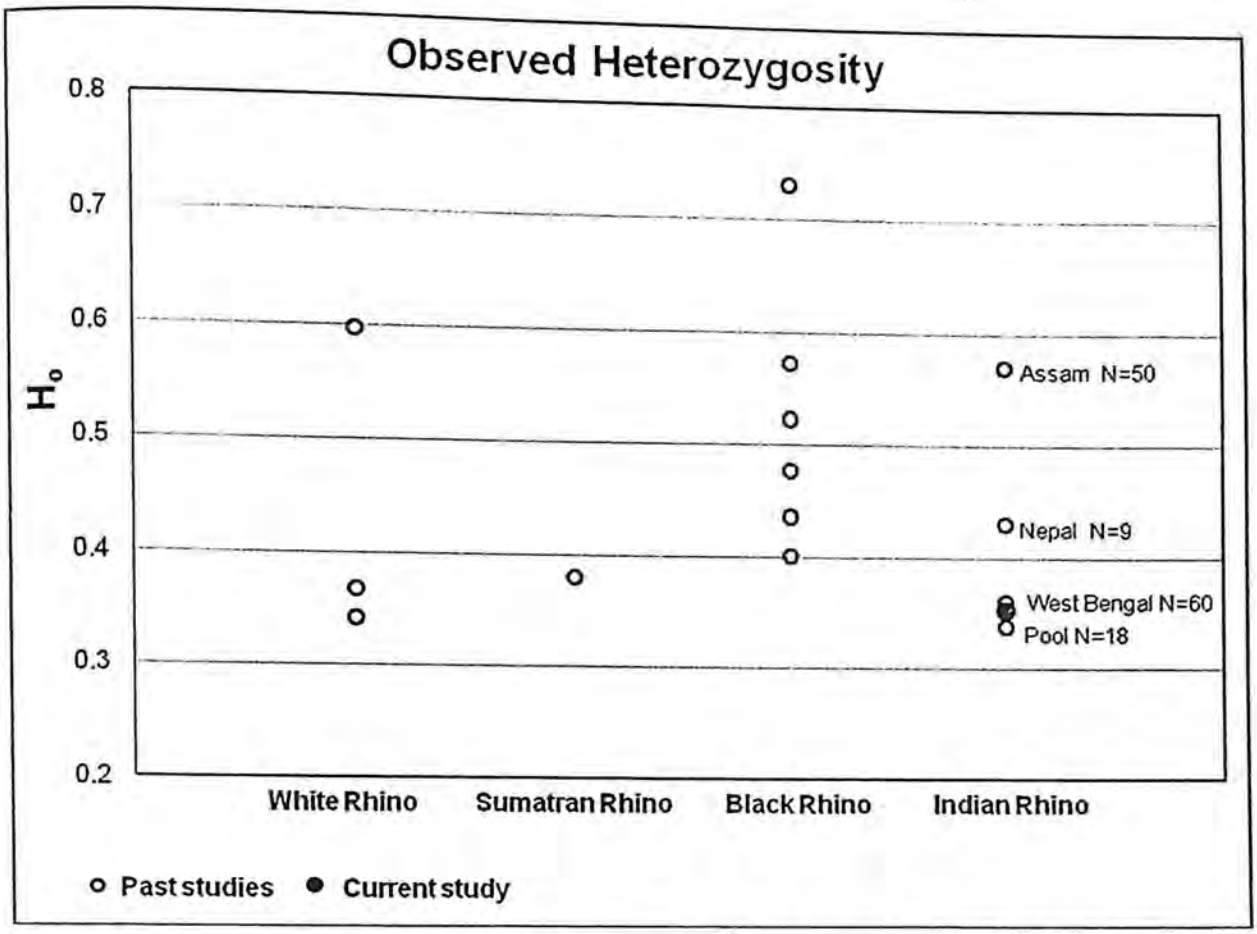

Fig. 3: Comparison of H_o in different rhinoceros species (N = No. of samples taken for analysis)

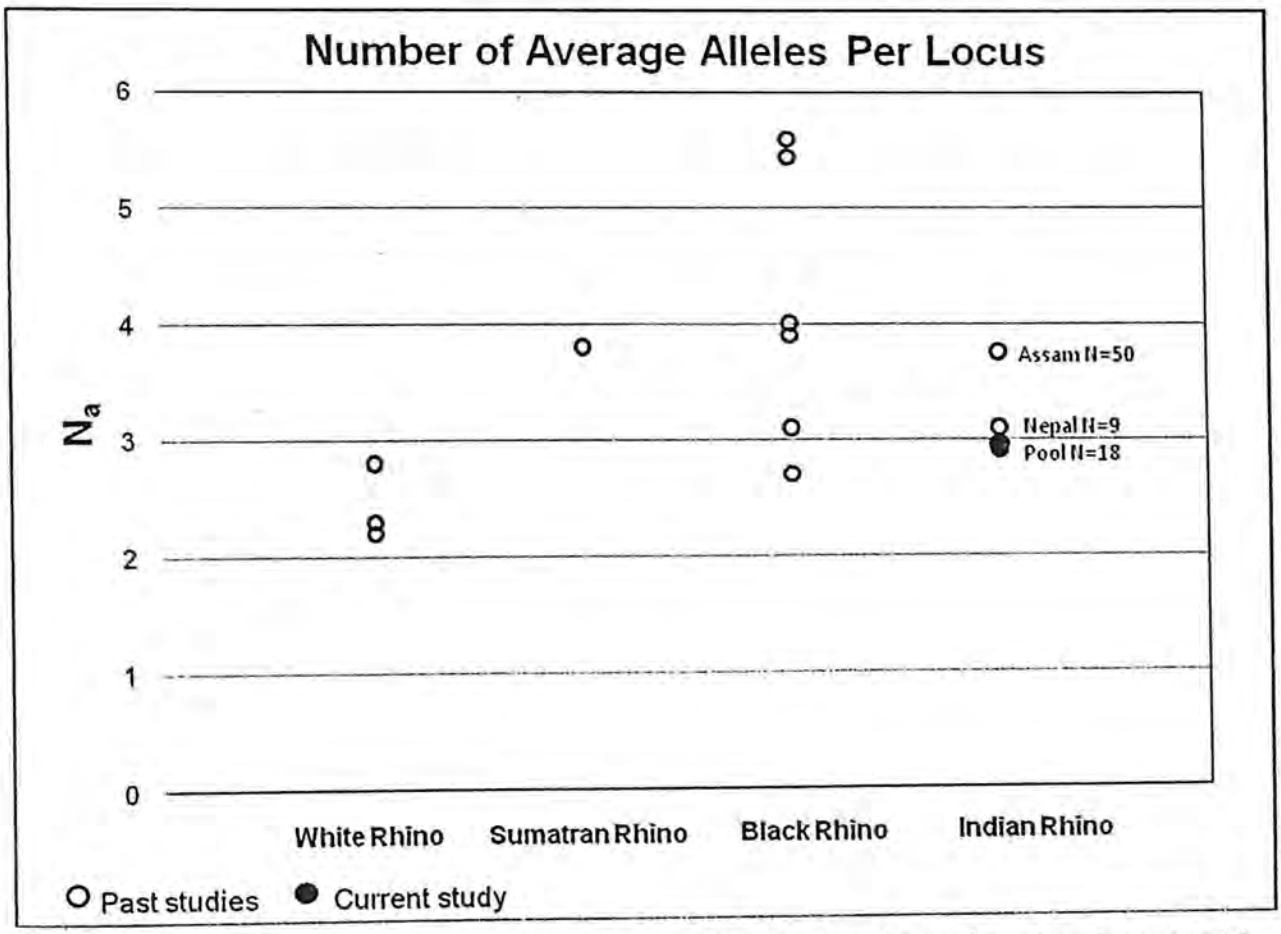

Fig. 4: Comparison of N_a in different rhinoceros species (N = No. of samples taken for analysis)

3.1.4. Discussion

The reintroduced greater one-horned rhinoceros population in Dudhwa National Park carries low genetic diversity in comparison to the ancestral sub-populations in Assam and Nepal. Mega-herbivores tend to have lower genetic variability than smaller mammals because their demographic characteristics, such as rarity, low fecundity and lengthy generation period (Eisenberg 1981), retards its accumulation (McCracken and Brennan 1993). This fact was reflected in published literature concerning the genetic diversity in various populations of different rhinoceros species (Table 1; Fig. 3; Fig. 4). Therefore, results of the genetic diversity evaluation of this small confined population corroborate with our expectations, given that the population is isolated and has risen from very few founder members.

Inbreeding has been proven to reduce germinal cell viability and fertility in an array of taxonomic groups (Wright 1977). Under this purview, Ballou and Ralls (1982) reported adverse effects of inbreeding in populations of 12 ungulate species (in each population the $F_{IS} > 0.196$). Present rhinoceros population is highly inbred ($F_{IS} = 0.390$), wherein inbreeding is likely to accumulate in future. However, the negative consequences of inbreeding in this population are yet to be ascertained and are superficially non-observable (Zschokke and Baur 2002). Baur and Studer (1995) have documented increased juvenile mortality in inbred captive rhinoceros population, which was later considered as an artefact of low sample size as Zschokke and Baur (2002) failed to confirm it in a similar study.

Zschokke *et al.* (2011) calculated significant genetic differentiation between the Assam and Nepal sub-populations which they reasoned to be due to lengthy period of isolation. They further suggested that since these two populations are partially genetically incompatible, they should never be intermixed and should be treated as different management units. Using the studbook data, Zschokke and Baur (2002) supported this idiographic notion with higher infant mortality rate in the captive population resulting from cross-breeding between Assam and Nepal sub-populations. Their conclusions were challenged by Pluhacek *et al.* (2007) who analysed both the captive and free ranging greater one-horned rhinoceros population demographics and suggested that the cross breeding is rather an effective tool to enhance the genetic diversity in the remnant populations of this species.

Both the sub-populations of greater one-horned rhinoceros occupy same ecoregion (*Terai* and *Duar*) with all the environmental and habitat variables similar. These sub-populations were once continuous till fifteenth century (Laurie 1978; Dinerstein and McCracken 1990), following which the anthropogenic factors isolated their ranges into present substantial populations in Assam and Nepal (Rookmaaker 1984). In Dudhwa National Park, these two populations were mixed together and successfully breeding since past 28 years. In the current research, we did not find the suspected outbreeding level (no negative F_{IS} value) indicating that cross-breeding the two populations is not detrimental as far as outbreeding is concerned. Over the years, these two sub-populations have acquired unique genetic identity to certain extent (Zschokke *et al.* 2011) but the phenotypic consequences of outbreeding has been nullified (see Pluhacek *et al.* 2007) and therefore, we propose that treating the two populations as separate management unit is not completely justified.

Moreover, it has become certain from the dendrogram that ideal random mating is overruled and mate-choice mating system must be prevalent in the population. The NJ dendogram, constructed for this population, presented a single large cluster (cluster 2 in Fig. 2) pointing towards the high phylogenic relatedness among majority of individuals. It also suggests that the population has evolved through non-panmixia (non- random mating). Considering the socio-biology of greater one-horned rhinoceros, this condition is likely to occur since the dominant male does not allows other males to breed in its vicinity and fights among males are often fatal for one opponent (Dinerstein and Price 1991; Zschokke and Baur 2002). Our results tallied with the forest staff's experience that only one male from the founder population sired most progenies. Cluster 1 and cluster 3 are less related and comprise of seven individuals. Conclusively, it shows that few of the satellite males are also successfully breeding in the population falling in clusters 1 and 3.

In the view of genetic diversity loss in the present population, we suggest that new individuals should be translocated, preferably from Assam as they carry more genetic variability in form of heterozygosity and allelic richness as compared to Nepal sub-population (Zschokke *et al.* 2011). We support the proposal to construct a new rhinoceros reintroduction area in *Bhadi-Churella taal* area (Sinha *et al.* 2010) to reintroduce more rhinoceros in Dudhwa National Park.

Combining the understanding of genetic status and male dominance behaviour, it is suggested that the dominant male in the present population should be kept with new females in second proposed fenced area. New males should be released in the existing RRA to allow breeding with the present females. The following procedure will bring in new alleles in the gene pool enhancing the genetic diversity in this population. Scientific management of this population, using recent advanced techniques, is necessary for the growth and sustainability of greater one-horned rhinoceros population in Dudhwa National Park.

REFERENCES

Abramov KG 1961 *Voprosy organizatsii I metody ucheta resoursov fauny nazmnyh. [Proceedings of a symposium on orgnization and methods of registration of ground vertebrates]. Izdatelstvo ANIMA USSR.* (In Russian.).

Alibhai SK, Jewell ZC & Towindo SS 2001 Effects of immobilization on fertility in female black rhino (*Diceros bicornis*). *Journal of Zoology* **253(3)** 333—345.

Alibhai SK, Jewell ZC & Law PR 2008 Identifying white rhino (*Ceratotherium simum*) by a footprint identification technique, at the individual and species levels. Endangered Species Research **4** 219—225.

Allendorf FW 1986 Genetic drift and the loss of alleles versus heterozygosity. Zoo Biology **5** 181—190.

Al-Otaibi SA & Fahmi AI 2011 Genetic Variation in Captive Herd of Arabian Oryx Using RAPD and ISSR Markers. African Journal of Biotechnology **10(27)** 5251—5262.

Aziz T 1990 Some studies on the behaviour of the Indian One-Horned Rhinoceros (*Rhinoceros unicornis*) in the Dudhwa National Park U.P., Master of Philosophy, Thesis, Aligarh Muslim University, Aligarh.

Ballou J & Ralls K 1982 Inbreeding and juvenile mortality in small populations of ungulates: a detailed analysis. *Biological Conservation.* **24** 239—272.

Barnes RFW 2001 How reliable are dung counts for estimating elephant numbers? *African Journal of Ecology* **39** 1—9.

Barnes RFW 2002 The problem of precision and trend detection posed by small elephant populations in West Africa. African Journal of Ecology **40(2)** 179—185.

Barnes RFW, Beardsley K, Michelmore F, Barnes KL, Alers MPT & Blom A 1997 Estimating Forest Elephant Numbers with Dung Counts and a Geographic Information System. *The Journal of Wildlife Management* **61(4)** 1384—1393.

Barnes RFW & Barnes KL 1992 Estimating decay rates of elephant dung piles in forest. African Journal of Ecology **30** 316—321.

Barnes REW & Dunn A 2002 Estimating forest elephant density in Sapo National Park (Liberia) with rainfall model. African Journal of Ecology **40** 159—163.

Barnes RFW & Jensen KL 1987 How to count elephants in forests. *IUCN African Elephant and Rhino Specialist Group* **1** 1—6.

Barnes RFW, Majam JM, Boateng BA & Ohemeng JA 2006 The survival of elephant dung piles in relation to the forest canopy & slope in Southern Ghana. *Pachyderm* **41** 37—43.

Basappanavar CH 1988 *Counting tigers. Sanctuary* **7** 77—85.

Bhupathy S 1991 Blotch structure in individual identification of Indian python (*Python molurus molurus* Linn.) and its possible usage in population estimation. Journal of the Bombay Natural History Society **87** 399—404.

Bist SS 2003 An overview of the methods for enumeration of wild elephants in India. Gajah **22** 67—70.

Bonnell ML & Selander RK 1974 Elephant seals: genetic variation and near extinction. Science **184** 908—909.

Borner M 1979 A field study of the Sumatran rhinoceros (*Dicerorhinus sumatrensis*), Fischer. PhD dissertation, University of Basel, Switzerland.

Borthakur U, Das PK & Talukdar BK 2012 Population genetic monitoring of Greater One-Horned Rhino (*Rhinoceros unicornis*) in Gorumara National Park, West Bengal, India. WGP, Aaranyak, Technical report no. **9** 1—18.

Botstein D, White RL, Skolnick M & Davis RW 1980 Construction of a Genetic Linkage Map in Man Using Restriction Fragment Length Polymorphisms. *American Journal of Human Genetics* **32** 314—331.

Boulinier T, Nichols JD, Sauer JR, Hines JE & Pollock KH 1998 Estimating species richness: the importance of heterogeneity in species detectability. Ecology **79** 1018—1028.

Brodie JF, Muntifering J, Hearn M, Loutit B, Loutit R, Brell B, Uri-Khob S, Williams NL & Preez PD 2010 Population recovery of black rhinoceros in north-west Namibia following poaching. Animal Conservation **2011** 1—9.

Buckleton J, Triggs CM & Walsh SJ 2005 Forensic DNA Evidence Interpretation. (Florida: CRC Press)

Cardillo M, Mace GM, Jones KE, Bielby J, Bininda-Emonds ORP, Sechrest W, Orme CDL and Purvis A. 2005. Multiple Causes of High Extinction Risk in Large Mammal Species. Science **309** 1239—1241.

Caro TM 1999 Conservation monitoring: estimating mammal densities in wooded habitats. Animal Conservation **2** 305—315.

Champion HC & Seth SK 1968 A revised list of Forest types of India. (New Delhi: Government of India Press)

Charlesworth D, Charlesworth B (1987) Inbreeding depression and its evolutionary consequences. Annual Review of Ecology and Systematics **18** 237—268.

Choudhury SR 1970 Let us count our tiger. *Cheetal* **14(2)** 41—51.

Choudhury SR 1971 The tiger tracer. *Cheetal* **13** 27—31.

Choudhury SR 1972 Tiger census in India Part I and II. Cheetal **15** 67—84.

Corbett J 1944. Maneaters of Kumaon. (London: Oxford University Press).

Cunningham J, Morgan-Davies AM & O'Ryan C 2001 Counting Rhinos from Dung: Estimating the number of animals in a reserve using microsatellite DNA. South African Journal of Science **97** 294—295.

Currier MJPP, Sheriff SL & Russel KR 1977 Mountain lion population and harvest near Canon City, Colorado. Colorado division, Wildlife Special Report 42.

Dawson S & Dekker AJFM 1992 Counting Asian elephant in forests: A techniques manual. (Bangkok: RAPA publications) 23—37.

Dinerstein E & McCracken GF 1990 Endangered Greater One-Horned Rhinoceros Carry High Levels of Genetic Variation. *Conservation Biology* **4(4)** 417-422.

Dinerstein E & Price L 1991 Demography and Habitat Use by Greater One-Horned Rhinoceros in Nepal. The Journal of Wildlife Management 55(3) 401-411.

Dinerstein E 2003 The return of the Unicorns (New York: Columbia University Press)

Dutta AK 1991 Unicornis (New Delhi: Konark Publications)

Eggert LS, Eggert JA & Woodruff DS 2003 Estimating population sizes for elusive animals: the forest elephants of Kakum National Park, Ghana. Molecular Ecology **12** 1389—1402.

Eisenberg JF 1981 The Mammalian Radiations: An analysis of trends in evolution, adaptation and behaviour. (Chicago: University of Chicago Press) 509.

Fay JM 1991 An elephant (*Loxodonta africana*) survey using dung counts in the forests of the Central African Republic. Journal of Tropical Ecology **7(01)** 25—36.

Fernando P, Polet G, Foead N, Ng LS, Pastorini J, Melnick DJ: Genetic diversity, phylogeny and conservation of the Javan rhinoceros (*Rhinoceros sondaicus*) 2006. *Conservation Genetics* **7** 439—448.

Fitzhugh EL & Gorenzel WP 1985 *Design and analysis of mountain lion track surveys. In Cal-Neva wildlife transactions, Western Section*. Bleich, V. C. (Ed.). Sacramento, CA. Wildlife Society 78—87.

Florescu A, Davila JA, Scott C, Fernando P, Kellner K, Morales JC, Melnick D, Boag PT, Van Coeverden De Groot P 2003 Polymorphic microsatellites in white rhinoceros. *Molecular Ecology Notes* **3** 344—345.

Flynn RW & Abdullah MT 1983 Distribution and number of Sumatran rhinoceros in the Endau Rompin region of peninsular Malaysia. Malay. Nat. J. **36** 219—247.

Frankham R, Ballou JD & Briscoe DA 2002 Introduction to Conservation Genetics. (Cambridge: Cambridge University Press)

Franklin IR 1980 Evolutionary change in small populations. ME Soule and B. A. Wilcox (eds) Conservation biology: an evolutionary-ecological perspective. Sinauer, Sunderland, Massachusetts 135—149.

Garner A, Rachlow J & Hicks JF 2005 Patterns of genetic diversity and its loss in mammalian populations. Conservation Biology **19** 1215—1221.

Gilpin ME & Soule ME 1986 Minimum viable populations: The processes of species extinction. In: Conservation Biology (ed. Sunderland MA) 19—34.

Gogate MG, Joshi PA, Gore AP & Paranjape SA 1989 *Tiger pugmark studies: a statistical perspective.* Tech. Bulletin No. 2, Directorate, Project Tiger, Melghat, Paratwada (Maharashtra).

Goodman D 1987 The demography of chance extinction. Soule ME (eds) Viable populations for conservation. Cambridge University Press, Cambridge, England 9—43.

Gopalaswamy AM, Royle JA, Delampady M, Nichols JD, Karanth KU & Macdonald DW 2012 Density estimation in tiger populations: combining information for strong inference. *Ecology* **93(7)** 1741—1751.

Goswami VR, Madhusudan MD & Karanth KU 2007 Application of photographic capture–recapture modelling to estimate demographic parameters for male Asian elephants. Animal Conservation **10(3)** 391—399.

Grigione MM & Burman P 2000 What is revealed in a mountain lions heel: using heel shape to ascertain identity. *Trans.West. Sect. Wildl. Soc.* **36** 21—26.

Harihar A, Pandav B & Goyal SP 2009 Density of leopards (*Panthera pardus*) in Chilla range of Rajaji National Park, Uttaranchal, India. *Mammalia* **73** 68—71.

Hariyadi ARS, Priambudi A, Setiawan R, Daryan D, Yayus A & Purnama H 2011 Estimating the population structure of Javan rhinos (*Rhinoceros sondaicus*) in Ujung Kulon National Park using the mark recapture method based on video and camera trap identification. *Pachyderm* **49** 90—99.

Harley EH, Baumgarten I, Cunningham J & O'Ryan C 2005 Genetic variation and population structure in remnant populations of black rhinoceros, *Diceros bicornis*, in Africa. Molecular Ecology **14** 2981—2990.

Harris RB, Junior JW, Amish SJ, Pereira AB, Godinho R, Costa V & Luikart G 2010 Argali Abundance in the Afghan Pamir Using Capture–Recapture Modelling From Fecal DNA. Journal of Wildlife Management **74(4)** 668—677.

Henschel P & Ray J 2003 Leopards in African Rainforests: Survey and Monitoring Techniques, Global Carnivore Program, Wildlife Conservation Society. (<http://www.savingwildplaces.com/media/file/low-leopard.pdf>)

Jachmann H 1991 Evaluation of four methods for estimating elephant densities. African Journal of Ecology **29** 188—195.

Janecka JE, Munkhtsog B, Jackson RM, Naranbaatar G, Mallon DP & Murphy WJ 2011 Comparison of noninvasive genetic and camera-trapping techniques for surveying snow leopards. *Journal of Mammology*. **92(4)** 771—783.

Javed S, Qureshi Q & Rehmani A R 1999 Conservation status and distribution of Swamp Francolin in India. Journal of Bombay Natural History Society **96** 16—23.

Jayarajan O 1983*a* Pugmark census. *Cheetal* **25** 44—51.

Jayarajan O 1983*b* Pugmark census. *Cheetal* **25** 3—6.

Jewell Z 2013 Effect of Monitoring Technique on Quality of Conservation Science. *Conservation Biology* **27(3)** 501—508.

Jewell ZC, Alibhai SK & Law PR 2001 Censusing and monitoring black rhino (*Diceros bicornis*) using an objective spoor (footprint) identification technique. Journal of Zoology **254** 1—16.

Kalinowski ST, Taper ML & Marshall TC 2007 Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Molecular Ecology **16** 1099—1006.

Kandel RC & Jhala YV 2008 Demographic Structure, Activity Patterns, Habitat Use and Food Habits of *Rhinoceros unicornis* in Chitwan National Park, Nepal. Journal of the Bombay Natural History Society **105(1)** 5—13.

Karanth KU & Nichols JD 1998 Estimation of tiger densities in India using photographic captures and recaptures. Ecology **79** 2852—2862.

Karanth KU & Nichols, JD 2000 Ecological status and conservation of tigers in India. Final Technical Report to the Division of International Conservation, US Fish and Wildlife Service, Washington DC and Wildlife Conservation Society, New York, Center for Wildlife Studies, Banglore, India.

Karanth KU 1993 How many tigers? Field censuses in India. *Hornbill* **1** 2—9.

Karanth KU 1995 Estimating Tiger *Panthera tigris* Populations From Camera-Trap Data Using Capture Recapture Models. Biological Conservation **71** 333—338.

Karanth KU, Nichols JD & Kumar NS 2004 Sampling Rare or Elusive Species (eds) Thompson WL (Washington DC: Island Press)

Karanth KU, Nichols JD, Seidensticker J, Dinerstein E, Smith JLD, McDougal C, Johnsingh AJT, Chundawat RS & Thapar V 2003 Science deficiency in conservation practice: the monitoring of tiger populations in India. *Anim. Conserv.* **6** 141—146.

Karanth, KU 1987. Tigers in India: a critical review of field censuses. Tilson RL & Seal US (eds) *Tigers of the world: the biology, biopolitics, management and conservation of an endangered species* (New Jersey: Park Ridge) 118—133.

Kohn MH & Wayne RK 1997 Facts from feces revisited. Trends in Ecology and Evolution **12** 223—227.

Kohn MH, York EC, Kamradt DA, Haught G, Sauvajot RM & Wayne R 1999 Estimating population size by genotyping faeces. Proceedings of the Royal Society of London. Series B, Biological Sciences **266** 657—663.

Kremen C, Merenlender AM & Murphy DD 1994 Ecological monitoring: a vital need for integrated conservation and development programs in the tropics. Conservation Biology **8** 1—10.

Kutilek MJ, Hopkins RA, Clinite EW & Smith TE 1983 Monitoring population trends of large carnivores using track transacts. In *Renewable resource inventories for monitoring changes and trends* 104—106.

Lahan P & Sonowal 1973 Kaziranga Wildlife Sanctuary, Assam. Journal of the Bombay Natural History Society **70(2)** 245—277.

Laing ST, Buckland TS, Burn RW, Lambie D & Amphlett A 2003 Dung and Nest Survey: Estimating Dung Decay Rates. Journal of Applied Ecology **14(6)** 1102—1111.

Lande R & Barrowclough GF 1987 Effective population size, genetic variation, and their use in population management. ME Soule (eds) Viable populations for conservation. (Cambridge: Cambridge University Press) 87—123.

Langella O 2002 POPULATIONS 1.2. 28. Population genetic software (individuals or populations distances, phylogenetic trees). CNRS, France.

Laurie A 1981 Behavioural ecology of the Greater one-horned rhinoceros (*Rhinoceros unicornis*). *J. Zool. London* **196** 307—341.

Lizcano M, D J & Cavelier J 2000 Densidad poblacional y disponibilidad de habitat de la danta de montana (*Tapirus pinchaque*) en los Andes centrales de Colombia. *Biotropica* **32** 165—173.

Lu-Na Y & De-Xing Z 2004 Effects of sample size on various genetic diversity measures in population genetic study with microsatellite DNA markers. Current Zoology **50(2)** 279—290.

Lukacs PM & Burnham KP 2005a Review of capture recapture methods applicable to non-invasive sampling. Molecular Ecology **14** 3909—3919.

Lukacs PM, Eggert LS & Burnham KP 2007 Estimating Population Size from Multiple Detections with Non-Invasive Genetic Data. *Wildl. Biol. Pract.* **3(2)** 83—92.

MacKenzie DI & Nichols JD 2004 Occupancy as a surrogate for abundance estimation. Animal Biodiversity and Conservation **27(1)** 461—467.

Marques FCF, Buckland ST, Goffin D, Dixon CE, Borchers DL, Mayle BA & Peace AJ 2001 Estimating Deer Abundance from Line Transect Surveys of Dung: Sika Deer in Southern Scotland. Journal of Applied Ecology **38(2)** 349—363.

McCracken GF & Brennan EJ 1993 Genetic variation in the greater one-horned rhino and implications for population structure. In: Ryder OA (ed.) Rhinoceros Biology and Conservation. Zoological Society of San Diego, San Diego CA, 228—237.

Mckelvey KS & Schwartz MK 2004 Genetic Errors Associated with Population Estimation Using Non-Invasive Molecular Tagging: Problems and New Solutions. Journal of Wildlife Management **68(3)** 439—448.

Mckelvey KS & Schwartz MK 2005 DROPOUT: A program to identify problem loci and samples for non-invasive genetic samples in a capture-mark-recapture framework. Molecular Ecology Notes **5** 716—718.

Merenlender M, Woodruff DS, Ryder O, Kock R & Váhala J 1989 Allozyme variation and differentiation in African and Indian rhinoceroses. *The Journal of heredity* **80** 377—382.

Mondol S, Karanth KU, Kumar NS, Gopalaswamy AM, Andheria A & Ramakrishnan U 2009 Evaluation of non-invasive genetic sampling methods for estimating tiger population size. Biological Conservation **142(10)** 2350—2360.

Morales JC, Andau PM, Supriatna J, Zainuddin Z & Melnick DJ 1997 Mitochondrial DNA Variability and Conservation Genetics of the Sumatran Rhinoceros. Conservation Biology **11(2)** 539—543.

Morley RC & Aarde RJV 2006 Estimating abundance for a savanna elephant population using mark–resight methods: a case study for the Tembe Elephant Park, South Africa. Journal of Zoology **271** 418—427.

Nei M 1987 Molecular evolutionary genetics. (New York: Columbia University Press)

Nichols JD 1992 Capture-recapture models: using marked animals to study population dynamics. *BioScience* **42** 94—102.

Norton JE & Ashley MV 2004 Genetic Variability and Population Differentiation in Captive Baird's Tapirs (*Tapirus bairdii*). Zoo Biology **23** 521—531.

Nowell K & Jackson P 1996. *Wild* cats: status survey and conservation action plan. Gland, Switzerland IUCN.

O'Brien SJ, DE Wildt, M Bush, Caro TM, Fitzgibbon C, Aggundey I & Leakey RE 1987 East African cheetahs: evidence for two population bottlenecks? Proceedings of the National Academy of Sciences of the United States of America. **84** 508—511.

Otis DL, Burnham KP, White GC & Anderson DR 1978 Statistical inference for capture data from closed populations. Wildlife Monographs **62** 135.

Palsbøll PJ, Allen J, Bérubé M *et al.* 1997 Genetic tagging of humpback whales. *Nature* **388** 767—769.

Panwar HS 1979*a* A note on tiger census technique based on pugmark tracings. *Indian For.* (Special Issue) 70—77.

Panwar HS 1979*b* Population dynamics and land tenure of tigers in Kanha National Park. *Indian For.* (Spec. Issue) 18—36.

Peakall ROD & Smouse PE 2006 GENALEX 6.5: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* **6(1)** 288—295.

Pearse DE, Eckerman CM, Janzen J & Avise JC 2001 A genetic analogue of 'mark-recapture' methods for estimating population size: an approach based on molecular parentage assessments. Molecular Ecology **10** 2711—2718.

Pemberton JM & Smith RH 1985 Lack of biochemical polymorphism in British fallow deer. Heredity **55** 199—207.

Penny M 1987 *Rhinos: Endangered Species* (Kent: Christopher Helm)

Petit E & Valiere N 2006 Estimating Population Size with Non-invasive Capture-Mark-Recapture Data. Conservation Biology **20(4)** 1062—1073.

Pimm SL, Gittleman JL, McCracken GF, and Gilpin ME 1989 Plausible alternatives to bottlenecks to explain reduced genetic diversity. TREE **4** 176—178.

Plumptre AJ 2000 Monitoring mammal populations with line transect techniques in African forests. Journal of Applied Ecology **37** 356—368.

Pluhacek J, Sinha SP, Bartos L & Sipek P 2007 Parity as a major factor affecting infant mortality of highly endangered Indian rhinoceros: Evidence from zoos and Dudhwa National Park, India. *Biological Conservation* **139** 457—461.

Ramakrishnan U, Santosh JA & Sukumar R 1991 Censusing Elephants in Forests. Proceedings of an International Workshop Southern India. Asian Elephant Conservation Centre of IUCN/SSC Asian Elephant Specialist Group.

Rexstad E & Burnham KP 1991 User's guide for interactive program CAPTURE. Color. Cooperative Fish and Wildlife Research Unit.

Rishi V 1997 Monitoring tiger populations by impression-pad method. *Indian Forester* **123** 583—600.

Rookmaaker LC 1984 The former distribution of the Indian Rhinoceros (*Rhinoceros unicornis*) in India and Pakistan. *J. Bombay Nat. Hist. Soc.* **80(3)** 555—563.

Sale JB & Singh S 1987 Reintroduction of greater Indian rhinoceros into Dudhwa National Park. *Oryx* **21(02)** 81—84.

Sankhla K 1978 *Tiger! The story of the Indian tiger.* (Calcutta: Rupa publications)

Sawarkar V B 1987 Some more on tiger tracks. *Cheetal* **28** 1—8.

Scandura M, Capitani C, Iacolina L & Marco A 2006 An empirical approach for reliable microsatellite genotyping of wolf DNA from multiple non-invasive sources. Conservation Genetics **7** 813—823.

Schenkel R & Schenkel-Hulliger L 1969 The Javan rhinoceros (*Rhinoceros sondiacus* Desm.) in Udjung Kulon nature reserve: its ecology and behaviour. Field study 1967 and 1968. Actatropica **26** 97—135.

Scott C, Foose T, Morales JC, Fernando P, Melnick DJ, Boag PT, Davila J, Van Coeverden De Groot PJ 2004 Optimization of novel polymorphic microsatellites in the endangered Sumatran rhinoceros (*Dicerorhinus sumatrensis*). *Molecular Ecology Notes* **4** 194—196.

Scott CA 2008 Microsatellite Variability In Four Contemporary Rhinoceros Species: Implications For Conservation. Master of Science Thesis. Queen's University Kingston, Ontario, Canada.

Santiapillai C 1990 Status of the Javan Rhino in Ujong Kulon National Park. Tiger Paper. 1—8.

Schipper J 2007 Camera-trap avoidance by Kinkajous *Potos flavus*: rethinking the "non invasive" paradigm. *Small Carnivore Conservation* **36** 38—41.

Sharma S, Jhala Y & Sawarkar VB 2005 Identification of individual tigers (*Panthera tigris*) from their pugmarks. *Journal of Zoology* **26** 7—9.

Silviera L, Jacomo ATA & Diniz-Filho JAF 2003 Camera trap, line transect census and track surveys: a comparative evaluation. Biological Conservation **114** 351—355.

Singh A 1972. A note on tiger census. *Cheetal* **15(3)** 24—26.

Singh A 1984. *Tiger! Tiger!* (London: Jonathan Cape)

Singh LAK 1999 Tracking tigers: guidelines for estimating wild tiger populations using the pugmark technique. New Delhi, India. WWF Tiger Conservation Program.

Singh R, Pandey P, Saxena L, Qureshi Q, Sankar K & Goyal SP 2012 Comparison of tiger population estimated using non-invasive techniques of pugmark, camera trap and DNA based analysis of hair and scat in Ranthambhore Tiger Reserve. Phase-II: Estimation of tiger population. Final report submitted to Wildlife Institute of India, Dehradun.

Sinha SP, BC Sinha & Sawarkar VB 2010 Twenty five years of Rhino Reintroduction Programme. (Germany: Lambert Academic Publishing)

Silver SC, Ostro LET, Marsh LK, Maffei L, Noss AJ, Kelly MJ, Wallece RB, Gomez H & Ayala G 2004 The use of camera traps for estimating jaguar (*Panthera onca*) abundance and density using capture/recapture analysis. *Oryx* **38** 1—7.

Subedi N, Jnawali SR, Dhakal M, Pradhan NMB, Lamichhane BR, Malla S, Amin R & Jhala YV (in Press) Population status, structure and distribution of the greater one-horned *Rhinoceros unicornis* in Nepal. Oryx

Talukdar BK, Emslie R, Bist SS, Choudhury A, Ellis S, Bonal BS, Malakar MC, Talukdar BN & Barua M 2008 *Rhinoceros unicornis.* In: IUCN 2012. IUCN Red List of Threatened Species. Version 2012.2. <www.iucnredlist.org>. Downloaded on 13 June 2013.

Tchamba, M.N. (1991) Defaecation by the African forest elephant (*Loxodonta africana cyclotis*) in the Santchou reserve, Cameroon. *Nature et Faune* **7** 27—31.

Tiwari PC & Joshi B 1997 *Wildlife in the Himalayan foothills: Conservation and Management* (Indus Publishing)

Thomas L, Laake JL, Strindberg S, Marques FFC, Buckland ST, Borchers DL, Anderson DR, Burnham KP, Hedley SL, Pollard JH, Bishop JRB & Marques TA 2006 Distance 5.0. Release 2. Research Unit for Wildlife Population Assessment, University of St. Andrews, UK. http://www.ruwpa.st-and.ac.uk/distance/

Thompson WL 2004 Sampling rare or elusive species: concepts, designs, and techniques for estimating population parameters (Washington, DC: Island Press)

Van Dyke FG, Brocke RH & Shaw HG 1986 Use of road track count as indices of mountain lion presence. *J. Wildl. Manage.* **50** 102—109.

Van Oosterhout C, Hutchinson WF, Wills DPM & Shipley P 2004 MICROCHECKER: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes **4** 535—538.

Van Strien NJ 1985 The Sumatran rhinoceros (*Dicerorhinus sumatransis*, Fisher) in the Gunung Leuser National Park, Sumatra, Indonesia, its distribution, ecology and conservation. Privately published, Doorn, Netherlands.

Varma S 2006 Methodologies for studying some aspects of ecology and conservation of the Asian elephants (*Elephas maximus*). In: Capacity building on elephant research and conservation issues in northeast India. Aaranyak, Guwahati and Asian Elephant Research and Conservation Centre (A division of Nature Conservation Foundation-ANCF), Bangalore, India.

Vidya TNC , Varma S, Dang XN, Thanh TV, Sukumar R 2007 Minimum population size, genetic diversity, and social structure of the Asian elephant in Cat-Tien National Park and its adjoining areas, Vietnam, based on molecular genetics analyses. *Conservation Genetics* **8** 1471—1478.

Waits LP, Luikart G & Taberlet P 2001 Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. Molecular Ecology **10** 249—256.

Wegge P, Pokheral CP & Jnawali SR 2004 Effects of trapping effort and trap shyness on estimates of tiger abundance from camera trap studies. *Animal Conservation* **7(3)** 251—256.

Westemeier RL, Brawn JD, Simpson SA 1998 Tracking the long-term decline and recovery of an isolated population. *Science* **282** 1695—1698.

White GC & Burnham KP 1999 Program MARK: survival estimation from populations of marked animals. Bird Study 46 Supplement 120—138.

Wilberg MJ & Dreher BP 2004 GENECAP: A program for analysis of multilocus genotype data for non-invasive sampling and capture-recapture population estimation. *Molecular Ecology Notes* **4(4)** 783—785.

Williams BK, Nichols JD & Conroy MJ 2001 Analysis and Management of Animal Populations. (New York: Academic Press)

Williams BK, Nichols JD & Conroy MJ 2002 Analysis and Management of Animal Populations. (San Diego: Academic Press)

Wing LD & Buss IO 1970 Elephants and forests. Wildlife Monographs **19** 1—92.

Woods JG, Paetkau D, Lewis D, McLellan BN, Proctor M & Strobeck C 1999 Genetic tagging of free-ranging black and brown bears. Wildlife Society Bulletin **27** 616—627.

Wright S 1977 Evolution and genetics of populations. 3. Experimental results and evolutionary deductions (Chicago: Chicago University Press) 44—96.

Zalewski A 1999 Identifying sex and individuals of pine marten using snow track measurements. *Wildl. Soc. Bull.* **27** 28—31.

Zar JH 1974 Biostatistical analysis (New Jersey: Prentice Hall).

Zschokke S, Armbruster GFJ, Ursenbacher S, Baur B 2011 Genetic differences between the two remaining wild populations of the endangered Indian rhinoceros (*Rhinoceros unicornis*). *Biological Conservation* **144** 2702—2709.

Zschokke S & Baur B 2002 Inbreeding, outbreeding, infant growth, and size dimorphism in captive Indian rhinoceros (*Rhinoceros unicornis*). Canadian Journal of Zoology **80** 2014—2023.

Zschokke S, Gautschi B & Baur B 2003 Polymorphic microsatellite loci in the endangered Indian rhinoceros, *Rhinoceros unicornis*. Molecular Ecology Notes **3** 233—235.