MOVEMENT ECOLOGY OF SWAMP DEER (*RUCERVUS DUVAUCELII*) ALONG THE UPPER GANGETIC PLAINS OF NORTH INDIA

Thesis submitted for the award of the degree of

Doctor of Philosophy

in

WILDLIFE SCIENCE

by

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to

Saurashtra University
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Under the supervision of

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January 2021

भारतीय वन्यजीव संस्थान
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CERTIFICATE

This is to certify that the thesis by **Mr. Shrutarshi Paul** entitled **"Movement ecology of swamp deer (*Rucervus duvaucelii*) along the upper Gangetic plains of north India"** is an original and independent research work submitted to the **Saurashtra University, Rajkot (Gujarat)**, for the award of the degree of **Doctor of Philosophy in Wildlife Science**.

Mr. Shrutarshi Paul has put more than six semesters of research work embodied in this thesis under my guidance and supervision. The work presented in this thesis has not been submitted to any other University or Institute for the award of any degree, diploma or distinction.

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I certify that the research work was appreciated by all who were present, and the comments made by the faculty and researchers have been appropriately included in the thesis.

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DECLARATION

I hereby declare that the work conducted under the thesis entitled **"Movement ecology of swamp deer (*Rucervus duvaucelii*) along the upper Gangetic plains of north India"**, is a record of original and independent research work done by me and subsequently submitted for the award of the degree of **Doctor of Philosophy in Wildlife Science** to the **Saurashtra University, Rajkot (Gujarat)**. This research work has been carried out under the guidance and supervision of Dr. Samrat Mondol, Scientist-E and co-supervision of Dr. Bivash Pandav, Scientist-F of Wildlife Institute of India, Dehradun. The work has not formed the basis for the award of any other degree, diploma or any other qualification. I also declare that the thesis embodies my own work, analysis, observation, understanding and the particulars given in it are true to the best of my knowledge.

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Introduction

1.1 Background and review of literature:

Large herbivores play critical roles in shaping ecosystems through various ecological functions (nutrient cycling, seed dispersal etc.) that affect forest structure, regeneration and benefit other species (Danell et al., 2006; Ripple et al., 2015). In recent time habitat destruction, resource depletion, hunting and human-animal conflict have resulted in massive declines of the world's large herbivores (Ceballos et al., 2017; Lindsey et al., 2017; Ripple et al., 2015) such that about 60% of them are threatened with extinction (Ripple et al., 2015; Trouwborst, 2019). The effects of such anthropogenic factors towards their extinction are further exacerbated by their relatively low population densities, unique habitat requirements and slow life history characteristics (Ripple et al., 2016; Wallach et al., 2015). While significant global conservation efforts helped in population recovery of some of the charismatic large herbivores (African rhinoceros- Emsile and Brooks, 1999; Asian elephant, gaur and sambar- Madhusudan, 2004; black rhinoceros- Brodie et al., 2011; Asian elephant and one-horned rhinoceros-Williams, 2011; elk, European bison and red deer- Sylven et al., 2012), it has mostly been targeted within protected areas, that represents only ~13% of the total land surface area (Chape et al., 2005; UNEP WCMC, 2012). The challenges are even more acute for habitat-specialist large herbivores that are distributed outside protected areas along the human-livestock-wildlife interfaces, where implementing regular management efforts like the protected areas are difficult (Karanth et al., 2010; Kshettry et al., 2020). Although these non-protected areas harbour significant mammalian biodiversity (Dorji et al., 2019; Harihar, 2011; Punjabi and Rao, 2017) and act as essential corridor habitats for free-ranging animals (Neelakantan et al., 2019;

Sunderraj et al., 1995), these areas are often overexploited (land conversion, grazing, unregulated poaching and hunting practices etc.) (Macdonald et al., 2013; Ripple et al., 2015) and experience strong human-wildlife conflict (crop raiding, damage to infrastructures and injuries to humans) (Gubbi et al., 2014; Kissui, 2008). Conservation of such large herbivores in these non-protected areas is dependent on accurate information on distribution, habitat status and other biological parameters. The Indian subcontinent retains one of the highest diversity of large herbivores in south and southeast Asia (Ahrestani et al., 2011; Johnsingh et al., 2004). The large herbivore species assemblages are currently facing severe conservation challenges in the subcontinent with 12 of the 15 terrestrial large herbivores classified as ‘‘Threatened’’ by IUCN (Ripple et al., 2014, 2015).

The swamp deer (*Rucervus duvaucelii)* or “Barasingha” is one such obligate swampy grassland dwelling large megaherbivore endemic to India and Nepal (Qureshi et al., 2004). It is a large 5-6 tined antlered cervid and its scientific name commemorates the famous French naturalist Alfred Duvaucel (Cuvier, 1823) (Figure 1.1). Historically swamp deer was widely distributed throughout Indo-Gangetic plains and the lowlands flanking the southern Himalayas from Pakistan to Bangladesh through India (Groves, 1982; Sankaran, 1989; Schaller, 1967). With current population size of less than 5000 individuals globally (Qureshi et al., 2004; Tewari and Rawat, 2013a) and a declining trend across its range (Duckworth et al., 2015), they are restricted to isolated pockets of northern, northeastern and central India and southwestern Nepal (Qureshi et al., 2004). Now considered as vulnerable by IUCN Red List (Duckworth et al., 2015), the species is listed in Appendix I in CITES and Schedule I of Wildlife Protection Act of

India (1972, i.e. highest level of protection). Three subspecies of swamp deer (based on morphological characteristics) are described across their range. The northern subspecies *Rucervus duvaucelii duvaucelii* is found in the north Indian states of Uttar Pradesh and Uttarakhand along with Nepal. The hard ground barasingha *Rucervus duvaucelii branderi* (Ellerman and Morrison-Scott, 1951; Pocock, 1943) is found as a single population in central India and the eastern subspecies *Rucervus duvaucelii ranjitsinhi* is found in the state of Assam (Groves, 1982; Qureshi et al., 2004). The ecology of *Rucervus duvaucelii*, specifically habitat use, feeding habits, diet, herd size and threats are fairly well studied in Jhilmil Jheel Conservation Reserve (JJCR), Dudhwa Tiger Reserve, Kanha Tiger Reserve and Kaziranga Tiger Reserve (Qureshi et al., 2004; Tewari and Rawat, 2013a, b, c, d, e) (Figure 1.2).

Figure 1.1 A herd of swamp deer at Kishanpur Wildlife Sanctuary

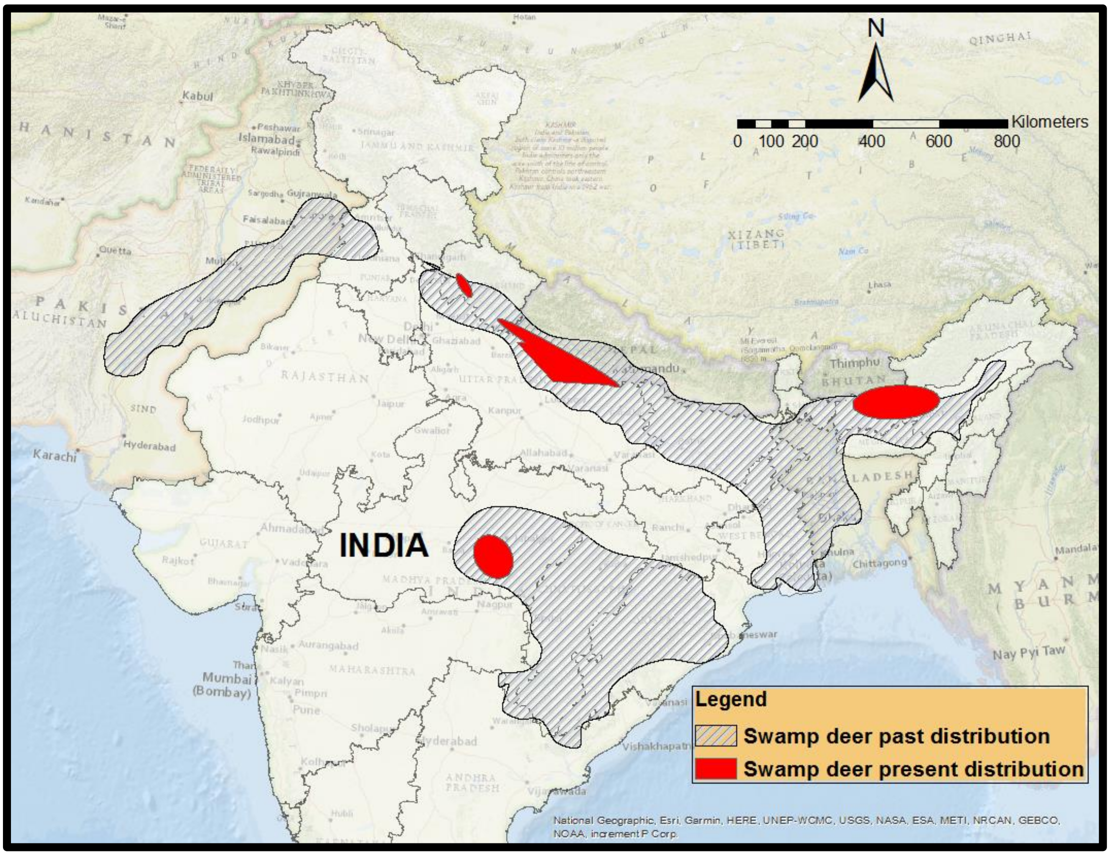

Figure 1.2 Past and present distribution of swamp deer in Indian subcontinent

Swamp deer are mainly grazers and feed primarily on aquatic grasses, foremost on *Saccharum* spp., *Imperata cylindrica*, *Narenga porphyrocoma*, *Phragmites karka, Oryza rufipogon, Hygroryza* sp. and *Hydrilla* sp. (Lydekker, 1890; Tewari and Rawat, 2013c). The breeding season lasts from September to April and they give birth to single fawn after a gestation period of 240–250 days from August to November (Schaller, 1967). All swamp deer populations in India have declined in recent time due to increased human pressure and changing land use practices (Qureshi et al., 2004). The long-term viability of the remaining populations is under serious threat from habitat loss and degradation leading to isolated populations, disturbance from heavy livestock grazing and poaching (Qureshi et al., 2004). *Rucervus duvaucelii duvaucelii* is the most abundant of all subspecies and retains about 80% of the global swamp deer population (Qureshi et al., 1995, 2004). The northern swamp deer has been reported from two swamp deer habitat blocks: the upper block restricted within the Sharda river basin and the lower block around the river Ganga. The upper habitat block is connected to the southern Nepal population, the stronghold of the subspecies. In addition, the Sharda basin swamp deer population mostly reside inside protected areas of Dudhwa and Pilibhit Tiger Reserve and receive good protection. Most of the ecological information on swamp deer is known from specific parts of the Sharda river habitat block (Qureshi et al., 2004).

However, swamp deer information from the lower habitat block around upper Gangetic plains of Uttar Pradesh and Uttarakhand is limited. The Ganga habitat block represents the westernmost distribution of the swamp deer, restricted to two reported protected areas, Jhilmil Jheel Conservation Reserve (JJCR) and Hastinapur Wildlife Sanctuary (HWLS) (Khan et al., 2003; Tewari and Rawat, 2013a). Following the discovery of Jhilmil Jheel population in 2005 by Sinha and Chandola, a number of research works have led to important management steps in conserving the only known population in Uttarakhand. There are reports of swamp deer presence in Bijnor Barrage area of Hastinapur Wildlife Sanctuary and below along river Ganga in Uttar Pradesh but comprehensive information on swamp deer distribution and ecology at a landscape level is lacking. Moreover, most of the areas along Ganga are human-dominated and receive limited protection measures. Additionally, individually each of these two populations (JJCR and HWLS) is not large enough to support viable swamp deer populations for long, and their conservation will strongly depend on how they are managed as one intermixing population with sufficient connectivity between them.

Without such connectivity there is a high chance of inbreeding within populations, thus decreasing their potential to survive changing environmental conditions. Gene flow and movement are intricately related and are important drivers of species survival (Cayuela et al., 2020). Observations of fluctuating swamp deer numbers in Jhilmil Jheel Conservation Reserve hint to the fact that these animals venture out in the non-protected areas outside the reserve. While they receive good protection measures in JJCR, but they are highly susceptible to poaching once they leave the reserve. In such a fragmented human-dominated landscape, the active movement routes and habitat are needed to be identified so that adequate protection measures can be taken. The biggest concern though for the swamp deer is the loss of habitats. According to recent assessments, grasslands are one of the most marginalized ecosystems in India (Task Force on Grasslands and Deserts report, 2006), with a loss of ~20 million hectares area between 1880-2010. With the ‘‘Green Revolution’’ movement in India, there has been a significant shift to irrigation-based agricultural practices, leading to fast decline in grassland habitats (Vanak et al., 2017) across north India. Given this ongoing devastation, it is important to assess the current and past habitat status in this area. Although ecology, spatial distribution, behaviour and population estimates of swamp deer have been studied inside protected areas, there is no information on distribution, movement patterns, genetic variation and habitat status of swamp deer thriving in the Gangetic habitat block. Understanding such ecological processes in a human-dominated landscape is critical and would help prioritizing effective future management strategy for the species across its distribution.

Using multidisciplinary tools, this doctoral study aims to uncover basic ecological information on swamp deer (distribution, gene flow, movement pattern and habitat status) living in a mosaic of protected and non-protected areas along Ganga. This doctoral work focuses on the urgent need for swamp deer and its habitat conservation at a landscape level covering the entire stretch of Ganga between JJCR and HWLS in the upper Gangetic plains. Conservation at a landscape level is more effective than protected area-based approach for long-term survival of any species (Wikramanayake et al., 2011). Through this landscape level approach, this doctoral work tries to identify the wetlands/grasslands in this area and make its conservation a priority. This doctoral work aims to project swamp deer as an umbrella species of these wetlands so that other animals like hog deer, otter and wetland birds could be protected. This thesis would be the first of its kind in this landscape and would help us to identify critical management changes required to ensure effective long-term swamp deer survival and prevent its local extermination from this landscape.

1.2 Study area:

The study area for this doctoral work covers the upper Gangetic plains of north India, mainly along river Ganga, between Jhilmil Jheel Conservation Reserve and Hastinapur Wildlife Sanctuary. However, for comprehensive assessment of distribution in the states of Uttarakhand and Uttar Pradesh, surveys were also conducted along river Sharda (Figure 1.3).

For assessing distribution of swamp deer in the upper Gangetic plains 253188 km^2 area was selected covering five districts of Uttarakhand (Dehradun, Haridwar, Pauri-Garhwal, Nainital and Udham Singh Nagar) and entire state of Uttar Pradesh. This area covers the floodplains of the Ganga river and a number of its tributaries like Sharda, Ghaghra, Gandak, Khoh, Ramganga, etc. including a complex network of riverine-grassland and grassland-forest systems (Figure 1.3). This region hosts the only existing populations of northern swamp deer (*Rucervus duvaucelii duvaucelii*) subspecies in India (Qureshi et al., 2004). The primary vegetation in the grassland patches comprises of *Typha* sp., *Phragmites* sp. and *Saccharum* spp. (Tewari and Rawat, 2013a). Other larger animals in the study area include hog deer (*Axis porcinus*), spotted deer (*Axis axis*), nilgai (*Boselaphus tragocamelus*), smooth-coated otter (*Lutrogale perspicillata*), fishing cat (*Prionailurus viverrinus*) and wetland birds such as sarus crane (*Antigone antigone*), black-necked stork (*Ephippiorhynchus asiaticus*), lesser adjutant (*Leptoptilosj avanicus*), Pallas's fish eagle (*Haliaeetus leucoryphus*) and bar-headed goose (*Anser indicus*) (Grimmett et al., 2013). The area experiences extreme variation in temperature (0°C to 50°C) throughout the year and receives a significant amount of rainfall during July-October (average rainfall of 1025 mm) with occasional flooding (Verma et al., 2019).

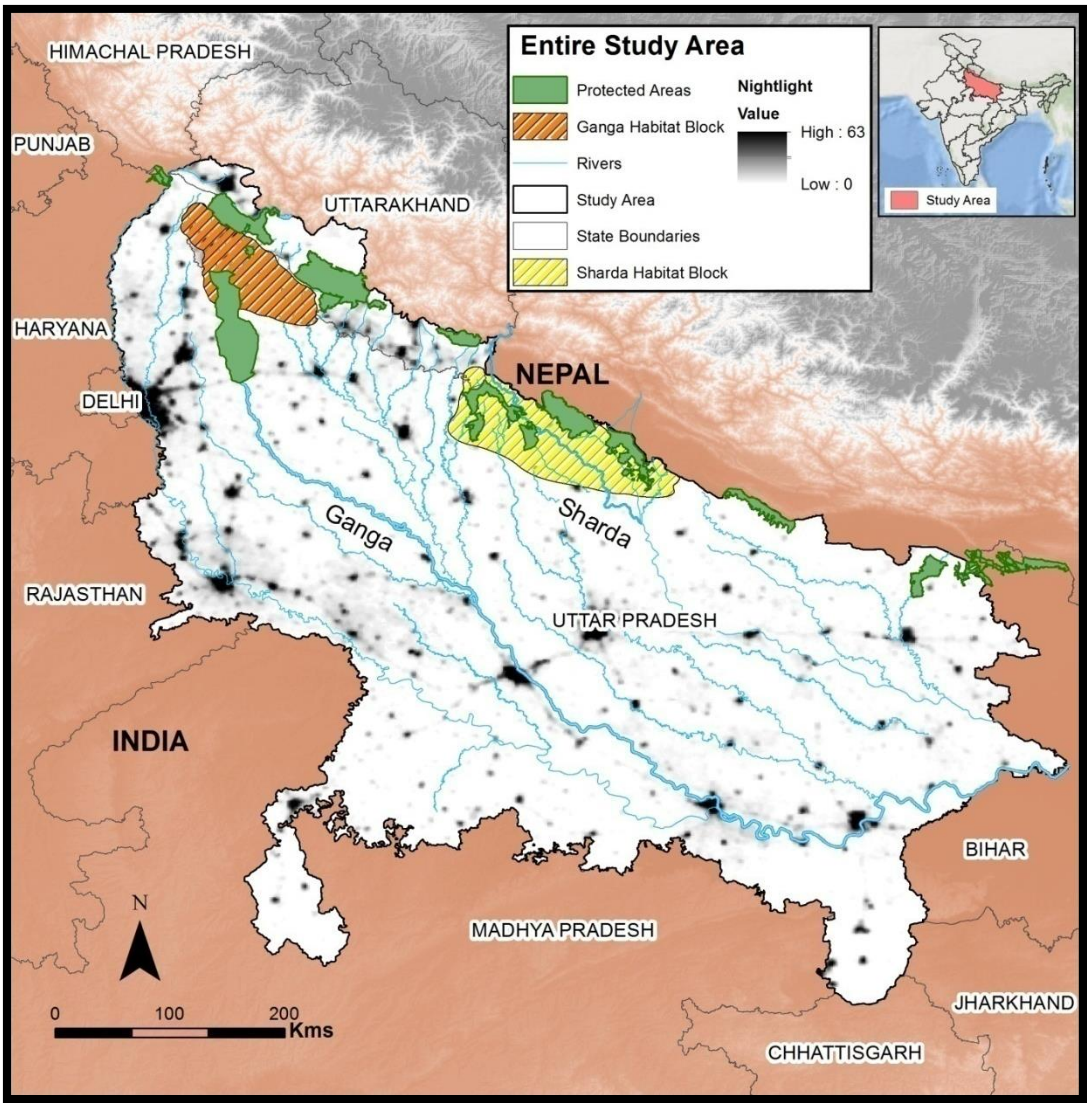

Figure 1.3 The study area map across the states of Uttarakhand (selected areas) and Uttar Pradesh, north India, highlighting both Ganga and Sharda habitat blocks

Our intensive study area includes only the area around river Ganga, covering a maximum of 5 km on either side of the river between Haridwar and Garhmukhteshwar (Figure 1.4). This is the westernmost distribution of swamp deer in its range and thus this habitat block demands separate attention. The major rivers in this region include Banganga and Solani, which join Ganga on the right bank. The two protected areas in this landscape include Jhilmil Jheel Conservation Reserve and Hastinapur Wildlife Sanctuary. Jhilmil Jheel is a wetland (oxbow lake) located along river Ganga, in

Rashiabad range of Haridwar Division, Uttarakhand. It encompasses an area of 37.83 km² with elevation varying between 200 to 250 m above mean sea level. This area is dominated by grasslands, mixed moist deciduous forests and secondary scrublands. Annual rainfall averages about 1300 mm and is most prominent during June-September (monsoon). Temperature goes up to 44°C in May and drops to 2°C in January (Tewari and Rawat, 2013a).

The Hastinapur Wildlife Sanctuary is situated between 28° 46' and 29° 35’N latitude and 77° 30’ and 78°30’ E longitude in Uttar Pradesh state of India. It encompasses an area of 2073 km² along the banks of Ganga covering five districts (Muzaffarnagar, Bijnor, Meerut, Hapur and Amroha) of Uttar Pradesh. The altitude of the sanctuary varies between 130 and 150 m above the mean sea level. The sanctuary is the sole protected area in India dedicated to the conservation of the endangered Gangetic grassland biome. Of the total sanctuary area, tall wet grasslands occupy 6% and short wet grasslands occupy 4%. Dry scrub grasslands and plantations occupy 5% and 2% respectively. The rest of the sanctuary is completely human-dominated. Climatically, three distinct seasons namely summer (mid-March to mid-June), winter (October to mid-March) and monsoon (mid-June to September) prevail in this sanctuary. Temperature reaches about 45°C in May and June whereas December and January record near 0°C. The study area receives an annual precipitation of about 1200 mm (Khan et al., 2003).

This landscape also contains non-protected areas between JJCR and HWLS covering both the states of Uttarakhand and Uttar Pradesh. This area mainly has croplands, human settlements and some fragmented grasslands along the Ganga, Banganga and Solani.

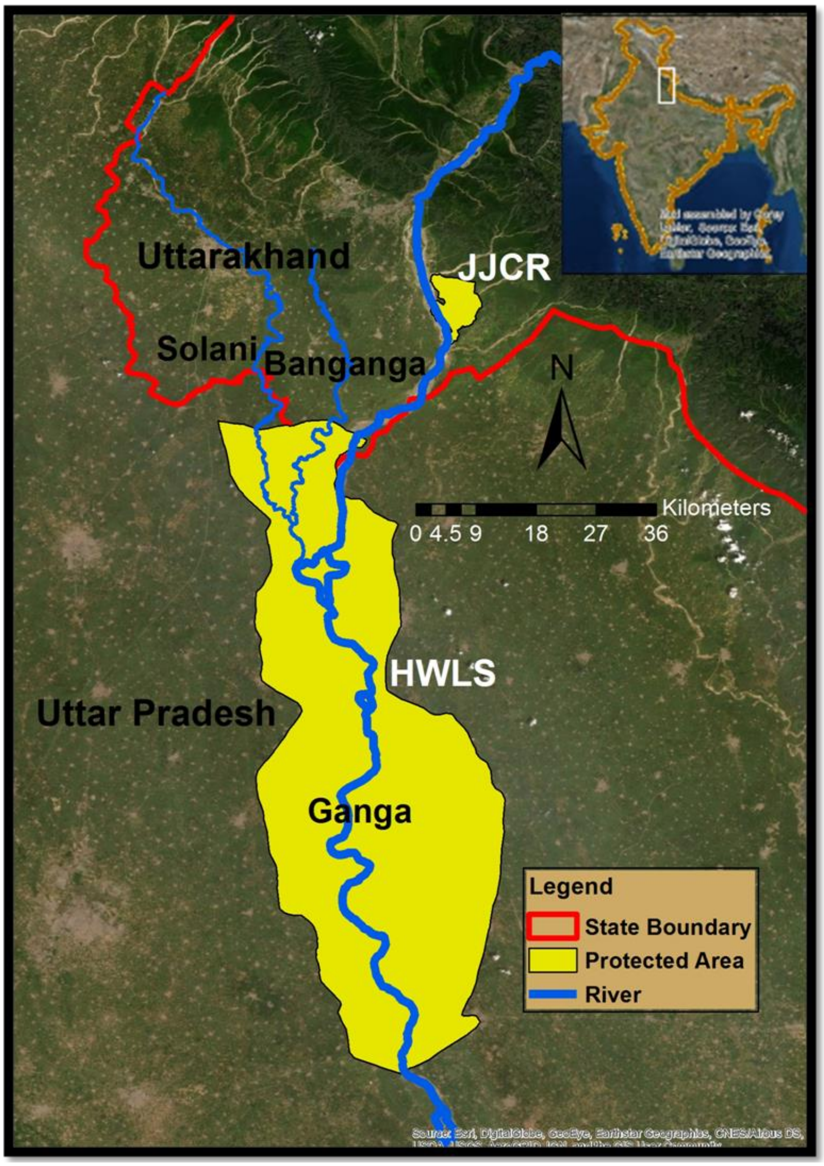

Figure 1.4 Intensive study area between JJCR and HWLS along Ganga covering both Uttarakhand and Uttar Pradesh

1.3 Objectives:

The proposed study aims to use a combination of ecological, molecular and GIS tools to understand the distribution, movement patterns, genetic variation and habitat dynamics of swamp deer in the upper Gangetic plains. The main idea of my thesis work was to generate baseline data on swamp deer ecology outside protected areas. The objectives of my doctoral work are:

- Distribution and status of swamp deer in the upper Gangetic plains of north India
- Analysing movement patterns of swamp deer in this landscape
- Assessing inbreeding status and viability of swamp deer in the upper Gangetic plains
- Assessment of current habitat availability and quantification of habitat change over the years for swamp deer in the study area

1.4 Research questions:

The specific questions of this doctoral study are:

- What is the fine-scale distribution of swamp deer and its habitat in the Ganga habitat block?
- What are the potential threats that these swamp deer face specifically outside protected areas?
- How has the grassland dynamics changed in this landscape in the recent past?
- Is the landscape genetically connected despite habitat fragmentation?

• Which are the active migration routes that the swamp deer is using and how are they utilising different habitats during their migration?

• What will be the effective practical conservation strategies for long-term survival of this species?

1.5 Thesis structure:

Limited information was available on swamp deer ecology in the upper Gangetic plains of north India before I embarked upon this work. My doctoral work revolves around the distribution, movement, gene flow and habitat assessment of swamp deer population of the upper Gangetic plains. I believe my work is the first landscape level study on swamp deer and its grassland habitats in the Gangetic plains, specifically highlighting the aspect of conservation of swamp deer habitats outside protected areas.

The 1st chapter of my thesis provides insight into the fine-scale distribution of swamp deer and grasslands along Ganga between Jhilmil Jheel Conservation Reserve and Hastinapur Wildlife Sanctuary assessed from pilot survey in the upper Gangetic plains. The 2nd chapter delves into unraveling the distributional limit of swamp deer in the entire upper Gangetic plains within the states of Uttarakhand and Uttar Pradesh using species distribution modeling and extensive field validations.

The 3rd and 4th chapters aim to look into the gene flow and movement pattern of swamp deer in this region using molecular tools and radio telemetry.

The 5th chapter analyses the habitat currently available for swamp deer and evaluates the change in dynamics of grasslands in the last 30 years.

In the conclusion, I have summarized the key findings of this thesis, discussed the implications and the impacts this work has had on swamp deer conservation.

Each chapter in this thesis has been appended with proper references added to the end of each chapter as separate sections. This entire doctoral work significantly upgrades the knowledge on swamp deer ecology in the upper Gangetic plains. Overall, the implications of this work are not only restricted to swamp deer conservation but also the grassland habitats which host a large number of other mammals, bird and other biodiversity. Unfortunately, they are under enormous pressure of conversion to croplands (a rapidly ongoing phenomenon across north India) and I believe my doctoral work will bring critical focus on long-term protection of these patches. In addition, this work also points everyone towards conservation "outside protected areas", another emerging area of work for conservation biologists globally.

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Chapter 1:

Pilot assessment of the distribution and status of northern swamp deer along Ganga in the upper Gangetic plains

2.1 Introduction:

The swamp deer or barasingha is the largest grassland dwelling cervid of the Indian subcontinent. The northern subspecies is found in a few isolated localities in southwestern Nepal and northern India. Within India, the northern swamp deer is restricted to few pockets of the Gangetic plains within the states of Uttarakhand and Uttar Pradesh, India (Qureshi et al., 2004). Specifically, along river Ganga, limited information is available on swamp deer distribution. Only Jhilmil Jheel Conservation Reserve (JJCR) in Uttarakhand has reports of swamp deer presence. This population was rediscovered in 2005 and subsequently it became a conservation reserve (Sinha and Chandola, 2006). Swamp deer congregate in the meadows of JJCR during the summer months and then migrate during the monsoons. Anecdotal reports suggest that they move along Ganga through heavily modified landscape. Their unknown whereabouts during the monsoon led to speculation of presence of swamp deer and its habitat outside JJCR. In the upper Gangetic plains, there are also reports of swamp deer presence from Bijnor Barrage area of Hastinapur Wildlife Sanctuary (HWLS) which is 53 km downstream from JJCR (Khan and Khan, 1999). This sanctuary was established in 1986 for the conservation of swamp deer but there is lack of fine-scale information about its distribution in this sanctuary. The entire landscape between these two protected areas is completely human-dominated and no information on presence of swamp deer or its habitat was available. Surveys carried out in 1966 revealed sporadic presence of swamp deer in certain grassland patches along Ganga (Schaller, 1967). The last swamp deer status report, based on a survey conducted along the Ganga river in 1995, found no conclusive evidence of its presence between Jhilmil Jheel

Conservation Reserve and Hastinapur Wildlife Sanctuary (Qureshi et al., 2004), despite anecdotal reports from local communities of swamp deer sightings in some wetland patches. A population of 100 individuals has been sighted along Banganga river in 2006 but the current status of this population is uncertain (Tewari and Rawat, 2013). Given the paucity of knowledge of fine-scale distribution coupled with unknown whereabouts of Jhilmil Jheel population, it is critical to evaluate the subspecies' distribution and status in this region to support its conservation.

In this chapter, we assessed the current status of *Rucervus duvaucelii duvaucelii* along both banks of the upper Ganga and its tributaries. We surveyed the entire stretch of Ganga from Jhilmil Jheel Conservation Reserve to Bijnor Barrage area of Hastinapur Wildlife Sanctuary and adjoining areas, including some tributaries of Ganga looking for current swamp deer occurrence, potential species habitats and documented the threats and status of these habitats.

2.2 Study area:

Swamp deer are known to prefer wetlands (also called "Swamps" or "Tals" locally) and flooded grasslands (Tewari and Rawat, 2013). Thus, our study area included the swampy grasslands found along stretch of upper Ganga and adjoining areas. We surveyed both left and right banks along Ganga between Jhilmil Jheel Conservation Reserve (total area of 37.83 km^2) and Bijnor Barrage area of Hastinapur Wildlife Sanctuary (total area of 2073 km^2) and grasslands along three of the tributaries of Ganga: rivers Solani and Banganga in the right bank and river Ramganga in the left bank of Ganga. Overall, our study area encompassed the geographic locations between 29°45'5.06"N, 78°16'20.79"E in the west to 29°35'50.65"N, 77°53'7.64"E in the east, and 29°50'15.08"N, 78°10'10.41"E in the north to 29°22'24.54"N, 78°1'8.68"E in the south, respectively, covering a total area of 1061 km^2 excluding the banks of Ramganga. We surveyed additional 63.44 km^2 area along the banks of Ramganga river.

2.3 Methods:

2.3.1 Survey methods:

As last survey report between Jhilmil Jheel Conservation Reserve and Hastinapur Wildlife Sanctuary showed no conclusive evidence of swamp deer presence (Qureshi et al., 2004) and no information on current status of swamp deer habitat is available, we decided to first gather information on evidences of swamp deer and their habitat from local communities living close to Ganga in our study area. The local communities included mostly villagers, cattle herders, farmers, boatmen and fishermen. Overall, information regarding swamp deer habitat and presence was opportunistically collected using unstructured questionnaire survey from a total of 42 people (11 villagers, 16 cattle herders, 8 farmers, 3 boatmen and 4 fishermen, respectively). Each of these interactions spanned about 10-20 minutes duration, where following information were enquired: (a) Residential status; (b) Occupation; (c) General mammalian diversity around their respective area; (d) Location of swamps or grasslands in the area, if any; (e) Information of swamp deer presence and specific identifying characteristics used in identification; (f) Approximate time and day of the sightings; (g) Presence of antler/s in the area; (h) Willingness to locate the antler/s, if they are found in the area; (i) Crop raiding related conflict in the area; and (j) Any hunting/poaching incidences known from that area. We also used Google Earth imageries to identify grassland patches to corroborate with the information on swamp deer habitat provided by the local residents along the Ganga and its tributaries within our study area. This information revealed that most of the grassland areas are currently restricted to 2-3 km on both sides of Ganga and its tributaries and are extremely fragmented along both banks of the entire 53 km stretch of Ganga between Jhilmil Jheel Conservation Reserve and Hastinapur Wildlife Sanctuary. Subsequently, we decided to conduct focused survey covering maximum of 5 km distance on both banks of Ganga and its tributaries along the entire stretch. We divided the entire study area into 10 survey zones taking account of the presence of grasslands, road network, tributary network, distribution of croplands and human habitations and surveyed each of these zones extensively on foot, vehicle or boat depending on the habitat characteristics for swamp deer presence (Figure 2.1). The entire survey was conducted between December 2015 to November 2016, barring the monsoon season between July-October 2016, covering a total distance of about 4692 km throughout the study area. Survey effort ranged from 362.51-643.53 km in different survey zones. Each zone was surveyed three independent times over a period of 15-18 days between 6.00 a.m. and 6.00 p.m. with occasional night surveys for potential swamp deer sighting.

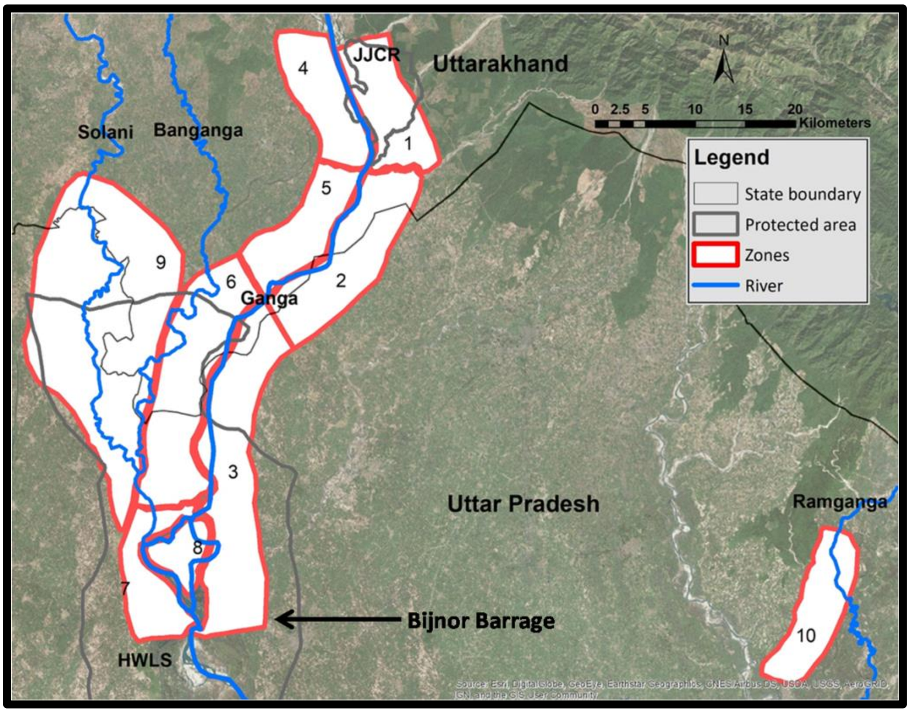

Figure 2.1 Survey zones between JJCR and HWLS along Ganga covering both Uttarakhand and Uttar Pradesh

The left bank of the Ganga was divided into three major survey zones: (i) the first zone (Survey Zone 1) comprises JJCR, which is saucer-shaped swampy grassland and surrounding areas in Uttarakhand. This region is dominated by vegetation of *Typha* sp. and *Phragmites* sp. (Tewari and Rawat, 2013); (ii) the second zone (Survey Zone 2) is a grassland situated 15 km downstream from JJCR along Ganga stretching to a distance of 26 km, locally called as Amichand, Nangal and Bhuria Sot spread across Uttarakhand and Uttar Pradesh. This area is non-protected, dominated by vegetations of *Sacchurum* spp., *Typha* sp. and interspersed with croplands; and (iii) the third zone (Survey Zone 3) is further south, downstream of Amichand extending up to HWLS in Uttar Pradesh. This region is filled with human settlements and crop fields, and devoid of any swampy areas (Figure 2.2).

Similarly, the right bank of Ganga was divided into five survey zones: (i) the first area (Survey Zone 4) comprises the region opposite to JJCR, which is non-protected, mostly human-dominated and interspersed with few grassland and wetland patches, located in Uttarakhand; (ii) the second area (Survey Zone 5) is a non-protected wetland downstream of the first area, adjacent to Ganga and locally known as Ranjitpur in Uttarakhand. This area harbours a huge swamp dominated with *Typha* sp. vegetation; (iii) the third area (Survey Zone 6) is further downstream along the Ganga non-protected and mostly dominated by human settlements, croplands, extremely fragmented grassland and wetland patches stretching across Uttarakhand and Uttar Pradesh; (iv) the fourth area (Survey Zone 7) along the right bank comprises parts of HWLS and adjoining regions in Uttar Pradesh. This wildlife sanctuary possibly harbours a large number of swamp deer along Ganga. The sanctuary also has some human settlements and croplands; and (v) the fifth survey zone (Survey Zone 8) is a number of river islands found in the riverbed close to HWLS in Uttar Pradesh (Figure 2.2). These river islands are dominated by patches of *Saccharum* spp. and *Typha* sp. vegetations.

Based on the information on potential swamp deer sighting provided by local communities, we also surveyed the grasslands along some of the tributaries of Ganga. First, about 10 km inland on the right bank of Ganga we found grassland and a big swamp near river Solani (Survey Zone 9) across Uttarakhand and Uttar Pradesh. This area is surrounded by human habitations, with potentially some connectivity with another Phragmites-dominated swamp locally known as Jogga Jheel downstream south of this region (Figure 2.2). Next, we focused on the left bank of Ganga along its tributary river Ramganga (Survey Zone 10) in Uttar Pradesh (Figure 2.2). This survey was motivated by a local newspaper report on swamp deer poaching in that region. Following interviews with the local community, we conducted an extensive survey and found a sizeable swamp in an area called Jamanpur. This swamp is present on both banks of Ramganga river and dominated with *Typha* sp. and *Phragmites* sp. vegetation. This swamp is completely surrounded by human habitations from all sides and possibly isolated from other swamp deer harbouring areas. Survey zone 1 covering Jhilmil Jheel Conseration Reserve in Uttarakhand is a protected area. Similarly, zones 7 and 8 in Uttar Pradesh fall entirely within protected area of Hastinapur Wildlife Sanctuary. However, only some parts of survey zones 3, 6 and 9 are within the boundary of Hastinapur Wildlife Sanctuary, and the entire area covering survey zones 2, 4 and 5 are completely non-protected.

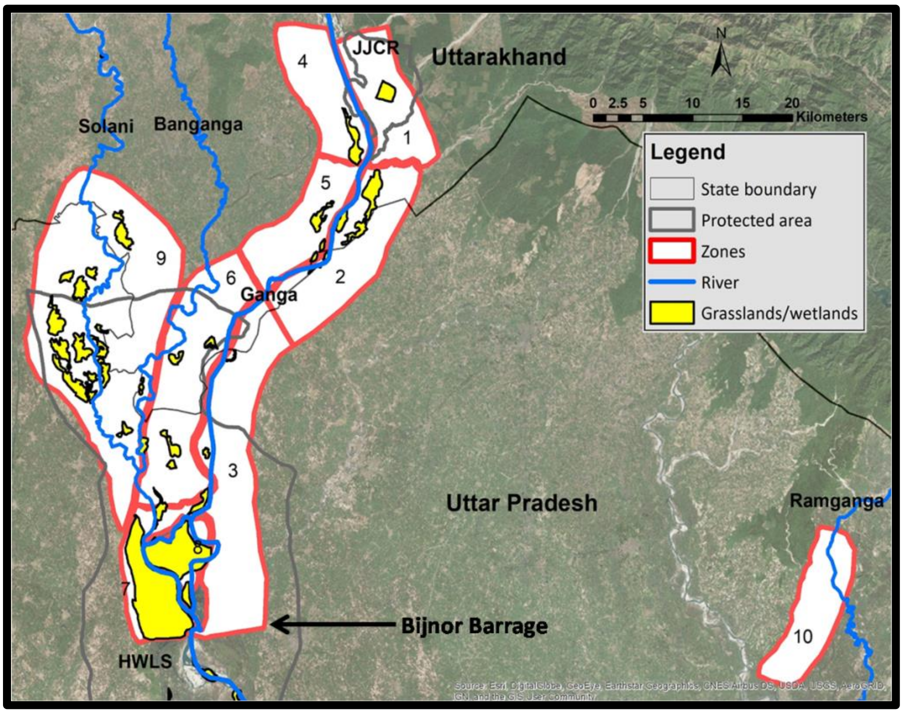

Figure 2.2 Swamp deer habitats along Ganga between JJCR and HWLS

During the survey, we considered direct sighting, presence of carcass and swamp deer specific signs (antlers, hoof marks, dung pellets etc.) as evidences of swamp deer presence in any area (Figure 2.3). Direct sighting and carcass were considered the best evidence of swamp deer existence, followed by presence of antler, hoof marks and faecal pellets in the field. Swamp deer antlers are very distinct from other cervid species found in the landscape. An adult swamp deer antler is mostly 5-6 tined and branches in a dichotomous manner in the upper half of the beam, whereas sambar, spotted deer and hog deer antlers are mostly three tined. Similarly, swamp deer hoof marks can be easily identified in the field from their distinctive splayed pattern due to evolutionary adaptation of living in swampy areas. Swamp deer faecal pellets are generally bigger in size than other sympatric cervids (hog deer) or domestic animals (sheep, goat etc.) found in this area, but this was not explicitly used as presence evidence alone. In the field, swamp deer presence was considered when at least two of the three signs (antler, hoof marks and faecal pellets) were found in a given area. Subsequently, we also conducted DNA-based species identification in the laboratory from different survey zones to further confirm swamp deer presence.

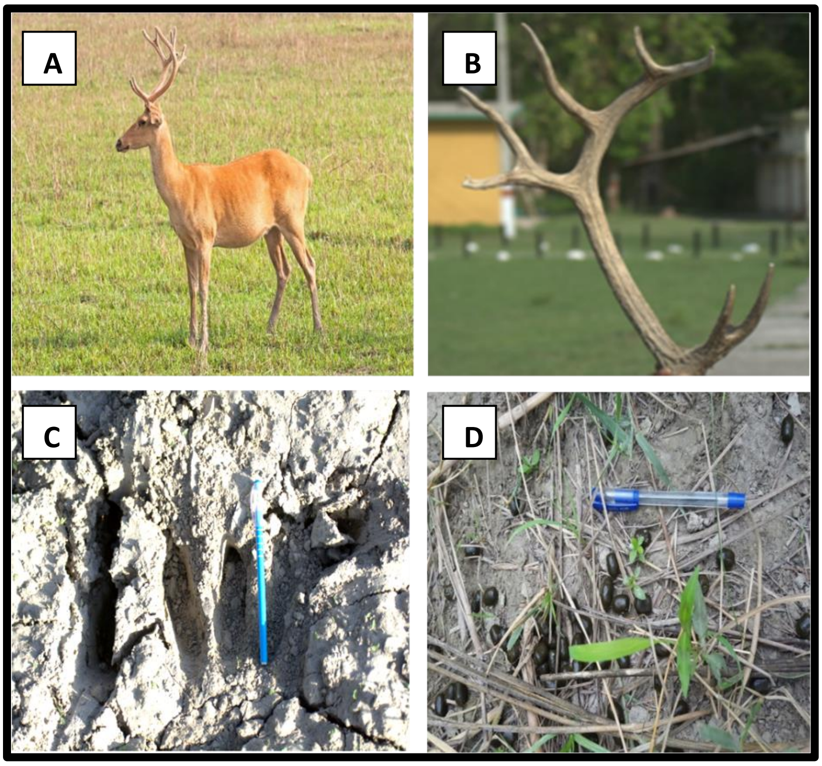

Figure 2.3 Signs considered as swamp deer evidence during survey: (A) Direct sighting (B) Antler (C) Hoofmark (D) Genetically identified faecal samples

During our surveys, we recorded GPS locations of all swamp deer presence evidence and collected biological samples (dung pellets, antlers, opportunistic tissue etc.) from all surveyed areas. Along with occurrence data, we have also opportunistically collected information on other important ecological and habitat parameters (disturbances, poaching evidences etc.). Presence of undisturbed *Typha* sp., *Phragmites* sp. and *Saccharum* spp. patches was an indicator of potential swamp deer habitat, while presence of livestock and humans in grassland were considered signatures of disturbance. Poaching related information were either provided by local residents or gathered through opportunistic evidences (for example, skinned hide) during surveys in our study area.

2.3.2 DNA-based swamp deer identification:

Although swamp deer pellets are larger than other grassland cervids (hog deer), domestic ungulates (sheep, goat) and distinctive from bovids (nilgai) found in this region, there are often uncertainties associated with pellet morphology-based species identification for similar body-sized animals. Identifying swamp deer pellets collected from marshy areas is easy as these areas are inaccessible to most other species. However, because swamp deer co-occur with other ungulates in many areas, we used DNA-based species identification to confirm species presence in our study. We randomly selected 104 pellets from surveyed zones and extracted DNA by swabbing the outer layer of each pellet (Ball et al., 2007). We amplified a 374 bp fragment of herbivore-specific mitochondrial cytochrome b gene described in Gupta et al. (2014) following published protocols. The PCR products were visualized in 2% agarose gel, cleaned with Exo-SAP mixture and then sequenced using forward primers in an ABI 3500XL bioanalyzer (Applied Biosystems). Sequences were aligned using MEGA v6.0 (Tamura et al., 2013) and visually examined for missense or frame-shift mutations. The aligned sequences were then matched against Genbank database for species confirmation.

2.4 Results:

2.4.1 Swamp deer survey:

While reporting our survey results, we will present swamp deer distribution patterns in each survey zone described in the "Methods" section. We had three such survey zones along the left bank of Ganga, five along the right bank and two along different tributaries of Ganga (Figure 2.4).

(i) The "Survey Zone 1" comprising of JJCR and surrounding areas has probably the highest number of swamp deer individuals in the state of Uttarakhand (Figure 2.4). Despite anthropogenic pressures in the form of proximity to a village, swamp deer is still common in this area as proven by frequent direct sightings. We counted up to 162 swamp deer individuals in the month of May 2016 in JJCR. Apart from sighting, a large number of biological materials in form of antlers and pellets were collected from this area (Table 2.1).

(ii) Our surveys in the "Survey Zone 2" along the left bank of Ganga known as Amichand, Nangal and Bhuria Sot showed both direct as well as indirect evidences of swamp deer presence (Figure 2.4). Our questionnaire surveys with local communities revealed regular presence of swamp deer in this area. We found swamp deer specific signs (characteristic splayed hoof marks) in different places across this zone and two carcasses as direct evidence. In addition, we also recovered swamp deer antlers and fresh pellets as biological samples (Table 2.1).

(iii) The "Survey Zone 3" is devoid of any typical swamp deer habitat. During our survey, we did not find any suitable habitat or indirect/direct signs of swamp deer in this zone.

(iv) The "Survey Zone 4" in the right bank of Ganga did not provide us with any direct or indirect evidences of swamp deer presence. Our questionnaire surveys with local communities revealed historical presence of swamp deer in this area, but currently no swamp deer is found here.

(v) The “Survey Zone 5” covers a non-protected wetland situated adjacent to Ganga river and directly opposite to Amichand grassland (Survey Zone 2). During our survey, we collected swamp deer antlers and fresh pellets from this area (Figure 2.4; Table 2.1).

(vi) Despite historical records of existence, the “Survey Zone 6” showed scanty evidences of swamp deer presence during our survey (Figure 2.4). Local communities reported occasional sighting of swamp deer in this area and we collected few pellets and found swamp deer signs (Table 2.1).

(vii) The “Survey Zone 7” in the right bank comprising HWLS and adjoining regions possibly harbours a large population of swamp deer. During our survey of this large area, we sighted swamp deer and collected large numbers of biological materials in the form of antlers and pellets (Figure 2.4; Table 2.1).

(viii) The final block of “Survey Zone 8” in the right bank of Ganga consists of a number of small grassland-dominated river islands. We recovered a carcass and collected fresh pellets and antlers as evidences of swamp deer presence in these river islands (Figure 2.4; Table 2.1).

(ix) Among the two tributaries of Ganga we first surveyed the “Survey Zone 9” on the right bank along river Solani. The grassland and wetland patches along this river showed direct and indirect evidences of swamp deer presence. We recovered a carcass, antlers and pellets from this area (Figure 2.4; Table 2.1).

(x) Our last survey area “Survey Zone 10” along the Ramganga river revealed a previously unreported swamp deer population about 58 km away from JJCR on the left bank of Ganga. We have direct sighting of swamp deer along with antler and pellet samples from this area (Figure 2.4; Table 2.1). During our surveys we did not find any evidences of swamp deer presence beyond this area.

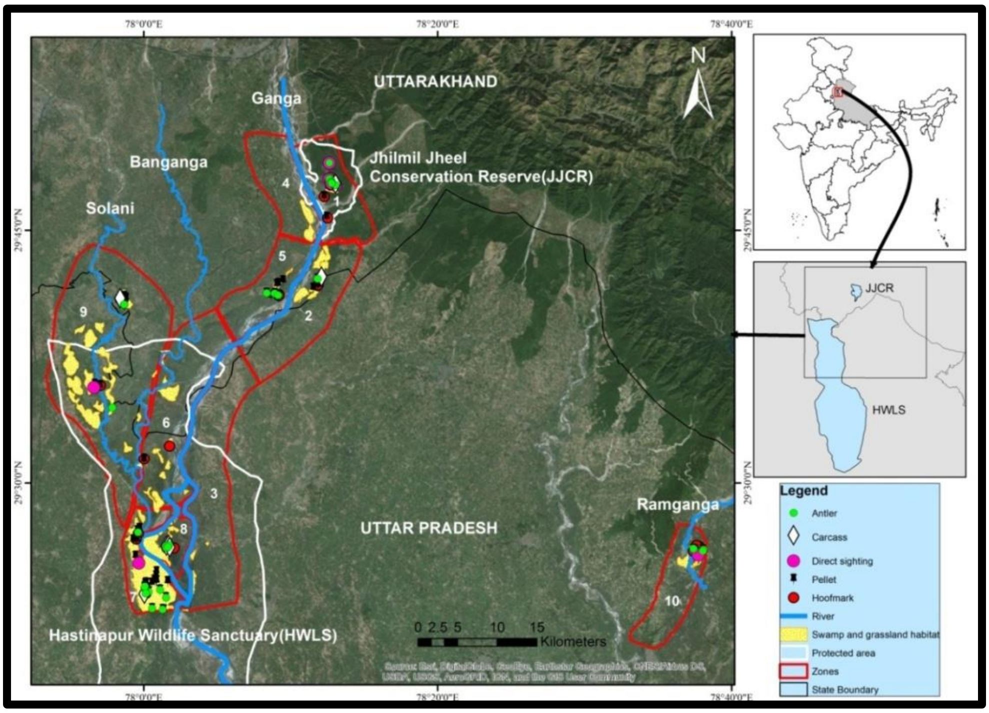

Figure 2.4 The swamp deer survey area between the Jhilmil Jheel Conservation Reserve and the Bijnor Barrage area of Hastinapur Wildlife Sanctuary in the upper Gangetic plains, north India. The map shows the survey zones, swamp and grassland habitats and locations of direct (sightings of live animals and carcasses) and indirect (antler, hoofmark, genetically identified faecal pellet) swamp deer evidences.

Table 2.1 Details of swamp deer surveys in the upper Gangetic plain during December 2015 to November 2016. Information on zone-wise area, survey effort, habitat characteristics, direct and indirect evidences of swamp deer presence and disturbance records are presented for each zone.

Area	Survey zones	Survey timings	Area (in km^2)	Survey effort (in km)	Names of local area with swamp deer evidences	Habitat characteristics	Direct sighting	Carcass	Antler	Hoofmark	Pellet	No. of pellet selected (swamp deer confirmed through DNA)	Disturbance records
Left bank of Ganga	Survey Zone 1	Dec 2015-Mar 2016	79.95	474.73	Jhilmil Jheel Conservation Reserve	• Wetland area with large swamp • Grasslands dominated with *Typha* sp., *Phragmites* sp.	Yes (n= 127-162)	1	61	Yes	1301	20(10)	Livestock grazing
	Survey Zone 2	Jan, Mar, Nov 2016	112.21	499.72	Amichand, Nangal, Bhuria Sot	• Patchy grassland habitat with agricultural fields • Grasslands dominated with *Typha* sp., *Phragmites* sp. and *Saccharum* spp.	No	3	3	Yes	43	6(5)	Sand mining and poaching
	Survey Zone 3	Apr-Jun 2016	177.68	409.04	No swamp deer evidences found	• Human-dominated area with croplands	No	0	0	No	0	0(0)	No swamp deer evidences found
Right bank of Ganga	Survey Zone 4	Feb, May, Jun 2016	71.74	362.51	No swamp deer evidences found	• Human-dominated area with croplands • Small patches of wetlands with *Typha* sp .present	No	0	0	No	0	0(0)	No swamp deer evidences found
	Survey Zone 5	Mar, Apr, Jun 2016	69.48	437.68	Ranjitpur	• Small wetland area surrounded by human habitations • Wetland dominated with *Typha* sp.	No	0	4	Yes	52	14(13)	Livestock grazing and habitat exploitation
	Survey Zone 6 (including Banganga river)	Apr, May, Jun 2016	144.70	502.94	Balia Khadar	• Grasslands habitat interspersed with agricultural fields • Grasslands dominated with *Saccharum* spp.	No	0	0	Yes	4	4(1)	Livestock grazing, habitat encroachment and agriculture
	Survey Zone 7	Jan, May, Jun 2016	65.65	560.70	Bijnor Barrage	• Big swamp with contiguous grassland habitat • Grasslands dominated with *Typha* sp., *Phragmites* sp.	Yes (n=12)	1	120	Yes	272	25(20)	Livestock grazing and poaching
	Survey Zone 8	Mar, Jun, Nov 2016	31.31	389.18	Raulighat	• River islands with grassland habitat • Grasslands dominated with *Typha* sp., *Phragmites* sp.	No	1	11	Yes	8	4 (0)	Livestock grazing, habitat encroachment, agriculture and poaching
Tributary (Solani and Banganga river)	Survey Zone 9	Mar, Jun, Nov 2016	308.69	643.53	Joggawala, Almawala	• Highly fragmented swampy habitat with *Typha* sp. • Human-dominated area with croplands	Yes (n=1)	1	7	Yes	28	16(07)	Habitat encroachment, agriculture and poaching
Tributary (Ramganga river)	Survey Zone 10	Apr, May, Jun 2016	63.44	412.57	Near Afzalgarh	• Swampy habitat surrounded by human habitations • Wetland dominated with *Typha* sp.	Yes (n=9)	0	9	Yes	26	15(14)	Livestock grazing, habitat encroachment, agriculture and poaching

2.4.2 Molecular identification of swamp deer:

We randomly selected 104 potential swamp deer pellet samples collected from different survey zones for molecular species identification. NCBI matching analyses of the sequences generated from successfully amplified samples revealed that 70 pellet samples belong to swamp deer. In addition, few sequences were found to be originating from sambar (n= 1), spotted deer (n= 6), hog deer (n= 1) and nilgai (n= 2). Details of zone-wise genetically identified swamp deer samples are provided in Table 2.1. The Genbank accession numbers for species-wise sequences of all identified species are MF143831-MF143910. 24 samples did not amplify during PCR possibly due to degraded DNA quality.

2.4.3 Habitat status and disturbances:

We found most number of swamp deer individuals and good habitat in Jhilmil Jheel Conservation Reserve (survey zone 1). However, our survey showed signs of anthropogenic pressures in the form of livestock grazing within both protected areas of Jhilmil Jheel Conservation Reserve and Hastinapur Wildlife Sanctuary and habitat conversion within Hastinapur Wildlife Sanctuary. Most of the areas covering survey zones 3, 6, 8 and 9 within the boundary of Hastinapur Wildlife Sanctuary receive very limited protection measures for swamp deer or their habitats. In the remaining non-protected survey zones major problems are poaching, crop depredation-related retaliatory killings and other forms of anthropogenic disturbances (Figure 2.5). For example, the grassland patches in "Survey Zone 2" in the left bank of Ganga are disturbed by heavy vehicles from sand mining (Figure 2.5D) activities. Similarly, in "Survey Zone 3" all small grassland patches have been converted to croplands, leading to no suitable habitats for the species now. Along the right bank of Ganga, we found that survey zone 4 has minimal grassland habitats left for swamp deer. Our questionnaire surveys with local communities revealed historical presence of swamp deer in this area, but surveys showed no current evidences of swamp deer in this area. The survey zone 6 showed scanty evidences of swamp deer presence during our survey. Local communities reported occasional sighting of swamp deer on the bank of Banganga river in this area, which is corroborated by presence of hoof marks and faecal pellets (Table 2.1). In survey zones 5 and 8, local communities reported incidences of swamp deer poaching activities and crop-raiding related retaliatory killings by the farmers (Figure 2.5). The grassland and wetland patches along Solani river (Survey zone 9) are completely fragmented and surrounded by human habitations and the status of swamp deer connectivity with other areas cannot be ascertained currently. The survey area along the Ramganga river (Survey zone 10) revealed occasional poaching incidences and potentially poor connectivity with other swamp deer populations. Overall, we calculated the total habitat of swamp deer to be 106 km^2 between JJCR and Bijnor Barrage. There are 52 grassland patches with areas ranging from 0.12 km^2 to 32.57 km^2. The biggest swamp deer habitat patch is the wetland in Bijnor Barrage area (32.57 km^2).

Figure 2.5 Potential threats for swamp deer: (A) Livestock grazing (B) Poaching (C) Habitat encroachment (D) Sand mining

2.5 Discussion:

Our survey of northern swamp deer subspecies *Rucervus duvaucelii duvaucelii* range in the upper Gangetic plain revealed their presence in multiple areas along the whole stretch of Ganga, starting from Jhilmil Jheel Conservation Reserve (Uttarakhand) to Bijnor Barrage area of Hastinapur Wildlife Sanctuary (Uttar Pradesh). We also found swamp deer presence along Solani and Banganga rivers, with a previously unreported population near Afzalgarh, Uttar Pradesh along Ramganga river (tributaries of Ganga). The presence of this subspecies across our survey zones was confirmed through direct sighting, presence of carcasses, antlers, hoofmarks and genetically identified pellets collected from the field. We found swamp deer evidences in eight of the 10 surveyed zones (Table 2.1), where local communities also suggested their presence. These new findings support finer scale swamp deer occurrence when compared to the earlier survey reports in this region (Qureshi et al., 2004), and add hope for future survival of this species. However, it is important to point out that swamp deer occurrence downstream of Bijnor Barrage as well as along other tributaries of Ganga is very much possible and future search along these areas may reveal further new areas with swamp deer presence.

Our results also suggest very patchy distribution of this subspecies in relatively small areas across the states of Uttar Pradesh and Uttarakhand. Though we have not conducted any population estimation in this study, our surveys indicate small population size of swamp deer in this region. Seasonal movement of swamp deer from Jhilmil Jheel Conservation Reserve indicates their capability of moving to other areas. Such seasonal and flood-driven movement patterns were observed in central Indian and northern swamp deer populations, respectively (Martin and Gopal, 2015).

Although our survey revealed much finer scale distribution of swamp deer in the upper Gangetic plains, except Jhilmil Jheel Conservation Reserve and parts of Hastinapur Wildlife Sanctuary, all other swamp deer bearing areas receive very limited protection measures. As all these areas have possibly small, seasonally fluctuating swamp deer numbers, their future survival will depend on their management as one metapopulation with stable demographic and genetic parameters. They are currently facing severe threats of habitat loss from conversion of wetlands to agricultural fields, livestock grazing and occasional poaching. There are also tremendous anthropogenic pressures on these grasslands habitats as they are probably the most fertile land in northern India.

Only more attention towards habitat conservation, anti-poaching activities and public awareness can improve the fragmented status and increase populations of this enigmatic species in this landscape. Conservation and management of *Rucervus duvaucelii duvaucelii* as an “umbrella species” in the non-protected areas will also have a long-lasting impact on conservation of swamp habitats in upper Gangetic plains. The conservation and management of the species living in these habitats outside protected areas bring new challenges and require particular attention towards involving local communities for their future survival. Directed efforts from government initiated large-scale programs like “National Mission for Clean Ganga (NMCG)” on reviving and protecting the swampy grassland patches along Ganga would greatly help future swamp deer recovery. These swampy grasslands or wetlands are home to variety of endangered fauna like hog deer, otter, fishing cat, herpetofauna, and a number of resident and migratory birds. Swamp deer is the largest cervid present in the wetlands along Ganga and its conservation and management are immediately required to save these critical habitats for the subspecies and other endangered fauna in this landscape.

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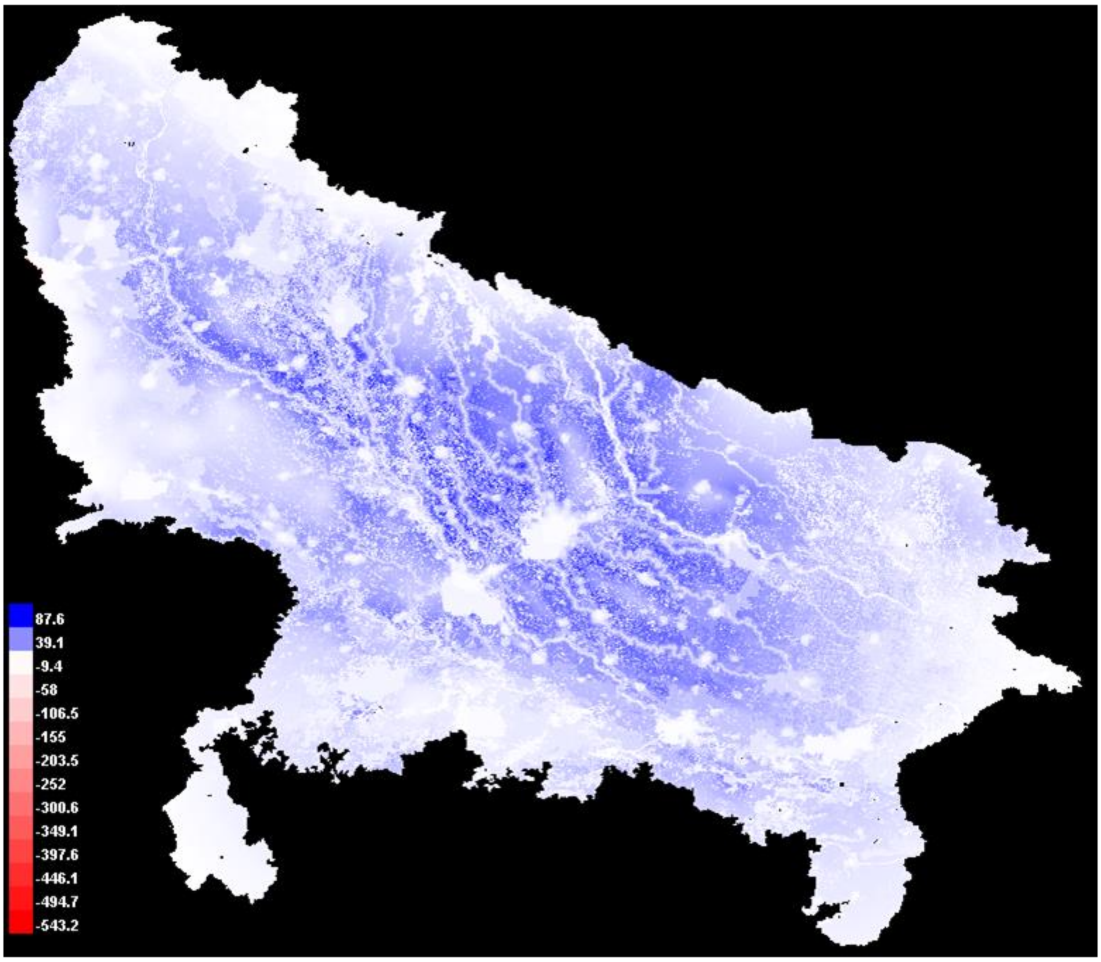

Chapter 2:

Modeling fine-scale distribution of swamp deer across Uttarakhand and Uttar Pradesh

3.1 Introduction:

Large herbivores have undergone rapid population declines and range contractions resulting in ~60% being threatened with extinction. Majority of the threatened species reside in developing countries where major threats include hunting, land-use change, and resource depression by livestock. The swamp deer is the most extinction-prone megaherbivore of the Indian subcontinent due to their inherent species biology and various anthropogenic activities and lack of knowledge about accurate information on distribution, population size and various habitat parameters pose a significant conservation challenge (Karanth et al., 2010). However, generating such information is difficult particularly for the northern swamp deer due to their presence in a mosaic of protected and non-protected areas surrounding human settlements (Jathanna et al., 2003; Linkie et al., 2013; Marshal, 2016). Given that only about 5% of land comes under protected area category within India (Wildlife Institute of India, 2020), it is critical to generate detailed information on distribution of this habitat-specialist large herbivore and identify important habitats outside protected areas for their long-term conservation. The northern swamp deer distribution within the upper Gangetic plains is broadly divided into two habitat blocks: a) the habitat block along the Sharda river basin in the state of Uttar Pradesh covering a mosaic of protected (Pilibhit Tiger Reserve, Kishanpur Wildlife Sanctuary, Dudhwa National Park and Katerniaghat Wildlife Sanctuary) and unprotected areas surrounding them and b) habitat block along river Ganga covering protected areas in Uttarakhand (Jhilmil Jheel Conservation Reserve) and Uttar Pradesh (Hastinapur Wildlife Sanctuary) along with surrounding unprotected, patchy grassland habitats (Qureshi et al., 1995; 2004, see 1st chapter).

Encroachment pressures on the grasslands (only 2% of the entire Gangetic floodplains) (Dinerstein, 2003) from one of the most highly dense human populations (about 800 people/km^2 compared to 382 people/km^2 in India, Census of India, 2011) living in this region and scanty information on exact distribution and habitat of swamp deer status pose significant conservation challenges (Duckworth et al., 2015; Midha and Mathur, 2010; see 1st chapter).

In the 1st chapter, we reported a detailed assessment of the northern subspecies presence and habitat status in the Gangetic block. This indicates promising signs for this subspecies, but a landscape wide assessment of distribution is essential for developing better management plans for swamp deer and its remaining grassland habitats. Even within the Sharda habitat block which is a stronghold of this species, fine-scale distribution pattern of swamp deer is unknown till date. Swamp deer is only reported from a few renowned swamps but for a species which shows flood-driven movements, it is important to identify all the seasonal habitats for the species. However, conducting surveys in a human-dominated landscape is logistically challenging and probably requires a combination of different approaches to assess the fine-scale distribution pattern of this subspecies.

In this chapter, we assessed *Rucervus duvaucelii duvaucelii* distribution covering protected and non-protected areas across the upper Gangetic plains, within the boundaries of Uttarakhand and Uttar Pradesh. We used multidisciplinary approaches involving scientifically designed rigorous field surveys in Ganga (data from 1st chapter) and Sharda block, distribution modeling and subsequent field validations to assess current swamp deer distribution and status of their habitats. Further, we have identified four new “Priority Conservation Areas” that are crucial for future swamp deer conservation planning.

3.2 Study area:

This study was conducted covering about 253188 km^2 area across selected districts of Uttarakhand (Dehradun, Haridwar, Pauri-Garhwal, Nainital and Udham Singh Nagar) and the entire state of Uttar Pradesh. The area covers the floodplains of the Ganga river and a number of its tributaries like Sharda, Ghaghra, Ramganga etc. including a complex network of riverine-grassland and grassland-forest systems (Figure 3.1). This region hosts the only existing population of northern swamp deer (*Rucervus duvaucelii duvaucelii*) in India (Qureshi et al., 2004). The primary vegetation in the grassland patches comprises *Typha* sp., *Phragmites* sp. and *Saccharum* spp. (Tewari and Rawat, 2013). Larger animals in the study area include swamp deer (*Rucervus duvaucelii duvaucelii*), hog deer (*Axis porcinus*), spotted deer (*Axis axis*), nilgai (*Boselaphus tragocamelus*), smooth-coated otter (*Lutrogale perspicillata*), fishing cat (*Prionailurus viverrinus*) and iconic wetland birds such as sarus crane (*Antigone antigone*), black-necked stork (*Ephippiorhynchus asiaticus*), lesser adjutant (*Leptoptilos javanicus*), Pallas’s fish eagle (*Haliaeetus leucoryphus*) and bar-headed goose (*Anser indicus*) (Grimmett et al., 2013). The area experiences extreme seasonal variations in temperature (0°C-50°C) and receives an average annual rainfall of 948 mm (502.4-2113.9 mm) with occasional flooding during July-September (Verma et al., 2019).

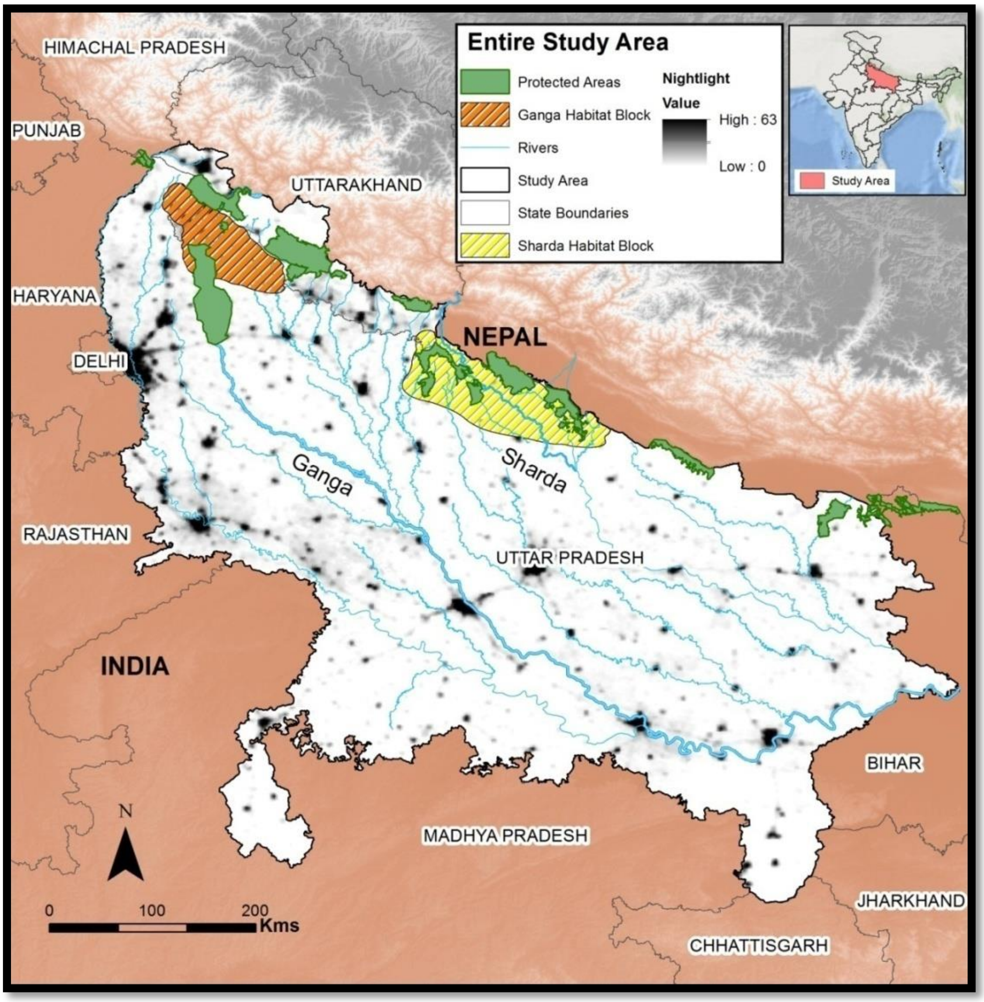

Figure 3.1 The entire study area map across the states of Uttarakhand (selected area) and Uttar Pradesh, north India covering both Ganga and Sharda habitat blocks

3.3 Methods:

3.3.1 Species Distribution Modeling (SDM) for swamp deer using MaxEnt:

3.3.1.1 Generation of swamp deer occurrence points

We used MaxEnt (Maximum Entropy) species distribution algorithm version 3.3.3k (Phillips et al., 2006) to model the potential distribution of swamp deer in the study area. Maximum Entropy is one of the most widely used algorithms for species distribution with presence-only data (Elith et al., 2006). For best performance of the algorithm, it is critical to have unbiased, error-free species presence points for initial calibration of the models (Kramer-Schadt et al., 2013). Biased survey design including inadequate survey efforts, sporadic or clumped location information, erroneous presence data etc. affect model accuracy (Costa et al., 2015; Kramer-Schadt et al., 2013). We conducted systematic intensive surveys to generate accurate swamp deer presence points from both Ganga and Sharda river habitat blocks for model calibration. Details of the survey and fine-scale swamp deer distribution at the Gangetic habitat block from Jhilmil Jheel Conservation Reserve (JJCR) in Uttarakhand to Bijnor Barrage area of Hastinapur Wildlife Sanctuary (HWLS) in Uttar Pradesh is described in the 1st chapter.

In Sharda block, swamp deer presence has been reported from a few well-known swamps within protected areas only. For a comprehensive detailed assessment of swamp deer distribution in Sharda block, we adopted a similar combined approach of opportunistic unstructured questionnaire surveys (see 1st chapter) and Google Earth imageries (Hu et al., 2013) to locate possible areas (both protected and adjoining non-protected) where the species was found. As most of the potential habitats in this region are protected, the questionnaire surveys were targeted towards Forest Department staff and occasionally with local cattle herders, boatmen and fishermen. All information indicated that majority of the grassland patches are currently restricted within 10 km areas on both banks of the rivers Mala (Pilibhit Tiger Reserve), Sharda (Pilibhit Tiger Reserve and Kishanpur Wildlife Sanctuary), Suheli (Dudhwa National Park) and Ghaghra (Katerniaghat Wildlife Sanctuary). Subsequently, we intensively surveyed both banks of the selected stretch of these rivers covering both protected as well as non-protected areas. The surveys were conducted using vehicle, boat or on foot depending on the area during the months of May of 2016 and 2017, covering a total area of around 6000 km^2.

During surveys, we collected geo-tagged information on direct (sighting, carcass etc.) as well as indirect (antlers and genetically identified dung pellets) evidences of swamp deer presence. Swamp deer antlers are quite distinctive from other coexisting cervids in this landscape (sambar, hog deer, chital and barking deer) (see 1st chapter). However, swamp deer pellets are difficult to identify based on morphology and we conducted molecular species identification using species-specific primers for different cervids (described in 3rd chapter). Remaining non-amplified samples were tested with ungulate specific primers (Gupta et al., 2014) to identify species.

3.3.1.2 Fine-tuning of species occurrence data for model calibration

Combining the earlier records from upper Gangetic habitat block (n= 169, see 1st chapter) and new surveys at Sharda basin habitat block (n= 139, 2 carcasses, direct sightings, 51 genetically identified pellets and 79 antlers), we gathered 308 confirmed swamp deer location points from the entire landscape. Upon plotting these locations in a map, we found that many confirmed swamp deer locations were clustered within the fragmented grassland patches, leading to spatial autocorrelation (around 4.4 points/km^2 grid). Such spatial patterns may lead to overfit model response to covariates, thereby limiting the model's prediction accuracy (Boria et al., 2014; Kramer-Schadt et al., 2013). Calibration and evaluation localities that are close to each other might lead to inflated values of performance (Hijmans, 2012; Veloz, 2009). To reduce such effects, we selected a single presence record in every one km^2 area (as the species is mostly restricted to habitat patches of around one km^2 currently within the grassland-cropland mosaics within this landscape) using Spatially Rarefy Tool of SDM tool Box (Brown, 2014) in ArcGIS 10.2.2. We used 69 such records for species distribution modeling from a total of 308 points in the Gangetic plains of Uttarakhand and Uttar Pradesh.

3.3.2 Selection of covariates:

Based on swamp deer species biology, we initially selected numerous climatic, vegetation, topographic and anthropogenic variables to model swamp deer distribution. Bioclimatic variables are regularly used in defining species environmental niches (Booth et al., 2014; Bradie and Leung, 2017) and we downloaded 30 arc-second spatial resolution (or one km^2 spatial resolution) data of four such variables from WorldClim-Global Climate Data (www.worldclim.org/bioclim). The topographic covariates in the form of "Elevation" and "Slope" layers were downloaded from the USGS website (www.usgs.gov). The "slope" was calculated using ArcToolbox, Spatial Analyst in ArcGIS 10.2.2 (ESRI, Redlands, CA, USA). We also selected Normalized Difference Vegetation Index (NDVI) of March and May 2017, distance from water, nightlight and human population density (anthropogenic variables), rescaled to one km^2 resolution as covariates (Table 3.1).

Correlated variables were removed as it might lend more statistical weight to one of them, leading to model overfitting (Dormann et al., 2013). We used ''Pearson correlation coefficient'' to identify highly correlated variables, where a threshold value of 0.75 was selected to choose the final covariates (Kalboussi and Achour, 2017; Pearson et al., 2002). After initial testing of all parameters, final species distribution modeling was run using the following variables: annual mean temperature, annual precipitation, NDVI-March 2017, NDVI-May 2017, nightlight, distance from water, human population density and elevation (Table 3.1 and 3.2). Elevation was selected despite having high correlation with annual mean temperature, as it is an essential ecological factor restricting swamp deer distribution within the Gangetic plains (being an obligate grassland species of the plains, swamp deer are not found in undulating, hilly areas).

Table 3.1 Details of all environmental and ecological covariates used in northern swamp deer distribution modeling

Category	Variables	Data source	Abbreviation	Units/Range	Variables selected
Bioclimatic	Annual Mean Temperature	WorldClim (https://worldclim.org/data/bioclim.html)	Bio1	°C	Yes
	Annual Precipitation		Bio12	Mm	Yes
	Precipitation of Wettest Month		Bio 13	Mm	No (High correlation with Bio 12)
	Precipitation of Wettest Quarter		Bio 16	Mm	No (High correlation with Bio 12)
Vegetation	NDVI March (2017)	MODIS V6 (https://earthexplorer.usgs.gov/)	NDVI March	-1 to +1	Yes
	NDVI May (2017)		NDVI May	-1 to +1	Yes
Demographic	Nightlight	Defence Metrological Satellite Programme-Operational Line Scan System	Nightlight	0 to 63	Yes
	Distance from Water	Waterbodies digitized in Earth Explorer and Euclidean distance calculated	Dist_Water	M	Yes
	Human Population Density	SEDAC (https://sedac.ciesin.columbia.edu/)	Population Density	people/km^2	Yes
Topographic	Elevation	ASTER DEM (https://earthexplorer.usgs.gov/)	DEM	M	Yes
	Slope		Slope	0 to 90	No (High correlation with DEM)

Table 3.2 Results of multi-collinearity tests (Pearson correlation coefficient, r) among the covariates to identify highly correlated variables

Layer	**Bio 1**	**Bio 12**	**Bio 13**	**Bio 16**	**DEM**	**Distance from water**	**NDVI March**	**NDVI May**	**Nightlight**	**Population Density**	**Slope**
Bio 1	**1.000**	-0.657	-0.612	-0.617	**-0.873**	0.105	-0.020	-0.475	-0.029	0.112	-0.715
Bio 12	-0.657	**1.000**	**0.944**	**0.983**	0.426	-0.100	0.069	0.415	-0.082	-0.120	0.437
Bio 13*	-0.612	**0.944**	**1.000**	**0.969**	0.462	-0.031	-0.012	0.328	-0.080	-0.196	0.452
Bio 16*	-0.617	**0.983**	**0.969**	**1.000**	0.437	-0.030	0.028	0.367	-0.078	-0.142	0.443
DEM	**-0.873**	0.426	0.462	0.437	**1.000**	0.232	-0.143	0.278	0.035	-0.203	**0.774**
Distance from Water	0.105	-0.100	-0.031	-0.030	0.232	**1.000**	-0.298	-0.209	-0.076	-0.254	0.056
NDVI March	-0.020	0.069	-0.012	0.028	-0.143	-0.298	**1.000**	0.146	-0.136	0.137	-0.089
NDVI May	-0.475	0.415	0.328	0.367	0.278	-0.209	0.146	**1.000**	0.036	0.037	0.186
Nightlight	-0.029	-0.082	-0.080	-0.078	0.035	-0.076	-0.136	0.036	**1.000**	0.413	-0.027
Population Density	0.112	-0.120	-0.196	-0.142	-0.203	-0.254	0.137	0.037	0.413	**1.000**	-0.163
Slope*	-0.715	0.437	0.452	0.443	**0.774**	0.056	-0.089	0.186	-0.027	-0.163	**1.000**

Highly correlated variables ($-0.75 \leq$ Values ≥ 0.75) are highlighted in bold.

*Rejected correlated covariates

3.3.2.1 Model parameters, performance and selection

We developed and tested 10 different MaxEnt models by modifying the default parameters in order to derive the best model (Aryal et al., 2016) for swamp deer distribution. Use of default parameters can produce an over-complex or over-simplistic model (Merow et al., 2013; Warren and Seifert, 2011). MaxEnt models are sensitive to the model parameter “regularization multiplier” value (default=1), which determines the output distribution (Elith et al., 2010). A smaller parameter value results in a more localized output distribution, while a larger value generates a more spread out, less localized predictions (Phillips, 2017). We developed models with 10000 background sampling points as environmental space (Wilting et al., 2010) with different regularization parameter values (0.5-5). We randomly assigned the presence localities as training and testing dataset (70% as training, 30% as test). Rest of the settings for each parameter were kept default in each run. To select the best-fitted regularization multiplier, we used the Corrected Akaike information criterion (AICc) based evaluation method and selected the model with the lowest AICc value (Cordellier and Pfenninger, 2009; Williams et al., 2017). The evaluation of different regularization multiplier values has been done using the software ENMTOOLS version 1.4.4 (Warren and Seifert, 2011).

After selecting the best-fitted regularization multiplier (Model with lowest AICc value), we ran the final model with 10 replicates of 5000 iterations in MaxEnt to derive the average model (Phillips et al., 2006). An auto feature limiting function was used to fit the species environmental curves and to train the MaxEnt models. The minimum probability of MaxEnt prediction was set to “Minimum training presence Logistic threshold" (MTPLR) (Montemayor et al., 2014). We used the logistic model output, which displays a suitable value from 0 (unsuitable) to 1 (suitable). To check which variables were the most important for model building, a Jackknife analysis was done (Phillips, 2017).

To assess qualitative characterization of the final species distribution model, we used Receiver operating characteristic (ROC) area under curve (AUC) values (Phillips et al., 2006). AUC value of a model ranges from 0 to 1, where 0.90 to 1.00 range is considered as a good fit (Araújo et al., 2005; Hemsing, 2010).

3.3.3 Swamp deer validation surveys:

The MaxEnt prediction gave a probability distribution map of swamp deer in the upper Gangetic plains of north India. To confirm model predictions, we conducted validation surveys in selected areas of this landscape. We selected the completely human-dominated lower part of Hastinapur Wildlife Sanctuary (downstream of Bijnor Barrage along Ganga) and Sohagibarwa Wildlife Sanctuary (in Sharda habitat block) in Uttar Pradesh for validation surveys. Hastinapur Wildlife Sanctuary is probably the most critical swamp deer habitat in the Ganga habitat block and earlier extensive surveys were conducted till Bijnor Barrage (see 1st chapter). We surveyed the entire stretch of Ganga from Bijnor Barrage till the southern boundary of Hastinapur Wildlife Sanctuary (about 100 km), covering all grassland patches found on either side of the river during May 2017 and April 2018. On the other hand, Sohagibarwa Wildlife Sanctuary is the easternmost protected area predicted by the model, known to harbour large patches of grassland habitat and historical presence of swamp deer (Ghimire et al., 2019). Apart from field-based information (direct sighting, antler and genetically confirmed pellets), we have also collected secondary information (newspaper report, personal communications etc.) on swamp deer presence in the southernmost predicted regions of our study area. To assess the accuracy of model prediction, we extracted pixel values of confirmed unique (duplicates removed) swamp deer presence points (primary, secondary and habitats) and considered those points with more than Minimum training presence Logistic threshold (MTPLR). The formula used to calculate accuracy is:

(Number of GPS points with pixel value more than MTPLR X 100) / (All GPS points of swamp deer presence)

3.3.4 Identification of Priority Conservation Areas:

Earlier work on the *Rucervus duvaucelii duvaucelii* showed fragmented, but fine-scale distribution of the subspecies in the northern part of the Ganga habitat block (till Bijnor Barrage) (see 1st chapter). As we generated detailed information on the subspecies entire range (covering both Ganga and Sharda habitat blocks) and habitat status based on the initial surveys, distribution modeling and validation surveys, it was meaningful to identify critical ‘‘Priority Conservation Areas’’ where immediate management attention is required. This entire landscape is a mosaic of protected and non-protected areas with very high pressures from anthropogenic interventions. Between these two habitat blocks there are differences in grassland coverage area, management regimes and human densities. We used a few criteria to select such ‘‘Priority Conservation Areas’’ across the entire landscape covering both protected and non-protected habitats. The criteria were: (a) presence of grassland habitats (through field surveys and GIS approaches, see 1st chapter); (b) results from the MaxEnt model predictions; (c) confirmed species presence in the area; and (d) strategic position of the areas within this human-dominated mosaic landscape to ensure continuation of the species seasonal migration (Martin and Gopal 2015; see 1st chapter). Most importance was given to species and grassland presence, followed by the other two criteria.

3.4 Results:

3.4.1 Swamp deer initial survey in Sharda habitat block:

We found much fine-scale swamp deer occurrence evidence than earlier reported (Qureshi et al., 2004) across majority of surveyed areas in the Sharda river habitat block, including all the prominent protected areas (Pilibhit Tiger Reserve, Kishanpur Wildlife Sanctuary, Dudhwa National Park and Katerniaghat Wildlife Sanctuary) (Figure 3.2). Additionally, we found presence of the species in non-protected grasslands of South Kheri and North Kheri Forest Divisions of Uttar Pradesh. Apart from swamp deer, we also identified the presence of other cervids (hog deer, chital, sambar etc.) in the grassland habitats. We collected a total of 225 biological samples (two swamp deer carcasses, 144 pellets and 79 confirmed swamp deer antlers) and identified 132 pellets out of which 51 belonged to swamp deer and 70, 10 and one to hog deer, chital and sambar, respectively. Remaining 12 samples did not provide any results during molecular species identification.

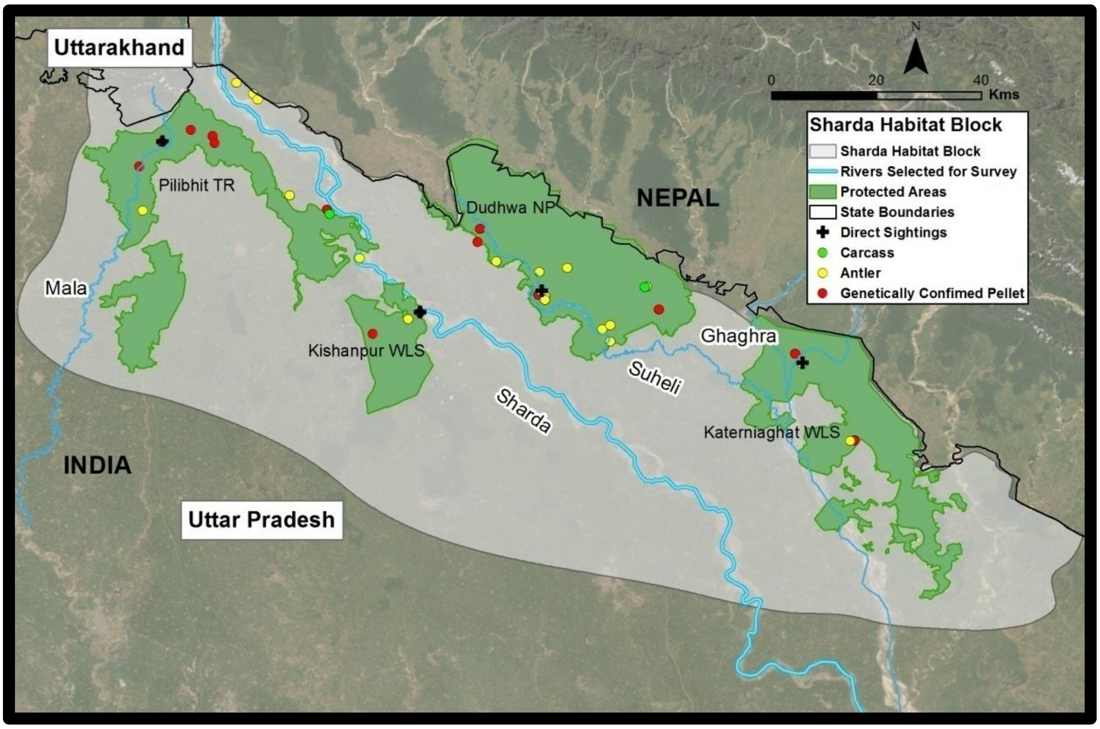

Figure 3.2 The locations of the confirmed swamp deer evidences within Sharda habitat block. Different evidence types (direct and indirect) are presented in the figure legends.

3.4.2 MaxEnt prediction of swamp deer distribution:

Out of the 10 different models with different regularization multipliers (RM 0.5-5), the model with RM 1.5 showed lowest AICc Value (1183.32) and was selected (Table 3.3). We pooled the prediction probabilities (above MTPLR=0.067) to create high (0.6-1), moderate (0.4-0.6) and low probability (0.067-0.4) occurrence zones. The high probability of swamp deer was predicted to be along the banks of rivers of both Ganga and Sharda blocks (Ganga, Kosi, Ramganga, Sharda, Suheli, Ghaghra and Baur) (Figure 3.3). In the Gangetic habitat block, the highest probabilities were found to be upstream of Bijnor Barrage along river Ganga, Banganga and Solani. The moderate zones included majority of the above-mentioned areas in addition to basins of Gomti, Gaula, Devha, Baigul, Ganga River (downstream of Bijnor Barrage) and some parts of Suhelwa Wildlife Sanctuary and Sohagibarwa Wildlife Sanctuary. The low probabilities of occurrence were found to be along river Yamuna and Hindon on the west; rivers like Rapti, Kuwano and some scattered areas of Suhelwa and Sohagibarwa Wildlife Sanctuary in the east and few areas further downstream south along Ganga (Figure 3.3). Out of the total predicted areas (3% of study area) for swamp deer distribution (7650 km^2), 3413 km^2 falls within protected areas of Uttarakhand and Uttar Pradesh.

Table 3.3 Model selection based on lowest Small Sample Corrected Akaike Information Criterion (AICc) value

Model No.	**Regularization Multiplier (RM)**	**Log Likelihood**	**Akaike Information Criterion (AIC score)**	**Small Sample Corrected Akaike Information Criterion (AICc score)**	**Bayesian Information Criterion (BIC score)**
1	0.5	-547.61	1165.22	1243.97	1242.91
2	1	-562.56	1181.13	1222.77	1243.27
3*	1.5	-570.33	1172.65	1183.32	1208.17
4	2	-574.89	1185.78	1199.74	1225.73
5	2.5	-578.39	1182.78	1189.52	1211.63
6	3	-580.61	1199.22	1215.05	1241.39
7	3.5	-587.35	1198.70	1204.37	1225.33
8	4	-591.73	1205.45	1210.17	1229.87
9	4.5	-596.97	1215.94	1220.66	1240.36
10	5	-600.87	1221.73	1225.59	1243.93

*selected model

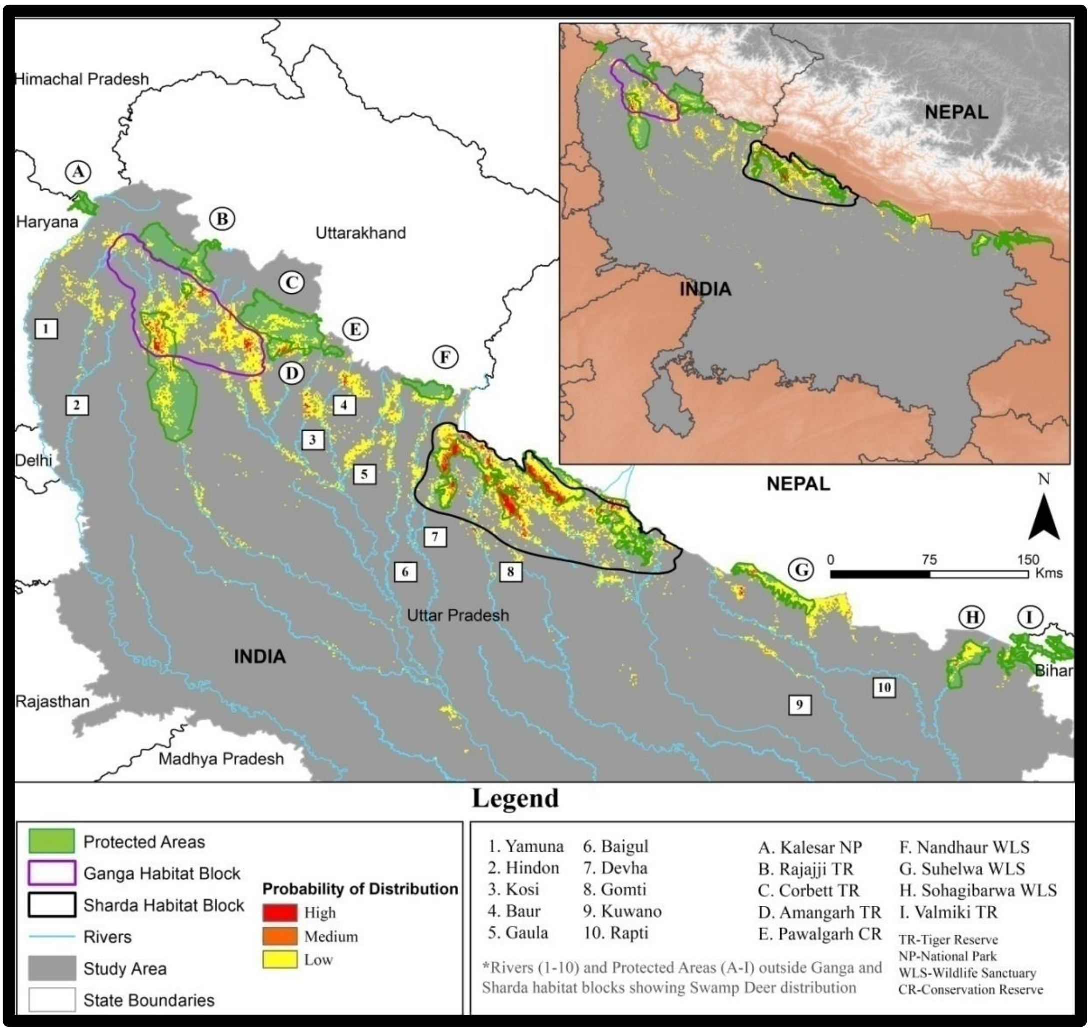

Figure 3.3 Map showing the predicted distribution of swamp deer through MaxEnt modeling. The major rivers and protected areas outside Ganga and Sharda habitat blocks are highlighted and marked.

The Jackknife prediction of the final selected model showed that the NDVI-May had highest contribution (37.1%), followed by annual mean temperature (24.5%), distance from water (18.6%), nightlight (8%), NDVI-March 2017 (7.1%) in predicting swamp deer distribution. The remaining 4.7% is contributed by human population density, DEM and annual precipitation. Majority of the predicted areas were having an elevation of about 200m, and mean annual temperature range between 22ºC- 25ºC. MaxEnt results showed best prediction at a NDVI value of 0.45 during March and 0.6

in case of May. Distance from water showed a negative relationship with swamp deer distribution after a distance of seven km from the rivers. Both nightlight and human population density were negatively related to the probability of distribution. The AUC value of the final selected model was 0.992 with a standard deviation of 0.001, indicating excellent prediction for swamp deer distribution (Elith et al., 2006) (Figure 3.4 and 3.5).

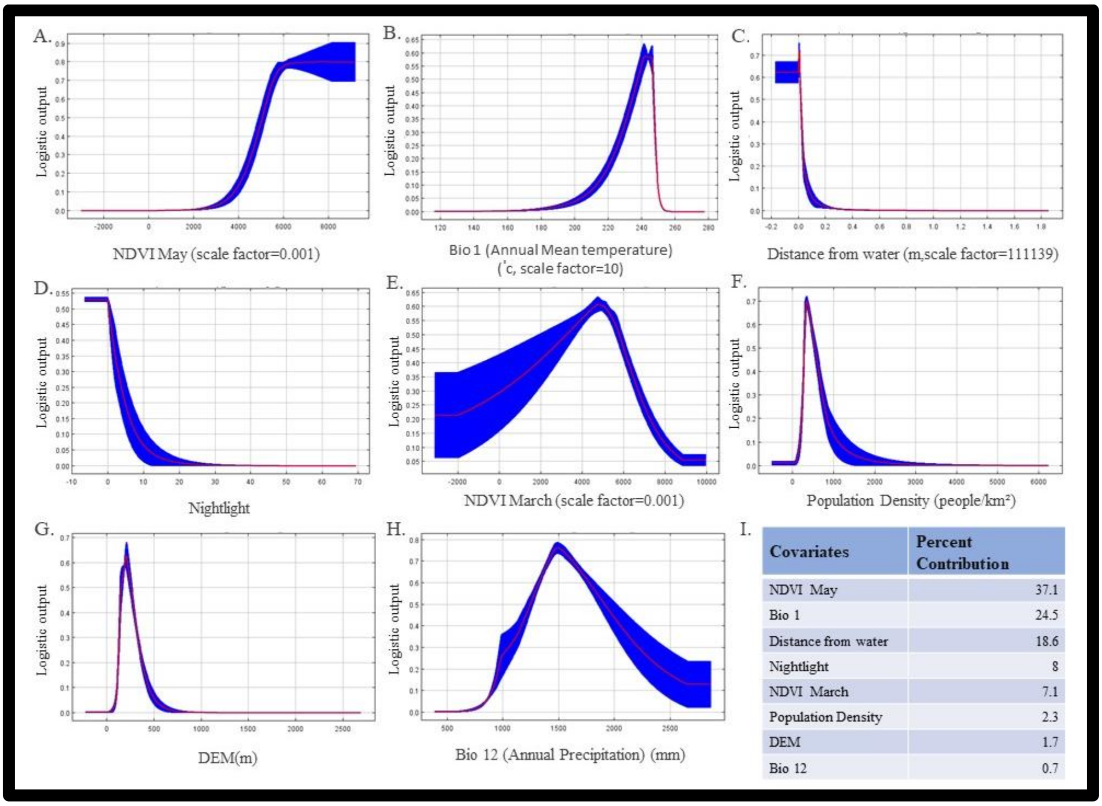

Figure 3.4 Figure showing the response curves of eight covariates used in the final MaxEnt model for swamp deer distribution predictions. Figures A-H shows the response curves, whereas I list the estimates of relative contributions of the respective covariates in the MaxEnt predictions.

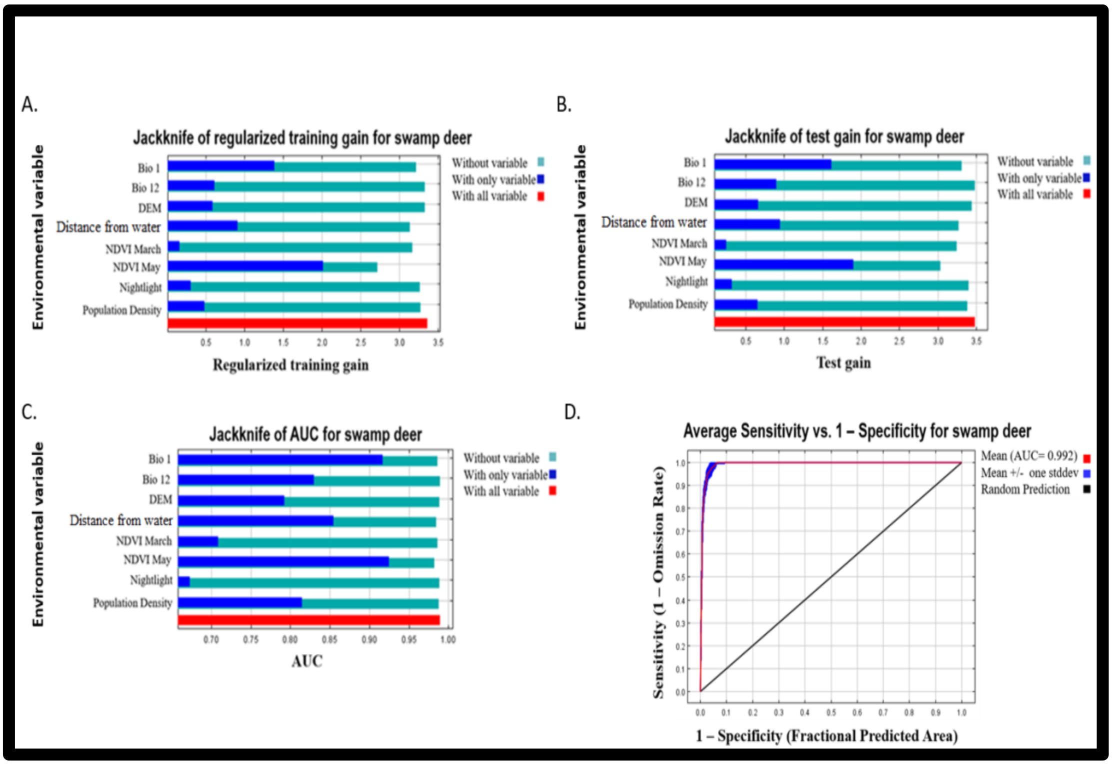

Figure 3.5 Jackknife tests of variable importance and Receiver operating characteristic (ROC) curve for predicted distribution of swamp deer: (A) Jackknife test using training gain (B) Jackknife test using test gain (C) Jackknife test using AUC on test data (D) ROC curve for the training omission rate and predicted area averaged over the replicate runs

3.4.3 Validation surveys:

Our survey in the lower part of Hastinapur Wildlife Sanctuary revealed the presence of fragmented grassland patches with swamp deer evidence (Figure 3.6B). In addition to direct sighting (n=1) and shed antlers (n=6), molecular analysis of pellet samples (n=163) confirmed presence of swamp deer (n=82), hog deer (n=18), nilgai (n=25), barking deer (n=3) and domestic goat (n=4) (Table 3.4). Rest 31 samples did not amplify during Polymerase Chain Reaction (PCR). In Sohagibarwa Wildlife Sanctuary, our surveys revealed *Typha* sp. and *Phragmites* sp. dominated grasslands but no evidence of swamp deer (Table 3.4, Figure 3.6D). Additionally, we validated the southernmost predicted location along Ganga (locally known as Kannauj, Figure

3.6C) through secondary sources (direct sighting and media reports). Considering all these evidences (n=32 unique occurrence points), we obtained a model accuracy of 84.37% after ground validation. Our validation surveys approximately expand the known range of swamp deer by 1125 km² along Ganga in the south.

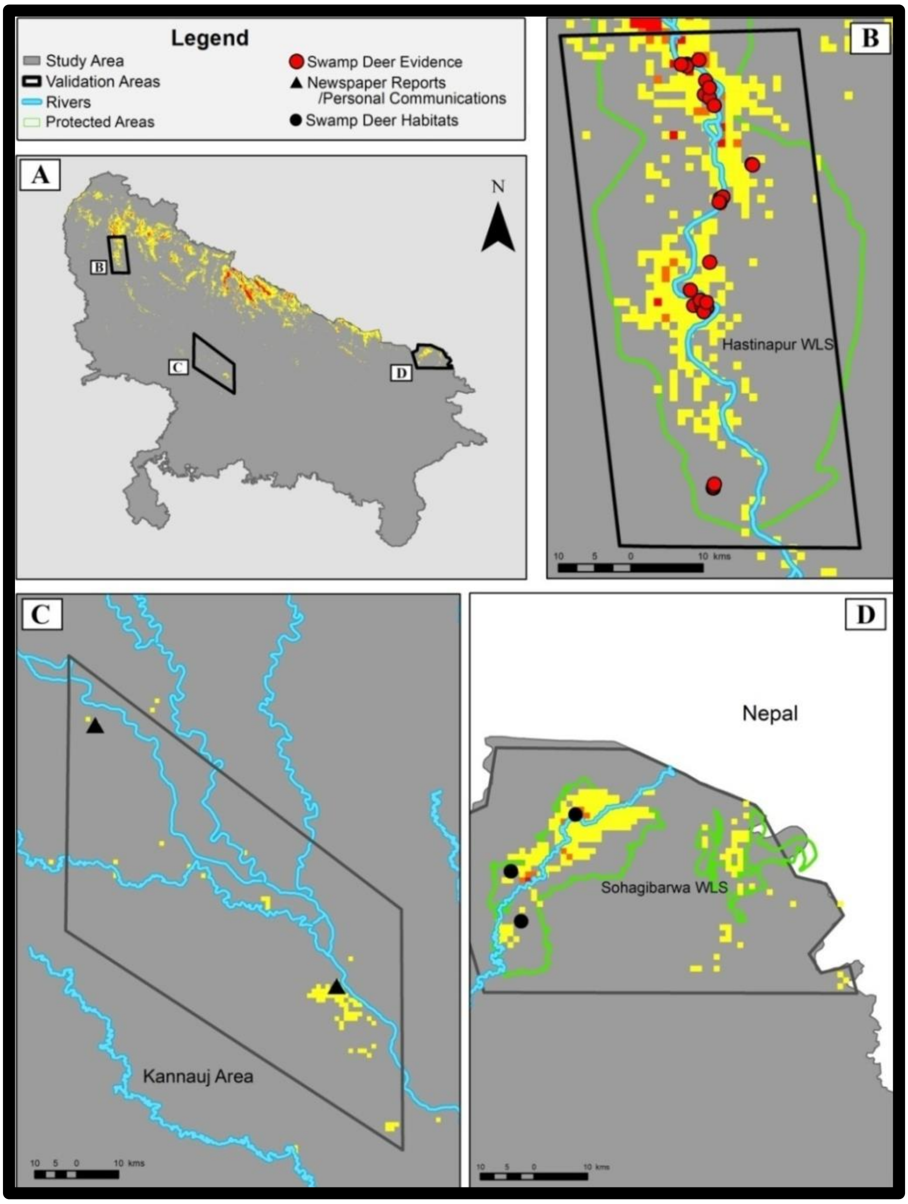

Figure 3.6 Map showing the swamp deer evidence locations within the surveyed areas predicted through MaxEnt models. Within the figure: (A) shows three selected validation areas (B) Validation area 1- Part of Hastinapur Wildlife Sanctuary below Bijnor Barrage (C) Validation area 2- Kannauj along Ganga (D) Validation area 3- Sohagibarwa Wildlife Sanctuary

3.4.4 Habitat status and disturbances:

Majority of the grasslands and its fauna within the protected areas of Sharda habitat block are undisturbed and receive proper protection. However, parts of Pilibhit Tiger Reserve and Katerniaghat Wildlife Sanctuary are facing anthropogenic disturbances like overgrazing and habitat conversion, and the non-protected areas in the Sharda block are experiencing rapid habitat encroachment (Table 3.4). The grasslands in Sohagibarwa Wildlife Sanctuary are protected, undisturbed and hold potential for future relocation of swamp deer.

Similarly, we found that the grasslands downstream of Bijnor Barrage in Hastinapur Wildlife Sanctuary (Ganga habitat block) are facing severe pressures from overgrazing, habitat conversion and occasional poaching (Table 3.4). The remaining habitats found downstream river Ganga are highly fragmented and future viability of swamp deer cannot be ascertained at this point.

Table 3.4 Details of swamp deer surveys in the Sharda habitat block, Sohagibarwa Wildlife Sanctuary and lower part of the Ganga habitat block during May 2016-April 2018. Information on block-wise area, habitat characteristics, direct and indirect evidences of swamp deer presence and disturbance records are presented for each area.

Survey blocks	Protected area/ Outside protected area	Major survey areas	Names of local area with swamp deer evidences	Survey timings	Habitat Characteristics	Direct sighting (n=occasion)	Carcass	Antler	Swamp deer confirmed pellet	Disturbance records
Sharda Habitat Block	Protected area	Pilibhit Tiger Reserve	Mala, Mohaf, Barahi,and Haripur Ranges, Lagga-Bagga	May 2016-April 2017	• Tracts of grassland dominated by *Phragmites* sp. along Mala river • Grasslands dominated with *Saccharum* spp. in Lagga-Bagga area near Sharda river	1	1	23	14	Minimal disturbance recorded
		Kishanpur Wildlife Sanctuary	near Ulla river, Jhaditaal, Jhadiphata		• Big wetlands comprising of *Typha* sp., *Phragmites* sp. and *Saccharum* spp.	1	0	5	7	No disturbance recorded
		Dudhwa National Park	Kakrahataal, Changanala, Chaprataal, Newelkharah, Bake taal, Bhaditaal, Nagrataal, Chibkura, Karmaihan, Madriyantaal. Bhadraulataal		• Big wetlands along Suheli river comprising of *Typha* sp., *Phragmites* sp. and *Saccharum* spp.	3	1	46	18	Large tracts of undisturbed habitats
		Katerniaghat Wildlife Sanctuary	Kereluaphata, Bara beily, Sijouli range		• *Phragmites* sp. dominated river islands along Ghaghra river	2	0	2	5	Some anthropogenic activities along Ghaghra river
	Non-protected area	South Kheri Forest Division	Sampurnanagar		• Human-dominated with interspersed grasslands and croplands	No	0	1	3	Livestock grazing, habitat encroachment and agriculture
		North Kheri Forest Divsion	Majgai range		• Wetland comprising of *Phragmites* sp. and *Typha* sp.	No	0	2	4	Some anthropogenic activities
Lower Gangetic Habitat	Protected area (Hastinapur	Bijnor Forest Division	Bijnor and Chandpur range	May 2017, April 2018	• Human-dominated area with croplands • Small river island patches dominated with *Saccharum* spp. • Small isolated wetland patch also present	No	0	1	19	Habitat encroachment and agriculture

Block (validation)	Wildlife Sanctuary)	Meerut Forest Division	Hastinapur range		• Small wetland area surrounded by human habitations • Wetland dominated with *Typha* sp. *and Saccharum* spp.	1	0	1	35	Livestock grazing and habitat exploitation
		Muzaffarnagar Forest Division	Jansat range		• Grasslands habitat interspersed with agricultural fields • Grasslands dominated with *Saccharum* spp. • Small fragmented swamps also present	No	0	1	16	Livestock grazing, habitat encroachment and agriculture
		Hapur Forest Division	Kopla Jheel, Garh range		• Small swamps of *Typha* sp. and *Phragmites* sp.	No	0	3	12	Livestock grazing, habitat encroachment
Predicted Eastern Habitat Block (validation)	Protected area (Sohagibarwa Wildlife Sanctuary)	Nichlaul and Maharajganj range	None found	March 2018	• Highly fragmented swampy habitat with *Typha* sp. • Human-dominated area with croplands	No	0	0	0	Habitat encroachment, agriculture and poaching

3.4.5 Priority Conservation Areas:

Based on the specific selection criteria (see section *3.3.4*), we identified four swamp deer "Priority Conservation Areas" covering both habitat blocks in this region (Figure 3.7-3.10; Table 3.5). Of these four selected areas, three (one protected and two non-protected areas) are in the Ganga habitat block, whereas one (non-protected areas) is in the Sharda habitat block. The details of these areas are as follows:

1. Priority Conservation Area 1: This is a ~193 km² area along Ganga between Jhilmil Jheel Conservation Reserve, Uttarakhand and Hastinapur Wildlife Sanctuary, Uttar Pradesh (Figure 3.7; Table 3.5). We quantified ~14 km² grassland habitat (~7% coverage) within this area with abundant evidence of swamp deer. The entire area is non-protected and acts as a potential migration route between the above-mentioned protected areas (see 1st chapter).

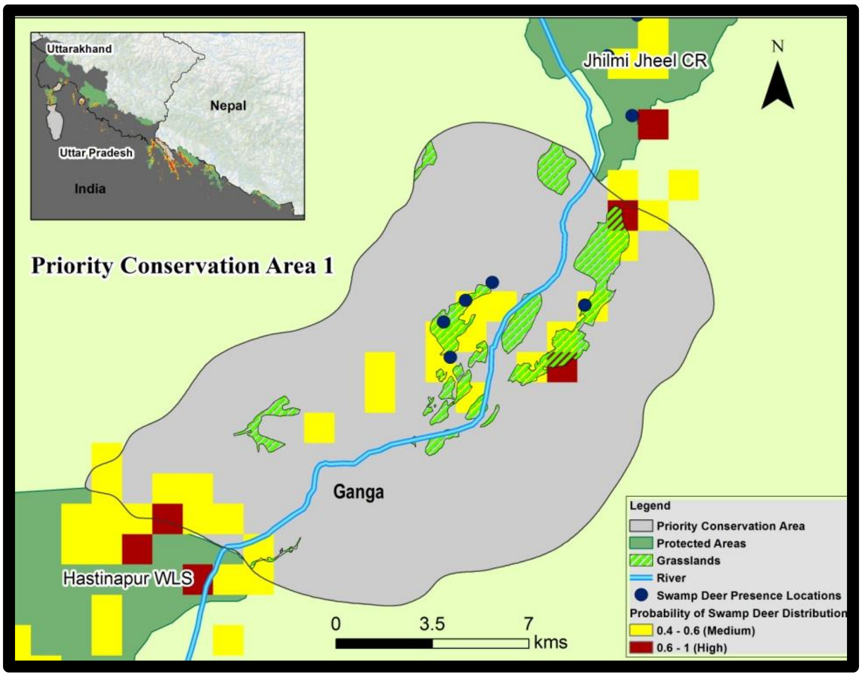

Figure 3.7 Priority Conservation Area 1: The stretch of Ganga between Jhilmil Jheel Conservation Reserve and Hastinapur Wildlife Sanctuary

2. Priority Conservation Area 2: This area covers the lower part of Hastinapur Wildlife Sanctuary downstream to Bijnor Barrage (1526 km²) (Figure 3.8; Table 3.5). This is a protected area with large human habitations within its boundary. We quantified ~73 km² grassland habitat (~4% coverage) with swamp deer evidence from different parts.

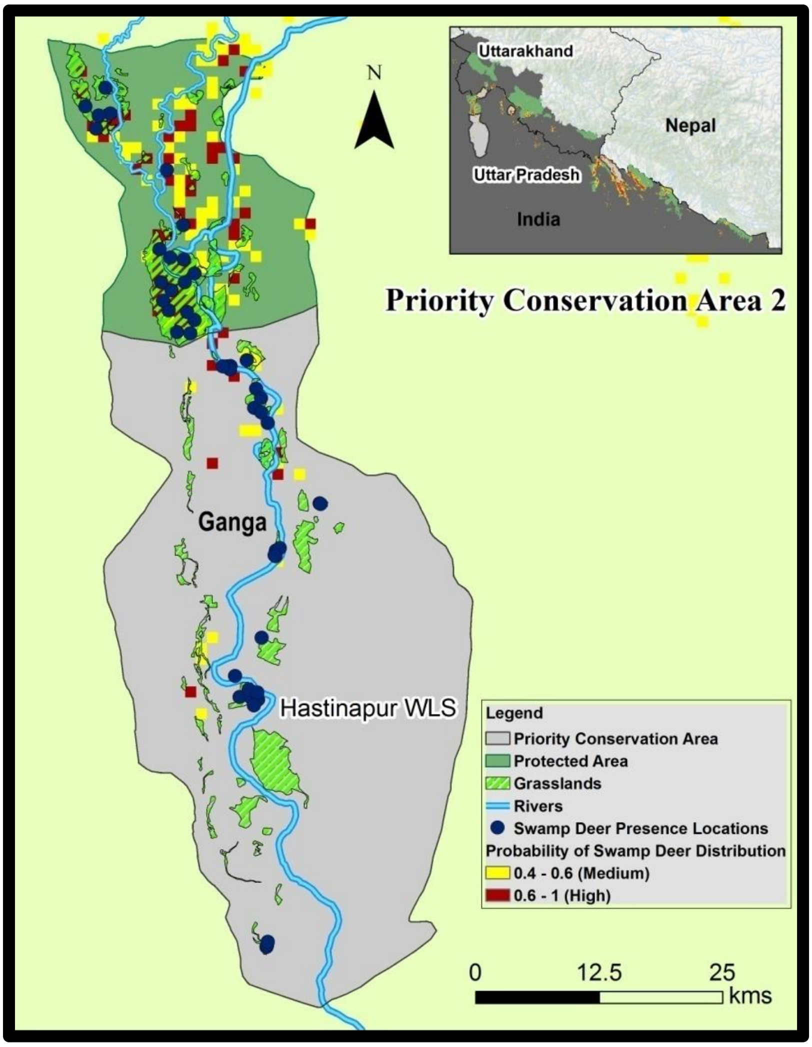

Figure 3.8 Priority Conservation Area 2: The lower part of Hastinapur Wildlife Sanctuary below Bijnor Barrage

3. Priority Conservation Area 3: This is an isolated, recently reported swamp deer population (see 1st chapter) along Ramganga river in Jamanpur, Afzalgarh, Uttar Pradesh (Figure 3.9; Table 3.5). This is a 139 km² area with ~3km² grassland habitat (~2% coverage) and retains a small population of swamp deer (see 1st chapter).

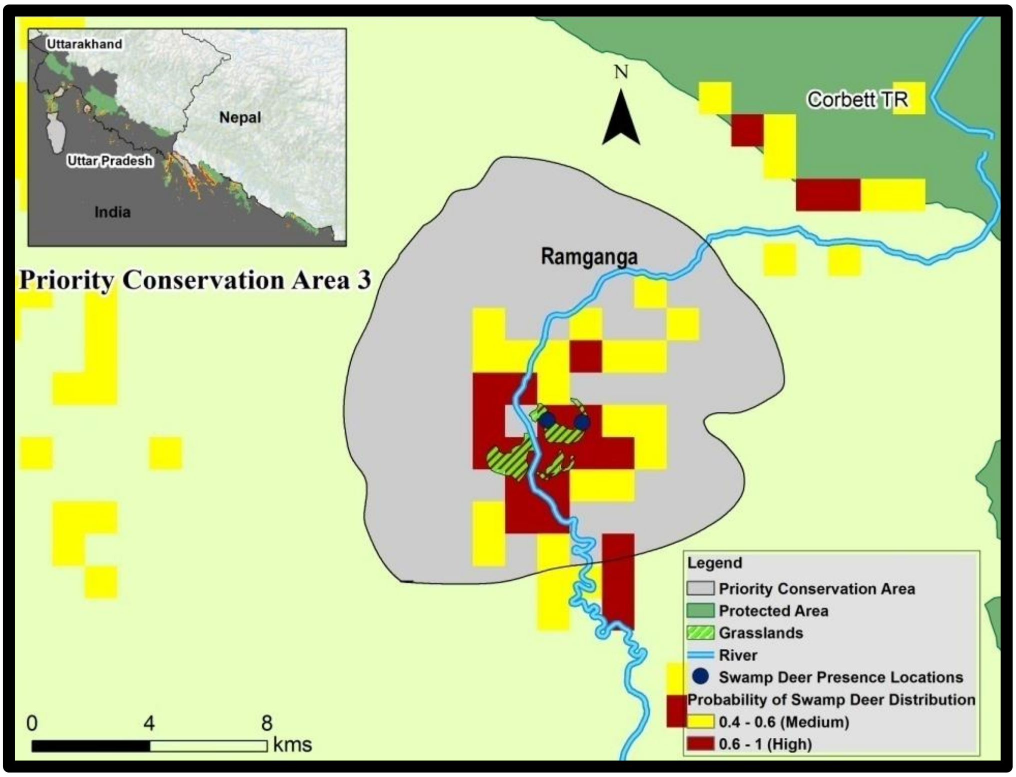

Figure 3.9 Priority Conservation Area 3: The isolated grassland area on the bank of Ramganga near Jamanpur, Afzalgarh

4. Priority Conservation Area 4: This is a 555 km² unprotected riverine landscape along Sharda river (Figure 3.10; Table 3.5). It retains ~136 km² grassland habitat (~24% coverage) and connects the Shuklaphanta Wildlife Sanctuary, Nepal with Kishanpur Wildlife Sanctuary, Uttar Pradesh, and possibly acts as the main corridor to connect the Nepal and north Indian population.

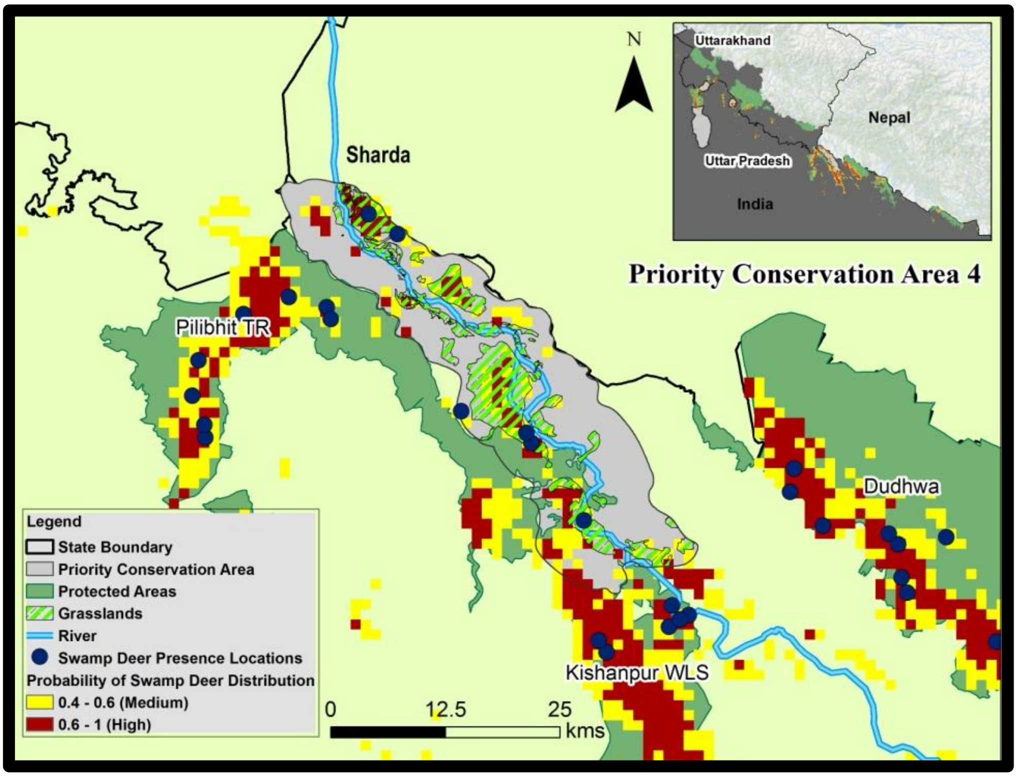

Figure 3.10 Priority Conservation Area 4: The unprotected area around Sharda river between Shuklaphanta Wildlife Sanctuary, Nepal and Kishanpur Wildlife Sanctuary, India

Table 3.5 Attributes of selection criteria for four identified "Priority Conservation Areas"

Priority Conservat-ion Area	**PCA area (km²)**	**Number of swamp deer evidences**	**Grassland coverage**	**Area covered by high and moderate prediction values**	**Strategic location importance**
Area 1	193	22	7%	13%	Connecting Hastinapur WLS and Jhilmil Jheel CR
Area 2	1526	89	4%	2%	Important protected area of Ganga block
Area 3	139	20	2%	26%	Isolated patch
Area 4	555	18	24%	26%	Connecting Kishanpur WLS in India to Shuklaphanta WLS in Nepal

3.5 Discussion:

In this chapter, we assessed fine-scale distribution of the northern swamp deer (*Rucervus duvaucelii duvaucelii*) using species distribution modeling and subsequent ground-truthing surveys across the subspecies' range in India. Compared to the Ganga habitat block, our surveys show that the Sharda block still retains considerably large and undisturbed patches of grassland habitat with reports of significant swamp deer population (Duckworth et al., 2015; Qureshi et al., 2004), thereby acting as a primary stronghold for the northern subspecies. During our surveys, we found large numbers of swamp deer evidence in Pilibhit Tiger Reserve, Kishanpur Wildlife Sanctuary and Dudhwa National Park, whereas fewer signatures from Katerniaghat Wildlife Sanctuary. However, the non-protected areas along the rivers harboring grasslands are experiencing a lot of disturbance and require urgent conservation attention. Anecdotal reports from some of these areas (Kishanpur Wildlife Sanctuary of India and Shuklaphanta Wildlife Sanctuary of Nepal) indicate that they are connected by regular swamp deer movements involving non-protected areas but the exact routes and patterns of habitat use are not known. Future studies should focus on using radio telemetry and genetic tools to understand these aspects. The Sharda habitat block is an integral part of the contiguous central Terai landscape and hosts the majority of the swamp deer population (Qureshi et al., 2004; Singh, 1978). It will be important to focus on estimating population size, assess critical viable habitats and corridors in this landscape for future conservation of swamp deer.

During this study, we have carefully designed the swamp deer distribution modeling and used relevant parameters based on the species ecology. Although it is known that species distribution modeling for habitat-specialists yields better predictions (Connor et al., 2018; Rhoden et al., 2017), our study design had a focal area-based modeling approach, accurate presence locations (primary data), ecologically meaningful covariates with a temporal correspondence to occurrence locations and appropriate spatial scale for prediction (Araújo and Guisan, 2006; Aubry et al., 2017; Beerkircher et al., 2009; Costa et al., 2015; Tye et al., 2017; Williams et al., 2012). It is essential to point out that we did not use species absence data during analyses as it was challenging to determine true absence from these mosaic habitats consisting of grassland and croplands. However, we think that for swamp deer this would not have any impact on the results as the species is only restricted within 5-10 km on either side of the rivers present in this region (data from 1st chapter). Overall, our model showed very accurate predictions (AUC value of 0.992), which is expected for a habitat-specialist species (Connor et al., 2018) (Figure 3.5). Finally, we conducted extensive validation surveys to confirm the accuracy of the distribution predictions by the models. Although most of the species distribution modeling studies result in identification of new areas of occurrences (Hernandez et al., 2006), the validations are primarily restricted to comparison of training data subsets with predictions (Fois et al., 2018; Pearson et al., 2007) and not on-field validations (Greaves et al., 2006; Jime'nez-Valverde et al., 2011).

Out of the 11 initial ecological/environmental covariates (eight in the final model) used, NDVI May, annual mean temperature, distance from water, nightlight and NDVI March were found to be the most important factors (~95%) in explaining swamp deer distribution (Figure 3.4 and 3.5). In the entire Gangetic plains, the croplands possess a lion's share of the Landuse Landcover in terms of area. Thus we chose NDVI May and NDVI March that represent the grasslands-cropland mosaics, where the March NDVI indicates indices of both vegetation types during the pre-harvesting period when it is difficult to differentiate them (thus overlapping NDVI values). However, during May, after harvestation of the crops (wheat and sugarcane) the same NDVI values are only represented by grasslands. Thus, NDVI May showed a strong positive relation with probability of swamp deer presence and was the highest contributor in explaining the species distribution. We used NDVI as a surrogate for grassland delineation in our analyses (comparatively lower accuracy) as detailed ground-truthed visual classification of the grasslands was not available. As a result, certain areas like Suhelwa Wildlife Sanctuary showed low prediction probabilities, though previous reports suggest absence of tall wet, swampy grasslands (Chanchani et al., 2014). The prediction has been driven majorly by distance to rivers and NDVI as the area has many rivers flowing through the sanctuary, but further surveys should be conducted to ascertain the current status.

Our validation surveys confirmed swamp deer presence in the lower part of Hastinapur Wildlife Sanctuary and identified only grassland habitats in Sohagibarwa Wildlife Sanctuary. During the surveys, we identified highly fragmented habitat patches with comparatively less swamp deer evidence below the Bijnor Barrage area of Hastinapur Wildlife Sanctuary along river Ganga. This area is highly human-dominated and needs immediate conservation attention for future survival of the species. There are sporadic reports of swamp deer presence further south along Ganga (Figure 3.6) and extensive surveys need to be conducted to ascertain viable habitat and population status. Within

Sohagibarwa Wildlife Sanctuary, we identified large patches of wet grasslands without any swamp deer evidence. Earlier studies reported swamp deer presence in this region during the 1960s (Ghimire et al., 2019) and given the protection status of this sanctuary, it can be a suitable area for potential reintroduction in future.

Finally, we identified four critical "Priority Conservation Areas" that demand immediate management attention for long-term survival of the species in this landscape (Figure 3.7-3.10). All of these selected areas are either corridor habitat (Priority Conservation Area 1 and 4, (Figure 3.7 and 3.10) or important populations that need careful management. Three of these habitats are in the Ganga habitat block, where habitat loss and other anthropogenic factors have resulted in extremely fragmented swamp deer habitats in recent times (see 1st chapter). The Priority Conservation Area 1 (between Jhilmil Jheel Conservation Reserve and Hastinapur Wildlife Sanctuary), is the only connecting habitat between these protected regions (Figure 3.7). Despite having unprotected status, this area has a grassland habitat coverage of ~7% which is similar to Jhilmil Jheel Conservation Reserve and Hastinapur Wildlife Sanctuary (3% and 7%, respectively) and retains swamp deer evidence. Swamp deer from Jhilmil Jheel Conservation Reserve are known to migrate seasonally (see 1st chapter) and most likely use this area to move downstream Ganga. However, this area currently faces a lot of human disturbances (see 1st chapter) and if these grassland habitats are lost, the two protected areas will become isolated. Conservation efforts should strongly focus on public awareness programs and community involvement through developing this area as a ''Conservation or Community Reserve'' (based on existing land use and land-tenure data) in near future to ensure long-term survival of this subspecies. However, in case of the Priority Conservation Area 2 (downstream to Bijnor Barrage area, (Figure 3.8) the challenges are different. This region is within a protected area (Hastinapur Wildlife Sanctuary) and thus the species status should be better here. A large part of the sanctuary (particularly downstream of Bijnor Barrage) is heavily human-dominated (including large villages and towns) and possibly cannot be reverted back to natural grassland habitats. We suggest further detailed, fine-scale mapping of this region to demarcate the human-dominated areas and rationalize the boundary to include only grassland habitats for focused habitat management. The third Priority Conservation Area is an isolated swamp deer population in the eastern border of the Ganga habitat block (Figure 3.9). This habitat patch is entirely surrounded by human habitats and needs urgent attention. This area is also the closest to the Sharda habitat block populations and future investigation should focus on understanding the historical connectivity between these two habitat blocks. Within the Sharda habitat block, we identified one priority area that is a critical migration corridor to connect the Nepal population with the Indian swamp deer population. While most of the swamp deer habitat in this block is within a number of protected areas and thus receive better protection (unlike the Ganga habitat block), this critical corridor is mostly unprotected (Figure 3.10). Given that this is the world's largest global intermixing population of swamp deer, protecting this corridor by converting this area as a ''Conservation or Community Reserve'' possibly will be the only way to maintain this metapopulation. If this corridor is lost due to human impacts, the Indian population might get genetically isolated from the Nepal population.

It is noteworthy to point out that the importance of these “Priority Conservation Areas” should not be misconstrued as purely based on grassland coverage/extent alone. The fragmented grassland habitats outside protected areas are easy targets for habitat conversion in this high human-dense landscape, and majority of the selected priority areas have low overall grassland coverages (~2-24% of the respective areas, Table 3.5). This can be argued against our suggestion of creating “Priority Conservation Areas”. However, these areas have critical importance when looked at swamp deer specific requirements (essential habitat for seasonal migration, feeding ground, fawning etc.) and they are essential to ensure long-term persistence of the species in this landscape. Thus, they need to be considered as critical parts of the entire landscape for swamp deer survival. According to recent assessments, grasslands are one of the most marginalized ecosystems in India (Report of the Task Force on Grasslands and Deserts, 2006), with a loss of ~20 million hectares area between 1880-2010. The ‘‘Green Revolution’’ movement of India has changed the agricultural practices, leading to fast decline in grassland habitats (Vanak et al., 2017) across north India. These remaining grassland patches are the last resort to the habitat-specialist biodiversity, and appropriate management interventions are required urgently to protect the ecosystem.

During the early 20th century, the swamp deer was found throughout the Indo-Gangetic plains (Duckworth et al., 2015) but they are currently considered to have the highest extinction probability among all Indian megaherbivores (Karanth et al., 2010). However, details of their population size and habitat status have been rather scanty due to logistical challenges in generating population-level information in human-dominated landscapes across the Gangetic plains. The result of this chapter provides a detailed insight of swamp deer distribution and highlights the importance of the riverine-grassland systems of northern India, where a considerable amount of biodiversity coexists with a very high human population (Uttar Pradesh ranks first among the states of India according to Census 2011). While all this new information is quite promising for the species, there is still a pressing need to focus on population enumeration and grassland management, particularly those present in the "Priority Conservation Areas" described here. Such efforts would also generate critical information for other co-occurring species (for example, hog deer, fishing cat, Bengal florican etc.) of the grasslands/wetlands and help in their conservation. We suggest using swamp deer as an umbrella species to strengthen habitat management and conservation for the otherwise marginalized grassland habitats in this landscape. This work also emphasizes the need for similar studies to generate critical information for other locally or regionally endemic, habitat-specialist species that occur outside protected areas for their conservation.

3.6 References:

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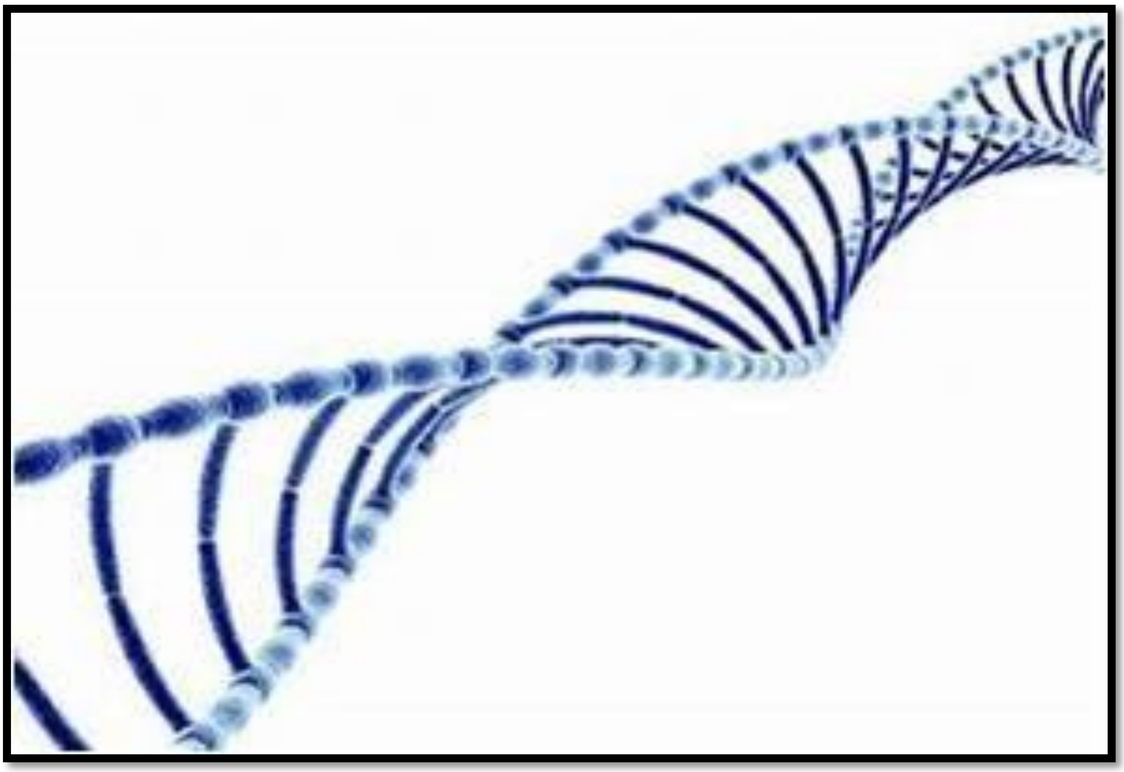

Chapter 3:

Genetic status and connectivity of swamp deer along river Ganga

4.1 Introduction:

Unprecedented habitat loss and fragmentation can have catastrophic effects on biodiversity (Novacek and Cleland, 2001). As expansion of human land use is occurring at such a rapid pace, most natural populations are likely to be negatively affected, specifically those residing outside protected areas (Craul et al., 2009; Epps et al., 2007; Fahrig, 2003). Along with reduction in total area of suitable habitat, landscape modification can lead to habitat fragmentation which splits populations into potentially smaller, isolated and non-functional units (Frankham et al., 2002; Frankham, 2006). This can result in reduced gene flow and movement which affects the survival potential of the population and enhancing its risk of extermination (Frankham et al., 2002; Keller and Waller, 2002).

Swamp deer is one such megaherbivore that has experienced rapid habitat fragmentation and loss in the last 100 years and is currently residing in isolated pockets in north, central and eastern India (Qureshi et al., 2004). The survey on northern swamp deer carried out in 2015-2016 showed much fine-scale presence of the subspecies than earlier known in the non-protected grasslands and surrounding areas along Ganga river between Jhilmil Jheel Conservation Reserve (JJCR) and Hastinapur Wildlife Sanctuary (HWLS) (see 1st and 2nd chapter). We also recorded a total of 91 fragmented grassland patches covering an area of 175.34 km^2 that is available to swamp deer. Further during our survey, we recorded evidences of ongoing habitat conversion that shed uncertainties over the future of this swamp deer population along Ganga. One fundamental question which needs immediate attention is the extent of present genetic connectivity of swamp deer among the fragmented grassland patches between JJCR and HWLS. Gene flow and movement are intricately related to each other and enhanced gene flow is often facilitated by unhindered movement (Doherty and Driscoll, 2017). A population with hindered movement and considerable inbreeding of individuals might result in clustering of related individuals to restricted areas (Doherty and Driscoll, 2017). As swamp deer were seen to be showing seasonal migration along Ganga, it was important to map the spread of highly related individuals in the landscape to get a qualitative indication about recent movement between Jhilmil Jheel and HWLS. However, generating such information for this elusive, grassland-living cervid at landscape level is difficult. Usage of techniques such as camera trapping become ineffective for swamp deer individual identification due to absence of unique morphological characteristics and physical tagging opportunities are limited due to logistical constraints, high expenditures and small sample size. In such a scenario, genetic methods have potential to generate population level information such as functional genetic connectivity (Pujolar et al., 2013), population size estimation (Mondol et al., 2009), reproductive fitness (Girman et al.,1997; Sillero-Zubiri et al.,1996), movement patterns (Epps et al., 2007; Gour et al., 2013) for supporting conservation of cryptic species living in a mosaic of protected and non-protected areas (Mondol et al., 2013). Microsatellite markers are the method of choice to assess the population genetic structure and population connectivity in natural and anthropogenic influenced populations (Manel et al., 2003). However, till date very limited studies on population genetic connectivity of swamp deer in the Ganga block have been carried out. A detailed assessment of landscape level genetic assessment can further help in evaluating the viability of this population and prioritizing effective future management strategy for the species across its distribution.

In this chapter, we describe a set of molecular markers to unambiguously identify individuals of swamp deer in the upper Gangetic plains of the northern part of India. Additionally, we have also developed species and sex identification markers for swamp deer and four other coexisting cervids (sambar, chital, hog deer, barking deer) in this landscape. Given the difficulties in faecal morphology-based species identification for overlapping body-sized animals (Costa et al., 2016; Hibert et al., 2008; Laguardia et al., 2015), it was necessary to develop a cheap, accurate and robust species and sex identification approach for these coexisting cervids. Our objectives in this study were, to (1) develop and validate species-specific markers and molecular sexing from a set of field-collected reference biological samples (tissue, antler and pellet) belonging to five cervid species; (2) test these assays on faecal pellets collected under a pilot northern swamp deer survey conducted in the upper Gangetic plains; (3) standardise a panel of microsatellite markers for unambiguous individual identification of swamp deer and finally; (4) use these microsatellite markers on samples collected from the swamp deer survey for assessing the genetic variation and inbreeding status of swamp deer in this landscape; (5) map spatial position of genetically related individuals to assess recent movement events in this area.

4.2 Study area:

The study area included Ganga and Sharda habitat block of the upper Gangetic plains of northern Indian states of Uttarakhand and Uttar Pradesh, covering a total of around 5000 km^2 mosaics of protected and non-protected areas (see in chapter 1 and 2). However, an intensive study area surrounding the Ganga specifically between JJCR and Garhmukteshwar, HWLS was selected for assessing the genetic status of swamp deer.

4.3 Methods:

4.3.1 Target species for species and sex identification:

The upper Gangetic plains covering the northern Indian states of Uttarakhand and Uttar Pradesh support an assemblage of cervid species (sambar, swamp deer, chital, hog deer and barking deer) (Johnsingh et al., 2004) which in spite of having ecological niche preferences, coexists in some areas. Swamp deer (vulnerable) and hog deer (endangered) have restricted distribution and are listed under "Schedule I", while sambar (vulnerable), chital (least concern) and barking deer (least concern) are widely distributed and listed as "Schedule III" under Wildlife Protection Act of India (1972).

4.3.2 Sample collection:

We collected a large number of samples from wild to standardize and test the novel species-specific markers for all target species. The entire sample collection procedure was divided into two phases. First, we surveyed specific areas in the upper Gangetic plains within Uttarakhand and Uttar Pradesh where each of the target species are found and opportunistically collected tissue, antler and pellet samples from selected areas. A total of 103 reference samples (swamp deer-22, sambar-19, chital-30, hog deer-26 and barking deer-6) have been collected in this phase. These samples were collected from following regions: Jhilmil Jheel Conservation Reserve (swamp deer, chital, sambar); Haridwar Forest Division (sambar, hog deer); Rajaji Tiger Reserve (sambar, chital); Lansdowne Forest Division (sambar); Ramnagar Forest Division (chital and barking deer) in Uttarakhand and Hastinapur Wildlife Sanctuary (swamp deer, hog deer); Pilibhit Tiger Reserve (hog deer); Dudhwa National Park (swamp deer); Katerniaghat Wildlife Sanctuary (hog deer) in Uttar Pradesh. In addition, we received one chital and barking deer tissue from Wildlife Institute of India's tissue repository, two hog deer pellets from Jaipur Zoo, Rajasthan and one barking deer pellet from Kalesar Wildlife Sanctuary, Haryana.

In the second phase, we used 737 probable swamp deer samples (477 pellets, 260 antlers) collected during survey from Jhilmil Jheel Conservation Reserve to Garhmukhteshwar area of Hastinapur Wildlife Sanctuary (described in 1st and 2nd chapters) for conclusive species identification using the assays developed in this study. Further we used these samples for analysing the genetic status of swamp deer along Ganga.

4.3.3 Molecular markers:

4.3.3.1 Species identification

We developed a novel species-specific gel-based assay to identify swamp deer and other co-occurring cervids in this landscape. To design species-specific primers, we downloaded complete mitochondrial genome sequences of all five target cervid species as references from GENEBANK (Swamp deer NC_020743.1, Sambar DQ989636.3, Chital DQ989636.3, Hog deer JN632600.1, Barking deer AF527537.1) and aligned with MEGA v6.0 (Tamura et al., 2013). These aligned sequences were screened visually and unique species-specific variations for all species were identified. Finally, primers were designed manually using the reference sequences, considering following criteria: (1) amplicon size of <300bp to assure amplification success from poor quality samples; (2) design multiple primers from widely-spaced mitochondrial DNA regions from each species to increase species ascertainment success and (3) vary amplicon sizes for two primers designed for same species to increase multiplexing capacity. A total of 15 primer pairs were designed for the five targeted species: four for swamp deer, three for sambar, three for chital, three for hog deer and two for barking deer.

4.3.3.2 Molecular sexing

We selected two earlier described sexing markers (SRY and Amelogenin genes) developed for sika deer (Takashi et al., 1998; Yamauchi et al., 2000) and standardized them as a multiplex system for molecular sexing of all targeted cervid species.

4.3.3.3 Individual identification

We developed molecular tools to identify swamp deer individuals from various types of DNA sources like tissue, antlers and pellets. So far, only one study had used individual-level information to look at phylogeography of swamp deer covering three subspecies in India (Kumar et al., 2017). However, to understand inbreeding status from a small and potentially inbred population, we need to select polymorphic nuclear markers that could differentiate among related individuals in the population. We standardised a panel which is capable of individual identification at landscape levels with high precision. The entire process involved marker selection, rigorous testing and standardisation before finalising a microsatellite panel for individual identification of swamp deer.

We initially screened a set of 48 microsatellites developed for red deer (Coulson et al., 1998). The markers were initially selected based on repeat motifs (di or tetra motif), allele size range and polymorphism in red deer and other species. First, we tested these markers on known swamp deer samples (2 tissues, 24 antlers and 18 pellets) to estimate amplification success and amplicon sizes in swamp deer. We adopted a cost-effective end-labelling of PCR products involving a fluorescently labelled universal primer in combination with locus-specific primers modified with universal M13 primer sequence tails to screen the final panel of microsatellites (Csencsics et al., 2010). We optimised these same markers to select the best ones based on the following criteria: i) marker polymorphism and heterozygosity; ii) amplification success; iii) ease in allele calling; iv) stable allele characteristics and v) multiplexing ability (Hoffman and Amos, 2005; Johnson et al., 2014; Linacre et al., 2011; Pompanon et al., 2005). Specifically, any primer sets with abnormal and inconsistent amplification profiles with multiple samples and long amplicon sizes were removed. While allele calling, we identified primers with low “relative fluorescence units” (RFU) (less than 100) and multiple peaks. These primers were rejected from further analyses as they can lead to erroneous allele calls (Hoffman and Amos, 2005; Pompanon et al., 2005). To finalise the multiplexing panels, we compared the peak characteristics of each marker in a multiplexing mode versus a single plexing one and any marker showing considerable deviations was discarded.

With the final selected panel, we altogether used 260 antler samples and 160 pellet samples (samples selected uniformly over the entire landscape) collected from the survey for determining genetic status of the species.

4.3.4 DNA extraction:

The DNA extraction method for different sample types (tissue and pellet) followed the protocol given in Mondol et al. (2013) with slight modification in case of antlers. All tissue samples were macerated using sterile blades and incubated overnight with 300µl of ATL solution (QIAGEN Inc.) and 25µl of Proteinase K (20 mg/ml), followed by spin-column extraction protocol of the tissue DNA kit. Final elution was performed twice using 100µl of 1X TE preheated to 70°C.

All field-collected antlers were cut around 2cm from the base using sterile saw blades and the powder was collected in sterile eppendorf tubes. Broken antler pieces were drilled using sterile driller and the powder was collected in eppendorf tubes. We weighed 20mg of the powder in an eppendorf tube and decalcified by incubating in 0.5M EDTA (pH 8) solution in an overhead rotor for two days (Gupta et al., 2012). The tubes were then centrifuged and the supernatant containing EDTA was discarded and lysis was performed at 56ºC with 40µl of Proteinase K (20 mg/ml) and 400µl of ATL solution (QIAGEN Inc.) for a week with intermittent vortexing, followed by spin-column extraction protocol of the tissue DNA kit. Final elution was performed twice using 100µl preheated 1X TE buffer.

All field-collected frozen pellets were thawed at room temperature and the top layer of each pellet was swabbed using sterile swabs (Himedia). The swabs were placed in sterile eppendorf tube and lysed with 300µl of ATL solution (QIAGEN Inc.) and 25µl of Proteinase K (20mg/ml) overnight. The lysed swab was discarded, and subsequent extraction was completed following standard spin-column protocol from the DNA tissue kit (QIAGEN Inc.). One negative control was included for each set of extraction (n=11) to monitor any possible contamination. All extracted DNA samples were stored in -20°C until further processing.

4.3.5 PCR standardization:

4.3.5.1 Species identification

All initial PCR standardizations were done using genetically confirmed samples (tissue, antlers and pellets) of all five target species. We generated mitochondrial Cytochrome b sequences (see 1st chapter) to confirm seven swamp deer, four sambar, four chital, five hog deer and three barking deer samples from the initial field-collected samples and tested all species-specific primers on this set of genetically identified reference samples. Following annealing temperature standardization for each species, PCR reactions were done for 10μl reaction volume using 4μl multiplex buffer (QIAGEN Inc.), 1μl of BSA (4 mg/ml), 1μl (2.5 μM) of primer, 2μl of 1:10 diluted DNA extracts and 2μl of RNAse free water. The PCR conditions included an initial denaturation (95°C for 15 min); 45 cycles of denaturation (95°C for 30 sec), annealing (T_a for 40 sec) and extension (72°C for 40 sec), followed by a final extension (72°C for 10 min). To track possible contamination, adequate negative controls were set up. To test the consistency of our primers, each species-specific primer was tested three independent times for all five species to monitor cross-species amplification. The amplified products were checked in 2% agarose gel. Any primer showing cross-species amplification was discarded. Post PCR standardization, PCR products were cleaned by Exonuclease (Thermo Scientific, Waltham, USA) and Shrimp Alkaline Phosphatase (Amresco, Solon, USA) mixture and sequenced using forward primers in an ABI 3500XL bioanalyzer (Applied Biosystems). All sequences were aligned in

MEGA v6.0 to visually examine any frame-shift and matched against GENEBANK database for species confirmation. If any species had more than one primer that provided species-specific bands, we standardized them as a multiplex system. Once PCR conditions for the primer sets were standardized using the reference samples, we amplified the selected species-specific primers with the remaining samples that were collected during the first phase of cervid reference sampling (swamp deer=15, sambar=15, chital=27, hog deer= 23 and barking deer=5). Post amplification, representative samples from each species were randomly selected (swamp deer=7, sambar=5, chital=12, hog deer=10 and barking deer=2) and were sequenced for species reconfirmation. Finally, we tested the swamp deer primers on all 737 field-collected samples from survey to check the large-scale field applicability of the assay.

4.3.5.2 Molecular sexing

For initial standardization we selected five tissue samples of known sex and four antlers (confirmed males) covering all target species. PCR reactions were performed in 10µl reaction volume using 4µl multiplex buffer (QIAGEN Inc.), 4µg of BSA, 0.25µM of primer mix and 2µl of 1:10 diluted DNA extracts. The PCR conditions included an initial denaturation (95°C for 15 min); 45 cycles of denaturation (95°C for 30 sec), annealing (56°C for 40 sec) and extension (72°C for 40 sec), followed by a final extension (72°C for 10 min). Negative controls were also included to check for contamination. The amplified products were checked in 2% agarose gel. Post temperature standardizations, we tested the sexing assay with two pellet DNA samples of each species. Finally, all field-collected samples that produced positive species-specific results were tested for sex identification. All samples were repeated three times to confirm the results and only samples with two same results were finally considered.

4.3.5.3 Individual identification

For standardization of microsatellites using M13 technique, PCR amplification was carried out in 10μl reaction volumes containing 4μl Qiagen multiplex PCR master mix (QIAGEN Inc.), 0.2μM forward primer, 0.1μM unlabelled reverse primer, 0.2μM M13 labelled universal primer, BSA (4mg/ml) and 2μl of the DNA extract. A negative control was always put to monitor contamination. The PCR reaction included an initial denaturation at 95°C for 15 min; 40 cycles of denaturation at 95°C for 30 sec, annealing at 57°C for 40 sec, extension at 72°C for 40 sec; final extension at 72°C for 20 min. PCR products were visualized in a 2% agarose gel and were pooled according to their size and label for genotyping. 1μl of the pooled PCR product was mixed with HIDI formamide and Liz 500 size standard and ran within an automated sequencer ABI3100XL (Applied Biosystems). The fragment lengths were scored manually using the program GENEMARKER (Soft genetics Inc., Pennsylvania, United States) (Holland and Parson, 2011). Each reaction was repeated three times for ensuring high accuracy. Once initial standardisation was done, the selected directly tagged microsatellite markers were multiplexed and used to generate data for the remaining samples. All samples that showed positive amplification in 50% loci during first PCR were repeated twice.

4.3.6 Data quality control for microsatellites:

Data quality is absolutely critical when working with low quality DNA samples (pellets). We adopted a selection process to minimise error after scoring for microsatellite allele with software GENEMARKER. We considered the following points for error reduction (i) all antler and pellet samples were genotyped thrice successfully at all loci, but those samples for which we failed to determine proper allele sizes for a selected number of loci (based on average amplification of no of loci/sample and $P_{ID(sibs)}$ value) were dropped from further analysis (ii) samples which showed fluctuations in genotypes in one of the three repeats were genotyped for a fourth time to validate. Finally, samples with identical genotypes in three distinct trials were considered for downstream analysis**.** After allele calling, we generated a "Quality index" protocol where identical calls in all three repeats were scored as "1" and if there was a mismatch in one of the repeats (amplification failure, allelic dropouts and false alleles), it was scored as "0" (Miquel et al., 2006; Modi et al., 2018). Quality index was calculated as summation of the three scores of each repeat divided by number of repeats. A value of 0.66 or more (at least two out of three repeats should have identical calls) was taken as a threshold for further downstream analysis. We calculated amplification success and false allele rates following Broquet and Petit (2004). We calculated Allele Dropout Rate (ADO) as the ratio between the observed number of amplifications in heterozygotes with one allele loss and the number of positive amplifications of the heterozygotes. We used the EM algorithm in FreeNA software to determine the null allele frequency (Chapuis and Estoup, 2007; Dempster et al., 1977).

4.3.7 Data analysis:

We used the CERVUS software to identify identical genotypes (recaptures) in our entire data set by comparing all the allele calls for all samples (Kalinowski et al., 2007). After removing all the recaptures, we calculated the $P_{ID(sibs)}$ for all the individuals using GIMLET (Valière, 2002). Alleles present in extremely low frequencies (less than 10% in the entire data set) were rechecked before final confirmation. ARLEQUIN was used for determining Hardy-Weinberg equilibrium and linkage disequilibrium (Excoffier et al., 2005).

4.3.7.1 Population structure detection

We used a Bayesian clustering approach implemented in program STRUCTURE 2.3.4 (Pritchard et al., 2000) to identify structuring of populations in our data. We ran STRUCTURE software 10 independent times for each cluster value (K) between 1 and 10. A correlated allele frequency model was run with a total of 450,000 iterations and a burn-in of 50,000. The optimal value of K was assessed using the "Evanno" method in STRUCTURE HARVESTER web version (Earl and VonHoldt, 2011).

4.3.7.2 Inbreeding and relatedness analysis

Assessment of inbreeding is crucial for a species living outside protected areas as they provide insight into the current genetic health and future viability of the population (Sweigart et al., 1999). We used ARLEQUIN to find out FIS or inbreeding coefficient. Further, we used program ML-RELATE (Kalinowski et al., 2006) to calculate maximum likelihood estimates of pair-wise relatedness and relationship categories between individuals from genotypic data. Relatedness (r) among individuals is measured by the fraction of alleles that are identical by shared descent. Common categories of relationships include first-degree relatives (r=0.5 as in case of parents or full siblings), and second-degree categories (as in case of half-siblings, r=0.25). Mean relatedness values can range from 0 to 1, where 0 implies no relatedness between sampled individuals (Blouin, 2003). We selected pairwise individuals with relatedness more than 0.6 and 100% amplification (reducing chances of any error) and plotted them on the map to see if related individuals have area specificity or not.

4.4 Results:

4.4.1 Validation of species-specific primers:

We designed 15 pairs of primers for five target cervid species and tested them initially on a set of 23 reference samples (Table 4.1). Out of all the markers only six primers (two for swamp deer and one each for sambar, chital, hog deer and barking deer) showed species-specific band patterns (Table 4.2; Figure 4.1). The remaining primers showed cross-species or non-specific amplifications during testing and thus were discarded. Sequencing of these products produced accurate results when matched against GenBank database for species identification. For swamp deer, testing both primers as multiplex assay always produced expected band patterns from reference samples and no cross-amplifications were found with any other species.

Table 4.1 Details of samples collected as references and from swamp deer field survey and the success rate for species and sex identification

Species	Sample Type	References & Test Samples	Swamp Deer Survey	Success Rate	*Samples for Sex Identification	Success Rate
Swamp Deer (n=759)	Tissue	3 (Reference= 1, Test= 2)	0	84.2%	3	84.16%
	Antler	5 (Reference= 1, Test= 4)	260		--	
	Pellet	14 (Reference= 5, Test= 9)	477		357	
Sambar (n=19)	Tissue	4 (Reference=1, Test=3)	--	84.12%	4	80%
	Antler	1 (Reference=1, Test=0)	--		--	
	Pellet	14 (Reference=2, Test=12)	--		11	
Chital (n=31)	Tissue	4 (Reference=1, Test=3)	--	93.55%	4	87.5%
	Antler	5 (Reference=1, Test=4)	--		--	
	Pellet	22 (Reference=2, Test=20)	--		20	
Hog Deer (n=28)	Tissue	3 (Reference=1, Test=2)	--	92.86%	3	77.27%
	Antler	4 (Reference=1, Test=3)	--		--	
	Pellet	21 (Reference=3, Test=18)	--		19	
Barking Deer (n=8)	Tissue	1 (Reference=1, Test=0)	--	75%	1	83.33%
	Antler	0	--		--	
	Pellet	7 (Reference=2, Test=5)	--		5	

*Samples for Sex Identification- Here only species identified samples were considered for sex identification

Table 4.2 Details of species-specific primers developed for five cervid species in this study

Species	Primers selected	mtDNA region	Sequence	Amplicon size	T_a
Swamp deer	2	D-loop	CATAGCCACAAGCTCTAG	107	50°C
			ATGAATATTATTGTAGGG		
		D-loop	ACATAACACATTTTATGCGC	123	50°C
			GTACTATAAATAATAGTATG		
Sambar	1	D-loop	AATCGCCCACTCCTTGTAGT	284	52°C
			AGGGGGGGGAAATATAGGTC		
Chital	1	D-loop	CCATGCTTATAAGCATGTACC	213	57°C
			AATAGCTACCCCCACAGTTT		
Hog deer	1	NADH subunit 2	CATCAATTGCCCACATAGGT	196	60°C
			GTAATGAGAATTAGGACAGTC		
Barking deer	1	NADH subunit 4	CAAGTCACTAATTGCATAC	171	52°C
			CTGTGGATTCGTTCATAGCC		

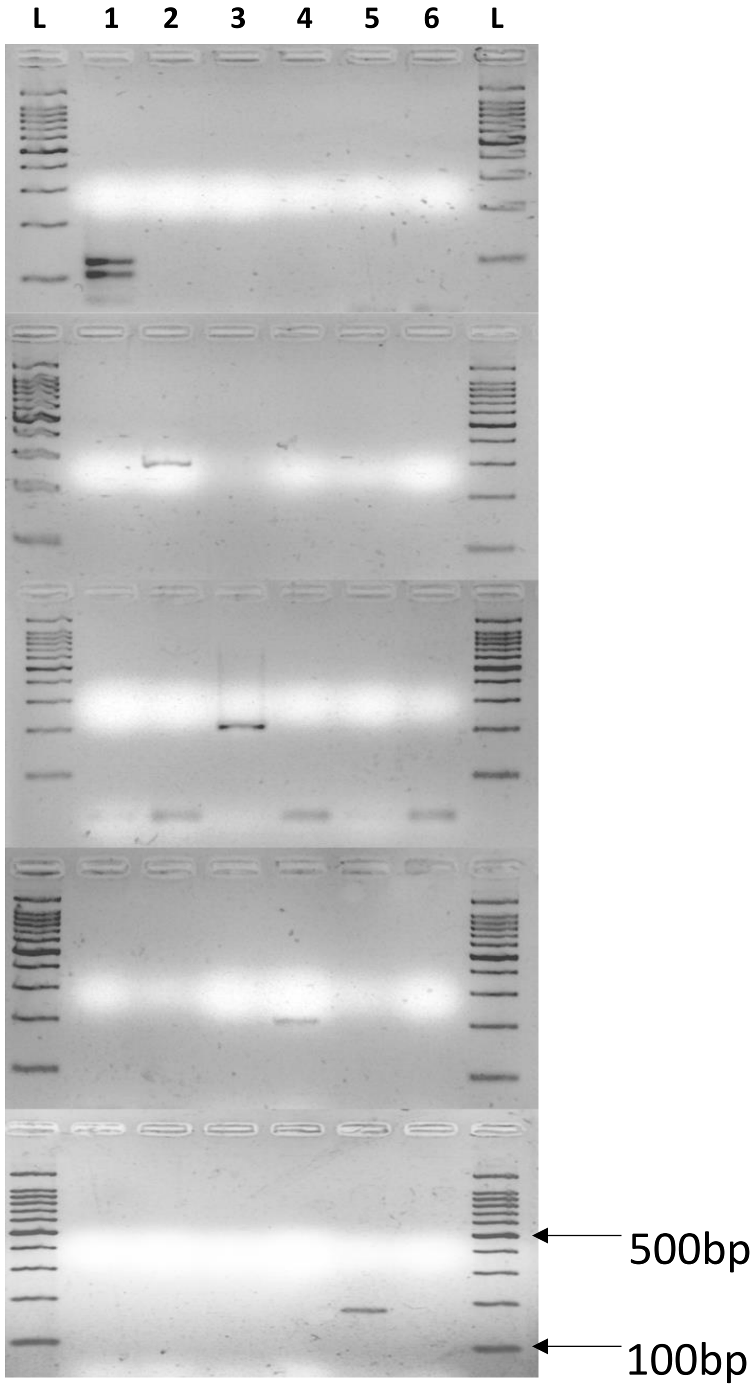

Figure 4.1 PCR amplification of species-specific primers for the five cervids. L: Ladder, Lane 1: Swamp deer (107 and 123bp), Lane 2: Sambar (284bp), Lane 3: Chital (213bp), Lane 4: Hog deer (196bp), Lane 5: Barking deer (171bp), Lane 6: Negative PCR, L: Ladder

Next, we tested these six primers with the remaining samples that were collected during the first phase of cervid reference sampling (Table 4.1). From all these samples collected as reference samples from both phases, species-wise success rates were found to be: swamp deer- 95.45% (n= 22), chital-93.55% (n=31), hog deer-92.86% (n= 28), sambar-84.21% (n=19) and barking deer-75% (n=8), respectively (Table 4.1; Figure 4.2A-B). Sequencing of representative 36 samples confirmed respective target species (GENEBANK accession No. MH04507-MH045150).

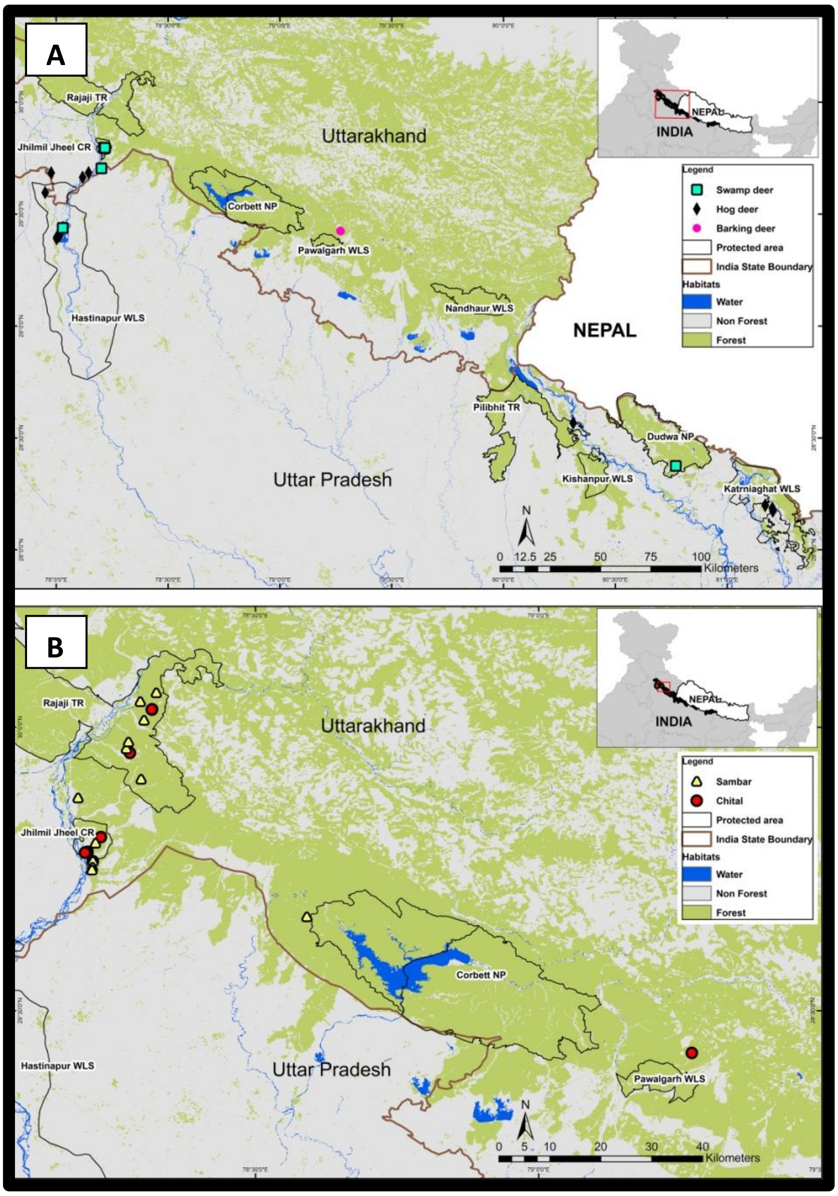

Figure 4.2 Locations of reference samples of all target species from the upper Gangetic plains of northern India: (A) Represents the sample locations of swamp deer, hog deer and barking deer (B) Represents the locations of sambar and chital

Finally, from all field-collected swamp deer survey samples (n= 737), 617 (83.71%) were successfully assigned to swamp deer using the species-specific multiplex assay (Figure 4.3).

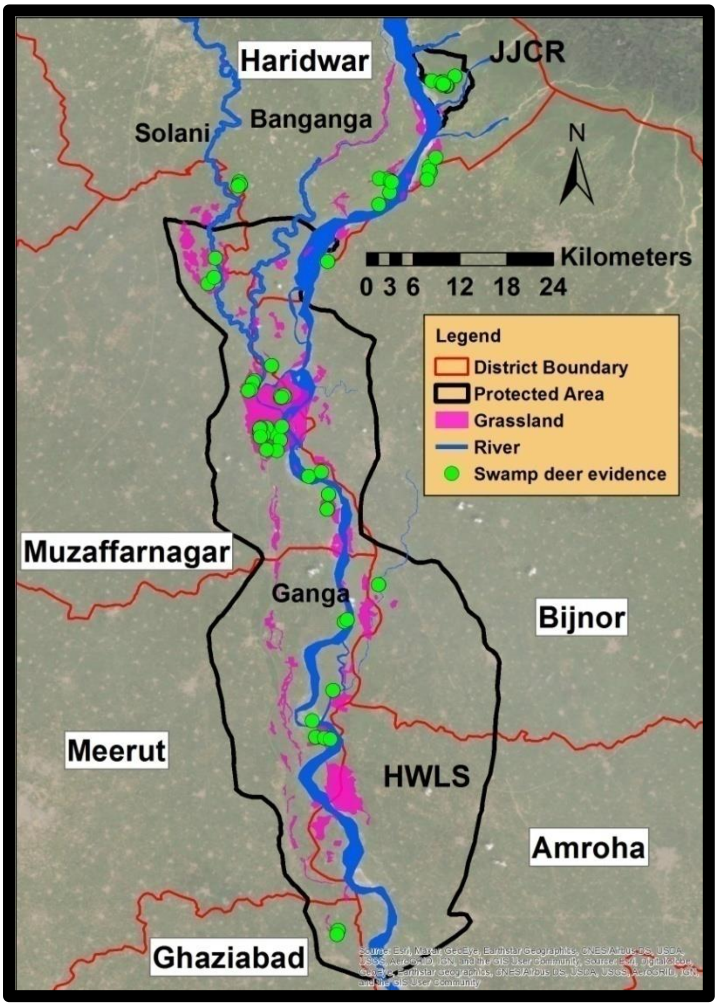

Figure 4.3 Locations of collected swamp deer evidences (antlers and pellets) during 2015-16 survey

4.4.2 Molecular sexing:

The sexing multiplex PCR showed a three-band pattern (two bands of 219bp and 165bp from Amelogenin and one band of 104bp from SRY) for males and a single band (219bp from Amelogenin) for females for all reference samples. Same band pattern was observed for all five species (Figure 4.4). Overall success rate for sex determination from all species identified samples was 82.5% (Table 4.1 for species-wise success rates). The male: female ratio was found to be 3:1 for all the swamp deer pellet samples.

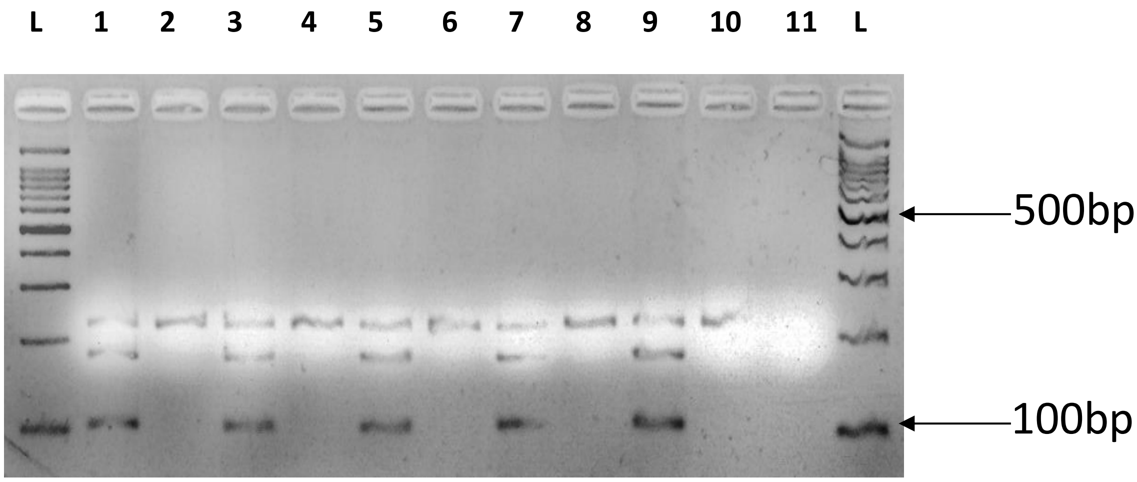

Figure 4.4 PCR amplication of amelogenin fragment (219bp and 165bp) and SRY fragment (104bp) for five cervid species. Lanes 1, 3, 5, 7, 9 are males, Lanes 2, 4, 6, 8, 10 are females. L: Ladder, Lane 1 and 2: Swamp deer, Lane 3 and 4: Sambar, Lane 5 and 6: Chital, Lane 7 and 8: Hog deer, Lane 9 and 10: Barking deer, Lane 11: Negative PCR, L: Ladder

4.4.3 Individual identification and genetic status:

After initial screening of 48 microsatellites, 16 loci were selected. Finally, when we screened additional faecal and antler samples, we found out inconsistent amplification of three loci Ceh 61, Ceh 64 and Ceh 76 and a final panel of 13 microsatellite markers was selected and used to generate data on 260 antlers and 160 pellet samples. Amplification success rates and levels of polymorphisms for these 13 loci were assessed after data validation using multiple repeats. Overall, the average amplification success was varying from 85.02% to 95.12% with average being 91.23%.

The overall mean frequency of null allele was found to be 0.06. We did not find any evidence of large-scale allelic dropouts in our data. The mean allelic dropout rate was 0.09 (range 0.03-0.18) and the mean frequency of false alleles for all the 13 loci was 0.08 (range 0.04-0.14). All the loci were moderately polymorphic with a mean of 6 alleles (varying between 2 and 13 alleles) and had expected and observed heterozygosity values of 0.60 and 0.51 respectively (Table 4.3). None of the loci deviated from the Hardy-Weinberg equilibrium and we found no strong linkage disequilibrium. Loci-wise summary of observed and expected heterozygosity, success rates, number of alleles, allelic size range and genotyping errors in the final selected panel are shown in Table 4.3.

Table 4.3 Summary statistics of 13 microsatellite loci used for swamp deer

Sl No.	**Locus**	**Success rate (%)**	**No of alleles**	**Allelic size range**	**H_E**	**H_O**	**Null allele**	**Allelic dropout rate**	**False allele rate**	**$P_{ID(sibs)}$**
1	CEH53	87.34	13	48	0.84	0.58	0.123	0.056	0.098	$3.43*10^{-1}$
2	CEH33	91.06	12	24	0.81	0.55	0.136	0.036	0.144	$1.23*10^{-1}$
3	CEH82	93.84	11	26	0.81	0.65	0.084	0.059	0.115	$4.43*10^{-2}$
4	CEH56	91.87	7	12	0.7	0.6	0.057	0.056	0.142	$1.92*10^{-2}$
5	CEH35	89.08	5	8	0.66	0.34	0.179	0.064	0.123	$8.85*10^{-2}$
6	CEH50	95.01	5	8	0.61	0.53	0.047	0.061	0.072	$4.41*10^{-3}$
7	CEH52	85.02	8	18	0.59	0.5	0.053	0.084	0.059	$2.25*10^{-3}$
8	CEH58	89.78	6	22	0.56	0.46	0.081	0.109	0.056	$1.20*10^{-3}$
9	CEH75	92.45	4	8	0.53	0.59	0.000	0.079	0.046	$6.79*10^{-4}$
10	CEH71	88.85	2	8	0.46	0.62	0.000	0.188	0.084	$4.20*10^{-4}$
11	CEH47	93.61	6	14	0.42	0.34	0.072	0.166	0.089	$2.62*10^{-4}$
12	CEH43	95.12	4	6	0.42	0.44	0.000	0.116	0.057	$1.67*10^{-4}$
13	CEH51	92.92	3	10	0.37	0.37	0.019	0.130	0.063	$1.12*10^{-4}$
Mean		91.23	6.62	16.31	0.60	0.51	0.065	0.093	0.088	

Based on data quality, we only considered samples that amplified in 10 of the 13 panel loci for individual identification. This threshold of 10 loci was decided based on the statistical support ($P_{ID(sibs)}$ value of 1 in 10000 siblings) produced by these loci. As maximum number of swamp deer in a reported population is about 2500 individuals, this value is statistically robust for individual identification in the Gangetic plains. Out of the total 420 field-collected samples tested with 13 markers, 305 samples produced ten or more loci data and were used in downstream analyses. Following analysis with CERVUS, we identified 273 (226 males and 47 females) unique swamp deer individuals from the entire data.

Exploratory analysis with programme STRUCTURE indicates presence of two distinct and one mixed genetic cluster (K=2, based on 10 independent runs) in this landscape. Our data reveals that although swamp deer populations between Jhilmil Jheel Conservation Reserve and Garhmukhtehwar have two distinct lineages, they are not spatially segregated. This result concludes that the swamp deer population between JJCR and HWLS is genetically connected and probably is a single population (Figure 4.5).

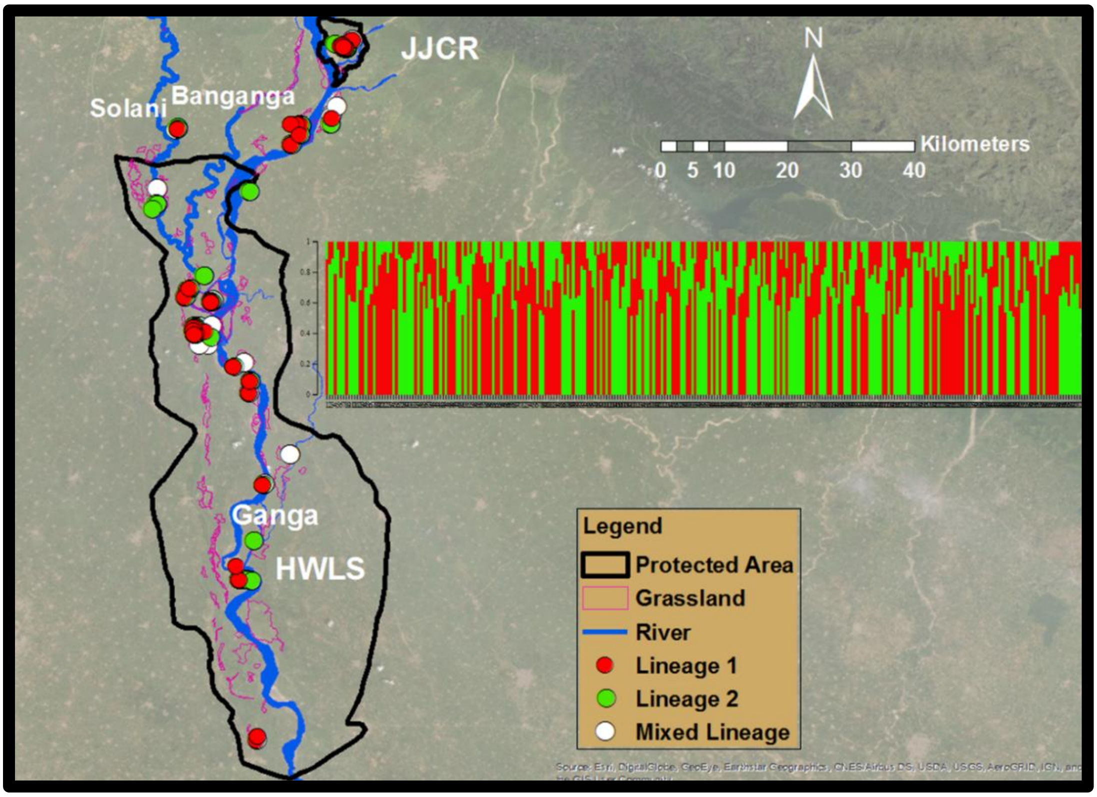

Figure 4.5 Swamp deer population structure analysed with STRUCTURE software using 13 microsatellite data. Number of genetic clusters (K=2), was determined with the Evanno method. The map shows spatial distribution of two distinct and one mixed genetic clusters in this landscape.

Overall, we found low average relatedness value of 0.07 (range 0.0001-0.93) in the entire landscape. When locations of related individuals were plotted on the map, the relatedness analysis showed that some related individuals (relatedness>0.6) are spread across the landscape at varying distances supporting the STRUCTURE data and indicating recent movement in this landscape (Figure 4.6). Overall, the FIS value (inbreeding co-efficient) was 0.099 indicating minimal inbreeding, probably maintained by genetic connectivity in this landscape.

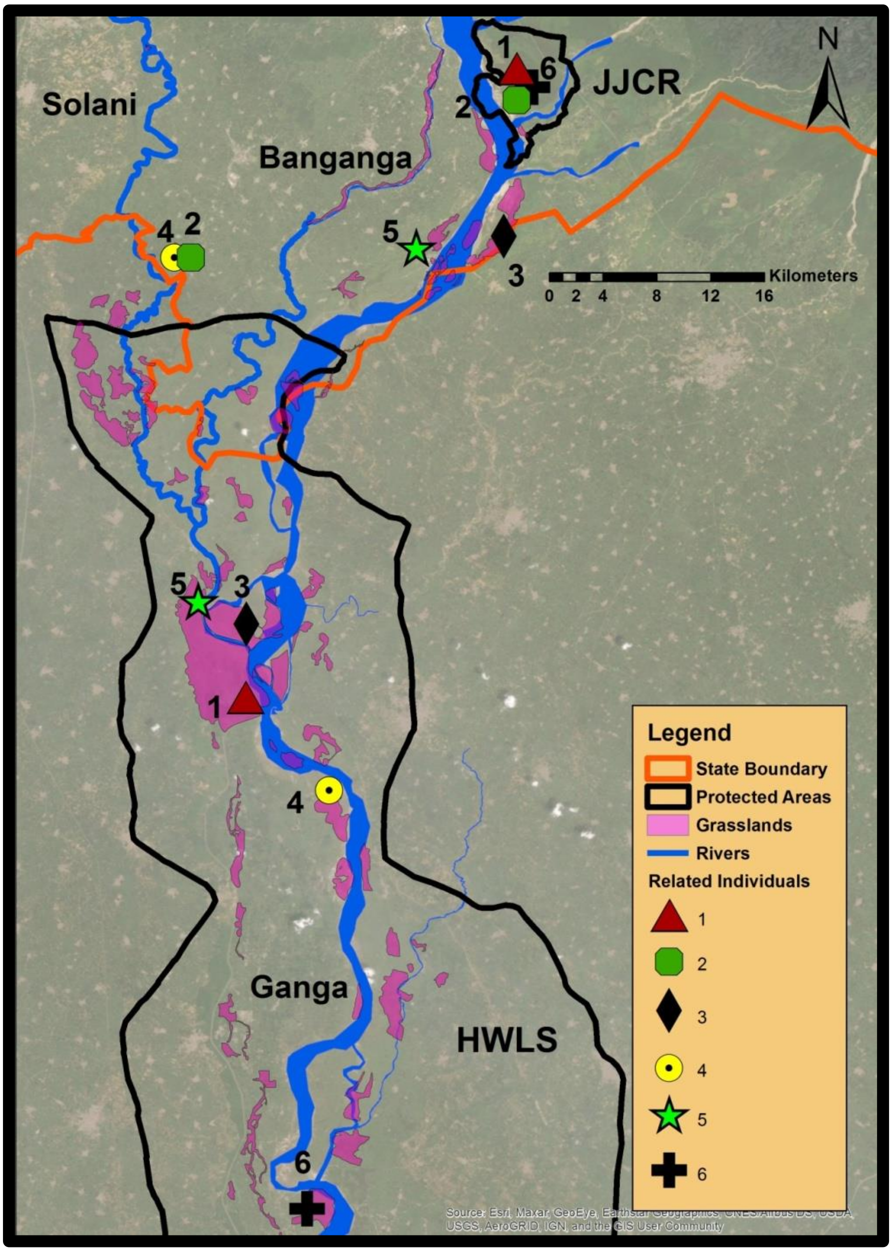

Figure 4.6 Distribution of related individuals (r>0.6) sampled in our survey. Each pair of related individuals (total six pairs) are indicated by same symbols.

4.5 Discussion:

We developed simple, fast and cost-effective species, sex and individual identification approaches for swamp deer. Species-specific and sex markers were standardised for a group of five coexisting cervid species and tested on field-collected samples from north Indian landscape. The selected mitochondrial DNA primers amplified only the target cervid species and showed no cross-amplification with others. The smaller amplicon sizes resulted in 75-84.2% and 77.27-87.5% success in species and sex identification, respectively, from field-collected samples of different target species. These approaches facilitate fast screening of large number of field-collected samples of various qualities, reduce species/sex misidentification and are cheaper than traditional sequencing or RFLP-based methods (Höss et al., 1992; Mukherjee et al., 2007; Palomares et al., 2002; Paxinos et al., 1997). Such molecular approaches are definitely advantageous for a group of coexisting species when compared to more error-prone faecal morphology-based species identification, where faecal dimensions are affected by overlapping body sizes (Laguardia et al., 2015). These approaches also have important forensic use for molecular identification of antler products (powder, pieces etc.) and solving species identification cases of illegal hunting that are prevalent in this human-dominated landscape (Harihar and Pandav, 2012).

To the best of our knowledge, this is the first study targeting species identification of five Asiatic cervid species with different distributional ranges. Earlier similar genetic tools have been used to study neotropical sympatric deer species (Costa et al., 2016; Duarte et al., 2016; Gonzalez et al., 2009; Woodruff et al., 2014) but no work on multiple coexisting deer species is done in sub-tropical regions of Asia. We were very careful while designing the species-specific molecular assays for multiple coexisting species with similar body sizes. For example, we designed multiple markers/species and used a large number of reference samples of different quality during the initial testing and standardization of the primers followed by sequencing confirmation of amplified products to ensure unambiguous species identification for all five species. For swamp deer, the multiplex species identification system was efficient in unambiguous species identification (84.2%). However, it is important to point out that all the target species have distribution outside our study area (upper Gangetic plains of northern India) and subsequent use of these primers to other regions would require similar standardization with samples from respective habitats due to possible population substructure. We strongly recommend using reasonably large number of reference samples before implementing these species identification markers in other regions for respective species.

Similarly, our two marker based multiplex sexing approach showed higher success rate and reduced rate of sex misidentifications or “false negatives” of males due to allelic dropout from Y chromosome from field-collected samples. This sexing approach showed an amplification success rate of 84.03% from poor quality faecal DNA samples (swamp deer), higher than other studies on various ungulate species (Oliveria and Duarte, 2013- 43.7%, Pelizzon et al., 2016- 64.4%). Positive results from all five target species suggest that this multiplex sexing PCR might be useful in other cervids also. However, addition of another X chromosome marker to this assay would make this assay more sensitive in sexing from poor quality DNA sources. We found a male biased sex ratio (3:1) while testing the sexing assay on faecal pellets of swamp deer. Earlier studies on other deer species (Oliveria and Duarte, 2013; Pajares et al., 2007; Sharma et al., 2015) also have shown similar male-biased population patterns for respective species in different parts of the world. Majority of our identified individuals were from antlers and this resulted in a sex-biased result. Future studies should focus on systematic sampling across the species habitat along with this molecular sexing assay to get valuable information on sex ratio at landscape level.

For individual identification of swamp deer from DNA samples, we adopted a microsatellite panel standardisation procedure that offers some advantages over other methods. M13 techniques allowed us to test 48 microsatellites and genotype them using only four universal tagged primers instead of 48 individual tagged primers. The rigorous testing of the loci using large number of DNA samples of varying quality (tissue, antler and pellets) also allowed us to omit problematic loci which showed in-consistent amplications. M13 technique has limitations in terms of multiplexing loci of overlapping size. Thus, final panel consisting of 13 markers was further standardized using individual forward labelled primers into four multiplex reactions which enabled us to shorten time and cost considerably. The overall genotyping error frequency was found to be <0.1 from swamp deer samples, which is below the threshold considered by other studies (Modi et al., 2018; Smith and Wang, 2014). The microsatellite panel standardised had a very low $P_{ID}(_{Sibs})$ value which gives it a high-resolution power of separating related individuals. We identified 273 unique individuals in the entire landscape using our 13 microsatellite panel. There is no information about the strength of the total population between Jhilmil and Garhmukhteshwar, but we assume we have been to sample at least 50-60% of the entire population. Our results provide us a rough idea that at least more than 300 individuals survive in the entire landscape. As animal counting in Jhilmil Jheel suggests a maximum of 170 individuals during May, this indicates prevalence of other resident populations of swamp deer in other parts of the landscape. Future radio-collaring studies and robust population count methods will shed some light on the population size of swamp deer along Ganga. Though our initial survey along Ganga revealed fragmented swamp deer habitats, genetic data is showing functional exchange of genes between these fragmented patches in this landscape. The STRUCTURE data clearly indicate presence of two distinct lineages and one mixed lineage but presence of all the lineages throughout the landscape indicates that there is connectivity between Jhilmil Jheel and Garhmukhteshwar area of Hastinapur Wildlife Sanctuary. The Ramganga population shows genetic similarity with the Ganga population. Whether it is connected to the Ganga population or has been recently isolated from it can't be inferred at the moment. When mapping highly related individuals, we only took only those individuals which had all 13 loci amplification to avoid erroneous conclusions. Presence of highly related individuals (>0.6) distributed across the landscape supports connectivity and recent movement. However, we did not find presence of related individuals among Ganga population and Ramganga population. Further sampling along Ramganga and the areas between Ganga and Ramganga will be crucial to understand the complete population dynamics in this region. As the Ramganga population lies between Ganga and Sharda habitat blocks, future investigation should focus on understanding the historical connectivity between these two habitat blocks. The level of inbreeding was not significant enough, probably maintained by connectivity in this area.

In summary, we present a set of molecular tools to carry out simple and quick species and sex identification for an assemblage of cervids along with individual identification for swamp deer in upper Gangetic plains of northern India. This landscape retains some of the most important grassland and wetland habitats in the subcontinent and is currently facing severe anthropogenic interventions in the forms of habitat conversion for agriculture, resource competitions with livestock, unregulated hunting etc. (Harihar, 2011; Harihar and Pandav, 2012). Such human-dominated habitats are refuge to a large number of species, but it is difficult to generate critical population-level information (for example, presence/absence, abundance, demography etc.) with traditional methods. For example, photographic captures and line transects are logistically difficult to implement (accessibility, theft, vandalism and visibility) in tall grassland habitats interspersed with human-dominated areas. This chapter demonstrates how molecular tools can help us gather crucial information at species/population level for a relatively understudied species. As all swamp areas have possibly small, seasonally fluctuating swamp deer numbers, their future survival will depend on their management as one metapopulation with stable demographic and genetic parameters. Current genetic status adds hope for the survival of swamp deer in such a challenging landscape. Even under such burgeoning human interventions, swamp deer continue to thrive in this human-dominated landscape. Further long-term genetic and radio-collaring study will be needed to get accurate information on landscape level population estimation, sex-ratio within the herds, signatures of genetic relatedness and movement events that could provide deeper understanding of swamp deer biology in a human-dominated landscape. Given the rate at which grasslands are diminishing in this landscape, such synergistic approach will be the key to long-term survival of swamp deer.

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Chapter 4:

Movement patterns of swamp deer in Ganga habitat block

5.1 Introduction:

Migration and seasonal movements are an integral part of a species life history and are vital for individual reproduction, fitness and survival (Stearns, 1992). Animal migration is increasingly becoming an endangered phenomenon as rapid urbanisation, habitat loss and fragmentation are resulting in disruption of species migration routes and resource utilisation patterns (Balme et al., 2009; Bolger et al., 2008; Both et al., 2006). This problem is even more acute if migration routes of the animals fall outside the realm of protected areas (Ojwang et al., 2017), where ecological constraints in the form of habitat or resource availabilities, landscape heterogeneity and human disturbance regimes are further squeezing the movement routes and putting animals on brink of extermination (Paton et al., 2017).

Swamp deer is one such mobile species which has been reported to show seasonal migrations (Martin and Gopal, 2015). In the upper Gangetic plains, there are anecdotal reports that the Jhilmil Jheel swamp deer population of Uttarakhand move out of the reserve into non-protected areas during the rainy season. They also have been sighted in the grassland patches along Ganga, downstream of JJCR by local communities. Our field observations in JJCR from January 2016 to July 2018 revealed that swamp deer start to congregate during late winter and numbers peak around May (Figure 5.1). Once the monsoon arrives, the animals start moving out of the area into unknown territories. The initial survey and later genetic analysis have revealed that fragmented non-protected grassland patches along Ganga between JJCR and HWLS still harbour swamp deer with genetic connectivity between them (see 1st and 3rd chapter). Genetic relatedness analysis indicates recent movement between the fragmented grassland patches. However, the exact movement routes and the fine-scale pattern of usage of grassland patches along Ganga remain unknown.

In this chapter, we analyse the movement patterns and habitat use of swamp deer along Ganga. Specifically, we identified the movement routes, estimated different movement parameters, habitat use, persistence in different habitats and directionality of movement in different areas of this non-protected landscape. These findings would be crucial in highlighting the importance of swamp deer habitats in non-protected areas and help in devising effective strategies to safeguard swamp deer and grasslands.

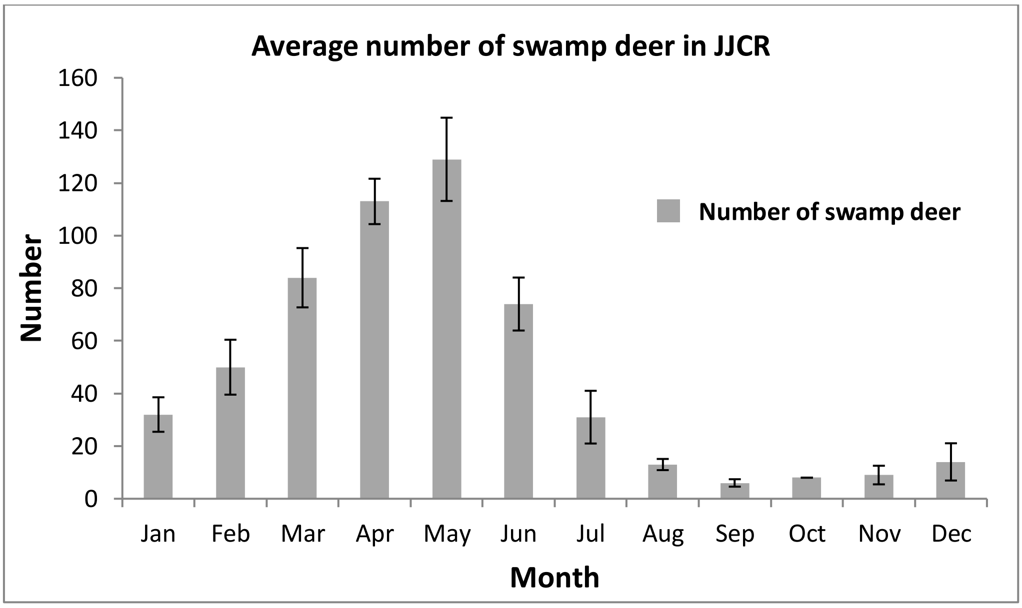

Figure 5.1 The average number of swamp deer in JJCR (2016-2018)

5.2 Study area:

The study area includes the grasslands along Ganga between JJCR and HWLS (see chapter 1, 2 and 3 for details).

5.3 Methods:

5.3.1 Capture and radio-collaring (Drive-net method):

We considered various methods for physical capture (Drop-nets, Drive-nets, Pop-up nets, Cannon-nets, Box traps, Clover traps etc.) and its associated procedures and risks (Schemnitz et al., 2009). Capture methods that are conspicuous (e.g., Drop-nets, Box traps, Clover traps) are susceptible to vandalism, specifically in disturbed areas. Other problems with some of these established capture techniques include difficulty in setting or moving traps in swamps (e.g., Drop-nets, Box traps), potential for the trap to damage multiple animals (e.g., Drop-nets, Net guns, Cannon-nets, Rocket-nets) and high risk of capture myopathy (e.g., Clover traps, Box traps) (Haulton et al., 2001; Peterson et al., 2003). Some of these capture methods can be expensive (e.g., Cannon-nets, Net guns, Clover traps) and lack the selectivity in animal capture.

Drive-net method is a technique for capturing and restraining free-ranging herding ungulates (Kock et al., 1987). In this case, camouflaged nets are put up at strategic positions covering a considerable distance. Then the animals are cornered and driven towards the net by a drive team. A restraining team remains hidden in the vegetation near the nets who starts restraining the animal once it hits the nets and gets entangled. Drive-net has been reported to be a safe and efficient method to capture wild ungulates with good success rates (López-Olvera et al., 2008).

Drive-net method for capturing swamp deer was narrowed down based on its successful and effective use in similar species elsewhere. Drive-nets had been effectively used in capturing swamp deer in Nepal (Youtube, 2016, *https://*www.youtube.com*/watch?v=gXwjemTw-Ng*), Saiga antelope in Mongolia (Berger et al., 2010), urban white-tailed deer in Florida (Locke et al., 2004) and mountain sheep (Kock et al., 1987). In the following section, we have discussed the necessary components of a physical capture operation (Drive-nets, physical deterrents, collars).

5.3.1.1 Drive-nets

We selected three drive-nets of 30m length each with mesh size of 4X4 inches and height of 10 feet and they were set up in a Phragmites patch used by the swamp deer. The nets were hoisted on bamboo poles (Figure 5.2A) positioned at six meter distance from each other. Around three feet of the net length was kept lying on the ground and remaining seven feet of net was hoisted on the poles. The nets were anchored on nail head on top of the pole. The efficacy of net to drop on impact was tested repeatedly. Five poles were used to hoist a single net (Figure 5.2B).

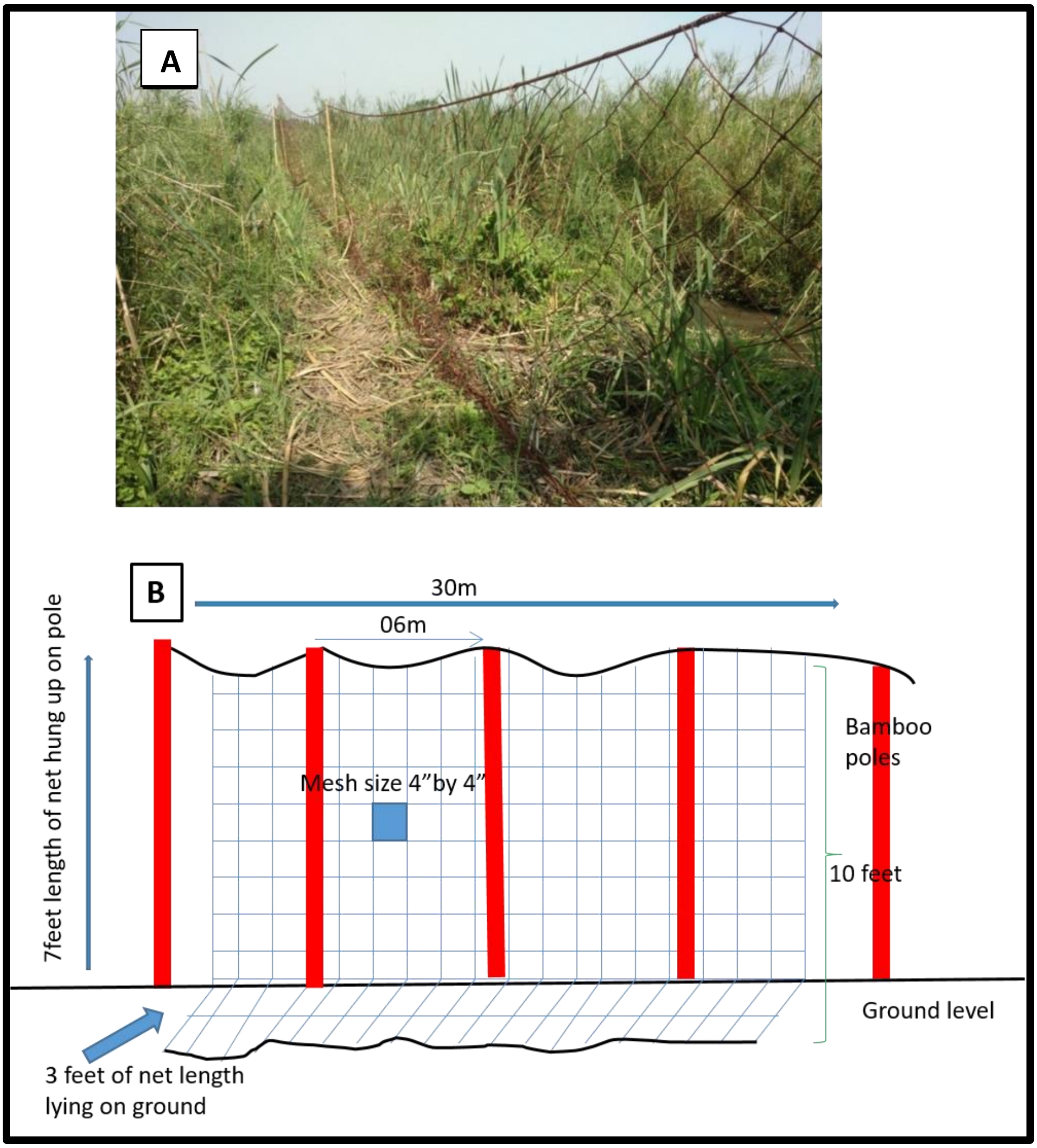

Figure 5.2 Physical capture method for collaring swamp deer: (A) Erected drive-net camouflaged within the Phragmites thicket (B) Graphical representation of drive-net system depicting length, mesh size and bamboo pole lengths

5.3.1.2 Cost-effective physical deterrents

As a physical deterrent to stop animals running haywire, a cost-effective locally made barrier was set up. The periphery of the entire ground (length 3.2 km) starting from the two sides of the nets was covered using the cost-effective barriers. The barriers were comprised of torn pieces of around 700 gunny bags attached to the 3.2 km rope (Figure 5.3A-B).

Figure 5.3 Physical deterrents used in swamp deer capture: (A) Deterrents being made using gunny bag fragments (B) Deterrents deployed in the field to cordon off all the exit routes for swamp deer during drive-net capture operation

5.3.1.3 Collars

In our study, we used Vertex Plus GPS collars which can provide data at a fine-scale temporal resolution. It had a VHF sensor and was coupled with external sensors and UHF ID Tags. All GPS, activity, temperature, proximity and external sensor data measured by the collar were stored in the non-volatile onboard memory. These collars had an option of Iridium two-way communication through which activity, mortality and temperature data were stored with every GPS fix.

5.3.1.4 Field operation

The collaring procedure involved initial monitoring of the resting and foraging grounds and temporal activities of swamp deer. Prior to collaring exercise, dry drives using humans and goats were attempted in the nearby village to see the efficacy of the nets. Finally, on 22nd May and 30th June we successfully captured two hinds using the drive-net method (Figure 5.4A). The animals were restrained and were immediately collared with GPS satellite collars (Figure 5.4B-C). During the entire operation, we monitored the physiological parameters and they were found to be within normal limits. The individuals were blindfolded and administered with mild doses of tranquilizer to minimise the stress. Biological samples (blood and hair) were collected and stored for further analysis. The swamp deer were released from the entanglements of the net and administered with reversal drugs. The radio-signals of the deployed collars were checked for proper functioning.

Figure 5.4 Collaring of swamp deer in JJCR: (A) Animal restrained by team of experts (B) and (C) Animal blindfolded and kept in contact with water to avoid stress and hyperthermia during fitting of collars

5.3.2 Post collaring data analysis:

5.3.2.1 Movement route and basic movement parameters

A high-resolution data at two hour interval enabled us to calculate basic parameters which characterise the movement pattern at a fine-scale. A *trajectory* is a record of sequence of locations travelled by an animal. We plotted the entire path trajectories of the two collared animals individually to get an overview of their moving patterns and movement routes in the landscape. Step length (distance between consecutive locations), speed, net displacement (Euclidian distance between the starting and the final point of the trajectory), maximum linear distance, migration type are some of the primary parameters that accurately convey pertinent information about the movement biology and behavioural states of a particular animal (Dodge et al., 2008, Edelhoff et al., 2016; Patterson et al., 2008).

We created trajectory paths and calculated step length, displacement, speed, total distance using ArcMET (Arc-GIS 10.2.2) and Microsoft Excel. The movement type was identified using the net squared displacement method in Excel (Bunnefeld et al., 2011).

5.3.2.2 Home range estimation

Brownian bridge movement model (BBMM) estimates the path of an animal's movement probabilistically from successive pairs of locations taking into account the time between locations, the distance between locations and the Brownian motion variance that is related to the animal's mobility (Horne et al., 2007). A BBMM unlike Kernel Density Estimator (KDE) assumes that locations are not independent. Using the BBMM, we estimated home ranges of two collared swamp deer during the entire period of GPS data records (May 2018-July 2019). The BBMM requires (1) sequential location data (2) estimated error associated with location data and (3) grid cell size assigned for the output utilization distribution. We selected a grid cell size of 30m for the analysis. Using the BBMM package (http://cran.opensourceresources.org), we calculated BBMM home range in the R software (Horne et al., 2017). We prepared 50% BBMM and 95% BBMM to represent the core area of use and the standard home range size, respectively (Fischer et al., 2013) and plotted them on the Land Use Land Cover (LULC) images (categorised into a) cropland; b) grassland; c) waterbody; d) forest; e) scrubland; f) settlement) of our study area along Ganga.

5.3.2.3 Habitat selection and use

Habitat selection procedure based on the availability can be defined at the level of landscape/study area (First-order selection), the home range of an individual animal or group (Second-order selection) and usage of specific components within the home range (Third-order selection) (Buskirk and Millspaugh, 2003; Johnson, 1980; Krausman, 1999). Fine-scale habitat preference assessment can be analysed using Ivlev's electivity index (Ivlev, 1961; Jhala et al., 2009). The Ivlev's index is calculated as:

Ivlev's electivity index = (u-a)/ (u+a)

where, u= proportion of GPS points in a particular LULC class (use); a= proportion of a particular LULC class available (availability)

The electivity value ranges from -1 to +1 whereas -1 signifies habitat avoidance and +1 indicates exclusive use, while 0 represents habitat used in proportion to availability (Ivlev, 1961; Kong et al., 2018). In our case, a total of 8790 GPS data locations from two radio-collared female swamp deer during the period of May 2018-July 2019 spread out between JJCR and Bijnor Barrage area, HWLS were used for habitat selection. Firstly, we plotted the proportion of points in each LULC class to get a qualitative idea of habitat selection (habitat availability not considered). For quantitative habitat selection, we considered the 1st and 2nd order selections. For the 1st order selection, we defined availability of a particular habitat as proportion of that habitat in the landscape between JCCR and Bijnor Barrage, HWLS i.e. covering area up to five km on either side of Ganga, Banganga and Solani. The landscape area for the 1st order was selected based on the collared animals' movement routes and ability to disperse (data from spread of related individuals), For 2nd order selection, we considered proportion of a particular habitat within 95% BBMM and 50% BBMM home ranges as availability. For particular habitat use, the proportion of radio telemetry locations in that habitat within the landscape (1st order) and home ranges (2nd order) were calculated in ArcGIS 10.2.2. The habitat preference was analysed by the Ivlev's index in Microsoft Excel. We also plotted the proportional use versus availability of different LULC classes (specifically grassland, cropland and waterbodies) at the three scales (landscape, 95% BBMM and 50% BBMM) in Microsoft Excel.

5.3.2.4 Temporal continuity of trajectory paths in different habitats

In a landscape where there are different types of LULC present, it is critical to evaluate how the swamp deer utilises its time in different habitats. To understand this, we conducted a trajectory path continuity analysis where all the successive temporal points can be connected if it is in a single habitat i.e. the particular swamp deer individual persists in that habitat (Figure 5.5A). However, the landscape around Ganga has prime habitats in patches (grasslands) and suboptimal habitats/habitats (presumably waterbody/croplands used as transit) situated intermixed with prime habitats. In such a scenario, temporally isolated points might occur in suboptimal habitats/habitats which swamp deer sporadically access (Figure 5.5B). In ArcGIS 10.2.2, we extracted the data locations of collared individuals covering each habitat type. Using the trajectory path tool in ArcMet (ArcGIS 10.2.2), we created trajectory movement paths and evaluated the proportion of points that could not be joined temporally (isolated points) in each habitat class (Wall, 2014).

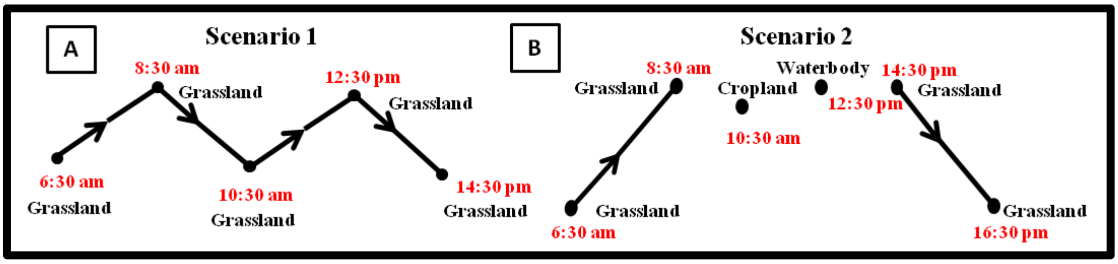

Figure 5.5 Two hypothetical scenarios for depicting temporal continuity of movement trajectories in single habitat: (A) Temporal continuity maintained in grasslands (B) Temporal continuity in grassland disrupted due to presence of isolated points in other habitats (croplands and waterbodies)

5.3.2.5 Assessment of directionality in movement patterns in this landscape

In a fragmented landscape, the animal movement patterns might show varying directionality on a spatial and temporal scale. High directionality values are characterised by high speed and low turning angles i.e. the animal moves in a straight line. Velocity grid algorithm summarizes movement trajectories in order to visualize and quantify directionality, fine-scale patterns of movement and speed statistics at the landscape-scale and identifies spatially clustered regions of identical movement behaviour in cartographic form. This provides the different space use patterns of a species in a human-dominated landscape (Wall et al., 2013). We generated velocity dot products (indicator of directionality) at 500m resolution grid scale in ArcMET (velocity grid tool) (Arc-GIS 10.2.2) using formula

$$\frac{\sum_{i \neq j}^{N} |\cos \theta_{ij}|}{\sum_{0}^{N-1} n}$$

where, ϴij is the angle between track segment j and segment i and the summation of the absolute cosine value is made over N segments.

The average dot product value ranges between 0 to 1 and describes the alignment of the animal tracks, where 0 signifies undirected movement and 1 signifies directed movement. The directionality and speed of collared individuals were calculated throughout the landscape as well as within each raster grid cell. The vector polyline consists of a single line emanating from the centre of each grid cell in the direction of the mean heading (Wall, 2014). For each grid, the length of the vector was scaled according to mean speed within that grid. The colouring of the vectors indicates similarity in track direction as calculated using the mean of the absolute value of track dot products (red = low; green = high). The grids were plotted on LULC maps to get an idea of fine-scale patterns of directionality in different areas of this landscape.

5.3.2.6 Group dynamics and activity pattern of swamp deer outside the protected areas

We intensively monitored the movement of swamp deer after radio-collaring. As the areas outside Jhilmil Jheel are non-protected, we wanted to generate baseline data on group dynamics as well temporal use of these human-dominated areas.

There was difficulty in sighting swamp deer inside the tall wet grasslands as accessibility to these areas during monsoon was limited. Based on the core area usage of the two collared swamp deer, we deployed cameras in surrounding accessible areas in three sessions between July 2018 and June 2019 where activity and group dynamics were monitored (376 trap nights). We analysed temporal activity of humans and swamp deer by Oriana software 2.0 (Gómez-Ortiz et al., 2019) and the temporal activity overlap with CamTrap package implemented in R software (Niedballa et al., 2016). Significance in temporal activity difference between swamp deer and human was assessed using Watson U2 test (Rubio et al., 2017).

5.4 Results:

5.4.1 Movement routes and basic movement parameters:

Two collared swamp deer were extensively followed and tracked for 14 (May 2018-July 2019) and 11 months (June 2018-May 2019) (Table 5.1). The path trajectories of both the collared individuals were computed and mapped for the entire period (Figure 5.6A-B). Individual 1 after being collared in Jhilmil moved along Ganga and settled in the non-protected grasslands of Uttarakhand-Uttar Pradesh border, 14 km downstream of Jhilmil Jheel (locally known as Amichand) (Figure 5.6A, 5.17). It also frequently used the grasslands of river islands situated two km further south of Amichand (Figure 5.6A, 5.17). Net squared displacement analysis showed that this animal had "mixed migratory" movement type, where the animal did not return back to its original area but a nearby area (Figure 5.7A). Individual 2 after being collared in JJCR, crossed the Ganga and halted at multiple grassland patches before finally reaching a human-dominated grasslands patch (locally known as Sukhapur) situated around 28km downstream of JJCR on the border of HWLS (Figure 5.6B, 5.17). On the onset of winter, in early 2019 it made its journey back to Jhilmil Jheel, thus showing a "migratory" movement type (Figure 5.7B). The maximum linear distance travelled, average step length, speed and other parameters for both the collared individuals are summarised in Table 5.1.

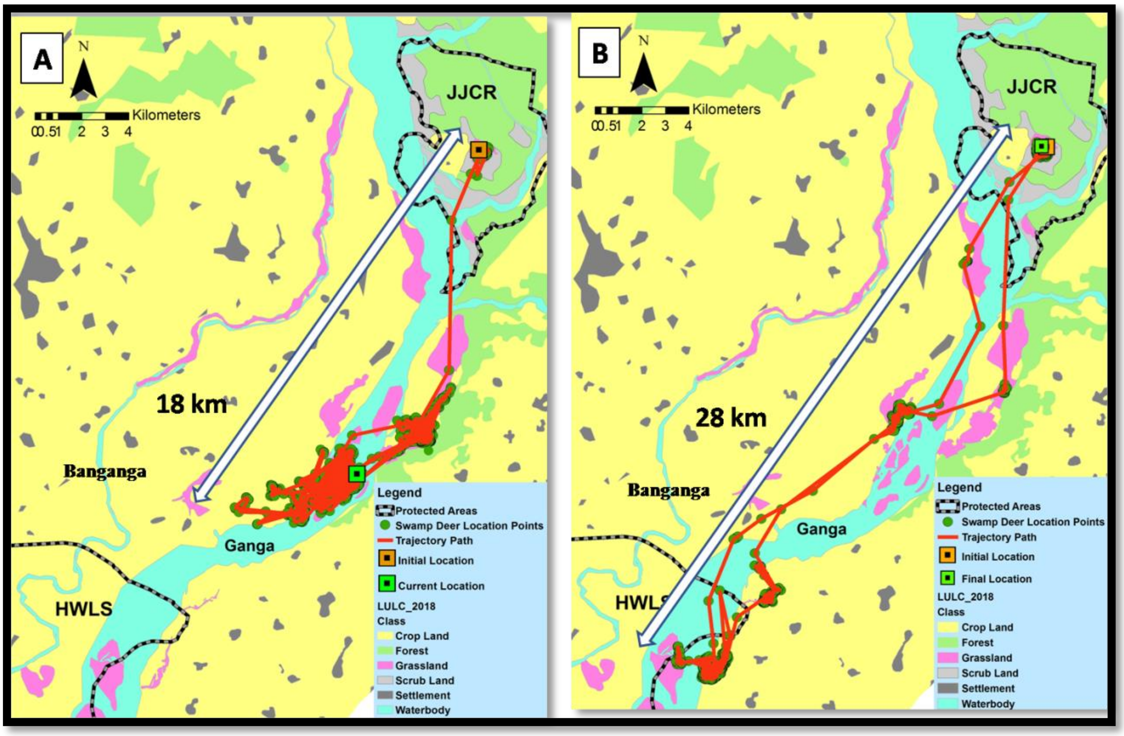

Figure 5.6 The movement routes of both the collared individuals: (A) Route of individual 1 over a period of 14 months (May 2018-July 2019) (B) Route of individual 2 over a period of 11 months (June 2018-May 2019)

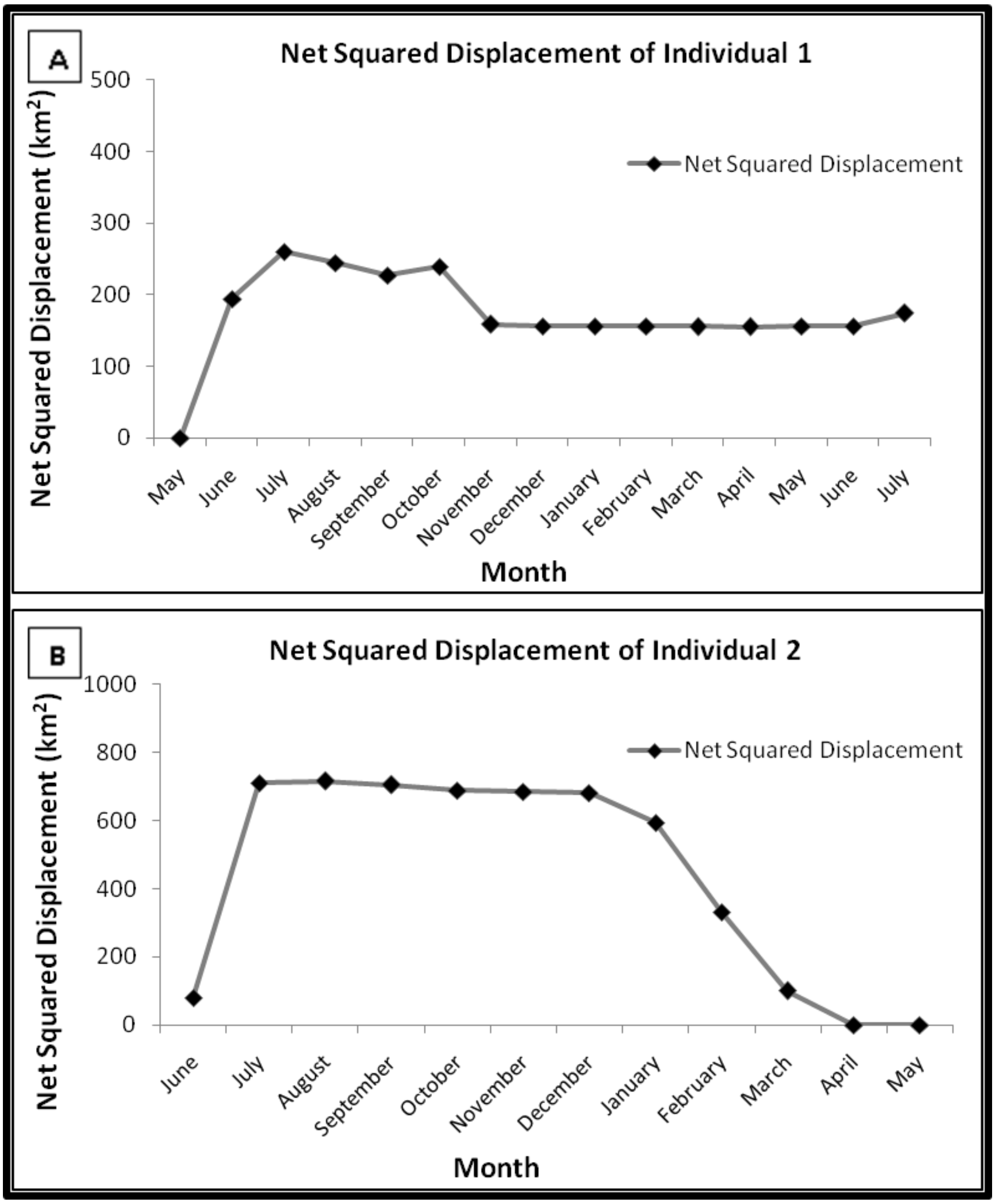

Figure 5.7 Demonstration of movement type through Net Squared Displacements: (A) Individual 1 showing "Mixed Migratory" movement type (B) Individual 2 showing "Migratory" movement type

Table 5.1 Basic movement parameters calculated for the two collared individuals

Movement Parameters	**Individual 1** (tracking duration- 14 months)	**Individual 2** (tracking duration- 11 months)
Mean step length (km)	0.117	0.082
Mean speed (km/hr)	0.058	0.040
Maximum linear distance travelled from starting location (km)	18	28
Total distance (km)	584.3	309.3
Total displacement (km)	15.2	0.227
Migration Type	Mixed migratory	Migratory

5.4.2 Home range estimation:

For individual 1, BBMM analysis revealed a 95% usage area of 8.22 km^2 and 50% usage area of 0.58 km^2 (Figure 5.8A). The 2^{nd} collared swamp deer showed a 95% and 50% usage area of 12.33 and 1.47 km^2 respectively (Figure 5.8B). Within the 95% BBMM for both the animals, grassland had a spatial coverage of 32% followed by croplands and waterbodies. But within 50% BBMM home range, spatial coverage of grasslands was significantly higher (around 62%). The size of the core home ranges and spatial coverages of different LULC types within 50% BBMM suggest that swamp deer is majorly restricted to fragmented small grassland patches along the Ganga.

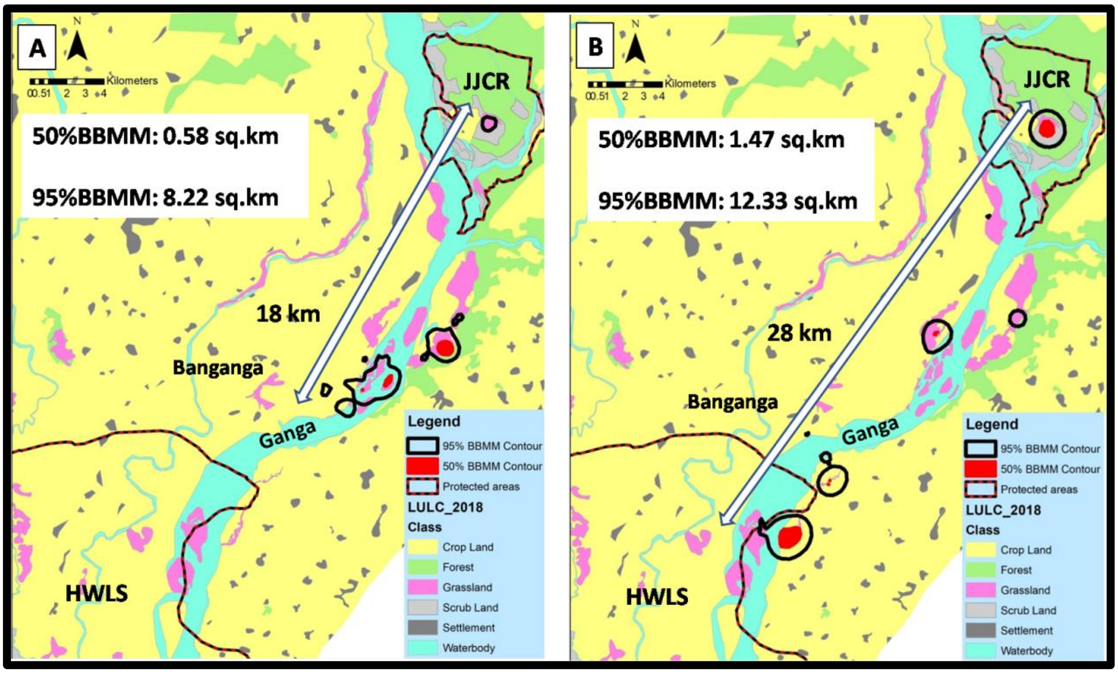

Figure 5.8 BBMM home ranges (95% and 50%) of collared individuals. The home ranges have been plotted on a LULC map to showcase intensive usage of different habitats: (A) Individual 1 (B) Individual 2

5.4.3 Habitat selection and use:

When we plotted the proportion of raw points falling within each LULC class type, we observed that 80% of the GPS points of both individuals fell on grasslands (Figure 5.9).

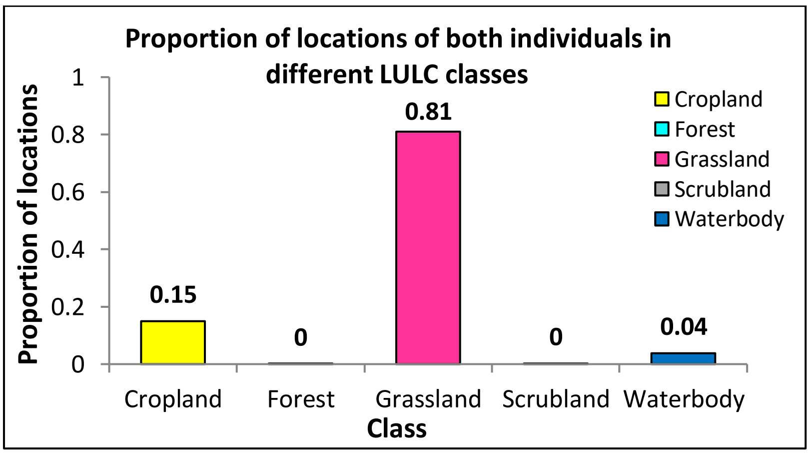

Figure 5.9 Proportion of locations of both collared individuals in different LULC classes

Ivlev's index analysis suggested that both the animals preferred grasslands (positive values) under the three scales of analysis (landscape level, 95% and 50% BBMM) (Figure 5.10A-B). Interestingly, the value of Ivlev's index for both the individuals was highest for the landscape level followed by 95% and 50% BBMM home range levels. Forest, scrublands and waterbodies were actively avoided. However, croplands were also avoided though their Ivlev's index value suggests sporadic use. When we plotted the proportion of use versus availability of different LULC classes (grassland, cropland and waterbodies) in three different scales, we obtained similar results. The proportion of grassland usage was more than its availability in all the scales of analysis, but at landscape level, the use was highest compared to its availability (Figure 5.11A-B).

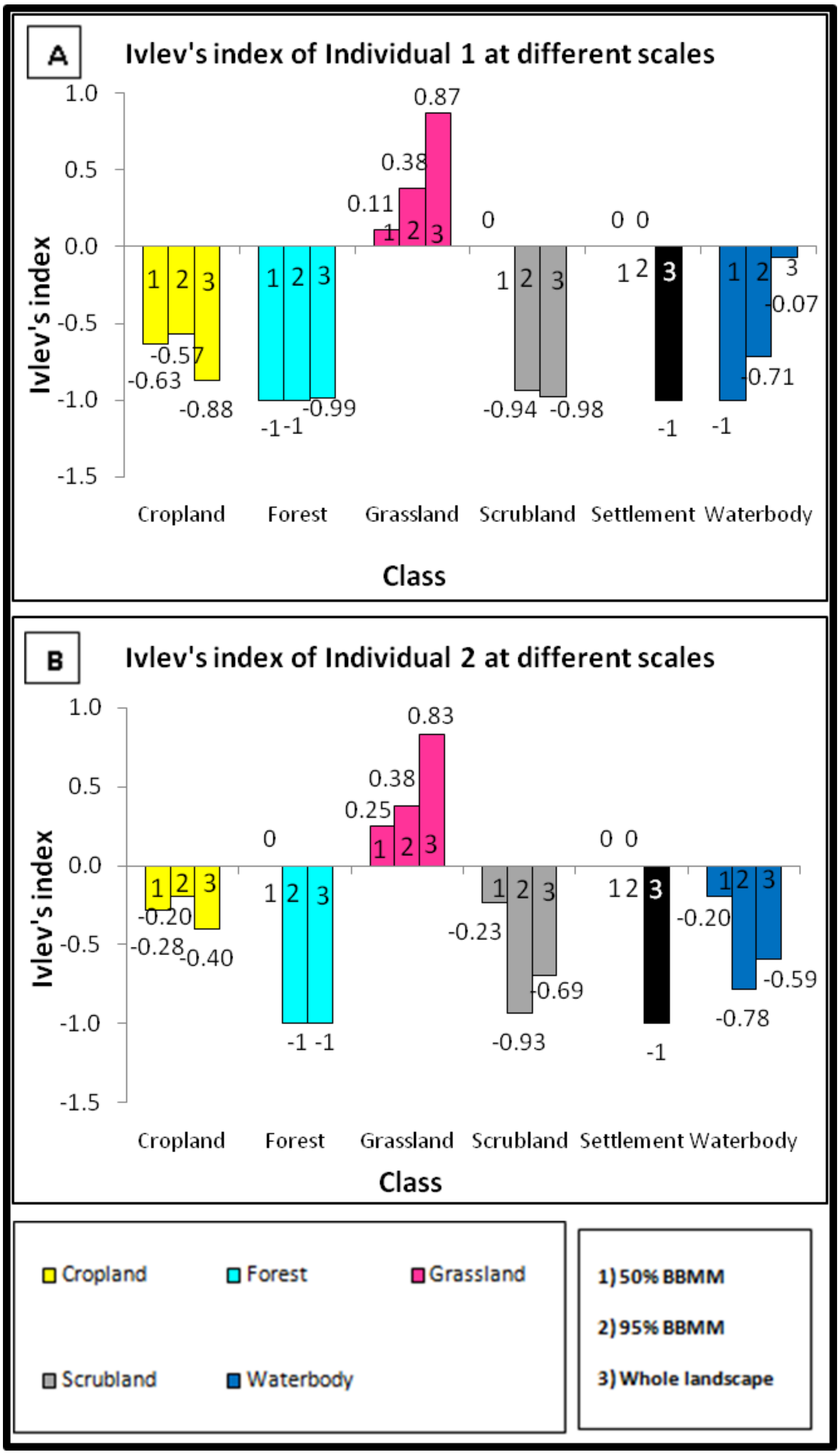

Figure 5.10 Ivlev's index calculated for each LULC class type in three different scales (50% BBMM, 95% BBMM and landscape level) (A) Individual 1 (B) Individual 2

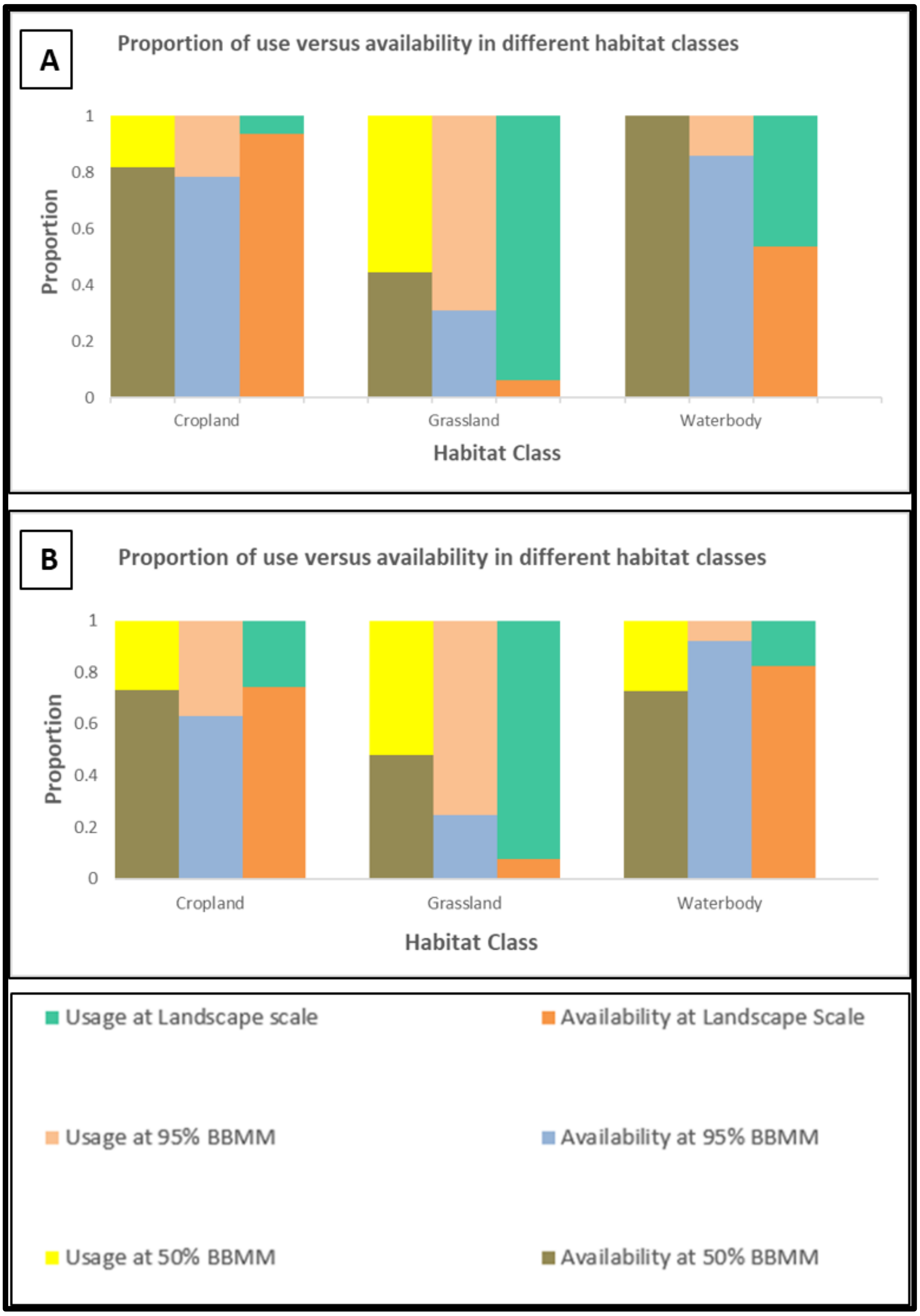

Figure 5.11 Proportion of habitat availability versus use in croplands, grasslands and waterbodies calculated in three different scales (50% BBMM, 95% BBMM and landscape level): (A) Individual 1 (B) Individual 2

5.4.4 Temporal continuity of trajectory paths in different habitats:

For both the individuals, we found that the continuity of the trajectory paths was highest in grasslands (82% of the locations was temporally connected for individual 1 and 93% for individual 2) followed by croplands (60% for individual 1 and 87% for individual 2) and waterbodies (44% for individual 1 and 57% for individual 2) (Table 5.2; Figure 5.12A-B and Figure 5.13A-B). Croplands and waterbodies had lots of temporally isolated points as the swamp deer either visited them sporadically or used them as a transit between two grassland patches.

Table 5.2 Proportion of points which could not be temporally connected in each habitat class for both the collared individuals

	Individual 1			Individual 2		
Habitat	**Original points**	**Path Points**	**% of points lost**	**Original points**	**Path Points**	**% of points lost**
Cropland	292	174	40.4	1187	1036	12.7
Grassland	5174	4234	18.2	2521	2353	6.7
Waterbody	355	156	56.1	67	38	43.3

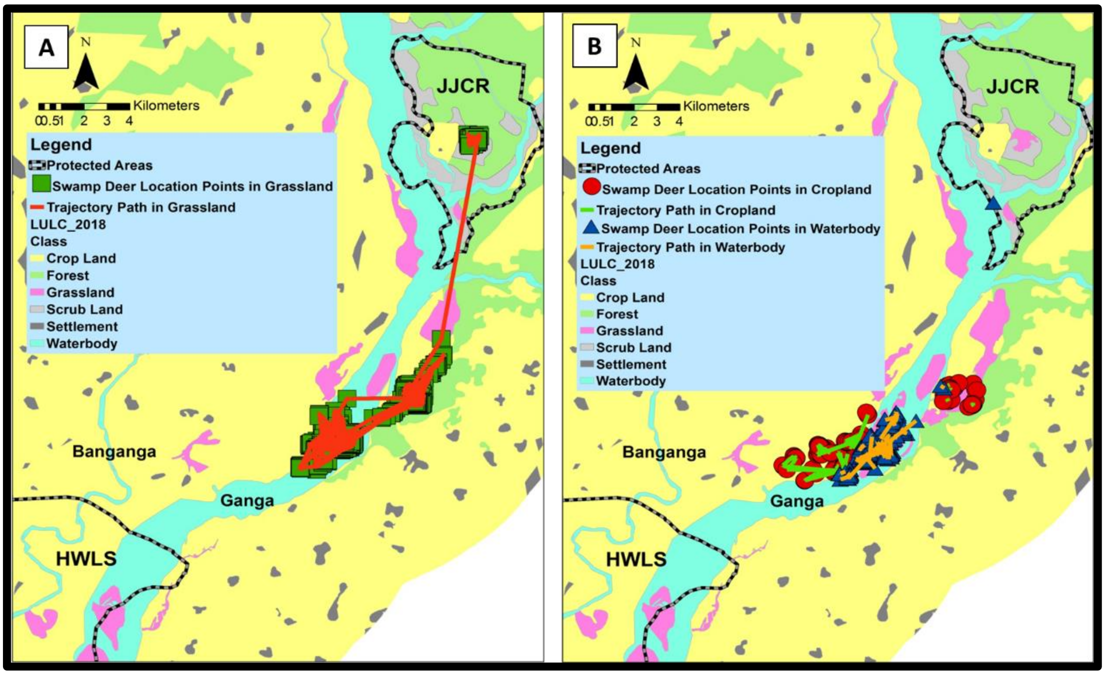

Figure 5.12 Representation of continuous (connected by lines) and disrupted trajectory paths (isolated points) for individual 1: (A) Trajectory paths in grassland (B) Trajectory paths in cropland and waterbody

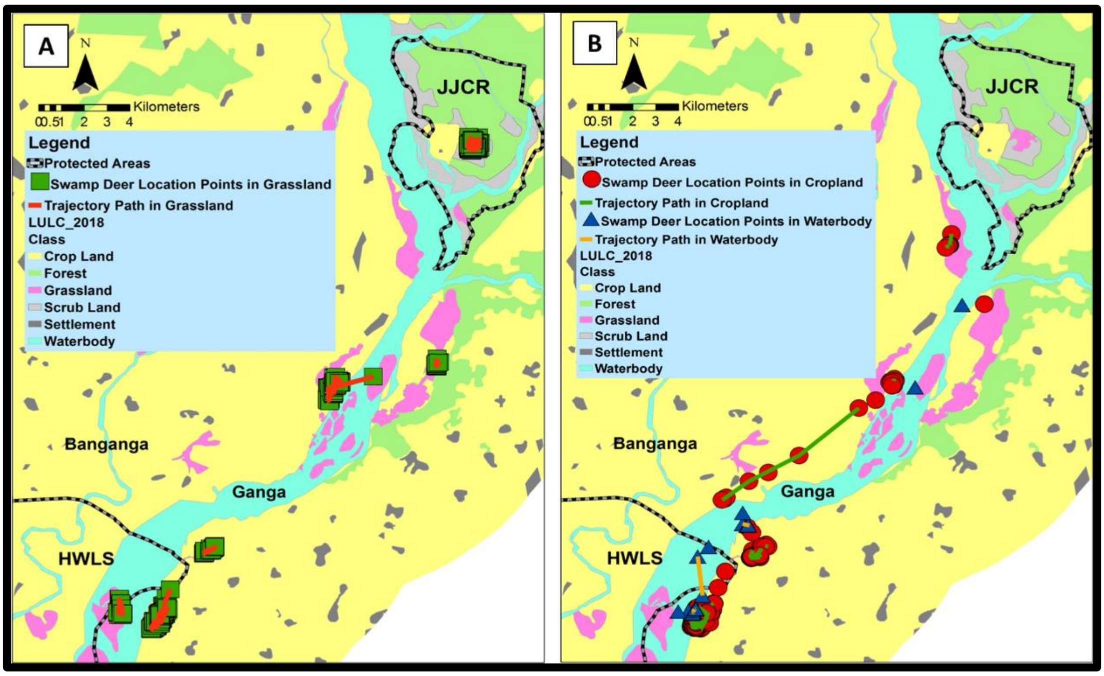

Figure 5.13 Representation of continuous (connected by lines) and disrupted trajectory paths (isolated points) for individual 2: (A) Trajectory paths in grassland (B) Trajectory paths in cropland and waterbody

5.4.5 Assessment of directionality in movement patterns in this landscape:

Movement directionality analysis showed a total of 73 directed grids ranging from 0.55 to 0.99 (low to high) in case of individual 1 in the entire landscape (Figure 5.15A, C and D) while for individual 2 there were 39 grids with values ranging from 0.43 to 0.98 (Figure 5.15B). The dot product indices (indicator of directionality) were pooled into low directionality grids (0.55-0.76 for individual 1 and 0.43-0.69 for the individual 2) and high directionality grids (0.76-0.99 for individual 1 and 0.69-0.98 for individual 2) based on mid-point values of the two distributions. The mean speed within the high directionality grids (indicated by length of vectors) was 0.54 km/hr for individual 1 and 0.36 km/hr for individual 2 while the respective values in the low directionality grids were 0.18 km/hr and 0.10 km/hr. When the grids were plotted on the LULC map, darker coloured grids representing high directionality (above 0.76 for the individual 1 and 0.69 for the individual 2) fell in grassland-cropland and grassland-waterbody interfaces (Figure 5.15A-D). Lighter coloured grids representing low directionality fell on grasslands. Speeds represented by length of output vectors emanating from the grids were also more in interface areas of grasslands-croplands or grassland-waterbodies (less vegetation cover) than within the grassland patches (more vegetation cover).

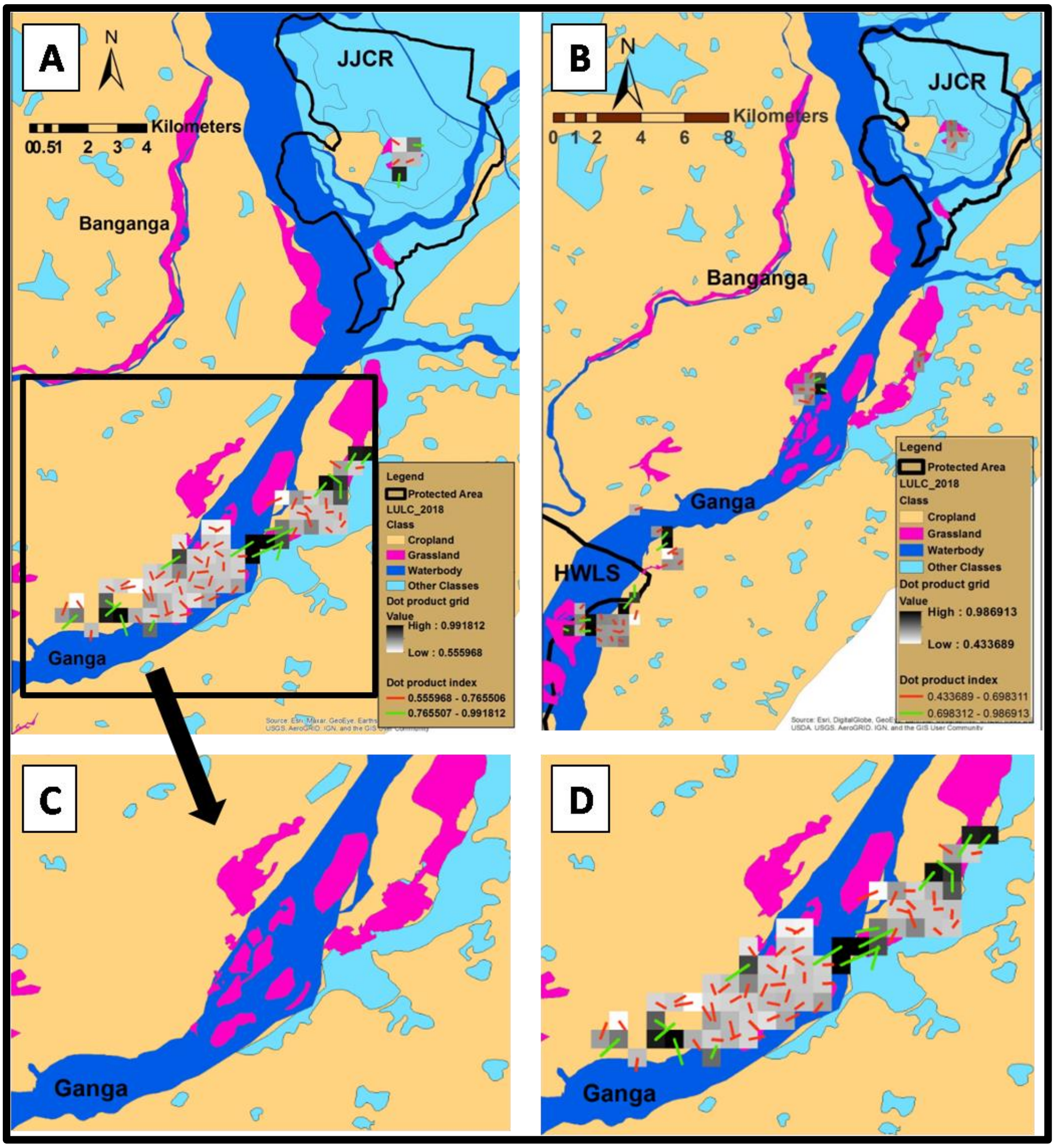

Figure 5.14 Directionality grids generated at 500 m scale on LULC map. Vector length indicates the mean speed and the colouring indicates similarity in track direction. The "Directed" class is represented in darker grids and "Undirected" class is represented in lighter grids: (A) Individual 1 (B) Individual 2 (C) and (D) represents zoomed view of the LULC map without and with the grids of individual 1 overlaid on it

5.4.6 Group dynamics and activity pattern of swamp deer outside the protected areas:

Camera trap data suggests that the swamp deer are present in small groups (3-4 individuals/group). (Figure 5.15C). From the camera trap images, it was understood that these fragmented grasslands are critical rutting and fawning grounds (Figure 5.15A-B). Most of the captures of swamp deer were during the night between 6:00pm to 6:00am (Figure 5.16A) whereas human activities were recorded during the day (Figure 5.15D), especially between 7:00am and 4:00pm (Figure 5.16B). The difference in temporal activity between human and swamp deer was statistically significant ($U2= 7.41$ $p<0.001$). An activity overlap analysis shows only a mere 21% overlap between swamp deer and humans (Figure 5.16C).

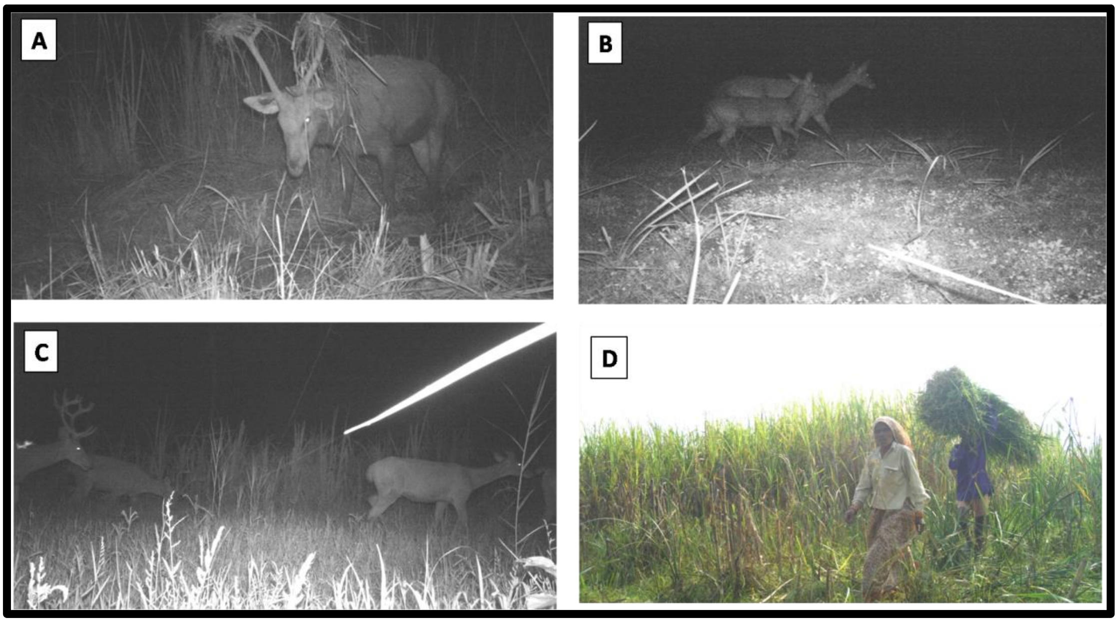

Figure 5.15 Images obtained from camera-traps laid in non-protected grasslands along Ganga during the period July 2018-June 2019 (three sessions): (A) Stags in rut (B) Presence of fawns (C) Swamp deer operating in small groups (D) Human activity during the daytime

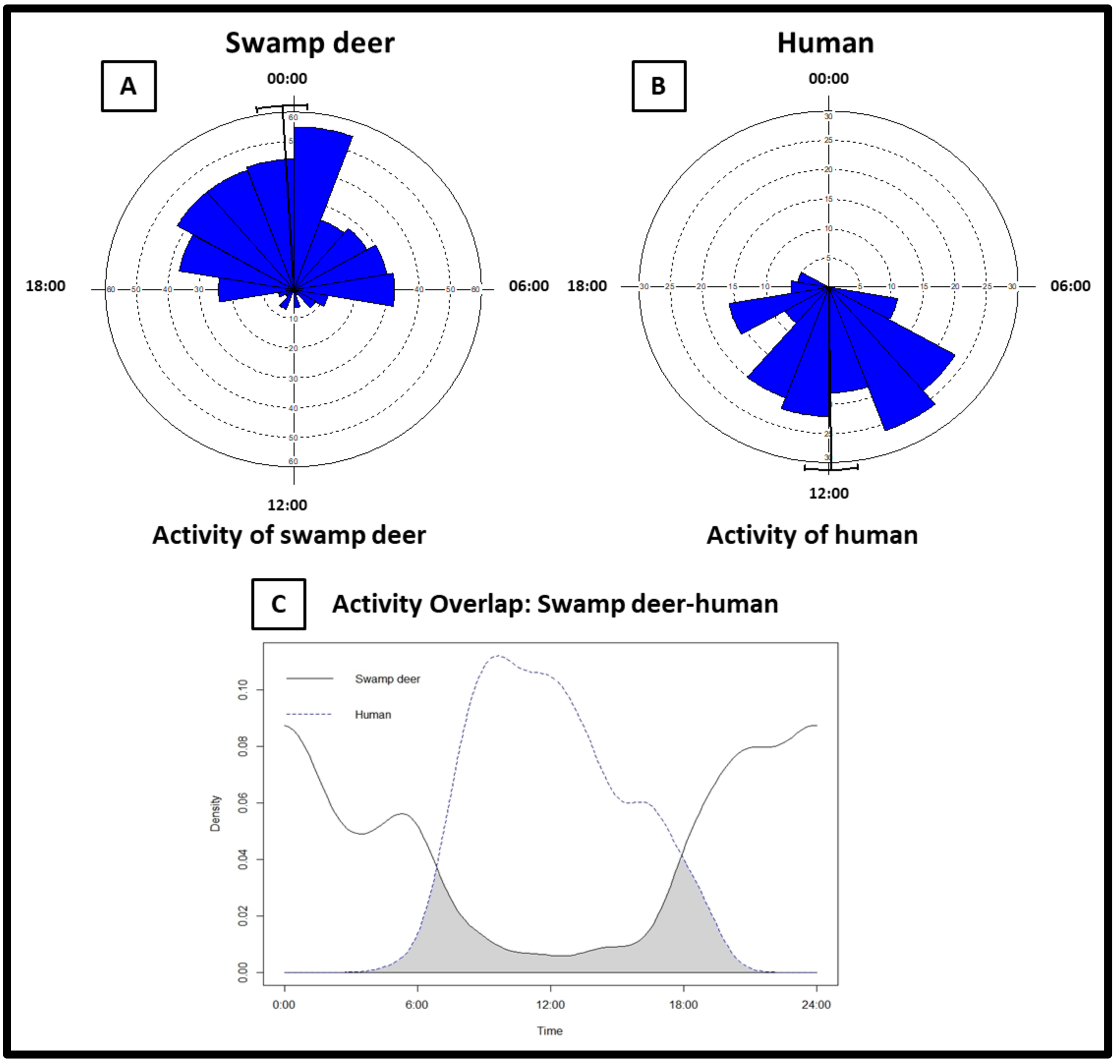

Figure 5.16 Temporal activity patterns: (A) Swamp deer (B) Human (C) Temporal activity overlap between swamp deer and human

5.5 Discussion:

This chapter focuses on the movement ecology of swamp deer in the upper Gangetic plains of north India. We investigated and generated critical information on movement routes, home ranges, habitat usage and fine-scale movement patterns of the swamp deer in this landscape. The collaring of swamp deer is a critical step in the conservation of this species, as there was no information on the movement ecology of this species, thriving in the human-dominated landscape around Ganga. Though surveys revealed presence of swamp deer in grassland patches outside Jhilmil Jheel, the active migration routes, stopover sites and the significance of those sites were unknown. Drive-net technique was successfully implemented to collar two swamp deer hinds. This technique has not been used to collar animals in India in a large scale, but it emerged as a useful technique to collar species, particularly herding animals that live in inaccessible habitats.

The movement pattern displayed by the two collared individuals validated the previously generated genetic data which showed connectivity between Jhilmil and Hastinapur WLS. The movement data identified critical stopover sites and important grassland patches (Nangal grassland, Ranjeetpur Jheel, river islands near Ranjeetpur, Sukhapur grassland) between Jhilmil Jheel and Bijnor which both swamp deer actively used (Figure 5.17). The swamp deer is a resilient species thriving in the minimal habitats but for maintenance as a metapopulation between Jhilmil Jheel and Hastinapur, these grasslands should receive immediate attention for ensuring proper functioning as corridors in future. Some of the habitats are still suitable for swamp deer (Nangal grassland) while Sukhapur and Amichand are highly degraded owing to conversion and overgrazing and require immediate attention (Figure 5.17). Our future efforts should focus on communicating with the local communities and other stakeholders for reviving these degraded grasslands.

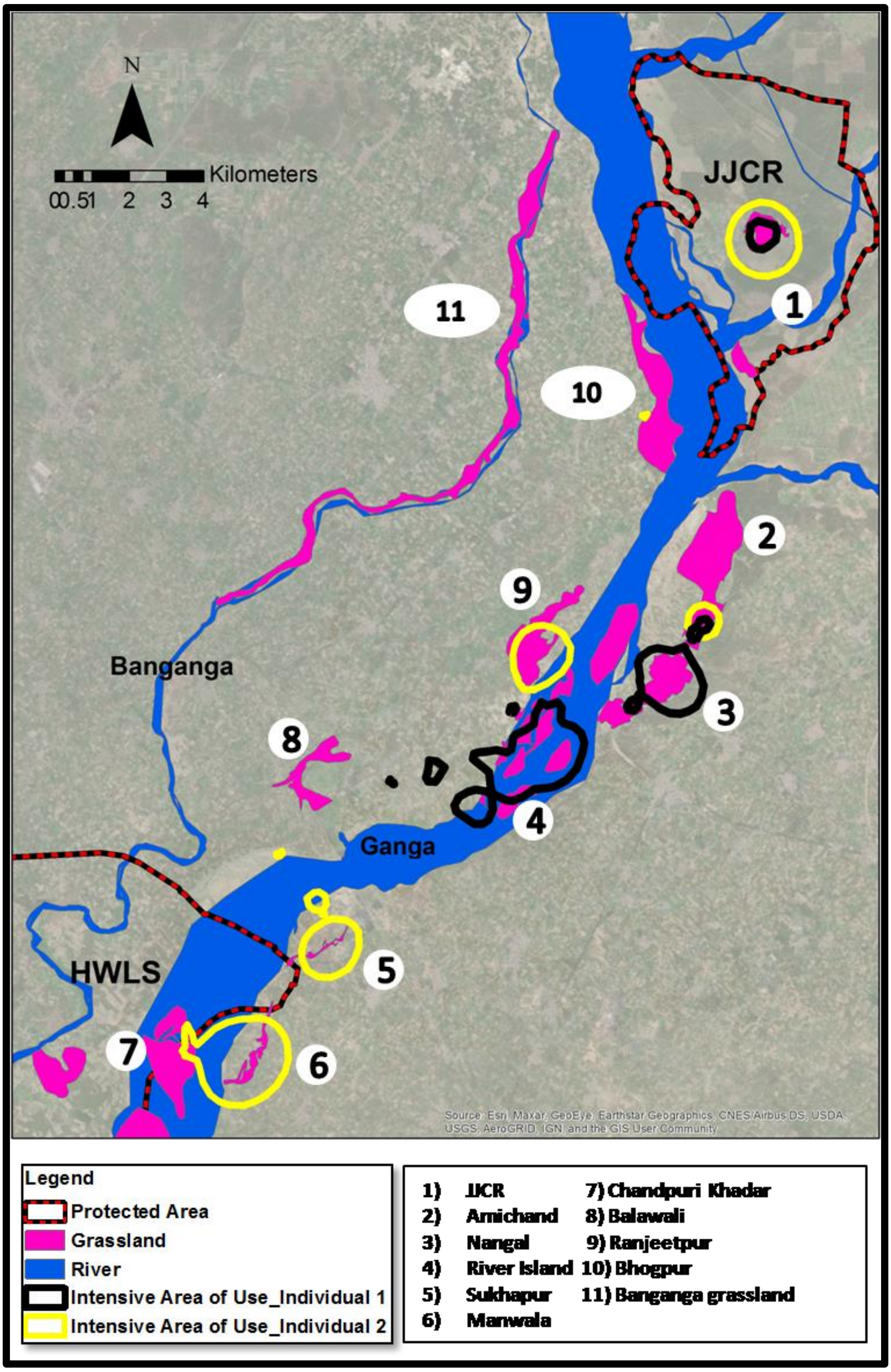

Figure 5.17 Grassland usage pattern by swamp deer along Ganga

We recorded that core area usage for swamp deer was 0.58 and 1.47 km^2 for the two individuals. Grasslands formed a major part of their core home ranges which indicate presence of extremely fragmented habitats left for swamp deer in this human-dominated landscape. The habitat usage patterns for both these individuals at three scales indicated that swamp deer used grasslands much more in comparison to its availability. As availability of grasslands at a landscape level was severely compromised (grasslands make up only 5% of the study area), the value of Ivlev's index was highest in this case compared to 95% and 50% BBMM.

The fine-scale movement patterns of the species reinstate the importance of grasslands in swamp deer conservation. Our trajectory path continuity analysis suggests that swamp deer spend considerable time in the grasslands and sporadically visit cropland (for foraging or transiting) and waterbodies (for transiting between grasslands). The swamp deer show less directed movement within the grasslands as it can forage with movement characterised by low speed and more turning angles. However, while transiting between two different grassland patches, they move through the interfaces of grassland-croplands and grassland-waterbodies with directed movement characterised by high speed and low turning angles. Lack of vegetation cover is one characteristic of such directed grids which probably drives faster movement events. We also recorded from our trajectories that absence of vegetation continuity forces swamp deer to traverse more distances in open areas. In one instance, individual 1 travelled nine km in single movement event due to minimal vegetation cover (tall grasslands) in its movement route. With ongoing fragmentation, the swamp deer are highly vulnerable to poaching or coming in conflict while transiting between grassland patches and thus disruption of their movement routes is a possibility in the near future. Croplands (mainly sugarcane) which are the major LULC class in this area can facilitate movement at certain time of the year as they provide cover but owing to their harvesting after winters, they cannot act as prime habitats all year round.

Our camera trapping exercise in the non-protected grasslands provided us with many interesting aspects of swamp deer ecology. Unlike Jhilmil Jheel where around 150 swamp deer congregate, the animals operate mainly in groups of 4-5 individuals in the non-protected grasslands patches. This is probably a strategy where splitting into groups enables them to maximise resource use from these small grassland patches and avoid conflict with humans. The non-protected grasslands between JJCR and HWLS emerged as critical fawning and breeding grounds for the barasingha. Though we did not get any evidence of fawns present with our collared individuals, we found fawns accompanying other females during August in these grasslands. Interestingly, sighting incidences of fawns in Jhilmil Jheel are very rare which point to presence of fawning grounds for the swamp deer outside JJCR. The swamp deer showed complete temporal segregation from human in the non-protected grasslands and such strategies probably enabled them to thrive amidst humans.

Our collaring data generates baseline information on ecology of swamp deer living outside protected areas but there are further fine-scale informations that need addressing. We could only collar female swamp deer and their movement patterns and habitat use may vary from males. Also, much of the information is generated from a very small sample size (n=2) and is thus prone to individualistic fluctuations. The landscape between JJCR and HWLS has many swamp deer harbouring areas but we have collared individuals only from JJCR. Future large-scale radio-collaring studies involving males and females from multiple sites in the landscape can shed light on the dynamicity of the entire population, major fawning and breeding grounds and habitat utilisation during different seasons.

Swamp deer is an animal that has evolved in the floodplains of north Indian rivers (Qureshi et al., 2004). As these rivers change course, the grasslands also show shift in their spatial positions with time (Midha and Mathur, 2010). For such an animal, movement is an integral part of their ecology and thus should be taken into account while making management recommendations. Habitat management is key but information about major hindrances to their movement routes, proper utilisation of available resources and breeding biology should be integrated with habitat management. All our analysis and findings indicate that these grassland patches continue to be critical in supporting a viable population of swamp deer, but ongoing habitat conversions demand urgent management plans involving different stakeholders around Ganga for future protection of swamp deer.

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Chapter 5:

Evaluation of grassland habitats and their temporal dynamics in past 30 years along the upper Gangetic basin

6.1 Introduction:

Grasslands play critical role in supporting biodiversity, global food production, medicines, climate regulation, water purification and nutrient cycling which greatly benefit mankind (Cagaš et al., 2009; Carlier et al., 2009; O'Mara, 2012, Rossi et al., 2019). Unfortunately, relentless anthropogenic exploitation in the form of conversion and grazing has turned grasslands into one of the most jeopardized ecosystems globally (Ceballos et al., 2010). The loss and fragmentation of grasslands have caused species extinctions, environmental resource depletion and altered ecosystems (Clark, 1989; White et al., 2000; Zavaleta and Hulvey, 2004).

In the northern part of the Indian subcontinent, a huge expanse of grasslands and swamplands once covered the basins of Ganga, Indus and Brahmaputra (Dudgeon, 2000; Thapar, 1997). Given the extreme fertility associated with these areas, most of these grasslands have been converted into agricultural lands and settlements, leaving fragments that are heavily used by people and livestock. Following the eradication of malaria in 1950s, the loss of grasslands was particularly alarming (Johnsingh et al., 2004). Moderate usage by human can be beneficial as fire, flood, cutting and grazing contribute to the maintenance of healthy grasslands (Menke, 1992; Xu and Guo, 2015). However, conversions for agriculture and plantations, development, grass harvesting, overgrazing, excessive burning have severely put the grasslands at risk of extermination. The swampy grasslands in the Ganga habitat block have also suffered unprecedented decline such that they cover only 2% of the entire Gangetic plains (Dinerstein, 2003). Despite fragmentation, the grasslands support a plethora of wildlife like swamp deer, hog deer, fishing cat, sarus crane etc. Given that majority of this block is outside protected areas, they are always vulnerable to conversion and overgrazing. A number of dams have also been built in this region in the last 50 years which has altered the volume and flow of Ganga river (Khan et al., 2018), thus impacting grassland formation and maintenance of permanent wetlands.

The grassland management along Ganga is dependent on the conservation of the largest animal inhabiting it, the iconic swamp deer. Our surveys in the Ganga habitat block suggest that the swamp deer are restricted to highly fragmented grasslands composed of mainly *Phragmites* sp., *Typha* sp., *Saccharum spontaneum* and *Saccharum bengalense* etc. We reported 52 patches of grasslands that cover a meagre 102.33 km^2 of grassland between Jhilmil Jheel and Bijnor Barrage (see 1st chapter). The condition is worse downstream of Bijnor Barrage (see 2nd chapter). Interestingly, genetic analysis suggests that the remnant grasslands continue to serve as critical corridor for swamp deer between JJCR and HWLS and help in maintaining genetic connectivity (see 3rd chapter). Radio-collaring data suggests movement of swamp deer along Ganga and extensive usage of grasslands between JJCR and HWLS (see 4th chapter). Till now there is no information on extent of grasslands and other major LULC classes in the Gangetic habitat block, the species composition of these grasslands and how the grasslands and other landuse landcovers have changed over the years. This information would be crucial in understanding the complex temporal dynamics of swamp deer habitats in the upper Gangetic plains.

In this current chapter, we have quantified the extent of spatial coverage and species composition of barasingha habitats and assessed the changes in grassland and other LULC classes in the last 30 years using a combination of GIS and field surveys. We used Landsat (1985, 1995, 2005, 2015) and field collected data to quantify the current swamp deer habitats and their historical change dynamics. We used Normalised Difference Vegetation Index (NDVI) of four major grassland species and field data (vegetation plots) to classify the grasslands. Such information will be crucial for devising a long-term integrative habitat management plan for safeguarding the swamp deer and other endangered grassland/wetland flora (fishing cat, hog deer, grassland birds etc.).

6.2 Study area:

Our study encompasses the Ganga habitat block from Haridwar in the north and Garhmukteshwar in the south, spanning an area of 3173 km^2. A buffer of eight km around the river Ganga, Solani and Banganga was taken to delineate our study area (Figure 6.1) as grassland patches are restricted to 1-2 km from the rivers. The study area included 1677 km^2 of protected areas of Hastinapur Wildlife Sanctuary (HWLS) and Jhilmil Jheel Conservation Reserve (JJCR) and 1496 km^2 of non-protected area. Overall study area encompassed geographic locations between 29°79'99''N, 78°21'71''E to 28°77'34''N, 78°13'57''E. The other details of the study area are provided in Chapters 1 and 2.

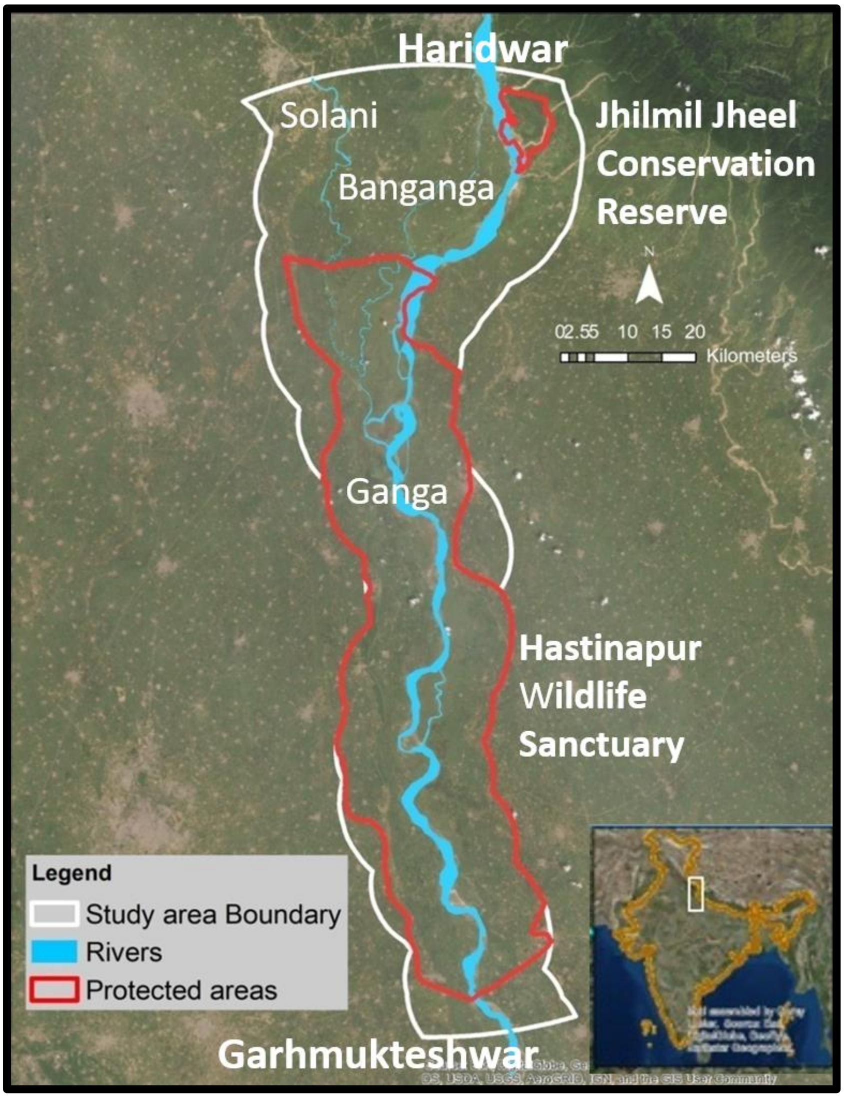


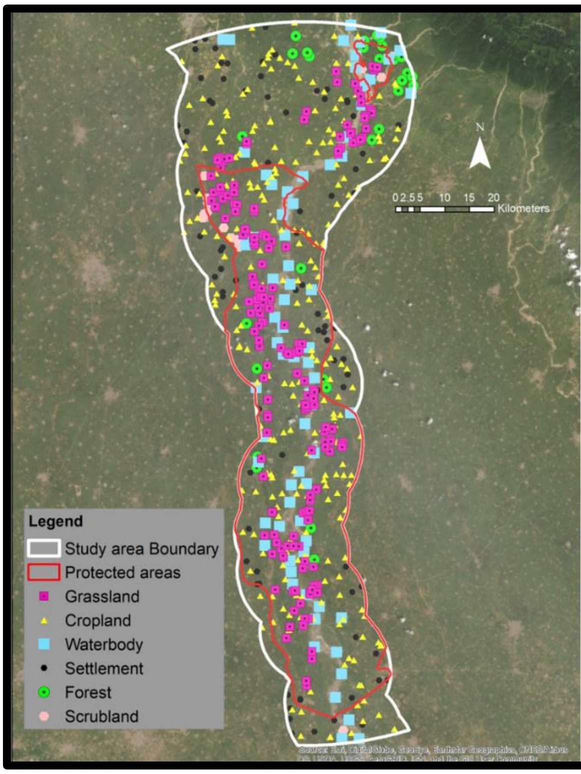
Figure 6.1 Study area stretching from Haridwar to Garhmukteshwar, with a buffer of eight km around rivers Ganga, Banganga and Solani

6.3 Methods:

6.3.1 Grassland present status - LULC map of 2015:

<u>*6.3.1.1 Field survey*</u>

To assess the extent of spatial coverage of swamp deer habitats and other major Land Use and Land Cover (LULC) classes in our study area, we conducted extensive field surveys in 2015 to gather information about different LULC classes. From different areas of this landscape we collected a total of 656 points covering mainly six types of LULC classes, i.e. cropland, grassland, waterbody, settlement, forest and scrubland (Figure 6.2).



Class	Number of GPS Points
Cropland	253
Forest	40
Grassland	196
Scrubland	27
Settlement	80
Waterbody	60
	656

Figure 6.2 Spatial distribution of surveyed points covering different Land Use and Land Cover types in the study area

<u>*6.3.1.2 Image processing, LULC classification and analysis*</u>

We downloaded Landsat images (USGS) of 2015 (Landsat 8) with 30 m resolution. Radiometric correction of images was done to obtain better results. Visual interpretation technique was applied to Landsat images using spectral characteristics (size, tone, texture) of different classes and field knowledge (Rawashdeh, 2012). Main features of the landscape were considered while generating LULC map. The classification was done at a scale of 1:50000 (Rawashdeh, 2012) and six above-mentioned LULC classes were categorized. Once the classification was done, we conducted further ground truthing by generating random points and validating them. Accuracy of classification was defined as:

(Number of points classified correctly/ Total no of points surveyed) *100

From the attribute table generated for each LULC class, the area of each class was calculated.

6.3.2 Grassland classification:

6.3.2.1 Vegetation field sampling

We conducted field survey in the month of April 2018 throughout the grasslands of study area between Haridwar and Bijnor barrage in the upper Gangetic plains and recorded the main species of the grasslands. Vegetation composition of the grassland (only major species like *Typha* sp., *Phragmites* sp., *Saccharum spontaneum* and *Saccharum bengalense*) was assessed by plot sampling method (Cavender et al., 2014; Oliver et al., 2012). Total 98 (5X5 m^2) training plots were laid randomly throughout the field area complying with vegetation heterogeneity i.e. areas with more heterogeneity in terms of species composition were covered with more plots. Individual numbers of pre-selected plant species were counted (individual stem or tuft). In case of homogeneous areas, 1X1 m^2 plots were laid at each corner of 5X5 m^2 plots and the species were counted and later extrapolated.

6.3.2.2 Image processing and NDVI classification

Normalized Difference Vegetation Index (NDVI) (El-Gammal et al., 2014) determines a value for the amount of green area i.e. quantity of vegetation and its state of health or vigor of growth (Eastman et al., 2013). It is a useful tool to characterize a vegetation where mathematical combination of several bands or spectral transformations of two or more bands are designed to enhance and characterize vegetation cover (Bannari et al., 1995). Swamp deer mainly uses grasslands and swamps characterized by *Typha* sp., *Phragmites* sp., *Saccharum spontaneum* and *Saccharum bengalense* (Tewari and Rawat, 2013). Preliminary surveys and Google Earth imageries suggested a difference in surface reflectance values of these four different grasses. For image classification of grasslands, we downloaded Landsat images (USGS) acquired in 2018 (Landsat 8) with 30 m resolution. We conducted radiometric correction of two tiles (Path 146, row 040 for zone 43 and path 146, row 039 for zone 44) before merging them (Schroeder et al., 2006). Among all different 11 bands of Landsat 8 we chose only two bands i.e. band 4 (visible red) and band 5 (near-infrared) for calculating NDVI which is defined as

$$\mathbf{NDVI = (NIR - R) / (NIR + R)}$$

where, R and NIR imply surface reflectance averaged over red (λ – 0.6 µm) and near-infrared (NIR) (λ – 0.8 µm) regions of the spectrum, respectively (El-Gammal et al., 2014; Jiang et al., 2006). NDVI values range between -1.0 and +1.0 where, very low values represent water bodies, barren areas and sands; moderate values specify shrub and grassland whereas temperate and tropical rainforests are indicated by high values (El-Gammal et al., 2014; Gross, 2005). We masked only the grassland areas from obtained NDVI image for further analysis (Figure 6.3A). Then we trained NDVI with the GPS points that were collected during the field survey of April month (Figure 6.3B) and reclassified our NDVI into major vegetation types.

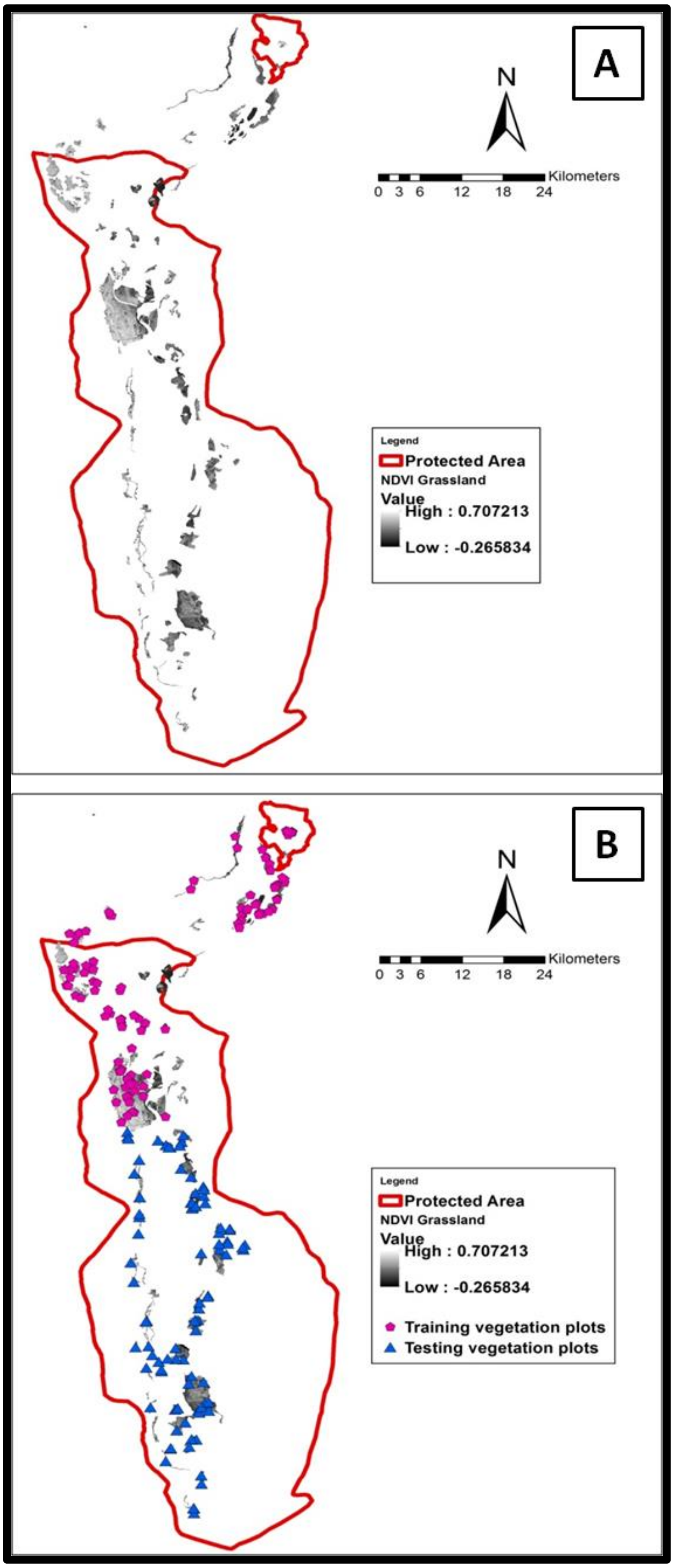


Figure 6.3 Spatial distribution of vegetation plots in the entire study area: (A) The grasslands were only chosen for vegetation plotting (B) The distribution of training (pink) and testing plots (blue) within the grasslands

6.3.2.3 Accuracy assessment and data analysis

To assess the accuracy of our NDVI classification, we conducted further ground truthing in May 2018 in another part of the study area i.e. south of Bijnor Barrage up to Garhmukhteshwar. We laid a total of 111 plots following same protocol of our earlier vegetation sampling method (Figure 6.3B). The overall accuracy of our vegetation classification was defined as

(Number of vegetation plots classified correctly / Total no of plots laid) *100

For assessing the accuracy of our field sampling, we conducted a clustering analysis and estimated relative abundance of concerned species in the vegetation plots. Non-metric Multidimensional Scaling (NMDS) is one such clustering analysis approach which produces an ordination based on a distance or dissimilarity matrix (Cavender et al., 2014). To represent the pairwise dissimilarity between the major classes in terms of species composition, as closely as possible in an ordination of low-dimensional space, we performed NMDS analysis (Scatterplot3d and Vegan package in R). For assessing the relative abundance of each species, we ran Box Plot in R package and Kruskal-Wallis test was performed to show the significance of differences in mean values between four classes (Reinecke et al., 2013). We then calculated the area coverage of different types of vegetation classes in the study area using Arc-GIS 10.2.2 and Microsoft excel.

6.3.3 Change detection -Trends in LULC changes in last 30 years:

6.3.3.1 Image processing and LULC classification

Land Use Land Cover (LULC) is highly dynamic and always subjected to natural and anthropogenic changes. To detect the changes of swamp deer habitats in the upper Gangetic plains between Haridwar to Garhmukteshwar in last 30 years we downloaded Landsat images 2005 (Landsat 7), 1995 & 1985 (Landsat TM) with 30 m resolution and radiometric correction of images was done. Visual Interpretation Technique was used to generate LULC maps of 1985, 1995, 2005 using 2015 classification (current year) as reference.

6.3.3.2 Data analysis

The total area of different LULC classes was calculated on a GIS domain (Arc-GIS 10.2.2). The data was partitioned in relevant ways and net change in area, total change in area of grassland and cropland within non-protected and protected areas were further analyzed in Microsoft Excel. Analysis of interchange of classes was performed using the Union Tool in Arc-GIS 10.2.2 and Microsoft Excel. Google Earth imageries were used for visual interpretation of dynamic temporal changes within particular area.

6.4 Results:

6.4.1 Grassland present status - LULC map of 2015:

The LULC map of 2015 consisted of six different categories (cropland, grassland, forest, waterbody, settlement and scrubland) and after extensive field verification, accuracy of our classification was found to be 91% (Figure 6.4A). It was found that

76% of the study area was covered by croplands while grassland coverage only 6% (Figure 6.4B). The croplands were spread all across the landscape while the grasslands were present in fragments, specifically along waterbodies. There were 438 settlements (4% coverage) spread across the landscape. Scrublands and forests spatially covered around 7% of this landscape (Figure 6.4B).

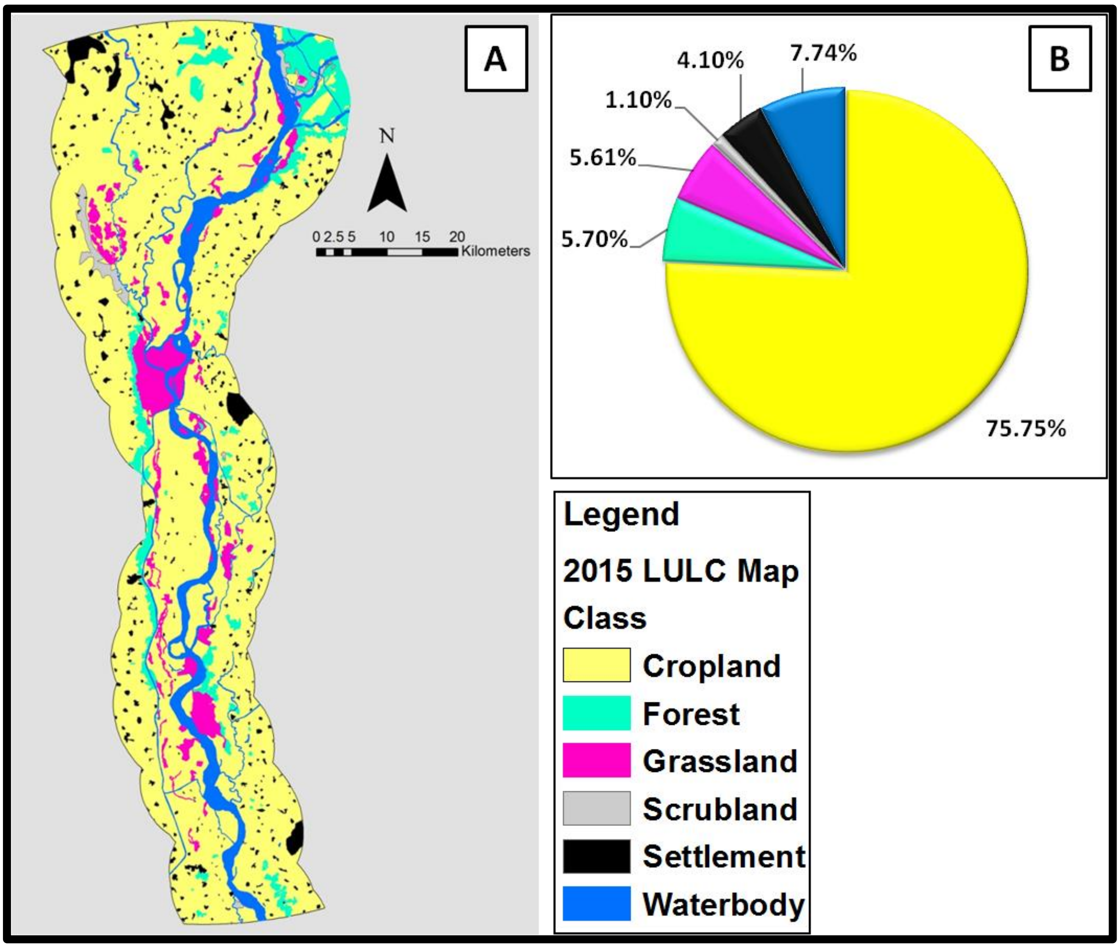


Figure 6.4 LULC map of the study area: (A) Present LULC map of 2015 depicting six different classes (B) The percentage wise area of different LULC classes within the landscape

6.4.2 Grassland classification:

6.4.2.1 The four grassland classes

Based on the four dominant species of grassland and distinct NDVI raster value, we classified the grasslands into four major classes (a) Saccharum (S)(NDVI value range:0.12-0.315) containing either *Saccharum spontaneum* or *Saccharum bengalense* or both (b) Saccharum Dominated Mix (SDM)(0.315-0.39) containing mainly *Saccharum* spp. and some *Phragmites* sp. or *Typha* sp. (c) Phragmites Dominated Mix (PDM)(0.39-0.44) containing mainly *Phragmites* sp. and some *Typha* sp., *Saccharum spontaneum* and *Saccharum bengalense* (d) Phragmites-Typha (PT)(0.44-0.56) containing exclusively *Phragmites* sp. or *Typha* sp. (Figure 6.5). As Phragmites and Typha showed similar reflectance values and found to be associated with each other, they could not be separately classified.

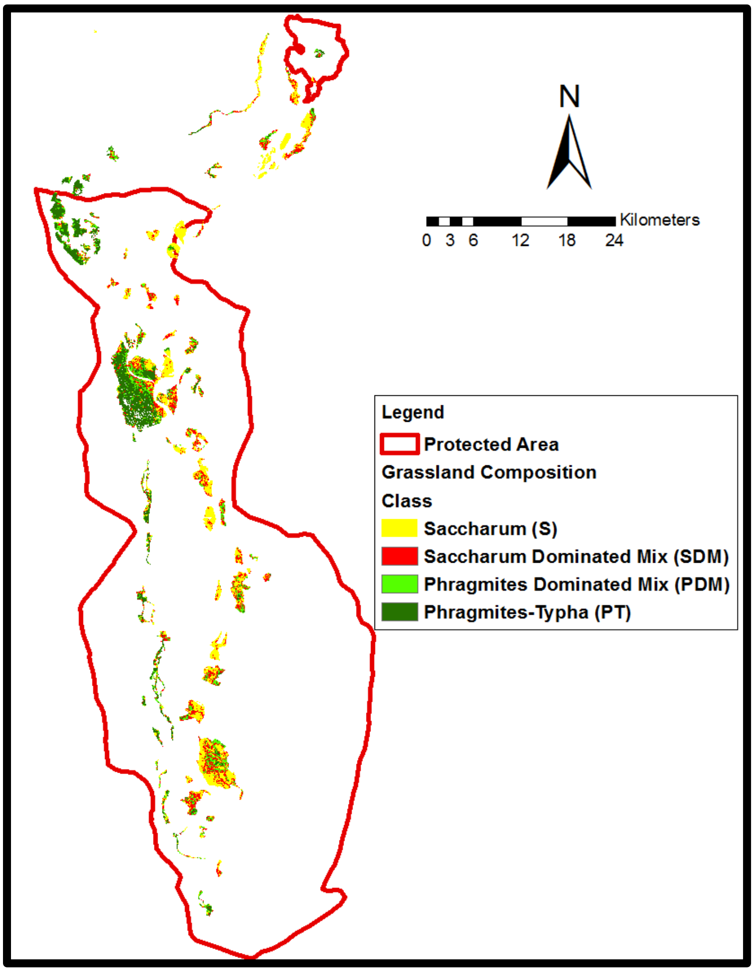


Figure 6.5 Vegetation cover map of the grasslands depicting the four types of grassland vegetation- Saccharum, Saccharum Dominated Mix, Phragmites Dominated Mix and Phragmites-Typha

6.4.2.2 Accuracy assessment and data analysis

We assessed the accuracy of our NDVI classification by calculating the percentage of testing plots that are correctly classified according to our NDVI classification (Table 6.1). Overall, out of 111 testing plots 96 were found to be correct with an accuracy of 86.40% (Table 6.1).

Table 6.1 Details of accuracy assessment of four different vegetation types

Grassland types	**Testing plots**	**Correctly classified plots**	**Accuracy**
Saccharum (S)	27	26	96.20%
Saccharum Dominated Mix (SDM)	49	41	84%
Phragmites Dominated Mix (PDM)	17	14	82.35%
Phragmites-Typha (PT)	18	15	83.30%
	111	96	86.40%

For accuracy assessment of plot data, we conducted a Non-metric multidimensional scaling analysis. NMDS analysis of our data clearly shows that the species composition of four vegetation classes- Saccharum (S), Saccharum Dominated Mix (SDM), Phragmites Dominated Mix (PDM) and Phragmites-Typha (PT) are distinct from each other. The stress value obtained was 0.13 which indicates good fit (Kruskal, 1964). However, there are some overlapping of different vegetation clusters especially in case of Saccharum (S) and Saccharum Dominated Mix (SDM), which indicate their similarity in species compositions (Figure 6.6).

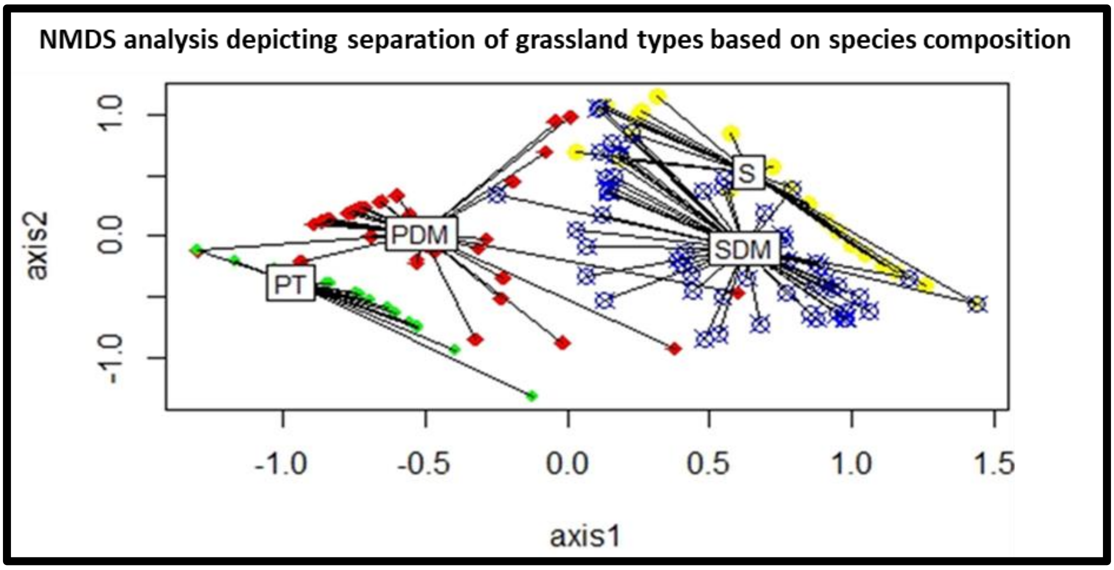


Figure 6.6 Non-metric Multidimensional Scaling Analysis displaying grassland vegetation classes in two dimensions where PT represents Phragmites-Typha (green), PDM-Phragmites Dominated Mix (red), SDM- Saccharum Dominated Mix (blue) and S- Saccharum (yellow)

The relative abundance of each of the four species in our vegetation plots was also calculated to assess the accuracy of our vegetation plot data. In Saccharum (S) class, *Saccharum spontaneum* has the highest mean abundance (0.85) followed by *Saccharum bengalense* (0.13), whereas *Phragmites* sp. and *Typha* sp. are merely present (<0.01) (Figure 6.7A). Saccharum Dominated Mix (SDM) and Phragmites Dominated Mix (PDM) types clearly showed that *Saccharum spontaneum* and *Phragmites* were more abundant (0.57 and 0.66 in respective plots) in the respective plots but other species were also present in both types (Figure 6.7B-C). In Phragmites-Typha (PT) class, most abundant species is *Phragmites* sp. (0.87), followed by *Typha* sp. (0.12). The other two plant species (*Saccharum spontaneum* and *Saccharum bengalense*) are completely absent in Phragmites-Typha class (Figure 6.7D). We found significant difference in species relative abundance of each vegetation class using Kruskal-Wallis test (χ^2 =96.6, $p<0.05$ for S; χ^2 =48.48, $p<0.05$ for SDM; χ^2=96.35, $p<0.05$ for PDM and χ^2=136.23, $p<0.05$ for PT).

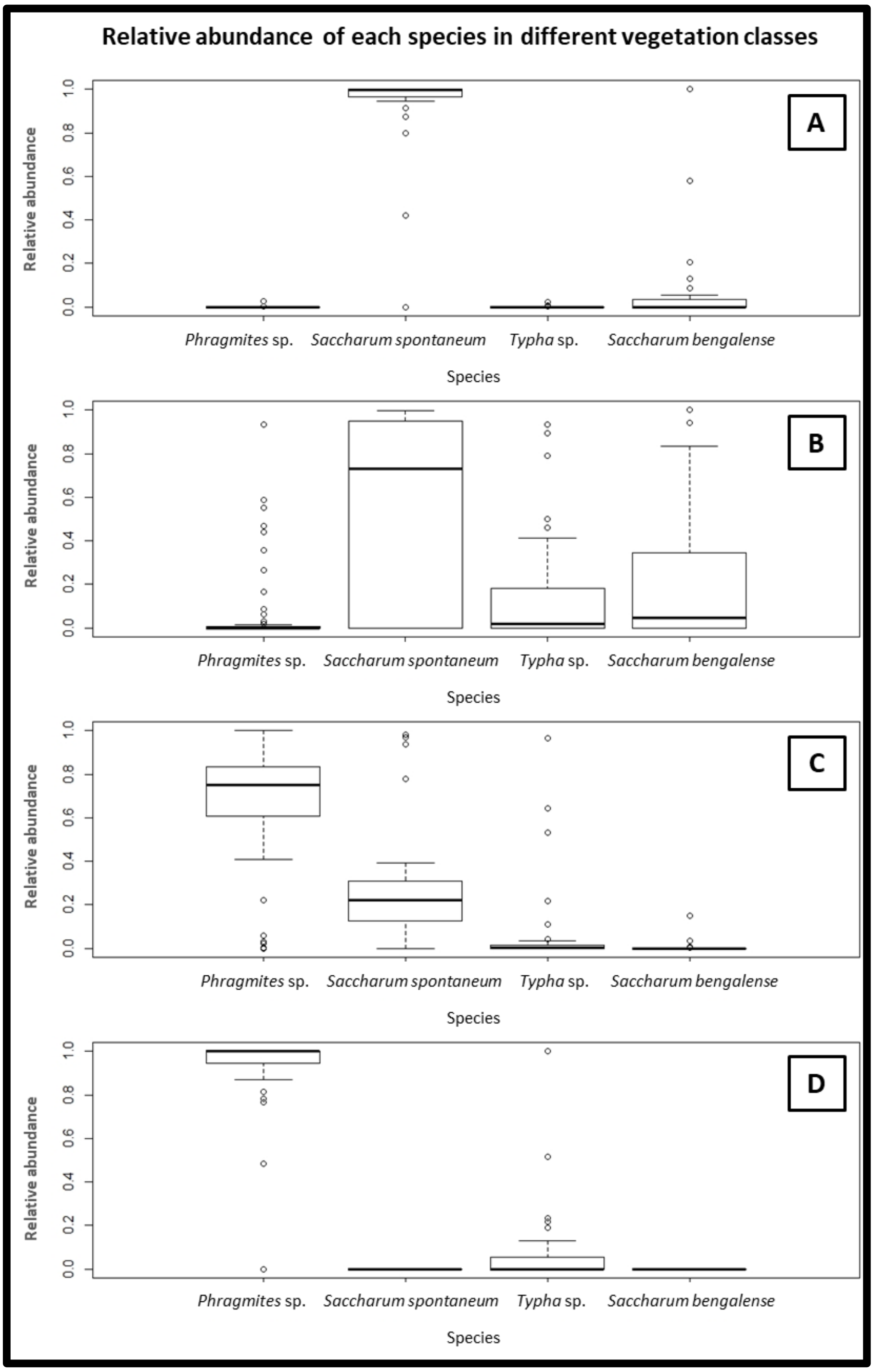


Figure 6.7 Relative abundance of each species (Phragmites sp., Saccharum spontaneum, Saccharum bengalense and Typha sp.) within the four vegetation classes: (A) Saccharum (B) Saccharum Dominated Mix (C) Phragmites Dominated Mix (D) Phragmites-Typha

Total area covered by four assigned vegetation classes was 144.04 km², among which Saccharum (S) was the dominant type covering 35% of the landscape (Figure 6.8). Similar pattern was obtained when the area coverages of each type were assessed within protected and non-protected areas (Table 6.2).

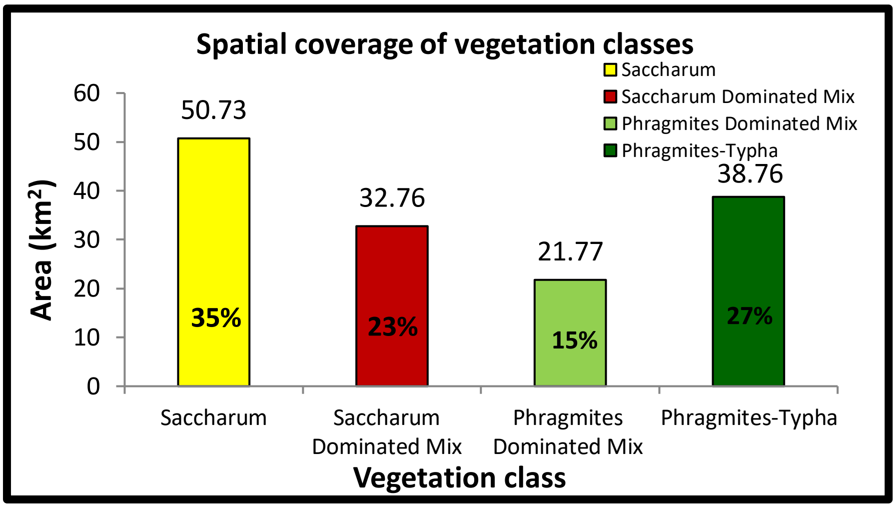


Figure 6.8 Area coverage of different vegetation classes in the entire landscape: Saccharum (yellow), Saccharum Dominated Mix (red), Phragmites Dominated Mix (light green) and Phragmites-Typha (dark green)

Table 6.2 Details of area covered by the four vegetation types in the protected areas, non-protected areas and the entire landscape

Vegetation Class	Protected Area (km^2)	Non-Protected Area (km^2)	Total Landscape (km^2)
Saccharum (S)	39.31	11.42	50.73 (35%)
Saccharum Dominated Mix (SDM)	27.95	4.82	32.77 (23%)
Phragmites Dominated Mix (PDM)	19.43	2.34	21.77 (15%)
Phragmites-Typha (PT)	35.74	3.02	11.42 (27%)

6.4.3 Change detection -Trends in LULC changes in last 30 years:

6.4.3.1 Change of percentage cover of each LULC class in last three decades

The historical changes of the six different Land Use and Land Cover classes were observed from 1985 to 2015 with snapshots of 1995 and 2005 between the initial and final years (Figure 6.9A-D). Area coverage of cropland was 69% in the year 1985 which increased to 76% in 2015 while the grassland area coverage decreased from 13% to 6% in 30 years (Figure 6.10A-D).

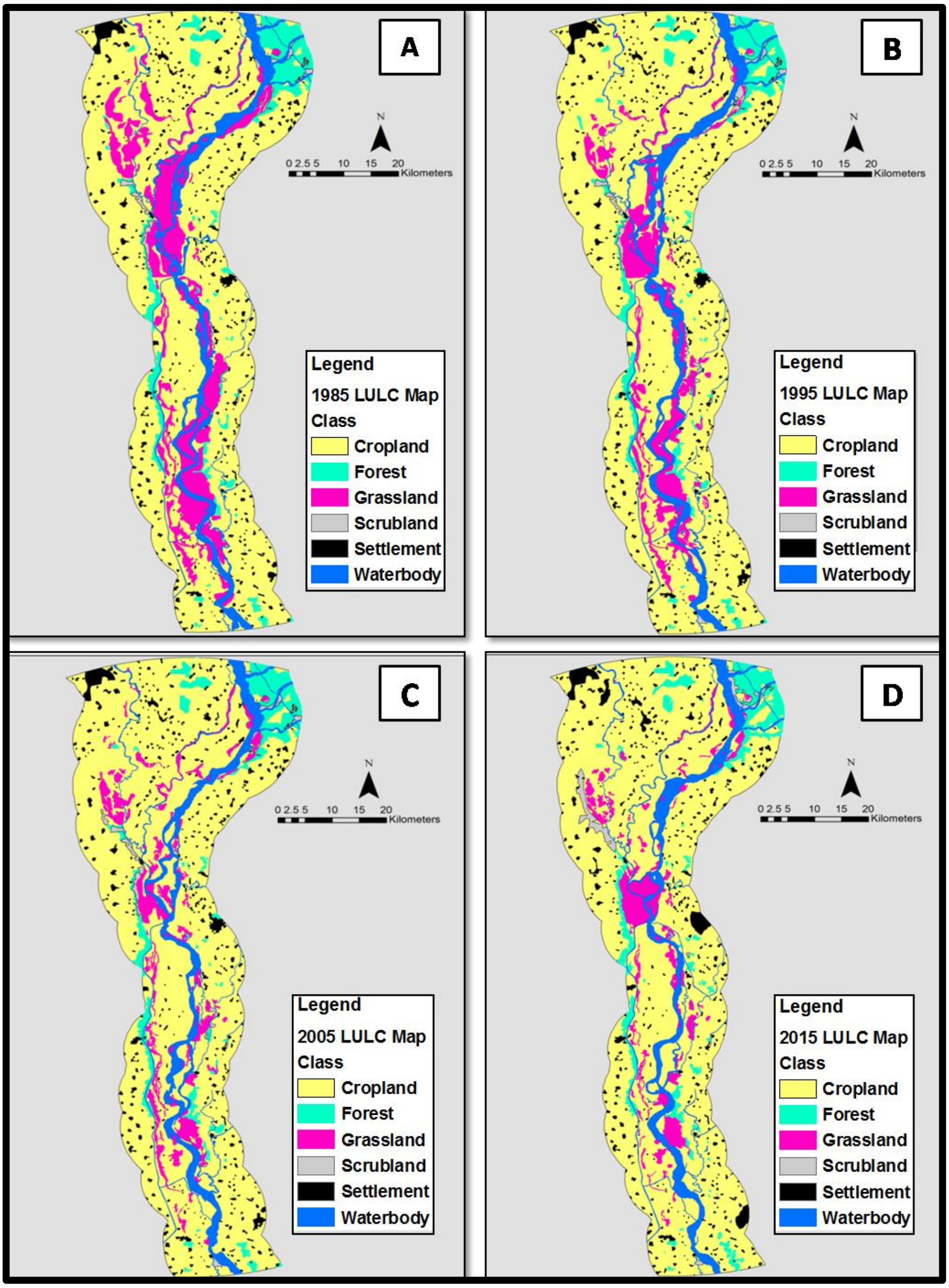


Figure 6.9 The change in LULC classes in the last 30 years in the upper Gangetic plains between Haridwar and Garhmukteshwar: (A) 1985 (B) 1995 (C) 2005 (D) 2015

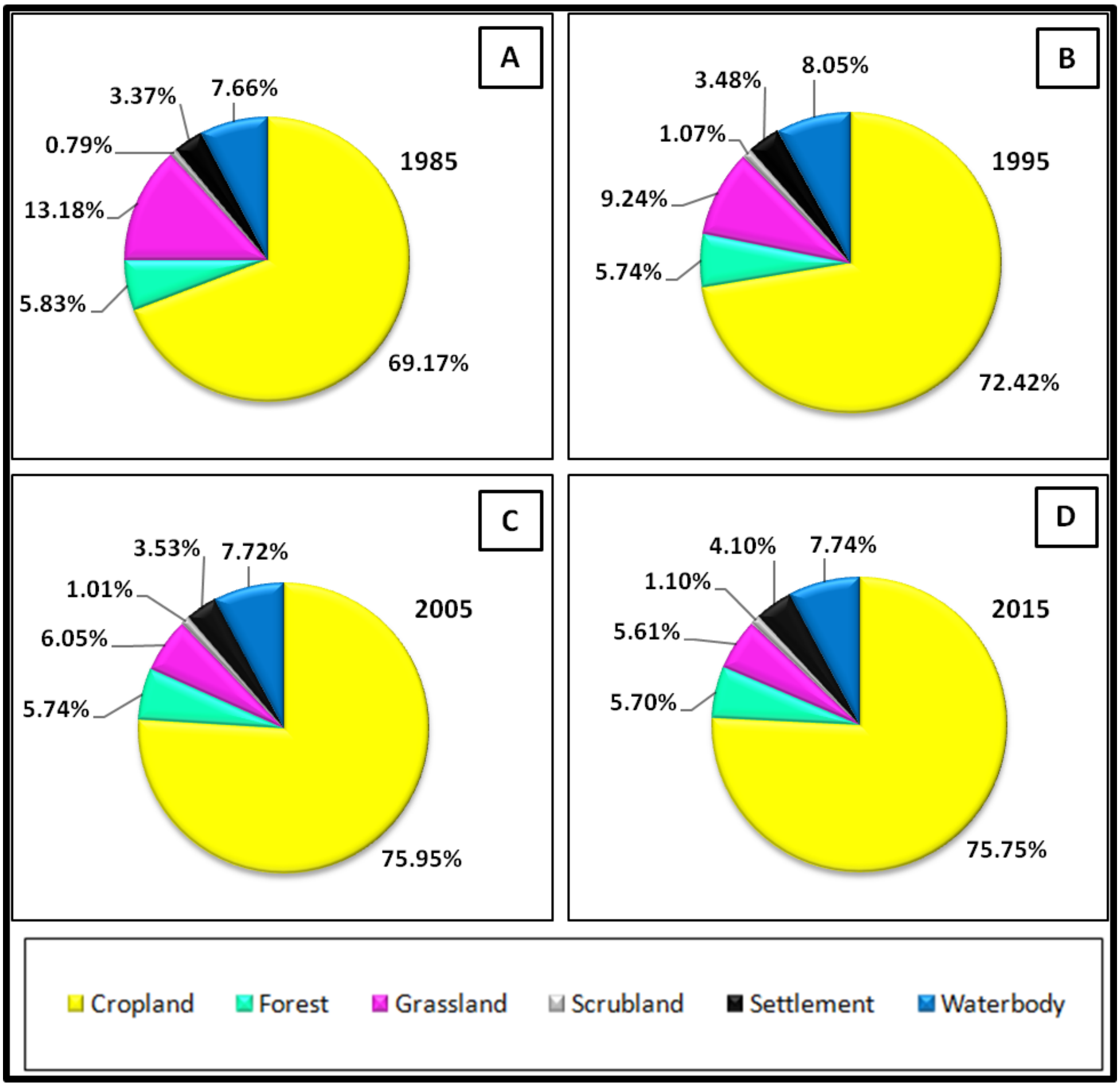


Figure 6.10 The percentage wise change of different LULC classes during the last 30 years in the upper Gangetic block: (A) 1985 (B) 1995 (C) 2005 (D) 2015

6.4.3.2 Analysis of variation in area of different LULC classes over 30 years

Upon analysing the net changes in area of different LULC classes over the last 30 years (1985, 1995, 2005 and 2015), we found that the majority of the changes occurred in grassland and cropland class (240 km^2 loss of grassland and 209 km^2 gain of cropland). The magnitude of change in the other classes was comparatively less than grassland and cropland (10 km^2, 23 km^2 and three km^2 gain in scrubland, settlement and waterbody respectively and four km^2 loss in forest) (Figure 6.11). For these reasons, only the changes in the grassland and cropland class were considered for further analysis.

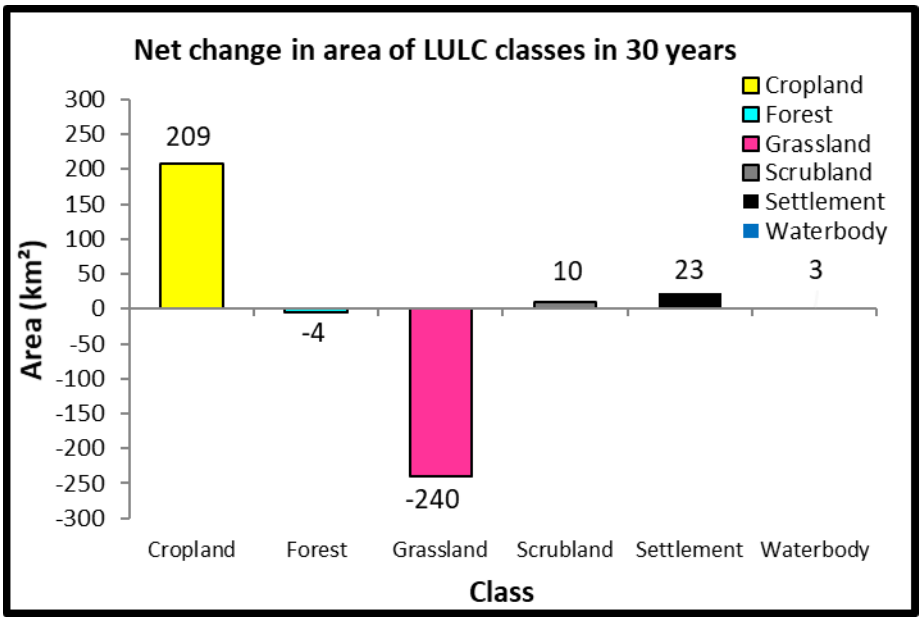


Figure 6.11 Net change in area of different LULC classes over the last 30 years

The 240 km^2 loss of grassland and 209 km^2 gain of cropland were the combination of both gain and loss from 1985 to 2015 (Figure 6.12A). The 240 km^2 net reduction of grassland in 30 years was the combination of 432 km^2 loss of grassland and 192 km^2 gain of grassland. Similarly, the 209 km^2 net gain of cropland in 30 years was a combination of 492 km^2 gain of cropland and 283 km^2 loss of cropland (Figure 6.12B).

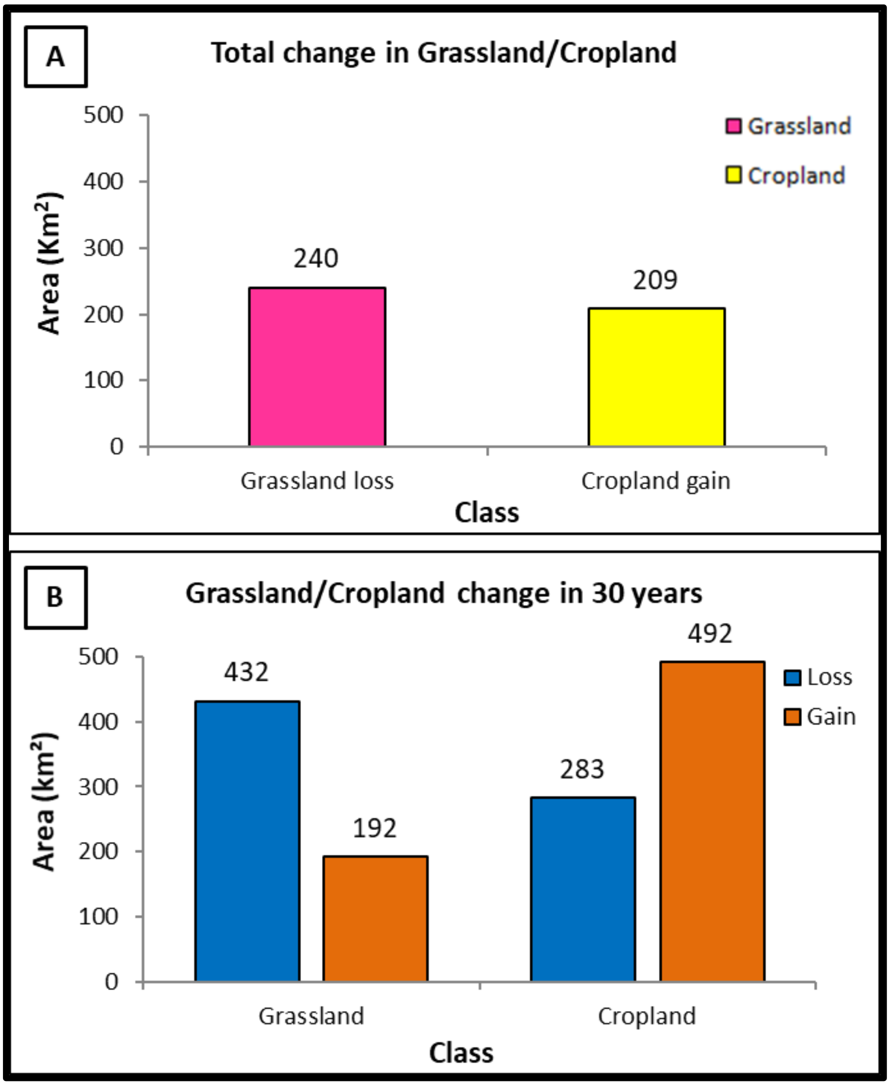


Figure 6.12 The net changes of two major LULC classes in the landscape: (A) Net loss of grasslands and net gain of croplands (B) The net changes of both classes have been splitted up into the individual components of loss and gain: Grasslands incurred a total net loss of 240 km² (432 km² of loss -192 km² of gain) while croplands increased by 209 km² (492 km² of gain -283 km² of loss)

<u>*6.4.3.3 Analysis of loss of grassland and gain of cropland*</u>

The study area had 418 km^2 area of grassland present in 1985 and it reduced to 178 km^2 in 2015 (57% reduction) (Figure 6.13A). At the same time 9% net gain was found in the cropland habitat from 1985 to 2015 (Figure 6.13B). As cropland was the dominant feature in this landscape during the entire study period, a 9% increase in 30 years is quite significant.

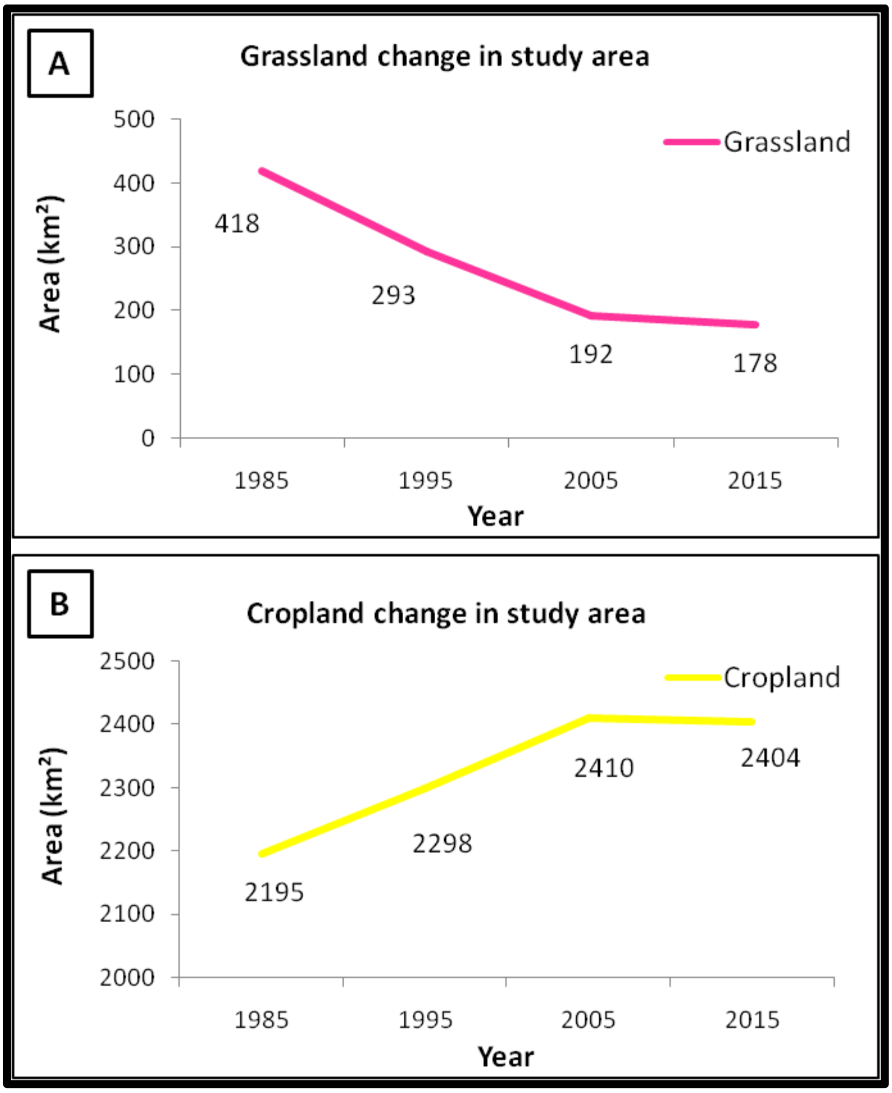


Figure 6.13 The change in area of two major LULC classes in the upper Gangetic block between 1985 and 2015: (A) Grassland (B) Cropland

As our study area encompasses a mosaic of protected and non-protected areas, we wanted to investigate the change in grassland dynamics within the protected and non-protected areas. Jhilmil Jheel Conservation Reserve was omitted from the analysis as it was formed in 2005. We found a reduction of grassland by 47 km^2 outside protected areas but the situation inside protected area of Hastinapur Wildlife Sanctuary was alarming. Hastinapur had 347 km^2 of grassland area in 1985 which reduced to 154 km^2 in 2015, thus incurring a net reduction of 193 km^2 (Figure 6.14A). Croplands also showed significant increase within HWLS (174 km^2) and a minor increase of 34 km^2 outside HWLS (Figure 6.14B).

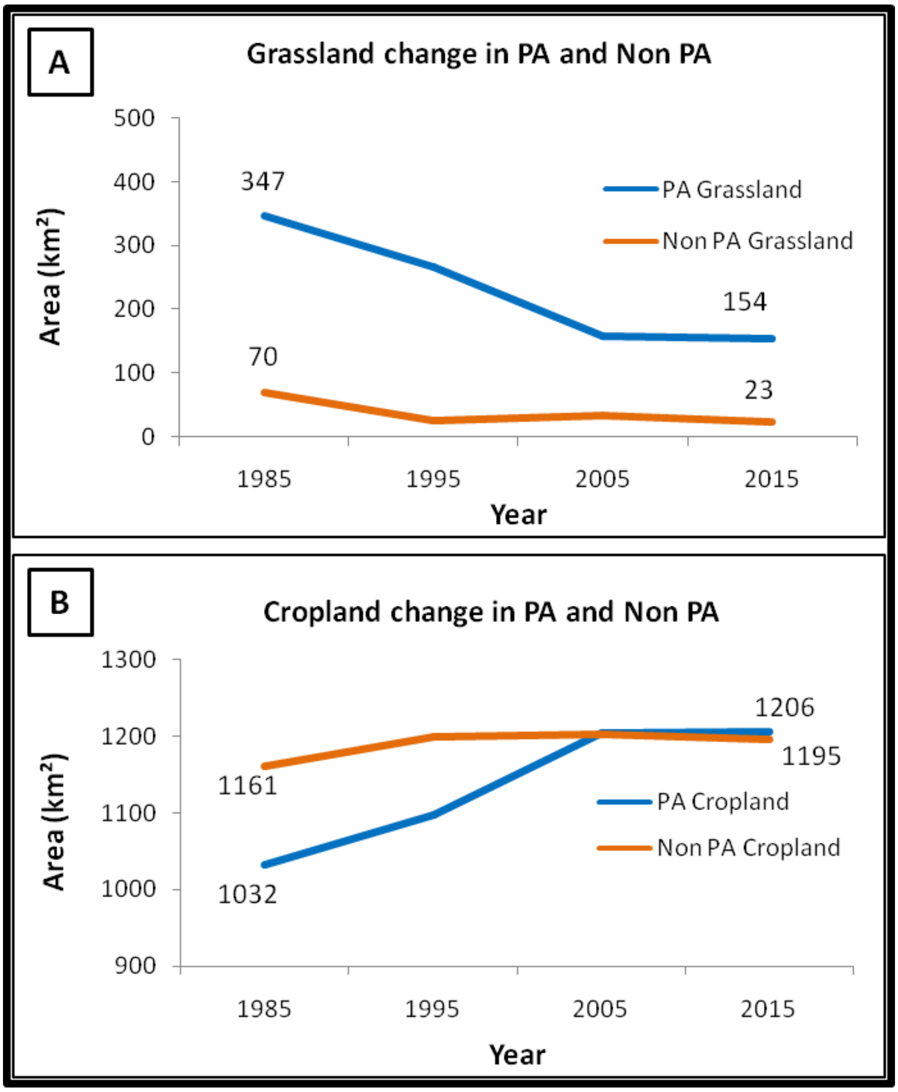


Figure 6.14 The change in area of two main LULC classes in the study area from 1985-2015: (A) Grassland in protected (blue line) and non-protected areas (orange line) (B) Cropland in protected (blue line) and non-protected areas (orange line)

6.4.3.4 Analysis of grassland gain and cropland loss

The changes in grassland and cropland (loss and gain in both cases) were teased apart decade wise to pinpoint the fine-scale time of grassland gain and cropland loss. It was observed that the loss of grassland and gain of cropland were predominant in the first two decades (1985-1995, 1995-2005) but loss and gain of grasslands in the last decade were similar (Figure 6.15A-B).

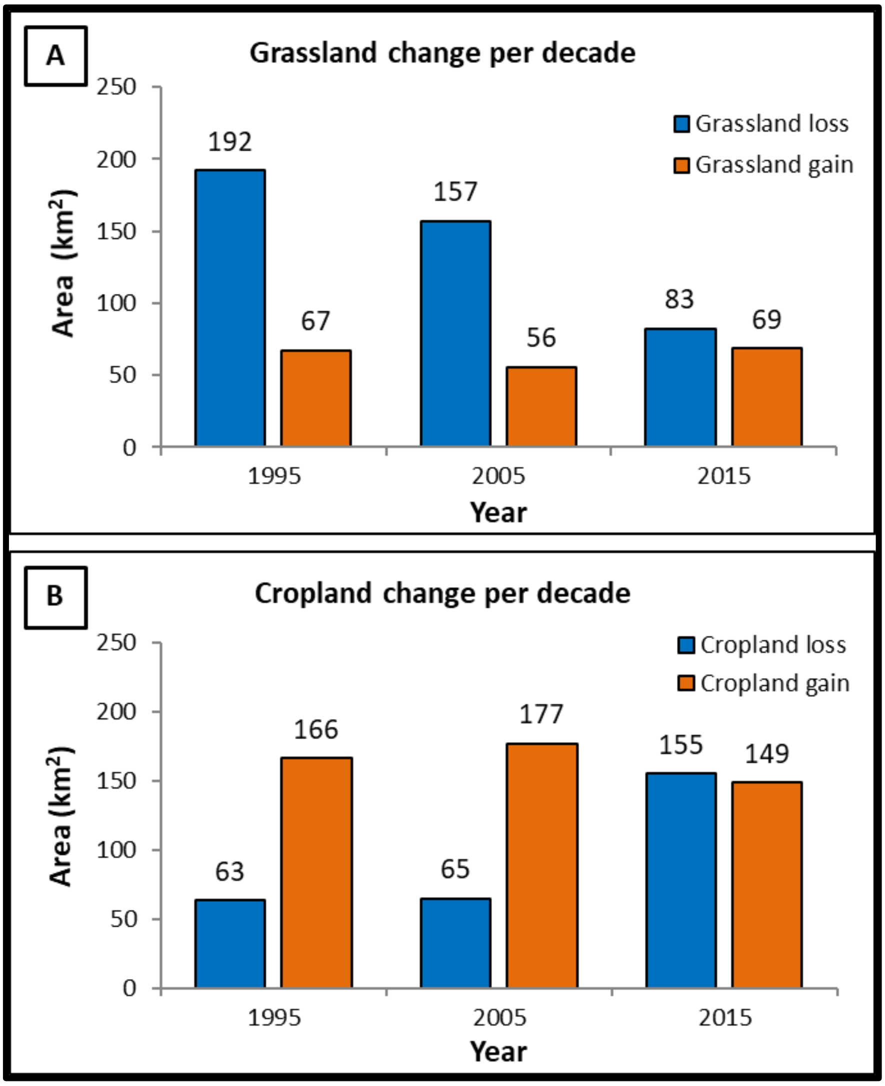


Figure 6.15 Fine-scale decade wise assessment of loss and gain of grassland and cropland in the landscape: (A) Grassland loss and gain status in 1995, 2005 and 2015 (B) Cropland loss and gain status in 1995, 2005 and 2015

The gain of grassland was recorded at multiple patches from 2005 to 2015 and one such instance has been described (Figure 6.16A-C). We find that due to shift in river courses in Rauli Ghat area of HWLS, new grassland was formed and survey in 2015 revealed swamp deer evidence in that patch (Figure 6.16D). However, upon subsequent surveys in 2017 and 2019, we documented increasing agricultural conversion of the grassland (Figure 6.16E-F).

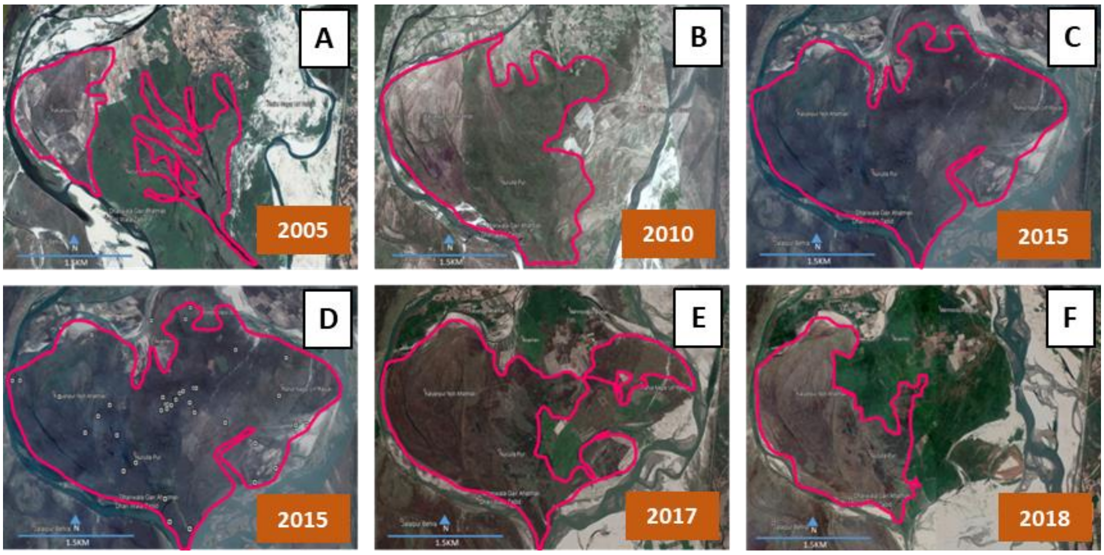


Figure 6.16 Dynamic changes of grassland in Rauli Ghat area of Hastinapur Wildlife Sanctuary from 2005 to 2019. The grassland area has been demarcated with pink boundaries. (A-C) Increase in grassland area from 2005 to 2015 (D) Swamp deer evidence within the grassland (E-F) Grassland conversion into cropland 2017 onwards

<u>6.4.3.5 Contribution of different LULC classes to grassland loss and cropland gain</u>

When we analysed the loss of grasslands (432 km^2) from 1985 to 2015 and the contributing factors to it (Figure 6.17A-B), we found that croplands contributed to 74% of grassland loss (Figure 6.18) while 65% of the gain in cropland is contributed by grassland (Figure 6.19).

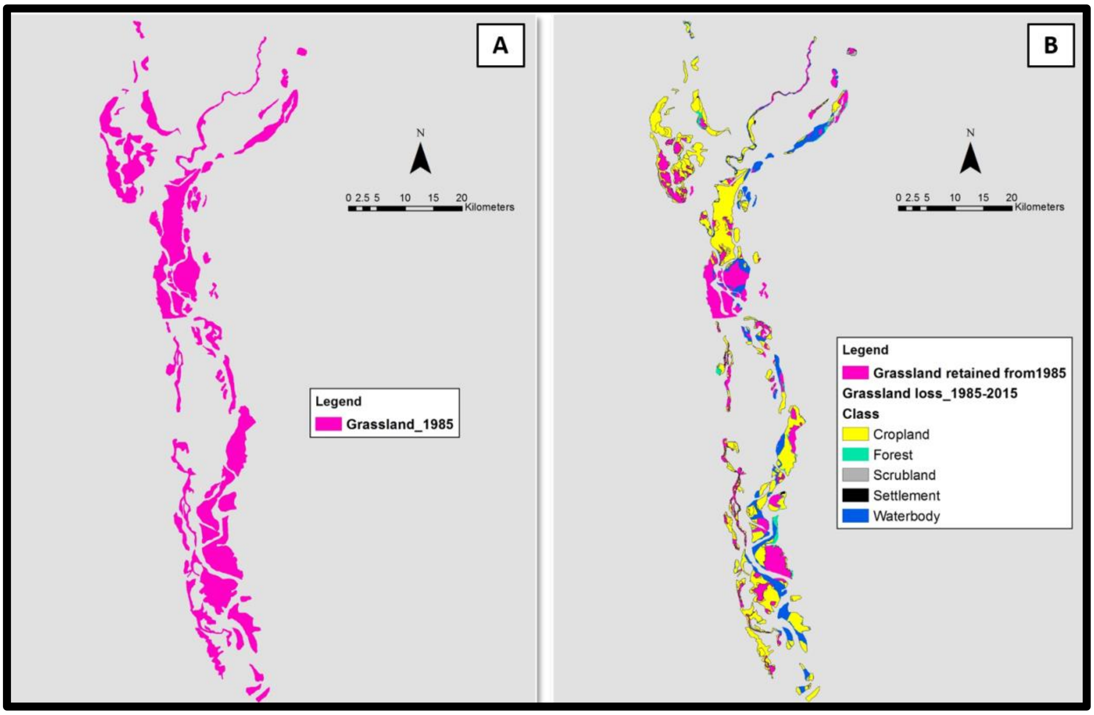


Figure 6.17 The change in grassland to other LULC classes from year 1985 to 2015: (A) The original grassland map of 1985 (B) The grasslands (pink) retained from 1985 in 2015 and grasslands converted (other colours) to other LULC classes in 2015

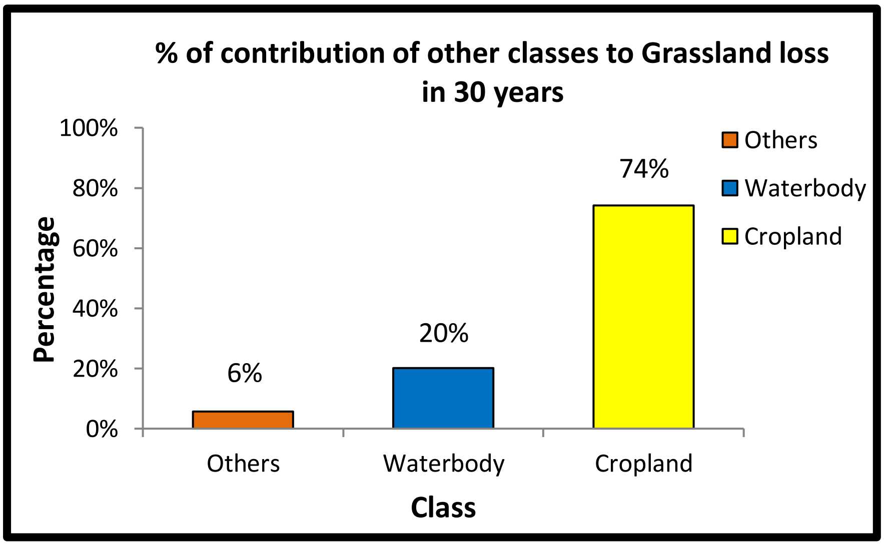


Figure 6.18 Proportional contribution of different LULC classes to grassland loss in 30 years within the study area

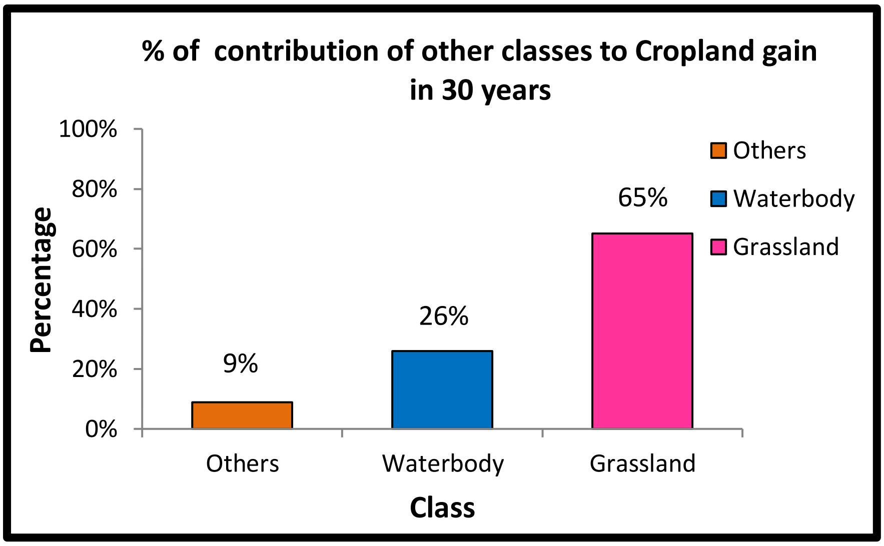


Figure 6.19 Proportional contribution of different LULC classes to cropland gain in 30 years within the study area

6.5 Discussion:

In this chapter, we have carried out a detailed assessment of present and past status of swamp deer habitats along Ganga in north India. We have mapped all the current swamp deer habitats, evaluated the grass species composition of these grasslands and assessed the change in grasslands over the last three decades in this landscape.

We generated the LULC map of the area using extensive robust surveys and visual interpretation of Landsat imageries. We opted for visual classification despite the recent popularity of digital classification as we had thorough knowledge about the study area (Puig et al., 2002). Visual interpretation has been found to show similar quality as digital classification for analysis of medium resolution satellite data (Puig et al., 2002). We classified the images into six different categories, as they were the major features of this landscape. Few orchards were merged with the croplands and sandbars were merged with waterbodies. In future, we can incorporate more classes when we analyse the swamp deer ecology in relation to LULC at a finer scale. We found croplands to be the most dominant feature of this landscape (75%) which supports livelihood of surrounding people. The fragmented grasslands and some inaccessible waterlogged swampy patches cover around 5% of the entire study area. These grasslands support populations of swamp deer, hog deer and other fauna but are also subjected to heavy encroachments and livestock grazing. The croplands also have settlements in their vicinity making this area completely human-dominated.

We have been able to broadly classify the grassland vegetation into four different types: Saccharum grasslands, Saccharum Dominated Mixed grasslands; Phragmites Dominated Mixed grasslands and Phragmites-Typha grasslands with high accuracy. Saccharum grasslands are the major grasslands in the landscape as altering river courses tend to keep the Saccharum grasslands afloat. For our grassland classification, we strategically placed plots taking ques from distinct NDVI values of four species and field knowledge (homogeneous areas of vegetation were covered using fewer plots than heterogeneous areas), thus enabling us to map the vegetation efficiently. In our grassland classification, we have generated vegetation data at a broad scale using only the four main grasses associated with barasingha biology. But apart from these four grassland species, other grasses are also sometimes present like *Cynodon* sp. and *Imperata* sp. which contribute to the overall reflectance values, thus incorporating errors. However, in our landscape, Cynodon and Imperata were sporadically present and did not influence the classification scheme significantly. It is also sometimes difficult to delineate other noises like varying presence of water in different grasslands which contributes to the overall NDVI value. We have classified NDVI based on

April-May data and incorporation of multi-season data in future would provide a more complete picture.

A detailed three decade long time-scale assessment of the grassland habitats proves that last of the Ganga grasslands are diminishing at an alarming rate as a result of conversion. Above 50% of grasslands in this landscape are lost in last three decades due to cropland expansion. The loss of 192 km^2 grassland in Hastinapur Wildlife Sanctuary in last 30 years is a serious cause of concern for swamp deer. A future prediction of grassland area in this landscape using the trend of grassland change in last 30 years data suggests that grassland will be reduced to 65 km^2 in 2025 which can have catastrophic effect on swamp deer (Figure 6.20). Various stochastic factors also contribute to grassland change, which has not been taken into account for this trend analysis. From field observations, we also documented plantation of Arjun, Eucalyptus on the newly formed grasslands by Forest Departments (Figure 6.21A-B). Although plantation prevents encroachment, but upon plantations the grasslands become uninhabitable for swamp deer. Therefore, plantation strategies should be revised in future but plantations within core swamp deer habitats should be avoided.

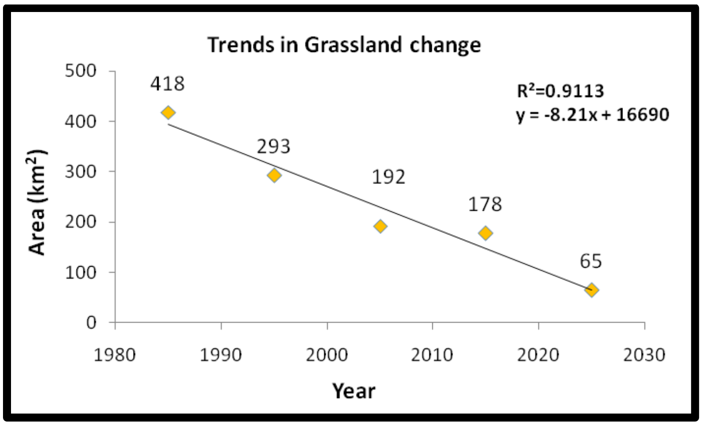


Figure 6.20 Linear regression prediction of grassland area in 2025 within the upper Gangetic habitat block using data from 1985-2015

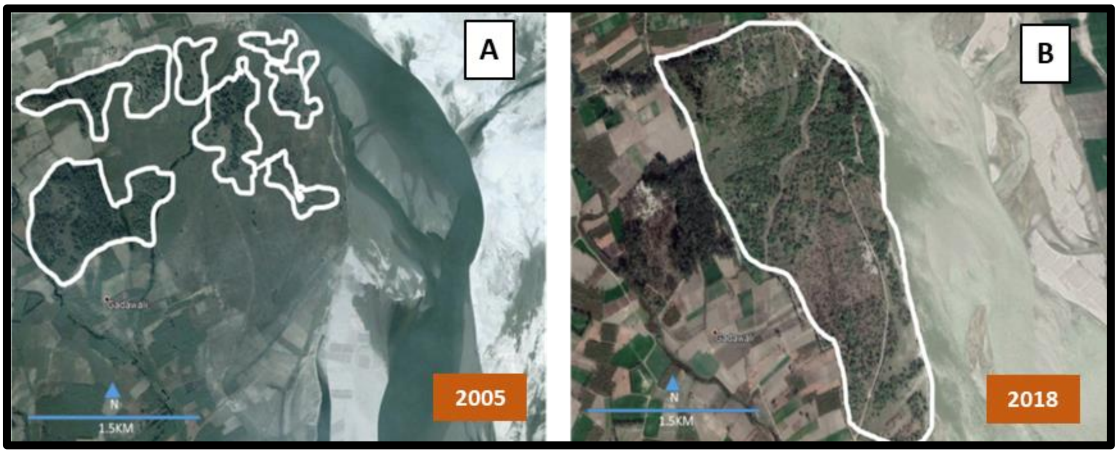


Figure 6.21 Conversion of grasslands to plantations: (A) The grasslands before plantation (white polygons) in 2005 (B) Grassland converted fully into plantation (white polygon) in 2018

According to recent studies, grasslands are one of the most marginalized and neglected ecosystems in India (Report of the task force on Grasslands and Deserts, 2006), with a loss of ~20 million hectares area between 1880 and 2010. The ‘‘Green Revolution’’ movement has brought about irrigation-based agricultural practices which has led to fast decline in grassland habitats (Vanak et al., 2017) across north India. The remaining grassland patches are subjected to immense anthropogenic pressures mainly in the form of livestock grazing (India is home to 535 million livestock population, the highest in the world) and encroachments (Chhabra et al., 2009; Livestock census 2019). There are some grassland patches along Ganga in this landscape which can revive back given grazing is checked. The land tenureship of new grasslands (formed due to river course changes) along Ganga is a problem. Such lands should be procured by Forest Departments to protect the habitats. The new grasslands in this landscape are critical for swamp deer and they have probably halted the swamp deer local extinction as these animals tend to colonise new patches quickly (as found in Rauli Ghat area). But we also recorded immediate encroachments in this area within five years which is a serious concern for future. The entire landscape between JJCR and HWLS should have grassland patches (even if fragmented) so that genetic connectivity and movement of swamp deer can be maintained. It is evident from our analysis that the future of swamp deer survival in this human-dominated landscape will lie in our ability to safeguard the remnant grasslands along Ganga.

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Key findings and conservation implications

7.1 Key findings:

The swamp deer is regarded as one of the most iconic species of the grasslands of the Gangetic plains. Swamp deer is reported from two major habitat blocks- the Sharda and the Gangetic habitat block. Majority of the work has been conducted in the Dudhwa Tiger Reserve of Sharda habitat block. Yet prior to this study, knowledge on swamp deer distribution and ecology in the Gangetic habitat block was limited. Surveys in 1967 and 2004 did not provide any conclusive evidence of swamp deer presence in this area (Qureshi et al., 2004; Schaller, 1967). A small population was reported in Bijnor Barrage area of Ganga in Hastinapur Wildlife Sanctuary in 1999 (Khan and Khan, 1999). It was only after the rediscovery of swamp deer in Jhilmil Jheel near Haridwar (Sinha and Chandola, 2006) this population along Ganga came into limelight. Further studies were concentrated only on Jhilmil Jheel population but a landscape-level assessment of swamp deer distribution and habitat status between Jhilmil Jheel and Hastinapur was lacking (Tewari and Rawat 2013a, b, c, d, e). The seasonal movement of swamp deer from Jhilmil Jheel and occasional sighting of swamp deer by local communities along Ganga formed the rationale for looking into the distribution, movement and habitat status of swamp deer in the Gangetic block for my PhD work.

A comprehensive pilot sign-survey along Ganga between Jhilmil Jheel Conservation Reserve and Hastinapur Wildlife Sanctuary covering both protected and non-protected areas revealed fine-scale presence of swamp deer in multiple fragmented patches along Ganga river. We found a completely unreported population of swamp deer along Ramganga river near Afzalgarh town in Uttar Pradesh. A total of 52 grassland patches covering 102.33 km^2 were identified between JJCR and HWLS. We reported habitat conversion, encroachments, poaching and sand mining as potential threats to swamp deer (Paul et al., 2018).

We further modeled the distribution of swamp deer in the entire Gangetic plains within the states of Uttarakhand and Uttar Pradesh taking location from surveys along Ganga as well as Sharda rivers. The survey along Sharda also revealed finer scale presence than earlier known (Paul et al., 2020; Qureshi et al., 2004). The predicted area of distribution for swamp deer covered only 3% of the total landscape. Grassland cover, annual mean temperature and distance to rivers were identified as important drivers of swamp deer distribution (contribution>80%). Further, we conducted ground validation of MaxEnt prediction in selected areas (accuracy- 84.37%) and reported new areas of swamp deer presence, most notably in the grasslands along Ganga south of Bijnor Barrage. All information from surveys, modeling and validation surveys enabled us to identify four "Priority Conservation Areas" that need critical management actions (Paul et al., 2020).

The viability of swamp deer population residing in the fragmented grassland patches depends on the gene flow and movement within this landscape. We thus investigated the genetic variation and connectivity of swamp deer within the Gangetic habitat block. We standardized molecular markers for species and sex identification of swamp deer from field collected faecal samples (Paul et al., 2019). For individual identification, we adopted M13 approach which helped us to screen a large number of microsatellite molecular markers and finalize a panel of 13, capable of statistically differentiating siblings ($P_{ID\ (sibs)}$ value $=1.12x10^{-4}$) within this population. From 420 samples (antlers and pellets), we identified a minimum of 273 individuals in the entire landscape. We found that the swamp deer population along Ganga between JJCR and HWLS is an intermixing population with low inbreeding (FIS=0.09). The dispersed spatial distribution pattern of highly related individuals in this landscape indicated movement of swamp deer among the fragmented grassland patches along Ganga.

We radio-collared two swamp deer hinds in JJCR and their movements were intensively followed over a year. The swamp deer moved along Ganga, extensively using the grassland patches between JJCR and HWLS which validated genetic connectivity in this landscape. We observed small home ranges (core area usage was 0.58 and 1.47 km^2 for the two individuals respectively) for the two collared swamp deer. Grasslands were predominantly used though their availability in the landscape was much less (Ivlev's index 0.8). Croplands were used sporadically and only for traversing between grassland patches. We documented swamp deer showing directional movement (high speed and low turning) outside the grassland patches as lack of cover forced faster movement events. Swamp deer were present in small groups in the non-protected areas and showed complete temporal segregation from humans (21% temporal overlap). The non-protected grasslands along Ganga were identified to be critical fawning and breeding grounds.

The last chapter discusses the current and historic grassland status in this landscape. We created a detailed Land Use Land Cover map of our entire study area using field data and classified images. Grasslands cover 5% of our landscape while the majority is occupied by croplands (76%). We classified the vegetation of these grasslands into four types based on the four predominant species found (*Saccharum bengalense,* *Saccharum spontaneum*, *Typha* sp. and *Phragmites* sp.) with 86% accuracy. Saccharum grasslands were major vegetation type found in our landscape. Our change detection analysis revealed that the Ganga habitat block has lost 57% of its grasslands in the last 30 years. The loss is more prominent within the protected areas of this landscape. Grassland to cropland conversion has accounted for 70% loss of grasslands. However, instances of increase in grasslands and decrease in croplands especially between 2005-2015 due to change in river flow were recorded, which highlights the dynamic nature of these Gangetic grasslands.

7.2 Management actions and recommendations:

In summary, my doctoral thesis generates baseline knowledge on swamp deer ecology in a non-protected human-dominated landscape. The key management actions that have been undertaken based on this study and future recommendations are described below.

1) The Ganga population is the westernmost distribution of swamp deer in its current range and is also situated approximately 200 km from the Sharda habitat block. Though this doctoral study did not engage in any population estimation, genetic data shows there are a minimum of 273 individuals in this landscape between JJCR and HWLS. Thus, this area should be protected as a separate conservation unit.

2) The findings of this doctoral work have identified the 32.57 km^2 area around Bijnor Barrage to be the largest wetland patch of the entire landscape. As this wetland supports considerable fauna like swamp deer, hog deer, fishing cat and huge flocks of wetland birds, State Government of Uttar Pradesh has put forward a proposal for this

wetland to be considered as a “Ramsar Site”.

3) Our surveys and radio-collaring data highlight the importance of grasslands between JJCR and HWLS in swamp deer conservation. Even as agricultural expansion has caused continuous grassland degradation, these remaining grasslands along Ganga have acted as a functional corridor for swamp deer movement in this landscape. Preliminary observations from movement data suggest a difference in movement patterns during different seasons. Both the animals showed extensive movement during the monsoon which hints to the corridor being dynamic or temporal (Bencharif, 2010). The enhanced movement of swamp deer during monsoons may be guided by flooding of natural habitats, presence of cover in the landscape (grass height reaches maximum within grasslands and sugarcane fields), dry fawning grounds and minimal human disturbances (as most areas are inaccessible). Future studies in this area should focus on how seasonal variation in resources, landscape features (vegetation cover, water volume in rivers), environmental covariates (precipitation) and anthropogenic covariates (human disturbance regimes) affect movement patterns in this landscape. Similar work done on elephants emphasizes the importance of seasonal variability of resources in influencing connectivity in a landscape (Osipova et al., 2018). Such fine-scale data could help in preserving the corridors and ensure safety passage for swamp deer from JJCR to HWLS.

4) A considerable fraction of this Ganga population resides in grassland fragments outside the protected areas. Swamp deer outside Jhilmil Jheel and Hastinpaur WLS are vulnerable to poaching and habitat loss and thus these grasslands need to be safeguarded. The grasslands in the Gangetic habitat block are all restricted to 2-3 km

on either side of Ganga, Banganga and Solani. Extensive patrolling in these grasslands is sufficient for monitoring poaching and illegal encroachment activities. Conservation and management of *Rucervus duvaucelii duvaucelii* as an umbrella species in unprotected areas would aid the conservation of swamp deer habitats in the upper Gangetic plains, but success will depend on the involvement of local communities. Directed efforts from government-initiated large-scale programmes such as the National Mission for Clean Ganga on reviving and protecting the swampy grassland patches along the Ganga could benefit swamp deer recovery.

5) Hastinapur WLS covers 2073 km^2 and its initial designated area covers parts of cities of Muzaffarnagar and Bijnor while the grasslands are only found along the rivers. This sanctuary is completely human-dominated and thus implementing all the rules and regulations of a sanctuary becomes a difficult task, ultimately hampering wildlife conservation. Based on the data from this thesis, the State Forest Department has put forward a proposal for realigning the boundaries to cover swamp deer habitats along rivers and exclude other human-dominated areas (Figure 7.1). Though 978 km^2 area loss will be incurred by the protected area, a pragmatic rational approach to safeguard the grasslands and its fauna is required.

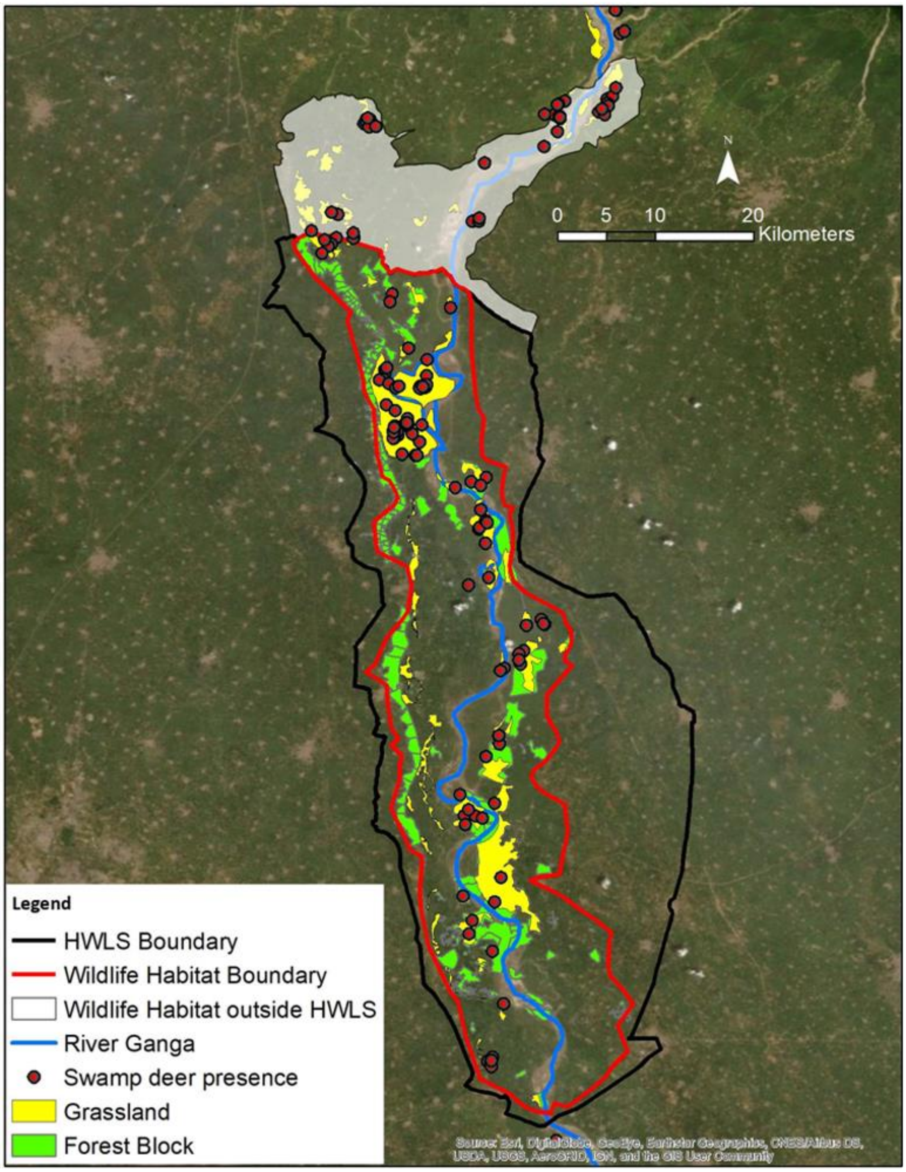

Figure 7.1 Proposed realignment of Hastinapur Wildlife Sanctuary boundary

6) Swamp deer is currently classified as "Vulnerable" according to IUCN (Duckworth et al., 2015), but slow life history, habitat specialisation, restricted and fragmented distribution range and a global population of less than 5000 makes it extinction prone. The status of a similar threatened species Eld's deer has been changed from Vulnerable to Endangered in 2008 (Timmins and Duckworth, 2008) and a similar relook into the conservation status of swamp deer is necessary.

Therefore, this doctoral work brings into focus the conservation of habitat-specific large herbivores outside protected areas worldwide. The implications of this work are much beyond swamp deer conservation as these grassland habitats host a large number of other mammals, birds and other biodiversity. Unfortunately, they are under enormous pressure of conversion to croplands (a rapidly ongoing phenomenon) and we believe that this study will bring critical focus on long-term protection of these patches. In a global scenario, the approach, results and inferences are relevant to other biologists interested in conservation of habitat-specialists living across human-dominated areas.

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List of publications:

Paul, S., Pandav, B., Mohan, D., Habib, B., Nigam, P. & Mondol, S. (2018) Current distribution and status of swamp deer *Rucervus duvaucelii duvaucelii* in the upper Gangetic plains of north India. Oryx 52, 646–653.

Biswas, S., Bhatt, **S., Paul**, S., Modi, S., Ghosh, T., Habib, B., Nigam, P., Talukdar, G., Pandav, B. & Mondol, S. (2019) A practive faeces collection protocol for multidisciplinary research in wildlife science. Current Science 116:1878-1885.

Paul, S., Ghosh, T., Pandav, B., Mohan, D., Habib, B., Nigam, P. & Mondol, S. (2019) Rapid molecular assays for species and sex identification of swamp deer and other coexisting cervids in human-dominated landscapes of the Terai region and upper Gangetic plains, northern India: implications in understanding species distribution and population parameters. Journal of Genetics 98, 44.

Paul, S., Sarkar, D., Patil, A., Ghosh, T., Talukdar, G., Kumar, M., Habib, B., Nigam, P., Mohan, D., Pandav, B., Mondol, S. (2020) Assessment of endemic northern swamp deer (*Rucervus duvaucelii duvaucelii*) distribution and identification of priority conservation areas through modeling and field surveys across north India. Global Ecology and Conservation 24, e10263.

List of conferences:

International conferences

Paul, S., Sarkar, D., Patil, A., Ghosh, T., Talukdar, G., Kumar, M., Habib, B., Nigam, P., Mohan, D., Pandav, B. & Mondol, S. (2018) Modeling spatial distribution of northern swamp deer subspecies (*Rucervus duvaucelii duvaucelii*) across upper Gangetic plains, India using survey and molecular data. Oral presentation at 55th Annual Meeting of the Association of Tropical Biology and Conversation 2018, Kuching, Sarawak, Malaysia, July 1-July 5, 2018.

Paul, S., Pandav, B., Habib, B., Nigam, P., Mohan, D. & Mondol, S. (2019) Movement patterns of endemic swamp deer across fragmented, human-dominated landscape of the northern Indian subcontinent. Poster presentation at Wildlife Research and Conservation, Berlin, Germany, September 30-October 2, 2019.

Paul, S., Pandav, B., Habib, B., Nigam, P., Mohan, D. & Mondol, S. (2019) The last of the grasslands along river Ganga of north India: Key to swamp deer movement and genetic connectivity in a human-dominated landscape. Oral presentation at British Ecological Society Annual Meeting, Belfast ICC, Northern Ireland, December 10-December 13, 2019**.**

Institutional conferences

Paul, S., Pandav, B., Habib, B., Nigam, P., Mohan, D. & Mondol, S. (2016) Status and distribution of swamp deer along Ganga river and its tributaries in north India. Oral presentation at XIIth Internal Annual Research Seminar, September 26-September 30, Wildlife Institute of India, Dehradun, India.

Paul, S., Pandav, B., Habib, B., Nigam, P., Mohan, D. & Mondol, S. (2017) Predicting swamp deer distribution in the upper Gangetic plains using a combination of survey and molecular approaches. Oral presentation at XIIIth Internal Annual Research Seminar, August 30-September 6, Wildlife Institute of India, Dehradun, India.

Paul, S., Pandav, B., Habib, B., Nigam, P., Mohan, D. & Mondol, S. (2018) Movement ecology of swamp deer along upper Gangetic plains of north India. Oral presentation at XXXIInd Annual Research Seminar, September10-September 20, Wildlife Institute of India, Dehradun, India.

Paul, S., Pandav, B., Habib, B., Nigam, P., Mohan, D. & Mondol, S. (2019) Grasslands on the wane: A three-decade long assessment of swamp deer habitat dynamics in the upper Gangetic plains. Oral presentation at XXXIIIrd Annual Research Seminar, August 19-August 27, Wildlife Institute of India, Dehradun, India.

ANNEXURE- I

Permits and ethical clearance:

All required permissions for our surveys and biological sample collection were provided by the Forest Departments of Uttarakhand (Letter no. 978/6–32/56 and 1875/C-32) and Uttar Pradesh (Letter no. 1127/23-2-12 (G), 2233/23–2–12 G and 3438/23-2-12 (G)).

The Forest Department of Uttarakhand (Letter no. 1-76/2017 WL) provided permission for capture and radio-collaring swamp deer in Uttarakhand.

ANNEXURE- II

Conference certificates:

Certificate of Recognition

This certificate is presented to

SHRUTARSHI PAUL

for having presented an abstract at the

55th Annual Meeting of the Association for Tropical Biology and Conservation

Linking Natural History and the Conservation of Tomorrow's Tropical Ecosystems

July 1-5, 2018 Pullman Kuching, Sarawak

Dr Ahimsa Campos-Arceiz
Organising Chairman
55th Annual Meeting of the Association for Tropical Biology and Conservation

Dr Mohd-Azlan Jayasilan
Co-Chair
55th Annual Meeting of the Association for Tropical Biology and Conservation

Leibniz-Institut für Zoo- und Wildtierforschung
IM FORSCHUNGSVERBUND BERLIN E.V.

Leibniz Gemeinschaft

LEIBNIZ-INSTITUT FÜR ZOO- UND WILDTIERFORSCHUNG (IZW) . Alfred-Kowalke-Str. 17 . 10315 BERLIN

Shrutarshi Paul
Wildlife Institute of India
Animal Ecology and Conservation Biology
Chandrabani, Dehradun, Uttarakhand, 248001
India

Berlin, 8th January 2020

Certificate of poster presentation

Dear Sir or Madam,

We confirm that Shrutarshi Paul presented the following poster at the Wildlife Research and Conservation 2019 conference in Berlin, Germany (30th September - 2nd October 2019):

Title: *Movement patterns of endemic swamp deer across fragmented, human-dominated landscape of the northern Indian subcontinent*

Authors: Shrutarshi Paul, Bivash Pandav, Dhananjai Mohan, Bilal Habib, Parag Nigam, Samrat Mondol

Sincerely,

J. Prügel

Josepha Prügel
(on behalf of the Conference Organising Committee)

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BES Annual Meeting
10 – 13 December 2019
Belfast, UK

This certificate confirms that:

Shrutarshi Paul

Attended the above Annual Meeting in Belfast and presented the below talk in the session Conservation Science and Policy:

Movement ecology and genetic connectivity of swamp deer (Rucervus duvaucelii duvaucelii) across human-dominated landscape of North India

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Current distribution and status of swamp deer *Rucervus duvaucelii duvaucelii* in the upper Gangetic plains of north India

Shrutarshi Paul*, Bivash Pandav*, Dhananjai Mohan, Bilal Habib
Parag Nigam and Samrat Mondol

Abstract The swamp deer *Rucervus duvaucelii* is the largest grassland-dwelling endemic cervid of India and Nepal. With a declining population trend across its range, this species is found in fragmented habitats of northern, north-eastern and central India and south-western Nepal. The northern swamp deer subspecies *Rucervus duvaucelii duvaucelii* occurs in small wetland patches across the states of Uttarakhand and Uttar Pradesh in India and has lost most of its habitat in the last century. Information about the distribution of the swamp deer in the upper Gangetic plains is limited, except in the Jhilmil Jheel Conservation Reserve in Uttarakhand and around the Bijnor barrage area of Hastinapur Wildlife Sanctuary in Uttar Pradesh. We surveyed the upper Gangetic plains between the Reserve and the Sanctuary, including some adjoining areas and three tributaries of the Ganges, to assess the status of these habitats, current swamp deer distribution and the threats faced by the species. We found several areas harbouring swamp deer within non-protected wetlands along the entire surveyed stretch of the upper Ganges and a previously unreported population in Uttar Pradesh. We documented major threats including habitat conversion, livestock grazing, poaching, conflict and other anthropogenic disturbances. We recommend community driven conservation and management of *Rucervus duvaucelii duvaucelii* in this fragmented landscape to ensure survival of this species and other threatened fauna of these wetlands and grasslands.

Keywords Conservation, habitat loss, Hastinapur Wildlife Sanctuary, Jhilmil Jheel Conservation Reserve, poaching, *Rucervus duvaucelii*, swamp deer, upper Gangetic plains

Introduction

The swamp deer *Rucervus duvaucelii* or barasingha is an obligate swampy grassland-dwelling large cervid endemic to India and Nepal (Qureshi et al., 2004). Historically swamp deer were widely distributed throughout the Indo-Gangetic plains and the lowlands flanking the southern Himalayas from Pakistan to Bangladesh and through to India (Schaller, 1967; Groves, 1982; Sankaran, 1989). With a current global population size of < 5,000 (Qureshi et al., 2004; Tewari & Rawat, 2013a) and a declining trend across its range (Duckworth et al., 2015), the swamp deer is restricted to isolated pockets in north, north-east and central India and south-west Nepal (Qureshi et al., 2004). The species is categorized as Vulnerable on the IUCN Red List (Duckworth et al., 2015) and is listed in Appendix I of CITES and Schedule I (highest level of protection) of the Wildlife Protection Act of India (1972). There are three subspecies of swamp deer. The northern subspecies *Rucervus duvaucelii duvaucelii* occurs in the north Indian states of Uttar Pradesh and Uttarakhand and in Nepal. The hard ground barasingha *Rucervus duvaucelii branderi* (Pocock, 1943; Ellerman & Morrison-Scott, 1951) occurs in a single population in central India, and the eastern subspecies *Rucervus duvaucelii ranjitsinhi* occurs in the state of Assam (Groves, 1982; Qureshi et al., 2004). All swamp deer populations in India have declined in the last century because of increased anthropogenic pressure and changing land use practices (Qureshi et al., 2004). The long-term viability of the remaining populations is under threat from habitat loss and degradation, leading to isolated populations, disturbance from livestock grazing and poaching (Qureshi et al., 2004).

Rucervus duvaucelii duvaucelii is the most abundant of the subspecies, comprising c. 80% of the global population (Qureshi et al., 1995, 2004). This subspecies occurs in small, fragmented populations across the states of Uttar Pradesh (Hastinapur Wildlife Sanctuary, Bijnor Forest Division, Pilibhit Tiger Reserve, Kishanpur Wildlife Sanctuary, Dudhwa National Park and Katerniaghat Wildlife Sanctuary) and Uttarakhand (Jhilmil Jheel Conservation Reserve) (Qureshi et al., 2004). It was thought to be extinct in the state of Uttarakhand, but was rediscovered in 2005 at Jhilmil Jheel Conservation Reserve (Sinha & Chandola, 2006), followed by reports of another small population in the Banganga wetland region, Uttarakhand (Tewari & Rawat, 2013a). The nearest known swamp deer habitat to these populations is c. 53 km downstream, in the area of Bijnor barrage in Hastinapur Wildlife Sanctuary, Uttar

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Pradesh (Khan & Khan, 1999; Duckworth et al., 2015). The area between these habitats is unprotected.

The ecology of *R. duvaucelii duvaucelii*, specifically habitat use, feeding habits, diet, herd size and threats, is well studied in and around Pilibhit Tiger Reserve, Kishanpur Wildlife Sanctuary, Dudhwa National Park and Katerniaghat Wildlife Sanctuary of Uttar Pradesh (Qureshi et al., 1995, 2004). These populations are also well protected inside the tiger reserves. However, populations in the upper Gangetic plains are less studied, except for those in the Jhilmil Jheel Conservation Reserve (Tewari & Rawat, 2013a–e). The last swamp deer status report, based on a survey conducted along the Ganges river in 1995, found no conclusive evidence of its presence between Jhilmil Jheel Conservation Reserve and Hastinapur Wildlife Sanctuary (Qureshi et al., 2004), despite anecdotal reports from local communities of swamp deer sightings in some wetland patches. Several smaller patches of swamp habitat are present between these two areas, but information on swamp deer status outside Jhilmil Jheel Conservation Reserve is poor. Swamp deer regularly congregate in the Reserve during summer and then migrate with the onset of monsoon. Anecdotal reports suggest they move towards the Ganges, through heavily modified landscapes. Given the paucity of information on the current distribution of the swamp deer in the upper Gangetic plains it is critical to evaluate the subspecies' status in this region to support its conservation.

In this study we assessed the current status of *R. duvaucelii duvaucelii* along both banks of the upper Ganges and its tributaries. We surveyed the entire stretch of the Ganges from Jhilmil Jheel Conservation Reserve to the Bijnor barrage area of Hastinapur Wildlife Sanctuary and adjoining areas, including some tributaries, searching for evidence of occurrence, assessing potential habitats and their status, and documenting threats faced by the species.

Study area

Swamp deer prefer wetlands (also called swamps or *tals* locally) and flooded grasslands (Tewari & Rawat, 2013a). The study area includes the swampy grasslands along a stretch of the upper Ganges and adjoining areas. We surveyed both the west and east banks along the Ganges between the Jhilmil Jheel Conservation Reserve (38 km^2) and the Bijnor barrage area of the Hastinapur Wildlife Sanctuary (2,073 km^2), and grasslands along three tributaries of the Ganges: Solani and Banganga rivers on the west, and Ramganga river on the east bank (Fig. 1). The study area covers 1,061 km^2, excluding the banks of Ramganga, where we surveyed an additional 63 km^2 area. The main vegetation in the grassland patches comprises *Typha* sp., *Phragmites* sp. and *Saccharum* sp. Larger animals in the study area include swamp deer, hog deer *Axis porcinus*, spotted deer *Axis axis*, nilgai *Boselaphus tragocamelus*, smooth-coated otter *Lutrogale perspicillata*, fishing cat *Prionailurus viverrinus* and wetland birds such as sarus crane *Antigone antigone*, black-necked stork *Ephippiorhynchus asiaticus*, lesser adjutant *Leptoptilos javanicus*, Pallas's fish eagle *Haliaeetus leucoryphus* and bar-headed goose *Anser indicus*.

Methods

Surveys

Because the last survey between Jhilmil Jheel Conservation Reserve and Hastinapur Wildlife Sanctuary found no conclusive evidence of swamp deer presence (Qureshi et al., 2004) and there is no information available on the current status of potential swamp deer habitat, we first gathered information from local communities living close to the Ganges. These communities consist mainly of villagers, cattle herders, farmers, boatmen (who provide transport services with small boats) and fishermen. We collected information about swamp deer presence and their preferred habitat from 42 people (11 villagers, 16 cattle herders, 8 farmers, 3 boatmen and 4 fishermen), using a semi-structured questionnaire survey. The interviews typically took 10–20 minutes, and the following information was collected: (1) the interviewee's residential status and (2) occupation, (3) general mammalian diversity in their area, (4) location of swamps or grasslands in the area, if any, (5) information on swamp deer presence and specific characteristics used for identification, (6) approximate time and day of any swamp deer sightings, (7) presence of antlers in the area, (8) willingness to locate the antlers, if any had been seen in the area, (9) conflict caused by swamp deer crop use in the area and (10) any hunting or poaching incidences known from the area. We also used Google Earth (Google, 2015) images to identify grassland patches to corroborate with the information on swamp deer habitat provided by the local residents.

This information revealed that most of the grassland areas are restricted to 2–3 km on both sides of the Ganges and its tributaries, and are extremely fragmented along both banks of the 53 km stretch between Jhilmil Jheel Conservation Reserve and Hastinapur Wildlife Sanctuary. Subsequently, we conducted a focused survey covering up to 5 km on both banks of the Ganges and its tributaries in this area. We divided the study area into 10 survey zones (Fig. 1) based on presence of grasslands, tributaries, road network, croplands and human habitations and surveyed each zone extensively for evidence of swamp deer presence. Surveys were conducted on foot, by road vehicle or boat, depending on the habitat characteristics, during December 2015–November 2016, excluding the monsoon season of July–October 2016. The total survey effort was c. 4,692 km

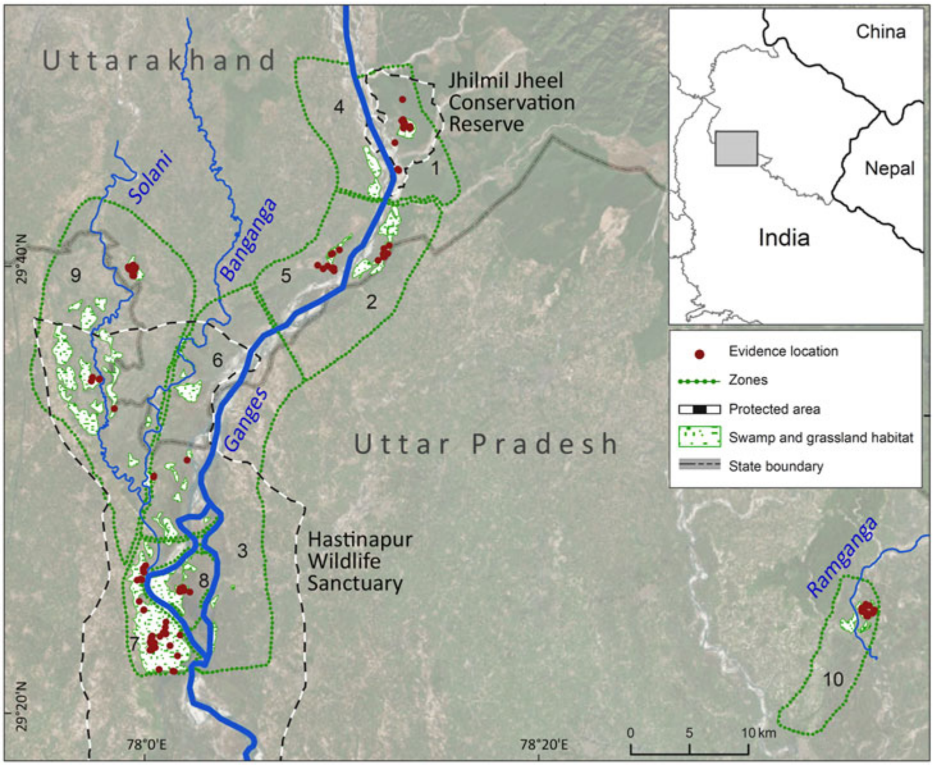

FIG. 1 The swamp deer survey area between the Jhilmil Jheel Conservation Reserve and the Bijnor barrage area of Hastinapur Wildlife Sanctuary in the upper Gangetic plains, north India. The map shows the survey zones, swamp and grassland habitats and locations of direct (sightings of live animals and carcasses) and indirect (antler, hoofmark and genetically identified faecal pellet) swamp deer evidence.

(Table 1). Each zone was surveyed three times over 15–18 days from 6.00 to 18.00, with occasional night surveys.

The east bank of the Ganges was divided into three survey zones: Zone 1 comprising Jhilmil Jheel Conservation Reserve, a swampy grassland and surrounding areas in Uttarakhand; Zone 2 comprising an area of grassland 15 km downstream and stretching to a distance of 26 km from the Reserve, in Uttarakhand and Uttar Pradesh; Zone 3 extending further south to the Bijnor barrage area of Hastinapur Wildlife Sanctuary in Uttar Pradesh.

The west bank of the Ganges was divided into five survey zones: Zone 4 comprising the area opposite Jhilmil Jheel Conservation Reserve in Uttarakhand, a mostly human-dominated landscape interspersed with a small number of grassland and wetland patches; Zone 5 covering a wetland south of Zone 4, adjacent to the Ganges in Uttarakhand; Zone 6 covering human-dominated areas, partly within the boundary of Hastinapur Wildlife Sanctuary, across Uttarakhand and Uttar Pradesh; Zone 7 including the Bijnor barrage area of Hastinapur Wildlife Sanctuary and adjoining regions in Uttar Pradesh; Zone 8 containing a number of river islands close to Zone 7 within Hastinapur Wildlife Sanctuary in Uttar Pradesh.

In addition, we surveyed the grasslands along three tributaries of the Ganges: the Solani and Banganga on the west bank and the Ramganga on the east bank. Zone 9 comprised swampy grasslands along the Solani, located c. 10 km from the Ganges, partly within Hastinapur Wildlife sanctuary and covering parts of Uttarakhand and Uttar Pradesh. The grasslands along the Banganga were covered by Zones 6 and 9. Zone 10 included the swampy areas on both banks of the Ramganga near the town of Afzalgarh. This survey was motivated by a local newspaper report of swamp deer poaching in that region. Following interviews with the local communities, we conducted surveys for swamp deer habitats and presence in this area.

The protection status varies between survey zones: Zone 1 covers Jhilmil Jheel Conservation Reserve, and Zones 7 and 8 in Uttar Pradesh lie entirely within Hastinapur Wildlife Sanctuary. But Zones 3, 6 and 9 are only partially within the boundary of the Sanctuary, and Zones 2, 4, 5 and 10 are entirely unprotected.

During the survey, we considered direct sightings of live animals, presence of carcasses, and swamp deer signs such as antlers, hoof marks and dung as evidence of swamp deer presence. Direct sightings and carcasses were considered the best evidence of presence, followed by antlers, hoof marks and faecal pellets. Swamp deer antlers are distinct from those of other cervid species occurring in the area. An adult swamp deer antler usually has five or six tines and branches in a dichotomous manner in the upper half of the beam, whereas sambar, spotted deer and hog deer antlers are typically three-tined. Swamp deer hoof marks can also be identified by their distinctive splayed pattern, an adaptation to living in swampy habitats. Swamp deer faecal pellets are generally larger than those of other sympatric cervids (hog deer) or domestic animals (sheep or goats) occurring in this area, but pellets were not considered as

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TABLE 1 Details of swamp deer *Rucervus duvaucelii duvaucelii* surveys in the upper Gangetic plains (Fig. 1) during December 2015–November 2016, with area, survey effort, habitat characteristics, direct and indirect evidence of swamp deer presence, and evidence of disturbance.

Survey zones	Survey dates	Area (km^2)	Survey effort (km)	Names of local areas with swamp deer evidence	Habitat characteristics	Direct sightings	Carcasses	Antlers	Hoofmarks	Faecal pellets	No. of pellets analysed (swamp deer DNA confirmed)	Disturbance
East bank of Ganges												
Zone 1	Dec. 2015–Mar. 2016	80	475	Jhilmeel Jheel Conservation Reserve	Wetland area with large swamp; grasslands dominated by *Typha* sp. & *Phragmites* sp.	Yes (n = 127–162)	1	61	Yes	1,301	20 (10)	Livestock grazing
Zone 2	Jan., Mar. & Nov. 2016	112	500	Amichand, Nangal, Bhuria Sot	Patchy grassland habitat with agricultural fields; grasslands dominated by *Typha* sp., *Phragmites* sp. & *Saccharum* sp.	No	3	3	Yes	43	6 (5)	Sand mining & poaching
Zone 3	Apr.–June 2016	178	409	No evidence of swamp deer	Human-dominated area with croplands	No	0	0	No	0	0 (0)	
West bank of Ganges												
Zone 4	Feb., May & June 2016	72	363	No evidence of swamp deer	Human-dominated area with croplands; small patches of wetlands with *Typha* sp.	No	0	0	No	0	0 (0)	
Zone 5	Mar., Apr. & June 2016	69	438	Ranjitpur	Small wetland area surrounded by human habitation; wetlands dominated by *Typha* sp.	No	0	4	Yes	52	14 (13)	Livestock grazing & habitat exploitation
Zone 6 (incl. Banganga river)	Apr., May & June 2016	145	503	Balia Khadar	Grasslands habitat interspersed with agricultural fields; grasslands dominated by *Saccharum* sp.	No	0	0	Yes	4	4 (1)	Livestock grazing, habitat encroachment & agriculture
Zone 7	Jan., May & June 2016	66	561	Bijnor barrage	Extensive swamp with contiguous grassland habitat; grasslands dominated by *Typha* sp. & *Phragmites* sp.	Yes (n = 12)	1	120	Yes	272	25 (20)	Livestock grazing & poaching

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Table 1 (*Cont.*)

Survey zones	Survey dates	Area (km^2)	Survey effort (km)	Names of local areas with swamp deer evidence	Habitat characteristics	Direct sightings	Car-casses	Antlers	Hoofmarks	Faecal pellets	No. of pellets analysed (swamp deer DNA confirmed)	Disturbance
Zone 8	Mar., June & Nov. 2016	31	389	Raulighat	River islands with grassland habitat; grasslands dominated by *Typha* sp. & *Phragmites* sp.	No	1	11	Yes	8	4 (0)	Livestock grazing, habitat encroach-ment, agriculture & poaching
Tributary (Solani & Banganga rivers)												
Zone 9	Mar., June & Nov. 2016	309	644	Joggawala, Almawala	Highly fragmented swampy habitat with *Typha* sp.; human-dominated area with croplands	Yes (n = 1)	1	7	Yes	28	16 (7)	Habitat encroach-ment, agriculture & poaching
Tributary (Ramganga river)												
Zone 10	Apr., May & June 2016	63	413	Near Afzalgarh	Swampy habitat sur-rounded by human habitation; wetland dominated by *Typha* sp.	Yes (n = 9)	0	9	Yes	26	15 (14)	Livestock grazing, habitat encroach-ment, agriculture & poaching

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conclusive evidence on their own. Swamp deer were considered to be present when at least two of the three indirect signs (antlers, hoof marks or faecal pellets) were found in an area.

During our surveys we recorded GPS coordinates of all locations with evidence of swamp deer presence, and collected dung pellets, antlers and tissue samples from carcasses from all surveyed areas. We also collected information on any disturbance and evidence of poaching. Presence of undisturbed grassland patches of *Typha* sp., *Phragmites* sp. and *Saccharum* sp. was considered an indicator of potential swamp deer habitat, whereas presence of livestock and humans in grassland was considered a sign of disturbance. Information about poaching came either from local residents or was gathered from direct evidence (e.g. skinned hides).

Identification using faecal DNA

Although swamp deer pellets are larger than those of other grassland cervids (hog deer) and domestic ungulates (sheep, goat), and distinct from those of bovids (nilgai) found in this region, species cannot always be identified with certainty based on pellet morphology alone. Pellets collected from marshy areas can usually be assigned to swamp deer because these areas are inaccessible to other species. However, because swamp deer co-occur with other ungulates in many areas, we used DNA-based species identification to confirm the species' presence. We randomly selected 104 pellets from surveyed zones and extracted DNA by swabbing the outer layer of each pellet (Ball et al., 2007). We amplified a 374 bp fragment of herbivore specific mitochondrial cytochrome b gene following published protocols (Gupta et al., 2014). The PCR products were visualized in 2% agarose gel, cleaned with Exonuclease (Thermo Scientific, Waltham, USA) and Shrimp Alkaline Phosphatase (Amresco, Solon, USA) mixture and then sequenced using forward primers in an ABI 3500XL bioanalyzer (Applied Biosystems, Waltham, USA). Sequences were aligned using *MEGA v6.0* (Tamura et al., 2013) and visually examined for missense or frame-shift mutations. The aligned sequences were then matched against the Genbank database for species confirmation.

Results

Surveys

We found evidence of swamp deer presence in all Zones except 3 and 4. We had sightings in Zones 1, 7, 9 and 10 and found carcasses in Zones 1, 2, 7, 8 and 9 (Table 1). We collected a large number of antlers and faecal pellets and recorded signs of hoof marks across all surveyed areas (Table 1). The results confirm the presence of swamp deer along both banks of the Ganges and all three of its tributaries surveyed, and included a previously unreported population c. 58 km from Jhilmil Jheel Conservation Reserve, near the town of Afzalgarh by the Ramganga river (Zone 10).

Faecal DNA

Genbank matching analyses of the sequences generated from successfully amplified samples revealed that 70 of the 104 pellet samples belonged to swamp deer (Table 1). A small number of sequences were identified as sambar ($n = 1$), spotted deer ($n = 6$), hog deer ($n = 1$) and nilgai ($n = 2$). The Genbank accession numbers for species-specific sequences of all identified species are MF143831–MF143910. Twenty-four samples did not amplify during PCR, possibly because of degraded DNA quality.

Habitat status and disturbance

We found the highest number of swamp deer and patches of suitable habitat in the Jhilmil Jheel Conservation Reserve (Zone 1). However, our survey showed signs of anthropogenic pressures in the form of livestock grazing in Jhilmil Jheel Conservation Reserve and Hastinapur Wildlife Sanctuary, and habitat conversion within the Sanctuary. Protection in the form of regular patrolling, grassland management and control of livestock grazing in swamp deer habitat is limited in most of Zones 3, 6, 8 and 9 within Hastinapur Wildlife Sanctuary. In the remaining unprotected survey zones the main threats are poaching, retaliatory killings related to crop depredation, and other forms of anthropogenic disturbances. For example, the grassland patches in Zone 2 on the east bank of the Ganges are disturbed by heavy vehicles used for sand mining. In Zone 3 all small grassland patches have been converted to croplands, so there is now no suitable habitat for swamp deer in this area. Along the west bank of the Ganges, Zone 4 has minimal grassland habitats remaining for swamp deer. Our interviews with local communities revealed historical presence of swamp deer in this area but our surveys did not find evidence of swamp deer. In Zone 6 we found little evidence of swamp deer presence. Local communities reported occasional sighting of swamp deer on the bank of the Banganga river, which is supported by presence of hoof marks and faecal pellets (Table 1). In Zones 5 and 8 local communities reported incidences of swamp deer poaching and crop-use related retaliatory killings by farmers. The grassland and wetland patches along the Solani river (Zone 9) are fragmented and surrounded by human habitation, and the status of swamp deer connectivity with other areas cannot be ascertained. The survey along the Ramganga river (Zone 10) revealed occasional poaching and potentially poor connectivity with other swamp deer populations.

Discussion

Our survey covering the range of the northern swamp deer subspecies *R. duvaucelii duvaucelii* in the upper Gangetic plains revealed its presence in several areas along the Ganges from the Jhilmil Jheel Conservation Reserve (Uttarakhand) to the Bijnor barrage area of Hastinapur Wildlife Sanctuary (Uttar Pradesh). We also found signs of swamp deer presence along the Solani and Banganga rivers and a previously unreported population near Afzalgarh, Uttar Pradesh, along the Ramganga river. The presence of this subspecies was confirmed through direct sightings, presence of carcasses, antlers, hoof marks and genetically identified faecal pellets. We found evidence of swamp deer in eight of the 10 surveyed zones (Table 1) where local communities suggested their presence, indicating that swamp deer are present in larger areas than previously reported for this region (Qureshi et al., 2004). Swamp deer may also occur downstream of Bijnor barrage and along other tributaries of the Ganges, and future surveys are required in these areas.

Our results reveal a patchy distribution of this subspecies in small areas across the states of Uttar Pradesh and Uttarakhand. Although we did not attempt to estimate population size, our surveys suggest the population in this region is relatively small. However, it is difficult to ascertain whether individuals in different survey zones are part of a meta-population, and information on swamp deer movement and gene flow rate in this landscape is not available. Seasonal movements of swamp deer from Jhilmil Jheel Conservation Reserve indicate their ability to move to other areas. Such seasonal and flood-driven movement patterns have been observed in central Indian and northern swamp deer populations (Martin & Gopal, 2015). Future studies, including tracking individuals with radio collars and analysing genetic data from antlers, pellets and tissue could provide additional information.

Although our survey revealed previously unknown habitats with swamp deer presence in the upper Gangetic plains, the habitat areas outside Jhilmil Jheel Conservation Reserve and parts of Hastinapur Wildlife sanctuary receive limited protection. Because all of these areas have potentially small, seasonally fluctuating numbers of swamp deer, survival of the subspecies will depend on its management as a meta-population with stable demographic and genetic parameters. Swamp deer in this region are threatened by habitat loss following conversion of wetlands to agricultural fields, livestock grazing and occasional poaching. The anthropogenic pressure on these grassland habitats is high because they are among the most fertile areas in northern India. Increased habitat conservation, anti-poaching activities and public awareness campaigns are needed to improve the status of these fragmented habitats and increase populations of this enigmatic species in this landscape. Conservation and management of *R. duvaucelii duvaucelii* as an umbrella species in unprotected areas would aid the conservation of swamp habitats in the upper Gangetic plains, but success will depend on the involvement of local communities. Directed efforts from government-initiated large-scale programmes such as the National Mission for Clean Ganga on reviving and protecting the swampy grassland patches along the Ganges could benefit swamp deer recovery. These swampy grasslands and wetlands are also home to other threatened species, and conservation and management is necessary to protect these critical habitats for all threatened fauna in this landscape.

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Author contributions Conception of the study: SM and BP. Field survey design: BP, SM and SP. Data collection: all authors. Writing: SM, SP and BP. Final edits: all authors.

Conflicts of interest None.

Ethical standards All required permissions for our surveys and biological sample collection were provided by the Forest Departments of Uttarakhand (Letter no. 978/6–32/56) and Uttar Pradesh (Letter no. 2233/23–2–12 G).

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RESOURCES

Rapid molecular assays for species and sex identification of swamp deer and other coexisting cervids in human-dominated landscapes of the Terai region and upper Gangetic plains, northern India: implications in understanding species distribution and population parameters

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Abstract. Burgeoning pressures of habitat loss is a major cause of herbivore decline across India, forcing them to coexist with humans in non-protected areas. Their conservation in such landscapes is challenging due to paucity of ecological and demographic information. The northern subspecies of swamp deer, *Rucervus duvaucelii duvaucelii*, is one such herbivore that lives across human-dominated landscapes in Terai region and upper Gangetic plains of north India. Here, we describe species-specific molecular markers and a cervid-specific molecular sexing assay for swamp deer and four other coexisting cervids sambar, chital, barking deer and hog deer. Our markers show species-specific band patterns and a high success rate of 88.21% in large number of field collected reference samples for all species. Faecal pellets from pilot swamp deer survey samples from upper Ganges basin show 93.81% success rate, and only 5.5% misidentification based on morphological characteristics. Our cervid-specific molecular sexing multiplex assay accurately ascertained 81.15% samples to respective sexes. These molecular approaches provide an easy, quick and cheap option to generate critical information on herbivore population parameters and aid their conservation in this mosaic of protected and non-protected grassland habitats.

Keywords. herbivore distribution; non-protected areas; species-specific assays; molecular sexing; swamp deer.

Introduction

The mammalian biodiversity of the Indian subcontinent is currently facing unprecedented anthropogenic interventions in the form of habitat destruction, changing land-use practices, poaching and man–animal conflict (Karanth *et al.* 2010). A study on 25 large mammal species across India indicates severe range contraction and decline in their population size (Karanth *et al.* 2010). Such a rapid loss of habitats has resulted in many species, specially herbivores, getting pushed from protected areas to human-dominated reserve forests and buffer zones, creating conservation challenges resulting from exploitation/utilization of resources and competition both by humans (grazing livestock, poaching and hunting practices) and herbivores (crop raiding and other conflicts) (Harihar 2011; Kumar *et al.* 2017). The future survival of these species depends on how current management practices deal with their coexistence and conflicts with humans outside protected areas.

Swamp deer or 'Barasingha' (*Rucervus duvaucelii*) represents one such vulnerable endemic herbivore species in India and Nepal. With a global population of about 5000 wild individuals, this obligate swampy grassland-dwelling cervid is facing severe decrease in habitat and population size across its range (Duckworth *et al.* 2015). Of the three known subspecies of swamp deer, the northern subspecies *R. duvaucelii duvaucelii* retains about 80% of global population (Duckworth *et al.* 2015). It is distributed across patchy habitats along the upper Gangetic plains and the Terai region in northern India and southern Nepal,

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covering both protected as well as unprotected areas (Qureshi *et al.* 2004). A recent survey on the northern swamp deer based on direct sighting, carcass, antlers and pellets has reported a much fine-scale presence of the subspecies in the non-protected grasslands and surrounding areas across the Ganga river (Paul *et al.* 2018), along with the occasional presence of four other cervids, namely sambar (*Rusa unicolor*), chital (*Axis axis*), hog deer (*Axis porcinus*) and barking deer (*Muntiacus vaginalis*). Morphological identification of swamp deer antlers from other cervids was easy (Geist 1998), but species identification from pellets based on morphology for these co-occurring cervids was challenging, particularly when there is limited information on the distribution and demography of these species outside protected areas in this landscape. Given the difficulties in faecal morphology-based species identification for overlapping body-sized animals (Costa *et al.* 2016), it is necessary to develop a low cost, accurate and robust species, and sex identification approach for these coexisting cervids.

In this paper, we describe a set of molecular markers to unambiguously identify species and sex of swamp deer and co-occurring cervids, namely sambar, chital, barking deer and hog deer in the Terai region and upper Gangetic plains. The objectives of this study were to: (i) develop and validate species-specific markers and molecular sexing from a set of field-collected reference biological samples (tissues, antlers and pellets) for five cervid species, (ii) test these assays on faecal pellets collected under a pilot northern swamp deer survey and (iii) identify errors in morphology-based species identification from the field-collected probable swamp deer samples. We believe that implementation of these new approaches will be useful in investigating critical ecological parameters like distributional limits, genetic composition, sex ratio, sex-biased dispersal and gene flow for these species in this landscape.

Materials and methods

Study area and species

This study was conducted in parts of the Terai region and upper Gangetic plains of northern Indian states of Uttarakhand and Uttar Pradesh, covering a total of around 5000 km^2 mosaics of protected and non-protected areas. This landscape is a complex of riverine–grassland and grassland–forest systems dominated by trees such as *Shorea robusta*, *Mallotus philippensis*, *Syzgium cumini*, *Bombax ceiba* etc. and grasses like *Saccharum spontaneum* (Rawat and Adhikari 2015) and supports an assemblage of cervid species (sambar, swamp deer, chital, hog deer and barking deer) (Johnsingh *et al.* 2004). Swamp deer (vulnerable) and hog deer (endangered) have restricted distribution in India and are listed under 'Schedule I', while sambar (vulnerable), chital (least concern) and barking deer (least concern) are widely distributed and are listed as Schedule III under the Wildlife Protection Act (1972).

Species-specific primer design

To design species-specific primers, we downloaded the complete mitochondrial genomes of all five target species from GenBank (swamp deer NC_020743.1, JN632696.1, DQ459338.1, EU921907.1; sambar DQ989636.3, NC_008414.3, EF035448.1; chital JN632599.1, JN093088.1, JN596149.1, NC_020680.1; hog deer JN632600.1, NC_020681.1, KM881625.1 and barking deer AF527537.1, AF042725.1, EF523654.1) and aligned them with MEGA ver. 6 (Tamura *et al.* 2013). These aligned sequences were screened visually and unique species-specific variations for all species were identified. Primers were designed manually considering the following criteria: (i) amplicon size of ≤300 bp to assure higher amplification success; (ii) design multiple primers for each species to increase species ascertainment success and (iii) vary amplicon sizes for two primers designed for the same species to increase multiplexing capacity. A total of 15 primer pairs were designed: four for swamp deer, three for sambar, three for chital, three for hog deer and two for barking deer.

Sample collection

During this study, we collected a large number of samples from the wild to standardize and test the novel species-specific markers for all target species. The entire sample collection procedure was divided into two phases. First, we surveyed specific areas in the Terai region and upper Gangetic plains within Uttarakhand and Uttar Pradesh, where each of the target species are found and opportunistically collected tissues, antlers and pellet samples from respective areas. A total of 103 reference samples (swamp deer-22, sambar-19, chital-30, hog deer-26 and barking deer-6) were collected in this phase. These samples were collected from the following regions: Jhilmil Jheel Conservation Reserve (swamp deer, chital and sambar); Haridwar Forest Division (sambar and hog deer); Rajaji Tiger Reserve (sambar and chital); Lansdowne Forest Division (sambar) and Ramnagar Forest Division (chital and barking deer) in Uttarakhand and Hastinapur Wildlife Sanctuary (swamp deer and hog deer); Pilibhit Tiger Reserve (hog deer); Dudhwa National Park (swamp deer) and Katerniaghat Wildlife Sanctuary (hog deer) in Uttar Pradesh. In addition, we received one chital and barking deer tissue from the Wildlife Institute of India's tissue repository, two hog deer pellets from Jaipur Zoo, Rajasthan, and one barking deer pellet from the Kalesar Wildlife Sanctuary, Haryana (table 1).

Table 1. Details of samples collected as references and swamp deer field survey and the success rate for species and sex identification.

Species	Sample type	Reference and test samples	Swamp deer survey	Success rate (%)	Samples for sex identification*	Success rate (%)
Swamp deer ($n = 124$)	Tissue	3 (Reference = 1, test = 2)	2	94.12	5	77.56
	Antler	5 (Reference = 1, test = 4)	13		–	
	Pellet	14 (Reference = 5, test = 9)	87		89	
Sambar ($n = 19$)	Tissue	4 (Reference = 1, test = 3)	–	84.12	4	80
	Antler	1 (Reference = 1, test = 0)	–		–	
	Pellet	14 (Reference = 2, test = 12)	–		11	
Chital ($n = 31$)	Tissue	4 (Reference = 1, test = 3)	–	93.55	4	87.5
	Antler	5 (Reference = 1, test = 4)	–		–	
	Pellet	22 (Reference = 2, test = 20)	–		20	
Hog deer ($n = 28$)	Tissue	3 (Reference = 1, test = 2)	–	92.86	3	77.27
	Antler	4 (Reference = 1, test = 3)	–		–	
	Pellet	21 (Reference = 3, test = 18)	–		19	
Barking deer ($n = 8$)	Tissue	1 (Reference = 1, test = 0)	–	75	1	83.33
	Antler	0	–		–	
	Pellet	7 (Reference = 2, test = 5)	–		5	

*Only genetically confirmed samples were considered for sex identification.

In the second phase, we used 102 probable swamp deer samples (87 pellets, 13 antlers and 2 tissues) collected during the survey from the Jhilmil Jheel Conservation Reserve to the Bijnor Barrage area of the Hastinapur Wildlife Sanctuary described in Paul *et al.* (2018) for conclusive species identification using the assays developed in this study. Only broken pieces of antlers that were difficult to ascertain to the species level morphologically during the surveys were used in this assay.

DNA extraction

DNA extraction from tissue samples was performed using DNeasy tissue kit (QIAGEN, Valencia, USA) extraction protocol. All field-collected frozen pellets were thawed at room temperature and the top layer of each pellet was swabbed using sterile swabs (Himedia). The swabs were placed in sterile Eppendorf tubes and lysed overnight with 300 μL of ATL solution (QIAGEN, Valencia, CA, USA) and 25 μL of Proteinase K (20 mg/mL). After overnight lysis, the swabs were discarded and subsequent extraction was completed following standard spin-column protocol from the DNA tissue kit (QIAGEN, Valencia, CA, USA).

All field-collected antlers were cut around 2 cm from the base using sterile saw blades or drilled (broken antlers) and the powder was collected in sterile Eppendorf tubes. A total of 2 mg of the powder from each antler was weighed and decalcified in 0.5 M EDTA (pH 8) solution in an overhead rotor for two days (Gupta *et al.* 2012). The supernatant containing EDTA was discarded and lysis was performed at 56°C with 40 μL of Proteinase K (20 mg/mL) and 400 μL of ATL solution (QIAGEN, Valencia, CA, USA) for a week with intermittent vortexing, followed by spin-column extraction protocol of the tissue DNA kit. For all extractions, one negative control was included for each set of extraction ($n = 11$) to monitor any possible contamination. All extracted DNA samples were stored at −20°C until further processing.

PCR standardization

All initial PCR standardizations were done using genetically confirmed samples (tissues, antlers and pellets). We confirmed seven swamp deer, four sambar, four chital, five hog deer and three barking deer samples using cytochrome-b sequencing (Gupta *et al.* 2014) and tested all species-specific primers on this set of reference samples. Following annealing temperature standardization for each species, PCR reactions were done for 10 μL reaction volume using 4 μL multiplex buffer (QIAGEN, Valencia, CA, USA), 1 μL of BSA (4 mg/mL), 0.25 μM of primer mix, 2 μL of 1:10 diluted DNA extracts and 2 μL of RNAse-free water. The PCR conditions included an initial denaturation (95°C for 15 min), 45 cycles of denaturation (95°C for 30 s), annealing (T_a for 40 s) and extension (72°C for 40 s), followed by a final extension (72°C for 10 min). Negative controls were included to monitor possible contamination. Each species-specific primer was tested three times independently with all five species DNA to monitor cross-species amplification. The amplified products were checked in 2% agarose gel. Any primer showing cross-species amplification was discarded. Amplified products were cleaned using Exo-SAP mixture, sequenced using forward primers and matched against Genbank database for species confirmation. If any species had more than one primer that provided species-specific bands, we standardized them as a multiplex system (table 2).

Following initial standardization, we amplified the selected species-specific primers with the remaining samples from the first phase of cervid reference sampling

Table 2. Details of species-specific primers developed for five cervid species in this study.

Species	Primers selected	mtDNA region	Sequence	Amplicon size	T_a (°C)
Swamp deer	2	D-loop	CATAGCCACAAGCTCTAG ATGAATATTATTGTAGGG	107	50
		D-loop	ACATAACACATTTTATGCGC GTACTATAAATAATAGTATG	123	50
Sambar	1	D-loop	AATCGCCCACTCCTTGTAGT AGGGGGGGGAAATATAGGTC	284	52
Chital	1	D-loop	CCATGCTTATAAGCATGTACC AATAGCTACCCCCACAGTTT	213	57
Hog deer	1	NADH subunit 2	CATCAATTGCCCACATAGGT GTAATGAGAATTAGGACAGTC	196	60
Barking deer	1	NADH subunit 4	CAAGTCACTAATTGCATAC CTGTGGATTCGTTCATAGCC	171	52

(swamp deer-15, sambar-15, chital-27, hog deer-23 and barking deer-5). Post amplification, representative samples from each species were randomly selected (swamp deer-7, sambar-5, chital-12, hog deer-10 and barking deer-2) and were sequenced for species reconfirmation.

Finally, we tested the swamp deer primers on all 102 samples from field survey. Any sample that did not amplify as swamp deer was further amplified with the other four species-specific primers to confirm species and misidentification percentages were calculated.

Molecular sexing

We multiplexed two molecular sexing markers described earlier (SRY and Amelogenin) (Takahashi *et al.* 1998; Yamauchi *et al.* 2000) and tested them on five known sex tissue samples and four antlers (confirmed males) from all five species. PCR reactions were performed in 10 μL reaction volume using 4 μL of multiplex buffer (QIAGEN), 3 μL of BSA (4 mg/mL), 0.25 μM of primer mix and 2 μL of 1:10 diluted DNA extracts with conditions including an initial denaturation (95°C for 15 min), 45 cycles of denaturation (95°C for 30 s), annealing (56°C for 40 s) and extension (72°C for 40 s), followed by a final extension (72°C for 10 min). Negative controls were included to monitor possible contamination. The amplified products were checked in 3% agarose gel. Finally, all field-collected samples that produced positive species-specific results were tested for sex identification. All samples were tested thrice to confirm the results and only samples with two identical results were finally considered.

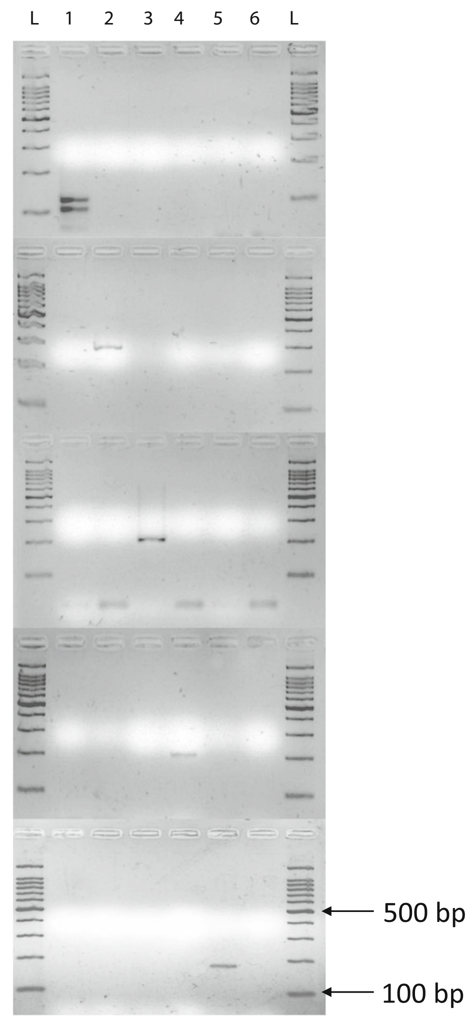
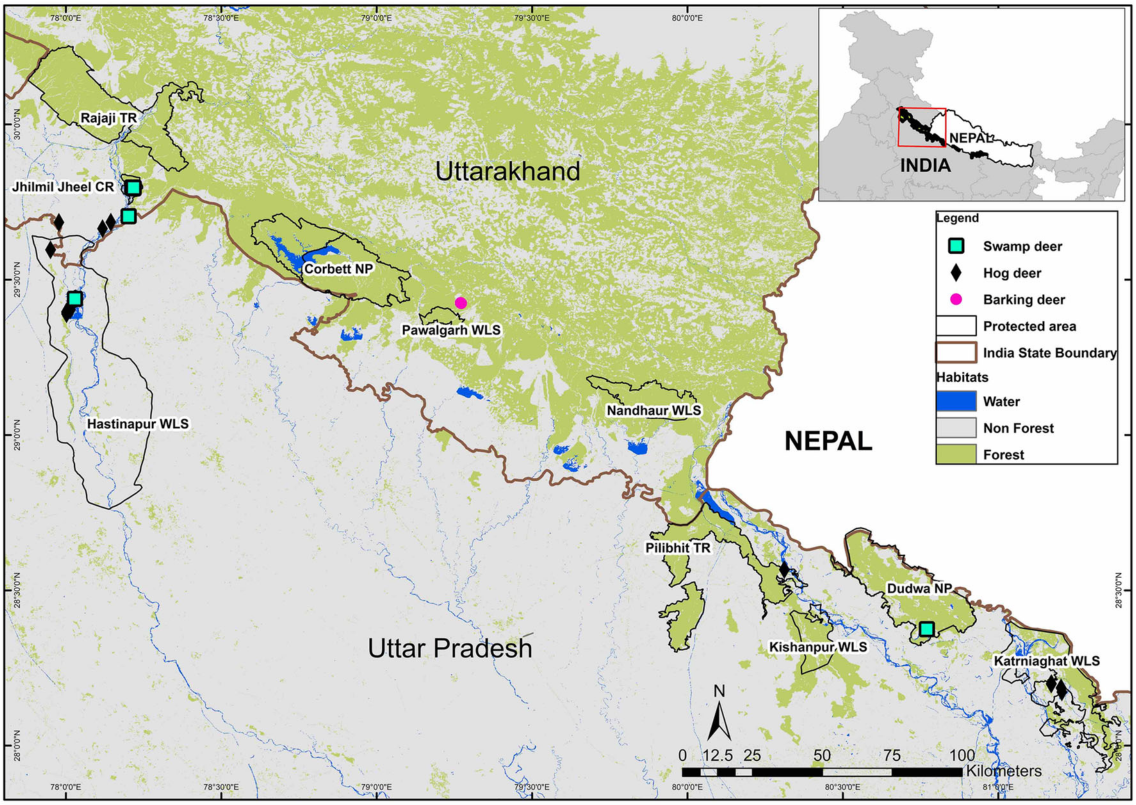


Figure 1. Gel representation of species-specific primers for the five cervids developed in this study. L: Ladder, Lane 1: Swamp deer (107 and 123bp), Lane 2: Sambar (284bp), Lane 3: Chital (213bp), Lane 4: Hog deer (196bp), Lane 5: Barking deer (171bp), Lane 6: Negative PCR, L: Ladder.

Results and discussion

Of the 15 pairs of primers designed, only six (two for swamp deer and one each for sambar, chital, hog deer and barking deer) showed species-specific band patterns (table 2; figure 1) from initial reference samples. Sequencing from representative samples resulted in accurate species identification when matched with the GenBank database. The remaining primers showed cross-species or nonspecific amplifications during testing and



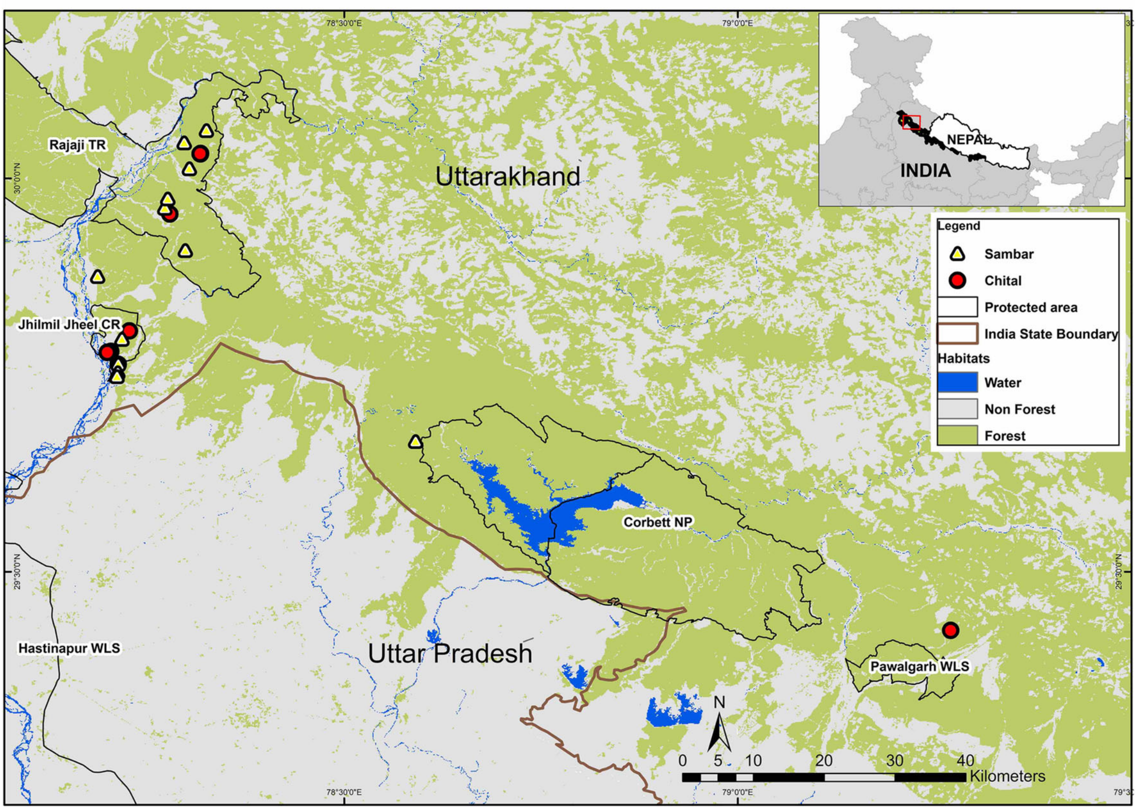


Figure 2. Locations of reference samples of all target species from the Terai region and upper Gangetic plains of northern India. (a) Represents the sample locations of swamp deer, hog deer and barking deer, and (b) represents the locations of sambar and chital.

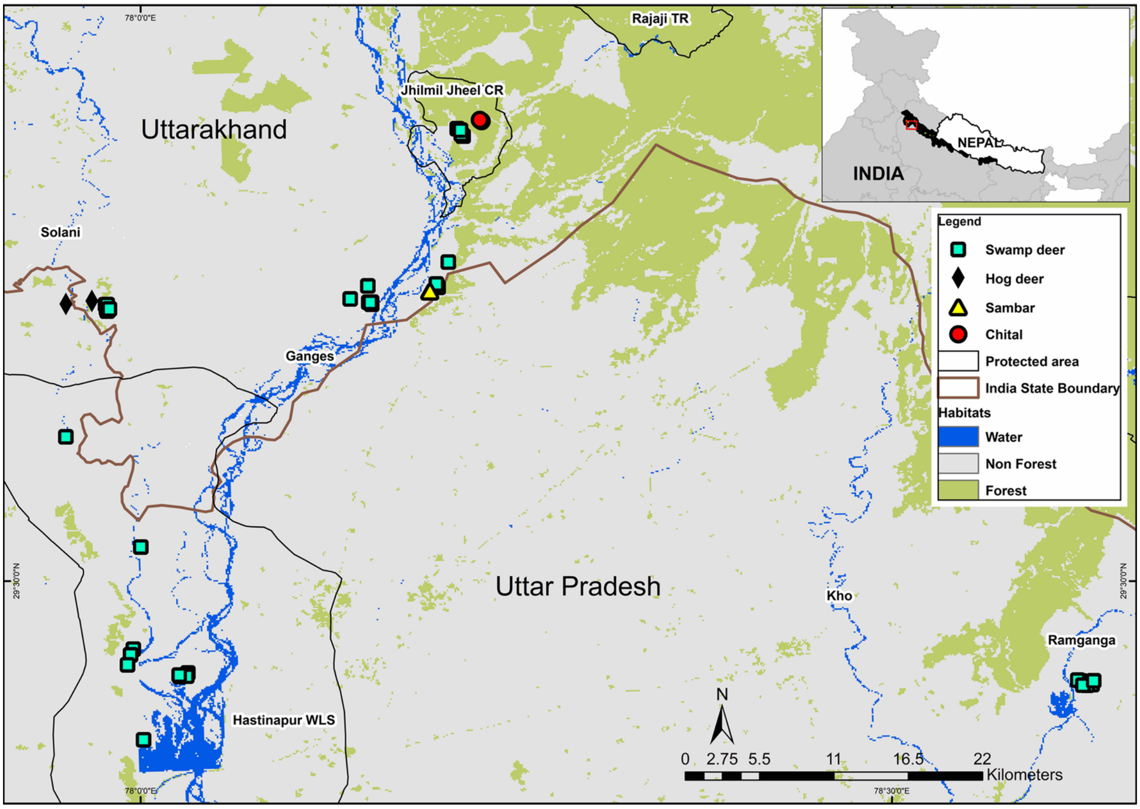


Figure 3. Locations of genetically identified swamp deer samples using the species-specific markers developed in this study. The figure also shows the locations of genetically confirmed hog deer, chital and sambar samples that were morphologically misidentified as swamp deer during the field survey.

were discarded. The finalized primers were subsequently tested on the remaining samples from the first phase of reference sampling ($n = 85$ from all species) with successful species-specific assignment ranging from 75 to 95.45% (figure 2, table 1). Sequencing of the representative 36 samples confirmed respective target species (GenBank accession no. MH045071–MH045150). Subsequently, any positive species-specific band patterns from samples collected during swamp deer survey was considered as originating from the respective species. Finally, from all field-collected swamp deer survey samples ($n = 102$), 91 (93.81%, tissue-2, antler-13, pellet-76) were successfully assigned to swamp deer using the species-specific multiplex assay. Of the remaining 11 samples, we assigned five pellets to sambar ($n = 1$), chital ($n = 2$) and hog deer ($n = 2$), respectively (figure 3).

The sexing multiplex PCR showed a three-band pattern (two bands of 219 and 165 bp from amelogenin and one band of 104 bp from SRY) for males and a single band (219 bp from amelogenin) for females, across all five species (figure 4). The overall success rate for sex determination from all species identified samples was 81.15% (table 1). The poor-quality pellet DNA samples showed a success rate of 72.22% in sex determination. The male:female ratio was found to be 5:1 for all field-collected samples from five cervids.

To the best of our knowledge, this is the first study targeting species identification of five coexisting Asiatic cervid species. Earlier, similar genetic tools have been used to study neotropical sympatric deer species (Gonzalez *et al.* 2009; Costa *et al.* 2016; Duarte *et al.* 2016) but no such work on multiple deer species has been done in the subtropical regions of Asia. The mitochondrial DNA primers designed in this study amplified only the target cervid species and showed no cross-amplification with others. The smaller amplicon sizes resulted in 75–94.12 and 81.15% success in species and sex identification, respectively, from field-collected samples (table 1). These approaches facilitate fast screening of large number of field-collected samples of various qualities, reduce species/sex misidentification and are of less cost than traditional sequencing or restriction fragment length polymorphism-based methods (Palomares *et al.* 2002; Mukherjee *et al.* 2007). Such molecular approaches are definitely advantageous for a group of coexisting species when compared to more error-prone faecal morphology-based species identification, where faecal dimensions are affected by overlapping body sizes (Costa *et al.* 2016). In this study, we obtained a 5.5% misidentification rate of three other cervid species pellets with targeted sampling of swamp deer from mostly wetlands and swampy habitat, indicating occasional usage of the area by other cervids. Although, we were able to

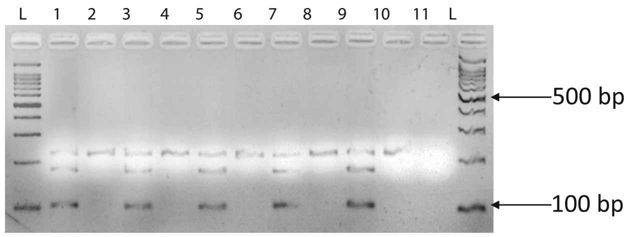


Figure 4. PCR amplication of Amelogenin (216bp and 165bp) and SRY markers (104bp) for five cervid species. The males show three bands and females show single band in the gel. L: Ladder, 1-2: Swamp deer (M and F), 3-4: Sambar (M and F), 5-6: Chital (M and F), 7-8: Hog deer (M and F), 9-10: Barking deer (M and F), 11- PCR negative.

unambiguously identify all five target species using these markers, it is important to point out that all the target species have distribution outside our study area (Terai region and upper Gangetic plains of northern India) and subsequent use of these primers to other regions would require testing them with samples from other respective areas. We strongly recommend using good number of reference samples before implementing these species identification markers in other regions for respective species to ensure a successful study.

Similarly, our two-marker based multiplex sexing approach showed a higher success rate and reduced the rate of sex misidentifications or 'false negatives' of males due to allelic dropout from Y chromosome from field-collected samples. This sexing approach showed an amplification success rate of 72.22% from poor-quality faecal DNA samples, higher than those of other studies on various ungulate species (Oliveira and Duarte 2013 (43.7%); Pelizzon *et al.* 2016 (64.4%)). However, addition of another X chromosome marker would make it a more robust sexing assay for poor-quality DNA sources. We found a male-biased sex ratio (5:1) while testing the sexing assay on field-collected samples of all target species in this study. Earlier studies on other deer species (Pajares *et al.* 2007; Oliveira and Duarte 2013; Sharma *et al.* 2015) also have shown similar male-biased genetic data for respective species in different parts of the world.

In summary, we present a set of molecular tools to carry out simple and quick species, and sex identification for an assemblage of cervids found in a landscape having a mosaic of protected and non-protected areas in the Terai region and upper Gangetic plains of northern India. This landscape retains some of the most important grassland and wetland habitats in the subcontinent and are currently facing severe anthropogenic interventions in the forms of habitat conversion for agriculture, resource competitions with livestock, unregulated hunting etc. (Harihar 2011; Kumar *et al.* 2017). It is difficult to generate critical population level information in such human-dominated habitats (e.g. presence/absence, abundance, demography etc.) with traditional methods. For example, camera traps and line transects are logistically difficult to implement (accessibility, theft, vandalism and visibility) in tall grassland habitats interspersed with human-dominated areas. This pilot swamp deer survey demonstrates that a faecal pellet DNA-based species and sex identification can help to generate important information at the species/population level. Further long-term genetic study to get regular information on landscape level population size estimates, sex ratio, genetic relatedness and dispersal events could provide a deeper understanding of species biology. Given the alarming rate of decline in herbivore habitats and populations at the global scale (Ripple *et al.* 2015), such a synergistic approach will be the key for their survival.

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Original Research Article

Assessment of endemic northern swamp deer (*Rucervus duvaucelii duvaucelii*) distribution and identification of priority conservation areas through modeling and field surveys across north India

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Non-protected grasslands

A B S T R A C T

Recent declines in large herbivores have led to significant conservation efforts globally. However, the niche-specific megaherbivores residing outside protected areas face more imminent extinction threats. Swamp deer, the obligate grassland-dwelling endemic cervid is the most extinction-prone megaherbivore in the Indian subcontinent. Limited information on distribution and habitat status pose significant conservation and management challenges for the remaining fragmented populations in north, north-east and central India. To this end, we combined exhaustive field surveys and Maximum Entropy (MaxEnt) modeling to generate the most detailed distribution map for the northern swamp deer subspecies. We used primary data from more than 6000 km^2 field surveys and eight ecologically relevant covariates for model predictions. Grassland cover, annual mean temperature and distance from water were the major factors that predicted the species distribution. Models predicted swamp deer distribution in only ~3% of the entire landscape, covering both protected (~1.4%) as well as non-protected (~1.6%) areas. Our validation surveys in some of these predicted areas confirmed swamp deer presence and indicated ~85% model accuracy. Finally, we identified four "Priority Conservation Areas" still retaining adequate grassland habitat and species presence that require immediate attention to ensure landscape-level population connectivity. These results highlight the importance of the marginalized grassland ecosystems of northern India that still retain high biodiversity. We suggest a swamp deer-centric conservation approach to protect these human-dominated habitats and emphasize in generating such information for other endemic, habitat-specialist species across the globe.

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1. Introduction

Large herbivores play critical roles in shaping ecosystems through various ecological functions (nutrient cycling, seed dispersal etc.) that affect forest structure, regeneration and benefit other species (Danell et al., 2006; Ripple et al., 2015). In recent time habitat destruction, resource depletion, hunting and human-animal conflict have resulted in massive declines of the world's large herbivores (Ceballos et al., 2017; Lindsey et al., 2017; Ripple et al., 2015) such that about 60% of them are threatened with extinction (Ripple et al., 2015; Trouwborst, 2019). The effects of such anthropogenic factors towards their extinction are further exacerbated by their relatively low population densities, unique habitat requirements and slow life history characteristics (Ripple et al., 2016; Wallach et al., 2015). While significant global conservation efforts helped in population recovery of some of the charismatic large herbivores (African rhinoceros- Emsile and Brooks, 1999; Asian elephant, gaur and sambar- Madhusudan, 2004; black rhinoceros- Brodie et al., 2011; Asian elephant and one-horned rhinoceros- Williams, 2011; elk, European bison and red deer- Sylven et al., 2012), it has mostly been targeted within protected areas, that represents only ~13% of the total land surface area (Chape et al., 2005; UNEP-WCMC, 2012). The challenges are even more acute for habitat-specialist large herbivores that are distributed outside protected areas along the human-livestock-wildlife interfaces, where implementing regular management efforts like the protected areas are difficult (Karanth et al., 2010; Kshettry et al., 2020). Although these non-protected areas harbor significant mammalian biodiversity (Dorji et al., 2019; Harihar, 2011; Punjabi and Rao, 2017) and act as essential corridor habitats for free-ranging animals (Neelakantan et al., 2019; Sunderraj et al., 1995), these areas are often overexploited (land conversion, grazing, unregulated poaching and hunting practices etc.) (Macdonald et al., 2013; Ripple et al., 2015) and experience strong human-wildlife conflict (crop-raiding, damage to infrastructures and injuries to humans) (Gubbi et al., 2014; Kissui, 2008). Conservation of such large herbivores in these non-protected areas is dependent on accurate information on distribution, habitat status and other biological parameters.

The Indian subcontinent retains one of the highest diversity of large herbivores in south and south-east Asia (Johnsingh et al., 2004; Ahrestani et al., 2011). The large herbivore species assemblages are currently facing severe conservation challenges in the subcontinent with 12 of the 15 terrestrial large herbivores classified as "Threatened" by IUCN (Ripple et al., 2015). A recent study by Karanth et al. (2010) has shown that within the subcontinent, the habitat-specific large herbivores (for example, swamp deer, rhino, wild buffalo etc.) are much more prone to extinction due to their inherent species biology and various anthropogenic activities and require accurate information on distribution, population size and various habitat parameters to ensure their future survival. However, generating such information is challenging for many of these species due to their low density and cryptic behaviour, particularly for those living in a mosaic of protected and non-protected areas surrounding human settlements (Jathanna et al., 2003; Linkie et al., 2013; Marshal, 2016). Given that only about 5% of land comes under protected area category within India (Wildlife Institute of India, 2020), it is critical to generate detailed information on distribution of these habitat-specialist large herbivores and identify important habitats outside protected areas for their long-term conservation.

The swamp deer or barasingha (*Rucervus duvaucelli*) exemplify such habitat-specialist endemic large herbivores of the south Asian region (Qureshi et al., 2004; Tewari and Rawat, 2013). This obligate swampy grassland-dwelling cervid was historically distributed throughout the Indo-Gangetic plains and the lowlands spanning across the southern Himalayas covering Pakistan, southern Nepal, India and Bangladesh (Groves, 1982; Sankaran, 1989; Schaller, 1967). However, it is now classified as "Vulnerable" according to IUCN due to severe population decline and range contraction during the last century (Duckworth et al., 2015). This has resulted in a global population size of <5000 individuals, restricted to isolated populations in north, north-east and central India and south-west Nepal (Qureshi et al., 2004; Tewari and Rawat, 2013). Among the three known subspecies (northern- *R. duvaucelii duvaucelii*, central- *R. duvaucelii branderi* and eastern- *R. duvaucelii ranjitsinhi*), the northern subspecies retains about 80% of the global population (Qureshi et al., 2004, 1995) and is distributed as small, fragmented populations across the states of Uttar Pradesh, Uttarakhand in India and southwestern Nepal (Khan and Khan, 1999; Paul et al., 2018). The Indian part of the northern swamp deer distribution is broadly divided into two habitat blocks: a) the habitat block along the Sharda river basin in the state of Uttar Pradesh covering a mosaic of protected (Pilibhit Tiger Reserve, Kishanpur Wildlife Sanctuary, Dudhwa National Park and Katerniaghat Wildlife Sanctuary) and unprotected areas surrounding them and b) habitat block along river Ganga covering protected areas in Uttarakhand (Jhilmil Jheel Conservation Reserve) and Uttar Pradesh (Hastinapur Wildlife Sanctuary) along with surrounding unprotected, patchy grassland habitats (Qureshi et al., 2004, 1995; Paul et al., 2018) (Fig. 1). Encroachment pressures on the grasslands (only 2% of the entire Gangetic floodplains) (Dinerstein, 2003) from one of the most highly dense human populations (about 800 people/km^2 compared to 382 people/km^2 in India, Census of India, 2011) living in this region and scanty information on exact distribution and habitat of swamp deer status pose significant conservation challenges (Duckworth et al., 2015; Midha and Mathur, 2010; Paul et al., 2018). A recent study by Paul et al. (2018) has reported a detailed assessment of the subspecies presence and habitat status in the Gangetic block, with previously unreported swamp deer harboring areas. This indicates promising signs for this subspecies, but a landscape wide assessment of swamp deer distribution is essential for developing better management plans for the species and its remaining grassland habitats. However, conducting surveys in this human-dominated landscape is logistically challenging and probably requires a combination of different approaches to assess the fine-scale distribution pattern of this subspecies.

In this study, we assessed *R. duvaucelii duvaucelii* distribution covering protected and non-protected areas across the upper Gangetic plains, within the boundaries of Uttarakhand and Uttar Pradesh. We used multidisciplinary approaches involving

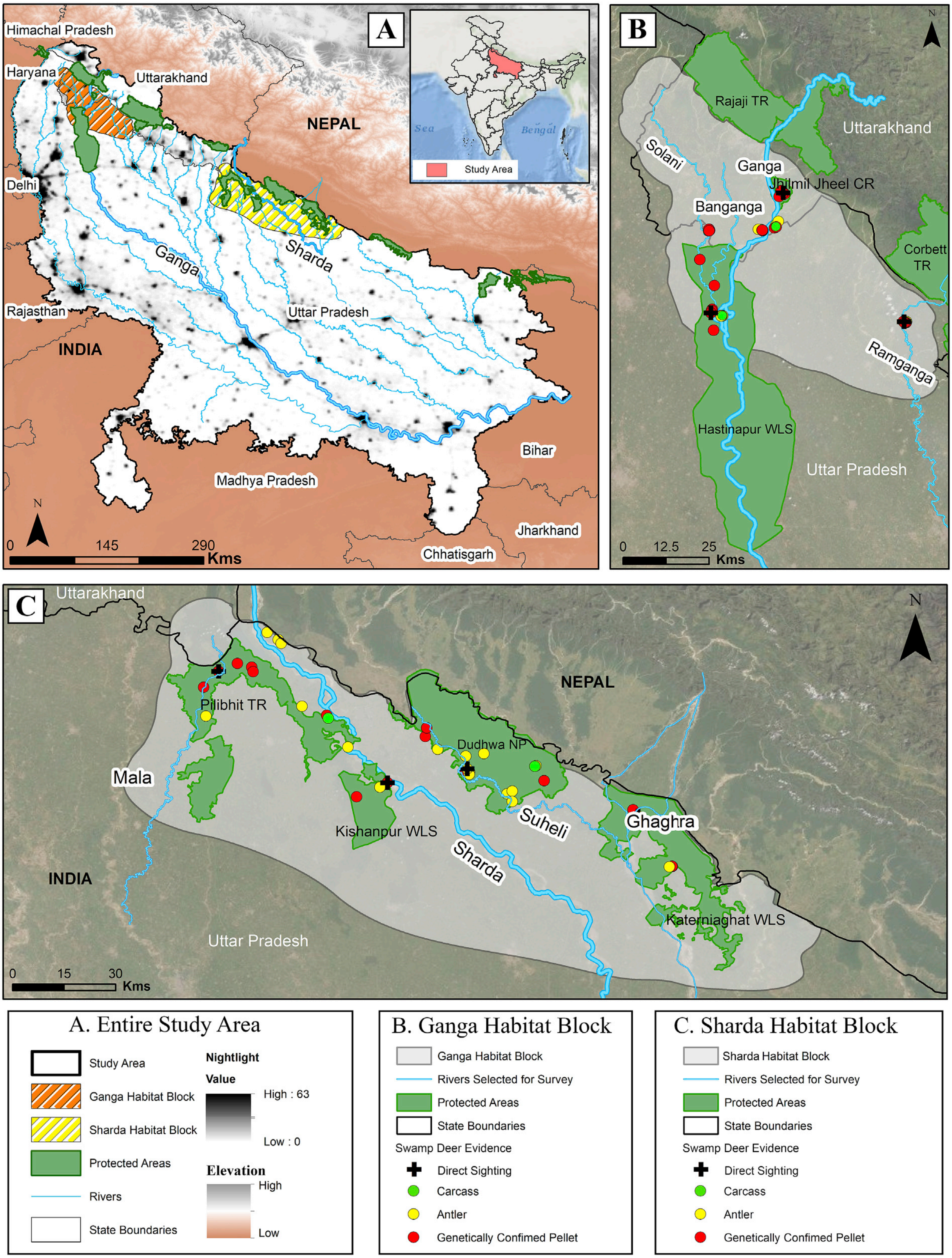

Fig. 1. The study area map across the states of Uttarakhand (selected area) and Uttar Pradesh, north India. The map shows (A) entire study area covering both Ganga and Sharda Habitat Blocks; (B) the locations of the confirmed swamp deer evidences within Ganga Habitat Block (data from Paul et al., 2018) and (C) the locations of the confirmed swamp deer evidences within Sharda Habitat Block. Different evidence types (direct and indirect) are presented in the figure legends.

scientifically designed rigorous field surveys in Ganga (data from Paul et al., 2018) and Sharda block, distribution modeling and subsequent field validations to assess current swamp deer distribution and status of their habitats. Further, we have identified four new "Priority Conservation Areas" that are crucial for future swamp deer conservation planning. The findings of this study would be necessary in developing long-term conservation strategies for swamp deer and the wetland/grassland habitats that host a number of other threatened fauna.

2. Materials and methods

2.1. Research permissions

All required permissions for swamp deer surveys and biological sample collection were provided by the Forest Departments of Uttarakhand and Uttar Pradesh (Letter no: 90/5, 978/6–32/56, 1127/23-2-12(G) and 2233/23-2-12(G)). Due to the non-invasive nature of sampling, no ethical clearance was required in this study.

2.2. Study area

This study was conducted covering about 25 3188 km^2 area across selected districts of Uttarakhand (Dehradun, Haridwar, Pauri-Garhwal, Nainital and Udham Singh Nagar) and the entire state of Uttar Pradesh (Fig. 1). The area covers the floodplains of the Ganga river and a number of its tributaries like Sharda, Ghaghra, Ramganga etc. (Fig. 1) including a complex network of riverine-grassland and grassland-forest systems. This region hosts the only existing populations of northern swamp deer (*R. duvaucelii duvaucelii*) in India (Qureshi et al., 2004). The primary vegetation in the grassland patches comprises *Typha* sp., *Phragmites* sp. and *Saccharum* spp. (Tewari and Rawat, 2013). Larger animals in the study area include swamp deer (*R. duvaucelii*), hog deer (*Axis porcinus*), spotted deer (*Axis axis*), nilgai (*Boselaphus tragocamelus*), smooth-coated otter (*Lutrogale perspicillata*), fishing cat (*Prionailurus viverrinus*) and iconic wetland birds such as sarus crane (*Antigone antigone*), black-necked stork (*Ephippiorhynchus asiaticus*), lesser adjutant (*Leptoptilos javanicus*), Pallas's fish eagle (*Haliaeetus leucoryphus*) and bar-headed goose (*Anser indicus*) (Grimmett et al., 2013). The area experiences extreme seasonal variations in temperature (7-40 °C) and receives an average annual rainfall of 948 mm (502.4–2113.9 mm) with occasional flooding during July–September (Verma et al., 2019).

2.3. Species distribution modeling (SDM) for swamp deer using MaxEnt

2.3.1. Generation of swamp deer occurrence points

We used MaxEnt (Maximum Entropy) species distribution algorithm version 3.3.3k (Phillips et al., 2006) to model the potential distribution of swamp deer in the study area. Maximum Entropy is one of the most widely used algorithms for species distribution with presence-only data (Elith et al., 2006). For best performance of the algorithm, it is critical to have unbiased, error-free species presence points for initial calibration of the models (Kramer-Schadt et al., 2013). Biased survey design including inadequate survey efforts, sporadic or clumped location information, erroneous presence data etc. affect model accuracy (Costa et al., 2015; Kramer-Schadt et al., 2013). We conducted systematic intensive surveys to generate accurate swamp deer presence points from both Ganga and Sharda river habitat blocks for model calibration. Details of the survey and fine-scale swamp deer distribution at the Gangetic habitat block from Jhilmil Jheel Conservation Reserve (JJCR) in Uttarakhand to Bijnor Barrage area of Hastinapur Wildlife Sanctuary (HWLS) in Uttar Pradesh is described in Paul et al. (2018). In Sharda block, swamp deer presence has been reported from a few well-known swamps within protected areas only. For a comprehensive detailed assessment of swamp deer distribution in Sharda block, we adopted a similar combined approach of opportunistic unstructured questionnaire surveys (see details in Paul et al., 2018) and Google Earth imageries (Hu et al., 2013) to locate possible areas (both protected and adjoining non-protected) where the species was found. As most of the potential habitats in this region are protected, the questionnaire surveys were targeted towards Forest Department staff and occasionally with local cattle herders, boatmen and fishermen. All information indicated that majority of the grassland patches are currently restricted within 10 km areas on both banks of the rivers Mala (Pilibhit Tiger Reserve), Sharda (Pilibhit Tiger Reserve and Kishanpur Wildlife Sanctuary), Suheli (Dudhwa National Park) and Ghaghra (Katerniaghat Wildlife Sanctuary). Subsequently, we intensively surveyed both banks of the selected stretch of these rivers covering both protected as well as non-protected areas. The surveys were conducted using vehicle, boat or on foot depending on the area during the months of May of 2016 and 2017, covering a total area of around 6000 km^2.

During surveys, we collected geo-tagged information on direct (sighting, carcass etc.) as well as indirect (antlers and genetically identified dung pellets) evidence of swamp deer presence. Swamp deer antlers are quite distinctive from other coexisting cervids in this landscape (sambar, hog deer, chital and barking deer) (Paul et al., 2019). However, swamp deer pellets are difficult to identify based on morphology and we conducted molecular species identification using species-specific primers for different cervids described in Paul et al. (2019). Remaining non-amplified samples were tested with ungulate specific primers (Gupta et al., 2014) to identify species.

2.3.2. Fine-tuning of species occurrence data for model calibration

Combining the earlier records from upper Gangetic habitat block (n = 169, Paul et al., 2018) and new surveys at Sharda basin habitat block (n = 139, 2 carcasses, 7 direct sightings, 51 genetically identified pellets and 79 antlers, Supplementary Table A1), we gathered 308 confirmed swamp deer location points from the entire landscape. Upon plotting these locations in a map, we found that many confirmed swamp deer locations were clustered within the fragmented grassland patches, leading to spatial autocorrelation (around 4.4 points/km^2 grid). Such spatial patterns may lead to overfit model response to covariates, thereby limiting the model's prediction accuracy (Boria et al., 2014; Kramer-Schadt et al., 2013). Calibration and evaluation localities that are close to each other might lead to inflated values of performance (Veloz, 2009; Hijmans, 2012). To reduce such effects, we selected a single presence record in every one km^2 area (as the species is mostly restricted to habitat patches of around one km^2 currently within the grassland-cropland mosaics within this landscape) using Spatially Rarefy Tool of SDM tool Box (Brown, 2014) in ArcGIS 10.2.2. We used 69 such records for species distribution modeling from a total of 308 points in the Gangetic plains of Uttarakhand and Uttar Pradesh.

2.4. Selection of covariates

Based on swamp deer species biology, we initially selected numerous climatic, vegetation, topographic and anthropogenic variables to model swamp deer distribution. Bioclimatic variables are regularly used in defining species environmental niches (Booth et al., 2014; Bradie and Leung, 2017) and we downloaded 30 arc-second spatial resolution (or one km^2 spatial resolution) data of four such variables from WorldClim-Global Climate Data (www.worldclim.org/bioclim). The topographic covariates in the form of "Elevation" and "Slope" layers were downloaded from the USGS website (www.usgs.gov). The "slope" was calculated using ArcToolbox, Spatial Analyst Tool in ArcGIS 10.2.2 (ESRI, Redlands, CA, USA). We also selected Normalized Difference Vegetation Index (NDVI) of March and May 2017, distance from water, nightlight and human population density (anthropogenic variables), rescaled to one km^2 resolution as covariates (Supplementary Table A2).

Correlated variables were removed as it might lend more statistical weight to one of them, leading to model overfitting (Dormann et al., 2013). We used "Pearson correlation coefficient" to identify highly correlated variables, where a threshold value of 0.75 was selected to choose the final covariates (Kalboussi and Achour, 2017; Pearson et al., 2002). After initial testing of all parameters, final species distribution modeling was run using the following variables: annual mean temperature, annual precipitation, NDVI-March 2017, NDVI-May 2017, nightlight, distance from water, human population density and elevation (Supplementary Table A2 and A3). Elevation was selected despite having high correlation with annual mean temperature, as it is an essential ecological factor restricting swamp deer distribution within the Gangetic plains (being an obligate grassland species of the plains, swamp deer are not found in undulating, hilly areas).

2.4.1. Model parameters, performance and selection

We developed and tested 10 different MaxEnt models by modifying the default parameters in order to derive the best model (Aryal et al., 2016) for swamp deer distribution. Use of default parameters can produce an over-complex or over-simplistic model (Merow et al., 2013; Warren and Seifert, 2011). MaxEnt models are sensitive to the model parameter "regularization multiplier" value (default = 1), which determines the output distribution (Elith et al., 2010). A smaller parameter value results in a more localized output distribution, while a larger value generates a more spread out, less localized predictions (Phillips, 2017). We developed models with 10 000 background sampling points as environmental space (Wilting et al., 2010) with different regularization parameter values (0.5–5). We randomly assigned the presence localities as training and testing dataset (70% as training, 30% as test). Rest of the settings for each parameter were kept default in each run. To select the best-fitted regularization multiplier, we used the Corrected Akaike information criterion (AICc) based evaluation method and selected the model with the lowest AICc value (Cordellier and Pfenninger, 2009; Williams et al., 2017). The evaluation of different regularization multiplier values has been done using the software ENMTOOLS version 1.4.4 (Warren and Seifert, 2011).

After selecting the best-fitted regularization multiplier (Model with lowest AICc value), we ran the final model with 10 replicates of 5000 iterations in MaxEnt to derive the average model (Phillips et al., 2006). An auto feature limiting function was used to fit the species environmental curves and to train the MaxEnt models. The minimum probability of MaxEnt prediction was set to "Minimum training presence Logistic threshold" (MTPLR) (Montemayor et al., 2014). We used the logistic model output, which displays a suitable value from 0 (unsuitable) to 1 (suitable). To check which variables were the most important for model building, a Jackknife analysis was done (Phillips, 2017).

To assess qualitative characterization of the final species distribution model, we used Receiver operating characteristic (ROC) area under curve (AUC) values (Phillips et al., 2006). AUC value of a model ranges from 0 to 1, where 0.90 to 1.00 range is considered as a good fit (Araújo et al., 2005; Hemsing, 2010).

2.5. Swamp deer validation surveys

The MaxEnt prediction gave a probability distribution map of swamp deer in the upper Gangetic plains of north India. To confirm model predictions, we conducted validation surveys in selected areas of this landscape. We selected the completely human-dominated lower part of Hastinapur Wildlife Sanctuary (downstream of Bijnor Barrage along Ganga) and Sohagibarwa Wildlife Sanctuary in Uttar Pradesh for validation surveys. Hastinapur Wildlife Sanctuary is probably the most critical swamp deer habitat in the Ganga habitat block and earlier extensive surveys were conducted till Bijnor Barrage (see Paul et al., 2018). We surveyed the entire stretch of Ganga from Bijnor Barrage till the southern boundary of Hastinapur Wildlife Sanctuary (about 100 km), covering all grassland patches found on either side of the river during May 2017 and April 2018. On the other hand, Sohagibarwa Wildlife Sanctuary is the easternmost protected area predicted by the model, known to harbor large patches of grassland habitat and historical presence of swamp deer (Ghimire et al., 2019). Apart from field-based information (direct sighting, antler and genetically confirmed pellets), we have also collected secondary information (newspaper report, personal communications etc.) on swamp deer presence in the southernmost predicted regions of our study area. To assess the accuracy of model prediction, we extracted pixel values of confirmed unique (duplicates removed) swamp deer presence points (primary, secondary and habitats) and considered those points with more than Minimum training presence Logistic threshold (MTPLR). The formula used to calculate accuracy is:

(Number of GPS points with pixel value more than MTPLR X 100) / (All GPS points of swamp deer presence)

2.6. Identification of Priority Conservation Areas

Earlier work on the *R. duvaucelii duvaucelii* showed fragmented, but fine-scale distribution of the subspecies in the northern part of the Ganga habitat block (till Bijnor Barrage) (Paul et al., 2018). As we generated detailed information on the subspecies' entire range (covering both Ganga and Sharda habitat blocks) and habitat status based on the initial surveys, distribution modeling and validation surveys, it was meaningful to identify critical "Priority Conservation Areas" where immediate management attention is required. This entire landscape is a mosaic of protected and non-protected areas with very high pressures from anthropogenic interventions. Between these two habitat blocks there are differences in grassland coverage area, management regimes and human densities. We used a few criteria to select such "Priority Conservation Areas" across the entire landscape covering both protected and non-protected habitats. The criteria were: (a) presence of grassland habitats (through field surveys and GIS approaches, Paul et al., 2018); (b) results from the MaxEnt model predictions; (c) confirmed species presence in the area; and (d) strategic position of the areas within this human-dominated mosaic landscape to ensure continuation of the species' seasonal migration (Martin and Gopal, 2015; Paul et al., 2018). Most importance was given to species and grassland presence, followed by the other two criteria.

3. Results

3.1. Swamp deer initial survey in Sharda habitat block

We found much fine-scale swamp deer occurrence evidence than earlier reported (Qureshi et al., 2004) across majority of surveyed areas in the Sharda river habitat block, including all the prominent protected areas (Pilibhit Tiger Reserve, Kishanpur Wildlife Sanctuary, Dudhwa National Park and Katerniaghat Wildlife Sanctuary). Additionally, we found presence of the species in non-protected grasslands of South Kheri and North Kheri Forest Divisions of Uttar Pradesh. Apart from swamp deer, we also identified the presence of other cervids (hog deer, chital, sambar etc.) in the grassland habitats. We collected a total of 225 biological samples (two swamp deer carcasses, 144 pellets and 79 confirmed swamp deer antlers) and identified 132 pellets out of which 51 belonged to swamp deer and 70, 10 and one to hog deer, chital and sambar, respectively. Remaining 12 samples did not provide any results during molecular species identification.

3.2. MaxEnt prediction of swamp deer distribution

Out of the 10 different models with different regularization multipliers (RM 0.5–5), the model with RM 1.5 showed lowest AICc Value (1183.32) and was selected (Supplementary Table A4). We pooled the prediction probabilities (above MTPLR = 0.067) to create high (0.6–1), moderate (0.4–0.6) and low probability (0.067–0.4) occurrence zones. The high probability of swamp deer was predicted to be along the banks of rivers of both Ganga and Sharda blocks (Ganga, Kosi, Ramganga, Sharda, Suheli, Ghaghra and Baur) (Fig. 2). In the Gangetic habitat block, the highest probabilities were found to be upstream of Bijnor Barrage along river Ganga, Banganga and Solani. The moderate zones included majority of the above-mentioned areas in addition to basins of Gomti, Gaula, Devha, Baigul, Ganga River (downstream of Bijnor Barrage) and some parts of Suhelwa Wildlife Sanctuary and Sohagibarwa Wildlife Sanctuary. The low probabilities of occurrence were found to be along river Yamuna and Hindon on the west; rivers like Rapti, Kuwano and some scattered areas of Suhelwa and Sohagibarwa Wildlife Sanctuary in the east and few areas further downstream south along Ganga (Fig. 2). Out of the total predicted area (3% of study area) for swamp deer distribution (7650 km^2), 3413 km^2 falls within protected areas of Uttarakhand and Uttar Pradesh.

The jackknife prediction of the final selected model showed that the NDVI-May had highest contribution (37.1%), followed by annual mean temperature (24.5%), distance from water (18.6%), nightlight (8%), NDVI-March 2017 (7.1%) in predicting swamp deer distribution. The remaining 4.7% is contributed by human population density, elevation and annual precipitation. Majority of the predicted areas were having an elevation of about 200m, and mean annual temperature range between 22-25 °C. MaxEnt results showed best prediction at a NDVI value of 0.45 during March and 0.6 in case of May. Distance from water

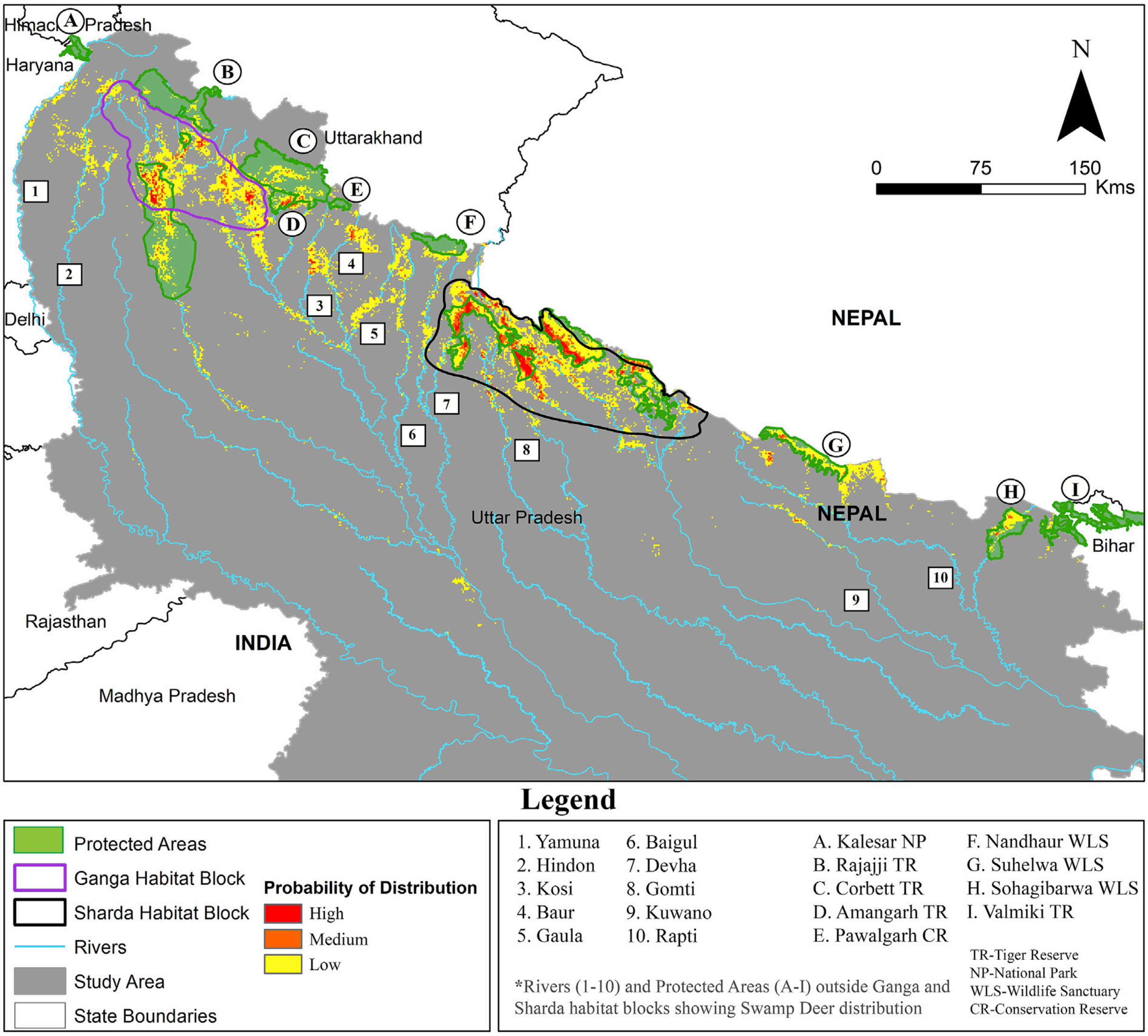

Fig. 2. Map showing the predicted distribution of swamp deer through MaxEnt modeling. The major rivers and protected areas outside Ganga and Sharda Habitat Blocks are highlighted and marked.

showed a negative relationship with swamp deer distribution after a distance of seven km from the rivers. Both nightlight and human population density were negatively related to the probability of distribution. The AUC value of the final selected model was 0.992 with a standard deviation of 0.001, indicating excellent prediction for swamp deer distribution (Elith et al., 2006) (Supplementary Fig. A1 and A2).

3.3. Validation surveys

Our survey in the lower part of Hastinapur Wildlife Sanctuary revealed the presence of fragmented grassland patches with swamp deer evidence (Fig. 3B). In addition to direct sighting ($n = 1$) and shed antlers ($n = 6$), molecular analysis of pellet samples ($n = 163$) confirmed presence of swamp deer ($n = 82$), hog deer ($n = 18$), nilgai ($n = 25$), barking deer ($n = 3$) and domestic goat ($n = 4$) (Supplementary Table A1). Rest 31 samples did not amplify during Polymerase Chain Reaction (PCR). In Sohagibarwa Wildlife Sanctuary, our surveys revealed *Typha* sp. and *Phragmites* sp. dominated grasslands but no evidence of swamp deer (Supplementary Table A1, Fig. 3D). Additionally, we validated the southernmost predicted location along Ganga (locally known as Kannauj, Fig. 3C) through secondary sources (direct sighting and media reports). Considering all these evidences ($n = 32$ unique occurrence points), we obtained a model accuracy of 84.37% after ground validation. Our validation surveys approximately expand the known range of swamp deer by 1125 km^2 along Ganga in the south.

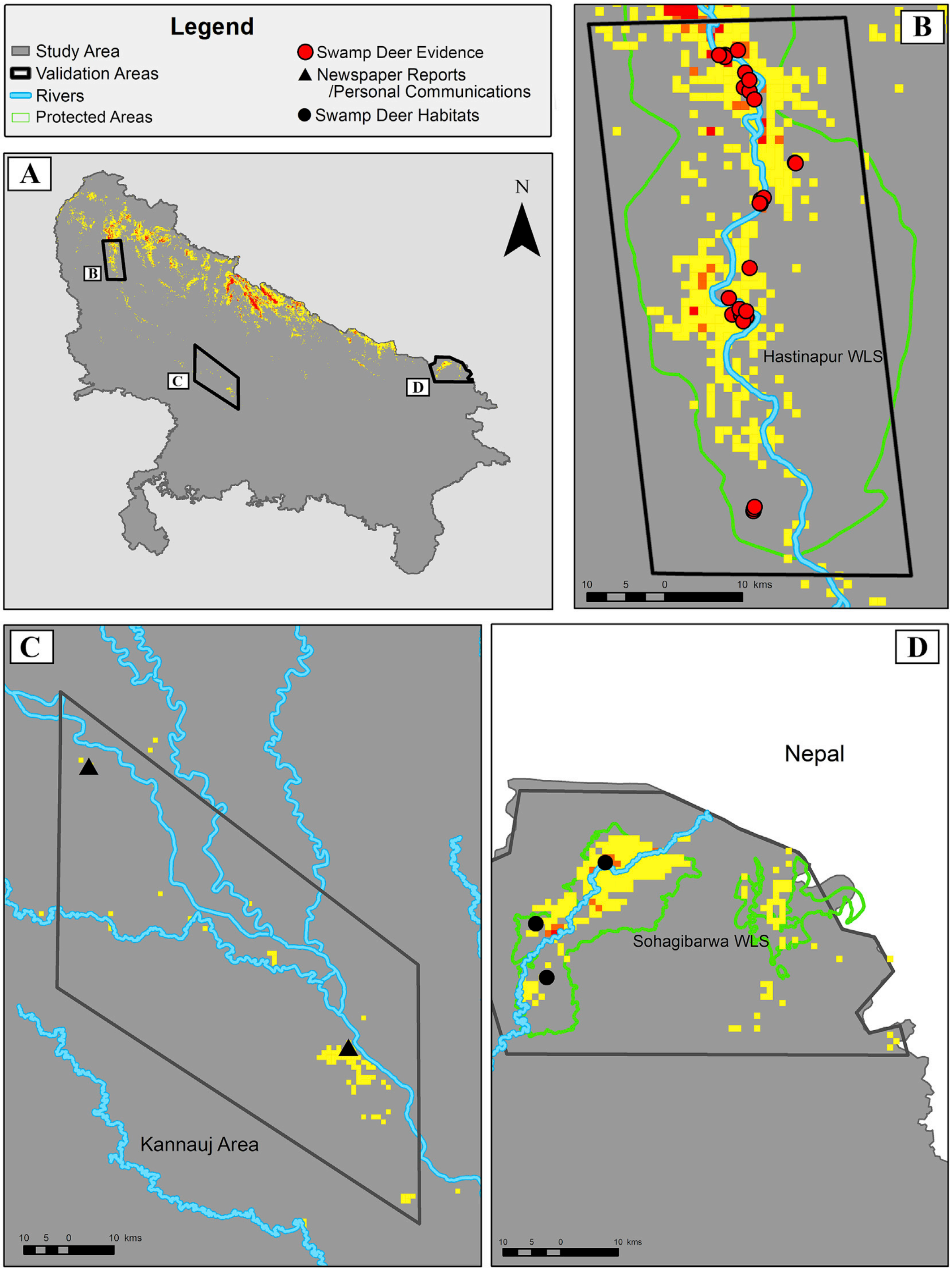

Fig. 3. Map showing the swamp deer evidence locations within the surveyed areas predicted through MaxEnt models. Within the figure, (A) shows three selected validation areas; (B) Validation area 1- Part of Hastinapur Wildlife Sanctuary below Bijnor Barrage, (C) Validation area 2- Kannauj along Ganga and (D) Validation area 3- Sohagibarwa Wildlife Sanctuary.

3.4. Habitat status and disturbances

Majority of the grasslands and its fauna within the protected areas of Sharda habitat block are undisturbed and receive proper protection. However, parts of Pilibhit Tiger Reserve and Katerniaghat Wildlife Sanctuary are facing anthropogenic disturbances like overgrazing and habitat conversion, and the non-protected areas in the Sharda block are experiencing rapid habitat encroachment (Supplementary Table A1). The grasslands in Sohagibarwa Wildlife Sanctuary are protected, undisturbed and hold potential for future relocation of swamp deer.

Similarly, we found that the grasslands downstream of Bijnor Barrage in Hastinapur Wildlife Sanctuary (Ganga habitat block) are facing severe pressures from overgrazing, habitat conversion and occasional poaching (Supplementary Table A1). The remaining habitats found downstream river Ganga are highly fragmented and future viability of swamp deer cannot be ascertained at this point.

3.5. Priority Conservation Areas

Based on the specific selection criteria (see 2.5), we identified four swamp deer “Priority Conservation Areas” covering both habitat blocks in this region (Fig. 4, Supplementary Table A5). Of these four selected areas, three (one protected and two non-protected areas) are in the Ganga habitat block, whereas one (non-protected areas) is in the Sharda habitat block. The details of these areas are as follows:

1. Priority Conservation Area 1: This is a ~193 km^2 area along Ganga between Jhilmil Jheel Conservation Reserve, Uttarakhand and Hastinapur Wildlife Sanctuary, Uttar Pradesh (Fig. 4A, Supplementary Table A5). We quantified ~14 km^2 grassland habitat (~7% coverage) within this area with abundant evidence of swamp deer. The entire area is human-dominated and non-protected and acts as a potential migration route between the above-mentioned protected areas (Paul et al., 2018).
2. Priority Conservation Area 2: This area covers the lower part of Hastinapur Wildlife Sanctuary downstream to Bijnor Barrage (1526 km^2) (Fig. 4B, Supplementary Table A5). This is a protected area with large human habitations within its boundary. We quantified ~73 km^2 grassland habitat (~4% coverage) with swamp deer evidence from different parts.
3. Priority Conservation Area 3: This is an isolated, recently reported swamp deer population (Paul et al., 2018) along Ramganga river in Jamanpur, Afzalgarh, Uttar Pradesh (Fig. 4C, Supplementary Table A5). This is a 139 km^2 area with ~3 km^2 grassland habitat (~2% coverage) and retains a small population of swamp deer (Paul et al., 2018).
4. Priority Conservation Area 4: This is a 555 km^2 unprotected riverine landscape along Sharda river (Fig. 4D, Supplementary Table A5). It retains ~136 km^2 grassland habitat (~24% coverage) and connects the Shuklaphanta Wildlife Sanctuary, Nepal with Kishanpur Wildlife Sanctuary, Uttar Pradesh, and possibly acts as the main corridor to connect the Nepal population with the north Indian population.

4. Discussion

To the best of our knowledge, this is the most exhaustive study conducted to assess the northern swamp deer (*R. duvaucelii duvaucelii*) distribution using species distribution modeling and subsequent ground-truthing surveys across the subspecies' range in India. Compared to the Ganga habitat block, our surveys show that the Sharda block still retains considerably large and undisturbed patches of grassland habitat with reports of significant swamp deer population (Duckworth et al., 2015; Qureshi et al., 2004), thereby acting as a primary stronghold for the northern subspecies. During our surveys, we found large numbers of swamp deer evidence in Pilibhit Tiger Reserve, Kishanpur Wildlife Sanctuary and Dudhwa National Park, whereas fewer signatures from Katerniaghat Wildlife Sanctuary. However, the non-protected areas along the rivers harboring grasslands are experiencing a lot of disturbance and require urgent conservation attention. Anecdotal reports from some of these areas (Kishanpur Wildlife Sanctuary of India and Shukla Phanta Wildlife Sanctuary of Nepal) indicate that they are connected by regular swamp deer movements involving non-protected areas but the exact routes and patterns of habitat use are not known. Future studies should focus on using radio telemetry and genetic tools to understand these aspects. The Sharda habitat block is an integral part of the contiguous central Terai landscape and hosts the majority of the swamp deer population (Qureshi et al., 2004; Singh, 1978). It will be important to focus on estimating population size, assess critical viable habitats and corridors in this landscape for future conservation of swamp deer.

During this study, we have carefully designed the swamp deer distribution modeling and used relevant parameters based on the species ecology. Although it is known that species distribution modeling for habitat-specialists yields better predictions (Connor et al., 2018; Rhoden et al., 2017), our study design had a focal area-based modeling approach, accurate presence locations (primary data), ecologically meaningful covariates with a temporal correspondence to occurrence locations and appropriate spatial scale for prediction (Araújo and Guisan, 2006; Aubry et al., 2017; Beerkircher et al., 2009; Costa et al., 2015; Tye et al., 2017; Williams et al., 2012). It is essential to point out that we did not use species absence data during analyses as it was challenging to determine true absence from these mosaic habitats consisting of grassland and croplands. However, we think that for swamp deer this would not have any impact on the results as the species is only restricted within 5–10 km on either side of the rivers present in this region (Paul et al., 2018; this study). Overall, our model showed very accurate predictions (AUC value of 0.992), which is expected for a habitat-specialist species (Connor et al., 2018) (Supplementary Fig. A2). Finally, we conducted extensive validation surveys to confirm the accuracy of the distribution predictions by the models. Although most of the species distribution modeling studies result in identification of new areas of occurrences (Hernandez et al., 2006), the validations are primarily restricted to comparison of training data subsets with

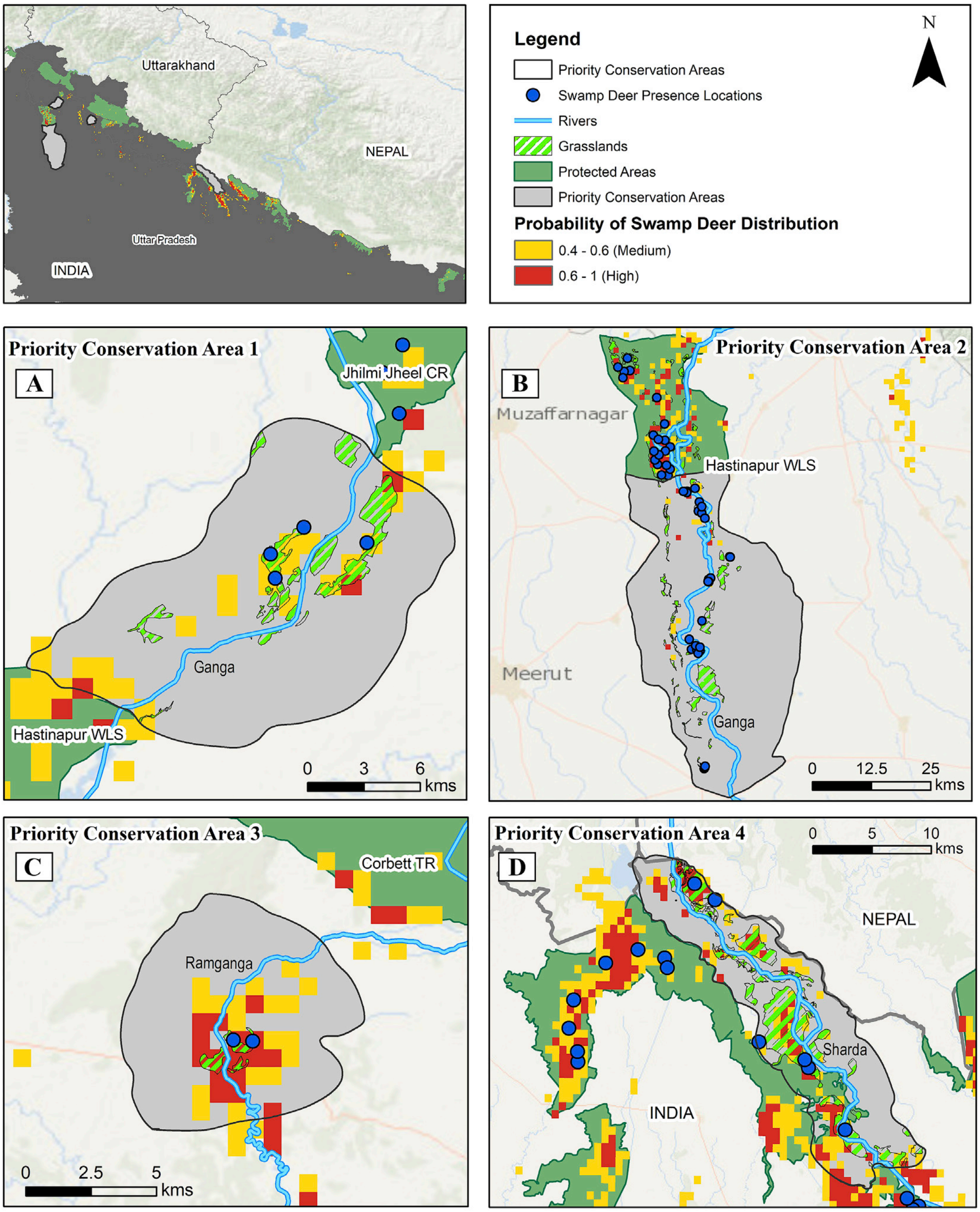

Fig. 4. Map showing the identified four "Priority Conservation Areas" in our study area. The priority areas identified are (A) The stretch of Ganga between Jhilmil Jheel Conservation Reserve and Hastinapur Wildlife Sanctuary; (B) The lower part of Hastinapur Wildlife Sanctuary below Bijnor Barrage; (C) The isolated grassland area on the bank of Ramganga near Jamanpur, Afzalgarh and (D) The unprotected area around Sharda river between Shuklaphanta Wildlife Sanctuary, Nepal and Kishanpur Wildlife Sanctuary, India.

predictions (Fois et al., 2018; Pearson et al., 2007) and not on-field validations (Greaves et al., 2006; Jime'nez-Valverde et al., 2011).

Out of the 11 initial ecological/environmental covariates (eight in the final model) used, NDVI May, annual mean temperature, distance from water, nightlight and NDVI March were found to be the most important factors (~95%) in explaining swamp deer distribution (Supplementary Fig. A1 and A2). In the entire Gangetic plains, the croplands possess a lion's share of the Landuse Landcover in terms of area. Thus we chose NDVI May and NDVI March that represent the grasslands-cropland mosaics, where the March NDVI indicates indices of both vegetation types during the pre-harvesting period when it is difficult to differentiate them (thus overlapping NDVI values). However, during May, after harvestation of the crops (wheat and sugarcane) the same NDVI values are only represented by grasslands. Thus NDVI May showed a strong positive relation with probability of swamp deer presence and was the highest contributor in explaining the species distribution. We used NDVI as a surrogate for grassland delineation in our analyses (comparatively lower accuracy) as detailed ground-truthed visual classification of the grasslands was not available. As a result certain areas like Suhelwa Wildlife Sanctuary showed low prediction probabilities, though previous reports suggest absence of tall wet, swampy grasslands (Chanchani et al., 2014). The prediction has been driven majorly by distance to rivers and NDVI as the area has many rivers flowing through the sanctuary, but further surveys should be conducted to ascertain the current status.

Our validation surveys confirmed swamp deer presence in the lower part of Hastinapur Wildlife Sanctuary and identified only grassland habitats in Sohagibarwa Wildlife Sanctuary. During the surveys, we identified highly fragmented habitat patches with comparatively less swamp deer evidence below the Bijnor Barrage area of Hastinapur Wildlife Sanctuary along river Ganga. This area is highly human-dominated and needs immediate conservation attention for future survival of the species. There are sporadic reports of swamp deer presence further south along Ganga (Fig. 3) and extensive surveys need to be conducted to ascertain viable habitat and population status. Within Sohagibarwa Wildlife Sanctuary, we identified large patches of wet grasslands without any swamp deer evidence. Earlier studies reported swamp deer presence in this region during the 1960s (Ghimire et al., 2019) and given the protection status of this sanctuary, it can be a suitable area for potential reintroduction in future.

Finally, we identified four critical "Priority Conservation Areas" that demand immediate management attention for long-term survival of the species in this landscape (Fig. 4). All of these selected areas are either corridor habitat (Priority Conservation Area 1 and 4, Fig. 4A and D) or important populations that need careful management. Three of these habitats are in the Ganga habitat block, where habitat loss and other anthropogenic factors have resulted in extremely fragmented swamp deer habitats in recent times (Paul et al., 2018). The Priority Conservation Area 1 (between Jhilmil Jheel Conservation Reserve and Hastinapur Wildlife Sanctuary, Fig. 4A) is the only connecting habitat between these protected regions. Despite having unprotected status, this area has a grassland habitat coverage of ~7% which is similar to Jhilmil Jheel Conservation Reserve and Hastinapur Wildlife Sanctuary (3% and 7%, respectively) and retains swamp deer evidence. Swamp deer from Jhilmil Jheel Conservation Reserve are known to migrate seasonally (Paul et al., 2018) and most likely use this area to move downstream Ganga. However, this area currently faces a lot of human disturbances (Paul et al., 2018) and if these grassland habitats are lost, the two protected areas will become isolated. Conservation efforts should strongly focus on public awareness programs and community involvement through developing this area as a "Conservation or Community Reserve" (based on existing land use and land-tenure data) in near future to ensure long-term survival of this subspecies. However, in case of the Priority Conservation Area 2 (downstream to Bijnor Barrage area, Fig. 4B) the challenges are different. This region is within a protected area (Hastinapur Wildlife Sanctuary) and thus the species status should be better here. A large part of the sanctuary (particularly downstream of Bijnor Barrage) is heavily human-dominated (including large villages and towns) and possibly cannot be reverted back to natural grassland habitats. We suggest further detailed, fine-scale mapping of this region to demarcate the human-dominated areas and rationalize the boundary to include only grassland habitats for focused habitat management. The third Priority Conservation Area is an isolated swamp deer population in the eastern border of the Ganga habitat block (Fig. 4C). This habitat patch is entirely surrounded by human habitats and needs urgent attention. This area is also the closest to the Sharda habitat block populations and future investigation should focus on understanding the historical connectivity between these two habitat blocks. Within the Sharda habitat block, we identified one Priority area that is a critical migration corridor to connect the Nepal population with the Indian swamp deer population. While most of the swamp deer habitat in this block is within a number of protected areas and thus receive better protection (unlike the Ganga habitat block), this critical corridor is mostly unprotected. Given that this is the world's largest global intermixing population of swamp deer, protecting this corridor by converting this area as a "Conservation or Community Reserve" possibly will be the only way to maintain this metapopulation. If this corridor is lost due to human impacts, the Indian population might get genetically isolated from the Nepal population.

It is noteworthy to point out that the importance of these Priority Conservation Areas should not be misconstrued as purely based on grassland coverage/extent alone. The fragmented grassland habitats outside protected areas are easy targets for habitat conversion in this high human-dense landscape, and majority of the selected priority areas have low overall grassland coverages (~2−24% of the respective areas, Supplementary Table A5). This can be argued against our suggestion of creating Priority Conservation Areas. However, these areas have critical importance when looked at swamp deer specific requirements (essential habitat for seasonal migration, feeding ground, fawning etc.) and they are essential to ensure long-term persistence of the species in this landscape. Thus, they need to be considered as critical parts of the entire landscape for swamp deer survival. According to recent assessments, grasslands are one of the most marginalized ecosystems in India (Report of the task force on Grasslands and Deserts, 2006), with a loss of ~20 million hectares area between 1880 and 2010. With the "Green Revolution" movement in India, there has been a significant shift to irrigation-based agricultural practices, leading to fast decline in grassland habitats (Vanak et al., 2017) across north India. These remaining grassland patches are the last resort to the habitat-specialist biodiversity, and appropriate management interventions are required urgently to protect the ecosystem.

5. Conclusion

During the early 20th century, the swamp deer was found throughout the Indo-Gangetic plains (Duckworth et al., 2015) but they are currently considered to have the highest extinction probability among all Indian megaherbivores (Karanth et al., 2010). However, details of their population size and habitat status have been rather scanty due to logistical challenges in generating population-level information in human-dominated landscapes across the Gangetic plains. The result of our study provides a detailed insight of swamp deer distribution and highlights the importance of the riverine-grassland systems of northern India, where a considerable amount of biodiversity coexists with a very high human population (Uttar Pradesh ranks first among the states of India according to Census, 2011). While all these new information is quite promising for the species, there is still a pressing need to focus on population enumeration and grassland management, particularly those present in the Priority Conservation Areas described here. Such efforts would also generate critical information for other co-occurring species (for example, hog deer, fishing cat, Bengal florican etc.) of the grasslands/wetlands and help in their conservation. We suggest using swamp deer as an umbrella species to strengthen habitat management and conservation for the otherwise marginalized grassland habitats in this landscape. This work also emphasizes the need for similar studies to generate critical information for other locally or regionally endemic, habitat-specialist species that occur outside protected areas for their conservation.

CRediT authorship contribution statement

Shrutarshi Paul: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization, Supervision. **Debanjan Sarkar:** Methodology, Software, Formal analysis, Writing - original draft, Visualization. **Abhilash Patil:** Methodology, Software. **Tista Ghosh:** Validation, Investigation, Writing - review & editing. **Gautam Talukdar:** Methodology, Resources, Writing - review & editing, Supervision. **Mukesh Kumar:** Writing - review & editing, Resources, Funding acquisition. **Bilal Habib:** Conceptualization, Writing - review & editing. **Parag Nigam:** Writing - review & editing. **Dhananjai Mohan:** Resources, Writing - review & editing, Supervision, Funding acquisition. **Bivash Pandav:** Conceptualization, Resources, Writing - review & editing, Project administration, Funding acquisition. **Samrat Mondol:** Conceptualization, Data curation, Resources, Writing - original draft, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare no conflict of interests.

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Appendix A. Supplementary data

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