Sustainable Forests & Livelihoods (SUFAL) Project
Forest Department
Ministry of Environment, Forest and Climate Change

Compendium of Wildlife Research under the SUFAL Innovation Grant

Volume 1

SUFAL

THE WORLD BANK
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This publication is an output from the Sustainable Forests & Livelihoods (SUFAL) Project implemented by Bangladesh Forest Department (BFD) under the Ministry of Environment, Forest and Climate Change.

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Dr. Farhina Ahmed
Secretary
Ministry of Environment, Forest and Climate Change
Government of the People's Republic of Bangladesh

Message

I am pleased to share this volume of research outputs generated under the SUFAL Innovation Grant (SIG) Program, an integral component of the Sustainable Forests and Livelihoods (SUFAL) Project of the Bangladesh Forest Department under Ministry of Environment, Forest and Climate Change. This compilation reflects a significant step forward in our collective efforts to ground forest management and wildlife conservation in robust scientific evidence.

Bangladesh is endowed with a remarkable diversity of flora and fauna, yet this heritage is under mounting pressure from rapid land-use change, habitat fragmentation, climate-induced stresses, and the growing interface between people and wildlife. These complex challenges cannot be addressed through traditional management practices alone. They require interdisciplinary inquiry, empirical data, and context-specific solutions that link ecological integrity with human wellbeing.

The SIG program was conceived precisely with this vision in mind. By offering competitive grants to researchers, universities, and specialized institutions, the program has created space for innovative ideas, rigorous fieldwork, and analytical research on Forestry, wildlife and biodiversity issues.

The findings presented here do not remain confined to academic discussion; they generate clear, actionable recommendations for managers, planners, and policymakers. They provide insights that can inform zoning and management plans, improve conflict mitigation measures, strengthen community participation, and enhance the resilience of forest, wildlife and ecosystems to climate and other anthropogenic pressures.

I wish to acknowledge the dedication of the researchers and their teams, the guidance provided by the various review committees, and the support of partner organizations and development partners who have contributed to the success of the SIG program. Their combined efforts have ensured that the studies uphold scientific rigor while remaining closely aligned with national conservation priorities and international commitments.

It is my expectation that this publication will be used widely by conservation practitioners, government agencies, academia, development partners, and civil society to inform decision-making and to inspire further research and innovation. As we strive to conserve Bangladesh's forest, wildlife, biodiversity and ecosystems for present and future generations, evidence-based knowledge such as that contained in this volume will remain one of our most valuable assets.

Dr. Farhina Ahmed

Md. Amir Hosain Chowdhury
Chief Conservator of Forests
Bangladesh Forest Department

Message

Forests and wildlife in Bangladesh play a vital role in maintaining ecological balance, supporting livelihoods, and contributing to national development. To manage these resources wisely, we need a clear understanding of forest dynamics, species ecology, ecosystem services, and the social context of resource use.

This publication represents significant research on both forestry and wildlife supported by the SUFAL Innovation Grant (SIG) Program of the Sustainable Forests and Livelihoods (SUFAL) Project. The studies compiled here reflect a strong commitment to grounding conservation and forest management in scientific evidence, practical field experience, and the realities faced by forest-dependent communities in Bangladesh.

The research presented in this volume responds to that need. It includes work on forest restoration and productivity, co-management and collaborative forest management, biodiversity assessment, species conservation, human–wildlife interaction, and climate-resilient management practices. Together, these studies demonstrate how well-designed research can guide more sustainable and inclusive management of forests and wildlife.

The importance of this publication lies in its ability to translate research findings into practical guidance. The results offer concrete suggestions for improving forest management plans, enhancing protection and restoration measures, designing and managing Reserved Forests and Protected Areas, and supporting communities that depend on forests and wildlife for their livelihoods. As such, this volume is intended to serve not only researchers and academics but also policymakers, forest managers, conservation practitioners, and development partners.

I hope that the insights and recommendations contained here will be widely used to strengthen evidence-based decision making in the forestry and biodiversity sectors of Bangladesh. This publication will help to formulate our future policies, improve field practices, and inspire further research and innovation. It will make a long lasting contribution to the conservation of our forests, biodiversity and wildlife for present and future generations.

Md. Amir Hosain Chowdhury

Gobinda Roy
Project Director
Sustainable Forests and Livelihoods (SUFAL) Project
Forest Department
Government of the People's Republic of Bangladesh

Foreword

Sustainable Forests and Livelihoods (SUFAL) Project has supported research and innovation through its Innovation Grant Program, designed to strengthen forestry and biodiversity knowledge in Bangladesh. SUFAL Innovation Grant (SIG) is one of the unique features of SUFAL Project guided by the Innovation Grant Manual. This mechanism was designed to promote scientific and applied research on forestry, wildlife, and biodiversity conservation, while also ensuring transparency, accountability, and quality in both the selection and implementation of research initiatives.

Through this competitive process, 34 research grants were awarded to researchers and institutions after rigorous evaluation. Each proposal and subsequent deliverable was carefully reviewed by the Deliverable Review and Comments Committee, the Research Proposal Evaluation Committee, and the Forestry and Wildlife Research Review Council. This multi-tiered scrutiny ensured that the work undertaken met high professional standards and directly addressed the needs and challenges in forestry and wildlife management.

SUFAL Project also organized result dissemination workshops, where researchers shared their findings with policymakers, practitioners, academics, and other stakeholders. These events served not only as a platform for knowledge exchange but also as an opportunity to foster collaboration and dialogue around future research priorities. Following this, the researchers submitted their final reports, marking the successful completion of all 34 studies under the SIG program.

To make these findings widely accessible, SUFAL is now publishing four volumes of research summaries: two focusing on forestry and two on wildlife. These volumes present the essence of the research in a concise and structured manner, allowing stakeholders, decision-makers, and development partners to draw practical lessons and insights. The outcomes documented here will help shape evidence-based policies, strengthen conservation practices, and guide further research and innovation in the forestry and biodiversity sector of Bangladesh.

I would like to extend my sincere gratitude to the dedicated researchers, the review committees, partner institutions, and the World Bank for their valuable support. Their commitment and contributions have made it possible to generate a body of knowledge that will serve the sector for years to come. This publication will not only enrich our understanding but also support Bangladesh for achieving its national priorities and international commitments related to forest, biodiversity and wildlife conservation.

Gobinda Roy

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Research Title

Integrated molecular techniques for the identification of illegally traded species and detection of species geographic origin

by
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CHAPTER I
INTRODUCTION

1.1 Problem statements

In recent decades, there has been a rapid and unprecedented decline in world biodiversity. Bangladesh is not an exception in this concern. 'Updating Species Red List of Bangladesh' is a massive milestone work in the conservation history of the country where 390 species (over 25%) were assessed under the Threatened category out of 1619 species (IUCN-Bangladesh, 2015a). In the Threatened category, it revealed that 0.32% of butterflies, 0.56% of fishes, 1% of reptiles, 0.6% of birds and 1% of mammals were Critically Endangered while, 6.9% butterflies, 1.85% fishes, 0.75% each of birds and mammals, 0.6% reptiles and an insignificant number of crustaceans and amphibians have been considered as Endangered. The most Vulnerable group appeared to be the butterflies with 4.63% species under this category and 1.5 % of freshwater fishes, 1% of reptiles and an insignificant number of other groups had been considered as Vulnerable (IUCN-Bangladesh, 2015a). We have already lost 31 species of mammals, birds and reptiles from our country since the last century and about 390 are nationally threatened (IUCN Bangladesh, 2015a). Along with the large number of invertebrates, butterflies also face the same fate and it is most distressing that even we do not know many species, as well as how many of them have already gone extinct (IUCN-Bangladesh, 2015a).

In the world, populations of fish, birds, mammals, amphibians and reptiles have declined on average by 60% in less than fifty years (between 1970 and 2014.) The main causes are loss of habitats and over-exploitation of species, including the illegal hunting and trading of animals (Tobe and Linacre, 2010; SWGWILD, 2012; https://www.wwf.org.uk/sites/default/files/2019-04/KS3_WWF_IllegalWildlifeTradeInvestigations.pdf accessed on 01.01.2024). Bangladesh has a big source of rare and endangered wild animals like turtle, tortoise, pangolin, gecko, birds and tiger for smuggling them out of the country. If the smuggling of these species continues, then these species will be extinct from Bangladesh in future.

Therefore, serious attention is required to save these species from becoming further threatened and heading for the next higher level of Critically Endangered or even in worst case, towards Regional Extinction (IUCN-Bangladesh, 2015a). According to data available from Wildlife Crime Control Unit (WCCU) under Bangladesh Forest department, law enforcement agencies rescued 37,039 wild animals and birds from across the country during the period of June 2012 to November 2016 (https://www.daily-sun.com/printversion/details/292686/2018/03/03/Wildlife-trafficking-continues-unabated 01.01.2024).

Poaching of tiger and prey has been identified as one of the major threats to tiger populations in Sundarbans (Karanth and Stith, 1999; Wikramanayake *et al.*, 2011; Aziz *et al.*, 2013). On the other hand, indiscriminate poaching of deer populations seriously hampers the whole ecosystem in the Sundarbans. Along with that poachers are also killing snakes, birds, otters etc. in this mangrove forest (https://www.observerbd.com/news.php?id=149195 accessed on 01.01.2024). Although illegal trading of butterflies is not known yet in Bangladesh, and we are afraid of only Critically Endangered (CR) species Sundarban crow's lone present in the mangrove forest (IUCN-Bangladesh, 2015b). Though illegal trade of butterflies are reported from India, China, Thailand and Malaysia (https://www.bbc.com/news/world-asia-china-41917630; https://www.newindianexpress.com/magazine/2010/oct/24/illegal-trade-of-butterflies-197722.html accessed on 01.01.2024).

In Bangladesh, it appeared that 31 species were Regionally Extinct while 390 species were assigned under the Threatened category by IUCN (IUCN-Bangladesh, 2015a). In addition, poaching and illegal trading make worsen the situation that leads them to extinction near future. To conserve the endangered species, forensic investigations and proper taxonomic identification is required. The standard method of utilization of morphological characters become challenging due to various factors such as phenotypical variations (Karthika *et al*., 2016). In this situation, mitochondrial DNA (DNA barcoding) has been extensively analysed and proven to be an important tool in species identification (Hebert *et al*., 2003a). A major feature of DNA barcoding is that it allows precise identification of animals (it's immature stages also) and of fragmentary body parts like scales, claws, hair etc. Mitochondrial gene such as Cytochrome c oxidase I (COI) and other molecular markers (Cytb, 16s rRNA, 12S rRNA and Control region) have been used to identify and discover new species as well.

1.2 Objectives of the Study

Prepare a priority list indicating the vulnerability of the most illegally traded and threatened animals of Bangladesh.

Collection of the samples of the most illegally traded and threatened animals.

Mitochondrial DNA genes (COI/Cytb/16s rRNA/ND5/Control region) will be generated from collected samples.

Molecular identification and determination of the geographic origin of the most illegally traded species from a local and global perspective.

Control of illegal trading and poaching of wildlife as a correct and quick means of identification.

Molecular data will be deposited in the BFD or national repository for further reference or use.

Molecular tools and techniques will be gathered and used to train BFD officials. # The required software or script will be provided to BFD officials.

BFD (allied stakeholders) officials will self-sustain to control illegal trading and poaching of wildlife.

1.3 Research Questions

Poaching and illicit trade pose a threat to biodiversity, particularly to endangered species. Notable and potentially driving local extinctions in the near future are the trade of endangered species such as tigers, small cats, pangolins, turtles, and tortoises (Aziz et al., 2017; WCS, 2018). A remarkable trade in shark fins and rays also occurs from Chittagong and Cox's Bazar. While international trade of tokay geckos is an emerging concern now a days in the country. The high prices reportedly paid for these reptiles indicate a strong demand resulting in international trade that could threaten the species in the future. Another endangered species is Ganges river dolphin, which is killed and poached frequently to sell their fat and oil in the local community (IUCN-Bangladesh, 2015; WCS, 2018). While, several domestic illegal trade routes are used in the country for the hornbills, mynas, parrots, bear cubs, pangolin, wild cats and freshwater turtles (WCS, 2018). An accurate identification of a species is necessary for several purposes, including biodiversity monitoring, forensic investigations, and the protection of endangered species.

In this context, morphological identification is sometimes ambiguous and time consuming as well as not possible if specimen is damaged, degraded (remain only scales, hairs, claws etc) and immature stages. In this

situation molecular identification (DNA barcoding) using mitochondrial DNA (mtDNA) may be a powerful tool to solve the problem precisely (Hebert *et al*., 2003a).

Currently, there is a lack of a thorough inventory of our illegally traded species in Bangladesh, which will provide an opportunity to enhance conservation and management efforts for illegally traded wildlife in the country.

It is necessary to compile a species list in order to priorities the generation of barcode genes and a reference database.

The collection of the specimen's sample is on the list for the acquisition of molecular data for controlling illegally trafficked animals and endangered wildlife.

Applying molecular data (mitochondrial DNA/genes) and bioinformatics for the precise and accurate identification and detection of species geographic origin in Bangladesh and global perspectives.

Accuracy is crucial when dealing with molecular data, therefore, a study based on several loci would be appropriate. The multiloci data collection comprises various mitochondrial genes, specifically COI, Cytb, 16s rRNA, ND5, and Control region.

In order to address the issue of illegal trading at a detailed level, it is necessary to integrate molecular data and create a wildlife forensic technique or tool that assists the Bangladesh Forest Department.

CHAPTER II
LITERATURE REVIEW

Conserving wildlife is a significant challenge for Bangladesh. This study reveals that a diverse range of wild animals and species in Bangladesh are currently facing significant threats. These threats include forestland encroachment, destruction of habitats, human activities in close proximity to forests, interactions between humans and animals, illicit hunting and trafficking, exclusion of local communities from conservation efforts, socio-economic and political challenges, as well as external issues such as the refugee crisis and cross-border animal migration. Currently, there has been a rise in environmental crimes, particularly illegal wildlife crimes, in Bangladesh in recent years, despite the presence of various laws such as the Wildlife (Conservation and Security) Act, 2012 (referred to as Wildlife Act 2012) and numerous international treaties, agreements, conventions, and protocols (Islam and Zheng, 2023).

Global biodiversity is threatened by wildlife trade. Targeted actions to avert species losses from unsustainable trade require specific trade patterns and processes. Bangladesh may be a global commerce lynchpin for some groups, but its role has been neglected. The trade of twelve live felid species was studied using interviews with actors in the trade, newspaper reports of seizure instances, and CITES export and import records for individual shipments from 2016 to 2021. IUCN classified one of the 12 wild felid species in trade as Endangered, four as Vulnerable, and three as Near Threatened. Live felids were mostly imported from South Africa and India (51% and 27%, respectively), while India and Myanmar dominated exports (20% and 15%). International live felid commerce involved nine lands, one airport, and one seaport. Current investigation implies that the surge in live felid import to Bangladesh may be for exotic pets. However, large differences in CITES data prevent clear interpretation of trade patterns, and additional work is needed to monitor and regulate wildlife trading in Bangladesh (Uddin *et al*., 2023).

Wildlife markets are hubs for illegal wildlife trade due to lack of surveillance and enforcement. To stop illegal wildlife trading, one must know the species trafficked, sources, and transport routes. From January to December 2019, wildlife marketplaces in Bangladesh were surveyed monthly to determine the abundance, diversity, and drivers of wildlife trafficking. The most traded orders were Passeriformes, Columbiformes, Psittaciformes, Artiodactyla, Carnivora, and Testudines. Tigers, crocodiles, and tortoises were traded in wildlife marketplaces. Hill markets and peri-urban markets sold mostly species from surrounding forests, whereas urban markets sold native and exotic species from abroad (Uddin *et al*., 2022).

Wildlife Crime Control Unit (WCCU) under Bangladesh Forest department has reported that law enforcement forces saved a total of 37,039 wild animals and birds from various locations in Bangladesh between June 2012 and November 2016 (https://www.daily-sun.com/printversion/details/292686/2018/03/03/Wildlife- trafficking-continues-unabated accessed on 01.01.2024).

According to several studies (Karanth and Stith, 1999; Wikramanayake *et al*., 2011; Aziz *et al*., 2013) tiger and prey poaching is a significant danger to Sundarbans tiger populations. Unfortunately, the Sundarbans ecosystem as a whole will suffer greatly from the careless killing of deer. Poachers are also destroying this mangrove forest's avian and wildlife populations, including snakes, birds, and otters (https://www.dhakatribune.com/bangladesh/238013/sundarbans-under-red-alert-to-stop- poaching accessed on 01.01.2024).

Currently, there is no recorded occurrence of illegal butterfly trading in Bangladesh. However, we are concerned about the presence of the critically endangered Sundarban crow, which is the only species of its kind found in the mangrove forest. Illegal butterfly trading is recorded in India, China, Thailand, and Malaysia (https://www.bbc.com/news/world-asia-china-41917630; https://www.newindianexpress.com/magazine/2010/oct/24/illegal-trade-of-butterflies- 197722.html accessed on 01.01.2024).

Comprehensive research has demonstrated that DNA barcoding has the ability to accurately identify a diverse array of animal species, such as mammals, birds, reptiles, amphibians, fishes, and crustaceans (Hebert *et al*., 2003a; Hebert *et al*., 2003b; Ivanova *et al*., 2012; Vences *et al*., 2012). Furthermore, DNA barcoding has been lately utilized in diverse domains such as health, food science, forensics, and conservation biology (Yang *et al*., 2018).

Bangladesh possesses a diverse range of biological diversity. The IUCN-Bangladesh conducted an assessment of 1,619 species. In the project "Updating Species Red List of Bangladesh," it was found that 31 species of mammals, birds, and reptiles had become extinct in the region over the past century, while 390 species have been classified as threatened (IUCN-Bangladesh, 2015a).The primary cause of these species' endangerment is the pressures of human population growth, which ultimately results in the destruction of forests and other habitats. Additionally, domestic and international wildlife commerce, as well as poaching within the nation, endangers these species. Illicit wildlife trade and poaching are pervasive issues in the country, according to numerous reports from NGOs, national and international media, and the Wildlife Crime Control Unit (WCCU).

Notable threats to local ecosystems, including small cats, tortoises, leopards, and pangolins, are traded in these species (Aziz *et al*., 2017; WCS, 2018). Additionally, shark fins and rays are traded extensively between Chittagong and Cox’s Bazar. Presently, the nation is experiencing a growing apprehension regarding the international trade of Tokay geckos (Olivier, 2013; Islam, 2023). The reportedly exorbitant prices paid for these reptiles are indicative of a robust demand that has led to international commerce, which may pose a future threat to the species. The Ganges river dolphin is an additional endangered species that is frequently poached and slain for its fat and oil, which are sold in the local community (WCS, 2018; IUCN-Bangladesh, 2015). Although the nation is home to various wildlife species such as freshwater turtles, hornbills, mynas, parrots, bear cubs, and pangolin, illicit trade routes for these animals also exist *(WCS, 2018).*

The global populations of fish, birds, mammals, amphibians, and reptiles have experienced an average decline of 60% during a span of fewer than fifty years, namely between 1970 and 2014. The primary factors contributing to this issue are habitat degradation and the excessive exploitation of species, which encompasses the unlawful hunting and trading of animals (Tobe and Linacre, 2010; SWGWILD, 2012; https://www.wwf.org.uk/sites/default/files/2019- 04/KS3_WWF_IllegalWildlifeTradeInvestigations.pdf accessed on 01.01.2024).

Howlader *et al*., (2015) described the Kalasgram Skittering Frog (Euphlyctis kalasgramensis), a dicroglossid from Bangladesh. Because of its conserved physical structure, E. kalasgramensis was once classified as the Indian Skittering Frog. This species has been found and reported throughout Bangladesh using DNA barcoding techniques, specifically targeting the mitochondrial gene 16s ribosomal RNA (16s rRNA) (Siammawii *et al*., 2022).

The only population of *Panthera tigris* that has adapted to mangrove forest habitat is found in the Sundarbans. 33 tiger samples from the Bangladesh Sundarbans were sequenced for 1263 base pairs (bp) of mtDNA across 4 mtDNA loci by Aziz *et al*. (2022). These sequences were then compared to 33 mtDNA haplotypes identified in all extant tiger subspecies. The authors identified three haplotypes in the Sundarbans tigers, two of which are shared with tiger populations residing in central Indian landscapes and one of which is exclusive to this population. The Sundarbans tigers were determined to be paraphyletic through phylogenetic analyses employing maximum likelihood and Bayesian inferences. This suggests that the Sundarbans population remains in close phylogenetic proximity to other populations of Bengal tigers, from which it departed approximately 26,000 years ago (Aziz *et al*., 2022).

In order to achieve species-level identification of ichthyofaunal diversity, a barcode dataset was generated from an amplification of approximately 655 base pairs of the mitochondrial cytochrome c oxidase subunit I (COI) gene of 29 endangered fish species. This study concentrates for the first time on COI-based molecular characterization of the endangered fish species of Bangladesh; as such, it may serve as a reference study for in-situ conservation and authentic identification of these species (Hossain *et al*., 2021).

Utilizing the mitochondrial cytochrome c oxidase subunit I (COI) gene as a marker, a comprehensive study characterizes the molecular makeup of marine and coastal vertebrates in Bangladesh. From 185 species constituting 146 genera, 74 families, 21 orders, and two classes of fishes, a cumulative of 376 mitochondrial COI barcode sequences were acquired. Authors can conclude that COI sequencing can be used as an authentic identification marker for Bangladesh marine fish species based on the present study's demonstration of high efficiency and fidelity in species identification and discrimination via DNA barcoding (Ahmed *et al*., 2021).

The technique of DNA barcoding has been utilized to accurately identify fish, aquatic molluscs, and crustaceans in the Sundarbans mangrove wetland. This involves amplifying two specific mitochondrial genes, namely COI and 16s rRNA, in order to create a reference library of the fish and shellfish species found in this unique ecosystem. This study successfully addressed the issue of misidentifying the mud crab species found in the Sundarbans. After being wrongly identified as Scylla serrata, this study cleared up the confusion over the Sundarbans mud crab species as Scylla olivacea (Habib *et al*., 2021).

The utilization of mitochondrial Cytochrome b gene in DNA barcoding effectively distinguishes H. litoralis from other closely related species due to a significant genetic divergence ranging from 13.9% to 27.8%. The sequences generated from H. litoralis in northeast India showed a slight divergence (1.5%) from the sequences generated in Bangladesh, suggesting that there may be genetic variability resulting from changes in the ecological niche (Kundu *et al*., 2018; Kundu *et al*., 2020).

In Bangladesh DNA barcoding has been employed to identify various species of diverse arena (Ghosh *et al*., 2019; Hossain *et al*., 2021; Ahmed *et al*., 2021**;** Siammawii *et al*., 2022; Hossain *et al*., 2022; Aziz *et al*, 2022; Akter *et al*., 2023; Mahjabin *et al*., 2023; Meem *et al*., 2023). Therefore it indicates a huge potentiality to identify species to lead to a better conservation and wildlife management in Bangladesh.

Accurate taxonomic identification is necessary for forensic investigations and the preservation of endangered species. Because of factors like phenotypic variability, the conventional approach to using morphological traits becomes difficult (Karthika *et al*., 2016). Hebert *et al*., (2003a) found that mitochondrial DNA (DNA barcoding) is a powerful tool for species identification in this context.

CHAPTER III

METHODOLOGY

3.1 Specimen (species) list

A specimen list of 579 species has been prepared by giving priority to illegally traded species and threatened species (IUCN Red List threatened species) in Bangladesh to generate DNA barcoding genes for species identification and proper management of illegally traded species as well as species conservation (IUCN-Bangladesh, 2015ab). Among them, we observed 26 species of mammals, 17 species of reptiles, 15 species of birds, and 2 species of crustaceans included in the list of illegally traded species in Bangladesh.

3.2 Established collaboration

For sample collection to generate molecular data, we established collaboration with the Bangladesh Forest Department (BFD), Wildlife Conservation Society (WCS), Bangladesh Bird Club, Halda River Research Laboratory, Bangabandhu Sheikh Mujib Safari Park, Gazipur and Dulahazara, Venom Research Centre (VRC), Deep Ecology and Snake Conservation Foundation, Bangladesh Bannayaprani Sheba Foundation, and IUCN Bangladesh.

3.3 Sample collection

Different field trips were conducted to collect samples of different species during the study period including Wildlife Rescue and Rehabilitation Centre (WRRC) or Wildlife Management and Nature Conservation Division, Khulna (22°49'45.1"N 89°32'03.3"E), Karamjal Wildlife Breeding Center, Sundarbans (22°25'41.3"N 89°35'25.1"E), Bangabandhu Sheikh Mujib Safari Park, Gazipur (24°10'11.8"N 90°23'50.3"E), Bangabandhu Sheikh Mujib Safari Park, Dulahazara (21°40'10.6"N 92°05'08.5"E), Lawachara National Park (Lawachara NP) (24°19'40" N 91°47'1" E), Baroiyadhala National Park (Baroiyadhala NP), Sitakunda (22°39'11.0"N 91°39'41.4"E), Madhupur National Park (24°42'14.3"N 90°04'39.0"E), Rajkandi Reserve Forest (Rajkandi RF) (24°15'55.8"N 91°55'16.6"E), Inani Reserve Forest, Cox's Bazar (21°13'47.4"N 92°03'23.8"E), Khagrachari Forest (23°05'58.0"N 91°59'26.3"E), Kudum Cave, Ukhiya (21°05'46.9"N 92°09'53.9"E), Bangladesh Bannoprani Sheba Foundation, Sreemangal (24°18'54.5"N 91°43'23.5"E), WCCU-Sreemangal (Appendix I). Besides, a few samples were collected from Chattogram University (22°28'02.5"N 91°46'50.6"E), Dinajpur (Pali Khanpur, Katla, Birampur) (25°20'34.8"N 88°57'36.0"E), Deep Ecology and Snake Conservation Foundation (23°53'16.0"N 90°15'44.3"E), Venom Research Centre (VRC), Chattogram Medical College Hospital, Chattogram (22°21'36.1"N 91°49'46.2"E), Nijhum Dwip (22°05'04.0"N 91°01'36.1"E), Rajshahi Zoo (24°22'09.6"N 88°34'14.8"E), Arunapalli, Nabinagar, JU campus and Savar areas. In addition, Dr. Mohammad Zahangir Alom of the Wildlife Conservation Society (WCS) provided us with some marine samples that were collected by our team from Khulna. Dr. Md. Manzoorul Kibria provided some samples from Halda River Research Laboratory. While IUCN-Bangladesh, a research team headed by Mr. A.B.M. Sarowar Alam provided us some bird samples from Tanguar Haor (25°08'24.1"N 91°05'18.0"E) and Sayam U. Chowdhury, also provided us some bird samples (Table 1).

3.3.1 Sample

We have collected a total of 280 samples of different species from different Institutions and field trips (Appendix I) (Table 1).

3.4 Sample labeling, vouchering and preserving

Immediately after collection, tissue samples were excised, labeled, and stored in 90% ethanol. After arrival at the DNA barcoding laboratory, Department of Zoology, Jahangirnagar University, the sample was kept at -20°C until further use and at -80°C for long-term storage (Fig. 1). A voucher specimen [in case of availability of sample size/volume, and this method (vouchering) was followed for all other samples in general] was also prepared from the same tissue sample, which was then transferred to 90% ethanol and stored at -80°C (Sharma et al., 2009; Gaur et al., 2017).The vouchering pattern for various animal groups was as follows: MBSV000 (Mammals of Bangladesh Sufal Voucher); BBSV0000 (Birds of Bangladesh Sufal Voucher); RBSV000 (Reptiles of Bangladesh Sufal Voucher); ABSV000 (Amphibia of Bangladesh Sufal Voucher); BFBSV000 (Butterflies of Bangladesh Sufal Voucher); CBSV000 (Crustaceans of Bangladesh Sufal Voucher). The voucher sample (depending on sample type) was preserved by dehydration and kept in a zip lock or cryovial, while some other samples were kept in 90% ethanol at -20°C until further use. Some samples were kept at -80°C for long-term preservation as well.

3.4.1 Feather sample

After collecting the feathers, they were placed in a sterile cryotube containing 70% ethanol. To avoid possible contamination, the feathers were handled only with sterile forceps. Samples were stored for several months at room temperature (or, more preferably at 4°C). Sample was also stored at -80°C for long-time storage (Gaur et al., 2017).

3.4.2 Fecal sample/Dung

Feces are the most preferred biological sample due to their ease of collection under natural conditions and minimum contact with the animals. During collection, the fecal sample was placed in a sterile 50 ml screw-cap container (Falcon tube). The sample is then oven dried at 55°C over night in the laboratory before being stored at room temperature for up to one year (De et al., 2020).

3.4.3 Scale/Claw

After collection, scale/claw was labeled and stored in 90% ethanol at -20°C until further use. For long-term storage, the sample was preserved at -80°C (Su et al., 2020).

3.4.4 Egg shell

After collection, egg shell was labeled and stored at room temperature. For long-term preservation, the sample was preserved in 90% ethanol and stored at -20°C.

3.4.5 Hair

The ideal number of hair samples is 8–12 strands of hair. Sterile forceps were used to handle hair sample. The sample was placed in a dry, clean plastic zip-lock bag and labeled accordingly. The zip-lock containing hair samples were kept at room temperature or in a refrigerator at -4°C until further use (Gaur et al., 2017).

Fig. 1. Vouchering and genomic DNA extraction at the DNA Barcoding Laboratory, Dept. of Zoology, JU

3.5 Genomic DNA extraction

During genomic DNA extraction, specimens were washed with distilled water from the preservation tube/zip-lock, rinsed well, and then crushed using a DNA Extraction Grinder (PRO200, PRO Scientific, Oxford, CT USA) adding Nuclei Lysis Solution (Promega, USA). To allow proper digestion of the sample, incubate the crushed extraction at 56°C overnight with Proteinase K (Thermo Scientific #EO 0491). Further digestion was accomplished by RNase A (Promega, USA) at 37°C for 30 minutes in a water bath. Then, Genomic DNA was extracted according to the protocol of the Wizard Genomic@ DNA Purification Kit (Promega, Madison, WI, USA). A NanoDrop 2000 UV-Vis Spectrophotometer (Thermo Scientific, USA) was used to determine the purity and yield of extracted genomic DNA (Fig.2). The results showed good- quality genomic DNA from most of the samples (Table 2). The extracted genomic DNA was kept at -20°C until further use in PCR and then sequencing. Following this method, genomic DNA from 236 samples was extracted. The details are given in Table 2.

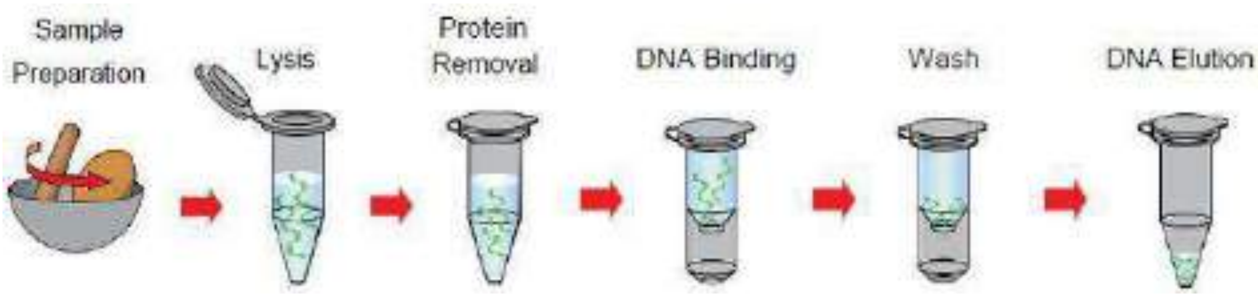

Fig. 2. Simplified DNA extraction process

3.6 Primer

For the PCR amplification of DNA barcode genes in different animal/groups, different sets of universal primers were chosen (Appendix II).

3.7 PCR amplification, gel electrophoresis, sequencing and gene submission

After Genomic DNA extraction from different samples, PCR amplification of the mitochondrial cytochrome c oxidase I (COI) gene region, cytochrome b (Cytb) gene, 16s rRNA, ND5 and the Control region was performed using different sets of primers depending on the animal samples (Fig. 3) (Appendix II). PCR was performed in 20 µL of Q2 Green PCR Master Mix (Promega, Madison, WI, USA) on a thermal cycler (Vetteii, USA). The cycle conditions varied depending on the animal/sample types. However, the standard protocol was as follows: initial denaturation (95°C for 5/7 min), 35/40 cycles of denaturation (95°C for 1 min), primer annealing (45-64°C for 1/1.5 min), and primer extension (72°C for 1 min), and a final extension (72°C for 4/7 min). The success of amplification was evaluated by 1% agarose gel electrophoresis under ultraviolet light (Bio Analyzer). The PCR product was further purified using Wizard@SV Gel and PCR Clean-up System Kit (Promega, Madison, WI, USA) and quantified by Nanodrop. After cleanup, the PCR product was sequenced using an ABI 3500 sequencer. The nucleotide sequences of Cytb and COI genes were deposited in the National Center for Biotechnology Information (NCBI) GenBank and their accession numbers is given in the Table 3.

3.8 Phylogenetic analysis

The sequences of the 122 samples were edited using Chromas version 2.6.2. The assembled sequences were aligned using the ClustalW multiple alignment function in BioEdit version 7.0 (Hall, 1999). Phylogenetic trees were constructed using MEGA10 with the Neighbor-Joining (Saitou and Nei, 1987; Kumar et al., 2018) methods with 1000 bootstrap replications.

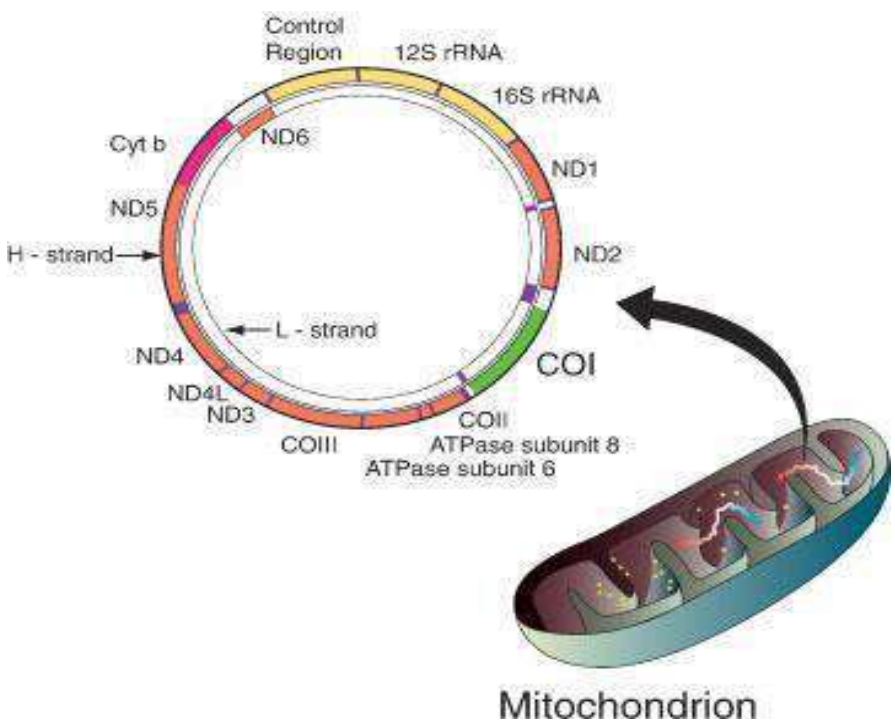

Fig. 3. Targeted regions (COI, Cytb, ND5, 16s rRNA, Control region) of the mitochondrial genome

CHAPTER IV
RESULT AND DISCUSSION

4.1 Generation of barcode genes and their analysis

The establishment of a comprehensive reference database, encompassing genetic information from various illegal traded and threatened species, a specimen list consisting of 579 species was initially prepared. The listed species were prioritized based on whether or not they were illegally trafficked or threatened in Bangladesh. Out of them, there are more than 60 species that are thought to be traded illegally in Bangladesh.

For sample collection to generate molecular data, a total of 280 samples of different species were collected by means of various scientific methods, and voucher specimens (using feather, hair, skin, scat, claw, egg shell etc.) were prepared accordingly (Table 1). The voucher sample (depending on sample type) preserved by dehydration and kept in a zip lock or cryovial while some other sample kept in 90% ethanol at -20°C until further use. Some samples were kept at - 80°C for long term preservation as well. The sample specimens included 51 mammals, 45 birds, 62 reptiles, 43 amphibians, 55 butterflies, 20 crustaceans and 4 other species (Manta Ray, Mudskippers and Hammer Head Shark) (Table 1).

Later, genomic DNA was extracted from 236 samples (49 mammals, 42 birds, 58 reptiles, 34 amphibians, 44 butterflies, 7 crustaceans and other 2 species) where the majority of the samples yielded high-quality genomic DNA, as assessed by Nanodrop (Table 2). Genomic DNA was subjected to PCR amplification utilizing universal primers from different sets (Appendix II). After sequencing of these PCR products, Basic Local Alignment Search Tool (BLAST) analysis showed that the barcode gene sequences of these 122 species matched those of respective species from different geographical areas (96%-100 similarity). Later nucleotide sequences of these COI, Cytb, 16s rRNA, ND5 and Control region genes of 122 samples were submitted to NCBI's Gene bank and obtained accession numbers (Table 3) (Appendix III). The submitted genes comprise 37 mammal species, 18 bird species, 22 reptile species, 13 amphibian species, 29 butterfly species, and 3 crustacean species (Table 3, Fig 4). Furthermore, among the 122 genes that were submitted into Gene bank, COI accounted for 50%, followed by Cytb at 37.7%, 16s rRNA at 10%, ND5 at 1%, and the Control region gene at 1% (Fig. 5).

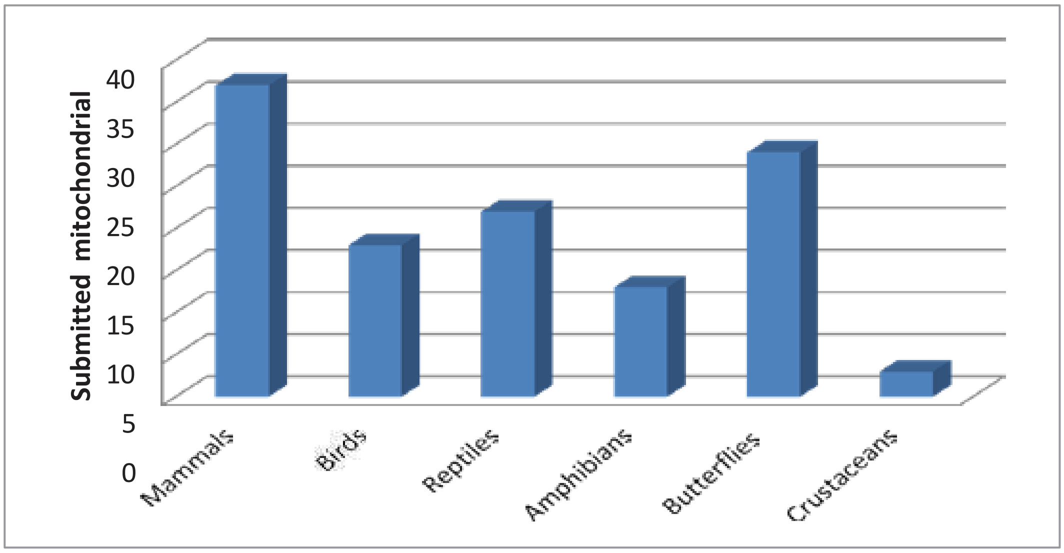

Fig. 4. Submitted mitochondrial barcode genes from different animal groups

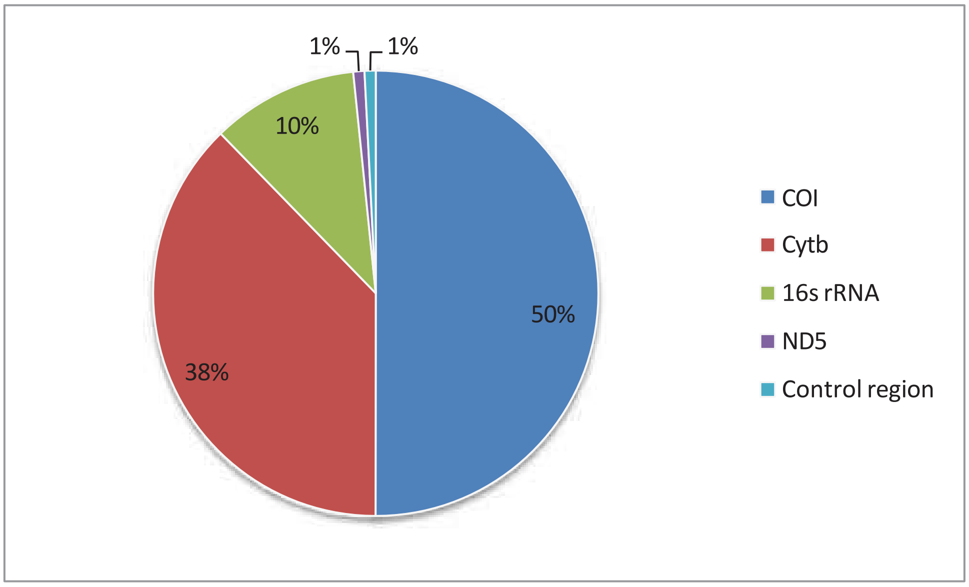

Fig. 5. Percentage of mitochondrial barcode genes

Mammals

A compilation of 51 species samples of Mammals was collected from various locations (Table 1). Following this, genomic DNA was isolated from 49 samples, with Nanodrop determining that the majority of the extracted samples contained high-quality genomic DNA (Table 2). Genomic DNA was amplified via PCR employing universal primers from distinct sets (Appendix II). After sequencing of these PCR products, nucleotide sequences of COI, Cytb, ND5 and Control region genes of 37 samples were submitted to NCBI's Genbank and obtained accession numbers (Table 3). Among the submitted genes, 10 species are critically endangered (CR), 7 species are endangered (EN), 4 species are vulnerable (VU), 4 species are near threatened (NT), 7 species are of least concern (LC), 3 species are data deficient (DD), and 2 species are not evaluated (NE) (Table 3). These molecular reference data is crucial for the accurate identification of unknown species. The DNA barcode serves as a unique identifier for a species, much like a product barcode in a store. By comparing the unknown DNA sequence to the reference data, researchers can quickly and accurately determine the species of the organism. This has applications in controlling illegal trade, forensics, and biodiversity studies.

In the present study, Cytb, ND5, and the Control region of *Panthera tigris tigris* (Bengal tiger), a CR species, were sequenced. Among these, Control region sequences (319 bp) in *P. t. tigris* is crucial to analyzing subspecies in a population. In this consequence, a phylogenetic tree was constructed using the NJ method and found that *P.t. tigris* from Bangladesh, India, and China belonged to one clade and *P.t. altaica* from Russia belonged to another clade (Fig 06). This result indicates that among sub-species levels, there are some differences in the control region. Therefore, the control region could be useful to characterize the sub population of *P. tigris.*

IUCN Bangladesh assessed the threat status of 10 species of primates, while most of the species are associated with illegal trade. In the present study, from 8 species of primates, we successfully generated the cytb gene sequences (Table 3). The phylogenetic tree revealed that 8 species in the present study share similarities with the same species, which originated from different geographic origins. Similar species stayed within the same clade, hence verifying the molecular gene sequences and the country of origin as well (Fig. 07). On the other hand, another phylogenetic tree was made using cytb to show the relationships among four species of cats: *Felis chaus*, *Pardofelis marmorata*, *Prionailurus viverrinus*, and *Prionailurus bengalensis*. The result showed that the four species shared similarities with their respective species, which originated from different geographic origins. Similar species stayed within the same clade as their country of origin (Fig. 08). Then again, the tree constructed by cytb for *Manis pentadactyla* showed that *M. pentadactyla* from Bangladesh and India remains one clade while *M. pentadactyla* from China and Taiwan constructs another clade (Fig 09). While the phylogenetic tree constructed for *Platanista gangetica* using cytb showed that *P. gangetica* from the Halda River and Sundarban of Bangladesh as well as India remain in the same clade with no differences between Halda River and Sundarban samples. On the other hand, *P. gangetica* from China showed a little distance (Fig 10). Future studies are required to uncover the relationship using different of gene sets.

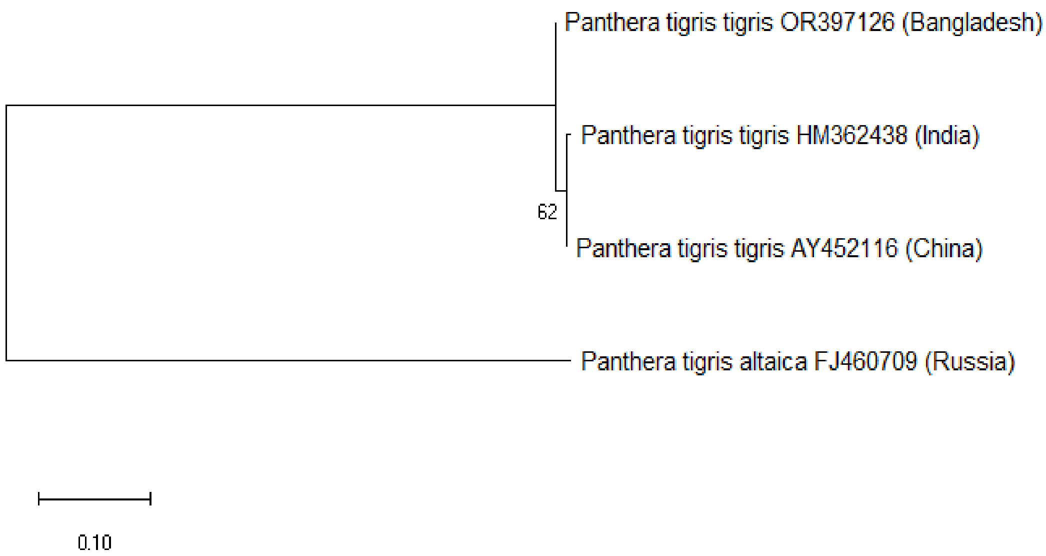

Fig. 6. Neighbor-Joining (NJ) tree of *P. t. tigris* based on Control region sequences. Bootstrap values are shown at the branching points.

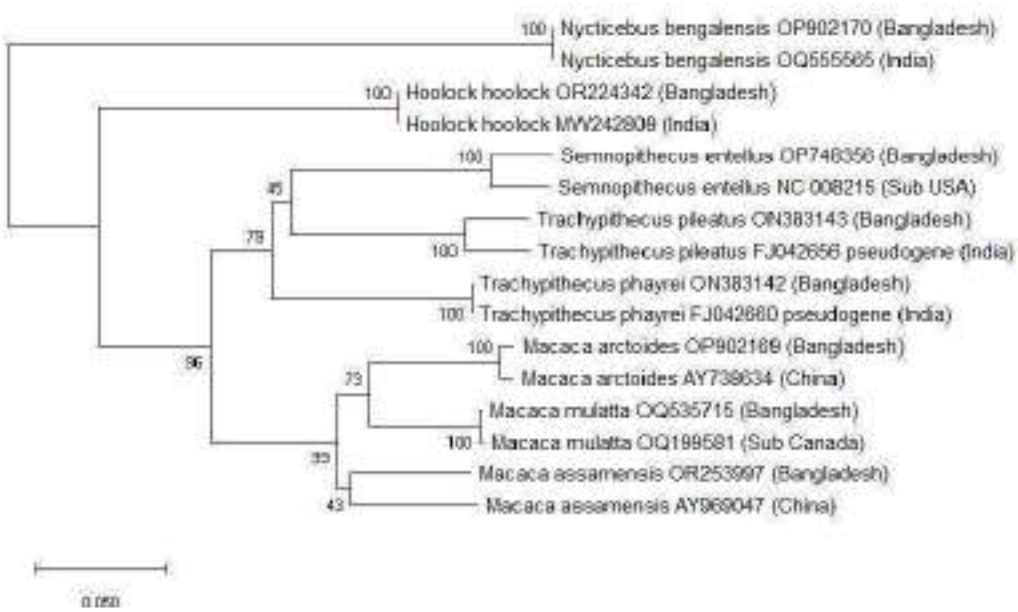

Fig. 7. NJ tree of 8 primates based on cytb gene sequences. Bootstrap values are shown at the branching points.

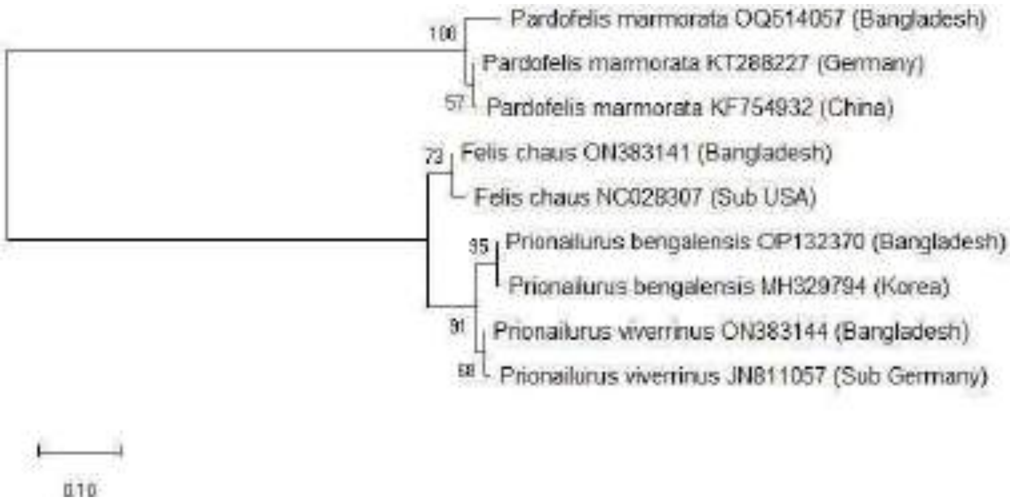

Fig. 8. NJ tree of 4 cats based on cytb gene sequences. Bootstrap values are shown at the branching points.

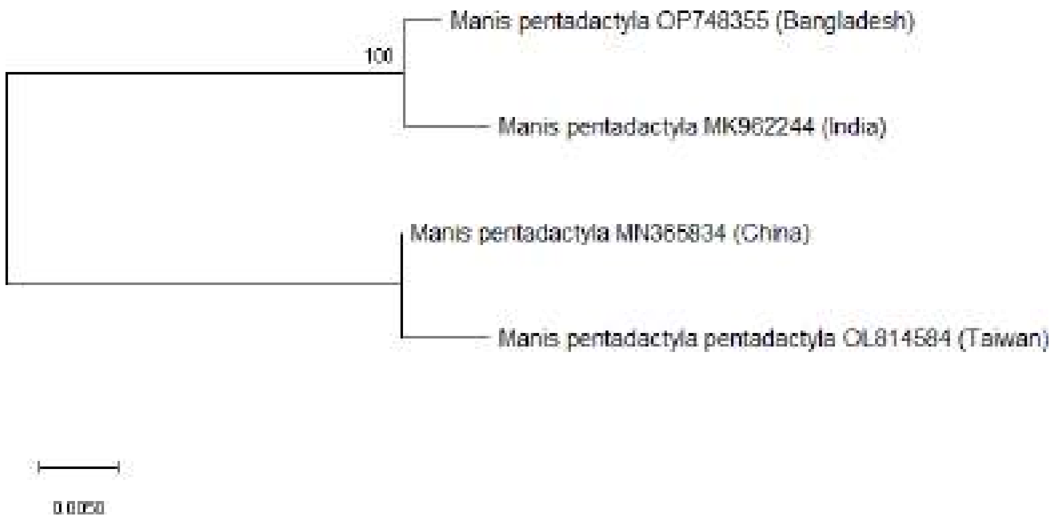

Fig. 9. NJ tree of *M. pentadactyla* based on cytb gene sequences. Bootstrap values are shown at the branching points.

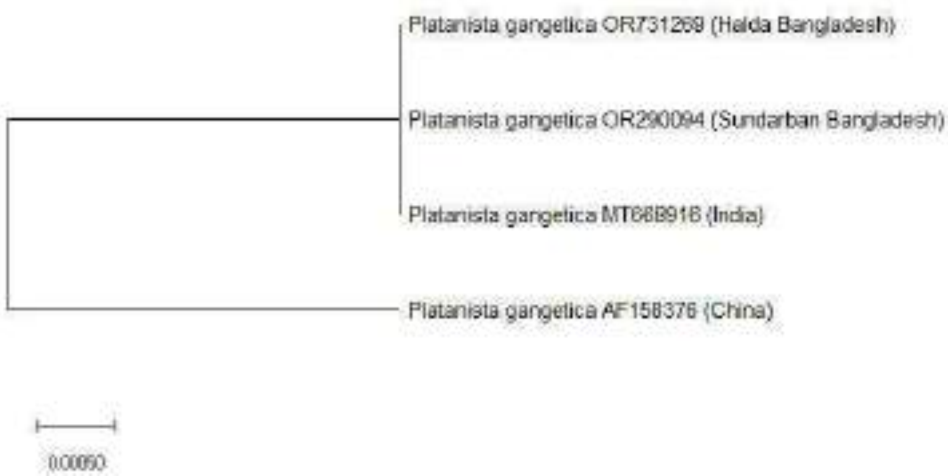

Fig. 10. NJ tree of *P. gangetica* based on cytb gene sequences.

Birds: A total of 45 samples of bird species from different areas of Bangladesh were collected (Table 1). Subsequently, genomic DNA was extracted from the aforementioned 42 samples, and Nanodrop analysis revealed that most of the samples contained genomic DNA of excellent quality (Table 2). The genomic DNA was amplified using PCR using universal primers (Appendix II). Following the sequencing of these PCR products, the nucleotide sequences of COI genes from 18 species were submitted to NCBI's Genbank (Table 3). The species whose genes were submitted to Genbank have the following IUCN threat category: 3 as VU, 1 as NT, and 14 as LC (Table 3).

The phylogenetic tree for *B. bicornis* using the COI gene was constructed using the NJ method and showed approximately identical clustering (Fig. 11). *B. bicornis* from Bangladesh, South Korea, and China belongs to the same cluster, while *B. rhinoceros* submitted from the USA belongs to another cluster (Fig. 11).

Moreover, another phylogenetic tree was constructed using the COI gene, and the result revealed that *D. javanica* from Bangladesh, India, and China belonged to the same cluster while

D. viduata from Argentina belonged to a different cluster (Fig. 12).

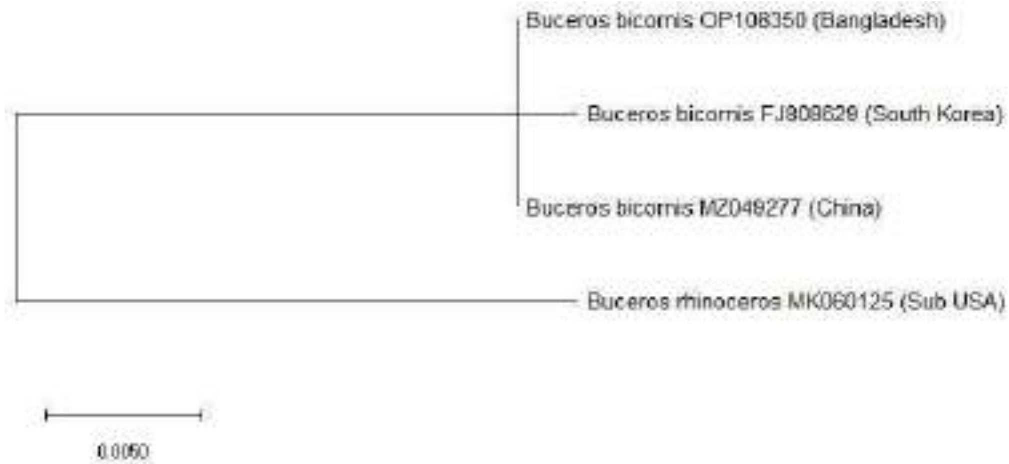

Fig. 11. NJ tree of *B. bicornis* based on COI gene sequences.

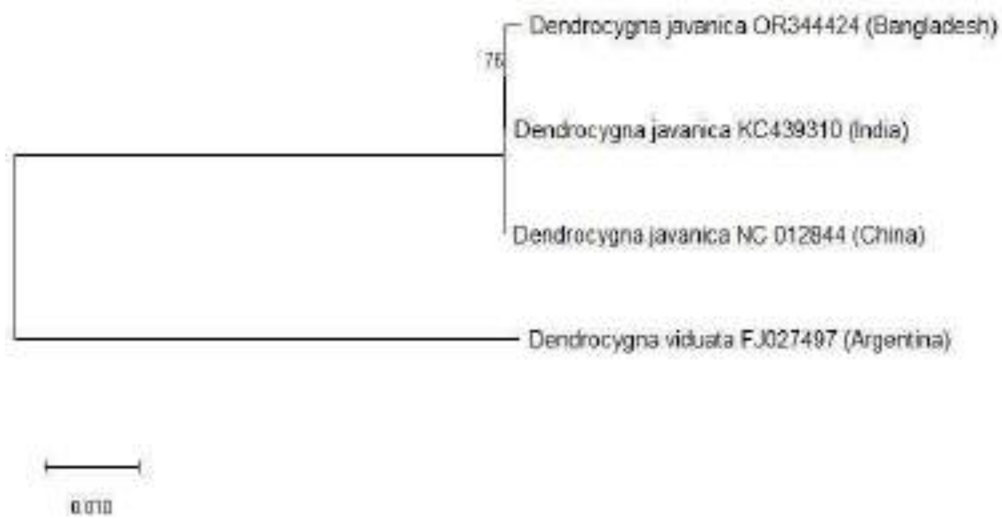

Fig. 12. NJ tree of *D. javanica* based on COI gene sequences. Bootstrap values are shown at the branching points.

Reptiles: From all around Bangladesh, 62 different samples of reptiles were collected (Table 1). Subsequently, genomic DNA was extracted from the 58 samples mentioned earlier (Table 2). Nanodrop analysis revealed that the majority of the extracted samples comprised genomic DNA of superior quality. By means of PCR, genomic DNA was amplified using universal primers obtained from separate sets (Appendix II). The nucleotide sequences of the COI and Cytb genes from 22 species were submitted to the NCBI's Genbank (Table 3). The IUCN threat status classifications for the species whose genes were submitted to Genbank are as follows: 1 species as regionally extinct (RE), 3 species as CR, 2 species as EN, 1 species as NT, 12 species as LC, 2 species as DD, and 1 species as NE (Table 3).

As for the species, *Indotyphlops porrectus* is the first confirmed record from Bangladesh that was submitted to the NCBI Genbank for the first time as well. *Cerberus rynchops* was also submitted to NCBI's Genbank for the first time (Table 3).

A phylogenetic tree was constructed using cytb for *Batagur baska*, and the results showed that *B. baska* from Bangladesh, India and Myanmar, were positioned in one cluster, while only one sample from Pakistan was differently positioned (Fig. 13).

In addition, a phylogenetic tree was constructed using the cytb of *Manouria emys* from Bangladesh, India, Taiwan, and the USA. The tree showed two clades, where the first clade included *M. emys* from Bangladesh and India, while samples from Taiwan and the USA constructed another cluster (Fig. 14). On the other hand, a phylogenetic tree for the cytb gene of *I. porrectus* was constructed using the NJ method. The tree analysis showed that *Typhlops syntherus, T. sylleptor, T. jamaicemsis* were resided in a cluster while *I. braminus, I. porrectus* and *T. tasymicris* were belonged separately (Fig 15).

Fig. 13. NJ tree of *B. baska* based on COI gene sequences. Bootstrap values are shown at the branching points.

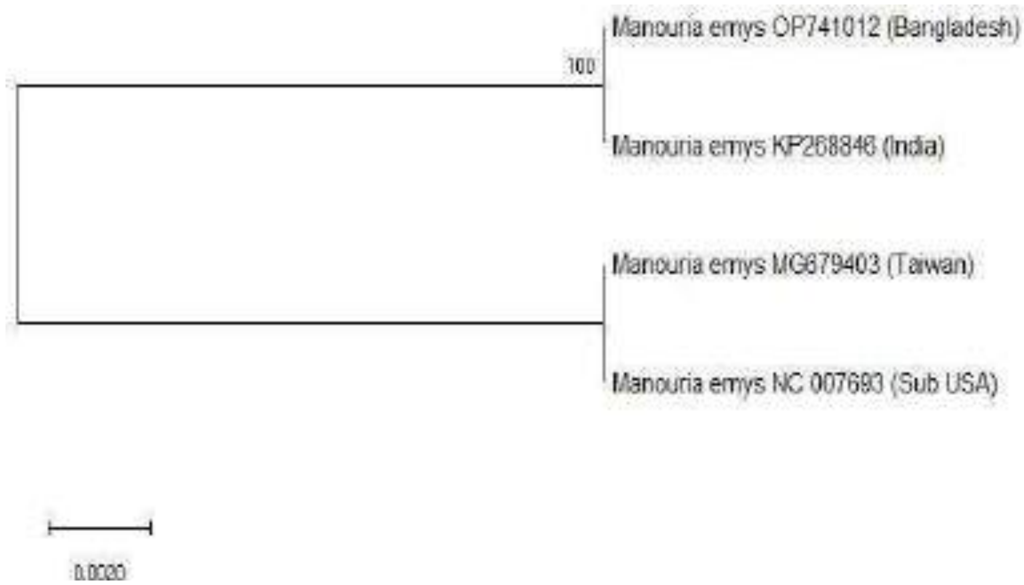

Fig. 14. NJ tree of *M. emys* based on COI gene sequences. Bootstrap values are shown at the branching points.

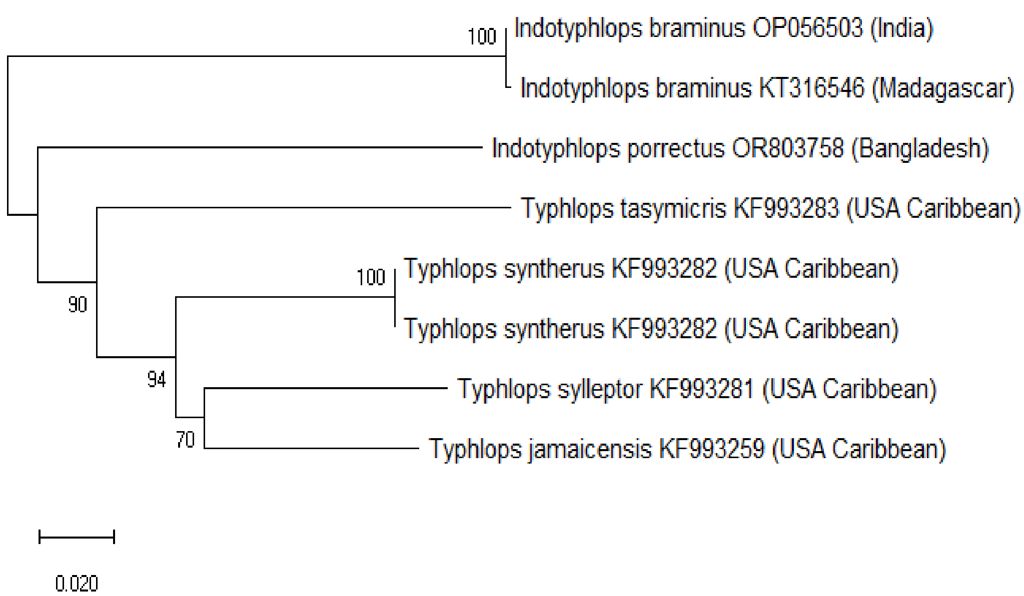

Fig. 15. NJ tree of *I. porrectus* based on COI gene sequences. Bootstrap values are shown at the branching points.

Amphibians: From different areas of Bangladesh, 43 different samples of amphibians were collected (Table 1). Subsequently, genomic DNA was extracted from the 34 samples (Table 2). Nanodrop analysis revealed that the majority of the extracted samples comprised genomic DNA of superior quality. By means of PCR, genomic DNA was amplified using universal primers (Appendix II). The nucleotide sequences of the 16s rRNA gene from 13 species were submitted to the NCBI's Gene Bank (Table 3). The species whose genes were submitted to Genbank have the following IUCN threat statuses: 1 species is classified as VU, 5 as NE and 7 as LC (Table 3).

A phylogenetic tree was constructed for 13 amphibians using 16s rRNA with the assistance of the NJ algorithm, which identified 2 major clades. The first clade included *Euphlyctis kalasgramensis*, *Hoplobatrachus tigerinus*, *Fejervarya orissaensis*, *Minervarya asmati*, *Minervarya pierrei*, *Microhyla berdmorei*, *Microhyla* *mymensinghensis*, *Uperodon globulosus*, *Duttaphrynus melanostictus* and the second clade included *Polypedates teraiensis, Polypedates leucomystax*, *Sylvirana lacrima* and *Hylarana tytleri*. All 13 species used in the tree analysis showed similarity with their respective species from different geographic origins (Fig. 16).

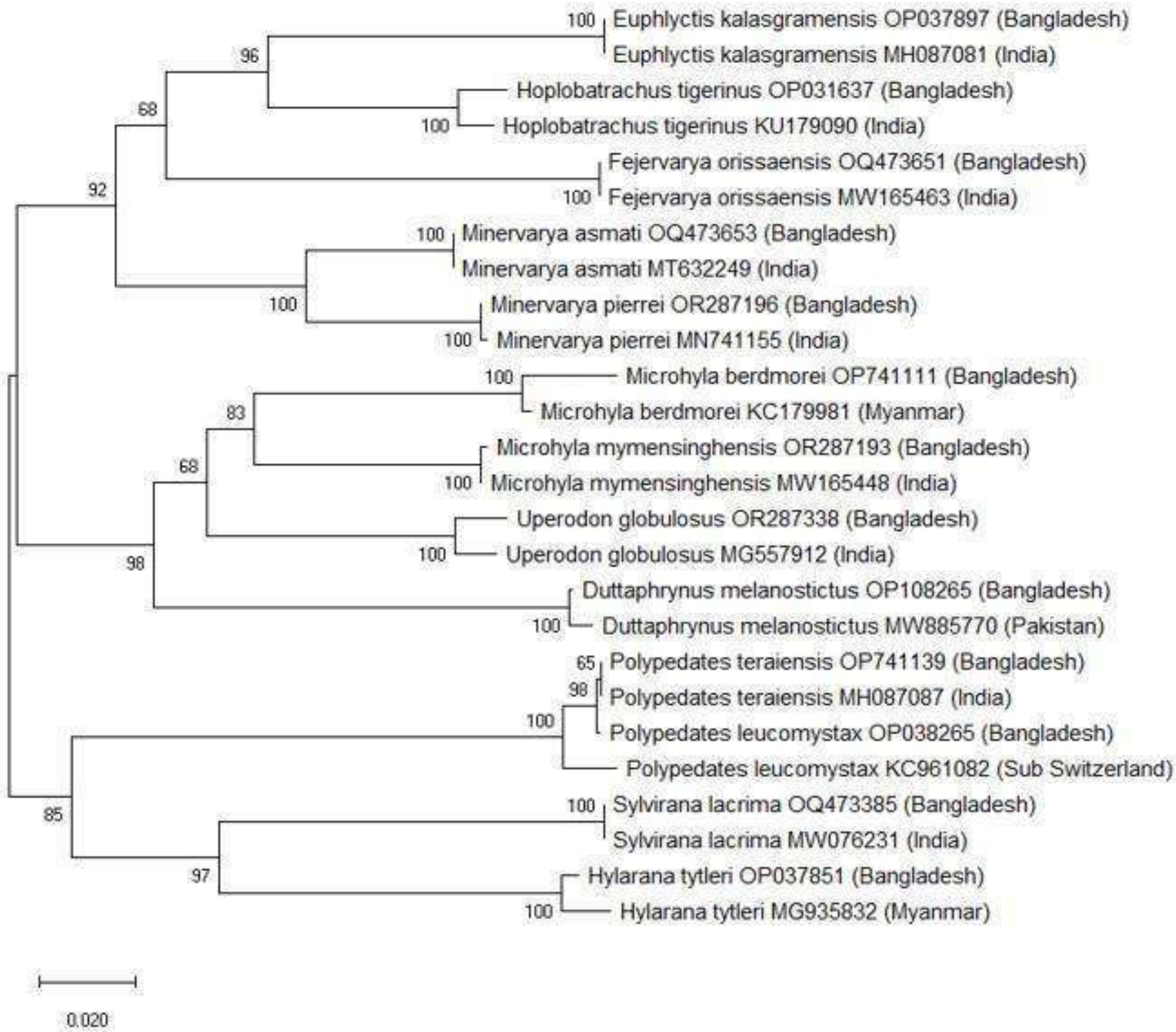

Fig. 16. NJ tree of 13 amphibians based on 16s rRNA gene sequences. Bootstrap values are shown at the branching points.

Butterflies: In case of butterflies, a total of 55 species were collected from different areas of Bangladesh (Table 1). Subsequently, genomic DNA was extracted from the 44 samples mentioned earlier (Table 2). Nanodrop analysis revealed that the majority of the extracted samples comprised genomic DNA of superior quality. By means of PCR, genomic DNA was amplified using universal primers (Appendix II). The nucleotide sequences of the COI gene from 29 species were submitted to the NCBI's Genbank along with the corresponding accession numbers subsequent to the sequencing of the PCR products (Table 3). The species whose genes were submitted to Genbank under threat have IUCN threat category (10 as EN, 13 as VU, 1 as DD and 5 as NE) (Table 3).

The NJ phylogenetic tree using the COI sequences of the 10 endangered butterfly species showed monophyletic entities that shared a common ancestor (Fig. 17). The tree revealed that similar species stayed within the same cluster, hence verifying the molecular gene sequences and the country of origin as well (Fig. 08). In the analysis, *Catapaecilma majo*r was submitted for the first time in NCBI Genbank, and resided a little distantly from the other nine endangered species in the tree (Fig. 17).

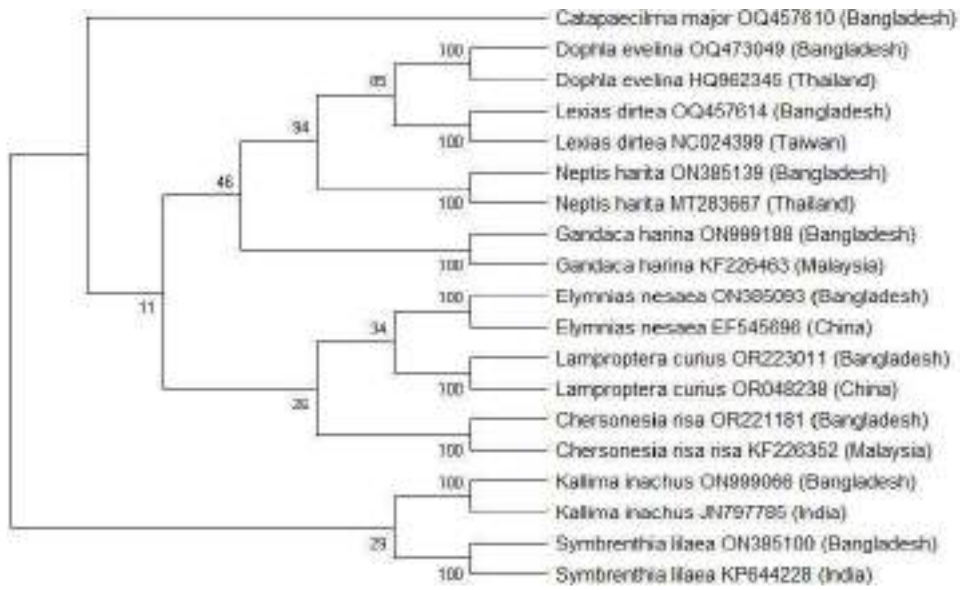

Fig. 17. NJ tree of 10 threatened butterflies based on COI gene sequences. Bootstrap values are shown at the branching points.

Crustaceans: A total of 20 species of different crustaceans were collected from different areas of Bangladesh (Table 1). Subsequently, genomic DNA was extracted from these 07 samples (Table 2). Nanodrop analysis revealed that the majority of the extracted samples comprised genomic DNA of good quality (Table 2). By means of PCR, genomic DNA was amplified using universal primers (Appendix II). The nucleotide sequences of the COI gene from 03 species were submitted to the NCBI's Gene Bank (Table 3). 1 species are classified as VU, 1 species as LC and 1 species as DD according to the IUCN threat status for the species whose genes were submitted to Genbank (Table 3).

The NJ tree constructed using the COI gene of *Carcinoscorpius rotundicauda* that showed all the specimen was originated from a common ancestor (Fig. 18). The phylogenetic tree revealed two main clades: the first clade included *C. rotundicauda* from Bangladesh, India, Malaysia, and Thailand, and the other clade included Indonesia and China (Fig. 18).

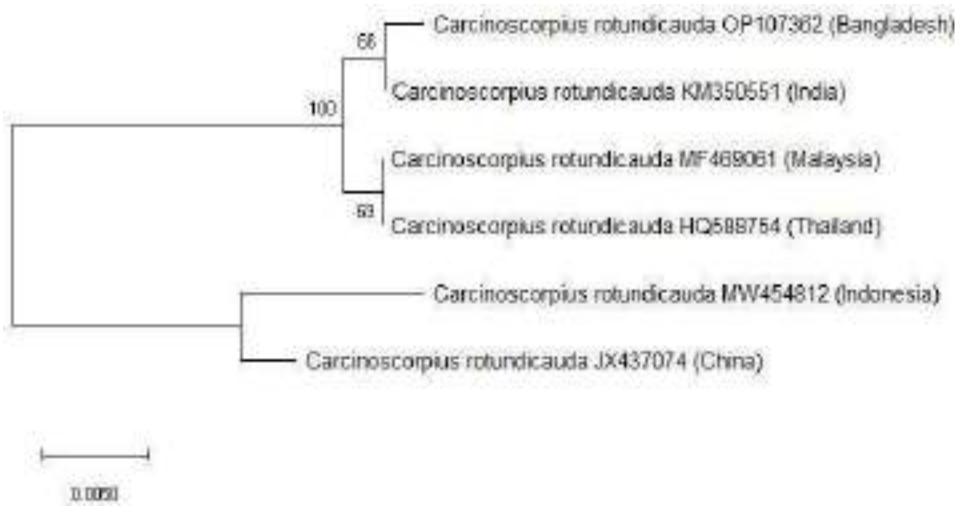

Fig. 18. NJ tree of *C. rotundicauda* based on COI gene sequences. Bootstrap values are shown at the branching points.

Molecular reference data (barcode genes) are needed to identify unknown species. An individual species that may be captured from illegally traded incidences can be uniquely identified by its barcode genes. In this process, by comparing an organism's unknown DNA sequence to reference data, researchers can identify a species precisely. We created limited (122 barcode genes) molecular reference data in this investigation and over 300 species remain to be done. Nonetheless, this can be used in forensics, biodiversity research, and the regulation of illicit trafficking in Bangladesh.

Arrange workshop and training session

Wildlife authorities often take on the responsibility of saving wildlife and proper management from poaching activities. However, the exact identification of species populations remains a challenge due to the limited availability of genomic information and methodologies. Hence, ten officials from the Bangladesh Forest Department (BFD) were extended invitations and subsequently attended a workshop and practical training session on October 18, 2023 (Fig. 19). The course primarily covered DNA barcoding, genomic DNA isolation, designing primers for DNA amplification, amplification of DNA using PCR, gel electrophoresis for visualization of DNA and further purification of the PCR product for sequencing. In addition to this, bioinformatics was also learned to edit DNA sequences, submit sequences to GenBank (discussed on NCBI, DDBJ and EMBL), nucleotide/genetic variation analysis using MEGA10, construct phylogenetic trees (NJ, ML methods) to find out the species country of origin, and conduct evolutionary distance model testing (Fig. 19).

Furthermore, throughout the project's duration, two training sessions were organized to train the personnel of the Sundarban East and West Forest Divisions and the Bangabandhu Sheikh Mujib Safari Park in Gazipur on the DNA barcoding process/information and sample collection techniques. Approximately 24 employees from each of these two locations attended the training (Fig. 20).

This workshop was conducted as part of the ongoing project (on the basis of the 6th and 7th deliverable). Both theoretical and practical information was imparted to educate participants on the wildlife management and forensic science perspectives in Bangladesh. Finally, molecular tools and techniques were used to train the BFD officials, and where necessary, software or scripts were provided to BFD officials. It is expected from this training workshop that BFD officials will self-sustain to control illegal trading and poaching of wildlife, as well as wildlife management in the country.

Fig. 19. Practical session and training workshop on DNA barcoding and bioinformatics at the DNA Barcoding Laboratory, JU

Training at WRRC, Khulna

Training at Bangabandhu Sheikh Mujib Safari Park, Gazipur

Fig. 20. Training workshop on DNA barcoding, and sample collection at WRRC, Khulna and Bangabandhu Sheikh Mujib Safari Park, Gazipur.

Conclusions

The project's main goals were to create and optimize DNA barcoding procedures for a variety of Bangladeshi illegal traded species. A comprehensive molecular reference library with genetic information from multiple locations will improve species identification and help trace species back to their origins. Due to these issues, a specimen list of 579 species was created to synthesize DNA-barcoding genes. Priority was given to species that are illegally traded or threatened in Bangladesh. In this view, we collaborated with the various institutions and conducted field trips, and finally, 280 species samples were obtained. The samples comprised 51 mammals, 45 birds, 62 reptiles, 43 amphibians, 55 butterflies, 20 crustaceans, and 4 others. After extracting genomic DNA from 236 samples, Nanodrop found that most provided high- quality DNA. Later, PCR amplification was performed using genomic DNA using universal primers from different sets. After sequencing the PCR products, 122 nucleotide sequences (of COI, Cytb, 16s rRNA, ND5, Control region) from 118 species were submitted to NCBI's Genbank, and received accession numbers. These genes come from 37 mammals, 18 birds, 22 reptiles, 13 amphibians, 29 butterflies, and 3 crustaceans. Among the submitted genes, *Cerberus rynchops* (Dog-faced Water Snake), *Indotyphlops porrectus* (Slender Worm Snake) and *Catapaecilma major* (Common Tinsel) were submitted for the first time in NCBI's Genbank. Moreover, a workshop and training session in the last quarter boosted the Bangladesh Forest Department's (BFD) ability to promote molecular species identification and detect species origins in Bangladesh and overseas.

Finally, by creating a DNA barcoding reference checklist, this initiative aims to aid national and international efforts to combat illegal wildlife trade, safeguard endangered species, and promote ethical and sustainable biodiversity use. Integrating molecular approaches improves our ability to protect Bangladeshi wildlife and habitats from emerging conservation threats.

Recommendations and Suggestions

Using molecular marker genes for the precise identification of animals can indeed be a valuable tool in combating illegal wildlife trade. DNA-based techniques can provide accurate and reliable information about the species and origin of a particular specimen, which is crucial for law enforcement and conservation efforts. Here are some recommendations and suggestions in which molecular markers can be utilized for this purpose in Bangladesh:

DNA Barcoding: DNA barcoding involves sequencing a short and standardized region of DNA to identify species. Establishing a reference database of DNA barcodes for various animal species in Bangladesh can aid in quick and accurate identification of confiscated specimens. A priority list of 579 species has been prepared, with only around 122 species barcoded. More samples should be barcoded so that molecular data is available for the identification of all animals to check for illegal trade and conservation of wildlife. Besides, population genetic study is one of the recent development through which variation between the population and sub-population of different animal group can easily be determined. Along with dispersion and migration of mammals, birds, reptiles, amphibians and butterflies in the country can easily be determined by studying population genetics.

Forensic DNA Analysis: Employ **forensic** DNA techniques to analyze biological samples obtained from the illegal trade, such as bones, scales, feathers, or other tissues. This can help determine the species, individual identity, and even the geographic origin of the specimens.

Genetic Fingerprinting: Develop genetic profiles or fingerprints for key species that are commonly targeted in illegal trade. This information can assist authorities in tracking down poaching hotspots and trade routes.

Wildlife DNA Database: Create and maintain a comprehensive database of wildlife DNA profiles. This can serve as a valuable resource for law enforcement agencies to cross-reference DNA samples and identify the source populations of confiscated specimens.

Capacity Building: Provide training and build the capacity of Bangladesh Forest Department that includes law enforcement officials, forensic experts (WCCU), and wildlife conservationists in molecular biology techniques. This includes training them to collect, process, and analyze DNA samples effectively.

Collaboration with Research Institutions: Collaborate with academic and research institutions to conduct genetic studies on local wildlife populations. This research can contribute to the development of genetic markers specific to Bangladeshi species. In this connection Bangladesh Forest Department will be connected with University's Departments, Animals farms, NGOs to collect sample, sequencing and overall checking illegal trade and poaching.

Integration with Existing Monitoring Systems: Integrate DNA analysis into existing wildlife monitoring and anti-poaching efforts. This can enhance the effectiveness of these programs by providing a molecular tool for species identification.

Strengthen Legislation: Enforce and strengthen wildlife protection laws, imposing severe penalties for illegal trade activities. This includes reviewing and updating existing laws to address emerging challenges. Bangladesh Forest Department including WCCU is trying to stop the illegal trade, and we should reinforce that.

Support Sustainable Livelihoods: Provide alternative livelihoods for communities that may be involved in illegal wildlife trade due to lack of economic opportunities. Sustainable development can reduce reliance on illegal activities.

International Collaboration: Work collaboratively with neighboring countries and international organizations to share information, intelligence, and best practices for combating trans- boundary illegal wildlife trade.

Engage Communities: Involve local communities in conservation efforts, making them stakeholders in protecting their natural resources. This can foster a sense of responsibility and ownership over wildlife protection.

Monitor and Regulate Markets: Keep a close eye on markets, both physical and online, where illegal wildlife trade may occur. Implement regulations and controls to curb such activities.

Public Awareness: Aware and educate the public about the use of molecular markers in wildlife conservation and law enforcement. This awareness can help reduce demand for illegal wildlife products and promote a culture of responsible consumption.

While molecular marker techniques are powerful tools, it's important to note that their successful implementation requires a combination of technical expertise, financial resources, and a commitment to collaboration among various stakeholders. Additionally, legal frameworks should be in place to support the admissibility of DNA evidence in court proceedings. By integrating molecular marker technologies into broader conservation strategies, Bangladesh can take significant steps toward curbing illegal wildlife trade and protecting its rich biodiversity.

Table 1. List of samples from different species that were collected in different parts of Bangladesh

Sl	Scientific Name	Common Name	Family	BD Status	Global Status
Mammals					
1	*Aonyx cinereus*	Small-clawed Otter (Sam-1)	Mustelidae	EN	VU
2	*Aonyx cinereus*	Small-clawed Otter (Sam-2)	Mustelidae	EN	VU
3	*Axis axis*	Spotted Deer (Sam-1)	Cervidae	LC	LC
4	*Axis porcinus*	Hog Deer	Cervidae	CR	EN
5	*Bandicota* sp.	Lesser Bandicoot Rat	Muridae	LC	LC
6	*Bos gaurus*	Gaur (Sam-1)	Bovidae	CR	VU
7	*Boselaphus tragocamelus*	Nilgai (Sam-1)	Bovidae	RE	LC
8	*Callosciurus pygerythrus*	Squirrel (Sam-1) (Irrawaddy Squirrel)	Sciuridae	LC	LC
9	*Canis aureus*	Golden Jackal	Canidae	LC	LC
10	*Elephas maximus*	Asian Elephant (Sam-1)	Elephantidae	CR	EN
11	*Elephas maximus*	Asian Elephant (Sam-2)	Elephantidae	CR	EN
12	*Felis chaus*	Jungle Cat (Sam-1)	Felidae	NT	LC
13	*Felis chaus*	Jungle Cat (Sam-2)	Felidae	NT	LC
14	*Funambulus* sp.	Squirrel (Sam-2)	Sciuridae	-	-
15	*Herpestes edwardsii*	Common Mongoose	Herpestidae	LC	LC
16	*Hoolock hoolock*	Hoolock Gibbon	Hylobatidae	CR	EN
17	*Hystrix brachyura*	Himalayan Crestless Porcupine	Hystricidae	LC	LC
18	*Hystrix indica*	Indian Crested Porcupine	Hystricidae	LC	LC
19	*Lutra lutra*	Common Otter (Sam-1)	Mustelidae	CR	NT
20	*Macaca arctoides*	Stump-tailed Macaque	Cercopithecidae	DD	VU
21	*Macaca assamensis*	Assamese Macaque	Cercopithecidae	EN	NT
22	*Macaca mulatta*	Rhesus Macaque	Cercopithecidae	VU	LC
23	*Manis pentadactyla*	Chinese Pangolin (Sam-1)	Manidae	CR	CR
24	*Manis pentadactyla*	Chinese Pangolin (Sam-2)	Manidae	CR	CR
25	*Muntiacus muntjak*	Barking Deer (Sam-1)	Cervidae	EN	LC
26	*Muntiacus muntjak*	Barking Deer (Sam-2)	Cervidae	EN	LC
27	*Mus musculus*	House Mouse	Muridae	LC	LC
28	*Neofelis nebulosa*	Clouded Leopard	Felidae	CR	VU
29	*Nycticebus bengalensis*	Bengal Slow Loris	Lorisidae	EN	VU
30	*Orcaella brevirostris*	Irrawaddy Dolphin	Delphinidae	NT	VU
31	*Paguma larvata*	Masked Palm Civet (Sam-1)	Viverridae	VU	LC
24	*Manis pentadactyla*	Chinese Pangolin (Sam-2)	Manidae	CR	CR
25	*Muntiacus muntjak*	Barking Deer (Sam-1)	Cervidae	EN	LC
26	*Muntiacus muntjak*	Barking Deer (Sam-2)	Cervidae	EN	LC
27	*Mus musculus*	House Mouse	Muridae	LC	LC
28	*Neofelis nebulosa*	Clouded Leopard	Felidae	CR	VU
29	*Nycticebus bengalensis*	Bengal Slow Loris	Lorisidae	EN	VU
30	*Orcaella brevirostris*	Irrawaddy Dolphin	Delphinidae	NT	VU

Sl	Scientific Name	Common Name	Family	BD Status	Global Status
31	*Paguma larvata*	Masked Palm Civet (Sam-1)	Viverridae	VU	LC
32	*Paguma larvata*	Masked Palm Civet (Sam-2)	Viverridae	VU	LC
33	*Panthera tigris tigris*	Bengal Tiger	Felidae	CR	EN
34	*Pardofelis marmorata*	Marbled Cat	Felidae	DD	VU
35	*Paradoxurus hermaphroditus*	Common Palm Civet	Viverridae	LC	LC
36	*Platanista gangetica*	Ganges River Dolphin (Sam-1)	Platanistidae	VU	EN
37	*Platanista gangetica*	Ganges River Dolphin (Sam-2)	Platanistidae	VU	EN
38	*Prionailurus bengalensis*	Leopard Cat	Felidae	NT	LC
39	*Prionailurus viverrinus*	Fishing Cat	Felidae	EN	EN
40	*Pteropus giganteus*	Bat UnID-3 (Indian Flying Fox)	Pteropodidae	LC	LC
41	*Rattus tanezumi*	Oriental House Rat	Muridae	LC	LC
42	*Rusa unicolor*	Sambar Deer	Cervidae	CR	VU
43	*Semnopithecus entellus*	Common Langur	Cercopithecidae	EN	LC
44	*Sus scrofa*	Common Wild Pig	Suidae	LC	LC
45	*Trachypithecus phayrei*	Phayre's Langur	Cercopithecidae	CR	EN
46	*Trachypithecus pileatus*	Capped Langur	Cercopithecidae	EN	VU
47	*Ursus thibetanus*	Asiatic Black Bear	Ursidae	CR	VU
48	*Viverricula indica*	Small Indian Civet	Viverridae	NT	LC
49	*Viverra zibetha*	Large Indian Civet	Viverridae	NT	NT
50	-	Bat UnID-1	-	-	-
51	-	Bat UnID-2 (Lesser Dawn Bat)	-	-	-
Birds					
1	*Anas acuta*	Northern Pintail	Anatidae	LC	LC
2	*Anas poecilorhyncha*	Indian spot-billed Duck	Anatidae	LC	LC
3	*Anthracoceros albirostris*	Oriental Pied Hornbill (Sam-1)	Bucerotidae	LC	LC
4	*Anthracoceros albirostris*	Oriental Pied Hornbill (Sam-2)	Bucerotidae	LC	LC
5	*Amaurornis phoenicurus*	White-breasted Waterhen	Rallidae	LC	LC
6	*Ardea cinerea*	Grey Heron (Sam-1)	Ardidae	LC	LC
7	*Buceros bicornis*	Great Hornbill (Sam-1)	Bucerotidae	VU	NT
8	*Dendrocygna javanica*	Lesser Whistling Duck	Anatidae	LC	LC
9	*Egretta garzetta*	Little Egret	Ardidae	LC	LC
10	*Eudynamys scolopaceus*	Asian Koel	Cuculidae	LC	LC
11	*Gallicrex cinerea*	Watercock	Rallidae	LC	LC
12	*Gallus gallus*	Red Junglefowl	Phasianidae	LC	LC
13	*Gorsachius melanolophus*	Malayan Night Heron	Ardidae	LC	LC
14	*Gracula religiosa*	Hill Myna (Sam-1)	Sturnidae	LC	LC
15	*Grus antigone*	Sarus Crane	Gruidae	RE	VU
16	*Gyps himalayensis*	Himalayan Griffon Vulture (Sam-1)	Accipitridae	LC	NT
17	*Haliastur indus*	Brahminy Kite	Accipitridae	LC	LC
18	*Halcyon smyrnensis*	White-breasted Kingfisher	Alcedinidae	LC	LC

Sl	Scientific Name	Common Name	Family	BD Status	Global Status
19	*Hydrophasianus chirurgus*	Pheasant-tailed Jacana	Jacanidae	LC	LC
20	*Ichthyophaga ichthyaetus*	Grey-headed Fish-eagle	Accipitridae	NT	NT
21	*Ichthyophaga* sp.	Eagle UnID-2 (Grey-headed Fish-eagle)	-	-	-
22	*Ketupa zeylonensis*	Brown Fish Owl	Srtigidae	LC	LC
23	*Larus brunnicephalus*	Brown-headed Gull	Laridae	LC	LC
24	*Leptoptilos javanicus*	Lesser Adjutant	Ciconidae	VU	VU
25	*Limosa limosa*	Black-tailed Godwit	Scolopacidae	NT	NT
26	*Lophura leucomelanos*	Kalij Pheasant	Phasianidae	VU	LC
27	*Loriculus vernalis*	Vernal Hanging Parrot	Psittacidae	LC	LC
28	*Mareca strepera*	Gadwall	Anatidae	LC	LC
29	*Microcarbo niger*	Little Cormorant	Phalacrocoracidae	LC	LC
30	*Milvus migrans*	Black Kite (Sam-2)	Accipitridae	LC	LC
31	*Netta rufina*	Red-crested Pochard	Anatidae	LC	LC
32	*Nycticorax nycticorax*	Black-crowned Night Heron (Sam-1)	Ardidae	LC	LC
33	*Nycticorax nycticorax*	Black-crowned Night Heron (Sam-2)	Ardidae	LC	LC
34	*Pavo cristatus*	Indian Peafowl	Phasianidae	RE	LC
35	*Porphyrio porphyrio*	Purple Swamphen (Sam-1)	Rallidae	LC	LC
36	*Psittacula alexandri*	Red-breasted Parakeet	Psittaculidae	LC	NT
37	*Psittacula krameri*	Rose-ringed Parakeet	Psittaculidae	LC	LC
38	*Rynchops albicollis*	Indian Skimmer	Laridae	CR	VU
39	*Spatula clypeata*	Northern Shoveler	Anatidae	LC	LC
40	*Spatula querquedula*	Garganey	Anatidae	LC	LC
41	*Spilopelia chinensis*	Eastern Spotted Dove	Columbidae	LC	LC
42	*Streptopelia decaocto*	Eurasian Collared Dove	Columbidae	LC	LC
43	*Streptopelia orientalis*	Oriental Turtle Dove	Columbidae	LC	LC
44	*Treron bicinctus*	Orange-breasted Green Pigeon	Columbidae	LC	LC
45	-	Eagle UnID-1	-	-	-
		Reptiles			
1	*Amphiesma stolatum*	Striped Keelback	Natricidae	LC	NE
2	*Argyrogena fasciolata*	Banded Racer [S-8 (VRC 9)]	Colubridae	LC	NE
3	*Batagur baska*	River Terrapin (Sam-1)	Geoemydidae	CR	CR
4	*Boiga cyanea*	Green Cat Snake	Colubridae	LC	NE
5	*Bungarus fasciatus*	Banded Krait	Elapidae	LC	LC
6	*Bungarus* sp.	Snake UnID-6 (DE) (*Bungarus* sp.)	Elapidae	-	-
7	*Bungarus* sp.	Snake UnID-6 (DE) (Snake-5) (*Bungarus* sp.)	Elapidae	-	-
8	*Calotes versicolor*	Common Garden Lizard (Sam-1)	Agamidae	LC	NE
9	*Calotes versicolor*	Common Garden Lizard (Sam-3)	Agamidae	LC	NE
10	*Caretta caretta*	Loggerhead Sea Turtle	Cheloniidae	DD	VU
11	*Cerberus rynchops*	Dog-faced Water Snake (Sam-2)	Homalopsidae	LC	LC

Sl	Scientific Name	Common Name	Family	BD Status	Global Status
12	*Coelognathus radiatus*	Copper-head Trinket Snake [S-4 (VRC 4)]	Colubridae	LC	LC
13	*Crocodylus palustris*	Marsh Crocodile	Crocodylidae	RE	VU
14	*Crocodylus porosus*	Salt-water Crocodile	Crocodylidae	EN	LC
15	*Daboia russelii*	Snake UnID-4 (Russell's Viper)	Viperidae	NT	LC
16	*Enhydris enhydris*	Snake UnID-3 (Common Smooth- scaled Water Snake)	Homalopsidae	LC	LC
17	*Eryx conicus*	Common Sand Boa	Boidae	DD	NE
18	*Fowlea piscator*	Checkered Keelback	Natricidae	LC	NE
19	*Gavialis gangeticus*	Gharial (Sam-1)	Gavialidae	CR	CR
20	*Gavialis gangeticus*	Gharial (Sam-2)	Gavialidae	CR	CR
21	*Gekko gecko*	Tokay Gecko (Sam-1)	Gekkonidae	LC	NE
22	*Gekko gecko*	Tokay Gecko (Sam-3)	Gekkonidae	LC	NE
23	*Gekko gecko*	Tokay Gecko (Sam-4)	Gekkonidae	LC	NE
24	*Gekko gecko*	Tokay Gecko (Sam-5)	Gekkonidae	LC	NE
25	*Geoclemys hamiltonii*	Black Spotted Pond Turtle	Geoemydidae	EN	VU
26	*Hardella thurjii*	Crowned River Turtle	Geoemydidae	EN	VU
27	*Hydrophis lapemoides*	Arabian Gulf Sea Snake [S-10 (VRC 11)]	Elapidae	DD	LC
28	*Hydrophis schistosus*	Hook-nosed Sea Snake	Elapidae	LC	LC
29	*Indotestudo elongata*	Elongated Tortoise	Testudinidae	CR	EN
30	*Indotyphlops porrectus*	Slender Worm Snake	Typhlopidae	NE	NE
31	*Lepidochelys olivacea*	Olive Ridley Sea Turtle	Cheloniidae	VU	VU
32	*Lissemys punctata*	Spotted Flapshell Turtle	Trionychidae	LC	LC
33	*Lycodon aulicus*	Snake UnID-5 (DE) (Common Wolf Snake)	Colubridae	LC	NE
34	*Manouria emys*	Asian Giant Tortoise	Testudinidae	CR	EN
35	*Nilssonia nigricans*	Black Softshell Turtle	Trionychidae	EN	CR
36	*Oligodon cyclurus*	Cantor's Kukri Snake *S-5 (VRC 6)]	Colubridae	LC	LC
37	*Oligodon kheriensis*	Coral Red Kukri Snake [S-6 (VRC 7)]	Colubridae	-	-
38	*Ophiophagus hannah*	King Cobra	Elapidae	VU	VU
39	*Pangshura tecta*	Indian Roofed Turtle	Geoemydidae	LC	LC
40	*Psammodynastes pulverulentus*	Snake UnID-1 (Common Mock Viper)	Lamprophiidae	-	-
41	*Psammophis condanarus*	Sand Snake [S-9 (VRC 10)]	Psammophiidae	-	-
42	*Ptyas mucosa*	Indian Rat Snake [S-1 (VRC 1)]	Colubridae	LC	NE
43	*Python molurus*	Indian Rock Python	Pythonidae	DD	LC
44	*Ptyas korros*	Chinese Rat Snake [S-2 (VRC 2)]	Colubridae	NT	NE
45	*Python bivittatus*	Burmese Python [S-7 (VRC 8)]	Pythonidae	VU	VU
46	*Rhabdophis subminiatus*	Red-necked Keelback [S-3 (VRC 3)]	Natricidae	NT	LC

Sl	Scientific Name	Common Name	Family	BD Status	Global Status
47	*Trimeresurus* sp.	Pit Viper	Viperidae	NE	LC
48	*Varanus bengalensis*	Bengal Monitor	Varanidae	NT	LC
49	*Varanus flavescens*	Yellow Monitor	Varanidae	NT	LC
50	-	Snake UnID-1	-	-	-
51	-	Snake UnID-2	-	-	-
52	-	Snake UnID-3	-	-	-
53	-	Snake UnID-4	-	-	-
54	-	Snake UnID-5	-	-	-
55	-	Snake UnID-6	-	-	-
56	-	Snake UnID-7	-	-	-
57	-	Unidentified Snake -1 [S-11 (VRC 12)]	-	-	-
58	-	Unidentified Snake -2 [S-12 (VRC 13)]	-	-	-
59	-	Skink UnID-3	Scincidae	-	-
60	-	Skink UnID-4	Scincidae	-	-
61	-	Turtle UnId-1	-	-	-
62	-	Turtle UnID-2	-	-	-
Amphibians					
1	*Duttaphrynus melanostictus*	Asian Common Toad	Bufonidae	LC	LC
2	*Euphlyctis kalasgramensis*	Kalasgram Skittering	Dicroglossidae	-	-
3	*Fejervarya nepalensis*	Frog UnID-6 (Nepal Cricket Frog)	Dicroglossidae	LC	LC
4	*Fejervarya* sp.	Frog UnID-50 (Orissa Frog)	Dicroglossidae	-	-
5	*Hoplobatrachus tigerinus*	Indian Bullfrog	Dicroglossidae	LC	LC
6	*Hylarana tytleri*	Yellow-striped Frog	Ranidae	LC	LC
7	*Microhyla mymensinghensis* (*Microhyla* sp.)	Mymensingh Microhylid Frog (Microhylid Frog)	Microhylidae	LC	LC
8	*Minervarya pierrei*	Pierre's Cricket Frog	Dicroglossidae	LC	LC
9	*Minervarya* sp.	Frog UnID-51 (Bangladeshi Cricket Frog)	Dicroglossidae	-	-
10	*Microhyla* sp.	Frog UnID-41 (Berdmore's Narrow- mouthed Frog)	Microhylidae	-	-
11	*Polypedates leucomystax*	Common Tree Frog	Rhacophoridae	LC	LC
12	*Polypedates teraiensis*	Frog UnID-5 (Terai Cricket Frog)	Dicroglossidae	LC	LC
13	*Sylvirana* sp.	Frog UnID-49 (Crying Stream Frog)	Ranidae	-	-
14	*Uperodon globulosus*	Balloon Frog	Microhylidae	VU	LC
15	-	Frog UnID-2	-	-	-
16	-	Frog UnID-7	-	-	-
17	-	Frog UnID-8	-	-	-

Sl	Scientific Name	Common Name	Family	BD Status	Global Status
18	-	Frog UnID-9	-	-	-
19	-	Frog UnID-10	-	-	-
20	-	Frog UnID-11	-	-	-
21	-	Frog UnID-12	-	-	-
22	-	Frog UnID-13	-	-	-
23	-	Frog UnID-14	-	-	-
24	-	Frog UnID-17	-	-	-
25	-	Frog UnID-20	-	-	-
26	-	Frog UnID-22	-	-	-
27	-	Frog UnID-23	-	-	-
28	-	Frog UnID-24	-	-	-
29	-	Frog UnID-25	-	-	-
30	-	Frog UnID-26	-	-	-
31	-	Frog UnID-27	-	-	-
32	-	Frog UnID-28	-	-	-
33	-	Frog UnID-29	-	-	-
34	-	Frog UnID-30	-	-	-
35	-	Frog UnID-31	-	-	-
36	-	Frog UnID-32	-	-	-
37	-	Frog UnID-39	-	-	-
38	-	Frog UnID-40	-	-	-
39	-	Frog UnID-42	-	-	-
40	-	Frog UnID-43	-	-	-
41	-	Frog UnID-44	-	-	-
42	-	Frog UnID-45	-	-	-
43	-	Frog UnID-46	-	-	-
Butterflies					
1	*Acytolepis puspa*	Common Hedge Blue	Lycaenidae	VU	NE
2	*Anthene emolus*	Common Ciliate Blue	Lycaenidae	VU	NE
3	*Arhopala perimuta*	Yellowdisc Tailless Oakblue	Lycaenidae	NE	NE
4	*Athyma nefte inara*	Colour sergeant	Nymphalidae	VU	NE
5	*Athyma ranga*	Blackvein Sergeant	Nymphalidae	VU	NE
6	*Athyma selenophora*	Staff Sergeant	Nymphalidae	DD	NE
7	*Badamia exclamationis*	Brown Awl	Hesperiidae	VU	NE
8	*Baoris farri*	Complete Paint-brush Swift	Hesperiidae	NE	-
9	*Catapaecilma major*	Common Tinsel	Lycaenidae	EN	NE
10	*Catochrysops panormus*	Silver forget-me-not	Lycaenidae	NE	NE
11	*Celaenorrhinus leucocera*	Common Spotted Flat	Hesperiidae	NE	-
12	*Chersonesia risa*	Common Maplet	Nymphalidae	EN	NE
13	*Chliaria othona*	Orchid Tit	Lycaenidae	VU	NE
14	*Choaspes benjaminii*	Indian Awlking	Hesperiidae	EN	NE

Sl	Scientific Name	Common Name	Family	BD Status	Global Status
15	*Cigaritis lohita*	Long-banded Silverline	Lycaenidae	VU	NE
16	*Curetis acuta*	Angled Sunbeam	Lycaenidae	NE	-
17	*Discolampa ethion*	Banded Blue Pierrot	Lycaenidae	VU	NE
18	*Dophla evelina*	Redspot Duke	Nymphalidae	EN	NE
19	*Elymnias nesaea*	Tiger Palmfly	Satyridae	EN	NE
20	*Euploea klugii*	King Crow	Danidae	VU	NE
21	*Eurema andersoni*	One-spot Grass Yellow	Pieridae	LC	LC
22	*Eurema blanda*	Three-spot Grass Yellow	Pieridae	LC	NE
23	*Gandaca harina*	Tree Yellow	Pieridae	EN	NE
24	*Gerosis bhagava*	Common Yellow-breasted Flat	Hesperiidae	VU	NE
25	*Graphium nomius*	Spot Swordtail	Papilionidae	EN	NE
26	*Heliophorus epicles*	Purple Sapphire	Lycaenidae	VU	NE
27	*Hypolycaena erylus*	Common Tit	Lycaenidae	VU	NE
28	*Jamides celeno*	Common Cerulean	Lycaenidae	LC	NE
29	*Junonia hierta*	Yellow Pansy	Nymphalidae	LC	LC
30	*Junonia orithya*	Blue Pansy	Nymphalidae	VU	NE
31	*Kallima inachus*	Orange Oakleaf	Nymphalidae	EN	NE
32	*Lamproptera curius*	White Dragontail	Papilionidae	EN	NE
33	*Lethe chandica*	Angled Red Forester	Satyridae	NE	NE
34	*Lexias dirtea*	Dark Archduke	Nymphalidae	EN	NE
35	*Loxura atymnus*	Yamfly	Lycaenidae	VU	NE
36	*Mycalesis perseus*	Common Bushbrown	Satyridae	VU	NE
37	*Neptis harita*	Dingiest Sailer	Nymphalidae	EN	NE
38	*Neptis soma*	Sullied Sailer	Nymphalidae	VU	NE
39	*Pantoporia paraka*	Perak Lascar	Nymphalidae	EN	NE
40	*Pantoporia sandaka*	Extra Lascar	Nymphalidae	NE	-
41	*Papilio helenus*	Red Helen	Papilionidae	VU	NE
42	*Rapala suffusa*	Suffused Flash	Lycaenidae	NE	-
43	*Remelana jangala*	Chocolate Royal	Lycaenidae	VU	NE
44	*Symbrenthia lilaea*	Common Jester	Nymphalidae	EN	NE
45	*Tanaecia julii*	Common Earl	Nymphalidae	VU	NE
46	*Tarucus ananda*	Dark Pierrot	Lycaenidae	NE	-
47	*Tarucus callinara*	Spotted Pierrot	Lycaenidae	EN	NE
48	*Tarucus nara*	Striped Pierrot	Lycaenidae	EN	NE
49	*Ticherra acte*	Blue Imperial	Lycaenidae	NE	NE
50	*Ypthima baldus*	Common Five Ring	Satyridae	VU	NE
51	*Ypthima huebneri*	Common Fourring	Satyridae	LC	NE
52	*Zizina otis*	Lesser Grass Blue	Lycaenidae	LC	NE
53	-	Butterfly UnID-1	Hespriidae	-	-
54	-	Butterfly UnID-2	Hespriidae	-	-
55	-	Unidentified Hespiridae (K-1)	-	-	-

Sl	Scientific Name	Common Name	Family	BD Status	Global Status
Crustaceans					
1	*Carcinoscorpius rotundicauda*	Mangrove Horseshoe Crab	Limulidae	VU	DD
2	*Ocypode macrocera*	Red Ghost Crab	Ocypodidae	LC	NE
3	*Pagurus bernhardus*	Common Hermit Crab	Paguridae	DD	NE
4	*Portunus sanguinolentus*	Three Spot Swimming Crab	Portunidae	VU	NE
5	*Scylla olivacea*	Mud Crab	Portunidae	LC	NE
6	*Scylla serrata*	Mangrove Crab	Portunidae	LC	NE
7	*Tubuca rosea*	Rosy Fiddler Crab	Ocypodidae	DD	NE
8	-	Crab UnID -1	-	-	-
9	-	Crab UnID -2	-	-	-
10	-	Crab UnID -3	-	-	-
11	-	Crab UnID -4	-	-	-
12	-	Crab UnID - 5	-	-	-
13	-	Crab UnID - 6	-	-	-
14	-	Crab UnID - 7	-	-	-
15	-	Crab UnID - 8	-	-	-
16	-	Crab UnID - 9	-	-	-
17	-	Crab UnID - 10	-	-	-
18	-	Crab UnID - 11	-	-	-
19	-	Crab UnID-12	-	-	-
20	-	Crab UnID-13	-	-	-
Others					
1	*Mobula* sp.	Manta ray	Mobulidae	NE	VU
2	*Periophthalmus koelreuteri*	Mudskipper (Sam-1)	Gobiidae	LC	NE
3	*Periophthalmus* sp.	Mudskipper (Sam-2)	Gobiidae	-	-
4	*Sphyrna* sp.	Hammer Head Shark	Sphyrnidae	NE	CR

Table 2. Genomic DNA extraction from samples of different species

Sl	Common Name	Scientific Name	Family	Body Part	Voucher	Genomic DNA extraction
Mammals						
1	Bengal Tiger (Sam-1)	*Panthera tigris tigris*	Felidae	Skin	MBSV001	6.5 ng/µl
2	Spotted Deer (Sam-1)	*Axis axis*	Cervidae	Hair	MBSV002	5.4 ng/µl
3	Sambar Deer	*Rusa unicolor*	Cervidae	Hair	MBSV003	8.3 ng/µl
4	Barking Deer (Sam-1)	*Muntiacus muntjak*	Cervidae	Hair	MBSV004	6.8 ng/µl
5	Nilgai (Sam-1)	*Boselaphus tragocamelus*	Bovidae	Feces	MBSV005	519.1 ng/µl
6	Asiatic Black Bear	*Ursus thibetanus*	Ursidae	Hair	MBSV006	31.0 ng/µl

Sl	Common Name	Scientific Name	Family	Body Part	Voucher	Genomic DNA extraction
7	Indian Crested Porcupine (Himalayan Crestless Porcupine)	*Hystrix indica* (*Hystrix brachyura*)	Hystricidae	Hard Scale	MBSV007	37.1 ng/µl
8	Ganges River Dolphin (Sam-1)	*Platanista gangetica*	Platanistidae	Skin	MBSV008	23.5 ng/µl
9	Stump-tailed Macaque	*Macaca arctoides*	Cercopithecidae	Hair	MBSV009	11.6 ng/µl
10	Asian Elephant (Sam-2)	*Elephas maximus*	Elephantidae	Dung	MBSV010	24.9 ng/µl
11	Asian Elephant (Sam-1)	*Elephas maximus*	Elephantidae	Hair	MBSV011	2.2 ng/µl
12	Jungle cat (Sam-1)	*Felis chaus*	Felidae	Hair	MBSV012	16.9 ng/µl
13	Phayre's Langur	*Trachypithecus phayrei*	Cercopithecidae	Hair	MBSV013	3.3 ng/µl
14	Capped Langur	*Trachypithecus pileatus*	Cercopithecidae	Hair	MBSV014	1.7 ng/µl
15	Masked Palm Civet (Sam-1)	*Paguma larvata*	Viverridae	Hair	MBSV015	3.5 ng/µl
16	Fishing cat	*Prionailurus viverrinus*	Felidae	Hair	MBSV016	4.5 ng/µl
17	Small Indian Civet	*Viverricula indica*	Viverridae	Hair	MBSV017	10.6 ng/µl
18	Large Indian Civet	*Viverra zibetha*	Viverridae	Skin	MBSV018	753.3 ng/µl
19	Irrawaddy Dolphin	*Orcaella brevirostris*	Delphinidae	Skin	MBSV019	4.2 ng/µl
20	Rhesus Macaque	*Macaca mulatta*	Cercopithecidae	Hair	MBSV020	14.7 ng/µl
21	Common Otter (Sam-1) (Small-clawed Otter)	*Lutra lutra* (*Aonyx cinereus*)	Mustelidae	Hair	MBSV021	21.5 ng/µl
22	Common Palm Civet	*Paradoxurus hermaphroditus*	Viverridae	Hair	MBSV022	20.7 ng/µl
23	Bengal Slow Loris	*Nycticebus bengalensis*	Lorisidae	Hair	MBSV023	21.8 ng/µl
24	Leopard Cat	*Prionailurus bengalensis*	Felidae	Hair	MBSV024	22.1 ng/µl
25	Barking Deer (Sam-2)	*Muntiacus muntjak*	Cervidae	Hair	MBSV025	17.9ng/µl
26	Hoolock Gibbon	*Hoolock hoolock*	Hylobatidae	Hair	MBSV026	10.9 ng/µl
27	Marbled Cat	*Pardofelis marmorata*	Felidae	Hair	MBSV027	33.2 ng/µl
28	Bat UnID-1 (Sam-1)	-	-	Hair	MBSV028	50.5 ng/µl
29	Bat UnID-2 (Sam-2) (Lesser Dawn Bat)	*Eonycteris* sp.	Pteropodidae	Hair	MBSV029	27.1 ng/µl
30	Assamese Macaque	*Macaca assamensis*	Cercopithecidae	Hair	MBSV030	3 ng/µl
31	Gaur (Sam-1)	*Bos gaurus*	Bovidae	Hair	MBSV031	2.1 ng/µl
32	Common Langur	*Semnopithecus entellus*	Cercopithecidae	Hair	MBSV032	1.1 ng/µl
33	Chinese Pangolin (Sam-1)	*Manis pentadactyla*	Manidae	Scale	MBSV033	4.1 ng/µl
34	Hog Deer	*Axis porcinus*	Cervidae	Hair	MBSV034	5.3 ng/µl
35	Squirrel (Sam-1) (Irrawaddy Squirrel)	*Callosciurus pygerythrus*	Sciuridae	Dry Skin	MBSV035	0.7 ng/µl
36	Squirrel (Sam-2)	*Funambulus* sp.	Sciuridae	Hair	MBSV036	1.4 ng/µl
37	Bat UnID-3 (Indian Flying Fox)	*Pteropus* sp. (*Pteropus giganteus*)	Pteropodidae	Wing	MBSV037	93.3 ng/µl

Sl	Common Name	Scientific Name	Family	Body Part	Voucher	Genomic DNA extraction
38	Lesser Bandicoot Rat (Oriental House Rat)	*Bandicota* sp. (*Rattus tanezumi*)	Muridae	Tail	MBSV038	1.3 ng/µl
39	House Mouse	*Mus musculus*	Muridae	Tail	MBSV039	103.7 ng/µl
40	Common Wild Pig	*Sus scrofa*	Suidae	Hair	MBSV040	2.1 ng/µl
41	Common Otter (Sam-3)	*Lutra lutra*	Mustelidae	Hair	MBSV041	2.3 ng/µl
42	Clouded Leopard	*Neofelis nebulosa*	Felidae	Dry Skin	MBSV042	6 ng/µl
43	Oriental House Rat	*Rattus tanezumi*	Muridae	Tail	MBSV043	1.3 ng/ul
44	Himalayan Crestless Porcupine (Sam-1)	*Hystrix brachyura*	Hystricidae	Spine	MBSV044	37.1 ng/µl
45	Ganges River Dolphin (Sam-2)	*Platanista gangetica*	Platanistidae	Fin	MBSV045	70.2 ng/µl
46	Golden Jackal	*Canis aureus*	Canidae	Muscle	MBSV046	94.3 ng/µl
47	Common Mongoose	*Urva edwardsii*	Herpestidae	Bone	MBSV047	41.8 ng/µl
48	Jungle Cat (Sam-2)	*Felis chaus*	Felidae	Hair	MBSV048	11.8 ng/µl
49	Small-clawed Otter (Sam-2)	*Aonyx cinereus*	Mustelidae	Hair	MBSV049	15.9 ng/µl
Birds						
1	Eastern Spotted Dove	*Spilopelia chinensis*	Columbidae	Feather	BBSV0001	13.9 ng/µl
2	Eurasian Collared Dove	*Streptopelia decaocto*	Columbidae	Feather	BBSV0002	8.0 ng/µl
3	Hill Myna (Sam-1)	*Gracula religiosa*	Sturnidae	Feather	BBSV0003	39.7 ng/µl
4	White-breasted Waterhen (Sam-1)	*Amaurornis phoenicurus*	Rallidae	Feather	BBSV0004	24.8 ng/µl
5	Purple Swamphen (Sam-1)	*Porphyrio porphyrio*	Rallidae	Feather	BBSV0005	34.7 ng/µl
6	Little Cormorant	*Microcarbo niger*	Phalacrocoraci dae	Feather	BBSV0006	19.3 ng/µl
7	Great Hornbill (Sam-1)	*Buceros bicornis*	Bucerotidae	Feather	BBSV0007	22.9 ng/µl
8	Lesser Adjutant	*Leptoptilos javanicus*	Ciconidae	Feather	BBSV0008	9.3 ng/µl
9	Lesser Whistling Duck	*Dendrocygna javanica*	Anatidae	Feather	BBSV0009	58.6 ng/µl
10	Grey Heron (Sam-1)	*Ardea cinerea*	Ardeidae	Feather	BBSV0010	9.2 ng/µl
11	Watercock	*Gallicrex cinerea*	Rallidae	Feather	BBSV0011	2.5 ng/µl
12	Brahminy Kite	*Haliastur indus*	Accipitridae	Feather	BBSV0012	6.4 ng/µl
13	Pheasant-tailed Jacana	*Hydrophasianus chirurgus*	Jacanidae	Feather	BBSV0013	1.7 ng/µl
14	Indian spot-billed Duck	*Anas poecilorhyncha*	Anatidae	Feather	BBSV0014	2.6 ng/µl
15	Asian Koel	*Eudynamys scolopaceus*	Cuculidae	Feather	BBSV0015	430.7 ng/µl
16	Garganey	*Spatula querquedula*	Anatidae	Feather	BBSV0016	11.7 ng/µl
17	Gadwall	*Mareca strepera*	Anatidae	Feather	BBSV0017	13.2 ng/µl

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18	Black-tailed Godwit	*Limosa limosa*	Scolopacidae	Feather	BBSV0018	04 ng/µl
19	Northern Pintail	*Anas acuta*	Anatidae	Feather	BBSV0019	10.3 ng/µl
20	Red-crested Pochard	*Netta rufina*	Anatidae	Feather	BBSV0020	14.3 ng/µl
21	Northern Shoveler	*Spatula clypeata*	Anatidae	Feather	BBSV0021	18.9 ng/µl
22	Vernal Hanging Parrot	*Loriculus vernalis*	Psittacidae	Feather	BBSV0022	24 ng/µl
23	Oriental Turtle Dove	*Streptopelia orientalis*	Columbidae	Feather	BBSV0023	7.4 ng/µl
24	Red Junglefowl	*Gallus gallus*	Phasianidae	Feather	BBSV0024	7.9 ng/µl
25	Oriental Pied Hornbill (Sam-1)	*Anthracoceros albirostris*	Bucerotidae	Feather	BBSV0025	12.1 ng/µl
26	Himalayan Griffon Vulture (Sam-1)	*Gyps himalayensis*	Accipitridae	Feather	BBSV0026	19.5 ng/µl
27	Black-crowned Night Heron	*Nycticorax nycticorax*	Ardidae	Feather	BBSV0027	15.8 ng/µl
28	Red-breasted parakeet	*Psittacula alexandri*	Psittaculidae	Feather	BBSV0028	44.1 ng/µl
29	Rose-ringed Parakeet	*Psittacula krameri*	Psittaculidae	Feather	BBSV0029	20.2 ng/µl
30	Brown-headed Gull	*Larus brunnicephalus*	Laridae	Feather	BBSV0030	38.1 ng/µl
31	Orange-breasted Green Pigeon	*Treron bicinctus*	Columbidae	Feather	BBSV0031	28.9 ng/µl
32	Kalij Pheasant	*Lophura leucomelanos*	Phasianidae	Feather	BBSV0032	19.8 ng/µl
33	Oriental Pied Hornbill (Sam-2)	*Anthracoceros albirostris*	Bucerotidae	Feather	BBSV0033	4.4 ng/µl
34	Eagle UnID-2 (Grey-headed Fish-eagle)	*Ichthyophaga* sp.	Accipitridae	Feather	BBSV0034	3.9 ng/µl
35	Little Egret	*Egretta garzetta*	Ardeidae	Eggshell	BBSV0035	10.4 ng/µl
36	Black-crowned Night Heron (Sam-2)	*Nycticorax nycticorax*	Ardeidae	Feather	BBSV0036	0.7 ng/µl
37	Eagle UnID-1	-	-	Feather	BBSV0037	4.6 ng/µl
38	White-breasted Kingfisher	*Halcyon smyrnensis*	Alcedinidae	Feather	BBSV0038	8.1 ng/µl
39	Grey-headed Fish-eagle	*Ichthyophaga ichthyaetus*	Accipitridae	Feather	BBSV0039	3.1 ng/µl
40	Brown Fish Owl	*Ketupa zeylonensis*	Strigidae	Feather	BBSV0040	3.2 ng/µl
41	Indian skimmer	*Rynchops albicollis*	Laridae	Feather	BBSV0041	22 ng/µl
42	Indian Peafowl (Sam-1)	*Pavo cristatus*	Phasianidae	Feather	BBSV0042	2.7 ng/µl
Reptiles						
1	Tokay Gecko (Sam-1)	*Gekko gecko*	Gekkonidae	Dry skin	RBSV001	38.1 ng/µl
2	Salt Water Crocodile	*Crocodylus porosus*	Crocodylidae	Scale	RBSV002	97.2 ng/µl
3	Marsh Crocodile	*Crocodylus palustris*	Crocodylidae	Egg Shell	RBSV003	47.2 ng/µl
4	Indian Rock Python	*Python molurus*	Pythonidae	Scale	RBSV004	203.2 ng/µl

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5	Common Garden Lizard (Sam-1)	*Calotes versicolor*	Agamidae	Dry skin	RBSV005	92.7 ng/µl
6	River Terrapin (Sam-1)	*Batagur baska*	Geoemydidae	Egg Shell	RBSV006	38.3 ng/µl
7	Black Spotted Pond Turtle	*Geoclemys hamiltonii*	Geoemydidae	Nail	RBSV007	13.9 ng/µl
8	Hook-nosed sea snake (Dog-faced Water Snake)	*Hydrophis schistosus* (*Cerberus rynchops*)	Elapidae (Homalopsidae)	Dry skin	RBSV008	32.9 ng/µl
9	Striped Keelback	*Amphiesma stolatum*	Natricidae	Scale	RBSV009	5.4 ng/µl
10	Pit Viper	*Trimeresurus* sp.	Viperidae	Dry skin	RBSV010	22.1 ng/µl
11	Indian Roofed Turtle (Sam-2)	*Pangshura tecta*	Geoemydidae	Nail	RBSV011	1.2 ng/µl
12	Banded Krait	*Bungarus fasciatus*	Elapidae	Scale	RBSV012	20.7 ng/µl
13	Elongated Tortoise	*Indotestudo elongata*	Testudinidae	Nail	RBSV013	10.3 ng/µl
14	Asian Giant Tortoise	*Manouria emys*	Testudinidae	Nail	RBSV014	7.1 ng/µl
15	Crowned River Turtle (Black Spotted Pond Turtle)	Hardella thurjii (Geoclemys hamiltonii)	Geoemydidae	Nail	RBSV015	5.7 ng/µl
16	Spotted Flapshell Turtle	*Lissemys punctata*	Trionychidae	Nail	RBSV016	9.1 ng/µl
17	Turtle UnId-1 (Roofed Turtle) (Sam-1)	*Pangshura tecta*	Geoemydidae	Nail	RBSV017	14.2 ng/µl
18	Snake UnID-1 (Common Mock Viper)	*Psammodynastes pulverulentus*	Lamprophiidae	Scale	RBSV018	96.7 ng/µl
19	Common Garden Lizard (Sam-3)	*Calotes versicolor*	Agamidae	Dry Skin	RBSV019	31.8 ng/µl
20	Olive Ridley Sea Turtle	*Lepidochelys olivacea*	Cheloniidae	Nail	RBSV020	9.5 ng/µl
21	Turtle UnID-2	-	-	Dry Skin	RBSV021	1 ng/µl
22	Black Spotted Pond Turtle	*Geoclemys hamiltonii*	Geoemydidae	Nail	RBSV022	5.7 ng/µl
23	Checkered Keelback	*Fowlea piscator*	Colubridae	Scale	RBSV023	98.7 ng/µl
24	Black Softshell Turtle (Bostami Turtle)	*Nilssonia nigricans*	Trionychidae	Dry Skin	RBSV024	21.8 ng/µl
25	Loggerhead Turtle	*Caretta caretta*	Cheloniidae	Nail	RBSV025	27.6 ng/µl
26	Bengal Monitor	*Varanus bengalensis*	Varanidae	Dry Skin	RBSV026	3.2 ng/µl
27	Gharial (Sam-1)	*Gavialis gangeticus*	Varanidae	Egg Shell	RBSV027	5.4 ng/µl
28	Tokay Gecko (Sam-3)	*Gekko gecko*	Gekkonidae	Dry Skin	RBSV028	2.5 ng/µl
29	Gharial (Sam-2)	*Gavialis gangeticus*	Gavialidae	Egg shell	RBSV029	6.4 ng/µl
30	Skink UnID-3	-	Scincidae	Dry skin	RBSV030	34.9 ng/µl
31	Skink UnID-4	-	Scincidae	Dry skin	RBSV031	8.3 ng/µl
32	King Cobra	*Ophiophagus hannah*	Elapidae	Dry skin	RBSV032	54 ng/µl

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33	Yellow Monitor	*Varanus flavescens*	Varanidae	Dry skin	RBSV033	26.7 ng/µl
34	Snake UnID-1 (Common Mock Viper)	*Psammodynastes* sp.	Pseudaspididae	Scale	RBSV034	96.7 ng/µl
35	Snake UnID-2 (Striped Keelback)	*Amphiesma stolatum*	Natricidae	Scale	RBSV035	23.8 ng/µl
36	Snake UnID-3 (Common Smooth-scaled Water Snake)	*Enhydris enhydris*	Homalopsidae	Scale	RBSV036	10.9 ng/µl
37	Snake UnID-4 (Russell's Viper)	*Daboia russelii*	Viperidae	Scale	RBSV037	143.7 ng/µl
38	Snake UnID-5(DE) (Common Wolf Snake)	*Lycodon aulicus*	Colubridae	Scale	RBSV038	14 ng/µl
39	Snake UnID-6 (DE) (*Bungarus* sp.)	*Bungarus* sp.	Elapidae	Scale	RBSV039	192.7 ng/µl
40	Indian Rat Snake [S-1 (VRC 1)]	*Ptyas mucosa*	Colubridae	Scale	RBSV040	13.3 ng/µl
41	Chinese Rat Snake [S-2 (VRC 2)]	*Ptyas korros*	Colubridae	Scale	RBSV041	6.9 ng/µl
42	Red-necked Keelback [S-3 (VRC 3)]	*Rhabdophis subminiatus*	Natricidae	Scale	RBSV042	4 ng/µl
43	Copper-head Trinket Snake [S-4 (VRC 4)]	*Coelognathus radiatus*	Colubridae	Scale	RBSV043	9 ng/µl
44	Cantor's Kukri Snake *S-5 (VRC 6)]	*Oligodon cyclurus*	Colubridae	Scale	RBSV044	72.8 ng/µl
45	Coral Red Kukri Snake [S-6 (VRC 7)]	*Oligodon kheriensis*	Colubridae	Scale	RBSV045	25.7 ng/µl
46	Burmese Python [S-7 (VRC 8)]	*Python bivittatus*	Pythonidae	Scale	RBSV046	33.3 ng/µl
47	Banded Racer [S-8 (VRC 9)]	*Ahaetulla fasciolata*	Colubridae	Scale	RBSV047	5.2 ng/µl
48	Sand Snake [S-9 (VRC 10)]	*Psammophis condanarus*	Psammophiidae	Scale	RBSV048	4.4 ng/µl
49	Arabian Gulf Sea Snake [S- 10 (VRC 11)]	*Hydrophis lapemoides*	Elapidae	Scale	RBSV049	70.4 ng/µl
50	Unidentified Snake -1	-	-	Scale	RBSV050	19 ng/µl
51	Unidentified Snake -2	-	-	Scale	RBSV051	3.1 ng/µl
52	Common Sand Boa	*Eryx conicus*	Boidae	Scale	RBSV052	4.1 ng/µl
53	Snake UnID-6(DE) (Snake-5) (*Bungarus* sp.)	*Bungarus.* sp.	Elapidae	Scale	RBSV053	149.8 ng/µl
54	Worm Snake	*Indotyphlops* sp.	Typhlopidae	Muscle	RBSV054	128.7 ng/µl
55	Tokay Gecko (Sam-4)	*Gekko gecko*	Gekkonidae	Skin	RBSV055	37.3 ng/µl
56	Tokay Gecko (Sam-5)	*Gekko gecko*	Gekkonidae	Skin	RBSV056	285.9 ng/µl

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57	Dog-faced Water Snake (Sam-2)	*Cerberus rynchops*	Homalopsidae	Scale	RBSV057	32.9 ng/µl
58	Green Cat Snake	*Boiga cyanea*	Colubridae	Scale	RBSV058	24.7 ng/µl
Amphibians						
1	Mymensingh Microhylid Frog (Microhylid Frog)	*Microhyla mymensinghensis* (*Microhyla* sp.)	Mcrohylidae	Skin	ABSV001	26.6 ng/µl
2	Indian Bullfrog	*Hoplobatrachus tigerinus*	Dicroglossidae	Skin	ABSV002	37.8 ng/µl
3	Yellow-striped Frog	*Hylarana tytleri*	Ranidae	Skin	ABSV003	14.5 ng/µl
4	Kalasgram Skittering	*Euphlyctis kalasgramensis*	Dicroglossidae	Skin	ABSV004	19 ng/µl
5	Common Tree Frog	*Polypedates leucomystax*	Rhacophoridae	Skin	ABSV005	165.2 ng/µl
6	Asian Common Toad	*Duttaphrynus melanostictus*	Bufonidae	Skin	ABSV006	149 ng/µl
7	Frog UnID-5 (Terai Cricket Frog)	*Polypedates teraiensis*	Rhacophoridae	Skin	ABSV007	35.5 ng/µl
8	Frog UnID-6 (Nepal Cricket Frog)	*Fejervarya nepalensis*	Dicroglossidae	Skin	ABSV008	69.4 ng/µl
9	Balloon Frog	*Uperodon globulosus*	Microhylidae	Skin	ABSV009	55.3 ng/µl
10	Frog UnID-2	-	-	Skin	ABSV010	21.6 ng/µl
11	Frog UnID-7	-	-	Skin	ABSV011	180.2 ng/µl
12	Frog UnID-8	-	-	Skin	ABSV012	46 ng/µl
13	Frog UnID-9	-	-	Skin	ABSV013	165 ng/µl
14	Frog UnID-10	-	-	Skin	ABSV014	54.7 ng/µl
15	Frog UnID-11	-	-	Skin	ABSV015	71 ng/µl
16	Frog UnID-12	-	-	Skin	ABSV016	21.6 ng/µl
17	Frog UnID-39	-	-	Skin	ABSV017	415 ng/µl
18	Frog UnID-40	-	-	Skin	ABSV018	445.5 ng/µl
19	Frog UnID-41 (Berdmore's Narrow-mouthed Frog)	*Microhyla* sp.	Microhylidae	Skin	ABSV019	75.8 ng/µl
20	Frog UnID-42	-	-	Skin	ABSV020	57.1 ng/µl
21	Frog UnID-43	-	-	Skin	ABSV021	63.1 ng/µl
22	Frog UnID-44	-	-	Skin	ABSV022	20 ng/µl
23	Frog UnID-45	-	-	Skin	ABSV023	90 ng/µl
24	Frog UnID-46	-	-	Skin	ABSV024	59.3 ng/µl

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25	Frog UnID-25 (Terai Tree Frog)	*Polypedates teraiensis*	-	Skin	ABSV025	26.6 ng/µl
26	Frog UnID-26	-	-	Skin	ABSV026	22.5 ng/µl
27	Frog UnID-27	-	-	Skin	ABSV027	37 ng/µl
28	Frog UnID-28	-	-	Skin	ABSV028	22.6 ng/µl
29	Frog UnID-29	-	-	Skin	ABSV029	12.5 ng/µl
30	Frog UnID-31	-	-	Skin	ABSV030	16.5 ng/µl
31	Frog UnID-49 (Crying Stream Frog)	*Sylvirana* sp.	Ranidae	Skin	ABSV031	10.4 ng/µl
32	Frog UnID-50 (Orissa Frog)	*Fejervarya* sp.	Dicroglossidae	Skin	ABSV032	26.2 ng/µl
33	Frog UnID-51 (Bangladeshi Cricket Frog)	*Minervarya* sp.	Dicroglossidae	Skin	ABSV033	15.8 ng/µl
34	Pierre's Cricket Frog	*Minervarya pierrei*	Dicroglossidae	Skin	ABSV034	69 ng/µl
Butterflies						
1	King Crow	*Euploea klugii*	Danaidae	Leg	BFBSV009	661.6 ng/µl
2	Common Yellow-breasted Flat	*Gerosis bhagava*	Hesperiidae	Leg	BFBSV028	85.6 ng/µl
3	Orange Awlet	*Bibasis jaina*	Hesperiidae	Leg	BFBSV034	17.3 ng/µl
4	Brown Awl	*Badamia exclamationis*	Hesperiidae	Leg	BFBSV037	1.5 ng/µl
5	Purple Sapphire	*Heliophorus epicles*	Nymphalidae	Leg	BFBSV077	106.2 ng/µl
6	Striped Pierrot	*Tarucus nara*	Lycaenidae	Leg	BFBSV080	5.3 ng/µl
7	Spotted Pierrot	*Tarucus callinara*	Lycaenidae	Leg	BFBSV081	1.6 ng/µl
8	Long-banded Silverline	*Cigaritis lohita*	Lycaenidae	Leg	BFBSV085	6.0 ng/µl
9	Common Cerulean	*Jamides celeno*	Lycaenidae	Leg	BFBSV086	7.8 ng/µl
10	Common Tit	*Hypolycaena erylus*	Lycaenidae	Leg	BFBSV088	1.6 ng/µl
11	Common Hedge Blue	*Acytolepis puspa*	Lycaenidae	Leg	BFBSV091	61.3 ng/µl
12	Common Ciliate Blue	*Anthene emolus*	Lycaenidae	Leg	BFBSV102	3.3 ng/µl
13	Lesser Grass Blue	*Zizina otis*	Lycaenidae	Leg	BFBSV128	4.7 ng/µl
14	Common Tinsel	*Catapaecilma major*	Lycanidae	Leg	BFBSV129	13.1 ng/µl
15	Yamfly	*Loxura atymnus*	Lycaenidae	Leg	BFBSV143	51 ng/µl
16	Chocolate Royal	*Remelana jangala*	Lycaenidae	Leg	BFBSV145	7.3 ng/µl
17	Banded Blue Pierrot	*Discolampa ethion*	Lycaenidae	Leg	BFBSV146	8.3 ng/µl
18	Orchid Tit	*Chliaria othona*	Lycaenidae	Leg	BFBSV151	16.6 ng/µl
19	Blue Pansy	*Junonia orithya*	Nymphalidae	Leg	BFBSV168	7 ng/µl
20	Sullied Sailer	*Neptis soma*	Nymphalidae	Leg	BFBSV170	14.8 ng/µl

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21	Common Maplet	*Chersonesia risa*	Nymphalidae	Leg	BFBSV171	0.2 ng/µl
22	Blackvein Sergeant	*Athyma ranga*	Nymphalidae	Leg	BFBSV179	8.7 ng/µl
23	Colour Sergeant	*Athyma inara*	Nymphalidae	Leg	BFBSV181	8.5 ng/µl
24	Staff Sergeant	*Athyma selenophora*	Nymphalidae	Leg	BFBSV185	4.1 ng/µl
25	Redspot Duke	*Dophla evelina*	Nymphalidae	Leg	BFBSV192	9.5 ng/µl
26	Dark Archduke	*Lexias dirtea*	Nymphalidae	Leg	BFBSV199	2.1 ng/µl
27	Dingiest Sailer	*Neptis harita*	Nymphalidae	Leg	BFBSV202	5.3 ng/µl
28	Perak Lascar	*Pantoporia paraka*	Nymphalidae	Leg	BFBSV215	14.2 ng/µl
29	Common Jester	*Symbrenthia lilaea*	Nymphalidae	Leg	BFBSV217	7.4 ng/µl
30	Orange Oakleaf	*Kallima inachus*	Nymphalidae	Leg	BFBSV223	25.6 ng/µl
31	Spot Swordtail	*Graphium nomius*	Papilionidae	Leg	BFBSV232	3.8 ng/µl
32	White Dragontail	*Lamproptera curius*	Papilionidae	Leg	BFBSV233	13.3 ng/µl
33	Red Helen	*Papilio helenus*	Papilionidae	Leg	BFBSV240	9.9 ng/µl
34	Tree Yellow	*Gandaca harina*	Pieridae	Leg	BFBSV257	11.3 ng/µl
35	Tiger Palmfly	*Elymnias nesaea*	Satyridae	Leg	BFBSV288	9.5 ng/µl
36	Common Fourring	*Ypthima huebneri*	Satyridae	Leg	BFBSV304	2 ng/µl
37	Blue Imperial	*Ticherra acte*	Lycaenidae	Leg	BFBSV311	73.8 ng/µl
38	Common Spotted Flat	*Celaenorrhinus leucocera*	Hesperiidae	Leg	BFBSV314	34.0 ng/µl
39	Silver Forget-me-not	*Catochrysops panormus*	Lycaenidae	Leg	BFBSV316	56.6 ng/µl
40	Extra Lascar	*Pantoporia sandaka*	Nymphalidae	Leg	BFBSV317	14.2 ng/µl
41	Suffused Flash	*Rapala suffusa*	Lycaenidae	Leg	BFBSV318	44.1 ng/µl
42	Complete Paint-brush Swift	*Baoris farri*	Hesperiidae	Leg	BFBSV319	1.2 ng/µl
43	Dark Pierrot	*Tarucus ananda*	Lycaenidae	Leg	BFBSV323	3.3 ng/µl
44	Angled Sunbeam	*Curetis acuta*	Lycaenidae	Leg	BFBSV325	1.3 ng/µl
Crustaceans						
1	Rosy Fiddler Crab	*Tubuca rosea*	Ocypodidae	Muscle	CBSV001	159.2 ng/µl
2	Mangrove Crab	*Scylla serrata*	Portunidae	Muscle	CBSV002	247.5 ng/µl
3	Red Ghost Crab	*Ocypode macrocera*	Ocypodidae	Muscle	CBSV003	77.8 ng/µl
4	Three Spot Swimming Crab	*Portunus sanguinolentus*	Portunidae	Muscle	CBSV004	18.1 ng/µl
5	Mangrove Horseshoe Crab	*Carcinoscorpius rotundicauda*	Limulidae	Muscle	CBSV005	90.3 ng/µl
6	Common Hermit Crab	*Pagurus bernhardus*	Paguridae	Muscle	CBSV006	216.7 ng/µl
7	Orange Mud Crab	*Scylla olivacea*	Portunidae	Muscle	CBSV007	247.6 ng/µl

Sl	Common Name	Scientific Name	Family	Body Part	Voucher	Genomic DNA extraction
Others						
1	Mudskipper (Sam-1)	*Periophthalmus koelreuteri*	Gobiidae	Skin	AFSV001	48.9 ng/µl
2	Mudskipper (Sam-2)	*Periophthalmus* sp.	Gobiidae	Skin	AFSV002	78.5 ng/µl

Table 3. Submission of the barcode genes to the NCBI's Gene Bank

Sl	Species name	Common Name	IUCN Status (BD)	GPS coordinates	Voucher No.	Barcode Gene Name	GenBank Accession No.
Mammals							
1	*Aonyx cinereus*	Small-clawed Otter	EN	24°18'57.1"N 91°43'24.5"E	MBSV049	*Cytb	OR261025
2	*Axis axis*	Spotted Deer	LC	22°25'41.6"N 89°35'25.0"E	MBSV002	Cytb	ON249125
3	*Bos gaurus*	Gaur	CR	21°40'06.1"N 92°05'15.3"E	MBSV031	Cytb	OP748226
4	*Canis aureus*	Golden Jackal	LC	25°20'34.8"N 88°57'36.0"E	MBSV046	Cytb	OR224343
5	*Elephas maximus*	Asian Elephant	CR	24°10'10.6"N 90°23'45.7"E	MBSV011	Cytb	ON249124
6	*Eonycteris spelaea*	Lesser Dawn Bat	DD	21°05'31.7"N 92°10'10.5"E	MBSV029	Cytb	OQ552534
7	*Felis chaus*	Jungle Cat	NT	22°49'44.8"N 89°32'02.7"E	MBSV012	Cytb	ON383141
8	*Hoolock hoolock*	Hoolock Gibbon	CR	24°18'54.4"N 91°43'23.3"E	MBSV026	Cytb	OR224342
9	*Hystrix brachyura*	Himalayan Crestless Porcupine	NE	24°10'11.2"N 90°23'52.6"E	MBSV044	Cytb	OQ535716
10	*Macaca arctoides*	Stump-tailed Macaque	DD	24°10'11.8"N 90°23'50.1"E	MBSV009	Cytb	OP902169
11	*Macaca assamensis*	Assamese Macaque	EN	21°40'06.6"N 92°04'50.2"E	MBSV030	Cytb	OR253997
12	*Macaca mulatta*	Rhesus Macaque	VU	24°19'03.9"N 91°46'52.8"E	MBSV020	Cytb	OQ535715
13	*Manis pentadactyla*	Chinese Pangolin	CR	24°10'14.9"N 90°23'49.0"E	MBSV033	Cytb	OP748355
14	*Muntiacus muntjak*	Barking Deer	EN	24°18'54.8"N 91°43'23.7"E	MBSV025	Cytb	OR228586
15	*Mus musculus*	House Mouse	LC	23°55'05.2"N 90°15'12.6"E	MBSV039	Cytb	OR204708
16	*Nycticebus bengalensis*	Bengal Slow Loris	EN	24°18'54.7"N 91°43'23.9"E	MBSV023	Cytb	OP902170
17	*Panthera tigris tigris*	Bengal Tiger	CR	22°15'01.4"N 89°14'31.0"E	MBSV001	Cytb	ON383140
18	*Panthera tigris tigris*	Bengal Tiger	CR	22°15'01.4"N 89°14'31.0"E	MBSV001	*ND5	OR397125
19	*Panthera tigris tigris*	Bengal Tiger	CR	22°15'01.4"N 89°14'31.0"E	MBSV001	Control Region	OR397126
20	*Paradoxurus*	Common Palm	LC	24°18'54.3"N	MBSV022	Cytb	OP132371

Sl	Species name	Common Name	IUCN Status (BD)	GPS coordinates	Voucher No.	Barcode Gene Name	GenBank Accession No.
	hermaphroditus	Civet		91°43'23.0"E			
21	*Pardofelis marmorata*	Marbled Cat	DD	24°18'54.6"N 91°43'23.8"E	MBSV027	Cytb	OQ514057
22	*Platanista gangetica*	Ganges River Dolphin (Sam 1, Halda)	VU	22°26'52.6"N 91°51'47.6"E	MBSV008	Cytb	OR731269
23	*Platanista gangetica*	Ganges River Dolphin (Sam 2, Sundarban)	VU	22°26'18.0"N 89°35'12.9"E	MBSV045	Cytb	OR290094
24	*Platanista gangetica*	Ganges River Dolphin	VU	22°26'52.6"N 91°51'47.6"E	MBSV008	*COI	OP131728
25	*Prionailurus viverrinus*	Fishing cat	EN	24°18'54.5"N 91°43'23.4"E	MBSV016	Cytb	ON383144
26	*Prionailurus bengalensis*	Leopard Cat	NT	24°18'55.2"N 91°43'24.0"E	MBSV024	Cytb	OP132370
27	*Pteropus giganteus*	Indian Flying Fox	LC	25°19'12.8"N 88°55'35.4"E	MBSV037	Cytb	OQ507392
28	*Rattus tanezumi*	Oriental House Rat	NE	25°19'12.9"N 88°55'35.3"E	MBSV043	Cytb	OQ507393
29	*Rusa unicolor*	Sambar Deer	CR	24°10'26.7"N 90°24'05.1"E	MBSV003	Cytb	OP748354
30	*Semnopithecus entellus*	Common Langur	EN	22°49'45.3"N 89°32'03.0"E	MBSV032	Cytb	OP748356
31	*Sus scrofa*	Common Wild Pig	LC	23°07'16.4"N 92°00'18.8"E	MBSV040	Cytb	OQ552533
32	*Trachypithecus phayrei*	Phayre's Monkey	CR	24°18'54.5"N 91°43'23.4"E	MBSV013	Cytb	ON383142
33	*Trachypithecus pileatus*	Capped Langur	EN	24°18'54.5"N 91°43'23.4"E	MBSV014	Cytb	ON383143
34	*Urva edwardsii*	Common Mongoose	LC	25°20'34.8"N 88°57'36.0"E	MBSV047	Cytb	OR224344
35	*Ursus thibetanus*	Asiatic Black Bear	CR	24°10'25.8"N 90°24'07.5"E	MBSV006	Cytb	OP132372
36	*Viverricula indica*	Small Indian Civet	NT	24°18'55.8"N 91°43'24.3"E	MBSV017	Cytb	ON383145
37	*Viverra zibetha*	Large Indian Civet	NT	23°51'57.1"N 90°17'06.6"E	MBSV018	Cytb	ON398720
				Birds			
1	*Amaurornis phoenicurus*	White-breasted Waterhen	LC	22°49'44.8"N 89°32'02.7"E	BBSV0004	COI	OQ028262
2	*Anas poecilorhyncha*	Indian Spot-billed Duck	LC	25°07'46.0"N 91°05'46.0"E	BBSV0014	COI	OP108135
3	*Ardea cinerea*	Grey Heron	LC	22°49'44.8"N 89°32'02.7"E	BBSV0010	COI	OR344385
4	*Buceros bicornis*	Great Hornbill	VU	24°10'20.8"N 90°23'44.2"E	BBSV0007	COI	OP108350
5	*Dendrocygna javanica*	Lesser Whistling Duck	LC	23°52'29.7"N 90°16'03.8"E	BBSV0009	COI	OR344424
6	*Eudynamys scolopaceus*	Asian Koel	LC	22°49'44.8"N 89°32'02.7"E	BBSV0015	COI	ON383968
7	*Gallicrex cinerea*	Watercock	LC	22°49'44.8"N	BBSV0011	COI	ON384046

Sl	Species name	Common Name	IUCN Status (BD)	GPS coordinates	Voucher No.	Barcode Gene Name	GenBank Accession No.
				89°32'02.7"E			
8	*Gracula religiosa*	Hill Myna	LC	22°49'44.8"N 89°32'02.7"E	BBSV0003	COI	ON325388
9	*Haliastur indus*	Brahminy Kite	LC	24°18'54.6"N 91°43'23.5"E	BBSV0012	COI	OP107363
10	*Haliaeetus ichthyaetus*	Grey-headed Fish-eagle	NT	21°40'23.7"N 92°05'11.1"E	BBSV0034	COI	OR344518
11	*Hydrophasianus chirurgus*	Pheasant-tailed Jacana	LC	25°07'46.0"N 91°05'47.0"E	BBSV0013	COI	ON384741
12	*Leptoptilos javanicus*	Lesser Adjutant	VU	24°10'14.9"N 90°23'43.6"E	BBSV0008	COI	ON359972
13	*Lophura leucomelanos*	Kalij Pheasant	VU	24°18'55.3"N 91°43'23.5"E	BBSV0032	COI	OR344507
14	*Microcarbo niger*	Little Cormorant	LC	22°49'44.8"N 89°32'02.7"E	BBSV0006	COI	OP108349
15	*Psittacula alexandri*	Red-breasted parakeet	LC	24°18'55.3"N 91°43'24.1"E	BBSV0028	COI	OP108346
16	*Psittacula krameri*	Rose-ringed Parakeet	LC	24°18'55.3"N 91°43'24.1"E	BBSV0029	COI	OP108348
17	*Spilopelia chinensis*	Eastern Spotted Dove	LC	22°49'44.8"N 89°32'02.7"E	BBSV0001	COI	ON326411
18	*Streptopelia decaocto*	Eurasian Collared-dove	LC	22°49'44.8"N 89°32'02.7"E	BBSV0002	COI	ON325305
Reptiles							
1	*Amphiesma stolatum*	Striped Keelback	LC	23°53'25.4"N 90°15'57.5"E	RBSV035	Cytb	OR334587
2	*Batagur baska*	River Terrapin	CR	22°25'40.3"N 89°35'20.2"E	RBSV006	Cytb	OR237804
3	*Boiga cyanea*	Green Cat Snake	LC	21°39'04.2"N 92°19'18.5"E	RBSV058	COI	OR398708
4	*Bungarus fasciatus*	Banded Krait	LC	24°18'54.3"N 91°43'22.8"E	RBSV012	COI	OP108274
5	*Cerberus rynchops*	Dog-faced Water Snake (New Submission)	LC	21°50'42.6"N 89°47'44.7"E	RBSV057	COI	OR437970
6	*Crocodylus porosus*	Saltwater Crocodile	EN	22°25'41.6"N 89°35'25.3"E	RBSV002	Cytb	ON398722
7	*Crocodylus palustris*	Marsh Crocodile	RE	24°10'15.6"N 90°23'41.0"E	RBSV003	COI	OP726382
8	*Daboia russelii*	Russell's Viper	NT	24°00'01.4"N 89°13'44.3"E	RBSV037	Cytb	OR334589
9	*Enhydris enhydris*	Smooth-scaled Water Snake	LC	23°53'18.0"N 90°16'04.0"E	RBSV036	Cytb	OR334588
10	*Eryx conicus*	Common Sand Boa	DD	23°49'10.2"N 89°40'51.2"E	RBSV052	Cytb	OR334590
11	*Fowlea piscator*	Checkered Keelback	LC	23°05'59.0"N 91°59'30.9"E	RBSV023	Cytb	OQ082557
12	*Gekko gecko*	Tokay Gekko	LC	22°30'34.5"N 92°12'30.0"E	RBSV001	Cytb	OP748353
13	*Geoclemys*	Black Spotted Pond	EN	21°40'05.2"N	RBSV022	COI	OP741017

Sl	Species name	Common Name	IUCN Status (BD)	GPS coordinates	Voucher No.	Barcode Gene Name	GenBank Accession No.
	hamiltonii	Turtle		92°04'48.0"E			
14	*Indotestudo elongata*	Elongated Tortoise	CR	21°40'05.2"N 92°04'48.0"E	RBSV013	COI	OP740994
15	*Indotyphlops porrectus*	Slender Worm Snake (New Submission)	NE	25°41'55.3"N 88°39'22.3"E	RBSV054	Cytb	OR803758
16	*Lissemys punctata*	Indian flap-shelled Turtle	LC	21°40'05.2"N 92°04'48.0"E	RBSV016	COI	OP741016
17	*Lycodon aulicus*	Indian Wolf Snake	LC	23°52'32.4"N 90°16'15.4"E	RBSV038	COI	OR398707
18	*Manouria emys*	Asian Giant Tortoise	CR	21°40'05.2"N 92°04'48.0"E	RBSV014	COI	OP741012
19	*Pangshura tecta*	Indian Roofed Turtle	LC	23°52'30.2"N 90°16'06.5"E	RBSV011	COI	OP108400
20	*Psammodynastes pulverulentus*	Common Mock Viper	LC	21°05'34.6"N 92°10'07.2"E	RBSV034	Cytb	OQ535718
21	*Python molurus*	Indian Rock Python	DD	24°10'18.2"N 90°23'35.4"E	RBSV004	Cytb	ON398721
22	*Trimeresurus erythrurus*	Red tail Pit Viper	LC	24°19'34.3"N 91°47'00.1"E	RBSV010	Cytb	OQ535717
			Amphibians				
1	*Duttaphrynus melanostictus*	Asian Common Toad	LC	23°52'51.1"N 90°16'04.8"E	ABSV006	*16s rRNA	OP108265
2	*Euphlyctis kalasgramensis*	Kalasgram Skittering	NE	24°15'57.8"N 91°55'22.2"E	ABSV004	16s rRNA	OP037897
3	*Fejervarya orissaensis*	Orissa Frog	NE	22°40'52.3"N 91°38'56.0"E	ABSV032	16s rRNA	OQ473651
4	*Hoplobatrachus tigerinus*	Indian Bullfrog	LC	23°52'04.8"N 90°17'06.1"E	ABSV002	16s rRNA	OP031637
5	*Hylarana tytleri*	Yellow-striped Frog	LC	23°52'33.6"N 90°16'05.0"E	ABSV003	16s rRNA	OP037851
6	*Microhyla berdmorei*	Berdmore's Narrow-mouthed Frog	LC	21°05'33.0"N 92°10'09.7"E	ABSV019	16s rRNA	OP741111
7	*Microhyla mymensinghensis*	Mymensingh Narrow-mouthed Frog	LC	24°15'57.8"N 91°55'22.3"E	ABSV001	16s rRNA	OR287193
8	*Minervarya asmati*	Bangladeshi Cricket Frog	NE	22°40'52.3"N 91°38'56.2"E	ABSV033	16s rRNA	OQ473653
9	*Minervarya pierrei*	Pierre's Cricket Frog	LC	23°52'50.7"N 90°15'54.7"E	ABSV034	16s rRNA	OR287196
10	*Polypedates leucomystax*	Common Tree Frog	LC	23°52'05.7"N 90°17'06.0"E	ABSV005	16s rRNA	OP038265
11	*Polypedates teraiensis*	Terai Tree Frog	NE	22°44'49.4"N 91°37'18.4"E	ABSV025	16s rRNA	OP741139
12	*Sylvirana lacrima*	Crying Stream Frog	NE	22°29'51.8"N 92°11'07.5"E	ABSV031	16s rRNA	OQ473385
13	*Uperodon globulosus*	Balloon Frog	VU	23°53'04.8"N 90°16'02.3"E	ABSV009	16s rRNA	OR287338
			Butterfly				

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1	*Acytolepis puspa*	Common Hedge Blue	VU	24°15'59.9"N 91°55'11.8"E	BFBSV091	COI	ON999056
2	*Athyma ranga*	Blackvein Sergeant	VU	24°19'40.1"N 91°47'03.1"E	BFBSV179	COI	
3	*Athyma nefte inara*	Colour Sergeant	VU	24°19'41.0"N 91°46'60.0"E	BFBSV181	COI	OQ469749
4	*Athyma selenophora*	Staff Sergeant	DD	24°19'40.1"N 91°46'59.9"E	BFBSV185	COI	OQ457608
5	*Anthene emolus*	Common Ciliate Blue	VU	24°07'32.3"N 91°26'46.3"E	BFBSV102	COI	ON999059
6	*Catochrysops panormus*	Silver Forget me Not	NE	24°07'33.0"N 91°26'45.4"E	BFBSV316	COI	OP703522
7	*Catapaecilma major*	Common Tinsel (New Submission)	EN	24°15'59.9"N 91°55'11.8"E	BFBSV129	COI	OQ457610
8	*Celaenorrhinus leucocera*	Common Spotted Flat	NE	22°29'23.5"N 92°10'41.3"E	BFBSV314	COI	OP703516
9	*Chersonesia risa*	Common Maplet	EN	22°29'51.9"N 92°11'07.5"E	BFBSV171	COI	OR221181
10	*Chliaria othona*	Orchid Tit	VU	24°19'40.1"N 91°46'59.9"E	BFBSV151	COI	ON385134
11	*Curetis acuta*	Angled Sunbeam	NE	22°29'51.9"N 92°11'07.5"E	BFBSV325	COI	OR221182
12	*Discolampa ethion*	Banded Blue Pierrot	VU	22°29'56.2"N 92°11'03.6"E	BFBSV146	COI	OR221195
13	*Dophla evelina*	Redspot Duke	EN	22°29'25.1"N 92°10'43.3"E	BFBSV192	COI	OQ473049
14	*Elymnias nesaea*	Tiger Palmfly	EN	24°15'57.2"N 91°54'48.6"E	BFBSV288	COI	ON385093
15	*Euploea klugii*	Brown King Crow	VU	24°19'40.1"N 91°47'04.9"E	BFBSV009	COI	ON385152
16	*Gandaca harina*	Tree Yellow	EN	24°15'57.8"N 91°55'22.2"E	BFBSV257	COI	ON999188
17	*Gerosis bhagava*	Common Yellow-breasted Flat	VU	22°30'14.8"N 92°09'09.7"E	BFBSV028	COI	OR224320
18	*Heliophorus epicles*	Purple Sapphire	VU	24°15'59.9"N 91°55'11.8"E	BFBSV077	COI	OP703531
19	*Kallima inachus*	Orange Oakleaf	EN	24°19'45.8"N 91°47'06.0"E	BFBSV223	COI	ON999066
20	*Lamproptera curius*	White Dragontail	EN	22°29'22.5"N 92°10'42.7"E	BFBSV233	COI	OR223011
21	*Lexias dirtea*	Dark Archduke	EN	22°29'51.9"N 92°11'07.5"E	BFBSV199	COI	OQ457614
22	*Loxura atymnus*	Yamfly	VU	22°29'17.6"N	BFBSV143	COI	OR224332

Sl	Species name	Common Name	IUCN Status (BD)	GPS coordinates	Voucher No.	Barcode Gene Name	GenBank Accession No.
				92°10'45.2"E			
23	*Neptis harita*	Dingiest Sailer	EN	24°19'40.1"N 91°47'03.1"E	BFBSV202	COI	ON385139
24	*Pantoporia sandaka*	Extra Lascar	NE	24°07'36.4"N 91°26'51.3"E	BFBSV317	COI	OQ073572
25	*Papilio helenus*	Red Helen	VU	24°19'40.1"N 91°47'01.0"E	BFBSV240	COI	ON385138
26	*Remelana jangala*	Chocolate Royal	VU	24°19'44.3"N 91°47'05.8"E	BFBSV145	COI	ON999057
27	*Cigaritis lohita*	Long-banded Silverline	VU	24°15'57.2"N 91°54'48.6"E	BFBSV085	COI	ON999134
28	*Symbrenthia lilaea*	Common Jester	EN	24°15'59.9"N 91°55'11.8"E	BFBSV217	COI	ON385100
29	*Ticherra acte*	Blue Imperial	NE	24°19'40.1"N 91°47'04.9"E	BFBSV311	COI	OQ518947
Crustaceans							
1	*Carcinoscorpius rotundicauda*	Mangrove Horseshoe Crab	VU	21°45'28.2"N 89°33'48.8"E	CBSV005	COI	OP107362
2	*Tubuca rosea*	Rosy Fiddler Crab	DD	22°25'41.8"N 89°35'25.8"E	CBSV001	COI	OP131731
3	*Scylla serrata*	Mangrove Crab	LC	22°25'41.6"N 89°35'29.0"E	CBSV002	COI	OR437977

* Cytb= Cytochrome b, *COI= Cytochrome oxidase subunit 1, *16S rRNA=Large subunit ribosomal RNA, *ND5= NADH dehydrogenase subunit 5

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Appendix I

Appx. I. Field activities during collection of samples

	
Karamjal, Sundarbans	WRRC, Khulna
	
Inani RF	Kaptai NP
	
Lawachara NP	Kudum Cave, Ukhiya

	
Baroiyadhala NP	Khagrachari Forest

Appendix II

Appx. II. List of primers for PCR

SEQUENCE NAME	SEQUENCE (5' to 3')	SCALE	LENGTH
TIF	ATAACCCCTCAGGAATGGTG	25 nmole	20bp
TIR	TGGCGGGGATGTAGTTATCA	25 nmole	20bp
L14841	AAAAAGCTTCCATCCAACATCTCAGCATG ATGAAA	25 nmole	35bp
L14724	CGAAGCTTGATATGAAAAACCATCGTTG	25 nmole	28bp
H15149	AAACTGCAGCCCCTCAGAATGATATTTGT CCTCA	25 nmole	34bp
LCO1490	GGTCAACAAATCATAAAGATATTGG	50 nmole	25bp
HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	50 nmole	26bp
Fishf1	TCAACCAACCACAAAGACATTGGCAC	50 nmole	26bp
Fishr1	TAGACTTCTGGGTGGCCAAAGAATCA	50 nmole	26bp
BirdF1	TTCTCCAACCACAAAGACATTGGCAC	25 nmole	26bp
BirdR1	ACGTGGGAGATAATTCCAAATCC	25 nmole	23bp
LepF1	ATTCAACCAATCATAAAGATATTGG	25 nmole	20bp
LepRI	TAAACTTCTGGATGTCCAAAAAATCA	25 nmole	20bp
BirdR2	ACTACATGTGAGATGATTCCGAATCCAG	25 nmole	35bp
BirdR3	AGGAGTTTGCTAGTACGATGCC	25 nmole	28bp
MamF1	TCAGCCATTTTACCTATGTTCAT	25 nmole	34bp
MamR1	ACTTCAGGGTGTCCGAAGAATCA	25 nmole	25bp
mcb398	TACCATGAGGACAAATATCATTCTG	25 nmole	26bp
mcb869	CCTCCTAGTTTGTTAGGGATTGATCG	25 nmole	26bp
16Sar-L	CGCCTGTTTATCAAAAACAT	25 nmole	20bp
16Sbr-H	CCGGTCTGAACTCAGATCACGT	25 nmole	22bp
Cox-I-F	TCAGCCATACTACCTGTGTTCA	25 nmole	22bp
Cox-I-R	TAGACTTCTGGGTGGCCAAAGAATCA	25 nmole	26bp
H16064-F	CTTTGGTTTACAAGAACAATGCTTTA	25 nmole	26bp
Gludg-R	TGACTTGAARAACCAYCGTTG	25 nmole	21bp
ND4-F	TGACTACCAAAAGCTCATGTAGAAGC	25 nmole	26bp
ND4-R	TACTTTTACTTCGATTTGCACCA	25 nmole	23bp
NADH5-F	TTACTAGGACTCCTCCTAGCC	25 nmole	21bp
NADH5-R	GAATAGGGTTGTGATGGCCCC	25 nmole	21bp
loop_F (Control R)	TAGCCCCACCATCAGCACCCAAAGC	25 nmole	25bp
loop_R (Control R)	AATGGGCCCGGAGCGAGAAGAGGTA	25 nmole	25bp
L14910	GACCTGTGATMTGAAAACCAYCGTTGT	25 nmole	27bp
H16064	CTTTGGCTTACAAGAACAATGCTTTA	25 nmole	26bp

Appendix III

Appx. III. A total of 122 mitochondrial genes from 118 animals were submitted to Genbank

Sl	Groups	COI	Cytb	16s rRNA	ND5	Control region	Total
1	**Mammals**	01	34	0	01	01	**37**
2	**Birds**	18	0	0	0	0	**18**
3	**Reptiles**	10	12	0	0	0	**22**
4	**Amphibians**	00	0	13	0	0	**13**
5	**Butterflies**	29	0	0	0	0	**29**
6	**Crustaceans**	03	0	0	0	0	**03**
	Total	**61**	**46**	**13**	**01**	**01**	**122**

Wildlife Diversity and Abundance in Relation to Habitat Type and Anthropogenic Activities in Baraiyadhala National Park

by

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CHAPTER I: INTRODUCTION

Bangladesh, located in South Asia, covers a total area of 147,570 square kilometers and is predominantly situated within the delta of the Ganges-Brahmaputra-Meghna River system, which is one of the largest river systems in the world. The northeastern and southeastern parts of Bangladesh are characterized by hilly terrain. Approximately 80% of the country consists of floodplains, 12% comprises hills, and 8% encompasses terraces or uplifted blocks (BBS 2011, http://www.bbs.gov.bd/Home.aspx). Additionally, Bangladesh has 106,613 square kilometers of marine area. Within the country, approximately 8-10% of the land area retains a good canopy cover, supporting around 5,000 species of angiosperm plants.

Due to the location of the country in the Indo-Malayan Realm it has become a habitat for diverse wildlife. Biogeographically, Bangladesh sits at the convergence of the Indian, Himalayan, and Indochinese sub-regions of the Oriental Realm or Regions (Khan 2015). This abundance of biodiversity is further amplified by the presence of various habitats and ecosystems, which have made the country favorable for diverse species.

The updated Red List of Bangladesh has evaluated 49 species of amphibians, 167 species of reptiles, 566 species of birds, 138 species of mammals, and 253 species of freshwater fishes within the vertebrate category. Additionally, assessments have been made for 253 species of freshwater fishes within the vertebrate category and 141 species of crustaceans and 305 species of butterflies within the invertebrate group (IUCN Bangladesh 2015).

Despite significant wildlife and habitat losses, Bangladesh continues to harbor relatively healthy populations of numerous wildlife species that hold global and national significance. Each of these species plays a crucial role in maintaining the delicate ecological balance within their respective ecosystems. According to the IUCN Red List, Bangladesh supports around 30 mammal species, 35 bird species, 27 reptile species, and 2 amphibian species that are globally threatened (IUCN 2015). This list includes flagship species, megafauna, and other important wildlife species of Bangladesh.

The south-east part of Bangladesh (Chattagaram and Chattagram Hill Tracts) includes Protected Areas (PAs), which are of special importance for conserving many rare and threatened wildlife species of the country. Baraiyadhala National Park is one of the PAs located in Mirsharai Upazila and has a diverse habitat type. Waterfalls, hill streams, creeks, steep hills, natural forests, bushlands, bamboo thickest made a unique composite habitat for many wildlife species. Species diversity and population density of wild animals in a particular area depends on its habitat type and vegetation structure. Systematic research on wildlife diversity in Protected Areas is scarce. It is very important to know the species diversity and their habitat preference, vegetation composition, potential threats to the habitat for the proper management of a protected area.

Due to the diverse landscapes of Baraiyadhala National Park, it is becoming a place of tourist attraction (Alam et al. 2009). Moreover, there are some ethnic community villages situated in and around Baraiyadhala National Park. The ethnic communities depend on bushmeat for their primary protein source and hunt a diverse group of animals. Hunting of rare and threatened animals, accelerating the threat of extinction.

The project entitled “Wildlife Diversity and Abundance in Relation to Habitat Type and Anthropogenic Activities in Baraiyadhala National Park” was supported by the innovative grant of the mother project ‘Sustainable Forests & Livelihoods (SUFAL)’. SUFAL project was implemented by the Forest Department of Bangladesh, Ministry of Environment, Forest and Climate Change, and was financially supported by The World Bank. The present research systematically quantified wildlife diversity and abundance in relation to habitat type and anthropogenic disturbances, threat assessment and threat mitigation measures in Baraiyadhala National Park. The findings of the research also came up with valuable policy implications highlighting wildlife diversity and abundance in heterogeneous habitats, conservation needs and ways to mitigate anthropogenic pressures on wildlife habitats. Determination of tourist pressure and measures to manage sustainable ecotourism will reduce the detrimental effect on wildlife habitats.

Objectives of the study

The specific objectives of the research were:

i) to quantify wildlife diversity and abundance in relation to habitat type and anthropogenic activities in Baraiyadhala National Park and establishing a database and monitoring system;

ii) to find out the threats to wildlife and their habitats in Baraiyadhala National Park;

iii) to come up with recommendation for the improvement of habitat quality, habitat connectivity and threat mitigation.

CHAPTER II: METHODOLOGY

Study Area

Baraiyadhala National Park lies between 22040.489´-22048´N latitude and 90040´-91055.979´E longitude and located in Sitakund and Mirsharai Upazilas of Chattogram district. The Park is located at 172 km southeast of Dhaka (the capital of Bangladesh) and 45 km northwest of Chattogram City. It is situated about 1.5 km west of the Dhaka-Chattogram Highway and 0.5 km west of Dhaka-Chattogram Railway Line (Map 1). The forest is under the jurisdiction of Baraiyadhala Forest Range of Chattogram North Forest Division. The park encompasses 2933.61 ha of land area under the Baraiyadhala Forest Range Office. The Park comprises three blocks: Baraiyadhala and Wahidpur Blocks under Baraiyadhala Forest Beat, and Kunderhat Block under Bartakia Forest Beat.

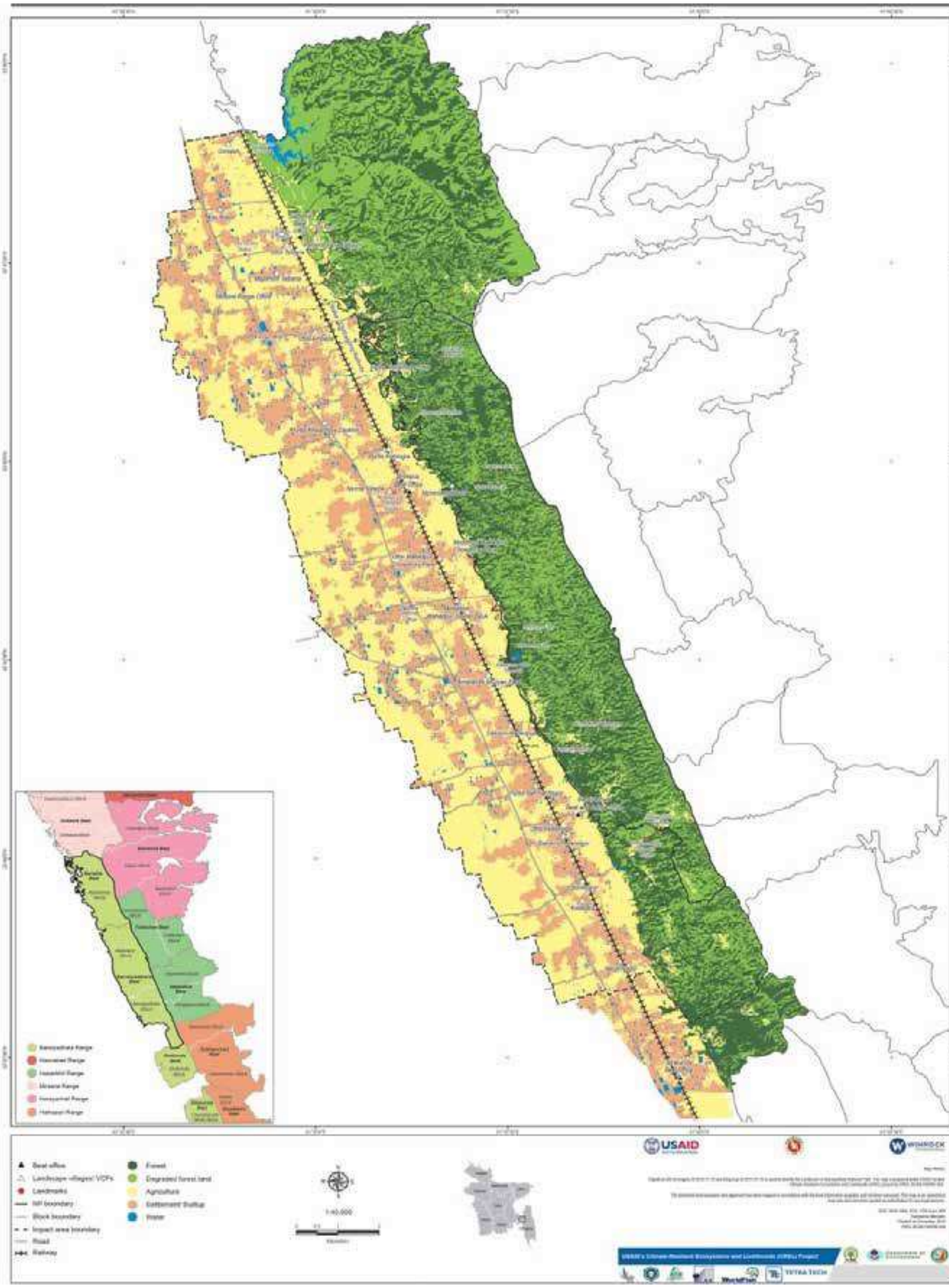
The topography of the National Park is very undulating and covers with a linear hill range (elongation north to south reaching an altitude up to 350 m). Hilly topography constitutes high (152 to 365 m), medium high (76 to 152 m) and lower hills (15 to 76 m). The landscape of the forest is characterized by hills, valleys, gullies and numerous streams and covered mainly by secondary degraded forests and plantations. Soils are yellowish brown to strong brown (high hills), strong brown to yellowish red (low hills) (Hassan 1994), and brown coloured foot hill (piedmont) soils are usual, slightly acid or neutral, with high base status (Champion et al. 1965).

Most of the streams are seasonal and dry up during off-monsoon season. There are many waterfalls in the National Park area of which Khoiyachara, Napityachara, Rupashi, Sohoshradhara, Sonaichari and Ujalia waterfalls are notable.

Forest habitat of Baraiyadhala National Park.

Methods

A combination of different well-established methods was used for the project. These systematic biodiversity survey methods were used for evaluating biodiversity of different protected areas of Bangladesh since 2010 (Feeroz et al. 2011, 2012, Feeroz 2013, 2014, Hasan et al. 2014, Khan 2018). The field surveys were conducted in different habitats of Baraiyadhala National Park between 06 October 2021 and 03 June 2023. A total of 78 days comprised of 389 man-days were spent in the field for data collection. A team of three experts and two / three field assistants were involved in data collection. Four local people were also involved in field data collection.



Source: Climate- Resilient Ecosystems and Livelihood, CREL Project

Map 1. Map of Baraiyadhala National Park.

Field activities during rainy season at Baraiyadhala National Park.

Wildlife Diversity and Abundance

Line Transect Sampling

Both temporary and permanent transect lines were set randomly covering all types of habitat. A total of 14 permanent line transects were set up for data collection (Map 3.2, Table 3.3). Four transect lines were set through the hill forest habitat (TL1, TL6, TL7, TL9), three transects through hill forests and streams (TL 2- TL5, TL8). Two transects were set in hill streams (TL 10 and TL11) and three through degraded hills (TL12 – TL14). Visual encounter survey was conducted on foot both in day and night. All the wild animals found were recorded from the both sides of transect. GPS coordination were used to calculate the total transect area covered in the survey. Existing trails or water creeks in the forest were used as a transect line.

Walking through transect line at Baraiyadhala National Park.

Quadrat Sampling

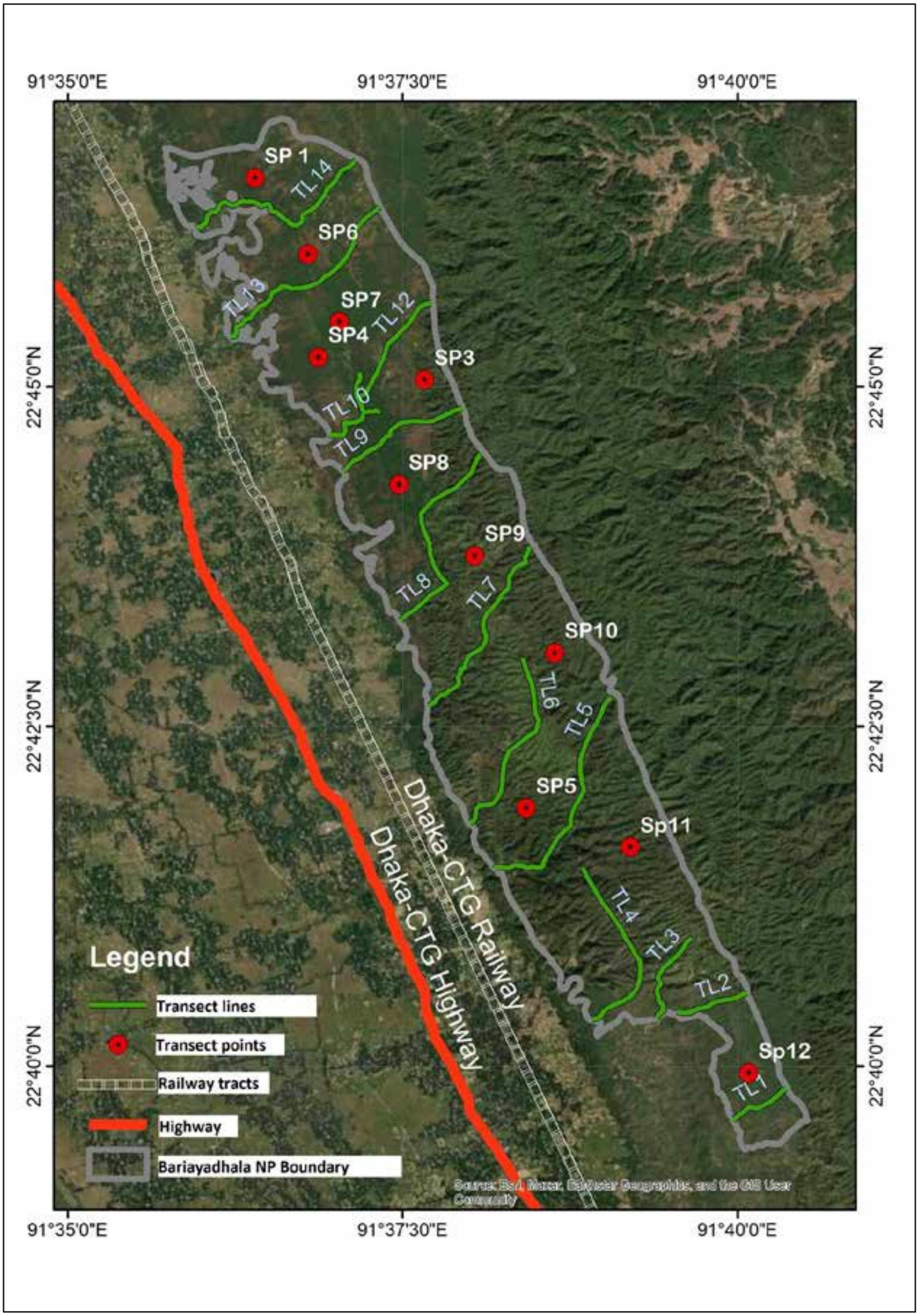
Randomly selected quadrates with fixed (10m × 10m) or variable size were taken in different habitats. Small sized wild animals like amphibians and reptiles were quantified using quadrat sampling. Animals found in each quadrat were recorded in the data sheet for further analysis.

Point Sampling

Permanent survey points were selected where line transect was not possible to use like in steep hills, hill tops and other confined areas. A total of 12 survey points were selected and regular survey was conducted within 100 meter-radius of the point (Map 2).

Camera Trapping

Camera trapping were carried at Baraiyadhala National Park for 15 months from January 2022 and March 2023. Camera traps were set up at 48 stations to cover the whole area of the national park (Map 3). Camera trap locations were un-baited and selected based on accessibility, terrain features, animal trails and animal signs (Ramesh et al. 2015, Marinho et al. 2018). A total of 2793 camera trap nights were spent for the survey.



Map 2. Transect lines and survey points in Baraiyadhala National Park.

The relative abundance index (RAI) was calculated for all the camera trapped animals based on the following formula (Jenks et al. 2011).

$$\mathrm{RAI} = \frac{\mathrm{A}}{\mathrm{N}} \times 100$$

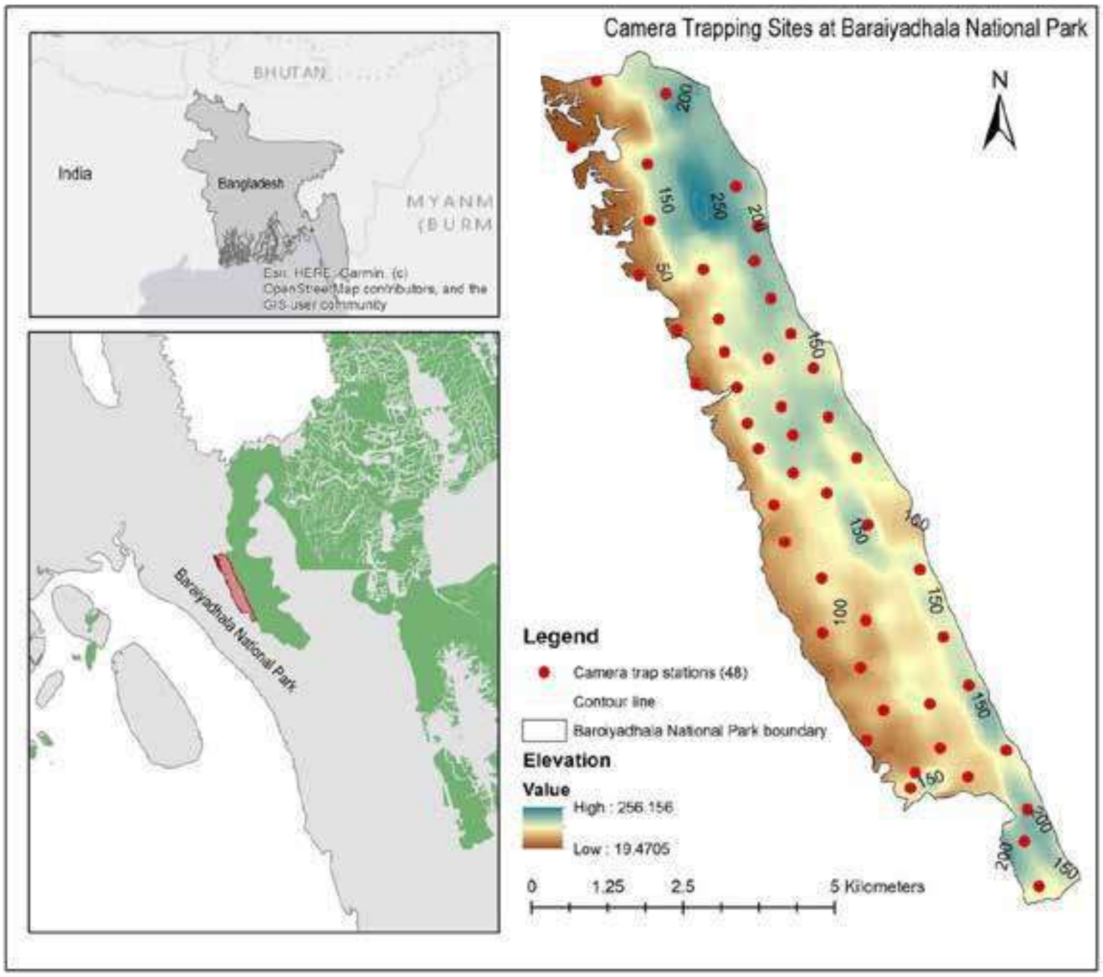
Where, ‘A’ represents the total number of captures of a species by all cameras, and ‘N’ equals to the camera trap days during the study period.

Density of camera trapped animals were estimated by Random Encounter Modeling (REM) (Rowcliffe et al., 2008).

$$\mathrm{D} = \frac{\mathrm{Y}}{\mathrm{H}} \cdot \frac{\pi}{\mathrm{v.r.}(2+\theta)}$$

Where, Y is the number of encounters (i.e. number of independent photographic sequences), H is the total camera survey effort, v is the average distance travelled by an individual during a day (day range), and r and θ are the radius and angle of the camera traps detection zone, respectively (Palencia et al. 2020).

Camera trapping activities in different habitats of Baraiyadhala National Park.



Map 3 Location of camera trap sites in Baraiyadhala National Park.

Box-trap Survey

Small metallic net box traps (14" x 6" x 6") were used to capture small mammals, particularly rodents which could not be covered sufficiently by line-transect sampling or camera trapping. A total of 10 box traps were set up in different habitats of Baraiyadhala National Park. Box traps were baited with potato, dry fish and biscuits. Box traps were placed in the evening and checked the capture in the early morning. Captured animals, if any were immediately released after recording necessary data.

Box-trap set up in different habitats to capture rodents.

Mist Netting

Mist nets are basically made of echo-protector nylon mesh. The mesh size of the mist net varies depending on the size of the targeted animal size. In this study, we used mist net for capturing bats as they are nocturnal and most elusive mammal, it was not possible to study these species only through direct observation.

Night Survey

Night survey was conducted with the aid of high-power torches and headlamps. Nocturnal and secretive animals which are likely to be missed in other surveys, can be sampled in this method. Probable habitats of nocturnal wild animals like amphibians, reptiles, nocturnal birds and mammals were thoroughly searched and data was recorded accordingly.

Activities during night survey at Baraiyadhala National Park.

Categorization of Wildlife Habitats

Wildlife habitats in Baraiyadhala National park has been categorized into five types on the basis of habitat conditions. These are, (i) Forest, (ii) Steep Hill (iii) Stream with water (iv) Stream without water and (v) Bushes and Bamboo thickets. Data were collected on the basis of habitat types and camera traps also set up in different habitats.

Anthropogenic Activities

Field Survey

Observations on anthropogenic activities were recorded during field surveys. Tourists' activities, activities by the local people, poacher activity and other sort of anthropogenic actions and their frequencies were recorded in the data sheet.

Quantification of anthropogenic activities

Anthropogenic activities were broadly categorized into two sub-heads; A. Tourist activities and B. Activities by the local people. These sub-categories were further divided into many segments in the predesigned questionnaire. Anthropogenic activities were also evident from the camera trap data. Quantification of anthropogenic activities was carried out from the field observation, camera trap data and questionnaire survey. A total of 600 questionnaire were surveyed among the tourists while 120 questionnaires were surveyed among the local people in and around the national park.

Questionnaire survey among the local people of Baraiyadhala National Park.

GIS mapping

GPS (Global Positioning System) coordinates were taken for every sighting of wild animals in the national park. GPS coordinates were also taken for all the camera trapping sites, transects, quadrates and survey points. These GPS coordinates were used to prepare distribution map of threatened wild animal species such as Red Serow, Southern Red Muntjac, Assamese Macaque, Small-clawed Otter and others.

Spatial autocorrelation is a statistical concept that examines the degree to which values of a variable tend to be similar or dissimilar across nearby spatial locations. In simpler terms, it explores whether nearby observations are more alike or different than those further away. This phenomenon is common in spatial data and can reveal underlying spatial patterns and relationships. Positive spatial autocorrelation occurs when similar values cluster together in space, suggesting a spatial pattern of similarity. Conversely, negative spatial autocorrelation arises when dissimilar values cluster together, indicating a pattern of dissimilarity. Understanding spatial autocorrelation is essential in various fields such as geography, urban planning, epidemiology, and ecology, as it provides insights into spatial relationships, spatial processes, and can inform decision-making processes related to spatially distributed phenomena.

The prediction maps of different species based on relative abundance index derived from the geostatistical interpolation including the variable of Semivariogram and spherical model type. The predictive efficacy of spatial interpolation methods exhibits variability across the relative abundance index of different species. To explore this variability, three distinct methods derived from spatial interpolation approaches such as Inverse Distance Weighting (IDW), Empirical Bayesian Kriging (EBK) and Ordinary Kriging (OK) and Simple Kriging (SK) were initially employed for relative abundance index. Subsequently, an evaluation was conducted, comparing the predictive capacities of these methods utilizing Root Mean Squared Standardized (RMSS) values. Finally, the highest predictive ability has been adopted from different interpolation methods based on RMSS. However, geostatistical interpolation methods predict more significant but prediction map shown flat while some map derived from deterministic method (IDW) which were visualized better.

Threat Identification and Management Guidelines

Threats to each of the nationally and globally threatened species were monitored through direct field observation, questionnaire survey and camera trapping. Data on each of the threatened species were recorded through field observations and photographs were taken through camera trapping which eventually helped to find out their relative abundance, habitat requirements and status of threats. Wildlife management guidelines for Baraiyadhala National Park were prepared on the basis of the findings of the project. The types and intensity of the particular threat to the threatened species were considered to prepare the guidelines.

CHAPTER III: RESULTS AND DISCUSSION

Status of Wildlife

The diverse habitats of Baraiyadhala National Park support a good number of wild animals. The checklists of mammals, birds, reptiles and amphibians were prepared based on the field observations and camera trapping data. A total of 54 species of mammals, 216 species of birds, 62 species of reptiles and 38 species of amphibians were recorded from Baraiyadhala National Park. This national park is the home of 14 globally threatened and 29 nationally threatened wildlife species, of which the population densities of some of the species were estimated. Species richness in particular habitat was also estimated. The species richness, population densities and relative abundance will act as the baseline for the long-term monitoring of Baraiyadhala National Park.

Many of the wildlife species recorded from Baraiyadhala National Park has only one or few previous records in Bangladesh. Some of the key wildlife species of this national park are Red Serow (*Capricornis rubidus*), Smooth-coated Otter (*Lutrogale perspicillata*), Small-clawed Otter (*Aonyx cinerea*), Chinese Pangolin (*Manis pentadactyla*), Marbled Cat (*Paradofelis marmorata*), Fishing Cat (*Prionailurus viverrinus*), Western Hoolock Gibbon (*Hoolock hoolock*), Phayre's Leaf Monkey (*Trachypithecus phayrei*), Bengal Slow Loris (*Nycticebus bengalensis*), Assamese Macaque (*Macaca assamensis*), Grey Peacock Pheasant (*Polyplectron bicalcaratum*), White-cheeked Partridge (*Arborophila atrogularis*), Elongated Tortoise (*Indotestudo elongata*), Cherrapunji Keelback (*Amphiesma xenura*), Water Monitor Lizard (*Varanus salvator*), Marbled Cascade Frog (*Amolophs marmoratus*), Green Cascade Frog (*Odorrana livida*), Crown Frog (*Megophrys parva*) and Reza Khan's Bush Frog (*Raorchestes rezakhani*).

Mammals

A total of 54 species of mammals under 22 mammalian families were recorded during the study period. Most of the mammals of Baraiyadhala National Park was rare (38.46%) and uncommon (17.31%) while 33.77% mammals were common (Figure 1). Family Muridae has the highest number of species (8 species) followed by Mustelidae and Cercopithecidae (5 species each). Among the recorded mammals, Southern Red Muntjac (*Muntiacus muntjak*), Hog Badger (*Arctonyx collaris*), Wild Boar (*Sus scrofa*), Assamese macaque (*Macaca assamensis*), Pig-tailed Macaque (*Macaca leonina*), Chinese pangolin (*Manis pentadactyla*), Small-clawed Otter (*Aonyx cinerea*) were also captured in the camera traps. The rest of the mammals were either directly observed or got animal sign in the field.

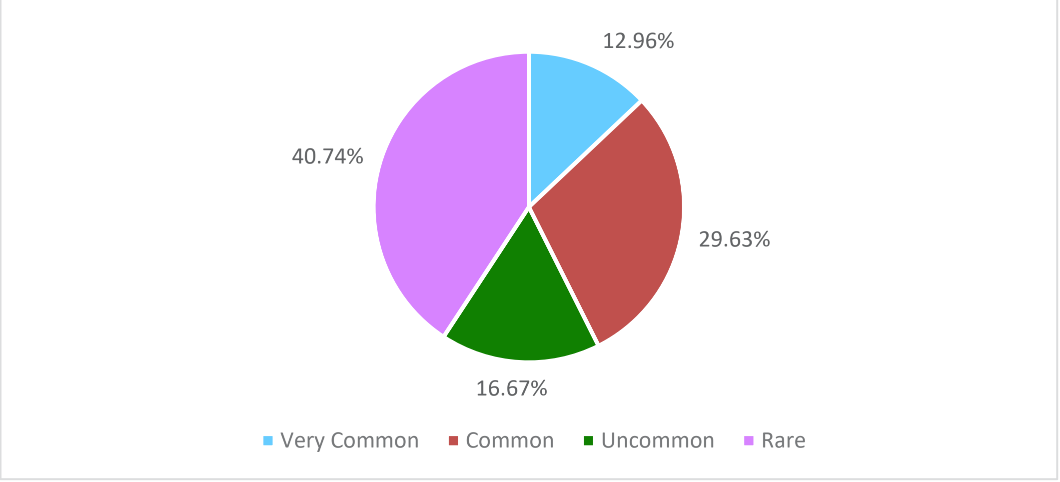


Figure 1. Status of mammals in Baraiyadhala National Park.

The relative abundance index (RAI) was the highest for Assamese Macaque (RAI =13.21), followed by Southern Red Muntjac (RAI =9.92), Wild Boar (RAI = 5.01), Northern Tree Shrew (RAI = 3.08) and Indian Crested Porcupine (RAI = 2.97) (Table 1). On the other hand, relative abundance of Chinese Pangolin (RAI = 0.07), Marbled Cat (RAI = 0.03), Small Indian Civet (RAI = 0.14), Fishing Cat (RAI = 0.14) and Yellow-throated Marten (RAI = 0.18) are low, indicating a very small population. Though the relative abundance of Capped Langur (RAI = 0.07) is low but as it is an arboreal species and our camera traps were set up focusing on the terrestrial species, this RAI value does not represent its real population in the national park.

		
Southern Red Muntjac	Burmese Red Serow	Wild Boar
		
Fishing Cat	Jungle Cat	Leopard Cat
		
Common Palm Civet	Large Indian Civet	Small Indian Civet

		
Hog Badger	Small-clawed Otter	Large-toothed Ferret Badger

Mammalian species captured in camera traps at Baraiyadhala National Park.

		
Assamese Macaque	Capped Langur	Rhesus Macaque
		
Pig-tailed Macaque	Indian Crested Porcupine	Crab-eating Mongoose
		
Asiatic Golden Jackal	Northern Tree Shrew	Long-tailed Climbing Mouse

		
Orange-bellied Himalayan Squirrel	Pallas's Squirrel	Hoary-bellied Squirrel

Mammalian species captured in camera traps at Baraiyadhala National Park.

A. Chinese Pangolin and B. Marbled Cat.

Distribution of some of the important mammals

Kernel Density map of the species was produced by using relative abundance index (RAI) data and respective GPS coordinates. This density map gives a clear idea about the distribution of a species in the national park. The distribution of some of the mammalian species are as follows.

Red Serow

Red Serow (*Capricornis rubidus*) is a nationally Endangered and globally Vulnerable species (IUCN Bangladesh 2015). There are very few reports of Red Serow from northeast and southeast regions of Bangladesh (Khan 2018, IUCN Bangladesh 2015). The photographic evidence of Red Serow in Baraiyadhala National Park confirms the occurrence of this species in Chattogram. Serows were mostly found in the steep hill areas of the national park. The population of Serow in this national park seemed very low (RAI = 0.90). Density (D) of Red Serow in Baraiyadhala National Park was 1.266 per square kilometer with the estimated population of 38 individuals. Habitat suitability index shows that only 9.42 sq km area of this national park is highly suitable for this species which is located at the southern portion of the national park (Map 4).

Table 1. Mammals recorded in the camera traps with their relative abundance index (RAI)

Sl. No.	Species Name	Scientific Name	RAI
1	Assamese Macaque	*Macaca assamensis*	13.21
2	Southern Red Muntjac	*Muntiacus muntjak*	9.92
3	Wild Boar	*Sus scrofa*	5.01
4	Northern Tree Shrew	*Tupaia belangeri*	3.08
5	Indian Crested Porcupine	*Hystrix indica*	2.97
6	Small-clawed Otter	*Aonyx cinerea*	2.79
7	Common Palm Civet	*Paradoxurus hermaphroditus*	2.65
8	Hog Badger	*Arctonyx collaris*	1.79
9	Pig-tail Macaque	*Macaca leonina*	1.58
10	Long-tailed Climbing Mouse	*Vandeleuria oleracea*	1.50
11	Jungle Cat	*Felis chaus*	1.40
12	Hoary-bellied Squirrel	*Callosciurus pygerythrus*	1.11
13	Red Serow	*Capricornis rubidus*	0.90
14	Rhesus Macaque	*Macaca mulatta*	0.86
15	Crab-eating Mongoose	*Heerpestes urva*	0.68
16	Asiatic Golden Jackal	*Canis aureus*	0.61
17	Leopard Cat	*Canis aureus*	0.47
18	Large-Indian Civet	*Viverra zibetha*	0.36
19	Pallas's Squirrel	*Callosciurus erythraeus*	0.29
20	Large-toothed Ferret Badger	*Melogale personata*	0.21
21	Yellow-throated Marteen	*Martes flavigula*	0.18
22	Fishing Cat	*Prionailurus viverrinus*	0.14
23	Small Indian Civet	*Viverricula indica*	0.14
24	Orange-bellied Himalayan Squirrel	*Dermomys lokriah*	0.11
25	Capped Langur	*Trachypithecus pileatus*	0.07
26	Chinese Pangolin	*Manis pentadactyla*	0.07
27	Marbled Cat	*Paradofelis marmorata*	0.04

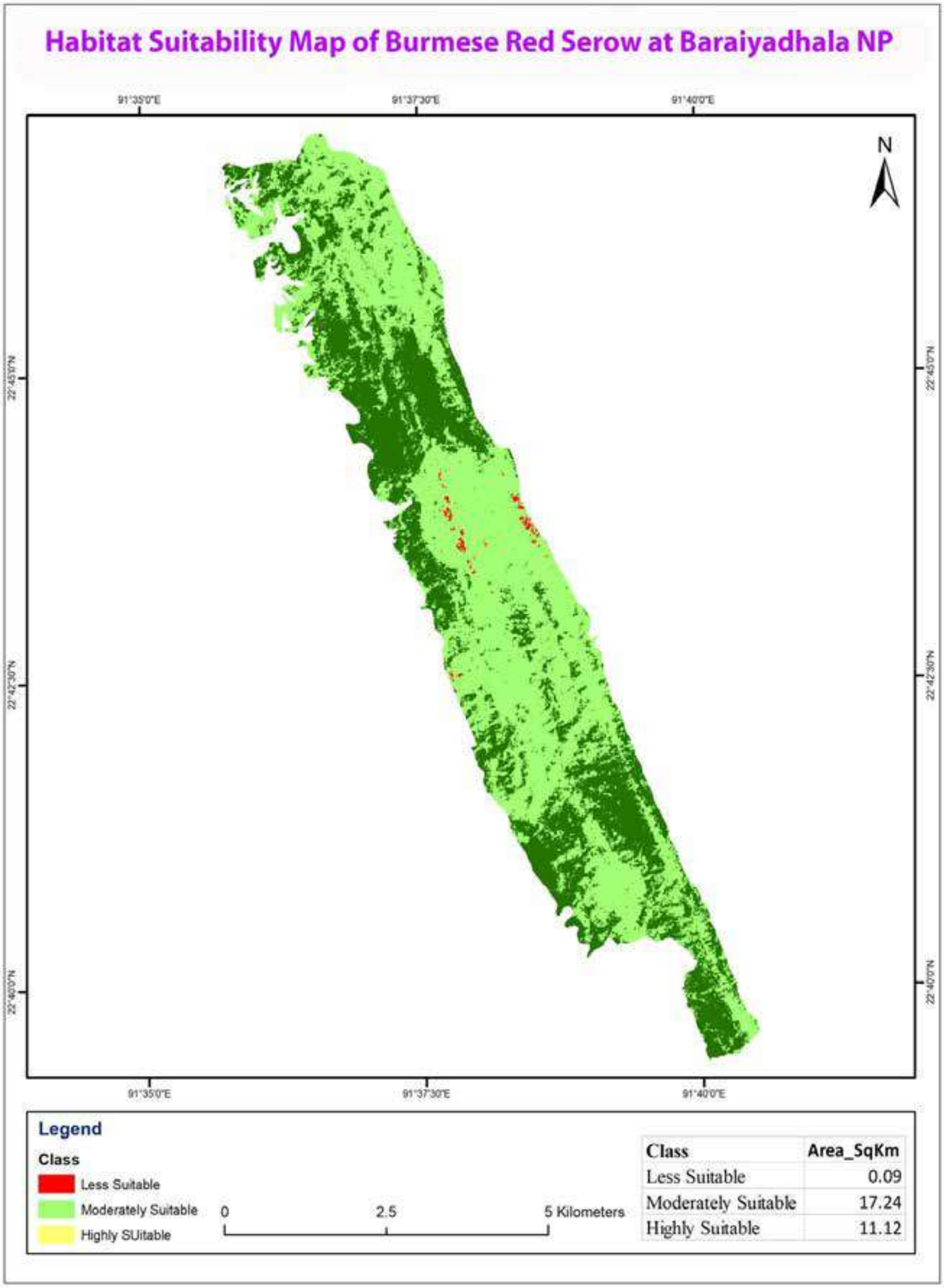
Red Serow mother with its calf at Baraiyadhala National Park.

Southern Red Muntjac

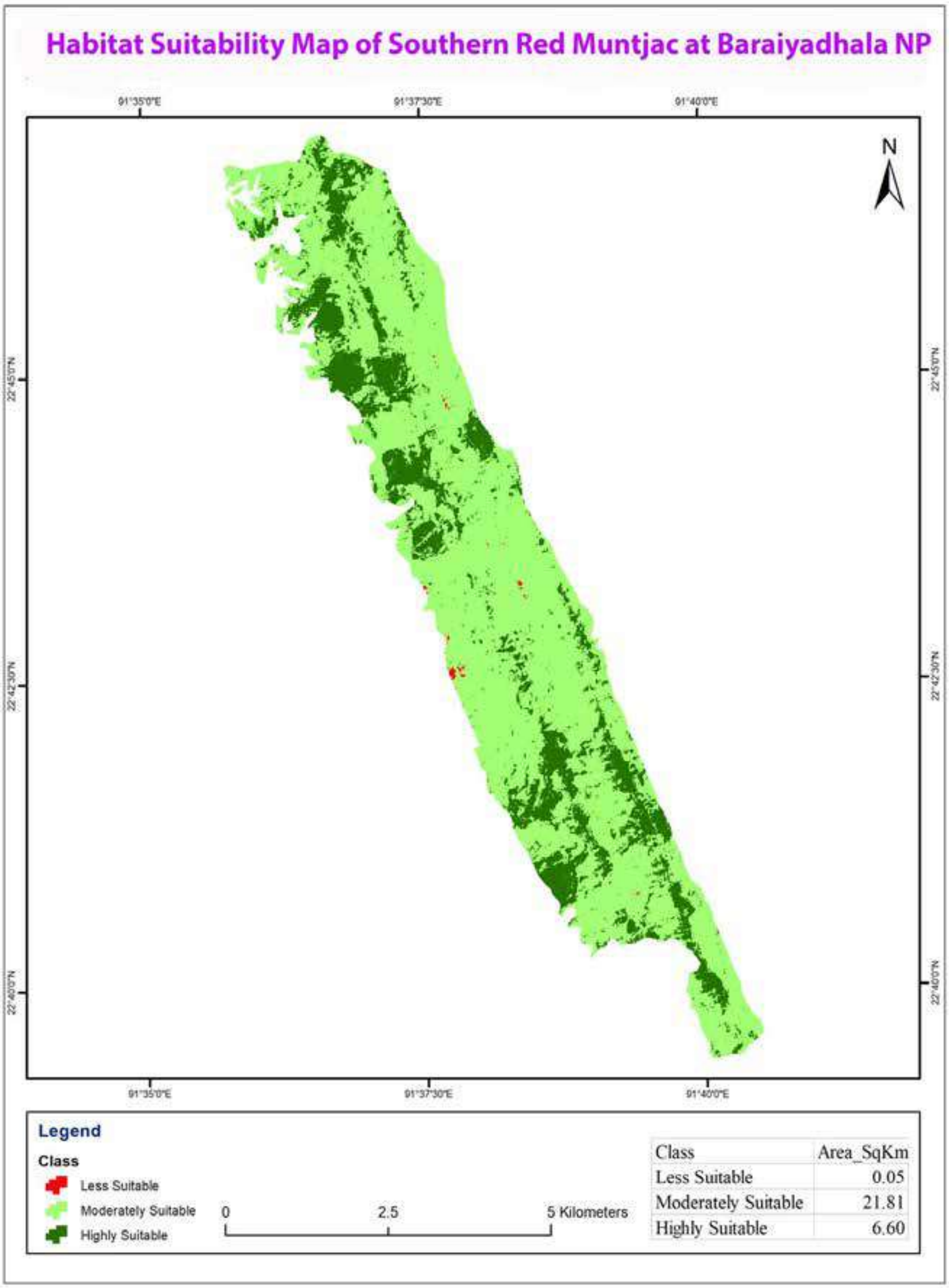
The only deer species found in Baraiyadhala National Park is the Southern Red Muntjac (*Muntiacus muntjak*). It is an Endangered species in Bangladesh (IUCN Bangladesh 2015). Southern Red Muntjac is distributed all over the national park area with the highest concentration in southern (Baraiyadhala Forest Range Office) and central part (Napityachara).

Southern Red Muntjac population in this national park seemed high (RAI = 9.92). From the camera trap data, it was revealed that Southern Red Muntjac population of this national park comprised of 47.49% male and 52.51% female. The density (D) of Southern Red Muntjac in Baraiyadhala National Park was 6.746 per square kilometer with the estimated population of 198 individuals. Habitat suitability index shows that about 6.60 sq km area of this national park is highly suitable while 21.81 sq km is moderately suitable for this species (Map 5).

Southern Red Muntjac (male) foraging at Baraiyadhala National Park.



Map. 4 Habitat suitability of Burmese Red Serow in Baraiyadhala National Park.



Map 5 Habitat suitability of Southern Red Muntjac in Baraiyadhala National Park.

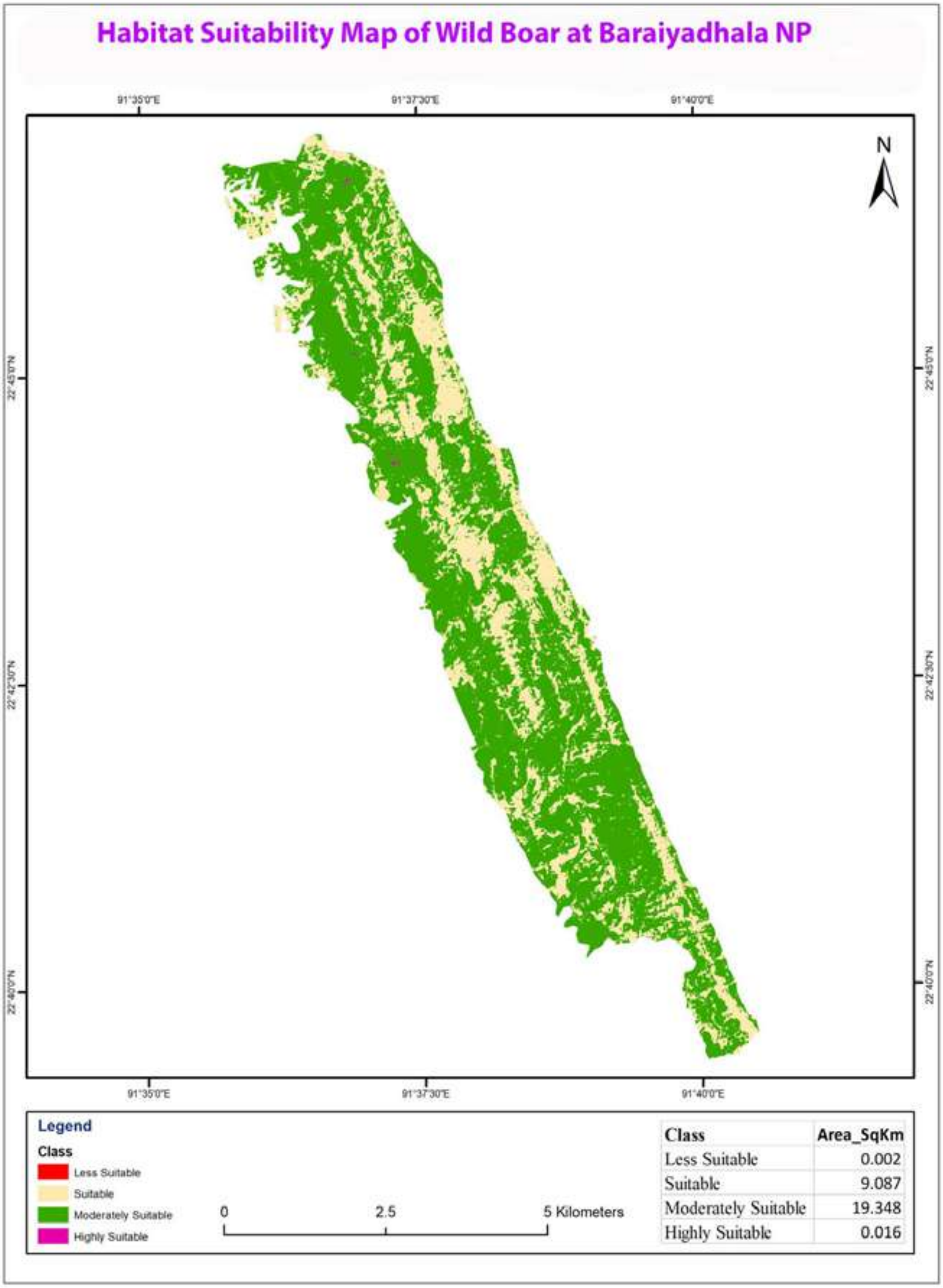
Wild Boar

In Bangladesh Wild Boar (*Sus scrofa*) is found in almost all the forests. A healthy population of Wild Boar is widely distributed all over the national park with the highest concentration at the southern part (Range Office) (Map 6). Wild Boar plays a vital ecological role by ploughing forest floor, exchanging nutrients to different tropic level of the ecosystem. As Wild Boar is a versatile omnivorous, they consume a wide range of food including rhizomes, roots, tuber, bulbs, shoots, barks, earthworms, insects, mollusks, bird eggs, lizards, snakes, frogs, garbage, carrion etc. They are also preyed upon by medium sized to large carnivores. Density (D) of Wild Boar in Baraiyadhala National Park was 4.677 per square kilometer with the estimated population of 138 individuals. Habitat suitability index shows that 9.067 sq km area of this national park is suitable while 19.349 sq km is moderately suitable for this species which is located all over the national park (Map 6).

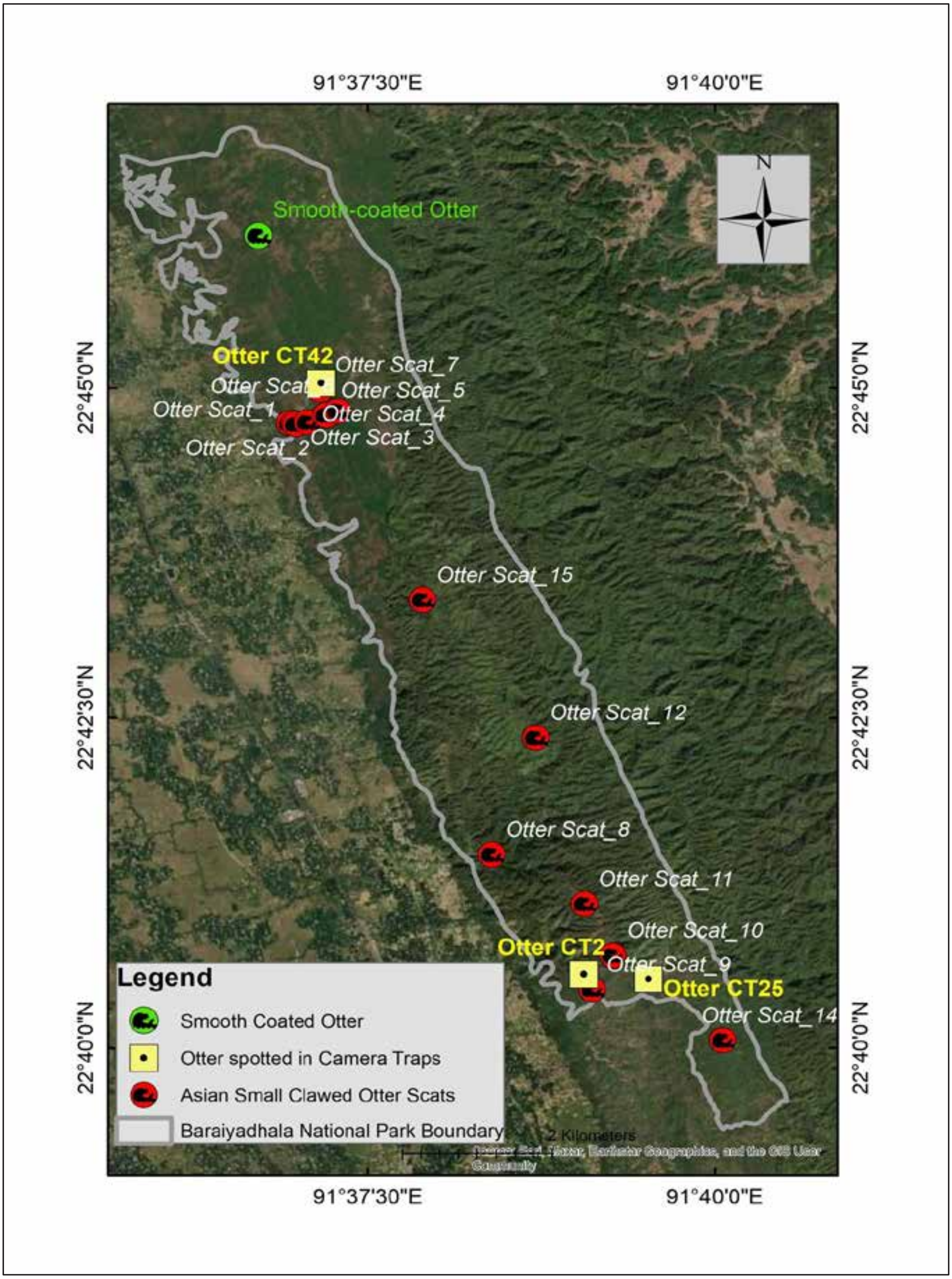
Plate 4.8 Wild Boar at Baraiyadhala National Park.

Otters

Otters are considered as the wetland ambassadors as they indicate the ecological health of the wetland ecosystem (Yoxon and de Silva 2017, Medhi et al. 2014, Hussain et al. 2011). Among the three species of otters in Bangladesh, two species *viz.* Smooth-coated Otter (*Lutrogale perspicillata*) and Small-clawed Otter (*Aonyx cinerea*) inhabits in Baraiyadhala National Park. Smooth-coated Otter and Small-clawed Otter are is Critically Endangered and Endangered respectively (IUCN Bangladesh 2015). Smooth-coated Otters are usually found in the north-western parts (close to Mohamaya lake) of the national park while Small-clawed Otters are distributed almost all over the national park with the highest concentration at the middle parts. The hill streams with large boulders makes a suitable habitat for the otters in this national park. Moreover, the hill streams support a diverse species of fishes, crabs, shells and frogs which provide food for otters throughout the year. Otters use these large boulders and clefts as a den. The relative abundance index (RAI = 2.79) of Small-clawed Otter in this national park indicates a good number of populations. Density (D) of Small-clawed Otter in Baraiyadhala National Park was 1.107 per square kilometer with the estimated population of 33 individuals.



Map 6. Habitat suitability of Wild Boar in Baraiyadhala National Park.



Map 7. Distribution of Small-clawed Otters and Smooth-coated Otters in Baraiyadhala National Park.

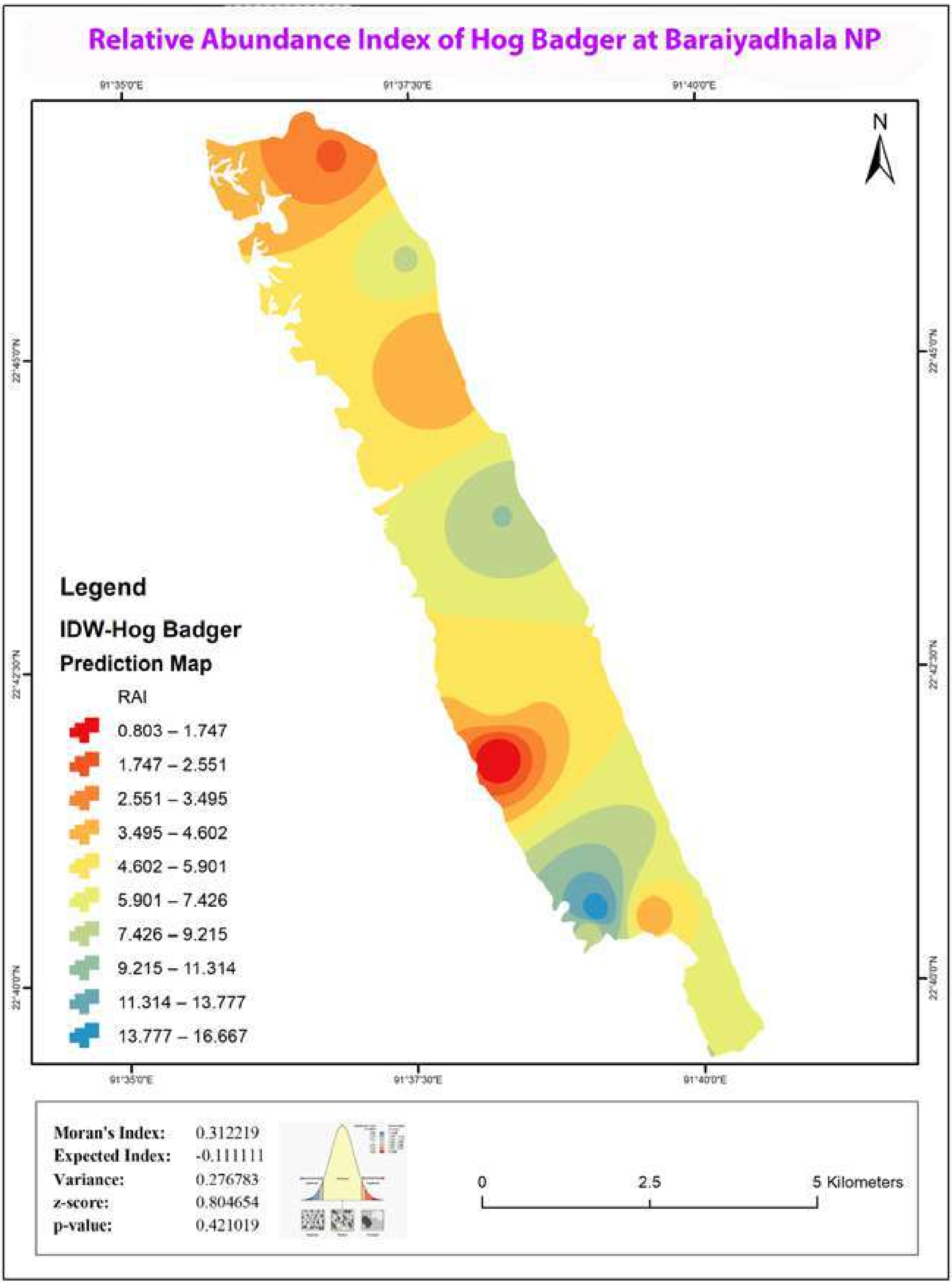
A troop of Small-clawed Otter at Baraiyadhala National Park.

Hog Badger

Hog Badger (*Arctonyx collaris*) is a Vulnerable species in Bangladesh (IUCN Bangladesh 2015). It is widely distributed throughout the national park. Camera trap data suggests that Hog Badger is active both at day and night time. Hog Badger is omnivorous in nature and diet mostly comprised of roots, fruits, earthworms and other small animals. The relative abundance index (RAI = 1.79) indicates that the population of Hog Badger in this national park is relatively low. Hog Badger in this national park is mostly distributed in the southern part (Map 8).

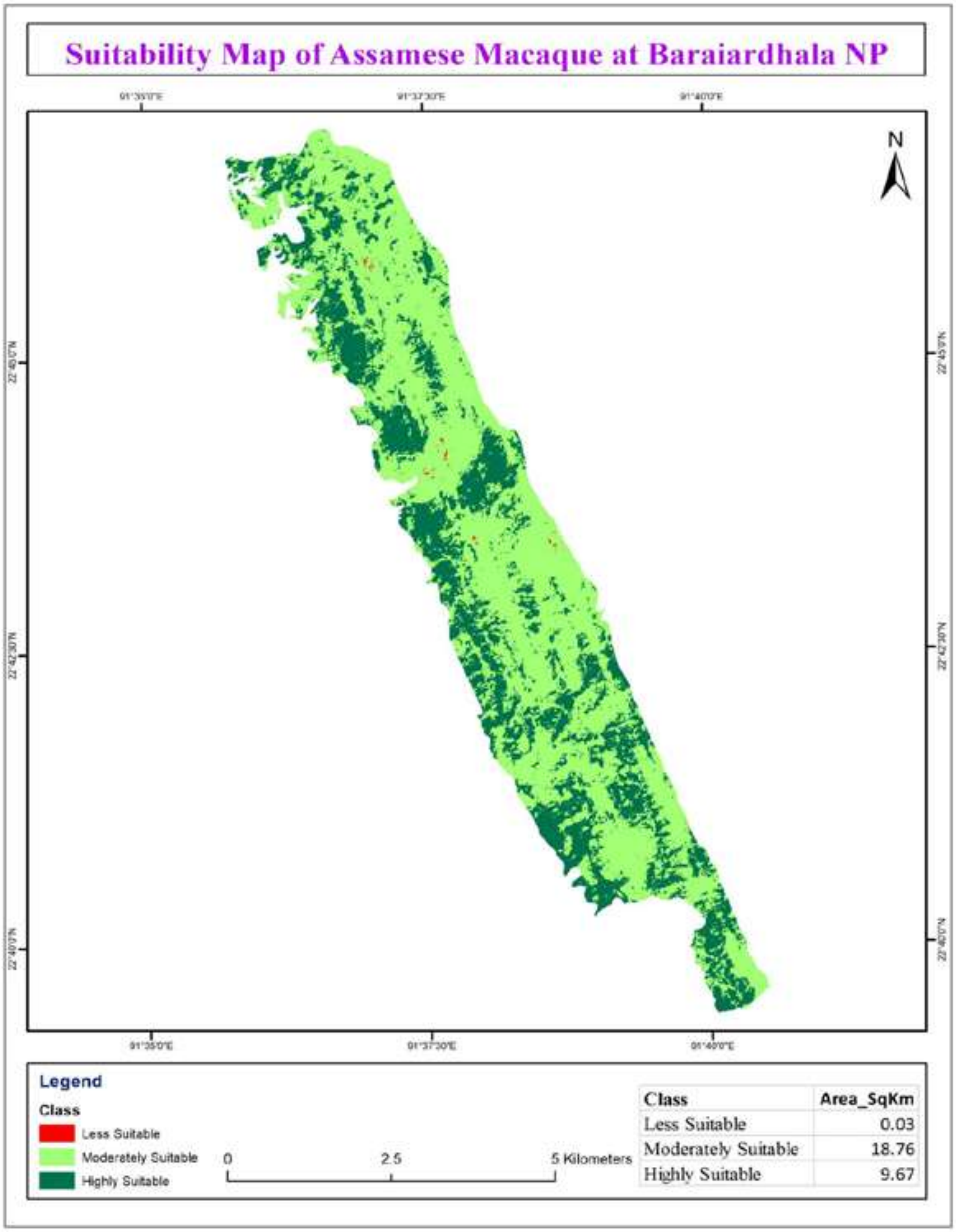
Assamese Macaque

Among the 10 non-human primates of Bangladesh, Assamese Macaque (*Macaca assamensis*) is one of the Endangered species (IUCN Bangladesh 2015). Very few information is available on its status, distribution and ecology in Bangladesh. In other range of its distribution, they mostly prefer high altitude mountains ranging from 200 m to 4000 m (Wada 2005, Srivastava and Mohnot 2001). Baraiyadhala National Park is a stronghold of this species with the highest relative abundance index (RAI = 13.21) among all the mammalian species. A total of 16 groups of Assamese Macaque with 234 individuals was recorded. Group size ranged from 9 to 20 individuals with the mean 14.63 ± 3.26. The density of Assamese Macaque in this national park was 7.98 individuals per km^2 with the estimated population of 234 individuals. Assamese Macaque was found all over the national park with the highest densities at the middle (Napityachara) (Map 9). Habitat suitability index shows that 9.67 sq km area of this national park is highly suitable while 18.76 sq km is moderately suitable for this species which is located all over the national park (Map 9).



Map 8 Distribution and density of Hog Badger in Baraiyadhala National Park.

Rhesus Macaque (*Macaca mulatta*) was comparatively less abundant in Baraiyadhala National Park. A total of four groups of rhesus macaque with 88 individuals was recorded. The hilly terrain and competition with sympatric Assamese Macaques would be one of the reasons of their low abundance compare to other forest areas of Bangladesh (Hasan et al. 2013). The abundance of Pig-tailed Macaque (*Macaca leonina*) was very low. Only one group of Pig-tailed Macaque with 34 individuals was recorded from this national park. The population of Phayre's Langur (*Trachypithecus phayrei*) was also very low. One group of Phayre's Langur with 11 individuals was recorded.



Map 9. Habitat suitability of Assamese Macaque in Baraiyadhala National Park.

Common Palm Civet

Common Palm Civet (*Paradoxurus hermaphroditus*) is a solitary nocturnal animal inhabits in forests as well as in human-influenced areas. It is omnivorous and primarily feeds on fruits and berries but also feeds on insects, crabs, fish and other small animals. They play a vital role in forest ecosystem by dispersing seeds. The relative abundance index (RAI = 2.65) of this species indicates a good population in Baraiyadhala National Park. It is distributed in almost all the habitats in this national park.

Crab-eating Mongoose

Among the three mongoose species in Bangladesh, Crab-eating Mongoose (*Herpestes urva*) is the rarest one. It has been recorded from the mixed evergreen forests of northeast and southeast (IUCN Bangladesh 2015). In Baraiyadhala National Park it was recorded mostly from the southern part (Baraiyadhala Range Office) and northern parts (Khoiyachara) (Map 4.12). The low relative abundance index (RAI = 0.68) of this species indicates low population in this national park.

Indian Crested Porcupine

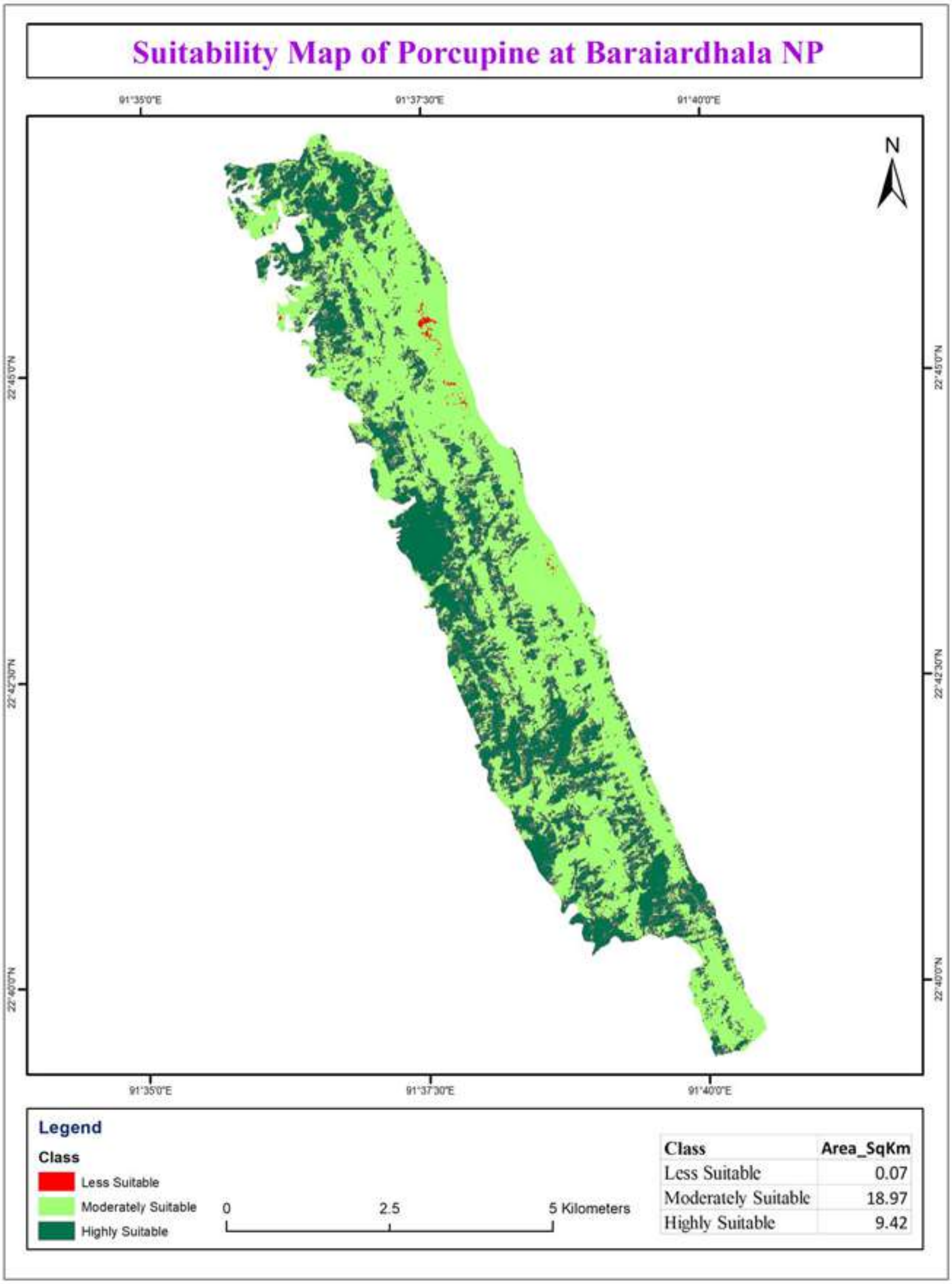
Indian Crested Porcupine (*Hystrix indica*) has been detected from 15 camera trap stations. The relative abundance index (RAI=2.97) of this species indicates that this species is fairly common in Baraiyadhala National Park (Map 4.13). Habitat suitability index shows that 9.42 sq km area of this national park is highly suitable while 18.97 sq km is moderately suitable for this species which is located all over the national park (Map 10).

Northern Tree Shrew

Northern Tree Shrew (*Tupaia belangeri*) is a Near Threatened species in Bangladesh and comparatively rare in the forests of Bangladesh. The relative abundance index (RAI = 3.08) of Northern Tree Shrew in this national park indicates a good number of populations which is distributed all over the national park. Habitat suitability index shows that 11.12 sq km area of this national park is highly suitable while 17.24 sq km is moderately suitable for this species which is located all over the national park.

Chinese Pangolin (*Manis pentadactyla*) is nationally and globally Critically Endangered species surviving in this national park. The population in this national park is very low; only two individuals was detected in camera traps with the low relative abundance index (RAI = 0.07).

Western Hoolock Gibbon (*Hoolock hoolock*) is another globally threatened species is also still surviving in this national park. This species is confined to the north-eastern part of the national park. One family group of this species with three individuals was observed and another group call was heard. Marbled Cat (*Pardofelis marmorata*) was captured in camera trap in only one occasion. It is a Data Deficient species in Bangladesh and previously recorded from a very few locations (IUCN Bangladesh 2015). Presence of this species in Baraiyadhala National Park indicates that habitats in this national park is still suitable to support medium sized carnivores. Leopard Cat (*Prionailurus bengalensis*) is recorded from seven camera trap stations.



Map 10. Habitat suitability of Indian Crested Porcupine in Baraiyadhala National Park.

The low relative abundance (RAI = 0.47) of this species indicates that population of this species in this national park is relatively low. Fishing Cat (*Prionailurus viverrinus*) is globally threatened; it was captured with three cubs in a single occasion. Though the stream habitats of this national park are suitable for fishing cat but the population of this species in this national park is very low (RAI = 0.14). Masked Palm Civet (*Paguma larvata*) has not been encountered in camera traps. It is mostly an arboreal species and probably very low population made this not to encounter in the camera traps. Local people support the presence of Dhole (*Cuon alpinus*) and Clouded Leopard (*Neofelis nebulosa*) in this national park but our camera trapping efforts failed to encounter these species, relatively very low population size could be the reason for this.

Karim and Ahsan (2016) recorded 29 species of mammals from Baraiyadhala National Park, while we have recorded 54 species from this national park during the study period. The present study recorded seven species of primates from this national park including Western Hoolock Gibbon, Phayre's Langur, Pig-tailed Macaque and Slow Loris. This study also confirmed the presence of two species of otters, *viz.* Smooth-coated Otter and Small-clawed Otter from this national park. Existence of Burmese Serow, Northern Tree Shrew, Hog-badger and Large-toothed Ferret Badger was also confirmed.

Birds

During the study period, a comprehensive record of the bird species present was made in Baraiyadhala National Park, totaling 262 species. These species belong to 21 avian orders (Figure 4.5) and 46 families. The majority of the birds observed in the park were classified as rare (42.75%) or uncommon (21%), while 30.15% of the birds were considered common (Figure 2). The order Passeriformes exhibited the highest species count with 138 species, followed by Coraciiformes with 23 species and Piciformes with 18 species. Among the families observed, Sylvidae displayed the greatest number of species, totaling 30. Of the recorded birds, 193 species were identified as resident birds, whereas 69 species were categorized as migratory.

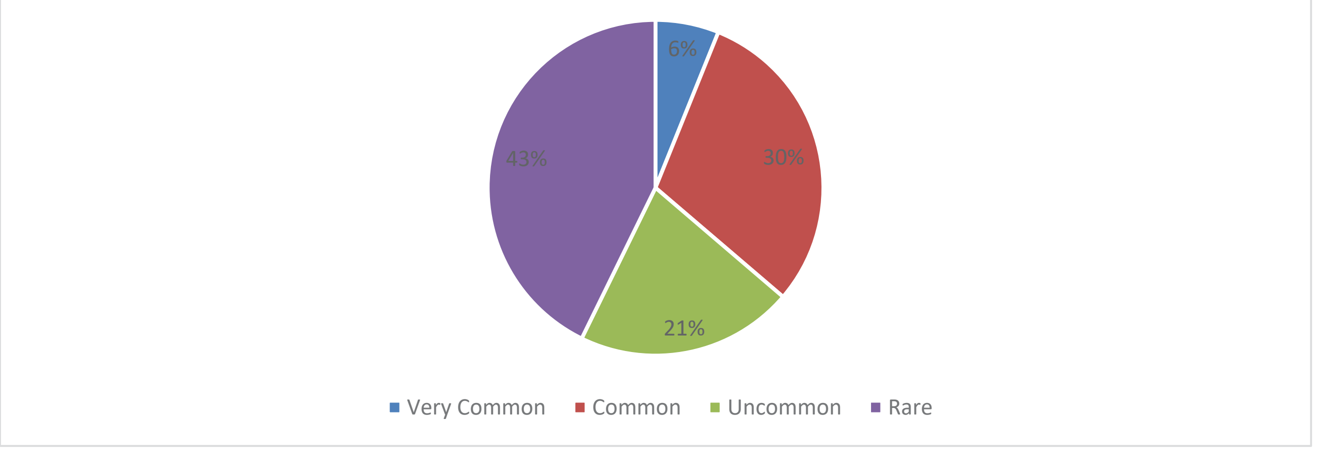


Figure 2. Status of birds in Baraiyadhala National Park.

A. Grey-peacock Pheasant and B. White-cheeked Partridge.

Baraiyadhala National Park boasts a remarkable array of bird species, encompassing a diverse range of types such as raptors, owls, cuckoos, ground-dwelling species, and frugivorous birds. Among the most frequently encountered bird species within the park are the Common Myna *Acridotheres tristis*, Red-vented Bulbul *Pycnonotus cafer*, Asian Pied Starling *Sturnus contra*, Black Drongo *Dicrurus macrocercus*, Oriental Magpie Robin *Copsychus saularis,* and Spotted Dove *Streptopelia chinensis*. The Red Jungle Fowl *Gallus gallus,* Kalij Pheasant *Lophura leucomelanos*, and Common Hoopoe *Upupa epops* are notable examples of ground-dwelling birds found in the national park. The abundance of ground-dwelling birds suggests a thriving population of insects or other food sources on the forest floor. Numerous birds of prey have been identified in Baraiyadhala National Park, including the Shikra *Accipiter badius*, Black Kite *Milvus migrans*, Brahminy Kite *Haliastur ordi*, Crested Serpent Eagle *Spilornis cheela*, and Black Baza *Aviceda leuphotes*, which were commonly observed near forested areas along streams where they can hunt small rodents, frogs, and fish from nearby water bodies. The national park is also home to various brood parasitic birds, such as the Asian Koel *Eudynamys scolopacea*, Common Hawk Cuckoo *Hierococcyx varius*, Indian Cuckoo *Cuculus micropterus*, Jacobin Cuckoo *Clamator jacobinus*, and Plaintive Cuckoo *Cacomantis merulinus*. Although these brood parasitic birds are challenging to spot, their presence is confirmed by their distinctive vocalizations. Additionally, several frugivorous bird species have been recorded in the national park, including the Rose-ringed Parakeet *Psittacula krameria*, Coppersmith Barbet *Megalaima haemacephala*, and Lineated Barbet *Megalaima lineata*, among others.

The forest is home to a significant number of resident bird species that are commonly found throughout the year. These resident species have adapted to the national park's habitat and can be observed consistently. Examples of resident birds in Barayadhala National Park include the Red-vented Bulbul, Indian Pond Heron *Ardeola grayii*, Chestnut-headed Bee-eater *Merops leschenaulti*, Asian Pied Starling, Black Drongo, Oriental Magpie Robin, Spotted Dove, White-throated Kingfisher *Halcyon smyrnensis* etc. These species are well-established within the forest and contribute to its avian biodiversity.

Kalij Pheasant

Black-napped Oriole

Spangled Drongo

White-rumped Shama

Some of the birds recorded from Baraiyadhala National Park.

Baraiyadhala National Park supports a good community of ground dwelling birds. Camera trapping efforts encountered a good number of Kalij Pheasant (*Lophura leucomelanos*) and Red Jungle Fowl (*Gallus gallus*). Three rare ground dwelling birds, *viz.* Grey Peacock Pheasant (*Polyplectron bicalcaratum*), White-cheeked Partridge (*Arborophila atrogularis*) and Barred Buttonquail (*Turnix suscitator*) are also found in this national park.

Reptiles

A total of 62 species of reptiles under 13 families was recorded during the study period. The reptilian community of Baraiyadhala National Park constitutes 58.067% (36 species) snakes, 35.48% (22 species) lizards and 6.45% (4 species) turtles & tortoises (Figure 3). Most of the reptiles of this national park was rare (51.61%) followed by uncommon (27.41%), common (12.90%) and very common (8.06%) (Figure 3). Family Colubridae possess the highest number of species (25 species) followed by Scincidae (8 species) and Gekkonidae (7 species) (Figure 4.13). Most of the snakes were found very rare in this national park. The reason might be because of the consumption of snakes by the local community. Same thing could be true for the turtles, only four turtle species were found.

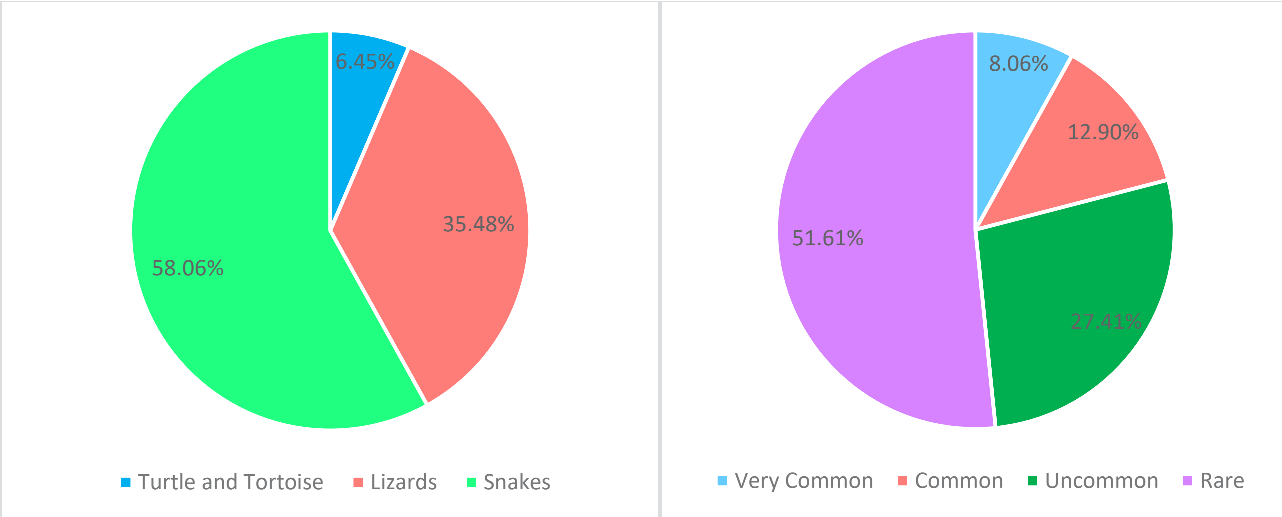


Figure 3. Proportion and local status of different groups of reptiles in Baraiyadhala National Park.

	
Red-necked Keelback	Common Garden Lizard
	
Bent-toed Gecko	Cherrapunji Keelback

Reptiles recorded from Baraiyadhala National Park.

Amphibians

A total of 38 species of amphibians under six families were recorded during the study period (Appendix 5). Among the recorded species 10.53% was very common, 23.68% common, 36.84% uncommon and 28.95% rare (Figure 4). The family Dicroglossiade has the highest number of species (12 species) followed by Ranidae and Rhacophoridae (9 species each) (Figure 4.15). Amphibians of Baraiyadhala National Park started their breeding activities at the beginning of the rainy season (May) and continue till August. Breeding amphibians were mostly found in the stagnant water in the national park.

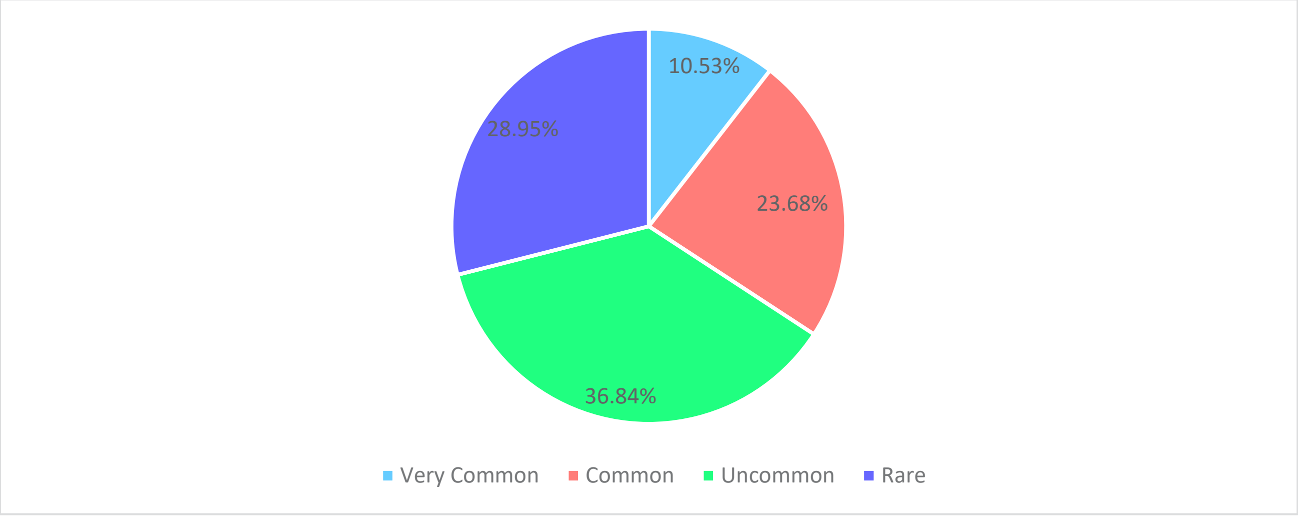


Figure 4. Status of amphibians in Baraiyadhala National Park.

	
Marbled Cascade Frog	Green Cascade Frog

Frogs recorded from Baraiyadhala National Park.

Two species of nationally threatened amphibian species, *viz.* Anderson's Bush Frog (*Philautus andersoni*) and Marbled Cascade Frog (*Amolops marmoratus*) were found in Baraiyadhala National Park. Green Cascade Frog (*Odorrana livida*) was recorded as a new frog species for Bangladesh from Baraiyadhala National Park during a previous survey (Hasan et al. 2021). The presence of this species in this national park extends the geographic range of this species ~ 145 km from the nearest population in Manipur and ~ 280 km from Mizoram of India, and is ~950 km from Dwana Mountain population in Myanmar. This is the only toxic frog in Bangladesh and its odiferous secretion makes allergic reactions to the skin. It is a nocturnal frog, strictly stream dweller frog and only found in the rocky stream and its vicinity. During daytime, they hid in the clefts of the stream rock. They started to appear with the sunset. The greatest number of individuals was found together near the waterfall area although a few numbers of individuals were found all over the stream area. Amplectant pairs were observed on the rock of the waterfall area between mid-July and end of August. *Odorrana livida* was found sympatric with Marbled Cascade Frog (*Amolops marmoratus*) and Crown Frog (*Xenophris parva*). Some individuals of Smith Litter frog (*Leptobrachium smithi*) and Berdmores Microhylid Frog (*Microhyla berdmorei*) were also observed in the same habitat. Marbled Cascade Frog (*Amolops marmoratus*) is also a stream dweller frog and was only found in the rocky stream during the study period. Anderson's Bush Frog (*Philautus andersoni*) was very rare in the national park and was only found few times during the survey period. The density of Marbled Cascade Frog was estimated 21.4 individuals / hectare while it was 24.8 individuals/ hectare for Green Cascade Frog and 6.2 individuals / hectares for Crown Frog. The density of Anderson's Bush Frog was 0.21 individuals / hectare.

Threatened Wild Animals

Baraiyadhala National park is the home of 30 species of nationally and globally threatened vertebrate wild animals. Mammals are the most threatened group of wild animals. A total of 17 mammal species are threatened out of 54 species of mammals recorded. Four species of mammals are Critically Endangered, 9 species Endangered and 4 species Vulnerable. Reptiles are the second most threatened group of animals with nine threatened species of which 1 species of Critically Endangered, four Endangered and Vulnerable species each. Two Vulnerable birds were recorded from this national park. One Endangered and one Vulnerable amphibian species also found in Baraiyadhala National Park (Table 2).

Table 2. List of threatened species found in Baraiyadhala National Park

Sl. No.	Common Name	Scientific Name	IUCN National	IUCN Global
Amphibians				
1	Anderson's Bush Frog	*Philautus andersoni*	EN	LC
2	Marbled Cascade Frog	*Amolops marmoratus*	VU	LC
Reptiles				
3	Green Fan-throated Lizard	*Ptyctolaemus gularis*	EN	LC
4	Spotted Flying Lizard	*Draco maculatus*	EN	LC
5	Spotted Supple Skink	*Lygosoma punctata*	EN	LC
6	Ring Lizard	*Varanus salvator*	VU	LC
7	Eyed Cat Snake	*Boiga siamensis*	EN	LC
8	Burmese Python	*Python bivittatus*	VU	VU
9	King Cobra	*Ophiophagus hannah*	VU	VU
10	Indian Leaf Turtle	*Cyclemys germeli*	VU	NE
11	Elongated Tortoise	*Indotestudo elongata*	CR	CR

Sl. No.	Common Name	Scientific Name	IUCN National	IUCN Global
Birds				
12	Kalij Pheasant	*Lophura leucomelanos*	VU	LC
13	Grey Peacock Pheasant	*Polyplectron bicalcaratum*	VU	LC
Mammals				
14	Assamese Macaque	*Macaca assamensis*	EN	NT
15	Pig-tailed Macaque	*Macaca leonina*	EN	EN
16	Rhesus Macaque	*Macaca mulatta*	VU	LC
17	Phayre's Leaf Monkey	*Trachypithecus phayrei*	CR	EN
18	Capped Langur	*Trachypithecus pileatus*	EN	VU
19	Bengal Slow Loris	*Nycticebus bengalensis*	EN	EN
20	Western Hoolock Gibbon	*Hoolock hoolock*	CR	EN
21	Smooth-coated Otter	*Lutrogale perspicillata*	CR	VU
22	Small-clawed Otter	*Aonyx cinerea*	EN	VU
23	Yellow-throated Marten	*Martes flavigula*	VU	LC
24	Hog Badger	*Arctonyx collaris*	VU	VU
25	Fishing Cat	*Prionailurus viverrinus*	EN	VU
26	Masked Palm Civet	*Paguma larvata*	VU	LC
27	Southern Red Muntjac	*Muntiacus muntjak*	EN	LC
28	Burmese Red Serow	*Capricornis rubidus*	EN	VU
29	Chinese Pangolin	*Manis pentadactyla*	CR	CR
30	Particoloured Flying Squirrel	*Hylopetes alboniger*	EN	LC

Wildlife Diversity and Abundance in Different Habitats

Vertebrate wildlife diversity was highest in the forest habitats followed by stream with water and bushes and bamboo thickets. A total of 368 species of wild animals were recorded from forest habitats followed by 211 species in stream with water and 194 species in bushes and bamboo thickets. The lowest species richness was found in steep hills habitat (58 species).

Anthropogenic Activities

Anthropogenic activities caused by the people are categorized into five types, *viz.* villagers, tourists, poachers, dogs and goats. Tourist activities were found the highest (43.18%) followed by villagers (35.06%). Poachers also active at a significant level (15.91%) in Baraiyadhala National Park (Figure 5). The Relative Abundance of tourist activities was also high (RAI = 4.84), followed by villagers (RAI = 3.93%) and poachers (RAI = 1.78) (Figure 6).

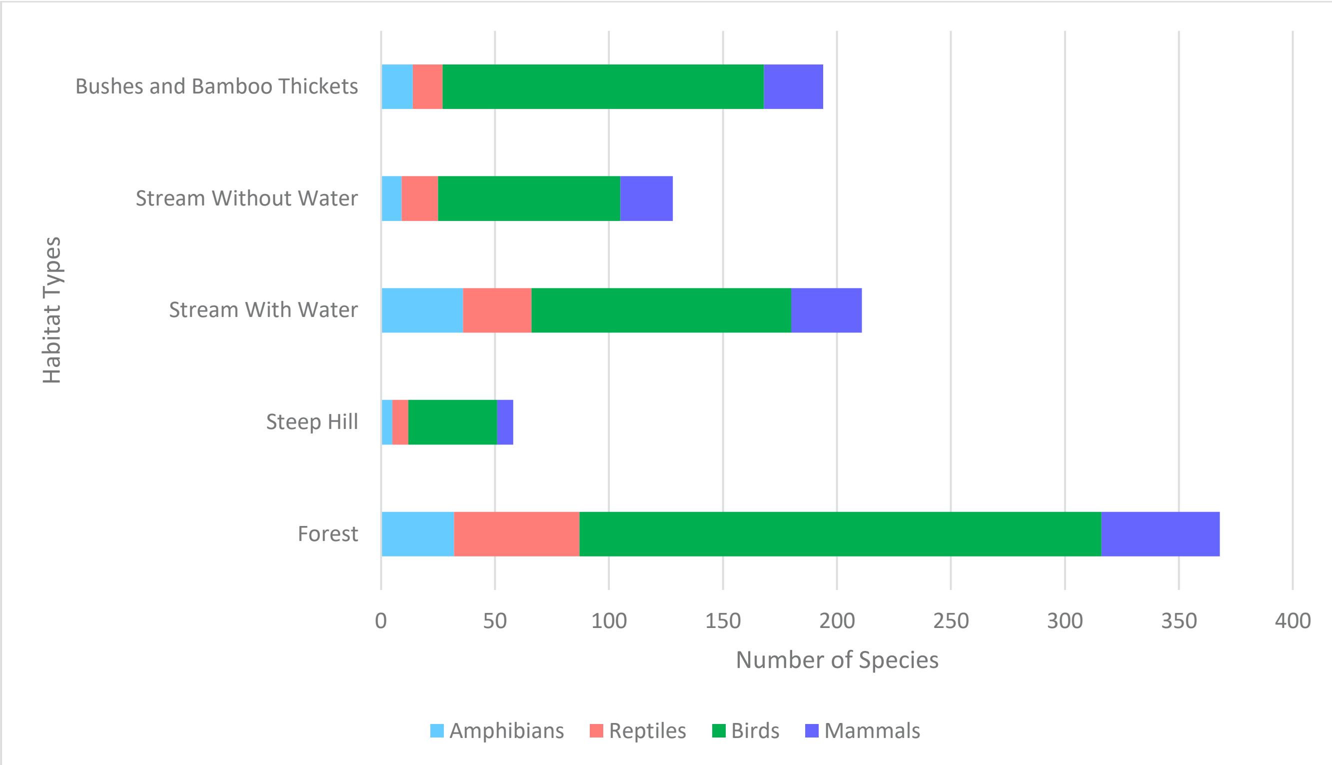
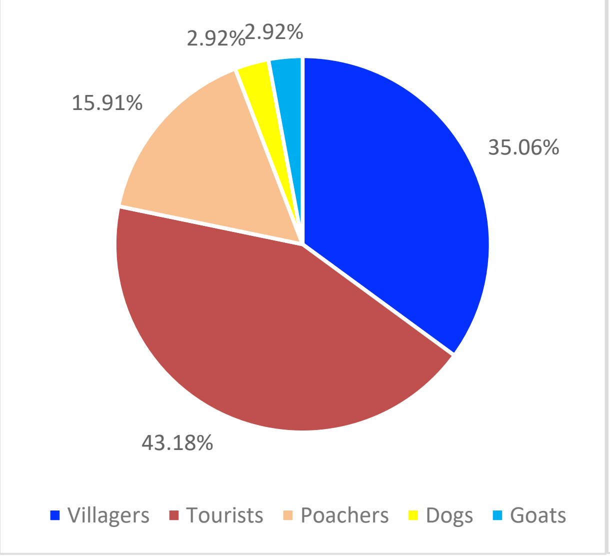


Figure 5. Species richness of different groups of wild animals in different habitats of Baraiyadhala National Park.

Hunters with dog in Baraiyadhala National Park.

Tourist activities in Baraiyadhala National Park.



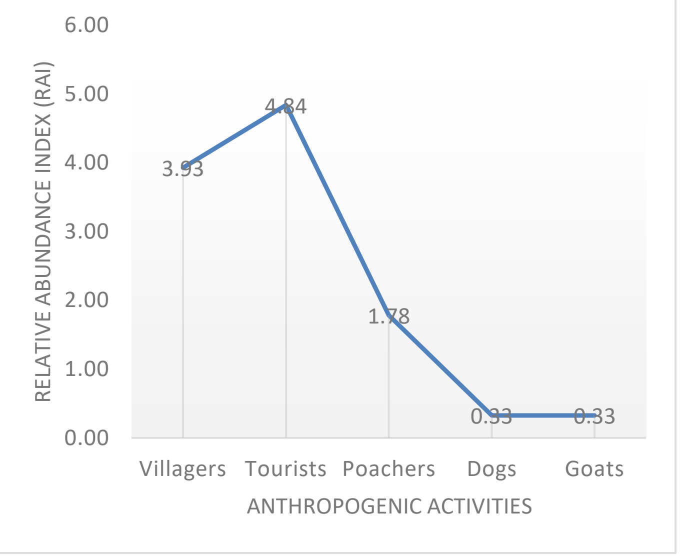


Figure 6. Percentage of anthropogenic activities in Baraiyadhala National Park.

Impacts of Tourist Activities on Wildlife and their Habitat

Tourists of Baraiyadhala National Park mostly concentrate at the waterfall area and the trail to reach there. Very few (2%) tourists visit other forest trails. Most of the tourists (> 95%) visit Baraiyadhala during monsoon and post monsoon (May to November), when waterfalls get enough water flow. The tourists start to visit the waterfalls from early morning (around 6.30 am) to dusk (around 7.00 pm). Our camera trap data supported that none of the large mammals like Southern Red Muntjac, Wild Boar and Red Serow appeared in front the cameras of at least 500 meters away from the tourist sites. Appearance of ground dwelling birds like Red Jungle Fowl and Kalij Pheasant also showed the same result. The same cameras captured the photos of those animals at night or when there was no tourist activity. Even the same cameras captured daytime photos of those animal during non-tourist season (December to March). This finding suggests that tourist activities have significant effect on the distribution of large mammals and ground dwelling birds at least for half a kilometer radius from the tourist sites. On the other hand, ground dwelling birds, Red Jungle fowl and Kalij Pheasant were found to forage at the tourist sites during early morning. It might be because of the leftover food from the tourists attracted these ground dwelling birds.

However, the data on tourist and other anthropogenic activities, their impact on wildlife distribution is still in the process of analyzing. Our draft final report will include more details about the impact of anthropogenic activities on wild animals of Baraiyadhala National Park.

Threats and Conservation Issues

The biodiversity of Baraiyadhala National Park is facing different kinds of anthropogenic threats. Threats to the forest and wildlife are categorized according to degree of disturbances. Baraiyadhala National park is the home of 30 species of nationally and globally threatened wild animals. It is very important to know the kinds of threats and their impacts on wild animals for the effective conservation measures.

Threats to Wildlife

Wildlife habitat degradation and alteration due to different anthropogenic activities, hunting and poaching and intentional forest firing are the major threats to the wildlife in Baraiyadhala National Park. The project team has documented different types of threats through field observations and camera trapping. Different types of threats were categorized and assessed on the basis of their respective impacts on wildlife, most of the threats are anthropogenic and can be eliminated or controlled (Table 3). Special emphasis was given to the impacts on nationally (IUCN Bangladesh 2015) and globally threatened wild animals.

Table 3. Types of threats, their nature and impacts on wildlife in Baraiyadhala National Park

Types of threats	Nature of impact	Potential level of impacts on wildlife [1 = low, 5 = high]
Fuelwood collection	Direct and Indirect	4
Collection of bamboo and sapling sticks	Direct and Indirect	4
Illegal logging	Direct and Indirect	3
Hunting and Poaching	Direct	5
Intentional forest fire	Direct and Indirect	5
Pollution	Direct and Indirect	3
Tourist Pressure	Indirect	2

Hunting and poaching

Hunting and poaching are the major threats to wildlife species in Baraiyadhala National Park which has direct impact on wildlife population. Local people hunt wild animals for meat and other body parts. Our camera trapped photos identified 49 individual poaching events (RAI = 1.78), which indicates intense hunting pressures for wildlife in this area. Poachers usually use guns and snares for the poaching of large mammals. Poachers also found to use domestic dogs to find out tortoises from the forest floor.

Two hunters with th eir guns at Baraiyadhala National Park.

Intentional Forest Fire

Intentional forest fire is very frequent and identified as a formidable threat to the wildlife population in Baraiyadhala National Park. Every year a significant portion of the forest has been burnt; in the dry season (March, April) of 2022 more that 50% of the forest area was burnt due to intentional forest firing. During dry season, the poachers use intentional fire to clean up undergrowth of the forest floor to increase visibility for hunting. They also use fire to frighten targeted animals in a particular direction where group of poachers wait for hunting. Intentional forest fire not only create threat to the mammals but also burnt-out bird nests, hibernating amphibians and reptiles and eventually destroy overall ecosystem.

Illegal logging

Very few numbers of mature trees are existing in Baraiyadhala National Park because of illegal logging. Illegal logging is still continuing to vanish the remaining few mature trees. Illegal logging has direct impact on loss and degradation of wildlife habitat. Illegal loggers also collect immature trees to make pole for Tin-shed house.

\Intentional forest fire at Napityachara region of Baraiyadhala National Park.

Fuelwood Collection

All households, living in and around the Baraiyadhala National Park, use fuel wood for daily cooking. They generally collect dead or fallen trees or branches, which do not contribute directly to the destruction of trees in the forest; but it does when they collect it for commercial purposes. The dependent local community earns their livelihood by selling these fuel wood to the local market.

Collection of bamboo sticks and saplings

The surrounding areas of Baraiyadhala National Park is well-known for vegetables cultivation especially for beans. Farmers use sticks (either bamboo or sapling) on the base of each bean plant to support its growth. Use of sapling stick in agriculture has direct impact on forest regeneration. Unsustainable collection of bamboo sticks and broom grass also have impacts on the destruction of forest undergrowth.

Tourist pressure

Tourists of Baraiyadhala National Park are mostly concentrated to the waterfall areas; thus, tourist activities and their movement have some direct impacts on the normal activity of stream dwelling diurnal animals. Though tourism pressure is not a major concern for Baraiyadhala National Park yet but tourist activities must be regulated. In some waterfall areas like Napityachara and Ruposhi Waterfall, tourists were found to cook food, doing barbeque parties which may create forest fire. Using fire in national park areas is also prohibited by the Act.

Pollution

Plastic pollution by the tourists particularly in the streams and waterfall areas are becoming a concern now a days. Tourists use plastic bottle and packet or bag to carry water and food which are thrown in the forest after use. Large amount of plastic waste pollutes and causes modification of the microhabitats especially for stream dwelling amphibians and fishes and crustacean those support food for other animals like otters, civets and fishing cat. Plastic pollution also has direct impact on amphibian breeding pools.

Plastic pollution in stream areas of Baraiyadhala National Park.

Poor institutional capacity

Manpower for the protection of Baraiyadhala National seems insufficient. Only six forest department officials are responsible for ensuring protection of this national park. The co-management committee at Baraiyadhala National Park was formed in 2012 but this committee is not functional now. Lack of both coordination and adequate funding made this co-management committee inactive. Logistics for the operation of Baraiyadhala National Park is also limited. Coordination among other law enforcing agencies also absent in the management system of Baraiyadhala National Park. Shortage of manpower, lack of proper logistics and coordination with very poor administration are creating opportunity for the poachers as well as unlawful activities inside the park.

CHAPTER V: CONCLUSIONS AND RECOMMENDATIONS

Conclusions

The forests in the Chattogram and Chittagong Hill Tracts are important for conserving many rare and threatened wildlife species of the country and Baraiyadhala National Park is also a part of that. Due to the presence of the beauty of waterfalls and forest, Baraiyadhala National Park is becoming an important tourist destination. Though tourism pressure is not a major concern for Baraiyadhala National Park yet but tourist activities must be regulated. The biodiversity of Baraiyadhala National Park is facing different kinks of anthropogenic threats including hunting and poaching and intentional forest firing. Local people hunt wild animals for meat and other body parts. Manpower for the protection of Baraiyadhala National seems insufficient.

Capacity building of the Forest Department by deploying sufficient manpower and logistics is an immediate need for the proper management of the national park. Involvement of local people through activating the co-management committee is recommended. Enhanced patrolling by involving local people to stop / minimize illegal hunting and poaching is also an urgent need for the conservation of elusive wild animals of this national park.

The study was conducted in Baraiyadhala National Park, Hazarikhil Wildlife Sanctuary and Sitakunda Hill Forest are continuous with this national park. Similar study should be conducted incorporating the whole landscape.

Recommendations

Management Action

As Baraiyadhala National Park is rich in biodiversity and has potential of ecotourism development, immediate conservation and management actions need to be taken because anthropogenic pressures have been increasing day by day. Some of the actions need to be implemented immediately such as zonation and boundary demarcation, habitat restoration, capacity building, improved patrolling, regular monitoring and research, sustainable ecotourism development, providing alternatives to fuelwood and bamboo sticks, formation of co-management committee and socio-economic developmental activities.

Zonation and Boundary Demarcation

Proper delineation of boundary and zonation should be completed in an urgent basis. The core zone should be kept for the preservation of flora and fauna together with the scenic beauty where all kinds of exploitation should be banned. On the other hand, the impact zone or buffer zone should be demarcated for the production of natural resources, grazing, cultivation and other activities to meet up the demands of local people. However, ecologically harmful establishment like brickfields, saw mills and industries should be banned in buffer zone. The national park boundary should be demarcated by permanent pilers or/and by the natural landmarks like streams or hills. The landmarks or streams should be plotted on the map by putting GPS coordinates. All the boundaries should be regularly monitored.

Habitat Restoration

In Baraiyadhala National Park most of the area is covered with bushes, bamboo thickets and small trees. There are very few patches with mature trees. Protection should be enhanced to encourage natural regeneration. Moreover, control of intentional forest fire to accelerate natural regeneration can restore wildlife habitats. Human aided regeneration program should be taken by collecting seed or seedling from the natural mother tree and to plant in open hills in the national park. Fruit yielding trees including figs for wildlife should give priority over timber producing trees. Denuded hills should cover with native plants to increase water holding capacity of the forest.

Capacity Building

Capacity building programs among the forest officials working in Baraiyadhala National Park should be taken. Manpower should be at least doubled with proper training so that they can perform not only management activities but also contribute to the scientific monitoring. Necessary logistics like vehicles, fire arms and other modern equipment like GPS, binoculars, digital camera, camera traps, laptop computer, should be ensured for monitoring and documentation.

Improved Patrolling

The conventional patrolling should be improved and modernized to control illegal activities including hunting and poaching, illegal harvest of forest products, intentional forest fire. Co-management lead community patrolling should be introduced to strengthen the regular patrolling activities of forest department. SMART (Spatial Monitoring and Reporting Tool) patrolling should be introduced in Baraiyadhala National Park as it seems successful in the Sundarbans.

Regular Monitoring and Research

In the current management system of Baraiyadhala National Park there is very little or no scope of regular research and monitoring but it is crucial for effective management plan of the national park. Basic monitoring should include GIS based record of forest crimes (e.g. hunting and poaching, illegal logging, encroachment etc.), human-wildlife conflicts and management implications. Forest health should be monitored based on the diversity and abundance of flora and fauna especially the abundance of indicator species (Feeroz 2011). Changes of vegetation cover and forest boundaries should be monitored by remote sensing with validation by ground-truthing. This report will serve as a baseline for flora and fauna in Baraiyadhala National Park. Any changes of forest health can be monitored by comparing this baseline information.

Ecotourism Development

Basic amenities for the tourists like public toilet, drinking water facilities, sitting arrangements are absent in Baraiyadhala National Park. There is no rest house of eco-cottage services in this national park. Though, home stay service has been introduced in two houses by an NGO but basic facilities are very poor there. A student dormitory can be established in order to allow economy accommodation for students. The existing trails should be properly maintained and plantation of indigenous plants in the trail will make the trail attractive as well as will ensure food and shelter for the wild animals. Some local young people especially the students can be trained as eco-tour guide which will facilitate tourists as well as will generate alternative income.

Providing Alternatives of Fuelwood

There is no electricity and gas pipeline in Baraiyadhala National Park. Local people are fully dependent on fuelwood collected from the forest. Even the restaurant owners also use fuelwood for cooking. Promoting improved cooking stove (that consume less fuelwood) can reduce fuelwood collection.

Formation of Co-management Committee

Co-management committee should be formed to ensure effective participatory management of the national park. As per the Government gazette co-management committee will get 50% share of the entry fee and this fund can be used for alternative income generation as well as for the community development.

Socio-economic Development Activities

Socio-economic development activities including renovation of roads, establishment of school, health clinic, and drinking water facilities should be taken in and around the Baraiyadhala National Park. As ecotourism already exists in this national park, more local people should be involved with this business. Local people should be encouraged to establish homestead gardens, plant nursery establishment, livestock rearing, fish culture and other small businesses. Government agencies and NGOs should be involved with the socio-economic development activities.

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Investigating Human-Fishing Cat Conflict and Exploring Mitigation Strategies in Northeastern Bangladesh

by

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CHAPTER I

INTRODUCTION

1.1 General Introduction

Bangladesh is recognized as a biodiversity-rich nation, documented to host a total of 1,619 species. This inventory includes 138 species of mammals, 566 species of birds, 167 species of reptiles, 49 species of amphibians, 253 species of freshwater fishes, 141 species of crustaceans, and 305 species of butterflies (IUCN Bangladesh, 2015a). Alarmingly, assessments indicate that approximately 25% of these species fall within IUCN Threatened categories, comprising 56 species classified as Critically Endangered and 390 species classified as Threatened (Vulnerable, Endangered, or Critically Endangered). The remaining assessed species are predominantly categorized as Least Concern. Furthermore, 31 species, including 11 mammals, 19 birds, and 1 reptile, are considered regionally extinct within Bangladesh (IUCN Bangladesh, 2015b).

The diverse aquatic ecosystems of Bangladesh encompass Haors – expansive, bowl-shaped seasonal wetlands that form in large depressions connected to rivers during periods of high discharge. These Haor ecosystems support over 653 documented freshwater species (BFD, 2017). Northeastern Bangladesh, falling under the Sylhet civil administrative division, comprises the districts of Moulvibazar, Habiganj, Sylhet, and Sunamganj.

Within these four districts, 217 distinct Haors/wetlands have been identified, collectively covering an area of approximately 6,155.56 hectares. This represents roughly 48% of the total land area of these Haor-centric districts (CEGIS, 2012). The wildlife dependent on these Haor ecosystems includes the Fishing Cat (*Prionailurus viverrinus*), Jungle Cat (*Felis chaus*), Golden Jackal (*Canis aureus*), various mongooses (Herpestidae), turtles, snakes, frogs, a vast array of freshwater fishes, and numerous bird species, including migratory ducks, geese, and waders. Freshwater fish are harvested extensively from Haors, beels (permanent water bodies), rivers, and ponds, including through man-made aquaculture practices. Communities within the wetland areas, particularly in northeastern Bangladesh, are often engaged in poultry farming. These farm areas are sometimes inhabited and used as breeding grounds by species such as jackals, Jungle Cats, and Fishing Cats. Poultry farmers commonly perceive the Fishing Cat as a significant threat to their chicken and duck rearing operations.

As its common name implies, the Fishing Cat (*Prionailurus viverrinus*) is intrinsically linked to aquatic ecosystems where fish constitute a primary food source. Morphologically, it is a stocky felid characterized by relatively short limbs and a short yet muscular tail. It is the only wild cat species in Bangladesh possessing an olive-gray to olive-brown fur coat. Its back is marked by longitudinal rows of elongated black spots, which form distinct lines along the neck region. The cheeks exhibit a paler coloration adorned with two prominent dark stripes. Fishing Cats are predominantly nocturnal and solitary hunters, preying on fish, birds, and small mammals. They are adept swimmers and preferentially inhabit wetlands where they actively forage for prey (Mukherjee et al., 2016). Globally classified as Vulnerable (Mukherjee et al., 2016), the Fishing Cat is considered Endangered within Bangladesh (IUCN Bangladesh, 2015b) and exhibits a wide distribution across various habitat types, though it shows a strong preference for areas rich in wetlands (SRCWP, 2015).

The Fishing Cat is currently facing an acute habitat crisis in recent decades, primarily driven by the widespread reduction and degradation of perennial water bodies throughout Bangladesh. Critical Fishing Cat habitats, particularly wetlands, have been extensively converted to agricultural land. Additional pressures include the destruction of homestead forests, expansion of housing and industrial facilities, reduced upstream water flows impacting wetland hydrology, and direct competition with humans for aquatic resources. These cumulative factors have significantly intensified the human-Fishing Cat conflict situation. Consequently, substantial numbers of Fishing Cats continue to be captured by local residents, leading to high annual mortality rates.

Physically, the Fishing Cat bears a resemblance to a leopard (albeit significantly smaller in size) and is commonly referred to as 'Bagh' (meaning tiger/leopard) within local communities. This perceived similarity contributes to frequent capture and killing by inhabitants of the Haor regions. However, the presence of Fishing

Cats plays a vital ecological role by controlling rodent populations (rats, mice) in agricultural fields and around homesteads.

1.1.1 Fishing Cat (*Prionailurus viverrinus*): An Overview

The Fishing Cat (Prionailurus viverrinus) is a medium-sized wild felid belonging to the family Felidae. Its range extends across South and Southeast Asia, including significant populations in India, Bangladesh, Nepal, Sri Lanka, Thailand, Cambodia, and potentially parts of Vietnam and Myanmar (Mukherjee et al., 2016; IUCN, 2024).

As its name suggests, the Fishing Cat is exceptionally adapted to an aquatic lifestyle. The coat features a remarkable two-layered structure crucial for aquatic thermoregulation. A dense, short undercoat lies next to the skin, acting like waterproof thermal underwear by preventing water penetration and retaining warmth. A second layer of longer guard hairs grows through the undercoat, providing the species' characteristic coloration and glossy sheen (ISEC, 2022).

i. **Hunting behaviour:** Fishing cat **is** primarily piscivorous (fish-eating), it is a skilled hunter in aquatic environments. Techniques include scooping fish out of the water with its paws, diving partially or fully submerged to catch prey, and patrolling the water's edge (Britannica, 2022; Sunquist & Sunquist, 2002). While fish are the primary prey, their diet also includes birds, small mammals, amphibians, reptiles, and crustaceans (Mukherjee et al., 2016).

Figure 1. Adult fishing cat

ii. **Habitat:** This species is strongly associated with wetland ecosystems. It inhabits a variety of waterside environments, including mangrove swamps, densely vegetated marshes (reed beds, cattails), oxbow lakes, rivers, streams, and areas near jungle or forest edges adjacent to water (Mukherjee et al., 2016; IUCN, 2024).

1.1.2 Distribution of the Fishing Cat (*Prionailurus viverrinus*)

The Fishing Cat exhibits a patchy and discontinuous distribution across South and Southeast Asia. Core populations are currently confirmed in Bangladesh, India, Sri Lanka, Nepal, Thailand, Myanmar, and Cambodia (Mukherjee et al., 2016; IUCN, 2024). While historically reported in Pakistan and parts of Indochina, its contemporary status in Pakistan is uncertain, and it is likely extinct in Vietnam and on the Indonesian island of Java (Willcox et al., 2015; Duckworth et al., 2005; IUCN, 2024). However, a population was confirmed in southwest Cambodia's Peam Krasop Wildlife Sanctuary (PKWS) during surveys conducted in 2009 and 2015, representing one of only two known locations for the species in Cambodia since 2003 (Thaung et al., 2015; IUCN Cat Specialist Group, 2020).

Their typical habitats encompass various water-associated environments, including marshy thickets, mangrove swamps, and densely vegetated riparian zones along rivers and streams (Mukherjee, 2013). They have been recorded at elevations up to 1,525 meters (5,000 feet) in the Himalayan foothills of India, utilizing dense vegetation near rivers and streams (Habib et al., 2013), and have also been observed utilizing degraded habitats such as areas near aquaculture ponds (Mukherjee, 2013).

A study on Java (prior to its inferred extinction there) found Fishing Cats restricted to coastal wetlands within 15 km of the ocean and below 20 meters elevation (Sunarto et al., 2008). A radio-telemetry study in Nepal's Shuklaphanta National Park revealed individuals were exclusively nocturnal, spending significant time in dense tall grasslands, sometimes at considerable distances from water. Female home ranges were documented at 4-6 km², while a single male's range was much larger at 16-22 km² (Dahal & Dahal, 2011).

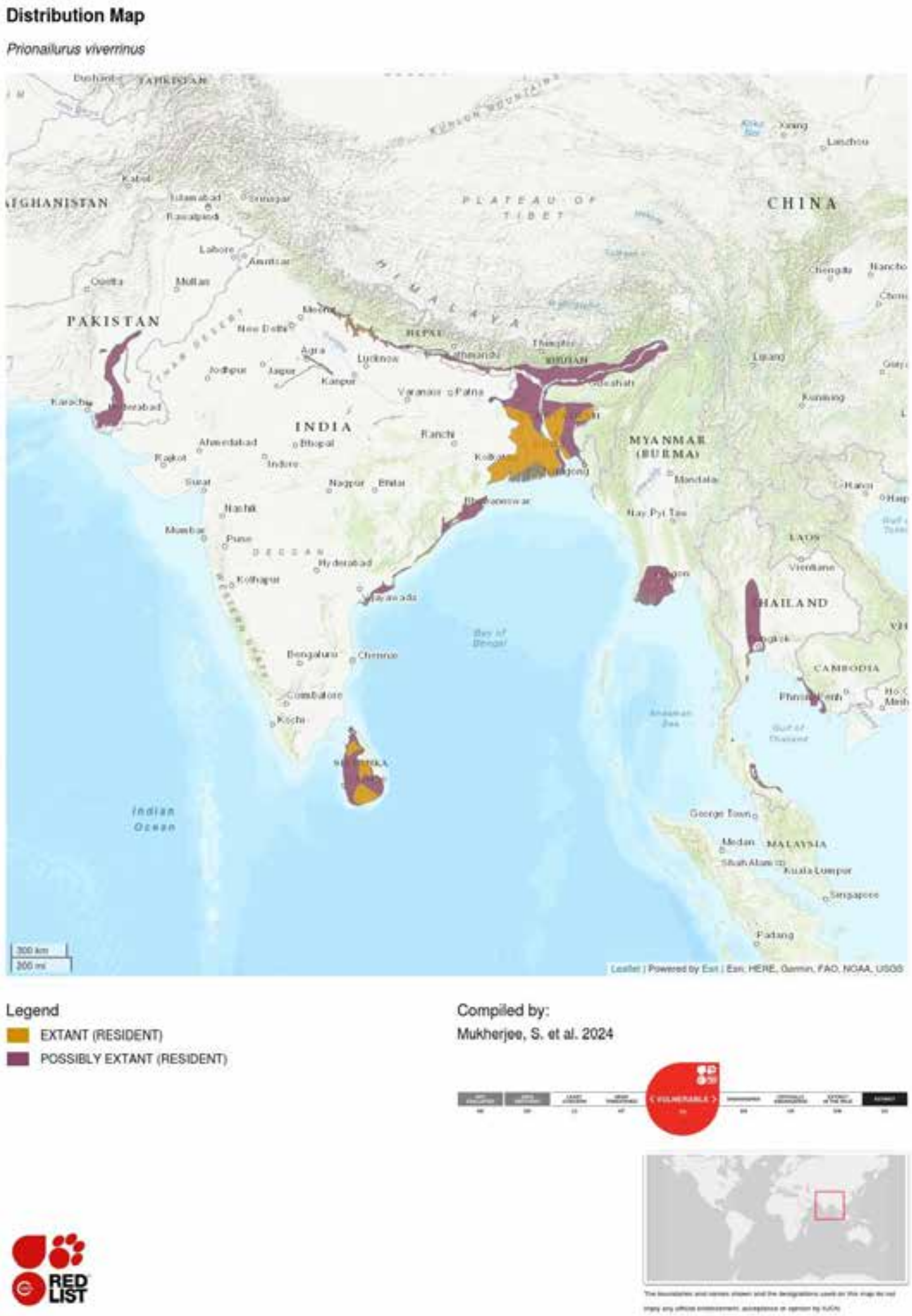

Figure 2. Distribution of the fishing cat across the world (IUCN, 2025).

1.1.3 Conflict

Conflict is a serious disagreement or argument between two or among several, typically a protracted, lasting one. According to the Merriam-Webster dictionary, conflict has been defined as competitive or opposing action of incompatibles: antagonistic state or action (as of divergent ideas, interests, or persons). The severe conflict situation normally originates in nature among the resource users, including animals, livestock, and human beings, when they use the same natural resources at a time.

1.1.4 Human-Fishing Cat Conflict

In Bangladesh's northeastern haor wetlands, human-fishing cat conflict intensifies seasonally as drying wetlands force cats into agricultural and human settlements (Siddiqui et al., 2021). This apex predator increasingly preys on domestic ducks and poultry during winter (December-January), triggering retaliatory killings by farmers who perceive them as threats to livelihoods (Fishing Cat Conservation Alliance [FCCA], 2022). A 2020-2024 study documented 63 conflict incidents involving 93 fishing cats across Sylhet, Moulvibazar, Habiganj, and Sunamganj districts, with Moulvibazar Sadar identified as the primary hotspot (38% of incidents) (FCCA, 2023). Alarmingly, 59% of affected cats were adults, and local persecution extends to kittens despite the species' Endangered status nationally (IUCN Bangladesh, 2015) and Vulnerable status globally (Mukherjee et al., 2016).

The conflict stems from large-scale wetland conversion (48% of northeast wetlands lost since 2000) and reduced fish stocks driving cats toward poultry farms (CEGIS, 2018). Baited metal cages account for 45% of captures, with mortality exceeding 70% without intervention (Siddiqui et al., 2021). Conservation responses include a rescue-release protocol achieving 88% adult survival and innovative nighttime kitten releases enabling 62% maternal retrieval (FCCA, 2023). However, sustained habitat protection remains critical: only 22% of key fishing cat habitats fall within protected areas, while community engagement programs reach just 15% of high-conflict villages (IUCN Bangladesh, 2020).

1.2 Problem Statement

To maintain viable fishing cat population through ensuring sustainable management of the existing aquatic wetland habitats in the country is now the priority activity identified by of all scientists, politicians, Natural Resource Managers, Wildlife Biologists now-a-days. As because the conflicting situation has been arisen much for using same wetlands by both people of the localities and the fishing cats. Thus, very unfortunately, there were a remarkable number of fishing cat captured cases recorded in last 5-10 years in the study area. So, conservation efforts should be taken immediately by identifying conflicts between human being and fishing cats.

For saving fishing cats from the verge of extinction and for ensuring its viable population the next generation the urgent need is to find out at least remnant potential fishing cat hotspots which are being still used both dry and wet seasons is necessary. We shall have to strengthen effective management with taking all possible conservation measures especially where the habitats are still existing without any scientific management. We should support our government to introduce integrated management where absent of any management systems or plan exists for reducing human- fishing cat conflict which cause premature death of this beneficial cat species unfortunately.

1.3 Objectives of The Study

In the foregoing context, the proposed study would assess the following to fulfill those optimum requirements, which were the objectives of the study too.

1) Defining and identifying Human-fishing cat conflicts that exist in the northeast Haor region, impacting their survival (specific objective).
2) Finding out their typical breeding habitats through trap cameras and satellite/radio collars, mapping the hotspots where they inhabit in different localities due to the seasonal variation, especially during monsoon and dry seasons, and mapping more conflict zones (specific objective).
3) Exploring recent threats, categorizing threats as low, medium, and high level or likely, unlikely, rare, etc., and describing site-specific mitigation measures and suggesting probable action(s) to be adopted by the concerned stakeholders (specific objective).

CHAPTER II

LITERATURE REVIEW

2.1 Distribution

The fishing cat is found in the entire grassland and low-lying areas in Bangladesh, India, Nepal, Sri Lanka, Thailand, Cambodia, Vietnam, and other countries of Southeast Asia. Population Status: Like other small carnivores, there have been very few studies on the status of the fishing cat (Lynda 2001). In Nepal, the Tarai region along the foot of the Himalaya, consisting of major wetlands and water systems, potentially supports large numbers of fishing cats (Lynda 2001). The population was assumed to be approximately 150-200 mature individuals of fishing cats in the Tarai of Nepal (Jnawali et al. 2011). However, the Tarai has been significantly impacted by human activities. It is believed that the population of the fishing cat in Southeast Asia is decreasing (Mukherjee et al. 2010). In Java, fishing cats appear only in isolated coastal wetlands in small numbers, and the absence of records during surveys 6 further inland beyond 15 km shows that it must be considered critically endangered (Nowell and Jackson 1996). Currently, it seems that the fishing cat no longer occurs in large parts of its range in India and possibly is extinct in Pakistan (Mukherjee et al. 2010). 90.6% of all fishing cat reports in Bangladesh were during the dry season between November and May, and 9.4% were during monsoon between July and September (Chowdhury et al., 2023). Similarly, in West Bengal, out of 27 fishing cat deaths in 2010 and 2011, 70.37% were in the dry season (Mukherjee et al. 2012).

2.2 Human-Fishing Cat Conflict Situation in Southeast Asia

Bangladesh is known as one of the key countries of the fishing cat (*Prionailurus viverrinus*), which is now recognized as a globally endangered species in response to its potentially rapid population declines in the last decade, primarily due to habitat loss (Sayam et al., 2015). They analyzed media coverage of two major daily newspapers and interviewed local forest officials and conservationists to understand human-fishing cat conflicts, the distribution of human-fishing cat conflicts, current management practices, and public perceptions. They collected a total of 82 fishing cat reports from news articles between January 2010 and March 2013. Collectively, these scientists were unable to determine the status of 38 fishing cats, and they suspect these individuals were rescued and released, killed, or otherwise died in captivity. In addition, the team found at least 10 jungle cats (*Felis chaus*) reported dead from all over the country and one leopard cat (*Prionailurus bengalensis*) reported trapped in Chauddagram, Cumilla, of Chattogram division during February 2012. Of all the media reports, 40.27% were confirmed deaths, 13.88% were rescued and released by the Forest Department, and the status of 45.83% remained undetermined, as there was no information on the conditions of the cats after being captured by locals, or they were taken into custody by the Bangladesh Forest Department (Sayam et al., 2015).

This research group suspected that the human-fishing cat conflicts have primarily occurred due to the degradation of wetland habitat and human encroachments. Shrinking habitat and food shortage have possibly driven these cats to move into human settlements, which compels the local community to react and kill fishing cats. However, during an annual hunting festival by the Santals, three fishing cats and three jungle cats were killed on the 24th of February 2012 in Khoksa upazila, Kushtia, by a group of 15 men of the tribal community.

South Asian wildlife habitat is susceptible to human encroachment for irrigation for agriculture, and aquaculture, and contamination by pesticides. Despite these, direct killing of fishing cats by humans for fur and meat, and poisoning and poaching by farmers for the taking of livestock are also great threats to the fishing cat (Lynda 2001). A survey shows that over 50 percent of Asian wetlands were facing moderate to high degrees of threat, which includes human settlement, drainage for agriculture, effluence, excessive hunting, woodcutting (Nowell and Jackson, 1996), and excessive fishing (Nowell and Jackson, 1996; Dahal and Daha, 2011). In Nepal, a high level of exploitation of wetlands is already increasing in the whole Tarai region with the increase in human population growth rate. In the late 1950s, human settlements rapidly increased throughout the region. Because of intensive farming, distinct blocks of wildlife habitat are interspersed, leading to habitat fragmentation (Smith, 1984), a major threat to the wild fauna. Although there is no permanent human settlement within Chitwan National Park (CNP), there is heavy pressure from adjoining villages for resource use (BPP, 1995a).

CHAPTER III

METHODOLOGY

3.1 Study Area

3.1.1 Geographic Scope

This study focuses on four districts within Sylhet Division: Moulvibazar, Habiganj, Sunamganj, and Sylhet (23°58'–25°12'N; 90°56'–92°30'E). These upazilas are further divided into 323 union parishads. Each union is roughly divided into 9 wards before going to village-level. There are roughly 10,185 villages in the Division. The region encompasses 12,298 km² of the Surma-Kushiyara floodplain, characterized by the haor wetland ecosystem—a network of 217 seasonally inundated depressions covering 6,155 km² (48% of the study area) (CEGIS, 2018; IUCN Bangladesh, 2015).

Geographically the region is surrounded by hillocks (known as tillas) from all three sides except its western plain boundary with the rest of Bengal. In the south of the region (Habiganj, Moulvibazar), eight hill ranges enter the plains of Sylhet running uniformly from the west to the east. They are: Raghunandan, Dinarpur-Shatgaon, Balishira, Bhanugach-Rajkandi, Hararganj-Singla, Patharia, Pratapgarh-Duhalia and Sorrispur-Siddheswar hill ranges. At the center of the region is also an isolated range known as the Ita Hills.

Figure 3. Geographical range of Sylhet division, showing Moulvibazar, Habiganj, Sunamganj, and Sylhet district (Source: Wikipedia).

The region is considered one of the most picturesque and archaeologically rich regions in South Asia. It is home to three national parks: the Lawachara National Park, Khadim Nagar National Park and Satchari National Park—as well as numerous smaller parks and forests such as the Ratargul Swamp Forest, Rema-Kalenga Wildlife Sanctuary. Its burgeoning economy has contributed to the regional attractions of landscapes filled with fragrant orange and pineapple gardens as well as tea plantations. The region has a tropical monsoon climate bordering on a humid subtropical climate at higher elevations. The rainy season from April to October is hot and humid with very heavy showers and thunderstorms almost every day, whilst the short dry season from November to February is very warm and fairly clear. Nearly 80% of the annual average rainfall of 4,200 millimetres (170 in) occurs between May and September.

3.1.2 Haor Ecosystem Dynamics and Protected Areas

Haors are bowl-shaped, floodplain wetlands fed by monsoon rains (June–October) and Himalayan rivers, creating depths up to 10 m. During dry months (November–April), these contract to fragmented beels (permanent water bodies) and marshes (Rahman et al., 2020). Key haors include Tanguar Haor (Sunamganj), Hakaluki Haor (Moulvibazar-Sylhet), Hail Haor (Moilvibazar). The protected areas of the study area include Lawachara National Park, Satchari National Park, Khadimnagar National Park, Barshijora Eco-Park, Tilagarh Eco-Park.

3.2 Methods

3.2.1 Fishing Cat Killing, Rescue, and Release Data Collection

To assemble a comprehensive dataset on fishing cat (*Prionailurus viverrinus*) mortality, injury, rescue, and release, we combined participatory field methods with systematic secondary-data gathering over the period January 2020 to June 2024. This multi-pronged approach ensured that both human–wildlife conflict events and subsequent interventions were captured across the haor landscape of Northeast Bangladesh.

3.2.1.1 Workshop and Expert Consultation

At the outset, a two-day threat-assessment workshop and consultation programme convened wildlife biologists, conservation practitioners, protected-area managers from the Bangladesh Forest Department, and representatives of relevant NGOs (UNDP, IUCN, WCS, WWF, Fishing Cat Survival Alliance, and the Mohamed bin Zayed Species Conservation Fund). Participants reviewed prevailing threats to fishing cats—ranging from poaching and accidental trapping to road-kills and habitat degradation—and helped to frame our threat categorization in line with IUCN Red List guidelines. They also refined the list of data sources and refined the scoring rubric for threat likelihood and severity.

3.2.1.2 Primary Data Collection

Interviews were conducted with farmers, fishers, and duck/poultry owners living adjacent to major rivers, canals, and haor wetlands, particularly in known conflict hotspots. Elicited first-hand accounts of fishing cat encounters, mortalities (e.g., adults and litters caught in snares or water traps), and community-led rescue efforts. Local residents were trained to report any captured or injured fishing cat to the nearest Forest Department office. Field teams (forest staff and university researchers) responded to reports, recorded GPS coordinates, and documented cause of injury or death (e.g. trap type, vehicle strike). Injured cats were transported to the Moulvibazar District Forest Office rescue center; release dates and locations were logged for those successfully rehabilitated. Trap cameras at feeding sites and riparian corridors alerted teams to capture or mortality events.

We maintained a live database—updated monthly—of all fishing cat conflict records, including date, location, age/sex (if known), injury type, and outcome (mortality vs. rescue vs. release).

3.2.1.3 Secondary Data Collection

We reviewed government reports and published rescue logs from the Wildlife Management and Nature Conservation Divisions in Sylhet (Moulvibazar headquarters), which tracked wildlife rescue and release events. Surveyed newspaper archives (2020–2024) and NGO press releases for documented fishing cat poaching or rescue cases. We compiled all recent (2020–2022) peer-reviewed studies, technical reports, and conference proceedings that mentioned fishing cat mortality or rehabilitation in the region. Established formal information-sharing links with Jahangirnagar University (Department of Zoology) and the Bangladesh Forest Department to verify incident dates and outcomes.

3.2.2 Data Collection through Community-Derived Information via FGDs and KIIs

To characterize and quantify human–fishing-cat conflict at the local scale, we employed two complementary participatory methods. By combining FGDs' group-level insights with KIIs' expert perspectives and quantitative questionnaire data, we achieved robust triangulation of human–wildlife conflict patterns (Fig. 8).

3.2.2.1 Focus Group Discussions (FGDs)

A minimum of 20 FGDs were convened across villages adjacent to known fishing-cat habitats. Each session gathered 8–12 participants—farmers, fishers, and duck-rearers—who collectively recounted recent conflict incidents, perceptions of threat, and community-led mitigation efforts. All discussions were audio-recorded (with consent) and key themes were transcribed and coded.

Figure 4. Conducting FGDs and KIIs during the study period: (a) Khadimnagar NP, Sylhet; (b) Azmiriganj, Habiganj; (c) Baniachang, Habiganj; (d) Naboganj, Habiganj; (e) Farhanga Beel, Habiganj; (f) Gandharbapur, Moulvibazar; (g) Satbeel Haor, Asharkandi, Sylhet; (h) Tahirpur, Sunamganj; (i) Bhatera, Moulvibazar; (j) Bangalibazar, Sunamganj.

3.2.2.2 Key Informant Interviews (KIIs) and Semi-structured Questionnaires

3.2.3 Data Collection by Camera Trapping

Camera trapping formed the cornerstone of our strategy to document fishing cat (*Prionailurus viverrinus*) presence, habitat use, and activity patterns across both inundated wetland basins and adjacent upland refugia. Beginning on 7 October 2023 and extending through June 2024, we installed 15 trail cameras (Dark OPS HD Pro X, PATRIOT, and Strike Force XD Pro) in a series of medium-sized (5–10 km²) study blocks. Each block was overlaid with a 1 km × 1 km grid, and we established one camera station per grid cell. By spacing stations at approximately 500 m to 1 km apart—adjusted for terrain complexity and pathway density—we ensured broad coverage while avoiding redundant overlap. This systematic arrangement yielded between 120 and 240 trap-nights at each site, depending on the length of deployment and seasonal travel constraints.

Our primary objectives were threefold. First, we sought to confirm monsoon-driven movements of fishing cats into seasonally flooded haor basins—specifically Hail Haor (Baikka Beel) and Hakaluki Haor—and their use of adjacent upland areas such as Lawachara National Park, Patharia Hill Reserve Forest, and surrounding tea estates. Second, we aimed to assess habitat use and spatial distribution during the dry season when water levels receded, thereby focusing on remaining terrestrial corridors and riparian habitats. Third, we targeted known conflict hotspots—fishery and duck-farm sites where local records indicated annual predation events and sporadic persecution—to quantify both presence and risk.

Figure 5. Camera-trap installation in: (a) Lawachara NP, Moulvibazar; (b) Baikka beel, Moulvibazar; (c) Jagannathpur, Sunamganj; (d) Satchari NP, Habiganj; (e) Khadimnagar NP, Sylhet.

At each station, cameras were mounted 25–30 cm above the ground on sturdy trees, secured with metal Anaconda chains, and oriented to minimize obstruction by vegetation and avoid direct sun glare. We configured each unit to capture three consecutive photographs per trigger event, followed by a 1-minute refractory period, and to concurrently record 30-second video clips. Infrared illumination ensured clear nighttime imagery without disturbing wildlife. All captures were time- and date-stamped automatically, allowing us to reconstruct fine-scale temporal activity patterns and align them with human activity cycles gleaned from concurrent community surveys.

Field teams visited stations every 15 to 20 days to replace batteries, exchange memory cards, and verify camera alignment and functionality. We logged each trap-night as a full 24-hour period of continuous camera operation. Over the course of the study, we accumulated more than 2256 trap-nights across the five focal landscapes. Photographic and video data were meticulously catalogued by site, camera ID, and timestamp. Spatial coordinates of each confirmed fishing cat sighting were integrated into a GIS database, enabling us to map occurrence points and infer movement corridors connecting inundated haor basins with upland forest patches.

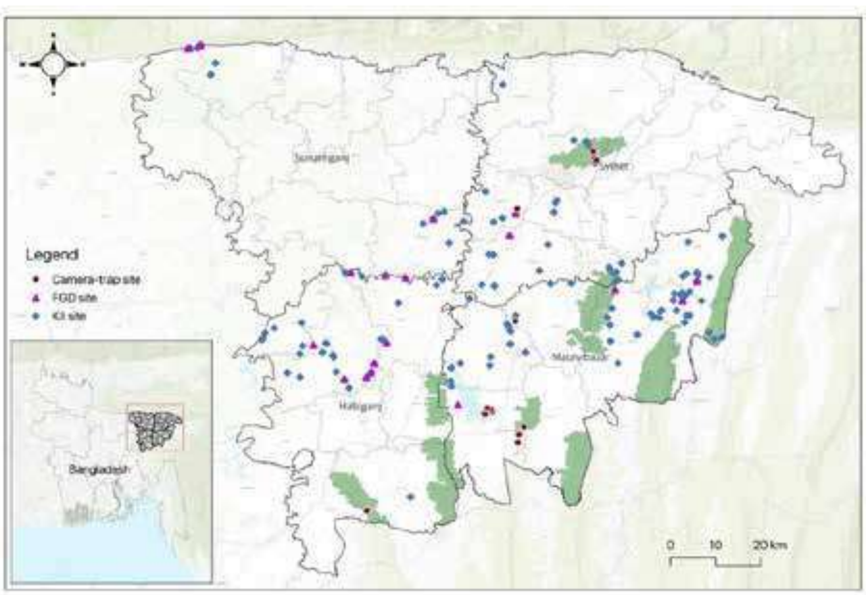

Figure 6. Location of conducting FGD, KII, and camera trap installation during the study period.

Finally, timestamped detections were used to generate diel activity histograms, measuring temporal overlap between fishing-cat activity and human presence—data critical for understanding conflict dynamics and for designing targeted mitigation measures.

3.2.4 Data Collection Using Satellite Collars

Our original protocol called for fitting two adult fishing cats—one male and one female—with high-frequency (144–145 MHz) satellite collars weighing between 200 and 250 g, thereby minimizing load while maximizing battery life and fix-rate. In practice, we succeeded in collaring a single adult male fishing cat that had been humanely captured by local residents in a known conflict zone. Prior to deployment, each collar was programmed with an auto-drop mechanism, designed to release automatically after a predetermined interval, ensuring that the device would fall to the ground and be recoverable without recapture.

In tandem with the satellite unit, we affixed a conventional Lotek™ radio-telemetry collar to the same individual. This dual-collaring approach allowed us to continue tracking movements via VHF signals after the satellite collar's battery life expired, providing uninterrupted monitoring at no additional data-download cost. The Lotek™ collars we employed are internationally recognized for reliability and precision; they delivered positional fixes at planned intervals (ranging from hourly during peak activity to four-hourly overnight) and performed consistently under dense canopy and across flooded floodplain habitats.

Once released, the collared fishing cat was monitored intensively for its first two weeks to confirm successful collar function and the animal's return to normal behavior. Thereafter, we retrieved satellite fixes remotely and conducted weekly VHF-tracking sorties using a handheld yagi antenna and receiver to verify locations, recover dropped collars, and ground-truth habitat features. From these data, we derived detailed maps of seasonal movements, computed home-range estimates using kernel density and minimum convex polygon methods, and identified critical habitat use zones—ranging from core riparian thickets to peripheral upland tea estates.

Moreover, by overlaying collar locations with land-use maps and ground observations, we documented forays into poultry farm and aquaculture pond areas, providing direct evidence of potential conflict hotspots.

Figure 7. Data collection using satellite collar— (a) Rescued male fishing cat for collaring; (b) Lotek radio collar (lite-track IR 250+); (c) Inspecting tranquilized fishing cat; (d) Observing fishing cat after collaring; (e) Collared fishing cat.

3.2.5 Research process flow

Schematic diagram showing the conceptual flow of the approach and methods leading to attaining the anticipated results. Brief description elaborating on the schematic diagram. The proposed research was mainly based on primary data collection from the field (Fig. 10).

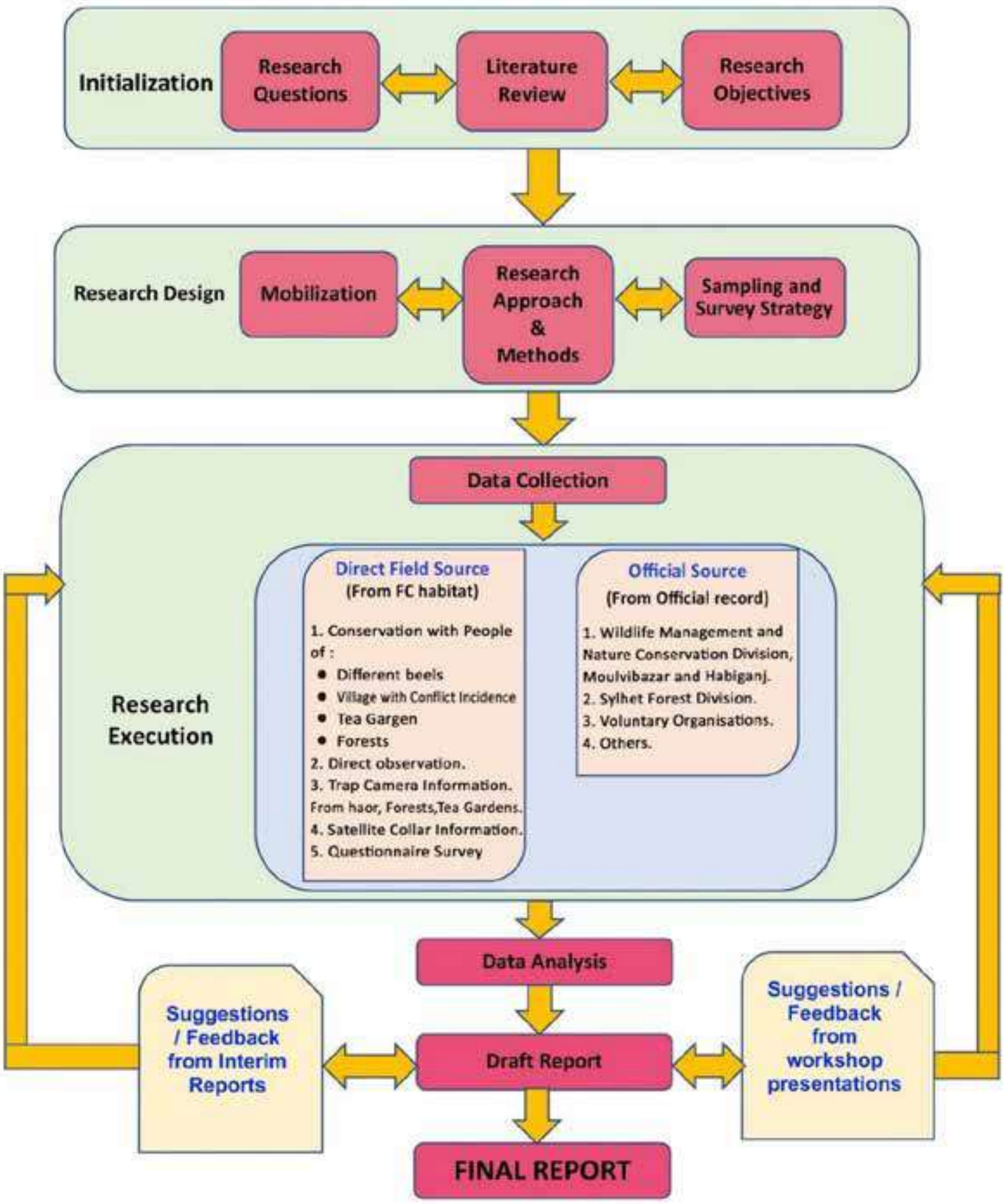

Figure 8. A diagrammatic view of the research process.

3.2.6 Data Analyses

Firstly, data analysis for capture-killing-rescue-release involved categorizing fishing cat encounters depending on the time of the day they were captured or killed, monthly and seasonal (Summer, winter and winter). Age was classified as adult and kitten while sex includes male, female and unknown gender on a yearly basis. Secondly, alive and dead fishing cats were separated yearly along with the number of dead fishing cats that died in rehabilitation facilities and were released back to the wild after recovery. Thirdly, captured or killed fishing cats were categorized depending on what methods people used to capture or kill them and the motive behind the incidents whether they occurred as intentional, opportunistic or accidental. Lastly, data were arranged depending on spatial and demographic status to evaluate the intensity of capturing or killing fishing cats and identify the hotspots in NE.

To assess the presence and activity patterns of fishing cats and other mammalian species across different locations in north-east Bangladesh, camera traps were strategically deployed in selected conflict-prone and ecologically significant areas. Each camera trap was placed at a fixed point and operated continuously for a defined period, capturing motion-triggered photographs and videos of animals that passed within its detection range.

All images and data obtained from the camera traps were systematically compiled and entered into a Microsoft Excel spreadsheet for further analysis. Each entry in the Excel file included metadata such as the date, time, location (with GPS coordinates where applicable), species identity (if identifiable), number of individuals, and any observable behavior. Special attention was given to accurately identifying individual species, particularly fishing cats (*Prionailurus viverrinus*), to determine their presence or absence in each study site.

To quantify the relative abundance of the recorded species, the Relative Abundance Index (RAI) was calculated for each species using the following formula:

$$\mathrm{RAI} = \left(\frac{\text{Number of independent detections of a species}}{\text{Total number of camera trap nights}}\right) \times 100$$

This index provides a standardized measure of how frequently each species appeared in the camera trap photos, allowing comparisons across locations and time periods.

Each photograph was examined carefully to distinguish individual species based on physical features, body size, coloration, patterns, and other distinguishing characteristics. A species checklist was developed separately for each camera trap location, providing an overview of the mammalian diversity in the surveyed areas. These checklists were instrumental in determining site-specific species richness and identifying key areas of conservation concern, especially in relation to fishing cat presence.

Moreover, beyond mere species detection, behavioral observations were also noted from the images captured by the camera traps. Several activities, including nocturnal foraging, scent marking, movement patterns, and interactions with other animals or human-related structures (such as poultry sheds or aquaculture ponds), were recorded whenever evident. These behavioral insights added an additional layer of ecological understanding, helping to interpret species' spatial use and conflict potential in the landscape.

All data collected from structured questionnaires, Focus Group Discussions (FGDs), and Key Informant Interviews (KIIs) were compiled and analyzed to assess the dynamics of human–fishing cat conflict and human perception about the fishing cat across four districts in northeastern Bangladesh. Responses from 32-question surveys were manually reviewed, coded, and entered into Microsoft Excel. Closed-ended answers (e.g., sighting frequency, seasonal patterns, conflict type, capture method) were analyzed using descriptive statistics such as percentages and frequency counts. Open-ended responses, including local names, ecological roles, reasons for conflict, and perceptions about fishing cats, were categorized thematically to identify common community narratives. Each response was linked with its GPS location for spatial mapping of conflict hotspots. Analysis focused on estimating the average number of adult and juvenile fishing cats captured or killed annually, identifying peak conflict seasons, and understanding capture methods—such as metal cage traps. Additional focus was given to evaluating community awareness of the Wildlife (Conservation and Security) Act, 2012, and the role of forest officials in rescue or release events. The combined quantitative and qualitative approach provided a clearer understanding of conflict trends, enabling evidence-based recommendations for conflict mitigation and conservation planning.

CHAPTER IV

RESULT AND DISCUSSION

4.1 Fishing cat killing, capture, rescue, and release (Objective 1)

4.1.1 Capture and killing of the fishing cat as a result of the conflict between humans and FC

The total number of fishing cat capture or death incidences recorded was 63, including 93 individuals from January 2020 to June 2024, among which 55 (59%) were adults and 38 (41%) were kittens throughout the study area (Fig. 1). Out of 55 captured/rescued adult FCs, 18 were male individuals, 14 were females, while most (61) of their gender could not be confirmed in this study. The highest number of fishing cat-human conflicts enlisted was 18, including 30 individuals that occurred in 2020, while it was the lowest (11 individuals) in 2024 (Fig. 12).

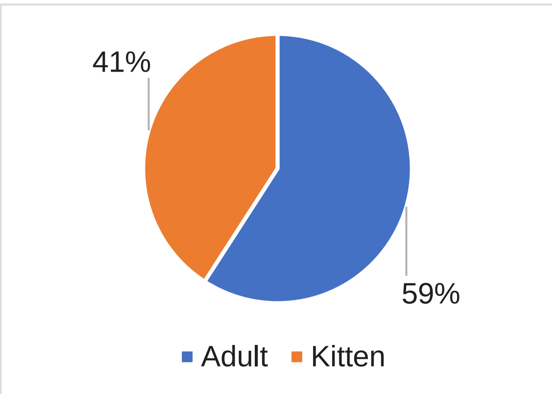

Figure 9. The ratio of enlisted fishing cats from 2020–2024 (June*).

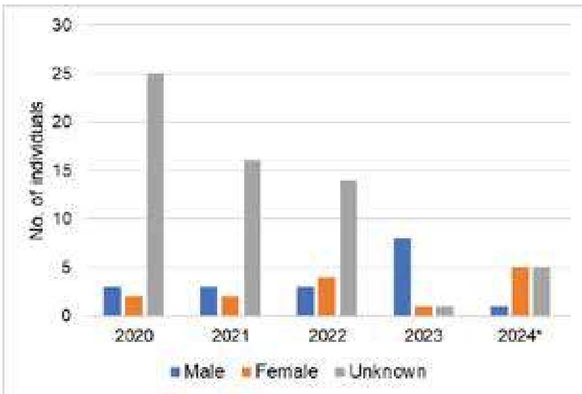

Figure 10. Fishing cat captured/death incidence from 2020–2024 (June).*

The highest capture numbers of fishing cats were observed in the winter season, with 51 individuals caught in 34 incidents. During summer, 16 individual fishing cats were captured in 12 separate incidents, while in monsoon, the number of individuals increased to 26, with 17 incidents reported.

Table 1. Seasonal distribution of fishing cat capture incidents and individuals

Season	No. of incidents	No. of individuals captured
Summer	12	16
Monsoon	17	26
Winter	34	51
Total	63	93

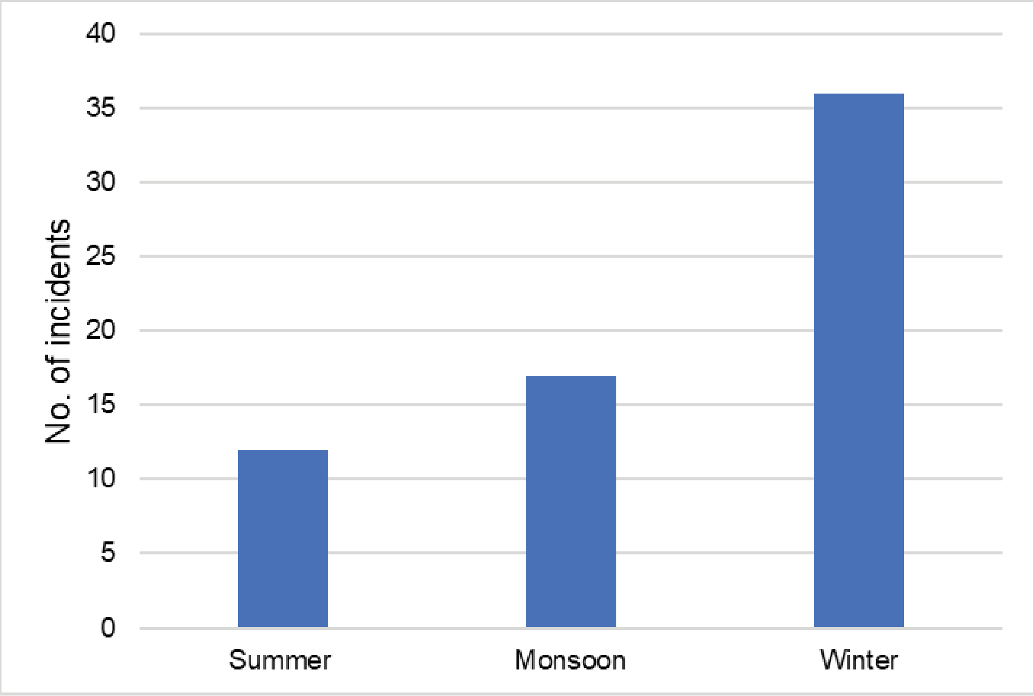

Figure 11. Seasonal fishing cat capture/death incidence from 2020–2024 (June*).

The study denotes that the highest number of fishing cats, i.e., 22 and 14 individuals were captured or killed in January and December, respectively while the lowest (2 individuals each) were in July and August (Fig. 3). The number of captured or killed FCs was highest (72.04%) in the late monsoon to the mid-dry season from October to February than mid-dry to late monsoon from March to September.

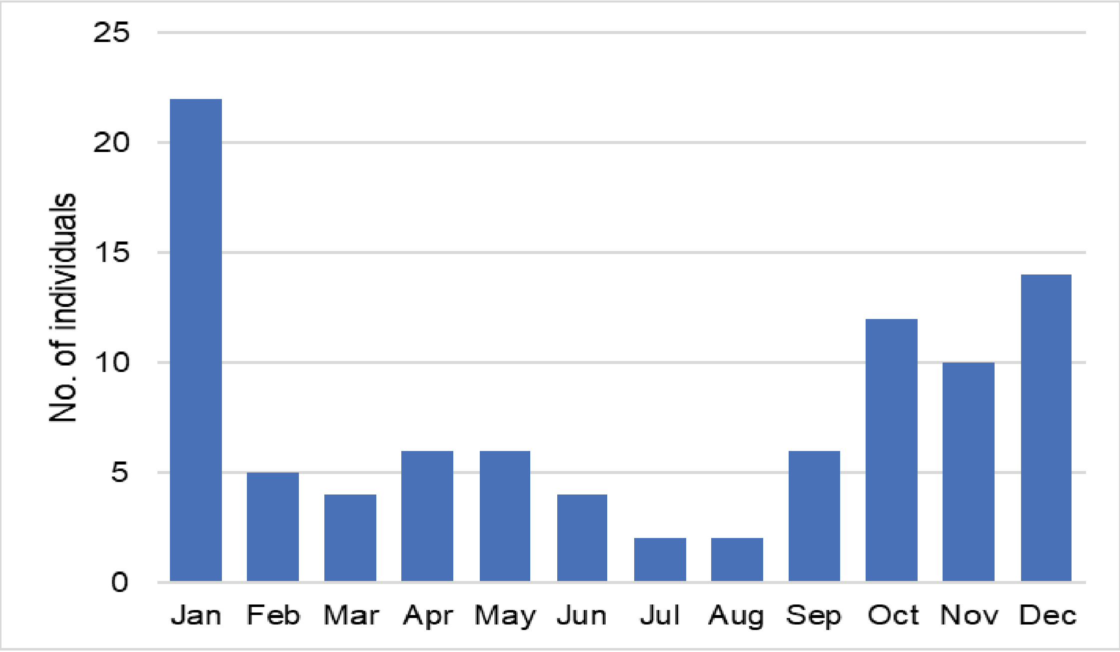

Figure 12. Monthly fishing cat capture/death incidence from 2020–2024 (June*).

It was also calculated that the highest (30 individuals) number of fishing cats were captured by various means between 00:01 and 6:00, whereas the lowest (1 individual) was between 18:01 and 20:00 (Fig. 4). Although there were 6 individuals found captured in early midnight between 20:01 and 22:00 as well as 4 individuals in midnight between 22:01 and 00:00, indicating that fishing cats move more in the early midnight and late night while movement was observed low in the evening and daytime.

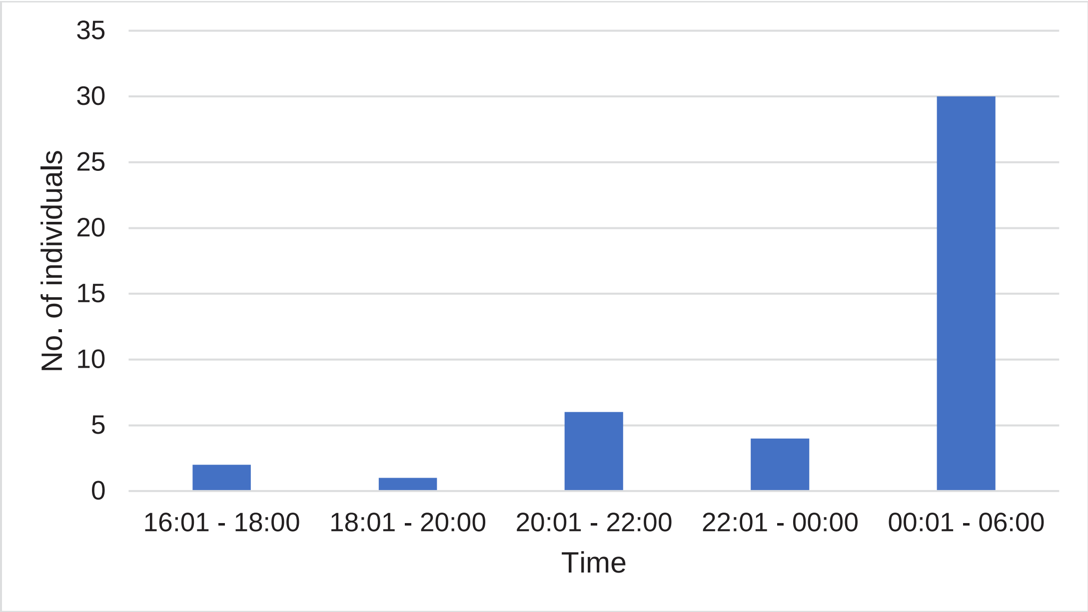

Figure 13. Time of fishing cat capture incidence from 2020–2024 (June*).

During the study, 13 incidents involving 38 individual fishing cat kittens were captured and recorded from January 2020 to June 2024. In total, 25 kittens were given back to their mother with the help and joint effort of the forest department and the local community as well as the support of the law enforcement agency. A maximum number of kittens (11 individuals) were confirmed to return to their mothers in 2022. On the contrary, a single kitten out of 14 was confirmed to meet its mother again captured in 2020.

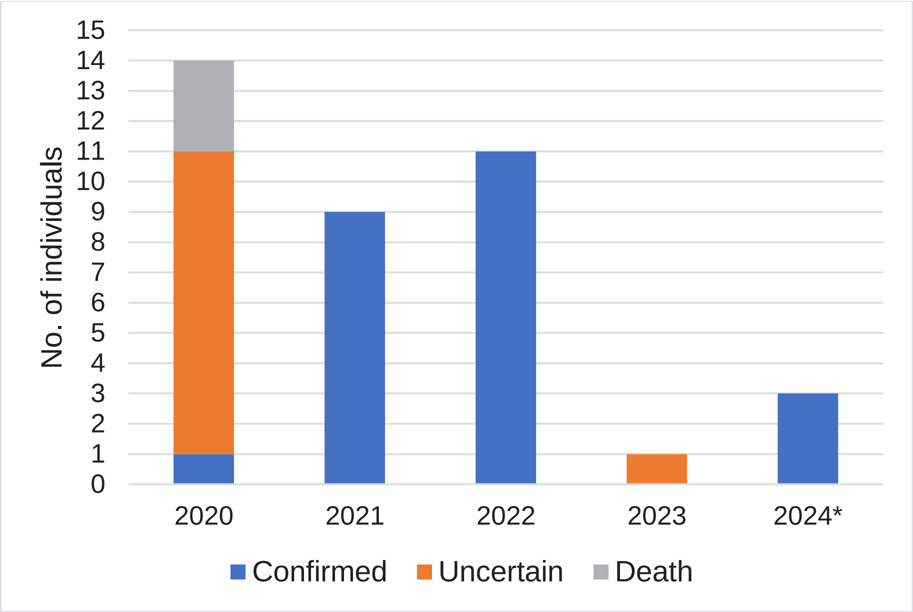

Figure 14. No. of individual fishing cat kittens released back to their mother (2020–2024)

Figure 15. (a) captured kitten near Osmani International Airport, Sylhet in 2020; (b) mother fishing cat with newborn kittens in the metal cage trap in Jagannathpur, Sunamganj; (c) a road accident on the Sreemangal-Moulvibazar highway in Moulvibazar took the life of a female fishing cat; (d) captured kittens were rescued from Kulaura, Moulvibazar; (e) releasing rescued fishing cat from Jaygirdarpur, Golapganj, Sylhet; (f) captured fishing cat was rescued from Doloipara, Dakshin Surma in Sylhet.

Among the captured fishing cats in 2020, 28 individuals were found alive, which was the highest along with 2 dead. On the contrary, 25 individuals were alive at the time of release and 3 of them were dead in 2020. Minimum (9) live capture occurred in 2023, along with 1 death incidence while 8 individuals were released and the rest of them were dead (Table 1).

Table 2. Year-wide fishing cats rescue and release records from 2020–2024*

Year	Rescued alive	Dead body recovered	Died in rehabilitation	Released
2020	28	2	3	25
2021	20	1	2	18
2022	18	3	2	16
2023	9	1	1	8
2024*	11	0	2	9

43 individuals (46.24%) of the FCs covering a major portion were trapped by the local people using metal cages with bait, while the number of fishing cats captured occurred through enclosing, netting, and road accidents were 25 (26.88%), 14 (15.05%) and 9 (9.68%) respectively (Fig. 6). 100% of the fishing cats survived, captured by enclosing and successfully released at the same place from where they were captured. Moreover, 81.72% of the captured fishing cats out of the total survived and were released in their respective areas.

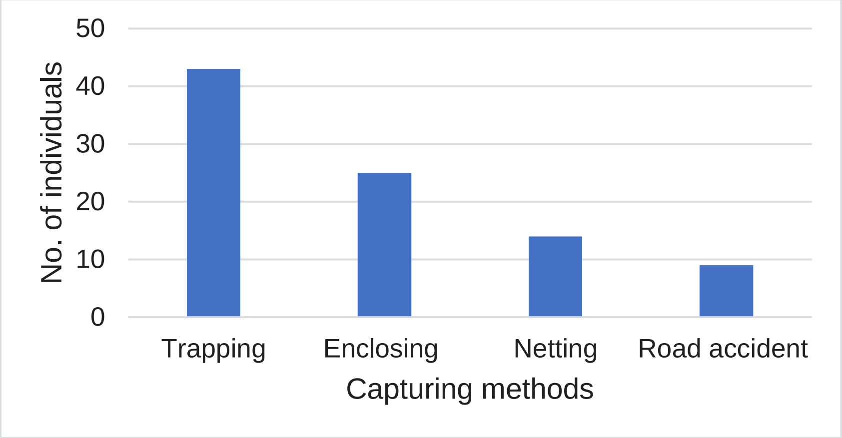

Figure 16. Capturing of fishing cats using different methods from 2020–2024.

The majority (15 individuals) of the fishing cats were captured intentionally in 2020, whereas only 4 individuals were in 2022. The highest (15 individuals) number of fishing cats were caught in 2022 employing several opportunistic ways, which was lowest in 2023 capturing only 1 individual.

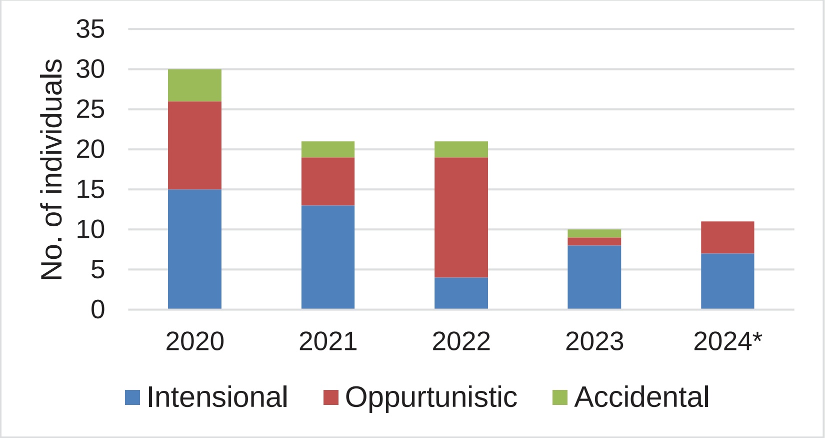

Figure 17. Human nature towards killing fishing cat.

A maximum number of fishing cats (35) were captured from the Moulvibazar district following Habiganj (26), Sylhet (17) and Sunamganj (15) (Fig. 7). Highest casualties of 13 individuals were found in Moulvi Bazar Sadar upazila of Moulvibazar district following Nabiganj (11) and Baniachong (8) upazila of Habiganj district and Jagannathpur (8) upazila of Sunamganj district (Table 2).

Table 3. Spatial and demographic status of rescued fishing cat in north-east, Bangladesh.

District	Upazila	No. of individuals			Age category		Conditions of rescued FC	
		Male	Female	Unknown	Adult	Kitten	Alive	Dead
Moulvibazar	Kamalganj	–	–	4	–	4	4	–
Moulvibazar	Kulaura	1	1	4	3	3	5	1
Moulvibazar	Moulvi Bazar Sadar	4	2	7	7	6	13	–
Moulvibazar	Rajnagar	–	–	6	2	4	6	–
Moulvibazar	Sreemangal	–	1	5	3	3	4	2
Sylhet	Balaganj	2	2	1	5	–	5	–
Sylhet	Beani Bazar	1	–	–	1	–	1	–
Sylhet	Bishwanath	1	–	1	2	–	2	–
Sylhet	Companiganj	–	–	2	2	–	2	–
Sylhet	Dakshin Surma	–	–	1	1	–	1	–
Sylhet	Fenchuganj	1	–	2	3	–	1	2
Sylhet	Golabganj	–	–	1	1	–	1	–
Sylhet	Sylhet Sadar	–	–	2	1	1	1	1
Habiganj	Baniachong	–	1	7	2	6	8	–
Habiganj	Chunarughat	–	–	3	–	3	3	–
Habiganj	Madhabpur	–	1	3	1	3	4	–
Habiganj	Nabiganj	–	–	–	10	1	–	–
Sunamganj	Derai	1	–	1	2	–	2	–
Sunamganj	Dharmapasha	1	2	–	3	–	3	–
Sunamganj	Jagannathpur	2	2	4	4	4	8	–
Sunamganj	Jamalganj	–	–	1	1	–	1	–
Sunamganj	Tahirpur	–	–	1	1	–	–	1

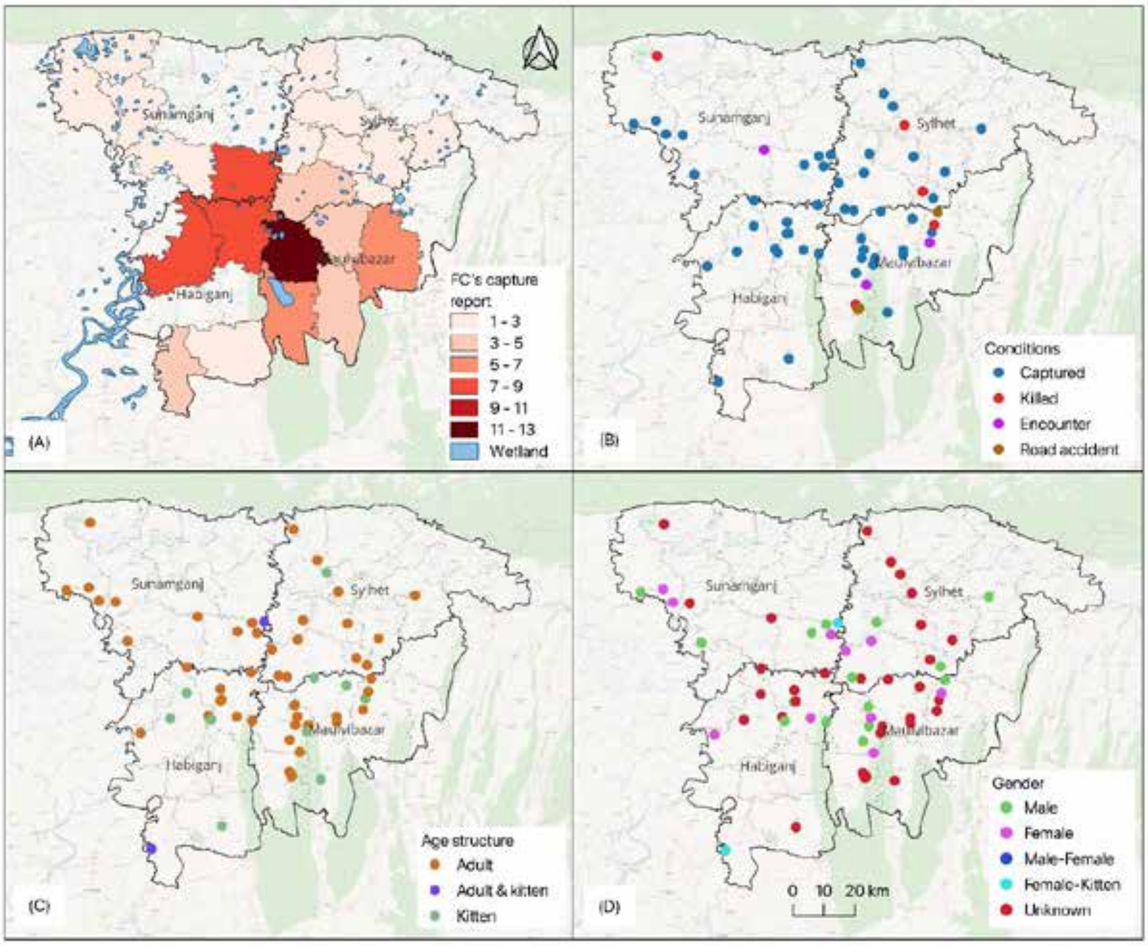

Figure 18. Fishing cat-human conflict occurrence, (A) fishing cat's capture report; (B) condition of fishing cats during seizing; (C) age structure of the fishing cats; (D) gender-wise capture distribution.

4.1.2 Discussion on Killing, Capture, Rescue and Release of the Fishing Cat

In the study region, we recorded 63 fishing cat capture or death events, including 93 individuals between January 2020 and June 2024, of which 55 (59%) were adults and 38 (41%) were kittens. Of FCs that were killed or captured, 19% were male and 16% were female; nevertheless, the sex of the majority of fishing cats (65%) could not be identified in our study due to the immediate disposal of the bodies after killing without identification. Besides, sex identification could not be possible to carry out for the majority of the kittens captured or killed because of their tenderness. Additionally, male individuals capturing events were more significant than females (Phosri et al., 2021). Vagrant habits of male fishing cats could be the reason for this high killing or capture rate in Bangladesh as well as in other countries considered as home ranges of fishing cats (Cutter, 2014; Ahmed et al., 2017). However, the limited information on the gender of fishing cats killed, captured, or rescued highlights the need for more research in this area. More data could help conservationists better understand the threats facing fishing cats and develop more effective conservation strategies.

The late monsoon to mid-dry season (October–February) showed the largest number (in 72.04% of cases) of fishing cat captures according to our study. Media reports on fishing cat incidents from 2010–2013 also found that 90.6% of all fishing cat reports occurred during the dry season (Chowdhury et al., 2015). A study in Hakaluki Haor suggested a similar pattern of human-fishing cat encounters from 2016–2019 where a higher number were reported in the dry season months of November to February (Eva et al., 2022). Another study in West Bengal, India, further showed a consistent result where 70.37% of fishing cat deaths in 2010–2011 occurred during the dry season (Mukherjee et al., 2012). This is because the shrinking water bodies during the winter season force fishing cats to search for prey in areas closer to human settlements, increasing the likelihood of conflict (Choudhury and Nishat, 2005; Rahman and Bhuiyan, 2020). The livelihood activities that occur in the study region during the dry season, including rice cultivation, chicken farming, cattle grazing, day work (in brick and crop fields) and fishing in waterbodies that persist in low depressions, were another reason for occurring conflict (Haider, 2013; Kamal et al., 2018).

We estimated that the greatest number of fishing cats (30 individuals) were caught by different methods between 00:01 and 6:00, while the smallest number (1 individual) occurred between 18:01 and 20:00. So, it can be proved that fishing cats move more in the early hours of the morning and late at night, while they move less in the evening and during the day, as documented by some earlier studies (Chakraborty et al., 2020; 2020; Sadik and Akash, 2024). However, some studies also showed the activity of fishing cats in the evening time, marked as a pick hour of their capture (Ganguly and Adhya). This suggests fishing cats may adjust their activity patterns with a peak hour at midnight to early in the morning to avoid humans, becoming more nocturnal in areas with high human activity.

The local people used metal cages with bait to trap and capture 46.24% of the FCs in the current study. They usually make these kinds of metal cages from the local market costing 8000–10000 BDT and install them beside their domestic farm to capture wild carnivores including fishing cats. Villagers in West Bengal, India also use the same types of trap cages to capture fishing cats (Adya, 2011). Wire-mesh traps have also been documented in South India (Naidu et al., 2015). Fish farmers in Koshi Tappu Wildlife Reserve, Nepal, reported using snares and live electric wires around their fish ponds for retaliatory killings of fishing cats (Mishra et al., 2021**a**). Furthermore, through enclosing 26.88% and using nets 15.05% of fishing cats were captured or killed in our study. When fishing cats were encountered, locals would spontaneously gather and beat FCs to death using available tools. This method was also observed in the killing of other carnivores in Bangladesh (Inskip et al., 2014) and India (Kolipaka, 2018). Road kills (9.68%) were seen in several areas in NE where at least 9 fishing cats were killed when crossing roads during our study. Mishra et al. (2021) also noted fishing cats as a victim of road kill.

Human-fishing cat conflicts were enlisted ranged from the highest (30 individuals) in 2020 to the lowest (11 individuals) in 2024. Of all the fishing cats that were caught in 2020, 28 were discovered alive, which was the highest number and two were found dead. However, three of them were deceased, while 25 were still living at the time of release. In 2023, there were at least 9 live captures and one fatality; 8 of the FCs were freed and the remaining individuals died. Fishing cats were usually kept in the Wildlife Rescue and Rehabilitation Centre of the Forest Department of Bangladesh after rescuing them for further assessment. However, the treatment facilities were not up to standard level before releasing them into the wild again. In the meantime, forest officials of NE seized several metal cages (at least 5) used as traps when they rescued fishing cats.

Simultaneously, the proactive role of forest officials in rescuing trapped fishing cats during our study time, awareness activities including the distribution of a fishing cat conservation leaflet, describing the importance of fishing cats in the ecosystem etc. were also executed which ultimately reduced the number of captures or killings of fishing cats. Forest officials, in collaboration with local conservation organizations, frequently reached capture sites promptly. They engaged with local communities, consulting and encouraging them to release the captured fishing cats at the same spot or ensure their safe rescue without causing injury. This proactive approach by forest officials was a significant observation within the study period.

In our study, people intentionally captured the majority of fishing cats, such as totaling 15 individuals in 2020, whereas the capture number decreased to only 4 individuals in 2022. These intentional captures or killings are a common theme, particularly in those areas where fishing cats and humans share space and resources (Mukherjee et al., 2016; Mishra et al., 2021**b**; Phosri et al., 2021). What is worth emphasizing is that most of the retaliatory killings are announced due to the sum of the fishing cats as dangerous for their livelihood (Adya, 2011; Kolipaka et al., 2019). Besides, fishing cats are blamed for preying on poultry, fish, goats and other domesticated animals (Mishra et al., 2021**a**; Phosri et al., 2021). Some people also kill fishing cats out of fear, sometimes misidentifying them as tiger cubs or other dangerous animals (Chowdhury et al., 2015; Sultana et al., 2022). Moreover, people sometimes capture fishing cats opportunistically as we recorded a minimum of 15 individuals to be caught in several locations in NE. An annual hunting festival by the Santal community where three fishing cats and three jungle cats were killed in February 2012 (Chowdhury et al., 2015). The festival involves hunting wildlife and the timing coincides with a period when fishing cats may be more vulnerable due to seasonal changes in their habitat. One study in Thailand found that poaching was a major threat to fishing cats, with 84% of radio-collared fishing cats being killed in an opportunistic manner (Mukherjee et al., 2016) and interviews revealed that poisoning was the most common cause of death in India (Mukherjee et al., 2012; Rana et al., 2022).

We separated our data depending on the District and Upazila level to pinpoint the hotspots of human-fishing cat conflict occurrence. The reasons behind the high and low conflict zones were also identified. The highest number (35) of fishing cats was captured in Moulvibazar district while Sunamganj stayed in the last position (15). We estimated Moulvi Bazar Sadar upazila of Moulvibazar district had the highest number of casualties, with 13 fishing cats, followed by Nabiganj (11) and Baniachong (8) upazila of Habiganj district and Jagannathpur (8) upazila of Sunamganj district.

The entire haor region of NE, encompassing the upazilas of high conflicting events in Moulvibazar, Habiganj and Sunamganj is a major contributor to fish production (CEGIS, 2012; Shaik and Islam, 2018). It boasts a fish habitat area of roughly 966,900 hectares, yielding approximately 0.432 million tons of fish annually (CEGIS, 2012). Which is why, haors with their intricate network of beels, rivers and canals offer an ideal environment for fishing cats to thrive (Khan and Ahmed, 2017). Baikka Beel specifically mentioned as a fishing cat's habitat as a significant number of fish culture farms present, exemplifies this relationship (Hasan et al., 2013). Its vast size and abundance of both natural and reared fish make it particularly attractive to this feline (Prosoil Foundation Consultant, 2016**b**; Khan and Ahmed, 2017). Also, haors particularly in these upazilas are characterized by their distinctive low-laying bowl-like shape, allowing for water accumulation during the rainy season (Talukder and Huda, 2021) which is lacking in other areas of our study. Fishing cats might have more easy access to their prey in these upazilas than others because of these low-depressed waterbodies.

Swamp forests are particularly significant in the Haor region, dominated by species like Hijal (*Barringtonia acutangula*) and Koroch (*Pongamia pinnata*). They are crucial for fish breeding, bird habitat and overall biodiversity (Prosoil Foundation Consultant, 2016**a**; Rahman, 2016) which act as a front ground for fishing cats. Besides, semi-evergreen forest cover surrounding wetlands plays a crucial role in their ecology (Thompson and Balasinorwala, 2010; Rahman et al., 2021). This connectivity provided by forest patches might allow fishing cats to move between different wetland areas. Meanwhile, the livelihoods of communities surrounding the haors are closely tied to these wetlands (Fox et al., 2013; Iqbal et al., 2015). They rely on them for fishing, agriculture, grazing livestock, and harvesting various resources (Dev, 2011; Prosoil Foundation Consultant, 2016**c**). That’s why, human activities within these landscapes can significantly impact fishing cat populations and the highest capture or killing occurs making these upazilas as hotspots for human-fishing cat conflict.

North-east Bangladesh is characterized by a pronounced human–fishing-cat conflict, driven primarily by the species’ incursions into poultry farms and occasional misidentification or fear-induced retaliation. Over the past four and a half years of systematic monitoring, local records indicate that an average of twelve adult fishing cats are captured annually across the four study districts, with a minimum of eight kittens likewise trapped or observed by residents each year. Notably, 70% of adult captures in this haor landscape result from metal-cage traps set around fishery ponds and duck farms; these devices are deployed ostensibly to protect valuable poultry and aquaculture assets. Although rare, documented cases include a solitary instance of a fishing cat preying upon a goat and four confirmed attacks upon human beings—incidents which, upon closer examination, were often precipitated by the inadvertent removal of a mother cat or her young, thereby provoking defensive or disoriented behavior.

4.2 Identifying Fishing cat habitats around the haor landscapes in the northeastern Bangladesh through camera trapping (Objective 2)

4.2.1. Findings from the captured camera trap data

4.2.1.1 Mammal’s diversity recorded in Lawachara National Park

We set 4 cameras in Lawachara National Park covering 151 Camera traps night (CTN) latitudes between N

24.282955° to 24.318468° and longitudes E 91.776599° to 91.789897°.

From the study, we got total 131 independent species records including 7 species of mammals in this study period. But there was not a single encounter of fishing cat in LNP, though other mammals (Table 1) have been captured in the camera trap such as following the list with independent records Crab-eating mongoose (9), Golden jackal (1), Leopard cat (5), Malayan crestless porcupine (6), Muntjac (78), Pig-tailed macaque (16), Rhesus macaque (7), Wild boar

(9) etc. Encounter rate of Muntjac and Pig-tailed macaque was significant. Relative abundance of muntjac was higher (51.66) followed by pig-tailed macaque (10.60), wild boar (5.96), crab-eating mongoose (5.96) and rhesus macaque (4.64). Presence of golden jackal was very low.

Figure 19. Camera trap captured Leopard cat (left) and Wild boar (right) in LNP.

From the previous record of Bangladesh Forest Department, a large number of fishing cats were released in Lawachara National Park which were rescued from nearest villages and haor or even from other districts but we didn't get any capture record from our current survey. They might have shifted to other places like to any wetlands or haor.

Table 4. RAI of mammals captured by camera trap in Lawachara National Park

Species ID	CTN	Encounter	RAI
Crab-eating mongoose	151	9	5.96
Golden jackal	151	1	0.66
Leopard cat	151	5	3.31
Malayan crestless porcupine	151	6	3.97
Muntjac	151	78	51.66
Pig-tailed macaque	151	16	10.60
Rhesus macaque	151	7	4.64
Wild boar	151	9	5.96

4.2.1.2 Mammal's diversity found in Satchari National Park

We were able to collect data from 132 camera traps night (CTN) and Total 11 mammalian species (Table 2) were detected by camera-trap in Satchari National Park though no single fishing cat evidence was detected in this area. Akash and others also did not find any fishing cat in Satchari National Park during his camera trapping event in 2020-21 (Akash et al. (2022).

Figure 20. Camera trap captured Malayan Crested Porcupine (top-left) and Large Indian Civet (top-right) and Common Palm Civet (bottom-left) in SNP.

The frequency of encounters with pig-tailed macaques was the highest, followed by large Indian civets, rhesus macaques, and wild boars. In terms of relative abundance, the following table denotes the value along with the encountered rate of the specieses.

Table 5. RAI of mammals captured by camera trap in Satchari National Park

Species ID	CTN	Encounter	RAI
Common palm civet	132	6	4.55
Crab-eating mongoose	132	4	3.03
Golden jackal	132	2	1.52
Leopard cat	132	5	3.79
Malayan crestless porcupine	132	12	9.09
Muntjac	132	98	74.24
Pig-tailed macaque	132	236	178.79
Rhesus macaque	132	112	84.85
Wild pigs	132	110	83.33
Large Indian civet	132	146	110.61
Malayan crested porcupine	132	9	6.82

4.2.1.3 Mammal's presence in Fisheries ponds near Baikka Beel

Another study was conducted around the fishery project near Baikka beel, Srimongal which is located in Moulvibazar district. We placed 7 camera traps in fisheries ponds next to baikka beel from latitudes 24.354527° to 24.347839° and longitudes 91.718452° to 91.704454°. The objective was to find out any presence of fishing cat there to catch fishes when the Baikka beel is flooded. In fact, the Baikka beel becomes overflooded and expanded in the monsoon and post monsoon period when catching fish id difficult for fishing cat. One of those trap cameras, we set under the light into the fisheries pond (GPS coordinates: 24.361584° to 91.709464°) to know the effect of light on fishing cat. From our study, we found that fishing cat avoided the light to catch their prey from the same location as unlike other camera trap data.Total camera-trap night was 502 and detected species only 4 species of mammals such as fishing cat (370 independent records), jungle cat (9 independent records), golden jackal (494 independent records) and Indian grey mongoose (1 record). Encounter rate of golden jackal was 494 which was higher than fishing cat which was 370, relative abundance of fishing cat was high (73.71) due to fish availability, relatively safe habitat for breeding and survival. Beside the relative abundance of golden jackal higher (98.41) than fishing cat.

Figure 21. Camera trap captured Golden Jackal (top-left) with fish in mouth and Fishing cat (top-right and bottom-left) in Fisheries pond near Baikka beel.

Table 6. RAI of wild mammals around Fisheries ponds near Baikka Beel

Species ID	CTN	Encounter	RAI
Fishing cat	502	370	73.71
Golden Jackal	502	494	98.41
Jungle cat	502	9	1.79
Indian grey mongoose	502	1	0.20

Encounter rate showed that photos of fishing cat were captured everyday meaning their frequent presence in that area. Moreover, presence of jungle cat and Indian grey mongoose were also detected in this area. A golden jackal also captured with a large fish in its mouth.

4.2.1.4 Mammal’s presence in Khadimnagar National Park

Khadimnagar National Park was established in 2006 under Bangladesh Wildlife (Preservation) Act,1974, which is situated at15 km away to the northeast of Sylhet City. The park area is about

678.80 ha with natural hill forest of the Khadimnagar Reserve Forest.

There is a primary estimation of 26 mammal species, such as the macaque, capped languor, fishing cat, civets, occurring in this forest. Three Camera-traps were installed from 16th of February to 18th of April 2024, to detect the fishing cat presence in this forest. Unfortunately, one of the Cameras was stolen during the study. Total Camera-trap-night was 124. Mammals such as Rhesus macaque, Crab- eating Mongoose, Ferret Badger, Golden Jackal, Large Indian Civet and Common palm Civet were detected, though there was no sign of fishing cats in any of the cameras deployed. Encounter of Common palm Civet was 9, which was higher. Relative abundance of Common palm Civet was higher (7.26) where it was lowest for Rhesus Macaque (0.81).

Table 7. RAI of wild mammals in Khadimnagar National Park

Species ID	CTN	Encounter	RAI
Rhesus macaque	124	1	0.81
Crab-eating Mongoose	124	4	3.23
Ferret Badger	124	3	2.42
Golden jackal	124	4	3.23
Large Indian Civet	124	3	2.42
Common palm Civet	124	9	7.26

4.2.1.5 Poultry farm at Doloipar, Sylhet under Shah Paran Thana near Khadimnagar NP

The Khadimnagar forest is surrounded by six tea estates. Although there is an ethnic group (the Patra Samproday) next to the forest, forest communities are not part of the Reserved Forest.

Peoples claimed that their livestock fisheries pond were attacked by fishing cat in several times. To detect whether it is fishing cat or other, two camera-traps were deployed in two different poultry farms in Doloipar village beside the forest. Both the cameras were there for 124 camera- trap nights in total, the distance between two cameras was approximately one kilometer. But no fishing cat were detected there too. Mammals such as Golden jackal, jungle cat and large Indian civet were found in cameras. Highest abundance was 100 for golden jackal whereas it was lowest for large Indian civet (3.23).

Table 8. RAI of wild mammals in and around Poultry farm at Doloipar, Sylhet under Shah Paran Thana

Species ID	CTN	Encounter	RAI
Golden Jackal	124	124	100.00
Jungle cat	124	7	5.65
Large Indian Civet	124	4	3.23

4.2.1.6 Farhanga Beel, Moulvibazar

Farhanga beel is located in Moulvibazar sadar. During monsoon, though this beel remain under water, various crops are cultivated and livestock are reared during dry season. There were several evidence of fishing cat presence from this area and often captured by the local people in recent years. People claimed that fishing cat attacks their domestics and commercial duck farms. Trap Cameras were deployed beside a local village dwellers farm from 15th of February to 16th of April, 2024, but could not captured any photographs of fishing cat. Abundance of both jungle cat (69.35) and golden jackal (56.45) were high in this area. It could be possible that fishing cat mainly remain at the surrounding area of this beel and sometimes they enter into locality in vain.

Table 9. RAI of wild mammals in Farhanga Beel, Moulvibazar

Species ID	CTN	Encounter	RAI
Golden Jackal	62	35	56.45
Jungle cat	62	43	69.35

4.2.1.7 Camera trapping in front of a Chicken farm at Rojokpur in Biswanath

We deployed 1 CT Infront of a Chicken farm to investigate the carnivore’s presence near the farm which is situated at Rojokpur union (N-24.82076 E-91.77482) in Bishwanath upazila. Placed camera for 40 Camera trap nights (CTN). This camera was active from 17:00 to 6:00 to avoid human disturbance and confidential issues for the women.

From our camera trapping, we got only jungle cat (4 times) and golden jackal (5 times) from this site. No Fishing cat were captured in this time. From the previous record, there is a capturing record of fishing cat by a trap in the same place.

For more interesting and amazing information is that, the jungle cat jumped many times into the cage of farm to catch the chicken but he failed because of strong nets and well set-up the poultry cage. From this study we may suggest to the authority of farm for making strong cages with net to mitigate human-carnivore conflicts. Maybe they can reduce their economic losses by attacking carnivores.

Figure 22. Jungle cat was waiting for its meal, meanwhile was trying to enter into the cage, but failed.

4.2.1.8 Camera trapping at Vatera, Kulaura tea garden

Bhatera Union, located in Kulaura upazila of Moulvibazar district, lies west of the scenic Hakaluki Haor. This area is known for its natural beauty, characterized by a mix of flat lands, hills, tea gardens, rubber plantations, haors (wetlands), canals, and rivers. Bhatera Union is also rich in natural resources such as natural gas, tea, and rubber.

Camera Trapping Survey: Bhatera has a long heritage and is known for its diverse ecology. To study the presence of fishing cats during the wet season, two camera traps (CTs) were set in the Mominchara tea garden near Hakaluki Haor. The survey was 124 camera trap nights (CTN). The coordinates for the traps were 24.64833 to 24.65234 latitude and 91.98524 to 91.98456 longitude.

Figure 23. Camera trap evidenced Fishing cat (top-left), Small Indian Civet (top-right), Golden Jackal (bottom-left), and Jungle cat (bottom-right) in Mominchara Tea garden, Vatera, Moulvibazar.

One camera trap was placed under a tea plant adjacent to the hills, while the other was near a fish pond in the tea garden area. The data collected revealed the presence of several species including Fishing cat (21%), Jungle cat (8%), Golden jackal (63%), Small Indian civet (1%) and Rhesus macaque (8%). The golden jackal appeared most frequently, while the small Indian civet was spotted only once. Continued monitoring would likely provide more insights into the region's wildlife.

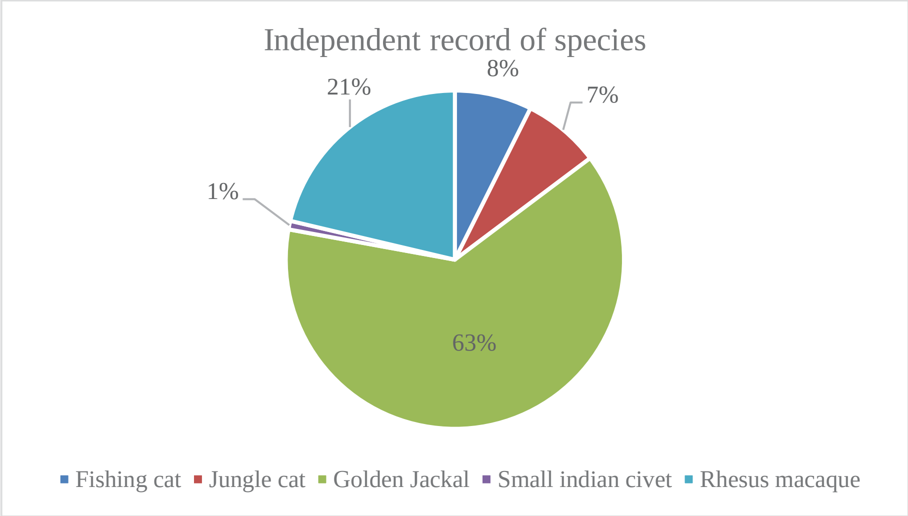

Figure 24. Captured species at vatera, Kulaura

4.2.1.9 Camera Trapping Report: Satchari National Park (Phase 02)

From July 12, 2024, to September 13, 2024, we conducted a second phase camera trapping in Satchari National Park, utilizing three trap cameras over a total of 189 camera trap nights (CTN). The goal of this survey was to assess the mammalian diversity and the overall condition of the habitat. Unfortunately, fishing cats were not detected during this phase again.

Species Detected: A total of five mammalian species were captured on this camera trapping:

Wild pig: 99 independent records (62% frequency of encounters), Large Indian Civet: 31 records (19%), Rhesus macaque: 27 records (17%), Leopard cat: 2 records, Pig-tailed macaque: 1 record.

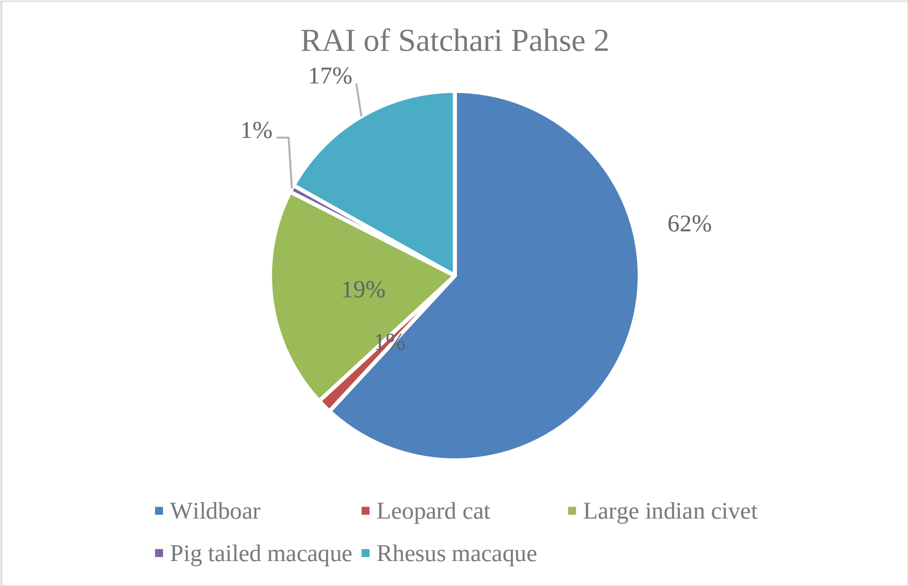

Figure 25. Relative abundance of SNP-02

4.2.1.10 Mammals record in Hakaluki haor (Malam beel) and Hail haor (Baikka beel)

It can be mentioned that we have had camera trap records which were collected from Malam beel, Hakaluki haor (69 trap nights), Moulvibazar during October 2020 to December 2020 and 12 encounters of fishing cats were recorded. That means fishing cat population is stable there. On the other hand, in August 2022, another 3 trap cameras were set (9 trap nights) at Baikka beel hijol-Koroch (Swamp spp.) forest patches where fishing cats were regularly photographed almost.

4.2.1.11 Mammals record in Borshijora eco-park

It can be also mentioned that we have had camera trap records whose were collected from Borshijora (254 trap nights), Moulvibazar and Tillagarh eco-park, Sylhet with 210 trap nights in 2022, satellite collar data applied on one individual male Fishing cat. Major findings are as follows:

Golden Jackals are most common. Wild pig, Jungle cat, Common Palm Civet, Rhesus macaque was also captured in the installed four Trap cameras. Although there was no single capture of any Fishing cat. These cameras were set there from August 2022 to October 2022.

Figure 26. Captured photos in Borshijora Eco-Park: (a) Golden Jackal; (b) Rhesus macaque; (c) Palm Civet; (d) Jungle Cat; (e) Wild boar.

4.2.1.12 Mammals observed in Tillagarh eco-park

Three Trap cameras were place at Tillagarh Eco-part in Sylhet for 2 months (September 2022 to November 2022). Like Borshijora Eco-Park there was no single Fishing cat was found but the Large Indian Civet, Palm Covet, Jackal, Rhesus macaque are more in numbers there. Which indicates that Fishing cat does not occupy the comparatively hillock areas and dense forest area like Borshijora Eco-park, Tillagarh Eco-park, Khadimnagar National Park, Satchari National park or even Lawachara National Park although forest officials have released 7 individuals in Borshijora, 4 individuals in Tillagars, 2 individuals in Khadimnagar, 1 individual in Satchari and 2 individuals of fishing cat in Lawachara national park in recent time since 2020. It should be noted that, time of the camera trap activity was chosen in monsoon when normally the haor areas become flooded completely and that time the fishing cats might stay in the adjacent forest with raised land. We explored fishing cat in the adjacent tea garden at vatera of Kulaura upazila, Patharia, Samanbag and Rashidpur of Baralekha upazila. In those case, we found fishing cat only in the Mominchara tea garden, vatera, Kulaura which is within only a kilometer away from Hakaluki haor where Patharia, Samanbag and Rashidpur tea gardens are little bit far away from Hakaluki haor.

Figure 27. Captured photos in Tillagarh Eco-Park: Common Palm Civet (top-left), Large Indian Civet (top-right), Rhesus macaque (bottom-left), and Golden Jackal (bottom-right).

4.2.3 Preliminary findings from the satellite collared fishing cat

An adult male fishing cat was captured on 27 January 2023 from the village Marukuna, office bazar, Moulvibazar Sadar upazila. We put satellite collar on this male fishing cat on 26 March 2023 and released as well at the place from where it was captured by metal cage trap three months ago. This cat named "Swadhin" was collared by the supervision and guidance of Professor Dr.

Figure 28. Collaring male fishing cat after tranquilization.

Mohammad Abdul Aziz, Dr. Ai Suzuki and veterinary surgeon Professor Dr. Md. Sultan Ahmed Tipu. I, myself and the research associates Mr. Aminur Rahman and Mr. Amir Hamza were present during satellite collar setting on the fishing cat and releasing session.

Figure 29. Tracking the collared fishing cat by radio antenna during study period.

Figure 30. *Fishing cat Swadhin's location on 28.3.2023*

Figure 31. *Movement of the collared fishing cat was traced (Location 2, Location 3)*

Figure 32. *Movement of the collared fishing cat was traced (Location 1)*

But unfortunately, this fishing cat was killed by the local people on 19 April 2023. From the track record, we observed that cat was survives only 25 days after releasing with satellite collar.

Figure 33. Retrieved bones of the collared fishing cat killed by the local people.

4.3 Investigating Human Perception of Fishing Cat through Interviews (Objective 2)

Assessing knowledge and attitude towards fishing cat in relation to mitigate Human-Fishing Cat Conflict around the haor landscapes in the northeastern Bangladesh through social survey (Semi-structured questionnaire is attached in appendix -1).

Among individual interviewees 211 persons well male where 9 were females (Fig. 36). Of which the major groups, 30% people engaged with business and farming attended in the interview, 29.55% were duck and poultry farmer, 9.09% were journalists (Fig. 37).

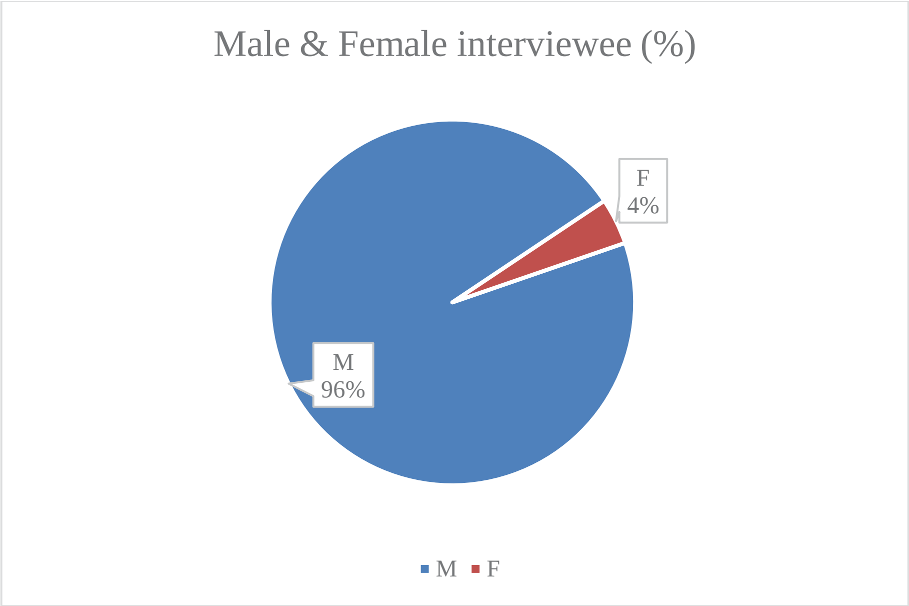

Figure 34. Percentage of Male and Female interviewee

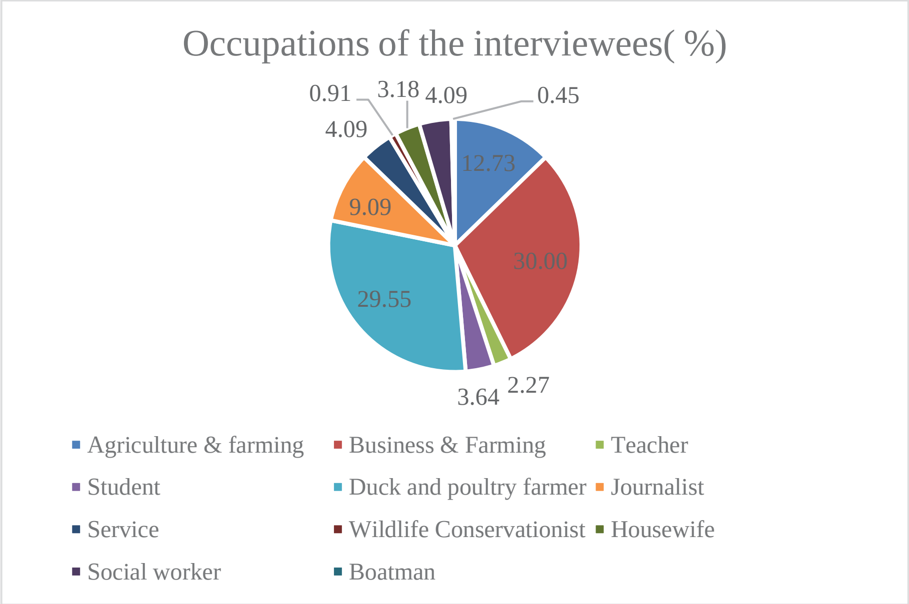

Figure 35. Occupation (%) of the interviewees

Again, out of the interviewees 84.55% have seen FC (Fig. 38). Interestingly, the majority (34.09%) people think FC as harmful (Fig. 39).

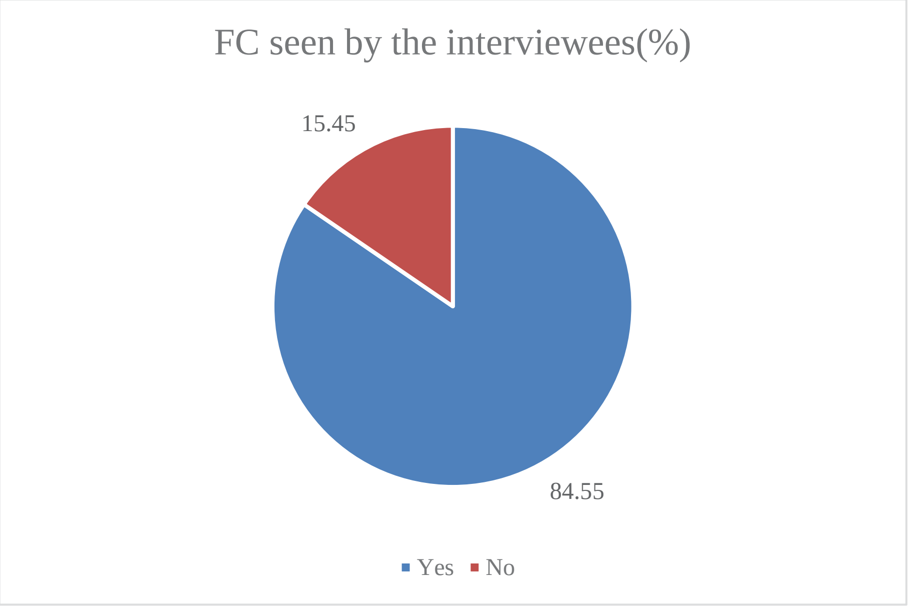

Figure 36. Percentage of people who have seen FC or not

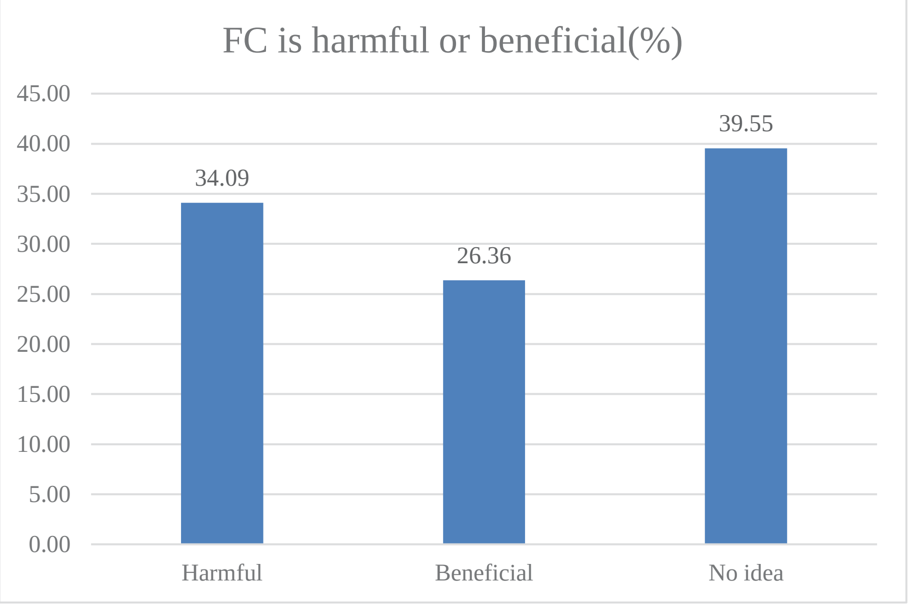

Figure 37. People's perception about FC as harmful or beneficial (%)

To know about the ideas of the interviewees regarding habitat of the fishing cat, we found that about 38.7 % opinions came where people expressed as the fishing cat inhabit in the agricultural field and haor, 28.8% in the bushy and jungle area, 11.8% opinion came as fishing cat inhabit near homestead and farm area, 8.4% in the pond area and interestingly 3.1% opinions came for the grave yard (Fig. 40). Chowdhury et al. has described that the fishing cat is a habitat specialist primarily associated with wetlands such as swamps, marshes, rivers, and mangroves. They have also been observed in agricultural fields, fish farms, and other non-protected areas such as rice fields, oil palm and coconut plantations, and along riverbanks (Chowdhury et al., 2015).

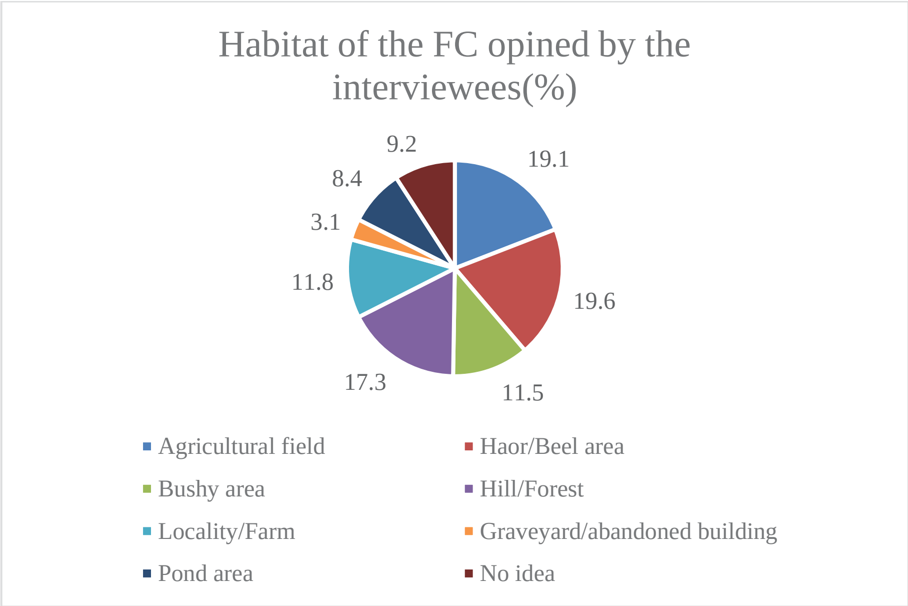

Figure 38. Habitat of the FC according to the interviewee

People claimed as the following foods that fishing cat used to eat (Fig.41). Mishra (2013) mentioned that the poaching of fishing cat was due to its preying on chicken, ducks, and fish and also for its meat and pelt (Mishra 2013).

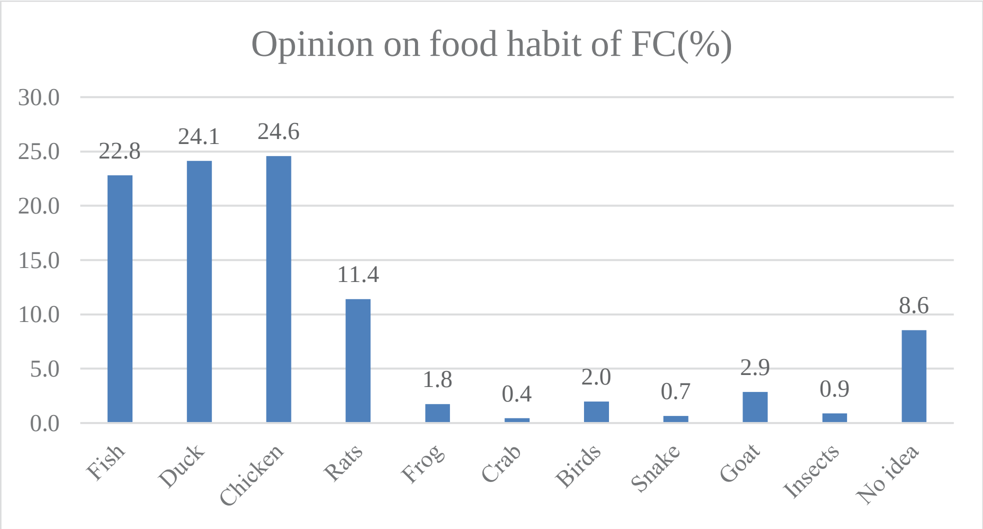

Figure 39. on FC's food habit

Among the all, 42.73% interviewees claimed that Fishing cat depends on livestock's (Fig. 42) although about 76.82 % interviewees did not see it by their own eyes (Fig. 43).

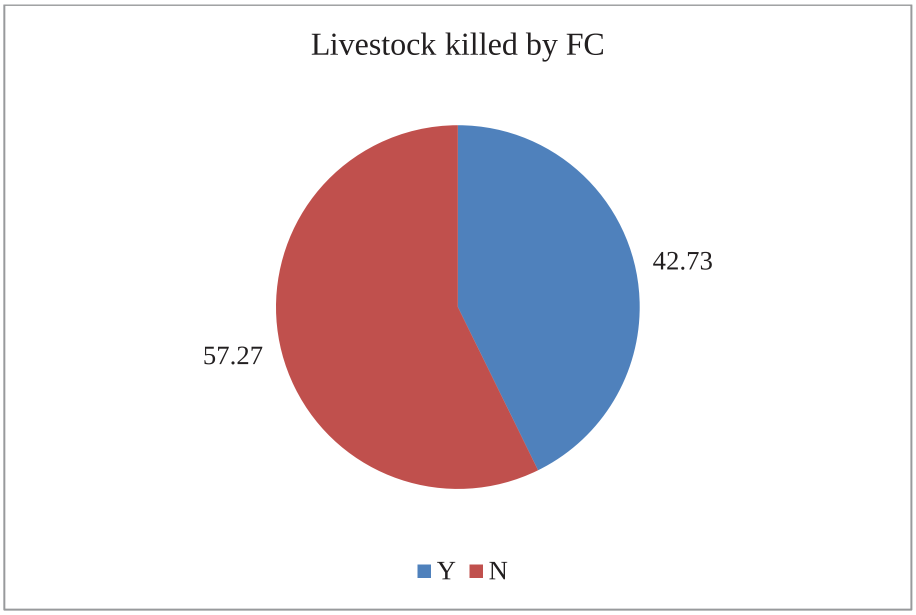

Figure 40. Livestock killed by FC opined by the interviewees

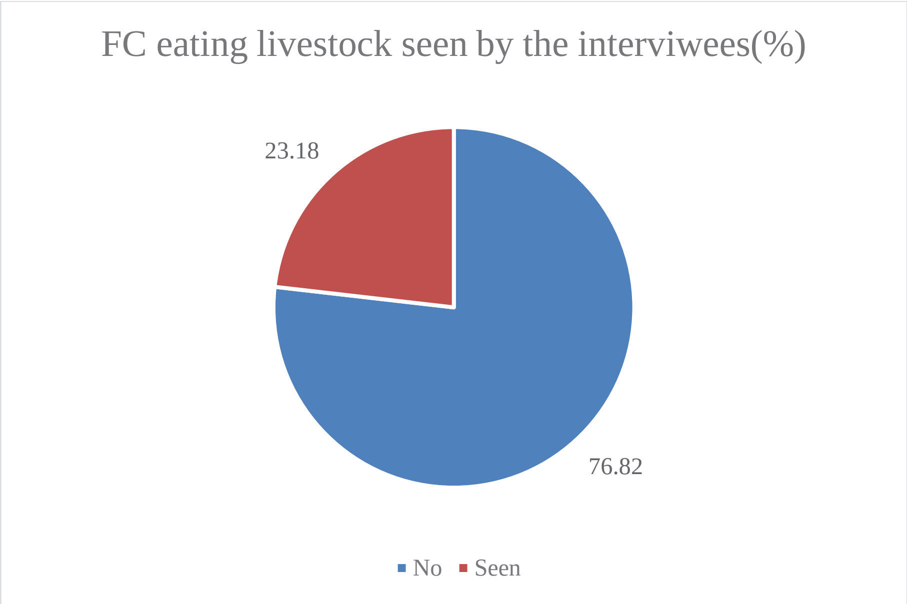

Figure 41. FC eating livestock seen by interviewees

About 8.9% opinion received as FC attacks human beings although majority (91.8%) do not think so (Fig. 44).

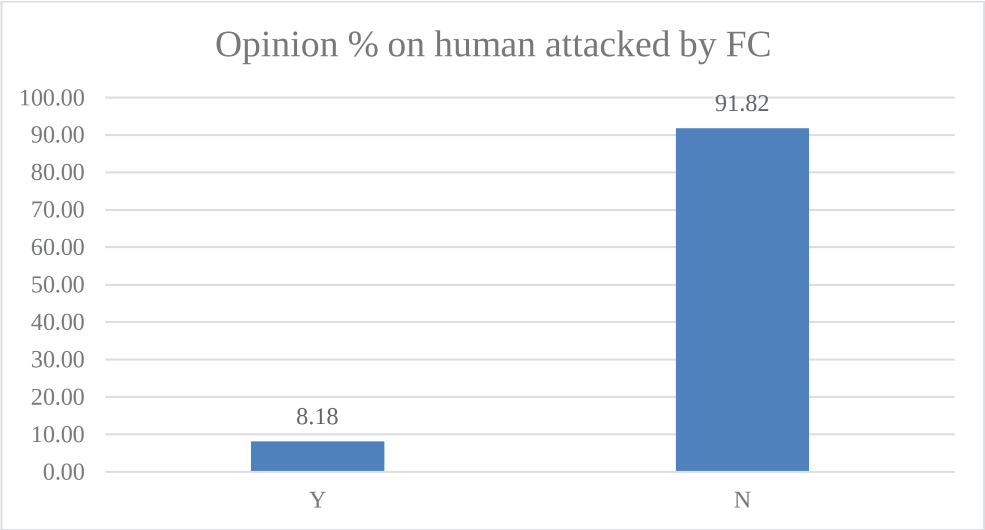

Figure 42. Opinion % on human attacked by FC

In terms of reasons of the killing or capture 39.04% opinion came as reason for killing ducks or chickens which is the highest on the other hand, fear (26.03%) proclaimed as the second reason (Fig. 45). In Nepal, a study revealed that poaching of fishing cat was due to its preying on chicken, ducks, and fish and also for its meat and pelt (Mishra 2013).

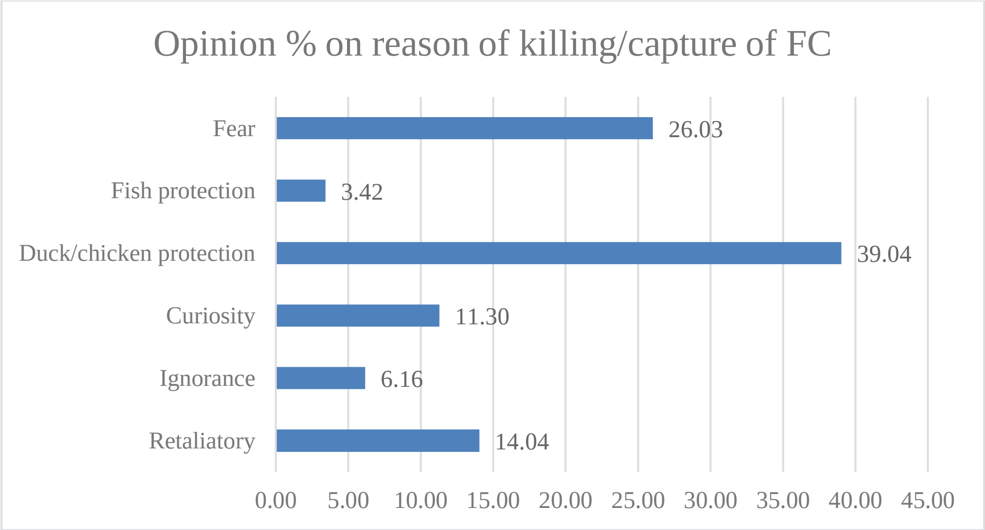

Figure 43. Opinion percentage on reason of killing/capture of FC

To know about the method used for capturing/killing of Fishing cat the interviewees were asked, more opinions come on using trap/metal cage trap (36.97%) and second one was cordon/gherao (24.64%) (Fig. 46).

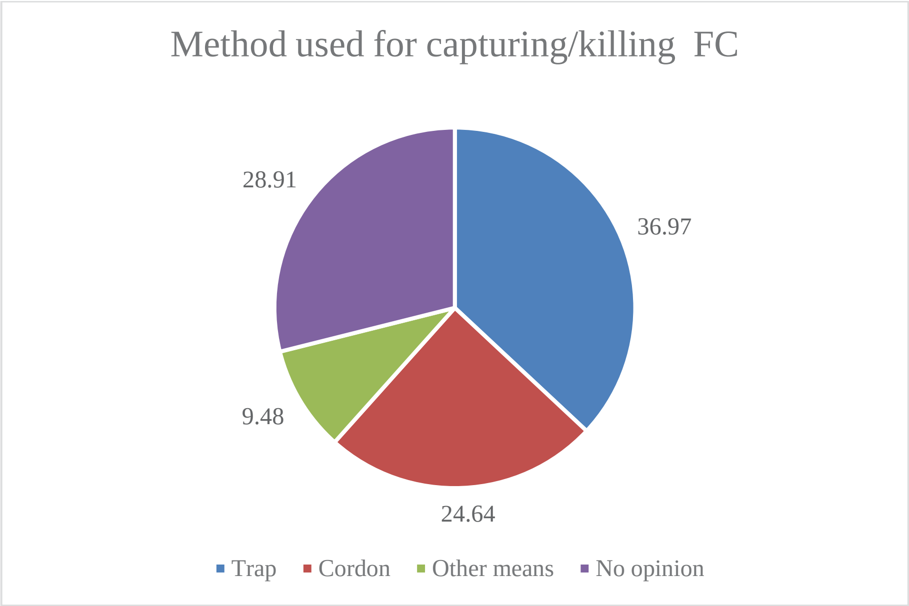

Figure 44. Method used for capturing/killing FC

Maximum people dislike fishing cat and it is 93.64% and only 6.36% people like fishing cat. This opinion expresses the conflicting situation between human and fishing cat in the haor landscape (Fig. 47).

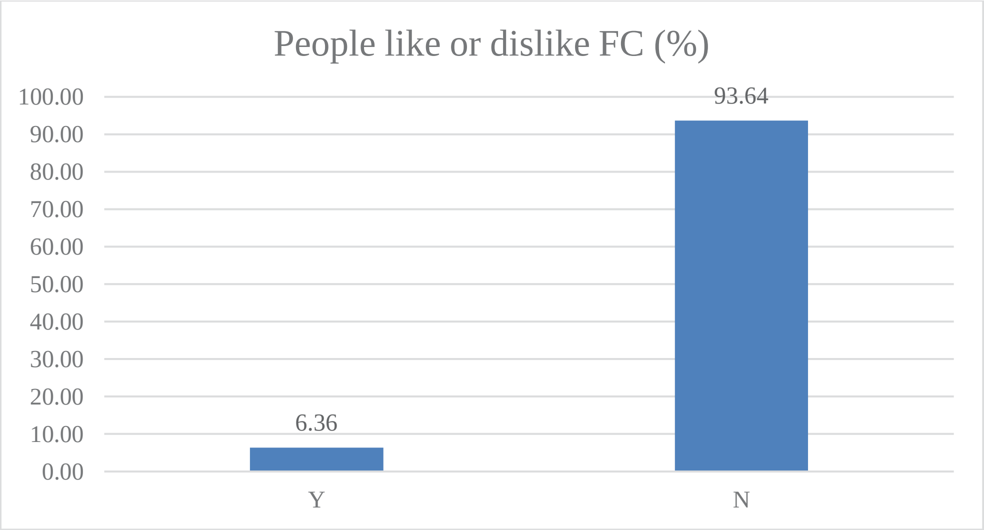

Figure 45. People like or dislike fishing cat

We asked 128 individual interviewees whether they know about the Wildlife (Conservation and Security) Act. 2012. In reply to this question, we observed only 58.59% people know a little bit about the wildlife law (Fig. 48) but unfortunately only 7.03% people are aware of the conviction or penalty (Fig. 49). Which are other reasons for the ignorance of the fishing cat survival.

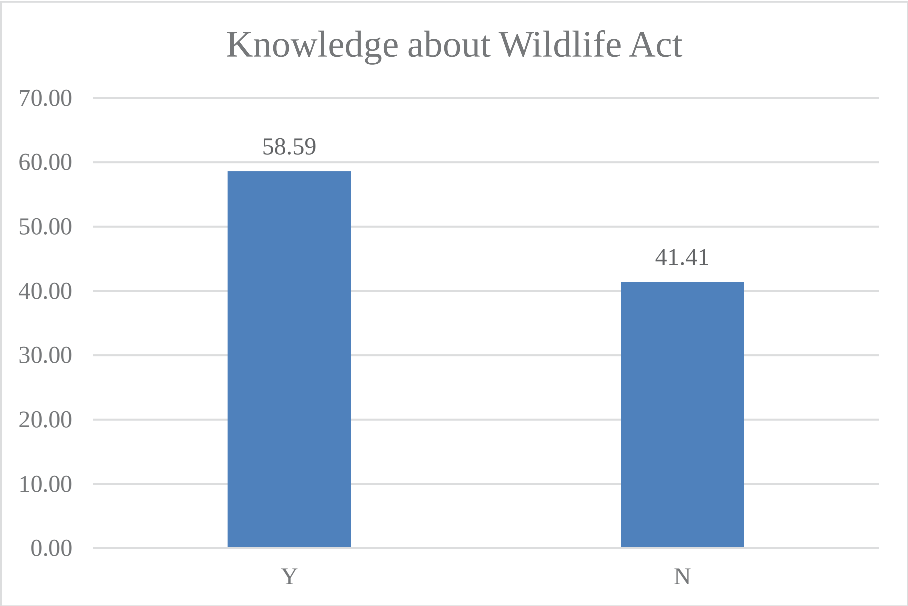

Figure 46. Knowledge about Wildlife Act

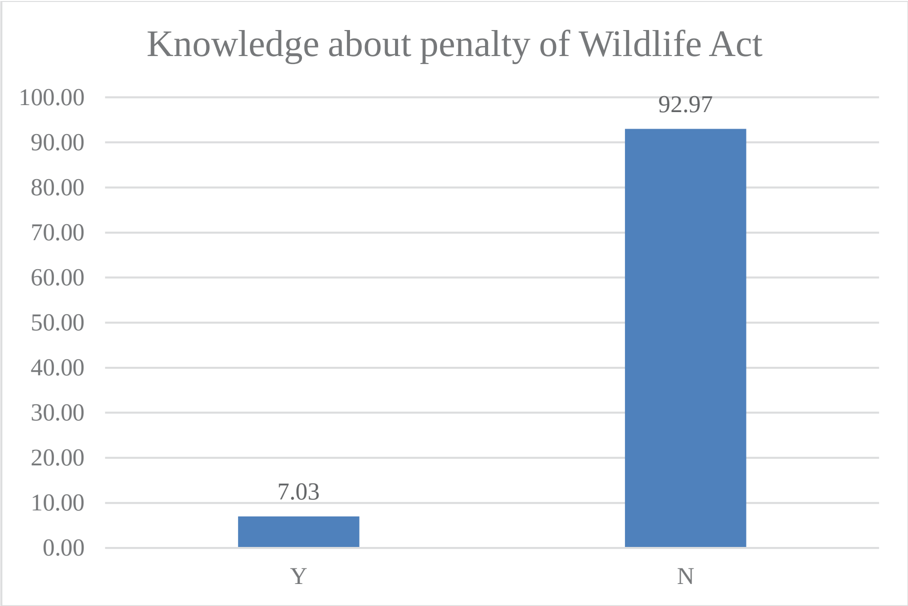

Figure 47. Knowledge about the penalty of Wildlife Act

About 27.27% people opined that government should emphasis on awareness creation where 12.7% people suggested to give compensation for the livestock damage and 1.36% people claimed both awareness and compensation (Fig. 50).

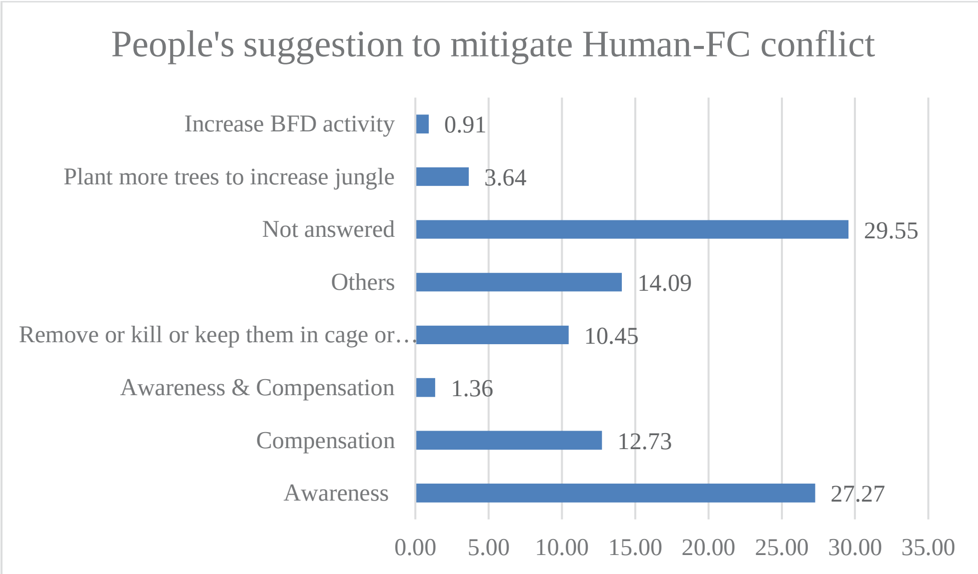

Figure 48. People's suggestion to mitigate human-FC conflict

On the other hand, we have received the responses from 20 focus group discussions for assessing knowledge and attitude towards fishing cat to mitigate Human-Fishing Cat conflict which are as follows. When we asked to focus groups, we observed the fishing cat is called as "bagh' and it 95%. Only one group expressed this vulnerable cat is "Bilai" means cat (Fig. 51).

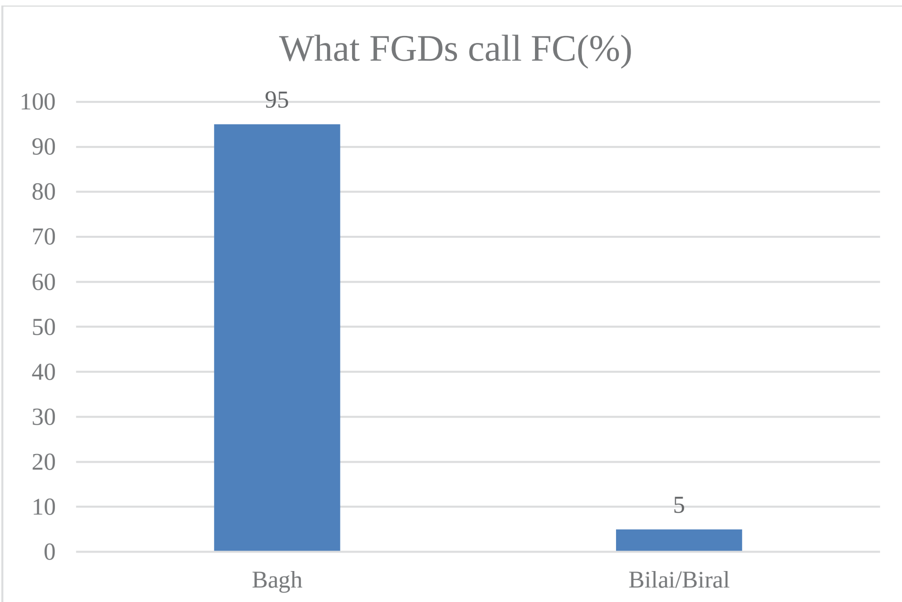

Figure 49. Fishing cat is familiar with "Bagh" mostly

Whether the groups think fishing cat is beneficial or not- that was asked to know. In reply to this, 65% opinion came as harmful whereas 25% opinion came as beneficial (Fig. 52).

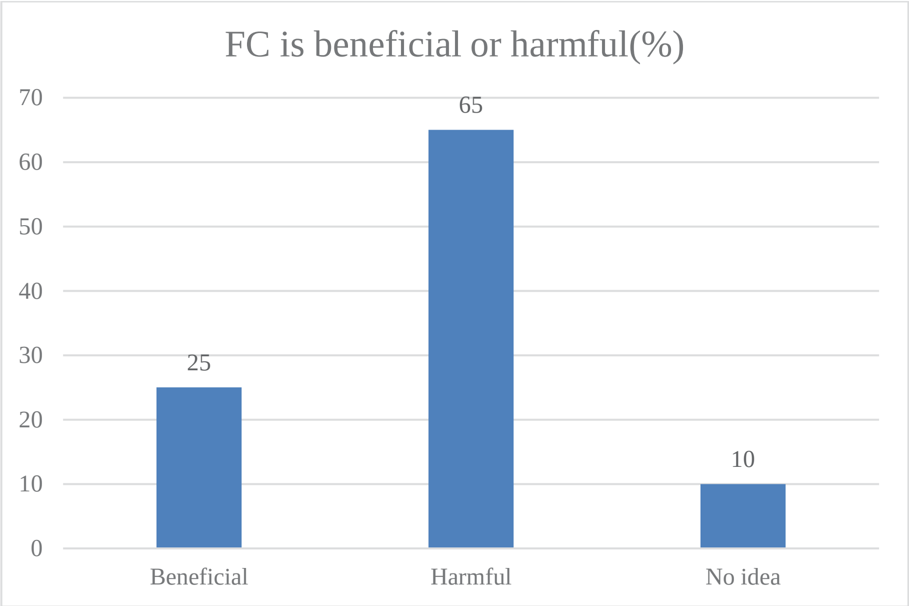

Figure 50. Fishing cat is beneficial to the environment or not

Regarding food habit of fishing cat the groups emphasized on poultry. That means 60.33% opinions came in such a way that fishing cat mostly depend on poultry food viz. chicken and duck. Fish came as second opinion (Fig. 53).

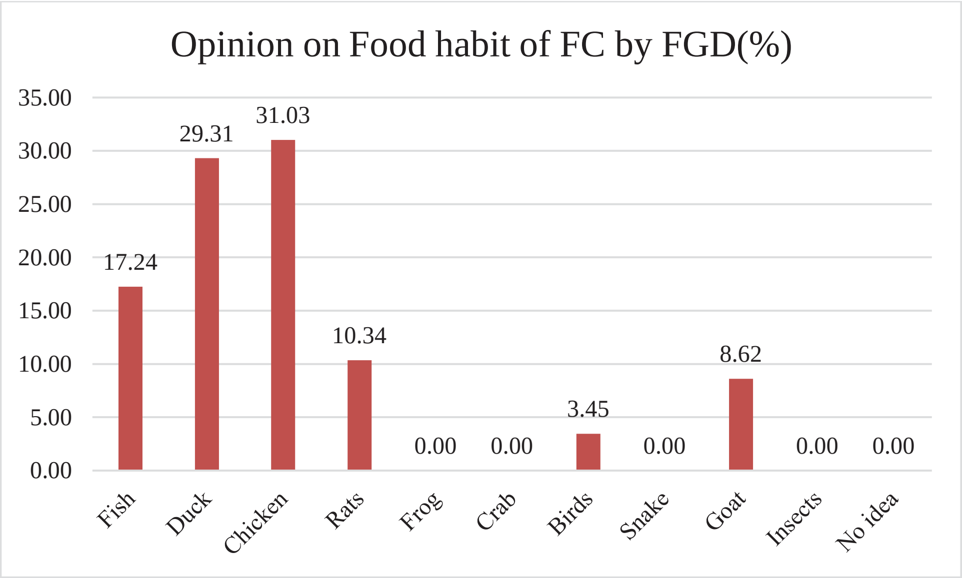

Figure 51. FGDs opinion on common food of fishing cat

Out of the 20 focus groups only two groups opined that fishing cat attack human being (Fig. 54).

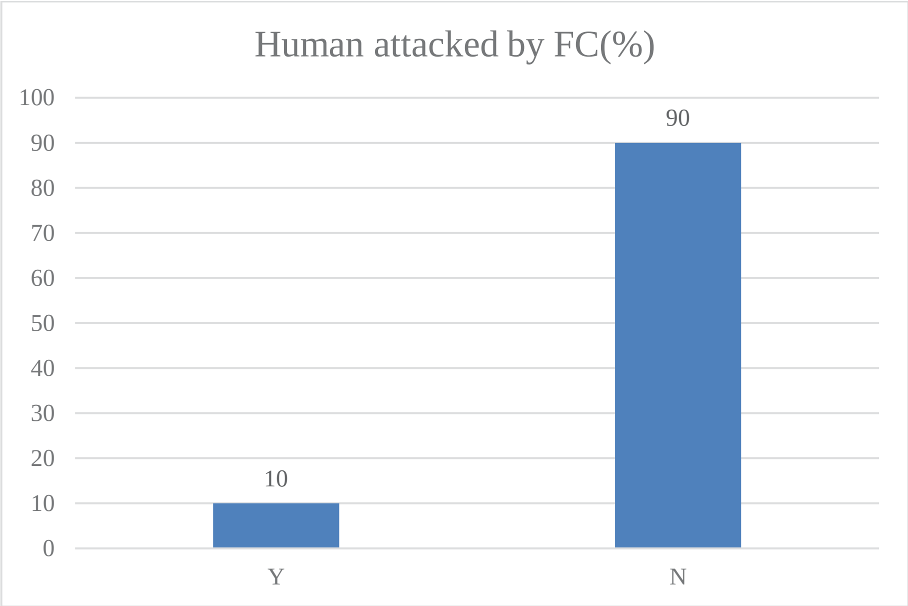

Figure 52. Focus group's knowledge about the human attack by the FC

For identifying the reasons of the capturing/killing fishing cat, opinions came again to protect the poultry which was as the principal reason (43.24%. Second reason was mentioned as the "fear" folled by "curiosity". The fourth reason was the retaliatory killing (Fig. 55). Among 80 interviewees, Kitipat et al. recorded 25 incidents of conflict, most relating to raids on poultry (n = 18)

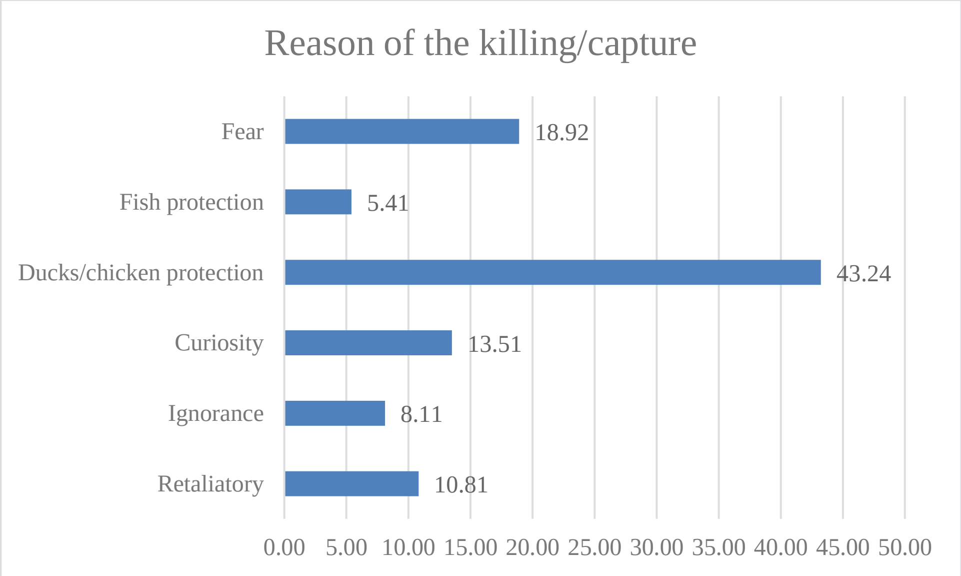

Figure 53. Reasons of killing/ capture of fishing cat

Unlike the general individual interviewee's maximum comments i.e. the comments of 95% groups were people dislike fishing cat (Fig. 56).

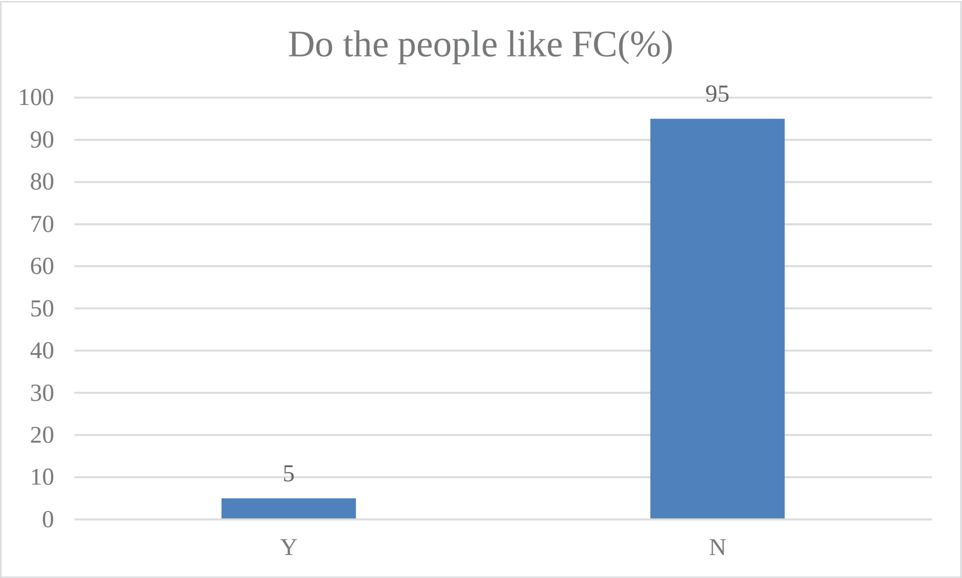

Figure 54. Whether people like or dislike FC

In case of the simple question like- what should government do to mitigate human-fishing cat conflict, groups answered as the following where compensation providing came as the principal suggestion (Fig. 57). Habitat restoring was mentioned as the second opinion where as some groups opined to put FC in the cage (15%).

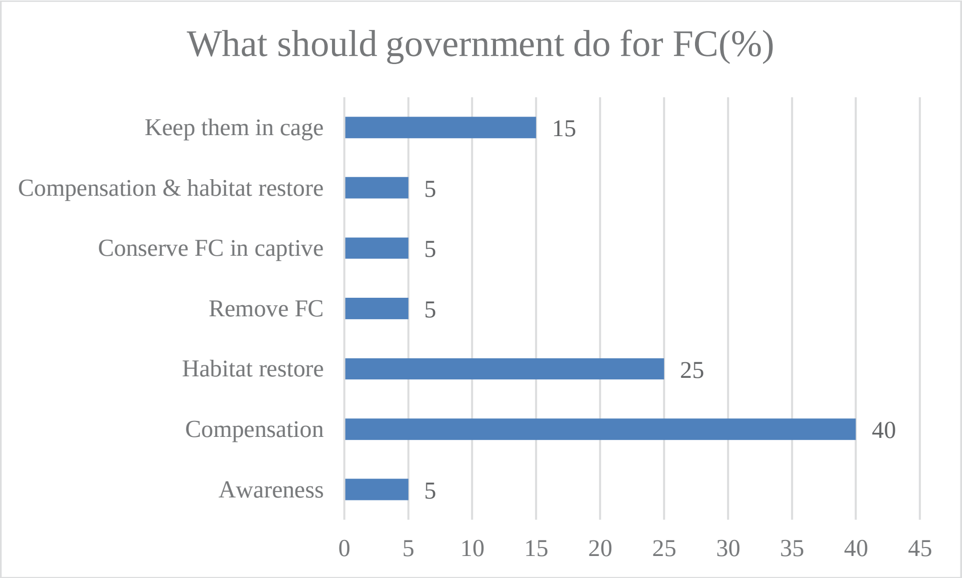

Figure 55. Suggestion to government to mitigate human- fishing cat conflict

To judge about the level of awareness on the Wildlife (Conservation and Security) Act,2012 or simply wildlife act, most groups are well known to the wildlife act although most groups are unknown the penalty for fishing cat capturing or killing (Fig. 58 & 59).

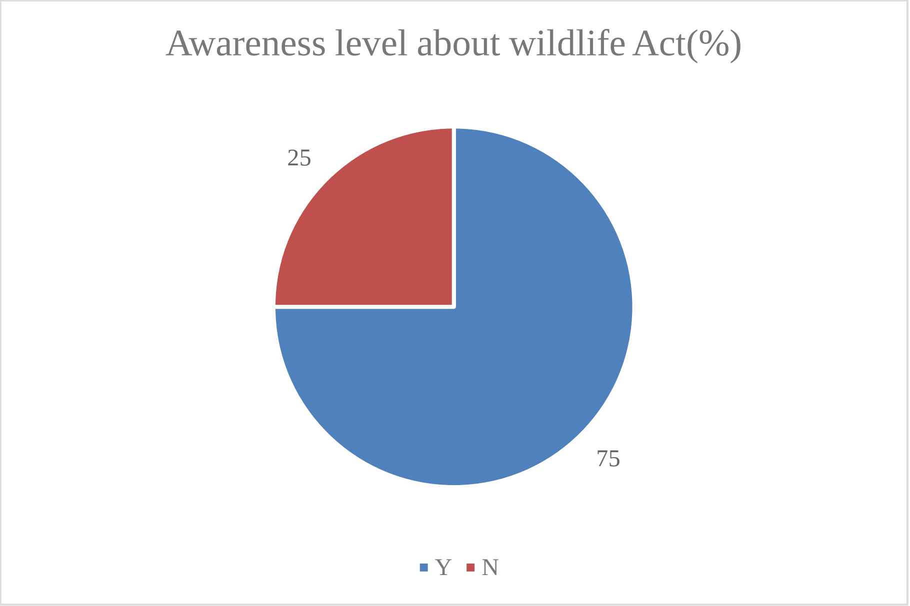

Figure 56. Awareness level about the wildlife Act

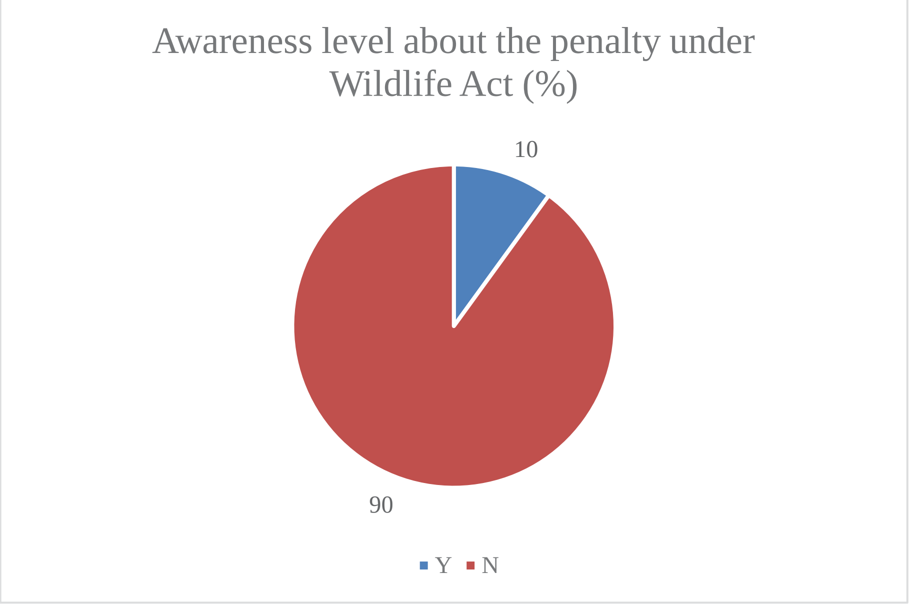

Figure 57. Awareness level about the penalty for killing/ capturing under wildlife Act

From the above-mentioned charts from the individual interviewee's thought and from the focus groups it is easily understood that there exists large knowledge gaps and misinterpretation about this poor cat. For example, people treat this cat as ferocious animal "Bagh" rather than a fish-eating small cat. About the major food of the fishing cat there again came a great mistake as poultry is the main food of them. People do not have any knowledge whether fishing cat would kill rat as their common food after fish and poultry. The ducks and chickens are actually as their opportunity when those are available. That is why fishing cat has become harmful to the locality. In fact, around 10-15 years back when haor people did not rear duck or chicken in the close vicinity of the beels/wetlands the fishing cat survived more in numbers, their population density was high then and there exists coexistence with the human with a harmonious condition rather than today's conflict situation. So, lack of knowledge and Awareness can be treated as a major challenge to maintain the viable population there. Based on the discussions, destroying or diminishing habitat like jungles in and around the wetlands cleaning all bushes around the homesteads, destroying reedlands etc. in and around wetland areas can be marked as another threat. Another important challenge is unknowing situation among local communities about the penalty of killing or capturing fishing cat under Wildlife Act and the application of the laws.

Suggestion on providing compensation has appeared as one of the prime tools to mitigate human- fishing cat conflict which came from the opinions of both individual interviewees and focus groups. So, government can really initiate the provision of compensating the loss and damage by the fishing cat especially for poultry farm affairs. People can also be suggested to set moderately strong and high fences around their poultry houses.

4.4 Ranking conservation threats and challenges of the Fishing cat and mitigation strategies (Objective 3)

During the study, we considered and matched **i**nternationally recognized threats to fishing cat as suggested by IUCN's species Red List (IUCN 2025) with our northeast Bangladesh sites. Here, we took help from our around 20 renowned wildlife biologists and practitioners to rank the conservation threats and challenges for fishing cat. Our experts have also sought out the way to resolve those. The undermentioned tables denote the ranking the threat and challenges as high, medium and low. The mitigation measures are also categorized as short time (1-3 years), medium term (1-6 years) and long term (1-10 years).

Table 10. Identified threats to fishing cat for maintaining its viable population in the north-east Bangladesh

Sl no.	**IUCN Threat Category to fishing cats**	**Level of threat as**			**Action required to mitigate as**		
	Sub-category	High	Medium	Low	Short term (1-3 years)	Medium term (1-6 years)	Long term (1-10 years)
1	**Biological resource use**						
	a. Direct killing		✓		1. Develop the conservation groups at village level 2. Local awareness Programme 3. Law enforcement 4. Endorsement of local conservation initiatives 5. Allocate budget for the fishing cat conservation 6. Impose stricter penalties for hunting of Fishing Cats 7. Provide incentives for reporting or killings	1. Develop strategic education plan at local schools 2. Local awareness 3. Law enforcement 4. Allocate budget for the fishing cat conservation 5. Punish killer and convey the message of punishment so that other people will be knowing that killing of Fishing cat is illegal	1. Local awareness programme 2. Change the social norm and remove misconception 3. Law enforcement 4. Conservation education 5. Mainstreaming fishing cat in media, education material, local lifestyle (e.g., bank notes, culture, etc.) 6. Establish fishing cat safe zone 7. Allocate budget for the fishing cat conservation
	b. Trapping	✓			1. Develop the conservation groups at village level 2. Awareness Programme 3. Legal action 4. Stricter enforcement of	1. Develop strategic education plan at local schools 2. Awareness 3. Law enforcement 4. Legal action 5. Regular monitoring, retrieve, seize and destroy the traps	1. Change the social norm and remove misconception 2. Law enforcement 3. Legal action 4. Regular monitoring, retrieve, seize

					laws and regulation 5. Trap removal campaigns 6. Monitoring at vulnerable zones 7. Regular monitoring, retrieve, seize and destroy the traps 8. Establish rapid response teams for rescuing injured or trapped Fishing Cats.	6. Establish wildlife rehabilitation centers for treatment and release	and destroy the traps 5. Find out the area and trappers 6. Awareness campaign
	c. Illegal trade			✓	1. Strengthen capacity of BFD 2. Legal action 3. Awareness Programme 4. Stricter enforcement of laws and regulation 5. Find out the trade route and regularly monitoring to rescue the cats 6. Try to capture the traders and punish them following laws 7. Crime hotspot identification 8. Use undercover operations to infiltrate smuggling networks	1. Law enforcement 2. Legal action 3. Find out the trade route and regularly monitoring to rescue the cats 4. Try to capture the traders and punish them following laws 5. Regular monitoring of the hotspots by the volunteers 6. Study the nature and scale of trade 7. Wildlife crime databases to track poaching trends. 8. Surveillance with BFD 9. Awareness in border areas	1. Develop monitoring system particularly to detect the demand for any parts of cats from inside and outside of the country including hotlines for reporting wildlife trade 2. Law enforcement 3. Legal action 4. Systematic monitoring of wildlife trade 5. Find out the trade route and regularly monitoring to rescue the cats 6. Try to capture the traders and punish them following laws 7. Regular monitoring of the hotspots by the volunteers 8. Change the plan as per findings (if there is a change in the hotspots) 9. Develop a project linked with study results 10. Execute the project/activities 11. Run social media

							campaigns highlighting the illegality and ecological harm of trade.
	d. Overfishing and harvesting aquatic resources		✓		1. Ensure full compliance with fishing regulation 2. Growing awareness for sustainable fishing and harvesting 3. Ensure natural protection in large aquatic habitat 4. Alternative livelihood practices 5. Law enforcement for fishing in Fishing cat safe zone 6. Fisherman thrown unused/old net into the haor/bills which should be clean regularly 7. Involve DoF/Local government to ensure that no illegal fishing gears are used, 8. Involve VCGs, RMOs in the wetland area to act as ambassador of fishing cat conservation 9. 8. Introduce community-managed fish sanctuaries	1. Strengthen the law enforcement on unsustainable fishing 2. Collaboration with Department of Fisheries 3. Declare some PA for this species 4. Growing awareness for sustainable fishing and harvesting 5. Law enforcement for fishing in Fishing cat safe zone 6. Release fish hatchlings in important areas 7. Declare critical wetlands and habitats as protected areas 8. Train communities in sustainable livelihoods (e.g., eco-tourism, organic farming) to reduce dependence on destructive practices. 9. Introduce sustainable fishing practices 10. Alternative income generation	1. Educate beel owners about fish stock dynamics in the context of economic benefit 2. Law enforcement 3. Protection of all aquatic PA in north-east 4. Declaration of OECM in north east 5. Growing awareness for sustainable fishing and harvesting 6. Law enforcement for fishing in Fishing cat safe zone 7. Establish a fish culture and release fish hatchlings in important areas 8. Multistakeholder consultations particularly with fisheries sectors to reduce the overfishing 9. Strengthen international agreements (e.g., Ramsar Convention) for wetland protection.
2	**Natural system modification**						
	a. Vegetation shrinkage (habitat destruction) in the wetlands	✓			1. Law enforcement 2. Need national integrated policy for all aquatic habitat management 3. Endorsement of local conservation initiatives 4. Stop over livestock grassing	1. Execute habitat restoration program following CREL project 2. Law enforcement 3. Need national integrated policy for all aquatic habitat management 4. Stop over livestock grassing and harvesting	1. Establish the landscape level management plan in terms of conservation unit, corridoes, and potential strongholds with appropriate level of law enforcement

					and harvesting of grasses for saving breeding and roosting sites of Fishing cat 5. Regular monitoring and mapping of the fishing cat habitat 6. Survey of existing vegetation 7. Restoration Plan preparation 8. Enforce wetland protection laws (e.g., Ramsar Convention, national wetland policies). 9. Provide incentives (e.g., payments, eco-jobs) for wetland restoration work 10. Need national integrated policy for all aquatic habitat management	of grasses for saving breeding and roosting sites of Fishing cat 5. Regular monitoring and mapping of the fishing cat habitat 6. Restoration 7. Protection 8. Involve VCG/RMO members to protect 9. Restore native plants grows edge of wetlands that support shelter and prey species 10. Mobilize locals to replant native vegetation in degraded areas.	2. Law enforcement 3. Need national integrated policy for all aquatic habitat management 4. Declaration of protected areas and OECMs 5. Stop over livestock grassing and harvesting of grasses for saving breeding and roosting sites of Fishing cat 6. Regular monitoring and mapping of the fishing cat habitat 7. Building small raised islands into the haor/bills for shelter of fishing cats. And declare no-go zone. So that during monsoon period they can stay there safely when they are bound to take shelter in adjacent villages. 8. Introduce adaptive management 9. Develop an action plan including all stakeholders 10. Media campaigns to reduce public demand for wetland conversion 11. Wetland restoration 12. Eco-sensitive zoning
	b. Homestead forest loss in the locality (including	✓			1. Awareness for saving homestead forests	1. Promote understanding of the protective functions of natural ecosystems by developing educational	1. Establish the landscape level management plan in terms of conservation

	backyard jungles)				2. Endorsement of local conservation initiatives 3. Survey of homestead where the thrive 4. Provision of giving Award to people who do not clear the homestead forests 5. Educate villagers on the ecological role of homestead forests 6. Identify & map remaining homestead forests important for Fishing cats	programs that focus on their roles in buffering wind, mitigating floods, and reducing the overall impact of natural disasters 2. Awareness for saving homestead forests 3. Regular monitoring of the hotspots by VGC and RMO members 4. Reward villages that maintain homestead forests (e.g., payments for ecosystem services). 5. Tree plantation drives 6. Backyard habitat conservation	unit, corridoes, and potential strongholds with appropriate level of law enforcement 2. Declaration of protected areas and OECMs 3. Awareness for saving homestead forests 4. Engage schools/youth groups to document Fishing Cat sightings in homestead forests. 5. Restore ponds with riparian vegetation to connect habitats
	c. Leasing of beels and dewatering	✓			1. Ensure full compliance with fishing regulation on water depth 2. Collaboration with Department of Fisheries 3. Commercial leasing must be stopped 4. Endorsement of local conservation initiatives 5. Minimum water retention system should be developed 6. Awareness creation about sustainable harvesting of fish 7. Establish aquatic sanctuary for all aquatic animals including fishing cat 8. Ban leasing of critical Fishing Cat habitats for aquaculture and agriculture 9. Increase fines and penalties for unauthorized filling of wetlands.	1. Strengthen the law enforcement on regulation (e.g. administrative suspension for beel leasing) and inspection system 2. Law enforcement 3. Encourage community-based leasing in all large water bodies 4. Awareness creation about sustainable harvesting of fish 5. Continue multi-departmental/ministries meeting and follow up the decisions 6. Train local "Wetland Guardian" groups to report leasing violations and dewatering 7. Pay farmers to conserve wetlands (e.g., Payments for Ecosystem Services - PES). 8. Monitoring 9. Wetland Co-management	1. Establish the landscape level management plan in terms of conservation unit, corridoes, and potential strongholds, and implement with appropriate level of law enforcement 2. Law enforcement 3. Declare periphery of all large beels and haor as ECA 4. Declaration of protected areas and OECMs 5. Awareness creation about sustainable harvesting of fish 6. Establish community-managed wetland trusts to oversee leasing 7. Divert leasing revenue into habitat restoration.

	d. Agricultural extension in the wetland	✓			1. Collaboration with Agriculture office 2. Compensation for not to agricultural extension 3. Law enforcement and monitoring 4. Zonation should be established and limit the agricultural expansion 5. Encourage 5-10m vegetated buffers around fields to provide cover for Fishing Cats and prey	1. Execute habitat restoration program following CREL project 2. Law enforcement and monitoring 3. Compensate farmers for leaving field edges wild for corridors and maintaining natural wetlands 4. Certification for farmers using non-lethal predator control and rearing wildlife-friendly livestock 5. Wetland land use policy	1. Establish the landscape level management plan in terms of conservation unit, corridoes, and potential strongholds, and implement with appropriate level of law enforcement 2. Law enforcement and monitoring 3. Proper zoning plan to demarked the agricultural land 4. Engage local communities as wildlife protectors through eco-tourism benefits
3	**Agriculture & Aquaculture**						
	a. Livestock farming & ranching in fishing cat habitat (in haor/beels)	✓			1. compensation for not to use the land 2. Alternative livelihood practices 3. Scientific studies to understand the impact of this practice on fishing cats 4. Law enforcement and introduce soft penalty 5. Establish "No-Grazing Zones" near core Fishing Cat habitats	1. Execute habitat restoration program following CREL project 2. Stringent protection of wetland areas 3. Law enforcement and introduce soft penalty 4. Certification for farmers using non-lethal predator control and rearing wildlife-friendly livestock 5. Introduce compensation to the livestock owners for not to use the land 6. Fence installation 7. Relocation of farms	1. Establish the landscape level management plan in terms of conservation unit, corridoes, and potential strongholds, and implement with appropriate level of law enforcement 2. Declaration of protected areas and OECMs 3. Law enforcement and introduce soft penalty 4. Livestock-Free Wetland Zones" managed by local cooperatives 5. Subsidies for zero-grazing systems
4	**Climate change & Severe weather**						
	a. Habitat shifting and alteration,		✓		1. Recognize the urgency of acquiring	1. Implement predictive modeling of habitat suitability and species	1. Maintain and enhance ecological

	Storm and flooding				ecological knowledge to inform timely and effective conservation planning and intervention 2. Awareness 3. Scientific studies to assess the impact of climate change on fishing cats and fishing cats' habitats 4. Building small raised islands into the haor/bills for shelter of fishing cats. And declare no-go zone. So that during monsoon period they can stay there safely when they are bound to take shelter in adjacent villages. 5. Set up rapid response teams to rescue stranded Fishing Cats post-flood. 6. Temporarily restrict fishing in storm-damaged areas to allow recovery.	range shifts informed by ecological data 2. Identification of climate vulnerable hotspots 3. Building small raised islands into the haor/bills for shelter of fishing cats. And declare no-go zone. So that during monsoon period they can stay there safely when they are bound to take shelter in adjacent villages. 4. Install flood sensors linked to SMS alerts for villagers and conservation teams.	connectivity in areas identified through predictive analysis, with a focus on providing covered spaces that facilitate safe movement 2. Conservation of climate vulnerable hotspots 3. Building small raised islands into the haor/bills for shelter of fishing cats. And declare no-go zone. So that during monsoon period they can stay there safely when they are bound to take shelter in adjacent villages. 4. Conservation insurance" funds to compensate farmers for flood-related habitat protection 5. Climate-resilient habitat plans 6. Early warning systems
5	**Transportation & service corridor**						
	a. Roads and railways		✓		1. Identify the pattern of roadkill on fishing cat population 2. Underpass in FC habitats 3. Scientific studies to understand the impact of this practice on fishing cats 4. Find out local migration route and set minimal speed for vehicles	1. Implement targeted awareness campaigns for relevant stakeholders, informed by data on high-risk locations, times of day, and seasons when roadkill are most likely to occur 2. Find out local migration route and set minimal speed for vehicles 3. Mark high-risk zones for priority mitigation (e.g., near beels/haors).	1. Develop, implement, and assess the effectiveness of both soft approaches, such as public awareness and education initiatives, and hard infrastructure measures, including structural road modifications

					5. Avoid wetland and fishing cat habitat when to build roads 6. Install wildlife warning signs near wetland crossings	4. Install fencing along roads to guide Fishing Cats to safe crossings 5. Use natural barriers (hedges, shrubs) to prevent wandering onto roads. 6. Signboards in sensitive zones 7. Compensation schemes 8. Rapid response teams	2. Find out local migration route and set minimal speed for vehicles 3. Conduct mandatory "Wildlife Impact Assessments" before construction 4. Compensate for fragmentation with artificial habitat creation

Table 11. Identified challenges for maintaining viable fishing cat population in the north-east Bangladesh

Sl no.	**Name of challenges**	**Level of challenges threat as**			**Action required to mitigate as**		
		High	Medium	Low	Short term (1-3 years)	Medium term (1-6 years)	Long term (1-10 years)
1	Lack of conservation awareness and knowledge about Wildlife Act,2012 among local communities	✓			1. Establish effective communication with local communities 2. Awareness Programme 3. Bill boards 4. Train personnel about fishing cat conservation 5. Formation of Fishing cat volunteer team 6. Village workshops and school programs using posters, videos, and storytelling in local dialects. 7. Distribute illustrated pamphlets explaining penalties for harming Fishing Cats.	1. 1.Design nation-wide strategic awareness programs such as social marketing 2. Awareness at local level 3. Formation of Fishing cat volunteer teams 4. Social media campaign 5. School/community sessions. 6. Leaflet	1. Integrate the school official curricula and include its contents within the textbook 2. Awareness at local level 3. Formation of Fishing cat volunteer teams 4. Integrate Fishing Cat conservation into national and regional wildlife policies. 5. Include Wildlife Act in school curricula (e.g., Bangladesh’s NCTB textbooks).

2	Low level capacity and less willingness for wildlife conservation in BFD	✓			1. Establish a recognition system where rangers are publicly awarded in nationally visible events, followed by opportunities such as invitations to deliver special talk—creating a framework that positions rangers as national heroes-protectors of national treasure. 2. Species specific training 3. Train personnel about fishing cat conservation 4. increase Logistic Support 5. Arrange Capacity Building Training 6. Incentives/ Risk Allowance 7. Ensure the willingness of the FD staff to work for the conservation by imposing service rule 8. Network of wildlife conservation volunteers 9. Field manuals in Bengali on species ID, rescue protocols, and Wildlife Act enforcement 10. Monetary rewards for staff who document Fishing Cat sightings or halt poaching	1. Establish a performance evaluation system that rewards conservation efforts, through promotions and/or recognition within FD 2. Capacity Building of FD 3. Species specific training 4. increase Logistic Support 5. Recruitment of manpower in wildlife handling 6. Arrange Capacity Building Training 7. incentives/ Risk Allowance 8. Ensure the willingness of the FD staff to work for the conservation by imposing service rule 9. Network of wildlife conservation volunteers 10. Conduct population surveys to identify key threats and hotspots. 11. Collaborate with law enforcement agencies to prosecute Wildlife Act violators 12. Wildlife staff training 13. Partnership with NGOs	1. Introduce transformation scheme for internal/intrinsic change in FD using implementation science 2. Capacity Building of FD 3. Species specific training 4. increase Logistic Support 5. Recruitment of manpower in wildlife handling 6. Arrange Capacity Building Training 7. Incentives/ Risk Allowance 8. Ensure the willingness of the FD staff to work for the conservation by imposing service rule 9. Network of wildlife conservation volunteers 10. Secure government and NGO funding for sustained conservation programs 11. Collaborate with universities and research institutions for ongoing studies.
3	Absence of proper protection/fences around the poultry house		✓		1. Promote awareness and dissemination of improved poultry	1. Awareness 2. Identify the ways that would deter fishing cats from predating on poultry	1. Conduct research on the future shifts in poultry dependence, secure the

					husbandry techniques 2. Scientific studies to assess whether chicken coups are the source of conflicts and, if so, to what levels 3. Ensure the protection 4. Promote the use of predator-proof poultry pens to reduce retaliatory killings. 5. Predator-proof fencing 6. Subsidy programs	3. Assess their efficacy 4. Ensure the protection	necessary funding, and, after considering scale effects, develop a robust long-term strategy. 2. Introduce the proven methods to fishers and farmers 3. Ensure the protection
4	Absence of the provision of compensation for poultry loss (N.B.Attacks by carnivores are perceived as a lower risk compared to other causes of poultry loss. For instance, addressing these other risks may help reduce local dissatisfaction. It is therefore recommended to conduct a study—such as a choice experiment—to better understand local perceptions and priorities.)	✓			1. Make compensation legislated in the next amendment of laws 2. Setting priorities 3. Amend the existing rules 4. Keep budget in the ADP/AWP of CMC/VCG/RMO 5. Compensate farmers for livestock losses to reduce hostility toward Fishing Cats	1. Revision of Wildlife Act 2. Setting priorities 3. Amend the existing rules 4. 4.Keep budget in the ADP/AWP of CMC/VCG/RMO	1. Conduct research on the future shifts in poultry dependence, secure the necessary funding, and, after considering scale effects, develop a robust long-term strategy 2. Devise ways to swiftly and methodically evaluate claims and disburse compensations 3. Perhaps, an app can be developed 4. Setting priorities 5. Amend the existing rules 6. Keep budget in the ADP/AWP of CMC/VCG/RMO

							7. Little compensation required
5	Overfishing from the haor/beel	✓			1. Ensure full compliance with fishing regulation, quotas set and close loopholes. 2. Awareness 3. Endorsement of local conservation initiatives 4. Ensure the existing rules/act's provisions are complied 5. Law enforcement and monitoring 6. Regulating fishing and fishing methods 7. Legal action and political commitment	1. Strengthen the law enforcement on unsustainable fishing 2. Law enforcement 3. Legal action and political commitment 4. Ensure the existing rules/act's provisions are complied 5. Law enforcement and monitoring 6. Fishing cooperatives 7. Seasonal bans	1. Educate beel owners about fish stock dynamics in the context of economic benefit, with taking into account prospect theory 2. Legal action and political commitment 3. Declaration of protected areas and OECMs 4. Law enforcement and monitoring 5. Ensure the existing rules/act's provisions are complied
6	Release of FC in elsewhere like Protected Area and different places which is far away from where the FC was captured	✓			1. Ensure field staff are well-informed about the habitat requirements and basic principles of releasing fishing cats 2. Train personnel about fishing cat conservation, rescue and release 3. Setting reintroduction guidelines 4. Carrying capacity assessment of an area before releasing 5. Population survey of FC 6. Preparation of release plan as per carrying capacity and population survey result 7. Ensue no diseased or	1. Develop a well-defined protocol for release procedures to ensure consistency 2. Strengthening capacity of FD 3. Build up rescue centers at different vital hotspots 4. Setting reintroduction guidelines 5. Carrying capacity assessment of an area before releasing 6. Population survey of FC 7. Preparation of release plan as per carrying capacity and population survey result 8. Ensue no diseased or injured FC are released 9. GPS tagging 10. Native zone release	1. Build a scientific knowledge base to guide the release of territorial carnivores, and systematically store the outcomes in a database to inform future efforts. 2. Setting reintroduction guidelines 3. Carrying capacity assessment of an area before releasing 4. Population survey of FC 5. Preparation of release plan as per carrying capacity and population survey result 6. Ensue no diseased or injured FC are released 7. Release FC in the same area or at

					injured FC are released		least similar habitat.
7	Soft penalty in the wildlife Act	✓			1. Create mechanism to ensure accountability and reduce undetected violations of killing by having information network before increasing the severity of penalties. Because, excessively harsh penalties risk driving carnivore killings underground 2. Stricter conservation measures 3. Wildlife Act Amendment and increase the penalty 4. Train forest officials & police to impose maximum 5. Engage local influencers (imams, teachers) to condemn wildlife crime. 6. Compensation 7. Should be given for poultry loss	1. Revision of Wildlife Act to increase the penalty 2. Track prosecution rates to measure impact. 3. Track conviction rates to hold officials accountable 4. Policy advocacy 5. Law amendment	1. Devise ways to swift prosecution 2. Revision of Wildlife Act to increase the penalty 3. Pilot strict enforcement in important districts (e.g., Sunamganj)
8	Scarcity of local fishes in haor/beels	✓			1. Program to release small fish fry of indigenous species (especially koi, sing, Magur) into open water bodies.	1. Fishing in the water using toxic chemicals must be legally stopped	
9	Poor implementation of ECA and PA rules	✓			1. Full implementation of ECA Management Rules, 2016 and Protected Area Management Rules, 2017	1. Full implementation of ECA Management Rules, 2016 and Protected Area Management Rules, 2017	1. Full implementation of ECA Management Rules, 2016 and Protected Area Management Rules, 2017

10	Law enforcement under political power	✓					1. Acquire political support in wildlife conservation
11	Habitat modification in response to the demand from overpopulated area	✓					1. Study on habitat modification in response to the demand from overpopulated area
12	Lack of ecological knowledge of habitat requirement	✓			1. Training the FD official and local people		
13	Conversion of wetland to fish farms	✓					1. Comply with the existing rules and regulations
14	Lacking of power to arrest offender in the Wildlife Act	✓					1. Amendment of Wildlife Act,2012

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion on fishing cat capture, rescue and release of adult and kittens

North-east Bangladesh does have profound human-fishing cat conflict situation. This conflict situation influences on the fishing cat capture or even killing. In an average there are at least 12 adult fishing cats are being captured per year in this northeast region of Bangladesh. Minimum yearly 8 kittens are also being captured or observed by the people of these 4 districts. In this haor region, about 70% adult fishing cats are captured by metal cage trap. From the capture records, it can be stated that for saving poultry farm people mainly used to catch fishing cats. There observed a single incidence that fishing cat attacks goat. Four confirmed cases were recorded where fishing cat attacked human being during the study period i.e. four and half years. But these incidences do have the reasons behind. The important findings were either kittens capture or the killing of female fishing cat that means removal of partner.

The remarkable observation is, by any reasons the adult fishing cats are being captured since time immemorial. The study period since 2020 to 2024 is not out of it. But one important thing was realized that during this study period the forest officials were more active to rescue the trapped fishing cat within short time. At least the forest officials along with the local conservation Organization used to attend the capture spot and tried to talk with the local peoples early as possible. Consulted with them and insisted on releasing the captured individual at the same spot or rescue the individual safely without any injury. Since this attitude of the forest officials was really present during the study time that we observed. in the long run the death casualty was very less.

In fact, keeping kittens to the place where they were found for ensuring to give those back to their mother, releasing the adult fishing cats at the same or nearby place from where those were caught or captured, attendance of forest officials to the capture site as early as possible etc. can make the situation better for ensuring conservation of any fishing cat. The wildlife Management and Nature Conservation Division, Sylhet(Headquarter-Moulvibazar) division actually practiced those in the Northeast Bangladesh during the study period. These were the best practices no doubt. Researcher Muntasir stated that In Bangladesh, between 2010–2013, at least 30 fishing cats were killed whereas, in 2021, Forest department personnel and citizen scientists were working together in northeast Bangladesh to turn the tide for this small cat now (Muntasir 2021).

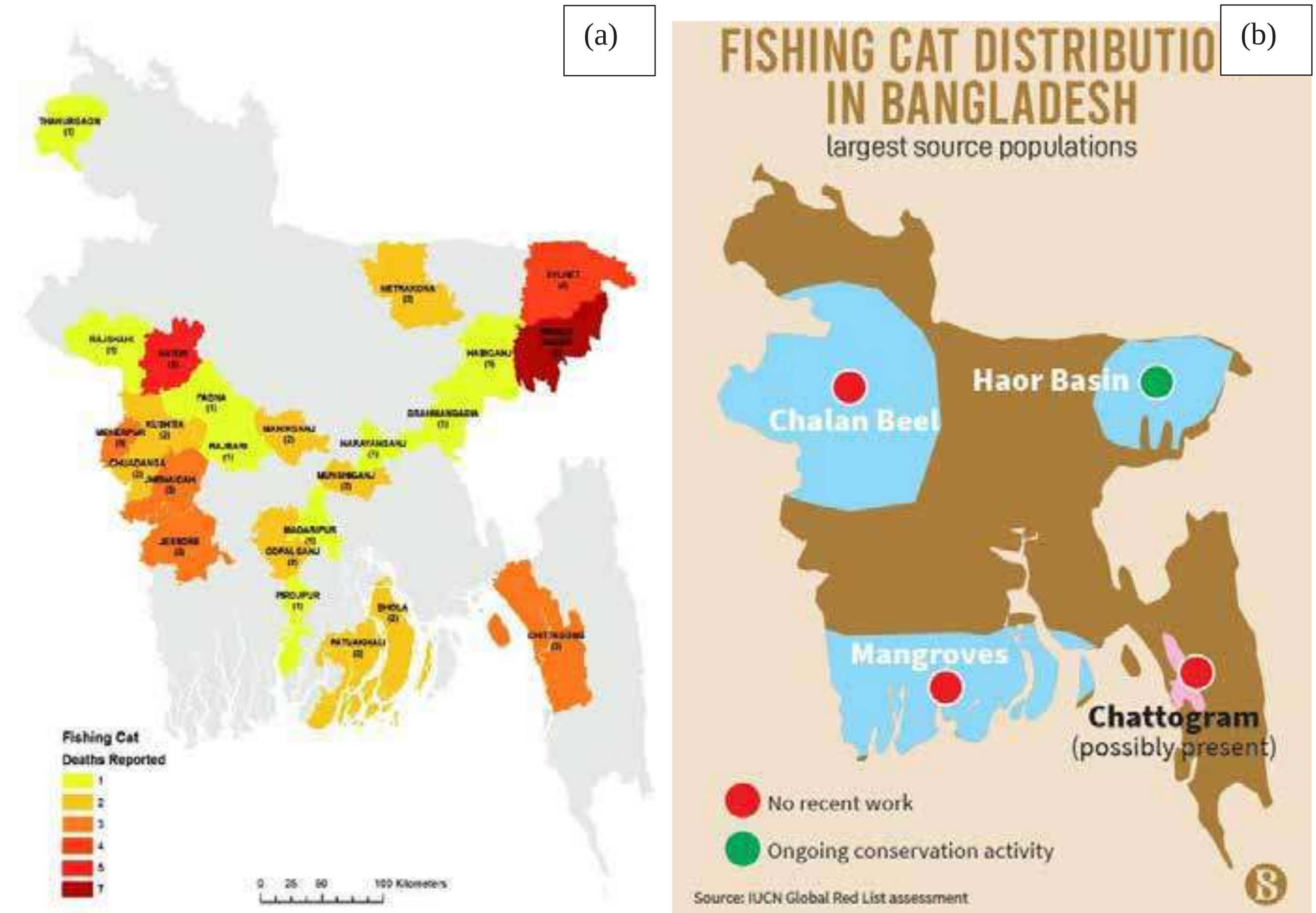

Figure 58. Comparison of fishing cat scenario in between 2013 and 2021: (a) Distribution of fishing cat in Bangladesh based on media reports between January 2010 and March 2013 (Sayam et al., 2015); (b) A tribute to Fishing cat-Distribution of Fishing cat (Akash, 2021)

This is also very important that if the management authority i.e. forest department wants to conserve fishing cats in their territory they should release the captured cat in the same place from where the individual was captured or caught. On the other hand, if it is not really possible to release the captured cat in the same place due to the disagreement of the local people then it can be released at a nearby place within one to two kilometers so that the fishing cat can go back to its own habitat.

Releasing elsewhere at a long distance or in the hilly or terrestrial forest that would occur a potential threat to that cat's survival. Especially if the cat is a lactating female one then fatality might happen to the kittens on the other hand, the mother would become a frustrated individual.

5.2 Recommendations and Suggestions for Conflict Mitigation

For the sake of fishing cat conservation, the following actions will definitely bring a positive result to mitigate human-fishing cat conflict.

1) Making rapid communication and building trust with local community through rescuing animals by Forest department as early as possible.
2) The moment the information comes to the forest department officials the telephonic communication should be established then and as early as possible someone should start going to the captured site.
3) It is better to release captured fishing cat in the night time at the same place from where it was captured or caught. If someone see any kittens, he or she should not displace them. Because at least in the evening the mother will take the kittens and will shift them to another safe place.

4) Metal cage trap should be seized so that it can be re-used for further capture of fishing cat.

5) To reduce the number of the capture the wildlife case shall be filed against the killer of the fishing cat.

6) The wall of the poultry farm should be made of concrete or at least should be of brick wall so the fishing cat cannot enter into the farm. Which will definitely reduce the conflicting situation.

7) Provision of giving compensation to the farm owner in case of poultry damages can be launched.

8) Build up information sharing hub with conservation groups.

9) Lighting can be provided around the fishing ponds to reduce conflict with FC.

10) Fishing cat must not be released in the small Protected area (PA) which are not at all the habitat of Fishing cat.

11) Aware people as- Fishing cats are not liable in all cases for making loss in poultry farms.

12) Legal action against the accused shall be taken.

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The Status, Distribution and Conservation of Colonial Waterbirds in Bangladesh

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CHAPTER I
INTRODUCTION

1.1 Context and Purposes of the Study

Waterbirds that are considered colonial assemble in groups for breeding purposes at sites known as colony sites. Aggregation of waterbirds breeding at such sites are called colonies (Kushlan, 1986). Technically it would take only two pairs nesting at a site to make a colony, but most range from hundreds to thousands of birds. The spatial limits of specific colony sites are sometimes hard to define precisely (Buckley and Buckley, 1980). Social nesting is a well-established behavior for the species under discussion, although there are differences in the extent of colonization. Most species find food in deep water or wetland environments, which include lakes, rivers, marshes, agricultural land, coastal lagoons, bays, and related riparian ecosystems. Due to their prominence in the aquatic food chain, they are regarded as reliable indicators of the health of ecosystems. In Bangladesh, heron colonies can be found all across the country. The majority of Ciconiiformes wading birds are dependent on wetland environments. Among the world's most productive ecosystems, wetlands are essential for preventing flooding, replenishing aquifers, absorbing nutrients, and reducing erosion. Furthermore, wetlands serve as habitat for a vast array of fauna, including fish, frogs, birds, mammals, insects, and plants (Buckton, 2007). Wetlands therefore contribute to the diversity of flora and fauna. Anthropogenic pressures, which can greatly influence the structure of the bird community.

The public and scientists have been fascinated with waterbirds for a long time due to their aesthetic appeal, quan tity, visibility, social behavior, recreational value, and economic significance. Recently, waterbirds have attracted attention as indicators of wetland quality, as well as success metrics for restoration and biodiversity in the area.

In Bangladesh, colonial waterbirds include herons, egrets, ibises, storks, gulls, terns, skimmers and cormorants. These waterbirds are very important for the wetland and agricultural ecosystem. These birds share the unusual characteristic of nesting in dense assemblages. Due to this tendency, they usually only reproduce in a few numbers of places, therefore the loss of a few breeding spots could have a significant impact on the population as a whole. In this research, our main focus would be to look for the diversity (waterbird colony location, numbers, plants used for nesting, species composition, activity status, and habitats occupied) and conservation status of colonial waterbirds in Bangladesh. We will also give emphasis on understanding the local perceptions on climate change impact in waterbird population, agriculture and fish production in nature, and community-based colonial waterbird conservation. This research work will also aim to establish networking with bird researchers, conservation activists/groups. In addition to that, this research work will also propose to bring small but ecologically important areas under protection terming Small Conservation Site/ Community Conserved Areas.

1.2 Problem Statements

The most significant threats to colonial waterbirds include human disturbance, predation, habitat loss, and contaminants. Protection of sensitive colonies depends on the availability of current locational information. Development of strategic conservation management plans to protect these species and breeding areas requires a broader understanding of population trends. These birds are particularly vulnerable to issues including human disturbance, predation, extreme weather, and competition for nesting sites because of their propensity of nesting in big flocks. They are threatened by habitat deterioration, environmental loss and contamination, shifting food webs, and other factors just like other birds. Bangladesh, one of the world's most climate-vulnerable nations, may be able to employ colonial waterbirds as a biological predictor. It has been established that birds are among the first organisms to respond to environmental change in the event of a climate change disaster. So, this research has the scope to study climate change effects on the waterbird colony engaging local community discussion.

Information and research are always needed to implement effective management. To prevent and alleviate conflicts with human activities, regular surveys and inventories are required on a regional basis to detect trends in the current state of the population. Research on species ecology is frequently required before effective management can be implemented, and studies of population demography are required for species that are experiencing population declines. It is also necessary to employ additional strategies to reduce conflicts between humans and birds. Human activity is probably one of the main factors restricting the number of some species, and on the other hand, people are mostly responsible for an increase in the number of potentially problematic species. It is crucial to keep educating the public and conservation management organizations about the significance of colonial waterbirds and their role. Many species are likely to continue to suffer from gradual incremental loss and degradation of habitats, and a conservation strategy for the protection of these birds throughout Bangladesh is recommended. These are no longer sufficient in the face of emerging issues, and to safeguard populations of the native colonial waterbirds, new approaches are required. Active management is likely necessary to maintain colonial waterbird populations for a long time. Active management includes actions like acquiring and restoring habitats, managing the food chain, and reducing chemical pollution, disturbances, competition, predation, and man-caused mortality. It also includes colony site protection and restoration programs. There is a constant need to enlighten the public, political figures, and government leaders to elicit and sustain public support for management. This study reviews the needs for further information, law enforcement, and education relative to the management of colonial waterbirds.

1.3 Objectives of the study

i. Determine the diversity (waterbird colony location, numbers, plants used for nesting, species composition, activity status, and habitats occupied) and conservation status of colonial waterbirds in Bangladesh.

ii. Investigate the climate change effect on waterbirds and understanding local perceptions, community-based colonial waterbird conservation and networking with bird researchers, conservation activists/groups in Bangladesh.

1. 4 Research questions

i. How many waterbird species do breed in Bangladesh (study area)? Where and when do they breed? What is the species composition in a waterbird colony in Bangladesh?
ii. What is the present status of waterbirds in Bangladesh (study area) and how waterbirds are coping with climate change in Bangladesh?
iii. What are the key threats and conservation challenges for the waterbirds in Bangladesh?
iv. Which plant species are most important for the nesting waterbirds in Bangladesh?
v. Is there any conflict between human and waterbirds at the colony site?
vi. Is there any impact of waterbirds in human livelihood? What is the local perception of climate change impact on waterbirds, agriculture and fish production in Bangladesh?

CHAPTER II

LITERATURE REVIEW

In Bangladesh, some works have been done on Asian Openbill such as conservation of storks and ibises in Bangladesh by M.A.R. Khan, 1984 (Tigerpaper 11(4): 2-4); conservation of storks and other waterbirds in Bangladesh by M.A.R. Khan, 1987 (Colonial Waterbirds, U.S.A. 10(2): 229-235); status and distribution of storks, ibises and spoonbills of Bangladesh by S.U. Sarker; M.A.R. Khan and A. Rashid, 1985 (ZUM Fliegn Geoboren No. 2, P.3). Confirmed breeding records of Asian Woollyneck *Ciconia episcopus* from Bangladesh by Mohammad Tarik Hasan and Prashant Ghimire, 2020 (ISSN 2710-1142 (online); *SIS Conservation*, 2020, **2**, 47-49). Lesser Adjutant breed in Bangladesh widely and no recent records after 1980 and a discovery of new colony sites in 2011 (Chowdhury S. U. & Sourav M. S. H. 2012). A small brochure on 'Introduction to bird colonies of North Bengal' (Dey, T.K., Karim, M. R. and Rahman, M. M., 2015), reported 50 bird colonies from 16 districts covering Rajshahi and Rangpur divisions. Recently, Bangladesh Forest Department funded primary research on bird colonies which mainly located 71 bird colonies (all kinds birds) from North Bengal (Rahman, M. M., 2021). This report also included three digital maps that showed 71 active and abandoned bird colonies (All bird species), of which only 21 are colonial waterbirds sites and rest of the colonies for other birds. and population was not estimated. However, that report did not clarify the definition of bird colonies, number of FGDs and awareness programs conducted in the region. That report also did not mention the population, bird diversity, nesting tree and habitat preferences etc.

This research would focus on identifying the breeding and night roosting waterbird colonies, representing heron, storks, cormorants, and a few breeding waders (Pratincoles, Terns) in 24 districts covering Rangpur, Rajshahi, Mymensingh and Sylhet divisions. This research work would also emphasize on recording species composition with an estimated population in each colony, record the plant species they prefer for nesting, monitor breeding ecology in a few selected nesting sites, explore the potentiality for declaring community conservation areas, and based on the research findings a national plan of action have been proposed.

CHAPTER III
METHODOLOGY

3.1 Study Area/Research Site

The study area is the North and north-eastern part of Bangladesh. About 57,379 km^2 (5,739,174 ha) and 39% of the country's total land area and comprises twenty-four districts of Rajshahi, Rangpur, Mymensingh and Sylhet divisions.

3.1.1 Location

The study area comprises four divisions (Rajshahi, Rangpur, Mymensingh and Sylhet divisions) including twenty-four districts.

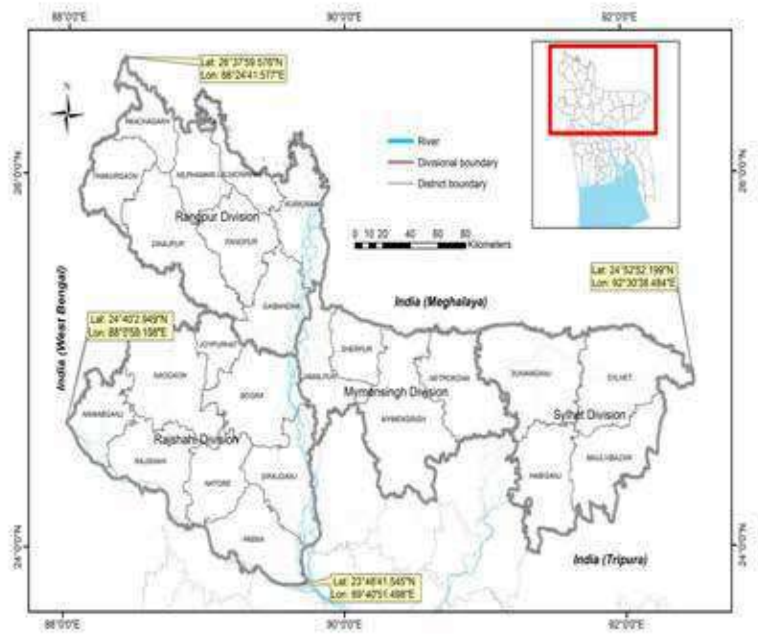

Figure 1: Map of the study area showing the four division (Rajshahi, Rangpur, Mymensingh and Sylhet divisions).

3.1.2 Climate

It has a humid, warm climate influenced by pre-monsoon, monsoon and post-monsoon circulations and frequently having heavy precipitation and tropical cyclones. The deltaic form of the country just lies above the sea level, and its funnel shaped estuary makes it vulnerable to cyclonic hazards i.e., strong winds, storm surges. Its climate is characterized by high temperature, heavy rainfall, humidity around 78% in most of the times, distinguished seasonal variation. Weather pattern has been erratic, cool weather, shorter dry season observed in recent years, it may be the temporary phenomenon or the beginning of the long-term changes because of global warming (Rashid, 1991). The mean annual temperature is around 25°C, mean monthly temperatures between 17°C in January and 29°C during April to May, the height temperatures between 38°C and 42.6°C (Figure 3). The average annual rainfall is about 2100 mm and 80% of the total rainfall occurs during May to September (Figure 6). The overall temperature increases in few last years (Figure 4 & Figure 5).

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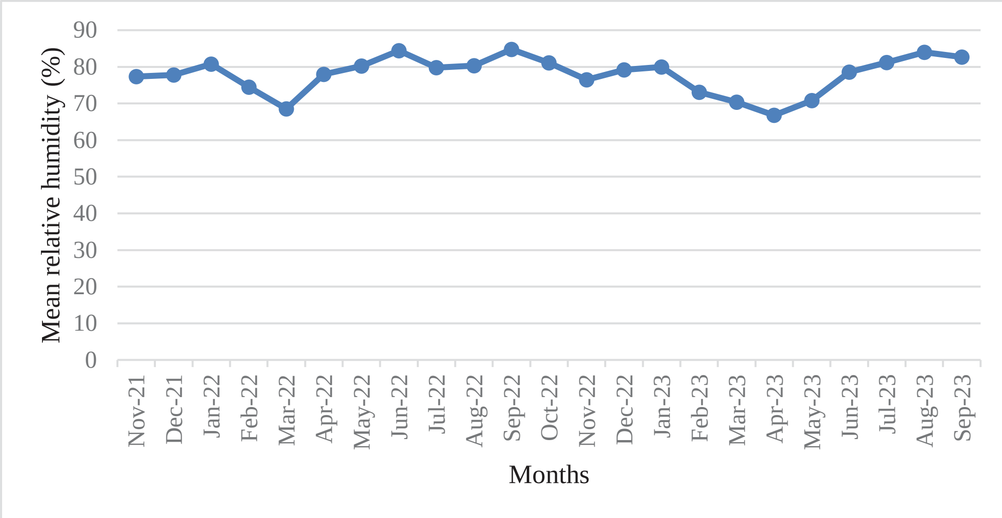


Figure 2: Mean relative humidity during the study.

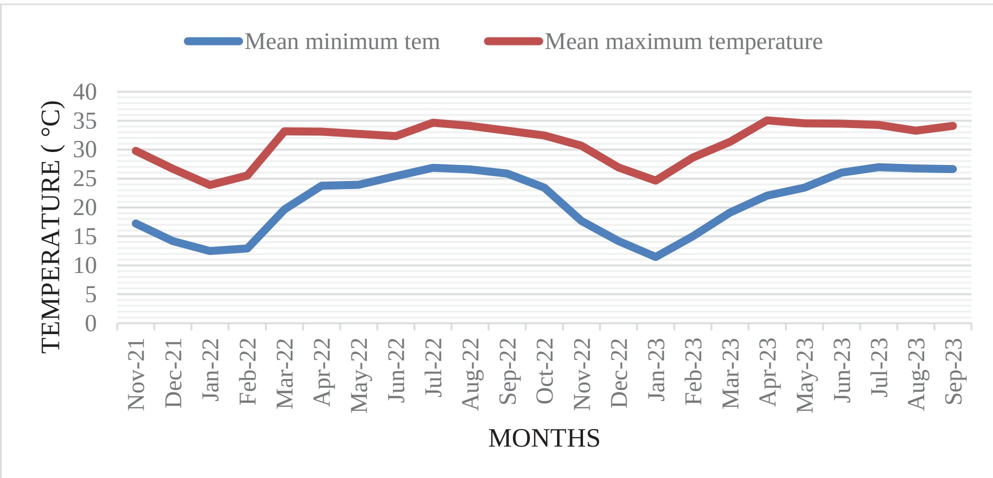


Figure 3: Mean maximum and minimum temperature during the study period.

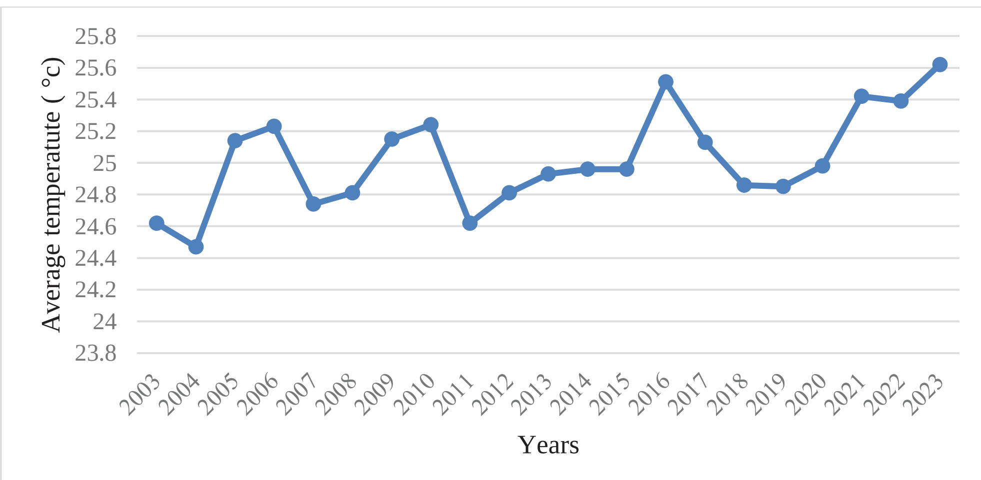


Figure 4: Yearly average temperature during the last twenty-one years of the study area.

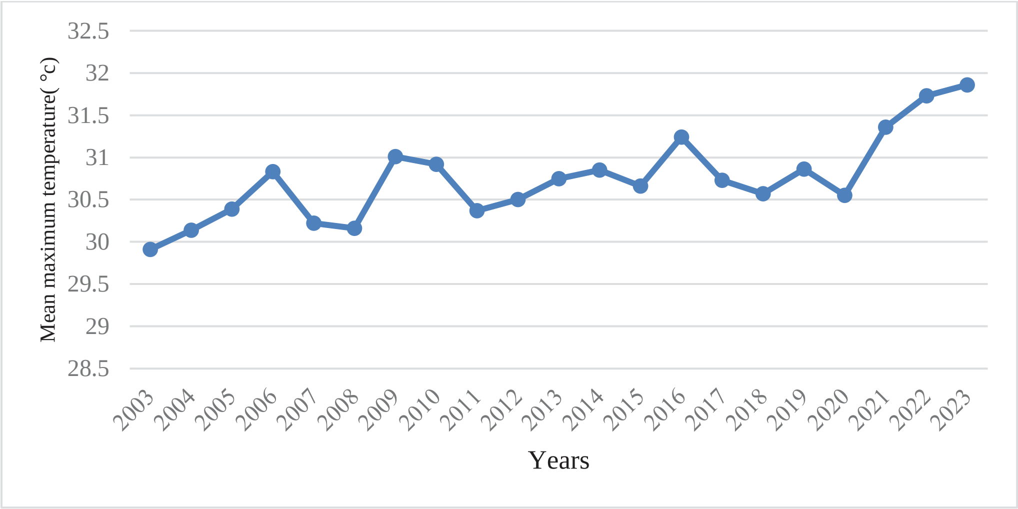


Figure 5: Mean maximum temperature during the last twenty-one years of the study area.

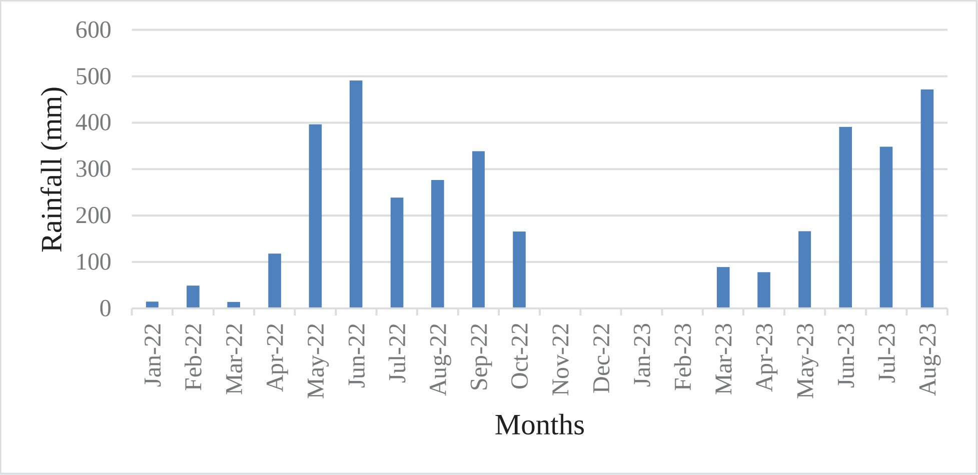


Figure 6: Monthly average rainfall during the study period.

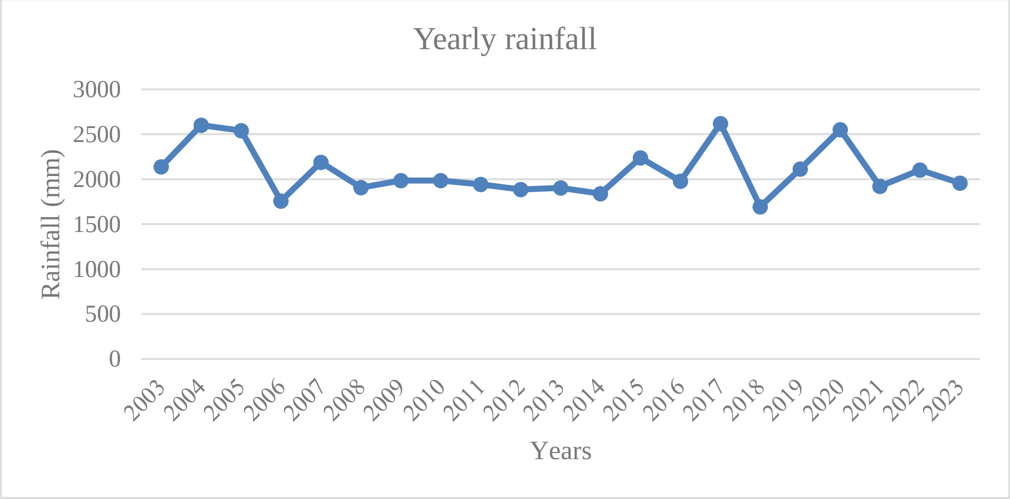


Figure 7: Yearly rainfall pattern of the study area.

3.1.3 Geology

The study area is in the Himalayan foothills, the Garo Hills of Meghalaya and some eastern part Tripura Hills piedmont; its land building process due to the Ganges and Brahmaputra rivers; Large area under Barind Tract of the North Bengal. The delta largely consisting of the big river- the Ganges and the Brahmaputra. (Rashid, 1991). Bangladesh is highly flood prone country and its topography is flat, the majority of the landmass laying 10 meters, rises in the average sea level, more area may be extremely vulnerable to flooding (GED, 2015). There is a major seasonal variation in the amount of water flow and it has a serious consequence for the morphology of the rivers. At the time of high flow, flooding, riverbank erosion, flash floods (Mohsenipour *et al*., 2018. The Teesta River originates from Himalayas and act as an important water source for irrigation. Mymensingh division classified into older floodplain and younger floodplains, and these floodplains are separated by Old Brahmaputra River; the younger floodplain consists of unconsolidated fine to medium sands with some silt and clay whereas the older floodplains consisting of clay and fine to medium sand (Akter *et.al*. 2018). The geological aspects of Sylhet Division are shaped by its location on the southern slopes of the Meghalaya Plateau and the presence of several rivers and hills. Sylhet is part of the Surma Basin, a region characterized by extensive sedimentary deposits. The basin is formed by the Surma and Barak rivers and their tributaries. The geology of the tea garden region, along with favorable climate conditions, makes it suitable for tea cultivation. The hilly terrain and well-drained soils contribute to the growth of high-quality tea bushes.

3.1.4 Vegetation

The Rajshahi region has a tropical monsoon climate, and the vegetation is influenced by the Ganges River and its tributaries. This area is a major rice-producing region, and extensive paddy fields are a common sight. Riverine vegetation includes grasses, shrubs, and trees adapted to the riparian environment. The region has only deciduous forests like Altadighi National Park. The vegetation of Greater Rangpur is very similar to Rajshahi division. The division has a mix of both deciduous and evergreen vegetation, with trees like mango, jackfruit, and bamboo being common. Some areas have deciduous forests and Sal is the dominant tree species. Some areas in Rangpur division have tea gardens, contributing to the country's tea production. The favorable climate and terrain support the cultivation of tea bushes. Mymensingh division exhibits diverse vegetation types influenced by its geographical features, climate, and soil conditions. Mymensingh is a significant rice-producing region, and rice fields are widespread. Sylhet Division is known for its scenic landscapes, hills, and diverse vegetation. The region's vegetation is influenced by its geographical features, climate, and elevation. This region is characterized by several hill ranges, including the Jaintia and Khasi Hills. These hills are covered with dense forests featuring a mix of evergreen and deciduous trees. The forested areas contribute to the region's biodiversity. The region has mixed deciduous forests in certain areas, featuring a variety of tree species that shed their leaves seasonally.

3.2 Methods

3.2.1 Selection of sampling method and site

Initially, in the project proposal, the field areas were a total of 64 districts covering whole Bangladesh. However, due to budget and time constraints from the Project Management Unit of SUFAL Project, the current proposal is now restricted to 24 districts. Moreover, in the project proposal, the field days were a total of 360 days (14-15 days per district). However, due to budget and time constraints from the Project Management Unit of SUFAL Project, the implemented research project was restricted to a total of 180 days. Which significantly impacts the overall project planning and data collection process. Each district was visited by at least two researchers along with a local field assistant. Average 7-9 days were given to collect data from each district.

Multi-stakeholder focused group discussions (FGDs) were organized in each district following Krueger (1988) guidelines. A combination of citizen science (newspaper, eBird, social & electronic media), youth conservation group members and chairman from union parishad (UP), district administration, and local conservation activists were engaged to identify waterbird colony locations. FGDs and semi-structured questionary were helped to understand the local perception of climate change effect on colonial waterbirds, fish and agriculture.

3.2.2 Data collection procedure

Schematic diagram showing the conceptual flow of the approach and methods leading to attaining the anticipated results. Survey team was formed of about one bird experts under the leadership of Principal Investigator to conduct the survey and initiate conservation measures for the waterbird colonies in Bangladesh. Citizen science using newspaper, eBird, social & electronic media, conservation activist group, community engagement and secondary data were used to primary deification of the colony sites. Some of the Union Parishads (UPs) were visited under 24 districts and a semi structured focused group discussion were arranged with UP members, and youth/conservation activist groups. Waterbird Conservation Group (WCG) were formed at large colony sites after discussing with UP members and social elites. Both breeding colonies and night roosts were identified and based on the collected data and findings a draft national action plan of colonial waterbirds was prepared and proposed to SUFAL Project of Forest Department.

Figure 8: Community engagement for identifying waterbird colony sites.

Figure 9: Community engagement and FGDs.

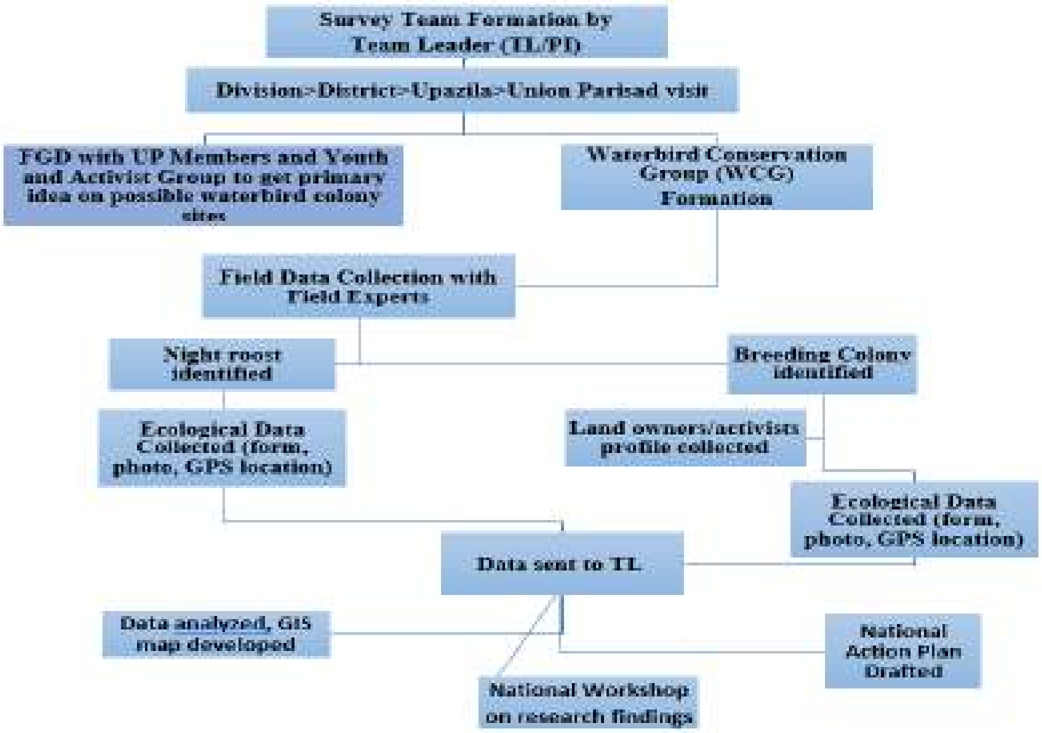


Figure 10: Research flow of the approach and methods leading to attaining the anticipated results.

3.2.3 Physical and chemical parameters measurements (if any) and the instruments used for measurement.

An extensive field survey was conducted during the early stages of the breeding season of the waterbirds. Identification of birds were done using field guides (Ali and Ripley, 1987; Grimmet *et al.*, 1999). We gather information on the number of nesting sites, nesting adults, number of nests, stage of nesting and nesting trees. Groups of breeding pairs are considered independent colonies if they are: separated from other groups within a continuous habitat by at least 400 m, or separated from other groups by a significant habitat discontinuity. We identified both the breeding and night roosts colonies that contains more than two thousands of birds during field visits. Field observations were made using binoculars (10x42), Spotting scope, GPS, measuring tape, scale, digital slide calipers, digital weight machine, laser rangefinder (Trupulse 200x laser), CCTV, trap camera, drone and digital camera. Separate data sheet was used to record each nesting colony.

Figure 11: Trail Camera installed at Woolly-necked Stork colony, Godagari, Rajshahi.

3.2.4 Analytical methods (Data)

3.2.4.1 Population Estimates – Complete direct counts and ground techniques such as transects are appropriate census techniques (Nettleship 1976; Hutchinson 1979; Erwin 1980). Population estimates are based on counts of active nests, and occasionally on the number of adults present. Active nests of these species are counted by a team of observers who move systematically through the colony recording the tree number, tree species and number of nests of each bird's species. Each tree containing a nest are marked with a tag bearing a unique number. The coordinates of nesting sites are mapped with Arc View GIS software. For ground nesters (Small Pratincole and Little Tern) census were conducted at the peak of their nesting period, generally the mid of March and first week of May, to determine their breeding population. Transects (3 m wide) were applied for Small Pratincole and Little Tern and observers carefully walk the transects counting all active nests with a manual handheld tally counter and mark each nest with a tag (bamboo stick) bearing a unique number to identify that is has been counted.

3.2.4.2 Habitat

Major habitat type of the colonial waterbirds was determined depending on major vegetation and foraging habitats of five-kilometer radius from each colony sites. Generally, the colonial waterbirds forage very close to nesting sites. Habitats were classified as six major categories i.e., Agriculture mixed, Agriculture rice, Homestead (Urban dominated areas), Mango orchard, Tea Garden and Wetlands.

3.2.4.3 Threat

Both natural and anthropogenic threats were determined, and each category divided in to four types depending on the intensity/degree of occurrences as very low (1-25% of intensity of occurrence), low (26-50% of intensity of occurrence), High (51-75% of intensity of occurrence) and very high (76-100% of intensity of occurrence).

3.2.4.4 People's perception on climate change and its impacts

To understand the local people's perception on climate change and its impacts on colonial waterbirds, agriculture and fish production in Bangladesh in the last 15 to 20 years, for this we engaged local communities adjacent to nesting colony sites and gathered their perspectives. This could involve conducting surveys, focus group discussions, and interviews with key informants, such as community leaders, farmers, fishers, and other stakeholders. More than 50 focused group discussions (FGD) and semi-structured questionnaire has been developed and conducted at different colonial sites or location of various age group with different local stakeholder. About 144 people participated/interviewed to understand the community perception about climate change and its impact on colonial waterbirds, agriculture and wetland ecosystem in Bangladesh.

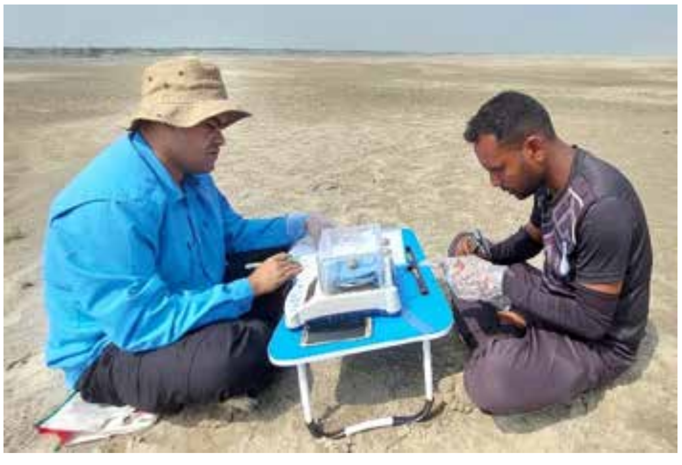


Figure 12: Nest count and egg morphometry at river bed of Padma, Rajshahi.

3.2.5 Statistical analysis

After the data collection, analysis of data was analyzed by various computer and statistical model. *Spatial Analysis:* Geospatial statistics and Geographic Information System (GIS) analysis to determine colony locations, habitat preferences, and distribution patterns of waterbird species. *Population Estimation:* Direct total nest count to estimate the abundance and population sizes of waterbird species. *Habitat Analysis:* Statistical techniques to correlate habitat characteristics (e.g., vegetation types) with nesting preferences. *Breeding Success Analysis:* Descriptive statistics to assess nesting success rates, clutch size, hatching success, fledgling success, etc. *Threat and conservation status Assessment:* Statistical analyses to quantify and prioritize threats (e.g., habitat loss, human disturbances) using survey data. Qualitative and quantitative analysis of survey data using statistical methods or factor analysis to understand perceptions on climate change, and its impact on colonial waterbird population, agriculture, and fish production.

CHAPTER IV
RESULT AND DISCUSSION

4.1 Identification and status of the colony sites

A total of 353 colony sites were identified from direct field visits, community engagement and different sources like news, journalists, NGOs, social media, eBird, and with the help of conservation activist groups. A total of 224 active breeding colonies were identified of which 45% colonies were identified through FGDs, 25% from direct sighting during field visit, 11% from personal communication, 7% birding communities, 7% through news (Print and electronic media), 2% from social media and 3% from Forest Department's record (Figure 13). Total 224 active nesting sites were identified of which 217 were tree nesting, 3 were tower nesting and 4 were ground nesting colonies (Figure 14). The tree nesting waterbird colonies inhabited a total of 149.53 hectare of land, of which 93% (139 ha) was privately owned and 7% (10.53 ha) was owned by the government (Figure 15).

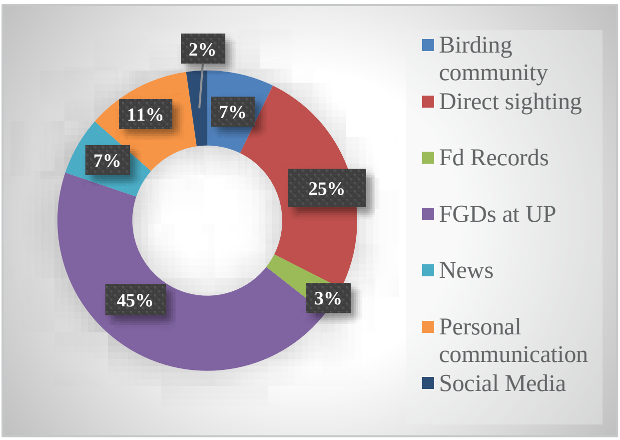


Figure 13: Mode of waterbird colonies identified.

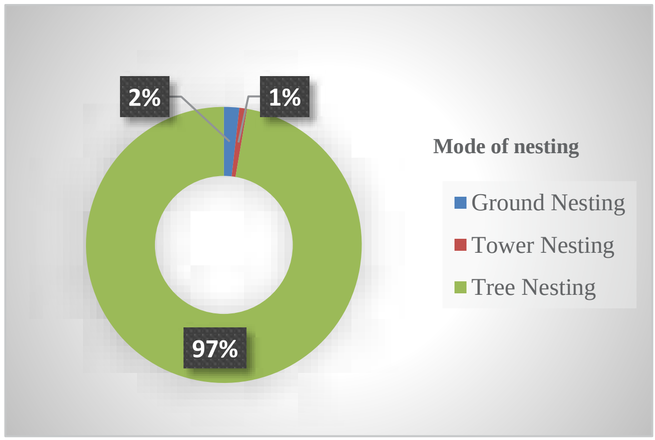


Figure 14: Types of nesting of the active colonies.

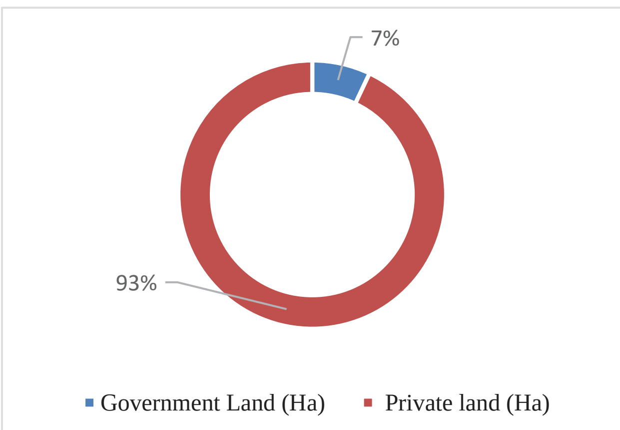


Figure 15: Occupied land areas of the tree nesting waterbird's colony.

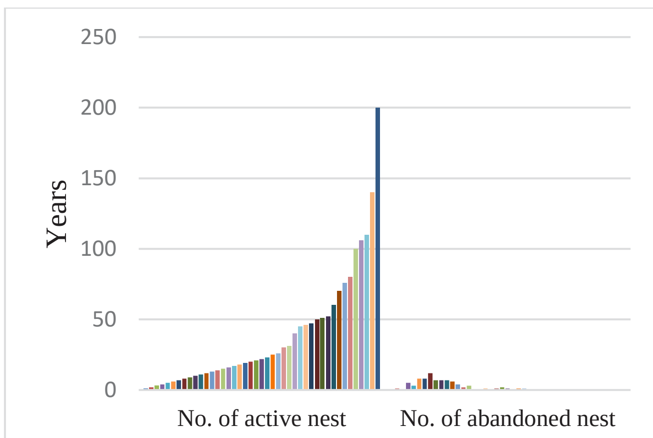


Figure 16: Number of years the colony sites used by the waterbirds.

4.2 Age of the nesting site

Nesting sites used by colonial waterbirds years after years until the habitat is useable for nesting and no major disturbances occurred. Generally, heron's and egret's colony used the same colony sites very long time even more than 100 years that found in Domar, Nilphamari. Active colonies range from 1 to 200 years old, but most of the colony sites (211 nos.) ranges from 5-20 years, six colonies were more than 100 years old and average age of the colonies were 6.67 years. The abandoned colonies range from 1 to 26 years old but average age were 1.86 years old (Figure 16).

Population status, species composition, habitat preference and distribution

4.3.1 Population status and species composition

During the field surveys, we have visited 353 waterbird colonies of which 271 colonies were active, and the population was estimated in 224 active breeding colonies. A total of 82,086 nests and 165,072 individuals of 16 species were estimated, with Cattle Egret accounting for the highest percentage (33.21%), followed by Little Cormorant (27.47%), Asian Openbill (11.04%), Intermediate Egret (7.45%), Great Egret (6.59%), Little Egret (5.78%), Black-crowned Night Heron (5.55%), Indian Pond Heron (1.39%), Small Pratincole (0.52%), Little Tern (0.46%), Glossy Ibis (0.21%), Black-headed Ibis (0.19%), Indian Cormorant (0.04%), Lesser Adjutant (0.01%), Oriental Darter and Asian Woollyneck, the lowest percentage among the colonial waterbirds (Table 1). Species composition among colonies were varied significantly from single to multi-species and it depends on bird species. Asian Openbill and Lesser Adjutant colonies were mostly single species. But most of the colony were composed of multi species. Generally, the tree nesting colony were formed by egrets, herons and cormorants; then the Asian Openbill join the colony. In most cases the Asian Openbill drive away the egrets, herons and cormorants.

4.3.2 Habitat preference and distribution

The most preferred habitat of the colonial waterbirds was agriculture rice. About 63% colonies were located in agriculture rice dominated areas, 16% colonies in wetland dominated areas, 12% colonies in agriculture mixed, homestead areas contain 4% of colonies, tea garden contains 3% colonies, river bed 1% and mango orchard contains 1% colony (Figure 17). Colonial waterbirds build its nets in different habitat types depending on food available and safe nesting place, but habitat preferences are species specific. In term of number of nests of all species, the agriculture rice habitat showed the height number of nesting abundance (65.43%), wetland showed second height number of abundance (18.54%), the third most preferred habitat was agriculture mixed, tea garden (3.63%), homestead habitat (3.01%), mango orchard preferred (1.43%) and the lowest preferred habitat was river bed (0.95%) (Table 2).

Table 1: Relative abundance of colonial waterbirds.

Sl. No.	Bird Species	Total number of nests	Population	Relative abundance
1	Asian Openbill	9012	18226	11.041%
2	Cattle Egret	27412	54824	33.212%
3	Great Egret	5444	10888	6.596%
4	Indian Cormorant	37	74	0.045%
5	Intermediate Egret	6153	12306	7.455%
6	Lesser Adjutant	7	28	0.017%
7	Little Cormorant	22679	45358	27.478%
8	Little Egret	4778	9556	5.789%
9	Night Heron	4585	9170	5.555%
10	Oriental Darter	4	8	0.005%
11	Pond Heron	1154	2308	1.398%

Sl. No.	Bird Species	Total number of nests	Population	Relative abundance
12	Small Pratincole	434	868	0.526%
13	Little Tern	382	764	0.463%
14	Black-headed Ibis	2	326	0.197%
15	Glossy Ibis	0	360	0.218%
16	Asian Woollyneck	3	8	0.005%
	Grand Total	82086	165072	100.00%

Among 16 species of colonial waterbirds of which 14 species found in agriculture rice habitat, 9 species in agriculture mixed and wetland habitat, 8 species found in homestead habitat, 7 species in mango orchard and tea garden habitats and only 2 species were found in river bed (Table 2).

Asian Woollyneck, Black-headed Ibis and Lesser Adjutant only prefer agriculture rice habitat. Agriculture rice habitat preferred by colonial waterbird species were Asian Openbill (80.28%), Cattle Egret (77.50%), Great egret (71.01%), Indian Pond Heron (70.52%), Indian Cormorant (69.76%), Little Egret (67.59), Intermediate Egret (67.50%), Little Cormorant (45.43%) and Black-crowned Night Heron (54.96) (Table 2). The agricultural land contains many waters reservoir and even inundated during breeding seasons that provides sufficient foraging habitats of the species. Little Tern and Small Pratincole only preferred river bed habitat because of they build nest on sandy with undisturbed char lands (new char lands of river) of major rivers. Asian Openbill mostly preferred agriculture rice habitat (80.28%) and following agriculture mixed (10.51%), homestead (5.21%), wetland (1.98%), tea garden (1.39%) and lowest preferred was mango orchard (0.60%). Cattle Egret highest preferred habitat was agriculture rice (77.50%), then wetland (10.93%), agriculture mixed (5.50%), tea garden (5.31%), mango orchard (1.37%) and lowest preferred habitat was homestead (0.36%). Great Egret mostly preferred agriculture rice (71.01%), subsequent preferred agriculture mixed (10.46%), wetland (8.07%), tea garden (5.31%), mango orchard (3,23%) and homestead (1.75%). Intermediate Egret mostly preferred habitat was agriculture rice (67.50%), wetland (18.07%), agriculture mixed (6.27%), tea garden (6.12%), mango orchard (1.37%) and lowest was homestead (0.65%). Little Egret preferred agriculture rice (67.59%), agriculture mixed (11.51%), wetland (10.52%), tea garden (8.13%), mango orchard (1.40%) and homestead (0.82%). Little Cormorant prefer agriculture rice (45.43%), wetland (42.70%), agriculture mixed (5.14%), homestead (3.33%), tea garden (3.07%) and mango orchard (0.30%). Indian Cormorant preferred agriculture rice (69.76%) and homestead (30.23%) habitat. Indian Pond Heron preferred agriculture rice (70.52%), wetland (22.87%), tea garden (5.03%) and agriculture mixed (1.56%). Black-crowned Night Heron preferred agriculture rice habitat (54.96%), homestead (22.31%), agriculture mixed (13.12%), wetland (5.71%) and mango orchard (3.88%).

The Asian Openbill colonies were found in only 16 districts i.e., Rajshahi, Chapainababganj Pabna, Naogaon, Joypurhat, Natore, Bogura, Sirajganj Gaibandha, Lalmonirhat, Nilphamari Panchagarh, Rangpur, Habiganj and Moulvibazar. Cattle Egrets, Intermediate Egrets, Great Egrets and Little Cormorants colonies were found in all the 24 districts, but Lesser Adjutant colony only found in Thakurgaon districts. Asian Openbill mostly found in Rajshahi and Rangpur division because of habitat preferences and safe nesting sites.

We identified and visited all 353-waterbird colonies of which 224 were active breeding, 47 were night roost and 82 were breeding abandoned colony. The highest number of colonies observed in Rajshahi division, 2nd height colonies in Sylhet division, 3rd in Rangpur and very few colonies in Mymensingh division. We identified and visited all 353 waterbird colonies and GIS-based map were created (Figure 24). The highest number of colonies were found in Rajshahi division, the second height colonies in Sylhet division, the third most in Rangpur and the lowest colonies in Mymensingh division. Among districts the highest number of nests observed in Naogaon district. Among the four division, highest percentage of colonial waterbirds were found in Rajshahi division

(40%), whereas the lowest percentage were the Mymensingh division (14%). The second height percentage of the colonial waterbirds were from the Sylhet division (29%) and third height were the Rangpur division (17%) (Figure 18). Six globally near threatened (NT) Asian Woollyneck Stork with three nests were found from the Rajshahi district. Twelve globally vulnerable Lesser Adjutant with six nests were found in Thakurgaon district and six from Rajshahi during breeding season, but no nest. The globally near threatened Oriental Darter with two nests from Rangpur district, two nests from Naogaon district and four nests from Moulvibazar districts. Nests of Little Tern and Small Pratincole were observed from River bed of Padma, Rajshahi. Four nests of globally near threatened Black-headed Ibis were observed in Naogaon district of Rajshahi division. About 360 Glossy Ibis regularly night roosts at three breeding sites of Asian Openbill in Rajshahi division. Indian Cormorant nesting was observed from two nesting sites of Rajshahi division. The highest number of Little Cormorant were found in Sylhet division followed by Rajshahi, Mymensingh and Rangpur. Intermediate Egret was most abundant in Sylhet followed by Rangpur, Rajshahi and Mymensingh. Great Egret was most abundant in Rajshahi followed by Rangpur, Sylhet and Mymensingh. Little Egret was most abundant in Rajshahi followed by Sylhet, Mymensingh and Rangpur. Asian Openbill mainly found in Rajshahi and Rangpur, but very few were observed in three nesting sites of Sylhet division.

Table 2: Number of occupied nests and habitat type in the study area

Bird Species	Habitat Type							
	Agriculture Mixed	Agriculture Rice	Homestead	Mango Orchard	Tea Garden	Wetland	River bed	Grand Total (Nests)
Asian Openbill	741	7278	474	0	223	296	0	**9012**
Lesser Adjutant	0	7	0	0	0	0	0	**7**
Asian Woollyneck	3	0	0	0	0	0	0	**3**
Black-headed Ibis	0	2	0	0	0	0	0	**2**
Cattle Egret	826	21755	106	575	935	3215	0	**27412**
Little Egret	201	4357	23	0	110	87	0	**4778**
Intermediate Egret	83	5850	0	0	54	166	0	**6153**
Great Egret	379	4879	14	0	60	112	0	**5444**
Indian Cormorant	0	37	0	0	0	0	0	**37**
Little Cormorant	35	3528	331	0	258	18527	0	**22679**
Night Heron	150	3940	340	108	0	47	0	**4585**
Pond Heron	96	319	0	0	0	739	0	**1154**
Oriental Darter	0	4	0	0	0	0	0	**4**
Small Pratincole	0	0	0	0	0	0	434	**434**
Little Tern	0	0	0	0	0	0	382	**382**
Grand Total (Nests)	**2514**	**51956**	**1288**	**683**	**1640**	**24005**	**816**	**82,086**

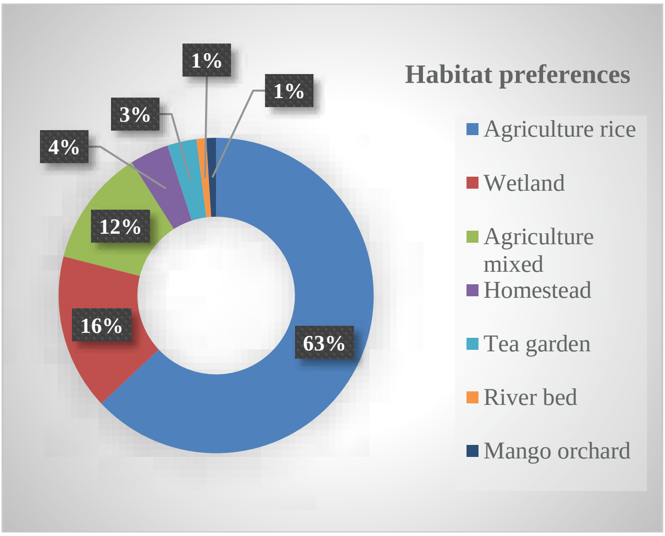


Figure 17: Habitat preferences of the colonial waterbirds.

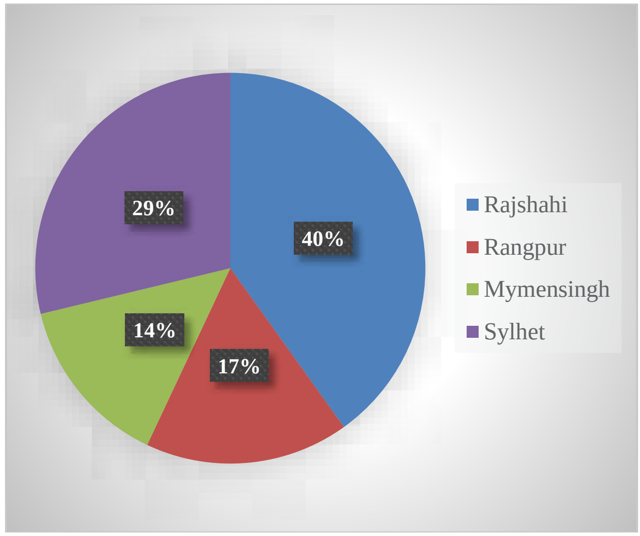


Figure 18: Colonial waterbirds abundance in four division.

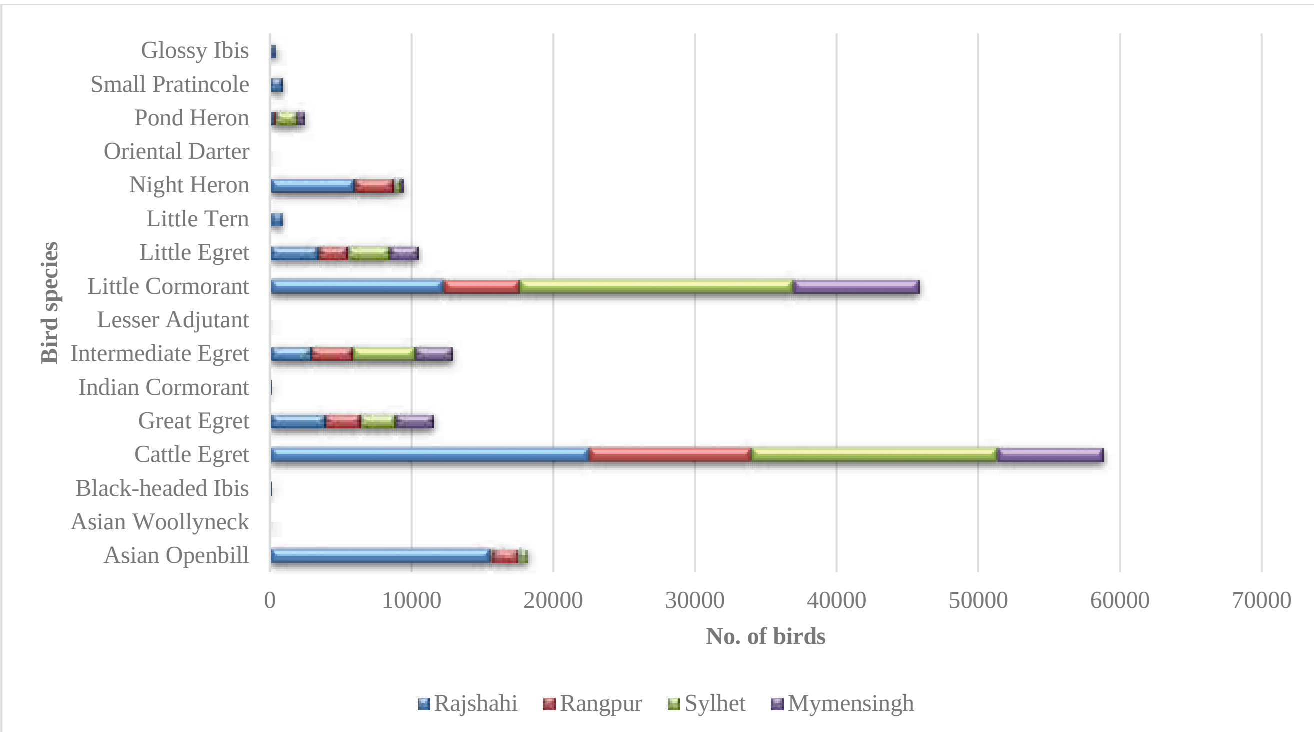


Figure 19: Colonial waterbirds abundance in four divisions.

4.3.2.1 Rajshahi division

A total of 16 colonial waterbird species comprising Cattle Egret (32.73%), Asian Openbill (22.59%), Little Cormorant (17.76%), Black-crowned Night Heron (8.63%), Oriental Darter, Lesser Adjutant, Black-headed Ibis and Asian Woollyneck were observed (Figure 20).

4.3.2.2 Rangpur division

A total of ten colonial waterbird species of which the highest number of Cattle Egret (39.52%), the second highest Little Cormorant (18.57%), the third highest Intermediate Egret (9.94%), the lowest was Oriental Darter

(0.01%), the globally vulnerable Lesser Adjutant was (0.04%) and it was the only breeding colony of the species in Bangladesh (Figure 21).

4.3.2.3 Sylhet division

A total of 49338 colonial waterbirds of nine species was observed of which Little Cormorant (39.08%) showing the highest abundance, the second abundance was Cattle Egret (35.07%), the third abundance was Intermediate Egret (9.02%) and lowest abundance was Oriental Darter (Figure 22).

4.3.2.4 Mymensingh division

Only seven out of sixteen colonial waterbird species of which the highest number of Little Cormorant (36.43%), the second highest Cattle Egret (30.72%), the third highest Great Egret (10.93%), the lowest was Black-crowned Night Heron (0.68%) (Figure 23).

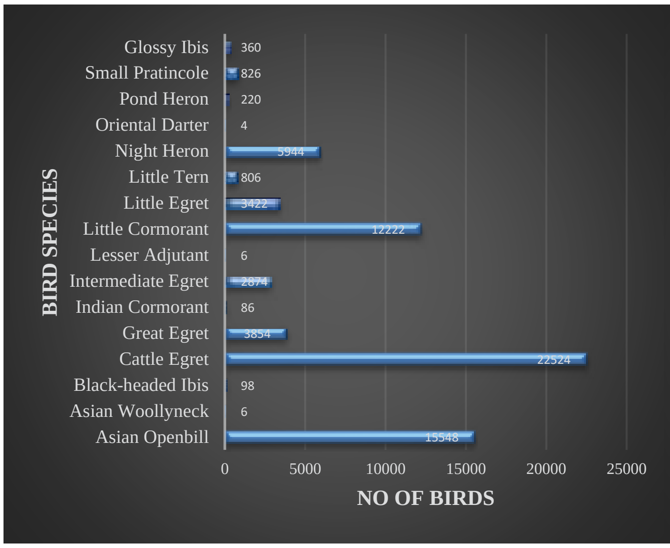


Figure 20: Colonial waterbird species abundance in Rajshahi division.

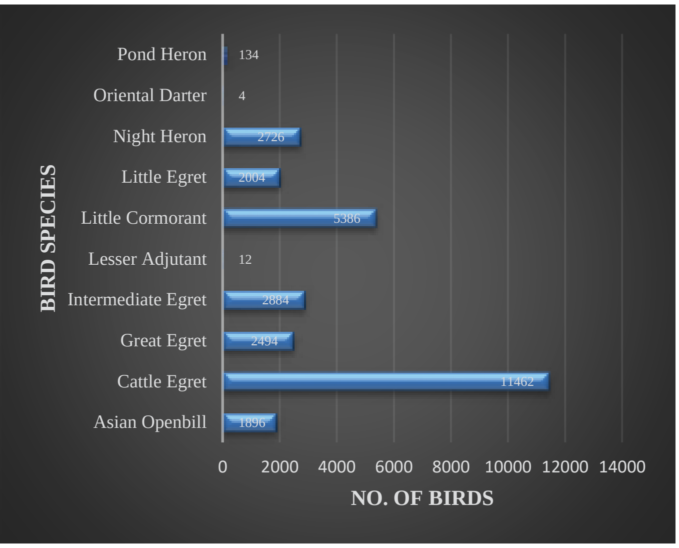


Figure 21: Colonial waterbird species abundance in Rangpur division

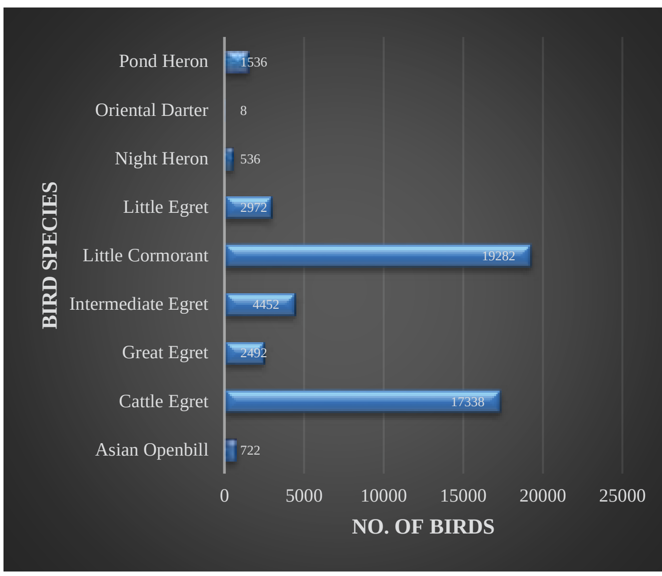


Figure 22: Colonial waterbird species abundance in Sylhet division.

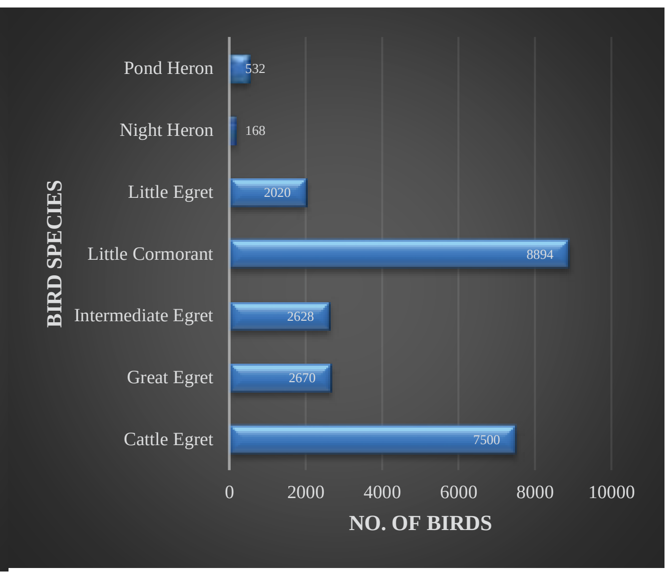


Figure 23: Colonial waterbird species abundance in Mymensingh division.

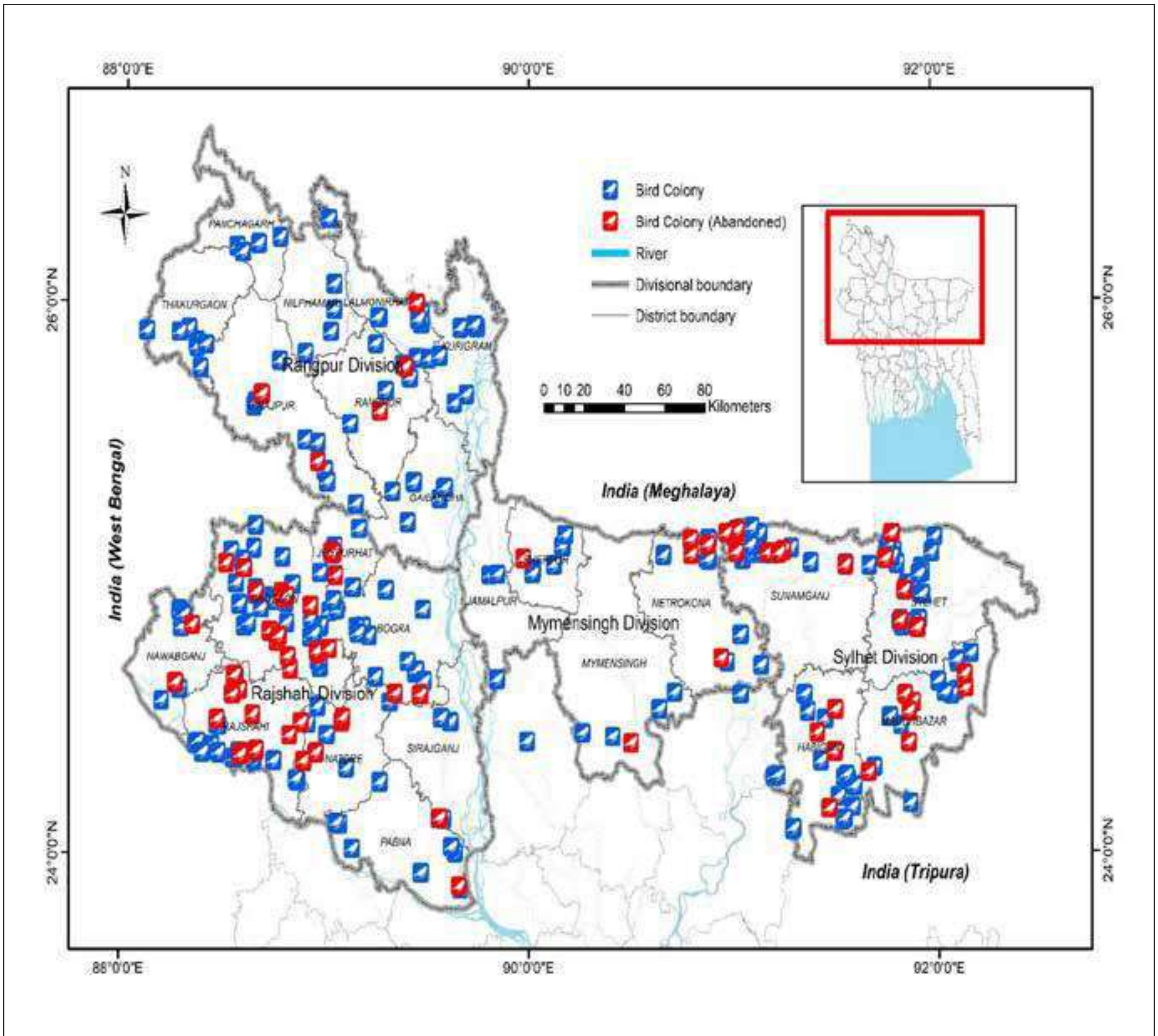


Figure 24: GIS-based distribution map of the nesting sites, and key habitats of colonial waterbirds in the study area (24 districts of Rajshahi, Rangpur, Mymensingh and Sylhet division).

4.4 Nesting plant species preferences and relationship of colonial waterbirds

Nesting tree/plant species preferences of colonial waterbirds were species specific and mostly depends on size of nest and duration of fledgling. A total 4090 plants/trees of 67 species preferred by the colonial waterbirds. The Shimul, Mango, Mahagoni, Kadam and Bot (Banyan) were the most preferred tree species of Asian Openbill; Cattle Egrets mostly preferred bamboos cluster and Little Cormorant mostly preferred Mahagoni, Barun and Bamboos. Mahagoni, Bamboos, Mango, Shimul, Koroch, Jackfruit, Kadam, Debdaru, Tetul, Raintree, Pakur, Neem, Arjan were the most preferred tree species for all tree nesting colonial waterbirds. The total number of trees/plants were 4090 and total number of nests were 81265 (Table 3) which showed linear relationship ($R^2 = 0.9514$) (Figure 25). Relationship between individual bird species there also have a positive linear relationship, in case of Little Egret ($R^2 = 0.9602$); Cattle Egret ($R^2 = 0.9243$), Great Egret ($R^2 = 0.9193$), Intermediate Egret ($R^2 = 0.8447$), Little Cormorant ($R^2 = 0.9569$), Night Heron ($R^2 = 0.8114$), Indian Pond Heron ($R^2 = 0.7718$), and Asian Openbill ($R^2 = 0.6369$) (Figure 26-33). There was a strong correlation between

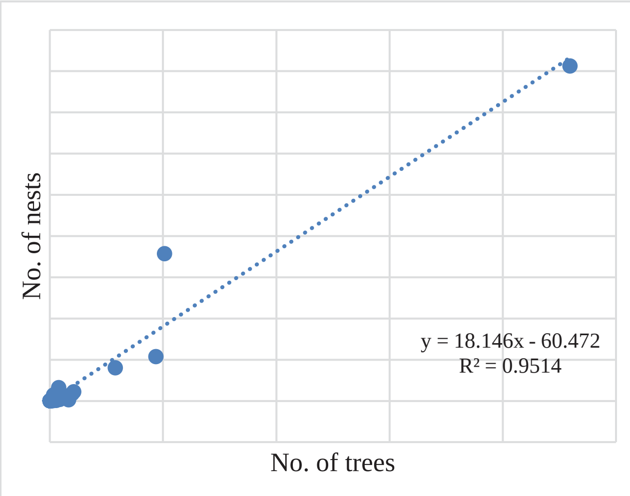
Asian Openbill and Little Cormorant (0.76); between Cattle Egret and Great Egrets (0.96), between intermediate Egret and Great Egret about 0.99; Intermediate Egret and Little Cormorant was 0.91; but there also have a negative correlation between Lesser Adjutant and other colonial waterbirds (Table 4).

Table 3: Species wise total number of trees and nests.

Bird Species	No. of trees/plants	Number of nests
Asian Openbill	768	9012
Cattle Egret	1813	27412
Great Egret	1167	5444
Indian Cormorant	3	37
Intermediate Egret	1348	6153
Lesser Adjutant	1	7
Little Cormorant	2191	22679
Little Egret	1061	4778
Night Heron	827	4585
Oriental Darter	2	4
Pond Heron	329	1154
Grand Total	**9510**	**81,265**

Table 4: Correlation between colonial waterbirds.

	Asian Openbill	Cattle Egret	Great Egret	Indian Cormorant	Intermediate Egret	Lesser Adjutant	Little Cormorant	Little Egret	Night Heron	Oriental Darter	Pond Heron
Asian Openbill	1										
Cattle Egret	0.29	1									
Great Egret	0.54	0.93	1								
Indian Cormorant	0.59	0.06	0.34	1							
Intermediate Egret	0.60	0.89	0.99	0.39	1						
Lesser Adjutant	0.08	-0.03	-0.03	-0.02	-0.04	1					
Little Cormorant	0.76	0.67	0.86	0.61	0.91	-0.04	1				
Little Egret	0.41	0.97	0.98	0.23	0.95	-0.03	0.78	1			
Night Heron	0.69	0.71	0.88	0.63	0.91	-0.04	0.93	0.83	1		
Oriental Darter	0.09	0.68	0.61	-0.03	0.57	-0.02	0.38	0.66	0.47	1	
Pond Heron	0.70	0.63	0.75	0.22	0.75	-0.03	0.71	0.67	0.63	0.24	1



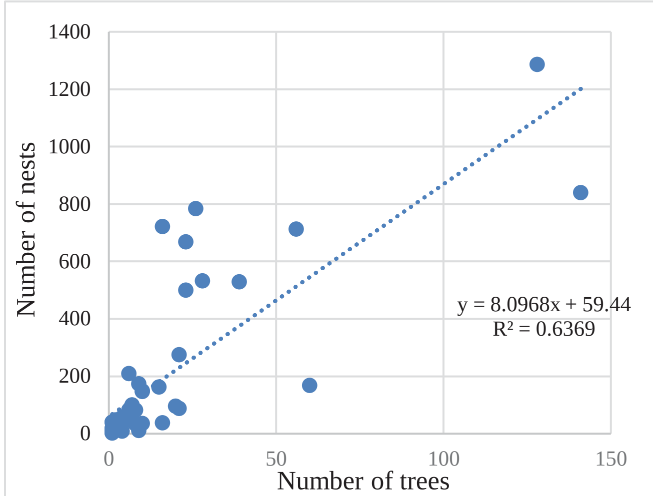
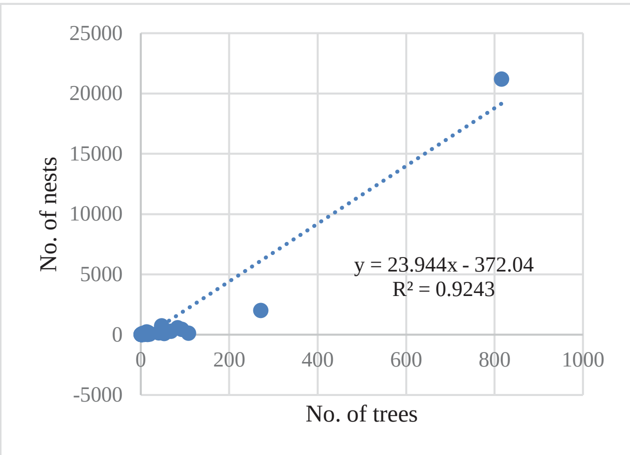


Figure 25: Linear relationship between total no. of trees and total no. of nests.

Figure 26: Linear relationship between the no. of trees and nests (Asian Openbill).



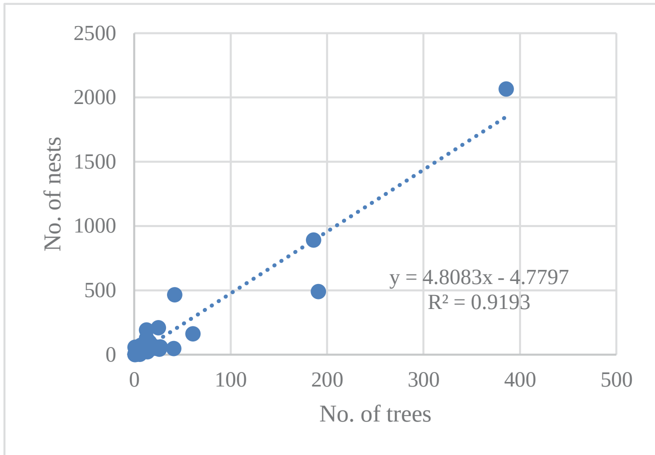
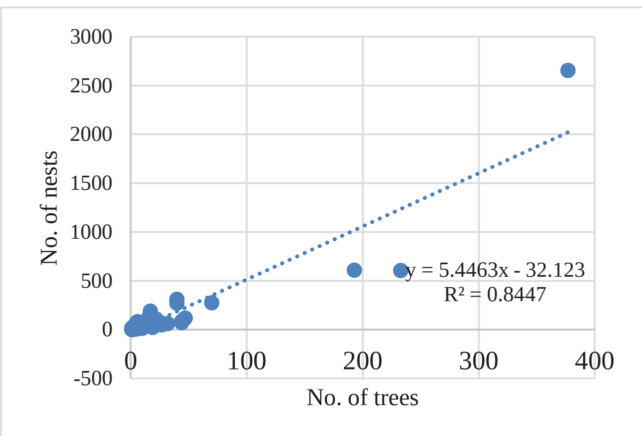


Figure 27: Linear relationship between the total no. of trees and nests (Cattle Egret).

Figure 28: Linear relationship between no. of trees and no. of nests (Great Egret).



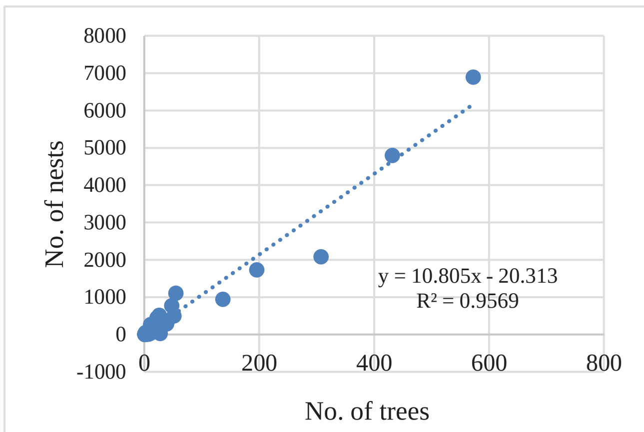


Figure 29: Linear relationship between no. of trees and no. of nests (Intermediate Egret).

Figure 30: Linear relationship between no. of trees and no. of nests (Intermediate Egret).

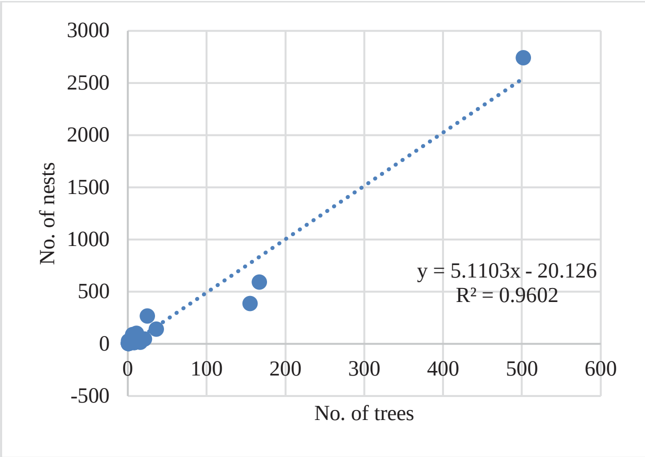


Figure 31: Linear relationship between no. of trees and no. of nests (Little Egret).

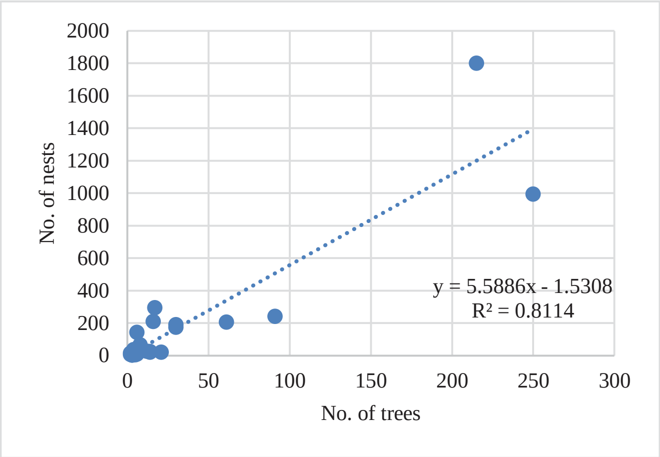


Figure 32: Linear relationship between no. of trees and no. of nests (Night Heron).

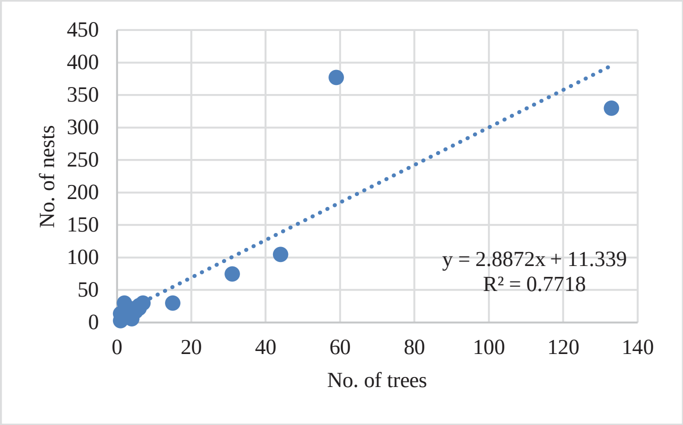


Figure 33: Linear relationship between no. of trees and no. of nests (Night Heron).

4.5 Nesting tree/plant height preferences of the colonial waterbirds

Colonial waterbirds, such as storks, herons, egrets, and cormorants, exhibit a variety of nesting tree height preferences depending on their species and environmental factors. Asian Openbills typically prefer to nest in tall trees, often at heights of 4.6 to 20 meters or more; the average lowest height (5 meter) of nesting at Lichu (Litchi chinensis) and average highest height of nesting at Shimul (*Bombax ceiba*) (Figure 34). Cattle Egrets often nest in trees at heights of 4 to 17 meters; the average lowest height (6.05 meter) of nesting at Boroi (*Ziziphus mauritiana*) and average highest height (17 meter) of nesting at African Mahagoni (Figure 35). Great Egrets often nest in trees similar in height to those chosen by Cattle Egret; the averave lowest height (5.20 meter) of nesting at Ghoraneem (*Melia azedarach*) and average highest height (16 meter) of nesting at Gab (*Diospyros discolor*) (Figure 36). Intermediate Egrets often nest in trees at heights of 5 to 17 meters; the average lowest height (5.10 meter) of nesting at Boroi (*Ziziphus mauritiana*) and average highest height (17 meter) of nesting at Bot/Banyan (*Ficus sp.*) (Figure 37). Little Egrets often nest in trees similar in height to those chosen by Cattle Egret; the average lowest height (5.10 meter) of nesting at Boroi (*Ziziphus mauritiana*) and average highest height (16 meter) of nesting at Gab (Diospyros discolor) (Figure 38). Little Cormorants often nest in trees at heights of 4.6 to 17 meters; the average lowest height (5 meter) of nesting at Coconut (Cocos nucifera) and average highest height (15.29 meter) of nesting at Bot/Banyan (*Ficus sp.*) (Figure 39). Night Heron often nest in trees at heights of 4.6 to 16 meters; the average lowest height (5 meter) of nesting at Coconut (*Cocos nucifera*) and average highest height (14.63 meter) of nesting at Eucalyptus (*Eucalyptus sp.*) (Figure 40). Indian Pond

Heron often nests in trees at heights of 6.5 to 18 meters; the average lowest height (5 meter) of nesting at Kamranga (*Averrhoa carambola*) and average highest height (17 meter) of nesting at Epil Epil (*Leucaena leucocephala*) (Figure 41).

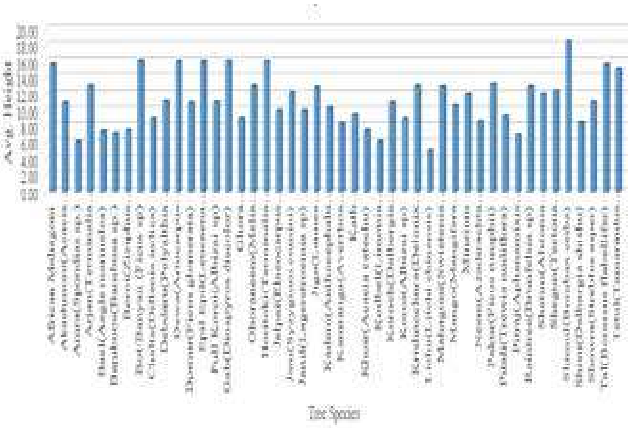


Figure 34: Average nesting height of the tree/plant preferred by Asian Openbill.

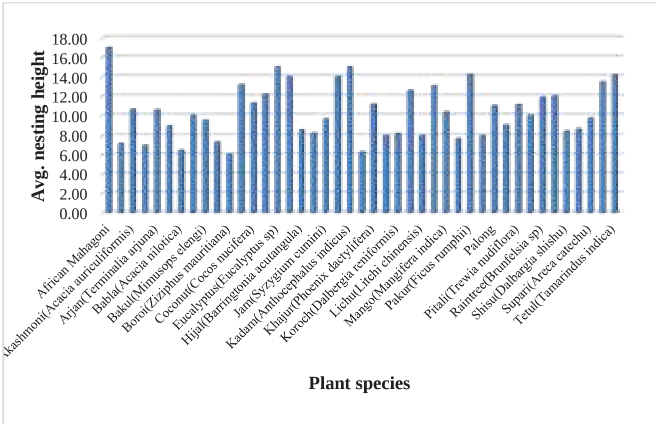


Figure 35: Average nesting height of the tree preferred by Cattle Egret.

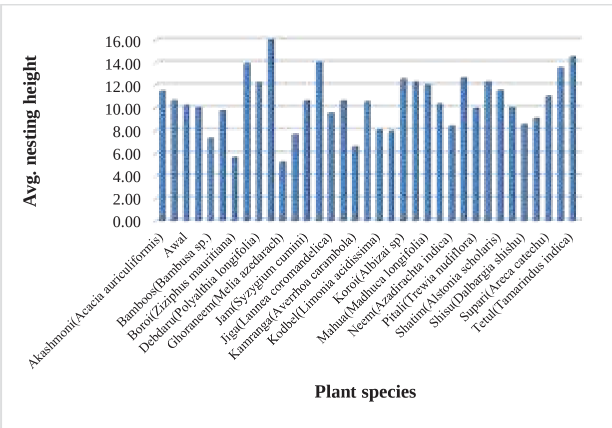


Figure 36: Average nesting height of the tree preferred by Great Egret.

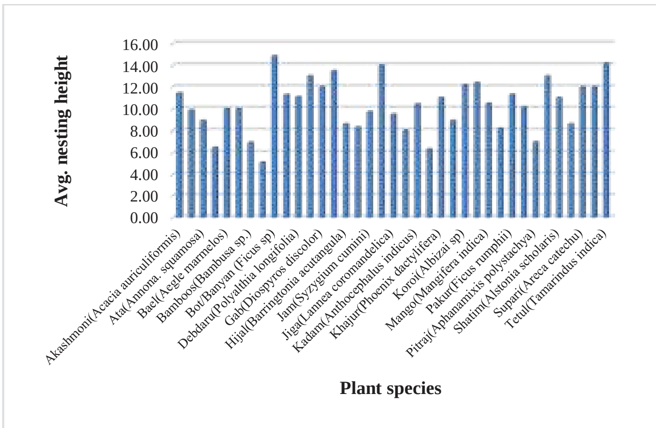


Figure 37: Average nesting height of the tree preferred by Intermediate Egret.

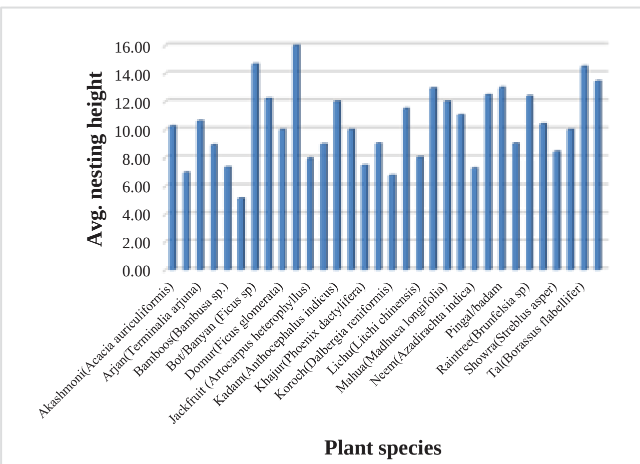


Figure 38: Average nesting height of the tree preferred by Little Egret.

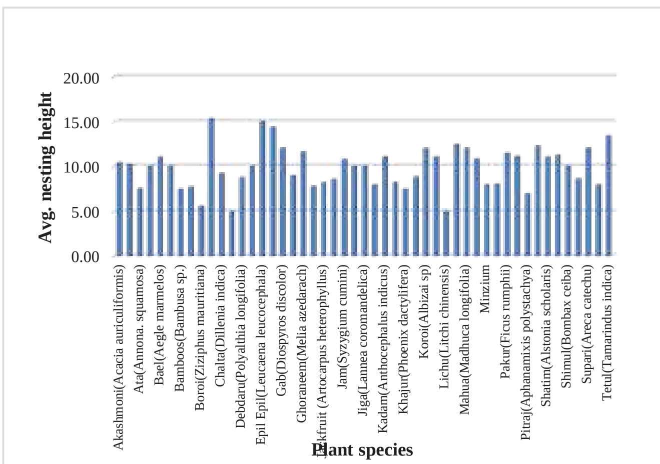


Figure 39: Average nesting height of the tree preferred by Little Cormorant

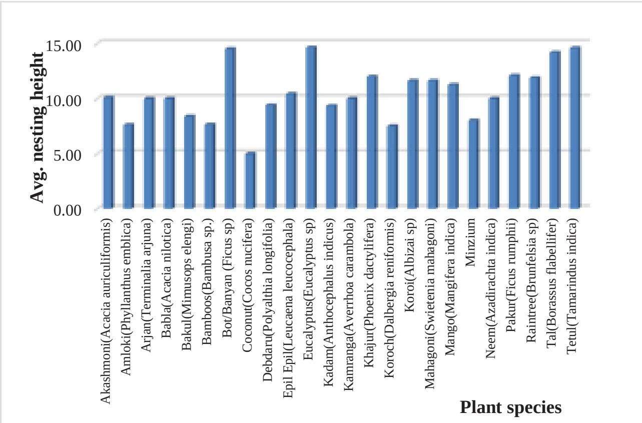


Figure 40: Average nesting height of the tree preferred by Night Heron.

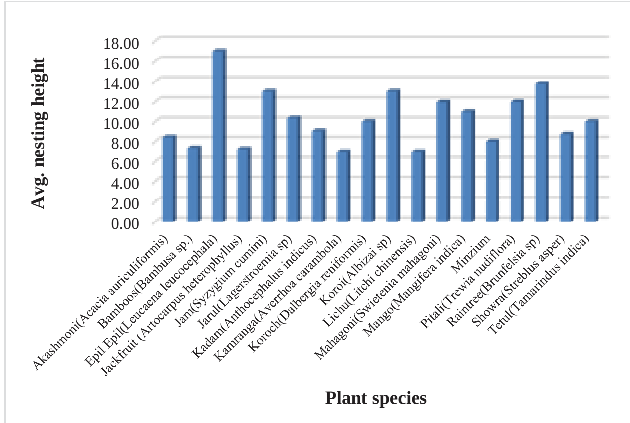


Figure 41: Average nesting height of the plant preferred by Pond Heron.

4.6 Threats and conservation status of colonial waterbirds

Colonial waterbird colonies are important breeding and nesting sites for a variety of waterbird species. These colonies provide critical habitat for these birds and play a vital role in maintaining healthy populations. However, they are also vulnerable to various threats that can disrupt their conservation efforts. Both anthropogenic and natural threats significantly impacted on breeding success of colonial waterbirds. Wetland drainage, resource damage and habitat destruction led to the loss of suitable nesting sites for colonial waterbirds. Human activities such as boating, fishing, and recreational activities near nesting colonies disturbed birds, causing them to abandon nests. Nest predators, including crows, snakes, foxes, cats, civets, raptors significantly impacted colony productivity by preying on eggs and chicks. Landowners' perceptions of waterbird colonies varied widely depending on their individual experiences, attitudes, and the specific circumstances of their land ownership. Some landowners may have had a positive perception of waterbird colonies and view them as a valuable part of their property. They may have appreciated the presence of diverse bird species and the ecological benefits they provide. About 28% colony protected by traditionally and humanity, 21% colony by good for environment, 18 % colony by social recognition, 11% colony by religious view, 6% colony by laws & order, 5 % colony protected only for personal recreation and 11% land owners don’t like but protect the colony (Figure 42). A large number of breeding colony were abandoned due to many reasons during the last 5 years (2018-2022). Total 80 breeding nesting sites were abandoned of which 46% were habitat disruption and habitat loss, 30% were habitat loss and disturbance, 11% were habitat loss and hunting, 5% for only hunting, 4% only for human disturbance, 1% for hunting and cutting of nesting trees/plants (bamboos) (Figure 43). Threats to colonial waterbirds categorized as anthropogenic threats and natural threats.

4.6.1 Anthropogenic threats

Anthropogenic threats observed about 90% (n-201) colony, natural threats occurred in 100% (n-222) colony and about 10% (n-21) colony safe from anthropogenic threats or no anthropogenic threats (Figure 45). Anthropogenic threats were sub categorized as Felling of nesting, Nest Stelling, Egg Steeling, Chick Stelling, Adult got hunted and poisoning (Figure 44). Felling of nesting trees were very frequent and most serious problem for colonies (Figure 45). It also affected the breeding colonies, That ultimately leads to habitat destruction and abandon of colonies. The second most threats were chick stealing; third most threats were adult bird got hunted and very less threats observed in nest stelling & eggs stealing (Figure 47-49). So, the nest and eggs of colonial waterbirds almost free from anthropogenic threats or negligible. In the felling of nesting trees 17% colony faced very high, 23% were high, 25% were low and 35 very low (Figure 46). Chick’s stealing was frequent in Sylhet and Mymensingh division; about 35% in very high categories, 20% as high, low 8% and very low 39% (Figure 47). Poisoned bait used for capturing adult waterbirds and categorized on degree of frequency as 22% high, 9% very high, 13% low and very low 56% (Figure 49).

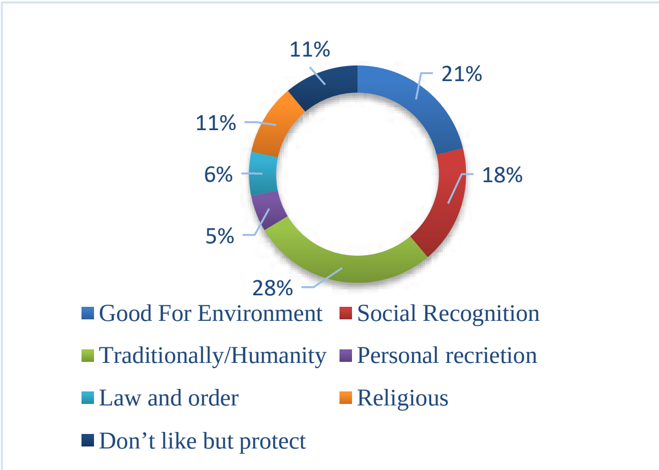


Figure 42: Land owners' perception on waterbird colony conservation.

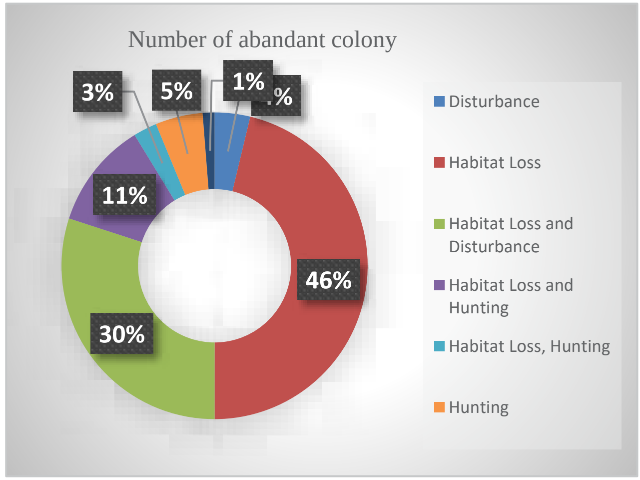


Figure 43: Reasons for colonial waterbirds nesting sites abandoned.

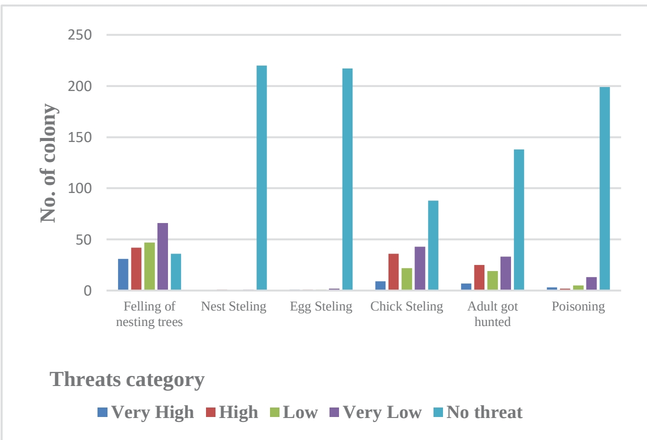


Figure 44: Anthropogenic threats to the colonial waterbirds in nesting sites.

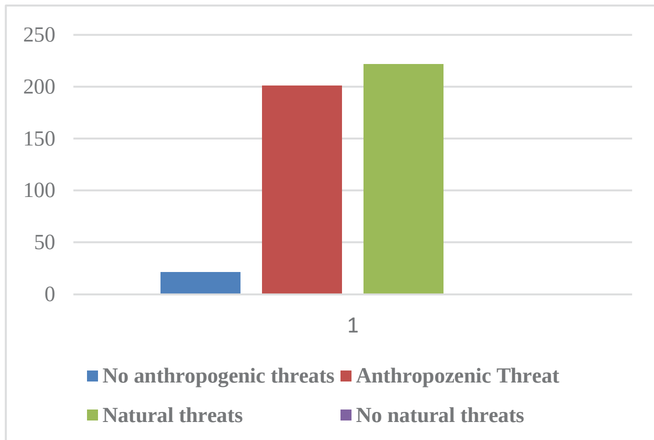


Figure 45: Threats of colonial waterbirds.

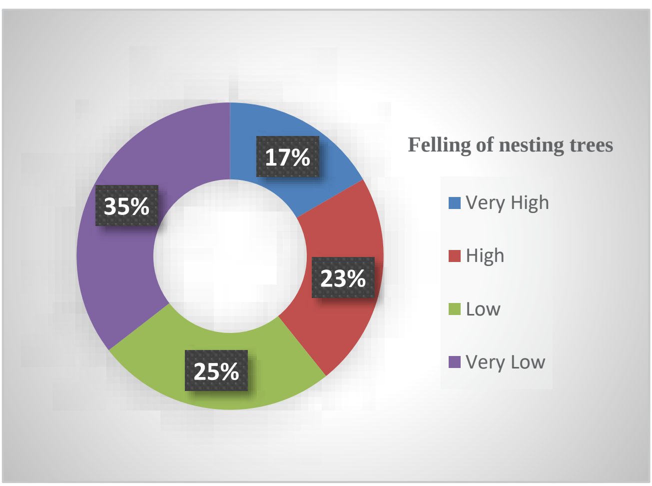


Figure 46: Felling of nesting trees.

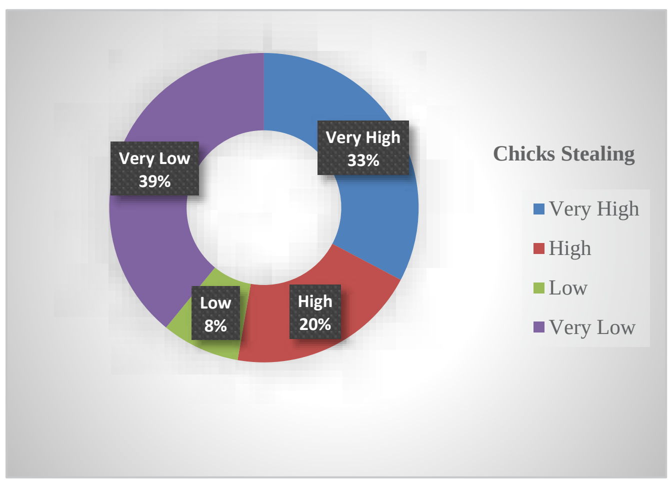


Figure 47: Chicks stealing of colonial waterbirds.

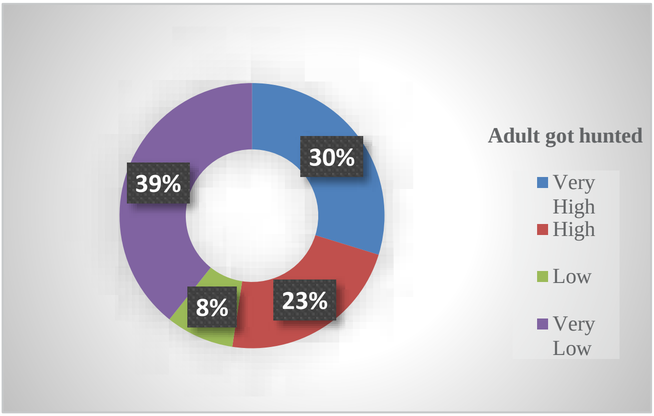


Figure 48: Adult bird hunted within or nearby colonies.

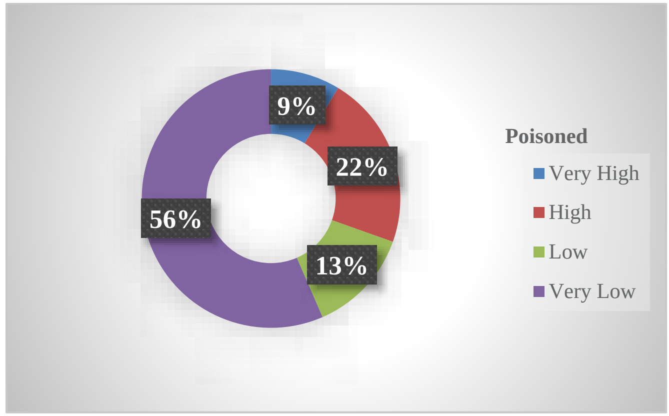


Figure 49: Poisoned bait used to capture the adult waterbirds.

4.6.2 Natural threats

Severe weather events, such as storms, cyclones, thunderbolt, predation, hailstorm, and heavy rainfall, can cause damage to nests and colonies. High winds, flooding, and erosion can lead to nest destruction, chick mortality, and the loss of suitable nesting sites. Natural threats observed in all the breeding colonies during the study period. Following natural threats were observed, a total of 222 breeding colonies were observed, of which storm was very frequent in 214 colonies, predation in 203 colonies, chicks fall from the nest in 220 colonies, thunderbolt in14 colonies and hailstorm occurred in 50 colonies (Figure 50). Breeding colony faced different degrees of intensity of storm, of which 46% were high, 30% very high, 14% low, 6% very low and 4% colonies have no such threats (Figure 51). Predation by Large-billed Crow, Fishing Cat, Black Kite, Snakes were observed and the degree of intensity greatly varies from location of the colonies; Very high 2%, high 8%, low 21%, very low 60% and almost free from predation 9% colonies (Figure 52). Chicks fall from the were very common phenomena, about 17 were high, very high 4%, low 41%, very low 32% and 6% colonies have no such threat (Figure 53).

Thunderbolt is a great threat to colonial waterbirds, thunderbolts were occurred in 14 colonies (Figure 54) during breeding season, that was caused huge mortality of Asian Openbill, egrets, cormorants and herons. About 300 waterbirds died including Asian Openbill in Kazipara bird colony, Khetlal, Joypurhat on 12 June, 2022 and 100 Asian Openbill died on 16 June 2022 at Mollan bird colony, Chapainawabganj. Hailstorm is a great serious threat to colonial waterbirds because of coloniality and leads to huge mortality of adult chicks with adult birds. Our field survey showed that 23% of the colonies faced the problem and 77% have no such mortality of birds (Figure 55).

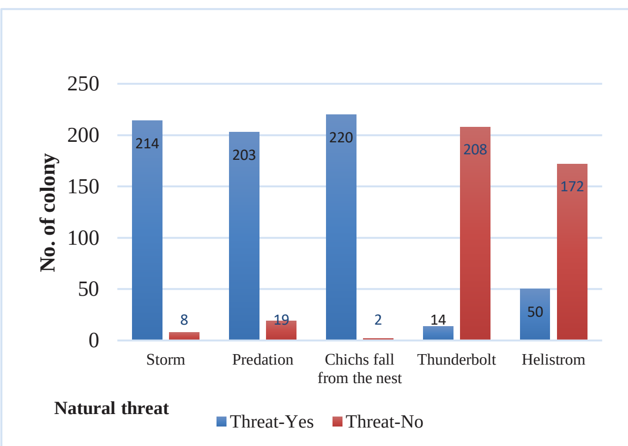


Figure 50: Natural threats of the colonial waterbirds.

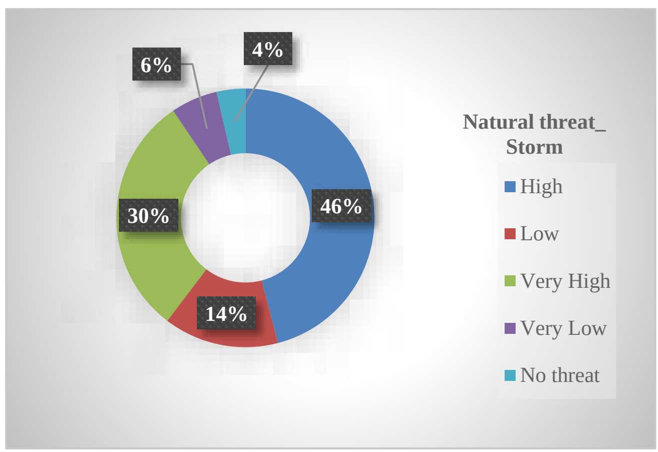


Figure 51: Storm has a great negative impact on colonial waterbirds.

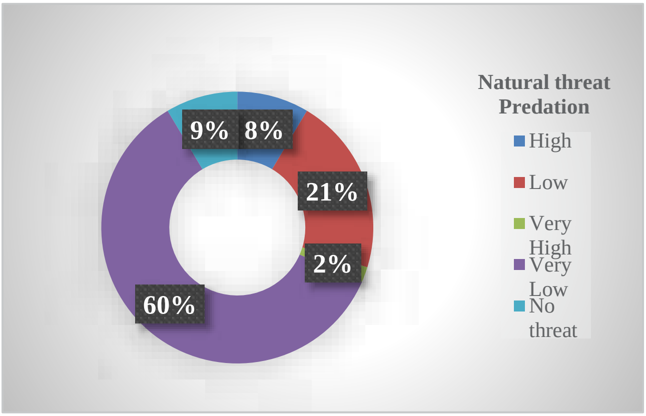


Figure 52: Predation of the colonial waterbirds.

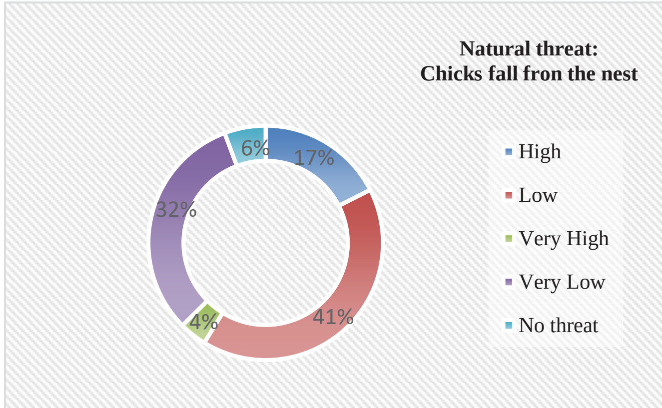


Figure 53: Chicks fall from the nest.

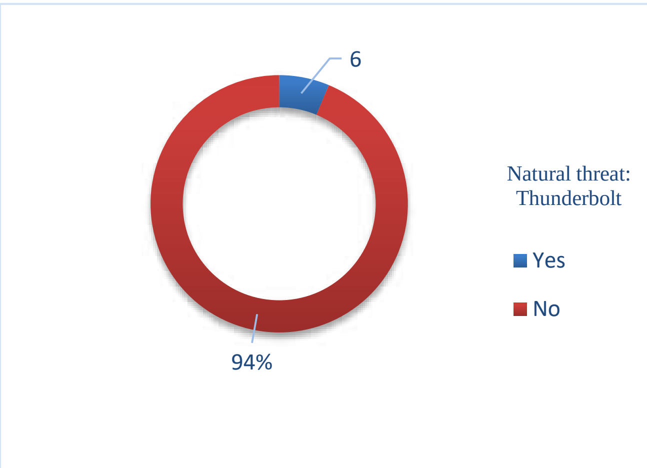


Figure 54: Thunderbolt has great negative impact on colonial waterbirds.

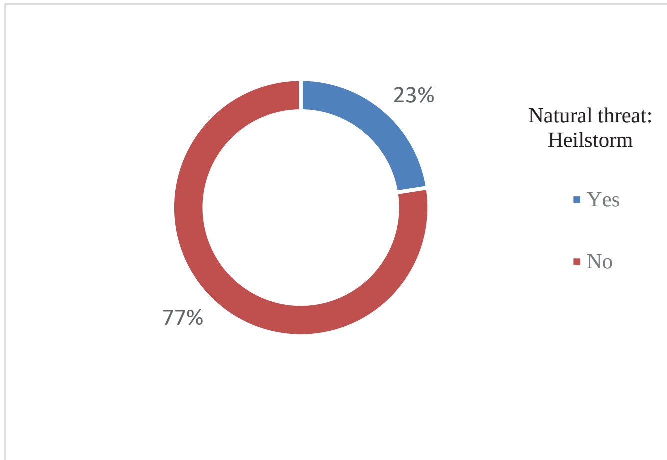


Figure 55: Large number of birds died due to hailstorm.

4.7 Disturbances faced by the local people during breeding season.

Numerous issues plagued the community, including odor, trouble moving, decaying trees and fish, health issues, roof damage, fruits that didn't grow in nesting trees, damage to other resources, etc. Among 222 nesting sites of which the strong stench was a very common issue in all colonies. Other issues included trees decaying in 118 colonies, roof damage in 45, people not getting fruit from fruiting trees in 38 colonies, difficulty moving or having trouble moving in 28, fish dying in 31 colonies, and no problems were observed in 21 colonies (Figure 56). Residents of colony sites experienced a range of issues and difficulties. These difficulties could differ based on a community's geography, socioeconomic status, and unique circumstances. Due to their gregarious behavior, which caused issues for the locals, waterbirds often constructed their nests in close proximity to or within human settlements with significant agglomeration.

4.8 Conservation measures

According to the locals, 214 (96.40%) colonies require habitat protection and improvement; they also require compensation 206 (92.97%); awareness 161 (72.52%); signboard or billboard installation 150 (67.57%); law enforcement 47 (21.17%); the establishment of a rescue center 46 (20.72%); the provision of watchers for 46 (20.72%); the construction of sheds on roads 26 (11.71%); and active collaboration 6 (2.70%), only 2 (0.90%) colonies require no intervention. (Figure 57). In addition, we calculated the annual cost of compensation for 206 colonies at Tk. 9527000. Local communities that lived close to bird colony sites were extremely significant to the conservation of these vital habitats. The long-term survival of bird colonies depended on recognizing their needs and integrating them into conservation initiatives. The importance of bird colonies, the species they hosted, and the ecological advantages they brought may have been the subject of educational programs and seminars that local communities desired. Local leaders, schools, and community members were engaged in educational and awareness-raising efforts. Habitat protection and improvement, compensation for resource damages, education and awareness, the installation of signboards or billboards at colony sites, the enforcement of laws to control bird hunting and poaching, the hiring of wildlife watchers to guard nesting sites, the construction of sheds on roads beneath nesting trees, and the act were all conservation measures suggested by local residents.

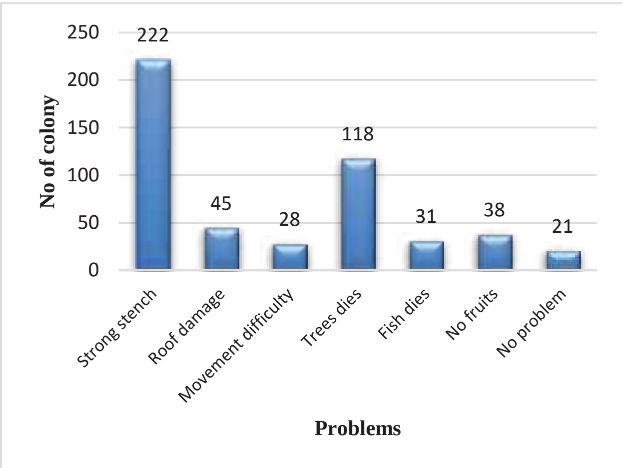


Figure 56: Problem faced by the communities at colony sites.

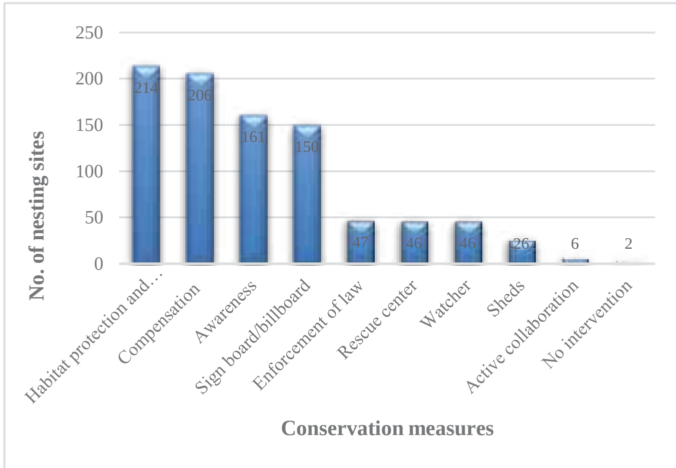


Figure 57: Community demand for conservation measures.

4.9 Breeding Ecology of Colonial Waterbirds

The breeding ecology of colonial waterbirds was fascinating and involved various aspects of their behavior, habitat selection, reproductive strategies, and interactions. The breeding ecology of colonial waterbirds involved a sequence of activities like colony formation, habitat selection, nesting sites, social structure, reproductive timing, egg laying and incubation, chick rearing, predator defense, fledging, and post-breeding dispersal, longevity, and reproductive success. The breeding chronology or the active breeding time differed from species to species (Table 5), but mostly preferred the rainy season when food was available.

Table 5: Active breeding months of colonial waterbirds.

Sl. No.	Species Name	Active breeding months											
		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1	Cattle Egret												
2	Little Egret												
3	Intermediate Egret												
4	Great Egret												
5	Asian Openbill												
6	Night Heron												
7	Pond Heron												
8	Little Cormorant												
9	Indian Cormorant												
10	Small Pratincole												
11	Little Tern												

Sl. No.	Species Name	Active breeding months											
		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
12	Lesser Adjutant												
13	Asian Woolly-neck												
14	Oriental Darter												
15	Black-headed Ibis												

4.9.1 Breeding ecology of Cattle Egret (Bubulcus ibis)

The Cattle Egret (*Bubulcus ibis*) is a common species of heron found in various parts of the world, including Africa, Asia, the Americas, and Europe. Their breeding ecology is interesting and adapted to their association with livestock and other large animals.

4.9.1.1 Habitat and nesting site selection: Cattle Egrets were highly adaptable birds that could be found in a variety of habitats. They were most commonly associated with open grasslands, wetlands, agricultural fields, and pastures where large herbivores like cattle and buffalo were present. They bred in colonies, and the size could vary from a few pairs to several hundred pairs, with the birds often nesting in trees and shrubs. The breeding colony was often associated with human settlement.

4.9.1.2 Breeding season: The timing of the breeding season could vary depending on the location and local climate conditions. Breeding typically occurred in April-July. If there were insufficient rains, then the breeding was delayed.

4.9.1.3 Courtship, nest building and Mating: Courtship displays involved mutual grooming, bill-touching, and various postures and vocalizations. They constructed relatively simple nests made of sticks and plant material in trees or shrubs or in other sheltered locations. The proximity to large mammals and human settlement provided protection from ground predators. Once pairs were formed, they engaged in copulation.

4.9.1.4 Egg-laying and Incubation: Cattle Egrets typically laid three to four eggs (Table 6). Both males and females took turns incubating the eggs, which usually took around 27-29 days. This shared incubation allowed one parent to guard the nest while the other foraged for food. The nesting success was 100%, and incubation success was 81.02% (Table 7).

Table 6: Clutch size of Cattle Egret.

Clutch size (No. of eggs/ nest)	No. of nest	No. of eggs
Clutch size 3 (Three eggs)	7	21
Clutch size 4 (Four eggs)	29	116
Total	**36**	**137**

Table 7: The fate of nest, eggs and breeding success of Cattle Egret.

Item/Criteria	Number	Breeding success/ failure (%)
Number of nests examined	36	-
Number of eggs counted	137	-
Number of eggs lost to predation	0 (unknown)	-
Number of eggs to unhatched	5	-
Number of eggs lost by storm	21	-
Number of eggs hatched successfully	111(eggs/chicks)	81.02%

Nest with eggs destroyed by storm	0	-
Nest Predation	0	-
Nest with viable eggs which hatched successfully	36 nests	100%
Nest with fledgling destroyed by storm	0	
Nest with viable fledgling	36 nests	100%
Number of chicks/fledglings lost by storm/fallen from the nests	24 chicks/fledgling	21.62%
Fledgling predation	0	-
Number of fledglings survived	87	78.37%
Final breeding success (Number of individuals survived successfully in ratio to clutch size)	**-**	**63.50 %**

Figure 58: Incubation of Cattle Egrets at Chanpukur Cathlic Charch Bird Colony, Patnitala, Naogaon.

Figure 59: Incubation of Cattle Egrets at Chanpukur Cathlic Charch Bird Colony, Patnitala, Naogaon.

4.9.1.5 Chick rearing: After hatching, the chicks were altricial, meaning they were born in a relatively undeveloped state and were dependent on their parents for food and warmth. Both parents fed the chicks by regurgitating partially digested food into their mouths. Fledgling survival was 78.37%, and final breeding success (number of individuals survived successfully in ratio to clutch size) was 63.50% (Table-7). Chicks fledged (left the nest) after about 35-45 days.

4.9.1.6 Diet: Cattle Egrets are primarily insectivorous and feed on a variety of insects, especially those flushed out by large mammals as they move through grasslands or disturb vegetation. They also fed on small vertebrates i.e., frogs, small fish and foraged in agricultural fields for insects and other prey.

4.9.1.7 Predator defense and post-breeding dispersal: While nesting colonies of Cattle Egrets benefited from the protection offered by cattle and humans, they were still vulnerable to predation by aerial predators such as Large-billed Crow, Snakes, Black kite, and other raptorial birds. Collective defense behaviors, such as mobbing intruders or aerial displays to distract predators, were common within the colony. After the breeding season, Cattle Egrets might have dispersed to different habitats, including wetlands, rice fields, and grasslands, depending on the availability of food resources. They were known for their nomadic and opportunistic foraging behavior.

4.9.1.8 Conservation challenge and recommendation: While Cattle Egret are not considered globally threatened, their populations can be impacted by nesting habitat degradation and poisoning baits and commercial hunting. Conservation efforts may include the protection of key breeding and foraging habitats and protect from hunting and chicks stealing and poisoning.

Figure 60: Feeding and fledging of Cattle Egrets at Chanpukur Cathlic Church Bird Colony, Patnitala, Naogaon.

Figure 61: Feeding and fledging of Cattle Egrets at Chanpukur Cathlic Church Bird Colony, Patnitala, Naogaon.

4.9.2 Breeding ecology of Asian Woolly-necked Stork *(Ciconia episcopus)*

The Asian woolly-necked stork (*Ciconia episcopus*) is a bird species native to South and Southeast Asia. While specific details about the breeding ecology of this species may vary depending on the region and habitat.

4.9.2.1 Habitat and nesting site selection:

Asian Woolly-necked Stork were typically found in Rajshahi and Chapainawabganj district very close to Padma River. Confirmed breeding record from this region and mainly they chose mobile communication tower that's height was about 50 m. Each tower only one nest. During our field study we got two more new nest, totaling three nests. They prefer habitats with shallow water, where they can easily access their primary food source, aquatic organism. Though they are colonial nesters, often in colonies with other waterbirds. The breeding colony associated with human settlement and mostly in agriculture rich habitats.

4.9.2.2 Breeding season: Breeding typically occurred in May-October.

4.9.2.3 Courtship, nest building and Mating: During the breeding season, male and female storks engaged in courtship displays, which included mutual bill-clattering, head bobbing, and other visual and vocal cues. Once a pair formed a bond, they worked together to build or refurbish a nest. The average nest diameter (core length) was 45.5 cm, and the total diameter was 63.5 cm. They constructed relatively simple oval nests made of sticks, grasses, leaves, and plant material in manmade telecommunication towers, as they preferred large and tall trees.

4.9.2.4 Egg-laying and Incubation: Asian Woolly-necked Storks typically laid four to five eggs (Table 8). The average length of the eggs was 62.9 mm, and the egg width was 47.84 mm. usually, the clutch size was completed in 6 to 9 days. Both the male and female shared incubation duties, with shifts that could last for several hours. Both males and females took turns incubating the eggs. Incubation began after the first egg was laid, and the incubation period was about 27-33 days. At least one bird stayed in the nest from the beginning of nest building until the young were grown, and usually, the female stayed in the nest. This shared incubation allowed one parent to guard the nest while the other foraged for food. The nesting success was 33.33%, and the incubation success was 10% (Table 9).

Table 8: Clutch size of Asian Woolly-necked Stork.

Clutch size (No. of eggs/ nest)	No of Nest	No. of eggs
Clutch size 1(One egg)	1	1
Clutch size 4 (Four eggs)	1	4
Clutch size 5 (Five eggs)	2	5
Total	**3**	**10**

Table 9: The fate of nest, eggs and breeding success of Asian Woolly-necked Stork.

Item/Criteria	Number	Breeding success/ failure (%)
Number of nests examined	3	-
Number of eggs counted	10	-
Number of eggs lost to predation	9	90%
Number of eggs to unhatched	0	0%
Number of eggs lost by storm	0	0%
Nest with eggs destroyed by storm	0	0%
Nest Predation	2	66.66%
Number of eggs hatched successfully	1 (eggs/chicks)	10%
Nest with viable eggs which hatched successfully	1 nest	33.33%
Nest with fledgling destroyed by storm	0	0%
Nest with viable fledgling	1 nest	33.33%
Number of chicks/fledglings lost by storm/fallen from	0	0%

the nests	chicks/fledgling	
Fledgling predation	0	0%
Number of fledglings survived	1	100%
Final breeding success (Number of individuals survived successfully in ratio to clutch size)	-	10 %

4.9.2.5 Predator defense and post-breeding dispersal: Asian Woolly-necked Storks could be vulnerable to predation by aerial predators such as Crows and Black Kite. During the field study, the camera trap results showed that two nests were predated by Large-billed Crows. Generally, one adult bird didn't leave the nest and guarded the nest all the time. So, no predator was allowed to destroy the eggs and nest. The failure of the two nests were mainly caused by human disturbances, because of the telecommunication tower were renovated for long time about 3 hours, so the both parents absent the nest and, in this time, the Large-billed Crows predated the nest (Figure 74 & 75). Adults engaged in mobbing behavior to drive away potential threats from the nest site. After the breeding season, Asian Woolly-necked left nest and dispersed to different habitats, but sometimes they visited the nesting tower.

4.9.2.6 Conservation challenge and recommendation: Asian Woolly-necked Stork is globally threatened, their populations impacted by nesting habitat degradation and predation due to human disturbances. Active collaboration with Telecommunication Company and guarding the nest during breeding season is very essential to protect the birds from its extinction.

Figure 62: Nest of globally threatened (NT) Woolly-necked Stork at Bidirpur, Godagari,

Figure 63: Eggs predation of Globally threatened (NT) Woolly-necked Stork at Bidirpur, Godagari, Rajshahi.

Figure 64: Eggs predation of Globally threatened (NT) Woolly-necked Stork at Bidirpur, Godagari, Rajshahi.

4.10 Understanding the climate change impact on agricultural and wetland ecosystems in Bangladesh

Climate change has significant impacts on agricultural and wetland ecosystems in Bangladesh. These impacts are perceived differently by different groups of people, including farmers, fishers, and local communities. For farmers, climate change can cause crop failures and reduce yields, which can lead to income loss and food insecurity. Changes in rainfall patterns and increased frequency of extreme weather events, such as floods, droughts, and storms, can also damage crops and infrastructure. For fishers, climate change can affect the availability and distribution of fish species, which can lead to reduced catches and income loss. By understanding local people's perspectives, policymakers and development practitioners can design more effective climate change adaptation and mitigation strategies that are relevant and responsive to the needs and concerns of local communities.

4.10.1 Sociodemographic information of the respondents

A total of 144 respondents of which 68 were farmer, 37 were business, 12 were teacher, 5 were student, 7 were housewife, 5 were job holder, 3 were UP chairman, 2 were member, 2 were driver, 1 was quad doctor, 1 was unemployed and 1 was father of catholic charge (Figure 56). Different ages from 22 years to 87 years old (Figure 66) and different sex group were participated in the study. About 5-60 years of experienced of the respondents (Figure 67) and the education level were primary to MSC/Masters (Figure 68). The number of respondents participated in this study depends on bird colony number and availability and willingness of the respondents; about 50% respondents were participated from Rajshahi division, 28% from Rangpur division, 13% from Sylhet division, and 9% from Mymensingh division (Figure 69 & 70).

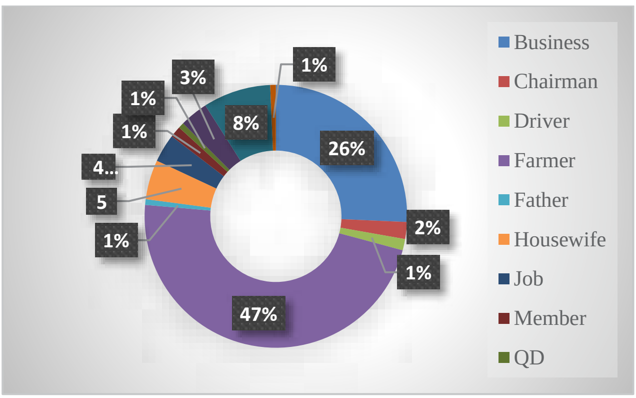


Figure 65: Occupation of the number of respondents.

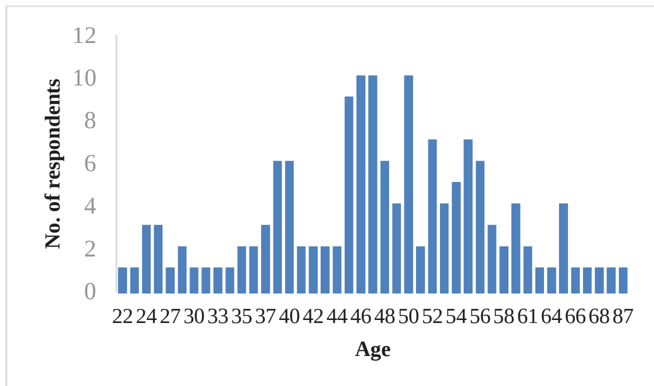


Figure 66: Age of the respondents.

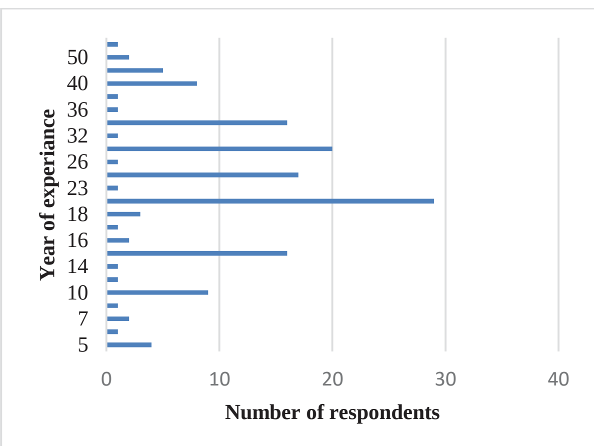


Figure 67: Year of experience of the number of respondents.

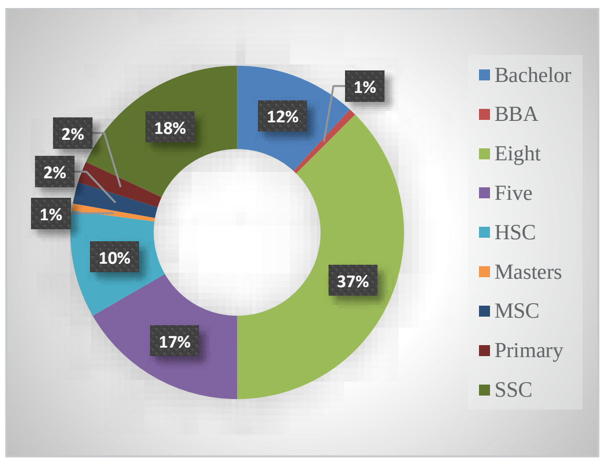


Figure 68: Education level of the number of respondents.

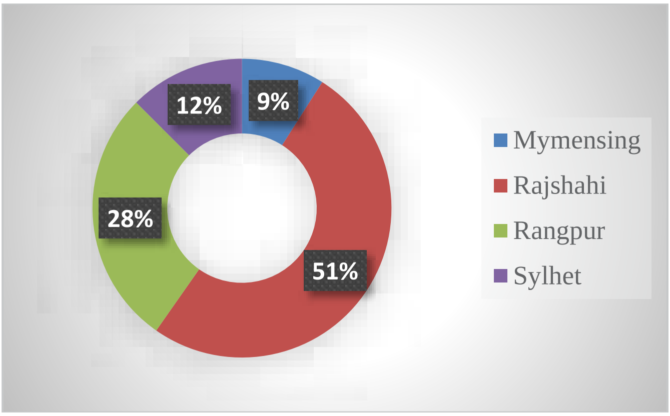


Figure 69: Respondents from four division.

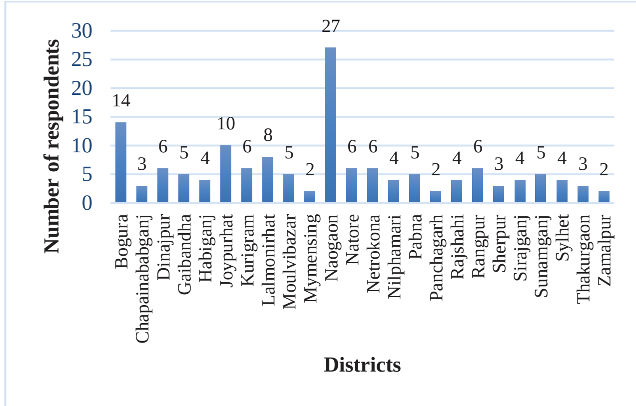


Figure 70: Number of respondents by district.

4.10.2 Climate change and its impact

About 80% respondents heard the name of climate change and 20% were not known about the climate change (Figure 71); and the respondents heard the name of climate from different sources (Figure 72).

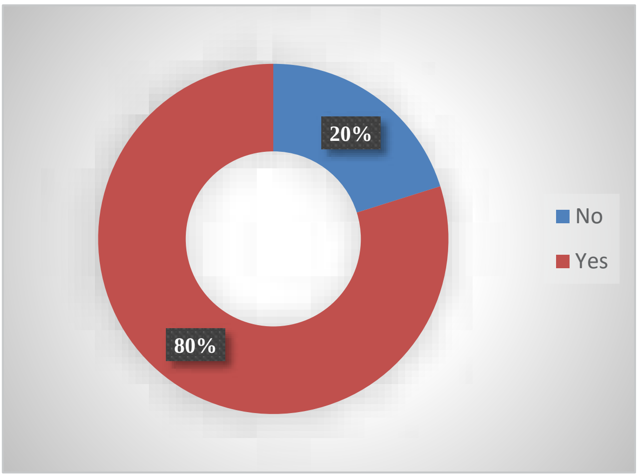


Figure 71: Heard the name of climate change by the respondents.

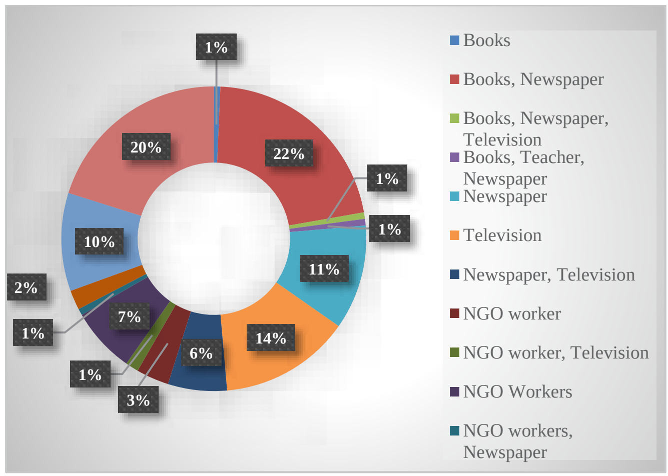


Figure 72: Heard the climate change issues from different sources by the respondents.

4.10.3 Frequency and effect of cyclone occurrences

About 71% respondents agree that the frequency of cyclone occurrences increased in last 15 to 20 years, 28% respondents did not notice and 1% was not agreed (Figure 73). About 68% respondents commented that the storms cause the birds' nests to collapse, the destruction of their eggs, the death of their young, and frequently the death of their adults. Additionally, 69% commented nesting trees were uprooted or broken, reducing the number of trees available for building nests, bird nest and eggs destruction, chicks and bird died; 2% agreed no effect of cyclone and 29% did not notice the effect of cyclone (Figure 74). Due to geographic location, Bangladesh frequently suffers a lot from tropical cyclones; generally, it occurs in early summer or late rainy season (Hossain I. and Mullick A. R. 2020). So, the locals have a good understanding of cyclones and its impact on waterbirds.

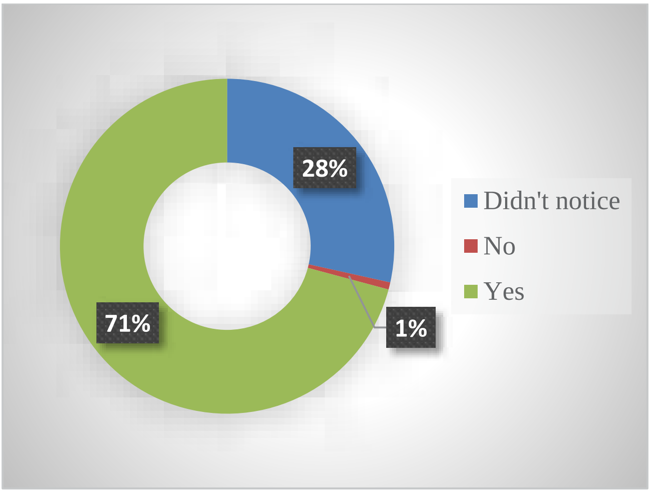


Figure 73: Frequency of cyclone occurrences increased.

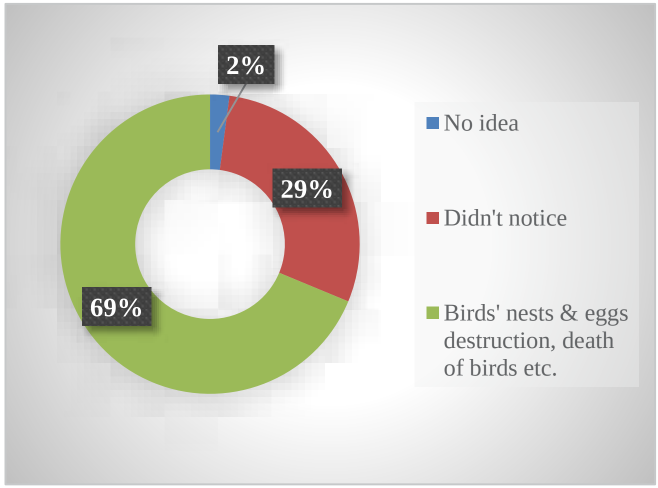


Figure 74: Effects of cyclone commented by the respondents.

4.10.4 Changes in rainfall pattern and Drought increases

About 86% respondents agreed to drought increased, 13% were didn't notice and 1% disagree to drought increased in last 15-20 years (Figure 75). According to respondents, about 70% commented that due to lack of rain, fish and frogs are decreasing in the fields, due to lack of food, chicks are dying and even birds are laying less eggs; 6% commented only food shortage for birds; 10% have no idea about effect of drought, and 14% didn't notice the effect of drought (Figure 76). About 76 % respondents agreed that the rainfall pattern has been changed during the last 15-20 years, 22% didn't notice and 2% dis agreed about rainfall pattern (Figure 77). The effect of changes in rainfall pattern affects the bird population (Figure 78). Of all natural disasters, droughts have the most effects and cause the most loss globally (Chunqiang, 2010). Northwestern part is the drought-prone region and it covers most part of the greater Dinajpur, Rangpur, Pabna, Rajshahi, Chapai Nawabganj, Bogura, Joypurhat and Naogaon district (Habiba *et al.*, 2011). In the northwest, groundwater provides around 75% of the irrigation water (Shahid and Hazarika, 2010). But inappropriate use of groundwater for home and agricultural irrigation contributes to the groundwater's declining trend (Habiba *et al*., 2012).

Considering changes in the climate and the reported rise in extreme weather events, there may be an increase in the frequency, severity, and length of droughts (Sivakumar et al., 2014; Peterson et al., 2013). Drought is a sneaky natural hazard that arises from less precipitation than usual or anticipated for an extended length of time (Wilhite, 2005). Bangladesh experiences droughts as a result of insufficient pre-monsoon rainfall, a delayed start to the rainy season, or an early monsoon departure (Shafie *et al*., 2009). The respondents have a very good idea about rainfall pattern and drought, the annual rainfall decreases in the last 21 (From 2003 to 2023) years, and the irregular rainfall during the study period (Figure 6). Respondents also have good idea regarding the effects of drought due to irregular rainfall. During our field visit, the sufficient rain triggered the breeding on May 2022, and in 2023 the breeding started on July due to sufficient rain on late June (Figure 6).

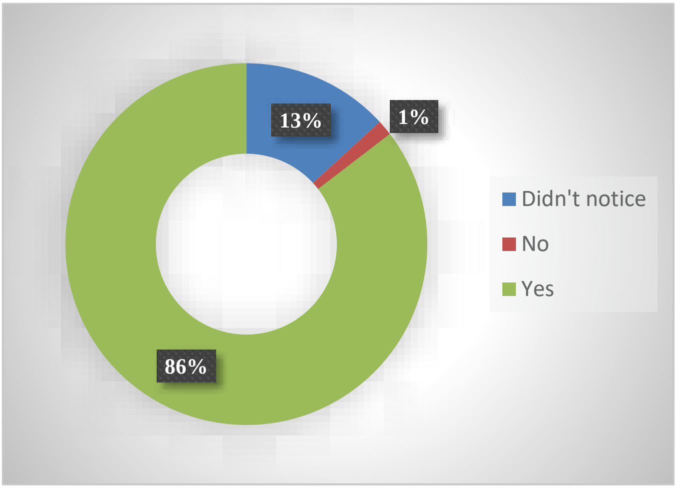


Figure 75: Drought increased commented by the respondents.

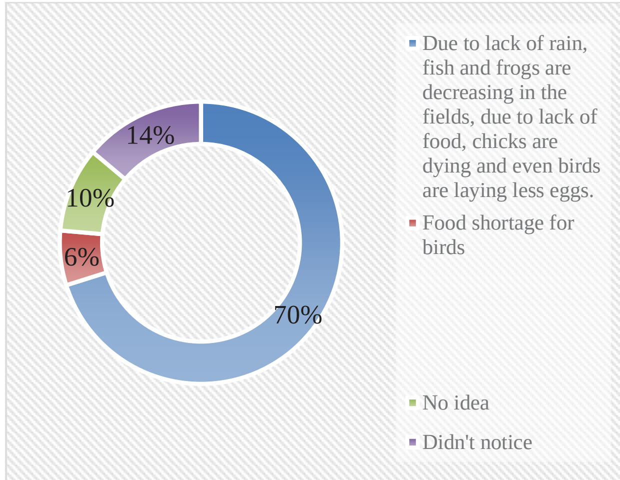


Figure 76: Effect of the drought commented by the respondents.

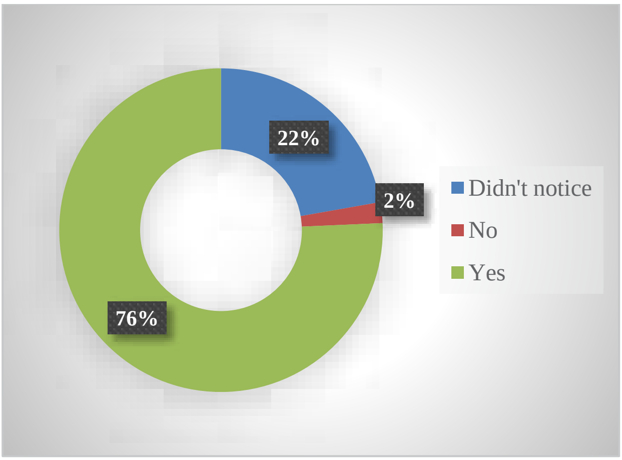


Figure 77: Changes the rainfall pattern commented by the respondents.

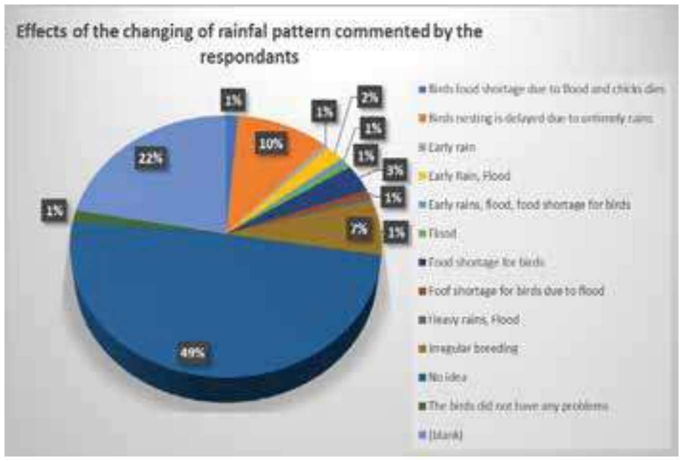


Figure 78: Effect of changing the rainfall pattern.

4.10.5 Present status of colonial waterbirds and how they are coping with climate change.

The population of colonial waterbirds decreasing 67%, stable 20%, increasing 10%, and 3% respondents have no idea about the status of colonial waterbirds (Figure 89). Most of the local respondents have no idea about climate change adaptation of colonial waterbirds (Figure 80). The local peoples have excellent perception on population trend of colonial waterbirds; because this study reveals that about 82 colony sited have been abandoned during the last five years (from 2018 to 2022), on the other hand very few new colonies were identified during these times. Most of the respondents have no perception on waterbirds coping with climate change and very few have good knowledge about the adaptation of colonial waterbirds with climate change effects. During our field visit it was observed that breeding activities were delayed due to irregular rains (Figure 6) and also the chicks were died due to lack of sufficient food because of insufficient rains.

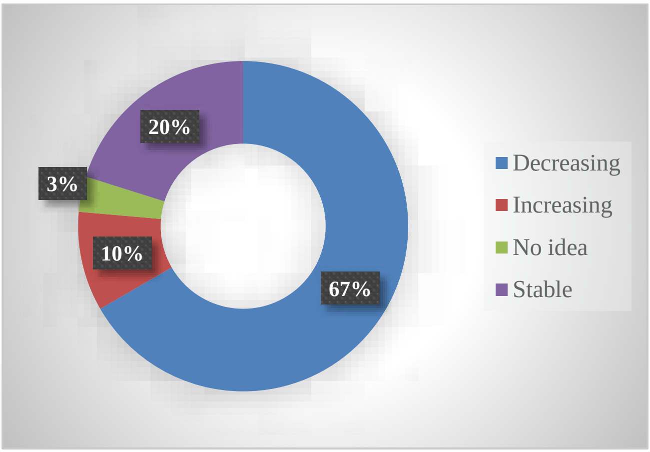


Figure 79: Population trend of colonial waterbirds commented by the respondents.

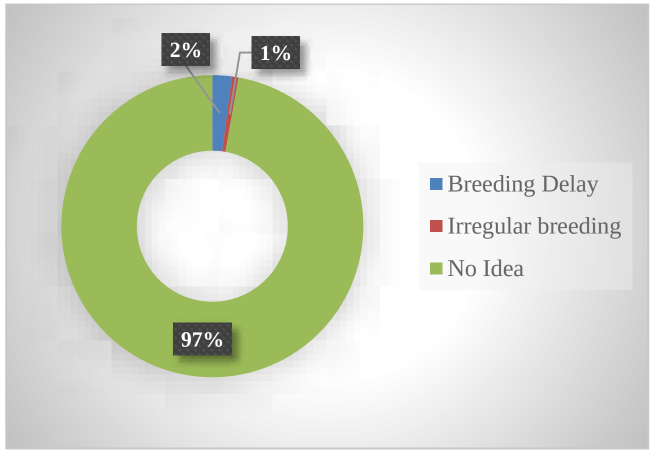


Figure 80: How colonial waterbirds are coping with climate change.

4.10.6 Effect of climate change in crop and fish production.

A total of 144 respondents of which 48 % agreed to negative impact in crop production, 33% have no idea and 19% believed no impact on agriculture because of hybrid varieties crop production increased (Figure 81). Native crop production decreasing about 93% due to various climatic factors but the exact reason was unknow to communities and about 7% crops were damaged by flood in Sylhet region due to early rains or flash flood from the upstream (Figure 82). Climate change has a great negative impact on fish production in nature agreed by 65% of respondents, only 2% disagreed and the rest 33% respondents have no idea (Figure 83). About 96% respondents commented that agricultural land, drains, and banks become dry and fish perish as a result of insufficient precipitation; 2% commented that there are fewer fish because of less rain and waters in the haor dries up quickly and only 1% commented that there are fewer fish because of less rain during fish breeding season (Figure 84). Bangladesh is one of the most climate vulnerable countries because of its geographical location and very prone to natural disasters; climate change accelerated the intensity and frequency of occurrences of drought, irregular rainfall, high temperature etc. that resulted from global warming that is directly and indirectly related to crop production (Kashem and Faroque, 2013). More than 80% of the country's arable land is used for rice cultivation, which is vulnerable to drought in three different growing seasons. Droughts in March and April also cause delays in crop planting during the monsoon season by impeding timely tillage and soil preparation (Paul, 1998). Food insecurity in cities and rural areas would be impacted by projections of a 2°C rise in global temperatures by 2100 (Vermeulen et al., 2012). Climatic impacts adversely affect food production, patterns of crop productivity, fishery (Nelson, 2009; Liverman and Kapadia, 2010). Rice and wheat production is likely to decrease 28 % and 68 % respectively due to a 1–2 °C increase in temperature (Hasan *et al.*, 2013). The countries temperature is generally increasing in the monsoon season (June, July and August) and average monsoon-season maximum and minimum temperatures show an increasing trend annually at the rate of 0.05 °C and 0.03°C, respectively (SAARC Meteorological Research Council (SMRC), 2003. / http://www.ipcc.ch). The overflow of the Ganges water causes severe floods and the underflow causes extreme drought (Afroz & Rahman, 2013). Drought is now a major issue for agriculture and a concern to food security because to the rising trend of mean maximum temperature (Mondal *et al.*, 2012). Drought typically affects aman rich throughout the kharif season, resulting in a significant yield loss (Dey et al., 2012). Droughts have a negative impact on crop productivity and harm 2.32 million hectares of crops annually (Habiba et al., 2011). The yield of rice was found to have declined by around 53.33% in the northwest region of Bangladesh, while the production of potatoes, jute, onions, and beans had decreased by 13.33%, 26.66%, 6.66%, and 4%, respectively, as a result of the drought (Habiba et al., 2012). The productivity of fish, hatchery operations, and fishing are all impacted in different ways by seasonal variations in temperature and precipitation (Haque 2007). Drought damages plant and animal species, lowers microbial activity, decreases soil organic matter contents, causes biodiversity loss, the extinction of some species, and increases disease incidence by introducing pathogens and parasites (Shaw et al., 2013). Local peoples have a good knowledge on climate change impact on crop and fish production. The annual average temperature and mean maximum temperature increases during the last twenty-one years of the study area (Figure 4 & 5); and the annual rainfall also decreases with irregular pattern (Figure 7) suggested that the climate is changing and may have negative impact on crop & fish production in nature.

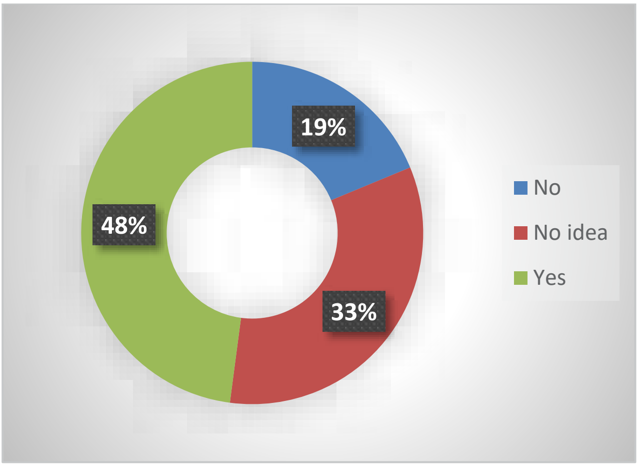


Figure 81: Effect of climate change on crop production commented by respondents.

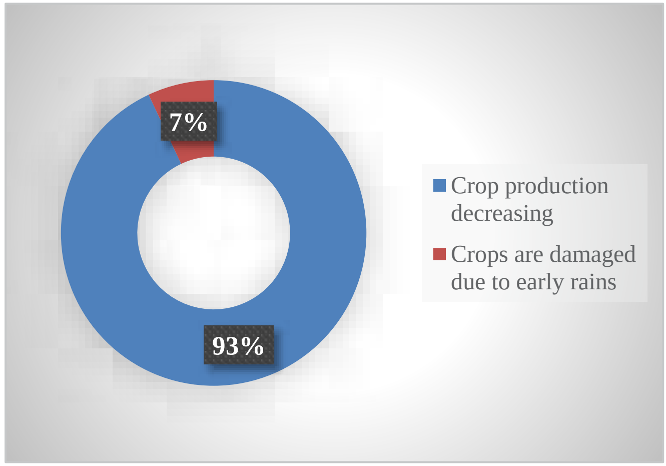


Figure 82: Climate change impact on agriculture commented by the respondents.

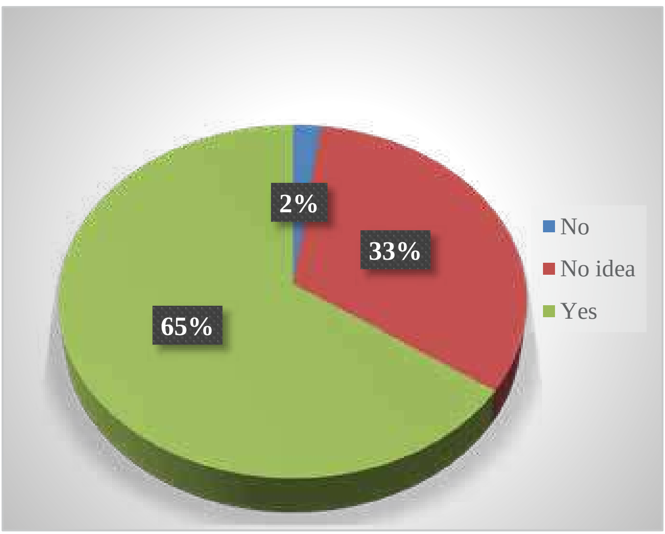


Figure 83: People's perception on climate change effect on fish production in nature

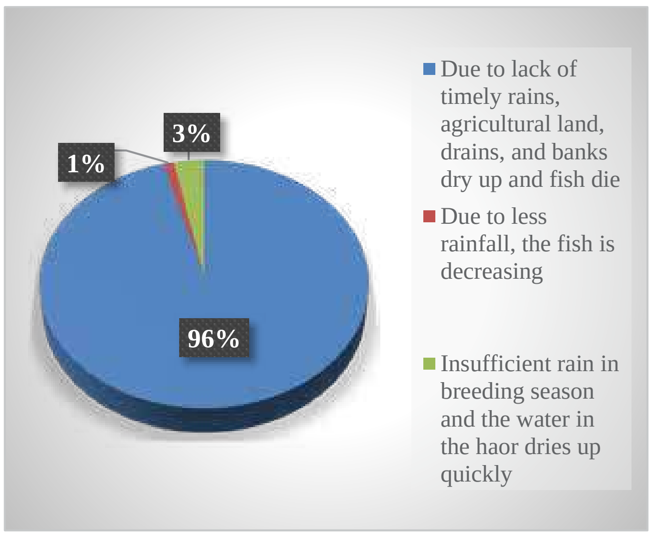


Figure 84: Climate change impact on fish production commented by the respondents.

4.11 Waterbird Conservation Groups (WCG) formation

Following discussions with the UP chairman and social elites, a total of 19 Waterbird Conservation Group (WCG) have been formed. Each group has nine members.

CHAPTER V
CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The Research Project entitled "The Status, Distribution, and Conservation of Colonial Waterbirds in Bangladesh" under SUFAL Innovative Grant Program, SUFAL project, Bangladesh Forest Department and funded by the World Bank has been contracted on 28 September 2021 to perform the activities of the project. In the project proposal, the field days were a total of 360 days (14-15 days per district) covering at least two breeding seasons of the colonial waterbirds. However, due to budget constraints from the Project Management Unit of SUFAL Project, the current and implemented proposal was restricted to a total of 180 days. Which significantly impacts the overall project planning and data collection process. To reflect the present allocated financial budget, now we have to limit field days to 5-7 core days per district.

After the commencement of the project on 29 September 2021, an inception report has been submitted on 28 October 2021; the 1st, 2nd, 3rd ,4th, 5th and 6th quarterly progress report were submitted on 1 January, 4 April, 2 August, 17 October 2022; 15 January and 18 April 2023. This draft final report covers the field activities that have been under taken during October 2021-July 2023 period. During this study a total of 353 colony sites with 82,086 nests and 16 species out of 21 in Bangladesh were identified. Some of them are globally threatened like Oriental Darter (NT), Glossy Ibis, Black-headed Ibis (NT), Woolly-necked Stork (NT) and Lesser Adjutant (VU). Threats and conservation measures have been identified. Local perception on climate change with its impact were identified; climate change has a great negative impact on the waterbird population, agricultural production and also immensely impacted fish production in nature. Habitat destruction and hunting greatly impacted on waterbird population. Most of the colony sites needed immediate conservation measures. So, we identified 163 potential sites and proposed them under a new conservation site approach to as 'Community Conservation Area. For protection of large colonies, we created 19 Waterbird Conservation Group (WCG) containing nine members. Strategic Multi-species Action Plan (2024-2033) to conserve Colonial Waterbirds in Bangladesh have been developed and submitted to the PMU, SUFAL Project for sustaining the research and conservation on colonial waterbirds in Bangladesh. The strategic conservation action plan is focused on the conservation of colonial waterbirds by safeguarding and enhancing their natural habitats, offering incentives and compensation for resource damages, protecting the nesting and roosting colonies, reducing conflict with local communities, and raising awareness, promote research and regular monitoring, and developing capacity for waterbird conservation.

5.2 Recommendations and Suggestions Guideline, strategy

Studying and conserving colonial waterbirds is crucial due to their ecological importance and the challenges they face. Some recommendations and suggestions given bellow.

Research on colonial waterbirds

1. Population survey: Conduct comprehensive surveys to assess the population size, distribution, and breeding habitats of colonial waterbirds in all regions of Bangladesh.
2. Ecological studies: Investigate the ecological requirements and nesting habits of different species. This can help in understanding their needs and updating the conservation strategies.
3. Research on potential avian diseases of waterbirds and human health hazards and mitigation.
4. Threat assessment: Continue to analyze the major threats these birds face, such as habitat loss, pollution, climate change, and human disturbances. Evaluate their impact on waterbird populations.

5. Collaborative studies: Foster collaborations among local universities, research institutions, and international organizations specializing in avian ecology and conservation to enhance research efforts and knowledge sharing.
6. Technology in monitoring: Utilize modern technologies like satellite tracking, drones, and remote sensing to study bird movements, migration, habitat changes, and population dynamics.

Conservation of colonial waterbirds

1. Habitat protection: Identify critical habitats and advocate for their protection through government policies and community involvement. This includes wetland conservation nearby colony sites, where many waterbirds congregate.
2. Community engagement: Involve local communities in conservation efforts through education, awareness programs, and incentivizing sustainable practices that benefit both people and birds.
3. Conflict mitigation: Develop strategies to minimize conflicts between waterbirds and human activities, through innovative solutions and community participation.
4. Strengthen law enforcement scope and capacity of the FD; and strengthen legal protection and revise or amending legislations relating to wildlife/waterbird conservation.
5. Monitoring and conservation actions: Implement long-term monitoring programs to track population trends and take proactive conservation measures when necessary, such as establishing protected areas.
6. International collaboration: Engage with international conservation bodies and initiatives, leveraging their expertise and resources for the conservation of waterbird species.
7. Advocacy and policy influence: Advocate for policies that safeguard waterbird habitats and their conservation need at local, national, and international levels.
8. Data sharing platforms: Establish national databases on colonial waterbirds and online platforms to facilitate data sharing among researchers, organizations, and policymakers, enhancing collaborative efforts and informed decision-making.
9. Innovation in conservation: Explore innovative solutions, such as artificial nesting sites or eco-friendly fishing techniques, to address specific challenges faced by waterbirds.

It is very urgent to take into account the proposed Strategic Multi-species Action Plan (2024-2033) to conserve Colonial Waterbirds in Bangladesh along with the proposed 163 Community Conservation Areas should be properly implemented. By combining robust research efforts with strategic conservation actions and community engagement, it's possible to protect and ensure the survival of colonial waterbirds in Bangladesh.

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Population status, habitat suitability and threats assessment of Smooth-coated Otter (*Lutragale perspicillata* Geoffroy St. Hilaire, 1826) in the Padma River basin.

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CHAPTER I

INTRODUCTION

The Bengal Basin, recognized as the world's largest delta, is shaped by the confluence of three major river systems: the Ganges, Brahmaputra, and Meghna. This vast hydrological network results in extensive lowland areas, leading to nearly half of Bangladesh being classified as wetlands (Khan, 1993; Hughes et al., 1994). These wetland ecosystems are critical for supporting a wide range of wildlife species, many of which face severe threats due to increasing anthropogenic pressures. Among these, otters are particularly vulnerable.

Globally, there are 13 recognized otter species, distributed across various regions except for Australia, Antarctica, and some isolated oceanic islands (Mason and Macdonald, 1986). In Bangladesh, three species have been documented: the Asian small-clawed otter (Aonyx cinereus), the Eurasian otter (Lutra lutra), and the Smooth-coated otter *(Lutrogale perspicillata).* Of these, the Smooth-coated otter is currently listed as Endangered within the country (IUCN Bangladesh, 2015).

Although two studies have previously explored traditional otter fishing practices and captive breeding of Smooth-coated otters (Feeroz et al., 2011a, b), and one study has focused on the population and feeding ecology of the Small-clawed otter in the Sundarbans (Aziz, 2018), there remains a critical research gap regarding the Smooth-coated otter's status in its natural habitat. The Padma River basin, particularly the stretch from Shibganj (Chapainawabganj) to Charghat (Rajshahi), has been identified as a potentially suitable habitat for this species. Despite occasional sightings in recent years, no comprehensive or systematic assessment of population status or threats has been conducted in this region. The proposed study aims to scientifically assess the population status, evaluate habitat suitability, and identify major threats to Smooth-coated otters within the Padma River basin. To address these knowledge gaps, this study employed a combination of direct field surveys using camera traps, sign surveys and local knowledge to estimate population presence and relative abundance. Habitat suitability was modeled using environmental variables in a species distribution modeling (SDM) framework, while threats were assessed through key informant interviews and structured community questionnaires followed by qualitative prioritization.

The Smooth-coated otter serves as a top predator in wetland ecosystems (Shenoy, 2005) and is widely recognized as a keystone species, instrumental in maintaining ecological balance through prey regulation and enhancing overall biodiversity. This species demonstrates distinct amphibious adaptations, enabling it to hunt both aquatic and terrestrial prey (Foster-Turley et al., 1990). Its diet includes fish, crustaceans, and other aquatic organisms, along with small terrestrial mammals, birds, and invertebrates emphasizing its ecological flexibility and crucial role within wetland food webs. As apex semi-aquatic carnivores, Smooth-coated otters are not only crucial in regulating fish populations but also serve as umbrella species meaning that their conservation inadvertently ensures the protection of entire wetland ecosystems and associated species. Understanding their status can therefore offer insights into the broader health of freshwater systems, making them a focal point for integrated river basin conservation strategies.

The availability of fish is a key determinant for the presence and persistence of Smooth-coated otters in a given habitat (Melquist and Hornocker, 1983). Moreover, they are recognized as biological indicators of wetland health due to their sensitivity to water quality, toxicity, and ecological disturbances (Earlings, 1972; Anoop and Hussain, 2004). Among the otter species recorded in Bangladesh, the Smooth-coated otter is the largest in body size and is distinguished by its notably sleek and smooth pelage (Francis, 2001).

Overexploitation of natural resources, destruction, and fragmentation of habitats, along with unsustainable practices such as overfishing, sand mining, and the operation of unregulated water vessels, are critically impacting the biodiversity of the Padma River basin. These activities introduce pollutants, including oil spills, industrial discharge, and waste from vessels, which significantly degrade water quality. Furthermore, the extensive use of chemical fertilizers and pesticides in agriculture contributes to nutrient pollution and eutrophication, further disrupting aquatic ecosystems and threatening the survival of sensitive species. To systematically identify and rank the threats faced by the species, the study employed a qualitative scoring approach based on perceived severity, frequency and reversibility as reported by stakeholders and field observations.

In addition to these direct anthropogenic pressures, many species in the Padma River basin face the threat of hunting, driven by traditional misconceptions about their medicinal or cultural value. This illegal and unsustainable exploitation not only reduces their populations but also impacts the ecological roles they play in maintaining ecosystem stability.

Studying cryptic and elusive species like the Smooth-coated otter poses significant challenges due to their nocturnal habits, aquatic mobility and often subtle field signs. These challenges necessitate the use of indirect methods such as trail cameras and community-based reporting to obtain reliable presence data, especially in large and dynamic riverine landscapes like the Padma basin.

In light of these pressures, the Padma River basin is facing significant ecological degradation, placing sensitive species like the Smooth-coated otter at serious risk. A science-based understanding of otter distribution, habitat preferences, and threat dynamics is urgently needed to inform targeted conservation actions and river management strategies. This study aims to generate such foundational knowledge to guide future conservation planning and policy interventions, ensuring the persistence of both otters and the vital ecosystem services they support.

1.1 Problem Statements

Bangladesh is globally recognized for its exceptional biodiversity, largely due to its strategic geographic position, favorable climate, and varied ecosystems. These factors collectively support an extraordinary range of wildlife species. However, in recent decades, the country has experienced mounting ecological pressures, consistent with global biodiversity decline trends. In response, both national authorities and international conservation organizations have initiated efforts aimed at mitigating these environmental challenges through policy, research, and habitat management.

Despite its rich biodiversity, conservation efforts in Bangladesh have paid relatively little attention to otters, even though they are often referred to as "ambassadors of wetlands" for their ecological importance (Gupta et al., 2016). The smooth-coated otter (*Lutrogale perspicillata*), in particular, is classified as "Vulnerable" on the IUCN Red List of Threatened Species (de Silva et al., 2015), with its global population estimated to have declined by approximately 30% over the past three decades (Pacifici et al., 2013). Similar conservation gaps have been documented across much of the species' range. For instance, studies from India (Hussain and Choudhury, 1997) and Southeast Asia (Duplaix and Savage, 2018) report population declines, habitat fragmentation and insufficient research on the species' ecological needs. These studies collectively emphasize the urgent need for site-specific, data-driven research to guide conservation.

Bangladesh is located close to the Indo-Burma region, which is known around the world as a biodiversity hotspot with more than 7,000 plant species found only in that area (Mittermeier et al., 1998). The country has a rich variety of plants and animals. However, its natural environment is facing serious problems due to pollution, habitat destruction, and uncontrolled use of natural resources (Nishat et al., 2002). Although conservation efforts have increased in recent years, species like the smooth-coated otter have not received enough research attention or support through policies.

According to the IUCN Bangladesh Red Data List (2015), all three otter species found in the country are listed as Endangered. Among them, the smooth-coated otter is especially understudied, with virtually no comprehensive scientific assessment regarding its population dynamics, habitat requirements, threats, or ecological role within Bangladesh's freshwater systems.

This species is undergoing a noticeable population decline throughout its range, mainly due to habitat destruction, fragmentation, and unsustainable exploitation (Hussain et al., 2008). As semi-aquatic, social carnivores, smooth-coated otters rely heavily on wetland habitats ecosystems that are increasingly disappearing at an alarming rate (Davidson, 2014). Wetlands offer essential resources such as food, shelter, and breeding grounds, and their degradation directly threatens the survival of otter populations.

The Padma River basin, one of South Asia's most ecologically significant wetland systems, is believed to be a potential stronghold for this species. Nevertheless, several human-induced threats compromise the otters' survival in this area. These include extensive sand mining operations that disrupt river substrates and reduce fish availability, overfishing, and the widespread use of harmful fishing methods such as gill nets and traps which not only deplete prey populations but also result in accidental otter fatalities. Pollution from oil spills and waste discharge from watercraft further contaminates the aquatic environment, while agricultural runoff laden with pesticides and fertilizers increases water toxicity and alters ecological balances through eutrophication.

Illegal hunting, motivated by traditional beliefs regarding the medicinal properties of otters and the illegal wildlife trade, poses another significant challenge. Such exploitation adds to the pressures on the population, already struggling due to habitat degradation. Otters are also highly susceptible to human disturbance, and increasing encroachment into wetland areas further hampers their natural behaviors and reproductive success. The illegal hunting and trade of otters for pelts or medicinal use have also been reported widely across South and Southeast Asia, contributing to significant population pressure. For example, Gomez et al. (2016) documented the high volume of illegal otter skin trade in parts of India, Nepal, and Indonesia, with Smooth-coated otters being the most heavily traded species.

The absence of detailed, data-driven research on these threats hinders the formulation of effective conservation strategies. Comprehensive ecological studies are urgently needed to understand the scale and impact of these issues. Conservation measures should prioritize habitat protection, strict regulation of resource use, reduction of pollution, and the promotion of sustainable practices through community engagement. Moreover, integrating local stakeholders into conservation initiatives can enhance awareness, reduce anthropogenic threats, and support coexistence between human communities and otters. Prakash et al. (2012) provided one of the first systematic accounts of Smooth-coated Otter monitoring along India's Cauvery River, highlighting threats from poaching, habitat alteration, dynamite fishing, and sand mining. They demonstrated effective threat-mitigation strategies through community-based stakeholder involvement and direct surveys using camera traps and sign detection

These regional studies reinforce the broader conservation crisis facing Smooth-coated otters, suggesting that the threats observed in the Padma River basin are representative of larger patterns across the species' range. However, site-specific ecological data, particularly in under-researched regions like Bangladesh, remain critically limited. Without immediate and coordinated conservation action, the continued existence of smooth-coated otters in the Padma River basin and across Bangladesh remains in jeopardy.

1.2 Objectives of the Study

Specific objectives are as follows:

i. To assess the population status of smooth-coated otter in the Padma River basin.
ii. To identify the suitable habitat and assess major threats of Smooth-coated otter.

1.3 Research Questions

Research questions are as follows:

i. Which types of habitats support smooth-coated otter to survive in the Padma River basin?
ii. What is the current population status of smooth-coated otter in the Padma River basin?
iii. What are the major threats declining the population of smooth-coated otter in the Padma River basin?

CHAPTER II

LITERATURE REVIEW

Freshwater ecosystems are highly biodiverse yet disproportionately threatened. Though representing less than 1% of the Earth's surface, they sustain about 12% of known species and nearly 30% of all vertebrates (Dudgeon et al., 2006; Reid et al., 2019; Darwall et al., 2018). These habitats deliver critical ecosystem services such as water purification, nutrient cycling, and flood regulation, while supporting flagship species like otters, gharials, and river dolphins (Dudgeon et al., 2006; Reid et al., 2019). Freshwater vertebrate populations have declined by over 80% since the 1970s, largely due to habitat degradation, pollution, over-exploitation, and climate impacts (Reid et al., 2019).

The smooth-coated otter *(Lutrogale perspicillata)* is a keystone semi-aquatic predator that relies on clean, fish-rich waters and riparian cover. Its sensitivity to environmental changes makes it an effective bio-indicator of wetland health (Anoop & Hussain, 2004; Foster-Turley et al., 1990). Globally classified as Vulnerable, and as Endangered in Bangladesh, its populations are declining due to habitat fragmentation, pollution, unsustainable fisheries, sand mining, and hunting (de Silva et al., 2015; IUCN Bangladesh, 2015).

Shuklaphanta National Park: Using 71 line transects along riverbanks and wetlands, researchers employed logistic regression to find that otter presence increased with canopy cover, wider waterways, and soft substrates (mud/sand), but decreased with higher disturbance and steeper banks (Awasthi et al., 2024)

Geruwa–Khaurahi River, Bardia NP: Sign surveys and habitat sampling along fixed transects showed otter signs clustered on sandy, vegetated shorelines with gentle slopes, indicating substrate and riparian cover are key factors (Gwachha et al., n.d.).

Community perception in Shuklaphanta buffer zones: Semi-structured interviews revealed widespread acknowledgement that agriculture, fishing pressure, pollution, and habitat modification degrade otter habitat, though community awareness of otters' ecological role was limited (Awasthi et al., 2024)

Bandhavgarh Tiger Reserve, Madhya Pradesh: Extensive camera trapping recorded the species' first photographic evidence in the reserve, confirming presence but indicating low density and emphasizing the need for targeted aquatic surveys (Rather et al., 2019).

Goa Estuary and riparian systems: Habitat selection studies in coastal/urbanized landscapes demonstrated that high nitrate and phosphate levels can indirectly limit otters by reducing prey fish, though in the study, measured pollution levels were below threshold.

Tamil Nadu estuarine research (2025): Estimated that threats including habitat modification, unsustainable fisheries, hunting, and salinity changes negatively influence site occupancy; emphasized combining fishery regulation and habitat management (Utthamapandian et al., 2025).

Bangladesh lacks systematic ecological studies on smooth-coated otters existing literature is limited to traditional fishing practices (Feeroz et al., 2011a; Feeroz et al., 2011b) and studies on the small-clawed otter in Sundarbans (Aziz, 2018). In contrast, neighboring Nepal and India show consistent patterns of threat riparian degradation, sand mining, overfishing, water pollution, and human-otter conflict and emphasize the importance of habitat-based, field-driven research. The methods proven effective in the region camera trapping, sign transects, habitat modeling, community surveys offer a robust blueprint for Bangladesh.

The species is heavily reliant on the availability of fish, unpolluted wetlands, and undisturbed riverbanks (Shenoy, 2005; Davidson, 2014). However, wetlands across South Asia are increasingly degraded due to agricultural runoff, damming, illegal hunting, and unregulated tourism (Hussain et al., 2008). As a result, otters face compounded threats ranging from accidental capture in fishing nets to habitat encroachment and persecution due to perceived conflict or use in illegal wildlife trade.

Recent studies have also underscored the utility of species distribution models (SDMs) and non-invasive field methods such as camera traps, sign surveys, and occupancy modeling for assessing habitat use and population status of elusive species like otters (Borah et al., 2014). These methods are particularly suitable for regions like the Padma River basin, where logistical constraints and fragmented habitats hinder extensive field coverage. Incorporating local ecological knowledge through community surveys and key informant interviews further enhances data robustness and contextual understanding (Bhandari et al., 2023).

Therefore, there is an urgent need for systematic, field-based research in Bangladesh focusing on the distribution, threats, and ecological role of smooth-coated otters. Such efforts are crucial not only for national biodiversity conservation but also for fulfilling broader regional and global commitments to freshwater ecosystem protection.

Regional studies from Nepal, India, and Sri Lanka demonstrate that smooth-coated otter populations are declining primarily due to anthropogenic threats that mirror those in Bangladesh's Padma River basin. They highlight habitat degradation, prey decline, pollution, and lack of awareness as consistent pressures. Methodologically, these studies validate the combined use of camera trapping, sign surveys, logistic and occupancy modeling, and species distribution mapping. Integrating ecology with community perception has enhanced understanding of threats and facilitated participatory conservation. Therefore, it is imperative that Bangladesh undertakes similarly rigorous, field-based ecological research to assess population status, habitat suitability and threats filling a critical knowledge gap and informing conservation strategies at a national and regional level.

CHAPTER III

METHODOLOGY

3.1 Study Area

3.1.1 Location

The Padma is a fast-flowing transboundary river originating from the Himalayas. It flows through India as the Ganga and enters Bangladesh at Thuthapara in Shibganj Upazila of Chapainawabganj District. From there, it flows southeastward to its confluence with the Meghna River.

The study area encompasses a section of the Padma River basin, situated within the administrative boundaries of Shibganj Upazila (Chapainawabganj District) and Charghat Upazila (Rajshahi District), in the northwestern region of Bangladesh. Geographically, it extends approximately between 24.85°N to 23.70°N latitude and 88.00°E to 88.60°E longitude, adjacent to the India-Bangladesh international border.

Considering the river's meandering path and the mapped boundary, the total length of the study area is approximately 160 km. The width of the study area varies depending on river morphology, floodplain extent, and the presence of braided channels. The narrowest sections, found in the mid-Rajshahi region, are around 2 km wide; moderate sections range between 4 to 6 km; and the widest areas, measuring up to 10–12 km, occur primarily in Godagari Upazila of Rajshahi and Chapainawabganj, where multiple river channels or confluences are present.

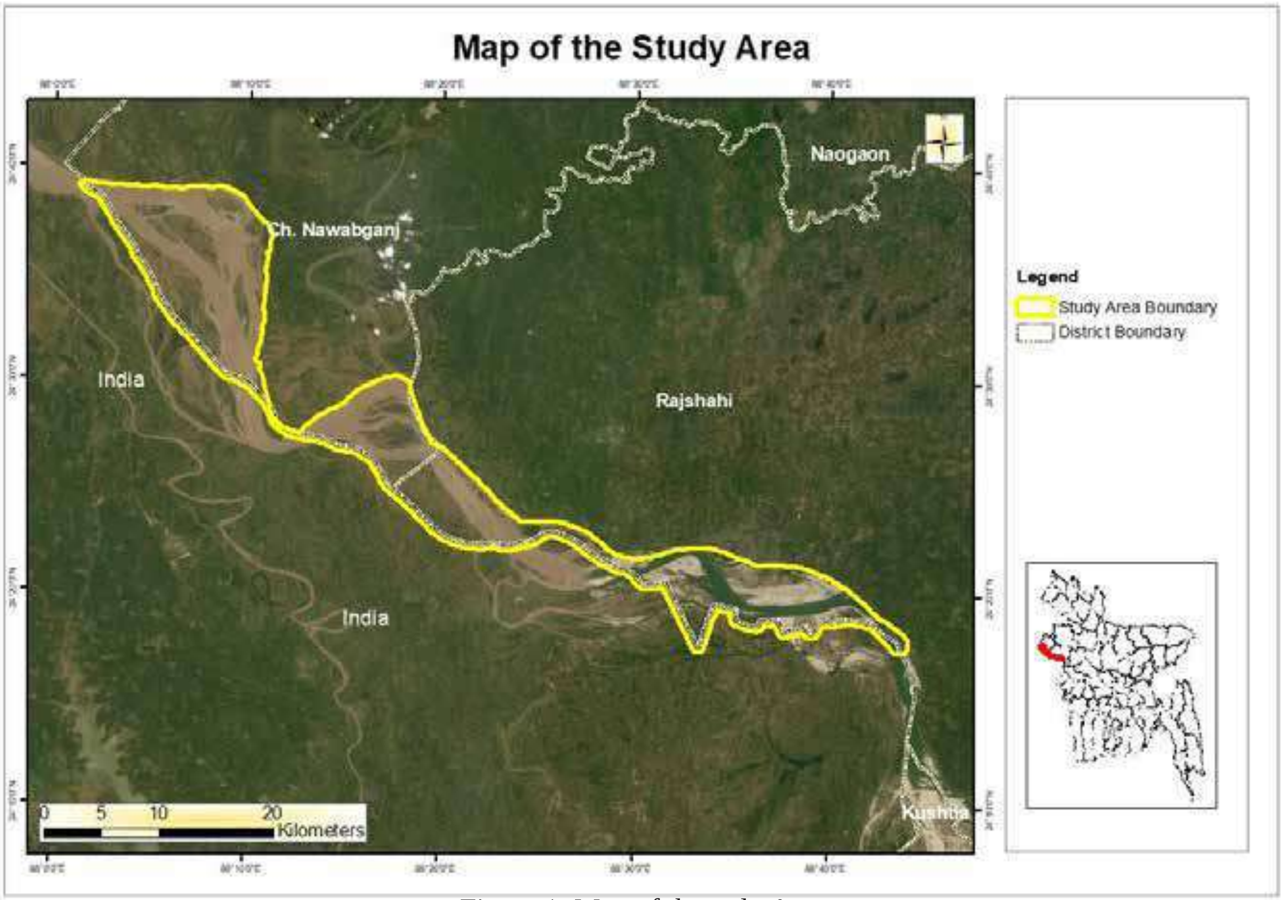


Figure 1: Map of the tudy Area

3.1.2 Climate

The study area is located within a tropical monsoon climate zone, marked by clear seasonal variation. The wet season typically spans from June to October, during which about 80–85% of the region's annual rainfall approximately 2000 mm is received (Rahman et al., 2021). This intense rainfall leads to high river discharge, often resulting in floods and widespread sediment transport. The dry season, from November to April, experiences significantly lower rainfall, leading to reduced water flow and occasional drought conditions (Khatun et al., 2020).

The Padma River, a major watercourse in Bangladesh and a distributary of the Ganges, displays a dynamic and braided morphology. Its wide riverbed often splits into multiple shifting channels, forming sandbanks and temporary islands called chars. These landforms are shaped by the river's high sediment load and variable flow patterns (Islam & Rashid, 2011). The chars, deep pools (beels and khals), and vegetated riparian margins provide a mosaic of aquatic and semi-aquatic habitats.

This diverse habitat structure supports a rich biodiversity, including several resident and migratory bird species. It also offers ideal conditions for semi-aquatic mammals such as the Smooth-coated Otter *(Lutrogale perspicillata)*. These otters depend on abundant fish populations and require undisturbed, vegetated riverbanks or islets for breeding and shelter (IUCN Bangladesh, 2015). Although the species is more commonly reported from the Sundarbans, suitable microhabitats along the Padma River may support local populations, especially where human disturbance is minimal.

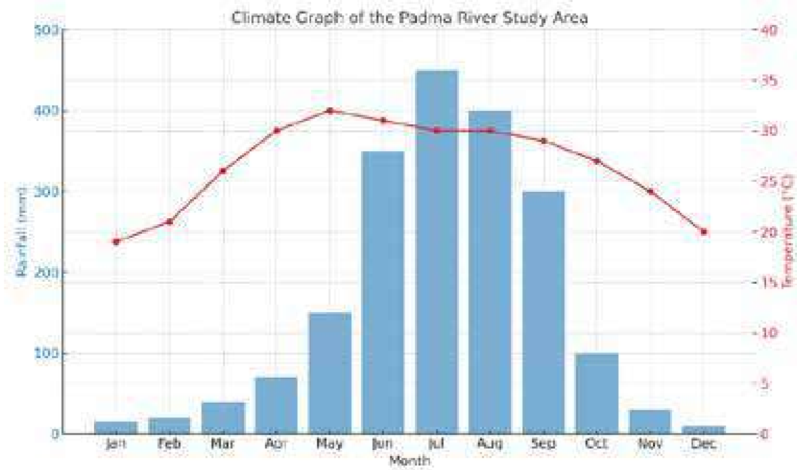


Figure 2: Rainfall and temperature patterns in the Padma River region, based on general descriptions from Rahman et al. (2021) and Khatun et al. (2020).

3.1.3 Vegetation

The natural vegetation of the Padma River floodplain is shaped by seasonal flooding, sediment deposition, and human activities such as agriculture and grazing. The area supports a dynamic mosaic of habitats including alluvial grasslands, reed beds, riverine sandbars (chars), and seasonally inundated wetlands. These ecosystems provide important ecological functions, such as erosion control, nutrient cycling, and habitat provision for both aquatic and terrestrial fauna.

The surveyed area includes both emergent and semi-aquatic vegetation along the riverbanks and natural colonization zones on the chars. The adjacent agricultural fields, often seasonally fallow or partially flooded, further contribute to habitat heterogeneity and may support transient or foraging wildlife species, including the smooth-coated otter.

The dominant wild plant species observed during field visits include:

Persicaria glabra – a common riparian herb thriving in moist, disturbed habitats; *Tamarix dioica* – a salt-tolerant shrub found on sandy riverbanks and flood-prone areas; *Ipomoea aquatica* – a semi-aquatic vine found in wetlands and waterlogged fields; *Xanhium strumarium* – an annual herb commonly found on disturbed soils; *Lippia alba* – a fragrant shrub occurring in moist riparian zones; *Persicaria odorata* – an aromatic herbaceous plant often found in wetlands; *Saccharum spontaneum* – a tall grass forming dense colonies in chars and floodplains; *Phragmites australis* – the common reed, dominant in wetland and marshy edges etc.

Other commonly encountered species include *Eichhornia crassipes* (water hyacinth) in stagnant waters, *Cynodon dactylon* (Bermuda grass), and various sedges (*Cyperus* spp.), which contribute to the wetland vegetation structure.

This varied vegetation provides shelter, denning materials, and foraging grounds for a wide range of faunal species, including otters, small mammals, birds, amphibians, and reptiles. The dense grasses and reeds, in particular, are important for otter holts and resting sites, especially in undisturbed char lands and less-accessible river margins (Appendix-7).

3.2 Study Design and Data Collection Overview

Assessing the population status, habitat suitability, and threats to the Smooth-coated Otter *(Lutrogale perspicillata)* in the Padma River basin required a multi-faceted and integrative research approach due to the region's complex and dynamic landscape. The study area includes a mosaic of habitat types such as agricultural lands, braided river channels, ephemeral sandbars (chars), deep stream networks, and densely populated rural settlements—each influenced by seasonal fluctuations and diverse human activities. These variations pose significant challenges in ecological monitoring and species detection.

To address these complexities, the research was structured into four sequential phases like data collection, data compilation, analytical processing and synthesis and interpretation of findings. Multiple complementary field methods were deployed to meet the study's three core objectives. Camera trap surveys were conducted to estimate otter population status and occurrence patterns, and the resulting presence data were also utilized in species distribution modeling (SDM) using MaxEnt to predict suitable habitats. Sign surveys (spraints, tracks, holts, and human disturbances) were used to validate otter presence and assess localized threats. Additionally, semi-structured questionnaire surveys with key local stakeholders were administered to gather community-based insights on otter occurrence, habitat condition, and perceived threats. This integrated methodology enabled triangulation of data sources and enhanced the robustness of ecological inference across spatial and social contexts.

3.2.1 Population Status Assessment

3.2.1.1 Camera Trap Survey

To assess the presence and population status of the Smooth-coated Otter *(Lutrogale perspicillata)*, motion-triggered camera traps were strategically deployed across suspected otter pathways, riverbanks, sandbars and riparian corridors within the Padma River basin. The deployment strategy was guided by preliminary sign surveys and local knowledge to maximize the probability of detection. A total of 10 trail cameras were used and overall trap effort was 250 trap nights.

Independent detection events were defined as otter photographs separated by at least 30 minutes at the same camera station to minimize pseudo-replication (O'Brien et al., 2003). The camera trap data enabled the estimation of species presence, activity patterns and relative detection frequency, but did not allow for individual identification due to the absence of unique pelage patterns in this species. Consequently, Relative Abundance Index (RAI) was employed as a proxy for otter density, calculated as the number of independent detections per 100 trap nights (Carbone et al., 2001; Anwar et al., 2011). This approach provides an indirect but informative estimate of spatial activity and abundance trends across the landscape.

While the RAI provides valuable comparative insights, its interpretation must consider inherent limitations, including non-individual-based detections and variable detection probabilities. The Random Encounter Model (REM) (Rowcliffe et al., 2008) offers an alternative density estimation method for unmarked species, but its application was constrained in this study due to the unavailability of key parameters such as animal daily movement rates and camera detection zone characteristics.

Figure 5: Deployment of camera traps by field staff.

Nevertheless, the camera trap survey remains a crucial tool in otter monitoring, offering undisturbed, continuous observations of otter behavior, spatial distribution, and habitat use critical for informing conservation planning in complex riverine landscapes. Moreover, the camera trap surveys offer a unique opportunity to study otter demographics, including age and sex ratios, providing valuable information for population assessments.

3.2.1.2 Questionnaire Survey:

To complement ecological field surveys and integrate local ecological knowledge into the assessment of *Lutrogale perspicillata* distribution, habitat use, and threats, a semi-structured questionnaire survey was administered among community members residing along the Padma River basin. This approach is increasingly recognized in conservation science for its ability to reveal species occurrence data, trends, and human-wildlife interactions in data-deficient regions (Anadón et al., 2009; White et al., 2005).

A total of 400 face-to-face interviews were conducted with key stakeholder groups, including fishermen, boatmen, riverine farmers, sand miners, and other individuals who regularly interact with the riverine environment. Participants were purposively selected across a broad age spectrum and from various professional and social backgrounds to capture a wide range of experiences and perceptions. The interviews were spatially distributed across the Padma River basin, covering diverse habitat types and land use zones where otter occurrence was suspected. The geographic coordinates of each respondent's location were recorded using GPS to facilitate spatially explicit analysis of otter sightings and threats.

The questionnaire included 30 open- and close-ended questions grouped into five thematic sections: (i) personal and socio-demographic information, (ii) past and recent otter sightings, (iii) frequency and seasonality of encounters, (iv) habitat types associated with sightings, and (v) perceived threats and human-wildlife conflict. Additional prompts allowed respondents to describe group sizes, activity periods, and behavior during sightings. Participants were also asked to identify otters using visual aids to minimize species misidentification a precaution supported in similar studies involving cryptic or sympatric species (Turvey et al., 2014).

All interviews were conducted in the local language, with informed verbal consent obtained prior to participation. Respondents were informed about the purpose of the study, the voluntary nature of their participation, and the confidentiality of their responses. The research protocol adhered to ethical guidelines for human-subject research in biodiversity studies (Newing, 2011) and approval was obtained from relevant institutional ethical review boards where applicable.

The questionnaire survey provided valuable qualitative and semi-quantitative insights into otter presence, habitat preference, temporal activity, and perceived population trends, while also revealing localized threats such as entanglement in fishing gear, habitat degradation, and direct persecution. These findings contributed critical human-dimension data to triangulate with camera trap and sign survey results, thereby supporting a more integrated and grounded conservation assessment.

Figure 4: Questionnaire survey being conducted in and around the study area.

3.2.2 Habitat Suitability Modeling

3.2.2.1 Data Preparation

To predict suitable habitats for the Smooth-coated Otter *(Lutrogale perspicillata)* across the Padma River basin, species distribution modeling (SDM) was conducted using the Maximum Entropy algorithm implemented in MaxEnt v3.4.4 (Phillips et al., 2006; Elith et al., 2011). A total of 23 presence records were used, compiled from validated sources including camera trap detections, confirmed otter signs (e.g., spraints, tracks) and verified local ecological knowledge gathered through semi-structured questionnaires. All presence points were georeferenced using handheld GPS units and spatial duplication was removed to avoid overrepresentation within grid cells (Merow et al., 2013).

An initial set of 24 environmental variables was considered based on known ecological correlates of otter distribution, including 19 bioclimatic variables (from WorldClim v2.1), topographic layers (elevation and slope from SRTM), vegetation indices (NDVI), hydrological layers (NDWI) and land cover data (Copernicus Global Land Cover). All raster layers were resampled to a 30-meter resolution, suitable for fine-scale modeling in riverine landscapes (Cianfrani et al., 2011; Yackulic et al., 2013).

To mitigate multicollinearity, pairwise Pearson correlation coefficients (r) were calculated and a threshold of $|r| \geq 0.7$ was applied. Further, a Variance Inflation Factor (VIF) analysis was performed to retain ecologically meaningful and independent predictors (Dormann et al., 2013). This process resulted in 11 non-collinear variables being used in the final model: annual mean temperature, isothermality, minimum temperature of the coldest month, temperature annual range, mean temperature of the driest quarter, annual precipitation, precipitation seasonality, elevation, slope, NDVI, and land cover. All layers were projected using EPSG:3857 (WGS 84 / Pseudo-Mercator) to ensure compatibility with MaxEnt and GIS analyses.

3.2.2.2 MaxEnt Modeling

To model the habitat suitability of the Smooth-coated Otter *(Lutrogale perspicillata)*, we employed the Maximum Entropy algorithm using MaxEnt software version 3.4.4, a robust and widely used tool for presence-only species distribution modeling (Phillips et al., 2006; Elith et al., 2011). This method estimates the probability distribution of maximum entropy, subject to environmental constraints derived from known presence locations.

Model Configuration and Settings

The model was trained using 23 confirmed presence records, derived from camera trap detections and sign surveys, with no duplicate records detected within the dataset. The replicated run type was set to "Bootstrap" with 100 replicates, allowing for robust estimation of prediction variability. The regularization multiplier was maintained at the default value of 1, and feature classes were automatically selected by the software based on the number of presence records and variable types.

Environmental predictors were resampled to a uniform 30-meter resolution and aligned to the EPSG:3857 (Web Mercator) projection. The model was configured to run for a maximum of 500 iterations with 10,000 background points. Random seed initialization was enabled to ensure variation across bootstrap replicates. The data partitioning split the presence data into 75% for training and 25% for testing, following best practices for model validation.

Environmental Variables

Initially, 24 bioclimatic and topographic variables were considered, including variables from the WorldClim dataset (Fick & Hijmans, 2017), remotely sensed NDVI and NDWI indices, land cover, and elevation/slope data. To minimize multicollinearity, a Pearson correlation analysis ($|r| > 0.7$) was performed, and only 11 variables were retained. These included 10 continuous variables and one categorical variable (land cover), ensuring a balance between ecological relevance and statistical independence (Dormann et al., 2012).

Model Evaluation and Output Interpretation

Model performance was evaluated using the Area Under the Curve (AUC) of the Receiver Operating Characteristic (ROC) plot, a threshold-independent metric widely used to assess the discriminatory power of SDMs. The model achieved a mean AUC > 0.9, indicating excellent predictive performance (Swets, 1988; Fielding & Bell, 2002).

The final habitat suitability map was generated using the logistic output format, which expresses habitat suitability on a scale from 0 (unsuitable) to 1 (highly suitable). Variable contribution analysis (Table 1) indicated that Precipitation Seasonality (23.9%), Land Cover (20.7%), and NDVI (14.9%) were the top contributors, suggesting that hydrological variation and vegetation productivity are key drivers of otter habitat distribution in the Padma River basin.

Variable Importance and Ecological Interpretation

The model output highlights the species' preference for areas with seasonally fluctuating but predictable precipitation patterns, dense vegetation, and specific land cover types such as riparian forests, marshes, and floodplains. These findings align with known ecological requirements of the species, which depends on structurally complex wetland habitats with abundant prey and shelter.

3.2.3 Threat Assessment

An integrative approach was employed to assess the threats to Smooth-coated Otters *(Lutrogale perspicillata)* in the Padma River basin by combining direct field evidence (sign surveys) with community knowledge (questionnaire surveys). This dual-method strategy helped triangulate findings and provided both spatially explicit and perception-based data to identify, categorize and prioritize key anthropogenic threats.

3.2.3.1 Sign Survey

To systematically document physical evidence of threats and otter activity, sign surveys were conducted along predetermined line transects placed in riparian zones, oxbow lakes, and wetland edges throughout the Padma River basin. Transects of 500–1000 meters in length were established in areas previously identified as potentially suitable habitats based on local reports and camera trap findings. The selection of transect locations was informed by accessibility, proximity to human activity, and diversity of habitat features.

During each survey, trained field personnel searched for and recorded otter signs including:

- Spraints
- Tracks or footprints
- Holts or dens
- Resting or grooming sites
- Scratch marks and slide marks on muddy banks
- Food remaining

In addition to otter-specific signs, observers documented visible threat indicators such as:

- Illegal fishing nets and traps
- Chemical pollution from the insecticides used in agriculture
- Sand and gravel extraction points
- Deforestation or vegetation clearance near shorelines
- Boat traffic indicators and bank erosion from motorized vessels

(a) (b) (c)

Figure 5: (a) Spraint (b) Foot Print (c) Food remaining of the species found during the sign survey.

Each sign or threat was georeferenced using a handheld Global Positioning System (GPS) unit to enable spatial overlay analysis with habitat variables in GIS. Observations were classified under broad threat categories following the IUCN-CMP Unified Classification of Direct Threats (Salafsky et al., 2008), including:

- Biological resource use (e.g., fishing, hunting, trapping)
- Pollution (e.g., plastic, sewage, agricultural runoff)
- Natural system modifications (e.g., sand mining, damming)
- Human intrusion and disturbance (e.g., boat traffic, tourism)

Where possible, threat locations were linked to proximate habitat features (e.g., vegetation type, bank structure, prey availability) and human land-use patterns, allowing the identification of spatial risk hotspots. A scoring system was developed to quantify the intensity of each disturbance. Each activity was assigned a score from 1 to 3 based on its perceived impact on otters and their habitats:

1 (Low): Minimal impact or localized threat.

2 (Moderate): Intermediate impact with broader implications.

3 (High): Severe and widespread impact.

The compiled data were analyzed to detect patterns and trends in the frequency, intensity, and spatial distribution of disturbances across the study area.

3.2.3.2 Questionnaire Survey (Threat Component)

To complement field-based threat assessments and incorporate the perspectives of local communities, the semi-structured questionnaire included a focused section on otter-related threats. Respondents comprising fishermen, boatmen, farmers, and other riparian residents were asked to identify and rank the primary threats facing otters in their locality. This section included questions on:

- Incidence and frequency of human-otter conflicts (e.g., otters damaging fish nets or preying on stocked fish)
- Perceived population trends and reasons for local decline (if any)
- Direct persecution (e.g., hunting, trapping, pet trade)
- Fishing practices (e.g., net type, fishing intensity, gear entanglement)
- River pollution sources (e.g., household waste, agricultural runoff, chemical effluents)
- Infrastructure impacts (e.g., bridges, sand mining, water extraction)

Figure 6: A sub-adult Smooth-coated Otter, killed by illegal fishing gear, was documented during the field survey.

Responses were analyzed using qualitative frequency analysis and ranking exercises. Threats were prioritized based on the proportion of respondents who cited each threat and the severity ascribed to it. This method allows for an evidence-based understanding of local perceptions and behaviors contributing to otter endangerment, while recognizing spatial variation across communities.

Ethical protocols were adhered to during interviews, including the use of verbal informed consent, assurance of respondent anonymity, and the voluntary nature of participation. This approach aligns with standard ethical guidelines in ethnobiological and conservation social science research (Newing et al., 2011).

3.3 Data Analysis

To derive ecologically meaningful insights from multi-source data, a comprehensive analytical framework was employed integrating geospatial, statistical, and modeling approaches. Spatial data from camera traps and field surveys will be georeferenced and analyzed using GIS tools to map species occurrence and MaxEnt (v3.4.4) for habitat suitability modeling. Habitat suitability of the species will be identified also by analyzing the sign survey data as well as the questionnaire survey data. To predict potential distribution zones of the species will be identified by correlating occurrence data with key environmental variables (e.g., vegetation cover, water availability, human disturbance, and prey abundance).

Simultaneously, qualitative and quantitative data from local community interviews and structured questionnaires will be analyzed to assess anthropogenic pressures, local attitudes, and perceived threats to otter populations. Descriptive statistics, cross-tabulations, and thematic content analysis will help to uncover patterns in perception and potential socio-ecological drivers influencing otter conservation in the region. Integrating ecological field data with social perception surveys provides a holistic lens to evaluate the conservation landscape, allowing for the identification of high-risk zones, conservation hotspots, and areas requiring community-based intervention. This multifaceted data analysis approach is critical for generating evidence-based recommendations for species-specific conservation action planning and policymaking.

3.3.1 Relative Abundance and Species Density Estimation

Data from camera trap deployments were used to calculate the Relative Abundance Index (RAI), Otter Detection Rate (ODR), and estimated population density of *Lutrogale perspicillata*. These metrics provided quantitative baselines for assessing otter occurrence and spatial activity.

RAI was computed as:

- RAI = (Number of Independent Detections/ Total trap Nights) X 100

ODR was calculated to assess camera-based detection success:

- ODR (%) = (Number of Camera Traps with Otter Detection/ Total Number of Camera Traps Deployed) X 100

3.3.2 Habitat Suitability Modeling (MaxEnt)

Habitat modeling was performed using the MaxEnt algorithm with the following settings:

- Bootstrap method with 100 replicates
- 75/25% partitioning for training and testing data
- Regularization multiplier set at 1.0
- 500 maximum iterations
- 10,000 background points

A total of 23 confirmed otter presence records were input alongside 11 ecologically relevant variables, pre-selected based on correlation analysis ($|r|<0.7$). Variables included NDVI, NDWI, precipitation seasonality, elevation, slope, and land cover. Model performance was evaluated using the Area Under the Curve (AUC) of the Receiver Operating Characteristic (ROC). The model yielded an AUC > 0.9, signifying excellent discriminatory capacity between suitable and unsuitable habitats. Jackknife tests and percent contribution metrics revealed precipitation seasonality (23.9%), land cover (20.7%), and NDVI (14.9%) as the most influential predictors.

Threshold-based binary maps (using the 10th percentile training presence threshold) were generated to delineate core suitable zones, which were later overlaid with threat maps and land-use features to identify critical conservation areas.

3.3.3 Habitat Use and Preference Analysis

Data obtained from camera trap photographs and otter signs have provided valuable insights into the habitat preferences of smooth-coated otters. Analysis of these records reveals that otters predominantly inhabit small canals, ditches, and other water bodies with dense vegetation cover, while showing a marked avoidance of the main river channel.

This preference for smaller, vegetated waterways is likely attributable to the availability of shelter, reduced human disturbance, and higher prey density in these microhabitats. These findings underscore the importance of conserving and restoring small-scale aquatic ecosystems, which are often overlooked in large-scale conservation efforts. Furthermore, understanding otter habitat preferences allows conservationists to focus habitat protection initiatives in areas that are most likely to support sustainable otter populations.

Figure 7: Suitable habitat of the species identified during the field survey.

3.3.4 Threat Analysis

To analyze anthropogenic disturbances affecting *Lutrogale perspicillata* in the Padma River Basin, data were collected through a triangulated approach combining semi-structured interviews, boat-based field observations and sign surveys. This method follows established practices for participatory conservation research and threat assessment in aquatic and carnivore studies (White et al., 2005; Salafsky et al., 2008).

Each reported or observed threat was geo-referenced using handheld GPS devices and classified into major categories, including:

- Illegal or harmful fishing practices (e.g., gillnets, electrofishing)
- Habitat degradation (e.g., riverbank encroachment, sand mining)
- Water pollution (e.g., pesticide runoff, sewage discharge)
- Navigation-related disturbances (e.g., oil spills, engine noise)

To quantify the severity of each threat, a scoring system (1 = low, 2 = moderate, 3 = high) was applied based on the perceived ecological impact, consistent with methods used in threat ranking for small carnivores and aquatic mammals (Khan et al., 2014; de Silva et al., 2015).

In parallel, ranking analysis and thematic coding of open-ended responses provided insight into community-level perceptions and spatial variation in threat intensity. High-impact threats identified included gillnet entanglement, agricultural runoff and sand mining, all of which are known to reduce prey abundance, degrade otter habitat and increase mortality risk (Hussain & Choudhury, 1997).

CHAPTER IV

RESULT AND DISCUSSION

4.1 Population Abundance and Spatial Distribution

4.1.1 Relative Abundance Index (RAI)

Otter abundance was assessed using the Relative Abundance Index (RAI), which is commonly used to represent the number of independent detections per 100 camera trap nights (O'Brien et al., 2003; Rovero & Zimmermann, 2016). A total of 56 independent smooth-coated otter detections were recorded over 250 trap nights, yielding an RAI of 22.4 detections/100 trap nights. This RAI suggests moderate otter activity across the Padma River basin.

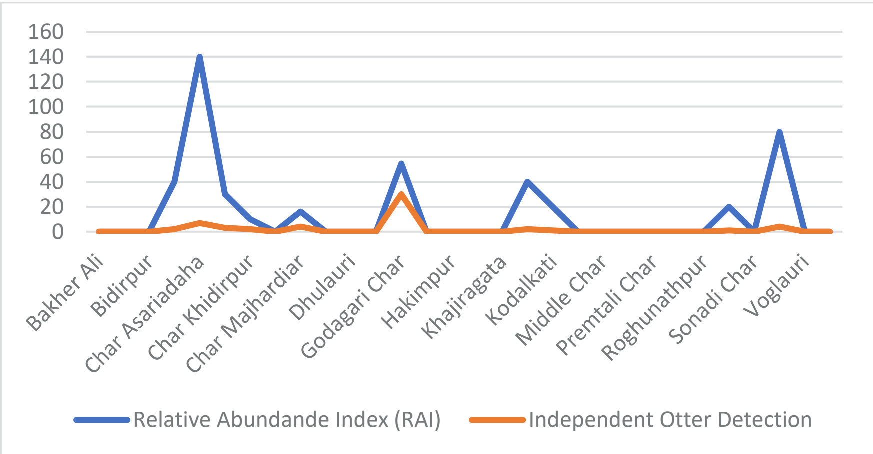


Figure 8: Relative Abundance Index (RAI) vs. Detection frequency of otters in different locations.

While RAI does not provide true population density, it serves as a comparative indicator of species abundance, especially when standardized survey efforts are applied (Carbone et al., 2006). High RAI values suggest areas of frequent otter activity, which may indicate higher habitat suitability or lower disturbance levels.

4.1.2 Otter Detection Rate (ODR)

Otter Detection Rate (ODR), calculated as the percentage of camera trap stations with detections, was 9.2%. This relatively low detection rate reflects a patchy distribution pattern across the landscape, potentially shaped by variable habitat conditions and human pressures.

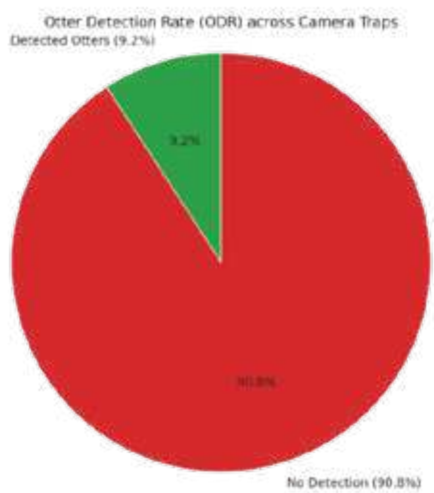


Figure 9: Otter detection rate across the camera traps

4.1.3 Spatial Patterns of Otter Activity

Distinct variation in detection frequency was observed among all the camera trap locations. Godagari Char recorded the highest detection (30 detections over 55 trap nights), followed by Char Asariadaha (7 detections over 5 nights). Several locations such as Bakher Ali, Bhubonpara, and Char Jhawbbona yielded no detections despite camera deployments.

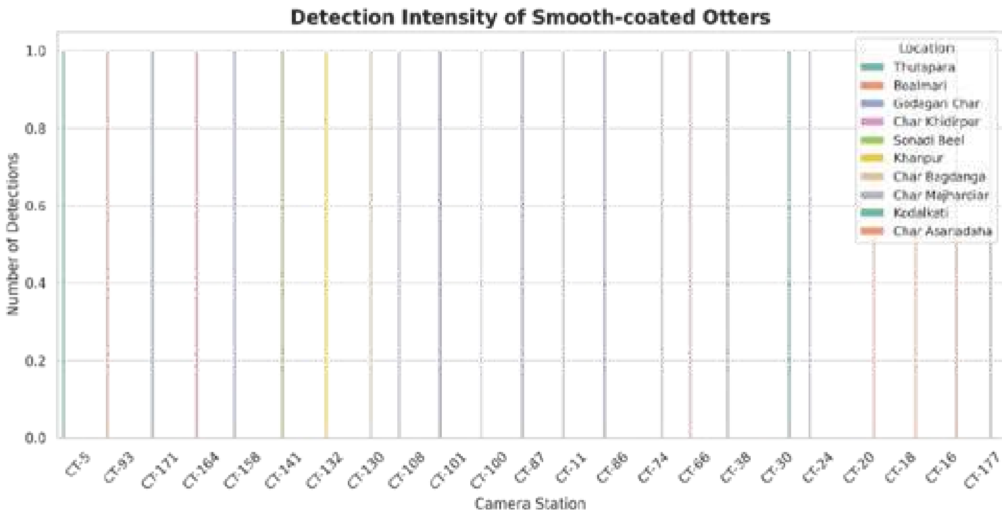


Figure 10: Detection intensity of the species in different location.

The uneven distribution of detections likely reflects habitat heterogeneity and anthropogenic influences. High-detection areas are characterized by intact riverine vegetation, minimal disturbance, and abundant prey resources. In contrast, sites with no detections may be affected by intensive fishing, riverbank modification, or boat traffic factors known to reduce otter occupancy (Hussain & Choudhury, 1997; Aadrean et al., 2014).

4.1.4 Comparison with Similar Studies

The RAI obtained in this study (22.4 detections/100 trap nights) is relatively high compared to similar research on smooth-coated otters. For instance, Nawab & Hussain (2012) reported an RAI of 12.5 in the Upper Ganges Basin, and Aadrean et. Al., (2010) documented in west Sumatra.

Such differences can be attributed to variations in river system dynamics, prey abundance and anthropogenic pressures. The Padma River basin, despite localized threats, appears to provide favorable habitat conditions for otters in certain zones, reinforcing its importance as a conservation priority area.

4.2 Habitat Suitability Assessment

4.2.1 Habitat suitability map for the smooth-coated (Maxent Result)

The resulting habitat suitability map (presented in Appendix- 2) (ensemble average of all model runs) displays predicted environmental suitability for the target species on a continuous scale from 0 to 1, where values represent the relative probability of suitable conditions. Grid cells approaching 1 indicate optimal habitat conditions, while values near 0 suggest unsuitable environments. For visual interpretation, the color gradient employs warm hues to denote areas of high suitability, contrasting with cool tones representing less favorable habitat conditions.

The current results of the maximum entropy modeling revealed distinct habitat preferences for the species. Among the assessed habitat types, "other waterbodies" exhibited the highest proportion of suitable habitat, with over 50% of these areas scoring a habitat suitability value of ≥ 0.5. Herb-dominated areas also showed considerable suitability, with 25.45% of their extent meeting the same threshold (suitability score ≥ 0.5). Sandy habitats followed, with 11.72% of their area classified as highly suitable (≥ 0.5). In contrast, croplands, rivers and khals, settlements, and tree-covered areas had lower proportions of suitable habitat, at 4.29%, 3.46%, 0.95%, and 0.82%, respectively.

However, when considering the total surface area of highly suitable habitat (suitability score ≥ 0.5), herb-dominated areas covered the largest extent (9,522,900 m²), despite not having the highest proportional suitability. Croplands ranked second in total suitable area (7,057,800 m²), followed by rivers and khals (6,259,500 m²) and sandy areas (6,040,800 m²). Other waterbodies, settlements and tree-covered areas contributed the least, with only 256,500 m², 237,600 m² and 61,200 m² of suitable habitat, respectively. These findings probably suggest that while certain habitat types (e.g., "other waterbodies") are more likely to support smooth-coated otters in high densities, others (e.g., "croplands" and "rivers and khals") may still provide substantial habitat due to their larger overall coverage in the landscape.

Suitable habitats for the species appear to be primarily concentrated in the central zone which is the "Godagari" area, particularly in a region that is almost entirely surrounded by water (Appendix 5). Satellite imagery (e.g., December 2016 and January 2017) suggests that this area periodically becomes an island, fully encircled by water during certain seasons, and later reconnects to the surrounding plains as water levels recede. This special location may help explain why the area functions as a habitat suitability hotspot. Its periodic isolation likely reduces disturbance and may provide a more secure environment for the smooth-coated otter. Additionally, the transition between aquatic and terrestrial zones may create an ecologically favorable environment, providing both refuge and foraging opportunities for the smooth-coated otter.

4.2.1.1 Interpretation of the obtained AUC value

The Maxent model for the smooth-coated otter achieved an AUC (Area Under the Curve) value of 0.926 with a standard deviation of 0.027 (Figure 3). The AUC is a measure of the model's ability to discriminate between suitable and unsuitable habitats. An AUC of 0.926 indicates excellent model performance, suggesting that the model reliably distinguishes areas where Lutrogale perspicillata is likely to occur from those where it is unlikely. The relatively low standard deviation (0.027) implies that the model's performance is consistent and stable across cross-validation or replicate runs.

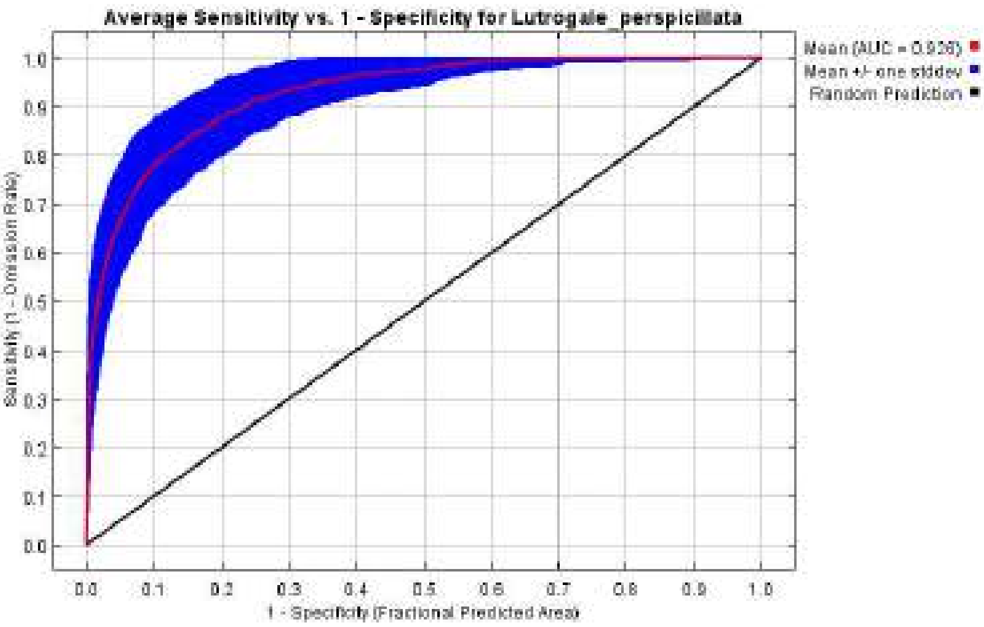


Figure 11. Predictive accuracy of smooth-coated otter habitat model (AUC = 0.926)

4.2.2 Habitat Suitability Classification based on respondent perceptions

Using responses on habitat encounter, preference, group size, and sightings, the suitability index was developed:

Table 2: Habitat suitability based on respondent perceptions.

Habitat Type	Suitability	Justification
Forest/Reedland/Bushland	High	Most frequently preferred habitat (189), supports group living, low disturbance
River branches/Canals/Ditches	High	High encounter rate (114), moderate human use, good for foraging
Char lands	Moderate	Used regularly (109), some disturbance from agriculture or grazing
Mainstream river	Low	Few encounters (55), high boat activity and sand mining
Agricultural lands	Unsuitable	Least encountered and least preferred habitat

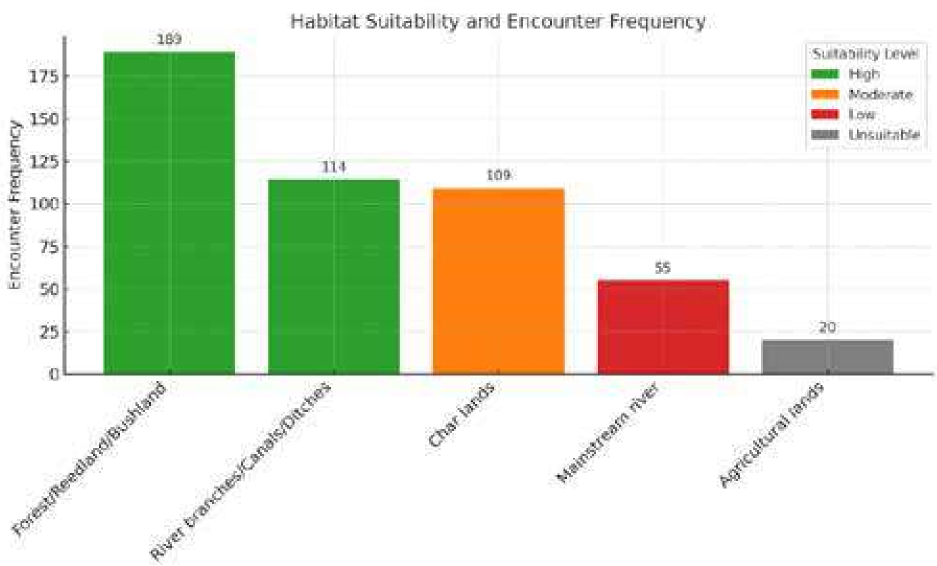


Figure 12: Habitat Suitability and Otter Encounter Frequency from Survey Responses

The habitat suitability for smooth-coated otters was assessed based on survey responses regarding sighting frequency, habitat preference, group behavior, and human disturbance. Forests, reed lands, and bushlands were classified as highly suitable, with 189 respondents identifying them as preferred habitats. These areas support group living and offer good cover and food availability with minimal disturbance, making them ideal for otters. River branches, canals, and ditches also received a high suitability rating. Reported by 114 respondents, these areas provide feeding opportunities and are moderately disturbed, allowing otters to use them regularly. Char lands were considered moderately suitable. Though frequently used (109 responses), they face seasonal disturbance from farming and grazing, which may limit their long-term suitability. Mainstream rivers were rated as low suitability due to fewer sightings (55 responses) and higher disturbance from boats and sand mining activities. Agricultural lands were marked as unsuitable, with very few sightings and low preference. These areas are highly disturbed and lack the necessary features (like cover or prey) that otters need.

4.2.3 Habitat Use and Preferences based on respondent perceptions

Respondents reported last observing otters in a variety of habitats. The highest number of sightings occurred in riverine offshoots such as canals, ditches, and small channels (n = 114; 28.5%), followed closely by char lands (seasonally exposed river islands) (n = 109; 27.3%), and natural vegetated areas like forests, grasslands, and reedlands (n = 93; 23.3%). Observations in the main river channel were lower (n = 55; 13.8%), with the least number recorded from agricultural lands (n = 29; 7.3%). When asked about perceived habitat preferences of otters, nearly half the respondents (n = 189; 47.3%) identified forested or reedy areas as most suitable, suggesting a strong association with semi-natural riparian zones. Other perceived preferences included the availability of fish (n = 90; 22.5%), presence of canals and ditches (n = 83; 20.8%), and to a lesser extent, char lands (n = 20; 5%), river mainstream (n = 11; 2.8%), and agricultural land (n = 6; 1.5%).

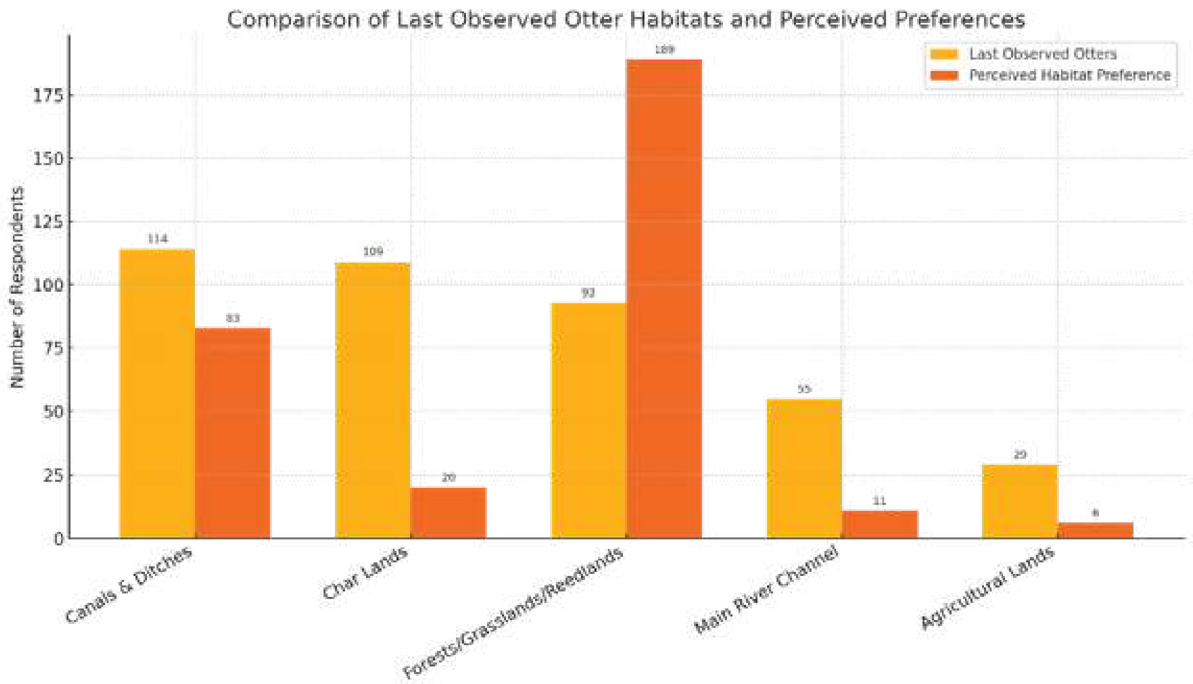


Figure 13: Relationship between last observed otter habitats and perceived habitat preferences.

These findings indicate that smooth-coated otters (*Lutrogale perspicillata*) in the Padma River basin exhibit clear selectivity for structurally complex riparian habitats with high prey availability and relatively low levels of disturbance. This aligns with previous research documenting the species' affinity for vegetated banks, wetlands, and backwater systems (Hussain et al., 2011; de Silva et al., 2015), which provide optimal conditions for foraging and denning.

4.2.3.1 Temporal Patterns of Sightings

The majority of otter sightings were reported during crepuscular periods early morning (n = 170; 42.5%) and evening (n = 135; 33.8%)—with a considerable number also reported at night (n = 94; 23.5%). Daytime sightings were almost negligible (n = 1; 0.3%), suggesting pronounced temporal avoidance of periods with peak human activity.

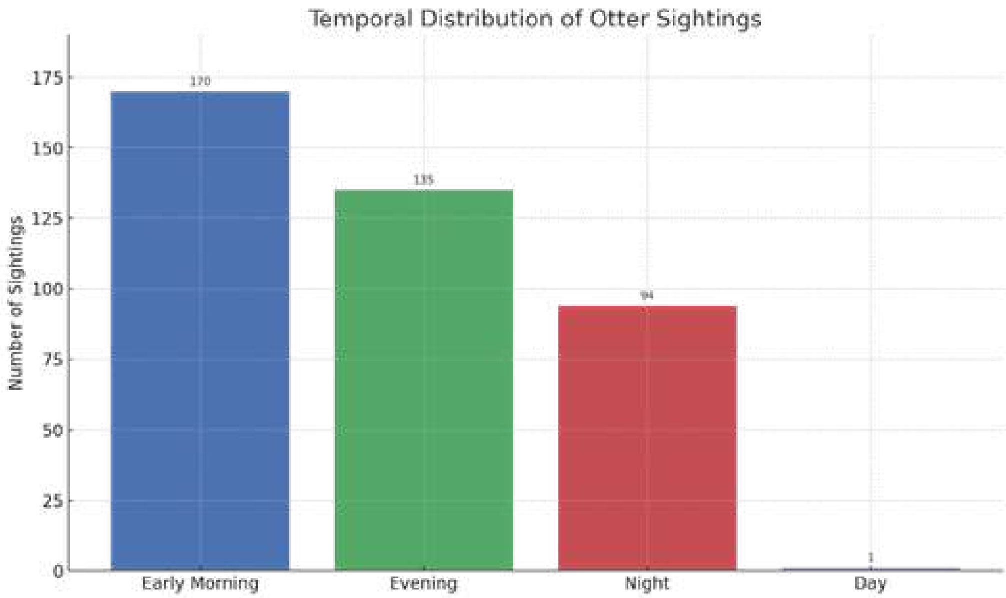


Figure 14: Temporal distribution of the otter sightings.

This crepuscular-to-nocturnal activity pattern likely represents an adaptive response to anthropogenic pressures, consistent with behavioral plasticity observed in other carnivore species inhabiting human-dominated landscapes (Gaynor et al., 2018). It also supports earlier observations of smooth-coated otters exhibiting temporal shifts to avoid direct encounters with humans while maximizing foraging efficiency during twilight hours (Anoop & Hussain, 2004).

4.2.3.2 Seasonality in Otter Presence

A marked seasonality in otter sightings was observed, with the monsoon period (June–September) accounting for the overwhelming majority of observations (n = 366; 91.5%). This period coincides with high river discharge, expanded floodplains, and elevated fish abundance due to breeding cycles of several fish species.

Otters are found more often in certain seasons, mainly because water levels change throughout the year. During the monsoon, more food is available and fewer people are around, making it easier for otters to find food and move around. The flooding also helps connect different areas, allowing otters to travel between habitats that are usually separated (Roos et al., 2015).

4.2.3.3 Group Size and Sociality

Group size observations further corroborate the social behavior of the species in this landscape. Most respondents (n = 282; 70.5%) reported sightings of groups containing 5–8 individuals, with the largest recorded group comprising 15 individuals. The prevalence of medium to large-sized groups suggests potential breeding aggregations or family units, particularly in optimal habitats during resource-abundant periods.

Smooth-coated otters are social animals, usually living in family groups that include a breeding pair and their young (Duplaix et al., 2015). The tendency to form larger groups in the Padma basin may indicate relatively intact social structures and suitable habitat patches capable of supporting extended family units.

4.2.3.4 Diet and Feeding Preferences

The otters' diet, as perceived by respondents, consisted predominantly of fish, with 81.3% indicating that otters consume all types of available fish. A slight preference for catfish over carps was reported, potentially reflecting prey availability or catchability in shallow water bodies. Additionally, 48 respondents (12%) noted that otters occasionally consumed other prey items such as crustaceans or amphibians. However, a substantial proportion

(35.5%) were uncertain about diet specifics, highlighting a need for more empirical dietary studies through scat or stable isotope analysis.

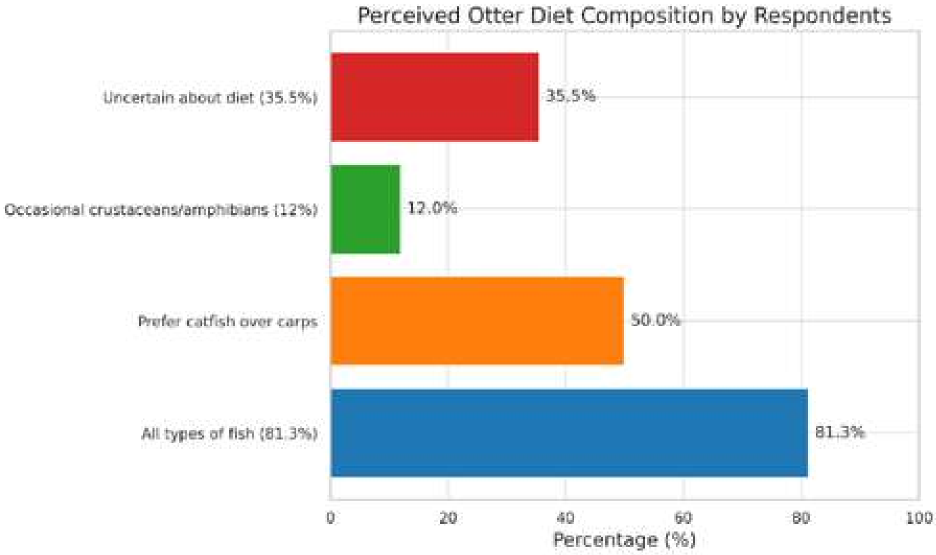


Figure 15: Community Perceptions of Otter Diet Composition

The wide variety of fish in the otters' diet supports their role as top predators in tropical freshwater systems. They may also eat other animals, like crustaceans or amphibians, especially when fish are harder to find or in poor-quality habitats (Prakash et al., 2012). This highlights the smooth-coated otter's importance in showing the health of rivers and the need to protect areas with plenty of prey.

4.3 Threat Assessment and Impact Scoring for Smooth-coated Otters

Based on perceived intensity and frequency, threats were scored as:

Table 1: Perceived Threats and Impact Scores for Smooth-coated Otters in the Padma River Basin.

Threats	Respondents (n)	Impact Score (Intensity)	Justification
Overfishing/ conflict with fishers	146	3	Most frequently cited threat; linked to direct human-wildlife conflict and prey depletion or food scarcity
Reducing forest or bushland or reed land	123	3	Reported as a major driver of habitat degradation and fragmentation
Agricultural expansion	119	2	Perceived as a significant cause of encroachment into riparian otter habitats.
Water pollution	6	1	Recognized as a minor but perceived as minor locally.
Sand mining	6	1	Reported less frequently, but has notable indirect impacts on riverine habitat.
Hunting	Not reported (assumed threat)	1	Not directly reported, but presumed present in other parts of the region.
Feral dogs or predators	Not reported	0	Not considered a relevant or observed threat in the surveyed areas.

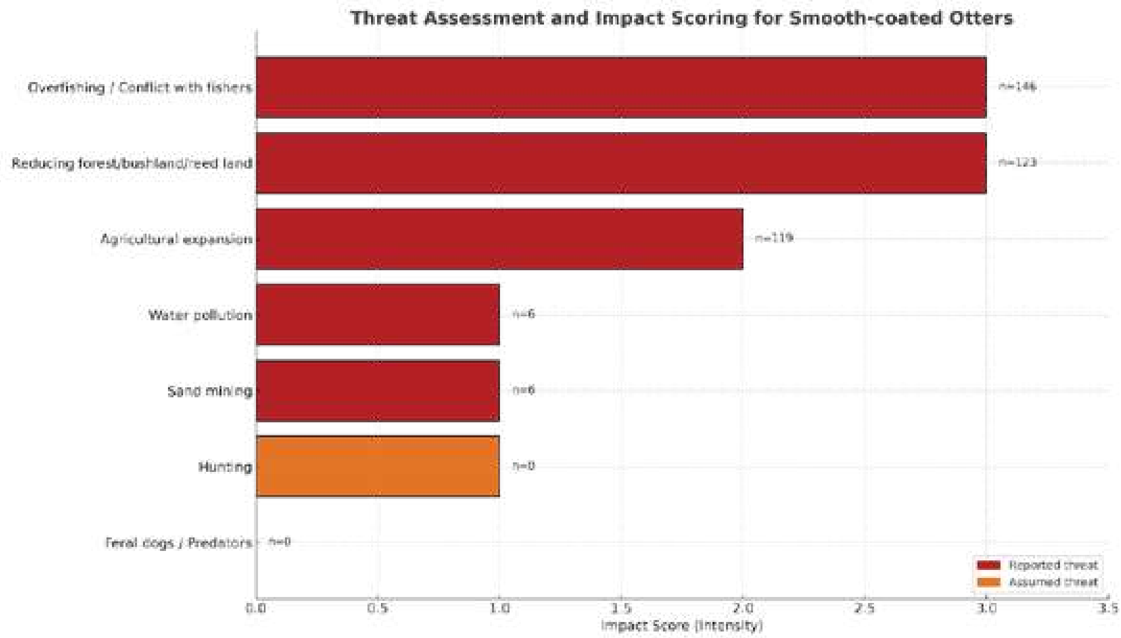


Figure 16: Perceived threats to Otter's habitat: Intensity vs Respondents frequency.

The threat assessment, derived from a structured questionnaire survey, reveals that overfishing and conflicts with fishers constitute the most critical threat to smooth-coated otters, as identified by 146 respondents. This reflects significant anthropogenic pressure on aquatic food resources and direct interactions with human livelihood practices. Habitat degradation, primarily through forest/bushland reduction (123 respondents) and agricultural expansion (119 respondents), emerged as prominent threats, reflecting the broader landscape transformation in the otters' habitat range. While water pollution and sand mining were cited by fewer respondents (n=6 each), their cumulative impacts, particularly on water quality and riverbank integrity cannot be overlooked. Interestingly, hunting was not reported in the survey, but it has been assigned an impact score due to its potential relevance in less-monitored regions of the study area. Lastly, threats from feral dogs or predators were not perceived to be of concern by any respondents, suggesting minimal interspecies conflict in this context.

4.3.1 Perceived Population Trend and Drivers of Change

Perceptions of local communities regarding the population trends of smooth-coated otters in the Padma River basin reveal significant concern over a possible decline. More than half of the respondents (n = 218; 54.5%) believed that otter populations are decreasing, whereas only a small minority perceived them as stable (n = 3; 0.75%). Interestingly, 65 individuals (16.3%) reported an increase in sightings over recent years, while a considerable proportion (n = 114; 28.5%) expressed uncertainty about the trend, highlighting gaps in awareness and the need for enhanced ecological literacy in the region.

Among those reporting a decline, the most frequently cited driver was habitat loss (n = 77), followed by overfishing (n = 53), agricultural expansion (n = 47), and general anthropogenic disturbances (n = 35). These factors reflect the intensifying human footprint across the river basin, leading to the degradation and fragmentation of otter habitats. The conversion of riparian vegetation to farmland, sand mining, bank erosion, and the encroachment of settlements are likely contributing to the loss of essential denning and foraging sites. Furthermore, overexploitation of fish stocks directly threatens the otters' primary food source, potentially leading to nutritional stress and reduced reproductive success. Agricultural encroachment often accompanied by pesticide runoff and alteration of natural water flow further diminishes habitat quality. Concurrently, increasing boat traffic, sand dredging, and unregulated tourism disturb core activity periods and spaces, forcing otters into marginal habitats with lower prey availability and greater exposure to human conflict. These community-derived insights mirror ecological findings from other regions in South and Southeast Asia, where anthropogenic pressures have led to significant population contractions in smooth-coated otters (de Silva et al., 2015; Nawab & Hussain, 2012).

Conversely, the 65 respondents who perceived a population increase attributed this trend primarily to the abundance of food (n = 39) and high reproductive rates (n = 17). These positive observations were often linked to areas where otters are known to exploit fish-rich water bodies with reduced human activity or where community-based conservation initiatives may have indirectly supported habitat and prey base recovery. The perception of local resurgence in such pockets may also reflect temporal fluctuations in habitat suitability driven by seasonal flooding and fish migration patterns, rather than long-term population growth.

Overall, the dominant perception of a declining trend, coupled with the identification of key anthropogenic threats, reinforces the urgency for targeted conservation action. Integrating local ecological knowledge with systematic population monitoring could enhance the effectiveness of otter conservation strategies in the Padma River basin.

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

The smooth-coated otter *(Lutrogale perspicillata)* in the Padma River basin is subject to a complex interplay of ecological dynamics and anthropogenic pressures that critically influence its population status and long-term viability. This study estimates the otter population to range between 519 and 1,037 individuals, with a Relative Abundance Index (RAI) of 22.4 detections per 100 trap nights, indicating a moderate but ecologically significant presence across the landscape.

Spatial analysis reveals a highly uneven distribution of otters, with areas such as Godagari Char and Char Asariadaha demonstrating higher detection rates, likely due to the persistence of natural vegetation, such as forest patches and dense bush cover, and comparatively lower human disturbance. In contrast, many char lands are extensively modified for agriculture and livestock grazing, contributing to habitat fragmentation and reduced otter presence.

Habitat preference data, combined with community insights, indicate that smooth-coated otters predominantly inhabit semi-natural riparian zones, including forested riverbanks, reed beds, and canal systems. However, these habitats are increasingly compromised by human activities, particularly overfishing, agricultural encroachment, and riparian habitat degradation, which collectively pose the most significant threats to otter populations.

Temporal and seasonal activity patterns underscore the importance of the monsoon season, during which enhanced food availability and increased hydrological connectivity appear to facilitate otter movement and habitat use. Local ecological knowledge further suggests that during the dry season, otters may migrate across the international border in search of more favorable conditions, including reduced anthropogenic pressure and greater prey abundance.

In conclusion, while the Padma River basin currently supports a moderately sized population of smooth-coated otters, their long-term persistence is jeopardized by habitat loss, resource competition, and escalating human-wildlife conflict. These findings highlight the urgent need for integrated conservation strategies, including habitat restoration, sustainable resource management, and transboundary cooperation, to ensure the survival of this vulnerable species in a rapidly changing landscape.

5.2 Conservation and Management Recommendations

The findings of this study emphasize the importance of evidence-based conservation planning for smooth-coated otters in the Padma River basin. Given their role as apex predators in freshwater ecosystems, otters contribute significantly to maintaining ecological balance. However, threats such as habitat destruction, water pollution, and anthropogenic disturbances pose significant challenges to their survival. To address these concerns, a multifaceted conservation approach is essential. Key recommendations include:

Identification of Key Habitats

The findings of this study identify specific zones within the Padma River basin that constitute key habitats for the smooth-coated otter *(Lutrogale perspicillata)*, particularly areas such as some areas in Godagari Char, Char Asariadaha, Char Majharrdia, Char Khanpur and Khidirpur where detection rates were consistently higher. Given the extensive anthropogenic pressure on riverine ecosystems stemming from overfishing, agricultural encroachment, sand mining and settlement expansion, it is neither feasible nor practical to enforce blanket protection across the entire basin.

Therefore, a targeted conservation approach is imperative. Areas identified as critical habitats, based on empirical data on otter presence, habitat quality, and anthropogenic threat levels, should be demarcated as high-priority conservation zones. These zones can serve as core habitat areas within a broader landscape-level conservation framework. The establishment of ecologically representative protected enclaves, potentially linked through riparian habitat corridors, would facilitate population connectivity, reduce local extinction risk, and enhance the species' resilience to environmental disturbances.

Habitat Protection and Restoration:

Priority should be given to the protection and restoration of key habitats such as forested reed lands, bushlands, and canals. These areas support the highest otter densities and are crucial for foraging, breeding, and sheltering. Restoration efforts should focus on reducing anthropogenic disturbances, such as agricultural encroachment, sand mining, and unregulated fishing practices, particularly in high-density zones.

Sustainable Fisheries Management:

Overfishing and human-otter conflict are critical threats to otter populations. Fisheries management strategies should be implemented to regulate fishing practices, particularly in otter-rich or key habitat areas. Use of illegal fishing gear such as fine meshed gill net, china duari etc., should be strictly controlled. Community-based management programs that involve local stakeholders, especially fishers, could help mitigate conflicts and ensure the sustainable use of fish stocks, which are essential for otter survival.

Regulation of Agricultural Expansion in Riverine and Char Landscapes

The unregulated expansion of agriculture in riverine and char areas within the Padma River basin poses a significant threat to the habitat integrity of the smooth-coated otter *(Lutrogale perspicillata)*. Conversion of riparian vegetation and wetlands into farmland not only leads to the loss of critical denning and foraging habitats, but also disrupts the natural hydrological processes essential for maintaining ecosystem connectivity. Intensive agricultural practicesoften accompanied by pesticide use, soil compaction, and livestock grazing further degrade the habitat quality and reduce prey availability for otters. As part of a conservation strategy, land-use zoning regulations should be introduced to restrict agricultural encroachment into ecologically sensitive areas, especially within identified otter habitats. Incentivizing sustainable agroecological practices in buffer zones and promoting community-led land stewardship can help balance local livelihoods with species conservation. Integrating these measures into watershed-level planning will be essential to mitigate habitat loss and ensure the long-term viability of otter populations in the basin.

Control Multiple Anthropogenic Pressures on Otter Habitats

The smooth-coated otter *(Lutrogale perspicillata)* in the Padma River basin is increasingly threatened by a convergence of anthropogenic pressures that degrade its natural habitat and disrupt its ecological behavior. Among the most critical threats are overfishing, which depletes the otter's primary food sources and leads to increased competition with humans, and habitat fragmentation, driven by sand mining, settlement expansion, and infrastructure development along riverbanks. Agricultural encroachment, particularly on char lands and riparian zones, further contributes to habitat loss through land conversion, pesticide runoff, and disturbance from livestock grazing. Unregulated boat traffic and the proliferation of illegal fishing gear such as fine-meshed gill nets, china duari etc., also pose a significant risk of accidental entanglement or displacement. Additionally, pollution from domestic waste, agricultural runoff, and industrial discharge leads to water quality degradation, undermining the health of aquatic ecosystems vital to otter survival. These pressures are compounded by a general lack of awareness, enforcement gaps, and limited conservation infrastructure in rural areas. Addressing this complex matrix of human-induced threats requires a holistic, multi-sectoral conservation approach, integrating sustainable development, habitat protection, and strong community engagement.

Monitoring and Research:

Ongoing monitoring using camera traps, field surveys, and GIS mapping is crucial for tracking otter population trends, habitat use, and the impact of conservation measures. Further research into otter diet and feeding behavior, including the analysis of spraints could provide more detailed insights into their ecological needs.

Promoting Community Awareness and Involvement:

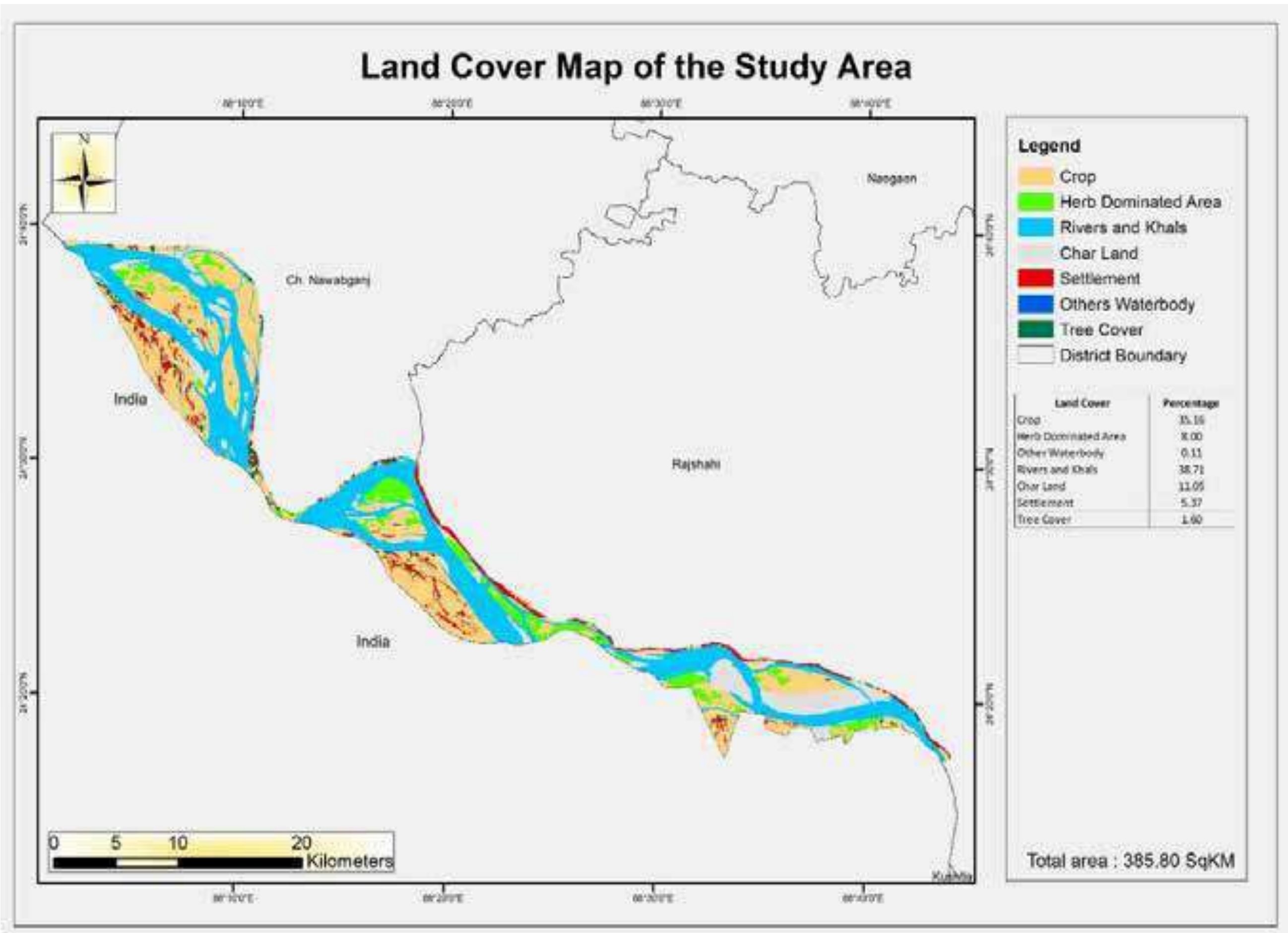
Educating local communities about the ecological role of otters and the benefits of conserving riverine habitats is essential for reducing anthropogenic pressures. Community-based conservation initiatives, such as promoting sustainable land-use practices and reducing conflicts, could contribute significantly to otter conservation.

Encouraging Eco-friendly Tourism:

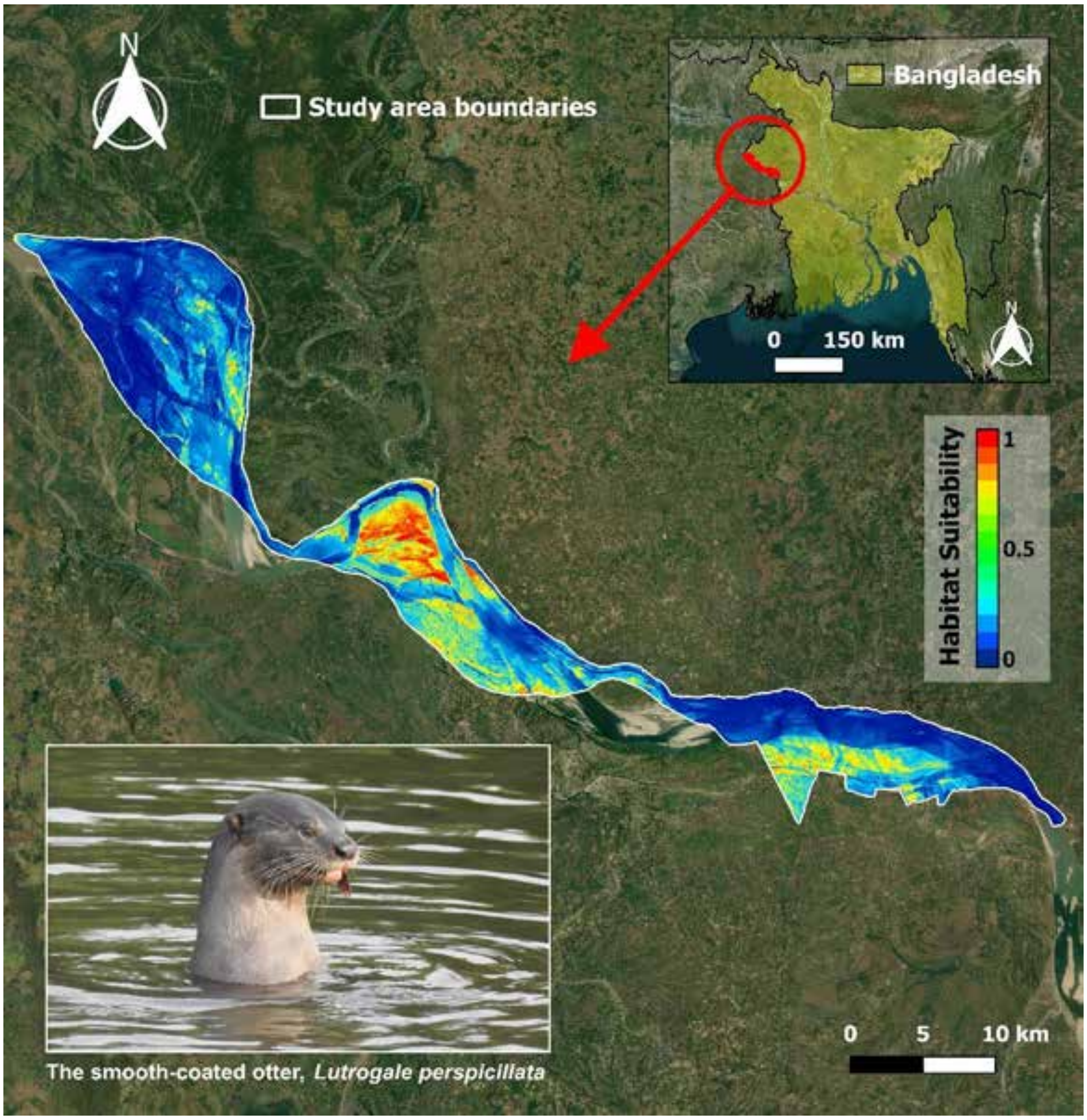
Promoting eco-tourism in the Padma River basin could raise awareness and generate funds for conservation efforts. Tourism should be carefully managed to avoid habitat disturbance, particularly in areas identified as critical for otter survival.

In conclusion, addressing the challenges faced by smooth-coated otters in the Padma River basin requires an integrated approach combining habitat restoration, sustainable resource management, community engagement, and robust monitoring. By implementing these recommendations, it is possible to enhance the conservation status of this vulnerable species and secure its future in one of South Asia's most vital riparian ecosystems.

Appendix 1: Land Use Map of the Study Area



Appendix 2: Habitat Suitability Map for the Smooth-coated Otter



The smooth-coated otter, *Lutrogale perspicillata*

Appendix 4: Camera Trap Photographs of Smooth-coated Otters


22 °C / 71 °F
04/25/2023 22:33:24


26 °C / 78 °F
23/09/2023 05:47:22


27 °C / 80 °F
23/09/2023 05:47:39


28 °C / 82 °F
23/09/2023 05:43:15


24 °C / 75 °F
20/10/2023 22:54:27


22 °C / 71 °F
22/10/2023 22:37:11


GardePro
24 °C / 75 °F
17/11/2023 17:49:39


GardePro
16 °C / 60 °F
17/02/2024 02:15:19


GardePro
33 °C / 91 °F
08/03/2024 05:49:17

Appendix 5: Photographs of Smooth-coated Otter Footprints




Appendix 6: Photographs of Smooth-coated Otter Spraints

Detection & Management of Tuberculosis (TB) at Captive-Wild Interface in Bangladesh

by

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CHAPTER-I: INTRODUCTION

1.1 Problem Statement

Globally, Tuberculosis (TB) is one of the most widespread zoonotic diseases and one of the leading causes of human and animal death. It is estimated that a total of 1.5 million people died from TB across the world in 2018 (Organization, 2019). Bangladesh is one of the high burden countries among the eight listed countries that accounted for two thirds of the total TB cases worldwide and ranked seventh in terms of global occurrence of TB (Organization, 2019).In animal, TB is widespread in domestic animals and has been widely documented in both captive and free ranging wildlife including non-human primates, elephants and other exotic ungulates, carnivores, marine mammals and psittacine birds in several countries in the world (Malama et al., 2013; Thapa et al., 2017). However, ecologic, environmental, or demographic factors including human–wildlife conflict contribute increased contact between animal & human resulting emergence of zoonotic diseases (Anwar et al., 2015). Moreover, South Asia is considered as a high-risk region for emerging infectious diseases that could originate in wildlife and creating a negative impact directly or indirectly on biodiversity conservation and public health (Thapa et al., 2017; Islam and Ahmed, 2019). In South Asia TB covers 34% of the global TB burden with 3 million of infection and a mortality of 0.4 million people in 2013(Chaudhary et al.). However, TB is a neglected disease both in animal and human health research with few confirmatory reports of zoonotic TB caused by *M. bovis* in the region (Thapa et al., 2017). In addition, the best of our knowledge, there are no reports of confirmed diagnosis of *M. bovis* infection in wildlife species found in the region including Bangladesh.

Tuberculosis is an emerging disease of free-ranging wildlife and posing continuous threat for survival of several captive wildlife species across the world. Recently, a novel *Mycobacterium tuberculosis complex (MTC)* has been detected from a wild chimpanzee in the Ivory Coast (Coscolla et al., 2013) and *M. orygis* to free-ranging rhinoceroses in Nepal (Thapa et al., 2017). Most importantly, TB is increasingly being identified in captive and free ranging wildlife population in South Asia and therefore wildlife TB is becoming one of the emerging challenges for conservation in this region (Thapa et al., 2017). It is also notified as serious zoonotic disease in Asian elephants as11- 25% of tested elephants in India and Nepal were positive for TB (Mikota and Maslow, 2011; Murphree et al., 2011) and it increases the risk to transmission of TB between wildlife population of Bangladesh because of having wildlife corridors with India. Recently TB infection (*M. bovis* and *M. tuberculosis*) have been detected in Spotted Deer (*Axix axix*), Gaur (*Bos gaurus*), Giraffe (*Cervus Camelopardalis*) and Common Eland (*Taurotragus oryx*) at Gazipur Safari Park and Bhawal National Park, Gazipur which is very alarming for wildlife conservation in this area (Chowdhury M. N. U, 2019; S. M. Shariul Hoque Belal, 2019). Moreover, It is speculated that transmission of TB infection between Rhesus monkey and cattle has been reported in Bangladesh (Rahim et al., 2017). Although TB has been reported both wild & captive wildlife population in India, Nepal and Sri Lanka. But TB situation is not yet accessed in Bangladesh (Verma-Kumar et al., 2012; Zachariah et al., 2017).

Bangladesh is a country of rich biodiversity and is a home of roughly 53 species of amphibian, 19 species of marine reptiles, 139 species of reptile, 628 species of birds (Singh, 2003). At present across the country around 10,000 wild animals of native and exotic species kept in captive management at safari parks, eco-parks, national parks and aviary of Forest Department for conservation & ecotourism. From official record, it is observed that a significant number of wild animals (i.e.320 birds, 81 mammals and 18 reptiles) died in these safari parks and eco- parks from different types of infectious diseases during last 04 years (i.e. 2016-

2019) and in most cases actual causes of death remain unknown due to lack of diagnostic facilities. However, from recent detection of TB at Gazipur Safari Park, it is commented by experts of veterinary medical board that TB infection could be transmitted from free ranging wild animals (Rhesus monkey, Jackal, fishing cats etc.) & neighbouring livestock population coexisting in safari parks area and further investigation is needed to prevent the transmission of TB in captive animals (Chowdhury M. N. U, 2019). Therefore, among the infectious diseases TB is becoming one of the emerging challenges for wildlife conservation in South Asia but its impact is still overlooked and underexplored in Bangladesh (Thapa et al., 2017). Considering the above- described circumstances, this proposed study will identify the nature & type of circulating TB organisms among captive wildlife population and associated risk factors link with the transmission of TB in captive animals at safari park, eco-parks and national parks.

1.2 Objectives of the Study

a) To investigate prevalence and distribution of Tuberculosis (TB) among wildlife species in Bangladesh.

b) To investigate types of Mycobacterium Tuberculosis Complex (MTBC) circulating at captive-wild interface in Bangladesh.

c) To formulate a feasible Tuberculosis Control & Management Guideline for safari parks, national parks, eco-parks and other protected areas.

1.3 Research Questions

a) What are risk factors associated with the transmission of TB in wildlife at safari park, eco-parks and national parks?

b) Which drugs (antibiotics) are sensitive for captive wild animals?

CHAPTER-II: LITERATURE REVIEW

2.1 Aetiology

Animal tuberculosis (TB) is a bacterial zoonosis of public health importance affecting wildlife, livestock, and humans throughout the world (Akhtar et al., 2023). It is a chronic granulomatous disease caused by significant number of infectious organisms like *Mycobacterium tuberculosis*, *Mycobacterium bovis*, *Mycobacterium bovis BCG*, *Mycobacterium canettii*, *Mycobacterium africanum*, *Mycobacterium pinnipedii*, *Mycobacterium microti*, *Mycobacterium caprae*, the *oryx bacillus*, and the recently discovered *Mycobacterium mungi* which are closely related species that form the *M. tuberculosis complex* (MTBC) (Katale et al., 2012). *Mycobacterium tuberculosis* and *M. bovis* are the most important species in the complex which commonly cause human and animal tuberculosis (TB), with concomitant negative consequences for human and animal health and economic costs (Katale et al., 2012).

2.2 Host Range

Cattle are considered the main host of M. bovis; however, some wildlife species are described to act as reservoirs, namely the African buffalo (*Syncerus caffer*) in South Africa, badger (*Meles meles*) in Britain, brushtail possums (*Trichosurus vulpecula*) in New Zealand, Canadian bison (*Bison bison*) in Canada, lechwe antelope (Kobus leche) in Zambia, white-tailed deer (*Odocoileus virginianus*) in United States, and wild boar (*Sus scrofa*) and red deer (*Cervus elaphus*) in the Iberian Peninsula. Furthermore, *M. bovis* was already reported in many other domestic and wild animal species, including diverse ungulates, carnivores and marsupials (Pereira et al., 2020). From the data of the World Animal Health Information Database (WAHID) Interface of World Animal Health Organization (OIE), the incidence of bovine and wildlife TB is low in Malaysia, Thailand, Myanmar and a few unverified cases in Vietnam. These include domestic goats, cattle, buffaloes, Asian elephant (*Elephas maximus*) and few unknown wildlife species (Che-Amat, 2018). Seropositivity also found in bears (*Melursus ursinus*) and elephants (*Elephas maximus*) of India (Sharma et al., 2022).

2.3 Transmission

Animal Tuberculosis can be transmitted in various ways. For instance, in air that is exhaled; sputum and urine, faeces as well as pus the bacteria can spread. Either direct contact or contact with infected animal excreta, aerosol inhalation can spread the disease that depends on the involvement of the species (Phillips et al., 2003).

The common mode of transmission is inhalation or ingestion. It is mainly by droplet infection, inhalation of dust contaminated by sputum, faeces, urine of infected animals. Thus, close housing and overcrowding along with improper management predisposes to the disease. Zebu (Brahman) type cattle are thought to be much more resistant to tuberculosis than European cattle. The dynamics of *M. bovis* transmission from an effective disseminator tuberculous animal to susceptible hosts are currently not clearly identified and are meagrely understood. Though, infection is principally confined to the respiratory system as bacteria aim to establishthe infection within the lung. Infected cattle are considered as possible source of infection as they shed significant amount of *M. bovis* through droplet nuclei in to the environmental and may act as source of intra-herd transmission (Van Rhijn et al., 2008).

Primarily, tuberculosis is a respiratory disease and is transmitted through mainly by air born route within and between species during close contact. In case of enterogenous route or ingestion through buccal mucosa, pharyngeal mucosa and intestinal mucosa the organism may enter into the animal body. It is also through the congenital route but is less common mode of acquiring infection. The infected bull may also transmit disease or through artificial insemination with the use of infected semen. The *M. bovis* is transmitted from animal to man through ingestion of unpasteurized dairy product, milk of infected cattle and undercooked meat which was recognised as a major public health problem (Verma et al., 2014).

2.4 Pathogenesis

Pathogenic mycobacteria survive and multiply in macrophages during the first stage by stabilizing the phagolysosome at PH 6.2-6.3 and staying in the endosomal recycling channel, where they can get iron. Avoiding acidity requires the cord factor. Phagosomes acquire lysosomal proteins but cannot merge with them. Monocytes are transported to the infection site in the second phase. Monocyte-to-macrophage differentiation maintains mycobacteria. When antigen-specific T cells grow, mycobacterial proliferation decreases. Release activates macrophages and removes the phagosome maturation block. Nitric oxide synthase produces mycobacteria-toxic radicals. After removing the impediment, the mycobacteria-containing vacuole can be acidified, speeding up the process. If immune cells fail to stop bacterium growth, the sickness progresses to the final stage. Infected macrophages produce large amounts of pro-inflammatory mediators such TNF, IFN-γ, IL-6, IL-11, IL-18, and IL-12. Anti- inflammatory cytokines include TGF- and IL-10. TB symptoms, including fever and wasting syndrome, stem from uncontrolled cytokine production. Wild animal tuberculosis is a zoonotic threat to people and domestic cattle. In animals, Inhaled *M. bovis* replicates in alveolar macrophages. After a lung infection spreads, lymph nodes in the mediastinum or elsewhere, such as the head and neck, may become infected. The host's immune system attacks the intruder cell by cell. Inflammation forms granulomas (Khurram et al., 2023).

Tubercles, or sores, characterize tuberculosis. After that, a laminar structure resembling an onion slicer emerges around the granuloma. The granuloma's core cells die, leaving dry cottage cheese-like debris. Biting wounds can spread TB in naturally infected groups. Pulmonary infection from infectious aerosols may take time to develop. Thus, a chronic illness develops, and the infected animal may exhibit a range of symptoms, from asymptomatic to severe overt disease with systemic pathology. Respiratory infections stay latent, unlike wound infections, which progress quickly and create sores. Badgers at any stage of sickness can harm vulnerable hosts. Badgers with many illnesses have best summarized the pathogenic chain. Grossly evident lesions are more common in the thoracic cavity, which contains the lungs, and in the head and body lymph nodes. The abdominal cavity is uninfected, although many other tissues and organs may be. Badgers disperse similarly. Saliva contains TB, which can infect bite wounds. Due to rapid progression, enlarged lesions, and extensive infection, pathogenesis may differ here. Infected bite sputum can cause tiny subcutaneous granulomas or large, skinless sores (Khurram et al., 2023).

2.5 Diagnosis

2.5.1 Ante-mortem Diagnosis

Where wildlife are indigenous protected species and postmortem samples are not frequently available, consideration may be given to trying to establish an ante-mortem diagnosis of tuberculosis. All the ante-mortem diagnostic procedures have limitations, but have the potential for determining not only the existence of tuberculosis within a population but also the prevalence of infection in that population. Specific and sensitive ante-mortem tests for a large range of species are increasingly required to ensure that wildlife moved to new game parks or zoological gardens are free of bovine tuberculosis and are not a source of infection for other animals. In particular, few tests can be used in pachyderms (De Lisle et al., 2002).

2.5.1.1 *Clinical Signs*

The majority of the course of infection, animals will be clinically normal. The most common clinical sign of disease is weight loss and this only occurs in the advanced stage of the disease in wild animals. Other clinical signs include swollen lymph nodes, especially of the head, discharging lymph node abscesses and signs associated with a tuberculous pneumonia such as coughing (Sharma et al., 2022).

2.5.1.2 *Tuberculin Test*

For antemortem diagnosis, single intradermal tuberculin test (SITT), single comparative intradermal tuberculin test (SCITT), and gamma interferon test (IFN-ɣ), which is regarded as a subsidiary test, are used (Bezos et al., 2014). These different analyses have their own shortcomings with regard to the results produced (Miller et al., 2019), but it is well accepted that they complement one another, especially when used synergistically; SCITT is regarded as a better diagnostic tool with the high specificity and moderate sensitivity, whereas the IFN-ɣ test has higher sensitivity with the lower specificity (Downs et al., 2018). The intradermal tuberculin test calculates dermal swelling basically due to CMI response 72 h after the introduction of purified protein derivative (PPD) in the skin of the neck or the caudal fold. The surface of the neck is more sensitive than the surface of the caudal fold; for this reason, more doses of PPD are required in the caudal fold region to increase its sensitivity like that of neck region (Schiller et al., 2010).

2.5.2 Post-mortem Diagnosis of MTBC

2.5.2.1 *Post-Mortem Lesions*

It is characterized by the formation of granulomas (tubercles) where bacteria have localized. These granulomas are usually yellowish and either caseous, or calcified, they are often encapsulated. In some species such as deer, the lesion tends to resemble abscesses rather than typical tubercles. Some tubercles are small enough to be missed by the naked eye unless the tissue is sectioned. In cattle, tubercles are found in the lymph nodes, particularly those of the head and thorax. It is common in the lungs, spleen, liver and the surfaces of body cavities. In disseminated case, lesions are sometimes founding on the female genitalia, but are rare on the male genitalia. In countries with good control programs, infected cattle typically have few lesions at necropsy. Most of those lesions found in lymph nodes associated with the respiratory system. However, small lesions can often be discovered in the lungs of these animals if the tissues are sectioned (Verma et al., 2014).

2.5.2.2 *Direct Smears*

For the rapid identification of MTBC infections, a direct smear is required for the detection of TB organisms from tissue samples with suspected infection followed by Ziehl–Neelsen (ZN) stain. The basic principle of the stain is that MTBC available in the smear can take up an aryl methane dye, resulting in acid-fast staining reaction, after treatment with a weak acid-alcohol solution. This is attributable to the mycolic acids that are present in the thick bacterial cell wall (Lekko et al., 2020).

2.5.2.3 *Histopathology*

In free-ranging wildlife, the diagnosis of Mycobacterium spp. relies on gross findings and histopathological analyses. One reliable method is histopathological analysis with hematoxylin and eosin staining (Silva et al., 2018). This technique has high specificity and the additional advantage of producing results within a few days. A correlation of 94% was found between the results obtained using histopathological analysis and culture methods, regarding an agreement on the diagnosis of *M. bovis* infection in cattle.

2.5.2.4 Bacterial Culture

Culture is regarded as the gold standard technique for diagnosing MTBC in animals, given its high specificity. However, it can occasionally produce false-negative results, and its sensitivity has reach only as high as 80% (Che'Amat et al., 2015). For the isolation of mycobacteria, three types of media are typically used. The first is egg-based media, such as Stonebrink's medium and Lowenstein–Jensen, with either glycerol or pyruvate. The second is agar-based media, such as Middlebrook 7H10 and 7H11, and tuberculosis blood agar medium, B83. The third type is liquid media, such as Middlebrook 7H9. The use of solid and liquid media together should maximize the sensitivity, an approach that has been approved for the isolation of mycobacteria in laboratories for human TB diagnosis globally (Gormley et al., 2014) (Issa et al., 2017).

2.5.2.5 Enzyme-Linked Immunosorbent Assay (ELISA)

The ELISA appears to be the most suitable of the antibody-detection tests and can be a complement, rather than an alternative, to tests based on cellular immunity. It may be helpful in anergic cattle and deer. An advantage of the ELISA is its simplicity, but sensitivity is limited mostly because of the late and irregular development of the humoral immune response in cattle during the course of the disease. Specificity is also poor in cattle when complex antigens such as tuberculin or *M. bovis* culture filtrates are used. However, a comparison of antibody levels to PPD-B and PPD-A has been shown to be useful in increasing specificity in the ELISA (Chambers, 2013).

2.5.2.6 Nucleic Acid Recognition Methods

PCR has been widely used for the detection of M. tuberculosis complex in clinical samples (mainly sputum) in animal cases and has recently been used for the diagnosis of tuberculosis in wild animals. The real time PCR determine the status of infection in animal for bovine tuberculosis as compare to the IFN gamma mRNA in blood culture. Another useful diagnostic method for bovine tuberculosis in cattle is RT-PCR (Gan et al., 2013). Multiplex PCR methods produce a higher rate of the detection of MTBC. These techniques amplify two or more target genes in a reaction simultaneously (Sharma et al., 2012).

2.6. Prevention and Control

Control of TB in wildlife reservoirs has relied on population reduction through increased hunting, trapping, or poisoning(Nugent et al., 2012) and vaccination (Buddle et al., 2011) (Palmer et al., 2014) and these strategies have met with mixed success. Efforts to reduce wildlife populations for disease control can be difficult and are often met with public criticism (Carstensen et al., 2011). Vaccination of either the wildlife reservoir or the livestock population is an anticipated alternative to culling (Buddle et al., 2011) (Chambers et al., 2011). Development of novel approaches to control diseases in livestock and wildlife, including TB which are both biologically relevant and acceptable to livestock owners is an important goal of One Health (Kaneene et al., 2014). Ultimately, successful control of TB in wildlife and livestock will reduce human infection, reduce losses to productivity and reduce market restrictions from countries where eradication programs are in place (Kaneene et al., 2014). Culturally appropriate education and active participation of livestock owners and other stakeholders is critical for the success of zoonotic disease control programs (Munyeme et al., 2010).

Wildlife disease detection and surveillance programs are rare (Siembieda et al., 2011) due to difficulties in enumerating and testing free-ranging wildlife populations. In instances where wildlife reservoirs are commonly hunted, surveillance programs have relied on post-mortem testing of hunter-harvested wildlife. However, when harvesting wildlife for surveillance is not feasible (e.g. rare or endangered species) programs involve trapping, sampling, and releasing animals to collect samples for immunological tests. Once detected, control programs for wildlife disease, including TB, can be difficult to implement and maintain, and are often unpopular (Santos et al., 2012). While culling infected wildlife is a useful strategy for reducing TB risk for livestock in many situations (Chambers et al., 2011), there have been instances where culling has had mixed impacts on livestock TB (Chambers et al., 2011). In fact, some critics have suggested that, given the economic costs and unpopularity of TB control in wildlife reservoirs and the successes of pasteurization and food hygiene, the costs far outweigh the benefits of control programs, and TB should not be considered a public health issue (Torgerson and Torgerson, 2010).

Using One Health approaches, particularly in sharing resources, training, and knowledge of laboratory and health care workers, should decrease this form of misdiagnosis. Refinement of currently-used tests for TB to improve sensitivity and specificity, particularly those that can be readily used in the field in developing countries and the development of new tests, are goals for TB research(Kaneene et al., 2014) .

Vaccination of livestock and wildlife for TB control is been investigated in developing countries and in countries with wildlife reservoirs of TB (Mbugi et al., 2012). In some instances, vaccination does not prevent infection, but reduces the burden of disease in the vaccinated wildlife (Chambers et al., 2011). With ongoing research to develop better vaccines and delivery methods, vaccination has been recognized as a future option for control of TB transmission between wildlife and livestock (Palmer et al., 2012).

Collaborative efforts between public health, agriculture, and wildlife professionals, with support from the public, are critical to the control of TB (Cunha et al., 2011). Lack of stakeholder support can seriously reduce the effectiveness of TB control programs, as seen in the control of TB in wild white tailed-deer in Michigan and Minnesota (Carstensen et al., 2011). Control programs have successfully reduced TB levels in wild deer in Minnesota with public acceptance and support while lack of cooperation with farmers and hunters in Michigan have made control programs more difficult to maintain (Carstensen et al., 2011).

CHAPTER-III: METHODOLOGY

3.1 Study Sites

1. Gazipur Safari Park
2. Bhawal National Park, Gazipur
3. Tilagar Eco-Park, Sylhet
4. Lawachara National Park, Moulvibazar
5. Cox's Bazar Safari Park
6. Aviary and Eco Park, Rangunia, Chattogram
7. Kaptai National Park, Chattogram
8. Karamjal Wildlife Breeding Center, Khulna
9. 05 Human Settlements Area of Rhesus Monkey-
    a) Old Dhaka City Area, Dhaka
    b) Dhamrai Upazilla, Dhaka
    c) Bormi, Sreepur, Gazipur
    d) Sylhet Town Area, Sylhet
    e) Charmuguria, Madaripur

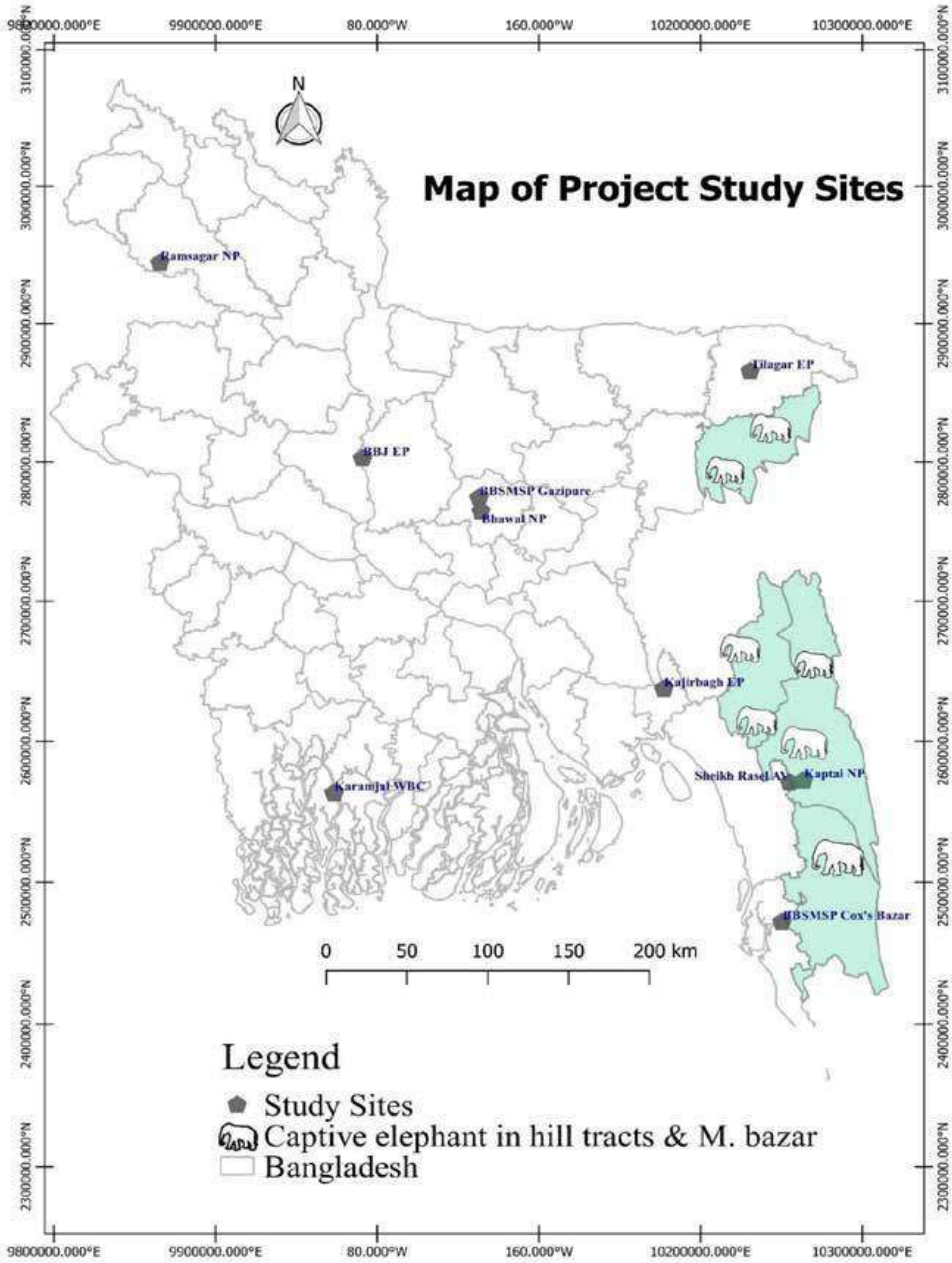

Figure: Map Showing Study Sites

3.2 Method

3.2.1 Selection of Study Sites

We have selected two safari parks, two national parks, one eco-parks and one aviary under Bangladesh forest department as recently TB has been documented in captive animals(Ahasan and Rahaman, 2007; Malama et al., 2013). Moreover, In these safari parks, national Park, eco parks and aviary more than 5000 animals and birds are kept under captive management and in a recent investigation *Mycobacterium Spp.* has been document in these wildlife settlement (Chowdhury M. N. U, 2019). However, 05 recognized human settlements (i.e. Old Dhaka city area, Dhamrai upazilla, Bormi, Sylhet town and Charmuguria) have been selected for sample collection from feral monkey population as human-monkey conflict reported in this areas that increase the likelihood of spillover of TB pathogens (Hasan et al., 2013). Karamjal is one of the important wildlife breeding center in Sundarbans and several type of wildlife species including spotted deer are kept under captive management in that center. Therefore, it is important to evaluate TB situation among these captive animals as free ranging wild monkey population are coexisting in the same habitat of the breeding center. Considering all this issue, above mentioned study areas are linked to our research program and best study area to investigate wildlife tuberculosis at captive-wild interface in Bangladesh.

3.2.2 Study Population

Captive wild animals & birds from safari parks, eco-parks, national parks, aviary and breeding centre were selected as a study population. Main species were Spotted Deer, Samber deer Gaur, Giraffe, Zebra, Elephant, Wildebeest, Blackbuck, Common Eland, Asiatic Black Bear, African Lion, Bengal tiger, Turtle & Tortoise, Crocodile, Wild Boar, Rhesus Monkey and Birds. Only species that are susceptible to TB were considered as study population. In addition, free ranging rhesus monkeys coexisting in park areas and human settlements were also considered as a study population.Sampling Technique

Simple random sampling technique was followed during sampling from captive species and both invasive and non-invasive samples were collected from TB susceptible animals and birds. Oral swab, blood, tissue, Trunk wash, faeces were mostly collected. But blood samples were collected from captive elephants and free ranging Rhesus monkeys. Before sample collection elephants are controlled by respective of each elephant. (Figure-1). However, a mobile animal capture trap was set up at the sampling site to collect samples from free-roaming monkeys, and various types of feed were used to attract monkeys. i.e. Banana, fried-rice and bread have been dispersed into the trap (Figure-2). Immediately after being trapped, monkeys were immobilized using blowpipe loaded with G-Ketamine and Xylazine.

Figure: 1- Restraining of Elephant by Mahut before Sample Collection

After Tranquilization oral swab and blood has been collected from the trapped monkeys. During tranquilization for short distance blowpipe and long-distance tranquilizing gun were used respectively to capture feral animal. WOAH wildlife health guidelines & protocol was followed for safe handling, capture and safe release of wildlife. Tranquilized monkeys were examined during anaesthesia and released after fully recovered from anaesthesia.

Figure-2: Use of Mobile Trap for Capturing Monkey

3.2.3 Sample Collection & Preservation Procedure

Basic supplies and equipment were used for sample collection depending on species being sampled and type of laboratory analyses. Sterile swab stick, leak-proof plastic bag, sterile vacutainer was used to collect specimens and all specimens were labelled with the species, collection date and type. For tissue specimen approximately 5-10 gm and 5 ml blood were collected by using sterile syringe for laboratory analysis. Only properly trained individual proficient in animal sampling techniques will be employed to collect blood samples from live animals. Immediately after collection, to avoid the coagulations of blood, serum samples were separated by centrifugation (5000-6000 RPM) using portable centrifuge machine.Each tissue sample from a deceased animal was collected using sterilized scissors, forceps, and plastic containers and bags. For preservation of samples in field, portable cooling box was used to maintain the cool chain (Figure-3). Finally, all samples were shifted and stored in freezer (- 20 ^{O}C) at the laboratory of the Central Pathology Department, Bangladesh Agricultural University (BAU), Mymensingh until Analysis.

Figure-3: Field Sample Collection & Preservation in Cool Box

3.2.4 Safeguard Issues & Biosafety Protocol for Sample Collection

Tuberculosis is a zoonotic disease, and to safeguard wildlife staff and community people against transmission of pathogens from wildlife animals, personal biosafety was strictly followed during sample collection. To ensure biosafety in the field, disposable gloves, face masks, and protective gowns were worn during sample collection, and different gloves were used for each sample collection to avoid cheek cross contamination. Used gloves, masks, PPE, syringes, and other items were placed in a biohazard bag immediately after sample collection, and hands were properly cleaned with soap and water. Finaly all biohazard material were disposed following appropriate scientific procedure.

Figure -4: Maintaining Biosafety Protocol to Safeguard against Zoonotic Transmission of

3.2.5 Laboratory Analytical Procedure

Rapid TB screening test i.e. Z-N staining was conducted initially for all types of swab samples
& trunk was and Polymerase Chain reaction (PCR) was applied to samples that reactive positive to Z-N staining. Bacteria from faecal samples were identified by using conventional PCR and qRT-PCR. Primarily from faecal sample Mycobacterium *spp.* were identified and analyzed by microbial DNA extraction followed by PCR (Polymerase Chain Reaction) from positive samples. DNA extraction was performed using Monarch® Genomic DNA Purification Kit (New England Biolabs, USA). Later on, to detect *Mycobacterium spp.* multiplex PCR was employed based on the amplification of 16S rRNA gene of *Mycobacterium complex.* Furthermore, uniplex PCR was carried out based on the amplification of H37Rv and MPB83 gene of *M. tuberculosis* and *M. bovis* respectively. The PCR primers were synthesized from a commercial source (AIT Biotech, Singapore). The primers used in this analysis are listed in Table-1**.** Electrophoresis was performed by using 1.5% agarose gel containing ethidium bromide (0.5 µg/ml) and images were captured in a transilluminator (Alpha imager, USA). For the evaluation of the size of amplicons, a 100 bp DNA ladder (TackIt, Invitrogen, USA) was used in the agarose gel.

Specimens that were found positive to PCR were subjected to incubation cultural media for isolation and species identification. At first all specimens were first homogenized using automated homogenizer. They were then decontaminated and concentrated using N-acetyl-L- cysteine (NALC)-NaOH-Na-citrate solution by following the Petroff's standard procedure (1). Two loops of processed specimens were inoculated on two Lowenstein-Jensen (L-J) slants and 500 µL in an MGIT 960 tube with a PANTA supplement (2). Cultures were incubated at 37 °C for up to eight weeks for L-J slants and 42 days for the MGIT 960 system. L-J slants were checked once per week and MGIT cultures were monitored continuously through the automated MGIT 960 instrument. If any specimens were found as culture positive, then the culture isolates were subjected to DNA isolation and PCR for species identification. All laboratory tests were be conducted in the laboratory of the pathology Department, Faculty of Veterinary Medicine, Bangladesh Agricultural University (BAU), Mymensingh with technical assistance from Tuberculosis laboratory, icddr,b Dhaka**.** During sample analysis SIG Evaluation Team and Director, Sheikh Kamal Wildlife Center, Gazipur visited at the laboratory of Pathology Department, BAU, and Mymensingh (Figure-5 & 6).

Figure 5: Demonstration of Laboratory Facilities to the SIG Evaluation Team by Dr. Munmun Pervin, Head, Department of Pathology, BA

U, Mymensingh.

Figure 6: Demonstration of TB Positive Samples to the Director, Bangladesh Wildlife Center, Gazipur by Professor Hadi Nur Ali Khan, Department of Pathology, BAU, Mymensingh & Co-PI, Detection & Management of Tuberculosis…. Bangladesh.

Table-1: Primer Sequences Used for Detection of Mycobacterium from Wild Animals

Target gene	Primers Name	Primer sequence (5´-3´)	Amplicon size
16sr RNA	MYCGEN- F1 MYCGEN- R1 MYCAV-R2 MYCINT-F2 TB 1-F3 TB1-R3	AGAGTTTGATCCTGGCTCAG TGCACACAGGCCACAAGGGA ACCAGAAGACATGCGTCTFG CCTTTAGGCGCATGTCTITA GAACAATCCGGAGTTGACA AGCACGCTGTCAATCATG	1030 (F1R1) Mycobacterium 180 (F1R2) *M. avium* 850 (F2R1) *M. Intracellulare* 372 (F3R3) *M. Tuberculosis* complex
H37Rv	Rv3479HPF Rv3479HPR	GAACTCACCGTCGGTGGTGA CCTTGCTCGATCTCTGCGTC	667 bp *M. tuberculosis*
MPB83	MPB83F MPB83R	CAGGGATCCACCATGTTCTTAGCGGGTTG TGGCGAATTCTTACTGTGCCGGGGG	600 *M. bovis*

3.2.6 Field Data Analysis

Field data analysis for risk factor determination on TB transmission among wildlife species is one of the important activities of this research program. To determine the potential risk factors for TB transmission in wild animal data were collected during sampling from all study sites using a prescribed questionnaire (Attached in appendix-1). Field data were collected from respondents through face-to-face interview (Figure-7 & 8) and respondent categories were veterinary surgeon, compounder, Lab. technician, animal keeper, wildlife scout, mahut, forester forest guard, visitors and community people. Details attached in table-2. Finally, all field and laboratory data were entered in MS Excel-2007 and exported into STATA™ 13.0 (Stata Corporation, College Station, TX, USA) for epidemiological analysis. Descriptive statistics was performed on TB prevalence data and distribution of Mycobacterium isolates. Multivariate logistic regression was applied to identify putative risk factors for TB transmission/infection in captive wildlife population at safari parks, eco-parks and national parks.

Table-2: Details of Data Collection

Sampling Sites	Respondent Category	Number of Data Collected
Gazipur Safari Park.	Veterinary Surgeon, Wildlife Supervisor, Compounder, Lab. Technician, Animal Keeper, Junior Wildlife Scout, Mahut and visitors.	23
Bhawal National Park, Gazipur	Forster, Wildlife Scout, Forest Guard, Gardener and Visitors.	18
Sylhet forest Division & Urban area	Forster, Forest Guard, wildlife Volunteer from PRADIKAR and Community People	21
Urban area of Dhamrai Upazila	FD Staff & Community People.	14

Sampling Sites	Respondent Category	Number of Data Collected
Cox's Bazar Safari Park.	Veterinary Officer, Compounder, Lab. Technician, Animal Keeper, Junior Wildlife Scout, Mahut and others.	21
Sheikh Rasel Aviary & Eco- Park, Chattogram.	FD Staffs and Visitors.	10
Kaptai Natioal Park, Chattogram.	Forster, Forest Guard and Wildlife Scout.	10
Barmi, Sreepur, Gazipur.	Community People & FD Staff.	12
Karamjal Wildlife Breeding Centre, Khulna	Local FD Staff and Visitors	10
Charmuguria, Madaripur	Local FD Staff and Community People	15
Moulavibazar District	Elephant Owners, Mahuths & Community People	14

Figure 7: Data Collection form FD Staff of the Study Sites.

Figure 8: Data Collection from Community People of the Study Sites.

3.2.7 Stakeholder (Field Staff) Training

A Day-long stakeholder (Field Staff) training on TB detection and sample collection techniques in wild animals were in conducted for relevant stakeholder in the study sites. Training participants were wildlife supervisor, Forester, Forest Guard, Wildlife Scout, Animal keeper, Laboratory Technician, Compounder, Mahut and community wildlife volunteer (Figure-9). The primary goal of the training is to educate wildlife field staff about basic management practices for TB-suspected/infected wild animals and prevention of transmission of zoonotic TB.

Figure 9: Speech By Mr. Imran Ahmed, Conservator of Forest, Wildlife & Nature Conservation Circle in inaugural Session of Field Staff Training Program at Safari Park, Gazipur.

3.2.8 Drafting of TB Management & Control Guidelines

Another important task of this study program was drafting TB management & control guidelines for the captive wildlife population of safari parks, eco-parks, and national parks. Initially situation analysis was conducted through review of literature and published reports on tuberculosis in wildlife. Finally, the findings from field data analysis and laboratory test results were used to develop first draft of TB management & control guidelines for captive wildlife populations. Later on, a validation workshop was conducted on 5th September 2023 at Wildlife Center, Gazipur for the finalization of drafted TB management & control guidelines. The day- long session was attended by relevant professional/experts, including wildlife veterinarians, wildlife biologists, and public health experts, academicians from public universities and staff from Bangladesh Forest Department (Figure-11). Director, Institute of Epidemiology Disease Control & Research (IEDCR), Dhaka was present as chief guest in inaugural session (Figure- 10-11). The purpose of this workshop was to review the proposed TB Control & Management Guideline and create a realistic guideline for captive populations at safari parks, national parks and eco-parks. Workshop observations and expert recommendations on drafted TB control guidelines were integrated into the final draft document.

Figure 10: Inaugural Speech by Prof. Dr. Tahmina Shirin, Director, Institute of Epidemiology, Disease Control and Research (IEDCR), Dhaka on Validation Workshop at Bangladesh Wildlife Center, Gazipur.

Figure 11: Workshop Participants of Finalization of TB Management & Control Guidelines at Bangladesh Wildlife Center, Gazipur.

CHAPTER-IV: RESULT AND DISCUSSION

4.1 Laboratory Analysis Finding

Laboratory analysis of biological samples is one of major activity of this research program. A total of 737 samples including swabs, blood, tissue, and faeces were analysed and among the tested specimens 19 case were confirmed by Z-N staining and molecular method (laboratory reports attached in appendice-1). *Mycobacterium tuberculosis complex, M. tuberculosis and M. bovis* were identified in these positive cases and infected wildlife species were Spotted Deer, Samber Deer, Goyal, Asiatic Elephant, Asiatic Black Bear, Wilde Beast and Rhesus Monkey and description given in table -3. In addition, *M. africanum* species was detected in 05 specimens from Spotted Deer, Goyal Wilde Beast and Common Eland in bacterial isolation & Line Probe Assay and illustrated in Table-4.

Table-3: Result of Molecular Analysis

Sl No.	Name of TB-Positive Species	Number of Animals	Mycobacterium Species Detected By PCR
1.	Asian Elephant	03	*Mycobacterium tuberculosis complex* *Mycobacterium tuberculosis* *Mycobacterium bovis*
2.	Asiatic Black Bear	02	
3.	Rhesus Monkey	05	
4.	Spotted Deer	04	
5.	Common Eland	01	
6.	Goyal	02	
7.	Samber Deer	01	
8.	Wilde Beast	01	

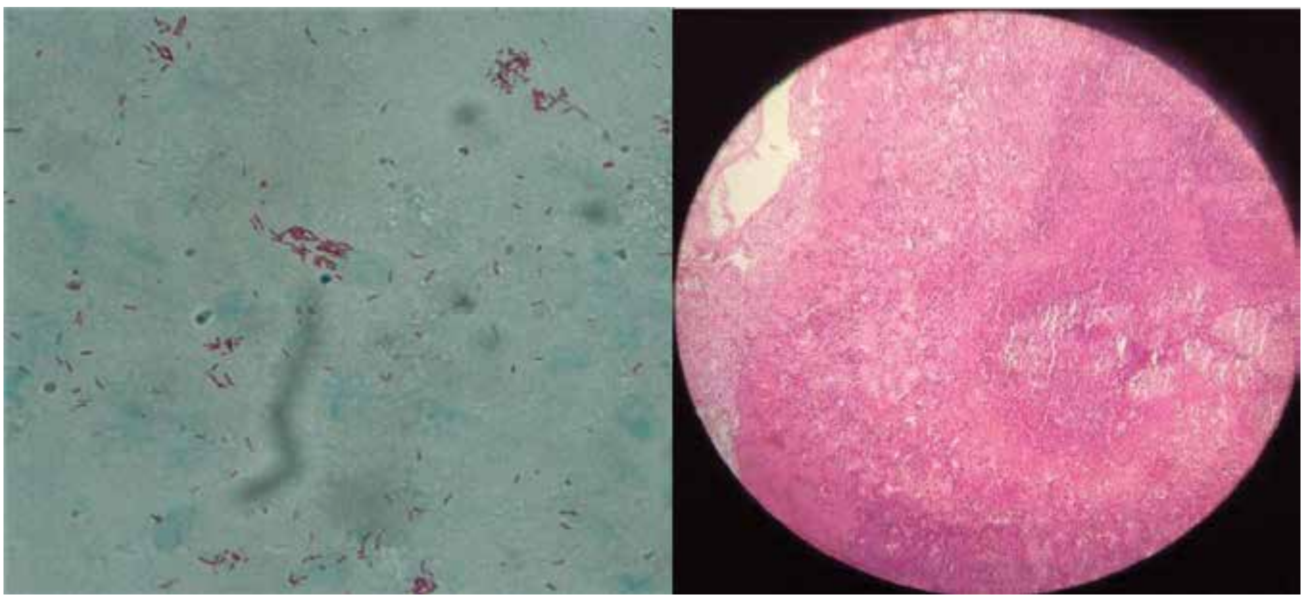

Figure 12: Microscopic & Histopathological Examination of TB Infected Tissue Sample

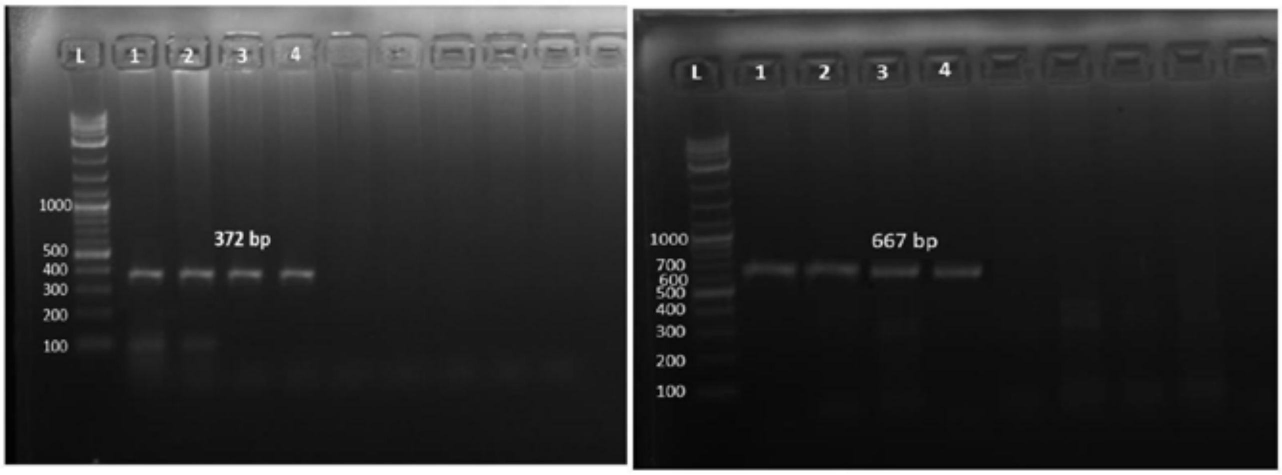

Figure13: PCR Detection of Mycobacterium tuberculosis complex (372bp) and Mycobacterium Tuberculosis (667bp) where sample placed in Lane1-4

Table-4: Laboratory Test Results for Culture Positive Isolates from Tissue Samples

Animal name	Specimen	MGIT Culture Result	*M. tuberculosis* Complex PCR result	GenoType MTBC test result
	Spleen	Negative	N/A	N/A
	Liver	Negative	N/A	N/A
	Kidney	Positive	Positive	Not done
	Heart	Positive	Positive	Not done
	Lungs	Positive	Positive	*M. africanum*
Wild Beast	Lungs	Negative	N/A	N/A
Common Eland	Spleen	Negative	N/A	N/A
	Liver	Negative	N/A	N/A
	Kidney	Negative	N/A	N/A
	Heart	Negative	N/A	N/A
	Lungs	Positive	Positive	*M. africanum*
Spotted Deer	Heart	Positive	Positive	Not done
	Liver	Positive	Positive	Not done
	Nodule	Positive	Positive	Not done
	Lungs	Positive	Positive	*M. africanum*
Goyal	Nodule	Positive	Positive	Not done
	Spleen	Negative	N/A	N/A
	Liver	Positive	Positive	Not done
	Heart	Positive	Positive	Not done
	Lungs	Positive	Positive	*M. africanum*
Spotted Deer	Kidney	Negative	N/A	N/A
	Heart	Negative	N/A	N/A
	Spleen	Negative	N/A	N/A
	Lungs	Positive	Positive	*M. africanum*
Bengal Tiger	Lungs	Negative	N/A	N/A
	Liver	Negative	N/A	N/A
	Heart	Negative	N/A	N/A
	Kidney	Negative	N/A	N/A
	Esophagus	Negative	N/A	N/A
	Spleen	Negative	N/A	N/A
Wild Beast	Lungs	Positive	Positive	Not done

To find out the sensitive drugs for wild animals a total of 131 swab samples were inoculated into growth media and positive bacterial growths were detected in 83 specimens. Drug sensitivity profile of bacterial isolates was assessed using the disc diffusion technique for 22 commonly used antibiotics. Finally, result drug sensitivity tests were recorded following standard reference value for zone of inhibition against each antibiotic discs. The following Table-5 summarizes the findings of antibiotic sensitivity testing:

Table-5: Summary of Findings of Antibiotic Testing

Sl No.	Species	Resistant Antibiotic	Sensitive Antibiotic	Reference Value for Zone of Inhibition
1.	Rhesus Monkey	Oxytetracycline, Gentamicin, Erythromycin, Azithromycin, Ampicillin, Ciprofloxacin, Moxifloxacin, Sulfamethoxazole.	Levofloxacin Streptomycin Chloramphenicol Neomycin, Doxycycline, Imipenem, Nitrofurantoin.	Oxytetracycline = 11 mm, Gentamicin =12 mm, Erythromycin = 13 mm, Azithromycin = 13, Amoxicillin =13mm, Ciprofloxacin =21mm, Streptomycin =11mm, Ceftiofur =17mm, Ceftriaxone =19mm, Sulfamethoxazole=12mm, Cefuroxime =14, Levofloxacin =13mm, Chloramphenicol =17mm, Ampicillin = 13mm, Enrofloxacin = 16mm, Moxifloxacin = 20mm, Doxycycline = 10, Neomycin = 12mm, Imipenem = 13mm, Meropenem =19mm, Nitrofurantoin =14mm, Nalidixic Acid =13mm.
2.	Parrot	Oxytetracycline, Amoxicillin, Ciprofloxacin, Cefuroxime, Levofloxacin, Enrofloxacin Moxifloxacin.	Gentamicin, Erythromycin, Azithromycin Cefuroxime, Doxycycline, Ampicillin, Chloramphenicol.	
3.	Mayna	Oxytetracycline, Amoxicillin, Ciprofloxacin, Ceftriaxone, Sulfamethoxazole, Cefuroxime, Levofloxacin.	Gentamicin, Erythromycin, Azithromycin, Chloramphenicol, Doxycycline, Neomycin, Imipenem	
4.	Goyal	Oxytetracycline, Azithromycin, Ciprofloxacin, Moxifloxacin, Sulfamethoxazole, Levofloxacin, Enrofloxacin.	Amoxicillin, Gentamicin, Streptomycin, Ceftiofur, Chloramphenicol, Ampicillin, Imipenem, Meropenem, Nitrofurantoin, Nalidixic Acid.	

Sl No.	Species	Resistant Antibiotic	Sensitive Antibiotic	Reference Value for Zone of Inhibition
5.	Kalim	Azithromycin, Amoxicillin, Ciprofloxacin, Cefuroxime, Levofloxacin, Ampicillin, Enrofloxacin.	Oxytetracycline, Gentamicin, Erythromycin, Streptomycin, Ceftiofur, Ceftriaxone, Sulfamethoxazole	
6.	Turtle	Oxytetracycline, Erythromycin, Azithromycin, Streptomycin, Levofloxacin, Neomycin, Meropenem.	Gentamicin, Ciprofloxacin, Ceftiofur, Ceftriaxone, Cefuroxime, Doxycycline, Imipenem, Nitrofurantoin.	
7.	Macaw	Oxytetracycline, Erythromycin, Amoxicillin, Ciprofloxacin, Streptomycin, Sulfamethoxazole, Ampicillin, Meropenem.	Gentamicin, Cefuroxime, Levofloxacin, Chloramphenicol, Enrofloxacin, Doxycycline, Imipenem, Nitrofurantoin.	
8.	Peacock	Oxytetracycline, Azithromycin, Amoxicillin, Ciprofloxacin, Levofloxacin, Moxifloxacin, Doxycycline.	Gentamicin, Erythromycin, Streptomycin, Ceftriaxone, Sulfamethoxazole, Chloramphenicol. Enrofloxacin, Nitrofurantoin	
9.	Ostrich	Erythromycin, Amoxicillin, Ciprofloxacin, Streptomycin, Ampicillin, Enrofloxacin.	Oxytetracycline, Gentamicin, Cefuroxime, Levofloxacin, Chloramphenicol, Doxycycline Imipenem, Nalidixic Acid.	
10.	Tortoise	Meropenem, Oxytetracycline, Erythromycin, Ciprofloxacin, Doxycycline, Neomycin.	Gentamicin, Azithromycin, Streptomycin, Ceftiofur, Ceftriaxone, Cefuroxime, Nitrofurantoin.	

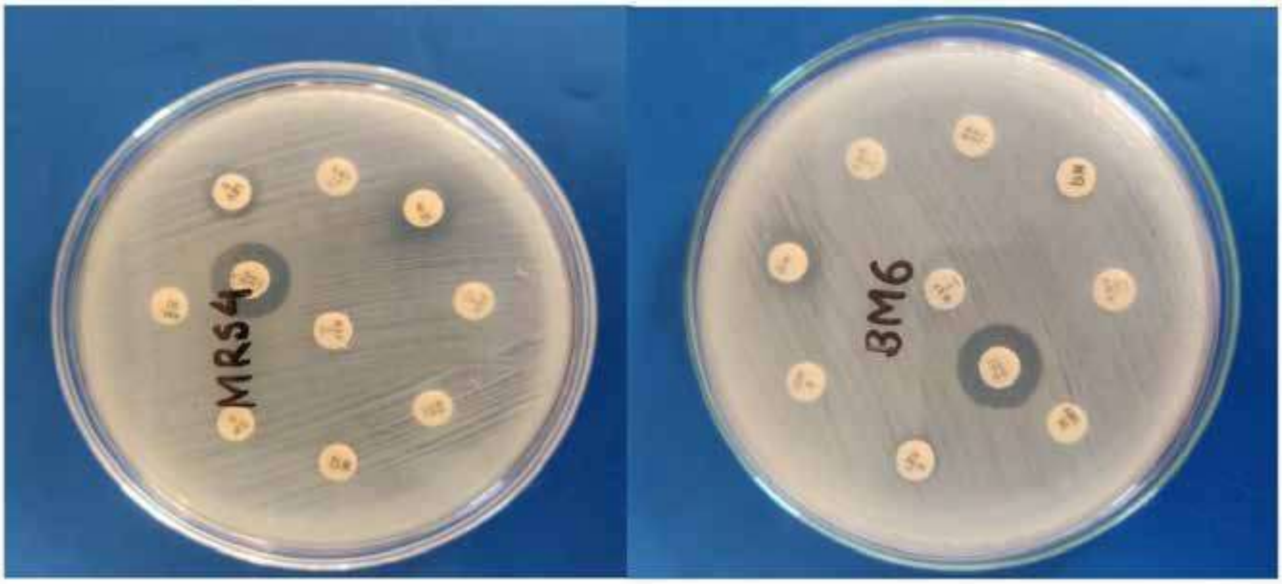

Figure-14: Implanted Antibiotic Discs in Muller Hinton Medium Showing Antibiotic Resistance

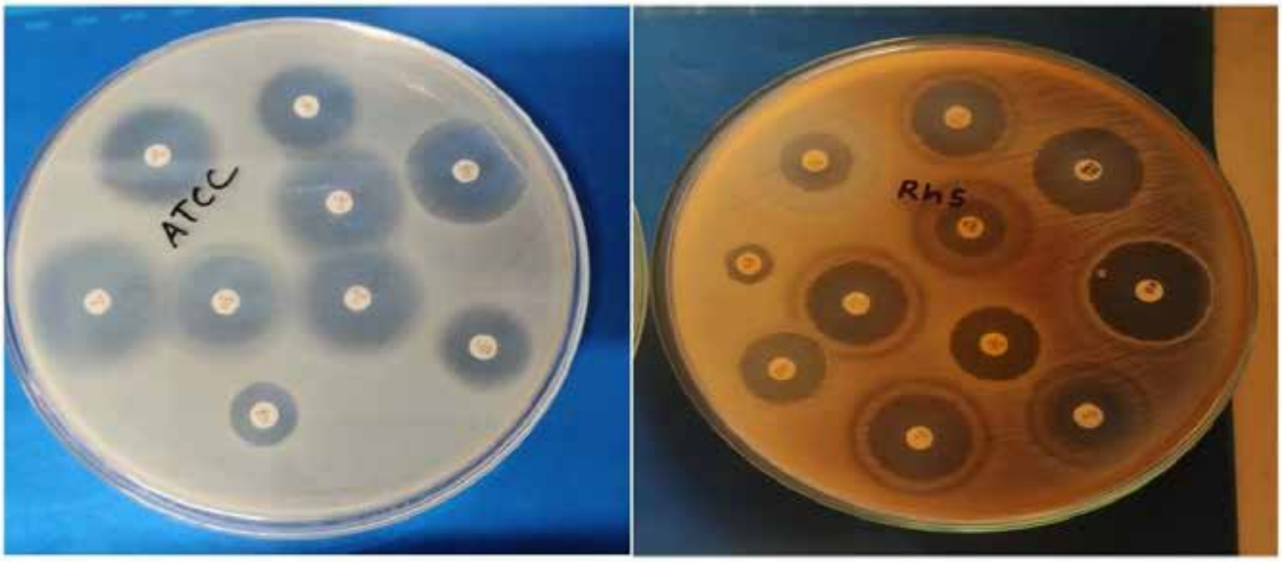

Figure-15: Implanted Antibiotic Discs in Muller Hinton Medium Showing Clear Zone of Inhibition

4.2 Findings of Field Data Analysis for TB Risk Factor

To determine the potential risk factors for TB transmission in wild animal Field data were collected from 84 respondents through face-to-face interview and respondent categories were veterinary surgeon, compounder, Lab. technician, animal keeper, wildlife scout, mahut, forester forest guard, visitors and community people. In epidemiological analysis presence of wild animals in enclosure, mixing of wild and captive animals, presence of TB infected animal, livestock grazing in park areas and sharing feed between feral and captive animals were identified as major risk factors for TB transmission/infection in captive wildlife population at safari parks, eco-parks and national parks. Among the wildlife settlement areas, Gazipur Safari Prak was recognized as vulnerable zone whilst among human settlements, Madaripur was designated as a high-risk region for TB transmission. A details list of risk factors for TB Transmission is given in Table-6.

Table 6: Risk Factors Associated with TB Infection in Wild Animals

Characteristics	OR	95% CI
Sampling sites		
karamjal wildlife breeding center, Khulna	Ref.	
Gazipur Safari Park	1.71	0.25-6.15
Charmuguria Eco-Park, Madaripur	5.71	1.02-31.85
Bhawal national park, Gazipur	1.22	0.22-6.54
Juri, Sylhet	1.26	0.14-20.33
Districts		
Khulna	Ref.	Ref.
Gazipur	1.52	0.34-6.79
Madaripur	1.84	0.15-21.98
Presence of wild animals		
No	Ref.	Ref.
Yes	2.24	0.51-9.81
Mixing of wild and captive animals		
No	Ref.	Ref.
Yes	1.38	0.52-3.64
Presence of sick animal in enclosure/ park		
No	Ref	
Yes	2.34	0.95-5.77
Having livestock		
No	Ref.	
Yes	0.39	0.05-3.03
Direct contact with workers during feeding		
No	Ref.	Ref.
Yes	3.65	0.48-27.69
Sharing feed between free range wild animals and captive wild animals		
No	Ref.	
Yes	1.38	0.52-3.64

4.3 Discussion

The laboratory analysis findings presented in this study provide crucial insights into the prevalence and molecular characterization of tuberculosis (TB) in wild animal populations, along with antibiotic sensitivity profiles of associated bacterial isolates. These findings are instrumental in understanding the epidemiology of TB transmission in wildlife and formulating effective management strategies to mitigate its spread in Bangladesh.

The utilization of various diagnostic techniques, including Z-N staining, PCR tests, culture, and Line Probe Assay, enabled comprehensive screening and identification of TB-positive animals (Thomas et al., 2021) in this study. The detection of *Mycobacterium tuberculosis complex, M. tuberculosis, and M. bovis* in the positive samples underscores the diversity of TB strains circulating among wildlife species (Gortazar et al., 2005) as well as identify the possible transmission possibility of TB in wild species. Additionally, the identification of co-infection with *M. africanum* species highlights the complexity of TB epidemiology in these populations (Silva et al., 2022). Hower, as many of the species imported to Bangladesh are from African countries it requires strong TB screening with long quarantine of all imported TB prone wild animals.

Molecular analysis revealed specific species affected by TB, with Asian Elephants, Asiatic Black Bears, Rhesus Monkeys, Spotted Deer, and other species showing positive results (Thomas et al., 2021). However, in context of infections in various species who resides in same safari park, rescue center might be due to cross contaminations through food and lodging in same encloser. Furthermore, the detection of *M. africanum* in certain wild animals emphasizes the importance of considering emergence of novel TB strains while assessing infection dynamics in wildlife (Damene et al., 2020).

The antibiotic sensitivity testing of bacterial isolates provides valuable information for designing treatment protocols in affected animals (Aljeldah, 2020). The identification of resistance to multiple antibiotics in certain species, such as Rhesus Monkeys, underscores the challenge of managing TB infections in wildlife populations (Miller et al., 2018). Conversely, the sensitivity of bacterial isolates to specific antibiotics offers opportunities for targeted therapeutic interventions in wild animals (Gormley and Corner, 2013).

The field data analysis identified several key risk factors associated with TB transmission in captive wildlife populations. Factors such as the presence of TB-infected animals, mixing of wild and captive animals, and sharing feed between feral and captive animals were identified as significant contributors to transmission (Yakubu et al., 2016). Furthermore, the determination of specific wildlife settlement areas and human settlements as high-risk zones underscores the importance of implementation of targeted surveillance and intervention efforts in these regions (Sichewo et al., 2019).

Overall, the integration of laboratory analysis findings and field data provides a comprehensive understanding of TB epidemiology in wild animal populations. These insights are essential for implementing evidence-based management strategies aimed at controlling TB transmission and safeguarding both wildlife and human populations from the associated risks. Continued surveillance and research efforts are warranted to monitor TB dynamics in wildlife and inform adaptive management approaches in response to emerging challenges.

CHAPTER-V: CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

The present study focused on investigation of TB cases in mostly captive wild animals and highlights the mycobacterial diversity among wildlife species in Bangladesh. A total of 24 cases were confirmed by PCR among the of 737 tested samples and *Mycobacterium Tuberculosis complex, M. tuberculosis and M. bovis* were detected in these specimens. Furthermore, a novel mycobacterium strain, *M. africanum* has been detected in specimens from Spotted Deer, Goyal, Common Eland and Wilde Beast. The finding of this novel strain in circulating Mycobacterium stains in wild animals poses a significant threat to wildlife conservation in Bangladesh. The highest incidence of TB was recorded in Gazipur Safari Park, Gazipur and the feral monkey population residing at Charmuguria, Madaripur among the study sites. Based on study findings and review of the literature, a TB Control & Management Guideline focused on captive species has been drafted. This guideline could be adopted in TB case management in zoological gardens. This is first time initiative by Bangladesh Forest Department to detect wildlife tuberculosis (TB) in collaboration with the Department of Pathology, Bangladesh Agricultural University, and Mymensingh. A further study be pursued as a matter of urgency to explore Tuberculosis transmission cycles in wild animals and to assess the threat of TB to wildlife health in Bangladesh.

5.2 Recommendations

1. Early diagnosis of Tuberculosis would significantly reduce both mortality & transmission rate of the valuable animals in the safari parks, national park eco-parks and aviary. So, a regular screening for TB infection in captive wild animals is recommended to maintain healthy, native animal population and conservation of endangered species as well.
2. To avoid zoonotic transmission, wildlife staff involved in wild animal care and management should be screened for tuberculosis on a regular basis.
3. To prevent transmission between healthy animals and visitors to safari parks and national parks, tuberculosis-positive animals should be immediately separated from animal enclosure or cages.
4. Tuberculosis has been detected in free-ranging monkeys, and an electric fence could be installed in all animal enclosures to prevent the entrance of feral animals into enclosures of captive wild animals at safari parks and national parks.
5. A further study be pursued as a matter of urgency to explore Tuberculosis transmission cycles in wild animals and to assess the threat of TB to wildlife in Bangladesh
6. In our current study PCR test protocols appeared to be sensitive and precise detection tool for identification of TB infection in wild animals. This technique can be applied for laboratory diagnosis of TB suspected case at safari park and national parks.
7. A well-equipped wildlife disease diagnostic laboratory is required to be established under the Bangladesh Forest Department for regular health screening of captive wild animals including tuberculosis testing.

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Appendix-1: Questionnaire

Detection and Management of Tuberculosis (TB) at Captive-Wild Interface in Bangladesh

QUESTIONNAIRE

Objectives:

a) To identify the risk factors associated with the transmission of TB between wildlife-livestock-human interfaces in Bangladesh.
b) To determine the prevalence and distribution of TB at wildlife-livestock interface in Bangladesh.
c) To formulate a feasible TB Prevention & management guideline for safari parks, national parks, Eco-parks and other protected areas.

For additional information or inquiry about the study or questionnaire please contact following investigators:

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Funded By:

Implemented by:

Respondent ID					Date of Recording DD/MM/YYYY	Recorded by:

A. Sampling Site information (Park/ Wildlife sanctuary/ Human habitat)

NO.	QUESTIONS	RESPONSE
A01	Name of Sampling Site	
A02	Name of Upazila	
A03	Name of District	
A04	GPS Location	Latitude : .... Longitude: ....

B. Demographic & Socio-economic Information of Respondent

NO.	QUESTIONS	RESPONSE
B01	Name	
B02	Sex	
B03	Age	
B04	Education level	
B05	Occupation (Designation, if applicable)	
B06	Number of family member(s)	
B07	Types of animal/ species handle?	
B08	Frequency of handling (daily/weekly)	
B09	Monthly income (amount in BDT)	

C. Information on Animal Management

NO.	QUESTIONS	RESPONSE
C01	Please state that the animal/bird/enclosure is directly under your supervision/ management?	....
C02	Please mention your regular duties briefly.	....
C03	Do you feed the animal directly?	☐Yes ☐ No
C04	If yes to C03, what feed/ration do you supply daily/weekly?	....

C05	Do you usually supply feed/ration daily to animal under your supervision?	☐Yes ☐ No
C06	If yes to C05, When and how many times do you supply feed daily?	☐ Morning …………………… ☐ Evening …………………… ☐ Others Schedule……………………
C07	How much time do you spend with animal or in the enclosure per day?	……………………………………
C08	Are you involved in cleaning of animal waste (Faeces/ Urine) in shed/enclosure?	☐Yes ☐ No
C09	If yes to C08, do you maintain protective measures during cleaning?	☐Yes ☐ No ☐ Not applicable
C10	Have you seen any wild animal in the enclosure/shed/park?	☐Yes ☐ No
C11	If yes to C10, which wild animals did you noticed?	…………………………………… ……………………………………
C12	If yes to C11, usually when you observe wild animals?	☐ Morning ☐ Evening ☐ Night
C13	Have you ever seen wild animal mixed up with captive animal?	☐Yes ☐ No
C14	If yes to C13, do feral and captive animals share feed/water?	☐Yes ☐ No
C15	Is there any sick animal in your enclosure/ park right now?	☐Yes ☐ No
C16	If yes to C15, please mention the species and number.	Birds: …………………………… …………………………………… Reptiles: …………………………… …………………………………… Mammals: …………………………… ……………………………………
C17	Please describe major symptoms you have observed?	…………………………………… …………………………………… …………………………………… …………………………………… …………………………………… ……………………………………
C18	Have you given any medications?	☐Yes ☐ No

C19	If yes to C18, please mention the treatment history briefly,	Antibiotic: .... Anthelmintic: .... Other symptomatic treatment: ....

D. Information about Tuberculosis & Other Zoonotic Diseases

NO.	QUESTIONS	RESPONSE
D01	Do you have any idea about zoonotic diseases?	☐Yes ☐ No
D02	If yes to D01, could you please mention name of some diseases?	....
D03	Are you aware of any diseases that have been transmitted to humans from wildlife/livestock?	☐Yes ☐ No ☐ Not Known
D04	If yes, could you please state the names of the diseases?	....
D05	Have you ever heard of Tuberculosis (TB)?	☐Yes ☐ No
D06	If yes to D05, How TB is transmitted? ***(Please tick (√) following symptoms)***	☐Feed ☐Direct contact ☐Urine ☐Faeces ☐Coughing ☐All the above ☐ Other please Specify.. ☐No Idea
D07	Do you think TB is transmitted through wild animals/livestock/birds?	☐Yes ☐ No ☐ No Idea
D08	If yes to D07, which animals/ birds?	....

D09	Have you or your family suffer from all or any of the following clinical signs? *(Please tick (√) following symptoms)*	☐ Coughing for 3 weeks or more ☐ Coughing up blood or mucus ☐ Chest pain or pain with breathing or coughing ☐ Loss of appetite ☐ Gradual weight loss. ☐ Fever ☐ Night sweats ☐ All the above ☐ Other please Specify………………………………… ☐ Not Known
D10	If yes to D09, where do you report first? *(Please tick (√) following symptoms)*	☐ Home remedy ☐ Village doctor ☐ Kabiraj ☐ Clinics/Hospitals ☐ Other please Specify………………………………… ☐ Not applicable
D11	Have you received any medication?	☐Yes ☐ No
D12	If yes to D11, please mention treatment history briefly.	…………………………………………………………… …………………………………………………………… ……………………………………………………………
D13	Do you think feral animals (monkey, Fox etc.) play role in transmission of tuberculosis in captive animal?	☐Yes ☐ No ☐ No Idea
D14	If yes to D13, what is your argument for that?	…………………………………………………………… …………………………………………………………… …………………………………………………………… ……………………………………………………………
D15	What are your opinions on wild animals/birds/ monkey? *(Please tick (√) following symptoms)*	☐ Wild animals/ birds/ monkey are benificial for nature/environment ☐ Wild animals/birds/monkey are benificial for us ☐ Wild animals/birds/monkey are harmful for us ☐ Wild animals/birds/monkey transmit diseases ☐ All the above
D16	What are your opinions on the existence of wildlife?	☐ Conserved ☐ Eliminated from human habitat

E. **Information on Livestock Farmer/Owner**

NO.	QUESTIONS	RESPONSE
E01	Have you any livestock?	☐Yes ☐ No
E02	If yes to E01, please mention the species and number.	☐ Cattle :..................... ☐ Buffalo :..................... ☐ Goat :..................... ☐ Sheep :..................... ☐ Chicken :..................... ☐ Duck :..................... ☐ Other please specify............................
E03	Types of farming system	☐ Intensive ☐ Semi-intensive ☐ Backyard
E04	What do you feed your animal daily?	....
E05	What is your main source of fodder/grass?	☐ Inside the park ☐ Outside the Park ☐ Other please specify................................
E06	Do you graze your animal?	☐Yes ☐ No
E07	If yes to E06, usually where are you graze them?	....
E08	Do your livestock come into contact with any wild animals while grazing?	☐Yes ☐ No
E09	If yes to E08, which animal has come into contact with?	....
E10	Please mention your animal grazing strategy	☐ Village ☐ Transhumant ☐ Interface
E11	What is your animal grazing systems?	☐ Free ☐ Tethering
E12	Have you noticed your livestock sharing feed and water while grazing?	☐Yes ☐ No
E13	If yes to E12, with which animal have you seen?	....
E14	Is your livestock grazing area adjacent to wildlife settlement/sanctuary?	☐Yes ☐ No
E15	If yes to E14, please mention the approximate distance?	....
E16	Have you heard about animal TB?	☐Yes ☐ No
E17	Have you seen any animals in your farm ever infected with TB?	☐Yes ☐ No

E18	If yes to E17, what are the symptoms? ***(please tick (√) following symptoms)***	☐ Weakness ☐ Loss of appetite ☐ Weight-loss ☐ Fluctuating fever ☐ Intermittent coughing ☐ Dyspnea ☐ Diarrhea ☐ Large prominent lymph node ☐ All the above ☐ Others please specify...................... ☐ Not applicable
E19	Have you given any medications?	☐Yes ☐ No
E20	If yes to E20, where did you get your medications? ***(Please tick (√) following)***	☐ Private Veterinarian ☐ Livestock hospital ☐ Kabiraj ☐ Quack ☐ Others please specify...................... ☐ Not applicable
E21	Do you take any precautions when caring for sick animals?	☐Yes ☐ No
E22	If not to E21, please mention the reasons. ***(Please tick (√) following)***	☐ Not affordable ☐ Low income ☐ Not habituated ☐ Not required ☐ No knowledge about use ☐ Don't believe ☐ All the above ☐ Others please specify...................... ☐ Not applicable
E23	Have you any comment?	....

....

Signature

Name of the Respondent...

Designation (if applicable) ....

Address...

APPENDIX-2: Pictures of Laboratory, Field Visit & Result Dissemination Workshop

Figure-16: Laboratory Activities Inspection by Md. Sobeder Islam, Director, Wildlife Center, Gazipur at Department of Pathology, Bangladesh Agricultural University, Mymensingh, Bangladesh on 15th March, 2023.

Figure-17: Laboratory Activities Inspection by Md. Sobeder Islam, Director, Wildlife Center, Gazipur at Department of Pathology, Bangladesh Agricultural University, Mymensingh, Bangladesh on 15th March, 2023.

Figure-18: Field Activities Inspection by Dr. Md. Zahirul Haque, DPD SFAL on 31th March, 2022.

Figure-19: Field Activities Inspection by Dr. Md. Zahirul Haque, DPD SFAL on 31th March, 2022.

Figure-20: Speech by Dr. Md. Golam Rakkibu, Khulna University & Member SIG Field Evaluation Team in Project Progress Meeting at Pathology Department, Bangladesh Agricultural University, Mymensingh on 20th June, 2022.

Figure-21: Project Progress Meeting with Visiting SIG Field Evaluation Team and Faculty Members at Pathology Department, Bangladesh Agricultural University, Mymensingh on 20th June, 2022.

Figure-22: Inaugural Session of Result Dissemination Workshop on the Research Project under SUFAL Innovation Grant Held on 20-21 January,2025 at Hoimonti Auditorium, Bana Bhaban Agargaon, Dhaka.

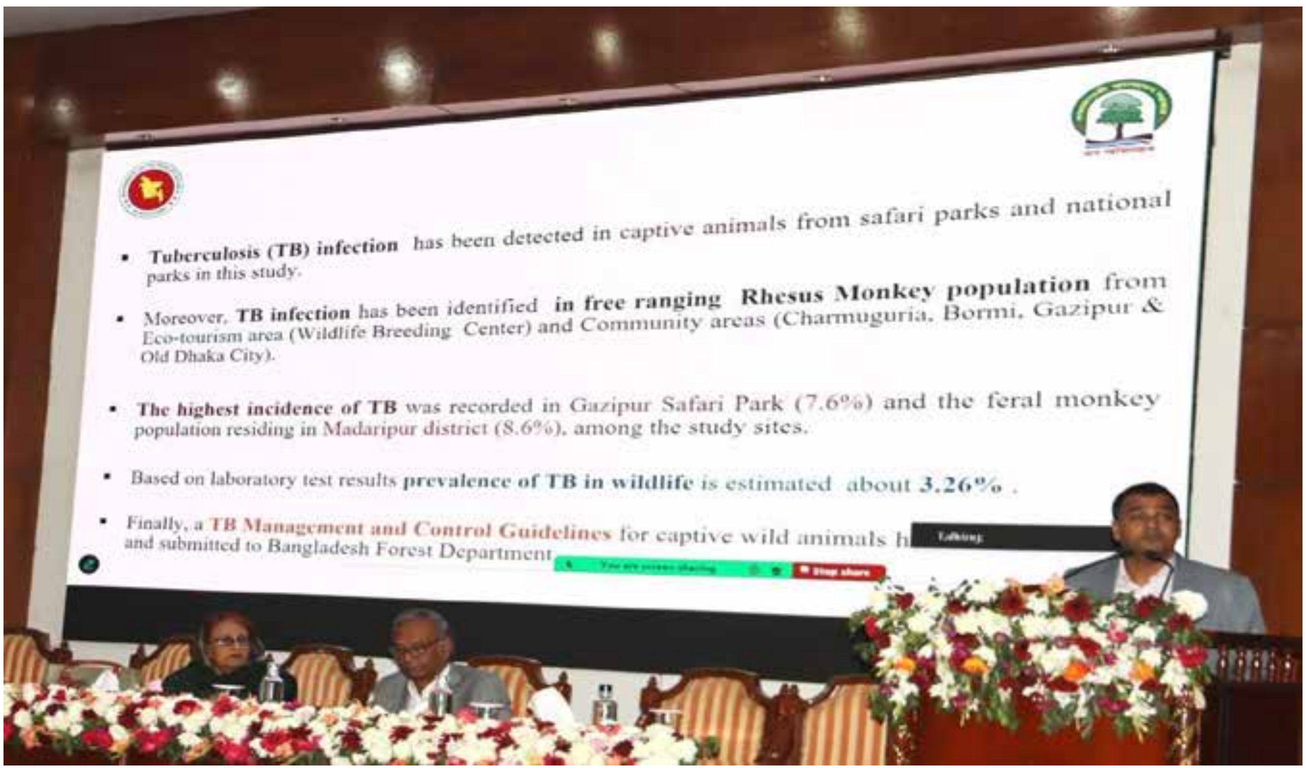

Figure-23: Dr. Mohammad Nizam Uddin Choudhury, Principal Investigator of the "Detection & Management of Tuberculosis (TB) at Captive-Wild Interface in Bangladesh" presented the study findings in the Result Dissemination Workshop held on 20-21 January, 2025 at Bana Bhaban Agargaon, Dhaka.

Annexure

Research Proposal Evaluation Committee (RPEC)

Serial No.	Name and Designation	Rank
01	Mr. Gobinda Roy, Project Director, Sustainable Forests and Livelihoods (SUFAL) Project, Bana Bhaban, Agargaon, Dhaka-1207.	Convener
02	Conservator of Forests, Wildlife & Nature Conservation Circle, Dhaka.	Member
03	Director, Bangladesh Wildlife Center, Gazipur.	Member
04	Professor Dr. Golam Rakkibu, Forestry and Wood Technology Discipline, Khulna University, Khulna.	Member
05	Dr. Noor Jahan Sarkar, Professor (Retd.), Department of Zoology, University of Dhaka, Dhaka.	Member
06	Dr. Md. Mahabubar Rahman, Divisional Officer, Silviculture Genetics Department, Bangladesh Forest Research Institute, Chittagong.	Member
07	Deputy Project Director-1, Sustainable Forests and Livelihoods (SUFAL) Project, Bana Bhaban, Agargaon, Dhaka.	Member-Secretary

Grant Review Committee (GRC)

Serial No.	Name and Designation	GRC Rank
01	Mr. Md. Amir Hosain Chowdhury, Chief Conservator of Forests, Forest Department, Bangladesh	Chairperson
02	Dr. Rafiqul Haider, Director, Bangladesh Forest Research Institute (BFRI), Chattogram	Member
03	Prof. Dr. Mohammed Mostafa Feeroz, Pro-vice chancellor (education), Jahangirnagar University.	Member
04	Professor Dr. A.Z.M Manzoor Rashid, Department of Forestry and Environmental Science, Shahjalal University of Science and Technology (SUST), Sylhet.	Member
05	DCCF, Planning Wing, Forest Department. Bana Bhaban, Agargaon, Dhaka-1207	Member
06	DCCF, Education and Training Wing, Forest Department, Bana Bhaban, Agargaon, Dhaka-1207.	Member
07	DCCF, Forest Management Wing, Forest Department, Bana Bhaban, Agargaon, Dhaka-1207.	
08	Mr. Gobinda Roy, Project Director, Sustainable Forest and Livelihood (SUFAL) Project, Bana Bhaban, Agargaon, Dhaka-1207.	Member-Secretary

Forestry Related Research Review Council (FRRRC)

Serial No.	Name and Designation	Rank
01	Md. Rakibul Hasan Mukul, Deputy Chief Conservator of Forests, Social Forestry Wing, Bana Bhaban, Agargaon, Dhaka	Convener
02	Dr. Mohammed Kamal Hossain, Professor (Retd.), Institute of Forest and Environmental Sciences, Chattogram University, Chattogram.	Member
03	Dr. Mohammad Al-Amin, Professor, Institute of Forest and Environmental Sciences, Chattogram University, Chattogram.	Member
04	Dr. Kazi Saiful Islam, Professor, Department of Urban and Rural Planning, Khulna University.	Member

Serial No.	Name and Designation	Rank
05	Dr. Saleh Ahmed Khan, Professor, Department of Botany, Jahangir Nagar University, Savar, Dhaka	Member
06	Dr. A.Z.M Monzoor Rashid, Professor, Department of Forestry and Environmental Science, Shahjalal University of Science and Technology, Sylhet.	Member
07	Mr. Mohammad Yosuf, Deputy Project Director-1, Sustainable Forest and Livelihood (SUFAL) Project, Ban Bhawan, Agargaon, Dhaka.	Member-Secretary

Wildlife Related Research Review Council (WRRRC)

Serial No.	Name and Designation	Rank
01	Mr. Md. Jahidul Kabir, Deputy Chief Conservator of Forests, Education and Training Wing, Bana Bhaban, Agargaon, Dhakap	Convener
02	Dr. Noor Jahan Sarkar, Professor (Retd.), Department of Zoology, University of Dhaka.	Member
03	Dr. Monirul Hasan Khan, Professor and Head of Department, Department of Zoology, Jahangirnagar University.	Member
04	Dr. Md. Zakir Hossain Hawlader, Professor, Biotechnology and Molecular Biology, University of Dhaka.	Member
05	Dr. Md. Shamsul Alam, Professor, Department of Fisheries Biology and Genetics, Bangladesh Agricultural University, Mymensingh.	Member
06	Mr. Md. Sanaullah Patwary, Conservator of Forests, Wildlife and Nature Conservation Area, Bana Bhaban, Agargaon, Dhaka.	Member
07	Mr. Md. Abdullah Abraham Hossain, Deputy Project Director-3, Sustainable Forests and Livelihoods (SUFAL) Project, Bana Bhaban, Agargaon, Dhaka	Member-Secretary

Deliverable Review and Comments Giving Committee

Serial No.	Name and Designation	Rank
01	Mr. Mohammad Yousuf, Deputy Project Director-1, Sustainable Forests and Livelihoods (SUFAL) Project, Bana Bhaban, Agargaon, Dhaka.	Chairperson
02	Mr. Ishtiaq Uddin Ahmad, Project Management Specialist, Sustainable Forests and Livelihoods (SUFAL) Project, Bana Bhaban, Agargaon, Dhaka.	Member
03	Mr. Md. Safiul Alam Chowdhury, Senior Monitoring & Evaluation Specialist, Sustainable Forests and Livelihoods (SUFAL) Project, Bana Bhaban, Agargaon, Dhaka.	Member
04	Mr. Md. Abdul Mabud, Senior Collaborative Forest Management Specialist, Sustainable Forests and Livelihoods (SUFAL) Project, Bana Bhaban, Agargaon, Dhaka.	Member
05	Dr. Md. Jahangir Hossain, Senior Livelihoods Specialist, Sustainable Forests and Livelihoods (SUFAL) Project, Bana Bhaban, Agargaon, Dhaka.	Member
06	Mr. Md. Mahbubur Rahman, Deputy Project Director-2, Sustainable Forests and Livelihoods (SUFAL) Project, Bana Bhaban, Agargaon, Dhaka.	Member
07	Mr. Md. Mehedi Hasan Khan, Assistant Project Director-3, Sustainable Forests and Livelihoods (SUFAL) Project, Bana Bhaban, Agargaon, Dhaka.	Member-Secretary

গণপ্রজাতন্ত্রী বাংলাদেশ সরকার

বন অধিদপ্তর