APPENDIX D BIODIVERSITY BASELINE METHODOLOGY

D BIODIVERSITY BASELINE METHODOLOGY

D.1 DESKTOP REVIEW

A desktop review was undertaken to provide an initial understanding of the habitat and species within the Project Area, areas of potential biodiversity importance that may be affected by the project and to identify where critical data gaps may exist. The gap analysis was used to guide follow-up field surveys undertaken in July and August 2024 within the Project area and its proximity, which, along with secondary data and expert consultation (***Section D.2***) allowed development of a reasonably complete baseline biodiversity data set adequate for completion of the ESIA and associated Critical Habitat Assessment (CHA). This process was used to identify habitat contiguity, habitats of conservation significance (e.g., protected areas, key biodiversity areas, etc.) and probable hotspots for migratory and congregatory species. A list of key data used in the desktop review are listed in ***Table D-1*****;** additional secondary sources are referenced as footnotes in the respective chapters.

TABLE D-1 KEY SECONDARY DATA SOURCES

No.	Source	Purpose	Link
1.	Integrated Biodiversity Assessment Tool (IBAT) for Business	The IBAT tool screens a potential location for ecological sensitivity and provides a list of species occurring within a 50 km radius. The tool also highlights potential Critical Habitat triggers by determining proximity to migration pathways, legally protected areas and key biodiversity areas. An IBAT analysis was carried out with respect to the Project, under license 35468-63737, from the 9 May 2024.	None; IBAT is a licensed product that ERM has obtained for the purpose of biodiversity screening across projects.
2.	IUCN Red List for Threatened Species online version	The IUCN Red List provides a list of threatened species by classifying them from Least Concern (LC) to Critically Endangered (CR) through an understanding of their global distribution, population numbers, and trends in population decline and stresses on the species. As part of the classification, the global distribution and habitat preference of the species is described.	https://www.iucnredlist.org/
3.	Birdlife Data Zone	BirdLife International maintains a database on Endemic Bird Areas (EBA) and Important Bird Areas (IBAs) that provides a list of species found in these designated areas, measure of sensitivity of these habitats and identifies migratory, congregatory and threatened species in the area.	http://datazone.birdlife.org/home

No.	Source	Purpose	Link
4.	ebird.org	ebird.org provides a geo-referenced list of identified bird species in a given area. The ebird.org database was used to confirm presence/absence of bird species within the Project landscape.	https://ebird.org/home
5.	UNESCO World Heritage Site	This provides an insight into the network of "Biosphere Reserve" and "Natural World Heritage Sites"	https://whc.unesco.org/
6.	Alliance for Zero Extinction Sites	To identify proximity to any declared Alliance for Zero Extinction Sites	https://zeroextinction.org/
7.	Global Biodiversity Information Facility (GBIF) and iNaturalist	GBIF is an international network and research infrastructure funded by the world's governments and aimed at providing anyone, anywhere, open access to data about all types of life on Earth.	https://www.gbif.org/ https://www.inaturalist.org/
8.	World Database on Protected Areas	The World Database on Protected Areas is one of the largest assembly of data on the world's terrestrial and marine protected areas.	https://www.protectedplanet.net/en
9.	Ramsar convention	The Convention on Wetlands is the intergovernmental treaty that provides the framework for the conservation and wise use of wetlands and their resources.	https://www.ramsar.org/

D.2 EXPERT CONSULTATION

ERM conducted consultations with various Bhutanese species and conservation experts in July and August 2024 to gain a deeper understanding of the biological landscape of the Project area (***Table* D-2**).

TABLE D-2 KEY EXPERTS CONSULTED

No.	Name	Designation	Expertise	Engagement format
1	Phub Dhendup	Chief Forestry Officer, Ministry of Agriculture and Forests, Sarpang	Wildlife Management and Human Wildlife Conflict	Physical meeting and email correspondence
2	Dorji Rabten	Chief Forestry Officer, Phibsoo Wildlife Sanctuary	Wildlife management, biodiversity and ecology	Physical meeting and email correspondence
3	Samten Wangchuk	Forest Officer, Royal Manas National Park	Wildlife management, biodiversity and ecology	Physical meeting and email correspondence

No.	Name	Designation	Expertise	Engagement format
4	Dorji Wangchuk	Forest Officer, Royal Manas National Park	Wildlife management, biodiversity and ecology	Physical meeting
5	Sonam Tshewang	Officer, The National Development Centre for Aquaculture	Fish diversity and ecology	Physical meeting
6	Drukpola	Program director of Ecological Conservation and Fish Species, Sarpang	Fish diversity and ecology	Physical meeting
7	Jigme Tshering	Chief of Gelephu Thromde	Ecology of Sarpang	Physical meeting
8	Sherab Gyeltshen	Environment Officer of Gelephu Thromde	Ecology of Sarpang	Physical meeting
9	Sherub Sherub	Professor at Ugyen Wangchuck Institute for Conservation and Environment	Ecology and species diversity of Sarpang	Online meeting
10	Kinley Tenzin (PhD)	Executive Director, Royal Society for Protection of Nature	Biodiversity and conservation initiatives	Physical meeting
11	Chogyal Tenzin	Senior Program Officer, Civil Society Organizations Authority	Funding management of biodiversity-related initiatives	Physical meeting
12	Ugyen Dorji	Dean of Student Affairs, College of Natural Resources, Royal University of Bhutan	Aquatic ecology	Physical meeting

D.3 SUPPLEMENTAL FIELD SURVEYS

Supplemental field surveys were undertaken to improve the quality of the baseline data and better inform the project ESIA and CHA targeted the following:

- Flora composition and habitat health;
- Faunal presence / absence including:
  - Mammals;
  - Birds;
  - Herpetofauna;
  - Ichthyofauna; and
  - Macrozoobenthos.

It should be noted that, due to survey limitations, the species counts likely underestimate the true population. While the survey confirms the presence of species observed, it cannot definitively rule out the presence of others that were not detected.

D.3.1 TERRESTRIAL SURVEY

The terrestrial flora and fauna survey was conducted in the monsoon season from 12 July - 29 August 2024. In Bhutan, the monsoon season typically starts in June and lasts until September, bringing heavy rainfall, especially in the southern regions. Post-monsoon rains can also occur in October and November.

For the purpose of biodiversity management, the Government of Bhutan has divided the country into 2424 monitoring grids of 4x4 km, called Biodiversity Monitoring Grids (BMG). Each BMG is assigned a numeric code, with grid numbers starting from 0001 to 2424. The survey area included the proposed road alignment and a 750 m assessment buffer either side of the Project. Considering the survey covered a smaller area along the proposed highway, a grid size of 1x1 km or 2x2 km (camera trap) was adopted instead of the 4x4 km described in the Biodiversity Monitoring and Social Survey Protocol of Bhutan[24], (***Table D-3***). The smaller grid ensures that biodiversity monitoring is tailored to the scale of the potential Impact Area and are nested within the country-level 4x4 grid.

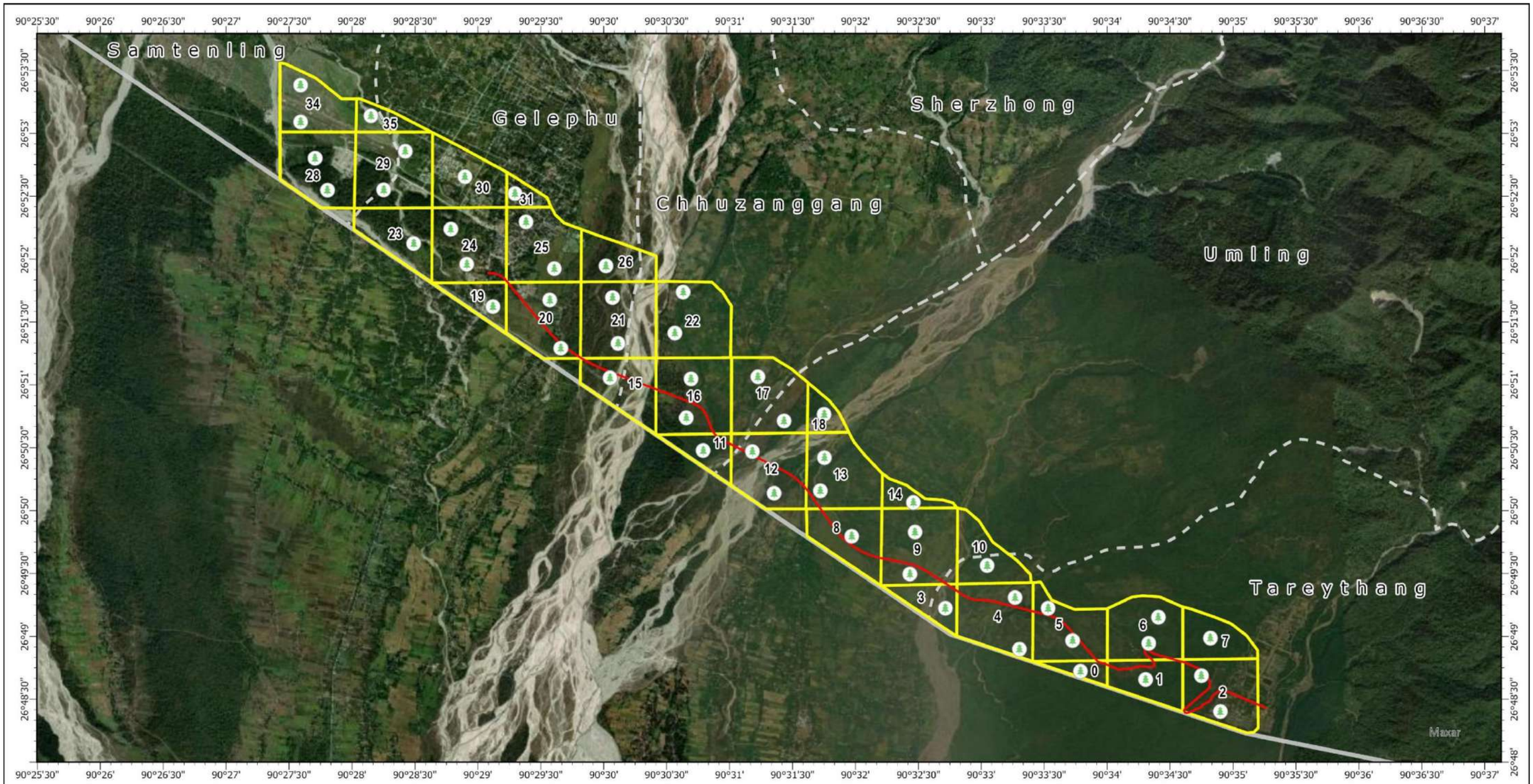
[24] DoFPS 2020. Biodiversity Monitoring and Social Surveying Protocol of Bhutan, Department of Forests and Park Services, Ministry of Agriculture and Forests, Thimphu, Bhutan.

TABLE D-3 TERRESTRIAL SURVEY METHODOLOGY

Survey	Date	Survey Technique	Survey Methods	Survey Effort
Flora (***Figure D-1***)	12 July – 02 August 2024	Quadrat survey	Inside the defined grid for the baseline survey, entering 200 m from the grid's boundary, two plots of 20x20 m were established as far as possible along the altitudinal gradient at 500 m apart. A vegetation plot inside the grid was chosen to represent the major forest type of the area. • Tree and shrub sampling: Both the trees (height > 1.3 m) and shrub species were recorded on the same data form as in Annexure 1 of the Flora Monitoring Protocol of Bhutan, 2020 (FMPB). Recorded data included Species name, Diameter at Breast Height (DBH), height, status, and stem type. • Herbs: a sub-plot size of 2x2 m quadrate was established and herbs data was recorded as in Annexure 2 of the FMPB, 2020. Species, height, and crown cover C (%) were noted. • Regeneration data collection: Regeneration data was collected inside the same herb plot (2x2 m). The data was collected as per Annexure 3 of the FMPB, 2020. Recorded data included Species, height, and age. • Epiphytic plants: Epiphyte data was recorded in the vegetation plot as per Annexure 4 of the FMPB, 2020.	71 vegetation plots were surveyed.
Bird (***Figure D-2***)	15 July – 02 August 2024	Transect survey	The survey aligns with the Bhutan Bird Monitoring Protocol (BBMP). The survey team walked along the existing path / road or proposed road alignment within the survey area in the morning (05:00 – 11:00) and evening (16:00 – 19:00) when birds are active. Birds were recorded when seen directly or from the call using the MacKinnon Listing Method. The MacKinnon List method listed all birds seen or heard in chronological order of detection.	A total of 33 grids were surveyed with a total of 58.65 km of transect.
Herpetofauna (***Figure D-3***)	13 July – 30 July 2024	Transect survey	The survey team walked along the transects from 8 am to 6 pm and occasionally 9 pm. Each transect lengths from 0.7 km to 3.2 km. Species presence was recorded either through visual encounter or sound. All the species detected or observed were photographed (mobile camera). The nocturnal Auditory Amphibian Counts method was used, which involved surveyors reaching the water bodies at night and listening to each wetland or water bodies. Those species with doubtful or unknown identities were captured/caught; amphibians and lizards with Frye net and hand with gloves and snake species using hook, tong or forked sticks, for proper observation and photography for identity confirmation. Night survey (19:00 – 22:00) was conducted for only transect in grid no. 9 given the inaccessibility from the base camp and Health and Safety risks related to elephants.	A total of 33 grids were surveyed with a total of 56.73 km of the transect.
Large and medium-sized mammals (***Figure D-4***)	13 July – 30 July 2024	Transect survey	The transect sampling was designed by the Distance for Windows software[25] with 1-2km transect line laid within a grid of 1x1 km covering the major habitat type. On occasion, a transect is continued into adjacent grid. A group of four (04) members were deployed for the data collection. Two (02) observers walked at the front (one observed the ground evidence and the other observed the tree canopy) followed by the data recorder. The security person is last. All sightings and signs, i.e., droppings, tracks, scrape marks, hairs, and scent marks were recorded along with a photo reference.	A total of 41.9 kilometers of transects were surveyed.
Mammals (***Figure D-5***)	13 July – 29 August 2024	Camera trap	A grid size of 2x2km was laid out along the Project Area spanning a length of 18 km. Camera trap Reconyx HC500 Hyperfire was used to capture five (05) consecutive images per trigger, during daytime and nighttime with no delay between triggers. Camera sensitivity was set to medium-high to detect small species. For each grid, two (02) camera stations were designated, with one camera installed at each station. Camera trap was placed at a height of 45-60 cm from the ground.	A total of 40 camera traps were installed with the number of trap nights of 17.26±2.06 per 2x2 km grid. Notably, three (03) camera traps were lost.

[25] Thomas, L., Buckland, S. T., Rexstad, E. A., Laake, J. L., Strindberg, S., Hedley, S. L., ... & Burnham, K. P. (2010). Distance software: design and analysis of distance sampling surveys for estimating population size. *Journal of Applied Ecology*, *47*(1), 5-14.

FIGURE D-1 TERRESTRIAL FLORA SURVEY



Legend

- Flora Plot Locations
- Preferred Road Alignment
- Biodiversity Survey Grid

Administrative Boundary

- Bhutan National Border
- Gewog Boundary

0 1.3 2.6 km

Scale: 1:60,000

Coordinate System: GCS WGS 1984

Service Credit: Esri, NASA, NGA, USGS, Esri, © OpenStreetMap contributors, TomTom, Garmin, Foursquare, METI/NASA, USGS

N

Locations of Flora Plot Survey

0737107 GMC ESIA Road and Bridges

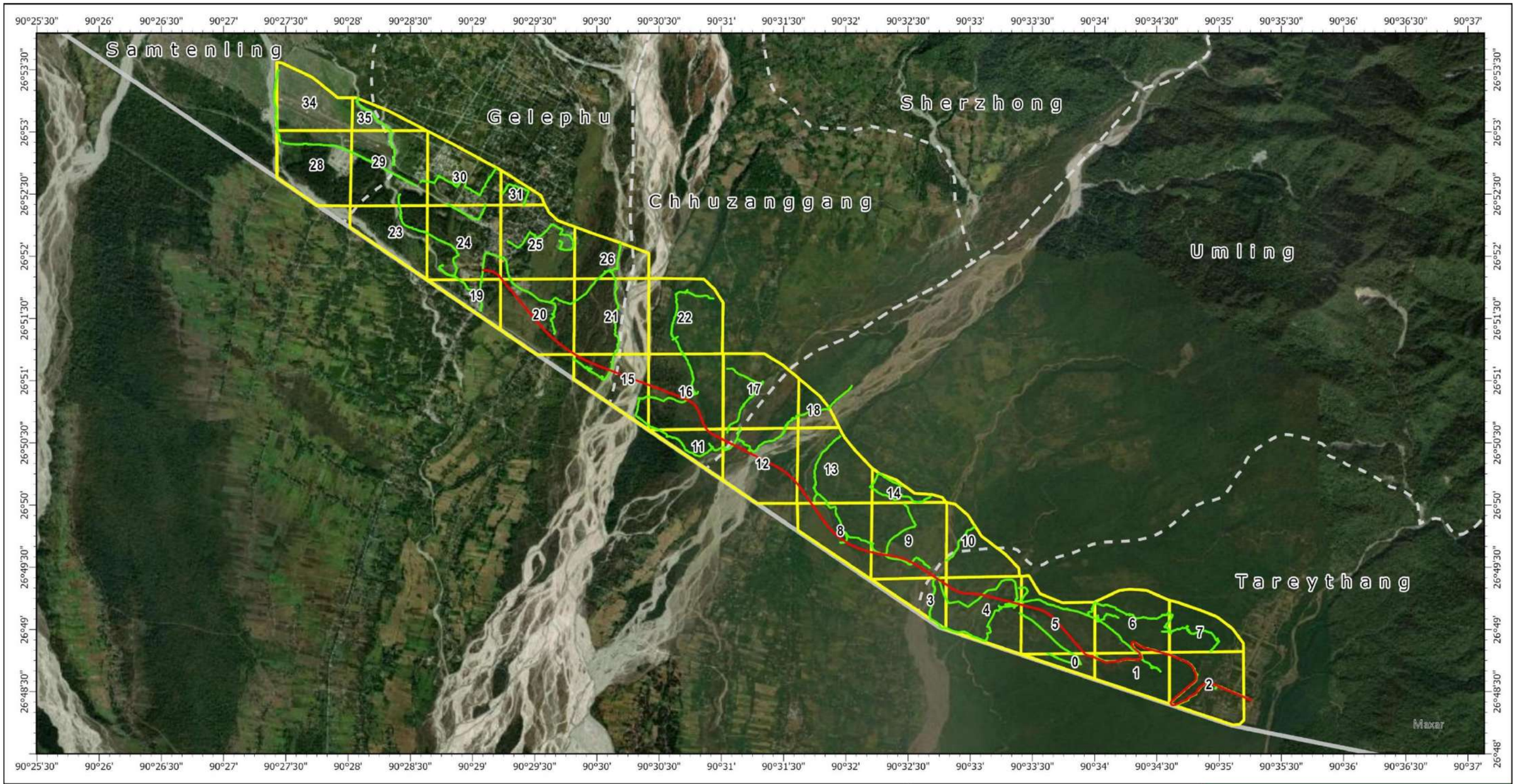
Date: 18/11/2024

Drawn By: KS

Review By: MS

ERM

FIGURE D-2 BIRD TRANSECT SURVEY



Legend

- Bird Transect Routes
- Preferred Road Alignment
- Biodiversity Survey Grid

Administrative Boundary

- Bhutan National Border
- Gewog Boundary

0 1.3 2.6 km

N

Scale: 1:60,000

Coordinate System: GCS WGS 1984

Service Credit: Esri, NASA, NGA, USGS, Esri, © OpenStreetMap contributors, TomTom, Garmin, Foursquare, METI/NASA, USGS

Locations of Birds Transect Survey

0737107 GMC ESIA Road and Bridges

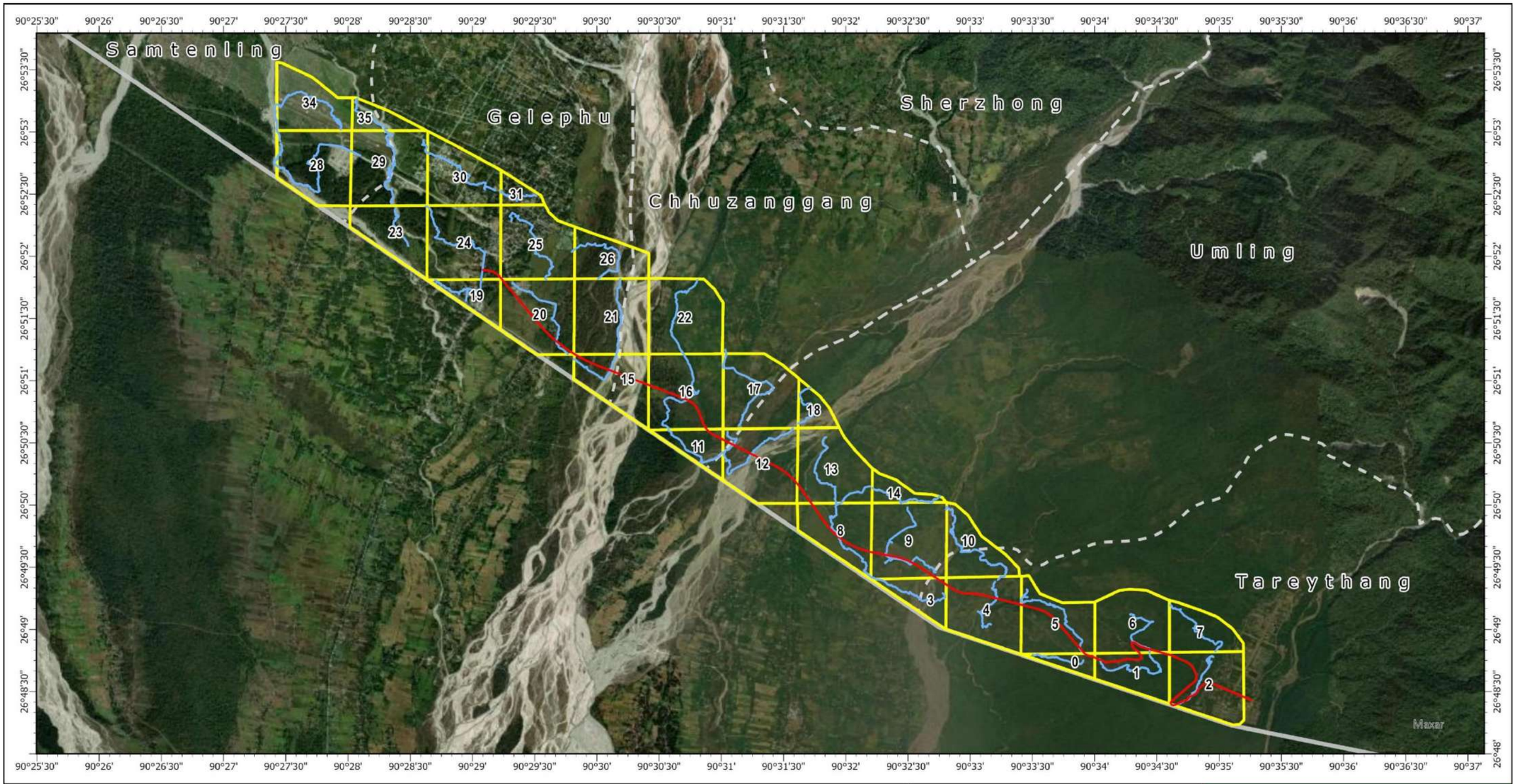
Date: 25/11/2024

Drawn By: KS

Review By: MS

ERM

FIGURE D-3 TERRESTRIAL HERPETOFAUNA TRANSECT SURVEY



Legend

- Herpetofauna Transect Routes
- Preferred Road Alignment
- Biodiversity Survey Grid

Administrative Boundary

- Bhutan National Border
- Gewog Boundary

0 1.3 2.6 km

Scale: 1:60,000

Coordinate System: GCS WGS 1984

Service Credit: Esri, NASA, NGA, USGS, Esri, © OpenStreetMap contributors, TomTom, Garmin, Foursquare, METI/NASA, USGS

N

Locations of Herpetofauna Transect Survey

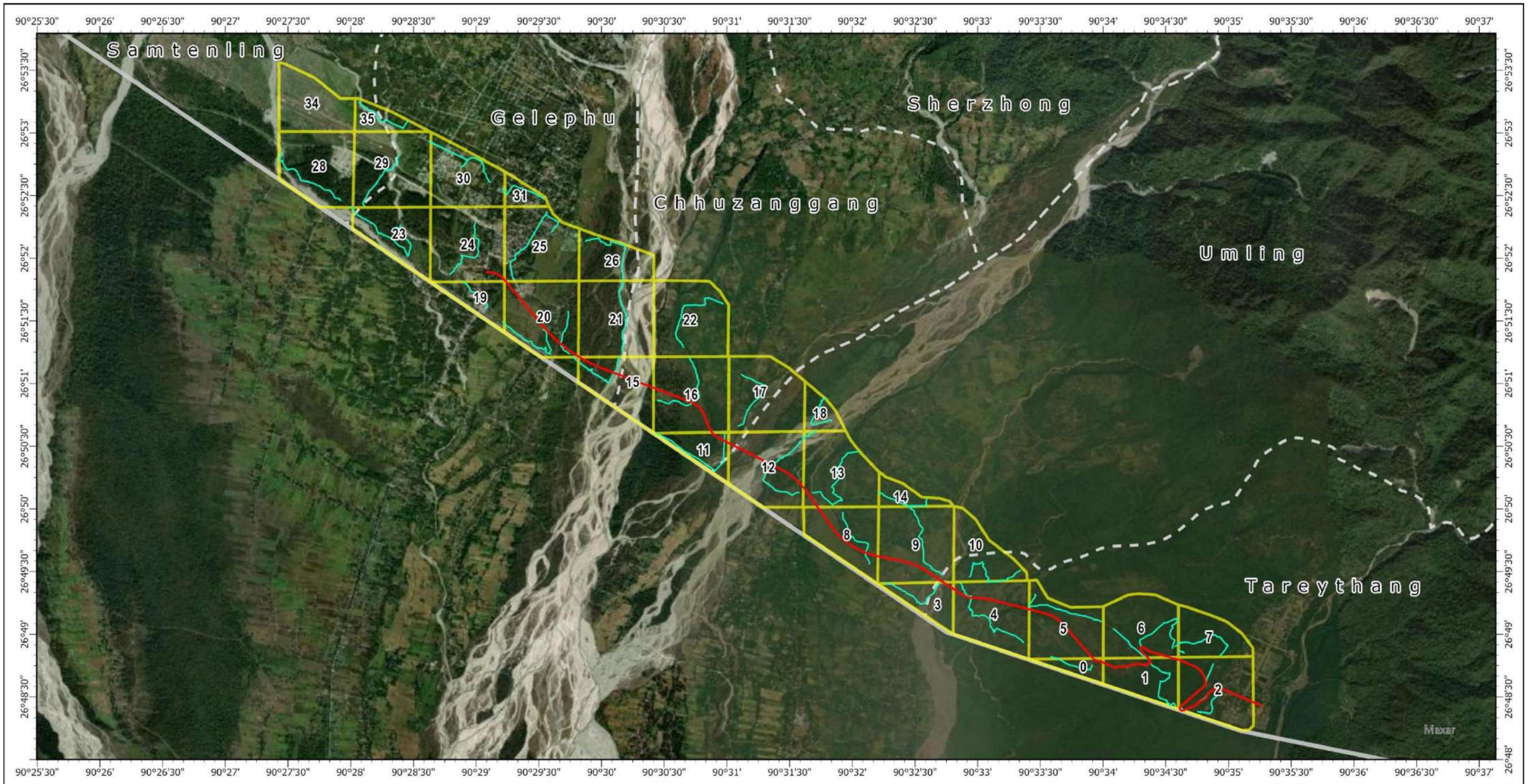
0737107 GMC ESIA Road and Bridges

Date: 25/11/2024

Drawn By: KS
Review By: MS

ERM

FIGURE D-4 TERRESTRIAL MAMMAL TRANSECTS SURVEY



Legend

- Mammal Transect Routes
- Preferred Road Alignment
- Biodiversity Survey Grid

Administrative Boundary

- Bhutan National Border
- Gewog Boundary

0 1.3 2.6 km

N

Scale: 1:60,000

Coordinate System: GCS WGS 1984

Service Credit: Esri, NASA, NGA, USGS, Esri, © OpenStreetMap contributors, TomTom, Garmin, Foursquare, METI/NASA, USGS

Locations of Mammal Transect Survey

0737107 GMC ESIA Road and Bridges

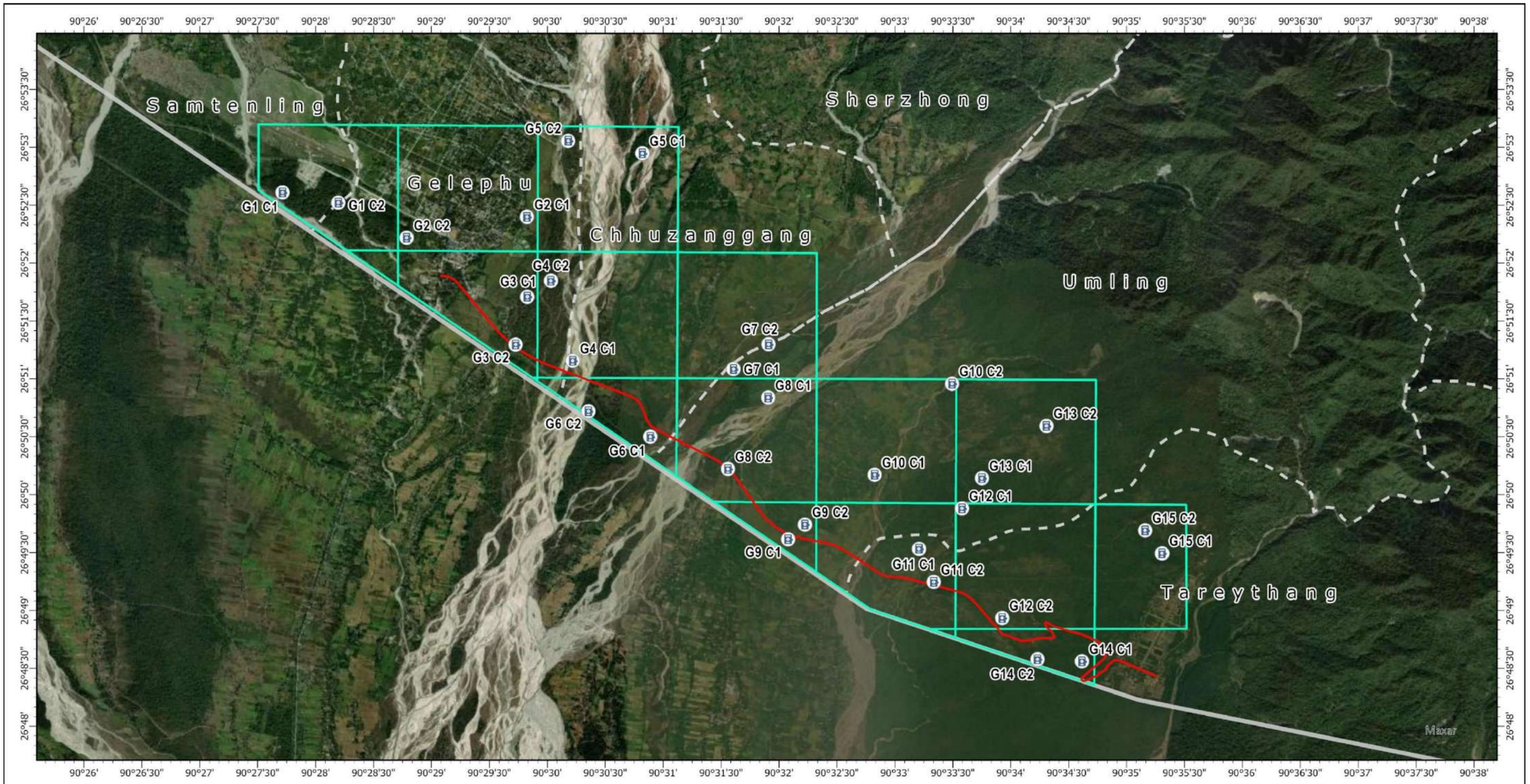
Date: 25/11/2024

Drawn By: KS

Review By: MS

ERM

FIGURE D-5 CAMERA TRAP SURVEY



Legend

- Preferred Road Alignment
- Camera Trap Locations
- Camera Trap Grid

Administrative Boundary

- Bhutan National Border
- Gewog Boundary

0 1.4 2.8 km

N

Scale: 1:65,000

Coordinate System: GCS WGS 1984

Service Credit: Esri, NASA, NGA, USGS, Esri, © OpenStreetMap contributors, TomTom, Garmin, Foursquare, METI/NASA, USGS

Data Source(s):
1. Divisional Forest Office, Sarpang

Locations of Camera Trap Installation

0737107 GMC ESIA Road and Bridges

Date: 25/11/2024

Drawn By: KS
Review By: MS

ERM

D.3.2 AQUATIC SURVEY

Samples were collected following a systematic random sampling technique (***Table D-4***). Sampling points were laid with one (01) km between each of the five (05) sampling Plots.[26] A 200 m transect line which is also known as sample stretch was laid in one (01) km sample distance for collection of data. [27] Plots 1, 4 and 5 were in upstream Mau River; Plot 2 was situated along a branch of the Taklai River; and Plot 3 was in upstream Taklai River (***Figure D-6***).

TABLE D-4 AQUATIC SURVEY METHODOLOGY

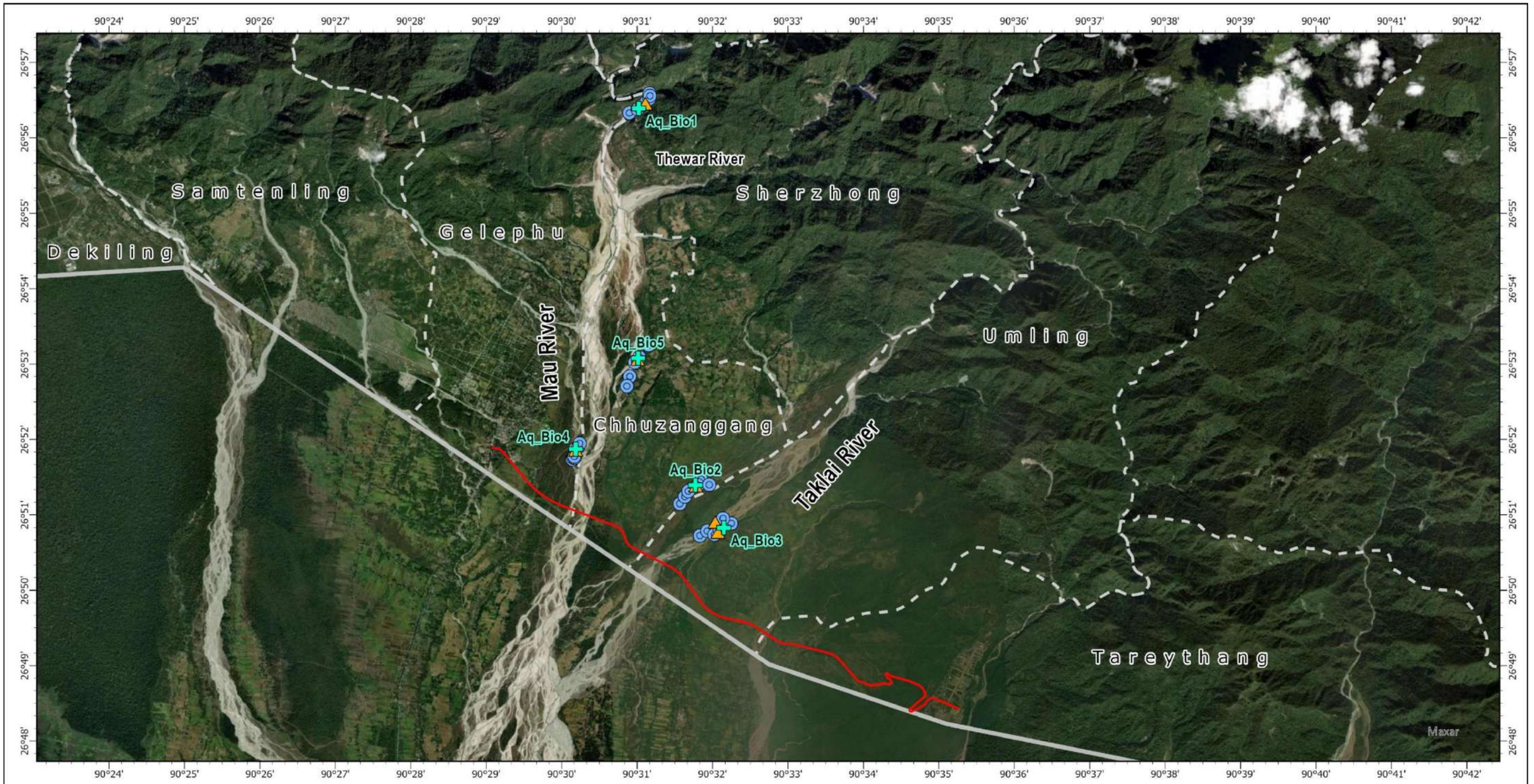
Survey	Date	Survey Technique	Survey Methods	Survey Effort
Fish sampling	5-8 August 2024	Multiple sampling methods	Fish were sampled using fishing gears like cast net, spinner hook, temporary river diversion, seine net and electro-shocker. The catch-and-release method was adopted. A cast net of three (03) meters radius was used for the sampling and the distance of two sample points was 50 meters each stretch covered up to one (01) km. Coupled with the cast net, wherever possible, a Seine net was used via the rock flip and kick sampling method. The coordinates, water depth and mean water velocity of the site where the fish were caught were recorded using an AVFM 6.1 Area Velocity Flow Meter.	Five (05) aquatic plots
Macroinvertebrate Sampling	5-8 August 2024	Kick sampling	Kick-sampling technique with a D-frame net (1x1 feet; 600 µm net mesh) was used to collect macroinvertebrates (Gretchen, 2007). Simultaneously, mosquito nets from the local market were also used to maximize the collection and effort. To guarantee that most macroinvertebrates were collected, the substrate was disturbed and scooped with the net multiple times. Three replicates of samples were collected from the sampling sites covering all representative habitat types: pool, riffle and run within the project sites.	Five (05) aquatic plots
Water quality	5-8 August 2024	Electrometric and instrumental analysis	A total of six water quality parameters, i.e., pH (electrometric), temperature (electrometric), conductivity (electrometric), TDS (electrometric), Turbidity (instrumental) and dissolved	Five (05) aquatic plots

[26] Gyeltshen, T. (2018). Assessment of fish diversity in Bhutan: A systematic approach. Journal of Bhutan Studies, 38, 45-60.

[27] Wangmo, T., & Rai, S. (2019). Sampling techniques for ecological studies in Bhutan. Bhutan Journal of Ecology and Conservation, 2(1), 15-25. Zhang, Y., Liu, H., & Chen, X. (2023). Urbanization impacts

Survey	Date	Survey Technique	Survey Methods	Survey Effort
			oxygen (Winkler methods with azide modification) were analyzed for the water samples collected from the two (02) sampling plots.	

FIGURE D-6 AQUATIC FAUNA SAMPLING STATIONS



Legend

- Fish Sampling Location
- Macroinvertebrate Sampling Location
- Aquatic Plot
- Preferred Road Alignment

Administrative Boundary

- Bhutan National Border
- Gewog Boundary

0 2.2 4.4 km

N

Scale: 1:100,000

Coordinate System: GCS WGS 1984

Service Credit: Esri, NASA, NGA, USGS, Esri, © OpenStreetMap contributors, TomTom, Garmin, Foursquare, METI/NASA, USGS

Data Source(s):
1. College of Natural Resources

Locations of Aquatic Fauna Survey

0737107 GMC ESIA Road and Bridges
Date: 25/11/2024
Drawn By: KS
Review By: MS

ERM