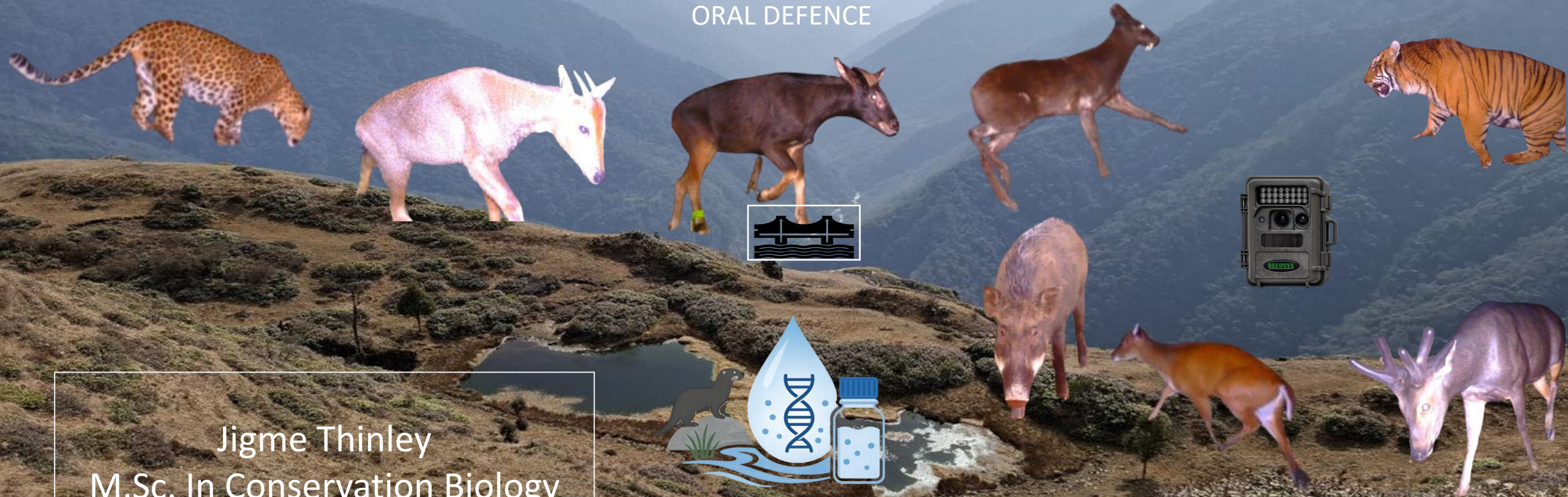




Diversity and Functional Connectivity of Mammals in Biological Corridor Seven (BC7), Bhutan



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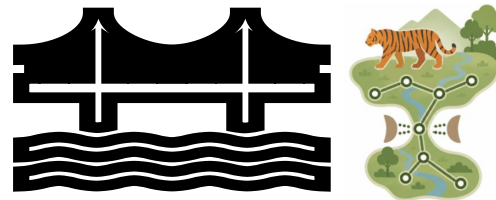


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1.1 Background

Mammal conservation depends on movement, site use, and ecological connectivity (Brodie et al., 2025; Hilty et al., 2020a).

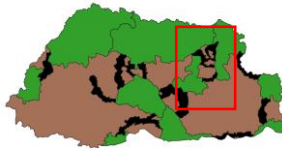
In mountain landscapes, topography, habitat heterogeneity, and human land use shape community structure and movement (Brodie et al., 2025; Penjor et al., 2024).

Bhutan provides a strong connectivity context, with $\geq 60\%$ of its forest area under constitutional mandate and a national PA–BC network (DoFPS, 2025).

BC7 links WCNP, PNP, and BWS across a multiple-use landscape in north-eastern Bhutan (DoFPS, 2025).

But corridor effectiveness cannot be assumed from structural linkage alone; it requires empirical ecological evidence (Poor et al., 2024; Rezvani et al., 2024).

BC7 is structurally important, but its functional effectiveness for mammals must be tested.



1.2 Problem statement

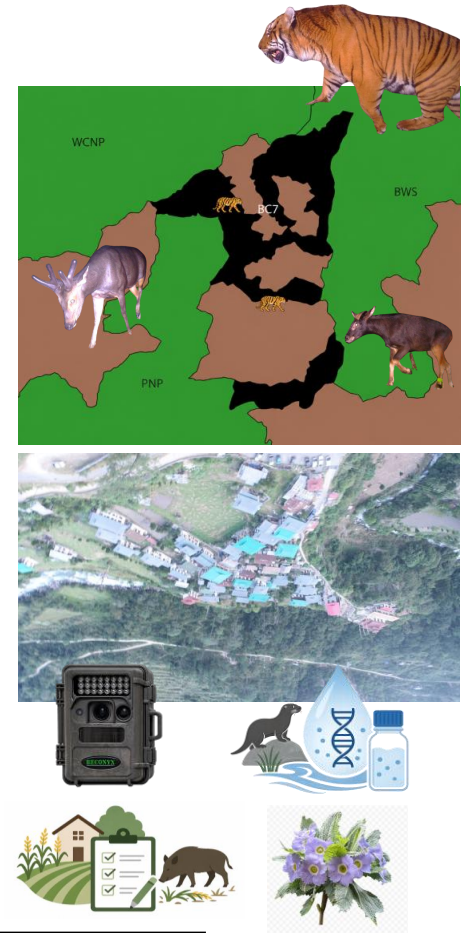
BC7 connects WCNP, PNP, and BWS, but its functional effectiveness remains unclear (DoFPS, 2025).

Structural corridor status alone cannot indicate actual mammal use, predator-prey site use, or movement constraints (Hilty et al., 2020a; Rezvani et al., 2024).

BC7 is a multiple-use landscape with ecological and social pressures, including roads and energy infrastructure development (MFD, 2023; Rai et al., 2021).

Previous studies in Bhutan addressed parts of corridor function, but not an integrated BC7 assessment (Tenzin et al., 2022; Letro et al., 2022).

Therefore, this study was needed to generate the ecological and social evidence required for targeted corridor management, mitigation planning, and long-term conservation of BC7.



1.3 Research questions and objectives

1. How diverse are mammals in BC7 and how is community structure organised?

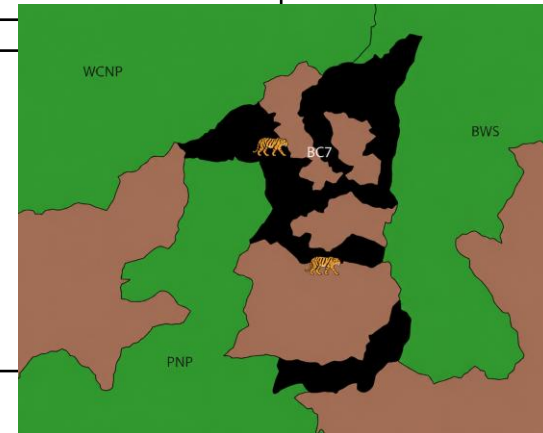
-Quantify mammal diversity, composition and functional groups in BC7.

2. How do predator and prey species use BC7 across space and time, and how do these patterns relate to environmental and social factors?

-Estimate occupancy, activity of predator and prey species, and relate these patterns to environmental and social factors.

3. To what extent does BC7 support tiger habitat suitability and connectivity?

-Map tiger habitat suitability and functional connectivity in BC7.



1.4 Significance of the study

Assesses BC7 as a multi-species corridor, not only a tiger corridor.

Integrates diversity, predator-prey relationships, and connectivity within a single landscape.

Links mammal activity with HWC.

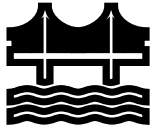
Uses a multi-method approach: camera traps, eDNA, spatial covariates and social surveys.

Applies occupancy modelling, prey-informed tiger modelling, and connectivity analysis.

Identifies pinchpoints and mitigation priorities for management.

Reduces the weakness of relying on one line of evidence in complex mountain landscapes.

This study provides an integrated ecological and social basis for evidence-based management of BC7.



2.0 Literature Review

Habitat loss and fragmentation threaten mammal persistence and ecosystem function (Hilty et al., 2020; Lacher et al., 2019).

Corridors matter when structural linkage translates into functional connectivity (Hilty et al., 2020).

Occupancy models help estimate site use while accounting for imperfect detection (Devarajan et al., 2020; MacKenzie et al., 2017).

Prey-informed connectivity is especially important in tiger landscapes (Letro et al., 2022; Sharma et al., 2025).

Camera traps and eDNA are complementary tools for corridor assessment (Deiner et al., 2017; Sherub et al., 2026).



In Bhutan, studies exist on inventory, prey occupancy, and conflict, but an integrated assessment has remained limited.



The literature supports an integrated, multi-method, prey-informed corridor assessment for BC7.

3.1 Study area

Location: northeast Bhutan

Linkage: WCNP, PNP, and BWS

Area: ~419 km²

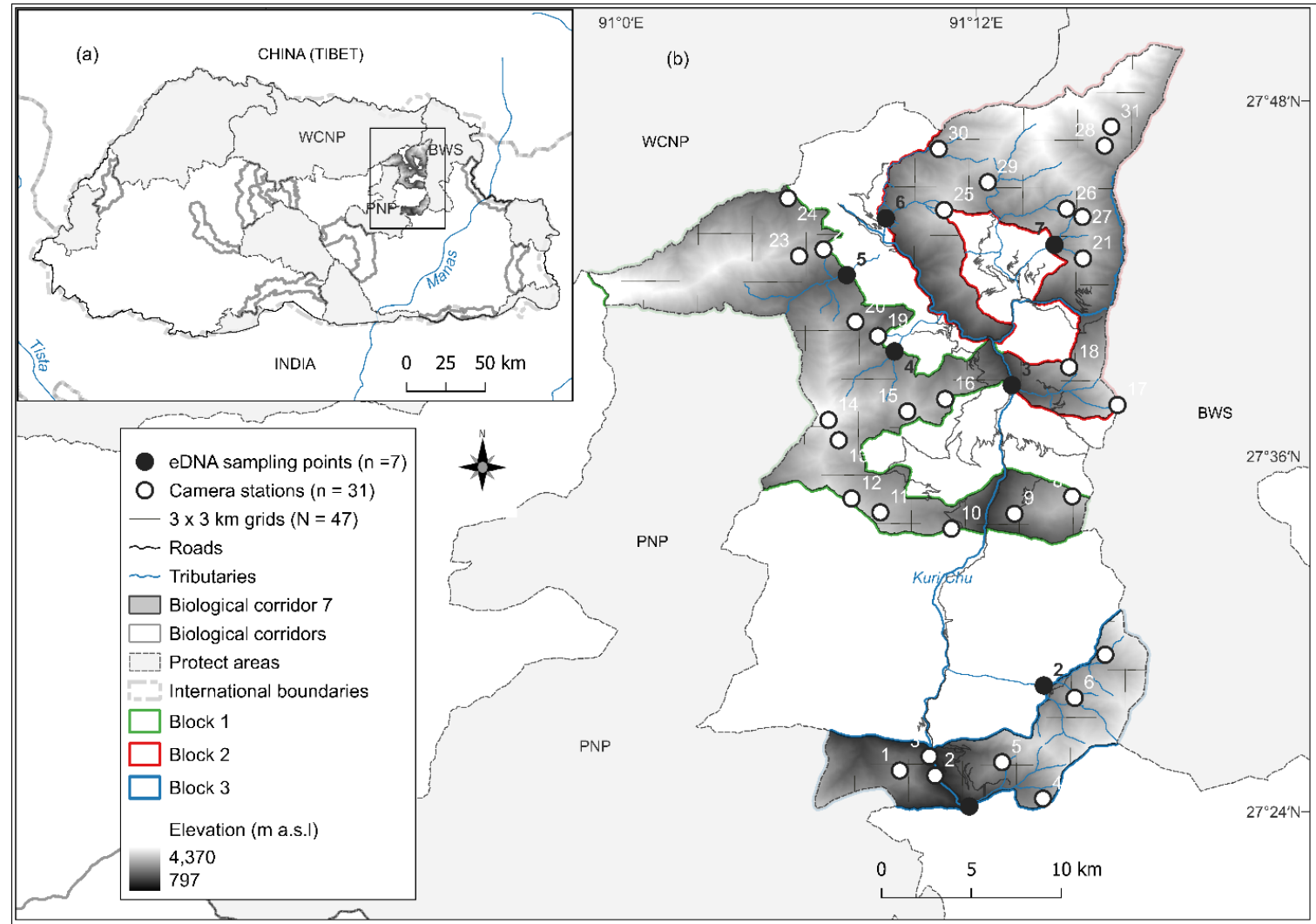
Elevation: 797–4,370 m a.s.l.

Temperature: 10.3–25.4°C

Precipitation: 34 mm (mean)

LULC: forest-dominated, multiple-use corridor

Includes: settlements (193), agriculture, grazing areas, roads, rivers, and streams



DoFPS, 2025; Mongar Forest Division (MFD), 2023; NCHM, 2025; Rai, 2022

Figure 3.1: Study area and sampling points

3.2 Sampling design

Field design at a glance

31

CT stations

62

cameras deployed

5,144

trap-nights

31

Vegetation plots

7

eDNA stream sites

117

HWC survey

Additional inputs

Environmental and anthropogenic spatial covariates (secondary)

Dhendup et al., 2019; DoFPS, 2023b; Letro et al., 2022; Sherub et al., 2026

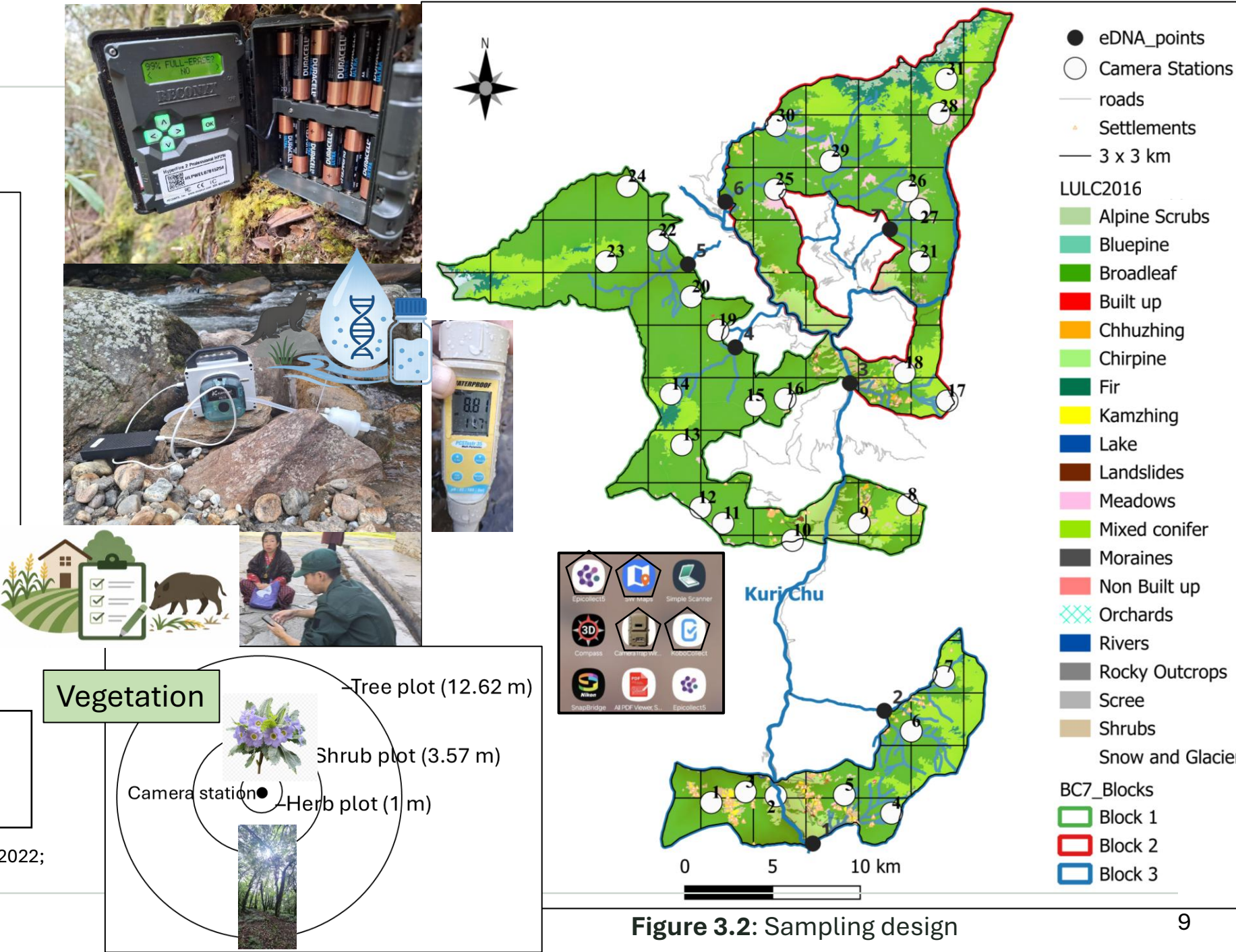


Figure 3.2: Sampling design

3.2.1 Conceptual framework

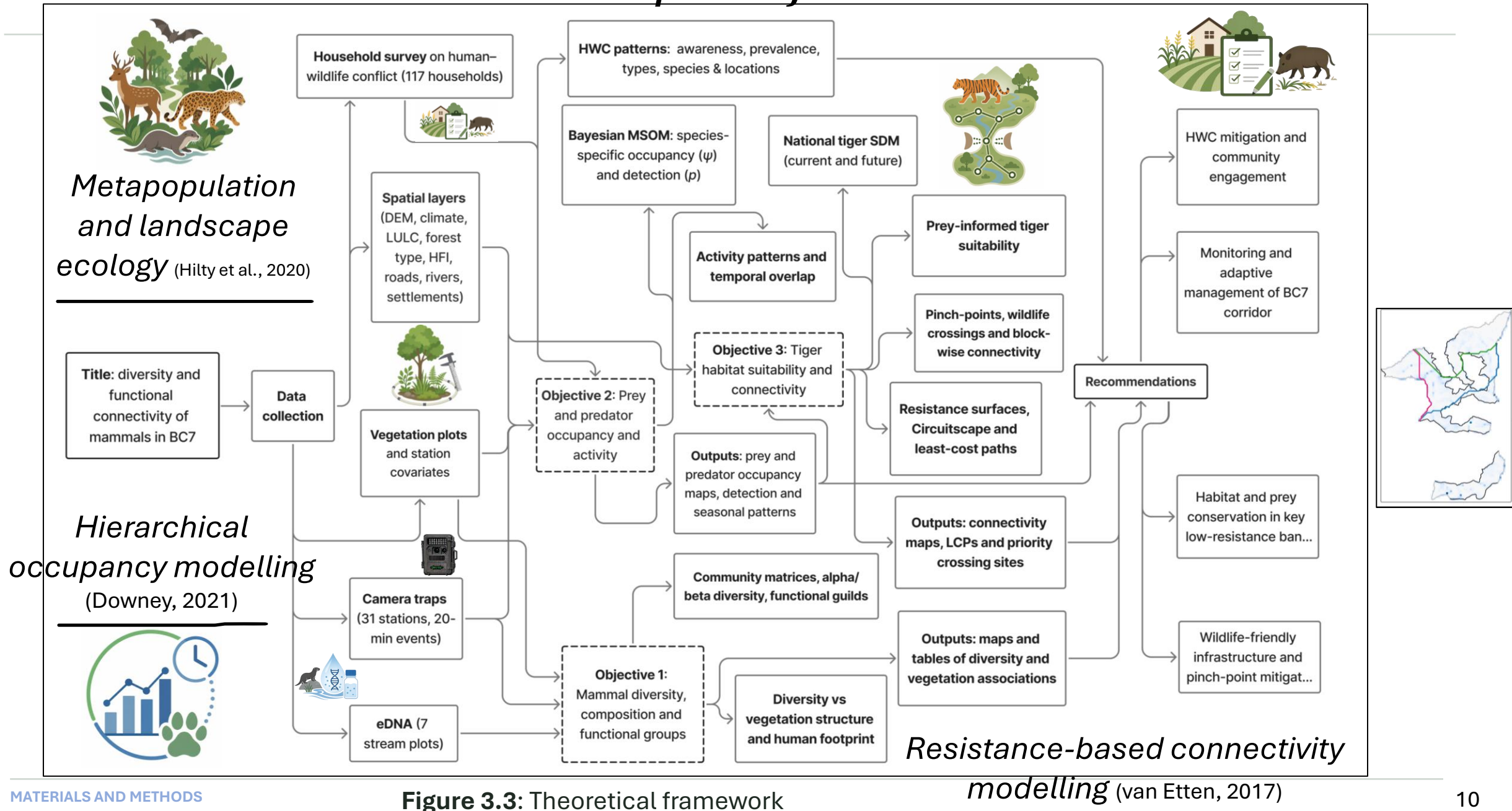


Figure 3.3: Theoretical framework

3.2.3 eDNA sampling, laboratory workflow, and bioinformatics

Field sampling

7 stream plots (P01–P07) in BC7

Sampled 3–6 Dec 2024

2 replicate/site

46–81 L filtered per replicate

Filters preserved in Longmire buffer
and kept cool

Field blanks and filtration blanks
were used



Laboratory workflow

Sterile pre-PCR and separate post-PCR workflow

Extraction blanks and PCR negative controls included

DNA extracted from preserved filters

PCR inhibition screened before library preparation

Two-step PCR with a short mammal mitochondrial marker

Amplicons cleaned, indexed, pooled, and sequenced on Illumina MiSeq

CNR Bhutan → ETH Switzerland

Bioinformatics

Cutadapt: primer trimming

DADA2: quality filtering and ASV inference

Chimaera removal

OBITools/ecotag: taxonomic assignment

Reference database curated with CRABS

Removed controls, human DNA, domestic taxa, and implausible detections

Final output: site × taxon presence–absence matrix

Jeunen et al., 2023; Polanco Fernández et al., 2021; Pont et al., 2018; Sherub et al., 2026

3.2.4 Vegetation and habitat surveys

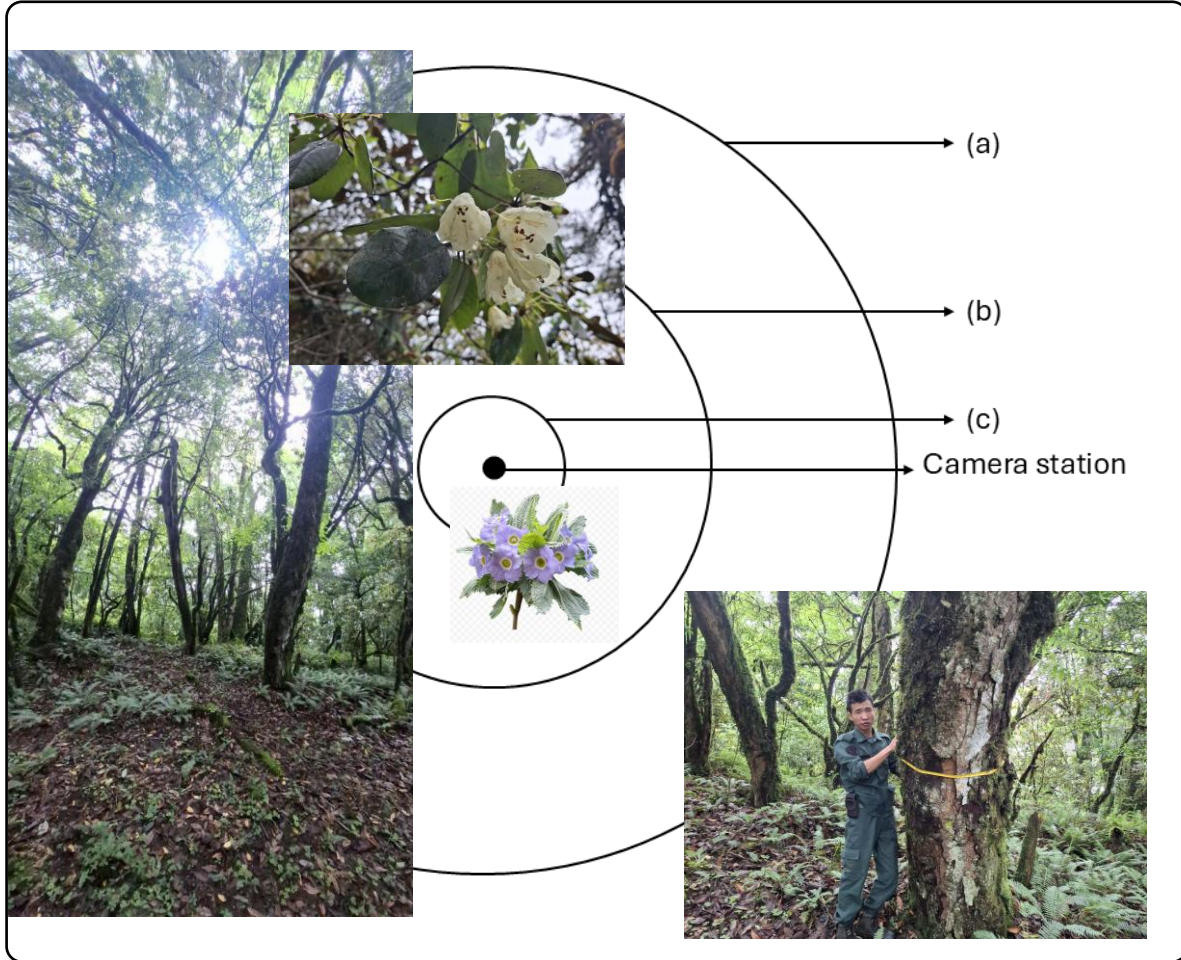


Figure 3.4: Vegetation survey plot design: (a) tree plot, (b) shrub plot and (c) herb plot

Survey workflow

Nested circular plots: trees 12.62 m radius, shrubs 3.57 m, herbs 1 m (McRoberts et al., 2010).

Tree measurements: all trees with DBH > 10 cm and height ≥ 1.3 m were identified and measured for DBH (DoFPS, 2023a).

Habitat variables: tree and shrub density, canopy cover, slope, aspect, elevation, and coordinates.

Terrain layers: slope and aspect were derived from FABDEM; canopy cover followed crown projection concepts (Hansen et al., 2013; Hawker et al., 2022).

Data capture: field data were recorded in ODK forms using KoboCollect (KoboToolbox, 2025).

Vegetation structure and terrain were measured at each CT station to support diversity and occupancy analyses.

3.2.5 Household survey on human–wildlife conflict



Survey scope

- 117/193 households surveyed from February to July 2025 (purposive sampling)
- Villages in 8 gewogs within or adjacent to BC7 were interviewed.



Questionnaire modules

- Wildlife-related damage experienced in the past 5 years.
- Damage types, reported species, and conflict locations.
- One questionnaire per household/respondent.

53/64: male to female (0.9:1) ratio

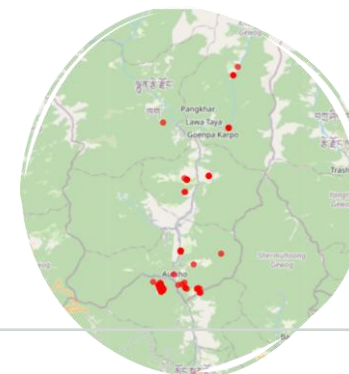
Mean age $\approx 46.0 \pm 12.4$ years

Data capture

- Responses were recorded on ODK forms using **KoboCollect**.
- Questionnaire included crop damage, livestock depredation, and location details.
- Species attribution was perception-based and used for socio-ecological interpretation.

HWC survey linked local conflict experience with species, locations, and BC7 management relevance.

KoboToolbox 2025; Letro & Fischer, 2020; Wangchuk et al., 2023



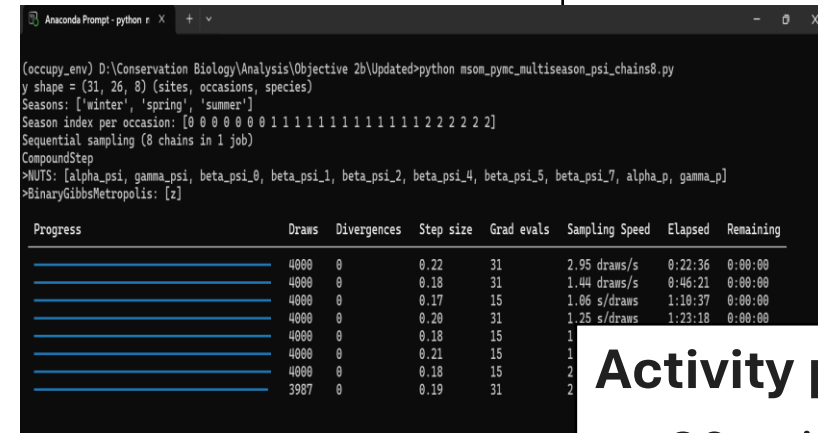
3.3.2 Occupancy and activity analysis

Species & detection histories (Amir et al., 2022)

6 prey: *M. muntjak*, *N. goral*, *C. sumatraensis*,
M. chrysogaster, *R. unicolor* and *S. scrofa*

2 predators: *P. pardus*, *P. tigris tigris*

31 stations × 26 weekly occasions (Jan–Jul 2025)



```
(occupy_env) D:\Conservation Biology\Analysis\Objective 2b\Updated>python mson_pymc_multiseason_psi_chains8.py
y shape = (31, 26, 8) (sites, occasions, species)
Seasons: ['winter', 'spring', 'summer']
Season index per occasion: [0 0 0 0 0 0 1 1 1 1 1 1 1 1 1 1 2 2 2 2 2]
Sequential sampling (8 chains in 1 job)
CompoundStep
>MUTS: [alpha_psi, gamma_psi, beta_psi_0, beta_psi_1, beta_psi_2, beta_psi_4, beta_psi_5, beta_psi_7, alpha_p, gamma_p]
>BinaryGibbsMetropolis: [z]

Progress                Draws  Divergences  Step size  Grad evals  Sampling Speed  Elapsed  Remaining
-----
1 4000 0        0.22      31         2.95 draws/s   0:22:36  0:00:00
2 4000 0        0.18      31         1.44 draws/s   0:46:21  0:00:00
3 4000 0        0.17      15         1.06 s/draws   1:10:37  0:00:00
4 4000 0        0.20      31         1.25 s/draws   1:23:18  0:00:00
5 4000 0        0.18      15         1
6 4000 0        0.21      15         1
7 4000 0        0.18      15         2
8 4000 0        0.18      15         2
3987 0        0.19      31         2
```

Bayesian multi-species occupancy model (**MSOM**) fitted in **PyMC** v5
(Downey, 2021):

checked multicollinearity (VIF > 5, | r | > 0.8)

$$\begin{aligned}\text{logit}(\psi_{is}) &= \alpha_{\psi,s} + x_i^T \beta_{\psi,s} \text{ (site covariates); } \text{logit}(p_{sr}) \\ &= \alpha_{p,s} + \gamma_{p,s,r} \text{ (seasonal detection: winter/spring/summer).}\end{aligned}$$

- Estimated occupancy (ψ) and detection probability (p)
- Model outputs summarised using means, 95% HDIs, ESS, and R-hat
- Model performance checked with posterior predictive checks and Brier scores

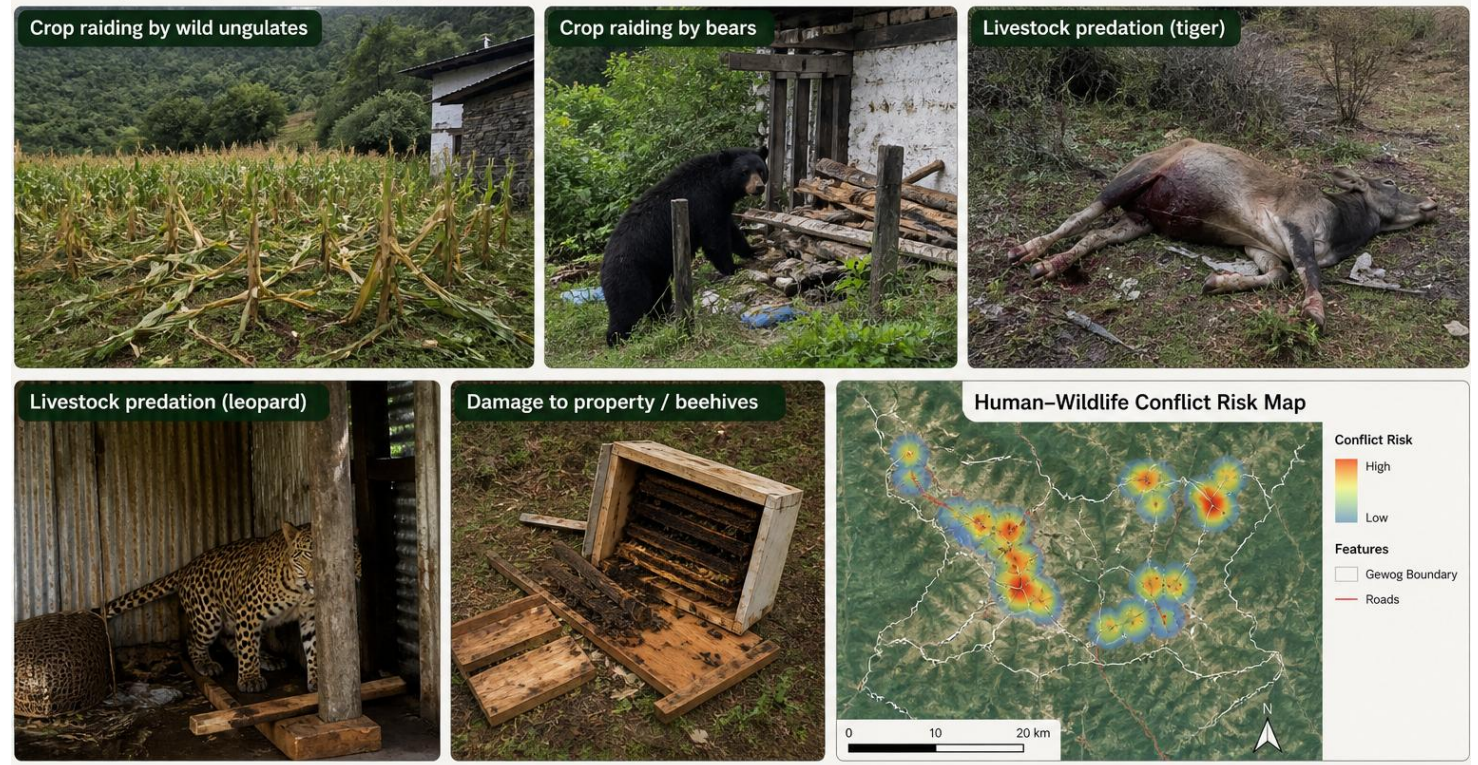
Activity patterns

- 20-min events → circular time; kernel density activity curves
- Coefficient of overlap between species and seasons

Niedballa, 2024; Rowcliffe et al., 2014

3.3.3 Human-wildlife conflict analysis

1. R (tidyverse, janitor); 117 complete records retained (Wickham et al., 2019)
2. Descriptive statistics by gewog, conflict type, species and location
3. Bar plots for conflict prevalence, conflict types, crop raiders, and livestock predators
4. Hotspot analysis and risk mapping



3.3.4 *Tiger habitat suitability and connectivity analysis*

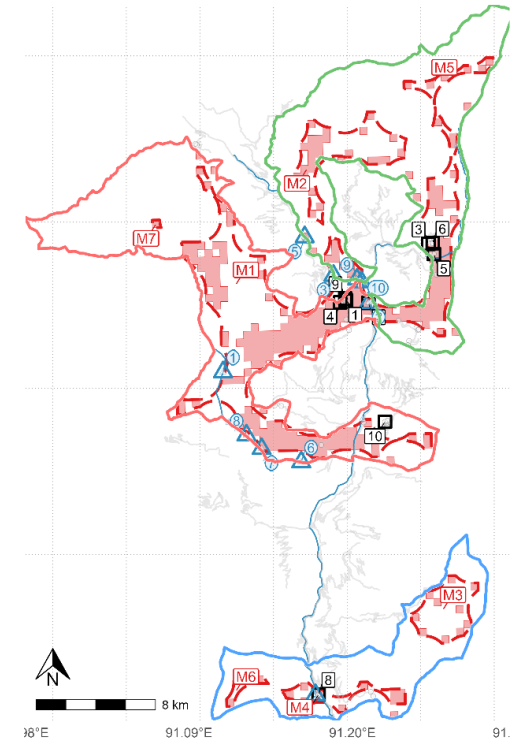
- Bayesian tiger SDM in PyMC v5 using Bhutan (68) and SA (2) presence–background data checked multicollinearity ($VIF > 5$, $|r| > 0.8$)
- Predictors: climate, topography, canopy, rivers, roads, PA status
- Predictions on a 500 × 500 m grid, clipped to BC7
- Combined with a six-prey occupancy surface to create prey-informed suitability
- Converted to conductance/resistance surfaces
- LCP, MST, corridor-enhanced comparisons, and block-wise tests
- Scenarios: Current, 2021–2050, 2051–2080 (CNRM-ESM2-1 SSP245)



Abril-Pla et al., 2023; Amir et al., 2022; Dickson et al., 2019; DoFPS, 2023; Downey, 2021; NCHM, 2025; van Etten, 2017; Zeller et al., 2012

3.3.5 Pinch-points and wildlife crossings

- Prey-informed passage surfaces from six ungulates (van Etten, 2017; Dickson et al., 2019).
- Top 5% of multi-species passage classified as pinch points (Dickson et al., 2019).
- Small fragments removed; nearby zones merged into macro pinch points (Dickson et al., 2019).
- Intersections with roads and rivers used to screen candidate crossing sites (OpenStreetMap contributors, 2025).
- Pinch-point cells were then polygonised, very small fragments ($< 0.25 \text{ km}^2$) were removed, and nearby patches were merged within a 1.5 km buffer to define macro pinch-point zones (Dickson et al., 2019).
- Block-wise summaries identified core movement areas and bottlenecks (Hilty et al., 2020).



4.1.1 Species composition

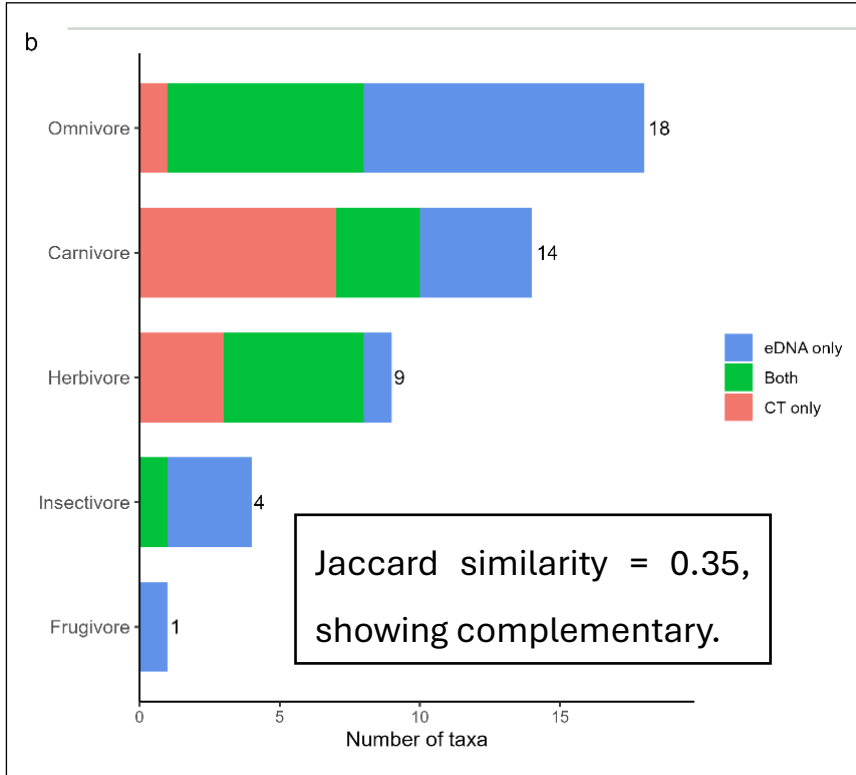
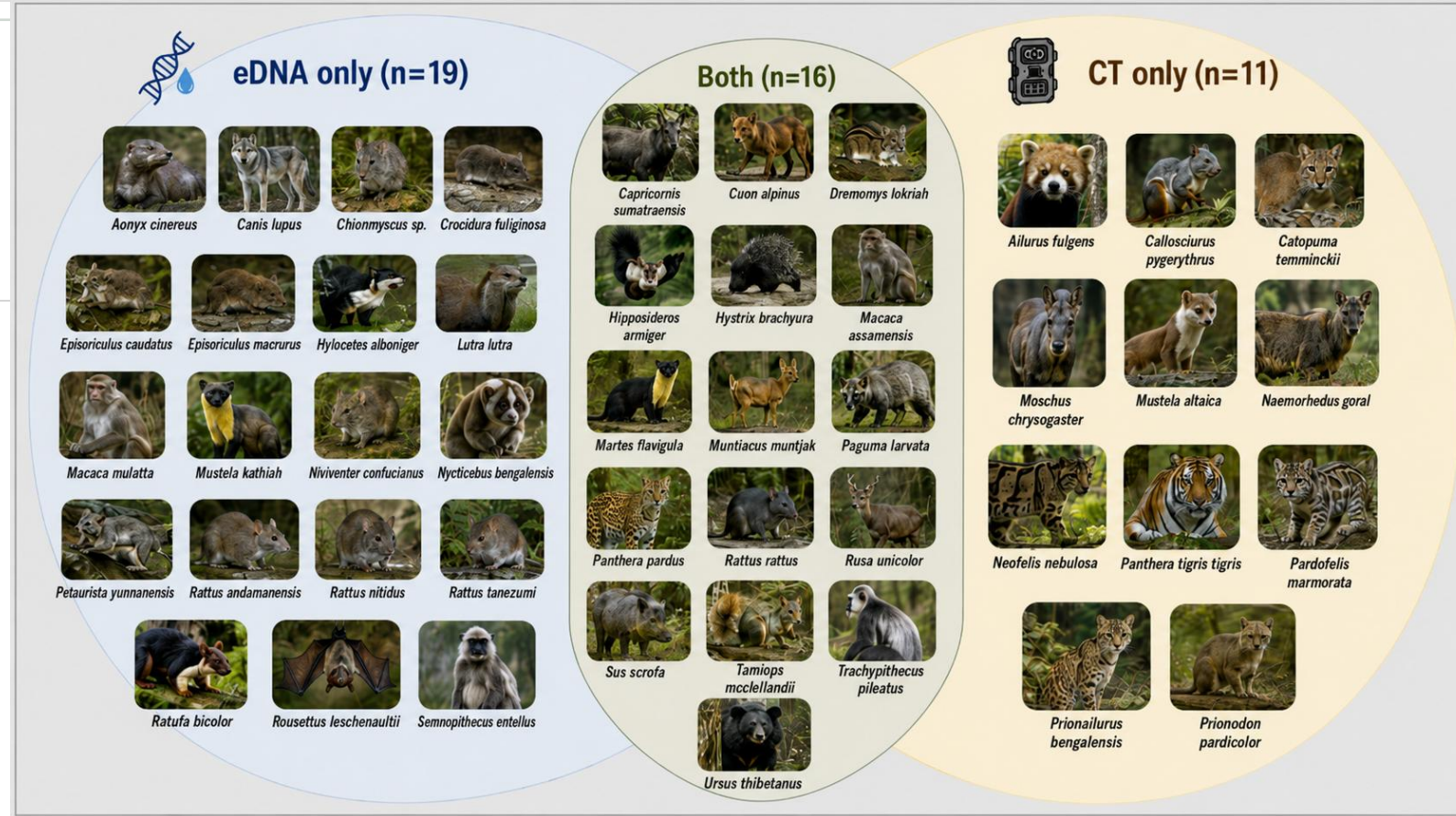
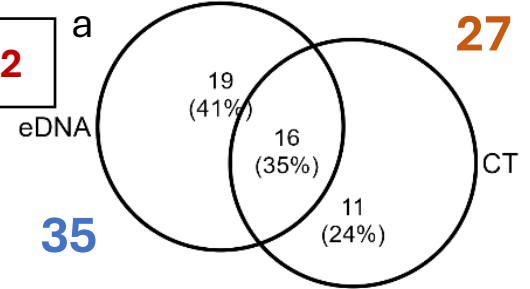


Figure 4.1: Species composition



CTs are better at detecting medium- to large-bodied terrestrial mammals, whereas eDNA can detect small, bat, and semi-aquatic taxa (Sherub et al., 2026; Tetzlaff et al., 2024).

Globally threatened = 12



4.1.2 Alpha diversity of mammals

eDNA mean $S = 17.6$, $H' = 2.77$, $1-D = 0.93$) > CT stations mean $S = 8.2$, $H' = 1.99$, $1-D = 0.84$).

Mann–Whitney $U = 159$, $p < .05$; Cliff's $\delta = 0.724$

Station richness differed among CT stations (Kruskal–Wallis $\chi^2 = 9.77$, $df = 2$, $p < .05$; $\varepsilon^2 = 0.28$).

Pairwise tests supported lower richness in open habitats than broadleaved forests (Holm-adjusted $p = .0148$).

eDNA broadened mammal detection, and broadleaved forests supported richer assemblages than open habitats (Letro et al., 2022; Seeber & Epp, 2022; Sherub et al., 2026).

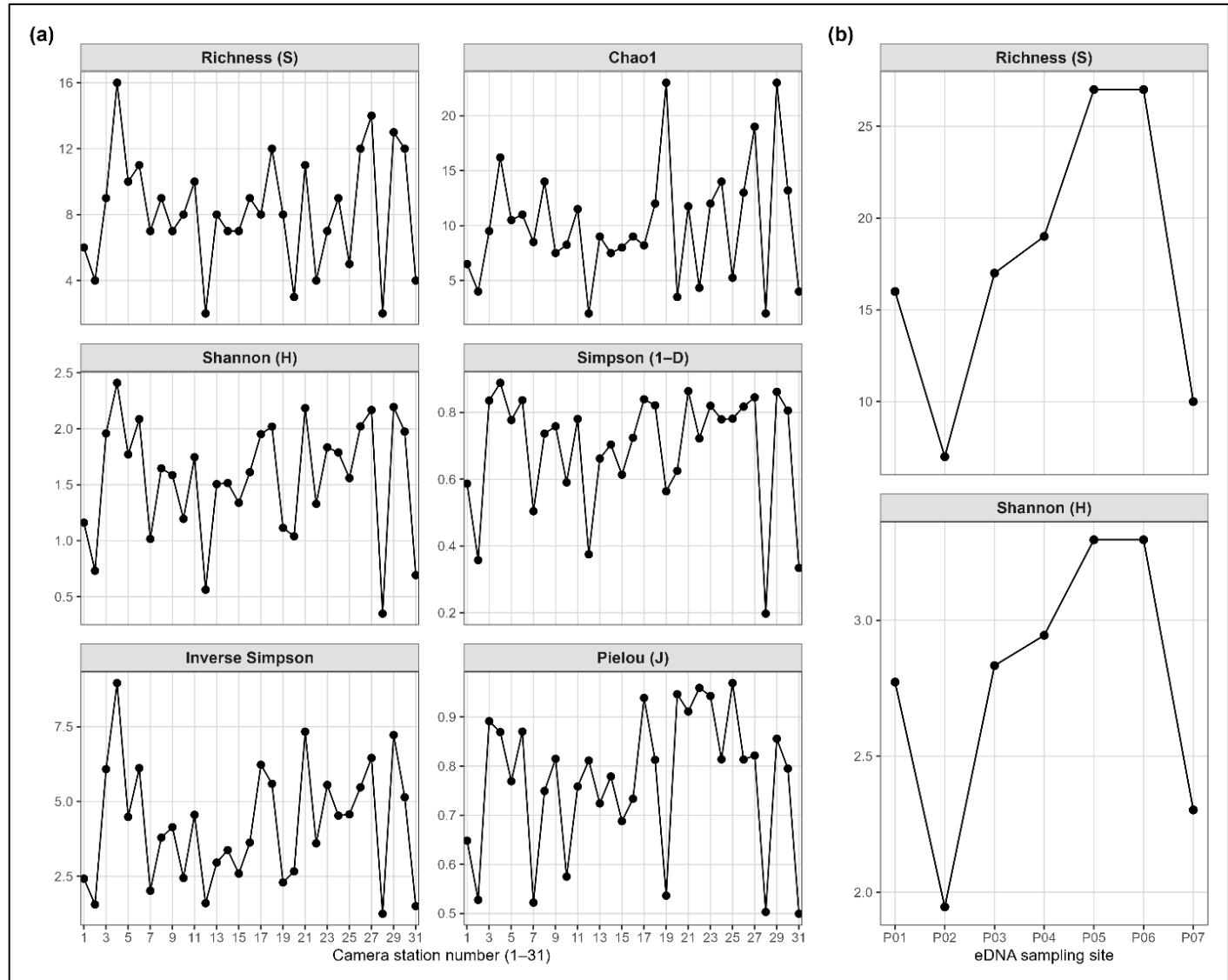


Figure 4.2: Alpha diversity patterns from camera traps and eDNA

4.1.3 Community composition

CT

Community composition did not differ among stations (PERMANOVA: $R^2 = 0.104$, $p > .05$)

eDNA

Showed a strong elevation-band signal (PERMANOVA: $R^2 = 0.657$, $p < .05$)

CT vs eDNA

Compositional variation (PERMANOVA: $R^2 = 0.188$, $p < .001$)

Sampling completeness was high for both methods (CT coverage = 0.989; eDNA coverage = 0.965)

Suggests complementary methods (Seeber & Epp, 2022; Sherub et al., 2026)

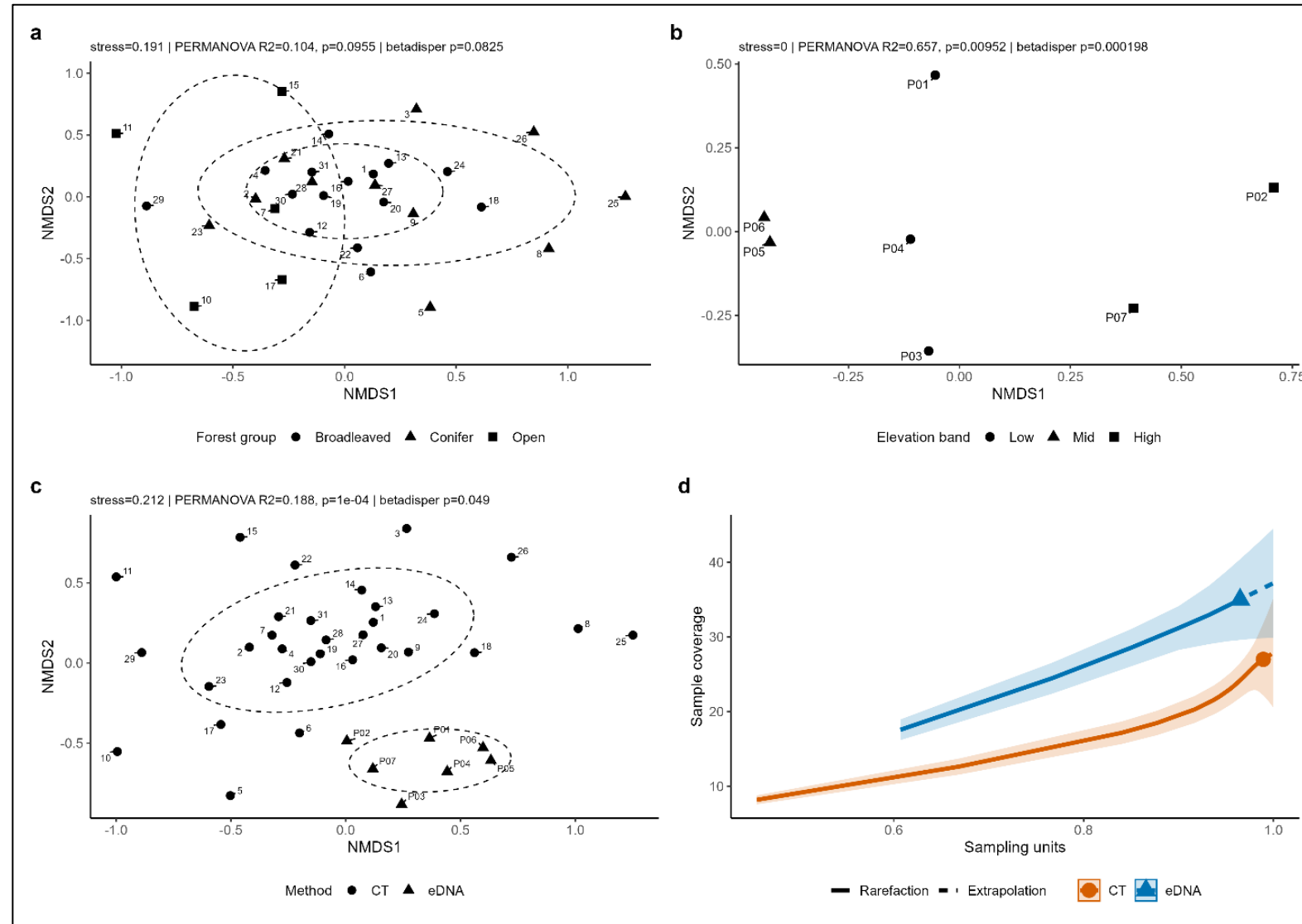


Figure 4.3: NMDS ordinations of mammal community composition in BC7

4.1.4 Relative abundance

CT detections were strongly prey-dominated (56 %), predator < 20%

eDNA top 10 taxa (66.8%) < CT top 10 (82.3%)

Supports complementary detection windows (Seeber & Epp, 2022; Sherub et al., 2026).

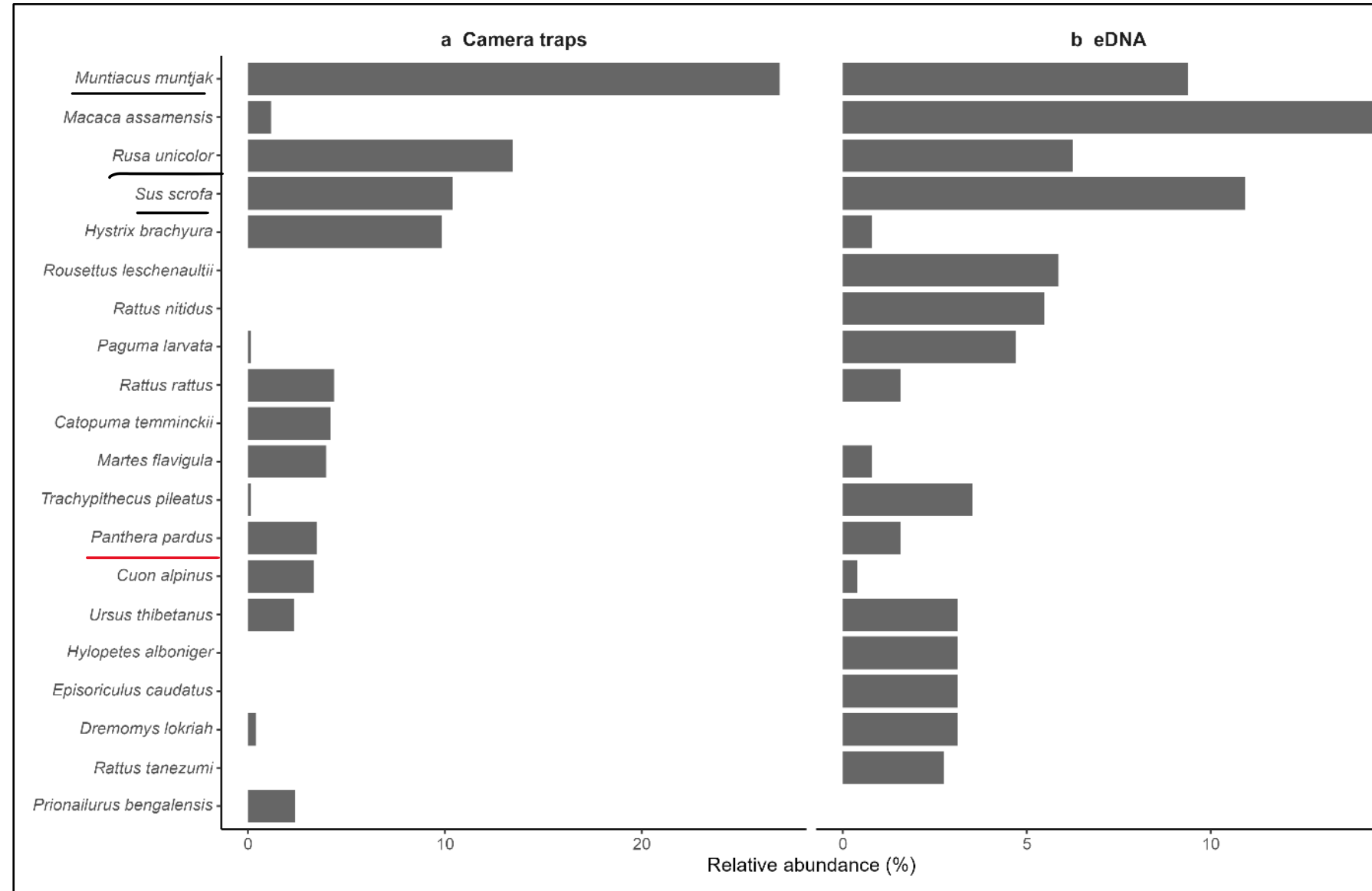


Figure 4.4: Relative abundance of mammals through CT and eDNA (top 20)

4.1.5 Naïve detection probabilities (CT vs eDNA)

CT (prey widespread)

barking deer ($p_{\text{naïve}} = .77$ (24/31)),
wild pig .71 (22), sambar .68 (21)

Carnivores were more restricted:

leopard .48 (15), tiger .06 (2)

In **eDNA**, barking deer, sambar and
wild pig were detected at all 7 sites

$p_{\text{naïve}}$ (shared species, $n = 16$)

– eDNA > CT (paired Wilcoxon, $V = 25$, $p = .025$)

– No strong correlation in species
ranking (Spearman $\rho = .13$, $p = .64$)

Methods are complementary

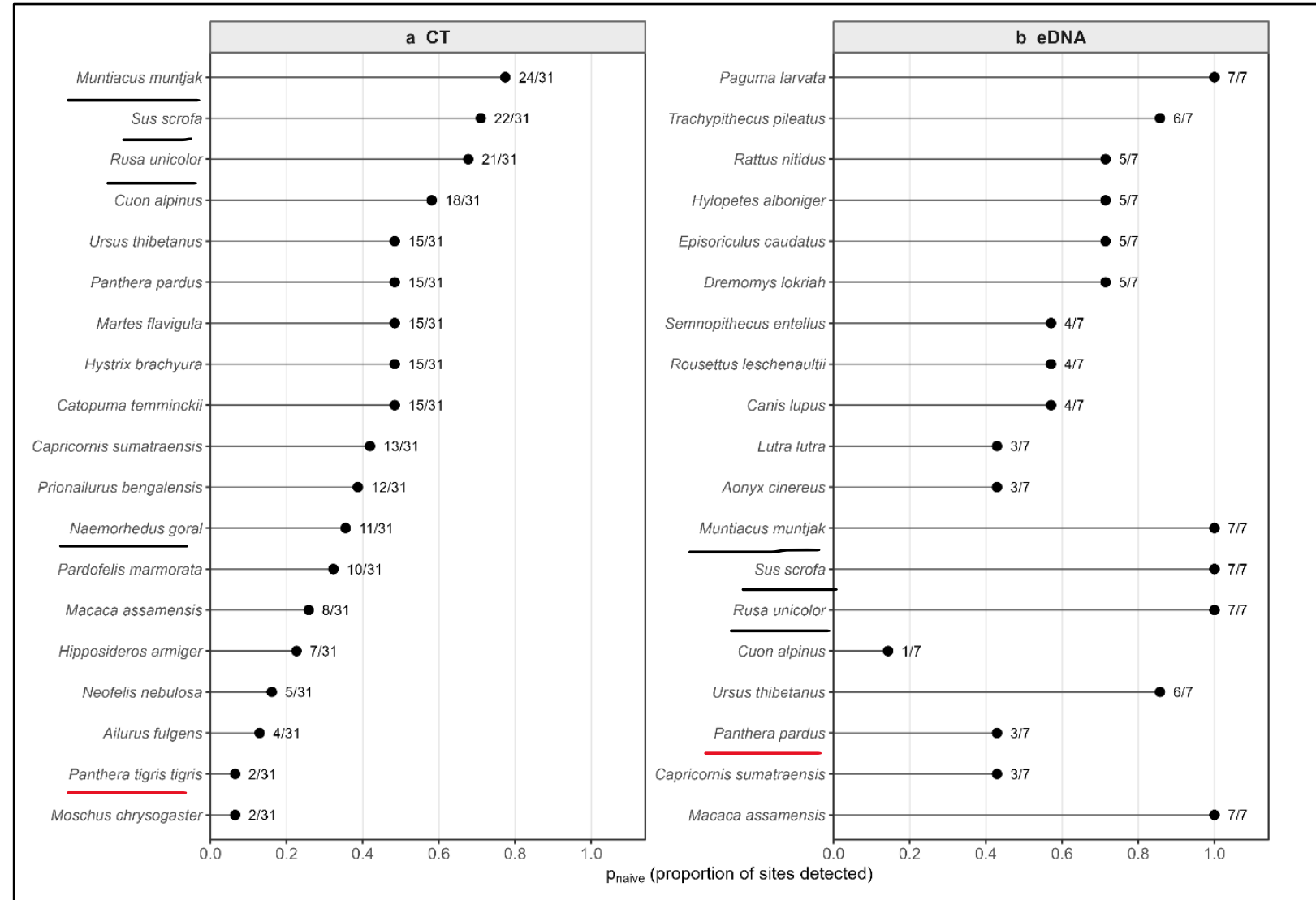


Figure 4.5: Naïve detection frequency ($p_{\text{naïve}}$) of mammals

4.2.1 Occupancy model performance

0

Divergences

1.02

Max R-hat

0.068

Overall Brier

720.6

Min bulk ESS

577

Min tail ESS

100%

PPC cells in 95%
HDI

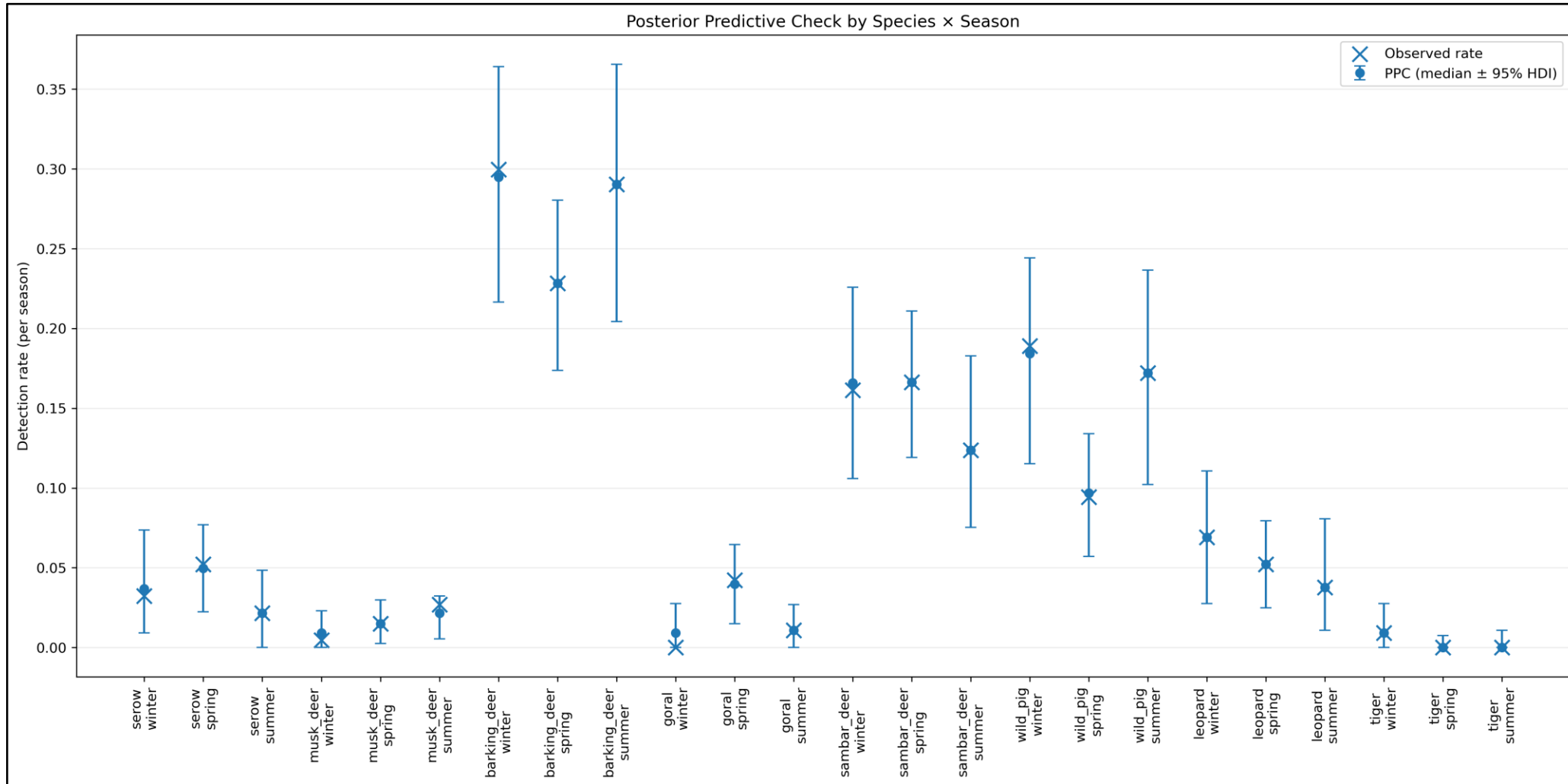


Figure 4.6: Posterior Predictive Check by species and season

Overall, the model fitted the data well, and the results can be interpreted with confidence.

4.2.2 Occupancy patterns

Prey species ($n = 6$): $\bar{\psi} = 0.53 \pm 0.26$

Predators ($n = 2$): $\bar{\psi} = 0.36 \pm 0.23$

Barking deer ($\bar{\psi} = 0.672$) > wild pig (0.615) > sambar (0.474) > leopard (0.446) > musk deer (0.062).

Seasonal occupancy

Prey: spring > winter > summer

Predator: winter > summer > spring

Could be due to fresh forage in spring (Srivastava & Kumar, 2018).

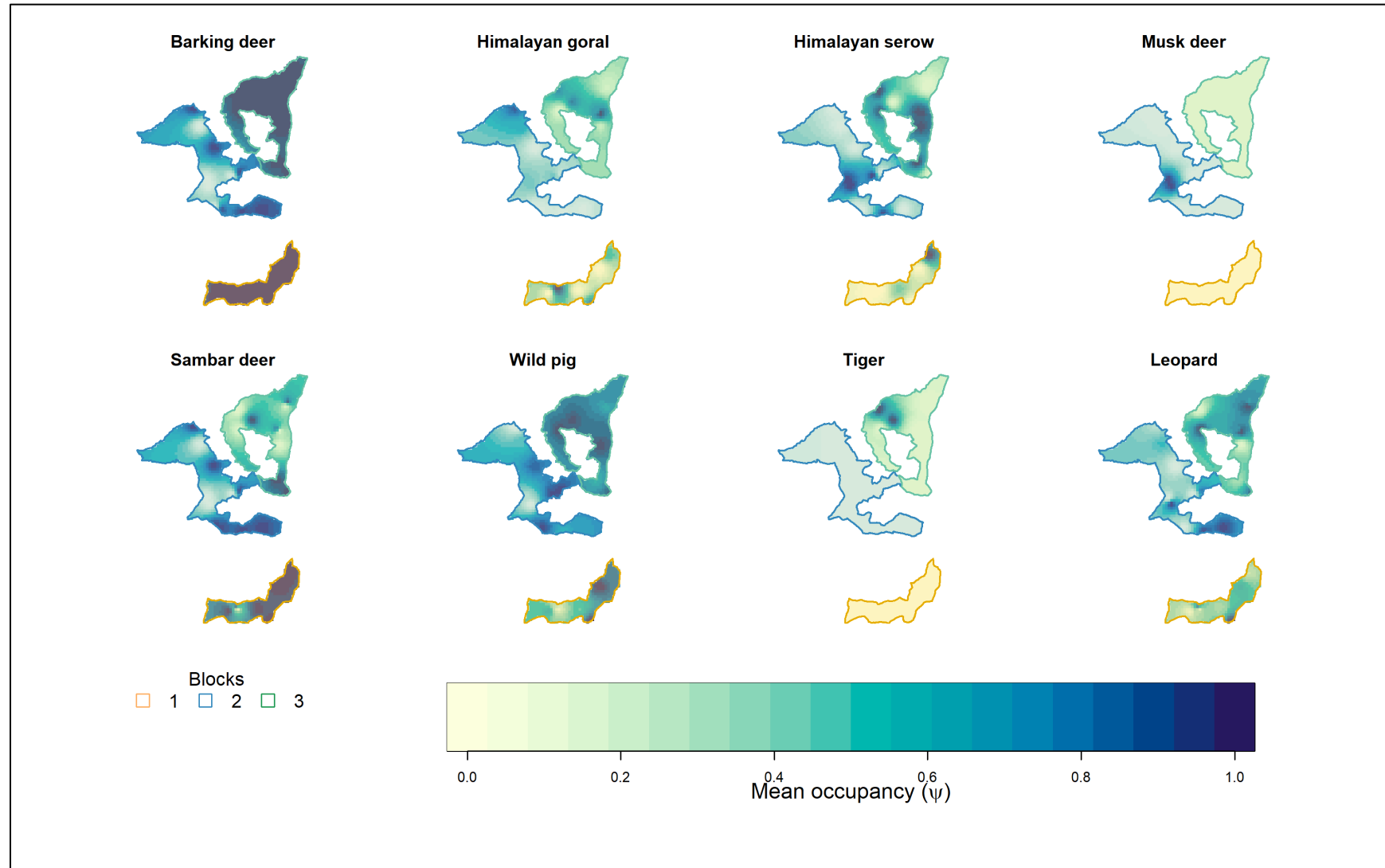


Figure 4.7: Mean occupancy (ψ) of predator and prey species across BC7

4.2.3 Detection probability

Barking deer had the highest detection probability ($\bar{p} = 0.387$)

Tiger and goral had the lowest detectability ($0.04, 0.02$)

Prey were more detectable than predators $\bar{p}$ prey = 0.197, $\bar{p}$ predator = 0.090, $\Delta p = 0.107$

Seasonal patterns were species-specific; predator-prey contrast was strongest in summer ($\Delta p = 0.224$)

BC7 is prey-supported, but species differ strongly in how widely they use the corridor and how easily they are detected.
Non-detection $\neq$ absence (Burton et al., 2015; MacKenzie et al., 2017)

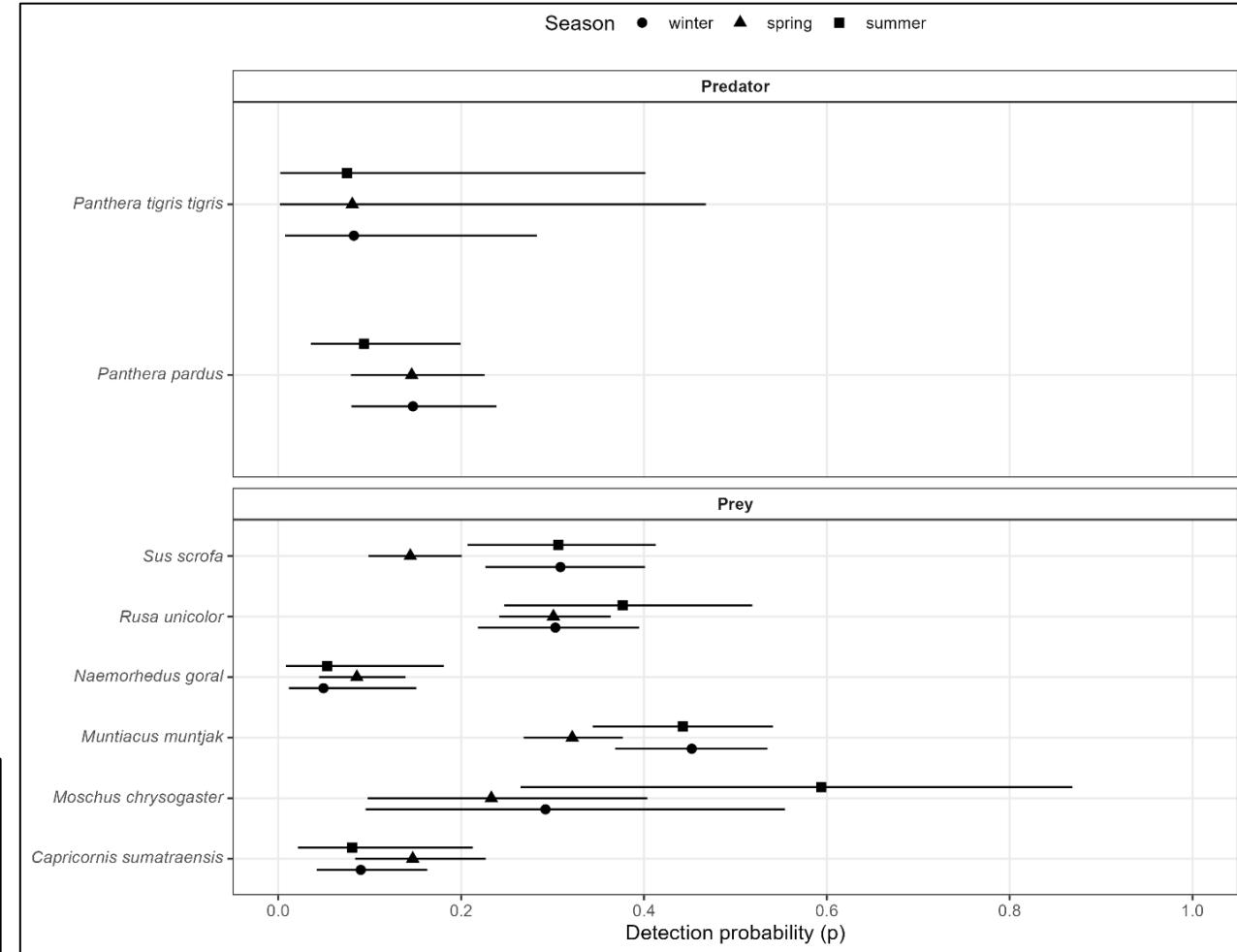


Figure 4.8: Species $\times$ season detection probability (p) estimates

4.2.4 Covariate effects

Goral: lower occupancy at higher elevation

Serow: higher occupancy at higher elevation, but higher occupancy also near roads

Sambar: positive effects of HFI, and precipitation

Leopard/sambar: negative canopy effect

Tiger and musk deer: wide uncertainty; limited covariate inference

BC7 occupancy is shaped by species-specific drivers, so management should be taxon-aware, not one-size-fits-all.

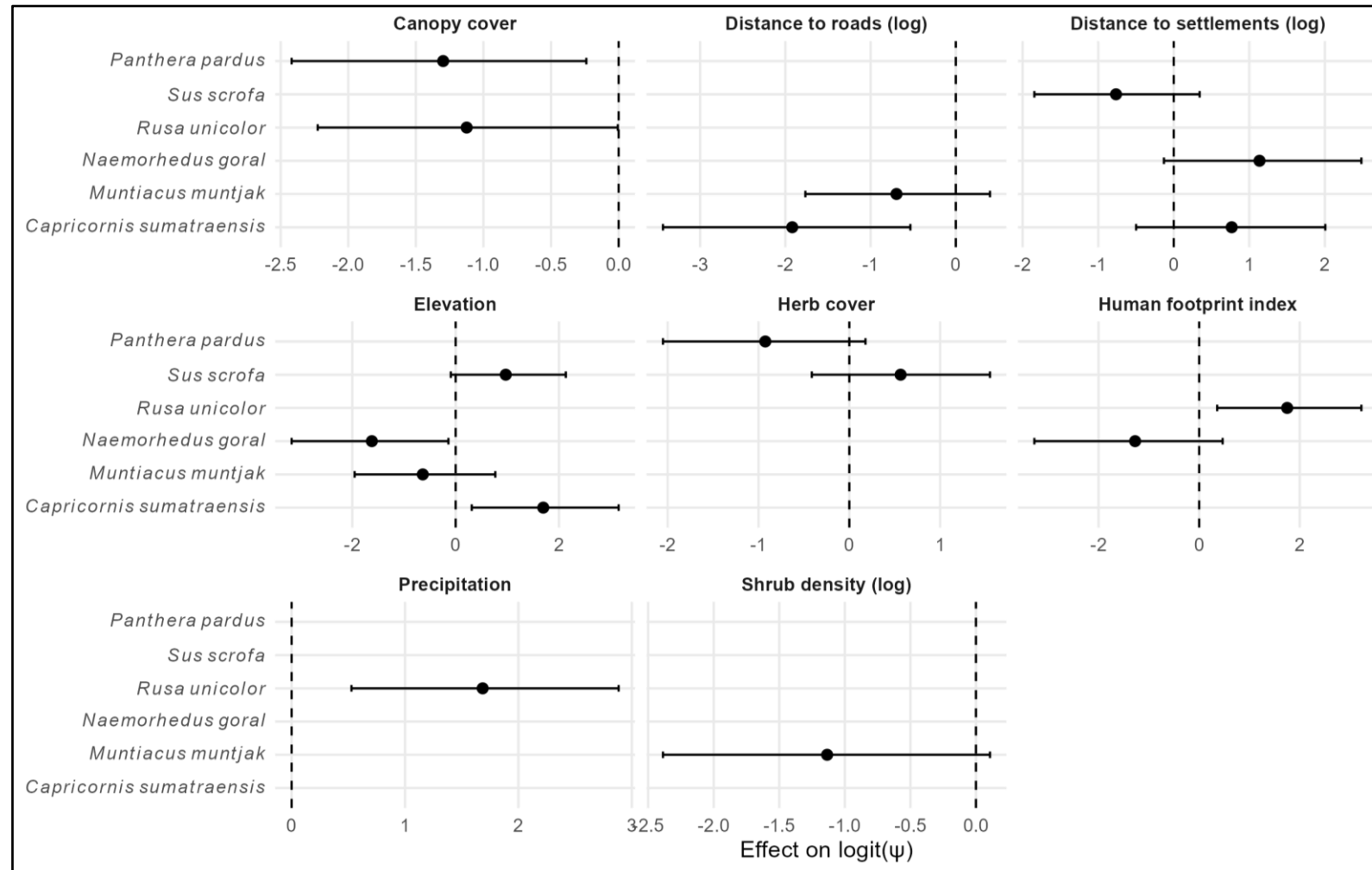


Figure 4.9: covariate effects on occupancy probability (ψ)

4.2.5 Temporal activity patterns

Barking deer: **diurnal–crepuscular**

Sambar and leopard: **nocturnal–crepuscular**

Wild pig: **Diurnal**

Diel-class tests showed leopard differed significantly from

Barking deer ($\chi^2 = 11.40$, $p < .05$, $V = 0.165$)

Wild pig ($\chi^2 = 24.68$, $p < .001$, $V = 0.255$)

but not from **sambar** or **serow**

BC7 supports substantial temporal alignment between leopard and several prey species, but wild pig shows greater temporal partitioning, suggesting more scope for time-based risk reduction

Leopard's nocturnal–crepuscular activity may partly reflect avoidance of daytime human activity in this multi-use corridor (Carter *et al.*, 2015; Gaynor *et al.*, 2018)

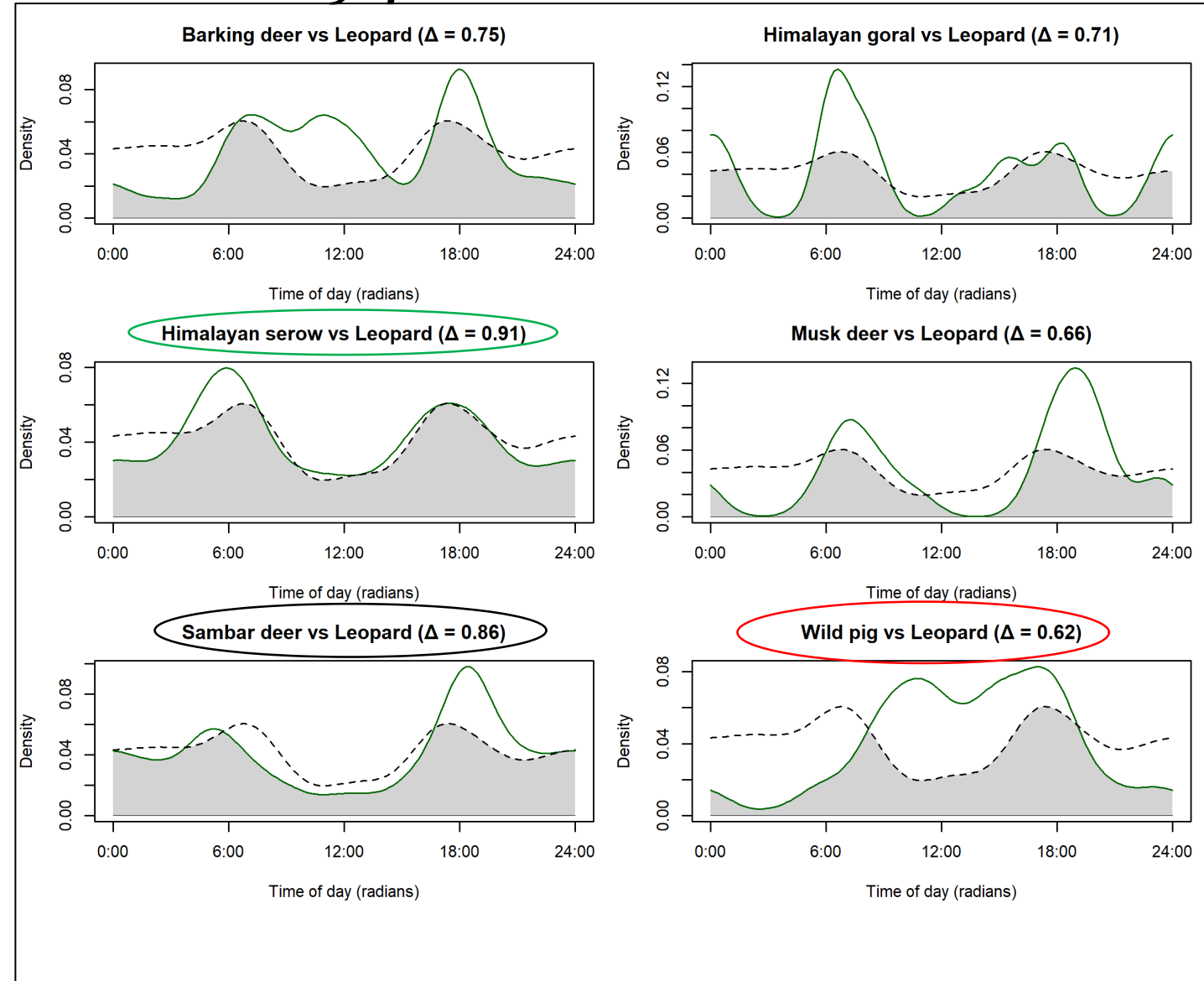


Figure 4.10: Activity overlap between leopard and six prey species

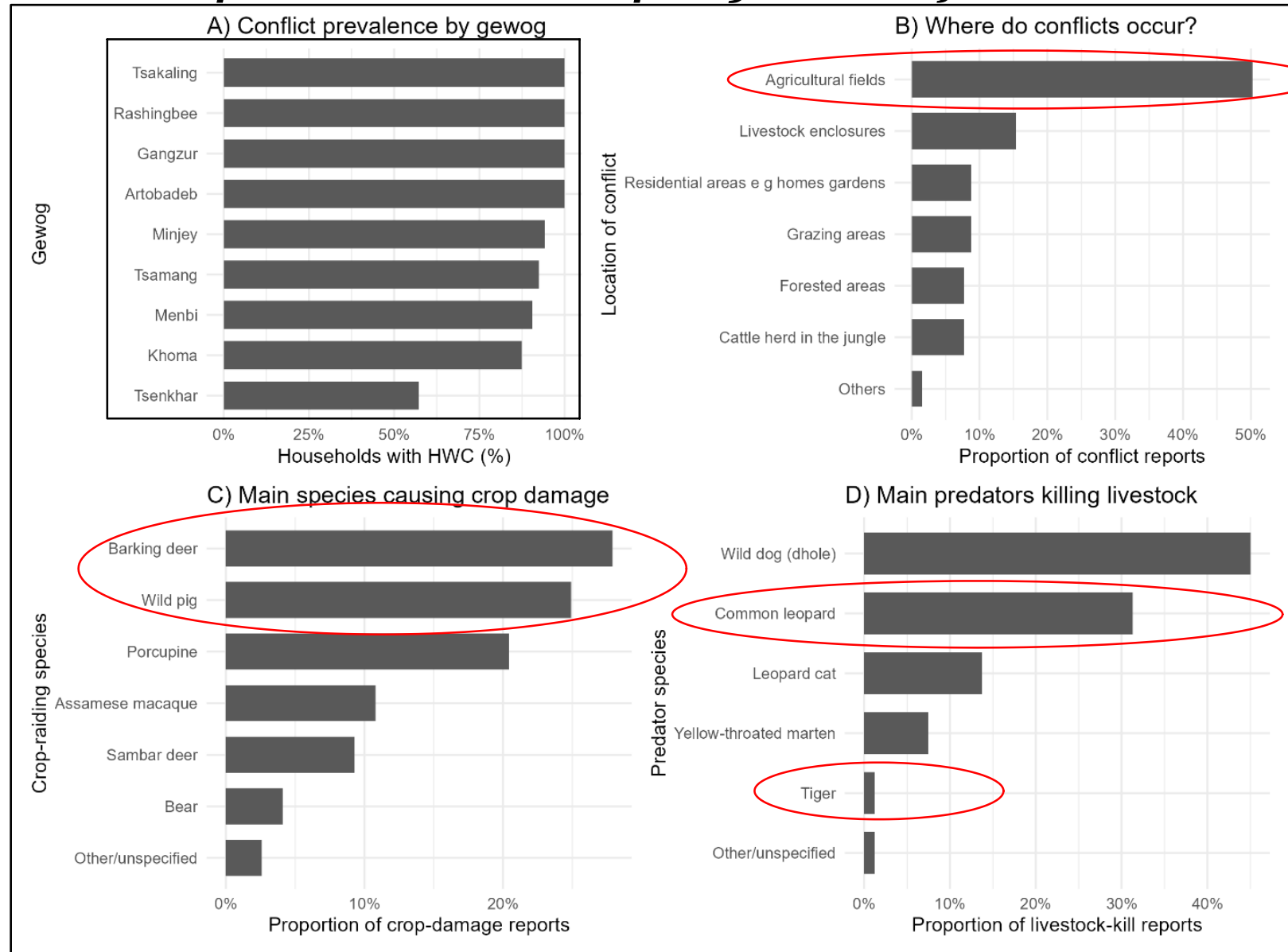
4.2.6 HWC in relation to predator and prey use of BC7

HWC was widespread: 102/116

households (87.9%; 95% CI [80.8–92.7%]) reported at least one conflict

Crop damage dominated conflict reports: 101/102 households; livestock depredation (50 households; 49.0%)

The same prey and carnivore assemblages that support corridor functionality are also the ones most involved in local conflict, making BC7 both an ecological corridor and a social interface. Letro et al. (2018)–BC8.



4.3.1 Model performance of Bayesian tiger SDM

0
Divergences

1.00
Max R-hat

4,841–7,778
Bulk ESS

3844-5794
Tail ESS

Low MCSE: reliable posterior estimates

Suitability increased slightly from current to future periods

Maps represent relative suitability, not confirmed tiger presence

Bayesian SDM provided a stable basis for inference, but its outputs are best interpreted as relative habitat-suitability surfaces rather than as direct evidence of tiger occurrence (MacKenzi et al., 2017).

4.3.2 Tiger habitat suitability in Bhutan and BC7

- Suitability was low overall but spatially structured
- In BC7, suitable habitat was limited but non-zero
- Under SSP245, suitability increased slightly over time
- Area above $\psi \geq .005$ increased from 65.8 km² to 68.2 km² and 70.5 km²
- Suitability remained higher at lower elevations (2100–2300 m), with no evidence of an upslope shift

BC7 held a small but persistent area of suitable habitat.
Deka et al. (2023)-East Himalaya

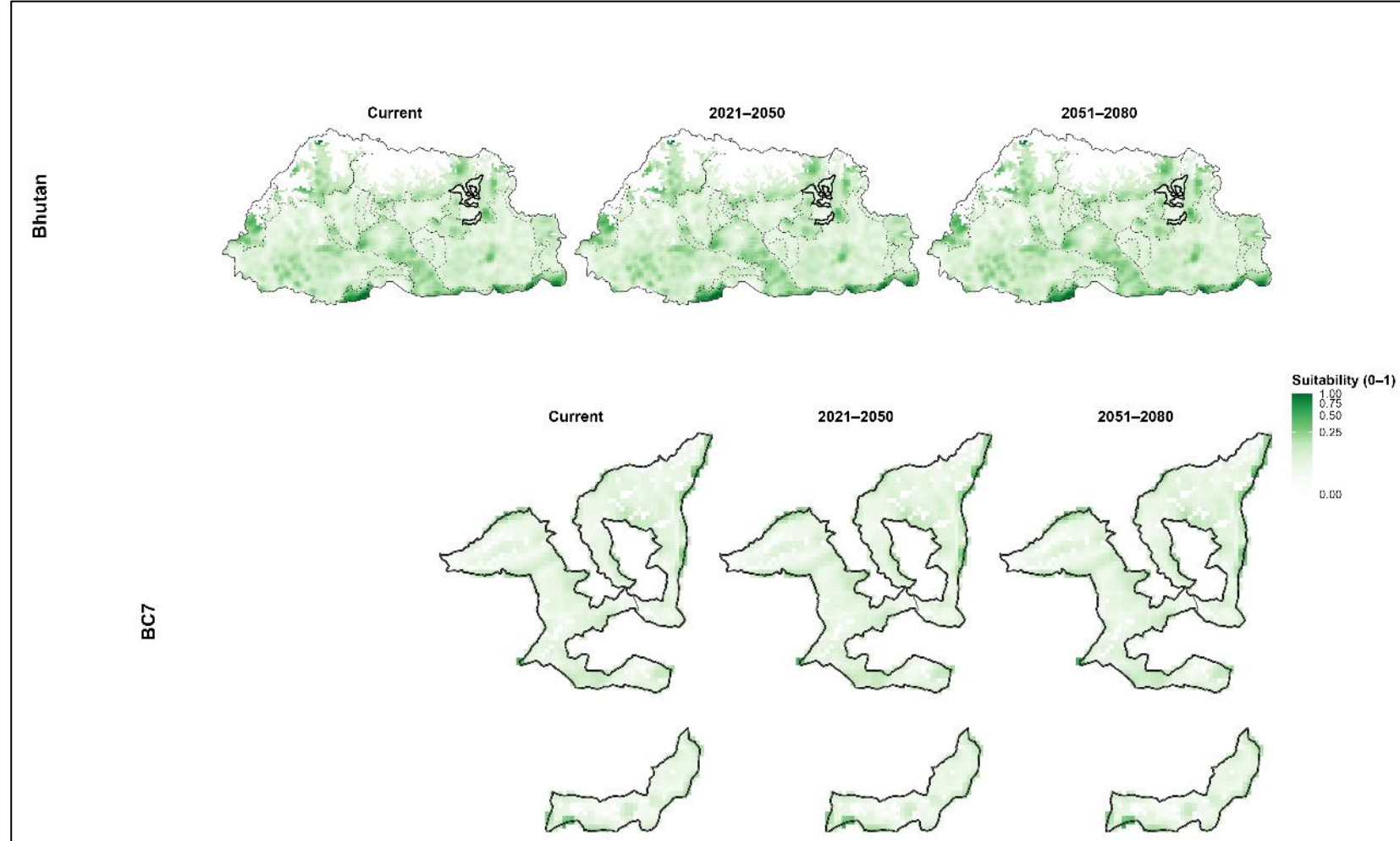


Figure 4.12: Tiger SDM suitability (scaled 0–1) across Bhutan (top row) and BC7 (bottom row) for Current, 2021–2050, and 2051–2080

4.3.3 Covariate effects on tiger habitat suitability

- Stronger effects were slope, distance to road, and PA status.
- Slope had a negative effect.
- PA status had a positive effect
- The road effect needs careful interpretation.
- It likely reflects landscape covariance, not road preference.
- BC7's value lies more in prey-rich, functionally connected patches than in uniformly high-quality tiger habitat.

The covariate and prey-informed results suggest that BC7 functions best as a corridor containing a narrower set of prey-rich, lower-elevation habitat patches rather than as uniformly suitable tiger habitat.

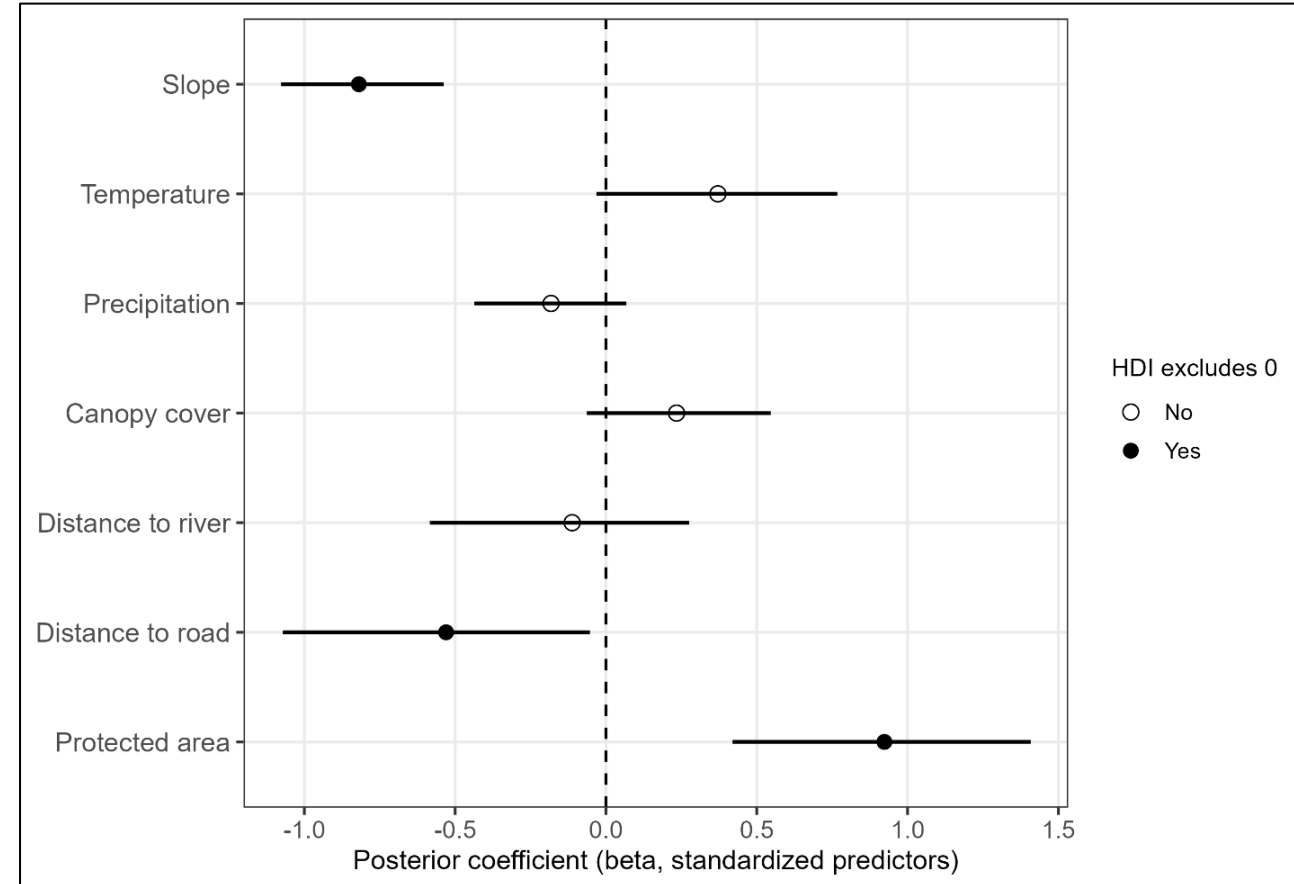


Figure 4.13: Posterior mean coefficients and 95% HDIs for covariates in the Bayesian tiger SDM

Sharma et al. (2025) reported that tiger occurrence is shaped by both prey availability and anthropogenic disturbance.

4.3.4 Current and future tiger pathways in BC7

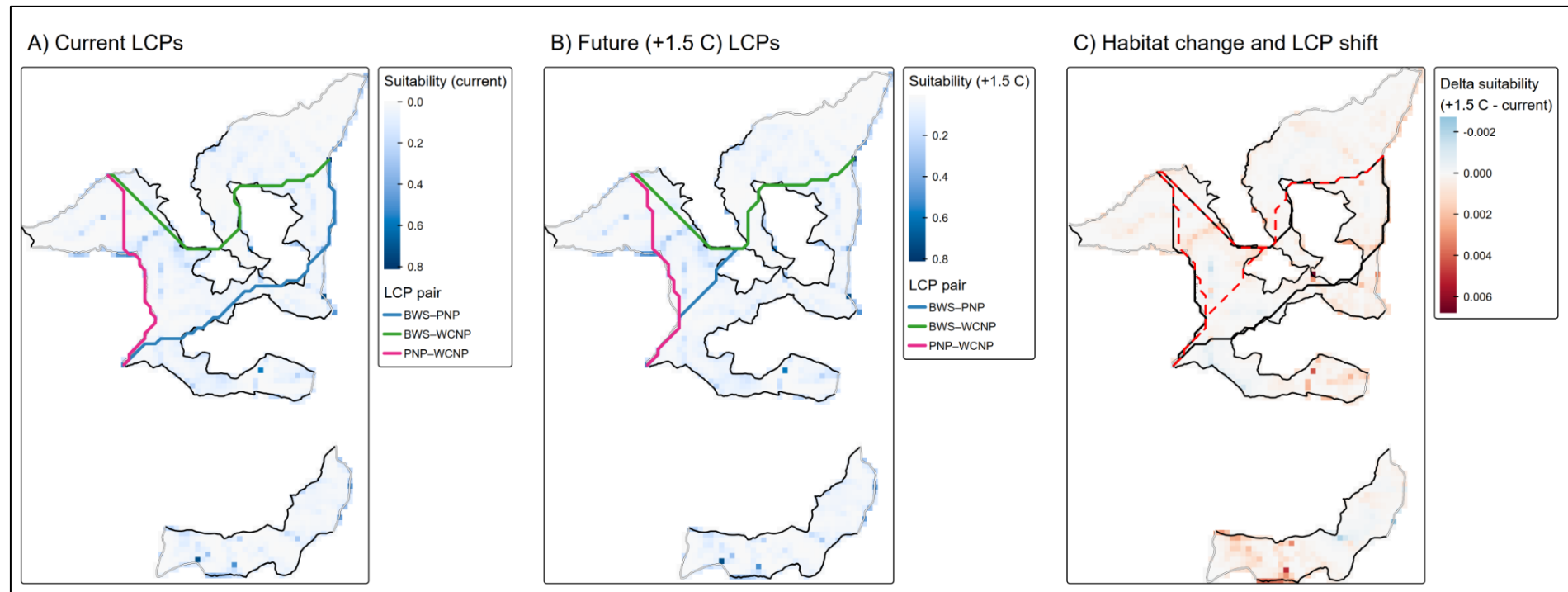


Figure 4.14: Habitat change surface (future minus Current suitability) with Current and future LCPs.

All three PA pairs remained connected under +1.5 °C.

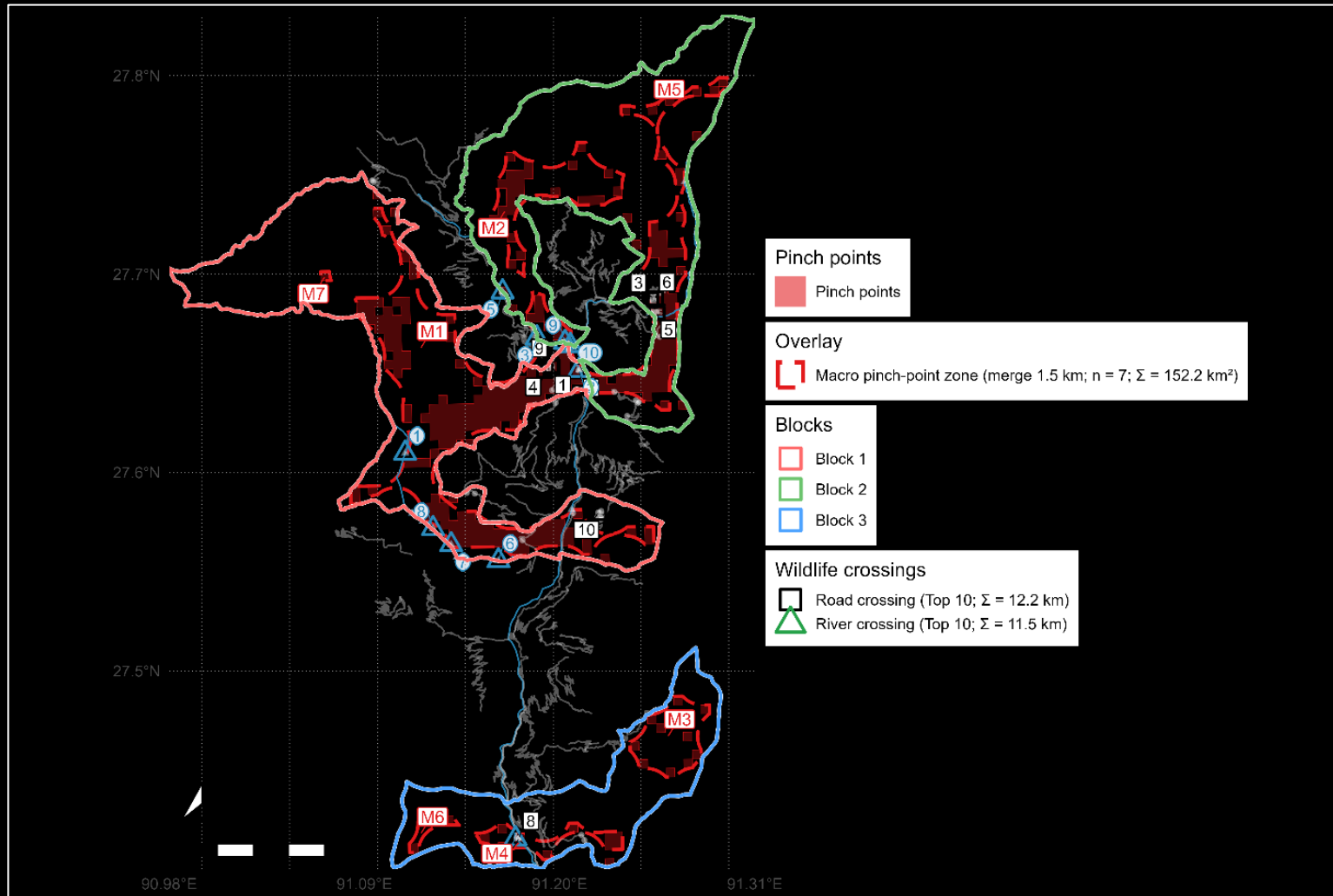
Path lengths became slightly shorter in the future.

The BWS-PNP linkage shows the strongest rerouting under +1.5 °C.

... indicates that the corridor persistence and pathway stability are not equivalent (Dickson et al., 2019; Hilty et al., 2020a)

4.3.5 Pinchpoints and wildlife crossings

Movement is concentrated into a relatively small set of high-value zones



7
macro pinch-
point zones

152.2
 km^2 pinch-
point area

29
road-conflict
segments

18
river-conflict
segments

Discussion

- BC7's future depends on keeping a few concentrated passage zones permeable.
- Short, high-risk segments offer efficient targets for crossing structures and traffic mitigation.
- This moves the study from corridor description to operational prioritisation.

Dickson et al., 2019; Soanes et al., 2024

5.1 Conclusion

RQ1

Mammal diversity

BC7 supports **46** recorded mammal taxa and functions as a multi-species corridor, showing ecological value beyond tiger movement alone

RQ2

Predator–prey use of BC7

Occupancy, seasonal site use, and movement were spatially uneven, with stronger use in prey-rich forests, riparian links, and a limited number of key passage zones.

RQ3

Tiger suitability and connectivity

BC7 showed limited but persistent suitability and connectivity for tigers, confirming its role as a functional landscape linkage.



If recommended conditions are maintained, BC7 can remain an important link within northeastern Bhutan's conservation network.

5.2 Recommendations

- Manage BC7 as a functionally heterogeneous corridor.
- Prioritise Blocks 1 and 2, especially Block 2.
- Maintain prey-rich lower- and mid-elevation forests (<2300 m).
- Protect riparian links and low-disturbance passage zones.
- Target short high-risk road and river segments first.
- Integrate HWC mitigation into corridor management.
- Continue CT – eDNA monitoring and field validation of pinchpoints.

BC7 management should be targeted, site-specific, and linked to continued monitoring.

5.3 Limitations

- CT and eDNA were complementary, not equivalent
- The study was time-bound, not long-term monitoring
- Rare species had sparse detections and wider uncertainty
- Occupancy and activity reflect site use, not abundance or demography
- HWC results include perception and recall bias
- Tiger SDM maps show relative suitability, not confirmed presence
- Pinchpoints and crossing sites are screening outputs, not final engineering designs



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Annexures: I Article evidence



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Prey Occupancy and Functional Connectivity in Biological Corridor Seven, Bhutan

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Abstract

Biological corridors with a healthy prey base support predator dispersal, yet studies that combine occupancy and connectivity remain limited. This study evaluated six ungulate prey species in Biological Corridor Seven, Bhutan. Thirty-one paired camera traps were deployed on a 3 km × 3 km grid in the first half of 2025, yielding 5,144 trap days and 822 independent events. A hierarchical Bayesian multi-species occupancy model was fitted in PyMC (v5) to estimate occupancy (ψ) and detection probability (p) across winter, spring and summer. Predicted occupancy was mapped onto a 500 m × 500 m grid ($N = 1,689$ cells) and analysed with random-walk connectivity to identify movement routes and pinch points. Generalists showed high and widespread occupancy ($\psi = 0.933\text{--}0.981$), with peak detectability in winter ($p = 0.185\text{--}0.320$). In contrast, specialists were concentrated in narrow upper-elevation areas ($\psi = 0.639\text{--}0.877$), peaking in spring ($p = 0.085\text{--}0.164$). Connectivity analysis isolated seven macro pinch points and numerous conflict segments (< 500 m long) where linear infrastructure is likely to restrict movement. Priority mitigation include vegetated overpasses on dry slopes and bridge-style underpasses or culverts at riparian conflicts. A tiger *Panthera tigris* was recorded at two locations, indicating the use of the corridor by large predators. However, further studies on predator occupancy employing telemetry or genetics are recommended. Nonetheless, this integrated occupancy–connectivity framework may serve as a reproducible basis for prioritising mitigation and guiding adaptive corridor management under growing infrastructure development and climate pressure.

Keywords: Bayesian model, biological corridor, climate, connectivity, occupancy, pinch point, prey, tiger

II Field Photos



Thank you!