Leibniz Gemeinschaft

Leibniz Institute for Zoo and Wildlife Research
in the Forschungsverbund Berlin e.V.

Studying terrestrial mammals in tropical rainforests

A user guide for camera-trapping and environmental DNA

Published by the Leibniz Institute for Zoo and Wildlife Research (Leibniz-IZW), June 2018

ISBN: 978-3-9815637-6-4

Front cover photo: Top row: Chinese serow *Capricornis milneedwardsii*, Bornean yellow muntjac *Muntiacus atherodes*, Bornean orangutan *Pongo pygmaeus*, Sun bear *Helarctos malayanus*; Second row: Leopard cat *Prionailurus bengalensis*; Banteng *Bos javanicus*; Banded civet *Hemigalus derbyanus*; Asian black bear *Ursus thibethanus*, Third row: Sunda pangolin *Manis javanicus*; Binturong *Arctictis binturong*, Indian civet *Viverra zibetha*, Malayan porcupine *Hystrix brachyura*; Yellow-throated marten *Martes flavigula*, Sunda clouded leopard *Neofelis diardi*, Asian elephant *Elephas maximus*, Annamite striped rabbit *Nesolagus timminsi*.

Back cover photo: Andrew R. Tilker

Chapter header photos:

1. **Contents**
   A family of elephant (*Elephas maximus*) taken in Tangkulap-Pinangah Forest Reserve
2. **Introduction**
   Bearded pigs (*Sus barbatus*) taken in Tangkulap-Pinangah Forest Reserve
3. **Methods and data collection**
   Pig-tailed macaques (*Macaca nemestrina*) taken in Tangkulap-Pinangah Forest Reserve
4. **Analytical methods**
   A red muntjac (*Muntiacus muntjak*) taken in Northern Kuamut Forest Reserve.
5. **Summary and perspectives**
   Red-shanked douc langur (*Pygathrix nemaeus*) taken in Bach Ma National Park, Vietnam.
6. **References**
   For the references photo: Mainland serow (*Capricornis milneedwardsii*) taken in Bach Ma National Park, Vietnam.

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Citation

This document may be cited as:

Abrams, J. F., Axtner, J., Bhagwat, T., Mohamed, A., Nguyen, A., Niedballa. J., Sollmann, R., Tilker, A. R. & Wilting, A. 2018. Studying terrestrial mammals in tropical rainforests. A user guide for camera-trapping and environmental DNA. Leibniz-IZW, Berlin, Germany.

A digital copy of this report along with supporting appendices are available at:

`http://www.leibniz-izw.de/userguide.html`

First release, June 2018

SPONSORED BY THE

Federal Ministry of Education and Research

Sunda clouded leopard, Deramakot Forest Reserve, Sabah, Malaysian Borneo. **Photo** Michael Gordon

Acknowledgements

All authors would like to thank the German Federal Ministry of Education and Research (BMBF FKZ: 01LN1301A) for funding the SCREENFORBIO project and the PT-DLR, in particular Paul Dostal for their support and scientific and administrative management of our project. We are grateful to the Leibniz-IZW for providing extensive financial, logistical, and in-kind support to the Junior Research Group: Biodiversity and Biogeography of Southeast Asia. We would like to extend a special thanks to Heribert Hofer und Miriam Brandt for their continuous support throughout the last years.

Our field work would not have been possible without the tremendous support of our in-country partners.

In Vietnam and Laos our project collaborated closely with the WWF-CarBi Project funded by the German Federal Ministry for the Environment, Nature Conservation, Building & Nuclear Safety and the KfW Development Bank. We enjoyed the close partnership with WWF throughout this project and thank many individuals, both in our study countries as well as in Germany. Specifically we thank Fanie Bekker, Stefan Ziegler, Aurelie Shapiro, Thinh Van Ngoc, Hung Luong Viet, An Nguyen Quang Hoa, Ben Rawson, Arnoud Steeman, Le Quoc Thien, Francois Guegan, Khamseng Homdouangxay, Amphone Phommachak, and Soukaseum Malychansy. We extend a special thanks to the two WWF-Forest Guards, Nguyen Van Sang and Dang Cong Vien, who led field teams in Vietnam. Pakham Outhankone helped lead a field team in Laos. Sang, Vien, and Pakham because not only good colleagues but also close friends. In all our study sites – Bach Ma National Park, Hue Saola Nature Reserve, Quang Nam Saola Nature Reserve, and Xe Sap National Protected Area – our fieldwork was supported by protected area staff. We would also like to extend a special thanks to the Katu people of Vietnam and Laos, particularly from the villages of A'ur (Vietnam) and Ban Pale and Ban Kalo (Laos). We also thank the Central Institute for Natural Resources Environmental Studies, particularly Assoc. Prof. Minh Le and Thanh Nguyen, for their assistance in arranging collection and export leech permits and the Access Benefit Sharing clearance for Vietnam. We thank the Biodiversity Conservation Agency of the Minister of Natural Resources and Environment for issuing the permits. In Laos, we thank the Ministry of Science and Technology for permitting us to export leech samples. We thank Rob Timmins and Will Duckworth for their assistance in identifying some camera-trap photos. Additional financial support to the fieldwork in Vietnam and Laos was provided by the Point Defiance Zoo & Aquarium, Safari Club International, and the Critical Ecosystem Partnership Fund. Andrew R. Tilker received support through a Fulbright scholarship granted through the US Department of State.

In Malaysia we thank our long-term partner the Sabah Forestry Department (SFD) for their continuous support. In particular, we thank Datuk Sam Mannan, Johnny Kissing and Peter Lagan. We also thank Albert Radin, Indra Purwandita, Herry Sunjoto, Hj. Hussin Tukiman and Ladwin Rupi from the SFD for their support. None of this work would have been possible without the help of numerous research assistants in Sabah. Thanks to Jereo Jaimin, Bandy Marinson, Azrie Petrus, Alexander Petrus, Reyner Rupin, Alex de Jellson Juis, Yoseff Julian Setiol, Mohd Nazri Rasinin, Maxius Maidi, Arson Asidin, Edify Gleam Jutin, Viktor Vitalis, Roslan Husin, Jeffendy Juani and Jacklan Juani. At the Forest Research Centre of Sabah Forestry Department we thank Robert Ong, Alexander Yuk Loong Hastie, Joseph Sikui and Eyen Khoo for collating and providing indispensable information about our study sites.

In addition, we thank Yayasan Sabah (Sabah Foundation) for supporting our survey in Northern Kuamut Forest Reseve. We are grateful to the Sabah Biodiversity Council for providing us an access license and license for export of biological resources. We thank Gary Albert, John Bakar and Quentin Phillips for their assistance in bird identifications. Mike Gordon for sharing his collection of Sunda clouded leopard photos taken in Deramakot and Tangkulap-Pinangah. Additional funding for fieldwork in Sabah Malaysia was provided by Point Defiance Zoo & Aquarium and the San Francisco Zoo.

THANK YOU to everyone in Vietnam, Laos, and Sabah Malaysia for supporting us throughout the last five years. We look forward continuing our collaboration with many of you in the current and upcoming projects. Methods developed under the SCREENFORBIO project and presented in this user's guide are currently replicated in additional sites in Vietnam and Malaysia.

In Vietnam, we expanded our surveys in collaboration with WWF-Vietnam within the project *Biodiversity monitoring in Quang Nam and Thua Thien Hue, Vietnam* funded by the U.S. Agency for International Development (USAID) through Management Systems International Inc. These surveys are led by the Leibniz-IZW PhD student Thanh Nguyen, who is supported by a Russel E. Train Fellowship from WWF-US. We currently collaborate with Save Vietnam's Wildlife and Vinh University on surveys in Pu Mat National Park. These surveys

are financially supported by the Mohamed bin Zayed Species Conservation Fund, The National Geographic Society's Committee for Research and Exploration, Ocean Park Conservation Foundation Hong Kong, and Point Defiance Zoo & Aquarium. An Nguyen is conducting surveys in collaboration with the Southern Institute of Ecology in southern Vietnam. For these surveys he received funding from Global Wildlife Conservation, Mohammed Bin Zayed Conservation Fund, Wroclaw Zoo, Auckland Zoo, and Sainte Croix Biodiversité.

In Malaysia we started camera-trapping surveys using the developed methodological framework in the Pasin Forest Management Unit in Sarawak. For this work we thank the team project leader Seth Wong, the Sarawak Forestry Department for issuing research permit, and WWF-Malaysia, particularly Jason Hon. Funding for Seth Wong is provided under the "Integrating conservation, economic development and well-being of communities across the Heart of Borneo Corridor" project supported by the German Federal Ministry for the Environment, Nature Conservation, Building & Nuclear Safety. We are grateful to the Ta Ann Holdings Berhad and particular to Nicholas Ting Kang Hwa, George Wong Tuong Nang, Ling Tiing Tchin, and Nelson Bijack James Chas.

In Sabah we currently continue our close collaboration with the Sabah Forestry Department and the Leibniz-IZW PhD student Roshan Guharajan is applying the tools of the SCREENFORBIO project in this work. Roshan Guharajan received a Elsa-Neumann PhD stipend from the state of Berlin and funding for the fieldwork from Point Defiance Zoo & Aquarium and the International Association for Bear Research and Management.

For the laboratory analysis of the leeches we are grateful to Jörns Fickel, head of the Department of Evolutionary Genetics, of the Leibniz-IZW for close cooperation and continuous support throughout the project. Furthermore, we thank Dorina Lenz, Anke Schmidt, and all other members from the Department of Evolutionary Genetics for their support. We would like to thank Alex Greenwood, head of the Department of Wildlife Diseases, for continuous cooperation. We also thank Ida Schnell, Thomas Gilbert, Kristine Bohmann, and Douglas Yu for the discussions on the laboratory and bioinformatics analysis.

We thank Beth Gardner for her support and insight with developing some of the analytical methods applied in this project.

RapidEye® satellite imagery was provided by Blackbridge Ltd as a part of the RapidEye Science Archive (RESA) programme of the German Aerospace Center through funding from the German Federal Ministry of Economy and Energy. We thank Martin Wegmann for advice on our remote sensing work.

For the case study 3.1.2 we are grateful to Tobias Kuemmerle und Matthias Baumann for their advice and expertise on handling and analysing the satellite image time series. For the case study section 3.2.3 John Mathai thanks the Sarawak Forest Department for issuing a research permit and Mr. Tsen Teck Fen of Samling for assistance and logistical support. John Mathai thanks all HOSCAP Borneo volunteers and fieldworkers and financial support for this study was provided by Care-for-Nature Trust Fund (administered by HSBC Trustee (Singapore) Limited), Ocean Park Conservation Foundation Hong Kong, Yaw Teck Seng Foundation, MAS Wings, Idea Wild and San Francisco Zoo.

We would also like to extend our gratitude to past and current members of the JRG for making this work possible. We are grateful to John Mathai, Seth Wong, Roshan Guharajan, Robert Brozovic, Robin Lietz, Alex Crampton-Platt, Lisa Hörig, Sebastian Wieser, Marie-Louise Kampmann, Niccolo Alfano, Johannes Bender and Thanh Nguyen for their support and assistance with data collection, identifying the thousands of camera-trap photos, and analysis.

We would like to thank our colleagues, Kathleen Röllig, Roshan Guharajan, Badru Mugerwa, and Thomas Gray for their comments on earlier version of this user's guide, which greatly improved all aspects of the work.

Foreword

The Leibniz Institute of Zoo and Wildlife Research (Leibniz-IZW) is Germany's premier wildlife research institute. Our mission is to conduct evolutionary wildlife research for the conservation of species and populations. A central focus of the work of the Leibniz-IZW is to understand and improve the adaptability of wildlife in the context of global change and, through this work, to support species conservation. Since a reliable assessment of wildlife populations forms the basis of all evidence-driven conservation initiatives, it gives me great pleasure to introduce this user guide on surveying and monitoring mammal communities in tropical rainforests as an important milestone for the Leibniz-IZW and for tropical rainforest monitoring and research. There is an urgent need for rigorous surveys and monitoring protocols for these ecosystems because they contain exceptional mammalian (and other forms of biological) diversity and a high proportion of threatened species across all taxa.

The Leibniz-IZW has a long history of pioneering non-invasive wildlife surveying tools. The systematic, standardised application of high-throughput approaches such as camera-trapping and analysing environmental DNA (eDNA) represent an important progress towards rigorous surveying and monitoring methods in tropical rainforests. In developing and refining both non-invasive methods, my colleagues are at the forefront of important emerging fields. Biologists have used camera-traps for decades, yet earlier studies often focused on single species and were limited in scope. Only within the last few years scientists have begun to analyse communities of terrestrial mammals at landscape scales in order to understand their role in complex ecosystems.

The use of eDNA, and specifically invertebrate-derived DNA (iDNA), in combination with high-throughput "metabarcoding" for the detection of terrestrial mammals is currently in its infancy. The much needed conceptual transition from detecting with confidence one particular species through iDNA to systematic species or biodiversity monitoring initiatives has only just begun. As a result, the section of the user guide on eDNA and iDNA is especially timely, as it provides a balanced overview of the opportunities and challenges of this method. I am very pleased to see that this user guide presents the most comprehensive and practical resource on camera-trapping and eDNA and iDNA published to date.

The research described in this user guide was mostly accomplished under the SCREENFORBIO project funded by the German Federal Ministry for Education and Research. Two major technical achievements were developed during this work. The first is the development of *camtrapR*, an "R"-based software application for managing large camera-trap datasets. This package has immediately established itself as a key platform for scientists and conservationists who work with camera-trap data. The second major technical achievement is the development of a laboratory workflow and bioinformatics pipeline for processing and analysing eDNA and iDNA metabarcoding samples. Although this workflow was only made available recently, I am convinced that it will be received with great interest and find wide application in the scientific and conservation communities.

This user guide is designed for practitioners. The often complex scientific topics are described in a language that is straightforward without losing scientific accuracy. It is our hope that it will be of value to a diverse audience, from students to conservationists to governmental organisations. It not only highlights the challenges of studying terrestrial mammals in tropical rainforests, but also guides readers through the numerous analytical tools available to manage and analyse the data collected by camera-traps and eDNA and iDNA. The case studies from the SCREENFORBIO project provide practitioners with real-world examples of how the methods and statistical tools can be applied to answer ecological and conservation-relevant questions. The case studies come from the evergreen rainforests of Malaysian Borneo and the Central Annamite Mountains of Vietnam and Laos. The selection of project sites, both from logistical and ecological perspectives, ensures that the methods and findings are of global relevance. Ecologically, the two study sites exemplify the three main threats to terrestrial mammals in tropical rainforests: habitat loss, habitat degradation (Sabah) and (often illegal) hunting (Central Annamites). Assessing how these threats impact mammal communities, and how stakeholders may improve forest management practices based on these

findings, is of great relevance to tropical rainforests worldwide.

The research presented in this user guide is the product of a close collaboration between Leibniz-IZW scientists and students and our partners from Malaysia, Vietnam and Laos:

- the Sabah Forestry Department and the Forest Research Center in Sabah;
- WWF-Vietnam, Bach Ma NP and the Hue & Quang Nam Saola Nature Reserves in Vietnam;
- WWF-Laos and Xe Sap NPA in Laos.

As the director of the Leibniz-IZW, I am very pleased to offer my gratitude to all partners for helping to make this project a success. The Leibniz-IZW has a keen interest in continuing our long-term research in these study regions, and we look forward to building on the collaborations established within the SCREENFORBIO project.

Despite numerous challenges facing biologists and conservationists working in tropical rainforests, emerging technologies and tools support our efforts to manage these ecosystems more sustainably and to use limited conservation resources more efficiently. I trust that this user guide will serve as a catalyst for future advancements on the techniques presented here, and as a foundation for practitioners as they develop their own projects of studying or conserving mammals in tropical rainforests.

Prof. Heribert Hofer DPhil

Director

Leibniz-IZW

Contents

i. INTRODUCTION

i.i Background

We are currently experiencing the **"sixth mass extinction"**, with global biodiversity loss estimated to be 100 – 1000 times higher than the pre-human background extinction rate (Chapin et al., 2000; Sachs et al., 2009). One of the most important factors driving this biodiversity crisis and threatening the diversity of life is the loss and degradation of habitat through unsustainable forest exploitation. This overexploitation negatively impacts ecosystem stability, functioning, and services provided to humans (Millennium Ecosystem Assessment, 2005; Naeem et al., 2009). Because biodiversity declines are linked to so many components of ecosystem services it is vital to develop strategies for the maintenance of sustainable ecosystems that preserve biological diversity (Balvanera et al., 2006; Cardinale et al., 2012; Mace et al., 2012).

Unfortunately, habitat destruction (both habitat loss and degradation) is progressing most rapidly in the tropics (Hansen & DeFries, 2004; FAO, 2010), where the centres or "hotspots" of biodiversity occur (Hamilton et al., 2010; Giam et al., 2011; Joppa et al., 2011) and where over 50 % of the planet's biodiversity is found. Although habitat loss and degradation impact almost all aspects of biodiversity, different taxonomic groups differ markedly in their vulnerability to forest ecosystem changes: a pan-tropical meta-analysis showed that species richness of mammals and amphibians declined more severely and at lower logging intensities than richness of invertebrates and birds (Burivalova et al., 2014).

In addition to unsustainable land use practices, mammals are highly threatened by a more direct, but less obvious threat: unsustainable hunting that has resulted of widespread "empty forest syndrome" – the loss of large mammals from ecological communities, leading to forests that are structurally complete but retain few large vertebrates (Redford, 1992; Harrison et al., 2016). The loss of larger terrestrial mammals may cause cascading effects, therefore impacting a wide range of species from different taxonomic groups (Bello et al., 2015). For example, the recruitment of carbon-rich canopy trees can be disrupted if the top-down control of herbivores and seed predators by vertebrate apex predators is missing (Terborgh et al., 2001), or if important, large-bodied seed dispersers go extinct (Bello et al., 2015), leading to the loss of important ecosystem services (Galetti & Dirzo, 2013). Apart from their keystone role for the ecosystem, some larger terrestrial mammals are also reliable indicators for anthropogenic processes that threaten the diversity and functioning of the entire natural ecosystem (Caro, 2010). Furthermore, many larger mammals represent charismatic flagship species ideally suited to increase ecotourism opportunities, the public acceptance and support of conservation and the idea of sustainable use of natural resources (Leader-Williams & Dublin, 2000; Caro, 2010). Despite their important role in ecosystem functioning and potential value for conservation efforts, large mammal populations are declining rapidly on the global scale in recent years (Ceballos, 2002; Ripple et al., 2016).

Although the negative impact of forest exploitation and degradation on mammalian biodiversity is well known, the specific consequences on species and species communities in most trop-

ical areas are largely unexplored. Anthropogenic changes affect species both by bottom-up factors (food, shelter, breeding sites) through habitat modifications and by top-down factors (predation) through hunting. The accepted wisdom demands in-depth ecological studies to provide the comprehensive data necessary to assess the impact of forest management strategies on these factors. This is not helpful in practical terms because collecting such a wealth of information is time-consuming, costly, labour-intensive, and usually only possible for single species or a small number of species and in small study areas. Current biodiversity assessments, on the other hand, often focus solely on the presence and number of species, and are frequently restricted to *ad hoc* approaches such as recording opportunistic sightings or rapid and non-standardised "surveys". The biases inherent in these approaches have been well documented. Furthermore, these non-standardised approaches often fail to take into account important aspects of biological communities, including species distribution, species population status, and community composition. The result is that the impacts of forest exploitation and degradation on biological diversity are insufficiently documented due to a lack of monitoring approaches that rigorously address bottom-up and top-down factors in species communities.

Standardised systematic survey and monitoring schemes geared to evaluate the impacts of management practices on biodiversity are usually only applied on small scales and in most cases by experienced scientists. Apart from these few projects, systematic monitoring is seldom implemented in the majority of tropical rainforests, in part because forestry practitioners or students have difficulties in developing appropriate survey and monitoring protocols. A standardised approach for systematic mammal biodiversity monitoring must be repeatable, cost effective, minimally susceptible to observer bias (Waldon et al., 2011) and applicable in tropical rainforest conditions. The very nature of tropical rainforest communities presents unique difficulties, because many tropical species are cryptic, nocturnal, or occur at low densities, prohibiting the application of methods based on direct observations (transect or point counts, distance sampling). Previous studies have shown that track surveys are an inappropriate method because many species cannot be unambiguously identified by their tracks. Moreover, dung samples from rainforest species are typically rare, due to the presence of dung beetles and high decomposition rates of faecal samples under the warm and humid conditions in tropical rainforests. Live trapping of most rainforest species is extremely challenging, requires special permissions and needs to be overseen by a veterinarian, not to mention that it is invasive and always carries the risk of capture- or anaesthesia-related fatalities.

To overcome these challenges and to study terrestrial mammal communities in tropical rainforests requires cost effective high-throughput methods that allow researchers to survey large areas (on the scale of several hundred km^2 in size). In the last decade two of these high-throughput methods have shown promising results:

1. **camera-trapping (Figure i.1) and**
2. **environmental DNA (eDNA), particularly invertebrate-derived DNA (iDNA) (Figure i.2)**

Although camera-trapping is a well-established method for studying secretive mammals in tropical forest ecosystems (Figure i.1) and several manuals and books about camera-trapping and study design have been published, projects are often designed for different goals – from simple detection of highly threatened taxa to density estimation of key species to assessing entire communities – and the different study designs often hinder broader-scale comparative analyses. Further, the lack of standardisation within most camera-trapping surveys as usually practiced prevents future follow-up surveys that form the cornerstone of any long-term monitoring programme.

In contrast to camera-trapping, e/iDNA studies are an emerging technique, and yet no best practice guidelines exist for their application. Currently, all publications that have applied these methods have focused solely on species detection, often with the objective of establishing or confirming the presence of rare or threatened species (Schnell et al., 2010; Calvignac-Spencer et al., 2013; Eva et al., 2016). While such studies can offer important practical information regarding the presence of conservation-priority species, because these approaches are not systematic or standardised, they cannot be applied within a rigorous monitoring context.

The two high-throughput methods camera-trapping and e/iDNA become even more powerful when combined with high-resolution earth observation technologies. Although earth observation is an effective tool to monitor biophysical processes on land and water, biodiversity cannot be directly measured using remote sensing. Connecting earth observation data with high-throughput biodiversity data requires the use of advanced statistical approaches. These approaches have opened new research avenues to biologists who seek to understand patterns of tropical biodiversity. From a conservation perspective, these approaches show promise as a way to track and report changes

Figure i.1: *Elusive tropical rainforest mammals caught on camera-trap from the* ScreenForBio *project. Vietnam / Laos (left panel) and Sabah, Malaysian Borneo (right panel). Mammal species in Vietnam, from top to bottom: Annamite striped rabbit* Nesolagus timminsi*; leopard cat* Prionailurus bengalensis*; red-shanked douc langur* Pygathrix nemaeus*; Annamite dark muntjac* Muntiacus rooseveltorum / truongsonensis*. Mammal species in Sabah, from top to bottom: Sunda clouded leopard* Neofelis diardi*; banded civet* Hemigalus derbyanus*; Sunda pangolin* Manis javanicus*; sun bear* Helarctos malayanus*.*

in biodiversity and ecosystem services across large spatial extents (Bush et al., 2017) – a commitment that the parties of the United Nations Convention on Biological Diversity (CBD) have agreed on within the Aichi Biodiversity Targets.

In summary, cost-efficient repeatable methods to track biodiversity changes are important for forest and wildlife managers to improve management practices and target conservation efforts at the local scale. Monitoring populations, particularly of threatened species, is a central component of global initiatives such as Reduced Emissions from Deforestation and Forest Degradation (REDD+) that explicitly require quantifiable biodiversity co-benefits. Similarly, certifications for sustainable forestry, for example by the Forest Stewardship Council (FSC), require the monitoring of threatened species and protection of High Conservation Value Forest (HCVF). Standardised biodiversity monitoring schemes are of great importance to local governments as there is an increasing need to track and report progress towards the Aichi Targets in a standardised and comprehensive way. However, many parties of the CBD currently struggle with reporting these and measurable indicators on the state of biodiversity are rarely presented. Although the lack of financial resources and commitment are central issues, the lack of standardised and repeatable approaches certainly contributes to the challenges for the CBD parties.

Figure i.2: *Different eDNA and iDNA sources.* ***Top left:*** *water (Valentini et al., 2016);* ***Top right:*** *mosquitos (Lura et al., 2012),* ***Bottom left:*** *flies (Rodgers et al., 2017);* ***Bottom right:*** *terrestrial leeches (Schnell et al., 2018).*

i.ii Introduction to the ScreenForBio project

Between 2013 – 2018, the German Federal Ministry for Education and Research funded a Junior Research Group at the Leibniz Institute for Zoo and Wildlife Research, Germany, to improve our ecological understanding of how Southeast Asian mammal communities respond to forest degradation through timber extraction (Sabah, Malaysian Borneo) and hunting (Central Annamites Landscape in Vietnam and Laos), the two main threats to mammalian biodiversity in the region (Figure i.3).

In both areas we surveyed three study sites with varying degrees of (past) logging (Sabah) and hunting and patrolling intensities (Vietnam / Laos) (Figure i.3). Both areas are covered by tropical evergreen rainforest and historically had similar mammalian communities. Despite these ecological similarities, there are also remarkable differences between our study sites, particularly with regards to accessibility. The sites in Sabah are characterised by flat terrain with few peaks above 500 m above sea level (a.s.l.). In contrast, the sites in Vietnam and Laos have extremely rugged terrain with elevations up to 2000 m a.s.l. Additionally, accessibility was easier in Sabah because of maintenance of roads for logging and silviculture activities. In Vietnam, access was often difficult, and it sometimes took days of travel to reach more remote camera-trap stations (Figure i.4). Taking into account the differences in accessibility and terrain was important for the second objective of our project: the development of a single systematic survey and monitoring approach for terrestrial mammals in two sites with drastically different logistical characteristics.

It was important to use the data we collected at discrete sampling stations to construct broad-scale maps of biodiversity across the study site landscapes. To do this, we collected data at stations using two high-throughput field methods – automated camera-trapping and iDNA using haematophagous leeches – and combined this information with earth observation data using advanced statistical modelling approaches. For camera-trapping, our fundamental objective was to streamline the process from standardised data collection and management to the analysis, and secondarily, to develop additional analytical pathways that provide insight into the status of mammalian biodiversity in our study sites. For iDNA, the main focus was to shift from the basic proof of principle that iDNA can be used to establish species presence to using the method

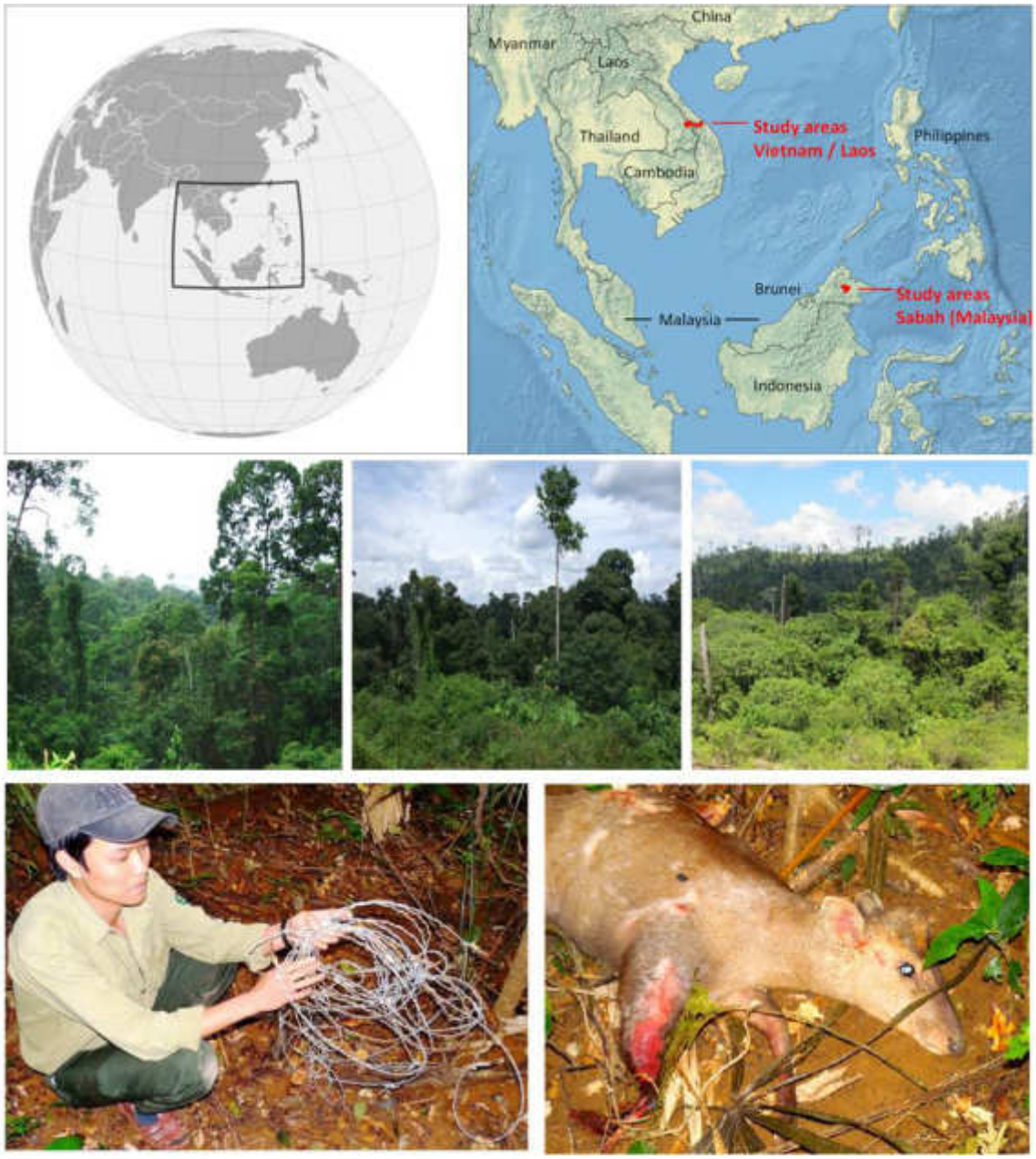

Figure i.3: ***Top:*** *Location of the study areas of the* SCREENFORBIO *project in Vietnam / Laos and Sabah, Malaysian Borneo.* ***Middle:*** *Photographs of the logged forest in Sabah, Malaysian Borneo. From left to right: Deramakot Forest Reserve, FSC certified and only applying Reduced Impact Logging (RIL) strategies for over 20 years; Tangkulap-Pinangah Forest Reserve regenerating from conventionally selective logging in 1990s; Northern Kuamut Forest Reserve conventionally selectively logged between 2004 and 2012.* ***Bottom:*** *A forest guard with snare traps removed from Bach Ma National Park in Vietnam (left) and a muntjac from the same area killed in a snare trap (right).*

as a robust biodiversity assessment and monitoring tool. To do this, we developed new sampling strategies, best practice guidelines in the laboratory, and a bioinformatics workflow. The biodiversity point samples, derived from the camera-traps and iDNA, were then interpolated using high-resolution earth observation data and species habitat associations to construct landscape-scale maps of distribution and species richness.

i.iii Aims and structure of this user guide

The aim of this user guide is to provide practitioners step-by-step instructions for biodiversity assessment and monitoring of tropical forest mammals using camera-traps and e/iDNA. This includes guidance on the project design and standardised methodologies for data collection, data management, laboratory and data analysis, which should enable users to produce standardised and

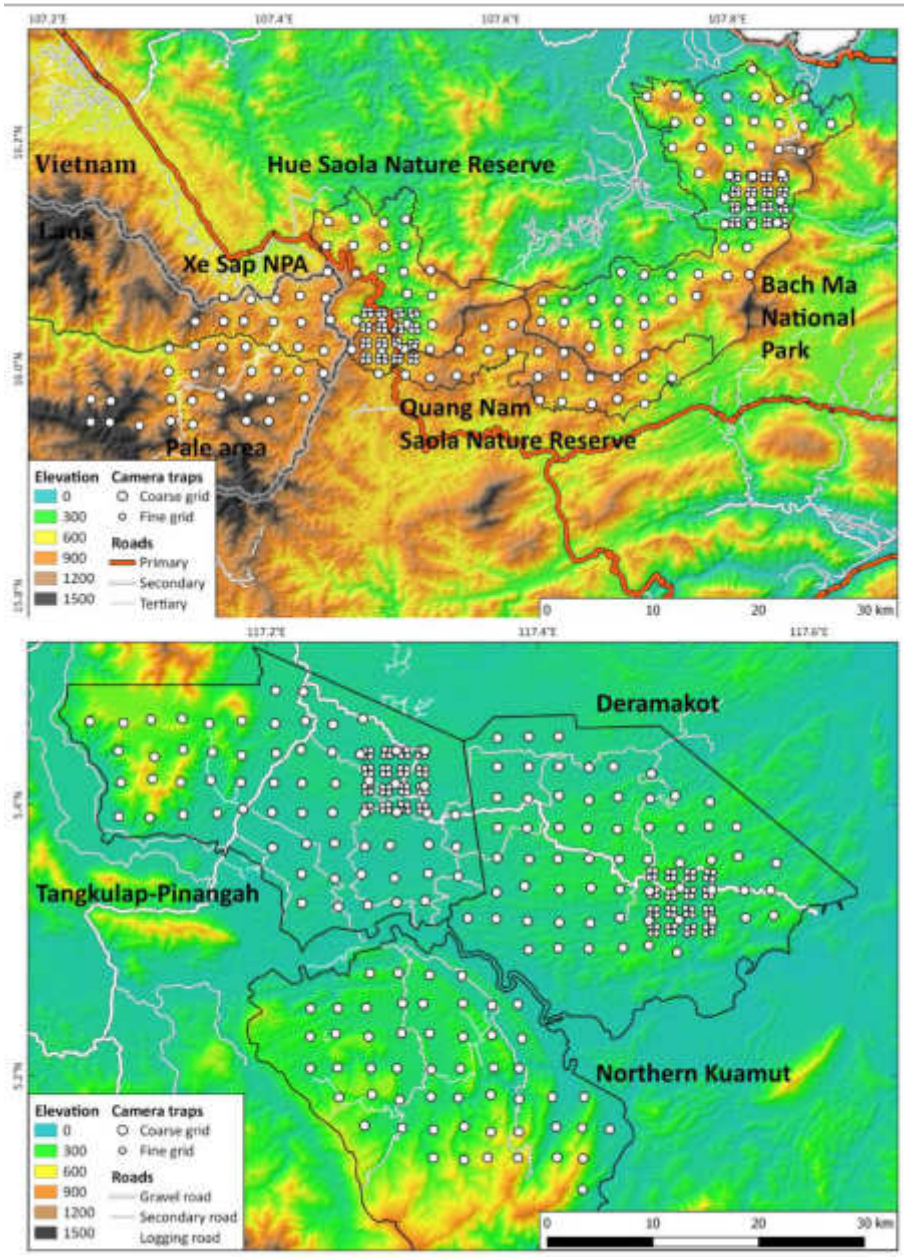

Figure i.4: *Study sites in Central Annamites in Vietnam and Laos (top) and Sabah, Malaysian Borneo (bottom). The maps show the locations of the camera-traps in the coarse grid (2.5 km spacing) and nested fine grid (500 m / 1 km spacing) in the study sites. Roads and the elevation indicate the drastically different logistical settings of both areas.*

more comparable biodiversity data collected in tropical rainforests.

In PART I METHODS AND DATA COLLECTION of this user guide we will first introduce the two main field methods, camera-trapping and iDNA, and highlight the advantages and disadvantages, of both techniques.

The introduction to methodologies is followed by an overview of the study design and data collection methods. In this section, we also include detailed step-by-step field protocols. We further present background information about the techniques and provide references to the available literature for further reading. Because both camera-trapping and e/iDNA surveys can accumulate large amounts of data – hundreds of thousands of individual photos can be generated from camera-trapping studies, and billions of sequence reads can be produced from e/iDNA surveys – we provide guidelines on the management of large datasets. We introduce the R package *camtrapR* (Niedballa et al., 2016) which we developed to manage camera-trapping data, and also provide bioinformatics scripts for the processing and species assignment of the billions of sequencing reads.

To understand species occurrence and the responses of species to environmental changes, a key

component of any camera-trapping or e/iDNA study is the collection of environmental data. Such data includes information collected in the field as well as remote sensing data. Thus, the second section of PART I details how we collected environmental data during this project. We also provide references to available earth observation datasets and guidance into how these data could be analysed with species occurrence datasets.

We recognize that the data collection described in PART I is largely site-specific and needs to be adjusted depending on the specific research questions or survey objectives. Nevertheless, we aim to keep our introduction broad in the hope that our experience can assist future projects and provide guidance to other practitioners.

In PART II ANALYTICAL METHODS we introduce the most common methods used to analyse camera-trapping and e/iDNA datasets.

We focus on methods that account for imperfect detection of animals. Because tropical forest mammals are never perfectly detected, we believe that it is critical to take imperfect detection into consideration during analyses. Ultimately, these methods allow us to disentangle confounding detection and occurrence processes, thereby providing unbiased estimates of key population parameters. Although we touch upon some basic model assumptions, we do not delve into the mathematical complexities of these models. Our main objective is to introduce the different approaches in a general way so that readers can choose the most appropriate analytical approach for their particular research questions. We provide numerous references for further reading, and encourage readers to use these resources to better understand the analytical component of a successful camera-trapping or e/iDNA survey.

In PART III CASE STUDIES we provide key examples from the SCREENFORBIO project.

These examples are taken from published articles as well as manuscripts currently in revision or preparation and provide an overview on the type of questions camera-trapping studies and e/iDNA studies can address. Of course, these case studies provide only a glimpse into the full range of possibilities for camera-trapping and e/iDNA surveys – a Google scholar search for camera-trapping yields over 10,000 publications. Nevertheless, this section provides relevant examples that demonstrate how camera-trapping and e/iDNA can be used to answer ecological questions and provide baseline measures for long-term monitoring programmes.

The final SUMMARY AND PERSPECTIVE section highlights the potential that these approaches have for the monitoring of terrestrial mammals in tropical rainforests, but also identifies areas in which further research is needed.

The rigorous design of our fieldwork is fundamental for evaluating population trends and, thus, to assess the impacts of different forest management practices and anti-poaching programmes. We have planned subsequent surveys to build upon the surveys completed under the SCREENFORBIO project, thereby allowing us to develop a long-term monitoring programme. We expect that upcoming work will further improve our approaches, and it is likely that new and innovative methods will become available in the future. However, baseline data must be established now, so that management practices can be evaluated and partied of the CBD can track and report their progress toward the Aichi Targets based on measurable indicators.

Sunset in Bukit Buyung, Deramakot Forest Reserve, Sabah, Malaysian Borneo. **Photo** Michael Gordon

1. METHODS AND DATA COLLECTION

PART I introduces the data required for mammal biodiversity surveys, as well as the two main methods, camera-trapping and e/iDNA used to collect these data. We provide protocols for camera-trap setup, data collection and data management guidelines for e/iDNA projects (e.g. leech collection and laboratory protocols), and advice on how to collect environmental covariates in the field (*in situ*) and via remote sensing (*ex situ*).

1.1 Camera-trapping

1.1.1 Introduction

The use of automatically-triggered cameras, commonly referred to as camera-traps, has revolutionised the field of wildlife ecology. At the most basic level, a camera-trap is a camera that takes a photograph (or video) when a sensor is triggered. Most commercially-available camera-traps are triggered by heat and / or motion and are most useful for medium- to large-sized terrestrial mammals (though they have also been applied to study other taxonomic groups). Camera-traps bring several advantages to wildlife studies. Because camera-traps are non-invasive, they are able to gather data without impacting the target species. Camera-traps are operational 24 hours a day and can work continuously for months at a time. Camera-traps therefore represent an ideal method for gathering data on tropical rainforest mammal species. These features also make camera-traps especially well-suited to studying rare and elusive species (*i.e.* Trolle & Kéry, 2005; Wilting et al., 2012; de Oliveira et al., 2016). Because the date and time of a photograph is recorded, information on activity patterns can be collected, and it allows biologists to have records of species detection or non-detection over a given timeframe. Information on the occurrence of a species or individuals within a species over a certain timeframe is a fundamental component of many analytical frameworks used in wildlife studies. By using repeated detection / non-detection surveys, it is possible to account for imperfect detection rates, and therefore establish more precise estimates of occupancy, density, and local abundance (see PART II and PART III). Other behavioural aspects, including interactions with other species (Linkie & Ridout, 2011), can also be explored. Finally, camera-traps have an important role to play in science communication and outreach, because they can provide visually stunning images of animals that are otherwise difficult to see or photograph in the wild.

The primary disadvantage to camera-traps is the relatively high startup cost. Quality camera-trap units typically cost between 250 – 600 USD, and most studies will require several – perhaps hundreds – of units for appropriate sampling effort. Purchasing cheap camera-traps, in our experience, is a poor investment because these units often give subpar performance and break down easily, especially in humid tropical rainforest conditions. Despite the initial startup cost, the method is more cost effective than many other approaches, as deployment, maintenance, and processing of data is relatively inexpensive. Another potential disadvantage is that camera-trapping as conventionally practiced is ideal to study ground-dwelling mammals and birds, but may under-detect highly arboreal species (Coudrat et al., 2014). Some species, including primates and arboreal civets, are almost never caught in camera-traps that are set at ground-level. However, it is worth noting that several recent studies

Advantages and disadvantages of camera-trapping

Advantages

- Non-invasive, does not impact the target species.
- Continuous, autonomous monitoring for weeks to months.
- Potential to gather large quantities of data in short time.
- Suitable for studying rare and elusive species where direct observation is difficult.
- Detection / non-detection information allow application of statistical techniques that account for imperfect detection.
- Little man-power required.
- Provides visually compelling photographs for communication and outreach.

Disadvantages

- Relatively high start-up costs.
- Difficult to find camera-trap models that are reliable in tropical rainforest conditions.
- Detections are mostly restricted to terrestrial mammals.
- Only suitable for medium-sized to large species – small mammals are easily missed.
- Large data sets to be managed.
- Image identification can be time-consuming.
- Individuals can only be identified in a few species (species with stripes or spots).
- Image quality is mediocre in many camera models (particularly infrared cameras).

have investigated the application of arboreal camera-trapping (Olson et al., 2012; Di Cerbo & Biancardi, 2013; Gregory et al., 2014).

Despite these disadvantages, camera-trapping is one of the most efficient, unbiased, and cost-effective non-invasive survey techniques for mammals in tropical rainforests and has, more than any other method, provided invaluable data on a wide range of species – from treeshrews (Giman et al., 2007) to tigers (Karanth & Nichols, 1998) and everything in-between.

1.1.2 Considerations in camera-trap studies

Specifics of camera-trap setup and study design depend on the objectives of the study. At the most fundamental level, camera-traps provide records of species presence. This information is often used to compile species lists, providing a community-level snapshot of the terrestrial mammal and bird species present in an area. Species lists may be adequate for some studies –for example, if the objective of the work is to confirm the presence of a particular rare or conservation-priority species. However, such lists do not take into account several fundamental issues of biological surveys, the most important of which is imperfect species detection. Indeed, some tropical rainforest mammals are so rare or elusive that failure to record the species provides little to no evidence of species absence. Not accounting for animals that are missed during a survey provides a biased perspective on the status and distribution of species within a community. To account for imperfect detection rates and obtain unbiased estimates of population parameters, biologists combine camera-trap data with advanced statistical modelling techniques.

In this manual, we present three methods that account for the imperfect detection of and varying detectability between species and individuals.

1. **Occupancy analyses** estimate the probability that a species is present at a site.
2. **Spatial capture-recapture models** estimate movement, density and abundance for animals that can be individually identified, for example, based on unique spot or stripe patterns (for more information on these analyses see PART II and PART III).
3. **N-mixture models** estimate local abundance of animals from counts of individuals.

We do not go into details on these analyses in this section. Rather, we aim to highlight certain aspects of survey design that need to be considered to meet the assumptions of these analytical approaches.

All three analytical approaches have basic assumptions related to camera-trap spacing and duration of the sampling period. For example, occupancy and N-mixture analyses require that animals are not photographed at multiple camera-trap stations (MacKen-

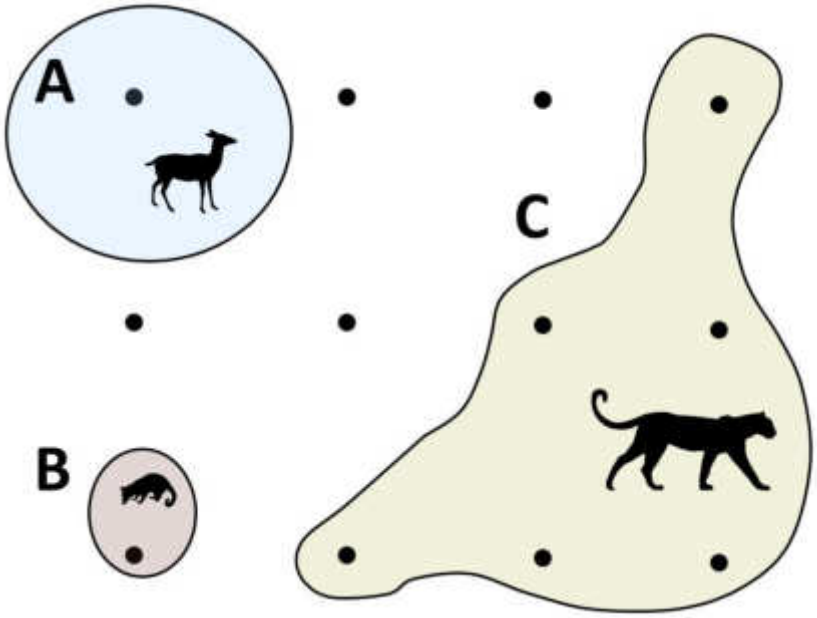

Figure 1.1: *Camera-trap spacing and the spatial closure assumption. Black circles represent camera-trap stations, coloured shapes represent animal movement patterns. For occupancy and N-mixture analysis, only one station should be within the home range of the target species (species A and B). For spatial capture recapture, multiple stations should be within the home range of the target species (species C).*

zie et al., 2002).This means that the spacing between camera-traps should be larger than the home range diameter of the species of interest (Figure 1.1). Studies on multiple species will require a compromise, which may necessitate removing some species from subsequent analyses, or employing more complex analytical methods accounting for lack of spatial independence. For example, for Southeast Asian species with large home ranges – such as large carnivores, elephants, and even wild pigs – spatial independence will be difficult to achieve. In our studies, we used a 2.5 km spacing, which ensured spatial independence of all but a few species in our study sites. Spatial capture-recapture, on the other hand, requires that individuals are photographed across multiple stations (Royle, 2004; Royle & Young, 2008). In this case, it is necessary to have multiple cameras within the home range of the species of interest (Figure 1.1). Again, exact spacing will depend on the movement behaviour of the focal species. Camera-trap spacing will be close for species with small home ranges and wider for wide-ranging species.

All three analyses require minimizing the probability that the population changes within the sampling period through births and deaths or immigration and emigration (see MacKenzie et al., 2002; Rota et al., 2009, for more information on the closure assumption). To meet assumptions of population closure, it is important to keep sampling periods relatively short. Appropriate time frames will depend on the study species. For our studies, we attempted to keep sampling periods close to 60 days. For some sites, particularly in the rugged terrain of Vietnam and Laos where logistical considerations make fieldwork difficult, we used longer durations (up to 120 days).

The spatial independence considerations mentioned above mean, in a practical sense, that consideration must be given to camera-trap station placement before a survey begins. An alternative to opportunistic camera-trapping surveys is a standardised or systematic approach. If the objective is to analyse camera-trap data within occupancy, N-mixture or capture-recapture frameworks, then an opportunistic or non-standardised survey design is not appropriate. Although there are different types of systematic survey designs, one of the most widely-used is a grid-based design. For our landscape-scale surveys, we overlaid a 2.5 km coarse grid onto our study sites, and placed a single camera-trap station (with two cameras per station) at the centrepoint of each square (Figure i.4 and 1.18). Spacing camera-trap stations systematically enabled us to collect data that could be analysed using both single-species (section 2.2, case studies section 3.1.1, 3.2, 3.2.1, 3.2.2 and 3.2.3) and community-level occupancy analyses (section 2.3, case study 3.3.1). A systematic design was also used for our studies that estimated local abundance (section 2.5, case studies section 3.4.1 and 3.6.1) and density (section 2.4, case studies section 3.5.1 and 3.6.1). Because density analysis requires individuals

Figure 1.2: *Example photographs showing camera-trap setup too close (top left), too far (top right), and correct (bottom in green box).*

to be photographed across multiple camera-trap stations, we used a nested fine grid survey designs with smaller trap spacing (500 m / 1 km spacing) to estimate density of medium-sized mammals (Figure i.4).

1.1.3 Camera-trapping field protocol

In this section we give step by step instructions on how to do the set up of camera-traps in a field. A summary of this field protocol, an example of a camera-trap datasheet and a checklist for the field can be download as a Word and pdf file[1].

Equipment needed (Figure 1.10)

(1) **Camera-trap unit.** Various makes and models are available.
(2) **Batteries.** We recommend using quality brand-name batteries such as Energizer or Duracell. Cheaper brands may result in poor camera performance.
(3) **SD cards**. Each card should permanently be labelled with an ID that matches the camera-trap ID.
(4) **GPS**. Used to navigate to the camera location and record the coordinates of each camera-trap station.
(5) **Digital camera.** Used to view the camera-trap test photos immediately after setup to ensure correct positioning.
(6) **Cable lock** or **bungee cord** to secure the camera to the tree. Cable lock provides theft deterrence.
(7) **Compass** for taking the bearing of the camera-trap (if the GPS unit does not have this function).
(8) **Machete** for cutting or clearing vegetation.

(1) Camera-trap settings

Always check the camera-trap settings before placing the camera-trap in the field. Important settings to check include:

- Date / time
- Set to photograph mode (we do not recommend video because video metadata are not standardised and date / time information can easily be lost from metadata)
- Maximum sensitivity

[1] `http://www.leibniz-izw.de/userguide.html`

Recommended settings for Reconyx 500 series cameras

- Motion sensor: On
- Sensitivity: High
- Pics per trigger: 3
- Picture interval: Rapidfire
- Quiet period: No delay
- Time lapse: Off
- Resolution: 3.1 MP
- AM period: Off
- Night mode: High quality
- PM period: Off

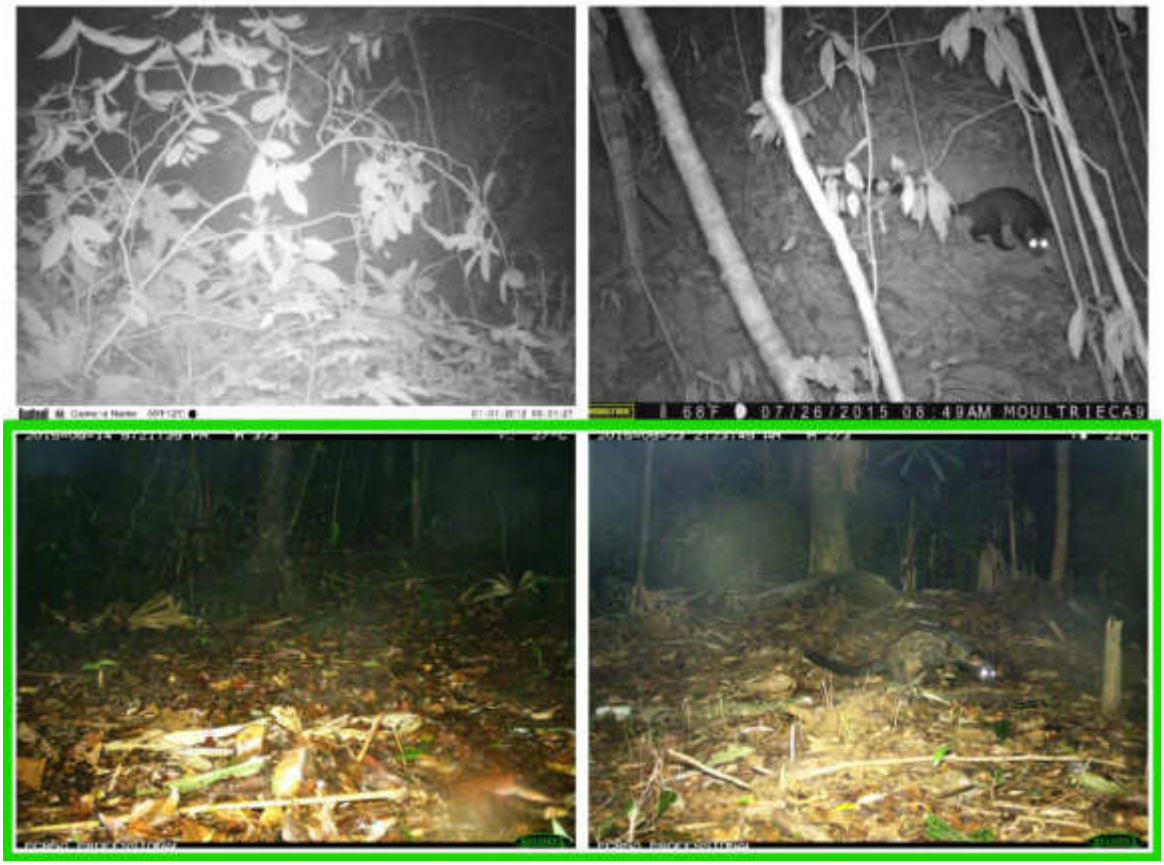

Figure 1.3: *Example photographs showing camera trap setup with too much vegetation (top) and just right (bottom in green box). Not clearing vegetation will cause many animals to be missed or can result in poor photos.*

- Minimum delay between photographs
- Set to take minimum 3 – 5 photographs in a sequence per trigger

(2) Selecting the camera-trap location

The systematic grid-based design should be viewed as a general guide for where to place camera-traps. However, placing each camera-trap at the exact UTM coordinates is not necessary and may often not be possible or desirable. Small discrepancies between the planned location and the actual site where the camera-trap is set will exist and are to be expected. The final location should be based on practical field conditions and on factors that will maximize detectability.

Exactly how much leeway is acceptable? For the coarse-grid survey design with 2.5 km between stations which we applied in our projects, we recommend setting the camera-trap as close as possible but at least within 500 m from the planned location. We recommend keeping a minimum of 2.0 km between stations at all times to approximate spatial independence. It is important that teams operating in adjacent areas communicate so that this minimum distance is maintained. For example, moving two neighbouring camera-traps 500 m towards each other would decreases the distance between stations to just 1.5 km.

Once the team is near the planned location, the process of searching for the specific camera-trap site begins. This process should not be rushed. Exact

Figure 1.4: *Example photographs showing camera-trap setup too high (top) and correct height (bottom in green box). Setting camera-traps above ~40 cm will result in a substantial portion of the animal community to be missed.*

camera placement is based on a variety of factors. The team should split and carefully search the surrounding area for places likely to yield the highest number of wildlife photos. At the macro-scale, look for landscape features that might funnel wildlife between two points. At the finer scale, look for signs of recent animal activity such as tracks or dung, as well as mud wallows, salt licks, or game trails. In some areas, water sources may be important in attracting wildlife. Man-made roads have also been shown to be conduits of wildlife activity for some species, especially large carnivores (Harmsen et al., 2010). In general, try to place the camera on flat terrain, as this will make it easier to optimize the field of detection. Avoid swampy or lowland areas that are prone to flooding. We recommend placing the camera 1.5 to 4 m from focus area (Figure 1.2). If the camera is setup closer than 1.5 m from the target area, the bright flash from the camera may "white out" the subject or a close up photo of the animal is taken, which often make species/individual identification difficult. If the setup is further than 4 m from the target area, the image might be too dark or there will be an insufficient level of detail for proper identification (especially for small mammals). Experience has shown that setting the camera-trap approximately 3 m from the target zone provides ideal results in most situations. In our case we set the two camera-traps per station within a 20 x 20 m plot (the same plot that we used for the vegetation survey, see section 1.3.3). If two camera-traps are used with the goal of increasing detection probability, rather than capturing both flanks of an animal, the cameras should be set facing in different directions, for example on different animal trails.

(3) Vegetation clearing

Once the camera location has been chosen, it is necessary to clear any vegetation that might affect the camera's performance or the quality of the resulting photographs. Vegetation, including grass and overhanging leaves, can both prevent the camera's sensor from triggering or can falsely trigger the camera. Furthermore, any obstructions can make it difficult to identify the photographed animal. Vegetation can also reflect the camera's flash, especially with infrared units, resulting in whited-out photos that make later identification difficult.

How much vegetation should be cleared? There is no straightforward answer, but a simple rule is to

Figure 1.5: *Incorrect (top left) and correct (top right) camera angles. In steep terrain, it is important to adjust the camera angle so that the sensor's field of view is parallel to the ground (bottom).*

make sure that the cameras field of vision is clear and that there is no major vegetation obstructing the field of view in the target zone (Figure 1.3). Of course, one should be careful not to modify the habitat too severely so as to deter wildlife from passing through the area. Because vegetation grows rapidly in the tropics, we recommend pulling up grass and other vegetation that you wish to remove by the roots. After this, it is often helpful to place a large flat rock or leaf just below the camera, which further prevents vegetation from growing in front of the camera's field of view, as well as to avoid mud splash on the camera during rainy days.

(4) Camera-trap set-up

To maximize the number of species detected, camera-traps should be placed at an appropriate height for the target species. We recommend setting cameras 20 – 40 cm above the ground on flat terrain (just below knee height). This allows for the capture of large, medium, and small mammals, although you likely will not be able to photograph the entire body of large mammals. Many camera-trap setups place cameras too high and as a result miss a significant portion of the terrestrial mammal and bird communities or juvenile individuals (Figure 1.4). The Annamite striped rabbit, for example, is only detected when camera-traps are placed close to the forest floor. When cameras are

placed in steep areas, the height at which the unit is placed on must be adjusted according to the specific situation (Figure 1.5).

(5) Adjusting the camera angle

As mentioned earlier, it is best practice to place the cameras in flat areas. However, we recognize that this is not always possible, especially in rugged mountainous terrain. The most important aspect for camera-trap setup is achieving the correct angle of the camera relative to the ground. The camera-traps' area of highest sensitivity is a straight horizontal plane extending out from the sensor. Therefore, the camera should be angled so that the sensitive area is parallel with the ground. The simplest way to do this is to angle the camera so that the resulting images are level within the frame (here it is important to make sure that you have a digital camera to check the photographs). If the ground slopes upwards or downwards perpendicular to the camera this will result in the sensitive area of the camera's motion sensor to point either down (towards the ground) or up (towards the sky) and a passing animal will not trigger the camera. To adjust the camera angle, we recommend using nearby materials, such as sticks or small stones, and wedging them between the camera-trap and the tree it is mounted to. You may need to trim the stick with a machete to get the width that gives you the desired camera angle.

(6) Testing the camera

Some cameras will include a test mode during which it is possible to move in front of the camera to test where on the trail an animal will trigger the camera. If the camera-trap model that you are using does not have this function, you can test the camera by turning it on. Have one team member move through the camera's detection field. It is important to remember that most animals are smaller than people and therefore it is most useful to crawl, rather than walk, in front of the camera during the test. We recommend moving through the detection field at close (one meter) and farther (3 m or more) ranges to ensure that the camera is positioned to detect targets at varying distances.

(7) Arm the camera-trap

Turn the camera-trap on. Write down the station ID, camera-trap ID, date and time, and compass bearing on the datasheet. Hold the sheet in front of the camera-trap and let it take several photographs. Lock the camera-trap. Please note that this step should be done after finishing the vegetation survey (see section 1.3.3).

1.1.4 Camera-trap data management overview

In the following sections we introduce the necessary steps that need to be taken for simple and efficient organization of the camera-trap data. We also introduce the R package* camtrapR *that was specially developed for the management of camera-trap data.

Large-scale camera-trapping projects can collect hundreds of thousand or even millions of photos in relatively short amounts of time. Such amounts of data require extensive automation of the data management workflow, as manual labour would be tedious, error-prone and time-consuming. Successful camera-trapping projects thus require efficient and reproducible processing and management of these enormous amounts of data to maximise the information obtained while minimising the time spent entering and tabulating data.

The main criteria for successful management of camera-trapping data are to provide a consistent data structure while flexibly accommodating different study designs, to provide analytical pipelines with strong automation of essential steps in order to minimise data entry errors, and to allow for the simple creation of input for ecological analyses.

In recent years, a number of different approaches for camera-trap data management have been published, giving users the freedom to choose software that suits their needs (Harris et al., 2010; Fegraus et al., 2011; Sundaresan et al., 2011; Sanderson & Harris, 2013; Tobler, 2014; Krishnappa & Turner, 2014; Zaragozí et al., 2015; Ivan & Newkirk, 2015; Bubnicki et al., 2016; Niedballa et al., 2016; Hendry & Mann, 2018; Ramachandran & Devarajan, 2018) . For a comparison of software for camera trap data management readers can refer to Niedballa et al. (2016); Scotson et al. (2017); Wearn & Glover-Kapfer (2017), or Ramachandran & Devarajan (2018).

As a part of the SCREENFORBIO project some authors of this user guide developed the R package *camtrapR* for camera-trap data management. Below we provide a short introduction in this R package. For further information please see the vignette published together with the R package (Niedballa et al., 2018) and the publication (Niedballa et al., 2016).

Figure 1.6: *Main steps of the workflow for managing camera-trapping data in the R package* camtrapR. *The five main steps of the workflow (grey box) process raw camera-trapping data and produce input for subsequent ecological analyses.*

1.1.5 Protocol *camtrapR*

The R package *camtrapR* is a toolbox for managing camera-trapping data. It implements a complete workflow for managing camera-trapping data, from storing raw data on the hard disk to preparing input for subsequent ecological analyses.

It was designed to seamlessly link data acquisition (camera-trapping) with well-developed tools for ecological analyses of camera-trapping data. It is implemented in the statistical software R and entirely relies on free and open-source software (Niedballa et al., 2018, 2016). Being implemented in R, *camtrapR* is a command line tool, requires some knowledge of the R language for statistical computing and does not have a graphical user interface. This is a potential hurdle for new user who need to get used to R first, but offers the advantage of great flexibility and seamlessly linking camera-trap data management with powerful data analyses provided by R packages for occupancy, spatial capture-recapture and activity analyses (e.g. *unmarked*, *secr*, *overlap*, *wiqid*).

The workflow is divided into five main steps and contains 23 different functions in total (Figure 1.6 and Table 1.1). It begins with storing the camera-trap images from memory cards on a computer's hard disk. *camtrapR* then assists in organising raw images and assigning species and individual identification, tabulates records of species and individuals, provides toosl for data exploration and visualisation and prepares input for subsequent ecological analyses, e.g. in occupancy or spatial capture-recapture frameworks.

One of the main aims of *camtrapR* was to provide maximum flexibility to accommodate different study designs. It works with any number of camera-traps per camera-trap station, and multiple cameras at camera-trap stations can be either paired or independent (see section 1.1.3). *camtrapR* supports user-defined tags (e.g. for species and individual identification, flank, sex, age class, behaviour, group size etc.), which can be assigned in photo management software. These tags are written into the image metadata, from where *camtrapR* can read them out. Users can thus assign relevant information to images flexibly and use these information for ecological analyses. It also aims to offer maximum flexibility when creating input for ecological analyses, namely creating detection histories for occupancy and spatial capture-recapture analyses, by giving users freedom to define occasion length and starting time.

camtrapR contains extensive documentation and vignettes, which serve as tutorials for the provided sample data[2] and demonstrate the usage of all functions. There is also a tutorial in Spanish available (López-Tello & Mandujano, 2017). A Google group[3] is maintained as a support forum for all questions related to *camtrapR*.

[2] `https://CRAN.R-project.org/package=camtrapR`
[3] `https://groups.google.com/forum/#!forum/camtrapr`

Table 1.1: *Functions of the camtrapR workflow for camera-trap data management (adapted from Niedballa et al., 2016).*

Function	Description
Image organization and management	
createStationFolders	Create directories for storing raw camera-trap images
timeShiftImages	Apply time shifts to JPEG images
fixDateTimeOriginal	Fix faulty DateTimeOriginal tag in Reconyx Hyperfire cameras (e.g. HC500)
imageRename	Copy and rename images based on station ID and image creation date
appendSpeciesNames	Add or remove species names from image filenames
addCopyrightTag	Add a copyright tag to images
Species/individual identification	
checkSpeciesNames	Check species names against the ITIS taxonomic database
createSpeciesFolders	Create directories for species identification
checkSpeciesIdentification	Consistency check on species identification
getSpeciesImages	Gather all images of a species in a new directory
Tabulating species records	
recordTable	Create a species record table from camera-trap images
recordTableIndividual	Create a single-species record table from camera-trap images with individual IDs
exifTagNames	Return metadata tags and tag names from JPEG images (for use in recordTable functions)
exiftoolPath	Add the directory containing exiftool.exe to PATH temporarily
Data exploration and visualisation	
detectionMaps	Generate maps of observed species richness and species detections by station
activityHistogram	Plot histograms of single-species activity
activityDensity	Plot kernel density estimations of single-species activity
activityRadial	Radial plots of single-species activity
activityOverlap	Plot two-species activity overlap and compute activity overlap coefficient
Creating input for subsequent analyses	
cameraOperation	Create a camera operation matrix
detectionHistory	Species detection histories for occupancy analyses
spatialDetectionHistory	Detection histories of individuals for spatial capture-recapture analyses
surveyReport	Summarize a camera-trapping survey

Setting up *camtrapR* on your computer

Setting up your computer to run *camtrapR* is easy and free. It runs under Windows, MacOS or Linux and entirely relies on free and open-source software. Essential components necessary to use *camtrapR* are the R statistical software[a], the *camtrapR* package[b] and Exiftool[c]. Exiftool is used internally to read and write metadata from and to images. The package vignette contains instructions on how to install Exiftool on your system[d].

digiKam[e] is a photo management and tag editor software which can be used for assigning metadata tags to camera trap images. Alternatively, proprietary software such as Adobe Bridge or Adobe Lightroom can be used for tagging images. These tags can be read out by *camtrapR*. RStudio[f] is an integrated development environment for R and simplifies working with scripts and data in R.

camtrapR runs well on standard computers or laptops, even older hardware. It has no particular hardware requirements apart from sufficient free disk space to hold camera trap images. If handling large amounts of images, stronger hardware will result in a smoother and faster workflow. Disk usage can be considerable, depending on the number of images, image size and quality. Assuming one image equals 1 Megabyte, 1000 images require 1 Gigabyte and 1 million images require 1 Terabyte of disk space. Keeping the raw images as a backup (which is advisable) doubles the required disk space. Depending on hardware, extracting image metadata from large quantities of images (in the range of hundreds of thousands) can take a few minutes, particularly if images are stored on HDD instead of SSD type hard disks.

[a] https://www.r-project.org/
[b] https://CRAN.R-project.org/package=camtrapR
[c] https://sno.phy.queensu.ca/~phil/exiftool/
[d] https://cran.r-project.org/web/packages/camtrapR/vignettes/ImageOrganisation.html
[e] https://www.digikam.org/
[f] https://www.rstudio.com/

1.2 Environmental and invertebrate-derived DNA

Surveying species based on traces of residual DNA from environmental samples (e/iDNA), offers a potential new tool for wildlife biologists. They are attractive due to their low sampling costs and the possibility to obtain high number of samples in relatively short time, but these tools are still in their infancies. In this section we introduce the concept of e/iDNA and provide guidance on the collection and analysis of e/iDNA samples and on legal aspects associated with the collection and export of e/iDNA samples. We focus in particular on terrestrial haematophagous leeches as an iDNA source.

1.2.1 Introduction

Monitoring or even detecting elusive or cryptic species in the wild can be challenging. As shown above, camera-trapping is one of the most efficient methods, but reliable camera-traps are costly and even the most expensive models will experience problems after periods of prolonged use. Furthermore, camera-traps are often stolen by illegal encroachers, likely out of fear that photos might provide evidence of their illegal activities. Although in general camera-traps are considered a non-invasive tool, they are sometimes recognised as something alien by the animals and are then examined out of curiosity or even attacked resulting, in additional camera losses (Figure 1.7). A more ideal technique for monitoring species occurrence would therefore record presence without any possibility – however small, as is the case for camera-trapping – of the animal being aware of the process.

Recent advances in molecular techniques have given rise to a new set of tools that allow species detections with no chance of impact on the target species. Environmental samples of water, soil or even air often contain remains of DNA from various organisms (Bonin et al., 2018). This environmental DNA (eDNA) can be collected and analysed with modern high-throughput DNA sequencing techniques. In these so-called DNA barcoding studies, certain fragments of the obtained DNA are amplified, sequenced, and then assigned to a certain species (Hebert et al., 2003). Identifying many different DNA barcodes simultaneously a single eDNA sample is called metabarcoding (Taberlet et al., 2012; Yu et al., 2012). The sequenced DNA fragments are usually very short (80

Figure 1.7: *Series of camera-trapping photographs of an Asian elephant damaging a camera-trap in Deramakot Forest Reserve, Sabah, Malaysian Borneo.*

to 600 base pairs) and have to show enough variation among species so that they can be assigned to a reference database of know sequences from various organisms that are expected to occur. The taxonomic identification of the DNA fragments is a critical step; therefore, a reliable reference database of correctly identified sequences is needed. However, for vertebrate species Barcode of Life Database (BOLD), based on the DNA barcode region, is currently the only available reference database, which at the moment contains only a fraction of the known species in the world (Mulcahy et al., 2018). For other genetic markers than COI, researchers have to rely on non-curated databases such as GenBank, which places the burden for metadata accuracy, including taxonomy, on the sequence submitters, with no restriction on sequence quality or veracity. Thus it is flawed with incorrectly identified sequences. **The use of multiple genetic markers might help to identify species with higher accuracy and can bypass the database gaps existing for most species and their genetic markers in the databases.**

A special case of these eDNA studies are studies on invertebrate derived DNA (iDNA) where genetic material of species other than the mammalian target species is extracted from sarco- or haematophagous invertebrates. Such invertebrates are ticks (Gariepy et al., 2012), blow or carrion flies (Lee et al., 2016; Calvignac-Spencer et al., 2013; Rodgers et al., 2017; Hoffmann et al., 2018), mosquitos (Schönenberger et al., 2016; Hopken et al., 2017; Townzen et al., 2008; Kocher et al., 2017) or leeches (Schnell et al., 2012; Tessler et al., 2018; Weiskopf et al., 2018). Many of these studies provided promising results indicating that iDNA will likely be a very useful tool for biodiversity surveys in the future (Schnell et al., 2012, 2015). In particular, the possibility for bulk collection and sequencing will allow screening of large areas and to minimise costs. In addition, these methods might also allow distinguishing very similar looking or even cryptic species, which cannot be unambiguously identified in camera-trap images.

In addition to these advantages there are also disadvantages of iDNA that researchers should be aware of. In bulk iDNA samples of many invertebrate specimens the variable time since each specimen has fed, results in differences in the level of degradation and the relative amount of target DNA within a sample. This makes eDNA and iDNA studies in general more comparable to ancient DNA samples, which have dealt with the problem of low quality and low target DNA amounts for several decades (Pääbo et al., 2004; Hofreiter et al., 2015). The low overall amount of target DNA compared to non-target DNA requires an enrichment step mainly done by amplification of the target fragment (amplicon) by polymerase chain reaction (PCR), to obtain enough target material for sequencing. This PCR step harbours several risks and problems, which can result in false positive (e.g. through contamination or volatile short PCR amplicons in the laboratory) or false negative (stochasticity in PCR process) results. Although laboratory standards to prevent and control for such false results are

Advantages & disadvantages of e/iDNA

Advantages:

- Low costs for sample collection.
- Sample collection is straightforward and does not require specific skills, enabling individuals such as rangers and research assistants to do the collection with minimal training.
- High numbers of samples can easily be collected (although this depends on the e/iDNA source).
- Suitable for long term monitoring if sample collection is standardised.
- If bulk samples are collected and processed via next-generation sequencing techniques, a high number of samples (several hundreds) can be processed within short time frames.
- Some cryptic species (species which cannot be distinguished with other methods such as camera-trapping) can be identified (given appropriate reference database).
- Smaller mammal species or even non-mammalian species such as frogs can be detected.

Disadvantages:

- High initial costs, depending on the availability of the molecular biology laboratories, and moderate costs once the laboratory workflow and system have been established.
- Collection, exportation and use of e/iDNA samples as a genetic resource requires specialised permits.
- Skilled personnel with laboratory and bioinformatics expertise is needed to process and analyse the samples.
- Many species are currently missing from the reference databases, increasing the risk of imperfect taxonomic assignments.
- DNA quantity and quality of the host is often low and requires specialised laboratory protocols.
- Species detection might be affected by host selection and host preferences of the invertebrate parasite.
- Spatial information about the host might be lost due to the movement of the invertebrate parasite.
- Sample collection can be severely affected by time of year (wet / dry season) or collection time (morning / afternoon).

well established in the field of ancient DNA, there are no best-practice guidelines for eDNA and iDNA studies, and thus few studies sufficiently account for such problems (but see Bonin et al., 2018). Furthermore, due to erroneous entries in reference databases, the incompleteness of reference databases, and the sequence similarity of related species within the target barcode, special care has to be taken during the taxonomic assignment. This increases the risk of false positives in a biodiversity survey which could reduce the confidence of stakeholders in such techniques and could potentially lead to false management decisions.

1.2.2 Legal aspects of e/iDNA studies

When working with e/iDNA, it is important to be aware of the legal requirements that regulate the handling and transfer of genetic material. To avoid complications—and potentially legal violations—researchers should be familiar with regulations before samples are collected. All projects should be conducted in full compliance with the local national laws (e.g. research, collection, and export permits) and international agreements such as the Convention on Biological Diversity (CBD) and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). As these regulations differ from country to country, it is the responsibility of the researcher or organization collecting the samples and / or conducting the genetic analysis to make inquiries about the necessary regulations. Obtaining this information can often be laborious and sometimes even frustrating, due to the complexities of administrative structures in some countries. Nonetheless, we believe that due diligence is required when working with genetic samples.

We would like to remind readers that any e/iDNA samples fall under the Nagoya Protocol. The Nagoya Protocol is part of the CBD and is a binding international agreement that came into effect in October 2014. The overall aim of the Nagoya Protocol is to share benefits from the use of genetic resources between a provider (country were the genetic material was collected) and a user (commercial or non-commercial use of any physical genetic resource). **It is important to note that the Nagoya Protocol**

Figure 1.8: *One advantage to using leeches as a source of iDNA is that they are often easy to collect in wet tropical rainforests.*

also applies for non-commercial research projects and conservation projects and is not restricted to the commercial use of genetic resources. Genetic resource is broadly defined as genetic material from any organism that has actual or potential value. This definition includes all e/iDNA samples, as iDNA samples are invertebrate specimens are thus direct genetic resources, but even environmental samples that contain DNA, such as soil and water.

To check if a particular country is a party of the Nagoya Protocol, visit the Access and Benefit-Sharing (ABS) Clearing-House website (https://absch.cbd.int/). Each party of the Nagoya Protocol is called to establish its own requirements to govern the access to genetic resources. Parties of the Nagoya Protocol have to declare their competent national authorities and as well as their National Focal Points. Contact information is provided for the National Focal Points.

Key requirements under the Nagoya Protocol include Prior Informed Consent (PIC) and Mutually Agreed Terms (MAT) documents that projects need to obtain from the provider countries. PIC is defined by the CBD as "the permission given by the competent national authority of a provider country to a user prior to accessing genetic resources, in line with an appropriate national legal and institutional framework." MAT is defined as "an agreement reached between the providers of genetic resources and users on the conditions of access and use of the resources, and the benefits to be shared between both parties." MAT therefore regulates and defines the limits of use of the genetic resource.

Obtaining the necessary permits and the documents required as a part of the Nagoya Protocol (if needed) can take time – in some cases a few months or even a year. Any e/iDNA project needs to be aware of this potential delay and plan accordingly. Our experiences show that obtaining the ABS clearance is both labour and time intensive, probably because the Nagoya protocol only came into force a few years ago and in many countries the national regulations are not yet well developed. We believe that this process will become easier once both providers and users are more familiar with the procedure.

1.2.3 Terrestrial leeches as a source for iDNA

One of the invertebrates that has revealed promising results as a biodiversity monitoring tool are terrestrial haematophagous leeches (Schnell et al., 2012; Tessler et al., 2018; Weiskopf et al., 2018). Terrestrial leeches occur in large parts of tropical and subtropical Asia, Australia and Madagascar (Schnell et al., 2015). Leeches are especially abundant and easy to collect during the rainy season. They appear to be opportunistic feeders (Tessler et al., 2018; Weiskopf

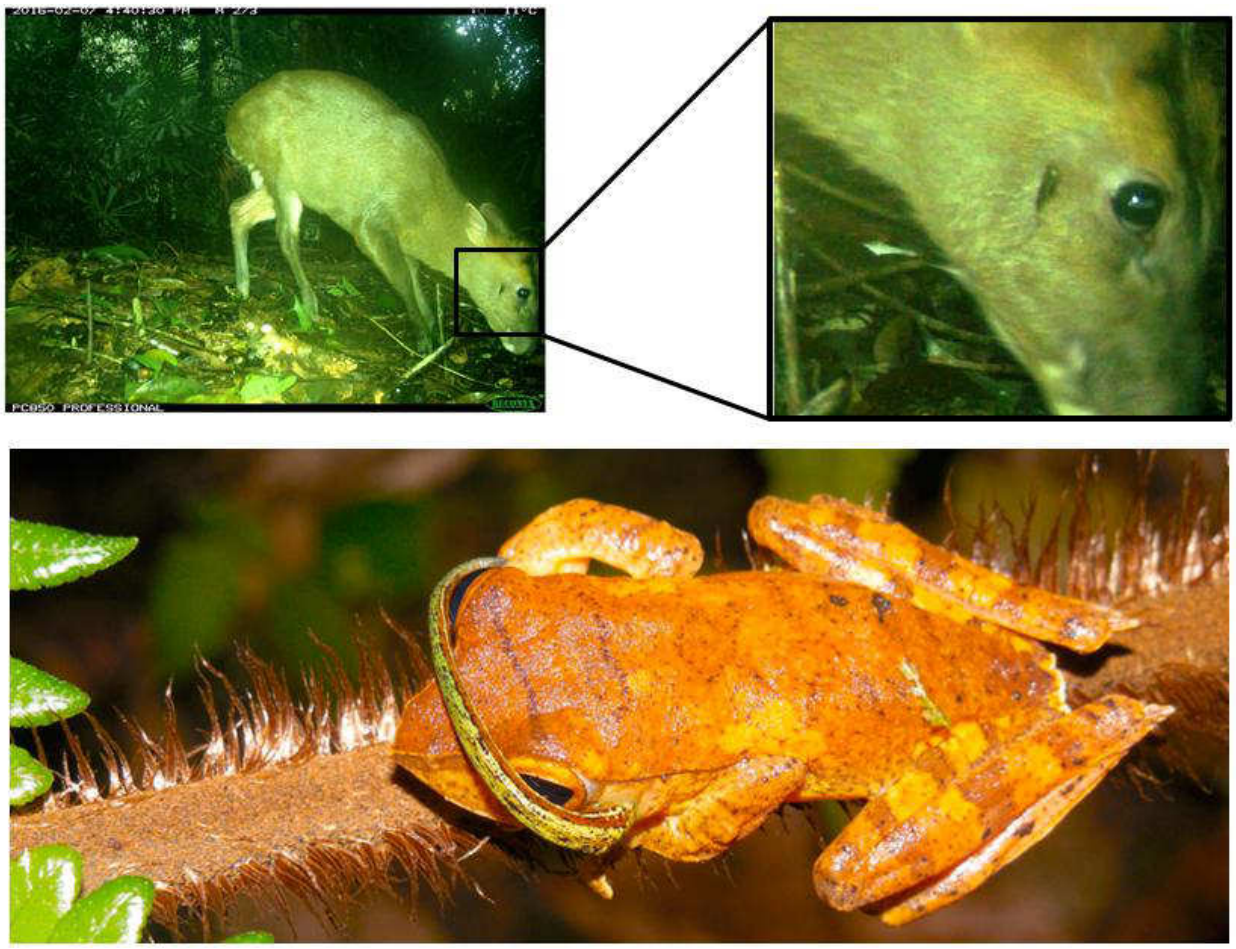

Figure 1.9: *Leeches sampling terrestrial vertebrate biodiversity; feeding on muntjac (top, taken from camera-trap photo) and frog (bottom).*

et al., 2018) sampling a wide range of vertebrates (see Figure 1.9), and thus give a more unbiased result compared to more selective feeders such as *Culex* mosquitos that prey mainly on birds (Farajollahi et al., 2011). Compared to other invertebrates, leeches ingest large amounts of blood per feeding, therefore providing more genetic material for laboratory analyses (Schnell et al., 2015). Despite these advantages, a number of uncertainties remain, in particular with respect to the relatively un-studied ecology of terrestrial leeches (Schnell et al., 2015). In the next sections we provide examples how leeches could be used in a rigorous way to systematically survey and monitor biodiversity.

1.2.4 Collection of leech samples

Terrestrial leeches are best sampled in predefined sampling plots. Because the spatial information (i.e. location of leech sample collections) is important for the subsequent analyses, it is important that leeches with different spatial attributes are not grouped together into one sampling tube. We suggest therefore to have rather small sampling plots (e.g. 20 x 20 m), but if the number of leeches in a plot is too low it might be necessary to increase the sampling area. The leeches should be transferred into collection tubes pre-filled with a fixative such as 90 % ethanol, or better yet, a nucleic acid preservative like a saturated ammonium sulphate solution (e.g. RNAlater^{TM}). We suggest the use of 25 ml collection tubes filled with 20 ml of RNAlater. We suggest that no more than 20 specimens should be collected in one tube, as a higher specimen numbers make it more difficult to detect rare species as a result of dilution effects. If there are more leeches available in a sampling plot at a given sampling occasion, they should be split into multiple collection tubes. For an occupancy analysis generally six to ten sampling occasions of each plot would be needed. Unfortunately, not all leech samples will amplify and can be sequenced successfully, leading to NAs in the dataset. Therefore, six replicates should be seen as a minimum to allow estimates on detection probabilities. It is essential to avoid contamination with human DNA during collection. Thus, leeches that have come into contact

with human skin should be discarded. Furthermore, we recommend that collectors wear latex gloves during the collection process (though we realize that this may not always be possible, particularly in difficult fieldwork conditions). Each collection tube should have a label inside giving the sampling location and date. We recommend labels are written in pencil, as ink may bleed or degrade in the buffer solution. We caution against labelling only on the outside of the tube, as our experience has shown that external labels may wear off. RNA integrity is best preserved by following recommendations: collected samples should be stored for 24 h at 4°C to allow the RNAlater to permeate into the collected leeches completely. After this, samples should be transferred to -20°C for long term storage. Repeated thawing and freezing of samples should be avoided. In case this procedure is not possible – for example, if teams are working in rugged field conditions for extended periods of time – try to protect samples from exposure to direct sunlight and keep them as cool as possible until they can be stored at -20°C.

1.2.5 Laboratory work

The laboratory workflow developed as a part of the SCREENFORBIO project was recently published on BioRxiv (Axtner et al., 2018) and the paper is currently in revision. Here we present a brief overview of the laboratory considerations that researchers should be aware of while working with e/iDNA samples.

Analysis of e/iDNA should minimize the risk of cross-sample or laboratory contaminations while simultaneously allowing for the processing of large sample volumes. To avoid contamination, the laboratory set-up should be analogous to ancient DNA studies with separated laboratories for the different processing steps (DNA extraction, pre-PCR, post-PCR and sequencing) and samples or materials should not be allowed to re-enter upstream laboratories at any point of the workflow. Complete labs should further be UV-irradiated at regular intervals (e.g. 4 h every night). We advise pipetting only under laminar-flow hoods or, at a minimum, at PCR work stations that can be easily cleaned and radiated with UV-light.

Due to the stochasticity of PCR, and in order to avoid false positive results, use of technical replicates is of great importance in metabarcoding studies (Bonin et al., 2018). Metabarcoding studies based on less than four PCRs replicates should be handled with care. As contamination can already occur during the extraction phase we split our samples directly after lysis into two technical replicates A and B (*extraction replicates*) and then ran all subsequent analyses independently for these two extraction replicates. We only accepted taxa present in both extraction replicates. As many species, especially rare species, are missing from the reference database, or their sequences are only available for a certain loci, the use of multiple markers increases probability for taxon identification in a sample. Furthermore, the use of multiple markers can increase the accuracy in taxonomic identification if two species do not show enough genetic differentiation in a marker. In our case, we used three mitochondrial markers: *16S*, *12S* and *cytochrome b* (*CytB*). For each of the three markers we made two PCR replicates per extraction replicate, resulting in twelve PCRs per sample (see Axtner et al., 2018, for details). We note that the performance of the longer markers *12S* (389 bp) and *CytB* (302 bp) was much poorer compared to the short *16S* (93 bp) marker. Although the longer markers increased the taxonomic resolution, the low amplification rates highlight the need to develop shorter *12S* and *CytB* markers for future studies.

The greatest risk of contamination during the laboratory analysis is through volatile PCR products. To mitigate against this risk, we developed a protocol that includes two rounds of PCR. In the first PCR, in which we amplify the target barcode region, we add the necessary sequencing primers as well as an individual sample tag (t1) as an overhang. Due to the individual sample tag no unlabelled PCR products were produced. In the second PCR, the primers matched the overhang of the first PCR primers sequences and added the necessary sequencing adapters, as well as a plate identifying tag (t2) (see Axtner et al., 2018, for more information on the PCR protocols). With only 24 sample tags t1 and 20 plate tags t2 it is possible to differentiate up to 480 samples. We used the Illumina MiSeq platform to sequence our PCR products.

1.2.6 Taxonomic assignment

The taxonomic identification of sequencing reads is one of the most fundamental, but also most challenging, steps when analysing e/iDNA in the laboratory. To identify a species in a sample it is necessary to have the appropriate reference sequence of that species in a reference database. But current databases contain only a fraction of the known species on earth. Furthermore, uncurated open-access databases such as Genbank, which many people use for taxonomic identification (e.g. Schnell et al., 2012), are flawed to an unknown degree by erroneous entries

that have wrong taxonomic annotations. Therefore, we made an effort to create a curated tetrapod reference database for our mitochondrial markers (see Axtner et al., 2018, for details on the bioinformatics steps to create clean reference databases).

Once the reference database has been established, there are several ways to perform a taxonomic assignment, ranging from simple methods such as a BLAST search to more complex model-based methods (see Bonin et al., 2018; Somervuo et al., 2017; Rodgers et al., 2017, for further details). For our studies, we used a Bayesian probabilistic method called PROTAX (Somervuo et al., 2017) because it allowed us to weigh the assignment for certain species expected to occur in the study areas. Our bioinformatic pipeline provided us both with a mean probability estimate for an assignment made by PROTAX, and also with a mean sequence similarity with the assigned reference sequence. Together with the number of sequencing reads, these two parameters were taken to make the final taxonomic assignments. However, the taxonomic assignment could not be automated completely and the results still need careful manual inspection (see Axtner et al., 2018, for details).

1.3 Environmental data collection

This section focuses on the collection of environmental data, which are essential for answering ecological questions about the distribution of species with respect to available habitat. We provide a detailed field protocol for* in situ *vegetation surveys and present* ex situ *remote sensing methods, data and data sources.

1.3.1 Introduction

Understanding the fundamental drivers of species occurrence is a central tenant of ecology. For centuries, great minds like Alfred Russell Wallace and Henry Walter Bates have sought to establish *what* species are found in an area and *why* they occur there. These same questions are being asked today by a new generation of tropical ecologists. Such questions are interesting from an academic perspective. However, given the unprecedented species extinction crisis occurring globally across the tropics, understanding species distribution also takes on a new, applied focus within the context of biodiversity conservation. By understanding how species respond to habitat degradation or hunting, stakeholders can make more informed conservation and management decisions.

Methods have changed with the times. While naturalists of the 18th and 19th centuries used observation or direct collection methods to establish *what* species occurred in an area, today's ecologists use standardised high-throughput survey methods such as camera-trapping and e/iDNA (sections 1.1 and 1.2). Where Victorian naturalists used descriptive science and intuition to attempt to understand *why* species occurred where they did, ecologists today rely on quantitative data and powerful statistical modelling techniques to answer these questions. Today, habitat-related information can be collected and then incorporated into these analyses as covariates. At the most basic level, covariates include anything that potentially influences species distribution and can be measured. Such information can be collected in the field, often referred to as *in situ*, or using remote-sensed data, often referred to as *ex situ*. An example of an *in situ* covariate could be data on vegetation density around a camera-trap location, while an example of an *ex situ* covariate could be using satellite imagery to landcover classes. Covariates often can include both environmental and anthropogenic factors. Habitat metrics, such as forest type or quality, may influence species occurrence in an area, but so too could prevalence of hunting, as assessed by distance to road or village. In our studies, we used a wide range of both environmental and anthropogenic factors. For example, in Sabah, moonrat occurrence was positively associated with forest quality and negatively associated with distance to plantation (see case study section 3.2.2), while in Vietnam and Laos, Annamite striped rabbit occurrence was significantly influenced by a proxy for past hunting pressure (see case study sections 3.2.1 and 3.4.1).

Ultimately, the type of covariate used in a given study – whether collected *in situ* or *ex situ*, or assessing environmental or anthropogenic factors – will depend on the questions being asked, and will differ with study sites and target species. Including covariates in analyses can provide critical insight into the factors influencing species occurrence. It allows ecologists to move from *what* and into *why*. We recommend choosing covariates before collecting data, and basing covariate selection on well-defined hypotheses whenever possible. For detailed information on how covariates can be incorporated into models that estimate occupancy or local abundance see PART II and PART III.

1.3.2 *In situ* habitat assessments

We conducted standard habitat assessments at each of our camera-trap locations. Our habitat assessments record information on both environmental and anthropogenic factors around our stations. We used the same data collection techniques for both of our study sites (Sabah, Malaysian Borneo and Central Annamites in Vietnam and Laos) so that we could make comparative studies between the two regions, and among different study sites within each region. **Likewise, we encourage other conservation scientists to standardize their covariate data collection whenever possible so that results are more comparable.**

Below we provide an example of our habitat assessment. There are numerous variables that can be measured and which might influence species occurrence. We cannot, of course, measure everything. Decisions on what to measure were also made with an understanding that time spent at each camera-trap station was limited. In the end, the variables we decided to measure represent a compromise between collecting as much relevant information as possible and keeping the assessment logistically feasible. Our assessment assesses major vegetative indexes (vegetation density and canopy cover) and presence or absence of key anthropogenic influences (logging or hunter snares). A trained team should be able to complete the habitat assessment in one hour.

1.3.3 Habitat assessment field protocol used in the SCREENFORBIO project

Equipment needed (Figure 1.10)

(1) **Compass** for taking the bearing of the plot.
(2) **GPS** for taking coordinate of the plot.
(3) **Camera** to take picture of canopy cover and understory vegetation density.
(4) **2 ropes with 20 m length each.** These ropes will be used to make a plot.
(5) **1 rope with 14.4 m length.** This rope will be used to guide surveyor from the centre to the corner of the plot.
(6) **1.5 m x 1 m orange tarpaulin** to measure the dense of understorey vegetation density.
(7) **Habitat assessment datasheet and pencil** to record data.

Figure 1.10: *Equipment needed for the habitat assessment.*

(1) Setting up the plot

At least two people are needed to complete the habitat assessment. One of the team members should have experience implementing the protocol. It is important to follow the same procedure and order when collecting data on leaf litter and vegetation. If the order is changed it can cause issues later when assigning the data to the sampling localities.

a) A 20 m x 20 m plot should be established at each camera-trap station using two 20 m ropes (or measuring tape), oriented along the four cardinal directions (North, East, South and West, Figure 1.11 and 1.12). If one camera is used per station, the centrepoint should be the camera-trap. If two cameras are used per station, the centrepoint should be the middle point between the two cameras.

Figure 1.11: *Establishing the vegetation survey plot. Two 20 m ropes are oriented along the North-South and East-West axes.*

b) Tie each end of the rope to a small tree or other inanimate object. It is important to ensure that you do not cut your way through the vegetation to the rope endpoint because understory vegetation density will be measured at each rope end, in all four directions.
c) Take the coordinates of the centerpoint with the

Figure 1.12: *Two 20 m ropes oriented along the four cardinal directions. The centerpoint is the middle of the two cameras (if two units are used per station) or the camera trap location (if one camera is used).*

GPS. It is important to use the Waypoint Averaging function to obtain a more accurate coordinate; the GPS error should be $\leq$ 5m. To do Waypoint Averaging, go to the Main Menu $\rightarrow$ Waypoint Averaging $\rightarrow$ Create Waypoint. The calculation will take several minutes—sometimes more than 10 minutes in total—and you should wait until sample confidence = 100 %. During this time, the GPS must remain stationary. Record the "distance adjusted" in the form or insert this information into the comment section when renaming the waypoint. After sample confidence = 100 % select $\rightarrow$ Done $\rightarrow$ Save.

d) Rename your waypoint to the camera-trap station name or other unique identification that you use to name your station. For instance, we named our camera-trap station with SDC XX, where S denotes country / state (Sabah), D for name of study site (Deramakot), C for survey name (coarse grid), and XX for the station number (01, 02, 03...). If you do not rename your waypoint, the GPS will automatically assign a number to your waypoint according to sequence. You can rename your waypoint in Waypoint Manager. In the note or comments box of the GPS waypoint you can add value of distance adjusted (e. g., 4M if it was 4 m) and then Save.

(2) Data collection

All information that needs to be collected at each plot should be written in the vegetation survey form and ideally the plot protocol should be attached together with the camera-trap form. The name of the observer and date for each visit should be recorded in the form so that questions can later be resolved (i.e. if it is difficult to read the handwriting for one of the forms). Details of data collected within each plot are described below:

a) General description about the site. Record unique features or notable components within the plot or nearby, such as presence of saltlick, water resources, human activity, or poaching sign.
b) Record any fruiting trees inside plot and if possible the name of the tree (scientific or local name).
c) Count tree stumps with diameter at breast height (DBH) >10 cm.
d) Count dead trees with DBH >10 cm. This includes standing and fallen trees; we recommend that these categories are recorded separately.
e) Count tree hollows below 1.5 m from ground.
f) Measure leaf litter percent cover in nine 1 m x 1 m subplots located at the centre, 10m in the cardinal directions (North, East, South & West), and at the corners (North East, South East, South West, North West) of the plot (Figure 1.13). It is best to standardize the order when measuring leaf litter and have all teams follow this order throughout the survey. Each subplot should be assigned with a value ranging from 0 to 4, with 0 representing no leaf litter, 1 representing 1 – 25 % leaf litter, 2 representing 26 – 50 % cover, 3 representing 51 – 75 % cover and 4 representing 76 – 100 % cover. As this is somewhat subjective, it is fundamental for each member of the team to have same understanding when they assign a value for each subplot. Using the same subplots, measure leaf litter depth at the centre and each corner of a

subplot and average this value.

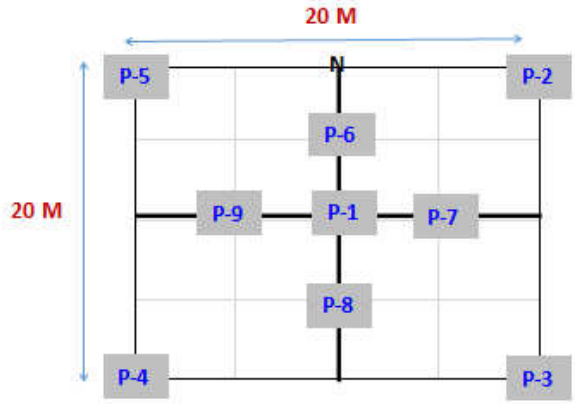

Figure 1.13: *Drawing shows locations of the subplot (P1, P2. . . .P9) to measure leaf litter cover and recommended order to collect the data.*

g) Take photos of canopy cover at the centre of the plot and at each of the four corners (North East, South East, South West and North West). Make sure you hold camera straight over your head, perpendicular to the ground. If you are standing next to a large tree, move aside so that the trunk is not in the image. If you are standing in dense vegetation, try to get a photo of the higher canopy strata. All photos should be taken in a standardised order throughout the survey. If possible use the same camera for all vegetation plots to ensure that the field of view and pixel resolution in all photos is the same.

h) Take photos of understory vegetation density against an orange tarpaulin (1.5 m height x 1 m width) held at ground level and 10 m from the plot center in the four cardinal directions (North, East, South, West) (Figure 1.14 and 1.15). The photographer should stand at the centre of the plot and use the zoom function to get a closer photo but also ensure that the tarpaulin does not go beyond the camera frame. If there are trees in the way, move slightly to the right or left. Be sure to make a note if you do move and try to estimate the distance from the original point. As mentioned earlier, do not cut any vegetation parallel to the ropes because this will change the natural understory vegetation.

1.3.4 Remote sensing-based *ex situ* environmental data

This section gives an overview of the potential of remote sensing technology for characterizing study sites and deriving meaningful habitat information for ecological analyses of camera-trapping and e/iDNA data. It briefly describes useful data sets, what information can be extracted, how to obtain these data and what software to use for analyses.

Figure 1.14: *Taking photo of understory vegetation using built-in camera in a GPS unit.*

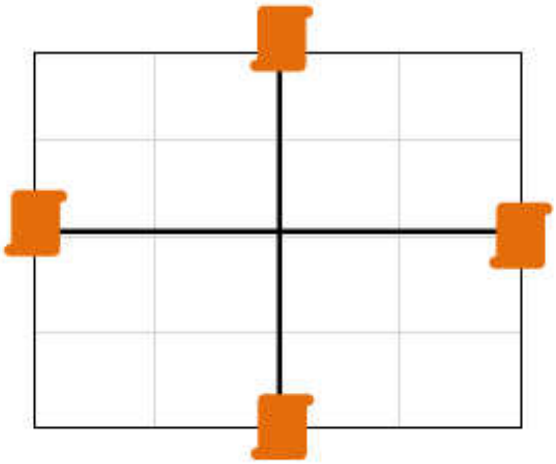

Figure 1.15: *Drawing shows four locations to take picture of understory vegetation.*

Remote sensing is a widely applied scientific method for collecting information about earth using airborne or space-borne technology such as drones or satellites. Satellite imagery and a variety of thematic data derived from these are useful data sources for conservation research (Turner et al., 2003; Pettorelli et al., 2018). These data can be processed to generate information related to ecological processes on the ground relevant for species distributions or human land use, as they continuously survey large areas of land. This chapter will cover a number or raster data sets that can provide useful information for ecological analyses in wildlife studies.

Satellite imagery is one of the most important and useful examples of remote sensing data, particularly in wildlife science and conservation. The spatial resolution differs between satellites, but is typically in the range of a few meters (see Table 1.2 for a selection of available satellite data). Freely available data typically have lower resolution than data from commer-

Deriving canopy cover and vegetation density from field photographs

Microhabitat vegetation characteristics have been shown to be an important predictor of species occurrence for some tropical mammal species. For our project, we assessed canopy cover and understory vegetation density around our camera-trap stations with digital photographs. Converting these photos into data that can be incorporated into analyses requires some processing. The free and open source software image manipulation software Gimp[a]. Below we provide a step-by-step guide for processing canopy cover and vegetation density photos in Gimp. Both workflows will produce binary (black and white) rasters with black representing vegetation and white being sky or gaps in understory vegetation, respectively. Canopy cover and vegetation density can be calculated from the classified binary images e.g. by automatically counting black (vegetation) and white (non-vegetation) pixels in the statistical software R.

Canopy cover

The basic idea is to convert the colour image into a binary black and white image with black representing foliage and white sky. The workflow is:

1. Open image in Gimp
2. Colours – Threshold
3. Adjust slider until sky and foliage are represented adequately (use preview function)
4. Image - Mode – Indexed (check box: Use black and white (1-bit) palette)
5. File - Export As
6. Save image as png file

Figure 1.16: *Example canopy cover photo (left) and processed image (right).*

[a] `https://www.gimp.org/`

cial satellites (30 m Landsat data and 10 m Sentinel data are freely available whereas some commercial satellites have resolutions <1 m). That said, lower resolution has advantages. It removes excessive detail and greatly reduces complexity, memory usage, and processing time compared to high-resolution imagery. High-resolution satellite data are useful for identifying fine-scale habitat associations, whereas lower resolution data are usually sufficient for identifying habitat associations at broad scales (Niedballa et al., 2015, see section 3.1.1). Unprocessed, raw satellite imagery is useful for planning field trips and creating visually appealing maps, but usually needs to be processed to derive meaningful habitat information for ecological analyses. Land cover classifications, the calculation of vegetation indices or change detection are common

Deriving canopy cover and vegetation density from field photographs (continued)

Vegetation density

Here the basic idea is similar to canopy cover, but involves an additional step of cropping the image to the extent of the flysheet that was being photographed in 10 m distance.

1. Open image in Gimp
2. Toolbox - Crop Tool
3. Crop image to boundary of orange flysheet (even if partly covered by vegetation).
4. Colours - Auto - Colour Enhance
5. Select - By Colour
6. Click into Red Area, keep clicking with Shift button pressed until all red areas are selected
7. Select the Bucket Fill Tool from the toolbox
8. In Tool settings, set Mode = Dissolve, Affected Area = Fill whole selection
9. Fill selection with white
10. Select – Invert to select only vegetation
11. Fill selection (foliage) with black
12. Selection – None to deselect
13. Image - Mode – Indexed (check box: Use black and white (1-bit) palette) to convert colour image to binary image
14. File - Export As
15. Save image as png file.

Figure 1.17: *Example vegetation density photo (left) and processed image (right).*

applications for deriving useful habitat information from satellite imagery.

By creating land cover classifications from satellite images, interpretability is greatly enhanced, as the complexity of the raw satellite images is reduced to a few distinct land cover classes. A typical land cover classification for a tropical forest contains classes such as dense forest, forest, shrub, grass, bare ground, or water. Depending on the area, other land cover types such as urban areas, agricultural areas or oil palm plantations can be present (see Figure 1.18 for an example of a land cover classification based on 5 m high resolution RapidEye data). Clouds and cloud shadows are commonly found in images and complicate

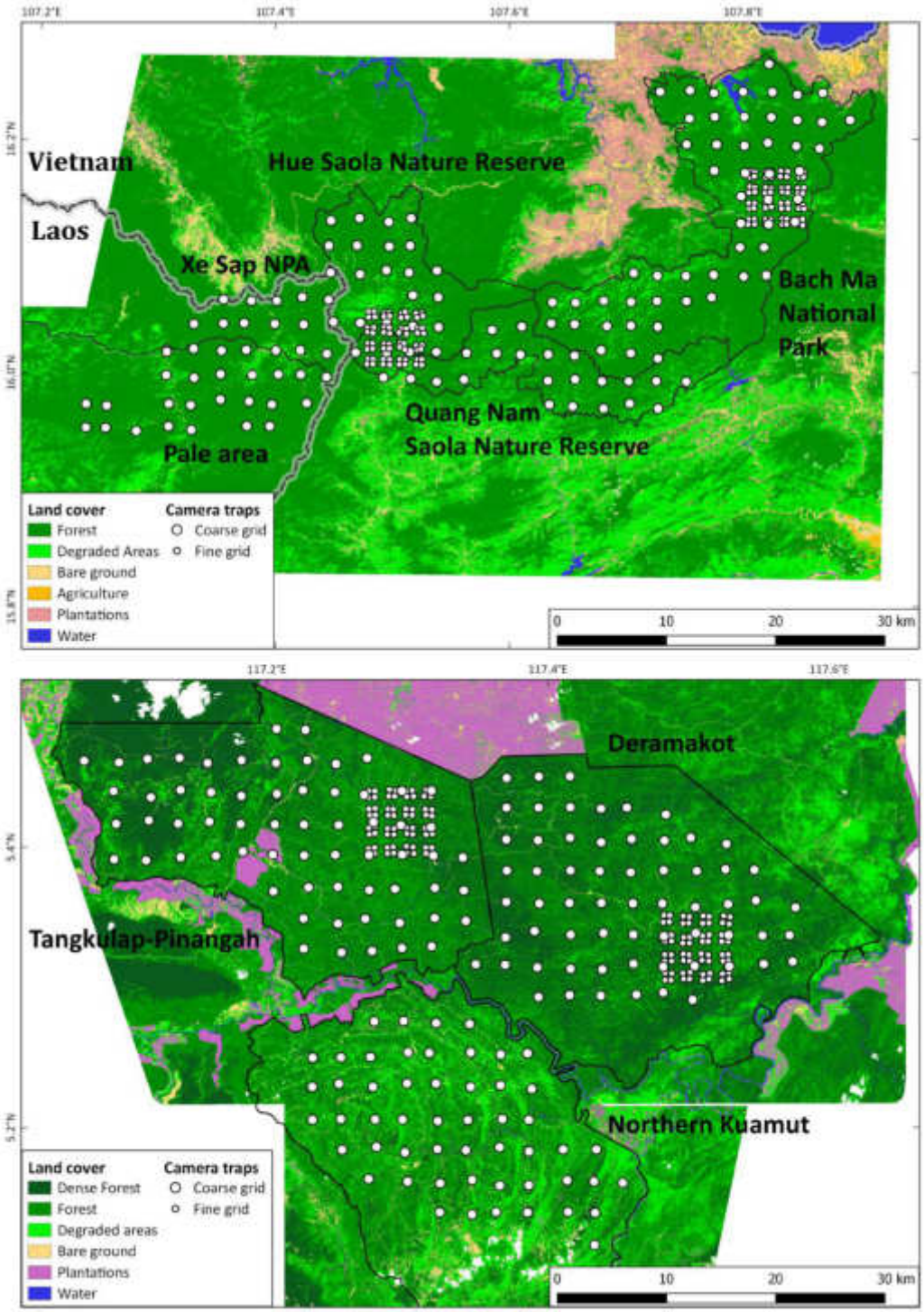

Figure 1.18: *Land cover classification of our two study areas in Central Annamites, Vietnam and Laos (top) and Sabah Malaysian Borneo (bottom). Land cover classification is based on a 5-m high resolution RapidEye satellite data (provided by Blackbridge under the RESA programme).*

analyses, particularly in the wet tropics. They can either be classified into distinct classes (and removed in analyses) or removed with data quality masks that are provided with satellite images). Classification can be done in a supervised way, by providing the algorithm with training areas with a known land cover type, or unsupervised, when the algorithms assigns pixels to classes according to their spectral reflectance values without the need for training areas, but without providing an ecological interpretation of the classes thus created. For most applications, supervised classification provides results that are easier to interpret. Irrespective of the classification approach, land cover classifications require ground-truthing for verification of land cover classes and accuracy assessment.

Vegetation indices are a data product derived from

Table 1.2: *Examples of optical Satellites for remote sensing used in ecological research (modified from Wegmann et al., 2016).*

Satellite	Spatial Resolution (m)	Swath width (km)	Data policy
WorldView 3	1.2	13	proprietary
Ikonos	3.2	11	proprietary
Quickbird	2.4	17	proprietary
Spot	1.5-6	60	proprietary
RapidEye	5	77	proprietary[1]
Landsat	30	185	free
Sentinel 2A	10-60	290	free
MODIS	250-1000	2330	free

[1] RapidEye data can be obtained freely for scientific and non-commercial purposes through the RapidEye Science Archive (RESA)

Software for processing geodata

Proprietary geographic information system (GIS) software is extremely expensive and may thus be unavailable for many small-scale projects. There is, however, free and open-source software for GIS analyses available, first and foremost the QGIS project[a]. It is a very powerful substitute for expensive proprietary software.

Users with coding experience can use the statistical software R[b] as a tremendously powerful substitute for GIS software. A number of R packages (e.g. raster, sp, rgdal) provide R with the ability to read, manipulate and save raster and vector geodata, making R an extremely powerful GIS platform.

Google's Earth Engine[c] provides cloud storage and computation capabilities using Google servers. As such it does not require installation or disk space for geodata. It is a suitable platform for planetary-scale analyses far beyond what is possible on desktop computers, but requires programming in JavaScript or Python.

[a] `https://www.qgis.org`
[b] `https://www.r-project.org/`
[c] `https://earthengine.google.com/`

satellite imagery (Pettorelli et al., 2011). There is a variety of vegetation indices, all of which are calculated from individual spectral bands of a multispectral image (e.g. the red and near infra-red channel for NDVI). They can, for example, provide a measure of the amount of photosynthetically active vegetation and thus give estimates of vegetation status. Care needs to be taken when interpreting these indices, as re-growth after logging can have equal or higher vegetation index values than pristine forests. If possible, time series of vegetation indices should be used instead of single scenes. The trajectories of vegetation indices can help reconstruct land cover history and guide interpretation.

Change detection can be applied to trace changes in vegetation or land cover, e.g. due to deforestation or afforestation, degradation or re-growth, urbanisation or flooding (Zhu, 2017). It requires satellite images from multiple points in time to detect changes between them. There is a variety of methods for conducting change detection analyses, but they all compare pixel values (e.g. reflectance or vegetation indices) at different points in time. For example, logging a forest will result in increased reflectance in the red channel (because there is less chlorophyll to absorb red light), a change that will be reflected when comparing the red channel or vegetation indices derived from the red channel from before and after logging. Change detection can provide powerful tools to trace changes in land cover and determine the time of land cover change.

Digital elevation models (DEMs) provide a digital representation of terrain in a raster. Elevation itself can be an ecologically relevant piece of information, but DEMs provide more information that just that. DEMs can be used to delineate rivers and ridges, calculate terrain measures like ruggedness, slope or topographic position, provide contour lines for use in

How to obtain remote sensing data

A wealth of remote sensing data that are commonly used in ecological analyses can be downloaded from just a handful of websites. Most websites require users to register, but provide data free of charge. The following websites provide the data described in this section (and a lot more). In addition, the GRASS-Wiki contains a comprehensive list of global datasets[a].

- **EarthExplorer**[b]
  Easy to use and very comprehensive, USGS EarthExplorer is one of the biggest and most complete websites for downloading remote sensing data and probably the best starting point. All data mentioned in the text (and more) are available from EarthExplorer, including the Landsat archive, SRTM digital elevation models, MODIS data and Sentinel-2 satellite imagery at up to 10 m resolution.
- **Global Forest Change 2000-2016**[c]
  Results from a time series analysis of global forest cover and change from 2000-2016 (Hansen et al., 2013). Tree cover as well as annual forest gain and loss are available. Analyses are based on Landsat satellite imagery at 30 m resolution (at the equator).
- **Libra**[d]
  An innovative Landsat image browser that makes finding and downloading Landsat 8 imagery very simple and accessible.
- **Global Urban Footprint**[e]
  A global raster of settlements and urban areas at approx. 12 m resolution for the years 2011-12. Data are free for scientific and non-commercial use, but need to be requested through the website.
- **NASA EOSDIS WorldView**[f]
  Near real-time global satellite data viewer which provides various thematic data for download.
- **Google Earth**[g]
  Global high-resolution satellite image in a 3D viewer. The desktop version Google Earth Pro allows overlaying geodata (both raster and vector) in various formats. Data are proprietary and cannot be downloaded.
- **Google Earth Engine**[h]
  A powerful cloud computing platform for processing geodata provided by Google. It contains the archive of Landsat and Sentinel satellite imagery, amongst other geodata, ready for analyses. Analyses in Earth Engine need to be written in JavaScript or Python programming languages.
- **RapidEye Science Archive**[i]
  The RapidEye Science Archive (RESA) programme provides free 5-m resolution RapidEye data to researchers of German research institutions and members of NGOs upon application.

[a] https://grasswiki.osgeo.org/wiki/Global_datasets
[b] https://earthexplorer.usgs.gov/
[c] http://earthenginepartners.appspot.com/science-2013-global-forest
[d] https://libra.developmentseed.org/
[e] http://www.dlr.de/eoc/en/desktopdefault.aspx/tabid-9628/16557_read-40454/
[f] https://worldview.earthdata.nasa.gov
[g] https://www.google.com/earth/
[h] https://earthengine.google.com/
[i] https://resa.blackbridge.com/

handheld GPS devices or hillshade rasters for high-quality maps.

Rivers and streams can be delineated automatically with GIS software. The process involves hydrologically correcting the DEM, determining the flow direction from every raster cell into neighbouring cells, summing up the number of upstream raster cells (equivalent to each cell's catchment area) and defining streams and rivers with a minimum catchment area threshold. Stream delineation is largely automated, e.g. in the QGIS tool r.watershed. Once rivers are delineated, they can be used to calculate distance to water, an often highly informative habitat covariate. Other terrain measures potentially interesting as habitat covariates are slope, aspect (the direction a slope is facing), topographic ruggedness or topographic po-

sition (e.g. for identifying ridges). All of these can be calculated with QGIS tools or the terrain function from the R package "raster". Likewise, elevation contour lines can easily be created with QGIS and are helpful on handheld GPS devices for orientation and navigation during field trips. Currently, the best freely available DEM is the SRTM data set, which is available as a gapless global raster in approximately 30 m resolution.

Innovative technologies such as LiDAR (Light Detection And Ranging) laser scanning can provide three-dimensional representations of habitats at very high resolutions, but are costly to obtain and difficult to work with for non-specialists. LiDAR-data have also been applied in conjunction with Landsat satellite imagery to predict aboveground biomass at 30-m resolution across the entire state of Sabah, Malaysian Borneo (Asner et al., 2018).

Labelled logs in Deramakot Forest Reserve, Sabah, Malaysian Borneo. **Photo** Azlan Mohamed

Western tarsier, Deramakot Forest Reserve, Sabah, Malaysia. **Photo** Michael Gordon

2. ANALYTICAL METHODS

In PART II we introduce three modelling methods that can be used to analyse camera-trap and e/iDNA data to obtain information about species distributions, habitat preferences, abundance and densities. All three of these methods account for the problem of imperfect detection that is associated with most collection methods.

2.1 Introduction to analytical methods

Early ecological research was largely from a naturalist perspective and qualitative in nature (Grinnell, 1904). However, it was those early studies that described biological patterns in terms of their relationships with geographical and/or environmental gradients (Grinnell, 1904; Murray, 1866; Schimper, 1903) that laid the foundation for modern day species distribution models (SDMs). An SDM is a numerical tool that combines observations of species occurrence or abundance at known locations with information on the environmental and/or spatial characteristics of those locations. Such numerical models were developed to provide a method to quantify ecological relationships and are now widely used across terrestrial, freshwater, and marine realms to gain ecological insights and to predict distributions across landscapes. Throughout the literature SDMs have also been called: bioclimatic models, climate envelopes, ecological niche models (ENMs), habitat models, resource selection functions (RSFs), and many more.

Traditionally, the go to analytical tools for SDMs were basic linear multiple regression and discriminant function analyses (Hosmer & Lemeshow, 2000; Guisan & Zimmermann, 2000), but that has changed in recent years. Over the past decade there has been rapid development of the statistical modelling tools available to ecologists to model species' distributions (Elith et al., 2006; Austin, 2007). This is mostly due to the increased computing abilities, but also due to implementation of analytical techniques from other scientific disciplines. These new techniques have been applied mostly to SDMs, combining concepts from ecological and natural history traditions with more recent developments in statistics and information technology.

Modern quantitative modelling and mapping of species distributions emerged when the new statistical methods from field-based habitat studies were linked with GIS-based environmental layers. Studies of species-habitat associations benefitted from new regression methods that accounted for the error in distributions of presence-absence and abundance data. Generalized linear models (GLMs) enabled pioneering regression-based SDMs and continue to be used in many current SDM methods (Manly & Sanderson, 2002; Phillips et al., 2006). Today, many methods are used to fit SDMs (Franklin et al., 2009). Parallel to the advances in statistical methods, there were rapid advances in physical geography. New methods and the development of geographic information systems (GIS) allowed for robust and detailed preparation of digital models of the earth's surface elevation, interpolation of climate parameters, and remote sensing of surface conditions.

Quantification of niche space at the species level is a first step toward predicting the distribution, occurrence, or abundance of wildlife species with SDM approaches. Data for SDMs can come in a number of forms and can represent spatially referenced oc-

currences or abundances. Counts, or densities, of animals within some defined area can be modelled as a function of environmental characteristics within that area. The large number of factors that characterize a habitat can often be reduced to a relative few that explain much of the variance in species responses. Although this technique is powerful, it must be used carefully because it can often obscure relationships between mechanism and response. Guisan & Zimmermann (2000); Stauffer et al. (2002); Guisan & Thuiller (2005); Richards et al. (2007) and Schröder (2008) provide reviews of SDMs and their potential for broader ecological insight.

Model parameters (coefficients) are most commonly estimated using maximum likelihood and represent the change in the response following a one-unit change in predictor. However, Bayesian statistical approaches (e.g. Gelman et al., 2004) have also become more common in ecological research in recent years (Clark, 2005; Ellison, 2004). All of the more traditional frequentist-based approaches described in this section can be analysed using a Bayesian approach. Bayesian analysis in ecology is often done using Markov Chain Monte Carlo (MCMC) with Gibbs Sampling (Casella & George, 1992), which can be easily implemented using the BUGS language in software such as WinBUGS (Lunn et al., 2000) or JAGS (Plummer, 2003). In the following section we focus on methods that are designed to account for the imperfect detection of species as both described survey methods (camera-trapping and e/iDNA) suffer from imperfect detection and detection probabilities that vary between species. Detection probability can be impacted by a number of site-specific parameters. Therefore, in our understanding accounting for varying detection probabilities and imperfect detection is of great importance when analysing camera-trapping and e/iDNA data. Specific requirements and assumptions of each modelling approach are described in the following sections.

2.2 Single-species occupancy models

Occupancy models are a class of hierarchical models for analysing species level detection/non-detection data. The strength of occupancy models lies in their ability to account for imperfect detection by separating the ecological process (a site being occupied by a species) from the imperfect detection process (detecting the species, given it is present). The detection process is conditional on the occupancy process because a species can only be detected when it is present (i.e. when a site is occupied). This separation into two levels or submodels makes occupancy models hierarchical models (Kéry & Royle, 2015). This chapter will focus on single-species, single-season occupancy models, whereas the following chapter will focus on multi-species or community occupancy models.

The classic approach to assess species occurrence is to spend time in the field trying to detect the species with various methods, such as camera-trapping or e/iDNA. For almost all species, detection will be imperfect in this scenario. In other words, a species that is present may go undetected in a survey. In the context of occupancy modelling, occupancy is the probability that a randomly selected site or sampling unit in an area of interest is occupied by a species (MacKenzie et al., 2002, 2006). Naïve occupancy estimates (the percentage of sites at which a species was detected and is thus known to be present) underestimate true occupancy if detection probability is <1 because species may occupy sites but are never detected.

Single-season occupancy models assume that the occupancy state of a site (i.e., occupied or not) remains constant over the course of a survey (assumption of closure), which has implications for study duration (see box: *Assumption of occupancy models* below). In the context of camera-trapping, the study duration is the period over which each camera-trap station was active. For iDNA surveys, specifying the study duration is more complex, as invertebrates can store the blood of their hosts, in the case of medicinal leeches for several months (Schnell et al., 2012; Kampmann et al., 2017). This means that the study duration is generally longer then the sampling period, a fact that is important to consider in respect to the closure assumption in the occupancy models (Schnell et al., 2015).

In order to estimate detection probabilities, the study period is subdivided into repeated "surveys", more commonly referred to as occasions. For camera-trapping, we typically used a few camera-trapping days as occasions, and for iDNA we use sampling replicates as occasions. The sequence of detections and non-detections at a site during these repeated occasions (the detection history) is represented as a vector of 1 for detection, 0 for non-detection, and NA if the site was not surveyed during an occasion (e.g. because cameras were not operational or a leech sample did not amplify). For example, a sequence "100" represents a detection on the first occasion and non-detections during the two subsequent occasions. The site is known to be occupied by the species be-

Assumptions of occupancy models

A number of basic assumptions must be met in order to successfully apply occupancy models to camera-trapping data. Violations of these assumptions may lead to biased estimates of occupancy and detection parameters, or underestimation of parameter variance. The main assumptions of site occupancy models are:

1. *Occupancy state is closed (closure):* occupancy status at sites (camera-trap stations, leech collection plot) does not change over the course of survey. If the assumption is violated, detection probability is underestimated and occupancy is overestimated.
2. *Sites and repeated visits are independent:* detections of species at different stations are independent, there is no spatial autocorrelation between stations and spacing between stations is large enough to ensure individuals can only be detected at one individual station; detections of species across repeated visits are independent (whether we detect the species on a given occasion does not influence whether we detect it on any other occasion). Failure to meet the independence assumption can lead to overly precise parameter estimates and/or biased estimates of habitat associations.
3. *Absence of false-positives:* All records in the detection histories are real and were identified correctly.
4. *No unexplained heterogeneity in occupancy.* Occupancy probability is either constant at all stations, or systematic variation of occupancy probability is explained by site covariates included in the model. Note that there will always be unexplained heterogeneity and no ecological study will ever fully meet this assumption.
5. *No unexplained heterogeneity in detectability.* Detection probability is either constant at all stations, or systematic variation of detection probability is explained by site or survey covariates included in the model. Analogously to assumption 4 there will also always be unexplained heterogeneity in detectability and no ecological study will ever fully meet this assumption.

Consequences of model assumptions for study design:

1. *Studies must be sufficiently short to approximate the closure assumption.* Although this depends on the ecology of the species, usually, no more than a few months of data are used. This is to ensure there is no local extinction or colonisation at sampling sites. If data are collected over longer time periods, dynamic occupancy models provide an alternative (MacKenzie et al., 2003).
2. *Camera spacing must be sufficiently large to ensure individuals cannot be photographed at several sites.* Techniques to correct for spatial autocorrelation in the data (e.g. conditional autoregressive models) might help overcome minor violations of this assumption in the data (Dormann et al., 2007; Ver Hoef et al., 2018).
3. *Occupancy models are very sensitive towards false positives, which can severely bias model estimates.* Thus, reliable species identification is very important. If misidentification is possible, methods to correct for this source of error should be considered (Miller et al., 2011).
4. *During field surveys, all habitat parameters thought to influence detection probability of the target species must be collected at adequate spatial scales and simultaneously with the camera-trapping study.* In addition, occasion-specific parameters may be helpful for explaining heterogeneity in detectability (e.g. weather conditions).

cause it was detected on the first occasion. A detection history "000" represents non-detection on all three occasions and may be the result of true absence (the site is not occupied), which makes detection impossible, or the site being occupied but the species not being detected on the three occasions. Missing observations in the detection histories (NAs, e.g. due to camera failure) do not affect the parameter estimation. The detection histories of all sites thus give a site-by-occasion matrix in which the rows represent sites and the columns represent occasions. This matrix is the primary input for single-species occupancy models.

Detection probability p is estimated from the detection histories and based on detection probability, occupancy probability Ψ can be estimated for sites at which the species was never detected. In the simplest case (the null model) detection probability p and occupancy probability Ψ are constant across sites and, for p only, across occasions. Usually, however, the relationship of p and/or Ψ with

habitat characteristic is of ecological interest. To investigate these relationships, p and Ψ can be modelled as functions of covariates. Occupancy covariates (covariates influencing Ψ) are site-specific, but have to be constant across occasions. Habitat characteristics such as canopy cover, vegetation density or land cover fall in this category. Detection covariates can be site-specific (like occupancy covariates), but they can also be occasion-specific. Occasion-specific covariates can differ between occasions, e.g. trapping effort or weather conditions. Multiple covariates can be used simultaneously in occupancy models, but more covariates require more data points (i.e., camera trap stations).

Occupancy models establish the relationship between the two probabilities and the covariates using generalized linear regression techniques. The models return intercept values and estimates for the effects of covariates on the logit scale together with their associated p-values. A one-unit change in parameter estimates. The logit link function is a transformation of probabilities (which are bound between 0 and 1) to a scale that can take any value between $\pm\infty$ for use in modelling. Logit probabilities can be back-transformed to probabilities which are bound between 0 and 1 using the inverse-logit function.

If we have multiple competing hypotheses, for example about different habitat associations, different models representing these hypotheses can be compared using model ranking and selection procedures. Commonly, the AIC (Akaike Information Criterion, Burnham & Anderson, 2002) of different models is used to rank models. The model with the lowest AIC values is the most parsimonious model, that is, the model that best describes data while using the lowest number of parameters. The relative importance of different models can be assessed using ΔAIC, which is the difference in AIC between a given model and the model with lowest AIC (often referred to as the top model). Models within a ΔAIC of 2 are considered to have essentially the same amount of support as the top model (Burnham & Anderson, 2002). In addition, the AIC weights represent the relative likelihood of a model and thus give an indication of the model's importance.

Using the habitat associations found in occupancy models, spatial predictions for species occupancy can be made for unsampled locations (for an example see PART III section 3.1.2 and 3.2.2). To do so, sampled locations must be representative of the larger area of interest, and the same covariate information used in the model must be available for the areas for which predictions are made. The most suitable data source to do so is remote sensing data, which offer continuous and extensive spatial coverage of study areas, in contrast to limited spatial extent of *in situ* habitat covariates. It is important to keep in mind that predictions to covariate values outside the range of those used in the occupancy model are mathematically possible, but because occupancy-covariate relationships can change depending on the range of covariate values considered (e.g. threshold effects), such predictions may be unreliable and should be avoided.

Occupancy models are implemented in frequentist (maximum likelihood) and Bayesian methods of statistical inference and can be computed in various software, e.g. the R packages *unmarked* and *wiqid* (Fiske & Chandler, 2011; Meredith, 2017), or standalone software such as PRESENCE or MARK.

2.3 Modelling a community of species

Species richness and diversity are central to community and macroecology and are frequently used in conservation planning, but cannot be estimated directly with single-species occupancy models presented in the previous section. The fundamental unit of all diversity metrics is a count of species, often combined with measures of abundance. However, surveys rarely detect all species during a survey, leading to underestimation of species diversity. Therefore, when measures such as species richness are of interest, these need to be estimated while accounting for the imperfect detection of species. Many classical indices of ecological diversity (e.g. Simpson index, Shannon index) ignore detection altogether by including observed species richness. Others account for undetected species primarily by controlling for sampling effort, such as Margalef's diversity index, sometimes resulting in serious overestimations and failing to disentangle the complex relationship between detection and occurrence. Community (or multi-species) occupancy models (Dorazio & Royle, 2005) are a method to estimate diversity that explicitly incorporate imperfect detection. Detection / non-detection data of multiple species collected at a number of survey locations through both camera-trapping and e/iDNA methods can be analysed in such a community modelling framework.

Community occupancy models have several advantages over fitting single-species occupancy models. Parameter estimates for rare species are naturally less precise than for common species and very rare species often cannot be analysed in

Model implementation, fitting, selection and testing

The implementation of community occupancy models starts with the generation of a site-by-species-by-occasion matrix, which can be obtained from data that have been collected through camera-trapping or e/iDNA. The site-by-species matrix is then paired with a corresponding site-by-environmental covariate matrix, generated from *in situ* and remote sensing data. The two datasets are combined statistically to infer the relationship of multiple species to environmental conditions.

Community occupancy models are usually implemented using Bayesian methods. There is currently no packaged community occupancy model software, therefore, multi-species occupancy models must be written by the user and run within a programming environment or precompiled Markov chain Monte Carlo programs such as the freely available JAGS or WinBUGS software, which use the Bayesian inference Using Gibbs Sampling (BUGS) language.

single species occupancy models. In multi-species models, however, information can be 'borrowed' from data-rich species to increase the precision of parameter estimates for rare species, by assuming that species-level parameters come from a common parametric distribution. Furthermore, via data augmentation, community occupancy models are able to estimate the number of species not detected at all (e.g. Bunge & Fitzpatrick, 1993; Williams et al., 2002). Although the first community occupancy models were developed over a decade ago, the application of hierarchical models to species communities, particular to camera-trapping and e/iDNA datasets is still relatively new, with both their limitations and potential yet to be fully explored.

Community occupancy models are based on the same basic sampling framework as single species occupancy models (see section 2.2): repeated detection/non-detection surveys are conducted at a number of sites within a single season. However, data now are collected on multiple species rather than for just a single species. The main assumptions of community occupancy models are the same as those for single-species occupancy models. In designing community occupancy models, however, it is important to make sure that the assumptions of closure and independence are met for all species.

A community occupancy model extends the system of linked, hierarchical models, describing the ecological and the observation process, by adding a third level to describe the sampling of individual species from the community. The resulting species-specific models are linked by assuming that species-specific parameters come from a common underlying distribution, governed by community (or hyper) parameters. The parameters for each species are treated as random effects that are drawn from the community hyperdistribution. Occupancy (Ψ) and detectability (p) can be modelled as a function of covariates, and regression coefficients can be modelled as species-specific random effects derived from the community-level hyperdistributions. Interpolation of point samples to continuous maps works similarly to single species models, see the Sollmann et al. (2017) paper in section 3.3.1 for an applied example.

2.4 Abundance and density estimate

One of the fundamental aspects of wildlife research and monitoring is to obtain estimates of abundance (number of individuals) of a species in an area. Abundance is a more sensitive measure of population status than the (binary) occupancy status. However, collecting the necessary data to estimate population sizes of species is challenging, especially for species that have large home ranges, occur at low population densities and live in logistically challenging environments such as dense tropical rainforests.

Spatial capture-recapture (SCR) models (also known as spatially explicit capture-recapture models – SECR) are hierarchical models applied to estimate density (i.e., abundance in a prescribed study area) while accounting for imperfect detection. Estimating density via SCR requires sampling a population using an array or grid of detectors (such as camera-traps) and individual identification of animals in a population. Individual identification can be derived from photographs (e.g. via unique coat patterns in spotted or striped species) or genetic methods. SCR also requires a study design that allows recaptures at different sampling sites (e.g. camera-trap stations) to allow estimation of the animal's movement. Hence, the spacing of the sampling grid needs to be close enough to allow recaptures of individuals at different sites.

Assumptions of spatial capture-recapture models

Spatial capture-recapture models make a few basic assumptions about the system under study and are robust to departures from some of these assumptions (Royle et al., 2014).

1. *Marks are not lost and are identified correctly*. There are, however, extensions to SCR for *unmarked* or partially identifiable populations (Chandler & Royle, 2013; Augustine et al., 2018). These models require larger datasets, which are often not available for rare and threatened species.
2. *Population closure*. The population is static and closed to changes during the study period (i.e. no recruitment or mortality, no entry or exit from the population). Meeting this assumption requires studies to be relatively short. Open population models can be used to relax this assumption and explicitly estimate population vital rates (Gardner et al., 2010).
3. *Individuals have static, randomly distributed activity centers*. In other words, home range centres are independent of each other and distributed in a spatially homogeneous way. This assumption can be relaxed when the density of home range centres varies as a function of habitat parameters and can be modelled using covariates (Royle et al., 2018). Open population models allow for variation in home range centres between surveys (Gardner et al., 2010). Home range centres that shift over the course of a study generally do not bias density estimates, but will lead to biased estimates of animal movement (Royle et al., 2016).
4. *Detection probability is a function of distance from activity center*. This detection model implies that home ranges are circular when detection probability decreases uniformly as a function of distance alone. Landscape metrics (e.g. terrain) can be included to relax the assumption of circular home ranges and account for home range geometries that vary with the structure of ecological landscapes (Royle et al., 2018).
5. *Independence of detections among and within individuals*. Encounters/detections of individuals are independent of one another. Encounters of an individual in a trap are independent of encounters in other traps or at other occasions. SCR models are relatively robust to minor deviations from this assumption (Royle et al., 2014).

SCR model allow researchers to flexibly incorporate various factors of interest affecting the detection process, movement, and abundance/density. Such factors can be site-specific (related to habitat) or individual covariates (e.g. sex or age class). In some species, individuals show great heterogeneity in their movement, e.g. male jaguars having much larger home ranges than females in Brazilian grasslands (Sollmann et al., 2011). Dominance structures can have similar effects on movement of individuals, with dominant males having larger home ranges than subdominant males for the Sunda clouded leopard (see PART III, section 3.5.1). Site-level covariates impacting detection probability, such as varying trapping effort between occasions, or set-up location (e.g. on/off road placement of the camera-trap) can be accounted for, too.

In SCR models, each individual is assumed to have an (unobserved) activity centre (conceptually, the home range centre during the study). The probability of detecting the individual is assumed to decline as a function of distance to the activity centre. SCR models use the locations of individual detections (e.g. camera-trap locations) to estimate the location of activity centres, the movement of individuals. This detection model explicitly describes variation in detection probability among individuals that arises from variation in exposure to trapping due to different levels of overlap of home ranges with the trapping grid (Efford, 2004; Borchers & Efford, 2008; Royle & Young, 2008). Non-spatial capture-recapture models cannot account explicitly for this source of heterogeneity in individual detection.

SCR models are a significant advancement compared to non-spatial capture-recapture models, particularly for density estimation. Abundance estimates from traditional capture-recapture models have no explicit spatial context; to derive density, users have to define the effective area sampled (the area covered by sampling effort plus an unknown area around, accounting for the fact that home ranges of sampled animals extend the trapping grid). Choice of how to estimate that area directly affects density estimates, making them somewhat arbitrary and difficult to compare across studies. In contrast, abundance in SCR is explicitly linked to a pre-defined area, called the state-

Assumptions of N-mixture models

1. *Population closure.* Sampled populations are closed with respect to recruitment, mortality and movement. Thus, the local abundance of individuals is assumed to remain constant throughout the study.
2. *Each individual in the local population is detected with a certain detection probability during each occasion.* This probability is the same for all individuals in the population.
3. *Local abundance follows a Poisson (or other appropriate parametric) distribution.* Variation in abundance across sampling locations can be incorporated explicitly via covariates (Royle, 2004).

space, the size of which, once chosen large enough, does not affect estimates of density. This is particularly advantageous for wide-ranging animals that are bound to occupy large areas outside of the trapping grid.

Camera-trapping in combination with (spatial) capture-recapture has been used to estimate density for a range of species such as tiger (Karanth, 1995; Karanth & Nichols, 1998), jaguar (Sollmann et al., 2011; Tobler et al., 2013), leopard (Hedges et al., 2015), Sunda clouded leopard (Wilting et al., 2012; Hearn et al., 2017), mainland clouded leopard (Mohamad et al., 2015), ocelot (Trolle & Kéry, 2003) and leopard cat (Mohamed et al., 2013). The data used as input for SCR comprise individual and trap level detection histories, and can be both counts (number of times an individual was detected at a given trap) or binary (detection/non-detection of an individual at a trap). Repeated sampling occasions are useful for collecting adequate amounts of data but are not technically necessary. For camera-trap studies, the entire study can be regarded as a single sampling occasion (unless there are temporal factors that affect detection probability). SCR models can be analysed using maximum likelihood estimation (Borchers & Efford, 2008), implemented, for example, in the R package secr (Efford, 2016) and oSCR (Sutherland et al., 2017); or in a Bayesian framework (Royle & Young, 2008). The Spatial Capture-Recapture book (Royle et al., 2014) provides detailed explanation about this approach with various examples to guide readers in conducting SCR analysis.

2.5 Local abundance

Spatial capture-recapture models require the reliable identification of unique individuals throughout the study. However, for most species in tropical rainforest – for example, almost all ungulate and carnivore species – individual identification based on camera-trap photos or metabarcoding data from e/iDNA is difficult or impossible. Alternative approaches have been developed to estimate abundance without the need for repeated individual identification of animals.

Royle & Nichols (2003) developed a model that uses repeated detection / non-detection data to estimate the abundance of a species across the study area. The model assumes that the probability of detecting a species at a given site depends on the abundance of that species at that site. However, it should be noted that the number of detections can also be inflated in camera-trap studies by the selection of the camera-trap location. For example, a camera-trap placed in front of a denning site or display area for pheasants will result in numerous species detections, often of the same individual, thus resulting in inflated local abundance estimates. When individuals can be counted, this problem can be circumvented by the application of an N-mixture model (also called Royle count model, Royle, 2004).

N-mixture models use repeated count data to estimate local abundance while accounting for imperfect detection. Although this method does not require repeated identification of all individuals across the entire study area (in contrast to spatial capture-recapture models), it does require identification of individuals recorded in each occasion at each sampling point. This restricts the application of N-mixture models to species in which individuals can be identified at least to some extent, such as the Annamite striped rabbit for which we present local abundance estimates in section 3.4.1. Heterogeneity in local abundance and detectability patterns can be accounted for using covariates.

N-mixture models use repeated count data to estimate local abundance while accounting for imperfect detection. Although this method does not require repeated identification of all individuals across the entire study area (in contrast to spatial capture-recapture models (see section 2.2), t does require identification of individuals recorded in each occasion at each sampling point. This restricts the application

of N-mixture models to species in which individuals can be identified, as is the case, for example, with Annamite striped rabbit in our study sites (see case study section 3.4.1). Heterogeneity in local abundance and detectability patterns can be accounted for using covariates.

The primary input for this model is a site-by-occasion matrix with replicated counts of individuals. If individuals cannot be counted, occupancy models provide an alternative as they only require binary detection / non-detection data (see sections 2.2 and 2.3 for more information on single-season and community occupancy analyses). N-mixture models can also be used as an alternative to spatial capture-recapture model if there are no recaptures or too few recaptures of individuals at different sampling locations, and if movement cannot be estimated (see case study section 3.6.1).

For both Royle-Nichols and N-mixture models the local abundance estimates cannot be transformed to density estimations, as the point estimates of abundance are not associated with a specific area. Thus, even though local abundance estimates allow a direct comparison between sites or years (in case the study design follows the same protocols), comparisons between studies are difficult.

Sunda giant squirrel, Deramakot Forest Reserve, Sabah, Malaysian Borneo. **Photo** Michael Gordon

Rainforest near the summit of Bach Ma mountain, Bach Ma national park, Vietnam. **Photo** Jürgen Niedballa

3. CASE STUDIES

In PART III we provide examples of applications of the methods described above within the SCREENFOR-BIO project. Of course, many additional scientific and conservation related questions can be addressed with data from the sampling design described in this user guide. The aim of this section is to provide examples how the different methods and tools described in PART I and PART II can be applied to answer ecological and conservation questions.

3.1 Environmental covariates and occupancy

3.1.1 Defining habitat covariates in camera-trap based occupancy studies

Niedballa, J., Sollmann, R., Mohamed, A., Bender, J. & Wilting, A. (2015). *Scientific Reports*, 5, 17041.

Background and methods

Understanding the distribution and habitat associations of wildlife species is a key topic in ecology, and important for their conservation. In species-habitat association studies, both the type and spatial scale of habitat covariates need to match the ecology of the focal species. Habitat covariates can be obtained *in situ* during field surveys or *ex situ* using remote sensing technology (see section 1.3.4), and their explanatory value in species-habitat association studies is not only determined by how relevant that piece of habitat information is for the species, but also by how well the spatial scale of the habitat information matches the habitat requirements and space use of the species. Spatial scale refers to grain size and extent of raster data. Grain size is the spatial resolution of raster data, and extent is the size of the habitat patch, also known as focal patch size (Wiens, 1989). To describe habitat adequately, we may need high resolution remote sensing data, rather than the more common satellite imagery of lower resolution (e.g. 30 m Landsat). In this study we used a land cover classification based on high-resolution (5 m) RapidEye satellite imagery for generating habitat covariates for species habitat association studies. The study was conducted using a camera-trapping data set collected between 2008 and 2010 in three commercial forest reserves in Sabah, Malaysian Borneo (see Figure 3.1).

The aims of this study were to assess the sensitivity of occupancy models to spatial resolution and focal patch sizes of land cover information around camera-trap stations. Analyses were conducted within an occupancy modelling framework to test the predictive power of RapidEye-based habitat covariates at different spatial resolutions (5 – 250 m) and extents (focal patch sizes, 10 – 500 m around sample points) on estimates of occupancy patterns of six small to medium sized mammal species/species groups. Covariates used were distance to water, distance to oil palm plantations, and forest score, an index of forest quality derived from land cover classes.

Results

High-resolution land cover information had considerably more model support for small, patchily distributed habitat features (water), whereas it had no advantage for large, homogeneous habitat features (oil palm plantations). On average, habitat covariates describing focal patches with a 50 m radius had most

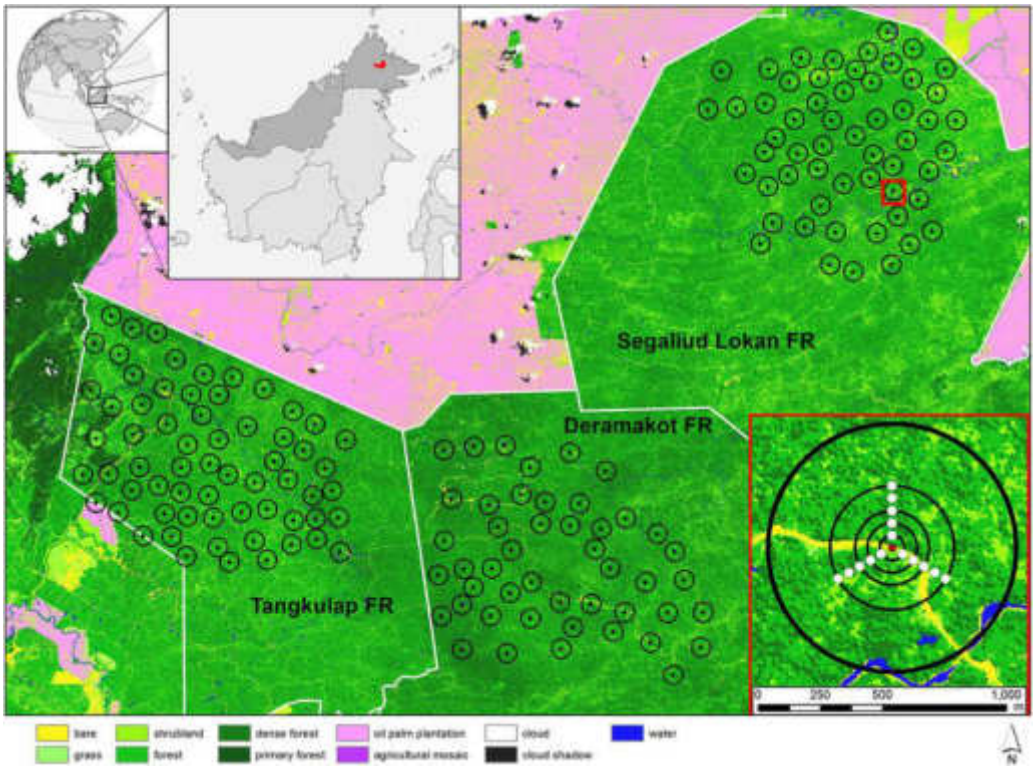

Figure 3.1: *Map of the study sites in Sabah, Malaysian Borneo. Points represent camera trap locations and circles in the main map represent 500 m radii around the camera traps. The inset shows a camera trap station (in red) and the circles represent focal patches of 10, 50, 100, 250 and 500 m radius (from Niedballa et al., 2015).*

support for the target species compared to smaller (10 m) and larger (100 – 500 m) radii.

Main conclusions

High-resolution satellite imagery (5 m) can provide habitat covariates for habitat association studies that explain observed species occupancy patterns much better than lower resolution data ($\geq$ 30 m). Particularly for small, patchily distributed land cover features in heterogeneous landscapes (e.g. water in a forest), the increased resolution is useful for identifying important habitat features that lower resolution data would miss. Additionally, remote sensed data provide more flexibility in defining appropriate spatial scales than *in situ* data, which we show to impact estimates of wildlife-habitat associations. We therefore recommend using high-resolution satellite imagery for creating habitat covariates, if available. If only habitat associations to large land cover features at broad scales are of interest (like distance to large oil palm plantations in this example), freely available satellite data (e.g. Landsat at 30 m resolution) would usually be sufficient). Given sufficient groundtruthing data, remote sensing data can be used as a surrogate for certain *in situ* measures, thus reducing field effort in logistically challenging environments and overall survey costs and effort.

3.1.2 Combining Landsat time-series and time-calibrated occupancy modelling to understand the impacts of logging of tropical forests on species distributions in space and time

Niedballa, J., Baumann, M. F., Kuemmerle, T, Mohamed, A., Hastie, A., Ong, R. & Wilting, A.

(currently in preparation, data shown are preliminary and might change prior to publication)

Background and methods

Understanding species distributions, how they vary over time, and how they are affected by human factors are central questions in ecology and conservation. Past habitat dynamics can affect today's species distributions, but data on these past habitat conditions and past species distributions are often not available. Species distribution models are applied to understand species occurrences in response to environmental conditions via habitat-related covariates. These covariates describe the species' environment and are commonly derived from satellite imagery. Historic time series of satellite imagery can help to understand past habitat dynamics and how they affect today's species distribution patterns (Potapov et al., 2015). They further

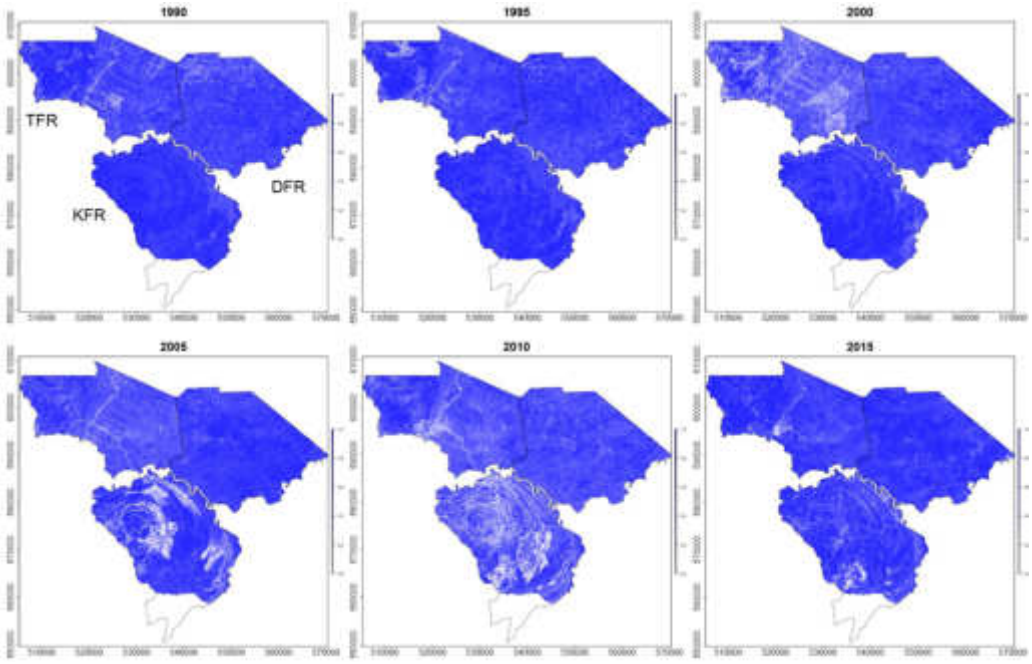

Figure 3.2: *Predicted occupancy probability of Bornean yellow muntjac 1990 – 2015 in 5-year steps in three forest reserves in Sabah, Malaysian Borneo. Blue indicates high occupancy, white low occupancy probability. Conventional logging in Tangkulap-Pinangah forest reserve (TFR) in the 1990s and in Northern Kuamut forest reserve (KFR) in the 2000s resulted in reduced occupancy probability there compared to relatively constant occupancy in Deramakot forest reserve (DFR) where reduced-impact logging is practiced.*

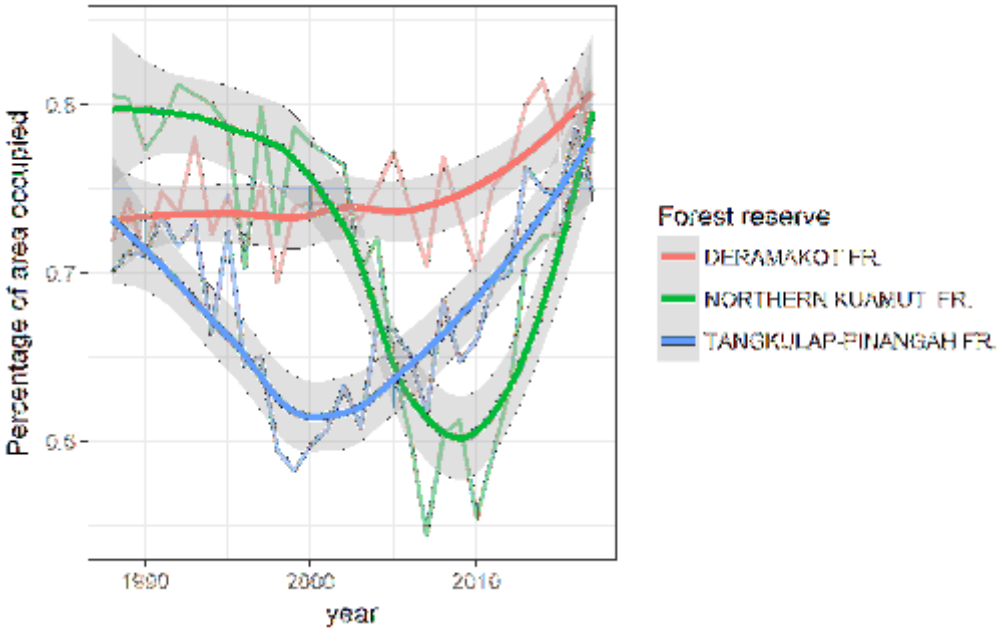

Figure 3.3: *Predicted percentage of area occupied of Bornean yellow muntjac in three forest reserves in Sabah, Borneo. Tangkulap-Pinangah FR was logged conventionally in the 1990s before logging ceased in 2001, whereas Northern Kuamut FR was conventionally logged in the 2000s. Deramakot FR was logged with reduced impact logging since 1997. Predicted occupancy is reduced as a consequence of conventional logging, but adverse impacts of logging on occupancy are much smaller when reduced impact logging is practiced.*

open the possibility to assess trajectories of species distributions in response to these past habitat changes.

We used systematic camera-trapping data comprising 180 camera-trap stations and 12,281 trap days from three commercial forest reserves (FR) in Sabah, Malaysian Borneo (see Figure i.4 and Figure 1.18). The study areas differ in land use history and cover a gradient of logging intensities from reduced impact logging in Deramakot FR to conventional logging at different stages of recovery in Tangkulap-Pinangah

and Northern Kuamut FRs, and consequently vary considerably in forest structure. We applied a 30 year time series of a Landsat-derived vegetation index, the Normalized Differenced Moisture Index (NDMI) to characterize habitat conditions during the study period as well as past logging activities and their impacts on species distributions over space and time. We chose this index as it was both sensitive to vegetation changes due to logging and remained constant in protected areas (Imbak Canyon and Danum Valley), where vegetation remained constant. We used data for 9 forest-dependent and 5 open-forest terrestrial mammal and ground-dwelling bird species analysed with single-species occupancy models using the R package *unmarked* (MacKenzie et al., 2006; Fiske & Chandler, 2011) to establish relationships of species with present habitat conditions and to extrapolate their response to past habitat conditions.

Results

NDMI was sensitive to logging-related habitat changes and allowed the characterization of past logging in time and space. The vegetation index decreased during logging and recovered to its original value afterwards. Using single-species occupancy models, we established a relationship with NDMI as measured during the study period for each species and, using these relationships, predicted distributions of each species over the past 30 years in response to logging in the study areas. The predictions showed that conventional selective logging activities in Tangkulap-Pinangah and Northern Kuamut FRs negatively impacted distributions of forest-dependent species, whereas reduced-impact logging in Deramakot FR greatly reduced these impacts. Furthermore, predicted occupancy of forest-dependent species increased after the cessation of logging activities (Figures 3.2 and 3.3). In contrast, open forest species showed an inverse pattern with increased occupancy during and shortly after logging activities compared to before and after logging.

Main conclusions

Our study highlights the power of the Landsat archive for reconstructing past habitat dynamics, such as from logging. Our data suggest that effects of continuous reduced impact logging activities on occupancy of forest-dependent tropical rainforest species are much less adverse than those of conventional selective logging. Although occupancy of forest-dependent species increased after cessation of conventional logging, the predicted impacts on occupancy were severe in some areas and thus increasing the risks of local extinctions. Our data show that combining time series of satellite data with camera-trap based biodiversity data is a powerful method to understand species distributions and responses to past and present habitat conditions and anthropogenic changes, particularly in challenging habitats such as tropical rainforests.

3.2 Single species occupancy

3.2.1 Data for a little-known endemic species caught in the Southeast Asian extinction crisis: the Annamite striped rabbit of Vietnam and Laos (part 1)

Tilker, A. R., Nguyen, A., Abrams, J. F., Bhagwat, T., Le, M., Nguyen, T.V., Nguyen, A. T., Niedballa, J., Sollmann, R., & Wilting, A. (in press), *Oryx*

Background and methods

The Annamite striped rabbit *Nesolagus timminsi* is found only in the Annamite Mountains on the border of Vietnam and Laos. The species was only discovered by science 25 years ago (Surridge et al., 1999; Averianov et al., 2000), and it remains among the least-known mammal species in Southeast Asia. The lack of information on the species is problematic from a conservation perspective because all terrestrial mammals in the Annamites are threatened by intensive poaching (Abramov et al., 2008; Gray, 2018). We conducted landscape-scale camera-trapping surveys and analysed results within an occupancy framework to better understand factors influencing occurrence and to establish the first conservation baseline for the species.

From 2014 – 16 we conducted intensive camera trapping across five areas in Vietnam and Laos. In Vietnam, study sites included Bach Ma National Park (NP), and the Thua Thien Hue and Quang Nam Saola Nature Reserves. In Laos, surveys were conducted in the eastern section of Xe Sap National Protected Area (NPA) and an adjacent ungazetted forest block to the south near the village of Ban Pale. Together these areas are approximately 900 km^2 in size. We set a total of 139 camera trap stations (see Figures i.4 and 1.18).

Information on potential drivers of species occupancy was collected both in the field and later using remote-sense data (see section 1.3). We assessed both environmental and anthropogenic factors. For example, around each camera trap station we measured

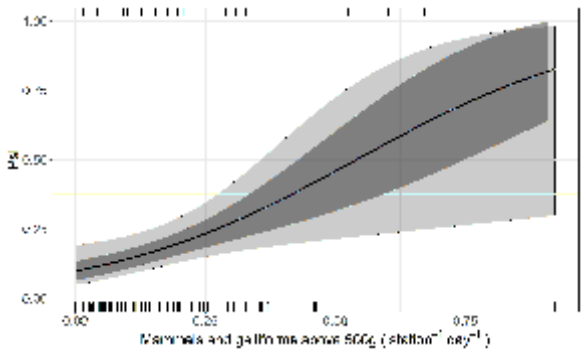

Figure 3.4: *Annamite striped rabbit occupancy (Ψ) modelled in relation to mammals and galliforms >500 g. We interpret this covariate to be an indication of past hunting pressure. Top rug: stations where Annamite striped rabbit was detected. Bottom rug: stations where Annamite striped rabbit was not detected. Modified from Tilker et al. Oryx (in press).*

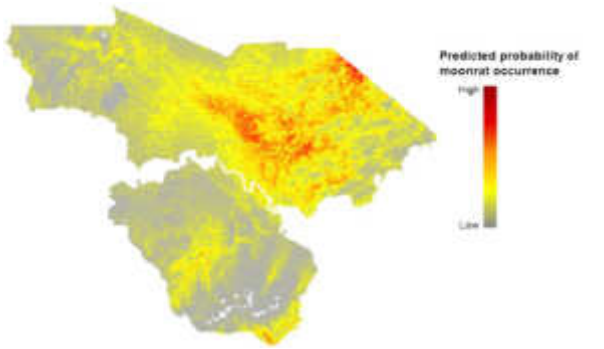

Figure 3.5: *Occupancy based predicted probability of occurrence of the moonrat across three study sites in Malaysian Borneo (modified from Brozovic et al. in revision).*

habitat characteristics and documented the presence of human signs. Landscape-scale covariates were obtained primarily from high-resolution RapidEye satellite imagery. Finally, because current species distribution may be influenced by past hunting pressure, we averaged the number of detections of hunting-sensitive animals (defined as all mammal and galliform species >500 g) for each station to create a photographic index that we interpret as an indication of past hunting pressure. We ran single-species occupancy models in the R package *unmarked* (Fiske & Chandler, 2011).

Results

Model selection by AIC suggested that the detection rate of mammals and galliforms >500 g had a positive effect on Annamite striped rabbit occurrence (Figure 3.4). No other covariate was found to be important.

Main conclusions

This study provides the first landscape-scale conservation baseline for the species and offers key insights into the factors influencing its occurrence. Follow-up surveys using the same study design can be used to assess change in occupancy and, by extension, provide information on population trajectory. The inability of current environmental or anthropogenic variables to explain occurrence provides compelling evidence that past hunting is a primary driver of current distribution. In our study sites, the Annamite striped rabbit exhibits characteristics of a "refugee species," in which anthropogenic pressures, rather than habitat preferences, drive distribution (Kerley et al., 2012). The results further highlight the importance of conservation measures to protect the species.

3.2.2 Ecology and occupancy of the moonrat *Echinosorex gymnura* in logged forests in Sabah, Malaysian Borneo

Brozovic, R., Abrams, J. F., Mohamed, A., Wong, S. T., Niedballa, J., Bhagwat, T., Sollmann, R., Mannan, S., Kissing, J., & Wilting, A. (under review).

Background and methods

The moonrat *Echinosorex gymnura* is an insectivore occurring from southern Myanmar and southern Thailand to Sumatra and Borneo. Very little is known about the species' ecology and habitat requirements. Large parts of its range have been severely deforested and degraded as a result of the conversion of forest to oil palm plantations and unsustainable logging practices. We conducted a systematic large-scale camera-trap survey in three forest reserves under different forest management strategies in Sabah, Malaysian Borneo (see Figure i.4 and 1.18). We recorded a total of 67 independent moonrat detections at 22 of 180 camera trap stations over 12,281 camera trap days.

We conducted single-season occupancy analyses (MacKenzie et al., 2006) in the R software v.3.4.1 (R Core Team, 2017) using the *unmarked* package v.0.12 (Fiske & Chandler, 2011). We used the parameter estimates from the best model to predict occupancy probability across the study landscape using *ex situ* covariates.

Results

We found that canopy cover and forest quality were positively associated with moonrat occurrence and were the most important determinants of the species' occupancy in our study area. Proximity to plantations (oil palm and industrial timber) and elevation negatively affected moonrat occurrence. Our occupancy prediction maps (Figure 3.5) indicate that Deramakot, the well-managed FSC-certified area has the highest moonrat occurrence, followed by Tangkulap-Pinangah, where logging ceased more than 10 years ago. The lowest occupancy estimates—some areas are likely completely devoid of moonrats—were predicted in Northern Kuamut, which was intensively logged using conventional logging methods between 2004 and 2012.

Main conclusions

Our results thus indicate that the moonrat strongly depends on high-quality lowland forest and is unlikely to occur close to plantations. Moonrat occupancy was highest in the sustainably managed forest reserve, suggesting that this species responds well to reduced-impact logging practices in contrast to conventional logging. Our first ecological data indicate that the moonrat may be more threatened by the continued conversion, degradation, and fragmentation of tropical lowland rainforests than previously assumed.

3.2.3 Fine-scale distributions of carnivores in a logging concession in Sarawak, Malaysian Borneo

Mathai, J., Sollmann, R., Meredith, M. E., Belant, J. L., Niedballa, J., Buckingham, L., Wong, S. T., Asad, S. & Wilting, A. (2017). *Mammalian Biology*, 86, 56-65.

Background and methods

Coarse-scale patterns of distribution and abundance of species are the outcome of processes occurring at finer spatial scales, hence the conservation of species depends on understanding their fine-scale ecology. For Bornean carnivores, little is known about fine-scale predictors of species occurrence and it is assumed that the two main threats to wildlife on Borneo, habitat disturbance and hunting, also impact their occurrence. To improve our understanding of the Borneo carnivore community, we used camera-trap surveys in a logging concession in northern Sarawak, Malaysian Borneo, characterised by gradients of both natural and anthropogenic covariates. We sampled in five blocks that were chosen to represent the range of elevation, logging regime, proximity and density of logging roads and settlements, and differences in forest disturbance and hunting pressure. Each block covered 15-20 km^2 and at each block, we sampled 20 points with two cameras and 20 points with a single camera to optimally use the available 60 cameras and maximize detection probability while accounting for logistical difficulties.

We recorded 498 independent events of 15 carnivore species over 14,814 camera trap nights. We had sufficient records to build occupancy models for seven species: Banded civet *Hemigalus derbyanus*, Hose's civet *Diplogale hosei*, masked palm civet *Paguma larvata*, leopard cat *Prionailurus bengalensis*, yellow-throated marten *Martes flavigula*, Malay civet *Viverra tangalunga* and short-tailed mongoose *Herpestes brachyrus*. We used the package *camtrapR* (Niedballa et al., 2016) for initial data preparation. To examine what influenced carnivore occupancy in our landscape, we constructed occupancy models in R using the package *unmarked* (Fiske & Chandler, 2011).

Results

We found forest disturbance to have a negative effect on the occurrence of Hose's civet, banded civet and yellow-throated marten. The probability of occupancy for banded civet was higher in more remote areas. The effect of logging on carnivore occupancy was the most mixed. Hose's civet and masked palm civet were negatively affected by proximity to roads, while Malay civet, short-tailed mongoose and leopard cat were more likely to occupy areas closer to roads. Canopy height, canopy cover, number of trees with holes and distance to nearest village also had varying effects on occupancy probability.

Main conclusions

We used a set of "stacked" single-species occupancy models to determine what factors influence carnivore occupancy in our study site. Our findings show that logged forests are able to provide valuable habitat for many carnivore species. Furthermore, our results highlight the need to consider habitat variables that are often overlooked, such as moss cover, as this was the most important factor influencing the occurrence of Hose's civet. Looking into such covariates may provide insight into habitat preferences of little-known species. As carnivore responses varied drastically

to our covariates, the stacked single species models provided a very useful approach for this study, as it permitted a species-specific analysis.

3.3 Community occupancy modelling

3.3.1 Quantifying mammal biodiversity co-benefits in certified tropical forests

Sollmann, R., Mohamed, A., Niedballa, J., Bender, J., Ambu, L., Lagan, P., Mannan, S., Ong, R. C., Langner, A., Gardner, B. & Wilting, A. (2017). *Diversity and Distributions*, *23*, 317-328.

Background and methods

The destruction of tropical rainforests leads to extreme losses of biodiversity (Brook et al., 2003; Gardner et al., 2009). In recent years financial incentives, such as certification or carbon storage payments, have been put into place to encourage the sustainable management of forests. In recent years, financial incentives, such as certification or carbon storage payments, have been put into place to encourage the sustainable management of forests. These incentives are assumed to have co-benefits for biodiversity conservation, a claim that remains little studied for rainforest mammals, which are particularly threatened, but challenging to survey. Here, we apply the community occupancy approach to mammal camera-trapping data collected in Sabah, Malaysian Borneo. Between 2008 – 2010 we collected camera-trapping data in three commercial forest reserves under different forest management (see Figure 3.1). A total of 37 mammal species were detected. Arboreal species with 2 or less detections and far ranging species, violating the spatial independence assumption (see text box on page 49) were removed reducing the number of species included in the analysis to 28.

We used a community occupancy framework to model the effect of three habitat covariates, with species-specific coefficients, on occupancy: (1) distance to water, (2) distance to oil palm plantation and (3) forest score.

Results

Many threatened species were positively associated with higher forest scores and thus occupied larger areas in the well-managed FSC-certified reserve. Species richness was higher in the certified site, particularly for threatened species. The certified reserve also had the highest aboveground biomass, leading to a positive relationship between biodiversity and carbon on the reserve-level (Figure 3.6). However, within reserves aboveground biomass was not strongly correlated with patterns of mammal richness (Spearman's ρ from 0.03 to 0.32).

Main conclusions

Community occupancy modelling in combination with camera-trapping presents a flexible and standardised tool to assess biodiversity and identify winners of sustainable forestry. Inferring patterns of species richness from camera-trapping carries potential for the objective designation of high conservation value forest, an important component of forest certifications or to receive carbon offset payments. Correlating species richness with aboveground biomass further allows evaluating the biodiversity co-benefits of carbon protection. We show that carbon stocks are not necessarily good predictors of biodiversity, particularly at local scales. The community occupancy framework based on multi-species camera-trapping detection/non-detection data provides an ideal tool to overcome the difficulties to rigorously quantify biodiversity co-benefits of forest certification and carbon storage payments.

3.4 Local abundance

3.4.1 Data for a little-known endemic species caught in the Southeast Asian extinction crisis: the Annamite striped rabbit of Vietnam and Laos (part 2)

Tilker, A. R., Nguyen, A., Abrams, J. F., Bhagwat, T., Le, M., Nguyen, T.V., Nguyen, A. T., Niedballa, J., Sollmann, R. & Wilting, A. (in press), *Oryx*

Background and methods

In addition to assessing landscape-scale occupancy of the little-known endemic Annamite striped rabbit *Nesolagus timminsi* (Abramov et al., 2008); see section 3.2.1 for more information on the study), we also conducted surveys at finer spatial scales (see Figures i.4 and 1.18)—one in Bach Ma National Park and one in the Thua Thien Hue and Quang Nam Saola Nature Reserves—to determine local abundance. 64 stations were set up in each phase, resulting in a total of 128 camera trap stations for both areas. Stations

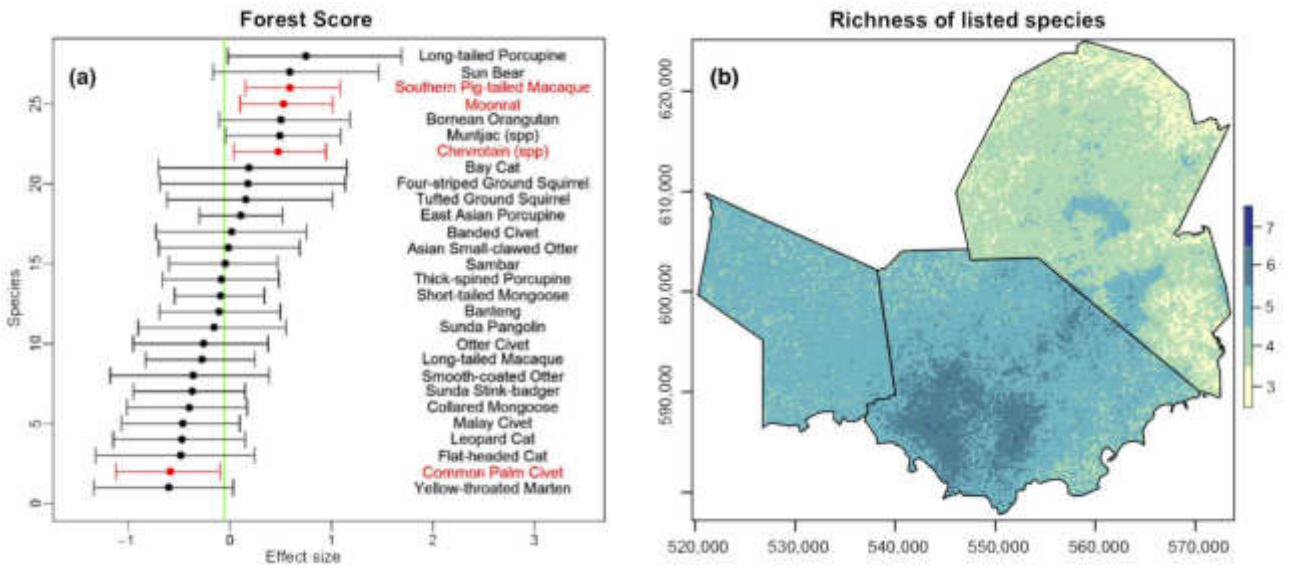

Figure 3.6: *Forest score covariate coefficients (mean and 95 % BCI in the logit scale) on occupancy probability of 28 mammal species (right) estimated with community occupancy models fit to camera-trap data from three forest reserves in Sabah, Malaysian Borneo. Predicted species richness of IUCN Red List species (right) (Figure modified from Sollmann et al., 2017).*

were arranged in a clustered design, with 16 camera trap clusters per survey spaced approximately 1.5 km apart. Each cluster then consisted of four camera trap stations, spaced approximately 500 m apart and arranged in a square. At each station two cameras were set facing each other to photograph both sides of a passing animal. The camera trap survey was designed to provide data that can be used to estimate local abundance for species that have individually-recognizable markings. Cameras were operational for a minimum of 60 days.

All camera trap photos were processed using *camtrapR* (Niedballa et al., 2016). We used N-mixture models (Royle, 2004) to estimate local abundance for Annamite striped rabbit from our fine-grid data. We identified individual rabbits using their unique striping patterns. Because we did not have any individual rabbit photographed at successive stations, we consider our data to be spatially independent (Kéry et al. (2005), see page 53). N-mixture models were run in the R package *unmarked* (Fiske & Chandler, 2011).

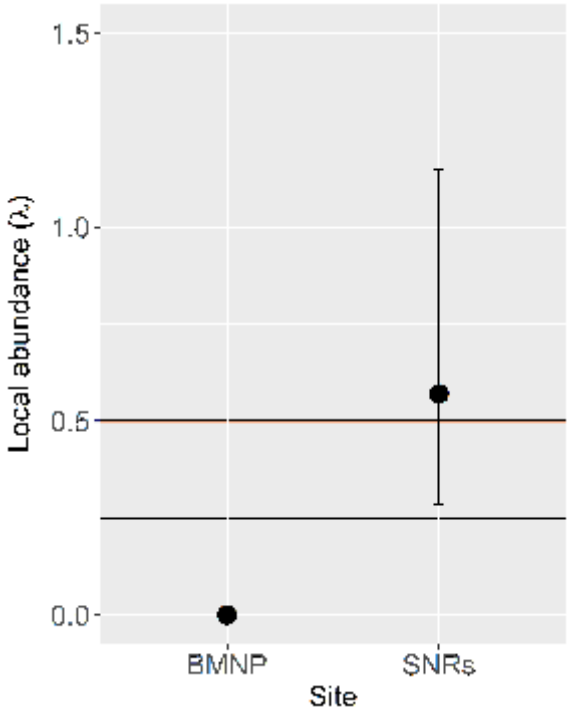

Figure 3.7: *Annamite striped rabbit local abundance (λ) in the Hue and Quang Nam Saola Nature Reserves (right) estimated through N-mixture model. Local abundance in Bach Ma NP (left) could not be estimated because there were no detections from this site; we interpret this to indicate that true abundance is zero or very close to zero. Modified from Tilker et al. Oryx (in press).*

Results

From 8,404 camera trapping nights, we obtained 54 independent detections of Annamite striped rabbit across 14 stations. All detections occurred in the Saola Nature Reserves, with no detections in Bach Ma NP. We identified a total of 27 individuals using striping patterns and other markings. The N-mixture model estimated an average local abundance (λ) in the Saola Nature Reserves of 0.57 (SE = 0.20) (Figure 3.7). Because we did not have detections from the camera trapping in Bach Ma NP, we could not estimate local abundance for this survey area. Given

that camera trapping effort in this area was the same as in the Saola Nature Reserves, we interpret the lack of records to indicate that true local abundance at the Bach Ma NP fine-grid survey area is zero or close to zero.

Main conclusions

The fact that we did not record any individuals in the Bach Ma NP survey indicates that the species is either extirpated or very rare in this area. The local abundance estimates from the Saola Nature Reserves provides the first abundance-based conservation baseline for the species and can be used to assess population changes over time. For the Annamite striped rabbit, the use of N-mixture analyses represents an effective way to obtain local abundance for species with individually-recognizable markings, and thus we expect that it can also be used successfully for other species in the rainforests of Southeast Asia.

3.5 Density

3.5.1 Counting Sunda clouded leopards with confidence: incorporating individual heterogeneity in density estimates

Mohamed, A., Sollmann, R., Wong, S. T., Niedballa, J., Abrams, J. F., Kissing, J., Mannan, S., & Wilting, A. (currently in revision)

Background and methods

The elusive Sunda clouded leopard *Neofelis diardi* is the largest of the five felid species found on Borneo. Within its range on Borneo and Sumatra, it is threatened by habitat loss and habitat degradation (Gaveau et al., 2007, 2014, 2016), and illegal hunting (Hearn et al., 2016). Due to these threats, the Sunda clouded leopard is classified as vulnerable on the IUCN Red List of Threatened Species (Hearn et al., 2015), with its Bornean and Sumatran subspecies being classified as endangered (Hearn et al., 2008; Sunarto et al., 2008). However, effective conservation management is hampered by the lack of essential information about the species and its ability to survive in human-modified landscapes, particularly within logging concessions where a large portion of the remaining populations are likely to occur (Wilting et al., 2012). This dearth of information is mainly due to the species' secretive behaviour and its occurrence in very low densities.

Previous studies estimating density for the species were unable to account for individual capture heterogeneity due to inadequate recaptures, particularly of females, possibly leading to biased density estimates. Here, we use data from large-scale camera-trapping surveys in three forest reserves with different management histories located in Sabah, Malaysian Borneo (see Figures i.4 and 1.18) to compare density estimates from models incorporating individual heterogeneity in detection with estimates from the null model to evaluate its potential bias.

We managed all camera trap data with *camtrapR* (Niedballa et al., 2016). We carried out individual identification of Sunda clouded leopards based on individual spot patterns and we identified the sex by looking at secondary sexual traits. We estimated population density of Sunda clouded leopards using SCR models implemented in the *secr* package (Efford, 2016) in R. We used data from coarse grid surveys for all three study areas to estimate Sunda clouded leopard densities. The spatial distribution of captures of all individuals indicated that heterogeneity in movement exists not only between males and females, but also among males. Therefore, the Sunda clouded leopard population may better be represented as a mixture of two types of individuals; dominant males who move over large areas and are detected more frequently, and subordinate males and females that range over smaller areas and are detected less frequently. Therefore, we also modelled individual heterogeneity in movement and baseline detection using a finite mixture model (Pledger, 2000) that assigns individuals to two latent groups. We compared results from the mixture model with estimates from a null model.

Results

In total, we obtained 98 independent records of Sunda clouded leopards over a total of 20,286 camera trapping nights. Densities estimates from null models for Deramakot, Kuamut and Tangkulap-Pinangah were 0.89 $\pm$ 0.42 individuals/100 km^2, 1.22 ($\pm$ 0.61) and 0.39 ($\pm$ 0.25) individuals/100 km^2, respectively. Model selection by AICc showed that the mixture model performed much better than the null model. The density estimates from the mixture models were 2.84 ($\pm$ 1.12), 2.43 ($\pm$ 1.09) and 1.27 ($\pm$ 0.69) for Deramakot, Kuamut, and Tangkulap-Pinangah, respectively. Nearly 92 % of all individuals in the population were assigned to a group with small movements (0.74 $\pm$ 0.11 km) and the remaining 8 % to a group with higher sigma (7.45 $\pm$ 1.75 km).

Main conclusions

We found that single-site data were too sparse to account for individual heterogeneity due to inadequate recaptures; thus, variation in detection among individuals could not be accounted for and only conservative null model estimates could be generated. However, aggregating data across study sites allowed us to account for individual heterogeneity and we estimated densities between 1.27 to 2.82 individuals/100 km^2. Similar densities found in well-managed FSCcertified forest and recently selectively logged forests reinforce that Sunda clouded leopards are quite resilient to forest disturbances. Our densities were two to three times higher than estimates from null models. In light of these findings, it is possible that earlier studies, which were all based on null models, underestimated the true density. The finding is also of great relevance for other wide-ranging carnivore species that occur at low densities.

3.6 Occupancy, local abundance and density: a comparison

3.6.1 Occupancy, local abundance and density: what can we estimate with camera-trapping data?

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(currently in preparation, results shown are preliminary and might change prior to publication)

Background and methods

Abundance and density are important measures of the state of a wildlife population, but are difficult to estimate for many species, particularly those occurring in tropical rainforests. Here, we used a large-scale camera-trapping survey conducted in two forest reserves in Sabah (Deramakot and Tangkulap-Pinangah, see Figures i.4 and 1.18), to investigate the relationship between occupancy, local abundance (Royle-Nichols and N-mixture models) and density (spatial capture-recapture; SCR) for the Malay civet *Viverra tangalunga* and the banded civet *Hemigalus derbyanus*. The model complexity and demands for necessary input data decreases as you move from density to local abundance to occupancy. Here, we are interested in how this change in complexity affects model performance and the trade-of between model complexity and effort. Both of our target species are individually identifiable, thus allow us to run SCR analyses and compare the unmarked approaches with more robust density estimates. Due to the differing model assumptions of occupancy / local abundance models – which require spatial independence of stations – and SCR – which require spatial recapture of individuals – we used the coarse grid sampling for the occupancy / local abundance models and the fine grid dataset for the SCR models. Therefore, our estimates are not directly comparable, but as our fine grid sites were nested within our coarse scale sites, we assumed that the overall abundance and occupancy patterns would be comparable.

Results

For the Malay civet and the banded civet, the top three covariates selected as important in occupancy, Royle-Nichols and N-mixture models were largely consistent. All analyses showed that Malay civets in Deramakot were 1.2 (occupancy), 1.4 (Royle-Nichols) and 1.9 (N-mixture) times more common than in Tangkulap-Pinangah, but these differences were not statistically significant (Figure 3.8). Spatial capture-recapture analysis, however, showed a significant difference in Malay civet density between Deramakot and Tangkulap-Pinangah (3.7 times greater in Deramakot). Occupancy, Royle-Nichols and N-mixture models showed that banded civet in Deramakot was significantly more common than in Tangkulap-Pinangah by a factor of 8.4, 16.6 and 15.7, respectively.

Main conclusions

Counter to our assumption, the local abundance models did not significantly improve our ability to detect differences between our study sites compared to occupancy models, as all models detected a significant difference for the banded civet, but not for the Malay civet. The SCR analyses, however, showed that density of the Malay civet in Deramakot was significantly higher than in Tangkulap-Pinangah. We acknowledge that the relationships between the measures are species- and probably site-specific and, thus, our findings are likely not representative of other species or locations. However, our results indicate that although occupancy models do not provide information about the abundance of species, this measure can be as powerful as local abundance models to assess habitat associations and compare species status among different areas. Repeated sampling in combination with dynamic occupancy models will likely allow conservationists and forest managers to monitor species trends

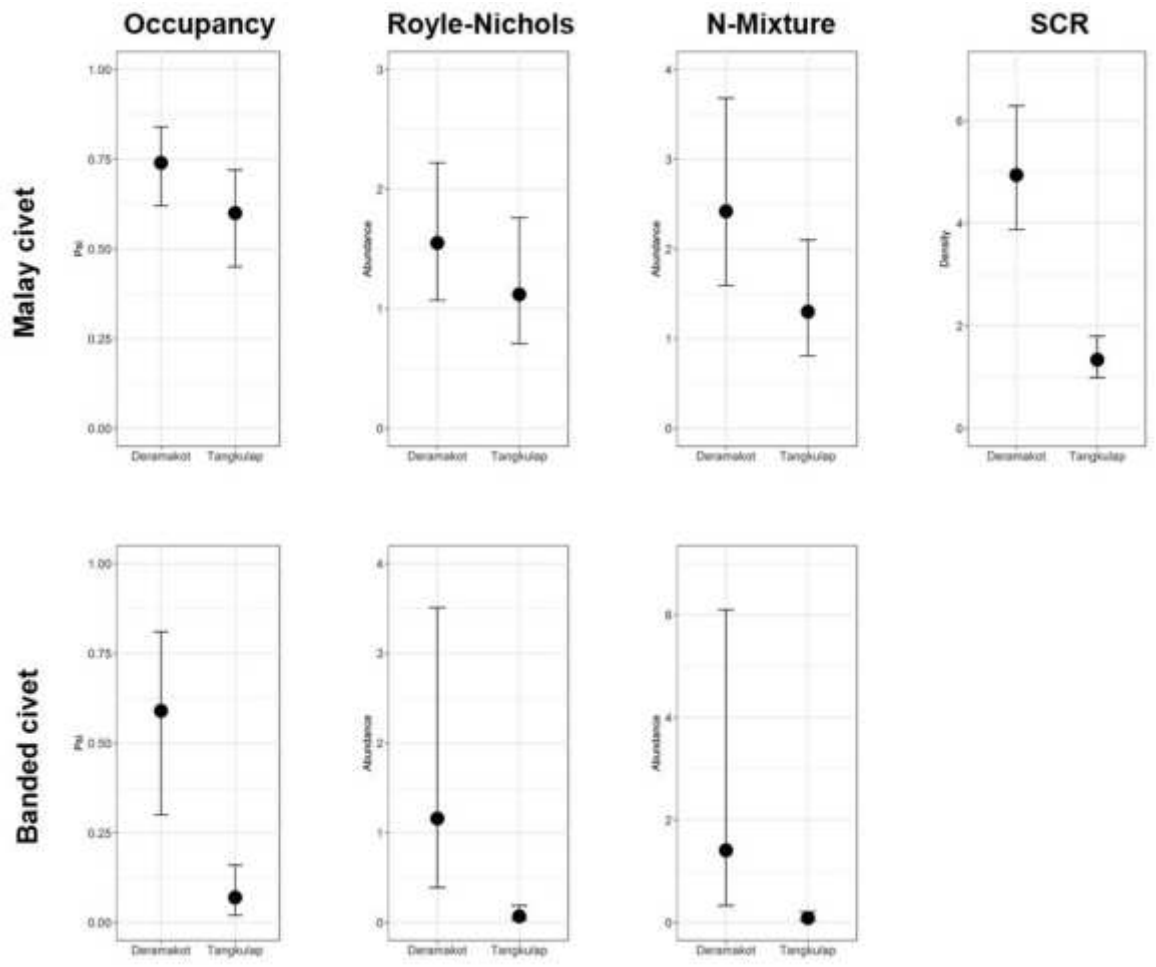

Figure 3.8: *Mean estimates with 95 % confidence intervals for occupancy, local abundance and density of Malay civet (top) and banded civet (bottom) for Deramakot and Tangkulap-Pinangah forest reserves.*

over time and, thus, evaluate their forest management practices.

3.7 iDNA as a tool for biodiversity assessments

3.7.1 Using leech-derived iDNA for mammal biodiversity assessment, monitoring and conservation

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(currently in preparation, results shown are preliminary and might change prior to publication)

Background and methods

Monitoring of threatened wildlife populations, particularly in biodiversity hotspots such as tropical rainforests, remains a challenge for biologists and conservationists as species of interest are often secretive and occur in remote areas. To overcome the difficulties in detecting species, there is a growing confidence that DNA-based methods could provide an effective means for detecting secretive and rare species (see section 1.2). In some cases, these DNA-based tools outperformed traditional methods, such as camera-trapping, as they also detected smaller species (Tessler et al., 2018), detected some species more frequently, or provided additional information, such as animal age (Kent, 2009). Although the first studies on iDNA are encouraging, the application if e/iDNA has been restricted to *ad hoc* opportunistic collections of invertebrates and the detection of vertebrates. In conservation, however, a single detection of a species is of limited use, as it does not allow for the monitoring of populations or biodiversity trends over time.

In this study we present an approach that makes the shift from the proof of principal that iDNA can be used to gather detection events, to using iDNA as a robust biodiversity assessment and monitoring tool. We used standardised collection of terrestrial haematophagous leeches in the vicinity of a system-

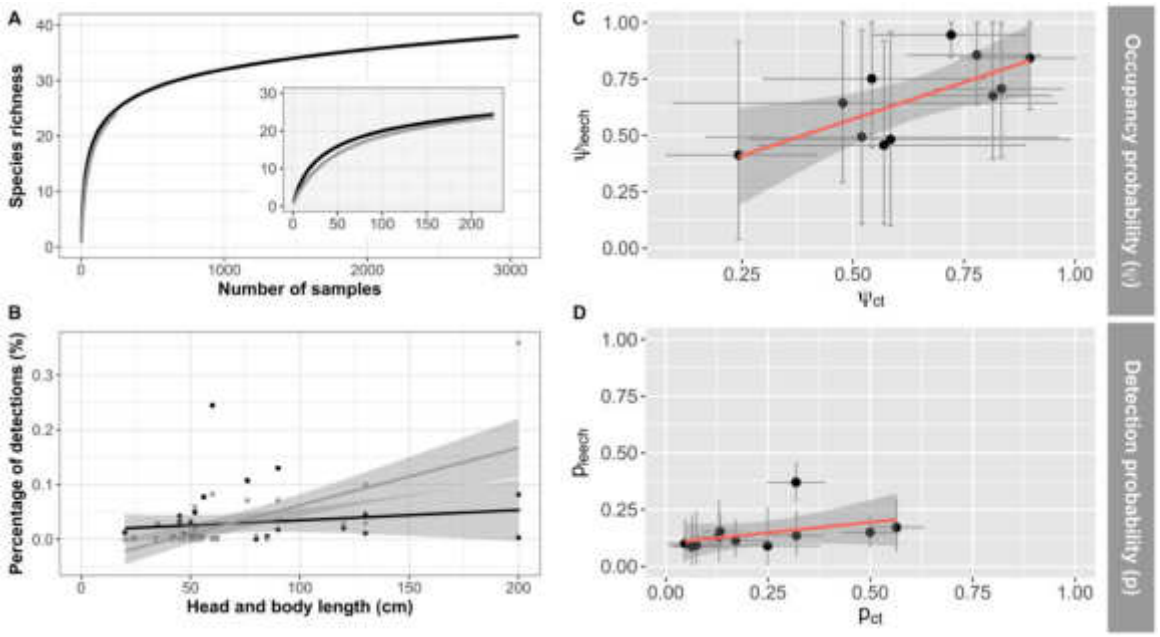

Figure 3.9: *Comparison of camera-trap and leech iDNA results.* ***(A)*** *Species-accumulation curves for the camera-trap (black line) and leech (grey line) surveys. The main plot shows the species-accumulation up to the maximum of 3177 camera-trap detections while the inset shows the accumulation up to the first 250 detections.* ***(B)*** *Correlation between detections and average body length of species. The number of detections in the leech surveys depends more on the size of the species, while detections in the camera-trap surveys were more independent of body size.* ***(C)*** *Occupancy and* ***(D)*** *detection probabilities estimated using null single-species occupancy models for camera-trap (x-axis) and leech (y-axis) surveys. Vertical and horizontal bars indicate 95 % Bayesian CIs. The leech and camera-trap occupancy results are positively, and significantly, correlated for panels C and D.*

atic camera-trap survey in Sabah, Malaysian Borneo, to test the efficiency of the iDNA approach. We analysed the iDNA derived mammalian detection events in occupancy models that account for imperfect detection (MacKenzie et al., 2002) and compared them to occupancy models of our concurrently collected camera-trap data.

Results

Overall, we detected a higher number of species with camera-traps, but the species accumulation curve suggests that with increased leech collection effort, more species could be detected (Figure 3.9A). Although our data confirmed the findings of earlier studies that smaller vertebrate species, which are often missed by camera-traps, can be detected via leeches, we found a much stronger detection bias in the leeches to larger bodied vertebrates such as the sambar than in camera-trapping (Figure 3.9B). This highlighted the importance of accounting for varying detection probabilities in iDNA studies, which may stem from unknown host preferences of these leeches, among other factors. When accounting for varying detectability between species, our leech-based occupancy models showed consistent predictions of species occurrence probabilities to those from our camera-trap based occupancy models (Figure 3.9C), suggesting that iDNA studies can provide occupancy estimates that are consistent with the long-established method of camera-trapping.

Main conclusions

This study provides the first empirical evidence for the potential and power of iDNA for monitoring wildlife populations and biodiversity. Our study suggests that systematic iDNA sampling and high-throughput iDNA sequencing coupled with modern ecological modelling tools can be used to assess biodiversity at landscape scales.

Misty morning on the main road in Deramakot Forest Reserve, Sabah, Malaysian Borneo. **Photo** Jürgen Niedballa

Pygmy elephant, Deramakot Forest Reserve, Sabah, Malaysian Borneo. **Photo** Michael Gordon

4. SUMMARY AND PERSPECTIVES

Global wildlife populations are continuing to decline at an alarming rate (Schipper et al., 2008; Butchart et al., 2010; Dirzo et al., 2014; Ceballos et al., 2015; Ripple et al., 2016; Benítez-López et al., 2017; Ceballos et al., 2017).

Because the drivers of global wildlife decline are powerful and complex, it is unlikely that the international community will be able to halt this ongoing decline within the coming decades unless strong and determined action is taken. Although the CBD Parties have committed themselves to the ambitious Aichi Biodiversity Targets (ABTs[1]), in practise the conservation of species and populations largely depends on targeted and local conservation efforts.

With an ever-growing global human population, it will not be possible to protect all remaining tropical rainforests from deforestation, degradation and hunting. Federal governments, NGOs and conservation scientists need to distinguish between conservation priority areas, where the limited financial resources for conservation can be allocated to maximise effectiveness of conservation interventions, and areas that can be used for human development. The availability of natural resources and the suitability of land for agriculture are important factors in making such decisions, but in line with international commitments, the conservation of tropical biodiversity must also be considered. Within the framework of conservation planning, scientists, conservationists and governments need to incorporate conservation goals into development strategies. However, it has taken many years of data collection and analysis to compile the data needed for the latest report of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES, 2018), and even with this immense effort, ground-truthed biodiversity data are missing from most biodiversity-rich areas of global conservation importance, which hinders effective conservation planning. While in some cases essential biodiversity variables (EBVs), which are considered to be promising metrics of holistic biodiversity (Skidmore et al., 2015; Pettorelli et al., 2016; Proença et al., 2016) can be assessed with satellite data (Pereira et al., 2013), ground-based survey efforts are needed to measure and thus monitor many of these quantities (Bush et al., 2017).

Forest managers also need ground-based biodiversity data to meet the requirements of international certification schemes. Assessing biodiversity co-benefits are a central component to REDD+ and to carbon offset projects under the Carbon, Community & Biodiversity Standards (CCB-Standards[2]). Rigorous monitoring of biodiversity – especially of threatened species – is also required by the Forest Stewardship Council (FSC[3]) and by the Round Table of Sustainable Oil Palm (RSPO[4]). In biodiversity-offset programmes the net biodiversity gain needs to be documented in a transparent way. Although of all these international programmes require the monitoring of biodiversity, the frameworks provide few specifics on how biodiversity data should be collected or analysed. In practise, this lack of standardisation often results in *ad hoc* survey efforts that do not collect data that can be analysed in a rigorous scientific manner, or in

[1] www.cbd.int/sp/targets

[2] www.verra.org/project/ccb-program

[3] www.ic.fsc.org/

[4] www.rspo.org

monitoring programmes that target one or two species under the assumption that these species are indicators for the overall status of biodiversity in a landscape. Assuming that some species serve as indicators for holistic biodiversity is risky, as numerous studies have shown that species respond in diverse ways to environmental changes (Urban et al., 2016; Sollmann et al., 2017). **While we acknowledge the need for site-specific biodiversity monitoring protocols, in our perspective a standardisation of the ground-based biodiversity surveys would be highly beneficial for all international certification schemes, as only standardised surveys will allow practitioners to continuously monitor biodiversity.**

This user guide provides guidance to practitioners on how to design and conduct the kinds of ground-based surveys that are needed for rigorous biodiversity monitoring. The two high throughput approaches for biodiversity assessment discussed in this user guide - camera-trapping and e/iDNA - provide fast and efficient ways to gather information on terrestrial mammals and, therefore, build comprehensive biodiversity datasets.

Camera-trapping provides unique and vital insights into the mostly hidden world of tropical rainforest mammals. One of the primary advantages to camera-traps is that they work autonomously and uninterruptedly 24/7 for weeks or months at a time. Despite the benefits of camera-trapping and its wide application to a variety of wildlife study questions, there are still a number of challenges that researchers face when employing this method.

1. Camera-traps are either costly and / or perform relatively poorly in the hot and humid climate of tropical rainforests. Many camera models are not well protected from small insects such as ants and thus failure rates of camera-traps remain high. We used one of the most expensive camera-trap models available, the Reconyx PC 850, and although performance was good during the first two years, after three years of intensive use in tropical rainforest conditions failure rates greatly increased. We tested a number of other white flash camera-trap models, but no model performed satisfactorily. Therefore, we cannot recommend any single camera-trap model as the perfect solution for camera-trap-based studies. However, the commercial camera-trap market is constantly evolving, and we are confident that new, more reliable models will become available in the future. One of the primary challenges, in our opinion, is that all camera-trap models currently available are designed for hunters and for use in temperate climate zones, and are thus not well suited for wet and humid tropical rainforest conditions. We hope that, in line with the camera-traps developed by the NGO Panthera, more camera-traps will be specifically designed for research purposes.
2. Loss and damage of camera-traps by animals or humans further increase the costs of camera-trapping studies and result in the loss of valuable biodiversity data. Damage by curious animals will always occur, and robust measures, such as steel housings, are not sufficient to keep away animals such as elephants. Damage and loss by humans will also happen if people legally or illegally enter the study sites. However, integrating local communities in the camera-trapping effort and informing them about the goals of the camera-trapping campaign might help to reduce camera-trap theft.
3. In addition to these logistic challenges, one of the main shortcomings of using camera-traps is that accurate abundance estimates are more challenging to obtain for species that cannot be individually distinguished based on unique stripe or spot patterns – which is the case for the majority of tropical rainforest mammal species. Because occupancy models do not require individual identification, we put a special emphasis on occupancy in this guide. Although occupancy models provide information on species presence or absence, rather than more 'informative' estimates of local or absolute abundance, we nonetheless believe that these models allow practitioners to address crucial questions related to the impacts of forest management on mammal communities. Such questions include:
    - Where do species occur?
    - How are species distributed?
    - Where are the highest concentrations of species?
    - Do species distributions change over time? (addressed using dynamic occupancy models)

While there is empirical and mathematical evidence that occupancy is related to abundance (Lawton, 1993; Gaston, 1996; MacKenzie & Nichols, 2004), we acknowledge that changes in abundance are not necessarily reflected in occupancy, especially at the local scale (e.g. Matthews et al., 2011; Efford & Dawson, 2012).

Within the last few years new analytical approaches to estimate abundance for species that cannot be individually identified have been developed (Random Encounter Model, Rowcliffe et al., 2008) and its extension, the random encounter and staying time (REST) model (Nakashima et al., 2018), camera-trap based distance sampling (Howe et al., 2017). We follow these developments with great interest and hope that they will contribute to the development of new analytical tools for density estimation without the need for individual identification within the next few years. In this user guide we decided, however, to focus on well-established methods, especially because empirical evidence about the reliability of these new methods under different field conditions is currently lacking. It is worth noting that these new methods require researchers to collect additional data in the field – for example, to take measurements to estimate the distance between the camera-trap and an animals in pictures – or make informed guesses for critical parameters – e.g., home range and daily movement rate for target species. Because we did not take these additional measurements in our field sampling protocols provided in PART I, we cannot give instructions on these aspects of data collection. We highlight that users who intend to apply these recently developed tools should make sure that the necessary additional data is collected.

In contrast to camera-trapping, e/iDNA applications for biodiversity monitoring are still in their infancy. More research is needed before this method can be applied in forest management and conservation as a rigorous biodiversity monitoring tool. However, developments in the field of e/iDNA are progressing rapidly, as can be seen from the fact that within the first few years after its establishment numerous studies on the use of invertebrate hosts as a source for vertebrate DNA have been published (Calvignac-Spencer et al., 2013; Gariepy et al., 2012; Hoffmann et al., 2018; Kocher et al., 2017; Lee et al., 2016; Rodgers et al., 2017; Schnell et al., 2012, 2018; Schönenberger et al., 2016; Lura et al., 2012; Tessler et al., 2018; Townzen et al., 2008; Weiskopf et al., 2018). Within the SCREENFORBIO project, we developed a laboratory and bioinformatics workflow, which will help to establish best practice guidelines for future e/iDNA studies. In our project, the workflow helped to reduce potential contaminations and, as a result, the occurrence of false positive detections was very unlikely. This workflow provided a foundation for analysing mammal occurrence data from leeches within the same analytical framework as camera-trap data (see case study section 3.7.1). Together with a more standardised and systematic sampling design (see section 1.3), we see great potential for e/iDNA to be incorporated into monitoring programmes in the upcoming years. However, to improve the method, additional basic research is needed.

1. Globally, the sequence reference datasets for species occurring in tropical rainforests remains poor. In the case of our study site in Sabah, Malaysia, the reference sequences for the barcoding markers that we used covered only half of the target species present in our study site. Nine of the 103 species had no sequence information available. Because a full sequence reference database is the basis for assigning sequencing reads to species, further barcoding initiatives are necessary to fill gaps in reference sequence databases, and thereby increase the power of future e/iDNA studies.
2. DNA extracted from e/iDNA sources is often of low quality due to DNA degradation and the low amounts of target DNA available relative to non-target DNA (e.g. from the invertebrate vector in iDNA studies). Therefore, although the short *16S* barcoding marker amplified better than the longer *12S* and *CytB* markers, sequence variability in this short fragment is low, often making the taxonomic assignment to species level difficult. We therefore recommend the use of multiple short fragments, as this helps to overcome some gaps in the reference database and increases the likelihood of species-specific taxonomic assignments. Although multiple markers increase the time and costs, their use will increase our confidence in the findings of any e/iDNA study.
3. For iDNA studies, it is important to obtain more insight into how the ecology of the invertebrate vectors influences the utility of the method. In the case of invertebrates that are able to fly large distances, the spatial information of the host remains unknown, and thus any spatial analysis at finer scales (such as occupancy models) will be difficult to conduct without violating major assumptions of the models. Although such information might not be as relevant for *ad hoc* surveys or for surveys aiming at compiling a list of species present, it is crucial for any long-term monitoring programmes that intend to assess changes of abundance or occupancy over time.

Despite the challenges associated with both camera-trapping and e/iDNA, they remain the best options for large-scale, rapid biodiversity surveys targeting terrestrial mammals in tropical

rainforests. Therefore, we believe that the application and use of camera-trapping and e/iDNA will continue to increase in the coming years. Only with wide application of these high-throughput techniques will we be able to compile the ground-truthed biodiversity data needed to employ rigorous monitoring programmes in priority areas.

These ground-truthed biodiversity data form the first of three cornerstones of a framework proposed by Bush et al. (2017) to assess and monitor biodiversity across landscapes:

1. Gather detection events of multiple species using high throughput approaches such as automated recording devices (e.g. camera-traps) or metabarcoding of e/iDNA.
2. Compile landscape-scale habitat information using earth observation data.
3. Connect these via sophisticated statistical tools to provide landscape maps of species and biodiversity distributions that can be tracked over time.

From our perspective, this protocol is a fast and efficient way to monitor the state of wildlife populations and their responses to conservation interventions. Locally, the application will help stakeholders to make informed conservation decisions and manage forests in a more sustainable way. If such assessments are conducted at landscape levels, states and countries will be able to generate well-informed reports about the state of biodiversity – important components of international certification schemes. The parties of the CBD agreed to report and track changes in their biodiversity and have committed to "take effective and urgent action to halt the loss of biodiversity in order to ensure that by 2020 ecosystems are resilient and continue to provide essential services…". It will take time for the tools and protocols described in this user guide to be implemented towards the formation of a global standardised biodiversity monitoring network. However, on local, provincial, and national scales, the tools may be implemented faster. We hope that this user guide will assist governments, NGOs, scientists, and students in designing and planning their projects and forest management studies. **Rigorously collected, standardised biodiversity data are the first step towards more reliable monitoring and sustainable management, and therefore towards achieving global goals such as the Aichi Biodiversity Targets.**

Imbak Canyon, near Maya falls.

Photo Michael Gordon

Binturong on a fig tree, Deramakot Forest Reserve, Sabah, Malaysian Borneo. **Photo** Michael Gordon

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ISBN: 978-3-9815637-6-4