International Journal of Primatology (2025) 46:842–874
https://doi.org/10.1007/s10764-025-00500-7

Passive Acoustic Cue Counting Surveys for Vocal Primates: A Field Test on the Critically Endangered Nosy Be Sportive Lemur (*Lepilemur tymerlachsoni*)

Luke D. Martin[1] · Herison Razafimanantsoa[2] · Eva S. Nomenjanahary[2] · Sylviane Volampeno[3] · Alice M. Richardson[4] · Robert D. Magrath[5] · Alison M. Behie[1]

Received: 5 February 2025 / Accepted: 9 June 2025 / Published online: 9 July 2025
© The Author(s) 2025

Abstract

Passive acoustic monitoring, which uses autonomous audio recorders in the field, offers a powerful, efficient and cost-effective survey method that samples continuously and non-invasively. But despite its potential, its application remains atypical in primate research. We conducted a field test of passive acoustic density estimation in a near range-wide survey of the Critically Endangered Nosy Be sportive lemur (*Lepilemur tymerlachsoni*). We used the sound level of target calls detected in audio recordings as a proxy for radial distance as part of a conventional cue-counting distance sampling framework. While the passive acoustic survey recorded a large number of calls (9,560 detected calls from 250 hr of audio recordings) and the sound-level-based distance prediction model performed well, the resulting density estimates using single calls as cues were implausibly high and had low precision, which we attribute to the inherent challenges of estimating the cue rate. Results were improved, however, when using call bouts, rather than single calls, as the unit of analysis (i.e., as "cues"). Concurrent line transect surveys provided a more plausible density estimate of 303.7 individuals/km^2 (95% confidence interval = 189.6–486.3, coefficient of variation = 0.18), corresponding to a global abundance estimate of *c*. 11,300 individuals. Our results highlight the potential and limitations of passive acoustic density estimation and provide critical information for the conservation of one of the world's most endangered primates.

Handling Editor: Onja H. Razafindratsima

Extended author information available on the last page of the article

Fintina:

Ny fanaraha-maso akôstika pasiva, izay mampiasa fitaovana firaketana feo mandeha ho azy any anaty ala, dia manome fomba fikarohana mahery vaika, mahomby ary tsy lafo, izay ahafahana manangona singa mitohy sy tsy mandrava ny tontolo iainana. Na dia eo aza izany lanjany, dia mbola mahasadaikatra ny fampiharana azy amin'ny fikarohana momba ny primata. Nanao andrana tany anaty ala izahay momba ny fanombanana ny hakitroky ny isan'ny trangalavaka amin'ny alalan'ny akôstika pasiva izay saika nanarona ny faritra manontolo any Nosy Be amin'ny akomba a tandindomin-doza any Nosy Be (*Lepilemur tymerlachsoni*). Nampiasanay ho solon'ny halavirana radialy ny haavon'ny feo amin'ireo antso kendrena hita tao anatin'ny fandraketana feo, ho ampahany amin'ny fomba fanao mahazatra amin'ny fanisam-peo sy fanombanana araka ny elanelana. Na dia nahazahoana antso maro aza ny fikarohana akôstika pasiva (antso 9560 voarakitra tao anatin'ny ora 250), ary tsara ny vokatry ny modely faminaniana miorina amin'ny haavon'ny feo, ny valin'ny fanombanana ny hakitroka izay niantehitra tamin'ny antso tokana dia avo loatra ary tsy dia nazava tsara, izay mety ho vokatry ny fahasarotana tsy azo ihodivirana amin'ny fanombanana ny tahan'ny antso. Nihatsara ny valiny rehefa nampiasaina ho toy ny singan'ny fanadihadiana ny andian'antso fa tsy ny antso tokana, izany hoe ho "fambara". Ny fanadihadiana mitohy tamin'ny alalan'ny lalana transekta dia nanome tombana mitombina kokoa momba ny hakitroka, izay 303,7 biby iray/km2 (fetran'ny fahatokisana 95% = 189,6-486,3; tahan'ny fiovaovana = 0,18), izay mitovy amin'ny isan'ny biby amin'ny ankapobeny izay 11300. Manasongadina ny valim-pikarohanay ny hery sy fetran'ny fampiasana ny akôstika pasiva,amin'ny fikarohana primate ary manome vaovao manan-danja ho an'ny fiarovana ny iray amin'ny karazana primata itandindonin-doza indrindra maneran-tany. ***The translated abstract was not copy-edited by Springer Nature.**

Keywords Passive acoustics · Distance sampling · Cue counting · Density · Primate conservation · Madagascar

Introduction

At its most basic, primate conservation requires an understanding of where primates live and in what numbers (Rylands *et al.*, 2008, 2020), but population estimates can be difficult and time-consuming to obtain (Campbell *et al.*, 2016). To estimate population density (number of individuals per unit area) and population size or abundance (density multiplied by area), we need reliable and, where possible, non-invasive survey techniques, appropriate for the species of interest (Campbell *et al.*, 2016). Traditional survey methods are based on visual observations (e.g., line transects, nest counts) or capture (e.g., mark-recapture), where primates (or their signs) need to be seen or captured to be counted (Kühl *et al.*, 2008; Plumptre *et al.*, 2013). Many primates can be difficult to detect visually, including species that are small, solitary, nocturnal, cryptic, rare, evasive, fast-moving, or canopy-dwelling (Maisels *et al.*, 2007; Turvey *et al.*, 2017), and most do not leave signs that can readily be counted (Plumptre *et al.*, 2013). Physical trapping has obvious welfare implications and will be infeasible in most cases (Fedigan, 2010). For conspicuously vocal primates, acoustic-based surveys, such

as triangulation (Vu *et al.*, 2016), point transects (Paddock *et al.*, 2020) or lure counts (Savage *et al.*, 2010), offer a way to overcome some of the constraints of visual detection. However, all of these traditional survey methods—visual- and acoustic-based—typically require several trained observers over many weeks or months, making them time- and labor-intensive and therefore expensive. The presence of observers can also affect primate behaviour and introduce survey biases, and expose primates to habituation and disease transmission (Wallis & Lee, 1999; Bessone *et al.*, 2023). Moreover, many primates occur in locations or environments that are difficult or even dangerous for observers to access and traverse. For these reasons, survey alternatives that can be conducted remotely, and that are efficient and cost-effective, are increasingly desirable (Sugai, 2020; Piel *et al.*, 2022).

Passive acoustic monitoring presents an alternative, and potentially safe and efficient, method of density estimation. In passive acoustic monitoring, autonomous audio recorders are deployed in an area to collect acoustic data at specified schedules and often for extended periods; the audio recordings are then processed (e.g., with bioacoustics software) for sounds of interest (e.g., calls made by the target species), which can be analysed similarly to other ecological or survey data (Browning *et al.*, 2017; Sugai *et al.*, 2019). In this way, it may be possible to automate data collection in acoustic-based surveys of vocal primates. By circumventing much of the need for observer presence in the survey area—researchers need only deploy, periodically maintain, and finally retrieve the audio recorders, and possibly collect ancillary data (Pèrez-Granados & Traba, 2021)—passive acoustics can thus reduce costs, survey biases and the negative impacts of observers on primate behaviour and health (Deichmann *et al.*, 2018; Sugai *et al.*, 2019; Piel *et al.*, 2022). Because audio recorders operate autonomously, recordings can be made at times and locations where it would otherwise be challenging for observers to work (e.g., at night, inaccessible habitats). Acoustic data can also be collected continuously and simultaneously from multiple locations, facilitating long-term and large-scale monitoring (Sugai *et al.*, 2020). Together, these advantages mean that passive acoustics can ensure the continuity of data collection in the midst of pandemics or other disruptions to fieldwork (Sugai, 2020). Finally, audio recorder hardware and bioacoustics software are increasingly affordable and accessible (Hill *et al.*, 2018; Gibb *et al.*, 2019). Of course, notwithstanding the advantages of remote sensing technologies, there remain important benefits to researcher presence at some field sites, including building local capacity and community engagement, influencing policy, and providing a safeguarding effect for primates and their habitats (Laurance, 2013; Bezanson & McNamara, 2019; Semel *et al.*, 2020).

One approach to estimating population density using passive acoustics is based on conventional cue counting (Marques *et al.*, 2013). Cue counting is an indirect form of distance sampling whereby observers record radial distances to detected cues for a predetermined length of time from a series of points placed randomly in a survey area (Buckland *et al.*, 1993, 2001; Buckland, 2006; Borchers *et al.*, 2010). A cue is something produced by the animals of interest, rather than the animals themselves, and while cues may be visual (e.g., whale blows), in terrestrial surveys they

are usually aural (e.g., calls, song bouts) (Thomas & Martin, 2006; Heidi-Jørgensen & Simon, 2007). The distribution of radial distances is used to model a detection function, that is, the probability of detecting a cue given distance from the point, thereby accounting for cues missed during the surveys (Marques *et al.*, 2009, 2011). Cue counting produces an estimate of the cue density (number of cues per unit time per unit area); to estimate animal density, the cue density needs to be divided by a "multiplier"—the cue rate (mean number of cues per animal per unit time)—derived from separate fieldwork. Conceptually then, cue counting can be considered a "close cousin" to point transects, and by extension line transects, but with a temporal component (Borchers *et al.*, 2010). The novelty of the passive acoustic cue counting approach, of course, is that audio recorders fill the role of human observers, but so long as radial distances from the recorders to detected cues can be estimated and certain key assumptions are met, reliable estimates of density can be obtained using standard distance sampling analyses (Marques *et al.*, 2013), and the methods remain accessible to even inexperienced bioacousticians (Pérez-Granados *et al.*, 2021).

As with other distance sampling techniques, passive acoustic cue counting relies on several key assumptions. These are (1) the distribution of distances to cues (whether detected or not) is known (this assumption is enforced by good survey design, so that cue/animal distribution is random with respect to points), (2) cues at or near the points are detected with certainty, (3) the locations of cues are unaffected by observer presence (this should not be an issue in passive acoustic surveys), (4) distances to detected cues are measured accurately, (5) the cue rate is an unbiased estimate of the animals present during the study (ideally, the cue rate is obtained from a large, random sample monitored at representative times and locations, synchronously with the cue counting survey), and (6) the detected distances are statistically independent (cues are not independent events, because successive cues from the same animal, or cues from more than one animal in a group, may be counted; however, distance sampling is robust to such violations and this should not bias results, although greater care is needed during analysis as the data are typically overdispersed) (Buckland *et al.*, 1993, 2001, 2008; Buckland, 2006; Marques *et al.*, 2011; Warren *et al.*, 2017; Howe *et al.*, 2018). A further assumption, relevant when using passive acoustic data, is (7) false-positive detections are correctly accounted for (e.g., by quantifying the performance of automated detection and classification algorithms where used) (Marques *et al.*, 2009, 2011, 2013). Notwithstanding the many assumptions, cue counting has several advantages compared to other distance sampling techniques such as line transects and point transects: animal movement does not generate bias (a cue is instantaneous and by definition does not move), silent animals at the point do not need to be detected, cues are recorded irrespective of whether the animal was previously detected, and it is not necessary to record group size (Buckland *et al.*, 1993, 2001; Plumptre *et al.*, 2013). Survey design considerations are addressed by Buckland *et al.* (1993, 2001, 2004).

Despite its use in other taxa, to our knowledge passive acoustic cue counting (or conventional cue counting with human observers) has not been used to systematically survey primate populations for density and abundance estimation (Plumptre *et al.*, 2013). This method has been used in surveys of cetaceans (Marques *et al.*,

2009, 2011), birds (Lambert & McDonald, 2014; Sebastián-González *et al.*, 2018), and elephants (Thompson *et al.*, 2009), generating reliable density estimates at generally reduced cost and effort. In primatology, however, the application of passive acoustic technology remains atypical (Piel *et al.*, 2022), though it has been used for some limited purposes, including monitoring vocal activity (e.g., temporal patterns and responses to environmental variables) and spatially locating vocalizing animals (Spillmann *et al.*, 2015; Piel, 2018; Ferrario *et al.*, 2024). As a proof of principle, a recent study experimentally estimated the detection space (i.e., sampling radius) of audio recorders in detecting calls of pale fork-marked lemurs (*Phaner pallescens*) and used this information and cue-based methods to generate plausible density estimates (Markolf *et al.*, 2022), albeit with no replication (a single recorder was used for one night to estimate density over 0.07 km^2, potentially limiting generalizability) and using non-concurrent cue rate data (which may not be representative for the survey period).

Like several other lemur genera, sportive lemurs (family Lepilemuridae, genus *Lepilemur*) are generally considered highly vocal, making them potential candidates for acoustic-based surveys (Méndez-Cárdenas *et al.*, 2008; Markolf *et al.*, 2022; but see Mandl *et al.*, 2018). *Lepilemur* spp. are small- to medium-sized, nocturnal, exclusively arboreal folivores, with 25 extant species distributed across Madagascar (Mittermeier *et al.*, 2023; Wilmet *et al.*, 2014). They are mostly solitary or, in some cases, pair-living (Wilmet *et al.*, 2014). Our study focuses on the Nosy Be or Hawks' sportive lemur (*L. tymerlachsoni*), which is endemic to the island of Nosy Be in north-western Madagascar. Its range encompasses Lokobe National Park and extends across the primary, secondary, and degraded forests and plantations of the wider Lokobe region (Sawyer *et al.*, 2015; Louis Jr *et al.*, 2020; Tinsman *et al.*, 2022). It is listed as Critically Endangered on the IUCN Red List owing to habitat loss and degradation from agricultural expansion, and from unsustainable levels of hunting (Louis Jr *et al.*, 2020). Population data for *L. tymerlachsoni* include small-scale density estimates (Bederu, 2014), as well as more recent encounter rate estimates (Tinsman *et al.*, 2022).

Here, we field test the reliability of passive acoustic cue counting in a near range-wide survey of *Lepilemur tymerlachsoni*. We applied a protocol developed by Sebastián-González *et al.* (2018) that uses the sound level of cues detected in audio recordings to estimate radial distance. The sound level of an acoustic signal, typically measured in decibels (dB) using bioacoustics software, is a measure of its energy at the point it reaches the recorder's microphone (Yip *et al.*, 2020). Sound levels show a predictable logarithmic decline with increasing distance from the source (Wiley & Richards, 1982). The protocol models this relationship between sound level and distance using a calibration dataset of target calls recorded at known distances, while accounting for the effects of weather variables on call transmission; a regression equation is then used in the cue counting survey to predict the radial distances of detected cues and hence the probability of detection (see Yip *et al.*, 2020 for discussion of alternative methods for developing calibration datasets). Sound-level-based distance prediction has been shown to be accessible, reliable and objective in both the field (Lambert & McDonald, 2014; Sebastián-González *et al.*, 2018; Graf & Hatlauf, 2021) and by simulation

(Yip *et al.*, 2020). To evaluate the reliability of our bioacoustic results, we also carried out concurrent line transect surveys of *L. tymerlachsoni* in the same survey area and compare our passive acoustic cue counting density estimates with those derived from the line transect surveys.

Methods

Study Site

The island of Nosy Be (321 km^2) is located in the Mozambique Channel, northwest of mainland Madagascar. It is part of the Sambirano Domain, which represents a transition zone between Madagascar's western dry deciduous forests and eastern rainforests (Goodman & Benstead, 2002). Nosy Be has a tropical climate, with a hot, wet season from approximately November–April and a cooler dry season from May–October (Macron *et al.*, 2016). Mean daily temperatures range from 23 °C in July–August to 28 °C in January–February (Birkinshaw, 1995). Mean annual rainfall is 2,250 mm (Andreone *et al.*, 2005). Lokobe National Park (7.4 km^2 terrestrial area), located in the southeast of the island, protects most of Nosy Be's remaining primary humid evergreen forest (Hasiniaina *et al.*, 2018). A mosaic of secondary and degraded forests, agricultural crops (e.g., ylang-ylang, rice) and small villages border the national park (Birkinshaw, 1995). Nosy Be exhibits a strong elevational gradient (0–430 m), with the primary forests of Lokobe occupying some of the highest altitudes (Tinsman *et al.*, 2022). The black lemur (*Eulemur macaco*) and Claire's mouse lemur (*Microcebus mamiratra*) are the other lemurs present on the island, which has been designated a priority area for lemur conservation (Schwitzer *et al.*, 2013, 2014).

Cue Definition

The definition of a cue is ultimately defined by the researcher, but it must be applied consistently between the cue rate estimation and the cue counting survey (Thomas & Martin, 2006). We used the 'ouah' and 'tchen-tchen' call types as the basis for our cues. The ouah is a short, broadband, single-element call, with a characteristic inverse u-shaped harmonic onset and downward frequency sweep (Fig. 1A.; Méndez-Cárdenas *et al.*, 2008; Seiler *et al.*, 2015; Mandl *et al.*, 2019). Ouah calls are considered common and sometimes form bouts (i.e., an extended series of calls), with each call in a bout typically separated by a few seconds (unpublished data; Mandl *et al.*, 2019). The tchen-tchen call is less common and structurally comprises two ouah calls given in quick succession (Fig. 1B.; Mandl *et al.*, 2019). While sex-based differences in the vocal repertoire and vocal activity have been observed in other *Lepilemur* species (Rasoloharijaona *et al.*, 2006; Mandl *et al.*, 2019), it is currently unknown whether age or sex influences the production of these specific call types in *L. tymerlachsoni*. Importantly, however, population estimates should remain unbiased provided that our estimated cue rate reflects the average calling behaviour of the population. By

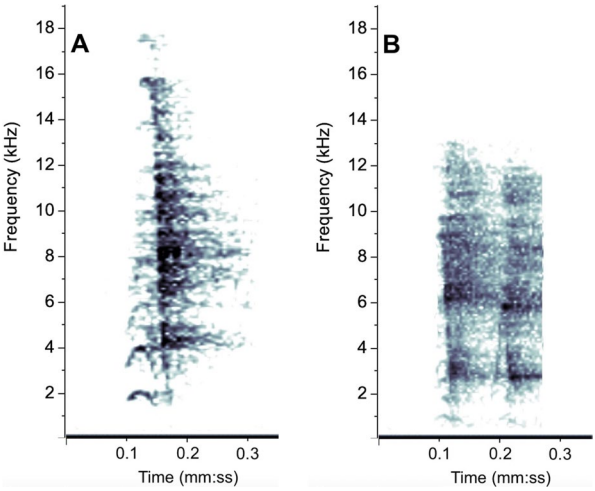

Fig. 1 Representative spectrograms of ouah (**A**) and tchen-tchen (**B**) calls of *Lepilemur tymerlachsoni*. Spectrograms were created in Raven Pro 1.6.4 (Hann window = 512 samples, discrete Fourier transform size = 512, hop size = 256 samples, grid spacing = 86.1 Hz, overlap = 50%) from recordings collected in the Lokobe region of Nosy Be, Madagascar, February–April 2023.

estimating this rate from a large, random sample of individuals, including those not calling during the sampling period, we inherently account for any differences in calling behaviour across age and sex classes (Marques *et al.*, 2013).

We primarily defined cues as single calls:

1) Ouah calls, including each call in an ouah bout, and
2) Tchen-tchen calls (two cues).

We also performed additional analyses using bouts as cues, representing an alternative unit of analysis. This approach is similar to that used in acoustic surveys of *Phaner pallescens* (Markolf *et al.*, 2022), whose calls also exhibit a bout structure. We defined bouts as a series of one or more ouah or tchen-tchen calls, where each call is separated by less than 10 s from the preceding call. For example, if a lemur produced a series of 20 ouah calls each separated by <3 s, stopped calling for 1 min, then produced another series of 15 ouah calls each separated by <3 s, then stopped calling altogether, we would count this as two bouts (and therefore two cues in the alternative analysis), whereas in the original analysis we would count this as 35 cues; if a lemur produced a single ouah call, then stopped calling altogether, we would count this as one bout (and therefore one cue, as in the original analysis). The 10-s threshold for defining bouts, although somewhat subjective, was informed by preliminary observations of *Lepilemur tymerlachsoni* calling patterns and the approach of Markolf *et al.* (2022). We did not distinguish whether we included the calls of one or more individuals in bouts, as this is difficult to ascertain. Thus, bouts might contain calls from more than one individual.

The ouah call can show some gradation with another call type, the 'chuck' call (Mandl *et al.*, 2019). However, ouah and chuck calls are usually straightforward to discriminate by ear and in spectrograms: ouahs by their harmonic onset, downward frequency sweep and higher maximum frequency; and chucks by their noisier structure, more upward frequency sweep, lower maximum frequency and tendency to be

compounded to form chuckle calls (unpublished data; Mandl *et al.*, 2019). We maintained a reference library of spectrogram images to promote consistency in identifying calls and their graded intermediaries in both the cue rate estimation and cue counting survey (Thomas & Martin, 2006). We excluded from our analyses all calls that we could not identify after aural and visual inspection of spectrograms (e.g., poor quality or obscured calls), taking into account the context.

Survey Design

We used the survey design feature in the software program Distance 7.5 release 2 (Thomas *et al.*, 2010) to create a systematic random layout for placing our line transects and audio recorders. First, we created a range map for *Lepilemur tymerlachsoni* using spatial data downloaded from the IUCN website (Louis Jr *et al.*, 2020), across which we superimposed a grid of points with a random starting position. We then extracted GPS coordinates of these points and used them to place 16 segmented parallel transect lines in the survey area (Fig. 2). We cut the transects in north-south straight lines, deviating only where necessary to avoid dangerous or inaccessible areas (e.g., ravines). We measured the transects with an open reel tape measure (mean transect length: 815 m, range: 633–1,125 m) and marked the centrelines with fluorescent flagging tape. The survey area included a variety of habitats, as well as both protected (national park) and unprotected areas (e.g., those managed by the village-based association *Vondron'Olona Ifotony* [VOI]). We abandoned attempts to place transects in the southern part of the national park as the rugged terrain made it too difficult to cut straight transects and raised safety concerns for nocturnal work.

We deployed ten autonomous audio recorders (Wildlife Acoustics Inc., Song Meter SM4) approximately evenly across the survey area (Fig. 2). We placed the recorders in suitable habitat *c.* 500 m along transects and away from potential sources of acoustic interference (e.g., rivers) (Browning *et al.*, 2017). We mounted the recorders to trees at a height of 2 m above ground. *Lepilemur tymerlachsoni* occupies a mean canopy height of 7.2 m and is infrequently observed above 10 m (Sawyer *et al.*, 2015; Tinsman *et al.*, 2022); the height of the recorders therefore represents a balance between the likely height of the target species and the physical constraints associated with deploying, maintaining and retrieving the recorders. We classified the vegetation in the vicinity of each recorder into one of three habitat classes:

1) Primary forest;
2) Secondary/degraded forest; and
3) Plantation, broadly following those described by Tinsman *et al.* (2022).

Data Collection

Data collection involved passive acoustic recordings, measurements of climatic variables (wind speed, rainfall), focal follows (to collect ancillary data necessary for the cue

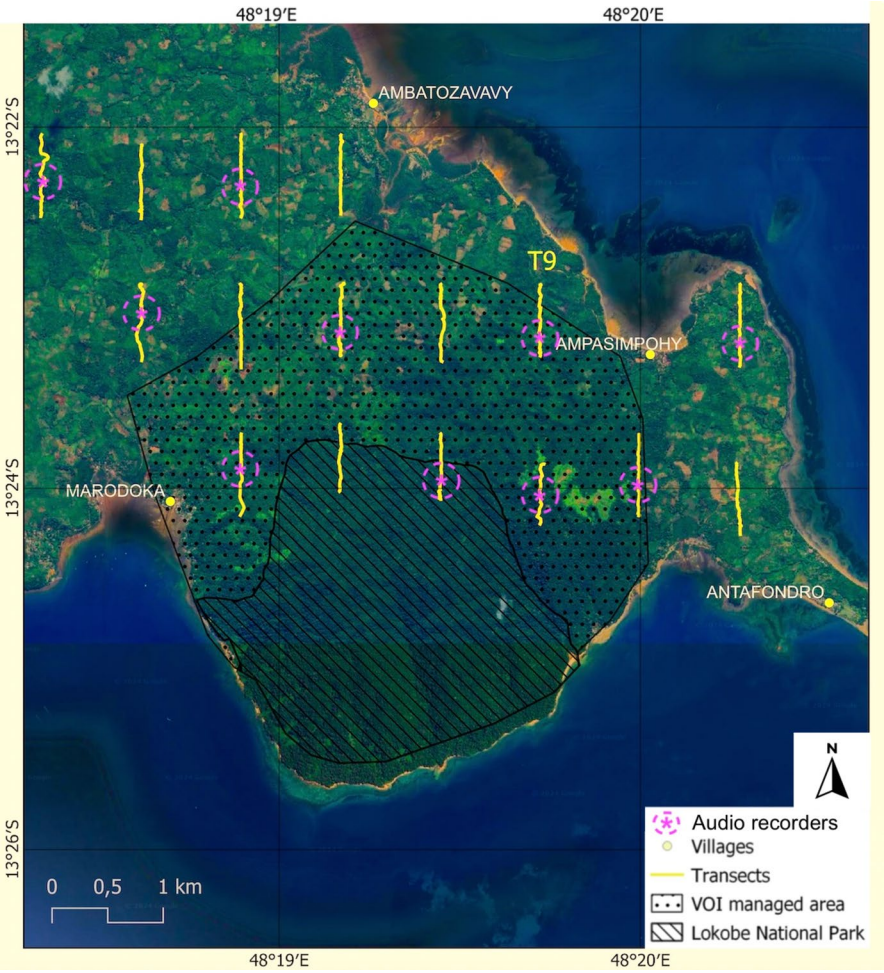

Fig. 2 Study site in the Lokobe region of Nosy Be, Madagascar, showing the systematic random layout of the line transects and the deployment locations of the ten audio recorders used for surveys of *Lepilemur tymerlachsoni* from February–April 2023. The VOI (Vondron'Olona Ifotony) is a Malagasy village-based association for forest management.

counting survey) and line transect surveys (to generate comparator density estimates for evaluating the passive acoustic cue counting density estimates). A total of three teams collected data, and on most nights at least two teams were active, conducting focal follows and line transect surveys in parallel and on different transects. Observers regularly rotated between teams and transects to minimize between-team variability (Peres, 1999). At the beginning of the field season in January 2023, LDM trained all observers on distance sampling theory (e.g., survey design, key assumptions), field protocols (e.g., equipment use, searching behaviour), and lemur identification (e.g., visual cues, call types). All sampling commenced a minimum of 72 hr after cutting the transects to minimize any effects of disturbance (Bessone *et al.*, 2023).

Passive Acoustic Cue Counting Survey – Audio Recordings

The ten audio recorders collected acoustic data for 4 to 5 hr per night from 1 February–5 April 2023, although the duration of the deployments varied (mean: 54 days, range 24–63 days). We initially set the recorders to record continuously each night for 4 hr, starting at astronomical twilight, but we later extended this to 5 hr for some recorders, starting 1 hr earlier, to capture earlier calling activity observed postdeployment (logistical constraints prevented this change to all recorders). These recording schedules coincide with the time and season (wet) when *Lepilemur* spp. are known to be most active, including vocally (Kappeler, 1997; Warren & Crompton, 1997; Müller *et al.*, 2000; Méndez-Cárdenas *et al.*, 2008; Mahaboubi *et al.*, 2015; Mandl *et al.*, 2019). The cue rate did not differ between 4 and 5 hr recordings, so we included recordings from both in our analyses. Song Meter SM4 audio recorders use two built-in omnidirectional microphones, and we recorded stereo uncompressed (.wav) 1-hr sound files. We set the sample rate to 44.1 kHz, gain to 16 dB, preamplifier gain to 26 dB, and we used a 220 Hz high-pass filter.

Wind and Rain Data

Climatic variables, such as rain and wind, may affect sound transmission, cue detection, and calling activity, and should be accounted for when using passive acoustics to estimate density (Bibby *et al.*, 1998; Sebastián-González *et al.*, 2018). We placed a digital rain gauge (Digitech, IC-XC0430) proximate to the survey area and paired hourly rainfall measurements with the audio recorders' 1-hr recording periods. We classified total rain for each 1-hr recording period into one of three classes:

1) None/light <1 mm/hr;
2) Moderate 1–4 mm/hr; and
3) Heavy >4 mm/hr.

We defined these classes to also allow straightforward classification in the field during focal follows (Sebastián-González *et al.*, 2018). We excluded from our analyses all passive acoustic cue count and focal follow data collected during heavy rain recording periods (*c.* 7% of all data), because heavy rain obscures most of the sounds in audio recordings. Observers measured the wind speed (km/hr) with an anemometer (Kestrel, 1000) at the start and end of each focal follow, categorizing wind speed into one of three classes:

1) None/light <15 km/hr;
2) Moderate 15–30 km/hr; and
3) Strong >30 km/hr.

However, wind speed was almost always negligible (<1 km/hr), so we did not exclude data based on wind speed nor measure its effects on the cue rate or sound-level-based distance prediction.

Focal Follows

From 17 February–31 March 2023, for six nights per week, we conducted focal follows of lemurs to simultaneously collect both the cue rate data and the calibration dataset for modelling the relationship between the sound level and distance of cues. Each night, commencing 1 hr prior to astronomical twilight, teams of two to three observers walked one to two transect lines, using headlamps to locate lemurs by reflective eye shine (from the *tapetum lucidum*). Where practicable, we used the low and red-light settings to minimize disturbance (Andriantsaralaza & Clarke, 2023). To the extent possible, we only located lemurs visually, and not aurally, so as not to bias the sample with louder or more vocally active focal individuals. For example, we kept to the transect line, maintained a consistent walking speed and searching behaviour and did not actively seek out vocalizing lemurs.

Once we located a lemur, we began a focal follow (Altmann, 1974). Occasionally, we located groups of up to four lemurs, in which case we included all readily visible individuals in the group as focals. This was feasible because *Lepilemur* spp. are often stationary when observed (Meyler *et al.*, 2012; Martin *et al.*, 2021) and because we recorded the focal follows with handheld audio recorders, allowing commentary on more than one individual and subsequent review and verification of the data. At the start of a focal follow, we recorded the time, number of focal individuals, habitat class, wind class and rain class. An observer would then start recording with a handheld Song Meter SM4 audio recorder, holding it at chest height with the front of the recorder oriented towards the focal individual/s, ensuring the focal individual/s remained roughly the same distance to its two side-mounted microphones. These handheld audio recorders used the same settings (sample rate, gain, preamplifier gain, and high-pass filter) as the unattended Song Meter SM4 recorders used for the passive acoustic cue counting survey. Observers remained quiet and, for the most part, stationary throughout the focal follows, and the lemurs appeared unaffected by observer presence. During a focal follow, we counted the number of ouah and tchen-tchen calls produced by each focal individual, and for each of these calls we measured the radial distance in meters from the handheld audio recorder to the vocalizing lemur with a laser rangefinder and verbally annotated this distance in the audio recording. To increase the range of distances recorded, observers variously approached and moved away from vocalizing focal individuals where possible. Where nonfocal lemurs were nearby and we heard them vocalizing, we verbally annotated their calls as 'nonfocal' to distinguish them in the audio recordings. We stopped a focal follow after 20 min, or sooner if we lost sight of the focal individual. At the end of a focal follow, we recorded the time, duration (to the nearest second), whether the wind or rain classes had changed and, if so, the predominant wind and rain classes, and then stopped the audio recording. Observers would then resume walking the transect line until we located a different lemur and began a new focal follow. This procedure continued until 4 hr after astronomical twilight, or until observers reached the end of the transect.

While the height of vocalizing animals may affect sound-level-based distance prediction, we did not record the vertical height of focal lemurs, nor test for its effects in our distance prediction modelling, because we considered such effects to likely be

minimal given the habitat use of *Lepilemur tymerlachsoni* (Alldredge *et al.*, 2010; Sawyer *et al.*, 2015; Tinsman *et al.*, 2022) and because the vertical height of calling animals in passive acoustic recordings will in most cases be unknown (Yip *et al.*, 2020). We randomized the order in which we sampled transects and the end of the transect we started from. In total, we sampled each transect between one and five times for the focal follows, with a mean sampling frequency of approximately 0.5 times per week per transect. We did not sample one transect owing to safety concerns (see T9, Fig. 2).

Line Transect Surveys

We conducted line transect surveys from 17 February–4 April 2023, six nights per week. Each night, commencing at astronomical twilight, teams of two to three observers slowly and quietly walked one to two transect lines at 1–2 km/hr. Observers used headlamps to visually locate lemurs by reflective eye shine, and aurally by their calls. We used binoculars to distinguish *Lepilemur tymerlachsoni* from other wildlife as required. Observers focussed search effort on and near the line, while stopping regularly to scan the survey area. We paused surveys during heavy rain. For each confirmed visual detection, we measured the perpendicular distance from the transect line to the lemur with a laser rangefinder and paced distances <4 m (the minimum range of the rangefinder). We recorded all detections individually, regardless of group size (e.g., if we located a group of three lemurs, we recorded three individual detections and measured perpendicular distances for each; Buckland *et al.*, 2001). We randomized the order in which we sampled transects. In total, we sampled each transect between two and five times for the line transect surveys (excluding transect T9), with a mean sampling frequency again of approximately 0.5 times per week per transect for this method.

Data Analysis

Sound File Calibration

To allow absolute measures of sound level in our analyses, we calibrated our sound files using the "end-to-end" approach and a signal of known sound pressure (https://www.ravensoundsoftware.com/knowledge-base/calibrating-recordings-in-raven-pro/). At deployment and retrieval for each of the ten audio recorders, we broadcast a 1-kHz tone towards the mounted recorder from a speaker (JBL, Clip 4) at 2-m height and horizontal distance and measured the sound level three times at each microphone using a handheld digital sound level meter (Digitech, QM-1589). We took the mean of the three measurements (A-weighted, fast response) to calibrate the sound files in Raven Pro 1.6.4 (K. Lisa Yang Center for Conservation Bioacoustics, 2023). This same calibration approach was applied to the sound files from the handheld audio recorders used for focal follows at the beginning and end of the field season.

Passive Acoustic Cue Counting Survey – Processing Audio Recordings

We processed the unattended audio recordings by manually scanning spectrograms for ouah and tchen-tchen calls. These calls were the acoustic units we used to define both single-call cues (for the primary analysis) and bout cues (for the alternative analysis). We considered manual call detection more appropriate than automated detection given our research goal was to field test the reliability of passive acoustic cue counting and not to quantify or exploit its efficiency or cost-effectiveness, and because of the generally superior performance of manual detection (Swiston & Mennill, 2009; Digby *et al.*, 2013; Truskinger *et al.*, 2013; Rocha *et al.*, 2015). To keep manual call detection feasible, we analysed a random subsample of 250 hr from the >2,400 total hours of audio recordings. For each of the ten audio recorders, we randomly selected 25 1-hr sound files, excluding those with predominant rain class heavy. We first calibrated each sound file in Raven Pro. We then aurally and visually scanned spectrograms of the left microphone (channel 1) recordings for these calls. For each detected call, we made a selection (i.e., a bounding box defining start and end times and lower and upper frequencies of the signal) and extracted the peak power density (dB FS/Hz) as a measure of the call's sound level. Where calls were obscured by nontarget signals that could affect the peak power density measurement, we drew the selection box over the unobscured part of the call with the highest peak power density. If it was not possible to isolate an unobscured part of the call for measurement, we excluded such calls from further analysis. We also annotated each selection with the habitat class in which we recorded the call. LDM analysed all sound files using the same spectrogram configuration (Hann window = 512 samples, discrete Fourier transform size = 512, hop size = 256 samples, grid spacing = 86.1 Hz, overlap = 50%) and comparable display settings, including spectrogram brightness, contrast and colour scheme. Following this procedure, we had a list of detected calls and their peak power density measurements for each subsampled 1-hr recording. This list then served as the basis for counting and estimating radial distances for single-call cues (our primary density estimation analysis) and bout cues (our alternative analysis).

Processing Focal Follow Audio Recordings

We reviewed each of the focal follow sound files by aural and visual inspection of spectrograms of the left microphone recordings in Raven Pro. First, we calibrated each sound file. We then identified and counted all ouah and tchen-tchen calls and bouts in the recording produced by the focal individual and made selections for each call. We used the count from the review if there was a discrepancy with the number of calls counted in the field. We annotated each selection with the distance to the vocalizing individual, the habitat class, the predominant wind and rain classes and extracted the peak power density measurement. We also noted whether other sounds (e.g., insect noise) partly obscured the selection and whether the call had overloaded the recording.

Cue Rate and Bout Rate Estimation

We estimated the mean number of single-call cues per lemur per minute (the "single-call cue rate") in two ways. First, we estimated it as the weighted mean of the single-call cue rates per focal follow, weighted by focal follow duration, with the variance calculated by using Cochran's approximation (Gatz & Smith, 1995). Second, we estimated it by using regression models and examined the effects of time of night and rain class on the rate. For this, we built generalized linear mixed models (GLMMs) in R version 4.0.0 (R Core Team, 2024) using the package *glmmTMB* (Brooks *et al.*, 2017). The null model used the number of single-call cues as the response variable, the logarithm of the decimal duration of the focal follows as an offset variable and focal follow ID as a random effect to account for individual variation. We also included recording period (5 levels representing consecutive 1-hr periods commencing 1 hr prior to astronomical twilight) and rain class (2 levels representing none/light and moderate) as categorical predictor variables in alternative models. We tested for multicollinearity between the categorical predictors by calculating variance inflation factor (VIF) values in the package *performance* (Lüdecke *et al.*, 2021) and found low correlation (VIF = 1.63), indicating that both could be retained in the models. Candidate GLMMs modelled the response using Poisson and negative-binomial (linear and quadratic parameterization) distributions and log link functions, both with and without zero-inflation. We based model selection on the lowest Akaike Information Criterion (AIC) values (Akaike, 1973) and inspection of mean-variance plots in the package *EcoCountHelper* (Cole *et al.*, 2022). We assessed goodness-of-fit with QQ-plots, nonparametric dispersion tests, outlier tests and zero-inflation tests of the simulated residuals using the package *DHARMa* (Hartig, 2022). We then calculated the estimated marginal mean of the single-call cue rate for the selected model with the package *emmeans* (Lenth, 2024). We also calculated the estimated marginal means of the single-call cue rate for each predictor variable in the best fitting model with both categorical predictors and performed post-hoc pairwise comparisons to determine any differences in the single-call cue rate between recording periods and rain classes.

We estimated the mean number of bout cues per lemur per minute (the "bout cue rate") as the weighted mean of the bout cue rates per focal follow, weighted by focal follow duration, using Cochran's approximation to estimate the variance.

Estimating Distances to Cues Using Sound Levels

We fit GLMMs in *glmmTMB* to model the relationship between the absolute sound level and distance of cues. We only used high-quality call selections in the analysis, omitting those that were obscured or overloaded. The null model used known radial distance as the response variable, sound level (peak power density) as the predictor variable and focal follow ID as a random effect to account for individual variation. We also included habitat class (3 levels representing primary forest, secondary/degraded forest, and plantation) and rain class as covariates in alternative models. Candidate GLMMs modelled the response with a Gaussian distribution and log link function. We selected the top model based on the lowest AIC values. We used simple

univariate linear regression to examine the extent to which the top model could predict radial distances for the set of known radial distances obtained from the focal follows. Following Yip *et al.* (2020), we calculated the distance estimation error (the absolute value of the difference between predicted distance and known distance) and the error bias (predicted distance minus known distance) for the top model and tested whether they differed from zero using one-sample *t*-tests. Additionally, we calculated the root mean square error. Finally, we used the package *ggeffects* (Lüdecke, 2018) to visualize the relationship between sound level and predicted radial distance and to estimate radial distance for each detected single-call cue based on its peak power density measurement as part of the density estimation process. For the additional analyses based on bouts, we estimated a single radial distance for each bout cue using the mean peak power density measurement of the call/s comprising the bout.

Passive Acoustic Density Estimation

We estimated density using the conventional distance sampling engine in program Distance (Thomas *et al.*, 2010). We pooled the detections for each point (audio recorder) and entered search time for all points as 1,500 (25 x 60-min subsampled recordings). We explored the dataset by plotting histograms of the radial distances, before grouping the distance data for analysis (a requirement under the adjusted AIC [QAIC] procedure below). We used left truncation at 5 m, because we could not reliably measure sound levels very close to the recorders owing to overloading (Marques, 2016), as well as right truncation of the largest 10% of distances (Buckland *et al.*, 2001; Thomas *et al.*, 2010). We then fit the following key functions and, to improve the flexibility and fit of the detection function, series adjustment combinations: uniform key function with 1, 2, and 3 cosine adjustments, half-normal key function with 0, 1, and 2 Hermite polynomial adjustments, and hazard-rate key function with 0, 1, and 2 cosine adjustments (Howe *et al.*, 2018). We selected from the candidate models using QAIC and the two-step model selection procedure outlined by Howe *et al.* (2018). The QAIC adjusts for overdispersed distance data, which are characteristic of cue counting surveys and which otherwise make goodness-of-fit tests invalid and cause AIC to select overly complex models. We calculated the variance of the density estimate using the delta method. We applied this density estimation procedure separately to both the analyses based on single-call cues and bout cues.

Line Transect Density Estimation

We again estimated density using the conventional distance sampling engine in program Distance (Thomas *et al.*, 2010). We pooled the visual detections for each transect line and entered effort as the line length multiplied by the number of times the line was surveyed (Buckland *et al.*, 2008). To better understand the dataset, we plotted histograms of the perpendicular distances with many cut points before grouping the distance data and right-truncating the largest 10% of distances for analysis. We then fit the following recommended key functions and series adjustment combinations: uniform key function with cosine series expansion, half-normal key function with cosine

and Hermite polynomial series expansion, and hazard-rate key function with simple polynomial series expansion (Buckland *et al.*, 2001; Thomas *et al.*, 2010). We used poststratification (i.e., grouping adjacent transects into strata) to reduce bias in the encounter rate variance estimation, which was appropriate given our systematic design (Fewster *et al.*, 2009). We selected from the candidate models by visually assessing model fit, chi-square goodness of fit tests and the lowest AIC values.

We determined statistical significance for all analyses at the $\alpha = 0.05$ level.

Ethical Note

This research was approved by the Australian National University's Animal Experimentation Ethics Committee (#A2022/30) and carried out in accordance with applicable national laws of Madagascar and Australia. Research permission was obtained from the Ministère de l'Environnement et du Développement Durable of Madagascar (#346/22/MEDD/SG/DGGE/DAPRNE/SCBE.Re). We consulted the International Primatological Society's Code of Best Practices for Field Primatology when developing and implementing the research. Our methods are noninvasive, and no lemurs were handled during the study. The authors declare that they have no conflict of interest.

Data Availability Statement The datasets generated and analysed during the current study contain sensitive location data for threatened species and raw audio recordings with potential privacy implications. Therefore, not all data are publicly available. However, subsets of the data, which exclude sensitive information, are publicly available in the Australian National University Data Commons.

Results

Cue Rate and Bout Rate

We calculated the single-call cue rate and bout cue rate using data from all focal follows, excluding heavy rain periods (focal follows = 390, total single-call cues = 1,982, total bout cues = 299, total observation time = 103 hr, focal follows with no cues = 358). The weighted mean single-call cue rate was 0.35 cues/min (standard error [SE] = 0.23). The GLMM-based mean single-call cue rate was also 0.35 cues/min (SE = 0.14). The selected GLMM was the null, which modelled the response with a negative-binomial distribution (quadratic parameterization) and log link function, to account for overdispersion common in count data, and without zero-inflation. Its baseline log rate (the intercept) was -1.0633 (± 0.418, $z = -2.54$, $P = 0.011$), which when exponentiated ($\exp(-1.0633) = 0.35$) gives the mean single-call cue rate. To account for individual variation, we included focal follow ID as a random effect, which showed a variance of 2.373e-08 (standard deviation [SD] = 0.000154). Pairwise comparisons of the single-call cue rate between recording periods and rain classes were not significant ($P \geq 0.24$). The weighted mean bout cue rate was 0.05 bouts/min (SE = 0.004).

Sound-Level-Based Distance Prediction

The calibration dataset comprised 1,463 high quality calls, which exceeded the sample size of 300 calls recommended by Yip *et al.* (2020) to minimize estimation error. The selected GLMM included sound level as the sole predictor variable (Table I). The sound level of calls was significantly associated with the radial distance of vocalizing lemurs: as this sound level increases, the predicted distances to vocalizing lemurs decrease (Table I; Fig. 3). The known radial distance of calls recorded during the focal follows was significantly associated with the predicted radial distance (Fig. 4; $F_{1, 1461} = 4{,}958$, $P < 0.001$, $R^2 = 0.77$). Distance estimation error was 2.43 m (95% confidence interval [CI] = 2.3–2.57), which was significantly greater

Table I Summary of the selected GLMM used to predict radial distances to *Lepilemur tymerlachsoni* cues based on sound levels.

Random effect (focal follow ID)	Fixed effects	Coefficient (± *SE*)	z-value	*P*
Variance = 0.1785 SD = 0.4225	(Intercept)	4.44 (± 0.11)	40.46	<0.001
	Sound level (peak power density)	−0.04 (± 0.001)	−31.89	<0.001

The GLMM modelled the response with a Gaussian distribution and log link function. Data were collected in the Lokobe region of Nosy Be, Madagascar, February–April 2023

SD = standard deviation

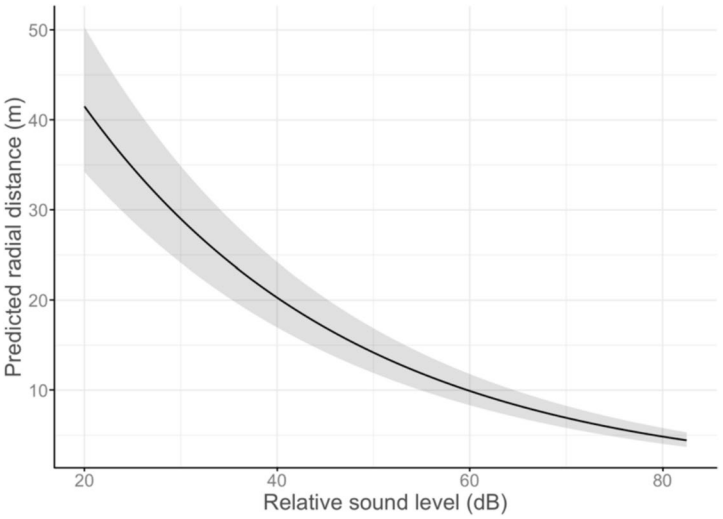

Fig. 3 Modelled relationship used to predict unknown radial distances to *Lepilemur tymerlachsoni* cues detected from the passive acoustic subsample based on sound levels. Data were collected in the Lokobe region of Nosy Be, Madagascar, February–April 2023. The minimum known distance used in the model was 3 m.

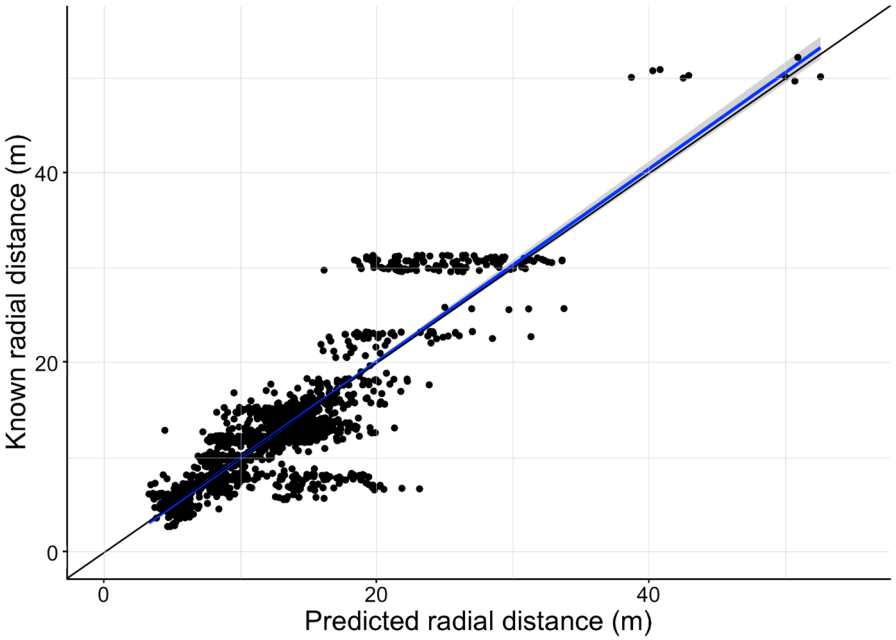

Fig. 4 Plot of known radial distances of *Lepilemur tymerlachsoni* calls recorded during focal follows in the Lokobe region of Nosy Be, Madagascar, February–April 2023, versus the radial distances predicted by the top model based on sound levels. The regression line is shown with a blue line and the 1:1 relationship with a black line.

than zero ($t = 35.69$, DF = 1,462, $P < 0.001$). The error bias was −0.02 m (95% CI = −0.21 to 0.16), which was not significantly different from zero ($t = -0.24$, DF = 1,462, $P = 0.81$). The root mean square error was 3.56 m.

Density Estimates

For the passive acoustic cue counting survey based on single calls, we detected a total of 9,560 single-call cues from 250 hr of search time (the random subsample). Of these, 8,762 cues entered the analysis after left and right truncation. The Distance analyses generated a density estimate of 2,752 individuals/km^2 (weighted mean single-call cue rate 95% CI = 822.7–9,205.4, coefficient of variation [CV] = 0.68, GLMM-based mean single-call cue rate 95% CI = 1,214.3–6,237, CV = 0.44). The half-normal key function with 2 Hermite polynomial adjustments was selected by QAIC and Howe *et al.* (2018)'s two-step procedure (Fig. 5A and B; detection probability = 0.31, effective detection radius = 13.89). The contributions of the detection probability, encounter rate and single-call cue rate to the overall variance in the density estimate were 0.6%, 5.7%, and 93.6% when using the weighted mean single-call cue rate and 1.5%, 13.9%, and 84.5% when using the GLMM-based mean single-call cue rate.

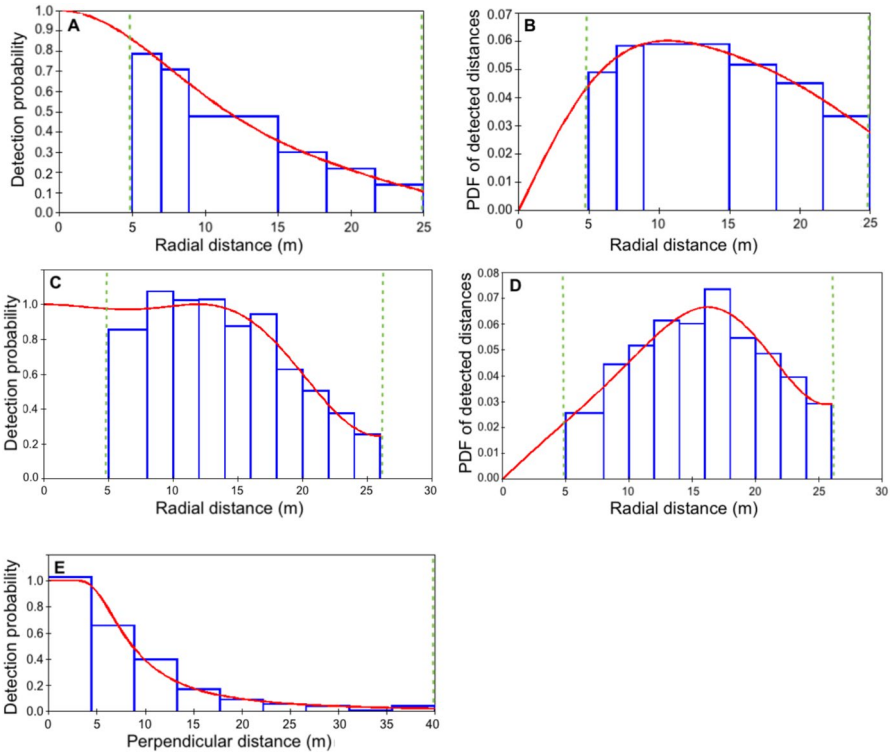

Fig. 5 Detection probabilities and probability density functions (PDF) from passive acoustic cue counting and line transect distance sampling surveys of *Lepilemur tymerlachsoni* conducted in the Lokobe region of Nosy Be, Madagascar, February–April 2023. (**A**) The detection probability as a function of radial distance, and (**B**) the PDF of detected distances, for the passive acoustic cue counting data based on single calls. (**C**) The detection probability as a function of radial distance, and (**D**) the PDF of detected distances, for the passive acoustic cue counting data based on bouts. (**E**) The detection probability as a function of perpendicular distance for the line transect data. The blue columns represent grouped detections (aural for the passive acoustic cue count, and visual for the line transects), the red curved lines represent the fitted models and the green vertical dashed lines represent the left (cue count only) and right truncation distances.

For the passive acoustic cue counting survey based on bouts, we detected a total of 916 bout cues from the same 250 hr of search time. Of these, 825 bouts entered the analysis after left and right truncation. The density estimate was 802 individuals/km^2 (95% CI = 442.2–1,453.3, CV = 0.31). The uniform key function with 3 cosine adjustments was selected by QAIC (Fig. 5C and 5; detection probability = 0.65, effective detection radius = 20.9). The contributions of the detection probability, encounter rate and bout cue rate to the overall variance in the density estimate were 93.2%, 0.1%, and 6.6%.

For the line transect surveys, we recorded a total of 395 visual detections from 46.5 km of total survey effort, which exceeded the recommended minimum sample size of 60–80 detections (Buckland *et al.*, 2001). After right truncation, 356 detections entered the analysis. The Distance analyses generated a density estimate of 303.7

individuals/km^2 (95% CI = 189.6–486.3, CV = 0.18). The hazard-rate key function with simple polynomial adjustment provided the best fit to the data (Fig. 5E; χ^2 = 7.7, DF = 6, P = 0.26, detection probability = 0.28, effective strip width = 11.03).

Discussion

This study found that passive acoustic cue counting, especially when using single calls as cues, likely overestimated the true density of *Lepilemur tymerlachsoni*, while the precision of the estimates was poor. We attribute this to the inherent challenges of estimating the cue rate. While passive acoustic cue counting shows promise for surveying vocal primates, further methodological refinements are needed for its successful application to this species. We propose that more reliable density estimates could be obtained through the use of animal-borne acoustic recording tags, which would control for potential observer effects and any calling suppression during focal follows and thus improve cue rate estimation, and by treating call bouts rather than single calls as the cues used in analysis.

Evaluation of Passive Acoustic Cue Counting for *Lepilemur tymerlachsoni*

Our line transect survey design and implementation followed best practice and yielded population estimates comparable with those reported previously and for other *Lepilemur* species. Specifically, we integrated sufficient randomization and replication, the survey effort and sample size were large, and our field protocols ensured the key assumptions of line transect distance sampling were met (Buckland *et al.*, 2001, 2010; Kühl *et al.*, 2008). Moreover, the distance data appear well-behaved (i.e., detection probability declines steadily with distance, and no evidence of heaping, evasive movement, or outliers; Buckland *et al.*, 2001; Fig. 5E) and the density estimate is made with reasonable precision (18% CV). The line transect density estimate (303.7 individuals/km^2) is broadly consistent with those of other sources from across northern and north-western Madagascar (Table II). Importantly, our raw encounter rate (8.5 individuals/km) is also consistent with the 7.8 *Lepilemur tymerlachsoni* individuals/km reported by Tinsman *et al.* (2022) following comprehensive reconnaissance walks of Nosy Be. While Bederu (2014)'s preliminary density estimate for *L. tymerlachsoni* (63/km^2) is much lower than ours, that survey comprised just four, non-random transects (along trails) in a small area of Lokobe and as such may be biased and/or non-representative of the broader region (Buckland *et al.*, 2010; de Andrade *et al.*, 2019). All in all, we consider our line transect density estimate to be reliable and thus suitable for evaluating the reliability of the passive acoustic cue counting density estimates.

Ideally, field tests of emerging survey methods like passive acoustic cue counting would be evaluated against populations where true density is known. Here, in the absence of known density, we are comparing *estimates* with *estimates* and therefore cannot say for certain whether either method is accurate. This raises the possibility

Table II Nonexhaustive list of published population densities of *Lepilemur* spp. from northern and north-western Madagascar.

Scientific name	Common name	Population density (N/km^2)	Location	Survey method	Reference
Lepilemur sahamalaza	Sahamalaza sportive lemur	154	Sahamalaza Peninsula	Line transect distance sampling	Hending *et al.* 2024
Lepilemur milanoii	Daraina sportive lemur	157	Loky-Manambato region	Line transect distance sampling	Salmona *et al.* 2014
Lepilemur mittermeieri	Mittermeier's sportive lemur	190	Ampasindava Peninsula	Line transect distance sampling	Wilmet *et al.* 2017
Lepilemur edwardsi	Milne-Edwards' sportive lemur	220	Mariarano forest	Line transect distance sampling	Ibouroi *et al.* 2013
Lepilemur ankaranensis	Ankarana sportive lemur	550	Ankarana Special Reserve	Simplified line transects (mean distances, including aural detections)	Hawkins *et al.* 1990

that the observed consistency of our line transect density estimate with previous studies using the same methods could be a result of shared biases within the line transect approach, rather than an indication of true reliability. Nevertheless, evaluating our passive acoustic density estimates against those from established methods still provides the most viable approach to assessing their accuracy without a known truth scenario (see Sebastián-González *et al.*, 2018 and Arranz *et al.*, 2023 for similar evaluation approaches).

Our passive acoustic cue counting density estimate using single calls as cues (2,752 individuals/km^2) is approximately an order of magnitude higher than the comparator line transect density estimate (303.7 individuals/km^2), with nonoverlapping confidence intervals. Moreover, this cue counting density estimate is not comparable with any of the *Lepilemur* spp. densities reported in the literature. This suggests it has likely overestimated the true density. The estimates also have very low precision, with coefficients of variation of 68% and 44%, suggesting they are unreliable.

Several factors could account for this apparent overestimation of true density. One potential reason is that we may have underestimated cue rates during focal follows. In cue counting, cue density is converted to animal density by dividing it by a cue rate "multiplier"; hence, a cue rate that is biased down will concomitantly bias animal density up, because the cue density is divided by a smaller number. While our focal follows collected cue rate data from a large, random sample of individuals, including silent individuals, both concurrently and across the same spatial and temporal scales as the cue counting survey (Warren *et al.*, 2017), we cannot rule this out as a source of potential bias. For instance, although not obviously apparent, it is possible that observer presence suppressed call (cue) rates of focal lemurs. Torchlight, for example, can affect the behaviour of some nocturnal primates (Nekaris *et al.*, 2008). A second possible factor is that using single acoustic elements as cues, given the lemurs often called in bouts, could inflate cue counts. That is, owing to chance and the bout structure of cues, our random subsample of audio recordings may have captured a more-than-usual number of cues, which would increase the cue density and concomitantly the animal density. Measurement error (as per key assumption 4: distances to detected cues are measured accurately) can also generate bias in density estimates; however, we consider this an unlikely source of bias in our case given the sound-level-based distance prediction model performed well, with relatively small distance estimation error (2.43 m) and negligible error bias (−0.02 m), and because the grouping of distance data somewhat relaxes this assumption (Buckland *et al.*, 1993).

In addition to the density estimate being an order of magnitude higher when using acoustic methods and single calls as cues, the variance in the estimates was also larger. The low precision can be attributed to the high variance in the individual single-call cue rates, largely driven by the bout structure of ouah calls (McCallum, 2005). In cue counting, the variation in the cue rate is one of several random components that will contribute to the overall variance in the density estimate, which in our case represented >84% of the total contribution. During our focal follows,

for instance, the majority of focal lemurs produced no cues (92%), some produced a few cues, whereas up to 22 cues/min were produced during bouts. Other sources have also found that the cue rate is the random component that contributes the most to the overall variance (Buckland, 2006, Marques *et al.*, 2011). For passive acoustic cue counting to produce results that are accurate and precise, and therefore meaningful to conservation managers, key requirements are that the cue rate can be reliably estimated, and that mean rates are relatively consistent between cue-producing individuals (i.e., small variance) (Warren *et al.*, 2017; see Marques *et al.*, 2013 for other considerations). Reliable estimation of cue rates, and assessing the consistency of mean rates, demands a thorough understanding of the target species' vocal repertoire and activity, yet such detailed information is not available for most taxa, including *Lepilemur* spp. Further studies on vocal primates' calling behaviour will be invaluable in this regard.

An alternative approach, which we explored, is to treat call bouts, rather than single calls, as the unit of analysis (i.e., as "cues"). This method yielded a density estimate (802 individuals/km^2) that, while still seemingly high, was much closer to and had overlapping confidence intervals with the comparator line transect density estimate and had improved precision (31% CV). Using bouts as the unit of analysis may therefore be more suitable in cue counting surveys of *Lepilemur* spp. and other taxa that produce calls in bouts, as it can reduce some of the variability in calling rates. However, this approach also presents significant limitations. Notably, the inability to distinguish the calls of multiple individuals in bouts detected in audio recordings creates a discrepancy between how bouts are counted when estimating the bout rate (i.e., calls from one individual are counted) and the actual survey (i.e., calls from multiple individuals are potentially lumped into a single bout). This discrepancy can lead to an underestimation of the bout density, and thus animal density, as simultaneous bouts from multiple individuals will be counted as a single bout. Another limitation is that defining a bout is somewhat subjective, and therefore different definitions may yield different results. Indeed, future studies could test how varying bout definitions affect cue counting density estimates. But despite these limitations, the improved precision and closer alignment with the line transect estimate suggests that the benefits of employing bouts as the unit of analysis may outweigh the drawbacks, resulting in more reliable density estimation.

Animal-borne acoustic recording tags offer a promising approach for accurately estimating cue rates, addressing challenges like the possible underestimation suspected in our study. These tags are an alternative to focal follows for collecting vocal activity data, and can improve both the accuracy and precision of cue rate (and bout rate) estimation by increasing sampling periods while controlling for potential observer effects (Couchoux *et al.*, 2015; Warren *et al.*, 2017). By deploying tags in a future field season at Nosy Be, an updated cue rate multiplier could be readily applied retrospectively to our existing dataset to refine the density estimates (Gunnlaugsson *et al.*, 2009). Such an approach would be feasible so long as the updated cue rate estimate is obtained from a large, random sample in the same survey area and, importantly, under similar conditions—specifically, during the wet

season when *Lepilemur* spp. are known to be more vocally active. Tagging animals is, however, more invasive, and the associated costs may necessarily limit the number of individuals that can be sampled. It may also be difficult to distinguish the calls of tagged individuals from those of conspecifics. Further research could also investigate whether a correction factor can be applied to the passive acoustic density estimates that adjusts for underestimation of the cue rate (Buij *et al.*, 2003). More broadly, automated detection and classification algorithms (i.e., "recognizers") can be employed in future passive acoustic cue counting surveys to more efficiently detect cues from audio recordings, noting that this necessitates estimation of an additional multiplier that accounts for the proportion of detections that are false positives (key assumption 7), as well as a correction for false negatives based on estimated recall. Studies quantifying the cost-effectiveness of passive acoustic cue counting would also allow conservation managers to better compare survey alternatives and weigh factors such as cost, precision and scale (Delisle *et al.*, 2023).

Conservation Implications for *Lepilemur tymerlachsoni*

Using our line transect data, we estimate the global population of *Lepilemur tymerlachsoni* to be *c*. 11,300 individuals. The area of forest habitat on Nosy Be has recently been estimated to cover 37.3 km^2 (Martin *et al.*, 2025); extrapolating our line transect density estimate (303.7 individuals/km^2) across this forested area provides this abundance estimate, which, given *L. tymerlachsoni* is locally endemic to Nosy Be, represents a global abundance estimate. This density estimate is moderately high compared to those reported for other *Lepilemur* species in the region (Table II). Potential drivers of this relatively high density, while currently unknown, might include effective protected area management at Lokobe or the absence of some key predators, such as the fosa (*Cryptoprocta ferox*), on Nosy Be compared to mainland Madagascar. These figures will inform future conservation status assessments and management plans, while providing a baseline for monitoring population changes over time.

Despite the relatively high population estimates reported here, the conservation outlook for *Lepilemur tymerlachsoni* remains fragile. Its geographic range is extremely restricted (extent of occurrence 72 km^2), which alone renders it amongst the world's most endangered primates (Mittermeier *et al.*, 2008). What little habitat there is continues to be cleared and degraded, and hunting persists (Louis Jr. *et al.*, 2020). Indeed, *L.* cf. *tymerlachsoni* has already been extirpated from the neighbouring island of Nosy Komba (Hyde Roberts & Daly, 2014). Lokobe is the only protected area in which *L. tymerlachsoni* occurs (Louis Jr. *et al.*, 2020), but because Lokobe is so small (7.4 km^2) it is doubtful whether it can ensure the species' long-term viability. A critical starting point for the conservation of *L. tymerlachsoni* is therefore safeguarding Nosy Be's unprotected forests, some of which are managed by village associations (VOI; Fig. 2). The tourism industry in Nosy Be is very strong, and there is real potential for community-led primate-watching for tourists to help protect these habitats and provide a source of income for local

communities (Martin *et al.*, 2025; Waters *et al.*, 2023). A more radical conservation strategy would be to reintroduce *L. tymerlachsoni* to Nosy Komba, subject to key requirements being met (e.g., habitat suitability assessments, long-term funding, post-release monitoring and protection, etc.; Baker, 2002). Surveys of Nosy Komba in 2013, while failing to locate any *L.* cf. *tymerlachsoni*, did identify large areas of suitable remaining habitat, indicating that hunting, rather than habitat disturbance, might have been the key driver in the lemurs' decline (Hyde Roberts & Daly, 2014). These so-called empty forests, in which primates have been extirpated by hunting, present opportunities for reintroduction so long as hunting can be controlled or replaced by alternative livelihoods (Beck, 2016).

Conclusion

At this stage, passive acoustic cue counting appears challenging for *Lepilemur tymerlachsoni* and perhaps other *Lepilemur* species for the reasons outlined, although improved estimates of the cue (or bout) rate, which could be obtained through the use of acoustic recording tags, may present a solution. The methods can be adapted for surveys of other vocal primates provided the cue rate can be reliably estimated with good precision (a key hurdle) and provided the target species is generally compatible with the protocol (i.e., it produces conspicuous loud calls, is amenable to focal follows, etc.). Otherwise, the line transect population estimates reported here provide critical information for the conservation of one of the world's most endangered primates.

Acknowledgments Financial support was provided by Re:wild's Lemur Conservation Action Fund and the Australian National University's Primate Conservation Travel Grant. We thank the directors of Lokobe National Park, Gérard Bakarizafy and Landisoa Randimbison; the staff of Madagascar National Parks, Franck Tanasi Tombomanana, Said Mohamad Assany, Omar Mohamad Assany and Joël Tianjara; our guides from the Comités Locaux du Parc at Nosy Be, Udo-Heiss Jaozafy, Tavandra Mohibo Mamoudou, Avilaza, Kadra Odile, Alphonsine Tina and Mohammad; Dr. Jean Freddy Ranaivoarisoa and the Mention Anthropobiologie et Développement Durable at the University of Antananarivo for their assistance with the research permit; the landowners and village presidents of Ambatozavavy, Ampasipohy, Antafondro, and Marodoka for allowing access to the survey area; and the editors and reviewers for their constructive comments on the manuscript. Thanks also to Dr. Anastasia Dalziell and Prof. Justin Welbergen for kindly loaning the audio recording equipment.

Author Contributions LDM: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, visualization, writing – original draft. HR: investigation, project administration. ESN: investigation, project administration, visualization, writing – translation, writing – review & editing. SV: project administration, supervision, writing – review & editing. AMR: formal analysis, supervision, writing – review & editing. RDM: methodology, supervision, writing – review & editing. AMB: methodology, supervision, writing – review & editing.

Funding Open Access funding enabled and organized by CAUL and its Member Institutions.

Declarations

Inclusion and Diversity Statement The author list includes contributors from the location where the research was conducted, who participated in study conception, study design, data collection, analysis, and/

or interpretation of the findings. Other collaborators, including those from local academic institutions, government and non-government organisations, are listed in the acknowledgements section.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

Akaike, H. (1973). Information theory and an extension of the maximum likelihood principle. In B. N. Petrov & F. Csaki (Eds.), *Second International Symposium on Information Theory* (pp. 267–281). Akadémiai Kiadó.

Alldredge, M. W., Simons, T. R., & Pollock, K. H. (2010). A field evaluation of distance measurement error in auditory avian point count surveys. *The Journal of Wildlife Management, 71*(8), 2759–2766.

Altmann, J. (1974). Observational study of behavior: Sampling methods. *Behavior, 49*(3/4), 227–267.

Andreone, F., Guarino, F. M., & Randrianirina, J. E. (2005). Life history traits, age profile, and conservation of the panther chameleon, *Furcifer pardalis* (Cuvier 1829), at Nosy Be, NW Madagascar. *Tropical Zoology, 18*, 209–225. https://doi.org/10.1080/03946975.2005.10531221

Andriantsaralaza, S., & Clarke, T. A. (2023). Recommendations for responsible lemur watching in Madagascar. In: S. Waters, M. F. Hansen, J. M. Setchell, S. M. Cheyne, R. A. Mittermeier, A. Ang, B. C. Aldrich, S. Andriantsaralaza, T. A. Clarke, A. Dempsey, K. M. Dore, K. T. Hanson, A. Kitegile, A. M. Maldonada, L. Maréchal, T. McKinney, C. R. R. Miranda, K. Niu, M. Svensson, ..., & A. M. Behie (Eds.), *Responsible Primate-Watching for Tourists*. IUCN SSC Primate Specialist Group Section on Human-Primate Interactions.

Arranz, P., Miranda, D., Gkikopoulou, K. C., Cardona, A., Alcazar, J., Aguilar de Soto, N., Thomas, L., & Marques, T. A. (2023). Comparison of visual and passive acoustic estimates of beaked whale density off El Hierro, Canary Islands. *The Journal of the Acoustic Society of America, 154*(4), 2469. https://doi.org/10.1121/10.0017921

Baker, L. R. (2002). Guidelines for nonhuman primate re-introductions. *Re-introduction NEWS: Special Primate Issue, Newsletter of the IUCN/SSC Re-introduction Specialist Group, 21*, 29–57. https://iucn-ctsg.org/wp-content/uploads/2018/01/RNews21_Jun02.pdf#page=29.

Beck, B. B. (2016). The role of translocation in primate conservation. In S. Wich & A. J. Marshall (Eds.), *An introduction to primate conservation* (pp. 241–256). Oxford University Press.

Bederu, M. (2014). Preliminary data on population density and habitat use of *Lepilemur tymerlachsonorum* in Lokobe National Park. *Lemur News, 18*, 2–4.

Bessone, M., Kühl, H. S., Hohmann, G., Herbinger, I., N'Goran, K. P., Asanzi, P., Da Costa, P. B., Dérozier, V., Fotsing, D. B. E., Ikembelo, B. B., Iyomi, D. M., Iyatshi, B. I., Kafando, P., Kambere, A. M., Moundzoho, B. D., Musubaho, L. K., & Fruth, B. (2023). Assessing the effects of survey-inherent disturbance on primate detectability: Recommendations for line transect distance sampling. *Primates, 64*, 107–121. https://doi.org/10.1007/s10329-022-01039-4

Bezanson, M., & McNamara, A. (2019). The what and where of primate field research may be failing primate conservation. *Evolutionary Anthropology, 20*(4), 166–178. https://doi.org/10.1002/evan.21790

Bibby, C., Jones, M., & Marsden, S. (1998). *Expedition field techniques: Bird surveys*. Royal Geographical Society.

Birkinshaw, C. R. (1995) *The importance of the black lemur,* Eulemur macaco *(Lemuridae, Primates), for seed dispersal in Lokobe Forest, Madagascar* (Publication No. 10046032) [Doctoral dissertation, University College London]. ProQuest Dissertations & Theses Global.

Borchers, D., Marques, T., Gunlaugsson, T., & Jupp, P. (2010). Estimating distance sampling detection functions when distances are measured with errors. *Journal of Agricultural, Biological, and Environmental Statistics, 15*(3), 346–361. https://doi.org/10.1007/s13253-010-0021-y

Brooks, M. E., Kristensen, K., Van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., Skaug, H. J., Mächler, M., & Bolker, B. M. (2017). glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R Journal, 9*(2), 378–400. https://doi.org/10.32614/RJ-2017-066

Brown, A., Garg, S., & Montgomery, J. (2019). Automatic rain and cicada chorus filtering of bird acoustic data. *Applied Soft Computing, 81*, 105501. https://doi.org/10.1016/j.asoc.2019.105501

Browning, E., Gibb, R., Glover-Kapfer, P., & Jones, K. E. (2017). *Passive acoustic monitoring in ecology and conservation*. WWF Conservation Technology Series. https://www.wwf.org.uk/sites/default/files/2019-04/Acousticmonitoring-WWF-guidelines.pdf

Buckland, S. T. (2006). Point-transect surveys for songbirds: Robust methodologies. *The Auk, 123*(2), 345–357. https://doi.org/10.1093/auk/123.2.345

Buckland, S. T., Anderson, D. R., Burnham, K. P., & Laake, J. L. (1993). *Distance sampling: Estimating abundance of biological populations*. Chapman and Hall.

Buckland, S. T., Anderson, D. R., Burnham, K. P., Laake, J. L., Borchers, D. L., & Thomas, L. (2001). *Introduction to distance sampling: Estimating abundance of biological populations*. Oxford University Press.

Buckland, S. T., Anderson, D. R., Burnham, K. P., Laake, J. L., Borchers, D. L., & Thomas, L. (2004). *Advanced distance sampling: Estimating abundance of biological populations*. Oxford University Press.

Buckland, S. T., Marsden, S. J., & Green, R. E. (2008). Estimating bird abundance: Making methods work. *Bird Conservation International, 18*, S91–S108. https://doi.org/10.1017/S0959270908000294

Buckland, S. T., Plumptre, A. J., Thomas, L., & Rexstad, E. A. (2010). Design and analysis of line transect surveys for primates. *International Journal of Primatology, 31*, 833–847. https://doi.org/10.1007/s10764-010-9431-5

Buij, R., Singleton, I., Krakauer, E., & van Schaik, C. P. (2003). Rapid assessment of orangutan density. *Biological Conservation, 114*, 103–113. https://doi.org/10.1016/S0006-3207(03)00015-6

Campbell, G., Head, J., Junker, J., & Nekaris, K. A. I. (2016). Primate abundance and distribution: Background concepts and methods. In S. Wich & A. J. Marshall (Eds.), *An introduction to primate conservation* (pp. 79–110). Oxford University Press. https://doi.org/10.1093/acprof:oso/9780198703389.003.0006

Cole, H. J., Gomes, D. G. E., & Barber, J. R. (2022). EcoCountHelper: An R package and analytical pipeline for the analysis of ecological count data using GLMMs, and a case study of bats in Grand Teton National Park. *PeerJ, 10*, e14509. https://doi.org/10.7717/peerj.14509

Couchoux, C., Aubert, M., Garant, D., & Réale, D. (2015). Spying on small wildlife sounds using affordable collar-mounted miniature microphones: An innovative method to record individual daylong vocalisations in chipmunks. *Scientific Reports, 5*, 10118. https://doi.org/10.1038/srep10118

de Andrade, A. C., Marques, T. A., & Buckland, S. T. (2019). Spider monkeys, the misunderstood assumptions of distance sampling and the pitfalls of poor field design. *Biodiversity and Conservation, 28*, 4119–4121. https://doi.org/10.1007/s10531-019-01872-y

Deichmann, J. L., Acevedo-Charry, O., Barclay, L., Burivalova, Z., Campos-Cerqueira, M., d'Horta, F., Game, E. T., Gottesman, B. L., Hart, P. J., Kalan, A. K., Linke, S., Do Nascimento, L., Pijanowski, B., Staaterman, E., & Aide, T. M. (2018). It's time to listen: There is much to be learned from the sounds of tropical ecosystems. *Biotropica, 50*(5), 713–718. https://doi.org/10.1111/btp.1259

Delisle, Z. J., McGovern, P. G., Dillman, B. G., Reeling, C. J., Caudell, J. N., & Swihart, R. K. (2023). Using cost-effectiveness analysis to compare density-estimation methods for large-scale wildlife management. *Wildlife Society Bulletin, 47*(2), e1430.

Digby, A., Towsey, M., Bell, B. D., & Teal, P. D. (2013). A practical comparison of manual and autonomous methods for acoustic monitoring. *Methods in Ecology and Evolution, 4*(7), 675–683. https://doi.org/10.1111/2041-210X.12060

Ellinger, N., & Hödl, W. (2003). Habiyat acoustics of a neotropical lowland rainforest. *Bioacoustics, 13*(3), 297–321. https://doi.org/10.1080/09524622.2003.9753503

Fedigan, L. M. (2010). Ethical issues faced by field primatologists: Asking the relevant questions. *American Journal of Primatology, 72*(9), 754–771. https://doi.org/10.1002/ajp.20814

Ferrario, V., Raimondi, T., De Gregorio, C., Carugati, F., Cristiano, W., Torti, V., Lewis, R. N., Valente, D., Williams, L. J., Raisin, C., Gamba, M., Von Hardenberg, A., & Giacoma, C. (2024). Singing in the rain! Climate constraints on the occurrence of indri's song. *American Journal of Primatology, 86*(10), e23673. https://doi.org/10.1002/ajp.23673

Fewster, R. M., Buckland, S. T., Burnham, K. P., Borchers, D. L., Jupp, P. E., Laake, J. L., & Thomas, L. (2009). Estimating the encounter rate variance in distance sampling. *Biometrics, 65*(1), 225–236. https://doi.org/10.1111/j.1541-0420.2008.01018.x

Forrest, T. G. (1994). From sender to receiver: Propagation and environmental effects on acoustic signals. *American Zoologist, 34*(6), 644–654. https://doi.org/10.1093/icb/34.6.644

Gatz, D. F., & Smith, L. (1995). The standard error of a weighted mean concentration—I. *Bootstrapping vs other methods. Atmospheric Environment, 29*(11), 1185–1193. https://doi.org/10.1016/1352-2310(94)00210-C

Gibb, R., Browning, E., Glover-Kapfer, P., & Jones, K. E. (2019). Emerging opportunities and challenges for passive acoustics in ecological assessment and monitoring. *Methods in Ecology and Evolution, 10*(2), 169–185. https://doi.org/10.1111/2041-210X.13101

Goodman, S. M., & Benstead, J. P. (2002). *The natural history of Madagascar*. The University of Chicago Press.

Graf, L., & Hatlauf, J. (2021). Distance estimation of howling golden jackals (Canis aureus) using relative sound level. *Mammal Research, 66*(4), 567–572. https://doi.org/10.1007/s13364-021-00587-2

Gunnlaugsson, T., Víkingsson, G. A., & Pike, D. G. (2009). Combined line-transect and cue-count estimate of sperm whale abundance in the North Atlantic, from Icelandic NASS-2001 shipboard survey. *NAMMCO Scientific Publications, 7*, 73–80.

Hartig, F. (2022). *DHARMa*: Residual diagnostics for hierarchical (multi-level/mixed) regression models (Version 0.4.6) [R package]. CRAN. https://CRAN.R-project.org/package=DHARMa

Hasiniaina, A. F., Scheumann, M., Rina Evasoa, M., Braud, D., Rasoloharijaona, S., Randrianambinina, B., & Zimmermann, E. (2018). High frequency/ultrasonic communication in a critically endangered nocturnal primate, Claire's mouse lemur (*Microcebus mamiratra*). *American Journal of Primatology, 80*(6), e22866. https://doi.org/10.1002/ajp.22866

Hawkins, A. F. A., Chapman, P., Ganzhorn, J. U., Bloxam, Q. M. C., Barlow, S. C., & Tonge, S. J. (1990). Vertebrate conservation in Ankarana Special Reserve, Northern Madagascar. *Biological Conservation, 54*(2), 83–110. https://doi.org/10.1016/0006-3207(90)90136-D

Heide-Jørgensen, M. P., & Simon, M. (2007). A note on cue rates for common minke, fin and humpback whales off West Greenland. *Journal of Cetacean Research and Management, 9*(3), 211–214. https://doi.org/10.47536/jcrm.v9i3.669

Hending, D., Randrianarison, H., Andriamavosoloarisoa, N. N. M., Ranohatra-Hending, C., McCabe, G., Cotton, S., & Holderied, M. (2024). Impact of forest fragmentation and associated edge effects on the population density of four nocturnal lemur species in North West Madagascar. *Animal Conservation, 27*(4), 522–537. https://doi.org/10.1111/acv.12929

Hill, A. P., Prince, P., Piña Covarrubias, E., Doncaster, C. P., Snaddon, J. L., & Rogers, A. (2018). AudioMoth: Evaluation of a smart open acoustic device for monitoring biodiversity and the environment. *Methods in Ecology and Evolution, 9*(5), 1199–1211. https://doi.org/10.1111/2041-210X.12955

Howe, E. J., Buckland, S. T., Després-Einspenner, M.-L., & Kühl, H. S. (2018). Model selection with overdispersed distance sampling data. *Methods in Ecology and Evolution, 10*(1), 38–47. https://doi.org/10.1111/2041-210X.13082

Hyde Roberts, S., & Daly, C. (2014). A search for the missing sportive lemurs of Nosy Komba, north west Madagascar. *Lemur News, 18*, 13–15.

Ibouroi, M. T., Schwitzer, C., & Rabarivola, J. C. (2013). Population density estimates of two endangered nocturnal and sympatric lemur species from the Mariarano Forest, northern Madagascar, using multiple approaches. *Lemur News, 17*, 49–54.

K. Lisa Yang Center for Conservation Bioacoustics at the Cornell Lab of Ornithology. (2023). *Raven Pro: Interactive Sound Analysis Software (Version 1.6.4) [Computer software]*. The Cornell Lab of Ornithology. Available from. https://www.ravensoundsoftware.com/

Kappeler, P. M. (1997). Determinants of primate social organization: Comparative evidence and new insights from Malagasy lemurs. *Biological Reviews, 72*(1), 111–151. https://doi.org/10.1017/S0006323196004999

Kühl, H., Maisels, F., Ancrenaz, M., & Williamson, E. A. (2008). *Best practice guidelines for surveys and monitoring of great ape populations*. IUCN SSC Primate Specialist Group.

Lambert, K. T. A., & McDonald, P. G. (2014). A low-cost, yet simple and highly repeatable system for acoustically surveying cryptic species. *Austral Ecology, 39*(7), 779–785. https://doi.org/10.1111/aec.12143

Laurance, W. F. (2013). Does research help to safeguard protected areas? *Trends in Ecology & Evolution, 28*(5), 261–266. https://doi.org/10.1016/j.tree.2013.01.017

Lenth, R. (2024). *emmeans*: Estimated marginal means, aka least-squares means (Version 1.10.2) [R package]. CRAN. https://CRAN.R-project.org/package=emmeans

Louis, E. E. Jr., Bailey, C. A., Raharivololona, B., Schwitzer, C., Wilmet, L., & Roos, C. (2020). *Lepilemur tymerlachsoni. The IUCN Red List of Threatened Species 2020: e.T136709A115585199.* https://doi.org/10.2305/IUCN.UK.2020-2.RLTS.T136709A115585199.en. Accessed Oct 2024.

Lüdecke, D. (2018). ggeffects: Tidy Data Frames of Marginal Effects from Regression Models. *Journal of Open Source Software, 3*(26), 772. https://doi.org/10.21105/joss.00772

Lüdecke, D., Ben-Shachar, M. S., Patil, I., Waggoner, P., & Makowski, D. (2021). performance: An R package for assessment, comparison and testing of statistical models. *Journal of Open Source Software, 6*(60), 3139. https://doi.org/10.21105/joss.03139

Macron, C., Richard, Y., Garot, T., Bessafi, M., Pohl, B., Ratiarison, A., & Razafindrabe, A. (2016). Intraseasonal rainfall variability over Madagascar. *Monthly Weather Review, 144*(5), 1877–1885. https://doi.org/10.1175/MWR-D-15-0077.1

Mahaboubi, S., Randrianambinina, B., Rakotondravony, R., Scheumann, M., Zimmermann, E., & Rasoloharijaona, S. (2015). First characterization of the vocal acoustics of Otto's sportive lemur *Lepilemur otto* (Craul *et al.*, 2007) with remarks on its abundance. *Lemur News, 19*, 16–21.

Maisels, F., Bout, N., Inkamba-Inkulu, C., Pearson, L., Aczel, P., Ambahe, R., Ambassa, E., & Fotso, R. (2007). New northwestern and southwestern range limits of De Brazza's monkey, Mbam et Djerem National Park, Cameroon, and Bateke Plateau, Gabon and Congo. *Primate Conservation, 22*(1), 107–110.

Mandl, I., Rabemananjara, N., Schwitzer, C., & Holderied, M. (2018). Evaluating the use of vocalization playbacks for lure counts of the rare nocturnal sportive lemur *Lepilemur sahamalaza*. *Primate Conservation, 32*, 109–122.

Mandl, I., Schwitzer, C., & Holderied, M. (2019). Sahamalaza sportive lemur, *Lepilemur sahamalaza*, vocal communication: Call use, context and gradation. *Folia Primatologica, 90*(5), 336–360. https://doi.org/10.1159/000493939

Markolf, M., Zinowsky, M., Keller, J. K., Borys, J., Cillov, A., & Schülke, O. (2022). Toward passive acoustic monitoring of lemurs: Using an affordable open-source system to monitor Phaner vocal activity and density. *International Journal of Primatology, 43*(3), 409–433. https://doi.org/10.1007/s10764-022-00285-z

Marques, T. A. (2016). A comment on Horcajada-Sánchez and Barja (2015): A cautionary tale about left truncation and density gradients in distance sampling. *Annales Zoologici Fennici, 53*(1–2), 52–54. https://doi.org/10.5735/086.053.0204

Marques, T. A., Thomas, L., Ward, J., DiMarzio, N., & Tyack, P. L. (2009). Estimating cetacean population density using fixed passive acoustic sensors: An example with Blainville's beaked whales. *The Journal of the Acoustical Society of America, 125*(4), 1982–1994. https://doi.org/10.1121/1.3089590

Marques, T. A., Munger, L., Thomas, L., Wiggins, S., & Hildebrand, J. A. (2011). Estimating North Pacific right whale *Eubalaena japonica* density using passive acoustic cue counting. *Endangered Species Research, 13*(3), 163–172. https://doi.org/10.3354/esr00325

Marques, T. A., Thomas, L., Martin, S. W., Mellinger, D. K., Ward, J. A., Moretti, D. J., Harris, D., & Tyack, P. L. (2013). Estimating animal population density using passive acoustics. *Biological Reviews, 88*(2), 287–309. https://doi.org/10.1111/brv.12001

Martin, L. D., Rowe, A. K., Nomenjanahary, E. S., Montaño, S. K., Wright, P. C., & Deppe, A. M. (2021). Population Estimates of Hubbard's Sportive Lemur (*Lepilemur hubbardorum*) at Zombitse-Vohibasia National Park, Madagascar. *Folia Primatologica, 92*(1), 70–78. https://doi.org/10.1159/000512559

Martin, L. D., Razafimanantsoa, H., Nomenjanahary, E. S., Volampeno, S., & Behie, A. M. (2025). First density estimates of the Endangered Claire's mouse lemur *Microcebus mamiratra* and recommendations for its conservation. *Oryx, 59*(1), 109–118. https://doi.org/10.1017/S0030605324000772

McCallum, D. A. (2005). A conceptual guide to detection probability for point counts and other count-based survey methods. In C. J. Ralph & T. D. Rich (Eds.), *Bird conservation implementation and integration in the Americas: Proceedings of the third international Partners in Flight conference 2002* (pp. 754–761). U.S Department of Agriculture Forest Service GTR-PSW-191.

Méndez-Cárdenas, M., Randrianambinina, B., Rabesandratana, A., Rasoloharijaona, S., & Zimmermann, E. (2008). Geographic variation in loud calls of sportive lemurs (Lepilemur ssp.) and their implications for conservation. *American Journal of Primatology, 70*(9), 828–838. https://doi.org/10.1002/ajp.20554

Meyler, S. V., Salmona, J., Ibouroi, M. T., Besolo, A., Rasolondraibe, E., Radespiel, U., Rabarivola, C., & Chikhi, L. (2012). Density estimates of two Endangered nocturnal lemur species from northern Madagascar: New results and a comparison of commonly used methods. *American Journal of Primatology, 74*(5), 414–422. https://doi.org/10.1002/ajp.21997

Mittermeier, R. A., Ganzhorn, J. U., Konstant, W. R., Glander, K., Tattersall, I., Groves, C. P., Rylands, A. B., Hapke, A., Ratsimbazafy, J., Mayor, M. I., Louis, E. E., Jr., Rumpler, Y., Schwitzer, C., & Rasoloarison, R. M. (2008). Lemur diversity in Madagascar. *International Journal of Primatology, 29*, 1607–1656. https://doi.org/10.1007/s10764-008-9317-y

Mittermeier, R. A., Reuter, K. E., Rylands, A. B., Louis, E. E., Jr., Ratsimbazafy, J., de Roland, L.-A.R., Langrand, O., Schwitzer, C., Johnson, S. E., Godfrey, L. R., Blanco, M. B., Borgerson, C., Eppley, T. M., Andriamanana, T., Volampeno, S., Andriantsaralaza, S., Wright, P. C., & Rajaobelina, S. (2023). *Lemurs of Madagascar*. Re: Wild Tropical Field Guide Series.

Müller, P., Velo, A., Raheliarisoa, E. O., Zaramody, A., & Curtis, D. J. (2000). Surveys of sympatric lemurs at Anjamena, north-west Madagascar. *African Journal of Ecology, 38*(3), 248–257. https://doi.org/10.1046/j.1365-2028.2000.00247.x

Nekaris, K. A. I., Blackham, G. V., & Nijman, V. (2008). Conservation implications of low encounter rates of five nocturnal primate species (*Nycticebus* spp.) in Asia. *Biodiversity and Conservation, 17*, 733–747. https://doi.org/10.1007/s10531-007-9308-x

Paddock, C. L., Bruford, M. W., & McCabe, G. M. (2020). Estimating the population size of the Sanje mangebey (*Cercocebus sanjei*) using acoustic distance sampling. *American Journal of Primatology, 82*, e23083. https://doi.org/10.1002/ajp.23083

Peres, C. A. (1999). General guidelines for standardizing line-transect surveys of tropical forest primates. *Neotropical Primates, 7*(1), 11–16. https://doi.org/10.62015/np.1999.v7.414

Pérez-Granados, C., & Traba, J. (2021). Estimating bird density using passive acoustic monitoring: A review of methods and suggestions for further research. *Ibis, 163*(3), 765–783. https://doi.org/10.1111/ibi.12944

Pérez-Granados, C., Barrero, A., Traba, J., Bustillo-de la Rosa, D., Reverter, M., & Gómez-Catasús, J. (2021). Assessment of cue counting for estimating bird density using passive acoustic monitoring: Recommendations for estimating a reliable cue rate. *Avian Conservation and Ecology, 16*(1), 11. https://doi.org/10.5751/ACE-01801-160111

Piel, A. K. (2018). Temporal patterns of chimpanzee loud calls in the Issa Valley, Tanzania: Evidence for nocturnal acoustic behavior in wild chimpanzees. *American Journal of Biological Anthropology, 166*(3), 530–540. https://doi.org/10.1002/ajpa.23609

Piel, A. K., Crunchant, A., Knot, I. E., Chalmers, C., Fergus, P., Mulero-Pázmány, M., & Wich, S. A. (2022). Noninvasive technologies for primate conservation in the 21st century. *International Journal of Primatology, 43*, 133–167. https://doi.org/10.1007/s10764-021-00245-z

Plumptre, A. J., Sterling, E. J., & Buckland, S. T. (2013). Primate census and survey techniques. In E. J. Sterling, N. Bynam, & M. E. Blair (Eds.), *Primate ecology and conservation: A handbook of techniques* (pp. 10–26). Oxford University Press.

R Core Team. (2024). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.R-project.org/

Rasoloharijaona, S., Randrianambinina, B., Braune, P., & Zimmermann, E. (2006). Loud calling, spacing, and cohesiveness in a nocturnal primate, the Milne Edwards' sportive lemur (*Lepilemur edwardsi*). *American Journal of Physical Anthropology, 129*(4), 591–600. https://doi.org/10.1002/ajpa.20342

Rocha, L. H. S., Ferreira, L. S., Paula, B. C., Rodrigues, F. H. G., & Sousa-Lima, R. S. (2015). An evaluation of manual and automated methods for detecting sounds of maned wolves (*Chrysocyon brachyurus* Illiger 1815). *Bioacoustics, 24*(2), 185–198. https://doi.org/10.1080/09524622.2015.1019361

Rylands, A. B., Williamson, E. A., Hoffmann, M., & Mittermeier, R. A. (2008). Primate surveys and conservation assessments. *Oryx, 42*(3), 313–314. https://doi.org/10.1017/S0030605308423050

Rylands, A. B., Mittermeier, R. A., & Williamson, E. A. (2020). Primate conservation – new reports from the field. *Oryx, 54*(6), 751–752. https://doi.org/10.1017/S0030605320000939

Salmona, J., Ralantoharijaona, T., Thani, I. M., Rakotonanahary, A., Zaranaina, R., Jan, F., Rasolondraibe, E., Barnavon, M., Beck, A., Wholhauser, S., Ranirison, P., Zaonarivelo, J. R., Rabarivola, C., & Chikhi, L. (2014). Daraina sportive lemur (*Lepilemur milanoii*) density and population size estimates in most of its distribution range: The Loky-Manambato region. *Lemur News, 18*, 16–19.

Savage, A., Thomas, L., Leighty, K. A., Soto, L. H., & Medina, F. S. (2010). Novel survey method finds dramatic decline of wild cotton-top tamarin population. *Nature Communications, 1*, 30. https://doi.org/10.1038/ncomms1030

Sawyer, R. M., Mena, H. E., & Donati, G. (2015). Habitat use, diet and sleeping site selection of *Lepilemur tymerlachsoni* in a disturbed forest of Nosy Be: Preliminary observations. *Lemur News, 19*, 25–30.

Schwitzer, C., Mittermeier, R. A., Davies, N., Johnson, S., Ratsimbazafy, J., Razafindramanana, J., Louis, E. E. Jr., & Rajaobelina, S. (Eds.). (2013). *Lemurs of Madagascar: A strategy for their conservation 2013–2016*. IUCN SSC Primate Specialist Group, Bristol Conservation and Science Foundation, and Conservation International.

Schwitzer, C., Mittermeier, R. A., Johnson, S. E., Donati, G., Irwin, M., Peacock, H., Ratsimbazafy, J., Louis, E. E., Jr., Chikhi, L., Colquhoun, I. C., Tinsman, J., Dolch, R., Lafleur, M., Nash, S., Patel, E., Randrianambinina, B., Rasolofoharivelo, T., & Wright, P. C. (2014). Averting lemur extinctions amid Madagascar's political crisis. *Science, 343*(6173), 842–843. https://doi.org/10.1126/science.1245783

Sebastián-González, E., Camp, R. J., Tanimoto, A. M., de Oliveira, P. M., Lima, B. B., Marques, T. A., & Hart, P. J. (2018). Density estimation of sound-producing terrestrial animals using single automatic acoustic recorders and distance sampling. *Avian Conservation and Ecology, 13*(2), 7. https://doi.org/10.5751/ACE-01224-130207

Seiler, M., Schwitzer, C., & Holderied, M. (2015). Call repertoire of the Sahamalaza sportive lemur, *Lepilemur sahamalazensis*. *International Journal of Primatology, 36*, 647–665. https://doi.org/10.1007/s10764-015-9846-0

Semel, B. P., Karpanty, S. M., Vololonirina, F. F., & Rakotonanahary, A. N. (2020). Eyes in the sky: Assessing the feasibility of low-cost, ready-to-use unmanned aerial vehicles to monitor primate populations directly. *Folia Primatologica, 91*(1), 69–82. https://doi.org/10.1159/000496971

Spillman, B., van Noordwijk, M. A., Willems, E. P., Setia, T. M., Wipfli, U., & van Schaik, C. P. (2015). Validation of an acoustic location system to monitor Bornean orangutan (*Pongo pygmaeus wurmbii*) long calls. *American Journal of Primatology, 77*(7), 767–776. https://doi.org/10.1002/ajp.22398

Sugai, L. (2020). Pandemics and the need for automated systems for biodiversity monitoring. *The Journal of Wildlife Management, 84*(8), 1424–1426. https://doi.org/10.1002/jwmg.21946

Sugai, L. S. M., Silva, T. S. F., Ribeiro, J. W., Jr., & Llusia, D. (2019). Terrestrial passive acoustic monitoring: Review and perspectives. *BioScience, 69*(1), 15–25. https://doi.org/10.1093/biosci/biy147

Sugai, L. S. M., Desjonquères, C., Silva, T. S. F., & Llusia, D. (2020). A roadmap for survey designs in terrestrial acoustic monitoring. *Remote Sensing in Ecology and Conservation, 6*(3), 220–235. https://doi.org/10.1002/rse2.131

Swiston, K. A., & Mennill, D. J. (2009). Comparison of manual and automated methods for identifying target sounds in audio recordings of Pileated, Pale-billed, and putative Ivory-billed woodpeckers. *Journal of Field Ornithology, 80*(1), 42–50. https://doi.org/10.1111/j.1557-9263.2009.00204.x

Thomas, L., & Martin, S. (2006). *Methods for estimating the density of cetaceans from fixed arrays of passive listening devices* [White paper]. lenthomas.org. https://lenthomas.org/papers/ThomasAndMartin2006.pdf

Thomas, L., Buckland, S. T., Rexstad, E. A., Laake, J. L., Strindberg, S., Hedley, S. L., Bishop, J. R. B., Marques, T. A., & Burnham, K. P. (2010). Distance software: Design and analysis of distance sampling surveys for estimating population size. *Journal of Applied Ecology, 41*(1), 5–14. https://doi.org/10.1111/j.1365-2664.2009.01737.x

Thompson, M. E., Schwager, S. J., Payne, K. B., & Turkalo, A. K. (2009). Acoustic estimation of wildlife abundance: Methodology for vocal mammals in forested habitats. *African Journal of Ecology, 48*(3), 654–661. https://doi.org/10.1111/j.1365-2028.2009.01161.x

Tinsman, J., Volampeno, S., Ganas-Swaray, J., Gann, D., Andrianirina, N., Chamizo, M., Ralazampirenena, C., Ranaivoarisoa, J. F., Ravoarisoa, H., Rivero, J., Zamora, A., & Gomes, C. M. (2022). Habitat use by the island lemurs of Nosy Be Madagascar. *American Journal of Primatology, 84*(3), e23362. https://doi.org/10.1002/ajp.23362

Truskinger, A., Cottman-Fields, M., Johnson, D., & Roe, P. (2013). Rapid scanning of spectrograms for efficient identification of bioacoustic events in big data. *2013 IEEE 9th International Conference on e-Science* (pp. 270–277). IEEE. https://doi.org/10.1109/eScience.2013.25

Turvey, S. T., Bryant, J. V., Duncan, C., Wong, M. H. G., Guan, Z., Fei, H., Ma, C., Hong, X., Nash, H. C., Chan, B. P. L., Xu, Y., & Fan, P. (2017). How many remnant gibbon populations are left on Hainan? Testing the use of local ecological knowledge to detect cryptic threatened primates. *American Journal of Primatology, 79*(2), e22593. https://doi.org/10.1002/ajp.22593

Vu, T. T., Tran, D. V., Giang, T. T., Nguyen, V. H., Nguyen, M. D., Nguyen, T. C., Tuyet, N. K., & Doherty, P. (2016). A mark-recapture population size estimation of southern yellow-cheeked crested gibbon *Nomascus gabriellae* (Thomas, 1909) in Chu Yang Sin National Park, Vietnam. *Asian Primates Journal, 6*(1), 33–42.

Wallis, J., & Lee, D. R. (1999). Primate conservation: The prevention of disease transmission. *International Journal of Primatology, 20*(6), 803–826.

Warren, R. D., & Crompton, R. H. (1997). A comparative study of the ranging behaviour, activity rhythms and sociality of *Lepilemur edwardsi* (Primates, Lepilemuridae) and *Avahi occidentalis* (Primates, Indriidae) at Ampijoroa, Madagascar. *Journal of Zoology, 243*(2), 397–415. https://doi.org/10.1111/j.1469-7998.1997.tb02790.x

Warren, V. E., Marques, T. A., Harris, D., Thomas, L., Tyack, P. L., Aguilar de Soto, N., Hickmott, L. S., & Johnson, M. P. (2017). Spatio-temporal variation in click production rates of beaked whales: Implications for passive acoustic density estimation. *The Journal of the Acoustical Society of America, 141*(3), 1962–1974. https://doi.org/10.1121/1.4978439

Waters, S., Hansen, M. F., Setchell, J. M., Cheyne, S., Mittermeier, R. A., Ang, A., Aldrich, B. C., Andriantsaralaza, S., Clarke, T. A., Dempsey, A., Dore, K. M., Hanson, K. T., Kitegile, A., Maldonado, A. M., Maréchal, L., McKinney, T., Ruiz Miranda, C. R., Niu, K., Svensson, M. …, & Behie, A. (2023). *Responsible Primate-Watching for Tourists*. IUCN SSC Primate Specialist Group Section on Human-Primate Interactions.

Wiley, R. H., & Richards, D. G. (1982). Adaptations for acoustic communication in birds: Sound transmission and signal detection. In D. E. Kroodsma, E. H. Miller, & H. Ouellet (Eds.), *Acoustic communication in birds* (pp. 131–181). Academic Press Inc.

Wilmet, L., Schwitzer, C., Devillers, P., Beudels-Jamar, R. C., & Vermeulen, C. (2014). Speciation in Malagasy lemurs: A review of the cryptic diversity in genus *Lepilemur*. *Biotechnology, Agronomy, Society and Environment, 18*(4), 577–588.

Wilmet, L., Schwitzer, C., Beudels-Jamar, R. C., Sonet, G., Devillers, P., & Vermeulen, C. (2017). Field data on the little known and endangered *Lepilemur mittermeieri*. *Primate Conservation, 31*(1), 17–25.

Yip, D. A., Knight, E. C., Haave-Audet, E., Wilson, S. J., Charchuk, C., Scott, C. D., Sólymos, P., & Bayne, E. M. (2020). Sound level measurements from audio recordings provide objective distance estimates for distance sampling wildlife populations. *Remote Sensing in Ecology and Conservation, 6*(3), 301–315. https://doi.org/10.1002/rse2.118

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Authors and Affiliations

Luke D. Martin[1] · Herison Razafimanantsoa[2] · Eva S. Nomenjanahary[2] · Sylviane Volampeno[3] · Alice M. Richardson[4] · Robert D. Magrath[5] · Alison M. Behie[1]

✉ Luke D. Martin
luke.martin@anu.edu.au

1 School of Archaeology and Anthropology, College of Arts and Social Sciences, The Australian National University, Canberra, Australia

2 Department of Anthropobiology and Sustainable Development, University of Antananarivo, Antananarivo, Madagascar

3 Mikajy Natiora Association, Antananarivo, Madagascar

4 Statistical Support Network, The Australian National University, Canberra, Australia

5 Division of Ecology and Evolution, Research School of Biology, The Australian National University, Canberra, Australia