1 Selective logging shows no impact on the dietary breadth of the fawn leaf-nosed bat
2 (*Hipposideros cervinus*)

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17 **Abstract**

18 Logging activities degrade forest habitats across large areas of the tropics, but the impacts
 19 on trophic interactions that underpin forest ecosystems are poorly understood. DNA
 20 metabarcoding provides an invaluable tool to investigate such interactions, allowing
 21 analysis at a far greater scale and resolution than has previously been possible. We
 22 analysed the diet of the insectivorous fawn leaf-nosed bat *Hipposideros cervinus* across
 23 a forest disturbance gradient in Borneo, using a dataset of ecological interactions from an
 24 unprecedented number of bat-derived faecal samples. Bats predominantly consumed
 25 insects from the orders Lepidoptera, Blattodea, Diptera and Coleoptera, and the
 26 taxonomic composition of their diet remained relatively consistent across sites regardless
 27 of logging disturbance. There was little difference in the richness of prey consumed in
 28 each logging treatment, indicating potential resilience of this species to habitat
 29 degradation. In fact, bats consumed a high richness of prey items, and intensive sampling
 30 is needed to reliably compare feeding ecology over multiple sites regardless of the
 31 bioinformatic procedures used.

32 Introduction

33 Logging is a common form of anthropogenic disturbance in forests, with over 90% of
 34 those in the tropics logged to some degree (Asner et al., 2009). Most logging undertaken
 35 in tropical forests is selective, which tends to favour removal of the largest, and highest-
 36 quality trees. While this disturbance can have lasting effects on forest structure
 37 (Milodowski et al., 2021) selective logging tends to be much less destructive than clear-
 38 felling.

39 Forest modification through logging is especially pronounced on the island of Borneo,
 40 which has lost half of its forest area since 1940 (Gaveau et al., 2014) and 62% of the
 41 remaining forest is classified as ‘degraded’ or ‘seriously degraded’ (Gaveau et al., 2016).
 42 Most studies of the impact this has on biodiversity have focussed on species composition
 43 (e.g. Edwards et al., 2011; Slade et al., 2011; Kitching et al., 2013; Struebig et al., 2013;
 44 Deere et al., 2018; Hayward et al., 2021). These often subtle changes to ecological
 45 communities can result in changes to ecosystem functioning (Ewers et al., 2015) and the
 46 structure of trophic networks (Hemprich-Bennett et al., 2020), indicating that selective
 47 logging may alter resilience to future perturbations. Understanding the ecological shifts
 48 that take place in degraded forest is of great importance for conservation, especially given
 49 the vast scale at which forest is managed for timber extraction globally.

50

51 Animal diet can differ between individuals of a species depending on numerous intrinsic
 52 and environmental factors. In insectivorous bats for example, inter-individual variation
 53 in diet appears to correlate with multiple factors, including wing morphology (Oliveira et
 54 al., 2020), sex (Burgar et al., 2014), reproductive condition (Czenze et al., 2018), season
 55 (Andriollo et al., 2019; Kolkert et al., 2020), geographic location (Czenze et al., 2018;

56 Vallejo et al., 2019), and habitat (Aizpurua et al., 2018; Hemprich-Bennett et al., 2020;
57 Tournayre et al., 2021). Such variation is of interest when because intraspecific
58 differences in the feeding behaviour of consumers can alter the abundance, community
59 composition and ecological functioning of their prey (Des Roches et al., 2018).

60

61 Intraspecific variation in diet is also an important consideration for research design. The
62 analysis of diet in a highly generalist species requires many observations to obtain a
63 representative sample. This can be especially true when studying the dietary ecology of
64 insectivorous bats through metabarcoding, as the technique gives an unprecedented level
65 of taxonomic resolution (Clare et al., 2009), highlighting variation which would not have
66 been apparent with morphological study. Inter-individual variation in bat diet is however
67 often obscured by the use of samples collected from underneath roosts, where numerous
68 bats are defecating (hereafter ‘roost-sourced’ samples) (Clare et al., 2014; Andriollo et
69 al., 2019) and samples cannot be linked to an individual. Obtaining faecal samples from
70 individually identifiable animals (hereafter ‘individual-sourced’ samples) is labour-
71 intensive due to the large trapping effort required, and so while many studies have used
72 individual-sourced samples (e.g. Czenze et al., 2018; Oliveira et al., 2020), their sample
73 sizes tend to be small. Mata et al (2018) used a dataset of individual-sourced samples to
74 analyse the importance of technical and biological replication on the dietary completeness
75 of *Tadarida teniotis* and reiterated the common rule of thumb that 20-50 such samples
76 per species is preferable, but stressed that higher sample sizes may be required for bat
77 species with greater dietary richness or intraspecific variation. The issue of sample size
78 is further complicated in networks generated from metabarcoding data because of
79 methodological considerations such as PCR primer bias and stochasticity (Alberdi et al.,

2018), and the influence of bioinformatic choices on the final data analysed (Hemprich-Bennett et al., 2021).

Here we use an unprecedented number of individually-sourced insectivorous bat faecal samples to test the hypothesis that selective logging alters the taxonomic composition and species richness of bats' diet. We also assess how sample size and bioinformatic parameters affect our inferences of insectivorous diet when using data derived from metabarcoding. Our evaluation focuses on the fawn leaf-nosed bat, *Hipposideros cervinus* - a cave-roosting insectivorous bat found throughout much of maritime Southeast Asia to northeastern Australia. Using high-duty cycle (HDC) echolocation, it is thought to use Doppler-shift compensation to detect the wingbeats of fluttering of prey such as moths (Bell and Fenton, 1984) against a cluttered backdrop (Schnitzler and Kalko, 2001; Lazure and Fenton, 2011). Although some bat species are negatively affected by logging, *H. cervinus* remains a dominant species in both old growth and logged forest in Borneo (Struebig et al., 2013; Hemprich-Bennett et al., 2020). It is not known whether bats such as *H. cervinus* respond to forest degradation by modifying their diets, or are able to maintain stable diets through prey selection or behavioural changes in foraging. We address three main predictions:

1) Taxonomic composition of the diet of *H. cervinus* is altered by rainforest degradation.

2) Individual bats are more specialised in logged forest sites than in primary forest.

3) Estimates of sampling completeness are heavily influenced by MOTU clustering threshold, quality-control methods used and the number of samples.

103 **Methods**

104 We sampled bats using six harp traps per night at four lowland tropical rainforest sites in
 105 Sabah, Malaysia, each <500m above sea level and limited seasonality. Two sites comprise
 106 mostly old growth rainforest (Danum Valley and Maliau Basin), and two sites have been
 107 subject to substantial logging disturbance (the Sabah Biodiversity Experiment and the
 108 Stability of Altered Forest Ecosystems Project) (Supplementary Table 1).

109 • Old growth rainforest:

110 ○ The Danum Valley Conservation Area (hereafter ‘Danum’) is a 438 km²
 111 region protected area of old growth rainforest in Sabah (Reynolds et al.,
 112 2011). Traps were erected in 2016 for ten nights in a 21-night period and
 113 2017 for ten nights in a 12-night period.

114 ○ The Maliau Basin Conservation Area (hereafter ‘Maliau’) is a 588 km²
 115 protected forest made up of lowland and hill forest, most of which has
 116 neither been logged nor inhabited in historical times. Traps were erected
 117 in 2016 and 2017 for ten nights in a 16-night period.

118 • Logged forest:

119 ○ The Stability of Altered Forest Ecosystems Project (hereafter ‘SAFE’) is
 120 a large area of degraded forest being converted to oil palm plantation, with
 121 fragments of forest retained for scientific study (Ewers et al., 2011). We
 122 sampled in the blocks ‘LFE’, ‘B’ and ‘C’, within the Ulu Segama Forest
 123 Reserve and Kalabakan area, during 2015, 2016 and 2017. Each block was
 124 sampled for a 5-night period, and then resampled at least 5 weeks later.

125 ○ The Sabah Biodiversity Experiment (Hector et al., 2011) (hereafter ‘SBE’)
 126 is an area of forest which was logged once in the 1950s and once in the
 127 2000s, and during the sampling period was in the early stages of
 128 enrichment replanting (Hector et al., 2011). Sampling took place over a
 129 total of 10 nights in a 20-night period in 2016.

130 Fieldwork, laboratory work and bioinformatics took place as previously described
 131 (Hemprich-Bennett et al., 2020). Briefly, bats were captured using harp traps erected
 132 along linear features such as streams and trails to target bat flyways. Sampling effort is
 133 summarised in Table 1. Faecal samples were processed by DNA extraction, PCR
 134 amplification of the CO1 gene using the primers described by Zeale et al (2011), and
 135 sequenced on an Illumina MiSeq. For complete methods see (Hemprich-Bennett et al.,
 136 2020).

137

Bioinformatics pipeline

139 Sequences were assembled into contigs using mothur (Schloss et al., 2009), and forward
 140 and reverse primers were removed using the galaxy web platform on the public server at
 141 usegalaxy.org (Afgan et al., 2016) sequence falling outside of a length of 155-159bp (2bp
 142 outside of the expected amplicon length) were excluded from analysis.

143

144 When processing the sequence data it is common to cluster sequences into MOTUs
 145 (Molecular Operational Taxonomic Units) (Floyd et al., 2002), on the basis of a given
 146 threshold of similarity, but the appropriate MOTU clustering thresholds required to best-
 147 represent the taxonomic diversity within metabarcoding samples are currently poorly

understood (Hemprich-Bennett et al., 2021). At high clustering thresholds routine sequencing errors may be falsely designated as distinct MOTU, artificially inflating the measured diversity and richness within a sample (Clare et al., 2016). Algorithms implemented using software such as LULU (Frøslev et al., 2017) have been proposed as a method of mitigating this, by combining probable duplicate MOTUs based on patterns of sequence similarity and cooccurrence.

To assess the impact of clustering threshold on the datasets analysed (Hemprich-Bennett et al., 2021) we generated datasets using MOTU clustering thresholds at ranges 91-98% similarity, using the Uclust algorithm (Edgar, 2010) as implemented in the QIIME platform (Caporaso et al., 2010). Representative sequences for each MOTU per clustering level were then compared to one another using BLAST+ (Camacho et al., 2009), with the resulting data being reduced in LULU (Frøslev et al., 2017) for quality control. All resulting bat-MOTU adjacency lists were then transformed into adjacency matrices using a custom perl script. These matrices were then split into multiple binary adjacency matrices by site. Networks were created by pooling samples from multiple years. To test prediction 2, separate analyses took place on networks both generated as composites of multiple years, and as separate networks for each site and year (see Table 1). All bioinformatic and statistical steps are recorded at

<https://github.com/hemprichbennett/hice>.

Prediction 1: Taxonomic composition of the diet of *H. cervinus* is altered by rainforest degradation

To analyse the prey taxa consumed by each bat, we used BLAST+ (Camacho et al., 2009) to compare all MOTUs to a library of all arthropod CO1 genes identified to species level

172 using the Barcode of Life Database on 28/03/2018 (BOLD) (Ratnasingham and Hebert,
173 2007) (3,319,062 sequences), and assigned them taxonomy in MEGAN 6 (Huson et al.,
174 2016) using the parameters in Salinas-Ramos *et al.* (2015). We then assigned MOTUs to
175 order and family level where possible, importing the resulting data into R for analysis,
176 and calculating the proportion of *H. cervinus* individuals per site consuming each
177 taxonomic order. To test the hypothesis that habitat type alters the order-level taxonomic
178 composition of the species' diet, we analysed the resulting values with a Chi-squared test.
179 The hypothesis was further tested using a permutational multivariate analysis of variance
180 test using distance matrices, and a non-metric multidimensional scaling ordination with
181 200 permutations using Bray-Curtis dissimilarity, both using the vegan package (Oksanen
182 et al., 2017) on datasets of the order-level diets of each individual bat. We also used a
183 similarity percentages analysis to identify the contribution of each taxonomic order to the
184 observed dissimilarity between sites and years, using Bray-Curtis dissimilarity.

185

186 We calculated correlations between the presence/absence of prey orders in faecal
187 samples, using the r package 'corrplot' (Wei and Simko, 2017), to identify both potential
188 significant correlations of prey consumption (e.g. bats that feed on Coleoptera may be
189 more likely to feed on Blattodea), and any potential taxonomic bias in PCR.

190 **Prediction 2: Individual bats are more specialised in logged forest sites than in old** 191 **growth forest**

192 We created binary bipartite networks for each sampling site and year at 95% similarity
193 clustering and quality control using LULU. In the networks each individual bat and
194 MOTU was classed as a distinct node. A criterion of 95% similarity was chosen for this
195 and all following analyses because it provided a balance between over and under-splitting

196 MOTUs (Hemprich-Bennett et al., 2021). Using the R package ‘bipartite’ (Dormann,
197 2011) in R 3.4.4 (R Core Team, 2017) these networks were then analysed using the
198 functions ‘specieslevel’, to calculate the degree of each bat (‘degree’ = the number of
199 prey nodes a bat consumes). Differences between the degree of individuals were
200 compared among sites using an ANOVA with Tukey’s HSD test.

201 **Prediction 3: Estimates of sampling completeness are heavily influenced by MOTU** 202 **clustering threshold and quality-control used**

203 Using networks generated at each clustering threshold between 91 and 98% similarity,
204 both with and without quality-control using LULU (Frøslev et al., 2017), we estimated
205 total MOTU richness and sampling completeness of the diet of *H. cervinus* at each site
206 and year using iNEXT (Hsieh et al., 2016), an R package for the interpolation and
207 extrapolation of species diversity using Hill numbers (Chao et al., 2014).

208

209 To assess how sample size affects assessments of bat diet, we generated multiple datasets
210 of n bats from each site, where n was a value of 10-100, increasing in increments of 10
211 (10, 20, 30, etc), with n bats taken at random from each site and the number of MOTUs
212 consumed in that sub-dataset calculated. This was repeated 100,000 times per site and
213 value of n , with the resulting data plotted in a violinplot.

214 Results

215 For the full sequencing run of multiple bat species (see Hemprich-Bennett et al., 2020)
 216 18,737,930 contiguous reads were output when assembling the paired-end files. After
 217 removing adapters and primers this was reduced to 10,064,815 sequences, which was
 218 then further reduced to 932,459 haplotypes after collapsing to haplotype, removing
 219 singletons and discarding sequences outside of 2bp of the expected read-length. For full
 220 counts of MOTUs before and after clustering with LULU, see Supplementary information
 221 2. Of these, 2,957,444 reads and 187,800 haplotypes were derived from *H. cervinus*
 222 samples and included in this study.

223 Prediction 1: Taxonomic composition of the diet of *H. cervinus* is altered by 224 rainforest degradation

225 The diet of the bat communities was dominated by insects from the orders Blattodea
 226 (especially family Ectobiidae), Diptera (especially family Cecidomyiidae) and
 227 Lepidoptera (Figure 1). The chi-squared test showed a non-significant effect of network
 228 identity on the order-level composition of a bat populations' diet ($\chi^2 = 0.16$, $df = 48$, $p >$
 229 0.05). The NMDS showed almost total overlap between the sites (Figure 2) with a stress
 230 of 0.21, showing poor convergence. The permutational multivariate analysis of variance
 231 test gave an R^2 of 0.014 for the explanatory power of site on bat diet. A total of 23
 232 arthropod orders were eaten based on the combined diets of all bats, with Blattodea,
 233 Coleoptera, Diptera and Lepidoptera collectively making up at least 79% of all MOTUs
 234 identified at each site. Positive correlations were observed between the occurrences of
 235 several taxa, with only Araneae and Hymenoptera being negatively correlated with the
 236 presence of one another (Supplementary information 3). Blattodea was the only taxon
 237 consistently observed to contribute significantly to inter-site dissimilarity scores (SAFE-

238 Maliau $p < 0.01$, SAFE-SBE $p = 0.014$, Maliau-SBE $p = 0.014$, SBE-Danum $p < 0.01$, see
239 Supplementary information 4). There was almost complete overlap between the different
240 years sampled at each site (Figure 2) and each site in 2016 (Figure 3).

241 **Prediction 2: Individual bats will be more specialised in logged forest sites than in**
242 **old growth forest**

243 When comparing networks with all years pooled together, significant differences
244 ($p < 0.05$) were only observed between Danum (old-growth) and SAFE (logged), and
245 between SAFE (logged) and SBE (logged).

246 **Prediction 3: Estimates of sampling completeness will be heavily influenced by**
247 **MOTU clustering threshold and quality-control used**

248 None of the networks were estimated as near to fully sampled, with all estimates placing
249 completeness at under 54% (Figure 4), with completeness estimates varying between both
250 sites and years. The number of MOTUs expected increased markedly with clustering
251 threshold when not using LULU for quality control, but this effect was dramatically
252 reduced when using LULU. This algorithm increased estimated sampling completeness
253 by reducing observed and estimated MOTU richness, and lowered the estimated number
254 of samples required to sample the community. Full counts can be found in Supplementary
255 information 2.

256

257 There was a positive correlation between the number of bats included in a dataset and the
258 number of MOTUs detected (figure 5)

259 Discussion

260 Logging is widespread in tropical forests, yet the consequences of this structural
261 disturbance for trophic interactions are poorly understood. Here we set out to assess how
262 the diet of a generalist insectivorous bat differs between old-growth and degraded forest
263 habitats. We observed broadly similar feeding habits in fawn leaf-nosed bats across forest
264 type with bats consuming many arthropod orders, particularly Blattodea, Coleoptera,
265 Diptera and Lepidoptera. Fawn leaf-nosed bats have extremely high dietary richness, with
266 many hundreds of samples being required to fully capture their diet.

267

268 We observed very little alteration in the taxonomic composition of the diet of *H. cervinus*.
269 We saw no significant difference between the consumption of prey at the order-level
270 between sites or years. This suggests that while northeast Borneo may possess high beta-
271 diversity of some insect species (Kitching et al., 2013), at coarse taxonomic levels there
272 is little spatial difference in the prey consumed by *H. cervinus*. Previous findings
273 suggested that, as high-duty cycle echolocators, *H. cervinus* primarily consumed flying
274 insects (Bell and Fenton, 1984; Link et al., 1986; Schnitzler and Kalko, 2001; Lazure and
275 Fenton, 2011), in particular Lepidoptera, Blattodea, Diptera and Coleoptera. The regular
276 presence of diverse families of spiders indicates a dietary contribution of these taxa
277 previously unknown in the Hipposideridae family of bats. Hipposiderids have been
278 observed gleaning stationary targets with fluttering wings (Bell and Fenton, 1984), but
279 the consumption of spiders would either suggest they are gleaning non-fluttering animals,
280 or taking them when ballooning as juveniles. Alternatively, the consumption of spiders
281 could be due to secondary predation: where the bat consumes a primary prey item which
282 has ingested a spider. This seems an unlikely explanation for our dataset, since predatory

arthropods other than Araneae are poorly represented in the MOTU dataset. In this study we used one of the most reliable primer sets for amplification of a wide range of digested arthropods (Zeale et al., 2011; Alberdi et al., 2018), but they are also reported to have taxonomic biases towards Diptera and Lepidoptera. However, we found no significant negative correlations between detecting Dipteran or Lepidopteran DNA in a sample, and the detection of any other prey order. This indicates that amplification of dipteran or lepidopteran DNA did not consistently inhibit the amplification of another taxonomic order during PCR, and that sequencing depth is sufficient.

291

There was no clear pattern of degree differing between logged and old growth habitats. This is in contrast to our previous findings in these study sites (Hemprich-Bennett et al., 2020), that the overall assemblage of bat species in these sites consistently had reduced degree in logged forest than old growth. The diversity of the overall bats' diet is likely due to the high diversity of prey available to them, and the lack of observed differences in diet between sites may indicate highly flexible foraging, with low impact of land-use change on their diets. Being able to forage adaptively, or fly long distances to viable feeding sites (Struebig et al., 2009) may enable them to remain abundant despite selective logging, while conspecific species experience population declines (Struebig et al., 2013). This species may, as a result, provide ecological redundancy and continue to contribute insectivory when more sensitive bat species have become locally extinct.

303

A crucial concern in network ecology is the minimum number of samples or observations required to characterise reliably the structure and identity of the interactions within a network (Nielsen and Bascompte, 2007; Rivera-Hutinel et al., 2012). This requirement is

307 complicated in studies utilising DNA metabarcoding as the number of nodes generated is
 308 dependent on the bioinformatic choices used to generate them. While MOTU approaches
 309 frequently apply a standard resolution to all nodes which helps control for variation in
 310 identification, altering MOTU clustering threshold will change the number of nodes and
 311 estimates of completeness, analogous to lumping taxonomy-based identifications to
 312 higher levels, but without a biological equivalent. We tested MOTU clustering and the
 313 use of LULU for quality-control and demonstrated that it was possible to alter estimates
 314 of sampling completeness greatly (Figure 4). However, when generating networks with a
 315 range of bioinformatics combinations, we observed that none exceeded an estimate of
 316 50% completeness and thus regardless of parameters used, obtaining the full estimate of
 317 *H. cervinus* diet would require several hundred samples per site, with the same likely true
 318 of many ecologically similar species. Altering MOTU clustering parameters has
 319 previously been shown to cause great variation in MOTU counts (Clare et al., 2016) and
 320 changes in numerous measures of network-level architecture (Hemprich-Bennett et al.,
 321 2021). The reduction in number of estimated MOTUs provided by LULU (Frøslev et al.,
 322 2017) is expected to be of great use in future metabarcoding-based studies to reduce
 323 spurious MOTU generation.

324

325 The dietary richness found here echoes previous studies (Clare et al., 2009; McCracken
 326 et al., 2012) but raises question about the capacity of bats to distinguish between prey
 327 types in detail (Neuweiler, 1990) and if this has implications for prey-choice. At the same
 328 time, our results highlight the substantial challenge of characterising the diets of this and
 329 other insectivorous bat species, especially in hyperdiverse ecosystems such as tropical
 330 rainforests. Their large dietary breadth is further highlighted by the fact that DNA
 331 extractions performed here were for pooled faecal samples from each individual bat, a

332 technique which Mata *et al.* (Mata et al., 2018) found underestimated the total richness
 333 of the diet per bat. Previous intensive studies of arthropod diversity in lowland tropical
 334 rainforest have failed to reach an asymptote (Novotný and Basset, 2000; Basset et al.,
 335 2012), and if bats are foraging opportunistically it is perhaps unsurprising that the
 336 taxonomic breadth of their diet is extremely large and nearly impossible to sample
 337 completely.

338

339 We demonstrate the vast richness of prey consumed by insectivorous bats in tropical
 340 rainforest and show that although quality-control steps in metabarcoding can reduce our
 341 estimates of the number of distinct prey items in a site, many hundreds of samples are
 342 required to collect a representative description of total diet. Although we focussed our
 343 sampling on a single species of insectivorous bat, some inferences likely also apply to
 344 similar species, and to other studies that use metabarcoding. The number of sites analysed
 345 in this study was low, but it has been shown here that this Hipposiderid species has a
 346 highly diverse diet; relying on cockroaches more than previously thought and potentially
 347 having a strategy of gleaning non-fluttering prey previously unknown in the family. This
 348 bat species is thus thought to exhibit low levels of dietary response to habitat degradation,
 349 potentially indicating reasons for their known versatility in the face of landscape
 350 modification.

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375 Works cited

- 376 Afgan, E., Baker, D., van den Beek, M., Blankenberg, D., Bouvier, D., Čech, M., et al.
377 (2016). The Galaxy platform for accessible, reproducible and collaborative
378 biomedical analyses: 2016 update. *Nucleic Acids Res.* 44, W3–W10.
379 doi:10.1093/nar/gkw343.
- 380 Aizpurua, O., Budinski, I., Georgiakakis, P., Gopalakrishnan, S., Ibañez, C., Mata, V.,
381 et al. (2018). Agriculture shapes the trophic niche of a bat preying on multiple
382 pest arthropods across Europe: Evidence from DNA metabarcoding. *Mol. Ecol.*
383 27, 815–825. doi:<https://doi.org/10.1111/mec.14474>.
- 384 Alberdi, A., Aizpurua, O., Gilbert, M. T. P., and Bohmann, K. (2018). Scrutinizing key
385 steps for reliable metabarcoding of environmental samples. *Methods Ecol. Evol.*
386 9, 134–147. doi:10.1111/2041-210X.12849.
- 387 Andriollo, T., Gillet, F., Michaux, J. R., and Ruedi, M. (2019). The menu varies with
388 metabarcoding practices: A case study with the bat *Plecotus auritus*. *PLOS ONE*
389 14, e0219135. doi:10.1371/journal.pone.0219135.
- 390 Asner, G. P., Rudel, T. K., Aide, T. M., Defries, R., and Emerson, R. (2009). A
391 Contemporary Assessment of Change in Humid Tropical Forests. *Conserv. Biol.*
392 23, 1386–1395. doi:<https://doi.org/10.1111/j.1523-1739.2009.01333.x>.
- 393 Basset, Y., Cizek, L., Cuénoud, P., Didham, R. K., Guilhaumon, F., Missa, O., et al.
394 (2012). Arthropod Diversity in a Tropical Forest. *Science* 338, 1481–1484.
395 doi:10.1126/science.1226727.
- 396 Bell, G. P., and Fenton, M. B. (1984). The use of Doppler-shifted echoes as a flutter
397 detection and clutter rejection system: the echolocation and feeding behavior of
398 *Hipposideros ruber* (Chiroptera: Hipposideridae). *Behav. Ecol. Sociobiol.* 15,
399 109–114. doi:10.1007/BF00299377.
- 400 Burgar, J. M., Murray, D. C., Craig, M. D., Haile, J., Houston, J., Stokes, V., et al.
401 (2014). Who's for dinner? High-throughput sequencing reveals bat dietary
402 differentiation in a biodiversity hotspot where prey taxonomy is largely
403 undescribed. *Mol. Ecol.* 23, 3605–3617. doi:10.1111/mec.12531.
- 404 Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., et al.
405 (2009). BLAST+: architecture and applications. *BMC Bioinformatics* 10, 421.
406 doi:10.1186/1471-2105-10-421.
- 407 Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., and Bushman, F. D.
408 (2010). QIIME allows analysis of high-throughput community sequencing data.
409 *Nat. Methods* 7, 335–336. doi:10.1038/nmeth.f.303.
- 410 Chao, A., Gotelli, N. J., Hsieh, T. C., Sande, E. L., Ma, K. H., Colwell, R. K., et al.
411 (2014). Rarefaction and extrapolation with Hill numbers: a framework for
412 sampling and estimation in species diversity studies. *Ecol. Monogr.* 84, 45–67.

- 413 Clare, E. L., Chain, F. J. J., Littlefair, J. E., and Cristescu, M. E. (2016). The effects of
414 parameter choice on defining molecular operational taxonomic units and
415 resulting ecological analyses of metabarcoding data. *Genome* 59, 981–990.
416 doi:10.1139/gen-2015-0184.
- 417 Clare, E. L., Fraser, E. E., Braid, H. E., Fenton, M. B., and Hebert, P. D. N. (2009).
418 Species on the menu of a generalist predator, the eastern red bat *Lasiurus*
419 *borealis*: using a molecular approach to detect arthropod prey. *Mol. Ecol.* 18,
420 2532–2542. doi:10.1111/j.1365-294X.2009.04184.x.
- 421 Clare, E. L., Symondson, W. O. C., Broders, H., Fabianek, F., Fraser, E. E., MacKenzie,
422 A., et al. (2014). The diet of *Myotis lucifugus* across Canada: assessing foraging
423 quality and diet variability. *Mol. Ecol.* 23, 3618–3632. doi:10.1111/mec.12542.
- 424 Czenze, Z. J., Tucker, J. L., Clare, E. L., Littlefair, J. E., Hemprich-Bennett, D. R.,
425 Oliveira, H. F. M., et al. (2018). Spatiotemporal and demographic variation in
426 the diet of New Zealand lesser short-tailed bats (*Mystacina tuberculata*). *Ecol.*
427 *Evol.* 8, 7599–7610. doi:10.1002/ece3.4268.
- 428 Deere, N. J., Guillera-Aroita, G., Baking, E. L., Bernard, H., Pfeifer, M., Reynolds, G.,
429 et al. (2018). High Carbon Stock forests provide co-benefits for tropical
430 biodiversity. *J. Appl. Ecol.* 55, 997–1008. doi:https://doi.org/10.1111/1365-
431 2664.13023.
- 432 Des Roches, S., Post, D. M., Turley, N. E., Bailey, J. K., Hendry, A. P., Kinnison, M.
433 T., et al. (2018). The ecological importance of intraspecific variation. *Nat. Ecol.*
434 *Evol.* 2, 57–64. doi:10.1038/s41559-017-0402-5.
- 435 Dormann, C. F. (2011). How to be a specialist? Quantifying specialisation in pollination
436 networks. *Netw. Biol.* 1, 1–20.
- 437 Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST.
438 *Bioinformatics* 26, 2460–2461. doi:10.1093/bioinformatics/btq461.
- 439 Edwards, D. P., Larsen, T. H., Docherty, T. D. S., Ansell, F. A., Hsu, W. W., Derhé, M.
440 A., et al. (2011). Degraded lands worth protecting: the biological importance of
441 Southeast Asia’s repeatedly logged forests. *Proc. R. Soc. B Biol. Sci.* 278, 82–
442 90. doi:10.1098/rspb.2010.1062.
- 443 Ewers, R. M., Boyle, M. J. W., Gleave, R. A., Plowman, N. S., Benedick, S., Bernard,
444 H., et al. (2015). Logging cuts the functional importance of invertebrates in
445 tropical rainforest. *Nat. Commun.* 6, 6836. doi:10.1038/ncomms7836.
- 446 Ewers, R. M., Didham, R. K., Fahrig, L., Ferraz, G., Hector, A., Holt, R. D., et al.
447 (2011). A large-scale forest fragmentation experiment: the Stability of Altered
448 Forest Ecosystems Project. *Philos. Trans. R. Soc. B Biol. Sci.* 366, 3292–3302.
449 doi:10.1098/rstb.2011.0049.
- 450 Floyd, R., Abebe, E., Papert, A., and Blaxter, M. (2002). Molecular barcodes for soil
451 nematode identification. *Mol. Ecol.* 11, 839–850. doi:10.1046/j.1365-
452 294X.2002.01485.x.

- 453 Frøslev, T. G., Kjølner, R., Bruun, H. H., Ejrnæs, R., Brunbjerg, A. K., Pietroni, C., et
454 al. (2017). Algorithm for post-clustering curation of DNA amplicon data yields
455 reliable biodiversity estimates. *Nat. Commun.* 8, 1188. doi:10.1038/s41467-017-
456 01312-x.
- 457 Gaveau, D. L. A., Sheil, D., Husnayaen, Salim, M. A., Arjasakusuma, S., Ancrenaz, M.,
458 et al. (2016). Rapid conversions and avoided deforestation: examining four
459 decades of industrial plantation expansion in Borneo. *Sci. Rep.* 6, 32017.
460 doi:10.1038/srep32017.
- 461 Gaveau, D. L. A., Sloan, S., Molidena, E., Yaen, H., Sheil, D., Abram, N. K., et al.
462 (2014). Four Decades of Forest Persistence, Clearance and Logging on Borneo.
463 *PLOS ONE* 9, e101654. doi:10.1371/journal.pone.0101654.
- 464 Hayward, R. M., Banin, L. F., Burslem, D. F. R. P., Chapman, D. S., Philipson, C. D.,
465 Cutler, M. E. J., et al. (2021). Three decades of post-logging tree community
466 recovery in naturally regenerating and actively restored dipterocarp forest in
467 Borneo. *For. Ecol. Manag.* 488, 119036. doi:10.1016/j.foreco.2021.119036.
- 468 Hector, A., Philipson, C., Saner, P., Chamagne, J., Dzulkifli, D., O'Brien, M., et al.
469 (2011). The Sabah Biodiversity Experiment: a long-term test of the role of tree
470 diversity in restoring tropical forest structure and functioning. *Philos. Trans. R.*
471 *Soc. Lond. B Biol. Sci.* 366, 3303–3315. doi:10.1098/rstb.2011.0094.
- 472 Hemprich-Bennett, D. R., Kemp, V. A., Blackman, J., Struebig, M. J., Lewis, O. T.,
473 Rossiter, S. J., et al. (2020). Altered structure and stability of bat-prey
474 interaction networks in logged tropical forests revealed by metabarcoding.
475 *bioRxiv*, 2020.03.20.000331. doi:10.1101/2020.03.20.000331.
- 476 Hemprich-Bennett, D. R., Oliveira, H. F. M., Comber, S. C. L., Rossiter, S. J., and
477 Clare, E. L. (2021). Assessing the impact of taxon resolution on network
478 structure. *Ecology* 102, e03256. doi:https://doi.org/10.1002/ecy.3256.
- 479 Hsieh, T. C., Ma, K. H., and Chao, A. (2016). *iNEXT: Interpolation and Extrapolation*
480 *for Species Diversity*. Available at: [http://chao.stat.nthu.edu.tw/blog/software-](http://chao.stat.nthu.edu.tw/blog/software-download/)
481 [download/](http://chao.stat.nthu.edu.tw/blog/software-download/).
- 482 Huson, D. H., Beier, S., Flade, I., Górski, A., El-Hadidi, M., Mitra, S., et al. (2016).
483 MEGAN Community Edition - Interactive Exploration and Analysis of Large-
484 Scale Microbiome Sequencing Data. *PLOS Comput. Biol.* 12, e1004957.
485 doi:10.1371/journal.pcbi.1004957.
- 486 Kitching, R. L., Ashton, L. A., Nakamura, A., Whitaker, T., and Khen, C. V. (2013).
487 Distance-driven species turnover in Bornean rainforests: homogeneity and
488 heterogeneity in primary and post-logging forests. *Ecography* 36, 675–682.
489 doi:10.1111/j.1600-0587.2012.00023.x.
- 490 Kolkert, H., Andrew, R., Smith, R., Rader, R., and Reid, N. (2020). Insectivorous bats
491 selectively source moths and eat mostly pest insects on dryland and irrigated
492 cotton farms. *Ecol. Evol.* 10, 371–388. doi:https://doi.org/10.1002/ece3.5901.

- 493 Lazure, L., and Fenton, M. B. (2011). High duty cycle echolocation and prey detection
494 by bats. *J. Exp. Biol.* 214, 1131–1137. doi:10.1242/jeb.048967.
- 495 Link, A., Marimuthu, G., and Neuweiler, G. (1986). Movement as a specific stimulus
496 for prey catching behaviour in rhinolophid and hipposiderid bats. *J. Comp.*
497 *Physiol. A* 159, 403–413. doi:10.1007/BF00603985.
- 498 Mata, V. A., Rebelo, H., Amorim, F., McCracken, G. F., Jarman, S., and Beja, P.
499 (2018). How much is enough? Effects of technical and biological replication on
500 metabarcoding dietary analysis. *Mol. Ecol.* 0. doi:10.1111/mec.14779.
- 501 McCracken, G. F., Westbrook, J. K., Brown, V. A., Eldridge, M., Federico, P., and
502 Kunz, T. H. (2012). Bats Track and Exploit Changes in Insect Pest Populations.
503 *PLoS ONE* 7, e43839. doi:10.1371/journal.pone.0043839.
- 504 Milodowski, D. T., Coomes, D. A., Swinfield, T., Jucker, T., Riutta, T., Malhi, Y., et al.
505 (2021). The impact of logging on vertical canopy structure across a gradient of
506 tropical forest degradation intensity in Borneo. *J. Appl. Ecol.*
507 doi:<https://doi.org/10.1111/1365-2664.13895>.
- 508 Neuweiler, G. (1990). Auditory adaptations for prey capture in echolocating bats.
509 *Physiol. Rev.* 70, 615–641. doi:10.1152/physrev.1990.70.3.615.
- 510 Nielsen, A., and Bascompte, J. (2007). Ecological networks, nestedness and sampling
511 effort. *J. Ecol.* 95, 1134–1141. doi:10.1111/j.1365-2745.2007.01271.x.
- 512 Novotný, V., and Basset, Y. (2000). Rare species in communities of tropical insect
513 herbivores: pondering the mystery of singletons. *Oikos* 89, 564–572.
514 doi:10.1034/j.1600-0706.2000.890316.x.
- 515 Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., et al.
516 (2017). *vegan: Community Ecology Package*. Available at: [https://CRAN.R-](https://CRAN.R-project.org/package=vegan)
517 [project.org/package=vegan](https://CRAN.R-project.org/package=vegan).
- 518 Oliveira, H. F. M. de, Camargo, N. F., Hemprich-Bennett, D. R., Rodríguez-Herrera, B.,
519 Rossiter, S. J., and Clare, E. L. (2020). Wing morphology predicts individual
520 niche specialization in *Pteronotus mesoamericanus* (Mammalia: Chiroptera).
521 *PLOS ONE* 15, e0232601. doi:10.1371/journal.pone.0232601.
- 522 R Core Team (2017). *R: A Language and Environment for Statistical Computing*.
523 Vienna, Austria: R Foundation for Statistical Computing Available at:
524 <https://www.R-project.org/>.
- 525 Ratnasingham, S., and Hebert, P. D. (2007). BOLD: The Barcode of Life Data System
526 (<http://www.barcodinglife.org>). *Mol. Ecol. Resour.* 7, 355–364.
527 doi:10.1111/j.1471-8286.2007.01678.x.
- 528 Reynolds, G., Payne, J., Sinun, W., Mosigil, G., and Walsh, R. P. D. (2011). Changes in
529 forest land use and management in Sabah, Malaysian Borneo, 1990–2010, with a
530 focus on the Danum Valley region. *Philos. Trans. R. Soc. B Biol. Sci.* 366,
531 3168–3176. doi:10.1098/rstb.2011.0154.

- 532 Rivera-Hutinel, A., Bustamante, R. O., Marín, V. H., and Medel, R. (2012). Effects of
533 sampling completeness on the structure of plant–pollinator networks. *Ecology*
534 93, 1593–1603. doi:10.1890/11-1803.1.
- 535 Salinas-Ramos, V. B., Herrera Montalvo, L. G., León-Regagnon, V., Arrizabalaga-
536 Escudero, A., and Clare, E. L. (2015). Dietary overlap and seasonality in three
537 species of mormoopid bats from a tropical dry forest. *Mol. Ecol.* 24, 5296–5307.
538 doi:10.1111/mec.13386.
- 539 Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., et
540 al. (2009). Introducing mothur: Open-Source, Platform-Independent,
541 Community-Supported Software for Describing and Comparing Microbial
542 Communities. *Appl. Environ. Microbiol.* 75, 7537–7541.
543 doi:10.1128/AEM.01541-09.
- 544 Schnitzler, H.-U., and Kalko, E. K. V. (2001). Echolocation by Insect-Eating Bats.
545 *BioScience* 51, 557–569. doi:10.1641/0006-
546 3568(2001)051[0557:EBIEB]2.0.CO;2.
- 547 Slade, E. M., Mann, D. J., and Lewis, O. T. (2011). Biodiversity and ecosystem
548 function of tropical forest dung beetles under contrasting logging regimes. *Biol.*
549 *Conserv.* 144, 166–174. doi:10.1016/j.biocon.2010.08.011.
- 550 Struebig, M. J., Kingston, T., Zubaid, A., Le Comber, S. C., Mohd-Adnan, A., Turner,
551 A., et al. (2009). Conservation importance of limestone karst outcrops for
552 Palaeotropical bats in a fragmented landscape. *Biol. Conserv.* 142, 2089–2096.
553 doi:10.1016/j.biocon.2009.04.005.
- 554 Struebig, M. J., Turner, A., Giles, E., Lasmana, F., Tollington, S., Bernard, H., et al.
555 (2013). Quantifying the Biodiversity Value of Repeatedly Logged Rainforests.
556 *Adv. Ecol. Res.* 48, 183–224. doi:10.1016/B978-0-12-417199-2.00003-3.
- 557 Tournayre, O., Leuchtman, M., Galan, M., Trillat, M., Piry, S., Pinaud, D., et al.
558 (2021). eDNA metabarcoding reveals a core and secondary diets of the greater
559 horseshoe bat with strong spatio-temporal plasticity. *Environ. DNA* 3, 277–296.
560 doi:https://doi.org/10.1002/edn3.167.
- 561 Vallejo, N., Aihartza, J., Goiti, U., Arrizabalaga-Escudero, A., Flaquer, C., Puig, X., et
562 al. (2019). The diet of the notch-eared bat (*Myotis emarginatus*) across the
563 Iberian Peninsula analysed by amplicon metabarcoding. *Hystrix Ital. J.*
564 *Mammal.* 30, 59–64. doi:10.4404/hystrix-00189-2019.
- 565 Wei, T., and Simko, V. (2017). R package “corrplot”: Visualization of a correlation
566 matrix. Available at: <https://github.com/taiyun/corrplot>.
- 567 Zeale, M. R. K., Butlin, R. K., Barker, G. L. A., Lees, D. C., and Jones, G. (2011).
568 Taxon-specific PCR for DNA barcoding arthropod prey in bat faeces. *Mol. Ecol.*
569 *Resour.* 11, 236–244. doi:10.1111/j.1755-0998.2010.02920.x.

570

571 **Tables**

572 Table 1. Trapping effort per site, in harp trap nights. One harp trap night is a harp trap
573 erected for a single night. Six harp traps were used per night, so a single night's trapping
574 was equal to six harp trap nights.

Sample Site	2015	2016	2017
SAFE	216	180	180
Danum	0	60	60
Maliau	0	60	60
SBE	0	60	0

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576 **Figures**

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579 Figure 1. The proportion of all individual bats within a sampling event found to consume each potential prey order. Diptera, Lepidoptera and Blattodea
 580 were the commonest prey items, with other prey orders being consumed rarely. The grey background shows locations in the plot where no arthropods of
 581 that order were detected in any bats.

582

583 Figure 2. Non-Metric Multidimensional Scaling ordination of the order-level consumption of individual bats across multiple years. The ellipses of each
 584 site show almost complete overlap. Stress was 0.21, indicating poor convergence. Danum and Maliau are old-growth sites, SAFE is a logged forest site.

Figure 3. Non-Metric Multidimensional Scaling ordination of the order-level consumption of individual bats in 2016. The ellipses of each site show almost complete overlap. Stress was 0.22, indicating poor convergence. Danum and Maliau are old-growth sites, SAFE and SBE are logged forest sites.

Figure 4. Completeness and richness for each network over a range of MOTU clustering thresholds, with and without use of LULU for post-clustering quality-control. Number of MOTUs is strongly positively correlated with clustering level when not using LULU for quality-control, reducing the estimated completeness of each network.

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Figure 5: Violinplots showing the distribution of the number of MOTUs consumed when reducing a dataset to n bats. With small datasets, sites appear to be rather similar in MOTU richness, but differences emerge as sample sizes increase.