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Methods matter: Different biodiversity survey methodologies identify contrasting biodiversity patterns in a human modified rainforest — A case study with amphibians

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1 **TITLE PAGE**

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3 **Methods Matter: Different Biodiversity Survey Methodologies Identify Contrasting**
4 **Biodiversity Patterns in a Human Modified Rainforest; a Case Study with Amphibians.**

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28 **ABSTRACT**

29 Understanding how well tropical forest biodiversity can recover following habitat change is
30 often difficult due to conflicting assessments arising from different studies. One often
31 overlooked potentially confounding factor that may influence assessments of biodiversity
32 response to habitat change, is the possibility that different survey methodologies, targeting
33 the same indicator taxon, may identify different patterns and so lead to different conclusions.
34 Here we investigated whether two different but commonly used survey methodologies used
35 to assess amphibian communities, pitfall trapping and nocturnal transects, indicate the same
36 or different responses of amphibian biodiversity to historic human induced habitat change.
37 We did so in a regenerating rainforest study site located in one of the world's most biodiverse
38 and important conservation areas: the Manu Biosphere Reserve. We show that the two survey
39 methodologies tested identified contrasting biodiversity patterns in a human modified
40 rainforest. Nocturnal transect surveys indicated biodiversity differences between forest with
41 different human disturbance histories, whereas pitfall trap surveys suggested no differences
42 between forest disturbance types, except for community composition. This pattern was true
43 for species richness, diversity, overall abundance and community evenness and structure. For
44 some fine scale metrics, such as species specific responses and abundances of family groups,
45 both methods detected differences between disturbance types. However, the direction of
46 differences was inconsistent between methods. We highlight that for assessments of
47 rainforest recovery following disturbance, survey methods do matter and that different
48 biodiversity survey methods can identify contrasting patterns in response to different types of
49 historic disturbance. Our results contribute to a growing body of evidence that arboreal
50 species might be more sensitive indicators than terrestrial communities.

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58 *Keywords:* amphibians; habitat disturbance; regeneration; tropical forest; indicators.

59 **1. INTRODUCTION**

60 There is a pressing need to better understand future biodiversity and conservation value of
61 tropical rainforest following human disturbance (Arroyo-Rodríguez et al. 2015; Dent and
62 Wright 2009; Kinnaird et al. 2003; Peres et al. 2006), especially as the Global Forest
63 Resources Assessment (FAO 2010) classifies just 36% of global forest cover as primary.
64 Despite regenerating landscapes representing the majority of remaining tropical forest, the
65 potential of such human-modified forests to provide important habitat for rainforest
66 biodiversity is contentious (Chazdon et al. 2009a, 2009b; Gibson et al. 2011). As human
67 populations in tropical countries increase and primary forest is converted to agricultural land
68 and later abandoned, some authors suggest that secondary forests will become increasingly
69 important for conservation (Anand et al. 2010; Arroyo-Rodríguez et al. 2015; Chazdon et al.
70 2009a; Durães et al. 2013; Irwin et al. 2010; Letcher and Chazdon 2009; Norris et al. 2010;
71 Tabarelli et al. 2010), while others suggest that the major conservation priority is to protect
72 remaining primary forest (Barlow et al. 2007a; Gibson et al. 2011; Sodhi et al. 2010); a
73 debate that has been widely discussed (Chazdon et al. 2009a; Dent and Wright 2009; Melo et
74 al. 2013).

75 Determining how well tropical forest biodiversity can recover is difficult (Gardner et al.
76 2010) as studies from different locations often produce contrasting results. There are many
77 potential reasons for different studies to identify contrasting patterns; including geographic
78 context, study scale, potential on-going human impacts, timeframe since disturbance
79 (Chazdon et al. 2009a) and a tendency to focus on overall species richness patterns (Anand et
80 al. 2010; Barlow et al. 2007a). However, one factor often overlooked is the potential for
81 different survey methodologies, targeting the same indicator taxon (such as amphibians,
82 butterflies and understory birds), to provide different results on the response of biodiversity
83 to habitat change (Barlow et al. 2007b). In the case of butterflies for example, line transect
84 studies carried out in a number of locations have suggested that butterfly biodiversity does
85 not show a significant degree of difference between human disturbed and primary forest
86 (Devy and Davidar 2001; Kudavidanage et al. 2012; Posa and Sodhi 2006). In contrast,
87 studies using traps undertaken at other sites suggest that butterfly biodiversity does show a
88 significant degree of difference between human disturbed and primary forest (Dumbrell and
89 Hill 2005; Ribeiro and Freitas 2012; Ribeiro et al. 2015). Additionally, bird studies carried
90 out in different locations and based upon different survey methodologies have also found
91 contrasting patterns (Barlow et al. 2007b). In some locations secondary forests display

92 similar biodiversity levels to primary forest based on mist net methodologies (Barlow et al.
93 2007b; Srinivasan et al. 2015; Waltert et al. 2005), but other studies using point counts have
94 suggested that secondary forest may have significantly lower levels than primary (Barlow et
95 al. 2007b; Carillo-Rubio et al. 2014). Understanding more about how these contrasting
96 patterns might relate to differences due to survey methodologies can therefore help to
97 improve our ability to assess the true value of regenerating tropical forests and better
98 understand the response of specific communities. Otherwise, assessments of a specific
99 community may under or overestimate the potential biodiversity value for such forests,
100 especially if the results from single surveys are over generalised (Barlow et al. 2007b).

101 One key taxonomic indicator group utilised to study the impacts of habitat disturbance in
102 tropical forests are amphibians, chosen due to their high conservation importance (31% of
103 evaluated species are threatened with extinction; IUCN 2015), and because they are key
104 components within their ecosystems (Ficetola et al. 2014; Hocking and Babbitt 2014).
105 Amphibians display a high level of sensitivity to disturbance due to low mobility, limited
106 dispersal capacity and narrow ecological requirements (Lawler 2010). Habitat change is
107 therefore likely to affect amphibians more severely than other vertebrate groups (Ficetola et
108 al. 2014); especially as small changes in vegetation structure can create significant alterations
109 to amphibian communities (Cortés-Gómez et al. 2013). As a result, habitat destruction and
110 fragmentation are among the leading causes of the global threat to amphibians (Catennazi and
111 Von May 2014; Eigenbrod et al. 2008), especially in tropical regions where levels of
112 diversity are highest (Ficetola et al. 2015).

113 So far, investigations using amphibians to assess rainforest biodiversity response to
114 habitat change often use different survey methodologies and describe contrasting patterns
115 from different locations. Hilje and Aide (2012), for example, utilised diurnal and nocturnal
116 visual searches and acoustic surveys in Costa Rica and found that even young regenerating
117 forest had similar amphibian species richness and composition to primary forest. In contrast,
118 Gardner et al. (2007), using terrestrial traps and diurnal visual searches to target leaf litter
119 amphibians in Brazil, found just two-thirds of primary forest amphibian species in
120 regenerating forest. Finally, Seshadri (2014) utilised quadrats to assess amphibian
121 biodiversity in selectively logged forests of southern India, detecting a 42% lower density of
122 amphibians than in primary forest; and even though species richness and composition were
123 converging with primary forest levels, the effects of logging were still detectable. These
124 results therefore raise the question of whether the lack of a consistent pattern in detected

125 amphibian responses is driven by site specific factors or whether such differences could be
126 caused by different methods that focus on different groups of amphibian communities.

127 Here we investigate whether two different but commonly used biodiversity survey
128 methodologies, pitfall trapping and nocturnal transects (Doan 2003; Dodd 2010; Heyer et al.
129 1994), find the same or different responses of amphibian biodiversity in areas with different
130 historic human induced habitat change. We do so in a regenerating rainforest study site
131 located in one of the world's most biodiverse and important conservation areas, the Manu
132 Biosphere Reserve, a UNESCO World Heritage Site designated to protect the globally
133 important Amazon rainforest and its biodiversity. Specifically, we quantified and compared
134 species richness, diversity, abundance, community structure and composition of amphibian
135 communities using both pitfall traps and nocturnal transect surveys, between areas of old
136 regenerating forest, following different types of historic human disturbance. We predict that
137 as each survey methodology likely targets a different subset of the amphibian community,
138 each method will likely show a different degree of biodiversity response to habitat
139 disturbance, or even display responses in opposing directions. Our null hypothesis would find
140 no difference in the degree of difference detected of biodiversity patterns for both survey
141 methodologies.

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144 **2. METHODS**

145 2.1 STUDY SITE

146 The study was carried out at the Manu Learning Centre (MLC) research station in the
147 Peruvian Amazon (71°23'28"W 12°47'21"S). The site (described in detail in Whitworth et
148 al. 2016a) is within the Manu Biosphere Reserve, which consists of a network of core
149 protected areas surrounded by areas designated as cultural buffer zones due to historically
150 high human impact, including extensive logging or clearance for subsistence agriculture. The
151 study site lay within one of these cultural buffer zones. It consists of ~800ha of regenerating
152 lowland tropical forest.

153 Three different anthropogenic disturbance types had occurred: 1) selective logging (SLR
154 – selectively logged and now regenerating forest), 2) complete clearance due to conversion to
155 agriculture for coffee and cacao (CCR – completely cleared and now regenerating forest), and
156 3) a mixed area that had historically consisted of a mosaic of small completely cleared areas
157 used for agriculture combined with selective logging of the adjacent forest (MXD – mixed
158 disturbance and now regenerating forest). Major human disturbance had started ~50 years
159 prior to the study and lasted for 20 years before systematic human disturbance activities were
160 abandoned in the 1980s. For 30 years following abandonment the site was left to regenerate,
161 and from 2003 the site was actively protected from further human disturbance. At the time of
162 the study the whole area was covered by closed canopy regenerating tropical forest.

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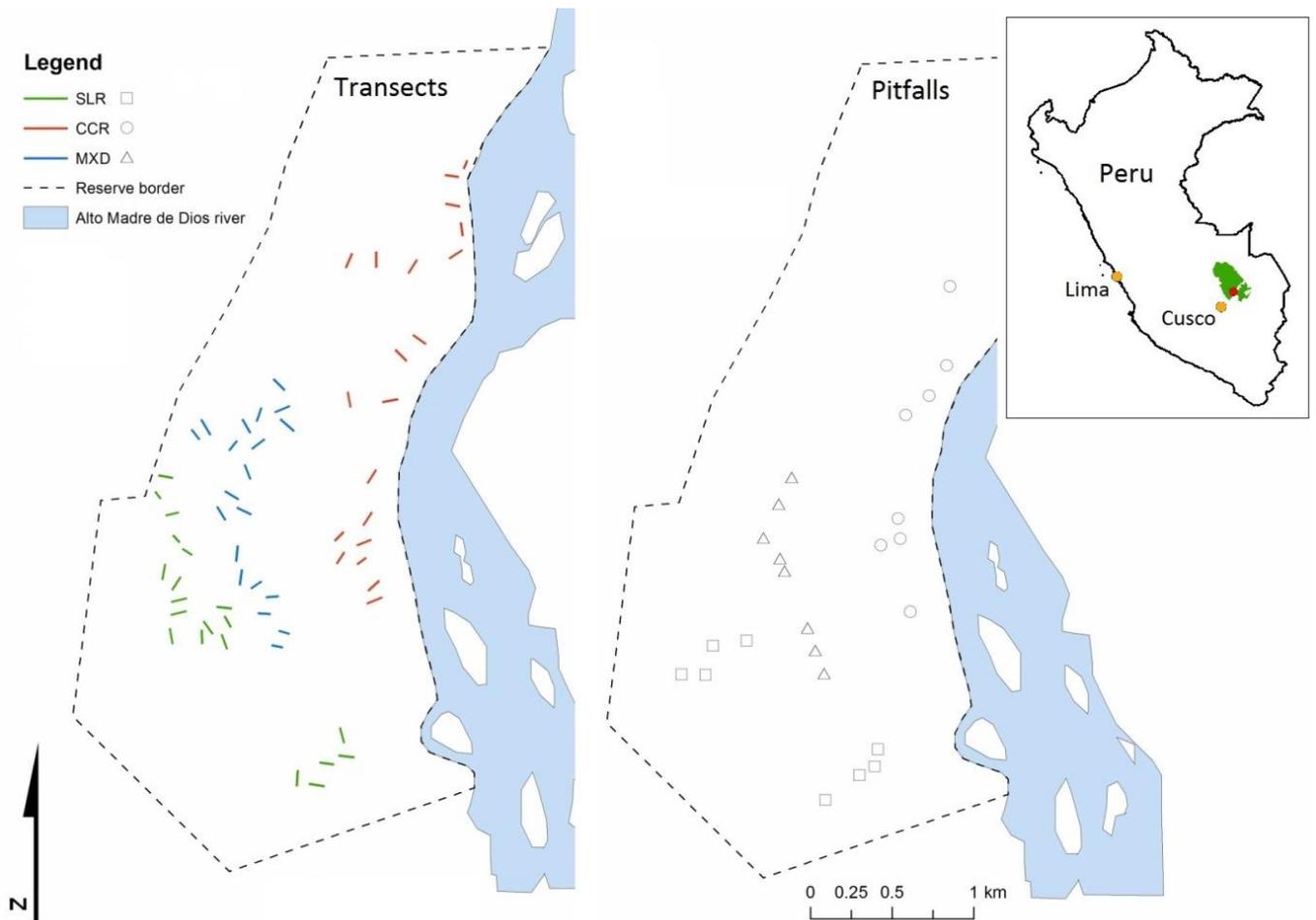
164 2.2 STUDY APPROACH, SAMPLING DESIGN, DISTURBANCE HISTORY AND HABITAT CLASSIFICATION

165 In order to test whether different methodologies indicate the same or different responses
166 of biodiversity to historic human induced habitat change, we used two different biodiversity
167 survey methods to compare detected within-site differences in relation to known differences
168 in human disturbance history. A regenerating rainforest study site was chosen where historic
169 human disturbance had varied across a relatively small area (~800 ha; see Whitworth et al.
170 2016a).

171 Initially the boundaries between the three different disturbance history types were
172 identified by two of the authors visiting the site to visually inspect it, with confirmation by
173 local guides who had expert local knowledge related to historic land-use of the study site. In
174 addition systematic vegetation structure surveys have been carried out at the site to assess
175 specific structural forest differences related to habitat class, and have confirmed the
176 subjective observations of consistent differences in forest structure (for details, see

177 Whitworth et al. 2016b). It was shown that even after 30 years of regeneration, past
178 selectively logged and now regenerating forest at the site had a higher forest canopy and
179 greater canopy cover, with an increased occurrence of epiphytes; whereas the past completely
180 cleared and now regenerating forest was characterised by the opposite trends, and a deeper
181 leaf litter.

182 Studying within site differences in biodiversity distribution across this small spatial scale
183 was used to avoid potential confounding effects due to large scale differences in climatic
184 variables or physical geography. We were confident that amphibians were not hindered in
185 dispersing across the site, as there were no geographic barriers, such as large rivers or
186 mountains dividing the site. We predicted that in the absence of any effects of differences in
187 historic disturbance, biodiversity would be distributed randomly across the site. As such, if
188 different historic human disturbance has differentially impacted biodiversity, we would
189 expect to see differences in current patterns across areas once subjected to different forms of
190 disturbance. To test whether different methodologies would detect different biodiversity
191 patterns, amphibians were surveyed across 36 sampling locations, 12 in each of the three
192 regenerating disturbance areas (Fig. 1). Following Demaynadier and Hunter (1998) all survey
193 locations were situated a minimum distance of 70m apart to ensure sampling independence.



194

195 **FIGURE 1** – The context of the study site (as indicated by a red circle) in the Manu
 196 Biosphere Reserve in SE Peru, and the study site highlighting amphibian transect and pitfall
 197 trap sampling locations.

198 Surveying was conducted through both wet and dry seasons between March 2012 and
 199 May 2014 in order to obtain an annual representation of community structures.

200 Methodologies were conducted simultaneously in order to avoid any bias in capturing a
 201 temporally different community due to the trapping method used at any particular time.

202 Sampling locations were situated at least 70m from a clear habitat edge or water body to
 203 reduce the influence of edge effects (Demaynadier and Hunter 1998). Forest streams were

204 present throughout all habitat types and as such were not expected to influence biodiversity
 205 patterns observed within this study. Due to the steep nature of the terrain and dense forest

206 habitat, sample sites were placed in areas that were accessible, yet away from existing trails,
 207 in order to avoid known detection biases associated with pre-existing trails (von May and

208 Donnelly 2009).

209

210 2.3 FIELD SURVEY METHODOLOGIES AND SAMPLING EFFORT

211 Pitfall traps were utilised due to their effectiveness in sampling terrestrial herpetofauna
212 (e.g. Beirne et al. 2013). Twenty four pitfall sampling locations were established throughout
213 the reserve: eight within SLR, eight within CCR and, and eight within MXD forest. The 25 m
214 long arrays consisted of four 25-litre buckets connected by eight metre lengths of drift fence,
215 40 cm in height. Pitfall traps were opened for a period of six days in each trapping session.
216 Each site had similar, although not identical level of survey effort due to weather and other
217 constraints, with a total effort of between 110 to 115 days of trapping at each site (900 days
218 of trapping per disturbance area; accumulating to 2700 trap days overall).

219 Nocturnal transects were used due to their known effectiveness in sampling arboreal and
220 semi-arboreal species of tropical forest herpetofauna (e.g. Bell and Donnelly 2006; Doan
221 2003) and are known to provide higher yields per unit effort than other sampling methods
222 (Bell and Donnelly 2006; Rödel and Ernst 2004). Sixty sampling locations (each consisting
223 of 100m long x 4m wide transects; surveyed up to two metres in height; Folt and Reider
224 2013) were established throughout the reserve: twenty transects within SLR, twenty within
225 MXD and twenty within CCR. For analysis purposes transects were aggregated into groups
226 of five transects, resulting in twelve independent sampling locations; four within each
227 disturbance area. All transects were surveyed at night, commencing at 20.00 h \pm 15 mins.
228 Transects were surveyed by a pair of searchers over a period of 25 mins (accumulating to
229 ~164 observer hours for the study). Each transect was surveyed between 13-22 occasions to
230 build a picture of the biodiversity at each survey location. The difference resulted due to
231 logistic constraints; half of the transects (10 within each disturbance area) were first installed
232 during 2012 and the second half were first installed in 2013. This meant that some transects
233 were more intensively surveyed than others, but since they were spread evenly between the
234 disturbance types following a balanced design this would not be expected to influence the
235 patterns identified. All transects, were studied throughout both dry (April-September) and
236 wet (October-March) seasons to avoid any potential temporal biases, and the order in which
237 transects were searched was randomised to avoid systematic sampling bias (Beirne et al.
238 2013).

239 Pitfall and transect search teams consisted of one experienced herpetologist and a trained
240 conservation volunteer. All amphibians encountered were identified in the field where
241 possible or later at the field centre (using the following resources: AmphibiaWeb 2012;
242 Beirne and Whitworth 2011; The Field Museum 2012 - von May et al. 2010; which led to the

243 production of a rapid colour guide for the study site, see Villacampa and Whitworth 2014).
244 Open pitfall traps were checked once daily between 08.00h and 13.00h. Lids were placed 10
245 cm above the buckets to prevent flooding during prolonged periods of heavy rain during the
246 trapping periods and then closed tight between sessions. Individuals caught in pitfall traps
247 were released approximately 40 m away from the trap site to reduce the probability of
248 recapture (Beirne et al. 2013; Trimble and van Aarde 2014). Individuals captured during
249 nocturnal transects were released behind the searchers, so that the same individual could not
250 be encountered twice within a survey (Beirne et al. 2013). Unidentifiable species were given
251 a temporary species label (e.g. “*Pristimantis spA*”) and a small number of individuals ($n \leq 4$)
252 of each unidentifiable species were euthanized with Lidocaine and fixed with 10% formalin,
253 then subsequently identified and stored at the herpetology department of the Natural History
254 Museum of the University of San Marcos (UNMSM) in Lima. Owing to the previous
255 detection of a limited number of cases of chytridiomycosis within the study site (Kosch et al.
256 2012) codes of good practice to prevent disease transmission were strictly adhered to. This
257 was achieved by the systematic cleaning of tools and equipment, and sterile bags were used
258 when handling amphibians.

259

260 2.4 ANALYSIS METHODOLOGIES

261 In order to test whether different methods detected significantly different responses in
262 areas with different disturbance history we calculated species richness, diversity, community
263 structure, community composition and overall abundance for each disturbance history type,
264 and compared the patterns detected by each survey methodology (Bruton et al. 2013; Hu et
265 al. 2013). Species strongly associated with either wetland or large river habitat were excluded
266 from analysis due to the presence of significant wetland habitat within CCR disturbance type
267 and more of the main large river habitat being located along the outer edge of the CCR
268 disturbance type (see Appendix A for details of excluded species). This enabled the analysis
269 to focus upon forest interior associated species. Transect nights or pitfall survey sessions
270 were used as the sample units for calculating species richness estimates, species diversity and
271 rarefaction curves (Beirne et al. 2013).

272 To assess observed species richness levels and the extent to which survey effort had
273 detected as many species as were likely to be found within each disturbance type, we plotted
274 rarefaction curves for each sampling methodology using the Rich package (Rossi 2011) and
275 presented these graphically using program R (R Core Team 2012). Where sampling effort

276 detected fewer individuals in one area, we extrapolated the lower lying curves towards an
277 equal number of individuals for a clearer comparison of where observed richness values
278 would have projected given detection of an even number of individuals. Since the issue of
279 which species richness estimators are most effective for amphibian survey methodologies
280 remains unresolved (Veith et al. 2004), a variety of species richness estimators were
281 calculated; ACE, ICE Chao 1 and 2, Jackknife 1 and 2, Bootstrap and MMEans, as
282 recommended by Veith et al. (2004). The average of these estimators was then calculated for
283 each methodology across each disturbance type. Following Altman and Bland (2011), and
284 Gotelli and Colwell (2011) the 84% confidence intervals for the average estimated species
285 richness were calculated for each group in CCR, MXD and SLR disturbance types, as for
286 pair-wise comparison, non-overlapping intervals at this level suggests differences that would
287 be significant at $p < 0.05$ (MacGregor-Fors and Payton 2013). In order to verify any patterns
288 statistically we carried out a linear model for both pitfall traps and nocturnal transects, with
289 average estimated richness as the response term (calculated for each of the 24 pitfall locations
290 and each of the 12 transect groups) and disturbance history as a categorical fixed effect and
291 accounted for any effect from imbalance of survey effort between sampling locations by
292 including survey effort as a fixed effect (using package lme4, program R).

293 Species diversity was defined as the Shannon diversity index (Seshadri 2014; Trimble and
294 van Aarde 2014) and to present the effective number of species or 'true' diversity, presented
295 as the Exponential Shannon diversity (Magurran and McGill 2011). Repeating the analyses
296 using Fisher's Alpha or Simpson's diversity indices both showed the same pattern of results
297 and therefore are not presented. All richness and diversity estimators were calculated in
298 Estimate S (Colwell 2013). Data were analysed with linear models for both pitfall traps and
299 nocturnal transects, with Shannon diversity as the response term (calculated for each of the
300 24 pitfall locations and each of the 12 transect groups) and disturbance history as a
301 categorical fixed effect and again accounted for any effect from imbalance of survey effort
302 between sampling locations by including survey effort as a fixed effect (using package lme4,
303 program R).

304 In order to confirm that any potential spatial auto-correlation between survey locations
305 had been controlled for in the analysis, a Moran's I test was carried out in program R (R Core
306 Team 2012) on the residuals of each preferred model (where preferred to the null) to test if
307 there was any effect from spatial auto-correlation that might lead to pseudo-replication (ape
308 package; Paradis et al. 2004).

309 Community evenness and structure was compared by producing dominance-diversity
310 (Whittaker) plots using the vegan package (Oksanen et al. 2011) in program R (R Core Team
311 2012). Such plots compare the evenness of a community (e.g. Beirne et al 2013; Whitworth et
312 al. 2015). Significant differences in slope, and therefore significant differences in community
313 evenness, were assessed through the use of a linear model with log relative abundance as the
314 response term and an interaction between species rank and habitat type as continuous and
315 categorical fixed effects, respectively (Beirne et al. 2013). Results are reported as ΔG which
316 corresponds to absolute change in gradient between disturbance areas, whereby more
317 negative values denote steeper curves and thus less even assemblages.

318 Community composition between disturbance areas for each of the two survey
319 methodologies was assessed using non-metric multidimensional scaling (NMDS; using the
320 Bray-Curtis similarity measure). All stress values were relatively low (0.14 for transects and
321 0.22 for pitfall tarps) and so were displayed within just two dimensions. To assess the
322 statistical significance of observed differences in assemblage composition between different
323 disturbance areas we conducted analysis of similarities tests (ANOSIM; using 999
324 permutations, see Helbig-Bonitz et al. 2015). NMDS ordinations and ANOSIM tests were
325 carried out in the vegan package (Oksanen et al. 2011), in program R (R Core Team 2012).
326 For this part of the analysis a standardised survey effort across all sampling locations was
327 utilised, as compositions were assessed based upon sampling locations and so included only
328 data collected in 2013 and 2014.

329 In order to determine whether methods detected different changes in amphibian
330 abundance patterns, relative abundance values of the overall community and family groups
331 were calculated. Amphibian transect abundances are presented as the number of individuals
332 encountered over 100 transect nights (each night consisting of five 100x4m transects or
333 2000m² area surveyed per night) and pitfall trap abundances were calculated as the number of
334 individuals encountered per trap site, based on 200 nights of trapping; abundance analyses
335 were carried out on the sampling units of individual transect nights and pitfall trap nights. We
336 assessed whether abundances could be predicted by disturbance history through the use of
337 linear mixed models (only conducted where over 30 detections were made of a particular
338 family). A Poisson family distribution was utilised initially (package lme4; glmer function)
339 but significant levels of overdispersion were detectable within the models, a common feature
340 in count data (Zuur et al. 2009). A negative binomial family distribution with a log link
341 function was therefore used to account for this overdispersion (packages - R2admb and
342 glmmADMB; using function glmmadmb) (Trimble and van Aarde 2014; Zuur et al. 2009).

343 To account for repeat measures from transect groups and from pitfall arrays, transect group or
344 pitfall identification was added as a random effect. An analysis of variance (ANOVA) test on
345 the log likelihoods of models including and excluding disturbance history was used to
346 determine the significance of disturbance history as a predictor of abundance for each
347 method. We used the same GLMM model structure to test the effect of seasonality on the
348 overall encounter rates of amphibians, for both nocturnal transects and pitfall traps (following
349 seasonal months as detailed by Whitworth et al. (2016b) from the same study site; wet season
350 from October to March, and dry season from April to September). As the family
351 Craugastoridae, containing the genus *Pristimantis* (previously *Eleutherodactylus*), have
352 previously been identified as a key indicator group of good quality habitat by Pearman
353 (1997), we used the same GLMM model structure to assess the observed species richness
354 encountered within this group between disturbance areas, in addition to testing the abundance
355 of the group. In order to access ‘sample by species matrices’, see the data profile in Appendix
356 B.

357

358

359 **3. RESULTS**

360 Following the exclusion of wetland and large river associated amphibian species, 1306
 361 individuals of 37 species were recorded (Table 1). These included 792 individuals of 33
 362 species from nocturnal transects and 514 individuals of 22 species from pitfall traps.

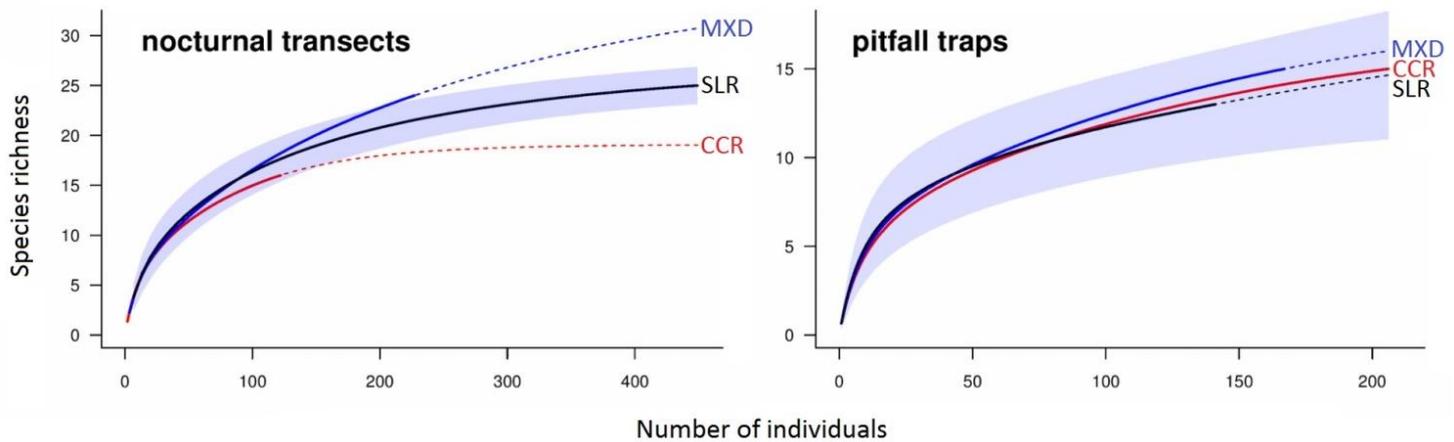
363
 364 **TABLE 1** – Observed and estimated species richness patterns based on different survey methodologies. A
 365 comparison of nocturnal transects and pitfall traps as amphibian survey methodologies. Survey effort for
 366 transects relates to the number of sampling nights (each night consisting of five 100x4m transects or 2000m²
 367 searched) and pitfall trap survey effort relates to the total number of individual trap sessions (each session
 368 consisting of one trap open for five trap nights).

	Habitat class	n ^a	Survey effort: samples	Observed species	Extrapolated species ^b	Species richness estimates								Sampling Coverage (%) ^d	Completeness (%) ^e	Unique species		
						ACE	ICE	Chao 1	Chao 2	Jackknife 1	Jackknife 2	Bootstrap	MMMean				Average ^c	
Amphibians	Nocturnal transects	SLR	449	67	25	25	26	27	26	26	29	29	27	26	27	92	76	5
		CCR	116	66	16	19	22	22	19	19	21	22	18	18	20	79	48	3
		MXD	227	64	24	31	35	39	34	36	34	40	28	26	34	70	73	1
		Total	792	197	33													
	Pitfall traps	SLR	140	180	13	15	18	18	14	21	17	20	15	13	17	77	59	3
		CCR	206	180	15	15	17	21	16	18	19	20	17	15	18	84	68	2
		MXD	168	180	15	16	26	22	19	19	20	22	17	15	20	74	68	2
		Total	514	540	22													

^a Number of individual records
^b Number of species estimated when curves extrapolated to the same number of encounters
^c Mean estimated species richness - classic Chao values were used in cases where CV>0.5
^d Sampling coverage defined as: $b/c \times 100$
^e Number of species observed as a percentage of combined species across all habitats

369 The average estimated species richness from nocturnal transects was highest in MXD
 370 disturbance type (34 ±3.21 species), followed by the SLR disturbance type with an estimated
 371 27 ±0.89 species and just 20 ±1.10 species in CCR (35% lower than SLR; Table 1). With
 372 non-overlapping 84% confidence intervals of average estimated species richness (SLR lower
 373 ci 26.35, upper ci 27.65; MXD lower ci 31.57, upper ci 36.43; CCR lower ci 19.22, upper ci
 374 21.03) these differences appeared significant for all disturbance areas (p<0.05). Figure 2
 375 shows that based on the extrapolated rarefaction curves of predicted species richness (Table
 376 2), MXD and CCR species richness values lie outside the 84% CI for the SLR curve. Linear
 377 modelling showed that using the transect methodology, disturbance history type was a good
 378 predictor of species richness with 27.4% of variation explained and that SLR locations had on
 379 average 8.2 (± 3.8 s.e) more species than CCR locations (d.f.=7, t=2.2, p=0.074). Survey
 380 effort across sampling locations was also found to have an effect on estimated species
 381 richness but did not significantly change the effect size of disturbance history.

382

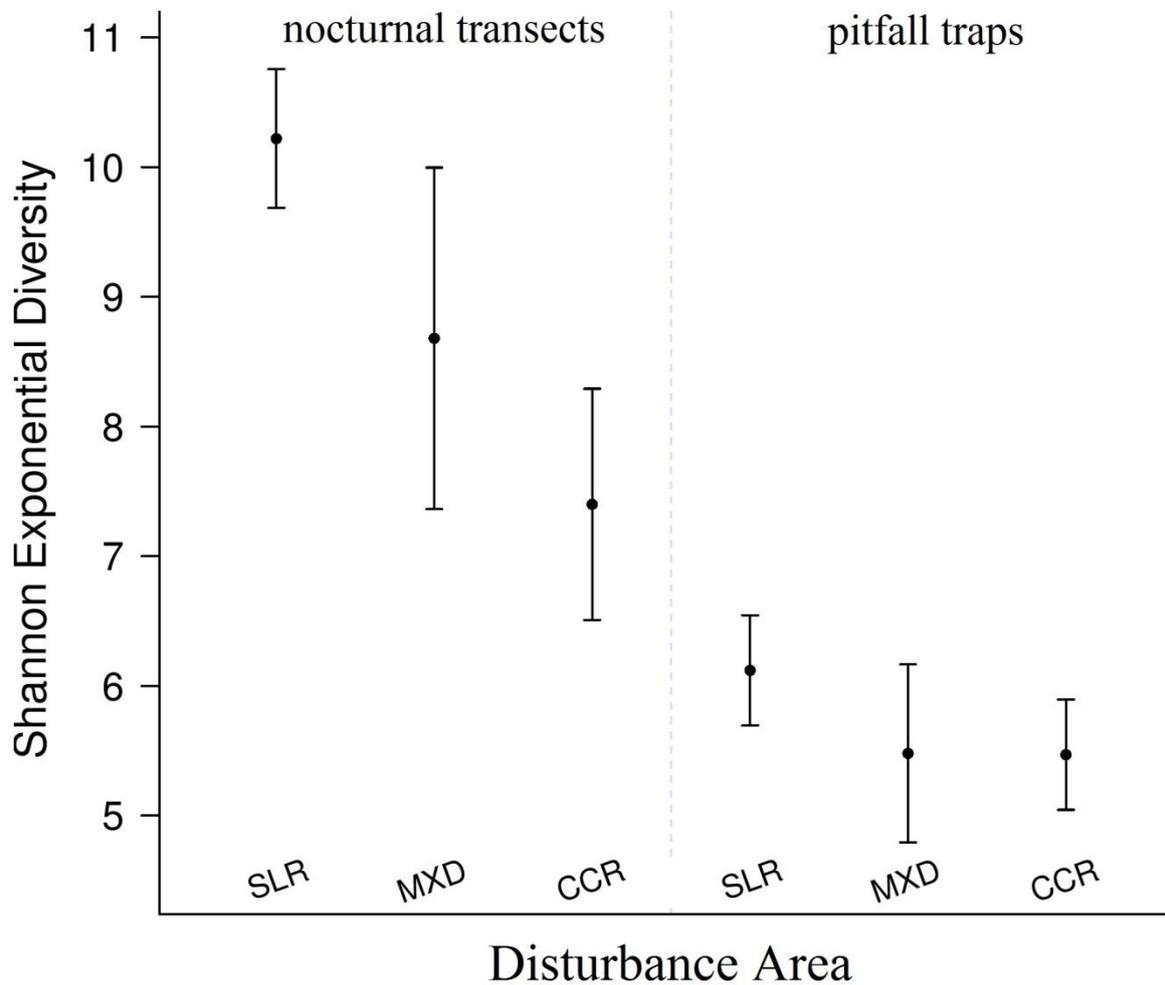


384 **FIGURE 2** – Amphibian species richness of regenerating rainforest with different disturbance histories for
 385 amphibian communities from the same area based on a) nocturnal transects and b) pitfall traps. Solid lines
 386 represent the observed number of individuals recorded and dashed lines represent predicted species richness
 387 based on extrapolated rarefaction curves. The grey shades represent 84% confidence intervals for SLR
 388 disturbance type (only the confidence intervals for SLR are plotted to provide clearer graphs).

389
 390 The average estimated species richness from pitfall traps did not appear to show any
 391 difference between disturbance areas; CCR = 18 ± 1.39 species, MXD = 20 ± 2.31 species and
 392 SLR = 17 ± 1.95 species, with overlap between 84% confidence intervals ($p > 0.05$; SLR lower
 393 ci 15.59, upper ci 18.41; MXD lower ci 18.31, upper ci 21.69; CCR lower ci 16.86, upper ci
 394 18.89). Disturbance history type was also a good predictor of species richness for pitfalls,
 395 explaining 26.95% of variation explained, but suggested the opposite patterns with on
 396 average $1.9 (\pm 1.02 \text{ s.e})$ fewer species in SLR than CCR (d.f. = 15, $t = -1.9$, $p = 0.08$). With
 397 the two methods predicting different directions for the species richness pattern this difference
 398 is significant ($p = 0.006$; when considering statistical significance where $p < 0.05$). Survey
 399 effort across sampling locations was found to have no effect on estimated species richness for
 400 pitfalls.

401 Overall Shannon diversity from nocturnal transects was higher in SLR than in CCR
 402 (Fig. 3) but not for pitfall traps. The MXD habitat displayed intermediate values of Shannon
 403 diversity between SLR and CCR disturbance areas. Linear modelling showed that using the
 404 transect methodology, disturbance history type explained 28.8% of variation for Shannon
 405 diversity (d.f. = 7, $t = 1.98$, $p = 0.095$). Survey effort across sampling locations was also
 406 found to have an effect but did not significantly change the effect size of disturbance history.
 407 However, linear modelling showed that when using the pitfall methodology, disturbance
 408 history type explained just 1.1% of variation for Shannon diversity (d.f. = 15, $t = -0.5$, $p =$

409 0.6). With the two methods predicting different directions for the diversity pattern this
 410 difference was close to significant ($p=0.057$).
 411

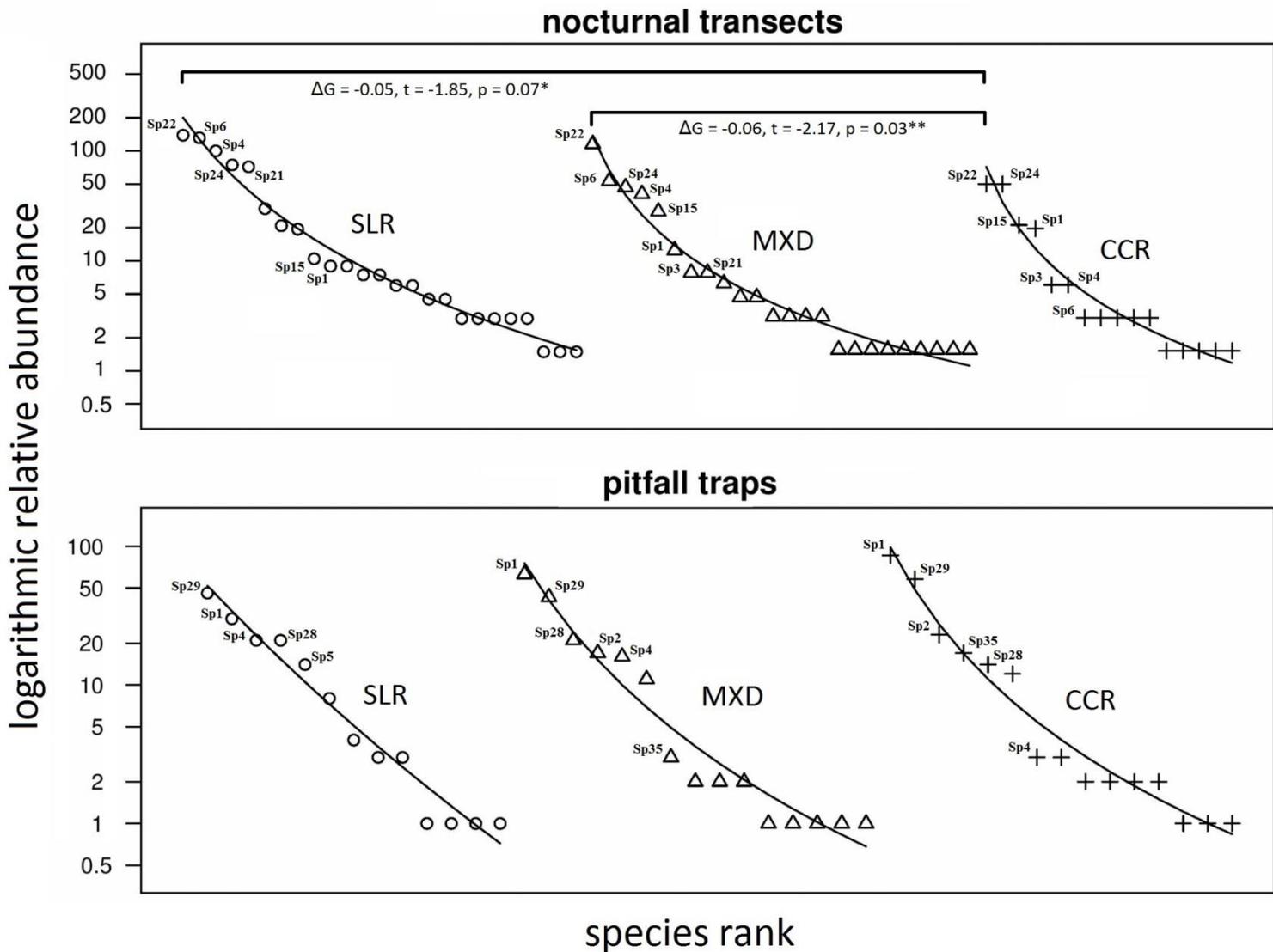


412

413 **FIGURE 3** - Shannon species diversity of regenerating rainforest with different disturbance histories for
 414 amphibian communities from the same area based on a) nocturnal transects and b) pitfall traps; with 84%
 415 confidence intervals.

416

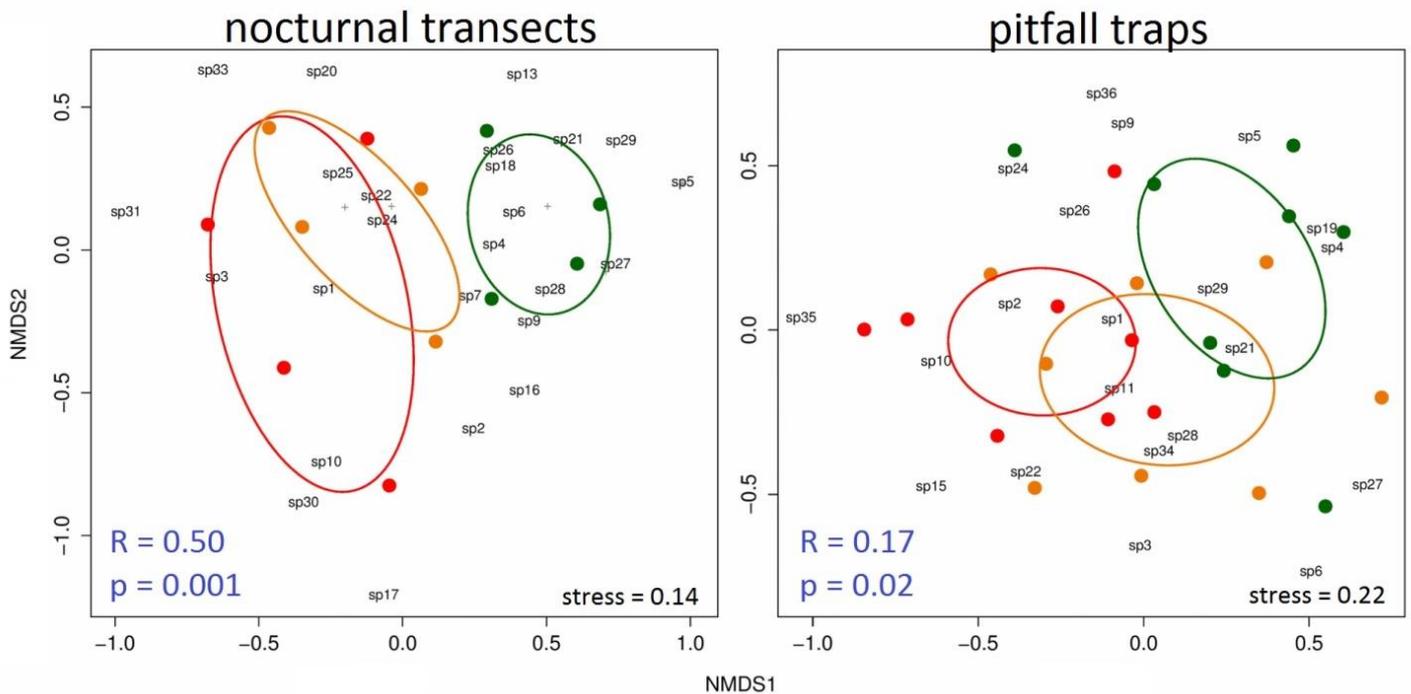
417 Testing of species richness and species diversity model residuals, from both transect
 418 and pitfall date, showed no evidence of spatial auto-correlation between samples with very
 419 low correlations (range from -0.30 to -0.007) and non-significant observed Moran's I values
 420 (range from $p=0.10$ to 0.79).



422 **FIGURE 4** – Dominance-diversity (Whittaker) plots for nocturnal transects and pitfall trap amphibian
 423 communities in regenerating rainforest with different disturbance histories. Species are represented by points.
 424 For each habitat the relative abundance of each species (n_i/N) was plotted on a logarithmic scale against the
 425 species rank ordered from most to least abundant. O = SLR, Δ = MXD and + = CCR. Linear models were used
 426 to determine if the slopes of SLR, MXD and CCR were significantly different, where ΔG denotes to absolute
 427 change in gradient from the comparative gradient and the * symbol denote the level of significance of the
 428 deviation where * = <0.1 – close to significance, ** = <0.05 – significant, and blank = >0.1 – not significant.
 429 The most dominant five species from SLR were indicated on each of the curves, along with any of the five most
 430 dominant species from MXD and CCR in order to investigate compositional shifts in the most frequently
 431 encountered or dominant species from each habitat; corresponding identifier codes are provided in Appendix A.

432 Dominance-diversity plots demonstrated that the amphibian community recorded by
 433 nocturnal transects supports a significantly more even assemblage (regular intervals between
 434 species) with more rare species (increased tail length) in SLR and MXD habitat, than in CCR

435 (Fig. 4). This difference was close to significance different between SLR and CCR ($\Delta G = -$
 436 0.05 , $t = -1.85$, $p=0.07$), and was significant between MXD and CCR ($\Delta G = -0.06$, $t = -2.17$,
 437 $p=0.03$). No significant difference in the evenness of the species composition was found for
 438 the community from pitfall traps between disturbance habitats (the lowest p-value CCR vs.
 439 SLR = 0.34).



441 **FIGURE 5** - Community composition NMDS plots of regenerating rainforest with different disturbance
 442 histories for amphibian communities from the same area, based on nocturnal transects and pitfall traps; species
 443 and corresponding codes are provided in Appendix A. The red circles = CCR sampling locations, orange circles
 444 = MXD sampling locations, and green circles = SLR sampling locations. The 12 sampling locations for transects
 445 represent groups of five 100m transects and for pitfalls, each of the 24 points represents a pitfall sampling
 446 location. Species points (+) and labels (e.g. sp1) were plotted using function orditorp in vegan package
 447 (Oksanen et al. 2011). This function will label an item only if this can be done without overwriting previous
 448 labels. If an item could not be labelled with text (priority was given to the most abundant species), it was
 449 marked as a point. Function ordiellipse in vegan package (Oksanen et al. 2011) was used to draw 95%
 450 confidence interval for disturbance classifications assigned to sampling locations. Stress values of the NMDS
 451 for two-dimensions are displayed, along with the respective R statistic and p-values from the associated
 452 ANOSIM analyses.

453
 454 The community composition analysis from NMDS plots and the associated ANOSIM
 455 analysis (Fig. 5) showed that community composition between disturbance areas was
 456 significantly different for both nocturnal transects ($R=0.50$, $p=0.001$) and pitfall trap ($R=0.17$,
 457 $p=0.02$) methodologies. However, the R statistic, which denotes the degree of difference

458 between communities, is higher for the communities assessed using nocturnal transects and
459 although the communities assessed by pitfall traps shows a significant p-value, the R statistic
460 is relatively low. The NMDS plots (Fig. 5) show that the SLR community for nocturnal
461 transects is completely distinct from both CCR and MXD communities (which show a
462 significant degree of overlap), whilst for pitfalls, the SLR community is only distinct from the
463 CCR community but shares some degree of overlap with the MXD community.

464 Differences in the most frequently encountered species between habitats were visible
465 from the dominance-diversity plots (Fig. 4) and the NMDS community composition plots for
466 both survey methodologies (for observed records and relative abundances of all species, see
467 Appendix A). For example, *Ameerega macero* (Am - both transects and pitfalls), *A. sp1* (As -
468 pitfalls), *Pristimantis carvalhoi* (Pc - transects) and the salamander, *Bolitoglossa caldwella*
469 (Ba - transects) all displayed a reduction in both abundance and community rank with
470 increasing disturbance (i.e. disturbance sensitive species). However, other species such as *P.*
471 *ockendeni* (Po - transects) and *P. reichlei* (Pr - transects) retained a high species rank despite
472 decrease in abundance (i.e. habitat generalists) and an association with more intensely
473 disturbed habitats. Some species, such as *Rhinella marina* (Pitfalls - Rm), *R. margaritifera*
474 (Rt - Pitfalls), *Osteocephalus castaneicola* (Oc - transects) and *Adenomera andrea* (Aa -
475 pitfalls) not only retained species rank but increased in abundance slightly in the habitat with
476 the most intense historic disturbance (i.e. disturbance specialists).

477 The overall relative abundance of amphibians from nocturnal transects was
478 significantly different between disturbance areas, highest in SLR and lowest in CCR (n/20ha
479 - the number of records per 100 transect nights, where five 100x4m transects or 2000m² are
480 surveyed per evening; n_{CCR}=176, n_{MXD}=355, n_{SLR}=670; df = 2, $\chi^2 = 19.47$, p=<0.001), whilst
481 overall amphibian abundance from pitfall traps showed no difference (n/200 trap nights;
482 n_{CCR}=229, n_{MXD}=187, n_{SLR}=156; df = 2, $\chi^2 = 4.15$, p=0.13; Table 2). Encounter rates of
483 amphibians were found to be higher in dry season months than wet season months; this was
484 true for both nocturnal transects (with ~4.4 fewer individuals found in the wet season for
485 every 10 survey nights or 2ha searched; p=<0.0001), and for pitfall traps (with ~2.1 fewer
486 individuals caught in the wet season per 50 single trap nights; p=0.042).

487 When considering different families encountered along nocturnal transects, the
488 Craugastoridae (p=0.005), Dendrobatidae (p=0.001) and Plethodontidae (p=<0.001) all
489 displayed a significantly different abundance between disturbance areas, whilst Hylidae did
490 not. Each group that displayed a difference was in highest abundance in SLR and lowest in

491 CCR. Pitfall traps found Dendrobatidae (p=0.001) and Leptodactylidae (p=0.009) to display
 492 significantly different abundances but in opposite directions, with Dendrobatidae in higher
 493 abundance in SLR and Leptodactylidae in higher abundance in CCR. Bufonidae displayed no
 494 difference in abundance between disturbance areas (p=0.83). The key indicator group of good
 495 quality habitat (Pearman 1997), the Craugastoridae, not only displayed a higher abundance in
 496 the less intensely disturbed habitat (SLR) but also displayed a higher observed species
 497 richness at the survey level in SLR forest; a result detectable from transects data (ANOVA
 498 result between disturbance history and the null model, p=0.005) but not from pitfall traps
 499 (ANOVA result between disturbance history and the null model, p=0.83).
 500

501 **TABLE 2** – The mean relative abundances for amphibian families in each disturbance type. Nocturnal transect
 502 relative abundances (RA) represent the number of individuals encountered per 100 survey nights (n/20ha; each
 503 transect night consisting of five 100x4m transects or 2000m² surveyed per night); pitfall trap relative
 504 abundances (RA) represent the number of individuals encountered per 200 trapping days at a given site. These
 505 are overall values for presentation; numbers at the sample level would be so small that whole numbers wouldn't
 506 be possible and decimal figures are not sensible for numbers of a species. Although the value is greater than our
 507 smallest search area, it is still smaller than the overall area covered within the study and so is not extrapolated to
 508 a degree outside the bounds of our overall research area. n = number of encounters; p = p-value relates to an
 509 ANOVA test on the log likelihoods of models including and excluding disturbance history; the * symbol
 510 denotes the level of significance where * = <0.01 and ** = <0.001; only conducted where Total n>30.

Family	Nocturnal transects								Pitfall traps							
	CCR		MXD		SLR		Total		CCR		MXD		SLR		Total	Statistical test
	n	RA	n	RA	n	RA	n	p	n	RA	n	RA	n	RA	n	p
Craugastoridae	67	102	122	191	243	363	432	0.005*	5	6	7	8	6	7	18	-
Hylidae	27	32	23	33	12	15	62	0.076	2	2	-	-	-	-	2	-
Dendrobatidae	8	12	31	48	72	107	111	0.001*	4	4	15	17	32	36	51	0.001*
Centrolenidae	-	-	4	6	6	9	10	-	-	-	-	-	-	-	-	-
Aromobatidae	-	-	-	-	-	-	-	-	16	18	4	4	1	1	21	-
Leptodactylidae	17	26	11	17	11	16	39	0.553	114	127	83	92	41	46	238	0.009*
Bufonidae	1	2	2	3	17	25	20	-	65	72	58	64	60	67	183	0.825
Plethodontidae	2	3	34	53	88	131	124	<0.001**	-	-	-	-	-	-	-	-
Total	116	176	227	355	449	670	792	<0.001**	206	229	168	187	140	156	514	0.125

511

512

513 **4. DISCUSSION**

514 We show that two different but commonly utilised survey methodologies identify
515 contrasting biodiversity patterns in a human modified rainforest, decades after initial
516 disturbance. The occurrence of contrasting patterns depending on methodology held true for a
517 variety of frequently utilised biodiversity measures; species richness, diversity, abundance,
518 community structure and community composition. Using nocturnal transects to assess
519 amphibian biodiversity suggested that historic clearance of tropical forest resulted in lower
520 levels of amphibian biodiversity and a greater disruption to community evenness and
521 composition, compared with forest once subjected to selective logging. Whereas pitfall traps
522 indicated no difference in amphibian species richness, diversity, abundance and community
523 evenness, and a lower level of dissimilarity in community composition between disturbance
524 areas than nocturnal transects. These results show how assessing the same taxonomic group,
525 at the same site, using different methods can suggest different relative biodiversity value
526 between disturbance types, which could ultimately therefore lead to over or underestimation
527 of the conservation value of different types of regenerating tropical forests.

528 Although previous studies have identified that survey methodologies often target subsets
529 of faunal communities (Sparrow et al. 1994) and have investigated the most efficient methods
530 (Doan 2003), few studies have systematically assessed the potential for different
531 methodologies to lead to contrasting conclusions in relation to biodiversity and conservation
532 value of regenerating forests (Barlow et al. 2007b). The results reported here focused on the
533 effect of methodology on detecting patterns in amphibian biodiversity; however, several
534 previous studies on other taxonomic groups, which focused on other questions, suggest that
535 such methodological effects may be important for biodiversity assessments more generally.
536 For example, Barlow et al. (2007b) utilised mist nets and point counts to assess the response
537 of bird communities to tropical forest disturbance in Brazil and found a contrasting response
538 of bird species richness. Bird species richness was higher in primary forest than in secondary
539 forest when point counts were used, but was equal to primary forest when mist nets were
540 utilised. In another avian study, mist netting found a negative response to the presence of an
541 unmarked Amazonian forest road, whilst bird point counts detected the opposite pattern, with
542 a greater biodiversity detected near to the road (Whitworth et al. 2015). Different biodiversity
543 response patterns using alternative survey methods have also been detected for butterflies, but
544 were conducted at different survey sites and not directly compared within the same study area
545 (Kudavidanage et al. 2012, Ribeiro et al. 2015).

546 A key aspect of the approach in this study is that the type of contrasting patterns
547 identified here can only be linked to methodological effects, because they were carried out
548 within the same study site. If data on different methods had come from different study sites
549 then it would have been much more difficult to disentangle the effects of study location. For
550 example, contrasting results from studies upon butterflies have been found across a variety of
551 locations (Devy and Davidar 2001; Dumbrell and Hill 2005; Kudavidanage et al. 2012; Posa
552 and Sodhi 2006; Ribeiro et al. 2015; Ribeiro and Freitas 2012), making it difficult to robustly
553 conclude that opposing patterns are related to landscape differences and not due to the
554 sampling methodology utilised.

555 Although confident that observed differences are an effect of different methodologies,
556 one alternative explanation for the results is the potential that the pitfalls and transects are in
557 different locations, and so results could be due to systematic differences in locations rather
558 than methods. However, since the pitfalls are interspersed within the network of transects this
559 should be unlikely. Although factors other than disturbance history type might be the cause
560 of the difference detected by nocturnal transects, in this specific study we are interested
561 specifically in what patterns the two different methods detect; and not the cause of the
562 differences. However, this study utilised a natural experiment approach in order to follow the
563 recommendations of Ramage et al. (2013) for avoiding potential pseudo-replication problems
564 in tropical forest ecology (Davies and Gray 2015), and we also examined whether spatial
565 autocorrelation of the sampling locations could be driving the biodiversity patterns detected.
566 The autocorrelation analysis confirmed that biodiversity patterns detected were not being
567 driven by spatial autocorrelation.

568 In addition to assessing overall patterns of biodiversity, we also investigated fine scale
569 metrics of the amphibian community, in the form of species specific response patterns and
570 abundances of family groups (as opposed to overall community structure and overall
571 abundance patterns). Although there is a growing body of literature investigating species
572 specific and functional groups in tropical forests for birds (De Coster et al. 2015; Edwards et
573 al. 2013; Hidas-Neto et al. 2012; Newbold et al. 2013) and plants (Ding et al. 2012; Carreño-
574 Rocabado et al. 2012), few studies exist for amphibians (Trimble and van Aarde 2014). These
575 fine scale metrics allowed for the detection of community structure and composition
576 differences between disturbance areas using both survey methodologies; with
577 increases/decreases in rank and abundance for some species. Although overall abundance of
578 the amphibian community from pitfalls did not show a difference between disturbance areas,
579 two of the three families tested did, albeit in opposite directions. We suggest therefore, that

580 fine scale metrics, which assess species specific responses or patterns of families, may be less
581 susceptible to show contrasting patterns between methodologies. However, this should be
582 considered with caution and requires further investigation, specifically in light of concerns
583 over the application of community similarity metrics to assess disturbance patterns across
584 landscapes, as suggested by Ramage et al. (2013).

585 We also found in contrast to previous studies (Aichinger 1987; Duellman 1995), that the
586 encounter rate of amphibians was higher during the dry than the wet season. However, this
587 might relate to the fact that seasons were broken coarsely into ‘wet’ and ‘dry’ as oppose to
588 multiple shorter periods as those by Duellman (1995). Additionally, survey sites in our study
589 were located to avoided water bodies and situated in typical forest habitat, whereas Aichinger
590 (1987) specifically targeted water habitat features. This potential difference in seasonality
591 patterns of encounter rates between typical forest habitat and specific water habitat features
592 requires further attention.

593 In addition to simply identifying that the different levels of impact upon biodiversity
594 linked to habitat change may be the result of alternative methodologies, it is also important to
595 understand more about why alternative methods indicate different patterns. Within this study
596 for example, we utilised two commonly used methodologies which target distinct subsets of
597 the overall amphibian community. Pitfall traps better target the terrestrial amphibian
598 community (i.e. Dendrobatidae, Leptodactylidae and Bufonidae; e.g. Beirne et al. 2013),
599 whereas nocturnal transects have been shown to be more efficient in detecting a wider
600 representation of the amphibian community (e.g. Beirne et al. 2013; Doan 2003), including
601 both terrestrial (i.e. Dendrobatidae and Leptodactylidae) and arboreal groups (i.e.
602 Craugastoridae, Hylidae and Plethodontidae). Our results related to overall patterns may
603 therefore suggest that arboreal amphibian communities (although some canopy dwelling
604 species are likely missed due to the limit of ground-based survey techniques) are more
605 sensitive to habitat disturbance than terrestrial communities. This is a pattern that has been
606 detected for a variety of invertebrates within tropical forests, including ants (Klimes et al.
607 2012), dung beetles (Tregidgo et al. 2010), and butterflies (Whitworth et al. 2016b). The
608 known key indicator group of good quality habitat (Pearman 1997), the Craugastoridae, are
609 more commonly encountered via transects as opposed to terrestrial based techniques, and are
610 a mostly semi-arboreal group (comprising mostly *Pristimantis* sp.). However, the mostly
611 terrestrial Dendrobatidae appeared well represented by both methodologies and indicated the
612 same abundance patterns in relation to historic disturbance. Contrastingly, Leptodactylidae
613 were better surveyed by pitfall traps, with only this method detecting a significantly different

614 abundance related to historic disturbance. This may be due to the detectability differences
615 within these families, which relates to morphological and functional traits (Mouillot et al.
616 2013). Dendrobatids are often bright coloured and conspicuous, therefore easy to spot on the
617 ground at night; whereas Leptodactylidae are brown cryptically coloured frogs, often located
618 in holes, more difficult to detect at night in the leaf-litter.

619 In conclusion, we show that the choice of survey methodology, for the same taxonomic
620 group, can suggest different biodiversity values of regenerating tropical forest, and as such,
621 methods matter in assessments of habitat disturbance upon biodiversity. Combining methods
622 is often the preferred approach whereby species inventories are required (Doan 2003), but
623 methods that target sensitive community sub-sets are preferable in the assessments of habitat
624 disturbance. This suggests that the use of different methods could be an important factor as to
625 why there are conflicting results and therefore conclusions regarding the biodiversity value of
626 secondary regenerating tropical forests (Chazdon et al. 2009a; Sloan et al. 2015). Increasing
627 our understanding about different methodologies targeting key indicator taxa and the patterns
628 they suggest can probably be best achieved by conducting side-by-side comparisons of
629 survey methodologies at the same study locations. Such studies are likely to be important if
630 we intend to better unravel the factors relating to how well tropical forest biodiversity can
631 recover from environmental change.

632

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