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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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ABSTRACT

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

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

1. INTRODUCTION

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

Determining how well tropical forest biodiversity can recover is difficult (Gardner et al. 2010) as studies from different locations often produce contrasting results. There are many potential reasons for different studies to identify contrasting patterns; including geographic context, study scale, potential on-going human impacts, timeframe since disturbance (Chazdon et al. 2009a) and a tendency to focus on overall species richness patterns (Anand et al. 2010; Barlow at al. 2007a). However, one factor often overlooked is the potential for different survey methodologies, targeting the same indicator taxon (such as amphibians, butterflies and understorey birds), to provide different results on the response of biodiversity to habitat change (Barlow et al. 2007b). In the case of butterflies for example, line transect studies carried out in a number of locations have suggested that butterfly biodiversity does not show a significant degree of difference between human disturbed and primary forest (Devy and Davidar 2001; Kudavidanage et al. 2012; Posa and Sodhi 2006). In contrast, studies using traps undertaken at other sites suggest that butterfly biodiversity does show a significant degree of difference between human disturbed and primary forest (Dumbrell and Hill 2005; Ribeiro and Freitas 2012; Ribeiro et al. 2015). Additionally, bird studies carried out in different locations and based upon different survey methodologies have also found contrasting patterns (Barlow et al. 2007b). In some locations secondary forests display similar biodiversity levels to primary forest based on mist net methodologies (Barlow et al. 2007b; Srinivasan et al. 2015; Waltert et al. 2005), but other studies using point counts have suggested that secondary forest may have significantly lower levels than primary (Barlow et al. 2007b; Carillo-Rubio et al. 2014). Understanding more about how these contrasting patterns might relate to differences due to survey methodologies can therefore help to improve our ability to assess the true value of regenerating tropical forests and better understand the response of specific communities. Otherwise, assessments of a specific community may under or overestimate the potential biodiversity value for such forests, especially if the results from single surveys are over generalised (Barlow et al. 2007b).

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

So far, investigations using amphibians to assess rainforest biodiversity response to habitat change often use different survey methodologies and describe contrasting patterns from different locations. Hilje and Aide (2012), for example, utilised diurnal and nocturnal visual searches and acoustic surveys in Costa Rica and found that even young regenerating forest had similar amphibian species richness and composition to primary forest. In contrast, Gardner et al. (2007), using terrestrial traps and diurnal visual searches to target leaf litter amphibians in Brazil, found just two-thirds of primary forest amphibian species in regenerating forest. Finally, Seshadri (2014) utilised quadrats to assess amphibian biodiversity in selectively logged forests of southern India, detecting a 42% lower density of amphibians than in primary forest; and even though species richness and composition were converging with primary forest levels, the effects of logging were still detectable. These results therefore raise the question of whether the lack of a consistent pattern in detected amphibian responses is driven by site specific factors or whether such differences could be caused by different methods that focus on different groups of amphibian communities.

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

2. METHODS

2.1 STUDY SITE

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

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

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

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

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

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

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

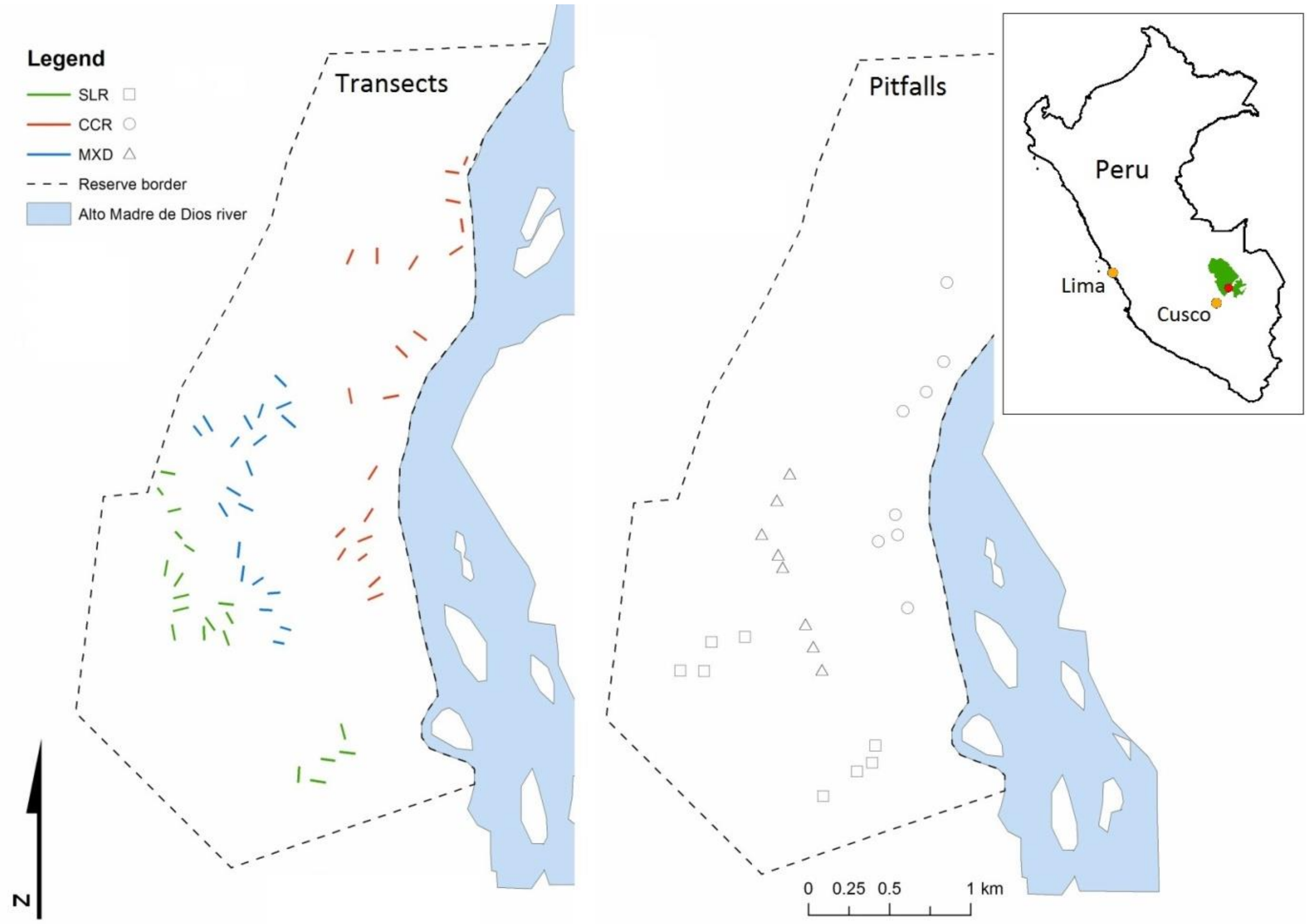

FIGURE 1 – The context of the study site (as indicated by a red circle) in the Manu Biosphere Reserve in SE Peru, and the study site highlighting amphibian transect and pitfall trap sampling locations.

Surveying was conducted through both wet and dry seasons between March 2012 and May 2014 in order to obtain an annual representation of community structures. Methodologies were conducted simultaneously in order to avoid any bias in capturing a temporally different community due to the trapping method used at any particular time. Sampling locations were situated at least 70m from a clear habitat edge or water body to reduce the influence of edge effects (Demaynadier and Hunter 1998). Forest streams were present throughout all habitat types and as such were not expected to influence biodiversity patterns observed within this study. Due to the steep nature of the terrain and dense forest habitat, sample sites were placed in areas that were accessible, yet away from existing trails, in order to avoid known detection biases associated with pre-existing trails (von May and Donnelly 2009).

2.3 FIELD SURVEY METHODOLOGIES AND SAMPLING EFFORT

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

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

Pitfall and transect search teams consisted of one experienced herpetologist and a trained conservation volunteer. All amphibians encountered were identified in the field where possible or later at the field centre (using the following resources: AmphibiaWeb 2012; Beirne and Whitworth 2011; The Field Museum 2012 - von May et al. 2010; which led to the production of a rapid colour guide for the study site, see Villacampa and Whitworth 2014). Open pitfall traps were checked once daily between 08.00h and 13.00h. Lids were placed 10 cm above the buckets to prevent flooding during prolonged periods of heavy rain during the trapping periods and then closed tight between sessions. Individuals caught in pitfall traps were released approximately 40 m away from the trap site to reduce the probability of recapture (Beirne et al. 2013; Trimble and van Aarde 2014). Individuals captured during nocturnal transects were released behind the searchers, so that the same individual could not be encountered twice within a survey (Beirne et al. 2013). Unidentifiable species were given a temporary species label (e.g. "*Pristimantis spA*") and a small number of individuals ($n \leq 4$) of each unidentifiable species were euthanized with Lidocaine and fixed with 10% formalin, then subsequently identified and stored at the herpetology department of the Natural History Museum of the University of San Marcos (UNMSM) in Lima. Owing to the previous detection of a limited number of cases of chytridiomycosis within the study site (Kosch et al. 2012) codes of good practice to prevent disease transmission were strictly adhered to. This was achieved by the systematic cleaning of tools and equipment, and sterile bags were used when handling amphibians.

2.4 ANALYSIS METHODOLOGIES

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

To assess observed species richness levels and the extent to which survey effort had detected as many species as were likely to be found within each disturbance type, we plotted rarefaction curves for each sampling methodology using the Rich package (Rossi 2011) and presented these graphically using program R (R Core Team 2012). Where sampling effort detected fewer individuals in one area, we extrapolated the lower lying curves towards an equal number of individuals for a clearer comparison of where observed richness values would have projected given detection of an even number of individuals. Since the issue of which species richness estimators are most effective for amphibian survey methodologies remains unresolved (Veith et al. 2004), a variety of species richness estimators were calculated; ACE, ICE Chao 1 and 2, Jacknife 1 and 2, Bootstrap and MMMeans, as recommended by Veith et al. (2004). The average of these estimators was then calculated for each methodology across each disturbance type. Following Altman and Bland (2011), and Gotelli and Colwell (2011) the 84% confidence intervals for the average estimated species richness were calculated for each group in CCR, MXD and SLR disturbance types, as for pair-wise comparison, non-overlapping intervals at this level suggests differences that would be significant at p=<0.05 (MacGregor-Fors and Payton 2013). In order to verify any patterns statistically we carried out a linear model for both pitfall traps and nocturnal transects, with average estimated richness as the response term (calculated for each of the 24 pitfall locations and each of the 12 transect groups) and disturbance history as a categorical fixed effect and accounted for any effect from imbalance of survey effort between sampling locations by including survey effort as a fixed effect (using package lme4, program R).

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

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

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

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

In order to determine whether methods detected different changes in amphibian abundance patterns, relative abundance values of the overall community and family groups were calculated. Amphibian transect abundances are presented as the number of individuals encountered over 100 transect nights (each night consisting of five 100x4m transects or 2000m^2 area surveyed per night) and pitfall trap abundances were calculated as the number of individuals encountered per trap site, based on 200 nights of trapping; abundance analyses were carried out on the sampling units of individual transect nights and pitfall trap nights. We assessed whether abundances could be predicted by disturbance history through the use of linear mixed models (only conducted where over 30 detections were made of a particular family). A Poisson family distribution was utilised initially (package lme4; glmer function) but significant levels of overdispersion were detectable within the models, a common feature in count data (Zuur et al. 2009). A negative binomial family distribution with a log link function was therefore used to account for this overdispersion (packages - R2admb and glmmADMB; using function glmmadmb) (Trimble and van Aarde 2014; Zuur et al. 2009).

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

3. RESULTS

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

TABLE 1 – Observed and estimated species richness patterns based on different survey methodologies. A comparison of nocturnal transects and pitfall traps as amphibian survey methodologies. Survey effort for transects relates to the number of sampling nights (each night consisting of five 100x4m transects or 2000m^2 searched) and pitfall trap survey effort relates to the total number of individual trap sessions (each session 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*	*Jacknife 1*	*Jacknife 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: $^{b/c}$*100

[e] Number of species observed as a percentage of combined species across all habitats

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

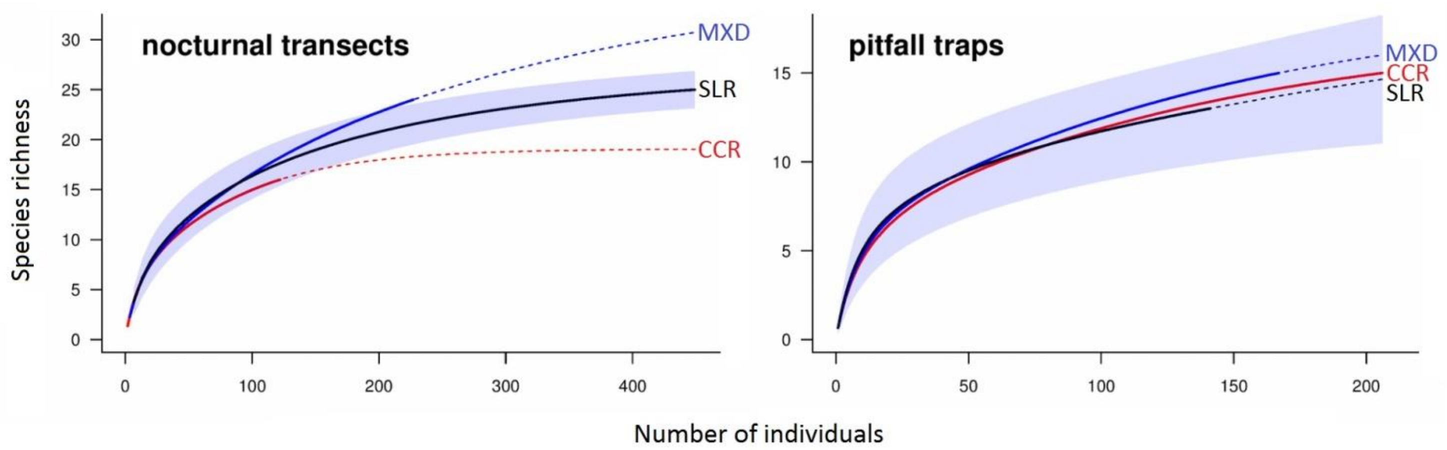

FIGURE 2 – Amphibian species richness of regenerating rainforest with different disturbance histories for amphibian communities from the same area based on a) nocturnal transects and b) pitfall traps. Solid lines represent the observed number of individuals recorded and dashed lines represent predicted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

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

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

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

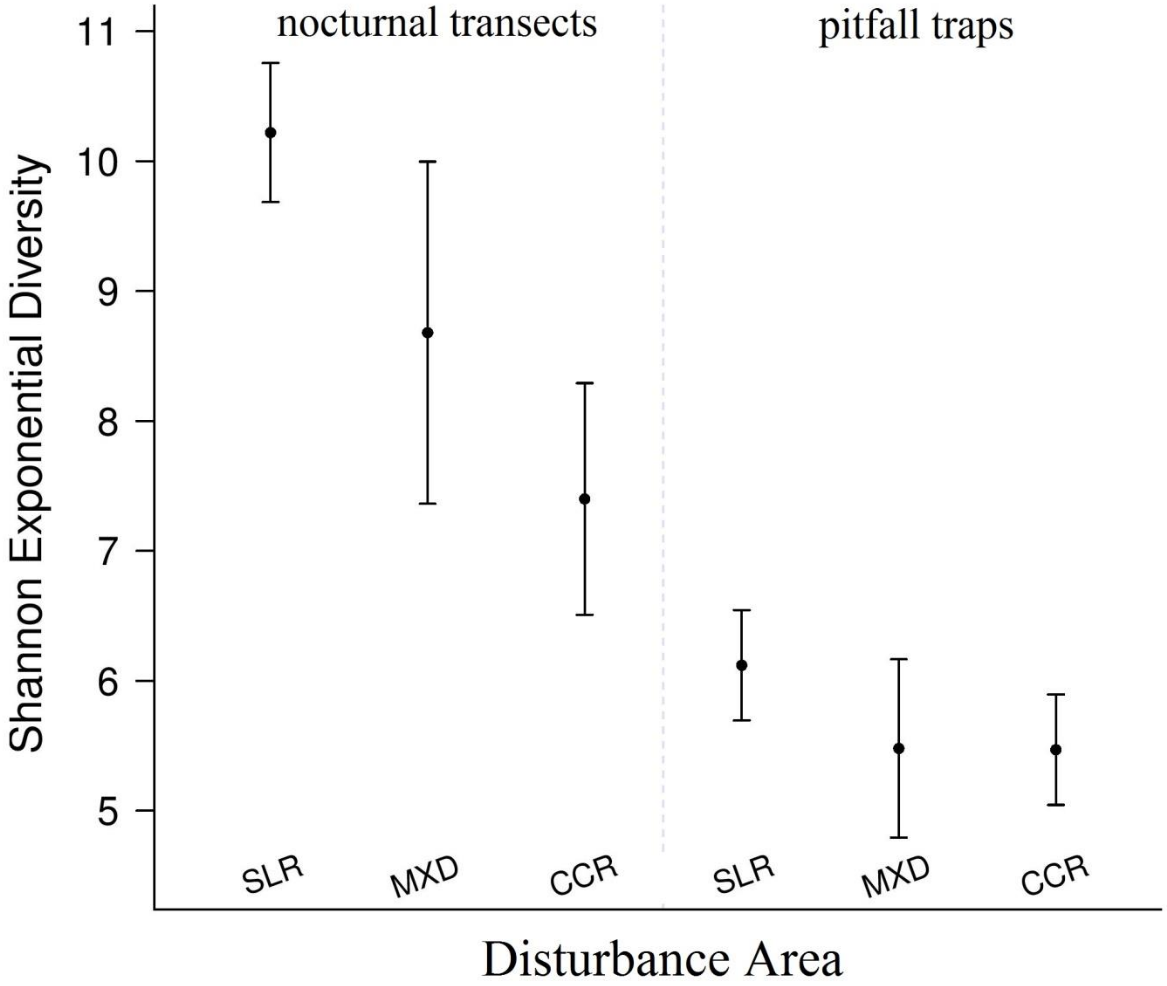

FIGURE 3 - Shannon species diversity of regenerating rainforest with different disturbance histories for amphibian communities from the same area based on a) nocturnal transects and b) pitfall traps; with 84% confidence intervals.

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

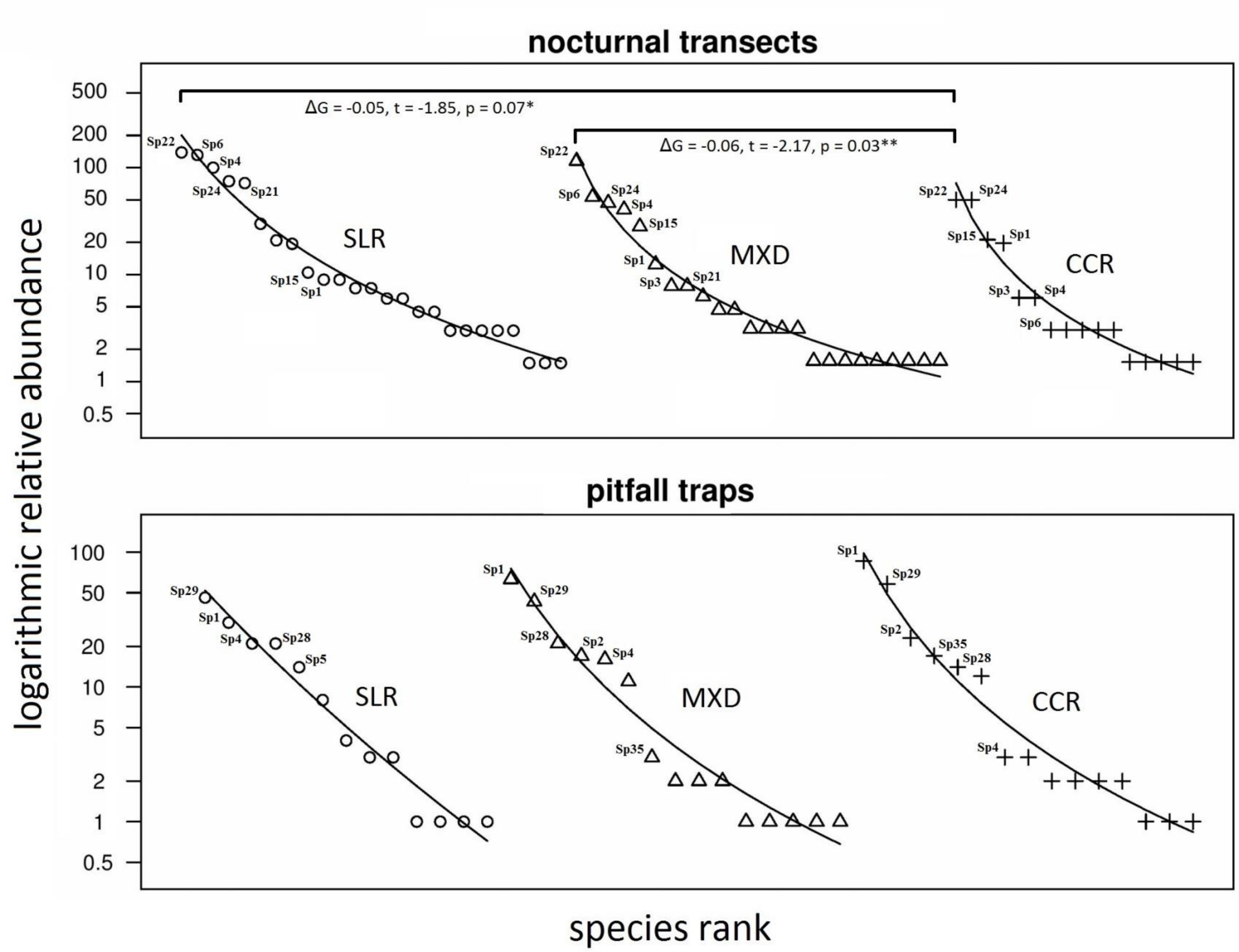

FIGURE 4 – Dominance-diversity (Whittaker) plots for nocturnal transects and pitfall trap amphibian communities in regenerating rainforest with different disturbance histories. Species are represented by points. For each habitat the relative abundance of each species (ni/N) was plotted on a logarithmic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

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

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

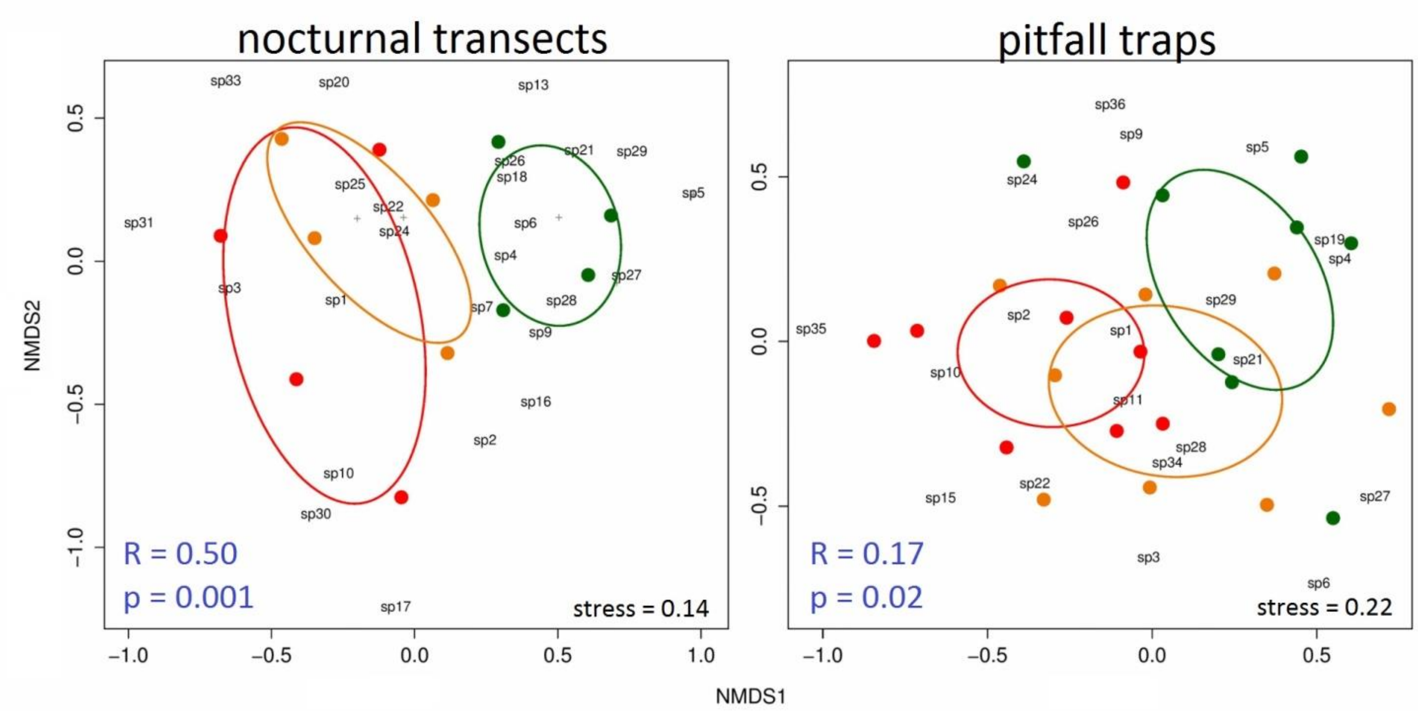

FIGURE 5 - Community composition NMDS plots of regenerating rainforest with different disturbance histories for amphibian communities from the same area, based on nocturnal transects and pitfall traps; species and corresponding codes are provided in Appendix A. The red circles = CCR sampling locations,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

The community composition analysis from NMDS plots and the associated ANOSIM analysis (Fig. 5) showed that community composition between disturbance areas was significantly different for both nocturnal transects (R=0.50, p=0.001) and pitfall trap (R=0.17, p=0.02) methodologies. However, the R statistic, which denotes the degree of difference between communities, is higher for the communities assessed using nocturnal transects and although the communities assessed by pitfall traps shows a significant p-value, the R statistic is relatively low. The NMDS plots (Fig. 5) show that the SLR community for nocturnal transects is completely distinct from both CCR and MXD communities (which show a significant degree of overlap), whilst for pitfalls, the SLR community is only distinct from the CCR community but shares some degree of overlap with the MXD community.

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

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

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

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

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

4. DISCUSSION

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

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

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

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

In addition to assessing overall patterns of biodiversity, we also investigated fine scale metrics of the amphibian community, in the form of species specific response patterns and abundances of family groups (as opposed to overall community structure and overall abundance patterns). Although there is a growing body of literature investigating species specific and functional groups in tropical forests for birds (De Coster et al. 2015; Edwards et al. 2013; Hidasi-Neto et al. 2012; Newbold et al. 2013) and plants (Ding et al. 2012; Carreño-Rocabado et al. 2012), few studies exist for amphibians (Trimble and van Aarde 2014). These fine scale metrics allowed for the detection of community structure and composition differences between disturbance areas using both survey methodologies; with increases/decreases in rank and abundance for some species. Although overall abundance of the amphibian community from pitfalls did not show a difference between disturbance areas, two of the three families tested did, albeit in opposite directions. We suggest therefore, that fine scale metrics, which assess species specific responses or patterns of families, may be less susceptible to show contrasting patterns between methodologies. However, this should be considered with caution and requires further investigation, specifically in light of concerns over the application of community similarity metrics to assess disturbance patterns across landscapes, as suggested by Ramage et al. (2013).

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

In addition to simply identifying that the different levels of impact upon biodiversity linked to habitat change may be the result of alternative methodologies, it is also important to understand more about why alternative methods indicate different patterns. Within this study for example, we utilised two commonly used methodologies which target distinct subsets of the overall amphibian community. Pitfall traps better target the terrestrial amphibian community (i.e. Dendrobatidae, Leptodactylidae and Bufonidae; e.g. Beirne et al. 2013), whereas nocturnal transects have been shown to be more efficient in detecting a wider representation of the amphibian community (e.g. Beirne et al. 2013; Doan 2003), including both terrestrial (i.e. Dendrobatidae and Leptodactylidae) and arboreal groups (i.e. Craugastoridae, Hylidae and Plethodontidae). Our results related to overall patterns may therefore suggest that arboreal amphibian communities (although some canopy dwelling species are likely missed due to the limit of ground-based survey techniques) are more sensitive to habitat disturbance than terrestrial communities. This is a pattern that has been detected for a variety of invertebrates within tropical forests, including ants (Klimes et al. 2012), dung beetles (Tregidgo et al. 2010), and butterflies (Whitworth et al. 2016b). The known key indicator group of good quality habitat (Pearman 1997), the Craugastoridae, are more commonly encountered via transects as opposed to terrestrial based techniques, and are a mostly semi-arboreal group (comprising mostly *Pristimantis sp.*). However, the mostly terrestrial Dendrobatidae appeared well represented by both methodologies and indicated the same abundance patterns in relation to historic disturbance. Contrastingly, Leptodactylidae were better surveyed by pitfall traps, with only this method detecting a significantly different abundance related to historic disturbance. This may be due to the detectability differences within these families, which relates to morphological and functional traits (Mouillot et al. 2013). Dendrobatids are often bright coloured and conspicuous, therefore easy to spot on the ground at night; whereas Leptodactylidae are brown cryptically coloured frogs, often located in holes, more difficult to detect at night in the leaf-litter.

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

ACKNOWLEDGMENTS

We first of all thank the Crees Foundation (www.crees-manu.org) and its director Quinn Meyer for supporting this research as part of their conservation and biodiversity monitoring programme. We gratefully acknowledge the financial support and encouragement of the TJMF Foundation who provided crucial support for this work through their Amazon Research Programme grant to the University of Glasgow. Thanks also to the Darwin Initiative for financial support of the Sustainable Manu project, a collaborative initiative between the Crees Foundation and The University of Glasgow that supported this research. Thanks also to the Ministerio de Agricultura of Peru for providing the permit to conduct research in Peru (Permit provided by the Ministerio de Agricultura of Peru; Permit Number ‘Codigo de Tramite’: 25397; Authorisation Number ‘Autorización No.’ 2904-2012-AG-DGFFS-DGEFFS).

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