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Amphibian diversity across three adjacent ecosystems in Área de Conservación Guanacaste, Costa Rica

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Abstract

Amphibians are the most threatened species-rich vertebrate group, with species extinctions and population declines occurring globally, even in protected and seemingly pristine habitats. These 'enigmatic declines' are generated by climate change and infectious diseases. However, the consequences of these declines are undocumented as no baseline ecological data exists for most affected areas. Like other neotropical countries, Costa Rica, including Área de Conservación Guanacaste (ACG) in north-western Costa Rica, experienced rapid amphibian population declines and apparent extinctions during the past three decades. To delineate amphibian diversity patterns within ACG, a large-scale comparison of multiple sites and habitats was conducted. Distance and time constrained visual encounter surveys characterised species richness at five sites - Murciélago (dry forest), Santa Rosa (dry forest), Maritza (mid-elevation dry-rain forest intersect), San Gerardo (rainforest) and Cacao (cloud forest). Furthermore, species-richness patterns for Cacao were compared with historic data from 1987-8, before amphibians declined in

31 the area. Rainforests had the highest species richness, with triple the species of their dry forest

32 counterparts. A decline of 45% (20 to 11 species) in amphibian species richness was encountered

33 when comparing historic and contemporary data for Cacao. Conservation efforts sometimes

34 focus on increasing the resilience of protected areas, by increasing their range of ecosystems. In

35 this sense ACG is unique containing many tropical ecosystems compressed in a small geographic

36 space, all protected and recognised as a UNESCO world heritage site. It thus provides an

37 extraordinary platform to understand changes, past and present, and the resilience of tropical

38 ecosystems and assemblages, or lack thereof, to climate change.

39

40 **KEY WORDS:** Amphibian declines; Costa Rica; species richness; forest habitats; Área de

41 Conservación Guanacaste; anurans.

42

1. INTRODUCTION

44

45   ONGOING BIODIVERSITY LOSS AND ITS ASSOCIATED IMPACTS ARE A major

46 global issue, with the current rate of extinctions unprecedented in recent time – over 1000 times

47 the probable natural background rate (Barnosky et al. 2011; Ceballos et al. 2017; Pimm et al.

48 2006; Pimm et al. 2014). This loss of species is changing and impoverishing ecosystems all over

49 the world (Hooper et al. 2012, Pimm & Raven 2000, Pimm et al. 1995) and is a major concern

50 for biologists and ecologists studying a wide range of taxa (Ehrlich 1995, Dirzo et al. 2014,

51 Janzen & Hallwachs 2020, Worm & Tittensor 2011), not to mention the tropical societies that

52 are losing their natural wild capital. At the vanguard of this current extinction spasm however are

53 amphibians, with more species threatened with extinction than any other major vertebrate taxon

54 (Stuart et al. 2004).

55

56   Amphibian diversity is strongly correlated with environmental conditions such as

57 precipitation, temperature, and available moisture. Available moisture can be measured as the

58 relation between potential and actual evapotranspiration and appears to be a major determinant

59 of amphibian diversity in Costa Rica (Savage 2002), with extreme humid conditions (where

60 precipitation greatly exceeds potential evapotranspiration) being associated with the highest

61 diversity of species. Temperature is another essential driver of Costa Rican amphibian diversity

62   and is reflected by changes in temperature along an altitudinal gradient – moving from cooler

63   temperatures at higher elevations to warmer ones at lower elevations. For example, 65% of Costa

64   Rican amphibians can be found within the premontane belt, potentially reflecting the overlap

65   between the lower temperature limits of upland species and upper limits of lowland species

66   (Savage 2002). However, this means that individuals are highly susceptible to changes in these

67   conditions (Bickford et al. 2010, Ficetola & Maiorano 2016, Ryan et al. 2015, Walls et al. 2013),

68   making them vulnerable to anthropogenic pressures.

69

70   There are approximately 8480 known amphibian species (Frost, 2022), 41% of which are

71   threatened with global extinction (IUCN 2018) and 43% have declining populations (Hof et al.

72   2011, Stuart et al. 2004,). Yet even these numbers are likely to be underestimated as our

73   knowledge of tropical amphibian diversity and density is so poor (Wake & Vredenburg 2008). It

74   is widely agreed that amphibians face a constellation of threats, with many working

75   synergistically to accelerate declines, including global climate change, habitat destruction and

76   alteration, invasive species, overexploitation, and infectious disease (Collins & Crump 2009).

77   Amphibian population declines have been noted as early as the 1950s (Houlahan et al. 2000) but

78   didn't receive broad attention until the 1980s (although see Alford et al. 2001), after several

79   localities experienced rapid population crashes, with many of these occurring in seemingly

80   pristine and protected areas (Stuart et al. 2004; Burrowes et al., 2004). These 'enigmatic'

81   declines were thought to occur due to a myriad of factors (Collins & Storfer 2003), but today two

82   main causal factors have since been recognised: the pathogenic fungus *Batrachochytrium*

83   *dendrobatidis* and climate change (Blaustein & Dobson 2006, Clare et al. 2016, Lips et al. 2006,

84   Lips et al. 2008, Pounds & Puschendorf 2004, Pounds et al. 2006, Rohr et al. 2008, Whitfield et

85   al. 2007).

86

87   Similar to other regions in tropical Central America, declines of Costa Rican amphibians

88   have occurred rapidly (within 2 - 3 yrs.) at elevations above 500 m (Young et al. 2001) and has

89   resulted in the extirpation of endemics found at higher elevations (Bolaños, 2002, Pounds et al.

90   1997). Área de Conservación Guanacaste (ACG), which protects 120,000 ha of dry, rain and,

91   cloud forest (and 43,000 ha of Pacific Ocean) in northwestern Costa Rica, (Janzen et al. 2016)

92   lost many amphibian species in the late 1980's, mostly in upland areas (Puschendorf et al. 2019).

93

94   Amphibian communities are already feeling the effects of climate change, both globally

95   (Blaustein et al. 2010, Corn 2005, Li et al. 2013) and within ACG. These impacts observed for

96   amphibians are mirrored by other taxa, with many lowland ACG species of both vertebrates and

97   invertebrates now being recorded at much higher elevations (Smith et al. 2014), whilst increased

98   droughts have led to widespread tree and epiphyte mortality (Powers et al. 2020). Furthermore,

99   Janzen and Hallwachs (2021) have witnessed a precipitous decline in insect numbers since they

100 first started working in ACG since 1963 and 1978, respectively. This trend they attribute to

101 climate change, specifically the expanded and irregular dry season in all three major ecosystems

102 present in ACG. The evidence is mounting that climate change is not an abstract event that will

103 impact the world and ACG in the future, but a catastrophe we are experiencing now. To

104 understand the future impacts of climate change, it is important to know the species that are most

105 at risk and their needs and characteristics.

106

107   To draw meaningful comparisons, document any potential shift in diversity and

108 distribution of species and define and measure conservation targets, temporal baseline data is

109 fundamental (Mihoub et al. 2017). Despite the well documented recent declines and extinctions

110 of amphibians across the globe, baseline data for many tropical places is still scant (Collen et al.

111 2008, Feeley & Silman 2010, Siddig, 2019). This well documented decline of tropical amphibian

112 diversity is based on a limited number of localities in better studied countries such as Australia,

113 Costa Rica, Panama, Ecuador and a few others (Pounds & Crump 1994, Richards et al. 1994,

114 Lips et al. 2006, Merino et al. 2006). Most of these declines have occurred at higher elevations,

115 but more recent work suggest lowland populations are not exempt, with declines tending to occur

116 over longer time periods (Whitfield et al. 2007, Ryan et al. 2014). Despite Costa Rica being one

117 of the better studied localities for amphibian declines, baseline data is still lacking for many

118 important areas - including ACG.

119

120   Several studies have investigated amphibian species richness within ACG, but these

121 tended to focus on a single forest type (Bickford 1994, Sasa & Solórzano 1995) and lacked

122 population level data. Identifying long-term population trends is essential for any conservation

123 endeavour but has proved difficult for most tropical amphibians due to the lack of historical

124 baseline data and overall disinterest in gathering it. The few studies (e.g., Acosta-Chaves et al.

125 2019, Ryan et al. 2014, Whitfield et al. 2007) that have incorporated long-term population data

126 have found large-scale declines in amphibian populations. Over a 35-year period in the lowland

127 rainforest of La Selva, Caribbean Costa Rica, Whitfield et al. (2007) documented a decline of

128 75% in terrestrial amphibian density since 1970. La Selva is a protected old-growth rainforest.

129 Here we are building on these initial studies and integrating abundance data in a large-scale

130 comparison of several sites and habitats within ACG, providing vital baseline data valuable for

131 understanding and anticipating long-term trends. Furthermore, by incorporating historic species

132 richness data for one of the ACG cloud forest sites, where species richness declined in the late

133 1980's, we hypothesise that some species recovery should be noted, mirroring similar species re-

134 discovery in many other sites in lower Central America, where declines occurred (García-

135 Rodríguez et al. 2012, Voyles et al, 2018).

136

137

138 **2 METHODS**

139

140 **2.1 Study sites**

141

142 We sampled five sites in ACG which included: Cacao (10°55'36.264"N;

143 85°28'5.8794"W; 1050 m above sea level (asl); cloud forest), San Gerardo (10°52'48"N;

144 85°23'20.3994"W; 573 m asl; rainforest), Maritza (10°57'727.0"N; 85°29'40.3"W; 590 m asl;

145 mid-elevation dry-rain forest intersect), Murciélago (10°54'3.6354"N; 85°43'45.444"W; 80 m

146 asl; dry forest) and Santa Rosa (10°50'16.7634"N; 85°37'7.2042"W; 289 m asl; dry forest;

147 Figure 1). All five sites are 4.5 - 37.5 km distance from each other. Murciélago has the highest

148 mean annual temperature, whereas Cacao has the lowest (Table 1). Cacao has the highest mean

149 annual precipitation and precipitation during the driest quarter, while Murciélago has the lowest

150 annual precipitation (Table 1). Santa Rosa and Murciélago are comprised of a mosaic of

151 relatively young dry forest in restoration from pastureland in the last three decades, with a few

152 remaining tiny patches of older growth forest that escaped logging and burning. San Gerardo is a

153 classical rainforest of 400 – 700 m elevation. Cacao and Maritza are both older forests, with a

154 mix of old-growth and regenerating forests. Average annual rainfall at these study sites can vary

155 and ranges between 1613.3 $\pm$ 17.44 mm and 2820 $\pm$ 56.35 mm (Mean $\pm$ *SD*; Fick & Hijmans

156 2017) with a major part of this variation due to hurricane years. The mean annual temperature

157 ranges between 20.74 $\pm$ 0.67 °C and 26.15 $\pm$ 0.18 °C (Mean $\pm$ *SD*; Fick & Hijmans 2017), with

158 a marked rainy season (May - December).

159

160

161 **2.2 |Sampling methods**

162

163 We collected data between the 09 August and 15 November 2017 (rainy season). At each

164 site, 10 X 100 m long transects were established – split evenly between terrestrial and riparian

165 habitats. Animals were captured within 2 m of the transect and extending 2 m in height. The

166 distance between transects varied between 100 m and 4 km, depending on terrain and

167 topography. We used distance and time constrained Visual Encounter Surveys (hereafter referred

168 to as 'VES'; Scott 1994, von May et al. 2010) for a duration of 40 minutes. We sampled three

169 quarters of the transects at night (1800 h – 0000 h) and the remainder during the day (1020 h –

170 1530 h) to account for both diurnal and nocturnal species. We used VES as most amphibian

171 species are nocturnal and previous studies have shown that VES's (Crump & Scott 1994) are

172 more effective than other methods when sampling at night (Doan 2003, Rödel & Ernst 2004) and

173 have been shown to be of equal effectiveness to other methods when sampling for amphibians

174 during the day (Doan 2003). VES are an effective tool for detecting several salamander species

175 of the Plethodontidae family (Grover 2006), however species in the genera *Nototriton* and

176 *Oedipina* are best sampled using cover object searches which can damage fragile habitats –

177 notably mosses and bromeliads. No specific efforts were therefore taken to conduct destructive

178 sampling of a fragile cloud forest ecosystem in search of salamanders.

179

180 On terrestrial transects amphibians located two meters either side of the transect centre

181 were captured, for a total width of four meters and on substrates up to two meters in height (von

182 May & Donnelly 2009). Captured individuals were placed in their own plastic bags with

183 substrate and water for moisture and labelled with a unique identification code and location on

184 the transect. Further biosecurity precautions (e.g., new gloves for each capture) were deemed

185   unnecessary due to the high prevalence of *B. dendrobatidis* and *Ranavirus* within the ACG

186   (Wynne 2018, Puschendorf et al. 2019). Most individuals were released at the end of the survey,

187   but some were brought back for further identification and released the next day back at the point

188   of capture.

189

190   We resampled transects at two-to-four-day intervals, with each transect sampled four

191   times during this study. After the transect was set up a minimum of two days were left before

192   surveying began, to minimise any impact from disturbance on sampling. We measured and

193   marked down every 10 m on transects using flagging tape which we collected at the end of the

194   study. GPS coordinates and elevation were collected at the midpoint of each transect using a

195   Garmin 60CSX. Annual mean temperature, annual precipitation and precipitation of the driest

196   quarter were extracted for each field site from WorldClim (version 1.4) at a 1 km^2 resolution

197   (Hijmans et al. 2005).

198

199   Historic data for Cacao was obtained from Arctos Collaborative Collection (MVZ 2018)

200   management solutions museum database. Data were collected by David Cannatella and David

201   Good over 23 days between July 1987 and January 1988 – with most sampling occurring in

202   August 1987 (For species list see Table S1). There was no standardised sampling, observers

203   walked through the forest collecting everything they came across (D. Cannatella pers. comm).

204   Historic data for Cacao is hereafter referred to as historic Cacao.

206

207   **2.3 Data analysis**

208

209   Unless otherwise stated, all statistical analysis was conducted in the R statistical

210   environment v4.1.2 (R Core Team 2022). We used the numbers equivalent approach as

211   suggested by Jost (2006, 2007) to describe patterns of beta diversity and community similarity

212   across sites using the package 'vegetarian' (Charney & Record 2012). β-diversity was analysed

213   based on the numbers equivalent of Shannon's diversity $^1D_\beta$ using the diversity order $q = 1$ which

214   considers the proportional abundance of each species in a community, without favouring either

215 rare or abundant species (Jost 2006). Ten thousand bootstrap replicates of the data were used to

216 determine standard error of β-diversity for each site.

217

218   We performed sample-based rarefaction analyses to compare patterns of species richness

219 between sites (Gotelli & Colwell 2001). Transect data were pooled across sites and the 'vegan'

220 package (Oksanen et al. 2017) was used to generate the subsequent comparisons. A sample-

221 based rarefaction curve was further used to compare species richness patterns between historic

222 and current data for Cacao.

223

224   To estimate inventories completeness, we used the nonparametric estimators of species

225 richness; ACE and Chao1 based on abundance data (Hughes et al., 2001, Jiménez-Valverde &

226 Hortal 2003), using EstimateS Program V9.1.0 (Chao, 1984, Chao & Lee 1992, Chao & Yang

227 1993, Chazdon et al. 1998, Colwell 2013, Colwell & Coddington 1994).

228

229   To compare species abundance patterns between sites, rank abundance curves (RAC)

230 were plotted (Magurran 2004) using the BiodiversityR package (Kindt & Coe 2005). The slope

231 of linear regression of an RAC expresses the evenness in abundance among species within an

232 assemblage and an analysis of covariance (ANCOVA) was used to compare differences in

233 evenness among sites. An abundant species was arbitrarily defined as those that were represented

234 by more than 12 individuals (which is approximately 2% of all individuals across the study). We

235 used the package brms (Bürkner 2017, 2018) to test for differences among sites in the rate of

236 decay in rank abundance slopes. We specified per-species abundance as an outcome variable,

237 with a negative binomial error structure. We included the interaction between rank and site as

238 fixed effects, allowing the slope of decay to vary by site. We assessed model fit using visual

239 inspection of mcmc chains, and posterior predictive checks. We determined differences between

240 sites in rates of abundance decay based on whether differences in 95% credible intervals of slope

241 parameters included zero. We used the Leave One Out Information Criterion (LOO-IC, Vehtari

242 et al. 2017, 2020) to perform a full model test of the maximal model against the intercept only

243 model (Forstemeier & Schielzeth 2011).

244

245   Multidimensional scaling (nMDS) ordination (k = 2, stress = 0.12) using the 'vegan'

246 package (Oksanen et al. 2022) was used to visualise the difference in community structure and

247 composition among sites. The nMDS plot is based on a Jaccard matrix, using species

248 presence/absence data. Additionally, the similarity percentage (SIMPER: Clarke & Warwick

249 2001) was calculated to identify the contribution of individual species to the dissimilarity of

250 amphibian community structure among sites. Moreover, a SIMPER analysis was also conducted

251 using the historic data for Cacao to understand the changes in community structure over time and

252 how this has affected inter-site relatedness. Abundance was analysed after a square root

253 transformation of the data. This was conducted using the 'vegan' package (Oksanen et al. 2017).

254

255   All code and datasets required for reproducing these results, including model fitting and

256 data visualisation, are provided online

257 (https://github.com/xavharrison/CostaRica_RankAbundance_2022).

258

3. RESULTS

259

260

261   During the surveys between August – November 2017, 660 individual amphibians from

262 37 species were recorded, all anurans, (Table S2). This represents 46.25% of known amphibian

263 species to occur in ACG (Table S3). The overall sampling effort was 267 person-hours

264 throughout the entire study. In total 50 transects were resampled four times for a total of 200

265 transects. Several other individuals and species were captured outside of standard sampling

266 (Table S4), but those have not been included in this analysis. *Duellmanohyla rufioculis*,

267 *Craugastor fitzingeri*, *Rhaebo haematiticus* and *Craugastor crassidigitus* were the most common

268 species, comprising 20.3%, 13.5%, 11.7% and 11.7% of the total captured. We recorded nine

269 amphibian families (all anuran), with three families represented by only a single species:

270 Microhylidae (*Hypopachus variolosus*), Phyllomedusidae (*Agalychnis callidryas*) and

271 Eleutherodactylidae (*Diasporus diastema*).

272

273   All sites had low similarity based upon species abundance (Horn index $\pm$ *SD*: 0.19

274 $\pm$ 0.17). The overall β-diversity for all sites combined was 3.16 $\pm$ 0.134 (^{1}D$_\beta$ $\pm$ *SD*), highest in

275   San Gerardo ($^1D_\beta = 3.27 \pm 0.26$) and lowest in Santa Rosa ($^1D_\beta = 1.23 \pm 0.11$). β-diversity for

276   the remaining sites was as follows; Cacao ($^1D_\beta = 2.02 \pm 0.11$), Maritza ($^1D_\beta = 1.98 \pm 0.16$) and,

277   Murciélago ($^1D_\beta = 2.14 \pm 0.20$).

278

279   The sample size was sufficient to characterise species richness for three of the five sites;

280   Cacao; San Gerardo and Santa Rosa, as the rarefaction curve approaches an asymptote (Figure

281   2a). The highest number of species was recorded in San Gerardo (rainforest) and the least in

282   Santa Rosa. In Cacao, a total of 20 species were recorded in the 1980's compared to only 11 in

283   2017, a decline of 45% (Figure 2b). Of the 11-species recorded in 2017, three of them were

284   absent from the 1987 data – *Craugastor fitzingeri*, *Hyalinobatrachium colymbiphyllum* and

285   *Smilisca baudinii*. Furthermore, the curve for the historic data failed to reach an asymptote,

286   suggesting that the inventory was incomplete at that stage and more species remained to be

287   discovered. This is supported by museum records and data collected and stored at Arctos

288   Collaborative Collection management solutions (MVZ 2018), which suggest a total of 39 species

289   are known to occur in Cacao (Table S5).

290

291   Overall estimates of completeness were highest for Santa Rosa (ACE = 85.71% and

292   Chao1 = 100%) and San Gerardo, which was predicted to be missing 7 species (Table 2). Cacao

293   had the lowest level of completeness (ACE = 68.75% and Chao1 = 64.71%), as 54.58% of all

294   individuals encountered were *Duellmanohyla rufioculis*.

295

296   Our Bayesian regression (Table 3), suggests that Cacao was found to have much higher

297   species abundances at lower ranks. Whilst all sites decayed at a similar rate (i.e. had similar

298   slopes), the site:rank interaction in the model revealed San Gerardo to have a much shallower

299   rate of decline (Figure 3, Figure S1). Low density species (represented by a single individual)

300   also mainly occurred in San Gerardo as well as Cacao. The abundance distribution in Murciélago

301   and Santa Rosa suggests that these sites today have less abundant species as compared with San

302   Gerardo (Figure 4). *Rhinella horribilis* was the most dominant species in both Murciélago and

303   Santa Rosa. In contrast the dominant species in Cacao and Maritza (*Duellmanohyla rufioculis*

304   and *Lithobates warszewitschii*) are not found in lowland sites (Savage 2002).

305

306 The nMDS shows a clear split between most of the sites. Santa Rosa and Murciélago are

307 the most similar sites, followed by Cacao and Cacao historic (Figure 4). Excluding Cacao

308 historic, San Gerardo was identified as the most unique site, but this was closely followed by

309 Maritza. However, including Cacao historic resulted in Maritza being the most unique. The

310 SIMPER analysis suggests that the community structure of the five sites is distinct from each

311 other, despite the short geographic distance between them (Table 4), with an average

312 dissimilarity of 83.20%. Murciélago and Santa Rosa were the least dissimilar sites, with a

313 dissimilarity of 60.97%, followed by Cacao and Maritza with a dissimilarity of 71.66%. Cacao

314 and Santa Rosa had the highest dissimilarity between sites at 96.37%. The SIMPER analysis

315 using the historic data for Cacao showed an increase in similarity between Cacao and the other

316 sites over the 30-year period (1987/8 – 2017). As expected, the historic data for Cacao was most

317 like contemporary Cacao, with a dissimilarity of 61.87%. All sites, except Santa Rosa,

318 experienced an increase in similarity between the two periods with Maritza experiencing the

319 biggest drop, with a decrease in dissimilarity from 81.77% to 71.66%. The dissimilarity between

320 Santa Rosa and Cacao increased between the two sampling periods, increasing from 75.35% to

321 96.37%.

322

323 **4. DISCUSSION**

324

325 Our analysis presented here reinforces that amphibian species richness is strongly

326 correlated with forest type. This pattern follows the diverging environmental conditions present

327 in each forest type, which has resulted in very different communities across ACG. Furthermore,

328 we observed a substantial decrease in amphibian species richness over time, at the relatively

329 undisturbed cloud forest site Cacao. This is further evidence for the widespread decline of

330 amphibians observed globally and in Costa Rica over the past several decades, and recovery is

331 still tenuous, if at all (Lips et al. 2006, Stuart et al. 2004, Whitfield et al. 2016).

332

333 Historic museum records kept at the Museo de Zoología, Universida de Costa Rica have

334 documented 80 species, consisting of 75 Anurans, one Gymnophiona and four Caudata within

335 ACG. We detected 37 species of anurans in the three main ACG ecosystems. Many ACG areas

336   have yet to be surveyed more than superficially; and will contain unrecorded or new species. For

337   example, during the pilot study we discovered *Agalychnis saltator* in Pitilla (Table S4), which

338   represents a substantial range expansion for this species and a species new to ACG. Furthermore,

339   new molecular approaches are revealing previously undescribed amphibian cryptic diversity

340   (Funk et al. 2012, Stuart et al. 2006) including in ACG frogs (e.g., Cryer et al. 2019). Finally,

341   sampling across seasons and years will be key to elucidating the full diversity of ACG

342   amphibians, with many species experiencing yearly fluctuations in population size (Marsh 2001)

343   and higher visibility in specific seasons (Laurencio & Fitzgerald 2010, Savage 2002).

344

345   Rainforests had the highest levels of amphibian species richness, which support previous

346   findings for Costa Rica (Savage 2002) and elsewhere (Duellman & Trueb 1994). The three forest

347   types sampled are in part defined by their evolutionary history, vegetation communities, previous

348   disturbance and stage of restoration, levels of precipitation, temperature and the annual actual

349   evapotranspiration (AET; Janzen et al. 2016). It has been demonstrated that a mixture of water

350   and energy variables are important in shaping amphibian species richness patterns in North

351   America, Europe, Asia and Central America (Currie 2001, Laurencio & Fitzgerald 2010,

352   Rodriquez et al. 2005). For example, Qian et al. (2017) found a strong positive correlation

353   between amphibian species richness and environmental variables such as precipitation, net

354   primary productivity, range in elevation and temperature; in 245 localities across China. These

355   findings demonstrate that environmental variables may play a role in constraining the species

356   richness at a site and constitutes the most plausible explanation for the differences between the

357   forest types. This is supported by the fact that dry forest sites, prior to disturbance, had much

358   lower levels of species richness and tended to be dominated by large-bodied generalists, such as

359   *Rhinella horribilis*, *Smilisca baudinii* and *Lithobates forreri* which have wide distributions and

360   are adapted to the seasonally xeric conditions of the dry forest. These anurans are less prone to

361   desiccation, as their large body size means that they have proportionally lower surface area to

362   body volume and thus lower rates of water loss than smaller bodied species (Duellman & Trueb

363   1994) This likely explains their higher abundances and dominance in the dry forest, which is

364   characterised by dry season high temperatures and less rain, especially during the dry season.

365   One such adaption to the xeric conditions of the dry forest is cocoon formation, as observed in

366   *Smilisca baudinii*, allowing them to survive long periods without rain (McDiarmid & Foster

367 1987). The similarity between Cacao and Maritza is likely due to the proximity of these two sites

368 (4.5 km) and that they occupy one continuous forest, albeit over an elevational gradient, rather

369 than environmental conditions – which are grossly different between the two sites.

370 *Duellmanohyla rufioculis* was only found at these two sites, whilst *Lithobates warszewitschii*

371 was far more abundant in these two sites than any other.

372

373 Weather conditions at different elevations are likely to play a significant role in

374 constraining diversity to a specific site and may explain the greater diversity found in San

375 Gerardo compared to Cacao. For many groups of organisms, including amphibians (Campbell

376 1999), diversity changes along an elevational gradient (e.g., McCain 2005, Navas 2003,

377 Terborgh 1971), following a bell-shaped curve. Species richness is relatively low at lower and

378 higher elevations, with the highest species richness recorded at mid-elevations. However,

379 endemism in the tropics is far more ubiquitous at high elevation sites; meaning they are of great

380 conservation priority – a consequence of these sites being far more insular (Savage 2002). The

381 results roughly follow this trend, with the average elevation of our transects in the most species

382 rich site, San Gerardo (573.32 m), between the elevation of the less diverse higher elevation site

383 (Cacao: 1050.17 m) and lower elevation sites (Santa Rosa: 289.2 m, Murciélago: 80.5 m).

384

385 Despite differences in the structure of the forest habitats, two species were found to occur in all

386 four, *Rhinella horribilis* and *Craugastor fitzingeri*. This is likely attributed to their generalist

387 nature and ability to adapt to human altered landscapes (Crawford et al. 2007). Only 11 species

388 were found at more than one site, but some exhibited far higher abundance in only one forest

389 type, such as *D. rufioculis* which was found at very high abundances in Cacao (131 individuals),

390 low abundances at Maritza (3 individuals) and absent from all other sites – a consequence of the

391 elevational range constraints and climatic requirements of this species (Savage 2002). Historic

392 declines may also play a role in the presence and absence of certain species at different sites, as

393 illustrated by *Craugastor ranoides*. This once widespread riparian species is likely highly

394 sensitive to *B. dendrobatidis* outbreaks (known populations of this species have disappeared

395 from most of its range in Costa Rica, and *B. dendrobatidis* was found responsible for the decline

396 of a highly-related species, *Craugastor punctariolus*; Ryan et al. 2008) and is likely only to

397 persist in Murciélago due to the areas status as a climatic refuge, where the environmental

398   conditions have helped prevent disease outbreaks (Puschendorf et al. 2009). However, this dry

399   forest peninsula is also subject to serpentinization (Sanchez-Murillo et al. 2014). This produces

400   hyperalkaline fluids, reaching a pH of > 11, which drain into the local streams in which these

401   frogs live. The potential effects of this pH change on the skin fungus and its resultant disease are

402   yet to be explored. In Cacao forest, alongside *Craugastor ranoides, Atelopus varius*, *Isthmohyla*

403   *tica*, *Craugastor andi*, *Duellmanohyla uranochroa* have also vanished and all salamanders are

404   now extremely uncommon. However, more intensive sampling during different years and

405   different seasons may reveal that these species persist, albeit in much lower numbers.

406

407   The steep decline in amphibian diversity in Cacao, over the 30-year period 1987/8 – 2017

408   is persistent and clearly recovery has been slow. A 45% reduction in species richness was

409   observed, with only 11 species recorded in 2017 compared to 20 in the 1980's, with far greater

410   sampling effort involved in 2017. The complete lack of salamanders on the transects was

411   especially notable, due to their historic ubiquity in the area and this finding aligns with the

412   declines reported by other studies on neotropical salamanders (Acosta-Chaves et al. 2015, Rovito

413   et al. 2009). In the early 1980's and 1990's, D.H. Janzen regularly encountered salamanders

414   under fallen, rotting tree stems (night and day) and on wet foliage at night, whilst searching for

415   caterpillars in the vicinity of Estacioón Biológica Cacao (800 – 1400 m) year-round. Since the

416   2000's none have been encountered by either D.H. Janzen or the parataxonomists on their daily

417   search for caterpillars. Although we cannot say with certainty that these salamanders are locally

418   extinct, if they are still present at Cacao it is likely at levels substantially below their pre-decline

419   numbers and recovery to these levels appears increasingly doubtful. The historic data supports

420   previous studies looking at herpetofauna diversity of sites at similar elevations (Scott 1976:

421   Puntarenas Province, Costa Rica). Cacao is comprised of mostly old growth forest with a few

422   patches of forest at various stages of regeneration, which makes these declines even more

423   alarming. But these declines match those experienced by other high elevation old growth forests

424   in the neotropics (Young et al. 2001). The limited data also demonstrates that there has been little

425   recovery of amphibian diversity following these declines. However, certain species appear to

426   have been less affected in the long-term than others, such as *C. crassidigitus*, *D. rufioculis and*,

427   *L. warszewitschii*, which despite experiencing similar declines, have since recovered and are now

428   the most visible of the Cacao amphibian community. A recent study by Acosta-Chaves et al.

429 (2019) found similar results with *C. crassidigitus* and *L. warszewitschii* now dominating the

430 amphibian community of Reserva de San Ramón, despite their almost absence in the 1990s.

431 Voyles et al. (2018), examined the temporal changes in detection rates of 12 riparian species at

432 three sites in Panama. Many of the species experienced rapid decreases during the epizootic

433 phase of the *B. dendrobatidis* outbreak. However, following the transition to the enzootic phase,

434 *B. dendrobatidis* prevalence decreased, concomitant to the recovery of several of the species;

435 including *L. warszewitschii* and *C. crassidigitus*. This suggests changes in host responses to

436 diseases.

437

438   A potential cause of these declines is the pathogenic fungus *B. dendrobatidis*, which has

439 been reported for several frog species on Cacao (Wynne 2018), although synergistic interactions

440 among different environmental variables may conceal individual effects (Navas & Otani 2007).

441 Scheele et al. (2019) suggest that *B. dendrobatidis* is responsible for the decline of 501

442 amphibian species and the potential extinction of 90 species, making it seem to be one of the

443 deadliest diseases for wild biodiversity. However, amphibian population collapses are not

444 occurring in isolation – they are part of a constellation of changes taking place in tropical old

445 growth forests (including Cacao), such as the decline of birds, lizards and insects, which are not

446 susceptible to *B. dendrobatidis* (Janzen & Hallwachs 2021, Lister & Garcia 2018, Pounds et al.

447 1999, M. Sasa unpubl. data, Zipkins et al. 2020, Zipkins & DiRenzo 2022), suggesting *B.*

448 *dendrobatidis* may not be the sole culprit of these observed declines. Cacao, as with many of the

449 other regions where declines have been documented, has gone through an ecological

450 homogenisation, with a large increase in similarity among sites following the declines (Smith et

451 al. 2009). This is likely to be an underestimate of dissimilarity as today we know that lowland

452 amphibian communities have also been suffering declines, just over a longer time period (Ryan

453 et al. 2008, Whitfield et al. 2007). However, baseline data is only available for Cacao.

454

455   Documenting long-term declines is only possible through the collection of baseline data

456 (e.g., Ryan et al. 2008, Whitfield et al. 2007). The observation of a substantial decline in

457 amphibian diversity within an old growth forest in ACG was only possible because of data

458 collected several decades prior, by an expedition from the University of California, Berkeley.

459 Other sites examined in this study may have experienced similar declines to that of Cacao,

460 however we lack the data to empirically support this. ACG is in a unique position to provide a
461 platform for understanding changes, past and present, and the resilience, or lack thereof, of
462 tropical ecosystems and assemblages to climate change.
463

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