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Edwards, A

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

Alex W. Edwards^{1*}, Xavier A. Harrison^{2*}, M. Alex Smith³, Maria Marta Chavarria⁴, Mahmood Sasa⁵, Daniel H. Janzen⁶, Winnie Hallwachs⁶, Gerardo Chaves⁵, Roberto Fernandez⁷, Caroline Palmer¹, Chloe Wilson¹, Alexandra North¹, Robert Puschendorf^{1**}

¹ School of Biological and Marine Sciences, University of Plymouth, Plymouth, PL4 8AA, UK

² Centre for Ecology and Conservation, Exeter University, UK.

³ Department of Integrative Biology, University of Guelph, Guelph, ON, Canada.

⁴ Programa de Investigación, Área de Conservación Guanacaste, Aptdo. 169-5000, Liberia, Costa Rica.

⁵ Escuela de Biología, Universidad de Costa Rica, San Pedro, Costa Rica.

⁶ Department of Biology, University of Pennsylvania, Philadelphia, PA, USA.

⁷ Guanacaste Dry Forest Conservation Fund,

*equal first authors

**Corresponding author: robert.puschendorf@plymouth.ac.uk

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

43 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 ± 17.44 mm and 2820 ± 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 ± 0.67 °C and 26.15 ± 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 km² 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. This work was carried out
205 under CONAGEBIO Permit number R-036-2013-OT- CONAGEBIO.

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

259 **3. RESULTS**

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 rufiocularis*,
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 ± 0.134 (${}^1D_{\beta} \pm$ 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, Universidad 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 rufiocularis* 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. rufiocularis* 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 serpentization (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 Estación 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. rufiocolis* 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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465

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471

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