

Amphibian diversity across three adjacent ecosystems in Costa Rica

Commented [VAC1]: I think the title could be a little more informative adding the ACG or Guanacaste province or at least Northern 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 - Murciélagos (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 the area. Rainforests had the highest species richness, with triple the species of their dry forest counterparts. A decline of 45% (20 to 11 species) in amphibian species richness was encountered when comparing historic and contemporary data for Cacao. Conservation efforts sometimes focus on increasing the resilience of protected areas, by increasing their range of ecosystems. In this sense ACG is unique containing many tropical ecosystems compressed in a small geographic space, all protected and recognised as a UNESCO world heritage site. It thus provides an extraordinary platform to understand changes, past and present, and the resilience of tropical ecosystems and assemblages, or lack thereof, to climate change.

KEY WORDS: Amphibian declines; Costa Rica; species richness; forest habitats; Área de Conservación Guanacaste; anurans.

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2 INTRODUCTION

ONGOING BIODIVERSITY LOSS AND ITS ASSOCIATED IMPACTS ARE A major global issue, with the current rate of extinctions unprecedented in recent time – over 1000 times the probable natural background rate (Barnosky et al. 2011; Ceballos et al. 2017; Pimm et al. 2006; Pimm et al. 2014). This loss of species is changing and impoverishing ecosystems all over the world (Hooper et al. 2012, Pimm & Raven 2000, Pimm et al. 1995) and is a major concern for biologists and ecologists studying a wide range of taxa (Ehrlich 1995, Dirzo et al. 2014, Janzen & Hallwachs 2020, Worm & Tittensor 2011), not to mention the tropical societies that are losing their natural wild capital. At the vanguard of this current extinction spasm however are amphibians, with more species threatened with extinction than any other major vertebrate taxon (Stuart et al. 2004).

Amphibian diversity is strongly correlated with environmental conditions such as precipitation, temperature, and available moisture. Available moisture can be measured as the relation between potential and actual evapotranspiration and appears to be a major determinant of amphibian diversity in Costa Rica (Savage 2002), with extreme humid conditions (where

precipitation greatly exceeds potential evapotranspiration) being associated with the highest diversity of species. Temperature is another essential driver of Costa Rican amphibian diversity and is reflected by changes in temperature along an altitudinal gradient – moving from cooler temperatures at higher elevations to warmer ones at lower elevations. For example, 65% of Costa Rican amphibians can be found within the premontane belt, potentially reflecting the overlap between the lower temperature limits of upland species and upper limits of lowland species (Savage 2002). However, this means that individuals are highly susceptible to changes in these conditions (Bickford et al. 2010, Ficetola & Maiorano 2016, Ryan et al. 2015, Walls et al. 2013), making them vulnerable to anthropogenic pressures.

There are approximately 8480 known amphibian species (Frost, 2022, 41% of which are threatened with global extinction (IUCN 2018) and 43% have declining populations (Hof et al. 2011, Stuart et al. 2004,). Yet even these numbers are likely to be underestimated as our knowledge of tropical amphibian diversity and density is so poor (Wake & Vredenburg 2008). It is widely agreed that amphibians face a constellation of threats, with many working synergistically to accelerate declines, including global climate change, habitat destruction and alteration, invasive species, overexploitation, and infectious disease (Collins & Crump 2009). Amphibian population declines have been noted as early as the 1950s (Houlahan et al. 2000) but didn't receive broad attention until the 1980s (although see Alford et al. 2001), after several localities experienced rapid population crashes, with many of these occurring in seemingly pristine and protected areas (Stuart et al. 2004; Burrowes et al., 2004). These 'enigmatic' declines were thought to occur due to a myriad of factors (Collins & Storfer 2003), but today two main causal factors have since been recognised: the pathogenic fungus *Batrachochytrium dendrobatidis* and climate change (Blaustein & Dobson 2006, Clare et al. 2016, Lips et al. 2006, Lips et al. 2008, Pounds & Puschendorf 2004, Pounds et al. 2006, Rohr et al. 2008, Whitfield et al. 2007).

Similar to other regions in tropical Central America, declines of Costa Rican amphibians have occurred rapidly (within 2 -3 yrs.) at elevations above 500 m (Young et al. 2001) and has resulted in the extirpation of endemics found at higher elevations (Bolaños, 2002, Pounds et al. 1997). Área de Conservación Guanacaste (ACG), which protects 120,000 ha of dry, rain and,

cloud forest (and 43,000 ha of Pacific Ocean) in northwestern Costa Rica, (Janzen et al. 2016) lost many amphibian species in the late 1980's, mostly in upland areas (Puschendorf et al. 2019).

Amphibian communities are already feeling the effects of climate change, both globally (Blaustein et al. 2010, Corn 2005, Li et al. 2013) and within ACG. These impacts observed for amphibians are mirrored by other taxa, with many lowland ACG species of both vertebrates and invertebrates now being recorded at much higher elevations (Smith et al. 2014), ~~whilst, whilst~~ increased droughts have led to widespread tree and epiphyte mortality (Powers et al. 2020). Furthermore, Janzen and Hallwachs (2021) have witnessed a precipitous decline in insect numbers since they first started working in ACG since 1963 and 1978, respectively. This trend they attribute to climate change, specifically the expanded and irregular dry season in all three major ecosystems present in ACG. The evidence is mounting that climate change is not an abstract event that will impact the world and ACG in the future, but a catastrophe we are experiencing now. To understand the future impacts of climate change, it is important to know the species that are most at risk and their needs and characteristics.

~~In order to~~To draw meaningful comparisons, document any potential shift in diversity and distribution of species and define and measure conservation targets, temporal baseline data is fundamental (Mihoub et al. 2017). Despite the well documented recent declines and extinctions of amphibians across the globe, baseline data for many tropical places is still scant (Collen et al. 2008, Feeley & Silman 2010, Siddig, 2019). This well documented decline of tropical amphibian diversity is based on a limited number of localities, in better studied countries such as Australia, Costa Rica, Panama, Ecuador and a few others (Pounds & Crump 1994, Richards et al. 1994, Lips et al. 2006, Merino et al. 2006). Most of these declines have occurred at higher elevations, but more recent work suggest lowland populations are not exempt, with declines tending to occur over longer time periods (Whitfield et al. 2007, Ryan et al. 2014). Despite Costa Rica being one of the better studied localities for amphibian declines, baseline data is still lacking for many important areas, including ACG.

Several studies have investigated amphibian species richness within ACG, but ~~these~~ ~~tended~~these tended to focus on a single forest type (Bickford 1994, Sasa & Solórzano 1995) and

lacked population level data. Identifying long-term population trends is essential for any conservation endeavour but has proved difficult for most tropical amphibians due to the lack of historical baseline data and overall disinterest in gathering it. The few studies (e.g. Ryan et al. 2014, Whitfield et al. 2007, [Acosta-Chaves et al. 2019](#)) that have incorporated long-term population data have found large-scale declines in amphibian populations. Over a 35 year period in the lowland rainforest of La Selva, Caribbean Costa Rica, Whitfield et al. (2007) documented a decline of 75% in terrestrial amphibian density since 1970. La Selva is a protected old-growth rainforest. Here we are building on these initial studies and integrating abundance data in a large-scale comparison of several sites and habitats within ACG, providing vital baseline data valuable for understanding and anticipating long-term trends. Furthermore, by incorporating historic species richness data for one of the ACG cloud forest sites, where species richness declined in the late 1980's, we hypothesise that some species recovery should be noted, mirroring similar species re-discovery in many other sites in lower Central America, where declines occurred (García-Rodríguez et al. 2012, Voyles et al, 2018).-

2 METHODS

2.1 Study sites

We sampled five sites in ACG which included: Cacao (10°55'36.264"N; 85°28'5.8794"W; 1050m above sea level (asl); cloud forest), San Gerardo (10°52'48"N; 85°23'20.3994"W; 573m asl; rainforest), Maritza (10°57'727.0"N; 85°29'40.3"W; 590m asl; mid-elevation dry-rain forest intersect), Murciélago (10°54'3.6354"N; 85°43'45.444"W; 80m asl; dry forest) and Santa Rosa (10°50'16.7634"N; 85°37'7.2042"W; 289m asl; dry forest; Figure 1). All five sites are 4.5 - 37.5 km distance from each other. Murciélago has the highest mean annual temperature, whereas Cacao has the lowest (Table 1). Cacao has the highest mean annual precipitation and precipitation during the driest quarter, while Murciélago has the lowest annual precipitation (Table 1). Santa Rosa and Murciélago are comprised of a mosaic of relatively young dry forest in restoration from pastureland in the last three decades, with a few

remaining tiny patches of older growth forest that escaped logging and burning. San Gerardo is a classical rainforest of 400-700m elevation. Cacao and Maritza are both older forest, with a mix of old-growth and regenerating forests. Average annual rainfall at these study sites can vary and ranges between 1613.3 ± 17.44 mm and 2820 ± 56.35 mm (Mean $\pm$ SD; Fick & Hijmans 2017) with a major part of this variation due to hurricane years. The mean annual temperature ranges between 20.74 ± 0.67 °C and 26.15 ± 0.18 °C (Mean $\pm$ SD; Fick & Hijmans 2017), with a marked rainy season (May - December).

2.2 | Sampling methods

We collected data between the 09 August and 15 November 2017 (rainy season). At each site, 10 X 100 m long transects were established – split evenly between terrestrial and riparian habitats. Animals were captured within 2m of the transect and extending 2m in height. The distance between transects varied between 100 m and ~~4~~four km, depending on terrain and topography. We used distance and time constrained Visual Encounter Surveys (hereafter referred to as ‘VES’; Scott 1994, von May et al. 2010) for a duration of 40 minutes. We sampled three quarters of the transects at night (1800 h – 0000 h) and the remainder during the day (1020 h – 1530 h) to account for both diurnal and nocturnal species. We used VES as most amphibian species are nocturnal and previous studies have shown that VES’s (Crump & Scott 1994) are more effective than other methods when sampling at night (Doan 2003, Rödel & Ernst 2004) and have been shown to be of equal effectiveness to other methods when sampling for amphibians during the day (Doan 2003). VES are an effective tool for detecting several salamander species of the Plethodontidae family (Grover 2006), however species in the genera *Nototriton* and *Oedipina* are best sampled using cover object searches which can damage fragile habitats – notably mosses and bromeliads. No specific efforts were therefore taken to conduct destructive sampling of a fragile cloud forest ecosystem in search of salamanders.

On terrestrial transects amphibians located two meters either side of the transect centre were captured, for a total width of four meters and on substrates up to two meters in height (von May & Donnelly 2009). Captured individuals were placed in their own plastic bags with

substrate and water for moisture and labelled with a unique identification code and location on the transect. Further biosecurity precautions (e.g., new gloves for each capture) were deemed unnecessary due to the high prevalence of *B. dendrobatidis* and *Ranavirus* within the ACG (Wynne 2018, Puschendorf et al. 2019). Most individuals were released at the end of the survey, but some were brought back for further identification and released the next day back at the point of capture.

We resampled transects at two-to-four-day intervals, with each transect sampled four times during this study. After the transect was set up a minimum of two days were left before surveying began, ~~in order to~~ minimise any impact from disturbance on sampling. We measured and marked down every 10 m on transects using flagging tape which we collected at the end of the study. GPS coordinates and elevation were collected at the midpoint of each transect using a Garmin 60CSX. Annual mean temperature, annual precipitation and precipitation of the driest quarter were extracted for each field site from WorldClim (version 1.4) at a 1 km² resolution (Hijmans et al. 2005).

Historic data for Cacao was obtained from Arctos Collaborative Collection (MVZ 2018) management solutions museum database. Data were collected by David Cannatella and David Good over 23 days between July 1987 and January 1988 – with most sampling occurring in August 1987 (For species list see Table S1). There was no standardised sampling, observers walked through the forest collecting everything they came across (D. Cannatella pers. comm). Historic data for Cacao is hereafter referred to as historic Cacao.

2.3 Data analysis

Unless otherwise stated, all statistical analysis was conducted in the R statistical environment v4.1.2 (R Core Team 2022). We used the numbers equivalent approach as suggested by Jost (2006, 2007) to describe patterns of beta diversity and community similarity across sites using the package ‘vegetarian’ (Charney & Record 2012). β -diversity was analysed based on the numbers equivalent of Shannon’s diversity ${}^1D_\beta$ using the diversity order $q = 1$ which

considers the proportional abundance of each species in a community, without favouring either rare or abundant species (Jost 2006). Ten thousand bootstrap replicates of the data were used to determine standard error of β -diversity for each site.

We performed sample-based rarefaction analyses to compare patterns of species richness between sites (Gotelli & Colwell 2001). Transect data were pooled across sites and the ‘vegan’ package (Oksanen et al. 2017) was used to generate the subsequent comparisons. A sample-based rarefaction curve was further used to compare species richness patterns between historic and current data for Cacao.

To estimate inventories completeness, we used the nonparametric estimators of species richness; ACE and Chao1 based on abundance data (Hughes et al., 2001, Jiménez-Valverde & Hortal 2003), using EstimateS Program V9.1.0 (Chao, 1984, Chao & Lee 1992, Chao & Yang 1993, Chazdon et al. 1998, Colwell 2013, Colwell & Coddington 1994).

To compare species abundance patterns between sites, rank abundance curves (RAC) were plotted (Magurran 2004) using the BiodiversityR package (Kindt & Coe 2005). The slope of linear regression of an RAC expresses the evenness in abundance among species within an assemblage and an analysis of covariance (ANCOVA) was used to compare differences in evenness among sites. An abundant species was arbitrarily defined as those that were represented by more than 12 individuals (which is approximately 2% of all individuals across the study). We used the package brms (Bürkner 2017, 2018) to test for differences among sites in the rate of decay in rank abundance slopes. We specified per-species abundance as an outcome variable, with a negative binomial error structure. We included the interaction between rank and site as fixed effects, allowing the slope of decay to vary by site. We assessed model fit using visual inspection of mcmc chains, and posterior predictive checks. We determined differences between sites in rates of abundance decay based on whether differences in 95% credible intervals of slope parameters included zero. We used the Leave One Out Information Criterion (LOO-IC, Vehtari et al. 2017, 2020) to perform a full model test of the maximal model against the intercept only model (Forstemeier & Schielzeth 2011).

Multidimensional scaling (nMDS) ordination ($k = 2$, stress = 0.12) using the ‘vegan’ package (Oksanen et al. 2022) was used to visualise the difference in community structure and composition among sites. The nMDS plot is based on a Jaccard matrix, using species presence/absence data. Additionally, the similarity percentage (SIMPER: Clarke & Warwick 2001) was calculated to identify the contribution of individual species to the dissimilarity of amphibian community structure among sites. Moreover, a SIMPER analysis was also conducted using the historic data for Cacao to understand the changes in community structure over time and how this has affected inter-site relatedness. Abundance was analysed after a square root transformation of the data. This was conducted using the ‘vegan’ package (Oksanen et al. 2017).

All code and datasets required for reproducing these results, including model fitting and data visualisation, are provided online (https://github.com/xavharrison/CostaRica_RankAbundance_2022).

3 RESULTS

During the surveys between August – November 2017, 660 individual amphibians from 37 species were recorded, all anurans, (Table S2). This represents 46.25% of known amphibian species to occur in ACG (Table S3). The overall sampling effort was 267 person-hours throughout the entire study. In total 50 transects were resampled four times for a total of 200 transects. Several other individuals and species were captured outside of standard sampling (Table S4), but those have not been included in this analysis. *Duellmanohyla rufiocularis*, *Craugastor fitzingeri*, *Rhaebo haematiticus* and *Craugastor crassidigitus* were the most common species, comprising 20.3%, 13.5%, 11.7% and 11.7% of the total captured. We recorded nine amphibian families (all anuran), with three families represented by only a single species: Microhylidae (*Hypopachus variolosus*), Phyllomedusidae (*Agalychnis callidryas*) and Eleutherodactylidae (*Diasporus diastema*).

All sites had low similarity based upon species abundance (Horn index $\pm$ SD: 0.19 $\pm$ 0.17). The overall β -diversity for all sites combined was 3.16 ± 0.134 (${}^1D_{\beta}$ $\pm$ SD), highest in

San Gerardo (${}^1D_{\beta} = 3.27 \pm 0.26$) and lowest in Santa Rosa (${}^1D_{\beta} = 1.23 \pm 0.11$). β -diversity for the remaining sites was as follows; Cacao (${}^1D_{\beta} = 2.02 \pm 0.11$), Maritza (${}^1D_{\beta} = 1.98 \pm 0.16$) and, Murciélago (${}^1D_{\beta} = 2.14 \pm 0.20$).

The sample size was sufficient to characterise species richness for three of the five sites; Cacao; San Gerardo and Santa Rosa, as the rarefaction curve approaches an asymptote (Figure 2a). The highest number of species was recorded in San Gerardo (rainforest) and the least in Santa Rosa. In Cacao, a total of 20 species were recorded in the 1980's compared to only 11 in 2017, a decline of 45% (Figure 2b). Of the 11-species recorded in 2017, three of them were absent from the 1987 data – *Craugastor fitzingeri*, *Hyalinobatrachium colymbiphylum* and *Smilisca baudinii*. Furthermore, the curve for the historic data failed to reach an asymptote, suggesting that the inventory was incomplete at that stage and more species remained to be discovered. This is supported by museum records and data collected and stored at Arctos Collaborative Collection management solutions (MVZ 2018), which suggest a total of 39 species are known to occur in Cacao (Table S5).

Overall estimates of completeness were highest for Santa Rosa (ACE = 85.71% and Chao1 = 100%) and San Gerardo, which was predicted to be missing 7 species (Table 2). Cacao had the lowest level of completeness (ACE = 68.75% and Chao1 = 64.71%), as 54.58% of all individuals encountered were *Duellmanohyla rufiocularis*.

Our Bayesian regression (Table 3), ~~suggests~~, suggests that Cacao was found to have much higher species abundances at lower ranks. Whilst all sites decayed at a similar rate (i.e. had similar slopes), the site:rank interaction in the model revealed San Gerardo to have a much shallower rate of decline (Figure 3, Figure S1). Low density species (represented by a single individual) also mainly occurred in San Gerardo as well as Cacao. The abundance distribution in Murciélago and Santa Rosa suggests that these sites today have less abundant species as compared with San Gerardo (Figure 4). *Rhinella horribilis* was the most dominant species in both Murciélago and Santa Rosa. In contrast the dominant species in Cacao ~~and Maritza and~~ Maritza (*Duellmanohyla rufiocularis* and *Lithobates warszewitschii*) are not found in lowland sites (Savage 2002).

308
309 The nMDS shows a clear split between most of the sites. Santa Rosa and Murciélago are
310 the most similar sites, followed by Cacao and Cacao historic (Figure 4). Excluding Cacao
311 historic, San Gerardo was identified as the most unique site, but this was closely followed by
312 Maritza. However, including Cacao historic resulted in Maritza being the most unique. The
313 SIMPER analysis suggests that the community structure of the five sites ~~are~~is distinct from each
314 other, despite the short geographic distance between them (Table 4), with an average
315 dissimilarity of 83.20%. Murciélago and Santa Rosa were the least dissimilar sites, with a
316 dissimilarity of 60.97%, followed by Cacao and Maritza with a dissimilarity of 71.66%. Cacao
317 and Santa Rosa had the highest dissimilarity between sites at 96.37%. The SIMPER analysis
318 using the historic data for Cacao showed an increase in similarity between Cacao and the other
319 sites over the 30-year period (1987/8 – 2017). As expected, the historic data for Cacao was most
320 like contemporary Cacao, with a dissimilarity of 61.87%. All sites, except Santa Rosa,
321 experienced an increase in similarity between the two periods with Maritza experiencing the
322 biggest drop, with a decrease in dissimilarity from 81.77% to 71.66%. The dissimilarity between
323 Santa Rosa and Cacao increased between the two sampling periods, increasing from 75.35% to
324 96.37%.

325

326 4 DISCUSSION

327 Our analysis presented here found that amphibian species richness was strongly
328 correlated with forest type. This pattern follows the diverging environmental conditions present
329 in each forest type, which has resulted in very different communities across ACG. Furthermore,
330 we observed a substantial decrease in amphibian species richness over time, at the relatively
331 undisturbed cloud forest site Cacao. This is further evidence for the widespread decline of
332 amphibians observed globally and in Costa Rica over the past several decades, and recovery is
333 still tenuous, if at all (Lips et al. 2006, Stuart et al. 2004, Whitfield et al. 2016).

334

335 Historic museum records kept at the Museo de Zoología, Universidad de Costa Rica have
336 documented 80 species, consisting of 75 Anurans, one Gymnophiona and four Caudata within
337 ACG. We detected 37 species of anurans in the three main ACG ecosystems. Many ACG areas
338 have yet to be surveyed more than superficially; and will contain unrecorded or new species. For

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example, during the pilot study we discovered *Agalychnis saltator* in Pitilla (Table S4), which represents a substantial range expansion for this species and a species new to ACG. Furthermore, new molecular approaches are revealing previously undescribed amphibian cryptic diversity (Funk et al. 2012, Stuart et al. 2006) including in ACG frogs (e.g. Cryer et al. 2019).

Rainforests had the highest levels of amphibian species richness, which support previous findings for Costa Rica (Savage 2002) and elsewhere (Duellman & Trueb 1994). The three forest types sampled are in part defined by their evolutionary history, vegetation communities, previous disturbance and stage of restoration, levels of precipitation, temperature and the annual actual evapotranspiration (AET; Janzen et al. 2016). It has been demonstrated that a mixture of water and energy variables are important in shaping amphibian species richness patterns in North America, Europe, Asia and Central America (Currie 2001, Laurencio & Fitzgerald 2010, Rodriguez et al. 2005). For example, Qian et al. (2017) found a strong positive correlation between amphibian species richness and environmental variables such as precipitation, net primary productivity, range in elevation and temperature; in 245 localities across China. These findings demonstrate that environmental variables may play a role in constraining the species richness at a site and constitutes the most plausible explanation for the differences between the forest types. This is supported by the fact that dry forest sites, prior to disturbance, had much lower levels of species richness and tended to be dominated by large bodied generalists, such as *Rhinella horribilis*, *Smilisca baudinii* and *Lithobates forreri* which have wide distributions and are adapted to the seasonally xeric conditions of the dry forest. These anurans are less prone to desiccation, as their large body size means that they have proportionally lower surface area to body volume and thus lower rates of water loss than smaller bodied species (Duellman & Trueb 1994); this likely explains their higher abundances and dominance in the dry forest, which is characterised by dry season high temperatures and less rain, especially during the dry season. One such adaption to the xeric conditions of the dry forest is cocoon formation, as observed in *Smilisca baudinii*, allowing them to survive long periods without rain (McDiarmid & Foster 1987). The similarity between Cacao and Maritza is likely due to the proximity of these two sites (4.5 km) and that they occupy one continuous forest, albeit over an elevational gradient, rather than environmental conditions – which are grossly different between the two sites.

Duellmanohyla rufiocularis was only found at these two sites, whilst *Lithobates warszewitschii* was far more abundant in these two sites than any other.

Weather conditions at different elevations are likely to play a significant role in constraining diversity to a specific site and may explain the greater diversity found in San Gerardo compared to Cacao. For many groups of organisms, including amphibians (Campbell 1999), diversity changes along an elevational gradient (e.g. McCain 2005, Navas 2003, Terborgh 1971), following a bell-shaped curve. Species richness is relatively low at lower and higher elevations, with the highest species richness recorded at mid-elevations. However, endemism in the tropics is far more ubiquitous at high elevation sites; meaning they are of great conservation priority – a consequence of these sites being far more insular (Savage 2002). The results roughly follow this trend, with the average elevation of our transects in the most species rich site, San Gerardo (573.32 m), between the elevation of the less diverse higher elevation site (Cacao: 1050.17 m) and lower elevation sites (Santa Rosa: 289.2 m, Murciélago: 80.5 m).

Despite differences in the structure of the forest habitats, two species were found to occur in all four, *Rhinella horribilis* and *Craugastor fitzingeri*. This is likely attributed to their generalist nature and ability to adapt to human altered landscapes (Crawford et al. 2007). Only 11 species were found at more than one site, but some exhibited far higher abundance in only one forest type, such as *D. rufiocularis* which was found at very high abundances in Cacao (131 individuals), low abundances at Maritza (3 individuals) and absent from all other sites – a consequence of the elevational range constraints and climatic requirements of this species (Savage 2002). Historic declines may also play a role in the presence and absence of certain species at different sites, as illustrated by *Craugastor ranoides*. This once widespread riparian species is likely highly sensitive to *B. dendrobatidis* outbreaks (known populations of this species have disappeared from most of its range in Costa Rica, and *B. dendrobatidis* was found responsible for the decline of a highly-related species, *Craugastor punctariolus*; Ryan et al. 2008) and is likely only to persist in Murciélago due to the areas status as a climatic refuge, where the environmental conditions have helped prevent disease outbreaks (Puschendorf et al. 2009). However, this dry forest peninsula is also subject to serpentinization (Sanchez-Murillo et al. 2014). This produces hyperalkaline fluids, reaching a pH of > 11, which drain into the local streams in which these

Commented [VAC3]: Probably the reason is because elevation as predictor is not the only factor that determinates the species diversity there; to considere Cacao is the lowest continental clouded forest in CR, even when at the same elevation in other areas we have a different premontane forest type (it is not what we classically found when survey at 1000 msnm, compare with the species list of [Silencio de los Angeles Cloud Forest](#) at this elevation, for example, with 26 species while Cacao has just 13 in the same time period). You are comparing tropical dry forest against premontane moist with Pacific influence and premontane with Caribbean influence. Biogeography is also another factor, Guanacaste would be expected to has less species than Tilaran, Central o Talamanca at the same elevation...

frogs live. The potential effects of this pH change on the skin fungus and its resultant disease are yet to be explored. In Cacao forest, alongside *Craugastor ranoides*, *Atelopus varius*, *Isthmohyla tica*, *Craugastor andi*, *Duellmanohyla uranochroa* have also vanished and all salamanders are now extremely uncommon.

The steep decline in amphibian diversity in Cacao, over the 30-year period 1987/8 – 2017 is persistent and clearly recovery has been slow. A 45% reduction in species richness was observed, with only 11 species recorded in 2017 compared to 20 in the 1980's, with far greater sampling effort involved in 2017. The complete lack of salamanders on the transects was especially notable, due to their historic ubiquity in the area and this finding aligns with the declines reported by other studies on neotropical salamanders (Rovito et al. 2009). In the early 1980's and 1990's, D.H. Janzen regularly encountered salamanders under fallen, rotting tree stems (night and day) and on wet foliage at night, whilst searching for caterpillars in the vicinity of Estación Biológica Cacao (800 – 1400m) year-round. Since the 2000's none have been encountered by either D.H. Janzen or the parataxonomists on their daily search for caterpillars. The historic data supports previous studies looking at herpetofauna diversity of sites at similar elevations (Scott 1976: Puntarenas Province, Costa Rica). Cacao is comprised of mostly old growth forest with a few patches of forest at various stages of regeneration, which makes these declines even more alarming. But these declines match those experienced by other high elevation old growth forests in the neotropics (Young et al. 2001). The data also demonstrates that there has been little recovery of amphibian diversity following these declines. Although certain species seem to have been less affected by the declines, such as *C. crassidigitus*, *D. rufiocularis* and, *L. warszewitschii* which experienced similar declines to other species, but have since recovered and are now the most visible of the Cacao amphibian community. A recent study by Acosta-Chaves et al. (2019) found similar results with *C. crassidigitus* and *L. warszewitschii* now dominating the amphibian community of Reserva de San Ramón, despite their almost absence in the 1990s. Voyles et al. (2018), examined the temporal changes in detection rates of 12 riparian species at three sites in Panama. Many of the species experienced rapid decreases during the epizootic phase of the *B. dendrobatidis* outbreak. However, following the transition to the enzootic phase, *B. dendrobatidis* prevalence decreased, concomitant to the recovery of several of the species;

Commented [VAC4]: This result is similar to what reported Acosta Chaves et al. 2015 in a clouded forest of Talamanca

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

A potential cause of these declines is the pathogenic fungus *B. dendrobatidis*, which has been reported for several frog species on Cacao (Wynne 2018), although synergistic interactions among different environmental variables may conceal individual effects (Navas & Otani 2007). Scheele et al. (2019) suggest that *B. dendrobatidis* is responsible for the decline of 501 amphibian species and the potential extinction of 90 species, making it seem to be one of the deadliest diseases for wild biodiversity. However, Amphibian population collapses are not occurring in isolation – they are part of a constellation of changes taking place in tropical old growth forests (including Cacao), such as the decline of birds, snakes, lizards and insects, which are not susceptible to *B. dendrobatidis* (Janzen & Hallwachs 2021, Lister & Garcia 2018, Pounds et al. 1999, M. Sasa unpubl. data), suggest *B. dendrobatidis* may not be the sole culprit of these observed declines. Cacao, as with many of the other regions where declines have been documented, has gone through an ecological homogenisation, with a large increase in similarity among sites following the declines (Smith et al. 2009). However, this is likely to be an underestimate of dissimilarity as today we know that lowland amphibian communities have also been suffering declines, just over a longer time period (Ryan et al. 2008, Whitfield et al. 2007). However, baseline data is only available for Cacao.

Documenting long-term declines is only possible through the collection of baseline data (e.g. Ryan et al. 2008, Whitfield et al. 2007). The observation of a substantial decline in amphibian diversity within an old growth forest in ACG was only possible because of data collected several decades prior, by an expedition from the University of California, Berkeley. Other sites examined in this study may have experienced similar declines to that of Cacao, however we lack the data to empirically support this. ACG is in a unique position to provide a platform for understanding changes, past and present, and the resilience, or lack thereof, of tropical ecosystems and assemblages to climate change.

Acknowledgments

Commented [VAC5]: [Zipkin et al. 2020](#), [Zipkin et al. 2022](#) on snake decline and cascade effect on food web should be a must here...instead of "unpublished data".

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