

## **Effects of Forest Fragmentation on Feather Corticosterone Levels in an Amazonian Avian Community**

Authors: Bicudo, Thiago, Anciães, Marina, Arregui, Lucia, and Gil, Diego

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## Research Papers

### EFFECTS OF FOREST FRAGMENTATION ON FEATHER CORTICOSTERONE LEVELS IN AN AMAZONIAN AVIAN COMMUNITY

### EFFECTOS DE LA FRAGMENTACIÓN DEL BOSQUE SOBRE LOS NIVELES DE CORTICOSTERONA EN PLUMAS EN UNA COMUNIDAD DE AVES AMAZÓNICAS

Thiago BICUDO<sup>1</sup>\*, Marina ANCIÃES<sup>1</sup>, Lucia ARREGUI<sup>2</sup> and Diego GIL<sup>2</sup>

**SUMMARY.**—In the Amazon, the construction of hydroelectric dams is an emergent driver of biodiversity loss, creating numerous land-bridge islands, most of them unable to sustain an assemblage of bird species comparable to the intact forest. Although we understand the effects of forest fragmentation on species richness and distribution, we still need to uncover the physiological mechanisms underlying the success of organisms living in disturbed habitats. In this study, we used feather corticosterone levels as a measurement of physiological indicators of stress, evaluating whether corticosterone levels mirror the effects of habitat fragmentation on species occurrence. Since data suggest that smaller islands can reduce habitat suitability, increasing stress in birds that live within them, we predicted that birds living in smaller islands would present increased feather corticosterone levels. We captured birds in 13 islands of varying size and in two continuous forests and analysed feather corticosterone levels of 265 individuals from eight different species. Overall, our findings did not support the hypothesis that corticosterone varies in relation to island size, except for the Guianan Antworbler *Hypocnemis cantator*, which presented the predicted pattern: decreasing feather corticosterone levels with increasing island size. These differences suggest that species respond differently to stressors driven by fragmentation. Further studies are necessary to assess the reliability of corticosterone levels as a physiological measurement of stress and to determine which parameters are useful to understand how insularisation caused by human activities may influence the resistance of avian populations to habitat disturbances. —Bicudo, T., Anciães, M., Arregui, L. & Gil, D. (2020). Effects of forest fragmentation on feather corticosterone levels in an Amazonian avian community. *Ardeola*, 67: 229-245.

**Key words:** Amazon hydropower, corticosterone, glucocorticoid, habitat loss, islands, physiological stress.

<sup>1</sup> Laboratório de Biologia Evolutiva e Comportamento Animal, Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, Amazonas, Brasil.

<sup>2</sup> Departamento de Ecología Evolutiva, Museo Nacional de Ciencias Naturales (CSIC), Madrid, Spain.

\* Corresponding author: bicudotks@gmail.com

**RESUMEN.**—En la Amazonia, la construcción de represas hidroeléctricas es un factor emergente en la pérdida de biodiversidad debido a la formación de numerosas islas, la mayoría incapaces de sostener comunidades de aves comparables a las de los bosques intactos. Si bien entendemos los efectos de la fragmentación de los bosques en la riqueza y distribución de las especies, aún necesitamos descubrir los mecanismos fisiológicos relacionados con el éxito de los organismos que viven en hábitats perturbados. En este estudio, utilizamos los niveles de corticosterona en las plumas como una medida del estrés fisiológico, evaluando si estos niveles reflejan los efectos de la fragmentación del hábitat sobre las especies. Dado que los datos previos sugieren que las islas más pequeñas pueden reducir la adecuación del hábitat y aumentar así el estrés en las aves que viven en ellas, predecimos que las aves en las islas más pequeñas tendrían niveles más altos de corticosterona en las plumas. Capturamos aves en 13 islas de diferentes tamaños y dos bosques continuos, y estimamos los niveles de corticosterona en plumas de 265 individuos de ocho especies distintas. En general, nuestros resultados no respaldaron la hipótesis de que la corticosterona varía con el tamaño de la isla, excepto para *Hypocnemis cantator*, que presentó el patrón esperado: disminución de los niveles de corticosterona en las plumas con el aumento del tamaño de la isla. Estas diferencias sugieren que las especies responden de manera diferente a los estresores causados por la fragmentación. Se necesitan más estudios para evaluar la fiabilidad de los niveles de corticosterona como una medida del estrés fisiológico y para determinar qué parámetros son útiles para comprender como la insularización causada por las actividades humanas puede influir en la resistencia de las poblaciones de aves a las alteraciones del hábitat. —Bicudo, T., Anciães, M., Arregui, L. y Gil, D. (2020). Efectos de la fragmentación del bosque sobre los niveles de corticosterona en plumas en una comunidad de aves amazónicas. *Ardeola*, 67: 229-245.

**Palabras clave:** corticosterona, estrés fisiológico, glucocorticoides, hidroeléctrica amazónica, islas, pérdida de hábitat.

## INTRODUCTION

Habitat fragmentation is recognised as a major threat to biodiversity, leading to long-term changes in the structure and function of fragments (Haddad *et al.*, 2015; Fahrig, 2017). In fragmented landscapes, patch area is one of the key factors influencing species richness, population abundance and community composition (MacArthur & Wilson, 1963; Fahrig, 2017). Small fragments (< 100-ha), can lose up to half of their species in less than 15 years of isolation, although species strongly differ in their probabilities of extinction (Ferraz *et al.*, 2003). The occurrence and survival of species is probably changed upon fragmentation, and area-sensitive species may decline or become extinct in small fragments (Dardanelli *et al.*, 2006).

Most of the studies on the impact of fragmentation in Amazonian birds have analysed a particular type of fragmentation, namely

habitat patches within a semi-permeable matrix of cattle pastures and abandoned cropland (Laurance *et al.*, 2002, 2011; Ferraz *et al.*, 2003, 2007; Wolfe *et al.*, 2015). A different and emergent driver of habitat fragmentation is the construction of hydroelectric dams, which can induce the flooding of huge forest surfaces that creates simultaneously numerous small land-bridge islands surrounded by water (Benchimol & Peres, 2015c; Lees *et al.*, 2016). The resulting non-forest matrix may act as a barrier to birds with low flight ability, hampering the dynamics of colonisation and extinction in this system (Aurélio-Silva *et al.*, 2016; Palmeirim *et al.*, 2017; Bueno & Peres, 2019).

In such archipelagos, habitat patches act like true islands, small ones being subject to a greater extinction of bird assemblages (Wolfe *et al.*, 2015; Aurélio-Silva *et al.*, 2016) and showing greater environmental degradation than larger ones due to strong edge effects

that alter forest microclimate, tree mortality and faunal diversity (Murcia, 1995; Benchimol & Peres, 2015a, c; Laurance *et al.*, 2018). Overall, the availability of trophic and structural resources is expected to decrease with diminishing island area (Palmeirim *et al.*, 2017).

At Balbina Dam, a major hydroelectric dam in Central Amazonia, bird assemblages that occupy islands and areas of intact forest vary predictably, with smaller and more isolated islands retaining a smaller number of species (Lees & Peres, 2006; Benchimol & Peres, 2015b; Aurélio-Silva *et al.*, 2016). For instance, fewer than 10% of all the islands created could hold a full assemblage of bird species comparable to the intact forest (Aurélio-Silva *et al.*, 2016). Similar patterns have been found in other taxonomic groups, including large terrestrial and arboreal vertebrates (Benchimol & Peres, 2015a), lizards (Palmeirim *et al.*, 2017), small mammals (Palmeirim *et al.*, 2018) and trees (Benchimol & Peres, 2015a), making forest fragmentation induced by hydroelectric dams a major threat to Amazonian biodiversity (Laurance *et al.*, 2002, 2011, 2018; Ferraz *et al.*, 2003, 2007; Stouffer *et al.*, 2009; Wolfe *et al.*, 2015).

Although we understand the effects of fragmentation on species richness and ecological-functional traits (Edwards *et al.*, 2013; Hamer *et al.*, 2015; Bicudo *et al.*, 2016; Pryde *et al.*, 2016; Bueno *et al.*, 2018), we still need to explore in depth the physiological mechanisms responsible for how organisms respond to such environmental alterations (Wingfield, 2013; Messina *et al.*, 2018). Physiological changes due to habitat fragmentation can be less obvious than those indicated by species presence/absence data or density. Nevertheless, they have been shown to be a useful way to provide immediate measures of the effects of human-induced environmental disturbance (Lucas *et al.*, 2006).

One of the most significant measures of physiological changes is provided by levels of corticosterone (CORT), the main glucocorticoid steroid of amphibians, reptiles, birds and some mammals (e.g. rodents), secreted by the cortex of the adrenal gland (Sapolsky *et al.*, 2000; Buchanan, 2000; Schoech *et al.*, 2011). When vertebrates experience situations with negative effects (e.g. agonistic interactions), the hypothalamic-pituitary-adrenal axis (HPA) is activated resulting in increased CORT synthesis (Romero *et al.*, 1998; Sapolsky *et al.*, 2000). Because CORT acts on several metabolic and behavioural pathways that help birds cope with environmental changes (Wingfield, 2002; Cockrem, 2007), this hormone is considered a biomarker by some authors (Fairhurst *et al.*, 2011; Warne *et al.*, 2015; Harris *et al.*, 2017; but see Madliger *et al.*, 2015). Although short-term elevations in CORT levels may have evolved as an adaptive defence mechanism to regulate homeostasis, chronically elevated levels have negative consequences for the survival of individuals (Sapolsky *et al.*, 2000; Blas, 2015).

In fragmented landscapes, birds may survive despite high levels of environmental stress caused by the use of suboptimal habitats (Mokross *et al.*, 2018) and lower food availability induced by edge effects (Zanette *et al.*, 2000). For instance, higher levels of CORT, due to habitat fragmentation, led to decreased quality and offspring survival in the Eurasian Treecreeper *Certhia familiaris* (Suorsa *et al.*, 2004). In addition, some studies have shown that even generalist bird species inhabiting recently logged forests may present higher levels of CORT than those living in undisturbed areas (Lucas *et al.*, 2006), suggesting that the stress response to habitat fragmentation is not exclusive to specialist birds (e.g., Suorsa *et al.*, 2003, 2004).

Glucocorticoids have been measured in free-living individuals of many species but an important methodological constraint is that

CORT levels increase very rapidly upon capture, and thus samples need to be taken in a very short time (Romero & Reed, 2005; Blas, 2015). This limitation has led to the development of less invasive techniques that rely on the long-term accumulation of CORT in tissues (Bortolotti *et al.*, 2008; Sheriff *et al.*, 2011; Romero & Fairhurst, 2016). In the case of birds, feathers are currently used for measuring levels of corticosterone (fCORT), which is incorporated and accumulated in growing feathers, when feather structures are being irrigated by blood (Fairhurst *et al.*, 2013; Jenni-Eiermann *et al.*, 2015). The use of fCORT was pioneered by Bortolotti and colleagues (Bortolotti *et al.*, 2008), since when there has been a great increase in the number of studies employing this way of assessing CORT levels (e.g., Legagneux *et al.*, 2013; Patterson *et al.*, 2015; Freeman & Newman, 2018; Ganz *et al.*, 2018; Bosholn *et al.*, 2019). This is because fCORT reflects an integrative measure of plasma CORT levels (Lattin *et al.*, 2011; Fairhurst *et al.*, 2013), providing a retrospective and cumulative record of the physiological response to ecological challenges (Bortolotti *et al.*, 2008; López-Jiménez *et al.*, 2017; Ganz *et al.*, 2018). In addition, feather collection is minimally invasive and samples can be easily stored for long periods of time (Fairhurst *et al.*, 2013; Hansen *et al.*, 2016; Romero & Fairhurst, 2016).

In the face of increased global habitat loss and fragmentation (Lucas *et al.*, 2006), we evaluated whether the well documented effects of habitat fragmentation upon species occurrence patterns are also mirrored by feather corticosterone levels. Since smaller islands are expected to present reduced or altered availability of food resources (Zanette *et al.*, 2000; Suorsa *et al.*, 2004), we predicted that this would lead to an increase of CORT levels to restore homeostasis (Suorsa *et al.*, 2004) and that this could be assessed in feather CORT (fCORT) levels.

With this less invasive method, we can evaluate physiological traits operating over

an ecologically meaningful time scale, including exposure and response to environmental disturbances (Blas, 2015), enabling us to assess the hidden effects of environment changes on animals (Berk *et al.*, 2016).

## MATERIALS AND METHODS

### *Study Area*

The study was conducted at the Balbina Hydroelectric Reservoir, within Amazonas state, Brazil, which was filled in 1986 by the damming of the Uatumã River, a left-bank tributary of the Amazon. Given the flat topography of the area, the resulting lake covers an area of approximately 312,900ha, including over 3,500 land-bridge islands ranging widely in size and shape, surrounded by freshwater (Benchimol & Peres, 2015a).

The vegetation of most islands consists of dense, closed-canopy, terra-firme forest with some small islands affected by edge-related windfalls and wildfires, that occurred during a late-1997 to early-1998 El Niño drought (Benchimol & Peres, 2015c). This has led to major environmental changes caused by fire, which further increases the adverse situations that the species living on these islands have suffered.

The annual mean temperature is 28°C and mean rainfall 2,376mm, with the wet season spanning November to April (IBAMA 1997). The islands are within or adjacent to areas of the Uatumã Biological Reserve (1°13'38"S, 59°27'28"W), a federal conservation unit that covers both the island landscape and the contiguous adjacent upland areas on the east bank of the Uatumã River.

### *Sampling design and feather collection*

For our study, we selected 13 islands from the Balbina reservoir, ranging in size from 13.6 to 673.4ha and situated at least 2km

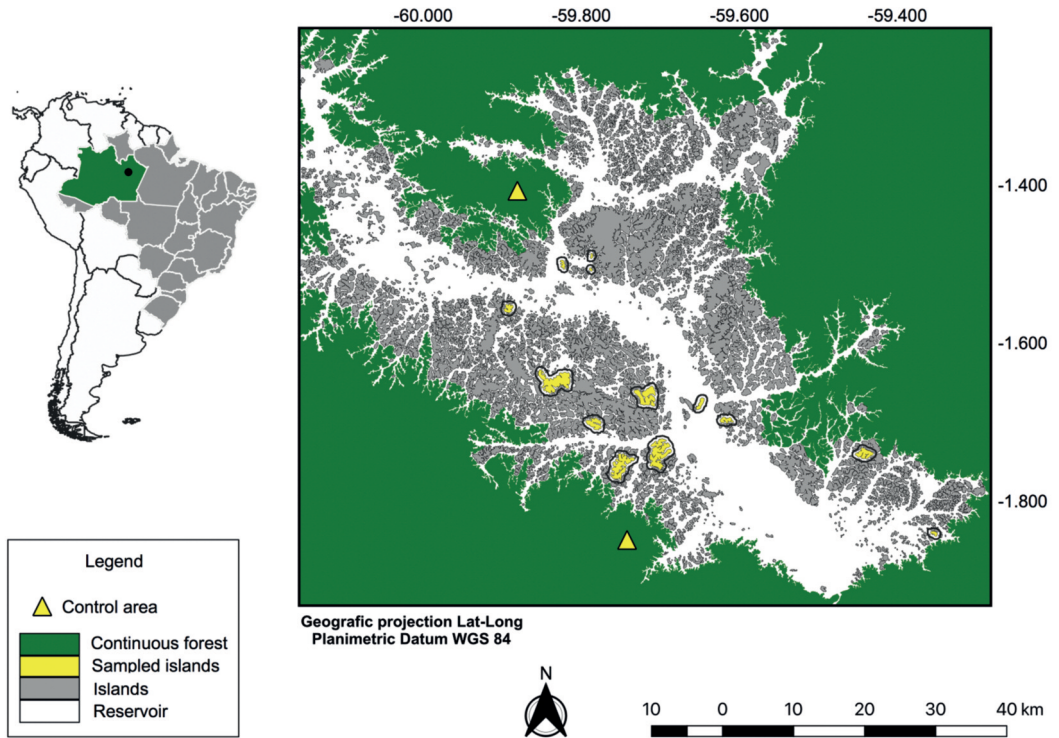


FIG. 1.—Spatial distribution of the study area and the 13 islands and two continuous forest sites (Control areas) surveyed in the Balbina Hydroelectric Reservoir, Amazonas state, Brazil.

[Distribución espacial del área de estudio destacando las 13 islas y las dos áreas de bosque continuo (áreas de control) ubicadas en el embalse de la central hidroeléctrica Balbina, estado de Amazonas, Brasil.]

from two continuous forest areas (CF) located on both sides of the lake (Control areas; Figure 1). We assigned area values of one order of magnitude greater than our two largest islands (i.e. 6,730.4ha, 3.83 in log) to the CF areas, following the logic of another study in the same area (Benchimol & Peres, 2015a).

To test the possibility of spatial correlation in fCORT data, we performed Mantel tests (mantel function of the vegan package for R, Oksanen *et al.*, 2015) for each species by constructing distance matrices for fCORT values and geographic locations of the forest areas (islands or CF). The results showed non-significant spatial correlation patterns

for all species (Supplementary Material, appendix 1, Table A2), and thus the forest areas sampled (islands or CF) were assumed to be spatially-independent units.

Although we lack data on dispersion and movement of birds between islands in our study area, we assumed a very low level of dispersion between islands due to the typically low dispersion and flight capacity observed for Amazonian understory birds (Moore *et al.*, 2008; Ibarra-Macias *et al.*, 2011).

Birds were routinely captured by a team of 2-3 people, using 14 Ecotone nylon mist-nets of 12 × 2.5m, mesh size: 32mm. Mist-nets were placed in the understory along a

continuous linear net-line (c. 200m) using previously established trails within each area, avoiding island edges by sampling at least 250m from the island boundaries. Mist-nets were left open for three consecutive days in each area (three sampling days \* 15 areas, 45 days of work), from 06:00h to 12:00h and inspected every half hour. The fieldwork was conducted from June to September 2016 and March to July 2017. Captured birds were identified to species level and ringed with standard metallic rings provided by CEMAVE (Centro Nacional de Pesquisa para Conservação das Aves Silvestres).

Feathers were only collected from adult birds (i.e. those that had reached sexual maturity in which the feather was produced through a moult process). We strived to sample the same feather types from each specimen to avoid possible bias due to differences in fCORT deposition (Harris *et al.*, 2016). Hence, assayed feathers were fully-grown and from the same locations in all birds captured: the eighth primary from both wings (numbered descendantly: innermost primary = 1) and the two outermost rectrices (tail feathers: right R6 and left R6). Feather numbering and classification follow Pyle (1997). In addition, we only collected feathers that showed no signs of abrasion. Feathers were stored in individual paper envelopes until fCORT levels were assayed in the laboratory.

We were unable to collect feather samples from all study sites for all species. Therefore, assays included a subsample of eight species for which we obtained sample sizes large enough for analyses at species level (12-58 individuals across forest areas of different sizes, either on islands or CF). The eight species represent understorey birds (Del Hoyo *et al.*, 2014), regarded *a priori* to be susceptible to habitat fragmentation (Stouffer *et al.*, 2009; Aurélio-Silva *et al.*, 2016; Bueno *et al.*, 2018; Laurance *et al.*, 2018) and that exhibit low dispersal ability through non-forest matrix habitats (Sekercioglu *et al.*,

2002; Laurance *et al.*, 2004; Moore *et al.*, 2008): Plain-brown Woodcreeper *Dendrocincla fuliginosa*, Wedge-billed Woodcreeper *Glyphorhynchus spirurus*, Chestnut-rumped Woodcreeper *Xiphorhynchus pardalotus*, White-crowned Manakin *Pseudopipra pipra*, Guianan Antwren *Hypocnemis cantator*, White-flanked Antwren *Myrmotherula axillaris*, Black-headed Antbird *Percnoscotola subcristata* and White-necked Thrush *Turdus albicollis*.

Except for the White-crowned Manakin, which is primarily frugivorous (but see, Fair *et al.*, 2013) the other species are insectivorous, the group most affected by habitat fragmentation (Sekercioglu *et al.*, 2002; Aurélio-Silva *et al.*, 2016; Laurance *et al.*, 2018). None of the selected species exhibit a well-marked annual moulting season and instead show peaks of moulting usually at the end of the dry season (December) and in the middle of wet season (March), generally following the peaks of the breeding cycle (Johnson *et al.*, 2012). It is not uncommon to find species moulting during each month, with substantial interspecific variation in both moult timing and duration (Johnson *et al.*, 2012; Stouffer *et al.*, 2013). Due to their limited dispersal ability (Laurance *et al.*, 2004; Van Houtan *et al.*, 2007; Moore *et al.*, 2008), and the fact that the species were present in the study area since the creation of the dam reservoir (Willis & Oniki, 1988), we expect that birds were equally exposed to stressors independent of age or timing of moult.

This study was approved by the Chico Mendes Institute for Biodiversity Conservation (license to TB, number 51536-5).

#### *Feather corticosterone measurement*

All fCORT measurements followed a slight modification of a published protocol (Bortolotti *et al.*, 2008). In brief, a

methanol-based extraction technique was used to extract CORT from feathers, since steroid hormones are generally soluble in polar alcohols such as methanol (Pötsch & Moeller, 1996).

All feather samples were equally processed, removing the calamus and mincing the rachis and vanes into pieces of less than 5mm, resulting in a highly homogeneous mixture. All feathers per individual were pooled and weighed on an analytical balance (A&D instruments, model FX-40) with 0.0001g of precision, to determine the feather mass available for fCORT extraction, and then transferred to a silanized glass tube (to increase hormone recovery).

We strived to obtain similar feather masses for each species, so that variation in the assay response due to feather mass would not bias within-species analyses, allowing for the representation of fCORT levels per unit mass (pg/mg) (Freeman & Newman, 2018). Six millilitres of methanol were added to the sample, and tubes were placed in a sonicating water bath at room temperature for 30min, followed by overnight incubation (19 hours) in a shaking 50°C water bath. Subsequently, the methanol was separated from the feather remains using a nylon syringe filter (0.45µ). Feather debris was washed twice with 2ml of methanol to recover residual extracts, which were added to the previous methanol extract. The pooled methanol extract was then placed in a 50°C water bath and evaporated under a stream of nitrogen. Extract residues were resuspended in 150µl steroid-free serum (DRG, Germany) and then vigorously vortexed for 10min in a multi-tube vortexer. Reconstituted samples were frozen at -20°C until assays were conducted to quantify CORT levels. Recovery of cold-spiked samples was 107% (SD = 5,75, n = 6). Linearity of the spiked sample was parallel to the expected dilution (expected dilution in logs:  $y = -0.1931x + 2.6743$ , spiked sample:  $y = -0.198x + 2.7149$ ).

To measure fCORT concentrations we used an enzyme immunoassay (DRG, Germany). Inter- and intra-assay variability was assessed using the coefficient of variation (CV) of known standards. Samples and standards were run in duplicate across nine assays with a mean intra-assay CV of 9.17% and inter-assay CV of 12.88%. Serial dilutions revealed parallel displacement to the standard curves. All samples were above detection limits (1.1pg/ml). All feather samples were randomised during the extraction and assay processes to avoid differences due to variation between extraction batches or assay plates. All fCORT analyses were performed at the Laboratory of Ecophysiology at the National Museum of Natural Sciences (MNCN) in Madrid (Spain).

#### *Statistical analysis*

Before testing specific effects of island size on fCORT levels, we ran a linear mixed model (LMM) to test for expected effects of feather mass on the amount of hormone present in feathers, as this variable may affect CORT levels (Lattin *et al.*, 2011; Jenni-Eiermann *et al.*, 2015; Berk *et al.*, 2016). We used feather mass as predictor of fCORT levels. Because this variable is generally species-specific, we also included species as random effects. In order to control for the effects of feather mass in fCORT levels, the residuals obtained from this analysis (see below) were used in all subsequent analyses involving fCORT. Given the highly skewed distribution of fCORT concentration and the irregular distribution of forest size (island and CF), these variables were log-transformed prior to analyses.

To test the hypothesis that fCORT levels increase with decreasing island size, we used a linear mixed model (LMM) pooling all species together, where the residuals obtained in the prior analysis (expected effects of feather



mass in fCORT levels) were used as response variable and log-transformed forest size as predictor. Since we expected the response to fragmentation to show species-specific differences, we used species and island as a random effect. Visual inspection of residual plots did not reveal deviations from homoscedasticity or normality.

Finally, in order to analyse effects of fragmentation on species-specific fCORT levels, we conducted LMMs for individual species, using fCORT ( $\mu\text{g mg}^{-1}$ ; log-transformed) as response variable, log-transformed forest size

(islands or CF) and feather mass as fixed predictors, and island identity as random factor.

All statistical analyses were conducted using R 3.4.0 (R Development Core Team, Vienna). Linear mixed models were fitted using restricted maximum likelihood, lmer function in the lme4 package (Bates *et al.*, 2015). The goodness of fit of linear mixed models was based on the marginal  $R^2$  and a conditional  $R^2$  (based on maximum likelihood) giving the variance explained by fixed effects, and both fixed and random effects, respectively, using the r.squaredGLMM

TABLE 1

Species-specific statistics of linear mixed models, for each species sampled at the Balbina reservoir. Statistically significant probability values shown in bold type.

Species	Common name	N	fCORT (Average)
<i>Myrmotherula axillaris</i>	White-flanked Antwren	37	18.43
<i>Dendrocincla fuliginosa</i>	Plain-brown Woodcreeper	20	3.73
<i>Pseudopipra pipra</i>	White-crowned Manakin	58	10.01
<i>Glyphorhynchus spirurus</i>	Wedge-billed Woodcreeper	43	5.82
<i>Hypocnemis cantator</i>	Guianan Antwarbler	28	14.68
<i>Percnostola subcristata</i>	Black-headed Antbird	12	6.79
<i>Turdus albicollis</i>	White-necked Thrush	25	3.85
<i>Xiphorhynchus pardalotus</i>	Chestnut-rumped Woodcreeper	42	5.36

function in the MuMIn package (Nakagawa & Schielzeth, 2013; Barton, 2019). In our models, p-values were based on the Satterthwaite approximation to degrees of freedom, lmerTest package (Kuznetsova *et al.*, 2017).

## RESULTS

We measured the fCORT levels of 265 individuals (225 from islands and 40 from CF) from eight different species ( $X \pm SD = 33.0 \pm 14.7$ ; Supplementary Material, appen-

dix 1, Table A1). The fCORT concentrations ( $\text{pg mg}^{-1}$ ; log-transformed) were negatively correlated with feather mass when all species were pooled in the analysis (t-Student test:  $t_{6,66} = -4.96$ ,  $P = 0.01$ ). However, individual models per species showed that feather mass did not affect fCORT levels at the species level, possibly because of the reduced variation in assayed feather mass due to our laboratory protocol (Table 1).

Our hypothesis that fCORT would vary among islands according to their size was not met for the overall dataset (Table 2). The

TABLE 1 (cont.)

[Resultados de los modelos lineales mixtos para cada especie muestreada en el embalse de Balbina. Los números en negrita representan valores de probabilidad que alcanzaron significación estadística.]

fCORT (SD)	Parameters	Estimated	SE	t value	Pr(> t )
5.46	Island size ( $\log_{10}$ )	-0.06	0.04	-1.50	0.14
	Feather mass	-0.04	0.02	-1.55	0.13
1.80	Island size ( $\log_{10}$ )	0.10	0.07	1.37	0.20
	Feather mass	-0.01	0.06	-0.15	0.88
4.64	Island size ( $\log_{10}$ )	0.04	0.03	1.15	0.25
	Feather mass	-0.00	0.02	-0.27	0.78
1.90	Island size ( $\log_{10}$ )	-0.01	0.04	-0.27	0.78
	Feather mass	-0.02	0.03	-0.86	0.39
4.93	Island size ( $\log_{10}$ )	-0.10	0.05	-2.01	<b>0.05</b>
	Feather mass	-0.00	0.04	-0.02	0.97
3.60	Island size ( $\log_{10}$ )	0.09	0.08	1.19	0.34
	Feather mass	0.05	0.04	1.16	0.28
1.21	Island size ( $\log_{10}$ )	-0.05	0.05	-1.12	0.30
	Feather mass	-0.00	0.01	-0.44	0.65
2.79	Island size ( $\log_{10}$ )	0.01	0.05	0.29	0.76
	Feather mass	-0.01	0.02	-0.49	0.62

TABLE 2

Linear mixed model statistics for all eight species pooled (265 individuals) to explain residual fCORT levels.

[Resultados del modelo lineal mixto para la muestra de las ocho especies agrupadas (265 individuos) para explicar los niveles residuales de fCORT.]

Parameters	Estimate	Std. Error	t value	Pr(> t )
Intercept	-0.01	0.09	-0.13	0.89
Island size ( $\log_{10}$ )	0.00	0.03	0.13	0.89

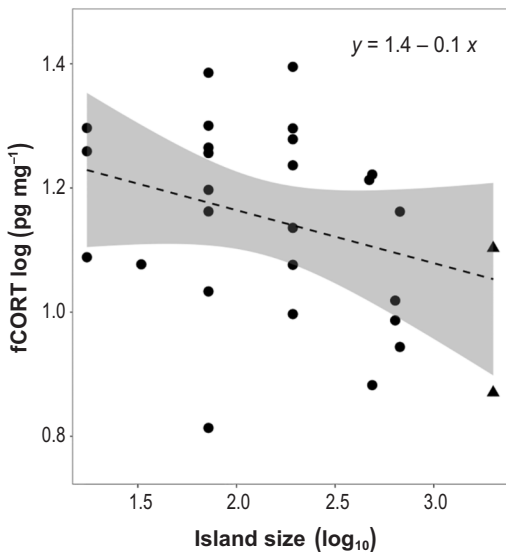


FIG. 2.—Relationships between log fCORT levels and log island size in the Guianan Antwarbler *Hypocnemis cantator* ( $N = 28$ ) sampled at the Balbina reservoir, Amazonas state, Brazil. The shaded area represents the 95% confidence interval and the triangles represent the continuous forest (CF) fCORT values.

[Relación entre los niveles de fCORT (log) y el tamaño de isla (log) en *Hypocnemis cantator* ( $N = 28$ ) muestreados en el embalse de la presa hidroeléctrica de Balbina, estado de Amazonas, Brasil. El área sombreada representa el intervalo de confianza del 95% y los triángulos los valores de fCORT en los bosques continuos (CF).]

response in fCORT of each species to forest size was heterogeneous. Thus, we ran individual models for each species (Table 1), finding only a significant island size-related estimate for only one species, the Guianan Antwarbler: which showed increasing levels of fCORT in smaller forests (Table 1; Figure 2), with a marginal and conditional  $R^2$  of 0.13. All the other species showed no significant association between fCORT levels and forest size.

## DISCUSSION

Habitat fragmentation may induce reductions in species diversity that may in turn affect the individual fitness of the individuals that survive in this area. Using a true island system to examine the effects of forest habitat fragmentation in levels of feather corticosterone (fCORT) in Amazonian birds, we tested the hypothesis that fCORT levels would be affected by island size, expecting fCORT to increase with decreasing island size.

Our results did not support our expectations of overall higher fCORT levels in smaller forests, when all species were pooled in the same analysis. As such, fCORT was not affected by island size across species. This is in contrast to previous analyses in

other species where habitat fragmentation was found to lead to elevated stress levels, as measured by increased CORT concentration within fragments (Suorsa *et al.*, 2003, 2004; Lucas *et al.*, 2006; Leshyk *et al.*, 2012).

Birds living in habitat fragments are expected to experience stressful situations, such as lower food supply and greater exposure to predators, more frequently, which can lead to chronic stress (Romero, 2004; Rich, 2005; Blas, 2015). However, contrary effects may occur, as found in a study comparing island and mainland populations of the Eurasian Blue Tit *Cyanistes caeruleus* where the results suggest that islands may select for lower CORT levels, perhaps because of lower rates of intraspecific aggression (Müller *et al.*, 2007).

The lack of observed effects in our general analysis can be due to several reasons. First, since the Balbina archipelago was formed 28 years ago, birds may have adapted to these new conditions, i.e. individuals may have already responded to the new biotic and abiotic settings on islands. It is known that fragmentation leads to the extinction of highly area-sensitive species (Ferraz *et al.*, 2003; Stouffer *et al.*, 2009; Bregman *et al.*, 2014; Aurélio-Silva *et al.*, 2016). Thus, those species that we still find in smaller fragments may be those with a higher level of resilience. A second possibility is that birds are still stressed in the fragmented landscape, but they show a downregulation of glucocorticoids, with a return to baseline levels as a strategy to avoid the deleterious effects of chronic CORT elevation (Romero, 2004; Rich, 2005; Blas, 2015). Indeed, it has been shown that chronic stress may result in both increasing and decreasing patterns of glucocorticoid secretion according to species (Dickens & Romero, 2013).

Alternative explanations that may help to interpret the observed lack of association between forest size and fCORT levels include the consequences of the variation in

moulting dynamics among species in our fCORT data, given that individuals may moult at different periods within the year, thus potentially presenting seasonal variation in fCORT. In this regard, asynchronous moulting periods in tropical environments may make fCORT-based studies less reliable in these regions than in temperate areas, where birds have more synchronised moulting periods. Another possibility is the relatively small sample size. Despite the large sampling effort, only a relatively small number of individuals of each species could be captured. Although we do not have *a priori* expectations of effect sizes, given the moderate effects that are typical of biological processes (Møller & Jennions, 2002), a larger sample size would have been desirable to avoid potential type-II errors.

In spite of the lack of relationship between fCORT levels and forest size for the data pooled across species, we did find the expected association in the Guianan Antwren (Thamnophilidae). A small percentage of the population of this species showed increasing fCORT levels with decreasing forest size. This raises the question of what is special about this species in comparison to the others analysed, in particular, the other two Antbird species (Black-headed Antbird and White-flanked Antwren), for which we found no relationship between CORT and forest size. Decreased food availability in smaller islands is a first possibility. Antbirds typically feed on small insects and other arthropods taken from twigs and foliage in the understory of the forest, and some species specialise in following army ants (Del Hoyo *et al.*, 2014). However, we could not find any clues in the scant bibliography on this species. For instance, a study in French Guiana showed that the prey size taken by the Guianan Antwren is very similar to that of other antbirds of similar size, such as the White-flanked Antwren (Thiollay, 1988). In addition, we found no differentiation of this

species in its isotopic nitrogen and carbon signatures (authors' unpublished data). Other aspects of its life history traits, such as the existence of male-female duets in this species (Seddon & Tobias, 2006), may set this species apart in terms of social requirements. However, song duets are also present in the other Antbirds that we studied. Finally, a previous study of area sensitivity in these islands showed that, although species richness was lower in small islands, the Guianan Antwren was not particularly affected by island size (Aur lio-Silva *et al.*, 2016). This runs counter our finding, and suggests that apparent population resistance may hide reductions in individual physiological condition.

The strong differences in fCORT levels found among species were largely due to the negative relationship between feather mass and fCORT levels, by which larger species had larger feathers, and thus lower fCORT levels. After removing the effect of feather mass, differences among species in fCORT levels remained but they were no longer related to body mass, but may rather have been due to differences in ecological and life-history related attributes (Hau *et al.*, 2010). Lastly, given that fCORT is an indirect measure of hormonal loads, which may be affected by a complex suite of factors, including feather abrasion and sunlight exposure (Freeman & Newman, 2018), it would be premature to try to use this measurement as a *prima facie* indication of differences between species in their responses to environmental changes.

To conclude, we found weak evidence for the hypothesis that habitat fragmentation leads to increased levels of glucocorticosteroid production in this set of Amazonian species. Out of the eight species studied, only one, the Guianan Antwren, showed the expected pattern. This pattern shows that different species respond differently to stressors, perceiving the same stimulus to be more or less of a threat (Cockrem, 2013),

thus generating different physiological responses by species to the same stimulus, as some species are more area-sensitive than others (Sekercioglu *et al.*, 2002; Anjos, 2006; Dickens & Romero, 2013; Bueno *et al.*, 2018). Further studies are necessary to assess the reliability of this technique, and to determine which parameters are useful to understand how human-driven insularisation affects avian population resilience.

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## SUPPLEMENTARY ELECTRONIC MATERIAL

Additional supporting information may be found in the on-line version of this paper. See volume 67(2) on [www.ardeola.org](http://www.ardeola.org)

**Table A1.** Information on all individuals sampled (N = 265) in all areas (N = 15) of the Balbina reservoir, Amazonas state, Brazil. Under island size, CF = Continuous Forest sites (control area).

**Table A2.** Mantel correlations of  $CORT_f$  level dissimilarities with geographic distances in 15 survey sites at the Balbina reservoir, Amazonas state, Brazil.

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