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Research paper

Sampling baseline androgens in free-living passerines: Methodological considerations and solutions

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ABSTRACT

Obtaining baseline hormone samples can be challenging because circulating hormone levels often change rapidly due to the acute stress of capture. Although field protocols are established for accurately sampling baseline glucocorticoid concentrations, fewer studies have examined how common sampling techniques affect androgens levels. Indeed, many studies focused on understanding the functional significance of baseline androgen levels use sampling methods known to activate the endocrine responses to stress. To understand how different field sampling protocols affect plasma androgen levels, we measured the androgen response to two types of capture stressors in a free-living tropical bird, the wire-tailed manakin (*Pipra filicauda*). First, we subjected males to a standardized capture and restraint protocol lasting either 15 or 30 min. Second, males were passively captured in nets that were filmed (to establish exact duration of time between capture and blood sampling) and checked every 30 min. The first study showed that circulating plasma androgen levels decreased significantly following both 15 and 30 min of restraint in a cloth bag, with a trend for the 30 min samples to be lower than the 15 min samples. Further, the change in androgen levels was dependent on an individual's initial androgen levels, with the individuals with the highest initial levels registering the largest declines. The results of the second study suggest that hanging in a mist net for extended periods of time also leads to a decrease in circulating androgen levels, but this effect was weaker than that of capture and restraint in a cloth bag. Our findings demonstrate that, overall, circulating androgen levels decrease in response to common sampling techniques; a finding that has important implications for studies measuring baseline androgen levels in free-living birds. Future studies should prioritize sampling individuals immediately upon removal from the mist net, as handling and restraint have a strong negative effect on circulating androgen levels. When constant monitoring of the mist net is not possible, investigators should use video cameras to record the amount of time an individual spends in the net prior to blood sampling and then statistically control for the effect of this variable in analyses.

1. Introduction

The ability to accurately quantify individual baseline levels of circulating hormones in vertebrate field studies is essential to understanding how the endocrine system mediates phenotypic differences and, ultimately, the fitness trade-offs that constrain life history strategies (Ketterson and Nolan, 1999; Williams, 2008; Zera and Harshman, 2001). Because the endocrine system is highly responsive to environmental cues, a number of extrinsic factors, including perturbation, can induce transient changes in circulating hormone levels (McEwen and Wingfield, 2003; Silverin, 1998; Wingfield et al., 1998). Although these acute changes in hormone levels facilitate temporary adjustments in physiology and behavior to cope with environmental challenges (Sapolsky et al., 2000; Wingfield, 1985), they can also introduce noise into datasets that may obscure meaningful variation in baseline hormone levels (Small et al., 2017). The ability to accurately quantify individual variation in baseline hormone levels therefore requires a working knowledge of how perturbation, including routine capture and sampling events, affects circulating levels of the hormone of interest. Indeed, accurately quantifying baseline hormone levels in free-living vertebrates is challenging because the acute stress of capture and handling is known to cause rapid changes in plasma hormone levels (e.g. Deviche et al., 2010; Krause et al., 2014; Romero and Romero, 2002). Further consideration of how routine capture and sampling methods can alter circulating levels of various hormones across a range of species will: 1) increase our ability to recognize and account for

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sources of variation in measures of baseline hormone levels, 2) inform useful modifications to field sampling protocols, and 3) broaden our understanding of adaptive variation in vertebrate stress responses.

Avian field studies have been integral in advancing our knowledge of how the vertebrate endocrine system responds to perturbation (Wingfield et al., 1998; Wingfield, 2012). Studies of endocrine stress responses in birds typically employ a capture and restraint protocol in which individuals are blood sampled immediately after capture to obtain a baseline hormone sample, held in a cloth bag, and resampled at designated time points to measure stress-induced changes in hormone levels (Wingfield et al., 1992). Most of these studies have focused on the responsiveness of the hypothalamic-pituitaryadrenal (HPA) axis, and in nearly all cases, capture and restraint induces a robust increase in plasma corticosterone concentrations (Breuner et al., 2008; Cockrem and Silverin, 2002; McEwen and Wingfield, 2003; Silverin, 1998; Wingfield et al., 1992). However, mounting evidence suggests that capture stress can also alter the circulating levels of multiple other hormones including testosterone, dihydrotestosterone, progesterone, and prolactin (Angelier et al., 2007; Chastel et al., 2005; Deviche et al., 2010; Gratto-Trevor et al., 1992; Krause et al., 2014; Verreault et al., 2008; Wingfield et al., 1982). Given the rather pervasive effect of capture and restraint stress on circulating hormone levels, it is necessary to evaluate sampling approaches to ensure that field efforts effectively measure baseline (i.e., pre-capture) hormone levels and, whenever possible, to account for variation introduced by capture stress. For example, studies of the adrenocortical response in birds frequently operate under the assumption that a sample acquired within 3 min of capture effectively measures pre-capture corticosterone levels (Romero and Reed, 2005). Yet, a recent study on Florida scrub jays (*Aphelocoma coerulescens*) found that plasma corticosterone levels may increase in as little as 2 min after capture (Small et al., 2017), a result similar to a previous study on European starlings (*Sturnus vulgaris*, Dawson and Howe, 1983). Other studies have revealed that the timing and magnitude of capture-induced changes in corticosterone levels can vary among individuals according to capture method (e.g., using a potter trap versus a mist net), sampling regime (e.g., how long a bird is left in a trap or net before blood sampling), and prior experience with capture and handling (Angelier et al., 2010; Carroll et al., 2016; Romero and Romero, 2002). These studies of the corticosterone response to capture stress underscore the need for further scrutiny of how capture and sampling efforts affect circulating levels of other hormones.

Numerous studies on free-living male birds have demonstrated that capture and restraint can cause circulating levels of androgens to decrease (Chiver et al., 2014; Davies et al., 2016; Deviche et al., 2012, 2014, 2016, 2017; Gratto-Trevor et al., 1992; Li et al., 2012, 2017; Moore et al., 2002; Silverin, 1998; Wingfield et al., 1982). Although an increase in androgen levels following capture and restraint was reported in one field study by Gratto-Trevor et al. (1992), this finding was based on very small effect sizes (androgen increases of 8–93 pg/ml in n = 3 males) and provides limited evidence that the directionality of the androgen response to stress in free-living male birds is variable. Whether and how strongly male androgen levels respond to capture stress, however, has been found to be context dependent. More specifically, capture and restraint was shown to have no effect on androgen levels in non-breeding male birds (i.e., when the hypothalamic-pituitary-gonadal (HPG) axis is inactive, Deviche et al., 2016) and, during the breeding season, male birds with higher initial androgen levels exhibited a greater decrease in androgen levels in response to capture and restraint than those with lower initial levels (Deviche et al., 2012, 2014). Further study is ultimately needed to identify the intrinsic and extrinsic factors that influence variation in the responsiveness of the avian HPG axis to perturbation.

For decades, studies of androgens in free-living birds have regarded samples collected within 10 min of capture as representative of baseline androgen levels, an approach often based on the findings of Wingfield and Farner (1976). Given that only a few additional studies have measured the effect of capture stress on androgens in birds, it is possible that the 10-minute rule is inappropriate for some species. For example, significant changes in androgen levels occurred within 10 min of capture in rufous-winged sparrows (*Peucaea carpalis*, Deviche et al., 2012). More time-stringent sampling regimes may indeed reduce artificial variation in baseline hormone data. Furthermore, no study has yet evaluated whether different capture methods and sampling regimes have variable effects on circulating androgens. Finally, additional studies from species inhabiting diverse environments and with variable life history strategies are needed to broaden our understanding of how selection pressures have shaped responsiveness of the avian HPG axis to perturbation.

In this study, we examined changes in circulating androgen levels in response to acute stress in free-living wire-tailed manakins (*Pipra filicauda*). First, we measured the effect of a standardized capture and restraint protocol on circulating androgen levels in male manakins. Then, to determine the influence of a common capture technique (i.e., capture in a mist net) on androgen levels, we passively captured males and examined whether the amount of time that they were in a mist net before blood sampling (hereafter 'net time') influenced individual variation in plasma androgen levels. To quantify net time, we filmed mist nets with GoPro video cameras and calculated the difference between the exact capture time and sampling time from videos of each sampling event. This novel approach is an effective method for accurately documenting capture events and is broadly applicable to other hormone studies where passive capture without constant net monitoring is necessary. Net time can subsequently be used in an attempt to statistically control for variation in hormone data induced by differences in net time or as a justification for excluding individuals from analyses with long capture times that are more likely to exhibit stress-induced (as opposed to baseline) androgen levels.

2. Methods

2.1. Study species, location, and field methodology

We conducted our research on wire-tailed manakins (*Pipra filicauda*) at the Tiputini Biodiversity Station, Orellana province, Eastern Ecuador (0° 83′ S, 76°08′ W, 190–270 m elevation). The wire-tailed manakin is a lekking, Neotropical sub-oscine bird that is found in lowland tropical rainforests throughout the northwestern Amazon Basin (Schwartz and Snow, 1978). In this study, males were passively captured using mist nets that were set up in territories the day before the experiment and opened before dawn the day of the experiment. All individuals were banded with both a numbered aluminum leg band and a unique combination of two or three colored leg bands. Birds were sampled during the population's peak breeding period (November to March) over the course of three field seasons (2014–2015, 2015–2016, 2016–2017). Male wire-tailed manakins exhibit delayed plumage maturation and do not obtain their definitive plumage until their third year of life (Ryder et al., 2008). Additionally, males do not obtain territories until they molt into their definitive plumage, although the age of obtaining a territory varies from 3 to 7 years (Ryder et al. unpublished data). Using both plumage characteristics and behavioral observations (following Ryder et al., 2008), we grouped males into four status classes (territory-holder, definitive floater, pre-definitive floater, and formative floater) that have previously been found to differ in age, behavior, plasma androgen levels, and, among floaters, plumage characteristics (Ryder et al., 2008, 2011a, 2011b).

2.2. Measuring the androgen response to a capture and restraint protocol

During the 2014–2015 field season, we measured the androgen response to capture and restraint following the protocol described in Wingfield et al. (1995). First, nets were watched from ~8–10 m away and, upon capture, a blood sample of approximately 75 µL was

collected within 3 min of capture using heparinized capillary tubes following brachial venipuncture with a 27-gauge needle. Birds were then placed in a cloth bag and randomly selected to be restrained in the bag for either 15 min or 30 min. Following restraint, a second blood sample of equal volume was collected. Capillary tubes were capped with Critoseal® and stored in a cooler with an ice pack (2–3 h) until centrifuged for 5 min, after which the plasma was removed and its volume measured to the nearest 0.2 µL using a Hamilton syringe. Plasma was then stored in ~100% EtOH following Goymann et al. (2007).

2.3. Measuring the androgen response to capture in a mist net

During the 2015–16 and 2016–17 field seasons, we assessed how the time a captured bird spent suspended in a mist net influenced circulating androgens by sampling birds that spent variable amounts of time in a mist net before the blood sample was collected. First, 10–15 mist nets were set up within a lek, and on each net a GoPro HERO4 Silver camera was attached to the base of one of the supporting poles using the GoPro Jaws: Flex Clamp. The wide-angle setting of the GoPro camera allows for the entirety of the net to be seen in the field of view so that exact capture times could be determined from video footage. After opening the net and turning on the cameras, we stated the time into the camera and left the area. Nets were subsequently checked every 30 min. All birds captured were sampled within 3 min of extraction from the net, and the exact time at which blood samples were acquired was stated into the camera. We determined the capture time for each bird by adding the amount of recording time elapsed between the time the net was opened and the time the bird was captured. We then quantified "net time" by calculating the difference between the time an individual was captured and the time the blood sample was acquired. The majority of individuals (n = 320) were sampled between 06:00 and 10:00 h, but some (n = 34) were sampled between 11:00 and 16:00 h. Sample storage in the field and processing followed the procedures described in Section 2.2.

2.4. Hormone assay

After each field season, we quantified total plasma androgen levels following double extraction with dichloromethane (average extraction efficiency for 2014–2105, 2015–2016, and 2016–2017: 68%, 63%, and 72% respectively) using a direct radioimmunoassay (following Moore et al., 2002; Wingfield et al., 1991). The average plasma volume was 32.97 ± 0.44 (mean ± SEM) and we ran samples in singlets to increase the detection probability. Androgen concentrations were adjusted for individual extraction efficiency. For each assay, samples from the same individual were grouped together, but the order of individuals was random. We calculated the intra-assay coefficient of variation among standards within an assay and the inter-assay coefficient of variation among these standards across the three assays. The intra-assay coefficient of variation was 13.8%, 6.6%, 11.6% for each field season (2014–2015, 2015–2016, 2016–2017, respectively), and the inter-assay coefficient of variation was 8.9%. The average detection limit for the assays was ~0.22 ng/mL, and all measured samples that fell below the assay's detection limit were assigned the detection limit for that assay.

2.5. Statistical analyses

For the capture and restraint experiment, androgen data were analyzed using a linear mixed model with sample type (baseline, 15 min, or 30 min) as a categorical fixed effect and individual as a random effect using the *lme4* package in the program R (Bates et al., 2015; R Core Team, 2015). Tukey's post-hoc comparisons were used to determine if average androgen levels significantly differed between each group (i.e., baseline, 15 min, or 30 min). All androgen levels were log-transformed to meet model assumptions prior to statistical analyses.

To analyze how net time affected androgen levels, we built linear mixed models with both additive and interactive effects of the time of day and net time. Time of day and net time were modeled as a fixed effect and, to account for repeated measures across two different field seasons, individual and year (i.e., 2015–2016 or 2016–2017) were modeled as random effects (individual with 2 captures n = 45, 3 captures n = 32, 4 captures n = 18, 5 captures n = 2). All models, including the null model, contained male status class as a fixed predictor variable, as previous work has documented a significant effect of a male's social status on androgen levels (Ryder et al., 2011b). Candidate model sets were symmetrical with respect to all predictor variables (Doherty et al., 2012). We ranked models using Akaike's Information Criterion (corrected for small sample size, AICc) and determined the relative likelihood of each model using model weights (w_i; Burnham and Anderson, 2002). Model effects are presented as standardized β parameter estimates and 95% confidence intervals. As above, all androgen levels were log-transformed prior to statistical analyses and fitted values were back-transformed prior to graphing in Program R (R Core Team, 2015).

3. Results

3.1. Capture and restraint experiment

We collected baseline and stress-induced samples from 24 males, of which 13 were restrained for 30 min and 11 were restrained for 15 min. Stress-induced androgen levels following restraint were significantly lower than baseline levels at both the 15 min and 30 min time points ($ß_{15} = -0.472$, $t_{25} = -2.332$, $p = 0.028$; $ß_{30} = -1.054$, $t_{25} = -5.623$, $p < 0.0001$). Additionally, there was a near-significant decrease in androgen levels between the 15 min and 30 min groups ($ß_{PM15\text{-}PM30} = -0.5817$, $z = -2.194$, $p = 0.069$, Fig. 1). To further examine whether the magnitude of stress-induced changes in androgen levels depended on initial androgen levels or the duration of restraint, we built a linear model with the change in androgen levels as the response variable and an interaction term between initial androgen levels

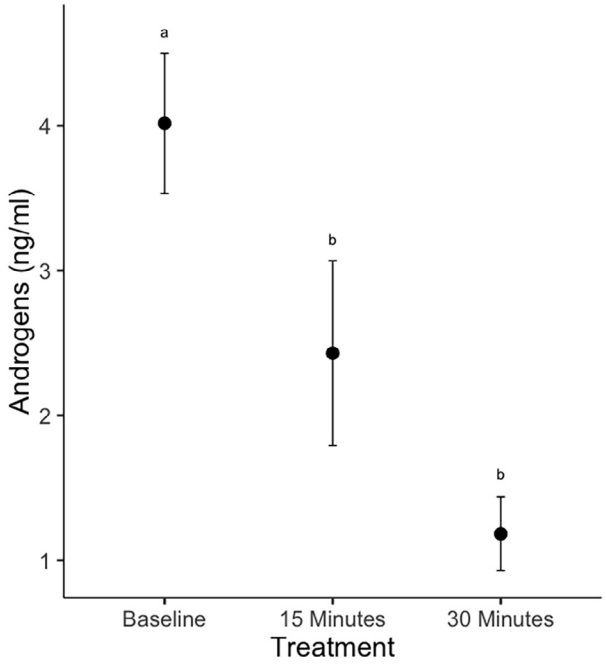

Fig. 1. Comparisons of androgen levels (untransformed) in male wire-tailed manakins sampled within 3 min of capture (baseline) and again after 15 min or 30 min of restraint in a cloth bag. A measurable decrease in circulating androgens occurred after 15 min; this decline was more pronounced after 30 min. Different letters above points denote significance differences ($p < 0.05$).

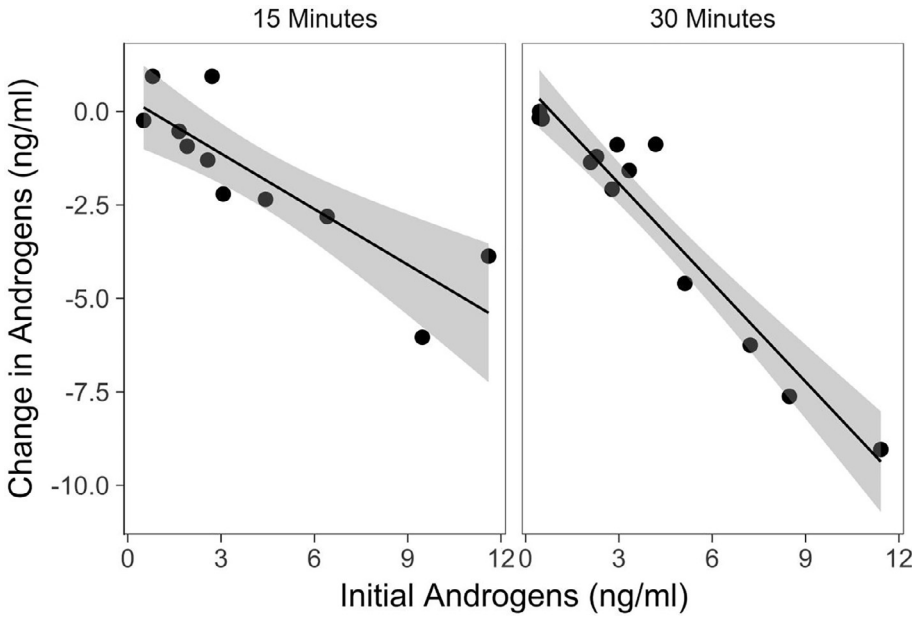

Fig. 2. The magnitude of the change in androgen levels (untransformed) following capture and either 15 or 30 min of restraint in relation to an individual's initial androgen levels. The change in androgen levels was negatively related to initial androgen levels ($\beta_{initial\ androgens} = -0.50$, 95% CIs = -0.27, -0.72), but this effect was dependent on the duration of the restraint ($\beta_{initial\ androgens*duration} = -0.39$, 95% Cis = -0.13, -0.64).

and restraint time as predictors. The magnitude of the change in androgen levels was positively associated with initial androgen levels (Fig. 2, $\beta_{initial\ androgens} = -0.50$, 95% CIs = -0.27, -0.72, $p < 0.001$), but this effect was found to be dependent on the duration of restraint (Fig. 2, $\beta_{initial\ androgens*duration} = -0.39$, 95% CIs = -0.13, -0.64, $p < 0.01$).

3.2. The androgen response to capture in a mist net

We measured the net time and obtained blood samples for 354 individuals (mean net time ± SEM = 17.4 min ± 0.93, range: 1–72 min). The top statistical model explaining variation in androgen levels included the factors status, year, and net time ($w_i = 0.75$, Table 1). This model suggests that net time negatively influences circulating androgen levels (Fig. 3, $\beta_{tElap} = -0.02$, 95% CIs = -0.039, -0.01) and that territory-holding males had higher testosterone levels than the three floater classes ($\beta_{formative\ floater} = -1.46$, 95% CIs = -1.81, -1.11; $\beta_{pre\text{-}definitive\ floater} = -1.16$, 95% CIs = -1.48, -0.84; $\beta_{definitive\ floater} = -0.27$, 95% CIs = -0.56, 0.03). Our results also suggest that the androgen response to capture does not depend on an individual's status, as the model with the interaction between status and net time was the lowest ranked model. Furthermore, we found no support for a time of day effect on androgen levels (Table 1). The effect of net time on androgen levels appeared to be driven by a relatively small number of samples (n = 7) collected > 40 min after capture. Although androgen levels do decline linearly with net time, the effect is not supported when samples from captures with long net times are removed from the analysis (Table 2).

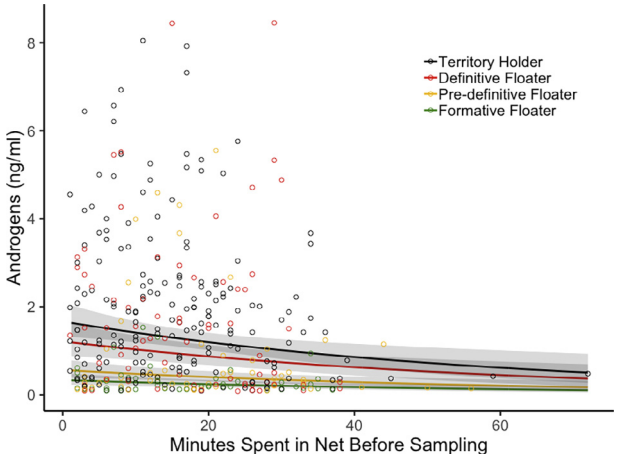

Fig. 3. A negative relationship exists between androgen levels and the time elapsed between capture and sample collection ($\beta_{tElap} = -0.02$, 95% CIs = -0.036, -0.011). The color of each point and each line represents one of the four status classes of male wire-tailed manakins that are known to differ in mean levels of androgens. Fitted values and their confidence intervals were back-transformed prior to graphing.

Table 1
AICc rankings for models examining the effect of net time (tElap), a male's status (Status), time of day (ToD), and year on androgen levels of wire-tailed manakins. *K* is the number of parameters in each model, ΔAICc is the difference in AICc score between each model and the best-fitting model, and w_i is the model weight.

	Model	K	ΔAICc[1]	w_i
1	~tElap + Status + Year	8	0.00	0.75
2	~Status + Year	7	2.67	0.20
3	~tElap + ToD + Status + Year	9	5.64	0.04
4	~ToD + Status + Year	8	7.48	0.02
5	~TOD * Status + Year	11	19.1	0.00
6	~tElap * Status + Year	11	23.40	0.00
7	~Status	6	34.38	0.00
8	~tElap + Status	7	35.61	0.00
9	~ToD + Status	7	41.11	0.00
10	~tElap + ToD + Status	8	42.66	0.00
11	~TOD * Status	10	54.11	0.00
12	~tElap * Status	10	59.24	0.00

[1] AIC = 1063.18 for the top ranked model.

4. Discussion

In this study, we measured the androgen response to two different acute stressors, a standardized capture and restraint protocol and capture in a mist net. Male wire-tailed manakins exhibited significant decreases in plasma androgen levels following capture and restraint (Fig. 1). Androgen levels also responded negatively, but more slowly, to capture and prolonged entanglement in a mist net (Table 2, Fig. 2). Overall, our results suggest that the effect of acute stress on androgen levels depends on the type and duration of the stressor. Moreover, these findings highlight how common methods used in avian field sampling

Table 2
AICc rankings for models examining the effect of net time (tElap), a male's status (Status), time of day (ToD), and year on androgen levels of wire-tailed manakins. In this analysis, 7 individuals with capture times greater than 40 min were excluded from the dataset. *K* is the number of parameters in each model, ΔAICc is the difference in AIC score between each model and the best-fitting model, and w_i is the model weight.

	Model	K	ΔAICc[1]	w_i
1	~Status + Year	7	0.00	0.75
2	~tElap + Status + Year	8	3.04	0.16
3	~ToD + Status + Year	8	4.77	0.07
4	~tElap + ToD + Status + Year	9	8.45	0.01
5	~TOD * Status + Year	11	16.46	0.00
6	~tElap * Status + Year	11	24.65	0.00
7	~Status	6	34.55	0.00
8	~tElap + Status	7	38.96	0.00
9	~ToD + Status	7	41.27	0.00
10	~tElap + ToD + Status	8	45.95	0.00
11	~TOD * Status	10	54.27	0.00
12	~tElap * Status	10	61.24	0.00

[1] AICc = 1042.32 for the top-ranked model.

protocols, including periodically checking nets and holding birds in bags, can themselves cause changes in circulating androgen levels and, ultimately, limit our ability to understand the functional significance of natural variation in baseline androgen levels.

For future studies to obtain accurate measures of baseline androgen levels, it is imperative that sampling protocols are designed to minimize and, when necessary, control for the influence of capture and restraint as well as net time on circulating hormone levels. Many avian field studies focused on measuring androgens use passive netting to capture birds, whereby nets are left unmonitored for extended periods of time. Often, such sampling regimes prioritize obtaining a blood sample within 10 min of extracting the bird from the net, but do not measure (or at least account for) the amount of time the bird spent in the net before being extracted and sampled (e.g., Day et al., 2007; DuVal and Goymann, 2011; Peters et al., 2001). When using such methods, sampling-induced variation in androgen levels may obscure meaningful relationships between phenotypic traits of interest and baseline hormone levels. For example, in a study by McGlothlin et al. (2007) on breeding dark-eyed juncos (*Junco hyemalis*), males were captured, placed in a cloth bag, and transported to another location before blood sampling. The authors reported a negative effect of handling and restraint time (range: 14–217 min) on circulating testosterone levels and also found no relationships between initial testosterone levels and male reproductive behaviors (parental care and aggression; McGlothlin et al., 2007), even though these behaviors have previously been described as being regulated by testosterone in this species (Ketterson et al., 1992; Schoech et al., 1998).

Future endocrine studies interested in measuring baseline androgen levels should use methods designed to avoid or control for potential sampling-induced changes in androgens. Foremost, we recommend taking blood samples immediately after removing an animal from a net or trap, as many studies on free-living birds, including ours, have shown that handling and restraint cause androgens to decrease (Chiver et al., 2014; Davies et al., 2016; Deviche et al., 2012, 2014, 2016, 2017; Li et al., 2012, 2017). For studies using passive, unmonitored netting protocols to capture birds, we recommend quantifying the amount of time between capture and blood sampling. The amount of time that an individual spends in the net or trap can then be included as a covariate in statistical models to help control for capture-induced variation in androgen levels. The methodology presented in this paper (i.e., filming the nets) provides a novel and simple way to measure the time between capture and sampling, especially when passive, unmonitored netting is preferred, as in cases where human presence or frequently checking nets modifies behaviors of interest (e.g., social interactions) and/or decreases capture rates. Moreover, using video cameras to quantify capture times presents an opportunity to increase daily capture rates by allowing for more nets or traps to be open simultaneously. This technique could also be applied to other trapping techniques in addition to mist nets, and to non-avian studies.

Although our results suggest that, for male wire-tailed manakins, being tangled in a mist net has a reduced effect on androgen levels compared to that of handling and restraint, and that this effect is most evident in birds with long net times, there are some important caveats to consider. Previous research has documented among-individual plasticity in endocrine responses to acute stressors (Cockrem and Silverin, 2002; Lendvai et al., 2014; Small et al., 2017), suggesting the rate and magnitude of a change in endocrine levels in response to perturbations varies among individuals. Indeed, the magnitude of a change in androgen levels following capture and restraint has been shown to depend upon an individual's initial androgen levels (Deviche et al., 2012). That is, individuals with higher initial androgen levels exhibit greater decreases in circulating levels than do individuals with lower initial levels (Deviche et al., 2012). In our study of how net time affects androgen levels, it was impossible to assess whether the observed effect of net time on androgen levels depended on initial hormone concentrations. However, in our capture and restraint experiment, the magnitude of the decline in androgen levels did depend on initial hormone levels (Fig. 2). It is therefore likely that the effect of net time on androgen levels consistently varies among individuals in manakins and in other species. Thus, although including net times (e.g., as acquired from video) as a covariate in statistical analyses can help control broadly for capture-induced variation in androgen levels, it cannot control for individual variation in the androgen response to capture stress. As such, watching nets and sampling birds immediately after capture is the most effective approach to reduce capture-induced variation in baseline androgen levels. Passive netting supplemented with video to record capture times provides an alternative only when the former method is not a tractable option. Additional capture and restraint studies at shorter time intervals (i.e., 5 and 10 min) would be useful to further our understanding of the sensitivity of androgen levels to acute stress and fine-tune methodologies for sampling baseline androgens.

Endocrine responses to acute stress are known to vary within and across species according to life history stage and life history strategy (Ricklefs and WIlkelski, 2002; Wingfield et al., 1998). For example, individuals that invest more heavily in current reproduction at the expense of survival and future reproductive opportunities have been found to exhibit reduced glucocorticoid responses to capture stress in some cases (Wingfield et al., 1995; Bókony et al., 2009). It is therefore plausible that androgen responses to stress may vary across and within species according to selection pressures and life history constraints. On the one hand, our study focused on a species that has a relatively low value of current reproduction (i.e., it is long-lived, has a long breeding season, and has low reproductive investment per attempt) and, as predicted, these male manakins exhibited a robust decrease in androgen levels in response to one type of stressor (i.e., capture, handling, and restraint). On the other hand, males exhibited a relatively attenuated androgen response to another form of stressor, entrapment in a mist net for a period of time. Currently, the degree to which variation in the strength of the androgen response to perturbation reflects differences in life history strategy is poorly understood. Similarly, the extent to which variation in the responsiveness of the HPG axis to acute stress is driven by individual differences in the reactivity of the hypothalamic-pituitaryadrenal axis is also poorly understood. Indeed, previous research has shown that androgen levels decrease as glucocorticoid levels increase (Moore et al., 1991; Moore et al., 2000; Deviche et al., 2010) and that multiple components of the HPG axis are sensitive to glucocorticoids (Orr and Mann, 1992; Dong et al., 2004; Deviche et al., 2010). A lack of a correlation in the magnitude of change in circulating levels of both hormones following acute stress (Deviche et al., 2014; Davies

et al., 2016), however, suggests the exact relationship is complex and may rely on multiple other mechanisms in addition to circulating glucocorticoids (Deviche et al., 2010; Deviche et al., 2017). Comparative studies aimed at addressing the influence of life history on endocrine responses to acute stress and mechanistic studies addressing the interactions between the HPA and HPG axes are promising directions for future research.

5. Conclusion

Measuring individual variation in baseline hormone levels is essential for environmental endocrine studies and our understanding of the evolution of life-history strategies (Ketterson and Nolan, 1999; Ricklefs and WIlkelski, 2002; Williams, 2008; Zera and Harshman, 2001). Here, we showed that circulating androgen levels in male wire-tailed manakins decline in response to capture and restraint, a pattern consistent with other studies on free-living male organisms. We also demonstrated that prolonged entanglement in a mist can also induce decreases in androgen levels. Thus, sampling protocols that do not properly account for the potential effects of capture and sampling on androgen levels risk a reduced ability to detect meaningful relationships between traits and androgens (e.g., McGlothlin et al., 2007; Day et al., 2007; Duval and Goymann, 2011). Moving forward, studies constrained to using sampling regimes with unmonitored, passive netting should incorporate both field and statistical methods that can help reduce and account for capture-induced variation in baseline androgen levels. The use of video described in this study provides a novel method for measuring the amount of time between capture and sampling, and thus the ability to account for artificial variation in androgen levels induced by the acute stress of capture and sampling.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ygcen.2018.07.017.

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