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Research article

Patterns of faecal steroids associated with reproduction in two Cracidae species: the blue-throated piping guan (*Pipile cumanensis cumanensis*) and the horned guan (*Oreophasis derbianus*)

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Abstract

Guans, curassows and chachalacas (family: Cracidae) are large-bodied, arboreal birds, native to tropical and subtropical Central and South America. Currently, 51 taxa are recognised, and many are listed by the IUCN as vulnerable or endangered. This study describes endocrine patterns associated with reproduction in two guan species, the blue-throated piping guan (*Pipile cumanensis cumanensis*) and the horned guan (*Oreophasis derbianus*). In total, 1895 faecal samples were collected from female and male piping guans (n=7 and n=6, respectively) and horned guans (n=2 and n=1, respectively) maintained at the Saint Louis Zoo. Concentrations of faecal oestrogen, progestagen, and androgen metabolites were quantified using commercially available enzyme immunoassays. Concentrations of faecal oestrogen and progestagen metabolites were higher in laying, compared to non-laying, females, and concentrations consistently rose prior to egg laying for both piping and horned guans. Faecal androgen concentrations of male piping guans varied by month, with the highest values measured from July to November. This study provides the first endocrine data for guans and confirms that faecal hormone analysis is an effective way to monitor reproduction, including egg laying and seasonal changes in steroid levels.

Introduction

Guans, curassows and chachalacas (family: Cracidae) are medium to large-bodied birds, with blunt wings, long tails and often distinctive crests or casques on the head or bill. Cracids are considered the most primitive of gallinaceous birds and are the only arboreal members of the Galliformes. Fifty-one taxa are currently recognised, with species inhabiting the sub-tropical and tropical regions of the Americas (Monroe and Sibley 1993). Little is known about the behaviour or life histories of many species due to their elusive nature and low population densities (del Hoyo et al. 1994; Brooks and Fuller 2006). However, Cracids are thought to play an important role as seed dispersers and seed predators in neotropical forests, and they are considered important bioindicators of forest quality (Santamaria and Franco 2000).

The Cracidae is regarded as one of the most endangered families of birds in the world, with many species threatened by habitat loss and hunting. Currently, 16 species are listed by the IUCN as "vulnerable" or "endangered"; an additional six species are listed as "critically endangered", and one is extinct in the wild. The Cracid Specialist Group has recommended *ex-situ* breeding programs be established to assist with conservation efforts (Brooks and Strahl 2000). Within the Association of Zoos and Aquariums (AZA) there are four managed breeding populations: blue-billed curassows (*Crax alberti*), horned guans (*Oreophasis derbianus*), northern helmeted curassows (*Pauxi pauxi*), and wattled curassows (*C. globulosa*). However, these captive populations each consist of fewer than 50 individuals, and efforts to improve reproduction are a priority for the Galliformes Taxon Advisory Group (TAG).

Endocrine monitoring is an important tool that can be used to assist with captive breeding (for a review, see Hodges et al. 2010). Hormones can be measured in a variety of sample types, but those collected non-invasively (saliva, urine and faeces) are often preferred because they allow for long-term monitoring without needing to disturb the animal. While faecal hormone analyses are commonly performed for mammalian species, endocrine analyses of avian species are often conducted with plasma samples (Wikelski et al. 1999; Cockrem and Silverin 2002). This may be due to the fact that many avian species produce faecal samples of small volume that can be prone to measurement error (Hayward et al. 2010). In addition, avian faecal samples are a mixture of faeces and uric acid and can require an additional enzyme hydrolysis step during the extraction process (Carere et al. 2003). However, blood sampling typically requires restraining an animal, and in a number of bird species handling stress can affect concentrations of circulating hormones, especially corticosterone and testosterone (Wilson et al. 1979; Gratto-Trevor et al. 1991). In contrast, faecal analysis has been shown to be a reliable and non-invasive method of assessing hormone concentrations for several avian species (Crofoot et al. 2003; Penfold et al. 2013; Brown et al. 2016).

In this study, we documented patterns of gonadal steroids associated with reproduction for two species of Cracids, the blue-throated piping guan (*Pipile cumanensis cumanensis*) and the endangered horned guan. Although blue-throated piping guans are listed as "least concern" by the IUCN, information from this species may help with captive breeding efforts of other closely related Cracids. Faecal samples were collected regularly from 13 piping guans and three horned guans maintained at Saint Louis Zoo; concentrations of faecal oestrogen, progestagen, and androgen metabolites were assessed using commercially available enzyme immunoassays. Based on previous studies of domestic hens (*Gallus gallus domesticus*) (Peterson and Common 1971, 1972), we predicted that female oestrogen and progestagen metabolite concentrations would be higher in laying versus non-laying females and that concentrations would increase prior to egg laying, with oestrogen concentrations rising before progestagens. For males, we predicted that faecal androgen concentrations would be highest during the breeding season, and that patterns would correspond with documented seasonal changes in sperm quality (DeMatteo et al. 1998).

This study presents the first endocrine data from any member of the Cracidae family. The methodology we outline could assist *ex-situ* breeding efforts by helping animal care staff predict the time of egg laying based on changes in hormone concentrations, determine whether individuals are sexually mature or post-reproductive, and identify infertile individuals.

Methods

Animals and housing

Piping guans

Study animals consisted of seven female and six male blue-throated piping guans housed at Saint Louis Zoo. Female guans of known age ranged from 1–15 years; the males ranged from 2–14 years, and all were considered of breeding age based on data from closely related species (Holmes and Sullivan 2015; Ingram and Sullivan 2015). Three females and two males were adults of unknown age. Animals were housed individually in off-exhibit habitats with cement flooring, measuring 3.1 m × 0.9 m × 2.1 m with 0.08 m × 0.01 m wire mesh, and several wooden perches approximately 1.5 m above the ground. Each female was provided with a straw basket (0.36 m × 0.36 m × 0.2 m) containing bark mulch bedding for egg laying. Birds could see individuals of the opposite sex through wire mesh but were not allowed access to mate. Each female's nest was checked daily for the presence of an egg. In captivity, females typically lay a clutch consisting of 2–3 eggs every 4–6 weeks (DeMatteo et al. 2004). When an egg was found, it was removed and often replaced with a dummy egg. Ambient temperature was maintained at 26–27°C, and the artificial light (full-spectrum) to dark cycle was set at 12L:12D. Individuals were fed 57 g fruits and vegetables daily, along with 170 g dry chow (females: Layena Performance Laying Ration; males: Game Bird Maintenance Chow, both Purina Mills, St. Louis, MO).

Horned guans

Three horned guans, one breeding pair and one unpaired female, were maintained on-exhibit at Saint Louis Zoo. The two females were 8 years of age; the male was 13 years old. The unpaired female was housed in a habitat measuring 7.6 m × 7.6 m × 7.6 m. The breeding pair was housed in a habitat with access to two enclosures (7.3 m × 5.5 m × 4.3 m and 5.5 m × 4.3 m × 4.3 m) that could be separated if needed. Both habitats contained mulch and were planted with a mix of tropical plants and ficus trees (*Ficus sp*.). Ambient temperature was maintained at 21–22°C from November through March and 24–25°C from April through October. The light-dark cycle was set to daylight hours, and light was provided by a combination of fluorescent and LED lights, as well as natural skylights. Birds were given 45 g fruit mix and 25 g Mazuri Exotic Gamebird Maintenance pellets (July through January) or Breeder pellets (February through June), along with kale, banana, grapes and avocado, twice daily. An elevated nest box (0.6 m × 0.6 m × 0.3 m) with bark mulch inside was provided along with wicker nest baskets. Nest boxes were checked daily, and two clutches were laid by the breeding pair. Typical clutch size for horned guans is two eggs, laid one day apart (Cornejo 2009). The eggs were removed and artificially incubated. Following hatching (June and August 2016), the chicks were hand reared.

Faecal collection and extraction

Piping guan samples were collected approximately once a week from August 1995 through November 1997, and horned guan samples were collected daily from January 2016 through June 2016. Differences in sampling frequency were the result of a concurrent study on piping guans (DeMatteo et al. 2004). All faecal samples were collected within 16 hours of defaecation and stored at −80°C prior to extraction using previously published methods (Dumonceaux et al. 2006). Briefly, samples were homogenised to evenly mix urates and faecal material. Approximately 0.5 g wet faeces was incubated at 37°C for 24 hours in 2.5 ml of modified phosphate-saline buffer and 25 µL of β-Glucuronidase/Arylsulfatase (Roche Diagnostics 10-127-698001) (Hartup et al. 2005). The following day, 2.5 ml of methanol was added to each sample, and the samples were shaken overnight. Liquid extracts were decanted, and solids were removed through centrifugation at 4000 g. The remaining supernatants were transferred to cryotubes and frozen at −80°C until assay. Faecal material was placed in a drying oven overnight at 100°C. Hormone concentrations were determined as ng/ml, and then divided by the dry weight of the extracted faeces to give the results as ng/g dried faeces.

Hormone analysis

Female samples were assayed for both faecal progestagen and oestrogen metabolites, while male samples were assayed for faecal androgen metabolites. Hormone concentrations were quantified using commercially available enzyme immunoassays (DetectX © Progesterone EIA K025, Estradiol EIA K030, Testosterone EIA K032, Arbor Assays). The detection limits of the progesterone assay were 50 pg/ml to 3200 pg/ml; the detection limits of the estradiol assay were 39 pg/ml to 10,000 pg/ml; the detection limits of the testosterone assay were 40 pg/ml to 10,000 pg/ml. Faecal extracts

were diluted 1:10 with assay buffer and run according to the kit directions.

For all assays, standards, samples and quality control pools were assayed in duplicate. In total, 27 progesterone, 26 estradiol, and 25 testosterone assays were performed. Mean intra-assay variation of duplicate samples was 9.9% for progesterone, 11.2% for estradiol, and 10.6% for testosterone. Mean inter-assay coefficients of variation for two quality control pools were 11.1% for progesterone, 10.3% for estradiol, and 10.7% for testosterone. Serial dilutions of piping and horned guan faecal extracts containing progesterone, oestrogen and testosterone were all parallel to the standard curve (test of equal slopes, P>0.10) (Zar 1996). Assay accuracy was assessed by adding a known amount of hormone to five faecal extracts that contained low values of hormone. Addition of known amounts at three dosage levels for piping guans resulted in recovery of 93.3±2.8% of added progesterone, 91.2±3.8% of added estradiol, and 92.4±2.9% of added testosterone. Recovery for horned guan samples resulted in 94.8±7.2% of added progesterone, 103.2±4.4% of added estradiol, and 101.1±6.3% of added testosterone.

Statistical analysis

All statistical analyses were performed using NCSS 9© (Kaysville, UT). Separate General Linear Models were used to investigate the relationships between hormone concentrations, egg laying and seasonality for piping guans. For all statistical models, animal ID and year of collection were included as random factors. To assess whether concentrations differed between laying and non-laying females, laying status (yes or no) was assigned as a fixed factor; to determine how concentrations changed in relation to egg laying, the sample date relative to clutch initiation (day the first egg in a clutch was laid) was assigned as a fixed factor. In both models, faecal hormone concentration (progestagen or oestrogen metabolites) was the response variable, and month of collection was included as a random factor. To investigate whether male hormone concentrations varied seasonally, month of collection was assigned as a fixed factor, and faecal androgen metabolite concentration was the response variable. Only descriptive statistics are presented for the horned guans due to the small number of individuals sampled.

Results

Female piping guans

Overall, 769 faecal samples were collected from seven female piping guans. Five females laid at least one egg, and two females failed to produce eggs; in total, 134 eggs were laid. Egg-laying females ranged in age from 1–15 years. The two females that failed to produce eggs were both adults of unknown age. Females laid eggs year-round, with the greatest number of eggs laid in July (n=18) and the fewest in April (n=4). The average number of eggs laid per laying female was 26.8±7.7 (range=2–50, median=27). Clutch sizes ranged from 1–3 eggs (mean±S.E.=1.5±0.1 eggs), which were laid, on average, 2.4±0.2 days apart (range=1–6, median=2). The average duration between subsequent clutches was 41.2±2.9 days (range=10–141, median=34).

Faecal oestrogen and progestagen metabolites were significantly higher in laying compared to non-laying females (oestrogen metabolites: F1,755=9.34, P=0.002; progestagen metabolites: F1,755=5.85, P=0.015), and concentrations of both hormones rose significantly in relation to egg laying (oestrogen metabolites: F90,506=1.60, P=0.001; progestagen metabolites: F90,506=3.26, P<0.0001) (Figure 1A). Because not all females were sampled on the same days in relation to egg laying, sample dates were first converted to days relative to clutch initiation (day the first egg in a clutch was laid). Data from all five laying females were then combined, and average daily faecal oestrogen and progestagen metabolite concentrations were calculated in relation to onset of oviposition (n=83 clutches). This allowed us to better visualise patterns of hormone production (Figure 2). Overall, faecal oestrogen metabolite concentrations increased starting approximately 13 days before oviposition and reached a maximum value 5 days before oviposition, and then declined to pre-laying levels. Faecal progestagen metabolite concentrations increased after faecal oestrogen concentrations, approximately 5

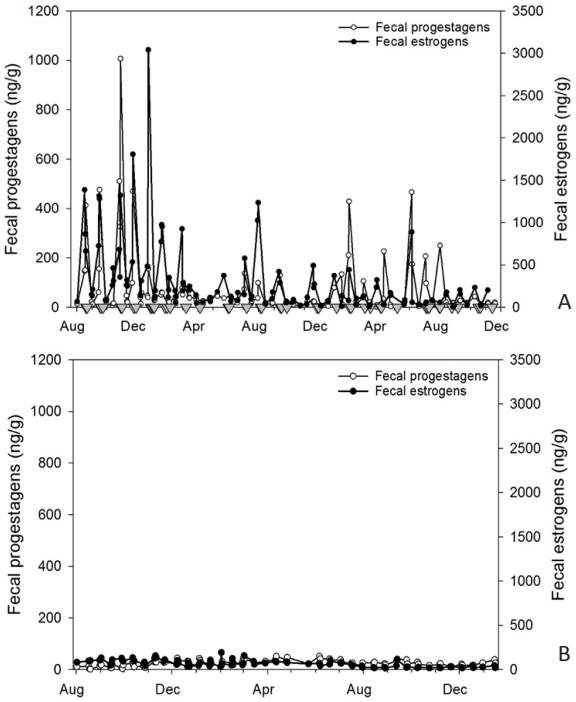

Figure 1: Faecal progestagen and oestrogen metabolite concentrations for (A) a female piping guan that produced 27 eggs (indicated by▼) over the course of 28 months; and (B) a female piping guan that failed to produce any eggs over the course of 18 months.

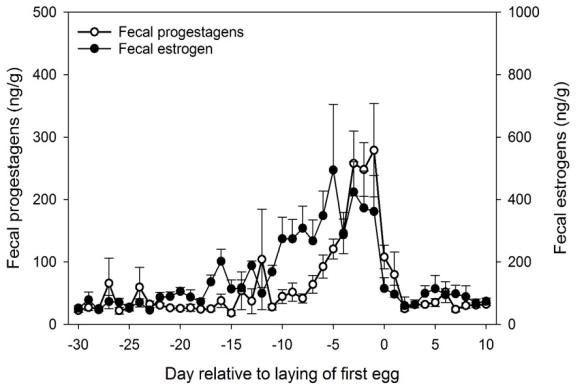

Figure 2: Daily faecal progestagen and oestrogen metabolite concentrations (mean±SEM) of female piping guans (n=5 females, 83 clutches) in relation to egg laying. Day 0 is the day the first egg in a clutch was laid.

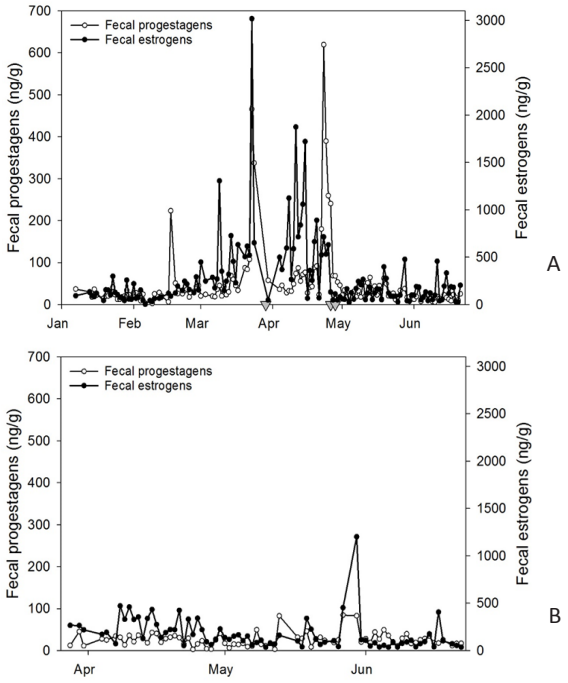

Figure 3: Faecal progestagen and oestrogen metabolite concentrations for (A) a female horned guan that produced two clutches (indicated by ▼); and (B) a female horned guan that failed to produce any eggs.

days prior to oviposition. Concentrations reached their maximum value the day prior to oviposition and then declined to pre-laying levels.

Between the onsets of oviposition, concentrations of faecal oestrogen metabolites averaged 83.2±3.6 ng/g, and concentrations of faecal progestagen metabolites averaged 39.9±2.5 ng/g; maximum concentrations averaged 423.8±97.1 ng/g for faecal oestrogen metabolites and 278.8±74.9 ng/g for faecal progestagen metabolites (n=83 clutches from 5 females). Average concentrations for the two non-laying females were 58.6±3.3 ng/g for faecal oestrogen metabolites and 21.9±1.1 ng/g for faecal progestagen metabolites, and no apparent hormone increases were detected (Figure 1B).

Female horned guans

Together, 213 faecal samples were collected from two female horned guans. The breeding female laid three eggs during the study. She produced two clutches, laid 28 days apart, one consisting of a single egg in March and the second consisting of two eggs in April (Figure 3A). The second female, who was housed singly, failed to lay any eggs (Figure 3B). Patterns of faecal hormones in relation to the onset of oviposition were similar to piping guans. Faecal oestrogen metabolites rose between 12 and 15 days before oviposition and declined to pre-laying levels on the day of oviposition. Concentrations peaked at 3017.0 ng/g, and averaged 802.4±146.5 ng/g. Similarly, faecal progestagen metabolite concentrations rose closer to oviposition, between 4 and 9 days, and declined shortly afterwards. Concentrations peaked at 619.4 ng/g, and averaged 325.0±58.3 ng/g. Between the onset of oviposition, faecal progestagen metabolites averaged 30.4±1.6 ng/g, and oestrogen metabolites averaged 178.6±13.2 ng/g. For the non-laying female, faecal progestagen metabolites averaged 25.9±1.9 ng/g, and oestrogen metabolites averaged 177.0±19.4 ng/g.

Male piping guans

Over two years, 769 faecal samples were collected from six male piping guans. Four ranged in age from 2–14 years, and two were adults of unknown age. Faecal androgen metabolites averaged 709.5±39.2 ng/g (range=10.1–13326.2 ng/g), and concentrations differed significantly between months of collection ($F_{11,769}=3.21$, $P=0.0003$). For most males, the lowest concentrations were measured in April and May; values then rose throughout summer, peaked between July and November, then declined during the winter and early spring (Figure 4). However, the two males of unknown age failed to show seasonal changes in faecal androgen concentrations.

Male horned guan

In total, 144 faecal samples were collected from the male horned guan. Faecal androgen metabolites ranged from 7.99–15,428.8 ng/g (mean=678.5±143.1 ng/g). Three sustained rises in faecal androgen metabolites were detected during the collection period (Figure 5). The first in March, which lasted approximately 21

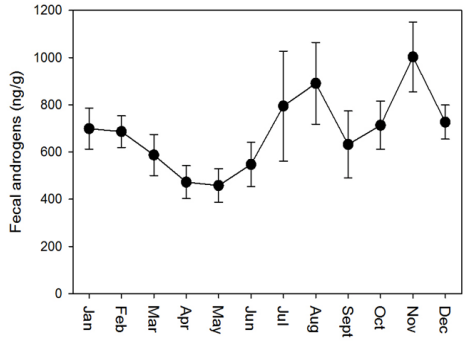

Figure 4: Faecal androgen metabolite concentrations (mean±SEM) of male piping guans (n=6) in relation to month of faecal collection.

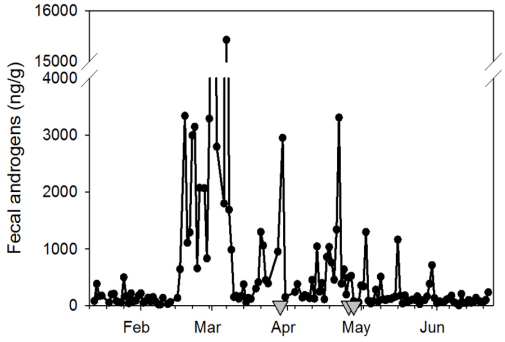

Figure 5: Faecal androgen metabolite concentrations of a male horned guan. Egg laying by the female is indicated by ▼.

days, occurred 8 days before a rise in his mate's faecal oestrogen concentrations was detected and 20 days before the first egg was laid. The two additional increases in faecal androgen metabolites lasted 9 and 16 days, respectively, and overlapped with egg-laying dates in April and May. Faecal androgen metabolite concentrations during these periods averaged 1956.8±444.8 ng/g; concentrations between these androgen rises averaged 169.7±17.6 ng/g.

Discussion

This study provides the first endocrine dataset for any member of the Cracidae family and demonstrates that hormone changes associated with egg laying and seasonality can be monitored in both piping and horned guans through faecal analysis. Females demonstrated clear increases in hormone concentrations corresponding with documented egg-laying dates. Male piping and horned guans had elevated faecal androgen concentrations during known breeding seasons.

In both piping and horned guans, faecal oestrogen and progestagen metabolites were significantly higher in laying versus non-laying females, and concentrations increased preceding oviposition. Similar findings have been reported for whooping cranes (*Grus americana*) (Brown et al. 2016), great hornbills (*Buceros bicornis*) (Crofoot et al. 2003), canaries (*Serinus canaria*) (Sockman and Schwabl 1999), and brown kiwi (*Apteryx mantelli*) (Jensen and Durrant 2006). In domestic chickens, elevated concentrations of luteinising hormone lead to the production of estradiol by follicular thecal cells. Production is highest in small, early stage follicles and declines in later-stage, yolky follicles (Etches 1996). Increased levels of estradiol have been shown to induce the synthesis and mobilisation of vitellogin and other yolk components and increase development of the oviduct (Etches 1996). Behaviourally, estradiol also stimulates food intake (Johnson 1986) and may be important for initiating female reproductive behaviour (Searcy 1992) and nest building (Hinde 1965). High concentrations of progesterone are produced by large, yolky follicles (Etches 1996), which stimulate a surge in luteinising hormone and trigger ovulation (Sharp 1980). In chickens, progesterone is also involved in the production of avidin, contraction of the myometrium, and shell formation (Yoshimura and Behr 1991), and behaviourally it is thought to play a role in nesting and incubation behaviour (Sharp and Lea 1996).

Differences in timing between peaks in faecal oestrogen and progestagens were observed for both piping and horned guans. Concentrations of faecal oestrogen metabolites rose approximately 2 weeks prior to oviposition, reached their maximum values several days prior to egg laying, and declined to pre-laying levels around the first day of egg laying. Concentrations of faecal progestagens rose after the increase faecal oestrogens, approximately 5 days prior to laying and declined several days after oviposition. Similar patterns have been described for brown kiwi (Jensen and Durrant 2006) and canaries (Sockman and Schwabl 1999) and likely correspond to differential hormone production by early and mature follicles.

In a majority of male piping guans, faecal androgen concentrations were highest from July through November, and then decreased throughout the winter and spring, matching observations of semen quantity and quality from a previous investigation. During regular semen collections, sperm quantity and quality were both found to decrease from mid-fall to mid-winter (DeMatteo et al. 1998). In the wild, little is known about the breeding season of blue-throated piping guans, but it is generally thought to occur during the rainy season (May to November for much of the species range) (del Hoyo et al. 1994). For the male horned guan, faecal androgen concentrations peaked in March, coinciding with the documented breeding season (Cornejo 2009). Two smaller rises in April and May were also detected, overlapping with elevated oestrogen levels in the female that preceded oviposition. Seasonal changes in faecal androgen concentrations have also been documented in European stonechats (*Saxicola torquata rubicola*) (Goymann et al. 2002), American kestrels (*Falco sparverius*) (Pereira et al. 2010) and graylag geese (*Anser anser*) (Hirschenhauser et al. 1999).

In our study, two female piping guans failed to lay eggs and two male piping guans failed to show a seasonal trend in androgen production. One possibility is that these individuals may have been pre-pubertal or post-reproductive, as these four birds were of unknown age. While the reproductive lifespan of piping guans has not been documented, egg-laying females in this study ranged from 1–15 years of age, and males ranged from 2–14 years of age. Similarly, blue-billed and wattled curassows can reproduce successfully from 2 to over 20 years of age and have been recorded as living into their late 20s or early 30s (Holmes and Sullivan 2015; Ingram and Sullivan 2015).

This study provides the first endocrine data for any species of guan and confirms that faecal hormone analysis is an effective way to monitor reproduction, including egg laying and seasonal changes in steroid hormones. Cracidae is considered one of the most endangered families of birds in the world, and the IUCN Cracid Specialist Group has recommended establishing *ex-situ* breeding programs to assist with conservation efforts (Brooks and Strahl 2000). The methods outlined here could contribute to those efforts by helping to assess fertility of captive populations of guans and other Cracid species.

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References

Brooks D.M., Fuller R.A. (2006) Biology and conservation of cracids. In: Cancino, L., and Brooks, D.M. (eds). *Conserving cracids: The most threatened family of birds in the Americas*. Houston, TX: Houston Museum of Natural Science, 11–26.

Brooks D.M., Strahl S.D. (2000) *Curassows, guans and chachalacas. Status survey and conservation action plan for cracids 2000–2004*. Cambridge, UK: IUCN/SSC Cracid Specialist Group.

Brown M.E., Converse S.J., Chandler J.N., Shafer C., Brown J.L., Keefer C.L., Songsasen N. (2016) Female gonadal hormones and reproductive behaviors as key determinants of successful reproductive output of breeding whooping cranes (*Grus americana*). *General and Comparative Endocrinology* 230: 158–165.

Carere C., Groothuis T.G.G., Möstl E., Daan S., Koolhaas J.M. (2003) Fecal corticosteroids in a territorial bird selected for different personalities: daily rhythm and the response to social stress. *Hormones and Behavior* 43: 540 – 548.

Cockrem J.F., Silverin B. (2002) Variation within and between birds in corticosterone responses of great tits (*Parus major*). *General and Comparative Endocrinology* 125: 197–206.

Cornejo J. (2009) Breeding programme for horned guan *Oreophasis derbianus* at Africam Safari, Mexico. *International Zoo Yearbook* 43: 136–148.

Crofoot M., Mace M., Azua J., MacDonald E., Czekala N.M. (2003) Reproductive assessment of the great hornbill (*Buceros bicornis*) by fecal hormone analysis. *Zoo Biology* 22: 135–145.

del Hoyo J., Elliot A., Saragatul J. (1994) *Handbook of the birds of the world. Volume 2: New World Vultures to Guinea fowl*. Barcelona: Lynx Editions, 638.

DeMatteo K.E., Asa C.S., Macek M.S., Snyder T.L. (1998) Use of the common piping guan (*Pipile c. cumanensis*) as a model for short- and long-term preservation of Cracid semen. In: Brooks, D.A., Olmos, F., and Begazo, A.J. (eds). *Biology and Conservation of the Piping Guans* (Aves: Cracidae). Houston, TX: Cracid Specialist Group, 33.

DeMatteo K.E., Karagiannis K.L., Asa C.S., Macek M.S., Snyder T.L., Tieber A.M., Parker P.G. (2004) Semen collection and artificial insemination in the common piping guan (*Pipile cumanensis cumanensis*): potential applications for Cracidae (Aves: Galliformes). *Journal of Zoo and Wildlife Medicine* 35: 447–458.

Dumonceaux G.A., Bauman J.E., Camilo G.R. (2006) Evaluation of progesterone levels in feces of captive reticulated giraffe (*Giraffa camelopardalis reticulata*). *Journal of Zoo and Wildlife Medicine* 37: 255–261.

Etches R. J. (1996) *Reproduction in Poultry*. Cambridge, UK: Cambridge University Press.

Gratto-Trevor C.L., Oring L.W., Fivizzani A.J. (1991) Effects of blood sampling stress on hormone levels in the semipalmated sandpiper. *Journal of Field Ornithology* 62: 19–27.

Goymann W., Möstl E., Gwinner E. (2002) Non-invasive methods to measure androgen metabolites in excrements of European stonechats, *Saxicola torquata rubicola General and Comparative Endocrinology* 129: 80-87.

Hartup B., Olsen G.H., Czekala N.M. (2005) Fecal corticoid monitoring in whooping cranes (*Grus americana*) undergoing reintroduction. *Zoo Biology* 24: 15–28.

Hayward L.S., Booth R.K., Wasser S.K. (2010) Eliminating the artificial effect of sample mass on avian fecal hormone metabolite concentration. *General and Comparative Endocrinology* 169: 117–122.

Hinde R.A. (1965) Interaction of internal and external factors in integration of canary reproduction. In: Beach, F.A. (ed.) *Sex and Behavior.* New York, NY: Wiley, 381–415.

Hirschenhauser K., Möstl E., Kotrschal K. (1999) Seasonal patterns of sex steroids determined from feces in different social categories of greylag geese (*Anser anser*). *General and Comparative Endocrinology* 114: 67–79.

Hodges K., Brown J.L., Heistermann M. (2010) Endocrine monitoring of reproduction and stress. In: Kleiman, D.G., Thompson, K.V., Baer, C.K. (eds.) *Wild Mammals in Captivity: Principles and Techniques for Zoo Management.* Chicago, IL: University of Chicago Press, 477–468.

Holmes C., Sullivan S. (2015) *Population Analysis and Breeding and Transfer Plan: Blue-billed Curassow (Crax alberti).* AZA Species Survival Plan® Red Program.

Ingram C., Sullivan S. (2015) *Population Analysis and Breeding and Transfer Plan: Wattled Curassow (Crax globulosa)*. AZA Species Survival Plan® Red Program.

Jensen T., Durrant B. (2006) Assessment of reproductive status and ovulation in female brown kiwi (*Apteryx mantelli*) using fecal steroids and ovarian follicle size. *Zoo Biology* 25: 25–34.

Johnson A.L. (1986) Reproduction in the female. In: *Avian Physiology.* New York, NY: Springer, 403–431.

Monroe B., Sibley C. (1993) *A World Checklist of Birds*. New Haven, CT: Yale University Press.

Penfold L.M., Hallager S., Boylan J., de Wit M., Metrione L.C., Oliva M. (2013) Differences in fecal androgen patterns of breeding and nonbreeding kori bustards (*Ardeotis kori*). *Zoo Biology* 32: 54–62.

Pereira R.J.G., Granzinolli M.A.M., Duarte J.M.B. (2010) Annual profile of fecal androgen and glucocorticoid levels in free-living male American kestrels from southern mid-latitude areas. *General and Comparative Endocrinology* 166: 94–103.

Peterson A.J., Common R.H. (1971) Progesterone concentration in peripheral plasma of laying hens as determined by competitive protein-binding assay. *Canadian Journal of Zoology* 49: 599–604.

Peterson A.J., Common R.H. (1972) Estrone and estradiol concentrations in peripheral plasma of laying hens as determined by radioimmunoassay. *Canadian Journal of Zoology* 50: 395–404.

Santamaria M., Franco A. (2000) Frugivory of Salvin's curassow in a rainforest of the Colombian Amazon. *Wilson Bulletin* 112: 473–481.

Searcy W.A. (1992) Song repertoire and mate choice in birds. *American Zoologist* 32: 71–80.

Sharp P.J. (1980) The endocrine control of ovulation in birds. In: Nöhring, R. (ed.) *Acta XVII Congressus Internationalis Ornithologici.* Berlin: Deutsche Ornithologen-Gesellschaft, 245–248.

Sharp P.J., Lea R.W. (1996) Central control of incubation behavior in birds. *Italian Journal of Anatomy & Embryology* 101: 83.

Sockman K.W., Schwabl H. (1999) Daily estradiol and progesterone levels relative to laying and onset of incubation in canaries. *General and Comparative Endocrinology* 114: 257-268.

Wikelski M., Hau M., Wingfield J.C. (1999) Social instability increases plasma testosterone in a year–round territorial neotropical bird. *Proceedings of the Royal Society B: Biological Sciences* 266: 551–556.

Wilson E.K., Rogler J.C., Erb R.E. (1979) Effect of sexual experience, location, malnutrition, and repeated sampling on concentrations of testosterone in blood plasma of *Gallus domesticus* roosters. *Poultry Science* 58: 178–186.

Yoshimura Y., Behr J.M. (1991) Localization of progesterone receptors in the shell gland of laying and nonlaying chickens. *Poultry Science* 70: 1246–1251.

Zar J.H. (1996) *Biostatistical Analysis,* 4th edition. Upper Saddle River, NJ: Prentice-Hall.