

Washington University in St. Louis

Washington University Open Scholarship

Undergraduate Research Symposium Posters

Undergraduate Research

Fall 2016

Patterns of fecal progestagens, estrogens, and androgens associated with reproduction in blue-throated piping guans (*Pipile cumanensis*)

Leslie Ann Sterling

Washington University in St Louis

Helen Clawitter

Saint Louis Zoo

Corinne P. Kozlowski

Saint Louis Zoo

Michael Macek

Saint Louis Zoo

Anne Tieber

Saint Louis Zoo

Follow this and additional works at: https://openscholarship.wustl.edu/undergrad_research



Part of the [Animals Commons](#), [Animal Studies Commons](#), [Environmental Studies Commons](#), [Ornithology Commons](#), and the [Zoology Commons](#)

Recommended Citation

Sterling, Leslie Ann; Clawitter, Helen; Kozlowski, Corinne P.; Macek, Michael; and Tieber, Anne, "Patterns of fecal progestagens, estrogens, and androgens associated with reproduction in blue-throated piping guans (*Pipile cumanensis*)" (2016). *Undergraduate Research Symposium Posters*. 77.

https://openscholarship.wustl.edu/undergrad_research/77

This Unrestricted is brought to you for free and open access by the Undergraduate Research at Washington University Open Scholarship. It has been accepted for inclusion in Undergraduate Research Symposium Posters by an authorized administrator of Washington University Open Scholarship. For more information, please contact digital@wumail.wustl.edu.

Patterns of fecal progestagens, estrogens, and androgens associated with reproduction in blue-throated piping guans (*Pipile cumanensis*)

Leslie A. Sterling¹, Helen Clawitter², Corinne P. Kozlowski², Michael Macek³, Anne Tieber³
¹Washington University in St. Louis; ²Research Department, Saint Louis Zoo; ³Bird Department, Saint Louis Zoo



Introduction



While fecal hormone analyses are routinely employed to monitor reproduction in mammals, few studies have utilized these techniques to investigate avian reproductive events. This study describes the hormone patterns associated with reproduction in the blue-throated piping guan (*Pipile cumanensis*), a less threatened relative of the critically endangered Trinidad piping guan (*P. pipile*).

Study Species

Blue-throated piping guans are members of the Cracidae family and are native to the dense, humid riverside forests of South America—specifically in the Guianas, Venezuela, Colombia, Brazil, and Peru. Similar in size to a turkey, guans are an average of 27 inches long from head to tail. Blue-throated piping guans are covered in black plumage with white patches on their wings and chests as well as striking white crests, and blue faces, beaks, and wattles. The International Union for the Conservation of Nature (IUCN) lists the status of the blue-throated piping guan as Least Concern due to its vast geographical range. However, it is approaching the criteria for Vulnerable as deforestation causes the population to decline. It is estimated that fewer than 10,000 mature individuals currently live in the wild.

Materials and Methods

Animals

Study animals were 7 adult female and 6 adult male blue-throated piping guans. Two of the 7 females did not lay eggs during the study period. Females and males lived in the Saint Louis Zoo’s Bird House and were housed individually.

Sample collection

Fecal samples were collected once per week from August 1995 to December 1997. Samples were frozen and maintained at -80°C until steroid hormone extractions were performed. A total of 1,525 fecal samples were collected from the 13 individual piping guans.

Fecal hormone extraction and assays

0.4-0.6 grams of fecal matter was placed in a pre-weighed scintillation vial, heated overnight at 37°C with 2.5 mL of phosphate-buffered saline and 25µl of β-glucuronidase, then shaken for a minimum of 16 hours after the addition of 2.5 mL of methanol. Liquid extracts were decanted from the solids into test tubes and centrifuged for an hour at 4,000 g to further remove any solid material. The resulting supernatants were transferred into cryovials and stored at -80°C until assay. Remaining fecal material in the scintillation vials was dried overnight at 100°C and weighed.

Fecal estrogen, progestagen, and androgen levels were analyzed using commercially available enzyme immunoassay kits (DetectX © Estrogen, Progesterone, and Testosterone EIAs, Arbor Assays). Concentrations were calculated in ng/mL hormone and converted to ng/g feces after dividing by the dry weight of the sample. Mean intra-assay variation of duplicate samples was 9.9% for progesterone, 11.2% for estradiol, and 10.6% for testosterone. Mean inter-assay variation for two quality control pools was 11.1% for progesterone, 10.3% for estradiol, and 10.7% for testosterone.

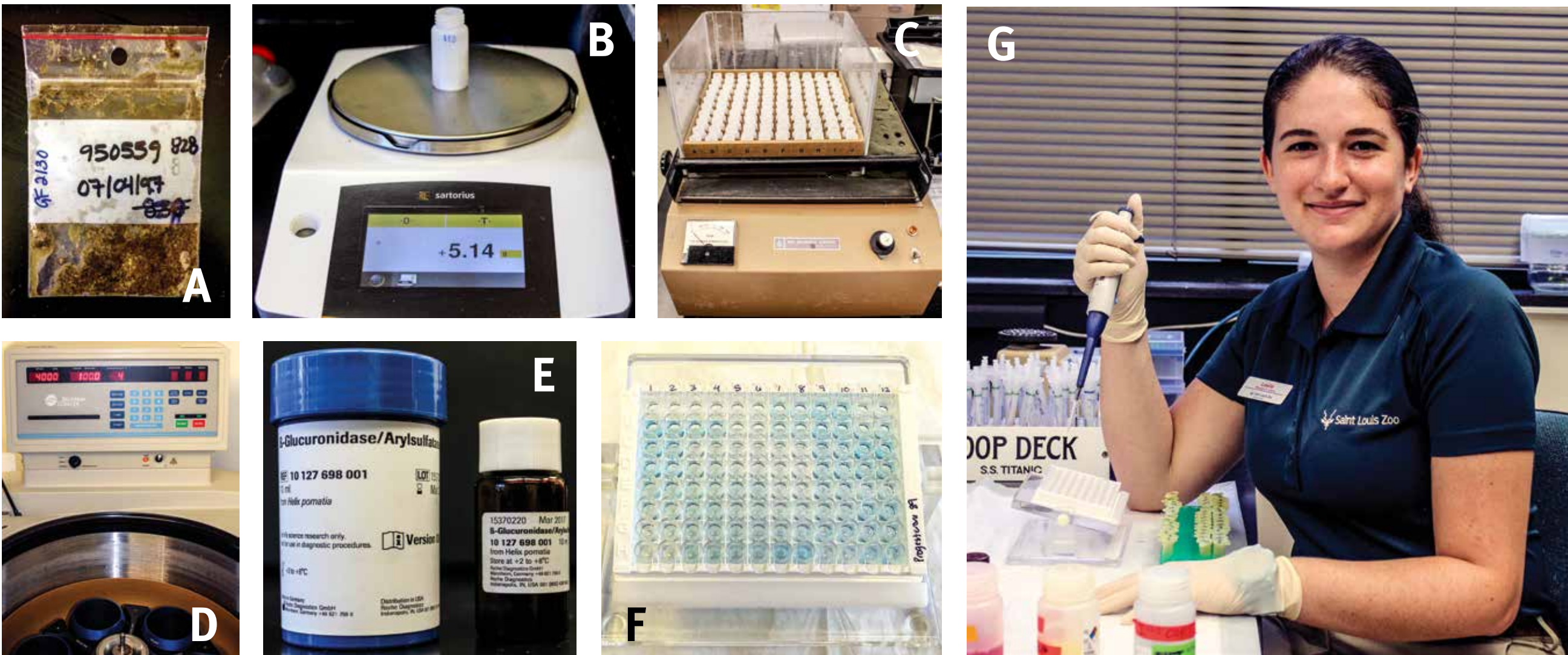


Figure 1: (A) Frozen fecal sample ready for extraction; (B) Digital scale used to weigh fecal material; (C) Fecal samples ready to be shaken overnight; (D) Fecal extracts in centrifuge; (E) β-glucuronidase; (F) Progesterone enzyme immunoassay; (G) Working in the lab.

Results



Images by Vladimír Motycka (A) and Greg & Yvonne Dean (B), Arkive.
Figure 2: (A) Blue-throated piping guan chick; (B) Adult female blue-throated piping guan.

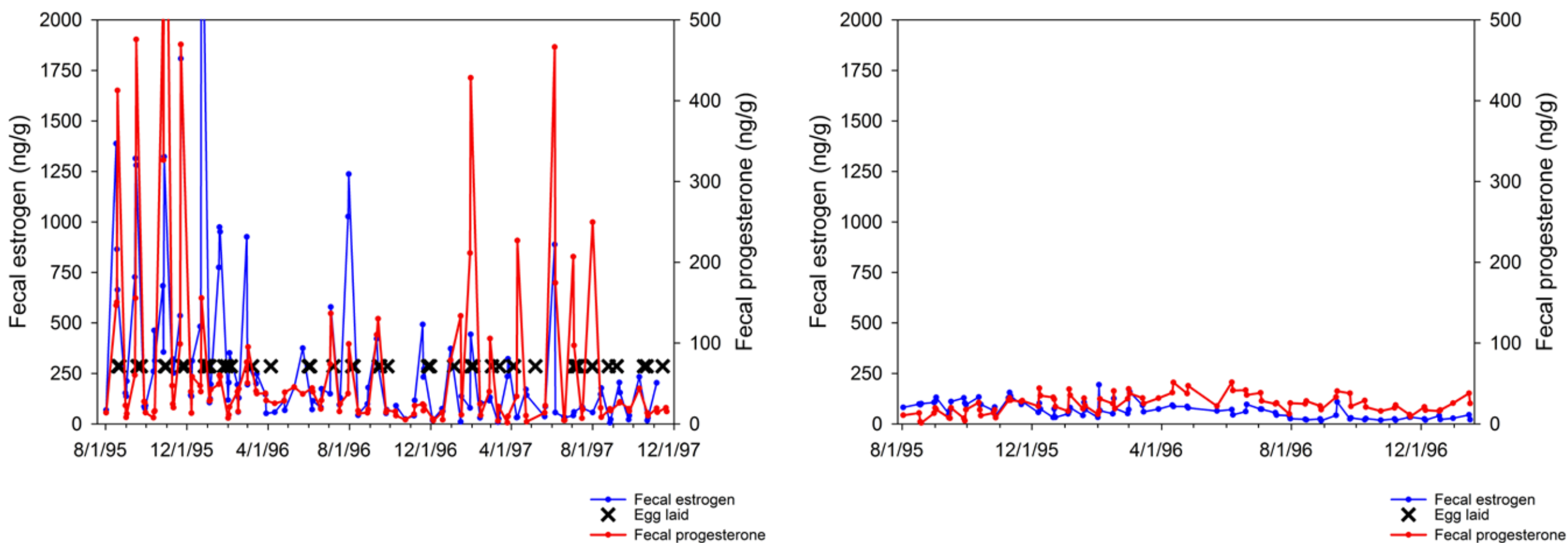


Figure 3: Fecal estrogen (red) and progestagen (blue) (ng/g) profile for Female 1. This female laid 49 eggs during the 28-month collection period. Egg laying events are noted with an “x”.
Figure 4: Fecal estrogen (red) and progestagen (blue) (ng/g) profile for Female 5. This female did not lay eggs during the collection period.

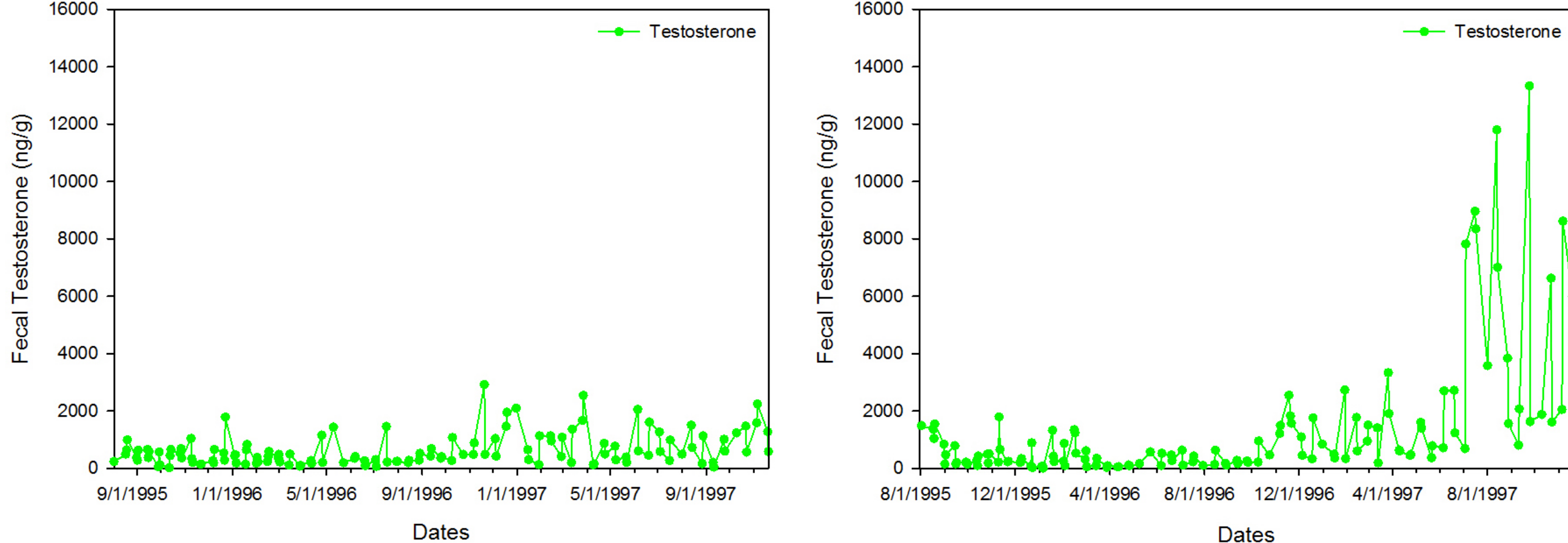


Figure 5: Fecal androgen (green) (ng/g) profile for Male 4. This male experienced minimal fluctuations in testosterone levels during the collection period.
Figure 6: Fecal androgen (green) (ng/g) profile for Male 5. This male experienced an increase in testosterone levels late in the summer of 1997.

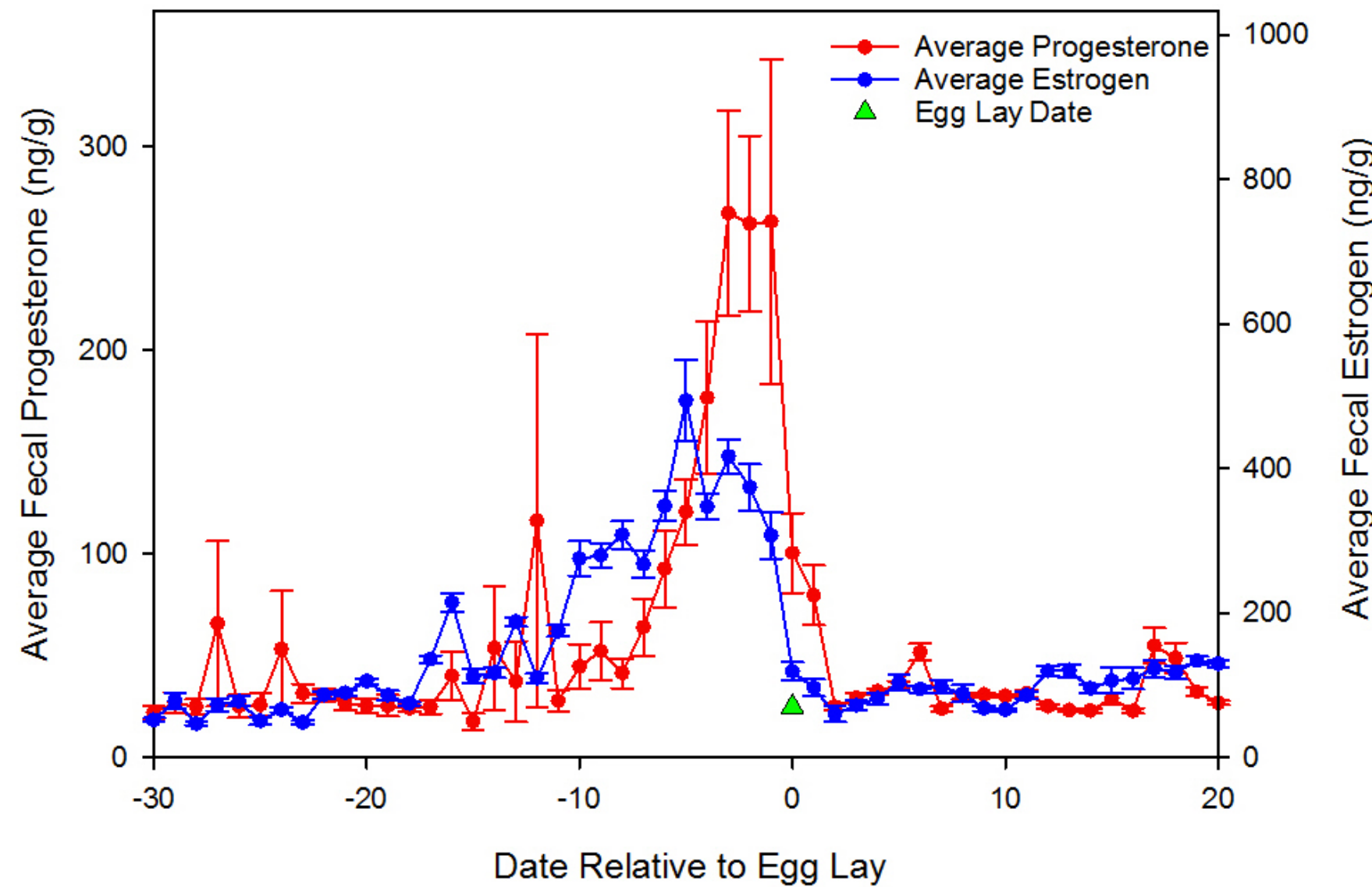


Figure 7: Average fecal estrogen (red) and progestagen (blue) (± S.E.) (ng/g) profiles for females (n=4) relative to egg lay date (△, t=0). Estrogen rises before progestagens, approximately 17 days before egg-laying and returns to baseline on the day of egg-laying. Progestagens rise about 11 days before egg-laying and returns to baseline 2 days after egg laying.

Table 1: Female and male blue-throated piping guan reproductive trends.

Mean Clutch Size (average ± S.E. eggs)	1.6 ± 0.07
Range of Clutch Sizes (eggs/clutch)	1 to 3
Mean Duration between Clutches (average ± S.E. days)	42.0 ± 2.8
Range of Durations between Clutches (days)	11 to 41
Month with Greatest Number of Eggs	July (n=18 eggs)
Month with Least Number of Eggs	March (n=5 eggs)
Month with Highest Average Male Testosterone Levels	July
Month with Lowest Average Male Testosterone Levels	December

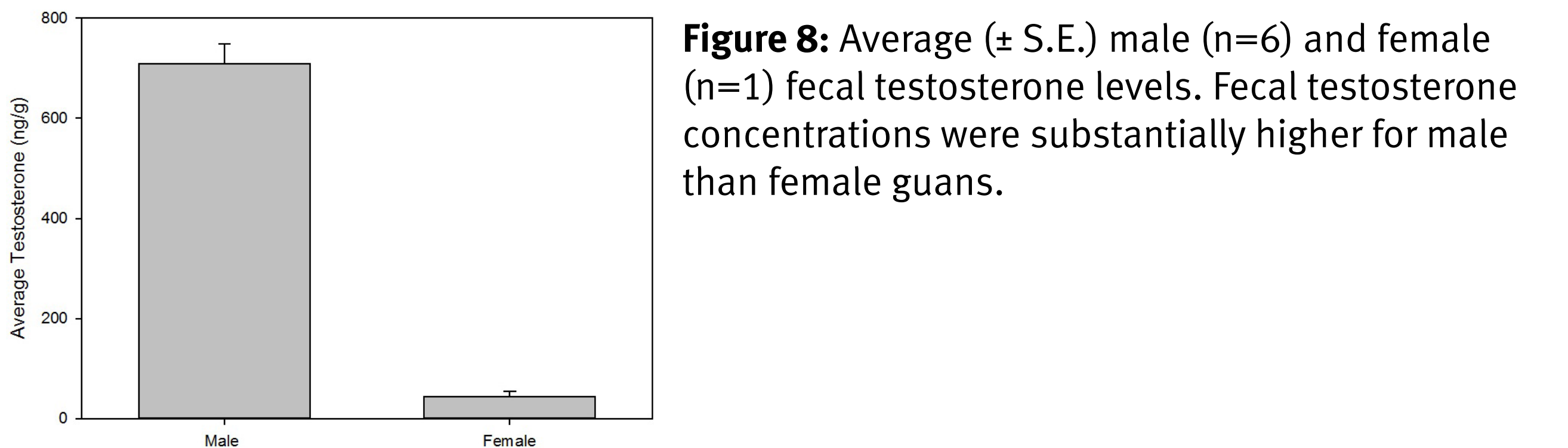


Figure 8: Average (± S.E.) male (n=6) and female (n=1) fecal testosterone levels. Fecal testosterone concentrations were substantially higher for male than female guans.

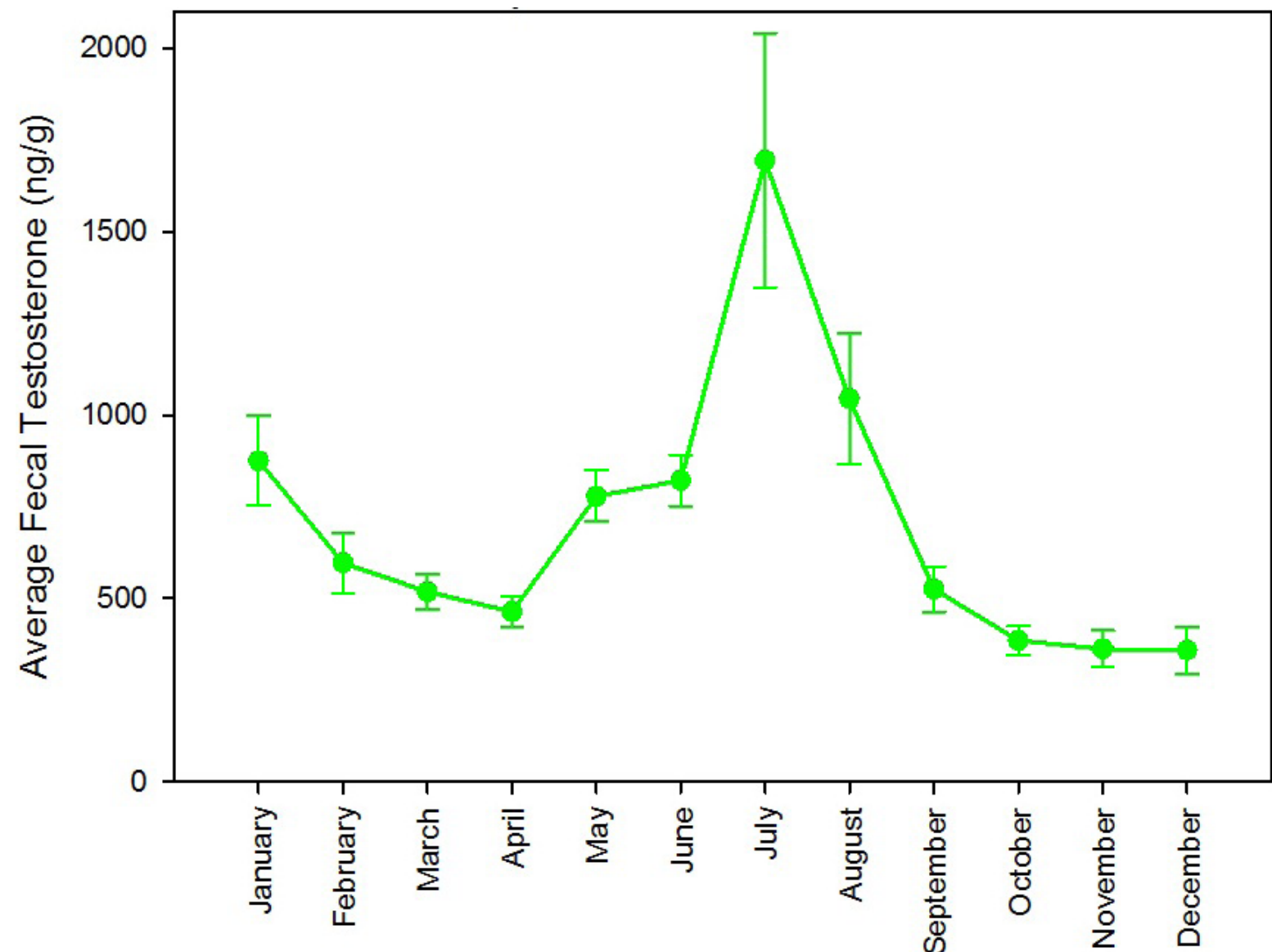


Figure 9: Average (± S.E.) monthly male fecal testosterone levels for males (n=6) and females (n=1). Fecal testosterone concentrations were substantially higher in the late spring and summer compared to fall and winter months.

Conclusions

- Estrogen concentrations for females ranged from 1.9–3041.3 ng/g (average ± S.E. = 121.2 ± 7.6 ng/g) with baseline values ranging from 1.9–230.1 ng/g. Progestagen concentrations for females ranged from 1.2–1,007.1 ng/g (average ± S.E. = 51.2 ± 3.3 ng/g) with baseline values ranging from 1.2–249.6 ng/g. Concentrations of both female reproductive hormones consistently increased prior to egg-laying days with peak concentrations ranging from 230.2–3041.3 ng/g for estrogens and 249.7–1,007.1 ng/g for progestagens.
- The average duration between subsequent laying events was 42.0 ± 2.8 days. Clutch sizes ranged from 1 to 3 eggs (average ± S.E. = 1.6 ± 0.1). Average estrogen and progestagen remain within baseline range until approximately 17 days prior to egg-laying event (T=0). Both hormones reach peak levels between 2 to 5 days prior to egg-laying and return to baseline between 0 to 2 days following egg-laying.
- Fecal androgen levels in males were substantially higher than females and ranged from 10.1–13,326.2 ng/g (average ± S.E. = 709.5 ± 39.2 ng/g). On average, male testosterone levels were consistently highest in July and lowest in December.
- This study is the first to document the reproductive endocrinology of the blue-throated piping guan and one of the few to employ non-invasive fecal hormone analyses to characterize avian reproduction.
- The methods used here may assist ex situ breeding programs for Trinidad piping guans, horned guans, and other endangered Cracidae species.

Acknowledgments

The authors would like to thank the Saint Louis Zoo’s Bird House keepers for sample collection and Scotti Holweger, Lily Malcolm, and Amber Stout for assistance with sample analysis.