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Testosterone and prolactin levels in incubating Mountain Plovers (Charadrius montanus)

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Abstract

The hormones prolactin (PRL) and testosterone (T) are linked to breeding behaviors such as territory defense, incubation, and brood-rearing in birds. The Mountain Plover (*Charadrius montanus*) is a shorebird with an uncommon parental care system in which males and females tend separate nests. We collected blood from incubating male (n = 38) and female (n = 33) Mountain Plovers in Montana to determine how circulating plasma PRL and T varied by sex, day of incubation, and across the nesting season. PRL levels were similar in males and females. There was no relationship between day of incubation or Julian day and circulating PRL for either sex. T concentrations were higher in males than in females and tended to decrease across the incubation season for both sexes.

Keywords

Incubation, Mountain Plover, prolactin, testosterone, uniparental care

Disciplines

Environmental Indicators and Impact Assessment | Environmental Monitoring | Natural Resources and Conservation | Natural Resources Management and Policy | Poultry or Avian Science | Sustainability | Water Resource Management

Comments

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Testosterone and Prolactin Levels in Incubating Mountain Plovers (*Charadrius montanus*)

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ABSTRACT.—The hormones prolactin (PRL) and testosterone (T) are linked to breeding behaviors such as territory defense, incubation, and brood-rearing in birds. The Mountain Plover (*Charadrius montanus*) is a shorebird with an uncommon parental care system in which males and females tend separate nests. We collected blood from incubating male (n = 38) and female (n = 33) Mountain Plovers in Montana to determine how circulating plasma PRL and T varied by sex, day of incubation, and across the nesting season. PRL levels were similar in males and females. There was no relationship between day of incubation or Julian day and circulating PRL for either sex. T concentrations were higher in males than in females and tended to decrease across the incubation season for both sexes. *Received 25 August 2015. Accepted 11 April 2016.*

Key words: incubation, Mountain Plover, prolactin, testosterone, uniparental care.

The Mountain Plover (*Charadrius montanus*) is an uncommon shorebird that nests in disturbed areas of the Great Plains and Great Basin (Knopf and Wunder 2006). Mountain Plovers have a rare parental care system (Graul 1973) in which each member of the pair tends its own nest without input from the other parent (Oring 1982, 1986). While both male and female Mountain Plovers tend individual nests unaided, and therefore have similar incubation and chick-rearing responsibilities, it is thought that the males arrive first at the breeding grounds in early to mid-April, establish loose territories, and compete for females (Graul 1973). Males tend the first clutch of eggs laid by the female, who then lays and tends a second clutch (Graul 1973). Earlier studies of the population ecology of Mountain Plovers in Montana have found differences in both nest (Dinsmore et al. 2002) and chick (Dinsmore and Knopf 2005) survival depending on which parent is caring for the young, with males tending to have greater nest survival and females greater chick survival.

Hormones such as prolactin (PRL; Angelier and Chastel 2009) and testosterone (T; Ketterson et al. 1992) are linked to many avian behaviors. T is usually positively correlated with territoriality, mate guarding, and nest-building and negatively correlated with parental behavior, whereas PRL is usually positively correlated with incubation behavior and care of young (reviewed in Vleck and Vleck 2011). In species where the female is the sole incubator, females have higher PRL levels than males (e.g., European Starling [*Sturnus vulgaris*], Dawson and Goldsmith 1982; Song Sparrow [*Melospiza melodia*], Wingfield et al. 1989). The opposite is also true in sex-role reversed species, like the Red-necked Phalarope (*Phalaropus lobatus*), where males are the sole incubators (Gratto-Trevor et al. 1990). In two species of shorebirds, PRL levels tend to peak during incubation and gradually decline throughout the breeding season (Semipalmated Sandpipers [*Calidris pusilla*] and Red-necked Phalaropes, Gratto-Trevor et al. 1990). In male Spotted Sandpipers (*Actitis macularius*), a species in which different individuals show either biparental or male uniparental care, males have greater increases in PRL levels during incubation than females (Oring et al. 1986), linked to increases in parental care behaviors, but artificial increases in T can negate these effects, leading to brood desertion (Oring et al. 1989). Male Wilson's Phalaropes (*Phalaropus tricolor*) have a marked drop in T at the onset of incubation, and levels remain low during the rest of the breeding period (Oring et al. 1988). In shorebirds where males must compete for females, the males have higher levels of T than females (Steiger et al. 2006), and the difference is greater

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in polygynous Pectoral Sandpipers (*Calidris melanotos*) than in monogamous Semipalmated Sandpipers (*Calidris pusilla*, Steiger et al. 2006).

The aim of this study was to quantify circulating PRL and T concentrations of incubating male and female Mountain Plovers. We predicted PRL levels would be similar for both sexes throughout incubation because of similar roles during that stage (Gratto-Trevor et al. 1990), but that T levels would be greater in males because of the greater role that males play in courtship and territory defense (Graul 1973).

METHODS

The Mountain Plover is a moderate-sized bird (90–110 g), sexually monomorphic, and drably-colored. Clutch size is usually three eggs (Knopf and Wunder 2006). The 29-day incubation period commences after the last egg is laid (Graul 1975). Nests in our study were in an ~3,000 km^2 area in north-central Montana, described in detail by Dinsmore et al. (2002).

We collected 77 plasma samples (38 males, 33 females, 6 unknown) from plovers during the 2006, 2007, and 2011 breeding seasons (6, 63, and 8 samples respectively). We collected blood samples from mid-May until early July by trapping the incubating adult at the nest, which was the only stage at which we were able to capture birds. Nest searching, monitoring, capture, handling, and banding techniques were similar across years and followed those described by Dinsmore et al. (2002). Trapping was done between 0800 and 2000 hrs Mountain Daylight Time [MDT]. Sex was determined molecularly from feather or blood samples (Avian Biotech International, Tallahassee, FL, USA) using techniques outlined in Dinsmore et al. (2002). We estimated the onset of incubation using egg floatation and back-dating (Westerskov 1950, Dinsmore et al. 2002). To examine seasonal changes in hormone concentrations, we set the earliest calendar day of nest initiation from the samples as Day 1; all other initiation dates were then scaled from this point (Day 1 = 23 May 2006). The last calendar day that we took blood samples was Day 47 (8 Jul 2007). Timing of the breeding seasons were consistent across years.

We drew blood samples from birds within 5 mins of capture to reduce the effects of handling stress (Gratto-Trevor et al. 1991). Samples from incubating plovers were taken by puncturing the ulnar vein using a 26-gauge needle and collecting <1 ml of blood (Fair et al. 2010). We collected blood opportunistically when the nest was first located. This approach yielded a well-distributed arrangement of samples across incubation; the mean was 12.3 days into incubation (range from day 1 to day 28 of incubation; SE = 0.83 days). Blood samples were kept on ice until that evening when the plasma was separated by centrifugation, and then frozen at –20°C. The 2006 and 2007 samples were stored at –80°C from August of the collection year until all were analyzed for PRL in December 2011 and May 2012 for T. Work was conducted under ISU's Institutional Animal Care and Use Committee protocol #5-06-6129-Q.

Circulating plasma PRL concentrations were determined with a commercial enzyme-linked immunosorbent assay (ELISA) kit (Model CH3956, TSZ ELISA, Framingham, MA, USA) that uses a 96-well microplate pre-coated with purified domestic chicken (*Gallus gallus domesticus*) PRL antibodies. Assays were run according to the kit directions, which included diluting plasma samples 1:5 using 10 μl of plasma. A standard curve based on chicken PRL ranged from 2.5–40 ng/ml. One plover plasma sample was diluted 1:5 and 1:2 and run with a trial standard curve to support the appropriateness of this kit for use with Mountain Plovers. The ratio of the calculated concentrations of these two dilutions from the standard curve was 1:2.59, similar to the expected 1:2.50. All other samples were analyzed singly in a single assay the following day.

Circulating plasma T concentrations were determined with a commercially available double antibody radioimmunoassay (RIA) kit (SKU 07189102, MP Biomedicals LLC, Santa Ana, CA, USA). Assays were run according to the kit protocol, although all volumes were reduced by three quarters to increase the number of tests. All samples were measured in duplicate in a single assay.

Hormone concentrations were ln-transformed to establish normality. We tested for differences in concentrations of PRL and T between incubating male and female Mountain Plovers, across the incubation period and among years using General Linear Models (LM) in R (R Core Team 2013). We constructed a single model for each hormone that

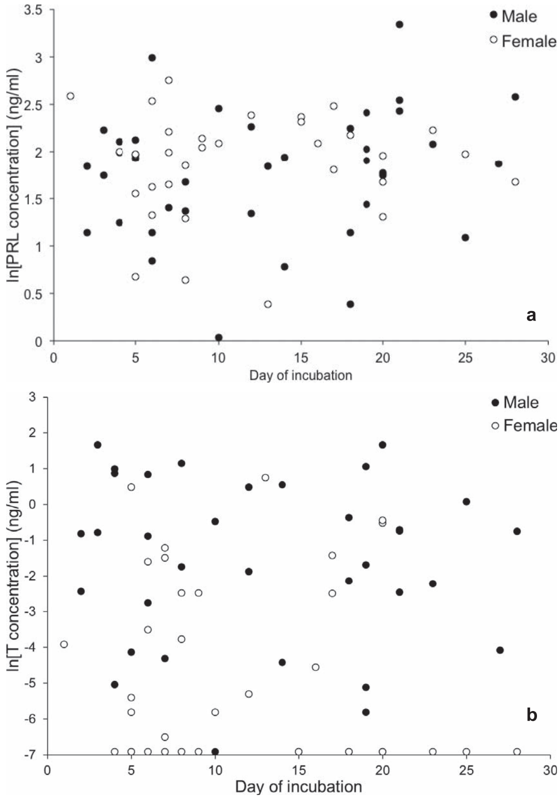

FIG. 1. Relationship between ln plasma prolactin (a) and testosterone (b) concentrations (ng/ml) and day of incubation. Plasma samples were collected from incubating male and female Mountain Plover (*Charadrius montanus*) in 2006, 2007, and 2011 in Phillips Co., MT, USA.

contained all explanatory variables of interest, without interactions, and excluding the birds of unknown sex when effects of sex were examined. Several samples ($n = 21$) produced concentrations of T that were below the lower limit of the standard curve (0.001 ng/ml) and so were adjusted to 0.001 for analyses. We used $\alpha = 0.05$ as the level of statistical significance for all statistical tests.

RESULTS

There were no differences in circulating PRL or T among the three years ($F_{2,71} = 0.13$, $P = 0.72$ and $F_{2,71} = 0.07$, $P = 0.80$ respectively). Plasma PRL levels were similar for incubating male and female Mountain Plovers ($F_{1,66} = 0.42$, $P = 0.52$). The back-transformed mean circulating plasma PRL concentration for incubating female Mountain Plovers was 6.49 ng/ml ($n = 33$) and ranged from 1.47–15.61 ng/ml. The back-transformed mean for males was 5.87 ng/ml ($n = 38$) and ranged from 1.04–28.17 ng/ml. Male Mountain Plovers had significantly higher concentrations of circulating T than females ($F_{1,66} = 13.77$, $P < 0.001$). The back-transformed mean concentration for males was 0.136 ng/ml (range from 0.001–5.326 ng/ml) and for females, 0.012 ng/ml (range 0.001–2.119 ng/ml).

The day of incubation did not have a significant effect on either PRL ($F_{1,66} = 1.10$, $P = 0.30$, Fig. 1a) or T ($F_{1,66} = 0.52$, $P = 0.47$, Fig. 1b) concentrations. While there was no relationship between Julian day of nesting season and PRL ($F_{1,66} = 0.51$, $P = 0.48$, Fig. 2a), there was evidence of a weak negative effect on T ($F_{1,66} = 3.83$, $P = 0.05$ Fig. 2b). Circulating PRL and T

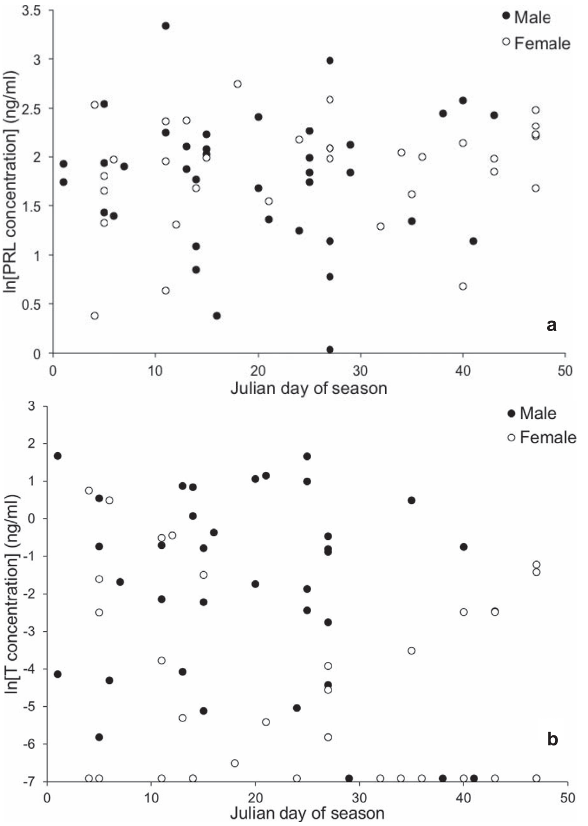

FIG. 2. Relationship between ln plasma prolactin (a) and testosterone (b) concentration (ng/ml) and Julian day of breeding season (Day 1 = 23 May and Day 47 = 8 July).

were not significantly correlated ($b = -0.668$, $P = 0.17$, $R^2 = 0.020$).

DISCUSSION

In this uncommon parental care system, male and female Mountain Plovers have equal, independent responsibilities during incubation, have similar incubation patterns (Skrade and Dinsmore 2012), and both develop brood patches (Knopf and Wunder 2006). So it is not surprising that there was no difference in the PRL levels of the two sexes. This is consistent with other studies in shorebirds in which both sexes incubate equally (Gratto-Trevor et al. 1990), although this is the first case in which PRL was measured in a species in which each member of the pair has its own nest. Plasma PRL levels in other shorebirds tend to increase at the start of incubation and gradually decline throughout the brood-rearing stage (Gratto-Trevor et al. 1990). Mountain Plover chicks are precocial, but the incubating parent remains with the brood until after the chicks are completely independent, performing anti-predator distraction displays and brooding the chicks (Graul 1975). It is likely that prolactin levels begin to decrease as chicks approach independence, although we were unable to sample birds except during incubation.

Early studies of Wilson's Phalaropes suggested that T concentrations were higher in females than in males in this sex-role reversed species (Höhn and Cheng 1967). However, more recent studies have found that the levels of circulating T are not reversed in sex-role reversed birds (Spotted Sandpipers, Fivizzani and Oring 1986; Wilson's Phalarope, Fivizzani et al. 1986; Red-necked Phalarope, Gratto-Trevor et al. 1990; African Black Coucals [*Centropus grillii*], Goymann and Wingfield 2004), and show patterns similar to socially monogamous bird species. In these species, males have higher levels of T than females in the pre-laying stage, although T levels can decrease significantly to similar levels depending on the degree of involvement of the male in incubation (reviewed in Vleck and Vleck 2011). Male Mountain Plovers in Montana establish territories and compete for females throughout incubation and defend their territories from other males to a greater extent than females do (PDBS and SJD, pers. obs.), which may be why males have persistently higher levels of T. However, we do not know the levels of circulating T at other stages of the nesting cycle, such as during the pre-laying, courtship phase, when T may be higher than it is during incubation.

The variance in PRL and T during incubation is intriguingly high among individual Mountain Plovers. However, many factors can affect circulating hormone levels including stress (Chastel et al. 2005, Kosztolányi et al. 2012), age (Angelier et al. 2006), and prior breeding experience (Angelier et al. 2006, Christensen and Vleck 2015); physical condition (Cherel et al. 1994, Criscuolo et al. 2006); and changes in clutch size (Delehanty et al. 1997). One possible explanation for the high variation within our samples may be the method of capture. Although we collected blood samples as quickly as possible (within 5 mins of capture), the very act of placing the trap on the nest may have stressed the birds, influencing their hormone levels. It will be challenging to determine the effects of these factors on hormone levels in this species or to obtain additional samples outside the incubation phase because of their behavior, declining numbers, and restricted breeding range. Such information, however, would be useful to better understand how hormones affect behavior in this unusual parental care system.

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Captive Hispaniolan Parrots (*Amazona ventralis*) can Discriminate Between Experimental Foods with Sodium Concentrations Found in Amazonian Mineral Licks

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ABSTRACT.—Wild Amazonian parrots likely consume soil to supplement sodium-deficient diets. However, it remains uncertain whether parrots can detect sodium at the concentrations found in mineral licks. During 3–8 day trials, we presented 20 captive Hispaniolan Amazon Parrots (*Amazona ventralis*) two foods with experimentally manipulated sodium concentrations. Parrots consumed more of the lower sodium food, confirming that they could distinguish between 891 ppm and 1,644 ppm available sodium—values well within the range of concentrations found in mineral licks in the wild. This study strongly suggests that wild parrots can use taste to detect salty soil

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