# Circulating breeding and pre-breeding prolactin and LH are not associated with clutch size in the zebra finch (*Taeniopygia guttata*)

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et al., 1994) and chick rearing (Angelier and Chastel, 2009; Miller et al., 2009; O'Dwyer et al., 2006). This mechanistic model was formulated based on several well-supported observations, namely that: (a) incubation behavior, tactile stimulation from the eggs, and plasma PRL levels reinforce each other in a positive feedback loop (El Halawani et al., 1984; Hall and Goldsmith, 1983); (b) rapid increases in PRL are temporally correlated with the onset of peak incubation behavior and the cessation of egg laying (Haftorn, 1981; Lea et al., 1981), and; (c) seasonal increases in the rate of incubation onset and plasma PRL are accompanied by seasonal declines in clutch size (Dawson and Goldsmith, 1985; Flint et al., 2006; Haftorn, 1981; Meijer et al., 1990; Müller et al., 2004). Potential anti-gonadal effects of PRL via inhibition of gonadotropin releasing hormone (GnRH) and luteinizing hormone (LH) have also been demonstrated in *in vitro* assays (El Halawani et al., 1984; Rozenboim et al., 1993; You et al., 1995), and are supported by evidence for anti-gonadal effects of PRL *in vivo* in some species (Bailey, 1950; Meier, 1969; Reddy et al., 2007), but not others (Buntin et al., 1999; Meier and Dusseau, 1968; Small et al., 2007). Much of the data used to support the PRL-based mechanistic model for clutch size determination however, is based on broad temporal correlations rather than direct experimental evidence,

cages ('pre-breeding', $n = 78$), and following pairing (females paired 13–17 days later), in the first experiment, on the day the third egg was laid ('breeding'; $n = 39$). Egg day three was selected based on experimental work which links the physiological mechanism for clutch size determination in zebra finches with the timing of the third laid egg (Haywood, 1993a,b). Blood samples for the bromocriptine experiment ($n = 38$) were also taken on the day the third egg was laid. In addition, in a follow-up study (~8 months following the bromocriptine experiment), females were bred and blood sampled for PRL measurement (but not LH) either on the day the second ($n = 28$) or fourth eggs ($n = 27$) were laid (days 2 and 4). Blood sampling was always carried out between 11:30 and 13:30 to minimize daily fluctuations in hormone levels. Birds were generally sampled within 1.5–5 min from the time of capture, and PRL and LH were not associated with estimated handling times. Blood samples were centrifuged at 5000$g$ for 5 min, and

## 3. Results

### 3.1. Relationship between pre-breeding LH, PRL and measures of body condition

There were no significant relationships between pre-breeding mass or hematocrit, i.e. measures of body condition, and pre-breeding LH ($F_{1,66} = 0.288$, $P = 0.594$ and $F_{1,66} = 0.128$, $P = 0.722$, respectively), or pre-breeding PRL ($F_{1,75} = 0.427$, $P = 0.516$; $F_{1,75} = 3.729$, $P = 0.057$, respectively; Table 1). However, pre-breeding PRL was weakly, but significantly and positively correlated with pre-breeding LH ($F_{1,65} = 4.272$, $r^2 = 0.05$; $P = 0.043$), including after removing values at the detection limits of the assay ($F_{1,55} = 3.46$, $r^2 = 0.091$; $P = 0.013$; Fig. 1).

### 3.2. Relationships between pre-breeding LH and PRL, and breeding hormone levels and reproductive traits

Compared to pre-breeding levels, LH was significantly higher during the 3-egg stage in breeding females (estimate for effect of breeding stage on LH ± S.E.: 0.098 ± 0.051 ng/mL, $df = 31$, $t = 2.38$, $P = 0.024$; Intercept: 0.265 ± 0.039, $df = 40$, $t = -9.77$, $P < 0.001$; Table 1). Furthermore, individual variation in LH was repeatable between pre-breeding and breeding stages ($R = 0.51$; 95% CI = 0.25, 0.77; $P < 0.002$). Pre-breeding LH was negatively correlated with laying interval after controlling for the time elapsed between pre-breeding blood sampling and subsequent pairing – females with higher pre-breeding LH had shorter intervals between pairing and laying of the first egg ($F_{2,31} = 15.52$, $P < 0.001$; Fig. 2). However, pre-breeding LH was not significantly correlated with either mean egg mass ($F_{2,30} = 1.66$, $P = 0.207$) or clutch size (Likelihood-ratio test: $\chi^2 = 0.011$, $df = 1$, $P = 0.915$) of the subsequent breeding attempt.

Breeding PRL levels at the 3-egg stage were markedly and significantly higher than pre-breeding levels (Estimate for effect of breeding stage on PRL ± S.E.: 180 ± 24 ng/mL, $df = 38$, $t = 19.17$, $P < 0.001$; Intercept: 23.07 ± 2.03, $df = 41$, $t = 37.21$, $P < 0.001$; Table 1). However, in contrast to LH, individual PRL levels were not repeatable between pre-breeding and breeding stages ($P > 0.90$). Log laying interval, egg mass, and clutch size were all independent of pre-breeding PRL levels ($P > 0.10$ in all cases).

### 3.3. Relationships between breeding LH, PRL and reproductive traits

Mean egg mass was significantly and positively correlated with body mass at pairing ($F_{1,39} = 5.72$, $P = 0.022$), but not laying interval ($F_{1,39} = 1.29$, $P = 0.264$). In contrast, clutch size was independent of mass at pairing (Likelihood-ratio test: $\chi^2 = 0.873$, $df = 1$, $P = 0.350$), but negatively correlated with laying interval (Likelihood-ratio test: $\chi^2 = 9.234$, $df = 1$, $P = 0.002$). Neither egg mass or clutch size was significantly correlated with individual variation in breeding plasma LH ($P > 0.15$ for both). Breeding plasma PRL on egg day 3 was significantly correlated with variation in mean egg mass ($F_{1,37} = 5.38$, $P = 0.026$) and clutch size ($\chi^2 = 9.17$, $df = 1$, $P = 0.002$; Fig. 3A), but these effects were inconsistent and skewed by several influential data points (i.e. high leverage points from clutch sizes outside the range normally observed in the wild [>7 eggs; Zann, 1996]). Within the normal range of clutch sizes, both mean egg mass and clutch size were independent of variation in breeding PRL at day 3 of egg-laying ($F_{1,34} = 0.004$, $P = 0.950$ and $\chi^2 = 0.227$, $df = 1$, $P = 0.634$, respectively; Fig. 3A). Similarly, in the follow-up study, variation in clutch size was independent of variation in breeding PRL on day 2 ($\chi^2 = 0.115$, $df = 1$, $P = 0.735$) and day 4 ($\chi^2 = 2.69$, $df = 1$, $P = 0.101$; data not shown) of egg-laying.

of breeding attempt on LH ± S.E.: −0.133 ± 0.029 ng/mL, $df = 31$, $t = -3.20$, $P = 0.003$; Intercept: 0.32 ± 0.045, $df = 34$, $t = 7.46$, $P < 0.001$; Table 1), but this effect was not different for the control group or either treatment (Breeding attempt *Treatment; Likelihood-ratio test:

($\chi^2 < 0.001$, $df = 1$, $P = 0.979$) or egg mass ($F_{1,30} = 0.345$, $P = 0.561$)

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