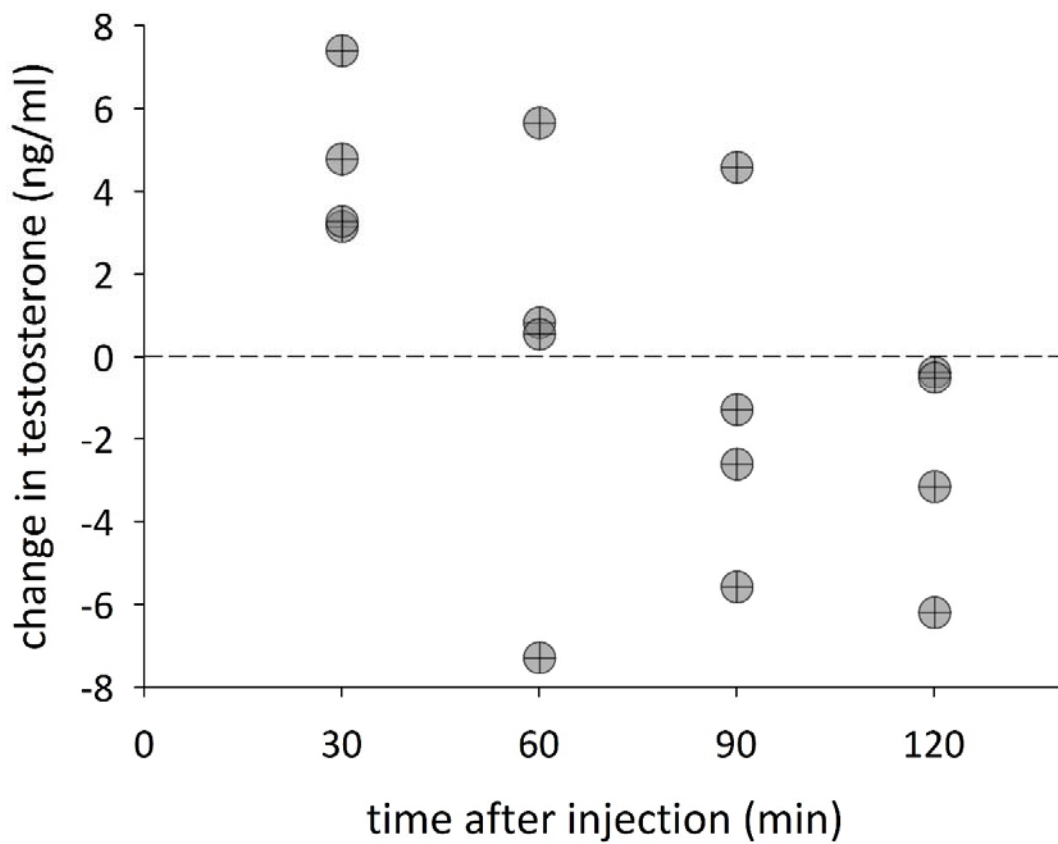


**Electronic supplementary material (Goymann and Flores Dávila: “Acute peaks of testosterone suppress paternal care: evidence from individual hormonal reaction norms”)**

***Duration of increased testosterone production after GnRH injections***

To validate the experimental protocol for this study, we first explored for how long a single injection of GnRH elicits a rise in circulating testosterone above baseline levels. So far, it was known that injections of GnRH increase testosterone of black redstarts for at least a period of 30 minutes [1-4]. Between April 15 and May 5, 2015 we captured 16 male black redstarts using mealworm-baited spring traps, and immediately after capture took a blood sample (ca. 100  $\mu$ l) to measure baseline testosterone concentrations. Then, each individual was injected with 1.25 $\mu$ g chicken GnRH-I (Bachem H 3106) in 50 $\mu$ l saline into the *pectoralis major* muscle (following the procedures described by [1, 2]). This dose of GnRH has led to the maximal release of testosterone in dark-eyed juncos [5], which is at least 25 % larger than the black redstart. After the injection birds were held in a cloth cage (h x w x l: 22 x 20 x 35 cm) with two perches and one bowl of water and one with mealworms to minimize stress. Then, each individual was bled (ca. 70 $\mu$ l) another time either 30 min (N = 4), 60 min (N = 4), 90 min (N = 4) or 120 min (N = 4) after the injection of GnRH, respectively. Plasma samples were analyzed for testosterone following the procedures described by Apfelbeck and Goymann [2]. The detection limit of the assay was 2.7pg/ml and the intra-assay of variation was 9.0%. The results confirmed earlier data that birds maintained elevated levels of testosterone until 30 min after the injection (Fig. S1). Three out of four birds still had elevated levels of testosterone 60 min after injection, one out of four birds had elevated levels of testosterone 90 min after the injection, and all birds showed decreased levels of testosterone compared to baseline 120 min after the injection (Fig. S1), indicating negative feedback of testosterone on the hypothalamic-pituitary-gonadal axis (leading to a decrease in circulating testosterone concentrations) within 60 to 90 min after capture. These results are similar to those of a previous study on dark-eyed juncos in which testosterone was still elevated after 60 min, but had returned to baseline 120 min after the GnRH injection [5]. Holding the birds in cloth cages for a period of 30 to 120 min before taking the second blood sample may have led to a stress-induced decline in testosterone, counteracting the effect of GnRH. Hence, the

validation protocol may underestimate the effect of GnRH may have on testosterone in birds that are immediately released after the injection of GnRH. Thus, the validation experiment represents a conservative estimate with respect to expected effects of GnRH inducing an increase in circulating testosterone.



*Fig. S1) Change in circulating testosterone concentrations depending on the time after the injection of 1.25µg GnRH, indicating that most of the birds show negative feedback regulation between 60 and 90 min after injection. Negative feedback of testosterone on the HPG-axis occurred in all birds after 120 min (N = 4 per time point).*

## References

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