

The Effect of Photoperiod on Adrenocortical Stress Response in Mountain Chickadees (*Poecile gambeli*)

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Birds respond to environmental changes by modulating their levels of plasma corticosterone, a stress hormone produced by the adrenal glands. Baseline levels of corticosterone and the magnitude of adrenocortical response to acute stress are known to vary seasonally. Photoperiod is one of several potential factors which could affect the seasonal modulation of corticosterone secretion. In this study, we examined the effects of photoperiod on baseline and acute-stress-induced levels of corticosterone in adult mountain chickadees (*Poecile gambeli*). We exposed 20 mountain chickadees to short days (8L:16D h) for 60 days, after which half of the chickadees were switched to long days (14L:10D h). We collected blood samples from both groups 33 days after the switching. Our results showed that photoperiod had no significant effect either on baseline levels of corticosterone or on the maximum levels reached during the adrenocortical response to acute stress. However, photoperiod had a significant effect on the way that chickadees responded to acute stress: birds maintained on long days reached significantly higher levels of corticosterone between 5 and 20 min after capture than birds maintained on short days, which reached their maximum levels of corticosterone between 20 and 50 min. Females reached significantly higher levels of corticosterone than males in response to acute stress. Our results suggest that factors other than photoperiod are responsible for the observed seasonal changes in baseline levels of corticosterone, whereas pho-

toperiod is directly involved in regulation of adrenocortical stress response. © 2002 Elsevier Science (USA)

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Birds respond to environmental changes by modulating their levels of plasma corticosterone, a stress hormone produced by the adrenal glands (Harvey *et al.*, 1984; Wingfield *et al.*, 1997, 1998; Silverin, 1998). Secretion of corticosterone resulting from environmental stressors such as food deprivation and unpredictable weather patterns appears to be highly beneficial because increased levels of corticosterone mediate behaviors directed toward immediate survival, such as active feeding, gaining extra fat reserves, abandoning reproductive activity, and migrating to more favorable habitats (Wingfield *et al.*, 1998; Silverin, 1998). However, baseline levels of corticosterone and the magnitude of adrenocortical response to acute stress are also known to exhibit seasonal variation (e.g., Wingfield, 1994; Wingfield *et al.*, 1998; Silverin, 1998).

In temperate zones, many wintering resident birds have to survive conditions of low ambient temperatures, short days, and limited and unpredictable food supplies. During this time, birds have been shown to increase their fat reserves, thus reducing the probability of death from starvation (Witter and Cuthill, 1993;

Pravosudov and Grubb, 1997). It is likely that winter behavioral strategies of birds are mediated by corticosterone (Astheimer *et al.*, 1992; Pravosudov *et al.*, 2001). For example, Silverin (1998) reported that willow tits (*Poecile (Parus) montanus*) have the highest baseline levels of corticosterone during the winter. There are several factors during the winter that might trigger elevated levels of baseline corticosterone: unpredictable food, short days, and low ambient temperatures. We have previously demonstrated that unpredictable and limited food supply results in significantly elevated baseline levels of corticosterone in mountain chickadees (Pravosudov *et al.*, 2001). The question remains as to whether food supply is the only factor stimulating increased secretion of corticosterone or whether other factors such as day length and/or ambient temperature could also cause baseline plasma corticosterone to increase.

It has been well established that sudden and unpredictable changes in the environment such as snow storms and a sudden drop in ambient temperature cause a significant increase in corticosterone levels, which far exceed the baseline levels (Wingfield *et al.*, 1997, 1998; Silverin, 1998). In many bird species, it has been demonstrated that high levels of corticosterone result in birds abandoning all their normal activities and initiating behaviors directed toward immediate survival (Wingfield *et al.*, 1998). Such a response may not always be beneficial, however, and in some circumstances, it would be more adaptive not to abandon all activities. For example, birds breeding in temperate zones, especially in higher latitudes, have limited time to complete their breeding cycle. In these birds, when resources are limited it may be advantageous to continue reproductive activities despite unpredictable environmental stress, and it has been hypothesized that adrenocortical stress response in such species could be suppressed during reproduction (Wingfield *et al.*, 1992, 1994, 1998). When birds have sufficient time to breed, it may be beneficial for them to abandon breeding activities during unpredictable stressful changes in the environment and thus a strong adrenocortical response to stress would be selected for (Wingfield *et al.*, 1992, 1994, 1998). Indeed, some species exhibit their strongest adrenocortical stress response during their reproductive season (Wingfield, 1994). Even within the same species, birds breeding in higher latitudes have been shown to have much

weaker adrenocortical stress responses than their conspecifics breeding in lower latitudes (e.g., Silverin *et al.*, 1997; Silverin and Wingfield, 1998).

Seasonal changes are usually associated with changes in several ecological variables such as photoperiod, ambient temperature, food supply, etc. All these factors can potentially contribute to changes in birds' baseline levels of plasma corticosterone and in their adrenocortical response to acute stress. To understand how birds regulate their levels of corticosterone, we need to understand which ecological factors are important. In this study, we test the effect of one of these factors, photoperiod, on the baseline levels of corticosterone and on adrenocortical stress response to acute stress in mountain chickadees (*Poecile gambeli*). We tested two predictions: (1) birds maintained on short, winter-like days have higher baseline levels of corticosterone than birds maintained on long, summer-like days and (2) photoperiod affects the magnitude of the adrenocortical response to acute stress in birds. We used mountain chickadees because these birds live at high elevations characterized by strong seasonal changes in environmental conditions.

METHODS

Twenty mountain chickadees were collected from different nestboxes in northern California and hand-raised in captivity during spring of 1998. Upon maturity, all birds were maintained individually in wire-mesh cages (60 × 42 × 60 cm) at a constant temperature (20°) and provided food (a mixture of shelled sunflower seeds, crushed peanuts, and mealworms) and water *ad libitum*. Before the experiment, all birds were maintained on long 14-h days (14L:10D h). We randomly assigned the birds into two groups, with 10 birds in each group. On February 16, 2000, all birds were switched to an 8L:16D h cycle corresponding to the shortest day length in the area. After being on a short day cycle for 60 days, one group of birds were switched to a long day cycle (14L:10D h) on April 15, 2000. After that, both groups of birds were maintained on their respective cycles (short and long days) for 33 days and we collected their blood samples on May 17 and 18, 2000. Both groups were maintained in the same room separated by a small chamber which

allowed for different light schedules for the groups while all other physical parameters were identical.

We collected four blood samples from a brachial vein of each bird, one bird at a time. Each bird was captured inside its cage and removed into a different room. The first sample was collected within 3 min of entering the cage, the second sample at 5 min, the third sample at 20 min, and the fourth sample was collected at 50 min after entering the cage. This is the standard procedure for testing adrenocortical stress response in a small bird with a body mass of less than 15 g (e.g., Wingfield *et al.*, 1995). The total amount of blood collected during the stress series did not exceed 1.5% of bird body mass, which should not cause an additional physiological stress to birds. All chickadees were held in cloth bags between sample collections. We used samples collected within 3 min of entering the cage to determine a baseline level of corticosterone because it has been shown that corticosterone levels usually do not start to elevate until 3 min after capture (Wingfield *et al.*, 1982; Kitaysky *et al.*, 1999). In this study the corticosterone levels did not increase significantly in response to handling within a 0- to 3-min interval after capture (regression analysis of corticosterone concentration on time since entering the cage, $F_{1,15} = 0.78$, $P = 0.38$). Blood was collected from 0900 to 1600 h and time of day had no significant effect on baseline levels of corticosterone (Regression analysis, $F_{1,15} = 0.13$, $P = 0.72$). Birds from both groups were bled at the same time of day and sampling was evenly distributed from 0900 to 1600 h to avoid time bias for either treatment group. There were no significant differences between the two groups in mean time of day when blood samples were taken (Mann–Witney U test, $z = 1.46$, $P = 0.16$). We collected blood in heparinized capillary tubes and then emptied them into 0.3-ml vials that were kept on ice. All samples were centrifuged within 2 h of blood collection; the collected plasma samples were frozen at -20° and then shipped in dry ice to University of Washington for radioimmunoassay analyses.

We measured concentrations of corticosterone after extraction of 5- to 20- μ l samples in dichloromethane. Recovery values of the extraction averaged 91.26% (range 80.4–100%). To avoid interassay variation we analyzed all samples during a single assay. Intraassay variance was 8% and sensitivity of the analysis was

7.8 pg/ml. For details of radioimmunoassay analyses see Wingfield and Farner (1975).

After collecting blood samples, all birds were sacrificed and their sex was determined. We weighed testes in males and oviducts in females to assess the effect of photoperiod, and we weighed the chickadees during blood collection.

We used an ANCOVA to compare baseline levels of corticosterone between the two treatment groups and to assess any potential sex differences. We included time of collecting blood as a covariate because it varied from 1 to 3 min. We used a repeated-measures ANOVA (PROC MIXED; SAS Institute, 1994) to compare the entire adrenocortical stress response including all four samples taken within 50 min of entering the cage. All assumptions of statistical tests were upheld and the significance level was set at 0.05.

RESULTS

Photoperiod had no significant effect on the body mass of chickadees (ANOVA, $F_{1,14} = 1.23$, $P = 0.29$) but males were significantly heavier than females in both treatment groups (males: 11.52 ± 0.58 g, $n = 9$; females: 10.84 ± 0.62 g, $n = 10$; ANOVA, $F_{1,14} = 9.22$, $P < 0.01$). Compared to birds maintained on short days, males that were housed on long days had more developed testes (testes mass: 2.8 ± 0.59 mg, $n = 5$ —short days; 46.67 ± 15.22 mg, $n = 4$ —long days; $P = 0.013$) and females had more developed oviducts (oviduct mass: 5.76 ± 0.63 mg, $n = 5$ —short days; 24.88 ± 1.45 mg, $n = 5$ —long days; $P < 0.001$).

Baseline levels of plasma corticosterone were not significantly different between the two treatment groups ($F_{1,12} = 4.19$, $P > 0.06$; Fig. 1). There also were no significant differences between males and females in the baseline levels of plasma corticosterone ($F_{1,12} = 0.62$, $P = 0.618$; Fig. 2).

There were no statistically significant differences between the two treatment groups in the magnitude of adrenocortical response to a standardized acute stress protocol and birds in both groups reached similar maximum levels of corticosterone (Treatment effect, Table 1, Fig. 1). Both groups significantly increased their levels of corticosterone during the stress protocol (Time effect, Table 1, Fig. 1). There was a significant

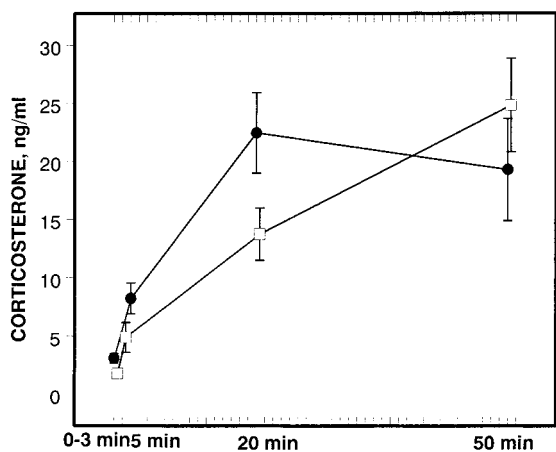


FIG. 1. Adrenocortical response to acute stress (mean \pm SE) in mountain chickadees maintained on short (open squares) and long (filled circles) days at 0–3, 5, 20, and 50 min after entering the cage.

time \times treatment interaction (Table 1), indicating that, although maximal levels of plasma corticosterone reached during the adrenocortical stress response were not significantly different between the treatments, there were differences in the way that the birds responded to stress (Fig. 1). Independent contrast analyses showed that corticosterone levels did not differ significantly between 3 and 5 min after entering the cage in both groups ($P > 0.08$) but birds in both groups increased their corticosterone levels significantly at 20 min after entering the cage ($P < 0.008$).

TABLE 1

Effect of Photoperiod (Treatment), Time Since Capture, and Sex on Adrenocortical Stress Response in Mountain Chickadees (Repeated-Measures ANCOVA)

Variable	F value	P value
Treatment	$F_{1,14} = 0.50$	0.489
Time	$F_{3,37} = 26.93$	< 0.001
Sex	$F_{1,14} = 5.49$	0.034
Treatment \times sex	$F_{1,14} = 1.36$	0.263
Treatment \times time	$F_{3,37} = 3.99$	0.015
Time \times sex	$F_{3,37} = 1.06$	0.377
Treatment \times time \times sex	$F_{3,37} = 1.73$	0.178

Between 20 and 50 min after entering the cage, birds maintained on long days did not change their corticosterone levels significantly ($P = 0.34$), whereas birds maintained on short days showed a significant increase ($P < 0.001$). Thus, birds maintained on short days reached the maximum concentration of plasma corticosterone between 20 and 50 min after capture (Fig. 1). Birds maintained on long days, on the other hand, reached their maximum levels of plasma corticosterone significantly faster, between 5 and 20 min after capture (Fig. 1). Birds maintained on long days also had significantly higher levels of corticosterone at 20 min after capture than birds maintained on short days (Independent contrast analysis, $P = 0.01$). There were also significant differences between the sexes in adrenocortical stress response, with females reaching significantly higher levels of plasma corticosterone compared to males (Table 1, Fig. 2). Independent contrast analyses showed that differences between males and females were significant for birds maintained on short days only ($P = 0.027$) and more specifically that females maintained on short days differed significantly from males only at 50 min after entering the cage ($P < 0.01$, Fig. 2). Differences between sexes at other times and in long-day treatment were not significant ($P > 0.06$). Body mass had no significant effect on adrenocortical stress response ($F_{1,13} = 0.22$, $P = 0.64$).

DISCUSSION

It is important to establish that the birds actually responded to the differences in photoperiod if one is

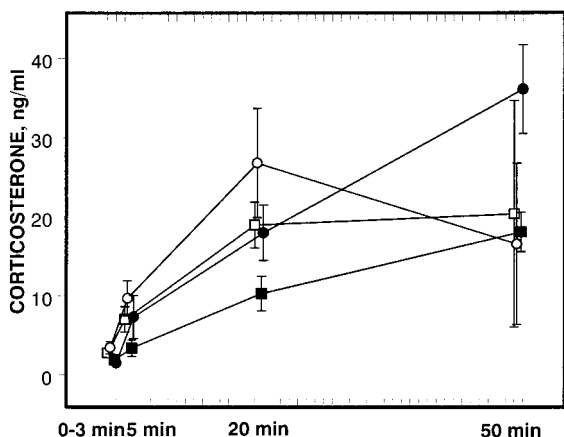


FIG. 2. Adrenocortical response to acute stress (mean \pm SE) in males (squares) and females (circles) mountain chickadees maintained on short (filled symbols) and long (open symbols) days at 0–3, 5, 20, and 50 min after entering the cage.

to claim that photoperiod has any or no effect on either baseline corticosterone levels or on the adrenocortical stress response. Our data on the increase of mass of testes and oviducts clearly indicated that birds did respond to an increase in day length.

While photoperiod resulted in physiological changes in the reproductive system of chickadees, it did not appear to trigger a predicted change in baseline levels of corticosterone. Seasonal changes in baseline levels of corticosterone in free-ranging willow tits, close relatives of the mountain chickadee, were reported by Silverin (1998): during short winter days, baseline concentrations of corticosterone appeared to exceed those observed during long summer days. Previously, Pravosudov *et al.* (2001) demonstrated that an increase in baseline levels of corticosterone in mountain chickadees is associated with unpredictable and limited food supply and, thus, such an increase might be independent of a change in day length. The result of the current study supports this prediction as we did not observe significantly higher baseline levels of corticosterone in birds maintained on short days compared to birds maintained on long days. We, therefore, suggest that an increase in baseline levels of corticosterone observed in parids during winter (Silverin, 1998) is likely to be independent of photoperiod and is associated with unpredictable and limited food (Pravosudov *et al.*, 2001) or a combined effect of limited food supply and low ambient temperature.

Our results suggest that photoperiod has a direct effect on adrenocortical stress response in the mountain chickadee in a way predicted for birds living in comparatively favorable conditions (Wingfield, 1994). Although maximum levels of corticosterone reached during the observed adrenocortical stress response did not differ significantly between the two groups, chickadees maintained on long days achieved their maximum levels of plasma corticosterone significantly faster than birds maintained on short days. Thus, reproductively active mountain chickadees might be more sensitive to environmental changes than wintering birds. Short-term environmental changes, such as an occasional snow storm or decrease in ambient temperature, although not very common, might occur during spring at high elevations at low latitudes. An increased sensitivity of reproductively active chickadees to a short-term deterioration of environmental conditions may allow them to redirect their activities away from reproduction and toward immediate sur-

vival. Such a response would be beneficial for mountain chickadees breeding in lower latitudes where they have sufficient time to successfully complete their breeding cycle once environmental conditions returned to normal (Wingfield, 1994).

Our data seem to contradict the field data on a closely related species, the willow tit, in which Silverin (1997) showed that adult birds have much weaker adrenocortical response to acute stress during the summer compared to the response of birds during winter. However, Silverin (1997) sampled willow tits during July when the majority of birds must have finished their breeding and started the postbreeding molt. In many bird species it has been shown that lowest adrenocortical stress response occurs in summer during molt (Romero *et al.*, 1998a,b; Romero and Ramage-Healy, 2000). Thus, whereas our study suggests that photoperiod facilitates sensitivity of chickadees to environmental stressors during the onset of breeding, such a response may become weaker after the breeding and during the molt. It is also possible that adrenocortical stress response is not always controlled by photoperiod. For example, in nonmolting starlings (*Sturnus vulgaris*), a switch to long days did not change their adrenocortical response to acute stress (Romero and Ramage-Healey, 2000). More studies are needed to understand factors controlling adrenocortical stress response in birds.

Supporting our previous findings in wild-caught mountain chickadees (Pravosudov *et al.*, 2001), we found that females maintained on short days reached significantly higher levels of corticosterone during their adrenocortical stress response than males. It is not clear why there are differences between males and females. As females are smaller than males and always socially subordinate to males in parids (Pravosudov *et al.*, 1999), they are more vulnerable to variability in the environment. As a result, females usually carry higher levels of expensive body fat than males during the winter (Pravosudov *et al.*, 1999). Perhaps females have stronger adrenocortical responses to stress so that they can respond faster and more strongly than males to increase their probability of survival during the nonbreeding season. Clearly we need more studies to understand why sexes might differ in their adrenocortical response to stress. In other passerines, for example, it has been shown that males reach higher levels of corticosterone than females during their adrenocorti-

cal response to stress during breeding (Silverin *et al.*, 1997; Silverin and Wingfield, 1998).

In our study, we used hand-raised birds, which might have altered their physiological responses due to a long period of captivity with highly predictable conditions. However, these birds had a typical physiological gonadal response to a change in photoperiod. Also, baseline levels of corticosterone and adrenocortical response to acute stress of handling and restraint in wild-caught mountain chickadees maintained on short days (Pravosudov *et al.*, 2001) are similar to those observed in the short-day treatment group in this study. Thus, hand-raising had no significant effect on adrenocortical function in mountain chickadees.

In summary, our results suggest that photoperiod is not responsible for increased baseline levels of corticosterone observed in some birds during the winter but it does affect adrenocortical response to acute stress in mountain chickadees in a manner predicted for species breeding in lower latitudes. Together with our previous study (Pravosudov *et al.*, 2001), these results show that, of the two possible factors which could potentially regulate seasonal changes in avian adrenocortical function, unpredictable food, but not photoperiod, has an effect on baseline concentration of corticosterone. On the other hand, photoperiod, but not food supply, affects adrenocortical stress response to acute stress. More experiments are needed to fully understand the seasonal dynamic in adrenocortical activity in animals. In particular, it is possible that a third factor—ambient temperature—which also varies seasonally, could affect both baseline concentration of corticosterone and adrenocortical stress response. It would also be interesting to investigate the combined effect of all three seasonal factors: food supply, photoperiod, and ambient temperature, which could jointly affect adrenocortical function in birds.

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REFERENCES

- Astheimer, L. B., Buttemer, W. A., and Wingfield, J. C. (1992). Interactions of corticosterone with feeding, activity and metabolism in passerine birds. *Ornis Scand.* **23**, 355–365.
- Harvey, S., Phillips, P. G., Rees, A., and Hall, T. R. (1984). Stress and adrenal function. *J. Exp. Zool.* **232**, 633–645.
- Kitaysky, A. S., Wingfield, J. C., and Piatt, J. F. (1999). Dynamics of food availability, body condition and physiological stress response in breeding black-legged kittiwakes. *Func. Ecol.* **13**, 577–584.
- Pravosudov, V. V., and Grubb, T. C., Jr. (1997). Energy management in passerine birds during the non-breeding season: A review. *Curr. Ornithol.* **14**, 189–234.
- Pravosudov, V. V., Grubb, T. C., Jr., Doherty, P. F., Bronson, C. L., Pravosudova, E. V., and Dolby, A. S. (1999). Social dominance and energy reserves in wintering woodland birds. *Condor* **101**, 880–884.
- Pravosudov, V. V., Kitaysky, A. S., Wingfield, J. C., and Clayton, N. S. (2001). Long-term unpredictable foraging conditions and physiological stress response in mountain chickadees (*Poecile gambeli*). *Gen. Comp. Endocrinol.* **123**, 324–331.
- Romero, L. M., Soma, K. K., and Wingfield, J. C. (1998a). Changes in pituitary and adrenal sensitivities allow the snow bunting (*Plectrophenax nivalis*), an arctic-breeding song bird, to modulate corticosterone release seasonally. *J. Comp. Physiol. B.* **168**, 353–358.
- Romero, L. M., Soma, K. K., and Wingfield, J. C. (1998b). Hypothalamic–pituitary–adrenal axis changes allow seasonal modulation of corticosterone in a bird. *Am. J. Physiol.* **274**, R1338–R1344.
- Romero, L. M., and Remage-Healey, L. (2000). Daily and seasonal variation in response to stress in captive starlings (*Sturnus vulgaris*): Corticosterone. *Gen. Comp. Endocrinol.* **119**, 52–59.
- SAS Institute 1994. SAS/STAT Software. Release 6.09 and Release 6.08 maintenance enhancements for PROC MIXED. SAS Institute, Inc., Cary, NC.
- Silverin, B. (1997). The stress response and autumn dispersal behaviour in willow tits. *Anim. Behav.* **53**, 451–459.
- Silverin, B. (1998). Stress responses in birds. *Poultry Avian Biol. Rev.* **9**, 153–168.
- Silverin, B., Arvidsson, B., and Wingfield, J. C. (1997). The adrenocortical responses to stress in breeding willow warblers *Phylloscopus trochilus* in Sweden: Effects of latitude and gender. *Func. Ecol.* **11**, 376–384.

- Silverin, B., and Wingfield, J. C. (1998). Adrenocortical responses to stress in breeding pied flycatchers *Ficedula hypoleuca*: Relation to latitude, sex and mating status. *J. Avian Biol.* **29**, 228–234.
- Wingfield, J. C. (1994). Modulation of the adrenocortical response to stress in birds. In “Perspectives in Comparative Endocrinology” (K. G. Davey, R. E. Peter, and S. S. Tobe, (Eds.), pp. 520–528. Natl. Res. Council Canada, Ottawa.
- Wingfield, J. C., and Farner, D. S. (1975). The determination of five steroids in avian plasma by radioimmunoassay and competitive protein bindings. *Steroids* **26**, 311–327.
- Wingfield, J. C., O’Reilly, K. M., and Astheimer, L. B. (1995). Modulation of the adrenocortical responses to acute stress in arctic birds: A possible ecological basis. *Am. Zool.* **35**, 285–294.
- Wingfield, J. C., Smith, J. P., and Farner, D. C. (1982). Endocrine responses of white-crowned sparrows to environmental stress. *Condor* **84**, 399–409.
- Wingfield, J. C., Vleck, C. M., and Moore, M. C. (1992). Seasonal changes of the adrenocortical response to stress in birds of Sonoran Desert. *J. Exp. Zool.* **264**, 419–428.
- Wingfield, J. C., Deviche, P., Sharbaugh, S., Astheimer, L. B., Holberton, R., Suydam, R., and Hunt, K. (1994). Seasonal changes of the adrenocortical responses to stress in redpolls, *Acanthis flammea*, in Alaska. *J. Exp. Zool.* **270**, 372–380.
- Wingfield, J. C., Breuner, C., and Jacobs, J. (1997). Corticosterone and behavioral responses to unpredictable events. In “Perspectives in Avian Endocrinology” (S. Harvey and R. J. Etches, Eds.), pp. 267–278. J. Endocrinol. Ltd., Bristol.
- Wingfield, J. C., Maney, D. L., Breuner, C. W., Jacobs, J. D., Lynn, S., Ramenofsky, M., and Richardson, R. D. (1998). Ecological bases of hormone–behavior interactions: The “emergency life history stage.” *Am. Zool.* **38**, 191–206.
- Witter, M. S., and Cuthill, I. C. (1993). The ecological costs of avian fat storage. *Phil. Trans. R. Soc. London B* **340**, 73–90.