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Experimental mate-removal increases the stress response of female house sparrows: The effects of offspring value?

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Abstract

Vertebrates secrete elevated levels of glucocorticoids in response to various stressors, which mobilize energetic reserves but concurrently interfere with reproduction. In accordance with life-history theory, recent evidence suggests that the corticosterone response to stress is modulated according to the value of the brood. Since brood value is positively related to parental care, the stress response modulation may be either the consequence of offspring value (e.g. large broods have high fitness potential — the brood value hypothesis) or the consequence of parental workload (e.g. large broods are energetically demanding for the parents — the workload hypothesis). In this experiment, we aimed at experimentally separating the effects of brood value and workload and to confront the latter two hypotheses. To do so, we captured the male parents from breeding pairs of house sparrows (*Passer domesticus*) and took them in captivity for 48 h. During the absence of males, mate-removed females made more food deliveries than controls (increased workload) but were unable to fully compensate the lack of their mate, thus their chicks were in worse condition (reduced brood value) than control chicks. After the experimental period, mate-removed females responded more strongly to the standardized stressor than controls. In both groups, the corticosterone response to stress was negatively related to the nestlings' mass gain. These results provide experimental support for the brood value hypothesis, i.e. that individuals may actively modulate their stress response (either down- or upwards) with respect to the value of their current reproduction.
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Introduction

A key paradigm in life-history evolution is the trade-off between current and future reproduction (Williams, 1966). Parents have to balance their investment between current reproduction and survival with respect to the current offspring value and future breeding opportunities (Stearns, 1992).

Hormones have been proposed to mediate such life-history trade-offs (Sinervo and Svensson, 1998; Ketterson and Nolan, 1999). For instance, corticosterone, the hormone secreted by birds and rodents in response to various survival risks (e.g. food limitation, predator attack or inclement weather, commonly referred to as stress) promote behaviors that enhance immediate survival, but concurrently interfere with reproduction (reviewed by Wingfield and Sapolsky, 2003). As stress-induced corticosterone levels alter the resource allocation between reproduction and survival, measuring the hormonal response to a standardized stress is an effective tool to study the proximate mechanisms by which the costs and benefits of this life-history decision are assessed and integrated within the animals.

For example, future breeding opportunities are central in determining the current reproductive effort (Maynard Smith, 1977; Székely et al., 2000). When the hormonal response to a standard capture-handling stress were compared between two populations of willow warblers (*Phylloscopus trochilus*), it turned out to be lower in the population where the breeding season is very short and therefore the probability for renesting is minimal (Silverin et al., 1997). It was suggested that in the latter population suppression of the stress response was favoured to ensure that the current reproduction is not inhibited by elevated corticosterone levels (Silverin et al., 1997). Numerous studies have found similar differences in the hormonal stress response between breeding

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individuals of different populations, sexes or reproductive stages, and these results were interpreted as modulation of the stress response, i.e. 'protecting' the current reproduction from the inhibiting effects of corticosterone (reviewed by Wingfield and Sapolsky, 2003). In a life-history context, this reaction has been considered as a reproductive investment in the current offspring when its value is high relative to survival and future reproduction (Jessop, 2001; Wingfield and Sapolsky, 2003).

Recently, we tested the latter hypothesis by manipulating brood value via the number of offspring, and we showed that house sparrows (*Passer domesticus*) tending enlarged broods responded less strongly to a standard stressor than those tending reduced broods (Lendvai et al., 2007). These results provide experimental support for the idea that individuals actively modulate their stress response with respect to the value of current reproduction (the 'brood value hypothesis').

However, there is a natural co-variation between the brood value and the parental workload: the energetic demand on parents tending more or larger offspring is higher because the young require more parental provisioning than fewer or smaller offspring. Paradoxically, mounting an intensive response to stress may be costly from an energetic perspective, because it may increase the risk of mortality from impaired body maintenance (McNamara and Buchanan, 2005). Thus individuals whose energetic resources were mainly devoted to parental duties are expected to show a modest response to stress (the 'workload hypothesis'). In line with this argument, several studies have found that acute stress response is down-regulated during energetically costly non-breeding periods, such as molting or migration (Holberton et al., 1996; Romero, 2002).

Therefore, parents having broods of high value may derive to benefits from suppressing their stress response: first, because the brood has high fitness potential and second, because it is energetically demanding. The aim of this study was to experimentally disrupt this natural covariation between the value of the brood and parental workload and test the predictions of the 'brood value' and the 'workload' hypotheses. To do so, we removed male parents from breeding house sparrow pairs for 2 days, after which we measured the females' response to a standard stress protocol. House sparrows have biparental care, thus the removal of the male parent decrease substantially the food delivered to the chicks. Previous studies have shown that female house sparrows tend to increase their own feeding activity in response to an experimental decrease of their mates' feeding contribution, but are apparently unable to fully compensate the lack of the male parent (Hegner and Wingfield, 1987; Mazuc et al., 2003; Schwagmeyer et al., 2002). Hence, the mate-removal was expected to produce a double effect: (i) a short-term increase in workload of temporary widowed females, and (ii) reduced brood value due to decreased growth and condition of the chicks. In this way, the mate removal was expected to cause opposite effects on brood value and workload. According to the brood value hypothesis, we predicted that mate-removed females would respond more strongly to a stressor than control ones, and that the stress response would be negatively related to brood value. Conversely, according to the workload hypothesis, we predicted that mate-removed females

would show lower stress response than controls and that stress response would be negatively related to the workload.

Methods

Study species and population

The study was carried out between March and July 2006, on a free-living population of house sparrows that breed in nest boxes in Chizé (46°08′50″N, 0°25′29″W), France (Chastel et al., 2003). A large proportion of the adults used in this study were first captured either in previous years or during the pre-breeding period using mist nets and marked with a unique metal ring and color combination. Nest boxes were monitored daily to determine laying dates, clutch sizes, hatching dates, and the number of hatchlings.

Experimental protocol

Male parents were captured in their nest on day 7 after hatching (day 0). At capture, males were measured and randomly assigned to either the removal or the control treatment. Males in the removed group were housed in individual cages ($30 \times 40 \times 50$ cm high) where food and water were provided *ad libitum*. Food consisted of a mixture of seeds and a protein rich cat food that were regularly provided on the study site for other captive animals and which was often consumed by house sparrows to feed their chicks and themselves. Cages were placed indoor at ambient temperature and natural lighting conditions and they were isolated from other sparrows. Males in the control group were released within an hour after the capture. After capturing the male, we ringed the chicks and measured their tarsus length (± 0.1 mm), wing length (± 1 mm) and body mass (± 0.1 g).

Female parents were captured in their nest on day 9, 48 h after capturing their mate. To measure the stress response of females, we used a standard capture-handling-restraint protocol (Wingfield, 1994). Immediately after capture, a small blood sample (50–100 µl) was collected from the brachial vein and the time required to collect the sample was recorded. Mean handling time was 2.57 ± 0.09 (S.E.M.) min, maximum 3.8 min.

After collection of the initial (baseline) blood samples, females were placed in cloth bags, while the chicks were measured and weighed again. Subsequent blood samples were collected from the females 30 min following the first blood sample, when corticosterone levels are known to be at their maximum for this species (Breuner and Orchinik, 2001; Lindström et al., 2005; Romero et al., 2006).

Blood samples were kept on ice and centrifuged (5000 rpm ($1396 \times g$), 6 min) as soon as possible. Plasma was separated and stored at -20 °C until further analyses. After the second blood was collected, we measured the females' tarsus length, wing length and body mass before releasing the birds. Males were released from captivity shortly after releasing their mates. All but four parents (one mate-removed female, one control female and one captive and one control male) were observed to return to their nest and to resume parental activities.

To measure workload, we carried out behavioral observations for 1–2 h total at each nest both before and after mate removal. We aimed at collecting two observations before mate-removal (day 6 and 7), and two observations after capturing the male (day 8 and 9). Observations lasted 1 h, and whenever it was possible, they were carried out early in the morning and mid-day, with alternating the two time periods on consecutive days. The daily scheduling of samples was based on the recommendations of Schwagmeyer and Mock (1997) to choose the hours that best predicted daily food delivery rates. During the observations we recorded several behavioral variables; here we focus on the number of food deliveries made by each parent (called thereafter feeding rate). Feeding rates were defined as the number of food deliveries per hour and per chick. Mean feeding rates were calculated for the periods before and after mate-removal.

Corticosterone assay

Total plasma corticosterone levels were determined by radioimmunassay at the Centre d'Études Biologiques de Chizé following the procedure detailed in Lormée et al. (2003).

Total plasma corticosterone was measured in samples (50 µl) after ethyl ether extraction by radioimmunoassays using a commercial antiserum, raised in rabbits against corticosterone-3-(Ocarboxy-methyl) oxime bovine serum albumin conjugate (Biogenesis, UK). Cross-reaction was 9% with 1-desoxycorticosterone and less than 0.1% with other plasma steroids. Duplicate aliquots (100 µl) of the extracts were incubated overnight at 4 °C with 8000 cpm of ^{3}H-Corticosterone (Amersham Pharmacia Biotech-France) and antiserum. The bound and free corticosterone were separated by adding dextran-coated charcoal. After centrifugation, the bound fraction was counted in a liquid scintillation counter. Minimal detectable corticosterone levels were 0.4 ng/ml (lowest measurement: 1.26 ng/ml) Hormone concentrations were run in one assay (coefficient of intra-assay variation was 6.7%, $n=6$ duplicates).

Data processing and statistical analysis

We had 14 mate-removed and 20 control broods. Brood sizes ranged from three to five chicks in this study. Feeding rates of the females and the growth of the nestlings was analyzed by general linear models. To analyze chick growth, we used wing length and body mass as dependent variables, measured at day 7 vs. day 9. We chose wing length instead of tarsus length to measure chick growth because at this nestling stage primary wing feathers still grow linearly and their growth is reduced in response to food restriction, whereas tarsus length is already close to the asymptotic length and its growth does not reflect variations in the food supplies (Lepczyk and Karasov, 2000). It is noteworthy that nestling period is very short (13–16 days) and compensatory growth does not occur in house sparrow nestlings which suggests that the actual growth rate of the chicks represents their physiological limits (Lepczyk and Karasov, 2000). To assess the body condition of the chicks and the adults, we used body mass as the dependent variable in a GLM model, controlling for wing length as a covariate (Gosler et al., 1998). In the analyses of the body size, growth and body condition of chicks, we used their mean per brood.

To determine whether the capture-restraint protocol elevated corticosterone levels, we used a repeated measures GLM, with the first vs. the second blood sampling as a within-subject factor. The effects of the treatment and other co-variates on the stress response were analyzed using GLM models with the rate of increase from baseline to maximum corticosterone levels per minute (ng/ml/min) as dependent variable (Silverin et al., 1997; Lindström et al., 2005). The latter measure of stress response was highly correlated with the maximum levels of corticosterone ($r=0.920$, $P<0.001$), and the statistical analyses of these variables gave almost identical results. As the date, the breeding status (first or second brood in the season) and the brood sizes were not under direct experimental control, but may affect brood value, we included these effects in our models of stress response regardless of their statistical significance. To account for seasonal variation in the corticosterone levels, we used the date of female capture as covariate in the models. For marginally non-significant results ($0.05<P>0.1$), we give the effect size and its 95% confidence intervals (CI), i.e. the parameter estimates ±1.96 S.E. (Colegrave and Ruxton, 2003). Data processing and statistical analyses were performed in the **R** computing environment (R Development Core Team, 2006).

Results

Pre-manipulation differences between experimental groups

Feeding effort by the female before mate-removal did not differ between treatment groups ($F_{1,31}=1.89$, $P=0.178$; Table 1). There was no difference in the brood sizes between the groups (Mann–Whitney $U=165.5$, $P=0.337$; Table 1). Neither body mass, nor tarsus and wing length of the chicks differed between the male-removed and control groups (body mass: $F_{1,31}=0.032$, $P=0.858$, wing: $F_{1,31}=0.083$, $P=0.775$, tarsus: $F_{1,31}=0.817$, $P=0.372$; Table 1).

Effects of manipulation on parental behavior and nestling condition

After the mate removal, female feeding effort was higher in the male-removed than in the control group ($F_{1,32}=6.912$, $P=0.013$, Fig. 1A). Despite the increased feeding rate of mate-removed females, their chicks tended to grow more slowly (measured by wing growth) and gained significantly less mass than control chicks (wing growth: $F_{1,32}=3.189$, $P=0.083$, effect size (CI): -1.25 (-2.63 to 0.12) mm; mass gain: $F_{1,32}=11.461$, $P=0.002$, Fig. 1B). As a consequence, at day 9, body mass and body condition (mass controlled for wing length) of chicks was significantly lower in male-removed than in control broods (body mass: $F_{1,32}=7.798$, $P=0.008$, condition: $F_{1,31}=5.542$, $P=0.025$; Table 1). However, chicks in the two groups were of similar size at day 9 (wing length: $F_{1,32}=1.763$, $P=0.193$, tarsus length: $F_{1,32}=0.035$, $P=0.852$; Table 1).

Corticosterone levels and body condition of females

Wing length was not different between mate-removed and control females ($F_{1,30}=0.428$, $P=0.517$). Body condition of females did not differ between the treatment groups ($F_{1,29}=0.848$, $P=0.364$). Corticosterone levels measured at the initial bleeding were not related to handling time ($F_{1,27}=3.113$, $P=0.090$, effect size (CI): 1.63 (-0.18 to 3.44) ng/ml; Table 1). In addition, we found no significant linear or curve–linear relationship between the time of day of capture and the baseline corticosterone ($F_{1,27}=1.128$, $P=0.297$). Baseline corticosterone levels was also unrelated to brood size, and did not differ between the treatment groups (brood size: $F_{1,30}=0.192$, $P=0.663$, treatment: $F_{1,30}=0.632$, $P=0.432$). Circulating plasma levels of corticosterone increased markedly following capture, handling and restraint ($F_{1,33}=322.28$, $P<0.001$; Table 1). The rate of corticosterone increase was higher in the morning and evening hours than midday ($F_{1,31}=7.894$, $P=0.008$). Therefore, in the following analyses of stress response, we included the time of day and its square in the models to control for this quadratic relationship.

Table 1
Comparisons of raw data of experimental groups

Response variable	Experimental groups	
	Mate-removed	Control
Variables before mate-removal		
Feeding rate of females (feedings/h/chick)	2.81±0.29	2.25±0.19
Brood size	3.93±0.16	4.2±0.19
Nestling body mass (g)	18.24±0.56	18.25±0.50
Nestling wing length (mm)	29.76±0.73	30.33±1.00
Nestling tarsus length (mm)	16.48±0.19	16.18±0.21
Variables after mate-removal		
Nestling body mass (g)	18.19±0.77	20.92±0.62
Nestling wing length (mm)	39.32±0.82	41.14±0.99
Nestling tarsus length (mm)	17.67±0.15	17.62±0.21
Hormone levels		
Baseline corticosterone levels (ng/ml)	4.84±0.78	5.32±0.48
Maximum corticosterone levels (ng/ml)	42.79±3.61	37.94±2.31

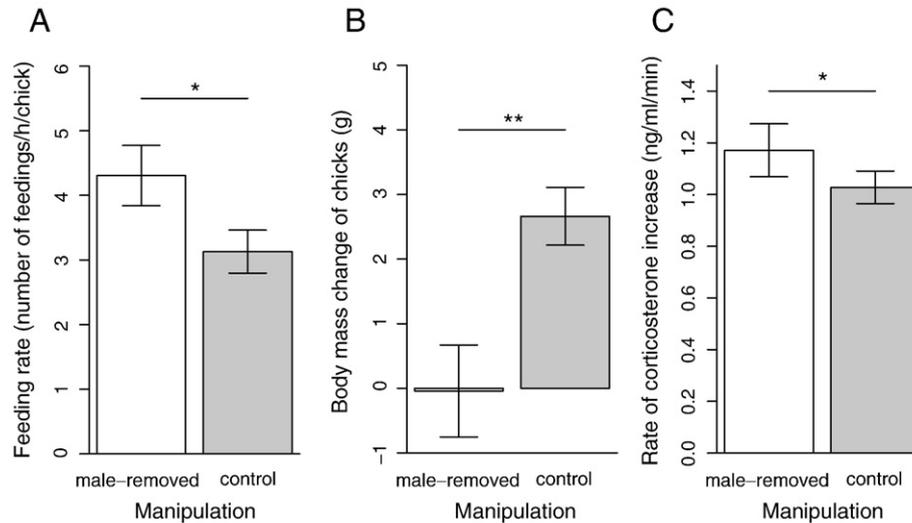Means±S.E. are presented for mate-removed ($N=14$) and control ($N=20$) females.

Fig. 1. Effects of the 2-day experimental male-removal on the (A) workload (feeding rate) of the female parents, (B) on the development of the chicks and (C) on the stress response of the female parents. (A) Females whose mate had been removed ($N=14$) had significantly higher feeding rate than control females ($N=20$). * $P<0.05$. (B) Body mass change of nestlings during male-removal. Chicks in male-removed broods ($N=14$) gained less mass than control chicks ($N=20$). ** $P<0.005$. (C) Stress response of female parents after the 2-day experimental male-removal. Stress response is expressed as the increase from baseline to maximum corticosterone levels per minute (ng/ml/min). Females whose mate had been removed ($N=14$) responded more strongly to the capture and handling stress than control females ($N=20$). * $P<0.05$.

Females in the mate-removed group showed higher stress response than control females ($F_{1,26}=4.302$, $P=0.048$, Fig. 1C). Capture date, brood size and the females' breeding status (whether they tended their first or the second brood in the season) did not explain significant variation in the magnitude of stress response (season: $F_{1,26}=0.186$, $P=0.669$, brood size: $F_{1,26}=0.100$, $P=0.754$, breeding status: $F_{1,26}=0.696$, $P=0.411$). Moreover, in both treatment groups the stress response was negatively related to the nestlings mass gain ($F_{1,26}=4.212$, $P=0.050$, Fig. 2).
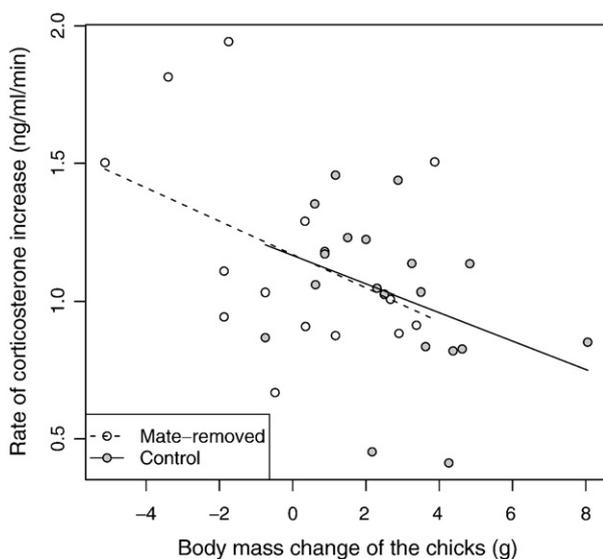

Fig. 2. Body mass change of nestlings (brood means) and the stress response of the female parent. Stress response is expressed as the increase from baseline to maximum corticosterone levels per minute (ng/ml/min). In both treatment groups, the stress response was negatively related to the chicks mass change ($N=14$ mate-removed females, $N=20$ control females). $F_{1,26}=4.212$, $P=0.050$.

Wing growth of the nestlings was not related to the female parent's stress response ($F_{1,26}=0.034$, $P=0.854$).

Body condition of females was not related to their stress response ($F_{1,26}=0.109$, $P=0.743$). Finally, neither the actual parental feeding rates (after the mate removal) nor the change in the feeding rates (after–before mate removal) were related to the magnitude of stress response (feeding rate: $F_{1,26}=0.110$, $P=0.741$; change in feeding rate: $F_{1,26}=0.096$, $P=0.758$).

Discussion

In this study we performed a temporary mate-removal to disrupt the natural co-variation between brood value and parental effort and to test which component better explains the modulation of the stress response.

Our manipulation was effective, first because the development of chicks in uniparental broods was weakened compared with control chicks. Nestlings in male-removed broods gained less mass during the 2-day male-removal than control chicks, moreover in several uniparental broods the chicks actually lost body mass. As a consequence, they were in worse body condition at 9 days of age than control chicks. Their growth was also inferior as their wings tended to grow more slowly (though this difference remained marginally non-significant). Nevertheless, it is plausible that during such a short period of mate-removal (48 h), it is the body mass change of the nestlings that best reflects the variations in their nutritional state; which in turn may be assessed by parents using begging intensity as a cue (Saino et al., 2003). Second, our manipulation also affected the females' parental workload, with mate-removed females making more food deliveries than controls. We found no difference in the body condition between the treatment groups, but this is what one would expect in house sparrows, because females have been

shown to deplete their energy reserves during incubation and the first days of the nestling period (Moreno, 1989; Chastel and Kersten, 2002). As parental feeding is an energetically costly behavior (Drent and Daan, 1980), the extra feeding necessitated by the manipulation must have substantially increased the energetic demand of mate-removed females.

We found that mate-removed females responded more strongly to a standard capture-handling stress than control females. We also found that in both treatment groups, the females' stress response was negatively related to their nestlings' mass gain. These results are most consistent with the 'brood value hypothesis', which proposes that individuals can modulate their stress response with respect to the value of the current reproduction (Wingfield et al., 1995, Lendvai et al., 2007). Our result also corroborates an earlier study which showed that in the pied flycatcher (Ficedula hypoleuca), single females (whose mates probably deserted the brood) had more robust stress response than females assisted by their mates (Silverin and Wingfield, 1998). Nevertheless, this is the first study to experimentally demonstrate that the modulation of stress response may involve not only suppression, but individuals experiencing a reduction in the value of their current reproduction may upregulate their hormonal response to stress.

One may argue however, that the disappearance of the males may represent a social stress for the females, for example because of the harassment of other males. Although we observed several floater males on the nestboxes of mate-removed females, their presence did not explain the stress response of the females (results not reported). Today, we have very limited information about how the disruption of pair bond affects the activity of the hypothalamic–pituitary–adrenal axis. Remage-Healey et al. (2003) found that experimental separation of breeding pairs resulted in an elevation of baseline corticosterone levels in captive zebra finches (Taeniopygia guttata). Recently, Angelier et al. (2007a) also found elevated baseline corticosterone after changing mate in the black-legged kittiwake (Rissa tridactyla). In our study, however, we found a different pattern, i.e. experimental groups differed not in their baseline, but in their stress-induced corticosterone levels. Baseline and stress-induced corticosterone levels mirror different life-history demands (Landys et al., 2006), and due to the conflict between reproduction and high corticosterone levels, it is the stress-induced level that may better reflect reproductive investment. Therefore our results suggest that the experimental differences in the stress response are not caused by the mate-removal per se. Moreover, the negative relationship between the stress response of the females and the body mass change of their chicks is also consistent with the brood value hypothesis, but does not support the idea of social stress.

Although the females in the mate-removed group actually increased their parental effort, they were essentially making the 'best of a bad job' (Dawkins, 1980). In the absence of the male parent, abandoning the nest would entail the certain loss of the whole brood, whereas even an increased parental effort does not suffice to avoid a reduction in the brood value. So, while these females apparently increased their parental effort, they may have 'devaluated' their offspring as shown by their increased

corticosterone response. In this study only one mate-removed and one control female abandoned her brood, but the stressful period in our study was short (hardly more than 30 min) and the acute stress response may reflect the general susceptibility to stress. Prolonged stress or experimental administration of corticosterone may induce nest abandonment (Silverin, 1986; Cherel et al., 1988, Cyr and Romero, 2007). Therefore, when a reproductive individual faces an increased risk of mortality, the stress-induced corticosterone levels may serve as an internal cue that incorporates the costs and benefits of the current reproduction and therefore may be used as a major mediator of behavioral decisions. Future studies should investigate the detailed behavioral consequences of endogenous corticosterone levels to understand the evolution of hormone-mediated reproductive decisions.

When mounting a stress response, an individual is also faced with a resource allocation dilemma. While a robust stress response may effectively prepare the organism to cope with the actual stressor, allocating too much resources to it may cause a delayed mortality due to build up of damages (e.g. diseases, oxidative stress, etc; McNamara and Buchanan, 2005). Therefore, this optimal resource allocation problem may affect stress responses. Although this resource allocation trade-off may be more important for chronic stress, the same rationale applies for acute stress response as well. For example, reduced corticosterone responses associated with poor energetic condition have been observed in the American redstart (Setophaga ruticilla) and in black-legged kittiwakes (R. tridactyla); and it has been proposed that by suppressing their hormonal response these birds may reduce the corticosterone-induced protein catabolism, and therefore may conserve their energy reserves (Marra and Holberton, 1998; Kitaysky et al., 1999). In our study, mate-removed females had to spend more energy on parental duties than expected, therefore according to the 'workload hypothesis' we predicted that they would reduce their stress response, but we found the opposite difference between the treatment groups. However, one may argue that (contrary to the workload hypothesis), unexpectedly increased energetic demand of mate-removed females may be perceived as stressful and therefore should elicit an increased stress response. In any way, females' feeding effort was not related to their stress response and we found no difference in baseline corticosterone levels between the experimental groups. The change in feeding rate from prior to after mate-removal was also unrelated to the stress response. This measure incorporates the males' contribution as well, because the females' change in feeding rates is the highest in those pairs where the males' pre-manipulation feeding activity was high.

We found a quadratic relationship between time of day and stress response. Whatever the adaptive significance of such daily pattern in the stress responses may be, it seems consistent with a previous study, where the corticosterone response to capture stress of captive house sparrows tended to be the lowest during midday (Rich and Romero, 2001). In any way, we statistically controlled for this relationship, therefore this relationship did not confound the effects of manipulations.

Admittedly, the effects of mate-removal on the stress response were rather weak. We think that this is because our

experimental design was a compromise between statistical power and ethical issues: we tried to minimize the risk of nest abandonment of either parent while detecting the effects of treatment. Our manipulation mimicked a realistic situation: from several populations, female house sparrows have been reported to raise their chicks on their own, because the males were predated or were polygynous and deserted the brood (Veiga, 1990; Summers-Smith, 1963; Lendvai and Chastel, personal observations). Had we removed the male parent for several days, probably we would have seen a more pronounced drop in the chicks' growth, additional chick mortality and probably a more striking difference in stress response between the treatment groups. Our best model explained nearly 40% of the total variance (adjusted $R^2 = 0.390$), so many other factors may also influence the corticosterone stress response.

Taken together, in this study female house sparrows were faced with a reproductive conflict: they had to increase parental effort to raise a brood of reduced value. In this situation, parents increased their corticosterone response to stress, which suggests that stress response may be modulated not only down- but also upwards according to the current value of reproduction. This result suggests that elevated corticosterone levels may play a major role in life-history decisions. Therefore the ability to modulate these hormone levels in any direction may be an important adaptation for birds breeding in changing environments. However, other physiological mechanisms may also take part in the physiological machinery involved in the mediation of life-history trade-offs. For instance, the plasma levels of corticosteroid-binding globulin (CBG) are thought to reduce the amount of effective corticosterone available to cells (the 'free hormone hypothesis'; Mendel, 1989). Therefore, corticosterone and CBG may interact to regulate behavior (Love et al., 2004). In the house sparrows, CBG levels did not change during the 30 min of acute stress response (Breuner et al., 2006), thus it is unlikely that CBG levels would have altered our conclusions. On the other hand, CBG levels may have influenced baseline corticosterone levels. Other hormones may be also involved in the stress response. Prolactin that directly affects parental behavior in birds may be especially relevant (Chastel et al., 2005; Angelier et al., 2007b). Integrating these effects in future studies could help us better understand how animals can cope with reproductive conflicts (Love et al., 2004).

Acknowledgments

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