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PHYSIOLOGICAL CONDITION AND BREEDING PERFORMANCE OF THE GREAT TIT

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Abstract. The energy expenditure associated with breeding may have detrimental effects on the breeder's physiology. Some studies have focused on the relationship between breeding performance and health status, but information on the link with biochemical variables reflecting susceptibility to oxidative stress is scarce. Over two years (2005 and 2006), using several morphological, biochemical and hematological variables, we measured the association between several measures of breeding (laying date, clutch size, mean egg weight, brood size, number of fledglings, and mean weight before fledging) and health in the Great Tit (*Parus major*). The effect of raising a second brood on the breeders' physiological condition was also studied. Females' body-condition index tended to be positively correlated with breeding performance, whereas in males the correlation was negative. Females laying later had lower hematocrits and higher glutathione peroxidase activity (GSH-Px), and those raising larger broods had also higher GSH-Px activity and tended to have lower plasma protein. The effect of raising a second brood was reflected mostly in body reserves but varied by sex. Our study suggests that trade-offs between breeding activity and physiological condition of the sexes differ and that hematocrit and GSH-Px are sensitive indicators of the physiological condition of breeding females.

Key words: breeding performance, *double breeding*, *glutathione peroxidase*, *hematocrit*, Parus major, *physiological condition, Great Tit*.

Condición Fisiológica y Desempeño Reproductivo en *Parus major*

Resumen. El gasto energético relacionado a la reproducción puede tener efectos negativos sobre la fisiología del que se reproduce. Algunos estudios se han enfocado en la relación entre el desempeño reproductivo y el estado de salud, pero es escasa la información sobre la conexión con las variables bioquímicas que reflejan la susceptibilidad al estrés oxidativo. Durante dos años (2005 y 2006) y usando varias variables morfológicas, bioquímicas y hematológicas, medimos la asociación entre varias medidas reproductivas (fecha de puesta, tamaño de la puesta, peso medio de los huevos, tamaño de la nidada, número de volantones y peso medio antes de dejar el nido) y el estado de salud en *Parus major.* También se estudió el efecto de criar una segunda nidada sobre la condición fisiológica del que cría. El índice de condición corporal de las hembras tendió a estar positivamente correlacionado con su desempeño reproductivo, mientras que en los machos la correlación fue negativa. Las hembras que pusieron sus huevos más tarde tuvieron menos hematocritos y más alta actividad de peroxidasa glutatión (GSH-Px), y aquellas que criaron nidadas mayores también tuvieron actividad más alta de GSH-Px y tendieron a tener menos proteínas plasmáticas. El efecto de criar una segunda nidada se reflejó principalmente en las reservas corporales, pero éste varió entre sexos. Nuestro estudio sugiere que la relación costo-beneficio entre la actividad reproductiva y la condición fisiológica difiere entre los sexos y que los hematocritos y la GSH-Px son indicadores sensibles de la condición fisiológica de las hembras reproductivas.

INTRODUCTION

Breeding is energetically costly (Drent and Daan 1980), and organisms face a energy trade-off between reproduction and maintenance of good health (Ots and Hõrak 1996, Hõrak et al. 1998b). Several studies have examined the association between measures of breeding performance, effort, or both and the physiological condition of breeders. In these studies physiological condition was measured in terms of body mass (Ryser 1989, Hillström 1995, Merilä and Wiggins 1997, Gebhardt-Henrich et al. 1998), immune function (Deerenberg et al. 1997, Nordling et al. 1998, Cichoñ et al. 2001, Hasselquist et al. 2001), including leukocyte counts (Gustafsson et al. 1994, Bachman 2003, Dubiec et al. 2005), hematocrit (Moreno et al. 2002), heterophil/lymphocyte ratio (Ots and Hõrak 1996), and plasma protein content (Masello and Quillfeldt 2004). A few

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studies, however, have suggested that the cost of reproduction might be evaluated in terms of susceptibility to oxidative stress (Alonso-Alvarez et al. 2004, 2006) because greater physical activity, due to a large production of ATP, can generate reactive oxygen species (ROS) that may damage macromolecules and lead to cell senescence (Monaghan et al. 2009). Because those studies were performed in captivity, however, little is known about how reproduction affects the oxidative state and antioxidant defenses in wild birds.

We explored the associations between several measures of breeding (laying date, mean egg weight, clutch and brood size, number of fledglings, and mean weight before fledging) and physiological condition in the Great Tit (*Parus major*), evaluated while the birds were raising broods. The physiological variables considered were (a) morphological—body-condition index, (b) biochemical—total plasma protein (protein), and glutathione peroxidase activity (GSH-Px) in red blood cells, and (c) hematological—hematocrit (HCT), hemoglobin index in red blood cells (Hb index), count of white blood cells (WBC), and the heterophil/lymphocyte ratio (H/L). This study will enable us to further evaluate to what extent physiological condition is influenced by breeding and to explore which physiological functions are traded off during breeding. We also assessed the effects of raising a second brood on the Great Tit's physiological condition.

Body-condition index and total plasma protein reflect nutritional reserves (Brown 1996). The major component of total plasma protein, albumin, is related to long-term protein intake (Payne and Payne 1987); however, in the event of dehydration or infection/inflammation, when plasma protein increases because of a reduction in plasma volume or an increase in globulins, respectively (Lewandowski et al. 1986, Campbell 1995), high levels of total plasma protein do not indicate good health. Glutathione peroxidase in red blood cells makes up part of the organism's antioxidant defense mechanism (Sies 1993) against ROS which, are generated not only by metabolic activity but also by the biotransformation of xenobiotics and processes such as inflammation (Kappus 1987, Ames et al. 1993, Parkinson 1996). This enzyme may increase in response to an increase in ROS (Halliwell and Gutteridge 1990, Ahmad et al. 2000) to prevent oxidative stress, which has been suggested as a mediator of the trade-off between breeding performance and survival in birds (Alonso-Alvarez et al. 2004, 2006, Wiersma et al. 2004).

Hematocrit is the relative volume of red blood cells in total blood volume. Both hematocrit and hemoglobin reflect the oxygen-carrying capacity of the blood and are related to amount of muscular activity (Saino et al. 1997) and metabolic rate (Kostelecka-Myrcha et al. 1993); High values are usually associated with high anabolic capacity and consequently health and good nutrition (Rattner et al. 1987, Averbeck 1992, Bańbura et al. 2007, but see Dawson and Bortolotti 1997) because both hematocrit and hemoglobin are costly to produce and parasites may decrease their levels, leading to anemia (Dein 1986, Campbell 1995). However, very high values of hematocrit may result from dehydration (Campbell 1995). The count of white blood cells (WBC) indicates the overall state of the organism's immune function and presence of infection/inflammation (Campbell 1995, Norris and Evans 2000). The heterophil/lymphocyte ratio (H/L), often used as an indicator of stress in birds (Gross and Siegel 1983, Maxwell 1993), increases as a response to factors such as psychological stress related to fear and social disruption, food restriction, temperature stress, and noise, among others (Vleck et al. 2000, Ruiz et al. 2002).

In southeastern Estonia, Hõrak et al. (1998a) found that the body mass, plasma protein, and WBC of Great Tits decrease through the breeding season. They enlarged broods experimentally, which caused an increase in the breeder's H/L ratio and HCT over those of controls (Hõrak et al. 1998b). Therefore, in comparison to birds reproducing poorly, we expect birds investing more in reproduction (i.e., reproducing earlier, laying heavier eggs, laying larger clutches, raising larger broods, producing more and heavier fledglings) to have (a) lower body-condition index and (b) lower plasma protein because of a decrease in nutritional condition, (c) higher GSH-Px activity to balance the increased production of ROS caused by the higher metabolic rate, (d) higher HCT and (e) Hb index because of increased muscular activity and consequent oxygen demand (Saino et al. 1997, Moreno et al. 2002), (f) lower WBC because of decreased energy devoted to immune function (Hõrak et al. 1998a, but see Dubiec et al. 2005), and (g) higher H/L, a stress indicator found to increase in Great Tits raising heavier fledglings (Ots and Hõrak 1996). Because these correlations between life-history traits and physiological condition might vary with breeding conditions (e.g., year of study) and age of the breeder we also tested for these effects in the analyses.

We expect birds to be in poorer physiological condition when raising a second broods than when raising the first brood because of a deterioration of environmental conditions as the season progresses and the cumulative energetic costs of a second clutch (Christe et al. 2002).

METHODS

FIELD METHODS

This study was conducted during the breeding seasons of 2005 and 2006 at Choupal (40° 13′ N, 8° 27′ W), in a 79-ha mixed deciduous wood on the periphery of Coimbra, Portugal, bordered by the Mondego River on the south and crossed by several ditches. The flora is very rich and includes plane trees (*Platanus* sp.), black poplars (*Populus nigra*), ash (*Fraxinus angustifolia*), nettle-tree (*Celtis australis*), common alder (*Alnus glutinosa*), common sallow (*Salix atrocinerea*), white willow (*Salix alba*), wych elm (*Ulmus glabra)*, and some other exotic species such as eucalyptus (*Eucalyptus* sp.), maple trees (*Acer* sp.), cedars (*Cedrus* sp.), acacias (*Acacia* sp.), and redwood (*Sequoia sempervirens*). The breeding season of 2005 was drier than that of 2006 (Norte et al. 2009b): the mean temperature and precipitation ± SD between March and June were 16.4 ± 4.2 °C and 34.5 ± 27.5 mm in 2005, 16.5 ± 3.4 °C and 58.8 ± 52.6 mm in 2006.

We monitored nest boxes occupied by breeding Great Tits in 2005 ($n = 26$) and 2006 ($n = 28$) from the start of the breeding season onward to determine the date of laying, mean egg weight, clutch size, brood size, number of fledglings, and mean pre-fledging weight. Laying date refers to the day when the first egg was laid, hatching date to the day when the first egg hatched. Mean egg weight was obtained from a sample of two or three random eggs weighed before the clutch was completed and incubation began (before they were warm) as a measurement of a female's reproductive investment. Mean pre-fledging weight is based on the body mass of 14-day-old nestlings (hatching date = day 0). Parents were caught at the nest when feeding 6- to 11-day-old nestlings; they were aged (one year or older), banded, weighed, their tarsus was measured, and a blood sample was taken (100–150 µL). We recorded time of sampling to the nearest hour to control in subsequent analyses for possible circadian rhythms affecting the variables being studied. We calculated body-condition index as the residuals of the linear regression of weight on tarsus length (Brown 1996; see Green 2001 for assumptions underlying the use of this index as a measure of condition). Some of the blood was smeared on a slide in a thin film, and the remainder was frozen for later analysis.

LABORATORY ANALYSES

The blood samples were centrifuged for 10 min at 3200 revolutions min^{-1} for the plasma to be separated from the red blood cells. Hematocrit was measured as the percentage of the length of the capillary tube occupied by the red blood cells in relation to the total length of the capillary tube occupied by all blood. Total plasma protein (mg mL^{-1}) was measured by the Bradford protein assay, based on the dye-binding procedure of Bradford (1976). For measurements of glutathione peroxidase activity (µmol min^{-1} g^{-1} hemoglobin), in a hemolysate of red blood cells, we used the method of Paglia and Valentine (1967) modified by da Silva and dos Santos (1991), which consists in measuring the rate of formation of oxidised glutathione (GSSG) at 340 nm as NADPH is converted to $NADP^+$. Glutathione peroxidase activity and total protein were measured in a Microplate reader (Sunrise Inst.).

The hemoglobin index of red blood cells (g L^{-1}) stands for the hemoglobin content of a 4× diluted hemolysate of red blood cells (prepared for the measurement of GSH-Px activity) and was measured by the cyanmethemoglobin method at a wavelength of 540 nm (van Kampen and Zijlstra 1961) with a commercial kit (BioSystems S.A.). Blood smears from each bird were air-dried and stained by the May–Gründwalds–Giemsa procedure and scanned under 1000× magnification. White blood cell count stands for the estimation of the number of white blood cells in approximately 10000 erythrocytes. The H/L ratio was measured on the basis of the examination of 50 white blood cells, because the repeatability, according to Lessells and Boag (1987), of the measurements of 50 and 100 white blood cells is very high ($r = 0.94 \pm 0.01$, $F_{1, 109} = 31.86$, $P < 0.001$). Norte et al. (2008) addressed the repeatability of physiological measures in the Great Tit and method-dependent variation in measurements in detail.

STATISTICAL ANALYSES

The H/L ratio was logarithm-transformed and WBC was square-root-transformed for normality. We analyzed the sexes separately because investment in reproduction might differ by sex.

Relationships between breeding and physiological variables. We evaluated the relationships between measures of breeding and physiology with a general linear model (GLM). The explaining variables included laying date, clutch size, brood size, mean egg weight (for females only), number of fledglings, and mean pre-fledging weight (for both females and males). For males, we used only these last two variables, reflecting fledging success, because males are less likely than females to influence the outcome of the nest before hatching. The number of days from hatching (time elapsed between hatching and sampling) and time of sampling (hour) were included as covariables. For each physiological measure we built a model containing the minimum number of significant predictors. First, we carried out a univariate analysis, testing each of these variables one by one, and any variable whose univariate test had $P < 0.25$ was a candidate for the multivariate model (Hosmer and Lemeshow 2000). We fitted the multivariate model (entering all variables with $P < 0.25$), examined the importance of each variable in the model with the Wald statistic, and retained those variables with $P < 0.10$. Any possible interactions between the significant predictors and the age of the breeder (one year or older) and year (2005 or 2006) were also tested and included in the model if significant, because the costs of reproduction might differ by age class and year (Sanz et al. 2002, Stjernman et al. 2004). These models were built on the basis of only first clutches and only one random observation per individual. Sample sizes varied from 38 to 42 for females and from 39 to 44 for males, depending on the physiological variable.

Physiological variables of birds raising first and second broods. We compared the physiological variables (body-condition index, plasma protein, GSH-Px, HCT, Hb index, WBC, and H/L, computed as their residuals on time of sampling) of males and females raising first and second broods for both 2005 and 2006 with a paired *t*-test. Statistical analyses were carried out in JMP 6.0.0 (SAS Institute 2005). Throughout, results means are presented ± SD.

TABLE 1. Final models for the effects of the breeding performance of female and male Great Tits on physiological variables. Final models include only variables with $P < 0.10$. No measures of breeding affected the remaining physiological variables significantly. Only first clutches were used in the analyses. Sample sizes varied from 39 to 41. For variable units see Table 2. Age (1 year) modeled in relation to the category of older adults.

	Body-condition index			Protein			GSH-Px			HCT		
Variable	Estimate	*F*	*P*	Estimate	*F*	*P*	Estimate	*F*	*P*	Estimate	*F*	*P*
Females												
Date of laying							0.05	9.9	0.008	−0.09	12.3	0.001
Clutch size	0.16	3.5	0.07									
Brood size				−2.56	3.5	0.07	0.72	7.8	0.003			
Mean pre-fledging weight of young	0.13	3.9	0.06									
Age (1 year)										−0.89	4.3	0.05
Day of measurement	−0.12	3.8	0.06									
Males												
Number of fledglings	−0.10	2.9	0.096									
Age (1 year)	−0.21	2.9	0.096									

RESULTS

RELATIONSHIPS BETWEEN BREEDING AND PHYSIOLOGICAL VARIABLES

We found females' body-condition index, protein, hematocrit, and glutathione peroxidase activity to be correlated with breeding effort, whereas in males only body-condition index was correlated with breeding effort.

Age of the breeder affected HCT in females (Table 1) and Hb index in males ($F = 8.54$, $P = 0.006$) because adult females had higher HCT and adult males had a higher Hb index than did first-year birds. The body-condition index of adult males was higher than that of first-year birds but not quite significantly (Table 1).

Both the Hb index and GSH-Px differed significantly by year in males ($F = 6.8$, $P = 0.013$ and $F = 6.2$, $P = 0.017$, respectively) because the Hb index was lower and GSH-Px was higher in 2005. There was also a significant interaction between age and year affecting the H/L ratio of males ($F = 7.1$, $P = 0.011$) because in first-year birds the H/L ratio was higher in 2005 than in 2006.

Females laying larger clutches and fledging heavier offspring tended to have a higher body-condition index, but those raising larger broods tended to have lower plasma protein. Females laying earlier had significantly higher HCT (Fig. 1a) and lower GSH-Px activity (Fig. 1b). GSH-Px was also positively correlated with brood size (Table 1, Fig. 1c). Males fledging more offspring tended to have a lower body-condition index (Table 1).

PHYSIOLOGICAL VARIABLES OF BIRDS RAISING FIRST AND SECOND BROODS

Means and standard deviations of measures of breeding comparing first and second broods are presented in Table 2. Females had a significantly lower body-condition index ($t_{1,11} = -2.4$, $P = 0.04$) and hematocrit ($t_{1,12} = -9.6$, $P < 0.0001$) when raising a second brood than when raising the first brood (Table 3). Males raising second broods had significantly higher protein ($t_{1,9} = 2.4$, $P = 0.038$) and a tendency to have a higher body-condition index ($t_{1,9} = 2.0$, $P = 0.08$; Table 3). In the other physiological variables adults raising first and second broods did not differ significantly.

TABLE 2. Means and standard deviations of measures of breeding of first and second broods of the Great Tit at the Choupal woods, Coimbra, Portugal, in 2005 and 2006.

	First brood		Second brood	
Variable	Mean ± SD	*n*	Mean ± SD	*n*
Date of laying (days)[a]	99.6 ± 5.3	14	151.2 ± 4.8	13
Clutch size (no. eggs)	5.9 ± 1.3	14	5.3 ± 1.0	14
Brood size (no. chicks)	5.1 ± 1.2	14	4.3 ± 1.2	14
No. fledglings	4.7 ± 1.5	14	3.0 ± 1.2	14
Mean egg weight (g)	1.64 ± 0.13	11	1.64 ± 0.14	7
Mean pre-fledging weight (g)	16.3 ± 1.4	14	15.9 ± 1.4	12

[a] Day 1 = 1 January.

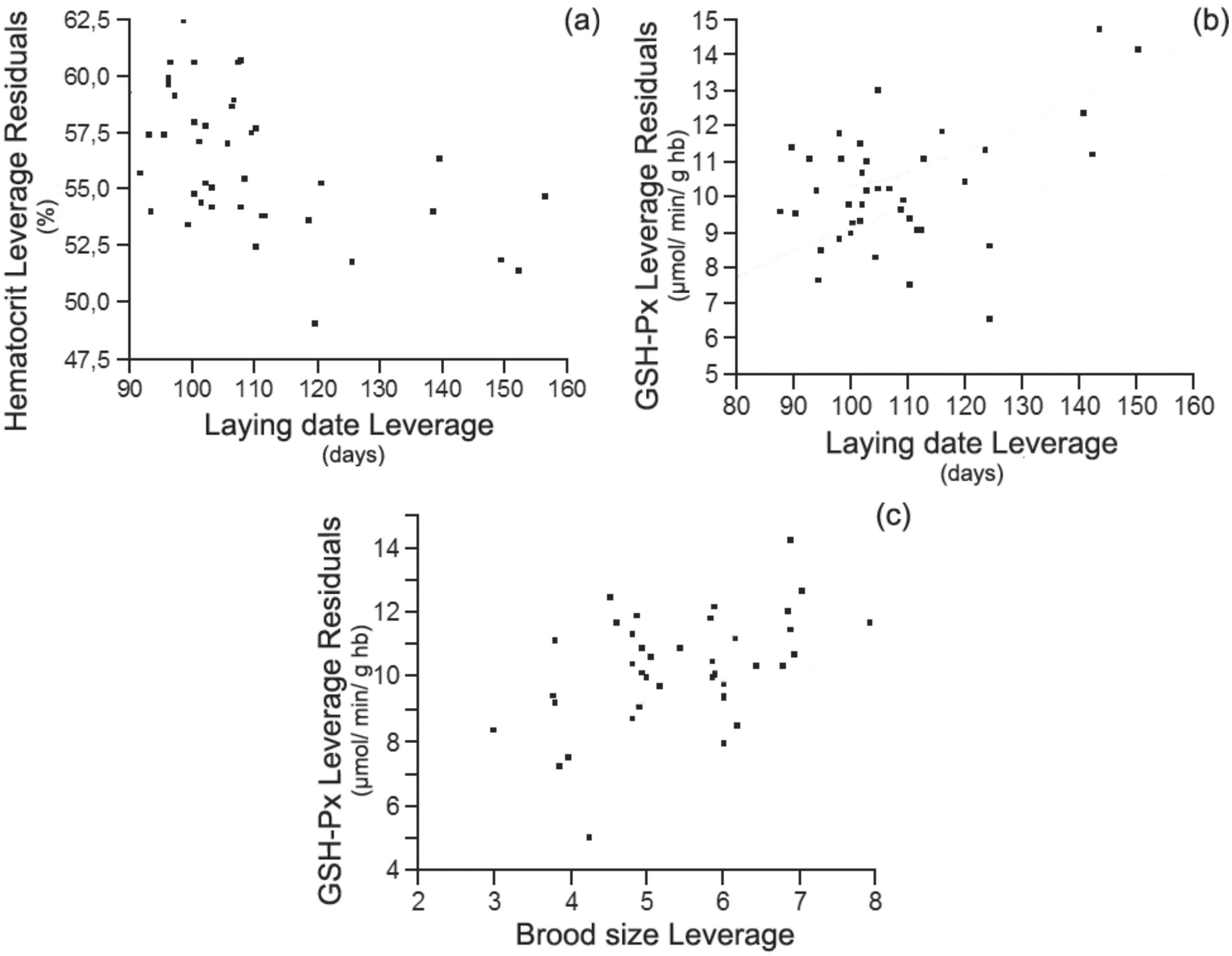

FIGURE 1. Leverage plots (point-by-point composition of the test for each effect showing, for each point, what the residual would be with and without the effect in the model) for the relationship in female Great Tits between (a) laying date and HCT, (b) laying date and GSH-Px, and (c) brood size and GSH-Px.

TABLE 3. Means and standard deviations of morphological, biochemical, and hematological variables of female and male Great Tits raising first or second broods. For explanation of variables see text. Body mass included for comparison.

	Females					Males				
	First brood		Second brood			First brood		Second brood		
Variable	Mean ± SD	*n*	Mean ± SD	*n*	*t* (*P*)	Mean ± SD	*n*	Mean ± SD	*n*	*t* (*P*)
Body mass (g)	16.66 ± 0.80	14	16.36 ± 0.59	14	−2.31 (0.04)	17.22 + 0.80	10	17.64 + 1.03	10	2.1 (0.04)
Body-condition index	0.25 ± 0.69	12	−0.16 ± 0.55	14	−2.40 (0.04)	−0.20 ± 0.80	10	0.21 ± 1.02	10	1.94 (0.08)
Protein (mg ml^{-1})	70.81 ± 11.04	14	67.77 ± 11.94	14	−1.21 (0.25)	64.00 ± 5.97	10	70.23 ± 12.97	10	2.43 (0.04)
GSH-Px (µmol min^{-1} g^{-1} hb)	9.89 ± 1.60	14	11.12 ± 1.60	14	1.68 (0.12)	10.68 ± 2.34	10	12.06 ± 1.16	10	1.59 (0.15)
Hematocrit (%)	58.00 ± 2.48	13	53.66 ± 3.08	14	−9.62 (<0.0001)	55.47 ± 2.55	10	52.75 ± 4.45	10	−1.82 (0.10)
Hb index (g L^{-1})	27.93 ± 6.21	13	26.12 ± 4.94	14	−0.93 (0.37)	29.77 ± 3.94	10	27.77 ± 6.19	10	−1.05 (0.32)
WBC	12.62 ± 6.37	13	13.07 ± 5.21	14	0.54 (0.60)	10.3 ± 4.57	10	10.8 ± 6.37	10	0.25 (0.81)
H/L	3.76 ± 1.51	14	3.54 ± 0.74	14	−0.07 (0.30)	0.51 ± 0.47	9	0.45 ± 0.42	8	−0.49 (0.64)

DISCUSSION

The results of our study are in agreement with the view that the sexes' trade-offs between breeding activity and physiological condition might differ (Hillström 1995, Hõrak et al. 1998a, Kilgas et al. 2006). Of all the physiological variables studied, only body-condition index tended to be correlated with breeding performance in males. In females, however, morphological (body-condition index), biochemical (plasma protein and GSH-Px activity), and hematological variables (HCT) were correlated with measures of breeding effort. Endocrine differences, the sexes' different contributions to brood rearing, and females' additional costs of egg laying and incubation might be responsible for the costs of reproduction differing by sex. Furthermore, we found the direction of the relationship between body-condition index and breeding performance of the sexes to differ. Males fledging more offspring had a lower body-condition index, probably as a consequence of higher energy expenditure depleting nutritional reserves, but females laying larger clutches and fledging heavier offspring had a higher body-condition index. This difference suggests the sexes adopt different breeding strategies, with females optimizing their breeding output according to their physiological state. Consequently, only females in good body condition were able to increase their breeding success.

We found that females in good physiological condition were also able to start breeding early, as has been reported as a successful strategy in several other species (Verhulst and Tinbergen 1991, Siikamäki 1998). Only females in good condition mobilize a large amount of nutrients for egg formation early in the season when the food may be scarcer: we found that early-breeding females had higher hematocrits, an anabolic indicator (Hõrak et al. 1998b), and lower glutathione peroxidase activity, revealing lower exposure to ROS, than females breeding later. Hematocrit is also negatively correlated with laying date in the Magellanic Penguin (*Spheniscus magellanicus*; Moreno et al. 2002). The lower GSH-Px activity of females laying earlier suggests that they were not challenged by high production of ROS, possibly because of low levels of infection, which are known to increase ROS (Ames et al. 1993), supporting the hypothesis that early-and late–breeding females differ in quality. Another explanation, which does not contradict the first, is that females that laid their eggs later probably raised their broods during a period less favorable in terms of food availability or increased parasite pressure, depressing their physiological condition.

In females, brood size was negatively correlated with plasma protein and positively correlated with GSH-Px activity, suggesting that raising a large brood may decrease females' nutritional reserves and increase their exposure to ROS as a result of a high metabolic rate and muscular activity associated with feeding the brood. This inference is in agreement with the results of Bize et al. (2008), who reported that Alpine Swifts (*Apus melba*) with larger clutches and better hatching success had increased antioxidant defenses. As pointed out by Monaghan et al. (2009), experimental studies focusing on the effects of reproduction on oxidative stress would be of great help in clarifying the role of oxidative stress in mediating life history trade-offs. Also, because oxidative stress can be inferred only by measuring both sides of the homeostatic equilibrium between ROS and antioxidant defenses, a parallel measure of ROS or oxidative damage, in addition to the GSH-Px we used to quantify antioxidant defense, would yield a more comprehensive view of the bird's oxidative state.

This study suggests that annual differences and inherent conditions should be taken into account when the relationships between breeding performance and physiological variables are assessed. The breeding season of 2005 was drier and possibly poorer for breeding in terms of food availability, because mean pre-fledging weight was lower than in 2006 (Norte et al. 2009b). This difference probably caused males' poorer physiological condition in 2005, which was revealed in a lower Hb index, higher GSH-Px activity, and higher H/L ratio. Higher levels of parasite infection (e.g., *Plasmodium* sp.; Norte et al. 2009a) in 2005 might have decreased males' Hb index and exposed them to higher levels of ROS (Ames et al. 1993). The higher level of H/L, a stress indicator (Gross and Siegel 1983, Maxwell 1993), in one-year-old males in 2005 also supports the assumption that males were in poorer physiological condition that year.

PHYSIOLOGICAL VARIABLES OF BIRDS RAISING FIRST AND SECOND BROODS

Raising a second brood also affects the physiology of males and females differently. Females raising second broods had lower body-condition indices and hematocrit than when raising first broods. Possibly because females' physiological condition was poorer when they attempted a second brood, the size of second clutches and broods was generally lower. In the House Martin (*Delichon urbicum*), Christe et al. (2002) found that hematocrit decreased from first to second clutches in both sexes, in partial agreement with our results.

We found that males raising second broods had higher levels of protein and a nearly significantly higher body-condition index. It is possible that the period of raising a second brood may be less stressful for males than for females because for males stress is likely to decrease through the breeding season, as Hõrak et al. (1998a) reported from pre-laying (courtship and mate guarding) to brood rearing. However, Kilgas et al. (2006) found that rearing a second brood was less stressful for both sexes of the Great Tit, as showed by a decrease in the H/L ratio of both males and females. They explained their results as a consequence of decreased competition during that period resulting from decreased density of breeding pairs. But they also found that females' fat decreased while they were raising second broods, suggesting lower body reserves during that period (Kilgas et al. 2006).

Because we did not manipulate breeding effort or sample before and after brood rearing repeatedly, it is difficult to know if some physiological variables represent a response to the energy expenditure associated with breeding or if the breeding performance is a result of the condition of the bird. As we evaluated the adults' physiological condition when their nestlings were 6 to 11 days old, we assume that the observed values of physiological variables were a consequence of the effort of breeding. However, as some of the evaluated variables were significantly repeatable in spring (e.g., hematocrit; Norte et al. 2008), they should reflect the birds condition over an extended period such as the breeding season. In summary, our study suggests that hematocrit and GSH-Px are sensitive indicators of the physiological condition of breeding females and that differences between the sexes have to be taken into account when the costs of reproduction are evaluated because of the sexes' possibly different sensitivities and breeding strategies. Also, yearly fluctuations in environmental conditions should not be disregarded because poor years may have detrimental effects on the physiological condition of breeders.

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