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Effects of Sibling Competition on Growth, Oxidative Stress, and Humoral Immunity: A Two-Year Brood-Size Manipulation

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ABSTRACT

We investigated the effects of ecological context (by comparing data from two consecutive years) and experimentally manipulated nestling developmental conditions (large vs. small brood size) on immune function (immunoglobulin Y [IgY]) and oxidative stress in nestling European starlings *Sturnus vulgaris*. On the basis of annual differences in chicks' morphological traits and body masses close to fledging, we established that 2007 was a relative low-quality year and 2008 was a relatively high-quality year. Total antioxidant capacity (TAC) was significantly lower in experimentally enlarged broods, but only in the low-quality year (2007). Total oxidant status (TOS) was independent of brood size in both years but was 45% higher in the low-quality year. Consequently, plasma oxidative status (the ratio between TOS and TAC) was higher in 2007. In contrast, plasma IgY levels were higher in the experimentally enlarged broods and in the high-quality year (2008). Thus, immune function and oxidative stress showed inverse relationships with developmental conditions and annual variation in year quality. Finally, TOS and TAC were positively correlated, but only in the low-quality year (2007), and there was no relationship observed between IgY and markers of oxidative stress. Our results demonstrate the importance of taking into account year effects or ecological context when assessing environmental effects on physiological mechanisms underlying the life-history traits of chicks, such as oxidative stress.

Introduction

Environmental conditions, such as resource availability or sibling competition, that are imposed on altricial nestlings during early development have been shown to constrain a wide range of traits that ultimately shape adult fitness (Metcalfe and Monaghan 2001; Love and Williams 2008; Partecke and Schwabl 2008; Uller 2008). Several studies have suggested that immune function and oxidative stress might be especially affected by the early developmental environment (Saino et al. 1997; Hõrak et al. 1999; Ilmonen et al. 2003; Brzek and Konarzewski 2007; Tschirren et al. 2009). Saino et al. (1997) showed that body mass and T-cell-mediated immune response were negatively correlated with increasing natural brood size (BS) in nestling barn swallows (*Hirundo rustica*). Similarly, nestling great tits (*Parus major*) responded to experimentally increased BS by exhibiting lower growth rates (Tinbergen and Boerlijst 1990) and reduced T-cell responses (Hõrak et al. 1999) compared with chicks raised in decreased-size broods. Costantini et al. (2006) proposed that intrabrood sibling competition might shape another component of offspring development, oxidative stress. Oxidative stress is defined as an imbalance between the production of reactive oxygen species (ROS) and antioxidant compounds (Finkel and Holbrook 2000). ROS are physiological by-products of metabolic processes; their unstable and very reactive nature causes damaging effects on biomolecules (Alonso-Alvarez et al. 2004). Oxidative stress occurs when the toxic effects of ROS cannot be buffered by endogenous (enzymatic and nonenzymatic) and exogenous antioxidants (Surai 2002), such as vitamin C, tocopherols, or carotenoids (although the latter group was recently shown to act as minor antioxidants in birds; Costantini and Møller 2008). In unmanipulated broods of wild kestrel chicks (*Falco tinnunculus*), oxidative stress increased (i.e., there was an increase of reactive oxygen metabolites concomitant with a decrease in serum antioxidant barrier) with increasing BS (Costantini et al. 2006). Nevertheless, this correlational study did not control for maternal effects. Although maternal antioxidants did not seem to affect the resistance to oxidative stress of yellow-legged gull chicks (*Larus cachinnans*) at hatching (Kim et al. 2010), they were shown to positively influence chick growth (Biard et al. 2005). To disrupt the natural covariation between BS and parental quality, Alonso-Alvarez et al. (2006, 2007) experimentally manipulated the BSs of captive zebra finches (*Taeniopygia guttata*). They found that antioxidant capacity, as measured by the resistance of red blood cells to a radical attack, was

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significantly weaker in chicks whose BSs were increased. However, they assessed only one component of oxidative stress (the antioxidant capacity), and this does not fully characterize the resulting redox balance (Costantini 2008; Costantini and Verhulst 2009; Monaghan et al. 2009). Therefore, more experimental studies presenting integrated measures of oxidative stress are needed to provide a more comprehensive view of oxidative stress as a life-history trait potentially mediating trade-offs (Hõrak and Cohen 2010).

Although von Schantz et al. (1999) proposed that an activation of the immune system might generate an increase in oxidative stress, strong evidence for this is largely still lacking (Costantini 2008). While some studies have reported that immune activation directly elicits oxidative damage in chicks (Costantini and Dell'Omo 2006) and adult birds (Bertrand et al. 2006; Torres and Velando 2007; van de Crommenacker et al. 2010), other studies have failed to demonstrate a causal link between these two parameters (Alonso-Alvarez et al. 2004; Perez-Rodiguez et al. 2008). Therefore, more studies are needed to fully understand the reciprocal relationships between oxidative stress and immune function (Costantini 2008; Costantini and Møller 2009; Monaghan et al. 2009) and the effect that early developmental conditions have on these traits and their interaction. One reason for the lack of consistency between different studies might be that most studies are performed during only one breeding season, and often without a known "ecological context," for example, if the study was conducted in a "good" versus a "bad" year. It is known that resource-based trade-offs may be compensated for in years of adequate resource abundance in chicks (e.g., Chin et al. 2005).

To date there have been no experimental studies of the importance of environmental effects during rearing and year effects (annual variation) on both immunocompetence and oxidative stress during growth in free-living chicks. Here we experimentally manipulated (decreased or enlarged) broods of European starling chicks (*Sturnus vulgaris*) within the naturally observed range for BS in this species during two consecutive years. Chicks were randomly exchanged between experimental broods to randomize genetic and parental effects on nestling growth, immune function, and oxidative stress. Humoral immunity was assessed through plasma concentrations of immunoglobulin Y (IgY), and plasma oxidative status was calculated as the ratio between plasma total oxidant status (TOS) and plasma total antioxidant capacity (TAC). First, we predicted that chicks raised in enlarged broods would exhibit reduced growth rates and lower IgY levels but higher plasma oxidative status (i.e., a greater imbalance between TOS and TAC) than chicks raised in decreased-size broods. Second, we predicted that if these physiological traits were mediated by resource allocation–based trade-offs, then these effects would be magnified during years of lower quality. Specifically, our experimental manipulation allowed us to test a recent proposal that there should be a trade-off between investment in immune function and oxidative stress (Halliwell and Gutteridge 2007).

Material and Methods

Species and Study Site

Fieldwork was performed between April and July of 2007 and 2008 on a nest-box-breeding population of European starlings *Sturnus vulgaris* at the Davistead dairy farm in Langley, British Columbia, Canada (49°10′N, 122°50′W), under a Simon Fraser University animal care permit (659-B), following the guidelines of the Canadian Council on Animal Care. European starlings are hole-nesting birds that lay four to six blue-green eggs per clutch. The female incubates the eggs by herself for 10–11 d, while both parents provision the chicks; chicks fledge 18–20 d after hatching. Our study site consisted of wooden nest boxes (190 boxes in 2007 and 163 in 2008), mounted on farm buildings and on posts in large fields, that were used annually by breeding starlings. Nest boxes were checked daily to determine clutch initiation and clutch completion dates as well as the laying sequence of eggs and the growth of chicks from hatching to fledging. Chicks used in this study were raised by primary females (females who benefit from paternal care from polygynous partners), and all hatched between April 25 and April 28 of both years.

Experimental Design and Sampling Protocol

During both years, BS was manipulated after hatching on the basis of females' initial clutch sizes. By manipulating both BS and female quality, we could create high- and low-quality environmental conditions for the chicks. On the basis of the experimental removal of the first clutch, it was demonstrated in this species that, independent of female body mass, the largest clutches are produced by high-quality females (Christians et al. 2001). Following this "quality hypothesis," females that raised decreased-size broods of three chicks laid significantly more eggs than did females that raised increased-size broods of six chicks, independent of the year (initial clutch size: 5.95 ± 0.05 eggs, $N = 21$ and 5.33 ± 0.14 eggs, $N = 18$, respectively; t-test: $t = 4.45$, $P < 0.0001$). Nevertheless, initial clutch size did not differ significantly between years within each group (decreased BS: 6.00 ± 0.00 eggs, $N = 9$ in 2007 and 5.92 ± 0.08 eggs, $N = 12$ in 2008; increased BS: 5.37 ± 0.26 eggs, $N = 8$ in 2007 and 5.30 ± 0.15 eggs, $N = 10$ in 2008; two-way ANOVA: group: $F_{1,35} = 18.67$, $P = 0.0001$; year: $F_{1,35} = 0.30$, $P = 0.58$; group × year: $F_{1,35} = 0.001$, $P = 0.98$). Moreover, clutch size was also manipulated after clutch completion, and each female was given five eggs to incubate.

At hatching, chicks were randomly exchanged between decreased-size and increased-size broods with similar hatch dates. Only those broods for which all chicks successfully fledged were selected for and presented in this study. The experiment was performed on 171 chicks over two consecutive years: 75 chicks in 2007 and 96 chicks in 2008. In 2007, the sizes of nine broods were decreased and eight were enlarged, versus 12 and 10, respectively, in 2008. Each year, chick growth was followed throughout the chick-rearing period by recording the body mass of each nestling (±0.01 g) at 5, 10, and 15 d after hatching. At 10 d of age, each nestling was metal-banded. When chicks were 15 d of age, tarsus and wing lengths were recorded and a blood sample was taken (from the brachial vein). Blood was collected in heparinized capillary tubes that were immediately stored on ice, samples were centrifuged within 2 h (5,000 rpm), and plasma was frozen and stored at −20°C. Plasma samples were subsequently used to assess IgY levels and plasma oxidative status within 2 mo after collection.

Plasma Analysis

TAC was measured, using a modification to the commercially available Randox TEAC assay described by Erel (2004), where the reduced ABTS (2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)) molecule is oxidized to $ABTSS^+$, using hydrogen peroxide (H_2O_2) alone in acidic medium (the acetate buffer, 30 mmol L^{-1}, pH = 3.6). While $ABTSS^+$ is diluted with a more concentrated acetate buffer solution at high pH values (0.4 mol L^{-1}, pH = 5.8), its deep green color is spontaneously and slowly bleached. Nonenzymatic antioxidants (of both dietary and endogenous sources) present in the plasma sample accelerate the bleaching rate to a degree that is proportional to their concentrations. This reaction can be monitored spectrophotometrically, and the final absorbance is inversely related to the TAC of the sample. The reaction rate is calibrated with Trolox (Sigma-Aldrich, 238813), a widely used standard for TAC measurement assays, and the assay results are expressed in mmol Trolox equivalent L^{-1} in reference to a standard curve. The TAC assay had an intraassay variability of 2.96% ($N = 15$ duplicates) and an interassay variability of 3.56% ($N = 25$ duplicates).

Total oxidant status (TOS) was measured as described in humans by Erel (2005) and, more recently, in avian studies (Maurice et al. 2009; Travers et al. 2010). TOS assesses both H_2O_2 components (that can break down in important prooxidants and can also be indicators of the superoxide dismutase enzyme's activity) and lipid hydroperoxides (markers of prior lipid damage that still maintain prooxidant properties; Erel 2005). Briefly, oxidants present in the plasma sample oxidize the ferrous ion–o-dianisidine complex to the ferric ion. The oxidation reaction is enhanced by glycerol molecules, which are abundantly present in the reaction medium. The ferric ion creates a colored complex with xylenol orange in an acidic medium. The color intensity, which can be measured spectrophotometrically, is proportional to the total amount of oxidant molecules present in the sample. The assay is calibrated with H_2O_2, and the results are expressed in μmol H_2O_2 equivalent L^{-1} in reference to a standard curve. The TOS assay had an intraassay variability of 8.75% ($N = 14$ duplicates) and an interassay variability of 6.79% ($N = 25$ duplicates). Plasma oxidative status was subsequently calculated as the ratio between TOS and TAC. High ratio values reflect high oxidative stress levels.

Immunoglobulins are the most important serum proteins involved in humoral immune responses in birds (Roitt et al. 1998). Plasma IgYs were assessed, using a sensitive enzyme-linked immune absorbent assay (ELISA) method. Commercial antichicken antibodies were used as reported by Martinez et al. (2003). We adapted this method for European starling chicks by determining the appropriate plasma dilution (1 : 4,000). IgY levels were expressed in units of absorbance.

Statistical Analyses

Statistical analyses were conducted using PASW Statistics 18.0.2 (SPSS, Chicago, IL). Values are presented as mean ± standard error (SE). Since IgY levels, plasma TOS, and oxidative status were not normally distributed (Kolmogorov-Smirnov test, $P < 0.05$), appropriate transformations were applied to meet parametric assumptions before parametric tests were used. Generalized linear mixed models (GLMMs) were used to test for the effects of group (decreased vs. enlarged broods) and year (2007 and 2008; fixed factors; full-factorial model) on morphological and plasmatic parameters (dependent variables; i.e.,

Table 1: Effects in European starling (*Sturnus vulgaris*) chicks, using generalized linear mixed models, of brood-size manipulation (group: decreased or enlarged brood) and year (2007 or 2008) on tarsus and wing lengths; body mass at 5, 10, and 15 d of age; and daily growth rates between 5 and 10 d of age and between 10 and 15 d of age

	Group			Year			Group × Year			Parameter Estimates ± SE			
Dependent Variable	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	Intercept	Group	Year	Group × Year
Tarsus length (mm)	43.1	.06	.80	46.67	20.4	<.001	53.19	.02	.90	34.56 ± .21	−.03 ± .32	−1.02 ± .30	−.06 ± .47
Wing length (mm)	48.96	3.39	.07	57.23	3.99	.05	63.68	1.85	.18	78.51 ± 1.10	3.67 ± 1.63	−.75 ± 1.46	−3.14 ± 2.30
Body mass (g):													
At 5 d old	53.69	9.93	.003	60.09	.16	.69	67.15	.76	.39	30.72 ± 1.23	2.97 ± 1.85	−1.68 ± 1.67	2.30 ± 2.64
At 10 d old	21.14	12.93	.002	16.43	1.77	.20	16.16	.29	.60	65.11 ± 1.03	5.07 ± 1.63	−.97 ± 1.54	−1.34 ± 2.49
At 15 d old	66.02	11.73	.001	61.56	13.29	.001	67.47	.001	.97	75.24 ± 1.06	4.18 ± 1.64	−4.43 ± 1.55	−.08 ± 2.46
Daily growth rate ($g\ d^{-1}$):													
Between 5 and 10 d of age	57.84	.004	.95	65.79	3.28	.07	72.80	3.92	.05	6.94 ± .15	.32 ± .22	.02 ± .20	−.62 ± .31
Between 10 and 15 d of age	55.43	.02	.89	72.27	3.59	.06	75.69	.2	.65	2.01 ± .24	−.14 ± .35	−.55 ± .28	.21 ± .46

Note. Parameter estimate values are given for the following fixed effects: for group, decreased; for year, 2007; and for group × year, decreased × 2007.

tarsus and wing lengths, body mass [at different stages], daily growth rate, IgY levels, TAC, TOS, and plasma oxidative status). Linear mixed-effect models (LMEs) were used to assess the relationships between TAC (dependent variable) and TOS (covariate) during each year. For each GLMM and LME, the maternal identity was used as a random factor to account for the repeated measures (within the same nest and between years). Sample sizes differed between analyses because of a lack of and/or poor quality of plasma.

Results

BS Manipulation and Year Effects on Morphological Traits and Growth Rates

While tarsus lengths were not significantly different among groups, chicks raised in 2007 had significantly smaller tarsi than chicks raised in 2008, independent of the sizes of their broods (GLMM; Table 1; Fig. 1*A*). Although wing length tended to be lower in enlarged broods than in decreased ones, and also lower in 2007 than in 2008, there was no significant effect of group or year on that trait (GLMM; Table 1; Fig. 1*B*). At 5 and 10 d of age, chicks raised in enlarged broods exhibited significantly lower body masses than did chicks raised in decreased-size broods (−12% and −7%, respectively), independent of the year (GLMM; Table 1). At 15 d of age (the stage at which the chicks' blood was sampled), enlarged-brood chicks were still significantly lighter than the decreased-brood chicks (−5.3%) and, independent of treatment, chick body mass was significantly lower in 2007 than in 2008 (−6%; GLMM; Table 1; Fig. 1*C*). Daily growth rates for the period between 5 and 10 d of age and between 10 and 15 d of age were not significantly different between groups or years (GLMM; Table 1), although the latter value was lower in 2007 (2007: 1.31 ± 0.15 g day^{-1}, $N = 75$; 2008: 1.95 ± 0.11 g day^{-1}, $N = 96$).

BS Manipulation and Year Effects on Plasma Oxidative Status and IgY Levels

TAC was significantly affected by BS manipulation but not by year, while the interaction of the main effects was significant (GLMM; Table 2). GLMMs run for each year showed that in 2007, TAC was significantly lower in chicks from enlarged broods than in chicks from decreased broods (1.31 ± 0.14 and 1.82 ± 0.13 mmol Trolox equivalent L^{-1}, respectively; GLMM: $F_{1,57.01} = 15.23$, $P < 0.0001$), while it was not affected by BS manipulation in chicks raised in 2008 ($F_{1,12.66} = 0.29$, $P = 0.60$; Fig. 2*A*). However, while TOS was not significantly affected by BS manipulation, it was 45% higher in 2007 than in 2008 (GLMM; Table 2; Fig. 2*B*). Likewise, plasma oxidative status was significantly higher in 2007 than in 2008 (by 48%), independent of BS manipulation (GLMM; Table 2; Fig. 2*C*).

There were significant effects of group and year on humoral immunity. During both years, chicks raised in enlarged broods showed significantly higher IgY levels than did those raised in decreased-size broods (by 20% in 2007 and by 13% in 2008; GLMM; Table 2; Fig. 2*D*). Moreover, IgY levels were significantly higher in 2008 than in 2007 (by 30%), independent of group (GLMM; Table 2; Fig. 2*D*).

Finally, there was a positive significant relationship observed between TOS and TAC in 2007 (LME: $t = 5.72$, $N = 75$, $P < 0.0001$; Fig. 3*A*) but not in 2008 ($t = 1.42$, $N = 94$, $P = 0.16$; Fig. 3*B*), which further illustrates that chicks exhibiting the highest oxidant statuses also showed the highest antioxidant capacities during low-quality years, independent of BS manipulation. Finally, there was no significant relationship between IgY levels and TAC, TOS, or plasma oxidative status within

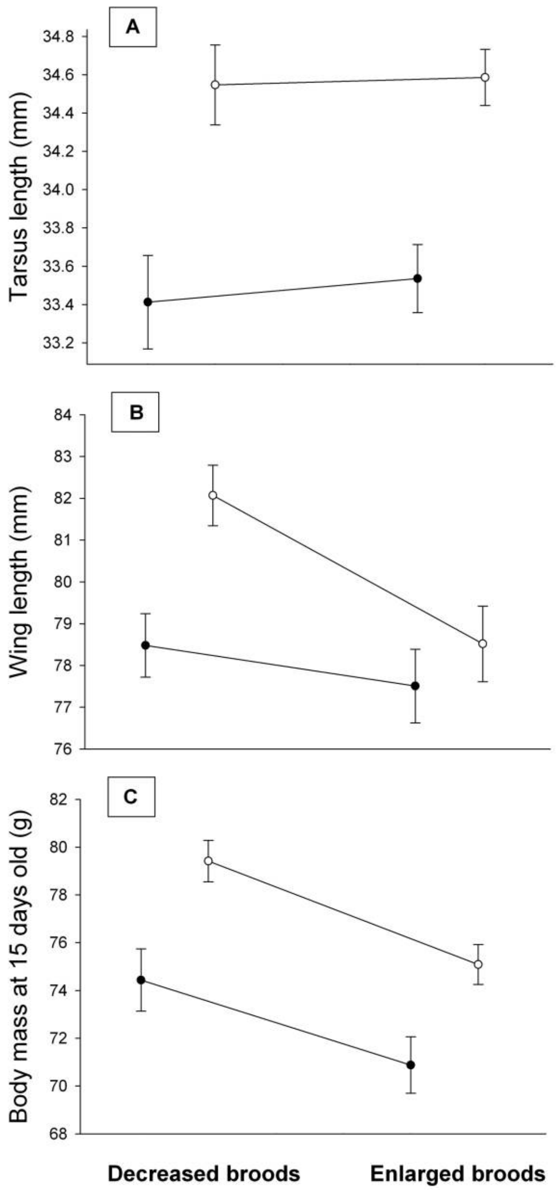

Figure 1. Effects of brood-size manipulation (decreased vs. enlarged broods) on (*A*) tarsus and (*B*) wing lengths and on (*C*) body mass in 15-d-old European starling (*Sturnus vulgaris*) chicks raised during two consecutive years, 2007 (*filled circles*) and 2008 (*open circles*). Values represented are means ± SE.

Table 2: Effects in European starling (*Sturnus vulgaris*) chicks, using generalized linear mixed models, of brood-size manipulation (group: decreased or enlarged brood) and year (2007 or 2008) on plasma total antioxidant capacity (TAC), total oxidant status (TOS), plasma oxidative status (ratio between TOS and TAC), and immunoglobulin Y (IgY) levels

	Group			Year			Group × Year			Parameter Estimates ± SE			
Dependent Variable	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	Intercept	Group	Year	Group × Year
TAC (mmol Trolox equivalent L^{-1})	51.66	5.47	.02	61.54	2.43	.12	67.96	5.86	.02	1.66 ± .08	−.01 ± .12	−.33 ± .10	.40 ± .16
TOS (μmol H_2O_2 equivalent L^{-1})[a]	58.50	.26	.61	77.23	46.85	<.0001	79.34	2.76	.10	2.31 ± .04	−.05 ± .06	.22 ± .05	.14 ± .08
Plasma oxidative status[a]	56.27	.66	.42	73.38	62.82	<.0001	76.57	.00	.98	1.09 ± .04	−.03 ± .06	.34 ± .05	−.00 ± .08
IgY level[b]	60.11	6.08	.02	67.12	50.49	<.0001	74.38	.11	.74	3.45 ± .26	.76 ± .38	2.05 ± .35	−.18 ± .55

Note. Parameter estimate values are given for the following fixed effects: for group, decreased; for year, 2007; and for group × year, decreased × 2007. IgY level data are presented in arbitrary units.

[a] Data were $\log_{10}$ transformed before statistical analysis.

[b] Data were inverse transformed before statistical analysis.

each experimental group of each year (data not shown; LME, $P > 0.05$ in all cases).

Discussion

With this study, we aimed to assess the combined effects of annual variation in year quality and developmental conditions on biomarkers of oxidative stress and humoral immune function in nestling European starlings. First, we showed that chicks raised in experimentally enlarged broods had lower TAC, but only during one of the study years (2007). BS manipulation did not influence TOS, but TOS was higher, independent of BS, in 2007. Consequently, plasma oxidative status was higher in 2007 than in 2008. Second, IgY level, which is a marker of humoral immune function, was higher in 2008 and, unexpectedly, was higher in chicks in experimentally enlarged broods compared with those in smaller broods. Finally, TAC and TOS were positively correlated only in 2007, while there were no relationships between biomarkers of oxidative stress and immune function (IgY) in either year. Our results therefore demonstrate that year effects can confound BS effects when assessing the impacts of sibling competition on chicks' growth and diverse physiological traits such as oxidative stress. This emphasizes the influence of year effects—that is, the "ecological context"—over BS on oxidative stress, and it raises the question of the nature of the physiological mechanisms that could be responsible for variations in oxidative stress.

Broods of European starling chicks were consistently manipulated during two consecutive years (2007 and 2008). Significant differences in morphological traits (tarsus and wing lengths), body mass, and daily growth rates were observed between the two study years; all of these traits had significantly lower values in 2007 than in 2008. Various parameters such as food quality and quantity, parasite pressure, or weather conditions could, acting alone or in synergy, influence chicks' morphological traits. Although the proximal factors responsible for these interyear differences were not controlled for in our study and remain to be identified, we defined 2007 as being a year of lower quality than 2008.

In this study, TAC was lower only in experimentally enlarged broods that were raised during a low-quality year (2007). On the other hand, while TOS did not vary with increasing BS, it was significantly affected by year. Indeed, chicks raised during a low-quality year showed a higher TOS than those raised during a higher-quality year (2008). Consequently, plasma oxidative status, defined as the ratio between pro- and antioxidants, was significantly higher in 2007 than it was in 2008, independent of BS. These results do not support our a priori predictions, and they contrast with the results of some previous studies. For example, oxidative stress was higher in larger broods of wild kestrels (Costantini et al. 2006) and European starlings (Costantini et al. 2010). Similarly, 20-d-old zebra finches raised in experimentally enlarged broods showed a weaker resistance of red blood cells to a free-radical attack, that is, a higher susceptibility to oxidative stress, than chicks raised in decreased-size broods. In the latter study, the physiological effects induced by BS manipulation were maintained for up to 40 d (Alonso-Alvarez et al. 2007) and had long-term effects on birds' quality (Alonso-Alvarez et al. 2006). In our study, because antioxidant levels did not significantly vary between years, the higher levels of plasma oxidative status we observed during the low-quality year could have depended on variations in TOS that significantly differed among years. This suggests that the presence of antioxidants might not be the main limiting factor controlling oxidative stress, at least in growing birds. Other avian studies have also shown that higher oxidative stress levels result from an increase in prooxidants, but with no apparent change in antioxidants (Cohen et al. 2007; van de Crommenacker et al. 2010). Nevertheless, our results do not allow us to rule out the importance of antioxidants or to infer the physiological mechanisms regulating antioxidant levels. Indeed, equal antioxidant levels between years could result from different responses to variations in oxidative damage among years. As previously suggested, an upregulation of antioxidant defenses that is concomitant with their mobilization could result in no net change in TAC (van de Crommenacker et al. 2010). Accordingly, Hõrak et al. (2007) reported a positive correlation between uric acid levels (which are believed to explain a high percentage of variance in TAC [Cohen et al. 2007]) and lipid

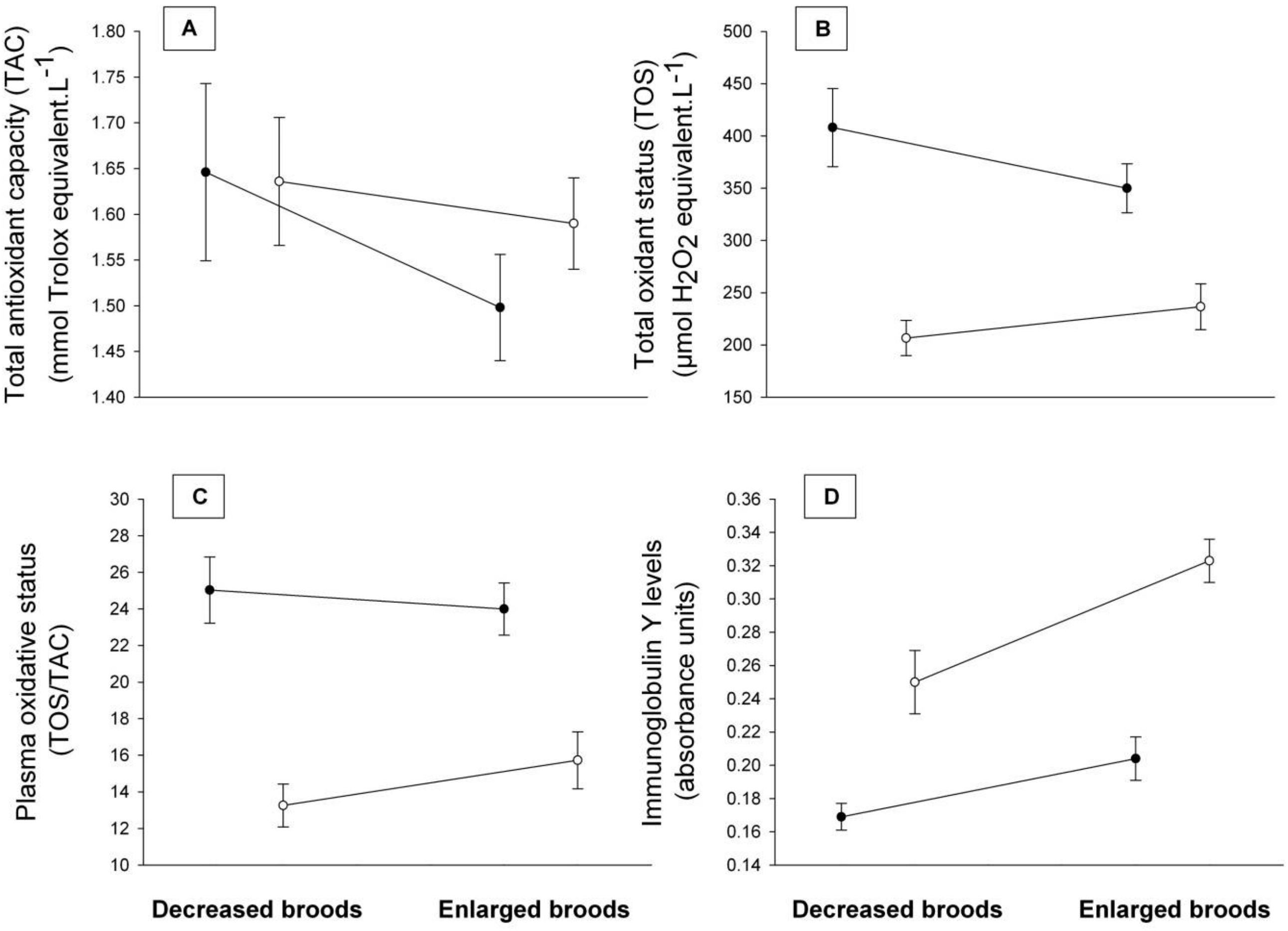

Figure 2. Effects of brood-size manipulation (decreased vs. enlarged broods) on plasma concentrations of (*A*) total antioxidant capacity (TAC), (*B*) total oxidant status (TOS), (*C*) plasma oxidative status (ratio between TOS and TAC), and (*D*) immunoglobulin Y in 15-d-old European starling (*Sturnus vulgaris*) chicks raised during two consecutive years, 2007 (*filled circles*) and 2008 (*empty circles*). Values represented are means ± SE.

peroxidation (marker of oxidative stress–derived damage) in captive greenfinches (*Carduelis chloris*) secondary to the activation of the immune system. This further illustrates the activation of the endogenous antioxidant machinery consecutive to the induction of a free-radical attack. We tried to address this question by examining the relationship between TOS and TAC between years. In 2007, there was a significant positive relationship between the two parameters, implying that chicks with the highest TOS also had the highest TAC, while in 2008 the latter relationship was not significant. Therefore, the antioxidant machinery might have been activated only during the low-quality year, when the production of prooxidant compounds was higher. Although the latter mechanism might help to buffer the increasing production of oxidants and limit oxidative stress during a low-quality year, one can only suppose it might entail an energetic cost (Cohen et al. 2008; reviewed in Costantini 2008). In this study, the TOS was significantly higher during the low-quality year than during the higher-quality year. Although chicks showed similar antioxidant levels between years, the positive relationship between TOS and TAC that was observed during the low-quality year might suggest that the antioxidant machinery was activated during that year to counteract the high TOS levels and to prevent the individual from sustaining high oxidative stress. While such an upregulation could contribute to limiting oxidative stress, it might have induced an energetic cost.

If there is resource-allocation trade-off between immunity and growth, we predicted that chicks raised under more stressful developmental conditions, such as resource limitation or increasing competition in larger broods, should exhibit lower immune capacity (Naguib et al. 2004). In this study, humoral immunity was accordingly lower during a low-quality year, which could be, among other factors, attributable to lower nutritional conditions during that year. Using a feeding regime manipulation, Brzek and Konarzewski (2007) showed that food-restricted nestling sand martins (*Riparia riparia*) exhibited

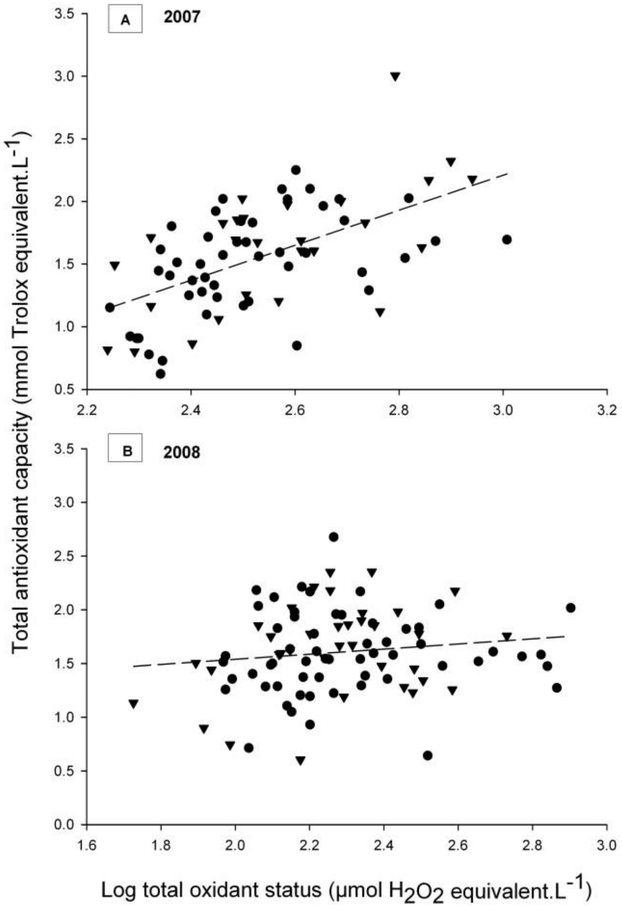

Figure 3. Relationship between total oxidant status and total antioxidant capacity in 15-d-old European starling (*Sturnus vulgaris*) chicks raised in (*A*) 2007 and (*B*) 2008 in two different experimental environments, decreased broods (*triangles*) and enlarged broods (*circles*).

a significantly weaker T-cell-mediated immune swelling response than nestlings fed ad libitum. However, in this study, chicks raised in experimentally enlarged broods showed higher IgY levels than did chicks raised in smaller broods during both years, contradicting our prediction. Other studies have also found a positive relationship between BS and immune function. For example, the T-cell-mediated immune response of domesticated zebra finch chicks raised in experimentally large broods was significantly stronger than that measured in chicks raised in small broods (Tschirren et al. 2009). Likewise, late-hatched barn swallow nestlings had lower body mass but a stronger humoral immunity (plasma immunoglobulin concentration) and a stronger T-cell-mediated immune response than their early-hatched siblings (Saino et al. 2001). However, these latter results are contradictory to those of cross-fostering experiments describing lower T-cell responses following a BS increase in great tits (Hõrak et al. 1999) and zebra finches (Naguib et al. 2004; Alonso-Alvarez et al. 2006). One alternative explanation for our results might relate to parasite load, on the basis of the fact that immunoglobulin levels could reflect not only immune capacity but also an immune response to an ongoing infection (reviewed in Morales et al. 2004), while parasite exposure was shown to increase with increasing BS in great tits (Norris et al. 1994) and tree swallows (*Tachycineta bicolour*; Shutler et al. 2004). Accordingly, small ground finches (*Geospiza fuliginosa*) facing naturally higher parasite abundance showed greater humoral immunity (Lindström et al. 2004). By contrast, nestling pied flycatchers (*Ficedula hypoleuca*) that were experimentally highly infested with mites showed lower immunoglobulin levels than did nestlings that were less exposed or not exposed to mites (Moreno et al. 2008). Moreover, a recent study provided evidence for a trade-off between resources available to body maintenance and IgY in fasting captive female mallards (*Anas platyrhynchos*; Bourgeon et al. 2010).

Costantini and Møller (2009) suggested that there might be a trade-off between investment in immune function and oxidative stress, although available data were not sufficient to validate or invalidate this proposal. On the one hand, immune-challenged birds experienced an increased production of reactive oxygen metabolites in studies of wild Eurasian kestrel nestlings (Costantini and Dell'Omo 2006) and adult homing pigeons (*Columba livia*; van de Crommenacker et al. 2010), a higher susceptibility to oxidative stress in a study of captive zebra finches (Bertrand et al. 2006), and higher lipid peroxidation in a study of captive greenfinches (Hõrak et al. 2007). On the other hand, Perez-Rodiguez et al. (2008) did not report any effect of cell-mediated immune activation on total plasma antioxidant status or lipid oxidative damage in male red-legged partridges (*Alectoris rufa*). In our study, independent of BS manipulation, chicks in broods that were raised during a high-quality year showed higher immunoglobulin levels but lower plasma oxidative statuses compared with those in broods raised during a year of lower quality. Moreover, independent of the year effect, immunoglobulin levels also significantly increased with increasing sibling competition, while plasma oxidative status was not significantly affected by BS manipulation. In addition, we observed no significant relationships between immunoglobulin levels and TAC, TOS, and plasma oxidative status when we ignored the effects of both year and BS. These correlational results do not seem to be in line with the idea of a trade-off between these physiological traits. Although birds' immune systems were not challenged in this study, our results do not provide any evidence to support the hypothesis of a trade-off between immune function and oxidative stress. In our study, variations in IgY among years do not seem to be driven by changes in antioxidant capacity, oxidant status, or plasma oxidative status, and vice versa.

In conclusion, BS enlargement imposed morphological or growth costs on wild starling chicks but it did not significantly affect oxidative stress. Unexpectedly, increasing sibling competition had a positive influence on humoral immunity. In addition, independent of BS manipulation, physiological costs were systematically emphasized during a lower-quality year (2007). Namely, plasma oxidative status was significantly higher in 2007, independent of BS. Even if the levels of antioxidants did not significantly vary among years, their production might have been upregulated during a low-quality year to cope with the subsequent increased production of oxidants, and this mechanism might be energetically costly. Future studies should take ecological context into account when inferring about proximal factors explaining variations in oxidative stress. Whether the structural and physiological costs imposed by sibling competition will lead to long-term effects on chicks' survival and/or fitness remains to be documented.

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