Sex dependence of environmental sensitivity differs qualitatively between growth and oxidative stress in jackdaw nestlings

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Summary
Developmental conditions can have profound fitness consequences, which may be sex dependent. Little is known of the underlying mechanisms, but oxidative stress is a promising candidate. We manipulated brood size in jackdaws *Corvus monedula*, and studied effects on growth and two oxidative stress parameters: total antioxidant capacity and oxidative damage.To our best knowledge, this is the first study examining the effects of experimentally manipulated environmental conditions during early development on both growth and oxidative stress under natural conditions. Growth was reduced in enlarged broods, and this effect was significantly stronger in daughters. Total antioxidant capacity was lower in enlarged broods, irrespective of sex, although the effect was most pronounced in sons. This could indicate more oxidative stress in enlarged broods. However, oxidative damage in daughters was unaffected and in sons it was significantly higher in reduced broods. Although levels of oxidative stress were related to growth, growth by itself did not explain the relation between oxidative stress and brood size manipulation. We suggest that energy turnover, and hence ROS production, of nestlings in reduced broods was higher, and hence oxidative protection was up-regulated. In sons compensation through this up-regulation was apparently not sufficient as indicated by the higher oxidative damage in reduced broods. To infer which sex is most susceptible to environmental conditions during early development, growth is often used as fitness proxy. Our finding that it varied between traits which sex was the most affected (growth in daughters, physiology in sons) suggests that this approach could lead to erroneous conclusions. Measuring fitness rather than a fitness proxy is required to resolve this issue.

Introduction

Environmental conditions experienced during early development can have profound effects on fitness prospects in many animal species (Lindström 1999; Cam, Monnat & Hines 2003; van de Pol et al. 2006) including humans (Lummaa 2003). For example, individuals reared under impaired conditions may have lower survival rates (Dijkstra et al. 1990; Metcalfe & Monaghan 2001; Lummaa & Clutton-Brock 2002) acquire lower quality territories (Verhulst, Perrins & Riddington 1997; van de Pol et al. 2006) and/or have lower reproductive potential through decreased fertility or attractiveness (Haywood & Perrins 1992; Gustafsson, Qvarnström & Sheldon 1995; de Kogel & Prijs 1996; but see Naguib, Heim & Gil 2008).

Considering the broad and long-lasting effects of early environmental conditions on the life history of adults, surprisingly little is known about mechanisms underlying these effects, in particular under natural conditions. In some cases a direct effect of body size seems evident, for instance when there is size-dependent resource holding potential (see Briffa & Sneddon 2007 for review), but in other cases mechanisms are probably more subtle. Environmental conditions experienced during early development affected for example standard metabolic rate at adulthood (independent of body size) (Verhulst, Holveck & Riebel 2006; Criscuolo et al. 2008), nestling (Saino, Calza & Møller 1997; Fargallo et al. 2002; Parejo, Silva & Aviles 2007; Bonisoli-Alquati et al. 2008) and adult (Birkhead, Fletcher & Pellatt 1999) immunity and song quality (Nowicki, Searcy & Peters 2002; Spencer et al. 2003).

The finding that rearing conditions affect so many aspects of the adult phenotype suggests there are developmental effects beyond simply growing to a smaller size. The self-construction process of growth may somehow be executed less well, e.g. with more construction errors, when rearing conditions are poor. Pro-oxidants (e.g. free radicals and peroxides) are the inevitable byproducts of aerobic metabolism. Their high reactivity can result in damage to lipids, proteins and nucleic acids. Antioxidant defense systems prevent such damage, and consist of enzymatic antioxidants such as superoxide dismutase and catalase that work primarily within mitochondria at the site of free-radical production (Barja 2004) and micromolecular antioxidants such as vitamins E and C, uric acid and glutathione that function both in tissues and in the bloodstream (Finkel & Holbrook 2000; Costantini 2008; Cohen, Hau & Wikelski 2008). Oxidative stress, defined as the rate at which oxidative damage is generated (Costantini & Verhulst 2009), results from the imbalance between pro-oxidant production and the capability of an individual to defend itself against pro-oxidants (Ames, Shigenaga & Hagen 1993; Beckman & Ames 1998; von Schantz et al. 1999; Finkel & Holbrook 2000; Kregel & Zhang 2007; Costantini 2008). Because of its (DNA-)damaging effect, oxidative stress leads to physiological deterioration and has been linked to disease and senescence (Beckman & Ames 1998; Finkel & Holbrook 2000). Thus, oxidative stress could potentially play a mediating role in the effect of rearing conditions on fitness prospects (Monaghan, Metcalfe & Torres 2009), for example when antioxidant defenses are impaired when growing up in poor rearing conditions.

The sensitivity to environmental conditions often differs between daughters and sons (Råberg, Stjernman & Nilsson 2005; Dubiec, Cichon & Deptuch 2006; Arnold et al. 2007; Alonso-Alvarez, Bertrand & Sorci 2007; Rowland et al. 2007). A sex difference in environmental sensitivity is interesting because it may create selective pressure to invest in a specific sex in response to state, condition and/or environment of the parents, thereby facilitating the evolution of adaptive sex allocation (Clutton-Brock, Albon & Guinness 1985; see also Benito & Gonzalez-Solis 2007). Sex dependent environmental sensitivity can not be attributed to differential effects on food acquisition alone,

because Martins (2004) showed in the sexually size mono-morphic zebra finch that growth rates of females were lower in conditions of restricted food when compared to males subjected to the same restriction protocol. This suggests more subtle differences between the sexes in their response to adverse circumstances, that could involve different tolerance of oxidative stress but this has to our knowledge not been studied.

To study the interplay between sex, early environmental conditions, growth and oxidative stress, we manipulated brood size in free-living jackdaws. The trade-off between the number and quality of offspring is one of the foundations of life history theory (Lessells 1991). Increasing or decreasing the number of young thus allowed us to compare the effect of environmental conditions during the rearing period on growth and oxidative stress of nestlings. We measured body mass and tarsus length up to fledging. After two-third of the nestling period had passed, shortly after the period of peak growth, we took blood samples to measure total antioxidant capacity (TAC) and oxidative damage in plasma. At the same age we measured resting metabolic rate (RMR) overnight for a subset of nestlings because it provides an index of the energy budget (Speakman 2000), and possibly also of the rate at which ROS are generated. We expected growth to be impaired in nestlings reared in enlarged broods, and the sub-optimal growth conditions experienced in enlarged broods could result in impaired anti-oxidant defenses (Blount et al. 2003; Costantini & Dell'Omo 2006; Rubolini et al. 2006; Isaksson et al. 2007) and thus higher oxidative stress.

Methods

Study population

We studied free-living jackdaws, a hole breeding semi-colonial bird species, during the breeding seasons of the years 2005 until 2008 in the colony at the Biological Centre in Haren (the Netherlands), a semi-urban environment (36 nest boxes), and 5 smaller colonies located in a more rural area 5–10 kilometers south of Haren (5–20 nest boxes). Due to relative low occupation rates at some of these sites, data were pooled for the colonies outside the Biological Centre (OTHER).

Nest boxes were checked daily, starting in the first week of April, until the clutch was complete, and eggs were numbered with a felt tip pen. For the BC colony, clutches were moved to an incubator 1–2 days before the estimated hatching date (temperature 37.7°C, humidity 75%), to determine from which egg a chick had hatched. Upon finding, hatchlings were placed in their original nest (for details see Salomons et al. 2006). At the other colonies nests were checked daily for hatched eggs, starting one day before estimated hatching date. Hatchlings were weighed and a blood sample (10-20ml) was taken by clipping the tip of a toe nail for sexing and future DNA-analysis. Sex was determined by PCR analysis of blood samples (Griffiths et al. 1998). The reliability of this method was confirmed using adult birds of known sex ($N > 50$). The clipping of a nail tip does not interfere with nestling growth. The clipped nail is identifiable up to fledging, and we used this to identify the chicks within broods.

Brood size manipulation

We manipulated brood size at day 5 or day 6 (day of hatching = day 1). Nests with only one chick alive in the nest at the day of manipulation were not used, as these nests could only be enlarged. Pairs of nests were selected with similar hatch date and clutch size, and randomly assigned to an enlarged or reduced treatment. For reduced broods, two chicks were moved to same aged broods in the enlarged treatment. In 2005 and 2006 a full cross-foster scheme was applied where three chicks were moved from the reduced brood to the enlarged brood and one chick from the enlarged brood was moved to the reduced brood.

Growth and survival
The survival of the chicks in the nest was checked every 5 days (day of hatching = day 1). At day 10, 20 and 30 the chicks were also weighed and tarsus- and wing length (day 20 and 30) were measured. As body mass is a combined measure of size and condition, we separated these components in our analysis by replacing body mass in the model by tarsus and residuals of the regression of mass over tarsus. At day 30, shortly before fledging, the chicks were ringed.

Oxidative stress analysis
At day 20, a blood sample was taken from the chicks by puncture of the brachial vein and collected in heparinized capillary tubes. Samples were then transported on ice, centrifuged and plasma was stored at -20°C within hours. Samples were analyzed immediately after each breeding season. TAC was measured by the OXY-Adsorbent test (Diacron, Grosseto, Italy) which uses colorimetric determination to quantify the ability of the antioxidant barrier to cope with the oxidant action of hypochlorous acid (HOCl). The intensity of the colored complex was measured with a spectrophotometer (Beckman Coulter DU530) at 505nm and 546nm. We used the mean of the values obtained at both wavelengths as measure of a chick's TAC levels. Analysis was according to specifications provided by the manufacturer, with some minor changes (volume: buffer 500 µl, chromogen 10 µl, calibrator 10 µl, sample 10 µl / dilution: calibrator 1:200, sample 1:200 / Incubation 10 minutes at 37°C). All results are expressed as µmol/ml HClO neutralized per volume of serum.

The level of oxidative damage was measured by the d-ROMs test (Diacron, Grosseto, Italy), which quantifies the level of hydroperoxides, which are derived from damage to both lipids and proteins (Alberti et al. 2000; Iamele, Fiocchi & Vernocchi 2002). This method uses colorimetric determination to quantify the ability of the plasma to oxidize a chromogen, producing a complex whose color intensity is proportional to the pro-oxidative status. The protocol was slightly adjusted from factory specifications provided by the manufacturer (buffer 400 µl, chromogen 4 µl, calibrator 10 µl, sample 40 µl / incubation 90 minutes at 37°C). Absorbance was measured with a spectrophotometer (Beckman Coulter DU530) at 505nm and 546nm. Mean value calculated using measurements at both wavelengths was taken as measure of oxidative damage. All results are expressed as Carratelli Units (1 CARR U is equivalent to 0.08 mg/dl H_2O_2).

Metabolic rate
In 2006 metabolic rate of a subset of 26 nestlings (BC only) was measured at the age of 20 ±2 days overnight at 28°C (well within the thermoneutral zone for adult jackdaws (Gavrilov & Dolnik 1985)) in the dark using indirect calorimetry. Chicks were moved from the nest just before sunset and kept in a Plexiglas box (10 x 10 x 15 cm) in an open air flow system (flow rate set to deliver 60 l/h) for measuring rates of O_2 consumption and CO_2 production (for further technical details see Wiersma, Salomons & Verhulst 2005). Nestlings were returned to the nest at sunrise the next morning. To prevent parental desertion 1–2 chicks would remain in the nest to be measured the next night.

Statistical analysis
We analyzed our data using mixed models, incorporating random effects to avoid pseudoreplication effects. We used JMP (version 7.0.1, SAS Institute Inc.) for all analyses except for the survival of nestlings where we used MLWiN (version 2.0.2, Rasbash et al. 2005). Siblings are not statistically independent; therefore nest was added as a random factor to all models. Where applicable the effect size (Cohen's d, see Nakagawa & Cuthill 2007) is reported. The BC colony was less productive than the other, more rural, colonies. These differences between colonies were reflected in our estimates of growth, TAC and oxidative damage. On average, growth was reduced at the BC (mass at age

20 days: $t_{1,176} = -5.0$ $P < 0.001$; tarsus at age 20 days: $t_{1.104.1} = -5.6$ $P < 0.001$) and also levels of both TAC ($t_{1,66.5} = -4.43$ $P < 0.001$) and oxidative damage ($t_{1,60.1} = -2.45$ $P = 0.02$) were lower at the BC colony. To account for these differences, colony was added as a random factor to all models.

Results

We manipulated brood size of 90 nests, containing in total 325 nestlings ($N = 14$, 24, 17, 35 broods for 2005-2008 respectively). At manipulation, there were no significant differences between enlarged and reduced broods in either laying date, hatching date, clutch size, brood size or nestling mass (all $P > 0.1$). For 76 nests two chicks were either removed from or added to the original brood size. In one occasion three chicks were moved from reduced to enlarged. In 12 nests only one chick could be moved because there were only two (viable) chicks left in the nests assigned to the reduced treatment at the time of the manipulation. Averaging over years and colonies, there were on average 2.1 ±0.1 and 5.3 ±0.2 chicks in reduced and enlarged broods respectively after manipulation. Levels of TAC and oxidative damage were measured in 186 nestlings at the age of 20 days in 2006 and 2008, of which 160 were reared in manipulated broods. Sex was successfully determined for 95% of these chicks. As not all parameters were available for all the individual chicks sample sizes vary slightly between analyses. We tested whether being raised by either natural or foster parents affected growth or oxidative stress parameters, but this was not the case (all $P > 0.1$), and this factor is therefore not presented in subsequent analyses.

Growth

When pooling all measurements after manipulation, the body mass of chicks reared in enlarged broods was lower compared to chicks in reduced broods (Figure 3.1A; $t_{1,208.2} = -5.4$, $P < 0.001$; tested as in Table 3.1A but without the sex * manipulation interaction). The manipulation effect on body mass growth was significantly stronger in daughters compared to sons (see interaction in Table 3.1A). Note that there was also a significant sex * age interaction, indicating that daughters and sons have different growth trajectories. The manipulation effect was significant in both sexes when analyzed separately (reduced vs enlarged: daughters $t_{1,107.8} = -4.6$ $P < 0.001$; sons $t_{1,92.7} = -2.6$ $P = 0.01$). At the age that oxidative stress was measured (20 days), the effect size for daughters ($d = -0.87$) was more than double the effect size in sons ($d = -0.34$).

Tarsus length, a measure of structural body size, was smaller for chicks in enlarged broods (Figure 3.1B; $t_{1,184.5} = -2.0$, $P < 0.05$; tested in model as in Table 3.1B without the sex * manipulation interaction). Unlike for body mass, there was no significant sex * manipulation interaction effect on tarsus length (Table 3.1B). However, on closer inspection it was found that at day 10 there was hardly an effect visible of the manipulation itself on tarsus length, most likely due to the relatively short period after the manipulation in combination with the fact that tarsus length is not a very flexible trait. When the analysis was restricted to later ages, i.e. the ages 20 and 30 days, there was indeed a significant sex * manipulation effect ($t_{1,185.6} = 2.0$, $P < 0.05$), which was due to there being a larger manipulation effect on tarsus length of daughters (Figure 3.1B; $t_{1,106} = -1.9$, $P = 0.06$; effect size at 20 days: $d = -0.46$) compared to sons ($t_{1,66.2} = -0.8$, $P = 0.4$; effect size at 20 days: $d = -0.20$).

Fledging probability was significantly lower in enlarged broods (Wald test; ΔDev = 7.8, $P < 0.01$; tested using logistic regression with colony and nest as random terms, and mass at manipulation and hatch date as fixed effects). There was a trend that fledging probability was higher for sons compared to daughters (Wald test; ΔDev = 3.2, $P = 0.07$), but the manipulation effect was independent of sex (Wald test; ΔDev = 2.3, $P = 0.13$).

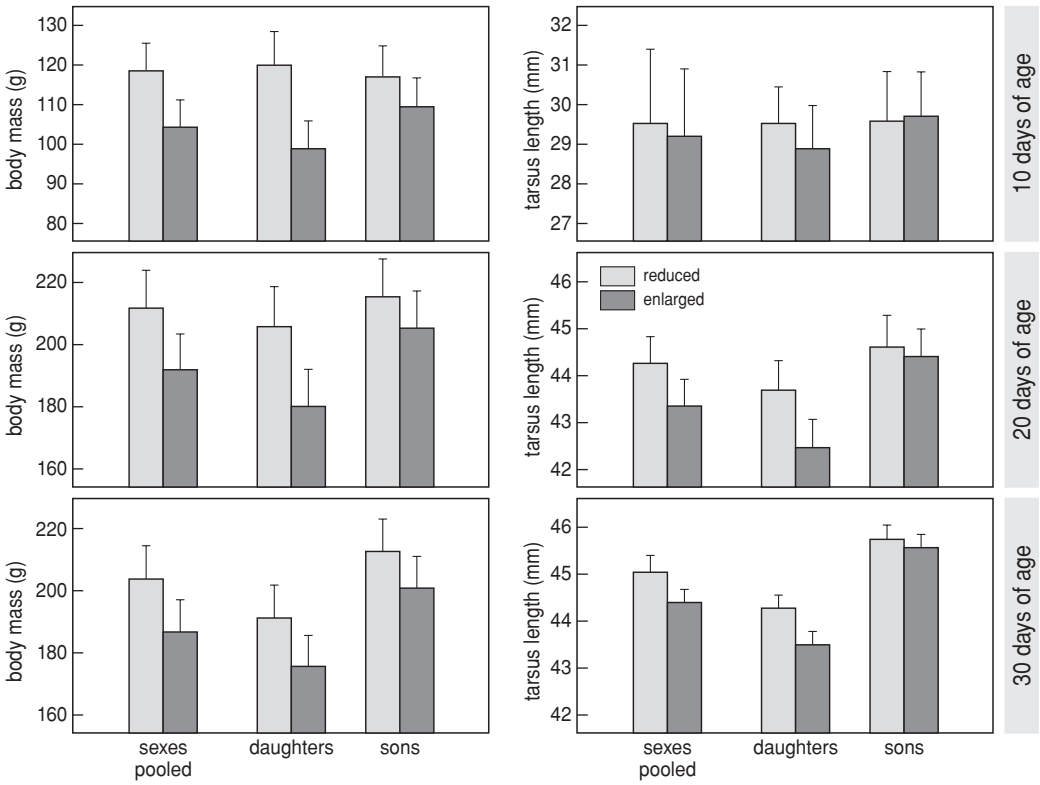

Figure 3.1. Brood size manipulation and growth, measured in body mass and tarsus length. Average and standard error are shown for sexes pooled and for daughters and sons separate.

Total antioxidant capacity

TAC was significantly lower in nestlings reared in enlarged broods (Fig. 3.2; $t_{1,148.5} = -2.1$ $P = 0.04$), and did not differ between daughters and sons. The sex * manipulation interaction was not significant ($t_{1,143.7} = -1.0$ $P = 0.3$), although the manipulation effect was substantially larger in sons ($d = -0.38$; $t_{1,70.1} = -1.6$, $P = 0.1$) as compared to daughters ($d = 0.10$; $t_{1,62.9} = 0.5$, $P = 0.6$).

Since both TAC and growth up to day 20 were higher in reduced broods, it is possible that the manipulation effect on TAC is due to the growth difference between treatments. We therefore tested whether TAC level was correlated to growth up to day 20 when TAC was measured. The correlation between TAC and body mass at the time of sampling was positive and bordered on significance (Table 3.2A), and manipulation was no longer significant in either sex when added to this model (Table 3.2A). Thus, the effect of the brood size manipulation on levels of TAC seemed to be the result of differences in growth between the treatments. Mass is determined by size as well as other factors such as energy stores, and to separate the effect of these components on TAC we replaced body mass in the model by tarsus length and the residual body mass from a regression on tarsus (including the sex * tarsus interaction). Again manipulation was rejected from the final model, which did however contain a significant interaction between residual

Table 3.1. Analysis of the effect of brood size manipulation on growth.

Dependent	Term	Estimate (Standard Error) [1)]	*DFDen*	*t* Ratio	Prob>|t|
(A) Mass	Intercept	172.3 (10.0)	8.8	17.3	<0.001
	Manipulation [Enlarged]	-20.6 (4.0)	219.6	-5.2	<0.001
N = 267 / 231 / 180 at ages 10 / 20 /30	Sex [Son]	8.7 (4.5)	209.3	2.0	0.05
	Age		424.1	-22.4	<0.001
	Age [10 days]	-53.8 (1.7)			
	Age [20 days)	32.7 (1.8)			
	Age * Sex		425.1	-2.9	<0.001
	Age [10 days] * Sex	-10.2 (2.4)			
	Age [20 days] * Sex	4.2 (2.5)			
	Manipulation * Sex	11.5 (5.3)	213.9	2.2	0.03
(B) Tarsus	Intercept	39.0 (0.8)	10.7	47.4	<0.001
	Manipulation [Enlarged]	-0.9 (0.4)	191	-2.2	0.03
N = 186 / 222 / 178 at ages 10 / 20 /30	Sex [Son]	0.8 (0.5)	183.6	1.8	0.08
	Age		330.7	-1.8	0.08
	Age [10 days]	-8.4 (0.1)			
	Age [20 days]	4.0 (0.1)			
	Age * Sex		338.0	-2.6	<0.01
	Age [10 days] * Sex	-0.7 (0.2)			
	Age [20 days] * Sex	0.4 (0.2)			
	Manipulation * Sex	0.7 (0.5)	184.4	1.2	0.2

[1)] Estimates of categorical variables represent deviation from reference category (between brackets)

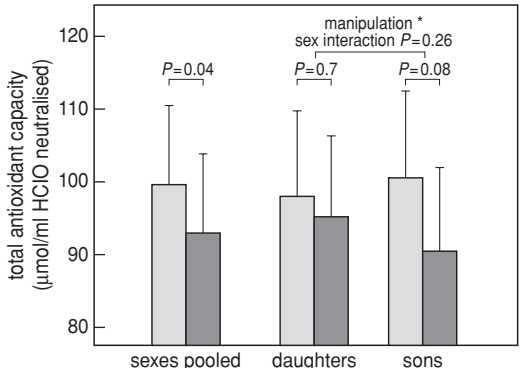

Figure 3.2. Brood size manipulation and total antioxidant capacity. TAC was lower in nestlings reared in enlarged broods (dark grey bars) compared to reduced broods (light grey bars).

Table 3.2. Analysis of the effect of brood size manipulation on total antioxidant capacity.

Dependent	Term	Estimate (Standard Error) [1)]	*DFDen*	*t* Ratio	Prob>|t|
(A) Total antioxidant capacity	Intercept	74.8 (13.5)	5.7	5.5	<0.01
	Body mass	0.1 (0.05)	146.4	1.9	0.06
	Rejected terms				
	Manipulation [Enlarged]	-4.6 (3.6)	149.4	-1.3	0.20
	Sex [Son]	-4.4 (3.1)	134.0	-1.4	0.16
	Body mass * Sex		137.7	-1.0	0.32
	Body mass [Daughter]	0.08 (0.08)			
	Body mass [Son]	0.16 (0.07)			
	Manipulation * Sex		144.2	-1.3	0.20
	Manipulation [Daughter]	0.7 (5.2)			
	Manipulation [Son]	-7.7 (6.6)			
(B) Total antioxidant capacity	Intercept	97.1 (9.5)	1.0	10.3	0.06
	Residual mass	0.02 (0.1)	146.8	0.2	0.83
	Sex [Son]	-1.7 (2.9)	127.4	-0.6	0.56
	Residual Mass * Sex	0.3 (0.1)	137.1	2.3	0.02
	Rejected terms				
	Tarsus	0.73 (1.0)	145.3	0.7	0.47
	Manipulation [Enlarged]	-3.6 (3.5)	141.4	-1.0	0.30

[1)] Estimates of categorical variables represent deviation from reference category (between brackets)

body mass and sex (Table 3.2B). This interaction was caused by a significantly positive correlation between residual mass and TAC in sons and absence of any relationship in daughters (Figure 3.3). Tarsus length was not related to levels of TAC. Thus, in sons residual mass was more important than structural size in explaining variation in levels of TAC, while in daughters neither mass component was correlated with TAC.

Oxidative damage

Levels of oxidative damage were somewhat lower in nestlings reared in enlarged broods (Figure 3.4), but this effect was not statistically significant ($t_{1,144.6} = -1.50$ $P = 0.1$). Oxidative damage levels did not differ significantly between daughters and sons, but there was a significant manipulation * sex interaction ($t_{1,141.2} = -2.2$ $P = 0.03$). When the sexes were tested separately, the manipulation effect was significant in sons (73.4 ±2.1 vs. 67.0 ±1.7; $d = -0.56$; $t_{1,36.8} = -2.3$ $P = 0.03$), but not in daughters (69.0 ±2.3 vs 70.1 ±1.6; $d = 0.10$; $t_{1,71.0} = 0.5$ $P = 0.6$).

There was no correlation between oxidative damage level and body mass at the time of sampling (Table 3.3A). Like in the analysis of TAC level, we replaced body mass in the model by tarsus length and residual body mass, as well as their interactions with sex. Residual body mass was rejected from this model, whereas the interaction between sex and tarsus was statistically significant (Table 3.3B). This interaction was caused by a negative correlation in sons ($t_{1,42.7} = -1.75$ $P = 0.09$), whereas the

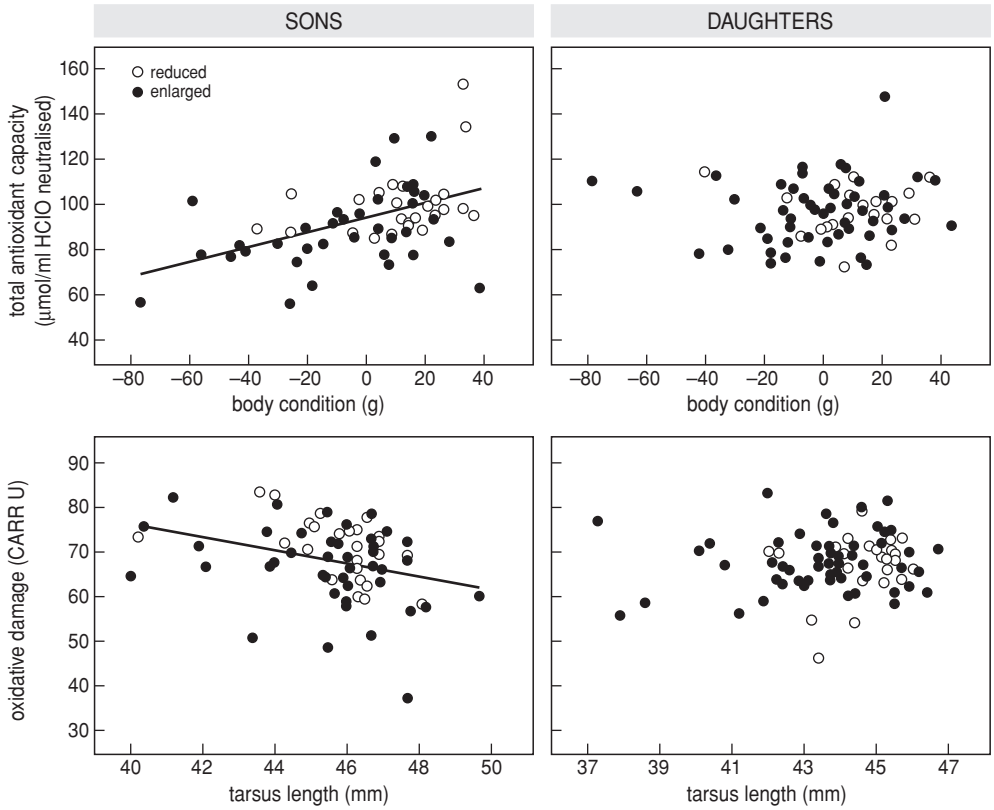

Figure 3.3. Partial residual plots of relation between total antioxidant capacity and residual body mass (top) and oxidative damage in relation to tarsus length (bottom) in sons (left) and daughters (right) in reduced broods (open circles) and enlarged broods (closed circles).

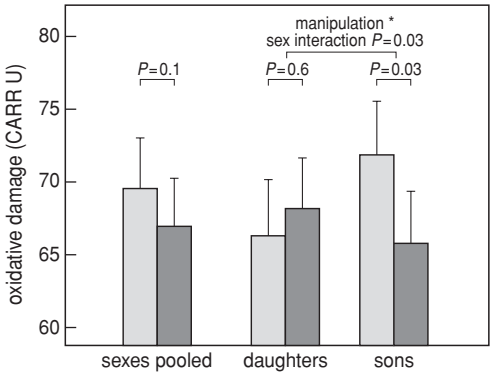

Figure 3.4. Oxidative damage in relation to brood size manipulation in sons and daughters. Light grey bars: brood size reduced; Dark grey bars: brood size enlarged.

Table 3.3. Analysis of the effect of brood size manipulation on oxidative damage.

Dependent	Term	Estimate (Standard Error) [1)]	*DFDen*	*t* Ratio	Prob> |t|
(A) Oxidative damage	Intercept	66.1 (3.9)	1.8	16.9	<0.01
	Sex [Son]	5.5 (2.9)	141.1	1.9	0.06
	Manipulation [Enlarged]	2.0 (2.6)	143.1	0.8	0.45
	Manipulation * Sex	-7.8 (3.5)	141.2	-2.2	0.03
	Rejected terms				
	Body mass	-0.04 (0.04)	128.6	-1.1	0.3
	Body mass * Sex		128.7	-1.5	0.1
	Body mass [Daughter]	0.006 (0.047)			
	Body mass [Son]	-0.063 (0.039)			
(B) Oxidative damage	Intercept	39.8 (33.0)	136.5	1.2	0.2
	Sex [Son]	6.6 (2.8)	138.5	2.3	0.02
	Manipulation [Enlarged]	2.3 (2.7)	140.5	0.9	0.4
	Manipulation * Sex	-8.3 (3.6)	137.1	-2.3	0.02
	Tarsus	0.6 (0.7)	144.0	0.8	0.4
	Tarsus * Sex	-2.1 (0.8)	119.6	-2.5	0.01
	Rejected terms				
	Residual mass	-0.01 (0.05)	136.4	-0.3	0.8
	Residual mass * Sex		124.8	0.9	0.4
	Residual mass [Daughter]	-0.05 (0.07)			
	Residual mass [Son]	0.02 (0.06)			

[1)] Estimates of categorical variables represent deviation from reference category (between brackets)

correlation in daughters was negligible (Fig. 3.3; $t_{1,68.8} = 0.47$ $P = 0.6$). Hence, in contrast to TAC, structural growth instead of residual body mass had the higher explanatory value for oxidative damage level. However, structural growth could not explain the differences between the treatment groups since the manipulation effect remained significant when tarsus length was controlled for (Table 3.3B).

Resting metabolic rate

RMR was higher in nestlings from reduced broods (Figure 3.5A; $t_{1,21.7} = -3.7$ $P < 0.01$). This effect was the same for daughters and sons (sex * manipulation: $t_{1,21.2} = -0.3$ $P = 0.8$). RMR is a measure of whole body energy consumption, whereas levels of TAC and oxidative damage are measured as concentrations. Therefore RMR should be controlled for body mass when comparing it to concentrations. Controlling for body mass, by adding it as a covariate to the model explaining RMR, revealed that neither TAC nor oxidative damage were related to RMR (TAC: $t_{1,23.0} = -1.2$ $P = 0.2$; Oxidative damage: $t_{1,17.9} = 0.6$ $P = 0.6$). Furthermore, the brood size manipulation effect on RMR was, in both sexes, fully explained by differences in body mass between treatment groups (Figure 3.5 B).

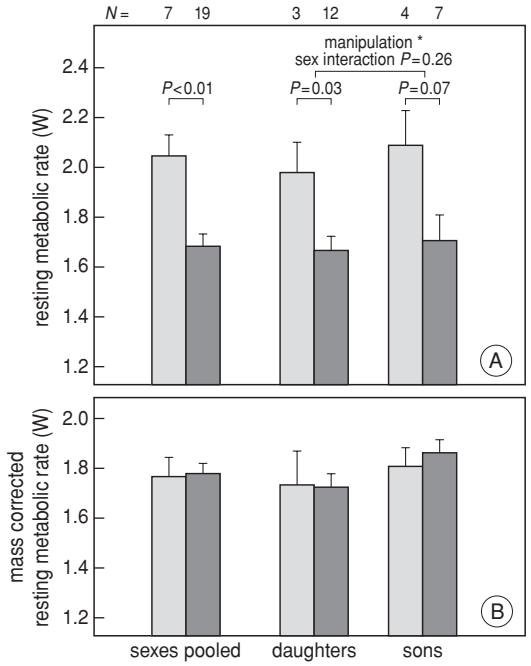

Figure 3.5. Resting metabolic rate (W) in 20 day old nestlings in relation to sex and brood size manipulation (light grey bars: reduced brood size; dark grey bars: enlarged brood size). (A) RMR. (B) RMR corrected for body mass.

Discussion

Rearing conditions, in the form of manipulated brood size, affected growth and oxidative stress parameters of nestling jackdaws, and we summarized these findings by calculating standardized effect sizes for each of these traits (Figure 3.6).

Both measures of growth (body mass, tarsus length) were lower in chicks reared in enlarged broods, but these effects were stronger in daughters compared to sons. Mass at fledging predicts subsequent survival probability in many bird species (for reviews see Magrath 1991; Schwagmeyer & Mock 2008) including jackdaws (Verhulst & Salomons 2004). Thus, our brood size manipulation is likely to have had a substantial effect on offspring survival prospects. We further anticipate that such a survival effect is likely to be stronger for daughters than for sons when we can assume that a possible mass * sex interaction with respect to survival is weak. Thus on the basis of growth it seems justified to tentatively conclude that daughters are more vulnerable to brood size effects than sons.

Sex dependent effects of brood size manipulations on growth and nestling survival have been reported more often (see Råberg et al. 2005 for review). Which sex is most susceptible to the manipulation varies between species however, or even between studies on the same species (Råberg et al. 2005). This indicates that sex per se does not determine susceptibility to environmental conditions. Sex dependent responses to manipulations could result from competition, e.g. the larger sex being least susceptible, or an increase of resource requirements with size, in which case the larger sex would be more susceptible. In jackdaws, males are the larger sex (Figure 3.1) and our finding that growth of sons was less susceptible to brood size enlargement than growth of daugh-

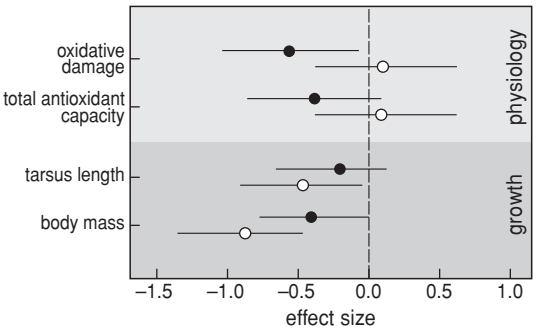

Figure 3.6. Effect size (Cohen's d) of manipulation on growth (tarsus and body mass) and physiology (total antioxidant capacity and oxidative damage) of daughters (open dots) and sons (closed dots) at the age of 20 days. Solid lines represent 95% confidence intervals. Calculated from t-statistic using the power module in the statistical software package Statistica. Dashed line indicates no effect.

ters could indicate that they do better in the competition between siblings. Sex biased parental feeding patterns could also cause these effects (Nishiumi et al. 1996; Lessells 1998; Magrath et al. 2004), when parents preferentially feed sons when food is short in supply. However, there may also be a sex difference in the efficiency with which food is converted to mass in the face of food shortage, as reported for zebra finches (Martins 2004). Given the latter finding, the conclusion that differences in the effect of the manipulation on growth reflect differences in energy consumption between sons and daughters may be premature. Sex dependent effects of brood size manipulations on growth could also arise because the sexes differ in how they change their resource allocation between growth and other processes when per capita provisioning rate declines as usually happens when brood size is enlarged.

The effects of the brood size manipulation were not limited to growth, but also affected other aspects of nestling physiology, as shown by the two oxidative stress related assays that we applied. The total antioxidant capacity is important in preventing oxidative damage, and the level of this protection mechanism was reduced in enlarged broods. Although the interaction between sex and manipulation did not reach significance, the effect size for sons was substantially larger than it was for daughters (Figure 3.6). The reduction in TAC could indicate that chicks (sons in particular) reared in enlarged broods allocated fewer resources to defense mechanisms against oxidative damage (Bortolotti et al. 2000; Costantini & Dell'Omo 2006). This was supported by the fact that the effect of the manipulation on levels of TAC could be entirely explained by differences in body condition (residual body mass over tarsus).

Surprisingly, the decreased antioxidant capacity level did not result in higher oxidative damage levels in nestlings in enlarged broods. In daughters the oxidative damage level was not affected by manipulated brood size, but in sons the oxidative damage level was actually lower in enlarged broods (Figure 3.6). This same pattern was observed when comparing colonies. While growth rate and nestling survival were highest at the OTHER colonies, levels of both TAC and oxidative damage were also higher. In fact, there turned out to be even a positive correlation between TAC and oxidative damage ($t_{1,178.1} = 2.0$ $P = 0.04$). This confirms that when studying oxidative stress it is insufficient to determine levels of oxidative protection (Costantini & Verhulst 2009), probably because oxidative protection will often be up regulated in response to increased ROS exposure (Barja 2002; Costantini 2008). Using correlational data, Nussey et al (2009) recently showed in free living Soay sheep (Ovis aries) that increased growth rate was positively correlated with oxidative damage level, in agreement with our experimental result. We therefore tested whether the manipulation effects on growth, as expressed in mass and size, could explain the increase in oxidative damage in nestlings in reduced broods. Although tarsus length was correlated to oxidative damage level, it did not explain the brood size manipulation effect. Moreover, within treatment groups there was a negative correlation between tarsus length and oxidative damage level in sons. This, in combination with the finding that the effect of manipulation on oxidative damage was observed in sons only, whereas growth estimates were instead most affected in daughters, makes it less likely that growth in the period before our measurements per se affected oxidative damage levels.

The higher level of oxidative damage in nestlings in reduced broods could reflect increased free radical production through higher daily energy expenditure. For instance, it could be that nestlings in reduced broods, similarly to nestlings of blue tits *Parus caeruleus* (Dubiec et al. 2006) and Eurasian kestrels Falco tinnunculus (Fargallo et al. 2002), allocated more resources to immune function and that, in experimentally enlarged broods, the immune

function competes with other physiological functions, such as growth (Saino et al. 1997; Hõrak et al. 1999). In our study this should then result in differential allocation of energy towards somatic maintenance between nestlings in enlarged and reduced broods. Also, higher body mass (Daan et al. 1989) as well as higher growth rates (Dietz & Drent 1997) are accompanied by a higher level of metabolic rate, as was shown by Massemin and colleagues in nestlings of the Eurasian kestrel (Massemin et al. 2002). Thus, it can be expected that ROS production was higher in nestlings in reduced broods a result of increased energy metabolism. Levels of RMR were indeed found to be higher in nestlings in reduced broods. However, when RMR was corrected for body mass, to correctly compare it to our estimate of oxidative damage, there was no additional brood size manipulation effect on RMR (Figure 5b). It is worth mentioning however that RMR comprises only a part of total daily energy expenditure (DEE), which we did not measure, and it is not unlikely that the relation between RMR and DEE differed between treatments (Wiersma et al. 2005), with chicks in small broods having a higher DEE/RMR ratio compared to chicks in large broods.

Oxidative damage level was higher in nestlings reared in reduced broods (Figure 3.4), which is not what one would expect given that birds reared in reduced broods generally have better fitness prospects. However, a similar pattern was observed in Soay sheep lambs, where the level of oxidative damage was positively associated with growth rate (Nussey et al. 2009), which in turn is positively associated with fitness prospects (Cluttonbrock et al. 1992). These data suggest that investment in growth entails costs in terms of oxidative damage, and further research is needed to assess whether individuals that combine a high growth rate with low oxidative damage have better fitness prospects than individuals in which both growth rate and oxidative damage are high. What our data do show is that although, compared to daughters, growth of sons was less affected by brood size manipulation, an effect on physiology was only observed in sons (Figure 3.6). This sex dependent response to the brood size manipulation shows a resemblance to the study on brood size manipulation effects in blue tit nestlings by Dubiec et al (2006), who showed that, with respect to growth daughters responded stronger to the manipulation, whereas immune function (response to PHA) was more affected in sons. Obviously, studies on the effect of rearing conditions can be highly dependent on the parameter of interest. Moreover, daughters and sons may differentially trade-off growth against other (physiological) parameters like immunity and somatic maintenance and repair.

Sex differences in the trade-off between growth and physiological condition could be induced when selective pressures towards larger body size are stronger in one of the sexes, which in the case of the jackdaw is supported by the fact that sexual size dimorphism has evolved, with males being the larger sex. This selective pressure could arise for example through an effect of body size on social dominance, as in jackdaws, dominance rank has been shown to affect future survival and reproductive success (Henderson & Hart 1995; Verhulst & Salomons 2004) and the rank of a breeding pair is primarily dependent on the status of the male (Röell 1978). In jackdaws adult (structural) body size is almost fully determined at fledging (Fig. 3.1), therefore growth during the nestling phase may be most important for sons. Larger body size has been shown to be positively associated with social dominance in two other corvids (carrion crow, Corvus corone corone (Richner 1989) and rooks Corvus frugilegus (Røskaft 1983)). However, in jackdaws such a relationship between social dominance and body size was not found (Henderson & Hart 1995; Verhulst & Salomons 2004), although sample sizes in both studies were modest. Nevertheless, investing in growth under sub-optimal conditions, at the

expense of other physiological costs, may be optimal for jackdaw sons, whereas for daughters body size may be less important and hence more resources are allocated towards physiological maintenance and repair. Ultimately, this may even result in equal effects of the brood size manipulation on fitness for both sexes. To our knowledge, only three studies that show sex dependent manipulation effects on growth also investigated effects on subsequent offspring survival (Oddie 2000; Råberg et al. 2005; Nicolaus et al. 2009). Strikingly, in all three studies the sex * manipulation interaction with respect to first year recruitment was not statistically significant. Hence earlier conclusions that one sex was more susceptible to an environmental condition than the other may have been premature. Clearly, more studies are needed to test whether sexes differ in sensitivity to environmental conditions during early development in terms of fitness, which is ultimately the only relevant parameter in this context.

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