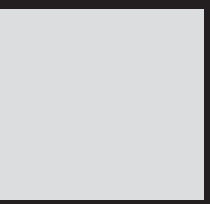


Reproductive effort, parental condition and oxidative stress in a free-living corvid

H. Martijn Salomons
Simon Verhulst

Summary
During life, each individual is faced with the fact that available resources (i.e. time, effort and energy) in any particular environment are finite. As a result, resources allocated to one trait or activity are no longer available for investment in other traits or activities. In order to maximize fitness, iteroparous species should therefore balance the resources allocated to the offspring (e.g. time, food) against investment in traits that increase their own survival (e.g. somatic maintenance and repair). We conducted a brood size experiment aimed at providing more insight into these mechanisms, by assessing its effect on several physiological indicators of body condition and survival. Brood size enlargement resulted in an increase in parental effort, because cumulative brood mass production approximately doubled. Nonetheless, effects on physiological indicators of parental condition were weak. Haematocrit levels were slightly lower in parents rearing enlarged broods, but body mass, buffy coat and levels of anti-oxidant protection and oxidative damage were not significantly affected. Since there was a significant effect of treatment on offspring quality, this might indicate that although parents increased their reproductive effort after brood size enlargement, there was a limit to this increase. Although the amount of parental effort was as such less than optimal to provide for the offspring, parents rearing enlarged broods by limiting their effort seemingly avoided detrimental effects on their own expected fitness benefits. However, data directly linking brood size manipulation to actual fitness costs of both parents and offspring are required to draw conclusions on this issue.

Introduction

Life history theory predicts that individuals trade-off current reproductive effort against future reproduction to maximize fitness (Williams 1966; Charnov & Krebs 1974; Stearns 1992; Roff 1992). This trade-off could result from the fact that resources invested in reproduction are no longer available for self-maintenance and somatic repair. Another possibility is that a certain activity (i.e. increased metabolism) itself has negative consequences for other traits. One mechanism via which this trade-off can occur is oxidative stress resulting from increased daily energy expenditure in combination with sub-optimal levels of anti-oxidant protection (Costantini 2008; Monaghan et al. 2009). Pro-oxidants (e.g. free radical oxygen species; ROS) are the inevitable byproducts of aerobic metabolism (Balaban et al. 2005). Their high reactivity can result in damage to lipids, proteins and nucleic acids. Antioxidant defense systems, are made up 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 et al. 2008). The imbalance between pro-oxidant production and the capability of an individual to defend itself against and/or repair damage caused by these pro-oxidants leads to oxidative stress (Ames et al. 1993; Beckman & Ames 1998; von Schantz et al. 1999; Finkel & Holbrook 2000; Kregel & Zhang 2007; Costantini 2008).

To test the hypothesis that oxidative stress is a mediator of the trade-off between current and future reproductive output, we manipulated brood sizes of free-living jackdaws, by either addition or removal of nestlings. Brood size manipulations are a classical and popular tool to manipulate parental effort and data from other field experiments have shown that they do indeed increase the energetic demands of parental birds (Røskaft 1985; Gustafsson & Sutherland 1988; Verhulst 1995; Nilsson 2002). There have been a limited number of studies investigating effects of increased reproductive effort on oxidative stress. However, these only focused on either concentrations of specific antioxidants (Wiersma et al. 2004) or the resistance of the blood to oxidative stress (Alonso-Alvarez et al. 2004; Alonso-Alvarez et al. 2006; Bertrand et al. 2006). As levels of anti-oxidant capacity may actually be up-regulated as a reaction to increased levels of pro-oxidants (Barja 2002; Costantini & Verhulst 2009), it remains unclear whether high levels of anti-oxidant capacity indicate either low or high levels of oxidative stress. Therefore, measuring only anti-oxidant capacity, like in the aforementioned studies, is insufficient to make firm conclusions about actual levels of oxidative stress. For this reason we measured not only levels of total anti-oxidant capacity (TAC), but also levels of reactive oxygen metabolites which are formed through the interaction between ROS and somatic tissue (primarily lipids) and can be used as an indicator of pro-oxidative status and oxidative damage.

Brood size manipulation has been shown to lower parental fitness and survival in several species (e.g. Reid 1987; Dijkstra et al. 1990a; Moreno et al. 1995; Jacobsen et al. 1995; Tinbergen & Verhulst 2000). To study the possible interplay between body condition, oxidative stress and reproductive effort, we measured not only levels of oxidative stress for each individual in this study, but also several estimates of body condition parameters. By definition, measures of (physiological) state can only be considered a condition index when they are associated with fitness (i.e. reproduction and survival). Firstly, we measured body mass, which after correction for structural body size (in our case tarsus length), can be used as an index of condition (Vandermeer & Piersma 1994). Residual body mass is very commonly used, and higher values are generally seen as

an indicator of good condition because of their association with higher fitness prospects (e.g. Ebbinge & Spaans 1995; Cichon et al. 1998; Vleck & Vleck 2002; Verhulst et al. 2004), but see (Houston & McNamara 1993; Verhulst 1998). Secondly, we measured haematocrit (i.e. the proportion of red blood cells per volume of blood). Haematocrit largely determines the oxygen transport capacity (Gentry et al. 1997; Burness et al. 1998; Hammond et al. 2000), and there are several indications that high haematocrit indicates good condition in birds (see Fair et al. 2007 for a review) . Observational studies report that haematocrit is typically lower in birds presumed to have suffered food shortage (Svensson & Merilä 1996; Piersma et al. 2000; Møller & Petrie 2002; Sánchez-Guzmán et al. 2004; Jenni et al. 2006). Furthermore, experimentally increased egg production, which exhausts resources, resulted in a decrease in haematocrit in the great skua *Stercorarius skua* (Kalmbach et al. 2004). And oystercatchers *Haematopus ostralegus* captured in winter were more likely to be found dead in the following year when their haematocrit was low (Verhulst et al. 2004). Our third parameter of body condition is the buffy coat, which is given by the proportion of cells other than erythrocytes (primarily leukocytes) per volume of blood (Wardlaw & Levine 1983). Buffy coat indicates acute or chronic infections (Harrison & Harrison 1986; Gustafsson et al. 1994) and can therefore be expected to be higher in birds with low condition. There are a few observational studies on free-living birds that do indeed show a relation between buffy coat and body mass (Verhulst et al. 2002; Møller & Petrie 2002), reproduction (Moreno et al. 1998) and survival (Verhulst et al. 2004). While collared flycatchers *Ficedula albicollis* rearing experimentally enlarged broods had more blood parasites and a larger buffy coat (Gustafsson et al. 1994).

Previously, it has been shown that social dominance plays an important role in the life history of jackdaws affecting especially reproductive success and sex allocation (Henderson & Hart 1995; Verhulst & Salomons 2004; Salomons et al. 2008). Interestingly, reproductive success in the colony also observed in this study was found to be impaired in high ranked individuals. This effect was found to be primarily mediated by a lower body condition of females paired to high ranked males. Hence, we were interested to see whether the position in the hierarchy also affected levels of oxidative stress of adult jackdaws.

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 a colony at the Biological Centre (BC) in Haren (the Netherlands), a semi-urban environment (36 nest boxes), and five smaller colonies located in a more rural area 5–10 kilometers south of Haren (colony size ranging from 5 to 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).

During the nestling period, both the male and the female were caught in their nest box (simultaneously when possible) using remote controlled trap doors, when their nestlings were 5 days old and a second time 15 days later. Immediately after capture a blood sample (~500 µl) was taken using puncture of the brachial vein and collected in heparinized capillary tubes. Samples were then transported on ice, haematocrit (amount of red blood cells relative to total plasma volume) and buffy coat (amount of leukocytes and thrombocytes relative to total plasma volume) were measured with a caliper after centrifugation (Hettich Zentrifugen, Mikro 12–24; 8 minutes at 8000 rpm) and plasma was stored at –20°C within hours. Body mass and size (tarsus, head and bill) were measured and birds new to the colony were individually marked with color

rings and a metal numbered ring. As a rule birds were released within 20 min after capture. All animals were handled in strict accordance with good animal practice, and all animal work was conducted under license from the Animal Experiments Committee of the University of Groningen (# D4071).

Brood size manipulation

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. Length and width of the eggs were measured to the nearest 0.1 mm, and egg volume (V, in cm3) was estimated using the formula: $V = \pi A^2 L K / 6$, where A is width, L is length and for jackdaws K = 0.00096 (Soler 1988). For the BC colony, clutches were moved to an incubator one to two 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 (10–20 µl) was taken by clipping the tip of a toe nail for sexing and future DNA-analysis. The clipping of a nail tip does not interfere with nestling growth. The clipped nail is identifiable by a blunt tip up to fledging, and we used this to identify the chicks within broods until they were ringed at the age of 30 days.

We manipulated brood size at day five or day six (day of hatching = day one). Nests with only one chick alive in the nest at the day of manipulation were not used in the analysis, as these nests could only be enlarged. Parents were randomly assigned to an 'enlarged' or 'reduced' treatment in their first 'manipulation year'. Parents stayed on this treatment in consecutive years, to study long-term effects of increased reproductive effort. For 'reduced' broods, two chicks were moved to same aged broods in the 'enlarged' treatment.

Oxidative stress analysis

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 anti-oxidants in the plasma 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 TAC levels. Analysis was according to specifications provided by the manufacturer, with some minor changes (volume: buffer 500ml, 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.

Oxidative damage was measured by the d-ROMs test (Diacron, Grosseto, Italy). This kit 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 the amount of oxidative damage. All results are expressed as Carratelli Units (one CARR U is equivalent to 0.08 mg/dl H_2O_2).

Social dominance

Agonistic interactions were recorded during March and the first half of April, until the first egg in the colony was laid. Conflicts are resolved in different ways, either through displacement, threat or physical fighting and these were all scored to obtain a rank-order (Röell 1978). To stage conflicts, food was offered in

small pits (Ø10 cm) approximately 10 m from the nearest nest box. The success in agonistic interactions of an individual bird was calculated using "David's score" (David 1987; Gammell et al. 2003). We then scaled rank between 0 and 1 (most and least dominant male respectively) because the number of birds in the hierarchy differed slightly between years (for further details see Salomons et al. 2008).

Statistical analysis

We analyzed our data using mixed models, incorporating individual and year as random effects to avoid pseudo-replication. We used JMP (version 7.0.1, SAS Institute Inc.) for all analyses.

Results

Brood size manipulation

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 $P > 0.1$). Averaging over years and colonies, there were on average 1.9 ±0.9 and 5.2 ±1.3 chicks in reduced and enlarged broods after manipulation. Although mortality of nestlings in enlarged broods at the BC colony was higher compared to the other colonies (Figure 5.1A), average brood size at day 20 of 'enlarged' broods was still higher compared to 'reduced' broods at all colonies (BC 2.5 ±0.2 vs. 1.1 ±0.2; $t_{1,43} = 4.4$ $P < 0.001$; OTHER 5.2 ±0.1 vs. 2.2 ±0.2; $t_{1,38} = 14.0$ $P < 0.001$). To provide an estimate of reproductive effort we summed the increase in body mass of all nestlings in the brood after brood size manipulation up until the age of 20 days when the parents were caught. Reproductive output of parents rearing enlarged broods was twice that of parents rearing reduced broods (Figure 5.1B; 617.0 ±44.6 g vs. 298.1 ±45.1 g; $t_{1,79} = 5.0$ $P < 0.001$). Although the difference was smaller at the BC colony (BC 306.7 ±36.7 g vs. 181.2 ±35.8 g OTHER 912.5 ±35.8 g vs. 427.3 ±37.6 g; Interaction: $t_{1,77} = -4.93$ $P < 0.001$) brood mass production of parents rearing enlarged broods was still significantly higher at both colonies when tested separate (BC $t_{1,39} = 2.6$ $P = 0.01$; OTHER $t_{1,38} = 8.9$ $P < 0.001$).

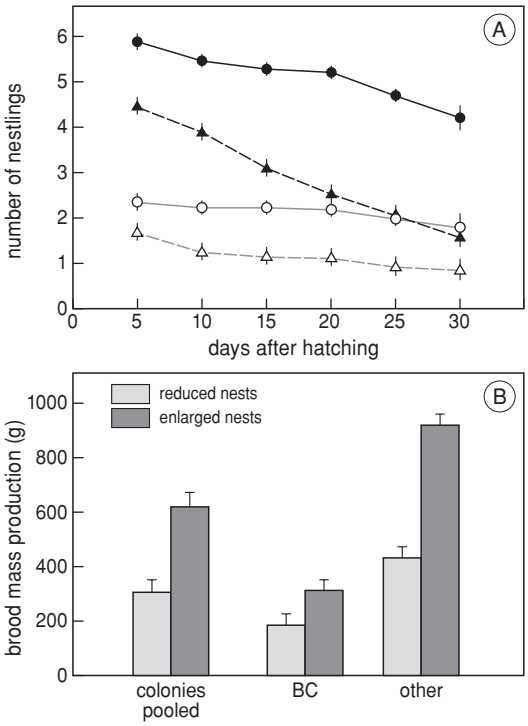


Figure 5.1. Change in parental effort after brood size manipulation. Although the number of offspring per nest was lower at the BC (triangles) than at the other colonies (circles), within colonies the total number of nestlings throughout the rearing period (A) was always higher in enlarged nests (closed symbols) and cumulative brood mass production (B) was also substantially higher in enlarged nests (dark grey bars) compared to nests reduced (light grey bars) in size.

We caught 185 different adults at least once during the breeding seasons of 2005-2008. In total we collected 312 blood samples either at day 5 or day 20. In 40 cases an individual was only caught at day 5, in 70 cases an individual was only caught at day 20. For 106 adults a

sample was available for both days within the same year. We manipulated brood size of 88 nests, where at least one of the parents was caught during the nestling period (N = 14, 23, 19, 32 broods for 2005-2008 respectively), 95 parents whose brood was manipulated were caught twice within the same year ('enlarged': N = 52, 'reduced': N = 43).

Body condition

Body condition (residual body mass over tarsus) was lower at day 20 compared to day 5, but not significantly different between parents rearing either 'enlarged' or 'reduced' broods in either sex (enlarged: –0.8 ±1.4 g vs reduced: –0.4 ±1.4 g; $t_{1,145.4} = -0.3$ $P = 0.8$). Testing only those individuals that were caught at both day 5 and day 20 showed that, on average, parents lost 3.7±0.7g of body mass ($t_{1,145} = -4.9$ $P < 0.001$) over this period, and that females lost more mass than males (Figure 5.2A; 5.5 ±1.0g vs. 1.7 ±1.0 g; $t_{1,145.7} = -2.7$ $P < 0.01$). Parents rearing 'enlarged' broods lost slightly more mass compared to parents rearing 'reduced' broods (–6.1 ±1.4 g vs. –4.6 ±1.4 g), but this difference was not statistically significant ($t_{1,114.7}$ = –1.0 $P = 0.3$). There was also no effect of the manipulation on body mass change when the sexes were tested separately, although males seemed a bit more affected compared to females (difference between 'enlarged' and 'reduced' in males: –3.0 ±2.4 g and females: 0.3 ±2.3 g) this was far from significant ($t_{1,114.1}$ = 0.8 $P = 0.4$).

Repeatability of measurements of haematocrit and buffy coat within the same individual were 43.3 ±0.07% ($F_{97,169} = 3.1$ $P < 0.001$) and 30.5 ±0.07% ($F_{97,169} = 2.2$ $P < 0.001$) respectively (tested using measurements at all years and stages of reproduction). During breeding, haematocrit levels were higher in females than in males (0.49 ±0.003 vs 0.48 ±0.003; $t_{1,159.2} = -3.1$ $P < 0.01$). This was mostly caused by the fact that in females, haematocrit level was higher at day 20 compared to day 5 (0.50 ±0.005 vs 0.48 ±0.005; $t_{1,70.7} = 6.6$ $P < 0.001$), while in males

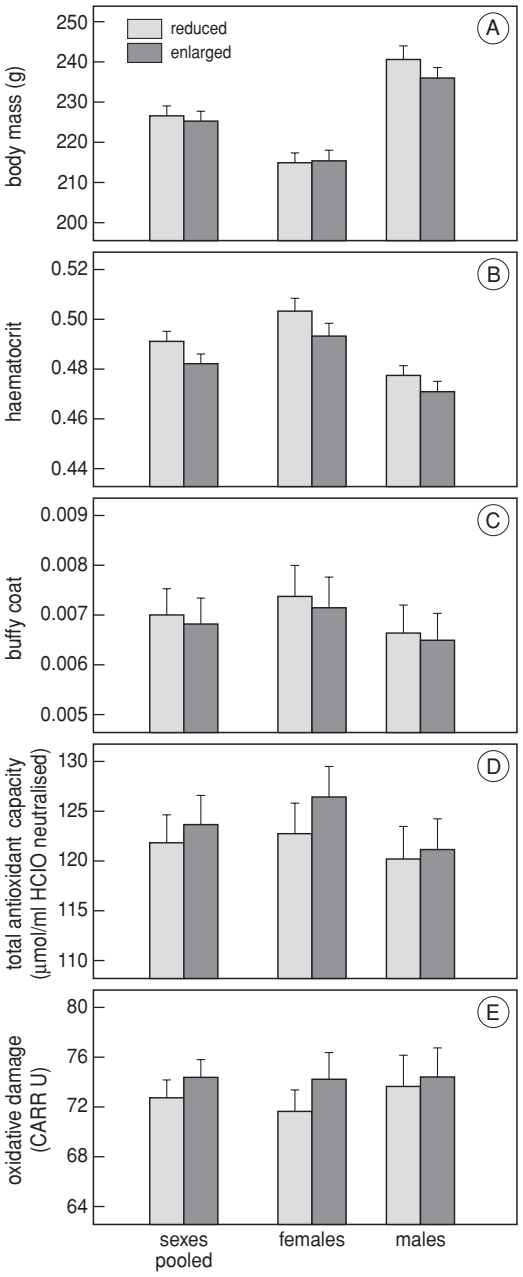


Figure 5.2. Physiological differences between parents rearing broods reduced (light grey bars) and enlarged in size (dark grey bars).

haematocrit levels did not change (0.48±0.003 vs. 0.48 ±0.003; $t_{1,69.8} = -0.2$ $P = 0.9$). Correcting for sex there was a trend that haematocrit levels at day 20 were higher in parents rearing 'reduced' broods (Figure 5.2B; sexes pooled; 0.50 ±0.004 vs 0.49 ±0.004; $t_{1,129.1} = -1.9$ $P = 0.06$). There was no significant sex * manipulation interaction. Adding haematocrit level at day 5 to the model (limiting the sample size to only those individuals that were caught twice during the rearing period) did not change this result quantitatively but the difference between the treatment groups for the sexes combined did become significant (sexes pooled; 0.51 ±0.005 vs 0.48 ±0.005; $t_{1,77.4} = -2.6$ $P = 0.01$). Buffy coat did not differ between day 5 or day 20 (0.007 ±0.0006 vs 0.007 ±0.0006; $t_{1,142.7} = 0.3$ $P = 0.8$), but was higher in females compared to males (0.007 ±0.0005 vs 0.006 ±0.0006; $t_{1,80.9} = -2.9$ $P < 0.01$). Testing only individuals for which a sample was available at both days also showed that buffy coat did not change between day 5 and day 20 in both sexes (0.007 ±0.0006 vs 0.007 ±0.0006; $t_{1,171.3} = -0.7$ $P = 0.5$). And there was also no effect of brood size manipulation (Figure 5.2C; 0.007 ±0.0007 vs 0.008 ±0.0007; $t_{1,96.9} = 0.5$ $P = 0.6$).

Oxidative stress

Levels of oxidative stress were estimated by levels of TAC and oxidative damage of the plasma. Within individuals, repeatability of these measurements (tested using measurements at all years and stages of reproduction) was moderate for TAC (20.0±0.07%; $F_{93,158} = 1.7$ $P = 0.002$) and somewhat lower for levels of oxidative damage (6.4 ±0.07%; $F_{93,158} = 1.2$ $P = 0.2$) suggesting a more dynamic (short-term) character of these parameters compared to haematocrit and buffy coat. In females, the level of TAC was marginally lower at day 5 compared to day 20 (119.3 ±3.1 vs 122.5 ±3.1; $t_{1,59.3} = 1.6$ $P = 0.1$). In males these levels also did not differ significantly (123.4 ±2.1 vs 122.3 ±2.1; $t_{1,46.8} = -0.5$ $P = 0.6$). The level of TAC at day 20 was independent of brood size manipulation (Figure 5.2D; $t_{1,117.7} = 1.0$ $P = 0.3$). The level of oxidative damage at day 5 and 20 were not correlated (Figure 5.3B; $t_{1,103.6} = 0.8$ $P = 0.4$), but decreased significantly over the nestling period (day 5: 77.5 ±1.1 day 20: 72.9 ±1.1; $t_{1,129.7} = -3.7$ $P < 0.001$). This decrease was independent of brood size manipulation ($t_{1,101} = 0.6$ $P = 0.6$). Interestingly, TAC at day 5 did have some predictive value on the level of oxidative damage at day 20. Individuals with higher levels of TAC at day 5 had lower levels of oxidative damage 15 days later (Figure 5.3E; $t_{1,100.2} = -2.5$ $P = 0.01$). This fit was higher than the correlation between TAC and oxidative damage within samples (Figure 5.3C,D; day 5: $t_{1,142.2} = 1.2$ $P = 0.2$; day 20: $t_{1,169} = 1.8$ $P = 0.08$).

There was a positive correlation between haematocrit and the level TAC (Figure 5.4A; $t_{1,300.8} = 3.6$ $P < 0.001$). This could not be explained through an effect of body condition as we found at the same time a trend towards a negative correlation between residual body mass and the level of TAC ($t_{1,180} = -1.8$ $P = 0.08$). We found no correlation between haematocrit and the level of oxidative damage (Figure 4B; $t_{1,265.8} = -1.3$ $P = 0.2$). However, our data did show a negative correlation between haematocrit measured at day 5 and the level of oxidative damage 15 days later at day 20 ($t_{1,88.6} = -2.6$ $P = 0.01$). Although the relation between haematocrit level at day 20 and oxidative damage at the same day was still negative, the slope was much less steep and far from significant ($t_{1,135.5} = -0.4$ $P = 0.7$). This may suggest that there was a delay in the increase of levels of reactive oxygen metabolites in the plasma.

When levels of TAC and oxidative damage were expressed as concentration per volume of blood instead of per volume of plasma, the correlation between these two parameters was much higher than for the uncontrolled values (day 5: $r = 0.14$; $t_{1,137.5} = 2.3$ $P = 0.03$; day 20: $r = 0.24$; $t_{1,165.8} = 3.5$ $P < 0.001$), as can be expected since they were multiplied with the same factor. More importantly, the concentration of TAC in the blood was significantly higher in females

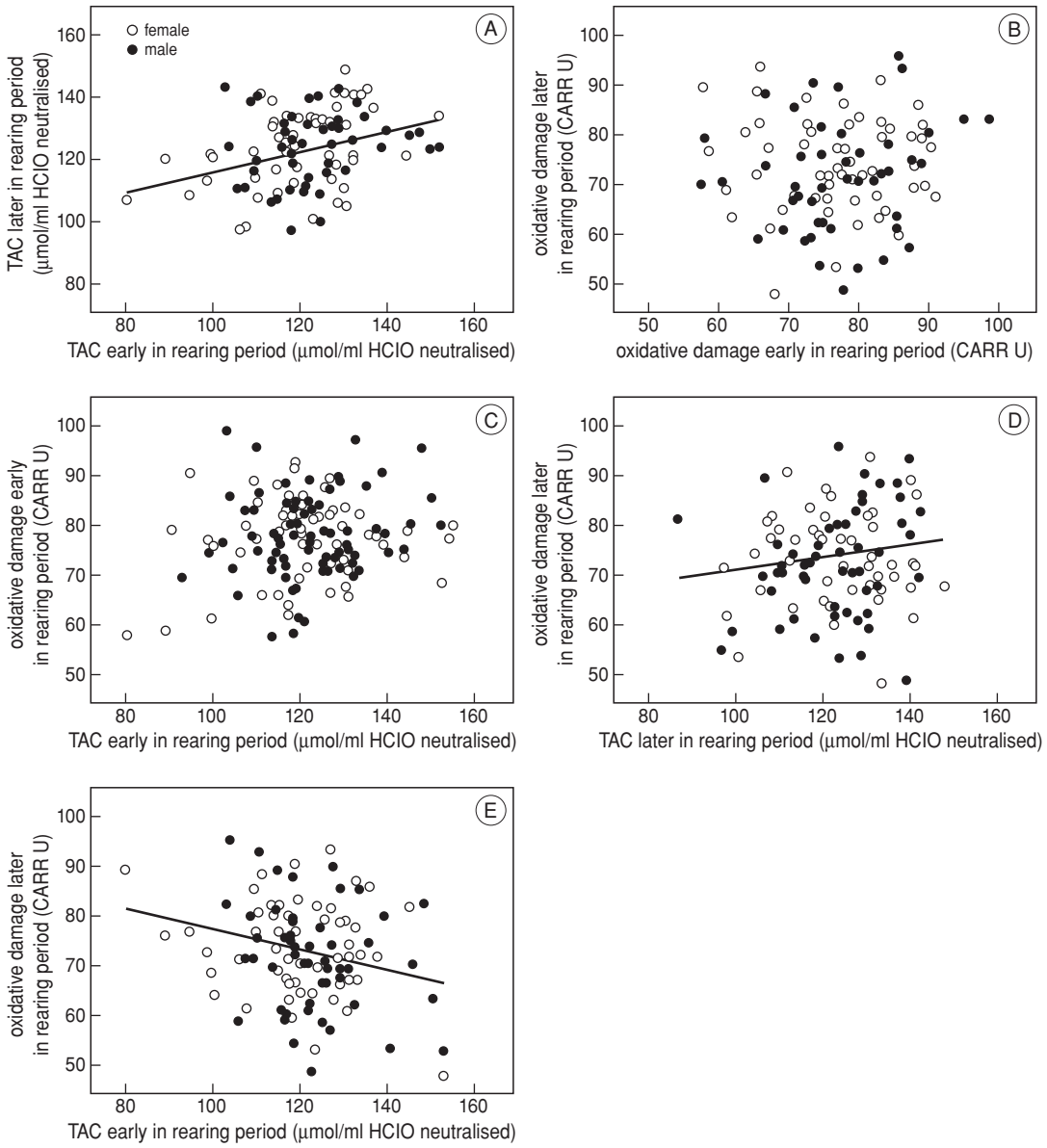


Figure 5.3. Correlations between levels of total anti-oxidant capacity and oxidative damage early and late in the rearing period for female (open symbols) and male (closed symbols) parents.

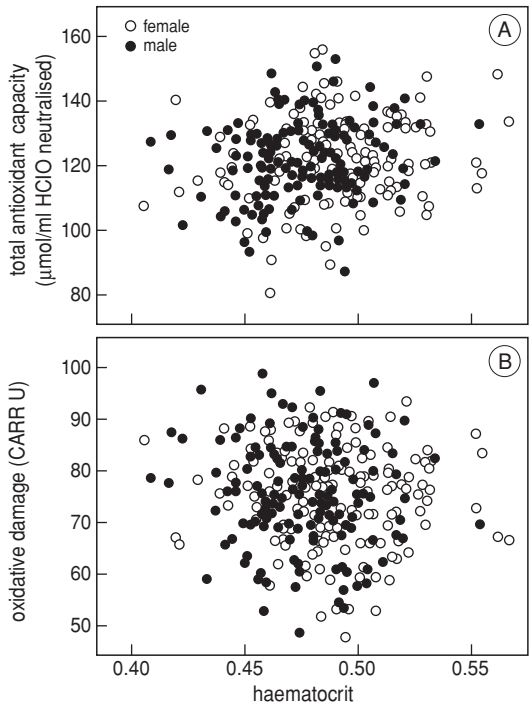
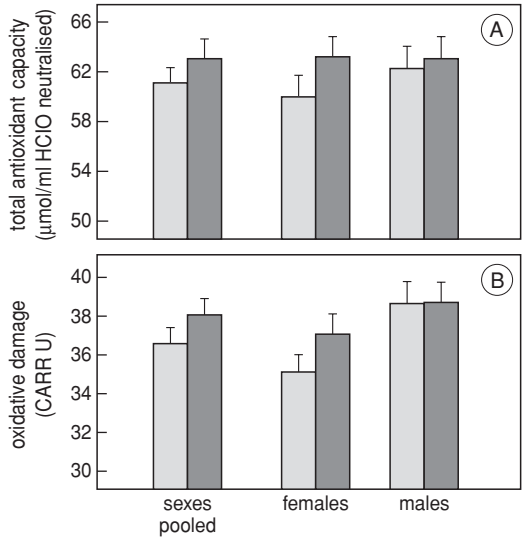


Figure 5.4. Correlation between haematocrit and the level of total anti-oxidant capacity and oxidative damage for female (open symbols) and male (closed symbols) parents.



rearing enlarged broods (Figure 5.5A; 63.0 ±1.8 vs 59.7±1.8; $t_{1,60.1} = 5.0$ $P = 0.03$). Correcting the level of oxidative damage for haematocrit showed that these levels tended to be higher in females rearing enlarged broods (Figure 5.5B; 37.0 ±0.9 vs 34.9 ±0.9; $t_{1,54.3} = 1.6$ $P = 0.1$). There was no significant difference between males rearing either 'enlarged' or 'reduced' broods in both TAC and oxidative damage expressed as concentration per volume of blood.

Social dominance

Males with a high position in the social hierarchy (BC data only) had higher body mass (independent of tarsus) during the rearing period (data pooled for day 5 and day 20) than subordinates (Figure 5.6B; $t_{1,68.5} = -2.1$ $P = 0.04$). This suggested that these males were in better condition. However, haematocrit levels seemed to contradict this, as these were significantly lower in high ranked males (Figure 5.6D; $t_{1,43.8} = 2.6$ $P = 0.01$). Buffy coat was not related to social rank in males, but in females buffy coat (log transformed) at day 5 was higher in more dominant individuals (Figure 5.6E; $t_{1,17.4} = -3.4$ $P < 0.01$). This suggested that these females were in lower condition; however body mass during the nestling period was not correlated with social rank in females (Figure 6A). There was a significant quadratic relation between social dominance and haematocrit in females early in the rearing period, with females of intermediate rank having the highest haematocrit (Figure 5.6C; $t_{1,28.5} = -2.1$ $P = 0.04$). However, this relation was caused by very low haematocrit values for the least dominant females, while there was little difference between high and intermediate ranked females. At day 20 the difference in buffy coat between females of different rank had disappeared (Figure 5.6F).

Figure 5.5. Concentration of total anti-oxidant capacity (A) and oxidative damage (B) per volume of blood for parents rearing broods reduced (light grey bars) or enlarged in size (dark grey bars).

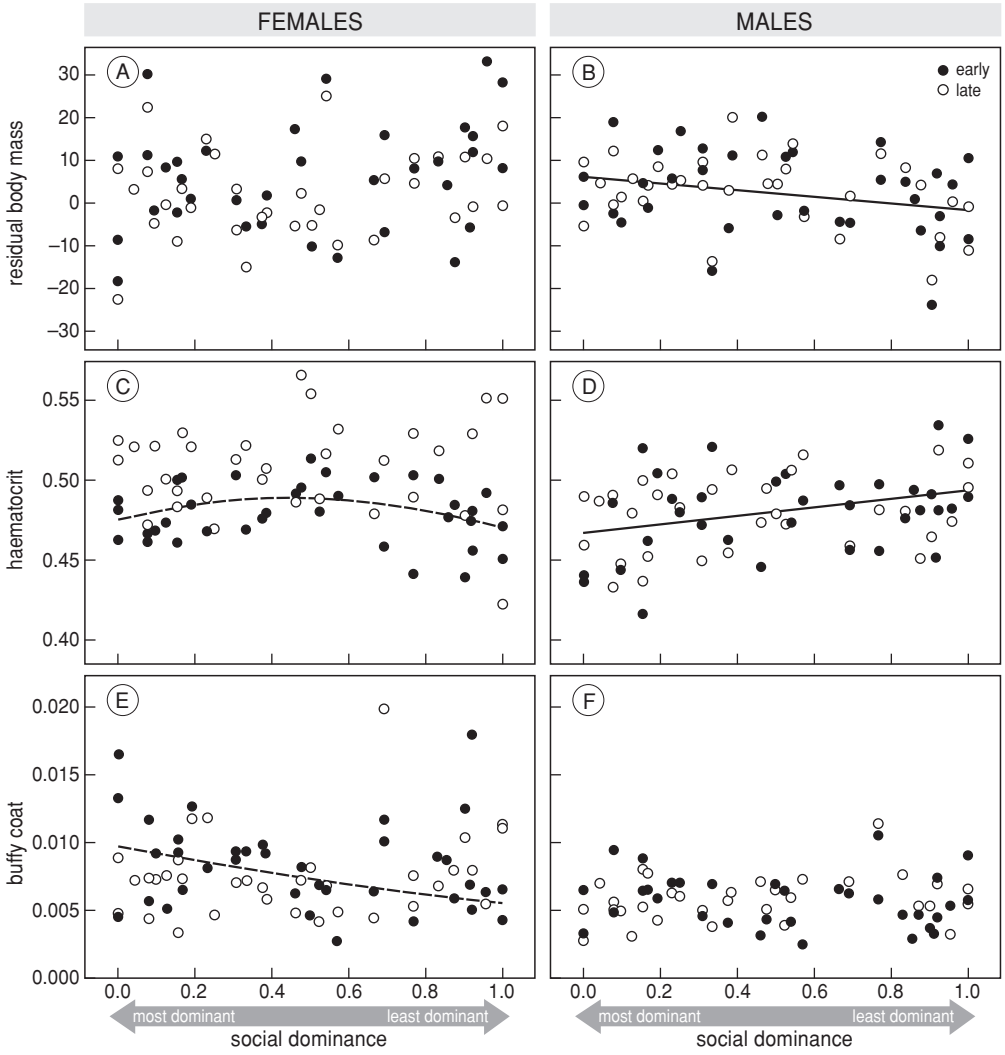


Figure 5.6. Correlation between social dominance and residual body mass, haematocrit and buffy coat of female (left) and male (right) parents early (closed symbols) and late (open symbols) in the rearing period.

Although buffy coat seemed to indicate that females mated to dominant males were in poorer condition early in the rearing period, levels of oxidative damage seemed to contradict this as these were instead higher in females mated to subdominant males (Figure 5.7C; $t_{1,29.3} = 2.4$ $P = 0.02$). Similar to buffy coat this difference had disappeared by day 20 (Figure 5.7D). Although some of the other graphs shown in Figure 5.7 seem rather suggestive, the level of oxidative damage was not related to social dominance in males, and also TAC was not significantly related to social dominance in both sexes.

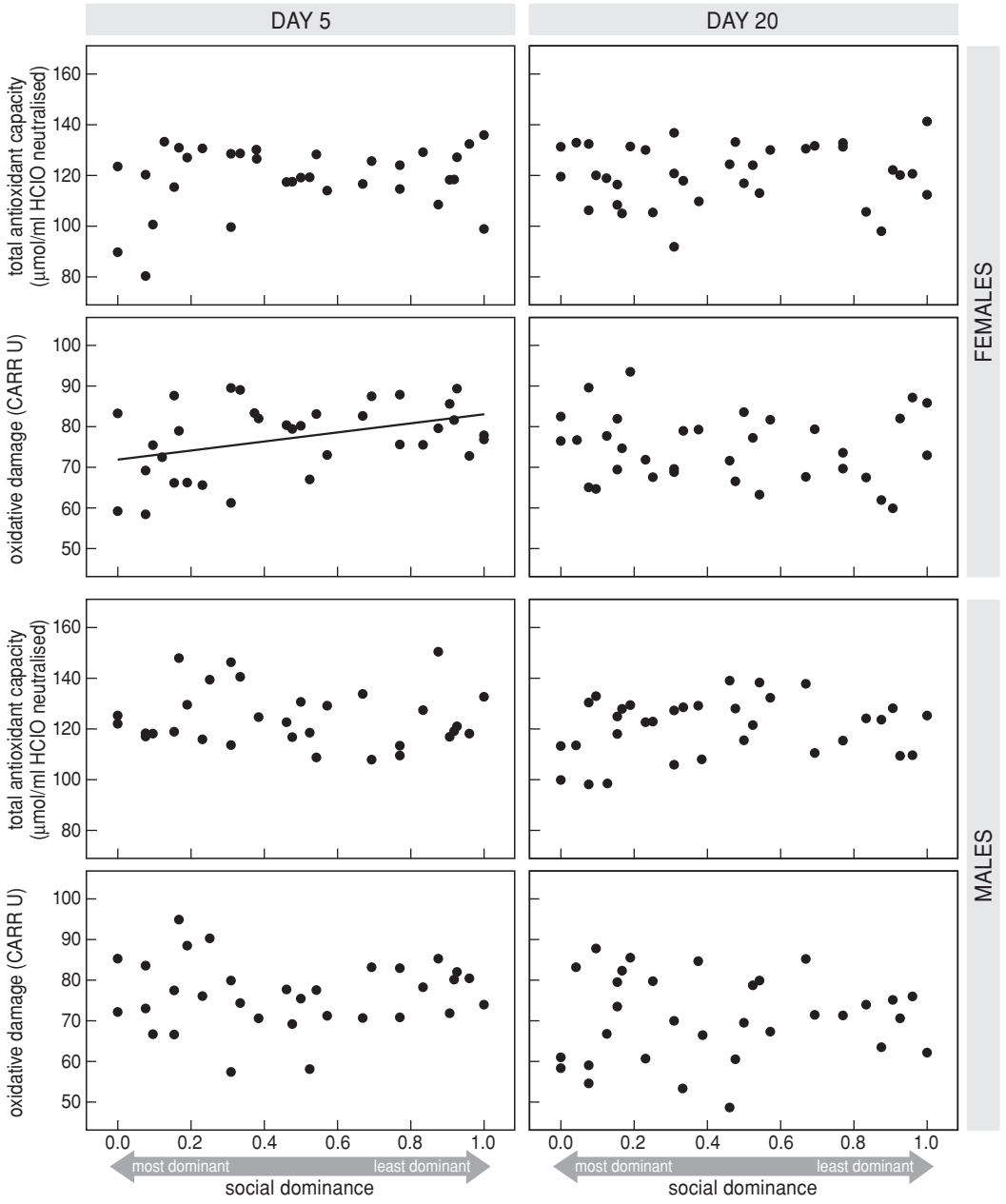


Figure 5.7. Correlation between social dominance and levels of total anti-oxidant capacity and oxidative damage of female (top) and male (bottom) parents early (left) and late (right) in the rearing period.

Discussion

In this study we tested the effect of increased parental effort on oxidative stress levels and parental body condition by experimentally manipulating brood size. Enlarging brood size substantially increased the number of nestlings reared by the breeding pair compared to pairs rearing nests reduced in size (Figure 5.1A). Because jackdaws are crop-feeders, it was not possible to determine the amount and composition of the food brought to the nest box by the parents preventing direct estimation of parental effort. However, total brood mass production for 'enlarged' broods (between day 5 and the moment we captured the parents and took our 'condition measurements' at day 20) was twice that recorded for 'reduced' broods (Figure 5.1B). We can conclude from this that although average quality of nestlings from 'enlarged' broods was lower, parental effort of birds rearing 'enlarged' broods was probably substantially higher. In drawing this conclusion we make the assumption that brood mass produced reflects the amount of food provided by the parents. Although recent evidence from an experimental mouse study supported such an assumption (Schubert et al. 2009), there are at the same time several reasons why brood mass would not, or at least not proportionally, be related to food consumption. For instance, begging rate will presumably be higher in enlarged broods as a result of increased sibling competition over the available resources and several studies have shown that this can interfere with growth rate (Kilner 2001; Neuenschwander et al. 2003), even when the total amount of food received was similar (Rodriguez-Girones et al. 2001). Furthermore, as nestling growth competes with other physiological traits like immunity (Saino et al. 1998; Soler et al. 2003) or somatic maintenance (Salomons et al. 2009c), nestlings could save energy by investing less in such physiological traits when resources are limited (Moe et al. 2005). Thus, there can be differences in the efficiency of growth depending on resource availability. Nevertheless, we believe that parental effort was higher for enlarged broods, given the substantial difference in brood mass production compared to reduced broods.

There was a decrease in residual body mass between day 5 and day 20. Although this decrease was found in both sexes, in females the effect was stronger. There was however no difference in body mass loss between parents rearing 'enlarged' or 'reduced' broods. Haematocrit levels also changed during the rearing period, but only in females. Haematocrit levels were higher at day 20 compared to day 5. Interestingly, haematocrit levels were slightly higher in parents rearing broods that were reduced in size, in agreement with our expectations that parental body condition should be higher after brood reduction. This finding was similar to results from two studies on great skuas (Kalmbach et al. 2004) and zebra finches (Birkhead et al. 1998) where respectively experimentally increased egg production and increased exercise (in combination with reduced food intake) also resulted in lower haematocrit. Alternatively, the increase in haematocrit could be induced by an increase in activity resulting in an increased demand for oxygen carrying capacity (Hõrak et al. 1998). However, this should lead to results opposite to what we found, as activity is expected to be higher for parents rearing 'enlarged' broods indicated by a higher cumulative brood mass production. Higher buffy coat indicates acute or chronic infections (Harrison & Harrison 1986; Gustafsson et al. 1994) and thus indicates lower body condition. Although in an earlier study in collared flycatchers *Ficedula albicollis* it was found that parents rearing experimentally enlarged broods had an elevated buffy coat (Gustafsson et al. 1994), we did not find a similar effect in our study. Also, there was on average no difference between samples taken either early or late in the rearing period.

Hypothetically, the increase in parental effort following brood size enlargement should lead both to an increase in energy expenditure

and metabolism (Deerenberg et al. 1995; but see Verhulst & Tinbergen 1997), presumably leading to an increased production of free radicals, as well as to a decrease in the allocation of resources towards somatic maintenance. We measured levels of total anti-oxidant capacity and oxidative damage in the plasma to obtain an estimate of somatic maintenance (i.e. defense against oxidative damage) and the susceptibility to oxidative damage. Although the level of TAC did not change over the rearing period, the level of oxidative damage decreased between day 5 and day 20. The repeatability within the reproductive bout of both TAC and oxidative damage were lower compared to our other estimates of body condition. This suggests a more short-term character of these parameters compared to these other estimates. Interestingly, the level of oxidative damage at day 20 was correlated to both TAC and haematocrit 15 days before. With regards to our brood size manipulation however, both the level of TAC and oxidative damage were unrelated to treatment.

TAC as well as oxidative damage was expressed as concentration per volume of plasma. Theoretically, it is possible that the total amount of anti-oxidants and reactive oxygen metabolites per volume of blood is more important than the concentration per volume of plasma. When expressed per volume of blood, levels of TAC in females were significantly higher when brood size was enlarged. Thus, it seems that females rearing 'enlarged' broods allocated more resources to somatic maintenance in the form of protection against oxidative damage. This result was in contrast to a study by Wiersma et al (2004) showing that an increase in reproductive effort in zebra finches was associated with a decrease in the concentration of specific antioxidants. However, considering our finding that parents rearing enlarged broods did less well in terms of fledgling quality (Salomons et al. 2009a; Salomons et al. 2009c), we feel that the increase in TAC in females rearing an enlarged brood also had a negative rather than a positive explanation. Levels of TAC were likely to be up regulated as a consequence of increased levels of pro-oxidative status (Barja 2002; Costantini 2008; Monaghan et al. 2009). Although the effect was not significant, our data indeed suggested that the concentration of oxidative damage per volume of blood was also somewhat higher in females rearing enlarged broods, but more data are required before a conclusion can be reached.

Earlier studies had shown that social dominance had a large effect on reproductive success in jackdaws breeding at the BC colony (Verhulst & Salomons 2004). Not only was fledging production much lower for individuals with a high social rank, but also the quality of the fledglings produced by these individuals was much lower. This rather counterintuitive result seemed primarily to be caused by a reduced female body condition early in the breeding season. Surprisingly, in this study we did not find the same pattern of lower body mass of females paired to high ranked males. However, we still found evidence that the physiology of these females was affected by their position in the hierarchy. Early in the rearing period, levels of oxidative damage were lower. This would indicate that these females were subject to lower levels of oxidative stress, perhaps because she is provisioned less by her partner and hence has reduced energy expenditure. At the same time however, the buffy coat of these females was larger compared to lower ranked females, indicating that these females suffered from acute or chronic infections (Harrison & Harrison 1986; Gustafsson et al. 1994). These differences had disappeared by day 20, suggesting that the detrimental effect of social dominance may be especially pronounced early in the breeding season during which the female is largely dependent on her partner for food provisioning.

So far, it is unclear why the effect of social dominance on female body condition was somewhat more subtle in the data from the latter years as compared to our earlier findings (Verhulst & Salomons 2004). There is however

some circumstantial evidence that the observed effects of social dominance in the BC colony may be affected by colony density (e.g. number of breeding pairs), in the sense that they may be more pronounced in years with higher density. Although our analyses failed to show a significant interaction effect between breeding density and social dominance on maternal body condition, the number of breeding pairs did decrease by 35% in recent years compared to the years of our first study (1998 and 2000). Therefore, we can not rule out this possibility.

The observed sex differences in the effect of brood size manipulation on parental body condition (in particular residual body mass and haematocrit) may be partly explained by the different tasks performed by the adults, especially early in the rearing period. In jackdaws, all egg incubation is performed by the mother, who during that period (~18 days, see Salomons et al. 2006) mostly relies on food provisioning by her partner. The mother will continue to spend most of their time in the nest box for the first 5–10 days after hatching (pers. obs.). After this period she will increasingly leave the nest to assist the father in food provisioning of the offspring. Thus, there is a sex difference in labor intensity at least until our first measurements five days after hatching. It is not at all unlikely that the increase in maternal food provisioning during the rearing period is dependent on brood size and or nestling condition and therefore may also be affected by our brood size manipulations.

Assuming that parental effort was indeed increased, surprisingly we found either no or only small effects of our brood size manipulations on the parents. A possible explanation could be that the increase in reproductive effort incurred by our brood size enlargement was not large enough, allowing parents to fully cope with the increased workload. Thus, it would seem that parents under 'normal' circumstances do not produce the optimal number of offspring. However, we have shown in an earlier study that enlarging brood size reduced growth and physiological condition of nestlings (Salomons et al. 2009a; Salomons et al. 2009c) indicating that the parents rearing enlarged broods did not provide optimal growth conditions for the offspring. Considering that we nonetheless did not find large effects of increased parental effort raises the question why these parents did not work harder for their offspring.

It is predicted that the extent to which the trade-off between current reproductive effort and future reproduction is biased toward parental survival depends on the longevity of the species of interest (Williams 1966). Maximum lifespan in our colony exceeded 15 years (unpublished data). And, estimates of survival of jackdaws were around 80%, indicating a breeding lifespan of around five years (Verhulst & Salomons 2004). Therefore, the jackdaw can be characterized as a species with a medium lifespan and, as such, allocation of available resources may be more biased towards the parents compared to shorter lived species. Unfortunately, data directly linking brood size manipulation to actual fitness costs of both parents and offspring are required to draw conclusions on this issue. A second reason why parents rearing enlarged broods may not have been providing optimal care for their offspring is that parents were not able to provide more food due to a time limitation. Such an effect was earlier found after brood size manipulations in great tits (Tinbergen & Verhulst 2000). Further study is needed to find out how our brood size manipulations affected parental behavior. These data are also required to establish how much more food the parents gathered when rearing an enlarged brood in jackdaws, but especially whether, and at what time scale, the increase in effort affected parental condition.

Acknowledgements
We thank Ellis Mulder for her help analysing the samples for levels of oxidative stress. H.M.S. and S.V. were supported by a Vici-grant to S.V.