Parental energy and fitness costs in birds

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CHAPTER 7

REPRODUCTIVE EFFORT DECREASES ANTIBODY RESPONSIVENESS

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ABSTRACT

The prevalence and intensity of parasitic infection often increases in animals when they are reproducing. This may be a consequence of increased rates of parasite transmission due to reproductive effort. Alternatively, endocrine changes associated with reproduction can lead to immunosuppression. In captive Zebra Finches *Taeniopygia guttata*, reproductive effort was manipulated through brood size. Enhanced effort was found to affect the production of antibodies towards sheep red blood cells. In addition, activity of zebra finches was manipulated independently of parental care. Experimentally increased daily workloads in activity reward schedules also suppressed antibody production. Thus, we show that not just the reproductive state, but the increased activity that accompanies reproduction is associated with immunocompetence. We suggest that reduced immunocompetence as a consequence of increased reproductive effort may be an important pathway for the life history cost of reproduction. This mechanism may be sufficient to explain the increased parasitism observed in reproducing animals.

INTRODUCTION

Reproductive effort entails the allocation of time and nutritional resources by parents to the production of offspring. Life history theory predicts that reproductive effort leads to negative fitness consequences for the parents in terms of reduced survival and future reproductive output, *i.e.*, a cost of reproduction (Williams 1966; Trivers 1974). The trade-off between current reproductive effort and parental survival is particularly well documented in birds (Lindén & Møller 1989; Dijkstra *et al.* 1990). The physiological basis for a cost of reproduction is, however, poorly understood. Current explanations focus on physiological deterioration (Drent & Daan 1980), manifest as accelerated senescence (Partridge 1987), and ecological factors, such as increased risks of predation (Magnhagen 1991).

Recent studies have shown that increased reproductive effort can be associated with a greater susceptibility of the parents to parasitism (Festa-Bianchet 1989; Møller 1993; Norris *et al*. 1994; Richner *et al.* 1995). The mechanism underlying this association remains to be elucidated. Increased parasitism may stem from either increased exposure or susceptibility of parents to parasites, or from reduced immunological defence mechanisms. Increased exposure might result from additional foraging activity or reduced maintenance activities such as preening (Clayton 1991), or from enhanced parasite infection in the enlarged broods employed in these studies. Reduced immunocompetence could related to a) the endocrine changes that accompany reproduction (Grossman 1984; Bhalla 1989; Marsh 1992), or b) a reallocation of resources that are drawn upon to support reproduction (Chapter 8). An epidemiological analysis in wild American Kestrels (*Falco sparverius*) has shown that the intensity of blood parasitism in females was negatively correlated with peripheral blood leukocyte levels and immunoglobulin G (= antibody) concentration, two measures of general immune function (Apanius 1991). However, this result may be interpreted as a reflection of parental condition, and is not necessarily related to the level of parental effort.

Here, we test the hypothesis that increased effort leads to depressed immune function, as measured by a generic antibody response to a non-pathogenic challenge. We first tested non-breeding and non-working (= control) birds for their response to the sheep red blood cell (SRBC) antigen, and to repeated blood sampling. Subsequently, we investigated whether antigen-specific immune responses vary with parental effort, experimentally manipulated in two ways. In Experiment 2, we altered reproductive effort by manipulating the number of offspring (brood sizes of 2, 4 and 6) in caged Zebra Finches (*Taeniopygia guttata*) and then measured antibody responses of the parents. By such manipulations it has been established previously that brood size affects an array of behavioural and fitness-related variables: parental time budgets are altered, and female body weight, offspring survival and body weight at independence are reduced by increased brood size. Parental rates of daily energy expenditure (DEE) of females (Chapter 5), and the time interval till the next reproduction are increased (Chapter 4). These studies verified that brood size manipulations influenced both parental effort, current reproductive success and future reproductive decisions.

In Experiment 3, we increased daily workloads of zebra finches through experimental manipulation of hopping activity outside the context of reproduction. These workloads were shown to negatively affect body mass during the experiment, and subsequent timing of reproduction (Chapter 6), and thus mimic the effects of brood size manipulation.

In a fourth experiment, we provided additional nutritional resources to breeding birds with a (control) brood size of 4 nestlings to test whether immune responses of breeding birds are affected by such additional resources. Food supplementation did not affect parental time

allocation (one measure of parental effort), but reduced parental weight loss during the nestling phase, as well as reproductive interval, while offspring weight at independence was enhanced (Deerenberg unpublished data).

METHODS

Antigen-specific immune responses

Reproducing birds (Experiment 2: n=43 individuals; Experiment 4: n=16) were immunized on day 16 of the nestling period, when parental care was assumed to be at its peak level. Working birds (Experiment 3: n=11) were immunized when they had been subjected to a workload schedule for at least one week. Adult birds were injected intraperitoneally with a washed suspension of sheep red blood cells (SRBC). The suspension contained $5 \cdot 10^7$ sheep red blood cells in 100 μl of sterile phosphate buffered saline (PBS). Blood samples and body weights were taken prior to immunization, and on 5 and 8 days post-immunization (control birds, Experiment 1), or on 6 and 9 days post-immunization (birds in experiments 2-4). Plasma was separated, and the cells were heat inactivated by incubation at 56 °C for 30 min. A 20μl aliquot of plasma was diluted 1:1 in PBS (titer=0), and then serially diluted (titers 1-12) in 96-well round-bottomed microtiter plates (supplier). A 20 μl aliquot of 2% SRBC-PBS suspension was then added to each dilution, and incubated at 37 °C for 60 min. Titers were scored as the inverse of the dilution that contained sufficient antibodies to haemagglutonate SRBC, and are based on a ^{2}log scale (Hay & Hudson 1989).

Corticosterone

To assess potential interference of immunosuppressive action of stress hormones, corticosterone concentrations were measured in Experiments 1 and 2. In Experiment 1, 16 birds were sampled for corticosterone. In Experiment 2, we had to pool the blood plasma remaining after antibody assay of day 0, 6, and 9 to obtain samples large enough for the hormone assay (n=41). Corticosterone was extracted from 30 μl plasma via solid-phase extraction with acetonitrile on Baker SPE C_{18} 1-ml column (Baker, the Netherlands). Quantification of plasma hormone was performed by high-pressure liquid chromatography in combination with ultraviolet detection (HPLC-UVD; Scheurink *et al.* 1990)

Manipulation of parental effort

Experiment 1 A random sample of 2 male and 9 female zebra finches that were kept in same gender groups (*c.* 10 birds) was tested for immune responses to sheep red blood cells (SRBC). These non-breeding, and non-working birds were housed in 40·80·40 cm cages, fed on a standard dry seed diet. Food and water was available *ad libitum*.

Experiment 2 23 Pairs of captive zebra finches were housed separately in cages (40·80·40 cm) provided with nestbox and nesting material at a constant room temperature of 25°C, and an L:D cycle of 14:10. They were provided with a standard dry seed mixture suitable for supporting reproduction. To manipulate reproductive effort, we transferred nestlings at 1-3 days of age to either enlarge (6 young) or reduce (2 young) broods relative to the control (4 young) brood size. For a larger sample of pairs, results on reproductive success, parental effort and energetics in relation to brood size are given and discussed in Chapter 5.

Experiment 3 To experimentally vary workload other than by manipulating brood size, we

manipulated hopping activity of zebra finches. Non-breeding birds were operantly conditioned to hop repeatedly between perches for a food reward. Two different workloads were imposed by adjusting the number of hops required for a fixed food reward. Compared to a non-working control group with a daily activity of *c.* 2000 hops, the mean daily number of hops was increased to on average 6158 and 8393 for low and high workload schedules, respectively. To test for immunocompetence, birds were again subjected to workloads after completion of the experiments described in Chapters 6 and 8.

Experiment 4 To improve the quality of the seed diet, eight families of control (4) brood size were given a high protein food supplement from 3 days after hatching onwards. The energy content of the two diets did not differ (22.1 *vs.* 23.6 $kJ \cdot g^{-1}$), while the N content was doubled (0.046 *vs.* 0.023 $g \cdot g^{-1}$ dry mass).

Data analysis

Immune responsiveness, *i.e.*, the fraction birds that produced detectable antibodies was analysed for differences among experimental groups with Chi-square (χ^2) tests. We tested the effects of several independent variables (brood size, gender, body mass, DEE) on responsiveness in log-linear models and F-ratio tests on the change in deviance when a variable was added to the model. Differences among experimental groups in antibody concentrations (titers) were tested with one-way analysis of variance (ANOVA). Effects of immunization and responsiveness on body mass was analysed with repeated measures ANOVA. Data on responsiveness are presented as means and 95% CI based on the binomial distribution.

RESULTS

Experiment 1

Non-breeding, non-working zebra finches had no detectable anti-SRBC antibodies prior to the experiment. All birds consistently produced haemaglutinating (HA) antibodies upon immunization (100%, n=11). The concentration (= titers) of detectable antibodies on subsequent sampling days were 4.59 (sd=3.52, n=11), and 7.45 (sd=2.55, n=11) on day 5 post-immunization (PI) and day 8 PI, respectively.

Corticosterone levels were on average 1.27 $ng \cdot ml^{-1}$ (sd=0.66, n=16).

Body mass declined during the immune response (Day 0: 15.68 g, sd=1.87; Day 8: 14.26 g, sd=1.97, n=11). Although body weights were significantly reduced due to immunization (paired t-test: $T_{10}=5.75$, $P<.001$), it is unlikely that the

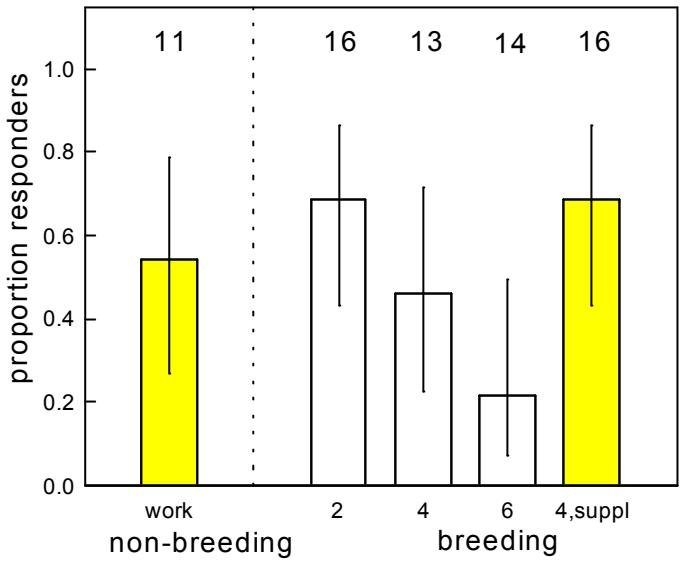

Figure 1. Proportion of zebra finches producing haemagglutinating antibodies against sheep red blood cells as a function of reproductive status, diet, or work load. Non-breeding and non-working birds all responded. Workload was manipulated by hopping activity; reproductive status was manipulated by brood size; diet refers to protein supplemented diet. Numbers above columns refer to sample size, and error bars indicate 95% confidence intervals based on binomial expectation.

immunization protocol, or the antibody response negatively affected the birds in the long term, because a reduction in body mass is a common, however temporary, aspect of the immune response (Beisel 1977).

Experiment 2

20 Out of 43 parent zebra finches showed a positive response to immunization. Average responsiveness in breeding birds (47%) was lower compared to birds in experiment 1 (χ_1^2=10.3, P=.001). The proportion of responsive breeding birds declined from 69% in parents raising two nestlings to 21% in parents raising six nestlings (Figure 1). Thus, the proportion of responsive birds was affected negatively by experimental brood size ($F_{1,41}$=7.02, P=.01). Responsiveness was not associated with gender ($F_{1,40}$=0.20), body mass ($F_{1,40}$ =.02), or DEE ($F_{1,28}$=1.94) prior to immunization.

Titers, *i.e.*, the quantity of anti-SRBC antibodies of responsive birds are given for the three experimental brood sizes in Figure 2. Titers of reproductive birds were not different from the control group (experiment 1) on day 5/6 PI ($F_{1,29}$=0.29), but were significantly lower on day 8/9 PI ($F_{1,29}$=14.84, P<.001). Intensity of the antibody response in responsive breeding birds did not vary with brood size, and was also not associated with gender, body mass or DEE prior to immunization (Table 1A).

Corticosterone concentrations in plasma samples are depicted in Figure 3 for responsive and non-responsive birds in each brood size category. The overall average concentration of corticosterone in breeding birds was rather similar to that of the birds

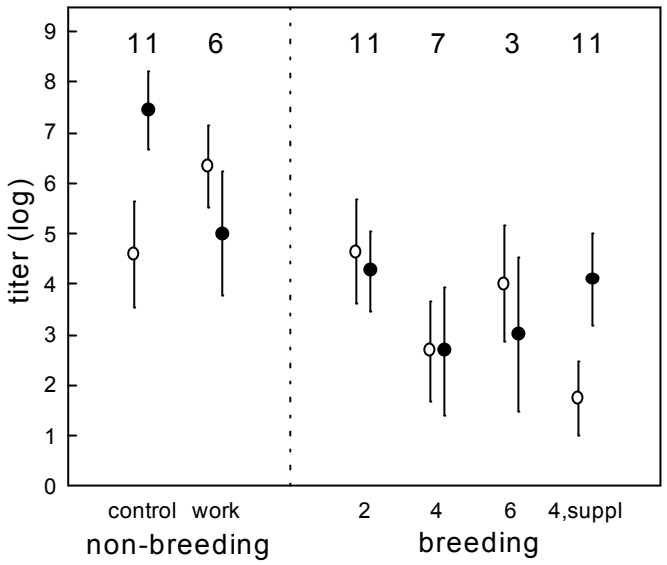

Figure 2. Intensity (= titer) of antibody response against sheep red blood cells on 6 days post-immunization (PI, open symbols), and on 9 days PI (filled symbols). Mean (log) titer o f responsive birds in each experimental group, and standard errors are depicted.

in Experiment 1 at 1.31 $ng \cdot ml^{-1}$ (sd=0.87, n=41). Corticosterone varied with experimental brood size (*b*) in a quadratic fashion (null model: deviance=31.46, df=40; *b*: ∂deviance=2.77, ∂df=1, P=.06; b^2: ∂deviance=5.74, ∂df=1, P=.02). Although there was a tendency towards a direct association between corticosterone plasma concentrations (*c*) and immune responsiveness (null model: deviance=56.62, df=40; *c*: ∂deviance=2.93, ∂df=1, P<.10), this association disappeared when we controlled for the effect of experimental brood size (*b*) on responsiveness (*b*: ∂deviance=5.73, ∂df=1, P=.02; *c*: ∂deviance=1.36, ∂df=1, ns).

Zebra finches were immunized at the end of the nestling period, and parents usually increase in mass during the subsequent days when their young fledge (Chapter 5). Intra-individual changes in body mass of immunized birds (n=42) after immunization were therefore compared to weight changes of non-immunized birds (n=76) in the same experiment (Figure 4). Initially (day 0), birds that were immunized did not differ in mass from birds that were not subjected to immunization ($F_{1,112}$=2.51). However, body mass of parent birds varied significantly with experimental brood size ($F_{2,112}$=3.90, P=.02). Controlling for the effect of experimental brood size ($F_{2,112}$=2.79, P=.07), immunization of birds negatively affected subsequent recovery of body mass during the nine days of the immune response measured ($F_{1,112}$=5.43, P=.02).

To test whether responsiveness was related to changes in body mass, we compared intra-individual mass changes of responsive and non-responsive birds (Table 2). Average mass change after immunization amounted to 0.05 g (sd=0.69, n=20) and 0.24 g (sd=0.57, n=22) for responsive and non-responsive birds,

Table 1. Linear regressions of titers (= concentration of anti-SRBC antibodies) of responsiv e birds on gender and on body mass prior to immunization, and, facultatively, on brood siz e and parental daily energy expenditure (DEE). A. Experiment 2. B. Experiment 3. C. Experiment 4.

	(increase in) deviance	(increase in) df	P	(increase in) deviance	(increase in) df	P
A.	--- Titer day 6 ---			--- Titer day 9 ---		
null model	168.95	19	<.001	140.80	19	<.001
brood size	5.07	1	ns	7.84	1	ns
gender	1.25	1	ns	1.80	1	ns
DEE ($kJ \cdot day^{-1}$)	0.67	1	ns	5.56	1	ns
null model (DEE)	106.86	13	<.001	98.00	13	<.001
DEE ($kJ \cdot day^{-1}$)	0.29	1	ns	4.74	1	ns
B.	--- Titer day 6 ---			--- Titer day 9 ---		
null model	19.33	5	<.001	46.00	5	<.001
gender	0.08	1	ns	0.75	1	ns
mass day 0 (g)	2.51	1	ns	10.34	1	ns
DEE ($kJ \cdot day^{-1}$)	8.30	1	ns	6.80	1	ns
C.	--- Titer day 6 ---			--- Titer day 9 ---		
null model	60.18	10	=.04	92.91	10	=.001
gender	0.98	1	ns	0.08	1	ns
DEE ($kJ \cdot day^{-1}$)	4.74	1	ns	6.83	1	ns

respectively. However, intra-individual mass changes neither differed significantly from zero ($F_{1,36}$=2.54), nor were they affected by responsiveness of the birds ($F_{1,36}$=1.05).

Experiment 3

As among the breeding birds, not all zebra finches subjected to workload schedules produced detectable antibody titers when they were immunized with SRBC (55%, n=11; Figure 1). Responsiveness in working birds was reduced relative to responsiveness of the zebra finches in experiment 1 (χ_1^2=6.47, P=.01). On the low workload schedule, 50% (n=6) of the birds produced detectable antibodies; on the high workload schedule 60% (n=5) of the birds showed a immune response. There was no significant difference in responsiveness between the work schedules (χ_1^2=0.11). Average responsiveness of working birds was similar to the average in all

(non-food supplemented) breeding birds (χ_1^2=0.23), and did not differ significantly from the fraction responsive birds in any of the brood size manipulation categories (Small: χ_1^2=0.56; Intermediate: χ_1^2=0.48; Large: χ_1^2=2.93, P=.09). There was also no

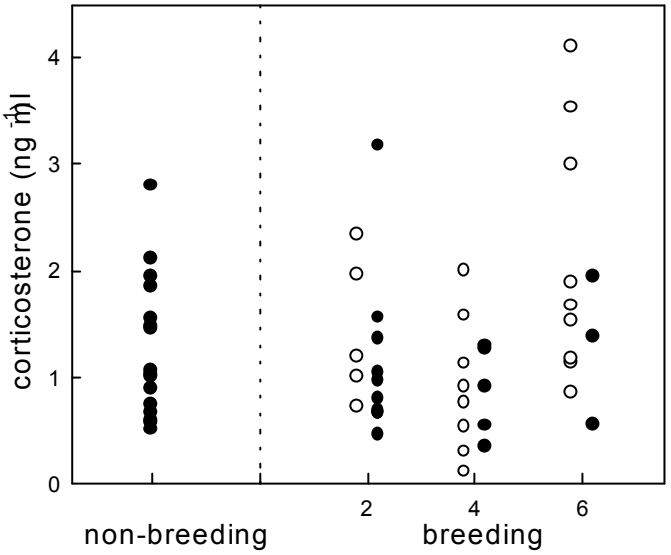

Figure 3. Plasma corticosterone concentrations of control birds (non-breeding and non-working, Experiment 1), and of parents raising broods of manipulated sizes (Experiment 2). Open symbols represent birds that responded to immunization with SRBC with antibody production; filled symbols indicate birds that failed to produce antibodies.

association between responsiveness and gender ($F_{1,9}$=2.52), body mass ($F_{1,9}$ =2.02) or DEE ($F_{1,9}$=0.07) prior to immunization.

Titers of responsive birds in the working experiment are shown in Figure 2. The antibody titers of working birds were not significantly higher than of all (not food supplemented) breeding birds (Day 6: $F_{1,24}$=3.34, P=.08; Day 9: $F_{1,24}$=1.16). Intensity of the antibody response was not related to gender, body mass or DEE prior to immunization (Table 1B).

Average change in body mass after immunization (Table 2) was 0.03 g (sd=0.55, n=11), which neither deviated from zero ($F_{1,9}$=0.02), nor differed between responsive and non-responsive birds ($F_{1,9}$=0.31). In this experiment, there was no control group of birds that were not subjected to immunization.

Experiment 4

The proportion of food-supplemented parents responding to immunization (69%, n=16) was reduced in comparison with non-breeding birds (χ_1^2=4.91, P=.03). In this experiment, we only tested parents rearing four nestlings. Antibody responsiveness was not enhanced (χ_1^2=1.51) by the diet quality enhancement relative to parents raising four nestlings on the standard seed diet. Within the pairs that received a high-protein food supplement, responsiveness did not vary with gender ($F_{1,14}$=0.29), and was not associated with body mass prior to immunization ($F_{1,14}$=0.17).

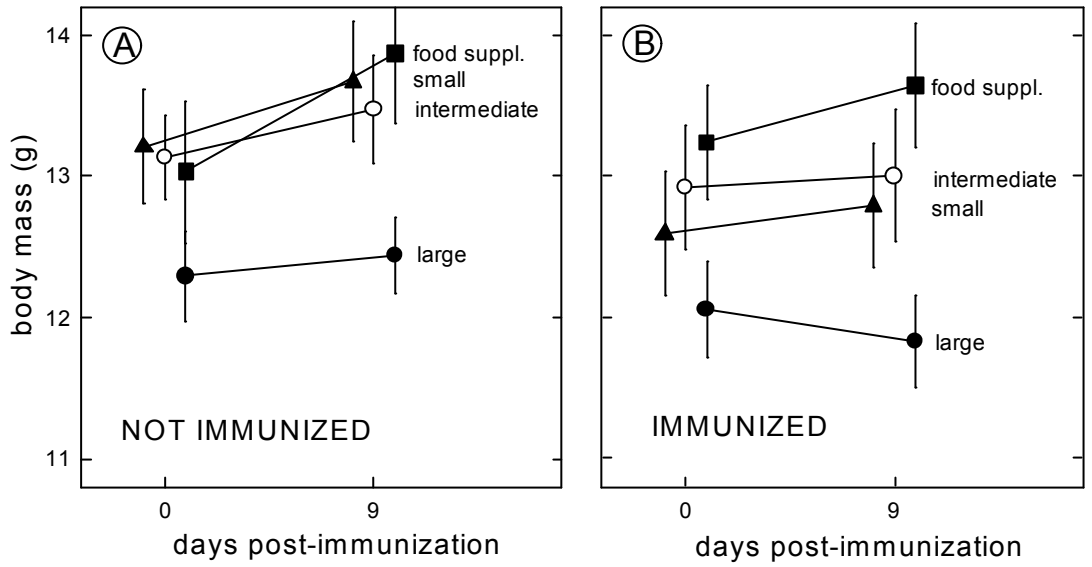

Figure 4. Body weights of breeding zebra finches (Experiments 2 and 4) on day 16 and day 25 of nestling age, corresponding to day 0 of immunization (= before) and day 9 post - immunzation. Means and standard errors are given. A. Breeding birds in Experiments 2 and 4 that were not subjected to the immunization protocol. B. Birds in Experiments 2 and 4 that were immunized.

Average titers of antibodies on day 6 and day 9 post-immunization of responsive birds are depicted in Figure 2. Titers of food supplemented birds did not differ from

titers of birds raising broods of the same size (Day 6: $F_{1,15}$=0.57; Day 9: $F_{1,15}$=0.84). Among food supplemented parents, titers of responsive birds were not associated with gender, and body mass prior to immunization (Table 1C).

As in Experiment 2, changes in body mass after immunization (n=16) were compared to weight changes of birds in the experiment that were not subjected to immunization (n=8; Figure 4). There were no significant differences between immunized birds and birds that were not subjected to immunization, neither in initial body mass (Day 0: $F_{1,22}$=0.0), nor in subsequent change in body mass ($F_{1,22}$=1.96).

Body mass changes of immunized birds are given in Table 2. Average weight change did not differ significantly from zero ($F_{1,14}$=3.10, P=.10), and there were no differences between responsive and non-responsive birds in recovery of mass after immunization ($F_{1,14}$=0.03).

DISCUSSION

In a group of non-breeding, non-working birds, it was shown that zebra finches responded to immunization with SRBC by producing high titers of haemagglutinating antibodies. Surprisingly, reproductive and working zebra finches did not consistently produce detectable antibodies upon immunization. It was thus demonstrated that this trait was phenotypically controlled. This study examined several ecological and behavioural factors that might influence

antibody responsiveness.

Table 2. Average body mass prior to immunization (= Day 0) and after immunization on day 9 of zebra finches in three experiments of manipulation of parental effort. Birds were grouped according to whether they produced detectable anti-SRBC antibodies (*responsive*), or not (*non-responsive*). Sample sizes, and means ± standard deviations are given.

	responsive	non-responsive
Experiment 2:		
sample size	20	22
mass day 0 (g)	12.45 ± 1.72	12.26 ± 1.37
mass day 9 (g)	12.51 ± 1.53	12.55 ± 1.68
Experiment 3:		
sample size	6	5
mass day 0 (g)	13.78 ± 1.21	12.89 ± 0.89
mass day 9 (g)	13.66 ± 0.76	12.96 ± 0.93
Experiment 4:		
sample size	11	5
mass day 0 (g)	13.26 ± 1.71	12.90 ± 1.70
mass day 9 (g)	13.70 ± 1.62	13.44 ± 2.29

The proportion of zebra finches responding to immunization with sheep red blood cells (SRBC) was reduced incrementally in birds raising manipulated broods of increasing size. As shown elsewhere, experimentally manipulated brood size affected the level of parental effort, as assessed by various behavioural and physiological measurements (Chapter 5). Our results on immune responses provide a sufficient explanation for increased blood parasitism observed in birds with increasing, experimentally manipulated, brood sizes (Møller 1993; Norris *et al.* 1994; Gustafsson *et al.* 1994; Richner *et al.* 1995).

Non-breeding birds that were subjected to workload schedules also showed a reduced responsiveness. This result is consistent with studies reporting depressed humoral immunity in humans or animals undergoing strenuous physical exercise (review in: Fitzgerald 1988). Thus, our data show that antibody responsiveness of zebra finches depends on the demands imposed on the animal. Because all experimental groups were treated identically (handling and blood sampling), except with respect to brood size, it is unlikely that other factors can account for these results. Both breeding and working birds reduced their response rates, therefore the data on experimentally manipulated levels of activity presented here suggest that increased physical activity associated with parental effort influenced antibody responsiveness of the parents negatively.

We were unable to establish a difference in in antibody responsiveness between birds maintained on low and high workloads. On the high workload, Daily Energy Expenditure (DEE) was slightly reduced, in spite of increased hopping activity (Chapters 6 and 8). We have shown that the rather similar DEE on the two workloads is due to compensatory reduction of metabolic rate during inactivity (Chapter 8). Thus the present results are consistent with the hypothesis that immune responsiveness is affected by overall DEE rather than specifically by total daily activity.

To our knowledge, this is the first experimental demonstration that workload associated with reproductive effort influences immune function negatively. One previous study has examined leukocyte and immunoglobulin levels in birds with manipulated broods (Gustafsson *et al*. 1994). In this review on reproduction and infectious diseases in the Collared Flycatcher, (*Fidecula albicollis*), Gustafsson reported increased parasitic infection rates concomitantly with symptoms of an increased activity of the immune system in parents with manipulated brood sizes. Because the species had natural infections with blood parasites, these seemingly contradictory results may be expected, *e.g.*, when the enhanced immunoactivity reflects a response to the increased parasitic infection, the relapse of which may originally have been due to impaired immune function, which in turn was a consequence of manipulated parental effort. In our study, we used disease-free animals in a controlled environment to demonstrate the influence of reproductive effort on one aspect of the adaptive immune system of parents.

Numerous studies have shown that immunosuppression causally increases host susceptibility to parasitic infections (review in: Folstad & Karter 1992). Reproducing birds during the phase of nestling care have usually low, or increasing, levels of gonadal steroids, while circulating levels of corticosterone may be increased (Vleck & Priedkalns 1985; Hegner & Wingfield 1986a, b; Logan & Wingfield 1995). Corticosterone is associated with high metabolic demands (Wingfield 1984a, b), or metabolic stress (Siegel 1980), and exhibits immunosuppressive action (reviews in: Munck & Guyre 1991; Marsh 1992). Corticosterone analyses showed that the level of this hormone was significantly affected by the brood size manipulations, with slightly increased levels in parents of both small and large broods relative to intermediate brood sizes. The only other study on brood size manipulations and hormone concentrations reported similar results, *i.e.*, a tendency of increased levels of corticosterone in female parents of large broods (Hegner & Wingfield 1987). Corticosterone concentrations were not different, however, between responsive and non-responsive birds within each group. These findings appear to rule out the possibility that suppressed immune responses result directly from differential stress factors imposed on the parents by manipulation of brood size.

Birds in the experiments were faced with increased energetic demands due to reproductive behaviour (nourishing young) or hopping activity. However, on average there were no corresponding changes in daily energy expenditure (DEE), except for females in the brood size manipulation experiment. The range of DEE-values manipulated through activity (Experiment 3) was similar to values of parental DEE measured in the brood size manipulation experiment (2) (Chapters 5 and 8). It was thus not unexpected that DEE was not directly associated with responsiveness, or with titers of anti-SRBC antibodies. Therefore, we suggest that the increased level of physical activity, whether or not associated with reproduction, required that the parents reallocated resources between various somatic compartments. This is dramatically demonstrated by body mass reductions of parents during the course of the reproductive cycle (Chapter 5), and by major reductions in maintenance energy of birds subjected to various workloads (Chapter 8).

While parents usually increase in weight around fledging of their young, immunized

parents showed suppressed weight gain. It is unlikely that reduced weight gain was entirely due to blood sampling (*c.* 300μl in nine days). We could not show differences in mass change between responsive and non-responsive birds in any of the experiments. A reduced body mass, corresponding with minimized food intake, is a normal finding in immunoresponsive animals (*e.g.*, Murray & Murray 1979; Klasing *et al.* 1987), and immunological processes thus rely primarily on stored resources (Beisel 1977). Antigen-specific immune responses require rapid lymphocyte proliferation, and draw heavily on protein resources. Following an immunological challenge, there is a homeostatic response of increased protein breakdown in muscle tissue, thereby supplying the amino acids for the increased protein synthesis of the immune response in liver and other immune tissues (Klasing & Austic 1994 a,b). These mechanisms explain the absence of weight gain in responsive birds only. The lack of positive body mass changes of non-responsive birds may be explained, if non-responsive birds had insufficient body reserves either to respond to the immune challenge, or to increase in weight. Apparently, there is a trade-off between maintenance and immune function, and both traded off against demands due to reproduction. This re-direction of parental resources may be a significant causal component of the cost of reproduction phenomenon.

Protein malnutrition has been shown repeatedly to impair immune function (*e.g.* Lochmiller *et al.* 1993). In view of the crucial involvement of stored proteins in the immune response mentioned above, the supplement of high protein food was expected to enhance immune responses. Unexpectedly, increasing the quality of the diet by supplementation with high protein food had also little influence on either responsiveness or weight changes, although responsiveness was still reduced compared to control birds. Apparently, protein availability was not limited or crucial in parents nourishing their young, or in working birds.

Why should immunocompetence decline at a time when the physiological performance of birds, *i.e.*, parents is at a premium? Typically, birds did or did not respond. However, when responding, the intensity of the production of antibodies did not vary among experimental groups. Initially, we expected birds to adjust the quantity of their response to the amount of resources available. An insufficient antibody production may be of limited effectiveness. Rather than an effect of insufficient resources, reduced responsiveness may be a strategy. Conditional on general prevalence of parasites and host susceptibility, a bird that does not react to (parasitic or other) infection may incur smaller fitness costs than when it cuts down on resources allocated to reproduction, and thereby reducing its reproductive output (Forbes 1992). We surmise that the depression of immune function and a concomitant increased susceptibility to parasitism of parents is part of a larger adaptive syndrome in which resources are re-allocated from self-maintenance to reproduction to maximize lifetime reproductive success.

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