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Survival costs of reproduction in the blue tit (*Parus caeruleus*): a role for blood parasites?

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One of the central tenets in life-history theory is that there is a trade-off between current and future reproduction (i.e. a cost of reproduction). The mechanism for this cost of reproduction is, however, largely unknown. One hypothesis is that the high workload during reproduction compromises resistance to parasites and that the resulting increase in parasitaemia has negative effects on the prospects of future survival. Although empirical evidence for a negative relationship between reproductive effort and parasite resistance exists, the causal relationships between reproductive effort, parasite resistance and future reproduction are still unclear. We use a path analytical approach to investigate whether a change in parasite resistance (as measured by intensities of infections by the blood parasite *Haemoproteus*) after manipulation of reproductive effort, translates into altered survival in female blue tits. Our results show a negative relationship between reproductive effort and parasite resistance, although evident only in first-year breeders. Moreover, we found survival costs of reproduction in first-year breeders. These costs were, however, not mediated by the blood parasite studied.

Keywords: cost of reproduction; reproductive effort; parasite intensity; survival; *Haemoproteus*; *Parus caeruleus*

1. INTRODUCTION

Despite the fundamental importance of reproductive costs (i.e. the reduction in future reproduction as a result of current reproductive effort; Williams 1966) in life-history theory, empirical evidence for mechanisms that can mediate such costs is largely missing (cf. Nilsson & Svensson 1996). Recent studies have concentrated on physiological mechanisms, in particular the relationship between reproductive effort and parasite resistance (Sheldon & Verhulst 1996). The theory posits that a high workload has a suppressive effect on the immune system, thereby reducing the ability to resist or control parasite infections (Gustafsson *et al.* 1994). Unbound by the control of the immune system, parasites can potentially cause a reduction in host survival and/or future fecundity.

To demonstrate that parasites may play an important role as mediators of reproductive costs, all steps in the chain of events between reproductive effort and survival and/or future fecundity should be verified (Norris & Evans 2000). So far, quite a few studies have investigated the first part in this chain of events; that is the relationship between reproductive effort and parasite resistance. Several of these studies have, through brood size manipulations, been able to demonstrate a negative relationship between workload and either some measure of immune responsiveness (e.g. Deerenberg *et al.* 1997; Nordling *et al.* 1998; Cichon *et al.* 2001) or parasite status (e.g. Norris *et al.* 1994; Richner *et al.* 1995; Allander 1997; Nordling *et al.* 1998). A few studies have also found a negative correlation between parasite status and survival (i.e. the second step in the chain; for

example, Richner *et al.* 1995; Nordling *et al.* 1998; Dawson & Bortolotti 2000). However, to our knowledge, no study has yet investigated whether the change in parasite resistance after manipulation of reproductive effort is casually related to costs of reproduction. Altering reproductive effort may affect not only parasite resistance but also other traits, for example, body condition or metabolic rate (Sanz & Tinbergen 1999; Nilsson 2002), which may then affect survival. In other words, the problem is that there is only an observational relationship between parasite status and survival. Experimental manipulations provide the best way to investigate causal relationships between variables. However, at least for endoparasites, these are difficult to perform (but see Merino *et al.* 2000). Under such circumstances, one may use a path analytical approach, to identify direct and indirect relations among variables (Mitchell 2001). The relative importance of these different relationships or pathways can then be compared. That is, to investigate the relative importance of parasites as mediators of costs of reproduction, one can include alternative pathways, such as the 'direct' effect of reproductive effort (i.e. through unmeasured variables) and the indirect effects of other variables, such as body condition, in the model and in that way statistically control for these pathways when investigating the effect of parasites on survival.

We used this approach to investigate whether blood parasites of the genus *Haemoproteus* serve as mediators of reproductive costs in female blue tits (*Parus caeruleus*). In addition, we investigated whether costs of reproduction were mediated by body mass, because body mass is a frequently used proxy for overall condition (Brown 1996). For this purpose, we experimentally altered the reproductive effort of female blue tits, through brood size manipulations, and

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investigated the concomitant effects on parasite intensity, body mass and survival. This allowed us to estimate the relative importance of the direct pathway between reproductive effort and subsequent survival, and the two indirect pathways (i.e. through parasite resistance or body mass).

2. MATERIAL AND METHODS

(a) *Study species and area*

This study was conducted over 3 years (2000–2002) in a nest-box breeding population of blue tits. In this species, sex can be determined on the basis of the presence/absence of a brood patch, and age (second calendar year birds, i.e. born the previous calendar year, 2Y, or third calendar year birds or older, 3Y+) on the basis of the colour of the primary coverts (Svensson 1992). Egg laying has begun by the end of April and young fledge in the first half of June.

The study area is Revinge (64 km²), a grassland area 20 km east of Lund, southern Sweden, where approximately 450 nest-boxes are erected in small forests and groves. For a description of general field methods see Nilsson (1994).

(b) *Parasite*

The most common blood parasite in our population is *Haemoproteus majoris* (overall prevalence ca. 80%; M. Stjernman, personal observation). Haematozoans of the genus *Haemoproteus* occur mainly in birds and have been reported in ca. 45% of all bird species examined (Bennett *et al.* 1982). The main vectors are biting midges (Ceratopogonidae) in which the sporogonic (i.e. sexual) cycle takes place, producing infectious sporozoites. Upon an infectious bite from the vector, sporozoites are transferred to the host and one or a few cycles of asexual reproduction (schizogony) occur in the tissues (Desser & Bennett 1993). A proportion of the resulting merozoites invade red blood cells and transform into gametocytes that are infective to the vector. Only gametocytes are detectable in the blood.

The prepatent period of *Haemoproteus* has been estimated to ca. 14 days (Desser & Bennett 1993). That is, from the time of an infectious bite of a vector it takes 14 days before gametocytes appear in the blood and thus can be detected by microscopy. Considering that the time period between manipulation of reproductive effort and sampling of the birds for parasites in our study was only 12 days (see § 2c,d), it seems highly unlikely that new infections acquired as a result of the manipulation can be detected. This is also corroborated by our data showing that only five birds changed infection status during this period (and only four going from non-infected to infected). Most probably, these few changes were changes in very low intensities rather than changes in infection status (i.e. infected or non-infected). We therefore chose to study the change in parasite intensity owing to altered reproductive effort, rather than change in infection status. Thus, we included only infected birds (i.e. birds with parasites on at least one of the sampling occasions) in the analyses.

Other blood parasites that also occur in our blue tit population include *Leucocytozoon*, *Plasmodium* and *Trypanosoma* (M. Stjernman, personal observation). However, the prevalence of these parasites is low and intensities are too low to be accurately determined by microscopy. We therefore consider only *Haemoproteus* in this study.

(c) *Experiment*

Brood size manipulations were performed on day two post-hatch (day of hatch = day 0) by asymmetric exchange of young between nests. To accommodate the need of another study to create equal proportions of own and foster young in each nest, we

used a cross-fostering design in the years 2000 and 2001 (Råberg *et al.*, unpublished data). We created pairs of nest-boxes, matched by hatch date and clutch size (\pm two eggs). From one of the nest-boxes in a pair (enlarged), one-third of the young were transferred to the other nest-box (reduced) and two-thirds of the young from this nest-box were transferred back to the first nest-box. In 2002, we did not exchange chicks reciprocally between nest-boxes, but moved only one-third of the chicks from one nest to another. In all years, nests not belonging to a pair were used as controls as long as they hatched within the same time-frame as manipulated nests.

(d) *Measurements*

Because we wanted to study the change in parasite status as a result of the experiment, we captured birds both before and after the manipulation. We caught females after 7 days of incubation, measured body mass (to the nearest 0.1 g) with a Pesola spring balance and took a blood sample (ca. 60 μ l) from the jugular vein using a 1 ml syringe (needle 29G \times 1/2; pre-manipulation sample). Males are difficult to catch during incubation (i.e. before the experiment), which is why we include only females in this study. On day 14 post-hatch, females were again caught and we measured tarsus length (to the nearest 0.1 mm, using digital callipers), wing length (to the nearest 0.5 mm, using a ruler) and body mass. On this occasion, a new blood sample was taken (post-manipulation sample). From the blood samples, a small drop of blood was smeared onto a glass slide. Slides were fixed in methanol for at least 3 min within hours from sampling and later stained with modified Giemsa stain (Accustain, Sigma Diagnostics, diluted 1:20 in ddH₂O) for 1 h. Infection status (infected/non-infected) and intensity of infection were determined by counting the number of gametocytes of *Haemoproteus* in 10⁴ red blood cells in \times 1000 magnification under oil immersion. Parasite intensities were log-transformed ($\ln(\text{parasite intensity} + 1)$) before statistical analysis to meet the requirements of parametric tests. The repeatability (Lessells & Boag 1987) of intensity measures was very high, both when counting the same smear twice ($R = 0.988$, $F_{30,31} = 166.16$, $p < 0.0001$), and when counting different smears made from the same blood sample ($R = 0.970$, $F_{9,10} = 65.50$, $p < 0.0001$).

To confirm that the brood size manipulation affected reproductive effort, females were equipped with a transponder on the tarsus upon first capture. On day 9 post-hatch, an antenna connected to a data logger was fitted to the entrance hole of the nest-box, and the number of feedings was recorded for 24 h. Feeding effort measured in this way has previously been shown to be positively related to field metabolic rate (Nilsson 2002) and should therefore reflect the workload of nestling feeding females.

To determine survival, nest-boxes were visited at night once or twice during the following winter (January–March) to catch sleeping birds, and birds breeding in nest-boxes in the area were caught during the breeding seasons of 2001–2003. A bird was considered to have survived if it was alive at least in the first winter after the focal breeding attempt.

(e) *Dataset*

We performed brood size manipulations on first clutches from 166 nests during the 3 years of the experiment. To ensure that the reproductive effort was manipulated throughout most of the feeding period, nests with severe brood reduction, or where one of the parents disappeared between hatching and day 14 (presumably owing to predation in most cases) were excluded. Moreover, females with polygynous males, and females that laid a second clutch, were also excluded. This reduced the dataset to 129 nests.

Control nests ($n = 95$) were selected on the same basis as experimental nests. Because the study was conducted over several years, the same individual may enter the dataset more than once, which introduces the problem of pseudoreplication. In those cases, data from 1 year were selected at random. Finally, for reasons described in § 2b, we included only infected females. Hence, the final dataset consists of 51 females from 2000, 75 females from 2001 and 26 females from 2002, in total 152 females (41 enlarged, 70 controls and 41 reduced).

(f) Statistical analysis

Body masses of nestling-feeding females were related to the time of day when measurements were taken (linear regression: pre-manipulation body mass: $R^2 = 0.05$, $F_{1,150} = 7.92$, $p = 0.006$; post-manipulation body mass: $R^2 = 0.12$, $F_{1,150} = 19.80$, $p < 0.0001$). We therefore used the residuals from these regressions as a measure of body mass in all analyses. However, for the sake of brevity, we will continue to refer to these residuals as body mass.

The effects of brood size manipulation on parasite intensity and body mass were analysed with ANCOVA and the effects of parasite intensity and body mass on survival with logistic regression, in both cases with brood size manipulation as a covariate. For both parasite intensity and body mass, pre-experimental values explained a large part of the variation in post-experimental values (parasite intensity: $R^2 = 0.58$, $F_{1,150} = 208.82$, $p < 0.0001$; body mass: $R^2 = 0.29$, $F_{1,150} = 60.51$, $p < 0.0001$). When investigating the role of parasites in mediating reproductive costs, we are interested in how altered workload affects parasite intensity, and how this *change* in parasite intensity in turn affects future survival (Sanz *et al.* 2002). We therefore included pre-manipulation values for parasite intensity and body mass in the models investigating the relationship between brood size manipulation, parasite intensity, body mass and survival.

To illustrate the relative importance of direct and indirect relationships between reproductive effort and survival we summarized the analyses in a path model (Mitchell 2001). Path coefficients were estimated as standardized partial regression coefficients from multiple regressions or ANCOVAs. These coefficients are presented in a path diagram (figure 3). The indirect effects of the brood size manipulation on survival (i.e. through parasite intensity or body mass) were obtained by multiplying path coefficients along the respective pathways (Kingsolver & Schemske 1991) and their standard error (SE) was calculated using the formula $(SE_Z/Z)^2 = (SE_A/A)^2 + (SE_B/B)^2$, where $Z = A \times B$.

As the study was conducted over several years, we tested for main and interaction effects of year (as a categorical variable) in all analyses. However, these effects were included in final models only if significant. Logistic regressions were performed using PROC LOGISTIC in SAS 8.2 (SAS Institute 1999). All other analyses were performed in SPSS 11 (SPSS Inc., Chicago, IL, USA).

3. RESULTS

Before the experiment, there were no differences in clutch size, brood size, lay date, hatch date, body mass or parasite intensity between experimental groups, neither for the age classes separately nor combined (table 1).

(a) Effect of manipulation on reproductive effort

Our manipulation of brood size significantly altered the feeding frequency of female blue tits. Females feeding reduced broods decreased their number of feedings by

Table 1. Mean (s.d.) pre-manipulation values for the different experimental groups. (There were no differences between experimental groups in any of the variables, neither for the age classes separately nor combined (all $p > 0.17$; in the analyses, body mass is corrected for time of the day, and parasite intensities are ln-transformed, see § 2). 2Y, second calendar year birds, 3Y+, third calendar year birds or older. For lay date and hatch date, day 1 = 1 April. Brood size is the number of hatched young and parasite intensity is the number of parasites per 10^4 red blood cells. Sample sizes for all variables except brood size are: 2Y birds, reduced $n = 27$, control $n = 46$, enlarged $n = 22$; 3Y+ birds, reduced $n = 14$, control $n = 24$, enlarged $n = 24$, control $n = 27$, control $n = 43$, enlarged $n = 21$; 3Y+ birds, reduced $n = 14$, control $n = 23$, enlarged $n = 18$.)

age	treatment	clutch	lay date	hatch date	brood size	parasite intensity	body mass
2Y	reduced	11.33 (1.39)	30.3 (4.0)	53.2 (4.3)	10.89 (1.45)	182.3 (170.0)	13.57 (0.65)
	control	10.85 (1.61)	29.3 (4.8)	51.7 (4.4)	10.15 (1.92)	154.4 (126.3)	13.47 (0.49)
	enlarged	11.09 (1.19)	28.7 (4.3)	51.0 (4.5)	10.68 (1.36)	155.5 (126.2)	13.61 (0.55)
	reduced	11.71 (1.14)	27.8 (3.3)	50.6 (3.1)	10.86 (1.35)	57.4 (51.0)	13.74 (0.68)
3Y+	control	11.79 (1.50)	27.2 (6.5)	50.2 (5.8)	11.26 (1.81)	59.0 (58.6)	13.86 (0.73)
	enlarged	11.68 (1.20)	28.1 (4.4)	51.4 (4.1)	11.05 (1.51)	95.4 (125.3)	13.91 (0.38)
	reduced	11.46 (1.31)	29.4 (3.9)	52.3 (4.1)	10.88 (1.40)	139.6 (152.4)	13.63 (0.65)
	control	11.17 (1.62)	28.6 (5.5)	51.2 (4.9)	10.55 (1.94)	121.7 (116.7)	13.60 (0.61)
total	11.37 (1.22)	28.4 (4.3)	51.1 (4.3)	10.85 (1.42)	127.6 (127.9)	13.75 (0.49)	

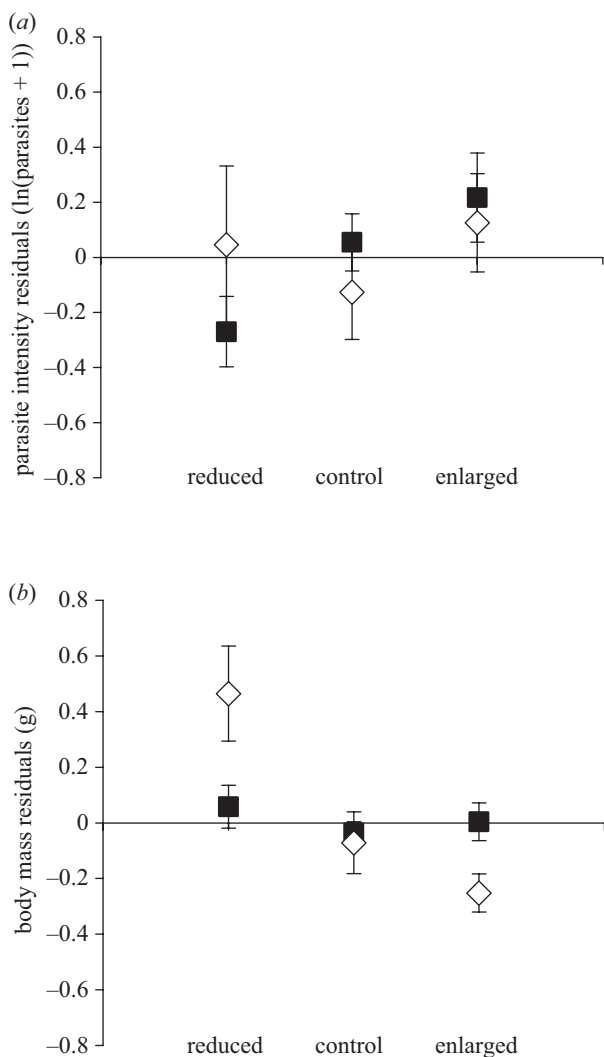


Figure 1. (a) Parasite intensity residuals and (b) body mass residuals for female blue tits belonging to the three different experimental groups. Residuals are from ANCOVAs where post-manipulation values (day 14) are the dependent variable and year (2Y birds only) and pre-manipulation values are the independent variables. Symbols (filled squares, 2Y birds; open diamonds, 3Y+ birds) show mean values (\pm s.e.m.). Sample sizes for 2Y birds: reduced $n = 27$, control $n = 46$, enlarged $n = 22$, and for 3Y+ birds: reduced $n = 14$, control $n = 24$, enlarged $n = 19$.

17.5% compared with controls, whereas females with enlarged broods increased their feeding effort by 30.0% compared with controls (reduced, $n = 28$: mean (s.d.) = 306 (86) feedings per 24 h, control, $n = 37$: 371 (93) feedings per 24 h, enlarged, $n = 29$: 482 (119) feedings per 24 h; $F_{1,90} = 42.47$, $p < 0.0001$). There was no overall difference between age classes in feeding effort ($p = 0.45$), and no interaction between treatment and age ($p = 0.86$). Further evidence suggesting that our manipulation was successful in altering reproductive effort is presented in electronic Appendix A.

(b) Age differences in parasite intensity

The two age classes differed in parasite intensities, 2Y birds having higher intensities both pre- ($F_{1,150} = 17.74$, $p < 0.0001$) and post-manipulation ($F_{1,150} = 29.14$, $p < 0.0001$). Such an age difference in intensities of blood

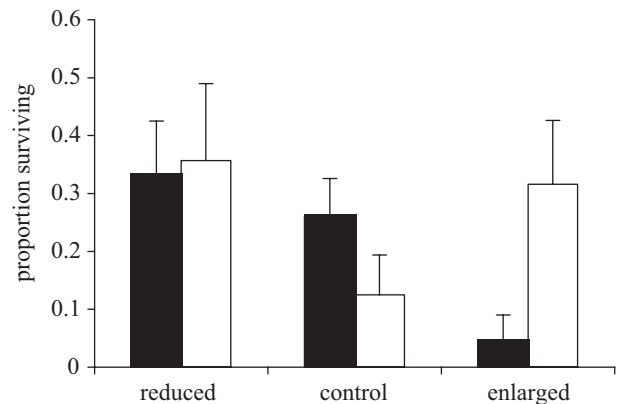


Figure 2. Survival of female blue tits to at least the first winter after the brood size manipulation. Bars denote average probability (\pm s.e.m.) of survival for 2Y (black) and 3Y+ (white) birds. Sample sizes as in figure 1.

parasite infection has previously been found (e.g. Allander & Bennett 1994; Sundberg 1995; Allander 1997; Sol *et al.* 2000) and is probably caused by the development of immunity (Sol *et al.* 2003). We therefore analysed the two age classes separately.

(c) Effect of brood size manipulation on parasite intensity, body mass and survival

In 2Y birds, we found a significant positive relationship between brood size manipulation and parasite intensity ($F_{1,88} = 6.08$, $p = 0.016$), controlling for significant effects of year ($F_{2,88} = 3.74$, $p = 0.028$), intensities before the manipulation ($F_{1,88} = 110.01$, $p < 0.0001$), and a significant interaction between year and pre-manipulation intensities ($F_{2,88} = 3.13$, $p = 0.049$; figure 1a). By contrast, post-manipulation parasite intensity in 3Y+ birds was not affected by the brood size manipulation (treatment: $F_{1,54} = 0.12$, $p = 0.73$; pre-manipulation intensity: $F_{1,54} = 41.12$, $p < 0.0001$; effect of year non-significant and excluded; figure 1a).

Manipulating brood size had a strong negative effect on body mass in 3Y+ birds (treatment: $F_{1,54} = 15.17$, $p = 0.0003$; pre-manipulation body mass: $F_{1,54} = 15.79$, $p = 0.0002$; effect of year non-significant and excluded; figure 1b). For 2Y birds, however, no significant effect of brood size manipulation on body mass could be detected (treatment: $F_{1,90} = 0.42$, $p = 0.52$; year: $F_{2,90} = 11.61$, $p < 0.0001$; pre-manipulation body mass: $F_{1,90} = 82.26$, $p < 0.0001$; figure 1b).

Finally, the brood size manipulation had a significant effect on the survival probability in 2Y birds such that increasing or decreasing brood size decreased or increased, respectively, the survival probabilities (survival regressed on treatment: Wald $\chi^2 = 5.11$, d.f. = 1, $p = 0.024$; figure 2). In 3Y+ birds no such relationship could be found (Wald $\chi^2 = 0.01$, d.f. = 1, $p = 0.93$; figure 2).

(d) Relative effects of parasite intensity, body mass and brood size manipulation on survival

In 2Y birds, in a logistic regression including treatment, pre- and post-manipulation values of parasite intensity, and body mass, only treatment had a significant effect on survival (Wald $\chi^2 = 5.23$, d.f. = 1, $p = 0.022$; figure 3a).

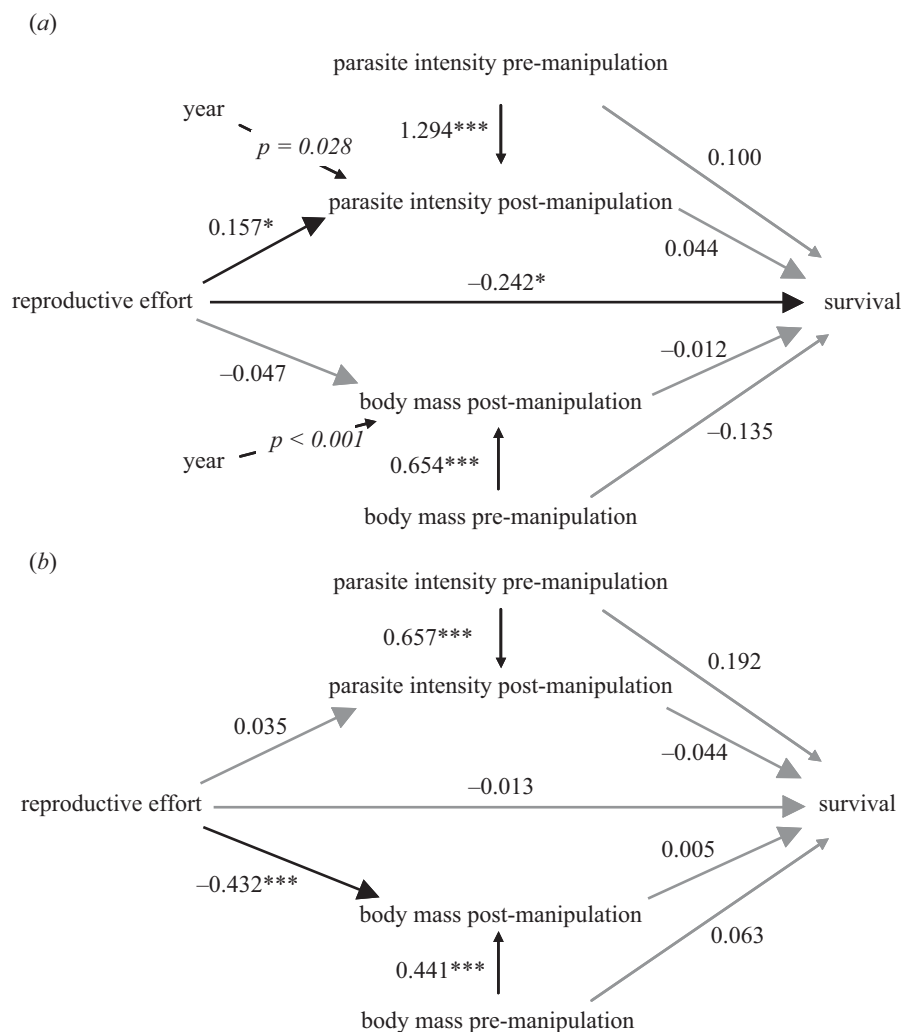


Figure 3. Path diagram showing the relationship between brood size manipulation (reproductive effort), parasite intensity and body mass before and after the manipulation, and survival to the first winter after the experiment. (a) 2Y birds, (b) 3Y+ birds. Path coefficients (black arrows significant, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$; grey arrows non-significant) are standardized partial regression coefficients from multiple regressions or ANCOVAs. Thin arrows indicate variables included in the models when estimating coefficients (with accompanying standardized partial regression coefficients for continuous, and p -values for categorical variables). In (a) a significant interaction between year and pre-manipulation parasite intensity is also included when estimating the relationship between brood size manipulation and post-manipulation parasite intensity (see § 3c). Sample sizes as in figure 1.

By contrast, in 3Y+ birds, neither brood size manipulation nor parasite intensity nor body mass affected survival (all $p > 0.20$; figure 3b).

(e) Path model

We summarized the results from the analyses above in a path model (figure 3). In 2Y birds, the indirect effect (s.e.m. within parenthesis) of reproductive effort on survival via parasites is 0.007 (0.026), and through body mass 0.001 (0.026), which should be compared with the direct effect of -0.242 (0.103) (figure 3a). The corresponding indirect effects in 3Y+ birds are -0.002 (0.008) through parasites and -0.002 (0.076) through body mass, compared with -0.013 (0.159) for the direct effect (figure 3b).

4. DISCUSSION

Through our manipulation of reproductive effort we were able to demonstrate a reproductive cost in the form of

reduced survival. There are as yet only a few studies that have found survival costs of reproduction in passerine birds (reviewed in Golet *et al.* 1998). In our study, the cost was only evident in 2Y birds, even though both age classes responded similarly to the manipulation in terms of feeding effort. Thus, our data suggest the interesting possibility that 3Y+ birds are better able to deal with potential problems of a high workload. This age difference could be mediated through 2Y birds being less skilful in foraging for antioxidants (cf. Senar & Escobar 2002), thus suffering a higher level of oxidative stress (cf. von Schantz *et al.* 1999) than 3Y+ birds. Younger birds are also, on average, subordinate to older birds (e.g. Lahti *et al.* 1996) and hence more sensitive to unfavourable environmental conditions (Gosler & Carruthers 1999). Such differences in sensitivity could possibly also explain differences in the effect of reproductive effort on survival (Verhulst 1998).

The question is then what mediates this cost in 2Y birds. We found that reproductive effort was positively correlated with a change in parasite intensity. This result is in accordance with several previous studies (Allander 1997; Siikamäki *et al.* 1997; Nordling *et al.* 1998). However, a comparison of the indirect and direct pathways from reproductive effort to survival showed that the survival costs were not mediated by the blood parasite studied (figure 3). Thus, even though we found a negative relationship between effort and the ability to control parasite intensities, these parasites do not seem to affect survival, as the indirect effect of reproductive effort on survival through parasites is very small compared with the direct effect (only the latter was statistically significant). This result is in line with the general view that parasites of the genus *Haemoproteus* are relatively benign (Atkinson & van Riper 1991). However, some recent studies have reported reduced survival associated with *Haemoproteus* parasitism in birds (Nordling *et al.* 1998; Dawson & Bortolotti 2000; Hörak *et al.* 2001; Sol *et al.* 2003). There are several possible explanations for these differences between studies. First, there might be spatial and/or temporal differences between populations and species of hosts and parasites in their resistance and virulence, respectively (Ebert 1994; Kraaijeveld *et al.* 1998; Lively 1999). A second possibility is that there is a non-linear relationship between parasite intensity and survival (Behnke *et al.* 1992). Such a relationship might be expected because different types of immune response (i.e. humoral versus cell-mediated or rather T_H1- versus T_H2-types of response) are effective against different types of parasite (Janeway & Travers 1996). In light of the antagonistic relationship between these different types of response (Jankovic *et al.* 2001; Erf 2004), a host that mounts an effective response against one parasite might have reduced ability to control another (Biozzi *et al.* 1984). In a situation in which several different types of parasite can infect a host, intermediate resistance against a particular parasite should be optimal, producing a nonlinear (bell-shaped) relationship between parasite intensity and survival.

Even if we could not find any evidence for *Haemoproteus* to be an important mediator of survival costs of reproduction, they may mediate costs in the currency of future fecundity. At present we do not have sufficient data to perform such an analysis. It is also important to note that although we were unable to find effects of these parasites on the survival of adult birds, they may still be an important selective force in juveniles when the immune system in general is poorly developed and/or before immunological memory to these parasites has been developed (Atkinson & van Riper 1991; Apanius 1998).

The reproductive cost that we observed can neither be explained by a negative effect on body condition as measured by the change in body mass. In 2Y birds, the amount of work invested in nestling feeding did not affect body mass. By contrast, we found a strong effect of workload on body mass in 3Y+ birds, suggesting that age classes differ in their strategic decisions during breeding. However, body mass did not affect survival in either age class, and therefore cannot be a mediator of the reproductive cost that we found.

We conclude that none of the indirect pathways we investigated could explain the survival costs in 2Y birds. Thus, it still remains to unravel the identity of the factor(s) that can explain the link between reproductive effort and

survival. We suggest three possibilities here. First, the negative relationship between reproductive effort and resistance against *Haemoproteus* parasites suggests that we cannot exclude the immune system as a link to costs of reproduction. It is possible, that other more virulent parasites are controlled by the same mechanisms as *Haemoproteus*, and that these other parasites mediate survival costs. Second, reproductive effort may affect the annual renewal of feathers (moult), an energy- and time-consuming activity (Lindström *et al.* 1993; Klaassen 1995) in potential conflict with the requirements of breeding (Siikamäki *et al.* 1994; Svensson & Nilsson 1997). Constraints imposed on the moult of birds may negatively affect the quality of the new feathers, resulting in increased energetic costs of thermoregulation during winter (Nilsson & Svensson 1996), which in turn can reduce survival probabilities. Third, a high metabolic rate, stemming from an increased investment in reproduction, increases the production of oxidative metabolites and free radicals (von Schantz *et al.* 1999; Alonso-Alvarez *et al.* 2004; Wiersma *et al.* 2004). Such oxidative stress increases the rate of cell destruction and tissue damage leading to senescence and death (Finch 1994; Perez-Campo *et al.* 1998).

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