Working for a living

Wiersma, Poppe

WORKING FOR A LIVING

Physiological and behavioural trade-offs in birds facing hard work

Popko Wiersma

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Chapter 1

Introduction

Popko Wiersma

Trade-offs and the costs of reproduction

Because any organism has to fulfil a myriad of different functions to stay alive and to reproduce, using numerous physiological processes that all draw from the available resources, compromises have to be made. Evolution results in a solution by optimising these trade-offs so that the fitness of the individual is maximised (Maynard Smith 1978a; Figure 1.1). Reproduction can be considered the key component of life against which other functions, that serve merely to survive, are traded-off. The trade-offs with reproduction, eventuating in the 'costs of reproduction' (Williams 1966), were the starting point of this thesis. A series of experiments was performed, on different bird species, both in the laboratory and in the field, to gain knowledge on the nature of these trade-offs, what the involvement of energy is, and how the energy budget is managed under conditions with varying energetic demands.

Fitness costs can be mediated by ecological factors (increased risk, reduced encounter probability of potential mates, etc.) or by physiological factors (body reserves, maintenance, protection against diseases, etc.). The costs of reproduction will vary with the reproductive effort, i.e. the rate at which resources (e.g. energy or time) are channelled into a reproductive event. However, reproductive effort is not easy to measure, partly because it is not clear what to measure in the first place. Usually, it is approximated by measuring energy expenditure during parental activities, such as incubation and food provisioning (e.g. Ricklefs & Williams 1984; Bryant 1988; Masman *et al*. 1989; Moreno 1989b; Mock 1991; Tinbergen & Dietz 1994). However, the fitness costs of individuals spending energy at equal rates might differ greatly, e.g. because their foraging efficiency and health status vary (Tinbergen & Verhulst 2000). What really counts is to what extent mechanisms for maintenance and protection of the soma suffer from channelling resources into reproduction and how this affects current and future reproductive success. Affected maintenance and protection mechanisms can be either behavioural or physiological. Examples from behaviour are vigilance to avoid predators, and preening to avoid wear and infections. Physiological mechanisms include cell replacement, DNA repair, immune responses and antioxidative protection (to protect against damaging oxygen radicals; Figure 1.1).

Although a trade-off may be the result of an energetic limitation, alternative limiting resources are of course possible. Time, nutrients and water are the most important other resources. Hence, energy management needs not be the driving force in life history evolution but can also be a product of other factors that are inescapably linked to energy (Ricklefs & Wikelski 2002). Nevertheless, studying the energetics of reproductive effort can give more insight into the mechanisms of the physiological constraints that underlie the trade-offs in life history evolution (Stearns 1992). In birds the study of energetics is particularly appropriate because in many cases they spend energy at very high rates during parental effort (both incubation and food provisioning), probably often close to a metabolic ceiling (Drent & Daan 1980; Williams 1996a; Tinbergen & Williams 2001). In the overview given by Hammond & Diamond (1997), metabolic scopes (i.e. maximum measured sustained metabolic rate/resting metabolic

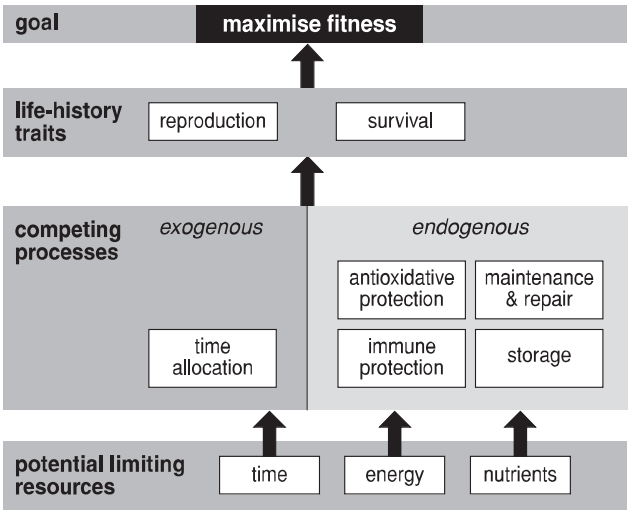

Figure 1.1 Chart showing physiological and ecological processes, competing for the same resources, that are treated in this thesis. Resources in short supply result in trade-offs between different processes and activities that are important for survival and reproduction. Ultimately, selection will lead to optimal outcomes of these trade-offs so that fitness is minimised. Oxidative stress is caused by reactive oxygen species that are formed as by-products of energy metabolism and by detoxification processes of the immune system. Maintenance & repair encompasses the repair or renewal of damaged cells, temperature regulation, the production of hormones and neurotransmitters, etc. Storage is the build up of energy for nutrient stores (fat, calcium, etc.) for later usage. Note that this chart is far from complete. Other processes include the availability of social partners and nest sites, water economy, etc.

rate (RMR)) in birds vary between 1.3 and 6.6 (mean 3.3 ± 0.25 (SE), n = 27). To scale these figures to something we humans find easier to appreciate, professional Tour de France cyclists, that cycled 3826 km, crossing 34 mountains, for a period of 22 days, spent 4.3 times BMR; the highest sustained metabolic scopes measured in humans (Westerterp *et al*. 1986). These cyclist can be considered to have worked on a maximum sustainable level, because they were taking in energy at maximum rates while not depending on body stores.

Energy management

In case energy is a limiting resource during reproduction or at other times, it will be beneficial to spend it as economically as possible. Then, maintenance and protection mechanisms should not be downregulated more than necessary. Part of this thesis discusses how energy is managed under increasingly strenuous conditions. This was also elicited by the evidence that showed that birds may reduce BMR and night-time energy expenditure under demanding conditions (Deerenberg *et al*. 1998; Bautista *et al*. 1998;

Nudds & Bryant 2001). Deerenberg *et al*. (1998) and Bautista *et al*. (1998) manipulated the costs of foraging by forcing, respectively, zebra finches *Taeniopygia guttata* and starlings *Sturnus vulgaris* to fly between two perches for a particular number of times before rewarding them with food. The birds were kept under these conditions for periods lasting several weeks. The zebra finches were on either a low or high costs schedule, having to fly 20 or 40 times, respectively, over 56 cm to gain 10 s access to food. The birds with low and high foraging costs had a BMR of, on average, 0.215 and 0.164 W, respectively. The starlings were alternately flying between perches 4.65 m apart and walking between perches 0.35 m apart. Birds on a low costs schedule made 2 flights per food reward and those on the high costs schedule 7.8. BMR of these birds were 0.814 and 0.493 W for low and high costs, respectively. Nudds & Bryant (2001) also made zebra finches fly more, but not for food, and measured a reduction in BMR in harder working birds too: BMR reduced from 0.63 to 0.54 W.

Presumably, saving energy through a BMR reduction leaves more energy for other processes and day-time activities. If energetic compensation through reduced BMR and night-time energy expenditure occurs regularly in free-living animals, the daily energy expenditure (DEE) is not simply proportional to the level of activity, as usually assumed in time-energy budgets (e.g. Ashkenazie & Safriel 1979; Williams & Nagy 1984; Bryant *et al*. 1985; Masman *et al*. 1988). In the studies of Deerenberg *et al*. (1998) and Bautista *et al*. (1998), DEE even decreased with increasing feeding activity! In **Chapter 2** and **4** we expand on the data of Deerenberg *et al*. (1998) and Bautista *et al*. (1998), applying other experimental conditions while manipulating foraging costs. Especially the application of variable reward rates instead of fixed, as in the experiments of Deerenberg *et al*. (1998) and Bautista *et al*. (1998), might have a large effect on food intake rates and energy budgets (Ferster & Skinner 1957; Fotheringham 1998). Fotheringham (1998) kept starlings in cages were they had to fly a number of times between perches to obtain food. He either applied fixed or variable food reward rates, meaning that the number of flights needed to obtain food was completely predictable (without variation) or with only the mean number of flights set while applying random variation around this mean. When the number of flights per reward was increased, the starlings with fixed reward rates became lighter, while those with variable reward rates did not. In our starling experiments we therefore applied variable reward rates. We think this also is closer to the natural situation, where variation and unpredictability in foraging success are likely to prevail. In **Chapter 2** we give an overview of results from comparable experiments to find out whether a general pattern emerges.

The starlings in our cages made many short flights to obtain their food. Flying over short distances, however, entails very high flight costs; in small birds over three times the predicted metabolic rate from aerodynamic models (Nudds & Bryant 2000). Therefore, the flying time of the starlings in our cages will have a major effect on their energy budget necessitating an accurate estimate of the energy expenditure during these short flights. We used labelled sodiumcarbonate to measure the ^{13}C isotope elimination rate in starlings flying in the cages (**Chapter 3**). This method was earlier applied in large animals, but was recently adapted to suit small animals as well

(Speakman & Thomson 1997; Hambly *et al*. 2002). The advantage over applying doubly labelled water is the fast elimination rate of the isotope, enabling measurements over very short time spans. This makes it possible to let the animal only perform one activity (flying, e.g.) during the measurement.

Because these experiments were performed on non-reproducing, captive birds, extrapolating to free-living birds that work for their offspring is precarious. We wanted to know whether birds with high energy expenditure rates caused by they are feeding nestlings might also reduce BMR and therefore measured BMR in free-living great tits *Parus major* during brood provisioning (**Chapter 5**). During two seasons we manipulated parental effort by altering brood sizes and measured BMR using a transportable oxygen meter. During one season we also measured DEE using doubly labelled water and measured the provisioning rates with video observations to quantify the effect of brood size on parental effort.

An alternative path of reasoning can be followed, however. A sustained increase in muscle exercise has been shown to be associated with enlargement of exercise organs, such as heart and muscles, in preparation for migration (Jehl 1997; Battley & Piersma 1997; Biebach 1998; Karasov & Pinshow 1998; Lindström *et al*. 2000), and because the size of metabolically active organs in part determines BMR (Daan *et al*. 1990; Piersma 2002), an increase in BMR in birds with increased foraging costs or parental costs could be envisaged as well. The increase in pectoral muscle size in migrating birds is not the result of training but endogenously regulated (Dietz *et al*. 1999), and hypothetically this could be the case in birds preparing for high parental demands. Similar relationships have been shown in lactating mammals. During lactation, the sustained metabolic rates of mammals with more than one pup, lay between 4.6 and 6.7, while BMR is shown to increase due to body composition changes (Speakman & McQueenie 1996; Koteja 1996; Hammond & Diamond 1997; Johnson *et al*. 2001a). Note that grey seals *Halichoerus grypus*, that are fasting during lactation, and hence not increasing the size of any digestive organ, reduce their maintenance metabolic rate during lactation (Mellish *et al*. 2000). Mellish *et al*. (2000) conclude that grey seals are using compensatory mechanisms to meet the high costs of lactation, that are apparently not associated with changes in lean body mass and behaviour.

Because data on the relationship between reproductive effort and BMR was lacking we studied this in captive zebra finches (**Chapter 6**). In the birds in the experiment to study BMR changes in response to a brood size manipulation (**Chapter 2**), we also looked for associations between pre-breeding BMR and (unmanipulated) reproductive output. As described above, a positive association between BMR and reproductive effort could be expected.

Protection mechanisms

An important category of functions that are involved in reproductive trade-offs are those that serve to protect against the many perils that animals meet (Figure 1.1; Sheldon & Verhulst 1996; von Schantz *et al*. 1999). Threats from the environment include fights with conspecifics or predators, accidents (e.g. collisions), diseases, parasites and toxins. They can lead to injuries, physiological damage, death or interfere with chemical, e.g. hormonal, processes. Also the organisms' own energy metabolism has negative side-effects due to the production of reactive oxygen species (ROS), such as oxygen free radicals (Jenkins 1988; Cadenas 1995; Finkel & Holbrook 2000). These noxious molecules cause so called oxidative stress by damaging DNA, proteins and lipids, leading to senescence (Chen *et al*. 1995; Felton 1995; von Schantz *et al*. 1999).

A range of protection mechanisms have evolved to cope with these perils. The immune system protects against intruding alien cells and molecules with a suite of specialised cells (Møller & Saino 1999; Whittow 2000). The immune system has two ways of doing this. The humoral immune response targets alien macromolecules with specific antibodies that are derived from B-lymphocytes (B-cells, originating in the bursa of Fabricius). This system has a 'memory', enabling an accelerated antibody response when the specific infection returns. The molecule eliciting an antibody response is called an antigen. The humoral immune response is most effective in destroying extracellular bacteria and viruses. The other category is called the cell-mediated immune response. It destroys virus-infected cells, parasites and cancer cells by non-specific T-lymphocytes (T-cells, originating in the thymus). T-cells are also important in the humoral immune system, because they stimulate the proliferation of B-cells.

The immune and detoxification (i.e. deactivation of chemicals) systems often use reactive metabolites and free radicals to deactivate the alien compounds, by which it also is a source of oxidative stress (von Schantz *et al*. 1999). ROS, that originate from the organism itself, can be detoxified by antioxidants. Exogenous antioxidants, such as carotenoids and vitamin C, are taken up from the diet (Sies & Stahl 1995; Stahl & Sies 1997), while antioxidant enzymes are produced inside the cells. Three types of antioxidant enzymes exist, namely superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (Ahmad 1995). Damage to DNA can be repaired to overcome harmful effects (Maldonado *et al*. 1996; Slupphaug *et al*. 1996), but little is known about the regulation of these processes.

Trade-offs between reproductive effort and protection mechanisms against diseases, parasites and oxidative stress were studied in captive zebra finches. The quality of immune responses was measured by challenging the birds' humoral immune system with antigens (sheep red blood cells, SRBC; **Chapter 7**). Experimentally increasing reproductive effort in zebra finches had earlier been shown to reduce a humoral immune response to SRBC (Deerenberg *et al*. 1997). In our experiments we wanted to find out whether this could have been due to an energetic trade-off. Therefore, we measured metabolic rates prior and after the immunisation. We also considered the possibility that an immune response is not energetically costly at all, but that it is rather the

maintenance of the immune system as a whole that is costly. In an experiment we manipulated brood sizes of zebra finches and measured the antibody responses after removal of the chicks. In that situation the birds were relieved from parental duties. If immuno-suppression would still be found in these birds, this would suggest that the immune system had been downregulated with increasing brood size, prohibiting an immediate response to the chick removal.

As an aspect of protection against oxidative stress we measured antioxidant enzyme activity (SOD and GPx) in zebra finch pectoral muscle tissue (**Chapter 8**). Oxidative stress has been intensely studies in the context of ageing and degenerative diseases (Austad 1997; Beckman & Ames 1998), but in relation to reproductive effort in vertebrates it is new. We manipulated brood sizes and let the birds feed their chicks for almost 3 weeks. We expected that the antioxidant activity would have been downregulated. The consequences of lower antioxidant activity levels would be more damage to DNA or other molecules. Like parasites and diseases, oxidative stress has the potential as a major selective force.

Time reallocation as a cost of reproduction

Investing time and energy in social and other behaviours may also be involved in trade-offs with parental effort. Potentially this can have strong fitness effects. Activities that may become affected are, for example, vigilance, increasing the risk of predation (Magnhagen 1991), or preening, increasing the risk of damage to feathers and infestation with parasites. These activities are already incorporated in the costs of reproduction as investments in maintenance and protection. Another category, however, is the time devoted to pursuit of extra-pair copulations (EPCs). There is considerable interest in the consequences of mating status and extra-pair fertilisations (EPFs) for the amount of parental care provided (Orians 1969; Searcy & Yasukawa 1989; Whittingham *et al*. 1992; Westneat & Sherman 1993; Slagsvold & Lifjeld 1994; Westneat & Sargent 1996; Bruun *et al*. 1997; Smith & Sandell 1998). But the other way around, consequences of the amount of parental care provided for the opportunities for EPFs, has received much less attention (Magrath & Komdeur 2003). Birds of (facultative) polygynous species, that spend more time on incubation, brooding or food provisioning, will have less time left for extra-pair liaisons and for mate guarding. The trade-off between pursuing EPCs and caring for the current clutch or brood will be affected by the size of the clutch and brood, because that is expected to affect how the clutch or brood is valued (in terms of fitness) by the parents (Delehanty & Oring 1993). Males attending large clutches or broods may have relatively less to gain from pursuing EPCs or attracting additional mates than males with small clutches or broods (Wright & Cuthill 1990; Whittingham 1993; Magrath & Elgar 1997).

We measured the time that male starlings spent on pursuing additional mates in relation to manipulated clutch size (**Chapter 9**). The starling is a semi-collonial hole-nesters, making it a very suitable species for this study. Both males and females incu-

bate the eggs and feed the nestlings. When a male is polygynous his part in parental care for a single clutch or brood is significantly lower (Pinxten & Eens 1994; Smith *et al*. 1995; Sandell & Smith 1996). Paired starling males trying to attract an additional mate must first occupy another nestbox. They usually sing very close to or in this nestbox using 'wing-waving' displays (Feare 1984; Eens *et al*. 1990) and carry green plant material into the nestbox when females are nearby (Gwinner 1997; Brouwer & Komdeur, submitted manuscript). We measured the time that males spent singing close to a neighbouring, empty nestbox during incubation of the first clutch, and the frequency of bringing green plant material into the nestbox (see Pinxten & Eens 1998). We expected that when we had enlarged a clutch the males would spend less time singing near and bringing green plant material to an adjacent empty nestbox, resulting in a negative relationship between the chance of getting an additional female and clutch size.

Chapter 2

Effects of foraging costs per reward on the energy budget, condition and reproduction of zebra finches

Popko Wiersma & Simon Verhulst

Abstract

We studied how costs of foraging affected components of the energy budget of zebra finches *Taeniopygia guttata*, because so far results were equivocal. Earlier studies showed that increasing foraging costs were compensated by night-time savings, resulting in a decrease in daily energy expenditure (DEE). However, this may have been due to the fixed foraging reward rates, because when applying variable reward rates birds were shown to maintain or increase DEE. We manipulated the foraging cost per seed found, applying variable reward rates, by adding 0, 25 or 75 g of chaff to 25 g of seeds. Night- and day-time energy expenditure were measured during 24-h sessions at 12, 22 and 32°C.

With increasing foraging costs per reward, the time spent foraging increased from 6 to 27% of the 10-h day (up to 40% at 12°C). At the temperature of the holding room (22°C), DEE decreased by 6.6% with increasing foraging costs (independent of temperature), which was due to a decrease in metabolic rate both during the day (by 5.6%) and night (7.7%). The decrease in DEE was more pronounced at low than at high temperatures. DEE changes were not caused by body mass changes, because foraging costs had no effect on mass. Energy was conserved by decreasing non-foraging activity during the day (measured with infrared sensors) when foraging costs increased (except at 12°C when non-foraging activity might have been negligible at all chaff/seed ratios). Body composition was apparently not affected because basal metabolic rate (BMR) and mass-specific BMR were not related to foraging costs. Because trade-offs may exist with maintenance and repair processes we measured, as possible indicators, the initiated growth of new tail feathers and, after put on *ad libitum* food again, of succeeding food intake and reproductive output (time delay until laying, clutch and brood size and fledgling number). We did not find effects of foraging costs on any of these parameters.

An overview of literature data showed that a common feature of studies using fixed reward rates in foraging costs manipulations, was a decrease in daily food intake and/or DEE with increasing foraging costs. In experiments using variable reward rates daily food intake and/or DEE remained constant, increased or decreased (this study). Although BMR was not measured in all studies, this is the only study showing a constant value, while in all other cases BMR decreased with increasing foraging costs. We conclude that effects of foraging costs on energy budgets remain elusive and that extrapolations to field conditions must be assessed with reservations.

Introduction

The assumption that the food intake rate positively affects fitness underlies most studies concerning foraging behaviour (Stephens & Krebs 1986; Stearns 1992). Usually, trade-offs within the energy budget are considered to link the two together. Studies that measure effects of food intake rates on the energy budget may therefore provide important insights into life-history decisions. Recent studies indicate that the relationships between food intake and energy expenditure are not clear-cut. Deerenberg *et al*. (1998) manipulated the foraging costs of captive zebra finches *Taeniopygia guttata* by forcing them to hop between perches before giving access to food. Daily energy expenditure (DEE) of these birds decreased with increasing foraging costs, which was the result of a reduced energy expenditure during the night and during the non-foraging time throughout the day. Bautista *et al*. (1998) manipulated foraging costs by varying the number of flights and walks starlings *Sturnus vulgaris* needed to make before being rewarded with food. They also found that DEE decreased with increasing foraging costs and that compensation for increased foraging activity took place by lowering the metabolic rate during the night. However, it has been shown that the way in which foraging is rewarded can have a crucial effect on the foraging behaviour and energy budget (Fotheringham 1998). Fotheringham, also working with starlings, showed that the slope of the relationship between daily food intake and foraging reward rate depended on the variation around the mean foraging success: when food was offered after completion of a fixed number of flights, daily food intake and body mass decreased, while both were unaffected when variation was applied around the average number of flights needed to obtain a reward. Wiersma *et al*. 2003a: (Chapter 4) also kept starlings at variable reward rates and actually showed an increase in DEE with increasing foraging costs. Apparently, cognitive processes may lead to different foraging decisions (see discussion in Kacelnik & Bateson 1996). Hence, identical mean foraging efficiencies that differ only in their variation, may result in very different energy budgets. We hypothesise that the discrepancy in the effect of foraging effort on DEE between Fotheringham's (1998) experiments and those of Deerenberg *et al*. (1998) and Bautista *et al*. (1998) can be explained by the variation in the foraging reward rates. In the latter two studies the fixed reward rates may have led to negative relationships between foraging costs and DEE.

We tested whether zebra finches would compensate for increased foraging costs by reducing nigh-time energy expenditure while applying variable reward rates. We mixed seeds through chaff according to three different chaff/seed ratios to manipulate the search time and hence foraging costs. This method was earlier used by Lemon (1991; 1993) and Lemon and Barth (1992). The total amount of food offered was identical for all chaff/seed ratios, but only the probability of finding seeds was altered. We think that this set-up better resembles natural foraging circumstances than the starling and zebra finch experiments and that it might therefore elicit more natural foraging decisions. This manipulation is in essence the same as the manipulation of food reward rates with variation around the mean as performed by Fotheringham (1998)

and likewise, we would have expected DEE not to decrease with a lowering of the food reward rate (i.e. increasing chaff/seed ratio).

Lemon (1991; 1993) and Lemon and Barth (1992) tested experimentally how foraging costs were related to fitness. They manipulated chaff/seed ratios offered to groups of captive zebra finches. By monitoring the life-time reproductive success of groups of birds held under different food conditions they showed that life-time reproductive output and adult survival declined when the foraging costs per reward increased. To explain the results, Lemon (1993) constructed energy budgets, and found that a margin of excess energy resulted in more fledglings and an extended life span. Although the time that was spent on foraging varied considerably, Lemon (1993) did not find differences in DEE between the experimental groups, possibly because night-time savings concealed differences in day-time energy expenditure. Therefore, we measured effects of food intake rates on night- and day-time energy expenditures. By using within-individual comparisons we maximised statistical power. We will give an overview of the effect of variability in foraging reward rates on the energy budget as reported in the literature.

The conditional occurrence of energetic compensation begs the question why birds not always reduce night-time energy expenditure to the minimal level. Apparently, there are costs to a metabolic rate reduction exceeding levels found under benign conditions. Possibly, energy is reallocated from maintenance and repair processes to foraging. As an indicator of these processes we tested whether experimentally induced feather growth was affected by the manipulation of foraging costs. This method has been shown to reveal nutritional stress and effects of parental effort (e.g. Grubb *et al*. 1991; White *et al*. 1991; Jenkins *et al*. 2001). Additionally, we also tested for persisting effects of manipulated foraging costs on reproductive output. Deerenberg and Overkamp (1999) studied long-term effects of elevated work loads on later reproduction in the zebra finch. They found a delay in the time until laying of the first egg, as had earlier been found in the same species experiencing a reduction in intake rate during breeding (Lemon & Barth 1992). We tested whether reproductive output was also engendered immediately after stopping the foraging costs manipulation.

Methods

We manipulated the foraging costs per reward following Lemon (1993) by mixing the food (tropical seed mixture) with chaff, thereby increasing search time per seed obtained. We applied three different treatments, mixing 25 g seeds with 0, 25 or 75 g of chaff respectively. Fresh food and chaff was provided every other day.

Twenty-four single-sex pairs of wild-type zebra finches (12 pairs of each sex) were kept in cages measuring 40x80x40 cm (hxwxd). Ambient temperature was between 22 and 24°C. After a week on a particular chaff/seed ratio we measured energy expenditure for 22-23 hours in an open-flow respirometer. These measurements were repeated at 3 different temperatures (12, 22 and 32°C) at 3-day intervals. During these meas-

urements the pair had access to food in the same chaff/seed ratio as in the preceding week. On the basis of literature we predicted that 32°C was within the thermoneutral zone (Calder 1964), 22°C resembles the temperature in the holding room, and measurements at 12°C were done to test whether night-time saving would increase when overall energetic demands increased.

After completion of the last respirometer measurement the chaff/seed ratio was changed, and after a week the three metabolic measurements were repeated. All pairs were exposed to all three chaff/seed ratios. We arranged the order of chaff/seed ratios presented, and of the temperatures at which the pairs were measured in such a way that treatments and measurement order were not correlated.

The measurements were done on the two birds of a pair simultaneously to increase measurement precision and because the zebra finch is a gregarious species, which may be more at ease when housed with a conspecific. The two birds were separated by a transparent partition, so they could see and hear each other but not interact physically. Measurements of oxygen consumption (using a paramagnetic Servomex Xentra 4100 analyser), carbondioxide production (Servomex 1440) and air flow rate (Brooks 5850S mass-flow controllers) were stored every 6th minute. Dry air was pumped through the 24-l, Plexiglas respirometer boxes at a rate of 36 l/h. The air was dried over a molecular sieve (3Å, Merck). The metabolic rate (MR) was calculated from the oxygen consumption rate using the RQ dependent conversion factor as given by Brody (1945). All MR measurements were divided by 2 to get values for a single bird. Before and after the measurement we measured body masses to the nearest 0.1 g.

Of a subset of sessions we made 1.5-h video recordings of the feeding behaviour, starting at the onset of the light period (9:00). The activity of the birds was further automatically recorded with passive infrared sensors (PIRs) fit inside the respirometer cages. The PIR measurements did not distinguish between the movements of the two individuals. Pairs were always measured in the same box.

Following this experiment we made 24 female-male pairs and kept them on the different chaff/seed ratios for 6 weeks (8 pairs for each seed-chaff ratio). Of these birds we plucked the left and right outer tail feather to measure the growth rate of the newly formed feathers. The length of the growing feathers was measured with a ruler after 12 d, and subsequently at 4 to 8-d intervals. Forty-two days after plucking the tail feathers the newly formed feathers were plucked and weighed to the nearest 0.1 mg. The left and right tail feather were compared to check for fluctuating asymmetry (e.g. Møller 1990). We measured the amount of food consumed by recovering and weighing the seeds remaining in the food tray 48 h after presenting the food. This was repeated two more times at weekly intervals.

After 6 weeks all pairs were put on *ad libitum* food and were given a nest box and nesting material. This enabled us to measure long-term effects of the chaff/seed ratio on reproductive output. We recorded day of laying the first egg, clutch size, egg mass, brood size and fledgling number.

Most data were analysed using Generalised Linear Mixed Models. This allowed us to take into account that individuals were measured repeatedly. When only one

measurement per individual or pair was analysed, Generalised Linear Models (GLM) were used. Chaff/seed ratio was entered as a covariate, coded 0, 1 or 3. Statistically significant variables (including 2-way interactions) were selected using backward, stepwise deletion of non-significant variables. Poisson regressions were applied for count data, using Statistix (v. 7, Analytical Software). All other analyses were done with SPSS (v. 11.0, SPSS Inc.).

Results

Feeding time and activity

The time spent foraging increased with increasing chaff/seed ratio and the increase was greater at low temperatures (Figure 2.1). Of the 90-minutes observation time up to 50% was spent on foraging when both temperature and food availability were low. At higher temperatures and with *ad libitum* food, only around 5% of the time was spent foraging. The total activity time, as registered with the PIRs, showed a quite different picture (Figure 2.1). While foraging time always increased with in-creasing foraging costs, this was only the case for total activity time at 12°C. There was still a relationship between the total activity time and chaff/seed ratio when feeding time was also in the model (Figure 2.2), and an analysis indicates that, at least at the two lower temperatures, the non-foraging activity decreased towards higher chaff/seed ratios, while foraging time increased.

We tried to estimate the costs of foraging by regressing the MR on the observed feeding time using the raw, 6-min interval data of the respirometer. There was however no significant relationship between the two ($F_{1,453} = 0.03$, $P = 0.86$, controlling for temperature). This was probably caused by the large volume of the respirometer box (24 l). With an air flow rate of 36 l/h it would need at least 40 min to replace the entire volume of air. This leads to large washout effects on changes in gas concentration and, hence, weak correlations between metabolic rate and the quickly fluctuating activity.

Body Mass and DEE

Body mass was not related to chaff/seed ratio (Figure 2.1). There was a slight decrease in body mass through time during the first leg of the experiment, equalling -15.9 ± 3.4 mg/d ($F_{1,190} = 22.6$, $P < 0.001$). Over the whole period of respirometer measurements (34 days) this was on average 0.54 g. Within the 3 measurement sessions (for measuring at the 3 different temperatures) mass decreased in the course of time with a steeper slope then between the session, namely -136 ± 13 mg/d ($F_{1,189} = 13.3$, $P < 0.001$).

Daily energy expenditure decreased with increasing chaff/seed ratio and was higher at lower temperatures (Figure 2.3). This was not solely due to body mass variation, because the effect remained when mass was added to the model.

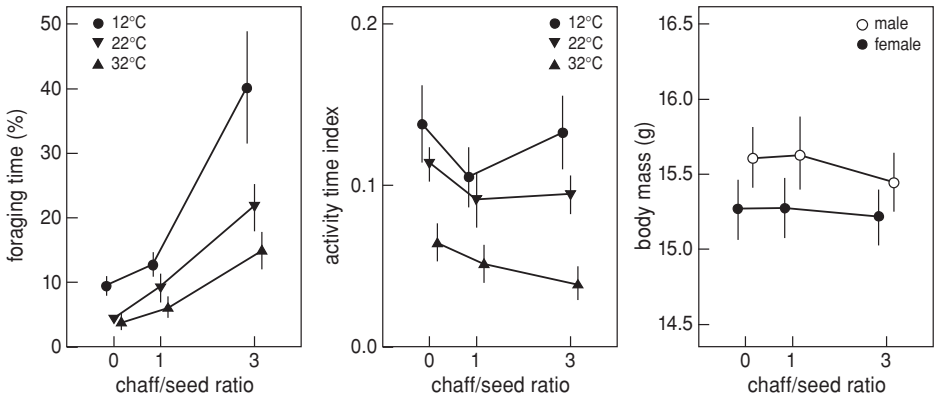

Figure 2.1 Left panel: proportion of time spent foraging between 9 and 10:30 AM is related to chaff/seed ratio ($F_{1,33}$ = 22.4, P < 0.001), body mass ($F_{1,57}$ = 4.78, P = 0.033) and the interaction of temperature and chaff/seed ratio ($F_{1,56}$ = 15.0, P < 0.001). Middle panel: the time spent on activities (including foraging) during the light period was related to temperature ($F_{1,83}$ = 53.9, P < 0.001), chaff/seed ratio ($F_{1,82}$ = 4.15, P = 0.045) and there was a trend to a relationship with chaff/seed ratio squared ($F_{1,82}$ = 3.19, P = 0.078). Right panel: body masses of the birds on the different chaff/seed ratios as measured prior to the respirometer measurements. No significant relationship with chaff/seed ratio was apparent ($F_{1,169}$ = 2.23, P = 0.14).

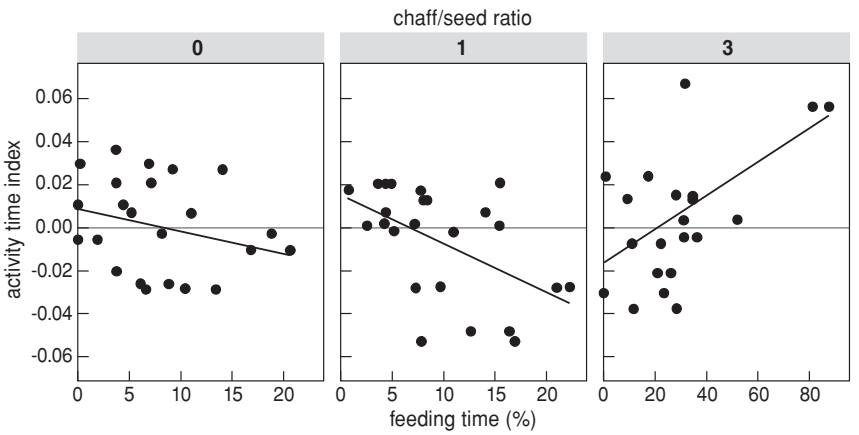

Figure 2.2 Residual of the activity time index during the light period (registered with Passive Infrared Sensors fit inside the respirometer box), correcting for temperature, for the 3 chaff/seed ratios, plotted against the percentage time spent feeding between 9:00 and 10:30 AM. At the two low chaff/seed ratios total activity time decreased with increasing foraging time, but at the highest ratio total activity time increased (temperature: $F_{1,46}$ = 47.5, P < 0.001; chaff/seed ratio: $F_{1,17}$ = 12.5, P < 0.005, ratio^2: $F_{1,18}$ = 9.72, P < 0.01, feeding time: $F_{1,44}$ = 3.66, P = 0.062, interaction feeding time and ratio: $F_{1,45}$ = 8.49, P < 0.01). Deleting the two rightmost points in right panel did not change the conclusions.

Mass-specific metabolic rate during the entire day also decreased with decreasing foraging costs ($F_{1,86} = 13.2$, $P < 0.001$), and decreased with temperature ($F_{1,86} = 2468$, $P < 0.001$). The average mass of the pair of birds was used for this measure.

Both day- and night-time energy expenditure were reduced at higher chaff/seed ratios (Figure 2.4). This reduction was stronger at low temperatures. The effect was not solely caused by body mass changes, because adding this to the model did not make the effect of chaff/seed ratio disappear. Mass-specific MR during the night clearly decreased as well when foraging costs were higher (chaff/seed ratio, $F_{1,85} = 18.9$, $P < 0.001$; temperature, $F_{1,85} = 1370$, $P < 0.001$; their interaction, $F_{1,85} = 5.72$, $P = 0.019$).

The changes in basal metabolic rate (BMR; Figure 2.5), estimated by the minimum night-time values measured at 32°C at 6-min intervals, were not significantly related to chaff/seed ratio. Mean BMR was 0.216 ± 0.004 W, and mean mass-specific BMR 14.2 ± 0.3 mW/g (Figure 2.5), which was also independent of the food manipulation.

Feather growth

Tail feather growth from 12 to 42 days after plucking was not related to chaff/seed ratio (Figure 2.6). The measured tail feather growth was maximal at day 20 (Figure 2.6). Analysing only data from that day did not reveal effects of chaff/seed ratio, sex and their interaction either. The final length of the tail feathers was not related to chaff/seed ratio and length of the previous feather, but there was a difference between

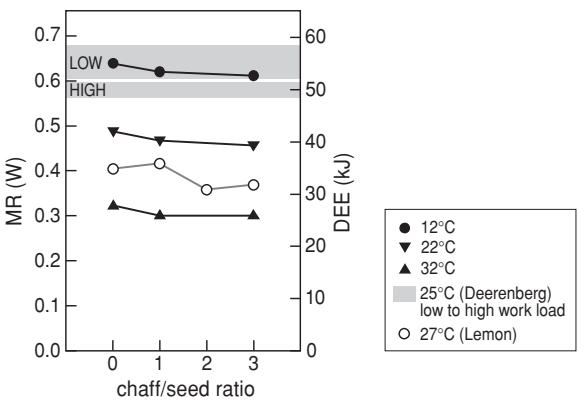

Figure 2.3 Average metabolic rate (MR, left axis) during 24-h period was related to chaff/seed ratio ($F_{1,85} = 22.7$, $P < 0.001$), temperature ($F_{1,85} = 3057$, $P < 0.001$) and body mass (negative relationship, $F_{1,93} = 9.77$, $P < 0.005$). Also plotted is the metabolisable energy intake as measured by Lemon (1993) in zebra finches on similar chaff/seed regimes (they found no significant differences between treatments). The grey bars indicate energy expenditure ranges of zebra finches on low or high foraging costs regimes, manipulated by the number of hops between perches needed to get access to food (Deerenberg *et al*. 1998). The temperatures at which the measurements were done are given in the legend.

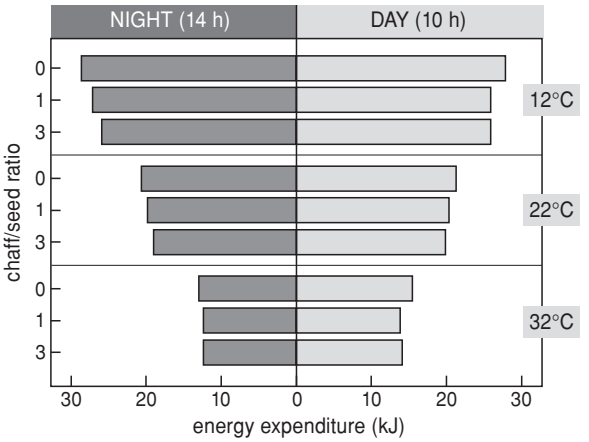

Figure 2.4 Energy expenditure during the day and the night, as measured in 24-h sessions in a respirometer, of zebra finches kept on different chaff/seed ratios at ca. 22°C, and measured at different temperatures. Day-time energy expenditure (kJ/10 h) was related to chaff/seed ratio ($F_{1,85}$ = 15.5, P < 0.001), temperature ($F_{1,85}$ = 2265, P < 0.001) and body mass ($F_{1,93}$ = 5.83, P = 0.018). Night-time energy expenditure (kJ/14 h) was related to temperature ($F_{1,84}$ = 1393, P < 0.001), chaff/seed ratio ($F_{1,84}$ = 22.0, P < 0.001), their interaction ($F_{1,84}$ = 5.74, P = 0.019) and to body mass ($F_{1,87}$ = 11.8, P < 0.001).

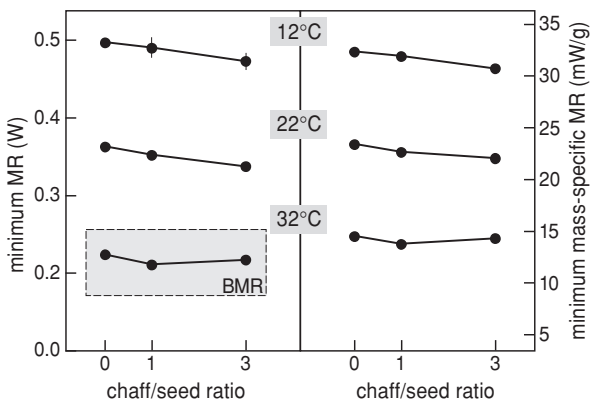

Figure 2.5 Left: minimum values of metabolic rate measured during the night. The values at 32°C equal BMR (although some measurements indicate that 32°C may be marginally below the thermoneutral zone, own obs.). BMR is not related to chaff/seed ratio ($F_{1,23}$ = 0.26, P = 0.62). Right: minimum values of mass-specific metabolic rates, calculated using the mean body mass of the two simultaneously measured birds. Mass-specific BMR was not related to chaff/seed ratio ($F_{1,23}$ = 0.02, P = 0.89), but at 12 and 22°C mass-specific MR was related to chaff/seed ratio.

the sexes (chaff/seed ratio, $F_{1,20} = 2.37$, $P = 0.14$; length previous feather, $F_{1,26} = 0.29$, $P = 0.59$; sex, $F_{1,19} = 5.03$, $P = 0.037$). Female feathers were 1.36 ± 0.61 mm, or 3.6%, shorter than the males', while the previously plucked feathers did not differ in length ($F_{1,6} = 0.02$, $P = 0.90$).

The final mass of the two regrown tail feathers was also not related to chaff/seed ratio ($F_{1,15} = 0.03$, $P = 0.86$). There tended to be a difference in sexes ($F_{1,13} = 4.28$, $P = 0.059$), while this trend did not exist in the tail feathers plucked at the start of the experiment ($F_{1,6} = 0.07$, $P = 0.80$). Feather mass was strongly related to the mass of the first plucked feather and to sex, but again not to chaff/seed ratio (previous feather, $F_{1,22} = 14.1$, $P < 0.001$; sex, $F_{1,12} = 6.11$, $P = 0.029$; chaff/seed ratio effect, $F_{1,12} = 0.12$, $P = 0.74$). The new female feathers were 0.285 ± 0.112 mg, or 7%, lighter than the male feathers.

As a measure of fluctuation asymmetry we compared the left and right tail feather. The difference in mass between the left and the right feather was not related to chaff/seed ratio ($F_{1,20} = 1.45$, $P = 0.24$; sex, $F_{1,20} = 2.67$, $P = 0.12$) and neither was the difference in length ($F_{1,20} = 0.45$, $P = 0.51$; sex, $F_{1,20} = 1.20$, $P = 0.28$).

Successive food intake and reproduction

Food intake rates during the 3 days immediately after termination of the food manipulation did not differ between chaff/seed ratios, but decreased slightly in all 3 groups in the course of those 3 days (chaff/seed ratio, $F_{1,21} = 1.12$, $P = 0.30$; day number, $F_{1,47} = 12.1$, $P < 0.005$).

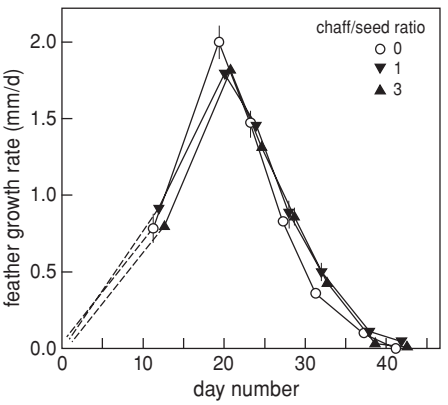

Figure 2.6 Average growth rates of the new, outer two tail feathers following plucking of outer tail feathers on day 0. The points depict the growth rates calculated over the preceding time period. The 3 lines depict the different chaff/seed ratios. There were no significant differences between chaff/seed ratios ($F_{1,53} = 0.016$, $P = 0.90$; sex, $F_{1,53} = 0.00$, $P = 0.98$; interaction, $F_{1,53} = 0.001$, $P = 0.93$). No effect of chaff/seed ratio was found when looking only at day 20 (where values were maximal; $F_{1,5} = 2.61$, $P = 0.17$).

There were no relationships between chaff/seed ratio and laying interval. Poisson regression:($z = 1.03$, $n = 20$, $P = 0.30$), clutch size ($z = 0.92$, $n = 24$, $P = 0.36$), brood size ($z = 0.74$, $n = 24$, $P = 0.46$) and fledgling number ($z = 0.00$, $n = 24$, $P = 1.0$). The average mass of the laid eggs also did not differ between chaff/seed ratios (GLM: $F_{1,19} = 0.82$, $P = 0.38$).

Discussion

Mixing the seeds with chaff increased the foraging time considerable. In the holding cages foraging time will have increased from about 4 to 22% of the day (Figure 2.1: 22°C). Nevertheless, DEE did not increase, but instead decreased by 6.6% from 46.1 to 43.0 kJ/d (Figure 2.3: 22°C). We could show that this decrease was due to reductions in energy expenditure during both the light and the dark period (Figure 2.4). At night, at 22°C, energy expenditure decreased by 7.7% and during the day by 5.6%, which is 43 and 57% of the change in DEE, respectively (note that the light period lasted 10 h). We also showed that the changes in metabolic rates were independent of body mass, because mass did not vary significantly with chaff/seed ratio (Figure 2.1), and including mass in the analysis together with chaff/seed ratio confirmed an effect on MR, and because mass-specific metabolic rate decreased with increasing chaff/seed ratio.

When Lemon (1993) increased the amount of chaff mixed with the seeds, he did not detect significant differences in DEE. However, the trend was in accordance with our results (Figure 2.3). In his experiments Lemon relied on between individual comparisons, rather than on within individual comparisons as in our study, and the resulting lower statistical power might explain the discrepancy. Furthermore, the energy budgets constructed by Lemon (1993) were constructed indirectly, using a summation of separately measured existence metabolism values of fasting birds and foraging costs. Assuming that the daily metabolisable energy intake in his experiment actually did decrease with increasing chaff/seed ratios, does not change his budget qualitatively. However, the differences in the surplus of the energy budget, by which he explains the variation in reproductive success and adult survival, become larger. Actually, the budget would have become negative at chaff/seed ratios of 2 and 3, which should have resulted in the usage of nutritional stores.

We made budgets for the birds in our experiment, making use of the foraging cost estimated by Lemon (1993). He arrived at a marginal cost of 0.583 W. This value results in foraging costs ranging between 3 and 16% of DEE, or between 9 and 32% of the day-time energy expenditure (Figure 2.7). From Figure 2.7 it is noticeable that the energy that remained for other activities besides foraging was very limited. The budget was even slightly negative at 12°C at the low chaff/seed ratio, indicating the birds might have used some of their nutritional stores. Body mass changes during the respirometer measurements did not give additional support for the outcome of the energy budget: although the decrease was greater at lower temperatures, it did not depend on the chaff/seed ratio (temperature, $F_{1,213} = 14.3$, $P < 0.001$; chaff/seed ratio, $F_{1,213} =$

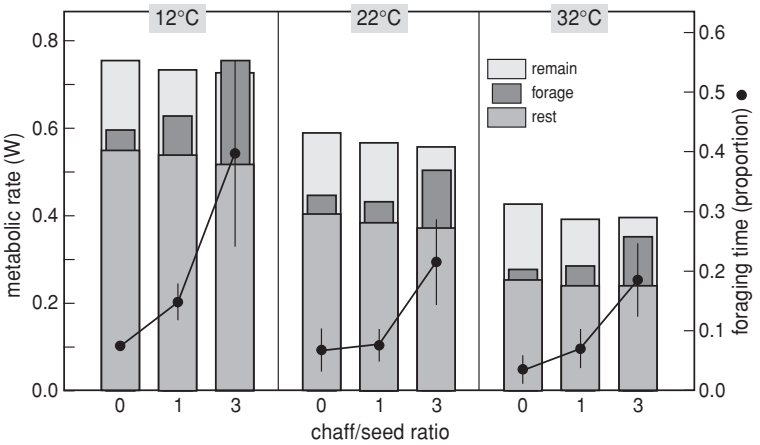

Figure 2.7 Energy budget during the 10-h day-time period at different chaff/seed ratios and temperatures as measured in a respirometer. Resting metabolic rate (rest) is the average of the measurements during the dark period (MR_{night}). Foraging costs were calculated using the estimate of 0.583 W of Lemon (1993). 'Remain' indicates the difference between DEE and the summation of resting metabolism and foraging metabolism (negative at 12°C and chaff/seed ratio '3'). The proportion (±SE) of time spent foraging during the first 90 min of the light period is shown by the dots and the scale on the right axis.

0.83, $P = 0.36$). Therefore, adjustments in energy intake and expenditure alone, made up for changes in the energy budget.

So how did our birds lower their energy expenditure while spending more energy on foraging? We see four possibilities: 1) body temperature might have dropped, 2) energy might have been reallocated away from physiological processes, 3) physical activity levels might have been reduced or 4) heat generated through activity might have been used for thermoregulation. The decrease in the minimum levels of mass-specific MR at the lower two temperatures (Figure 2.5) suggests that some physiological processes were downregulated, either by lowering body temperature or in other ways. Because hypothermia is a common trait of small bird species (McKechnie & Lovegrove 2002), it is likely to have occurred in our birds too. Nevertheless, non-foraging activity levels also had changed. Comparing foraging activity and total activity as depicted in Figure 2.1 shows that the increase in foraging time with increasing chaff/seed ratio was not matched by the change in total activity. Clearly, total activity decreased from chaff/seed ratio '0' to '1', and than levelled off (at 22°C), implying that non-foraging activity decreased. We could confirm this statistically by relating the activity time to chaff/seed ratio and feeding time in one model (Figure 2.2). It showed that the interaction between feeding time and chaff/seed ratio was significant. We think that the positive correlation seen at the high chaff/seed ratio is due to the fact that very little time is spent on other activities besides foraging so that changes in these other activities are overshadowed by foraging activities. A trade-off between activities was earlier seen in

other bird species that were made to increase their feeding effort (Tiebout 1991; Fotheringham 1998), as well as in two small rodent species (Perrigo 1987). Whether heat substitution took place is unknown, but it has been demonstrated in some studies (Zerba & Walsberg 1992; Bruinzeel & Piersma 1998).

The effect of the variability of foraging success rates on BMR is undecided (Table 2.1). Most studies found a reduction in BMR with a decreasing, fixed foraging success. Starlings on a decreasing, but variable reward rate did also lower BMR (Wiersma *et al*. 2003a: Chapter 4), while the zebra finches in this study did not. Unfortunately, Fotheringham (1998) did not measure BMR in his experiments. A reduction in BMR with increasing day-time activity was found in hormone-treated white-crowned sparrows *Zonotrichia leucophrys* (Wikelski *et al*. 1999).

Although Lemon and Barth (1992) showed that an increase in chaff/seed ratio had negative effects on reproduction and adult survival, we could not detect differences in growth rate and end mass of newly formed tail feathers (Figure 2.6), and did not detect fluctuating asymmetry, which we measured as indicators of resources put into maintenance and repair processes. Food consumption during the days immediately following the food manipulation did not differ between chaff/seed ratios, giving no indication that the birds needed to compensate earlier deficiencies. We did also not find effects on reproduction following the food manipulation. Lemon & Barth (1992) showed that the time delay between two broods increased with increasing chaff/seed ratio. Deerenberg & Overkamp (1999) also showed long term effects of the manipulated foraging reward rate: birds that previously had worked harder for their food (after the manipulation had stopped and they were on *ad libitum* food again) showed a delay in the time until nesting. We did not see such a long term effect in our birds. DEE of our birds (at the temperature of the holding cages) was around 25% below that of Deerenberg & Overkamp's (1999; Figure 2.3). Apparently, the increase in foraging time and costs was not demanding enough to lead to long term negative effects.

The manner in which food is offered to the animals can have great consequences for the energy budget. A distinction that has to be made is between a fixed and variable foraging reward. Animals experiencing a fixed reward rate receive food after a fixed number of actions (pecks, flights, etc.) while on a variable schedule only the mean of the number of actions needed is fixed, but with variation around this mean. What the zebra finches in this study experienced is comparable with a variable reward rate: the time needed to encounter a seed followed some positively skewed probability distribution, which' mean depended on the amount of chaff mixed with the seeds. In a study on starlings, Bautista *et al*. (1998) rewarded the birds with food after a fixed number of flights or walks, and showed that DEE decreased with decreasing foraging success rate, while the time spent foraging increased considerably. In other studies that manipulated foraging success using fixed success rates the results were similar: DEE or food intake (of starlings, zebra finches and two hummingbirds species) did not increase with decreasing foraging success (Table 2.1). Also in two rodent species food consumption decreased with decreasing foraging success (Table 2.1). However, when the reward rate is variable, a different picture arises: starlings that had to fly between perches a number

Table 2.1 Summary of results from experiments in which foraging reward rate was manipulated (without altering the predictability) in a closed economy system. The responses to a decrease in foraging reward rate are shown. Reward rates could either be fixed (without variation) or variable (only mean fixed). Increases and decreases are depicted with '+' and '-', while '0' means no change. Indicators in brackets refer to trends ($P < 0.1$). Where no result was given the cells are left empty.

	reward rate	foraging activity	mass	non-foraging activity	BMR or RMR	daily food intake	DEE
starling [1] *Sturnus vulgaris*	variable	+	0	-		0	
starling [2] *Sturnus vulgaris*	variable	+	-		-	+	+
zebra finch [3] *Taeniopygia guttata*	variable	+	0			0	0
zebra finch (this study) *Taeniopygia guttata*	variable	+	0	-	0	-	-
starling [1] *Sturnus vulgaris*	fixed	+	-	-		-	
starling [4] *Sturnus vulgaris*	fixed	+	-		-	-	-
zebra finch [5] *Taeniopygia guttata*	fixed	+	-	(-)	-	-	-
steely-vented hummingbird [6] *Amazilia saucerottei*	fixed	+		-	-*	-	-
fork-tailed emerald [6] *Chlorostilbon canivetii*	fixed	+		-	-*	-	-
domestic pigeon [7] *Columba livia*	fixed	+	-		-**	-	
house mouse [8] *Mus domesticus*	fixed	0	0	-		-	
deer mouse [8] *Peromyscus maniculatus*	fixed	0	0	-		-	

*perching MR (not directly measured), **inferred from body temperature, [1]Fotheringham (1998), [2]Wiersma (2003a: Chapter 4), [3]Lemon & Barth (1992), [4]Bautista *et al*. (1998), [5]Deerenberg *et al*. (1998), [6]Tiebout (1991), [7]Rashotte & Henderson (1988), [8]Perrigo (1987)

of times to be rewarded with food, did not eat less with a decreasing, but variable, foraging reward rate (Fotheringham 1998; Wiersma *et al*. 2003a: Chapter 4). It seemed that the birds were more motivated to forage under variable than fixed conditions. Our results do not fit well in this picture however: foraging success was variable, but DEE did not increase with increasing foraging effort. Possibly, the chaff/seed ratio we offered was not high enough, and in combination with a relatively cheap foraging mode, did not necessitate increasing DEE. This is supported by the lack of an effect on body mass of the chaff/seed ratio. Nevertheless, the studies shown in Table 2.1 that

applied fixed foraging success rates might have had essentially different outcomes, had variable rates been used. An extrapolation of these results to field conditions must therefore be assessed with reservations.

Acknowledgements

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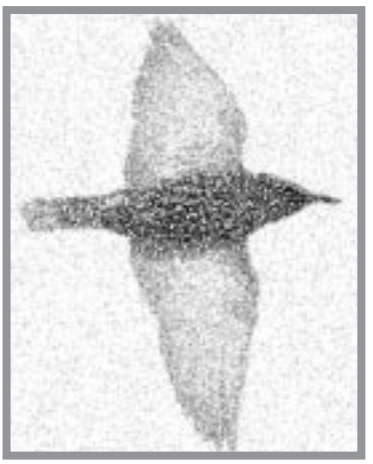
Chapter 3

The energetic cost of short flights in starlings *Sturnus vulgaris* measured using the labelled bicarbonate technique.

Catherine Hambly, Popko Wiersma, Simon Verhulst, E. Jean Harper & John R. Speakman



Abstract

The ^{13}C labelled bicarbonate technique was used to measure the energy cost of short flights in starlings *Sturnus vulgaris*. The technique was validated in 5 individuals at temperatures ranging from 1 to 30°C. The ^{13}C isotope elimination rate (k_c), after injection with $NaH^{13}CO_3$, was multiplied by the bicarbonate pool size (N_c) and the product, k_cN_c, was compared to the metabolic rate (O_2 consumption and CO_2 production) measured simultaneously by respirometry. The closest relationship between k_cN_c and both VO_2 and VCO_2 (ml/min) occurred between 15 and 30 minutes after injection (r^2 = 0.84 and 0.85 for VO_2 and VCO_2 respectively). The relationships between k_cN_c and both VO_2 and VCO_2 were used to predict energy expenditure during flight for birds injected with ^{13}C labelled bicarbonate flying between perches 5.0 m apart. Flight costs averaged 20.5 ± 0.9 W from a total of 27 flying sessions across 9 individuals. Wing beat frequency and flight speeds were measured in each flying session. Wing beat frequency averaged 10.8 ± 0.2 Hz and was negatively related to the flight cost, however, there was no significant relationship between flight cost and flight speed which averaged 3.9 ± 0.1 m/s. Birds were housed in 5.4 m long flight cages for 4 days and had to fly between perches located at either end to obtain food rewards. Average daily energy expenditure (DEE) was 152.1 ± 8.6 kJ/d which was calculated by combining the diurnal metabolism at rest measured using indirect calorimetry, BMR, combined with the flight cost estimated here. The average daily metabolisable energy intake from food was 166.1 ± 9.4 kJ/d, which in all cases exceeded the daily energy output.

Introduction

During foraging, the flights of birds are typically short and throughout the foraging period there will be many take-offs and landings. The energetic cost of this type of flight tends to be higher than travelling flights (Nudds & Bryant 2000). The flight cost measured in zebra finches *Taeniopygia guttata* flying over short distances, below the speed for minimum power requirements, and including many take-offs and landings, was over 3 times the values predicted using aerodynamic models (Nudds & Bryant 2000) such as those formulated by Pennycuick (1975), Berger & Hart (1974) or Masman & Klaassen (1987).

Power curves are one of the main sources of information for estimating the energy cost of short flights. There are 2 types of power curve: mechanical and metabolic. The mechanical power curve (Pennycuick 1968; Pennycuick 1969) shows a U-shaped pattern with a single flight speed, that provides the lowest flight cost. Above and below this flight speed the power requirement for flight is elevated. Direct metabolic measurements of flight costs however tend towards a J-shaped curve and there is little change in energy expenditure between hovering flight where the bird is stationary and intermediate flight speeds (Rayner 1990; Ellington 1991). Starlings, however, had no significant change in flight cost during long flight periods with speeds between 6 and 18 m/s (Torre-Bueno & Larochelle 1978), which is contradictory to both curves although more recently wind tunnel studies of flight costs in starlings do match the predictions more closely (Ward *et al*. 2001). These contrasting results, in addition to the fact that the cost of short flights are expected to exceed those of prolonged flights, necessitate measurement of the cost of short flights using experimental techniques, rather than relying on the predictions from power curves.

There are both direct and indirect methods for measuring the energetic cost of flight. One direct method involves measuring heat dissipated from the body surface using thermographic images (Ward *et al*. 1999). There are also indirect methods including mask respirometry (Tucker 1968; Tucker 1969; Klaassen *et al*. 2000), body mass loss (Hussell 1969), or stable isotope techniques such as doubly labelled water (DLW) (Speakman 1997; Kvist *et al*. 2001) or a relatively new approach, the ^{13}C-labelled bicarbonate technique which has only recently been applied to flight (Hambly *et al*. 2002).

Flight cost measurements are an important component of the time budget estimates of avian daily energy expenditure (DEE) because flight is the most energetically expensive daily activity (Bryant 1997). DEE estimates using time-energy budgets (TEB) may be considerably improved with more accurate flight cost predictions because DEE is predicted by multiplying the energetic cost for each activity measured in the laboratory, by the time spent each day conducting that activity (Kendeigh *et al*. 1977). The accuracy of this technique can be assessed by simultaneously measuring the DEE using methods such as measuring the daily energy intake from food consumption (Kunz 1974; Collins 1983) or expenditure using doubly labelled water (Utter & Lefebvre 1973; Tatner & Bryant 1986; Speakman 1997). Errors when constructing time and energy budgets can be as large as 20% when using food intake (Koplin *et al*.

1980), or DLW (Weathers & Nagy 1980; Weathers *et al*. 1984) as there are many factors that can affect the energy expenditure for a particular activity e.g. seasonal changes in temperature and wind speed (Buttemer *et al*. 1986). Moreover, if a bird is only conducting short flights, which may have a higher cost than predicted using power curves, the DEE will be underestimated.

In this study, flight costs were measured for starlings conducting short flights (using the ^{13}C labelled bicarbonate technique). Contrary to the DLW technique, labelled bicarbonate enables measurements of energy expenditure over very short time frames. Previous measurements on starlings that had to fly between perches 7.0 m apart estimated that the cost of these short flights was between 45.5 and 52.3 W (Bautista *et al*. 1998) which is high compared to sustained flight in a wind tunnel (19.4 W, Ward *et al*. 2001). This flight cost was measured indirectly using DLW and time budgeting techniques, and the resulting estimate was between 2.6 and 5.2 times the predicted flight costs using aerodynamic theory (at 45 to 92 times BMR).

The aim of this study was to validate the ^{13}C labelled bicarbonate technique in starlings by comparing the isotope elimination rate of ^{13}C in breath with O_2 consumption and CO_2 production measured by indirect calorimetry. We then aimed to measure the cost of short flights in this species, and compare these measurements to other flight energy measurements in starlings.

Methods

The ^{13}C labelled bicarbonate technique was used to measure energy expenditure in starlings. In this technique the isotope is injected intraperitoneally as ^{13}C labelled $NaHCO_3$, and the ^{13}C mixes in the bicarbonate pool and is expired in CO_2. The rate of isotope elimination depends on the metabolic rate, however the size of the bicarbonate pool is small, and therefore the isotope elimination rate is rapid, allowing energy expenditure to be measured over short periods.

To obtain a standard dilution curve, a fixed volume of 0.2 ml of 0.29 M $NaH^{13}CO_2$ solution was injected, along with varying volumes (between 5.0 and 0.5 ml) of CO_2 gas, into 10 ml vacutainers (Becton Dickinson, Vacutainer Systems Europe). Three replicates were made for each volume of CO_2. The vacutainers were placed in an oven at 60°C for 4 days to equilibrate, after which 0.5 ml of the resulting gas was extracted and injected into new vacutainers. This 0.5 ml of resulting gas was admitted to an isotope ratio mass spectrometer (Micromass ISOCHROM mG), which uses a gas chromatograph column to separate nitrogen and CO_2 in a stream of helium gas, before analysis by isotope ratio mass spectrometry. The enrichment (delta) of ^{13}C:^{12}C was measured as the ratio of the minor to major beam currents of the samples, compared with a reference gas of known enrichment (after Lajtha & Michener 1994) which had previously been characterised relative to the IAEA international standards 309 a and b.

Validation

A validation study was conducted on 5 starlings to examine the relationship between the log converted ^{13}C isotope elimination rate in breath and both O_2 consumption and CO_2 production measured using indirect calorimetry. The birds were placed in a respirometry chamber in an indirect calorimetry set-up at a flow rate of 100 l/h regulated using a Mass Flow Controller (5850S, Brooks). Background ^{13}C enrichment was measured by collecting gas samples from the out flow of the chamber through a 19 gauge needle directly into 10-ml vacutainers. The birds were then removed from the chamber and injected intraperitoneally with a weighed volume (to the nearest 0.0001 g) of approximately 0.6 ml of 0.29 M sodium bicarbonate ($NaH^{13}CO_3$) and placed immediately back into the chamber. Over the following 60 minutes, oxygen consumption was measured with an oxygen gas analyser (Servomex Xentra 4110) and CO_2 production was measured using a CO_2 gas analyser (Servomex 1440). Air was dried throughout the system using columns of micro sieve (3Å, Merck), and gas samples were collected from the outflow of the chamber into vacutainers each minute as previously described. The birds underwent this procedure on 3 separate occasions with the chamber incubated initially between 1 and 5°C and subsequently at 15 and 30°C to increase the range of metabolic rates observed. Gas samples were shipped immediately to Aberdeen University where they were analysed using isotope ratio mass spectrometry within 5 days of collection. For each measurement we determined the isotope elimination rate (k_c), which was the gradient of the log converted isotope enrichment with time. Metabolic rate and k_c were calculated initially for all the data after the isotope had become equilibrated within the bicarbonate pool. There was no significant relationship between isotope elimination rate and metabolism over the whole time period and therefore both k_c and metabolic rate were recalculated over sequential 15-minute intervals to locate the closest relationship between them, and thus indicating the optimal time interval for flight measurements.

Flight Costs

Flight cost was measured in 9 individuals. For each flight experiment the birds were brought in from their holding pen and placed individually in one of 8 flight cages (5.4x0.7x0.8 m, lxbxh). Water was available *ad libitum*. The birds had previously been trained to fly between two perches at either end of the cage a pre-determined number of times to obtain one food pellet (Europa Eel, Trouw). Each perch had microswitches to register whether the bird had landed on it, and the delivery of the food was controlled by a PLC (programmable logic controller), which was linked to automatic food dispensers (Model 442 893, Campden Instruments Ltd, Sileby, Leics, UK). A PC was used to record the number of flights and food rewards obtained by the birds each day. To obtain the food pellet the bird had to hop onto a third perch which was attached to a balance (Model P-1040, Tedea Huntleigh, UK), through which body mass data was automatically recorded. In addition to counting the number of pellets rewarded to each bird, the mass of food consumed each day accounting for spillage, was also measured on a 2-figure balance.

After 4 days, the birds had become accustomed to the feeding regime (on average 1 pellet every 2.6 return flight) at which point we measured their flight cost using the labelled bicarbonate technique (Hambly *et al*. 2002). This experimental set-up required a portable system for collecting breath samples under similar conditions as used in the validation in close vicinity of the flight cages. The same chamber was used and air was pumped through using a Charles Austin pump at the same rate of airflow (100 l/h). Air was dried using micro sieve and air samples were collected from the outflow each minute into 10-ml vacutainers. Prior to injection, background samples from the outflow of the chamber were collected and then the bird was injected intraperitoneally with the same volume (0.6 ml) of labelled sodium bicarbonate solution. The bird was immediately placed back in the chamber and breath samples were collected each minute until 14 minutes after injection (the predetermined optimal time from the validation). The bird was then removed and placed in the flight cage where it was gently encouraged to fly for approximately 2 minutes (total flight time ignoring the short periods between landing and take-off). The flight time was carefully recorded to the nearest second. If the bird did not achieve 2 minutes of flight within the time designated by the validation experiment of 15 minutes then the bird was recaptured and the final flight time achieved was used.

After flight the bird was quickly recaptured and returned to the measurement chamber where air samples were collected for a further 10 minutes. Following the final sample collection the bird was placed back into its own flight cage where it resumed the feeding regime. Each flight was video recorded using a high-8 video camera for analysis of wing beat frequencies and flight speeds. Each bird underwent 2 or 3 separate flight measurements and in addition a control experiment was carried out on each individual. During this control experiment the protocol previously described was followed, but instead of the birds flying over the designated time period, they were placed in a darkened box for 15 minutes and then returned directly to the measurement chamber for the final collection of outflow air samples. This enabled us to examine whether handling stress has an affect on the flight cost estimates.

Daily Energy Expenditure Calculations

The number of flights conducted each day was averaged over a four-day period for each of the 9 individuals. The average number and rate of food rewards was also calculated for this period to enable calculations of the energy intake for each individual. The birds were travelling between 5.4 and 19.8 km per day in the flight cages and the daily metabolisable energy intake (MEI) in kJ/d was calculated using earlier measurement of the water content of the pellets (9%), energy content of the pellets (24.492 kJ/g dry mass) and the assimilation quotient (0.83).

This daily metabolisable energy intake was compared to predictions of daily energy expenditure generated from adding the daily flight cost for each bird to the combination of the day-time and night-time resting energy expenditure. Resting energy expenditure for the period of daylight was estimated from equations generated during the validation. To formulate these equations, metabolism was regressed against the cham-

ber temperature at the time of measurement. During the day the average temperature was 21°C (Figure 3.3). Average night-time metabolic rate for starlings was measured in a different experiment, using the same respirometer as described in the validation (Wiersma *et al*. 2003a: Chapter 4).

Data Analysis

Means are shown ± the standard error. In the analysis linear regressions, generalised linear models (GLM) and paired *t*-tests were applied using Minitab 11 software.

Results

Validation

Isotope enrichment increased rapidly until it reached a plateau at an average of 9 minutes after injection (Figure 3.1). The enrichment of the isotope then declined over the next 50 minutes until it approached the pre-measured background enrichment. Isotope elimination rate (k_c) was the gradient of the log converted isotope enrichment with time, and it was calculated over sequential 15 minute time intervals after injection. The isotope elimination rate was then multiplied by the body bicarbonate pool size (N_c). N_c was calculated using the standard dilution curve, which was generated by injecting a fixed volume of the labelled sodium bicarbonate solution into vacutainers with varying volumes of CO_2 gas. The estimate for N_c was achieved by substituting the enrichment at the plateau of each isotope elimination curve from the validation into

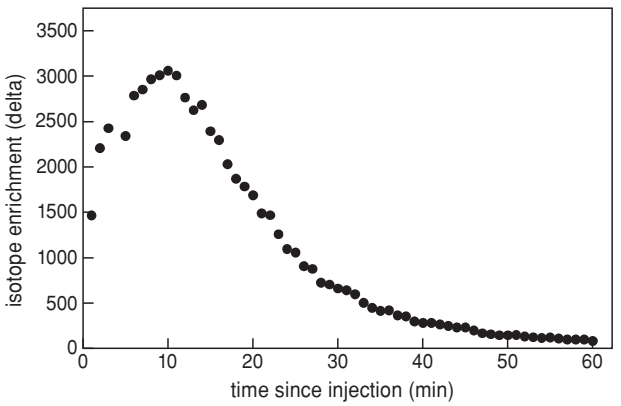


Figure 3.1 Isotope enrichment in exhaled air against time since injection. The ^{13}C isotope mixed with the bicarbonate pool until an equilibrium was reached between the isotope incorporation to the pool and isotope elimination from the pool with CO_2 in breath. At equilibrium a plateau was observed, which in this example, occurred at 10 minutes after injection. The isotope was then gradually eliminated over the following 50 minutes until it approached the pre-measured back-ground level of ^{13}C.

the least squares fit linear regression equation generated from plotting the log converted volume of CO_2 gas injected against the resulting log-converted isotope enrichment of the gas (Figure 3.2).

Metabolic rate decreased linearly with increasing temperature (Figure 3.3). O_2 consumption (VO_2) ranged from 3.5 to 6.4 ml/min while CO_2 production (VCO_2) ranged from 2.4 to 4.7 ml/min. The relationship with temperature was significant for both VO_2 and VCO_2 (regression: VO_2 $F_{1,13} = 22.7$, $P < 0.001$, VCO_2 $F_{1,13} = 19.9$, $P = 0.001$; Figure 3.3). The average respiratory quotient at thermoneutral in these 5 individuals was 0.7 ± 0.003. The metabolic rate was compared to the simultaneous isotope elimination rate, multiplied by body bicarbonate pool size after conversion from moles to ml using the gas constant. This relationship was first estimated for the data collected between 15 and 60 minutes after injection to include the period after the plateau had been reached until the isotope had been eliminated, which gave a poor relationship between metabolism and k_cN_c. The comparison was then repeated for sequential 15-minute time intervals after injection. k_cN_c increased linearly with increased metabolism and the interval between 15-30 minutes provided the closest relationship for both O_2 consumption and CO_2 production (Figure 3.4). Both of these relationships were highly significant (regression: VO_2 $F_{1,13} = 62.7$, $P < 0.001$, VCO_2 $F_{1,13} = 67.1$, $P < 0.001$) and could then be used to predict VO_2 and VCO_2 during flight given a known k_cN_c. The significance of the relationship was not enhanced due to the repeated measurements in different individuals (one-way ANOVA: VCO_2 $F_{4,13} = 1.92$, $P = 0.19$, VCO_2 $F_{4,13} = 2.75$, $P = 0.10$).

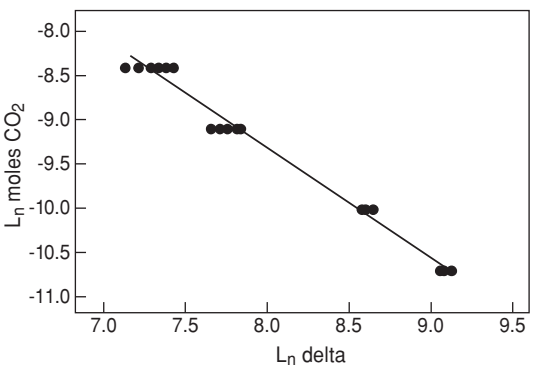


Figure 3.2 Relationship between the equilibrium enrichment of 0.2 ml of 0.29 M labelled bicarbonate with varying amounts of CO_2. The volumes of CO_2 added in moles were log-converted and plotted against the log-converted enrichment values. The relationship was linear ($r^2 = 0.99$) and was described by $y = 0.63 - 1.24x$. The equation for the relationship was used to calculate the size of the body bicarbonate pool (N_c) in moles, and subsequently ml of CO_2, given the known equilibrium isotope enrichment in each bird.

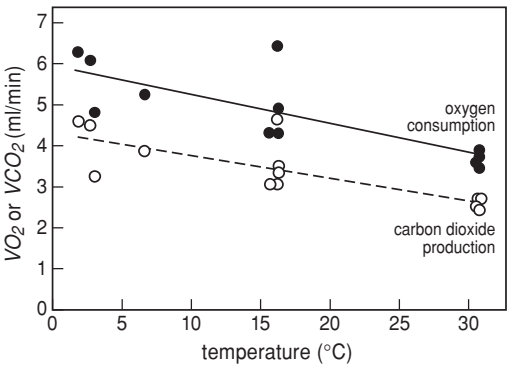


Figure 3.3 Relationship between the rates of oxygen consumption and carbon dioxide production measured with indirect calorimetry, and the temperature inside the metabolic chamber during each validation experiment. Least squares regressions are shown for VO_2 ($y = 5.99 - 0.07x$, $r^2 = 0.65$), and VCO_2 ($y = 4.31 - 0.05x$, $r^2 = 0.62$).

Flight Cost

As in a previous study (Hambly *et al*. 2002) a linear regression provided the best fit between the log converted isotope enrichment and time before flight, while a second order polynomial regression provided the best fit after flight (Figure 3.5). This was not true for the control experiments where a linear regression provided the best fit after the rest period, and the isotope enrichment values were much higher than the measurements made after the birds had conducted a flight. The small difference between the isotope elimination before and after the rest period in control birds indicates that flight costs were not greatly elevated due to handling stress. To account for the time spent resting during the flight period, we calculated the total duration of rest and flight activity. To simplify analysis, we then treated the data as if all flight activity had taken place in the middle of the flight phase and was preceded and succeeded by resting periods of equal duration (Hambly *et al*. 2002). The regression equations in the flights experiments were forward and back extrapolated to the time when the flight started and ended, thus accounting for the time spent on the perches.

The gradient between these two extrapolated points, predicted for the beginning and end of flight, was the isotope elimination rate during the flight period (Table 3.1). N_c was calculated for each flight using the enrichment at the plateau and interpolating it onto the regression equation in Figure 3.2. VO_2 and VCO_2 were calculated for the flight period by interpolating k_cN_c onto the validation equations, and these values were converted to energy expenditure in Watts using the RQ calculated for each flight. The average O_2 consumption during flight was 61.3 ± 2.8 ml/min and CO_2 production was 47.3 ± 2.2 ml/min. These values were 16.4 and 18.1 times the resting VO_2 and VCO_2 respectively, measured at thermoneutral (30°C) during the validation experiment. Flight cost in this sample of birds was 20.5 ± 0.9 W, and the average RQ from the pre-

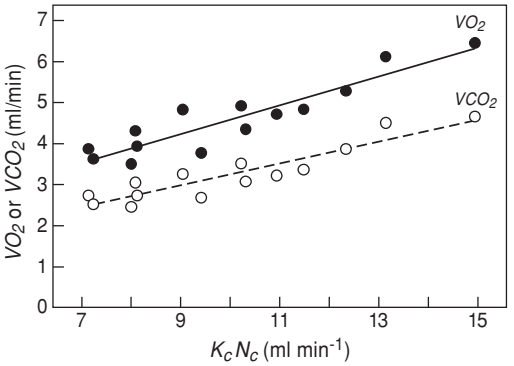


Figure 3.4 Results of the validation experiment relating isotope elimination rate (k_c), corrected for body bicarbonate pool size (N_c), to simultaneously measured VO_2 or VCO_2 between 15 and 30 minutes after injection. Both VO_2 and VCO_2 were significantly related to kcNc. (VO_2, $y = 0.35x + 1.11$, $r^2 = 0.84$, and VCO_2, $y = 0.27x + 0.54$, $r^2 = 0.85$).

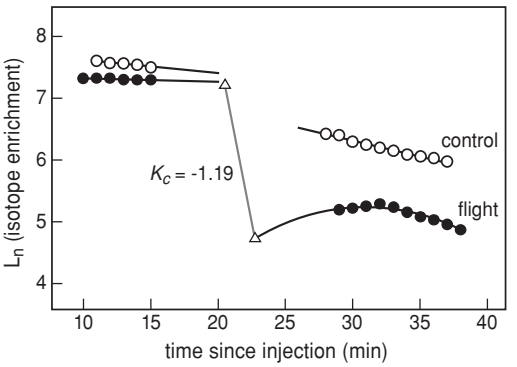


Figure 3.5 Method used for calculating the isotope elimination rate during flight. A linear regression was fitted through the enrichment of the samples collected before flight, while in all cases a second order polynomial regression provided the best fit after flight. The lines were extrapolated to the start and end of flight and in this case the isotope elimination rate was estimated to be 1.19. A much smaller difference in the isotope enrichment measured before and after flight was observed in the control experiment, and a linear regression also provided the best fit after flight for each control.

diction was 0.77 (Table 3.1). Flight cost was averaged over the 3 flights in each individual. There was no significant difference in flight cost between the individuals (one-way ANOVA $F_{6,20} = 0.6$, $P = 0.8$). Flight cost was also not significantly related to body mass over this range (64.6 - 76.5 g; regression $F_{1,24} = 0.18$ $P = 0.68$; Figure 3.6).

The videotapes were used to examine the relationship between flight cost and wing beat frequency and flight speed. The film was slowed to show individual frames which were recorded at 25 frames per second and the number of wing beats and the time taken to fly between perches was measured. The average speeds and wing beat fre-

Table 3.1 Raw data of the isotope elimination rates during the flights and the corrections for body bicarbonate pool size. Also included, are the results when k_cN_c was interpolated onto the 2 validation equations, and the resulting VO_2 and VCO_2 predictions. The respiratory quotient (RQ), measured during flight using these predictions, was used to convert the metabolism to energy expenditure (W) during flight.

Bird/run	k_c	k_cN_c	Metabolism (ml O_2/min)	Metabolism (ml CO_2/min)	RQ	Flight cost (W)
a1	1.1	170.6	60.5	46.6	0.771	20.24
a2	0.9	137.2	48.9	37.6	0.769	16.32
a3	0.9	150.5	53.5	41.2	0.770	17.88
b1	0.6	123.9	44.3	34.0	0.769	14.76
b2	1.2	250.4	88.3	68.2	0.772	29.59
b3	1.1	195.9	69.4	53.5	0.771	23.20
c1	0.7	160.9	57.2	44.0	0.770	19.10
c2	1.2	158.6	56.4	43.4	0.770	18.83
c3	1.5	238.6	84.2	65.0	0.772	28.21
d1	1.7	237.1	83.7	64.6	0.772	28.03
d2	1.1	152.7	54.3	41.8	0.770	18.14
g1	0.7	141.4	50.4	38.8	0.769	16.81
g2	1.2	182.9	64.8	50.0	0.771	21.68
h1	1.5	218.2	77.1	59.5	0.772	25.81
h2	1.4	186.1	65.9	50.8	0.771	22.05
h3	1.0	165.3	58.7	45.2	0.770	19.61
i1	1.2	230.6	81.4	62.8	0.772	27.26
i2	0.7	202.6	71.7	55.3	0.771	23.98
i3	0.6	119.5	42.7	32.8	0.768	14.24
j1	1.1	147.4	52.4	40.4	0.770	17.51
j2	0.9	120.2	43.0	33.0	0.768	14.33
j3	1.1	149.7	53.3	41.0	0.770	17.79
k1	0.9	182.6	64.7	49.9	0.771	21.65
k2	0.8	114.6	41.0	31.2	0.768	13.68
k3	0.8	184.8	65.5	50.5	0.771	21.90
			61.3 ± 2.8	47.3 ± 2.2	0.77 ± 0.0003	20.50 ± 0.93

quencies (WBF) were calculated for a minimum of 10 flights between individual perches for each bird (Figure 3.7, Table 3.2). WBF averaged 10.8 ± 0.2 Hz and was significantly negatively related to the average flight cost (regression $F_{1,20} = 12.09$, $P < 0.01$) but there was no significant relationship with flight speed which averaged 3.89 ± 0.08 m/s (regression $F_{1,20} = 1.57$, $P = 0.23$). Flight speed was not significantly related to WBF (regression $F_{1,20} = 1.71$, $P = 0.2$).

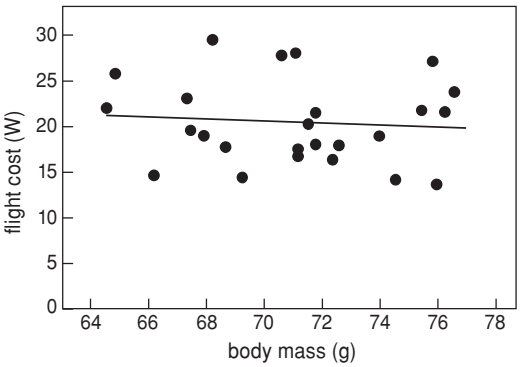


Figure 3.6 Flight cost was not significantly related to body mass over this range (regression, $y = 28.77 + 0.12x$, $F_{1,24} = 0.18$, $P = 0.68$).

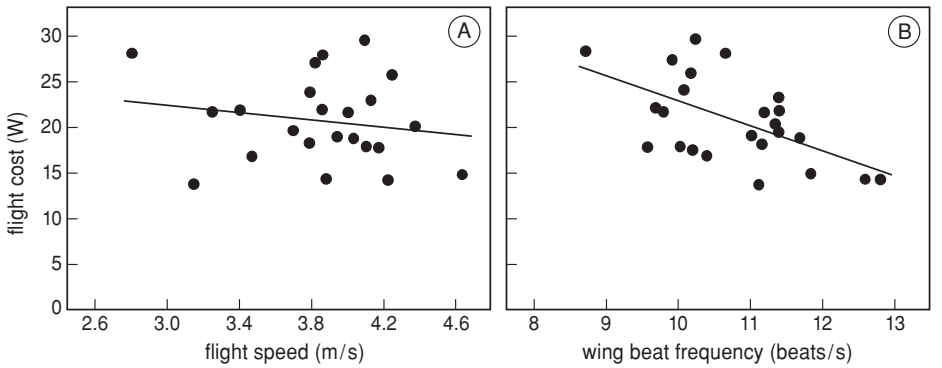


Figure 3.7 (a, b). Flight cost in starlings was significantly related to the average wing beat frequency measured over the flight (regression, $y = 50.12 - 2.73x$, $F_{1,20} = 12.09$, $P < 0.01$), however there was no significant relationship with flight speed (regression, $y = 28.63 - 2.05x$, $F_{1,20} = 1.57$, $P = 0.23$).

Table 3.2 Average wing beat frequencies (Hz), flight speeds (m/s) and flight costs for each individual.

Bird ID	WBF (Hz)	(±SE)	Speed (m/s)	(±SE)	Flight Cost (W)	(±SE)
a	10.8	(0.4)	4.3	(0.1)	18.2	(1.1)
b	11.2	(0.5)	4.3	(0.2)	22.5	(4.3)
c	10.5	(0.9)	3.6	(0.4)	22.1	(3.1)
d	10.9	(0.3)	3.8	(0.04)	23.1	(4.9)
g	10.1	(0.3)	3.7	(0.3)	19.3	(2.4)
h	10.4	(0.5)	3.9	(0.2)	22.5	(1.8)
i	10.9	(0.8)	3.8	(0.03)	21.8	(3.9)
j	10.9	(1.0)	4.2	(0.02)	16.5	(1.1)
k	11.3	(0.1)	3.3	(0.1)	19.1	(2.7)

Daily Energy Expenditure Calculations

Birds were conducting on average 2182 ± 296 flights per day and were therefore travelling an average of 10.9 ± 1.5 km each day. The rate that birds received rewards averaged 5.2 ± 0.4 flights per pellet. With each pellet weighing 0.02 g and the energy content of the food being 24.5 kJ/g (dry mass), the net energy intake was calculated to be 166.1 ± 9.4 kJ/d assuming there was no spillage.

All birds maintained or increased their body mass during the study period. The estimated DEE, as estimated from time-energy budgets and the flight cost estimate, was 152.9 ± 8.6 kJ/d which is 2.32 times BMR (i.e. 0.763 W from Bautista *et al*. 1998). In all but one individual, the daily energy intake was significantly higher than the daily energy output as estimated from the TEB and the estimated flight cost (Table 3.3; paired *t*-test $t = 2.9$, $P = 0.02$). As most components for the time energy budget are assumed to be accurate, such as the resting energy expenditure and night-time energy expenditure measured using respirometry, then the unknown factor is flight cost. As the DEE prediction is close to the MEI estimate, which is also assumed to be accurate as all components are measured individually, then there is confidence in the use of labelled bicarbonate for measuring flight cost in this species.

Table 3.3 Estimates of daily energy intake compared to daily energy expenditure. DEE was calculated from a combination of the flight cost, metabolism at rest at 21°C, (calculated from Figure 3.2), and the average metabolism measured during the night in starlings.

Bird	Number of flights	Distance travelled (km)	Time spent in flight (hours)	Daily food intake (g wet)	Metabolisable energy intake (kJ/d)	Estimated daily flight cost (kJ/d)	Estimated daily metabolism (kJ/d)	Daily energy expenditure (kJ/d)	Difference between intake and output
a	2402.0	12.0	0.9	9.5	175.2	56.2	95.1	151.4	23.8
b	1077.5	5.4	0.4	7.9	146.9	31.3	94.8	126.1	20.8
c	3955.3	19.8	1.4	12.1	224.1	112.5	95.5	208.0	16.2
d	2508.5	12.5	0.9	9.3	172.0	74.7	95.2	169.9	2.1
g	2864.5	14.3	1.0	9.1	168.3	71.1	95.2	166.4	1.9
h	1743.0	8.7	0.6	8.1	150.9	50.6	95.0	145.6	5.4
i	1669.3	8.3	0.6	8.0	148.8	47.0	95.0	142.0	6.8
j	2165.0	10.8	0.8	9.9	182.9	46.2	95.1	141.3	41.6
k	1246.0	6.2	0.5	6.8	125.3	30.7	94.9	125.5	-0.2

Discussion

The energy costs of short flights in the starlings in this study were high compared to other estimates of flight costs in this species. For example Ward *et al*. (1999) estimated flight costs in starlings to be between 7.8 and 9.6 W for a bird flying at 22.8°C and 10.2 m/s using thermal imaging techniques and between 10.4 and 14.9 W using mask respirometry in the same bird (Ward *et al*. 2001). Torre-Bueno & La Rochelle (1978) measured the flight cost of starlings to be 8.9 W using wind tunnel respirometry. The birds in our study however were flying at much lower speeds (average 3.9 m/s) due to the restrictions of the size of the flight cage, and their flight cost (average 20.5 W) was over double these previous values.

Flight cost of starlings had been measured previously using a very similar flight cage but of slightly shorter distance (Bautista *et al*. 1998). Bautista *et al*. (1998) examined how starlings coped with changes of food availability. There were 2 treatments, one of which gave food rewards after fewer flights between perches than the other. Doubly labelled water and night time BMR measurements were used to measure daily energy expenditure, which in turn was used to calculate the flight costs. The cost of flight was estimated to be 52.3 and 45.5 W in the easy and hard treatments respectively. These values are over double the values measured in our study. An additional study by Westerterp & Drent (1985) also measured energy expenditure in starlings conducting short flights using DLW from which they predicted a flight cost of approximately 34 W. The reason for these overestimations using DLW is due to the extent of extrapolation. The birds were only flying for up to 4% of the total measurement period which therefore requires a great deal of extrapolation to predict flight cost for 100% of the period. Using labelled bicarbonate may eliminate the extrapolation errors.

Using Pennycuick's mechanical power curve (1968; 1969; 1989), the optimal flight speed was predicted to be 6.5 m/s. The starlings in this study were flying at flight speeds considerably lower than this value and were therefore predicted to have a higher mechanical flight cost. Many studies have been conducted on birds and bats flying in wind tunnels to produce metabolic power curves and test the aerodynamic theory (Torre-Bueno & Larochelle 1978; Masman & Klaassen 1987; Rayner 1990; Ward *et al*. 2001). Torre-Bueno & LaRochelle (1978) and Ward *et al*. (2001) both constructed metabolic power curves for starlings, however, they only measured flight costs at speeds higher than 6.3 m/s.

Birds, that have to manoeuvre below the minimum power speed, will have an increased flight cost (Tatner & Bryant 1986). In a study by Nudds & Bryant (2000), zebra finches flew between perches 5.46 m apart and their flight cost was measured using DLW and a time budgeting technique. They found that the flight costs measured was over 3 times greater than the predictions from aerodynamic models (Berger & Hart 1974; Pennycuick 1975; Masman & Klaassen 1987). We measured flight costs of 2.7 greater than predicted using Pennycuick's (1989) model for the range of speeds observed, using an efficiency of 0.23 for mechanical flight. Nudds & Bryant (2000) used data from different species of birds which had been measured during short

flights, to generate an allometric equation to predict the cost of short flights from body mass. Using this equation the present starlings, which had an average body mass of 71.1 g, had a predicted average short flight cost of 24.8 ± 0.22 W which was slightly but significantly higher than the average flight cost we measured. (paired *t*-test $t = 4.40$, $P < 0.001$). This indicated that although the energetic cost of flight seems high related to sustained flight in wind tunnels (Torre-Bueno & Larochelle 1978; Ward *et al*. 1999) these values are not unreasonable compared to other species flying over short distances at low speeds.

Flight cost was not significantly related to body mass, however the range in individual body mass was small in comparison to seasonal variation. This was also the case in a study on zebra finches using this technique (Hambly *et al*. 2002). Kvist *et al*. (2001) recently showed that the flight cost in red knots *Calidris canutus* with increased loads, was substantially less than aerodynamic predictions. The range in body mass would therefore have to be large before you would expect to observe a significantly elevated flight cost.

In this study, flight speed was not significantly related to WBF over this small range of speeds (2.8 to 4.6 m/s). An increase in WBF with flight speed has only been only observed over a larger range. Tobalske (1995) reported WBF increased from 13.3 to 16.2 Hz when flight speeds increased from 8 to 18 m/s. Extrapolating Tobalske's (1995) results to the flight speed of our starlings predicted WBF to be 10.7 Hz, which corresponds to the value of 10.8 Hz measured in this study at 3.9 m/s. In starlings, (Tobalske 1995) and black-billed magpies *Pica pica* (Tobalske & Dial 1996), the amount of time that the birds spent in energy saving glides or bounds during flight, significantly decreased at lower speeds. The birds in the present study conducted no obvious energy saving methods during their flights because of the short distance travelled and this may also have contributed to the high flight cost.

In all but one individual, the daily energy intake was significantly higher than the daily energy output. But minor deviations from the estimated resting metabolism during the day and the night, can explain the discrepancies Also some of the birds increased their body mass during the study and therefore the excess energy that some individuals took in was likely to become fat stores. Bautista *et al*. (1998) found that starlings spent 144 kJ/d on the 'easy' treatment and 107 kJ/d on the 'hard' one using the doubly labelled water technique combined with night-time BMR measurements. DLW has been found to be accurate for measuring energy expenditure in starlings and only underestimated CO_2 production by an average of 7.4% compared to gravimetric and energy balance methods in this species (Williams 1985). Our birds were undergoing a similar feeding rate as the 'easy' treatment and the average daily energy expenditure calculated using a time budgeting technique with known resting energy expenditure and flight energy expenditure of 20.5 W was very similar at 152.9 ± 8.56 kJ/d. As the DLW technique has been measured to be accurate in starlings, it gives confidence in the predicted values for flight cost measured in this paper.

The present study had two purposes. First to assess the use of the ^{13}C labelled bicarbonate technique to measure short-term energy expenditure in starlings and second to

measure flight costs over short distances in this species. It was successful in that in the validation there was a close relationship between metabolism measured by indirect calorimetry and the isotope elimination rate. There was also correspondence between the measurements for flight cost and daily energy expenditure with other studies on starlings.

Acknowledgements

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Chapter 4

Metabolic adjustments to increasing foraging costs of starlings in a closed economy

Popko Wiersma, Martijn Salomons & Simon Verhulst

Summary

Knowledge of the physiological consequences of variation in food availability is required to understand behavioural and life history responses to such variation. To study physiological consequences of food availability animals are usually subjected to caloric restriction, thereby reducing the upper limit to the energy budget. The relevance of this approach to free-living animals is questionable however, because under natural conditions low food availability often results in higher foraging costs, and everything else remaining equal this results in a *higher* energy budget. We manipulated food availability by varying the foraging costs per reward and studied effects on energy allocation and the total budget of captive starlings *Sturnus vulgaris*. Birds in a closed economy earned their food by flying between two perches. The probability of a reward was set at three different levels, thereby creating a poor, intermediate and rich environment. Birds flew 4 times more (i.e. 2.3 h per day) in the poor environment, as compared with the rich environment, and increased daily energy expenditure (DEE) with 40% to 220 kJ/d, equivalent to 3.7xBMR (comparable to free-living parents rearing young). Body mass, BMR and pectoral muscle size were reduced in the poor environment. Nocturnal energy expenditure was further reduced in the poor environment, by reaching the BMR level earlier in the night. BMR expressed on a mass-specific basis remained unchanged. Calculations show that in the poor environment the energy demands could not be met with flight costs of 20.5 W measured previously in a rich environment. Flight costs derived indirectly from the energy budget were estimated at 17.5 W. Flight costs were probably lower in the poor environment due to lower body mass. By reducing body mass by 20%, and economising during sleep, the birds achieved savings of 37% in their DEE. Without these savings, a DEE substantially higher than measured in free-living parents rearing young would be required to remain in energy balance. Surprisingly little data exist to verify whether free-living animals use the same tactics to survive periods with low food-availability.

Introduction

Large variations in food supply are the rule in nature, and are thought to exert a major selection pressure on birds (Newton 1998). Surprisingly few experimental data quantify how birds adapt physiologically or behaviourally to this variation. Although individual-based models of animal behaviour often include the variation in the environment (weather, food source; Houston & McNamara 1993), in the absence of critical data, the role of acclimation to these changes is not adequately dealt with (e.g. Sutherland 1996; Stillman *et al*. 2000). Yet, knowledge about how physiological or metabolic acclimation relate to foraging reward rate is essential for making predictions about behavioural choices of animals, for example when animals should leave a site in which food availability decreases. Recently this topic has been emphasised in studies describing changes in daily energy expenditure (DEE) with variation in foraging reward rate (Tiebout 1991; Deerenberg *et al*. 1998; Bautista *et al*. 1998). Evidently, the response to diminishing food conditions has many dimensions: resting metabolic rate may be altered, mass is reduced and consequently flight and thermoregulation costs may change. Therefore, DEE is not simply positively associated with the effort needed to obtain the food, and, in fact, DEE sometimes decreases when foraging reward rate diminishes (e.g. Deerenberg *et al*. 1998; Bautista *et al*. 1998). Thus total expenditure is not simply proportional to the level of activity.

Birds that face a declining foraging success have to spend more time and energy foraging for the same amount of food, ultimately leading to an elevated DEE. Unlike caloric restriction, where DEE is experimentally decreased, DEE is expected to be an accelerating function of foraging costs, because the extra energy spent foraging must also be acquired, which again increases foraging time and energy expenditure, and so on (Figure 4.1). Surprisingly, in laboratory experiments, increasing foraging costs resulted in DEE reductions (Bautista *et al*. 1998), resembling results of caloric restriction

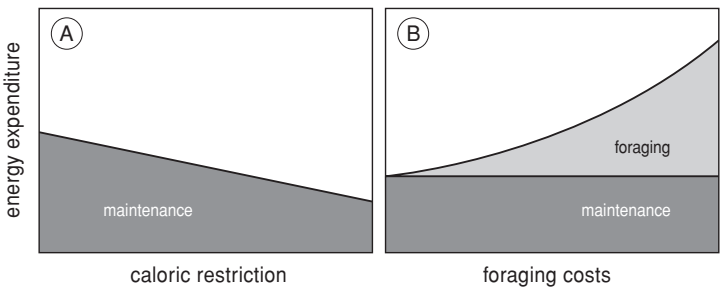

Figure 4.1 Depiction of relationships between energy expenditure and changes in food availability in the absence of physiological adjustments. Energy spent on maintenance includes resting metabolism and basal (non-foraging) activities. A. Caloric restriction results in a decrease in energy expenditure. B. When foraging reward rates decline, more time is spent foraging and more energy will be spent. Because the extra energy needed also has to be covered by foraging, energy expenditure is an accelerating function of foraging costs.

experiments (e.g. Daan *et al*. 1989). instead of working harder to increase food intake, as expected under natural conditions, the birds avoid costly activities. A possible explanation for the intuitively unexpected result is pointed out by Fotheringham (1998): in his experiments starlings ate less and decreased body mass with a decreasing foraging reward rate when the number of flights needed for a food pellet was fixed. But when using variable reward rates (with only the mean fixed) they did not eat less or decrease body mass. Fotheringham speculated that cognitive processes, such as motivation or memory, cause this difference. Because food reward rates experienced by free-living animals will typically be variable, applying variable foraging reward rates in laboratory studies may proof essential when extrapolating the results to natural conditions.

We studied captive starlings foraging in environments differing in foraging costs, to quantify the dimensions over which starlings adapt their physiology to the harshness of their environment. We manipulated foraging costs by changing the probability of obtaining a reward after a return flight, thereby yielding a variable foraging reward rate. We measured mass changes, time and energy budgets and components thereof and pectoral muscle size in a poor, intermediate and rich environment.

Methods

Flight cages

Starlings were individually housed in 8 flight cages (5.45x0.68x0.80 m, lxwxh). We manipulated foraging reward rate by adjusting the number of flights that had to be made between two perches, 5.00 m apart, before obtaining food. Switches on the flight perches were attached to a computer, registering when a bird had landed. A return flight was rewarded with a food pellet with a probability set by the experimenter, which yields a variable reward rate. The pellet was released into a small tray in front of a feeding perch, which could be reached by hopping 20 cm from the nearby flying perch.

The feeding perch was attached to a balance (P-1040 load cell, Tedea Huntleigh, UK), and body mass data were automatically stored in a PC at 1-s intervals. Communication with the balances was established using a RS232 serial multiport (C218, Moxa Technologies, Taiwan). Pellet dispensers were operated by a PLC (DL205, Koyo) while the activity data were stored in a PC.

The L:D cycle was 14:10 and food could only be obtained during the light period. To facilitate taking measurements at the end of the starlings' working day, the light period was from 00:00 to 14:00. The night started and ended with a 10-min period of dim light. Fresh drinking water was always present on the cage floor, and water for bathing was present one day per week. As a source of complementary nutrients, two mealworms were given three times each week, except during the periods of energy intake measurements. Average temperatures were 16.3 ± 0.1°C (± SE) during the night and 17.1 ± 0.0°C during the day.

Food pellets (Trouvit Europe Eel, Trouw, France) consisted of proteins (44%), fat (30%) and fibres/ashes (20.0%), complemented with vitamins and minerals (manufac-

turer's specifications). Fresh mass of one pellet was 0.020 g, with a water content of 4%. Energy content was 24.7 kJ/g dry mass; one pellet therefore contained 474 J.

Experimental protocol

The experiment was performed from December 2000 to March 2001 with 8 males which had been caught in the wild, and housed in an outdoor aviary prior to the experiment. All birds had prior experience with the system. At the start of the experiment all birds experienced a foraging reward rate of 2.0 return flights per pellet (f/p), further referred to as the 'rich environment'. After one week the foraging reward rate of 4 birds was gradually decreased to 6.3 f/p during a period of 3 weeks; the 'poor environment'. The other 4 birds remained in the rich environment. One bird kept losing weight when on a rate of 5.6 f/p, and this bird was therefore kept on a foraging reward rate of 5.0 f/p. The birds stayed on these schedules for 2 weeks, and during the last days of this period energy expenditure measurements were taken on all 8 birds. Next, the rich and poor feeding conditions were gradually changed to the opposite state. In this transition period all birds remained on a foraging reward rate of 4.0 f/p ('intermediate environment') for one week to measure their energy budget. Subsequently, foraging reward rates of the birds formerly in the rich conditions was steadily decreased during a period of 4 weeks, until one bird was on a rate of 6.3, one at 5.6 and one at 5.0 f/p. The fourth bird died halfway through the experiment and was left out from all analyses. The other 4 birds were gradually brought to a rich, 2.0 f/p regime.

Metabolic measurements

To measure BMR the birds were taken from their cages at the end of the light period and kept for the night in an open air flow system for measuring rates of O_2 consumption and CO_2 production. Starlings are post-absorptive after 60-75 min (Karasov 1990; Levey & Karasov 1994). During a measurement, a bird was sitting on a perch inside a dark Plexiglas box of 24 l at a temperature of 26.5°C, which is within the thermoneutral zone (Kendeigh *et al*. 1977; Biebach 1979; Biebach 1984). The air flow rate was controlled by mass-flow controllers (5850S, Brooks, The Netherlands) and set to 40.0 l/h. In- and out-flowing air was dried with molecular sieve (3Å, Merck, Germany). Gas analysis was done with a paramagnetic O_2-analyser (Servomex Xentra 4100) and CO_2-analyser (Servomex 1440). Measurements were recorded at 9-min intervals. The rate of oxygen consumption was calculated from these measurements and converted to the energy equivalent, while correcting for the respiratory quotient, according to Brody (1945). BMR was taken to be the minimum value of a 30-min running mean. Body mass was measured before and after respirometry.

Because the temperatures in the flight cages (on average 16.8°C) were within the thermoneutral zone (Kendeigh *et al*. 1977), respirometer measurements could be applied for night-time energy expenditure (E_{night}, kJ/10 h) estimates in the flight cages without temperature corrections.

To estimate the metabolic rate of the starlings during the day when not flying, a series of trials were undertaken where a group of starlings were restricted to cages

measuring 80x40x40 cm (lxwxh), precluding flight activities. Eighteen birds had *ad libitum* food, and an additional 6 birds were restricted to 70% of the *ad libitum* food intake per day. These individual trials lasted ca. 7 days, at the end of which their metabolic rate was monitored for 24 h by respirometry.

Daily energy expenditure

DEE was measured through food consumption and faeces production. Food consumption was measured by weighing the food in the pellet dispensers at 48-h intervals. All faeces were collected from the cages, and from the plastic sheets covering the floors. The sheets were wiped clean with moist towels, of which the dry mass was known. Faeces and towels were dried for 3 days at a temperature of 70°C and weighed to estimate faeces production. Energy content of dried food samples and of individual faeces samples were measured with a bomb calorimeter (C5000, IKA, Germany). DEE was calculated from the metabolisable energy intake (MEI) and body mass changes, according to

$$DEE = I - E - P,$$

where I is the gross energy intake, E the energy excreted and P the energy cost of tissue accumulation or energy catabolised from stored tissue, all in kJ/d. P was estimated to equal mass change x -18.0 kJ/g from energy budget data from captive starlings (S. Verhulst, unpublished data). The assimilation efficiency was calculated as gross energy intake minus energy content of the faeces divided by gross energy intake.

Body mass and pectoral muscle thickness

Body mass was measured automatically to the nearest 0.1 g each time a bird was on the feeding perch, and with an ordinary balance whenever a bird was handled. In the analyses we used the average mass during the last hour of the active period. Relative pectoral muscle thickness was measured using a 'muscle meter' (Max-Planck-Research-Centre for Ornithology, Seewiesen), which measured, to the nearest 0.1 mm, the distance from the breast surface to a virtual plane perpendicular to the sternum crest, 3.0 mm sideways of the sternum. Three measurements were taken at the location where the sternum protruded furthest from the centre of the body, and the average value was used.

Foraging currencies

It is usually assumed that decision rules in foraging have evolved to maximise particular foraging currencies. Physiological changes may affect the realised values of these currencies, thereby providing an ultimate explanation for such changes. We calculated two foraging currencies: net rate and efficiency of energy intake. Net rate was computed as the difference between the rate of metabolisable energy gain and the rate of energy expenditure during a foraging cycle (Watts). Efficiency was computed as the ratio of rate of metabolisable energy gain and the rate of energy expenditure during a foraging cycle (dimensionless). A foraging cycle included the flying time needed to

obtain one pellet, the perch time following each of these flights and the time for handling and eating a pellet. Perch time and handling time were not measured and we estimated them both to be 2 s. Within reasonable limits the length of these intervals had only minor effects on the results. Energy expenditure during a foraging cycle was the sum of flight costs (MR_{fly}) and metabolic costs during perching and handling of the food (MR_{nonfly}) as shown in Table 4.1.

Statistics

All mean values are given ± the standard error of the mean. We used generalised linear models (GLM) for the analyses, except where otherwise stated. In the statistical analyses we always controlled for individual differences by including individual as a factor in the models. SPSS (v. 10.0, SPSS Inc.) was used for all statistical calculations.

Results

Activity and energy budget

The number of flights and flight time per day increased fourfold with decreasing foraging reward rate (Table 4.1). Flight speed in the poor environment was 4% lower than in the rich environment. Food intake increased significantly with decreasing foraging reward rate, and the assimilation efficiency was not associated with foraging reward rate. Independent of foraging reward rate ($F_{2,12} = 0.06$, $P = 0.95$) body mass decreased slightly during the measurement periods (-0.22 ± 0.095 g/d), and thus birds used on average 3.95 ± 1.72 kJ/d from their body stores (range -12.15 to 18.90 kJ/d). Combining data on food consumption, assimilation efficiency and mass change, we found that DEE was negatively related with foraging reward rate: birds in the poor environment spent 43% more energy compared to those in the rich one (2.56 vs. 1.78 W respectively). There was no effect of treatment order on DEE (Figure 4.4; repeated measures GLM: $F_{2,4} = 1.36$, $P = 0.36$).

BMR decreased by 20% from the rich to the poor environment (Table 4.1). BMR was positively related to body mass ($F_{1,13} = 35.3$, $P < 0.001$). The slope of the within-individual regression of log(BMR) and log(body mass) was 1.02 ± 0.17 W/g. Metabolic rate decreased in the course of the night (Figure 4.2) and in intermediate and rich conditions reached minimum values after 6-8 h. In the poor environment, minimum values were reached earlier, after ±4 h. When we calculated the slopes of MRs plotted against time for each measurement session, these slopes decreased with decreasing foraging reward rate (Figure 4.2; $F_{2,12} = 17.6$, $P < 0.001$), resulting in a larger difference between treatments in E_{night} than in BMR. BMR decreased by 20% from the rich to the poor environment, but E_{night} decreased by 27%. Treatment order had no effect on BMR ($F_{2,4} = 1.58$, $P = 0.31$).

Mass-specific MR decreased, and differed between treatments, in the same way as whole-body MR (Figure 4.2). Mass-specific BMR (BMR_{ms}), however, was independent of foraging reward rate (Table 4.1).

Table 4.1 Mean measurement values (± SE) of 7 starlings measured successively at different food availabilities. Subscript 'fixed' refers to estimates based on the measured flight cost of 20.5 W from Hambly *et al*. (*in prep*.: Chapter 3), while subscript 'estimated' refers to calculated costs based on our DEE measurements, time budgets and E_{nonfly} estimates. The variables with subscript 'nonfly' refer to the light period when the birds were not flying. BMR_{ms} stands for mass-specific BMR. Flight speed was not measured at the intermediate reward rate. Test statistics result from GLMs with foraging reward rate and bird id entered as factors.

	FORAGING REWARD RATE			
	high	intermediate	low	
MASS AND FLIGHT MUSCLE				
body mass (g)	79.8 ± 2.4	72.7 ± 2.1	64.2 ± 1.4	$F_{2,12}$ =19.2, P<0.001
muscle depression (mm)	2.31 ± 0.24	3.21 ± 0.17	3.39 ± 0.25	$F_{2,12}$ =11.0, P<0.005
ACTIVITY				
travel distance (km/d)	7.84 ± 0.38	20.38 ± 2.16	31.84 ± 2.74	$F_{2,12}$ =49.9, P<0.001
fly time (min)	32.2 ± 2.1	85.4 ± 9.3	136.1 ± 12.8	$F_{2,12}$ =49.9, P<0.001
fly speed (m/s)	4.08 ± 0.08		3.92 ± 0.09	$F_{1,203}$=19.0, P<0.001
MEASURED ENERGETICS				
assimilation efficiency	0.826 ± 0.010	0.843 ± 0.014	0.840 ± 0.016	$F_{2,12}$ =0.71, P=0.51
MEI (kJ/d)	149.8 ± 5.6	189.2 ± 20.3	215.5 ± 9.5	$F_{2,12}$ =7.45, P<0.01
DEE (kJ/d)	153.8 ± 5.1	192.3 ± 16.2	220.2 ± 10.1	$F_{2,12}$ =12.1, P<0.005
E_{night} (kJ/d)	37.79 ± 1.01	31.92 ± 1.22	27.54 ± 1.17	$F_{2,12}$ =17.1, P<0.001
BMR (W)	0.861 ± 0.019	0.776 ± 0.019	0.692 ± 0.029	$F_{2,12}$ =18.6, P<0.001
BMR_{ms} (mW/g)	10.85 ± 0.40	10.69 ± 0.27	10.76 ± 0.28	$F_{2,12}$ =0.12, P=0.89
ENERGY BUDGET ESTIMATES				
$E_{fly(fixed)}$ (kJ/d)	39.66 ± 2.62	105.04 ± 11.47	167.36 ± 15.75	
$E_{nonfly(fixed)}$ (kJ/d)	76.37 ± 3.43	55.35 ± 6.05	25.30 ± 7.45	
$MR_{nonfly(fixed)}$ (x BMR)	1.84 ± 0.10	1.59 ± 0.19	0.87 ± 0.26	
DEE - fixed costs (kJ/d)	24.62 ± 3.96	11.83 ± 6.38	-10.90 ± 7.52	
$MR_{nonfly(estimated)}$ (W)	1.70 ± 0.05	1.44 ± 0.05	1.24 ± 0.05	
$MR_{nonfly(estimated)}$ (x BMR)	1.97 ± 0.04	1.85 ± 0.05	1.79 ± 0.01	
$MR_{fly(estimated)}$ (W)	16.99 ± 2.37	17.90 ± 1.56	17.50 ± 0.78	
$E_{fly(estimated)}$ (kJ/d)	33.63 ± 5.98	95.39 ± 16.85	140.43 ± 9.81	
$E_{nonfly(estimated)}$ (kJ/d)	82.40 ± 2.17	65.00 ± 2.44	52.23 ± 2.06	

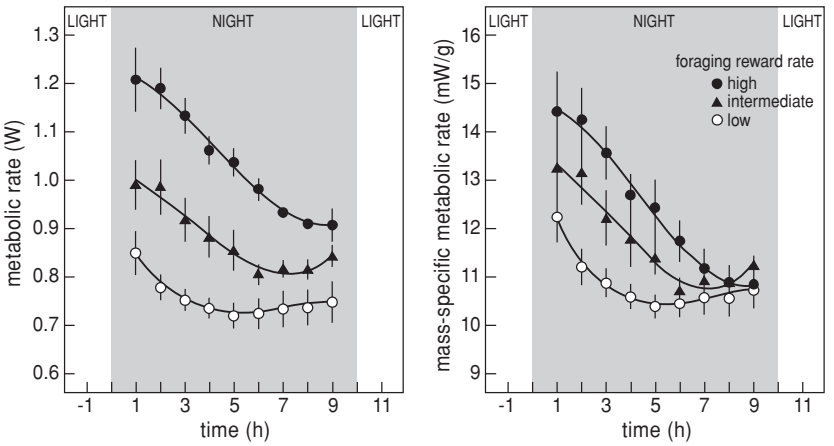

Figure 4.2 The hourly averaged metabolic rates (A) and mass specific metabolic rates (B) (± SE) of starlings during the night at different foraging reward rates (night from 0 to 10 h, i.e. 14:00 to 00:00 local time). MR and mass-specific MR steadily decreased during the night, but the minimum value (or BMR) was reached sooner when foraging reward rate was lower.

Body mass and pectoral muscle size

Birds responded to changes in foraging reward rate with drastic changes in body mass (Table 4.1). Birds that experienced the poor conditions had reduced body mass by 15.6 ± 3.1 g compared to the rich environment. The range in body mass changes was large: from 3.8 to 26.1 g (Figure 4.3). We found a large difference between body masses of birds with different starting treatments (Figure 4.3). Birds in the poor environment lowered body mass with 23.4 ± 1.5 g when coming from the rich environment. Birds that started in the poor environment increased body mass with only 9.7 ± 2.5 g when in the rich environment. Note that initial body mass in both groups was closely comparable (Figure 4.3).

In the poor environment the pectoral muscle was ca. 1 mm thinner than in the rich environment. Between the intermediate and rich environment there was no difference (paired *t*-test, $t_6 = 0.62$, $P = 0.56$).

Flight costs

An important part of the energy budget is made up of the flight costs. If flight costs remained constant under all conditions, the energy expended flying would have been quadrupled ($E_{fly(fixed)}$ in Table 4.1), due to the 4-fold increase in flight time.

Flight costs of starlings were earlier measured in the same system by Hambly *et al*. (*in prep*.: Chapter 3) using labelled bicarbonate, a method enabling short term energy expenditure measurements (Speakman & Thomson 1997; Hambly *et al*. 2002). The empirical value for metabolic rate during the short flights averaged 20.5 ± 0.93 W (n = 25). These estimates were made under rich conditions only, and we therefore made

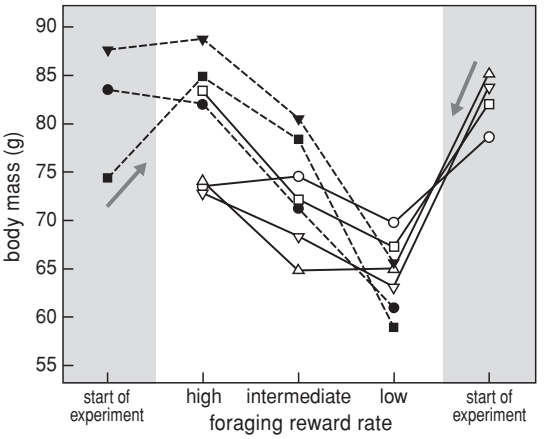

Figure 4.3 Body masses of individual birds measured during the last hour of the active period at different foraging reward rates. Closed symbols connected with broken lines depict birds that started the experiment in the rich conditions (chronological from left to right), while open symbols connected with continuous lines depict those that started in poor conditions (chronological from right to left). Repeated measures GLM showed that treatment and order of treatment both had an effect on mass: treatment, $F_{2,4} = 47.0$, $P < 0.005$, order, $F_{2,4} = 10.5$, $P = 0.025$.

independent estimates of flight costs for all three experimental treatments from the DEE, activity and respirometer measurements by completing the budget with the flight costs.

To calculate an energy budget where E_{fly} is the remainder of the budget, we had to derive the energetic costs during the day-time when not flying. For the night we used measured values E_{night}. For the day-time, when a bird was not flying we used the values from 24-h respirometer measurements on another group of starlings. These birds stayed at 20.8 ± 0.2°C, were either on *ad libitum* food or mildly food-rationed, had drinking water available and experienced a L:D cycle of 12:12. We used the same respirometer boxes as in our current measurements, which were too small to allow much activity, and most of the time was spent sitting. From these measurements we calculated MR_{day} and MR_{night}. MR_{day} was correlated with mass ($r = 0.44$, $P = 0.032$), and MR_{day} and MR_{night} were strongly correlated (Figure 4.5). Therefore, we calculated the ratio of MR_{day} to MR_{night}, i.e. 1.62 ± 0.03, and applied this figure to our current data to predict MR_{day}. On the basis of Aschoff & Pohl's (1970) allometric relationship for passerines a ratio of 1.42 for RMR_{α}/RMR_{ρ} would have been expected. Because they used measurements of birds in the dark our value is expected to be higher. Using the estimated MR_{day} in the budget, resulted in average flight costs of 17.5 ± 0.9 W. The estimated flight cost was not associated with foraging reward ($F_{2,12} = 0.08$, $P = 0.92$). As will be expanded in the discussion, there are independent data confirming the general level of this flight cost factor in our experimental set-up, at least in the poor environment.

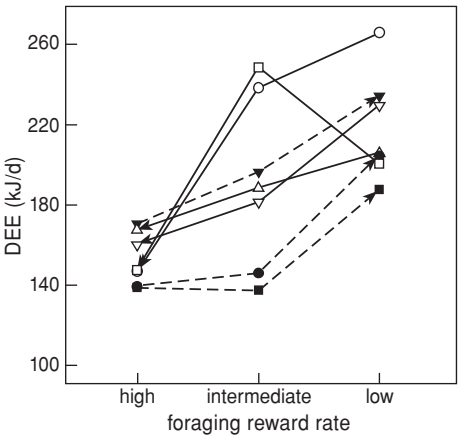

Figure 4.4 Daily energy expenditure at different foraging reward rates. Closed symbols connected with broken lines depict birds that started the experiment in the rich conditions, while open symbols connected with continuous lines depict those that started in poor conditions. The arrowheads indicated the chronological order of measurements. Symbols correspond to those in Figure 4.3.

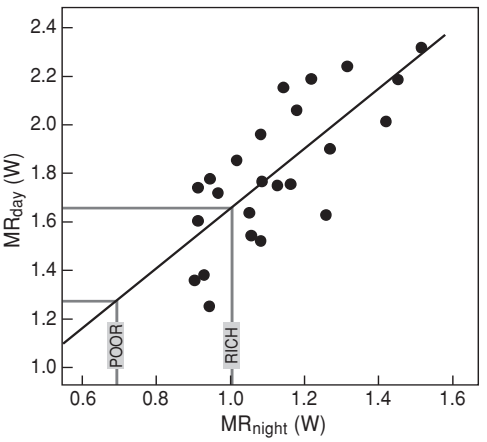

Figure 4.5 Association between average metabolic rates (MR, measured at ±20.8°C) of starlings measured during the day (MR_{day}) and the night (MR_{night}) during a 24-h respirometer measurement. MR_{day} and MR_{night} were strongly correlated ($r = 0.75$, $n = 24$, $P < 0.001$, controlling for mass). Also shown is the regression line. The dotted lines show the average values of MR_{night} for our birds and the estimated values of MR_{day} in the rich and poor environment.

Foraging currencies

Different foraging currencies were calculated to determine whether they might have driven foraging decisions. The net rate of energy intake decreased from 49.8 to 13.3 W from rich to poor conditions, and the efficiency decreased from 6.45 to 2.52 (Table 4.2). For these estimates we used the measured MR_{fly} values of 20.5 W (Hambly *et al.* in prep.: Chapter 3) in the rich and intermediate environment and the estimated value of 17.5 W in the poor environment. Under all conditions the birds would be foraging at higher net rates and efficiencies if flight and maintenance costs would be lower. If retaining the high mass as it was in the rich environment, in the poor environment the net intake would have been 12.4 instead of 13.3 W, while the efficiency would have been 2.17 instead of 2.52. Considering these foraging currencies, we calculated that in the rich environment the occurrence of a negative net rate or efficiency would be below 0.01% per reward. On the other hand, in the poor environment an efficiency below 1 would occur in ±5.7% of the rewards (22-30 per day) and a negative net rate with ±4.7% (18-25 per day).

Table 4.2 Estimates of two currencies during foraging, i.e. net rate of energy intake and the efficiency of energy intake, in environments varying in their mean food reward rates. For the poor environment currencies were also estimated assuming the bird had maintained the same (higher) mass as in the rich environment. Time for handling and eating the food and for turning on the perch were both estimated at 2 s. For MR_{fly} we used 20.5 W in the rich and intermediate environment and 17.5 W in the poor environment.

	net intake (W)		efficiency	
environment	actual mass	high mass	actual mass	high mass
rich	49.8		6.45	
intermediate	21.2		3.17	
poor	13.3	12.4	2.52	2.17

Discussion

DEE

DEE increased from 154 to 220 kJ/d from the rich to the poor environment (Figure 4.4), which is within the lower end of the range of brood provisioning starlings, i.e. 200-331 kJ/d (Westerterp *et al.* 1982; Ricklefs & Williams 1984). To our knowledge, this is the first experiment where DEE was elevated by manipulating foraging conditions, providing a tool for studying the physiology of hard work.

The DEE we measured can be compared with two earlier experiments with starlings. In the flight cages of Bautista *et al.* (1998) DEE was 144 kJ in the rich environment, and DEE decreased to 107 kJ in the poor environment. These low values are in agreement with the lower time spent foraging in their experiment. In Fotheringham's

(1998) experiments, the maximum DEE was approximately 169 kJ/d (estimated from gross food intake rates in birds with variable reward rates: ca. 19 g/d, and energy content and assimilation efficiency from Bautista *et al*. (1998), who used the same food). This is comparable to the DEE of our birds in the rich environment (Table 4.1). The poor environment in our experiment was much harsher than the poorest environment in Fotheringham's experiments. The difference in DEE is further in accordance with the much lower flight times in Fotheringham's experiment: his birds flew ±4 km in the poorest environment, only 13% of the realised flight time in the poor environment in our experiment.

Body mass and pectoral muscle size

Body mass was on average 15.6 g (19.5%) lower in the poor environment, and reached levels below that usually measured in free-living starlings (Cramp & Perrins 1994). The association between body mass and foraging effort is in agreement with field studies: Starlings reduce mass during breeding (Westerterp *et al*. 1982; Ricklefs & Hussell 1984), and similar mass reductions have been shown in several other birds species (Moreno 1989). Swaddle & Biewiener (2000) showed that captive starlings reduced mass when they were forced to engage in flight exercise (1 h daily), and suggested that birds were regulating a lower body mass to reduce flight costs. Lind & Jacobsson (2001) showed a mass reduction in tree sparrows *Passer montanus* when experimentally increasing wing loading, possibly also to decrease flight costs. These effects are however much smaller than found in this study. Food supplementation and incubation extension experiments strongly suggest that the mass changes found in breeding birds function to reduce flight costs and are not merely a consequence of the high work load (Cavitt & Thompson 1997; Slagsvold & Johansen 1998; Cichon 2001).

While our birds decreased in mass when the environment was made poorer, Fotheringham (1998) did not find body mass changes with decreasing foraging reward rate (using a variable reward rate, as we did), but the minimum reward rate was much lower in our experiment.

BMR

Energy was saved by reducing BMR by 19.6% (14.6 kJ/d) under poor conditions (Table 4.1). Proportionally, the savings during the entire night were even larger (Table 4.1), because the metabolic rate decreased more quickly at a low foraging reward rate (Figure 4.2). If the birds under poor conditions had maintained E_{night} values they had in the rich environment, they would have had a 37% higher energy expenditure during the night.

In other food manipulation experiments animals showed similar energy savings by reduction of basal or resting metabolism when foraging reward rate was limited (Tiebout 1991; Deerenberg *et al*. 1998; Bautista *et al*. 1998). A similar reduction of RMR was observed in zebra finches forced to increase their daily flight time (Nudds & Bryant 2001). A number of non-experimental studies also showed the occurrence of savings. Chilean hummingbirds *S. sephanoides* spent less energy during the night when during day-time energy output had been high (López-Calleja *et al*. 1997). Captive star-

lings lost significantly more mass during a night in spring and summer than in winter (Meijer *et al*. 1994), which suggests energy saving behaviour during winter nights when day-time energy expenditure is likely to be high. These results are consistent with effects of caloric restriction (Daan *et al*. 1989; Ramsey *et al*. 2000).

BMR_{ms} did not differ between treatments. In contrast, Bautista *et al*. (1998) and Deerenberg *et al*. (1998; using zebra finches), found reductions in BMR_{ms} in birds that had to work harder for their food. The constant BMR_{ms} in our starlings is remarkable considering the large range in body mass: on average 20% lower mass under poor conditions. Either the proportional body composition did not undergo major changes (which seems unlikely given that the birds became lighter, not smaller), or energy was saved in other ways, e.g. by lowering body temperature (T_b), in which case the constant BMR_{ms} may be coincidence. Hypothermic responses seem to be common in birds (McKechnie & Lovegrove 2002). For example, night-time hypothermia has been shown in pigeons *Columbia livia* when foraging reward rates decrease (Rashotte & Henderson 1988), and also food deprivation often causes hypothermia (Daan *et al*. 1989; Reinertsen 1996). The finding that night metabolism reached BMR earlier in the night in a poorer environment (Figure 4.2) could also be due to an earlier drop in T_b.

Although intraspecific and intra-individual relationships between BMR, body mass and sizes of some organs have been shown (Daan *et al*. 1989; Piersma *et al*. 1996; Meerlo *et al*. 1997), data are scarce (see overview in Battley *et al*. 2001). In general, the slope of the relationship between BMR and body mass is steeper in intra-individual and intraspecific allometric relationships, than in interspecific relationships, which is in agreement with the steep slope we found.

In contrast with these results, it has been shown that animals may change body composition leading to an increase in BMR when there is a higher demand for sustained energy output, e.g. for migration, lactation or maintenance (Piersma & Lindström 1997). This is opposite to the decreasing BMR of our starlings that increased their foraging effort. Clearly, birds make physiological adjustments differently under different ecological circumstances, but it is not evident what triggers these different responses. Inevitably, there is also a cost to reducing BMR, otherwise it is difficult to understand why birds not always save energy during the night, thereby conserving this energy for other activities. Possibly, physiological protection and maintenance processes are less efficient, and escaping predators may be less effective.

Flight costs

To assess energy allocation to foraging we need estimates of the flight costs. Because the length of the flight cages was only 5 m, measurements on starlings in steady-state flight are not applicable (Nudds & Bryant 2000). Hambly *et al*. (*in prep*.: Chapter 3) measured flight costs of starlings of 20.5 W in our flight cages and found no relation between flight cost and body mass (range 64.6 to 76.5 g, average 71.1 g). This flight cost is high compared to earlier measurements of starling flight costs in wind tunnels and in the field (Ward *et al*. 1999; Ward *et al*. 2001), i.e. 7.8-14.9 W (Torre-Bueno & Larochelle 1978; Westerterp & Drent 1985). The difference may be due to the fact that

in our experiment the average speed was only 4.0 m/s, well below the 6-18 m/s of starlings during sustained flight (Torre-Bueno & Larochelle 1978; Ward *et al*. 2001).

Assuming that 20.5 W is the best estimate for our experiments, our birds would have spent 39.7 and 167.4 kJ/d on flying in the rich and the poor environment, respectively ($E_{fly(fixed)}$ in Table 4.1). However, in the poor environment the value is too high to fit the energy budget. A decrease in body mass is expected to result in a lower MR_{fly} (Pennycuick 1975; Rayner 1979), although the savings may be smaller than earlier thought (Kvist *et al*. 2001) and have not been confirmed for the starling (Hambly *et al*. *in prep*.: Chapter 3). Using the (interspecific) allometric equation of Nudds & Bryant (2000) for the range of body masses of our starlings, the metabolic rate during flight should decline by 17%, from the rich to the poor environment. This is very close to our budget results (entailing a savings of about 15%, from 20.5 W to 17.5 W).

Effect of treatment order

Birds that started off in the poor environment did not fully recover when they were later brought in a rich environment: their body masses remained below those of birds starting at rich conditions (Figure 4.3). We can only speculate regarding the causes of this pattern, but possibly they had reduced the size of internal organs to such a degree that recovery was hampered. The reduced BMR of the lighter birds (see above) might be a consequence of this body composition change. This scenario would also imply differences in assimilation efficiencies between the two groups under the conditions they entered last. Indeed assimilation efficiencies of the birds that started in the rich environment tended to be higher (by 6%). But the difference is far from significant, al-though the effect size of 1.25 is considered very large (Cohen 1988), suggesting that this deserves further study.

Treatment order had no effect on DEE or BMR. Apparently the size of metabolically active tissues, E_{night} and the foraging effort were not affected by the difference in start treatment.

Starving in the midst of plenty?

Figure 4.6 summarises our findings and illustrates the effects of the metabolic changes the starlings made when the food conditions changed. By lowering mass in the poor environment, and hence maintenance metabolism and probably flight costs, the birds achieved a flight time reduction of 34%, and a 37% reduction in DEE compared with the expected DEE in the absence of such responses.

Reduced flight costs and maintenance metabolism resulted in ca. 15% reduction in DEE, but the flight time reduction brings about the greatest energy savings, namely ca. 24%. The 27% energy saved during the night gives rise to a further 5% lower DEE.

Our birds spent 3.7xBMR in the poor environment, which is close to the suggested limit of sustained energy expenditure of 4xBMR (Drent & Daan 1980), and within the range of food provisioning birds (Daan *et al*. 1990). DEE in the poor environment (220 kJ/d) was within the range of values reported from starlings in the field. Still, the birds were very light compared to wild birds. So why did the birds not forage more, so that they could maintain the energy balance?

In theory, the birds may have been time-constrained. However, in the poor environment birds spent only 16.2% of the light period on flying. This increases to 42% of day-time spent on foraging when food handling time is taken into account. Using our energy expenditure measurements (Table 4.1), we calculated that to maintain mass in the poor environment, the flight time should have increased by 52%. Including turning time on the perch and food handling time this would add up to 65% (Figure 4.6). This still leaves 35% 'free time', and since there were no other notable time-consuming activities, we conclude that time was not a limiting factor.

Energy expenditure in the poor environment could have been constrained by a digestive bottleneck (Kersten & Visser 1996) which could have prevented them from increasing their DEE to maintain mass. Because starlings are already post-absorptive after 60-75 minutes (Karasov 1990), we assume that when we measure intake rates expressed per hour, we obtain intake rates equal to, or below, maximum sustainable rates. The average, hourly consumption rate during a day was 28 to 40 pellets/h for the different birds, well below the maximum hourly consumption rates (range 49-70 pellets/h). This suggests that DEE was not constrained by a digestive bottleneck.

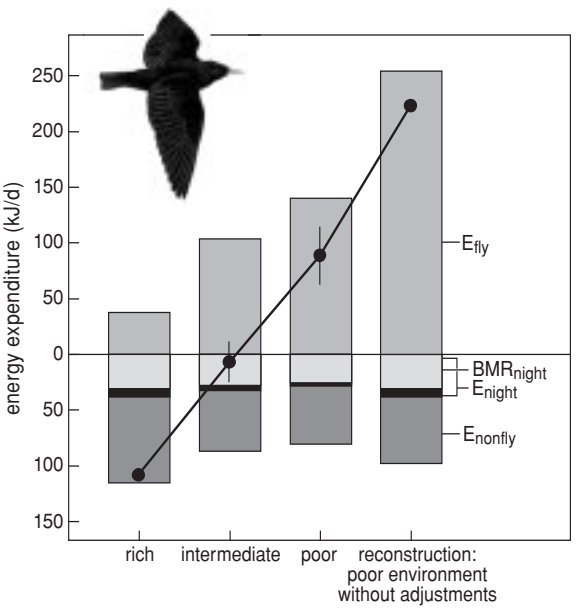

Figure 4.6 Energy budgets at different foraging reward rates and, at the far right, the estimated budget in the poor environment of a starling that maintained a high body mass as in the rich environment. E_{fly} is the energy spent on flying, BMR_{night} is the energy spent during the night on BMR only, E_{night} is the total energy spent during the night, and E_{nonfly} is what is spent during day-time when not flying. The broken lines in the hypothetical energy budget indicate the surplus energy that would have been spent due to the extra time spent flying (and less time spent not flying), and the extra energy that is spent on flying due to the body mass increase. The total DEE budget is shown on top of each bar.

A negative energetic foraging efficiency might have been another reason why mass was not maintained in the poor environment. We calculated foraging efficiency and net intake rate in the different environments for birds with their actual mass and the mass maintained in the rich environment. The effect of body mass (via flight and resting metabolism costs) on the currencies were rather small (Table 4.2). Clearly both currencies decreased considerably when foraging reward rate decreased, but no substantial improvement was made in the poor environment by the lowering of flight costs and resting metabolism. Certainly, no currency would drop to unsustainable levels. There is, therefore, no indication that effects on foraging efficiency were driving the mass changes of the birds.

Other possibilities for sub-optimal performance might be some form of exhaustion. We did not notice symptoms indicating exhaustion, such as difficulties to fly or lethargic behaviour, but cannot rule out that such effects would have occurred when birds had increased their foraging effort to the level required to maintain mass. Also cognitive processes might affect the starlings' foraging effort. A lack of reinforcement may lead to reluctance to start the next foraging bout (Ferster & Skinner 1957). We circumvented this problem by applying variable reward rates (Fotheringham 1998), but cannot exclude the possibility that further modifications in the reward schedule would result in even higher work rates in the poor environment.

Although our starlings changed their BMR dramatically, BMR_{ms} remained the same under all conditions. Apparently, no major changes in body composition had occurred. The experimental protocol followed by our starlings can be looked upon as a training scheme for endurance training, and we see similarities with studies on human exercise physiology. Westerterp (2001) points out that 'novice' trainees for the half-marathon (a) lose body mass and (b) concomitantly lower night-time metabolism. Whether mass-specific metabolism decreased is unclear. Only the well trained, professional sports(wo)men achieve an increase of BMR at the same mass (hinting that a suite of changes are involved). It seems our starlings 'acclimate' to a training programme in much the same way as 'average' human beings do. In contrast, birds rearing young have recently been shown to increase BMR when parental effort increased following brood size enlargement (Nilsson 2002), although another study was unable to replicate this effect (Wiersma & Tinbergen 2003: Chapter 5). The discrepancy with the present study may be caused by a lack of the right stimuli (nestlings) and/or the absence of hormone controlled processes (e.g. for flight muscle growth). This suggests that the response to changing work levels may differ in the course of the annual cycle, depending on the state of the animal.

When trying to understand the effect of food availability on animal behaviour it is important to be aware of the flexibility of the energy budget. The substantial energy saving that may be achieved through physiological adjustments may have significant consequences for individual based modelling exercises, which explore the relationship between food supply, individual behaviour and population dynamics. Surprisingly, field data on the relationship between foraging costs per reward and DEE or BMR still have to be collected.

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Chapter 5

No nocturnal energetic savings in response to hard work in free-living great tits

Popko Wiersma & Joost M. Tinbergen

Abstract

We measured energy expenditure in free-living great tits *Parus major* during the active (day) and the inactive period (night) with the aim of determining whether great tits compensate for energy costs made during periods of high activity in periods of low activity. If such compensation occurs, inferences from measurements of energy expenditure over a 24-h period, with regard to the energy costs of the different behavioural elements, may obscure relations between parental effort and energy expenditure. Also, energy budgets, based on estimates of time budgets combined with a fixed cost for each behavioural category observed, may be unreliable if animals are able to compensate for energy costs made during periods of high activity in periods of low activity.

Laboratory studies have revealed a reduction in resting metabolic rate (RMR) when birds are forced to work harder during the day, but it has not yet been investigated whether such nocturnal savings are also made under natural conditions.

We manipulated brood size in a free-living population of great tits to create a difference in the demands of the nest, measured effort (feeding visits) and daily energy expenditure (DEE). In order to test whether compensation occurred we measured both DEE over 24 h, and resting metabolic rates (RMR) of female great tits at night. DEE and feeding rate differed between the experimental groups, being higher in females rearing enlarged broods, but we did not find evidence of nocturnal saving.

Introduction

It has recently been discovered that birds, when pressed to work for their food, reduced night-time energy expenditure. Evidence of such energy compensation comes from laboratory studies on zebra finches *Taeniopygia guttata* and common starlings *Sturnus vulgaris*, by Deerenberg *et al*. (1998) and Bautista *et al*. (1998), respectively. They made birds work for food, during a period of ca. 4 weeks, and showed that nocturnal mass-specific metabolic rate was reduced when activity was experimentally increased. These findings may have repercussions for life history studies.

Life history theory hinges crucially on the premise that organisms make costs in order to reproduce. The price of reproduction is paid for through a declining future reproductive output. The theory considers parental effort, the work parents do for their current offspring, as the reason for this declining future reproductive output. However, effort has many dimensions, since it represents anything the parents do for their current offspring. Empiricists have, for a long time, investigated factors limiting reproductive output. As a starting point, parental effort was substituted by feeding rate but more recently by parental energy expenditure. Stimulated by the work of Hails & Bryant (1979) and Drent & Daan (1980) the research concentrated on the question whether and how energy expenditure limits reproductive output.

Empirical studies have concentrated on the effect of brood size manipulation on energy expenditure, with the expectation that energy expenditure would increase with brood size. However, correlations between energy expenditure and brood size are sometimes much less pronounced than expected, or even absent (Verhulst & Tinbergen 1997; Wright *et al*. 1998; Tinbergen & Verhulst 2000). Williams (1987), Hails & Bryant (1979) and Moreno *et al*. (1995) could only show a correlation between feeding rate and DEE in one of the sexes. In his overview, Bryant (1988) shows that five out of nine studies cannot detect a positive relationship between energy expenditure and nest visit rate. Also Moreno (1989b) could not detect any effect on energy expenditure of brood size manipulation in both parents in northern wheatears *Oenanthe oenanthe*. Tinbergen & Verhulst (2000) questioned the role of energy expenditure on the cost of reproduction. They could not show a difference in female DEE between the control and enlarged broods, whereas birds with enlarged broods did show a lower probability of starting a second clutch. Therefore, they concluded that DEE per se is probably not causally involved in the cost of reproduction.

We argue that empirical studies of the effect of brood size on energy expenditure (discussed above) may fail to find a relationship due to night-time compensation of increased work rate during the active phase. Hence, estimates of parental work rate based on energy expenditure measurements over a 24-h period, as taken by doubly-labelled water, may give misleading results. Moreover, as mentioned by Deerenberg *et al*. (1998), translation of time budgets to energy budgets will be inadequate if night-time compensation occurs.

Published studies of night-time compensation were carried out in the laboratory, which may not mimic adequately the natural conditions with respect to the possibili-

ties the birds have to adjust their energy budgets. Therefore, we decided to study the occurrence of night-time reduction of energy expenditure in response to increased daytime work in the field. We chose a well-known system; great tits caring for offspring. Work rate of the parents, as measured by provisioning rate and DEE, can, in the study population chosen, be altered by brood size manipulation (Sanz & Tinbergen 1999). We measured night-time energy expenditure using oxygen consumption, and from the same individuals we estimated the 24-h energy turnover using doubly-labelled water.

Methods

The experiment was carried out in 1997 and 1998 in the Lauwersmeer, located in the north of The Netherlands. Four woods were used, consisting of plots of varying sizes containing either coniferous or deciduous trees. Coniferous plots were usually bordered by deciduous trees and shrubs. In total 200 nestboxes were present. The boxes were fixed to the trees at a height of ca. 2 m.

Nestboxes were checked regularly during the breeding season to assess clutch size, first day of incubation, number of hatchlings and fledglings, and date of hatching and fledging. During the nestling period, when the chicks were around 10 days old, attempts were made to catch both parents using a spring trap placed inside the nestbox. All nestlings and newly caught adults were ringed and morphometric measurements were taken. For tarsus length we measured the length of the tibiotarsus with callipers (resolution 0.1 mm) and the length of the third primary was measured using a stopped ruler (1 mm; Svensson 1992). Body mass was measured with a spring balance (0.1 g).

We manipulated the brood size by adding or removing three chicks when they were 2 days old. Triplets of matching nests were selected on the basis of hatching date (maximum difference 1 day), clutch size (maximum difference two eggs) and location. Thirteen nest pairs were in the same wood, and four pairs were in separate, but nearby, woods. Chicks were moved between two of the nests, leaving one nest serving as a control. For the metabolic measurements presented in this paper only the nests with reduced and enlarged broods were used. In total, we took measurements of 17 pairs of nests. Figures concerning timing of the breeding season and numbers of eggs and nestlings are given in Table 5.2.

The resting metabolic rate was measured when the nestlings were on average 12 days old. DEE of female parents, and feeding rates of both parents, were measured, in 1997 only, immediately prior to, or after the RMR measurement.

At night, between 22:00 and 00:00, two birds were caught in their nestboxes and weighed. They were held in a respirometer until sunrise, i.e., 04:00-04:45, and then returned to their nestboxes. In 1997, the oxygen consumption rate was first measured at ambient temperature (average 14.9°C, range 10.1-20.3) and after ca. 2 h the temperature was increased to, on average, 23.4°C (range 19.6-27.1). In 1998, only a measurement at a high temperature was taken (average 27.8°C, range 23.5-30.4). The high

temperatures varied due to differences in ambient temperature and battery capacity. The respirometer boxes were darkened with black plastic sheets. Although it was difficult to ascertain whether the birds were stressed during the measurements, we have some indications that they were not. A few times the birds were seen sleeping in the boxes when these were uncovered again after measurement. At other times, when this was not observed, we cannot be sure if they had been sleeping or not, because the disturbance of uncovering the box might have alarmed and woken them. Strong activity can be seen from erratic changes to the oxygen consumption readings. There were always long periods of steady values in the measurements, indicating that the bird inside the box was not moving much.

When the bird could be caught quickly, body temperature was measured with a microthermistor connected to a datalogger (Squirrel 1259, Grant Instruments, Cambridge), inserted ca. 1.5 cm in the cloaca (accuracy 0.2°C). When the time it took to insert the thermistor and take a reading was too long (more than 10 s) to yield a reliable measurement, the data were omitted from analyses.

In 1997 only, daily energy expenditure of female parents was measured using doubly-labelled water (D_2O^{18}, further referred to as DLW). The females were caught in the nestbox during the night following the day that the chicks were 11 days old (range 10-13). The birds were injected intraperitoneally with 0.10 or 0.12 ml DLW and an initial blood sample was taken from a brachial vein 1 h later. Twenty-four h later we tried to recapture the bird to take the final blood sample. A detailed description of the DLW technique used can be found in Tinbergen & Dietz (1994). Because frequently we did not succeed in recapturing the birds 24 h later (see results), some birds were caught again with a trap early the next morning. In these cases, the time interval over which energy expenditure was measured was longer and to ensure acceptable accuracy in the analyses we changed from injecting with 0.10 to 0.12 ml of DLW. The measurements over longer time intervals were corrected by linear interpolation to a 24-h period. We corrected for the proportion of inactive time in the measurement period, assuming the birds were inactive from 20:40-05:00 (own obs., and see Tinbergen & Dietz 1994), and assuming that the metabolic rate in the active period was 1.96 times that in the inactive period (Tinbergen & Dietz 1994). DLW analyses were carried out at the Centre for Isotope Physics (University of Groningen, The Netherlands). For the calculations we assumed that the body water content was 66% (Mertens 1987) and that the RQ was 0.75.

In the beginning of the 1997 season, the oxygen measurement was performed immediately after the final DLW-blood sample was taken. However, due to the disturbance, frequently the birds were not present in the nestbox that night, and a final blood sample could not be taken, or only after trying to trap the bird the following morning. We then chose to do the oxygen measurement immediately after the DLW injection and prior to taking the initial blood sample. This ensured that we had the oxygen measurement at least.

Feeding activity was observed directly with video cameras. In the morning following the DLW injection the nestbox visits were recorded for 3 h. The cameras were

positioned ca. 10 m from the nestbox, preferably in or behind a bush and covered as well as possible with leaves and grass. The recordings were analysed to determine the feeding rate of both male and the female.

Nocturnal metabolism measurements were made with a two-channel, car-battery powered, transportable respirometer. The portable oxygen analyser (Servomex model 570A, Crowborough, UK) had an accuracy of ±0.1% (manufacturer's specification). The measurement accuracy is, in fact, higher because the difference between the measured channels and a reference channel (outside air) was used instead of an absolute value. The zero value of the oxygen analyser was calibrated with nitrogen gas (<0.001% O_2) before, and, in 1997 only, after each measurement. Outside air (assumed 20.95% O_2) was used to calibrate the so-called span of the meter. In 1997 we used a floating ball flow meter (Sho-Rate model 1355, Brooks Instrument, Veenendaal, The Netherlands) with an accuracy of 0.2 l/h (manufacturer's specification). These meter readings were adjusted to standard temperature and pressure. In 1998, mass flow controllers were used (model 5860S, Brooks Instrument) with an accuracy of 0.12 l/h (manufacturer's specification). Air flow rates through the respirometer chambers (volume 1.7 l) were, on average, 19 l/h in 1997 and, owing to deploying mass flow meters, 12 l/h in 1998. Because at night the birds were probably mainly assimilating fat, an RQ of 0.75 and an energy equivalent of the O_2 consumption rate of 19.83 kJ/l (Schmidt-Nielsen 1997) were assumed. As RMR value we took the lowest point from a stable period of measurements.

Ambient temperature and the temperature inside the respirometer chamber (T_{box}) were recorded continuously with thermistors (accuracy 0.2°C, manufacturer's specification). O_2-, flow- (in 1998 only) and temperature values were recorded every minute with a digital data logger (Squirrel 1259). The temperature inside the respirometer boxes could be increased by putting power on a high capacity resistor fixed to the lid on the inside of the box.

Unfortunately, skipping the calibration at the end of the respirometer measurements in 1998 resulted in incomparable data sets. This was caused by a non-linear drift of the meter output, probably due to temperature changes inside the oxygen analyser. In fact, the oxygen consumption measured in 1998 would be on average 11% lower when applying a correction factor calculated from the 1997 calibration data. The variation in this factor is, however, too large to make accurate corrections. The average correction factor corresponds well with the actual difference between years (9%, Table 5.1). Consequently, comparison of data from the two years is not possible. Since two females of an experimental triad were measured simultaneously, time related measurement biases were avoided.

Variation in nocturnal energy expenditure was analysed using residuals of RMR, fur-ther called RMR_{resid}, as measured at the high temperatures. These were calculated using backward stepwise regression analysis, entering variables for which we did not control fully in the experimental set-up, i.e., tarsus length, third primary length, respirometer box temperature, body mass and year. This resulted in a model that included box temperature and year as best predicting variables, with a r^2 of 0.35 (Table 5.1).

Table 5.1 Results of regression analysis with resting metabolic rate (W), or mass specific resting metabolic rate (W/g), as measured at the high temperatures, as dependent variable. T_{box} is the temperature inside the respirometer chamber (°C) and year has a value of 0 (1997) or 1 (1998).

	b ± SE	t_{41}	P
dependent variable: RMR			
Intercept	0.522 ± 0.074	7.02	< 0.0001
T_{box}	-0.00836 ± 0.00315	-2.65	< 0.05
year	0.0877 ± 0.0191	-4.61	< 0.0001
dependent variable: SRMR			
Intercept	0.0273 ± 0.0043	6.25	< 0.0005
T_{box}	-0.000369 ± 0.004365	-2.00	0.052
year	0.00393 ± 0.00019	-3.51	< 0.005

The fact that temperature was still affecting the metabolic rate means that the birds were not within the thermoneutral zone at all (high) temperatures. Neither the squared value of T_{box} (to check for a curvilinear relationship), nor the interaction between year and T_{box} added significantly to the model.

Mass specific RMR (W/g), further called SRMR, as measured at high temperatures, was also related to respirometer box temperature and varied between years ($r^2 = 0.24$, Table 5.1).

Values following the means and parameter estimates are the standard errors.

Results

Effects on broods

There were no differences in reproductive parameters between the manipulated pairs before the manipulation was performed (Table 5.2). After the manipulation, when the nestlings were 7 days old, the brood size was 6.2 in the reduced broods and 11.8 in the enlarged broods. The mass difference between enlarged and reduced broods was 79.5 g (74% of the reduced brood mass). Also in each separate year the brood masses differed (t -tests, 97: $t_{19} = -9.13$, $P < 0.0005$; 98: $t_{14} = -9.98$, $P < 0.0005$). More birds fledged from the enlarged broods, although mortality was higher in the latter (paired t-test: $t_{16} = -8.0$, $P < 0.0005$). On average 1.7 nestlings died between 0-14 days old (day of hatching = 0) in an enlarged brood, and 0.2 in the reduced.

The body mass of the individual nestlings, at 14 days old, was higher in the reduced broods than in the enlarged broods (Table 5.3). Wing and tarsus length was not significantly different. When comparing the data of the 2 years, no difference in the effect of the manipulation on body mass can be shown (ANOVA on mass differences and with year as factor: $F_{1,33} = 0.04$, $P = 0.8$).

Because the combined DLW and O_2 measurements meant elaborate handling of the birds we could imagine that their behaviour would be changed as a consequence, resulting in lower feeding rates and lower growth rates of their young. Therefore, the mass of these nestlings was compared with those of unmeasured parents. For young of unmeasured parents, a selection of the data with the same range of hatching dates and nestling numbers as the nestlings with measured parents was used. This selection yielded 74 broods, while 25 broods with a measured parent were available. The body masses of nestlings 14 days old were not significantly affected by the energy measurements of a parent (generalised linear model with mean nestling mass in brood as dependent factor and controlling for number of nestlings and date of hatch: $F_{1,95} = 1.79$, $P = 0.18$).

Table 5.2 Means and standard errors of various characters of the experimental broods in 1997 and 1998. Reduction and enlargement of broods was performed at nestling age of 1 day, and consisted of a transfer of three individuals. Test statistics resulted from paired *t*-tests. Only those broods are included of which also respirometry measurements were collected.

	reduced	enlarged	*t*_	*P*
hatching date (day in May)	17.9 ± 2.4	18.0 ± 2.4	-1.46	0.16
clutch size	9.9 ± 0.4	9.8 ± 0.4	0.32	0.75
hatchling number	9.3 ± 0.3	9.0 ± 0.4	0.86	0.40
nestling number (day 7)	6.2 ± 0.3	11.8 ± 0.4	-14.8	< 0.0005
brood mass (day 14; g)	106.5 ± 5.3	186.0 ± 7.4	-14.5	< 0.0005
number fledged	6.1 ± 0.3	10.3 ± 0.6	-7.98	< 0.0005

Table 5.3 Mean body measurements of nestlings, averaged per brood, at nestling age of 14 days, from enlarged and reduced broods. Experimental nests of which no energetic measurements were collected are included. Standard error of means between brackets. Statistics result from paired sample *t*-tests.

	reduced	enlarged	*t*	*P*
hatching date (day in May)	17.9 ± 2.4	18.0 ± 2.4	-1.46	0.16
clutch size	9.9 ± 0.4	9.8 ± 0.4	0.32	0.75
hatchling number	9.3 ± 0.3	9.0 ± 0.4	0.86	0.40
nestling number (day 7)	6.2 ± 0.3	11.8 ± 0.4	-14.8	< 0.0005
brood mass (day 14; g)	106.5 ± 5.3	186.0 ± 7.4	-14.5	< 0.0005
number fledged	6.1 ± 0.3	10.3 ± 0.6	-7.98	< 0.0005

Feeding rates

Feeding rates were measured in 1997. In order to avoid effects of age differences, a selection of pairs was made in which both broods of a pair were of exactly the same age before testing the difference (14 out of 17). The rates were found to be higher in enlarged broods both in females and in males (Table 5.4). The difference between the sexes in their reaction to the manipulation was tested by comparing the differences between reduced and enlarged broods in feeding rates between males and females (paired *t*-test: $t_8 = 1.19$, $P = 0.27$), and by testing the relative differences in feeding rates between reduced and enlarged broods (paired *t*-test: $t_8 = 1.08$, $P = 0.3$). Thus, no difference between the sexes was apparent, in accordance with previous studies.

Table 5.4 Average feeding rates with standard errors of means of female, male and both parents, with experimentally reduced or enlarged brood sizes. The rates were measured as the number of nest visits per hour and originate from single 3-h observations. The age of the nestlings at the measurement was on average 11.6 ± 0.12 days. The test statistics were based on paired sample *t*-tests.

category	reduced	enlarged	t_{13}	*P*
female	10.1 ± 2.88	22.8 ± 4.20	-6.72	< 0.001
male	12.4 ± 2.04	20.4 ± 2.82	-2.12	0.054
both parents	22.4 ± 2.76	43.2 ± 4.92	-4.54	< 0.01

DEE

Average daily energy expenditures over 24 h as measured with DLW of females with reduced or enlarged broods were 77.5 ± 3.6 kJ/d and 84.2 ± 3.5 kJ/d, respectively. A paired *t*-test showed an almost significant effect of the brood size manipulation ($t_9 = -2.12$, $P = 0.063$, Figure 5.1, Table 5.5). Body masses of the birds did not differ between the two categories (Table 5.5).

RMR and mass specific RMR

Because at a certain point we changed the order of DLW and oxygen consumption measurements (see methods section), we first tested whether this had affected the outcome of RMR. This does not seem to be the case (generalised linear model, controlling for T_{box}: $F_{1,12} = 0.45$, $P = 0.51$).

RMR_{resid} (see Table 5.1) were used for the pairwise comparison of the two manipulation categories (Figure 5.2, Table 5.5). The paired RMR_{resid} did not differ significantly (mean reduced = 0.00231 ± 0.0107 W, enlarged = 0.00193 ± 0.00888 W, $t_{16} = 0.04$, $P = 0.97$). The average of both residuals does not equal zero, because they were calculated using a more extensive data set. The average RMR which the regression model (Table 5.1) predicts, adjusted to the average values of 1997, are 0.347 ± 0.0078 W and 0.353 ± 0.0087 W, respectively (Table 5.5). Also in each separate year there was no

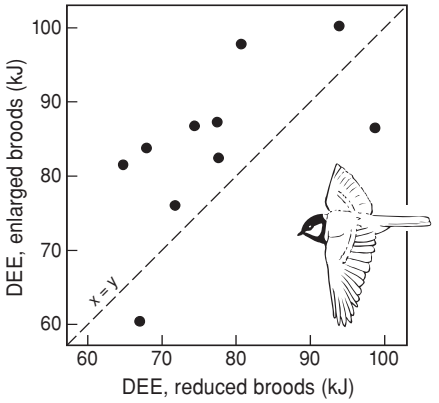

Figure 5.1 Daily energy expenditure of females tending reduced or enlarged broods with nestling of 11 days old. Each dot represents data of two females of a manipulation pair. The dotted line represents the line of equality.

Table 5.5 Results of energy measurements on adult female great tits tending a reduced or enlarged brood. RMR_{pred} stands for RMR adjusted to the year 1997 and adjusted for respirometer box temperature. All other abbreviations are explained in the text. All test statistics were based on paired sample *t*-tests.

category	reduced	enlarged	*t*(df)	*P*
DEE (kJ/d)	77.5 ± 3.6	84.2 ± 3.5	-2.12 (9)	0.063
RMR_{pred} (W)	0.347 ± 0.0079	0.353 ± 0.0088	-1.17 (16)	0.3
RMR_{resid} (W)	0.00231 ± 0.0107	0.00193 ± 0.00888	0.04 (16)	1.0
$SRMR_{resid}$ (W/g)	$3.07 \times 10^{-4} \pm 6.20 \times 10^{-4}$	$-9.20 \times 10^{-5} \pm 5.81 \times 10^{-4}$	0.57 (16)	0.6
EE_p (kJ)	11.7 ± 0.79	12.2 ± 0.79	-0.53 (6)	0.6
EE_α (kJ)	64.1 ± 4.0	72.0 ± 5.5	-2.52 (6)	0.045
body mass (g)	17.5 ± 0.20	18.0 ± 0.22	-1.47 (16)	0.16

difference (97: t_7 = -0.50, P = 0.6, 98: t_8 = 0.20, P = 0.8). A power analysis shows that the difference between the two groups could maximally be 0.034 W (α= 0.05, power = 0.80, SE of difference = 1.113, n = 17; Buchner *et al.* 1997). This is about 11% of BMR.

The paired residuals of $SRMR_{resid}$ (see Table 5.1) did not differ either. The mean value for the reduced broods was $3.07 \times 10^{-4} \pm 6.20 \times 10^{-4}$ W/g, and for the enlarged $-9.20 \times 10^{-5} \pm 5.81 \times 10^{-4}$ W/g (t^{16} = 0.57, P = 0.6; Table 5.5). Also in each separate year there was no difference (97: t_7 = -0.17, P = 0.9, 98: t_8 = 0.79, P = 0.5).

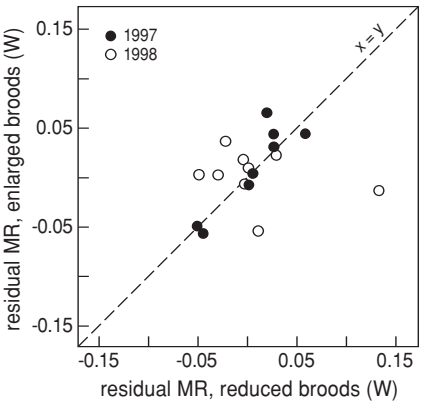

Figure 5.2 Residual metabolic rates of adult female great tits during the nestling period. Residuals result from a linear regression with minimum metabolic rate as dependent variable and the temperature of the respirometer box and year as predicting variables. Each dot represents a paired sample of a bird attending a reduced or an enlarged brood.

Day and night time metabolism

Even if RMR is unchanged, the energy expenditure over longer periods may differ, e.g., due to an earlier decline, or delayed rise of metabolic rate. Therefore, we also analysed variation in the amount of energy expended during the entire resting phase. We did so by using the metabolism measurements at the low temperatures (in 1997 only), which are very close to ambient temperature; T_{box} was on average 1.3 ± 0.52°C higher than the actual ambient temperature. A correction was made to predict RMR at the ambient temperature, according to the next equation:

$$EE_{\rho} = RMR \times (T_{body} - T_{a\rho})/(T_{body} - T_{box}) \times 3.6 \times 8.34$$

This equation assumes a constant heat conductance value and body temperature (Scholander curve). Body temperature (T_{body}) was set at 39.42°C (average of 18 cloacal measurements), $T_{a\rho}$ is the ambient temperature during the dark period of the DLW measurement, and T_{box} is the temperature in the respirometer box when RMR was measured. The factor 3.6 is applied to convert Watts to kJ/h, and this was multiplied by 8.34, the average length of the inactive period in hours (Tinbergen & Dietz 1994). This yields a value for the total amount of kJ spent during the night. Subtracting this from the DLW measurement of DEE gave an estimate of the amount of energy spent during the active period (EE_{α}). EE_{ρ} is a very conservative estimate because the birds will for part of the night have a metabolic rate above the resting value, e.g. due to heat increment of feeding.

The total energy expenditure during the night was not correlated with the energy expenditure during the day (r = -0.35, n = 16, P = 0.18; Figure 5.3). In a paired test

the day- and night-time energy expenditure of females rearing reduced or enlarged broods were compared. After selection of the paired samples (i.e., with both DLW and O_2 measurements performed without problems) seven pairs remained. EEρ did not differ between the manipulation categories ($t6$ = -0.53, P = 0.6), while EEα did ($t6$ = -2.52, P = 0.045; Table 5.5). EEα of females with reduced broods was 64.1 ± 4.0 kJ, and with enlarged broods 72.0 ± 5.5 kJ. EEρ of females with reduced broods was 11.7 ± 0.8 kJ and with enlarged broods 12.2 ± 0.79 kJ.

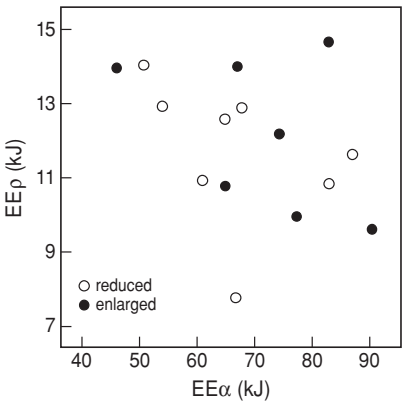

Figure 5.3 Relation between energy expenditure during the active phase (EE_α) and the resting phase (EE_ρ). Active phase expenditure is calculated as the DLW data minus an estimate of nocturnal energy expenditure based on the oxygen measurements and the air temperature.

Discussion

The average DEE values measured here are 12% lower than those measured in great tits in the same area in 1995 by Sanz & Tinbergen (1999). They found that female great tits with nestlings of 12 days old, rearing reduced broods, expended on average 86.3 ± 4.95 kJ/d (n = 5) and when rearing enlarged broods they expended 98.4 ± 6.36 kJ/d (n = 4). In our study the expenditure of females with reduced and enlarged broods was, respectively, 77.5 kJ and 84.2 kJ. We tested whether the difference between the two studies was significant, using a general linear model, with DEE of the reduced and enlarged broods treated as repeated measures, and with the two studies entered as factor. It showed that the results from the two studies do not differ significantly ($F_{1,12}$ = 3.26, P = 0.096), and that the overall effect of manipulation is significant ($F_{1,12}$ = 8.97, P = 0.011). The average DEE of both studies combined are 79.54 ± 3.07 for the reduced, and 88.26 ± 3.47 for the enlarged broods (n = 14).

The average feeding rates of females reported by Sanz & Tinbergen (1999) from the same area in 1995 were higher, i.e., 19.7 ± 3.0 (n = 10) and 34.7 ± 2.7 (n = 9) visits

per brood per hour for reduced and enlarged broods, respectively. In this study it was 10.1 ± 2.9 (n = 13) and 22.8 ± 4.2 (n = 13) visits per brood per hour (t-test, reduced: t_{21} = 2.27, P = 0.03, enlarged: t_{20} = 2.15, P = 0.04). Therefore, the tendency to lower DEE values found in this study may be the result of lower feeding rates. The most obvious causes for the lower feeding rates are food-related factors and temperature differences.

The average RMR we measured at high temperatures in 1997 was 0.325 ± 0.011 W (n = 24). Because, at least, some values were measured below the thermoneutral zone, BMR will be lower. We could only find one study (Hissa & Palokangas 1970) giving RMR values of great tits. They showed that the thermoneutral zone starts above 28°C, and that the metabolic rate, in summer, at temperatures between 29 and 33°C, is 0.37 W. This value is considerably higher than ours, more so considering the higher temperatures at which they measured. The difference may be related to the higher latitude of their study site (60 ± 310 vs 53 ± 060). BMR according to allometric predictions of Kendeigh *et al*. (1977) is 0.307 ± 0.003 W (n = 41), for a 17.3 g passerine bird in the resting phase in summer. The mean of our measured value of RMR is not different from the predicted value (t-test, t_{63} = 1.95, P = 0.056).

As a result of the brood size manipulation, the parent birds had to feed more nestlings in the enlarged broods (Table 5.2). Although the growth of the nestlings was negatively affected by the brood size change (Table 5.3), the rate at which the nestlings were fed by the female parent was 2.3 times higher in the enlarged broods (Table 5.4). As was the purpose of our experiment, parents with enlarged broods put more effort, measured as visits per hour, in feeding their young.

In accordance with the increased provisioning rate, DEE was 1.086 times (i.e., 6.7 kJ/d) higher in female parents with enlarged broods (Figure 5.1). No effect on metabolic rates, either RMR (Figure 5.2) or mass specific RMR, and on night-time energy expenditure (Figure 5.3) could be shown. Evidence for compensation for elevated energy output during the working period is therefore lacking.

Correlative analyses do not give any indication of reduced RMR in response to higher feeding efforts either. There was no correlation between the feeding rates of the females and their resting metabolic rate (r = 0.42, n = 20, P = 0.07). Although this value is close to significance, omission of a single point, with a very high feeding rate, weakens the correlation considerably. Temperature corrected SRMR is positively correlated with the feeding rate of the females (r = 0.45, n = 20, P = 0.045). But when a single outlier is omitted from the analysis it is not significant anymore (r = 0.22, n = 19, P = 0.4).

In spite of the fact that the feeding rates of the females are positively related to DEE (regression coefficient = 30.0 ± 11.3, t_{19} = 2.64, P = 0.016, where feeding rate is expressed in number per minute and DEE in kJ), the relationship between EE_ρ and feeding rate is not significant (t_{14} = 0.88, P = 0.4), while it is almost significant for EE_α (regression coefficient = 28.5 ± 14.2, t_{14} = 2.01, P = 0.064).

The lack of evidence for nocturnal energy savings in the great tit could be related to the small scope there may be for any substantial savings on a daily basis because

nights lasted only 8.5 h. Zebra finches reduced RMR by 18% when faced with a high workload and, all else being equal, and assuming BMR is reduced during the resting phase only, this would yield a saving of 2.8-4.2% on a daily basis (depending on the estimate of DEE used; Deerenberg *et al*. 1998). Starlings faced with a high workload reduced their BMR by 35.4%, which would yield a reduction of 9.5% on a daily basis (Bautista *et al*. 1998). The working period was 14 h in the zebra finches and only 8 h in the starlings, which explains part of the outcomes in total savings in the two studies. If the BMR reduction were applicable during the whole day, DEE would be, respectively, 8.4-12.7% and 16.2% lower.

When applying these figures to great tits, reducing RMR during the resting phase, would result in a 2.3% (zebra finch) to 4.6% (starling) reduction of DEE. Assuming that the BMR reduction is applicable during the whole day, these values are 6.5 and 12.8%, respectively. These reductions are in all cases below those estimated in zebra finches and starlings, but overall are fairly similar. Hence, the lack of scope for energy savings does not seem to be the explanatory factor. The most influential factor in reducing DEE in the zebra finches was apparently the decrease in activity during the day, when not foraging. Perhaps this is not a feasible option for the wild great tits tending a brood. The starling data show that substantial savings can be made through a reduction of RMR. However, these birds had also reduced their body mass considerably, namely by 18%. The decline in body mass in the zebra finches was only 3%. The great tits did not show a difference in body mass between the manipulation categories (17.5 ± 0.20 vs 18.0 ± 0.22 g for reduced and enlarged broods, respectively; Table 5.5).

Another cause for a lack of compensation in great tits could be that their intensity of energy output, i.e. scaled to their species specific BMR, was lower than the values in the other two studies. BMR of zebra finches is 0.21 W (own measurements), of great tits 0.31 W, or somewhat lower (this study), and of starlings 0.76 W (starlings in 'easy' condition in Bautista *et al*. 1998). The DEE values of these species in the three studies were ca. 2.8, 2.9 and 2.5xBMR, respectively. These values are very close and, therefore, it cannot be the case that differences in DEE, corrected for BMR, give rise to the difference in results.

It is not immediately clear what causes the difference in results between this study and the studies of Bautista *et al*. (1998) and Deerenberg *et al*. (1998). It is possible that the lab studies suffer from unnatural feeding conditions. The set-up of the systems was such that the birds did not experience any random variation in feeding success. This may result in cognitive constraints ('demotivation'), which would reduce foraging activity (Fotheringham 1998). If this is actually the case then those birds were starved, and starvation may lead to mass reduction of metabolic active tissue, and subsequently to a decline in BMR (Daan *et al*. 1989; Piersma *et al*. 1996a).

Another possible cause which may explain the lack of any effect on BMR of the brood size manipulation could be the effect of the annual phase the birds were in. During the reproductive phase, other decisions, including physiological ones, may be taken, because fitness costs of lowering RMR may vary.

There is also the possibility that the time allowed for accommodation to the hard-working situation was too short in the great tit experiment. The manipulation was performed when the nestlings were 2 days old and the measurements were done 10 days later. In the experiments of Deerenberg *et al*. (1998) and Bautista *et al*. (1998) the birds had more time to accustom themselves to the work levels to which they were submitted, i.e. at least 3 weeks.

We conclude that there are no indications that free-living great tits save energy by reducing their nocturnal energy expenditure in response to an increased energy expenditure after brood enlargement. The use of DEE as an estimate of parental work load in field studies remains valid.

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Chapter 6

Nocturnal metabolic rate and reproductive success in zebra finches

Popko Wiersma & Simon Verhulst

Abstract

We tested the hypothesis that there is a positive relationship between standard metabolic rate (SMR) and reproductive success using captive zebra finches *Taeniopygia guttata*. This hypothesis is based on the assumption that individuals that work harder can be expected to provision their offspring at a higher rate, and the earlier finding that SMR and work rate are positively associated. Independent of mass, males with high SMR were more likely to start breeding, and high male SMR was further associated with early laying and large clutch size. Female SMR was not correlated with breeding, laying interval or clutch size. We further measured body mass and hematocrit before and during breeding as potential physiological traits that may determine individual performance. Pre-breeding hematocrit was negatively associated with SMR and mass in males, opposite to most other species studied, but probability of breeding and clutch size were independent of hematocrit and mass in both sexes. Brood size has previously been shown to affect daily energy expenditure, and we manipulated brood size to two or six young to test whether birds would adjust their SMR. Parents did not adjust SMR or hematocrit significantly to brood size, although both increased (effect sizes 0.2-0.39). Thus our hypothesis was supported on a correlational level, but only weakly by the experiment.

Introduction

The hypothesis that energy expenditure may set a limit to reproductive output is intuitively plausible, because individuals that work harder can be expected to provision their offspring at a higher rate (Williams & Vézina 2001). It is thought that the maximum sustained or peak rate of energy expenditure is functionally linked with the basal metabolic rate, or BMR (Drent & Daan 1980; Peterson *et al*. 1990; Daan *et al*. 1991). This hypothesis is supported by experimental effects of induced changes in daily energy expenditure on BMR (Gelineo 1964; Arieli *et al*. 1979; Daan *et al*. 1989; Speakman & McQueenie 1996; Williams & Tieleman 2000). The link between BMR and maximum sustainable energy output can be understood considering that BMR is a reflection of the size of the 'metabolic machinery', i.e. organs such as heart, kidney, liver and intestines (Daan *et al*. 1989; Daan *et al*. 1990; Piersma *et al*. 1996a).

Although it seems plausible that there is a positive association between BMR and reproductive effort and, consequently, reproductive success, this has been little studied. In mammals there is some evidence for associations between metabolic rates and life-history variables on the interspecific level (Glazier 1985; McNab 1986b; Stephenson & Racey 1995), although other studies failed to find such a pattern (McNab 1986a; Read & Harvey 1989; Harvey *et al*. 1991; Stephenson & Racey 1995). Intraspecific studies of mammals failed to reveal any association between BMR and aspects of reproduction (Derting & McClure 1989; Earle & Lavinge 1990; Hayes *et al*. 1992; Stephenson & Racey 1993a; Stephenson & Racey 1993b; Johnson *et al*. 2001b). In birds no interspecific associations between BMR and life history traits have been found so far (Padley 1985; Trevelyan *et al*. 1990). However, Nilsson (2002) recently found a positive relationship between manipulated nestling provisioning rate and mass-corrected BMR in female marsh tits *Parus palustris*.

In this paper we test the hypothesis that individuals with a high standard metabolic rate (SMR) have a higher reproductive success. (We refer to SMR instead of BMR because we measured MR at temperatures just below the lower critical temperature.) First we measured SMR of captive zebra finches *Taeniopygia guttata* before the onset of reproduction, and investigated how pre-breeding SMR was associated with aspects of reproductive success. Female zebra finches increase their DEE in response to an increase in brood size (Deerenberg 1996), and SMR may also increase in response to increased demands of the brood (Johnson *et al*. 2001b; Nilsson 2002). To test this hypothesis we manipulated brood size, and measured SMR late in the nestling phase. In addition to SMR we measured body mass and hematocrit, because they are functionally associated with energy expenditure (Carpenter 1975; Hammond *et al*. 2000).

METHODS

Animals and housing

This study was carried out under license 2281 of the Animal Experiments Committee of the University of Groningen. A total of 78 wild-type, captive zebra finches were initially used in the experiment. All birds were at least two years of age. The birds had a blue or black plastic, numbered leg ring. They were housed in cages of 40x80x40 cm (hxwxd). Pairs were formed between June 2 and 6. Room temperature was on average 25°C, ranging from 23 to 27°C. Lights went on at 8:00 and off at 22:00, i.e. a LD cycle of 14:10 h. Food and water were present *ad libitum*. Food consisted of a mixture of dry seeds, and a teaspoon of fortified canary food was given 3 times per week as source of complementary nutrients, until completion of the clutch.

Chronology

Chronologically, the experiment was performed as follows: pair formation was followed, within 12 days, by pre-breeding metabolic, body mass and hematocrit measurements. A nest box and nesting material was offered 20 days after pair formation and brood size was manipulated when chicks were 1-3 days old. Then breeding metabolic rate, body mass and hematocrit measurements were taken when nestlings were 14-15 days of age (age estimation was accurate to ca. 0.5 d), and nestling size was measured when on average 20.7 d old.

Manipulation

The nest boxes were checked daily for nest building and eggs. A clutch was considered complete when no more eggs were laid on three consecutive days. After completion of the clutch, the eggs were checked regularly for hatching. Broods were manipulated when the chicks were 1-3 days old. Broods of two and six nestlings were created. In total 10 broods were formed having two nestlings and nine broods with six nestlings. The other pairs failed to produce a nest, eggs or young. Original and manipulated brood size were not correlated (r = -0.086, P = 0.73).

Measurements

Metabolic rates were measured by indirect calorimetry, using rate of oxygen consumption and carbon dioxide production. Bird were kept in a 1.7 l Plexiglas box, with a perch and drinking water, but without food, inside a climate room. Dry air was pumped through the boxes at a rate of 20 l/h. The air was dried over a molecular sieve (3Å, Merck, Darmstadt, Germany). Flow rates were measured with mass-flow controllers (Brooks, type 5850S, Veenendaal, The Netherlands). Oxygen concentration was measured with a paramagnetic analyser (Servomex, type Xentra 4100), as was the carbon dioxide concentration (Servomex, type 1440). We measured 8 birds per night, and each bird was measured for 1 min at 9-min intervals. The inlet air was also measured every 9 min. Oxygen consumption was calculated from the volume of dry air entering the respirometer box and the fractions of oxygen and carbon dioxide in the incoming

and outgoing dry air. Metabolic rate was calculated using the, RQ dependent, conversion factor for the oxygen consumption as given by Brody (1945). RQ was on average 0.726 ± 0.002 (±SE) and did not differ between sexes ($t_{77} = 0.52$, $P = 0.6$).

Metabolic rate was measured 1-3 weeks before offering nesting material (pre-breeding) and when nestlings were 14-15 days of age (breeding). The birds were put in the respirometer at 17:30 (pre-breeding) or 21:00 (breeding) and taken out at 8:00. A later starting time was used for breeding birds to minimise consequences for the chicks. Unfortunately, the different starting times impede direct comparisons between first and second measurement. Lights were off between 18:00 and 7:00. The chamber was regulated at two temperatures during the measurements: first the temperature was similar to the temperature inside their home cage, i.e. 25°C, to obtain estimates of their metabolic rate in the nest cages. These values were not used in this study. Between 22:30 and 3:30 the temperature was increased to on average 32.2°C. Metabolic rates were estimated by taking the average value between 00:15 and 3:30. We estimated that the food retention time in the digestive tract was ca. 1 h (Karasov 1990) and therefore assumed that the birds were post-absorptive at the time of measurement. Although we aimed to measure BMR (i.e. metabolic rate in the thermoneutral zone), there was still an effect of temperature on metabolic rate (Table 6.1). This was unexpected, because Calder (1964) reported that the lower critical temperature of zebra finches was 29.5°C. There was no indication that part of the measurements had been in the thermoneutral zone, because temperature squared did not have an additional effect in the model ($F_{1,72} = 2.66$, $P > 0.1$). In the statistical analyses we therefore controlled for ambient temperature, and we refer to our measurements as standard metabolic rate (SMR). One SMR value of the breeding measurements was considered an outlier (this value was 4.9 standard deviations above the mean), and this value was omitted from further analyses. This bird was probably active during large part of the night.

Body mass (resolution 0.1 g) was measured before each respirometer measurement. High mass can be due to high mass of nutritional stores, organs and muscles, and to large structural body size, and we therefore examined the effect of these variables separately. We used tarsus length (length tibiotarsus; resolution 0.1 mm) as a measure of size, and the residual of the regression of mass on tarsus as a measure of nutritional stores, muscle and organ sizes. Both before and during breeding, mass and tarsus were strongly related (before breeding: mass = -0.63 (±5.44) + 1.18 (±0.38) x tarsus, $F_{1,81} = 8.13$, $P < 0.01$, sex and interaction sex x tarsus: $F_{1,81} > 0.73$, $P > 0.1$; during breeding: mass = -13.92 (±7.22) + 2.01 (±0.52) x tarsus, $F_{1,43} = 16.3$, $P < 0.001$, sex and interaction sex x tarsus: $F_{1,43} > 0.52$, $P > 0.1$). Tarsus length squared or wing length (squared) did not significantly improve the regressions.

Hematocrit was measured ca. 10 h prior to the respirometer measurement. A blood sample was collected in two heparinised capillaries by puncturing the brachial vein with a needle. Capillaries were centrifuged for 10 min at 10000 rpm and height of the blood column, packed red blood cells and white blood cells was measured with a magnifying glass and digital sliding callipers to the nearest 0.1 mm. Hematocrit and buffy

coat thickness were calculated as the percentage of red and white blood cells, respectively, of the total blood column.

As measures of reproductive output we used laying incidence, i.e. whether breeding started at all, laying interval, clutch size, hatching incidence, i.e. whether one or more eggs hatched, and average nestling mass. Laying interval was defined as the time between receiving a nestbox and laying the first egg. Nestling size (mass, wing and tarsus length) was measured at an age of 20.7 ± 0.2 (±SE) d.

Statistical analyses

Although pairs were randomly created by us, due to accidental assortative mating, the pre-breeding size (tarsus and wing length, body mass and residual mass) and SMR of birds forming pairs could have been correlated, but this was not the case (all $r < 0.34$, all $P > 0.09$). Also during breeding the change in body mass, SMR and hematocrit were not correlated within pairs (all $P > 0.34$). Pair members were therefore statistically treated as independent values in the analyses. Chick development was analysed using hierarchical linear models (MLwiN version 1.10), with brood on the second level (Rabasch *et al*. 2000). In these models, *P*-values were based on the X^2-distributed changes in deviances. Models were selected using backwards deletion of the least significant term.

When experimental effects are expressed as 'effect size' we refer to Cohen's standardised effect size (Cohen 1988), which for a comparison of two groups is the difference between the means, divided by the mean standard deviation.

RESULTS

Pre-breeding

Before analysing the relationships between reproduction and the different state parameters (tarsus, residual mass, SMR and hematocrit), we briefly describe metabolic rates and the correlations between state parameters for the two sexes measured before the start of reproduction. Female SMR was 10.3% higher than male SMR (Table 6.1). At 32°C, the averages were 256 ± 5.7 mW for females and 232 ± 5.7 mW for males. Females tended to be heavier than males (females: 16.4 ± 0.32 g, $n = 39$; males: 15.6 ± 0.34, $n = 39$; $t_{76} = 1.59$, $P = 0.1$), but the effect of sex on SMR remained significant when tarsus length and residual mass were controlled for statistically (Table 6.1). To our knowledge only a few other studies found a differences between the sexes in BMR or SMR when mass was controlled for (Daan *et al*. 1989; Hammond *et al*. 2000).

Males with high hematocrit had low residual mass and low SMR (Figure 6.1). We corrected for the effect of residual mass in a partial correlation and the negative correlation between SMR and hematocrit remained significant ($r = -0.36$, $n = 36$, $P = 0.029$). However, the correlation of hematocrit with residual mass did not persist when controlling for SMR (partial correlation: $r = -0.14$, $n = 36$, $P = 0.39$), indicating that hematocrit was related to SMR and not to residual mass. Female hematocrit was

Table 6.1 Result of GLM analysis of pre-breeding measurements of SMR (mW) of 78 individuals in relationship to temperature, body mass, tarsus length and sex.

source	coefficient	*F*	*df*	*P*
intercept	1173 ± 335	12.5	1	< 0.001
temperature (°C)	-35.24 ± 10.32	11.67	1	< 0.005
sex (female=1, male=0)	19.70 ± 6.84	8.29	1	< 0.01
tarsus length (mm)	13.85 ± 5.64	6.03	1	< 0.05
residual mass (g)	9.22 ± 1.74	28.03	1	< 0.001
error			73	
total			78	

Note. Shown are parameter coefficient estimates ±SE. The r^2 of the model was 0.48.

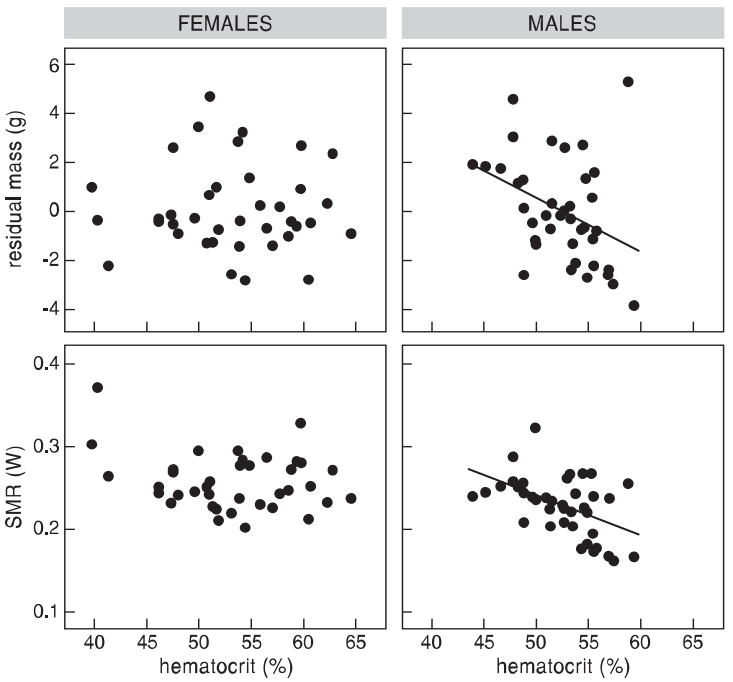

Figure 6.1 SMR (W) and residual mass (g) in relation to hematocrit in male and female birds prior to reproduction. Residual mass is the residual of the regression of body mass with tarsus length. Lines depict the statistically significant relationships. Male residual mass: r = -0.38, n = 39, P < 0.02; male SMR: r = -0.49, n = 39, P < 0.002; female residual mass: n = 38, P > 0.99; female SMR: n = 38, P = 0.15.

not correlated with residual mass or SMR (Figure 6.1), nor with the interaction term of both variables ($P = 0.31$).

Reproduction

Pairs of which males had a high pre-breeding SMR had more eggs in their nest (Figure 6.2). Tarsus length and residual mass, entered either singly or simultaneously could not explain this variation (Table 6.2; $t_{37} < 1.2$, $P > 0.22$). This regression includes clutch sizes of zero, because not all birds started breeding when they were given a nestbox. (We define breeders as pairs that had at least one egg in the nest.) SMR was higher in breeding males than in non-breeders (difference of 30 mW, or 15%; $t_{37} = 2.79$, $P < 0.01$). This was not due to confounding effects of size or mass, because tarsus and residual mass did not differ between breeders and non-breeders ($t_{37} < 1.09$, $P > 0.25$). We also analysed the variation between breeders and non-breeder using multiple logistic regression (with parameters hematocrit, pre-breeding SMR, residual mass and tarsus length of both sexes), and this yielded a model with only male pre-breeding SMR. Also among breeders there was a positive correlation between male pre-breeding SMR and clutch size ($r = 0.49$, $n = 27$, $P < 0.01$), and males with high pre-breeding SMR had short intervals until the start of laying (Figure 6.2). Tarsus length and/or

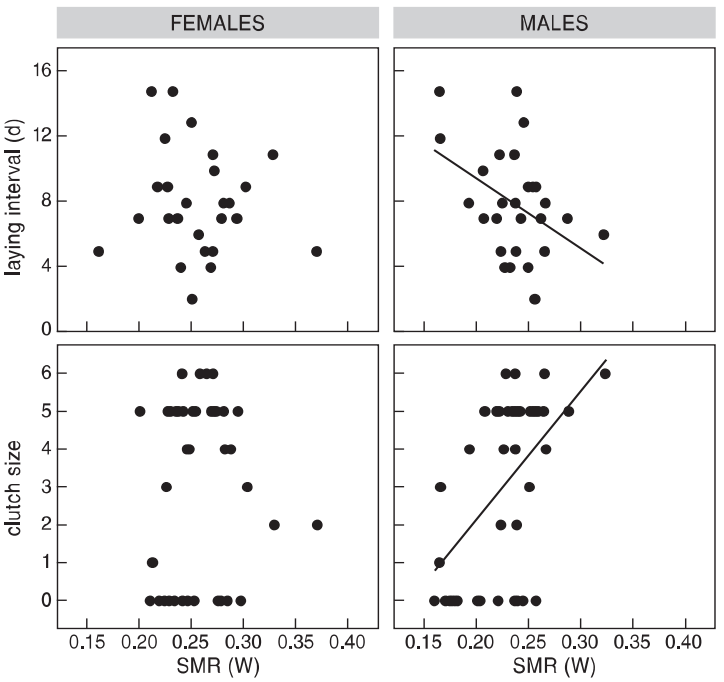

Figure 6.2 Laying interval (days between receiving a nestbox and the day the first egg was laid), and clutch size in relation to SMR measured prior to reproduction. Lines depict the significant relationships.

residual mass did not explain variation in laying interval. No effects of pre-breeding SMR on hatching success was found in either sex, but females with high pre-breeding residual mass and small tarsus were more likely to hatch their young. Female or male state parameter were not correlated with nestling mass (controlling for nestling age and brood size).

Females with high hematocrit had a longer interval until the start of laying. This increase was restricted to part of the range (Figure 6.3), and significantly more variation was explained when hematocrit squared was included in the model ($r^2 = 0.43$, hematocrit: $P < 0.04$, hematocrit^2: $P = 0.02$). Minimum laying interval was found at a hematocrit of approximately 47%.

Table 6.2 Associations between metabolism, body size, residual mass and hematocrit of male and female parents prior to the start of reproduction with parameters of subsequent reproductive behaviour and success.

		clutch size		laying interval (d)		eggs hatched?		nestling mass (g)	
	mean (±SE)	3.2 ± 0.4		7.9 ± 0.6		0.66		10.1 ± 0.3	
	sample size	39		27		27		17	
sex	variable	parameter	*t*	parameter	*t*	parameter	*t*	parameter	*t*
female	tarsus		-0.97		1.53	-2.79 ± 1.35	7.52**		1.26
	res. mass		0.32		-0.55	0.96 ± 0.50	5.80*		-1.17
	hematocrit		-0.46	0.214 ± 0.097	2.21*		1.11		1.96
	SMR		0.03		-0.2		1.26		1.18
male	tarsus		-1.66		1.12		1.22		1.78
	res. mass		-1.12		-0.74		0.01		-0.27
	hematocrit		0.35		-0.22		0.07		0.46
	SMR	35.09 ± 8.97	3.91***	-44.42 ± 18.23	-2.44*		1.12		0.18

Note. Parameter, *t* and *P*-values are based on backward stepwise linear or logistic regressions, performed for females and males separately. Only values of significant parameters are printed. Non-significant *t*-values were calculated by entering parameters singly in the final model. Also shown are the averages and their standard errors of the reproduction variables. Clutch size include clutches of 0. We checked whether the residuals of the regression with clutch size and male SMR deviated from a normal distribution, but this was not the case ($P = 0.7$). 'eggs hatched?' is a binary variable indicating whether at least one egg hatched. Analyses of nestling mass were performed with the per-brood mean of the age and brood size controlled nestling mass just prior to fledging.

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

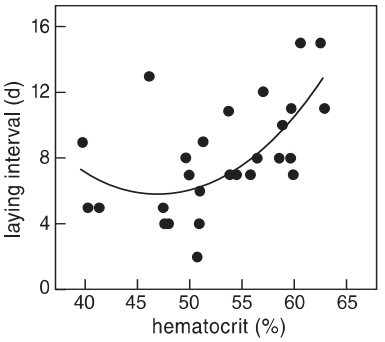

Figure 6.3 Female hematocrit and the laying interval (days between receiving a nestbox and the day the first egg was laid). The line shows the significant relationship with hematocrit and hematocrit^2.

Breeding

Parents seemed to be constrained in their chick feeding effort when brood size was large, since nestlings in broods of six chicks were 1.68 ± 0.31 g lighter than nestlings in broods of two ($X_1^2 = 13.49$, $P < 0.0005$). Wing length of nestlings in large broods was also reduced (-2.42 ± 0.86 mm, $X_1^2 = 7.48$, $P < 0.01$), while tarsus length was not affected (-0.30 ± 0.17 mm, $X_1^2 = 1.32$, $P = 0.25$). In four of the large broods and in none of the small broods, a nestling died (age 2-5 d), but these numbers are too low to allow statistical testing.

When investigating effects of brood size on parental mass and SMR we calculated the within-individual change between the pre-breeding measurement and the breeding measurement to control for individual variation (Table 6.3). All breeding birds became lighter (Figure 6.4), but birds rearing large broods lost 1.06 ± 0.49 g more than birds

Table 6.3 Differences in condition, SMR and hematocrit in parents with small or large brood and the change therein from pre-breeding to breeding.

	female		male	
	reduced	enlarged	reduced	enlarged
body mass (g)	13.33 ± 0.58 (8)	13.78 ± 0.55 (9)	13.74 ± 0.31 (10)	12.64 ± 0.50 (9)
change (g)	-2.08 ± 0.27 (8)	-3.33 ± 0.53 (9)	-2.28 ± 0.57 (10)	-3.16 ± 0.51 (9)
residual mass (g)	0.32 ± 0.29 (8)	0.11 ± 0.49 (9)	0.42 ± 0.34 (10)	-0.86 ± 0.32 (9)
relative SMR change[a]	-10.8 ± 14.2 (8)	-9.7 ± 18.7 (7)	-10.9 ± 15.9 (8)	-1.9 ± 12.9 (9)
hematocrit (%)	51.0 ± 1.3 (10)	53.3 ± 1.9 (9)	47.9 ± 1.3 (10)	47.8 ± 1.3 (9)
change (%)	-0.9 ± 2.2 (10)	0.5 ± 2.4 (9)	-4.7 ± 0.9 (10)	-3.7 ± 1.5 (9)

Note. Shown are parameter values ±SE with sample size given in parentheses. The individual change in body mass is identical to the change in residual mass, which is therefore omitted.
aThe absolute values of SMR changes were overestimated due to a change in the time of start of the measurement. SMR values were corrected for temperature variation.

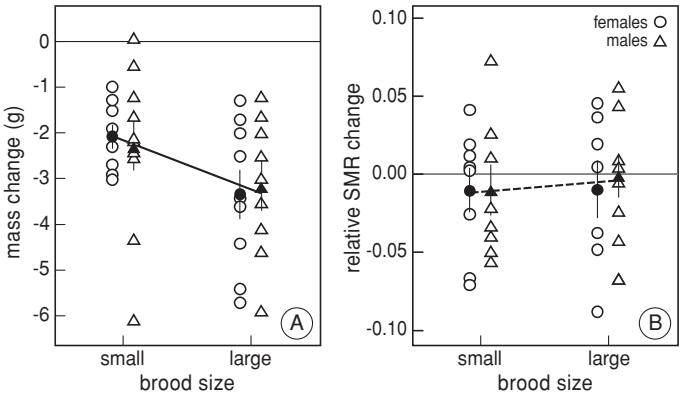

Figure 6.4 Change in SMR and residual mass in female and male individuals from before reproduction until when the chicks were 14-15 d of age, plotted against the manipulated brood size. The absolute values of the SMR changes could not be calculated, but comparisons between categories are valid. The black dots depict the average values of both females and males with their associated standard error. Only for residual mass the relationship is significant.

rearing small broods ($t_{34} = 2.17$, $P = 0.037$), in agreement with the result of Deerenberg (1996). Mass change did not differ between the two sexes. The change in SMR was not significantly associated with brood size (Figure 6.4; controlling for sex, $F_{1,29} = 1.13$, $P = 0.30$), although effect size was reasonable at 0.39. Controlling for mass change did not alter this result.

The change in hematocrit between the pre-breeding and breeding was independent of manipulated brood size (controlling for sex, $F_{1,34} = 0.46$, $P = 0.50$). Hematocrit decreased from pre-breeding (51.8 ± 0.8%) to breeding (47.9 ± 0.9%) in males (paired *t*-test: $t_{19} = 4.66$, $P < 0.001$), but not in females (51.9 ± 1.4% to 52.6 ± 1.1%; paired *t*-test: $t_{18} = 0.41$, $P = 0.68$). In the non-breeders there was no change in hematocrit in either sex (both $P > 0.24$). The sex difference in response to breeding was significant (interaction sex and breeding incidence: $F_{1,48} = 4.49$, $P = 0.039$; model included main effects).

Discussion

We aimed to test the hypothesis that a positive relationship exists between SMR and reproductive success. This hypothesis was based on the assumption that individuals that expended more energy could potentially provide their offspring with more resources, and the positive association between SMR and daily energy expenditure in comparative studies. Our results are mixed, in the sense that clear effects were found one-way only: experimentally increased reproductive effort had no significant effect on SMR, but we found that there were significant correlations between male SMR and the probability that a pair started laying, clutch size and the laying interval (Figure 6.2). To

our best knowledge, this is the first study to demonstrate an association between pre-breeding nocturnal metabolic rate and aspects of reproduction in birds. The associations with SMR were independent of mass, because in the regression models (Table 6.2) tarsus length and residual mass did not explain additional variation, and SMR remained significant when mass was added to the model.

Female zebra finches increased daily energy expenditure with increasing brood size (Deerenberg 1996), but we found no significant effect of brood size manipulation on SMR in either sex (Figure 4). Effect size of this difference in SMR was intermediate at 0.39, but the statistical power to detect such an effect was only 0.22, which is rather low. Given the sample size, an effect size of 1.03 (53 mW) was detectable with 80% power, suggesting that if an effect of brood size on SMR exists it is likely to be smaller than 53 mW, i.e. ca. 20% of SMR. Although the effect that we found was not significant, it was of similar magnitude as the effect size of 0.50 (n = 39) found in a similar experiment with marsh tits (Nilsson 2002). Both values are substantially higher than the effect size of -0.01 (n = 17) found in a comparable study in free living great tits *Parus major* (Wiersma & Tinbergen 2003: Chapter 5). When these values are combined in a meta-analysis (following Hedges & Olkin 1985, pp. 230-232), we find that overall there is a trend that BMR (SMR) is adjusted in response to brood size manipulation, which doesn't quite reach significance (z = 1.75, P = 0.08). It is clear that more studies are needed to verify whether there really is an adjustment of BMR (SMR) in response to brood size, and how the large interspecific differences can be explained.

In addition to lack of statistical power, there could be several biological explanations for the absence of an effect of brood size on SMR. In a number of studies on non-reproducing birds and mammals, and reproducing brown long-eared bats *Plecotus auritus*, an experimental elevation of exercise level induced a reduction in SMR (Deerenberg *et al*. 1998; Bautista *et al*. 1998; McLean & Speakman 2000; Nudds & Bryant 2001; Westerterp 2001). The BMR reduction was interpreted as energy saving behaviour to cope with the increased work load, and in our study adjustment of SMR may have been masked by saving mechanisms acting during the night. Alternatively, a brood size effect on DEE in our study may have been insufficient to induce a detectable effect on SMR. Although our data on mass of offspring and parents indicate that parental provisioning was constrained with increasing brood size (Table 6.3), we were unfortunately not able to measure energy expenditure to verify that DEE increased as found by Deerenberg (1996).

Average hematocrit tended to be higher in birds rearing large broods (effect size 0.29 in males, 0.20 in females), in reasonable agreement with the significant effect size of 0.49 found in great tits (Hõrak *et al*. 1998). To our knowledge there are no other studies of the association between hematocrit and manipulated brood size. The increase in hematocrit with increasing brood size is in agreement with other studies showing a positive association between flight effort and hematocrit (Carpenter 1975; Saino *et al*. 1997; Verhulst *et al*. 2002), and is probably an adaptation to accommodate the increased oxygen demands of e.g. the flight muscles.

The absence of associations of clutch size with female mass, tarsus or condition, is in agreement with earlier results obtained in zebra finches (Williams 1996b). The hematocrit of female birds typically decreases around the time of laying (Silverin 1981; Keys *et al*. 1986; Morton 1994), and our result (Figure 6.3) is consistent with this find-ing. Male hematocrit was negatively correlated with SMR and residual mass (Figure 6.1), suggesting that birds with high hematocrit were in worse shape, although there was no direct correlation between male hematocrit and reproductive success. These results are surprising, because hematocrit is usually found to be lower in birds with lower (residual) mass (Svensson & Merilä 1996; Piersma *et al*. 1996b; Piersma *et al*. 2000; our unpublished personal observations in oystercatchers *Haematopus ostralegus* and starlings *Sturnus vulgaris*), and correlations between hematocrit and metabolic rate are usually positive (Burness *et al*. 1998; Hammond *et al*. 2000). The cause of the negative correlations is not clear, but could in theory be the result of dehydration of birds with high RMR, although water was always present *ad libitum* for all birds. However, if the correlations were caused by dehydration this would also have resulted in a positive correlation between hematocrit and the buffy coat (proportion of white blood cells), but no such correlation was found ($r = 0.02$, $n = 38$, $P = 0.9$).

The correlations between SMR and breeding, laying interval and clutch size were restricted to males (Figure 6.2), and since the female lays the eggs this is somewhat unexpected. In zebra finches and other species, females invest more in reproduction when their males are more attractive (Sheldon 2000; Verhulst 2003). BMR has been found to increase in response to testosterone treatment, at least in some species (e.g. in house sparrows *Passer domesticus*; Buchanan *et al*. 2001), suggesting that males with high SMR might have had high testosterone levels. Since testosterone stimulates sexual behaviour, males with high SMR may have been more sexually active (e.g. better singers or nest builders), inducing a higher investment of their mates. Obviously, direct observations of sexual behaviour and testosterone are necessary to substantiate these speculations.

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Chapter 7

Brood size and immunity costs in zebra finches

Simon Verhulst, Bernd Riedstra & Popko Wiersma

Abstract

We tested three hypotheses that explain why birds rearing experimentally enlarged broods have lower antibody responses to a novel antigen, using zebra finches inoculated with sheep red blood cells (SRBC) compared to saline controls.

1. Compensatory cellular immunity: The humoral immune response is slow compared with cellular immunity, and removal of SRBC through up-regulated cellular immunity could pre-empt an antibody response. However, cellular immune response to PHA decreased with increasing brood size, allowing rejection of this hypothesis.

2. Costs of antibody-production: Chicks in large broods grow less well, and birds with large broods may favour resource allocation to chicks at the expense of immune function when producing antibodies is costly. SRBC suppressed metabolic rate in the hours following immunisation, but there was no effect in the following night, or at any time 4 and 8 days later. Fitness costs were measured by repeatedly immunising parents with SRBC while rearing young. Chick growth, parental condition, and subsequent reproduction of the parents were not affected by SRBC. We conclude that costs of an antibody response cannot explain the trade-off between brood size and antibody-formation.

3. Costs of immune system maintenance: Costs of an antibody response may be negligible but maintaining a system enabling antibody-formation may be very costly, and birds rearing large broods may have downregulated this system. Based on this hypothesis we predicted that antibody-formation would still be reduced in parents rearing large broods when immunised after the chicks had been removed, because restoring the system requires time, and our results confirmed this prediction. We suggest that the trade-off between brood size and antibody-formation is attributable to downregulation of the capacity to produce antibodies in birds rearing large broods.

Introduction

Parasites shape many aspects of behaviour and life histories (Loye & Zuk 1991; Clayton & Moore 1997). Resistance to parasites has therefore drawn increasing attention from evolutionary ecologists. An experimental increase in brood size generally results in increased rates of parasitism (Richner *et al*. 1995; Ots & Hõrak 1996; Oppliger *et al*. 1996; Allander 1997; Nordling *et al*. 1998; Sanz *et al*. 2002). The immune response to a novel antigen decreases with increasing brood size (Deerenberg *et al*. 1997; Nordling *et al*. 1998; Moreno *et al*. 1999), suggesting that reduced resistance could explain at least part of the brood size effect on parasitism. Thus resource allocation to immune function may be important in mediating life-history trade-offs such as the costs of reproduction (Moreno 1993; Gustafsson *et al*. 1994; Sheldon & Verhulst 1996; Zuk 1996; Owens & Wilson 1999).

Understanding why parents rearing experimentally enlarged broods have lower antibody (ab)-responses may yield insights relevant for understanding host-parasite interactions. We used zebra finches inoculated with sheep red blood cells (SRBC), in which a trade-off between brood size and antibody-responsiveness has previously been demonstrated (Deerenberg *et al*. 1997), to test three hypotheses that could explain reduced ab-responses in birds rearing large broods:

1. Compensatory cellular immunity. The humoral immune system is slow, taking several days to produce measurable levels of antibodies. In the mean time antigens are also attacked and removed by the cellular immune system (Roitt *et al*. 1996). When cellular immunity is up-regulated, this could theoretically remove antigens before antibodies are produced. Thus enhanced cellular immunity could explain lower ab-production of birds rearing large broods. We compared cellular immunity (PHA-responsiveness) between birds rearing small and large broods to test this hypothesis (Experiment 1).

2. Costs of ab-production. Resource competition between offspring and self-maintenance could lie at the heart of the trade-off between brood size and ab-responsiveness. We tested this hypothesis using two different experiments. In experiment 2A we quantified the consequences of inoculation with SRBC for energy expenditure (Demas *et al*. 1997; Svensson *et al*. 1998). From an evolutionary perspective only fitness costs are relevant. We therefore examined the effect of repeated SRBC inoculations of breeding birds on offspring growth and subsequent reproduction of the parents (experiment 2B). Immunopathological and oxidative damage may constitute a cost of immune function (Westneat & Birkhead 1998; Råberg *et al*. 1998; von Schantz *et al*. 1999), and if these are important in our system this should be expressed in current and/or future reproductive success.

3. Costs of immune system maintenance. Costs of an ab-response could be low, but maintaining an immune system required to produce high ab-responses may be costly (Fair *et al*. 1999; Williams *et al*. 1999; Schmid-Hempel 2003). On the basis of this hypothesis we predict that ab-formation would still be reduced in parents rearing large broods when immunised after the chicks had been raised, because recovery of the immune system to its original state requires time. We tested this prediction (Experiment 3).

Methods

Housing and breeding conditions

Birds were housed in 80x40x40 cm (lxhxd) cages, with two perches, water, cuttlebone, and food *ad libitum* (mixture of tropical and grass seeds). A protein supplement was provided three times per week, except during the experiments (in experiments with breeding birds protein supplementation was stopped when egg laying started). Rooms were approximately 22°C, humidity was >60%, and lights were on 14 h per day (6:30 to 20:30). Breeding pairs were supplied with a nest box and nest material. Nests were checked regularly to monitor the start of laying, clutch size and the number of hatchlings. Chicks were transferred between broods 1-3 d after hatching to create broods with 2 or 6 young (experiment 1), or 2, 4 or 6 young (experiments 2B and 3). In experiment 2, but not in experiments 1 and 3, there was still a correlation between original and manipulated brood size, due to the low numbers of broods available simultaneously. As in earlier brood size manipulation studies (Deerenberg *et al*. 1996), there were significant effects of brood size on chick growth in all experiments, but for brevity we do not report these data.

Cellular and humoral immunity

A humoral immune response was induced with an intra-peritoneal injection of 0.1 ml 2% SRBC suspended in phosphate buffered saline (PBS) as described by Deerenberg *et al*. (1997). Controls were injected with PBS only. Blood samples (100-150 μl) were taken from the *Vena jugularis*, centrifuged at 12477 g for 10 min, and plasma was stored at -20°C until analysed. Antibodies were measured in duplicate using haemagluttination (Hudson & Hay 1989). Titers were scored as the inverse of the highest dilution at which antibodies were detectable and are based on a base 2 log scale. Blood samples were taken six days after immunisation, when antibody titers reach maximum values (our unpublished observations). Birds injected with PBS had no measurable SRBC antibodies (Deerenberg *et al*. 1997; Birkhead *et al*. 1998; our unpublished observations). Only primary immune responses are reported in this paper.

A cellular immune response was induced by injecting 40 μg phytohaemagglutinin (PHA-P, L8754, Sigma, in 0.2 ml PBS) intradermally into one wing web. PHA is mitogenic to T-lymphocytes. The other wing web was injected with 0.2 ml PBS. Before injection and after 24 h we measured the thickness of both wing webs to the nearest 0.01 mm using a spessimeter (Mitutoyo, 2046F-60). The PHA response was calculated by subtracting the change in thickness of the PBS injected wing from the change in thickness of the PHA injected wing (Smits *et al*. 1999).

Metabolic rate

During measurements birds were in a transparent Plexiglas chamber of 21.6 l. Food was present *ad libitum*. No water was provided to facilitate drying the air. Light regime and temperature were similar to standard housing conditions. Birds were weighed and moved in or out of the respirometer between 12:00 and 15:00. We used measurements obtained between 16:00 and 12:00 the following day.

Air flow through the respirometer chambers was set to ca. 18 l/h in experiment 2 when there was one bird per chamber, and to ca. 24 l/h when there were two birds per chamber. Air flow was measured and controlled with mass-flow controllers (Brooks Instrument, Model 5850E, accuracy 0.02%). Air was dried with molecular sieve (8Å, Merck) before oxygen measurement with an AMETEK Applied Electrochemistry analyser (model S-3A/II, accuracy 2%) and carbon dioxide measurement with an infrared analyser (BINOS-IR 1.2, accuracy 2.5%). Accuracy values according to manufacturer's specifications. Oxygen consumption was calculated according to Hill (1972), and metabolic rate was calculated using the RQ dependent energy equivalent of the oxygen consumed (kJ/l O_2) following Schmidt-Nielsen (1997).

Passive infrared (PIR) sensors recorded movement 340 times per 360 seconds. We defined 'activity' as the proportion of samples with movement. Individual sensors differed in sensitivity, and in the second session in experiment 2A we corrected for these differences prior to analysis. In the first session of experiment 2A birds were always measured with the same PIR sensors and because we made within individual comparisons no correction was necessary.

Statistical analyses

When controlling statistically for experimental treatment, responses of pair-members were not correlated in any of the tests. Pair members were therefore treated as independent samples. All means are shown with their standard error.

Experiment 1: Compensatory cellular immunity

Broods with two (n = 10) or six (n = 9) nestlings were created. PHA/PBS was injected in both parents when the chicks were 17 d old. One female died due to unknown causes before the PHA response was measured, and one measurement failed, presumably because the injection was not successful (Figure 7.1). This case was removed from the analysis, but this did not change the result.

Experiment 2: Costs of ab-response

EXPERIMENT 2A: ENERGY COSTS

The energy costs of an antibody response was measured in two separate immunisation sessions, which differed mainly in the timing of metabolic measurements relative to immunisation. In the first session, 20 males were housed individually, of which 10 were injected with SRBC and 10 with PBS. Metabolic measurements were performed 5 days before and for 20 h immediately following immunisation. In the second session birds were housed in single-sex pairs (n = 16 pairs, 8 of each sex, 4 controls and 4 immunised). Metabolic measurements were done on the paired birds together (separated by wire mesh) to increase accuracy. Metabolic rate and activity were measured four times: six and two days before immunisation, and four and eight days after immunisation. The first measurement served to accustom the birds to the protocol, and is not further discussed. The second measurement served as within-pair control.

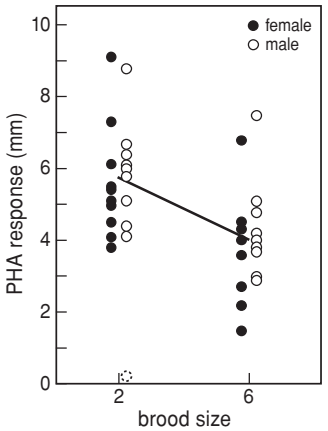

Figure 7.1 Brood size and response to PHA. One measurement in which there was no response at all, presumably because the injection failed, is depicted by the broken-lined dot.

EXPERIMENT 2B: FITNESS COSTS

Pairs of broods with the same brood size were selected, and in each dyad one randomly chosen pair was immunised three times with SRBC, on day 7, 9 and 11 after hatching. The other pair served as control and was injected with PBS on the same days. Nestlings were weighed before the first injection (day 7), and before blood sampling (day 13). When chicks are 7-13 d growth is approximately linear (our unpublished data). After the young fledged the nest box was removed. The young were measured (mass, tarsus, wing length) and removed when 35 d old. One week later a second blood sample was taken from the adults, and all birds were immunised once with SRBC. The initial purpose was to investigate the build-up of immunological memory, but the antibody titers at immunisation were too high to provide a meaningful test, and the memory data will not be presented. After the final blood sample was taken, the pairs were again supplied with a nest box, and reproduction was monitored as before.

Experiment 3: Costs of immune system maintenance

Trios of broods were formed, and chicks were distributed so that independent of the original brood size in each trio there were broods with 2, 4 or 6 chicks. Nestlings and parents were weighed at the time of manipulation. At 16 d after hatching, the young were weighed and measured, and removed from the cage. The parents were also weighed at this time. One day later the parents were weighed again, and immunised with SRBC, and a blood sample was taken six days later.

Results

Experiment 1: Compensatory cellular immunity

PHA response was significantly weaker in birds rearing large broods (Figure 7.1; $F_{1,34} = 12.0$, $P = 0.001$), also when the sexes were analysed separately (females: $F_{1,16} = 6.11$, $P = 0.025$; males: $F_{1,16} = 5.95$, $P = 0.027$). There was no difference between the sexes ($F_{1,32} = 0.9$, $P = 0.3$) and no brood size x sex interaction ($F_{1,32} = 0.08$, $P = 0.8$).

Experiment 2: Costs of ab-response

2A ENERGY COSTS

Of the immunised birds in the first session (short term effects) 70% produced SRBC-antibodies, and mean antibody-titer was 5.1 ± 1.4 ($n = 10$, including non-responders), indicating we were successful in eliciting an immune response. Mass, metabolic rate and activity did not differ between control and immunised birds during the control measurements (*t*-tests, all $P > 0.3$). We calculated the difference between the post-immunisation and individual control values measured 5 days before immunisation. SRBC reduced metabolic rate in the afternoon following injection ($t_{18} = 2.16$, $P < 0.05$), but this effect disappeared over the next two time intervals (Figure 7.2B; night: $t_{18} = 1.14$, $P = 0.18$; morning: $t_{18} = 0.64$, $P = 0.53$). Detailed analysis revealed that the decrease in metabolic rate had disappeared after approximately nine hours (Figure 7.2C; immunisation was at 12:46, SE = 0.14 h). An immunisation effect on activity can obscure metabolic effects (see e.g. Gentry *et al*. 1997). However, activity levels in the afternoon and the next morning were not affected by immunisation (Figure 7.2A; $t_{18} < 0.2$, $P = 0.9$). Controlling statistically for the change in activity did not change the results.

All immunised birds in the second session (effects 4 and 8 days after immunisation) produced SRBC-antibodies. Mean antibody-titer was 4.47 ± 0.62 ($n = 16$). Metabolic rate and activity (afternoon or morning) during the control measurements did not differ between experimental groups (*t*-tests; all $t_{14} < 0.8$, $P > 0.5$). The change in metabolic rate (afternoon, night, and morning) was independent of immunisation at 4 and 8 days after immunisation (Figure 7.2B; *t*-tests: all $t_{14} < 1.14$, $P > 0.27$). Activity level was significantly reduced in immunised birds during the afternoon on day 4 after immunisation (Figure 7.2A; $t_{14} = 3.12$, $P < 0.008$), but not at any other time (all $t_{14} < 1.1$, $P > 0.3$). Controlling for changes in activity when testing immunisation effects on metabolic rate did not change the results.

2B FITNESS COSTS

We used 16 control broods, and 15 experimental broods in this experiment. In total, 28/30 (93%, sexes combined) of the immunised birds responded with the formation of SRBC-antibodies. We verified whether there was an association between brood size and ab-response. Because we took two samples (6 and 35 d after immunisation), we used repeated measures ANCOVA. Ab-response declined significantly with broods size in females (Figure 7.3B; $F_{1,12} = 6.3$, $P < 0.03$), but not in males (Figure 7.3A; $F_{1,12} =$

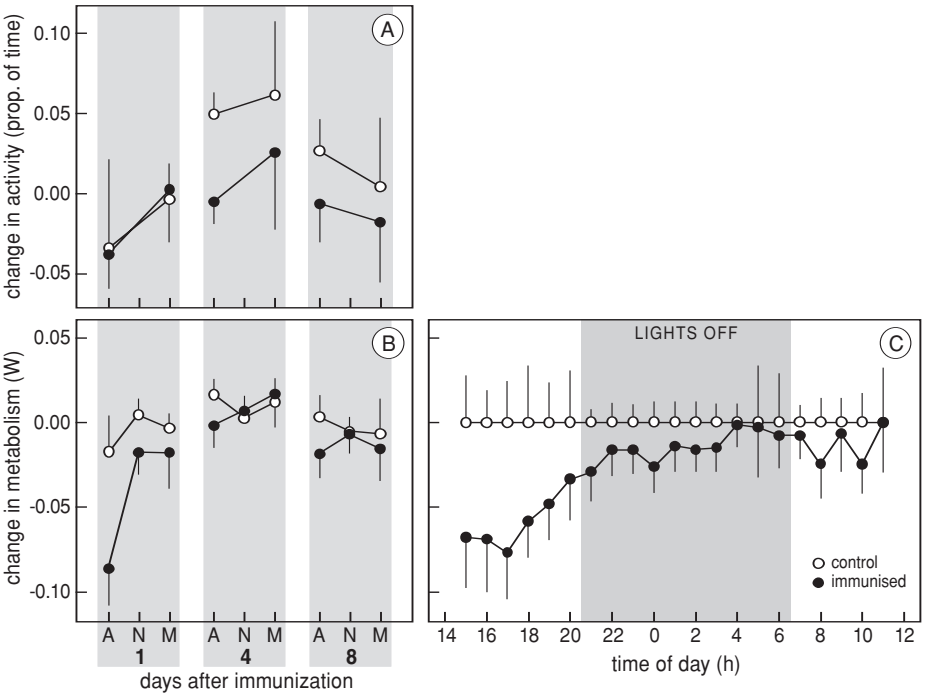

Figure 7.2 Metabolism (B, C; in W / bird) and activity (A) of control and immunised birds at different times after immunisation. A and C: The abscissa indicates time since immunisation, showing data separately for the afternoon (A), night (N), and morning (M). Data shown are mean differences (± SE) between the measurement at the indicated time, and the control measurement prior to immunisation. In C the change in metabolism of the controls was set to zero. Data experiment 2A.

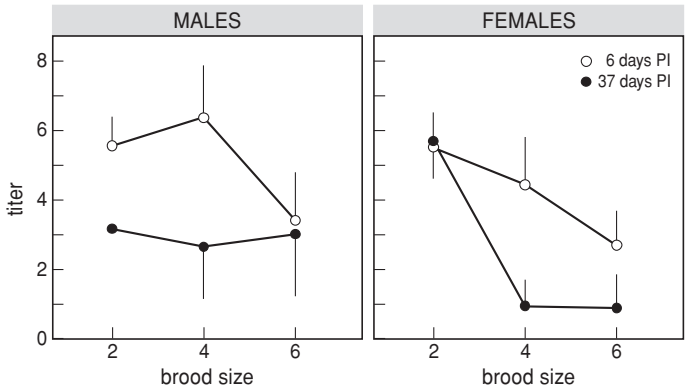

Figure 7.3 Brood size and SRBC-antibody titers in A. Males, and B. Females. PI = Post Immunisation. Data experiment 2B.

0.6, $P = 0.4$). However, the brood size effect was significant when sexes were pooled ($F_{1,26} = 4.6$, $P < 0.05$), and there was no significant sex x brood size interaction ($F_{1,24} = 0.7$, $P = 0.4$). Brood size before and after manipulation were correlated in this experiment, and to compare effects of natural and artificial brood size variation we replaced brood size with brood size at manipulation and the number of young added/removed (these variables summed is the manipulated brood size). Both parameters approached significance (brood size at manipulation: $F_{1,25} = 3.73$, $P < 0.07$, number of chicks added/removed: $F_{1,25} = 3.14$, $P < 0.09$), but, more importantly, the slopes of these effects were indistinguishable (difference < 5%).

Nestling mass at time of the first immunisation did not differ between control and immunised pairs (immunisation: $F_{1,28} = 0.01$, $P = 0.9$; controlling for brood size: $b = -0.34 \pm 0.13$ g/young ($F_{1,28} = 6.9$, $P < 0.02$). Controlling for brood size, growth in the experimental period (age 7-13 d) was not affected by the immunisations (Figure 7.4A; $b = 0.04 \pm 0.09$ g/young/d ($F_{1,28} = 0.2$, $P = 0.7$). Nestling mortality in the experimental period was too low to warrant statistical analysis (3/122 chicks died). Chick mass at 35 d was also independent of immunisation (Figure 7.4B; $F_{1,29} = 0.7$, $P = 0.4$). Thus there was no evidence of any effect of parental immunisation on development of the chicks.

The costs of an immune response could be paid through subsequent reproduction (e.g. via an effect on parental state), but neither clutch size, nor the interval between the first egg of a new clutch and the time a nest box was offered were affected by immunisation (Figure 7.4C; laying interval: Mann Whitney-U-test, $P > 0.9$; clutch size: $t_{24} = 0.64$, $P = 0.5$). Controlling for previous brood size did not change these results.

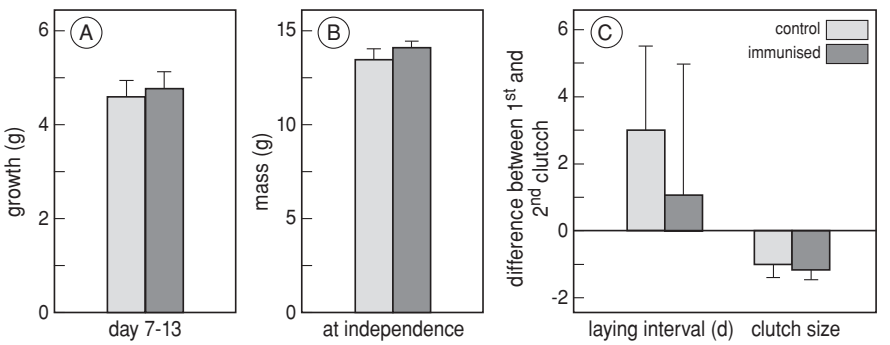

Figure 7.4 A. Growth (+ SE), and B. mass at 35 d of chicks reared by control (light bar), or immunised (dark bar) parents. C. Laying interval and clutch size of clutches produced after immunisation treatment, expressed as the difference between values at the first and the second clutch. Positive values indicate that the second clutch was later / larger. Data experiment 2B.

Mass-changes of immunised birds

Energy costs of immune function could reveal itself through an effect on energy reserves (Ots *et al*. 2001). In experiment 2A mass change from the immunisation to the blood sampling 6 days later was independent of immunisation (session 1: $t_{18} = 1.56$, $P = 0.14$; session 2: $t_{18} = 1.41$, $P = 0.17$), and effects were opposite in the two sessions (Table 7.1). Looking at other intervals after immunisations, or analysing sexes separately, does not change these results (Table 7.1). In experiment 2B (brood rearing birds) there was also no significant effect of immunisation (Table 7.1; repeated measures ANOVA: $F_{1,60} = 0.4$, $P = 0.5$). Although a weak effect may be present in females, restriction of the analysis to females did not change results (repeated measures ANOVA: $F_{1,29} = 2.3$, $P = 0.14$).

Experiment 3: Costs of immune system maintenance

We pooled the data from experiment 3 with the data from the control group of experiment 2B, in which birds were also immunised after the young were removed (see methods). There was a negative association between brood size and the proportion of birds forming SRBC-antibodies (Figure 7.5; logistic regression: $F_{1,94} = 5.1$, $P < 0.03$). The brood size effect on ab-formation did not differ between experiments 2B and 3 (experiment: $F_{1,93} = 0.1$, $P = 0.8$; experiment x brood size interaction: $F_{1,92} = 0.1$, $P = 0.7$). Neither experimental effect, nor the mean level differed between the sexes

Table 7.1 Mass (g, SE in brackets) at immunisation and subsequent mass change relative to mass at immunisation (time = 0).

				time (d) after immunisation				
exp.	sex	n	treatment	0	2	4	6	8
1a	Males	10	control	15.6 (0.54)			0.72 (0.22)	
		10	immunised	15.9 (0.69)			0.12 (0.20)	
1b	Males	8	control	14.4 (0.56)		0.19 (0.11)	-0.15 (0.12)	-0.38 (0.13)
		8	immunised	14.8 (0.68)		0.33 (0.14)	0.05 (0.08)	-0.30 (0.17)
	Females	8	control	15.4 (0.88)		0.15 (0.11)	0.04 (0.11)	-0.05 (0.20)
		8	immunised	15.1 (0.67)		0.34 (0.53)	0.15 (0.12)	-0.03 (0.57)
	Total	16	control	14.9 (0.52)		0.17 (0.08)	-0.06 (0.08)	-0.21 (0.12)
		16	immunised	14.9 (0.46)		0.33 (0.26)	0.10 (0.07)	-0.16 (0.29)
2	Males	16	control	13.6 (0.35)	-0.23 (0.08)	-0.28 (0.11)	-0.19 (0.21)	
		15	immunised	14.2 (0.44)	-0.09 (0.18)	-0.25 (0.17)	-0.15 (0.30)	
	Females	16	control	15.5 (0.56)	-0.13 (0.06)	0.02 (0.11)	-0.04 (0.26)	
		15	immunised	14.2 (0.38)	-0.23 (0.07)	-0.37 (0.12)	-0.26 (0.19)	
	Total	32	control	14.5 (0.37)	-0.18 (0.05)	-0.13 (0.08)	-0.12 (0.16)	
		30	immunised	14.2 (0.28)	-0.16 (0.09)	-0.31 (0.11)	-0.20 (0.18)	

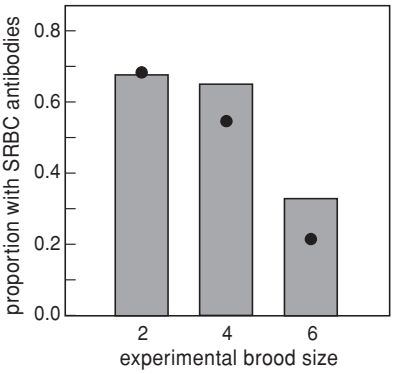

Figure 7.5 Proportion of birds with SRBC-antibodies six days after immunisation (sexes pooled). Bars: proportions responders when parents were immunised after the young were removed (for brood sizes 2, 4, 6, n = 34, 31, 32 respectively). Data experiment 3 and 2B (control group only). Proportion responders in presence of young added for comparison (dots, data Deerenberg *et al*. 1997).

(sex: $F_{1,93} = 0.2$, $P = 0.7$; sex x brood size interaction: $F_{1,92} = 0.1$, $P = 0.8$). For comparison, the data of Deerenberg *et al*. (1997) are also shown in Figure 7.5. The brood size effect did not differ significantly between the two studies (study: $F_{1,136} = 0.6$, $P = 0.4$; brood size x study interaction: $F_{1,135} = 0.4$, $P = 0.5$), and was highly significant in this combined analysis ($F_{1,137} = 9.9$, $P < 0.002$).

The persistence of the brood size effect on ab-responsiveness may be explained by an effect of brood size on body mass (data experiment 3 only). We controlled for sex in subsequent analyses; the interaction between sex and brood size was in no case significant. Parents with large broods lost more mass during brood rearing than parents with small broods (Figure 7.6; $b = -0.16 \pm 0.05$ g/young ($F_{1,62} = 12.3$, $P < 0.001$; sex: $F_{1,62} = 0.2$, $P= 0.6$). Mass gain over the 24 h after the brood was removed increased with brood size ($b = 0.08 \pm 0.03$ g/young ($F_{1,62} = 7.2$, $P < 0.01$; sex: $F_{1,62} = 6.8$, $P < 0.02$). Consequently, body mass was independent of brood size at immunisation ($F_{1,62} = 3.1$, $P = 0.1$; sex: $F_{1,62} = 5.2$, $P < 0.03$). Further mass change until blood sampling was also independent of brood size (Figure 7.6; $F_{1,62} = 0.1$, $P = 0.7$; sex: $F_{1,62} = 28.7$, $P < 0.0001$). Thus the persistence of the brood size effect on ab-responsiveness was not due to effects on mass.

Discussion

Our study was based on the effect of brood size on ab-responsiveness reported by Deerenberg *et al*. (1997), and it is therefore important that we replicated this result. Practically all birds formed antibodies in response to repeated immunisation, but brood size had an effect on the strength of the response (Figure 7.3). This extends the results of Deerenberg *et al*. (1997), which were restricted to brood size effects on the

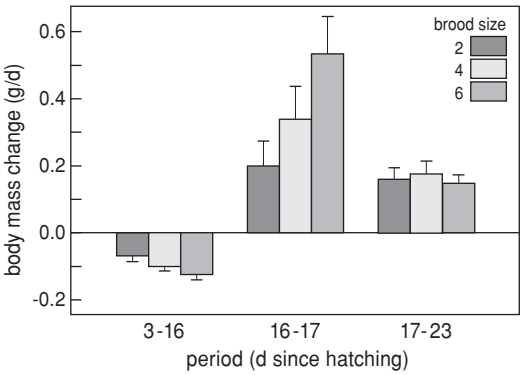

Figure 7.6 Parental mass change (g/d ± SE) in three periods following brood size manipulation (sexes pooled). Chicks removed at age 16 d. Parents immunised at age 17 d, and blood sample was taken at age 23 d. Bars indicate parents rearing 2, 4 or 6 young (n = 24, 19, 22, respectively). Data experiment 3.

proportion of responders. Cellular immunity also decreased with increasing brood size (Figure 7.1), providing further evidence of a trade-off between reproductive effort and immunocompetence.

We tested three hypotheses that could explain why birds with large broods have lower ab-responsiveness. Compensatory cellular immunity is an unlikely explanation, because PHA responsiveness decreased with brood size. The metabolic consequences of SRBC were small, and restricted to the first few hours after inoculation (Figure 7.2). Furthermore, the direction of the effect was opposite to what we had expected, since the birds decreased their metabolic rate, although this is in agreement with the decrease in food intake of sick animals (e.g. Murray & Murray 1979). The lack of strong effects of immunisation on metabolic rate is in agreement with the results obtained in other avian species (Siegel *et al*. 1982; Henken & Brandsma 1982; Svensson *et al*. 1998; Hõrak *et al*. 2003; but see Ots *et al*. 2001), suggesting that the metabolic costs of mounting a humoral immune response are negligible. This is in agreement with the results of theoretical calculations for poultry in which the costs of immune function were found to be small compared with growth and egg production (Klasing 1998).

Although the metabolic consequences of an immune response were small, the costs could be higher when measured in another currency, e.g. protein or amino acid turnover (Klasing & Austic 1984; Parry Billings *et al*. 1992; Lochmiller & Deerenberg 2000). Ultimately only fitness costs are relevant, but repeated inoculation of parents rearing young had no effect on parameters strongly related to fitness in free-living birds (Figure 7.4). Fitness costs of resistance may be expressed in harsh environments only (Kraaijeveld & Godfray 1997; Moret & Schmid-Hempel 2000), but reduced immune responses with increasing brood size indicates parental performance was constrained, suggesting a harsh environment. Immunopathological and oxidative damage may constitute a cost of immune function (Westneat & Birkhead 1998; Råberg *et al*. 1998; von

Schantz *et al*. 1999), but these processes were apparently not of sufficient magnitude to cause fitness costs. Thus, at least in the system that we studied, such damage is unlikely to be important. In contrast to the present paper, several recent studies reported that immunisation reduced reproductive performance of breeding females (Ilmonen *et al*. 2000; Råberg *et al*. 2000; Bonneaud *et al*. 2003). More work will be needed before this variation can be understood.

The costs of immune function may lie in the maintenance of a system enabling abformation, rather than in immune system deployment. The negative association between brood size and immune function persisted after brood rearing (Figure 5), in agreement with this hypothesis. We therefore suggest that the trade-off between brood size and antibody-formation is attributable to down-regulation of the capacity to produce antibodies in birds rearing large broods. Results from two experiments that differed in the length of the brood-rearing period and in the interval between chick removal and inoculation were indistinguishable. This suggests that the brood size effect on parental state does not disappear very rapidly (i.e. in days), which is in agreement with the observation that costs of reproduction can reveal themselves months after parental care has ended (Gustafsson & Sutherland 1988; Daan *et al*. 1996; Verhulst 1998).

Independent support for the significance of the costs of immune system maintenance come from artificial selection experiments on immune function, which reported negative associations between immune function and fitness related parameters (Kraaijeveld & Godfray 1997; Verhulst *et al*. 1999; Webster & Woolhouse 1999; Schmid-Hempel 2003). Since these animals were not allocating resources to an induced immune response when fitness-parameters were measured, it follows that the fitness consequences were due to investment in the capacity to respond. Future studies of immunity costs should distinguish between the maintenance and deployment costs of the immune system.

Acknowledgements

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Chapter 8

Bird sacrifice oxidative protection for reproduction

Popko Wiersma, Colin Selman, John R. Speakman & Simon Verhulst

Summary

We tested whether there is a trade-off between reproductive effort and protection against oxidative damage in birds. Processes of oxidative metabolism generate reactive oxygen species (ROS) as by-products, which may cause oxidative damage to DNA, proteins and lipids (oxidative stress) which is considered to play a major role in senescence. A suite of endogenous and exogenous antioxidants can deactivate ROS, thereby protecting against oxidative damage, and we measured the activity of two endogenous antioxidant en-zymes, SOD and GPx, in zebra finches. We manipulated reproductive effort by changing their brood sizes to either 2 or 6 young and measured enzyme activity in the pectoral muscle after 19-20 d of brood provisioning. We scaled the activity of SOD and GPx to daily energy expenditure, assuming this provides an index of ROS production. Scaled SOD and GPx activity decreased with increasing brood size by 28% and 24% respectively. This effect was identical in the two sexes, but arose in different ways: males did not change their DEE, but had lower absolute enzyme activity, and females increased their DEE, but did not change absolute enzyme activity. This result supports the suggestion that oxidative stress plays a role in mediating life-history trade-offs.

Introduction

The rate of senescence, defined as a decrease in residual reproductive value with advancing age (Hamilton 1966; Partridge & Barton 1996), varies sharply, even between closely related species of similar size (Austad 1997). Such variation is theoretically at least partly understood: the rate of extrinsic mortality (i.e., mortality due to factors over which the organism has limited control, such as harsh winters and predation), determines the optimal allocation of resources between reproduction and somatic maintenance (Rose 1991; Kirkwood & Austad 2000). More specifically, optimal resource allocation to reproduction increases with increasing risk of extrinsic mortality. Recent comparisons between (Keller & Genoud 1997; Ricklefs 1998) and within species (Austad 1993; Stearns *et al*. 2000) confirm that slower rates of senescence evolve when the risk of ex-trinsic mortality is low and that reproductive effort declines when the risk of extrinsic mortality declines. An experimentally induced increase in reproductive effort generally leads to a decrease in residual reproductive value (Reid 1987; Gustafsson & Pärt 1990; Dijkstra *et al*. 1990; Jacobsen *et al*. 1995; Daan *et al*. 1996; Siikamäki *et al*. 1997; Verhulst 1998; Nager *et al*. 2001), and this 'cost of reproduction' is conceptually similar to an acceleration of senescence. This provides a link between comparative studies on lifespan variation and intraspecific studies on reproductive rate. It seems possible that the mechanisms underlying senescence and the costs of reproduction are to some extent identical. One potential mechanism shared by these processes, oxidative stress, is the subject of this study.

Metabolic processes that consume oxygen continuously produce a wide variety of reactive oxygen species (ROS), which damage DNA, proteins and lipids (Cadenas 1995; von Schantz *et al*. 1999). Accumulating oxidative damage ('oxidative stress') is thought to be one of the key mechanisms in causing cellular senescence and death (Harman 1956; Austad 1997; Beckman & Ames 1998). Oxidative damage can potentially be prevented through a suite of antioxidants (Felton 1995), such as carotenoids, vitamin C and a number of endogenous antioxidant enzymes, that convert ROS into less reactive molecules. Higher resource allocation to somatic maintenance and repair in long-lived species could be expressed in a relatively high investment in defences against ROS. However, attempts to link antioxidant enzymes to lifespan in comparative analyses have shown unexpected trends: antioxidant enzyme activity is lower in long-lived species (Pérez-Campo *et al*. 1998; Barja 2002). Species also differ in O_2 metabolism per unit tissue, and in ROS production per O_2 volume that is metabolised (Holmes *et al*. 2001). Since variation in antioxidant enzyme activity can only be interpreted in the context of the ROS production (Barja 2002), this complicates the comparative approach. We used the alternative of phenotypic manipulation, which ensures an identical genetic and physiological background of groups differing in residual reproductive value. We manipulated reproductive effort in birds, and measured the activity of antioxidant enzymes. To our knowledge this is the first experimental test of a trade-off between reproduction and oxidative protection.

To manipulate reproductive effort we altered brood size of captive zebra finches *Taeniopygia guttata* to either two or six young. Zebra finch parents (in particular females) adjust their metabolic rate to the altered brood size (Deerenberg 1996). More importantly, captive zebra finches suffer a cost of reproduction (a decrease in residual reproductive value) when brood size is increased (Deerenberg *et al*. 1996). To test whether parents sacrifice oxidative protection for parental care we quantified the effect of brood size on the activity of two antioxidant enzymes, superoxide dismutase (SOD) and glutathione peroxidase (GPx; for practical reasons we did not measure catalase, which is the third major antioxidant enzyme). SOD transforms superoxide ($\cdot O_2$), that is formed in the mitochondria, to hydrogen peroxide (H_2O_2), which can be metabolised to water and molecular oxygen by GPx and catalase (Finkel & Holbrook 2000). Given a particular level of oxidative protection, the oxidative damage incurred will depend on the exposure to ROS and we therefore scaled the activity of antioxidant enzymes to daily oxygen consumption measured in an earlier study (Deerenberg 1996). Because ROS production does not depend on enzymatic reactions, the oxygen consumption rate provides an index of the ROS production rate (Finkel & Holbrook 2000), especially within individuals.

Methods

Animals and housing

A total of 43 pairs of wild-type zebra finches of at least 1 year old started in the experiment. All birds were marked with a numbered blue or black ring. Pairs were housed in cages of 40x80x40 cm (hxwxd) and given nesting material 16-20 days after pair-formation. Birds that had not build a nest after a week, or had not laid eggs within a week after nest completion were paired up with other partners, or were removed from the experiment.

Room temperature was on average 25°C (range 23-27°C), and the LD cycle was 14:10. Food (tropical seed mixture) and water were present *ad libitum*. A teaspoon of egg food was given 3 times per week to complement the diet, until completion of the clutch.

Manipulation

Nestboxes were checked daily for nest building and eggs. Clutches were checked regularly to record the hatching date, and broods were manipulated to contain 2 (n = 10) or 6 (n = 9) nestlings when the chicks were 1-3 days old. Brood size after manipulation was not correlated with original brood size (r = -0.086, P = 0.73).

Antioxidant enzyme assays

Birds were killed by cervical dislocation when their nestlings were 19-20 days old. Pectoral muscle samples were immediately immersed in liquid nitrogen within 80 s after death and stored at -70°C. The samples were thawed prior to analysis and homo-

genised in 20 volumes of ice cold 50 mM phosphate buffer (pH 7.4). The suspension was centrifuged at 3200 g for 20 min at 5°C and the supernatant fraction was used for antioxidant enzyme measurement.

We measured total activity of SOD and of the Se-dependent isozyme of GPx. Both enzymes have been shown to be very important in preventing oxidative stress (Gauthier 1987; de Haan *et al*. 1998). SOD activity was measured by the inhibition of pyrogallol autoxidation at 25°C, and was followed kinetically at 420 nm (Marklund & Marklund 1974). One unit of SOD activity was defined as the amount of enzyme that caused 50% inhibition of pyrogallol autoxidation. GPx activity was measured at 25°C by following NADPH oxidation spectrophotometrically at 340 nm in the presence of reduced glutathione (GSH) and H_2O_2 (Paglia & Valentine 1967). One unit of GPx was defined as the amount of enzyme that oxidises 1 μM of NADPH per minute. To correct for spontaneous reactions in the absence of enzyme, blanks were run without sample and then subtracted form the assay values (López-Torres *et al*. 1991).

Absorbance changes were measured using a SPECTRAmax Plus microplate spectrophotometer (Molecular Devices Corp., Sunnyvale, USA) and analysed with SOFTmax Pro software (Molecular Devices Corp.). All enzyme activities are expressed per mg protein (Selman *et al*. 2000; Selman *et al*. 2001). Protein contents of the samples were measured using the Bradford method (Bradford 1976).

Energy expenditure

Parental energy expenditure was measured using doubly labelled water (Speakman 1997) by Deerenberg (1996) in an experimental design identical to the design used in the present study. DEE was measured when the chicks were 14-16 days old. Based on these data we estimated that male DEE was 53.3 kJ/d, independent of brood size, and female DEE was 48.8 and 67.5 kJ/d for broods of two and six young, respectively.

Statistical analyses

Nestling measurements were compared using multi-level models to account for the statistical dependency within nests, while *t*-tests or generalised linear models were applied in all other cases. All tests were performed using SPSS (v. 11.0, SPSS Inc.). Protein and antioxidant enzyme measurements in males and females of pairs were not correlated (protein: $r = 0.14$, $n = 17$, $P = 0.58$; SOD: $r = 0{,}36$, $n = 17$, $P = 0.15$; GPx: $r = 0.14$, $n = 17$, $P = 0.60$), and were therefore treated as independent samples in the analyses. All averages and parameter estimates are shown together with standard errors.

Results

We did not directly measure the effect of brood size on parental effort or energy expenditure in this experiment, but data on parental mass and nestling growth provide an indication of whether parents rearing large broods were constrained when rearing a large brood. Indeed, independent of sex, parents rearing large broods lost 1.06 ± 0.49 g more mass from the onset of breeding to the day that the chicks were 17 d old than birds rearing small broods ($t_{34} = 2.17$, $P = 0.037$). Furthermore, nestlings in broods of six chicks were 1.68 ± 0.31 g lighter than nestlings in broods of two ($X_1^2 = 13.49$, $P < 0.0005$). Wing length of nestlings in large broods was also reduced (-2.42 ± 0.86 mm, $X_1^2 = 7.48$, $P < 0.01$), while tarsus length was not affected (-0.30 ± 0.17 mm, $X_1^2 = 1.32$, $P = 0.25$). In agreement with earlier brood size manipulation experiments in zebra finches (Deerenberg *et al*. 1996), the data on offspring growth indicate that parents rearing large broods were constrained in their chick feeding abilities, and the data on parental mass indicate that more resources were allocated to reproduction.

Relative GPx activity (enzyme activity scaled to daily energy expenditure; U/ml O_2 d^{-1}) was 24% lower, and relative SOD activity was 28% lower in birds rearing larger broods. Both effects were statistically significant and independent of sex (Figure 8.1). Absolute GPx activity decreased from 3581 ± 303 (2 young) to 3213 ± 273 (6 young), but this was not a significant change ($t_{32} = 0.88$, $P = 0.39$). Absolute SOD activity decreased significantly with increasing brood size from 1080.1 ± 88.9 (2 young) to 888.1 ± 80.7 (6 young; $t_{33} = 2.06$, $P = 0.037$). Because absolute GPx activity did not

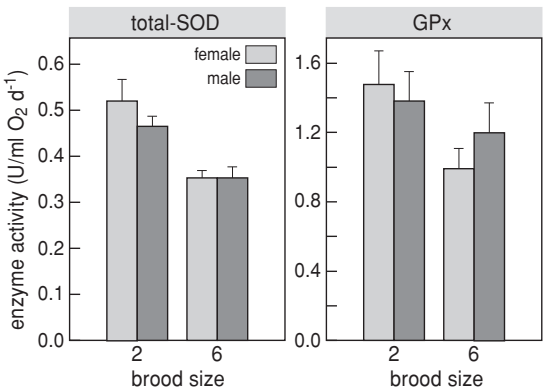

Figure 8.1 Total superoxide dismutase (SOD) and glutathione peroxidase (GPx) activity in pectoral muscles expressed per whole body O_2 consumption per day of males and females rearing broods of 2 or 6 young. Relative activity of both enzymes decreased with increasing brood size (mean U/ml O_2 d^{-1}, SOD: 2 young: 0.490 ± 0.026, $n = 19$; 6 young: 0.355 ± 0.014, $n = 16$; $t_{33} = 4.40$, $P < 0.001$; GPx: 2 young: 1.42 ± 0.12, $n = 19$; 6 young: 1.08 ± 0.10, $n = 15$; $t_{32} = 2.09$, $P = 0.045$).

change, the decrease in relative GPx activity was largely due to the increase in O_2 consumption rate in the birds rearing larger broods. The decrease in relative SOD activity was due to both an increase in O_2 consumption rate and a decrease in SOD activity (see Figure 8.2).

Relative enzyme activity was lower when brood size was large in both sexes, but these effects arose in different ways (Figure 8.2). In females, absolute enzyme activity was only marginally lower when brood size was large, but the relative enzyme activity decreased due to an increase in DEE. In males however there was no significant effect of brood size on DEE, but a significant decrease in enzyme activity, and thus also a decrease in relative enzyme activity.

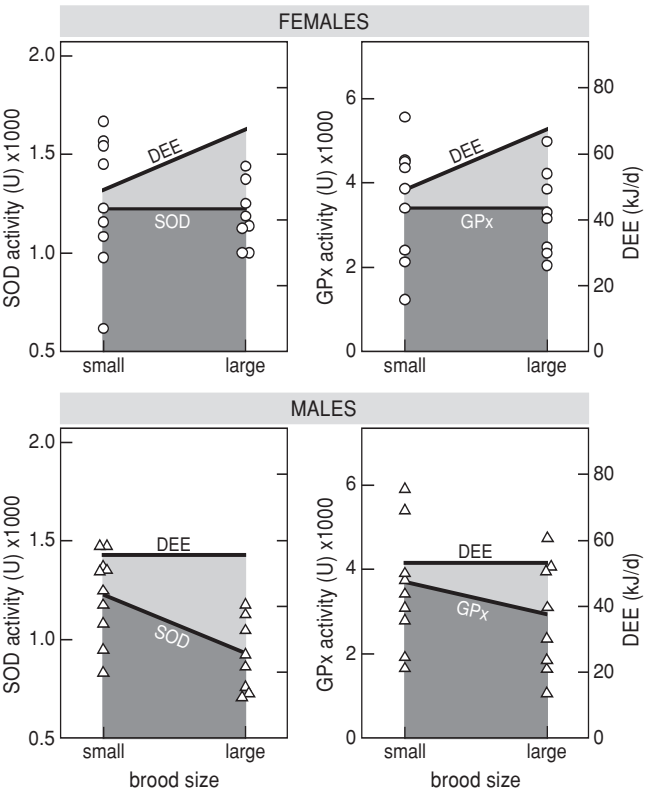

Figure 8.2 SOD and GPx activity and daily energy expenditure (DEE) for females and males rearing experimentally created small or large broods. Average SOD activity decreased with brood size from 1252.2 ± 113.0 to 1182.9 ± 56.9 in females (t_{15} = 0.53, P = 0.60), and from 1220.8 ± 62.0 to 932.0 ± 63.7 in males (t_{17} = 3.22, P = 0.005). Average GPx activity for females with 2 and 6 nestlings was 3534 ± 464 and 3277 ± 359, respectively (t_{15} = 0.43, P = 0.67), and for males 3622 ± 421 and 3140 ± 445, respectively (t_{15} = 0.77, P = 0.45). The daily oxygen consumption data are from Deerenberg (1996).

Discussion

Individuals are thought to invest in reproduction at the expense of their future reproductive output (Dijkstra *et al*. 1990), and, on a different level, species can be characterised by their place in the lifespan/reproduction spectrum, ranging from very short adult lives with high reproductive rates to very long lives with very low annual investment in reproduction (Ricklefs & Wikelski 2002). Oxidative stress resulting from parental effort has been suggested as an important mechanism mediating this trade-off, both between and within species. Our result, that one of the main defences against ROS (i.e. the activity of two endogenous antioxidant enzymes) was reduced relative to the potential for generating ROS in zebra finches when brood size was experimentally increased, supports this suggestion. To our knowledge this is the first experimental demonstration of a trade-off between reproductive effort and oxidative protection.

Our result can be compared with comparative studies that reported a negative correlation between lifespan and antioxidant enzyme activity (Pérez-Campo *et al*. 1998). The predicted effect on the basis of comparative studies is a *positive* relationship between brood size and antioxidant activity, because individuals rearing large broods have lower residual reproductive value. This is opposite to the effect we actually observed. Pérez-Campo *et al*. (1998) suggest that ROS production is lower in species with a longer lifespan, explaining why they could live longer despite having lower antioxidant enzyme activity. This explanation seems plausible, but does create a serious complication for comparative studies, because it is not clear how antioxidant activity should be scaled to energy expenditure when comparing species. Using phenotypic manipulations to induce variation in residual reproductive value avoids this problem, because different experimental groups then have identical genetic and physiological backgrounds.

In both sexes oxidative protection was reduced in parents rearing large broods. In males this was due to a decrease in enzyme activity, but in females this was mainly due to an increase in energy expenditure (Figure 8.2). This is interesting, because it indicates that the two sexes may have had different strategies to cope with the experimental increase in workload. Sexual differences in adjustment of energy expenditure to manipulated brood size has previously been found in other bird species (Moreno *et al*. 1995; Verhulst & Tinbergen 1997). Unfortunately we were not able to measure energy expenditure directly in our experiment, but such data could potentially reveal further variation in strategies to rear a large brood, e.g. between young and old individuals. Such data would also be important to provide a more direct estimate of the effect of brood size on relative antioxidant enzyme activity.

The decrease in (relative) activity of antioxidant enzymes with increasing brood size indicates a decrease in the ability to neutralise ROS. This can logically be expected to result in an increase in oxidative damage, but this remains to be tested explicitly, partly because the activity of other antioxidants and of DNA repair processes will have confounding effects. Direct measurements of the effect of reproductive effort on oxidative damage, in combination with estimates of the effect of oxidative damage on the

residual reproductive value, are required to assess the quantitative importance of oxidative damage in mediating life history trade-offs. Elevated damage by ROS may induce degradation of repair systems, so knowing how these respond to elevated ROS following brood manipulation will be important in understanding the ultimate impact on damage. However, initial results in invertebrates (*Drosophila melanogaster, Caenorhabditis elegans*) imply that oxidative damage, specifically the level of superoxide, does play a role in limiting lifespan (Golden *et al*. 2002). Nevertheless, other aspects of physiological maintenance and repair may also be downregulated when more resources are allocated to reproduction, possibly further contributing to acceleration of senescence. For example, experimental enlargement of brood size has been shown to depress humoral and cellular immunity in various bird species, including the zebra finch (Sheldon & Verhulst 1996; Deerenberg *et al*. 1997; Nordling *et al*. 1998; Moreno *et al*. 1999). Ultimately an integrative approach, combining different components of physiological maintenance and repair, may be required to provide quantitative insight in the relative contribution of different processes to the effect of increased effort on the residual reproductive value.

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Chapter 9

Paternal care and male mate attraction effort in the European starling is adjusted to clutch size

Jan Komdeur, Popko Wiersma, & Michael Magrath

Abstract

In facultative polygynous birds with biparental care, a trade-off may occur between male parental care and attraction of additional mates. If there is a cost associated with reduced male parental care, the relative benefit of mate attraction may be predicted to decrease as the size of a male's clutch or brood increases. We tested this prediction in monogamous pairs of facultatively polygynous European starlings *Sturnus vulgaris*. The larger the clutch, the more time the male spent incubating and the less time he spent attracting an additional female (i.e. singing near and carrying green nesting material into adjacent empty nestboxes). Reduced paternal incubation resulted in lower overall incubation (the female did not compensate) and lower hatching success. Immediately after experimental reduction of clutches, males spent significantly less time incubating and more time singing and carrying greenery, and vice versa for experimentally enlarged clutches. Males with experimentally reduced clutches attracted a second female more often than males with experimentally enlarged clutches. This is the first study, to our knowledge, to provide experimental evidence for an adjustment of paternal care and male mate attraction effort to clutch size. However, a trade-off between paternal nestling provisioning and mate attraction was not revealed, probably due to the absence of unpaired females by that time in the breeding season. Experiments showed that the relative contribution of the male and female to nestling provisioning was unrelated to brood size.

Introduction

Males of many bird species contribute to one or more aspects of parental care, such as incubation of eggs and feeding of the young (Clutton-Brock 1991). In many of these species, males also seek additional mates (Yasukawa & Searcy 1982; Hannon 1984; Breiehagen & Slagsvold 1988; Dunn & Hannon 1991; Veiga 1992; Kempenaers 1994; Slagsvold & Lifjeld 1994; Sandell & Smith 1996) or copulations with females other than their social mate (extra-pair copulations; EPCs), which can result in extra-pair fertilisations (EPFs) (Birkhead *et al*. 1990; Gibbs & Faaborg 1990; Kempenaers *et al*. 1992; Birkhead & Møller 1992; Dixon *et al*. 1994; Westneat & Webster 1994; Wetton *et al*. 1995). Currently, there is considerable interest in the consequences of mating status and EPFs for the amount of parental care provided. Most research has focused on paternal care in relation either to a change in mating status (Orians 1969; Searcy & Yasukawa 1989; Slagsvold & Lifjeld 1994; Bruun *et al*. 1997; Smith & Sandell 1998), or to a perceived loss of paternity in their nest (Whittingham *et al*. 1992; Westneat & Sherman 1993; Westneat & Sargent 1996). However, the amount of care provided by the male may also vary inversely in relation to his opportunities to attract additional mates (Emlen & Oring 1977) or EPCs (Trivers 1972; Maynard Smith 1978b; Beecher & Beecher 1979; Patterson *et al*. 1980; Westneat *et al*. 1990). So far, this latter prediction has received less attention (Whittingham 1993; Smith 1995; Cucco & Malacarne 1997; Magrath & Elgar 1997; Smith & Härdling 2000). One factor that may affect the benefits of paternal care is clutch or brood size. Males attending large clutches (broods) may have relatively less to gain from pursuing EPCs or attracting additional mates than males with small clutches (broods) (Westneat 1988; Wright & Cuthill 1990; Westneat *et al*. 1990; Whittingham 1993; Magrath & Elgar 1997; Smith & Härdling 2000). Consequently, the size of the clutch or brood may influence the conflict between paternal care and mate attraction. Given that males, compared with females, typically have greater opportunities for increasing their reproductive success through EPCs or by attracting additional mates and may therefore pay a higher cost in terms of lost fitness by providing care, additional eggs in a clutch should be of greater value to males than females (Smith & Härdling 2000). The only empirical support for a clutch-size-related response comes from a study on fairy martins *Hirundo ariel*, in which males with smaller than average clutches contributed less to incubation and were more responsive to the proportion of fertile females in colonies than males with larger clutches (Magrath & Elgar 1997). Until now, there has been no experimental support for the effects of clutch or brood size on male participation in parental care on the one hand, and mate attraction, or extra-pair mating effort, on the other hand (Webster 1991; Smith 1995; Magrath & Elgar 1997; Smith & Härdling 2000). In this study on the European starling *Sturnus vulgaris*, we test experimentally for the existence of such a trade-off.

The facultatively polygynous European starling is a semi-colonially breeding, hole-nesting passerine. Starlings have communal feeding areas and males defend only the nest hole, not the food resource (Feare 1984). Monogamous males contribute substan-

tially to both incubation and feeding of the young and provide significantly more care than polygynous males (Pinxten & Eens 1994; Smith *et al*. 1995; Sandell & Smith 1996). EPCs occur with already mated females, usually neighbours (Pinxten & Eens 1990; Pinxten *et al*. 1993; Smith & von Schantz 1993; Pinxten & Eens 1994). Males engaging in extra-pair courtship approach non-mate females very closely (usually high up in trees) and then start singing to invite copulation (Pinxten & Eens 1990; Pinxten & Eens 1997). Conversely, paired males trying to attract an additional mate must first occupy an additional nestbox. They usually sing very close to or in this nestbox using 'wing-waving' displays (Feare 1984; Pinxten & Eens 1990; L. Brouwer and J. Komdeur, personal communication) and carry green nesting materials into the nestbox when females are in close proximity to the nestbox (Pinxten *et al*. 1993; Gwinner 1997; L. Brouwer and J. Komdeur, personal communication). The frequency of male singing close to an empty nestbox during incubation of the first clutch is positively associated with the acquisition of a secondary mate (Merkel 1978; Cuthill & Hindmarsh 1985; Eens *et al*. 1990; Mountjoy & Lemon 1991; Eens *et al*. 1991; Smith 1995; Pinxten & Eens 1998; this study). We considered only breeding pairs that were monogamous before the onset of clutch and brood size manipulations and that had one empty nestbox within 2 m of their own nestbox (i.e. within the territory already defended by the male, Smith 1995). This study was designed to answer four questions: (I) does clutch or brood size affect the amount of parental care?; (II) what are the benefits of paternal care in terms of relieving the female partner and hatching and fledging success?; (III) does clutch or brood size influence paternal care and mate attraction effort?; and (IV) does this opportunity for EPCs or polygyny influence the amount of paternal care? The first three questions were examined experimentally by manipulating clutch and brood sizes and nestbox availability, and by monitoring the male and female reproductive behaviours before and after manipulations.

Methods

Study population and observations of focal pairs

The starlings were studied at three colonies (52, 34 and 26 nestboxes) at Vosbergen, near Groningen (The Netherlands) from 14 April to 30 June 1999 and from 17 March to 28 June 2001. The colonies were separated by at least 500 m. Each colony consisted of uniform nestboxes, situated ca. 6 m apart at a height of 2.5 m. During incubation, most males and females were colour marked and, during the nestling phase, the remainder were captured and colour marked. We observed all breeding pairs during the entire breeding cycle (1999: 47 pairs; 2001: 31 pairs) to quantify reproductive behaviour and male mating status (monogamous or polygynous). The nestboxes were checked daily between 07:00 and 10:00 for the presence of eggs and start of incubation (determined by sensing the egg temperature). For each egg within a clutch, the laying date, and, if hatched, the hatching date, were determined by numbering the eggs with indelible ink. Intraspecific brood parasitism (the presence in a nest of two or more new

eggs in one day, Yom-Tov 1980) was not observed in our population. Observations on incubation and nestling provisioning were related to the start of incubation (i-day 0) and to hatching of the first egg (h-day 0), respectively. All nests with a clutch were monitored at i-day 5 for 90 min between 10:00 and 13:00, the time window when males perform most singing to obtain a secondary mate or EPCs (Smith 1995; Pinxten & Eens 1997; Pinxten & Eens 1998). Telescopes, situated 50-60 m away from the focal box, allowed proper detection and identification of individuals as they arrived at and departed from their nest. When birds were not colour marked, the sexes were distinguished by bill coloration and plumage characteristics of breast and abdomen (Feare 1984). For each sex, we measured incubation attendance (proportion of time spent in the nestbox) and recesses (proportion of time spent outside nestbox). Ambient temperature was also recorded during each incubation watch, because temperature has previously been shown to affect male and female contributions to incubation (Smith *et al*. 1995). From i-day 12 until h-day 6, each clutch was checked three times daily (between 08:00 and 18:00) for hatching and for the presence of unhatched eggs. Nestlings were individually marked by clipping the nails of specific toes immediately after hatching, and were individually colour ringed between 8 and 11 days of age. All nests with broods were monitored at h-day 12 for 90 min between 10:00 and 13:00, following the same protocol as above. For each sex, we measured the frequency at which food was delivered to the young.

During each observation in 1999, we collected additional data for each focal male on their opportunities for polygyny and EPCs. Given that most polygynous males hold secondary females in the closest neighbouring nestbox (63.2%, $n = 19$ (Pinxten *et al*. 1989); 77.5%, $n = 40$ (Smith *et al*. 1994); 71.0%, $n = 7$ (1999 data of this study)), we quantified the opportunity for polygyny by the availability of an empty nestbox on either side of the focal nestbox. If these nestboxes were occupied, we evaluated whether these neighbouring females were fertile and hence provided the possibility for extrapair matings. Females were considered to be fertile from 6 days before they laid their first egg until the day when they laid their penultimate egg (Arvidsson 1992; Møller 1994). In 2001, we manipulated the availability of empty nestboxes (see next section).

Manipulation of nestbox availability and observations on mate attraction effort

For the analyses of a trade-off between parental care and attraction of a secondary mate, only breeding pairs were used that had an opportunity to attract an additional female. These are breeding pairs with one empty nestbox available within 2 m of the occupied nestbox. Among the 34 observed monogamous males during the 1999 season, only 10 males had one and 4 males had two empty neighbouring nestboxes available. During the 2001 season, we increased this sample through manipulation of nestbox availability to give each of the 31 monogamous males an equal opportunity to attract an additional female. On i-day 1, we removed all nestboxes within a radius of 8 m from the focal nestbox and provided the focal male with one extra nestbox. The new nestbox was placed within 1.5-2 m of the original one, i.e. within the territory already defended by the male to avoid affecting the cost of defending nestboxes (Smith 1995).

During all incubation watches of these males (14 in 1999 and 31 in 2001) and food provisioning watches (12 watches in 1999 and 24 in 2001), we monitored the focal male's (always colour ringed) efforts to attract secondary females and gain EPCs. These observations were conducted by two observers simultaneously; one observed the nestbox and the other the focal male. The focal male's effort to attract an additional mate was scored as the duration of singing with 'wing-waving' within 2 m of the empty nestbox (hereafter termed 'nestbox singing') and the frequency of carrying green material into the nestbox. The focal male's effort to obtain EPCs was scored as the duration of singing high up in the trees within 5 m of a non-mate female (hereafter termed 'EPC singing').

Clutch size manipulations

Twelve sets of three monogamous pairs were selected (four in 1999 and eight in 2001) that had laid clutches of the same size (either five or six eggs) on the same date and had one empty nestbox within 2 m of their own nestbox. At all nests, incubation attendance of both sexes was measured on i-day 5 for 90 min between 10:00 and 13:00. Each of the three pairs of observers present was randomly assigned a nestbox. Within each set, on i-day 5 (half way through the incubation period of 12 days) between 14:00 and 16:30, one clutch was enlarged by three eggs, one was reduced by three eggs and one remained at the same size (control). Experimental and control treatments were randomly assigned to nestboxes. To control for potential effects of foster-egg appearance on discrimination abilities of starlings, all nests, including controls, had similar fractions of new eggs. The manipulations within each set were always conducted by one person, keeping the other observers ignorant of the manipulation treatment. The manipulated nests were observed again on i-day 6 for 90 min between 10:00 and 13:00. Of each pair within each set, mate attraction and EPC effort of the focal male and the amount of fresh green nesting material in the neighbouring nestbox (for methods, see previous section), were monitored during the observation periods before and after clutch manipulations. At i-day 11 (1 to 2 days before hatching), the exchanged eggs ware returned to their original clutch, thus recreating the original clutch sizes.

Brood size manipulations

Nine sets of three monogamous pairs were selected (four sets in 1999 and five sets in 2001) that had broods of the same size (either five or six nestlings) of which the first young hatched on the same day and had one empty nestbox within 2 m of their own nestbox. All nests were monitored and manipulated at h-day 12 (half way through the nestling period of 20 days) and monitored again on h-day 13. For observations and manipulations, we used the same protocol and the same observation hours as described in the previous section. We measured the number of feeding visits for each sex, mate attraction effort and EPC effort of the focal male (for methods, see above).

Data analyses

Each pair produced only one clutch and observations in 1999 and 2001 involved different pairs to avoid duplications. For each pair, the laying date of the first egg was related to the date when the first egg in the colony was laid in that year (laying date 1). Total incubation attendance is expressed as the sum of attendance by both parents. The male's relative contribution to either incubation or food provisioning is expressed as his contribution to total incubation or total food provisioning, respectively. For either sex, the change in incubation attendance was calculated as the attendance at i-day 6 minus attendance at i-day 5, the change in food provisioning rate as provisioning rate at h-day 13 minus provisioning rate at h-day 12, and in both cases corrected for the change in incubation attendance and food provisioning rate at the control clutch or brood. For males, the change in mate attraction effort (nestbox singing duration and frequency of carrying green material) and EPC effort (EPC singing duration) were calculated in the same way. Because most variables deviated from normality, we used non-parametric statistics for most of our analyses, but we describe the data with least squares linear fits. All parametric analyses were based on arcsine-transformed data. The relation between male nestbox singing and carrying frequency of green material on the one hand, and the acquisition of a secondary mate on the other hand, were analysed by stepwise forward logistic regression. A variable enters into the equation only if the probability (P) associated with the G-test on the decrease in scaled deviance ('D') is less than 0.05. Means are expressed with standard errors, probability values are two-tailed and the null hypothesis was rejected at $P < 0.05$. Statistical analyses were performed using SPSS (version 10.0), and MLwiN (Rabasch *et al*. 2000) was used for binomial hierarchical models with nested data (for the analyses of hatching and fledging success). In the latter cases, t-tests were performed using the parameter estimate with its standard error and the degrees of freedom calculated from the number of cases at the nest level (i.e. the number of nests) minus the number of estimated parameters.

Results

Trade-off between incubation attendance and singing activity

(I) Effect of clutch size

The clutch sizes of observed nests varied from four to seven eggs, with a mean of 5.4 ± 0.9 (n = 65). Commonest clutch sizes were five (43.0%) and six (34.0%). Male incubation attendance and total incubation attendance increased significantly with clutch size, whereas female incubation attendance decreased with clutch size (Figure 9.1A). The relative contribution to incubation by the male increased significantly with clutch size (Figure 9.1A). Although male and female incubation attendances were inversely correlated, the female does not fully compensate for reduced paternal incubation (Figure 9.1A). Male and female incubation attendance were independent of year, date and temperature during the incubation observation (general linear model: male: $F_{1,61}$ = 0.02, P = 0.90, $F_{1,61}$ = 2.70, P = 0.11 and $F_{1,61}$ = 0.19, P = 0.66, respectively;

female: $F_{1,61} = 0.04$, $P = 0.85$, $F_{1,61} = 0.55$, $P = 0.46$ and $F_{1,61} = 0.09$, $P = 0.77$, respectively; relative male incubation attendance: $F_{1,61} = 0.002$, $P = 0.97$, $F_{1,61} = 2.76$, $P = 0.10$ and $F_{1,61} = 0.58$, $P = 0.45$, respectively). Of the 41 clutches, which remained constant in size during the entire incubation period (65 clutches - 24 size manipulated clutches = 41 clutches), male contribution to incubation explained most of the variance in hatching success (binomial hierarchical linear model; male incubation: $t_{39} = 4.27$, $P < 0.001$). A decreased male contribution to incubation of clutches was associated with the likelihood of hatching failure. After controlling for male incubation attendance, female incubation attendance and clutch size had no effect on hatchability (female incubation: $t_{37} = 0.73$, $P = 0.47$; clutch size: $t_{37} = 1.51$, $P = 0.14$). Given this important role of male incubation for hatching success, males with larger clutches may be expected to allocate more time to incubating the clutch and less time to attracting additional mates or pursuing EPCs.

Considering only those males with at least one empty nestbox within 2 m of their own nestbox, there are two lines of evidence for a trade-off between incubation attendance and attraction effort of an additional mate. The larger the clutch, the more time spent incubating and the less time spent singing near the empty neighbouring nestbox, and the lower the frequency of carrying green nesting material into the empty nestbox by the focal male (Figure 9.1B). Three of the four males that had two empty nestboxes within 2 m of their own nestbox tried to attract an additional partner and always used the same empty nestbox (the other empty nestbox was not used). Hence, these four males are henceforward classified as males with one empty nestbox. Within individual males, the average amount of singing time near the empty nestbox was significantly higher than the average amount of singing time to obtain EPCs (mean nestbox singing: 6.3 ± 1.1%; mean EPC singing: 1.9 ± 0.4%; Wilcoxon paired-sample test: $Z = -3.72$, $n = 45$, $P < 0.001$). The male's EPC singing effort was independent of clutch size ($r^2 = 0.04$, $n = 45$, $P = 0.214$). Of the 21 males on which singing observations were conducted and whose clutches remained constant in size during the entire incubation period (45 males with singing observations - 24 males with size manipulated clutches = 21 males), five males had acquired a secondary female (all in the neighbouring nestbox) during the incubation period of the primary female (males were seen attending both nests). Both the amount of time spent singing near the empty nestbox and the carrying frequency of green nesting material by these males were positively associated with the chance of becoming polygynous at a later stage (nestbox singing explained most of the variance: $D_1 = 11.81$, $n = 21$, $P < 0.001$; carrying greenery (controlled for nestbox sing-ing): $D_1 = 5.95$, $n = 21$, $P = 0.015$). The experimental clutch size manipulations confirmed the plasticity of this trade-off within individual males. One day after the experimental reduction of clutch size, male incubation attendance had decreased and both nestbox singing activity and carrying frequency of greenery had increased significantly compared with the day before, and *vice versa* for males of experimentally increased clutch sizes (Figures 9.2A and 9.3). By contrast, incubation attendance, sing-ing activity and carrying frequency of greenery of control males remained the same during corresponding periods (Figure 9.2A). The larger the decrease in incuba-

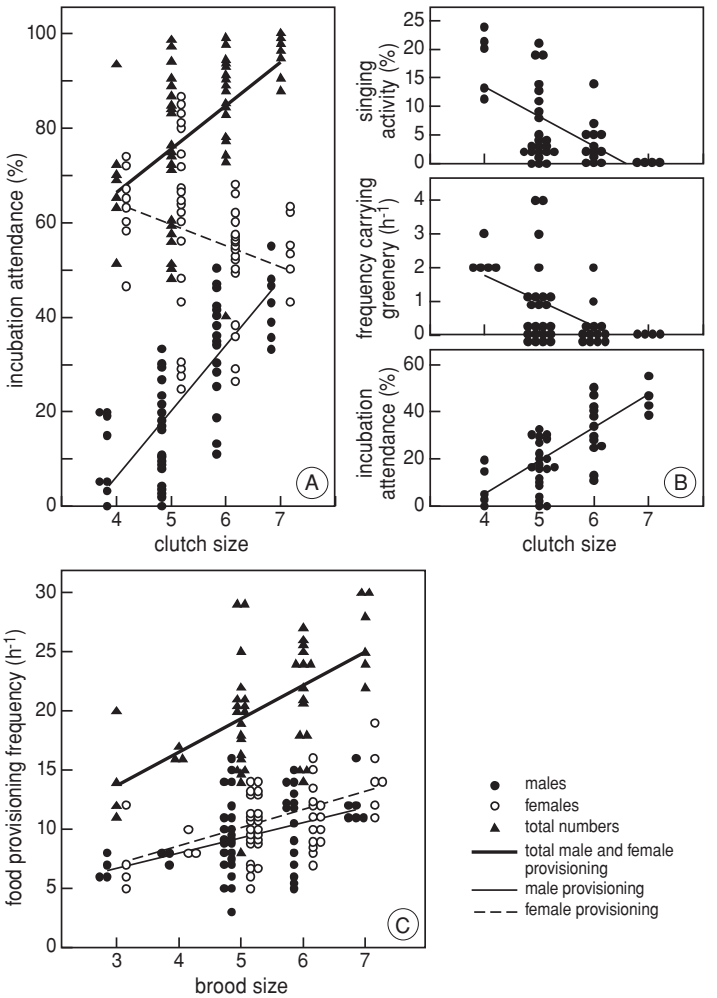

Figure 9.1A Incubation attendance at day 5 of incubation (*y*-axis) in relation to natural clutch size (*x*-axis) by monogamous male and female starlings (male: $y = 13.84x - 51.32$; $r^2 = 0.56$, $n = 65$, $P < 0.001$; female: $y = -4.47x + 80.79$; $r^2 = 0.06$, $n = 65$, $P = 0.048$; total: $y = 9.28x + 29.01$; $r^2 = 0.22$, $n = 65$, $P < 0.001$). The relative contribution to incubation of the male (*y*) increased significantly with clutch size ($y = 0.13x - 0.44$; $r^2 = 0.43$, $n = 65$, $P < 0.001$).

B. Nestbox singing activity, frequency of carrying greenery into the empty nestbox and incubation attendance (*y*-axis) of the same males at day 5 of incubation in relation to natural clutch size (*x*-axis) (nestbox singing: $y = -5.13x + 33.84$; $r^2 = 0.35$, $n = 45$, $P < 0.001$; green carrying: $y = -0.73x + 4.68$; $r^2 = 0.27$, $n = 45$, $P < 0.01$; incubation: $y = 13.83x - 50.08$; $r^2 = 0.57$, $n = 45$, $P < 0.001$).

C. Food provisioning frequency by monogamous male and female starlings on day 12 after hatching of the first egg (*y*-axis) in relation to natural brood size (x-axis) (male: $y = 1.30x + 2.80$; $r^2 = 0.18$, $n = 52$, $P < 0.005$; female: $y = 1.55x + 2.30$; $r^2 = 0.28$, $n = 52$, $P < 0.001$; total: $y = 2.84x + 5.10$; $r^2 = 0.69$, $n = 52$, $P < 0.001$). The relative contribution to food provisioning of the male was independent of brood size ($r^2 = 0.01$, $n = 52$, $P = 0.98$).

tion attendance, the more pronounced the increases in both singing time near the empty nestbox and carrying frequency of green nesting material into the empty nestbox, by the focal male (Figure 9.2A). Males with control or enlarged clutches remained monogamous through-out the breeding season, whereas 33.0% of males with reduced clutches had attracted an additional female into their empty neighbouring nestbox during the incubation period of their first female. This difference in the chance of becoming polygynous was significant (Kendall's W-test: $X^2_2 = 8.00$, $n = 13$ sets, $P = 0.018$). In contrast to male incubation, clutch size manipulations had no effect on female incubation attendance (Figure 9.2A). However, one day after the experimental reduction of clutch sizes, a decrease in male incubation attendance was associated with an increase in female incubation attendance, and vice versa for enlargement of clutch sizes (Figure 9.2A).

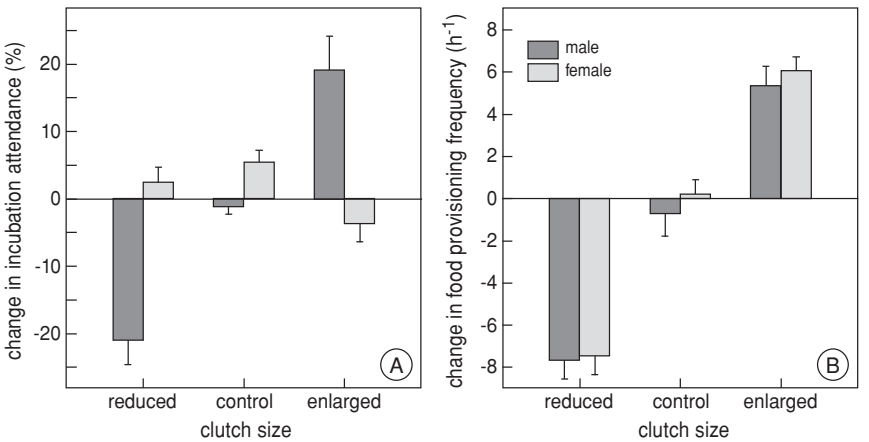

Figure 9.2A The influence of clutch size manipulations on change in percentage incubation attendance time within individual male and female starlings (male: $Z = 3.06$, $n = 12$ sets, $P < 0.005$; female: $Z = 1.25$, $n = 12$ sets, $P = 0.21$). One day after the experimental reduction or enlargement of clutch sizes, the change in male incubation attendance (y) was inversely associated with the change in female incubation attendance (x) ($y = -1.27x - 2.19$; $r^2 = 0.23$, $n = 24$, $P = 0.019$).
B. The influence of brood size manipulations on change in food provisioning rate within individual male and female starlings (male: $Z = 2.67$, $n = 9$ sets, $P < 0.01$; female: $Z = 2.68$, $n = 9$ sets, $P < 0.01$). One day after the experimental reduction or enlargement of brood sizes, male food provisioning rate had changed to the same extent as female food provisioning rate compared with the day before ($y = 0.93x - 0.47$; $r^2 = 0.88$, $n = 24$, $P < 0.001$). For the analyses of A and B only those males were included which had one empty nestbox within 2 m of their own nestbox. Black bars represent males and open bars represent females.

(II) EFFECT OF OPPORTUNITY FOR POLYGYNY

The investment in incubation attendance of the male was significantly associated with his opportunity for polygyny. Male incubation attendance (controlled for the effect of clutch size) was negatively related to the number of empty neighbouring nestboxes on either side of the focal nestbox (Figure 9.4), but unrelated to the number of fertile females in neighbouring nestboxes of the focal nestbox ($F_{2,32} = 1.14$, $P = 0.34$).

Brood size and trade-off between food provisioning and singing activity

The brood sizes of monogamous breeding pairs varied from three to seven nestlings, with a mean of 5.3 ± 0.1 ($n = 52$). The commonest brood sizes were five (46.0%) and six (29.0%). Across broods, the food provisioning rate of both male and female increased significantly with brood size (Figure 9.1C). However, the relative contribution to food provisioning of the male was independent of brood size (Figure 9.1C). In addition, mean total food provisioning per nestling decreased significantly with brood size

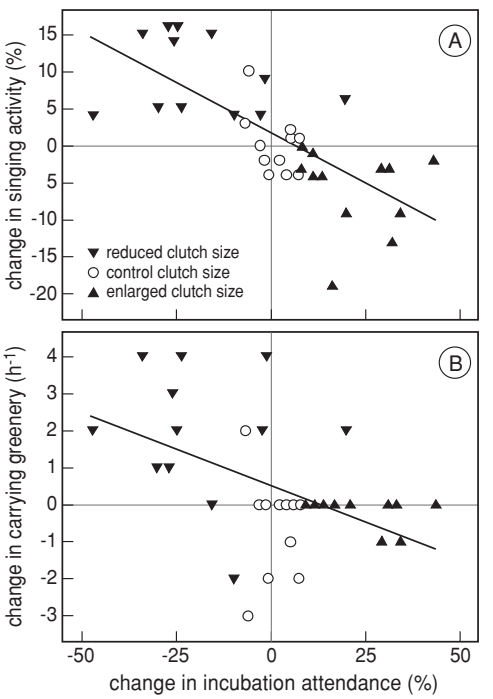

Figure 9.3A The influence of clutch size manipulations on change in percentage incubation attendance time (*x*-axis) and changes in percentage nestbox singing time (*y*-axis) within individual male starlings (nestbox singing: y = -0.28x - 1.61; $r^2 = 0.50$, $n = 36$, $P < 0.001$; $Z = 3.07$, $n = 12$ sets, $P < 0.005$).

B. The influence of clutch size manipulations on change in percentage incubation attendance time (*x*-axis) and carrying greenery (*y*-axis) within individual male starlings (green carrying: $y = -0.038x - 0.44$; $r^2 = 0.22$, $n = 36$, $P < 0.005$; $Z = -2.58$, $n = 12$ sets, $P = 0.010$). For the analyses of A and B the same males were included, all of which had one empty nestbox within 2 m of their own nestbox.

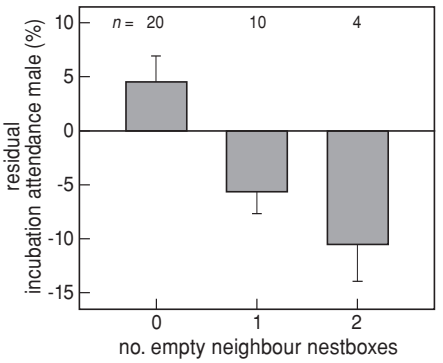

Figure 9.4 The association between polygyny potential and residual male incubation attendance (controlled for clutch size) at day 5 of incubation during the 1999 breeding season (polygyny potential expressed as the presence of 0-2 empty neighbour nestboxes on either side of the focal nestbox; $F_{2,32} = 7.59$, $P < 0.005$).

($r^2 = 0.09$, $n = 52$, $P = 0.028$). Of the 34 broods, which remained constant in size during the entire nestling period (52 broods - 18 size manipulated broods), the rate of food provisioning by either parent, controlling for brood size, had no effect on fledging success (binomial hierarchical linear model; male feeding: $t_{31} = 0.30$, $P = 0.77$; female feeding: $t_{31} = 0.17$, $P = 0.87$; brood size in both cases: $t_{31} > 0.51$, $P > 0.60$).

Considering only monogamous males with one empty nestbox within 2 m of their own nestbox, there was no evidence for the existence of a trade-off between food provisioning rate and mate attraction effort by the male. First, males were never observed singing near the empty nestbox during the observations on food provisioning ($n = 52$). Second, these males were never observed carrying green nesting material into the empty nestbox. Third, one day after the experimental reduction of brood size, both male and female food provisioning rate had decreased to the same extent compared with the day before, and, vice versa, for experimentally enlarged broods (Figure 9.3B). During this period, unpaired and fertile females were absent from the colonies.

Discussion

Parental care in relation to clutch or brood size

In species with uniparental incubation, there is some evidence that larger clutches are perceived as more valuable. In Wilson's phalaropes *Phalaropus tricolor* with unipaternal incubation, males are more likely to abandon experimentally reduced clutches (Delehanty & Oring 1993), while female European barn swallows *Hirundo rustica* increased attendance to experimentally enlarged clutches (Jones 1987). In species with biparental incubation, there are only two studies that have considered male incubation in relation to clutch size. In the fairy martin, with fertile females present, male incubation attendance was positively associated with clutch size (Magrath & Elgar 1997).

However, in captive breeding zebra finches *Taeniopygia guttata*, male incubation was independent of clutch size (Delesalle 1986), but this could be due to the fact that males had no access to other fertile females because pairs were isolated, physically and visually, in cages. In the starling, male incubation attendance increased markedly in relation to clutch size, while female incubation declined. However, the observed increase in male contribution to larger clutches could also be explained by several other factors, other than the greater reproductive value of the clutch *per se*. (I) Elevated energetic costs of incubating a larger clutch (reviewed by Thomson *et al*. 1998), causing the male to perform a greater share of incubation to relieve the female. An increase in the metabolic cost of incubating larger clutches has been shown for the starling (Biebach 1984) and several other species (e.g. blue tit *Parus caeruleus*, Haftorn & Reinertsen (1985); great tit *Parus major*, Mertens (1977); zebra finch, Vleck (1981); Bengalese finch *Lonchura striata*, Coleman & Whitall (1988)). These studies indicate that the cost of additional eggs is greatest at low ambient temperature. If metabolic cost is an important consideration, and given that in our study female incubation attendance decreased with clutch size, then relative male incubation attendance should increase with declining ambient temperature as the energetic cost of incubation increases. However this was not the case. (II) Assortative pairing, in which males paired to females that produce larger clutches are of higher quality, contributing more to paternal care (e.g. moorhen *Gallinula chloropus*, Petrie 1983). However, in our study population, the relative male contribution declined with experimentally reduced clutch size, re-futing the effect of assortative pairing on male incubation attendance. (III) The production of eggs has been shown to be costly, which may affect parental care (Carey 1996). The production of a larger clutch may affect female condition, thus females are more likely to decrease incubation to regain energy losses (Carey 1996) and the males are responding to this reduction by increasing their investment (Smith & Härdling 2000). Although our clutch size manipulation experiments demonstrated that the change in female incubation attendance was inversely associated with the change in male incubation attendance (Figure 9.2A), this cannot be caused by differential female production costs. In our experiment, we compared similar initial clutch sizes of presumed similar production costs.

In a range of biparental species, relative male contribution to nestling feeding varies with brood size. Some studies show that relative male contribution increases with brood size (Grundel 1987; Westneat 1988; Carey 1990; Wright & Cuthill 1990; Sanz 1997), others reported no change in relative contribution (Leffelaar & Robertson 1986; Breitwisch *et al*. 1986; Moreno 1987; Jones 1987; Smith *et al*. 1988; Verhulst & Tinbergen 1997) and, in a few species, relative male contribution declines with brood size (Hegner & Wingfield 1987; Buitron 1988). In contrast to the male starling's relative incubation attendance, the male's relative contribution to nestling feeding in our data was independent of brood size, suggesting that the reproductive value of the brood was similar for the male and female of pairs (see also next section).

Clutch size and sexual conflict

Since clutch size is a female trait, because a male has little opportunity of influencing clutch size directly, this trait will evolve to the optimum value of females. However, the optimal clutch size from a female's perspective will depend on the amount of paternal care that she expects her mate to provide. The sexual conflict over parental care will, in turn, be affected by clutch or brood size, since a larger clutch or brood makes male parents more valuable in species with biparental care (Smith & Härdling 2000). However, so far it has been difficult to deduce whether the amount of paternal care is affected by a trade-off between attracting additional mates and the importance of male care for the fitness of primary clutches or broods. Evidence for a trade-off between male incubation and mate attraction comes from only two studies (empirical evidence: fairy martin, Magrath & Elgar 1997; experimental evidence: European starling, Smith 1995). Our study is, to our knowledge, the first to offer an experimental demonstration and quantification that clutch size affects the amount of parental care and mate attraction effort in males (Westneat 1988; Wright & Cuthill 1990; Westneat *et al*. 1990; Whittingham 1993; see also Magrath & Elgar 1997). (I) We showed that the amount of time that males spent incubating was inversely related to singing activity near the empty nestbox, carrying frequency of green nesting material into the empty nestbox (Figure 9.1B) and the chance of becoming polygynous. The amount of incubation had no effect on male singing time to obtain EPCs. (II) Reduction of clutch size resulted in males spending significantly more time singing near the empty nestbox and carrying greenery than males in the control group (and *vice versa* for males that had clutches enlarged; Figure 9.2A). Clutch size manipulation also influenced the chances of males becoming polygynous. (III) We found that the greater the reduction in male incubation attendance in the experimental group, the larger the increase in nestbox singing activity and carrying frequency of greenery (and *vice versa* for males that increased incubation attendance) (Figure 9.3). However, there is no evidence for the existence of a trade-off between male care for nestlings and mate attraction effort. This is, perhaps, unsurprising because unpaired and fertile females were absent during the nestling period.

Males should only be expected to allocate less time to attracting additional mates or pursuing EPCs if there is a cost to reduced male participation in incubation (Smith & Härdling 2000). In starlings, there is a cost of lower male attendance of the clutch because (I) there is incomplete compensation in incubation effort between males and females. Females do not fully compensate for experimentally reduced male assistance (Wright & Cuthill 1989; Pinxten *et al*. 1993; this study; Figure 9.2A); and (II) reduced male contribution to incubation (controlled for clutch size) significantly increases the likelihood of hatching failure. This study concludes that, at the proximate level, the resolution of the trade-off between paternal care and mate attraction effort is adjusted to clutch size. This study has shown that a trade-off between paternal and mate attraction effort is the main explanation for the increase in male contribution to incubation with clutch size. We expect that similar clutch-size-related sex differences in parental care patterns occur in any species that exhibit biparental care and that are known to exhibit EPFs or facultative polygyny.

Acknowledgements

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Chapter 10

Working for a living: physiological and behavioural trade-offs in birds facing hard work

Popko Wiersma

Cost of reproduction

Experimental data generate a picture that birds are reluctant to increase energy expenditure (Wiersma & Tinbergen 2003: Chapter 5; Wiersma & Verhulst 2003a: Chapter 2; Wiersma *et al*. 2003a: Chapter 4). By decreasing night-time energy expenditure, body mass, flight costs or non-foraging activity birds are able to achieve appreciable savings on their daily energy budget. Apparently, spending more energy entails disadvantages. To find out what these are, one can look for effects of experimentally induced increases in work levels. The most widely used experimental avenue to achieve this is by manipulating brood size. By reducing or enlarging broods parent birds are 'provoked' to adjust the energy they put into food provisioning. The advantage of manipulating parental effort is that fitness costs can relatively easily be measured through their current, and additionally, future reproductive success. Although detrimental effects of increased parental effort on adult survival have been shown, the nature of the mechanisms involved remains elusive. The immune system is potentially an important mediator in the costs of reproduction (Sheldon & Verhulst 1996). To what extent energy plays a role in a trade-off with the immune system was the focus of Chapter 7 (Verhulst *et al*. 2003). We also explored a novel potential candidate that might play an important role in mediating costs of reproduction, namely the production of oxygen free radicals and associated molecules in energy metabolism processes (Wiersma *et al*. 2003b: Chapter 8). In addition to these factors we have examined an aspect of competition for time (Komdeur *et al*. 2002: Chapter 9).

Time reallocation as a cost of reproduction

In addition to the physiological trade-offs, such as those with immunological and antioxidant processes, time also has to be allocated between different activities. Time invested in parental effort cannot be spent on other activities, including social behaviour. Activities that may become affected are, for example, vigilance, increasing the risk of predation (Magnhagen 1991), or preening, increasing the risk of damage to feathers and infestation with parasites. Also the time devoted to conspecifics may be affected. Birds of (facultative) polygynous species, that spend more time on incubation, brooding or food provisioning, will have less time left for extra-pair liaisons and for mate guarding. The trade-off between pursuing extra-pair fertilisations and caring for the current clutch or brood will be affected by the size of the clutch and brood, because that is expected to affect how the clutch or brood is valued (in terms of fitness) by the parents (Delehanty & Oring 1993). Males attending large clutches or broods may have relatively less to gain from pursuing extra-pair copulations or attracting additional mates than males with small clutches or broods (Wright & Cuthill 1990; Whittingham 1993; Magrath & Elgar 1997; Magrath & Komdeur 2003).

As we expected, we found that the male starlings' *Sturnus vulgaris* pursuit for additional mates was negatively related to manipulated clutch size (Komdeur *et al*. 2002:

Chapter 9). Paired starling males trying to attract an additional mate must first occupy another nestbox. They usually sing very close to or in this nestbox, using 'wing-waving' displays (Feare 1984; Eens *et al*. 1990) and carry green plant material into the nestbox when females are nearby (Gwinner 1997; Brouwer & Komdeur, submitted manuscript). The frequency of male singing close to an empty nestbox during incubation of the first clutch is positively associated with the acquisition of a secondary mate (Pinxten & Eens 1998). We found that when we had enlarged a clutch the males indeed spend more time incubating, while spending less time singing near and bringing green plant material to an adjacent empty nest box. This resulted in a negative relationship between clutch size and the probability of getting an additional female.

Immunosuppression as a cost of reproduction

It has been repeatedly shown that challenging the immune system with antigens leads to a reduction in parental effort. Råberg *et al*. (2000) showed that blue tits *Parus caeruleus* immunised with diphtheria-tetanus vaccine (DPT) reduced nestling feeding rate. DPT vaccinated pied flycatchers *Ficedula hypoleuca* had reduced breeding success (Ilmonen *et al*. 2000). House sparrows *Passer domesticus* injected with lipopolysaccharide were more likely to desert their nest, and those that continued breeding raised fewer fledglings (Bonneaud *et al*. 2003). Apparently, when challenged, resources that were earlier expended on brood provisioning are channelled into immune processes to eradicate the alien substances.

Conversely, parental effort has also been shown to suppress immune responses, further underlining the conclusion that reproductive effort and immune responses compete for the same resources. Nordling *et al*. (1998) and Cichoń *et al*. (1998) manipulated reproductive effort of collared flycatchers *Ficedula albicollis* and found a reduced response to Newcastle disease virus vaccine and sheep red blood cells (SRBC) antigens, respectively. Moreover, Nordling *et al*. (1998) also found an increased intensity of *Haemoproteus* infections in collared flycatchers when reproductive effort was increased, and this was associated with higher mortality rates.

Although it is often assumed that mounting an immune response is energetically costly, actually measuring this is difficult due to the many confounding effects (changes in behaviour, food intake, etc.). Lochmiller & Deerenberg (2000) conclude from the data available in the literature that the associated energetic costs of mild immune challenges represent increments of 15-30% of resting metabolic rate. Measurements of the basal metabolic rate (BMR) after immunisation showed a 9% increase in free-living great tits *Parus major* challenged with SRBC (Ots *et al*. 2001), 29% of RMR in house sparrows challenged with a cellular immune response eliciting mitogen (PHA) (Martin *et al*. 2003) and 27% of RMR in laboratory mice *Mus musculus* challenged with the antigen keyhole limpet hemocyanin (Demas *et al*. 1997). However, Svensson *et al*. (1998) did not find a significant BMR increase in captive blue tits challenged with DPT, nor did Henken & Brandsma (1982) challenging domestic chickens *Gallus gallus* and Hõrak *et al*. (2003) challenging greenfinches *Carduelis chloris* with SRBC. We measured the

energetic costs of a humoral immune response in zebra finches *Taeniopygia guttata* (Verhulst *et al*. 2003: Chapter 7). The birds were immunised with either SRBC, a novel antigen, or injected with a physiological salt (PBS) as a control. The metabolic rate was not affected by the antigen immunisation around the time when maximum antibody numbers circulate in the body, which is ca. 6 days after injection (Verhulst *et al*. 2003: Chapter 7). However, earlier, immediately after immunisation there was an effect on metabolic rate, although not in the expected direction (Figure 10.1). We found a reduction in metabolic rate that was greatest during the hour immediately following the immunisation (10%) and vanished during the following 9 h. Recently, a decrease in metabolic rate after immunisation has also been found in ruffs *Philomachus pugnax* (Luisa Mendes, personal communication). As far as we are aware, similar results have not been published before, but reductions in metabolic rate may also have remained undetected due to the rapid disappearance of the effect.

The decrease in metabolic rate after immunisation that we measured does not fit the assumption that the formation of antibodies is energetically costly. A reduction in energy expenditure, however, agrees with the often reported reduction in locomotory activity as an effect of an immune challenge (Bonneaud *et al*. 2003; Hõrak *et al*. 2003). This might be linked to the reduction in food intake which is also a typical effect of an immune challenge (Lochmiller & Deerenberg 2000). We did not detect an effect of immunisation on the zebra finches' activity, though. The conflicting results of the effect of immunisation on metabolism as presented above, may be due to the differences in antigens used, dosage, health or condition of the animals, behavioural reaction to the immunisation, etc. It is important to bear in mind that energy will not be the only resource the immune system is drawing upon. The demand for proteins may be

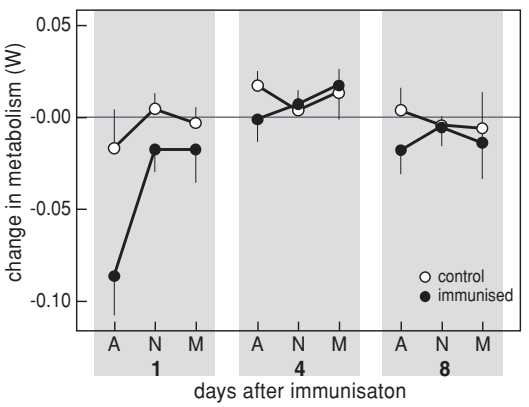

Figure 10.1 Changes in metabolic rate (in W/bird) of control and immunised birds at different times after immunisation. On the *x*-axis time since immunisation is indicated, showing data separately for the afternoon (A), night (N), and morning (M). Data shown are mean differences (±SE) between the measurement at the indicated time, and the control measurement prior to immunisation.

high, since nitrogen balance during an immune reaction is usually negative (Lochmiller & Deerenberg 2000). In addition, immune reactions are accompanied by high levels of reactive oxygen species (ROS), such as free oxygen radicals, that are damaging to DNA, proteins and fat (von Schantz *et al*. 1999).

Although the formation of antibodies has been suggested to be costly (Fair *et al*. 1999; Williams *et al*. 1999), high costs may also arise from maintenance of the immune response generating system. In that case it could be expected that the immune system would be downsized when it is not expected to be needed or when other processes drawing from the same resources gain priority. Then, instead of being the result of direct competition for resources between antibody production and parental effort, the reproductive trade-off with immune function, as has been found in birds (Nordling *et al*. 1998), could also be an effect of a downsized, low-capacity, immune system.

We tested whether reproductive effort resulted in an immune system with a smaller capacity. Assuming that if a brood size manipulation led to changes in the size or physiological make-up of the antibody-producing system, we expected that the effect of the manipulation on antibody production would not immediately disappear when the birds suddenly did not have to take care of a brood anymore. Indeed, the reduction in immune activity lingered after experimentally relieving the birds from parental duties (Figure 10.2). This was not an effect of body mass, since, at the time of immunisation, this was not related to manipulated brood size. The relationship of the immune response with brood size that we found was very similar to that found by Deerenberg *et al*. (1997) who had immunised zebra finches with SRBC while still provisioning their brood (Figure 10.2). The suggested flexibility of the size of the immune system is supported by the variation found in spleen mass, and the associated cell-mediated immune activity, between the breeding and non-breeding season in various bird species (Møller *et al*. 2003). Possibly, the immune system possesses the same adaptive flexibility as many other organs, in particular the nutritional organs and the 'exercise' organs (e.g. heart, muscles), that vary in size according to their need (Piersma 2002; Biebach & Bauchinger 2003).

Oxidative stress as a cost of reproduction

In addition to immunosuppression, as a component of maintenance and repair processes that are traded-off with reproductive effort, we studied protection against oxidative damage. Reactive oxygen species (ROS), i.e. molecules containing or generating oxygen free radicals, cause damage to fat, protein and DNA molecules, thereby impairing the functioning of cells. The damaging potential of ROS is termed oxidative stress. Because all metabolic processes that use oxygen, including the immune and detoxification systems, are continuously producing ROS (von Schantz *et al*. 1999; Finkel & Holbrook 2000), oxidative stress is inescapable. Fortunately, the effects of oxidative stress can be alleviated by a suite of endogenous and dietary anti-oxidants that scavenge for these noxious molecules (Ahmad 1995). In parallel with immunosuppression, we

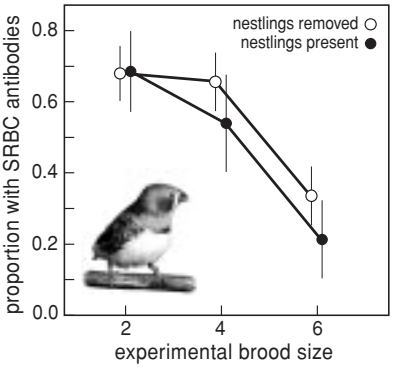

Figure 10.2 Proportion (± SE) of birds with SRBC-antibodies six days after immunisation (sexes pooled). Open dot: Proportion responders when parents were immunised after the young were removed (for brood size 2, 4, 6, n = 34, 31, 32 respectively). Immunisation took place one day after the young were removed closed dot: Proportion responders when young remained present shown for comparison (from Deerenberg *et al*. 1997).

hypothesised that endogenous antioxidant activity might be suppressed when reproductive effort increases, due to the costs involved in producing the antioxidants. This then could result in a decline in future reproductive output. Experimentally increasing parental effort generally results in a decline in future reproductive potential. Interpreting this decline as due to an acceleration of senescence (Gustafsson & Pärt 1990; Kirkwood & Austad 2000), links the studies on ageing with those on reproductive costs.

Oxidative stress has been extensively studied in the context of ageing and degenerative diseases (Ahmad 1995; Austad 1997), mostly using a proximate approach, or, when in an evolutionary context, usually on an interspecific level (see Box). However, the interaction with physical effort, and more particular parental effort, may make it an essent-ial component of life-history evolution and hence life-history decisions of individuals. We showed that captive zebra finches rearing experimentally created large broods showed lower relative activities of the antioxidant enzymes SOD and GPx than zebra finches with small broods (Wiersma *et al*. 2003b: Chapter 8). The antioxidant activity was expressed relative to the oxygen consumption rate, because that determines, at least in part, the rate at which ROS are produced. This implies that a higher reproductive effort incurs the cost of less protection against oxidative damage, leading to more damage to DNA, proteins and fats. However, changes in only one component of the oxidative stress complex are difficult to interpret. To assess the potential rate at which damage might occur information on the ROS formation rate is needed as well. We measured 2 of the 3 antioxidant enzymes, but other, mostly exogenous, antioxidant agents exist (Clarkson & Thompson 2000). Therefore, measurements on the actual oxidative damage will be essential for the interpretation of experimental results.

BOX: The disposable soma theory

Senescence is a decline in the state of the organism with age that is manifested through a reduction in the rate of survival and a decline in reproductive output rate (Partridge & Barton 1996). Theories formulated to explain variation in senescence, and presumably rates of oxidative damage, are mostly of a proximate nature. The disposable soma theory, formulated by Kirkwood (1977; Kirkwood & Holliday 1979), intends to explain the variation in ageing rates in evolutionary terms. It states that there is a trade-off between resources allocated to reproduction and to damage prevention and repair processes, and that this trade-off is mediated through extrinsic mortality rates. For example, when rates of extrinsic mortality are high, spending a great deal of resources on maintenance and repair would be a waste, because death will strike soon anyway. If more resources would have been spent on reproduction instead, fitness would have been higher. The disposable soma theory also supports comparative results by giving a functional explanation for the low rates of ageing and long life spans of species that have to some extent succeeded in escaping predators, namely birds and bats (Austad 1993). Birds have a four times longer maximum life spans than mammals with the same BMR or body mass (Austad & Fischer 1991).

Energy mamagement under demanding conditions

If energy is a limiting resource for important life-history traits, it is advantageous to manage energy as economically as possible. In this view it is worthwhile to measure energy budgets, because this may give insights into the behavioural decisions that animals make. A good example of the use of an energy budget in explaining behavioural decisions and limitations is given by de Leeuw's (1997) study on diving ducks wintering in the (Lake) IJsselmeer. By quantifying the costs for diving, flying and maintenance he defines limits to distances between roost and foraging sites. Another good example is the study on kestrels *Falco tinnunculus* by Masman *et al.* (1988), quantifying the energy budget throughout the annual cycle and approximating the energetic consequences of changes in the annual timing. In general, knowledge on energy budgets makes it possible to consider behavioural alternatives, both on a daily and annual scale, to make statements about optimisation of behaviour (i.e. diet, time budgets, reproduction, etc.) and maximisation of fitness.

To balance their daily energy budget animals have to gather food during their active period to cover the energy expenditure of the whole day and to store energy to cover the energy expenditure during the inactive period. The most straightforward way to balance the energy budget in demanding periods (e.g. when food availability is low, at low temperatures, or during build up of energy stores) is by extension of the foraging time or reduction of energy expenditure during the inactive period (McNab 2002).

Extension of the foraging time to increase the daily energy intake is limited by the available foraging time and the rate at which food can be processed (Kirkwood 1983; Weiner 1989; Lindström 1991; Kersten & Visser 1996). Increasing the food processing rate requires that the digestive system is modified to cope with the higher processing rates. Remarkable examples of the flexibility of the digestive system have been found in a range of species (Piersma & Lindström 1997). Because BMR partly reflects the size of metabolically active organ systems (the 'metabolic machinery'), a size increase of the digestive system will usually manifest itself by a BMR increase (Piersma 2002).

This process of adapting to (or even anticipating) periods of demanding conditions by enlarging particular organ systems seems to contrast with processes in animals that reduce their metabolic rate when they encounter demanding conditions. Reducing BMR by hypothermia when thermoregulatory demands are high, is well documented and seemingly quite common in small birds (Reinertsen 1989; McKechnie & Lovegrove 2002). But other energetically demanding conditions, for example when facing high foraging costs or when caring for a brood, have not received much attention when it comes to physiological adjustments. Among the few reported cases are reductions in nocturnal energy expenditure after a reduction in foraging success in hummingbird *Trochilidae* species (Tiebout 1991; López-Calleja *et al*. 1997), birds renowned for their hypothermia abilities (Krüger *et al*. 1982; McNab 2002). Yet, mass, and concurrent, BMR reductions that are not likely to be completely explained by hypothermia have been found in starlings (Bautista *et al*. 1998; Wiersma *et al*. 2003a: Chapter 4) and zebra finches (Deerenberg *et al*. 1998; Nudds & Bryant 2001; Wiersma & Verhulst 2003a: Chapter 2) when forced to increase the daily flight time. This suggests that BMR reductions (whether or not resulting from body composition changes) in response to high energetic demands are more wide-spread than was earlier taken into consideration.

Moreover, the reductions in energy expenditure as shown in zebra finches and starlings are not restricted to the night: in response to a reduction of the foraging reward rate, the energy expended during the entire day (DEE) may be economised as well (Deerenberg *et al*. 1998; Bautista *et al*. 1998; Wiersma & Verhulst 2003a: Chapter 2). That animals, forced to work harder for their food, reduce their total energy expenditure is a startling result, and makes one wonder whether the flexibility in the energy budget has been appreciated to its true extent. Many mammals and birds spend more than 60% of their active period at rest (Herbers 1981), and therefore changes in metabolism during this period have a potential for appreciable savings.

Joint effects of work on BMR and DEE

To study the effects of varying work levels on the energy budget of individual animals, partitions of this budget have to be measured simultaneously at different work loads. This, however, is a rare amalgamation. Often DEE is measured under different conditions, while BMR is estimated from allometric relationships or from prior measurements. When effects on BMR are measured, often DEE is not of direct interest. DEE values from experimental studies where work levels were manipulated, either by fora-

ging reward rate or parental demand, and where the effects on both DEE and BMR (or SMR) and on body mass were measured are shown in Figure 10.3. The figure shows that when birds increase the time spent flying and foraging, DEE does not necessarily increase too. In some cases DEE actually decreases with increases in work levels (*st1, zf1-3*). Work and DEE are therefore not interchangeable terms. Comparing the DEE data with a large number of field measurements assembled by Bryant & Tatner (1991) shows that the energy expenditure levels are not particularly high (Figure 10.4). Only the zebra finches (*zf3*) and our starlings (*st2*), both flying for food, had reasonably high energy expenditure rates. Our starling data also happen to represent the largest DEE increase of the data set and actually the starling was the only species that showed an increase in the mass-specific DEE (not shown).

Figure 10.5A-C shows the concurrent changes in body mass, mass-specific BMR (i.e. BMR divided by body mass), BMR and DEE in response to an increase in work level. To account for between-experiment differences between overall work levels and for between-species differences, the changes in DEE are shown in percentages instead of absolute values. Changes in (mass-specific) BMR are also expressed in percentages, to allow for between-species comparisons. Body mass often decreased; and in one starling study it did plummet quite dramatically (*st1*; Figure 10.5A). DEE and body mass were not associated in a straightforward way: changes in mass and DEE seem to be related according to a quadratic relationship. Clearly, there is a great deal of flexibility in both DEE and (mass-specific) BMR, and they can change in either direction with increasing work levels (Figure 10.5B, C). The positive association between changes in mass-specific BMR and DEE borders significance. The changes in mass-specific BMR are most likely

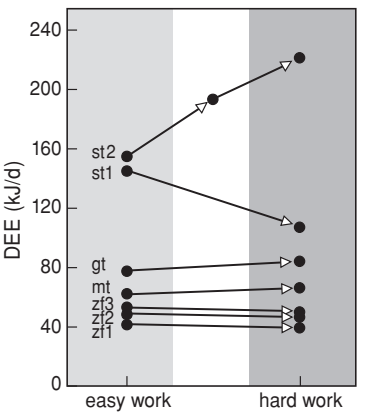

Figure 10.3 Daily energy expenditure of birds at different manipulated work levels. zf: zebra finches, *zf1*: Wiersma & Verhulst 2003a: Chapter 2, *zf2*: own unpublished results from manipulation of foraging reward rate by mixing chaff through seeds, *zf3*: Deerenberg *et al.* 1998, st: starling, *st1*: Bautista *et al.* 1998, *st2*: changes from low to intermediate and intermediate to high foraging reward rates (Wiersma *et al.* 2003a: Chapter 4), gt: great tit (Wiersma & Tinbergen 2003: Chapter 5), mt: marsh tit (Nilsson 2002).

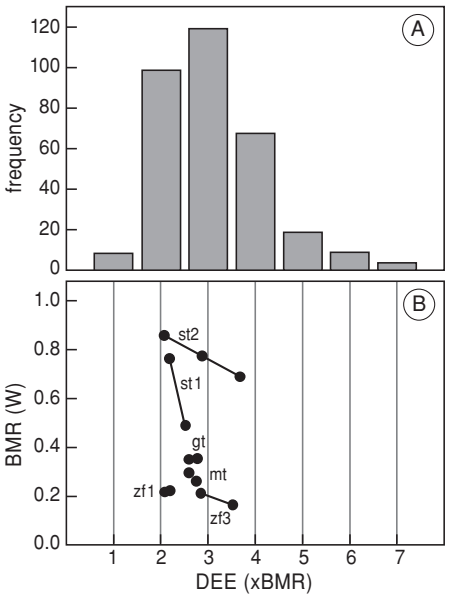

Figure 10.4 Comparison of DEE values presented in Figure 10.3 and an extensive set of field data. **A**) Frequency distribution of energy expenditure during breeding expressed as a multiple of BMR. Data for birds rearing nestlings only (n = 323, 19 species; graph from Bryant & Tatner 1991). **B**) Absolute DEE and BMR values of same birds shown in Figure 10.3, except for *zf2* of which SMR instead of BMR values were measured. The birds are either working for food or for their nestlings. zf: zebra finches, *zf1*: Wiersma & Verhulst 2003a: Chapter 2, *zf3*: Deerenberg *et al*. 1998, st: starling, *st1*: Bautista *et al*. 1998), *st2*: Wiersma *et al*. 2003a: Chapter 4, gt: great tit (Wiersma & Tinbergen 2003: Chapter 5), mt: marsh tit (Nilsson 2002).

due to body composition or body temperature changes. In most experiments BMR of the birds decreased and the marsh tit *Parus palustris* (*mt*) was the only species that increased BMR (Nilsson 2002). The association between BMR and DEE changes seems to be positive, confirming the generally reported positive relationships between BMR and DEE (Daan *et al*. 1991; Koteja 1991; but see Ricklefs *et al*. 1996). Nudds & Bryant (2001) also measured a reduction in BMR in zebra finches that were stimulated to increase their daily flight time. Unfortunately they did not measure DEE of these birds.

When analysing the association between BMR and DEE there might be a problem of inherent dependency due to the fact that BMR is incorporated in DEE (Ricklefs *et al*. 1996). To compare independent parameters, BMR (in kJ/d) can be subtracted from DEE, resulting in the energy expenditure of activity (ACT). The resulting data are shown in Figure 5D. ACT has no apparent association with BMR, suggesting that DEE and BMR are affected independently. Whether this is the appropriate combination to consider depends on how BMR and DEE are functionally related. One option is that DEE is 'generated' by an intensification of the maintenance processes that make up BMR ('shared pathways model' in Ricklefs *et al*.'s (1996) terminology). Alternatively, DEE is the summation of BMR and ACT, where BMR and ACT use separate metabolic pathways ('parti-

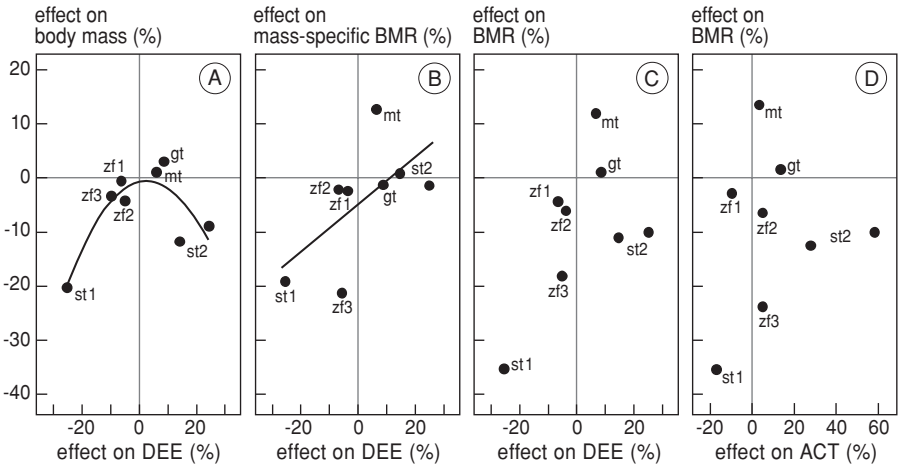

Figure 10.5 Changes in the energy budget and body mass when conditions are manipulated from low to high work levels. Associations between changes in DEE and A body mass, **B** mass-specific BMR and **C** BMR and **D** between changes in activity metabolism (ACT, i.e. DEE – BMR, both in kJ/d) and BMR in various bird species that were experimentally submitted to different work levels. The dots labelled *mt* and *gt* depict experimental data from brood size manipulations, while the others are from foraging reward rate manipulations. *zf*: zebra finches, *zf1*: (Wiersma & Verhulst 2003a: Chapter 2), *zf2*: own unpublished results from manipulation of foraging reward rate by mixing chaff through seeds, *zf3*: (Deerenberg *et al*. 1998), st: starling, *st1*: (Bautista et al. 1998), *st2*: changes from low to intermediate and intermediate to high foraging reward rates (Wiersma *et al*. 2003a: Chapter 4), *gt*: great tit (Wiersma & Tinbergen 2003: Chapter 5), *mt*: marsh tit (Nilsson 2002). Correlation analyses DEE and mass (partial correlation with DEE2, controlling for DEE): r = -0.85, P = 0.017, DEE and mass-specific BMR: $r = 0.66$, $P = 0.077$, DEE and BMR: $r = 0.58$, $P = 0.14$, ACT and BMR: $r = 0.18$, $P = 0.67$.

tioned pathways model'). Ricklefs *et al*. (1996) warn that in the second situation BMR and DEE are statistically dependent (an increase in BMR inevitably results in an increase in DEE), while in the first situation this would not be the case. Unfortunately, Ricklefs *et al*. (1996) do not specify which physiological and biochemical mechanisms might be involved in creating 'different physiological states' for active and resting animals.

Suggested mechanisms resulting in a shared pathway are the make-up and size of the organs that constitute the metabolic machinery of an organism (Daan *et al*. 1990; Piersma *et al*. 1996a; Piersma 2002). In this view, the make-up and size of the organs are related to their minimum and sustained metabolic output, reflected in BMR and DEE. This scenario of a shared pathway results in a multiplicative model, where DEE can be considered an 'inflated BMR' with the extent of inflation depending on the initial size of the 'BMR-balloon'. In this multiplicative scenario, that could be termed a 'shared sources model', BMR and DEE are dependent, as in the additive 'partitioned pathways model'. Both scenarios make a case for analysing the association between BMR and ACT instead of DEE (Figure 10.5D). A 'shared sources model' is compatible with the 'aerobic capacity' hypothesis (Bennett & Ruben 1979), stating that variation

in maximum sustained metabolic rates corresponds with the capacity of underlying metabolic and physiological systems and is therefore functionally linked with BMR (Drent & Daan 1980; Peterson *et al*. 1990; Daan *et al*. 1991). This link would be caused by the relatively high metabolic rates of some organs (in particular heart, kidney, liver and intestines) that vary in size in keeping with long-term energetic demands (Daan *et al*. 1990; Piersma *et al*. 1996a; Piersma 2002).

Explaining the pattern(s) in Figure 10.5 is complicated by the mixture of different experimental systems used that may have led to different food intake rates. First, there is a difference in the type of work: brood food provisioning or foraging. The birds caring for a brood tend not to decrease BMR and body mass with increased work levels, while the birds working for food do decrease BMR and body mass with increased work levels (Figure 10.5A, C), although this difference is not apparent in mass-specific BMR (Figure 10.5B). Second, when foraging involves mainly flying, foraging decisions can have large consequences for the energy expenditure, and hence food intake may differ from birds foraging with cheaper locomotory means. In the experiments where the birds had to fly for food (*zf3, st1* and *st2*), DEE had increased most, while mass tended to decrease most (Figure 10.5). Third, the presence or absence of variation in the foraging reward rates can have important consequences (Fotheringham 1998), as will be discussed below.

Because a large brood size is associated with a high parental effort, we hypothesised that having large exercise organs could be beneficial for raising successful nestlings. Because of the link between BMR and the size of the exercise organs (Piersma 2002), we measured whether birds with a high BMR would have a high reproductive output (Wiersma & Verhulst 2003b: Chapter 6). (Because the measurement temperature was slightly below the lower critical temperature, we refer to these measurements as SMR, not BMR.) In both sexes, SMR and manipulated brood size were not correlated. Also the change in SMR from just before nest building to brood provisioning was not affected by manipulated brood size, nor was SMR before nesting correlated with original clutch size. Nilsson (2002) reported an increase in BMR in marsh tits with experimentally enlarged broods and we did not find such an effect in great tits (Wiersma & Tinbergen 2003: Chapter 5; Figure 10.5C). It is possible that the increases in parental effort in zebra finches and great tits were not strong enough to necessitate an enlargement of exercise organs, but on the other hand the marsh tits had a similar increase in DEE than the great tit (Figure 10.3). Birds that rely stronger on flight during food provisioning (e.g. many kestrels *Falco* spec. and swallows *Hirundo* spec.) might show more pronounced effects of brood size on body composition and BMR.

Decisions concerning energy management may differ between the reproductive and the non-reproductive season. This might explain why BMR of the birds working for their own food decreased their BMR while BMR did not change in the free-living great tits studied by us (Wiersma & Tinbergen 2003: Chapter 5) and increased in the marsh tits studied by Nilsson (2002) at elevated levels of parental effort (Figure 10.5C). One could argue that the increase in work levels that was achieved by enlarging the brood size may have been modest, perhaps not necessitating the saving of energy during the night. However, a BMR increase, as measured in the marsh tits, would then not be

expected. Energy savings may bear a direct fitness cost, because suppressing DEE would not enhance the nestlings' growth and condition, while the potential gain in the future reproductive output is unlikely to outweigh the costs incurred by the current brood, even more so, given that there is a risk of not having a next brood at all.

Variability in the foraging reward rate

Fotheringham (1998) studied starlings in cages where they had to collect food by flying back and forth between two perches. By manipulating the number of flights needed to receive food he could change the food reward rate (i.e. foraging efficiency) and measure the consequences for daily food intake, body mass, activity patterns and more. He also manipulated the variability of the food reward rates by adding random variation to the average number of flights needed to obtain food. This revealed major effects: when the foraging reward rate was fixed (without variation and thus entirely predictable) a decrease in the reward rate resulted in a decrease in daily food consumption and body mass, while using variable food reward rates (random but with fixed mean value), daily food consumption and body mass were not reduced. The difference is explained in terms of cognitive processes: although the average intake rate while foraging would have been identical, a variable success rate seems to offer a preferred stimulus to forage. It may in some way be related to differences in memorising fixed versus variable time intervals, as hypothesised by the scalar expectance theory (Giraldeau 1997). The difference between fixed and variable reward rates was actually already a research topic fifty years ago in the psychology tradition typified by application of the 'Skinner Box' (Ferster & Skinner 1957).

Whatever the mechanism behind the effect of variability, it is likely that variable foraging reward rates are the norm under natural conditions, while experiments are typically conducted using fixed reward rates (Table 10.1). This makes it important to find out whether nocturnal and/or day-time energy expenditure reductions also occur under more natural foraging conditions. We compared energy budgets from Bautista *et al.*'s (1998) starling experiment, who applied fixed reward rates, with those of our starlings that experienced variable reward rates (Wiersma *et al*. 2003a: Chapter 4). From Fotheringham's (1998) results we expected that, because of the variable reward rates, DEE would not decrease with a decreasing reward rate, as was the case in Bautista *et al.*'s (1998) study. Actually, DEE *increased* from 154 to 220 kJ/d with a decreasing reward rate (Figure 10.6, and '*st2*' in Figure 10.5). Our starlings further showed a major decrease in body mass (Figure 10.5A), probably largely due to reduced fat reserves and partly due to reduction of the flight muscle size (Wiersma *et al*. 2003a: Chapter 4). The starlings in the study of Bautista *et al*. (1998) also strongly decreased their body mass and reached levels that must have been close to their starvation mass. At the same time mass-specific BMR decreased with decreasing fixed reward rates, while this remained constant in our starlings (Figure 10.5B). Apparently, in these two studies the birds had different body compositions at the high work levels. The starlings kept with variable reward rates also achieved higher working levels (3.7xBMR) than the birds in the other studies. It seems that with fixed reward rates the starlings are

merely starving while with variable reward rates the birds adjust to the higher demands. Because Bautista *et al*.'s (1998) starlings had an average DEE of 144 kJ/d, which is quite similar to Wiersma *et al*.'s (2003a: Chapter 4) starlings, differences in overall work levels in the two experiments seem not to be responsible for the different results. Note, however, that the experimental effects of work on DEE have shown that 'work' and DEE are not synonymous (Figure 10.3).

The opposite effect on DEE and on body mass in our starlings compared to the results from experiments using fixed reward rates in zebra finches, starlings and two hummingbird species (Tiebout 1991; Deerenberg *et al*. 1998; Bautista *et al*. 1998; Figure 10.5C) support Fotheringham's (1998) conclusions. However, two experiments with zebra finches, manipulating foraging reward rate, also applied variable reward rates (by mixing chaff through seeds), while DEE and body mass decreased with decreasing foraging reward rate (*zf1* and *zf2* in Figure 10.5). Clearly, the presence or absence of variability in the food reward rate is not an adequate explanation for the variation in the effect of foraging reward rates on DEE in all situations.

Table 10.1 Summary of DEE measurements from experiments with birds or mammals in which foraging reward rate was manipulated in a closed economy system. The responses to a decrease in foraging reward rate are shown. DEE may have been inferred from daily food intake measurements. Reward rates could either be fixed (without variation) or variable (with variation but with fixed mean). Increases and decreases are depicted with '+' and '-', while '0' means no change.

	reward rate	DEE	source
starling *Sturnus vulgaris*	variable	+	Wiersma *et al*. (2003a):Chapter 4
starling *Sturnus vulgaris*	variable	0	Fotheringham (1998)
zebra finch *Taeniopygia guttata*	variable	0	Lemon & Barth (1992)
zebra finch *Taeniopygia guttata*	variable	-	Wiersma & Verhulst (2003a): Chapter 2
starling *Sturnus vulgaris*	fixed	-	Fotheringham (1998)
starling *Sturnus vulgaris*	fixed	-	Bautista *et al*. (1998)
zebra finch *Taeniopygia guttata*	fixed	-	Deerenberg *et al*. (1998)
steely-vented hummingbird *Amazilia saucerottei*	fixed	-	Tiebout (1991)
fork-tailed emerald *Chlorostilbon canivetii*	fixed	-	Tiebout (1991)
domestic pigeon *Columba livia*	fixed	-	Rashotte & Henderson (1988)
house mouse *Mus domesticus*	fixed	-	Perrigo (1987)
deer mouse *Peromyscus maniculatus*	fixed	-	Perrigo (1987)

Energy saving mechanisms

Although the increase in DEE with a lowering of the food reward rate in our starlings does not reveal energy saving measures, this is far from the truth. Savings have actually been achieved in key components of the energy budget, but they have become obscured in the energy budget's total. With the detailed measurements on the time-energy budget of the starlings (Figure 10.6) we are able to calculate alternative scenario's. We reconstructed the energy budget of a starling working for food under poor conditions, while it would have retained the body mass actually measured under the rich regime. Because the higher body mass results in a higher BMR and in higher flight costs, the energy budget would rise significantly. Without the savings the energy budget under poor conditions would increase not be 220, but 353 kJ/d, which equals ca. 4.7xBMR (Figure 10.6, rightmost bar). Partly, this predicted increase would be due to the higher resting metabolic rate (including BMR), but above all, most extra energy is spent in flight. This is the result of the greatly extended foraging time in combination with the higher flight costs, resulting from the higher body mass (Wiersma *et al*. 2003a: Chapter 4). The increase in energy expenditure will amplify energy expenditure further, because this demands an additional increase in foraging time.

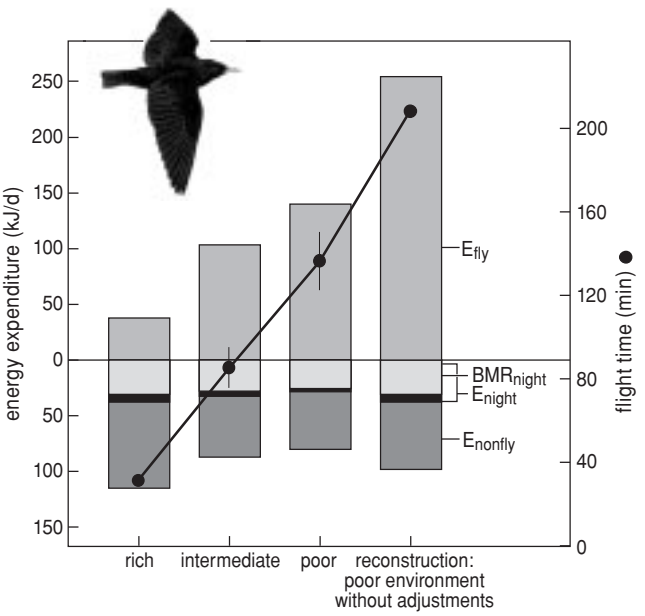

Figure 10.6 Daily energy budget and flight time of captive starlings at different foraging reward rates. Seven birds were kept alternately in a rich, intermediate or poor environment where they had to fly, respectively, 2, 4 or ±6 times back-and-forth between perches that were 5 m apart to obtain food. E_{fly} is the energy spent during flight, BMR_{night} the energy spent during the 10-h night on a BMR level, while E_{night} is what is spent in total during the night. E_{nonfly} is the energy spent during day-time while not flying. The rightmost bar represents the predicted energy budget of a bird under poor conditions that has retained the same mass, BMR and flight costs as a bird under rich conditions. Also its predicted flight time is shown. See Wiersma *et al*. (2003a: Chapter 4) for more details.

Zebra finches foraging at different reward rates showed a small but significant decrease in DEE with increasing work levels (Figure 10.7, *zf1* and *zf2* in Figure 10.5). These birds did not have to fly large distances for their food, but were searching for seeds between large quantities of chaff. Therefore, foraging was not as energetically laborious as in the case of the starlings. Body mass of the birds was not affected by food reward rate, and presumably, lowering body mass would have had no significant effect on the foraging costs. BMR and mass-specific BMR were not related to food reward rate either. Foraging time increases considerably with decreasing foraging reward rates, as did the energy spent on foraging, but total energy expenditure during daytime nevertheless decreased (Figure 10.7). Although BMR did not vary with food reward rate, the total energy expenditure during the night decreased with decreasing reward rate. At temperatures below the thermoneutral zone, SMR and mass-specific SMR decreased with decreasing reward rate too, implying that the birds might have lowered their body temperature. The energy expenditure during the day is mainly adjusted by the energy spent on other activities (labelled 'remainder' in Figure 10.7). This was confirmed by activity measurements using infrared sensors.

An overview of mechanisms that can be used to control energy expenditure is given in Table 10.2. Several of these methods have been shown to be used by birds that were pressed to perform. Reducing time spent on costly activities has the greatest potential for economising energy expenditure, especially when the bird can cut back on flight time. A reduction in body mass is often shown when foraging reward rates decrease. This can have a considerable impact on flight costs. By lowering body mass, resting metabolism may be reduced significantly too, but this depends on the nature of the

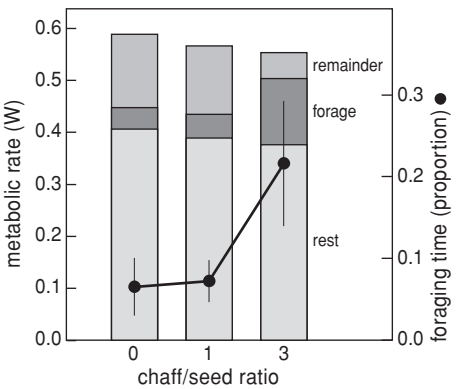

Figure 10.7 Energy budgets (bars) and foraging times (dots: means ±SE) of zebra finches during the 10-h day-time period. The birds were foraging for seeds that were offered *ad libitum*, or mixed with chaff according to chaff/seed mass-ratios of 1 or 3 to manipulate foraging reward rates. Energy expenditure was measured by respirometry at 22°C. Average BMR was 0.216 W and independent of foraging reward rate. The partition of the energy budget labelled 'remainder' is made up of the energy expenditure not accounted for by resting or foraging. See Wiersma & Verhulst (2003a: Chapter 2) for details.

mass loss. If only stores are usurped, that mainly consist of fat with a very low metabolism (Scott & Evans 1992), BMR will not be affected. Size reductions of reserves (muscle tissue) and of the 'metabolic machinery' are necessary to reduce BMR, but these obviously may comprise major consequences for the animal. In addition, the mass-specific metabolism of the 'metabolic machinery' may be altered (Weber & Piersma 1996), but probably, the achieved saving would be negligible. Hypothermia is regularly used in some bird species (McKechnie & Lovegrove 2002), and seems to be an effective way of reducing energy expenditure during the resting phase. It also seems to be mainly restricted to small birds, presumably because they can increase their body temperature quicker than large-sized birds. We have some indications that birds in our foraging experiments might have reduced night-time body temperature to some degree (Wiersma & Verhulst 2003a: Chapter 2; Wiersma *et al*. 2003a: Chapter 4), but we are lacking actual measurements.

In this thesis I restricted myself to a proximate view of the costs of reproduction, sometimes even outside a reproductive setting. We studied how energetic constraints might affect different trade-offs, and hypothesise how this could affect life-history traits. Nevertheless, although fitness is the currency by which we ultimately have to explain animal physiology and behaviour, without knowledge of how various resources are divided between physiological processes and behavioural activities, a real understanding of life-history theory will not emerge. In this thesis I have touched upon the changes in energy management and a few critical, physiological trade-offs under different conditions, to learn about the role of energy in 'physiological decisions'. The substantial variability in the energy budget that emerged has to be considered when making predictions about animal behaviour. But unfortunately we often do not understand the variation we see, such as that in Figure 10.5. Will a bird economise when it is forced to work harder for its food? Perhaps even to such an extent that DEE decreases? The answer is still not evident. Fortunately, progress might be expected with fairly basic experiments, such as studying a single species' foraging intensity and daily energy budget while manipulating along the various axes that make up the environment.

Table 10.2 Animals working hard for a living: overview of measures to economise energy expenditure. The targets between brackets are only weakly affected by the particular method of saving.

Saving measures	Saving targets
reduce time spent on costly activities (e.g. foraging)	DEE
reduce size of stores (e.g. subcutaneous fat)	locomotory costs; BMR
reduce size of reserves (e.g. pectoral muscles)	locomotory costs; BMR
reduce body temperature	BMR
reduce size of 'metabolic machinery' (e.g. intestines, liver)	BMR; (locomotory costs)
reduce mass-specific metabolism of 'metabolic machinery' (e.g. liver, kidneys)	BMR

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Werken voor de kost: fysiologische en gedragsmatige afwegingen in hard werkende vogels

Popko Wiersma

Nadelige effecten van hard werken

Niet alleen sommige mensen werken hard, veel andere dieren werken minstens zo hard. Er hangt veel van af hoe hard iemand werkt, omdat het mede bepaald hoe succesvol hij of zij is. Bij veel dieren kun je het succes meten aan het aantal nakomelingen en hun conditie. In de natuur wordt hierop geselecteerd: individuen die lang leven en veel nageslacht hebben, hebben een hoge 'fitness' (Figuur 1). Ook in vogels wordt, onder andere, geselecteerd voor ouders die een grote reproductieve inspanning leveren, door bijvoorbeeld hard te werken voor hun jongen. Hierdoor zullen zij veel uitvliegende jongen hebben die zelf ook weer nageslacht produceren.

Maar hoe meet je hoe hard een dier werkt? Je zou het kunnen uitdrukken in de tijd die wordt besteed aan bepaalde activiteiten, maar natuurlijk moet je dan wel rekening houden met wat die activiteit inhoudt; na een dag typen of bessen uit een boom eten zal de lichamelijke vermoeidheid een stuk minder zijn dan na een dag schoffelen of een nest met jongen te eten geven. Wat nodig is om de werkniveaus van verschillende dieren of individuen met elkaar te vergelijken is een maat die niet afhankelijk is van het soort werk. Zo'n maat is het dagelijkse energieverbruik (**DEE**), dat gewoonlijk wordt uitgedrukt in kilojoules per dag. Hoe harder er wordt gewerkt, des te meer energie er wordt uitgegeven en des te meer er moet worden gegeten. Nu is het probleem nog niet helemaal opgelost, want als individuen of diersoorten namelijk in grootte van elkaar verschillen zullen ze alleen daardoor al verschillen in hoeveel energie ze uitgeven. Dit kan worden omzeild door het energieverbruik uit te drukken in het aantal keer basisstofwisseling of basaalmetabolisme (**BMR**), omdat dat een maat is die rekening houdt met grootteverschillen. BMR is het energieverbruik van een dier tijdens zijn slaap bij een behaaglijke temperatuur terwijl er geen eten meer wordt verteerd. BMR is enigszins vergelijkbaar met het benzineverbruik van een stationair draaiende automotor,

terwijl DEE kan worden vergeleken met het benzineverbruik van een auto die de hele dag is gebruikt. Een DEE dat gelijk is aan vier keer BMR wordt beschouwd als een hoge waarde, dat meet je alleen bij een hard werkend dier. Vogels die jongen in het nest verzorgen besteden vaak energie op dat niveau. Om een indruk te krijgen van de werkdruk van deze vogels kunnen we hun energieverbruik vergelijken met dat van hard werkende mensen. Klaas Westerterp heeft het energieverbruik gemeten van wielrenners die deelnamen aan de Tour de France. Deze sporters leveren dik drie weken lang topprestaties en hun gemiddelde DEE is gelijk aan 4 tot 5 keer BMR. Harder werken gedurende zo'n lange periode kan eigenlijk niet omdat mensen niet meer kunnen eten, en dus niet meer energie kunnen opnemen. Gedurende een korte periode kan wel harder worden gewerkt, maar dat gaat dan ten koste van de lichaamsreserves (voornamelijk vet), wat leidt tot een afname in lichaamsgewicht. Vogels, bijvoorbeeld, kunnen tijdens het vliegen wel een energieverbruik van 20 keer BMR hebben, maar dat houden ze niet lang vol en daarna moet er weer veel gegeten worden.

Toch werkt het ene individu harder voor zijn of haar nakomelingen en brengt meer nakomelingen groot (ofwel: heeft een groter reproductief succes dan het andere). Waarom werken niet alle individuen zo hard als de hardst werkende? Heeft hard werken misschien ook nadelige consequenties? Hoewel rust roest en arbeid adelt, zijn er inderdaad ook kosten verbonden aan noeste arbeid.

Een kostenpost is bijvoorbeeld de kleinere hoeveelheid tijd die aan het zoeken naar voedsel voor eigen gebruik kan worden besteed als er meer voedsel naar een nest met jongen wordt gebracht. Ook nadelig is de verminderde tijd die beschikbaar is om 'vreemd te gaan'. Spreeuwen, bijvoorbeeld, zijn facultatief polygaam; dat betekent dat veel mannetjes proberen hun aantal nakomelingen te verhogen door als ze de kans krijgen ook jongen bij een ander vrouwtje dan hun partner te krijgen. Maar als ze meer tijd moeten steken in het verzorgen van hun eerste nest met eieren of jongen hebben ze minder gelegenheid om de aandacht van andere vrouwtjes te trekken. Ook een voorbeeld van een kostenpost is de toename van het risico om gepakt te worden door een roofvogel of een andere predator, als gevolg van verminderde oplettendheid tijdens het voeren van de jongen.

Andere kosten van hard werken vinden hun oorsprong in stofwisselings- ofwel fysiologische processen. Doordat er veel energie (of bepaalde nutriënten) opgebruikt wordt tijdens het harde werken kan dat niet worden besteed aan de bescherming en het onderhoud van het lichaam. Verschillende fysiologische processen concurreren dus met elkaar om dezelfde middelen (Figuur 1). Als er veel van de beschikbare middelen besteed wordt aan het voeren van jongen kan dit ten koste gaan van, bijvoorbeeld, de werking van het immuunsysteem van de ouders. Andere processen die concurreren om dezelfde middelen zijn o.a. de bescherming tegen reactieve zuurstofsoorten (waarover later meer), het opruimen van schadelijke stoffen door bijv. de lever, herstel van schade aan cellen en opslag van reserves om moeilijke tijden door te komen (bijv. vet, eiwitten en calcium;).

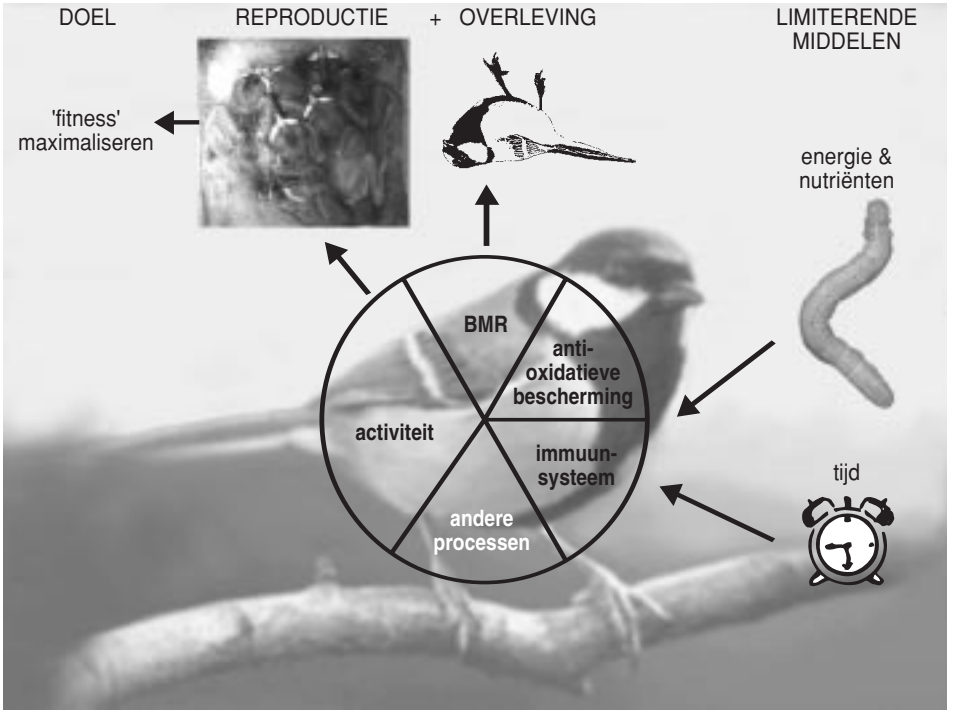

Figure 1 Schematische weergave van de onderlinge afhankelijkheid van fysiologische processen en gedragskeuzes van een dier. Omdat deze processen, zoals weergegeven in de taartdiagram, concurreren om dezelfde middelen (tijd, energie, nutriënten) moeten optimale afwegingen worden gemaakt omtrent de keuze hoeveel geïnvesteerd moet worden in elk proces. Dit bepaalt uiteindelijk het reproductieve succes en de overlevingskansen van de ouders. Het uiteindelijke 'doel' is het maximaliseren van de 'fitness', wat neerkomt op het produceren van zo veel mogelijk reproducerende jongen in de toekomstige populatie.

Energiehuishouding

Als energie een beperkende factor is voor veel fysiologische processen in het lichaam, zou je verwachten dat het goed is om zo zuinig mogelijk met energie om te springen, zodat alle processen optimaal kunnen blijven werken. Hoe vogels hun energiehuishouding 'managen' als ze hard moeten werken is het onderwerp van een aantal hoofdstukken in dit proefschrift. Eerdere studies aan voor eten werkende zebravinken en spreeuwen in gevangenschap, door Charlotte Deerenberg en Luis Miguel Bautista en hun medewerkers, lieten verrassenderwijs zien dat het dagelijkse energieverbruik *lager* was als de vogels harder moesten werken voor hun eten. In deze experimenten moesten de vogels gedurende enkele weken al hun eten zelf verdienen door heen en weer te vliegen tussen twee stokken. Het aantal vluchten dat ze moesten uitvoeren om een beloning te krijgen kon worden aangepast om zodoende de vogels hard of minder hard te

laten werken. De verlaging in DEE werd o.a. bewerkstelligd door een lager basaalmetabolisme (BMR), waardoor ze tijdens de nacht minder energie uitgaven. Echter, James Fotheringham heeft laten zien dat de *manier waarop* spreeuwen voer verdienen met werken een sterk effect had op hoe hard zij werkten en op hun lichaamsgewicht. Als de voedselbeloning 100% voorspelbaar was verlaagden de spreeuwen hun lichaamsgewicht en hun dagelijkse voedselopname als ze gedwongen werden meer te vliegen voor hun eten. Als daarentegen de voedselbeloning gedeeltelijk onvoorspelbaar was handhaafden de spreeuwen hun lichaamsgewicht en voedselopname als ze meer moesten vliegen voor hun eten. De eerder genoemde experimenten waar vogels hun DEE verlaagden met een toename in werk gebruikten voorspelbare beloningsregimes. Maar omdat dit in de natuur gewoonlijk in zekere mate onvoorspelbaar zal zijn, waren de beloningsregimes in onze experimenten ook onvoorspelbaar.

Zebravinken lieten we harder werken door hun voer (zaden) te mengen met verschillende hoeveelheden kaf (**Hoofdstuk 2**). Als er meer kaf door hun voer was gemengd nam hun foerageertijd toe van 40 tot 130 minuten per dag. Ook deze vogels verlaagden hun DEE enigszins door hun BMR een klein beetje te verlagen, maar vooral door minder energie uit te geven tijdens de periodes dat ze niet foerageerden. Waarschijnlijk besteedden de vogels minder tijd aan andere activiteiten dan foerageren, zoals bijvoorbeeld poetsen, en zaten ze meestal stilletjes op hun stok. Spreeuwen lieten we werken door ze 5 meter tussen twee stokken heen-en-weer te laten vliegen voordat ze beloond werden met een voedselpellet (**Hoofdstuk 4**). Een aantal spreeuwen hoefde slechts gemiddeld twee keer heen-en-weer te vliegen voor een beloning, terwijl anderen gemiddeld zes keer heen-en-weer moesten. (Na verloop van tijd wisselden de spreeuwen van schema en deden we onze metingen nog een keer.) Onder de relatief gemakkelijke omstandigheden (2 keer heen-en-weer) vlogen de spreeuwen ±32 minuten per dag en legden ze ±8 km af. Onder zware omstandigheden vlogen ze ±136 minuten per dag en vlogen ze in totaal ±32 km. In tegenstelling tot de hard werkende spreeuwen van Bautista gaven zij *wel* meer energie uit als ze harder moesten werken. Dit is misschien wel het eerste labexperiment waarin het is gelukt om de DEE van dieren te laten toenemen door hun activiteit te manipuleren. Hoewel hun energieverbruik behoorlijk toenam door de manipulatie (van 154 tot 220 kilojoules per dag) bespaarden de spreeuwen toch ook heel veel energie. Ten eerste verlaagden ze hun BMR, waardoor ze 's nachts zuiniger waren. Maar de grootste slag hebben ze geslagen door een verlaging van de vliegkosten, wat veroorzaakt werd door een afname in lichaamsgewicht. Hoe lichter een vogel is des te gemakkelijker blijft hij in de lucht. Als de spreeuwen hun gewicht en hun nachtelijke energieverbruik niet hadden veranderd zou hun energieverbruik flink hoger zijn geweest: niet 220 maar 353 kilojoules per dag. Een besparing van 40% maar liefst!

In een ander experiment hebben we het energieverbruik van de spreeuwen in de vliegkooien tijdens het vliegen gemeten met behulp van stabiele isotopen (**Hoofdstuk 3**). Door de spreeuwen te injecteren met gelabeld bicarbonaat en ze dan een tijdje te laten vliegen, kan aan de hand van de afname in bicarbonaat (meteen na het vliegen gemeten in de uitgeademde lucht) berekend worden hoeveel energie ze hebben uitge-

geven tijdens het vliegen. Op die manier maten we vliegkosten van gemiddeld 20.5 Watt, wat een hele hoge waarde is (ongeveer 25 keer BMR). De vliegkosten van de spreeuwen in het experiment dat hiervoor werd beschreven, waarin ze moesten vliegen om eten te verdienen, waren een stuk lager als ze hard moesten werken. Naar schatting gaven ze tijdens het vliegen ongeveer 15% minder energie uit, nl. 17.5 Watt.

Omdat dit tot nu toe allemaal studies zijn geweest aan werkende vogels in kooien was het van belang om ook metingen aan wilde vogels te doen, aangezien vogels in gevangenschap misschien wel heel anders reageren dan in het wild levende vogels. Daarom hebben we metingen gedaan aan voor hun jongen werkende koolmezen (**Hoofdstuk 5**). We hebben het aantal jongen in het nest vergroot of verkleind zodat sommige ouders genoodzaakt waren vaker voer naar hun nest te brengen dan anderen. Met behulp van videocamera's hebben we gemeten hoe frequent elke ouder (beide ouders hadden een unieke combinatie van kleurringen) de nestkast bezocht, om te controleren of ouders met meer jongen ook daadwerkelijk harder werkten. DEE is gemeten met de zogenaamde zwaar-water methode. Een bepaalde hoeveelheid zwaar water ($D_2{}^{18}O$) wordt geïnjecteerd in de vogels die vervolgens weer vrij wordt gelaten. Door na 24 uur de vogel weer te vangen en de concentraties van D(euterium) en ^{18}O in het bloed te meten kan berekend worden hoeveel CO_2 ze hebben geproduceerd gedurende de afgelopen 24 uur. Deze hoeveelheid CO_2 is een maat voor de hoeveelheid energie die ze hebben verbruikt gedurende de afgelopen dag. BMR van de koolmezen is 's nachts in het veld gemeten met een mobiele zuurstofmeter, waarmee het zuurstofverbruik kan worden gemeten van een vogel in een kleine luchtdichte kamer. Het zuurstofverbruik is ook een maat voor het energieverbruik. We vonden dat de koolmezen met vergrootte broedsels meer energie uitgaven gedurende een dag, maar dat hun BMR niet verschilde van die van mezen met verkleinde broedsels. Er was dus geen aanwijzing voor energiebesparing bij in het wild levende koolmezen.

In plaats van een verlaging van BMR, worden ook wel BMR-verhogingen gemeten als dieren harder moeten werken. Recentelijk vond Jan-Åke Nilsson een verhoging in BMR in glanskoppen (een mezensoort) die extra jongen in hun nest hadden gekregen. Dit is dus tegengesteld aan de verwachting zoals ik die hierboven beschreef. Toch is ook dit wel te verklaren. Veranderingen in BMR zijn doorgaans geassocieerd met een verandering in de grootte van bepaalde organen (zoals lever, nieren, hart en dunne darm). En orgaangrootte kan sterk fluctueren in de loop van een jaar. Zo vergroten vogels die zich klaar maken voor de langeafstandstrek hun vliegspieren en hart om beter te kunnen presteren tijdens de lange vlucht. Daarom is het ook mogelijk dat vogels die harder moeten werken voor hun jongen, hun spieren, hart en misschien nog andere organen vergroten, om zodoende harder te kunnen werken voor hun kroost. In dat geval zouden we kunnen verwachten dat vogels met een hoger BMR harder kunnen werken en dus meer eieren zullen leggen en zwaardere jongen kunnen grootbrengen.

Om dit te kunnen testen hebben we de relatie tussen BMR en verschillende maten van het broedsucces van zebravinken in gevangenschap bekeken (**Hoofdstuk 6**). We vonden dat mannetjes met een hoog BMR een grotere kans hadden een legsel te hebben en ook dat zij eerder en meer eieren hadden. BMR van de vrouwtjes was niet

gecorreleerd met wel of niet leggen, het aantal dagen voordat werd begonnen met leggen en de legselgrootte. Het lijkt er op dat eigenschappen van het mannetje invloed hebben op de eileg. Mogelijk reageert het vrouwtje op de kwaliteit van haar partner: hoe hoger het BMR van haar partner, hoe beter hij is, en daarom is ze geneigd eerder en meer eieren te gaan leggen.

Beschermingsmechanismen

Beschermingsmechanismen zijn broodnodig. Er loeren altijd en overal gevaren die ontweken moeten worden, zoals gevechten met soortgenoten, ziektes, parasieten en gifstoffen. Onvoldoende bescherming kan leiden tot verwondingen, fysiologische schade, verstoring van chemische en hormonale processen of zelfs de dood. Afgezien van alle gevaren van buitenaf, produceert het lichaam zelf ook gifstoffen in de vorm van reactieve zuurstofsoorten (**ROS**). ROS is een verzamelnaam voor vrije zuurstofradicalen en aanverwante moleculen. Zij spelen een belangrijke rol bij veroudering en in het ontstaan van sommige ziekten doordat zij schade veroorzaken aan het erfelijk materiaal (DNA) en aan eiwitten en vetten. ROS ontstaan continu als een bijproduct van fysiologische processen die zuurstof gebruiken. Hoe meer energie er wordt omgezet, dus ook als er harder wordt gewerkt, des te meer van deze 'giftige' moleculen er ontstaan. Het lichaam wordt beschermd tegen deze moleculen door antioxidanten die ROS omzetten in minder gevaarlijke stoffen. Antioxidanten kunnen via het voedsel opgenomen worden (bijv. vitamine C en carotenoïden), maar in het lichaam zelf worden ook antioxidant-enzymen gemaakt die dicht bij de bron, in de cellen, hun werk doen.

Wij hebben onderzocht of de bescherming, dat is de activiteit van antioxidanten, te lijden heeft onder een toename van reproductieve inspanning (**Hoofdstuk 8**). We hebben de activiteit van twee antioxidant-enzymen (SOD en GPx) in de vliegspier gemeten in zebravinkouders met 2 of met 6 jongen. Omdat de omvang van de door ROS veroorzaakte schade afhangt van zowel de antioxidant-enzym-activiteit als de hoeveelheid gevormde ROS, moet de enzymactiviteit uitgedrukt worden ten opzichte van de ROS-productie. Als een indicatie van deze productie hebben we zuurstofverbruikmetingen gebruikt. Het bleek inderdaad dat de relatieve activiteit van beide antioxidantenzymen lager was in vogels met een groter broedsel. Verwacht mag worden dat vogels die een grotere inspanning leveren dus meer schade oplopen aan DNA en andere moleculen, en, doordat ze daardoor in een slechtere conditie verkeren, eerder dood gaan, en in de toekomst minder nageslacht zullen produceren.

Ook de werking van het immuunsysteem verslechtert wanneer de reproductieve inspanning toeneemt. In een populatie withalsvliegenvangers lieten Dag Nordling en medewerkers zien dat vogels met meer jongen een grotere kans hadden om geïnfecteerd te worden met vogelmalaria en dat de kans om dood te gaan was toegenomen. Charlotte Deerenberg en medewerkers hebben laten zien dat zebravinken die extra jongen kregen toegewezen een verminderde immuunreactie hadden na een injectie met een onschadelijke lichaamsvreemde stof (rode bloedcellen van schapen). De lichaams-

vreemde cellen initiëren de aanmaak van antilichamen die de schapencellen opruimen. Als de immuunreactie niet goed werkt, en in plaats van onschuldige schapencellen waren er schadelijke bacteriën of parasieten in de bloedbaan gekomen, is de kans groot dat de vogel ziek wordt. Wat de oorzaak van de afname in de kwaliteit van de immuunreactie is, als de reproductieve inspanning toeneemt is niet bekend. De meest voor de hand liggende oorzaak is een verminderde investering van energie in het maken van antilichamen en andere ziektebestrijdende cellen als gevolg van een grotere investering in het verzorgen van de jongen. Dit impliceert dat het genereren van een immuunreactie veel energie kost. We hebben dit onderzocht in zebravinken (**Hoofdstuk 7**). We hebben het energieverbruik van zebravinken gemeten (in een zuurstofmeter) na een injectie met rode bloedcellen van schapen, maar we vonden geen toename in het energieverbruik. Daarentegen was een uur na de injectie het energieverbruik met 10% gedaald! Het ziet er daarom naar uit dat deze immuunreactie niet erg veel energie kost, maar hoe het komt dat het energieverbruik daalt is niet duidelijk. Mogelijk worden de vogels minder actief als het immuunsysteem zijn werk gaat doen.

Een andere oorzaak voor een verminderde immuunreactie als de reproductieve inspanning toeneemt is een verkleining van het systeem dat de immuunreactie creëert. Met het systeem worden de organen en weefsels bedoeld die de antilichamen en andere immuuncellen (witte bloedcellen) produceren. Dit zou kunnen worden verwacht als het *onderhoud* van het immuunsysteem kostbaar is en niet zozeer het produceren van antilichamen. Een toename in de investeringen in reproductie gaat dan mogelijk ten koste van de kwaliteit van het immuunsysteem, wat verklaart waardoor de immuunreacties minder strek zijn. We hebben voor deze hypothese ondersteuning gevonden in een experiment met zebravinken (**Hoofdstuk 7**). Ook in dit experiment kregen de vogels meer of minder jongen te verzorgen, maar we hebben de kwaliteit van het immuunsysteem gemeten een dag nadat we de jongen (van 16 dagen oud) uit het nest hadden gehaald. Zodoende hoefden de ouders niet meer te werken voor hun jongen en was er dus geen sprake meer van reproductieve inspanning. Toch was in deze vogels de immuunreactie ook lager in de vogels die meer jongen hadden gehad. Er was dus een naijleffect van de vergrootte inspanning. Omdat verwacht mag worden dat aanpassingen aan het immuunsysteem enige tijd vergen, zou dit naijleffect verklaard kunnen worden door een verkleining van het immuunsysteem tijdens het verzorgen van de jongen.

Reallocatie van tijd

Reproductie kost niet alleen energie en nutriënten, maar ook tijd. Tijd die aan het bebroeden van eieren of voeren van jongen wordt besteed kan niet worden gebruikt voor andere doeleinden, zoals het produceren van jongen bij een tweede partner. We hebben in de Vosbergen, een landgoed nabij Groningen, onderzoek gedaan aan spreeuwen die daar in nestkasten broeden (**Hoofdstuk 9**). Spreeuwen hebben één partner waarmee ze een nest hebben en zowel de mannetjes als de vrouwtjes bebroeden de

eieren. We hebben het gedrag vergeleken van mannetjes met legsels die wij verkleind of vergroot hadden. Mannetjes met vergrootte legsels besteedden meer tijd aan broeden. Dat kunnen we verklaren doordat een groot legsel meer 'waard' is, omdat dat waarschijnlijk meer uitvliegende jongen op zal leveren. Waar we het aantal eieren van de ouders hadden verkleind, besteedden de mannetjes minder tijd aan broeden en meer tijd aan het verwerven van een tweede vrouwtje dat ook een legsel met hem zou willen produceren in een nabijgelegen nestkast. Een mannetje met een klein legsel heeft relatief meer te winnen (in de vorm van nageslacht) met een 'buitenechtelijke' relatie.

Vele processen bepalen dus samen hoeveel tijd en energie er in reproductie wordt gestoken. Omdat deze middelen maar een keer kunnen worden uitgegeven moeten er afwegingen worden gemaakt. Uit de experimenten met voor eten werkende zebravinken en spreeuwen blijkt dat deze vogels zeer terughoudend zijn in het verhogen van hun energiebudget. Het lijkt dat alles uit de kast wordt getrokken om maar zo weinig mogelijk energie uit te geven. De spreeuwen, waarmee het ons het beste is gelukt om ze hard te laten werken, wisten tot bijna 40% aan energie te besparen, voornamelijk door hun lichaamsgewicht te verlagen. Andere mechanismen waarmee bespaard zou kunnen worden zijn: minder tijd besteden aan relatief dure activiteiten (bijvoorbeeld, baltsen en poetsen), de lichaamstemperatuur verlagen en de activiteit van sommige organen reduceren, door ze bijvoorbeeld kleiner te laten worden. Er valt nog heel veel te onderzoeken op dit gebied; we kunnen bijvoorbeeld nog niet goed voorspellen hoe een vogel in het veld zijn energiebudget aan zal passen als het meer moeite moet doen om aan eten te komen. Wel is duidelijk uit de hier beschreven experimenten dat vogels veel mogelijkheden hebben om zich aan te passen aan nieuwe omstandigheden. Gelukkig kunnen we met niet al te ingewikkelde metingen en experimenten nog veel meer inzicht krijgen in hoe dieren reageren als ze harder moeten werken.

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