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Short-term, but not long-term, increased day time workload leads to decreased night time energetics in a free living song bird

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Running title: Work load and energetics in a songbird

Summary Statement: Birds provisioning offspring can either increase metabolic processes or reduce energy spend on maintenance. We show that a song bird does the latter, but only in the short term.

Abstract

Reproduction is energetically expensive and to obtain sufficient energy, animals can either alter their metabolic system over time to increase energy intake (increased-intake hypothesis), or reallocate energy from maintenance processes (compensation hypothesis). The first hypothesis predicts a positive relationship between basal metabolic rate (BMR) and energy expenditure (DEE) because of the higher energy demands of the metabolic system in rest. The second hypothesis predicts a trade-off between different body functions, with a reduction of the BMR as a way to compensate for increased daytime energetic expenditure. We experimentally manipulated the workload of wild pied flycatchers by adding or removing chicks when chicks were 2 and 11 days old. We then measured the feeding frequency (FF), DEE and BMR at day 11, allowing us to assess both short- and long-term effects of increased workload. The manipulation at day 2 caused an increase in FF when broods were enlarged, but no response in DEE or BMR, while the manipulation at day 11 caused an increase in FF, no change in DEE and a decrease in BMR in birds with more chicks. Our results suggest that pied flycatchers adjust their workload but that this does not lead to a higher BMR at night (no support for the increased-intake hypothesis). In the short-term we find that birds reallocate energy with a consequent reduction of BMR (evidence for the compensation hypothesis). Birds thus resort to short-term strategies to increase energy expenditure, which could explain why energy expenditure and hard-work are not always correlated in birds.

Introduction

From an energetic point of view, reproduction is an expensive annual-cycle stage that largely determines the fitness of an individual. Costs of reproduction in birds, for example, not only involve the physiological costs of egg production by the female, but also of courtship, territory defence and offspring rearing, that may involve both males and females (Tinbergen and Verhulst, 2000; Visser and Lessells, 2001). The debate over how animals meet the increased energy demands required for reproduction spans many years and has been investigated in birds (Daan et al., 1990; Drent and Daan, 1980; Moreno and Sanz, 1994; Nilsson, 2002; Tinbergen and Verhulst, 2000; Vézina et al., 2006; Wiersma and Tinbergen, 2003) and mammals (Bennett and Ruben, 1979; Ricklefs et al., 1996; Speakman et al., 2004).

It has been hypothesised that two strategies for increasing workload are available: animals could increase the rate of energy expenditure, which requires remodelling the metabolic machinery over time (*increased-intake hypothesis*) (Burness et al., 2001; Drent and Daan, 1980; Nilsson, 2002;

Speakman et al., 2004; Tinbergen and Verhulst, 2000). Alternatively, energy is relocated from maintenance processes, increasing the energy expenditure during the active phase, while decreasing it during the resting/recovery phase (*compensation hypothesis*) (Bennett and Ruben, 1979; Deerenberg et al., 1998; Nilsson, 2002; Vézina et al., 2006; Wiersma and Tinbergen, 2003), without an increase in the total daily energy expenditure.

Which of the strategies is adopted determines the form of the relationship between basal metabolic rate (henceforth, BMR) and daily energy expenditure (henceforth, DEE). When animals increase their energy expenditure under increased workload through an adaptation of the metabolic machinery (*increased-intake hypothesis*) it is expected they will have an increased BMR because the costs of organ maintenance during rest will be higher (Nilsson, 2002). Because this strategy requires adaptation of the animals' metabolic machinery, it may be a more long-term strategy. But when birds trade-off a higher energy expenditure during the day against a reduced maintenance processes at night (such as maintenance of immunological defence or DNA repair systems (Burness et al., 2001; Tinbergen and Verhulst, 2000), BMR is expected to decrease during the resting phase, (*compensation hypothesis*) (Deerenberg et al., 1998; Wiersma and Tinbergen, 2003) while DEE remains the same.

Although the two hypotheses have been explored (Burness et al., 2001; Deerenberg et al., 1998; Nilsson, 2002; Wiersma and Tinbergen, 2003), it is not clear whether animals would adopt one strategy over the other. The literature is contradictory (Nilsson, 2002; Wiersma and Tinbergen, 2003), suggesting that both strategies could be in place and that animals alternate between them over different breeding stages (Nilsson, 2002), but no study has experimentally tested this. Such experiment should take into account both short- (compensation) and long-term (increased-intake) adjustments that individuals can make.

In the present study, we used a migratory songbird, the pied flycatcher, to investigate how animals can maintain a higher workload during breeding, via either short or long-term adjustments which, allow them to sustain higher levels of activity. We manipulated workload by adding or removing chicks at two different moments: during early or late breeding, when chicks were 2 and 11 days old, respectively, and measured feeding frequency (henceforth, FF), DEE and BMR at day 11 (which is around three days before fledging). These consecutive manipulations allowed us to distinguish the different strategies pied flycatchers use as short- or long-term adjustments to work harder.

Material and Methods

Experimental rationale

We manipulated workload via manipulation of brood sizes when chicks were two days old (henceforth, D2) by giving the nests -2 (n=18), +0 (n=18), or +2 (n=9) chicks, and then allowed parents to adapt to the increased brood size until the chicks were 10 days old (henceforth, D10). During the night between D10 and D11, we performed a second brood size manipulation in 18 of the manipulated pairs, by enlarging their broods with either +2 chicks (9 out of 18 pairs of the group that had +0 chicks from D2 onwards) or +4 chicks (9 out of 18 pairs of the group that had -2 chicks from D2 onwards) (see also table 1) to investigate whether parents would respond differently to an immediate increase in brood size compared to a long term increase. The remaining nests were used to assess the effects of the first D2 brood size manipulation and as a control for the second manipulation on D11. Measurements of feeding frequency (FF) were taken at D10 and D11 (to be able to measure within pair changes in FF before and after the D11 manipulation), daily energy expenditure (DEE) at D11 and basal metabolic rate (BMR) in the night of D11.

If birds responded to the manipulations via an adjustment of their metabolic machinery, with a consequent increase in their BMR (increased-intake hypothesis), we expected that BMR would be higher in enlarged broods (+2) and lower in reduced broods (-2) in relation to control broods (+0) (Fig. 1A: comparison between the groups -2 D2/ +0 D11: light blue points, +0 D2/ +0 D11: blue points and +2 D2/ +0 D11: purple points).

If birds responded to the manipulations by relocating energy from their maintenance processes, with a consequent decrease in their BMR (compensation hypothesis), we expected a decrease in the BMR of the groups that had their brood enlarged at D11, in relation to the broods that were not enlarged at D11 (Fig. 1B: comparison between the groups -2 D2/+4 D11: red points, +0 D2/+2 D11: pink points, and combination of -2,+0,+2 D2/+0 D11: black points).

Study species and allocation to the experiment

The pied flycatcher (*Ficedula hypoleuca*) is a long-distance migratory bird, that, similarly to other bird species, display a considerable variation in individual oxygen consumption rate (Roskaft et al., 1986). The experiment was conducted in 2006 with a wild population of Pied Flycatchers in the Hoge Veluwe National Park (The Netherlands 5°51'E 52°02'N), which is part of a long-term research project (Tomotani et al., 2018).

Nests were regularly checked for egg laying to assess laying dates and clutch sizes. When females were incubating, we randomly selected 45 nests with six or seven eggs (the most common clutch sizes for the Hoge Veluwe) and allocated them to the treatment groups (Table 1). Because not all eggs were viable, we

corrected brood sizes upon hatching by adding same-age chicks from nests that were not allocated to a treatment in order to match the brood size to the original number of incubated eggs. At day 12 after hatching all nestlings were weighted using a spring balance as a measure for their fledging weight.

All procedures were carried out under Licences of the Animal Experimental Committee of the Royal Netherlands Academy of Sciences (KNAW), protocol number CTO 06.01. The experiment had no impact on the breeding success of the population, with no increase in chick mortality or desertion rates after treatments were applied (for more details on number of animals, see Table 1).

Feeding frequency (FF)

When chicks were seven days old (D7), both adults were caught, weighed and equipped with a passive integrated transponder (PIT)-tag glued to three colour rings. Then, when chicks were eight days old (D8), we placed a transponder reader (software: Trovan LID650/LID665/LID1260, version V607) around the opening of the nest box, which recorded when birds entered the box with a 20-second lag between readings. FF was calculated as the average number of nest visitations per hour and measured on D10 and D11. On both days, we used the readings between 5h 00m and 19h 00m, as in the morning most birds (83%) started feeding between 5h 00m and 6h 00m and in the evening the birds were caught for the BMR measurements (see below).

Daily energy expenditure (DEE)

We measured daily energy expenditure (DEE) for each bird with the doubly-labelled water technique ($D_2^{18}O$) (Lifson and McClintock, 1966; Moreno et al., 1995; Moreno and Sanz, 1994; Nagy, 1980) from the evening of D10 until 24 h later. At the end of D10, males and females were caught on average at 19h 32m (± 24 min; range 18h 55m - 21h 10m), and were injected intraperitoneally with 0.105 ± 0.0005 ml of a mixture containing 65% of $H_2^{18}O$, 97 atom% and 35% of D_2O , 99.9 atom%. Each bird was then placed in a small bag for 68 ± 3 min to allow equilibration of the isotopes in the bird's body fluids. After this period, we took three blood samples (15 μ l) from the brachial vein (puncturing the vein only once), which were stored in flame-sealed heparinised capillary tubes. Then, on average at 20h 33m (± 30 min, range 19h 20m - 21h 50m) of the next day, birds were recaptured and blood sampled (3x 15 μ l).

Blood samples were analysed for ^{18}O and D concentrations at the Centre for Isotope Research of the University of Groningen (Guidotti et al., 2013). Body water volume was deduced from the dilution space of ^{18}O (Schoeller et al., 1986), using the extrapolation method. Daily CO_2 (from which the

average daily metabolic rate (ADMR) in ml CO₂/g/h is derived) was determined from fractional turnovers of the two isotopes using the equations by (Lifson and McClintock, 1966). CO₂ production was converted to energy expenditure (kJ/d) by assuming a respiratory quotient of 0.8 and energetic equivalent of 27.8 kJ per litre of exhaled carbon dioxide (te Marvelde et al., 2011). The isotope enrichment of the blood samples was corrected for the natural background isotopic abundance of the body fluids. The latter was determined using blood samples from four non-injected individuals.

BMR measurements

On D11 birds were kept overnight in a metabolic chamber for BMR measurements. BMR was measured as the average minimum oxygen consumption in an open-circuit respirometer located in a field shed in the study area (from 22h 00m until 6h 00m). Each bird was placed in an individual sealed metabolic chamber (2.2 dm³) which was placed in the dark inside a climate cabinet at 25.5±0.15 °C (which is within the thermoneutral zone). H₂O and CO₂ were removed from the inlet air (blown into the animal chamber) respectively with Drierite® (6 mesh, Sigma-Aldrich Chemie b.v., Zwijndrecht, The Netherlands) and Ascarite® (5–20 mesh, Fluka, Zwijndrecht, The Netherlands). Air flow rate was set to 250 ml min⁻¹ with flowmeters (Brooks Instrument b.v., Ede, The Netherlands), placed before the chamber and previously calibrated using a soap bubble method (Bubble-O-Meter, LLC, Dublin, OH, USA). Oxygen content of outlet air was measured with an oxygen analyser (Servomex 4100, Servomex BV, Zoetemeer, The Netherlands) (see also Caro and Visser, 2009). Readings were recorded during pre-set periods (six minutes for each channel, with readings each 15 seconds, every bird was measured every 48 min). Temperature, air flow rate and air concentrations of O₂ were measured, based on inlet and outlet air. Oxygen consumption was calculated based on Hill (1972), and converted to energy expenditure (kJ) assuming an appropriate respiratory quotient (RQ) of 0.8 for insectivorous birds, and an energetic equivalent of 20 kJ per litre of oxygen consumed (Weir, 1949). BMR was calculated as the lowest value of the mean measurements after 0h 00m, without the first minute of each set of six minutes.

In the morning of D12 we removed the PIT-tags and released the birds near their nest boxes. The absence of the adults in the night of D11 was assumed not detrimental to the chicks' thermoregulation since they are able to thermoregulate themselves at this age (which is just around three days before fledging).

Data analyses

When birds were raising chicks without a partner ($n=7$), they were excluded from all analyses. We also excluded cases in which we observed a high BMR variation overnight caused by equipment malfunction (two nights). Statistical analyses were performed in R version 3.4.3 (R_Core_Team, 2017) in the “lme4” (Bates et al., 2015) and “pbkrtest” (Halekoh and Højsgaard, 2014) packages and were carried out separately for both parts of the experiment.

First, we tested whether the brood size manipulations at D2 led to a change in FF, DEE and BMR. We used linear mixed-effect models and performed three separate analyses with FF (at D11), DEE and BMR as response variables and included treatment (-2, 0, +2) as fixed effect. For this analysis, we only used birds that were not manipulated at D11 (so, +0 chicks at D11). Because we expected that an increase in brood size would lead to an increase in FF, DEE and BMR, we used ordered heterogeneity tests (Rice and Gaines, 1994) to calculate the p -values that take into account the expected order of treatments.

Then, we tested the effect of the brood size manipulations at D11. As before, we used linear mixed-effect models and performed three separate analyses with the within pair difference in FF between D10 and D11, DEE and BMR as response variables and treatment at D11 (0, 2 or 4 chicks added at D11) as fixed effects. We used ordered heterogeneity tests (Rice and Gaines, 1994) to calculate the p -values that take into account the expected order of treatments (either increase or decrease in BMR with a higher number of chicks).

~~Finally, we tested the relationship between FF and DEE and between DEE and BMR, including the treatment effects of D2 and D11~~

In all models, to control for other variables that could affect our response variables, we also included the sex of the parent (as a main effect and in interaction with treatment), the original brood size (at D1) and the day that chicks were 10 days old, to control for changes in effort over the season, as fixed effects. We also included nest box as random effect, since both parents of the same nest were measured. To control for effects of the weather, we obtained measurements of wind speed (m/s), average ambient temperature ($^{\circ}\text{C}$), number of hours of sun, sun shine duration (hours) at D11 from a weather station at De Bilt, close to the Hoge Veluwe study area, and performed a Principal Component Analysis on all these weather variables. PC1 loadings were mainly related to temperatures and sun duration, while PC2 loadings were related to rainfall and wind. We then included the principal components 1 and 2 as fixed effects in our models. Finally, for all analyses with BMR as response variables, we also included the mass of the bird in the morning as fixed effect and the number of the metabolic chamber where we measured BMR as random effect. We defined the minimal model using

backwards variable selection but always keeping the nuisance variables in the models (sex of the bird, number of chicks at day 1, weather PC1 and PC2, day of the year and mass of the bird in the morning).

Results

Feeding frequencies (FF) at D11 significantly differed between the -2, +0 and +2 treatments at D2, with treatment +2 having a higher and treatment -2 a lower FF than the control +0 ($F_{2,16.78} = 2.57$; $rsPc = 0.89$; p -value = 0.02; Fig. 2A). After the brood size manipulation at D11, the difference between the FF at day 10 and 11 was significantly different between the treatments, with +4 treatment having the largest and the +0 treatment the smallest increase in FF ($F_{2,31.16} = 7.62$; $rsPc = 0.99$; p -value < 0.01; Fig. 2D). In both cases, there was no significant interaction between treatment and sex of the bird (Treatment D2: $F_{2,20.29} = 0.54$; p -value = 0.59; Treatment D11: $F_{2,33.24} = 2.32$; p -value = 0.11).

Daily energy expenditure (DEE) did not differ between treatments at D2 ($F_{2,11.10} = 0.83$; $rsPc = 0.54$; p -value = 0.10; Fig. 2C), nor between treatments at D11 ($F_{2,27.72} = 0.94$; $rsPc = 0.25$; p -value = 0.30; Fig. 2E). Again, there was no significant interaction between treatment and sex (Treatment D2: $F_{2,14.21} = 0.76$; p -value = 0.49; Treatment D11: $F_{2,29.62} = 2.01$; p -value = 0.15).

Basal metabolic rate (BMR) did not differ between treatments at D2 ($F_{2,14.34} = 0.42$; $rsPc = 0.17$; p -value > 0.05; Fig. 2D). However, there was a significant decline in BMR when broods were enlarged after treatment at D11, with the lowest BMR value for the +4 treatment and the highest for the +0 treatment ($F_{2,19.84} = 3.46$; $rsPc = 0.95$; p -value = 0.02; Fig. 2F). There was no significant interaction between treatment and sex (Treatment D2: $F_{2,3.29} = 0.81$; p -value = 0.52; Treatment D11: $F_{2,33.24} = 2.32$; p -value = 0.05).

Mean within brood chick weight at D12 differed between D2 treatments (comparing -2/0, 0/0 and +2/0 treatments) with the -2/0 being the heaviest and the +2/0 chicks the lightest, although the effect size was very small ($F_{2,36.05} = 2.03$; $rsPc = 0.85$; p -value = 0.03, estimates: -2 = -2.93 ± 3.39 , +0 = -3.39 ± 3.36 , +2 = -3.75 ± 3.43). While there was no difference between D11 treatments ($F_{2,37.18} = 0.34$; $rsPc = 0.15$; p -value = 0.4).

Discussion

We experimentally tested whether wild pied flycatchers respond to a short- and long-term increase in day-time workload (i.e. an increase in their feeding frequency; FF), in their daily energy expenditure (DEE) and basal metabolic rates (BMR). For both the short- and the long-term manipulation we find

that increasing the number of nestlings experimentally leads to an increase in FF, hence our treatment successfully increased the day-time workload.

In the long-term manipulation (D2 treatment), pied flycatchers with enlarged broods fed their offspring more often than birds with reduced broods but this adjustment in FF did not lead to a change in DEE or BMR. This argues against a long-term adjustment that would allow the birds to increase their energy expenditure (increased-intake hypothesis). It remains unclear how the birds could feed more frequent without increasing their energy expenditure (see also Appendix). Note that the increase in FF did not completely compensated the increase in the number of chicks as there was a reduction in fledgling mass at D12 with the increased brood size on D2.

In the short-term manipulation (D11 treatment), pied flycatchers increased their FF, reduced their BMR and showed no change in their DEE. This indicates that pied flycatchers compensate on the short term for an increased number of chicks in their nests (compensation hypothesis). It is difficult to translate the reduction in BMR (with about 1.5 kJ/24 h) into a gain in energy for food provisioning. The average DEE (whole day) is 80 kJ/24 h and BMR (night-time) is 24 kJ/h, leaving 56 kJ for all day-time activities, but we do not know how much of this is allocated in food provisioning. In the unlikely case that the entire 56 kJ is allocated to provisioning, the gain after a night-time saving would just be 3%, but if only 25% is allocated to provisioning, the gain would be much more substantial at 10%.

Similar to the present experiment, Nilsson (2002) investigated the effects of increased workload on free-living marsh tits (*Poecile palustris*) by increasing their brood size. He found evidence supporting the energy-intake hypothesis: a few days after having their workload increased, birds had a higher energy expenditure and basal metabolic rate. Costs of breeding in this case, thus have not come from a trade-off but from the consequences of sustaining a high metabolic rate *per se* (Nilsson, 2002). Wiersma & Tinbergen (2003) also manipulated brood size of great tits (*Parus major*) when chicks were two days old and then measured BMR and DEE when chicks were 12 days old. They found that BMR decreased with the increased workload but the relation was not significant and thus did not find evidence for the compensation hypothesis. It is important to stress, however, that both these studies do not necessarily exclude the possibility that compensation occurred. In order to test for compensation it is important to also measure BMR shortly after the workload manipulation, because the two processes are not mutually exclusive and, therefore, both increased-intake expenditure on the long- and compensation on the short-term may be in place.

Deerenberg et al. (1998) manipulated workload by forcing the birds to work harder for their food. Results of their (laboratory) study supported that, under work-for-food conditions, zebra finches would compensate their day-time activity with reduced nocturnal expenditure. They argued that

compensation could be profitable if the environment does not allow an increase in energy intake, for example, limited food availability or high foraging risks. Similarly, Nilsson (2002) suggested that increasing the food intake is only feasible when the foraging cost is low, which seem to be the case for the marsh tits in his study site. For the pied flycatchers in the Hoge Veluwe, although there is now a mismatch between timing of chick hatching and the caterpillar biomass peak (Both et al., 2006), fitness did not correlated with the timing of breeding relative to the food peak (Visser et al., 2015). Therefore, low food availability does not seem to be a convincing explanation for the use of compensation rather than increased-intake by pied flycatchers responding to a high workload.

The results of our experiment suggest that pied flycatchers may work at their physiological limit and are constrained in their ability to adjust organs over time to increase energy expenditure. They thus resort to the short-term strategy of trading off current and future reproduction. This pattern is curious, since long-distance migrants are known to increase their energy intake well beyond the normal rate when they are preparing to migrate (Lindstrom and Kvist, 1995). However, such capacity could also depend on the stage of the annual cycle (Weber and Piersma, 1996) and outside the migration period long-distance migrants may have a lower flexibility to adjust their energetic expenditure via physiological modifications. In the light of the combined results from this and previous studies (Deerenberg et al., 1998; Nilsson, 2002; Wiersma and Tinbergen, 2003) differences across species and studies could be caused by species-specific physiological limitations and/or ecological constraints, for example, differences of migratory and non-migratory birds. If the strategy of compensation is specific to certain species or environmental conditions, it may explain why DEE reflects parental effort in a few cases but not others (Bryant, 1988; Wiersma and Tinbergen, 2003). Therefore, studies using DEE as a measure of parental effort should also take into account whether their focus species uses nocturnal compensation as an energy-saving strategy because, if they do, results may be blurred as a consequence.

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Competing interests

No competing interests declared

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320 **Data Availability**

321 Data supporting this manuscript will be available at the Dryad Digital Repository upon publication.

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Legends

Figure 1: Predictions of the experiment based on the two hypotheses: A) According to the increased-intake strategy, BMR increases with increased workload, and thus BMR is expected to increase for parents of nests that were given more chicks at day 2. This pattern is obtained by comparing broods that were not further manipulated at day 11. Colours represent the different treatments: light blue = -2 chicks at D2, blue = +0 chicks at D2, purple = +2 chicks at D2. **B)** According to the compensation strategy, the brood enlargement when chicks are 11 days old will lead to a decrease in BMR compared to birds with no additional brood size manipulation. This pattern is obtained by using all broods, but the +0 treatment at day 11 comprises nests from all treatments at day 2 (the same as in figure A), while +2 treatment only includes the +0 treatment at D2 and +4 treatment only includes the -2 treatment at D2. Colours represent the different treatments: black = +0 chicks at D11, pink = +2 chicks at D11, red = +4 chicks at D11.

Figure 2: The effects of brood size manipulations on day 2 (A-C) and day 11 (D-F) on the feeding frequency (A & D), daily energy expenditure (B & E) and basal metabolic rate (C & F). Mean and s.e.m. per treatment group at day 2 (a-c) and day 11 (d-f) (calculated using the residuals of the model without treatment effect) are plotted. **Treatment D2:** **a)** feeding frequency at day 11 (sample sizes: -2 chicks = 16; +0 chicks = 14; +2 chicks = 14); **b)** Daily energy expenditure (kJ/day) at day 11 (sample sizes: -2 chicks = 14; +0 chicks = 10; +2 chicks = 9) and **c)** basal metabolic rate (kJ/day) at day 11 (sample sizes: -2 chicks = 11; +0 chicks = 8; +2 chicks = 10). This treatment only compares broods that were not further manipulated at day 11. Colours represent the different treatments: light blue = -2 chicks at D2, blue = +0 chicks at D2, purple = +2 chicks at D2. **Treatment D11:** **d)** difference in feeding frequency between day 10 and day 11 (sample sizes: +0 chicks = 40; +2 chicks = 15; +4 chicks = 17); **e)** Daily energy expenditure (kJ/day) at day 11 (sample sizes: +0 chicks = 33; +2 chicks = 12; +4 chicks = 15) and **f)** basal metabolic rate (kJ/day) at day 11 (sample sizes: +0 chicks = 29; +2 chicks = 9; +4 chicks = 13). This treatment compares all broods, but the +0 treatment at day 11 lumps nests from all treatments at day 2 (-2, +0 and +2 chicks at D2), while +2 treatment only includes the +0 treatment at D2 and +4 treatment only includes the -2 treatment at D2. Colours represent the different treatments: black = +0 chicks at D11, pink = +2 chicks at D11, red = +4 chicks at D11.

Tables

Table 1: An overview of the main characteristics of the treatments.

Treatment D2	Treatment D11	Number of nests	Number of females	Number of males	Average no. of chicks on D10 (min/max)	Average no. of chicks on D11 (min/max)
-2	+0	9	9	8	4.5 (4/5)	4.5 (4/5)
-2	+4	9	9	8	4.3 (4/5)	7.8 (7/9)
+0	+0	9	8	6	5.6 (3/7)	5.5 (3/7)
+0	+2	8	8	8	6.1 (6/7)	8.0 (7/9)
+2	+0	9	8	8	8.35 (6/9)	8.2 (5/9)

Remarks: When the minimum or maximum number of chicks is lower than expected, based on the starting number of chicks (six or seven) and the treatment, this means that some chicks have died during the experiment.

Figure 1

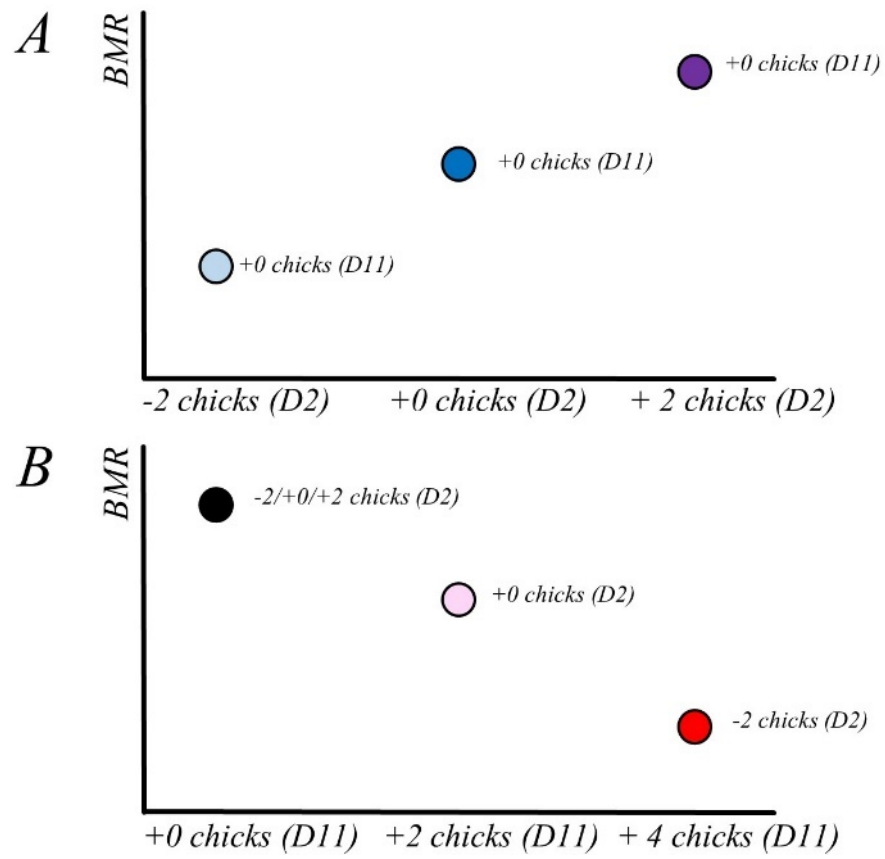
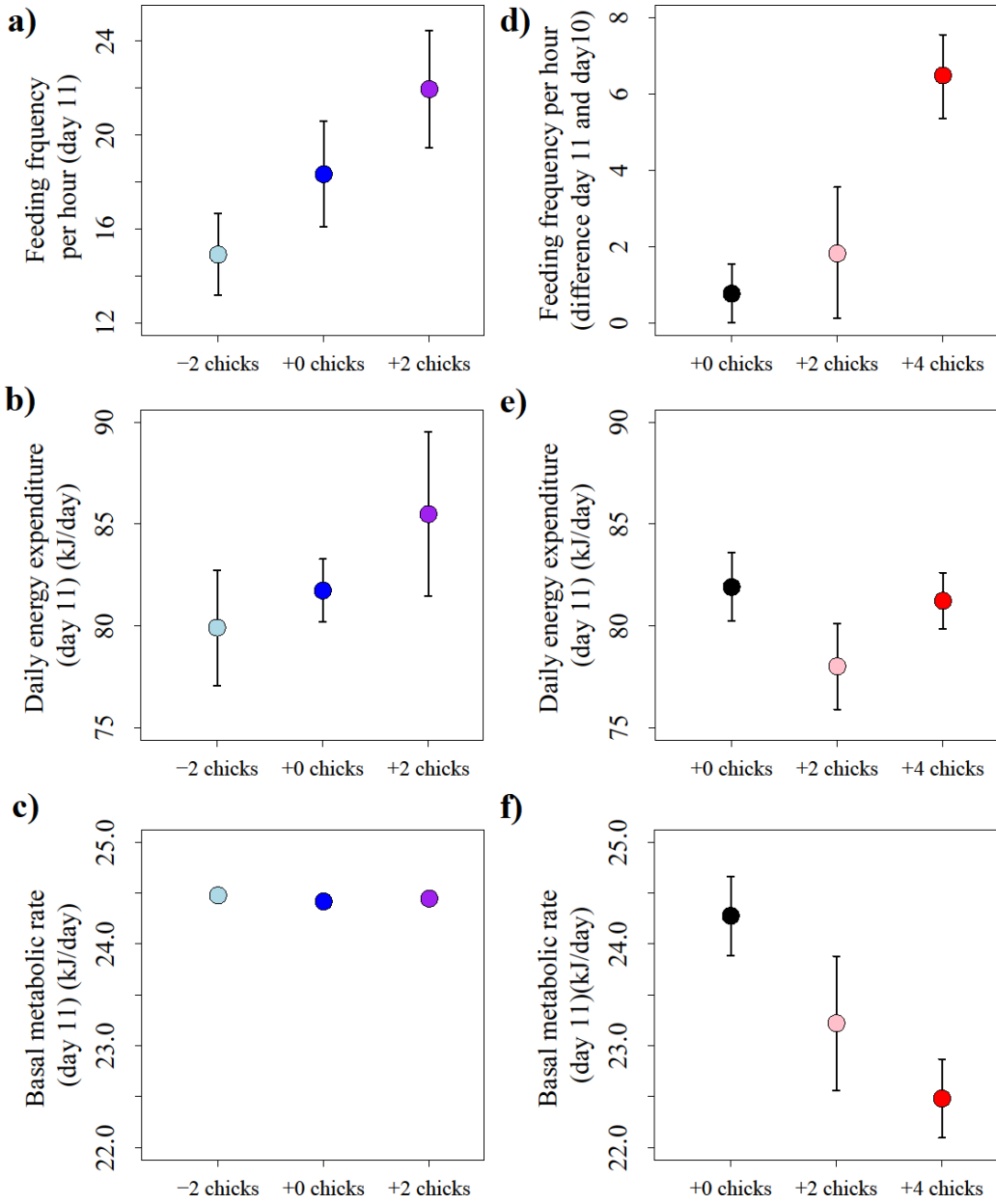


Figure 2



Appendix

A common measurement of the classical life history trade-off of current and future reproductive success is how much parents work to raise their offspring (Stearns, 1992; Wiersma and Tinbergen, 2003; Williams, 1966). Parental effort can be measured as how often individuals feed their offspring, which should translate into the amount of energy an individual spends. Many studies, however, do not find a correlation between FF and DEE or it is inconsistent across studies or individuals (Bryant, 1988; Burness et al., 2001; Moreno et al., 1995; Tinbergen and Verhulst, 2000) but see (Nilsson, 2002; te Marvelde et al., 2011) and Williams (2012) for a revision, This raises questions on the role of energy expenditure on the costs of reproduction (Tinbergen and Verhulst, 2000; Wiersma and Tinbergen, 2003).

We tested for a correlation between FF and DEE but found none ($F_{1,49.75} = 2.01$; p -value = 0.16; slope: 0.20 ± 0.13 , Fig. A1A) nor between DEE and BMR ($F_{1,49.75} = 31.26$; p -value = 0.16; slope: 0.02 ± 0.05 , Fig. A1B). These two patterns were also found on previous studies using birds to measure FF, DEE and/or BMR (Bryant, 1988; Ricklefs et al., 1996; Tinbergen and Verhulst, 2000). Because DEE measurements consist in a sum of the parental effort over 24h, if birds compensate for a higher effort during the day by reducing their energy expense at night, DEE may give a misleading picture of parental effort (Wiersma and Tinbergen, 2003).

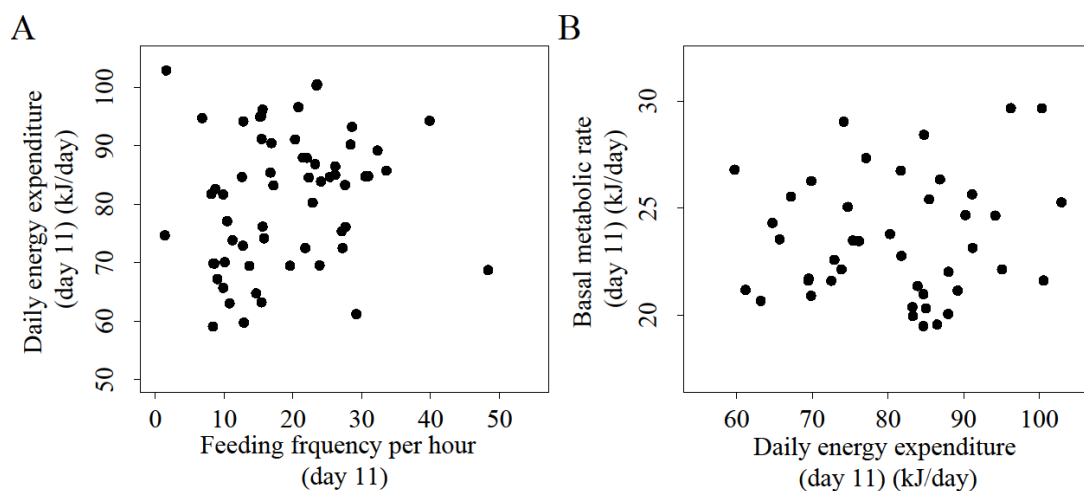


Figure A1: Relationship between **A)** daily energy expenditure (kJ/day) and feeding frequency per hour and **B)** daily energy expenditure (kJ/day) and basal metabolic rate (kJ/day) at day 11.