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RESEARCH ARTICLE

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

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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 at 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 found 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.

KEY WORDS: *Ficedula hypoleuca*, Increased-intake hypothesis, Compensation hypothesis, Basal metabolic rate, Daily energy expenditure, Feeding frequency

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, involve the physiological costs not only 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. (1) 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). (2) Alternatively, energy could be 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 (DEE).

Which of the strategies is adopted determines the form of the relationship between basal metabolic rate (BMR) and 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 animals trade off a higher energy expenditure during the day against 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 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 an 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 parental workload by adding or removing chicks at two different times: during early breeding or late breeding, when chicks were 2 and 11 days old, respectively, and measured feeding

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frequency (FF), DEE and BMR at day 11 (which is around 3 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.

MATERIALS AND METHODS

Experimental rationale

We manipulated parental workload via manipulation of brood sizes when chicks were 2 days old (henceforth, D2): −2 chicks (*n*=18 pairs), +0 chicks (*n*=18 pairs) or +2 chicks (*n*=9 pairs). Parents were then allowed to adapt to the change in brood size until the chicks were 10 days old (D10). During the night between D10 and day 11 (D11), we performed a second brood size manipulation in 18 of the manipulated pairs, by enlarging their broods with either +2 chicks (*n*=9 out of 18 pairs of the group that had +0 chicks from D2 onwards) or +4 chicks (*n*=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 with 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 FF were taken at D10 and D11 (to enable measurement of within-pair changes in FF before and after the D11 manipulation), DEE at D11 and 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), +0 D2/+0 D11 (blue) and +2 D2/+0 D11 (purple)].

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

Study species and allocation to the experiment

The pied flycatcher, *Ficedula hypoleuca* ([Pallas] 1764), is a long-distance migratory bird that, similar to other bird species, displays a considerable variation in individual oxygen consumption rates (Røkaft 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 size for the Hoge Veluwe) and allocated them to the treatment groups (Table 1). Because not all eggs were viable, we corrected brood size 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 weighed using a spring balance as a measure for their fledging mass.

All procedures were carried out under licence from 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 the number of animals, see Table 1).

FF

When chicks were 7 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 8 days old

Table 1. Overview of the main characteristics of the treatments

Treatment		No. of	No. of	No. of	Mean no. of chicks (min./max.)	
D2	D11	nests	females	males	D10	D11
−2	+0	9	9	8	4.5 (4/5)	4.5 (4/5)
	+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)
	+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)

A lower minimum or maximum number of chicks than expected, based on the starting number of chicks (6 or 7) and the treatment, indicates that some chicks have died during the experiment.

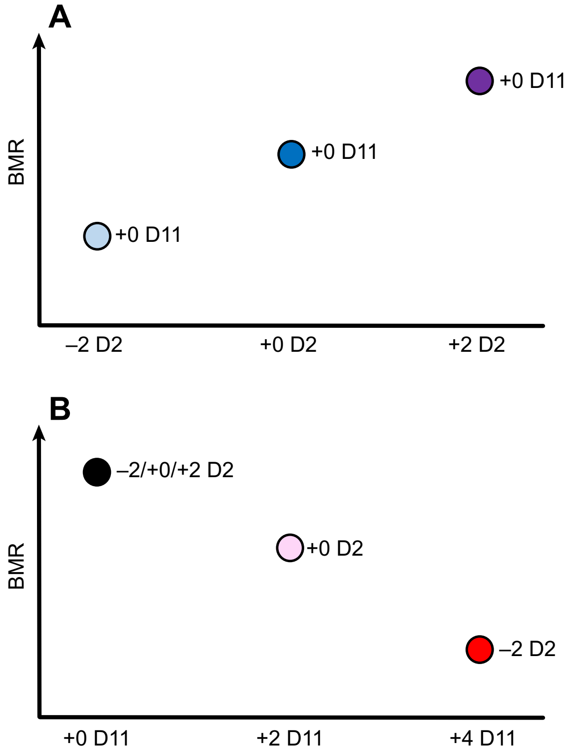

Fig. 1. Predictions based on the two hypotheses: increased intake and compensation. (A) According to the increased-intake strategy, basal metabolic rate (BMR) increases with increased workload, and thus BMR is expected to increase for parents that were given more chicks to care for on day 2 (D2). This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

(D8), we placed a transponder reader (Trovan LID650/LID665/LID1260 software, version V607) around the opening of the nest box, which recorded when birds entered the box, with a 20 s 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 05:00 h and 19:00 h, as in the morning most birds (83%) started feeding between 05:00 h and 06:00 h, and in the evening the birds were caught for BMR measurements (see below).

DEE

We measured 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 at 19:32 h (mean, ±24 min; range 18:55–21:10 h), 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 µl) from the brachial vein (puncturing the vein only once), which were stored in flame-sealed heparinised capillary tubes. Then, at 20:33 h (mean, ±30 min, range 19:20–21:50 h) the next day, birds were recaptured and blood samples taken (3×15 µl).

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 in ml CO_2 g^{-1} h^{-1} was derived) was determined from fractional turnover of the two isotopes using the equations of Lifson and McClintock (1966). CO_2 production was converted to energy expenditure (kJ day^{-1}) by assuming a respiratory quotient of 0.8 and energetic equivalent of 27.8 kJ l^{-1} 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 22:00 h until 06:00 h). Each bird was placed in an individual sealed metabolic chamber (2.2 dm^3) which was placed in the dark inside a climate cabinet at 25.5±0.15°C (which is within the thermoneutral zone). H_2O and CO_2 were removed from the inlet air (that was 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 the 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 (6 min for each channel, with readings each 15 s; every bird was measured every 48 min). Temperature, air flow rate and air concentration of O_2 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 l^{-1} of oxygen consumed (Weir, 1949). BMR was calculated as the lowest value of the mean measurements after 00:00 h, excluding the first minute of each set of 6 min.

On 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 as they are able to thermoregulate themselves at this age (which is around 3 days before fledging).

Data analysis

Birds that raised chicks without a partner (n=7) 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 (http://www.R-project.org/) 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 a fixed effect. For this analysis, we only used birds that were not manipulated at D11 (i.e. +0 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 *P*-values that take into account the expected order of treatments (r_sP_c).

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 *P*-values that take into account the expected order of treatments (either an increase or a decrease in BMR with a higher number of chicks).

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 a random effect, as both parents of the same nest were measured. To control for effects of the weather, we obtained measurements of wind speed (m s^{-1}), average ambient temperature (°C), number of hours of sun (h), number of hours of rain (h) and amount of rain (mm) 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 temperature and sun duration, while PC2 loadings were related to rainfall and wind. We then included the principal components (PC)1 and 2 as fixed effects in our models. Finally, for all analyses with BMR as the response variable, we also included the mass of the bird in the morning as a 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

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, r_sP_c=0.89, P=0.02; Fig. 2A). After the brood size manipulation at D11, the difference between the FF at day 11 and day 10 was significantly different between the treatments, with the +4 treatment having the largest increase and the +0 treatment the smallest increase in FF ($F_{2,31.16}$=7.62, r_sP_c=0.99, P<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=0.59; treatment D11: $F_{2,33.24}$=2.32, P=0.11).

DEE did not differ between treatments at D2 ($F_{2,11.10}$=0.83, r_sP_c=0.54, P=0.10; Fig. 2B) or between treatments at D11 ($F_{,27.72}$=0.94, r_sP_c=0.25, P=0.30; Fig. 2E). Again, there was no significant interaction between treatment and sex (treatment D2: $F_{2,14.21}$=0.76, P=0.49; treatment D11: $F_{2,29.62}$=2.01, P=0.15).

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

Mean within-brood chick mass at D12 differed between D2 treatments (comparing −2/0, 0/0 and +2/0 treatments), with −2/0 chicks being the heaviest and +2/0 chicks the lightest, although the effect size was very small ($F_{2,36.05}$=2.03, r_sP_c=0.85, P=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, r_sP_c=0.15, P=0.4).

DISCUSSION

We experimentally tested whether wild pied flycatchers respond to a short- and long-term increase in daytime workload (i.e. an increase in their FF) in terms of their DEE and BMR. For both the short- and long-term manipulation, we found that increasing the number of nestlings experimentally led to an increase in FF, hence our treatment successfully increased the daytime workload.

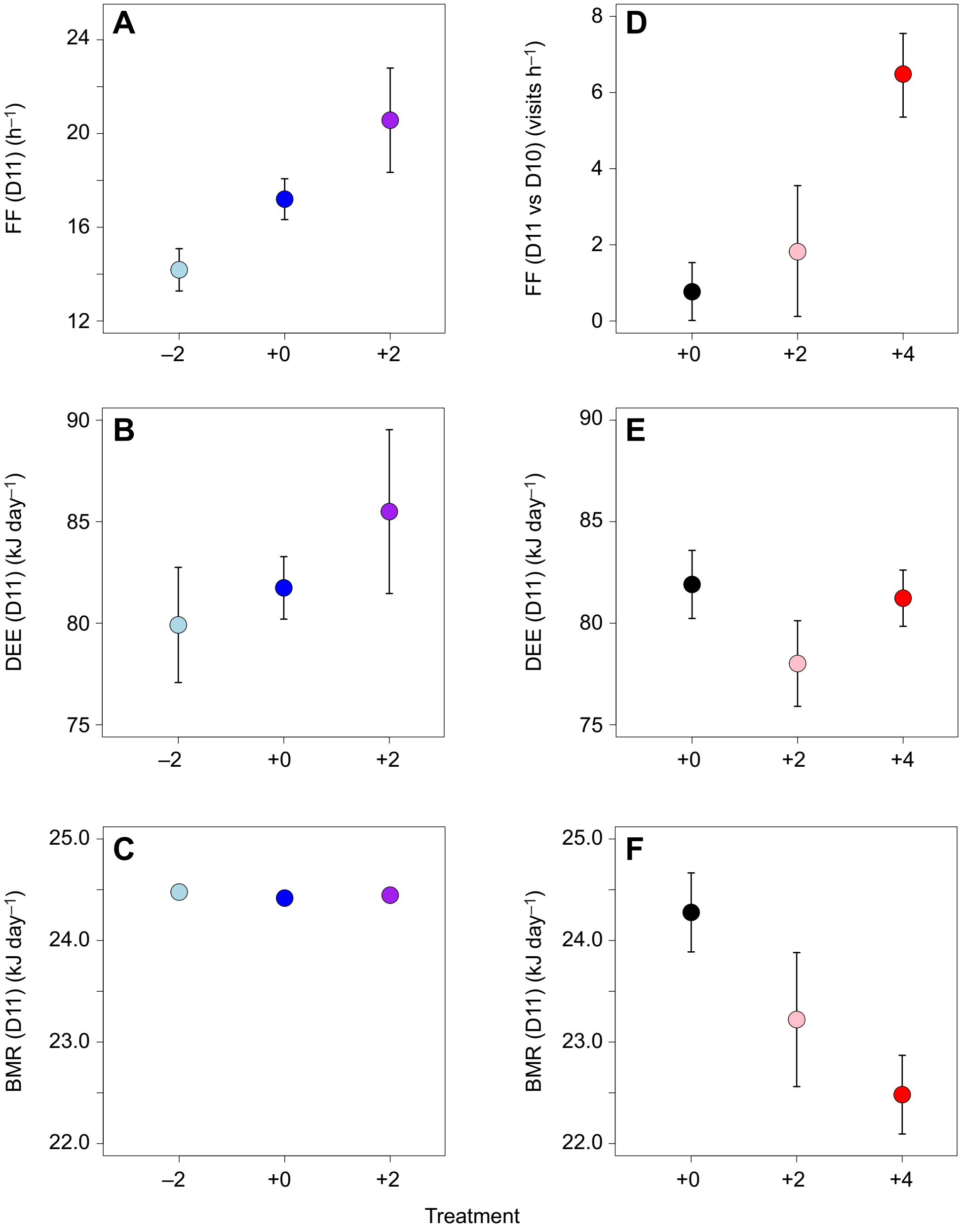

Fig. 2. The effects of brood size manipulation on D2 and D11 on feeding frequency (FF), daily energy expenditure (DEE) and BMR. (A,D) FF, (B,E) DEE and (C,F) BMR. Means±s.e.m. per treatment group at D2 (A–C) and D11 (D–F) (calculated using the residuals of the model without treatment effect) are plotted. Treatment D2: (A) FF at D11 (sample size: −2 chicks n=16, +0 chicks n=14, +2 chicks n=14); (B) DEE at D11 (sample size: −2 chicks n=14, +0 chicks n=10, +2 chicks n=9); and (C) BMR at D11 (sample size: −2 chicks n=11, +0 chicks n=8, +2 chicks n=10). This treatment only compares broods that were not further manipulated at D11. 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 FF between D11 and D10 (sample size: +0 chicks n=40, +2 chicks n=15, +4 chicks n=17); (E) DEE at D11 (sample size: +0 chicks n=33, +2 chicks n=12, +4 chicks n=15); and (F) BMR at D11 (sample size: +0 chicks n=29, +2 chicks n=9, +4 chicks n=13). This treatment compares all broods, but the +0 treatment at D11 lumps nests from all treatments at D2 (−2, +0 and +2 chicks at D2), while the +2 treatment only includes the +0 treatment at D2 and the +4 treatment only includes the −2 treatment at D2. Colours represent the different treatments: black, +0 D11; pink, +2 D11; red, +4 D11.

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 frequently without increasing their energy expenditure (see Appendix). Note that the increase in FF did not completely compensate for the increase in the number of chicks as there was a reduction in fledgling mass at D12 with the increase in 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 in the short term for an increase in the number of chicks in their nests (compensation hypothesis). It is difficult to translate the reduction in BMR (of 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^{-1}, leaving 56 kJ for all daytime activities, but we do not know how much of this is allocated to 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 BMR. 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 and Tinbergen (2003) also manipulated the brood size of great tits (*Parus major*) when chicks were 2 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 relationship was not significant and thus they did not find evidence for the compensation hypothesis. It is important to stress, however, that these two 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 in the long term and compensation in the short term may take place.

Deerenberg et al. (1998) manipulated the workload of zebra finches by forcing them to work harder for their food. Results of their (laboratory) study supported that, under work-for-food conditions, zebra finches compensate for their daytime 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, when there is limited food availability or a high foraging risk. Similarly, Nilsson (2002) suggested that increasing food intake is only feasible when the foraging cost is low, which seems to be the case for the marsh tits at his study site. For the pied flycatchers in the Hoge Veluwe, although there is now a mismatch between the timing of chick hatching and the caterpillar biomass peak (Both et al., 2006), fitness did not correlate 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 their 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, as 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, such as differences between 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 the focus species uses nocturnal compensation as an energy-saving strategy because, if it does, the results may be blurred as a consequence.

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 in terms of how often individuals feed their offspring, which should translate into

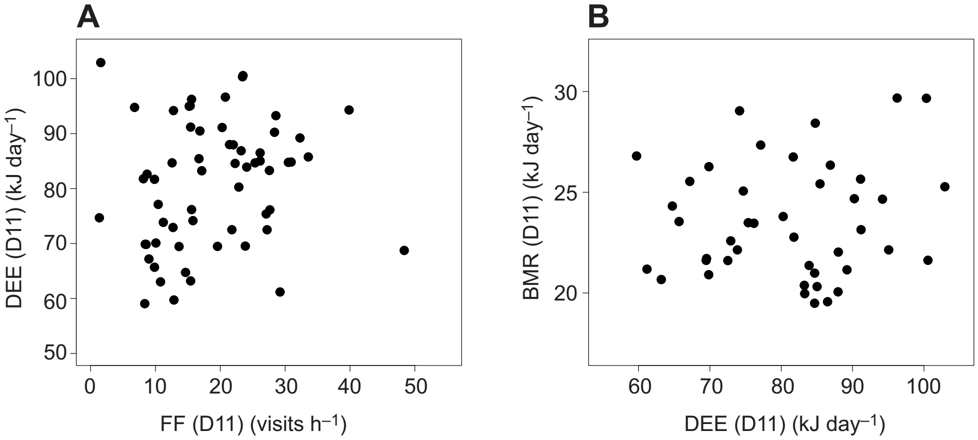

Fig. A1. Relationship between DEE and both FF and BMR at day 11. (A) DEE and FF; (B) DEE and BMR.

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; Williams, 2012). This raises questions on the role of energy expenditure in the cost 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=0.16, slope: 0.20±0.13; Fig. A1A), nor was there a correlation between DEE and BMR ($F_{1,49.75}$=31.26, P=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 of the sum of the parental effort over 24 h, if birds compensate for a higher effort during the day by reducing their energy expenditure at night, DEE may give a misleading picture of parental effort (Wiersma and Tinbergen, 2003).

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: M.E.V.; Methodology: M.E.V., A.B., H.M., L.t.M.; Formal analysis: M.E.V., C.v.D., B.M.T., P.G.; Investigation: C.v.D., A.B., H.M., L.t.M.; Writing - original draft: M.E.V., C.v.D., B.M.T.; Writing - review & editing: A.B., H.M., P.G.; Visualization: B.M.T.; Supervision: M.E.V.

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Data availability

Data are available from the Dryad Digital Repository (Visser et al., 2019): https://doi.org/10.5061/dryad.pp77bs0

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