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

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19

20 **Keywords:** *Ficedula hypoleuca*, Increased-intake Hypothesis, Compensation Hypothesis, Basal  
21 Metabolic Rate, Daily Energy Expenditure, Feeding Frequency.

22 **Running title:** Work load and energetics in a songbird

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

25

26

27

28 **Abstract**

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

46

47 **Introduction**

48 From an energetic point of view, reproduction is an expensive annual-cycle stage that largely  
49 determines the fitness of an individual. Costs of reproduction in birds, for example, not only involve  
50 the physiological costs of egg production by the female, but also of courtship, territory defence and  
51 offspring rearing, that may involve both males and females (Tinbergen and Verhulst, 2000; Visser and  
52 Lessells, 2001). The debate over how animals meet the increased energy demands required for  
53 reproduction spans many years and has been investigated in birds (Daan et al., 1990; Drent and Daan,  
54 1980; Moreno and Sanz, 1994; Nilsson, 2002; Tinbergen and Verhulst, 2000; Vézina et al., 2006;  
55 Wiersma and Tinbergen, 2003) and mammals (Bennett and Ruben, 1979; Ricklefs et al., 1996;  
56 Speakman et al., 2004).

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

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

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

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

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

89

90

91 **Material and Methods**

92 *Experimental rationale*

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

104 If birds responded to the manipulations via an adjustment of their metabolic machinery, with a  
105 consequent increase in their BMR (increased-intake hypothesis), we expected that BMR would be higher  
106 in enlarged broods (+2) and lower in reduced broods (-2) in relation to control broods (+0) (Fig. 1A:  
107 comparison between the groups -2 D2/ +0 D11: light blue points, +0 D2/ +0 D11: blue points and +2 D2/  
108 +0 D11: purple points).

109 If birds responded to the manipulations by relocating energy from their maintenance processes, with a  
110 consequent decrease in their BMR (compensation hypothesis), we expected a decrease in the BMR of the  
111 groups that had their brood enlarged at D11, in relation to the broods that were not enlarged at D11 (Fig.  
112 1B: comparison between the groups -2 D2/+4 D11: red points, +0 D2/+2 D11: pink points, and  
113 combination of -2,+0,+2 D2/+0 D11: black points).

114

115 *Study species and allocation to the experiment*

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

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

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

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

131

### 132 *Feeding frequency (FF)*

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

141

### 142 *Daily energy expenditure (DEE)*

143 We measured daily energy expenditure (DEE) for each bird with the doubly-labelled water technique  
144 ( $D_2^{18}O$ ) (Lifson and McClintock, 1966; Moreno et al., 1995; Moreno and Sanz, 1994; Nagy, 1980) from  
145 the evening of D10 until 24 h later. At the end of D10, males and females were caught on average at 19h  
146 32m ( $\pm 24$  min; range 18h 55m - 21h 10m), and were injected intraperitoneally with  $0.105 \pm 0.0005$  ml of  
147 a mixture containing 65% of  $H_2^{18}O$ , 97 atom% and 35% of  $D_2O$ , 99.9 atom%. Each bird was then placed  
148 in a small bag for  $68 \pm 3$  min to allow equilibration of the isotopes in the bird's body fluids. After this  
149 period, we took three blood samples (15  $\mu$ l) from the brachial vein (puncturing the vein only once),  
150 which were stored in flame-sealed heparinised capillary tubes. Then, on average at 20h 33m ( $\pm 30$  min,  
151 range 19h 20m - 21h 50m) of the next day, birds were recaptured and blood sampled (3x 15  $\mu$ l).

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

155 average daily metabolic rate (ADMR) in ml CO<sub>2</sub>/g/h is derived) was determined from fractional  
156 turnovers of the two isotopes using the equations by (Lifson and McClintock, 1966). CO<sub>2</sub> production  
157 was converted to energy expenditure (kJ/d) by assuming a respiratory quotient of 0.8 and energetic  
158 equivalent of 27.8 kJ per litre of exhaled carbon dioxide (te Marvelde et al., 2011). The isotope  
159 enrichment of the blood samples was corrected for the natural background isotopic abundance of the  
160 body fluids. The latter was determined using blood samples from four non-injected individuals.

161

#### 162 *BMR measurements*

163 On D11 birds were kept overnight in a metabolic chamber for BMR measurements. BMR was  
164 measured as the average minimum oxygen consumption in an open-circuit respirometer located in a  
165 field shed in the study area (from 22h 00m until 6h 00m). Each bird was placed in an individual sealed  
166 metabolic chamber (2.2 dm<sup>3</sup>) which was placed in the dark inside a climate cabinet at 25.5±0.15 °C  
167 (which is within the thermoneutral zone). H<sub>2</sub>O and CO<sub>2</sub> were removed from the inlet air (blown into  
168 the animal chamber) respectively with Drierite®(6 mesh, Sigma-Aldrich Chemie b.v., Zwijndrecht, The  
169 Netherlands) and Ascarite® (5–20 mesh, Fluka, Zwijndrecht, The Netherlands). Air flow rate was set to  
170 250 ml min<sup>-1</sup> with flowmeters (Brooks Instrument b.v., Ede, The Netherlands), placed before the  
171 chamber and previously calibrated using a soap bubble method (Bubble-O-Meter, LLC, Dublin, OH,  
172 USA). Oxygen content of outlet air was measured with an oxygen analyser (Servomex 4100, Servomex  
173 BV, Zoetemeer, The Netherlands) (see also Caro and Visser, 2009). Readings were recorded during  
174 pre-set periods (six minutes for each channel, with readings each 15 seconds, every bird was measured  
175 every 48 min). Temperature, air flow rate and air concentrations of O<sub>2</sub> were measured, based on inlet  
176 and outlet air. Oxygen consumption was calculated based on Hill (1972), and converted to energy  
177 expenditure (kJ) assuming an appropriate respiratory quotient (RQ) of 0.8 for insectivorous birds, and  
178 an energetic equivalent of 20 kJ per litre of oxygen consumed (Weir, 1949). BMR was calculated as the  
179 lowest value of the mean measurements after 0h 00m, without the first minute of each set of six  
180 minutes.

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

184

#### 185 *Data analyses*

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

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

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

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

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



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

220

## 221 **Results**

222 Feeding frequencies (FF) at D11 significantly differed between the -2, +0 and +2 treatments at D2, with  
223 treatment +2 having a higher and treatment -2 a lower FF than the control +0 ( $F_{2,16.78} = 2.57$ ;  $rsPc =$   
224  $0.89$ ;  $p$ -value =  $0.02$ ; Fig. 2A). After the brood size manipulation at D11, the difference between the FF  
225 at day 10 and 11 was significantly different between the treatments, with +4 treatment having the  
226 largest and the +0 treatment the smallest increase in FF ( $F_{2,31.16} = 7.62$ ;  $rsPc = 0.99$ ;  $p$ -value  $< 0.01$ ; Fig.  
227 2D). In both cases, there was no significant interaction between treatment and sex of the bird  
228 (Treatment D2:  $F_{2,20.29} = 0.54$ ;  $p$ -value =  $0.59$ ; Treatment D11:  $F_{2,33.24} = 2.32$ ;  $p$ -value =  $0.11$ ).

229 Daily energy expenditure (DEE) did not differ between treatments at D2 ( $F_{2,11.10} = 0.83$ ;  $rsPc = 0.54$ ;  $p$ -  
230 value =  $0.10$ ; Fig. 2C), nor between treatments at D11 ( $F_{2,27.72} = 0.94$ ;  $rsPc = 0.25$ ;  $p$ -value =  $0.30$ ; Fig.  
231 2E). Again, there was no significant interaction between treatment and sex (Treatment D2:  $F_{2,14.21} =$   
232  $0.76$ ;  $p$ -value =  $0.49$ ; Treatment D11:  $F_{2,29.62} = 2.01$ ;  $p$ -value =  $0.15$ ).

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

239 Mean within brood chick weight at D12 differed between D2 treatments (comparing -2/0, 0/0 and  
240 +2/0 treatments) with the -2/0 being the heaviest and the +2/0 chicks the lightest, although the effect  
241 size was very small ( $F_{2,36.05} = 2.03$ ;  $rsPc = 0.85$ ;  $p$ -value =  $0.03$ , estimates: -2 =  $-2.93 \pm 3.39$ , +0 =  $-3.39$   
242  $\pm 3.36$ , +2 =  $-3.75 \pm 3.43$ ). While there was no difference between D11 treatments ( $F_{2,37.18} = 0.34$ ;  
243  $rsPc = 0.15$ ;  $p$ -value =  $0.4$ ).

244

## 245 **Discussion**

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

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

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

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

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

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

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

290 ~~A common measurement of the classical life history trade-off of current and future reproductive~~  
291 ~~success is how much parents work to raise their offspring (Stearns, 1992; Wiersma and Tinbergen,~~  
292 ~~2003; Williams, 1966). Parental effort can be measured as how often individuals feed their offspring,~~  
293 ~~which should translate into the amount of energy an individual spends. Many studies, however, do~~  
294 ~~not find a correlation between FF and DEE or it is inconsistent across studies or individuals (Bryant,~~  
295 ~~1988; Burness et al., 2001; Moreno et al., 1995; Tinbergen and Verhulst, 2000), see Williams 2012 for~~  
296 ~~a revision), raising questions on the role of energy expenditure on the costs of reproduction~~  
297 ~~(Tinbergen and Verhulst, 2000; Wiersma and Tinbergen, 2003). This is also the case for the present~~  
298 ~~study as no correlation was found between FF and DEE (Fig. 5a), which was supported by the lack of~~  
299 ~~correlation between DEE and BMR (Fig. 5b). These two patterns were also found on previous studies~~  
300 ~~using birds to measure FF, DEE and/or BMR (Bryant, 1988; Ricklefs et al., 1996; Tinbergen and Verhulst,~~  
301 ~~2000). Because DEE measurements consist in a sum of the parental effort over 24h, if birds~~  
302 ~~compensate for a higher effort during the day by reducing their energy expense at night, DEE may give~~  
303 ~~a misleading picture of parental effort (Wiersma and Tinbergen, 2003)~~

304 The results of our experiment suggest that pied flycatchers may work at their physiological limit and  
305 are constrained in their ability to adjust organs over time to increase energy expenditure. They thus  
306 resort to the short-term strategy of trading off current and future reproduction. This pattern is curious,  
307 since long-distance migrants are known to increase their energy intake well beyond the normal rate  
308 when they are preparing to migrate(Lindstrom and Kvist, 1995). However, such capacity could also  
309 depend on the stage of the annual cycle (Weber and Piersma, 1996) and outside the migration period  
310 long-distance migrants may have a lower flexibility to adjust their energetic expenditure via  
311 physiological modifications. In the light of the combined results from this and previous studies  
312 (Deerenberg et al., 1998; Nilsson, 2002; Wiersma and Tinbergen, 2003) differences across species and  
313 studies could be caused by species-specific physiological limitations and/or ecological constraints, for  
314 example, differences of migratory and non-migratory birds. If the strategy of compensation is specific  
315 to certain species or environmental conditions, it may explain why DEE reflects parental effort in a few

316 cases but not others (Bryant, 1988; Wiersma and Tinbergen, 2003). Therefore, studies using DEE as a  
317 measure of parental effort should also take into account whether their focus species uses nocturnal  
318 compensation as an energy-saving strategy because, if they do, results may be blurred as a  
319 consequence.

320

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325

### 326 **Competing interests**

327 No competing interests declared

328

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333

### 334 **Data Availability**

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

336

337

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424

425

426 **Legends**

427

428 **Figure 1: Predictions of the experiment based on the two hypotheses: A)** According to the increased-  
429 intake strategy, BMR increases with increased workload, and thus BMR is expected to increase for  
430 parents of nests that were given more chicks at day 2. This pattern is obtained by comparing broods  
431 that were not further manipulated at day 11. Colours represent the different treatments: light blue =  
432 -2 chicks at D2, blue = +0 chicks at D2, purple = +2 chicks at D2. **B)** According to the compensation  
433 strategy, the brood enlargement when chicks are 11 days old will lead to a decrease in BMR compared  
434 to birds with no additional brood size manipulation. This pattern is obtained by using all broods, but  
435 the +0 treatment at day 11 comprises nests from all treatments at day 2 (the same as in figure A),  
436 while +2 treatment only includes the +0 treatment at D2 and +4 treatment only includes the -2  
437 treatment at D2. Colours represent the different treatments: black = +0 chicks at D11, pink = +2 chicks  
438 at D11, red = +4 chicks at D11.

439

440 **Figure 2: The effects of brood size manipulations on day 2 (A-C) and day 11 (D-F) on the feeding**  
441 **frequency (A & D), daily energy expenditure (B & E) and basal metabolic rate (C & F).** Mean and  
442 s.e.m. per treatment group at day 2 (a-c) and day 11 (d-f) (calculated using the residuals of the model  
443 without treatment effect) are plotted. **Treatment D2: a)** feeding frequency at day 11 (sample sizes: -  
444 2 chicks = 16; +0 chicks = 14; +2 chicks = 14); **b)** Daily energy expenditure (kJ/day) at day 11 (sample  
445 sizes: -2 chicks = 14; +0 chicks = 10; +2 chicks = 9) and **c)** basal metabolic rate (kJ/day) at day 11 (sample  
446 sizes: -2 chicks = 11; +0 chicks = 8; +2 chicks = 10). This treatment only compares broods that were not  
447 further manipulated at day 11. Colours represent the different treatments: light blue = -2 chicks at D2,  
448 blue = +0 chicks at D2, purple = +2 chicks at D2. **Treatment D11: d)** difference in feeding frequency  
449 between day 10 and day 11 (sample sizes: +0 chicks = 40; +2 chicks = 15; +4 chicks = 17); **e)** Daily energy  
450 expenditure (kJ/day) at day 11 (sample sizes: +0 chicks = 33; +2 chicks = 12; +4 chicks = 15) and **f)** basal  
451 metabolic rate (kJ/day) at day 11 (sample sizes: +0 chicks = 29; +2 chicks = 9; +4 chicks = 13). This  
452 treatment compares all broods, but the +0 treatment at day 11 lumps nests from all treatments at day  
453 2 (-2, +0 and +2 chicks at D2), while +2 treatment only includes the +0 treatment at D2 and +4  
454 treatment only includes the -2 treatment at D2. Colours represent the different treatments: black =  
455 +0 chicks at D11, pink = +2 chicks at D11, red = +4 chicks at D11.



456 **Tables**

457 **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)

458

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

462

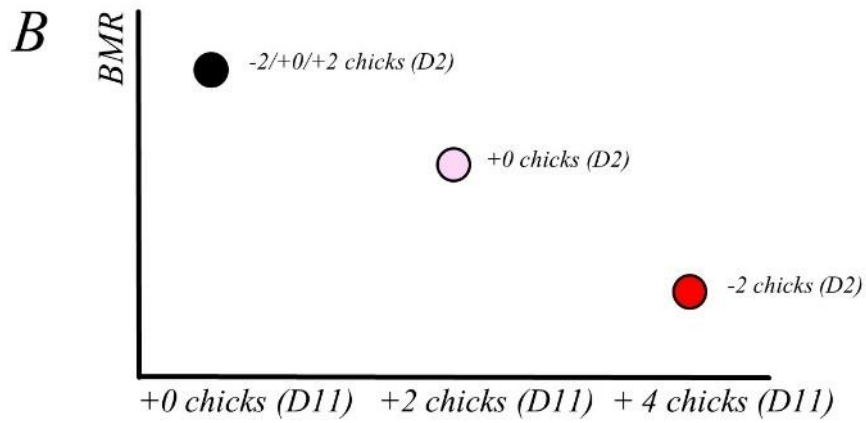
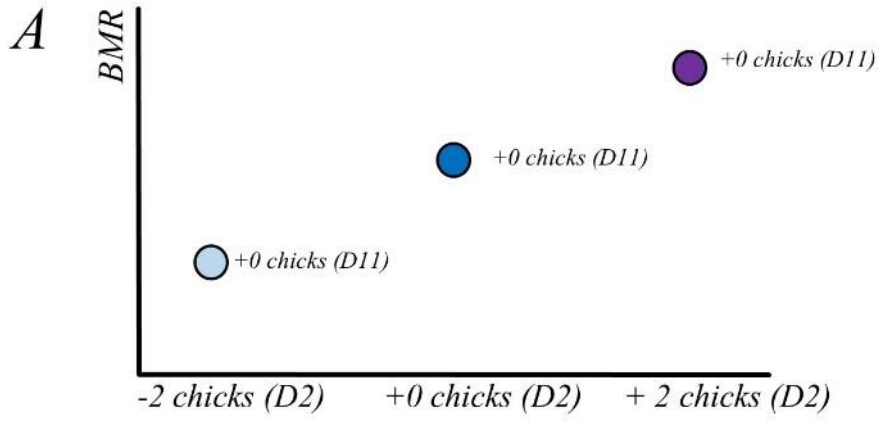
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466 **Figure 1**

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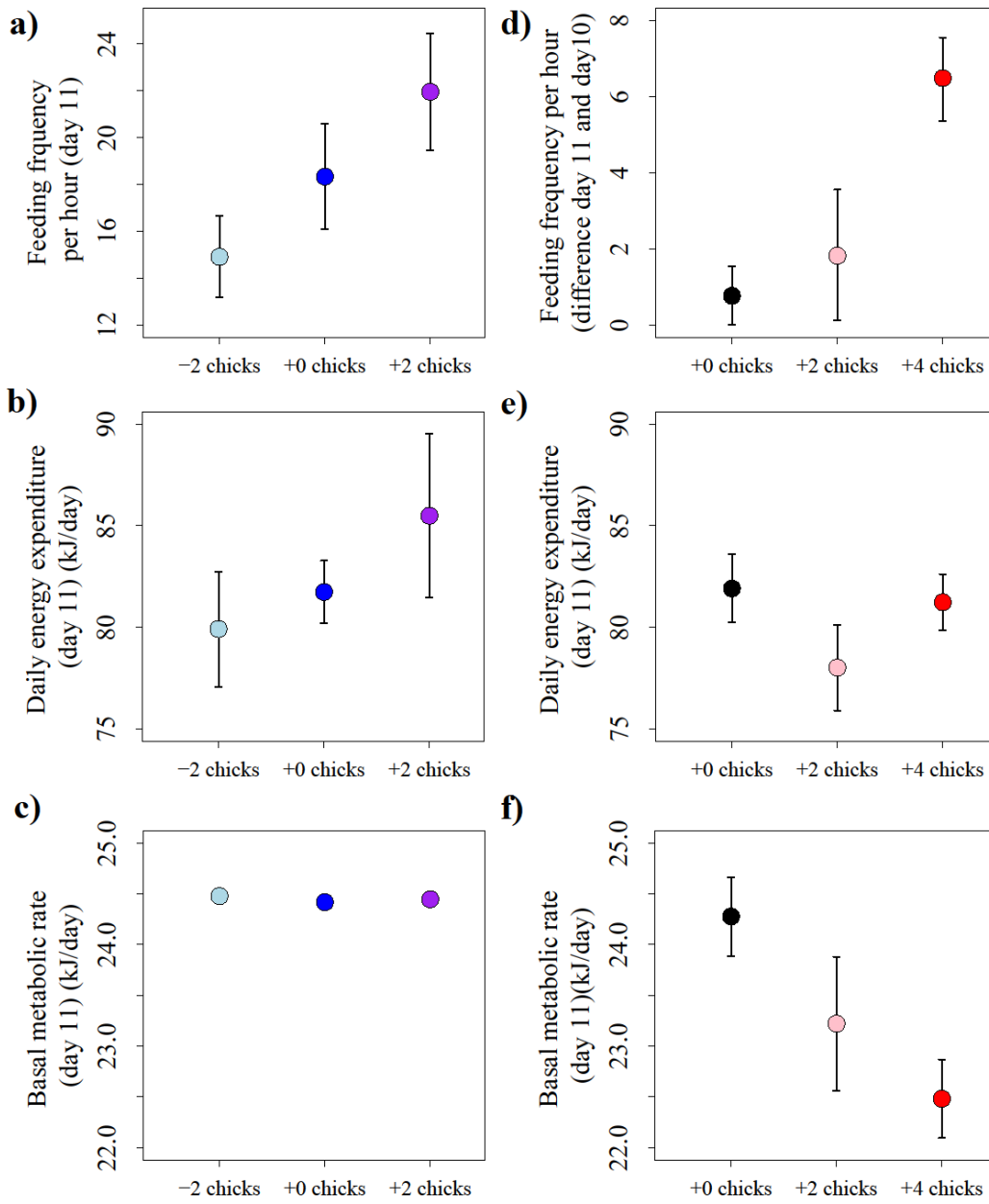


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470 **Figure 2**

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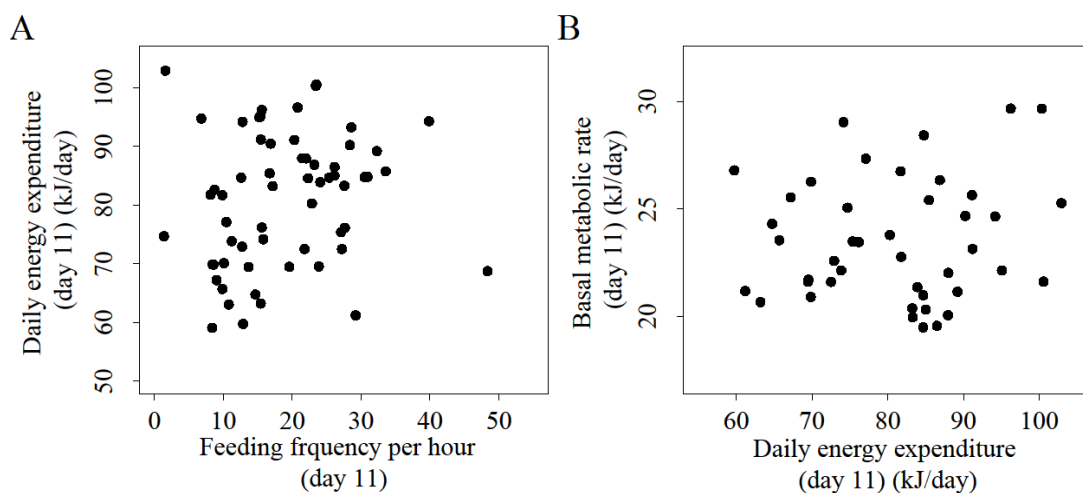


472

473 Appendix

474 A common measurement of the classical life history trade-off of current and future reproductive  
475 success is how much parents work to raise their offspring (Stearns, 1992; Wiersma and Tinbergen,  
476 2003; Williams, 1966). Parental effort can be measured as how often individuals feed their offspring,  
477 which should translate into the amount of energy an individual spends. Many studies, however, do  
478 not find a correlation between FF and DEE or it is inconsistent across studies or individuals (Bryant,  
479 1988; Burness et al., 2001; Moreno et al., 1995; Tinbergen and Verhulst, 2000) but see (Nilsson, 2002;  
480 te Marvelde et al., 2011) and Williams (2012) for a revision, This raises questions on the role of energy  
481 expenditure on the costs of reproduction (Tinbergen and Verhulst, 2000; Wiersma and Tinbergen,  
482 2003).

483 We tested for a correlation between FF and DEE but found none ( $F_{1,49.75} = 2.01$ ;  $p$ -value = 0.16; slope:  
484  $0.20 \pm 0.13$ , Fig. A1A) nor between DEE and BMR ( $F_{1,49.75} = 31.26$ ;  $p$ -value = 0.16; slope:  $0.02 \pm 0.05$ , Fig.  
485 A1B). These two patterns were also found on previous studies using birds to measure FF, DEE and/or  
486 BMR (Bryant, 1988; Ricklefs et al., 1996; Tinbergen and Verhulst, 2000). Because DEE measurements  
487 consist in a sum of the parental effort over 24h, if birds compensate for a higher effort during the day  
488 by reducing their energy expense at night, DEE may give a misleading picture of parental effort  
489 (Wiersma and Tinbergen, 2003).



490

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