

**Pied flycatcher nestlings incur immunological but not growth begging costs**

Journal:	<i>Behavioral Ecology</i>
Manuscript ID	BEHECO-2015-0467.R1
Manuscript Type:	Original article
Keywords:	Begging, Signaling, Communication, Handicap Principle, <i>Ficedula hypoleuca</i>

SCHOLARONE™  
Manuscripts

Pre-Review Only

1  
2  
3  
4 **1 Pied flycatcher nestlings incur immunological but not growth begging costs**

5  
6  
7 2 Short title: Nestling begging costs

8  
9  
10 **3 LAY SUMMARY**

11  
12 4 Nestling begging signals should be costly in order to limit the benefits of selfish  
13 deception to parents. Exactly which cost begging entails has been hotly debated in the  
14  
15 5  
16  
17 6 past. Here we show that begging impairs the immune system of pied flycatcher  
18  
19 7 nestlings. It also reduced nestling growth but nestlings were able to recover while  
20  
21 8 resting at night. Since most of the controversy was about growth costs, this study may  
22  
23  
24 9 help to settle the debate.  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

10 **ABSTRACT**

11 Many theoretical models on the evolution of nestling begging assume this behavior is  
12 costly, so that only nestlings in real need of food would profit from giving intensive  
13 signals to parents. However, evidence accumulated for the last two decades is either  
14 contradictory (growth costs) or scant (immunological cost). Here, we experimentally  
15 test the existence of both costs in pied flycatcher (*Ficedula hypoleuca*) nestlings, a  
16 species in which parents appropriately respond to honest begging signals. Nestlings  
17 were paired by nest of origin and similar body mass. In each pair, a nestling was forced  
18 to beg for 51 s/meal, while the other begged for only 3.4 s/meal, both receiving the  
19 same amount of food. Simultaneously, the nestling immune response to an antigen  
20 (phytohaemagglutinin) was measured. Experimental nestlings showed reduced  
21 immunocompetence compared to control chicks, which in this species could be regarded  
22 as a genuine direct cost. High-begging nestlings also gained less mass during the  
23 daylight activity hours. However, they lost less mass while resting at night, resulting in  
24 similar mass gains for both groups across the whole daily cycle. This suggests that  
25 negative effects of excess begging upon mass gain can be compensated for by nestlings,  
26 thus avoiding the negative fitness consequences (i.e. cost) of a retarded growth. Mixed  
27 results found in previous studies may reflect interspecific differences in compensatory  
28 changes in mass gain. But if such differences do not map into fitness consequences,  
29 they may be of little help to answer the question of whether begging entails direct  
30 growth costs.

31

32

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4 33 **INTRODUCTION**

5  
6 34 Compared with other vertebrates, altricial nestlings show an extremely fast postnatal  
7  
8 35 development (Erickson et al. 2001) and parents must sustain high rates of food  
9  
10 36 provisioning in order to fulfill offspring demands (Starck and Ricklefs 1998). Food  
11  
12 37 transfer from parents to nestlings is largely mediated by begging signals, a complex  
13  
14 38 array of postures, vocalizations and colorful mouth structures affecting both the amount  
15  
16 39 of food delivered and how it is allocated among the brood (Kilner and Johnstone 1997;  
17  
18 40 Budden and Wright 2001; Kilner 2002). Suboptimal growth may have long-lasting  
19  
20 41 negative consequences upon fitness (Lindström 1999; Metcalfe and Monaghan 2001).  
21  
22 42 Therefore, there is an evolutionary incentive for nestlings to secure food at the expenses  
23  
24 43 of their nestmates, especially when food becomes insufficient or unpredictable, as it is  
25  
26 44 often the case (Leech & Leonard 1996). Nestlings may be selected to obtain a  
27  
28 45 disproportionate share of the food delivered, or coerce parents into bringing food at  
29  
30 46 rates that benefit them, but are harmful to parental fitness (Royle et al. 2002; Hoover &  
31  
32 47 Reetz 2006). For these reasons, there is wide consensus among behavioral biologists  
33  
34 48 that begging signals have evolved within this evolutionary scenario of conflicting  
35  
36 49 interests among family members (Trivers 1974; Kilner and Hinde 2008).  
37  
38 50 Parents are known to rely on begging signals to make decisions about how much food  
39  
40 51 deliver to the nest and how distribute it among the brood (Budden and Wright 2001,  
41  
42 52 Searcy and Nowicki 2005). On the other hand, there is evidence that begging signals  
43  
44 53 reliably covary with nestling hunger in a finely-graded, informative fashion (Kilner and  
45  
46 54 Johnstone 1997; Searcy and Nowicki 2005). Moreover, nestlings usually beg below  
47  
48 55 their maximum capacity (Chappell and Bachman 2002), despite being able of escalating  
49  
50 56 begging intensity in response to factors other than nutritional need (e.g. nestmate size or  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4 57 begging, Rodríguez-Gironés et al. 2002). Such evidence seems at odds with the  
5  
6 58 reasonable expectation that nestlings are selected to overplay signals in order to secure  
7  
8 59 more food, despite the potential harm caused to other family members (review in Mock  
9  
10 60 et al. 2011).

11  
12  
13 61 Theoretical models have found two plausible evolutionary routes that may lead to a  
14  
15 62 stable resolution of this conflict, depending on the behavioural mechanism underlying  
16  
17 63 parental feeding decisions. First, nestlings may engage in a scramble competition of  
18  
19 64 signals and parents passively allocate food to the offspring presenting the greatest  
20  
21 65 stimulus (Macnair and Parker 1979). Second, nestlings may display ‘honest’ signals and  
22  
23 66 parents actively monitor offspring begging because it conveys information about their  
24  
25 67 nutritional need or hunger (which is cryptic to parents), then distributing food according  
26  
27 68 to their assessment of the signals of different chicks (Godfray 1991). Both mechanisms  
28  
29 69 of parental allocation may be relevant to parent-offspring communication in multiple  
30  
31 70 broods (Royle et al. 2004; Andrews and Smiseth 2013). Moreover, both mechanisms  
32  
33 71 may lead to a stable evolutionary equilibrium where parents give more food to nestlings  
34  
35 72 that beg at higher levels and nestlings display reliable signals of need, provided that  
36  
37 73 more intense begging entails a cost that reduces nestling fitness in direct proportion to  
38  
39 74 the degree of signal escalation (Parker et al. 2002; Royle et al. 2002). In this scenario,  
40  
41 75 begging cost is an increasing function of signal intensity (Godfray 1991; Parker et al.  
42  
43 76 2002) and offspring differing in nutritional need have different optimal signaling levels  
44  
45 77 at equilibrium because of the differential marginal benefits accrued from obtaining a  
46  
47 78 given amount of extra food via more intensive (costly) begging (Maynard Smith and  
48  
49 79 Harper 2003; Royle et al. 2004). Honest begging by nestlings is also predicted by  
50  
51 80 theoretical signaling models in which nestlings in different nutritional condition would  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4 81 differ in the cost incurred by begging escalation, for example if satiated nestlings that  
5  
6 82 pretend to be hungry, begging at a higher (out-of-equilibrium) level for the same  
7  
8 83 amount of food, pay a special cost (Hurd 1995; Számadó 1999; Lachmann et al. 2001).  
9  
10 84 A final possibility is that begging is totally cost-free and an honest equilibrium is  
11  
12 85 reached because parents and offspring share overlapping interests that allow a partially  
13  
14 86 informative communicative exchange (Maynard Smith 1994; Bergstrom and Lachmann  
15  
16 87 1998; Lachmann et al. 2001). The last possibility has not yet been explored in detail by  
17  
18 88 empirical studies (Számadó 2011) but, although theoretically feasible, it may not fully  
19  
20 89 apply to the problem of how finely graded, informative begging signals may evolve  
21  
22 90 under manifest sibling competition (Brilot and Johnstone 2003; Maynard Smith and  
23  
24 91 Harper 2003; but see Lachmann et al. 2001).  
25  
26  
27  
28  
29 92 Two such putative begging costs have so far been explored in some detail. First, noisy  
30  
31 93 begging may attract eavesdropping predators to the nest (McDonald et al. 2009; Haff  
32  
33 94 and Magrath 2011). This cost would be shared by all members of the brood, as long as  
34  
35 95 predators would not be selective on those nestlings begging louder, but usually kill the  
36  
37 96 entire brood. Second, nestlings begging more intensively might incur direct individual  
38  
39 97 costs, such as a higher metabolic expenditure, increased attentiveness, reduced time to  
40  
41 98 sleep or lower digestive efficiency (Moreno-Rueda 2007; Grodzinski et al. 2009). Some  
42  
43 99 models predict different results depending on whether the cost of begging is shared or  
44  
45 100 individual (e.g. Macnair and Parker 1979; Harper 1986; Motro 1989; Godfray and  
46  
47 101 Parker 1992), and it is not entirely clear whether predation costs could guarantee signal  
48  
49 102 honesty (Godfray 1995; but see Parker et al. 2002).  
50  
51  
52  
53  
54 103 The most obvious way in which begging could directly reduce nestling fitness is  
55  
56 104 increasing metabolic expenditure. Begging signals involve buoyant physical  
57  
58  
59  
60

1  
2  
3  
4 105 performance (vigorous posturing and calling) that must be metabolically sustained.  
5  
6 106 Increased metabolic expenditure may have negative fitness consequences because it can  
7  
8 107 be both energetically demanding (Chapell and Bachman 2002) and cause oxidative  
9  
10 108 stress (Costantini 2014)

11  
12  
13 109 Considerable effort has been devoted to solve the question of whether begging is  
14  
15 110 sufficiently costly in terms of energetic expenditure, but results are still inconclusive.  
16  
17  
18 111 First, several studies found that metabolic rate measured as oxygen consumption  
19  
20 112 increased ca. 27% above resting metabolic rate during begging but this comprised a tiny  
21  
22 113 fraction (<0.25%) of the total daily energy budget which could be easily compensated  
23  
24 114 for by a few extra feedings (Chappell and Bachman 2002). Such energetic demands  
25  
26 115 might, however, be of biological importance considering the limited metabolic scope of  
27  
28 116 developing nestlings, and could still impact growth negatively (Verhulst and Wiersma  
29  
30 117 1997), especially under conditions of suboptimal food abundance (Leech and Leonard  
31  
32 118 1996). This idea stimulated a second wave of empirical studies covering different bird  
33  
34 119 species (five passerines and a dove) aimed at finding whether actively growing nestlings  
35  
36 120 that were forced to beg at high rates incurred a growth cost, but again with mixed,  
37  
38 121 inconclusive results. Some studies (Kilner 2001; Rodríguez-Gironés et al. 2001) found a  
39  
40 122 negative effect of begging upon nestling growth, while others (Rodríguez-Gironés et al.  
41  
42 123 2001; Leonard et al. 2003) did not. Sometimes, different studies on the same species  
43  
44 124 arrived at opposite conclusions (Kedar et al. 2000; Moreno-Rueda 2010; Moreno-Rueda  
45  
46 125 and Redondo 2011, 2012; Soler et al. 2014).

47  
48  
49 126 Several explanations have been advanced to account for this confusing mixture of  
50  
51 127 empirical results. Species may differ in begging effort (Kilner 2001), growth rates and  
52  
53 128 peak energy demands (Rodríguez-Gironés et al. 2001; Leonard et al. 2003), alternative  
54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4 129 non-begging ways of sibling competition (e.g. jostling, Chappell and Bachman 2002;  
5  
6 130 Moreno-Rueda 2007), or allocation to different parts (e.g. growth versus immunity) of  
7  
8 131 the energy budget (Moreno-Rueda 2010). For example, in small insectivorous birds,  
9  
10 132 nestlings may be less likely to incur growth costs due to their short begging bouts and  
11  
12 133 tight growth constraints (Kilner 2001). Also, hole-nesting species, being less  
13  
14 134 constrained by predation costs, may have evolved more flamboyant begging signals  
15  
16 135 (Briskie et al. 1999) and also rely more on alternative ways of sibling competition (e.g.  
17  
18 136 physical interference for favorable positions close to the nest entrance), and thus may  
19  
20 137 better afford the cost of an experimentally induced begging effort. Finally, discrepancies  
21  
22 138 between studies may result from differences in experimental setups, e.g. diet (Moreno-  
23  
24 139 Rueda and Redondo 2012), or the time scale over which measurements of costs are  
25  
26 140 made (Soler et al. 2014).

27  
28  
29  
30  
31 141 More recently, some studies have explored the possibility that begging affects  
32  
33 142 metabolically demanding processes other than growth, such as immune function  
34  
35 143 (Buchanan et al. 2007), in house sparrows *Passer domesticus* (Moreno-Rueda 2010;  
36  
37 144 Soler et. al 2014), southern shrikes *Lanius meridionalis* (Moreno-Rueda and Redondo  
38  
39 145 2011, 2012) and magpies *Pica pica* (Moreno-Rueda et al. 2012). All these studies found  
40  
41 146 that intense begging reduces immune function, providing less controversial evidence  
42  
43 147 than studies on growth costs but the number of species tested is still small. Clearly,  
44  
45 148 there is a need to increase the diversity of the data set before any firm conclusion can be  
46  
47 149 reached.

50  
51 150 In this study, we contribute experimental evidence supporting the existence of begging  
52  
53 151 costs in nestlings of the pied flycatcher (*Ficedula hypoleuca*), a small hole-nesting,  
54  
55 152 insectivorous passerine. Begging by nestling pied flycatchers reliably covaries with  
56  
57  
58  
59  
60



1  
2  
3  
4 153 nutritional need (Wright et al. 2010) and affects allocation of parental feedings  
5  
6 154 (Gottlander 1987). Hence, begging can be regarded as a stable, honest signaling system  
7  
8 155 in this species. Body mass at fledging is a powerful predictor of subsequent survival  
9  
10 156 until reproduction (Potti et al. 2002), so there is an incentive for signal overplay too.

11  
12  
13 157 In this study, experimental nestlings were forced to beg for longer than their control  
14  
15 158 nestmates for the same amount of food. The rationale for this experimental design lies  
16  
17 159 on the following assumptions:

18  
19  
20 160 1) Nestlings are free to choose their optimal (equilibrium) begging level which is  
21  
22 161 determined by the differential benefits of food according to need and a cost which  
23  
24 162 depends on begging effort, but not need (i.e. we assume a differential benefit (Godfray  
25  
26 163 1991; Johnstone 1997), rather than a differential cost signaling model (Számadó 1999;  
27  
28 164 Lachmann et al. 2001; Számadó 2011). By manipulating begging effort while holding  
29  
30 165 need constant (equal food amounts given to similar-size nestlings on an identical time  
31  
32 166 schedule) we expect to find measurable variations in begging cost.

33 167 2) By focusing on growth and immune costs, we assume that all nestlings, irrespective  
34  
35 168 of their need, are constrained to pay similar marginal costs (Getty 2006) for a given  
36  
37 169 deviation in begging effort (Számadó 2011). Cost is an intrinsic property of the signal  
38  
39 170 caused by the unavoidable physical activity required to produce it, and says nothing  
40  
41 171 about whether such cost is either an 'efficacy' or a 'strategic' cost (Maynard Smith &  
42  
43 172 Harper 2003). In other words, even if we assume that begging may be costly at  
44  
45 173 equilibrium (because needy offspring have to expend more in muscular activity), such  
46  
47 174 cost should be regarded as an investment (Getty 2006) rather than a 'handicap' (Grafen  
48  
49 175 1990). Therefore, we expect a *difference* (Bergstrom & Lachmann 1998) in cost  
50  
51 176 between experimental treatments which represents the cumulative marginal costs of  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4 177 deviating from an equilibril, freely chosen begging effort by control nestlings to a  
5  
6 178 higher, further away from equilibrium, enforced begging effort by experimental ones.  
7  
8  
9 179 3) The only meaningful way to test that signal costs are balancing signal overplay at  
10  
11 180 equilibrium (assumption 1) above) is to force nestlings to beg outside their natural  
12  
13 181 signaling range (Moreno-Rueda 2007; Lachmann et al. 2001; Számadó 2011). However,  
14  
15 182 precisely which signal components should be experimentally altered, and to what  
16  
17 183 extent, are open empirical questions, nonetheless because too large experimental  
18  
19 184 alterations outside the natural range of options that nestlings can choose may provide  
20  
21 185 significant, but biologically meaningless results. In response to variations in parental  
22  
23 186 feeding rates, begging rates by pied flycatcher nestlings may vary by orders of  
24  
25 187 magnitude within a given period of time (say, hours) but begging bouts by individual  
26  
27 188 nestlings in a given feeding visit are much less variable and, by definition, are equal or  
28  
29 189 shorter than the duration of the begging bout of the whole brood. We assume that the  
30  
31 190 key component of begging effort is the duration of begging bouts, rather than the rate of  
32  
33 191 begging bouts per hour, because nestlings have the opportunity to rest and be fed in the  
34  
35 192 interval between two successive feeding visits (which may alter the benefit/cost balance  
36  
37 193 between successive bouts) and parents can more easily compare the effort of different  
38  
39 194 nestlings begging simultaneously during the same bout. Therefore, we forced  
40  
41 195 experimental nestlings to beg for much longer begging bouts while summing hourly  
42  
43 196 rates within the range of natural broods.  
44  
45  
46  
47  
48  
49 197 Results in this study showed that experimental nestlings experienced a reduced T-cell  
50  
51 198 mediated immune response. Begging also affected nestling mass gain, but only in the  
52  
53 199 short term. This finding may shed light on the complexity of growth costs and help  
54  
55 200 putting into perspective the apparently contradictory results found in previous studies.  
56  
57  
58  
59  
60

1  
2  
3  
4 201 **MATERIALS AND METHODS**  
5

6  
7 202 The study was carried out during the spring of 2013 in an extensively studied pied  
8  
9 203 flycatcher population at La Hiruela (central Spain; details in Potti and Montalvo 1990;  
10  
11 204 Camacho et al. 2015). The study area is an old oak (*Quercus pyrenaica*) forest provided  
12  
13 205 with nest boxes. Nests were inspected regularly to determine the exact date of hatching  
14  
15 206 (day 1).  
16

17  
18 207 During the previous year, we had obtained samples of parent and nestling behavior by  
19  
20 208 placing miniature cameras (Sony Go-Pro) inside nest boxes at eleven 5 or 6-chick  
21  
22 209 broods when nestlings were 7 days old. A decoy camera of identical external  
23  
24 210 appearance was placed during the preceding 24 h to ensure parental habituation. From  
25  
26 211 these video recordings, we measured rates of parental visits and begging bouts by the  
27  
28 212 whole brood by using JWatcher 1.0 software (Blumstein and Daniel 2007). This dataset  
29  
30 213 served to ensure that our experimental setup induced excess begging in experimental  
31  
32 214 nestlings in a way similar to other studies (Kilner 2001; Kedar et al. 2000; Leonard et  
33  
34 215 al. 2003; Moreno-Rueda 2010; Moreno-Rueda and Redondo 2011, 2012; Soler et al.  
35  
36 216 2014) but within the range of natural begging rates in wild broods.  
37  
38

39  
40 217 The experiment was performed with 40 chicks from 20 nests, starting when nestlings  
41  
42 218 were 7 days old, at their peak of daily mass gain. In the afternoon of the day before the  
43  
44 219 experiment, we took one pair of nestlings of similar body mass from the nests, leaving  
45  
46 220 at least three nestlings to prevent parental desertion. Nestlings were placed in a warm  
47  
48 221 chamber and carried to a nearby laboratory. Transportation lasted about 20 min. On that  
49  
50 222 afternoon, nestlings were conditioned to a begging stimulus (a playback of a parental  
51  
52 223 feeding call recorded from the same population) while fed *ad libitum*.  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4 224 We randomly assigned one nestling of each pair of nestmates to either a high begging  
5  
6 225 (HB) or a low begging (LB) treatment. Nestlings were maintained isolated in small (5  
7  
8 226 cm diameter) cups lined with a cotton fabric. While resting, nestlings were covered with  
9  
10 227 a duster, simulating brooding by the mother. This procedure precluded nestlings from  
11  
12 228 begging between trials. Heating was provided by bulb lamps whose distance to artificial  
13  
14 229 nests could be regulated in order to maintain a temperature close to 35 °C within nest  
15  
16 230 cups. Temperature was monitored with a probe digital thermometer. Nestlings were  
17  
18 231 grouped by treatment at opposite sides of the laboratory to minimize interference caused  
19  
20 232 by spontaneous begging. Both sides were chosen randomly (but not swapped between  
21  
22 233 treatments) and were at equal distances from sources of environmental noise and  
23  
24 234 vibrations, such as the laboratory entrance and the testing chamber (see below). Both  
25  
26 235 groups of nestlings were kept under identical conditions of illumination, temperature  
27  
28 236 and humidity. All this ensured that both groups of nestlings were similarly impacted by  
29  
30 237 environmental stress factors which could potentially affect immune response (Romero  
31  
32 238 2004). The whole begging session started at 08:00 (local hour) and ended at 20:45.  
33  
34 239 During the night, artificial nests were covered with a cardboard opaque to dim light.  
35  
36 240 Previously, nestlings were weighed with a digital balance (Sartorius®; accuracy 0.01 g).  
37  
38 241 We estimated the food to be ingested by nestlings according to their mass during the  
39  
40 242 experimental day, following the allometric relationship calculated by Weathers (1996):  
41  
42 243  $\text{daily food to be consumed} = 0.98 \times M^{0.814}$ , where M is nestling body mass in grams.  
43  
44 244 Daily food intake was divided into 18 equal portions corresponding to the 18 begging  
45  
46 245 trials, which were performed every 45 min during a 12:45 h begging session. Any  
47  
48 246 deviations from expected food intake during a trial were compensated for in subsequent  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4 247 trials. Food consisted in the alternation of dipteran larvae and tiny omelette chunks that  
5  
6 248 were weighed individually.  
7  
8

9 249 During each feeding trial, nestlings were carried to an adjacent room and stimulated to  
10  
11 250 beg by using the same stimulus as before (feeding call playback). However, while LB  
12  
13 251 nestlings were fed immediately after gaping, HB nestlings were repeatedly stimulated to  
14  
15 252 beg for 1 min before being fed. Five begging trials, starting at 09:30 and evenly  
16  
17 253 distributed every 135 minutes were recorded with a digital video camera Handycam  
18  
19 254 HDR-XR155E (Sony®). A trained observer transcribed the video recordings into  
20  
21 255 continuous numeric sequences of ordinal begging ranks using JWatcher 1.0 software  
22  
23 256 (Blumstein and Daniel 2007). Postural begging ranks were categorized in ascending  
24  
25 257 order of vigor and muscular activity: 1 (gaping, tarsi flexed), 2 (gaping, neck extended,  
26  
27 258 tarsi flexed), 3 (gaping, neck extended, body up) and 4 (gaping fully stretched on  
28  
29 259 extended tarsi, sometimes including wing flapping) (modified from Redondo and Castro  
30  
31 260 1992). Time spent begging (at any postural intensity) and mean ordinal rank of postural  
32  
33 261 intensity were computed for each nestling at each recorded begging trial. We then  
34  
35 262 computed average begging time and average postural rank for each nestling as  
36  
37 263 individual measures of begging effort. Some nestlings failed to beg at all during some  
38  
39 264 trials, particularly during the central hours of the day. To measure individual  
40  
41 265 repeatability across trials we selected four recorded trials (excluding the central one at  
42  
43 266 14:00 h) where all nestlings begged except three LB nestlings at three different trials.  
44  
45 267 Begging failures were computed as zero for estimating average time begging and  
46  
47 268 postural intensity but were omitted for repeatability analyses.  
48  
49  
50  
51  
52

53 269 Body mass of nestlings was measured three times, at 8:00 h at the start of the begging  
54  
55 270 session (mass 1), at 21:00 at the end of begging trials (mass 2), and the next day, at  
56  
57  
58  
59  
60

1  
2  
3  
4 271 08:00 h, exactly 24 hours after the first measurement (mass 3). Nestlings were weighed  
5  
6 272 only after they had defecated. When a nestling produced a fecal sac within the next 15  
7  
8 273 minutes after it was weighed, it was discounted. Diurnal mass gain during the whole  
9  
10 274 begging session was estimated as mass 2 minus mass 1. Nocturnal (negative) mass gain  
11  
12 275 was estimated as mass 3 minus mass 2. Total daily mass gain was estimated as mass 3  
13  
14 276 minus mass 1. We computed mass lost attributable to metabolic expenditure ( $M_{EE}$ )  
15  
16 277 during begging as  $M_{EE} = M_I - M_G - M_F$ , where  $M_I$  is food mass ingested,  $M_G$  is mass  
17  
18 278 gained, and  $M_F$  is the mass of feces. Then, we calculated the difference  $D_{EE} = M_{EE}(HB)$   
19  
20 279 -  $M_{EE}(LB)$  between pairs of nestmates as an estimate of the marginal metabolic cost of  
21  
22 280 begging (Kilner 2001). Two nestlings (one HB and one LB) vomited between two  
23  
24 281 consecutive trials and were excluded from  $M_{EE}$  analyses. Fecal sacs were not weighed  
25  
26 282 individually but collected in closed containers and kept refrigerated until weighed. Two  
27  
28 283 measures of cumulative fecal mass were taken, at the end (21:00 h) and ca. the middle  
29  
30 284 (13:00 h) of the whole begging session.

31  
32  
33  
34  
35 285 We also measured how the experimental treatment affected cell-mediated immune  
36  
37 286 response. Immediately before the onset of the experiment (07:30 h), we injected into the  
38  
39 287 left patagium of each chick 0.2 mg of phytohaemagglutinin (PHA-P, L-8754, Sigma  
40  
41 288 Aldrich) diluted in 0.04 ml of isotonic phosphate buffer (following Moreno et al. 2005).  
42  
43 289 PHA-P is an innocuous protein which induces an immune response in birds causing a  
44  
45 290 swelling of the patagium skin that is positively correlated with the strength of the T-cell  
46  
47 291 mediated immune response (Kennedy and Nager 2006), although other components of  
48  
49 292 the immune system are also involved (Martin et al. 2006). Previously, we had measured  
50  
51 293 (three times) the patagium thickness with a pressure-sensitive micrometer (Mitutoyo®;  
52  
53 294 accuracy: 0.01 mm). At the end of the day (21:00 h) and at the end of the experiment  
54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4 295 (24 h later), we again measured the patagium thickness, calculating the T-cell mediated  
5  
6 296 immune response as the difference between measurements. Patagium measurements  
7  
8 297 were performed by a trained person blind with regard to nestling treatment.  
9

10  
11 298 The day after the experiment, nestlings were fed *ad libitum* again, marked with non-  
12  
13 299 toxic waterproof ink and returned back to their nests during the morning. On the  
14  
15 300 following days, we regularly checked nests to monitor the fate of chicks used in the  
16  
17 301 experiment. With one exception, all nestlings looked well until they were ringed when  
18  
19 302 13 days old. One nestling died of starvation some days after the experiment had  
20  
21 303 concluded.  
22  
23

24  
25 304 For statistical analyses, we performed Linear Mixed Effects Models of Restricted  
26  
27 305 Maximum Likelihood (REML-LMM; Zuur et al. 2009), by using the package "nlme"  
28  
29 306 (Pinheiro et al. 2012) in R (R Development Core Team 2012). In each model, nest of  
30  
31 307 origin was introduced as a random factor to control for variance among nests, thus  
32  
33 308 avoiding problems of statistical independence (Hurlbert 1984). In some models, initial  
34  
35 309 body mass and food consumed were introduced as predictors to examine possible  
36  
37 310 effects on the dependent variables. For every model, we checked for homoscedasticity  
38  
39 311 (residuals vs. fitted plots), and log-transformed the variable "time begging" in order to  
40  
41 312 fulfill homoscedasticity requirements. We also checked visually for normality of  
42  
43 313 residuals (normal quantile plots), which never deviated from a normal distribution  
44  
45 314 according to Kolmogorov-Smirnov tests (Quinn and Keough 2002). Means are given  
46  
47 315 with one standard error (SE). The complete dataset can be found in Supplementary  
48  
49 316 Material.  
50  
51

52  
53  
54 317 The experimental procedure was approved by the CSIC Ethical Committee (ref.  
55  
56 318 CGL2011-29694) and the Andalusian Committee of Animal Experimentation (ref.  
57  
58  
59  
60

1  
2  
3  
4 319 2011\_03Potti) to comply with Spanish and European legislation on the protection of  
5  
6 320 animals used for scientific purposes.  
7

## 8 9 321 **RESULTS**

10  
11 322 The experimental treatment succeeded at making HB nestlings beg for much longer  
12  
13 323 bouts (51.2 s) than LB nestlings (3.4 s), and also at higher postural intensities (Table 1).  
14  
15 324 Most of the variation in begging time and intensity was explained by treatment (Table  
16  
17 325 2). Individual repeatabilities within treatment across four begging trials were low, which  
18  
19 326 comes at no surprise considering that treatment involved the experimenter either  
20  
21 327 enforcing (HB) or preventing (LB) begging by nestlings. Video recordings from nests  
22  
23 328 showed that pied flycatcher broods in the wild begged with short begging bouts similar  
24  
25 329 to LB nestlings (4.8 s  $\pm$  1.23 SE, total range 0.1-21.5, range of mean values per brood  
26  
27 330 3.1-8.5). Therefore, begging effort per bout of HB nestlings substantially exceeded the  
28  
29 331 natural range in this species (Fig. 1). Since experimental nestlings were stimulated to  
30  
31 332 beg once every 45 min, hourly begging rates of HB nestlings (68 s/h) were within the  
32  
33 333 range of wild broods (135 s/h  $\pm$  29.4 SE, range 21-344).  
34  
35  
36  
37  
38

### 39 334 **The effect of begging on cell-mediated immune response**

40  
41 335 Chicks begging for longer and with higher intensity had a smaller diurnal immune  
42  
43 336 response than LB nestlings (Table 1). Food consumed or initial body mass did not affect  
44  
45 337 initial immune response ( $F_{1, 19} = 2.48, P = 0.13; F_{1, 19} = 2.85, P = 0.11$ , respectively),  
46  
47 338 and their inclusion in the model did not remove the significant effect of treatment on  
48  
49 339 initial immune response ( $\chi^2 = 27.80, P < 0.001$ ). Final immune response remained  
50  
51 340 significantly smaller in HB nestlings than in LB nestlings (Fig. 2). Again, food  
52  
53 341 consumed and initial body mass did not affect final immune response (respectively,  $F_1$ ,  
54  
55  
56  
57  
58  
59  
60



1  
2  
3  
4 342  $_{19} = 2.51, P = 0.13; F_{1, 19} = 0.26, P = 0.62$ ), and their inclusion in the model did not  
5  
6 343 qualitatively affect the results ( $\chi^2 = 10.05, P = 0.0015$ ). Immune response decreased  
7  
8 344 during the night (paired t-test,  $t = -2.36, P = 0.023$ ), irrespective of treatment (Table 1).  
9  
10 345 Food consumed and initial body mass did not affect the change in immune response  
11  
12 346 during the night (respectively,  $F_{1, 19} < 0.01, P = 0.95; F_{1, 19} = 0.85, P = 0.37$ ), and their  
13  
14 347 inclusion in the model did not qualitatively alter the results, although rendered them  
15  
16 348 marginally non-significant ( $\chi^2 = 2.97, P = 0.08$ ).

### 349 **The effect of begging on mass gain and metabolic expenditure**

350 There were no differences in initial body mass or food consumed between high begging  
351 (HB) and low begging (LB) nestlings (Table 1). Nestlings in the HB treatment gained  
352 significantly less mass than LB nestlings during the diurnal phase of active begging  
353 when begging trials took place (diurnal mass gain; Table 1). Diurnal mass gain was not  
354 significantly affected by the amount of food consumed ( $F_{1, 19} = 0.46, P = 0.51$ ) or initial  
355 body mass ( $F_{1, 19} = 0.24, P = 0.63$ ). Therefore, the effect of treatment on diurnal mass  
356 gain remained significant when the amount of food consumed and initial body mass  
357 were included in the model ( $\chi^2 = 7.82, P = 0.005$ ). However, total mass gain during the  
358 24 h period did not significantly differ between treatments (Table 1). Neither was total  
359 mass gain during 24 h affected by food consumed ( $F_{1, 19} = 0.02, P = 0.89$ ) or initial  
360 body mass ( $F_{1, 19} = 1.06, P = 0.32$ ), and their inclusion in the model did not change the  
361 results ( $\chi^2 = 0.20, P = 0.65$ ). The reason for the discrepancy between diurnal and total  
362 mass gain was that HB nestlings lost significantly less mass during the night resting  
363 phase than LB nestlings (Table 1). In this case, initial body mass negatively influenced  
364 nocturnal mass loss ( $\beta = -0.39, F_{1, 19} = 6.82, P = 0.017$ ). There was no effect of food  
365 consumed ( $F_{1, 19} = 2.19, P = 0.15$ ). Nonetheless, the effect of treatment on nocturnal

1  
2  
3  
4 366 mass loss remained significant even after controlling for initial body mass and  
5  
6 367 consumed food ( $\chi^2 = 4.84$ ,  $P = 0.028$ ). Summarizing, HB nestlings grew less during the  
7  
8 368 daylight, active begging time but also lost less mass during the night, resulting in  
9  
10 369 similar growth rates for both treatments during a 24 h period (Fig. 3).

11  
12  
13 370 There were no differences in metabolic expenditure  $M_{EE}$  according to treatment (Table  
14  
15 371 1).  $M_{EE}$  comprised a larger fraction of diurnal mass gain in HB than in LB nestlings  
16  
17 372 ( $40.0\% \pm 4.04$  and  $32.5\% \pm 5.08$ , respectively) but differences were not significant ( $F_{1,17} = 1.16$ ,  $P = 0.29$ ). The mean marginal metabolic cost of begging ( $D_{EE}$ ) was  $0.09$  g ( $\pm$   
18  
19 373  $0.087$  SE,  $N = 18$ ) of mass lost by HB nestlings attributable to begging.  $D_{EE}$  was neither  
20  
21 374 correlated with differences between nestlings in a pair (HB-LB) in time begging ( $r = -$   
22  
23 375  $0.10$ , NS) nor differences in postural intensity ( $r = 0.05$ , NS).  
24  
25  
26  
27  
28

### 29 377 **The effect of begging on fecal mass production**

30  
31  
32 378 Experimental treatment had no effect on total fecal mass excreted at the end of the  
33  
34 379 begging session. However, HB nestlings excreted a marginally ( $P = 0.061$ ) larger  
35  
36 380 fraction of cumulative fecal mass during the first half of the begging session than LB  
37  
38 381 nestlings (Table 1). Among HB nestlings, the amount of time spent begging had a  
39  
40 382 positive, marginally significant effect on the amount of feces excreted during the first  
41  
42 383 half of the begging session, after controlling for food intake ( $\beta = 0.39$ ,  $F_{1,19} = 3.98$ ,  $P =$   
43  
44 384  $0.062$ ) but this trend was not evident in the control LB group ( $\beta = -0.04$ ,  $F_{1,19} = 0.02$ ,  $P =$   
45  
46 385  $0.88$ ). This suggests that begging had a mild, short-term effect upon the digestive  
47  
48  
49 386 dynamics of nestlings.  
50

### 51 52 387 **DISCUSSION**

53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4 388 Compared with their LB nestmates in the control group, experimental HB nestlings  
5  
6 389 invested a great deal in begging effort. Begging bouts of LB nestlings (3.4 s) were  
7  
8 390 similar in duration to begging bouts at wild nests (5 s). Sustained begging bouts of HB  
9  
10 391 nestlings were 15 times longer (51 s) and well above the maximum duration of begging  
11  
12 392 bouts recorded in the wild (21.5 s). It is reasonable to assume that variation in the  
13  
14 393 duration of begging bouts recorded at natural broods may in part reflect variations in  
15  
16 394 nestling nutritional need, with well-fed chicks begging at shorter durations. Nestlings in  
17  
18 395 this study received ca. 0.5 g of food per g of body mass over a 24 h period (Table 1).  
19  
20 396 This food amount is above the average estimated for a 7-days old, 9 g pied flycatcher  
21  
22 397 nestling in the wild (0.44 g per g of body mass) and close to the highest food intake  
23  
24 398 (0.67 g/g) established in a previous experimental study testing the effects of nutritional  
25  
26 399 need upon begging and digestion (Wright et al. 2010). Therefore, as both LB and HB  
27  
28 400 nestlings could be considered as belonging to a well-fed category of signalers, the long  
29  
30 401 begging bouts performed by HB nestlings, above the range recorded at natural broods,  
31  
32 402 can be considered as out-of-equilibrium signals (Számadó 2011).  
33  
34  
35  
36  
37  
38 403 Hourly begging rates of experimental nestlings were within the range observed at wild  
39  
40 404 broods. Some previous studies on growth costs also reported keeping hourly begging  
41  
42 405 rates of enforced HB nestlings within the natural range of wild broods (Kilner 2001;  
43  
44 406 Leonard et al. 2003; Moreno-Rueda et al. 2012). Others did not report natural begging  
45  
46 407 rates but adjusted their testing schedule to approach natural rates of feeding visits by  
47  
48 408 parents (Kedar et al. 2000; Moreno-Rueda 2010; Moreno-Rueda and Redondo 2011,  
49  
50 409 2012). Comparatively, the HB/LB ratio of begging duration in this study (15) was  
51  
52 410 higher (6, Kilner 2001; 3.5 and 5, Rodríguez-Gironés et al. 2001; 6, Leonard et al. 2003;  
53  
54 411 6.7, Moreno-Rueda et al. 2012) or similar (16.7 Moreno-Rueda and Redondo 2011; 14.1  
55  
56  
57  
58  
59  
60

1  
2  
3  
4 412 Moreno-Rueda and Redondo 2012) to those reported by previous studies. Only in two  
5  
6 413 of them did the HB/LB time ratio exceeded that in this study (24, Moreno-Rueda 2010;  
7  
8 414 36, Soler et al. 2014). In summary, experimental HB nestlings in this study were forced  
9  
10 415 to beg at least as hard as in studies that did show a growth cost (Kilner 2001;  
11  
12 416 Rodríguez-Gironés et al. 2001; Moreno-Rueda and Redondo 2011; Moreno-Rueda et al.  
13  
14 417 2012). Mass gain measured during 24 h in the laboratory (0.86 g) was lower than in  
15  
16 418 the field at the same age (1.36 g), which is common for hand-reared wild birds and can  
17  
18 419 be attributed to general stress caused by husbandry practices (Flammer and Clubb 1994)  
19  
20 420 even if fed with high-quality diets, as in this study.  
21  
22  
23

24 421 Excess begging experimentally induced had a two-fold impact upon nestling  
25  
26 422 physiology, reducing both the mass gain during activity hours and immune response.  
27  
28 423 Such effects can hardly be explained in terms of metabolic expenditure (measured as  
29  
30 424 mass lost during the begging session). Metabolic expenditure was similar between HB  
31  
32 425 and LB nestlings, either in absolute terms or as a fraction of diurnal mass gain and  
33  
34 426 differences in begging effort between pairs of nestlings had no effect upon mass lost  
35  
36 427 attributable to begging. The small, non-significant effect of excess begging upon  
37  
38 428 metabolic expenditure is consistent with previous respirometry studies (Chappell and  
39  
40 429 Bachman 2002). As an alternative to energetic expenditure, vigorous physical  
41  
42 430 exercising associated to intensive begging may cause oxidative stress (Noguera et al.  
43  
44 431 2010; Boncoraglio et al. 2012; Costantini 2014). Both growth and immune function  
45  
46 432 contribute to the production of free radicals (Alonso-Álvarez et al. 2007; Costantini and  
47  
48 433 Møller 2009; Sorci and Faivre 2009), and nestlings sustaining a high begging effort may  
49  
50 434 be able to momentarily downregulate other oxidative processes in order to keep up with  
51  
52 435 oxidative balance (Moreno-Rueda et al. 2012). A final possibility is that it is not the  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4 436 physical effort of begging *per se* what is immunosuppressive , but rather the  
5  
6 437 physiological processes leading nestlings to beg with greater effort (Buchanan et al.  
7  
8 438 2007). Some studies have found that endogenous levels of testosterone and  
9  
10 439 glucocorticoids may simultaneously promote more intense begging and reduce both  
11  
12 440 growth and immunocompetence in pied flycatchers (Goodship and Buchanan 2006,  
13  
14 441 2007) and other birds (Quillfeldt et al. 2006; Buchanan et al. 2007; Loiseau et al. 2008).  
15  
16  
17 442 The fact that experimental nestlings in this study begged not only for longer, but also at  
18  
19 443 higher postural intensities, is consistent with this last possibility. Effects of endogenous  
20  
21 444 hormones on begging, growth and immune response may vary among different species  
22  
23 445 (Smiseth et al. 2011) and this variation could provide an additional explanation for the  
24  
25 446 different results obtained in previous studies about the impact of begging on growth.  
26  
27  
28 447 T-cell mediated immune response was highly impacted by experimental treatment at the  
29  
30 448 end of the begging session (Cohen's  $d = 1.15$ ) and also, but with a milder effect, 24  
31  
32 449 hours later (Cohen's  $d = 0.64$ ). This change is expected in phytohaemagglutinin assays  
33  
34 450 (Navarro et al. 2003) as a result of a rapid (3-12 h) transient infiltration of heterophyls  
35  
36 451 and lymphocytes in the injected tissue, later followed by macrophage infiltration (ca. 24  
37  
38 452 h), the swelling typically remitting after 48 h (Smits et al. 1999; Martin et al. 2006).  
39  
40  
41 453 Experimentally induced excess begging has been shown to impair T-cell mediated  
42  
43 454 immune response in each and every species tested so far: house sparrows (Moreno-  
44  
45 455 Rueda 2010; Soler et al. 2014), magpies (Moreno-Rueda et al. 2012), southern shrikes  
46  
47 456 (Moreno-Rueda and Redondo 2011, 2012), and pied flycatchers (this study). By  
48  
49 457 contrast, Romano et al. (2011) found that female barn swallow (*Hirundo rustica*)  
50  
51 458 nestlings injected with an antigen (LPS, a bacterial lipopolysaccharide) increased their  
52  
53 459 begging intensity. However, their study was not specifically designed to test the effect  
54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4 460 of enforced begging on immune response and chicks were allowed to beg freely. This  
5  
6 461 detail is of critical importance, as long as marginal begging costs predicted by signaling  
7  
8 462 models can be evaluated only by manipulating nestlings into begging above their  
9  
10 463 preferred (out-of-equilibrium) levels (Kilner 2001; Számadó 2011). Furthermore,  
11  
12 464 Romano et al. (2011) measured the begging response of nestlings two days after the  
13  
14 465 inoculation, when nutritional condition of nestlings (body mass, feather quality and  
15  
16 466 gape coloration) had already deteriorated as a result of the immune challenge.  
17  
18 467 Therefore, begging response in that study might be affected by both immune challenge  
19  
20 468 and nestling condition (Jacob et al. 2011). One potential drawback of studies showing  
21  
22 469 an impact of enforced begging upon immune response is that, to make experimental HB  
23  
24 470 nestlings beg with greater effort, they were stimulated more than their LB nestmates  
25  
26 471 (Moreno-Rueda 2010; Moreno-Rueda and Redondo 2011, 2012; Moreno-Rueda et al.  
27  
28 472 2012; Soler et al. 2014; this study). More stimulation may cause stress, which may  
29  
30 473 elevate glucocorticoid levels and depress immune response (Saino et al. 2003). Some  
31  
32 474 studies on growth costs (Kedar et al. 2000; Kilner 2001) also suffer from the same  
33  
34 475 drawback but not others (Rodríguez-Gironés et al. 2001; Leonard et al. 2003). There is  
35  
36 476 not an obvious relationship in these studies between stimulation schedule and whether  
37  
38 477 they found a significant impact on growth (Kilner 2001; Rodríguez-Gironés et al. 2001)  
39  
40 478 or not (Kedar et al. 2000; Leonard et al. 2003). Glucocorticoids may affect both growth  
41  
42 479 and immune response simultaneously (Saino et al. 2003), which suggests that  
43  
44 480 differential stimulation may not be the only cause of reduced immune response in HB  
45  
46 481 nestlings. However, the possibility that experimenter-induced stress may affect results  
47  
48 482 in studies of immunological costs of begging should be addressed in future studies.  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4 483 If, as suggested by this and previous studies, there is a generalized immunological  
5  
6 484 impact of excess begging, this effect could be qualified as a genuine direct begging cost  
7  
8 485 of the type hypothesized by begging models. First, cost should affect the nestling fitness  
9  
10 486 function by reducing viability (Számadó 2011). Downregulating immune function may  
11  
12 487 reduce nestling survival prospects in case of a parasitic infection. Pied flycatcher  
13  
14 488 nestlings from Iberian populations suffer from a high (above 20 %) prevalence of  
15  
16 489 haematozoan and arthropod nest-dwelling ectoparasites (Merino and Potti 1995, 1996).  
17  
18 490 Nestlings showing a reduced immune response are worse at coping with an eventual  
19  
20 491 parasitic infection (Tschirren et al. 2007; Pitala et al. 2010), and indeed have a reduced  
21  
22 492 viability in the long term (Cichoń and Dubiec 2005; Bowers et al. 2014). A second  
23  
24 493 assumption of signaling models is that marginal begging costs should not be offset by  
25  
26 494 marginal benefits (i.e. extra food), thus rendering fitness returns negative for nestlings  
27  
28 495 begging above the honest equilibrium level (Számadó 2011). T-cell immune response at  
29  
30 496 fledging is a better predictor of survival to first reproduction than body mass or  
31  
32 497 condition in pied flycatchers (Moreno et al. 2005). This implies that gaining mass by  
33  
34 498 begging intensively may be of little use if immune function is impaired as a result of  
35  
36 499 signal overplay. Furthermore, it is not immediately obvious whether obtaining extra  
37  
38 500 food would compensate for an impaired immune response. In an experimental study in  
39  
40 501 which we simultaneously manipulated marginal benefits and costs of extra begging, we  
41  
42 502 found that southern shrike nestlings receiving 30% extra food were able to compensate  
43  
44 503 for the impact of excess begging on growth, but immune response was affected  
45  
46 504 independently of the amount of food ingested (Moreno-Rueda and Redondo 2012). We  
47  
48 505 therefore conclude that excess begging is costly for pied flycatcher nestlings as a result  
49  
50 506 of impaired immunocompetence.  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4 507 The impact of excess begging on mass gain was large at the end of the begging session  
5  
6 508 (Cohen's  $d = 0.65$ ) but had virtually vanished the morning after (Cohen's  $d = 0.06$ ).  
7  
8 509 Nestlings that had begged intensively and gained less mass during the daylight hours  
9  
10 510 were capable of a compensatory regulation of mass loss while resting later at night. At  
11  
12 511 least two possible mechanisms might be involved in the compensatory phase of  
13  
14 512 nocturnal mass loss. First, a large fraction (ca. 3/4) of a nestling energy budget is  
15  
16 513 allocated to dissipative processes such as maintenance and thermoregulation (Verhulst  
17  
18 514 and Wiersma 1997). During the night, HB nestlings may have reduced basal  
19  
20 515 metabolism or heat production in favor of growth (Moe et al. 2004; Vézina et al. 2009;  
21  
22 516 Killpack and Karasov 2012). Second, experimental treatment may have affected  
23  
24 517 digestive dynamics, allowing HB nestlings to delay food assimilation. For example,  
25  
26 518 pigeons are able to postpone digestion at low temperatures in order to use the extra heat  
27  
28 519 from digestion-related thermogenesis (Laurila et al. 2003). Unfortunately, we did not  
29  
30 520 keep a continuous record of the daily changes in fecal production throughout the  
31  
32 521 begging session neither we measured fecal production the morning after. But we found  
33  
34 522 a marginally significant trend for HB nestlings to excrete a lower fraction of the total  
35  
36 523 fecal mass on the second half of the begging session. In canaries *Serinus canaria*,  
37  
38 524 enforced begging during a relatively short (6 h) trial also increased fecal sac production  
39  
40 525 (Kilner 2001). Alternatively, the lower diurnal mass gain of HB nestlings may have  
41  
42 526 been a consequence of a lower digestive efficiency (Kilner 2001; Budden & Wright  
43  
44 527 2008; Grodzinski et al. 2009; Wright et al 2010) that would have been compensated for  
45  
46 528 during the night, when begging ceased.  
47  
48  
49  
50  
51  
52  
53 529 Does excess begging have an impact on pied flycatcher nestling mass gain? The answer  
54  
55 530 to this question will depend on the time scale of measurement. It did after 13 h of  
56  
57  
58  
59  
60



1  
2  
3  
4 531 begging, but not 11 h afterwards. Other studies have also found begging effects upon  
5  
6 532 mass gain to be dependent on time scale or age. For example, canaries showed an  
7  
8 533 impact of begging on  $M_{EE}$  at 8 days, but not at 6 or 10 days, and HB-LB differences in  
9  
10 534 mass gain during a 24 h period also varied with age (Fig. 2 in Kilner 2001). Magpies  
11  
12 535 sustaining high begging rates for three consecutive days also showed an impact on mass  
13  
14 536 gain at 24 h, but not at 48-72 h (Moreno-Rueda et al. 2012). In shrikes, the impact of  
15  
16 537 begging on mass gain over 24 h depended on the amount and quality of ingested food  
17  
18 538 (Moreno-Rueda & Redondo 2011, 2012). And house sparrows showed an impact after  
19  
20 539 60 h of sustained begging effort but not at 6, 12, 72, 84 and 108 h (Kedar et al. 2000;  
21  
22 540 Moreno-Rueda 2010; Soler et al. 2014). Two of these studies found an impact of excess  
23  
24 541 begging upon body condition measured as residuals of a regression of body mass  
25  
26 542 against wing length at 72 h (Kedar et al. 2000) and between 48 and 108 h (Soler et al.  
27  
28 543 2014). However, this last finding may prove difficult to interpret in biologically  
29  
30 544 meaningful terms. First, body condition measured as Model I regression residuals may  
31  
32 545 suffer from several statistical hindrances, such as slope overestimation and lack of  
33  
34 546 allometric linearity, especially for growing nestlings (Green 2001). Second, variations  
35  
36 547 in nestling body mass relative to structural size may reflect patterns of mass allocation  
37  
38 548 to different body parts in response to a variety of environmental conditions (Potti 1999,  
39  
40 549 2000; Szép and Møller 2000).

41  
42  
43  
44  
45  
46 550 The possibility exists that mixed results found in previous studies on the effect of  
47  
48 551 begging on nestling growth are not simply the outcome of differences in experimental  
49  
50 552 setups, but actually reflect the ability of nestlings to show flexible variations in mass  
51  
52 553 gain in response to excess begging according to different circumstances. But if begging  
53  
54 554 impact on mass gain can be compensated for under many situations then it fails to  
55  
56  
57  
58  
59  
60

1  
2  
3  
4 555 qualify as a genuine direct cost (Számadó 2011). This raises the interesting question of  
5  
6 556 why growth in some species is more easily affected by begging than in others.  
7  
8 557 However, begging-induced growth variations may not offer a universal explanation for  
9  
10 558 the cost predicted by signaling models. Immunological costs seem a promising avenue  
11  
12 559 for future studies, particularly those aimed at testing differential benefit signaling  
13  
14  
15 560 models.  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

For Review Only

**REFERENCES**

- Alonso-Alvarez C, Bertrand S, Faivre B, Sorci G. 2007. Increased susceptibility to oxidative damage as a cost of accelerated somatic growth in zebra finches. *Funct Ecol.* 21:873-879.
- Andrews CP, Smiseth PT. 2013. Differentiating among alternative models for the resolution of parent–offspring conflict. *Behav Ecol.* 24:1185-1191.
- Bergstrom CT, Lachmann M. 1998. Signaling among Relatives. III. Talk is Cheap. *P Natl Acad Sci USA.* 95:5100-5105.
- Blumstein DT, Daniel JC. 2007. *Quantifying Behavior: the JWatcher Way.* Sunderland, USA: Sinauer Associates, Inc.
- Boncoraglio G, Caprioli M, Saino N. 2012. Solicitation displays reliably reflect oxidative damage in barn swallow nestlings. *Behav Ecol Sociobiol.* 66:539-546.
- Bowers EK, Hodges CJ, Forsman AM, Vogel LA, Masters BS, Johnson BG, Sakaluk SK. 2014. Neonatal body condition, immune responsiveness, and hematocrit predict longevity in a wild bird population. *Ecology.* 95:3027-3034.
- Brilot BO, Johnstone RA. 2003. The limits to cost-free signalling of need between relatives. *Proc Biol Sci.* 270:1055-1060.
- Briskie JV, Martin PR, Martin, TE. 1999. Nest predation and the evolution of nestling begging calls. *Proc Biol Sci.* 266:2153-2159.
- Buchanan KL, Goldsmith AR, Hinde CA, Griffith SC, Kilner RM. 2007. Does testosterone mediate the trade-off between nestling begging and growth in the canary (*Serinus canaria*)?. *Horm Behav.* 52:664-671.
- Budden AE, Wright J. 2001. Begging in nestling birds. *Curr Ornithol.* 16:83-118.

- 1  
2  
3  
4 Budden AE, Wright J. 2008. Effects of feeding frequency on nestling begging and  
5 digestion. *Ibis*. 150:234-241.  
6  
7  
8  
9 Camacho C, Canal D, Potti J. 2015. Testing the matching habitat choice hypothesis in  
10 nature: phenotype-environment correlation and fitness in a songbird population. *Evol*  
11 *Ecol*. 29:873-886.  
12  
13  
14 Chappell MA, Bachman GC. 2002. Energetic costs of begging behaviour. In: Wright J,  
15 Leonard ML, editors. *The evolution of begging: Competition, cooperation and*  
16 *communication*. Dordrecht, Netherlands: Kluwer Academic Publishers. p. 143-162.  
17  
18  
19 Cichoń M, Dubiec A. 2005. Cell-mediated immunity predicts the probability of local  
20 recruitment in nestling blue tits. *J Evol Biol*. 18:962-966.  
21  
22  
23  
24  
25  
26 Costantini D. 2014. *Oxidative stress and hormesis in evolutionary ecology and*  
27 *physiology*. Berlin Heidelberg, Germany: Springer Verlag.  
28  
29  
30  
31 Costantini D, Møller AP. 2009. Does immune response cause oxidative stress in birds?  
32 A meta-analysis. *Comp Biochem Physiol A*. 153:339-344.  
33  
34  
35  
36 Erickson GM Rogers KC, Yerby SA. 2001. Dinosaurian growth patterns and rapid  
37 avian growth rates. *Nature*. 412:429-433.  
38  
39  
40  
41 Flammer K, Clubb SL. 1994. Neonatology. In: Ritchie BW, Harrison GJ, Harrison LR,  
42 editors. *Avian medicine: principles and application*. Lake Worth, Florida: Wingers  
43 Publishing. p. 805-841.  
44  
45  
46  
47 Getty T. 2006. Sexually selected signals are not similar to sports handicaps. *Trends Ecol*  
48 *Evol*. 21:83-88.  
49  
50  
51  
52 Godfray HCJ. 1991. Signalling of need by offspring to their parents. *Nature*. 352:328-  
53 330.  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3  
4 Godfray HCJ. 1995. Signaling of need between parents and young: parent-offspring  
5  
6 conflict and sibling rivalry. *Am Nat.* 146:1-24.  
7  
8 Godfray HCJ, Parker GA. 1992. Sibling competition, parent-offspring conflict and  
9  
10 clutch size. *Anim Behav.* 43:473-490.  
11  
12 Goodship NM, Buchanan KL. 2006. Nestling testosterone is associated with begging  
13  
14 behaviour and fledging success in the pied flycatcher, Ficedula hypoleuca. *Proc Biol*  
15  
16 *Sci.* 273:71-76.  
17  
18 Goodship NM, Buchanan KL. 2007. Nestling testosterone controls begging behavior in  
19  
20 the pied flycatcher, Ficedula hypoleuca. *Horm Behav.* 52:454-460.  
21  
22  
23  
24 Gottlander K. 1987. Parental feeding behaviour and sibling competition in the pied  
25  
26 flycatcher Ficedula hypoleuca. *J Avian Biol.* 18:269-276.  
27  
28  
29 Grafen A. 1990. Biological signals as handicaps. *J theor Biol.* 144:517-546.  
30  
31 Green A.J. 2001. Mass/length residuals: measures of body condition or generators of  
32  
33 spurious results?. *Ecology.* 82:1473-1483.  
34  
35 Grodzinski U, Hauber ME, Lotem A. 2009. The role of feeding regularity and nestling  
36  
37 digestive efficiency in parent-offspring communication: an experimental test. *Funct*  
38  
39 *Ecol.* 23:569-577.  
40  
41 Haff TM, Magrath RD. 2011. Calling at a cost: elevated nestling calling attracts  
42  
43 predators to active nests. *Biology Lett.* 7:493-495.  
44  
45  
46 Harper AB. 1986. The evolution of begging: sibling competition and parent-offspring  
47  
48 conflict. *Am Nat.* 128:99-114.  
49  
50 Hoover JP, Reetz MJ. 2006. Brood parasitism increases provisioning rate, and reduces  
51  
52 offspring recruitment and adult return rates, in a cowbird host. *Oecologia.* 149:165-173.  
53  
54  
55 Hurd PL. Communication in discrete action-response games. *J theor Biol.* 174:217-222.  
56  
57  
58  
59  
60

- 1  
2  
3  
4 Hurlbert SH. 1984. Pseudoreplication and the design of ecological field experiments.  
5  
6 Ecol Monogr. 54:187-211.  
7
- 8 Jacob S, Rieucan G, Heeb P. 2011. Multimodal begging signals reflect independent  
9  
10 indices of nestling condition in European starlings. Behav Ecol. 22:1249-1255.  
11
- 12 Johnstone RA. 1997. The evolution of animal signals. In: Krebs JR, Davies NB, editors.  
13  
14 Behavioural ecology: an evolutionary approach. Oxford, UK: Blackwell. p. 155–178.  
15
- 16 Kedar H, Rodríguez-Gironés MA, Yedvab S, Winkler DW, Lotem A. 2000.  
17  
18 Experimental evidence for offspring learning in parent-offspring communication. Proc  
19  
20 Biol Sci. 267:1723-1727.  
21  
22
- 23 Kennedy MW, Nager RG. 2006. The perils and prospects of using phytohaemagglutinin  
24  
25 in evolutionary ecology. Trends Ecol Evol. 21:653-655.  
26  
27
- 28 Killpack TL, Karasov WH. 2012. Growth and development of house sparrows (Passer  
29  
30 domesticus) in response to chronic food restriction throughout the nestling period. J Exp  
31  
32 Biol. 215:1806-1815.  
33  
34
- 35 Kilner RM. 2001. A growth cost of begging in captive canary chicks. P Natl Acad Sci  
36  
37 USA. 98:11394-11398.  
38  
39
- 40 Kilner RM. 2002. The evolution of complex begging displays. In: Wright J, Leonard  
41  
42 ML, editors. The evolution of begging: Competition, cooperation and communication.  
43  
44 Dordrecht, Netherlands: Kluwer Academic Publishers. p. 87-106.  
45
- 46 Kilner RM, Hinde CA. 2008. Information warfare and parent-offspring conflict. Adv  
47  
48 Stud Behav. 38:283-336.  
49
- 50 Kilner RM, Johnstone RA. 1997. Begging the question: are offspring solicitation  
51  
52 behaviours signals of need?. Trends Ecol Evol. 12:11-15.  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4 Lachmann M, Számadó S, Bergstrom CT. 2001. Cost and conflict in animal signals and  
5 human language. P Natl Acad Sci USA. 98:13189-13194.  
6  
7

8 Laurila M, Hohtola E, Saarela S, Rashotte ME. 2003. Adaptive timing of digestion and  
9 digestion-related thermogenesis in the pigeon. Physiol Behav. 78:441-448.  
10  
11

12 Leech SM, Leonard ML. 1996. Is there an energetic cost to begging in nestling tree  
13 swallows (Tachycineta bicolor)?. Proc Biol Sci. 263:983-987.  
14  
15

16 Leonard ML, Horn AG, Porter J. 2003. Does begging effort affect growth in nestling  
17 tree swallows, Tachycineta bicolor? Behav Ecol Sociobiol. 54:573-577.  
18  
19

20 Lindström J. 1999. Early development and fitness in birds and mammals. Trends Ecol  
21 Evol. 14:343-347.  
22  
23

24 Loiseau C, Sorci G, Dano S, Chastel O. 2008. Effects of experimental increase of  
25 corticosterone levels on begging behavior, immunity and parental provisioning rate in  
26 house sparrows. Gen Comp Endocrinol. 155:101-108.  
27  
28

29 Macnair MR, Parker GA. 1979. Models of parent-offspring conflict. III. Intra-brood  
30 conflict. Anim Behav. 27:1202-1209.  
31  
32

33 Martin II LB, Han P, Lewittes J, Kuhlman JR, Klasing KC, Wikelski M. 2006.

34 Phytohemagglutinin-induced skin swelling in birds: histological support for a classic  
35 immunoecological technique. Funct Ecol. 20:290-299.  
36  
37

38 Maynard Smith J. 1994. Must reliable signals always be costly?. Anim Behav. 47:1115-  
39 1120.  
40  
41

42 Maynard Smith J, Harper D. 2003. Animal signals. Oxford, UK: Oxford University  
43 Press.  
44  
45

46 McCarty JP. 1996. The energetic cost of begging in nestling passerines. Auk. 113:178-  
47 188.  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3  
4 McDonald PG, Wilson DR, Evans CS. 2009. Nestling begging increases predation risk,  
5  
6 regardless of spectral characteristics or avian mobbing. *Behav Ecol.* 20:821-829.  
7  
8 Merino S, Potti J. 1995. High prevalence of hematozoa in nestlings of a passerine  
9  
10 species, the pied flycatcher (*Ficedula hypoleuca*). *Auk.* 112:1041-1043.  
11  
12 Merino S, Potti J. 1996. Weather dependent effects of nest ectoparasites on their bird  
13  
14 hosts. *Ecography.* 19:107-113.  
15  
16 Metcalfe NB, Monaghan P. 2001. Compensation for a bad start: grow now, pay later?.  
17  
18 *Trends Ecol Evol.* 16:254-260.  
19  
20 Mock DW, Dugas MB, Strickler SA. 2011. Honest begging: expanding from signal of  
21  
22 need. *Behav Ecol.* 22:909-917.  
23  
24 Moe B, Brunvoll S, Mork D, Brobakk TE, Bech C. 2004. Developmental plasticity of  
25  
26 physiology and morphology in diet-restricted European shag nestlings (*Phalacrocorax*  
27  
28 *aristotelis*). *J Exp Biol.* 207:4067-4076.  
29  
30  
31 Moreno J, Merino S, Sanz JJ, Arriero E, Morales J, Tomás G. 2005. Nestling cell-  
32  
33 mediated immune response, body mass and hatching date as predictors of local  
34  
35 recruitment in the pied flycatcher *Ficedula hypoleuca*. *J Avian Biol.* 36:251-260.  
36  
37 Moreno-Rueda G. 2007. Is there empirical evidence for the cost of begging?. *J Ethol.*  
38  
39 25:215-222.  
40  
41  
42 Moreno-Rueda G. 2010. An immunological cost of begging in house sparrow nestlings.  
43  
44 *Proc Biol Sci.* 277:2083-2088.  
45  
46  
47 Moreno-Rueda G, Redondo T. 2011. Begging at high level simultaneously impairs  
48  
49 growth and immune response in southern shrike (*Lanius meridionalis*) nestlings. *J Evol*  
50  
51 *Biol.* 24:1091-1098.  
52  
53  
54  
55  
56  
57  
58  
59  
60



1  
2  
3  
4 Moreno-Rueda G, Redondo T. 2012. Benefits of extra begging fail to compensate for  
5 immunological costs in southern shrike (Lanius meridionalis) nestlings. PLoS One  
6 7:e44647.  
7  
8  
9

10 Moreno-Rueda G, Redondo T, Trenzado CE, Sanz A, Zúñiga JM. 2012. Oxidative  
11 stress mediates physiological costs of begging in magpie (Pica pica) nestlings. PLoS  
12 One 7:e40367.  
13  
14  
15

16  
17 Motro U. 1989. Should a parasite expose itself? (Some theoretical aspects of begging  
18 and vigilance behavior). J Theor Biol. 140:279-287.  
19  
20

21 Navarro C, Marzal A, De Lope F, Møller A P. 2003. Dynamics of an immune response  
22 in house sparrows Passer domesticus in relation to time of day, body condition and  
23 blood parasite infection. Oikos. 101:291-298.  
24  
25  
26

27  
28 Noguera JC, Morales J, Pérez C, Velando A. 2010. On the oxidative cost of begging:  
29 antioxidants enhance vocalizations in gull chicks. Behav Ecol. 21:479-484.  
30  
31

32 Parker GA, Royle NJ, Hartley IR. 2002. Begging scrambles with unequal chicks:  
33 interactions between need and competitive ability. Ecol Lett. 5:206-215.  
34  
35  
36

37 Pinheiro J, Bates D, DebRoy S, Sarkar D, R Development Core Team. 2012. nlme:  
38 Linear and Nonlinear Mixed Effects Models. R package version 3.1-103.3.1.  
39  
40

41 Pitala N, Siitari H, Gustafsson L, Brommer JE. 2010. Costs and benefits of  
42 experimentally induced changes in the allocation of growth versus immune function  
43 under differential exposure to ectoparasites. PLoS One 25:e10814.  
44  
45  
46

47  
48 Potti J. 1999. Environmental factors and sexual differences in mass and condition of  
49 nestling pied flycatchers Ficedula hypoleuca. Ecoscience. 6:19-24.  
50  
51

52 Potti J. 2000. Sexual size dimorphism and sources of variation in the growth of wing  
53 feathers in nestling pied flycatchers (Ficedula hypoleuca). Ardeola. 47:37-47.  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3  
4 Potti J, Dávila JA, Tella JL, Frías Ó, Villar S. 2002. Gender and viability selection on  
5 morphology in fledgling pied flycatchers. *Mol Ecol.* 11:1317-1326.  
6  
7  
8 Potti J, Montalvo S. 1990. Ocupación de áreas con nidales por el Papamoscas Cerrojillo  
9 (*Ficedula hypoleuca*). *Ardeola.* 37:75-84.  
10  
11  
12 Quillfeldt P, Masello JF, Strange IJ, Buchanan KL. 2006. Begging and provisioning of  
13 thin-billed prions, *Pachyptila belcheri*, are related to testosterone and corticosterone.  
14  
15  
16 *Anim Behav.* 71:1359-1369.  
17  
18  
19 Quinn GP, Keough MJ. 2002. Experimental design and data analysis for biologists.  
20  
21  
22 New Cambridge, UK: Cambridge University Press.  
23  
24 R Development Core Team. 2012. R: A language and environment for statistical  
25 computing. <http://www.R-project.org>  
26  
27  
28 Redondo T, Castro F. 1992. Signalling of nutritional need by magpie nestlings.  
29  
30  
31 *Ethology* 92:193-204.  
32  
33 Rodríguez-Gironés MA, Zúñiga JM, Redondo T. 2001. Effects of begging on growth  
34 rates of nestling chicks. *Behav Ecol.* 12:269-274.  
35  
36  
37 Rodríguez-Gironés MA, Zúñiga JM, Redondo T. 2002. Feeding experience and relative  
38 size modify the begging strategies of nestlings. *Behav Ecol.* 13:782-785.  
39  
40  
41 Romano A, Rubolini D, Caprioli M, Boncoraglio G, Ambrosini R, Saino N. 2011. Sex-  
42 related effects of an immune challenge on growth and begging behavior of barn  
43 swallow nestlings. *PLoS One.* 6:e22805.  
44  
45  
46  
47 Romero, L.M. 2004. Physiological stress in ecology: lessons from biomedical research.  
48  
49  
50 *Trends Ecol Evol.* 19: 249–255.  
51  
52  
53 Royle NJ, Hartley IR, Parker GA. 2002. Begging for control: when are offspring  
54 solicitation behaviours honest?. *Trends Ecol Evol.* 17:434-440.  
55  
56  
57  
58  
59  
60

- 1  
2  
3  
4 Royle NJ, Hartley IR, Parker GA. 2004. Parental investment and family dynamics:  
5 interactions between theory and empirical tests. *Popul Ecol.* 46:231–241.  
6  
7  
8 Saino N, Suffritti C, Martinelli R, Rubolini D, Møller AP. 2003. Immune response  
9 covaries with corticosterone plasma levels under experimentally stressful conditions in  
10 nestling barn swallows (*Hirundo rustica*). *Behav Ecol.* 14:318-325.  
11  
12  
13 Searcy WA, Nowicki S. 2005. The evolution of animal communication. Princeton,  
14 USA: Princeton University Press.  
15  
16  
17 Smiseth PT, Scott MP, Andrews C. 2010. Hormonal regulation of offspring begging  
18 and mediation of parent-offspring conflict. *Anim Behav.* 81:507-517.  
19  
20  
21 Smits JE, Bortolotti GR, Tella JL. 1999. Simplifying the phytohaemagglutinin  
22 skin-testing technique in studies of avian immunocompetence. *Funct Ecol.* 13:567-572.  
23  
24  
25 Soler M, Ruiz-Raya F, Carra LG, Medina-Molina E, Ibáñez-Álamo JD, Martín-Gálvez  
26 D. 2014. A long-term experimental study demonstrates the costs of begging that were  
27 not found over the short term. *PLoS One* 9:e111929.  
28  
29  
30 Sorci G, Faivre B. 2009. Inflammation and oxidative stress in vertebrate host–parasite  
31 systems. *Philos T R Soc B.* 364:71-83.  
32  
33  
34 Starck JM, Ricklefs RE. 1998. Avian growth and development: evolution within the  
35 altricial-precocial spectrum. Oxford, UK: Oxford University Press.  
36  
37  
38 Számadó S. 1999. The validity of the handicap principle in discrete action-response  
39 games. *J theor Biol.* 198:593-602.  
40  
41  
42 Számadó S. 2011. The cost of honesty and the fallacy of the handicap principle. *Anim*  
43 *Behav.* 81:3-10.  
44  
45  
46 Szép T, Møller AP. 2000. Exposure to ectoparasites increases within-brood variability  
47 in size and body mass in the sand martin. *Oecologia.* 125:201-207.  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4 Trivers RL. 1974. Parent-offspring conflict. *Am Zool.* 14:249-264.

5  
6 Tschirren B, Bischoff LL, Saladin V, Richner H. 2007. Host condition and host  
7  
8 immunity affect parasite fitness in a bird–ectoparasite system. *Funct Ecol.* 21:372-378.

9  
10 Verhulst S, Wiersma P. 1997. Is begging cheap?. *Auk* 114:134-134

11  
12 Vézina F, Love OP, Lessard M, Williams TD. 2009. Shifts in metabolic demands in  
13  
14 growing altricial nestlings illustrate context-specific relationships between basal  
15  
16 metabolic rate and body composition. *Physiol Biochem Zool.* 82:248-257.

17  
18 Weathers WW. 1996. Energetics of postnatal growth. In: Carey C, editor. *Avian*  
19  
20 *energetics and nutritional ecology.* New York, USA: Chapman and Hall. p. 461-496.

21  
22 Wright J, Karasov WH, Kazem AJ, Gonçalves IB, McSwan E. 2010. Begging and  
23  
24 digestive responses to differences in long-term and short-term need in nestling pied  
25  
26 flycatchers. *Anim Behav.* 80:517-525.

27  
28  
29 Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM. 2009. *Mixed effects models*  
30  
31 *and extensions in ecology with R.* New York, USA: Springer.

32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

## FIGURE LEGENDS

Figure 1. Duration of sustained begging bouts per trial by High-Begging experimental nestlings (HB), Low-Begging control nestlings (LB) and broods in the wild. Shown are medians, interquartile range (boxes) and 10th-90th percentiles (error bars).

Figure 2. Changes in T-cell mediated immune response (patagium swelling) to an antigen (PHA) of High-Begging (HB) and Low-Begging (LB) nestlings after 13 and 24 h of inoculation. Error bars are SE around means.

Figure 3. Changes in body mass of High-Begging (black dots) and Low-Begging (open dots) at the onset of the experiment (0 h), after 13 h of active begging (diurnal phase) and 24 h afterwards. Asterisks indicate significant differences ( $P = 0.005$ ) between groups. Error bars are SE around means.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

## TABLES

Table 1

Mean  $\pm$  SE for each variable measured in the study and the effect of Treatment (fixed), controlling for Nest (random).

	LB	HB	Treatment
	(n = 20)	(n = 20)	<i>F</i>
Initial body mass (g)	7.79 $\pm$ 0.21	7.76 $\pm$ 0.22	0.05 <sup>ns</sup>
Consumed food (g)	3.93 $\pm$ 0.08	3.96 $\pm$ 0.07	0.60 <sup>ns</sup>
Feces mass (g)	1.25 $\pm$ 0.08	1.32 $\pm$ 0.10	0.32 <sup>ns</sup>
Feces mass at 13:00 h (%)	24.1 $\pm$ 2.85	30.6 $\pm$ 1.91	3.96 <sup>ns</sup>
Time begging (s/trial)	3.43 $\pm$ 0.32	51.18 $\pm$ 2.57	662.7***
Begging postural intensity	1.78 $\pm$ 0.07	2.14 $\pm$ 0.04	21.70***
Diurnal mass gain (g)	2.02 $\pm$ 0.06	1.85 $\pm$ 0.06	7.46**
Nocturnal mass gain (g)	-1.16 $\pm$ 0.05	-1.00 $\pm$ 0.04	5.21*
Total mass gain (g)	0.87 $\pm$ 0.09	0.85 $\pm$ 0.07	0.02 <sup>ns</sup>
Metabolic expenditure (g)	1.78 $\pm$ 0.11	1.73 $\pm$ 0.08	0.24 <sup>ns</sup>
Diurnal immune response (mm)	4.21 $\pm$ 0.19	3.41 $\pm$ 0.19	28.30***
Nocturnal immune response (mm)	-0.43 $\pm$ 0.21	-0.15 $\pm$ 0.12	1.90 <sup>ns</sup>
Final immune response (mm)	3.78 $\pm$ 0.22	3.25 $\pm$ 0.14	11.31**

*F* values from Restricted Maximum Likelihood Estimation Linear Mixed Models

(REML-LMM). Degrees of freedom *df* = 1,19, except for Metabolic Expenditure (*df* = 1,17). Time Begging was log transformed prior to analyses.

*P*-values: \* *P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001, ns for not significant.

Table 2

ANOVA estimation of variance components due to Treatment (fixed) and Nestling (random) of Time Begging and Postural Intensity.

Effect	Time Begging			Postural Intensity	
	df	MS	<i>F</i>	MS	<i>F</i>
Treatment	2	6989.65	7642.64***	315.28	1223.29***
Nestling(Treatment)	38	0.92	3.64***	0.26	2.54***
Error	117	0.25		0.11	
Repeatability (intraclass correlation coefficient)					
HB		0.45***		0.52***	
LB		0.22*		0.33***	

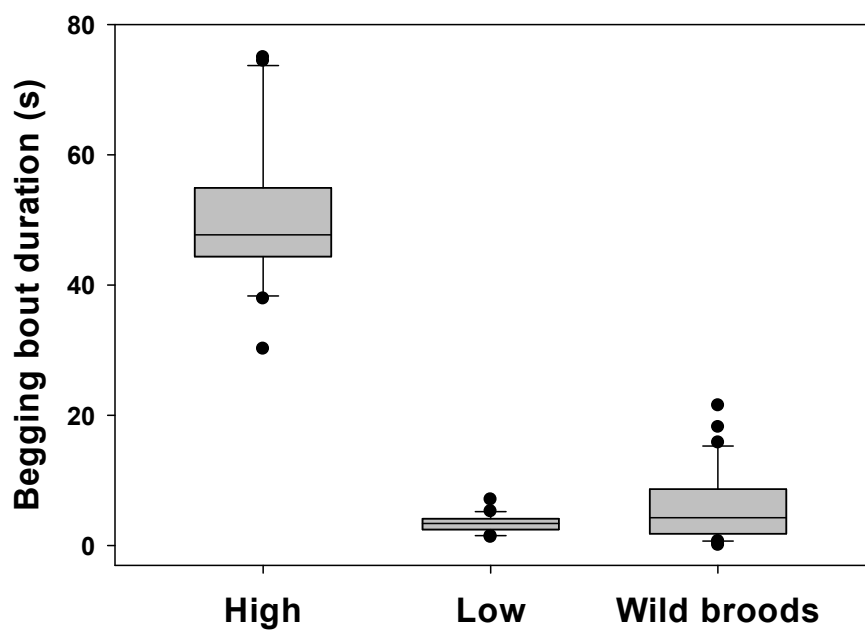
Repeatability of begging variables across four begging trials for each treatment level (HB and LB) indicates the fraction of total variance explained by Nestling (random).

Time begging was log transformed prior to analyses.

*P*-values: \*  $P < 0.05$ , \*\*\* $P < 0.001$ .

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

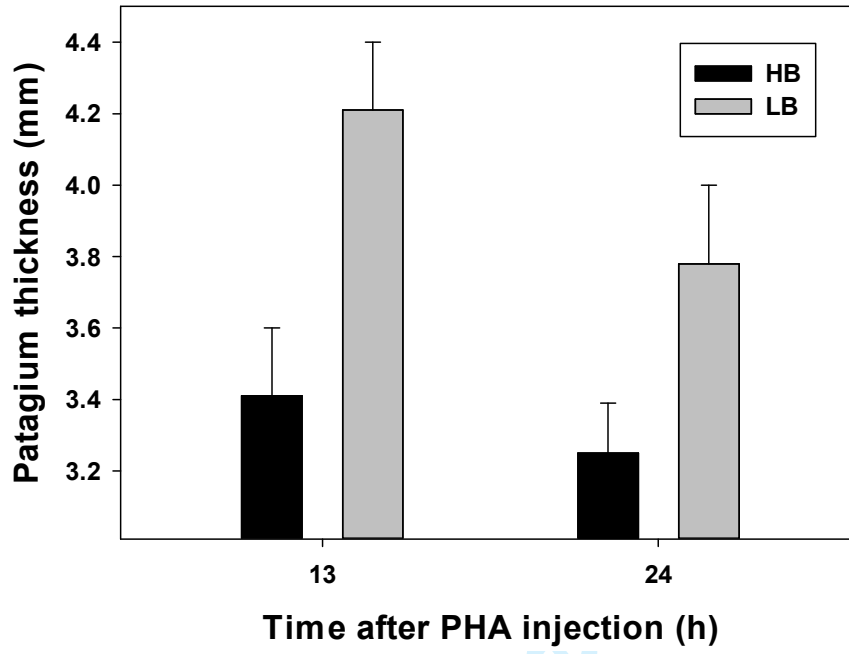
Figure 1



view Only



Figure 2

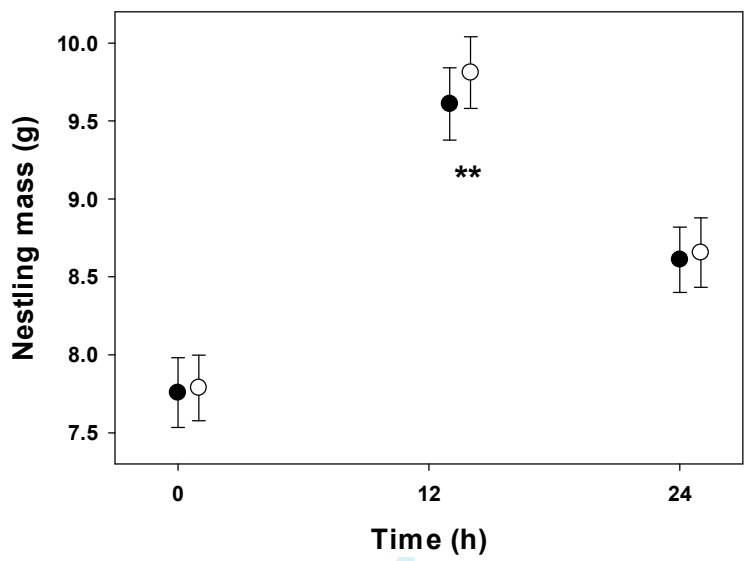


ew Only

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

Figure 3



Review Only

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1 **LAY SUMMARY**

2 Nestling begging signals should be costly in order to limit the benefits of selfish  
3 deception to parents. Exactly which cost begging entails has been hotly debated in the  
4 past. Here we show that begging impairs the immune system of pied flycatcher  
5 nestlings. It also reduced nestling growth but nestlings were able to recover while  
6 resting at night. Since most of the controversy was about growth costs, this study may  
7 help to settle the debate.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

8

For Review Only