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Nest-dwelling ectoparasites reduce begging effort in Pied Flycatcher (*Ficedula hypoleuca*) nestlings

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Running title: Ectoparasites reduce begging effort

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29 Parasitised nestlings are expected to increase begging effort to obtain additional resources to
30 compensate for those sequestered by parasites. However, begging is expected to be costly and
31 chicks harbouring parasites may find it more difficult to attain high begging levels.
32 Consequently, we predicted that, for the same level of nutritional need, parasitised nestlings
33 should invest less in begging than non-parasitised ones. We tested this prediction by
34 measuring begging in Pied Flycatcher (*Ficedula hypoleuca*) nestlings parasitised with
35 haematophagous mites (*Dermanyssus gallinoides* and *D. gallinae*) and blowfly larvae
36 (*Protocalliphora azurea*) and subjected to different levels of food deprivation –therefore,
37 controlling for their short-term nutritional need. As predicted, parasitised nestlings begged for
38 a shorter time than non-parasitised ones, especially when very hungry. Consequently, our
39 results suggest that time begging may indicate not only the level of need, but also nestling
40 parasitism status.

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42 **Keywords:** begging, blowflies, ectoparasites, mites.

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48 Parasites, by extracting resources from their hosts and decreasing their fitness, are an
49 important ecological and evolutionary force (Schmid-Hempel 2011). Vertebrates harbour
50 several ectoparasitic arthropods, which feed mainly on blood. In birds, many haematophagous
51 ectoparasites inhabit nests, feeding on nestlings whose fitness may consequently be reduced
52 drastically (Møller *et al.* 2009). Nestlings, however, may increase resource consumption by
53 elevating begging levels (Kilner & Johnstone 1997), thus compensating –at least partially–
54 the losses due to parasitism. In fact, Great Tit (*Parus major*) and Pied Flycatcher (*Ficedula*
55 *hypoleuca*) nestlings increase begging when parasitised (Christe *et al.* 1996, Cantarero *et al.*
56 2013). These studies, however, did not control for the nutritional need of nestling and argued
57 that the increased begging in parasitised nestlings was simply due to their higher need
58 compared with unparasitised nestlings. Parasites, nonetheless, not only affect nutritional
59 condition, but also nestling physiology (e.g., reduced haematocrit [Potti *et al.* 1999] and
60 thermogenesis capacity [Simon *et al.* 2004], increased stress response [Lobato *et al.* 2008],
61 reduced antioxidant capacity [López-Arrabé *et al.* 2015]).

62 Begging behaviour is costly in different ways, including energetic expenditure (Kilner
63 2001), generation of oxidative stress (Moreno-Rueda *et al.* 2012) and reduced immune
64 response (Moreno-Rueda & Redondo 2012). Consequently, begging should be especially
65 costly for parasitised nestlings, since they are in worse physiological and nutritional condition
66 than non-parasitised ones. On this basis, we hypothesised that, for the same need of food,
67 parasitised nestlings should invest less in begging than non-parasitised ones. We tested this
68 prediction in Pied Flycatcher nestlings parasitised by the haematophagous mites *Dermanyssus*
69 *gallinoides* and *D. gallinae*, and by larvae of the blowfly *Protocalliphora azurea*. Parasitism
70 by these arthropods negatively impacts nestling fitness by reducing their growth and survival
71 (Merino & Potti 1995), the latter even affecting reproduction when they reach adulthood

72 (Potti 2008). We experimentally examined the begging behaviour (typified as latency to beg,
73 postural intensity, and time spent begging) of nestlings from ectoparasite-infested and non-
74 infested nests in response to a standard stimulus (playback of a parent feeding call), and at
75 different, but standardized levels of food deprivation.

76

77 **METHODS**

78 The study was carried out in 2011 and 2013 in an intensively studied Pied Flycatcher
79 population breeding in nest-boxes near La Hiruela (Central Spain; more details in Camacho *et*
80 *al.* 2013). Nest-boxes were regularly inspected to determine hatching date (day 1). When
81 nestlings were 8-days old, we took 36 nestlings of intermediate size from 18 nests (one pair of
82 nestlings with similar body mass per nest) in the afternoon, always leaving in the nests at least
83 three nestlings to prevent parental desertion. Nestlings were placed in a warm chamber and
84 taken to a nearby laboratory, which took about 20 min. Nestmates were kept together in
85 artificial nests lined with cotton fabric, covered by a duster in a quiet room to avoid begging
86 in response to visual or acoustic stimulus unrelated to the experiment. Given that parasites
87 analysed in this study are nest-dwelling, nestlings were free of parasites in the lab. Heat was
88 provided by bulb lamps and temperature inside nest cups was monitored with a probe digital
89 thermometer and kept at ca. 36°C. Upon arrival to the lab, nestlings were conditioned to a
90 begging stimulus and fed *ad libitum* with commercial dipteran larvae just after playing a
91 parental feeding call recorded from the same population. We made sure that all nestlings were
92 conditioned to the stimulus and begged similarly to how they behave in their nests (based on
93 nest video recordings, see Redondo *et al.* 2016).

94 On the following morning, nestlings were stimulated to beg with the parental feeding
95 call at 7:30, 8:00 and 8:30 h while being fed *ad libitum* in order to eliminate differences in
96 nutritional need among nestlings. Our rationale for this is that, for measuring begging

97 behaviour, it is important that all nestlings are completely satiated at the start of the
98 experiment, so that any difference found is not due to initial differences in need of food. To
99 quantify begging behaviour, we established four begging trials at 15, 30, 60, and 90 min
100 intervals since the last meal. Begging trials consisted of stimulating begging in a standardized
101 way, by playing a recording of an adult Pied Flycatcher feeding call. Given that sleeping
102 sometimes prevented responses to the playback feeding call, this was played thrice in each
103 trial. Nestling behaviour was recorded with a digital camera Handycam HDR-XR155E. By
104 using the JWatcher 0.9 software (Blumstein & Daniel 2007), we measured from video
105 recordings: (1) Latency to beg, as the time taken for nestlings to open the gape to beg since
106 the onset of the playback stimulus. (2) Time begging, as the time each nestling spent begging.
107 Once nestlings started to beg, they were allowed to beg without interference until they
108 voluntarily ceased begging. And (3) begging intensity, by establishing five categories of
109 postural intensity (following Redondo & Castro 1992): 0 (no response), 1 (gaping, tarsi
110 flexed), 2 (gaping, neck extended, tarsi flexed), 3 (gaping, neck extended, body up), and 4
111 (gaping on fully stretched feet and tarsi, sometimes including wing flapping). Note that these
112 categories represent a gradient of increased energy invested in begging, which correlates well
113 with need levels and the probability of being fed (Gottlander 1987). The analysis of begging
114 behaviour was blind regarding parasitism status.

115 When the experiment ended (at midday), nestlings were fed *ad libitum* again,
116 individually marked with non-toxic felt pens, and took back to their nests. In total, nestlings
117 were in the lab for less than 24 hours. On the following days, we regularly checked nests to
118 monitor the fate of nestlings used in the experiments, re-marking them when necessary. All
119 experimental nestlings fledged successfully, except three (one from unparasitised and two
120 from parasitised nests), which died from natural causes. Therefore, no nestling was damaged
121 or died as a consequence of our experiment. When nestlings were 13 days-old, they were

122 ringed, weighed and measured (tarsus length). Moreover, we scored nests as parasitised or not
123 by haematophagous mites following Merino & Potti (1995): nestlings were placed in a white
124 cotton bag during 5 min, and then we recorded whether any mite was left by nestlings in the
125 cotton bag. This estimation of mite occurrence is tightly correlated with the number of mites
126 in the nest, as revealed by fine examination of nesting material with Berlese funnels (Merino
127 & Potti 1995). This fine analysis of parasites estimation showed however that “absence of
128 mites” did not necessarily imply their complete absence, but rather a low parasitic load, at
129 levels for which mites have no detectable effects on nestling fitness (Merino & Potti 1995). In
130 any case, nests in which mites were categorized as “present” should be interpreted as having
131 considerably higher (with detrimental effects for nestlings) parasitic load than nests in which
132 mites were categorized as “absent” (Merino & Potti 1995). After fledglings left the nest, the
133 complete material of the nest was examined and the presence of blowfly larvae and pupae was
134 recorded. We are confident that such estimations reflect parasite load at the time begging was
135 measured, although we estimated parasite load 5-15 days (5 days for mites, 10-15 for
136 blowflies) after, considering: 1) the time these parasites need to complete their cycles (10-36
137 days, Gold & Dalhsten 1989, Bruneau *et al.* 2001), and 2) in infected nests, blowflies and
138 mites are usually detected at an early stage of the nestling period (3-5 days) and at a
139 parasitism level similar to that found at the end the nestling period (personal observations).

140 Statistical analyses were performed with Linear Mixed Effects Models of Restricted
141 Maximum Likelihood (REML-LMM; Zuur *et al.* 2009), by using the package "nlme"
142 (Pinheiro *et al.* 2012) in R (R Development Core Team 2012). In each model, “Nestling
143 identity” nested in “Nest of origin”, at the same time nested in “year” (Gelman & Hill 2007),
144 was introduced as a structured random factor to control for variance among years, nests and
145 repeated measures of nestlings. As fixed predictors, we used “Deprivation” (four levels: 15,
146 30, 60, and 90 min since the last feeding), “Parasitism status” (four levels: uninfected, only

147 mites, only blowflies, and both mites and blowflies), and their interaction. However, given
148 that there were no differences in the dependent variables among nests parasitised by mites,
149 blowflies or both (ANOVA, $F_{2,27} < 1.40$, $P > 0.25$ for all begging variables and levels of food
150 deprivation; Fig. S1), we repeated the analyses with two levels for “Parasitism status”:
151 uninfected versus infected. Dependent variables were “Latency”, “Intensity”, and “Time
152 begging”. For every model, we checked for homoscedasticity and normality of residuals.
153 Means are given with one standard error (SE). Main effects were tested with a type III model,
154 which is recommended for unbalanced designs (Quinn & Keough 2002, p. 243).

155 The study was licensed by the Madrid government. Experimental procedures were
156 approved by the CSIC Ethical Committee (ref. CGL2011-29694) and the Andalusian
157 Committee of Animal Experimentation (ref. 2011_03Potti) to comply with Spanish and
158 European legislations on the protection of animals used for scientific purposes. The complete
159 dataset is available in Table S1.

160

161 **RESULTS**

162 Eight nestlings came from nests infected with mites only, 10 from nests infected with
163 blowflies only, 12 from nests simultaneously infected by the two ectoparasites, and six from
164 nests that remained uninfected (total sample size, $n = 18$ nests). Nests harboured a mean of
165 9.18 ± 6.26 mites (range: 0-71) and 5.45 ± 2.57 blowflies (range: 0-29). There were no
166 differences according to parasitism status in either hatching date ($F_{3,14} = 0.49$, $P = 0.70$),
167 brood size ($F_{3,14} = 0.70$, $P = 0.57$), or body mass at 8 days of nestlings used in the study ($F_{3,14}$
168 $= 0.64$, $P = 0.60$). For the nestlings that survived until fledging (33 of 36 nestlings), there
169 were no differences in tarsus length at day 13 ($F_{3,29} = 1.24$, $P = 0.31$), according to the
170 presence of mites, blowflies or both. However, there was a trend for nestlings in non-

171 parasitized nests to weigh more at day 13 than those in parasitized nests ($F_{3, 29} = 2.89$, $P =$
172 0.052), suggesting that ectoparasites had a detrimental effect on nestling growth.

173 Irrespective of parasitism status, time begging and begging intensity increased with
174 hunger (Table 1; Fig. 1). As expected, the time spent begging was significantly shorter in
175 nestlings reared in parasitised than in uninfected nests (Table 1; Fig. 1a). Differences in
176 begging time were especially marked at the highest level of food deprivation, as indicated by
177 the significant interaction deprivation*parasitism (see Table 1). That is, when nestlings were
178 very hungry (90 min), nestlings from uninfected nests begged for longer than those from
179 infected nests (Fig. 1a). In fact, after 90 min of food deprivation, differences in begging time
180 among nestlings differing in parasitism status were highly significant (REML-LMM, $\chi^2 =$
181 10.85, $P < 0.001$), while differences were not significant for the trials at 30 and 60 min since
182 the last feeding ($P > 0.65$), and only were closed to significance at 15 min ($\chi^2 = 3.42$, $P =$
183 0.064). In contrast to begging time, parasitism status had no effect on begging postural
184 intensity or latency (Table 1; Figs. 1b and 1c). Findings were qualitatively similar when we
185 repeated the statistical models with 4 levels of parasitisation (uninfected, only mites, only
186 blowflies, and both; Table S2; Fig. S1).

187

188 **DISCUSSION**

189 Our study shows that nestlings from parasitised nests begged for shorter than non-parasitised
190 nestlings in a similar state of nutritional need, especially at the highest level of food
191 deprivation. This suggests that the effect of parasitism on begging may be exacerbated at
192 higher levels of begging effort. Parasitism, on the other hand, had no detectable effect on the
193 postural intensity or latency of begging. Therefore, it seems that parasitism has no detectable
194 effect on the neural and muscular capacity of nestlings, but it limits the time they may invest
195 in begging. Begging time, in fact, seems to be the variable most related to begging effort and

196 begging costs (Moreno-Rueda & Redondo 2011). Our study, however, is correlational and
197 sample size is limited, and thus we cannot rule out the possibility that an unknown factor (e.g.
198 nest site quality or microclimate) is simultaneously affecting parasite prevalence and begging
199 behaviour, resulting in the relationship found here.

200 At first glance, our findings contrast with those of Cantarero *et al.* (2013) in a nearby
201 population of Pied Flycatchers, in which nestlings in parasitised nests (with mites, blowflies,
202 and fleas) begged more intensely than non-parasitised ones; similar findings were found in
203 Great Tits (Christe *et al.* 1996). In these studies, increased begging effort of parasitised
204 nestlings could be explained by their presumably higher nutritional need, as long as
205 parasitised nestlings were in more need than unparasitised nestlings. Therefore, to ascertain
206 the effect of parasites on begging, it is necessary to control for short-term needs. Our findings
207 are novel because we controlled for short-term nutritional need, and by doing so, we found
208 that parasitised nestlings begged less than non-parasitised nestlings for the same level of food
209 deprivation. Similarly, O'Connor *et al.* (2014) found that Darwin's Finch (*Geospiza*
210 *fuliginosa*) nestlings parasitised with larvae of the fly *Philornis downsi* begged less than non-
211 parasitised nestlings. That is, nestlings in bad condition may increase begging effort to some
212 degree to increase food consumption and compensate for their mass loss due to parasitism
213 (Christe *et al.* 1996, Cantarero *et al.* 2013), but this is probably not possible when physical
214 condition is much deteriorated (O'Connor *et al.* 2014). Along this line, we found that the
215 largest differences between parasitised and non-parasitised nestlings in begging time occurred
216 when nestlings' need was highest.

217 The underlying mechanisms of reduced begging effort in parasitised nestlings are still
218 unclear. An energetic cause may be invoked (Moreno-Rueda & Redondo 2011), consistent
219 with a higher effect of parasitism on begging at higher levels of begging effort. Nonetheless,
220 nestlings did not differ in body mass at the time the experiment was performed, which

221 suggests that they had similar energy reserves. Alternatively, the reduction in begging might
222 be mediated by hormones since, for example, corticosterone levels have been reported to
223 increase with parasitism (Lobato *et al.* 2008). However, corticosterone also increases begging
224 effort (Loiseau *et al.* 2008), so their potential effects would markedly contrast with our
225 findings. A third possibility is that the reduction in begging effort is mediated by oxidative
226 stress. One of the main responses against ectoparasites is inflammation, which is associated
227 with the release of several pro-oxidant substances, such as nitric oxide (NO[·]) or superoxide
228 anion (O₂⁻) (reviewed in Sorci & Faivre 2009). Indeed, parasitism depletes antioxidant
229 defences in Pied Flycatcher nestlings (López-Arrabé *et al.* 2015). Begging behaviour implies
230 considerable neural and muscular activity, which is also associated with the release of pro-
231 oxidant molecules (Costantini 2014), and some studies have found evidence of oxidative
232 stress associated with begging (Noguera *et al.* 2010, Moreno-Rueda *et al.* 2012). In such a
233 situation, parasitised nestlings may pay a cost in the way of increased oxidative stress.
234 Therefore, parasitised nestlings would benefit from reducing begging effort in order to
235 keeping their oxidative balance within safe limits. Finally, another possibility is that the
236 reduction in begging is due to a trade-off with the immune response. Birds frequently show an
237 immune response against ectoparasites (review in Owen *et al.* 2010), and begging impairs
238 immune response in our population (Redondo *et al.* 2016). Consequently, it is possible that
239 parasitised nestlings reduced begging in order to avoid negative consequences of impaired
240 immune response.

241 An important implication of our findings is that time employed in postural begging
242 may indicate not only nutritional need, but also nestling parasitism status. Parasitised
243 nestlings are of less reproductive value for parents (Forbes 1993), and thus parents may gain
244 useful information on nestling quality from begging signals (Mock *et al.* 2011), and respond
245 accordingly by feeding preferentially less parasitised broods (Saino *et al.* 2000). Health

246 signalling by nestlings is usually associated to mouth colour (e.g. Saino *et al.* 2003) and it
247 actually indicates parasitism by haematophagous mites (*Pellonyssus reedi*) in House Sparrow
248 (*Passer domesticus*) nestlings (Dugas & Doumas 2014). Our study, nevertheless, shows that
249 time begging may also inform about parasitism status. Therefore, time begging should no
250 longer be viewed as only an indicator of need, as it may also indicate aspects of individual
251 quality (see Mock *et al.* 2011).

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253

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359 **Table 1.** Results of Linear Mixed Effects Models of Restricted Maximum Likelihood
360 (REML-LMM) analysing the effect of “Deprivation” (4 levels: 15, 30, 60, and 90 min since
361 the last feeding), “Parasitism status” (2 levels: uninfected vs. infected) and its interaction on
362 “Postural Intensity”, “Time begging”, and “Latency to beg”. In each model, “Nestling
363 identity” (n = 36) is nested within “Nest of origin” (n = 18), and nested within Year, and
364 introduced as a structured random factor to control for variance among years and nests and the
365 repeated measured of nestlings.

366

	χ^2	d.f.	P
a) Time begging			
Deprivation	35.82	3	< 0.001
Parasitism status	3.91	1	0.048
Deprivation * Parasitism status	16.83	3	< 0.001
b) Postural intensity			
Deprivation	9.67	3	0.022
Parasitism status	0.02	1	0.88
Deprivation * Parasitism status	1.34	3	0.72
c) Latency to beg			
Deprivation	5.59	3	0.13
Parasitism status	0.50	1	0.48
Deprivation * Parasitism status	2.80	3	0.42

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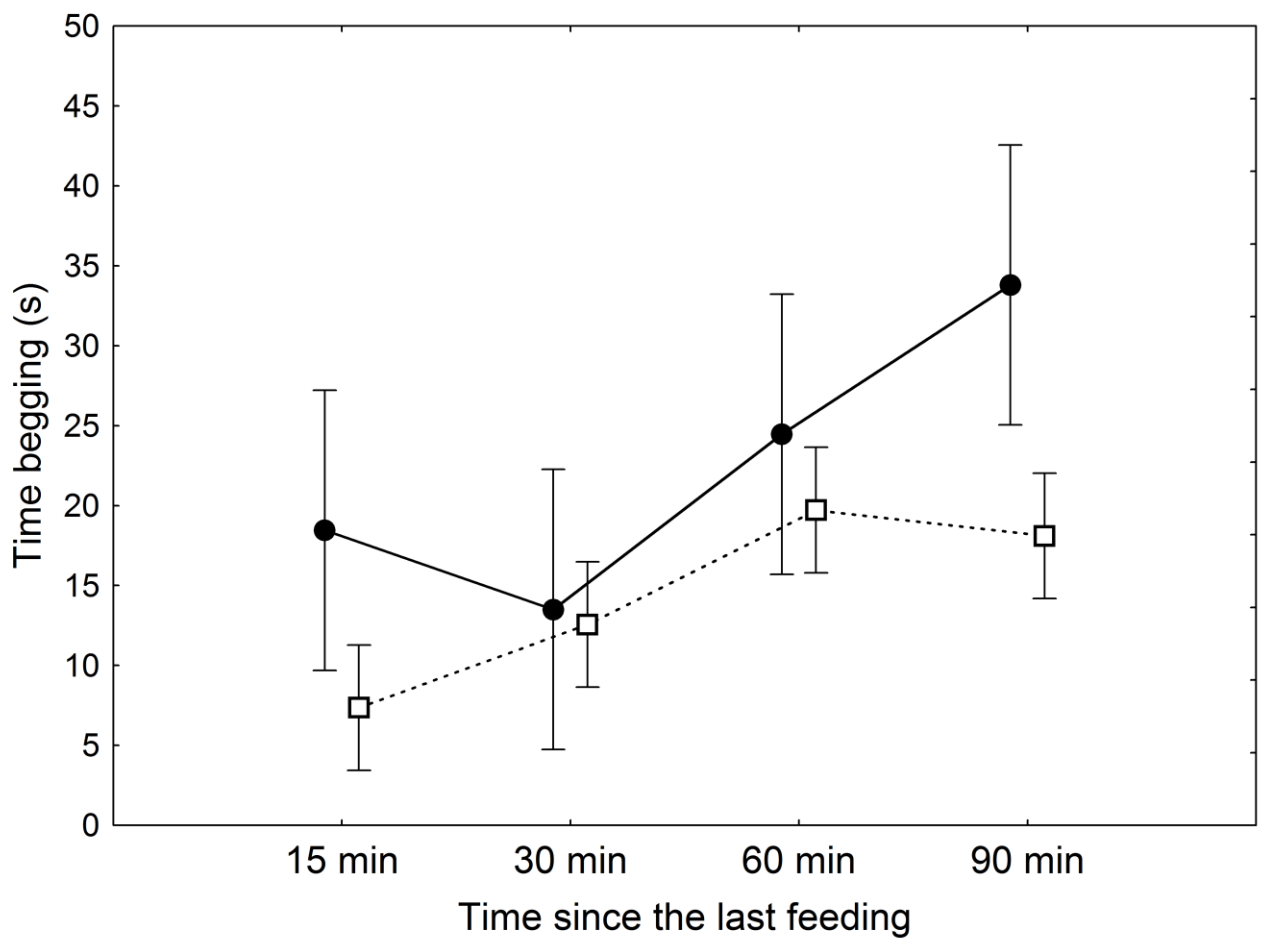
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373 **Figure 1.** Average values, with 95% Confidence Intervals, for Time begging (a), Begging
374 postural intensity (b), and Latency (c), according to time since the last feeding (hunger) and
375 prevalence of parasites in the nest (black circles: uninfected; white squares: infected). Sample
376 size: 6 uninfected nestlings, 30 infected nestlings.

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378 a)



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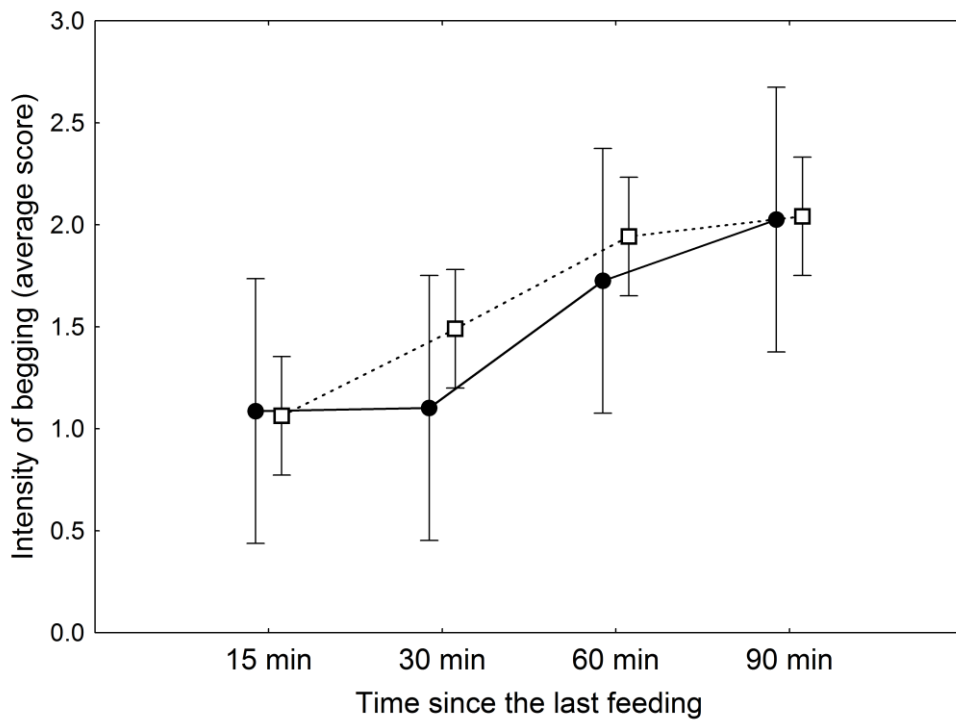
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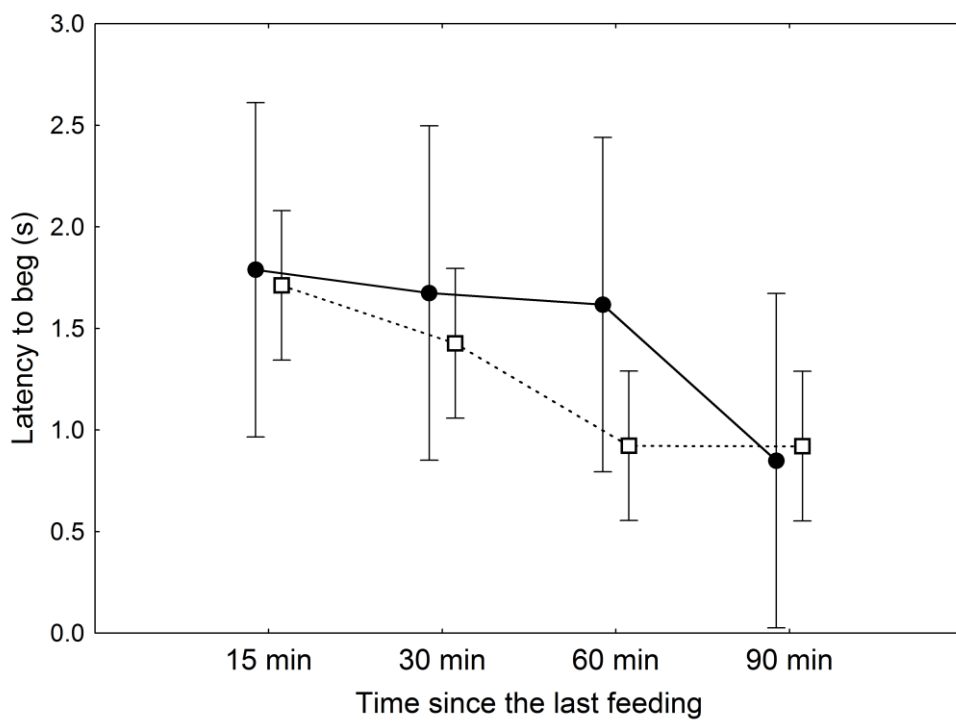
386 b)



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389 c)



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