

1 ABSTRACT

2 Mass regulation in small birds is based on simultaneously minimising starvation and predation risk,
3 but the mechanisms birds use to assess starvation risk are still debated. Whilst we know that birds
4 anticipate periods of unpredictable food availability/energy expenditure (e.g. the winter and night)
5 by increasing their fat reserves, we do not know whether this anticipation involves learning. This
6 study investigated whether birds could learn to use a light cue that predicted a period of food
7 unavailability, to adaptively regulate their foraging and/or body weight. Sixteen captive starlings
8 (*Sturnus vulgaris*) were subjected to 42 days of an irregular schedule of food deprivation that
9 involved depriving them of food for 5 hours on 20 pseudo-randomly chosen days. Birds were
10 randomly allocated to two treatment groups for which a 30-minute period of reduced ambient light
11 either provided perfect information (Predictable) or no information (Unpredictable) about upcoming
12 food deprivation. Both groups of birds increased their dawn body mass over the period of the
13 experiment, consistent with a response to unpredictable food deprivation. However, no differences
14 in either foraging behaviour or dawn body mass emerged between the groups, suggesting that the
15 Predictable birds were unable to learn to use the light cue to initiate anticipatory foraging ahead of
16 food deprivation. Furthermore, both groups immediately decreased their foraging behaviour in
17 response to the onset of the light cue, suggesting that starlings do not have an evolved anticipatory
18 foraging response to low light levels. Further work is needed to test alternative cues and designs
19 before any general conclusions can be drawn regarding the flexibility of anticipatory foraging.

20 KEYWORDS

21 anticipatory foraging, associative learning, body mass, fat regulation, food intake, unpredictable
22 fasting, winter storm

24 INTRODUCTION

25 For many animals, carrying body fat has costs and benefits: too little fat and they starve (Ketterson
26 & King, 1977; Stuebe & Ketterson, 1982); too much fat and they increase their likelihood of
27 predation (Blem, 1975; Brodin, 2001; Witter & Cuthill, 1993). Theoretical models show there is an
28 optimum level of body fat that minimises the combined risk of starvation and predation, and that
29 this optimum will vary depending on environmental conditions (Lima, 1986; McNamara & Houston,
30 1990). A key prediction from these theoretical models is that if perceived starvation risk is lower,
31 body masses will decline to reduce predation risk. Conversely, if perceived starvation risk is higher,
32 body masses will increase to reduce starvation risk. Corroboration of these predictions comes from
33 multiple field and laboratory studies on passerine birds (Cuthill et al., 2000; Hudin et al., 2016;
34 Witter et al., 1995).

35 There is empirical evidence that passerine birds not only adjust body masses in response to current
36 starvation risk, but that they also strategically regulate their body fat in anticipation of future
37 starvation risk. Well-studied examples of anticipatory regulation include fat gain prior to winter and
38 nightfall. In winter, food availability and energy expenditure are less predictable than in other
39 seasons, and small birds increase foraging intensity and body fat levels in autumn (King & Mewaldt,
40 1981; McEwan & Whitehead, 1984; Pienkowski et al., 1979) to buffer against upcoming periods of
41 forced fasting and/or increased energy expenditure (Blem, 1976). Similarly, overnight starvation
42 presents a significant survival risk for many small birds and they increase foraging intensity and body
43 fat levels immediately before dusk to mitigate this risk (Houston et al., 1993; Polo & Bautista, 2006;
44 Witter & Cuthill, 1993). Despite their rapidity, short-term changes in fat within a day can be large
45 and comparable to fat changes in winter (Meijer et al., 1994). For example, in European starlings
46 (*Sturnus vulgaris*) the amount of weight gained prior to dusk may represent 6-14% of their total
47 dawn body mass, which is similar to the amount of weight gained prior to winter corresponding to
48 11% of autumn body mass (Cuthill et al., 2000; Meijer et al., 1994).

49 While we know that anticipatory regulation of body fat occurs, the mechanisms that govern it are
50 poorly understood (Kelly et al., 2002). We do not know whether anticipatory regulation is an
51 inflexible evolved response to reliable natural cues, or whether birds can respond to novel cues by
52 associative learning. There is evidence that the amount of body fat deposited in anticipation of
53 winter reflects long-term average energy demands from past winters (Biebach, 1996; Evans, 1969).
54 Yet even with the added buffer provided by winter fattening, the actual fat reserves for many small
55 birds only allows for a very small period of disruption to foraging by unusually severe winter storms
56 (Carey & Dawson, 1999). For example, in starlings, the amount of additional fat carried during winter
57 can only buffer against a single 24-hour period without food (Meijer et al., 1994). Consequently, it
58 has been suggested that any mechanism involved in anticipatory winter fattening ought to be
59 flexible, so that fat levels can be adjusted in response to short-term fluctuations in energy
60 requirements or food availability (Blem & Shelor, 1986). Such a mechanism could use reliable cues
61 of upcoming food unavailability (Lima, 1986), such as weather changes that signal approaching
62 storms. Rapid, anticipatory adjustments of foraging behaviour and body fat could then occur, much
63 in the same manner as the daily patterns of fat changes.

64 We have good reason to suspect that birds may be able to make flexible short-term, anticipatory
65 adjustments in this way. In an opportunistic study, Middleton (1982) observed American goldfinches
66 (*Spinus tristis*) flocking to bird feeders in the hour preceding a harsh snow storm. Inclement weather
67 can severely reduce feeding opportunities (Graber & Graber, 1979) and may cause significant
68 mortality to bird populations (Carey & Dawson, 1999). Middleton (1982) showed that the foraging
69 effort of the goldfinches was much greater in the hour before the storm compared to similar time-
70 periods on non-stormy days and that the masses of birds captured during the storm were greater
71 than in the days preceding it. He speculated that an increased mass and a full gut would help
72 protect the birds from starvation and the low overnight temperature. Later work has provided some
73 experimental support for the idea that birds undergo short-term increases in body fat in response to
74 cues of upcoming storms, such as increased rainfall (Kelly et al., 2002), reduced ambient

75 temperature (Krams et al., 2010) and reduced barometric pressure (Breuner et al., 2013; Metcalfe et
76 al., 2013). However, with the exception of barometric pressure, it could be that the increases in
77 body fat observed were responses to the increased energy demands provided by the meteorological
78 changes themselves as opposed to adjustments in anticipation of increased starvation risk.
79 Furthermore, these studies shed no light on whether birds have acquired knowledge of cues of
80 storms by natural selection or by individual learning.

81 The aims of the current study were to test experimentally the hypothesis that birds can learn to use
82 an environmental cue to anticipate and prepare for upcoming food deprivation. We used a
83 laboratory experiment in order to eliminate confounds often present in natural environments. We
84 studied European starlings, since there is strong evidence for body mass regulation in response to
85 laboratory manipulations of food availability and energy expenditure in this species (Bednekoff &
86 Krebs, 1995; Cuthill et al., 2000; Witter et al., 1995). Starlings were exposed to an environment in
87 which food was occasionally (approximately every two days on average) unavailable for a period of 5
88 hours. The birds were randomly allocated to two treatment groups. In the Predictable group an
89 environmental cue perfectly predicted the periods of food unavailability and in the Unpredictable
90 group the same cue was completely uninformative. By only manipulating the informativeness of the
91 cue (via its correlation with subsequent food deprivation), we were able to keep constant the
92 frequency, duration and sequence of food deprivation to ensure that the level of environmental
93 harshness did not differ between treatment groups (c.f. Cuthill et al., 2000). The cue that we used
94 was an instant drop in the ambient light intensity that lasted 30-minutes in duration. This cue was
95 chosen to be an ecologically plausible predictor of storms, since it is possible that birds might be
96 more prepared to learn ecologically relevant cues (Seligman, 1970). Furthermore, the cue was
97 chosen so as not to change the energy expenditure of the birds themselves, in order to allow us to
98 study true anticipatory fattening as opposed to a direct response to increased energy expenditure.
99 Since the birds that we used were hand-reared in the laboratory (Nettle et al., 2017) and had never
100 been housed outside, they had no exposure to storms, and hence no opportunity prior to the

101 current experiment to learn an association between a sudden drop in ambient light and food
102 unavailability.

103 If the birds learnt to respond adaptively to the light cue during our experiment, we predicted the
104 following: 1) increased foraging activity following the onset of the cue for the Predictable group only;
105 2) increased food consumption following the onset of the cue for the Predictable group only; 3)
106 *lower* dawn body masses for the Predictable group relative to the Unpredictable group, reflecting
107 the fact that only the Predictable group could restrict their adaptive weight gain to the period
108 immediately prior to the period of food unavailability. In addition, we predicted an emergence of
109 differences between groups over time, reflecting the time needed to learn the association between
110 cue and food unavailability. Alternatively, if starlings have an unlearnt response to a reduction in
111 ambient light that has evolved because low light often precedes periods of food unavailability in
112 natural environments, we predicted the following: 1) increased foraging activity following the onset
113 of the cue for both groups; 2) increased food consumption following the onset of the cue for both
114 groups; 3) no difference in dawn body masses between groups. In addition, we predicted an
115 immediate difference in foraging behaviour and food consumption for both groups following the
116 onset of the cue, reflecting the fact that the response to the cue was not learnt. Finally,
117 independent of whether the birds showed any learnt or unlearnt response to the cue, we predicted
118 that all birds should show a gradual increase in dawn body mass reflecting the initial unpredictable
119 food deprivation present in both groups.

120

121 METHODS

122 *Husbandry and Housing*

123 *Ethics statement*

124 The current study was approved by the ethical review committee at Newcastle University and
125 complied with the Association for the Study of Animal Behaviour's guidelines for use of animals in
126 research. The study was carried out under UK home office Project Licence number PPL70/8089. The
127 removal of starlings from the wild prior to this study was approved by Natural England, under licence
128 number 20121066.

129 *Subject historical information*

130 Experimental animals were 16 starlings (*Sturnus vulgaris*), 8 males and 8 females, that comprised
131 four families of four siblings. At the time of the current experiment, the birds were three years of
132 age. The birds were taken from nests on day five post-hatching and hand reared to adulthood as
133 part of a previous study that involved manipulating the amount of food they were given and the
134 effort required to receive it during the nestling period (Nettle et al., 2017). This manipulation was
135 not part of the current investigation and was counterbalanced along with sex between treatment
136 groups of the current experiment.

137 *Room setup*

138 Prior to the current experiment, birds were group-housed in indoor aviaries supplied with ad lib food
139 and water. Birds were caught from the aviary and transferred to individual cages in a separate room
140 for the duration of the experiment. A single room was used to standardise any room-effects
141 between treatment groups. The temperature in the room was ~20 °C and the humidity was ~41%.
142 Due to the size restriction of the experimental room, the 16 birds were divided into two consecutive
143 replicates of eight, each consisting of four birds per treatment group. The experimental room
144 contained eight 75 x 45 x 45cm cages that were placed against the walls in stacks of two. The
145 position of the birds in the room was counterbalanced between treatment groups. Every bird was
146 provided with a water bath, two drinkers, two perches, and one food bowl. Food (Special Diets
147 Services 'Poultry Starter (HPS)' domestic chick crumb) were available ad lib except during
148 deprivation periods.

149

150 *Light regime*

151 The lighting in the room was remotely controlled and consisted of four vertical halogen corner lights
152 and four evenly spaced halogen ceiling lights. During the experiment, the light-dark schedule was set
153 at 15L:9D. This was identical to the schedule that the birds have been maintained on since fledging,
154 although here dawn and dusk were delayed by four hours for experimenter convenience. In order to
155 simulate a dawn, the lights came on at 0950 every morning and increased in intensity every minute
156 until they reached their maximum at 1000 (Figure 1). In the evening, the lights started dimming to
157 simulate dusk at 0050 and incrementally decreased in intensity until they turned off at 0100.

158 *Experimental Design*

159 *Habituation phase*

160 The experiment started with a cage habituation phase of six days. By the start of the experimental
161 phase all birds were eating a minimum of 8 g of domestic chick crumb (hereafter referred to as
162 'food') per day.

163 *Experimental phase*

164 The experimental phase lasted for 42 days during which the birds were subjected to 20 periods of
165 food deprivation. All food deprivation periods lasted for five hours and, if they occurred, always took
166 place between 1100-1600. Days where food deprivation took place were pseudo-randomly
167 allocated in a way that ensured all birds were deprived for a total of 20 days, and the distribution of
168 occurrence of one, two and three days of consecutive deprivation was identical for both treatment
169 groups (Table 1). The difference between treatment groups came from the informativeness of a light
170 cue preceding the deprivation period. The light cue, when it occurred, was an immediate reduction
171 in light intensity from 100% (340 lx) to 30% for all lights that started at 1030 and ended at 1100
172 when light intensity was returned to 100% (Figure 1). The Predictable treatment group always

173 received this light cue prior to food deprivation. The Unpredictable treatment group received the
174 light cue on 10 of the deprivation days (50%) and on 10 non-deprivation days (Table 1).
175 Consequently, the light cue was completely informative for the Predictable group, but completely
176 uninformative for the Unpredictable group, which was thus a “truly random” control group
177 (Rescorla, 1967, 1988). This design ensured that both treatment groups received near-identical
178 experience of food deprivation, but differed in the informativeness of the cue.

179 *Daily experimental procedure*

180 Ten minutes before dawn (0950), any remaining food was removed from the cages and exchanged
181 with a fresh bowl of food (Figure 1). Five minutes before dawn, two cameras were placed on tripods
182 and were positioned facing the cages at a distance of 1m. Before leaving the room, a curtain was
183 drawn between the cages to reduce social facilitation of foraging behaviour. From dawn to 1030,
184 the birds were free to eat ad lib food. On cue days (see Table 1) the light cue started at 1030 and
185 lasted for 30 minutes. At 1100, food was either removed (on deprivation days: Table 1) or otherwise
186 exchanged for a new bowl. Cameras were also removed at this point. At 1600, a new bowl of food
187 was given, regardless of the day. Daily husbandry also took place at this time. From 1600 onwards,
188 the birds were left undisturbed for the remainder of the day and night.

189 *Outcome Variables*

190 Three outcome variables were measured: foraging time and food intake to assess the presence of
191 any adaptive increase in foraging behaviour; and body mass to assess the impact of any change in
192 foraging behaviour on body mass regulation.

193 *Foraging time*

194 The amount of time that starlings foraged was recorded via video camera during the 30 minutes
195 between the time when a light cue could start (1030) and the time when food deprivation could
196 begin (1100). We also recorded this behaviour in the 40 minutes between dawn (0950) and when

197 the light cue could start (1030) to provide a baseline level of foraging effort for each bird on each
198 day; this measure was used as a control variable in our analyses. Filming took place every day to
199 facilitate habituation and to avoid associations with the presence of the cameras. A representative
200 subset of video footage was analysed for foraging behaviour (Table 1). Video footage from five
201 evenly distributed cue days and four evenly distributed non-cue days was analysed (Table 1; cue
202 days: 17, 26, 32, 36 and 42; non-cue days: 16, 22, 28 (Predictable birds only), 31 (Unpredictable birds
203 only) and 34). These days were chosen as there was no deprivation on the preceding days, which
204 reduced the likelihood of any behavioural effects of the deprivation from carrying over into the
205 foraging behaviour recorded on video. Thus, across the entire experiment we analysed nine days'
206 worth of video footage for each bird. A bird was designated as foraging when the tip of its beak was
207 below the rim of the food bowl.

208 *Food intake*

209 Food intake was measured every day during the period from dawn (0950) until the onset of possible
210 food deprivation (1100). Food intake was not measured during the period of the cue only (1030-
211 1100), as this would have required the food bowl to be exchanged at the time of the light cue,
212 introducing an additional cue. Consequently, food intake was a less precise measure of adaptive
213 foraging behaviour than time spent foraging because it additionally includes foraging that occurred
214 before the onset of the cue. We also measured food intake between 1600-0100 to provide a
215 baseline level of food consumption for each bird on each day; this measure was used as a control
216 variable in our analyses.

217 *Dawn body mass*

218 For the purpose of this experiment, dawn body mass was measured as a proxy for fat reserves. To
219 obtain a precise measure of body mass maintained outside of the food deprivation periods, the birds
220 were weighed before dawn when the gut was empty. Weighing took place 10-30 minutes before
221 dawn at the start of each phase and on every third day of the experimental phase. Birds were caught

222 by hand in the dark, placed in a weighing cone and weighed in grams on a digital scale to two
223 decimal places. The first nine days of experimental data were excluded from the subsequent analysis
224 owing to a change in experimental protocol between replicates (pre-dawn weighing did not start
225 until day nine for the first replicate but was undertaken for the entirety of the second replicate).
226 Thus, across the entire experiment each bird underwent 12 separate mass measurements. Our
227 analyses of body mass involved three control variables that are likely to have contributed to
228 between- and within-individual variation in mass: tarsus length to control for individual differences
229 in skeletal size; sex, because male starlings are heavier than females; and the number of days since
230 the last period of food deprivation. Tarsus length was measured when skeletal growth was complete
231 (day 56 post-hatching) with digital callipers; in the current study we used the mean of two replicate
232 measurements of each leg.

233 *Statistical Analysis*

234 All data analyses were undertaken in R version 3.3.2 (R Core Team, 2016). The raw data files and the
235 R script are available at the Zenodo repository (van Berkel et al., 2018). We fitted linear mixed
236 models using the package lme4 (Bates et al., 2015). Error distribution was Gaussian and all models
237 used in the analysis were checked to satisfy the assumptions of normally distributed residuals and
238 homogenous variance of residuals across the fitted values of the model. Maximum-likelihood
239 estimation was employed throughout.

240 We fitted three different linear mixed models, one for each outcome variable. The fixed effects
241 included in each model are given in Table 2. Experimental variables included: the continuous fixed
242 effect of “Day” and the categorical fixed effects of “Treatment” (Predictable or Unpredictable) and
243 “Cue” (Present or Not present). We included all 2-way and, where relevant, 3-way interactions
244 between experimental variables. Control variables included were: the continuous effect of “Baseline
245 foraging” (time spent foraging between 0950 and 1030; model 1); the continuous effect of “Baseline
246 consumption” (how much a bird had eaten on the previous day between 1600 and 0100; model 2);

247 the continuous effect of “Tarsus” length, the categorical effect of “Sex” (male or female) and the
248 continuous effect of “Days since last deprivation” (model 3). Two random effects, the individual bird
249 ID and its natal nest were included in all three models.

250 Significance testing was carried out by the likelihood ratio test (LRT), which compares the change in
251 deviance when a term is excluded from the model with the χ^2 distribution with 1 degree of freedom.
252 We assumed a criterion for significance of $P < 0.05$ with 95% confidence intervals (CIs) also shown.

253

254 RESULTS

255 The output of the final models detailing the significance of all fixed effects and interactions are given
256 in Table 2. Findings of relevance to our hypotheses are described below. Note that we also repeated
257 our three models on a subset of data from day 36 onwards and excluded the fixed effect of “Day”.
258 This was to reveal any differences in our outcome variables at the end of the experiment without the
259 need to consider interactions with the “Day” variable. As these results were in-line with our existing
260 model results, they are not reported here.

261 *Foraging time*

262 Figure 2 shows the time spent foraging between 1030 and 1100 (i.e. the period when a cue could
263 occur) over the course of the experiment. If the birds learnt the association between the cue and
264 food deprivation and used this information to adjust their foraging behaviour between 1030 and
265 1100 in anticipation of deprivation, then foraging time during this period should have increased over
266 time for the Predictable group only, on cues days only. However, contrary to the learning
267 hypothesis, the critical 3-way interaction between day, treatment and cue was not significant (Table
268 2). Although Figure 2 suggests that the Predictable group increased their post-cue foraging
269 behaviour over time, this non-significant increase was present on both cue and non-cue days and
270 the interaction between day and treatment was also not significant (Table 2). Alternatively, if the

271 birds had an unlearnt activating response to low ambient light levels, then post-cue foraging time
272 should have been higher from the start of the experiment in both the Predictable and Unpredictable
273 groups. Although there was a significant main effect of cue, contrary to predictions, birds foraged
274 significantly less on days when the cue was present compared to days when it was not present
275 (Figure 2; Table 2).

276

277 *Food Intake*

278 Figure 3 shows food intake between 0950 and 1100 (i.e. the period in the morning prior to when
279 food deprivation could occur) over the course of the experiment. If the birds learnt the association
280 between the cue and food deprivation and used this information to adjust their food consumption in
281 anticipation of deprivation, then morning food intake should have increased over time for the
282 Predictable group only, on cues days only. However, the critical interaction between day, treatment
283 and cue was not significant (Table 2). Alternatively, if the birds had an unlearnt activating response
284 to low ambient light levels, both groups should have immediately increased their food intake in
285 response to the cue. Although there was a significant main effect of cue (Table 2), the starlings ate
286 less food on cue days than they did on days when the cue was not present (Table 2).

287

288 *Dawn Body Mass*

289 Figure 4 shows dawn body condition over the course of the experiment. Birds should respond to
290 unpredictable food deprivation by gaining weight. In line with this prediction, the main effect of
291 experimental day was significant (Table 2) and both groups gained body mass over the course of the
292 experiment. If the birds learnt to use the cue to adjust foraging efforts in anticipation of
293 deprivation, dawn body mass should increase less in the Predictable group, as these birds should
294 have less need to insure against unpredictable food deprivation. However, the critical interaction

295 between treatment and day was not significant (Table 2) and both groups showed similar mass
296 change trajectories (Figure 4).

297

298 DISCUSSION

299 We examined whether European starlings were able to use a light cue to anticipate and prepare for
300 upcoming food deprivation via short-term adjustments to their foraging behaviour and body masses.
301 Our design employed a light cue that either perfectly predicted subsequent food deprivation
302 (Predictable) or provided no information about food deprivation (Unpredictable). Both experimental
303 groups gradually increased their dawn body masses over the course of the experiment in line with a
304 strategic adjustment to the periods of food unavailability experienced by both groups. However,
305 there was no difference in the rate of weight gain between groups, as would have been predicted if
306 the Predictable group learnt anticipatory foraging in response to the cue and hence had less need to
307 insure themselves against starvation at other times. Furthermore, time spent foraging and food
308 intake immediately prior to deprivation actually decreased for both experimental groups on cue days
309 relative to non-cue days. Thus, our results are consistent with the idea that starlings were unable to
310 learn to use the light cue and also, did not have an evolved activating response to low light levels
311 that allowed them to prepare for upcoming periods of food deprivation.

312 We found that food intake and time spent foraging decreased for both experimental groups
313 following exposure to the light cue. This difference was present from the beginning and did not
314 change as the experiment progressed. Our interpretation of this result is that it is likely that there
315 was something intrinsic to the light cue that caused this difference, independent of the information
316 the cue provided. However, it is not clear whether it was the sudden drop in light levels or the low
317 intensity of ambient light itself that decreased foraging efforts for our starlings. Our findings are
318 opposite to a previous experiment that showed that wild-caught house finches (*Haemorhous*
319 *mexicanus*) increased their foraging behaviour in response to lower luminance (Fernández-Juricic &

320 Tran, 2007). Possible explanations for this discrepancy are that in the house finch experiment, high
321 and low light conditions were different compared to our experiment (we used artificial lighting at
322 illuminance levels of 340 lx and 102 lx to simulate sunlight and shade from storm clouds respectively,
323 whereas in the house finch experiment they used natural sunlight and shade, and so not only could
324 absolute levels of illuminance be higher, but also the relative difference between the two
325 conditions). Other explanations include the fact that starling vision may be restricted to a narrower
326 luminance range than for house finches (Martin, 1986), and that wild starlings inhabit more open
327 habitats than house finches and may have evolved to associate light levels, predation risk and thus
328 willingness to forage in a different way (Devereux et al., 2006). Furthermore, the starlings used in
329 our study have lived their whole lives in indoor aviaries with stable, uniform light levels and so the
330 light cue was something highly unusual for them, which could explain the resultant decline in
331 foraging effort.

332 We also found no difference in dawn body mass between treatment groups over time, which is
333 unsurprising given that there was no evidence of anticipatory foraging behaviour in the Predictable
334 group. Both groups increased dawn body masses over time, which is potentially due to both groups
335 being subjected to an environment with unpredictable food availability. We are unable to
336 definitively say that the increase in body mass was due to a variable food supply as no control group
337 (with no food deprivation) was present; however, in another experiment of similar duration where
338 the food supply was kept constant (*unpublished data*), we found that the rate of weight gain in long-
339 term individually caged starlings was only 66% of that reported here. Thus, our results align with
340 previous studies on starlings (Cuthill et al., 2000; Witter et al., 1995) and great tits (*Parus major*)
341 (Bednekoff & Krebs, 1995), that showed increased weight in response to an unpredictable food
342 environment.

343 In addition to the findings we report here, we observed rapid feeding behaviour prior to the removal
344 of the food bowl, which seemed to occur more frequently as the experiment progressed. This

345 behaviour may have been anticipatory as the starlings could have made the association between the
346 presence of an experimenter and food deprivation. If so, one possibility is that starlings were able to
347 anticipate and act to mitigate against upcoming food deprivation, but that they did not learn the
348 intended association with the cue in this experiment. Why this should be so is not clear, as our
349 experiment was designed to offer the optimum conditions for associative learning to take place. We
350 used a combination of delay (light level) and trace conditioning (light reduction). We also prevented
351 other stimuli from blocking or overshadowing the light cue by ensuring it was the only salient
352 stimulus that occurred prior to food deprivation. Our choice of cue was also designed to be
353 ecologically relevant (Garcia & Koelling, 1966), as even though our captive birds had never
354 experienced a sudden reduction in ambient light prior to food deprivation, they had experienced a
355 gradual reduction in light prior to night where food is effectively unavailable to this diurnal species.
356 Similarly, the mean ratio of the signal length to the inter-stimulus interval was 1:88 for our
357 Predictable treatment group, close to the 1:90 ratio that has been used to promote rapid acquisition
358 of conditioned responses with rats (Gallistel, Fairhurst, & Balsam, 2004). Finally, it is unlikely that
359 time of day was used by the birds to adjust their morning feeding behaviour instead of the intended
360 light cue, as we would have expected to see both treatment groups gradually increase their foraging
361 effort over the course of the experiment, regardless of cue presence.

362 Although the current study yielded a negative result, it is possible that a different design would have
363 produced evidence for flexible, short-term, strategic weight adjustment of the type we were hoping
364 to see. For example, maybe we did not train the birds for long enough in the current experiment for
365 them to acquire the desired association, or maybe the light cue was insufficiently salient to the birds
366 (although it did impact their foraging behaviour). Maybe making excess fat more costly (perhaps by
367 increasing cues of predation) would increase the benefits of anticipatory foraging in the Predictable
368 group. Or maybe birds are constrained to learn about some cue other than light (e.g. atmospheric
369 pressure) that predicts periods of food unavailability (Breuner et al., 2013). Further procedural
370 limitations include the fact that although the onset of the cue preceded the consequence, it did not

371 overlap as is usually the case with delay conditioning, which could have negatively affected learning.
372 A possible improvement on our design would be to pair other signals with our light cue to potentiate
373 the informativeness or non-informativeness of our light cue in a discrimination procedure.
374 The question of whether birds can learn cues of future food deprivation therefore needs further
375 investigation before definitive conclusions can be drawn. However, if the current result holds up, it
376 appears that although starlings respond to experienced periods of food deprivation by gaining
377 weight, they may not be able to make rapid anticipatory adjustments in response to learned cues of
378 future food deprivation. The rapid adjustments of foraging behaviour and body fat before a
379 snowstorm observed by Middleton could be explained as a direct response to increased energy
380 expenditure (caused by falling temperatures or increased wind) as opposed to the information
381 provided by putative cues of the upcoming storm. More generally, the mechanism behind strategic
382 fat regulation may be relatively inflexible. This could have important implications for how successful
383 birds will be in the face of rapid environmental change, such as the introduction of artificial street
384 lighting (Navara & Nelson, 2007) and the increased frequency of severe storms predicted to occur
385 due to climate change (Beniston et al., 2007).

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394 DATA ACCESSIBILITY

395 The raw data and R script from this experiment are publicly available at:

396 <https://doi.org/10.5281/zenodo.1193788> (van Berkel et al., 2018).

397 COMPETING INTERESTS

398 We have no competing interests to declare.

399 AUTHORS' CONTRIBUTIONS

400 MVB wrote the first draft of the manuscript; MVB and JD carried out the experiment; MVB, MB and
401 DN conceived the experiment. All authors contributed to the final draft.

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516

517

519 Table 1. Experimental design and schedule of measurements made

Day	Light cue	Deprivation		Measurements			
		Predictable	Unpredictable	Video C	Video N	Food intake	Dawn mass
1	X	X	X			X	
2						X	
3			X			X	X
4	X	X				X	
5			X			X	
6			X			X	X
7	X	X				X	
8	X	X	X			X	
9	X	X				X	X
10			X			X	
11	X	X				X	
12	X	X	X			X	X
13	X	X	X			X	
14			X			X	
15						X	X
16					X	X	
17	X	X		X		X	
18						X	X
19	X	X	X			X	
20	X	X	X			X	
21						X	X
22			X		X	X	
23	X	X	X			X	
24	X	X				X	X
25						X	
26	X	X		X		X	
27			X			X	X
28			X		X*	X	
29						X	
30	X	X				X	X
31					X*	X	
32	X	X	X	X		X	
33						X	X
34			X		X	X	
35						X	
36	X	X	X	X		X	X
37	X	X	X			X	
38			X			X	
39	X	X				X	X
40	X	X				X	
41						X	
42	X	NA	NA	X		X	X

520 Notes: crosses indicate: 1) days on which a light cue was given; 2) days on which five hours of food
521 deprivation occurred; 3) cue days used in video analysis (Video C); 4) non-cue days used in video
522 analysis (Video N); 5) days on which food intake 0950-1100 was measured; and 6) days on which
523 dawn body mass was measured. *days 28 and 31 were used in the video analysis for the Predictable
524 and Unpredictable group respectively. The experiment ended at 1100 on day 42.

525

526

527 Table 2. Model parameter estimates for predictors of foraging time, food intake and dawn body
 528 mass.

Model	Response variable	Random effects	Fixed effects	Estimate	SE	LRT	p-value	CI 2.5%	CI 97.5%
1	Time spent foraging 1030-1100 (mins)	Natal nest / Bird	Day:CuePresent:TreatmentUnpredictable	0.05	1.05	0.002	0.96	-2.02	2.12
			Day:CuePresent	0.26	0.52	0.24	0.62	-0.78	1.29
			Day:TreatmentUnpredictable	-0.30	0.50	0.35	0.56	-1.29	0.70
			CuePresent:TreatmentUnpredictable	12.45	8.13	2.32	0.13	-3.60	28.51
			Day	0.01	0.25	0.003	0.96	-0.49	0.52
			CuePresent	-17.34	4.12	16.60	<0.001	-25.47	-9.21
			TreatmentUnpredictable	7.81	6.36	1.42	0.23	-5.60	21.02
			Baseline foraging	0.31	0.04	46.31	<0.001	0.23	0.39
2	Food intake 0950-1100 (g)	Natal nest / Bird	Day:CuePresent:TreatmentUnpredictable	0.001	0.01	0.01	0.92	-0.02	0.02
			Day:CuePresent	0.03	0.004	0.43	0.51	-0.01	0.01
			Day:TreatmentUnpredictable	-0.01	0.004	3.55	0.06	-0.02	0.0003
			CuePresent:TreatmentUnpredictable	0.13	0.09	2.04	0.15	-0.05	0.30
			Day	-0.003	0.002	1.60	0.21	-0.01	0.001
			CuePresent	-0.20	0.05	19.88	<0.001	-0.29	-0.11
			TreatmentUnpredictable	0.14	0.29	0.24	0.62	-0.47	0.76
			Baseline consumption	0.04	0.01	15.72	<0.001	0.02	0.06
3	Dawn body mass (g)	Natal nest / Bird	Day:TreatmentUnpredictable	0.01	0.01	1.15	0.28	-0.01	0.04
			Day	0.05	0.01	49.34	<0.001	0.04	0.06
			TreatmentUnpredictable	0.10	1.65	0.004	0.95	-3.44	3.60
			Days since last deprivation	0.02	0.06	0.10	0.75	-0.09	0.13
			Tarsus	2.74	0.95	5.78	0.02	0.62	4.71
			SexMale	3.47	1.64	3.81	0.05	-0.01	6.96

529 Fixed effects with p-values ≤ 0.05 are shown in bold.

530

531

532 FIGURE CAPTIONS

533 Figure 1. Schematic of light regime for cue and non-cue days with the deprivation period shown in
534 grey shading. 1) Prior to dawn at 0950 food was removed and exchanged with a new bowl and video
535 recording started. Any weighing occurred at this time. 2) At 1030, the light cue was given or not,
536 depending on the day, and lasted for 30 minutes during which the birds were free to eat ad lib food.
537 3) At 1100, food was either removed or exchanged with a new bowl, depending on whether the
538 birds were deprived that day. Video recording was stopped at this time. 4) At 1600 a new bowl of
539 food was given, regardless of events earlier in the day and birds were free to eat ad lib food until
540 lights off at 0100 (not shown).

541 Figure 2. Mean time spent foraging in the 30 minutes after the time when a cue could be given
542 (1030-1100). Data is shown over time for both a) the Predictable (n=8) and b) Unpredictable (n=8)
543 treatment groups on days when a light cue was present or absent. Note that n = 4 on day 28 and
544 n=4 on day 31 as one of each treatment groups had a deprivation event on the day preceding these
545 two days, preventing us from including all birds in the analysis. The footage for Predictable and
546 Unpredictable groups was analysed on day 28 and 31, respectively. Between-bird SE error bars are
547 shown.

548 Figure 3. Mean food intake in the morning before deprivation could occur (0950-1100). Data are
549 shown over time for both a) the Predictable (n=8) and b) Unpredictable (n=8) treatment groups on
550 days when the light cue was present or absent. Between-bird SE bars are shown.

551 Figure 4. Mean body condition at dawn. Data are shown over time for the Predictable (n=8) and
552 Unpredictable (n=8) treatment groups. Data before day 9 is omitted as it was deemed non-
553 comparable between replicates (pre-dawn weighing did not start until day nine for the first replicate
554 but was undertaken for the entirety of replicate two). Note: data are presented as body condition
555 and not body mass to control for adult tarsus length, a measure of skeletal size. Between-bird SE
556 error bars are shown.

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