Can starlings use a reliable cue of future food deprivation to adaptively modify foraging and fat reserves?

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

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

(Cuthill, Maddocks, Weall, & Jones, 2000; Hudin et al., 2016; Witter, Swaddle, & Cuthill, 1995).

There is empirical evidence that passerine birds not only adjust body masses in response to current starvation risk, but that they also strategically regulate their body fat in anticipation of future starvation risk. Well-studied examples of anticipatory regulation include fat gain prior to winter and nightfall. In winter, food availability and energy expenditure are less predictable than in other seasons, and small birds increase foraging intensity and body fat levels in autumn (King & Mewaldt, 1981; McEwan & Whitehead, 1984; Pienkowski, Lloyd, & Minton, 1979) to buffer against upcoming periods of forced fasting and/or increased energy expenditure (Blem, 1976). Similarly, overnight starvation presents a significant survival risk for many small birds and they increase foraging intensity and body fat levels immediately before dusk to mitigate this risk (Houston, McNamara, & Hutchinson, 1993; Polo & Bautista, 2006; Witter & Cuthill, 1993). Despite their rapidity, short-term changes in fat within a day can be large and comparable to fat changes in winter (Meijer, Möhring, & Trillmich, 1994). For example, in European starlings, *Sturnus vulgaris*, the amount of

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weight gained prior to dusk may represent 6—14% of their total dawn body mass, which is similar to the amount of weight gained prior to winter corresponding to 11% of autumn body mass (Cuthill et al., 2000; Meijer et al., 1994).

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

We have good reason to suspect that birds may be able to make flexible short-term, anticipatory adjustments in this way. In an opportunistic study, Middleton (1982) observed American goldfinches, *Spinus tristis*, flocking to bird feeders in the hour preceding a harsh snow storm. Inclement weather can severely reduce feeding opportunities (Graber & Graber, 1979) and may cause significant mortality to bird populations (Carey & Dawson, 1999). Middleton (1982) showed that the foraging effort of the goldfinches was much greater in the hour before the storm compared to similar time periods on days without storms and that the masses of birds captured during the storm were greater than on the days preceding it. He speculated that an increased mass and a full gut would help protect the birds from starvation and the low overnight temperature. Later work has provided some experimental support for the idea that birds undergo short-term increases in body fat in response to cues of upcoming storms, such as increased rainfall (Kelly et al., 2002), reduced ambient temperature (Krams et al., 2010) and reduced barometric pressure (Breuner, Sprague, Patterson, & Woods, 2013; Metcalfe, Schmidt, Bezner, Guglielmo, & MacDougall-Shackleton, 2013). However, except for barometric pressure, it could be that the increases in body fat observed were responses to the increased energy demands imposed by the meteorological changes themselves as opposed to adjustments in anticipation of increased starvation risk. Furthermore, these studies shed no light on whether birds have acquired knowledge of cues of storms by natural selection or by individual learning.

The aims of the current study were to test experimentally the hypothesis that birds can learn to use an environmental cue to anticipate and prepare for upcoming food deprivation. We used a laboratory experiment to eliminate confounds often present in natural environments. We studied European starlings, since there is strong evidence for body mass regulation in response to laboratory manipulations of food availability and energy expenditure in this species (Bednekoff & Krebs, 1995; Cuthill et al., 2000; Witter et al., 1995). Starlings were exposed to an environment in which food was occasionally (approximately every 2 days on average) unavailable for a period of 5 h. The birds were randomly allocated to two treatment groups. In the Predictable group an environmental cue

perfectly predicted the periods of food unavailability and in the Unpredictable group the same cue was completely uninformative. By only manipulating the informativeness of the cue (via its correlation with subsequent food deprivation), we were able to keep constant the frequency, duration and sequence of food deprivation to ensure that the level of environmental harshness did not differ between treatment groups (cf. Cuthill et al., 2000). The cue that we used was an instant drop in the ambient light intensity that lasted 30 min. This cue was chosen to be an ecologically plausible predictor of storms, since it is possible that birds might be more prepared to learn ecologically relevant cues (Seligman, 1970). Furthermore, the cue was chosen so as not to change the energy expenditure of the birds themselves, to allow us to study true anticipatory fattening as opposed to a direct response to increased energy expenditure. Since the birds that we used were hand-reared in the laboratory (Nettle et al., 2017) and had never been housed outside, they had no exposure to storms, and hence no opportunity prior to the current experiment to learn an association between a sudden drop in ambient light and food unavailability.

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

METHODS

Ethical Note

The study adhered to ASAB/ABS guidelines for the use of animals in research. Birds were taken from the wild under Natural England permit 20121066 and the research was completed under Home Office licence PPL 70/8089, with approval of the local ethical review committee at Newcastle University. After the completion of the current experiment the birds were retained in the laboratory for further studies. At the time of writing, the birds are alive at Newcastle University.

Husbandry and Housing

Subject historical information

Experimental animals were 16 starlings, eight males and eight females, that comprised four families of four siblings. At the time of the current experiment, the birds were 3 years of age. The birds were taken from nests on day 5 posthatching and hand reared to

adulthood as part of a previous study that involved manipulating the amount of food they were given and the effort required to receive it during the nestling period (Nettle et al., 2017). This manipulation was not part of the current investigation and was counterbalanced along with sex between treatment groups of the current experiment.

Room set-up

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

Light regime

The lighting in the room was remotely controlled and consisted of four vertical halogen corner lights and four evenly spaced halogen ceiling lights. During the experiment, the light:dark schedule was set at 15:9 h light:dark. This was identical to the schedule that the birds had been maintained on since fledging, although here dawn and dusk were delayed by 4 h for experimenter convenience. To simulate a dawn, the lights came on at 0950 every morning and increased in intensity every minute until they reached their maximum at 1000 (Fig. 1). In the evening, the lights started dimming to simulate dusk at 0050 and incrementally decreased in intensity until they turned off at 0100.

Experimental Design

Habituation phase

The experiment started with a cage habituation phase of 6 days. By the start of the experimental phase all birds were eating a minimum of 8 g of domestic chick crumb (hereafter referred to as 'food') per day.

Experimental phase

The experimental phase lasted for 42 days during which the birds were subjected to 20 periods of food deprivation. All food deprivation periods lasted for 5 h and, if they occurred, always took place between 1100 and 1600. Days where food deprivation took place were pseudorandomly allocated in a way that ensured all birds were deprived for a total of 20 days, and the distribution of occurrence of 1, 2 and 3 days of consecutive deprivation was identical for both treatment groups (Table 1). The difference between treatment groups came from the informativeness of a light cue preceding the deprivation period. The light cue, when it occurred, was an immediate reduction in light intensity from 100% (340 lx) to 30% for all lights that started at 1030 and ended at 1100 when light intensity was returned to 100% (Fig. 1). The Predictable treatment group always received this light cue prior to food deprivation. The Unpredictable treatment group received the light cue on 10 of the deprivation days (50%) and on 10 nondeprivation days (Table 1). Consequently, the light cue was completely informative for the Predictable group, but completely uninformative for the Unpredictable group, which was thus a 'truly random' control group (Rescorla, 1967, 1988). This design ensured that both treatment groups received near-identical experience of food deprivation but differed in the informativeness of the cue.

Daily Experimental Procedure

Ten minutes before dawn (0950), any remaining food was removed from the cages and exchanged with a fresh bowl of food (Fig. 1). Five minutes before dawn, two cameras were placed on
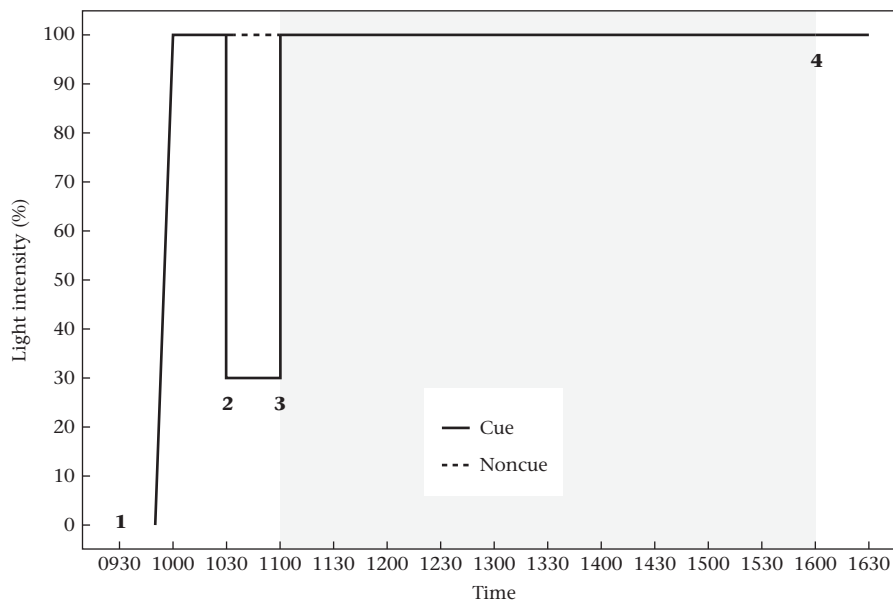
Figure 1. Schematic of light regime for cue and noncue days with the deprivation period shown in grey shading. (1) Prior to dawn at 0950, food was removed and exchanged with a new bowl and video recording started. Any weighing occurred at this time. (2) At 1030, the light cue was given or not, depending on the day, and lasted for 30 min during which the birds were free to eat ad libitum food. (3) At 1100, food was either removed or exchanged with a new bowl, depending on whether the birds were deprived that day. Video recording was stopped at this time. (4) At 1600 a new bowl of food was given, regardless of events earlier in the day and birds were free to eat ad libitum food until lights off at 0100 (not shown).

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

Crosses indicate: (1) days on which a light cue was given; (2) days on which 5 h of food deprivation occurred; (3) cue days used in video analysis (Video C); (4) noncue days used in video analysis (Video N); (5) days on which food intake from 0950 to 1100 was measured; and (6) days on which dawn body mass was measured. The experiment ended at 1100 on day 42.
* Days 28 and 31 were used in the video analysis for the Predictable and Unpredictable group, respectively.

tripods and were positioned facing the cages, 1 m away. Before leaving the room, the experimenter drew a curtain between the cages to reduce social facilitation of foraging behaviour. From dawn to 1030, the birds were free to eat ad libitum food. On cue days (see Table 1) the light cue started at 1030 and lasted for 30 min. At 1100, food was either removed (on deprivation days: Table 1) or otherwise exchanged for a new bowl. Cameras were also removed at this point. At 1600, a new bowl of food was given, regardless of the day. Daily husbandry also took place at this time. From 1600 onwards, the birds were left undisturbed for the remainder of the day and night.

Outcome Variables

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

Foraging time

The amount of time that starlings foraged was recorded via video camera during the 30 min between the time when a light cue could start (1030) and the time when food deprivation could begin (1100). We also recorded this behaviour in the 40 min between dawn (0950) and when the light cue could start (1030) to provide a baseline level of foraging effort for each bird on each day; this measure was used as a control variable in our analyses. Filming took place every day to facilitate habituation and to avoid associations with the presence of the cameras. A representative subset of video footage was analysed for foraging behaviour (Table 1). Video footage from 5 evenly distributed cue days and 4 evenly distributed noncue days was analysed (Table 1; cue days: 17, 26, 32, 36 and 42; noncue days: 16, 22, 28 (Predictable birds only), 31 (Unpredictable birds only) and 34). These days were chosen as there was no deprivation on the preceding days, which reduced the likelihood of any behavioural effects of the deprivation from carrying over into the foraging behaviour recorded on video. Thus, across the entire experiment we analysed 9 days' worth of video footage for each bird. A bird was designated as foraging when the tip of its beak was below the rim of the food bowl.

Food intake

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

Dawn body mass

For this experiment, dawn body mass was measured as a proxy for fat reserves. To obtain a precise measure of body mass maintained outside of the food deprivation periods, the birds were weighed before dawn when the gut was empty. Weighing took place 10–30 min before dawn at the start of each phase and on every third day of the experimental phase. Birds were caught by hand in the dark, placed in a weighing cone and weighed (g) on a digital scale to two decimal places. The first 9 days of experimental data were excluded from the subsequent analysis owing to a change in experimental protocol between replicates (predawn weighing did not start until day 9 for the first replicate but was undertaken for the entirety of the second replicate). Thus, across the entire experiment each bird underwent 12 separate mass measurements. Our analyses of body mass involved three control variables that are likely to have contributed to between- and within-individual variation in mass: tarsus length to control for individual differences in skeletal size; sex, because male starlings are heavier than females; and the number of days since the last period of food deprivation. Tarsus length was measured when skeletal growth was complete (day 56 posthatching) with digital callipers; in the current study we used the mean of two replicate measurements of each leg.

Statistical Analysis

All data analyses were undertaken in R version 3.3.2 (R Core Team, 2016). The raw data files and the R script are available at the Zenodo repository (van Berkel, Bateson, Nettle, & Dunn, 2018). We fitted linear mixed models using the package lme4 (Bates, Maechler, Bolker, & Walker, 2015). Error distribution was Gaussian and all models used in the analysis were checked to satisfy the assumptions of normally distributed residuals and homogeneous variance of residuals across the fitted values of the model. Maximum-likelihood estimation was employed throughout.

We fitted three different linear mixed models, one for each outcome variable. The fixed effects included in each model are given in Table 2. Experimental variables included the continuous fixed effect of 'Day' and the categorical fixed effects of 'Treatment' (Predictable or Unpredictable) and 'Cue' (Present or Not present). We included all two-way and, where relevant, three-way interactions between experimental variables. Control variables included were the continuous effect of 'Baseline foraging' (time spent foraging between 0950 and 1030; model 1), the continuous effect of 'Baseline consumption' (how much a bird had eaten on the previous day between 1600 and 0100; model 2), the continuous effect of 'Tarsus' length, the categorical effect of 'Sex' (male or female) and the continuous effect of 'Days since last deprivation' (model 3). Two random effects, the individual bird ID and its natal nest, were included in all three models.

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

RESULTS

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

Foraging Time

Fig. 2 shows the time spent foraging between 1030 and 1100 (i.e. the period when a cue could occur) over the course of the experiment. If the birds learnt the association between the cue and food deprivation and used this information to adjust their foraging behaviour between 1030 and 1100 in anticipation of deprivation, then foraging time during this period should have increased over time for the Predictable group only, on cues days only. However, contrary to the learning hypothesis, the critical three-way interaction between day, treatment and cue was not significant (Table 2). Although Fig. 2 suggests that the Predictable group increased their postcue foraging behaviour over time, this nonsignificant increase was present on both cue and noncue days and the interaction between day and treatment was also not significant (Table 2). Alternatively, if the birds had an unlearnt activating response to low ambient light levels, then postcue foraging time should have been higher from the start of the experiment in both the Predictable and Unpredictable groups. Although there was a significant main effect of cue, contrary to predictions, birds foraged significantly less on days when the cue was present compared to days when it was not present (Fig. 2, Table 2).

Food Intake

Fig. 3 shows food intake between 0950 and 1100 (i.e. the period in the morning prior to when food deprivation could occur) over the course of the experiment. If the birds learnt the association between the cue and food deprivation and used this information to adjust their food consumption in anticipation of deprivation, then morning food intake should have increased over time for the Predictable group only, on cues days only. However, the critical interaction between day, treatment and cue was not significant (Table 2). Alternatively, if the birds had an unlearnt activating response to low ambient light levels, both groups should have
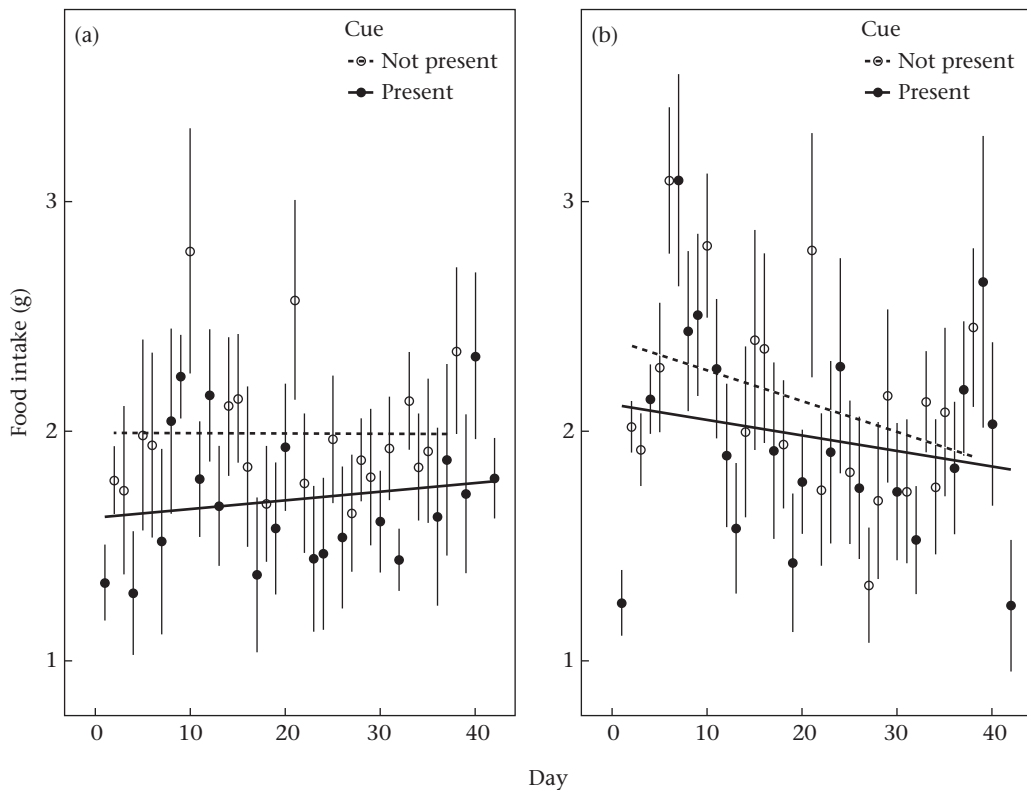
Table 2
Model parameter estimates for predictors of foraging time, food intake and dawn body mass

Model	Response variable	Random effects	Fixed effects	Estimate	SE	LRT	P	CI 2.5%	CI 97.5%
1	Time spent foraging 1030–1100 (min)	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**

Fixed effects with P values ≤0.05 are shown in bold.
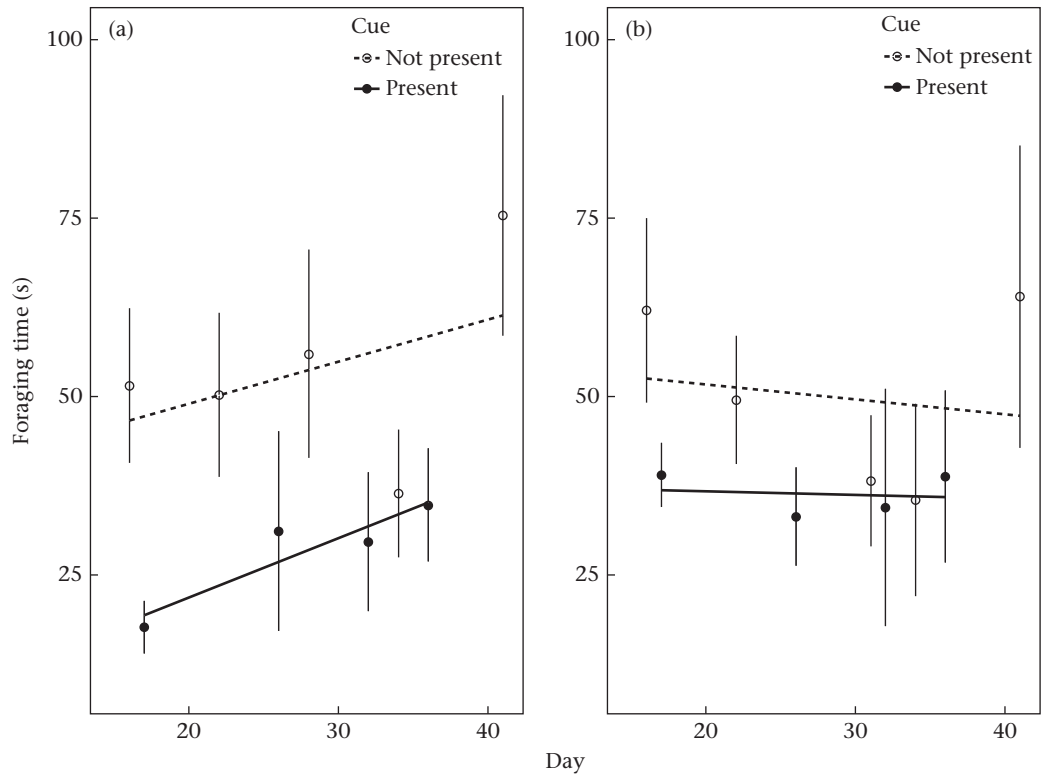
Figure 2. Mean time spent foraging in the 30 min after the time when a cue could be given (1030–1100). Data are shown over time for both (a) the Predictable ($N=8$) and (b) Unpredictable ($N=8$) treatment groups on days when a light cue was present or absent. Note that $N = 4$ on both day 28 and day 31 as one of each treatment groups had a deprivation event on the day preceding these 2 days, preventing us from including all birds in the analysis. The footage for Predictable and Unpredictable groups was analysed on days 28 and 31, respectively. Between-bird SE error bars and regression lines are shown.

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

immediately increased their food intake in response to the cue. Although there was a significant main effect of cue (Table 2), the starlings ate less food on cue days than they did on days when the cue was not present (Table 2).

Dawn Body Mass

Fig. 4 shows dawn body condition over the course of the experiment. Birds should respond to unpredictable food deprivation by gaining weight. In line with this prediction, the main effect of experimental day was significant (Table 2) and both groups gained body mass over the course of the experiment. If the birds learnt to use the cue to adjust foraging effort in anticipation of deprivation, dawn body mass should increase less in the Predictable group, as these birds should have less need to insure against unpredictable food deprivation. However, the critical interaction between treatment and day was not significant (Table 2) and both groups showed similar mass change trajectories (Fig. 4).
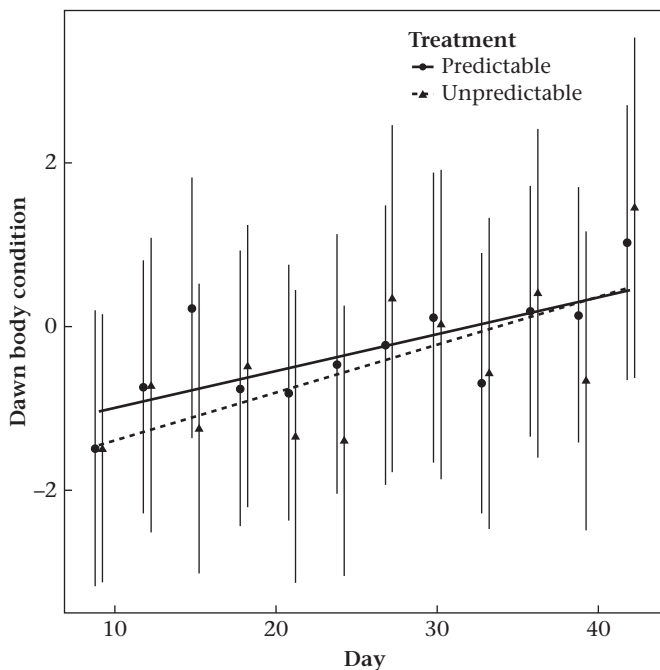
DISCUSSION

We examined whether European starlings were able to use a light cue to anticipate and prepare for upcoming food deprivation via short-term adjustments to their foraging behaviour and body masses. Our design employed a light cue that either perfectly predicted subsequent food deprivation (Predictable) or provided no information about food deprivation (Unpredictable). Both experimental groups gradually increased their dawn body masses over the course of the experiment in line with a strategic adjustment to the periods of food unavailability experienced by both groups. However, there was no difference in the rate of weight gain between groups, as would have been predicted if the Predictable group learnt anticipatory foraging in response to the cue and hence

Figure 4. Mean body condition at dawn over time for the Predictable (N=8) and Unpredictable (N=8) treatment groups. Data before day 9 are omitted as replicates were not comparable (predawn weighing did not start until day 9 for the first replicate but was undertaken for the entirety of the second replicate). Body condition is body mass after controlling for adult tarsus length, a measure of skeletal size. Between-bird SE error bars and regression lines are shown.

had less need to insure themselves against starvation at other times. Furthermore, time spent foraging and food intake immediately prior to deprivation decreased for both experimental groups on cue days relative to noncue days. Thus, our results are consistent with the idea that starlings were unable to learn to use the light cue and did not have an evolved activating response to low light levels that allowed them to prepare for upcoming periods of food deprivation.

We found that food intake and time spent foraging decreased for both experimental groups following exposure to the light cue. This difference was present from the beginning and did not change as the experiment progressed. Our interpretation of this result is that it is likely that there was something intrinsic to the light cue that caused this difference, independent of the information the cue provided. However, it is not clear whether it was the sudden drop in light levels or the low intensity of ambient light itself that decreased foraging effort for our starlings. Our findings are opposite to a previous experiment that showed that wild-caught house finches, *Haemorhous mexicanus*, increased their foraging behaviour in response to lower luminance (Fernández-Juricic & Tran, 2007). Possible explanations for this discrepancy are that in the house finch experiment, high and low light conditions were different compared to our experiment (we used artificial lighting at illuminance levels of 340 lx and 102 lx to simulate sunlight and shade from storm clouds, respectively, whereas in the house finch experiment they used natural sunlight and shade, and so not only could absolute levels of illuminance be higher, but also the relative difference between the two conditions). Other explanations include the possibility that starling vision may be restricted to a narrower luminance range than for house finches (Martin, 1986), and that wild starlings inhabit more open habitats than house finches and may have evolved to associate light levels, predation risk and thus willingness to forage in a different way (Devereux, Whittingham, Fernández-Juricic, Vickery, & Krebs, 2006). Furthermore, the starlings used in our study have lived their whole lives in indoor aviaries with stable, uniform light levels and so the light cue was highly unusual for them, which could explain the resultant decline in foraging effort.

We also found no difference in dawn body mass between treatment groups over time, which is unsurprising given that there was no evidence of anticipatory foraging behaviour in the Predictable group. Both groups increased dawn body masses over time, which is potentially due to both groups being subjected to an environment with unpredictable food availability. We are unable to definitively say that the increase in body mass was due to a variable food supply as no control group (with no food deprivation) was present; however, in another experiment of similar duration where the food supply was kept constant (Andrews et al., 2018), we found that the rate of weight gain in long-term individually caged starlings was only 66% of that reported here. Thus, our results align with previous studies on starlings (Cuthill et al., 2000; Witter et al., 1995) and great tits, *Parus major* (Bednekoff & Krebs, 1995), which increased their weight in response to an unpredictable food environment.

In addition to the findings we report here, we observed rapid feeding behaviour prior to the removal of the food bowl, which seemed to occur more frequently as the experiment progressed. This behaviour might have been anticipatory as the starlings could have made the association between the presence of an experimenter and food deprivation. If so, one possibility is that starlings were able to anticipate and act to mitigate against upcoming food deprivation, but that they did not learn the intended association with the cue in this experiment. Why this should be so is not clear, as our experiment was designed to offer the optimum conditions for associative learning to take place. We used a combination of

delay (light level) and trace conditioning (light reduction). We also prevented other stimuli from blocking or overshadowing the light cue by ensuring it was the only salient stimulus that occurred prior to food deprivation. Our choice of cue was also designed to be ecologically relevant (Garcia & Koelling, 1966), as even though our captive birds had never experienced a sudden reduction in ambient light prior to food deprivation, they had experienced a gradual reduction in light prior to night where food is effectively unavailable to this diurnal species. Similarly, the mean ratio of the signal length to the interstimulus interval was 1:88 for our Predictable treatment group, close to the 1:90 ratio that has been used to promote rapid acquisition of conditioned responses with rats (Gallistel, Fairhurst, & Balsam, 2004). Finally, it is unlikely that the birds used time of day to adjust their morning feeding behaviour instead of the intended light cue, as we would have expected to see both treatment groups gradually increase their foraging effort over the course of the experiment, regardless of cue presence.

Although the current study yielded a negative result, it is possible that a different design would have produced evidence for flexible, short-term, strategic weight adjustment of the type we were hoping to see. For example, maybe we did not train the birds for long enough in the current experiment for them to acquire the desired association, or maybe the light cue was insufficiently salient to the birds (although it did impact their foraging behaviour). Maybe making excess fat more costly (perhaps by increasing cues of predation) would increase the benefits of anticipatory foraging in the Predictable group. Or maybe birds are constrained to learn about some cue other than light (e.g. atmospheric pressure) that predicts periods of food unavailability (Breuner et al., 2013). Further procedural limitations include the fact that although the onset of the cue preceded the consequence, it did not overlap as is usually the case with delay conditioning, which could have negatively affected learning. A possible improvement on our design would be to pair other signals with our light cue to potentiate the informativeness or noninformativeness of our light cue in a discrimination procedure.

The question of whether birds can learn cues of future food deprivation therefore needs further investigation before definitive conclusions can be drawn. However, if the current result holds up, it appears that although starlings respond to periods of food deprivation by gaining weight, they may not be able to make rapid anticipatory adjustments in response to learned cues of future food deprivation. The rapid adjustments of foraging behaviour and body fat before a snowstorm observed by Middleton (1982) could be explained as a direct response to increased energy expenditure (caused by falling temperatures or increased wind) as opposed to the information provided by putative cues of the upcoming storm. More generally, the mechanism behind strategic fat regulation may be relatively inflexible. This could have important implications for how successful birds will be in the face of rapid environmental change, such as the introduction of artificial street lighting (Navara & Nelson, 2007) and the increased frequency of severe storms predicted to occur due to climate change (Beniston et al., 2007).

Authors' Contributions

M.V.B wrote the first draft of the manuscript; M.V.B. and J.D. carried out the experiment; M.V.B., M.B. and D.N. conceived the experiment. All authors contributed to the final draft.

Declaration of Interest

We have no competing interests to declare.

Data Accessibility

The raw data and R script from this experiment are publicly available at: https://doi.org/10.5281/zenodo.1193788 (van Berkel et al., 2018).

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