

2012-06-23

# Water bathing alters threat perception in starlings

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<http://hdl.handle.net/10026.1/3000>

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10.1098/rsbl.2011.1200

Biology Letters

The Royal Society

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11 The majority of bird taxa perform water bathing, but little is known  
12 about the adaptive value of this behaviour. If bathing is important  
13 for feather maintenance then birds that have not bathed should  
14 have poorer feather condition, compromised escape ability and  
15 therefore increased responsiveness to cues of predation. We  
16 conducted two experiments examining the behaviour of captive  
17 starlings responding to conspecific alarm calls. Birds that had no  
18 access to bathing water showed a decreased willingness to feed and  
19 increased their vigilance behaviour following an alarm call. We  
20 argue that birds denied access to bathing water interpreted an  
21 ambiguous cue of threat as requiring more caution than birds that  
22 had access, consistent with higher levels of anxiety. Our results  
23 support the provision of bathing water for captive birds as an  
24 important welfare measure.

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27

28 **Keywords:** bathing, European starling, *Sturnus vulgaris*, threat  
29 perception, animal welfare

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31

32 **1. INTRODUCTION**

33

34 Bathing in water is a trait common to the majority of bird taxa  
35 [1,2], but little research has been conducted into its adaptive value  
36 [1-6]. If bathing is essential for the maintenance of plumage  
37 condition, then we can derive some predictions. Birds that have not  
38 bathed should have impaired flight performance, their escape ability  
39 should be compromised and consequently, they should be more  
40 responsive to signals of predation threat.

41

42 Captive European starlings (*Sturnus vulgaris*) denied access to  
43 bathing water collide with more objects but fly more quickly during  
44 escape flights [7]. Separate experiments have shown that starlings  
45 housed in cages without environmental enrichments (including  
46 bathing water) are more likely to interpret ambiguous stimuli as  
47 indicating a negative future outcome [8,9]. These findings suggest  
48 that lack of access to bathing water may alter threat perception in  
49 starlings. To test this hypothesis more directly, we examined the  
50 behaviour of caged starlings responding to a conspecific alarm call  
51 [10]. This call signals that a predator may be present but it is  
52 ambiguous as to the predator's location or identity. We predicted  
53 that starlings previously denied access to bathing water should take  
54 longer to begin feeding and have elevated vigilance levels on  
55 hearing a conspecific alarm call.

56

57 **2. MATERIAL AND METHODS**

58 We used 20 starlings for experiment 1 and 24 for experiment two.  
59 In both experiments, replicates of four birds were housed  
60 individually in visually isolated cages. Bark-covered kitten food and  
61 drinking water from wall-mounted drinkers were provided ad lib.  
62 [10]. All birds were given a large plastic tray; for half of them this  
63 was filled daily with clean water. Bathing was not directly observed,  
64 but was evinced by wet cage papers and reduced water levels.

65

66 Birds were given three days to settle and then, on a test day,  
67 deprived of food for two hours. The laboratory lights were  
68 subsequently switched off and a bark-filled food bowl containing 10  
69 mealworms (a preferred food) was placed in each cage. The bark  
70 increased the difficulty of the foraging task to induce a foraging-  
71 vigilance trade-off. The experimenter left the room and after 5  
72 minutes an acoustic stimulus was played; on its completion the  
73 lights were switched on and the birds' behaviour recorded on digital  
74 video.

75

76 For both experiments, the acoustic stimulus comprised a starling  
77 alarm call [10] played using an Apple Nano ipod (frequency  
78 response: 20Hz to 20kHz  $\pm$  3dB) and Yamaha YST-M20DSP active  
79 speakers (frequency response: 70Hz - 20kHz  $\pm$  3dB). The sound  
80 pressure level was standardised to a peak amplitude of 75 dB,  
81 measured at the perch in each cage that was furthest from the  
82 speakers (all were equidistant from the speakers). Birds in  
83 experiment 2 were also subjected to a control starling 'threat' call, a  
84 signal given in mild agonistic conspecific encounters. Call types  
85 were presented individually on consecutive days in a  
86 counterbalanced repeated-measures design. Additionally, in  
87 experiment 2 all baths were removed prior to the lights being  
88 switched off to ensure there was no motivation for birds to move in  
89 order to bathe.

90  
91 We scored the following behaviours using The Observer (XT v8.0,  
92 Noldus): latency to move; latency to begin feeding; duration of the  
93 first feeding bout; duration of each period spent with the bill  
94 continuously below horizontal during this bout (head-down bout  
95 duration); the duration of each period spent with the bill  
96 continuously above horizontal during the first feeding bout (head-up  
97 bout duration); the frequency of transition of the bill from below to  
98 above horizontal during the first feeding bout (head-up rate).

99  
100 Unfortunately the birds could not be acoustically isolated and  
101 auditory disturbances occurred both outside and within the  
102 laboratory (e.g. some birds emitted alarm calls in response to the  
103 experimenter). Any birds that experienced such disturbance before  
104 trials or during the trials were excluded. The recordings for two  
105 birds for one of the call-types in experiment 2 allowed latencies to  
106 be scored, but the video quality was not satisfactory for scoring  
107 vigilance. Hence, the final sample sizes were 14 birds for  
108 experiment 1 and 10 for experiment two.

109  
110 To reduce our dependent variables we dropped latency to move  
111 since this was highly correlated with latency to feed (exp 1:  $r =$   
112  $0.530$ ,  $p = 0.051$  [the strength of this correlation was greatly  
113 reduced by the data from one subject]; exp. 2, alarm call:  $r =$   
114  $0.999$ ,  $p < 0.001$ ; exp. 2, 'threat' call:  $r = 0.978$ ,  $p < 0.001$ ). We  
115 entered the remaining measures (transformed to ensure normality)  
116 into a principal component analysis (PCA, using PASW Statistics for  
117 Mac v18.0.3, SPSS Inc.) assuming no rotation (the results also held  
118 under an assumption of orthogonal/oblique relationships between  
119 factors).

120  
121

### 122 3. RESULTS

123 The PCA yielded two factors for both experiments 1 and 2 (table 1).  
124 For experiment 1, we employed a multivariate analysis of variance  
125 with the two factors as the dependent variables. Bathing had a  
126 significant effect on the subjects' behaviour ( $F_{2,11} = 5.503$ ,  $p =$   
127  $0.022$ ; Fig. 1). This was limited to the first factor where bathing had  
128 a large effect as judged by the effect size estimate, Hedges'  
129 unbiased estimator  $d$  [11] (factor 1: no bath group  $\bar{x} = -0.68 \pm$   
130  $0.76$ , bath group  $\bar{x} = 0.68 \pm 0.72$ ;  $F_{1,12} = 11.565$ ,  $p = 0.005$ ,  $d =$   
131  $1.702$ ; factor 2: no bath group  $\bar{x} = -0.09 \pm 1.09$ , bath group  $\bar{x} =$   
132  $0.09 \pm 0.98$ ;  $F_{1,12} = 0.113$ ,  $p = 0.742$ ;  $d = 0.168$ ). Due to the  
133 mixed design in experiment 2, we conducted separate linear mixed  
134 model analyses, using an unstructured covariance matrix, for each  
135 ( $\log_{10}$  transformed) PCA factor (Fig. 2). For factor 1 the minimal  
136 model included significant effects of bathing treatment, acoustic  
137 stimulus type and acoustic stimulus presentation order (table 2).  
138 For factor 2 there was a significant effect of the acoustic stimulus  
139 presentation order, together with a significant interaction effect for  
140 acoustic stimulus type\*acoustic stimulus presentation order (table  
141 2).

142  
143 Bathing appeared to increase factor 1 scores in experiment 1 and  
144 decrease them in experiment 2. However, the factor 1 weightings  
145 for both experiments were qualitatively equivalent (duration of first  
146 feeding bout aside): latency to feed varied positively with the  
147 average duration of each head-up bout but varied inversely with the  
148 head-up rate per minute and the average duration of each head-  
149 down bout (table 1). Hence, bathing had qualitatively the same  
150 effect in both experiments.

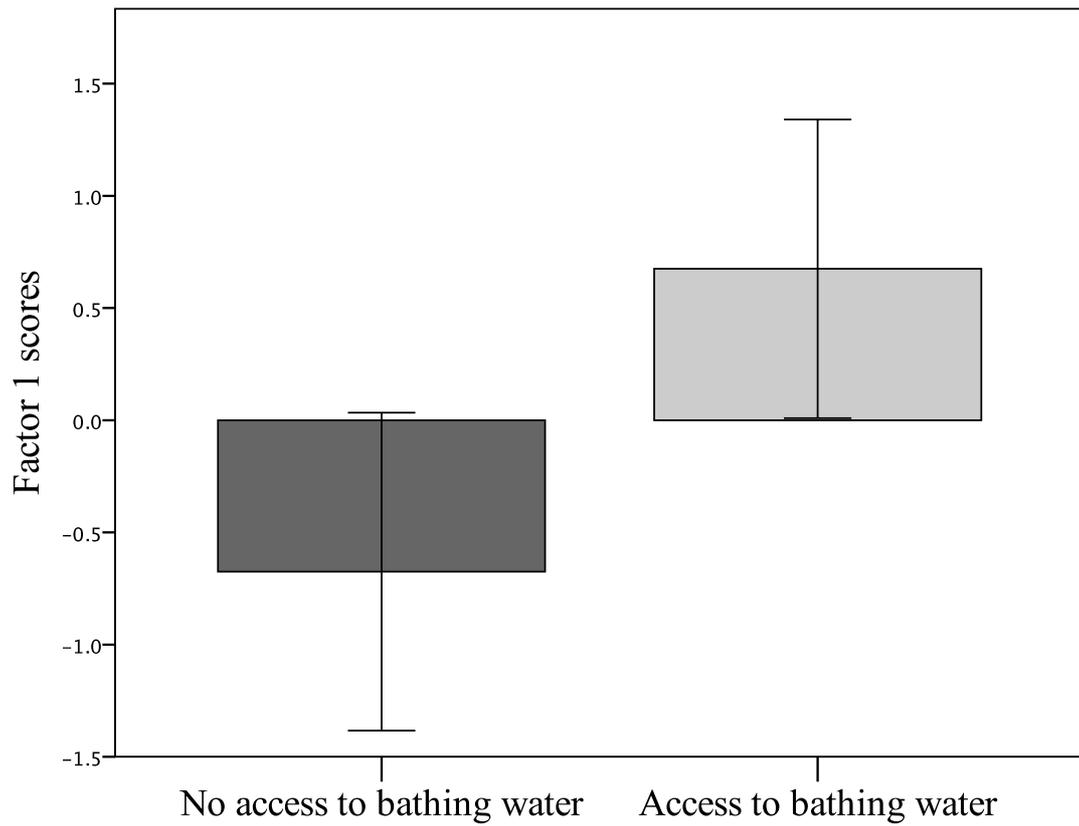
151

### 152 4. DISCUSSION

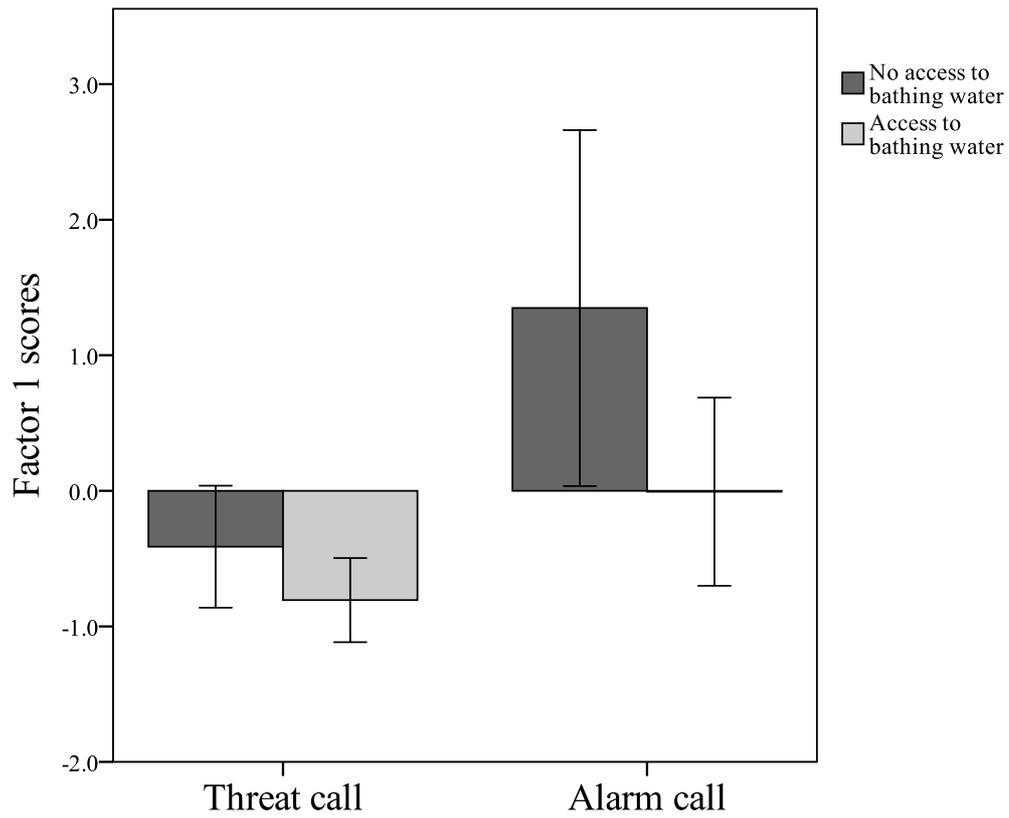
153 Access to bathing water had a large and significant effect on a  
154 behavioural factor that captures sensitivity to threat in captive  
155 starlings. Bathing caused birds to decrease their latency to feed,  
156 decrease the average duration of each head-up scanning bout,  
157 increase the average duration of each head-down feeding bout and  
158 increase their head-up rate. Thus birds given access to bathing  
159 water were more willing to feed in the face of an ambiguous threat  
160 performing shorter, albeit more frequent, vigilance bouts. This  
161 indicates two possibilities: either birds that bathed interpreted the  
162 ambiguous threat signalled by the acoustic stimuli as being less  
163 dangerous; or they were more motivated to move/feed. The latter  
164 is unlikely since all birds were fed ad libitum until the day of testing.  
165 Taking away water baths during testing in experiment 2 also  
166 removed any potential confound of motivation to bathe in the group

Table 1. Principal Component Analysis results for both experiments.

Behavioural measure	Experiment 1		Experiment 2		Effect of bathing on factor 1 (experiment 1/experiment 2)
	Factor 1 loading	Factor 2 loading	Factor 1 loading	Factor 2 loading	
Latency to feed	-0.642	-0.205	0.772	-0.155	↓/↓
Head-up bout duration	-0.688	0.696	0.917	-0.077	↓/↓
Head-up rate	0.836	-0.070	-0.367	0.903	↑/↑
Head-down bout duration	0.823	0.477	-0.392	-0.453	↑/↑
Duration of first feeding visit	0.014	0.918	0.745	0.462	↑/↓



**Figure 1.** Birds with access to bathing water had significantly higher factor 1 scores indicating reduced vigilance in experiment 1. Error bars represent 95% CI.



**Figure 2.** Birds with access to bathing water had significantly lower factor 1 scores indicating reduced vigilance in experiment 2. Note that these are untransformed scores ( $\log_{10}$ -transformed scores were employed for the analysis). Error bars represent 95% CI.

Table 2. Linear mixed model analysis results for experiment 2.

Significant model terms (minimal model) <sup>†</sup>	Factor 1			Factor 2		
	<i>F</i> -ratio (df)	Coefficient estimate <sup>‡</sup>	<i>p</i> -value	<i>F</i> -ratio (df)	Coefficient estimate <sup>‡</sup>	<i>p</i> -value
Bathing	17.062 <sub>(1,7.4)</sub>	-0.221	0.004*	0.004 <sub>(1,7)</sub>	0.002	0.953
Acoustic stimulus type	24.296 <sub>(1,8)</sub>	0.193	0.001*	5.089 <sub>(1,7)</sub>	-0.175	0.059
Bathing*Acoustic stimulus type	0.777 <sub>(1,8)</sub>	0.084	0.404	0.001 <sub>(1,7)</sub>	0.001	0.978
Acoustic stimulus presentation order	14.321 <sub>(1,7)</sub>	-0.147	0.007*	4.455 <sub>(1,7)</sub>	-0.234	0.073
Acoustic stimulus type*Acoustic stimulus presentation order	NS and excluded from model			10.246 <sub>(1,7)</sub>	-0.205	0.015*

<sup>†</sup>Full model included: acoustic stimulus type, bathing treatment and acoustic stimuli presentation order, and all two-way interactions. Terms were removed sequentially by highest *p*-value, but the experimental factors and their interaction were retained. \* indicates significance at the  $\alpha=0.05$  level. <sup>‡</sup>Coefficient comparisons for main effects are given as: no bathing water vs. bathing water; threat call vs. alarm call; alarm call heard second vs. alarm call heard first.

167 given access to bathing water (though no subjects did so during  
168 testing in experiment 1).

169  
170 We argue that birds denied access to bathing water interpreted an  
171 ambiguous cue of threat as requiring more caution than birds that  
172 had access because their ability to cope with threats was impaired.  
173 This is consistent with flight trials [7] which suggested that birds  
174 with no access to bathing water considered escape from potential  
175 threat to be more important than avoiding physical harm from  
176 collisions. We tentatively propose that the findings from both  
177 studies may be due to differences in feather condition caused by a  
178 combination of bathing and preening. In any case, the effect of  
179 bathing must be short-term since bathing water was only removed  
180 for three days and had previously been provided ad libitum.

181  
182 The alarm call elicited a greater defensive response than the 'threat'  
183 call, but the bathing manipulation had a significant effect on the  
184 response to both. A priori we predicted that the 'threat' call would  
185 not be perceived as a sign of imminent physical danger so the  
186 bathing manipulation should have had no effect. There are two  
187 possibilities: either the 'threat' call contains some connotation of  
188 potential harm; or the bathing manipulation more generally  
189 changed the perception of the experimental context. Previous  
190 experiments showed that starlings also respond more defensively to  
191 white noise than to the same 'threat' call [10]. Thus it may be the  
192 experimental context that the birds perceive as ambiguously  
193 threatening, rather than the 'threat' call per se. Further experiments  
194 are required using no acoustic stimulus to address this possibility.

195  
196 European Union legislation regarding laboratory birds advises that  
197 bathing water should be available either constantly or on a regular  
198 basis, depending on the species concerned (revised Appendix A of  
199 ETS 123, Council of Europe Convention). More specific guidelines  
200 exist suggesting the constant provision of shallow water baths for  
201 starlings [12]. This recommendation is based on a notion that  
202 bathing is important for feather maintenance and on the anecdotal  
203 observation that starlings are enthusiastic bathers. However, of 106  
204 research articles featuring captive starlings, only 15 reported  
205 provision of water for bathing [13]. Our findings suggest that when  
206 baths are not provided, starlings may have a continual bias in their  
207 perception of ambiguously threatening situations (e.g. ambient  
208 noises) arising from a perceived increase in their vulnerability to  
209 predation. We therefore hypothesise that long-term lack of access  
210 to bathing water may be a cause of chronic stress and/or anxiety-  
211 like symptoms in captive starlings [14]. However, further  
212 experiments are required in order to demonstrate any potential  
213 long-term impact (e.g. permanent changes in willingness to alarm-

214 call; changes in baseline and stress-induced corticosterone levels).  
215 Whatever the long-term consequences, in the short-term at least,  
216 the provision of bathing water for starlings (and arguably, other  
217 water-bathing bird species) is clearly of welfare importance given  
218 the large effect of bathing water availability on threat perception  
219 that we have demonstrated.

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224 We thank Michelle Waddle, Mark Whittingham, Dan Blumstein and  
225 an anonymous reviewer. This work was supported by a grant  
226 awarded to MB by the Biotechnology and Biological Sciences  
227 Research Council (BB/E012000/1).

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- 230 1 Simmons K.E.L. 1964 Feather maintenance. In: *A new*  
231 *dictionary of birds* (ed. Thomson A.L.), pp. 278-286. New  
232 York: McGraw-Hill.
- 233 2 Slessers M. 1970 Bathing behavior of land birds. *Auk* **87**, 91-  
234 99.
- 235 3 Van Rhijn J.G. 1977 Processes in feathers caused by bathing  
236 in water. *Ardea* **65**, 126-147.
- 237 4 Elowson A.M. 1984 Spread-wing postures and the water  
238 repellency of feathers : a test of Rijke's hypothesis. *Auk* **101**,  
239 371-383.
- 240 5 Oswald S.A., Bearhop S., Furness R.W., Huntley B., Hamer  
241 K.C. 2008 Heat stress in a high-latitude seabird: effects of  
242 temperature and food supply on bathing and nest attendance  
243 of great skuas *Catharacta skua*. *J. Avian Biol.* **39**, 163-169.
- 244 6 Murphy S.M., Braun J.V., Millama J.R. 2011 Bathing behavior  
245 of captive Orange-winged Amazon parrots (*Amazona*  
246 *amazonica*). *Appl. Anim. Behav. Sci.* **132**, 200-210.
- 247 7 Brilot B.O., Asher L., Bateson M. 2009 Water bathing alters  
248 the speed-accuracy trade-off of escape flights in European  
249 starlings. *Anim. Behav.* **78**(4), 801-807.
- 250 8 Bateson M., Matheson S.M. 2007 Removal of environmental  
251 enrichment induces 'pessimism' in captive European starlings  
252 (*Sturnus vulgaris*). *Anim. Welfare* **16**, 33-36.
- 253 9 Matheson S.M., Asher L., Bateson M. 2008 Larger, enriched  
254 cages are associated with 'optimistic' response biases in  
255 captive European starlings (*Sturnus vulgaris*). *Appl. Anim.*  
256 *Behav. Sci.* **109**, 374-383.
- 257 10 Brilot B.O., Normandale C.L., Parkin A., Bateson, M. 2009 Can  
258 we use starlings' aversion to eyespots as the basis for a novel  
259 'cognitive bias' task? *Appl. Anim. Behav. Sci.* **118**, 182-190.
- 260 11 Nakagawa, S., Cuthill, I.C. 2007 Effect size, confidence  
261 interval and statistical significance: a practical guide for  
262 biologists. *Biol. Rev.* **82**,= 591-605.
- 263 12 Hawkins P. et al. 2001 Laboratory birds: refinements in  
264 husbandry and procedures. *Lab. Anim.* **35**, S1-S163.
- 265 13 Asher L., Bateson M. 2008 Use and husbandry of captive  
266 European starlings (*Sturnus vulgaris*) in scientific research: a  
267 review of current practice. *Lab. Anim.* **42**, 111-126.
- 268 14 Bateson M, Brilot, B.O., Nettle, D. 2011 Anxiety: an  
269 evolutionary approach. *Can. J. Psychiat.* **56**(12), 707-715.