

1 **Individual egg camouflage is influenced by**  
2 **microhabitat selection and use of nest materials in**  
3 **ground-nesting birds**

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24 **Abstract**

25 Camouflage is a widespread strategy to avoid predation and is of particular importance  
26 for animals with reduced mobility, or those in exposed habitats. Camouflage often relies  
27 on matching the visual appearance of the background, and selecting fine-scale  
28 backgrounds that complement an individual's appearance is an effective means of  
29 optimising camouflage. We investigated whether there was an active selection of  
30 microhabitats and nest materials in three ground-nesting birds (pied avocet, Kentish  
31 plover and little tern) to camouflage their eggs using avian visual modelling. Plovers  
32 and avocets selected substrates in which their eggs were better camouflaged, and that  
33 choice was done at an individual level. Terns have lighter, less spotted eggs, and while  
34 they did select lighter background than the other species, their eggs were a poor match  
35 to their backgrounds. The worse matching of the tern eggs was likely due to a  
36 compromise between thermal protection and camouflage because they breed later, when  
37 temperatures are higher. Finally, the addition of nest materials improved egg  
38 camouflage in terms of luminance, although the materials reduced pattern matching,  
39 which may be associated with the different roles that the nest materials play. Active  
40 selection of substrates at an individual level may be crucial to improve nest success in  
41 species that nest in exposed sites.

42 **Significance statement**

43 Many bird species nest on the ground at sites with no vegetation cover where their nests  
44 are exposed to visual predators. We studied whether individual females chose nest  
45 substrates that improved the camouflage of their eggs. Using images of nests and avian  
46 vision modelling, we found that the choice of nesting substrates in two species was done  
47 at the individual level, so that egg camouflage was optimised. In addition, such species  
48 were even able to improve egg camouflage by the addition of materials into the nests.  
49 However, this pattern was not observed in another species with paler and less spotted  
50 eggs, which may reflect a trade-off between camouflage and overheating of eggs  
51 because it breeds later in the season. Although individuals may try to choose substrates  
52 in which their eggs are better camouflaged, other factors that compromise offspring  
53 survival may hamper this.

54

55 **Key words:** Crypsis • background matching • habitat choice • eggshells • pattern  
56 matching

**57 Introduction**

58 Evading detection is essential for individual survival, either for protection from  
59 predators or to go unnoticed by potential prey, making camouflage the most common  
60 coloration strategy in the animal kingdom (Stevens and Merilaita 2009). One way to  
61 avoid detection is by matching the background against which animals can be seen,  
62 which means that camouflage involves an interaction between the environment and the  
63 animal's phenotype (Endler 1978; Manríquez et al. 2009; Stevens and Merilaita 2011).  
64 However, habitats are not uniform even at small spatial scales, and this may favour  
65 individuals that choose specific sites in which their camouflage is enhanced according  
66 to the vision of their predators or prey. Many animals are also able to adaptively alter  
67 their phenotypic appearance to match their backgrounds (Ryer et al. 2008; Barbosa et al.  
68 2012; Duarte et al. 2017), meaning that there are two potential main ways for animals to  
69 improve their individual-level camouflage: behaviourally electing microhabitats that  
70 enhance camouflage (Colwell et al 2011; Uy et al. 2017), and/or by using materials  
71 from the microhabitat to cover and decorate the animal's body (Hölldobler and Wilson  
72 1986; Stachowicz and Hay 2000; Hultgren and Stachowicz 2008; Lee et al. 2014;  
73 Mayani-Parás et al. 2015; Ruxton and Stevens 2015). Finally, another solution to  
74 improve crypsis is by manipulating the backgrounds, as for example some birds do  
75 covering the eggs with materials from the surroundings (Amat et al. 2012; Troscianko et  
76 al. 2016a). To date, relatively few studies have addressed whether behavioural selection  
77 of microhabitats and/or substrate modification by individuals in relation to their own  
78 appearance enhances camouflage (but see for example: Solís and de Lope 1995; Lovell  
79 et al. 2013; Kang et al. 2015), and even fewer studies have modelled the relevant visual  
80 systems (Marshall et al. 2016; Stevens et al. 2017).

81           The fitness of any ground-nesting bird depends on its own survival, and on that  
82 of its offspring, meaning that a number of factors influence their anti-predator behaviour  
83 and reproductive strategies (Fontaine and Martin 2006; Amat et al. 2017). Many  
84 Charadriiform birds (shorebirds and allies) nest at ground level in sites with little or no  
85 vegetation cover, because in such sites incubating adults are less vulnerable to predators  
86 (Amat and Masero 2004; Cunningham et al. 2016). However, when adults flush (flee)  
87 from nests because of predator disturbance, unattended eggs may remain vulnerable to  
88 both predation and overheating due to direct solar radiation (Montevecchi 1976; Grant  
89 1982; Amat and Masero 2007; Gómez et al. 2016; Wilson-Aggarwal et al. 2016; Amat  
90 et al. 2017). One way with which ground-nesting birds in exposed sites may improve  
91 nesting success is by matching egg appearance to that of the laying substrates (Lee et al.  
92 2010; Troscianko et al. 2016b). However, this may not be an easy task for shorebirds  
93 that breed in unpredictable sites, compelling the same individuals to breed in sites  
94 located hundreds of kilometres away, not only between breeding seasons, but also  
95 within the same breeding season (Stenzel et al. 1994; Figuerola 2007). In addition, at  
96 such sites shorebirds may encounter a variety of habitats, in which case they may use  
97 different strategies to match the appearance of their eggs with the nesting backgrounds.  
98 First, shorebirds might theoretically improve camouflage by laying eggs with similar  
99 coloration and patterning to those of the sites in which they breed. Given that egg  
100 coloration and patterning seem to be primarily genetically controlled (Gosler et al.  
101 2000; see Fig. 2 in Skrade and Dinsmore 2013), it is unlikely that females may show  
102 plasticity to produce eggs that match the characteristics of their habitat. A different  
103 solution is to select microhabitats according to individual eggshell appearance, so that  
104 the eggs are better camouflaged, as Lovell et al. (2013) found under lab conditions in  
105 Japanese quail, *Coturnix japonica*. These authors concluded that the females were able

106 to “know where to hide their eggs” because they matched the substrate luminance  
107 (perceived brightness) well. However, studies undertaken under natural conditions are  
108 needed, where multiple selective agents may play different roles at the same time  
109 (Underwood and Sealey 2002; Kilner 2006; Cherry and Gosler 2010; Maurer et al.  
110 2011). Additionally, given that it may be difficult to find sites in which the eggs would  
111 be well camouflaged, another possibility for parents is to manipulate the nesting sites  
112 themselves by choosing nest materials that improve the camouflage of the clutch (Solís  
113 and de Lope 1995; Amat et al. 2012; Bailey et al. 2015; Troscianko et al. 2016a).

114         Here, we studied whether nest camouflage is improved through selection of  
115 microhabitat, not only at the species-level, but within species at an individual-level.  
116 Three ground-nesting bird species were chosen, all breeding in a similar habitat where  
117 their nests are exposed: Kentish plover *Charadrius alexandrinus*, pied avocet  
118 *Recurvirostra avosetta*, and little tern *Sternula albifrons*. In addition to species-level  
119 and individual-level matching of egg appearance to microhabitats, we predicted that egg  
120 camouflage should be improved by the addition of materials into the nest scrapes, and  
121 that this should be accomplished both at species- and individual-level. Finally, we  
122 expected that as the three species have to respond to different selective drivers that may  
123 compromise embryo survival (e.g., they may experience different thermal regimes),  
124 such drivers may compromise camouflage (Gómez et al. 2016), which would determine  
125 the apparent maladaptive choice of sites (poorer camouflage) by some species, in spite  
126 of nesting in the same area as the others.

## 127 **Materials and methods**

128

### 129 *Study sites and species*

130 Our study was conducted in 2014 in a 15 ha saltpan at Cádiz Bay Natural Park, southern  
131 Spain (36° 30' 53.4" N 6° 09' 23.3"W). We photographed 30, 37, and 18 nests of pied  
132 avocet, Kentish plover and little tern, respectively. These species make scrapes on the  
133 ground into which they add some materials (e.g., pebbles, mollusc shells, plant  
134 fragments; del Hoyo et al. 1996). It was not possible to record data blind because our  
135 study involved focal animals in the field.

136 At our study site, the pied avocet and the Kentish plover usually start nesting in  
137 late March–early April. The number of pied avocet nests increases rapidly early at the  
138 start of the breeding season and declines sharply; however, the number of Kentish  
139 plover nests increases slowly and continuously until a peak that is usually around mid-  
140 May. On the other hand, the little tern breeds later than the other two species (Fig. S1  
141 Electronic Supplementary material [ESM]), facing more stressful hotter conditions (Fig.  
142 S2 ESM).

143

### 144 *Eggshell colour and camouflage using digital image processing*

145 Protocols for photography and image analysis closely followed previous studies (e.g.  
146 Troscianko and Stevens 2015; Troscianko et al. 2016a). Nests were photographed using  
147 a Nikon D7000 camera (fitted with a 105mm Micro-Nikkor lens, which transmits  
148 ultraviolet, UV) converted to full spectrum sensitivity by removal of its UV and IR  
149 blocking filter (Advanced Camera Services Limited, Norfolk, UK), replacing it with a  
150 quartz sheet to allow quantification of colour throughout the avian visible spectrum.  
151 Human-visible spectrum photographs were taken through a Baader UV-IR blocking

152 filter (Baader Planetarium, Mammendorf, Germany), transmitting only visible spectrum  
153 light from 420 to 680 nm, and UV photographs were taken with a Baader UV pass  
154 filter, transmitting UV light from 320 to 380 nm. This resulted in five image layers:  
155 longwave (LW), mediumwave (MW), shortwave (SW) and two ultraviolet (UV) layers  
156 (from the camera's red and blue channels). Each image included a Spectralon  
157 reflectance standard (Labsphere, Congleton, UK) reflecting light with a flat spectral  
158 reflectance of 40% between 300 and 700 nm. All photographs were taken at f/8,  
159 ISO400, in RAW format between 09:00 and 11:00 h (GMT) to ensure that lighting  
160 conditions were comparable between photographs. The camera was mounted on a tripod  
161 1.6 m high, and positioned at 1m from the focal nest.

162 Images were calibrated following Stevens et al. (2007) and Gómez and Liñán-  
163 Cembrano (2017). Briefly, we linearized and equalized the images using the toolbox  
164 released by Troscianko and Stevens (2015). We processed the images and transformed  
165 them to cone catch images so we were able to model the visual systems. Birds have four  
166 single cone types used in colour vision (sensitive to LW, MW, SW and UV light;  
167 Cuthill 2006), and additional double cones thought to be used in luminance vision  
168 (Osorio and Vorobyev 2005). Because the avocet, plover and tern are likely violet  
169 sensitive (VS) (Ödeen et al. 2010; Ödeen and Håstad 2013), we generated cone-catch  
170 images using peafowl sensitivities (*Pavo cristatus*; Hart 2002; a commonly used model  
171 for VS colour vision). Cone-catch images were generated using a widely used and  
172 tested image mapping approach, which is highly accurate for modelling cone catch  
173 responses (Stevens and Cuthill 2006; Stevens et al. 2007; Pike 2011; Troscianko and  
174 Stevens 2015), resulting in images corresponding to the LW, MW, SW, VS and double  
175 cone receptors. We inferred the visual system of the pied avocet, Kentish plover and  
176 little tern from that of closely phylogenetically related species following Ödeen et al.



177 (2010). These authors did not quantify the absorbance of oil droplets, which may  
178 modify the outcome of models (Bowmaker 1977). However, colour discrimination in  
179 bright light, as in the site where the three species nest, is mostly dependent on the visual  
180 pigment (UVS or VS) and little on the ocular media (Lind et al. 2014).

181         Using Image J (Schneider et al. 2012) we selected three ROIs (regions of  
182 interest): the eggs, the nest, and the microhabitats. We manually selected all of the eggs  
183 that were in the image (1-4). After that, we created a circle that encompassed the eggs  
184 and grew it by 500 pixels to create our nest ROI (eliminating the eggs and including a  
185 small area around them). The remaining part of the picture was selected as the  
186 microhabitat (i.e., after removing the grey standard, nest, and eggs). All images were  
187 scaled using the width of the grey standard (32 mm) as a reference to properly compare  
188 pattern camouflage.

189         In the visual model we calculated luminance differences, colour differences, and  
190 pattern differences. Luminance (based on the double cones) and colour (single cones)  
191 differences were calculated using 'just noticeable differences' based on a widely-used  
192 model whereby receptor noise limits visual discrimination (JNDs; Vorobyev and Osorio  
193 1998). Values of JND lower than 1 could not be distinguished by possible predators  
194 (Siddiqi et al. 2004), between 1- 3, small differences could be appreciated under good  
195 light conditions, and higher than 3 mean that two substrates could be easily  
196 differentiated by predators. Weber fractions were calculated based on peafowl cone  
197 ratios of (shortest to longest) 1 : 1.9 : 2.2 : 2.1 (Hart 2002), with a Weber fraction of  
198 0.05 for the most dominant channel. Pattern differences were calculated from the  
199 average in absolute differences in bandpass energy spectra (for further details see  
200 Troscianko and Stevens 2015; Troscianko et al. 2016a, 2017). Differences were  
201 calculated between eggs and the nest, as well as between eggs and microhabitats. We

202 used the visual system of the three species (avian VS) to model the perception of the  
203 incubating adults and how they select the laying substrates (nest materials and  
204 microhabitats). Although some nest predators have different visual systems to those of  
205 the species that we studied (e.g. gulls, which are UVS), we did not use the visual  
206 systems of nest predators because our study was not on nesting success, but on the  
207 choice of substrates and nest materials by plovers, avocets and terns according to their  
208 own perceptual visual system.

209         To describe how the eggs reflected more or less energy in the visible range of  
210 the sun spectrum we calculated the total reflectance of the eggs, nests and microhabitats  
211 using the reflectance images that were previously obtained (linearised and equalised).  
212 The total reflectance was measured by averaging the three camera bands in the visible  
213 (R-v, G-v and B-v). Moreover, within the egg (placed in the nest as it was projected in  
214 the image, not removing it) we differentiated between the pigmented maculation and the  
215 background colour using a thresholding algorithm. Separating eggs into maculated  
216 regions using simple thresholding inevitably leads to slightly shaded regions of the egg  
217 being erroneously classified as maculation. We therefore used a difference-of-Gaussians  
218 method that removed spatial information at a large scale (the scale of the whole egg),  
219 calculating the difference between this and the fine-scale image, then thresholding this  
220 image (code available by contacting with us). This allows identification of egg  
221 maculation without perfectly diffuse lighting conditions. All eggs were processed with  
222 the same threshold and Gaussian filter scales to ensure consistency. “Proportion of  
223 spottiness” was calculated as the total area of spots divided by the entire eggshell area  
224 and multiplied by 100 (projected in the image).

225

226 *Chimeric experiment*

227 As we could not exchange clutches between nests, we carried out a chimeric experiment  
228 (this process generated 870 chimerical nests of pied avocet, 1332 of Kentish plover and  
229 306 of little tern). To test whether the choice of sites depended on the individual  
230 characteristics of eggs (luminance, coloration and patterning), we virtually placed the  
231 eggs of every entire individual clutch in the nests/microhabitats of the remaining  
232 individuals of the same species (Fig. 1). For example, we “placed” the Kentish plover  
233 eggs of nest 1 in the images of nests and microhabitats of each one of the remaining  
234 Kentish plovers (2-37), after “removing” the eggs of the latter nests.

235

### 236 *Statistical analyses*

237 General linear models (GLM) and General linear mixed models (GLMM) were used to  
238 test the hypotheses, with normal error distribution and using the identity link function.  
239 We used GLM to compare interspecific differences in total reflectance of eggs (all the  
240 eggs in every clutch as a unit) and substrates, and proportion of spottiness (mean of all  
241 the eggs in every clutch). Tukey post-hoc tests were used after for multiple comparisons  
242 between species. GLMMs and Tukey post-hoc tests were used to test differences in the  
243 three response variables related to camouflage (luminance, colour and pattern  
244 differences) among species and substrates (nest materials and microhabitat), with nest  
245 identity included as a random factor. Those response variables were transformed using  
246 the square root to improve residuals' normality. GLMM models were also used to  
247 analyse intraspecific comparisons (see Chimeric experiment), including clutch identity  
248 and substrates (nest and microhabitat) identity as random factors to avoid  
249 pseudoreplication. All statistical analyses were carried out in R statistical software  
250 version 3.2.2 (R Core Team 2015) and significance level was set at 0.05.

251

## 252 **Results**

253 The eggs of the little tern had higher total reflectance (Table 1,  $F_{2,82} = 12.33$ ,  $p < 0.001$ ,  
254 M. R-sq: 0.2311, Adj. R-sq: 0.2124) and were less spotted ( $F_{2,82} = 13.14$ ,  $p < 0.001$ ,  
255 M. R-sq: 0.24, Adj. R-sq: 0.23), than those of the pied avocet and Kentish plover (see  
256 Fig. 2). The nest materials and microhabitats selected by the little terns were in  
257 accordance with the total reflectance values and had higher values than those selected  
258 by the other two species (Table 1).

259 In terms of background matching, the little tern had the least camouflaged eggs  
260 (Fig. 3; see ESM Table S1). In general, its eggs did not match the nest materials and the  
261 microhabitats as well as the other two species, and this was found for the three response  
262 variables (luminance, colour, and pattern, Fig. 3). In fact, little tern's eggs were the most  
263 conspicuous in terms of coloration and they surpassed the threshold for which two  
264 objects would likely be perceptually differentiated (i.e., its colour JND  $> 3$ ). On the  
265 contrary, the pied avocet and the Kentish plover selected nest materials and  
266 microhabitats in which their eggs were better camouflaged.

267 All three species added materials to the nest (e.g. small pebbles, shells, twigs,  
268 etc.). To test whether this addition of materials improved the camouflage of the egg, we  
269 made comparisons between the camouflage of eggs-nests and eggs-microhabitats (Table  
270 2). The eggs of the pied avocet and the little tern were a better achromatic match to the  
271 additional nest materials than the microhabitat. In terms of coloration, the pied avocet  
272 also chose nest materials that more effectively matched their eggs in comparison with  
273 the microhabitat. On the contrary, the Kentish plover's nest materials were a worse  
274 match to the eggs than their microhabitats, while there were no differences in the little  
275 tern. Regarding pattern matching, we found the opposite effects of luminance

276 comparisons: the added nest materials were a worse match to the eggs than the  
277 microhabitats in all three species.

278         Lastly, in the chimeric experiment we found that eggs of individual pied avocets  
279 and Kentish plovers were better camouflaged against their own nest materials and  
280 microhabitats than if the eggs had been laid in the substrates selected by their  
281 conspecifics (Table 3). Differences appeared in the luminance and the chromatic  
282 variables rather than in the patterning, but individual Kentish plover also chose specific  
283 microhabitats that matched the patterning of its own eggs better. On the contrary, the  
284 eggs of the little tern were similarly camouflaged on their own nest as on nests of their  
285 conspecifics, suggesting that they do not select the substrates (either the microhabitat or  
286 the nest materials) at an individual level (Table 3).

287

## 288 **Discussion**

289 Pied avocet and Kentish plover eggs effectively matched the materials that adults added  
290 into the nest scrape, as well as the corresponding microhabitats, under the perceptual  
291 visual system of both species. In fact, the choice of microhabitats and nest materials was  
292 not only undertaken at the species level, but also at the individual level, which may be  
293 particularly important for nest survival (Lee et al. 2010; Troscianko et al. 2016a; but see  
294 Stoddard et al. 2016). This result supports the findings of Lovell et al. (2013) and  
295 Stevens et al. (2017), who found under laboratory and field conditions, respectively,  
296 that individual females selected substrates to lay their eggs based on egg coloration and  
297 patterning to improve camouflage. However, in our study the lighter and less spotted  
298 eggs of the tern did not as effectively match their nest microhabitats and nesting  
299 material, and their camouflage was worse than that of the other two species. Besides,  
300 adults did not choose the substrates based on the individual characteristics of eggs.

301           We found that nest materials did not improve all components of camouflage  
302 similarly. Overall, except in Kentish plover, the other species added materials that  
303 matched better the luminance of the eggs but this in turn decreased their pattern match,  
304 whereas for colour we did not find the same results for the three species (nest materials  
305 increased camouflage in the avocet, worsened it in the plover, but there were no  
306 differences in the tern). Matching the eggs in terms of luminance could be more  
307 advantageous than colour matching to reduce predation rates on eggs of ground-nesting  
308 birds (Troscianko et al. 2016b). However, nest materials may have multiple roles  
309 besides camouflage and they could still be advantageous for other functions, for  
310 example in maintaining an appropriate thermal environment in nests (Reid et al. 2002;  
311 Mayer et al. 2009; Carroll et al. 2015), or they could keep the eggs above water if the  
312 scrape is flooded during rainy periods (Moreno et al. 1995). Therefore, the choice of  
313 optimal nesting materials could be affected by other factors in addition to camouflage  
314 and some of these may represent a compromise similar to the trade-offs known to affect  
315 eggshell coloration (Gómez et al. 2016). With these strategies (selection of  
316 microhabitats and addition of nest materials), together with other behavioural strategies  
317 to reduce egg detection (Amat et al. 2012; Ekanayake et al. 2015, Mayani-Parás et al.  
318 2015; Wilson-Aggarwal et al. 2016; Amat et al. 2017), ground nesting birds could  
319 compensate for the lack of high levels of individual plasticity in egg characteristics  
320 (Gosler et al. 2000; Skrade and Dinsmore 2013) that other organisms exhibit to match  
321 specific habitats (Stevens et al. 2015; Polo-Cavia and Gomez-Mestre 2017; Edelaar et  
322 al. 2017). These behavioural strategies would facilitate the nesting of individual  
323 shorebirds in very distant sites, among which the coloration and pattern of the substrates  
324 may differ substantially, and in particular would facilitate camouflage within habitats  
325 that are visually variable.

326           How do egg characteristics contribute to camouflage in the three species? As  
327 well as in other animals (Ortolani 1999), several studies have shown the importance of  
328 the spottiness for egg camouflage in birds, given that more spotted eggs are better  
329 camouflaged (Montevecchi 1976; Kilner 2006; Gómez et al. 2016; Troscianko et al.  
330 2016). Therefore, the lowest proportion of spottiness of tern eggs, as well as their lighter  
331 eggshells, may be a reason for their inferior camouflage. Interestingly, Stoddard et al.  
332 (2016) reported similar findings to ours, as they found that the camouflage of least terns  
333 *Sternula antillarum* eggs was worse than that of snowy plovers *Charadrius nivosus*.  
334 Obviously, phylogenetic factors could explain interspecific differences in egg coloration  
335 and spottiness, as the avocet and plover are more closely related (Baker et al. 2007).  
336 However, solar radiation could affect egg appearance not only at congeneric levels  
337 (Gómez et al. 2016) but also at a population level (Lathi 2008). Nevertheless,  
338 accounting for phylogenetic factors would likely not explain why little terns selected  
339 substrates that matched their eggs poorly, given that avoiding nest detection is so  
340 important for egg survival (Lee et al. 2010; Troscianko et al. 2016b). A plausible  
341 explanation could be related to the more stressful thermal conditions that the little tern  
342 presumably faces during its breeding season (ESM Fig. S2), so having lighter and less  
343 spotted eggshells, together with selecting lighter substrates, would be advantageous to  
344 reduce risks of overheating when the nests are left unattended by adults, even if this  
345 compromises egg camouflage (Montevecchi 1976; Mayer et al. 2009; Gómez et al.  
346 2016). Additionally, it has been shown that little tern may adjust the distance at which  
347 the adults flush from the nest when a predator is approaching depending on the level of  
348 egg camouflage (Amat et al. 2017), so this behaviour may compensate for the poorer  
349 matching and reduce nest detection.

350 Our results support the findings of Lovell et al. (2013) on domestic quail, in that  
351 the selection of laying substrates was undertaken at an individual level according to the  
352 characteristics of eggshells, at least for Kentish plover and pied avocet. Our results are  
353 also in accordance with those of Stevens et al. (2017), which showed that individual  
354 ground-nesting birds improved the camouflage of their eggs by choosing appropriate  
355 backgrounds. They found differences among species, as we have found in this study,  
356 which indicates the importance of studying egg camouflage and habitat selection in  
357 different species and habitats to gain insights into the strategies used by different  
358 species. On the other hand, adding nest materials to the scrapes improved the  
359 achromatic camouflage but worsened the pattern camouflage of the eggs, which could  
360 be explained because the materials added are likely important for other functions in  
361 addition to camouflage. The apparent need to respond to other selective drivers that may  
362 compromise embryo survival, such as the risk of overheating, may explain why the  
363 camouflage of little tern eggs was worse than that of the other species. This highlights  
364 the importance of carrying out studies with wild species that face different stressful  
365 factors.

366

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372

### 373 **Author contribution statement**



374 JG designed the study and analysed the images. JT and MS contributed with materials  
375 and image analyses. JG, MC, AP-H, CR and JAA collected the field data. JG analysed  
376 the dataset, and wrote the manuscript. All authors contributed on later manuscript  
377 versions.

378

### 379 **Compliance with ethical standards**

380

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387

### 388 **Conflict of interest**

389 The authors declare that they have no conflict of interest.

390

### 391 **Ethical approval**

392 All applicable institutional and national guidelines for the care and use of animals were  
393 followed and approved by Comité Ético de Bienestar Animal from EBD-CSIC  
394 (reference CEBA-EBD\_2011\_01).

395

### 396 **Data availability**

397 All relevant data are available from the CSIC Institutional Data  
398 (<https://digital.csic.es/handle/10261/167693>).

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570 **Table 1** Apparent reflectances (estimated means  $\pm$  standard errors) of eggshells, nests  
 571 and microhabitats of pied avocet (A, n = 30), Kentish plover (Kp, n = 37) and little tern  
 572 (Lt, n = 18). P-values (p) were calculated with a Tukey post-hoc test to the results of  
 573 different general linear models (significant p-values are in bold)  
 574

	Total Reflectance (%)		
	EGGS	NESTS	MICROHABITAT
Pied Avocet	11.35 $\pm$ 0.53	14.39 $\pm$ .91	13.88 $\pm$ 0.92
Kentish plover	11.30 $\pm$ 0.48	16.74 $\pm$ 0.95	15.99 $\pm$ 0.95
Little tern	15.92 $\pm$ 0.97	22.80 $\pm$ 1.86	22.95 $\pm$ 1.96
p	Kp - A = 0.998	Kp - A = 0.184	Kp - A = 0,240
	<b>Lt - A &lt; 0.001</b>	<b>Lt - A &lt; 0.001</b>	<b>Lt - A &lt; 0.001</b>
	<b>Lt - Kp &lt; 0.001</b>	<b>Lt - Kp = 0.007</b>	<b>Lt - Kp = 0.002</b>

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580 **Table 2** Model-adjusted means ( $\pm$ SE) of differences in camouflage (luminance, colour  
581 and pattern) between the eggs and the nest materials and the eggs and the microhabitats,  
582 and p-values (Tukey post-hoc test) for pied avocet (n = 30), Kentish plover (n = 37) and  
583 little tern (n = 18) according to the peafowl visual system. Significant p-values are in  
584 bold  
585

		586		
		Luminance	Colour	Pattern <sup>587</sup>
Pied Avocet	Egg-nest	0.39 $\pm$ 0.06	1.67 $\pm$ 0.25	5100 $\pm$ 317
	Egg-microhabitat	0.48 $\pm$ 0.06	1.82 $\pm$ 0.26	3488 $\pm$ 262
	p	<b>0.002</b>	<b>0.047</b>	<b>&lt;0.001</b>
Kentish plover	Egg-nest	0.62 $\pm$ 0.05	2.43 $\pm$ 0.27	4725 $\pm$ 275
	Egg-microhabitat	0.61 $\pm$ 0.05	2.24 $\pm$ 0.26	3907 $\pm$ 250
	p	0.750	<b>0.017</b>	<b>&lt;0.001</b>
Little tern	Egg-nest	0.61 $\pm$ 0.07	4.26 $\pm$ 0.51	6847 $\pm$ 399
	Egg-microhabitat	0.71 $\pm$ 0.07	4.45 $\pm$ 0.52	4948 $\pm$ 403
	p	<b>0.004</b>	0.196	<b>&lt;0.001</b>

588 **Table 3** Results of intra-specific comparisons for pied avocet, Kentish plover and little  
589 tern in a chimeric experiment (see material and methods). Model-adjusted means ( $\pm$ SE)  
590 of differences in camouflage (luminance, colour and pattern) between egg-nest and nest-  
591 microhabitat (GLMM) according to the peafowl visual system, and p-values (Tukey  
592 post-hoc test) are shown. Significant p-values are in bold

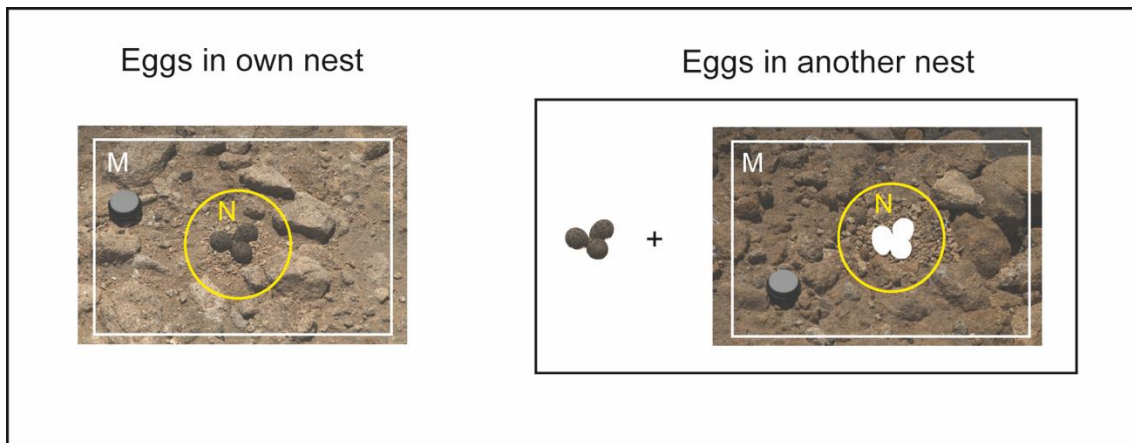
593

		Pied avocet		Kentish plover		Little tern	
		Original nest	Chimeric nest	Original nest	Chimeric nest	Original nest	Chimeric nest
Luminance	Egg-nest	0.39 $\pm$ 0.056	0.57 $\pm$ 0.049	0.62 $\pm$ 0.051	0.69 $\pm$ 0.044	0.61 $\pm$ 0.073	0.63 $\pm$ 0.063
		<b>p &lt; 0.001</b>		<b>p = 0.007</b>		p = 0.56	
	Egg-microhabitat	0.48 $\pm$ 0.057	0.59 $\pm$ 0.050	0.61 $\pm$ 0.051	0.68 $\pm$ 0.044	0.71 $\pm$ 0.073	0.74 $\pm$ 0.063
		<b>p &lt; 0.001</b>		<b>p = 0.006</b>		p = 0.57	
Colour	Egg-nest	1.53 $\pm$ 0.200	1.91 $\pm$ 0.220	2.10 $\pm$ 0.247	2.39 $\pm$ 0.248	3.76 $\pm$ 0.631	3.97 $\pm$ 0.59
		<b>p &lt; 0.001</b>		<b>p = 0.024</b>		p = 0.515	
	Egg-microhabitat	1.68 $\pm$ 0.220	2.00 $\pm$ 0.230	1.87 $\pm$ 0.220	2.34 $\pm$ 0.242	4.07 $\pm$ 0.684	4.05 $\pm$ 0.604
		<b>p = 0.006</b>		<b>p &lt; 0.001</b>		p = 0.953	
Pattern	Egg-nest	5100 $\pm$ 359	5102 $\pm$ 320	4680 $\pm$ 312	4828 $\pm$ 281	6847 $\pm$ 537	6917 $\pm$ 482
		p = 0.990		p = 0.322		p = 0.783	
	Egg-microhabitat	3488 $\pm$ 297	3607 $\pm$ 269	3878 $\pm$ 284	4195 $\pm$ 262	4949 $\pm$ 457	5119 $\pm$ 415
		p = 0.398		<b>p = 0.020</b>		p = 0.439	

600 FIGURE LEGENDS

601

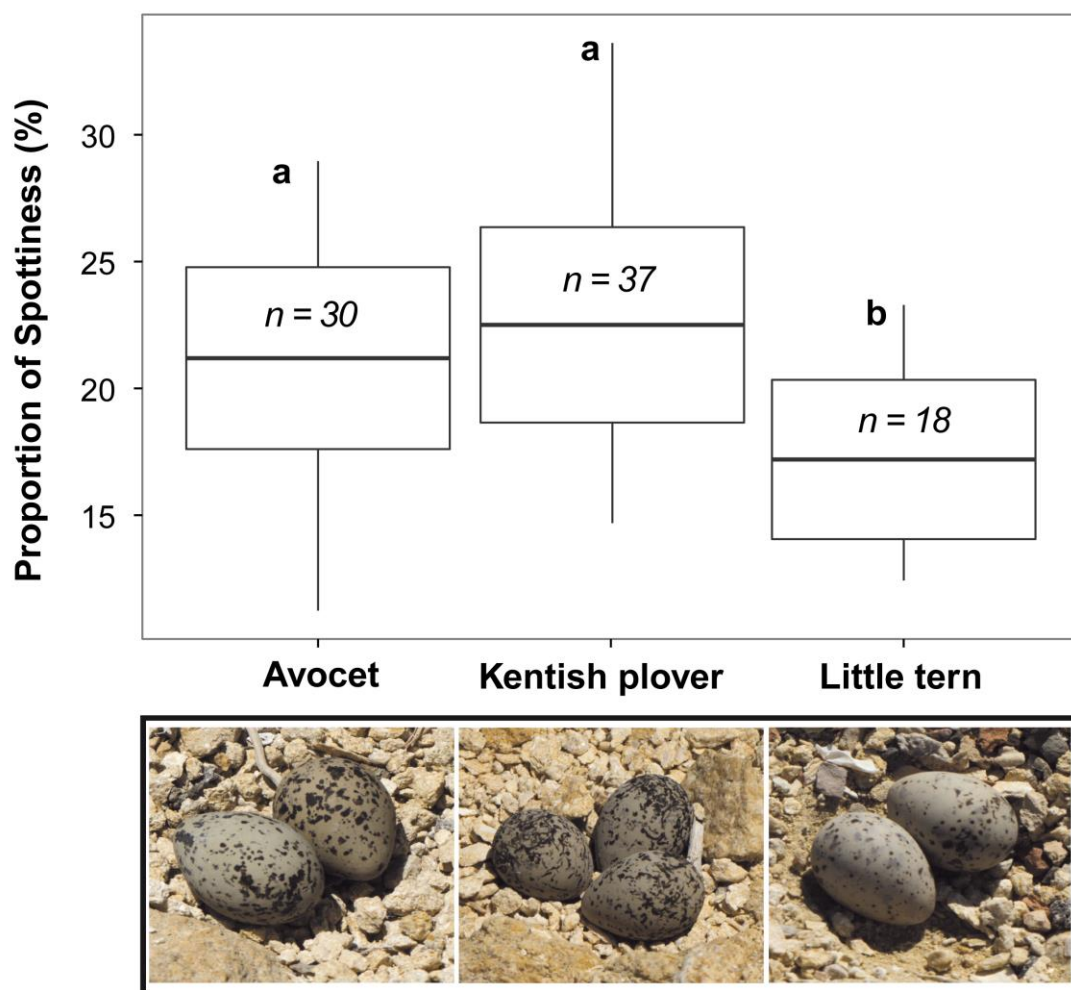
602 **Fig 1** Diagram representing the procedure employed for the chimeric experiment using  
603 photographs of nests of pied avocet (n = 30), Kentish plover (n = 37) and little tern (n =  
604 18). The eggs of every clutch were virtually deposited in the nests of all other  
605 conspecifics. The camouflage of eggs was estimated with respect to the nests (N) and  
606 microhabitats (M). A grey standard was placed in every picture to standardise light  
607 conditions, but was not included in the microhabitat area in the analyses of digital  
608 images



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610

611 **Fig 2** Proportion of spottiness (percentage of eggshell surface covered by spots,  
612 average of the eggs in every clutch) in three ground-nesting birds (sample mean, 95%  
613 confidence intervals, and minimum and maximum values; sample sizes are also shown).  
614 The images were linearised and standardised, using a grey standard, to control for the  
615 lighting conditions. After obtaining a reflectance image, contrast and exposure were  
616 changed equally for the three images to improve visualisation. Different letters denote  
617 significant differences



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620 **Fig 3** Differences in achromatic (luminance), chromatic (colour) and pattern  
621 camouflage between the eggs and the nest (left) and between the eggs and the  
622 microhabitats (right), in pied avocet (n = 30), Kentish plover (n = 37) and little tern  
623 (n=18), under the peafowl visual model. The higher the values (estimated means  $\pm$  SE)  
624 the worse the camouflage. Values of differences lower than 1 (dashed line) in luminance  
625 and colour could not be distinguished by possible predators, between 1 - 3, small  
626 differences could be appreciated under good light conditions and higher than 3 (solid  
627 red line) mean that two substrates could be easily differentiated. Different letters denote  
628 significant differences

