

25 Abstract

26 In ground-nesting birds egg colour and appearance may have evolved due to opposite
27 selection pressures. Pigmentation and spottiness make the eggs darker and have been
28 suggested to improve camouflage. However darker and more spotted eggs may reach
29 higher temperatures when not attended by adults and receiving direct sunlight, which
30 may be lethal for embryos. Some authors suggested that this trade-off may not exist
31 because eggshell pigments mainly reflect in the infrared region of the solar spectrum,
32 but have not considered that wavelengths in the visible part of the spectrum may also
33 contribute to overheating. To test the occurrence of a trade-off between camouflage and
34 overheating of eggs, we took digital images to analyse colour and camouflage in 93
35 nests of four shorebird species (two stilts and two plovers) in two regions (tropical and
36 mediterranean sites). We predicted that these species (closely related) may have evolved
37 different eggshell designs depending on solar radiation, which is supposed to be
38 stronger in the Tropics. To record egg temperatures, we placed Japanese quail eggs in
39 natural nests of shorebirds, and registered temperatures using a datalogger. We found
40 that darker and more spotted eggs reached higher temperatures than lighter ones, and
41 that after controlling for environmental temperatures, eggs overheated more in the
42 Tropics, likely because of a more intense solar radiation. We also found that tropical
43 shorebirds' eggshells have darker spots and lighter backgrounds. Overall, darker eggs
44 were better camouflaged. Taken together, our results show that the benefits of
45 increasing pigmentation of eggshell backgrounds and spottiness for a better camouflage
46 are counteracted by the increased risks of overheating when eggs remain exposed to
47 direct solar radiation.

48

49 Key words

50 background camouflage, crypsis, egg temperatures, eggshell pigmentation, nests

51

52

53 **Introduction**

54

55 Solar radiation is a very important factor in animal ecology, determining not
56 only the colour with which animals are perceived, but also, depending on its intensity,
57 the amount of radiation absorbed by an individual (Clusella-Trullas et al. 2009, Arenas
58 et al. 2014). Colouration is important to explain evolution by natural (e.g. concealment
59 from predators) and sexual (e.g., communication) selection. As colouration is related to
60 the amount of energy absorbed or reflected at different wavelengths, body temperatures
61 may be affected by body colour. The evolution of colouration as an adaptation to cope
62 with temperature is likely when differences in body temperature lead to fitness
63 advantages (Umbers et al. 2013).

64 Many bird species nest on the ground in sites without cover in which the eggs
65 remain exposed to direct solar radiation when the nests are unattended. At high ambient
66 temperatures ($> 30\text{ }^{\circ}\text{C}$) the eggs may reach critical temperatures for embryogenesis if
67 they are unattended for a few minutes (Grant 1982, Webb 1987, Amat and Masero
68 2004a, Amat and Masero 2007), or the adults may face hyperthermia while incubating
69 (Grant 1982, Amat and Masero 2004a, Amat and Masero 2009), which may result in
70 lowered fitness because nests may be deserted (Salzman 1982, Amat and Masero
71 2004a). Why birds do not nest in the shade to avoid the risk of adverse high
72 temperatures may seem puzzling, given that covered sites may be readily available.
73 However, incubating adults detect more easily approaching predators from exposed than

74 from covered sites, thus incurring a lower risk of being themselves depredated (Grant
75 1982, Maclean 1984, Koivula and Rönkä 1998, Amat and Masero 2004b).

76 Nesting in exposed sites poses another problem to ground nesting birds beyond
77 the costs of hyperthermia that incubating adults have to face. Namely, eggs may be
78 more easily detected by predators when the nests remain unattended. To overcome this,
79 the eggs of ground nesting birds are usually cryptically coloured (Underwood and
80 Sealey 2002, Kilner 2006). A way in which crypsis may be achieved is by laying
81 spotted eggs with pigmented backgrounds (Kilner 2006, Cherry and Gosler 2010).
82 Variations in pigmentation according to substrate colour have been documented in
83 several animals (Blanco and Bertellotti 2002, Sánchez et al. 2004, Hargeby 2005,
84 Morgans and Ord 2013, Kang et al. 2014, Stevens et al. 2014), a strategy with which
85 predation risk is reduced (Lloyd et al. 2000, Lee et al. 2012, Skrade and Dinsmore
86 2013).

87 Not only does solar radiation possibly act as a stressor for birds incubating in
88 exposed sites, but it may also have driven the evolution of colour and degree of
89 spottiness of their eggs (Lathi 2008, Maurer et al. 2011). This is likely because,
90 according to the colour-mediated heating hypothesis, more pigmented animals (i.e. with
91 lower brightness) heat quicker than less pigmented ones when receiving direct solar
92 radiation (Heath 1975, Montevecchi 1976, Clusella-Trullas et al. 2009, Geen and
93 Johnston 2014). Therefore, as Montevecchi (1976) proposed, due to conflicting selective
94 pressures, ground nesting birds may have to trade-off the pigmentation of their eggs,
95 with predation favouring pigmentation and overheating opposing pigmentation.

96 Two are the main pigments that produce the great variety of eggs designs:
97 protoporphyrin and biliverdin (Kennedy and Vevers 1976). The first molecule produces
98 the red-brown tones in the background of the eggs and is the main constituent of the

99 spottiness of ground-nesting bird eggs (Kennedy and Vevers 1976; Mikšík et al. 1996).
100 On the other hand, biliverdin is responsible of blue-green colour of the eggs and is
101 found in a lower proportion in eggs of those species. The experiments conducted so far
102 to demonstrate the effect of egg colour on egg temperature have not used naturally
103 coloured eggshells. Instead, eggshells were artificially painted (Montevecchi 1976,
104 Magige et al. 2008), which may not reflect adequately the thermal properties of eggs
105 (Underwood and Sealy 2002, Kilner 2006). Bakken et al. (1978) hypothesized that the
106 protoporphyrin pigment found in eggs of many avian species, contrary to other
107 pigments like melanins, reflect high rates of infrared wavelengths, thus reducing the risk
108 of overheating. The results of this study may have led other authors to incorrect
109 conclusions, because other parts of the spectrum (as the visible) could also be important
110 for overheating. Moreover there is a study by Westmoreland et al. (2007) that have
111 diminished the importance of the colour-mediated heating hypothesis. They tested
112 experimentally the effects of colouration on overheating using natural eggs of three
113 species of blackbirds (tree-nesting birds that use cup-nests) and they did not find an
114 effect of pigmentation on egg temperatures. Yet, in such experiment the eggs were
115 exposed to direct solar radiation during one hour, a period that normally may exceed the
116 periods that adults naturally spend outside their nests and during which egg
117 temperatures could reach equilibrium, independently of their pigmentation (see
118 Discussion). Here, to analyse whether there is a trade-off in pigmentation in ground
119 nesting birds, we used an approach suggested by Kilner (2006). Namely, we (1)
120 compared the proportion of eggshell spottiness and egg background colour in two pairs
121 (each pair from the same genus) of shorebird species (Charadrii) nesting in
122 environments differing in solar radiation, and then (2) analysed if egg colour affected
123 temperatures and camouflage. For testing how eggshell colouration affects overheating

124 and model eggshell temperatures we used Japanese quail eggs. We predicted that darker
125 eggs (i.e. lower brightness) should be better camouflaged, but should also reach higher
126 temperatures when exposed to direct sunlight. In addition, for a same colouration,
127 overheating should be higher in the site with more intense solar radiation.

128

129 **Materials and methods**

130

131 *Study sites, species and field protocols*

132

133 Our study was conducted in Costa Rica and Spain in 2010 and 2011 (Sites, see
134 supplementary material). In Costa Rica we studied Wilson's plover *Charadrius wilsonia*
135 and black-necked stilt *Himantopus mexicanus*, and in southern Spain Kentish plover
136 *Charadrius alexandrinus* and black-winged stilt *Himantopus himantopus*. All are
137 ground nesting species, which make scrapes into which they add materials (e.g.,
138 pebbles, mollusc shells, plant fragments). Modal clutch sizes are 3 for the plovers and 4
139 for the stilts (Colwell 2010).

140 We usually found the nests by watching the adults when they returned to their
141 nests after having been flushed. Once we arrived at the nests we took a photo (with a
142 resolution of 3888×2592 pixels), on which we measured later eggshell colour and
143 spottiness. We used a Canon EOS 450D camera, equipped with Canon EFS 18-55 mm
144 macrozoom lens. We took photographs approximately 50 cm above the nests with the
145 white balance set manually. Images were taken under sunny conditions, between
146 9:00–11:00 h, and were standardized using a white balance (Lastolite Ezybalance, 30
147 cm).

148 We used Japanese quail *Coturnix japonica* eggs (n = 11) to record temperatures
149 between 12:00 – 15:00 h in empty nests of the four shorebird species. Quail eggs are
150 protoporphyrin-based, spotted and of similar size to shorebird eggs, but intraspecific
151 variation in pigmentation and spottiness is larger than in shorebird eggs.

152

153 *Eggshell colour and spottiness*

154

155 We quantified the colour of eggshells (EG), nests (N) and nest surroundings (S),
156 as well as the degree of eggshell spottiness. For this, we used Adobe Photoshop CS4
157 (Adobe, San Jose, CA, USA). In the eggshells we recorded the colour of both spots and
158 backgrounds, for which we used the eyedropper tool in Photoshop, which was set at 51
159 × 51 pixels with a resolution of 72 pixels/inch. Values were recorded in both RGB (red,
160 green, blue) and $L^*a^*b^*$ (L = lightness, a = red/green, b = yellow/blue, CIE) colour
161 spaces (Hunt and Pointer 2011). RGB values vary from 0 (darkest) to 255 (lightest),
162 thus higher values mean lighter (i.e. brighter) colours. We took readings at spaced
163 points on the images, noting whether the readings were on EG (spots or background), N
164 and S. Five values were recorded for every category, which were averaged. The
165 proportion of surface covered by spots (proportion of spottiness) in eggshells (see
166 Figure S1, supplementary material) was quantified also in Adobe Photoshop CS4 using
167 the histogram palette. For this, we selected an area of 250 x 250 pixels in one of the
168 eggs of each nest chosen randomly. Then, we selected the area covered by the spots
169 using a mask threshold by luminosity and recorded the number of pixels covered by
170 spots. By inverting the selection, we recorded the same parameters in the eggshell area
171 not covered by spots, which is called the background. Egg spottiness was estimated as

172 the area (pixels) covered by spots relative to total sampled eggshell area (62500 pixels),
 173 and expressed as a proportion.

174

175 *Camouflage*

176

177 The degree of camouflage was estimated by quantifying colour differences between two
 178 substrates, for which we used the equation:

$$179 \quad \Delta E = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2} \quad (\text{Nguyen et al 2007, Hunt and Pointer 2011}).$$

180 The larger ΔE , the lower the similarity in colouration between the substrates. We chose
 181 the $L^*a^*b^*$ colour space of the Commission International de l'Eclairage to quantify
 182 differences because it closely approximates and linearly correlates with human vision
 183 (Stevens et al. 2007, Lovell et al. 2013). We made three types of comparisons: between
 184 EG and N (ΔE_{EG-N}), EG and S (ΔE_{EG-S}), and N and S (ΔE_{N-S}).

185

186 *Temperatures*

187

188 Quail eggs (n = 11) were emptied and filled with plaster of Paris, which has a
 189 thermal conductivity very similar to that of natural eggs (Ward 1990). We inserted 30-
 190 36 gauge nickel-chromium/nickel-aluminum thermocouple probes (Omega
 191 Engineering, Inc., Stamford, CT, USA) into the model eggs.

192 The quail eggs were placed in 138 empty shorebird nests (46 in tropical sites and
 193 92 in mediterranean sites). We placed two model quail eggs in each nest, one little
 194 spotted (light egg) and another heavily spotted (dark egg), which were not in contact
 195 between them. Ambient temperature was measured at exposed sites about 1 m from
 196 nests, and 5 cm above ground level, using the same type of thermocouple probes as for

197 eggs. All probes were connected to an Omega HH147U datalogger, programmed to
 198 record temperatures every second during 5–min periods. We registered temperatures
 199 during such periods because they are similar to those spent by incubating shorebirds
 200 outside their nests during the hottest parts of the day (Grant 1982, Hoffmann 2005), and
 201 also because, under hot conditions ($> 30\text{ }^{\circ}\text{C}$), eggs in unattended nests may reach lethal
 202 temperatures for embryos in just two minutes (own unpubl. data). We chose maximum
 203 temperatures because of the importance of these on the survival of the embryo.

204 The colour and proportion of spottiness of quail eggs were measured on digital
 205 photographs as explained above. We expected that differences in egg temperature
 206 between light and dark quail eggs were only affected by eggshell colour and spottiness,
 207 and not by any other feature of eggs. To account for any differences between eggs not
 208 related to their pigmentation, we recorded temperatures of both types of eggs in the
 209 shade, in which case we did not expect differences between light and dark eggs. In
 210 addition, we compared egg volumes of dark and light quail eggs to eliminate the
 211 possibility that differences in temperature between both groups of eggs were due to
 212 differences in size. All quail eggs were measured using digital callipers (length [l] and
 213 breath [b] to the nearest 0.1mm), and their volumes (V) estimated as

$$214 \qquad V = K_V \times lb^2$$

215 where K_V is the volume coefficient, which for avian eggs is 0.507 (Hoyt 1979).

216 The datalogger has four input channels, and we recorded the temperature of
 217 every individual egg in each one of the channels, which served to check that
 218 temperatures were not affected by the channel to which the thermocouples were
 219 connected.

220

221 *Statistical analyses*

222

223 When comparing mean values, and the data met normality and
224 homoscedasticity, Student's t-test was used. If these criteria were not met, Mann-
225 Whitney U-test was chosen instead. General linear models (GLM) were fitted to test if
226 there were relationships between background camouflage and the overall eggshell
227 colouration. In these models ΔE was the response variable and overall eggshell colour
228 (RGB) the explanatory variable. Generalized linear mixed models (GLMM) were used
229 to test whether maximum egg temperatures were affected by the input channel (random
230 factor) of the datalogger, as well as to test differences in temperature between dark and
231 light quail eggs in natural shorebird nests. In this last case, the response variable was
232 maximum egg temperature and the independent variables were maximum
233 environmental temperature, colour of quail eggs (dark or light) and region
234 (Mediterranean and Tropics). Egg identity was considered as random factor (to control
235 for repeated measures with the same eggs) and no interactions among factors were
236 found. Analyses were carried out in R (R Core Team 2013) and significance level was
237 set at 0.05.

238

239 **Results**

240

241 *Egg spotting and colour: tropical vs. mediterranean species*

242

243 A comparison between congeneric species showed that eggs of the
244 mediterranean Kentish plovers were slightly more spotted than eggs of tropical
245 Wilson's plovers, though the difference was not statistically significant (Table 1). On

246 the contrary, eggs of the tropical black-necked stilt were significantly more spotted than
 247 eggs of the mediterranean black-winged stilt (Table 1).

248 In the case of the plover species (see images in supplementary material, Fig. S2),
 249 overall colouration of eggs was darker in the Kentish plover than in the Wilson's plover
 250 (Table 1), but the difference between RGB of the eggshell's background and RGB of
 251 the spots was larger in Wilson's than in Kentish plover (Figure S3 supplementary
 252 material), indicating that the contrast between eggshell background and spots was
 253 greater in the Wilson's plover (Table 1). A similar result about differences between
 254 background and spottiness was found for the stilts, although in this case the overall
 255 colour of the egg was similar (Table 1).

256

257 *Background matching camouflage*

258

259 Plovers

260

261 Kentish plover eggs appeared better camouflaged than Wilson's plover eggs with
 262 respect to N (ΔE_{EG-N} , Table 1) but not to S (ΔE_{EG-S}). However, Kentish plover nests
 263 were more conspicuous when compared with the surroundings than those of Wilson's
 264 plover (ΔE_{N-S} , Table 1). GLMs show that, in Wilson's plover, darker eggshells are
 265 better camouflaged with respect to both N (Fig. 1; $r^2 = 0.50$, $p = 0.0015$) and S ($r^2 =$
 266 0.65 , $p < 0.001$). However, such relationships were not found for the Kentish plover (all
 267 $p > 0.1$). No significant relationships were found between ΔE_{N-S} and RGB eggshell
 268 colour for either plover species (all $p > 0.6$).

269

270 Stilts

271

272 No significant differences were found between stilts in ΔE_{EG-N} , ΔE_{EG-S} and ΔE_{N-S} values
 273 (Table 1). In the tropical black-necked stilt, there were linear relationships between
 274 ΔE_{EG-N} and ΔE_{EG-S} and the RGB values of the eggs (Fig 2, $r^2 = 0.74$, $p < 0.001$ and $r^2 =$
 275 0.78 , $p < 0.001$, respectively), so that darker eggshells were better camouflaged.
 276 However, in the black-winged stilt only in the case of ΔE_{EG-N} ($r = 0.4585$, $p = 0.036$).
 277 For both species there was no relationship (all $p > 0.15$) between ΔE_{N-S} and RGB of the
 278 eggshells.

279

280 *Egg temperatures in relation to spottiness and colouration*

281

282 The proportion of spottiness (mean \pm SD) was greater in the dark (0.81 ± 0.104 ,
 283 $n = 5$) than in the light (0.21 ± 0.173 , $n = 6$) quail eggs (Figure S4, supplementary
 284 material; Mann-Whitney U-test, $U = 30$, $p = 0.004$) used to record temperatures in
 285 natural shorebird nests. Although the background colour of dark eggs (182.8 ± 13.8)
 286 was slightly darker than that of lighter ones (199.6 ± 19.8), the difference was not
 287 statistically significant (Mann-Whitney U-test, $U = 5$, $p = 0.082$). There was a
 288 significant difference in the colour of spots, being darker in dark (73.6 ± 14.3) than in
 289 light eggs (105.6 ± 27.0) (Mann-Whitney U-test, $U = 3$, $p = 0.030$).

290

291 GLMM results showed that when the quail eggs were exposed to direct sunlight
 292 during 5 min in shorebird nests ($n = 138$), dark eggs reached higher maximum
 293 temperatures than light ones ($t = 5.55$, $p < 0.001$; Fig. 2 and see also Table S1 and S2,
 294 supplementary material). In addition, for a same ambient temperature, quail eggs
 295 overheated more in tropical than in mediterranean sites ($t = 3.35$, $p = 0.0028$; Figure 2).

295 These differences in egg temperature were likely only due to differences in
296 colouration and spottiness between both categories of eggs, as there were no colour-
297 related differences in maximum temperatures reached by eggs when they were in the
298 shade (dark eggs: 32.7 ± 0.28 °C; light eggs: 32.6 ± 0.30 °C; Mann-Whitney U-test, $U =$
299 17 , $p = 0.776$). In addition, there were no differences in the size of eggs that could have
300 affected the rates of overheating. Indeed, the volume of light quail eggs (12.0 ± 1.38
301 cm^3) was similar to that of dark eggs (11.4 ± 1.01 cm^3) (Mann-Whitney U-test, $U = 19$,
302 $p = 0.537$). Temperatures of quail eggs recorded by each one of the input channels of
303 the datalogger were not different ($F_{3,30} = 2.6$, $p = 0.066$).

304

305 **Discussion**

306 The main results of our study regarding the effect of egg colouration on egg
307 temperatures are that darker eggs overheat more quickly than lighter ones, and that more
308 pigmented eggs, hence darker, are better camouflaged. Therefore, our results support a
309 trade-off between overheating and camouflage on shorebird eggshell colouration.

310 Although it has been suggested that overheating is unlikely to have a selective
311 influence on avian egg appearance (Ruxton 2012), our results support the contrary, as
312 we found that dark eggs reached higher temperatures than light ones when exposed to
313 direct sunlight during 5–min periods. Such periods are similar to those spent by
314 incubating shorebirds outside their nests during the hottest parts of the day (Grant 1982,
315 Hoffmann 2005). A previous study, in which natural eggs were also used, did not find
316 that temperatures of eggs exposed to direct sunlight were related to egg pigmentation
317 (Westmoreland et al. 2007). However, in such study, eggs of cup-nester songbirds
318 placed on trees received solar radiation during one hour. Because of this, the result of
319 Westmoreland et al. (2007) may not be biologically meaningful for ground-nesting

320 birds given that parents likely do not allow their eggs to remain exposed to adverse hot
321 conditions during extended periods, given the fatal consequences of overheating for the
322 embryo (Grant 1982, Webb 1987, Maurer et al. 2011). Indeed, for the hottest part of the
323 day eggs were not left uncovered by plovers and stilts during >1 min in hot
324 environments (Grant 1982). Even in cooler environments incubation recesses in ground
325 nesting birds last about 10 min, and those lasting >1 h are very rare (<1% of all daytime
326 recesses, MacDonald et al. 2013). Likely, when exposed to direct solar radiation during
327 long periods, eggs of similar size may reach similar equilibrium temperatures,
328 independently of their colouration, because heat dissipation mechanisms may not be
329 enough to overwhelm heavy heat loads, which may explain the results of Westmoreland
330 et al. (2007). Despite their results they suggest that opposite results may be found in
331 ground nesting birds, as we found. Yet, colouration may be important when eggs are
332 exposed to direct sunlight during short periods, because darker eggs may heat more
333 quickly than lighter eggs, as our results support. In addition, our results also indicated
334 that for similar ambient temperature, internal egg temperatures were greater at the
335 tropical sites than at the Mediterranean perhaps because of the more intense solar
336 radiation in the Tropics (Wallace and Hobbs 2006).

337 Baken et al. (1978) showed that the protoporphyrin of avian eggshells reflects a
338 high percentage of the sun's energy in the infrared zone of the spectrum, and this
339 diminished the overheating risk of eggs exposed to direct sunlight in comparison to
340 melanin pigments. Some authors have focused on this result to minimize the importance
341 of overheating as a selective agent on eggshell colouration (e.g., Mikšik et al. 1996,
342 Ruxton 2012), but have not taken into account that around 43 % of the sun energy falls
343 in the visible part of the spectrum (Gueymard 2004), which means that darker eggshells,

344 even containing protoporphyrin, could absorb more energy and heat the eggs faster
345 when receiving direct sunlight than lighter eggshells.

346 Thus, we found support for Montevecchi's (1976) hypothesis, in that more
347 pigmented eggs were better camouflaged, but also overheated more when they received
348 direct sunlight. However, there were some differences in the eggshell colouration
349 between shorebird species. As indicated by Ahlgren et al. (2013), due to multiple
350 stressors, animals have to trade-off their responses to different threats, or alternatively
351 respond only to the most severe stressor. Different types of responses in relation to the
352 severity of a particular stressor may account for some of the interspecific differences
353 that we found depending on the region. Indeed, Wilson's plovers and Black-necked
354 stilts breeding in tropical environments, suffering more intense solar radiation, have
355 eggshells with lighter backgrounds than those of their congeneric mediterranean
356 species, which may indicate that overheating may be a more severe stressor than
357 predation for tropical shorebirds. Although the eggshell spots of the tropical species
358 were darker than those of the mediterranean species, the spots cover around 30% of the
359 total eggshell surface (Table 1 and Table 2), so their contribution to overheating may be
360 lower than that of the eggshell background.

361 Even so, if darker colours incur greater risks of overheating, why are eggshell
362 spots of tropical shorebirds darker than those of mediterranean ones? One potential
363 advantage is that by increasing the contrast between the colouration of eggshell
364 background and spots, disruptive camouflage may be facilitated (Kang et al. 2014,
365 Stevens and Merilaita 2011). Thus, the thermal environment, through its effects on risk
366 of egg overheating, may affect the reliance on different egg camouflage strategies
367 (background matching and/or disruptive camouflage) to counteract nest detection by
368 predators.

369 Differences in the species' biology may lead to additional different strategies of
370 nest camouflage. Stilts usually nest in colonies (Pierce 1996) and plovers usually do not
371 (Wiersma 1996), which could affect the response against predators approaching their
372 nests. Colonial nesting birds may mob and attack in group approaching predators, which
373 may reduce predation risks (Montevecchi 1979, Whittam and Leonard 2000). This may
374 determine variation in the time that eggs remain uncovered and the relative importance
375 of overheating as a stressor, which may be less critical for stilts than for plovers. In
376 addition, the eggs of stilts are larger than those of plovers, so that smaller plover's eggs
377 would overheat faster. This may explain why stilts have darker and more spotted
378 eggshells.

379 To conclude, in this study we have found that more pigmented eggs may suffer
380 overheating but at the same time are better camouflaged. Lighter eggshells are selected
381 where solar radiation is more intense, as in the Tropics. The relative importance of the
382 two evolutionary drivers suggested by Montevecchi (1976) to affect egg colouration,
383 would affect how shorebirds trade-off their responses to the stressors. Shorebirds may
384 move hundreds of kilometers between breeding attempts not only between but also
385 within seasons (Stenzel et al. 1994, Figuerola 2007), and within females egg colour and
386 degree of spottiness are genetically based (Gosler et al. 2000). Likely because of the
387 variability in colouration in nesting substrates, matching of egg colour may be better
388 achieved with the materials added to the nest rather than with the surroundings (Mayer
389 et al. 2009). However, the materials added to nests may also make the nests more
390 conspicuous with respect to surroundings, as in Kentish plover, which suggests that the
391 materials may also play other roles (Holwell 1979, Mayer et al. 2009, Amat et al. 2012).
392 Therefore, more studies are necessary to demonstrate if the colouration patterns that we

393 found here are also found in other bird species that rely on egg camouflage to diminish
394 the risk of predation of their nests.

395

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410

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- 553
- 554

555 Table 1. Colour and camouflage comparisons between two pairs of congeneric species:
 556 Kentish plover vs. Wilson's plover, and black-winged stilt vs. black-necked stilt.
 557 Overall eggshell colouration (RGB eggs), proportion of spottiness (proportion of
 558 eggshell surface covered by spots) and differences between background colour (BACK)
 559 and spottiness colour (SPOT) are shown ($\Delta\text{RGB}_{\text{BACK-SPOT}}$). Higher RGB values reflect
 560 lighter colours. Relating to camouflage, the table shows contrasts (ΔE) between the
 561 three different substrates: eggs (EG), nest (N) and surroundings (S). Higher values of
 562 ΔE reflect worse background camouflage. T-values (t), degrees of freedom (df) and p-
 563 values (p) are shown. * Mann-Whitney U test was used instead of Student's t-test, then
 564 the t-value corresponds to U-value.
 565

	Kentish plover (n = 41)	Wilson's plover (n = 17)	t	df	p
	Mean \pm Std. Dev.	Mean \pm Std. Dev.			
RGB eggs	120.33 \pm 20.28	139.25 \pm 22.28	-3.14	56	0.003
Prop. of spottiness	0.30 \pm 0.08	0.27 \pm 0.09	1.54	56	0.128
$\Delta\text{RGB}_{\text{BACK-SPOT}}$	47.30 \pm 30.47	75.62 \pm 26.99	-3.26	56	0.001
$\Delta E_{\text{EG-N}}$	12.84 \pm 7.60	18.16 \pm 10.25	-2.19	56	0.033
$\Delta E_{\text{EG-S}}$	15.44 \pm 7.50	20.03 \pm 11.67	-1.79	56	0.079
$\Delta E_{\text{N-S}}$	11.69 \pm 7.57	7.47 \pm 6.36	2.02	56	0.048

566

	Black-winged stilt (n = 14)	Black-necked stilt (n = 21)	t	df	p
	Mean \pm Std. Dev.	Mean \pm Std. Dev.			
RGB eggs	112.71 \pm 19.77	112.05 \pm 29.70	0.08	33	0.937
Prop. of spottiness	0.30 \pm 0.11	0.39 \pm 0.12	-2.28	33	0.029
$\Delta\text{RGB}_{\text{BACK-SPOT}}$	40.21 \pm 25.40	74.03 \pm 16.26	41*		0.000*
$\Delta E_{\text{EG-N}}$	13.36 \pm 7.42	12.53 \pm 13.34	0.24	33	0.816
$\Delta E_{\text{EG-S}}$	16.15 \pm 10.18	15.03 \pm 11.48	0.30	33	0.764
$\Delta E_{\text{N-S}}$	15.43 \pm 11.24	7.89 \pm 2.99	101*		0.126*

567

568 FIGURE LEGENDS

569

570 Figure 1. Background matching camouflage between eggs and nests (ΔE_{EG-N}) of the
571 mediterranean (Kentish plover and black-winged stilt, black dots) and tropical
572 (Wilson's plover and black-necked stilt, emptied triangles) species. Lighter (higher
573 RGB values) eggshells had lower degree of camouflage (higher ΔE_{EG-N} values). Kentish
574 plover: $y = 1.53 + 0,094*x$; $p = 0.11$, $r^2 = 0.063$. Wilson's plover: $y = -27.14 + 0.32*x$;
575 $p = 0.0015$, $r^2 = 0.50$. Black-winged stilt: $y = -6.04 + 0.17*x$; $p = 0.037$, $r^2 = 0.21$.
576 Black-necked stilt: $y = -30.66 + 0.39*x$; $p < 0.001$; $r^2 = 0.74$

577

578 Figure 2. Results of a GLMM of the effect of maximum environmental temperature, egg
579 colour (dark or light) and region (Tropics or Mediterranean) on maximum temperature
580 reached by quail eggs when exposed to direct sunlight during 5-min periods in
581 shorebird nests. (A) Linear relationship between maximum quail egg temperatures
582 exposed to direct solar radiation and maximum environmental temperatures (black line
583 with grey confidence intervals), indicating the threshold of egg temperatures $>40^\circ\text{C}$ that
584 are very critical for embryos. And partial effects of egg colouration (B) and region (C).
585 Eggs reached higher temperatures (mean \pm SE) in the Tropics than in the
586 Mediterranean, and darker eggs heated more than lighter ones. Each partial effect
587 controls for the other independent variables in the model.



