A trade-off between overheating and camouflage on shorebird eggshell colouration

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Abstract

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

Key words

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

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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 °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   Johston 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          $\Delta E = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$ (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   Engeneering, 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 ℃), 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]

213   breath [b] to the nearest 0.1mm), and their volumes (V) estimated as

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

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 $\Delta E_{\text{EG-N}}$, $\Delta E_{\text{EG-S}}$ and $\Delta E_{\text{N-S}}$ values

273   (Table 1). In the tropical black-necked stilt, there were linear relationships between

274    $\Delta E_{\text{EG-N}}$ and $\Delta E_{\text{EG-S}}$ 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 $\Delta E_{\text{EG-N}}$ ($r = 0.4585$, $p = 0.036$).

277   For both species there was no relationship (all $p > 0.15$) between $\Delta E_{\text{N-S}}$ and RGB of the

278   eggshells.

279

280   *Egg temperatures in relation to spottiness and colouration*

281

282   The proportion of spottiness (mean ± 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   GLMM results showed that when the quail eggs were exposed to direct sunlight

291   during 5 min in shorebird nests (n = 138), dark eggs reached higher maximum

292   temperatures than light ones (t = 5.55, p < 0.001; Fig. 2 and see also Table S1 and S2,

293   supplementary material). In addition, for a same ambient temperature, quail eggs

294   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., Mikšík 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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412

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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 ($\Delta RGB_{BACK\text{-}SPOT}$). 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)			
	Mean ± Std. Dev.	Mean ± Std. Dev.	t	df	p
RGB eggs	120.33 ± 20.28	139.25 ± 22.28	-3.14	56	0.003
Prop. of spottiness	0.30 ± 0.08	0.27 ± 0.09	1.54	56	0.128
$\Delta RGB_{BACK\text{-}SPOT}$	47.30 ± 30.47	75.62 ± 26.99	-3.26	56	0.001
$\Delta E_{EG\text{-}N}$	12.84 ± 7.60	18.16 ± 10.25	-2.19	56	0.033
$\Delta E_{EG\text{-}S}$	15.44 ± 7.50	20.03 ± 11.67	-1.79	56	0.079
$\Delta E_{N\text{-}S}$	11.69 ± 7.57	7.47 ± 6.36	2.02	56	0.048

566

	Black-winged stilt (n = 14)	Black-necked stilt (n = 21)			
	Mean ± Std. Dev.	Mean ± Std. Dev.	t	df	p
RGB eggs	112.71 ± 19.77	112.05 ± 29.70	0.08	33	0.937
Prop. of spottiness	0.30 ± 0.11	0.39 ± 0.12	-2.28	33	0.029
$\Delta RGB_{BACK\text{-}SPOT}$	40.21 ± 25.40	74.03 ± 16.26	41*		0.000*
$\Delta E_{EG\text{-}N}$	13.36 ± 7.42	12.53 ± 13.34	0.24	33	0.816
$\Delta E_{EG\text{-}S}$	16.15 ± 10.18	15.03 ± 11.48	0.30	33	0.764
$\Delta E_{N\text{-}S}$	15.43 ± 11.24	7.89 ± 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 >40ºC that

584   are very critical for embryos. And partial effects of egg colouration (B) and region (C).

585   Eggs reached higher temperatures (mean ± 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.