

20 SUMMARY

21 The magnetic field, the sun, the stars and the polarization pattern of visible light
22 during twilight are important cues for orientation in nocturnally migrating songbirds.
23 As these cues change with time and location on Earth, the polarization pattern was
24 put forward as a likely key reference system calibrating the other compass systems.
25 Whether this applies generally to migratory birds is, however, controversially
26 discussed. We used an experimental approach in free-flying birds to study the role of
27 polarization for their departure direction in autumn. Experimental birds experienced a
28 90° shift of the band of maximum polarization during sunset, whereas control-birds
29 experienced the polarization pattern as under natural condition. Full view of the
30 sunset cues near the horizon was provided during the cue conflict exposure. Here we
31 show both the experimental and the control-birds being released after nautical
32 twilight departed consistently towards south-southeast. Radio telemetry allowed
33 tracking first 15 km of birds' way out, thus the intrinsic migration direction as chosen
34 by the birds was measured. We found no recalibration of the magnetic compass after
35 pre-exposure to a cue conflict between the natural magnetic field and the artificially
36 shifted polarization pattern at sunset. The lacking difference in the departure direction
37 of both groups may suggests that birds did not recalibrate any of the compass
38 systems during the experiment. As free-flying migrants can use all available
39 orientation cues after release, it remains unknown whether our birds might have used
40 the magnetic and/or star compass to determine their departure direction.

41

42 **Key words:** cue conflict, magnetic compass, migration, orientation, polarization pattern,
43 radio telemetry, songbirds, star compass

44 INTRODUCTION

45 Migrating birds use different cues for orientation, including the magnetic field of the Earth
46 (Wiltschko and Wiltschko, 1972), the position of the sun at sunset (Moore, 1987; Schmidt-
47 Koenig, 1990), light polarization patterns around sunset and sunrise (Able, 1982; Phillips and
48 Moore, 1992; Able and Able, 1993; Muheim et al., 2006b) and the position of the stars
49 (Wagner and Sauer, 1957; Wiltschko and Wiltschko, 1978), see also reviews by (Muheim et
50 al., 2006a; Wiltschko and Wiltschko, 2009). Since cue availability changes depending on a
51 bird's location on the Earth, time of day and season, it seems likely that the multiple compass
52 systems are regularly calibrated by a common reference system (Cochran et al., 2004;
53 Muheim et al., 2006a; Muheim et al., 2006b), thus that one of the multiple compasses is
54 dominant over the others. The polarization pattern during twilight has been proposed as a
55 candidate cue for the key reference system against which the other compass systems are
56 calibrated (Muheim et al., 2006b; Muheim et al., 2007; Muheim et al., 2008). However, it
57 remains unclear how universal this calibration strategy is (Wiltschko et al., 2008a; Wiltschko
58 et al., 2008b; Gaggini et al., 2010; Chernetsov et al., 2011).

59 To our knowledge, only three studies (Sandberg et al., 2000; Cochran et al., 2004;
60 Chernetsov et al., 2011) have monitored free-flying birds to test the orientation of birds, i.e.,
61 to track the departure directions after exposure to a cue conflict over the first part of their
62 journey. In addition Emlen and Demong (1978) found that birds released at migratory altitude
63 under clear sky condition flew in a straight line towards the seasonally appropriate migration
64 direction, whereas the concentration of birds' tracks was reduced under overcast situation. The
65 major advantages of tracking free-flying birds over Emlen funnel experiments are that (1)
66 Emlen funnel's drawbacks are overcome, (2) free-flying birds can phenotypically respond to
67 the environmental cues after the experiment during the departure, (3) the intrinsic migration
68 direction as chosen by the birds is detected, and (4) they move through space (Emlen and
69 Emlen, 1966; Nievergelt et al., 1999; Nievergelt and Liechti, 2000; Chernetsov et al., 2011).

70 Here, we test whether a 90° shift of the band of maximum polarization during sunset
71 determines the departure direction of a free-flying nocturnal songbird migrant, the northern
72 wheatear (*Oenanthe oenanthe* Linnaeus 1758, wheatear hereafter). Under the general
73 hypothesis that the polarization pattern at sunset and sunrise is a reference cue to adjust
74 alternative compass systems, we hypothesized that such a 90° shift of the band of maximum
75 polarization affects the birds' departure direction by on average of 90° in comparison to
76 control birds (Fig. 1).

77

78

79 MATERIALS AND METHODS

80 Study site and study species

81 The study was carried out on Helgoland, Germany, a small island (54°11'N, 07°55'E) in the
82 North Sea, during autumn migration 2008 (06.09.–6.10.) and 2009 (22.08.–29.09.). Its
83 distance to the nearest land is approximately 50 km in easterly to southerly directions. Thus,
84 birds having left the island can be considered as having resumed migration. Because of its
85 small size, the presence and absence of radio-tagged birds on the island can easily be
86 determined, and nocturnal exploratory flights are not misinterpreted as departure events
87 (Schmaljohann et al., 2011).

88 The wheatear is a nocturnal migrant (Schmaljohann et al., 2011; Schmaljohann and
89 Naef-Daenzer, 2011) and regularly occurs on the island during autumn migration
90 (Schmaljohann and Dierschke, 2005), but is not a regular breeding species there (Dierschke et
91 al., 2011). Therefore, all wheatears on the island can be treated as migrants. The birds were
92 caught with spring traps baited with mealworms (*Tenebrio molitor* Linnaeus 1758), measured
93 (wing length to the nearest 0.25 mm), weighted to the nearest 0.25 g using a Pesola balance
94 (Pesola AG, Switzerland), and banded with individual combinations of one reddish anodized
95 aluminium ring and four colour-rings. Age was estimated from the colour of the inner upper
96 mandible (Svensson, 1992). We considered only first-year birds to avoid any age effect and
97 we did not distinguish between subspecies, since both can be assumed to migrate in the same
98 southerly direction from Helgoland in autumn (Zink, 1973; Dierschke, et al. 2011).

99

100 Housing

101 The birds were kept indoors for several days (mean±s.d., 5±2 days) in separate cages (45.0 ×
102 34.0 × 23.5 cm) in a room at the Institute of Avian Research. The windows were covered with
103 baking parchments allowing birds to perceive the natural light conditions, but to prevent them
104 from receiving any information on the polarization pattern. Up to eight birds were stationed
105 simultaneously in the room. Mealworms and water were provided *ad libitum*. The wheatears
106 usually started feeding within several minutes after we had left the room as revealed by video
107 recording. Each morning (8.00–9.00 am) and evening (5.30–6.30 pm) the birds were weighted
108 and their fat score (Kaiser, 1993) and muscle score (Bairlein, 1994) determined. If a bird lost
109 weight during the day, it was not considered for the experiment and immediately released.

110

111 Experimental procedure

112 Experiments were only carried out on evenings with “good” migration conditions. We used
113 the free available weather forecast program windfinder (www.windfinder.com) to predict
114 weather conditions on evenings and selected nights with no rain and with predicted wind
115 speed of less than 7 m s^{-1} . Stronger winds are supposed to be assessed to be unfavourable by
116 migratory birds (Erni et al., 2002). Cloudiness of sky was not considered as the polarization
117 pattern is also visible when overcast (Hegedüs et al., 2007). Time and azimuth of local sunset
118 was taken from SunEarthTools (www.sunearthtools.com) with settings for Helgoland.

119 When all of the above criteria were met, we selected two birds in good and similar
120 body condition, i.e., fat (≥ 3) and muscle score (≥ 2), for the night’s experiment, see result
121 section for further details about the birds’ body condition. We attached a radio transmitter to
122 each bird about two hours before sunset (see Radio tracking below) and placed one of the
123 birds into the cardboard box of the experiment-cage and the other into the cardboard box of
124 the control-cage (see Exposure cages below). Each cage was covered with a cloth, allowing
125 the birds to accustom to the conditions for about 45 min. They were then transported, still
126 covered, to the exposure site ($54^{\circ}11'13''\text{N}$, $7^{\circ}52'30''\text{E}$) on a short-grassy hilltop (Fig. 2).
127 From there, the birds had a panoramic view of the horizon, slightly interfered by the highest
128 peak of the island, the Pinneberg (azimuth: $150\text{--}160^{\circ}$) and a small building (azimuth: 295--
129 305°).

130 There, the cages were placed horizontally and in a distance of 5 m from each other on
131 top of the hill and aligned relative to the sunset azimuth, as described below (see Exposure
132 cages). 30 min before sunset, the cages were uncovered and at least two persons surveyed the
133 cages from a distance of 50–100 m to detect any type of disturbance, which never occurred.
134 The birds were released out of the cages when the sun’s elevation was lower than 13.7° below
135 the horizon, i.e., after nautical twilight, so that stars, when not cloudy, were visible and the
136 skylight polarization pattern had disappeared, see Cochran et al. (2004) and Chernetsov et al.
137 (2011). The birds were then held in the open hand, so that they could decide by themselves
138 when and into which direction to depart. This lasted no longer than 5 min. The direction of
139 the hand and birds’ alignment on the hand were randomized. Radio tracking started the
140 moment the birds had taken off (see below).

141

142 Exposure cages

143 Two cardboard boxes (30 x 32 x 32 cm) had net-covered windows (14 x 14 cm) on all four
144 side walls. Each cardboard box was placed centrally into a larger wooden box (45 x 62 x 39
145 cm, exposure cage) with the windows congruent with the windows of the cardboard boxes

146 (Fig. 2). All windows of the wooden boxes were covered on the outside with a pseudo-
147 depolarizing filter (two sheets of Hostaphan polyethylene terephthalate, Hoechst AG,
148 Germany, aligned at an angle of 45° relative to each other, resulting in a depolarization of
149 90% with a 10–15% reduction of light intensity between 400 and 800 nm) as described in the
150 supporting online material by Muheim et al. (2006b). Thereby, we minimized effects on light
151 intensity changes caused by the interaction of the polarizing filters with the natural skylight
152 polarization. On the inside, the windows were covered with polarizing filters (linear polarizer
153 #POA1, 3Dlens Corporation, Taiwan, transmittance 38%, polarizing efficiency 99.98% at
154 wavelengths $\sim 400\text{--}700\text{nm}$).

155 In the control-cage, the polarizing filters were aligned so that the e-vector of
156 polarization simulated a polarization pattern that was in agreement with the natural condition
157 where the band of maximum polarization crosses the zenith at an angle of 90° relative to the
158 azimuth of the sun (Muheim et al., 2006b). The e-vectors of the polarization filters in the two
159 windows on the longitudinal axis (“northern” and “southern” window) were aligned
160 vertically, while the e-vectors on the lateral axis (“western” and “eastern” window) were
161 aligned horizontally, see supporting online material by Muheim et al. (2006b). Thus, the birds
162 experienced an artificial band of maximum polarization crossing the sky at 90° from the sun,
163 approximately going from “North” to “South”.

164 In the experiment-cage, the polarization filters were attached so that they simulated a
165 polarization pattern that was shifted by 90° relative to the natural one. The e-vectors in the
166 two windows on the longitudinal axis (“northern” and “southern” window) were aligned
167 horizontally, while the e-vectors on the lateral axis (“western” and “eastern” window) were
168 aligned vertically.

169 The cages were aligned so that the “western” window always pointed towards the
170 actual azimuth direction of the sunset. Thus, the cages were not exactly aligned along the
171 cardinal directions, but instead relative to the sunset position. In both boxes, the overhead
172 region of the sky was blocked from view within approximately $\pm 30^\circ$ of the zenith, so that the
173 bird had only reduced access to overhead cues of polarized light. We used only glue for fixing
174 and did not use any metal to construct the boxes or cages to prevent any influences of metal
175 components on the magnetic senses of the birds.

176

177 Radio tracking

178 Radio transmitters were constructed by the Swiss Ornithological Institute in cooperation with
179 the University of Applied Sciences Bern, Switzerland (Naef-Daenzer et al., 2005). Radio

180 transmitters, including battery and harness, weighed 0.8 g. The transmitters were attached to
181 wheatears using a Rappole-Tipton-type harness made from 0.5 mm elastic cord (Rappole and
182 Tipton, 1990). Length of leg-loops was adjusted individually to birds (Naef-Daenzer, 2007).
183 Since the lowest body mass of the tested wheatears was 20.9 g (mean±s.d., 29.8±3.8 g,
184 $N=42$), the mass of the radio transmitter represented less than 3.8% (mean: 2.7%) of the
185 birds' body mass. The relative load was, therefore, below the recommended 5% limit
186 (Cochran, 1980; Caccamise and Hedin, 1985). Potential adverse effects on the birds'
187 behaviour are insubstantial (Naef-Daenzer et al., 2001; Rae et al., 2009), and the increase in
188 flight costs is small (Irvine et al., 2007), though drag (Bowlin et al., 2010; Pennycuick et al.,
189 2011) as well as energy expenditure (Barron et al., 2010) increase. Transmitter life was about
190 30 days.

191 We used Yagi 3EL2 hand-held antennas (Vårgårda, Sweden) in combination with FT-
192 290RII receivers (Yaesu, Japan) to track the two birds per night. The detection range of the
193 radio transmitters was approximately 12–15 km (Schmaljohann et al., 2011; Schmaljohann
194 and Naef-Daenzer, 2011).

195 In those cases where the birds did not immediately depart from the island after release,
196 their location on the island was estimated by triangulation from subsequent observer
197 positions. During all departure events, the birds were radio-tracked from the “Oberland”, the
198 highest area on the island elevated 50–60 m above sea level. Departing birds were recorded
199 until loss of signal. According to the series of bearings of the departing birds, they left
200 Helgoland in a straight line. We used the last recorded direction before loss of signal as the
201 departure direction. The bearing accuracy of this method is $3\pm 5^\circ$ (mean±s.d., $N=49$), see
202 Schmaljohann et al. (2011). In the mornings after a departure, the whole island was searched
203 to see whether any departed bird had returned to the island, but no bird did so.

204 The birds' flight altitude could not be estimated with the radio telemetry method used.
205 As the island is very small, a potential parallax error in direction estimates is small compared
206 to the bearing accuracy of hand-held antennas (Kenward, 2001). Set-off distance between bird
207 and observer was < 500 m (see above); the parallax error in respect to a tracking distance of
208 15 km would be $< 2^\circ$.

209 From the start of exposure, we estimated cloud cover [x/8], visibility [0=horizon,
210 1=not to the horizon but beyond the dune (> 2.5 km), 2=to the dune (c. 1.5 km), 3=not to the
211 dune (< 1.5 km)] and the rainfall [0=no rain, 1=shower of rain, 2=continuous rain] every hour
212 on the hour and at departure events.

213

214 Departure fuel load and potential flight range

215 Individual lean body mass m_0 (g) was estimated following Schmaljohann and Naef-Daenzer
216 (2011) as:

217

$$218 \quad m_0 = 0.29 \cdot \omega - 6.85 \quad (1),$$

219

220 where ω is the individual wing length (mm).

221

222 Individual departure fuel load f relative to bird's lean body mass m_0 was calculated using
223 bird's departure body mass m taken just before radio tagging as:

224

$$225 \quad f = (m - m_0) / m_0 \quad (2).$$

226

227 Bird's potential flight range Y is a function of the individual departure fuel load f (Delingat
228 et al., 2008):

229

$$230 \quad Y = 100 \cdot U \cdot \ln(1 + f) \quad (3),$$

231

232 where U is wheatear's airspeed of 47 km h⁻¹ (Bruderer and Boldt, 2001).

233

234 **Statistics**

235 Statistics were calculated using the statistical software package R (R Development Core
236 Team, 2011). Directions were tested for uniformity with the Rayleigh test of uniformity
237 (Jammalamadaka and SenGupta, 2001). Circular-circular and circular-linear correlations
238 were calculated according to the methods described by (Jammalamadaka and SenGupta,
239 2001). The P-value for a circular-linear correlation was estimated by a randomization test
240 with 10000 runs each, see Crawley (2005) and Schmaljohann and Naef-Daenzer (2011).

241

242

243 **RESULTS**

244 **Conditions of experimental and control-birds**

245 Eight experiments were carried out in 2008 and 13 in 2009. None of the variables for body
246 condition differed significantly between experimental and control-birds, all Wilcoxon tests for

247 paired samples: $P > 0.22$: wing length (experimental birds: mean \pm s.d., 99.6 ± 3.8 mm, $N = 21$;
248 control-birds: 101.7 ± 3.6 mm, $N = 21$), fat score (4.0 ± 0.67 ; 3.8 ± 0.77), departure body mass
249 (29.8 ± 3.7 g; 29.7 ± 4.1 g) and departure fuel load (0.34 ± 0.13 ; 0.32 ± 0.15). The potential flight
250 ranges, which all were sufficient to reach the nearest mainland at a distance of 50 km, did not
251 differ between groups (experimental birds: 1350 ± 445 km; control-birds: 1276 ± 529 km;
252 Wilcoxon test for paired sample: $V = 130$, $P = 0.63$). Between year comparisons did not reveal
253 any significant difference in wing length and fat score (Mann Whitney U-test: $P > 0.83$), but in
254 departure body mass (2008: 28 ± 4.0 , $N = 16$; 2009: 31 ± 3.2 , $N = 26$), departure fuel load
255 (0.25 ± 0.13 ; 0.38 ± 0.12) and consequently flight range (1022 ± 491 km; 1492 ± 391 km; Mann
256 Whitney U-tests: $P < 0.02$).

257

258 Departure events

259 37 of the 42 wheatears departed in the course of the night of the experiment. Three
260 experimental and two control-birds remained on the island for another day. We determined
261 the departure direction for 33 ($N_{\text{experiment}} = 17$, $N_{\text{control}} = 16$) and tracked the duration of the
262 departure until loss of signal for 31 ($N_{\text{experiment}} = 17$, $N_{\text{control}} = 14$) out of these 37 departing
263 wheatears. The tracking duration could not be estimated for two control-birds, because the
264 onset of their departure flights remained unclear. In 13 experiments both the experimental and
265 control-bird departed from Helgoland during the same night of the experiment allowing pair-
266 wise comparisons between experimental and control-birds. There was no significant
267 difference in the tracking duration of the departure events between experimental and control-
268 birds (mean \pm s.d., 17 ± 6 min, $N = 17$; 17 ± 6 min, $N = 14$; Mann Whitney U-test: $W = 114$, $P = 0.86$;
269 pair wise comparison: 18 ± 6 min; 17 ± 6 min; Wilcoxon test for paired sample: $V = 33$, $P = 1$,
270 $N = 11$). Overall, the birds were tracked after setting off for 17 ± 6 min ($N = 31$). This together
271 with an assumed airspeed of 13 m s^{-1} (47 km h^{-1}) indicated a mean detection range of the
272 radio transmitters of 13 ± 4 km ($N = 31$).

273 Wheatears departed from the island between 3 and 270 min after they were released
274 from the orientation cages (experimental birds: mean \pm s.d., 104 ± 74 min, $N = 17$; control-birds:
275 88 ± 65 min, $N = 14$; Mann Whitney U-test: $W = 133$, $P = 0.60$; pair wise comparison: 106 ± 82
276 min; 82 ± 60 min; Wilcoxon test for paired sample: $V = 46$, $P = 0.28$, $N = 11$). There was no
277 significant difference between the experimental and control-birds in the timing of departure in
278 respect to sunset (experimental birds: mean \pm s.d., 215 ± 60 min, $N = 17$; control-birds: 185 ± 41
279 min, $N = 14$; Mann Whitney U-test: $W = 148$, $P = 0.26$; pair wise comparison: 214 ± 69 min;
280 190 ± 37 min; Wilcoxon test for paired sample: $V = 46$, $P = 0.28$, $N = 11$).

281

282 Departure directions

283 The mean departure direction of the experimental birds was unimodally orientated towards
284 141° (Rayleigh test of uniformity: $R=0.54$, $P<0.0001$, $N=17$; Fig. 3) and direction of control-
285 birds towards 154° (Rayleigh test of uniformity: $R=0.78$, $P<0.0001$, $N=16$; Fig. 3). Departure
286 directions and angular dispersion around the corresponding means did not differ significantly
287 between both groups (Watson-Williams test: $F_{1,31}=0.43$, $P=0.52$; Wallraff test: Kruskal-
288 Wallis- $\chi_1^2=1.33$, $P=0.25$), also when considering only nights in which both the experimental
289 (180° ; Rayleigh test of uniformity: $R=0.52$, $P=0.026$, $N=13$) and control-bird (159° ; Rayleigh
290 test of uniformity: $R=0.74$, $P=0.0003$, $N=13$) departed (Watson-Williams test: $F_{1,24}=0.02$,
291 $P=0.88$; Wallraff test: Kruskal-Wallis- $\chi_1^2=0.9$, $P=0.34$).

292 When each departure direction of the experimental birds was plotted as the deviation
293 from its corresponding control-bird, the deviations were uniformly distributed and not
294 significantly different from zero. Mean deviation was 4° (95% CI: $338^\circ-33^\circ$; Rayleigh test of
295 uniformity: $R=0.71$, $P<0.001$, $N=13$; Fig. 4). Absolute deviation was 40° (95% CI: $26^\circ-53^\circ$;
296 Rayleigh test of uniformity: $R=0.91$, $P<0.001$, $N=13$; Fig. 4) and therefore, significantly
297 different from an expected 90° shift.

298 Wind direction, wind speed, cloudiness and visibility at departure did not explain the
299 variation in the departure direction of experimental and control-birds (circular~circular
300 correlation: $P=0.32$, circular~linear correlations all: $F_{2,11}<1.1$, $P>0.6$, $N=13$). Departure fuel
301 load did neither affect the departure direction of experimental nor of control-birds (circular-
302 linear correlation: experimental birds: $F_{2,15}\approx 3.49$, $P=0.21$, $N=17$; control-birds: $F_{2,14}\approx 0.76$,
303 $P=0.70$, $N=16$) also when considering only birds departing within the same night
304 (experimental birds: $F_{2,11}\approx 2.97$, $P=0.27$, $N=13$; control-birds: $F_{2,11}\approx 0.36$, $P=0.85$, $N=13$).
305 Departure time after release was significantly correlated with departure fuel load in
306 experimental birds ($R_S=-0.50$, $P=0.043$, $N=17$) indicating that birds with slightly higher
307 departure fuel loads set off earlier in the night after being released than birds with lower
308 departure fuel load. This was not true for control-birds ($R_S=0.16$, $P=0.59$, $N=16$). Regarding
309 the birds setting off on the same night, departure time after release did neither correlate with
310 departure fuel load in experimental ($R_S=-0.41$, $P=0.16$, $N=13$) nor in control-birds ($R_S=0.04$,
311 $P=0.92$, $N=13$). There was no seasonal effect on departure direction (circular-linear
312 correlation, experimental birds: $F_{2,15}\approx 0.88$, $P=0.65$, $N=17$ and $F_{2,11}\approx 1.30$, $P=0.55$, $N=13$;
313 control-birds: $F_{2,14}\approx 0.20$, $P=0.91$, $N=16$ and $F_{2,11}\approx 0.15$, $P=0.92$, $N=13$).

314

315

316 DISCUSSION

317 The experimental and control-birds departed both consistently in south-southeasterly
318 directions with no significant difference in the bearing between the groups (Figs 3, 4). A
319 similar migratory direction was expected from earlier evidence on autumn migration by ring
320 recoveries pointing towards south-southwest from Helgoland (Dierschke et al., 2011). Hence,
321 we found no compass recalibration as a result of the exposure to the cue conflict between the
322 natural magnetic field and an artificially shifted polarization pattern at sunset. In the case of
323 recalibration, we would have expected a difference in the departure directions between the
324 experimental and control-birds of about 90°, either as a bimodal distribution (towards -90°
325 and +90°, cf. Muheim et al., 2006b) or a unimodal distribution (either towards -90° or +90°;
326 Figs 1, 4). Such shifts in orientation has been observed in Savannah (*Passerculus*
327 *sandwichensis*) and white-throated sparrows (*Zonotrichia albicollis*) tested in Emlen funnels
328 (Muheim et al., 2006b, 2009) and in free-flying *Catharus* thrushes (Cochran et al., 2004) after
329 experiencing similar cue conflict exposures. In these cases, the birds calibrated their magnetic
330 compass from the experienced sunset cues.

331 Our study is in agreement with a number of recent studies that did not find an effect of
332 cue conflict exposures on the orientation of the corresponding study species (Wiltschko et al.
333 2008a, Wiltschko et al. 2008b, Gaggini et al. 2010, Chernetsov et al., 2011; but see Muheim
334 et al. 2008). Similar to our study, the authors reported no compass calibration, but instead a
335 simple domination of magnetic or stellar cues over the sunset cues (Chernetsov et al., 2011).

336 Possible explanations for our results include that the experimental birds either did not
337 calibrate any of their compasses and showed (i) a domination of the magnetic compass or (ii)
338 a domination of the celestial compasses (most likely the star compass) when departing from
339 Helgoland, or alternatively that they (iii) recalibrated the celestial (sunset) compass by
340 magnetic cues and then used the magnetic compass to determine the departure direction, or
341 that they showed (iv) a recalibration of the magnetic compass by the polarized light cues and
342 then used their star compass to determine the departure direction. Thus, to be able to make the
343 distinction between these four possibilities, we would need to know which compass the
344 wheatears used when they determined their departure direction from Helgoland, i.e., whether
345 they relied on the magnetic or a celestial compass. The study by Cochran et al. (2004)
346 indicated that birds use their magnetic compass when departing at night after all sunset cues
347 had disappeared. Eleven of our wheatears (6 experimental and 5 control-birds) set off under \geq
348 4/8 overcast conditions showing no unusual departure directions (mean: 131°, 95% CI 85–

349 169°) in comparison to the others (mean: 155°, 95% CI 137–177°, $N=22$). Their departure
350 directions and angular dispersion around the corresponding means did not significantly differ
351 between the two groups (Watson-Williams test: $F_{1,31}=1.29$, $P=0.26$; Wallraff test: Kruskal-
352 Wallis- $\chi^2_1=0.21$, $P=0.65$). Thus, also our wheatears may have primarily used their magnetic
353 compass to determine their departure direction, supporting the hypothesis that the magnetic
354 compass might be dominant over the celestial compasses. However, this still does not allow
355 us to discriminate between scenario (i) and (iii), i.e., to determine whether the birds did not
356 calibrate any of their compasses, disregarding the conflicting cues during the cue conflict
357 exposure (i), or whether they recalibrated the celestial compasses relative to magnetic cues
358 during the exposure to the cue conflict between the natural magnetic field and the artificially
359 shifted polarization pattern at sunset (iii).

360 Explanations for the possible lack of calibration of any of the compass systems during
361 the experiment on Helgoland may or may not be related to hypotheses of a reversed cue
362 hierarchy (Muheim et al., 2008; Wiltschko et al., 2008a; Wiltschko et al., 2008b). It is highly
363 unlikely that the wheatears' migratory state can explain the ignorance of the cue conflict, as
364 departure directions of both groups agreed with the general seasonally appropriate migration
365 direction based on ring-recoveries (Dierschke et al., 2011). The birds were released at higher
366 sun elevations than they generally depart, and the timing of departure with respect to sunset
367 was within the range of their natural departures from Helgoland, although this reference data
368 refers to spring migration (Schmaljohann et al., 2011; Schmaljohann and Naef-Daenzer,
369 2011). Both the experimental and control-birds did not differ in their physiological condition,
370 their potential flight range and their timing of departure demonstrating clearly that the
371 individuals of both groups did not differ in any apparent factor influencing their departure
372 direction. Thus, we doubt that these factors might have driven wheatears to not calibrate their
373 compass systems.

374 A potential biasing influence of the island and the sea as an ecological barrier for
375 small land birds is also an unlikely explanation for the lack of response to the cue conflict.
376 Birds have been shown to react to an upcoming ecological barrier by a change in their
377 migration direction (Meyer et al., 2000), but the strength of this reaction depends strongly on
378 birds' body condition (Sandberg and Moore, 1996; Sandberg, 2003; Deutschlander and
379 Muheim, 2009; Schmaljohann et al., 2011; Schmaljohann and Naef-Daenzer, 2011). Lean
380 birds avoid crossing ecological barriers, whereas fit birds do migrate across large ecological
381 barriers (Schmaljohann and Naef-Daenzer, 2011). As all wheatears had on average potential
382 flight ranges of more than 1000 km, and as departure fuel load did not affect departure

383 direction in this study, the effect of the upcoming sea barrier on wheatears' departure
384 direction was most probably low, cf. Schmaljohann and Naef-Daenzer (2011). Likewise, the
385 landscape of the Courish Spit did not seem to influence the take-off directions of song
386 thrushes in a cue conflict experiment in comparison to the inland site of the same study
387 (Chernetsov et al., 2011).

388 We are confident that we can exclude problems with the experimental set-up, as the
389 experiments were carried out as in Muheim et al. (2006b), except that the birds were radio
390 tracked and experienced a longer cue conflict exposure, on average 167 min as compared to
391 60 min in Muheim et al. (2006b, 2009). Possibly, the wheatears were stressed because of the
392 longer stay in the exposure cages compared to other studies, e.g., Muheim et al. (2006b),
393 Wiltschko et al. (2008a) and Muheim et al. (2009). However, in Cochran et al.'s (2004) study,
394 the birds experienced equally long exposure times, but with the difference that Cochran et al.
395 (2004) exposed their birds to a shifted magnetic field under the natural sky, whereas we
396 exposed the birds in an artificially shifted polarization pattern in the natural magnetic field.
397 As the e-vector of the band of maximum polarization vertically intersects the horizon only at
398 the exact times of sunrise and sunset (the orientation axis of the e-vector at any point in the
399 sky at a $\pm 90^\circ$ angle to the sun is always perpendicular to the beeline to the sun), the birds may
400 have become confused by seeing this vertically aligned pattern at times far from sunset, and
401 therefore may have decided to ignore the pattern.

402 We cannot exclude that the birds at certain locations or under certain environmental
403 conditions pay no attention to conflicting compass cues altogether, e.g., after reaching a
404 transit site or an emergency land site where they do not intend to stop over. Likewise, in areas
405 with prominent landmarks or geographic features, the birds may calibrate their compasses
406 with each other upon arrival to the new site and then transfer the calibration information to
407 these landmark features and ignore consecutive cue conflicts. In our case, the wheatears very
408 likely had time to explore the island and probably even calibrate their compasses before
409 capture, because our catching effort does not justify assuming that wheatears were trapped on
410 their first day of arrival. When then exposed to the cue conflict in this familiar landscape, they
411 might have decided to simply ignore the conflicting cues, as has been suggested by Muheim
412 et al. (2008) as the reason for the ignorance of the silvereyes (*Zosterops lateralis*) in the study
413 by Wiltschko et al. (2008a).

414 Additionally, the calibration process might be a mechanism operating at the scale of
415 several days and/or calibration's outcome result might be verified during each sunset and
416 sunrise event so that solitary appearing aberrant cues are of no consequence for the general

417 orientation towards the seasonally appropriate migratory direction. Such an inert mechanism
418 would yield a relative robust way of calibrating the compass systems. However, if this is true,
419 we still need explaining why single cue conflicting events let to a recalibration of the compass
420 systems in former studies.

421

422 Conclusions

423 The lack of a response to the cue conflict exposure between the natural magnetic field and the
424 artificially shifted polarization pattern at sunset most likely indicates a simple domination of
425 one of the compasses, most likely the magnetic compass, over the other cues and an ignorance
426 of the cue conflict. Possible reasons for the lack of response to the cue conflict could be
427 manifold. However, three hypotheses resulting from our experiment are that (1) the
428 calibration process may operate at a slower pace, (2) the calibration process may be more
429 robust than formerly anticipated or (3) the rate of calibration may differ amongst species (Liu
430 and Chernetsov, 2012).

431

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440

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579 **Fig. 1.** Predicted departure directions from the study site, Helgoland (54°11'N, 7°55'E), for
580 experimental birds which have experienced a 90° shifted band of maximum polarization
581 during sunset and control-birds, see Materials and Methods for further information. The dot
582 indicates Helgoland. Sea barrier towards west is 525 km.

583

584 **Fig. 2.** Set-up of orientation experiment, here without birds. Exposure cages were positioned
585 on a short-grassy hilltop (54°11'13''N, 7°52'30''E) from where birds had a panoramic view
586 of the horizon, see Materials and Methods for further information. Scale bar is 50 cm. Photo:
587 Heiko Schmaljohann.

588

589 **Fig. 3.** Detected departure direction of a) experimental birds (mean: 141°, 95% CI: 111–178°;
590 Rayleigh test of uniformity: $R=0.54$, $P=0.0056$, $N=17$; black circles) and b) of control-birds
591 (mean: 154°, 95% CI: 135 – 175°; Rayleigh test of uniformity: $R=0.78$, $P<0.0001$, $N=16$; grey
592 circles; cf. Fig. 1). The mean direction of each distribution is represented by an arrow, whose
593 length is drawn relative to the radius of the circle=1. Corresponding 95% CIs are given as
594 dashed lines.

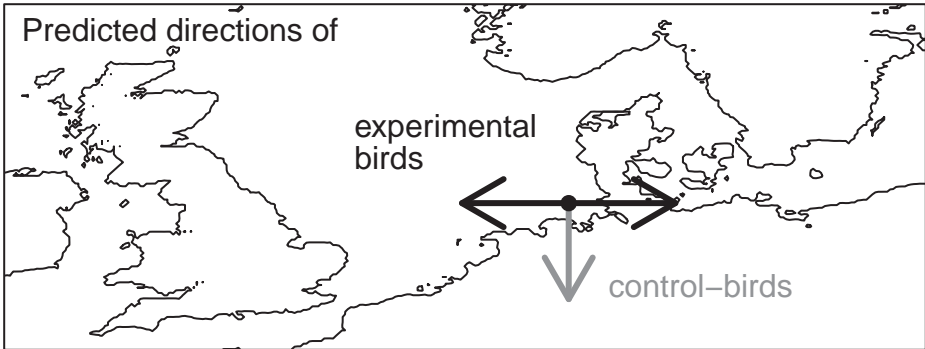
595

596 **Fig. 4.** Deviations from the departure directions of experimental and control-birds of the same
597 night (filled circles). Positive deviation indicates that experimental bird departed clockwise
598 from the corresponding control-bird. Negative deviation indicates that the experimental bird
599 departed anticlockwise from the corresponding control-bird. Mean deviation of the departure
600 directions was 4° (95% CI: 338–33°; Rayleigh test of uniformity: $R=0.71$, $P<0.001$, $N=13$)
601 and is represented by the thick arrow, whose length is drawn relative to the radius of the
602 circle=1. Dashed thick lines give corresponding 95% CI. Corresponding absolute deviation
603 (open circles, drawn slight away from the circumference for sake of clarity) was 40° (95% CI:
604 26°–53°; Rayleigh test of uniformity: $R=0.91$, $P<0.001$, $N=13$) and is represented by the
605 narrow arrow, whose length is drawn relative to the radius of the circle=1. Dotted thin lines
606 give corresponding 95% CI. If the experimental exposure had a significant effect on the
607 departure direction, the 95% CI of the absolute deviation should include the 90° value.

Predicted directions of

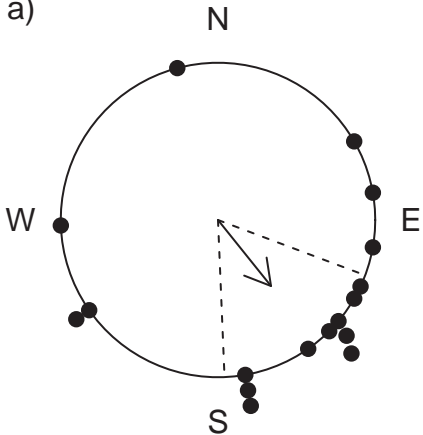
experimental
birds

control-birds





a)



b)

