RESEARCH ARTICLE

Response of a free-flying songbird to an experimental shift of the light polarization pattern around sunset

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SUMMARY

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

Key words: cue conflict, magnetic compass, migration, orientation, polarization pattern, radiotelemetry, songbird, star compass.

INTRODUCTION

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

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

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

MATERIALS AND METHODS
Study site and study species
The study was carried out on Helgoland, Germany, a small island (54°11′N, 07°55′E) in the North Sea, during autumn migration 2008 (6 September–6 October) and 2009 (22 August–29 September). Its
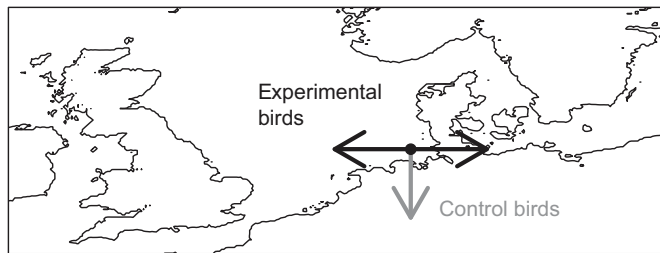
Fig. 1. Predicted departure directions from the study site, Helgoland (54°11′N, 7°55′E), for experimental birds that have experienced a 90 deg shifted band of maximum polarization during sunset and control birds; see Materials and Methods for further information. The black dot indicates Helgoland. Sea barrier towards the west is 525 km.

distance from the nearest land is ~50 km in easterly to southerly directions. Thus birds having left the island can be considered as having resumed migration. Because of the small size of the island, the presence and absence of radio-tagged birds can easily be determined, and nocturnal exploratory flights are not misinterpreted as departure events (Schmaljohann et al., 2011).

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

Housing

The birds were kept indoors for several days (5±2 days, mean ± s.d.) in separate cages (45.0×34.0×23.5 cm) in a room at the Institute of Avian Research. The windows were covered with baking parchment, allowing birds to perceive the natural light conditions, but to prevent them from receiving any information on the polarization pattern. Up to eight birds were stationed simultaneously in the room. Mealworms and water were provided *ad libitum*. The wheatears usually started feeding within several minutes after we had left the room, as revealed by video recording. Each morning (08:00–09:00 h) and evening (17:30–18:30 h) the birds were weighed and their fat score (Kaiser, 1993) and muscle score (Bairlein, 1994) determined. If a bird lost weight during the day, it was not considered for the experiment and immediately released.

Experimental procedure

Experiments were only carried out on evenings with 'good' migration conditions. We used the free available weather forecast program windfinder (www.windfinder.com) to predict weather conditions on evenings and selected nights with no rain and with predicted wind speed of less than 7 m s^{-1}. Stronger winds are supposed to be assessed as unfavourable by migratory birds (Erni et al., 2002). Cloudiness of sky was not considered as the polarization

pattern is also visible when overcast (Hegedüs et al., 2007). Time and azimuth of local sunset was taken from SunEarthTools (www.sunearthtools.com) with settings for Helgoland.

When all of the above criteria were met, we selected two birds in good and similar body condition [i.e. fat score (≥3) and muscle score (≥2)] for the experiment that night (see Results section for further details on body condition of the birds). We attached a radio transmitter to each bird about 2 h before sunset (see 'Radio tracking' section below) and placed one of the birds into the cardboard box of the experiment cage and the other into the cardboard box of the control cage (see 'Exposure cages' section below). Each cage was covered with a cloth, allowing the birds to acclimatize to the conditions for about 45 min. They were then transported, still covered, to the exposure site (54°11′13″N, 7°52′30″E) on a short grassy hilltop (Fig. 2). From there, the birds had a panoramic view of the horizon, slightly interfered by the highest peak of the island, the Pinneberg (azimuth: 150–160 deg) and a small building (azimuth: 295–305 deg).

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

Exposure cages

Two cardboard boxes (30×32×32 cm) had net-covered windows (14×14 cm) on all four side walls. Each cardboard box was placed centrally into a larger wooden box (45×62×39 cm, exposure cage) with the windows congruent with the windows of the cardboard boxes (Fig. 2). All windows of the wooden boxes were covered on the outside with a pseudo-depolarizing filter [two sheets of Hostaphan polyethylene terephthalate (Hoechst, Germany), aligned at an angle of 45 deg relative to each other, resulting in a depolarization of 90% with a 10–15% reduction of light intensity between 400 and 800 nm] as described in the supporting online material of Muheim et al. (Muheim et al., 2006b). We therefore minimized effects on light intensity changes caused by the interaction of the polarizing filters with the natural skylight polarization. On the inside, the windows were covered with polarizing filters [linear polarizer #POA1 (3Dlens Corporation, Taipei, Taiwan); transmittance 38%, polarizing efficiency 99.98% at wavelengths ~400–700 nm].

In the control cage, the polarizing filters were aligned so that the e-vector of polarization simulated a polarization pattern that was in agreement with the natural condition where the band of maximum polarization crosses the zenith at an angle of 90 deg relative to the azimuth of the sun (Muheim et al., 2006b). The e-vectors of the polarization filters in the two windows on the longitudinal axis ('northern' and 'southern' windows) were aligned vertically, while the e-vectors on the lateral axis ('western' and 'eastern' windows) were aligned horizontally; see supporting online material in Muheim et al. (Muheim et al., 2006b). Thus the birds experienced an artificial

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

band of maximum polarization crossing the sky at 90 deg from the sun, approximately going from 'north' to 'south'.

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

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

Radio tracking

Radio transmitters were constructed by the Swiss Ornithological Institute in cooperation with the University of Applied Sciences Bern, Switzerland (Naef-Daenzer et al., 2005). Radio transmitters, including battery and harness, weighed 0.8 g. The transmitters were attached to wheatears using a Rappole-Tipton-type harness made from 0.5 mm elastic cord (Rappole and Tipton, 1991). Length of leg-loops was adjusted individually to birds (Naef-Daenzer, 2007). Since the lowest body mass of the tested wheatears was 20.9 g (mean ± s.d., 29.8±3.8 g, N=42), the mass of the radio transmitter represented less than 3.8% (mean 2.7%) of the birds' body mass. The relative load was, therefore, below the recommended 5% limit (Cochran, 1980; Caccamise and Hedin, 1985). Potential adverse effects on the behaviour of the birds are insubstantial (Naef-Daenzer et al., 2001; Rae et al., 2009), and the increase in flight costs is small (Irvine et al., 2007), though drag (Bowlin et al., 2010; Pennycuick et al., 2012) as well as energy expenditure (Barron et al., 2010) increase. Transmitter life was approximately 30 days.

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

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

The flight altitude of the birds could not be estimated with the radiotelemetry method used. As the island is very small, a potential parallax error in direction estimates is small compared with the bearing accuracy of hand-held antennae (Kenward, 2001). Set-off distance between bird and observer was <500 m (see above); the parallax error with respect to a tracking distance of 15 km would be <2 deg.

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

Departure fuel load and potential flight range

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

$$m_0 = 0.29\omega - 6.85, \qquad (1)$$

where ω is the individual wing length (mm).

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

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

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

$$Y = 100U \ln (1 + f), \qquad (3)$$

where U is the airspeed of the wheatear of 47 km h^{-1} (Bruderer and Boldt, 2001).

Statistics

Statistics were calculated using the statistical software package R (R Development Core Team, 2011). Directions were tested for uniformity with the Rayleigh test of uniformity (Jammalamadaka and SenGupta, 2001). Circular–circular and circular–linear correlations were calculated according to the methods described by Jammalamadaka and SenGupta (Jammalamadaka and SenGupta, 2001). The P-value for a circular–linear correlation was estimated by a randomization test with 10,000 runs each (see Crawley, 2005; Schmaljohann and Naef-Daenzer, 2011).

RESULTS
Conditions of experimental and control birds

Eight experiments were carried out in 2008 and 13 in 2009. None of the variables for body condition differed significantly between experimental and control birds (all Wilcoxon tests for paired samples: P>0.22): wing length (experimental birds: mean ± s.d., 99.6±3.8 mm, N=21; control birds: 101.7±3.6 mm, N=21), fat score (4.0±0.67; 3.8±0.77), departure body mass (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

ranges, which were all sufficient to reach the nearest mainland 50 km away, did not differ between groups (experimental birds: 1350±445 km; control birds: 1276±529 km; Wilcoxon test for paired sample: V=130, P=0.63). Between-year comparisons did not reveal any significant difference in wing length and fat score (Mann–Whitney U-test: P>0.83), but did in departure body mass (2008: 28±4.0 g, N=16; 2009: 31±3.2 g, N=26), departure fuel load (0.25±0.13; 0.38±0.12) and consequently flight range (1022±491 km; 1492±391 km; Mann–Whitney U-tests: P<0.02).

Departure events

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

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

Departure directions

The mean departure direction of the experimental birds was unimodally orientated towards 141 deg (Rayleigh test of uniformity: R=0.54, P<0.0001, N=17; Fig. 3) and direction of control birds towards 154 deg (Rayleigh test of uniformity: R=0.78, P<0.0001, N=16; Fig. 3). Departure directions and angular dispersion around the corresponding means did not differ significantly between both groups (Watson–Williams test: $F_{1,31}$=0.43, P=0.52; Wallraff test: Kruskal–Wallis χ_1^2=1.33, P=0.25), also when considering only nights in which both the experimental (180 deg; Rayleigh test of uniformity: R=0.52, P=0.026, N=13) and control birds (159 deg; Rayleigh test of uniformity: R=0.74, P=0.0003, N=13) departed (Watson–Williams test: $F_{1,24}$=0.02, P=0.88; Wallraff test: Kruskal–Wallis χ_1^2=0.9, P=0.34).

When each departure direction of the experimental birds was plotted as the deviation from its corresponding control bird, the deviations were uniformly distributed and not significantly different from zero. Mean deviation was 4 deg (95% CI: 338 to 33 deg; Rayleigh test of uniformity: R=0.71, P<0.001, N=13; Fig. 4). Absolute deviation was 40 deg (95% CI: 26–53 deg; Rayleigh test
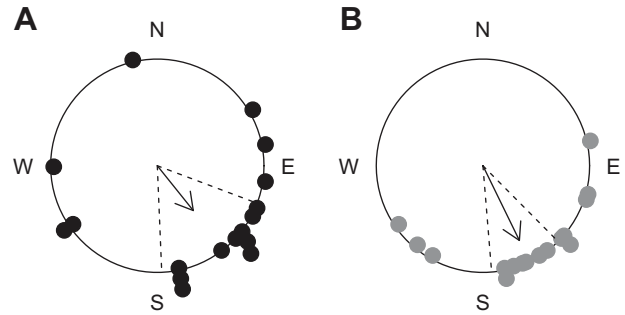
Fig. 3. Detected departure direction of (A) experimental birds (mean 141 deg, 95% CI 111–178 deg; Rayleigh test of uniformity: R=0.54, P=0.0056, N=17; black circles) and (B) control birds (mean 154 deg, 95% CI 135–175 deg; Rayleigh test of uniformity: R=0.78, P<0.0001, N=16; grey circles; cf. Fig. 1). The mean direction of each distribution is represented by an arrow, whose length is drawn relative to the radius of the circle (=1). Corresponding 95% confidence intervals are given as dashed lines.

of uniformity: R=0.91, P<0.001, N=13; Fig. 4) and therefore significantly different from an expected 90 deg shift.

Wind direction, wind speed, cloudiness and visibility at departure did not explain the variation in the departure direction of experimental and control birds (circular–circular correlation: P=0.32, circular–linear correlation: $F_{2,11}$<1.1, P>0.6, N=13). Departure fuel load did not affect the departure direction of either the experimental or control birds (circular–linear correlation: experimental birds: $F_{2,15}$=3.49, P=0.21, N=17; control birds: $F_{2,14}$=0.76, P=0.70, N=16) or when considering only birds departing within the same night (experimental birds: $F_{2,11}$=2.97, P=0.27, N=13; control birds: $F_{2,11}$=0.36, P=0.85, N=13). Departure time after release was significantly correlated with departure fuel load in experimental birds (R_S=–0.50, P=0.043, N=17), indicating that birds with slightly higher departure fuel loads set off earlier in the night after being released than birds with lower departure fuel load. This was not true for control birds (R_S=0.16, P=0.59, N=16). Regarding the birds setting off on the same night, departure time after release did not correlate with departure fuel load in experimental (R_S=–0.41, P=0.16, N=13) or control birds (R_S=0.04, P=0.92, N=13). There was no seasonal effect on departure direction (circular–linear correlation, experimental birds: $F_{2,15}$=0.88, P=0.65, N=17 and $F_{2,11}$=1.30, P=0.55, N=13; control birds: $F_{2,14}$=0.20, P=0.91, N=16 and $F_{2,11}$=0.15, P=0.92, N=13).

DISCUSSION

The experimental and control birds both departed consistently in south-southeasterly directions with no significant difference in the bearing between the groups (Figs 3, 4). A similar migratory direction was expected from earlier evidence on autumn migration by ring recoveries pointing towards south-southwest from Helgoland (Dierschke et al., 2011). Hence we found no compass recalibration as a result of the exposure to the cue conflict between the natural magnetic field and an artificially shifted polarization pattern at sunset. In the case of recalibration, we would have expected a difference in the departure directions between the experimental and control birds of about 90 deg, either as a bimodal distribution [towards −90 and +90 deg (cf. Muheim et al., 2006b)] or a unimodal distribution (either towards −90 or +90 deg; Figs 1, 4). Such shifts in orientation have been observed in Savannah (*Passerculus sandwichensis*) and white-throated sparrows (*Zonotrichia albicollis*) tested in Emlen funnels (Muheim et al., 2006b; Muheim et al., 2009)
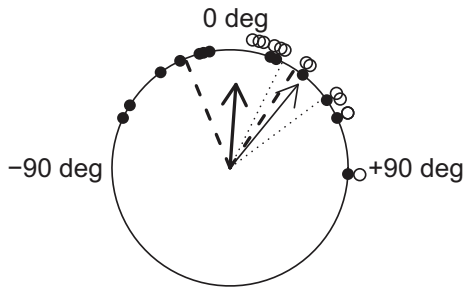
0 deg

−90 deg

+90 deg

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

and in free-flying *Catharus* thrushes (Cochran et al., 2004) after experiencing similar cue conflict exposures. In these cases, the birds calibrated their magnetic compass from the experienced sunset cues.

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

Possible explanations for our results include that the experimental birds either did not calibrate any of their compasses and showed (i) a domination of the magnetic compass or (ii) a domination of the celestial compasses (most likely the star compass) when departing from Helgoland, or alternatively that they (iii) recalibrated the celestial (sunset) compass by magnetic cues and then used the magnetic compass to determine the departure direction, or that they showed (iv) a recalibration of the magnetic compass by the polarized light cues and then used their star compass to determine the departure direction. Thus to be able to make the distinction between these four possibilities, we would need to know which compass the wheatears used when they determined their departure direction from Helgoland, i.e. whether they relied on the magnetic or a celestial compass. The study by Cochran et al. (Cochran et al., 2004) indicated that birds use their magnetic compass when departing at night after all sunset cues had disappeared. Eleven of our wheatears (6 experimental and 5 control birds) set off under ≥4/8 overcast conditions showing no unusual departure directions (mean 131 deg, 95% CI 85–169 deg) in comparison with the others (mean 155 deg, 95% CI 137–177 deg, N=22). Their departure directions and angular dispersion around the corresponding means did not significantly differ between the two groups (Watson–Williams test: $F_{1,31}$=1.29, P=0.26; Wallraff test: Kruskal–Wallis χ_1^2=0.21, P=0.65). Thus our wheatears may have primarily used their magnetic compass to

determine their departure direction, supporting the hypothesis that the magnetic compass might be dominant over the celestial compasses. However, this still does not allow us to discriminate between scenario (i) and (iii), i.e. to determine whether the birds did not calibrate any of their compasses, disregarding the conflicting cues during the cue conflict exposure (i), or whether they recalibrated the celestial compasses relative to magnetic cues during the exposure to the cue conflict between the natural magnetic field and the artificially shifted polarization pattern at sunset (iii).

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

A potential biasing influence of the island and the sea as an ecological barrier for small land birds is also an unlikely explanation for the lack of response to the cue conflict. Birds have been shown to react to an upcoming ecological barrier by a change in their migration direction (Meyer et al., 2000), but the strength of this reaction depends strongly on the body condition of the birds (Sandberg and Moore, 1996; Sandberg, 2003; Deutschlander and Muheim, 2009; Schmaljohann et al., 2011; Schmaljohann and Naef-Daenzer, 2011). Lean birds avoid crossing ecological barriers, whereas fit birds do migrate across large ecological barriers (Schmaljohann and Naef-Daenzer, 2011). As all wheatears had on average potential flight ranges of more than 1000 km, and as departure fuel load did not affect departure direction in this study, the effect of the upcoming sea barrier on the departure direction of the wheatears was most probably low (cf. Schmaljohann and Naef-Daenzer, 2011). Likewise, the landscape of the Courish Spit did not seem to influence the take-off directions of song thrushes in a cue conflict experiment in comparison with the inland site of the same study (Chernetsov et al., 2011).

We are confident that we can exclude problems with the experimental set-up, as the experiments were carried out as in Muheim et al. (Muheim et al., 2006b), except that the birds were radio tracked and experienced a longer cue conflict exposure, on average 167 min compared with 60 min in Muheim et al. (Muheim et al., 2006b; Muheim et al., 2009). Possibly, the wheatears were stressed because of the longer stay in the exposure cages compared with other studies (e.g. Muheim et al., 2006b; Wiltschko et al., 2008a; Muheim et al., 2009). However, in the study of Cochran et al. (Cochran et al., 2004), the birds experienced equally long exposure times, but with the difference that Cochran et al. exposed their birds to a shifted magnetic field under the natural sky, whereas we exposed the birds in an artificially shifted polarization pattern in the natural magnetic field. As the e-vector of the band of maximum polarization vertically intersects the horizon only at the

exact times of sunrise and sunset (the orientation axis of the e-vector at any point in the sky at a ±90 deg angle to the sun is always perpendicular to the beeline to the sun), the birds may have become confused by seeing this vertically aligned pattern at times far from sunset, and therefore may have decided to ignore the pattern.

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

Additionally, the calibration process might be a mechanism operating at the scale of several days and/or the outcome result of the calibration might be verified during each sunset and sunrise event so that solitary appearing aberrant cues are of no consequence for the general orientation towards the seasonally appropriate migratory direction. Such an inert mechanism would yield a relative robust way of calibrating the compass systems. However, if this is true, we still need to explain why single cue conflicting events led to a recalibration of the compass systems in former studies.

Conclusions

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

AUTHOR CONTRIBUTIONS

H.S. proposed the research idea and designed the research. H.S. and T.R. carried out the field work, wrote the manuscript and analysed the data. R.M. contributed to the design of the experimental set-up and B.N.-D. supported the radio tracking study. All authors contributed significantly to drafting and critically revising the manuscript and have read and approved the final version of the manuscript.

COMPETING INTERESTS

No competing interests declared.

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