The Journal of Experimental Biology 215, 2814-2819

doi:10.1242/jeb.069542

RESEARCH ARTICLE

The skylight gradient of luminance helps sandhoppers in sun and moon identification

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SUMMARY

To return to the ecologically optimal zone of the beach, the sandhopper *Talitrus saltator* (Montagu) maintains a constant sea–land direction based on the sun and moon compasses. In this study, we investigated the role of the skylight gradient of luminance in sun and moon identification under natural and artificial conditions of illumination. Clock-shifted (inverted) sandhoppers tested under the sun (during their subjective night) and under the full moon (during their subjective day) exhibit orientation in accordance with correct identification of the sun and the moon at night. Tested in artificial conditions of illumination at night without the artificial gradient of luminance, the artificial astronomical cue is identified as the moon even when the conditions of illumination allow sun compass orientation during the day. When the artificial gradient of luminance is added, the artificial astronomical cue is identified as the sun. The role of the sky gradient of luminance in sun and moon identification is discussed on the basis of present and past findings.

Key words: orientation, *Talitrus saltator*, sun compass, moon compass, skylight gradient of luminance.

Received 16 December 2011; Accepted 28 March 2012

INTRODUCTION

Sandhoppers use a unidirectional, non-vectorial orientation to return to a planar goal, the damp belt of sand they frequent during the day. It has long been known (Papi and Pardi, 1953; Pardi and Papi, 1953) that both the sun and the moon compasses are used by sandhoppers, like *Talitrus saltator* (Montagu), and the relationships between compass systems of orientation have been investigated (Ugolini et al., 1999b; Ugolini, 2003; Meschini et al., 2008). As pointed out previously (e.g. Pardi and Ercolini, 1986), the presence of the two compass systems of orientation is important for sandhoppers because they allow a rapid return to or escape from the damp belt of sand to avoid harmful biotic (predation) or abiotic (high sand temperature, sea storms) factors during the day or at night. Recently, we found a new celestial orienting factor used by sandhoppers in direction finding (i.e. the sea–land direction of the home beach); namely, the skylight gradient of luminance (Ugolini et al., 2009). Hence, sandhoppers can use the skylight gradient of luminance even though it allows a less precise directional choice than the other orienting cues (see also Ugolini, 2003). Experiments on compensation for the apparent movement of the sun have demonstrated that the sun compass works even at night (Pardi, 1954; Ugolini et al., 2002) following the '*Talitrus* model': when clock-shifted sandhoppers (12 h inverted) are released under the sun (during their subjective night), they are able to compensate for the apparent movement of the sun following an internal model in which the sun comes back to east from west, passing through south (Pardi, 1954; Ugolini et al., 2002). Therefore, the modality of sun compensation depends on the time of day and the subjective phase, but this is not so for sun identification: the sun is always correctly identified during the day and during the (subjective) night (Pardi, 1954; Ugolini et al., 2002; Ugolini, 2003).

However, different results were obtained in laboratory releases under artificial illumination that reproduced the scenario with a bright spot of light (the false sun or moon) and the artificial 'sky' by means of a white (opaline) Plexiglas dome (Ugolini et al., 1998; Ugolini et al., 2005). Sandhoppers tested during the day showed the correct direction based on solar orientation when the illumination of the spot of light and artificial sky exceeded a threshold (1.1–1.5 and 3–10 $\mu W\,cm^{-2}$, respectively) (Ugolini et al., 1998; Ugolini et al., 2005). When tested at night, sandhoppers constantly showed good orientation based on the moon compass whatever the intensity of the artificial spot of light and sky (Ugolini et al., 2005). These results suggest that moon identification is largely independent of the intensity of illumination. Hence, our research was to investigate the role of the skylight gradient of luminance in discrimination between the sun and moon, a crucial problem for the survival of sandhoppers.

MATERIALS AND METHODS

Adult individuals of *T. saltator* were collected from a beach in the Natural Park of Migliarino, San Rossore, Massaciuccoli (Pisa, Italy; 43°75′N, 10°30′E) in 2008 and 2009 (June–July) and in 2011 (September). The sea–land direction of the *Y*-axis (perpendicular to the shoreline) was 264–84 deg. After their capture, the sandhoppers were transferred to the laboratory and kept in Plexiglas boxes containing wet sand in conditions of room temperature and an artificial photoperiod (L:D) corresponding in phase and duration to the natural photoperiod. Food (universal dried fish food, SERA Vipan) was constantly available. The animals were tested within 20 days of their capture.

We carried out two types of release, as detailed below.

Experiments during the day and at night under natural sun/moon and sky with clock-shifted individuals

These releases were carried out to test the identification of the moon during the sandhoppers' subjective day. To phase-shift the chronometric mechanism regulating the compensation for the apparent motion of the sun by 12 h (inversion), we kept a group of sandhoppers in a room with constant temperature of 20°C for 15 days with an artificial photoperiod (12 h:12 h L:D) inverted with respect to the natural one: sunrise at 18:00 h, sunset at 06:00 h (Pardi, 1954; Ugolini et al., 2002).

To test the effectiveness of the phase shifting, we released sandhoppers during the day (from 09:30 h to 10:00 h), i.e. during their subjective night. Releases were carried out in Florence (43°46′N, 11°15′E). Night experiments were conducted about 30 km from Florence from 21:00 h to 23:00 h under a full moon (moon phase 100%) to render ineffective the influence of the albedo of the lights of Florence on the directional choice of sandhoppers. The experimental apparatus was a slightly modified version (Ugolini and Macchi, 1988) of that used elsewhere (Pardi and Papi, 1953). The device consisted of a transparent Plexiglas bowl (height 5 cm, diameter 20 cm) set on a goniometer placed on a circular transparent Plexiglas plate (diameter 30 cm) set horizontally on a tripod. A white Plexiglas screen (height 5 cm, diameter 30 cm) placed around the bowl blocked the view of the surrounding landscape but allowed vision of the sun/moon and sky.

To motivate sandhoppers to quickly make a directional choice (not easy in night experiments), we carried out releases in wet conditions (expected landward direction orientation 84 deg), created by putting natural seawater in the bowl where the animals were released (about 1 cm depth).

Luminance profiles of the natural sky during the day and at night are presented in Fig. 1 (profiles a and d).

Experiments in the laboratory at night and during the day with artificial light sources

The experiments were conducted in Florence during a new moon (moon phase 0%). The animals were tested in dry conditions (expected seaward direction for solar orientation 264 deg). In the laboratory, motivation of sandhoppers to orientate was achieved more easily than in the open field at night. Therefore, we opted for the dry experimental conditions because the directional choice of sandhoppers is more precise than in the water releases.

The laboratory treatments were carried out using a device described previously (Ugolini et al., 1998; Ugolini et al., 2005). The bowl containing the animals was covered by an opaline white Plexiglas dome (diameter 80 cm). The glazed internal surface of the dome (artificial sky) was illuminated by a fibre-optic illuminator (Schott KL1500). The end of the fibre bundle (diameter 8 mm) was housed in a tube at the centre of the bowl so that the light source was as close as possible to the centre of the dome. To render the illumination even more uniform, the end of the fibre was equipped with a negative lens (diameter 21 mm, focal length 11.7 mm). To test the role of the skylight gradient of luminance in discriminating between the sun and the moon, we reproduced an artificial gradient inside the dome similar to the gradient we found under the dome in natural conditions of sun and sky: the dome under natural conditions and the dome under artificial illumination had almost the same luminance ratio (although they differed in absolute luminance levels). To recreate the correct gradient of luminance inside the dome, we placed a hemicycle of grey gelatine filter (Medium Grey no. 210, Spotlight, Milano, Italy) on the fibre simulating the sky in a defocused position so that transition between the two hemicycles was gradual (Fig. 1, profile c). Of course, the grey filter was placed in the opposite hemicycle and with its diameter orthogonal to the azimuth of the artificial astronomical cue. A second fibre-optic illuminator, similar to the first one, was used to simulate the astronomical orienting cue. For this purpose, the dome was provided with a circular hole, located 45 deg above the horizon, in which the end of the fibre bundle was inserted (diameter 4.5 mm, angular size 0.32 deg). The luminance profile recorded in the dome under the artificial diurnal sky with the hemicycle of grey gelatine filter on the fibre simulating the sky is presented in Fig. 1 (profile c).

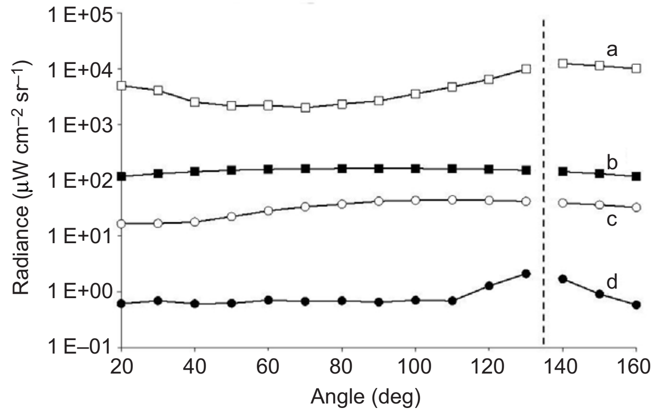

Fig. 1. Luminance profiles (log scale) along the solar meridian between 20 and 160 deg elevation angle (*x*-axis), measured (a) under the natural sky, (b) in the dome under the artificial diurnal sky without a gradient of luminance, (c) in the dome under the artificial diurnal sky with a gradient of luminance. To reproduce the gradient of luminance inside the dome for artificial day (c), we placed a hemicycle of grey gelatine filter on the fibre simulating the sky. (d) Natural sky at night with full moon visible (the night sky profile is multiplied by a factor of 1000 for graphical reasons). The measurement of luminance of the light sources was omitted because of spectroradiometer saturation problems. See Materials and methods for further explanations.

Therefore, the animals in the bowl could see the bright spot of light and the artificial sky. In releases carried out without the artificial gradient of luminance, the intensities of the spot of light and artificial sky were 68 and 172.125 $\mu W\,cm^{-2}$ (Fig. 1B), respectively. In releases with the spot of light and artificial sky switched on and the artificial intensity gradient present, the intensity of the spot of light was 68 $\mu W\,cm^{-2}$, whilst that of the artificial sky was 83.8 $\mu W\,cm^{-2}$ (Fig. 1, profile c). In releases with the spot of light switched off and the artificial intensity gradient present under the dome, the intensity of the artificial sky was 83.8 $\mu W\,cm^{-2}$.

Statistical methods and calculation of ephemerides

Statistical analysis of the circular distributions was carried out as reported elsewhere (Batschelet, 1981). The mean resultant vector was calculated for each distribution. The *V*-test was used to ascertain whether the distribution differed from uniformity ($P<0.05$ at least) by testing the mean direction against the expected direction for sun/moon orientation. The Watson U^2-test (two samples) was used to compare some circular distributions.

The ephemerides and azimuths of the sun and moon were calculated by the Horizons Web-Interface software of the Jet Propulsion Laboratory (California Institute of Technology, USA: http://ssd.jpl.nasa.gov/horizons.cgi).

Characterization of the experimental devices

The luminance was measured in steps of 10 deg with a spectroradiometer (Minolta CS1000) along the meridian passing by

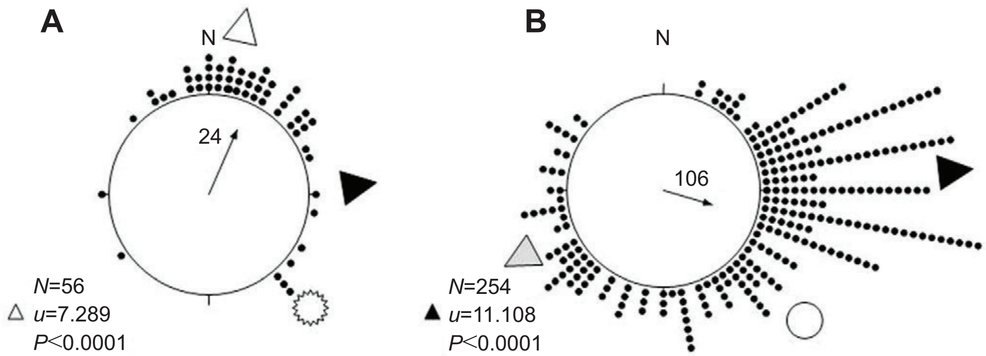

Fig. 2. Releases of clock-shifted (inverted) sandhoppers in seawater in natural conditions of (A) sun and sky and (B) moon and sky (moon phase: 100%). Black triangle, landward direction of the home beach; white triangle, expected direction for clock-shifted individuals based on solar orientation; grey triangle, expected direction for moon orientation during the day. The sun and moon symbols represent the mean sun or moon azimuth; black dots, directions of sandhoppers; black arrow, mean vector and angle (the length of the mean vector ranges from 0 to 1=radius of the circle). *N*, sample size; *u*, *V*-test with probability level *P*.

the spot of light. The measurement of luminance of the spot of light was omitted because of spectroradiometer saturation problems. The spectroradiometer was placed on a stand below the dome in the position occupied by the bowl and pointed at the interior of the dome. The same set-up was used for natural sky measurements. Graphs of luminance along the solar meridian are reported in Fig. 1 on a logarithm scale in order to better represent luminance differences. To evaluate the similarity of the luminance profile along the solar meridian in artificial conditions with respect to natural ones, we normalized the shape of each curve to the absolute value of luminance at the zenith. In Eqn 1 we define D as the absolute difference between two luminance profile curves $L(\vartheta)$, one of which is taken as the target (subscript 'nat'), expressed as a percentage:

$$D = \frac{\int_{0\,\mathrm{deg}}^{180\,\mathrm{deg}} \left| \ln\left[L_{\mathrm{lab}}(\vartheta)\right] - \ln\left[L_{\mathrm{nat}}(\vartheta)\right] \right| \mathrm{d}\vartheta}{\int_{0\,\mathrm{deg}}^{180\,\mathrm{deg}} \ln\left[L_{\mathrm{nat}}(\vartheta)\right] \mathrm{d}\vartheta} \times 100\,. \quad (1)$$

where L_{lab} is the luminance achieved in laboratory experiments and L_{nat} that in the natural environment. Differences between the two values (L_{lab} and L_{nat}) were calculated for each fixed angle (ϑ), summed together with an integral operation and expressed as a percentage of the area subtended under the $L_{\mathrm{nat}}(\vartheta)$ profile. With Eqn 1, the luminance profile of natural conditions of sun and sky was compared with that in the laboratory. The difference between the profile of natural conditions of sun and sky and the profile in artificial laboratory conditions was almost 18% (D=17.5). In fact, in natural conditions, the difference between solar and antisolar hemidomes is more evident than in laboratory conditions. Moreover, the difference between laboratory conditions with or without an artificial gradient of luminance is 4.5% (D=4.5). In cases where the calculation is limited to half the dome, we have of course a low difference in the solar hemidome ($D_{90\text{–}180}$=1.2) and a higher difference in the antisolar hemidome ($D_{0\text{–}90}$=7.9):

RESULTS

Experiments during the day and at night under natural sun/moon and sky with clock-shifted individuals

Individuals tested under the sun during their subjective night (Fig. 2A; see Fig. 1 for the sky luminance profile a) showed a mean direction in agreement with the direction expected for compensation of the sun's apparent movement at night (white triangle). Despite the clock shifting, individuals released at night (i.e. during their subjective day, Fig. 2B) under a full moon (see Fig. 1 for the sky luminance profile d) showed a mean direction in agreement with the expected direction based on the moon compass orientation at night (Fig. 2B, black triangle). However, a minority of individuals seemed to head in a direction in agreement with the landward orientation for moon compensation during the day (Fig. 2A, grey triangle).

Experiments in the laboratory at night and during the day with artificial light sources

These releases were carried out to test the influence of the skylight gradient of luminance on identification of the spot of artificial light as the sun or moon. Fig. 3 shows the results of night (left column) and day (right column) experiments with artificial illumination under the dome in the laboratory. Control experiments (absence of the artificial gradient of luminance) confirmed the orientation of sandhoppers towards the expected (seaward) direction, both at night (Fig. 3A) and during the day (Fig. 3B). It should be stressed that the correct direction of orientation at night (Fig. 3A) was determined by the sandhoppers on the basis of the moon compass mechanism, even though the illumination was the same as in the experiments during the day that allowed the sandhoppers to use the sun compass orientation mechanism.

Fig. 3C,D shows the results of releases in the presence of the artificial gradient of luminance (see Fig. 1 for luminance profile c). Of course, the illumination conditions were the same as for control tests only in the half dome. Whilst the tests during the day (Fig. 3D) produced a distribution similar to that of controls tested during the day, with only a modest deflection of the mean resultant vector (7 deg; Watson U^2 test, $U^2_{40,41}$=0.019, P=n.s.), Fig. 3C shows that the presence of the artificial gradient of luminance at night modified the directional choice of the talitrids by 135 deg with respect to the control releases (Fig. 3A), in agreement with the expected direction for orientation based on the sun compass at night.

Releases carried out under the same experimental conditions with the bright spot of light turned off (Fig. 3E,F) indicated that sandhoppers are still able to head in the expected direction based on the sun compass mechanism, albeit with an increased dispersion of individual directions. In particular, the increased dispersion shown in Fig. 3F with respect to that registered in Fig. 3E could be due to a photopositive component of the directional choice, which could be stronger in Fig. 3E (the expected direction falls in the more illuminated hemicycle) than in Fig. 3F.

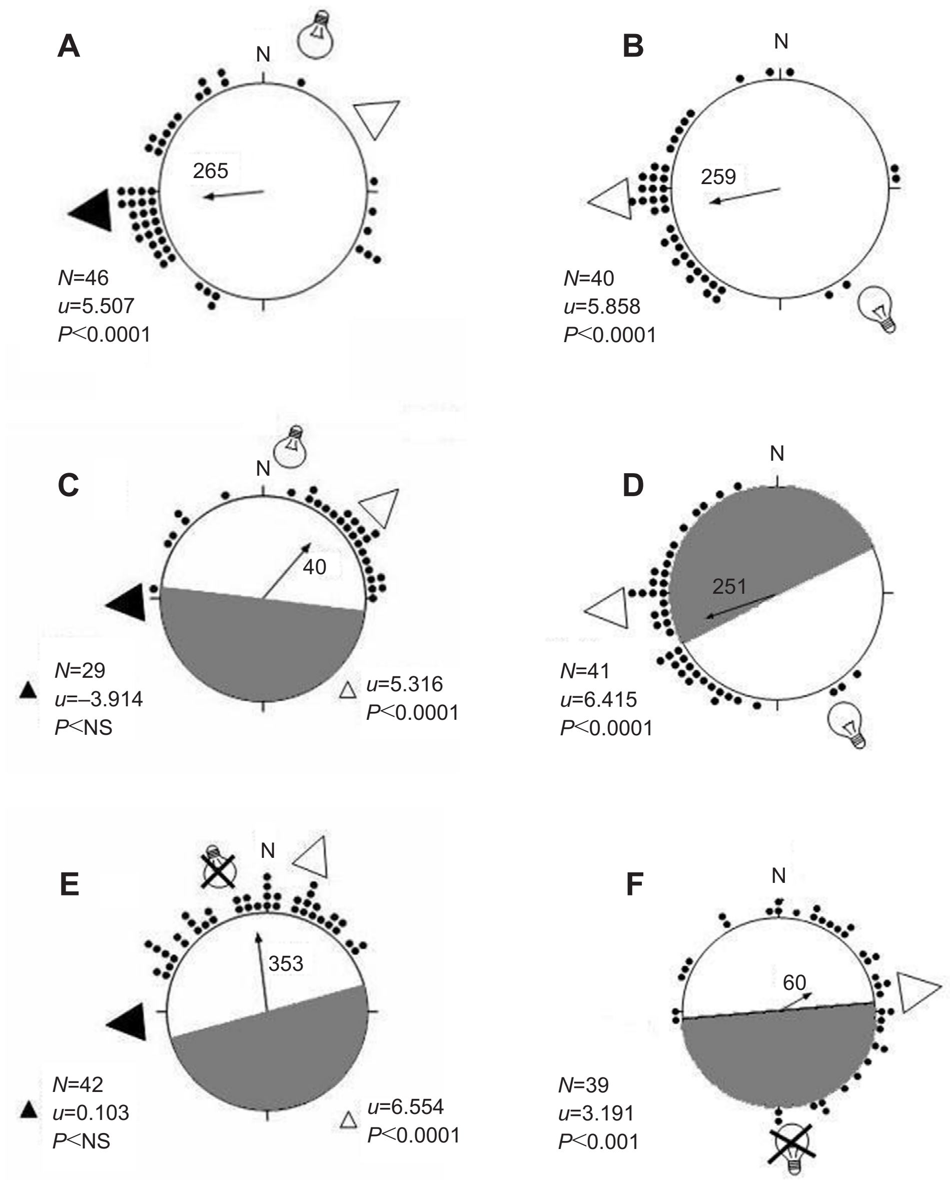

Fig. 3. Releases in dry conditions under the dome with artificial light. Left column, releases at night; right column, releases during the day. (A,B) control releases without the artificial gradient of luminance (spot of light 68 μW cm^{-2}, artificial sky 172.125 μW cm^{-2}); (C,D) releases with the spot of light and artificial sky switched on and the artificial intensity gradient present (spot of light 68 μW cm^{-2}, artificial sky 83.8 μW cm^{-2}); (E,F) releases with the spot of light switched off and the artificial intensity gradient present under the dome (spot of light 0 μW cm^{-2}, artificial sky 83.8 μW cm^{-2}). Black triangle, expected seaward direction for moon orientation; white triangle, expected seaward direction for sun orientation. Light bulb symbol, azimuth of the spot of light; grey area, position of the grey gelatine filter reproducing the intensity gradient of luminance. For further explanations, see Figs 1, 2.

DISCUSSION

Before discussing the results of the present study, we consider it useful to recall some of the results on sun and moon identification by sandhoppers reported in the literature.

(1) Sandhoppers tested under natural conditions of sun during the day show correct identification of the orienting factor (Pardi and Papi, 1953; Pardi and Ercolini, 1986); the same is well known for moon orientation at night (Papi and Pardi, 1953; Papi and Pardi, 1959; Papi, 1960; Enright, 1961; Enright, 1972; Ugolini, 2003; Ugolini et al., 1999a; Ugolini et al., 1999b; Ugolini et al., 2003) (see also present results).

(2) Clock-shifted sandhoppers tested during their subjective night under natural conditions of sun and sky correctly identify the sun (its apparent movement is compensated for following the '*Talitrus* model') (Pardi, 1954; Ugolini et al., 2002; Ugolini et al., 2007) (see also present results).

(3) Sandhoppers tested during the day under artificial conditions of light (in the dome) exhibit a correct directional choice based on sun orientation only when the artificial spot of light exceeds 1.1–1.5 μW cm^{-2} and the sky is illuminated (at least 3–10 μW cm^{-2}) (Ugolini et al., 1998; Ugolini et al., 2005). Otherwise, sandhoppers show a photopositive tendency or their directions are dispersed. This means that when the artificial light intensities in the dome are below the threshold for solar orientation, sandhoppers do not head in the direction expected for moon orientation during the day (i.e. the bright spot is not the moon) (Ugolini et al., 1998; Ugolini et al., 2005).

(4) However, clock-shifted (inverted) individuals tested at night (i.e. during their subjective day) under natural conditions of sky and moon correctly identify the moon, i.e. they do not take it to be the sun (this paper).

These results show a directional choice based on the moon compass at night even though the intensity of the moon (about 130–140 μW cm^{-2}) (Ugolini et al., 2007) theoretically exceeds the threshold to be identified as the sun. However, we should remember that good solar orientation in the laboratory requires an illuminated sky (Terracini-Debenedetti, 1958; Ugolini et al., 1998) and a gradient of luminance in the diurnal sky (this paper). Nevertheless, it is correct to note that a minority of individuals head in the direction expected for use of the moon during the day. Incidentally, this result obtained by testing clock-shifted individuals supports two previous findings. Firstly, the chronometric components of the two compass mechanisms are independent: one for the sun and one for the moon (Ugolini et al., 1999b), in contrast with the results of Meschini and colleagues (Meschini et al., 2008). In fact, the traditional clock shifting for the sun compass chronometric mechanism (i.e. faster or slower phase shift of the light:dark photoperiod with respect to the natural one) does not affect the chronometric mechanism for compensation of azimuthal variations of the moon (Ugolini et al., 1999b). Secondly, the chronometric mechanism of the moon compass works independently of the phase of the moon (Ugolini et al., 2007). However, we must still explain the directional choice of the minority of individuals in the direction expected for use of the moon during the day. We hypothesize that the minority component could be due to a bimodal tendency (towards 264 deg, in line with the sea–land axis of the home beach), which is slightly deflected because of a photopositive component due to the azimuth of the moon (Fig. 2B).

Nevertheless, we also must stress (see point 3 above) that it is not yet clear why sandhoppers tested during the day under artificial dim light (below the threshold of light intensity for solar orientation) do not identify the spot of light as the moon, whilst clock-shifted sandhoppers tested at night during their subjective day do. It should be noted that the apparatus we used to reproduce sun and moon compass orientation under artificial light is far from a complete representation of the natural conditions of sun, moon and sky (see Ugolini et al., 1998).

Moreover, the absence of an influence of the intensity of the spot of light on its identification as the moon is confirmed because of the following points.

(5) Sandhoppers tested at night under the natural or artificial sky and the artificial spot of light simulating the astronomical orienting cue head in the correct direction indicated by the moon compass mechanism. This is largely independent of the intensities of the light spot and artificial sky used in our tests (light spot 0.8–156 μW cm^{-2}, artificial sky 0–172 μW cm^{-2}) (Papi and Pardi, 1953; Papi, 1960; Ugolini et al., 1999a; Ugolini et al., 2005; Ugolini et al., 2007) (see also present results).

(6) Previous experiments carried out during the day under the dome demonstrate that the natural skylight gradient of luminance contributes to correct direction finding by sandhoppers even in the absence of direct vision of the sun (Ugolini et al., 2009).

(7) Sandhoppers tested in the dome at night under artificial illumination (light spot 68 μW cm^{-2}; artificial sky 172.1 μW cm^{-2}) with an artificial gradient of luminance identify the source of illumination as the sun (this paper). It should be noted that under the dome the orientation at night is based on the moon compass mechanism even when the bright spot and the artificial sky intensities would allow very good sun compass orientation in tests during the day (see point 6) (Ugolini et al., 2005; Ugolini et al., 2007) (see also present results). Moreover, releases carried out with the artificial gradient of luminance and the bright spot of light off are consistent with previous findings (see point 6), demonstrating that the gradient of luminance plays a role in direction finding, even though a phototactic component could be present. Identification of the moon based on the absence (or very reduced presence) of the gradient of luminance and its independence of the intensity of the light source is indirectly confirmed by the results of previous experiments in which the natural moon was replaced by an electric torch without regard to the azimuth of the natural moon or the intensity and spectral composition of light to which the sandhoppers were subjected (Papi, 1960; Ugolini et al., 1999a; Ugolini et al., 2005; Ugolini, 2003).

Finally, releases in artificial light with the sky illuminated, the spot of light switched on/off and the gradient of luminance reproduced under the dome confirm the importance of the difference in hemidome luminance in sun identification, despite the fact that the luminance ratio under the artificial sky between the half dome containing the spot of light ('solar' hemidome) and the opposite one ('antisolar' hemidome) is lower than that of the natural sky during the day. In fact, despite an overall difference of 18% (D=17.5) between the natural and artificial sky luminance profiles, the partial difference between the natural and artificial hemidomes indicates that the artificial sky is more uniformly illuminated, while in the natural sky the solar hemidome luminance is higher (D_{0-90}=9.8) than the antisolar one (D_{90-180}=19.0). In terms of absolute luminance, the open sky luminance is more than 2 orders of magnitude higher than the laboratory luminance (Fig. 1). Moreover, it is 7 orders of magnitude higher than the night sky luminance, and this could explain why the gradient of the open night sky, with a shape similar to the day one, is not perceived, as the luminance values could be too low. This hypothesis is supported by previous electroretinogram (ERG) findings [see fig. 3 of Ugolini et al. (Ugolini et al., 2010)] in which we demonstrated that the response height of the sandhopper's eye irradiated by 1.8×10^{14} quanta cm^{-2} s^{-1} (about 80 μW cm^{-2}) was less than 1 mV. Under –2.0 log light irradiance (0.8 μW cm^{-2}), the signals of each response were 'at the limit for detection above noise level'. Moreover, tests on orientation capacity carried out at sunset confirmed the good agreement with the ERG results [see fig. 4 of Ugolini et al. (Ugolini et al., 2004)].

Therefore, (a) the skylight gradient of luminance can be used by *T. saltator* in its direction finding (see also Ugolini et al., 2009) and (b) it is used to identify the sun at night. Of course, we must wonder about the adaptive value of the use of the gradient of luminance, as the sun at night is not characteristic of the latitudes of the *T. saltator* distribution area. Despite our findings demonstrating that the skylight gradient of luminance is used to identify the sun at night, we hypothesize that, on the beach, *T. saltator* uses the gradient of luminance during the day when it is in a position concordant with the direction of orientation indicated by the solar disc and/or when the solar disc is obscured by clouds or under a slightly overcast sky. In fact, preliminary measurements carried out under a completely overcast sky showed a difference of about 20% of luminance between hemidomes.

We must highlight, however, a number of previous experiments in which the solar disc was separated from the skylight gradient of luminance by the classic experiment of deflection of the sun's azimuth by a mirror (Santschi, 1911) [for sandhoppers, see Pardi and Papi (Pardi and Papi, 1953)], as well as the results of tests carried out during the day under artificial illumination in the dome without the artificial gradient of luminance. In both these conditions, sandhoppers usually showed a good orientation towards the expected direction based on the sun compass even though the gradient of luminance was practically absent [in the dome, see Ugolini et al. (Ugolini et al., 1998)] or not concordant (mirror experiments) with the directional indication of the sun compass.

Therefore, we must conclude that the skylight gradient of luminance is only one of the components on which sandhoppers base their identification of the sun and moon. Other factors are probably involved, such as the period of the day and some spectral characteristics of the two astronomical cues (e.g. Cohen et al., 2010). Finally, our results do not exclude the perception and use by sandhoppers of other celestial factors, e.g. the sky colour gradient, as demonstrated in ants and bees (Rossel and Wehner, 1986; Wehner, 1997) (see also Jensen, 2010), or the use of the skylight polarization pattern, as demonstrated in many arthropods (e.g. Wehner, 2001; Horvath and Varju, 2004).

ACKNOWLEDGEMENTS

Thanks are due to the Ente Parco Regionale di Migliarino, San Rossore, Massaciuccoli (Pisa) for authorization to conduct samplings and to two anonymous referees for their useful suggestions.

FUNDING

This research was funded by the Università di Firenze (local funds ex-60%) assigned to A.U.

REFERENCES

Batschelet, E. (1981). *Circular Statistics in Biology*. London: Academic Press.

Cohen, J. H., Cronin, T. W., Lessios, N. and Forward, R. B., Jr (2010). Visual physiology underlying orientation and diel behavior in the sand beach amphipod Talorchestia Longicornis. *J. Exp. Biol.* **213**, 3843-3851.

Enright, J. T. (1961). Lunar orientation of *Orchestoidea corniculata. Biol. Bull.* **120**, 148-156.

Enright, J. T. (1972). When the beachopper looks at the moon: the moon compass hypothesis. In *Animal Orientation and Navigation* (ed. S. R. Galler, K. Schmidt-Koenig, G. J. Jacobs and R. E. Belleville), pp. 523-555. Washington, DC: NASA SP-262.

Horvath, G. and Varju, D. (2004). *Polarized Light in Animal Vision*. Berlin, Heidelberg: Springer Verlag.

Jensen, K. K. (2010). Light-dependent orientation responses in animals can be explained by a model of compass cue integration. *J. Theor. Biol.* **262**, 129-141.

Meschini, E., Gagliardo, A. and Papi, F. (2008). Lunar orientation in sandhoppers is affected by shifting both the moon phase and the daily clock. *Anim. Behav.* **76**, 25-35.

Papi, F. (1960). Orientation by night: the moon. *Cold Spring Harb. Symp. Quant. Biol.* **25**, 475-480.

Papi, F. and Pardi, L. (1953). Ricerche sull'orientamento di *Talitrus saltator* (Montagu) (Crustacea, Amphipoda). II Sui fattori che regolano la variazione dell'angolo di orientamento nel corso del giorno. L'orientamento di notte. L'orientamento diurno di altre popolazioni. *Z. Vgl. Physiol.* **35**, 490-518.

Papi, F. and Pardi, L. (1959). Nuovi reperti sull'orientamento lunare di *Talitrus saltator* Montagu (Crustacea amphipoda). *Z. Vgl. Physiol.* **41**, 583-596.

Pardi, L. (1954). Esperienze sull'orientamento di *Talitrus saltator* (Montagu) (Crustacea, Amphipoda): l'orientamento al sole degli individui a ritmo nictiemerale invertito, durante la 'loro notte'. *Boll. Ist. Mus. Zool. Univ. Torino* **4**, 127-134.

Pardi, L. and Ercolini, A. (1986). Zonal recovery mechanisms in talitrid crustaceans. *Boll. Zool.* **53**, 139-160.

Pardi, L. and Papi, F. (1953). Ricerche sull'orientamento di *T. saltator* (Montagu) (Crustacea, Amphipoda). I. L'orientamento durante il giorno in una popolazione del litorale tirrenico. *Z. Vgl. Physiol.* **35**, 459-489.

Rossel, S. and Wehner, R. (1986). Polarization vision in bees. *Nature* **325**, 128-131.

Santschi, F. (1911). Observations et remarques critiques sur le mécanisme de l'orientation chez les fourmis. *Rev. Suisse Zool.* **19**, 303-338.

Terracini-Debenedetti, E. (1958). Orientational response of some Amphipods under artificial light. *Boll. Ist. Mus. Zool. Univ. Torino* **6**, 21-28.

Ugolini, A. (2003). Activity rhythms and orientation in sandhoppers (Crustacea, Amphipoda). *Front. Biosci.* **8**, s722-s732.

Ugolini, A. and Macchi, T. (1988). Learned component in the solar orientation of *Talitrus saltator* Montagu (Amphipoda: Talitridae). *J. Exp. Mar. Biol. Ecol.* **121**, 79-87.

Ugolini, A., Tiribilli, B. and Castellini, C. (1997). Artificial light and sun compass orientation in the sandhopper *Talitrus saltator* (Crustacea, Amphipoda). *J. Comp. Physiol. A* **182**, 43-50.

Ugolini, A., Melis, C. and Innocenti, R. (1999a). Moon orientation in adult and young sandhoppers. *J. Comp. Physiol. A* **184**, 9-12.

Ugolini, A., Melis, C., Innocenti, R., Tiribilli, B. and Castellini, C. (1999b). Moon and sun compasses in sandhoppers rely on two separate chronometric mechanisms. *Proc. Biol. Sci.* **266**, 749-752.

Ugolini, A., Tiribilli, B. and Boddi, V. (2002). The sun compass of the sandhopper *Talitrus saltator*: the speed of the chronometric mechanism depends on the hours of light. *J. Exp. Biol.* **205**, 3225-3230.

Ugolini, A., Fantini, T. and Innocenti, R. (2003). Orientation at night: an innate moon compass in sandhoppers (Amphipoda: Talitridae*). Proc. Biol. Sci.* **270**, 279-281.

Ugolini, A., Castellini, C. and Tiribilli, B. (2004). The orientation of the sandhopper *Talitrus saltator* during a partial solar eclipse. *J. Comp. Physiol. A* **190**, 855-859.

Ugolini, A., Boddi, V., Mercatelli, L. and Castellini, C. (2005). Moon orientation in adult and young sandhoppers under artificial light. *Proc. Biol. Sci.* **272**, 2189-2194.

Ugolini, A., Castellini, C. and Mercatelli, L. (2007). Moon orientation on moonless nights. *Anim. Behav.* **73**, 453-456.

Ugolini, A., Galanti, G. and Mercatelli, L. (2009). Difference in skylight intensity is a new celestial cue for sandhopper orientation (Amphipoda, Talitridae). *Anim. Behav.* **77**, 171-175.

Ugolini, A., Borgioli, G., Galanti, G., Mercatelli, L. and Hariyama, T. (2010). Photoresponses of the compound eye of the sandhopper *Talitrus saltator* (Crustacea, Amphipoda) in the ultraviolet-blue range. *Biol. Bull.* **219**, 72-79.

Wehner, R. (1997). The ant's celestial compass system: spectral and polarization channels. In *Orientation and Communication in Arthropods* (ed. M. Lehrer), pp. 145-85. Basel: Birkhäuser Verlag.

Wehner, R. (2001). Polarization vision-uniform sensory capacity? *J. Exp. Biol.* **204**, 2589-2596.