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Green land and blue sea: a coloured landscape in the orientation of the sandhopper *Talitrus saltator* (Montagu) (Amphipoda, Talitridae)

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Summary

The use of the landscape in the zonal recovery of *Talitrus saltator* (Montagu) was demonstrated in the past using natural and artificial landscapes. Here we evaluate the importance of colour in the landscape orientation of sandhoppers. Adult individuals of *T. saltator* were released in a Plexiglas bowl under the sun, with a view of an artificial landscape: a black cardboard strip or a pair of differently coloured filters, each occupying 180° of the horizon. Our results not only confirm the influence of the black and white artificial landscape-based compass cue on the zonal orientation of *T. saltator*, but also show that vision of a blue and green artificial landscape affects the direction of orientation; in fact, the orientation agreed with the directional indication of the landscape even when it contrasted with the sun compass indication. The same result was obtained with a blue–grey and green–grey landscape, but not with pairs of grey filters. Therefore, in the sandhoppers' visual world, a coloured landscape that matches the prevalent natural field colours greatly contributes to their directional choice.

Key words: orientation, landscape, colour, vision, sandhopper, *Talitrus saltator*.

Introduction

The orientation of *Talitrus saltator* along the sea–land axis of their home beach (the y axis) is mediated by numerous sensory cues. Some of them, like celestial and astronomical cues, are of a general nature, whereas others are local. Among location-independent compass cues, many species of sandhoppers possess sun, moon and magnetic compasses (see Pardi and Ercolini, 1986; Ugolini, 2001a; Ugolini, 2001b; Ugolini et al., 2002; Ugolini, 2003). Local cues are also important: through learning, sandhoppers can modify the innate compass reference direction to improve their orientation along the sea–land axis direction or when they are displaced to a differently orientated beach (Ugolini and Macchi, 1988; Ugolini et al., 1988; Ugolini et al., 1991). Of course, location-dependent compass cues play an important role in direction finding when the general factors are not available (e.g. cloudy days, moonless nights). Among local cues, the influence of natural or artificial landscape-based compass cues on sandhopper orientation has been frequently hypothesized and tested (Williamson, 1951; Williamson, 1954; Craig, 1973; Hartwick, 1976; Edwards and Naylor, 1987; Ugolini et al., 1986; Ugolini and Cannicci, 1991; Scapini, 1997; Scapini et al., 1997). The artificial landscape usually consisted of black rectangles of different heights occupying 180° on the periphery of a circular arena in which the sandhoppers were released (e.g. Ugolini et al., 1986). Therefore, tests of the landscape-based compass cues on sandhopper orientation were based on a difference in contrast between the two hemicycles: the landward and seaward ones.

The natural landscape, however, is coloured. Yet, we do not know very much about the visual capabilities and spectral sensitivity of sandhoppers. In *T. saltator* from Mediterranean coasts, the visual field in the horizontal plane is quite wide (Beugnon et al., 1987). Studies on the relationship between spectral filtering and solar orientation capacity (Ugolini et al., 1996), as well as preliminary electrophysiological investigations (Ugolini et al., 1996) (M. Lindstroem and A.U., unpublished data), suggested the presence of at least two pigments in the eye: one in the blue range, the other in the green.

Therefore, we decided to evaluate the influence of artificial coloured landscape-based compass cues on the sea–land axis orientation of *T. saltator*.

Materials and methods

We used adult individuals of *Talitrus saltator* (Montagu) collected on a beach near Albegna, Grosseto, southern Tuscany, with the sea–land axis direction towards the sea=268°.

After capture, the sandhoppers were transferred to the laboratory and kept in Plexiglas™ boxes containing wet sand. They were raised in conditions of natural temperature and with a light–dark cycle corresponding in phase and duration to the natural one. Food was constantly available and the sand was kept well aired and wet.

The experimental apparatus was similar to that used by Pardi and Papi (Pardi and Papi, 1953), modified by Ugolini and Macchi (Ugolini and Macchi, 1988). A transparent Plexiglas bowl (diameter 20 cm) was placed on a goniometer placed on a circular transparent Plexiglas plate (diameter 30 cm). We tested a total of 867 sandhoppers. Each animal was tested only once. Groups of 5–7 individuals were released at a time inside the bowl. Previous experiments demonstrated that group releases do not influence the directional choice of sandhoppers (Scapini et al., 1981).

The sandhoppers were released in dry conditions (the empty bowl) and they were previously dehydrated for a few minutes by exposure to direct sunlight. The bowl was covered with a sheet of transparent acetate to prevent the animals from escaping. A cylindrical, opal white Plexiglas™ screen (height 5 cm, diameter 30 cm) placed around the bowl blocked vision of the surrounding landscape but allowed the individuals to see the sun and sky. The observer could read the directions assumed by the tested animals directly below the apparatus.

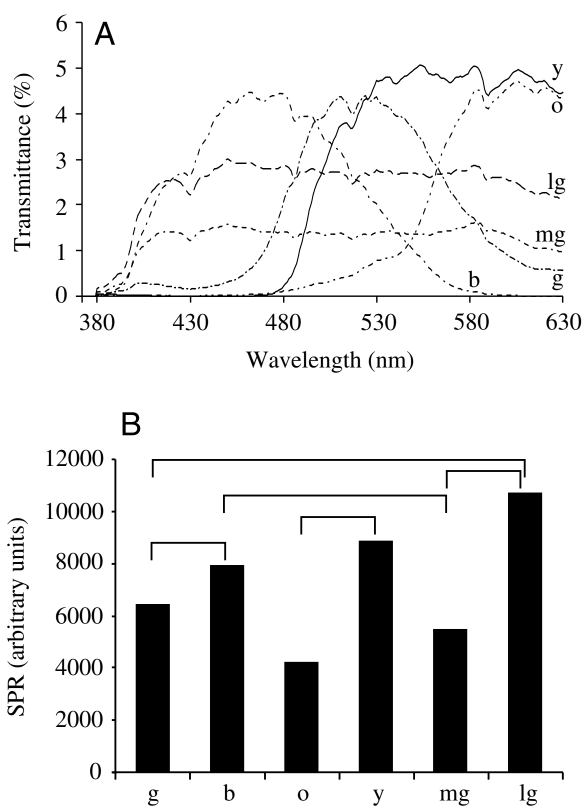

Fig. 1. (A) Transmittance diagrams of the gelatine filters used as the artificial landscape. (B) Sandhopper perceived radiance (SPR) expressed in arbitrary units for the blue (b), green (g), yellow (y), orange (o), light grey (lg) and medium grey (mg) gelatine filters. The filter pairs used in the experiments are also indicated. See text for further explanation of sandhopper perceived radiance.

The experiments were conducted in Florence, Italy, around midday, in spring–summer 2004 and 2005.

The following types of releases were performed. (1) Control sandhoppers were tested under the sun and blue sky, inside the bowl surrounded by the white screen (without vision of the landscape). (2) Experimentals were tested under the sun and blue sky, with vision of an artificial landscape. (a) Groups of animals were tested with vision of a black cardboard strip, 5 cm high (=19° from the centre of the bowl, the sandhoppers' release point), placed on the inside wall of the screen and occupying 180° of the horizon. (b) Sandhoppers were tested with vision of a coloured landscape. In each trial, two differently coloured gelatine filters (blue–green or yellow–orange) were always present inside the screen, each one occupying a hemicycle. The spectral transmittance of each gelatine filter (produced by Spotlight, Milan, Italy) was measured with a Perkin-Elmer λ900 spectrophotometer (Fig. 1A). We used blue and green to simulate the natural landscape (blue=sea and green=Mediterranean macchia), whilst yellow and orange were used as a 'nonsense' landscape since these colours are not prevalent in the natural habitat of sandhoppers. (c) To test if the animals' reactions to the blue–green landscape were due to real colour vision and not merely to a difference in the perceived radiance between hemicycles (the amount of light transmitted by the filters and perceived by the sandhoppers, see below), we also used achromatic (grey) gelatine filters. These two filters were used as a pair, or coupled with the blue and green filters: care was taken to substitute a grey filter of similar sandhopper perceived radiance (SPR, Fig. 1B) to the coloured filter, calculated as the

Table 1. *Goodness of orientation values of sandhopper releases carried out with vision of the sun and sky only (control) and with vision of an artificial landscape either agreeing with the natural disposition (black, green and medium grey towards land) or opposite to the natural situation*

	Goodness of orientation*		
	Artificial landscape position		
Artificial landscape colour	Natural	Opposite	Control
Black–white	0.763	–0.084	0.425
Blue–green	0.611	–0.672	0.347
Yellow–orange	0.343	0.824	0.748
Medium grey–light grey	0.543	0.529	0.389
Blue–medium grey	0.599	–0.500	0.542
Green–light grey	0.865	–0.243	0.703

*Values range from –1 to 1 (see text for further explanation).

In case of concordance with the natural situation, the direction indicated by the landscape position also agreed with the (seaward) direction indicated by the sun compass. For the pair of yellow–orange filters, we arbitrarily decided that the natural disposition corresponded to the orange hemicycle towards land.

integral of the product of filter transmittance and sandhopper spectral sensitivity functions (Ugolini et al., 1996):

$$\mathrm{SPR} = \int_{630\ \mathrm{nm}}^{330\ \mathrm{nm}} T(\lambda) \cdot s_{\mathrm{talitrus}}(\lambda)\mathrm{d}\lambda\ ,$$

where $T(\lambda)$ is the filter transmittance and $s_{\mathrm{talitrus}}(\lambda)$ is the sandhopper spectral sensitivity.

Each filter of every pair and the black cardboard strip were alternately placed in the seaward and landward hemicycle, corresponding to the seaward and landward direction of the home beach.

The analysis of circular distributions was carried out according to Batschelet (Batschelet, 1981). For each distribution, we calculated the mean angle α and the length of the mean resultant vector, **r**. The V test was used to assess if the mean vector of the data had a statistically significant vector component in the direction of the expected direction. To quantify the goodness of orientation, we used Batschelet's formula (Batschelet, 1981) (see p. 41) for the home component=**r**cosΔα, where **r** is the length of the mean vector and Δα is the difference in absolute value between the mean angle (α) and the reference (seaward) direction. To compare the angular dispersion and the angular deviation between circular distributions, we used the I and II Wallraff tests (Wallraff, 1979).

Results

In the experiments carried out with the black cardboard strip (Fig. 2A–C), when the simulated landscape was located in the land hemicycle (Fig. 2B), the seaward orientation improved with respect to the control distribution (Fig. 2A; Tables 1, 2),

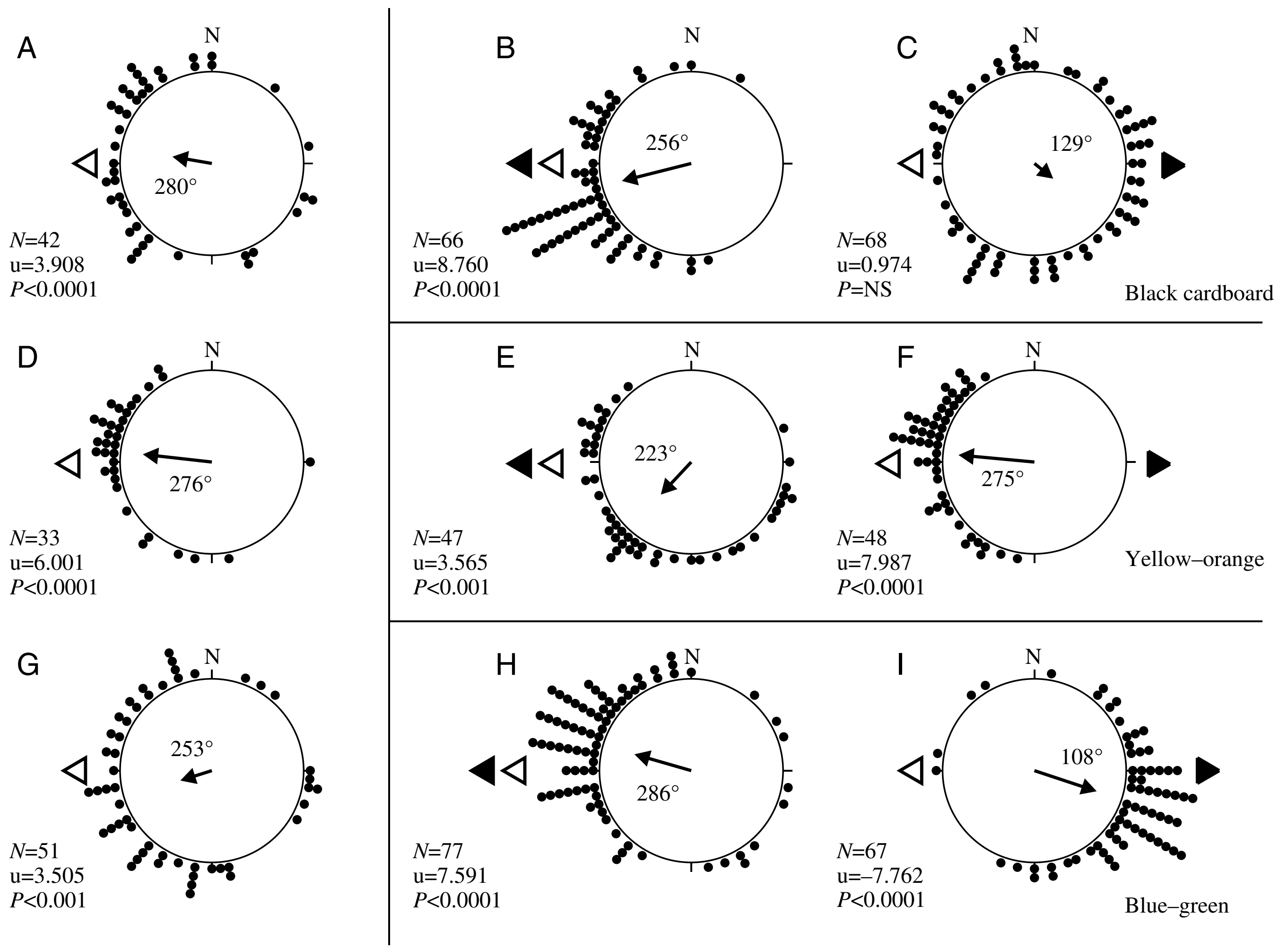

Fig. 2. Directional choice of sandhoppers in a range of coloured landscapes. (A,D,G) Controls with vision of the sun and sky only. (B,C) Black cardboard artificial landscape. (B) Black cardboard landward; (C) black cardboard seaward. (E,F) Yellow and orange artificial landscape. (E) Orange landward, yellow seaward; (F) orange seaward, yellow landward. (H,I) Blue and green artificial landscape. (H) Green landward, blue seaward; (I) green seaward, blue landward. White arrowhead, seaward direction for solar orientation; black arrowhead, seaward direction indicated by vision of the artificial landscape; dots, direction of movement of sandhoppers (one per dot); 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 value with the probability level of at least $P<0.05$.

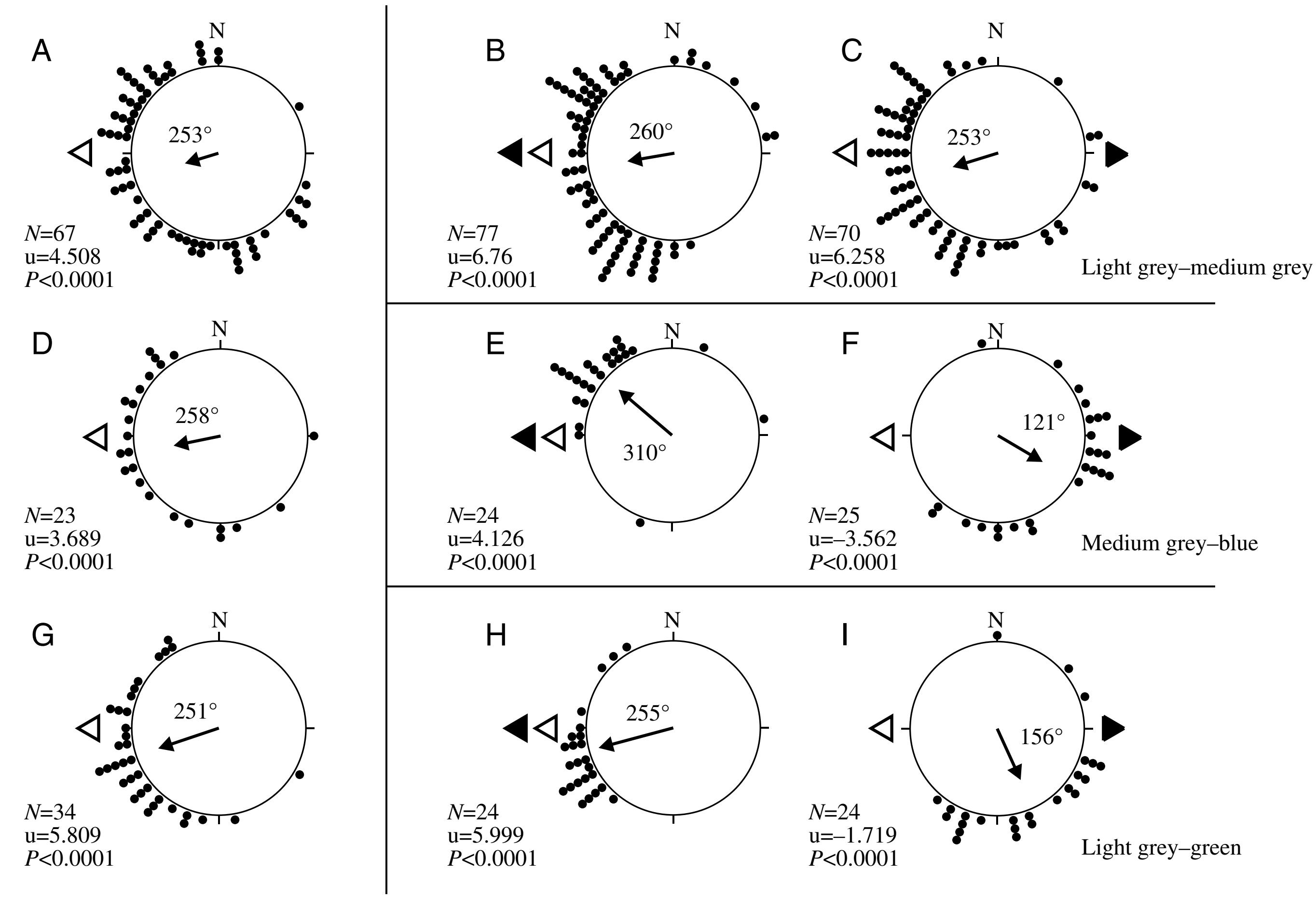

Fig. 3. Directional response of sandhoppers to variation in blue-green landscapes. (A,D,G) Controls with vision of the sun and sky only. (B,C) Medium and light grey artificial landscape. (B) Medium grey landward, light grey seaward; (C) reversed disposition. (E,F) Blue and medium grey artificial landscape. (E) Blue seaward, grey landward; (F) reversed disposition. (H,I) Light grey and green artificial landscape. (H) Green landward, grey seaward; (I) reversed disposition. For further explanation, see Fig. 2.

whereas it became worse when the black strip was placed in the seaward hemicycle (Fig. 2C; Tables 1, 2).

There was a modest effect on the sandhoppers' directional choice in tests with the pair of yellow and orange filters (Fig. 2D–F; Tables 1, 2): when the yellow hemicycle was placed seaward, the goodness of orientation became worse than that of the control releases and worse than when the same hemicycle was placed landward (Table 1). The comparison between control distributions and those with the yellow and orange gelatine strips reached statistical significance for both angular deviation and dispersion when the orange filter was positioned landward (Table 2). However, when the same filter was seaward, no comparison was significant (Table 2).

Sandhoppers tested with the pair of blue and green hemicycles (Fig. 2G–I) showed a constant preference for the blue, independently of its seaward or landward position (Fig. 2H,I, respectively; Tables 1, 2). It should also be noted that the sandhoppers' orientation significantly improved when they were tested with the blue and green gelatine strips, as compared to the control distribution (Fig. 2G; Tables 1, 2).

The pair of grey filters had no effect on the directional choice (angular deviation) of *T. saltator*, independently of their position with respect to the natural sea–land axis (Fig. 3A–C; Table 3); a modest effect on the angular dispersion was present only when the medium grey filter was positioned landward (Fig. 3A *vs* C; Table 3). There seemed to be an increase in the goodness of orientation with respect to that of control (Tables 1, 3) when the medium grey was seaward; however, the control distribution presented a particularly high dispersion (Fig. 3A), and the two values of goodness of orientation obtained in releases with different positions of the grey filters were very similar (Table 1).

When the blue and green filters were coupled with the medium grey and light grey filters, respectively (Fig. 3), the position of the coloured filters matched the natural disposition (blue seaward or green landward), the goodness of orientation improved (Table 1) even though the only significant difference between distributions was in the comparison of angular dispersion (Table 3D *vs* E, medium grey–blue filters). However, when the blue and the green filters were opposite to the natural situation, the goodness of orientation decreased or

Table 2. *Comparison between distributions of Fig. 2*

	Angular deviation		Angular dispersion	
	z	*P*	*z*	*P*
A *vs* B	–3.379	<0.001	–3.881	<0.001
A *vs* C	3.712	<0.001	2.344	<0.02
D *vs* E	2.802	<0.01	2.360	<0.02
D *vs* F	0.048	NS	0.452	NS
G *vs* H	–2.938	<0.01	–3.460	<0.001
G *vs* I	6.667	<0.00001	–3.854	<0.001

Letters correspond to those of Fig. 2.
z, Wallraff test value with the probability level, *P*.
See Fig. 2 for the sample size.

Table 3. *Comparison between distributions of Fig. 3*

	Angular deviation		Angular dispersion	
	z	*P*	*z*	*P*
A *vs* B	–1.248	NS	–1.601	NS
A *vs* C	–1.769	NS	–3.023	<0.01
D *vs* E	0.320	NS	–2.498	<0.02
D *vs* F	4.565	<0.00001	–0.175	NS
G *vs* H	–1.537	NS	–1.740	NS
G *vs* I	5.526	<0.00001	1.351	NS

See Table 2 for further explanations.

the direction of the mean vector was reversed: the comparisons between distributions for angular deviation were all highly significant (Tables 1, 3).

Discussion

The few studies carried out on the visual system of sandhoppers (Ercolini, 1964; Hallberg et al., 1980; Schmitz, 1992) and the absence of investigations of the neurobiology of vision in talitrid Amphipods prevent us from speculating about the photoreceptors that sandhoppers use in achromatic and colour vision (Osorio and Vorobyev, 2005). Nevertheless, tests carried out with the black and white landscape fully confirm the influence of vision of the artificial landscape on the sea–land axis orientation of *T. saltator*, at least as far as the difference in the sandhopper perceived radiance between hemicycles is concerned (Ugolini et al., 1986; Ugolini and Cannicci, 1991). It is also noteworthy that when the seaward direction indicated by the landscape position contrasted with that indicated by the sun compass, the sandhoppers produced a distribution where the mean vector of the data had no statistically significant vector component in the expected direction. This agrees well with previous findings (Ugolini et al., 1986) that vision of the black strip (14° high) did not invert the sandhoppers' mean direction, despite some differences among the populations tested.

Concerning the coloured landscape, we did not attempt to reproduce the 'real colour' of the landscape sandhoppers see in the field during their jumps along the *y* axis of the beach. We only tried to reproduce a scenario with the dominant colours: blue for the sea, green for the Mediterranean macchia. Despite this arbitrary choice, partly based on preliminary knowledge of the spectral sensitivity of *T. saltator* (Ugolini et al., 1996), it is evident that vision of a blue and green landscape greatly influences the direction finding: the direction of the mean vector agreed with the landscape directional indication, even when the latter contrasted with the sun compass indication. This did not occur when the sandhoppers were tested with the pair of light–medium grey filters (the sandhopper perceived radiance of the former was double that of the second). However, the inversion of the seaward orientation was still present when *T. saltator* was tested with the blue–grey or green–grey landscape (similar sandhopper perceived radiance, different colour).

Therefore, we conclude that differences in colour between the hemicycles are important for sandhopper orientation along the sea–land axis of the beach. Nevertheless, not every colour determined the same directional choice of *T. saltator*, even though there was some difference when the yellow filter was positioned in the landward or seaward hemicycle. We can speculate about the reason for the effect of the pair of yellow and orange filters. Considering that these two colours are not predominant in the sandhoppers' environment, the effect could be due to different sensitivity to – and thus perception of – the two colours by the *Talitrus* eye. In any case, the general effect is much less than that of the blue–green landscape.

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