



# OPEN The use of the skylight polarization gradient in young sandhopper's orientation

A. Ugolini<sup>1✉</sup>, Y. Yamahama<sup>2</sup>, T. Hariyama<sup>2</sup> & L. Mercatelli<sup>3</sup>

*Talitrus saltator* is known for its capacity to use celestial orienting cues (sun, moon, skylight spectra, and radiance gradients) to return to the damp belt of sand. adult individuals perceive radiance and/or color gradients more effectively when exposed to polarized light. expert (collected in the field) or inexperienced (born in the laboratory) young of this species exhibit zonal recovery using a solar or lunar compass. Our experiments aimed to test the use of skylight polarization gradient in the zonal orientation of expert or inexperienced young sandhoppers. Our observations of the rhabdom revealed that 14-day-old sandhoppers have thick and disordered microvilli. The microvillar arrangement becomes thinner and regular above 27-day-old sandhoppers. It is known that the microvilli of the rhabdom in the ommatidia are involved in polarized light reception, this suggests that *T. saltator* perceives polarized light efficiently from approximately one month of age. Sandhoppers of various ages were tested in a transparent Plexiglas bowl under an opaline Plexiglas dome. The bowl was covered by a blue gelatin filter with a grey filter and a linear polarizing filter positioned under the blue one in such a way as to occupy half of the upper surface of the Plexiglas bowl to create a linear polarization gradient. Results show that adult and expert young sandhoppers above a month old use the linear polarization gradient, whilst inexperienced young are unable to take any direction independently from their ages. Therefore, our results suggest that the use of the polarization gradient as an orientation factor needs learning in the field.

**Keywords** *Talitrus saltator*, Skylight polarization, Celestial orientation, Microvillar alignment

In the supralittoral environment, various stress factors affect organisms with efficient movements, enabling them to remain in or return to ecologically favorable areas e.g.<sup>1–3</sup>. Supralittoral talitrid amphipods have been the subject of numerous investigations begun in the 1950s<sup>4,5</sup> aimed at shedding light on the orientation factors and mechanisms used in returning to the damp belt of sand near the shoreline and their possible interrelationships<sup>6</sup>. The direction of the sea-land axis, characteristic of each population of sandhoppers, constitutes the set-point of the solar and lunar compasses and, although subject to the possibility of change due to learning<sup>7–10</sup>, is innate and heritable<sup>11–15</sup>.

In fact, the use of the sun, the moon and the natural magnetic field as compass references is integrated by local orientation factors, such as the slope of the substrate and the view of the landscape<sup>16–23</sup>. More recently it has been highlighted that the skylight gradient of luminance helps sandhoppers in sun and moon identification<sup>24</sup> and that the sky radiance and spectral gradient contribute, in a chronometrically compensated compass manner, to the correct directional choice of adult individuals of *Talitrus saltator*<sup>25–27</sup>.

Many animals, especially arthropods, perceive the polarized light of the sky and use the orientation of the e-vector to return to a particular place or more simply to maintain a straight course (e.g. see<sup>28–38</sup>). The mechanism that can determine the direction of the e-vectors is through the presence of visual pigments in the microvillar membrane of the ommatidia of the compound eyes of arthropods. To reflect the properties of this visual pigment, the microvilli must be long and rod-like and they must be aligned in parallel to perceive polarized light by the photoreceptor cell<sup>30,39</sup>.

Recent studies have shown that adult experts (i.e., those collected in the field) of *T. saltator* can perceive polarized light but do not use the e-vector pattern as a reference for orientation<sup>40</sup>. It was noted that adult sandhoppers use the perceived difference in the radiance of polarized light between the solar hemisphere and

<sup>1</sup>Dipartimento di Biologia, Università di Firenze, Via Romana 19, 50125 Firenze, Italy. <sup>2</sup>Institute for Nano-Suit Research, Hamamatsu University School of Medicine, 1-20-1 Handayama, Chuo-ku, Hamamatsu, Shizuoka 431-3192, Japan. <sup>3</sup>Istituto Nazionale di Ottica – CNR, Largo E. Fermi 6, 50125 Firenze, Italy. ✉email: alberto.ugolini@unifi.it

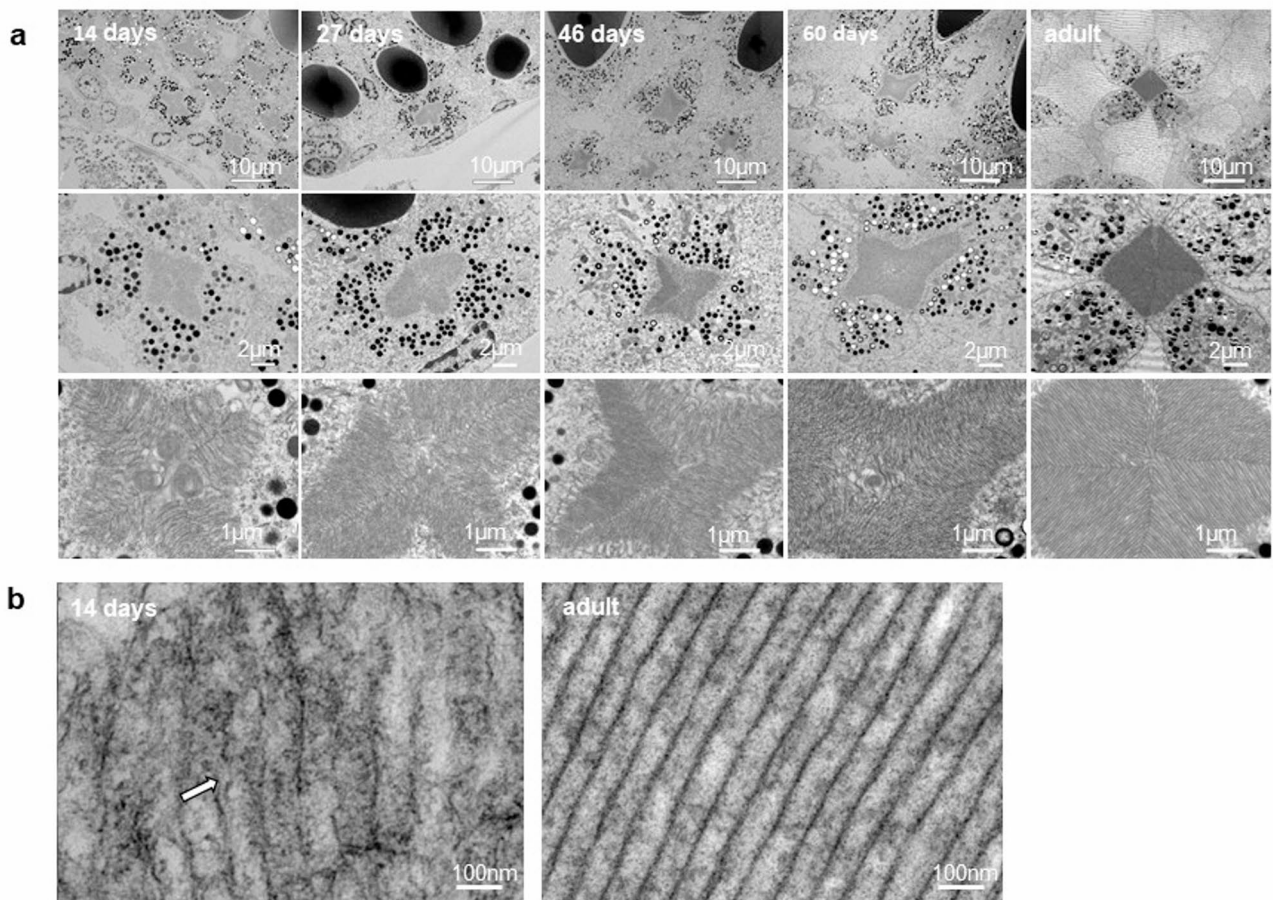
the one opposite the sun in a chronometric manner to determine the direction of the sea-land axis, or at least the direction of the sea and land hemicycles, of the beach of origin<sup>24,26</sup>.

In this paper, we aimed to investigate whether two young groups of *T. saltator*, both inexperienced (born in the laboratory) and experienced (captured in the wild), utilize the celestial polarization gradient in zonal recovery and to investigate the age-dependent structural changes of microvilli in their compound eye.

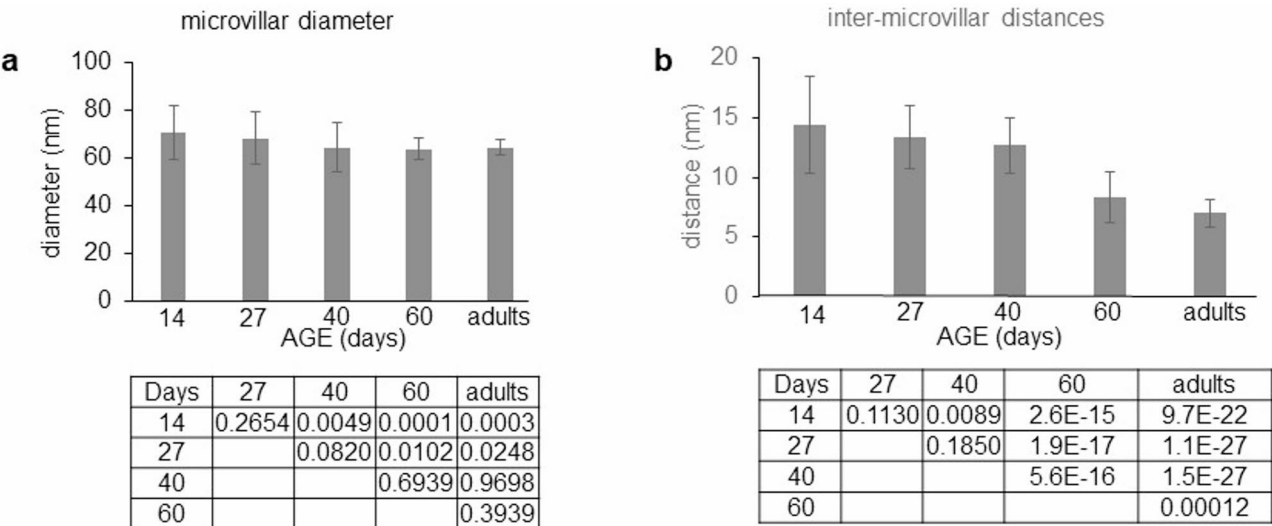
## Results and discussion

Cross-sections of photoreceptor cells (10, 2 and 1  $\mu\text{m}$  thickness) below the tip of the crystalline cone show that all ommatidia at 14, 27, 46 and 60 days of age in laboratory-born sandhoppers have five reticular cells with many microvilli (see also<sup>41,42</sup>) and many pigment granules (Fig. 1a). Cross-sections obtained in young sandhoppers 14–46 days old show similar features: the rhabdoms were cross-shaped, with the microvilli of each rhabdomere being concave toward the cell body, however, the shape of rhabdom of 60 days old *T. saltator* is square (cf<sup>42</sup>). Moreover, the notable age difference is that the microvilli forming the 14-day-old sandhoppers' rhabdoms were squiggly and disorganized (Fig. 1a left bottom photo, Fig. 1b), the microvilli appeared thicker than the others and there are many gaps between the adjacent microvilli (Fig. 1b arrow), and the 27, 46, and 60 days old microvilli were seemed also thicker than adults and sometimes possesses gaps.

However, the diameter of the microvilli significantly decreases and their alignment improves (i.e. the inter-microvilli distance decreases) with age from the juvenile to the adult forms (Fig. 2a, b). Meyer - Rochow and Reid<sup>43</sup> also found the diameter change of microvilli of a crab eye depending on age and claimed that researchers should consider the microvillar size of experimental animals' ages. Of course, further research is needed to determine the growing mechanism of microvilli, and the sensitivity change such as response sensitivity and polarization sensitivity. Based on our observations of aligned microvilli in each rhabdomere (Figs. 1a, b and 2) we hypothesize that young sandhoppers older than 30 days can perceive the polarized light, in fact microvilli diameter stabilizes after 27 days of age while their distance continues to decrease until adulthood.



**Fig. 1.** (a) Images of cross sections observed with a transmission electron microscope at 14, 27, 46, 60-day of age and adult. The vertical columns show different magnifications at the almost same areas. The microvilli become compact and more regular in outline, and the sizes of the rhabdom increase depending on the age of the sandhoppers. (b) Enlarged TEM examples of the microvillar difference between 14-day-old and adult *T. saltator*. The white arrow indicates one of the gaps between adjacent microvillar membranes.



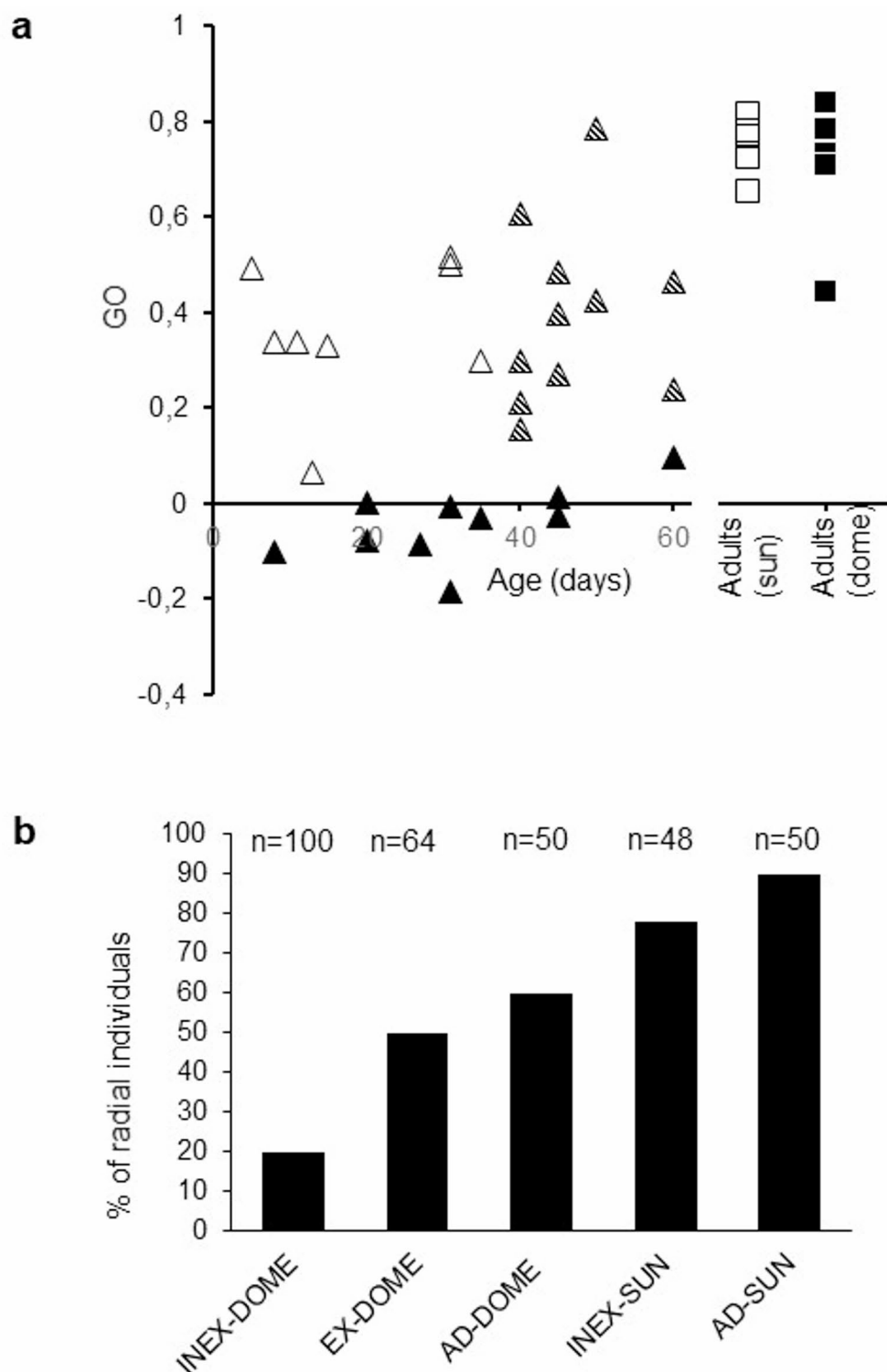
**Fig. 2.** The microvillar diameters and the inter-microvillar distances of *T. saltator* rhabdomere ( $n = 50$ ). **(a)**, the diameters of the microvilli were about 70 nm on the 14th and 27th days, but they became 65 nm at around from the 46th day to adult. **(b)**, the inter-microvillar distances become closer and tighter with growth: by day 46 they were about 12–14 nm, but by day 60 they were less than 7 nm apart. The tables show the probability levels obtained in comparisons between the means of two groups at different ages.

In the tests carried out in the dome, the comparison between goodness of orientation (GO) (see Materials and Methods) calculated for releases of inexperienced young vs. expert young shows that the latter are significantly better oriented towards the expected direction than the other group ( $U_{10,11} = 0$ ,  $P < 0.001$ , Mann-Whitney U test) (Fig. 3a). The same is for the comparison between inexperienced young and adults tested in the dome ( $U_{10,5} = 0$ ,  $P < 0.002$ , Mann-Whitney U test) and for the expert young vs. adults in the dome ( $U_{11,5} = 6$ ,  $P < 0.02$ , Mann-Whitney U test). In both cases the adults are better directed towards the sea (Fig. 3a).

A similar result is obtained by comparing inexperienced young tested in the dome vs. inexperienced young tested under the sun (Fig. 3a;  $U_{10,8} = 1$ ,  $P < 0.001$ , Mann-Whitney U test): the young tested under the sun are significantly better oriented than the inexperienced young tested in the dome. The inexperienced young tested under the sun, although discreetly directed towards the expected direction (Fig. 3a), show the usual greater dispersion compared to the expert adults who appear better oriented ( $U_{8,5} = 0$ ,  $P < 0.001$ , Mann-Whitney U test) (e.g. see<sup>14</sup>). The comparison between adults tested in the dome vs. adults tested under the natural sun ( $U_{5,5} = 12$ ,  $P > 0.20$ , Mann-Whitney U test) nor the comparison between expert young tested in the dome vs. inexperienced young tested under the sun ( $U_{11,8} = 41$ ,  $P > 0.20$ , Mann-Whitney U test) are not statistically significant.

Figure 3b shows that the inexperienced young sandhoppers tested under the dome have greater difficulty in heading in any direction compared to adults and expert young ones tested in the same condition (inexpert young vs. adults,  $G = 23.256$ ,  $df = 1$ ,  $P < 0.001$ ; inexperienced young vs. expert young  $G = 15.893$ ,  $df = 1$ ,  $P < 0.001$ , G test); however, this is not confirmed for expert young (expert young vs. adults,  $G = 1.120$ ,  $df = 1$ ,  $P > 0.35$ , G test) although adults show a slightly higher percentage of radially oriented individuals (= 60%, Fig. 3b). Even the comparison between inexperienced young and adults tested under the sun does not reach full statistical significance ( $G = 2.952$ ,  $df = 1$ ,  $0.05 < P < 0.1$ , G test), although adults reach 90% of radial individuals out of the total released ( $n = 50$ ). Finally, it is also interesting to note that the expert young tested under the dome have a lower percentage of radiality compared to the inexperienced ones tested under the sun ( $G = 8.643$ ,  $df = 1$ ,  $P < 0.01$ , G test).

Therefore, adult and young (expert) sandhoppers collected in the field use the polarization gradient to head towards the ecologically efficient direction (the sea, damp belt of sand of the shoreline). Adult sandhoppers tested under the sun and adults tested with the polarization gradient alone (in the dome) do not appear to be different in the GO, confirming what has already been recently published<sup>26</sup>. Laboratory-born young (inexpert) tested under the sun assume an orientation comparable to that of expert young tested in the lab, although the latter show greater difficulty in assuming a radial direction. The ability of inexperienced young tested under the sun to head towards the direction of the sea of the beach of origin has been known for some time<sup>11,12</sup> and it has also been widely demonstrated that the solar compass mechanism in *T. saltator* is innate and heritable (e.g. see<sup>8,13,14</sup>). The orientation ability of the young experts tested under the dome (in the presence of the polarization gradient but without the vision of the sun) can be hypothesized to depend on the association between the vision of the sun disc (solar orientation) with the polarization gradient. This association can be hypothesized to occur during the excursions sandhoppers undertake on the beach, and therefore subject to individual variability in learning ability. This lack of association, experimentally prevented for the inexperienced young tested under the dome, could explain why the (inexpert) young born in the laboratory tested with the polarization gradient alone are not able to assume an ecologically effective direction: the most of the inexperienced young is unable to take any direction: most of them turn around the perimeter of the bowl. Unlike the use of the sun and moon as orienting factors in the zonal recovery of adult and young sandhoppers, our results suggest that the use of the polarization gradient



**Fig. 3.** (a) Relationship between goodness of orientation (GO) and age of expert and inexperienced sandhoppers. Black squares (■), tests with adult individuals under the dome; open squares (□), tests with adult individuals under the sun. Black triangles (▲), tests with inexperienced young sandhoppers conducted under the dome; open triangles (△), tests with inexperienced young sandhoppers conducted under the sun. Dashed triangles (▤), tests with expert young sandhoppers conducted under the dome. (b) The graph shows the difficulty in assuming any radial direction during the experiment. INEX-DOME, inexperienced young individuals tested in the dome; EX-DOME, expert young tested in the dome; AD-DOME, adult sandhoppers tested in the dome; INEX-SUN, inexperienced young tested under the sun; AD-SUN, adult sandhoppers tested under the sun. See text for further explanations.



as an orientation factor is learned. On the other hand, it is worth remembering that alongside the innate basis of solar orientation, there is a strong learning component capable not only of improving orientation along the sea-land axis of sandhoppers but also of adapting the use of the solar compass to variations in the direction of the shoreline<sup>7–10</sup>.

## Materials and methods

### Collection and breeding

Adult and young sandhoppers, *Talitrus saltator*, were collected during spring and summer 2020–2023 on a sandy beach in the Regional Natural Park of Migliarino, S. Rossore, Massaciuccoli (Tuscany, Pisa, 43°40′03″N, 10°20′29″E, sea-land axis of the beach = 265°–85°) with entomology aspirators. In the rearing room, some of the adult sandhoppers were placed in pairs of different sexes in transparent plastic boxes (20 × 12 × 13 cm) containing wet sand, blotting paper, and dried fish food (SERA® Vipan, Heisenberg, Germany) ad libitum. The females were previously inspected using a binocular microscope to ensure the absence of eggs in the pouch. The possible presence of young born from each couple was monitored daily and the date of birth was recorded (i.e. the abandonment of the maternal pouch by the offspring). The other sandhoppers were separated into adults and young and placed in boxes identical to those described above. During their stay in the rearing room, all the sandhoppers were kept at a temperature of 25 ± 2 °C, with a 12-hour light:12-hour dark (12 L:12 D) cycle in phase with the natural photoperiod. They were not allowed to see the sun, sky or natural light.

The young sandhoppers collected in nature (experts) were tested at presumed ages between 40 and 60 days-old. To determine the age, we referred to Williams<sup>44</sup> by counting the number of articles of the flagellum of the second antennae. However, it is worth noting that this method offers a significant range of uncertainty since the molting, and consequently the number of articles of the flagellum, depend on numerous external environmental factors.

### Morphological observations

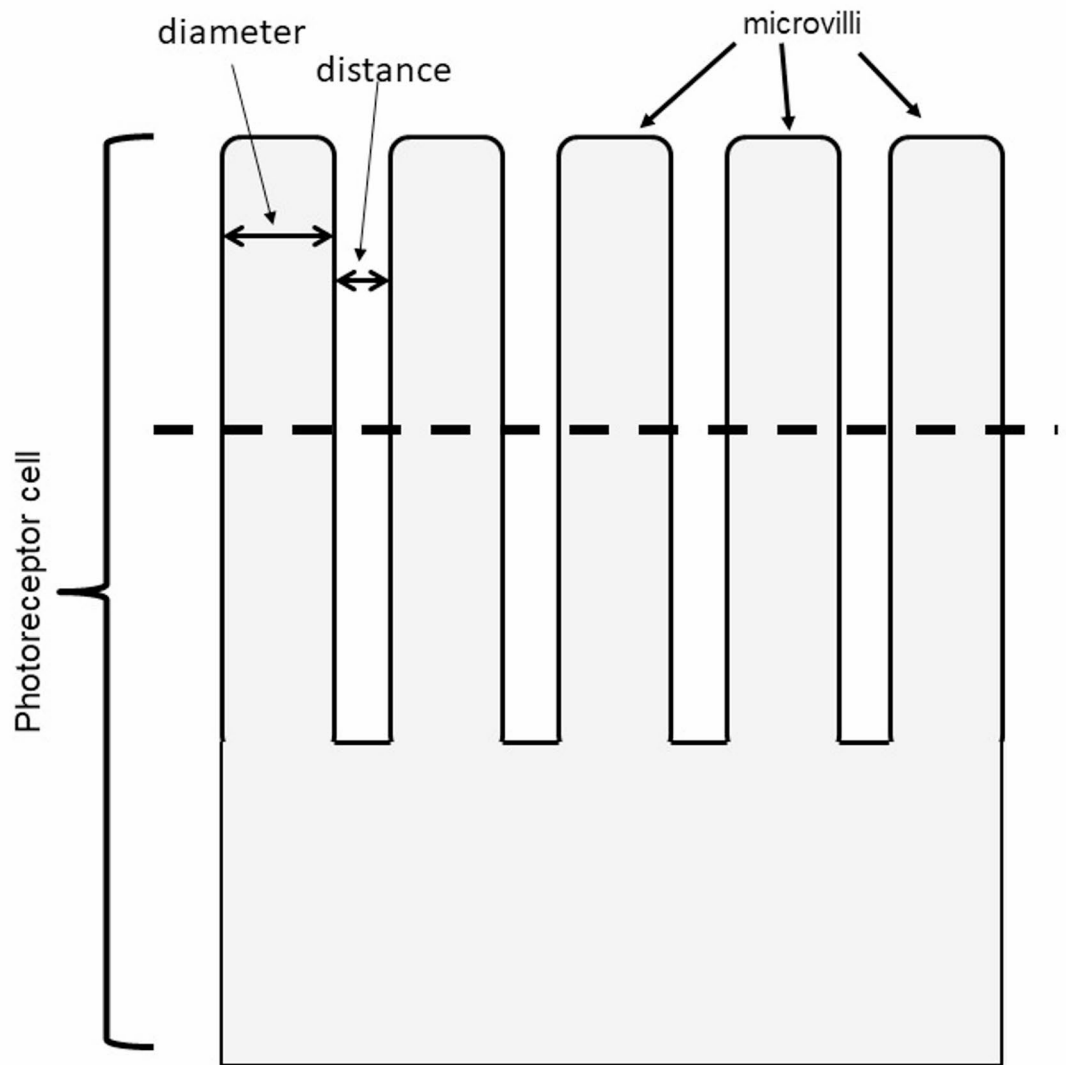
To investigate age-related morphological differences, we observed eye structure of *T. saltator* at 14, 27, 46, and 60-day-old born in the laboratory. The heads of five sandhoppers of each group were dissected in a prefixative solution, consisting of 2% paraformaldehyde and 2% glutaraldehyde in a 0.1 mol/L sodium cacodylate solution (pH 7.4). The heads were then rinsed in the buffer solution, and the eyes were removed using a razor blade. The eyes were postfixed for 2 h with 1% OsO<sub>4</sub> in the same buffer solution. The fixed tissues were dehydrated using a series of graded ethanol solutions, then transferred to propylene oxide, and finally embedded in 45% Araldyte-Quetol 812. For transmission electron microscopy, 70 nm thick sections were cut using a Leica UC7 ultramicrotome and then placed on a formvar-coated copper grid with a single hole. The samples were double-stained with 1% uranyl acetate solution for 5 min and 0.1% lead citrate solution for 3 min. The eyes of young animals on different days after birth and adults were observed using a JEOL JEM 1400-plus electron microscope.

To test the possible relationship between the age of sandhoppers and the shape of microvilli as found by Meyer-Rochow and Reid<sup>43</sup> in decapod crustaceans, we measured the diameter of microvilli and the distance between adjacent microvilli. We drew a straight line perpendicular to the microvilli's long axis and measured the internal space of each microvilli and the space between two adjacent microvilli. (Fig. 4).

We randomly chose 5 animals 14, 27, 46, 60 days old, and adults. Ten measurements were made on 5 TEM sections of different animals at each age. 50 cross-sectional microvillar diameter and width were compared by the Student *t*-test.

### Orientation tests

Laboratory-born young animals (inexperts) were tested under the sun and clear sky (5 to 40 days-old) and in the laboratory in a confined environment (5 to 60 days-old). Groups of a maximum of 10 individuals (adults, young experts, and inexpert young born in the laboratory) were tested under the sun or in the laboratory, in a confined environment, around local noon. Solar orientation tests were carried out using an apparatus described several times in the past (e.g. see<sup>7</sup>). Essentially, this consists of a transparent Plexiglas bowl (diameter = 18 cm) placed on a horizontal transparent plate (diameter = 28 cm) surrounded by an opaline Plexiglas cylinder (4 cm high) preventing the vision of the surrounding landscape. The bowl was empty, allowing sandhoppers to jump, walk or attempt to climb the sides of the bowl. A single direction for each individual was recorded 2 min after each release and established from freeze-framed images taken by a camera placed below the bowl. The sandhoppers were released only once. A schematic representation of the experimental setup is available in Fig. 5a. Laboratory releases were carried out in a darkened room using the same equipment used for similar experiments conducted on adult *T. saltator*<sup>26</sup>. The experimental setup consists of the transparent Plexiglas bowl covered by an opaline Plexiglas dome (diameter = 30 cm,) to diffuse the incoming artificial light on the bowl produced by a white LED Panel Lamp (100 W 12 V, Sumbulbs) driven with a controlled power supply (Elind mod. 32DP32) placed at a distance of 37 cm from the top of the bowl (Fig. 5b). Since previous experiments show three different peaks of ERG spectral sensitivity curves in *T. saltator* at 390, 430, and 450 nm<sup>42,45</sup> a blue gelatin filter (no 118 Light Blue, λ<sub>max</sub> = 450 nm) was placed on the Plexiglas bowl. Moreover, a grey filter (no 209, 0.3 optical density, transmittance = 46%, 400 nm < λ < 600 nm, SpotLight, Milan, Italy), and a linear polarizing filter (HN42, Polaroid – Knight Optical, UK), were cut in way as to occupy half of the upper surface of the Plexiglas bowl, joined together along the diameter and positioned above the bowl, under the blue filter (Fig. 5b). Transmittance of blue, grey and polarizing filters were measured by means of a Perkin-Elmer Lambda 1050 spectrometer with an experimental error of about 2%. Since a polarizing filter only lets through the incident light with polarization corresponding to the polarization axes of the filter itself, to equalize the amount of light perceived by *T. saltator* in the two halves of the bowl it was necessary to place a neutral density filter (grey filter) on the half opposite to that of the polarizing filter. The polarization axis is parallel to the junction line of the

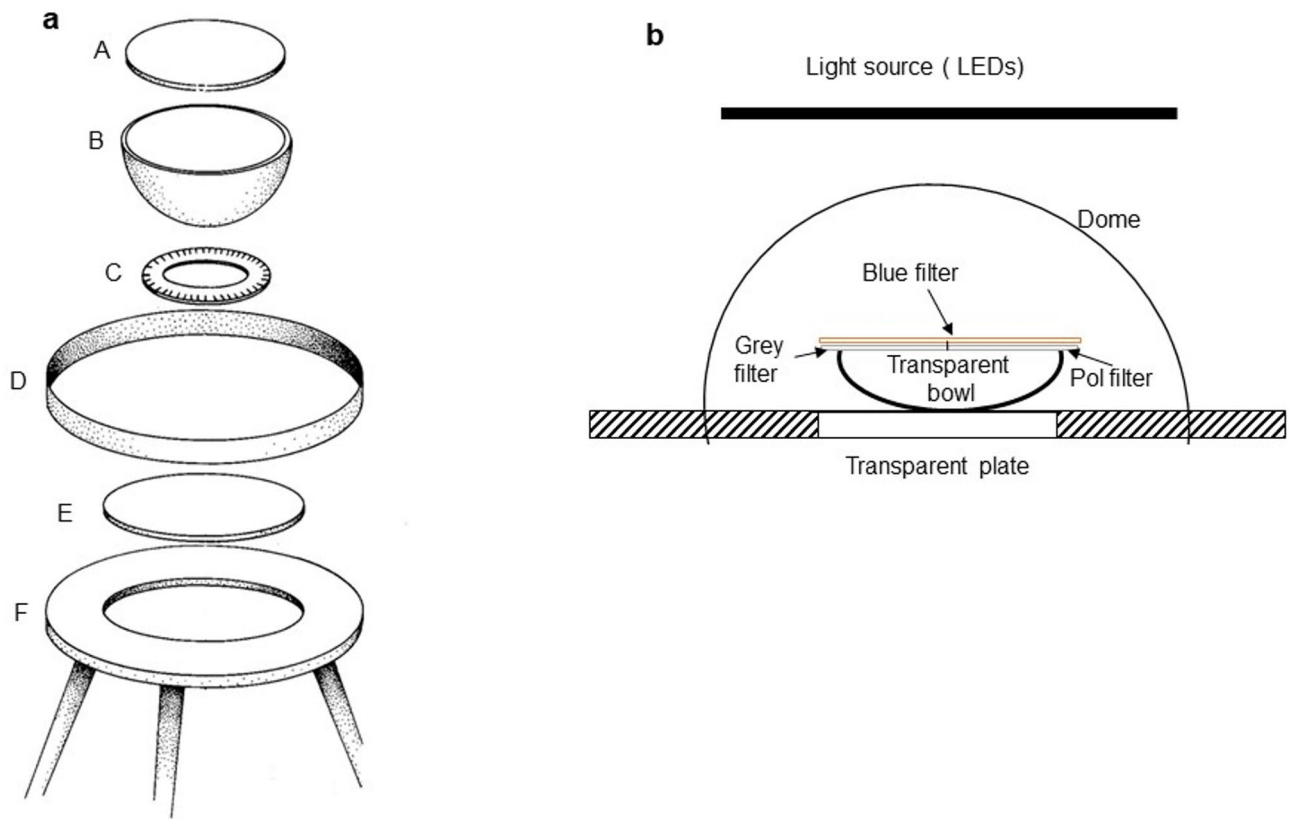


**Fig. 4.** Schematic drawing of a photoreceptor cell showing the measurements of microvillar diameters and inter-microvillar distances. The horizontal dashed line shows the level at which the measurements were made.

two filters. The irradiance measured under the filter package, was  $150 \mu\text{W}/\text{cm}^2$ . The two vertical lines in Fig. 6a show the “window” of wavelengths projected on the sandhoppers during the experiment: in fact, are limited below 400 nm by the spectrum emitted by the LED source (which does not contain shorter wavelengths), and above 600 nm by the cutoff of the blue filter. The radiance along the solar meridian was measured at  $10^\circ$  intervals with a radiometer (Ophir mod. Nova Display, head PD300UV) equipped with an optic fiber and an objective mounted on a miniaturized rotating holder with fixed  $10^\circ$  steps: the rotation took place perpendicularly respect to the horizontal plane, following the meridian which passes through the zenith. Measurements performed with the system radiometer-optic fiber-objective-rotating holder are affected by a total error of approximately 10%, taking in to account the radiometer sensitivity, system throughput and mounting accuracy. Thus, considering the experimental error we can conclude that measurements showed the equality of the two halves (Fig. 6b). Moreover, if we calculate the integral in the range 350–650 nm of the combination of source + blue filter + gray filter compared to the one of source + blue filter + polarization filter we achieve a difference  $< 4\%$  which can be explained with spectral differences and errors explained above. Therefore, the only orientation cue was the polarized light on one half of the artificial sky.

The motivation behind applying overhead visual stimulus divided into a polarized and unpolarized half is that natural sky presents the maximum of polarization orthogonal to the sun. The connection between the grey filter and the polarizer was positioned in the north-south direction ( $0^\circ$ – $180^\circ$ ), with the polarizer pointing east ( $90^\circ$ ). This arrangement was kept constant regardless of the time of the experiment. Therefore, we had to calculate the theoretical direction of orientation to the sea for each release.

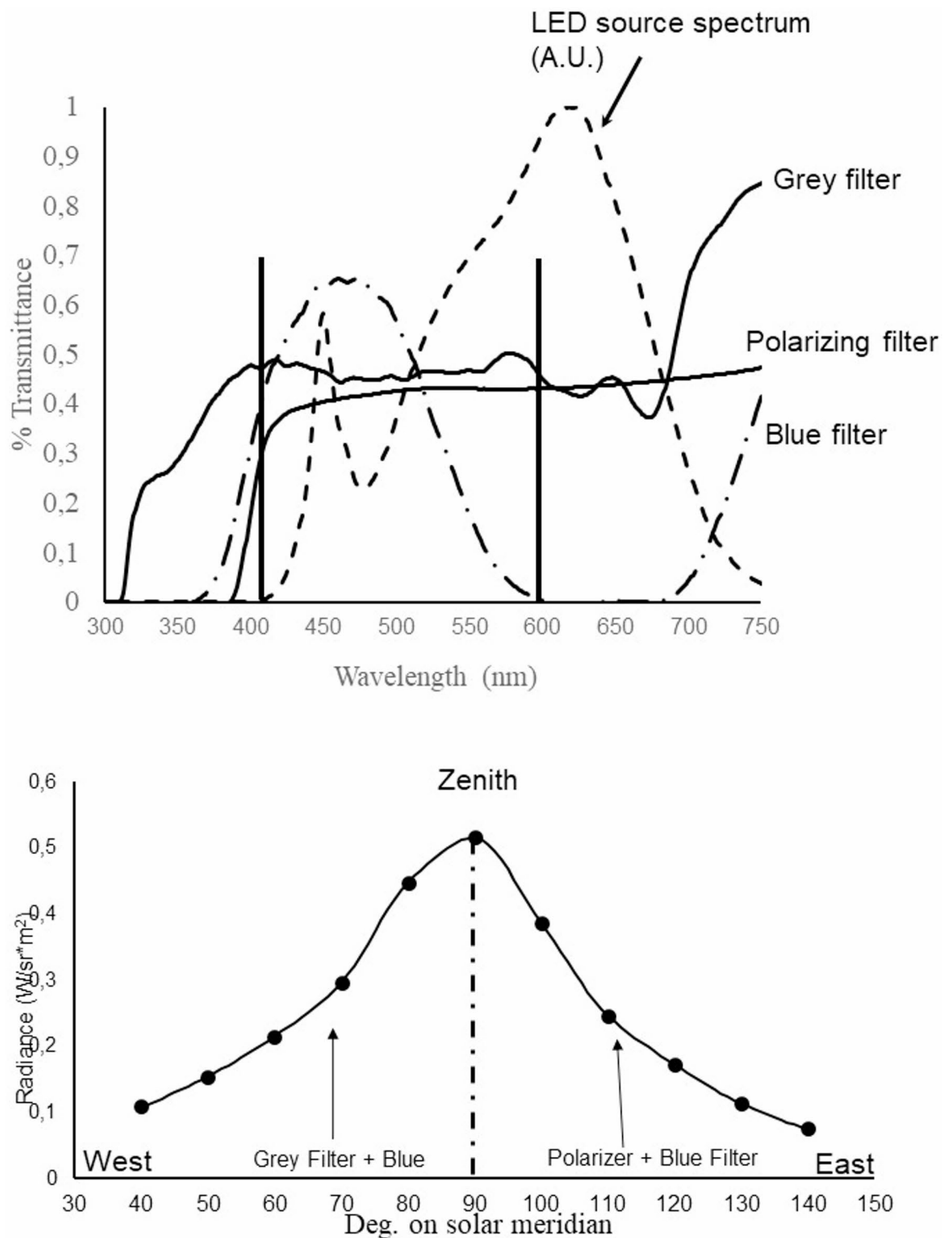
For each release, the “goodness of orientation” was considered,  $\text{GO} = r \cdot \cos(\alpha - \text{TED})$  (i.e. the “home component” defined by Batschelet<sup>46</sup>) where  $r$  and  $\alpha$  are, respectively, the length of the mean resultant vector and its mean angle calculated for each release (see<sup>46</sup>), the TED is the Theoretical Expected Direction towards which



**Fig. 5.** (a) Experimental set-up used in releases under the sun and natural sky (modified from<sup>7</sup>). A, upper closure of the bowl. B, Plexiglas bowl; C, goniometer; D, opaline Plexiglas screen; E, Plexiglas plate; F, aluminium tripod. (b) Schematic representation of the set-up used for experiments in the laboratory under the dome (from<sup>26</sup> modified). The dome was illuminated by an external light source. The upper side of the transparent bowl was covered with blue, grey and polarizing filters. The sandhoppers are released in the transparent bowl; the directions were recorded by a video-camera from below.

the sandhoppers should head for a perfect seaward orientation ( $265^\circ$  for the locality of collection). Comparisons between groups were carried out using the Mann-Whitney  $U_{n_1, n_2}$  test<sup>47</sup>.

To better document the possible “difficulty” for sandhoppers in making directional choices (not necessarily in the direction of TED), we also considered the number of individuals exhibiting radial orientation. That is, with the head pointed toward the outside of the bowl and the longitudinal body axis oriented no more than  $\pm 45^\circ$  from the radius of the bowl. The numbers of radial individuals were compared by using the G-test ( $P < 0.05$  at least)<sup>48</sup>.



**Fig. 6.** (a) Spectral transmittance of the filters and light source used. The black vertical bars show the “window” of wavelengths projected on the sandhoppers during the experiment. (b), radiance measured along the solar meridian of the dome. The West and East parts are equalized when the polarizing filter and the neutral density grey filter are both present. The irradiation along the solar meridian was measured at 10° intervals using a radiometer.

#### Data availability

Data are available upon request addressed to alberto.ugolini@unifi.it for orientation; to hariyama@hama-med.ac.jp for morphology; to luca.mercatelli@ino.it for spectral transmittance and irradiance.



Received: 6 March 2025; Accepted: 13 May 2025

Published online: 20 May 2025

## References

- Vannini, M. & Chelazzi, G. Adattamenti comportamentali Alla vita intertidale tropicale. *Oebalia NS*. **11**, 23–37 (1985).
- Raffaelli, D. & Hawkins, S. Intertidal ecology. (*London Chapman Hall*, ). (1996).
- Widdows, J. & Brinsley, M. Impact of biotic and abiotic processes on sediment dynamics and the consequences to the structure and functioning of the intertidal zone. *J. Sea Res.* **48**, 143–156 (2002).
- Pardi, L. & Papi, F. Die sonne Als Kompass Bei *Talitrus saltator*. *Naturwissenschaften* **39**, 262–263 (1952).
- Pardi, L. & Papi, F. Ricerche sull'orientamento Di *Talitrus saltator* (Montagu) (Crustacea-Amphipoda). I. L'orientamento Durante Il Giorno Di Una popolazione Del litorale Tirrenico. *Z. Verh. Physiol.* **35**, 459–489 (1953).
- Herrnkind, W. F. Movement patterns and orientation. In *The Biology of Crustacea Behavior and Ecology* Vol. 7 (eds Vernberg, F. J. & Vernberg, W. B.) 41–105 (Academic, 1983).
- Ugolini, A. & Macchi, T. Learned component in the solar orientation of *Talitrus saltator* (Montagu) (Amphipoda, Talitridae). *J. Exp. Mar. Biol. Ecol.* **121**, 79–87 (1988).
- Ugolini, A. & Scapini, F. Orientation of the sandhopper *Talitrus saltator* (Amphipoda, Talitridae) living on dynamic sandy Shores. *J. Comp. Physiol. A*. **162**, 453–462 (1988).
- Ugolini, A., Scapini, F., Beugnon, G. & Pardi, L. Learning in zonal orientation of sandhoppers in *Behavioural adaptation to intertidal life*. NATO Adv. Workshop Castiglione, (Eds. Chelazzi, G. & Vannini, M.) (Plenum Press, New York, 1988). (1987).
- Ugolini, A., Felicioni, S. & Macchi, T. Orientation in the water and learning in *Talitrus saltator* (Montagu). *J. Exp. Mar. Biol. Ecol.* **151**, 113–119 (1991).
- Pardi, L., Ercolini, A., Marchionni, V. & Nicola, C. Ricerche sull'orientamento degli Anfipodi del litorale: il comportamento degli individui allevati in laboratorio sino dall'abbandono del marsupio. *Atti Accad. Sci. Torino, I Cl. Sci. Fis. Mat. Nat.* **92**, 1–8 (1958).
- Pardi, L. Innate components in the solar orientation of Littoral amphipods. *Cold Spring Harbor Symp. Quant. Biol.* **25**, 395–401 (1960).
- Pardi, L. & Scapini, F. Inheritance of solar direction finding in sandhoppers: mass-crossing experiments. *J. Comp. Physiol.* **151**, 435–440 (1983).
- Scapini, F., Ugolini, A. & Pardi, L. Inheritance of solar direction finding in sandhoppers. II. Differences in arcuated coastlines. *J. Comp. Physiol. A*. **156**, 729–735 (1985).
- Ugolini, A., Fantini, T. & Innocenti, R. Orientation at night: an innate Moon compass in sandhoppers (Amphipoda: Talitridae). *Proc. R Soc. Lond. B*. **270**, 279–281 (2003).
- Williamson, D. I. Studies in the biology of Talitridae (Crustacea, Amphipoda): visual orientation in *Talitrus saltator*. *J. Mar. Biol. Ass. UK*. **30**, 91–99 (1951).
- Williamson, D. I. Landward and seaward movements of the sandhoppers *Talitrus saltator*. *Adv. Sci. (London)*. **11**, 71–73 (1954).
- Craig, P. C. Orientation of the sand-beach amphipod, *Orchestoidea corniculata*. *Anim. Behav.* **21**, 699–706 (1973).
- Hartwick, R. F. Beach orientation in Talitrid amphipods: capacities and strategies. *Behav. Ecol. Sociobiol.* **1**, 447–458 (1976).
- Ugolini, A., Scapini, F. & Pardi, L. Interaction between solar orientation and landscape visibility in *Talitrus saltator* (Crustacea: Amphipoda). *Mar. Biol.* **90**, 449–460 (1986).
- Edwards, J. M. & Naylor, E. Endogenous circadian changes in orientational behaviour of *Talitrus saltator*. *J. Mar. Biol. Ass. UK*. **67**, 17–26 (1987).
- Ugolini, A. Optic flow and sea - land orientation in the sandhopper *Talitrus saltator* (Montagu). *J. Exp. Biol.* **217**, 2041–2043 (2014).
- Ugolini, A. & Ciofini, A. Landscape vision and zonal orientation in the Equatorial sandhopper *Talorchestia martensii*. *J. Comp. Physiol. A*. **202**, 1–6 (2016).
- Ugolini, A., Galanti, G. & Mercatelli, L. The skylight gradient of luminance helps sandhoppers in sun and Moon identification. *J. Exp. Biol.* **215**, 2814–2819 (2012).
- Ugolini, A., Galanti, G. & Mercatelli, L. Difference in skylight intensity is a new celestial cue for sandhopper orientation (Amphipoda, Talitridae). *Anim. Behav.* **77**, 171–175 (2009).
- Ugolini, A., Hariyama, T., Wilcockson, D. C. & Mercatelli, L. The use of polarized light in the zonal orientation of the sandhopper *Talitrus saltator* (Montagu). *Zool Letters* **9:10** (2023).
- Ciofini, A., Mercatelli, L., Hariyama, T. & Ugolini, A. Sky radiance and spectral gradient are orienting cues for the sandhopper *Talitrus saltator* (Crustacea, Amphipoda). *J. Exp. Biol.* **224** (2), jeb239574 (2021).
- Waterman, T. H. Polarization sensitivity. In: *Handbook of sensory physiology. Vision in invertebrates*. (Ed. by H. Autrum). pp. 281–469. Vol. VII/6B. Springer-Verlag Berlin Heidelberg New York. (1981).
- Wehner, R. The ant's celestial compass system: spectral and polarization channels. In: *Orientation and Communication in Arthropods* (Ed. by M. Lehrer). pp. 145–187. Birkhauser Verlag Basel/Switzerland. (1997).
- Labhart, T. & Meyer, E. P. Detectors for polarized skylight in insects: a survey of ommatidial specializations in the dorsal rim area of the compound eye. *Microscopy Res. Tech.* **47**, 368–379 (1999).
- Horváth, G. & Vári, D. *Polarization Light in Animal Vision. Polarization Patterns in Nature* (Springer-, 2004).
- Dacke, M., Baird, E., El Jundi, B., Warrant, E. J. & Byrne, M. How Dung beetles steer straight. *Annu. Rev. Entomol.* **66**, 243–256 (2021).
- El Jundi, B., Smolcka, J., Baird, E., Byrne, J. & Dacke, M. Diurnal Dung beetles use the intensity gradient and the polarization pattern of the Sky for orientation. *J. Exp. Biol.* **217**, 2422–2429 (2014).
- Cronin, T. W., Johnsen, S., Marshall, N. J. & Warrant, E. J. *Visual Ecology* 405 (Princeton Univ, 2014).
- Patel, R. N. & Cronin, T. W. Mantis shrimp navigate home using celestial and idiothetic path integration. *Curr. Biol.* **30**, 1981–1987 (2020).
- Zittrell, F., Pfeiffer, K. & Homberg, U. Matched-filter coding of Sky polarization results in an internal sun compass in the brain of desert locust. *PNAS* **117**, 25810–25817 (2020).
- Homberg, U. et al. The Sky compass network in the brain of the desert locust. *J. Comp. Physiol. A*. **209**, 641–662 (2023).
- Wiltshko, R. & Wiltshko, W. W. Animal navigation: how animals use environmental factors to find their way. *Eur. Phys. Spec. Top.* **232**, 237–252 (2023).
- Israelachvili, J. N. & Wilson, M. Absorption characteristics of oriented photopigments in microvilli. *Biol. Cybernetics*. **21**, 9–15 (1976).
- Ugolini, A., Galanti, G. & Mercatelli, L. Do sandhoppers use the skylight polarization as a compass cue? *Anim. Behav.* **86**, 427–434 (2013).
- Ercolini, A. Sulla struttura Degli Occhi composti Di *Talitrus saltator* Montagu (Crustacea-Amphipoda). *Redia* **49**, 129–138 (1965).
- Ciofini, A., Yamahama, Y., Mercatelli, L., Hariyama, T. & Ugolini, A. Specializations in the compound eye of *Talitrus saltator* (Crustacea, Amphipoda). *J. Comp. Physiol. A*. **206**, 711–723 (2020).
- Meyer-Rochow, V. B. & Reid, W. A. Does age matter in studying the crustacean eye? *J. Comp. Physiol. B*. **319**, 319–324 (1996).
- Williams, J. A. The relationship between antennal segment number and moulting in *Talitrus saltator* (Montagu, 1808) (Amphipoda, Talitridae). *Crustaceana* **53**, 243–252 (1987).

45. Ugolini, A., Borgioli, G., Galanti, G., Mercatelli, L. & Hariyama, T. Photoresponses of the compound eye of the sandhopper *Talitrus saltator* (Crustacea, Amphipoda) in the ultraviolet-blue range. *Biol. Bull.* **219**, 72–79 (2010).
46. Batschelet, E. *Circular Statistics in Biology* (Academic, 1981).
47. Siegel, S. & Castellan, N. J. Jr. *Nonparametric Statistics for the Behavioral Sciences* 2nd Edn 399 (McGraw-Hill, Stat. Ser., 1988).
48. Zar, J. H. *Biostatistical Analysis* 2nd edn (Prentice Hall, 1984).

## Acknowledgements

We wish to thank the Ente Parco Naturale Regionale MSRM (Pisa, Italy) for the authorizations to sampling. This work was supported by JSPS KAKENHI Grant Number JP22 K18250 assigned to TH and RICATEN 2023 University of Florence funding assigned to AU.

## Author contributions

Design of the work AU, LM; acquisition, analysis, interpretation of data AU, LM for orientation, YY, TH for morphology; wrote and revised the paper AU, LM, YY, TH. All the Authors approved the submitted version.

## Declarations

## Competing interests

The authors declare no competing interests.

## Additional information

**Correspondence** and requests for materials should be addressed to A.U.

**Reprints and permissions information** is available at [www.nature.com/reprints](http://www.nature.com/reprints).

**Publisher's note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

**Open Access** This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>.

© The Author(s) 2025