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Outlanders in an Unusual Habitat: *Holothuria mammata* (Grube, 1840) Behaviour On Seagrass Meadows from Ria Formosa (S Portugal)

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Abstract

Holothuria mammata is one of the new target species from the Mediterranean and Atlantic. Usually, it inhabits rocky bottoms, staying in crevices and holes during the day and leaving them in the night for feeding on sandy bottoms. However, it can be found in unusual habitats such as seagrass with diurnal and nocturnal feeding. This study provides information for the first time on the behaviour, density and small scale distribution of *H. mammata* in a seagrass habitat from Ria Formosa (S Portugal). To reach these aims, a mark/recapture methodology was used. Abundance was estimated through R statistical software v.2.15.3 (package "Rcapture"). The minimum area method was applied in GRASS GIS v.6.4.2 for home range. Size distribution was estimated applying a Shapiro-Wilk test. Rayleigh test for randomness was applied to study the directionality of movements. A circular one-way ANOVA was used to test for differences in movement direction.

Capture probability was higher on seagrass than sand and the total length of the individuals ranged from 13 to 25 cm. Movement speed was between 4.7 and 14.7 m day^{-1}. Movements were not directional. *H. mammata* differs in its behaviour from the related *Holothuria arguinensis* occurring in the same habitat.

Keywords: sea cucumbers, behaviour, unusual habitat, movement, population dynamics

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Introduction

Catches of sea cucumbers from the Mediterranean Sea and NE Atlantic Ocean have been increased recently due to overexploitation of species inhabiting the Pacific and Indian oceans (González-Wangüemert, Aydin, & Conand, 2014; González-Wangüemert, Valente, & Aydin, 2015). Mainly, six species are caught in this geographical area (González-Wangüemert, Valente, Henriques, Domínguez-Godino, & Serrão, 2016): *Holothuria polii* (Delle Chiaje, 1824), *Holothuria tubulosa* (Gmelin, 1791), *Holothuria mammata* (Grube, 1840), *Holothuria arguinensis* (Koehler & Vaney, 1906), *Holothuria sanctori* (Delle Chiaje, 1823) and *Holothuria forskali* (Delle Chiaje, 1823). The market of sea cucumbers in European countries exporting to Asia is becoming more important each day (Aydın, Sevgili, Tufan, Emre, & Köse, 2011). In Spain, 6 companies are already exporting sea cucumbers to China, some of them with important profits around 1-2 millions $ US (González-Wangüemert, & Domínguez-Godino, 2016; González-Wangüemert et al., 2016). In Portugal, three companies are commercialising several sea cucumbers species including, *H. arguinensis, H. sanctori, H. forskali* and *H. mammata.* They offer supply ability among 2.000-50.000 kg/month and prices ranging from 70 to 350 euro/kg (González-Wangüemert, & Domínguez-Godino, 2016; González-Wangüemert et al., 2016). However, there is not legislation for fishing these new target species in European countries, therefore it is difficult to get official records of catches and prices (González-Wangüemert, & Domínguez-Godino, 2016).

In the last three years, the sea cucumber fishery in Turkey has also increased rapidly, with 555 tons in 2011 (80% *H. polii* and 20% *H. tubulosa* plus *H. mammata*) (González-Wangüemert et al., 2014). Sea cucumbers are fished by hookah, and a single diver catches around 2.000-3.000 individuals per day (Aydin, 2008). The current Turkish fleet (120 vessels) could collect around 720.000 sea cucumbers per day (González-Wangüemert et al., 2014). As a consequence, some signals of over-exploitation have been already detected, such as lost of the largest and heaviest individuals and a lower genetic diversity on the exploited populations (González-Wangüemert et al., 2015; González-Wangüemert, & Domínguez-Godino, 2016). This decrease on holothurian abundance could further lead to changes in nutrient recycling, bioturbation, habitat structuring and the food web (Francour, 1997; Uthicke, 1999, 2001a, 2001b; Bruckner, Johnson, & Field, 2003).

Holothuria mammata is distributed throughout the Mediterranean and NE Atlantic, including the continental Atlantic coast of Portugal and the Macaronesian Islands of the Azores, Madeira and Canary Islands (Borrero-Pérez, Gómez-Zurita, Wangüemert, Marcos, & Pérez-Ruzafa, 2010). It is one of the new target species for fisheries (González-Wangüemert et al., 2016). Despite the important role of these sea cucumbers species in the marine ecosystem and their commercial interest, scarce information has been published on its ecology, behaviour, biology and population dynamics. *Holothuria mammata* is a species with high affinity to rocky bottoms and shows mainly nocturnal activity, spending the light hours into crevices and leaving its diurnal refuge during the night for feeding (Borrero-Pérez et al., 2010; Navarro, García-Sanz, & Tuya, 2013a). However, this species can be also found in seagrass-sandy habitats at low densities in some areas of the Mediterranean and confined coastal lagoons from

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the southern Atlantic Ocean (González-Wangüemert, & Borrero-Pérez, 2012; González-Wangüemert et al., 2016).

This study aims to provide data on the population parameters (density, length, weight, spatial distribution), and behaviour characteristics of *H. mammata* in the Ria Formosa National Park (S Portugal), an unusual distribution area characterized by seagrass communities mixed with sandy bottoms (González-Wangüemert, & Borrero-Pérez, 2012; González-Wangüemert, Braga, Silva, Valente, Rodrigues, & Serrao, 2013; Siegenthaler, Cánovas, & González-Wangüemert, 2015). This information will be of value for future management of sea cucumber stocks in the Ria Formosa National Park.

Materials and Methods

Study Area

This study was carried out in a 50 x 60 meter area in the intertidal zone of the Ria Formosa (Figure 1). The Ria Formosa National Park (10.000 ha) is a tidal lagoon extending for 55 km along the south coast of Portugal. It consists of tidal flats and salt marches which are protected by a belt of dunes (Sprung, 1994). Ria Formosa harbours sandy, muddy and seagrass habitats with *Zostera noltii*, in the intertidal area and *Zostera marina* and *Cymodocea nodosa* in the subtidal zone (Malaquias, & Sprung, 2005). Seaweed communities consist mainly of *Ulva spp.* and *Enteromorpha spp* (Asmus, Sprung, & Asmus, 2000). Average depth is 3-4 m (channels are up to 20 m) with a tidal amplitude between 1.30 and 2.80 m (Sprung, 2001; Malaquias, & Sprung, 2005). The study area located along the coast, covered the stripe from the high shore level to the end of the intertidal zone. It consists of a sandy habitat (44.5 % coverage) and a seagrass habitat (35.6 % coverage). More details about the study area can be found in Siegenthaler et al. (2015).

Experimental Design

Holothurian abundance and distribution were assessed by means of a mark/recapture study performed at the beginning (period 1) and end (period 2) of April 2013. Captures and recaptures were made during periods of aerial exposure for 10 consecutive low tides per period. Tidal height at low tide was between 0.57 and 0.70 m during the first period and 0.36 and 0.60 m during the second one (source Instituto Hidrográfico). The whole study area was searched during each period of exposure (between 2 hours before low tide and 1 hour after it) and all holothurians found were marked *in situ* by means of scratching a code on their dorsal side (Reichenbach, 1999; Mercier, Battaglene, & Hamel, 2000; Navarro, García-Sanz, Barrio, & Tuya, 2013b; Siegenthaler et al., 2015) and released them at the same spot where captured. Although stress caused by marking and handling could result in increased initial activity (Shiell, 2006), scratching is considered as less invasive than other tagging methods (Conand, 1990; Kirshenbaum, Feindel, & Chen, 2006; Navarro, García-Sanz, & Tuya, 2014; Purcell, Agudo, & Gossuin, 2008; Schiell, 2006) and does not result in major behavioural changes (Mercier et al., 2000).

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Relative position (see Siegenthaler et al. (2015) for the methodology used), total length (by means of metric tape), time and type of substrate were recorded for each sea cucumber sampled. Temperature, salinity and weather information for the duration of the study can be also found in Siegenthaler et al. (2015).

Data Analyses

Abundance was estimated from the mark-recapture data by the use of Poisson regressions performed with the R statistical software v.2.15.3 package "Rcapture" (Baillargeon, & Rivest, 2012). Models for a closed population were used, considering the short duration of study and the low motility of the animals (Baillargeon, & Rivest, 2007). Models were fitted by using a combination of minimizing Akaike information criteria and standard error to a capture history consisting of absence/presence data. Profile likelihood confidence intervals based on log-linear distribution with the closest fit, were then used to estimate density (Baillargeon, & Rivest, 2007). The minimum area method (Worton, 1987) was applied in GRASS GIS v.6.4.2 (Neteler, Bowman, Landa, & Metz, 2012) for the calculation of the home range area of specimens that were recaptured for a minimum of 4 times. Quantum GIS v.1.8.0 (Quantum GIS Development Team, 2009) was used for the visualisation of the home ranges and distribution of *H. mammata*. Habitat preferences were described by using substrate information, which was collected during sampling (only one animal was recaptured at more than 1 type of habitat, in this case the most occurring substrate was chosen). A binominal test was used to check for equal distribution. Size distribution was estimated from all captured individuals, applying a Shapiro-Wilk test for normality. Median movement speed was calculated per individual and per period, which was assumed to be more representative than mean movement speed, due to infrequent movements over longer distances (Navarro et al., 2013b, 2014; Siegenthaler et al., 2015). Recapture probability was low (see the results section for details), limiting therefore the number of movements recorded. It was not possible to constrain the analysis to movements between consecutive tides (Siegenthaler et al., 2015), so all movements recorded within a period were used. Student's t-test was used looking for differences in movement speed between periods. The R statistical software v.2.15.3 package "circular" (Lund, & Agostinelli, 2013) allowed to manage the angular data of the specimens' movement directions. Rayleigh test for randomness was applied to study the directionality of the movements. The dataset included all time intervals and it was independent on the number of recaptures. A circular one-way ANOVA was used to test for differences in movement direction between habitats.

Results

Abundance and Length

A total of 30 individuals of *H. mammata* were captured during this study; 20 during the first period and 15 during the second one, being five specimens caught during both periods. Marking through scratching of *H. mammata*, was difficult due to its dark integument colour, which decreases the contrast

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of marks (supplementary material 1). Recapture probabilities were 46.67 % (period 1) and 40.00 % (period 2). Abundance estimates based on Mth Darroch and Mt models (see Baillargeon, & Rivest, 2007 for more information on the selected models), were 37 (CI: 23-78) and 16 (CI: 15-24) animals for periods 1 and 2, respectively. Total density estimates ranged from 53 to 123 individuals per ha (Figure 2). More animals were caught in the seagrass habitat than on sand (Seagrass: N = 31, Sand: N = 4; Binominal test: P < 0.00). Home ranges could only be estimated for 3 animals (Figure 2), showing a mean area of 34 m^2. Total length of *H. mammata* varied between 13 and 25 cm presenting a Gaussian distribution (Figure 3; Shapiro-Wilk normality test: W = 0.9773, P = 0.6692).

Movement Speed and Direction

Median movement speed varied between 4.7 m day^{-1} (period 1) and 14.7 m day^{-1} (period 2) and differed significantly between the two periods (Student's t-test t_8= 2.465, P < 0.05). Movement direction was random (Figure 4; Rayleigh test: Z = 0.2741, N = 18, P = 0.262), both during day (Rayleigh test: Z = 0.384, N = 7, P = 0.3692) and night (Rayleigh test: Z = 0.382, N = 11, P = 0.204), without differences between habitats (Circular one-way ANOVA $F_{1,16}$ = 0.5949, P = 0.4518).

Discussion

This study provides important insight on *H. mammata* from the Ria Formosa National Park, where its main habitat is an intertidal area characterized by a mixed seagrass meadow and sandy bottom. Its low density (53 to 123 ind. ha^{-1}) agrees with previous data obtained using visual census in the Ria Formosa between November 2012 and February 2013 (González-Wangüemert et al., 2013). This low density of *H. mammata* in Ria Formosa, linked with the presence of unusual habitats for its survival, limited the number of marked individuals in our study, but allowed us to obtain important insight about ecology, behaviour and population structure of this new target species. The density of *H. mammata* in Ria Formosa is significantly lower than that registered in Gran Canarias close to 1600 ind. ha^{-1} (Navarro, 2012; Navarro et al., 2013a). This lower density could be due to differences in habitats between the intertidal zone in the Ria Formosa and the fully submerged area in the Canary Islands (Siegenthaler et al., 2015). In the intertidal area of the Ria Formosa, *H. mammata* is much less abundant than another sea cucumber species belonging to *Holothuriidae* family, *Holothuria arguinensis*, showing densities close to 527-563 ind. ha^{-1}, (Siegenthaler et al., 2015). This fact could indicate that *H. mammata* is less adapted than *H. arguinensis* to intertidal lagoons and desiccation periods associated to low tides. In fact, its preferences for complex habitats with rocks and crevices (González-Wangüemert et al., 2014, 2016) are substantially different from the muddy intertidal habitat of the Ria Formosa. However, density estimates should be considered with care (specially in rocky bottoms) due to *H. mammata*'s cryptic nature (Navarro, 2012) and low recapture probability caused by the low readability of the marks. Marking by scratching is a common method used in sea cucumber mark-recapture studies (Reichenbach, 1999; Mercier et al., 2000; Navarro et al., 2013b, 2014; Siegenthaler et al., 2015), being

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considered less invasive and more effective than other methods such as glued tags, colouring agents, PIT tags and T-bar tags (Conand, 1990; Kirshenbaum et al., 2006; Shiell, 2006; Purcell et al., 2008; Navarro et al., 2014). However, based on our experience with the low readability of scratched marks on *H. mammata'* integument (previously commented in Methodology section), we suggest the search of other tagging methods for this species.

Holothuria mammata's preference for the seagrass habitat in Ria Formosa, agrees with results also obtained for *H. arguinensis* in the same geographical area, where seagrass could be providing shelter against UV radiation to this last species (González-Wangüemert et al., 2013; Siegenthaler et al., 2015). However, *H. mammata* does not show sheltered behaviour or offshore directionality in its movements during daytime. *H. mammata* has dark skin and nocturnal feeding (Navarro, 2012; González-Wangüemert et al., 2014), which might protect itself against direct sunlight. Its higher abundance in seagrass could be most likely related to its preference for complex habitats (Navarro, 2012; González-Wangüemert et al., 2014) and higher food availability, although tidal elevation might also play a secondary role which must be assessed in further studies.

Holothuria mammata size ranged from 13 to 25 cm total length in the Ria Formosa, which is comparable to results obtained from populations in Aegean Sea (Turkey) (10-28cm, González-Wangüemert et al., 2014); however, its maximum length was smaller than that (35 cm) registered for *H. mammata* at Gran Canarias (Navarro, 2012) or 43 cm for the same species in Peniche (W Portugal) (Henriques, 2015; González-Wangüemert et al., 2016). The low maximum length reached for this species in our study area might be a concern for future exploitation of this species in the Ria Formosa, since fishing pressure might reduce the largest size classes even more (Cariglia, Wilson, Graham, Fisher, Robinson, Aumeeruddy, Quatre, & Polunin, 2013; Purcell, Mercier, Conand, Hamel, Toral-Granda, Lovatelli, & Uthicke, 2013; González-Wangüemert et al., 2014, 2015). However, more studies assessing the stock status in this area must be performed to confirm this trend.

Animal movements are very useful for the management of sea cucumber stocks, for example sizing of not-take zones or the implementation of population surveys (Purcell, & Kirby, 2006; Shiell, & Knott, 2008). *Holothuria mammata* shows comparable movement speed to other related temperate sea cucumbers such as *H. arguinensis* (10 m day^{-1}, Siegenthaler et al., 2015) and *H. sanctori* (11 m day^{-1}, Navarro et al., 2013a). The movement speed of these temperate species is much faster than those from tropical species such as *Actinopyga mauritiana* (3 m day^{-1}, Graham, & Battaglene, 2004), *Apostichopus japonicus* (2 m day^{-1}, YSFRI, 1991), *H. fuscogilva* (2 m day^{-1}, Reichenbach, 1999), *H. scabra* (1.3 m day^{-1}, Purcell, & Kirby, 2006) and *Parastichopus californicus* (3.95 m day^{-1}, Da Silva, Cameron, & Fankboner, 1986). It should be stressed that mark/recapture methods use an approximation to the real movement which might lead to errors especially over longer time intervals. Movement speed could also be influenced by behavioural effects of marking (Graham, & Battaglene, 2004; Purcell, & Kirby, 2006;

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Navarro et al., 2013a, 2014). Nevertheless, due to this wider mobility, temperate sea cucumbers might require different management strategies than tropical species.

Coastal lagoons such as Ria Formosa, can act as hotspots and sources of genetic diversity (Rodrigues, Valente, & González-Wanguemert, 2015; Vergara-Chen, Rodrigues, & González-Wangüemert, 2015). Furthermore, these ecosystems harbour important habitats such as seagrass meadows (Siegenthaler et al., 2015) which are declining across the coast in Portugal over the last 20 years (Duarte, 2002; Orth et al., 2006; Cunha, Assis, & Serrão, 2013). A comparison between the results of this study and previous publications on *H. arguinensis* from Ria Formosa (González-Wangüemert et al., 2013; Siegenthaler et al., 2015), stresses that very related species can show differences in abundance, density and behaviour in the same ecosystem. Therefore, they might require different management strategies for fisheries, restocking actions or aquaculture development (Sale et al., 2005; Purcell, & Kirby, 2006; Siegenthaler et al., 2015).

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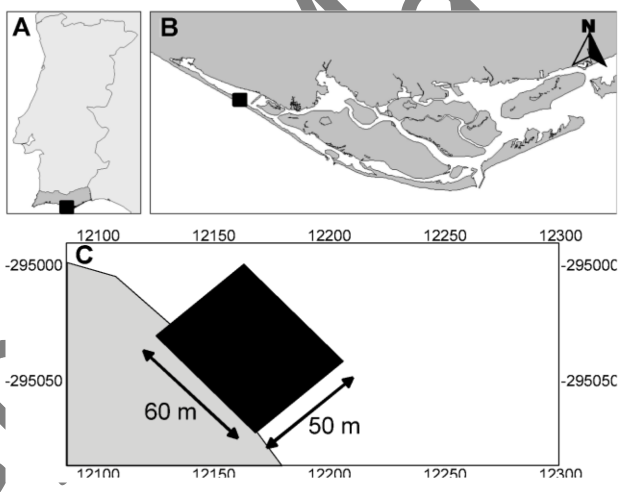

Figure 1. Location of the study area. Map of Portugal (A) showing the location of the Ria Formosa (B) and the study area (C). Projection: EPSG:3763 – ETRS89 / Portugal TM06

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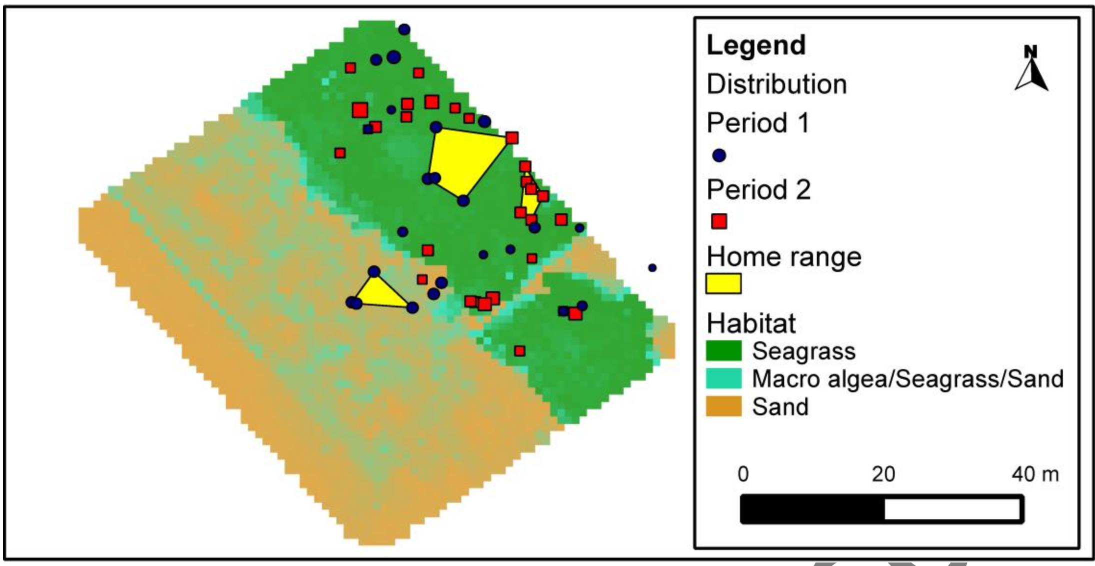

Figure 2. Distribution of captures (circles: period 1; squares: period 2) and home ranges (polygons) of *H. mammata*. Different specimens are indicated with different numbers. Differences in symbol size represent relative differences in sea cucumber length.

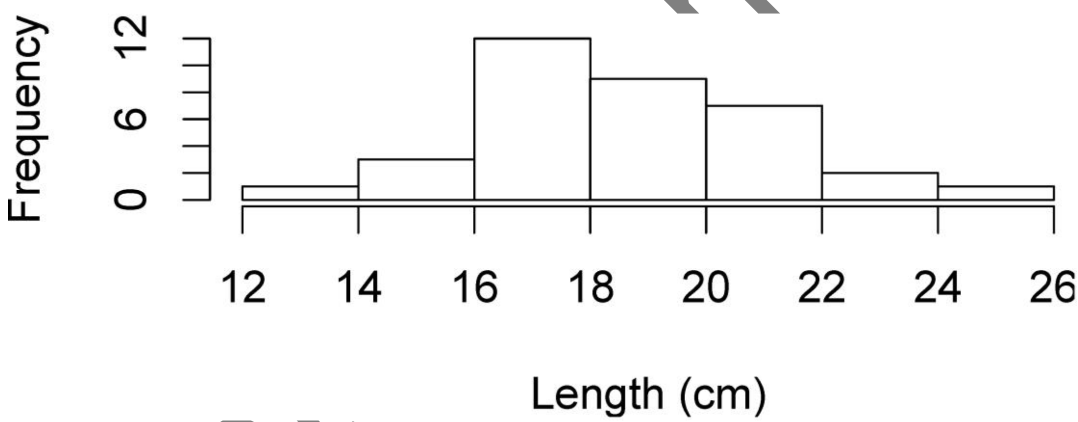

Figure 3. Length distribution of captured *H. mammata*.

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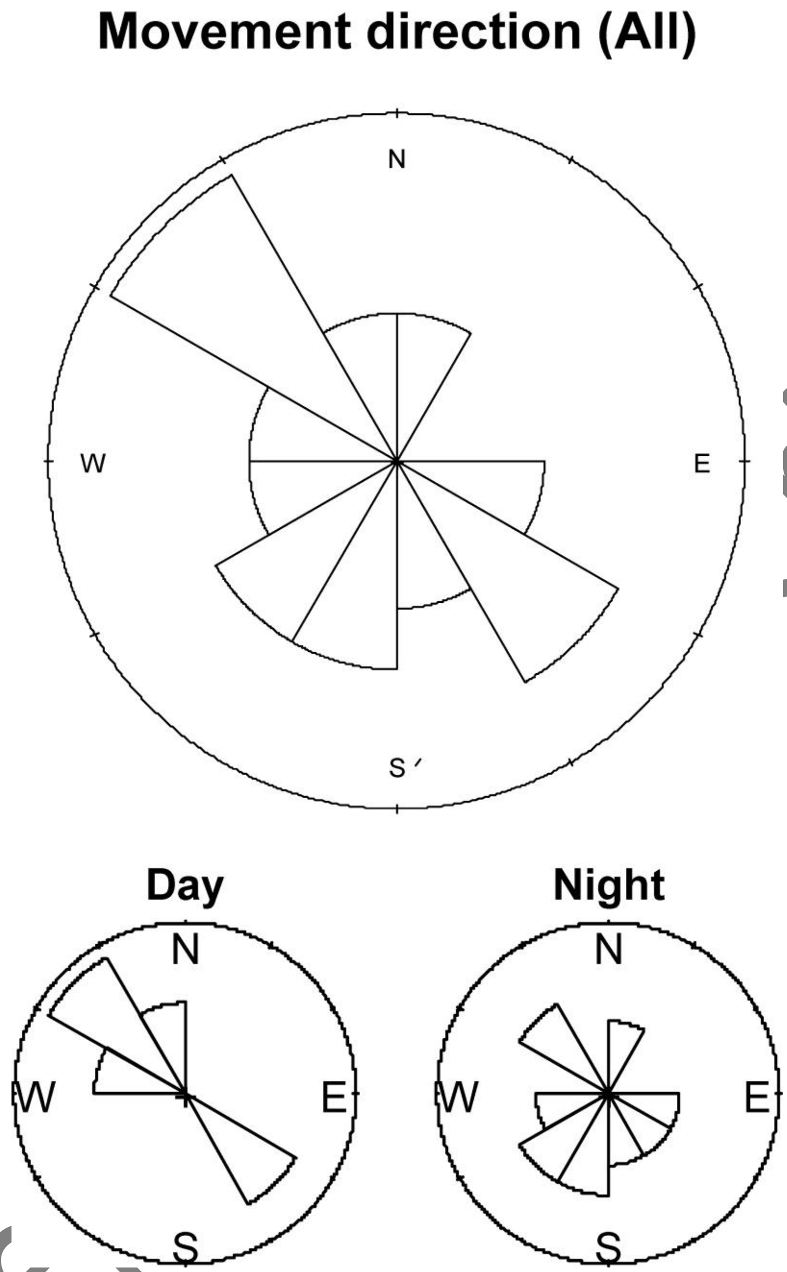

Figure 4. Orientation of *H. mammata*' movements (all) and during day/night. Length of the bars in the rose graphs represent the frequency in which the specimen moved in a certain direction.

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Supplementary material 1. Visibility of marks on *H. mammata*.