Spatial distribution patterns and movements of *Holothuria arguinensis* in the Ria Formosa (Portugal).

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Abstract

Holothurian populations are under pressure worldwide because of increasing demand for beche-de-mer, mainly for Asian consumption. Importations to this area from new temperate fishing grounds provide economic opportunities but also raise concerns regarding future over-exploitation. Studies on the habitat preferences and movements of sea cucumbers are important for the management of sea cucumber stocks and sizing of no-take zones, but information on the ecology and behaviour of temperate sea cucumbers is scarce. This study describes the small-scale distribution and movement patterns of *Holothuria arguinensis* in the intertidal zone of the Ria Formosa national park (Portugal). Mark/recapture studies were performed to record their movements over time on different habitats (sand and seagrass). *H. arguinensis* preferred seagrass habitats and did not show a size or life stage-related spatial segregation. Its density was 563 ind ha^{-1} and mean movement speed was 10 m per day. Movement speed did not differ between habitats and the direction of movement was offshore during the day and shoreward during the night. Median home range size was 35 m^{2} and overlap among home ranges was 84 %. *Holothuria arguinensis'* high abundance, close association with seagrass and easy catchability in the intertidal zone, indicate the importance of including intertidal lagoons in future studies on temperate sea cucumber ecology since those systems might require different management strategies than fully submerged habitats.

1. Introduction

Increasing demand of beche-de-mer is influencing sea cucumber populations all over the world. The traditional Asian demand of holothurian products for delicacies and medicines has resulted in the depletion of traditional fishing grounds in tropical areas. The fishing pressure is now moving towards temperate areas, including the Mediterranean Sea and Northeastern Atlantic Ocean (González-Wangüemert et al., 2013, 2014, 2015; Toral-Granda et al., 2008). Nowadays, global catch estimates are in the range of 100, 000 tonnes of sea cucumbers per year (Purcell et al., 2010).

The overfishing of sea cucumbers in the Indo-Pacific, has resulted in catch of new target species (*Holothuria tubulosa*, *H. polii*, *H. mammata*, *H. forskali* and *H. arguinensis*) from Mediterranean Sea and NE Atlantic Ocean (Aydin, 2008; González-Wangüemert et al., 2014; González-Wangüemert and Borrero-Pérez, 2012; Rodrigues et al., 2015; Sicuro and Levine, 2011). In the last three years, the sea cucumber fishery in Northern Turkey has increased rapidly, with 555 Tn in 2011 (80% *H. polii* and 20% *H. tubulosa* plus *H. mammata*) (González-Wangüemert et al., 2014). Sea cucumbers are fished by hookah facilities, a diver catches around 2.000-3.000 individuals per day (Aydin, 2008); the current Turkish fleet (120 vessels) can collect around 720.000 sea cucumbers per day (González-Wangüemert et al., 2014). As consequence, some signals of over-exploitation were already detected, showing loss of the largest and heaviest individuals and genetic diversity (González-Wangüemert et al. 2015). Scarce official information on sea cucumber fisheries from this geographical area is available due to catches are catalogued as "invertebrates" and/or obtained illegally. In Spain, more than 10 companies are exporting sea cucumbers to China (http://www.alibaba.com/countrysearch/ES/sea-cucumbersupplier.html); some of them with 1-2 millions $ US of total revenue. The main target species are *H. tubulosa*, *H. forskali* and *H. mammata.* In Portugal, three companies are selling sea cucumbers, mainly *Holothuria arguinensis*, *H. forskali* and *H. mammata*, offering supply ability among 2.000-50.000 Kg/month and prices oscillating among 70-350 euro/kg (http://www.alibaba.com/countrysearch/PT/sea-cucumber.html) depending on quality of product.

Holothurians are sensitive to over-exploitation due to a combination of biological (late maturity, low recruitment rate and density-dependent reproduction), and anthropogenic factors (the ease with which shallow water species can be harvested) (Bruckner et al., 2003). Dramatic reductions in sea cucumber abundance might cause concern about their ecological role in bioturbation, nutrient recycling and habitat structuring (Bruckner et al., 2003; Uthicke, 1999, 2001a, b), and also on the food-web, because sea cucumbers are a substantial biomass for some predators as fishes, starfishes and crustaceans (Francour, 1997).

Information on the ecology and behaviour of sea cucumbers is relatively scarce (Bruckner et al., 2003; Conand, 1990; Graham and Battaglene, 2004; Navarro et al., 2013, 2014), despite their economical and ecological importance. Studies on the habitat preferences and movements of sea cucumbers have demonstrated their utility on the management of sea cucumber stocks and sizing of no-take zones providing for example, parameters for surveys and restocking (Purcell and Kirby, 2006; Shiell and Knott, 2008).

Movement and distribution of sea cucumber can be influenced by several factors such as substrate type, organic matter availability, light intensity, depth and salinity (Dong et al., 2011; Hamel et al., 2001; Mercier et al., 2000; Navarro et al., 2013, 2014; Shiell and Knott, 2008; Slater et al., 2011). Movement speed is slow with averages of 2-5 m day^{-1} for *Holothuria fuscogilva*, *Holothuria whitmaei*, *Apostichopus japonicus* and *Actinopyga mauritiana* (Graham and Battaglene, 2004; Reichenbach, 1999; Shiell, 2006; YSFRI, 1991) and around 10 m day^{-1} for *Holothuria sanctori* (Navarro et al., 2013).

Behavioural studies on sea cucumbers are focused mainly on tropical species (e.g. Graham and Battaglene, 2004; Mercier et al., 2000; Purcell and Kirby, 2006; Reichenbach, 1999; Shiell, 2006; Shiell and Knott, 2008), but information on temperate species is still scarce (Navarro et al., 2014).

Holothuria arguinensis is a sea cucumber that is distributed along the east North Atlantic from Portugal to Morocco, Mauritania and Canary Islands (González-Wangüemert and Borrero-Pérez, 2012; Rodrigues, 2012), and some individuals have been found in the Mediterranean sea on the south-eastern Spanish coast recently (González-Wangüemert and Borrero-Pérez, 2012). This species is usually associated with sandy/rocky areas and seagrass meadows where it occurs from 0 m to 50 m depth (González-Wangüemert and Borrero-Pérez, 2012; Navarro, 2012; Rodrigues et al., 2015). In Ria Formosa National Park (South Portugal) this species is the most abundant holothurian and can be found in high densities exposed on the intertidal flats during low tide (González-Wangüemert et al., 2013). As was stressed before, *H. arguinensis* is one of the target species caught in Portugal to be exported mainly to

Asiatic countries. Nowadays, unless three companies are offering this species to be sold (http://www.alibaba.com/countrysearch/PT/sea-cucumber.html). The combination of its high nutritional value for human consumption (Roggatz, 2012), high densities of its populations and the ease with which this species can be harvested, makes it vulnerable to overexploitation such as another tropical species (Bruckner et al., 2003).

The ecology and behaviour of *H. arguinensis* have been studied recently but only on the Canary Islands (Spain), where this species is occupying habitats dominated by volcanic rocks and seagrass at 4-8m depth (Navarro, 2012; Navarro et al., 2013, 2014; Tuya et al., 2006). These habitats are substantially different from the tidal lagoon habitat of Ria Formosa national park, where only one study on *H. arguinensis* has been conducted to assess the population status of this species through a volunteer program doing visual census (González-Wangüemert et al., 2013). We aim to study the behaviour of *H. arguinensis* at small spatial scale in Ria Formosa Natural Park (South Portugal). To achieve this objective, we analysed the movement patterns of *H. arguinensis*, interpreted its spatial distribution across habitat-diverse areas, studied the home-range of this species and its relationship with the size of individuals, and estimated its density. This information would be very useful to further development of regulations to sea cucumber fishery in Ria Formosa (Natural Park, South Portugal) and along Portuguese coast. Also, these data have a valuable interest to develop the aquaculture biotechnology on *H. arguinensis*, which could supply part of the demand from Asiatic countries, with less impact on wild populations, and allow further restocking programs if they are necessary.

2. Material and Methods

2.1. Study area

Ria Formosa Natural Park is a tidal lagoon, consisting of tidal flats and salt marshes protected by a belt of dunes extending for 55 km along the south coast of Portugal (Sprung, 1994). Total surface of the lagoon is about 10 000 ha and the average depth is 3-4 m with a tidal amplitude of about 1.30 m at neap tide and 2.80 m at spring tide. Channels are up to 20 m deep (Malaquias and Sprung, 2005; Sprung, 2001). Habitats are covered mainly by either sand, mud and seagrass (intertidal: *Zostera noltii*, subtidal: *Zostera marina*, *Cymodocea nodosa* (Malaquias and Sprung, 2005)). *Z. noltii* biomass is high in the intertidal, showing low oscillations during the year (Asmus et al., 2000). Sandy habitats are generally associated with intertidal seaweed communities, consisting mainly on *Ulva spp.* and *Enteromorpha spp.* (Asmus et al., 2000).

Experiments were carried out in the intertidal zone of the Ria Formosa close to Praia de Faro (Fig. 1), covering an area along the coast from the high shore level to the end of the intertidal zone. The area was selected because of its high holothurian abundance (González-Wangüemert et al., 2013). Transects (60 m of length each one and parallel to the waterline) were walked during periods of aerial exposure and percentage of coverage by either seagrass, seaweed and sand were estimated for every cell in a 1 m^2 grid of the study area. Transects were separated 2 meters from each other and the observer recorded at both sides of the transects for 1 m distance. Although quadrants were not used for coverage estimates, the observer error was minimized by the use of only one sampler. A principal component analysis (PCA) was then used to summarize habitat variability across the study area by using spatial distribution of coverage information already described above. Each cell of the study area, can be then classified, interpreting the first component of the ordination. Analysis was performed using GRASS GIS v.6.4.2 (Neteler et al., 2012), and variables were represented by 1x1 m raster maps of each coverage type.

2.2. Mark/recapture experimental designing

A preliminary study was done and the methods retained were used in order to optimize the sampling design. The study was performed during two periods at the beginning and end of April 2013. Captures were made during periods of aerial exposure (between 2 hours before low tide and 1 hour after low tide) for 5 consecutive days (10 low tides per period). Tidal height at low tide varied from 0.57 to 0.70 m (first period) and 0.36 to 0.60 m (second period) (Instituto Hidrográfico: http://www.hidrografico.pt/previsao-mares.php). During each sampling, the whole study area was searched and all holothurians encountered were marked *in situ* and immediately released at the same spot where captured. Marking was done by scratching a code on their dorsal surface with a surgical scalpel (Mercier et al., 2000; Navarro et al., 2013, 2014; Reichenbach, 1999). The wound usually heals within 10 days, leaving a scar with the shape of the mark (Shiell, 2006; Supplementary Fig. S1). Scratched marks are visible up to a month with no indication of any considerable behavioural change (Mercier et al., 2000; Reichenbach, 1999). Other tagging methods such as glued tags, colouring agents, PIT tags and T-bar tags were considered less effective and often more invasive (Conand, 1990; Kirshenbaum et al., 2006; Navarro et al., 2014; Purcell et al., 2008; Schiell, 2006). Stress caused by handling and marking could result in a higher activity during the initial hours (Shiell, 2006). Reducing this effect by postponing the sampling for several hours after marking

(Navarro et al., 2013, 2014), was not deemed effective since the specimens were also handled during recaptures in those cases where the marks were difficult to read directly. Observations started directly after marking and the effect of handling was reduced by working *in situ* (Navarro et al., 2014). Recaptured individuals were directly released without remarking since repeated marking could increase the chance of infections and possibly behavioural changes. In the few cases that remarking was essential to maintain the readability of the mark (12 % of the animals recaptured were remarked once) the animals did not show a behavioural change. Total length, date, time, substrate, and relative position (see positioning section for further explanations), were recorded for every capture and recapture. The length was measured by metric tape at the moment of capture in order to prevent underestimations because of unpredictable contractions and evacuations of water (Hammond, 1982; Reichenbach, 1999). Temperature and salinity were measured twice per low tide with an Eutech Salt 6+ Salinity/temperature meter.

2.3. Positioning system

Reference position of an holothuria was estimated using the bearings relative to two fixed reference marks and applying linear trigonometric functions. Those references were placed at the shoreward corners of the study area. Angles were measured using the geographical north as zero reference, using a Topomarine Rescue 7x50 waterproof/floating binocular with internal compass. Flash lights were used at night to show the exact location of the captures. Angles were corrected for magnetic declinations for the study area during the sampling days (2.57º W) and magnetic deviations of the compass used prior to the calculations. Linear increment between angles in magnetic deviations was assumed.

Geographical coordinates of reference marks were located using a Garmin GPSmap 60CSx (European Terrestrial Reference System ETRS 1989 Datum, GRS 1980 spheroid, Transverse Mercator projection). Relative position of every specimen was then corrected by using the reference mark absolute positions. All calculations were done in R statistical software v.2.15.3 (R Development Core Team, 2013).

2.4. Data analyses

Size distribution was estimated from the dataset, which contained the length of all captured individuals. Shapiro-Wilk test was then applied to test for normality.

To estimate the holothurian density within the study area, capture-recapture data were encoded in absence/presence from the capture history, consisting on ones (captures) and zeros (misses). Population size was estimated by fitting several models, assuming a closed population (no migrants) due to the relatively slow-motion lifestyle of the species and the short duration of the study (Baillargeon and Rivest, 2007). Model selection was based on a combination of minimizing Akaike information criteria (AIC) and standard error. The final abundance was estimated using profile likelihood confidence intervals based on log-linear distribution with the closest fit.

Capture data was used to study habitat preferences. A grid (3 x 3 m cells) of the study area was used to summarize the capture data, allowing to analyse habitat preferences independently of the number of recaptures, and later test for differences between habitats for both presence of individuals and size. A total of 125 cells covered the seagrass area (>74% seagrass, < 5% sand) and 210 cells covered the sandy area (>74/% sand, < 5% seagrass). 23 cells covered the transition area between seagrass and sand but were not considered due to their low abundance. Cells were considered sampling units and independent from each other. 50 cells per habitat and tidal cycle were randomly selected and a generalized linear model (GLM) with a binomial distribution was applied to test for differences in presence/absence between the habitats. Average length per cell was also calculated for all cells containing any individual. One-way ANOVA was used by implementing a sequential sum of squares (type I) to compare length distribution using as factors "periods" and "habitat".

Movement speeds were only calculated for recaptures between consecutive tides and within the same habitat. Mean movement speed was biased towards higher values due to infrequent movements over longer distances, possibly influenced by the currents and the behavioural effects of marking (Navarro et al., 2013, 2014). Because of this skewed distribution, median speed for each individual was used. Differences in median movement speeds between habitats were then compared using one-way ANOVA, also considering differences between the two periods. Pearson correlation coefficient was used to explore the relationships between the individual length and movement speed.

Orientation of movement was tested on the dataset, containing all time intervals and independent of the number of recaptures. Angles were transformed into circular and Rayleigh test for randomness was applied. Circular one-way ANOVA was then used to test for differences in orientation between periods, habitats and day/night group of means, under the null hypothesis of same mean direction for all the group means (See: Lund and Agostinelli, 2013). Circular Pearson test for correlation was also used to explore the relationships with wind direction.

Movement patterns and home ranges were analysed by specimen which was recaptured for a minimum of 4 times within one period (movement patterns) or over both periods (home ranges). Movement patterns were described based on visual analysis of the data plotted on the habitat map and on linearity indices (net distance travelled/gross distance). Differences in occurrence of specific movement patterns between the two periods were tested using a binominal test for the movement patterns and a Mann-Whitney U test for the linearity indices. The minimum area method (which is based on the smallest area convex polygon that contains all capture points) was applied for the calculation of the home range area (Worton, 1987).

All analyses were done in R statistial software v.2.15.3, using packages "Rcapture" (Baillargeon and Rivest, 2012), which estimates parameters in capture-recapture experiments by the use of Poisson regressions (Baillargeon and Rivest, 2012), and "circular" (Lund and Agostinelli, 2013), which allows to manage angular data. Data met the normality and homoscedasticity requirement of ANOVA. Home range area and overlap between home ranges were calculated using GRASS GIS v.6.4.2 (Neteler et al., 2012) and visualized in Quantum GIS v.1.8.0 (Quantum GIS Development Team, 2009).

3. Results

3.1. Habitat description

The two study periods (8-12 April and 24-28 April 2013) differed significantly in hydrological conditions (Table 1). During the second period, the water was warmer and saltier, and the wind speed was higher than during the first one. The wind blew mainly from the West during the first period and from the North-North-West during the second one. Tidal height of low tide was higher during the first period.

The study area can be divided in two mayor habitats which were dominated by sand (44.5 % coverage) or seagrass (35.6 % coverage). Seaweeds covered 19.9 % of the area and generally occurred in the sandy habitat (Fig. 2A-C). The first component of the PCA explains 83.25 % of the variance for the habitat composition (Fig. 2D). Seagrasses were found in the North to North-East half of the study area and consisted of two patches that were separated by a sandy channel of 1-4 m wide. The sandy channel between the two seagrass patches recorded the same depth as the patches. Stronger currents were observed within the channel compared to the other areas during the upcoming and retreating tides.

3.2. Abundance and length

A total of 215 specimens of *H. arguinensis* were captured during this study with an average recapture probability of 73 % (Table 2). A small difference of 6 % in recapture probability was observed between the periods. 63 % of individuals which were caught during the second period, showed marks from being captured during the first period, but only 38% of the recaptures had a readable code. The Mh Chao (LB) and Mth Darroch models for population size estimation were selected based on AIC. Estimated numbers of *H. arguinensis* within the study area were 169 individuals (*CI*:

155-193) and 158 individuals (*CI*: 141-184), for period 1 and 2 respectively. Total density estimates range from 527-563 individuals ha^{-1}. Differences in presence between both seagrass (average number of cells present = 9) and sand (average number of cells present = 1) habitats, were significant (binomial GLM: $Z_{1799} = 9.338$, $P < 0.001$). Differences between average numbers of presences between periods (period 1 = 10, period 2 = 10; binomial GLM: $Z_{1799} = -0.618$, $P = 0.537$) and between day and night (day = 11, night = 9; binomial GLM: $Z_{1799} = -1.129$, $P = 0.259$), were not significant.

Length distribution of all captured *H. arguinensis* specimens showed a Gaussian distribution (Shapiro-Wilk normality test: X+SD = 20+5 cm, $W = 0.9921$, $N = 225$, $P = 0.2702$). Mean length differed slightly but significantly between the two periods (Period 1: X+SE = 22+0.4 cm; Period 2: X+SE = 20+0.5; ANOVA: $F_{1,273} = 13.55$, $P < 0.001$). This difference was not considered for further analysis, since the differences between the means were so small that they felt within the natural variation of contraction of the specimen. Mean length per cell did not differ between the habitats (ANOVA: $F_{1,497} = 2.30$, $P = 0.13$).

3.3. *Movement patterns and home ranges*

Median (X+SE) movement speed of *H. arguinensis* was 0.42+0.37 mh^{-1}, and did not differ between periods (ANOVA: $F_{1,89} = 1.948$, $P = 0.166$) nor habitats (ANOVA: $F_{1,89} = 0.024$, $P = 0.877$). Length and movement speed were independent and uncorrelated (Pearson correlation: $r_p = 0.07$, $N = 102$, $P = 0.50$), as well as movement speed between night and day (Paired samples T-test with log-transformation: $t_{51} = -1.79$, $P = 0.08$).

Directionality of movements differed significantly between day and night (Circular ANOVA: $F_{1,328} = 376.8$, $P < 0.001$; Fig. 3). Movements during the day were not random (Rayleigh test: $Z = 0.325$, $N = 217$, $P < 0.001$) and orientated towards the NNE (X+SD = 12.7+1.5°). Movements during the night were also not random (Rayleigh test: $Z = 0.375$, $N = 113$, $P < 0.001$) but were orientated in the opposite direction, the SSW (X+SD = 205.1+1.4°). Directionality was not correlated with wind direction (circular Pearson correlation: $r_p = -0.92$, $N = 377$, $P = 0.36$). Animals captured in both seagrass and sand habitats moved in NE (offshore) direction (Circular ANOVA: $F_{1,215} = 1.831$, $P = 0.178$; Fig. 3) during the day, and in SW (near shore) direction during the night (Circular ANOVA: $F_{1,111} = 3.719$, $P = 0.056$).

Several movement patterns were observed on *H. arguinensis* during this study (See supplementary Fig. S2 for a representation these patterns). The two most abundant patterns were directional (without showing a preference for a certain area; $N_{\text{period 1}} = 14$; $N_{\text{period 2}} = 3$) and clustered movements (showing a preference for a certain area in which recaptures do not show a directional movement but are clustered; $N_{\text{period 1}} = 1$; $N_{\text{period 2}} = 15$). Variations on directional movement were also observed: semicircular (directional towards the starting point; $N_{\text{period 1}} = 4$; $N_{\text{period 2}} = 1$) and zigzag (limited directionality and covering more area on the way; $N_{\text{period 1}} = 6$; $N_{\text{period 2}} = 3$). A combination of directional and clustered movement was observed regularly where the specimen showed directional movement towards or from an area where it clustered ($N_{\text{period 1}} = 12$; $N_{\text{period 2}} = 10$). *H. arguinensis* showed significantly more directional movement during the first period than during the second (Binomial test: $N = 17$, $P = 0.013$) and more clustered patterns during the second than during the first one (Binomial test: $N = 16$, $P < 0.001$). The ratio net distance travelled (X+SE =

Period 1: 11.5 + 1.0 m; Period 2: 5.3 + 0.6 m) per gross distance (X+SE = Period 1: 27.2 + 2.8 m; Period 2: 18.3 + 1.8 m) was significantly higher during the first period than during the second (Period 1: 0.56 + 0.03 m ; Period 2: 0.33 + 0.04 m; Mann-Whitney U test: $Z_{25,31} = -2.810$, $P < 0.01$).

Home ranges of *H. arguinensis* are shown in Fig. 4. Median (+SE) home range area was 35+10 m^2. On average, 84 % of each home range was overlapped. As visible in Fig. 5, the sandy channel between the seagrass patches was included in most of the home ranges. No correlation between the individual size and the home range area was observed (Pearson correlation: $r_p = 0.10$, $N = 69$, $P = 0.40$).

4. Discussion

The results of this study provide a valuable insight in the small scale distribution of *H. arguinensis*. Estimated densities of 527-563 ind. ha^{-1} are slightly higher than *H. arguinensis'* densities of ca. 250 (seagrass meadow) and 460 (macroalgal bed) ind. ha^{-1} measured on the Canary Islands by Navarro et al. (2012; 2014). Although the differences between studies could be also due to other locational differences than habitat type, these results clearly indicate the importance of intertidal lagoons such as the Ria Formosa for this species. Holothurian density varies per location within the Ria Formosa (González-Wangüemert et al., 2013) and the estimates of the present study are in line with the highest densities measured by those authors.

H. arguinensis shows a preference for the seagrass habitat over the sandy habitat. This result should, however, be considered with care since its preference could be confounded with tidal elevation. Nevertheless, the results of this study agree with habitat-dependent distributions observed in several holothurian and other echinoderm species (Entrambasaguas et al., 2008; Navarro, 2012). Navarro et al., (2014) found differences in *H. arguinensis*' densities between a seagrass meadow (2.5 ind. 100 m^{-1}) and a macroalgal bed (4.6 ind. 100 m^{-1}), but these differences were not significant, possibly due to the low number of replications included in the study. Multiple factors can influence the distribution of holothurians such as life stage (Eriksson et al., 2012; Mercier et al., 2000; Reichenbach, 1999), predation pressure (Andrew, 1993; Bartholomew et al., 2000; Hammond, 1982; Mercier et al., 1999), organic matter availability (Navarro et al., 2013) and light intensity (Dong et al., 2011; González-Wangüemert et al., 2013). Several holothurian species show a clear segregation between adults and juveniles, in which the juveniles utilize shallow seagrass habitats and larger individuals move to deeper sandy or hard substrate habitats (Eriksson et al., 2012; Mercier et al., 2000; Reichenbach, 1999). Since the mean length did not differ between habitats, no separation between *H. arguinensis'* adults and juveniles (< 15 cm length; pers. com. Jorge Domínguez-Godino) has been observed during our study. Life stage is, therefore, not considered to be an important factor in determining *H. arguinensis* distribution in our study area. Predation pressure is also not considered to be a major factor because of the high recapture rates, absence of sheltering behaviour (Navarro et al., 2014) and high presence of juveniles (compared to: Eriksson et al., 2010; Navarro, 2012) observed during our study. Organic matter availability (Navarro et al., 2013; Slater et al., 2010; Slater et al., 2011) might explain the major overlap of home ranges in the area within the sandy channel. The currents observed within it during the upcoming and retreating of the tide, might have increased the organic matter coming from adjacent areas and therefore, making this channel more suitable for holothurians due to a high availability of food. On the other hand, a recent study on *H. arguinensis* in the Canary Islands failed to show any connection between particulate organic matter consumption and type of habitat (Navarro et al., 2014) and no measurements on organic matter concentrations have been conducted during our study. Shelter against UV-irradiance might be the most likely candidate explaining the higher abundance of *H. arguinensis* in the seagrass-habitat area. Broad spectrum UV-absorbing bioactive compounds have indicated that high radiation can be a problem for intertidal sea cucumbers in day active species (Bandaranayake and Rocher, 1999). *H. arguinensis* can indeed show a mucilaginous skin irritation when exposed to high irradiance at low tide, which can be minimized by staying submerged or covered by vegetation (González-Wangüemert et al., 2013).

H. arguinensis moved 10 m day^{-1} within our study area. This speed is comparable to 8 m day^{-1} observed for this species by Navarro et al. (2014) and speed measured for the related *H. sanctori* (11 m day^{-1}, Navarro et al., 2013), both studied on the Canary Islands. Studies on other species show lower daily distances travelled; e.g. *A. mauritiana* (3 m, Graham and Battaglene, 2004), *H. fuscogilva* (2 m, Reichenbach, 1999), *Parastichopus californicus* (3.95 m, Da Silva et al., 1986), *H. scabra* (1.3 m, Purcell and Kirby, 2006) and *A. japonicus* (2 m, YSFRI, 1991). However, comparisons between studies should be considered with care since the mark/recapture method uses an approximation to the real movement and, it could be influenced by the method of marking (Graham and Battaglene, 2004; Navarro et al., 2013, 2014; Purcell and Kirby, 2006; Shiell, 2006).

The home range of 70 specimens of *H. arguinensis* was registered during 4 weeks. These home ranges were not very large (median: 35 m^2) but showed a high degree of overlap. There is scarce information about home range of sea cucumbers. Reichenbach (1999) recorded the home range of 2 individuals of *H. fuscogilva* for a duration of minimum 8 months with home ranges occupying 44 m^2 and 332 m^2 respectively.

Mark/recapture experiments are widely accepted because they can provide a set of population parameters as well as it can be applied on diverse groups of animals (Cunjak et al., 2005; Hestbeck et al., 1991; Lau et al., 2011; Pradel, 1996). Our results show that movement of *H. arguinensis* is independent of the habitat and directed offshore during the day and shoreward during the night. These movements could be explained by avoidance of exposure to UV. Seagrasses are located in the offshore part of the area, which is longer submerged during low tide, and thus is offering protection against sunlight. These results are in contrast to the results of Navarro et al. (2014) where *H. arguinensis'* movement direction was random and speed was faster on a seagrass meadow than on a macroalgal bed. Their study was conducted between 3-8m depth so protection against sunlight was likely not an important factor in their movements. In line with the results of these authors, no differences in activity were measured during day and night. Environmental conditions might also influence sea cucumber behaviour. Barkai (1991) showed, for example, that the two filter feeding holothurian species *Thyone aurea* and *Pentacta doliolum* cluster together in rough waters while *T. aurea* occupies more space in wave protected sites. During our study, the change from directional dominated movement at the start of April and clustered dominated movement and the end of that month was possibly related to the variable environmental conditions during that period. Unfortunately, it was not possible to include more that two study periods to test this hypothesis due to logistical constraints. Further studies on the effect of environmental conditions on holothurian behaviour in intertidal systems along a longer time period will be done to improve the knowledge on *H. arguinensis*.

Temperate holothurians are becoming an important resource for new fisheries (Aydin, 2008; González-Wangüemert and Borrero-Pérez, 2012; González-Wangüemert et al., 2013, 2014, 2015; Sicuro and Levine, 2011), being specially vulnerable in intertidal lagoons due to their ease of capture during low tide and the degradation of seagrass meadows (Cunha et al., 2013; Duarte, 2002; Orth et al., 2006), which provide shelter in those dynamic systems. This study on the behaviour of *H. arguinensis* indicates that future studies on the ecology of temperate sea cucumbers should include intertidal lagoons such as the Ria Formosa since those systems can host high densities of sea cucumbers and might require different management strategies (Purcell and Kirby, 2006; Shiell and Knott, 2008) since the behaviour of holothurian populations living in these lagoons can differ from those living in fully submerged habitats (Fig. 5).

5. Acknowledgements

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6. References

Andrew, N.L., 1993. Spatial Heterogeneity, Sea Urchin Grazing, and Habitat Structure on Reefs in Temperate Australia. Ecology 74, 292-302.

Asmus, R., Sprung, M., Asmus, H., 2000. Nutrient fluxes in intertidal communities of a South European lagoon (Ria Formosa) - similarities and differences with a northern Wadden Sea bay (Sylt-Rømø Bay). Hydrobiologia 436, 217-235.

Aydin, M., 2008. The commercial sea cucumber fishery in Turkey. SPC Beche-de-mer Inf. Bull., 40-41.

Baillargeon, S., Rivest, L.-P., 2007. Rcapture: loglinear models for capture-recapture in R. J. Stat. Softw. 19, 1-31.

Baillargeon, S., Rivest, L.-P., 2012. Rcapture: Loglinear Models for Capture-Recapture Experiments, http://cran.r-project.org/web/packages/Rcapture/Rcapture.pdf.

Bandaranayake, W.M., Rocher, A.D., 1999. Role of secondary metabolites and pigments in the epidermal tissues, ripe ovaries, viscera, gut contents and diet of the sea cucumber *Holothuria atra*. Mar. Biol. 133, 163-169.

Barkai, A., 1991. The effect of water movement on the distribution and interaction of three holothurian species on the South African west coast. J. Exp. Mar. Biol. Ecol. 153, 241-254.

Bartholomew, A., Diaz, R., J., Cicchetti, G., 2000. New dimensionless indices of structural habitat complexity : predicted and actual effects on a predator's foraging success. Mar. Ecol. Prog. Ser. 206.

Bellchambers, L.M., Meeuwig, J.J., Evans, S.N., Legendre, P., 2011. Modelling habitat associations of 14 species of holothurians from an unfished coral atoll: implications for fisheries management. Aquat. Biol. 14, 57-66.

Bruckner, A.W., Johnson, K.A., Field, J.D., 2003. Conservation strategies for sea cucumbers: Can a CITES Appendix II listing promote sustainable international trade. SPC Beche-de-mer Inf. Bull. 18, 24-33.

Conand, C., 1990. The fishery resources of Pacific island countries: Holothurians, FAO Fisheries technical paper. FAO, Rome, p. 145.

Cunha, A.H., Assis, J.F., Serrão, E.A., 2013. Seagrasses in Portugal: A most endangered marine habitat. Aquat. Biol. 104, 193-203.

Cunjak, R.A., Roussel, J.M., Gray, M.A., Dietrich, J.P., Cartwright, D.F., Munkittrick, K.R., Jardine, T.D., 2005. Using stable isotope analysis with telemetry or mark-recapture data to identify fish movement and foraging. Oecologia 144, 636-646.

Da Silva, J., Cameron, J.L., Fankboner, P.V., 1986. Movement and orientation patterns in the commercial sea cucumber *Parastichopus californicus* (Stimpson)(Holothuroidea: Aspidochirotida). Mar. Behav. Physiol. 12, 133-147.

Dong, G., Dong, S., Tian, X., Wang, F., 2011. Effects of photoperiod on daily activity rhythm of juvenile sea cucumber, *Apostichopus japonicus* (Selenka). Chin. J. Oceanol. Limnol. 29, 1015-1022.

Duarte, C.M., 2002. The future of seagrass meadows. Environ. Conserv. 29, 192-206.

Entrambasaguas, L., Pérez-Ruzafa, Á., García-Charton, J.A., Stobart, B., Bacallado, J.J., 2008. Abundance, spatial distribution and habitat relationships of echinoderms in the Cabo Verde Archipelago (eastern Atlantic). Mar. Freshw. Res. 59, 477-488.

Eriksson, H., Fabricius-Dyg, J., Lichtenberg, M., Perez-Landa, V., Byrne, M., 2010. Biology of a high-density population of *Stichopus herrmanniat* One Tree Reef, Great Barrier Reef, Australia. SPC Beche-de-mer Inf. Bull. 30, 41-45.

Eriksson, H., Jamon, A., Wickel, J., 2012. Observations on habitat utilization by the sea cucumber *Stichopus chloronotus*. SPC Beche-de-mer Inf. Bull. 32, 39-42.

Francour, P., 1997. Predation on holothurians: a literature review. Invertebr. Biol. 116, 52-60.

González-Wangüemert, M., Aydin, M., Conand, C., 2014. Assessment of sea cucumber populations from the Aegean Sea (Turkey): First insights to sustainable management of new fisheries. Ocean Coast Manage. 92, 87-94.

González-Wangüemert, M., Borrero-Pérez, G., 2012. A new record of *Holothuria arguinensis* colonizing the Mediterranean Sea. Mar. Biodivers. Rec. 5, 1-4.

González-Wangüemert, M., Braga, T., Silva, M., Valente, S., Rodrigues, F., Serrao, E., 2013. Volunteer programme assesses the *Holothuria arguinensis* populations in Ria Formosa (southern Portugal). SPC Beche-de-mer Inf. Bull. 33, 44-48.

González-Wangüemert, M., Valente, S., Aydin, M., 2015. Effects of fishery protection on growth and genetic structure of three target sea cucumber species. Hydrobiologia, 743, 65-74.

Graham, J.C.H., Battaglene, S.C., 2004. Periodic movement and sheltering behaviour of *Actinopyga mauritiana* (*Holothuroidea: Aspidochirotidae*) in Solomon Islands. SPC Beche-de-mer Inf. Bull. 19, 23-31.

Hamel, J.-F., Conand, C., Pawson, D.L., Mercier, A., 2001. The sea cucumber *Holothuria scabra* (Holothuroidea: Echinodermata): Its biology and exploitation as Beche-de-mer. Adv. Mar. Biol. Volume 41, 129-223.

Hammond, L.S., 1982. Patterns of Feeding and Activity in Deposit-Feeding Holothurians and Echinoids (Echinodermata) from a Shallow Back-Reef Lagoon, Discovery Bay, Jamaica. Bull. Mar. Sci. 32, 549-571.

Hestbeck, J.B., Nichols, J.D., Malecki, R.A., 1991. Estimates of Movement and Site Fidelity Using Mark-Resight Data of Wintering Canada Geese. Ecology 72, 523-533.

Kirshenbaum, S., Feindel, S., Chen, Y., 2006. A study of tagging methods for the sea cucumber *Cucumaria frondosa* in the waters off Maine. Fish. Bull. 104, 299.

Lau, D.C.C., Dumont, C.P., Lui, G.C.S., Qiu, J.-W., 2011. Effectiveness of a small marine reserve in southern China in protecting the harvested sea urchin *Anthocidaris crassispina*: A mark-and-recapture study. Biol. Conserv. 144, 2674-2683.

Lund, U., Agostinelli, C., 2013. Package ‘circular’. http://cran.r-project.org/web/packages/circular/circular.pdf.

Malaquias, M.A.E., Sprung, M.J., 2005. Population biology of the cephalaspidean mollusc *Haminoea orbygniana* in a temperate coastal lagoon (Ria Formosa, Portugal). Estuar. Coast. Shelf. S. 63, 177-185.

Mercier, A., Battaglene, S.C., Hamel, J., 1999. Daily burrowing cycle and feeding activity of juvenile sea cucumbers *Holothuria scabra* in response to environmental factors. J. Exp. Mar. Biol. Ecol. 239, 125-156.

Mercier, A., Battaglene, S.C., Hamel, J., 2000. Periodic movement, recruitment and size-related distribution of the sea cucumber *Holothuria scabra* in Solomon Islands. Hydrobiologia 440, 81-100.

Navarro, P., 2012. Biología y ecología de las holoturias [Echinodermata: Holothuroidea] de la isla de Gran Canaria (Atlántico Centro-Oriental), PhD Thesis. Universidad De Las Palmas De Gran Canaria Las Palmas de Gran Canaria, p. 239.

Navarro, P.G., García-Sanz, S., Barrio, J.M., Tuya, F., 2013. Feeding and movement patterns of the sea cucumber *Holothuria sanctori*. Mar. Biol. 160, 2957-2966.

Navarro, P.G., García-Sanz, S., Tuya, F., 2014. Contrasting displacement of the sea cucumber *Holothuria arguinensis* between adjacent nearshore habitats. J. Exp. Mar. Biol. Ecol. 453, 123-130.

Neteler, M., Bowman, M.H., Landa, M., Metz, M., 2012. GRASS GIS: A multi-purpose open source GIS. Environ. Modell. Softw. 31, 124-130.

Orth, R.J., Carruthers, T.J.B., Dennison, W.C., Duarte, C.M., Fourqurean, J.W., Heck, K.L., Hughes, A.R., Kendrick, G.A., Kenworthy, W.J., Olyarnik, S., Short, F.T., Waycott, M., Williams, S.L., 2006. A Global Crisis for Seagrass Ecosystems. Bioscience 56, 987-996.

Pradel, R., 1996. Utilization of capture-mark-recapture for the study of recruitment and population growth rate. Biometrics 52, 703-709.

Purcell, S.W., Agudo, N.S., Gossuin, H., 2008. Poor retention of passive induced transponder (PIT) tags for mark-recapture studies on tropical sea cucumbers. SPC Beche-de-mer Inf. Bull. 28, 53-55.

Purcell, S.W., Kirby, D.S., 2006. Restocking the sea cucumber *Holothuria scabra*: Sizing no-take zones through individual-based movement modelling. Fisheries Research 80, 53-61.

Purcell, S.W., Lovatelli, A., Vasconcellos, M., Ye, Y., 2010. Managing sea cucumber fisheries with an ecosystem approach, FAO Fisheries and aquaculture technical paper. No. 520. FAO, Rome, p. 157.

Purcell, S.W., Samyn, Y., Conand, C., 2012. Commercially important sea cucumbers of the world, FAO Species Catalogue for Fishery Purposes. FAO, Rome, p. 124.

Reichenbach, N., 1999. Ecology and fishery biology of *Holothuria fuscogilva* (*Echinodermata: Holothuroidea*) in the Maldives, Indian Ocean. Bull. Mar. Sci. 64, 103-114.

Rodrigues, N.V., 2012. New geographic distribution records for Northeastern Atlantic species from Peniche and Berlengas Archipelago. Life Mar. Sci. 29, 63-66.

Rodrigues, F., Valente, S., González-Wangüemert, M., 2015. Genetic diversity across geographical scales in marine coastal ecosystems: *Holothuria arguinensis* a model species. J Exp Mar Biol Ecol, 463, 158-167.

Roggatz, C.C., 2012. Health from the Ocean – Sea cucumbers as food of high nutritional value and source of bioactive compounds, M.sc. thesis, CCMAR. Universidada do Algarve, Faro, p. 39.

Shiell, G.R., 2006. Effect of invasive tagging on the activity of *Holothuria whitmaei* [Echinodermata: Holothuroidea]: A suitable mark-recapture method for short-term field studies of holothurian behaviour. Mar. Freshw. Behav. Physiol. 39, 153-162.

Shiell, G.R., Knott, B., 2008. Diurnal observations of sheltering behaviour in the coral reef sea cucumber *Holothuria whitmaei*. Fisheries Research 91, 112-117.

Sicuro, B., Levine, J., 2011. Sea Cucumber in the Mediterranean: A Potential Species for Aquaculture in the Mediterranean. Rev. Fish. Sci. 19, 299-304.

Slater, M.J., Carton, A.G., Jeffs, A.G., 2010. Highly localised distribution patterns of juvenile sea cucumber *Australostichopus mollis*. N. Z. J. Mar. Freshw. Res. 44, 201-216.

Slater, M.J., Jeffs, A.G., Sewell, M.A., 2011. Organically selective movement and deposit-feeding in juvenile sea cucumber, *Australostichopus mollis* determined in situ and in the laboratory. J. Exp. Mar. Biol. Ecol. 409, 315-323.

Sprung, M., 1994. Macrobenthic Secondary Production in the Intertidal Zone of the Ria Formosa - a Lagoon in Southern Portugal. Estuar. Coast. Shelf. S. 38, 539-558.

Sprung, M., 2001. Larval abundance and recruitment of *Carcinus maenas L*. close to its southern geographic limit: a case of match and mismatch. Hydrobiologia 449, 153-158.

Toral-Granda, V., Lovatelli, A., Vasconcellos, M., 2008. Sea cucumbers. A global review on fishery and trade., FAO Fisheries Technical Paper. No. 516. FAO, Rome, p. 317.

Tuya, F., Hernández, J.C., Clemente, S., 2006. Is there a link between the type of habitat and the patterns of abundance of holothurians in shallow rocky reefs? Hydrobiologia 571, 191-199.

Uthicke, S., 1999. Sediment bioturbation and impact of feeding activity of *Holothuria* (*Halodeima*) *atra* and *Stichopus chloronotus*, two sediment feeding holothurians, at Lizard Island, Great Barrier Reef. Bull. Mar. Sci. 64, 129-141.

Uthicke, S., 2001a. Interactions between sediment-feeders and microalgae on coral reefs: grazing losses versus production enhancement. Mar. Ecol. Prog. Ser. 210, 125-138.

Uthicke, S., 2001b. Nutrient regeneration by abundant coral reef holothurians. J. Exp. Mar. Biol. Ecol. 265, 153-170.

Worton, B.J., 1987. A review of models of home range for animal movement. Ecol. Model. 38, 277-298.

YSFRI, 1991. Training manual on breeding and culture of scallop and sea cucumber in China Part II. Fisheries Research Institute, Qingdao, China, pp. 47-79.

Figure 1. Location of the study area (■). A. Map of Portugal, B. Ria Formosa, C. study area (European Terrestrial Reference System ETRS 1989 Datum, GRS 1980 spheroid, Transverse Mercator projection).

Figure 2. Habitat distribution within the study area. A-C represent the percentage cover, expressed in colour intensity, per habitat. The habitat map (D) represents the first component of a PCA describing the habitat variability. Green-blue colours represent both the border area of seagrass with sand and the seaweed habitat.

Figure 3. Orientation of *H. arguinensis'* movements during day/night and within seagrass and sandy habitats. Length of the bars in the rose graphs represent the frequency in which the specimen moved in a certain direction. Differences in mean orientation between day and night were significant ($P < 0.05$) and independent of the habitat. Differences in mean orientation between habitats were not significant ($P > 0.05$).

Figure 4. Home ranges of *H. arguinensis*. The minimum area method was applied for the calculation of the home range area (Worton, 1987).

Figure 5. Summary figure of movement and abundance between the results obtained in this study and in Navarro et al. (2014). Bar graphs represent relative abundance (habitat: black: seagrass, grey macro algae; white: sand). Dashed lines represent relative movement speed. Solid lines represent movement direction.

Supplementary Figure S1. Readability of scratched marks over time. Photos show the marks of 3 *H. arguinensis* specimen directly after marking (A) and recapture (B). Time between recaptures is given above the photos.

Supplementary Figure S2. Movement patterns of *H. arguinensis*. A. Directional movement, B. Clustered movement, C. Zigzag and Circular Movement and D. Directional and Clustered movement. Different IDs represent different specimen.

Table 1. Environmental variability during the course of the study in both periods 1 (8-12 April) and 2 (22-28 April).

Table 2. Recapture probabilities over 215 specimens of *H. arguinensis* for both periods. The number of individuals recaptured with readable marks and unreadable marks during period 2 is given for specimen captured during both periods. The total average recapture probability was 73%. Period 1: 8-12 April, period 2: 22-28 April.

TABLES

Table 1. Environmental variability during the course of the study in both periods 1 (8-12 April) and 2 (22-28 April).

	Mean+SD		**One-way ANOVA**	
	Period 1	**Period 2**	$F_{1,18}$	***P***
Salinity	30.4+1.3	32.1+0.7	12.680	**
Water temperature (°C)	17.4+1.3	19.5+2.7	5.325	*
Tidal height low tide (m)	0.63+0.05	0.45+0.09	35.33	***
Wind speed (knots)	6.6+2.9	10.6+4.2	5.919	*
Wind direction (°)[1]	267+1	348+1	11.35	**

Significance codes: 0 < *** < 0.001 < ** < 0.01 < * < 0.05

[1] Circular Analysis of Variance

Table 2. Recapture probabilities over 215 specimens of *H. arguinensis* for both periods. The number of individuals recaptured with readable marks and unreadable marks during period 2 is given for specimen captured during both periods. The total average recapture probability was 73%. Period 1: 8-12 April, period 2: 22-28 April.

	Number of captures[1]	**Number of recaptures**[2]	**Percentage**
Total study	215	156	73
Period 1	147	105	71
Period 2	124	80	65
Mark readable		56	38[2]
Mark unreadable		38	26[2]

[1] Differences in counts between the total and the sum of both periods are caused by captures of period 1 being recaptured in period 2.

[2] Percentage of captures from period 1 that were recaptured in period 2.

Figure 1

FIGURES

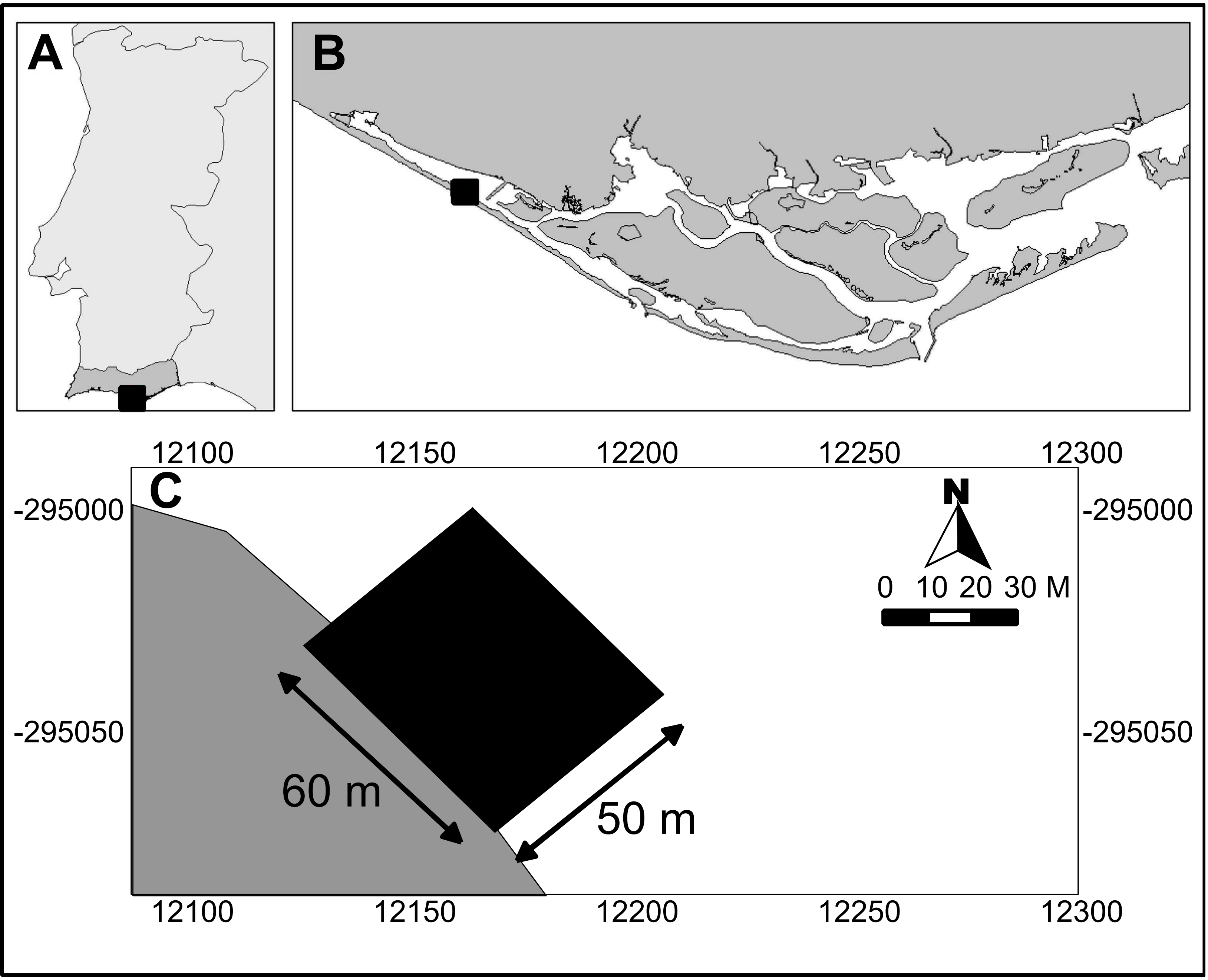


Figure 2

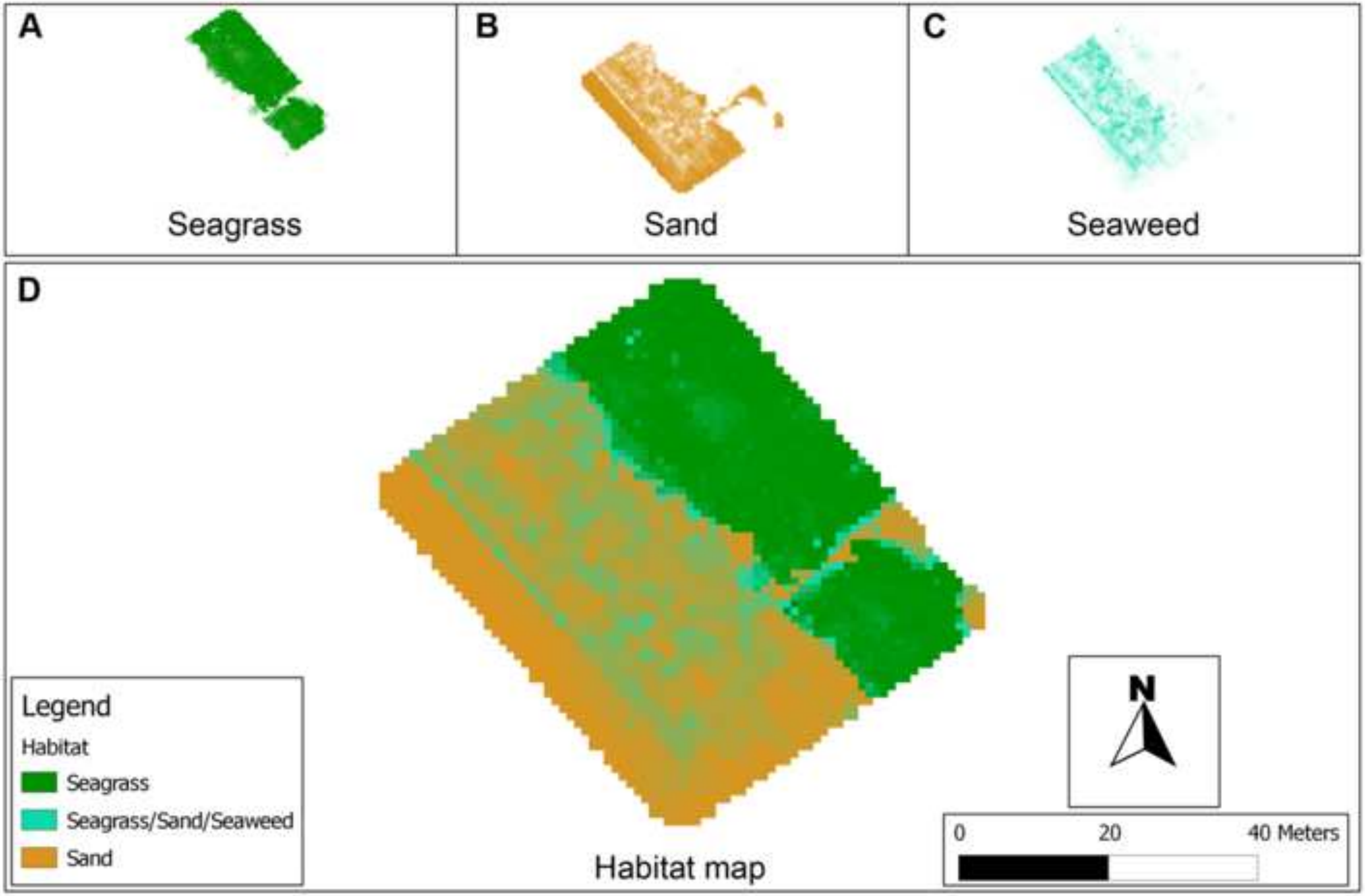
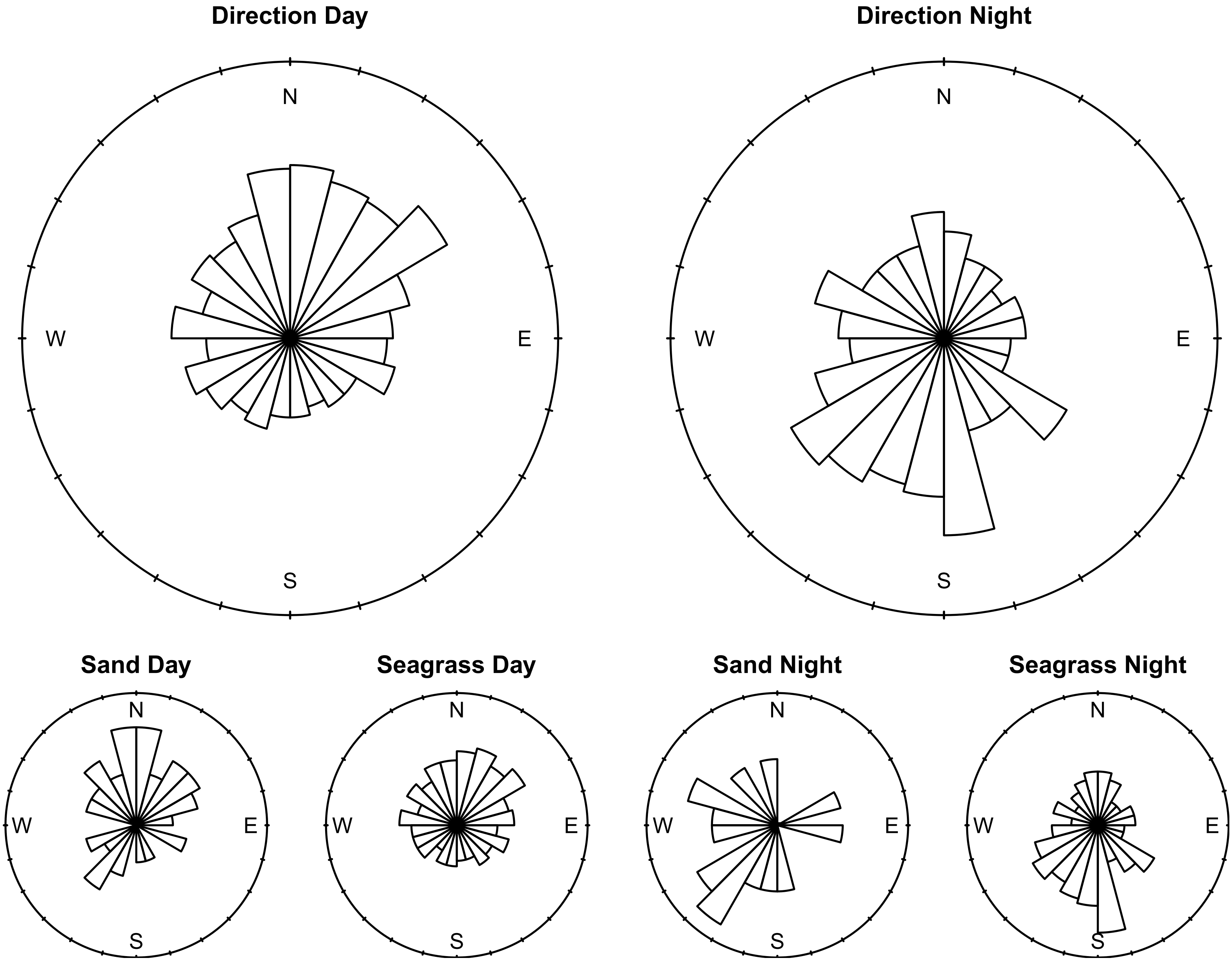

A
Seagrass
B
Sand
C
Seaweed
D
Habitat map
Legend
Habitat
Seagrass
Seagrass/Sand/Seaweed
Sand
N
0
20
40 Meters

Figure 3


Direction Day
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N
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Sand Day
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Seagrass Day
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Sand Night
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E
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Seagrass Night
N
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E
S

Figure4

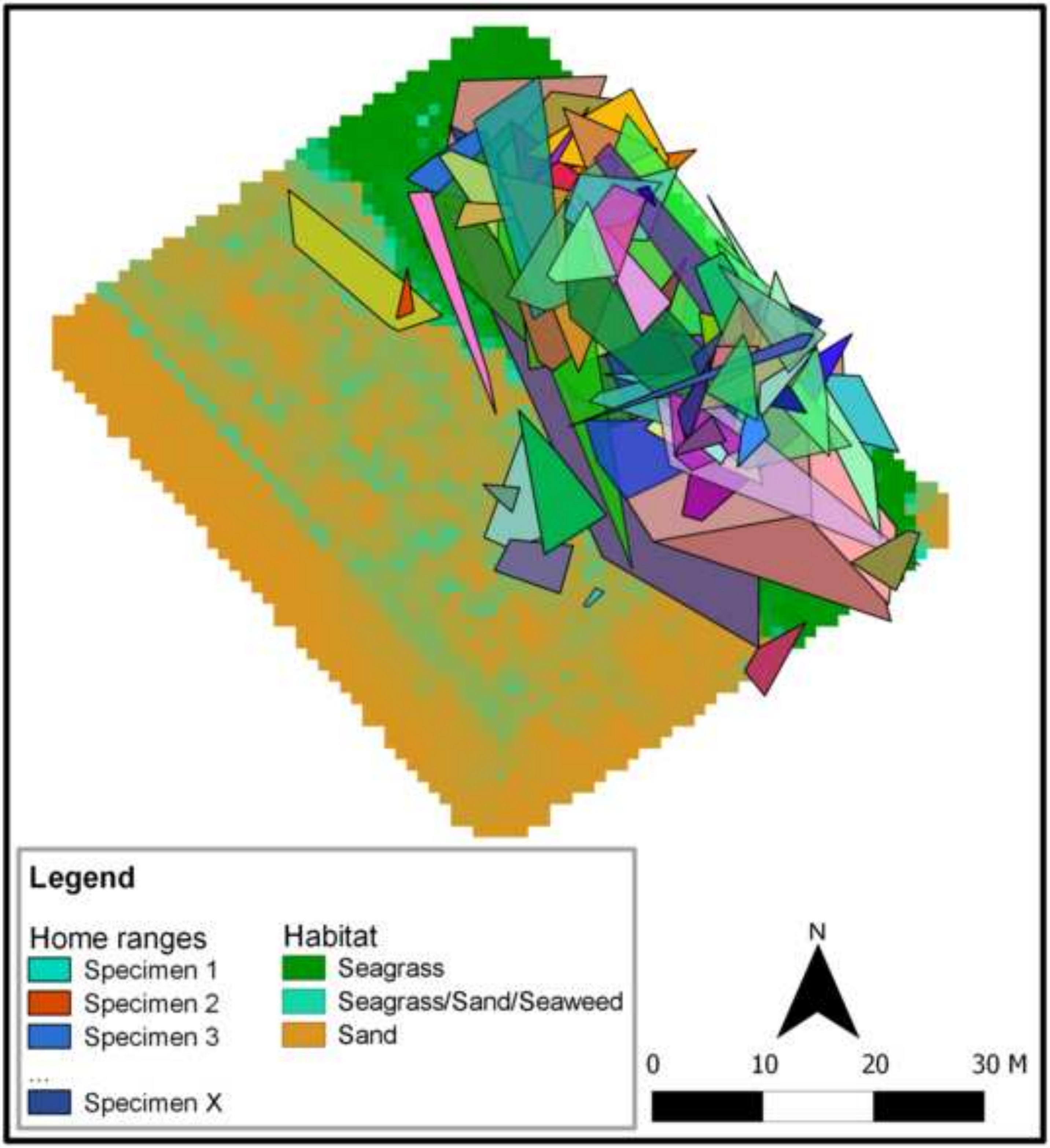
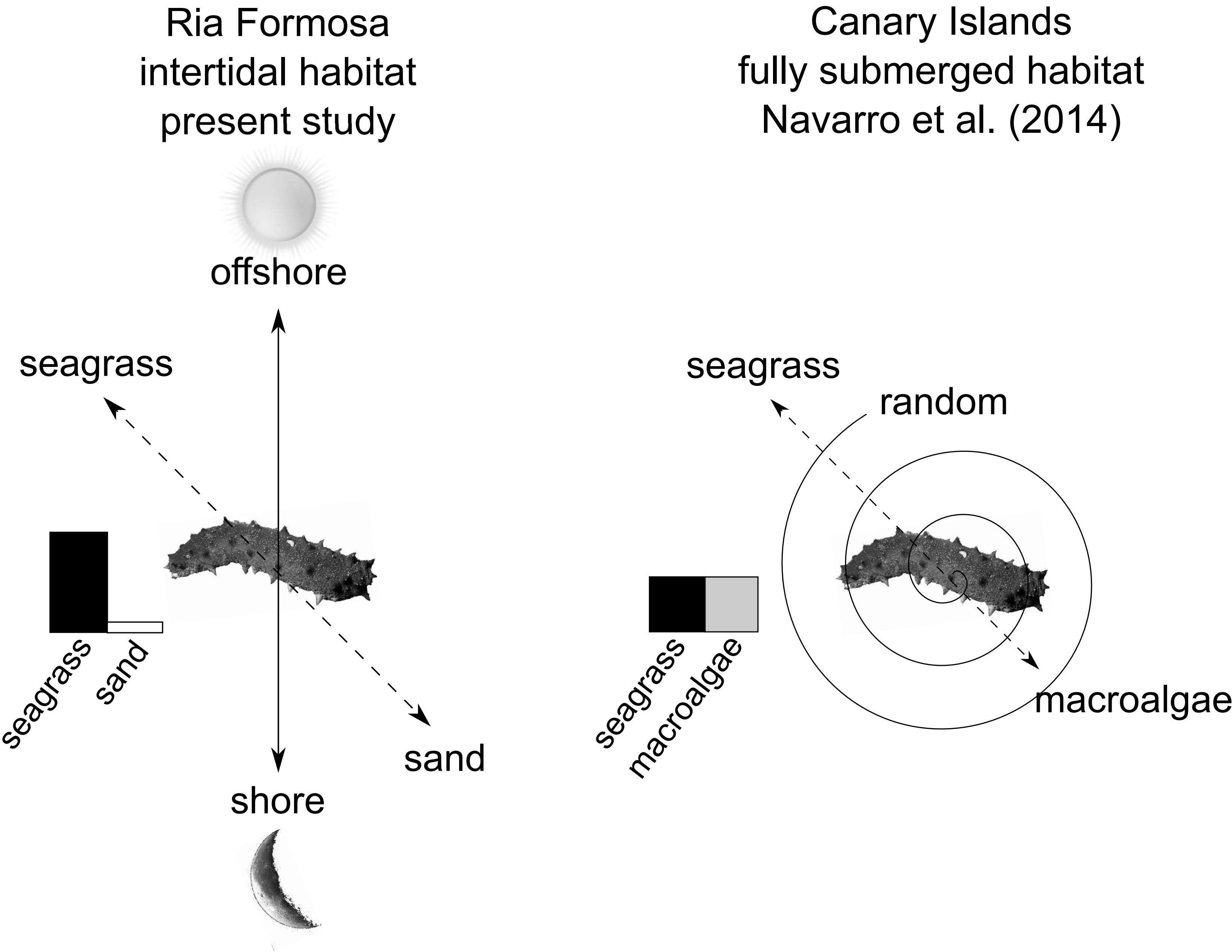

Legend
Home ranges
Specimen 1
Specimen 2
Specimen 3
...
Specimen X
Habitat
Seagrass
Seagrass/Sand/Seaweed
Sand
N
0
10
20
30 M

Figure 5


Ria Formosa
intertidal habitat
present study
offshore
seagrass
seagrass
sand
sand
shore
Canary Islands
fully submerged habitat
Navarro et al. (2014)
seagrass
random
seagrass
macroalgae
macroalgae