Comparing activity and space patterns of the European pond turtle, *Emys orbicularis* (L., 1758) in a Venice Lagoon wetland area: implications for conservation planning and management

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Abstract. Behavioural and spatial distribution analyses were quantified during a phase of activity and lethargy in a wild population of the European pond turtle inhabiting a protected internal wetland area of the Venice lagoon. The marked individuals (13 males and 16 females) provided informative radiotracking data to study differential patterns of activity, dispersion and habitat use between the two study periods ("October-November both 2019 and 2020" and "June-July 2020"). The differences in the movements behaviours and habitat selection were affected by period. Movements were higher in the period of activity than lethargy, but they were not influenced by sex and size. The presence of the European pond turtle in the transitional woodland/shrubs and brackish water valley habitats was significantly higher in the period of activity than lethargy. During the latter one, pond turtles were observed to brumate gregariously in a small area for brumation, usually in shallow water. In contrast, all individuals have changed water bodies during the activity period. Part of those movements has occurred towards aquatic habitat with higher salinities 1-17‰ (mean: 10.64‰). These findings provide a set of information to better understand the behavioural ecology of *Emys orbicularis* in the lagoon area. This is of relevance for management actions and for the conservation of this threatened species.

Keywords: behavioural ecology, habitat use, home range, movement, pond turtles, radiotracking.

Introduction

Reptiles have discrete times of the day and year when activity is concentrated (Tosini, Bertolucci and Foà, 2001; Dayananda, Jeffree and Webb, 2020), yet movement and activity are not always predictable. Movement, basking, nesting and brumation behaviours may be influenced by biotic (e.g., prey availability, reproductive condition, experience of individuals) and physical factors (e.g., climate, the attributes of the landscape, topography (Meeske, 2000; Meeske and Mühlenberg, 2004; Ficetola, Thuiller and Padoa-Schioppa, 2009; Vilardell-Bartino et al., 2015; Ottonello et al., 2017a; Canessa et al., 2016)). The understanding of these factors is extremely useful to minimize negative interactions between anthropogenic disturbance and wildlife (Novarini and Bano, 2019) or to assist wildlife managers in minimizing threats to endangered species and those of conservation concern (Brussard, 1991). A winning strategy is to analyse information on animal movements, use of space and activity patterns for understanding their life history and

how attributes of the home range size, movement rates and timing of movements interact with biotic and physical factors. Wetland landscapes present a system that is well suited for the examination of physical factors driving the behavioural patterns of those species adapted to these habitats and that are interesting for conservation planning. Wetlands are patchily distributed within terrestrial habitat, yet wetlands (especially temporary wetlands that occasionally or regularly dry) may vary in quality and so provide gradients in resource quality through space and time. This in turn affords a potential incentive to move and find a more suitable patch (Sayer and Davenport, 1991; Roe and Georges, 2008). Freshwater turtles are a special group among vertebrates that colonize aquatic and terrestrial environments. Their semi-aquatic behaviour allows us to study and understand the close connection between terrestrial and aquatic environments (Bodie and Semlitsch, 2000). The conservation of turtles not only depends on the quality of the aquatic habitat but also on the quality of the terrestrial habitat surrounding it. This makes the conservation of turtles a complicated and expensive undertaking (Turtle Conservation Fund, 2002). The Valle Averto Oasis, a protected internal wetland area of the Venice lagoon, is an excellent case study to assess how physical factors can have an influence on the biology, ecology and dynamics of the freshwater turtle populations. Wild populations of the European pond turtle, *Emys orbicularis* inhabit this area of mixed natural and abandoned fishing valley habitat. A recent study about the abundance estimation of the *E. orbicularis* population in this study site, have showed one of the most populated areas of the Italian peninsula (Liuzzo et al., 2021). On the national red list of IUCN, the conservation status of *E. orbicularis* was established "Endangered" (Andreone et al., 2013). Consequently, it was fully protected within the frame of the NATURA 2000 network. However, even though the species was reported along the inland margins of the Venice lagoon (Agapito Ludovici, Di Francesco and Di Tizio, 2013; Ficetola et al., 2013) with high density values recorded in the Valle Averto Oasis (Liuzzo et al., 2021) a lack of information still exists relating to basic life-history and only a few studies were carried out on its behavioural ecology (Lebboroni and Chelazzi, 1991; Zuffi et al., 2020). This may result in difficulties when designing effective management plans. The present study aims to provide new data on the use of aquatic and terrestrial habitats in a protected internal wetland area of the Venice lagoon, Valle Averto (Southern Lagoon) for a wild population occupying a system of freshwater wetlands and brackish valleys. Individual activities and space use were compared between a period of activity and lethargy.

A positive effect of the period of activity was predicted on basking behaviour, movement distance, and home range size, and it was hypothesized that more turtles would occur in the brackish valley area. In contrast, a positive effect of the period of lethargy was predicted on brumation behaviour and reduction of movement distance and home range size. The integration of these data with the existing literature on the ecology and life history of pond turtles can improve the *in-situ* conservation planning of *E. orbicularis* populations and emphasises the ecological requirements that must be met before turtle relocations.

Materials and methods

Study area

The Valle Averto Oasis is a protected area of the southern basin of the Venice lagoon established in 1988 and currently managed by the Italian Association for the World Wildlife Fund (WWF Italy Ong – ONLUS). The Oasis located within the municipality of Campagna Lupia Province of Venice, Italy (45°21′N, 12°09′E) has a surface of 500 hectares. Of these, about 83 ha are owned by WWF and include emerged lands, freshwater wetlands, two brackish lakes and saltmarshes. Salinity ranges between 0.5 and 12‰ depending on the season and distance of the study site from the sea (higher values were recorded during summer in water bodies near the lagoon) (Liuzzo et al., 2021). The study area included in the list of wetlands of international importance, according to the Ramsar Convention. It also lies within the Special Conservation Area of the middle and lower Venice

lagoon (ZSC IT3250030) and the Special Protection Area extended to the entire lagoon basin (ZPS IT3250046). Valle Averto is an area of mixed natural and abandoned fishing valley patch with dominant vegetation of *Phragmites australis*, *Typha latifolia*, association of *Lolio-Plantaginetea* in wetlands, and *Nymphaea alba*, *Potamogeton natans*, *Myriophillum spicatum* in water bodies. The upland area is defined by different habitat types: linear structures of hedges and tree spots, bushy or herbaceous vegetation with *Rubus ulmifolius* and dense reed vegetation with the association of *Puccinellio festuciformis-Phragmitetum australis* (Padoan and Caniglia, 2004).

Trapping and radiotracking

Sampling was carried out in the Nitticore (N) (45°21′ 23.54″N, 12°8′29.55″E), a canal with an alternate water flow and dense reed vegetation associated with *Tamarix* spp., on three occasions (end of September 2019, 2020 and June 2020) each consisting of three trapping days. Baited floating traps (n = 27, length = 60 cm, diameter = 30 cm, eye = 16 × 14 cm) with sardines were placed in the body water along the shoreline located 50 m apart from each other. Date of capture, trap number, sex, age (adult/juveniles), straight carapace length (SCL, sliding callipers to the nearest 0.1 mm) and body mass (BM, digital balance ± 1 g) were recorded, and only individuals with evident sexual characters were considered as adults (Zuffi and Gariboldi, 1995). At capture, after the morphometric measurements, each adult female was processed manually with palpation of the inguinal region in order to detect for any oviductal egg (Zuffi et al., 2005). The population structure was determined by dividing SCL data in size classes of 10 mm (Ottonello et al., 2017b; Liuzzo et al., 2021).

From total captures, only the adult freshwater turtles in an apparent good state of health (without morphological anomalies or aberrant locomotor performance) were tagged. More specifically, the individuals were captured, marked and fitted with radio-transmitters (TXE-124G Telenax, UK, Receiver YAESU FT-818ND, 148–152 MHz and Yagi antenna). The tagged individuals were monitored for 2-3 days in the quarantine area in order to assess the transmitter's influence. The quarantine area was composed of a fish tank (about 120 × 80 × 60 cm) filled with ∼30 cm water and submerged rocks for stable basking sites. Specifically, to minimize the marking impact, the possible pain, distress, or general interferences with natural turtles' behaviour was supervised and avoided (Gutema, 2015; Vecchio et al., 2018). Afterwards, the tagged turtles were released into the water body near the trap in which they were caught. Radio-transmitters were attached to the anterior upper carapace margin with resin epoxy and weighed between 2-3% of the pond turtle body mass. Transmitter frequencies were searched 5-6 days per week from the beginning of October to the end of November both 2019 and 2020, then from the half of June to the end of July 2020. A total of 29 different individuals were radio-marked: 7 (2 males, 5 females) in autumn 2019, 9 (5 males, 4 females) in summer 2020, and 13 (6 males, 7 females) in autumn 2020. Due to the COVID-19 pandemic's outbreak in the spring *E. orbicularis* activity,

it was not possible to collect location data for the early reproductive phase (March, April, May 2020). Nonetheless, overall, 702 fixes were recorded. Both homing and triangulation methods were used to locate the individuals, although priority was given to the homing method because it provides more precise locations.

Habitat use and behaviours

Habitat use, home range size and turtle behaviours were investigated during the period of activity and lethargy. These periods were analysed by dividing month data into two phases: a late reproductive period from the half of June to the end of July 2020 and a post-reproductive period from the early of October to the end of November both 2019 and 2020. At a finer scale, the first season might correspond to the late reproductive period where the peak of nesting activity has been exceeded (Masiero, 2015; Pesce, 2020; Marchand, Le Gal and Georges, 2021) whereas the second season corresponding with the early period of torpor (Cadi et al., 2004; Novotný et al., 2008). Specifically, habitat use was explored distinguishing five types of macrohabitats in the study area (fig. 1): (1) Woodland (W) rows of *Robinia pseudoacacia*, *Tamarix gallica* and *Rubus ulmifolius*; (2) Transitional Woodland/shrub (T) bushy or herbaceous vegetation with scattered trees; (3) Phragmites reeds (F) dense reeds vegetation with the association of *Puccinellio festuciformis-Phragmitetum australis*; (4) Brackish valleys (B) brackish water bodies with association of *Chaetomorpho-Ruppietum*; (5) Watercourses and Canals (C) water bodies with banks covered with reed vegetation. Each location was associated with the type of habitat and the percentage of individual pond turtles' locations was calculated in each habitat type. The main turtles' behaviours variation observed among sampling periods were also investigated in the water and on the canal banks. Focused observations on individual tagged turtles have also been conducted to assess the basking, floating, movements, and brumation behaviours. Specifically, these behaviours were explored and inferred by distinguishing five types of adults' behaviours in the study area: Five adults' behaviours were observed during the period of activity and lethargy: (1) Basking (B) turtles laying on a rock, bank, or another surface in order to completely dry off and soak up UV-rays, (2) Movement (M) general movements both in aquatic and terrestrial habitat, (3) the Movement caused by the radiotracking operator (Mo) behaviour affected by the radio-tracking operator that was removed from biological interpretation, (4) a Short period of stationary in the mud bottom (Ss) static turtles in the mud bottom of the water body (<5 h) (5) a Long period of stationary in the mud bottom (Ls) static turtles in the mud bottom after 5 hours without detecting movement. To obtain the phenological variation of the activity, also movement data were grouped in the period of activity (June, July 2020) and lethargy (October and November 2019, 2020), calculating the mean distance covered in each period. Total distance moved was estimated as the sum of the straight-line distances between sequential locations; this distance was then broken into movements in water and on land. Movement rates (m day^{-1}) and the minimum terrestrial distance were also determined on a bimonthly basis.
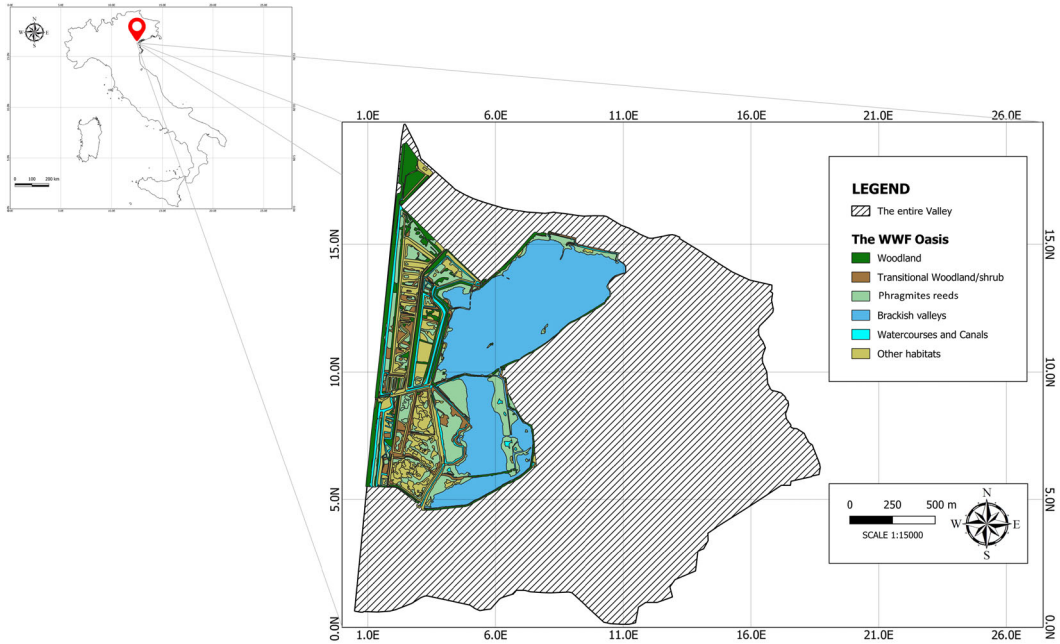
Figure 1. Territorial framework of Valle Averto and delimitation of the naturalistic oasis WWF. Map of land use types according to the European Union classification Corine Land Cover focused on the location sites (original map from Buffa, Ghirelli and Fantinato, 2013).

Statistical analysis

The location data were not normally distributed then the analyses to test for differences in macrohabitat use were performed with the non-parametric Kruskal-Wally test. In addition, a post hoc Dunn's test was carried out to compare the number of locations between the period of activity and lethargy. Home range sizes were estimated by using the minimum convex polygon method (MCP) and kernel density estimators to allow for comparison with other spatial distribution studies. The MCP is the most common method for estimating home ranges and consists of constructing the smallest convex polygon encompassing all known or estimated locations for the animal (Hayne, 1949). The Kernel Density Estimator (KDE) produces a utility distribution, which represents the probability that an animal will be in any part of its home range (Silverman, 1986; Worton, 1989; Seaman and Powell, 1996; Powell, 2000). More specifically, the MCP was calculated into R software following the functions of the package adehabitatHR (version 0.4.19) (Calenge, 2006) whereas the KDE was performed into QGIS version 3.4.9-Madeira software following the Silverman functions (Silverman, 1986). The results with the 95% and 50% contours were presented both for the MCP and fixed kernel estimator (table 1, fig. 3). The spatial autocorrelation was not eliminated in the point data because it has been suggested that doing so reduces biological relevance of home range estimates (De Solla, Bondurianski and Brooks, 1999), especially during the brumation period. Due to defects in one transmitter, fewer than 10 locations were produced by an individual. This individual was not included in the estimation of home range size even if a correlation was not observed between the number of locations and the size of the home range (Pearson Correlation test, $\rho = 0.362$, $P > 0.05$). Because home range data were not normally distributed (Shapiro-Wilk $W = 0.331$, $P < 0.001$ for the period of activity and $W = 0.732$, $P < 0.001$ for the period of lethargy), the Wilcoxon test was performed to assess significant differences in the home range area. The relationships between the size of an individual (weight at the time of release) and home range were investigated using a Kendall's Rank Correlation. Differences in home range size between sexes were also analysed, using the Mann-Whitney U test.

The behaviour frequency was measured as the number of observations per location. The differences in the number of the main behaviours (recorded during the individual locations) were analysed between the periods of activity and lethargy through the Wilcoxon test as data did not meet the parametric assumptions.

The GPS coordinates of locations were plotted into QGIS version 3.4.9-Madeira software and then the minimum linear distance was measured among these points. The differences in the individual distance covered by individuals among the period of activity and lethargy was tested by means of the Kruskal-Wally test as data did not meet parametric assumptions. Moreover, to compare the daily distance travelled among the periods, a post hoc Dunn's test was performed. Differences in the minimum terrestrial distance were also analysed between sexes and periods using the Wilcoxon test.

Table 1. Movement and space use estimates (mean ± 1 SD) for 12 male and 17 female *Emys orbicularis* in the Valle Averto Oasis, Venice lagoon. Differences in movement and space use estimates of *E. orbicularis* were calculated between the period of activity (A) and lethargy (L) period using the Minimum Convex polygon (MCP) and Kernel Density Estimation (KDE).

Parameters	Activity$_{(A)}$	Range$_{(A)}$	Lethargic$_{(L)}$	Range $_{(L)}$
MCP 95% (*ha*)	3.90 ± 3.05	0.15-9.47	0.13 ± 0.11	2×10^{-3}-0.42
MCP 50% (*ha*)	0.32 ± 0.21	0.04-0.7	0.03 ± 0.02	1×10^{-3}-0.09
KDE 95% (*ha*)	1.80 ± 1.44	$82 \times 10^{-4} \pm 3.46$	0.16 ± 0.15	$2 \times 10^{-3} \pm 0.26$
KDE 50% (*ha*)	0.24 ± 0.21	$14 \times 10^{-4} \pm 0.52$	0.0 ± 0.015	$1 \times 10^{-4} \pm 0.05$
Total movement (*m*)	82.63 ± 73.93	1.93-546.64	10.16 ± 9.86	0-67.64
Terrestrial movement (*m*)	65.03 ± 58.74	3.75-327.96	12.85 ± 11.84	0-61.21

Relationships between *E. orbicularis* movement distance and five predictor variables (mean water temperature, mean salinity, sex, turtle's weight, number of days radio-tracked) were investigated using multiple and simple linear regression models. The environmental variables were measured with a water thermometer (Hanna Instruments Checktemp® 1) and refractometer (Giorgio Bormac Mod. 106) at the same depth (20-40 cm below the water surface) and with the same frequency of locations. Relationships between covariates were assessed using Pearson correlation coefficients and Variance Inflation Factors (VIF) (Zuur, Ieno and Elphick, 2010). Although a value of 10 is often suggested as the threshold for VIF, a more stringent VIF threshold of three was selected because collinearity inflates *p-value*, making it more difficult to detect patterns (Zuur, Ieno and Elphick, 2010). Significant correlation involved four predict variables: relationships in pairs between mean temperature and mean salinity and between sex and turtle's weight. To avoid multicollinearity problem in the fitting process, independent variables was checked individually and sequentially based on the nested estimate procedure method (Lin, 2008). A backward stepwise multiple linear regression was performed on the turtle's monitored in 2020 to determine whether environmental and morphometric characteristics cause variation in turtle movement distance among occupied water bodies. Backward elimination of the independent variables based on AIC value was conducted in order to optimize and improve the parsimony of the regression model. Specifically, the sex and turtle' weight predict variables were removed following the algorithm of the R package "olsrr" (Hebbali, 2021). To meet assumptions of residual normality, *E. orbicularis* movement distance was transformed using natural logarithms. All linear regression assumptions were accepted following the algorithm of the R package "gvlma" (Peña and Slate, 2006). All statistical analysis was made using R v. 4.0.2 (R Core Team, 2020).

Results

Sampled individuals

A total of 36 captures (10 in the end of September 2019, 10 in the end of September 2020 and 16 at the beginning of June 2020) were obtained; among these 10 were already marked in previous studies (Liuzzo et al., 2021). Out of the examined individuals, 17 adults males, 16 adults females and 3 juveniles were collected with an overall capture sex ratio of [MM/(MM + FF)] = 0.5. This sample was characterized by a dominance of turtles with SCL between 130 and 149.9 mm (55.5%). The most represented range class for males was 130-139.9 mm (47.1%), whereas for females is 140-149.9 mm (44.1%). No presence of oviductal eggs was detected in the entire captures.

Habitat use and behaviours

A variation in the location percentages was recorded for each habitat type between the two study periods: 10.00% for W, 0.21% for T, 0.87% for F, 0.00% for B and 88.91% for C in the period of lethargy, whereas 13.55% (W), 4.20% (T), 2.96% (F), 11.44% (B) and 67.79% (C) during the period of activity. Significant differences were detected in the number of locations between the transitional woodland/shrubs (T) and brackish water valley (B) habitats ($K = 12.868$, $P < 0.001$ and $K = 9.894$, $P < 0.05$) that was significantly higher in the period of activity than in the lethargy (Dunn 'test, $P < 0.001$ and $P < 0.05$). Turtles exhibited high variation in total movement distance as well as in home range between period of activity and lethargy, with some moving as little as 67.64 m and using home ranges as small as 0.42 ha, while others traversed total distances up to 546.64 m and had home ranges as large as 9.47 ha (table 1). Significant differences in

home range areas were detected between the period of activity and lethargy ($W = 567.5 P < 0.001$) that were higher in the first than in the latter (fig. 3). No significant inter-sexual differences in home range size were found in each of the two investigated periods ($W = 306$, $P = 0.199$). Moreover, a significant correlation was not found between the home range size and individual size, considering its weight at release ($\tau = 0.007$, $P = 0.447$).

Five main adults' behaviours were observed during the period of activity and lethargy: Basking (B), Movement (M), the Movement caused by the radiotracking operator (Mo) a Short period of stationary in the mud bottom (Ss) a Long period of stationary in the mud bottom (Ls). In contrast no nesting and floating behaviours were detected during the study period. Basking was performed by the turtles out of the canal between 9 and 12 am when the water bodies had a temperature range of 21-28°C. Specifically, this behaviour was observed among reeds, among banks of *Tamarix gallica* and *Rubus ulmifolius*, individually or in groups. While the other behaviour activities were detected mainly in the water bodies. Significant differences were checked between the period of activity and lethargy in the main behaviours observed (table 2, fig. 2). The frequency of B, Ss and M behaviours was significantly higher during the period of activity whereas Ls behaviour was significantly more frequent in the in the period of lethargy (table 2). During the latter period pond turtles

Table 2. Significant differences in the main turtles' behaviours measured as the number of observations per location (Basking (B), Movement (M), a Short period of stationary in the mud bottom (Ss) a Long period of stationary in the mud bottom (Ls) [after 5 hours without detecting movement]) observed between the period of activity and lethargy (P).

Differences	Statistics test
$B \times P$	$W = 59$, $P < 0.05$
$M \times P$	$W = 58$, $P < 0.05$
$Ss \times P$	$W = 57.5$, $P < 0.05$
$Ls \times P$	$W = 5.5$, $P < 0.05$

were observed to brumate gregariously in a relatively homogenous 0.63 ha area within a 5-m distance from canals banks (fig. 3b). All pond turtles' overwintering locations were detected in the Nitticore canal with 10-100 cm water depth. The individuals were observed in places with shallow water (10-60 cm) with a 10-40 cm deep mud bottom.

Movements were highest in the phase of activity and declined in the lethargy (fig. 4). Movement distance among water bodies was positively related to water temperature ($F_{1,20} = 88.050$, $P < 0.0001$, $R^2 = 0.815$) and salinity ($F_{1,20} = 9.716$, $P < 0.005$, $R^2 = 0.330$) whereas sex, weight and the number of days radio-tracked variables did not contribute significantly to the movement distance (table 3). Salinity seems to be a secondary variable in explaining *E. orbicularis* movement distance. If used as the sole independent variable in a linear regression model this factor explained 33% of variation in movement distance between the period of activity and lethargy (fig. 4, table 3). The overwintering period end was detected in the threshold mean water temperature of about 14°C (fig. 4a) into canals with riparian vegetation. Significant differences were also observed in the minimum terrestrial distance covered between periods ($W = 972$, $P < 0.05$) which were significantly higher in the period of activity than in the lethargy. In contrast, no significant differences were detected in terms of terrestrial distance between sexes of turtles ($W = 483$, $P = 0.136$). Moreover, during the period of lethargy, no mass movement and turtle travel routes were observed, whereas during period of activity 6 out of 9 individuals have changed water bodies travelling towards aquatic habitat with higher salinities 1-17‰ (mean: 10.64‰) (fig. 3a).

Discussion

This study showed differential patterns of activity, dispersion and habitat use between the phase of activity and lethargy in the adult population
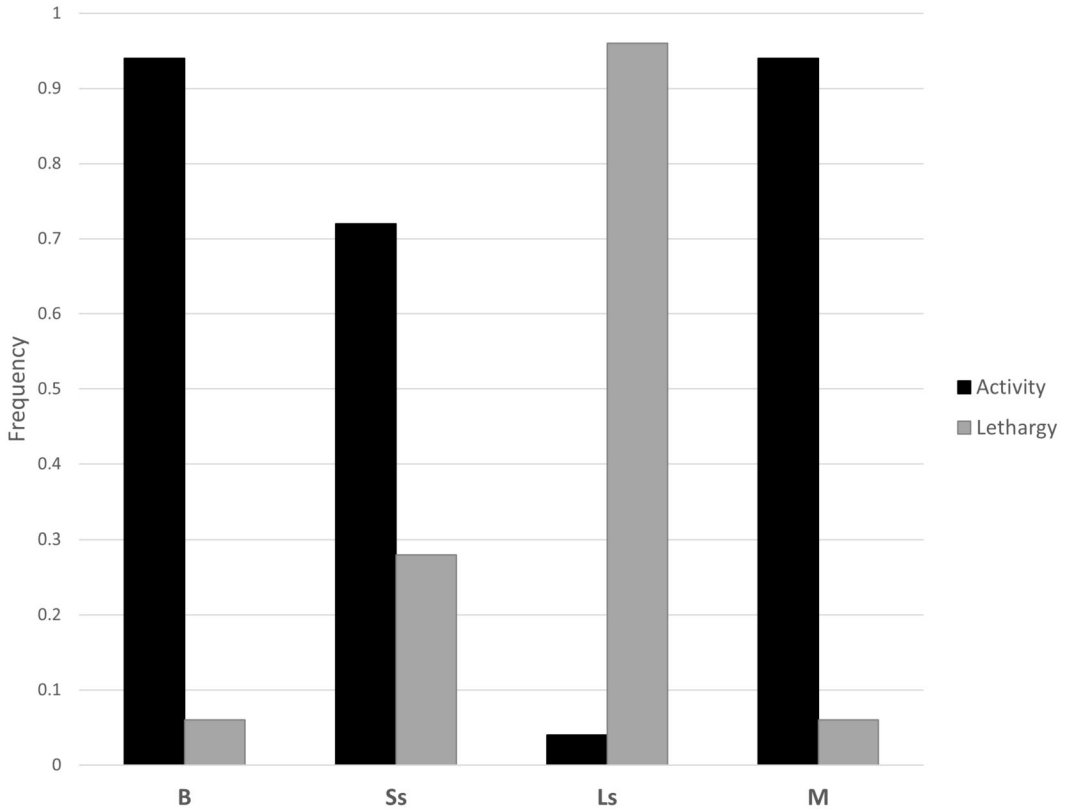
Figure 2. Phenological variation in the relative fraction of the main turtles' behaviours (Basking (B), Movement (M), a Short period of stationary in the mud bottom (Ss) a Long period of stationary in the mud bottom (Ls) [after 5 hours without detecting movement]) observed in the water and on the canal banks.

of *Emys orbicularis* living in a wetland area of the inner Venice Lagoon. The individual activity and space use patterns of the studied population showed a variation in the habitat preference and movements, marking a clear difference between a period of activity and lethargy. To date the habitat selection of *E. orbicularis* in Italy is poorly documented: nevertheless, although its distribution is linked to wide wetlands in plains, it seems to prefer marginal areas such as ditches, pools and ponds, avoiding large and deep bodies of water (Lebboroni and Chelazzi, 1991). Generally, emydid turtles, especially if living in ponds or canals, show attachment to more than one pool, probably to exploit alternative habitats in summer periods (Gibbons, 1970). Indeed, during the activity period, the population stud-

ied in the Valle Averto Oasis inhabiting a marshland with scattered canals performed more frequent overland trips (fig. 3a). This phenomenon could be associated to different feeding opportunities or different competition rates (e.g., presence or absence of fishes) among habitats, thus yielding different long-term survival (e.g., Canessa et al., 2016; Ottonello et al., 2017a). Studies on habitat preference have shown that to survive in brackish environments, freshwater turtles implement various behavioural, physiological, and morphological homeostatic mechanisms (Agha et al., 2018). In the absence of physiological adaptations, multiple freshwater turtle species show a flexible behaviour that allows them to temporarily occupy brackish water environments (Greenberg and Maldonado, 2006). Behavioural patterns include activ-
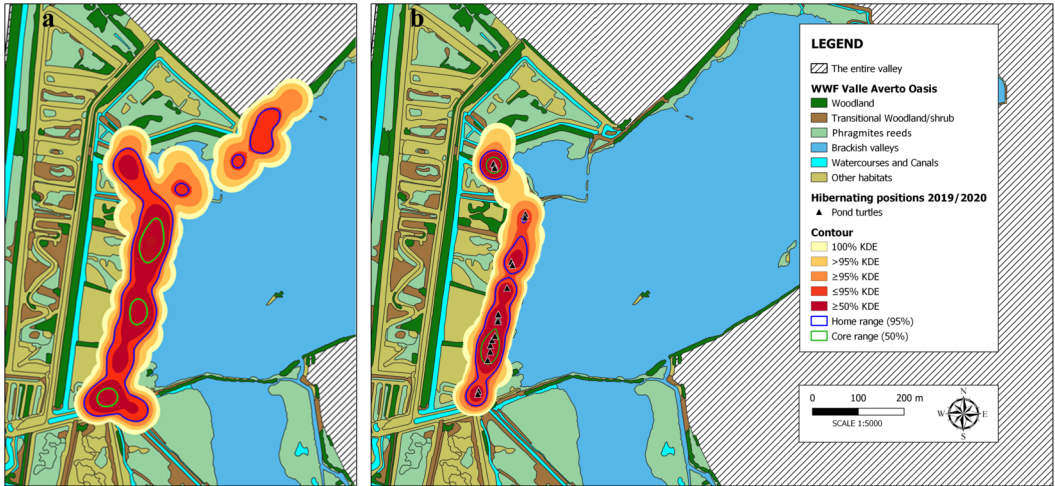
Figure 3. Differences on spatial distribution of *Emys orbicularis* between the period of activity (a) and lethargy (b) period using the Kernel Density Estimation (KDE). The KDE percentages represent the probability that *E. orbicularis* individuals will be in any part of its home range. The highest use areas were highlighted with blue (95% KDE) and green (50% KDE) contours. The hibernating positions of pond turtle's individual were plotted with triangle symbol (b). Map of land use types according to the European Union classification Corine Land Cover focused on the location sites (original map from Buffa, Ghirelli and Fantinato, 2013).

ities like movements between saline and fresh-water areas (Hart and Lee, 2006; Harden, Midway and Williard, 2015; Bower et al., 2016). In our study area, the percentage of locations in the transitional woodland/shrubs and brackish water valley was significantly different between the period of activity and lethargy. Specifically, out of the activity period, no or few individuals were located in the transitional woodland/shrubs and brackish water valley (fig. 3b). Moreover, salinity and water temperature also influenced turtles' overall patterns of movement distance (table 3). These results suggest that during the activity period *E. orbicularis* individuals might tend to move towards aquatic habitat with higher salinities. Similar results were obtained along the coast of the Caspian Sea where the preference of brackish water by *E. orbicularis* compared to *Mauremys caspica* (Gmelin, 1774) has been documented (Kami et al., 2006). Nonetheless, further research should address spatial distribution at a finer scale with several micro-habitat predictors to assess the preferential habitat and whether the salinity can be considered a robust driving parameter for

dispersion activity for this threatened *E. orbicularis* populations in a transitional water system as the lagoon.

Basking (B), the Short period of stationary in the mud bottom (Ss) and Movement (M) were the main behaviours observed in the activity period, whereas the Long period of stationary in the mud bottom (Ls) was more frequently detected in the lethargy period. These results confirm what has been observed in other populations of this species (Rovero and Chelazzi, 1996; Drechsler et al., 2018). The lack of floating activity might be related to few and discontinuous hydrophytes patches reported along canals of the Valle Averto Oasis (Tomè, 2010). The floating behaviour was strictly associated with *Myriophyllum* sp. zones in southern Tuscany (Italy) where the 94% of foraging and floating recordings has occurred in areas covered by this plant (Lebboroni and Chelazzi, 1991). The lower basking activity recorded in autumn differs from the study of Lebboroni and Chelazzi (1991), where the *E. orbicularis* individuals spent the most time in basking activity in early spring and autumn. This difference could result from local effect as basking
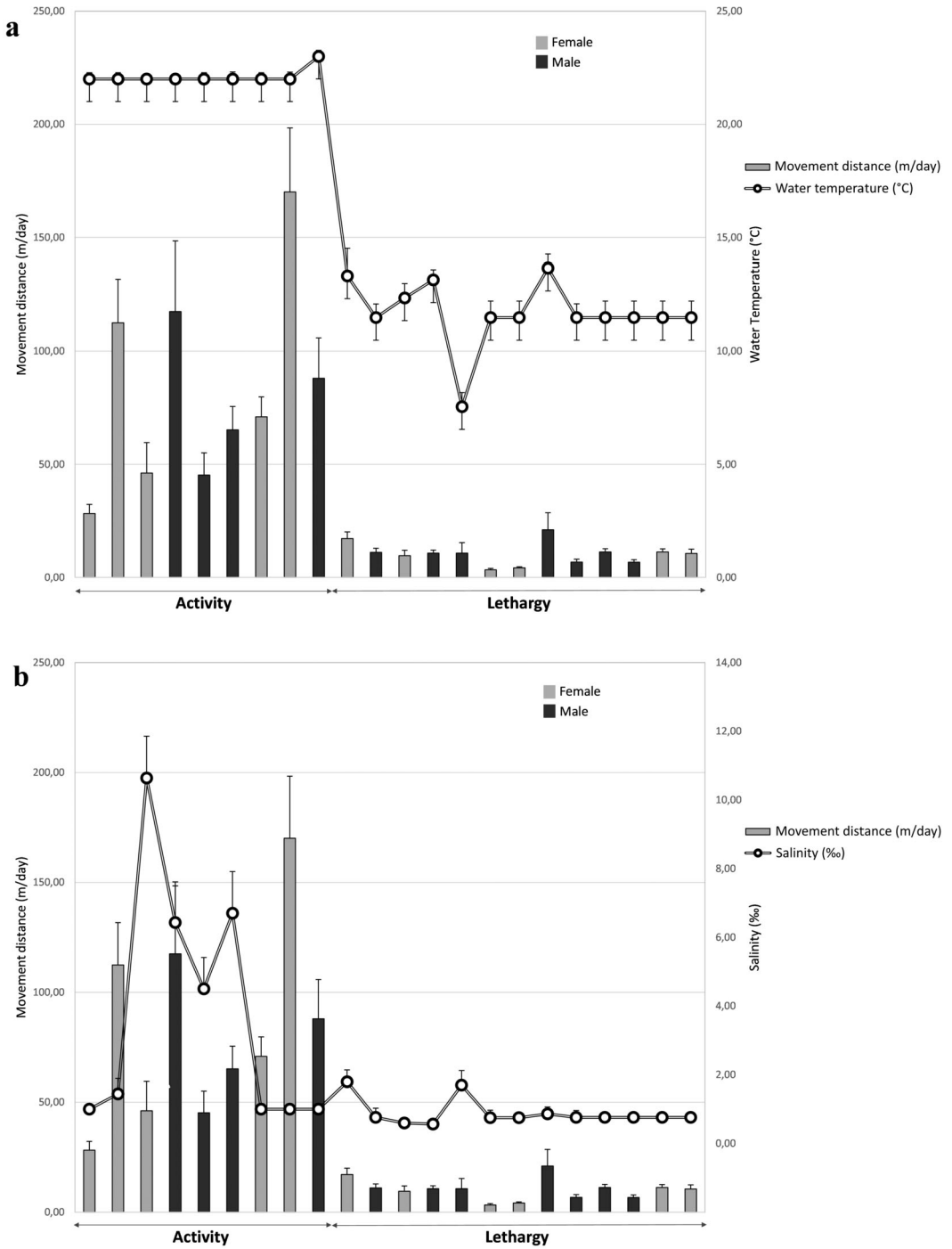
Figure 4. Relationships between movement distance (bars) and (a) water temperature (°C) and (b) salinity (‰) for *Emys orbicularis* studied with radiotelemetry during the period of activity and lethargy (the year 2020). Movement and environmental variables are mean ± 1 SE.

Table 3. Summary of multiple and simple linear regression models demonstrating the relationships between temperature, salinity and the movement distance of *Emys orbicularis* individuals located in the water bodies of Valle Averto Oasis.

Predictors	Movement distance					
	B	*C I*	*P*	*B*	*C I*	*P*
(Intercept)	1.24	1.04-1.43	**<0.001**	0.19	−0.23-0.62	0.351
Salinity	0.76	0.25-1.26	**0.005**			
Water temperature				0.08	0.07-0.10	**<0.001**
Days radio-tracked				−0.01	−0.02-0.00	0.185

Note: *B* values are the linear regression coefficients, *CI* values show the confidence interval of *B*.

behaviour can be influenced by time of day or by wetland features such as bank morphology, sun exposure, or the availability of basking logs (Di Trani and Zuffi, 1997; Cadi and Joly, 2003). For example, it is possible that in sunny water bodies turtles bask earlier in the day, and are therefore active during the hottest hours, while in shaded water bodies turtles could also bask during the central hours of the day. Such behavioural differences between wetlands could lead to underestimation of turtle presence in sunny wetlands surveyed during the hottest hours and should be detected from a significant interaction effect between the time of survey and sun exposure on turtle observation (Ficetola et al., 2004).

Sex and body size did not influence movements or use of space between the study periods. The lack of a body size effect may in part stem from our exclusion of immature animals from radiotelemetry, but our sample nevertheless included nearly the complete size range of adults from the population. Seasonal differences in movement and space use patterns between sexes, typical of many freshwater turtles are often attributed to the 'reproductive strategies hypothesis' of Morreale, Gibbons and Congdon (1984), which predicts males should become more active and traverse longer distances at times of peak breeding activity to increase encounters with females, and females should increase activity during peak nesting activity in search of the most suitable nesting sites. In this protected area, breeding occurs in March-April in *E. orbicularis* (Liuzzo et al., 2021), while nesting occurs from between the

end of May and June (Masiero, 2015; Pesce, 2020). However, analysing a portion of the activity period, the sexes did not differ significantly in movement distance and home range (fig. 4). Moreover, a long-distance migration to distant wetlands (e.g., 150 to 600 m from the home canal; (Rovero and Chelazzi, 1996)), for the specific purpose of nesting was not observed. The potential reasons of these observations might be related to location period. The sampling period might be lightly shifted towards the tail of reproduction behaviour, causing a bias in the inter-sexual movement difference. Nonetheless, our data of movement patterns have shown a late reproductive period, from the half of June to July when the turtles move for large distances (e.g., up to ∼600 m from the home water body) and a post-reproductive period, from October to November when the period of brumation begins. Similar observations have been reported for other parts of the range (Italy: Rovero and Chelazzi, 1996; Hungary: Farkas, 2000; Ukraine: Kotenko, 2000). This pattern was also confirmed by the home range analysis where the *E. orbicularis* individuals moved differently in wide areas between the period of activity and lethargy. Similar home ranges have been reported for *E. orbicularis* in France (Cadi and Joly, 2004), Italy (Lebboroni and Chelazzi, 2000), and Lithuania (Meeske and Mühlenberg, 2004), where they gradually increased the home ranges during their activity period; home ranges became wider in June, July, and August in France and Lithuania, whereas they decreased their movements in autumn. The duration of these periods is not fixed and can

be influenced by local climatic conditions (Cadi et al., 2004). The influence of habitat structure and weather cues may outweigh other competing intrinsic factors also thought to be influencing behaviour in *E. orbicularis*. For instance, temperature and salinity are important proximal cues driving some aspects of movement in *E. orbicularis*, a conclusion consistent with several studies of wetland animals (Wygoda, 1979; Donaldson and Echternacht, 2005; Todd and Winne, 2006). Our studied turtles migrated significant distances in response to two seasonal factors measured: water temperature and salinity (fig. 4, table 3). More specifically, the transition from the period of activity to lethargy was detected in the threshold mean water temperature of about 14°C (fig. 4a). A very similar results were also observed in Italy (Bruno, 1986) and in south-eastern Slovakia (Novotný et al., 2008). In the temperate zone, all turtle species brumate in response to temperature (Rollinat, 1934; Gibbons, Greene and Congdon, 1990; Parde, Hurstel and Lefevre, 1999), *E. orbicularis* mostly underwater (Cadi et al., 2004; Thienpont et al., 2004, present research). During brumation, movements are either absent or weak and increase as soon as weather conditions allow. In the overwintering period, our radio-tracked turtles have moved leading to an aggregation in a limited area of the study site (fig. 3b). This area was characterized by bushy or herbaceous vegetation with *Rubus ulmifolius* and dense reed vegetation with the association of *Puccinellio festuciformis-Phragmitetum australis*. Basically, a thick layer of decomposing dead leaves or radical shoots associated with the Phragmition plant community were the brumation sites recognized. This overwintering site was not essentially different from that found in other studies (Schneeweiss and Steinhauer, 1998; Parde, Hurstel and Lefevre, 1999). Aggregation of brumating turtles is also known in some other species (Brown and Brooks, 1994; Lewis and Ritzenhaler, 1997), but is not the rule in freshwater turtles (Plummer and

Burnley, 1997). It remains unclear which characteristics turtles might prefer or even recognize in a hibernaculum. However, the frequency and accuracy of site fidelity and the congregations of turtles in small, specific areas illustrate that sites are not chosen at random (fig. 3b). Moreover, evidence of overwintering groups of *E. orbicularis* was also recorded in Lithuania and Germany (Schneeweiss, Andreas and Jendretzke, 1998; Meeske, 2000). During the activity period, in contrast, 67% of the radio-tracked turtles have moved toward brackish lakes characterized by the association of *Chaetomorpho-Ruppietum* through transitional woodland/shrub with *Rubus ulmifolius* aggr. (fig. 3a).

The results obtained with this study, namely regarding the difference in the use of habitat, movements and behaviours pattern between period of activity and lethargy, will be essential to increase the understanding on what spatial portions and associated habitats of the investigated area should be included in a management plan for the conservation of this species. Our study reveals that both in the period of activity and lethargy, those areas with riparian vegetation were commonly used by the *E. orbicularis* individuals. The knowledge of this set of information could help to minimize the anthropogenic disturbance and/or to assist the wildlife managers in the timing of activities. For instance, current canals management practices in the Valle Averto oasis often include the removal of reeds; association of *Puccinellio festuciformis-Phragmitetum australis*, one of the most important overwintering microhabitats, are frequently cut over winter. This could seriously threaten overwintering pond turtles. Moreover, to predict, prioritize, and mitigate the anthropogenic impact and extreme weather events (e.g., flooding, droughts) on coastal freshwater species, species-specific salinity preferences and behavioural responses to salinity gradients should be understood. The detection of a gradient in water salinity along the water bodies of the inland wetland of the largest

lagoon of the Mediterranean might be an occasion to observe a new pattern of distribution and abundance of the European pond turtle across an apparently similar habitat. Quantitative data on habitat requirements for this threatened species are needed to better evaluate which areas are the most suitable, or what actions can improve habitat suitability. In the lagoon area, this may have influential consequences for the management and conservation of freshwater turtles.

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