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Management and conservation along the inland margins of the lagoon: the European pond turtle (*Emys orbicularis*) case study in a protected internal wetland area of the Southern Venice lagoon

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Coordinatore del Dottorato

Prof. Enrico Bertuzzo

Supervisore

Prof. Stefano Malavasi

Co-supervisore

Prof. Vincenzo Arizza

Dottorando

Mirko Liuzzo

Matricola 956430

Ai miei Genitori

CONTENTS

ABSTRACT

A freshwater turtle monitoring programme along the inland margins of the Venice lagoon was initiated in 2018 in collaboration with the World Wild Fund for Nature (WWF-Italy). The efforts were focused on the European pond turtle, *Emys orbicularis* which is potentially recognized as surrogate species (es. flagship or sentinel species) in the context of conservation biology. Despite this species is used as a shortcut to monitor or solve conservation problems its scientific knowledge is still under development and currently on debate, particularly in relation to its basic life-history, behavioural ecology and ecological requirements. Quantitative data analysing with a relevant number of individuals are required to increase the set of information on the biology, behaviour and habitat preference for the long-term survival of *E. orbicularis* populations.

In this work: (1) by estimating the *E. orbicularis* population density with the capture-recapture method in a Venice lagoon wetland area, it was possible to observe one of the most populated areas of the Italian peninsula (~250 ind./ha) (2) by analysing behavioural and dispersal patterns between the period of activity and lethargy in a wild population of the European pond turtle, it was possible to observe movements towards aquatic habitats with higher salinities 1-17‰ (mean: 10.64‰) in summer whereas a gregarious brumation and no mass movement behaviour were detected in autumn (3) by evaluating the difference in the distribution of *E. orbicularis* and some micro-habitat descriptors of the Valle Averto Oasis, it was possible to observe a positive effect of the emergent vegetation, salinity and turbidity on the occurrence and abundance of this species.

These indications could have value in optimizing conservation efforts by concentrating funding on management areas and improving landscape features that are most important to the species and for wetlands biodiversity.

GENERAL INTRODUCTION

Wetlands are considered key territories for biodiversity, and their survival strongly depends on water control (Sica et al. 2016). These areas are the most threatened on the planet and little is known from the scientific environmental research. The main threats result from morphological and hydrological changes, the immission of non-native species, chemical and biochemical pollution and many more (Baigún et al. 2008; Meineri et al. 2014; Burkart et al. 2021). The presence of these multidisciplinary issues makes the environmental management and conservation activity a complex field. Time and funding are often limited, so conservation biologists are looking for an innovative approach to operate between natural and social systems (Berkes et al. 2003). A successful strategy is to focus on species able to induce empathy toward wildlife, making them ideal for financial support (Sergio et al. 2008) (flagship species). The presence of a charismatic species, the European pond turtle (Fig. 1), combined with management actions and different pressures, including alien species, make the Valle Averto Oasis an excellent case study to assess how the various elements can have an impact on the biology, eco-ethology, habitat preference and dynamics of the population.

Figure 1 The European pond turtle, *Emys orbicularis* in the terrestrial habitat of the Valle Averto Oasis (Southern Venice lagoon)

Emys orbicularis and the single-species approach

The conservation and management of wildlife and environmental assets have commonly emphasized single-species (Block et al. 1999). Basically, the assumption was that managing for single species would provide suitable habitat, reducing the costs, for diverse other species as well. This assumption is clearly valid for some situations. However, measuring whether the single-species approach affects positively fauna is complicated and poorly studied (Mace 2001). To this aim, peculiar single species have been employed in conservation as flagship, sentinel, umbrella and focal species. The following examples show certain features of *Emys orbicularis* that might be used as a management tool to protect the freshwater fauna and habitat.

Flagship species are used for its empathic characteristics that facilitate the obtaining of funds, or enacting legislation (Caro and O'Doherty 1999). These species are often chosen from a list of threatened species (Possingham et al. 2002), possibly because those species already carry regulatory protection (Fleishman et al. 2000), which markedly promote conservation intervention. The IUCN (International Union for Conservation of the Nature) listed *Emys orbicularis* as "Near Threatened for Europe" (Tortoise & Freshwater Turtle Specialist Group 1996) meaning that the species depends on conservation efforts to prevent becoming threatened. Conservation status on national red lists depends on the country. For instance, in Italy, the conservation status of *E. orbicularis* on national red list of IUCN is "Endangered" (Andreone et al. 2013). Moreover, as *E. orbicularis* is listed in the Habitat Directive of the European Commission (Annexes II and IV) and in the Bern Convention (Annexe II), several conservation programmes were promoted in this country.

The European pond turtle occupies a interesting place among reptiles flagship species owing to its inherent charismatic appearance and behaviour. Conservation organizations use its frequently as poster species on reserve logos, magazine covers, and advertisements to attract funding and attention. For example, *E. orbicularis* was used as a symbol to promote a restoration and breeding program in Liguria (NW Italy). This conservation plan was implemented by different public Authorities, private entities, NGOs, volunteers and a LIFE+ Nature project was co-financed in the 2013 by the EU Community (LIFE EMYS - LIFE12 NAT/IT/000395) with a total amount of 1.123.496 € (Ottonello et al. 2014). A further recent example was represented by the LIFE project NAT-IT-LIFE URCA PROEMYS – "URgent Conservation Actions Pro *E. orbicularis* in Italy and Slovenia" with ~4.770.000€ in support of this species. The project aims to improve the conservation status of *E. orbicularis* in Italy and Slovenia while maintaining the genetic diversity of existing populations. Moreover, many European bodies are involved in a large number of *in situ* conservation projects, some useful links on current and former projects have been inserted in Table 1.

Table 1 Conservation projects planned in some countries of Europe

Country	*In situ* conservation links
Austria	https://www.sumpfschildkroete.at/
France	https://www.ecologie.gouv.fr/sites/default/files/PNA_Cistude_2020_2029.pdf
Germany	http://www.xn--sumpfschildkrte-ltb.de/ https://rlp.nabu.de/tiere-und-pflanzen/amphibien-und-reptilien/sumpfschildkroete/ https://niedersachsen.nabu.de/tiere-und-pflanzen/aktionen-und-projekte/sumpfschildkroete/index.html https://www.lubw.baden-wuerttemberg.de/-/europaeische-sumpfschildkroete-Emys-orbicularis-linnaeus-1758 https://www.stalu-mv.de/ms/Themen/Naturschutz-und-Landschaftspflege/Artenhilfsprogramm-Sumpfschildkr%C3%B6te/
Italy	http://www.lifeEmys.eu/en/ https://www.wwf.it/pandanews/wwf-life/progetti-e-iniziative/progetto-a-sostegno-testuggine-palustre-europea/
Latvia	https://webgate.ec.europa.eu/life/publicWebsite/index.cfm?fuseaction=search.dspPage&n_proj_id=4530
Spain	http://www.lifepotamofauna.org/www.lifepotamofauna.org/ca/que-es/especies/especies-autoctones.html#estany-box
Switzerland	http://www.karch.ch/karch/de/home/reptilien/reptilienarten-der-schweiz/europaische-sumpfschildkrote.html
Various countries	http://www.glis.lt/life/?pid=59&lang=en

Further demonstration of the good fundraising potential of *E. orbicularis* is its semi-natural centres of reproduction located in Europe (see Table 2). Ex situ areas are one possible option that can contribute to the conservation of this threatened species. In the last century, pond turtle's population suffered a strong decline mainly due to habitat loss, fragmentation and alteration. Many populations are now isolated, increasing the risk of population extinction, because of demographic or genetic isolation (Zuffi et al. 2010). "Emys centres" activities such as population monitoring, habitat management and restoration, reintroduction and captive breeding bring benefits to the population, the species, habitat and the social context. The sharing the knowledge and experiences in the breeding centres raises public awareness of the importance of wetlands to biodiversity conservation.

Table 2 *Emys* centres located in in some semi-natural areas of Europe

Country	***Emys* centre**
Italy	Centro *EMYS* di leca di Albenga (SV, Liguria)
	Centro *Emys* Piemonte (VC, Piemonte)
Switzerland	Centre *Emys*-Protection et Récupération des Tortues (Chavornay)
Germany	*Emys orbicularis* Schildkrötenfarm Völke (Karden)
Spain	Fundació *Emys* (Girona)

E. orbicularis is also used as a tool for education and dissemination. A short animated film (https://vimeo.com/442624384) was made in an educational partnership with Tour du Valat (a research institute for the conservation of Mediterranean wetlands) in Camargue (South of France) by a group of students of the MoPA (Computer Graphics Animation School in Arles). This short film graphically illustrates the impact of pollutants on the European pond turtle. The small native aquatic turtle is also presented as excellent sentinel species for evaluating the contamination of aquatic habitats in the Camargue. Turtles are often suggested as reliable sentinel species (also called "indicator species") owing to their position at the top of the food chain and to a number of life history traits (longevity, semiaquatic condition, nesting behaviour, etc.) (Bridges 2002) that make them particularly vulnerable to human-induced alterations of their supporting ecosystems. The effects of terrestrial habitat alteration that disturbs or eliminates nesting and refuge sites of freshwater turtles can also be severe (Author and Buhlmann 1995; Burke and Gibbons 1995). Notable examples include declines of the Chelonia fauna, which have become increasingly imperilled following damming, flow modification, and general degradation of river habitat. For instance, channelization and flow modification have been implicated in declines of *E. orbicularis* in Slovakia and Italy (Bonato et al. 2007; Horváth et al. 2021). Although these studies have revealed important findings that are essential for promoting sustainable management of the European pond turtle in the wetland of Europe, potential impacts of metal pollution on the European pond turtle have been poorly explored. *E. orbicularis* might provide useful information on local contamination. This species is a lo long-lived (> 40-80 yr) opportunistic predator (feeding on fish, amphibians, aquatic insect, gastropods, and crayfish) and scavenger (Ficetola and De Bernardi 2006; Ottonello et al. 2016a; Ziane et al. 2020), allowing to study bioaccumulation processes and long-term trends of contaminants. Moreover, *E. orbicularis* is sedentary, providing information on the contamination at a precise location.

In contrast to species indicators, which may help to select the location of a reserve, umbrella species can provide information on the size and configuration of protected areas (Sergio et al. 2008). The European pond turtle usually needs aquatic and terrestrial areas for foraging, breeding, brumation and dispersal activity (Lebboroni and Chelazzi 1991; Ottonello et al. 2016b; Liuzzo et al. 2021). These ecophysiological characteristics might identify *E. orbicularis* as umbrella species (Cadi et al. 2004; Maciantowicz and Bartt Lomiej Najbar 2004; Ottonello et al. 2017). However, to date, no results of an experimental animal study confirm whether the niche of *E. orbicularis* is quite large to contain the requirements of less demanding species. Despite its potential utility, the umbrella species concept is not without criticism among conservation biologists. Basically, it is considered improbable that the requirements of one species would encapsulate those of all other species (Noss et al. 1997; Basset et al. 2001; Hess and King 2002; Caro 2003; Favreau et al. 2006). To meet the conservation objectives, umbrella species should be chosen in fact at the appropriate scale, represent ecologically-linked taxa that share similar habitats (Fleishman et al. 2000; Caro 2003; Favreau et al. 2006), and in some cases should have similar life history traits or management requirements as the target group (Báldi 2003; Lovell et al. 2007). A more pragmatic solution could be to select a subset of “focal species”. This strategy, although the concept is related to umbrella species, differs in that it includes a suite of species, each of which is used to define the features of different landscape attributes that must be represented in the landscape (Lambeck 1997). The needs of these focal species will be also essential to increase the understanding of what spatial portions and associated habitats of the investigated areas should be included in a management plan.

The European pond turtle, *Emys orbicularis*

Taxonomy and geographic distribution

The members of the order Testudinata inhabiting the wetland of Europe are all attributed to families Geoemydidae and Emydidae. The latter one includes 11 genera restricted to America (mainly Nearctic region) (Seidel and Ernst 2017) and one monotypic genus endemic to Palearctic region: *Emys* Duméril, 1806 (Ernst and Barbour 1989). Fossil records, distributive and genetic data suggest that the common ancestor of the genus *Emys* might have arrived in Europe from North America in the Lower Miocene (Fritz 1998; Lenk et al. 1999). The genus *Emys* belonging to the primarily Nearctic family of Emydidae might have invaded Eurasia about 16 Mya via a trans-Beringian land bridge (Spinks and Shaffer 2009).

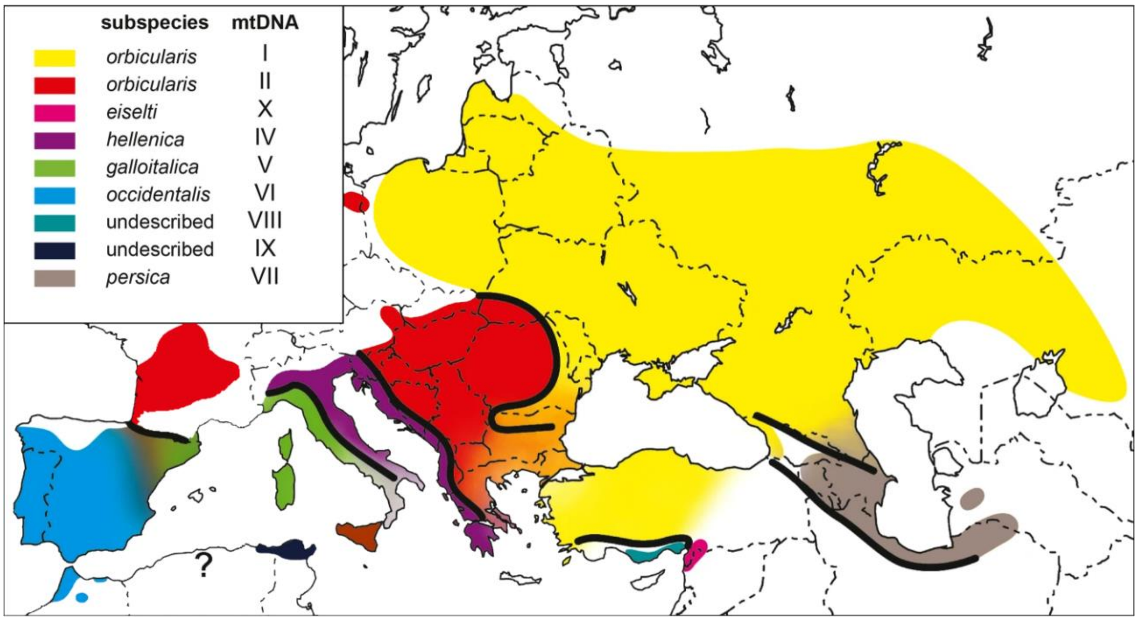

Figure 2 Territorial framework of *Emys orbicularis* and *E. trinacris* (brown) distribution. Different subspecies correspond to different colours and to distinct mtDNA lineages as shown in the legend. Merging colours indicate hybrid zones. The Violet box shows the subspecies of the study region. Question mark represents an isolated population of unknown genetic identity. Map modified from Pöschel et al. (2018).

The European pond turtle *Emys orbicularis* with the closely related *Emys trinacris* Fritz et al. 2005 are the only two representatives of the Emydidae family in Europe (Zuffi et al. 2010; Marrone et al. 2016). Many studies have investigated the phylogeny and biogeography of *E. orbicularis* (Lenk et al. 1999; Fritz et al. 2007; Spinks and Shaffer 2009; Stuckas et al. 2014) though the analysis of the mitochondrial and nuclear DNA sequences relating to cytochrome *b*: the results showed the existence of 9 different haplotypes, each of them characterized by a define distribution area (Pöschel et al. 2018) (Fig. 2).

Within the genus *Emys* occurs a higher diversity of mitochondrial haplotypes in the southern regions. It has been hypothesized that *Emys*, being a thermophilic species, would be confined to areas of refuge with relatively mild temperatures, such as the Mediterranean region or the Ponto-Caspian region: small and isolated refuges where the new mutations could be affirmed during the last glaciation. It was supposed that with the advent of Holocene *E. orbicularis* has recolonized more northerly parts of Europe and adjacent Asia from few refuges in the Balkans and the northern Black Sea/northern Caucasus region since in the respective mtDNA lineages has been confirmed in decreasing haplotype diversity with increasing distance to the refuge (long-distance dispersal model of Hewitt 1996) (Fritz et al. 2007). Sicily seems to have been a glacial refuge area, where *E. trinacris* remained possibly isolated from the congener *E. orbicularis* during this period.

In Italy, two subspecies are currently recognised: *E. o. galloitalica* Fritz, 1995 along the Tyrrhenian coast and *E. o. hellenica* (Valenciennes, 1832) on the Adriatic one (Pöschel et al. 2018). Southern Italy is a region where these two genetic lineages of *E. orbicularis* meet and hybridize (Vamberger et al. 2015). In close proximity occurs the Sicilian endemic cryptic species *E. trinacris* (Fritz et al. 2005; Pedall et al. 2011).

The current classification to the rank of species was confirmed by the phylogenetic analysis of Vamberger et al. 2015 in which no evidence was found for any gene flow between pond turtles from Sicily and adjacent mainland Italy.

Ecology and biology

Emys orbicularis is defined as a semi-aquatic species (Ficetola and De Bernardi 2006) being inhabiting both lentic (flowing) and lotic (stagnant) waters (Bonin et al. 2006) and its use the upland for nesting, hunting, aestivation, brumation and travelling (Naulleau 1992; Ottonello et al. 2005; Liuzzo et al. 2021). The species occupies static waters with mud bottom such as ponds, rivers and swamps, but can also be found in canals and other artificial water bodies (Lebboroni and Chelazzi 1991; Zuffi 2004; Bonin et al. 2006; Liuzzo et al. 2021). Furthermore, it is also possible to find pond turtles in altered habitats, like in old caves or abandoned fishing valley area (Zuffi and Rovina 2006; Liuzzo et al. 2021).

Populations of *E. orbicularis* are locally distributed across the internal Po valley and Venetian plain areas (Soccini and Ferri 2004). Specifically, it is located mainly on costal and lower parts of lowland, including lagoons, delta area and agricultural lands; it is more fragmentary in the mid lowland (Bonato et al. 2007) (Fig. 3). Active individuals have been observed from early March to late November, mainly between the beginning of April and mid-June (Pesce 2020; Liuzzo et al. 2021).

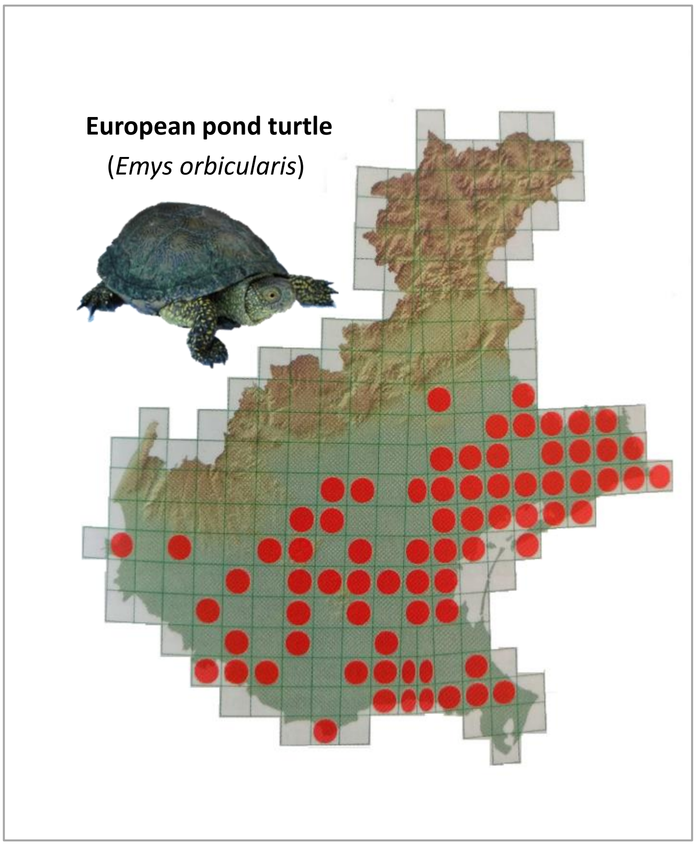

Figure 3 Distribution of *Emys orbicularis* in the Veneto region. Original map from Bonato (2007)

Due to the irreversible alteration of the wetland areas (eg., drainage of water, modification of banks, constant practices of mechanical control of riparian vegetation and removal of bottom sediments in canals) and the potential competition with *Trachemys scripta* (Thunberg in Schoepff 1792), most of the records of the European pond turtle come only from small isolated protected Venetian areas and scattered individuals found along canals, ditches and ponds (Semenzato 2007).

Despite extensive studies of biology and ecological knowledge of Italian populations of freshwater turtles in Italy (Seglie 2015; Ottonello et al. 2017; Zuffi et al. 2020; Liuzzo et al. 2021), information concerning density and population estimates on Adriatic coastal environments and populations are few and weak, although recently methodology studies have been published: present research, Mazzotti (1995), Ottonello et al. (2017), Zuffi et al. (2020). Local research on abundance, structure and dynamic of turtles population may facilitate short-term research projects that produce large data sets including, for example, information on habitat preference (Luiselli 2017), movements (Liuzzo et al. 2021) and thermal profiles (Di Trani and Zuffi 1997) but a comparison of abundance, density, population structure and survivorship in different populations may additionally present information on life history patterns (Olivier et al. 2010; Canessa et al. 2016).

AIM OF THE STUDY

Various bibliographic information is available on the presence of *Emys orbicularis* in the Veneto region (Ninni 1888; Padovan 2000; Bonato et al. 2007; Beggiato et al. 2019). However, these studies are often anecdotic and rather synthetic, and they are not very useful for the analysis of historical trends.

The general aim of this PhD project was to deepen the knowledge about the basic life history, behavioural ecology and habitat preference for a wild population of the European pond turtle which inhabits the inner water bodies of the Southern Venice lagoon, Valle Averto. Quantitative research on *E. orbicularis* distribution, ecology, dispersion and impact of invasive species are extremely important tools to generate strategies for the management and conservation of this threatened species. The results of these studies can list guidelines for planning and habitat management actions for turtles' conservation. A more objective approach based on quantitative data and statistical models can provide more accurate management indications (Lecis and Norris 2003 and references therein). In turn, these indications could have value in optimizing conservation efforts by concentrating funding on management areas and improving landscape features that are most important to the wild community fauna of the inland wetlands.

The PhD project and thus the thesis is organized in different parts and chapters each of which focuses on the *E. orbicularis* as a surrogate species for the conservation of the main wetland organisms and management of the study area. The research activity addressed the following aspects:

I. The study was focused on population size, structure and movements of mature individuals within the Valle Averto Oasis. The individual and population parameters were estimated using the capture-recapture method (Capture-Mark-Recapture-CMR). Moreover, preliminary data on metal contamination was investigated in the Valle Averto Oasis, and in particular, in the eggshells of the European pond turtle (*Emys orbicularis*) CHAPTER 1. POPULATION ABUNDANCE, STRUCTURE AND MOVEMENTS OF THE EUROPEAN POND TURTLE, *EMYS ORBICULARIS* (LINNAEUS 1758) BASED ON

CAPTURE-RECAPTURE DATA IN A VENICE LAGOON WETLAND AREA, ITALY

II. The study of the main behaviours (es. basking, movements and brumation) and home range size were conducted between the period of activity and lethargy in a wild population of the European pond turtle inhabiting the Valle Averto Oasis. In order to assess the behavioural ecology of this threatened species, a total of 29 different individuals were tagged and monitored using the radiotracking approach. CHAPTER 2. 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

III. The study aims to identify the possible differences in the distribution and abundance of *Emys orbicularis* and some habitat features and environmental parameters in the canal network of the Valle Averto Oasis, where strong variations in water flow with possible salinity gradient occur. Baited floating traps were used to determine turtle presence and relative abundance. CHAPTER 3. HABITAT FEATURES AFFECTING THE SPATIAL DISTRIBUTION OF *EMYS ORBICULARIS* (L., 1758) IN AN INTERNAL VENICE LAGOON WETLAND AREA: IMPLICATIONS FOR WATER AND TERRESTRIAL MANAGEMENT

STUDY SITE

The Valle Averto Oasis is a protected wetland 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, only 78.15 ha are currently owned by the WWF Italia and include emerged lands, freshwater wetlands, two brackish lakes and saltmarshes (Fig. 4).

The actions of the PhD project are implemented within the WWF Oasis of Valle Averto, an environment of extraordinary naturalistic interest. Valle Averto is famous for its flora and fauna peculiarities representative of the wetlands of the upper Adriatic. It is an important stopover and feeding site for many species of aquatic birds and migratory waterfowl. Moreover, as described in Chapter 1, it is inhabited by one of the largest European pond turtles, *Emys orbicularis* populations of the Italian peninsula (Mazzotti 1995; Seglie 2015; Zuffi et al. 2020; Liuzzo et al. 2021). Valle Averto is dominated by a 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).

In this study site, the water management is in some cases climatically induced, but in most cases is artificially managed. Massive freshwater input are canalized into the system for conservation purposes, especially to attract target bird species. This water management system produces a water salinity difference along a West-East gradient, increasing from the Eastern part of the lagoon. The salinity gradient is also related to considerable diversification of habitats, the different levels of the soil and to the type of substrate. Anthropic pressures and climate changes put the specific biodiversity of this dynamic environment at risk, thus, active protection actions are required to preserve it.

Recognized as an area of international importance under the Ramsar Convention in 1989, it was subsequently identified as a State Natural Reserve. It also lies within three Natura 2000 sites that affect the Venice lagoon (i.e. SPA IT 3250046, SAC IT 3250030 and SAC IT 3250031).

Site of primary importance for scientific research, a LIFE+ Nature project was co-financed in the 2018 by the EU Community (LIFE18 NAT/IT/001020 LIFE FORESTALL) and for years it has collaborated with the Department of Environmental Sciences, Informatics and Statistics of the Ca' Foscari University of Venice, with the University of Padua and the Istituto Zooprofilattico Sperimentale delle Venezie (Italian health authority and research organization for animal health and food safety).

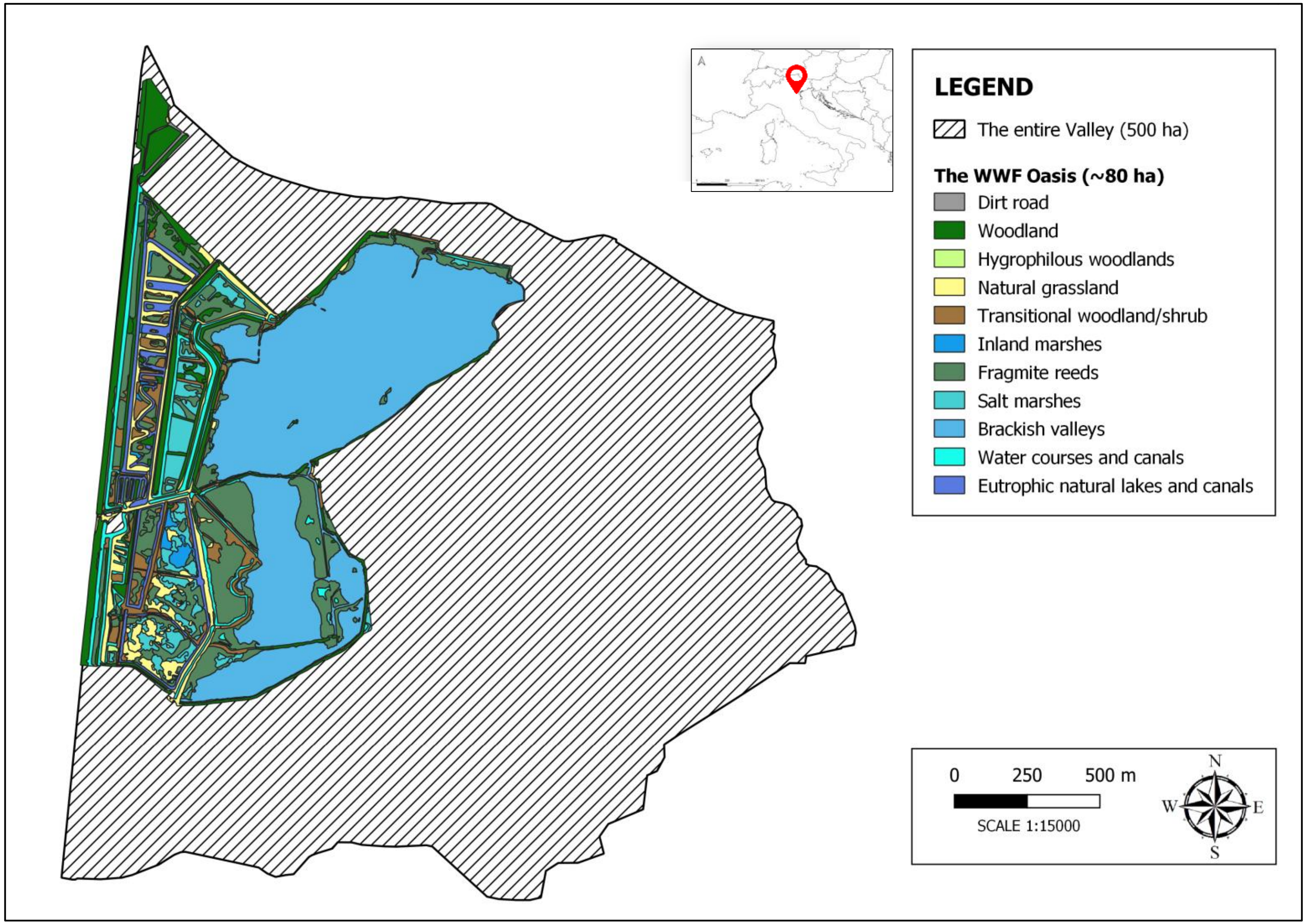

Figure 4 Location of the study site, the Valle Averto Oasis (Southern Venice lagoon) and delimitation of the naturalistic oasis WWF. Map of land use types according to the European Union classification Corine Land Cover (original map from (Buffa et al. 2013)).

CHAPTER 1. POPULATION ABUNDANCE, STRUCTURE AND MOVEMENTS OF THE EUROPEAN POND TURTLE, *EMYS ORBICULARIS* (LINNAEUS 1758) BASED ON CAPTURE-RECAPTURE DATA IN A VENICE LAGOON WETLAND AREA, ITALY

This chapter has been published in Ethology Ecology & Evolution [Liuzzo et al., 2021]. In close collaboration with Stefano Borella, Dario Ottonello, Vincenzo Arizza and Stefano Malavasi, the five authors have contributed to the research development. Specifically, M. Liuzzo and S. Malavasi conceived the idea and planned the methods. M. Liuzzo collected and analysed the data. M. Liuzzo and S. Malavasi wrote the manuscript. D. Ottonello, S. Borella and V. Arizza supervised the data collection and the study implementation. All authors provided a critical revision to the final manuscript. The contents of ecotoxicological nature are not present in the publisher's version.

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1.1 Introduction

In Italy, populations of *Emys orbicularis* (L. 1758) inhabit two main types of habitats: the "pond" system, consisting of one or more natural, shallow water bodies (ponds and marshy areas) with abundant water and riparian vegetation, and the "canal" habitat (Lebboroni and Chelazzi 1998), which is characterized by artificial canals of drainage waters, generally in open or marginal areas (Zuffi and Rovina 2006). The presence of *Emys* has been documented in the Italian region of Veneto since the second half of the 19th century. Lioy (1868) reported that *Emys lutaria* Link (1807) (an obsolete synonymous form of *Emys orbicularis*) was very rare in Veneto and possibly associated with a vivid trade of pet turtles. Ninni (1888) described afterwards that more than 200,000 turtles were caught in 5 months in the region of Venice, and many turtles from there entered via the trade Central Europe (von Fischer 1884; Dahms 1912). To date, in Veneto, while the populations along the coast are relatively abundant and mostly in an apparently healthy conservation state, the situation in the inland plain is rather different: most of the records come only from small isolated protected areas and scattered individuals found along canals, ditches and ponds. In fact, land reclamations, the intensifications of agricultural activities and the reduction of riparian vegetation, caused a decrease of suitable sites and the strong fragmentation of existing populations (Bonato et al. 2007). Consequently, as *E. orbicularis* is listed in the Habitat Directive of the European Commission (Annexes II and IV) and in the Bern Convention (Annexe II), several conservation programmes were promoted in this Italian region. However, even though the species was reported along the inland margins of the Venice lagoon (Agapito Ludovici et al. 2012; Fritz and Chiari 2013), a lack of information still exists relating to basic life-history and only a few studies were carried out on its ecology. In order to design efficient management actions for an endangered species, a solid knowledge of the biological and ecological requirements is needed. The present study aims to provide first data on population parameters and dispersion activity in a protected internal wetland area of the Venice lagoon, Valle Averto (Southern Lagoon) for a wild population inhabiting an area of mixed natural and abandoned fishing valley habitat. The focuses of the present contribution were to investigate: (1) the abundance of the pond turtle's population by means of CMR (Capture-Mark-Recapture) assessment, (2) the population structure based on the analysis of biometric parameters of captured and re-captured individuals, (3) the dispersal activity inferred by recapturing marked individuals.

1.1.1 Metals accumulation data in the eggshell of the European pond turtle

Turtles are also considered as a reliable bioindicator of environmental contamination compared to many other species. Specifically, pond turtles have one of the more extensive geographical ranges both for the Nearctic (Gibbons 1990) and Western Palearctic ecozones. They are often carnivorous species that may accumulate greater concentrations of hazardous chemicals through trophic transfer, and some species are relatively sedentary, making them useful for monitoring contaminants within a specific area (Yu et al. 2011).

The European pond turtle is a long lived and widely distributed freshwater species in Europe (Ficetola and De Bernardi 2004; Andreone et al. 2013), and it has been proposed as a suitable species for bio-monitoring pollution in aquatic ecosystems (Namroodi et al. 2017; Beau et al. 2019; Burkart et al. 2021). However, metal contaminant studies on the *E. orbicularis*, to the best of our knowledge, are very limited. It is important, from an eco-toxicological point of view, to investigate the extent to which metal toxicants accumulate in turtle eggs from the water bodies of the Valle Averto Oasis, because eggs of the European pond turtles may be an important food source for other wildlife, such as fox, marten, badger, crow and nightjar (Fig. 1.2B) (Zuffi 2000). Considering bioaccumulation and possible biomagnification of metals through food chains (Burger and Gibbons 1998; Bridges 2002), accumulation studies on metals in turtle eggs will help develop practical management strategies for protection of structures and functions of the aquatic ecosystem. In this section, the levels of heavy metals were investigated in the eggshells of the European pond turtle from the canals network of the Valle Averto Oasis.

1.2 Material and methods

1.2.1 *Population parameters*

Biometric and movement data were collected from April to September 2019 in Valle Averto Oasis, located in the Southern basin of the Venice lagoon, in the municipality of Campagna Lupia Province of Venice, Italy (45°21′N, 12°09′E) (Fig. 4). In order to represent the spectrum of the environmental variability and based on previous records of *E. orbicularis* in this study area (Masiero 2015; Chiarello 2018), three types of macrohabitats were selected: (1) Nitticore (N) (45°21′23.54"N, 12°8′29.55"E), a canal with an alternate water flow and a dense reed vegetation associated with *Tamarix* spp., (2) Cavalli (C) (45°21′2.17"N, 12°8′19.90"E), a eutrophic canal without an input water flow with free-floating surface communities of the *Hydrocharition* and with associations of large pondweeds (*Magnopotamion*), (3) Lago (L) (45°21′9.77"N, 12°8′46.48"E), a portion of brackish lake with association of *Chaetomorpho-Ruppietum* (Fig. 1.2C). These trapping sites had an average surface area of approximately 4 ha. The lake environment included almost 1.8 ha (2.7%) of the total area. Outside of this zone, the water was deeper, generally lacking suitable food items and it would appear not frequent by the freshwater turtles (M. Liuzzo unpubl. observations).

Salinity ranges between 0.5 and 12‰ depending on season and distance of the study site from the sea (higher values were recorded during summer in the water body near the lagoon). The three sampling sites are independent and not aquatic connected habitats as shown in Fig. 1.2C. The minor linear distance among dry land of study sites is 111.42 m between “N” and “C”, 570.74 m between “C” and “L”, 447.60 m between “L” and “N”. 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*.

Pond turtles were captured through baited floating traps with sardines (n = 27, length = 60 cm, diameter = 30 cm, eye = 16 × 14 cm), located 50 m apart from each other, during five sampling occasions (April, May, June, July and September 2019) of 3 trapping-days each. Date of capture, trap number, sex, age (adult/juveniles), straight carapace length (SCL, sliding callipers to the nearest 0.1 mm), width (CW), height (CH) 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). All captured turtles were individually marked by unique notches on their carapace (Servan 1986) (Fig. 1.1).

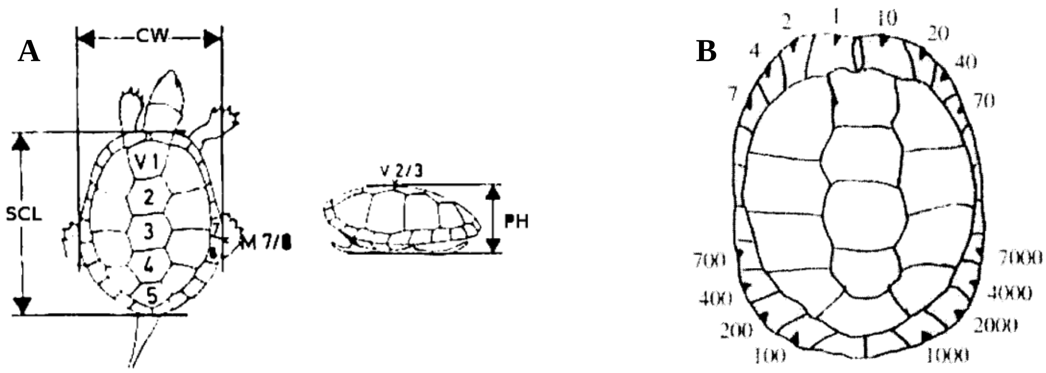

Figure 1.1 (A) Biometric measurements and (B) the Servan (1986) marking code for *Emys orbicularis*

For recaptured individuals, date, individual number, and trap number were recorded. Freshwater turtles were released into the water at the end of the session, near the trap in which they were caught. The movements of *E. orbicularis* were inferred by recapturing marked individuals during the sampling occasions.

1.2.2 *Ecotoxicological parameters*

Despite the good natural conditions, the Valle Averto Oasis is also known for the presence of disturbing factors (e.g., industrial and urban pollution sources) (see Tomè 2010) that could affect the environmental quality and the health of the freshwater turtles. In order to assess the impact of pollutants in the European pond turtle habitat, the eggshells of *E. orbicularis* were collected in June-July 2019 in the surrounding areas of the study sites (Fig. 1.3). All samples were stored and transported at 4 °C in ice coolers. Moreover, the samples have been transferred at the "Università degli Studi di Palermo" under the supervision of Professor Vincenzo Arizza (co-tutor of this project) for sample preparation and chemical metal analysis.

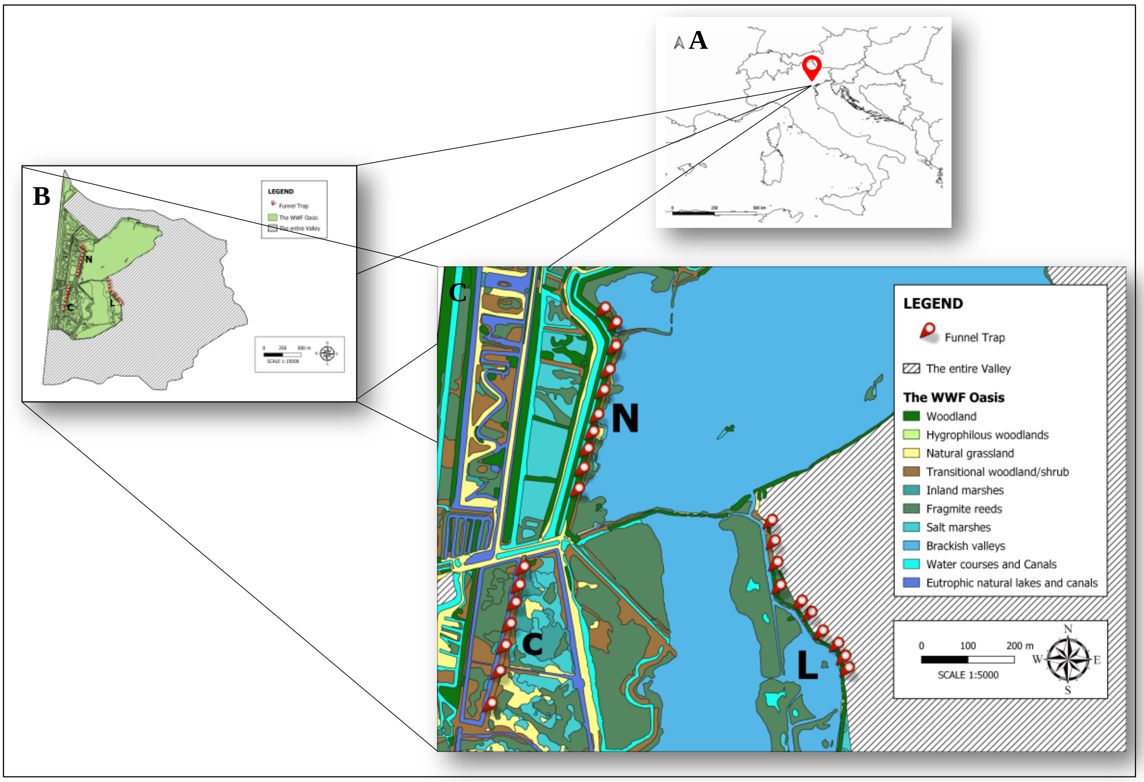

Figure 1.2 (A), (B) Territorial framework of Valle Averto and delimitation of the naturalistic oasis WWF. (C) Map of land use types according to the European Union classification Corine Land Cover focused in three study areas: Nitticore (N, canal habitat) Cavalli (C, eutrophic canal habitat) and Lago (L, brackish lake habitat) (original map from Buffa et al. (2013)).

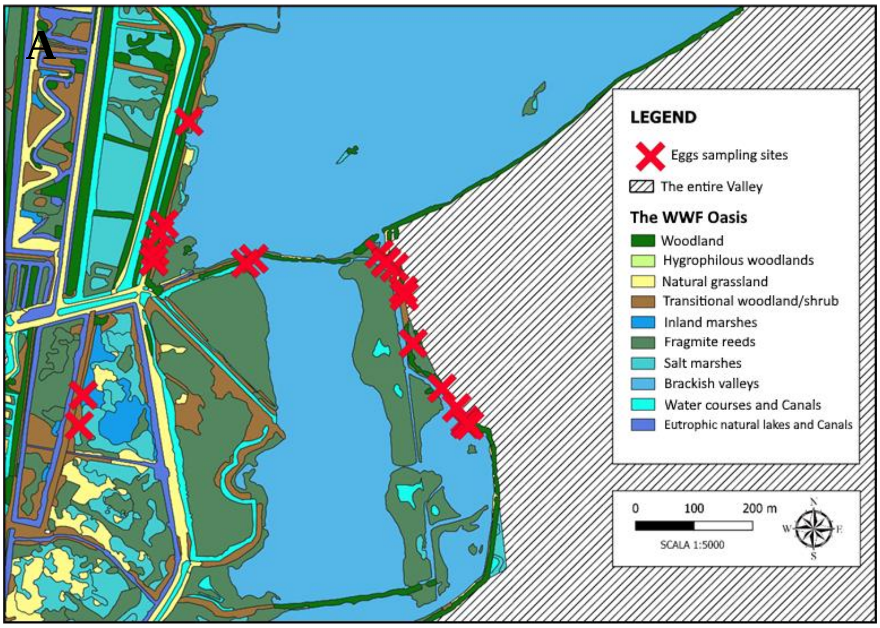

Figure 1.3 (A) *Emys orbicularis* clutches points discovered in the Valle Averto Oasis (B) example of *E. orbicularis* nest predation.

1.2.3 *Chemical analysis of metals*

The sample extraction for Fe, Zn, Cu, Mn, Ni, Cr, Pb, Co, V, Ag, Sn, As and Cd assessment was carried out by a digestion procedure using a Multiwave 3000 (Anton-Paar, Graz) in accordance with UNI EN 13805:2002. The samples digested were made up to 50 ml with Millipore deionised water until the ICP-MS analysis. Fe, Zn, Cu, Mn, Ni, Cr, Pb, Co, V, Ag, Sn, As and Cd levels were quantified by a 7700x series ICP-MS (Agilent Technologies, Santa Monica CA, USA). The operating conditions and instrumental settings are shown in Table 1.1. The analyses were carried out on the basis of calibration curves, constructed by the linear interpolation of at least 7 points corresponding to the readings of 7 standard solutions and white calibration, admitting a maximum error of 5% on the reading of the single standards and a correlation coefficient $r^2 > 0.999$.

Table 1.1 Strumental setting of the ICP-MS

Parameter	Set
RF-Power (W)	1550
Reflected power	< 5
Carrier gas flow (mL/min)	1.2
Plasma gas flow (L/min)	15
Auxiliary gas flow (mL/min)	1
Spray chamber	Water cooled double pass
Spray chamber temperature (°C)	2
Lens voltage (V)	4.5
Mass range (amu)	6-209
Mass resolution	0.7
Integration time points/ms	3
Points per peak	3
Replicates	4

1.2.4 *Data analysis*

The studied sub-population can be assumed to be “demographically open” during the experiment as evidence of significant migration and emigration movements were observed from and to the nearest wetland, despite sampling occurred in a short period. Additionally, the Closure Test (Stanley and Burnham 1999) was performed to test if the sub-population can be considered open. A loglinear model for open population capture-recapture studies (Rivest and Daigle 2004) was used to estimate population size, using the package Recapture (Baillargeon and Rivest 2007) in R (R Core Team 2020). Due to the short sampling period, any survivorship estimation was not addressed to the study areas. To estimate the abundance and density of pond turtle's population, the presence of heterogeneity was tested using a graphical approach as explained by Baillargeon and Rivest (2007). Moreover, to take care of the heterogeneity, the animals captured in May were removed from analyses as a significant decrease was observed in the number of captures possibly linked to low values in the solar radiation and air temperature during this sampling occasion (Pupins and Pupina 2009). The climatic parameters, based on the 2019 data of the meteorological station located in the Valle Averto Oasis (owned by the Regional Agency for Environmental Protection-ARPA) showed the lowest values of mean air temperature (12.2 °C) and mean solar radiation (17355 MJ/m2) in May as compared to April (14.2 °C; 23685.3 MJ/m2), June (25.5 °C; 26871.3 MJ/m2), July (22.9 °C; 21610 MJ/m2) and September (19.3 °C; 19685.3 MJ/ m2). The density was extrapolated as the ratio between the number of estimated individuals by the best fitting model and the water body surface of trapping sites (Otis et al. 1978; Efford et al. 2004). Data on sexual differences (males vs females) in size and body mass were firstly checked for normality with Shapiro-Wilk test and then analysed using a *t*-test. When data did not meet assumptions of normality and homogeneity of variance a Wilcoxon test was used. Population structure was analysed using a graphical approach by dividing SCL data in size classes of 10 mm. The sex ratio was calculated as the proportion of males in a sample (males/(males + females)), and not a ratio *sensu stricto* (males/females)).

For capture and recapture traps, the GPS coordinates were plotted into QGIS version 3.4.9-Madeira software and then the minimum linear distance was measured between these points. A χ^2 test was used to compare recapture differences per period and both sexes. Differences in the moved distance were tested between sexes, among weight classes (< 300 g, 300–400 g, > 400 g) and study sites (N-canal habitat, C-eutrophic canal habitat and L-brackish lake habitat). Because data was not normally distributed (Shapiro-Wilk W = 0.632, $p < 0.001$ for sex grouped data, W = 0.573, $p < 0.001$ for weight grouped data and W = 0.910, $p < 0.05$ for study sites grouped data), Mann Whitney and Kruskal-Wallis test was performed to evaluate significant differences in movement. Differences in the captures and recaptures were also investigated among the study sites. The capture data met parametric assumptions then the ANOVA test was applied. In addition, to compare the capture among the study areas, a post hoc Tukey test was performed.

Conversely, the recapture data were not normally distributed (W = 0.683, $p < 0.001$) then the analyses were performed with the non-parametric Kruskal-Wallis test. Moreover, a post hoc Dunn's test was carried out to compare the recapture among the study areas. Correlation between distance travelled and size of freshwater turtles was estimated through Kendall's tau rank correlation coefficients. The number of recaptures is higher than the number of individuals used for the computes of average distance intra-study sites, as the individuals that changed the water and the individuals recaptured in the same trap (without real evidence of movement) have been excluded. The recapture data by month (only cases of pond turtles being captured twice during a 3-day trapping effort) were investigated to compute the daily movements. Moreover, differences between pond turtles never recaptured and recaptured were explored in term of size class. All statistical analysis was made using R v. 4.0.2 (R Core Team 2020).

Graphic comparisons between different mean metal concentrations in eggshell samples that were collected at different sampling location were conducted using Microsoft Excel.

1.3 Results

1.3.1 *Population parameters*

A total of 336 captures (138 in April, 27 in May, 95 in June, 57 in July and 19 in September) were obtained in 2019. Out of the examined individuals, 172 males, 144 females and 20 juveniles were collected with an overall capture sex ratio [MM/(MM + FF)] of 0.5:0.5. The capture sex ratio differed significantly among periods ($\chi^2 = 11.348$, $p < 0.05$), with lowest values in September (0.2:0.5) as compared to April (0.6:0.5), May (0.5:0.5), July (0.5:0.5) and June (0.4:0.5) (Fig. 1.4). The number of individuals captured differed among study sites, being 136 freshwater turtles in Nitticore, 130 in Cavalli and 70 in Lago. The population was characterized by a dominance of freshwater turtles with SCL between 120 and 149.9 mm (68.7%) (Fig. 1.5). The most represented range class for males was 120-129.9 mm (45.5%), whereas for females is 140-149.9 mm (40.3%), and for juveniles: 70-79.9 mm (28.6%) (Fig. 1.5). Females were heavier (*t*-test = 25.552, $p < 0.001$) and bigger than males, both in terms of carapace length (*t*-test = 19.004, $p < 0.001$), width (W = 22712, $p < 0.001$), and height (W = 24444, $p < 0.001$) (Table 1.2). The sampling was highly shifted toward adults reflecting the low capture rate of juveniles, which represented only the 5.9% of the population sampled. The Closure test supports our hypothesis that the sub-population can be considered open during the experiment ($\chi^2 = 89.500$, $p < 0.01$). Following the loglinear approach of Cormack (1985, 1989) and Rivest and Daigle (2004), the abundance of sub-population was estimated as 1009.4 ± 365.2 individuals (deviance = 157.982, df = 4072, AIC = 365.066). This result gives a density estimation of about 252.3 ± 91.3 ind./ha. Out of 336 European pond turtles (*Emys orbicularis*) marked, 97 freshwater turtles were recaptured (28.9% return rate) at least once, 21 in April, 7 in May, 35 in June, 33 in July and 1 in September. Considering the multiple recaptures of the same individual, the total recapture events were 140 (41.6% return rate). The recapture rate was rather balanced between sexes: 47% for females and 41% for males. The low number of recaptured juveniles (n = 2) did not allow any statistical analysis for this group. Significant differences in distance were not observed between sexes, among weight classes and study sites (Table 1.3). On the contrary, significant differences were detected in the captures and recaptures among the study areas which were significantly higher in the two canals than in the brackish lake area (Table 1.4). No correlation was found between size and travelled distance ($\tau = 0.040$, $p = 0.570$).

No significant differences were observed in terms of size class between turtles that were marked and never recaptured and turtles that were recaptured (W = 9, p = 0.1).

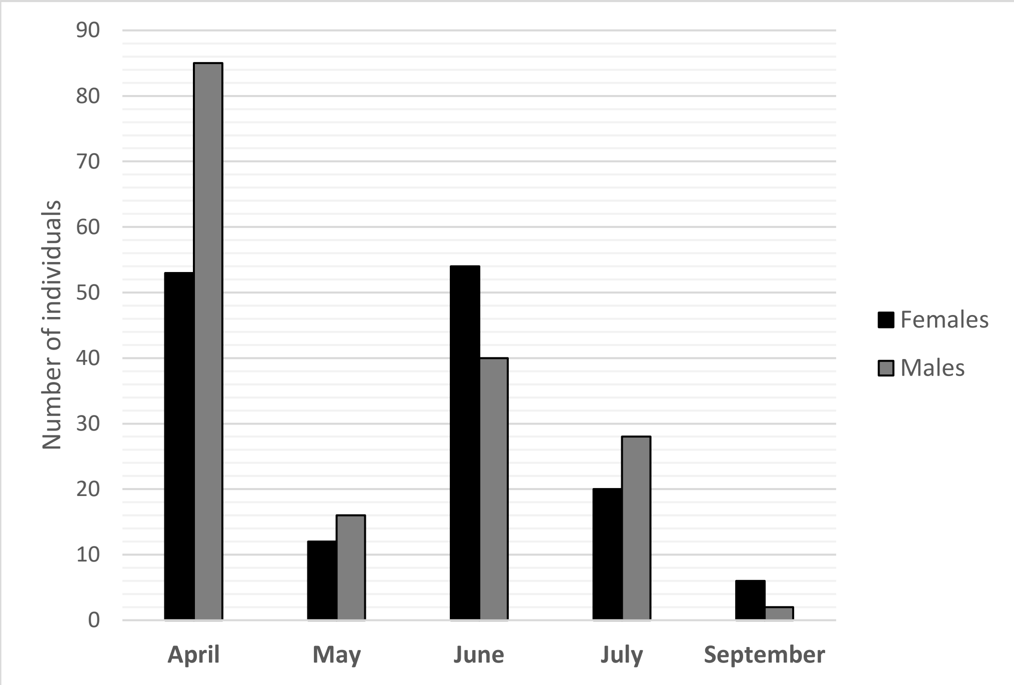

Figure 1.4 Number of adult males and females *Emys orbicularis* captured per month at the Valle Averto Oasis.

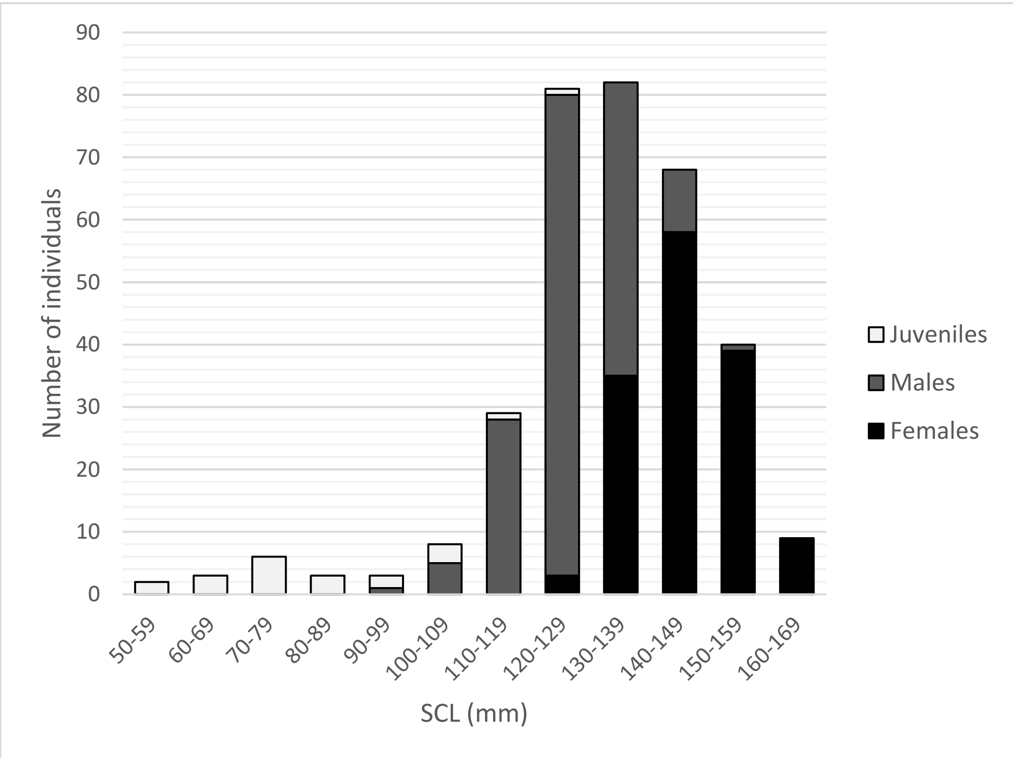

Figure 1.5 Straight carapace lengths (mm) of *Emys orbicularis* captured during the study at the Valle Averto: unsexed juveniles, males, and females.

Table 1.2 Mean values of major biometric measurements (in mm and g) for *Emys orbicularis*. SCL = straight carapace length; BM = body mass; CW = carapace width; CH = carapace height; SD = Standard deviation [172 ♂ adult, 144 ♀ adult and 20 Juveniles (J)].

Biometric Measurement	Sex	Mean ± SD	Min	Max
SCL	♀	145.3 ± 8.9	122.3	169.1
	♂	126.3 ± 8.7	99	156.6
	J	88.5 ± 21.4	52.1	120.3
BM	♀	542 ± 97.1	298.4	783.2
	♂	307.4 ± 57.4	157.6	445.3
	J	113.6 ± 63.1	27.3	252.5
CW	♀	107.6 ± 6.9	89.1	131.5
	♂	94.1 ± 10.9	42.5	109
	J	68.4 ± 12.8	43.7	90.1
CH	♀	61.02 ± 5.1	49.2	89.6
	♂	45.1 ± 5.0	35.8	92.4
	J	34.2 ± 6.3	22.1	44.7

Table 1.3 Mean values and differences of distance moved in meters (m) of *Emys orbicularis*: daily distance (in dark grey), movements covered inside of each study sites (in grey) and among study sites (in white).

Sex	Mean (m)	SD (m)	N	Differences	Statistics test
♀	141.49	67.95	9	Sex × Distance	W = 1214.5, $p = 0.447$
♂	81.08	41.80	15	Weight × Distance	K = 83.8, $p = 0.577$
J	69.70	37.34	2	Size × Distance	$\tau = 0.040$, $p = 0.570$
♀	146.79	117.08	29		
♂	125.43	112.34	31		
J	69.70	37.34	2		
♀	871.83	507.34	8		
♂	802.50	112.34	9		

A total of 17 (eight females and nine males) out of 97 freshwater turtles changed the water body during mating and egg-laying periods (from April to early September) showing a wide dispersion activity (Table 1.3, Fig. S1). The movements did not show a clear pattern, being apparently random without any preference for a specific study site. The migration required crossing dry land as the aquatic connection among the study sites was lacking.

A female freshwater turtle migrated in all three study sites, being recaptured 1502.32 m from the tagging site. On July and September 2019, two *E. orbicularis*, a juvenile and an adult male were observed while they were travelling along the north-south shore of the canal "Nitticore" (WGS84 Geographical coordinates respectively: 45°21′11.40"N, 12°8′26.66"E and 45°21′20.66"N, 12°8′28.70"E).

Table 1.4 Differences in percentages of total capture (in grey) and recapture (in white) were detected among the study areas. Statistics tests showed significant differences in the captures and recaptures among the study sites with a higher rate in the two canals (N and C) than in the brackish lake area (L).

Study sites	Captures (%)	Differences	Statistics test
Nitticore (N)	214 (44.9%)	Capture × Study sites	$F_{2,24} = 14{,}02$, $p < 0.001$
Cavalli (C)	183 (38.4%)		Tukey HSD, $p < 0.001$
Lago (L)	79 (16.7%)		
Nitticore (N)	78 (55.7%)	Recapture × Study sites	K = 8.61, $p < 0.05$
Cavalli (C)	53 (37.8%)		Dunn'test, $p < 0.05$
Lago (L)	9 (6.5%)		

1.3.2 *Ecotoxicological parameters*

A total of 23 clutches were discovered along the banks of the canals or in a portion of these (Fig. 1.3). Concentrations of the detectable metals in eggshell are shown in Fig. 1.6 and have a descending order of Fe > Zn > Cu > Mn > Ni > Cr > Pb > Co > V > Ag > Sn > As > Cd

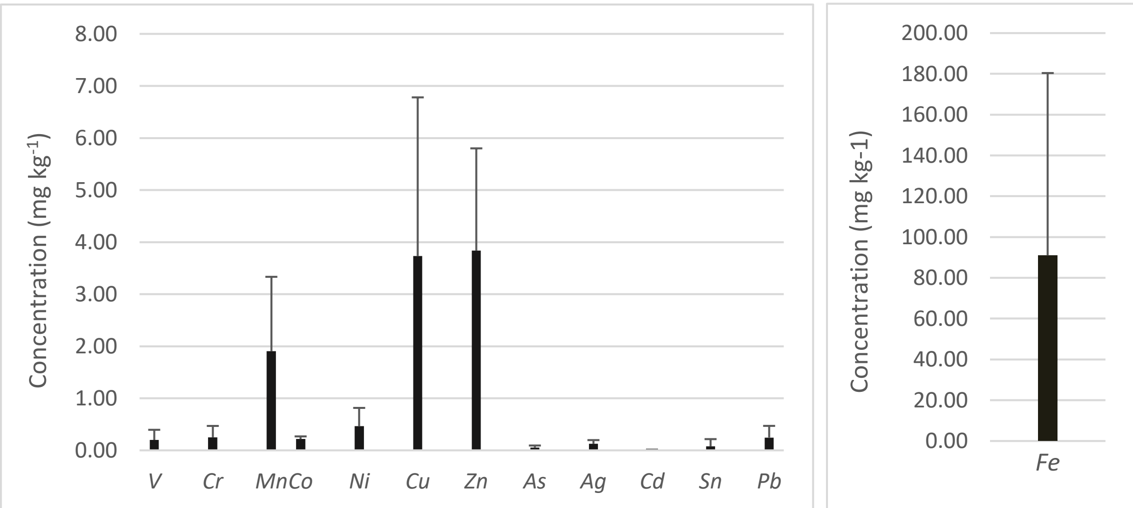

Figure 1.6 Concentration of metal accumulation in eggshell of the European pond turtle (*Emys orbicularis* during the sampling period. Values are means and standard deviations (n = 23 clutches).

1.4 Discussion

1.4.1 *Population parameters*

The abundance estimation of the *Emys orbicularis* population inhabiting the Valle Averto Oasis, here reported, might show one of the most populated areas of the Italian peninsula (Mazzotti et al. 2007; Seglie 2015; Zuffi et al. 2020). Population densities of *E. orbicularis* vary considerably, depending on the region (Fritz 2001, 2003). Generally, population abundances are high in southern Europe and in the centre of the species' range (Ayaz et al. 2008). This pattern, however, is also likely due to the different methods employed, ranging from few (Mazzotti 1995; Bayrakci et al. 2016) to multiple sampling occasions (Mazzotti et al. 2007; Olivier et al. 2010; Bayrakci and Ayaz 2014; present research), depending on the sampling area – from very small to many large areas (Ayaz et al. 2008; present research) – and, finally, to population abundance (Zuffi et al. 2020). Therefore, *E. orbicularis* population size is not easy to determine without a continuous sampling effort, an approach that is not standardized to date. Nevertheless, population density is a valuable estimator of population viability and of habitat carrying capacity, especially in the light of comparing different populations and of deriving baseline information for the species management, conservation and protection (Chelazzi et al. 2007). In Italy, the few studies elaborated were characterized by low numbers: 3-10 ind./ha of *E. orbicularis* were estimated with the linear transect census in the Bosco Mesola (Mazzotti et al. 2007) and 9.4 ind./ha in the Palude di San Genuario with the capture-recapture method (Seglie 2015). The density of Venetian freshwater turtle population, conversely, showed high values (252.3 ± 91.3 ind./ha) and apparently it is close to those living in Anatolia and the Black Sea region (242 ind./ha and 225 ind./ha) (Ayaz et al. 2007; Bayrakci and Ayaz 2014), those of *E. trinacris* in Sicily (290 ind./ha, Lo Valvo et al. 2008; 240 ind./ha, Ottonello et al. (2017)) or those of *E. orbicularis* of western Italy (from 146.7 to 183.6 ind./ha, Zuffi et al. 2020). Although at a smaller scale, dispersion events were observed among study sites in present research compared to Zuffi et al. (2020). However, as in the research of to Zuffi et al. (2020), the study sites are located along the coast and are characterised by mixed wetlands, marshes, artificial and natural canals. Moreover, the captures were carried out in one seasonal sampling long transects with the same captured method (CMR) showing a very similar recapture rate: 28.6% for to Zuffi et al. (2020) and 28.9% in present work. Therefore, densities are easily comparable in this case. According to Zuffi et al. (2020) multiple sampling sessions with high trapping points may represent the best approach to infer suitable information on the density patterns in this species. Likely, with this kind of approach, the carrying capacity of selected habitats could be derived quite easily, even if in many cases a complete habitat analysis has not been carried out.

The population structure was characterized by a size distribution that was markedly shifted towards adult age, with a bimodal distribution due to difference in size related to the sex. This trend was also observed in the congeneric *E. trinacris* in a protected area of south-western Sicily (Ottonello et al. 2017). The low number of juveniles was not essentially different from that found in other studies (Vamberger and Kos 2011; Fediras et al. 2017; Romanato et al. 2020; Scali et al. 2020) and could be associated with sampling bias due to lower detectability and different habitat use (Lebboroni and Chelazzi 1998; Zuffi et al. 2020), low recruitment rates, or a combination of both (Keller et al. 1998). The overall capture sex ratio of captured individuals was rather balanced, but it also was detected significant differences between the periods probably related to the different activity of males and females (Girondot and Pieau 1993). In *E. orbicularis*, the reproductive activity starts with spring emergence, with a peak of courtship behaviour and mating between the end of March and May (e.g. Mitrus and Zemanek 2004; Servan and Roy 2004). During this period the males are very active, moving along the wetland looking for reproductive females (Lebboroni and Chelazzi 1991). On the contrary, the females are very active in June, corresponding to the peak of egg-laying for Italian *E. orbicularis* (Zuffi et al. 2015). In our study area, a significant decrease in the number of captures for females and males was observed during May, which can be influenced by local climatic conditions (Cadi et al. 2004) possibly linked to low values in the solar radiation and air temperature during sampling occasion (Pupins and Pupina 2009), or by different behavioural phenomena.

The analysis of the movements did not show any difference between sexes at the level of spatial patterns, and the absence of "inter-sexual and inter-size classes differences" suggested a similar use of the freshwater habitat by the adults, as shown with radiotelemetry method for *E. orbicularis* in southern France and eastern Spanish lagoon (Cadi et al. 2004; Drechsler et al. 2018). In contrast with Drechsler et al. (2018), an increase movement activity was observed in June even though both study areas are lagoon habitats. This difference might be because the Spanish study area is surrounded by rice fields (in the south). These environments are not suitable for egg-laying as the females do not tend to make large movements (Drechsler et al. 2018).

Our findings suggest that European pond turtles reach higher densities in canals in comparison to brackish water bodies. This phenomenon could be associated with the habitat surrounding wetlands as *E. orbicularis* occurs more frequently and abundantly in permanent wetlands surrounded by woodlands rather than in brackish water near the coast (Ficetola et al. 2004). Moreover, different habitats could provide different feeding opportunities or different competition rates (e.g. presence or absence of fish), thus yielding different long-term survival (e.g. (Ottonello et al. 2016a; Canessa et al. 2016). Nonetheless, the "Lake" site is more extensive than the other study areas and the freshwater turtles could disperse more easily in this macro-habitat. Further investigations are required to test in detail for a difference in habitat selection. The canal is one of the two main habitat systems recorded for *E. orbicularis* in Italy (Lebboroni and Chelazzi 1998). More specifically, this habitat was favoured during the nesting activity with a highly significant propensity for north-south oriented canals (Zuffi and Rovina 2006). This tendency could be common also for Valle Averto Oasis. In the frame of the sampling activities aimed at investigating the movements of *E. orbicularis* in this study area, 21 out of 23 clutches were discovered exactly along the north-south shore of the canals or in a portion of these (Fig 1.2). The canal system is relatively common in several parts of the European distribution area (Italy: Lebboroni and Chelazzi 1998; Austria: Rössler 2000; Hungary: Farkas 2000; Ukraina: Kotenko 2000 ; France: Olivier et al. 2010). Special attention should be brought to these habitats, including both aquatic and terrestrial areas being a good practice reported in a lot of studies (Zuffi and Rovina 2006; Pereira et al. 2011) and confirmed in part by our results. Hence, quantitative radio-tracking studies (e.g. Joyal et al. 2001; Schabetsberger et al. 2004) are required to examine the range of migration and habitat use for the inland lagoon *E. orbicularis* populations.

1.4.2 *Ecotoxicological parameters*

Direct comparisons of metal accumulation in eggshell between different field studies were difficult because of different turtle species and metals involved. In a study of the red eared slider (*Trachemys scripta elegans*) from the Lower Illinois River concentrations of Mn and Cu in eggshell were generally lower than the concentrations in eggshell of the

European pond turtle in the Valle Averto Oasis. However, Zn, Sn, Cu, Cr, V, Pb, Ni and Cd concentrations in eggshell of the red-eared slider were higher than the concentrations in eggshell of the *E. orbicularis*. In another field study conducted in Oregon, Henny et al. (2003) analysed 16 trace elements in eggs of the western pond turtle (*Actinemys marmorata* Holman and Fritz 2001), with Cu, Mn and Zn above their detection limits. Interestingly the eggshells had very high average concentrations of Fe (~90 mg kg^{-1}). Similar metal concentration was found in Nile Crocodile eggs from the Kruger National Park, South Africa where a linear relationship of iron against inner eggshell thickness was confirmed (du Preez et al. 2018). The thickness of the shells plays a crucial role in gas and water exchange as well as in the effort required for the hatchling to emerge (Paik et al. 2009; du Preez et al. 2018). Concentrations of these metals, except for Zn and Fe, were generally comparable with the concentrations in eggshell of the European pond turtle. Concentrations of Zn (65 mg kg^{-1}) in the western pond turtle eggs were higher than the concentrations in the European pond turtle eggshell of our study. Likely, these differences in concentrations between this study and other documented studies may be associated with differences in species, diet, geographic location, contamination, and specific environmental conditions at each location (Tryfonas et al. 2006). To avoid speculation the relationship between metal concentrations in the habitat (es. based on superficial sediments and water samples) and the concentrations in turtles' eggs require to be elucidated in the future. Chemical compositions of different environmental matrices in the *E. orbicularis* habitat could clarify whether Mn and Cu concentrations would be a matter of concern. Geology determines the concentration of metals and metalloids in the natural background, although anthropogenic input from agriculture, wastewater, and wastes handling may increase the absorption of the metals above the background (Luoma 1983). It is well recognised that the predominant worldwide anthropogenic inputs of Mn, Fe and Cu to aquatic ecosystems can result from domestic wastewater, sewage sludge disposal, water by discharge from industrial facilities, or leachate from landfills and soil (US EPA 1979; Francis and White 1987; ARPAV 2019). This pioneering study provides some important baseline information in the European pond turtle habitat along the inland margins of the Venice lagoon, which will be essential for other future long-term studies. Future studies have to take into consideration also xenobiotic compounds as a potential source of anthropogenic impact and threat for *E. orbicularis*.

1.4.3 *Conclusion*

In conclusion, the European pond turtle is a good species model to apply management action in this area: it is a bioindicator and can occur in different aquatic habitats. However, despite the possible high abundance of *E. orbicularis* populations in the wetland area of the Italian lagoon, the information about its distribution in this area is lacking. This species has often been a victim of the operations of clearing the anthropized Venetian areas which had put the population at risk (Novarini and Bano 2019). Therefore, Valle Averto with the high densities values recorded could provide a rare opportunity to study *E. orbicularis* in the transitional water system. The detection of a gradient in water salinity along the water body might be an occasion to observe a different pattern of distribution of the European pond turtle across an apparently similar habitat. Spatial distribution studies analysing a large number of individuals are required to test for this hypothesis, providing a better understanding of the ecology of *E. orbicularis*. In the lagoon area, this may have important consequences for the management and conservation of this threatened species.

1.5 Supplemental data

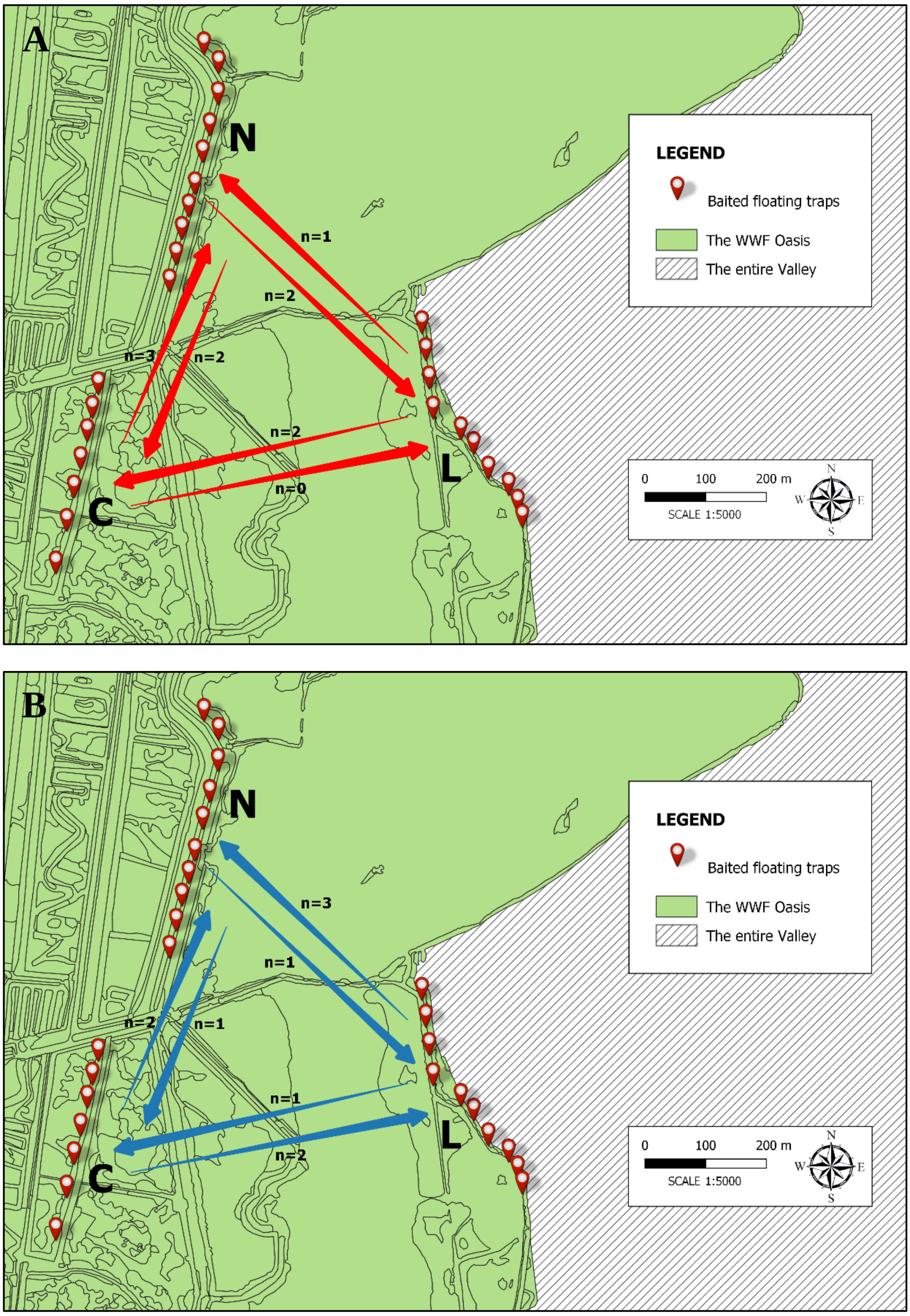

Figure S1 (A) Females (red arrows) and (B) males (blue arrows) capture movements (n = number of individuals) among the study sites: Nitticore (N, canal habitat), Cavalli (C, eutrophic canal habitat) and Lago (L, brackish lake habitat).

CHAPTER 2. 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

This chapter has been submitted to Amphibia-Reptilia. In close collaboration with Stefano Borella, Dario Ottonello, Vincenzo Arizza and Stefano Malavasi, the five authors have contributed to the research development as specified in the chapter 1.

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2.1 Introduction

Reptiles concentrate their activity on specific days and seasonal periods (Tosini et al. 2001; Dayananda et al. 2020), yet movement and activity are not always predictable. Movements, 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 landscape features, topography (Meeske 2000; Meeske and Mühlenberg 2004; Ficetola et al. 2009; Vilardell-Bartino et al. 2015; Ottonello et al. 2016a; Canessa et al. 2016). The understanding of these factors is central to reducing 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 can intermittently occur within the terrestrial habitat, yet wetlands (e.g., temporary wetlands that occasionally or regularly dry) may vary in size and condition providing different levels of resources through space and time. Consequently, this might be a potential stimulus to move and find a more suitable area (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 et al. 2000). The conservation of turtles not only depends on the quality of the aquatic habitat but also on the quality of the surrounding terrestrial habitat. 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 site. A recent study about the abundance estimation of the *E. orbicularis* population has shown one of the most populated areas of the Italian peninsula in this study site (Liuzzo et al. 2021). However, even though the species was reported along the inland margins of the Venice lagoon (Agapito Ludovici et al. 2012; 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 periods of activity and lethargy. To test for these hypotheses, the following aspects were investigated: (1) basking, movements and brumation behaviours, (2) temporal and spatial differences in the size of the home range utilized, (3) the main terrestrial and aquatic areas preferred by *E. orbicularis*, and (4) travelled distance and the variation of displacement in relation to water temperature and salinity. All these behavioural and spatial distribution analyses were quantified during a phase of activity and lethargy in the inland water bodies of the Valle Averto Oasis, testing for differences in their frequency and magnitude of changes.

2.2 Materials and Methods

2.2.1 *Trapping and radiotracking*

Sampling was carried out in the Valle Averto Oasis along the canal “Nitticore” (N) (45°21′23.54"N, 12°8′29.55"E) (Fig. 1.2C), a water body 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 with sardines (n = 27, length = 60 cm, diameter = 30 cm, eye = 16 × 14 cm) 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). The population structure was analysed by dividing SCL data in size classes of 10 mm (Ottonello 2017; Liuzzo et al. 2021). 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).

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 x 80 x 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 2020, it was not possible to collect location data for the early reproductive phase (March, April, May). 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.

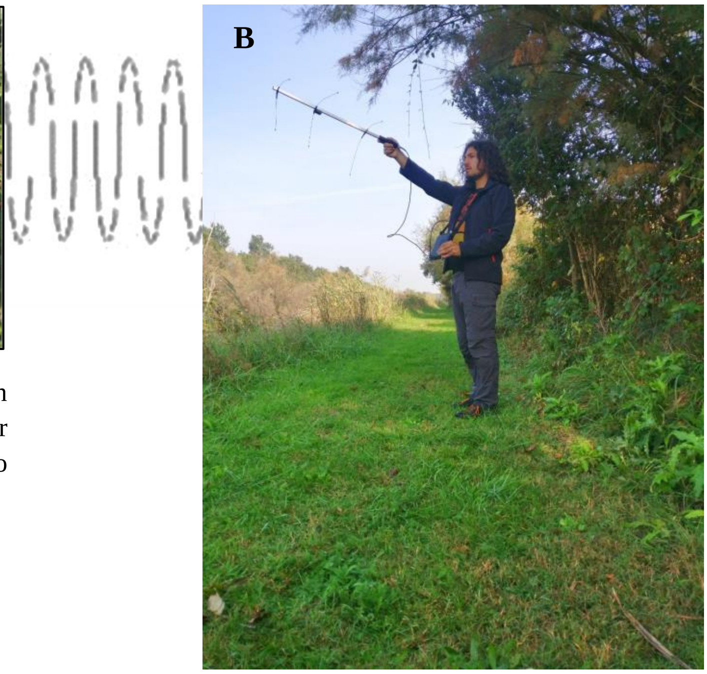


Figure 2.1 (A) An adult female European pond turtle fitted with a radio-transmitter and (B) an operator detecting the radio waves from transmitters

2.2.2 *Habitat use and behaviour*

Habitat use 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 et al. 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. 2.3B): (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 location data were not normally distributed then the analyses to test for differences in macrohabitat use were performed with the non-parametric Kruskal-Wallis 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 habitat use 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 1950). 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 (2019) 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 2.1, Fig. 2.3A-B). 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 et al. 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, $\rho = 0.362$, $p > 0.05$). The home range areas were also calculated for each pond turtle in the period of activity (June-July 2020) and lethargy (October-November 2019, 2020). Because 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), Wilcoxon test was performed to assess significant differences in 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. 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 and groups have also been conducted to assess the basking, floating, movements, and brumation behaviours. 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. Differences in home range size between sexes were also analysed, using the Mann-Whitney U test. All statistical analysis was made using R v. 4.0.2 (R Core Team 2020).

2.2.3 *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. 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-Wallis 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. 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 et al. 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 et al. 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).

2.3 Results

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) 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 [MM/(MM + FF)] of 0.5:0.5. 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.

Table 2.1 Movement and space use estimates (mean ± 1 SD) for 12 male and 17 female *Emys orbicularis* in the Valle Averto Oasis, Venice lagoon.

Parameters	Activity $_{(A)}$	Range $_{(A)}$	Lethargy $_{(L)}$	Range $_{(L)}$
MCP 95% (*ha*)	3.90 ± 3.05	0.15 - 9.47	0.13 ± 0.11	$2x10^{-3}$- 0.42
MCP 50% (*ha*)	0.32 ± 0.21	0.04 - 0.7	0.03 ± 0.02	$1x10^{-3}$- 0.09
KDE 95% (*ha*)	1.80 ± 1.44	$82x10^{-4}$± 3.46	0.16 ± 0.15	$2x10^{-3}$± 0.26
KDE 50% (*ha*)	0.24 ± 0.21	$14x10^{-4}$± 0.52	0.02 ± 0.015	$1x10^{-4}$± 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

2.3.1 *Habitat use and behaviour*

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 lethargy period, whereas 13.55% (W), 4.20% (T), 2.96% (F), 11.44% (B) and 67.79% (C) during the activity period. 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 2.1). 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. 2.3A-B). 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 (τ = 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**) and a Long period of stationary in the mud bottom (**Ls**) (after 5 hours without detecting movement). 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 in the water bodies. Significant differences were checked between the period of activity and lethargy in the main behaviours observed (Table 2.2, Fig. 2.3). The frequency of **B**, **Ss**, **M**, and **Mo** behaviours was significantly higher during the period of activity whereas **Ls** behaviour was significantly more frequent in the period lethargy (Table 2.2). During the latter period pond turtles were observed to brumate gregariously in a relatively homogenous 0.63 ha area within a 5 m distance from canals banks (Fig. 2.3B). 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.

Table 2.2 Significant differences in the main behaviours measured as the number of observations per location (Basking (B), Movement (M), the Movement caused by the radiotracking operator (Mo), a Short period of stationary in the mud bottom (Ss) and 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 x P	W = 59, $p < 0.05$
M x P	W = 58, $p < 0.05$
Mo x P	W = 51, $p < 0.05$
Ss x P	W = 57.5, $p < 0.05$
Ls x P	W = 5.5, $p < 0.05$

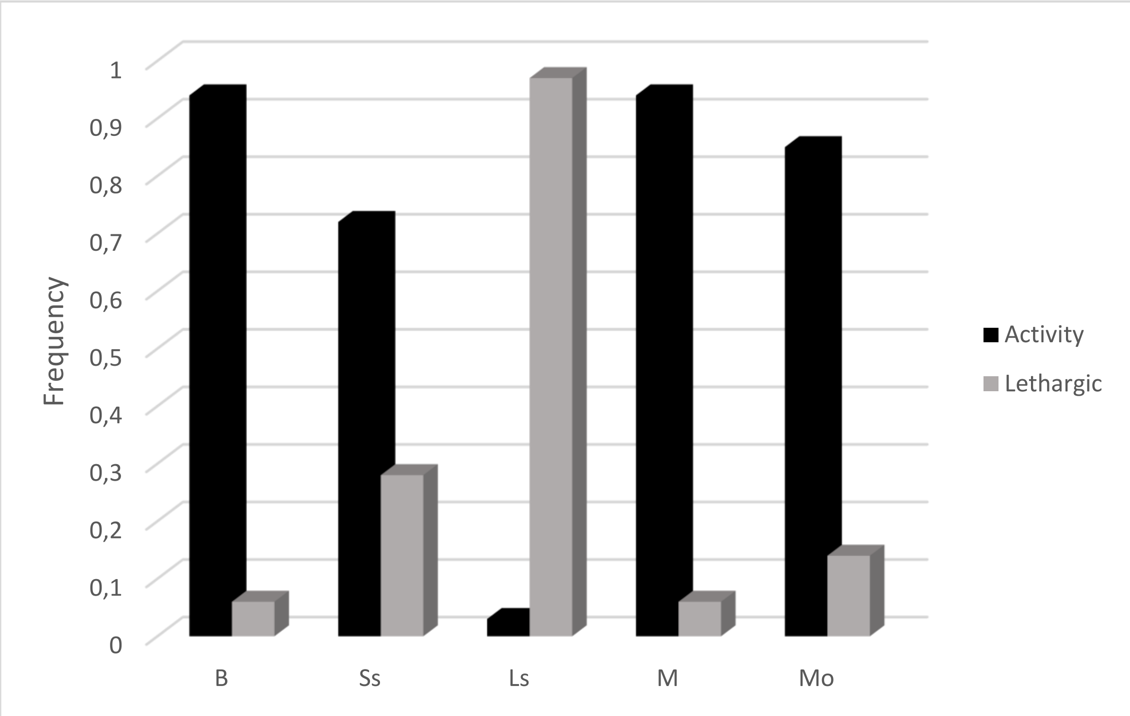


Figure 2.2 Phenological variation in the relative fraction of the main turtles' behaviours (Basking (B), Movement (M), the Movement caused by the radiotracking operator (Mo), a Short period of stationary in the mud bottom (Ss) and a Long period of stationary in the mud bottom (Ls) [after 5 hours without detecting movement]) observed in the water and on the canal banks.

2.3.3 *Movement*

Movements were highest in the phase of activity and declined in the lethargy (Fig. 2.3). 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 2.3). 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. 2.3; Table 2.3). The overwintering period end was detected in the threshold mean water temperature of about 14 °C (Fig. 2.3A) into canals with riparian vegetation. Significant differences were also observed in the minimum terrestrial distance covered between periods (W= 972, $p < 0.05$) that 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 behaviour and turtle travel routes were observed, whereas during the period of activity 6 out of 9 individuals have changed water bodies migrating towards aquatic habitat with higher salinities 1-17‰ (mean: 10.64‰) (Fig. 2.3A).

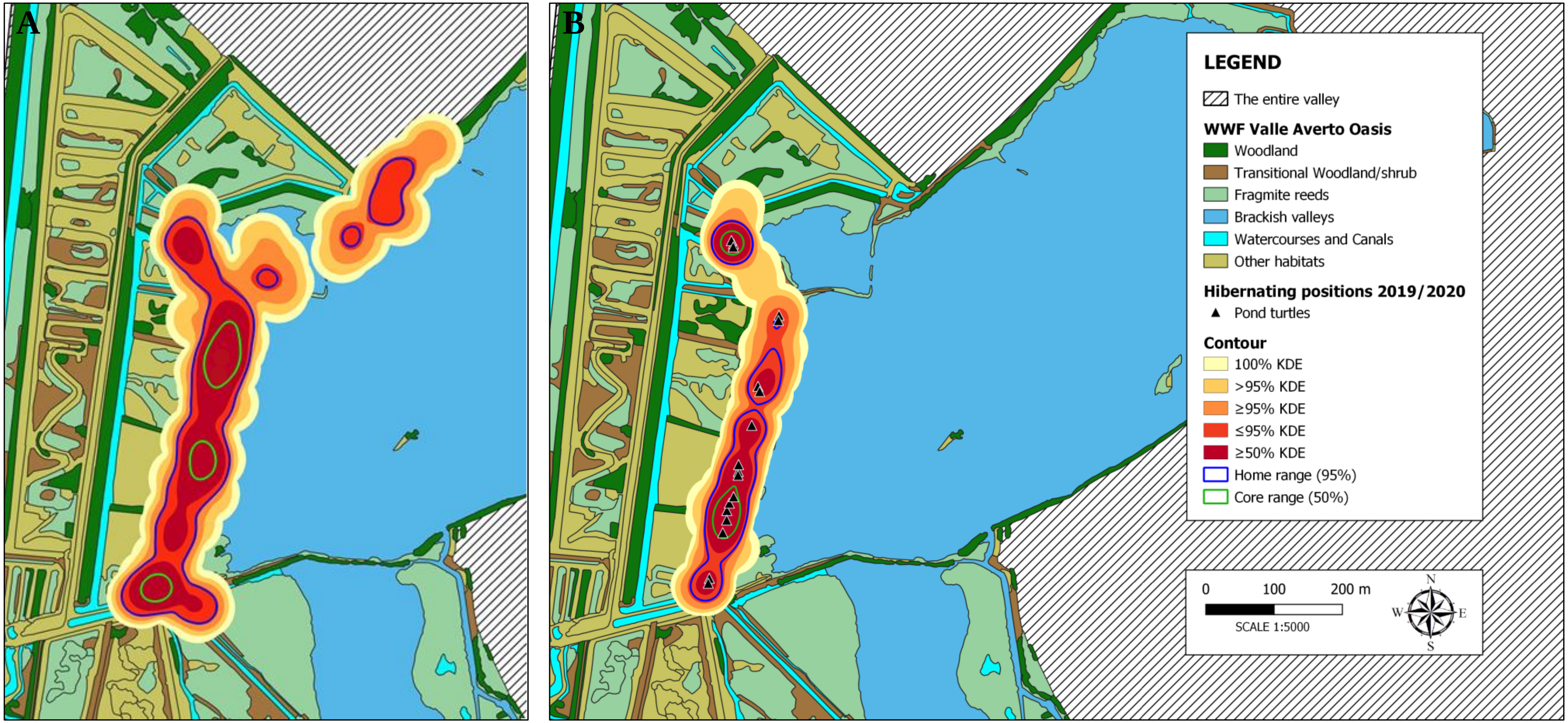


Figure 2.3 Differences on spatial distribution of *Emys orbicularis* between the period of activity (A) and lethargy (B) using the Kernel Density Estimation (KDE). The KDE percentages represent the probability that *E. orbicularis* individuals will be in any part of its home range. The highest use areas were highlighted with blue (95% KDE) and green (50% KDE) contours. The brumating 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 et al. 2013)

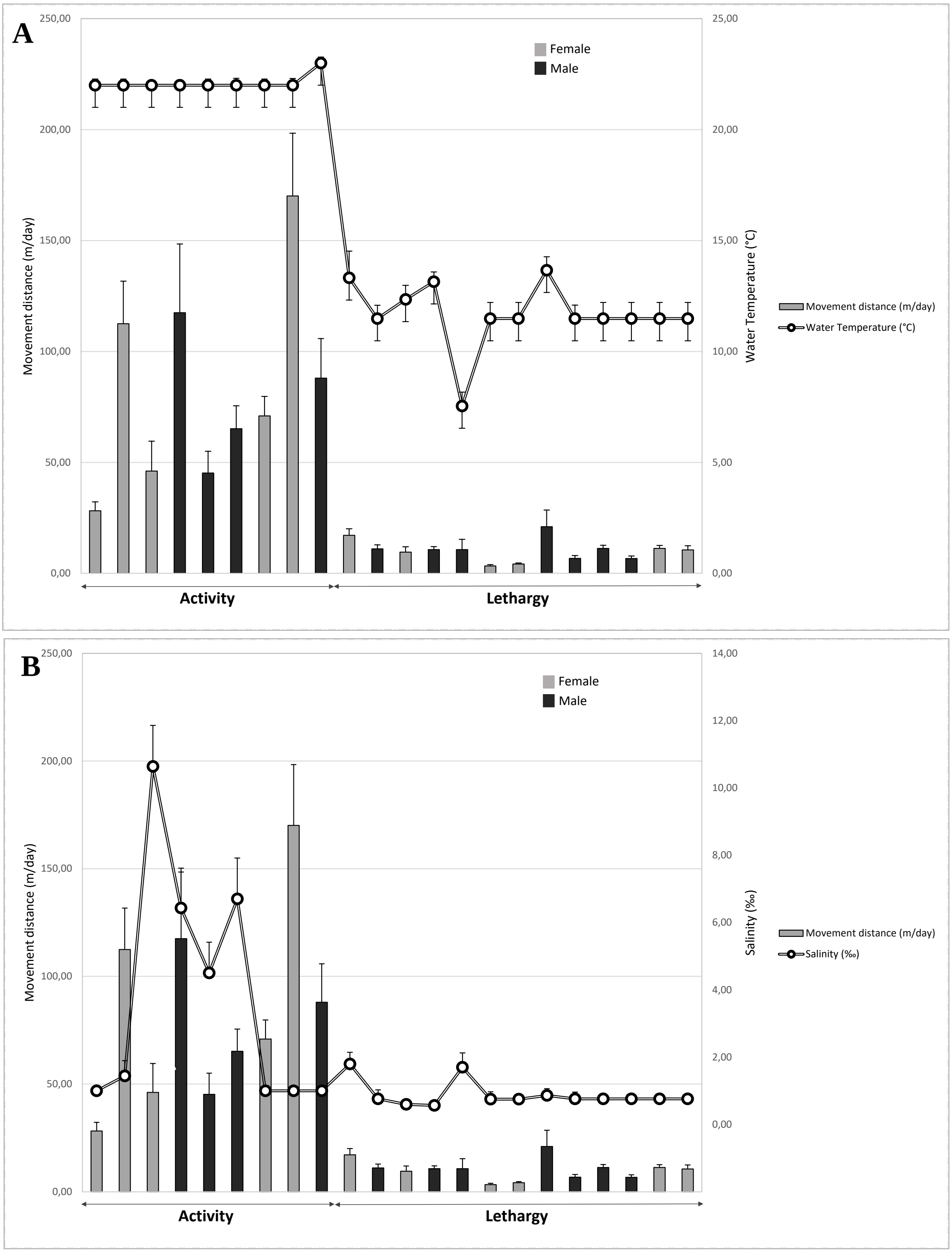


Figure 2.4 Relationships between movement distance (bars) and (A) water temperature (°C) and (B) salinity (‰) for *Emys orbicularis* studied with radiotelemetry during the period of activity and lethargy (the year 2020). Movement and environmental variables are mean ± 1 SE.

2.4 Discussion

This study showed differential patterns of activity, dispersion and habitat use between the late reproductive (activity phase) and the post-reproductive (lethargy phase) period in the population 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 studied in the Valle Averto Oasis inhabiting a marshland with scattered canals performed more frequent overland trips (Fig. 2.3A). 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. 2017). 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 activities like movements between saline and freshwater areas (Hart and Lee 2006; Harden et al. 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. 2.3B). Moreover, salinity and water temperature also influenced turtles' overall patterns of movement distance (Table 2.3). 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.

Table 2.3 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.

	Movement distance					
Predictors	*B*	*CI*	*p*	*B*	*CI*	*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*

Basking (B) and the Short period of stationary in the mud bottom (Ss) 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 might be associated with a local effect as basking behaviour can be affected by the time of day or by wetland features such as bank morphology, sun exposure, the availability of basking log or canal orientation (Di Trani and Zuffi 1997; Cadi and Joly 2003; Peterman and Ryan 2009).

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 sexual differences in movement and space use patterns, typical of many freshwater turtles are often attributed to the 'reproductive strategies hypothesis' of Morreale et al. (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. 2.4). 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 torpor 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 et al. 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. 2.4; Table 2.3). More specifically, the transition from the period of activity to lethargy was detected in the threshold mean water temperature of about 14 °C (Fig. 2.4A). 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 1984; Gibbons et al. 1990; Parde et al. 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. 2.3B). 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 et al. 1999). Aggregation of brumating turtles is also known in some other species (Brown and Brooks 1994; Lewis and Ritzenthaler 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. 2.3B). Moreover, evidence of overwintering groups of *E. orbicularis* was also recorded in Lithuania and Germany (Schneeweiss et al. 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. 2.3A). 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.

CHAPTER 3. HABITAT FEATURES AFFECTING THE SPATIAL DISTRIBUTION OF *EMYS ORBICULARIS* (L., 1758) IN AN INTERNAL VENICE LAGOON WETLAND AREA: IMPLICATIONS FOR WATER AND TERRESTRIAL MANAGEMENT

This chapter has been prepared for submission in a journal that deals with aspects of the spatial ecology. In close collaboration with Stefano Borella, Dario Ottonello, Vincenzo Arizza and Stefano Malavasi, the five authors have contributed to the research development as specified in the chapter 1. Matteo Costenaro has contributed to the field activities.

Mirko Liuzzo, Stefano Borella, Dario Ottonello, Matteo Costenaro, Vincenzo Arizza & Stefano Malavasi (2022): Habitat features affecting the spatial distribution of *Emys orbicularis* (L., 1758) in an internal Venice lagoon wetland area: implications for water and terrestrial management (*In prep*)

3.1 Introduction

Research on wetlands conservation is important because freshwater systems contain a great potential biodiversity, yet are particularly vulnerable and and poorly studied (Abell 2002; Martin 2003). Some of the potentially harmful threats are the morphological and hydrological changes to water bodies occur through regulations to provide water supplies and/or to improve water quality. However, despite global anthropogenic modifications of water flow and water extraction, how these changes affect freshwater fauna is complicated, poorly understood and, for many individual taxa, completely unknown (Bower et al. 2012). For instance, very few conservation indications exist on the relationships between *Emys orbicularis* distribution and water quality or the availability of terrestrial habitat, despite the generally acknowledged importance of upland habitat for freshwater turtles (Naulleau 1992; Semlitsch and Bodie 2003; Ficetola and De Bernardi 2006). The European pond turtle, *E. orbicularis*, is the most widespread of any freshwater turtle in Europe (Ficetola et al. 2004), but it has unfavourable conservation status and a negative population trend over most of its range. *E. orbicularis* is a generalist species and so can occur in a wide range of natural and managed water bodies, from permanent marshes and brackish lakes to canals and temporary ponds (Semenzato 2007). However, land reclamations, the intensifications of agricultural activities and the reduction of riparian vegetation, caused a decrease of suitable sites and the strong fragmentation of existing populations (Bonato et al. 2007). *E. orbicularis* is also present along the internal wetland of the Venice lagoon (Agapito Ludovici et al. 2012; Ficetola et al. 2013, Liuzzo et al., 2021). Most wetlands of the Venice lagoon are artificially managed for agricultural or recreational activities. This water management involves massive freshwater input into the system to keep marshes flooded during non-natural periods in order to attract targeted bird species for hunting or conservation. This water management has caused the emergence of new artificial wetlands with reduced or non-existent dry periods and caused a general reduction in salinity along the inland margins of the Venice lagoon. Specifically, the salinity gradient could play an important role in determining the distribution of nectonic species and can also prove to be one of the main stressors in these environments, directly affecting the growth of species, their distribution and the diversity of populations (Gebbani 2018). The Valle Averto Oasis, a protected internal wetland area of the Venice lagoon, is an excellent case study to assess which enviromental parameters can have an affect on the habitat suitability of the freshwater turtle populations. Wild populations of the European pond turtle, *E. orbicularis* inhabit this area of mixed natural and abandoned fishing valley patch. Recent studies about the abundance estimation and dispersion activity of the *E. orbicularis* population in this study area have shown one of the most populated areas of the Italian peninsula (Liuzzo et al., 2021). It is therefore important to understand the habitat requirement of the species in the Valle Averto Oasis and to investigate whether the salinity can be consider an influencing factor for the abundance and distribution of *E. orbicularis* in this region. Such knowledge could have value in optimizing conservation efforts by concentrating funding on management areas and improving landscape features that are most important to the species.

The present study aims to provide new data on habitat requirements in a protected internal wetland area of the Venice lagoon, Valle Averto (Southern Lagoon) for a wild freshwater turtle population occupying a system of freshwater wetlands and brackish valleys. Novel recent observations about the eco-ethology of this species have shown a movement pattern of *E. obicularis* towards water bodies with mesohaline condition in this transitional water system (Liuzzo et al. 2022, *submitted*). In consideration of this evidence, the preferential habitat and a possible positive effect of salinity on the distribution of this threatened species was investigated. To test for these hypotheses, the differences among three study sites selected within the wetland habitats of Valle Averto were analysed, and in particular the differences were tested in regards the following aspects: (1) abundance of *E. orbicularis* expressed as CPUE data and a number of micro-habitat descriptors, (2) presence of potential nektonic competitors (such as *Procambarus clarkii* Girard 1852 and *Ameirus melas* Rafinesque 1820) (3) population and individual parameters such as sex ratio, biometry and population structure.

3.2 Materials and Methods

3.2.1 *Sampling sites and methods*

The survey was carried out in a internal wetland area of the Venice lagoon, Valle Averto (Southern Lagoon, Italy). In order to represent the spectrum of the environmental variability and following a salinity gradient based on previous records of *E. orbicularis* in this study area (Masiero 2015; Chiarello 2018; Liuzzo et al. 2021), three types of water bodies were selected: (**1**) T-Cav (TC) (45°21'1.66"N, 12° 8'16.01"E), a canal with an alternate freshwater input flow and dense briars associated with tree cores (freshwater: 0-1‰), (**2**) T-Nit (TN) (45°21'2.33"N, 12°8'16.13"E), a canal with an alternate water flow and a dense reed vegetation associated with *Tamarix* spp., (oligohaline: 1-6‰) (**3**) T-Sal (TS) (45°21'34.30"N, 12° 8'44.65"E) a eutrophic canal without an input water flow colonized by halophytic grasses such as: *Puccinellia* spp. and rushes such as *Juncus* spp. (mesohaline: 6-17‰). Between 10 and 16 sampling stations were selected within each site, making a total of 42 sampling stations. Only the TC canal differed in the number of sampling station as the length of this canal was slighty different from the others Fig 3.1C. The 42 stations were sampled during 3 sampling occasions from May to July 2021 of 2 trapping-days each. Pond turtles were captured through baited floating traps with sardines (n = 42, length = 60 cm, diameter = 30 cm, eye = 16 × 14 cm), located 30 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), width (CW), height (CH) 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).

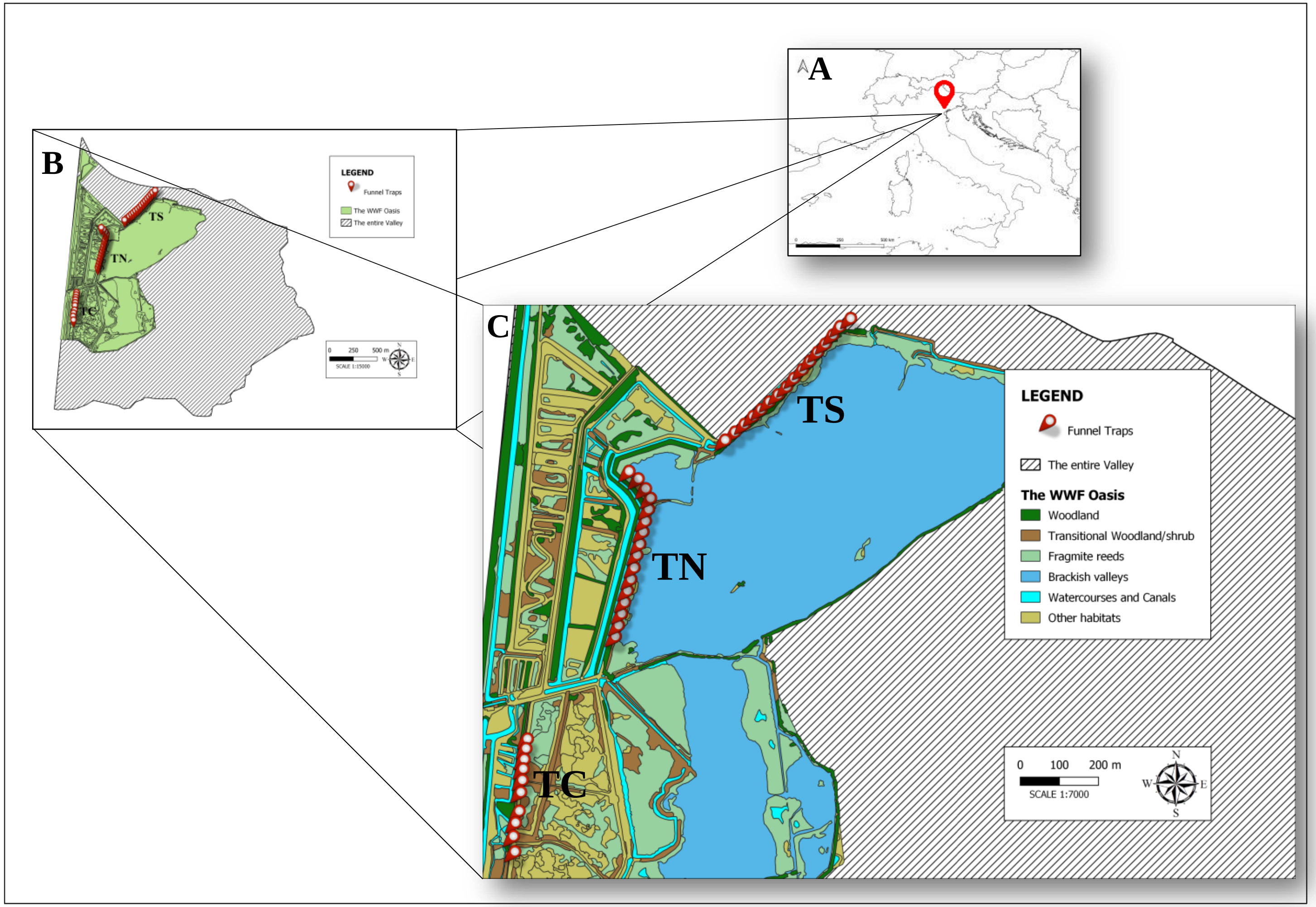


Figure 3.1 (A), (B) Territorial framework of Valle Averto and delimitation of the naturalistic oasis WWF.
(C) Map of land use types according to the European Union classification Corine Land Cover focused in three study areas: T-Cav (TC, freshwater), T-Nit (TN, oligohaline) and T-Sal (TS, mesohaline) (original map from (Buffa et al. 2013).

For each sampling station, 14 environmental variables were recorded (Table 3.1). Basking sites, emergent vegetation, sorrounding trees, grasses and scrubs were extracted for an approximately 10 m side transversal strip of the canals and the remainder variables were visually assessed by the same observer for an area of approximately 5 m radius surrounding the trap. Temperature, salinity and turbidity were measured with a water thermometer (Hanna Instruments Checktemp® 1), refractometer (Giorgio Bormac Mod. 106) and a HI93703C portable turbidimeter (Hanna Instruments, Eibar, Spain) at the same depth (20-40 cm belowthe water surface) and with the same frequency of trapping.

Table 3.1 Variables recorded at each sampling station (cont, continuous variables; ord, ordinal variables)

Variables $_{\textbf{(Type)}}$	**Units**	**Code**
Study site $_{(Ord)}$	Number of study sites	Site
Turbidity $_{(Cont)}$	Formazin Turbidity Units (FTU)	Turbidity
Salinity $_{(Cont)}$	1000 grammi/litro	Salinity
Water temperature $_{(Cont)}$	Celsius degree	Temperature
Amerius melas $_{(Cont)}$	Number of individuals	*A. melas*
Procambarus clarkii $_{(Cont)}$	Number of individuals	*P. clarkii*
Basking sites $_{(Ord)}$	**0**: Absent, **1**: Present	Banks
Surrounding trees $_{(Ord)}$	**0**: 0–25%; **1**: 25–50%; **2**: 50–75%; **3**: 75–100%	Tree%
Surrounding grass $_{(Ord)}$	**0**: 0–25%; **1**: 25–50%; **2**: 50–75%; **3**: 75–100%	Gras%
Surrounding scrub $_{(Ord)}$	**0**: 0–25%; **1**: 25–50%; **2**: 50–75%; **3**: 75–100%	Scrub%
Emergent vegetation $_{(Ord)}$	**0**: 0–25%; **1**: 25–50%; **2**: 50–75%; **3**: 75–100%	Em. Veg%
Dominant shoreline inclination $_{(Ord)}$	**0**: 0–25%; **1**: 25–50%; **2**: 50–75%; **3**: 75–100%	Inclination %
Maximum depth $_{(Ord)}$	**0**: 0–25%; **1**: 25–50%; **2**: 50–75%; **3**: 75–100%	Depth%
Sun exposure $_{(Ord)}$	**0**: 0–25%; **1**: 25–50%; **2**: 50–75%; **3**: 75–100%	Sun Expo. %

3.2.2 *Data Analysis*

Differences in the *Emys orbicularis* abundance and environmental parameters were tested among the study sites (TC, TN, TS). *E. orbicularis* abundance was calculated as Catch per unit effort (CPUE). The CPUE was calculated as the average capture for baited funnel trap with constant effort about 24 hours for each occasion (N_{mean}/trap). The authors are aware that this estimate of *E. orbicularis* abundance could be inaccurate since it has not been validated using capture-mark-recapture methods. However, it could be considered a reliable estimate of relative turtles' abundance among wetlands (Chelazzi et al. 2007; Chessman 2021).

Data were not normally distributed, then the analyses to test for the differences among the study sites were performed with the non-parametric Kruskal-Wallis test. In addition, a post hoc Dunn's test was carried out to compare the variables among the study areas. When data met assumptions of normality and homogeneity of variance the ANOVA test was applied. In addition, to compare the variables among the study areas, a post hoc Tukey test was performed.

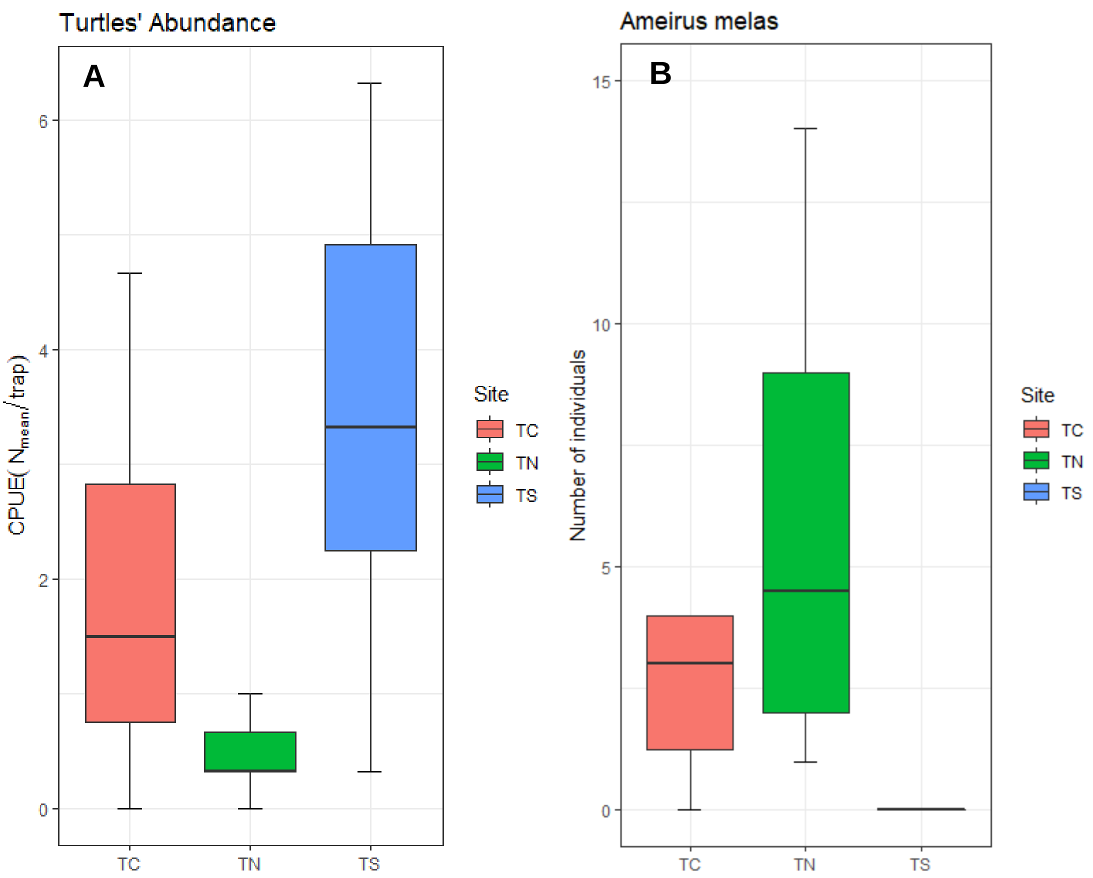
Population structure was analysed using a graphical approach by dividing SCL data in size classes of 10 mm (Ottonello et al. 2017; Liuzzo et al. 2021). The sex ratio was calculated as the proportion of males in a sample (males/(males + females)), and not a ratio *sensu stricto* (males/females)). A χ^2 test was used to compare capture differences per period and both sexes. Sexual differences in size and body mass were firstly checked for normality with Shapiro-Wilk normality test and then analysed using the Wilcoxon test. Differences in the captures were also investigated among the study sites. The capture data did not meet the parametric assumptions then the Kruskal-Wallis test was applied. In addition, to compare the capture among the study areas, a post hoc Dunn-test was performed. Body size (SCL) differences among the three micro-habitat were tested for both sexes using a one-way analysis of variance (ANOVA). This data meet all anova assumptions. All statistical analysis was made using R v. 4.0.2 (R Core Team 2020).

3.3 Results

3.3.1 *Differences between turtle distribution and habitat features*

The number of individuals and abundance differed among study sites (Fig. 3.2A, Table 3.2). The highest *E. orbicularis* abundances were observed in TS with more than 69% of the total captures (Fig. 3.2A). Significant differences in salinity, the presence of NIS-Non-Indigenous Species (*A. melas* and *P. clarkii*), turbidity, and emergent vegetation were observed among study sites (Fig. 3.2, Table 3.2).

Salinity, the presence of NIS and turbidity seem to be the most important variable in explaining *E. orbicularis* abundance. These environmental variables in the Dunn's test showed a marked significant difference among the study sites (Table 3.2). The levels of salinity and turbidity were significantly higher in TS than in TC and TN. The emergent vegetation was significantly higher in TS and TC than in TN. In contrast, the presence of allochthonous species was significantly more frequent in TC and TN than in TS (where no allochthonous species has been captured) (Fig. 3.2B-C, Fig. 3.3).



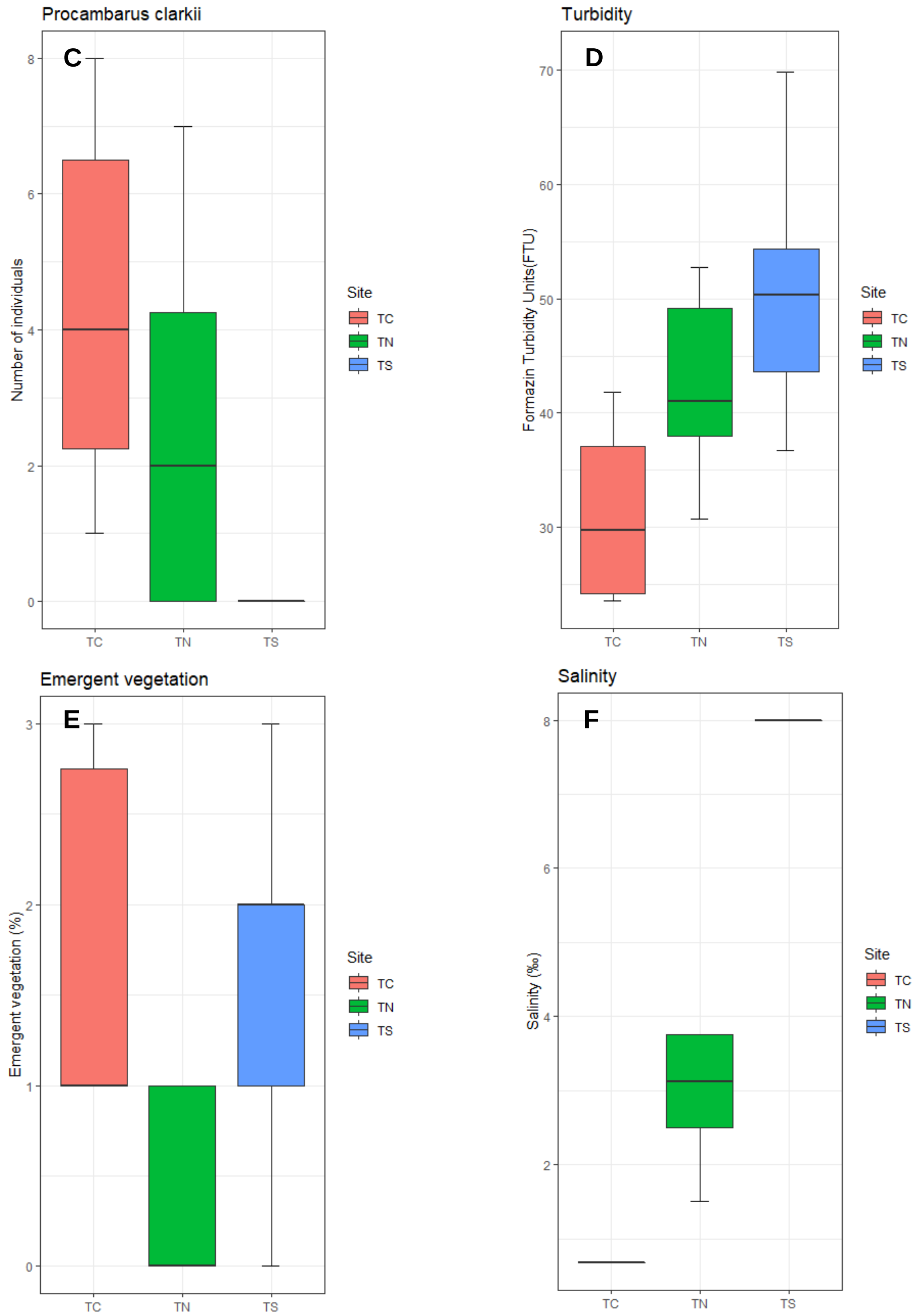


Figure 3.2 Differences in turtle's abundance (A), presence of *Ameirus melas* (B) and *Procambarus clarkii* (C), turbidity (D), emergent vegetation (E) and salinity (F) among the study areas: T-Cav (TC, freshwater), T-Nit (TN, oligohaline) and T-Sal (TS, mesohaline).

Differences (Variables x Study sites)	**Statistics test**
CPUE	K = 22.44, $p < 0.001$ Dunn'test, $p < 0.001$
Turtles' captures	K = 22.38, $p < 0.001$ Dunn'test, $p < 0.001$
Ameirus melas	K = 28.00, $p < 0.001$ Dunn'test, $p < 0.001$
Procambarus clarkii	K = 23.61, $p < 0.001$ Dunn'test, $p < 0.001$
Turbidity	$F_{2,39} = 16.56$, $p < 0.001$ Tukey HSD, $p < 0.001$
Emergent vegetation	K = 17.51, $p < 0.01$ Dunn'test, $p < 0.01$
Salinity	K = 39.05, $p < 0.001$ Dunn'test, $p < 0.001$

Table 3.2 Statistics tests showed significant differences in the population and environmental parameters among the study sites: T-Cav (TC, freshwater), T-Nit (TN, oligohaline) and T-Sal (TS, mesohaline).

3.3.2 *Population and individual parameters*

A total of 255 captures (88 in May, 83 in June, and 84 in July) were made in 2021; among these 14 were already marked in previous studies (Liuzzo et al. 2021). Out of the examined individuals, 152 adult males, 72 adult females, 19 juveniles and 12 unsexed turtles were collected with an overall capture sex ratio [MM/(MM + FF)] of 0.7:0.5. The capture sex ratio varied significantly among periods ($\chi^2 = 21.471$, $p < 0.01$), with the lowest values being in July (0.5:0.5) with respect to June (0.7:0.5) and May (0.8:0.5). The number of turtles trapped per period differed significantly both for females ($\chi^2 = 11.901$, $p < 0.01$) and for males ($\chi^2 = 13.681$, $p < 0.01$), with similar values in May and June; the lowest number of captures for females were in May and the highest in July, and for males – in July and May, respectively (Fig. 3.4).

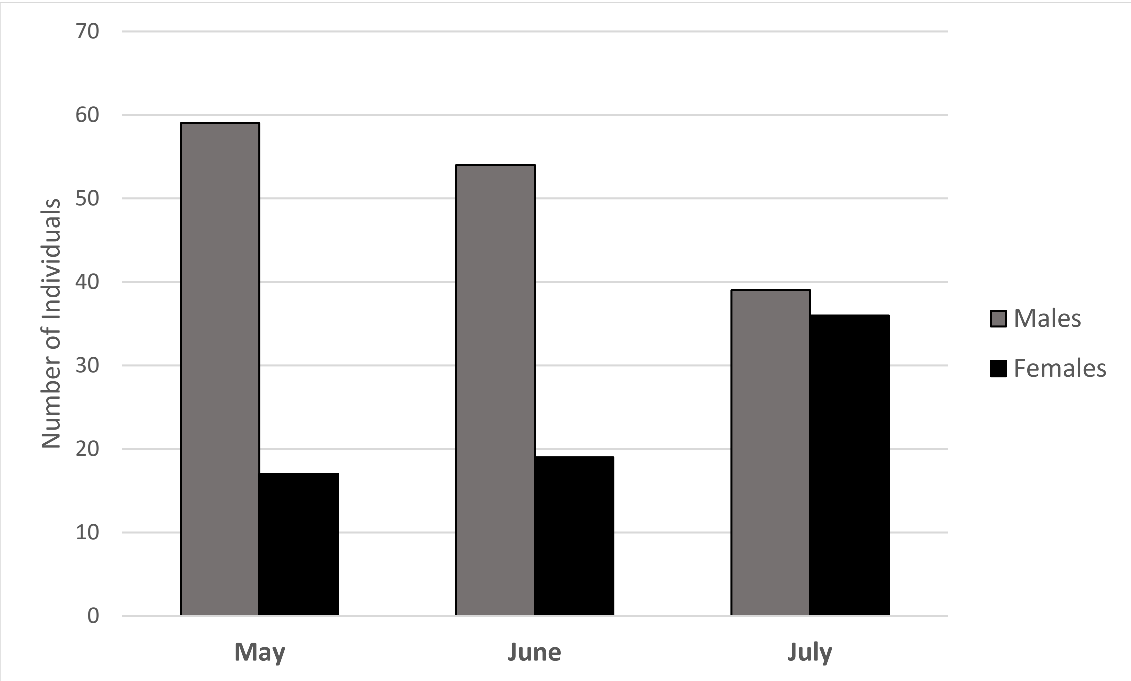


Figure 3.4 Number of adult males and females *E. orbicularis* captured per month at the Valle Averto Oasis.

Significant differences were detected in the captures among the study areas (K=11.679, $p < 0.01$) which were significantly higher in the mesohaline water (TS) than in the freshwater canal (TN) (Dunn' test, $p < 0.01$). The sampling was highly shifted toward adults reflecting the low capture rate of juveniles, which represented only the ~8% of the population sampled. The population was characterized by a dominance of turtles with SCL between 120 and 139.9 mm (~55%) (Fig. 3.5). The most represented range class for males was 120-129.9 mm (~45%), whereas for females it was 140-149.9 mm (~35%), and for juveniles: 90-109.9 mm (~51%). Sexual size dimorphism was evident, females were heavier (W= 10772, $p < 0.001$) and bigger than males, both in terms of carapace length (W= 10039, $p < 0.001$), width (W= 9537.5, $p < 0.001$), and height (W= 10868, $p < 0.001$). Males and females significantly differed for all biometrical data, even if no interaction between three micro-habitat and body size has been recorded (*Male*: $F_{2,133} = 1.058$, $p = 0.350$, *female*: $F_{2,67} = 1.630$, $p = 0.204$) (Table 3.4). The presence of oviductal eggs was detected in one adult female of the canal TN in June.

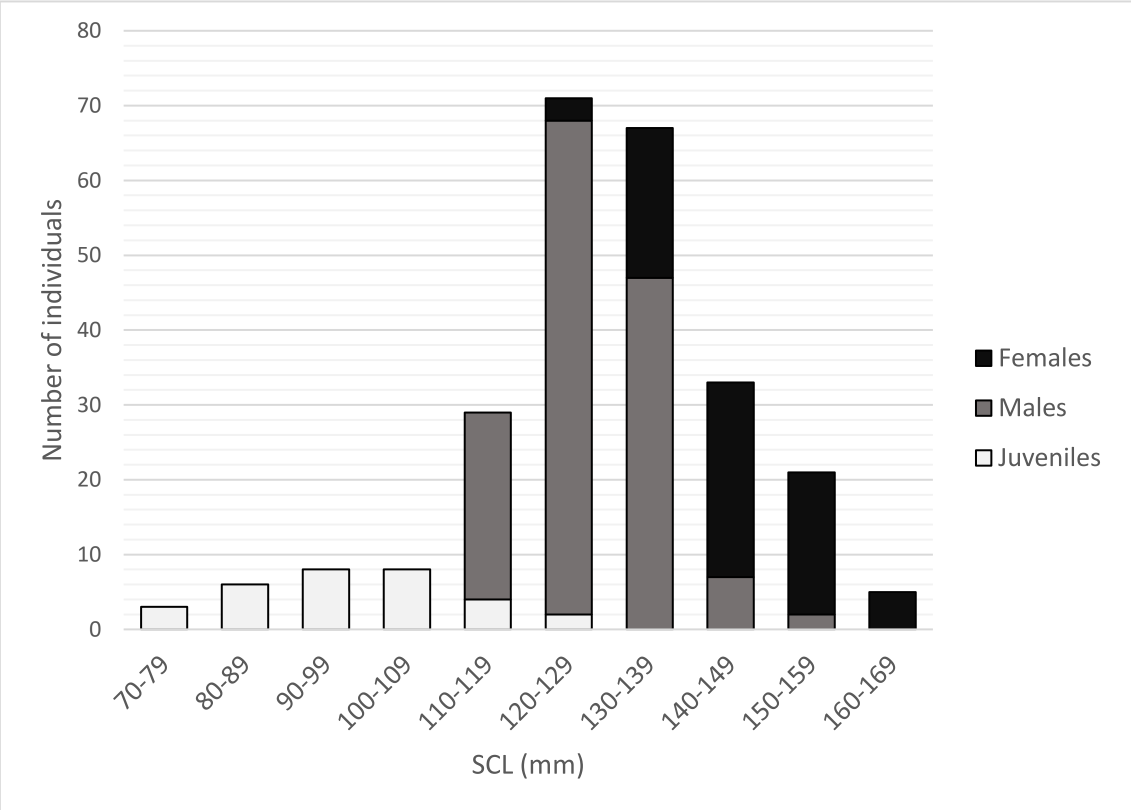


Figure 3.5 Straight carapace lengths (mm) of *E. orbicularis* captured during the study at the Valle Averto: unsexed juveniles, males, and females.

Table 3.4 Mean values of major biometric measurements (in mm and g) for *E. orbicularis* among the study sites (TC, TN, TS). SCL = straight carapace length; CW = carapace width; CH = carapace height; BM = body mass; SD = Standard deviation [152 ♂ adult, 72 ♀ adult and 19 Juveniles (J)].

Biometric measurement	***sex***	**TC**			**TN**			**TS**		
		N	***Mean***	***SD***	***N***	***Mean***	***SD***	***N***	***Mean***	***SD***
SCL	♀	21	141.29	8.42	9	144.50	13.37	42	146.53	8.58
	♂	29	125.45	6.97	12	126.49	9.70	111	127.26	9.22
	J	4	92.14	20.56	3	100.53	13.60	12	98.92	12.69
CW	♀	21	106.20	6.11	9	109.96	10.68	42	108.90	6.94
	♂	29	96.13	8.76	12	97.34	6.53	111	97.50	7.21
	J	4	75.86	15.34	3	80.19	12.28	12	79.38	8.31
CH	♀	21	59.29	3.60	9	59.84	4.70	42	61.05	4.55
	♂	29	46.66	3.92	12	45.19	3.06	111	45.50	4.04
	J	4	36.90	12.57	3	39.09	7.18	12	38.12	5.25
BM	♀	21	503.62	74.44	9	544.67	128.51	42	549.21	89.09
	♂	29	304.90	56.69	12	318.25	53.83	111	313.69	62.84
	J	4	152.00	122.91	3	179.00	80.58	12	176.58	57.97

3.4 Discussion

3.4.1 *Differences between turtle distribution and habitat features*

Our results show that the attributes of both water bodies and the habitat surrounding are important for the distribution of *E. orbicularis*. Interestingly, the emergent vegetation, salinity and turbidity are important habitats features for the presence and abundance of *E. orbicularis* in the inland margins of the Venice lagoon. The pronounced occurrence of *E. orbicularis* for sites with dense emergent vegetation might indicate a greater thermoregulation efficiency since individuals are directly exposed to the sunlight on these microhabitats. Specifically, the *Phragmition* plant community was also recognized both as a suitable brumation site and as a hibernaculum in this study area (Liuzzo et al. 2022 *submitted*). Moreover, the emergent vegetation may be a useful micro-habitat for hunting behaviour in terrestrial habitats. *E. orbicularis* often moves to land for different purposes, most probably including feeding activity (Ficetola and De Bernardi 2006), which in part could explain the preference for densely vegetated margins that would confer protection to turtles during movements between water and land.

As predicted in the previous studies (Liuzzo et al. 2022 *submitted*), the results show that salinity, within the range encountered in the Valle Averto oasis, positively affects the distribution of *E. orbicularis*. High salinity increased the capture probability of *E. orbicularis* in all sampling occasion. Kami et al. (2006) mentioned that *E. orbicularis* adult individuals are found preferentially in the littoral zone of marshes along the coast of the Caspian Sea. The presence of the European pond turtle was also detected in the threshold water salinity of about 17 ‰ in this study area (Liuzzo et al. 2022 *submitted*). Although more sites with high salinity would be necessary to assess clear salinity thresholds in the study region, the results of Liuzzo et al. (2022 *submitted*) and this research seem to show that *E. orbicularis* has an intermediate salinity tolerance between truly freshwater forms and the highly specialized estuarine terrapin (*Malaclemys* spp.). A similar salinity tolerance of Emydid turtles (*Pseudemys nelsoni* Car 1938 and *Trachemys decussata* (Gray 1831)) was also documented in the estuarine and insular environment (Dunson 1986). 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; Liuzzo et al. 2022 *submitted*). However, it remains unclear which characteristics freshwater turtles might prefer or even recognize in a brackish environment.

Body size also influences an individual turtles' salinity tolerance. For instance, net water loss is inversely proportional to body size, providing larger turtles with increased tolerance (Dunson 1986). The larger body sizes than their conspecifics from freshwater locales in addition to providing a selective advantage in salinity tolerance (Eisemberg et al. 2015) reduce the risk of predation and increase the capacity of individuals to migrate (Moll and Moll 2004). Nonetheless, significant relationship was not checked between the turtle body size and canals for both sexes in this study area. This phenomenon might be linked to a potential dilutive effect. During the activity period *E. orbicularis* individuals tend to move from freshwater towards mesohaline habitat in this nearby water bodies (Fig. 2.3A) (Liuzzo et al. 2022 *submitted*). Therefore, further research should address spatial distribution at a larger scale with continental and costal study sites to assess the magnitude of this possible difference.

Such as the emergent vegetation and salinity, even turbidity might be positively related to the presence and abundance of *E. orbicularis*. These environmental variables may be associated to minimization of predatory risks as important factors in general for the turtle's habitat selection (Pluto and Bellis 1986). The combination of the vegetation cover and turbidity may be correlated with anti-predatory requirements by turtles (Manzini et al. 2015). Nonetheless, these variables could not be linked exclusively to predation avoidance, and thus need further experimental confirmation (Zuffi 2000; Ficetola et al. 2004).

3.4.2 *Presence of NIS*

Ameirus melas and *Procambarus clarkii* were abundant in this study area. Both species of Nearctic origin were introduced in Europe either for commercial purposes or angling (Gherardi 2006; Sicuro et al. 2016). Natural spread and indiscriminate introductions have increased rapidly the distribution of *A. melas* and *P. clarkii* within the Iberian Peninsula, southern France and Italy (Garcia-de-Lomas et al. 2009; Holdich et al. 2009). Considering the current increase distribution of the *A. melas* and *P. clarkii* population and their ability to feed voraciously on a variety of prey from small aquatic macroinvertebrates to fish (Leunda et al. 2008; Sicuro et al. 2016), the impacts on estuarine ecosystem function and services might occur. The presence and abundance of these NIS are limited to wetlands characterized by oligohaline water bodies (Garcia-de-Lomas et al. 2009; Meineri et al. 2014). In fact, a negative effect was observed of the presence of NIS on *E. orbicularis* in the inland canals of the Venice lagoon. The total of captures of *P. clarkii* and *A. melas* individuals were obtained only in the freshwater and oligohaline canals. In contrast, the peak of *E. orbicularis* captures was observed in the mesohaline canal on all sampling occasions (69%). At the local scale, *E. orbicularis* tends to occupy sites where the invasive species (*P. clarkii* and *A. melas*) is less abundant. This pattern of distribution might be caused by "priority effects" (i.e. the repercussion that a particular species or lineages can have on the establishment of later-arriving immigrants in the community) (Incagnone et al. 2015). Biological interactions, e.g. predation, competition, are considered strong relations contributing to inhibit the establishment of new immigrants (e.g. Eitam et al. 2004; Uronen et al. 2007; Yawata et al. 2014); these interactions regulate the composition of a community and enhance its stability over time (Incagnone et al. 2015). The combination of high *E. orbicularis* abundance (Liuzzo et al. 2021), variation of the salinity level and the drastic diet shift toward a high intake of red swamp crayfish reported for the European pond turtle (Ottonello et al. 2017) might have resulted in marked spatial segregation between *E. orbicularis* and NIS in the internal water bodies of the Venice lagoon, at least regarding the scales analysed in this study.

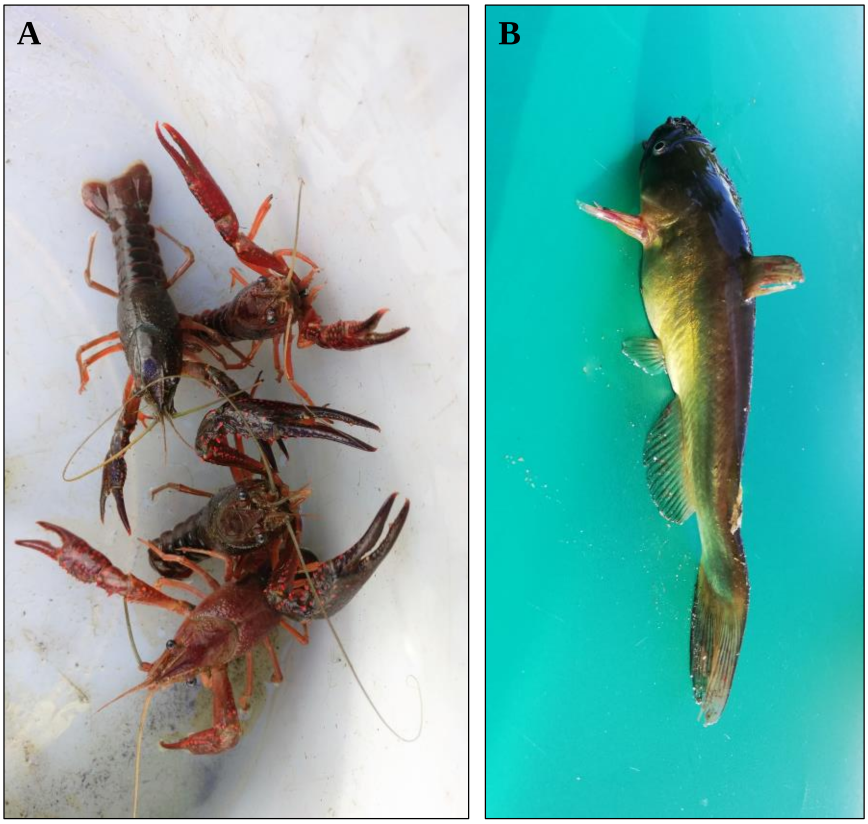


Figure 3.3 Left to right. Individuals of *Procambarus clarkii* (A) and *Ameirus melas* (B) captured with baited funnel traps among canals of the Valle Averto Oasis.

3.4.3 *Population and individual parameters*

The population structure was characterized by a size distribution that was markedly shifted towards adult age, with a bimodal distribution due to difference in size related to the sex. This trend was also observed in an Algerian *E. orbicularis* population (Fediras et al. 2017), in the congeneric *E. trinacris* in a protected area of southwestern Sicily (Ottonello et al. 2017) and in this same protected internal wetland of Venice lagoon (Liuzzo et al. 2021). The low number of juveniles was not basically different from that found in other studies (Vamberger and Kos 2011; Fediras et al. 2017; Scali et al. 2020) and could be associated with sampling bias due to lower detectability and different habitat use (Lebboroni and Chelazzi 1998; Zuffi 2000), low recruitment rates, or a combination of both (Keller et al. 1998). The overall capture sex ratio of captured individuals was shifted slightly in favour of males, but it also was detected significant differences with an opposite trend among the periods: males capture was higher in May and June (sex ratio 0.8 and 0.7 respectively), whereas the females and males capture were rather balanced in July (0.5) (Fig. 3.4). This result is probably related to the different activities of males and females (Girondot and Pieau 1993). In *E. orbicularis*, the reproductive activity starts with spring emergence, with a peak of courtship behaviour and mating between the end of March and May (e.g. Mitrus and Zemanek 2004; Servan and Roy 2004). During this period the males are very active, moving along the wetland looking for reproductive females (Lebboroni and Chelazzi 1991). Vice versa, females tend to return in water bodies after the terrestrial nesting activity (Zuffi et al. 2015; Liuzzo et al. 2021).

3.5 Management recommendations and Conclusions

The present study highlights a possible link between the abundance of *E. orbicularis* and water and terrestrial habitat in the Valle Averto Oasis (a protected wetland area of the South Venice lagoon). The most important result suggest (a) that increased salinity to a mesohaline condition facilitates the occurrence and abundance of *E. orbicularis* (b) that decreased salinity to freshwater and oligohaline condition promote the occurrence, establishment and expansion of NIS (specifically *Ameirus melas* and *Procambarus clarkii*). In light of these results, a possible solution for managers would be to limit freshwater inputs into the wetland systems and, if possible, to restore the natural processes involving summer drying and winter flooding in order to increase the overall salinity and thus reduce the reproductive success of exotic species. It is conceivable that current hydraulic management in Valle Averto tends to suppress natural dynamics and, consequently, contribute to the establishment of NIS. Therefore, research programs are needed for modelling studies quantifying within the different wetland types with respect to different flooding regimes and their effect to wildlife populations.

GENERAL CONCLUSION

The Valle Averto Oasis is recognised as a relict area in good natural conditions with a remarkable diversity among the wetlands of the upper Adriatic, yet the surrounding areas are mainly characterized by permanent baiting and agricultural fields. Agriculture activities are the world's largest user of freshwater and the main factor in degradation of surface and groundwater resources (Baigún et al. 2008; Ficheux et al. 2014). Changes in land use by these practices degrade habitat quality and influence the hydrological cycle of wetlands physically and chemically (Asselen et al. 2013; Sica et al. 2016). Thus, landscape alteration and fragmentation changes in the species composition, abundance and distribution, resulting in changes in the community structure (Rayfield et al. 2009; Hagen et al. 2012). In the last years, The European pond turtle (*Emys orbicularis*) and wetlands patches has shown important range reduction in Europe with increasing fragmentation of populations and the extinction of several relict populations (Kracauer Hartig et al. 1997; Servan 1999; Pereira et al. 2018). Moreover, this species is considered by conservation biologists as a good species model to apply management actions: both for its biological features and its ecological requirements (Ottonello 2017; Liuzzo et al. 2021). Herein a pioneer study on *E. orbicularis* density, behavioural ecology and spatial distribution was reported in a protected internal wetland area of the Venice lagoon, Valle Averto (Southern Lagoon) where strong variations in water flow with possible salinity gradient occur.

In this thesis, the following aims were investigated in order to minimize the anthropogenic disturbance and/or assist the wildlife managers in the management plans for the long-term survival of *E. orbicularis* habitat and populations: (1) The conservation status of the European pond turtle along the inland margins of the Venice lagoon (2) Dispersion activities, habitat use and main behaviours in this transitional water system (3) Habitat selection and whether salinity can be considered a robust driving parameter for the spatial distribution of this threatened species.

The conservation status of the European pond turtle along the inland margins of the Venice lagoon

A population of the European pond turtle was studied employing capture, marking and recapture (CMR) techniques to determine population density and abundance in natural and protected areas within the Valle Averto Oasis in southern Venice lagoon, Italy. The results indicated strong sexual size dimorphism, with males smaller than females. The abundance estimation of the *Emys orbicularis* population in this study site show one of the most populated areas of the Italian peninsula (~ 250 ind./ha). The studied populations were characterized by similar densities to those living in Anatolia and in the Black Sea region or those of *Emys trinacris* of western Sicily (about 240 turtles/ha; Bayrakci and Ayaz 2014; Ottonello 2017). The high presence of *E. orbicularis* is historically known in the region of Venice mainly on coastal portions, including lagoon, delta areas and agricultural lands (Bonato et al. 2007). Ninni (1888) reports that more than 200,000 turtles were caught in five months alone in the region of Venice, and many turtles from there entered via the trade Central Europe (von Fischer 1884; Dahms 1912). Nowadays, this species has often been a victim of the operations of clearing the anthropized Venetian areas, drainage of the water basins, modification of banks, and possible competition with the introduced Red-eared slider turtle which put the autochthonous populations at risk (Bonato et al. 2007; Novarini and Bano 2019). Moreover, few captures of the *E. orbicularis* juveniles were observed in the Valle Averto oasis. Considering the good status of the marsh environment, this could be related to the lack of suitable nesting sites and high nest predation rate. The high number of predated clutches (~23) collected along the shore of the canals in only one year is considered an uncommon event in some wetland areas (Zuffi 2000). In term of pollution, despite of the eggshell efficiency to prevent metals transfer from the environment to the embryo, this barrier is not always sufficient to completely inhibit metals transfer to the hatchlings, which result in their bioaccumulation in tissues. This transfer of metals, especially for the nonessential ones, can be the feasible cause of the genotoxic damage for hatchlings (Frossard et al. 2021). The preliminary analysis of the metal contamination in the eggshell of the European pond turtle showed the presence of essential metals, such as Fe, Mn and Cu. These metals are important to promote standard embryo growth, enabling normal functioning of both cellular and structural metabolisms as well as precise operation of several vital proteins (Eisler 1988; Pappas et al. 2006). However, the iron detected at elevated concentrations can be toxic (e.g., present research; du Preez et al. 2018) and can affect embryo development, survival, and hatching. Further investigation will be needed to explore the relationships with the habitat using multiple approaches (es. based on the metals analysis in superficial sediments, plants, and water samples).

Valle Averto with the high densities' values recorded might have a key role in the management and conservation of this threatened species. The conservation status in the areas occupied nowadays by the fishing valleys, completely limited by embankments along the border of the largest lagoon of the Mediterranean is virtually unknown. The fishing valleys have become more complex systems, not only made of aquaculture but also agro-production, agrotourism and eco-environmental conservation. This territorial framework might be a great resource for the European pond turtles' populations. Further analyses are in fact needed to explore the causes that may influence, the density, the population structure and the possibility of the presence of population dynamics between different inland water bodies of the Venice lagoon using models for open populations and multi-annual monitoring programs.

Dispersion activities, habitat use and main behaviours in this transitional water system

Quantitative radiotracking studies analysing a relevant number of individuals are required to increase the set of information on the behaviour and habitat preference for the surrogate species *Emys orbicularis*. To achieve this goal, some behavioural and dispersal events, and home range size were monitored between the period of activity and lethargy in a wild population of *E. orbicularis* inhabiting the Valle Averto Oasis. The results suggested that the differences in the movements, habitat selection, basking and brumation behaviours were affected by the period. Movements were higher in the activity period (a late reproductive phase from the half of June to the end of July) than the lethargy period (a post-reproductive period from the early of October to the end of November), but they were not influenced by sex and size. Basking was performed by the turtles out of the canal, among reeds, *Tamarix gallica* and *Rubus ulmifolius* of the banks, individually or in groups. The frequency of Basking and Movement behaviours was significantly higher during the activity period whereas the brumation behaviour was significantly more frequent in the lethargy period. The presence of the European pond turtle in the transitional woodland/shrubs and brackish water valley areas was significantly higher in the activity period than in the lethargy period. During the latter one period, pond turtles were observed to brumate gregariously in a small area for brumation, usually in shallow water. In contrast, during the activity period all individuals have changed water bodies. Part of those movements have occurred towards aquatic habitat with higher salinities 1-17‰ (mean: 10.64‰). The characterization of the most differences in the use of habitat, movements and turtles' behaviours between period of activity and lethargy will be crucial to increase the understanding of what ecological requirements for the European pond turtle should be included in a management plan for the habitat conservation. There often is a lack of knowledge of where, when and why species move. The field of movement ecology provide the knowledge needed to incorporate movements of species into management planning. This knowledge can also be used to develop management strategies that are flexible in time and space and may improve the effectiveness of management actions. Therefore, wildlife management and conservation may benefit by strengthening the link with movement ecology in minimizing the anthropogenic disturbance and/or assisting wildlife managers in minimizing the potential threats to endangered species.

Habitat selection and whether salinity can be considered a robust driving parameter for the spatial distribution of this threatened species

According to the results presented in Chapter 3, a possible interaction between the abundance of *E. orbicularis* and water and terrestrial habitat was detected in the Valle Averto Oasis. Specifically, a positive effect of salinity, emergent vegetation and turbidity on the occurrence and abundance of *E. orbicularis* was observed. In contrast, a negative effect of the salinity and presence of *E. orbicularis* on Non-Indigenous-Species (NIS) (specifically *Ameirus melas* and *Procambarus clarkii*) was detected.

In consideration of these results, *E. orbicularis* seems to have an intermediate salinity tolerance between truly freshwater forms and the highly specialized estuarine terrapin (*Malaclemys* spp). These observations suggest that the salinity gradient could be a major constraint in the dynamics of *E. orbicularis* populations. The capacity to distribute spatially into specific mesohaline water bodies could be a highly adaptive phenomenon in a transitional water system as the lagoon. For instance, similar adaptations of Emydid turtles (*Pseudemys nelsoni* and *Trachemys decussata*) to salinity tolerance was also documented in the estuarine and insular environment of the Nearctic region (Dunson and Seidel 1986).

While we are convinced that the potential relationship with salinity, emergent vegetation, turbidity and the presence of invasive species are the most important factors explaining our results, we cannot exclude other confounding factors that may play an important role. For instance, the water management has caused the emergence of new artificial wetlands with reduced or non-existent dry periods and caused a general reduction in the habitat types within the Valle Averto Oasis. Despite local anthropogenic modifications of the canal network with the installation of locks, how these changes alter freshwater fauna is complex, poorly understood, and, for the European pond turtle, virtually unknown. This lack of fundamental ecological knowledge produces an unobjective approach to conservation planning. Research programs based on quantitative data and statistical models quantifying the impact of the canals flooding regulation in the internal wetlands of the Venice lagoon are required to provide a more accurate management indication and their effect on wildlife populations.

RECOMMENDATIONS AND GUIDELINES FOR THE MANAGEMENT OF VALLE AVERTO

From the results of this thesis, the complexity in understanding and evaluating how the anthropogenic modifications of water flow alter freshwater fauna appears clear. The analyses of this thesis suggest that the management actions of the European pond turlte in this transitional water system habitat can be discerned in 3 main objectives:

1. Monitoring and eradication of the alien species, especially concerning *Procambarus clarkii* and *Ameirus melas*
2. Protecting and controlling the reed vegetation with the association of *Puccinellio festuciformis-Phragmitetum australis*
3. Regulation of freshwater input to restore the natural processes involving summer drying and winter flooding

A negative effect was detected of the presence of these alien species on *E. orbicularis* occurrence in the canal network of the Valle Averto Oasis. Moreover, the water bodies with mesohaline and oligohaline conditions would seem more suitable than freshwater canals. A possible solution to control and, if possible, to eradicate these alien species would be to limit freshwater inputs into the wetland systems and to use an innovative approach to improve the catchability for these target species. A successful strategy might be developing both aspects simultaneously: (1) restore the hydraulic current in line with the natural process of flooding in order to increase the overall salinity in summer and thus reduce the reproductive success of exotic species (Garcia-de-Lomas et al. 2009; Meineri et al. 2014) (2) combine traps and netting methods for controlling and understanding the structure and dynamics of the invasive populations (Garciá-de-Lomas et al. 2020).

The control of emergent vegetation (es. *Phragmite*s) involves cutting at the terrestrial shore of the reedbed in the Valle Averto Oasis. To assist wildlife managers in minimizing threats to *E. orbicularis*, cutting should be implemented in the beginning of March (before the mating activity) and at the middle of October (after the end of the breeding season). The first cutting improves the conditions for nesting and oviposition while the latter avoid disturbing the brumation and hibernation sites.

Thanks to this study, knowledge of the habitats and management of the inland wetlands of the Venice lagoon has been increased. The conservation status of the study sites directly targeted by the project was improved. The aquatic invasive species remains an unsolved problem today in this study site. However, these finding will be the baseline to design a long-term strategic plan for the eradication.

Emys spp. in the naturalistic WWF reserves

The presence of *Emys* spp. was confirmed in 36 out of 75 WWF Oasis within the "Natura 2000" network (~48%)**.** Specifically, *E. orbicularis* occurs in the Continental (10 out of 29 reserves, ~34.5%) and Mediterranean biogeographical regions (24 out of 41, ~58.5%) of the Italian peninsula. In contrast, *E. trinacris* occupies only the Mediterranean region in two WWF Oasis (Agapito Ludovici et al. 2012). Despite the different locations of the species, conservation and management actions can be an instrument to better protect species and habitat types under similar natural conditions across borders. For instance, the Valle Averto Oasis and the "lake Preola and Gorghi tondi" natural reserve (SW Sicily) show a sort of parallelism between the pond turtle taxa (*Emys orbicularis* and *Emys trinacris*) and the anthropogenic impact that insists on sites:

1. Contaminants impacts from agriculture and urban sources
2. The presence of the invasive species (es. *Procambarus clarkii, Cyprinus carpio* (L., 1758), *Gambusia holbrookii* Girard, 1859)

Concentrations of trace elements (Cd, Pb, As, V, Cr, Ni, Cu and Zn) were determined in superficial sediments and in muscle and hepatopancreas tissues of the red swamp crayfish *P. clarkii* from the "Lake Preola and Gorghi Tondi" Natural Reserve (Bellante et al. 2015). In contrast, preliminary data of contaminants were detected in the eggshells of the European pond turtle from the terrestrial habitat of the Valle Averto Oasis. Despite the sentinel species used for trace metals contamination being different, these data are needed in order to quantify the human impact of agricultural and urban activities on the ecosystem health of both natural reserves. The management actions could provide long-term protection to reduce the input of pollutants into water bodies. The production of buffer zones might be an advantageous management option that could offset the devastating impact of water pollution. In addition to its significance as a biofilter for preventing nutrients and pollutants from entering lakes or canals from the agricultural and urban area, the establishment of a buffer zone would enable the restoration and connectivity of natural potential habitats, contributing to overall biodiversity. For instance, a framework project that is recently concluded (end of November 2021) for the construction of a buffer zone included the establishment of a grass belt, and a belt of high and low vegetation (trees and shrubs) (Radosavljević 2021). However, the increase in shrub and tree cover might have consequences on the composition of animal communities and on population dynamics that will have to be evaluated over time.

The structure, abundance and dynamics of pond turtle populations can be also influenced by the occurrence of invasive species. A very clear difference was found between sites with Non-Indigenous-Species (NIS) and a site NIS-free both for "Lake Preola and Gorghi Tondi" and Valle Averto nature reserve. The presence of two alien fishes, *Gambusia holbrooki* and *Cyprinus carpio* seems to have in fact a true impact on the aquatic invertebrate community of Gorghi Tondi Medio-Alto system. Specifically, a significant difference was found between a site with allochthonous fishes and a site fish-free, with a more abundant and wide diet spectrum in the last one (Ottonello et al. 2016a). Also the invasive alien red swamp crayfish could have a high impact on wetlands ecosystems, such as decrease and change in macrophyte and macro-invertebrate communities and water quality (Rodríguez et al. 2003; Rodríguez-Pérez et al. 2016). In both nature reserves very few amphibians were found, probably due to the expansion of the *P. clarkii* (D'Angelo S; Liuzzo M pers. obs.). In addition, a negative effect of the occurrence of *E. orbicularis* on Non-Indigenous-Species (NIS) (specifically *A. melas* and *P. clarkii*) was detected in the Valle Averto Oasis. It is clear that these reserves are an example of multiple stressors: the concurrent impact of immission of non-native species and chemical and biochemical pollutans should be monitored with long-term studies. Simultaneous actions must be taken to avoid the spread of NIS in other water bodies and to study the direct and indirect impact of *P. clarkii*. Although species occur in different biogeographical regions, the needs of the Sicilian Pond turtle parallel the basic ecological needs of the European pond turtle (Zuffi 2000). Therefore, raising awareness about the threat from multiple stressors should promote experimental and observational programmes specifically addressing consequences of stressor interactions on wetland communities.

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APPENDIX A

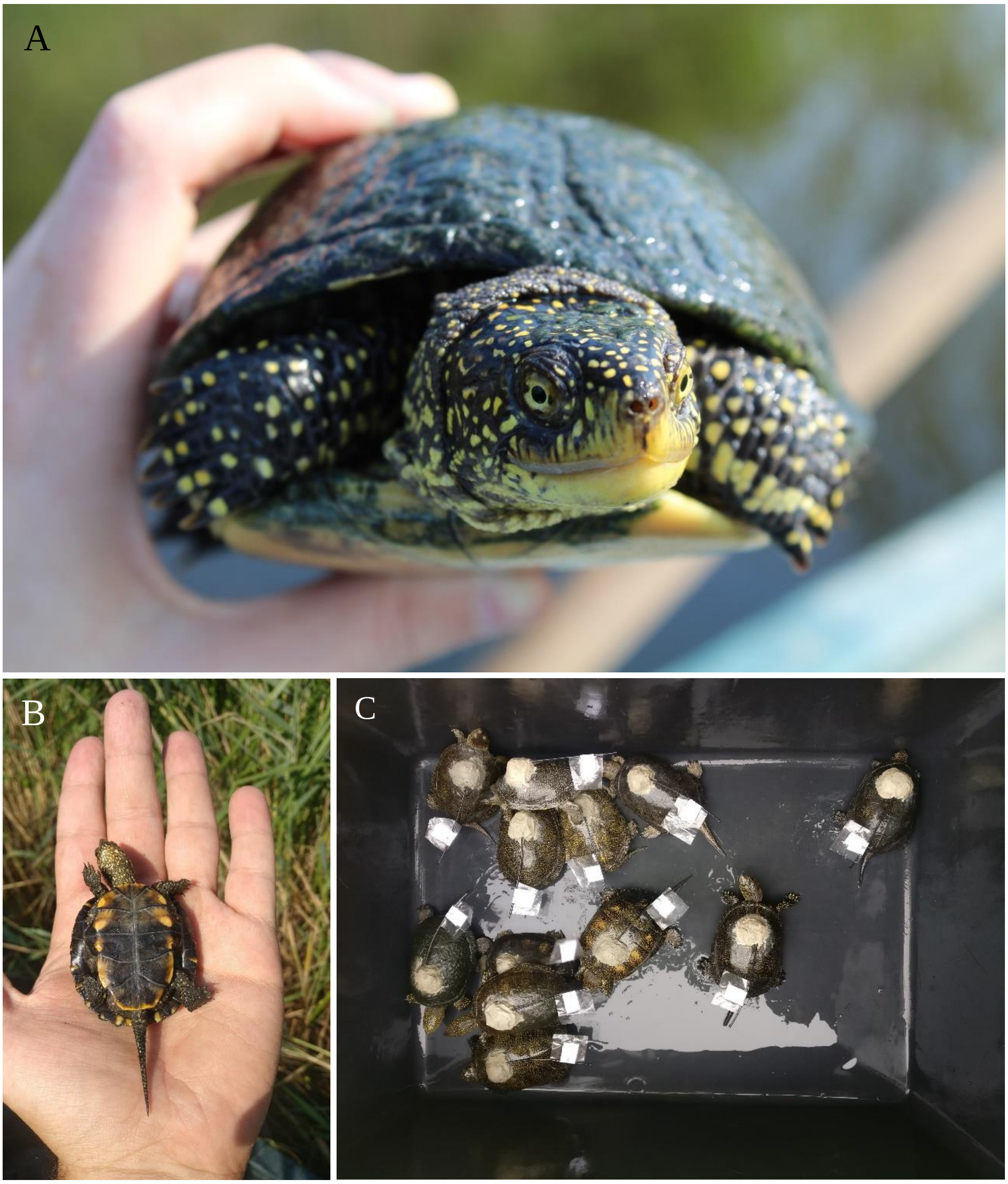

Figure A1 (A) Adult female turtle marked by unique notches on its carapace, (B) a juvenile turtle collected during the sampling activities for abundance estimation, and (C) adult turtles marked with transmitters for the radiotracking activity.

Studente: **Mirko Liuzzo** matricola: **946430**

Dottorato: **Scienze Ambientali**

Ciclo: **34°**

Titolo della tesi[1] :

Management and conservation along the inland margins of the lagoon: the European pond turtle (*Emys orbicularis*) case study in a protected internal wetland area of the Southern Venice lagoon

Abstract:

I. A freshwater turtle monitoring programme along the inland margins of the Venice lagoon was initiated in 2018 in collaboration with the World Wild Fund for Nature (WWF-Italy).

The efforts were focused on the European pond turtle, *Emys orbicularis* which is potentially recognized as surrogate species (es. flagship or sentinel species) in the context of conservation biology. Despite this species is used as a shortcut to monitor or solve conservation problems its scientific knowledge is still under development and currently on debate, particularly in relation to its basic life-history, behavioural ecology and ecological requirements. Quantitative data analysing with a relevant number of individuals are required to increase the set of information on the biology, behaviour and habitat preference for the long-term survival of *E. orbicularis* populations.

In this work: (1) by estimating the *E. orbicularis* population density with the capture-recapture method in a Venice lagoon wetland area, it was possible to observe one of the most populated areas of the Italian peninsula (~250 ind./ha) (2) by analysing behavioural and dispersal patterns between the period of activity and lethargy in a wild population of the European pond turtle, it was possible to observe movements towards aquatic habitats with higher salinities 1-17‰ (mean: 10.64‰) in summer whereas a gregarious brumation and no mass movement behaviour were detected in autumn (3) by evaluating the difference in the distribution of *E. orbicularis* and some micro-habitat descriptors of the Valle Averto Oasis, it was possible to observe a positive effect of the emergent vegetation, salinity and turbidity on the occurrence and abundance of this species.

These indications could have value in optimizing conservation efforts by concentrating funding on management areas and improving landscape features that are most important to the species and for wetlands biodiversity.

[1] Il titolo deve essere quello definitivo, uguale a quello che risulta stampato sulla copertina dell’elaborato consegnato.

II. Nel 2018 è iniziato un programma di monitoraggio delle testuggini palustri in un’area peri-lagunare della laguna di Venezia in collaborazione con *World Wild Fund for Nature* (WWF-Italia). Gli sforzi sono stati incentrati sulla testuggine palustre Europea, *Emys orbicularis*, considerata nel contesto della biologia di conservazione una *surrogate species* (es. specie bandiera, sentinella). Nonostante questa specie sia utilizzata come strategia di sintesi per monitorare o risolvere problemi di conservazione, le sue conoscenze scientifiche sono ancora in fase di sviluppo e attualmente in discussione, in particolare in relazione al ciclo vitale, all'ecologia comportamentale e ai requisiti ecologici. L'analisi di dati quantitativi con un numero rilevante di individui è pertanto necessaria al fine di aumentare l'insieme di informazioni sulla biologia, il comportamento e la preferenza dell'habitat per la sopravvivenza a lungo termine delle popolazioni di *E. orbicularis*.

In questo lavoro: (1) stimando la densità di una popolazione di *E. orbicularis* in una area umida della laguna di Venezia con il metodo della cattura-ricattura, è stato possibile osservare una delle aree più popolate della penisola italiana (~250 ind./ha) (2) analizzando i modelli di comportamento e di dispersione tra il periodo di attività e quello letargico in una popolazione selvatica di testuggine palustre Europea, è stato possibile osservare migrazioni verso habitat acquatici con più alti livelli di salinità in estate 1-17‰ (media: 10.64‰), mentre un’ibernazione gregaria e nessun comportamento migratorio di massa è stata rilevato in autunno (3) valutando la differenza nella distribuzione di *E. orbicularis* e di alcuni descrittori del microhabitat dell'Oasi di Valle Averto, è stato possibile osservare un effetto positivo della vegetazione emergente, salinità e torbidità sulla presenza e abbondanza di questa specie.

Queste indicazioni potrebbero avere valore per ottimizzare gli sforzi di conservazione concentrando i finanziamenti sugli aspetti di gestione e migliorando le caratteristiche paesaggistiche più importanti per la specie e per la biodiversità delle zone umide

Firma dello studente