Current situation and environmental factors affecting the distribution of *Emys orbicularis* in Sèquia Major (NE Iberian Peninsula) in syntopy with *Mauremys leprosa*

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This study describes the situation and population distribution of the European pond turtle (*Emys* orbicularis) in the protected area of Sèquia Major and adjacent area. Between 2013 and 2015, 77 *E. or*-bicularis were captured, with a sex ratio close to 1:1. Regarding the population structure, biometric data showed that the population is aging, with 79.22% of the captured animals being adults with an average carapace length of 141 mm in females and 137 mm in males. Different trophic parameters, competition with other species, habitat use and physico-chemical characteristics of the water were analysed. Results showed that the factors influencing the species distribution in the area were the vegetation coverage in ponds, the abundance of the aloctonous red swamp crayfish *Procambarus clarkii*, the presence of Mediterranean turtles (*Mauremys leprosa*), the trophic status of the water and the concentration of phosphates in the water. Very low recruitment was detected because of the temporary flooding of more than 80% of the nesting area, which potentially slows down the spreading of the population.

Key words: dispersion; distribution; Emys orbicularis; Mauremys leprosa; recruitment rate.

Situación actual y factores ambientales que afectan a la distribución de *Emys orbicularis* en la Sèquia Major (NE Península Ibérica) en sintopía con *Mauremys leprosa*. En el presente estudio se describe la situación y distribución de la población de galápago europeo (*Emys orbicularis*) en el espacio natural protegido de la Sèquia Major y sus zonas adyacentes. Entre los años 2013 y 2015 se capturaron 77 *E. orbicularis*, con una razón de sexos cercana a 1:1. Respecto a la estructura poblacional, los datos biométricos revelaron que la población está envejecida, siendo un 79.22% de los ejemplares capturados adultos, con una longitud media del caparazón de 141 mm en hembras y de 137 mm en machos. Se analizaron diferentes parámetros a nivel trófico, competencia con otras especies, uso del hábitat y la características físico-químicas del agua, obteniendo como resultado que los factores que influyen en la distribución son el nivel de cobertura vegetal en las lagunas, la abundancia de *Procambarus clarkii*, la presencia de galápago leproso (*Mauremys leprosa*), la categoría trófica del agua y la concentración de fosfatos en el agua. Se ha detectado una tasa de recluta-

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miento muy baja, debido a la inundación temporal de más del 80% de la zona de puesta, lo que podría contribuir a ralentizar la dispersión de la especie.

Key words: dispersión; distribución; Emys orbicularis; Mauremys leprosa; tasa de reclutamiento.

The European pond turtle (Emys orbicularis, Linnaeus 1758) is a semi-aquatic chelonian that inhabits preferably lentic aquatic environments (Ayres, 2009). It is distributed from north Africa to north Europe and central Asia (PODLOUCKY, 1997; FRITZ, 2001). Even though it has a wide distribution, effective population is declining, mainly due to the destruction, alteration and pollution of the habitats where it lives, and also due to the competition for resources with the exotic pond sliders, Trachemys scripta (SCHNEEWEISS et al., 1998; BALLASINA ĸ LOPEZ-NUNES. 2000: QUESADA, 2000; KELLER & ANDREU, 2002; CADI & JOLY, 2003; AYRES, 2009).

The distribution of E. orbicularis in the Iberian Peninsula is discontinuous and fragmented, with most of the records coming from isolated individuals or very small populations and being absent from large areas of the Peninsula (Keller & Andreu, 2002; Ayllón et al., 2010). In Catalonia (northeast Iberian Peninsula), E. orbicularis populations consist of few individuals (LLORENTE et al., 1995), which threatens their conservation. Nowadays, the species can only be found in seven locations: Baix Ter, Aiguamolls de l'Empordà, Estany de Banyoles, Estany d'Ivars and La Selva (Girona province), as well as at Delta de l'Ebre and Sèquia Major (Tarragona province). Only those from La Selva (RAMOS et al., 2009) and Sèquia Major (MARTÍNEZ-MARTÍNEZ, 2014) are natural populations, all the other populations being the result of reintroductions (Martínez-Martínez, 2013).

Sèquia Major is a coastal wetland zone originated by the sandy sediments transported by the Francolí River and it would be part of its delta, which is now occupied by the Tarragona harbour and by several chemical industries. This natural space was protected by the Catalonian Plan of Areas of Natural Interest (PEIN) in 1992, mainly because of the existence of a drainage canal built more than 400 years ago.

The E. orbicularis population from Sèquia Major has suffered many changes throughout history. The species was abundant in the middle of the last century, but the intensification of agriculture, the deterioration of water quality and the increase of urban pressure during the last decades caused the decrease of the effective population to the point that in the mid-1990s there were only about twenty specimens (MASCORT, 1998). Ten years later, researchers of the Universitat de Barcelona conducted a diagnosis of the species' situation, capturing 16 specimens in 2008 and only three in 2010 (M. Franch, personal communication).

In 2008, a golf course was established on the terrains surrounding Sèquia Major, where abandoned crop fields existed thus far. This change involved the addition of artificial aquatic habitat to the area with the creation of eight artificial ponds filled by the phreatic water and largely surrounded by wetland vegetation. This soil reconversion supposed the increase of the habitat potentially available for aquatic species; however, no data existed so far about how this change affected the *E. or-bicularis* population and if it took advantage of the new habitat.

This study gives new insights into a relict population of E. orbicularis that has survived to several changes in its habitat throughout history, remaining isolated from other northeaster Iberian populations, and which was even thought to be extinct (Mascort, 1998; Franch et al., 2008). This fact makes the study of the population from Sèquia Major of special interest. Furthermore, recent genetic studies show that all the specimens in the population have a haplotype VA, corresponding to the subspecies E. o. galloitalica. This homogeneity suggests that there have not been anthropic introductions, and that the nucleus constitutes a single metapopulawithout genetic inbreeding tion (Martínez-Silvestre & Soler, 2014). Thus, the location of the population from Sèquia Major at the southeaster edge of the E. o. galloitalica distribution makes its study even worthier. The aim of this study was to analyse the current situation of E. orbicularis in Sèquia Major and to determine the factors affecting its distribution. The obtained results were compared with the situation and distribution of the Mediterranean turtle (Mauremys leprosa) in the zone, a species subjected to the same habitat transformations as our study species.

MATERIALS AND METHODS

In order to study the current situation and distribution of *E. orbicularis* in the altered habitat of Sèquia Major (UTM co**Figure 1:** Location of the study area within Catalonia (small insert) and distribution of ponds inside and outside the protected area.



ordinates: X: 346973; Y: 4549454), an annual survey program was conducted from 2013 to 2015. The study area occupies an extension of 107 ha, of which 17.3 ha correspond to the PEIN of Sèquia Major (protected zone) and the remaining 89.7 ha correspond to the golf course (Fig. 1). This area is surrounded by anthropised areas. Area elevation varies from 0 to 16 m above sea level, with an annual average temperature ranging between 18 and 16°C.

To capture individuals, we used minnow traps of 50 cm diameter x 1 m long, baited with sardines. Other methods were used occasionally, such as the capture of specimens by scoop nets, crayfish trap, and even manually if they were on the land. Each sampling device consisted of 10 minnow traps operating simultaneously in 10 fixed points in a given water body (either a pond or a canal). Each sampling device was checked daily in the morning during a whole week. On a weekly basis, we changed the minnow traps to another water body until all of them (11 water bodies for a total 150 fixed points) were sampled. This procedure was repeated three times during the study, once per year. For each freshwater turtle captured, data registered were: species, UTM of capture location, sex, age, carapace length and body weight. In addition, all captured individuals were marked with ISO FDX-B (8 or 12 mm), FECAVA (12 mm) or AVID (12 mm) coded microchips (Avid Identification Systems, Norco, California, USA).

To calculate the age structure, individuals were categorized depending on their carapace length. Because in E. orbicularis populations from Italy (ZUFFI & FOSCHI, 2015) or east Spain (V. Sancho, personal communication) gravid females of 110 mm of carapace length have been observed, we considered all females above that size to be adults. To determine minimum size of males, we used the presence of secondary sexual characters. For M. leprosa, we followed the criterion of Keller (1997), who considered females larger than 140 mm of carapace length and males larger than 90 mm as mature individuals. The sex ratio skewness was analysed using the chisquared (χ^2) test, comparing the observed ration to an expected distribution of 50% of males and females.

To locate nesting area, we considered the place where neonates were captured. This location was refined by radiotracking nine adult females with VHF transmitters (Biotrack, Wareham, UK) attached to the carapace. In order to ensure the reproductive potential of the tracked females, the first nine females captured in 2014 with carapace lengths above 110 mm and weighing over 300 grams were the ones selected for radio-tracking.

Different variables that might be limiting factors for the presence of E. orbicularis in the ponds were taken into account. These variables were: habitat, feeding resources, physico-chemical parameters and trophic status of the water, and presence or absence of competitors. The habitat was characterized for each sampling point, registering the most abundant plant species and the associated vegetation coverage, which was categorized into abundant, present or absent. Feeding resources were estimated from amphibians and red swamp crayfish (Procambarus clarkii) captured in the traps, with the abundance of these species in each pond categorized as abundant, present or absent. Water physico-chemical parameters were analysed for each pond through colorimetric analyses to obtain the following variables: conductivity, pH, temperature, and concentration of nitrate, nitrite, ammonia and phosphate. All these data were collected on the same day to minimize environmental variations. Trophic status of the water was also analysed, using a Secchi disk, categorizing the classes into: oligotrophic, mesotrophic and eutrophic (CARLSON, 1977). The presence or absence of competitors (M. leprosa and Trachemys sp.) was recorded as a binomial

Variable	Code	Value	Parameter coding			
		value	DV1	DV2		
		Eutrophic	1	0		
Trophic status	TS	Mesotrophic	0	1		
-		Oligotrophic	0	0		
T 7 ()		Absent	1	0		
Vegetation	Cov	Abundant	0	1		
cover		Present	0	0		
Abundance of amphibians		Absent	1	0		
	AA	Abundant	0	1		
		Present	0	0		
		Absent	1	0		
Abundance of	AP	Abundant	0	1		
P. clarkii		Present	0	0		
Presence of	М	Presence	1			
M. leprosa	ML	Absence	0			
Presence of	TD	Presence	1			
Trachemys sp.	TR	Absence	0			

Table 1: Codification ofthe categorical variables inthe dummy variables (DV)used in the model.

variable with a value assigned to each trap depending on whether at least an individual of *M. leprosa* or *Trachemys* sp. was captured (presence of competitors) or no turtles other than *E. orbicularis* were captured (absence of competitors).

Statistical analysis to determine the influence of environmental factors on the occupancy of E. orbicularis was performed using a logistic regression to determine the probability of presence of *E. orbicularis* in each particular point. In order to integrate so many environmental variables (15), a Wald stepwise forward procedure was used to evaluate the best-fitting model and to find out the most influential variables for *E. orbicularis* presence. Categorical variables were coded as shown in Table 1. To select the best model, we focused on the one resulting in the lowest value of the -2 log likelihood, using an omnibus test to

prove that this reduction was statistically significant. In addition, the Nagelkerke R² was used to calculate the strength of the association between the response variable and the predictors. Finally, the global adjustment of the model was analysed with a Hosmer and Lemeshow test.

Results

We got 280 captures of *E. orbicularis* and 324 captures of *M. leprosa* (Table 2), as well as 31 *Trachemys* sp. individuals that were removed from the environment. The population structure of *E. orbicularis* shows that 79.22% of captured individuals were adults (Fig. 2). We found a sex ratio of 1.11:1 in favour of males, which was not statistically different from a balanced sex ratio (N = 61, χ^2 = 0.15, d.f. = 1, *P* = 0.696). The population structure of *M. leprosa* was characterized by a high density of in-

Table 2: Number of capturedindividuals and total numberof captures per species andyear during the study.

Species		2013	2014	2015	Total
Emys	New captures (individuals)	52	17	8	77
orbicularis	Total captures	133	78	69	280
Mauremys leprosa	New captures (individuals) Total captures	66 136	21 109	32 79	119 324
Trachemys sp.	Captures	7	12	12	31

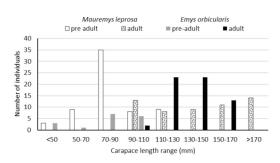


Figure 2: Population structure and size-class distribution of *Emys orbicularis* and *Maure-mys leprosa* in Sèquia Major.

dividuals with a pre-adult size (Fig. 2), with only 44.5% of the individuals identified as adults. Biometric data showed, as expected, that *E. orbicularis* females were larger than males. The minimum size of an *E. orbicularis* male displaying secondary sexual characters was 99 mm (Table 3).

Emys orbicularis occurred in a 58.3% of the surveyed territory, with 96% of captures occurring within the protected zone of the study area and in the two ponds adjacent to it, although this area of high occupancy of E. orbicularis it represents only the 16% of the surveyed area. In this same area, the percentage of M. leprosa captures was 80.8% (Fig. 3). Furthermore, M. leprosa occupied 95.48 % of the territory, its population was well distributed across the entire zone and there was only one pond where no individuals of this species were found (Fig 3). Regarding nesting area, five of the radio-tracked E. orbicularis females were found out of the water at dusk, in an area of approximately 1 ha. Furthermore, a female was observed digging in this area. The captures of neonates

Table 3: Biometrical data for *Emys orbicularis* population by sex and size-based age groups. Mean values and their standard deviations, as well as minimum (Min) and maximum (Max) values are shown.

	Carapace length (mm)					Body weight (g)					
	Ν	Mean	SD	Min	Max	Ν	Mean	SD	Min	Max	
Females	28	141.06	12.51	114	168	27	491.9	117.9	253	737	
Males	33	136.83	8.74	99	159	33	338.5	55.7	150	554	
Pre-adult size	16	63.51	19.05	15	96	16	77.3	44.4	4.8	176	



Figure 3: Maps of species abundance according to the number of captures per pond. Suggested nesting area for *Emys orbicularis* is also represented. For a correct visualization of the colours indicating abundance levels, consult the online version of the paper.

on the shores of the ponds surrounding this area where radio-tracked females were found supports its identification as nesting area (Fig. 3).

Regarding the environmental factors affecting the distribution of *E. orbicularis*, five of the 15 analysed variables were selected by the model. Among all the alternative models proposed by the logistic regression, the model chosen as the one showing the best adjustment, with the lowest -2 log likelihood value (model 5, Table 4) had the strongest association level between the response variable and the predictors, as shown by the Nagelkerke R²

value, and good data adjustment as indicated by the Hosmer and Lemeshow test, scoring a non-significant χ^2 . The selected model accurately predicted 84.7% of the cases (86.4% when *E. orbicularis* was present and 83.3% when it was absent), which is a really high percentage compared to the null model, which predicted a 56% of the cases. Abundance of vegetation cover, eutrophic and mesotrophic waters, and abundance of crayfish increased the probability of trapping *E. orbicularis*, while high levels of phosphates in the water and the absence of *M. leprosa* decreased such capture probability (Table 4).

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Table 4: Models constructed after stepwise forward lineal regression to identify environmental variables influencing the probability of capture of *Emys orbicularis*. Model selection was based in the lowest -2 log likelihood (-2LL) value. Selected model is indicated in bold characters. Nagelkerke R², as well as the results of the Howmer and Lemeshow (H & L) and the Omnibus tests are shown. PO4: concentration of phosphate in the water; see Table 1 for abbreviation of the other variables in the models.

M 11	E sus tis s	21.1	R ²	H & L			Omnibus		
Model	Equation	-2LL		X ²	df	Р	χ²	df	Р
1	-2.890 + 1.551 * Cov(Absence) + 3.701 * Cov (Abundant)	158.26	0.364	0	1	1.00	47.52	2	< 0.001
2	-2.645 + 2.259 * Cov(Absence) + 4.054 * Cov (Abundant) -1.585 * ML(Absence)	143.63	0.455	4.76	3	0.19	14.63	1	<0.001
3	-3.063 + 1.321 * Cov(Absence) + 3.297 * Cov (Abundant) + 0.587 * AP(Absence) +1.641* AP (Abundant) - 1.262 * ML(Absence)	132.53	0.518	7.35	6	0.29	11.10	2	0.004
4	-2.422 + 0.973 * Cov(Absence) + 3.608 * Cov (Abundant) - 0.604 * PO4 + 1.273 * AP (Absence) + 3.315 * AP(Abundant) - 0.818 * ML (Absence)	117.69	0.595	11.95	8	0.15	14.83	1	<0.001
5	-6,638 + 0.680 * Cov(Absence) + 4.430 * Cov (Abundant) – 0.549 * PO4 + 1.511 * TS (Eutrophic) + 3.525 * TS(Mesotrophic) + 1.897 * AP(Absence) + 4.060 * AP(Abundant) – 0.377 * ML(Absence)	105.14	0.655	4.64	8	0.79	12.54	2	0.002
6	-6.931 + 0.504 * Cov(Absence) + 4.423 * Cov (Abundant) – 0.575 * PO4 + 1.477 * TS (Eutrophic) + 3.649 * TS(Mesotrophic) + 2.138 * AP(Absence) + 4.304 * AP(Abundant)	105.64	0.653	7.19	8	0.52	-0.500	1	0.479

DISCUSSION

Body size and population structure

To analyse the results, biometrical data and population structure observed at Sèquia Major were compared with the nearest populations, including La Selva (RAMOS *et al.*, 2009), Peñíscola (BATALLER *et al.*, 2008) and Burriana (SANCHO & RAMIA, 2008). The *E. orbicularis* population structure at Sèquia Major is determined by the high percentage of adult individuals (79.22%). In Peñíscola and Burriana population structures are very similar, with 80% and 91% of captured specimens, respectively, identified as adults. These population structures are very different from the population of La Selva, which has 44.5% of adult individuals and 55.5% of juveniles, suggesting that our study population would have a structure bias toward adults. However, we must consider that potential differences between studies in sampling methodology could have resulted in variations of the catchability of neonates and juveniles. Both the type of traps and their location can affect the relative percentages of captures per age group, since adults and juveniles tend to use different habitats within the area of occupancy of the population (ZUFFI, 2000; MOSIMANN & CADI, 2004).

Sex ratio of Sèquia Major E. orbicularis population is virtually 1:1, with a number of captured males slightly higher than that of females. The populations of Burriana and La Selva have around twice as many males as females (sex ratios of 1.8:1 and 2.24:1, respectively), whereas in Peñíscola the sex ratio is 1:1.4 in favour of females. Regarding body size, there is a clear difference between the Sèquia Major population and the other ones. While in nearby populations the carapace length is around 112-119 mm for males and 116-124 mm for females, in Sèquia Major the mean values are considerably higher (129 mm for males and 141 mm for females), which would indicate that Sèquia Major is a beneficial habitat for pond turtles.

Nesting area and low recruitment

Radio-tracking was used to find the nesting area and helped to confirm the colonial character of *E. orbicularis* when selecting the nesting area (MAYOL, 1993). The nesting area was located in the middle of the protected zone. We have observed that 80% of this nesting area becomes flooded during periods of 3-5 days several times per year, which could destroy a significant part of the nests because *E. orbicularis* eggs only tolerate a short period of immersion in the water (MOLL & MOLL, 2000). This fact could explain the lack of recruit-

ment that would cause the aging of the population, as indicated by the low rate of capture of neonate and juvenile individuals compared to that of adults.

A low recruitment rate is common in *E*. orbicularis populations (Ayres, 2009); however, if biometrical data were cautiously used as an indicator of the age of individuals, we could consider that over 50% of them, especially females (60.7% with carapace length above 140 mm and body mass above 500 g), are old individuals, which would threaten the viability of the population. In the late 20th and early 21st centuries, areas surrounding Sèquia Major became intensively developed for tourism; a hotel resort and a promenade walkway were built between the study area and the sea. These constructions produced a blockage of the drainage canal, which began to flow underground from the Sèquia Major to the sea, causing the aforementioned flooding in some parts of Sèquia Major, including the nesting area of *E. orbicularis*. Two additional factors might affect population recruitment. First, occasional presence of badgers (Meles meles) and wild boars (Sus scrofa) was noticed in the area, and these two mammals can predate on nests of E. orbicularis (SALVADOR & PLE-GUEZUELOS, 2002). The second factor is the significant increase in abundance of fishing birds over recent years due to the release of carps (Cyprinus carpio) by humans. These birds are potential predators of neonates (Andreu & López-Jurado, 1998). Another factor that could have an effect on our study population, like the presence of invasive competitor Trachemys sp., was not found to directly affect, according to our statistical analysis, the pop-

ulation of *E. orbicularis*.

Factors affecting distribution

Regarding population distribution, estimated from the spatial distribution of captures, E. orbicularis was located within the protected zone, occupying half of the study area. The statistical analyses revealed that one of the most influential factors for the presence of E. orbicularis was the abundance of vegetation coverage. Emys orbicularis requires places that it can use as refuge (Segurado & Kunin, 2005; Se-GURADO et al., 2005; SEGURADO & FIGUEIRE-DO, 2007), a requirement that we have confirmed with the radio-tracking study in which we saw that the displacement of individuals took always place in areas with abundant vegetation coverage.

Another important factor determining distribution of E. orbicularis was the presence of red swamp crayfish. Emys orbicularis has an opportunistic diet (FRITZ, 2001) that varies on the basis of food availability (Ottonello et al., 2005; Ficetola & De BERNARDI, 2006). In Sèquia Major, the most abundant food is red swamp crayfish, which could explain why the abundance levels of this species also affect the presence of E. orbicularis. The trophic status of the water was also an indicator for the presence of E. orbicularis. The number of captures in mesotrophic and eutrophic ponds was much higher than in the oligotrophic ones. Oligotrophic ponds of Sèquia Major have considerable turbidity due to the abundance of carps (ZAMBRANO & HI-NOJOSA, 1999; РАККОS et al., 2003). Carps remove the soil of the pond, hindering the vision of turtles to get food. Mesotrophic and eutrophic ponds contain moderate amount of nutrients and algae, being a better environment for *E. orbicularis* than oligotrophic ones. Finally, we also found a negative relationship between the concentration of phosphates in the water and the presence of *E. orbicularis*, which could be reflecting the reported little tolerance of the species to contaminated habitats (SEGURADO & ARAÚJO, 2004).

Syntopy between E. orbicularis *and* M. leprosa

According to our analysis, the presence of M. leprosa is another indicator of the presence of E. orbicularis. This could be due to the ecological equivalence of the two species leading them to similar habitat requirements. In a scenario of few resources, the coexistence of species with similar ecological requirements would increase competition and hinder this association. However, the abundance of trophic resources in Sèquia Major facilitates the coexistence of both pond turtle species. Mauremys leprosa population seems more abundant than E. orbicularis population, as deduced by the higher number of captures.

Contrarily to *E. orbicularis*, the population of *M. leprosa* shows a high abundance of juveniles (1.26 juveniles per each adult individual), indicating a high recruitment rate. This might be due to several factors; for example, nesting patterns in *M. leprosa* have no colonial character (KELLER, 1997), so nests are distributed across the territory. This pattern can serve to partially avoid the negative effects of flooding on egg survival, and also to reduce the number of nests in the protected zone, where the pressure from nest predators could be

especially high. The location of M. leprosa neonates was more sparsely distributed than in E. orbicularis, and its number of captures triplicated that of E. orbicularis. Another factor is the different dispersion strategy used by both species; E. orbicularis has a territorial and aggressive behaviour, scattering when there is a high number of individuals per pond (MASCORT, 1992). Mauremys leprosa has a broader distribution than E. orbicularis (Fig. 3), with most adults captured in peripheral ponds, while juveniles being captured in the same ponds as E. orbicularis. This fact indicates that the dispersion of *M. leprosa* is usually marked by the movement of the breeding adults, leaving juveniles in optimal ponds for growth. Therefore, the low number of births and a territorial dispersion strategy make no need for E. orbicularis individuals to colonize new territories, as the number of individuals does not increase. In addition, the lack of connection between the new ponds and the absence of natural corridors with high vegetation coverage can also be factors restricting the dispersion of E. orbicularis.

General conclusions and management measures

The population of *E. orbicularis* has enough individuals to guarantee the survival of the species in the zone. The main problem for this population appears to be the low recruitment rate that leads to an aged population with apparent problems for dispersion and mobility. The fact that an important part of the nesting area is affected by flooding is likely to bring the number of births below the naturally expected rates.

Taking into account this study, the following urgent measures are recommended to conserve the population of E. orbicularis in Sèquia Major: 1) flooding in the nesting area should be prevented through the rehabilitation of drainage gates in order to recover the control of the phreatic levels. As a result, the recruitment rates are expected to increase to sustainable levels for the species; 2) the conservation, recovery and creation of natural corridors among water bodies would facilitate the movement of individuals and the colonization of new territories; and 3) introduced species, mainly carps, should be controlled in order to avoid their proliferation, since these species alter the structure and composition of the habitat. Exotic pond turtles should also be controlled because, although not directly affecting the presence of E. orbicularis, they may become a serious problem in the future.

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