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RED-EARED SLIDER: A THREAT TO INDIGENOUS FRESHWATER TURTLES IN AN IBERIAN CONTINENTAL WETLAND

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RESUMEN *El galápago de Florida: una amenaza para los galápagos autóctonos en un humedal continental del noreste ibérico*. Analizamos los cambios en la estructura y la dinámica de las poblaciones entre 2004 y 2015 de dos galápagos autóctonos, el galápago leproso (*Mauremys leprosa*) y el galápago europeo (*Emys orbicularis*), que conviven con la especie exótica invasora *Trachemys scripta elegans* en la Reserva Natural Dirigida de Los Sotos y Galachos del Ebro, un humedal continental del noreste ibérico. Se extrajeron 206 galápagos de Florida. Sin embargo, a pesar del esfuerzo de extracción, sus capturas se mantuvieron constantes en el tiempo. El galápago de Florida se reproduce en la naturaleza y prevalecen las hembras de gran tamaño, que podrían provenir en parte de cautividad. Las capturas de galápago leproso fueron aumentando: su estimación poblacional pasó de 36 a 90 ejemplares, manteniendo una proporción equilibrada de sexos y un predominio de ejemplares grandes. La estimación de galápago europeo se mantuvo en el tiempo (28 ejemplares). La proporción de ejemplares pequeños (juveniles) fue baja (8,8 % y 10,4 % respectivamente) en los galápagos autóctonos y mucho mayor (24,1 %) en el galápago de Florida, probablemente debido a su

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mayor tasa de reproducción. El seguimiento de las tres especies y la extracción de la especie exótica deben continuar para garantizar la viabilidad de los galápagos autóctonos y se deben desarrollar campañas de educación ambiental para evitar la liberación de nuevos galápagos exóticos en la naturaleza.

PALABRAS CLAVE *Trachemys scripta*. *Emys orbicularis*. *Mauremys leprosa*. Competición. Especies invasivas. Reserva Natural Dirigida de Los Sotos y Galachos del Ebro. Noreste ibérico (España).

ABSTRACT We analysed (2004-2015) the changes in population structure and dynamics of two native freshwater turtles —the Mediterranean pond turtle (*Mauremys leprosa* Schweigger, 1812) and the European pond turtle (*Emys orbicularis* L., 1758)— and the invasive American red-eared slider *Trachemys scripta* which co-exist in the Ebro Sotos and Galachos Managed Natural Reserve, a continental wetland in NE Iberia. Two hundred and six red-eared sliders were removed. In spite of removal efforts, captures still do not decrease overtime. The Florida red-eared slider breeds in the wild and large females prevail, some of which may come from captivity. Captures of Mediterranean pond turtle were increasing overtime, its population estimate grew from 36 to 90, maintaining a balanced sex ratio and a predominance of large specimens. The estimated number of European pond turtle was maintained overtime (28 specimens). The proportion of small native freshwater turtles (juveniles) was low (8.8 % and 10.4 % respectively) while there was more than twice as many red-eared sliders (24.1 %), probably due to their higher reproduction rate. The monitoring of the three species and the removal of the red-eared slider must proceed to ensure the viability of native freshwater pond turtles, and environmental education campaigns should be developed to prevent the release of new red-eared sliders in the wild.

KEYWORDS *Trachemys scripta*. *Emys orbicularis*. *Mauremys leprosa*. Competition. Invasive species. Ebro Sotos and Galachos Managed Natural Reserve. Northeast of Iberian Peninsula (Spain).

INTRODUCTION

The American red-eared slider *Trachemys scripta* Schoepff, 1792 is considered one of the most harmful exotic species for native fauna out of the worst 100 invasive species (Lowe *et al*., 2000). It is also one of the most commonly traded pet reptiles in Spain (Pérez-Santigosa *et al*., 2008), and the one that has occupied most territory (Martínez-Silvestre *et al*., 2015a). Originally from Southeast United States of America and Northeast Mexico, it was exported worldwide as a pet (Arvy and Servan, 1996; Barquero, 2001). Whether when they have escaped or they have been released, they have adapted well in many areas, generating well-documented impacts (Chen and Lue, 1998; Cady and Joly, 2004; Patiño-Martínez and Marco, 2005; Pearson *et al*., 2015). In the Iberian Peninsula, its main impact is its competitive superiority and the displacement of the native freshwater turtles, the Mediterranean pond turtle *Mauremys leprosa* Schweigger, 1812 (Vulnerable; Bertolero and Busack, 2017) and the European pond turtle *Emys orbicularis* Linnaeus, 1758 (Near Threatened; Van Dijk and Sindaco, 2004). These have suffered a considerable decline, due to the red-eared slider's competitive advantage (Cady and Joly, 2004; Polo-Cavia *et al*., 2014), and other reasons such as habitat loss and expansion of exotic invasive predatory fishes (Ayres, 2015; Díaz-Paniagua *et al*., 2015).

The red-eared slider has a larger size, an earlier sexual maturity, a longer daily and annual activity, longer laying periods, a larger fecundity, a more diversified diet and, only with respect to the European pond turtle, a larger tolerance to contamination and human presence (Andreu *et al*., 2003; Polo-Cavia *et al*., 2014; Cady and Joly, 2004; Patiño-Martínez and Marco, 2005; Marco *et al*., 2003; Pleguezuelos, 2002). Native freshwater turtles avoid areas with red-eared slider chemical secretions (Polo-Cavia *et al*., 2009; 2014). Red-eared sliders are more aggressive and dominant, they compete for food and basking places (Polo-Cavia *et al*., 2011; 2014) and occupy the best habitats (Franch i Quintana *et al*., 2007). This competition for basking places negatively affects native freshwater turtle's survival (Pérez-Santigosa *et al*., 2006, 2011).

These three above mentioned turtles have an adaptable diet to the available food resources. The red-eared slider has a wider trophic range and a more opportunistic diet, depending on the most abundant resource (Pérez-Santigosa *et al*., 2011). This could be a key aspect for the colonization of scarce resource habitats or abundant red-eared slider populations in comparison to natives (Pérez-Santigosa *et al*., 2011).

The red-eared slider also has morphologic and thermoregulatory advantages, with a more spherical shape than the Mediterranean pond turtle, which means a lesser surface-volume ratio and a higher thermal inertia, which facilitates heat retention (Polo-Cavia *et al*., 2014). It is more active in low temperature water compared to natives so it can start its reproductive cycle earlier. Also, the spherical shell reduces successful predations and is more effective when self-righting (Polo-Cavia *et al*., 2014). Its escape response also gives it some advantages. Therefore, red-eared sliders are more competitive than natives, in terms of reproduction, food, thermal regulation and morphology. Another important effect could also be seen in parasite and illness transmission. Lethargy and lack of movement appears in European pond turtle with trematode *Spirorchis elegans* infestation of the vascular system (Iglesias *et al*., 2015), an originally American parasite, which is common in its main host, the red-eared slider. This suggests a transmission to native species, with its negative consequences. Additionally, the opposite effect observed in intestinal infections due to native parasites (*Serpinema*), transmitted to the exotic turtles, has been described (Martínez-Silvestre *et al*., 2015b). Other parasites have been documented in the Mediterranean pond turtle (Meyer *et al*., 2015).

The aim of this work is to describe the abundance, size, adult sex ratio and trend of the two native and the invasive freshwater turtles living in sympatry in an Iberian continental wetland for eleven years, where the invasive species is being removed from the wild.

MATERIAL Y METHODS

Study area

The study area was the relict riverine habitat of the Ebro Sotos and Galachos Managed Natural Reserve, a protected area along the River Ebro near Saragossa, in the centre of Aragon, Spain, of 1,537 hectares, of which 40 % is riverine habitat (fig. 1). Near the Ebro River there are some *galachos*, which are small lagoons originated when the river abandoned old meanders to take a straighter line, the last ones were formed around 1960. The climate is sub-arid, with an average annual precipitation of 302 millimetres, most of which falls in May-June and October-November. Between 1994 and 2011, annual minimum and maximum temperatures were –9 °C and 43.1 °C, respectively. In 2017, the median (minimum-maximum) annual water temperature was 15.5 ºC (5.3-26.8 ºC) and conductivity 20 ºC was 1,671 μS/cm (372-2,479 μS/cm). Reed *Phragmites australis*, tamarisk *Tamarix africana*, poplar *Populus* spp., willow *Salix* spp., and ash *Fraxinus angustifolia* are predominant in the small, isolated, riverine habitats (Rivas and

Baselga, 2005). Also, the area is a Special Protection Area for Birds (SPA) under the European Union legislation, due to the presence of sedentary, migrant, and breeding birds such as the black-crowned night-heron *Nycticorax nycticorax* (Rivas and Baselga, 2005). In addition, it is part of two European Special Conservation Areas (SCA). SPA and SCA belong to the European Natura 2000 network.

Main fish species are the introduced carp *Cyprinus carpio* and Wels catfish *Silurus glanis*. Medium size mammals are: red fox (*Vulpes vulpes*), stone marten (*Martes foina*), genet (*Genetta genetta*) (all terrestrial), Eurasian otter *Lutra lutra* and European beaver (*Castor fiber*), both aquatic (Rivas and Baselga, 2005).

Sampling was undertaken in two small lagoons formed by abandoned meanders, on each side of the Ebro river, La Alfranca (5 ha, 780 m length) and La Cartuja (3.2 ha, 980 m length), separated by 2 kilometres, which are connected during floods (fig. 1).

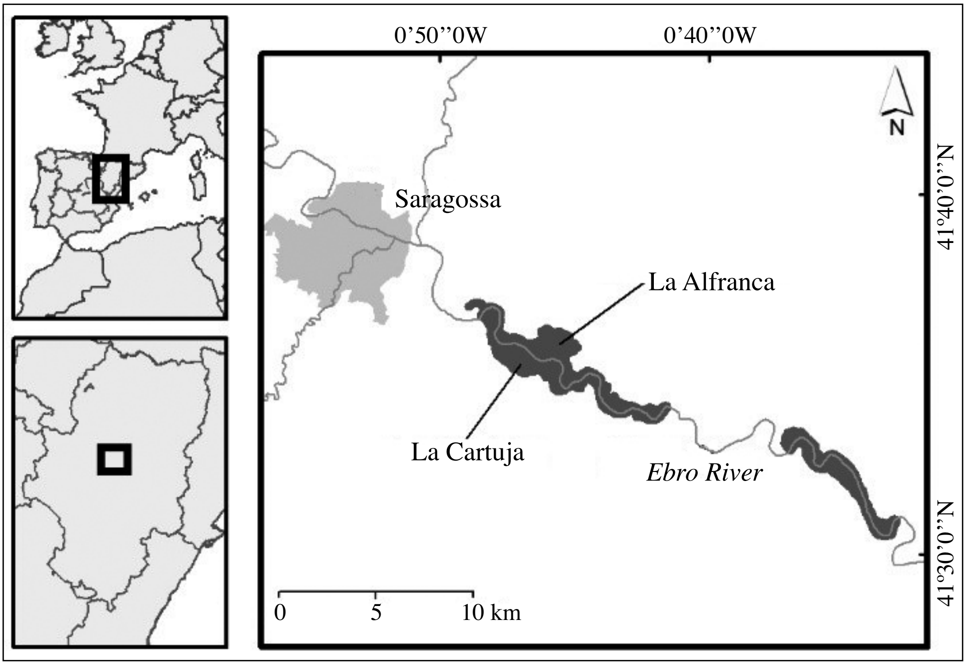

Fig. 1. Study area (right) and its position in Spain (left up) and in Aragon region (left down), the Ebro Sotos and Galachos Natural Reserve in dark grey with its two lagoons.

Methodology

We trapped continuously from April to August in 2004, 2005, 2006 and 2015, using 1 metre long floating nets baited with European pilchard *Sardina pilchardus*. Nets were checked two or three times per week. We weighed all captured turtles, and measured and marked native's shells in the field with lateral incisions following Holland (1991) code. Natives were released and red-eared sliders removed.

In 2004, 2005 and 2006 we used ten large nets, always in the same spots. In 2015 it was only possible to access six points, due to the impact of the location of heron breeding colonies, so we only used six large nets. In 2015, in part of the period we used six small nets (60 cm), close to the large ones, in order to obtain a capture effort more similar to previous years, by achieving a similar number of nets per day. To compare capture frequency, we only analysed data from a common annual trapping period from April 30 to August 7. Capture vs .non-capture was calculated with a Pearson X^2 test comparing the number of active nets multiplied by the number of days in which they were checked vs. the number of events in which different natives were captured. The comparison of capture frequency between years was used to estimate if population could increase or decrease.

The size of Native population was estimated using capture-recapture Lincoln-Petersen method (Seber, 1982). In 2005, 2006 and 2015 we undertook population estimations. Population could not be estimated in 2004 due to inconsistency in recapture data. To apply this method, we have considered April 30 to May 31 as the capture period and June 8 to August 7 as the recapture period. Both periods were established to make a roughly similar number of captures and recaptures. Other trappings were undertaken in 2010, 2011 and 2012, with the aim of removing red-eared sliders, using nets and basking traps, that we called unequal trapping effort. The trapping period and number and type of traps varied each year, so results from 2010, 2011 and 2012 years are not comparable to the other sampling.

Data treatment of the similar sampling effort

To determine population structure, we measured the straight carapace length (cm) with a gauge and we considered three length classes (small,

Table I. Size classes per turtle species (straight carapace length) (Keller, 1997; Outerbridge, 2008).

Size classes	*Mediterranean pond turtle*	*European pond turtle*	*Red-eared slider*
Small (mainly juveniles)	0-8.9 centimetres	0-10.9 centimetres	0-9.9 centimetres
Medium	9-16.9 centimetres	11-14.9 centimetres	10-17.9 centimetres
Large adults	> 17 centimetres	> 15 centimetres	> 18 centimetres

medium and large). The small class corresponds mainly with juveniles (table I). Sex ratio was only calculated for medium and large specimens, as it is difficult to assign to small ones.

The relation between variables was analysed through non-parametrical tests due to the lack of normality (U of Mann-Whitney, Kruskal-Wallis and Pearson X^2). We used IBM SPSS v. 19 statistical package.

Table II. Number of different turtle species caught (excluding recaptures) in the two lagoons separately.

La Alfranca				
	Mediterranean pond turtle	*European pond turtle*	*Red-eared slider*	*Total*
2004	20	27	1	48
2005	15	20	1	36
2006	17	13	4	34
2010	2	15	3	20
2011	4	9	0	13
2012	1	4	12	17
2015	11	18	0	29
La Cartuja				
2004	15	9	34	58
2005	19	4	17	40
2006	16	4	23	43
2010	14	2	4	20
2011	14	3	33	50
2012	14	4	42	60
2015	38	2	30	70

RESULTS

Overall sampling

The number of different species captured is presented in table II. We captured 204 red-eared sliders between 2004 and 2015 using both methodologies, the equal and the unequal trapping effort. Captures of the European pond turtle are concentrated in La Alfranca lagoon, where red-eared slider is scarce. In La Cartuja lagoon, red-eared slider is abundant and European pond turtles scarce. The level of occupation of Mediterranean pond turtles is similar in both lagoons.

Due to the marking and recapture of all autochthonous specimens, we found that nine marked Mediterranean pond turtles and one European pond turtle moved from one lagoon to the other in both directions between 2004 and 2006. For this reason, both lagoons were considered as part of one single population.

Equal trapping effort

Mediterranean pond turtle capture frequency increased between 2004 and 2015 ($X^2 = 23.384$, $p = 0.000$, d. f. = 1), while red-eared slider trend was

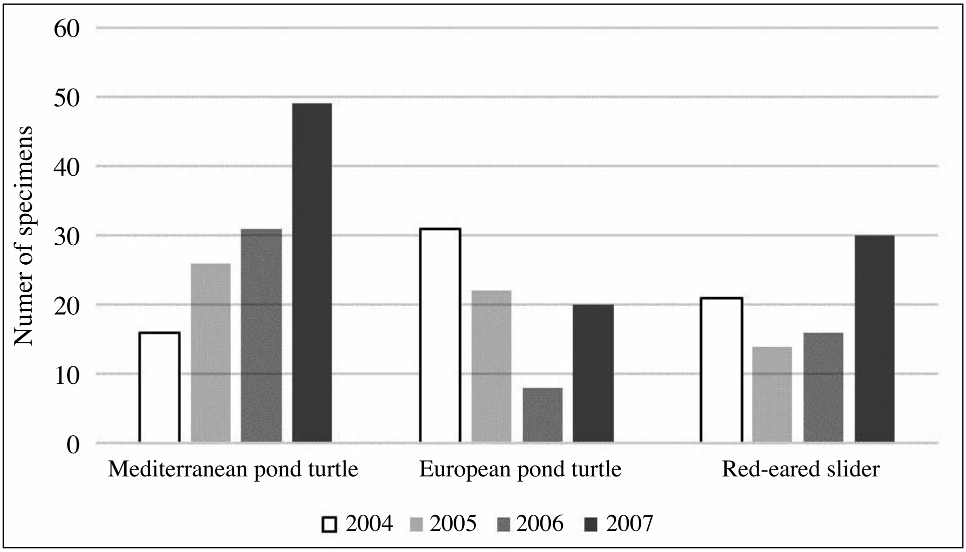

Fig. 2. Number of different freshwater turtle specimens captured between April 30 and August 7 in 2004, 2005, 2006 and 2015.

Table III. Population estimation of Mediterranean and European pond turtles. M, marked turtles during capture period; n, marked turtles during recapture period; R, recaptured turtles; N, estimated number of turtles; SD, standard deviation.

Year	*Species*	*M*	*n*	*R*	*N*	*N (without bias)*	*SD*
2005	Mediterranean pond turtle	16	14	6	37.3	36.4	11.5
2006	Mediterranean pond turtle	13	12	8	19.5	20.2	4
2015	Mediterranean pond turtle	21	36	8	94.5	90.4	29.5
2005	European pond turtle	12	14	6	28.0	27.9	8.6
2006	European pond turtle	3	2	0			
2015	European pond turtle	5	18	3	30	28.5	15.8

non-significant ($X^2 = 2.847$, $p = 0.092$, d. f. = 1) as well as European pond turtle ($X^2 = 1.675$, $p = 0.196$, d. f. = 1). However, European pond turtle capture frequency decreased between 2004 and 2006 ($X^2 = 10.609$, $p = 0.001$, d. f. = 1, fig. 2).

Autochthonous pond turtle population estimation showed similar trends to those of capture frequencies. Mediterranean pond turtle population estimation moved from 36 (2005), 20 (2006) to 90 (2015) whereas European pond turtle population was 28, both in 2005 and 2015 (table III).

Size structure

In the overview of the entire period of monitoring, small specimens (mainly juveniles) were more scarce in natives (8.8 % in Mediterranean pond turtle, 10.4 % in European pond turtle) than in the red-eared sliders (24.1 %), being more abundant in the red-eared slider compared with the Mediterranean pond turtle (Pearson test, $N = 226$, $X^2 = 9.777$, $p = 0.002$, d. f. = 1) or compared with the European pond turtle (Pearson test, $N = 175$, $X^2 = 5.827$, $p = 0.016$, d. f. = 1), with no differences between natives (Pearson test, $N = 243$, $X^2 = 0.168$, $p = 0.682$, d. f. = 1) (fig. 3).

In 2004 no red-eared slider smaller than 18 centimetres was captured. In the following years small specimens were found, particularly in 2006. In 2015 many large adults and some small red-eared sliders specimens were captured. Almost all European pond turtles were medium (mainly adults), even if there were also large ones, mainly in 2005 and 2015. Small (mainly

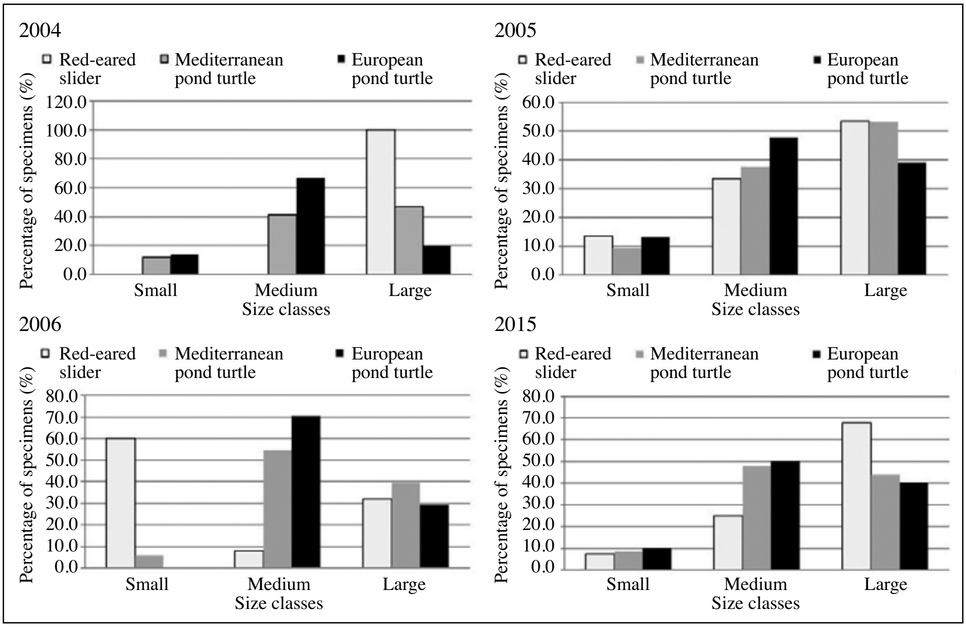

Fig. 3. Population structure of the captured freshwater turtles. White, red-eared slider; grey, Mediterranean pond turtle; black, European pond turtle.

juveniles) European pond turtles are scarce, absent in 2006. The majority of Mediterranean pond turtles were medium and large (fig. 3).

Biometry

Annual median shell size is stable in natives (European pond turtle, 14.0 centimetres, Kruskal-Wallis's test, $N = 96$; $H = 2{,}317$; $p = 0.509$; Mediterranean pond turtle, 15.9 centimetres, $N = 148$; $H = 1.312$; $p = 0.726$) and decreases in the red-eared slider between 2004 and 2006 (16.0 centimetres, Kruskal-Wallis's test, $n = 79$; $H = 13.670$; $p = 0.003$) (fig. 4).

Sex ratio

The sex proportion is balanced in Mediterranean pond turtle (50.4 % males), less balanced in European pond turtle (59.8 % males) and unbalanced in the red-eared slider (8.1 % males) (fig. 5). Mediterranean pond turtles

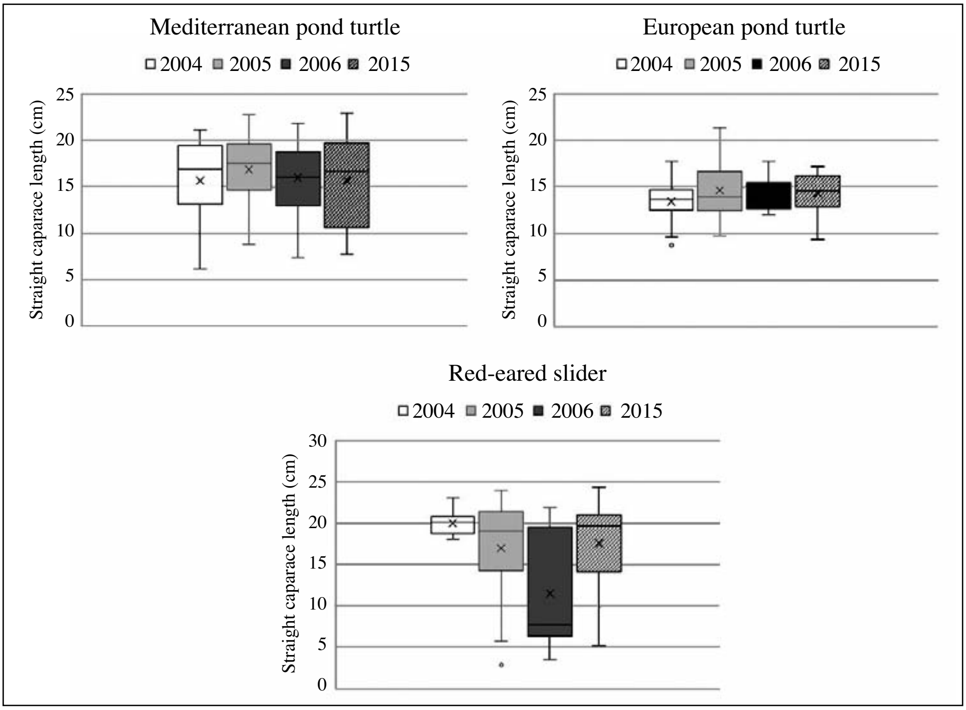

Fig. 4. Straight carapace length (centimetres) of Mediterranean pond turtle ($N = 148$), European pond turtles ($N = 96$) and red-eared slider ($N = 79$) captured. Whiskers express minimum and maximum values; box shows first (Q1) and third (Q3) quartiles; median (Q2) is the middle value in the box; *x* is the average.

showed a balanced sex ratio in all years. European pond turtles also showed non-significant differences in sex ratio, even in 2015 with 72.2 % males ($N = 18$; $X^2 = 3{,}556$, $p = 0.059$, d. f. = 1). However, the red-eared slider had a higher proportion of females in 2005 and 2015, ($N = 14$; $X^2 = 10{,}286$, $p = 0.001$, d. f. = 1; and $N = 24$; $X^2 = 16.667$, $p < 0.001$, d. f. = 1). In 2004 all red-eared sliders were females.

DISCUSSION

Turtle populations have a high adult longevity and survival with a moderate juvenile contribution (Wilbur and Morin, 1988; Congdon and Gibbons, 1983; Frazer *et al*., 1990). Nevertheless, these three freshwater

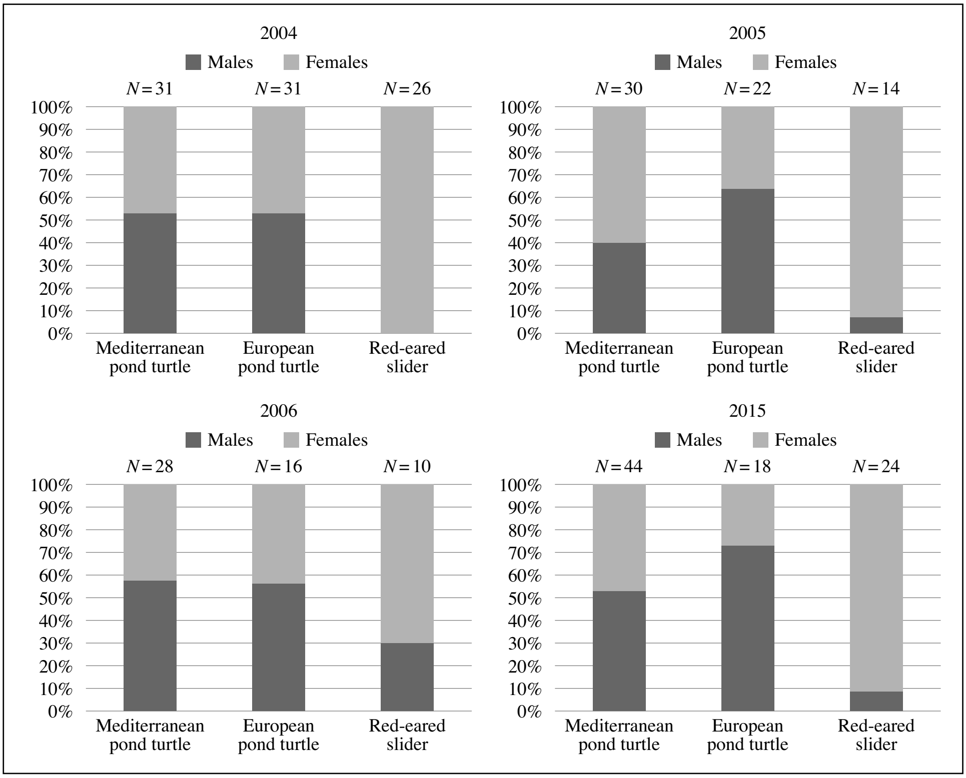

Fig. 5. Sex-ratio of medium and large (mainly adult) freshwater turtles.

turtles show different population characteristics in terms of abundance, size, sex ratio and trend.

The red-eared slider capture frequency is roughly stable over eleven years in our studied lagoons, in spite of removal efforts. In other areas, with a milder climate, the red-eared slider population size increased (Sancho and Lacomba, 2016) despite removals. The population could be even larger as sliders are trapped less effectively than natives (Pérez-Santigosa, 2007). The skewed sex ratio towards females happens in other introduced populations (Cadi *et al*., 2004; Franch i Quintana *et al*., 2007; Pérez-Santigosa *et al*., 2006; Pérez-Santigosa, 2007), due to incubation in breeding centres before skewed trade (Bull *et al*., 1982; Franch i Quintana *et al*., 2007). The presence of juveniles, especially in the last years of the study, demonstrates reproduction in the wild in our study area, similar to what has been recorded in Spain (Martínez-Silvestre *et al*., 1997; De Roa and Roig, 1998; Alarcos-Izquierdo *et al*., 2010; Valdeón *et al*., 2010). Size distribution and sex ratio revealed the arrival of new trade red-eared sliders, mainly big females, as the main growth factor rather than *in situ* reproduction (Maceda-Veiga *et al*., 2019; Martínez-Silvestre *et al*., 2015a). A global skewed sex ratio towards females and the relevance of large red-eared sliders, could be caused by continuous releases in the lagoons or, more likely, its arrival from the Ebro River, originated by releases from captivity, rather than *in situ* reproduction (Maceda-Veiga *et al*., 2019; Martínez-Silvestre *et al*., 2015a). Anyhow, the high growth rate of red-eared slider could imply that some of the large specimens were born in this region. The number of small red-eared slider specimens is double that of natives, which demonstrates higher reproductive rate than natives, due to competitive advantages of the exotic species (Cady and Joly, 2004; Franch i Quintana *et al*., 2007; Martínez Silvestre *et al*., 2015a; Polo-Cavia *et al*., 2014). Native turtles have a different situation. Both species originally live in sympatry, and negative interactions have not been confirmed in the wild (Keller, 1997).

Average size does not change throughout the years (Franch i Quintana *et al*., 2007; Keller *et al*., 1998; Valdeón, 2007), showing age stability. The Mediterranean pond turtle has a balanced sex ratio and a predominance of large sizes, similarly to other Iberian areas (Alarcos *et al*., 2009; Keller, 1997; Díaz-Paniagua *et al*., 2015). European pond turtles have predominantly average sizes and a high proportion of medium and large specimens (Alarcos *et al.,* 2008; Ayres, 2015; Sancho and Lacomba, 2001). Keller (1997) argues that when the European pond turtle reaches sexual maturity its size increases slowly, which could explain the abundance of this size. This does not happen with the Mediterranean pond turtle with its continued growth, particularly females (Keller, 1997). Small specimens are scarce but might be underestimated as they can escape from the nets (Pérez-Santigosa *et al*., 2006). However, they surely are heavily predated by herons and introduced fishes (Valdeón, 2007).

Competition with the red-eared slider could show a higher sensitivity of European pond turtle, that has been experimentally demonstrated in terms of survival and basking (Cadi and Joly, 2003; 2004). Even though, the red-eared slider also competes with the Mediterranean pond turtle (Polo-Cavia *et al*., 2011; 2014), displacing it to the worst habitats as they have higher reproductive potential, competing for basking places and food. Additionally, the Mediterranean pond turtle avoids the red-eared slider's chemical secretions (Franch i Quintana *et al*., 2007; Polo-Cavia *et al*., 2009; 2014). The higher sensitivity of the European pond turtle towards the red-eared slider could be the reason for its decrease and the stability of the Mediterranean pond turtle when living together in other studied southern Iberian lagoons (Pérez-Santigosa *et al*., 2006). This could explain the differences between the two lagoons studied in this paper: high red-eared slider density matches with low European pond turtle density. Furthermore, the extraction of red-eared slider in our study area could have favoured the increase of the Mediterranean pond turtle. The ability of the Mediterranean pond turtle to compete and survive in the same habitat as the red-eared slider has already been observed (Martínez-Silvestre *et al*., 2012).

Other factors negatively affecting the European pond turtle could be eggs, juvenile and adult predation by invasive fishes (Osorio and Alarcos, 2019; Valdeón, 2007) and mammals (Ayres, 2015); weight loss and high mortality (Cadi and Joly, 2003); diseases (Hidalgo-Vila *et al*., 2009; Iglesias *et al*., 2015) or longer hibernation periods (Pérez-Santigosa *et al*., 2013). The Mediterranean pond turtle better tolerates water eutrophication (Keller *et al*., 1995) and has a more diverse diet, which is an advantage compared with the European pond turtle and considering the competition with the red-eared slider (Keller and Busack, 2001).

The predominance of adult males among European pond turtles, which has already been observed (Keller, 1997; Alarcos *et al*., 2008; Ayres and Cordero, 2004), could be due to a more important predation of adult females during egg laying (Alarcos *et al*., 2008).

Overall, the red-eared slider seems to be a worth considering conservation problem for native freshwater turtles in the Ebro Sotos and Galachos Natural Reserve. Temperatures increase due to climate change could worsen this (Hansen *et al*., 2006; New *et al*., 2011). We recommend proceeding with the red-eared slider removal and carrying out environmental education campaigns to avoid releases in the wild, which is one of the main reasons for the expansion of the red-eared slider population.

CONCLUSIONS

- In spite of the considerable trapping effort (206 extractions), captures of red-eared sliders did not decrease nor increased during the study period. Reproduction occurs and large females predominate, which could come from captivity.
- On the contrary, captures of Mediterranean pond turtle increased, as well as its population size from 36 to 90. It had a balanced sex ratio and a predominance of large individuals.
- European pond turtle population size estimation was similar between 2005 and 2015 (28), with predominance of large individuals.
- Juveniles proportion was low in native freshwater turtles (8.8 % in the European pond turtle and 10.4 % in the Mediterranean pond turtle), and large in the red-eared slider (24.1 %), the later probably due to its higher reproductive rate.

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