

# Population traits of the invasive *Trachemys scripta elegans* (Reptilia: Testudines: Emydidae) (Wied-Neuwied 1838) at Mallorca (Balearic Islands, Spain)

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The importation of the red-eared slider (*Trachemys scripta elegans*) to Europe for the pet trade has caused frequent release of this alien turtle to the natural environment. Because of these actions, this alien turtle is currently present in wetlands of many countries worldwide. The reproduction of *T. s. elegans* has been cited in several localities of Mediterranean countries including our study area, s'Albufera de Mallorca (Muro, Spain). During the study 116 individuals were captured; 27 males, 46 females and 43 juveniles. This population was mostly composed of adults, with a statistically significant sexual dimorphism in body size and a female biased sex-ratio. The smallest reproductive female was 172,0 mm of straight carapace length (SCL), and no correlation was found between clutch size and female body length. The 88.9% of sexually mature females were reproductive females and 7.5% of these reproductive females had calcified eggs in their oviducts. The demographic and reproductive parameters analyzed showed a great capacity of colonization and reproduction of the red-eared slider in s'Albufera de Mallorca. The results obtained show the need to propose policies to eradicate the introduced populations of *T. s. elegans*, as well as to promote the sensitivity of the owners to avoid pet releases to the natural environment.

**Key words:** *Trachemys scripta elegans*, *Albufera de Mallorca*, *Natural Park*, *invasive species*, *demographic traits*, *reproductive biology*.

CARACTERÍSTIQUES POBLACIONALS DE LA INVASORA *TRACHEMYS SCRIPTA ELEGANS* (REPTILIA: TESTUDINES: EMYDIDAE) (WIED-NEUWIED 1838) A MALLORCA (ILLES BALEARS, ESPANYA). La importació de la tortuga de Florida (*Trachemys scripta elegans*) a Europa com a animal de companyia ha causat una alliberació freqüent d'aquesta espècie al lloc de tortuga al medi natural. Com a conseqüència, aquesta espècie de tortuga es troba present a zones humides de la majoria dels països d'arreu del món. La reproducció de *T. s. elegans* ha estat reportada a diferents localitats dels països mediterranis, incloent la zona d'estudi, s'Albufera de Mallorca (Muro, Espanya). Al llarg de l'estudi 116 individus foren capturats; 27 mascles, 46 femelles i 43 juvenils. Aquesta població estava composta majoritàriament per adults, amb un dimorfisme sexual estadísticament significatiu en la mida corporal, i una proporció de sexes enviada cap a les femelles. La femella reproductora més petita tenia 172,0 mm de longitud recta de la closca (SCL) i no es va trobar una correlació entre la mida de la posta i la longitud corporal. El 88.9% de les femelles eren sexualment madures i el 7.5 % d'aquestes tenia ous calcificats als seus oviductes. Els paràmetres demogràfics i reproductius analitzats mostren una gran capacitat de colonització i reproducció de la tortuga de Florida a s'Albufera de Mallorca. Els resultats obtinguts mostren la neces-

sitat de proposar polítiques per a eradicar les poblacions introduïdes de *T. s. elegans elegans*, així com promoure la consciència dels propietaris d'evitar l'alliberació d'animals de companyia al medi natural.

**Paraules clau:** *Trachemys scripta elegans*, *Albufera de Mallorca*, *parc natural*, *espècie invasora*, *característiques demogràfiques*, *biologia reproductiva*.

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## Introduction

Globalization and increase in animal trade in the last decades have contributed to the accidental or deliberate propagation of alien species to new distribution areas (IUCN, 2000; Manchester and Bullock, 2000; Di Castri, 2012). Introduction of alien species outside their natural distribution range is the second cause of global biodiversity loss after habitat fragmentation (Walker and Steffen, 1997; IUCN, 2000). It can alter resident communities through phenomena such as competitive exclusion, predation, transfer of parasites and hybridization with native species (Manchester and Bullock, 2000; Hidalgo-Vila *et al.*, 2009; Ayres *et al.*, 2013).

The natural distribution range of the red-eared slider (*Trachemys scripta elegans* (Wied-Neuwied 1838)) includes the Southeastern United States of America (Iverson, 1992), from Illinois down through the Gulf of Mexico (Burger, 2009). However, because of its massive sale as a pet and its release to the natural

environment for several decades, populations of this species are widely distributed worldwide (Lever, 2003). After turtle trade was banned in United States of America in 1975, turtle farms began to import specimens to Europe and Asia (Telecky, 2001). *T. s. elegans* was the most commercialized subspecies in Europe for several decades (Patiño-Martínez and Marco, 2005) until in 1997 the European Union banned its importation because of its invasive skills and its effect on native species (Balmori, 2014). For this reason, *T. s. elegans* has been introduced in part of America (Perry *et al.*, 2007), Asia (Chen and Lue, 1998), Africa (Newberry, 1984) and Europe (Cadi *et al.*, 2004; Pérez-Santigosa *et al.*, 2006, 2008) in the last decades. Nowadays, this species is considered to be among the 100 most invasive species worldwide (Lowe *et al.*, 2000) and one of the 20 most harmful invasive species in Spain (GEIB, 2006).

Reproductive populations have been reported in Mediterranean countries such as Spain (Pérez-Santigosa *et al.*, 2006, 2008), Italy (Ferri and Soccini, 2003) or France

(Prévot-Julliard *et al.*, 2003; Cadi *et al.*, 2004), in areas with suitable conditions for egg incubation (Pérez-Santigosa *et al.*, 2008). In Spain, reproduction of *T. s. elegans* under natural conditions was firstly reported in 1997 (Martínez-Silvestre *et al.*, 1997). In 2012, *T. s. elegans* populations had already covered all the Iberian Peninsula, the Balearic Islands and the Canary Islands (González, 2012). At the Balearic Islands the first record dated from 1993 in Mallorca (Rivera and Arrivas, 1993) and later its reproduction was reported at s'Albufera de Mallorca (Mas and Perelló, 2001; Pinya *et al.*, 2007). The occurrence of *T. s. elegans* has also been reported at Menorca (Rivera and Arrivas, 1993; González, 2012), Ibiza and Formentera (Pinya and Carretero, 2011). In Mallorca, the introduction of *T. s. elegans* and its reproduction is considered to threaten the European pond turtle *Emys orbicularis* (Linnaeus, 1758), mainly present in s'Albufera de Mallorca (Velo-Antón and Pinya, 2015).

*T. s. elegans* has a pronounced sexual dimorphism in body size in which females reach significant larger body sizes than males in the same population (Gibbons and Lovich, 1990). The degree of difference between body length in both sexes depends on several parameters such as sampling biases, sex ratio of the population, size in which individuals of each sex reach maturity, growth rates and predatory pressures (Gibbons and Lovich, 1990). In terms of reproduction, *T. s. elegans* is not characterized by a high reproductive potential, but instead high survival and longevity are the factors that keep populations stable (Cagle, 1950; Pérez-Santigosa *et al.*, 2006).

*T. s. elegans* can live in a wide variety of freshwater ecosystems but it prefers calm and shallow waters with abundant

vegetation and basking places (Gibbons and Lovich, 1990). *T. s. elegans* is able to colonize new areas and establish breeding populations in a short period of time (Pérez-Santigosa *et al.*, 2006). It is considered a competitor of two native species of turtles (*E. orbicularis* and *Mauremys leprosa* (Schweiger, 1812)) (Cadi and Joly, 2004; Pérez-Santigosa *et al.*, 2008), which are in regression in some areas of Spain, including the island of Mallorca (Pleguezuelos *et al.*, 2002; Velo-Antón and Pinya, 2015). In comparison with native species, specimens of *T. s. elegans* reach larger body lengths, and in terms of reproductive ecology they reach sexual maturity earlier, they have longer laying periods and they are more fecund and fertile (Arvy and Servan, 1998; Andreu *et al.*, 2003; Pérez-Santigosa *et al.*, 2008). The red-eared slider can compete for nesting sites against native species, basking places and food, and they can be involved in interference competition (Cadi and Joly, 2004). The exclusion of *E. orbicularis* from basking places by *T. s. elegans* has been recorded (Cadi and Joly, 2003) and asymmetrical responses to competition have been reported, with higher survival and growth of *T. s. elegans* compared to *E. orbicularis* (Cadi and Joly, 2004).

Studies based on the ecology of introduced populations provide crucial information for the correct management of this alien species in the natural environment. Demographic and reproductive parameters vary according to the population. For this reason, in order to guarantee a correct management, it would be necessary to analyze the demographic traits of each population. To date, demographic and reproductive traits of the population of *T. s. elegans* from Mallorca have not been analyzed, so its management is conditioned to the available information

from other populations. The present study aims to analyze the demographic and reproductive parameters of the population of *T. s. elegans* from Mallorca. With these parameters, it is intended to size the problematic that the introduction of this species produce and to contribute to the management for its eradication where this species coexists with the European pond turtle *E. orbicularis*.

## Materials and methods

### Study area

This study was carried out in s'Albufera de Mallorca, the largest wetland in Mallorca island (Balearic Islands, Spain) and where the only population currently known in Mallorca is located (Velo-Antón and Pinya, 2015). S'Albufera de Mallorca has an area of 1.646,48 hectares and it is localized in the northeast of the island, at the municipalities of Muro and Sa Pobla. It was declared Special Protected Area for birds under the European 2000 Network, Natural Park in 1988 and RAMSAR wetland in 1989 (Riddiford and Serra, 1996). S'Albufera de Mallorca consists of a system of artificial channels that collect water from an extensive hydro geographical basin. There are five main streams that lead the rainwater to the sea (Martínez-Taberner *et al.*, 1995). The wetland is mainly composed of reeds, with *Phragmites australis* and *Arundo donax* as dominant species (Riddiford, 2003; Ebejer, 2006). Predominant aquatic habitats contain fresh or brackish water, according to the proximity of the sea. It highlights for being one of the most important biodiversity hotspots of the Balearic Islands, mainly due to its condition of wetland (Pinya *et al.*, 2008).

### Sampling and data collection

*T. s. elegans* specimens were trapped with partial submerged traps baited with three species of fish (*Sardina pilchardus*, *Scomber scombrus* and *Spicara smaris*) used alternately according to their availability. Traps were tied to the shore and partial flotation was achieved by two floats located in both sides of the traps.

Twelve traps were distributed in Canal Ferragut (1), Canal Loco (3), Camí dels Senyals (2), Canal de Sa Senyora (2), Canal de Sa Siurana (2) and Canal des Sol (2). Sampling period lasted from June to September 2016 and traps were checked weekly. In addition, individuals captured in campaigns carried out by the Consorci de Recuperació de Fauna Silvestre de les Illes Balears (COFIB) during the years 2015 and 2016 were also analyzed. Information of date of capture were not recorded by the COFIB. Captured turtles were euthanized by veterinarians of the COFIB and preserved frozen until they were analyzed.

### Demographic traits and reproductive biology

Captured specimens were analyzed in detail to confirm the correct ascription to the subspecies *elegans*. Sex of adults was determined by observing external secondary sexual characters and the reproductive organs. Biometric parameters such as straight carapace length (SCL, mm), and body mass (g) were measured with a digital caliper and an electronic scale, respectively. Body condition index (BCI) was calculated as Fulton's K Index ( $BCI = [\text{body mass (g)} / \text{SCL}^3 \text{ (cm)}] \times 10^4$ ); Ricker, 1975; Koch *et al.*, 2007; Avens *et al.*, 2012).

The attainment of sexual maturity depends on body size, not age (Cagle, 1950; Pérez-Santigosa *et al.*, 2006, 2008). For this reason, all the individuals that

showed external secondary sexual characters were considered adults. In case of males, individuals larger than 100 mm SCL were classified as adult and sexually mature males (Cagle, 1950; Pérez-Santigosa *et al.*, 2006, 2008). In case of females, specimens larger than 170 mm SCL were considered adult and sexually mature (Cagle, 1950; Pérez-Santigosa *et al.*, 2006, 2008). Individuals smaller than these measures did not show external secondary sexual characters and were considered juveniles (Pérez-Santigosa *et al.*, 2006). After biometric parameters were recorded, adult specimens were dissected. For females the presence or the absence of eggs in their oviducts was registered. Sexually mature females were classified into reproductive or non-reproductive females according to whether they were egg-bearing females or not. Follicles and oviductal eggs were classified into categories according to their diameter and calcification. Follicles were classified in two categories according to their diameter:  $< 10$  mm (named as “small follicles”) and  $\geq 10$  mm (named as “enlarged follicles”) according to Congdon and Tinkle (1982). Oviductal eggs were classified in two categories according to their degree of calcification: partially and totally calcified.

### **Statistics**

Demographic traits of the population were analyzed. Size-frequency distribution of the population was plotted in order to analyze population composition. Sex ratio of adult individuals and sex ratio of the total of sexed individuals were analyzed by using  $\chi_1^2$  test. Mean, standard deviation (SD) and range (minimum-maximum) of SCL and BCI were calculated. A size-frequency distribution presented as a violin plot for adult males and adult females was plotted to analyze the age structure of adult

specimens. A Student’s t-test and a Wilcoxon test were used to compare SCL and BCI between both sexes, respectively. Life tables and a mortality curve based on the age-specific mortality rate ( $q_x$ ) were constructed to provide information about retrospective population dynamics.

Reproductive parameters of the population were also analyzed. The proportion of reproductive females versus sexually mature females was calculated, in order to obtain the reproductive frequency of females. The size of the smallest reproductive female was registered. Mean, standard deviation (SD) and range (minimum-maximum) of the number of eggs of each category per reproductive female were calculated. Correlations between SCL of the reproductive females and the number of enlarged follicles and oviductal eggs were analyzed with Spearman correlation coefficients. The two categories of oviductal eggs (partially and totally calcified eggs) were analyzed together in order to increase the population sample in the correlation test.

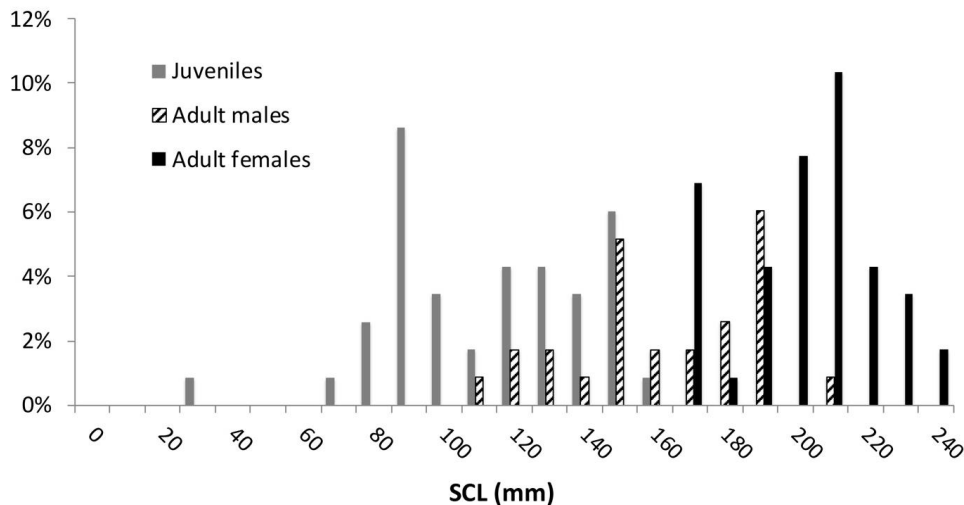
## **Results**

### **Sampling and data collection**

A total of 116 individuals were captured and analyzed to estimate the demographic and reproductive parameters of the population from s’Albufera de Mallorca. Regarding total number of specimens, 32 were captured from June to September of 2016 during the sampling period of the present study and 84 specimens were captured by the COFIB in invasive wildlife control campaigns during 2015 and 2016.

### **Demographic traits**

A total of 27 sexually mature males (larger than 100 mm SCL), 46 sexually mature females (larger than 170 mm SCL)



**Fig. 1.** Size-frequency distribution of the population of *T. s. elegans* captured in s'Albufera de Mallorca. Juveniles (males of < 100 mm SCL; females of < 170 mm SCL), adult males ( $\geq 100$  mm SCL) and adult females ( $\geq 170$  mm SCL) are represented.

**Fig. 1.** Distribució de la freqüència de talles de la població de *T. s. elegans* capturada a s'Albufera de Mallorca. Juvenils (mascles de < 100 mm SCL; femelles de < 170 mm SCL), mascles adults ( $\geq 100$  mm SCL) i femelles adultes ( $\geq 170$  mm SCL) són representades.

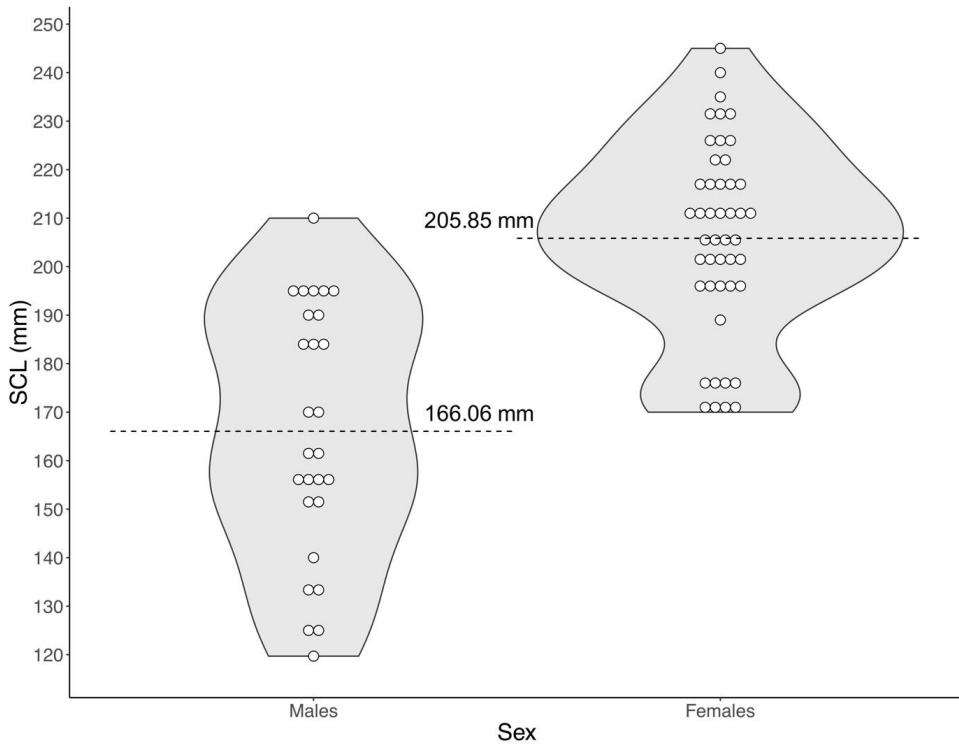
		n	$\bar{x} \pm SD$	Range
SCL (mm)	Males	27	166.06 $\pm$ 25.98	119.70 - 210.00
	Females	46	205.85 $\pm$ 19.68	170.00 - 245.00
	Juveniles	43	119.47 $\pm$ 27.82	32.00 - 160.00
	Total	116	164.57 $\pm$ 45.05	32.00 - 245.00
BCI	Males	27	1.39 $\pm$ 0.23	1.10 - 2.29
	Females	46	1.40 $\pm$ 0.17	1.00 - 1.77
	Juveniles	43	1.53 $\pm$ 0.24	1.08 - 2.14
	Total	116	1.45 $\pm$ 0.22	1.00 - 2.29

**Table 1.** Sample size (n), mean ( $\bar{x}$ ), standard deviation (SD) and range (minimum - maximum) of straight carapace length (SCL) and body condition index (BCI) of adult males, adult females and juveniles of the population sampled.

**Taula 1.** Mida de mostra (n), promig ( $\bar{x}$ ), desviació estàndard (SD) i rang (mínim - màxim) de la longitud recta de la closca (SCL) i l'índex de condició corporal (BCI) dels mascles adults, femelles adultes i juvenils de la població mostrejada.

and 43 juveniles were analyzed (Fig. 1, Table 1). Among these 43 juveniles, 28 were identified as females and the remaining 15 could not be classified. The largest nine males sampled had dark coloration and were considered melanistic individuals. Sex ratio of sexually mature

individuals was significantly biased to females ( $\chi_1^2 = 4.95$ ,  $P = 0.026$ ), with a proportion of 1.00:1.70 (males:females). Sex ratio of all sexed individuals was also significantly biased to females ( $\chi_1^2 = 21.87$ ,  $P = 2.91 \cdot 10^{-6}$ ), with a proportion of 1.00:2.74 (males:females).



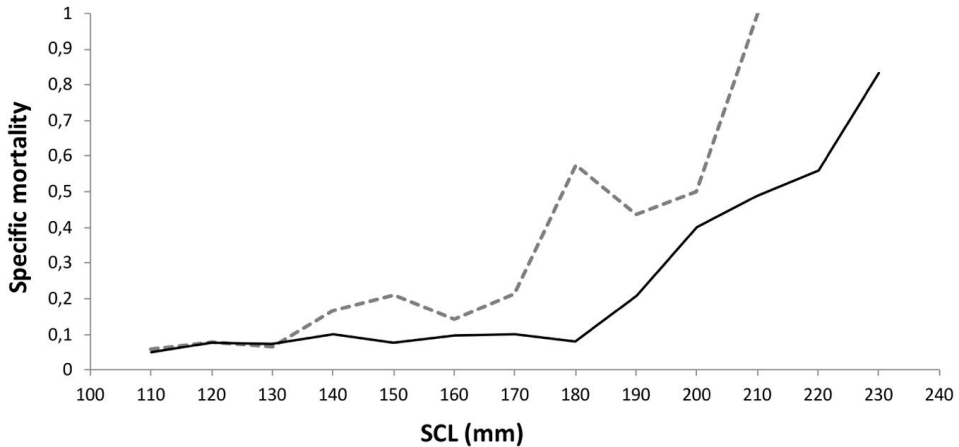
**Fig. 2.** Size-frequency distribution presented as violin plots for adult males and females of the population sampled. Dashed lines indicate mean of SCL of both sexes.

**Fig. 2.** Distribució de la freqüència de talles representada com a gràfic de violí per a mascles i femelles adultes de la població mostrejada. La línia discontinua indica el promig de la SCL d'ambdós sexes.

SCL values of the population ranged from 32.00 mm to 245.00 mm. Adult females reached greater sizes than adult males, attaining 245.00 mm SCL in comparison to 210.00 mm SCL of males. Adult females showed a higher mean value of SCL than adult males, with an average of 205.85 mm SCL in comparison to 166.06 mm SCL in males (Table 1, Fig. 2). Statistically significant differences were observed in SCL between both sexes ( $t = -6.88$ ,  $P = 1.775 \cdot 10^{-8}$ , Student's  $t$  test). BCI values of the entire population ranged from 1.00 to 2.29. Adult males reached greater values than adult females, however, mean

BCI values of adult males and females were 1.39 and 1.40, respectively (Table 1), so no statistically significant differences were found in BCI between males and females ( $W = 507$ ,  $P = 0.196$ , Wilcoxon test).

Specific mortality of males presented low values until the cohort 130 mm. It increased abruptly from the cohort 170 mm and it reached the maximum at the cohort 200, which indicated the maximum longevity of male individuals. Specific mortality of females increased with a steep slope from the cohort 170 mm, size at which females achieve adulthood. It assumed the maximum value at the cohort



**Fig. 3.** Mortality curve based on the age-specific mortality rate ( $q_x$ ) for males and females of the population sampled. Grey dashed line: males; black line: females.

**Fig. 3.** Corba de mortalitat basada en la taxa mortalitat específica de cada edat ( $q_x$ ) per a mascles i femelles de la població mostrejada. La línia discontinua gris pertany als mascles i la negra a les femelles.

230 mm, which indicated the maximum longevity of females in the studied population (Fig. 3).

### Reproductive biology

Up to 88.9 % of sexually mature females ( $\geq 170$  mm SCL) were egg-bearing females, so they were classified as reproductive females. Remaining females did not show follicle development or eggs in their oviducts although they were sexually mature, so they were classified as non-reproductive females. The smallest reproductive female was 172.00 mm SCL and from the cohort 210 mm all females were reproductive (Fig. 4).

Correlation coefficient obtained between the number of enlarged follicles and SCL of reproductive females indicated a weak positive relationship ( $P = 0.02$ ,  $\rho = 0.26$ , Spearman correlation coefficient). Correlation between the number of oviductal eggs (partially and totally calcified eggs) and SCL of reproductive

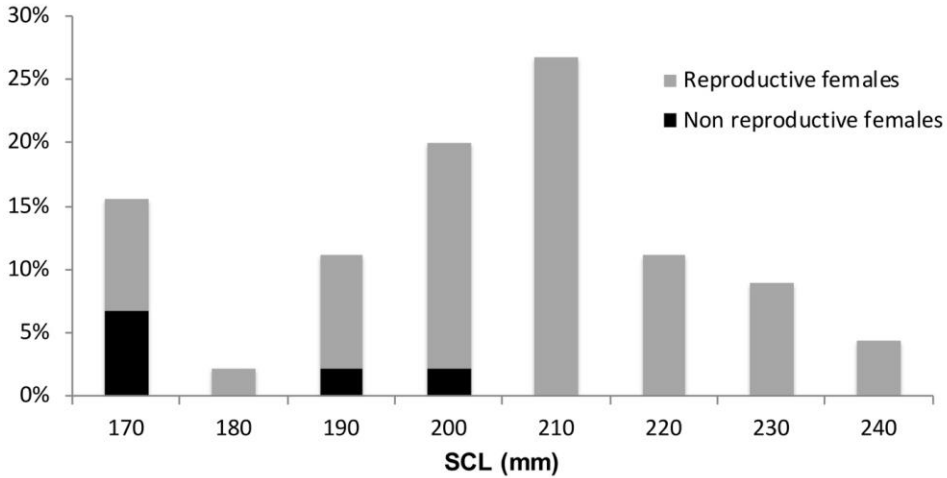
females was not statistically significant ( $P = 0.77$ , Pearson correlation coefficient). The mean ( $\pm$  SD) of “Enlarged follicles” category was  $11.05 \pm 6.50$  eggs. The mean ( $\pm$  SD) value of the “Totally calcified” category was  $11.67 \pm 4.04$  eggs (Table 2). Of all reproductive females, 7.5 % had totally calcified eggs in their oviducts. The sample size of egg-bearing females increased as size of eggs decreased.

## Discussion

### Demographic traits

Free-living individuals of *T. s. elegans* have been reported throughout Spain (Pleguezuelos *et al.*, 2002) and since the late 1990s its reproduction has been cited in many localities of the country (Martínez-Silvestre *et al.*, 1997; Díaz-Paniagua *et al.*, 2013), among them s’Albufera de Mallorca (Mas and Perelló, 2001; Pinya *et al.*, 2007).





**Fig. 4.** Body size-frequency distribution of adult females classified as reproductive and non-reproductive.

**Fig. 4.** Distribució de la freqüència de la mida corporal de femelles adultes classificades com a reproductiva i no reproductiva.

Categories	n	x ± SD
Small follicles (<10 mm)	40	128.68 ± 67.92
Enlarged follicles (≥10 mm)	80	11.05 ± 6.50
Partially calcified eggs	7	8.86 ± 3.39
Totally calcified eggs	3	11.67 ± 4.04

**Table 2.** Sample size of egg-bearing females (n), mean (x) and standard deviation (SD) of the number of follicles and oviductal eggs extracted per reproductive female. Follicles are classified in “small follicles” (<10 mm) and “enlarged follicles” (≥10mm), and eggs are distributed in two categories according to their calcification.

**Taula 2.** Mida de msotra de les femelles portadores d’ous (n), promig (x) i desviació estàndard (SD) del nombre de fol·licles i ous oviductals extrets per femelles reproductores. Els fol·licles foren classificats en “fol·licles petits” (<10 mm) i “fol·licles engrandits” (≥10mm), i els ous es distribuïren en dues categories d’acord amb el grau de calcificació.

At the studied area, a mainly adult population was found, and sex ratio of adults was significantly biased to females. The sex ratio of sexually mature individuals obtained in the present study (1:1.70) differed from the sex ratio observed in some native populations, which present a ratio of 1:1 (Cagle, 1950). In contrast, sex ratio is often unbalanced in both native populations (Gibbons, 1990; Tucker et al., 2008) and also in recently established and

introduced populations (Cadi et al., 2004; Patiño-Martínez and Marco, 2005; Pérez-Santigosa et al., 2006, 2008).

Significantly differences in SCL were observed between both sexes in the present study. Sexual size dimorphism occurs in natural populations (Cagle, 1950; Gibbons and Lovich, 1990) and introduced populations (Pérez-Santigosa et al., 2006). The degree of difference among both sexes varies due to several parameters such as the

sex ratio and the size at which each sex reach maturity (Gibbons and Lovich, 1990). A greater proportion of females and a larger size at which females reach sexual maturity (170 mm SCL compared to 100 mm SCL in males) can be some of the causes of the sexual size dimorphism observed in s'Albufera de Mallorca population.

In terms of body condition, a great variability in values of body condition index (BCI) was observed. This fact shows a great heterogeneity of the estimated parameter, which indicates low reliability as an indicator of the state of the population sampled. It has been observed that body condition varies throughout the year, according to age and sex (Koch *et al.*, 2007; Avens *et al.*, 2012). The information obtained from the specimens captured in the campaigns carried out by the COFIB in 2015 and 2016 did not include the date when the individuals were captured. For this reason, the temporal variable could have altered the interpretation of the body condition results.

Populations in their original distribution range present a population pyramid with greater representation in adult cohorts and a progressive decrease of individuals in late-age cohorts (Cagle, 1950). As the population analyzed in the present study had been introduced in a new territory, the population pyramid obtained did not present a balance between age groups nor between sexes. *T. s. elegans* was firstly cited in Mallorca in 1993 (Rivera and Arrivas, 1993) and its reproduction was firstly cited in 2001 (Mas and Perelló, 2001). Therefore, since 1993 or even previously specimens of this species are found in s'Albufera de Mallorca. In the last years the population from s'Albufera de Mallorca has increased due to the reproduction of sexually mature individuals and the releases of new adult specimens.

For this reason, the structure and dynamics of the population sampled were probably different to native populations.

Specimens released to the environment are usually large individuals. In s'Albufera de Mallorca releases of adult specimens can be demonstrated by the capture of nine melanistic males since melanism is a trait that indicates an advanced age in males (Cagle, 1950). For this reason, capture of melanistic males would be the result of the release of adults to the natural environment, given the impossibility of these old individuals having been born in the wild. Releases of adult specimens to the environment could explain the proportion of sexes biased to females in the studied population. It is because of females reach larger sizes than males (Cagle, 1950; Gibbons and Lovich, 1990), they would be more susceptible to be released to the natural environment than males. Another hypothesis is that during the sampling period females would be more susceptible to be captured than males. Adult males would decrease their activity against high temperatures before females. Reproductive females would have to complete their nesting period and would be exposed to be captured during a longer period of time than males (Pérez-Santigosa *et al.*, 2006). For this reason, more females than males would be captured.

### **Reproductive biology**

Juvenile individuals born in the natural environment were captured in the present study. In addition, a high percentage of sexually mature males and females were also captured. Results obtained are similar to other introduced populations in Spain, in which the smallest sexually mature female was 170.7 mm SCL (Pérez-Santigosa *et al.*, 2006, 2008). Results obtained in the present study are also similar to natural

populations, where males reach sexual maturity at 90-100 mm of plastron length (PL) and females at 150-195 mm PL (Cagle, 1950; Gibbons and Lovich, 1990).

A highly weak correlation between the number of follicles and SCL of reproductive females was found and no correlation was found among the number of oviductal eggs and SCL. Results obtained match with studies realized in introduced populations in Huelva (Spain), where SCL of females was not correlated with clutch size (Pérez-Santigosa *et al.*, 2008). However, clutch size is correlated with body length of females in some natural populations (Cagle, 1950; Congdon and Gibbons, 1983; 1985; Vogt, 1990).

The nesting season varies depending on years and localities. In native populations, the nesting season occurs between April and July (Cagle, 1950; Tucker, 1997). In these populations, females lay at least three different clutches per year with interesting periods of 14-22.5 days (Cagle, 1950; Tucker, 2001). In introduced populations in France the nesting period occurs between May and August (Cadi *et al.*, 2004) and in southern Spain it occurs between April and July (Pérez-Santigosa *et al.*, 2006, 2008). In the Spanish populations, oviductal eggs of different size found in dissected females suggest that three or more clutches per year could be laid (Pérez-Santigosa *et al.*, 2008). Follicles of different diameter and oviductal eggs with distinct degree of calcification were also observed in the reproductive females of the present study, which could mean several clutches per female each year (Congdon and Tinkle, 1982).

In native populations clutch size is between seven and nine eggs, although in some cases 20 eggs have been found (Cagle, 1950). In introduced populations, clutch size was between 4 and 15 eggs in

France (Cadi *et al.*, 2004) and the mean clutch size ( $\pm$  SD) was  $11.5 \pm 2.3$  eggs in southern Spain (Pérez-Santigosa *et al.*, 2008). In conditions of semi-captivity in Catalonia (Spain) the mean clutch size was six eggs (Martínez-Silvestre *et al.*, 1997). In the present work, an average of  $11.67 \pm 4.04$  totally calcified eggs per reproductive female was obtained. The number of totally calcified eggs in reproductive female's oviducts could indicate the mean size of a future clutch. It should also be considered that 7.5 % of reproductive females presented totally calcified eggs in their oviducts, which showed that they were in the final phase of the reproductive process. Many species of freshwater turtles do not reproduce annually in their natural distribution range. Only among 27.2 – 47.1 % of females of *T. s. elegans* species reproduce every year in some areas (Tucker, 2001). In the case of *Chrysemys picta*, 50 to 70 % of females reproduce annually (Congdon and Tinkle, 1982). In introduced populations in southern Spain, the reproductive frequency analyzed was higher, obtaining 80-85 % of reproductive females per year (Pérez-Santigosa *et al.*, 2008). In the present work, the reproductive frequency of females was 88.9 % and it was quite similar than the obtained in the mainland Spain. Thus, an increase in the reproductive potential can be observed in newly established populations relative to their original distribution range (Pérez-Santigosa *et al.*, 2008). This highest value can be caused by the younger age of females in introduced populations in comparison with native regions in which females can reach more than 30 years (Frazer *et al.*, 1990).

### **Management for conservation**

Results obtained in the present and previous studies, as in previous studies,

show that populations usually provide a limited number of juveniles to the natural environment because of the clutch size (Pérez-Santigosa *et al.*, 2006). Within this context, high survival and longevity of adults are the factors that keep a population stable (Cagle, 1950; Pérez-Santigosa *et al.*, 2006), in addition to the period of reproduction (Cagle, 1950).

Established populations of *T. s. elegans* show that this species can reach a great abundance in invaded areas, being able to exceed the abundance of populations of native chelonians (Martínez-Silvestre *et al.*, 2011). Several demographic and reproductive traits obtained in the present work indicate the great capacity of colonization and reproduction of *T. s. elegans* in s'Albufera de Mallorca. Some of these traits have been the high number of individuals captured, the high proportion of sexually mature specimens, the higher reproductive frequency of females relative to natural populations and the capture of juveniles born in the natural environment.

The red-eared slider has been reported in other areas of Mallorca island. However, the most abundant population with reproductive potential is located in s'Albufera de Mallorca (Pinya *et al.*, 2007). These data show that this Natural Park has the necessary conditions for the colonization and reproduction of the red-eared slider. It is important to consider that s'Albufera de Mallorca is a widely known wetland in the island, so it is susceptible to the release of pets (Pinya *et al.*, 2007). Five taxa of alien aquatic turtles including *Chelydra serpentina* Linnaeus 1758, *C. picta*, *Mauremys leprosa* (Schweiger, 1812) and *T. s. scripta* have been reported in s'Albufera de Mallorca, but *T. s. elegans* is the species with greater distribution and abundance (Pinya *et al.*, 2007).

Nowadays, information about introduced populations of *T. s. elegans* is only obtained from invasive wildlife control campaigns. Knowledge of demographic and reproductive parameters of these exotic populations provides essential information for their correct management. Through these data, it is possible to size the problematic that this species causes to the natural environment, as well as to monitor the effectiveness of the control actions carried out in managed populations.

Introduction of alien species can lead to loss of biodiversity (Walker and Steffen, 1997; IUCN, 2000), competition with native species and parasite transfer (Cadi and Joly, 2004; Pinya *et al.*, 2007; Velo-Antón and Pinya, 2015). In Mallorca, the introduction of *T. s. elegans* constitutes a major threat to native species, so it is necessary to implement management measures against this alien species. Essential actions of management include prevention actions, control actions and finally, environmental education and sensitization of the population. In terms of prevention actions, it would be necessary to reinforce the regulation of the importation of potentially invasive alien species. In terms of control actions, it would be necessary to propose policies to eradicate the introduced populations of *T. s. elegans*, including detection of individuals, capture of specimens and monitoring of the areas where the extraction has been carried out (Pérez-Santigosa *et al.*, 2006). In addition, eradication programs should continue for a period of 3-5 years to avoid the re-establishment of new populations (Pérez-Santigosa *et al.*, 2008). Finally, environmental education of citizens through informative campaigns would be of great importance to avoid the release of exotic

species to the natural environment (Pérez-Santigosa et al., 2006).

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