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Preliminary data on the structure of freshwater turtle populations (*Emys orbicularis* and *Mauremys leprosa*) in a stream in the Natural Park of Los Arribes del Duero (Zamora, Spain)

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Abstract: Although talking about the Iberian terrapins' decline has become frequent, there are no intensive studies about their ecology and conservation in Castilla y León. Both the European pond terrapin (*Emys orbicularis*) and the Mediterranean pond turtle (*Mauremys leprosa*) are widely distributed in the region, especially in its south-western part (Zamora and Salamanca). We present the results of a one year-monitoring in one population of each species in the Natural Park of Los Arribes del Duero (Zamora). The study was carried out along a 3 km stretch of stream. The area is more appropriate for *E. orbicularis*, with 78 captured individuals, whereas only 16 *M. leprosa* were found. The Mediterranean pond turtle prefers greater water bodies, restricted in the area to three man-made ponds. The activity of *E. orbicularis* begins earlier in the year, with an aestivation period, while *M. leprosa* remains active in summer. The population size of the European pond terrapin was estimated in 110 individuals according to the Jolly-Seber method of mark-recapture. This population would be quite old, with only 13% of immature individuals. This population structure motivates the priority in establishing conservation measures directed to protect adult individuals. This is the first study of freshwater turtle populations in Castilla y León. The information presented here, as well as in further studies with similar methodology, will be necessary to protect these species in the region.

Key words: Castilla y León, conservation measures, *Emys orbicularis*, *Mauremys leprosa*, population monitoring.

Resumen: Datos preliminares sobre la estructura de las poblaciones de galápagos (*Emys orbicularis* y *Mauremys leprosa*) en un arroyo del Parque Natural de Los Arribes del Duero (Zamora, España). – Aunque cada vez se habla con más frecuencia de una regresión de los galápagos ibéricos, en Castilla y León no existe ningún estudio detallado acerca de su ecología y conservación. Tanto el galápago europeo (*Emys orbicularis*) como el galápago leproso (*Mauremys leprosa*) están bien representados en la región, especialmente en su zona suroccidental (Zamora y Salamanca). Presentamos los resultados correspondientes a un año de seguimiento realizado sobre una población de cada especie en el Parque Natural de Los Arribes del Duero (Zamora). El estudio se llevo a cabo en un tramo de arroyo de 3 km de longitud. La zona es más apropiada para *E. orbicularis*, especie de la que se capturaron 78 individuos por los 16 de *M. leprosa*. El galápago leproso prefiere medios de cierta entidad, restringidos en el área a tres charcas artificiales. *Emys orbicularis* comienza su actividad anual antes que *M. leprosa*; además, posee un periodo de inactividad estival, mientras que *M. leprosa* se mantiene activo durante el verano. El tamaño de la población de galápago europeo se estimó en 110 individuos según el método de Jolly-Seber de marcaje-recaptura. Dicha población estaría bastante envejecida, con sólo un 13% de individuos subadultos. Esta estructura poblacional motiva la necesidad de establecer medidas orientadas a la protección de los individuos adultos. Este trabajo constituye el primer estudio poblacional de galápagos conocido en Castilla y León. La información recogida aquí, así como en estudios similares a desarrollar en el futuro, será clave para la conservación de ambas especies en la región.

Palabras clave: Castilla y León, *Emys orbicularis*, *Mauremys leprosa*, medidas de conservación, seguimiento de poblaciones.

INTRODUCTION

The interest in conservation of freshwater turtles has experienced a significant increase during the last years. These species are strongly linked to their habitats and consequently greatly affected by their deterioration, therefore, the conservation of these animals has become a priority in many regions of the planet. In the Iberian Peninsula, several cases of population decline have been reported for the two native species throughout their distribution range (e.g. ARAÚJO *et al.*, 1997; MASCORT, 1998; AYRES & CORDERO, 2002). In spite of this apparently poor conservation status, especially in the case of the European freshwater turtle (*Emys orbicularis*), studies of the biology and ecology of these species in the region are still very scarce, which makes it difficult to establish the appropriate conservation and management strategies.

Emys orbicularis is widely distributed in Europe, from the Mediterranean to the Baltic Sea, reaching central Asia at the eastern edge of its distribution; it also appears in northern Africa (see review in FRITZ, 2001). In the Iberian Peninsula it also presents a wide distribution, except in the Cantabrian coastal area, although this range is in fact highly fragmented and populations are quite isolated in most of the region (see review in KELLER & ANDREU, 2002). The species has been catalogued on a global scale as Nearly Threatened (NT) by the IUCN, but in Spain it has been recently proposed as Endangered (EN) (Asociación Herpetológica Española, unpublished data). The other native terrapin, the Mediterranean pond turtle (*Mauremys leprosa*) is, in Europe, exclusive to the Iberian peninsula and southern France. In northern Africa it is widely distributed in Morocco, Algeria and Tunisia. In the Iberian Peninsula it is absent from some areas of the northwest (DA SILVA, 2002).

The main threats for terrapin conservation are habitat degradation, water pollution and the loss of aquatic environments, the presence of introduced competitors such as *Trachemys scripta* (CADI & JOLY, 2003, 2004) and unintentional captures in crayfish traps (e.g. GÓMEZ-CANTARINO & LIZANA, 2000). Nevertheless, the main obstacle to its conservation in Spain is the lack of information about the species' real sensitivities to these threats. In the region of Castilla y León (central Spain), both *E. orbicularis* and *M. leprosa* have been proposed as Vulnerable species (LIZANA *et al.*, 2002) and their conservation is considered top priority. The information about terrapins in the region is restricted to isolated observations during the compilation of the atlas of distribution of amphibians and reptiles (PLEGUEZUELOS *et al.*, 2002) and thorough studies of population biology or conservation do not exist. In consequence, the terrapin conservation status in Castilla y León cannot be known with accuracy, although the reported decline of the species in the whole Iberian Peninsula leads to the supposition that the situation might be similar for this region.

In this study we present the first data obtained during the monitoring of two sympatric populations of *E. orbicularis* and *M. leprosa* in Castilla y León. Due to the characteristics of the study area, the information corresponds mainly to *E. orbicularis*. Nevertheless, we also present the results obtained for *M. leprosa*. The aim of this study is to establish a starting point in the analysis of the conservation status of terrapins in Castilla y León and the subsequent establishment of measures if necessary.

MATERIAL AND METHODS

The study was carried out along a 3 km stretch of the Las Carvitas stream in the south-west of the province of Zamora (Castilla y León, Spain) (41º 21’ N; 6º 20’ W), close to the border with Portugal. The area is located on the Mesomediterranean bio-climatic floor at 650 m above sea level. Vegetation around the stream is mainly constituted by grasslands, brooms, rockroses and holm oak woods and mature riparian forest does not exist. Although the area is mainly used for livestock it is not exposed to severe impacts. The stream is seasonal and becomes completely dry in summer with the exception of a few ponds that constitute the only permanent habitat for aquatic wildlife. These permanent waters include two artificial ponds (deep 3 m) of 20 and 30 m diameter in the stream headwaters. A third artificial pond with similar characteristics is located 120 m downstream. Finally, a few small pools within the stream bed complete the permanent waters, the rest of the stream being constituted by rapids and quite shallow water. At the lower edge of our study area there is a zone with high banks and rapids that constitute a barrier for terrapin movement along the stream.

The stream was sampled from March to September in 2004 and from March to May in 2005. Sixteen samplings were carried out. Samplings consisted in standardized transects along the stream combined with the installation of baited crayfish traps of 1.5 m long and 40 cm in diameter. Terrapins were thus captured both by hand and with traps. The seasonal activity of each species was estimated from the encounter rate in every sampling. The population reproductive phenology was recorded from the observation of mating or pregnant females. To detect pregnant females we touched the inguinal zone of animals, as described by ANDREU & VILLAMOR (1989).

The sex of the captured individuals was determined by the observation of sexual secondary characteristics, such as plastron concavity, distance from the vent to the base of the tail, and tail length and thickness. Individuals were considered immature if none of these characteristics allowed the determination of sex. Age was estimated from the count of growth rings (SEXTON, 1959). All the individuals were marked on the marginal shields following the methodology described in CAGLE (1939). Terrapins were also weighed with a Kern® MH5K5 balance (max = 5 kg; precision = 5 g) and several biometric measures such as carapace height, carapace straight length, carapace curved length, plastron length and total widths at the second, third and fourth plastron sutures were taken with a ACHA® 17-262 digital calliper (max = 300 mm; precision = 0.01 mm). Individuals were released immediately after marking and measuring.

Sexual dimorphism was analysed by comparing weights and lengths with a Kolmogorov-Smirnoff Z test. In addition, observations of colour and design were also compared between sexes. The population size was estimated from the marked individuals following the mark-recapture Jolly-Seber method for open populations (POLLOCK *et al.*, 1990). Finally, we recorded all the morphological abnormalities observed during the study as a possible indicator of the population status. SPSS® 11.5 for Windows was used for the statistical analyses.

RESULTS

Although both species were found in the study area, the population of *M. leprosa* presented a smaller distribution in the stream. This species was restricted to three large ponds at the headwaters. The only individual captured outside these ponds was found 70 m downstream. *Emys orbicularis* was found homogeneously distributed along the study area and appeared in large ponds, shallow waters and rapids.

Emys orbicularis population

Seventy-eight *E. orbicularis* individuals were found during 15 of the 16 samplings of the study. The seasonal activity showed a strong variation through the year (Fig. 1). Activity was higher in the spring. It dropped during summer and completely stopped in September. Sixty-eight (39 males and 29 females) of the captured individuals were identified as adults. Thus, the sex ratio was estimated in 1.34:1 in favour of males and the percentage of adult individuals in the population was 87.2%. The analysis of age classes from growth rings showed an old population with more than 50% of individuals 13 years old or older (Fig. 2). Sexual maturity seemed to be reached at 8 years in males and 10 years in females, although some 10 years old individuals were identified as immature. The distribution of age classes was quite coincident with that of the size classes based on the carapace length (Fig. 3). Minimum size at maturity would be about 95 mm for males and 111 mm for females, although some terrapins up to 125 mm-long did not present sexual characteristics. Age determination in the older individuals was difficult because of shield erosion, which made the observation of growth rings difficult. Nevertheless, females seemed to live longer than males, as they were more frequent in the older age classes (Fig. 2).

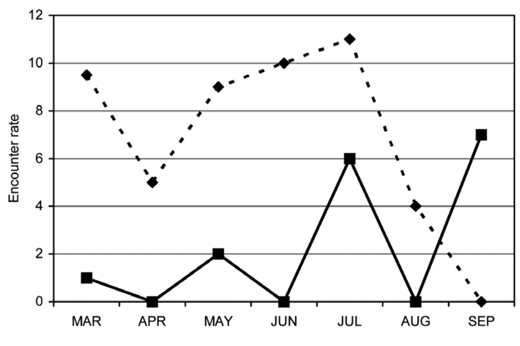


FIGURE 1. Monthly encounter rate of terrapin populations in the Las Carvitas stream estimated from the encounter rate. The continuous line corresponds to *Mauremys leprosa* activity and the dashed line to *Emys orbicularis*. Error bars are not represented because of the absence of replicates for some months.

FIGURA 1. Tasa de captura mensual de las poblaciones de galápagos en el arroyo de Las Cavitas. La línea continua corresponde a *Mauremys leprosa* y la línea discontinua a *Emys orbicularis*. No se representan las barras de error debido a la ausencia de réplicas para algunos meses.

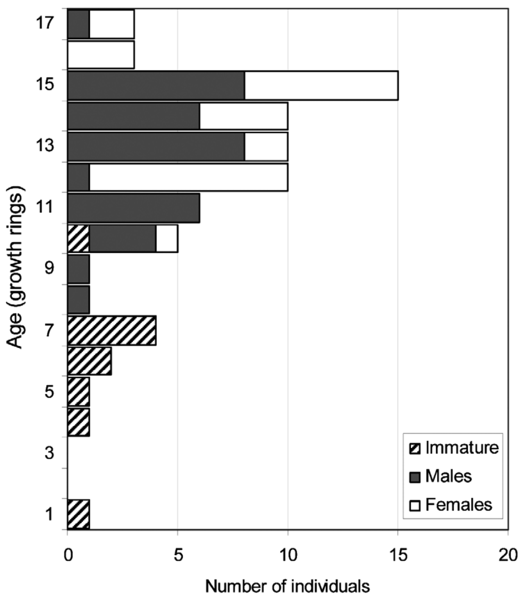


FIGURE 2. Population structure of *Emys orbicularis,* based on growth rings.

FIGURA 2. Estructura de la población de *Emys orbicularis* basada en los anillos de crecimiento.

All biometric measures and weight reflected sexual dimorphism; females always presented a greater size and weight than males (Table 1). The biggest terrapin captured was a female of 165 mm carapace length, 75.4 mm height and 770 g weight. The largest male was 154 mm long, 63 mm-high and weighed 615 g. Sexual dimorphism was also observed in body coloration and design. Males usually showed darker colorations in

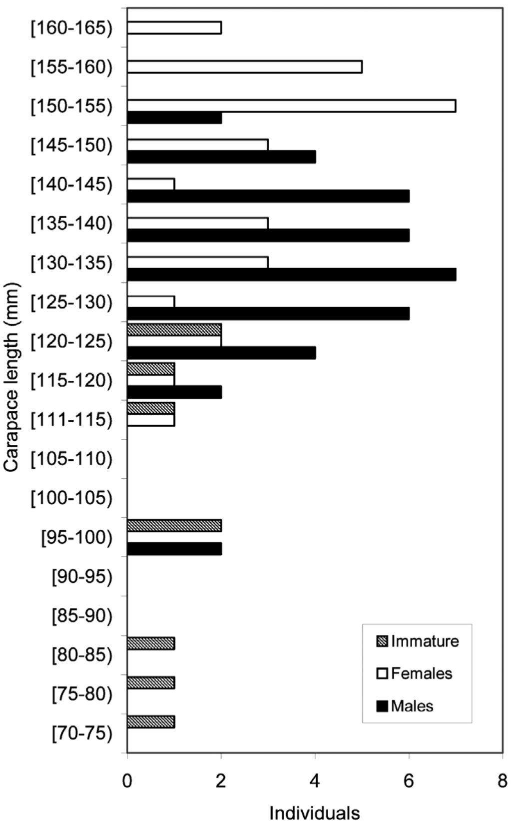


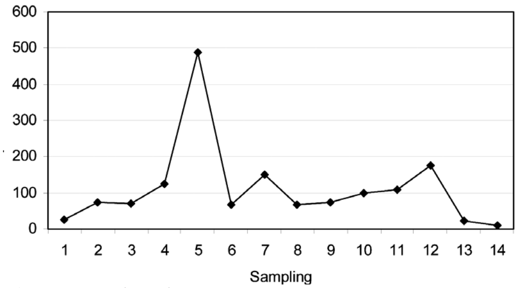
FIGURE 3. Distribution of size classes based on the straight carapace length in the *Emys orbicularis* population.

FIGURA 3. Distribución de las clases de tamaño basadas en la longitud recta del caparazón en la población de *Emys orbicularis*.

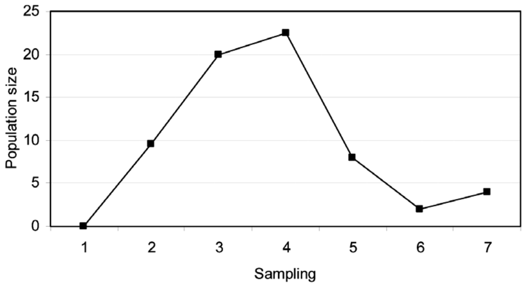
the plastron than females, while females often presented yellow shades in the lower jaw. The contrast of yellow and black colours in the upper part of the head was greater in females. Finally, we observed how most of the males in the population had red irises, while females did not. These patterns were based on simple observations and we could not quantify them, although these trends in sexual dimorphisms in the population were clear.

Three matings were observed in late March and early April, thus this would be the reproductive period in this population, which is confirmed by the fact that all the females with calcified eggs in the oviduct were captured in June. Nevertheless, the only hatchling still exhibiting the egg tooth was found in late March.

The total number of captures during the 15 positive samplings was 144. The estimated rate of marked population after these samplings was over 80%. The estimated population size for each sampling is shown in Fig. 4a. The maximum estimated was 488 individuals, although there are great differences between this maximum value and the rest of the estimations. Overall population size was estimated from all the samplings in 110 ± 31.66 individuals.



a) *Emys orbicularis*



b) *Mauremys leprosa*

FIGURE 4. Estimated population size on each sampling of (a) *Emys orbicularis* and (b) *Mauremys leprosa* from the Las Carvitas stream, based on the Jolly-Seber mark-recapture method.

FIGURA 4. Tamaño estimado en cada muestreo de las poblaciones de (a) *Emys orbicularis* y (b) *Mauremys leprosa* en el arroyo de Las Carvitas basado en el método de Jolly-Seber de marcaje-recaptura.

TABLE 1. Mean, maximum (max) and minimum (min) values of the biometric measures for each sex taken in the terrapin populations from Las Carvitas stream. CL: Carapace straight length, CC: Carapace curved length, PL: Plastron length, H: Total height, W2, W3, W4: Carapace width at plastron sutures, second, third and fourth respectively, W: Weight. Results of Kolmogorov-Smirnoff Z test to compare mean values between sexes are shown.

TABLA 1. Valores medios (mean), máximos (max) y mínimos (min) para cada sexo de las medidas tomadas en la poblaciones de galápagos del arroyo de Las Cavitas. CL: Longitud recta del caparazón, CC: Longitud curva del caparazón, PL: Longitud del plastrón, H: Altura total, W2, W3, W4: Anchura del caparazón al nivel de las suturas segunda, tercera y cuarta del plastron respectivamente, W: Peso. Se muestran los resultados de las pruebas Z de Kolmogorov-Smirnoff para comparar las medias entre sexos.

		Males				Females				Z	P
		N	Mean	Min	Max	N	Mean	Min	Max		
Emys orbicularis	CL (mm)	39	133.1	97.6	153.8	29	144.2	114.6	163.1	1.761	0.004
	CC (mm)	37	149.9	124.0	174.0	28	161.0	126.0	185.0	1.476	0.026
	PL (mm)	39	114.4	102.8	125.4	29	132.7	103.6	152.8	2.928	<0.001
	H (mm)	36	50.5	43.5	63.6	29	62.0	36.9	79.9	2.980	<0.001
	W2 (mm)	35	92.8	76.2	102.8	29	99.9	79.0	121.1	1.697	0.006
	W3 (mm)	35	103.3	77.4	114.3	26	112.8	92.0	131.9	2.065	<0.001
	W4 (mm)	39	107.6	91.2	117.9	29	117.6	94.0	141.1	2.333	<0.001
	W (g)	38	410.4	260.0	615.0	28	574.6	270.0	795.0	2.545	<0.001
Mauremys leprosa	CL (mm)	4	173.4	160.9	184.9	6	173.4	149.7	201.3	0.387	0.998
	CC (mm)	4	183.3	171.0	194.3	5	179.8	153.0	205.0	0.522	0.900
	PL (mm)	4	141.4	131.8	148.2	6	154.1	132.3	175.1	1.033	0.236
	H (mm)	3	56.4	54.0	58.1	6	64.8	55.5	69.8	1.179	0.124
	W2 (mm)	3	111.5	105.8	114.8	6	110.4	96.3	141.0	0.707	0.699
	W3 (mm)	4	118.4	108.5	123.0	6	124.6	109.6	153.4	0.775	0.586
	W4 (mm)	4	126.2	117.5	132.3	6	134.8	115.7	166.6	0.775	0.586
	W (g)	4	645.8	560.0	723.3	6	793.9	460.0	1265.0	1.033	0.236

During the study we observed some individuals showing abnormalities. The most common was septicemic cutaneous ulcerative disease, which consisted in small hemorrhages and ulcerations caused by bacterial infections, mainly on the plastron (see details in HARVEY-CLARK, 1998). These symptoms were observed in 22 individuals, one of which showed over 90% of plastron shields affected by ulcerations. Another common abnormality was the duplication of carapace shields, presented by six males and four females in the population. Shield duplication was mainly observed in the fourth and fifth vertebral shields.

Mauremys leprosa population

The capture rate showed *M. leprosa* as more active from late spring to mid-summer. The seasonal activity in this population was prolonged until later in the autumn than in the *E. orbicularis* population (Fig. 1). Only eight of the 16 samplings resulted positive for this species. The number of captured and marked individuals was 16, six of which were female, four male and six immature. Thus, the estimated sex ratio was 1.5:1 in favour of females.

Statistical differences between sexes were not detected in any of the measures, although females were clearly greater in plastron length, height and weight (Table 1). The absence of statistical significance is most probably due to the low sample size. The biggest captured female was 201 mm-long, 70 mm-high and weighed 1265 g, while the biggest male was 184 mm-long, 57 mm-high and weighed 723 g.

The total number of captures during the eight positive samplings was 24. The rate of marked population was estimated at almost 100%. The maximum estimated population size was 22.5 individuals (Fig. 4b), while the mean value obtained from all the samplings was 11.02±3.44 terrapins.

DISCUSSION

The two native terrapin species were found in the Las Carvitas stream. *E. orbicularis* seems to have a larger population and wider distribution than *M. leprosa*. The former is present in all the available habitats while the latter is restricted to the large permanent ponds where the two species are in syntopy. The presence of both species in the same location is quite usual in areas where their distribution ranges coincide (DA SILVA, 1993; KELLER, 1997). Nonetheless, only a few studies have reported spatial segregation between them. DA SILVA (1993) observed that *M. leprosa,* in the province of Badajoz (SW Spain), occupied larger water bodies than *E. orbicularis*, which concurs with what we have observed. On the other hand, BARBADILLO *et al*. (1999) pointed out that *M. leprosa* could also be present in shallow streams if there are pools that never become dry. KELLER (1997) found *E. orbicularis* almost exclusively in shallow waters in Doñana National Park (SW Spain); however, we have also found the species in permanent ponds.

The activity of *E. orbicularis* significantly decreased during late summer until it disappears in September. Some authors have reported an aestivation period that coincides with the drying up of water bodies (GALÁN & FERNÁNDEZ-ARIAS, 1993; ANDREU & LÓPEZ-JURADO, 1998; BARBADILLO *et al*., 1999). The species might be active again during early autumn after rain refills the pools and diurnal temperatures are still warm. Unfortunately we did not carry our any sampling after September so we could not check this possibility. The fact that *M. leprosa* maintained its activity during the summer could be related to its preference for permanent water bodies.

The population structure in *E. orbicularis*, with a high proportion of adult individuals, coincides with most of the other analysed populations of the species, both in the Iberian Peninsula (ANDREU & LÓPEZ-JURADO, 1998; AYRES & CORDERO, 2001; SANCHO & LACOMBA, 2001; CARRASCO *et al*., 2002) and other European countries (e.g. MAZZOTTI, 1995). The higher proportion of males observed in this population has also been reported in other cases, such as in Doñana National Park (KELLER, 1997). Nevertheless, in some other Iberian populations (CARRASCO *et al.,* 2002), as well as in the Po valley in Italy (MAZZOTTI, 1995), the proportion of females was higher, which would coincide with the results from our *M. leprosa* population. Three reasons might explain the higher proportion of males in the *E. orbicularis* population from the Las Carvitas stream: firstly, females could be exposed to a higher mortality rate than males due to their higher energetic investment in reproduction; secondly, females spend longer periods out of the water because of egg laying, which implies a higher predation risk. Finally, since females reach sexual maturity later than males, the sex ratio would be favourable to males if life expectancy were similar for both sexes; however, we have observed a higher proportion of females in the older age classes, which leads us to suppose that females live longer than males, as indicated by SCHNEEWEISS (2004).

The larger size of females with respect to males, found for both species, has been observed in many other populations. ANDREU & LÓPEZ-JURADO (1998) proposed that in *E. orbicularis* this sexual dimorphism could be due to the fact that females reach sexual maturity later than males, which is in agreement with our observations. Mature individuals invest most of their energy in reproduction, thus decreasing growth rate with respect to the immature stages (WILSON *et al*., 2003), which would imply a larger size of individuals with longer juvenile stages.

Overall the *E. orbicularis* population size was estimated at 110 terrapins, whereas the maximum calculated was 488 individuals. Most of the studies that estimate population sizes or densities suggest that the maximum value estimated, obtained under optimal conditions for the species studied, would probably be the best estimator for the real population size (e.g. CAROTHERS, 1973). However, since our estimations have been made from only the first field campaign, we can expect that the increase in the recapture rate during the samplings would be higher than it should be with regard to the real population size, although this hypothesis will have to be tested in future campaigns. Therefore, the overall estimated population size is the best indicator. Although the estimated value seems low, the sampled area was relatively small, so this estimation could indicate a good population status. MAZZOTTI (1995), using the Jolly-Seber method, estimated an *E. orbicularis* population size of 551 individuals in the Po valley, in a much larger area than that sampled in our study. The total number of individuals in the region of Galicia (NW Spain), where the species is critically endangered, could be even lower than the overall population size estimated in the Las Carvitas stream (AYRES & CORDERO, 2002). The population size of *M. leprosa* has been estimated from a very small number of captured individuals, so the results are not conclusive. We can conclude that this population requires conservation measures while further studies are carried out.

Matings in the *E. orbicularis* population were observed in March and April and the laying period, estimated from the detection of calcified eggs inside females, would be in June. This reproductive phenology coincides with what has been observed in other Iberian populations (ANDREU & LÓPEZ-JURADO, 1998; MERCHÁN & MARTÍNEZ-SILVESTRE, 1999). Nonetheless, the only hatching was found in March, still having the egg tooth, which means that the it had just hatched. This could be due to the existence of a second clutch in the reproductive cycle, which has recently been described in some populations of Southern Spain (ROQUES *et al*., 2006). In populations in Galicia, GALÁN & FERNÁNDEZ-ARIAS (1993) observed courtships in autumn, so egg laying would occur during the early spring. On the other hand, this hatchling found in March could come from the spring matings of the previous year. MITRUS & ZEMANEK (2003) reported the existence of hibernating hatchlings in *E. orbicularis* populations from northern Europe; however, the hatchling we have found should have passed the winter as an egg in stead of as a hatchling.

This study constitutes the first analysis of terrapin populations in the region of Castilla y León. Although the information presented here is insufficient, it does not seem that the *E. orbicularis* population from the Las Carvitas stream is subject to a regressive process. For example, the percentage of individuals showing abnormalities in the carapace shields, which could be an indicator of the population stress, is relatively small in comparison to what has been observed in other Iberian populations, where up to 75% of individuals were affected by these abnormalities (AYRES & CORDERO, 2004).

The small population size of *M. leprosa* could be due to the lack of appropriate habitats for the species rather than a regressive trend of the population.

From our study three main conclusions can be drawn in reference to the conservation of the native terrapin populations; first, it is essential to obtain data about population structure, size and dynamics to establish a starting point for monitoring populations. In addition, data about ecological requirements of the species should be obtained in order to improve the efficiency of habitat management and appropriate conservation measures as indicated by FICETOLA *et al*. (2004). Our observations about the differences between the two species, in habitat use, reproductive phenology and population structure support this proposal. Thus, the second conclusion is related to this matter: environmental heterogeneity should be promoted in order to favour the presence and establishment of both species. Finally, the third conclusion, supported both by our study and others, is the necessity of establishing measures directed mainly towards the conservation of the adult population (KELLER & ANDREU, 2002). The high mortality rates to which juveniles are exposed, the low recruitment rate and the long life expectancy of individuals make it essential to maintain the number of adults to protect these populations.

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