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Morphometry and biological cycle of a European pond turtle (*Emys orbicularis*) population from north-eastern Spain

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Abstract: Morphometry, biological cycle, structure and size of a population of European pond turtle (*Emys orbicularis*) from La Selva County (north-eastern Spain) were studied. A total of 182 individuals were captured from 1997 to 2002 during the activity period. The minimum size of a female with calcified eggs was 103.70 mm (NCL) and the minimum size of a male with secondary sexual characters was 88.90 mm. The results indicate the existence of allometry in six variables for the males (BW, HW, HH, MW2, PCL and HL2), and three for the females (MW1, HW and HL2). A significant difference was only found in the slope between males and females for the width of the third rear right marginal plate (MW1). Precloacal length (PCL) is clearly dimorphic, larger in males. Females have a broader carapace, a longer plastron and they are heavier than males. They also have a relatively greater bridge width (BW) and relatively shorter hindlimbs. The discriminant function shows a large percentage of correct classification (97.50%). The main habitat occupied by *Emys orbicularis* in the study area are ponds, drainage channels and small marshes. The percentage of immatures (55.49%) is very high. Estimated sex-ratio (♀♀:♂♂) was 1:2.24. Activity begins when water temperature is near 9° C about the second fortnight of February and ends at the end of October. The average population density estimated was 1030.60 individuals/ha ± 745.7. Finally, our results are compared with other European populations.
Key words: biological cycle, biometry, *Emys orbicularis*, population size, population structure.

Resumen: Morfometría y ciclo biológica de una población de galápago europeo (*Emys orbicularis*) del noreste de España. – Se ha estudiado el ciclo biológico, la estructura y tamaño de población y se ha realizado un análisis morfométrico de una población de galápago europeo (*Emys orbicularis*) localizada en la comarca de La Selva (NE España). Desde 1987 hasta 2002 se capturaron un total de 182 individuos durante el período de actividad. La hembra de menor talla con huevos calcificados fue de 103.70 mm (NCL), mientras que el macho de menor talla con los caracteres sexuales secundarios desarrollados fue de 88.90 mm. Los resultados indican que existe alometría en seis variables para los machos (BW, HW, HH, MW2, PCL y HL2) y tres para las hembras (MW1, HW y HL2). Entre machos y hembras únicamente la anchura de la tercera placa marginal posterior (MW1) muestra diferencias significativas en la pendiente. La longitud precloacal (PCL) es claramente dimórfica; mucho mayor en los machos. Además los machos muestran unas extremidades posteriores relativamente más cortas y una anchura de puente (BW) relativamente menor. Se ha obtenido una función discriminante con un alto porcentaje de clasificación (97.50%). El hábitat ocupado por la especie en el área de estudio son las charcas, canales de drenaje y pequeñas zonas inundadas. El porcentaje de inmaduros es muy alto (55.49%). La sex-ratio estimada (♀♀:♂♂) fue de 1: 2.24. La actividad se inicia cuando la temperatura del agua alcanza los 9° C, alrededor de la segunda quincena de febrero y finaliza hacia finales de octubre. La densidad media de población estimada fue de 1030.60 individuos/ha ± 745.7. Finalmente los resultados obtenidos se comparan con los de otras poblaciones europeas.
Palabras clave: biometría, ciclo biológico, *Emys orbicularis,* estructura poblacional, tamaño poblacional.

Introduction

The distribution range of *Emys orbicularis* comprises parts of northern Africa, the Mediterranean area and moderate climatic regions of Europe and the Middle East up to the Aral Sea. In the Iberian Peninsula the European pond turtle is present in scattered populations and it is only common in some localities. The species is in a very critical situation all along the Mediterranean coast (Keller & Andreu, 2002) and in Spain is considered rare or scarce and endangered (Andreu, 1997). Moreover, in Catalonia, it has a highly fragmented distribution with populations containing few individuals (Llorente *et al.*, 1995), and little is known about most aspects of the biology and basic ecology of these populations. In this area, Arribas (1991), Llorente *et al.* (1995), Bertolero (1997), Mascort (1998), and Mascort *et al.* (1999) provide some information on north-eastern populations. While some of these papers describe the distribution and conservation status of these populations (Arribas, 1991; Llorente *et al.*, 1995; Mascort, 1998) or are focused in taxonomical issues (Mascort *et al.*, 1999), only Bertolero (1997) provides some data of a mixed population from the Ebro Delta. In this paper we provide information on some hitherto unknown aspects of biometry, biology, population structure and population size of the best preserved population of European pond turtle in north-eastern Spain (Mascort, 1998).

Materials and Methods

The studied population of European pond turtle (*Emys orbicularis*) is located in La Selva County (2° 35' 31" E, 41° 49' 55" N) (Fig. 1), in a prelitoral depression with a Mediterranean climate with frequent winter thermal inversions. This population is made up of several subpopulations located mainly in ponds and drainage channels in the hydrographical basin of the Santa Coloma stream (Fig. 1). This area is considered as an Important Area for Spanish Herpetofauna (Mateo, 2002).

From 1997 to 2002 individuals were captured during their activity period by means of nets, baited funnel traps, and by hand (Pérez *et al.*, 1979; Plummer, 1979; Ross & Anderson, 1990; Keller, 1997). All

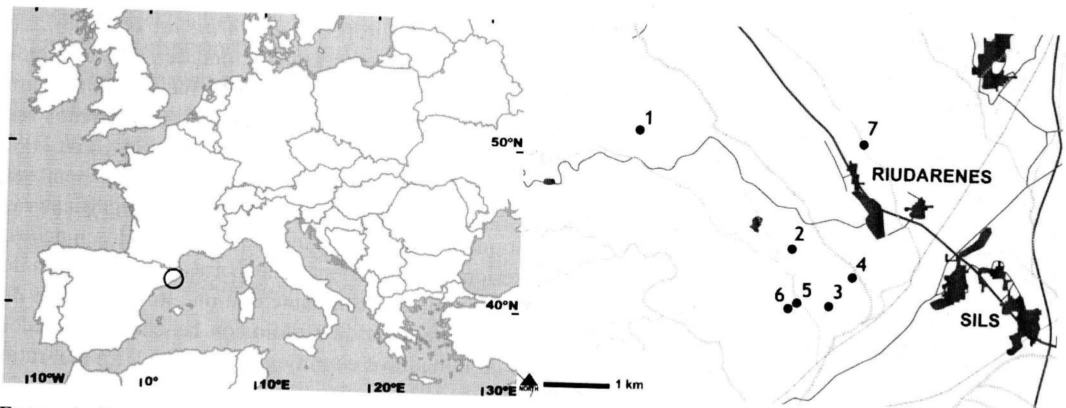

Figure 1. Study area and pond localization. 1: Can Cuní, 2: Can Calçada, 3: Prats de Mas Vern, 4: Pedrera Mas Vern, 5: Pont 3 Ulls, 6: Montcorb,7: Can Prats.

Figura 1. Área de studio y localización de las charcas. 1: Can Cuní, 2: Can Calçada, 3: Prats de Mas Vern, 4: Pedrera Mas Vern, 5: Pont 3 Ulls, 6: Montcorb, 7: Can Prats.

individuals were marked and released at the same site of capture. Morphometric data of 17 variables were collected (Fig. 2). The estimation of population size was carried out by means of the Lincoln-Petersen method. Other biological and ecological data were recorded in order to determine the life history traits.

Three size-age classes were considered: adult females, adult males and immature individuals. Adult females were considered all specimens greater than the minimum size of a gravid female. Abdominal palpation and low-intensity X-ray were used to detect gravid females. To determine the minimum size of males, secondary sexual characters were used. The immature class includes individuals with nucal carapace lenght (NCL) smaller than the minimum size considered.

In order to analyze the morphological characteristics for the three size-age classes the variables were transformed logarithmically. ANCOVA analysis were performed using as co-variable the total length and a multivariate analysis (PCA) was performed with the residuals of the regressions of the variables with logNCL as independent variable, to avoid the influence of size. The "varimax" method was used to rotate the axes. In order to characterise the different classes, a

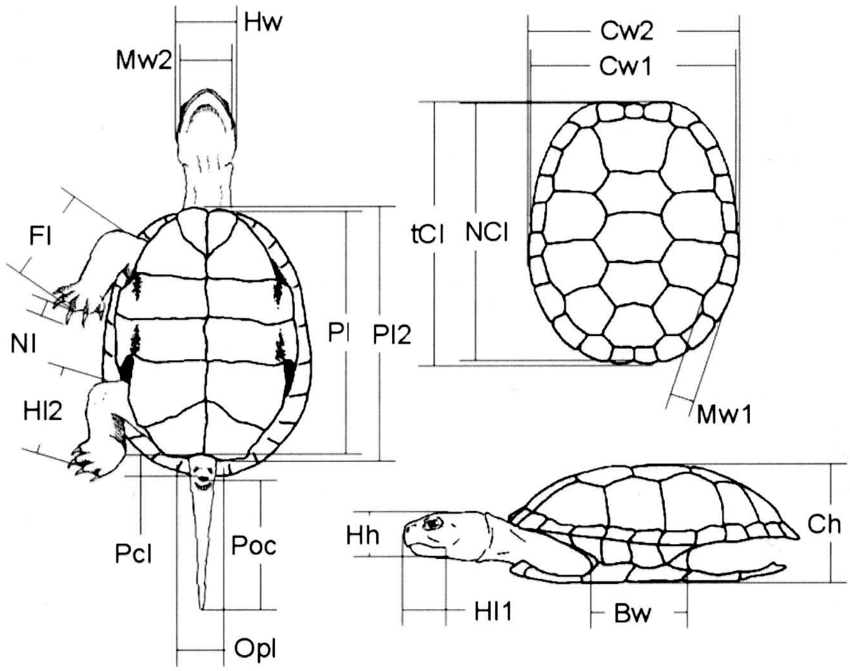

FIGURE 2. Morphometric variables measured. BW: bridge width, CH: carapace height, CW1: carapace width, CW2: maximal carapace width, FL: forelimb length, HH: head height, HL1: head length, HL2: hindlimb length, HW: head width, MW1: marginal width, MW2: mouth width, NCL: nucal carapace lenght, NL: nail length, PCL: precloacal length, PL: plastron length, POCL: postcloacal length, TCL: maximal carapace length, TW: total weight.

FIGURA 2. Variables morfométricas analizadas. BW: anchura del puente, CH: altura del caparazón, CW1: anchura del caparazón entre la segunda y tercera placas vertebrales, CW2: anchura máxima del caparazón, FL: longitud de la extremidad anterior derecha, HH: altura de la cabeza, HL1: longitud de la cabeza, HL2: longitud de la extremidad posterior derecha, HW: anchura de la cabeza, MW1: anchura de la tercera placa marginal posterior derecha, MW2: anchura de la boca, NCL: longitud nucal del caparazón, NL: longitud de la uña, PCL: longitud precloacal, PL: longitud del plastrón, POCL: longitud postcloacal, TCL: Longitud máxima del caparazón, TW: Peso.

canonical analysis was performed on the basis of the untransformed variables, considering the three size-age classes. A discriminating function was also estimated.

RESULTS

A total of 182 individuals were captured from 1997 to 2002 during the activity period. The minimum size of a female with calcified eggs detected was 103.70 mm (NCL) and the minimum size of a male with secondary sexual characters was 88.90 mm. These values were used to assign individuals to the different size-age classes. Of 182 turtles only 80 (45 immatures, 14 males and 21 females) were used for morphometric analyses, whereas the total sample (101 immatures, 56 females and 25 males) was included in the population structure and biological data study. Descriptive statistics for the 17 variables for the three size-age classes are shown in Table 1.

No significant differences between sexes in size (NCL) were found. However, significant differences between males and females in 8 of the 17 variables ($p < 0.05$) were found (Table 1). Table 2 presents the regression values obtained between all biometrical variables and carapace length (NCL). Only two variables for the males (HL1 and POCL) and three for the females (HL1, PCL and POCL) do not show a significant correlation with carapace length (NCL). The results indicate the existence of allometry (slope significantly different from 1) in six variables for the males (BW, HW, HH, MW2, PCL and HL2), and three for the females (MW1, HW and HL2) (Table 2). A significant difference was only found in the slope between males and females for the width of the third rear right marginal plate (MW1).

TABLE 1. Descriptive statistics of the 17 variables and classes considered (males, females and immatures). Asterisk (*) indicates significant differences between males and females ($p < 0.05$).

TABLA 1. Estadística descriptiva de las 17 variables y clases consideradas (machos, hembras e inmaduros). El asterisco (*) indica diferencias significativas entre machos y hembras ($p < 0.05$).

		Males					Females					Inmatures				
		N	Mean	SE	Min	Max	N	Mean	SE	Min	Max	N	Mean	SE	Min	Max
*	**TW**	14	231.21	76.03	117.00	390.00	21	282.81	76.50	187.00	436.00	45	93.36	54.32	3.00	197.00
	NCL	14	111.97	13.59	91.00	136.57	21	116.86	10.28	105.07	139.14	45	75.34	21.10	23.18	102.67
*	**CW1**	14	86.19	9.80	70.00	104.38	21	88.17	7.93	79.13	102.48	45	61.71	15.14	20.88	80.88
	CW2	14	90.74	10.31	73.00	107.74	21	92.19	8.75	80.74	109.97	45	63.91	15.67	21.24	84.37
*	**CH**	14	40.37	4.84	32.65	46.41	21	48.17	4.68	41.49	58.21	45	31.65	8.46	10.82	43.70
*	**MW1**	14	12.31	1.53	9.92	15.37	21	11.55	1.86	8.81	14.93	45	7.90	2.18	2.84	11.69
*	**BW**	14	26.48	2.12	23.00	29.70	21	31.11	4.17	20.99	41.69	45	20.02	6.23	4.19	29.60
	HW	14	21.13	0.91	18.00	25.71	21	21.54	1.32	19.58	24.65	45	5.52	3.35	7.62	20.47
	HH	14	16.41	0.98	14.86	18.46	21	16.56	1.51	4.77	21.00	45	12.29	2.59	6.20	17.00
	HL1	14	26.84	4.32	20.14	35.51	21	27.44	3.60	19.43	33.80	45	18.94	4.90	7.69	27.61
	MW2	14	17.47	1.79	14.98	20.32	21	17.86	1.55	15.18	20.21	45	13.00	3.21	5.90	18.81
*	**PL**	14	96.38	11.78	78.00	115.12	21	110.03	10.44	95.12	132.00	45	70.96	21.05	19.74	97.23
*	**PCL**	14	24.87	2.73	20.00	30.28	21	12.80	2.72	7.00	18.62	45	8.26	3.01	2.91	17.76
	POCL	14	46.80	6.49	37.31	56.16	21	51.04	8.73	21.48	65.01	45	39.05	8.88	14.26	52.32
	NL	14	7.54	1.58	4.00	9.52	21	7.10	0.94	5.00	8.58	45	4.67	1.52	1.34	7.20
	FL	14	30.25	3.73	25.73	36.54	21	30.02	3.80	22.12	38.00	45	20.10	6.08	6.45	31.01
*	**HL2**	14	44.95	4.38	38.00	51.21	21	43.85	2.73	39.00	50.79	45	29.12	8.53	9.12	43.22

TABLE 2. Determination coefficient (r^2), slope and intercept between the considered variables and carapace length (NCL). One asterisk (*) indicates no significant differences, crosses (†) indicate slope different than 1 ($p < 0.05$), and two asterisks (**) indicate significant differences in slope between males and females ($p < 0.05$).

TABLA 2. Coeficiente de determinación (r^2), pendiente y ordenada en el origen entre las variables analizadas y la longitud del caparazón (NCL). Un asterisco (*) indica diferencias no significativas, las cruces (†) indican diferencias significativamente distintas de 1 en la pendiente ($p < 0.05$) y dos asteriscos (**) indican diferencias significativas entre machos y hembras en la pendiente ($p < 0.05$).

	Males				Females				Inmatures		
	N	r^2	Slope	intercept	N	r^2	Slope	intercept	d.f.	F	p
TW	14	0.94	$2.71^{=3}$	-3.20	21	0.90	$2.88^{=3}$	-3.52	31	0.38	0.54
CW1	14	0.91	0.90	0.09	21	0.91	0.98	-0.09	31	0.64	0.43
CW2	14	0.94	0.92	0.08	21	0.92	1.04	-0.19	31	1.57	0.22
CH	14	0.81	0.89	-0.23	21	0.74	0.95	-0.28	31	0.10	0.75
MW1	14	0.71	0.85	-0.65	21	0.72	1.60*	-2.25	31	7.03	0.01**
BW	14	0.74	0.57*	0.26	21	0.50	1.12	-0.83	31	3.75	0.06
HW	14	0.81	0.65*	-0.01	21	0.63	0.55*	0.19	31	0.54	0.47
HH	14	0.34	0.28*	0.64	21	0.45	0.68	-0.18	31	3.61	0.07
HL1	14	0.18	0.55†	0.31	21	0.03	0.27†	0.88	31	0.31	0.58
MW2	14	0.81	0.74*	-0.28	21	0.65	0.81	-0.43	31	0.161	0.69
PL	14	0.96	0.99	-0.05	21	0.95	1.05	-0.14	31	0.65	0.42
PCL	14	0.31	0.49*	0.39	21	0.02	0.38†	0.32	31	0.03	0.86
POCL	14	0.09	0.34†	0.97	21	0.01	-0.22†	2.15	31	0.69	0.4
NL	14	0.58	1.51	-2.21	21	0.37	0.96	-1.14	31	1.39	0.24
FL	14	0.29	0.53	0.39	21	0.28	0.78	-0.14	31	0.45	0.50
HL2	14	0.87	0.74*	0.13	21	0.53	0.52*	0.56	31	2.59	0.12

Precloacal length (PCL) is clearly dimorphic, larger in males. The females have a broader carapace, a longer plastron and they are heavier than males. They also have a relatively greater bridge width (BW) and relatively shorter hindlimbs. In the PCA analysis, the males are clearly separated from the females and immatures on factor 1. In this axis the principal transformed variables implicated were PL, PCL and CH. Moreover, this factor only explains 19.41% of the variance.

In the canonical analysis, root 1 clearly separates the males from the females and immatures, fundamentally by precloacal length (PCL) and size (NCL) (Fig. 3). Root 2 separates the adults from the immatures by carapace height (CH), the most important variable in this axis. The coefficients of the discriminant functions and the percentages of correct classification using only three non-transformed variables (NCL, CH and PCL) are presented in Table 3. The global percentage of correct discrimination is very high (97.50%).

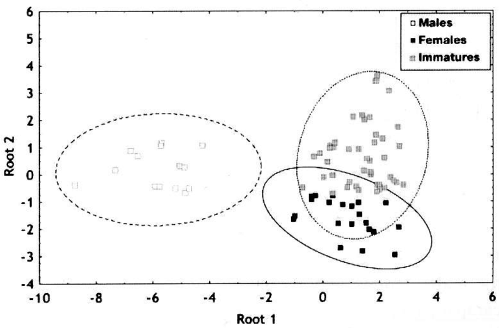

FIGURE 3. Canonical and discriminant analyses.
FIGURA 3. Análisis canónico y discriminante.

TABLE 3. Coefficients of the discriminant functions for males, females and immatures and the percent individuals correctly classified.
TABLA 3. Coeficientes de la función discriminante para machos, hembras e inmaduros y el porcentaje de ejemplares correctamente clasificados.

Variable	Males	Females	Immatures
NCL	0.65	0.06	0.03
CH	-1.48	0.79	0.68
PCL	2.83	0.12	0.05
Constants	-43.52	-24.38	-10.51

Group	%	Males	Females	Immatures
Males	100.00	14	0	0
Females	100.00	0	21	0
Immatures	95.56	0	2	43
Total	97.50	14	23	43

For the 182 individuals captured, 81 were adults (25 males, 56 females) and 101 were immature individuals (Fig. 4). Therefore the ratio adults: immatures was 1:1.25. This result indicates a large percentage of immatures (55.49%) in the population. Estimated sex-ratio (♀♀:♂♂) was 1:2.24.

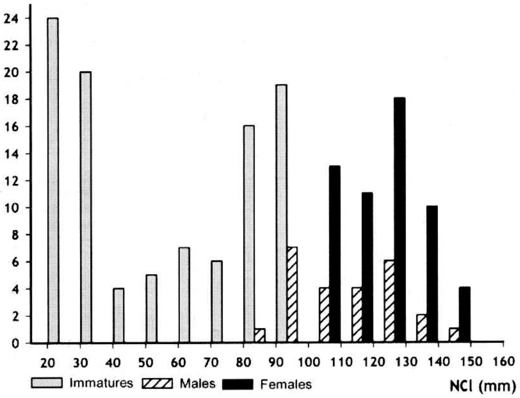

FIGURE 4. Population structure and size-class distribution for male, female and immature individuals. NCL: Nucal carapace length.
FIGURA 4. Estructura poblacional y distribución de clases de talla para machos, hembras e inmaduros. NCL: Longitud nucal del caparazón.

The main habitat occupied by *Emys orbicularis* in the study area consists of ponds, drainage channels, and small marshes. Lentic waters were the habitats presenting the greatest density of individuals. However, some isolated individuals have been detected in streamlets and drainage channels. The estimation of abundance in different subpopulations is presented in Table 4. The results obtained by direct counts are very similar to results obtained by the Lincoln-Petersen method.

Figure 5 shows the biological cycle of *Emys orbicularis* in the study area. Activity begins when minimum water temperature is near 9° C about the second fortnight of February. From March onwards an increase of activity can be detected. This period coincides with the spring emergence of the hatchlings, always after the rain periods with the rise in temperatures.

From the first fortnight of July, the observations of individuals decrease, according with the days of maximum air temperature. In this period the minimum water temperature reaches 20° C.

At the end of October, when water temperature decreases from 14° to 9° C, the observations of active individuals decrease considerably and the last ones are observed basking. The inactivity period starts when water temperature decreases below 9° C.

The mating period occurs between the second fortnight of March and the first of April. In this month, the minimum water temperature varies between 9 and 14° C. Gravid females were detected from the first fortnight of May until the first fortnight of July. Laying takes place between the second fortnight of June and the first one of July.

DISCUSSION

In the studied population, no significant

TABLE 4. Estimation of abundance in the different subpopulations studied obtained by Lincoln-Petersen method and visual censuses (direct counts). Pond numbers as in Fig. 1.
TABLA 4. Estimas de abundancia poblacional en las distintas subpoblaciones estudiadas obtenidas mediante el método de Lincoln-Petersen y censos visuales (conteo directo). Los números de las charcas coinciden con los indicados en Fig. 1.

MPond	Surface (m^2)	Captures (N)	Lincoln-Petersen Estimation		Direct Observation	
			N	D (ind/ha)	N_{Max}	D (ind/ha)
1 Can Cunill	218	60	45	2064	34	1560
2 Can Calçada	240	19	19	791	14	583
3 Prats Mas Vern	1670	75	33	198	22	132
4 Pedrera Mas Vern	210	11	-	-	7	333
5 Pont 3 Ulls	20	3	3	1500	3	1500
6 Montcorb	14	5	-	-	4	2857
7 Can Prats	200	9	12	600	16	800

	n	Mean	SE	Minimum	Maximum
Lincoln-Petersen Estimation	5	1030.60	745.70	198	2064
Direct Observation	7	1109.28	943.45	132	2857

differences in size were detected between males and females as in Doñana and norwestern Spanish populations (ANDREU, 1982; KELLER, 1996; KELLER *et al.*, 1997; AYRES & CORDERO, 2001). However, size differences have been detected in other European populations, where females are larger than males (CHEYLAN, 1992; FRITZ, 1995; FRITZ *et al.*, 1995; MAZZOTTI, 1995; ZUFFI & GARIBOLDI, 1995; TASKAVAK & REIMANN, 1998).

In agreement with the results obtained by other authors (ANDREU, 1982; MAZZOTTI, 1995; ZUFFI & GARIBOLDI, 1995; KELLER, 1997), significant differences were found between males and females in carapace height (CH). Precloacal length (PCL) is clearly also dimorphic, being much greater in males, as in the Doñana population (KELLER, 1997). The females have a wider carapace, larger plastron and they have more weight than males, like Italian populations (ZUFFI & GARIBOLDI, 1995).

In our population, females show greater width bridge (BW) than males and relatively shorter hindlimbs. Males show allometric growth of the head that becomes smaller with size. Allometric growth has also been

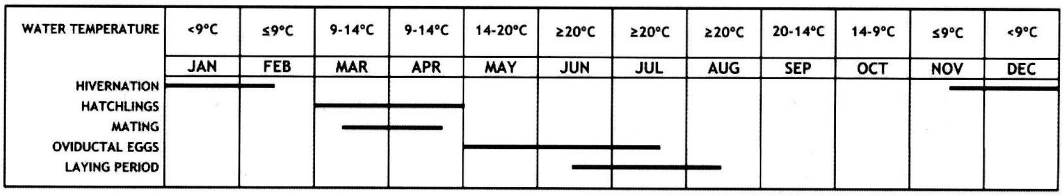

FIGURE 5. Biological cycle of studied population.
FIGURA 5. Ciclo biológico de la población estudiada.

observed in the variables related to secondary sexual traits (PCL and BW).

Only the width of the third marginal plate (MW1) shows a significant difference in slope between sexes. This feature is related to positive allometry in females and is consequence of different shape in the rear of the carapace of this sex. The PCA analysis reflects similar morphology between females and immatures. This result is confirmed by the canonical analysis.

The sex ratio biased towards females is very similar to that observed in France and Italy (LEBBORONI, 1989; SERVAN *et al.*, 1989; MAZZOTTI, 1995). In the Iberian Peninsula, the Doñana and northwestern populations present a male-biased sex ratio (KELLER, 1997; CORDERO & AYRES, 2004).

In general, adults are present in large proportion in natural populations of turtles (BURY, 1979; WILBUR & MORIN, 1988). IVERSON (1991) considers that detecting low recruitment is a common characteristic in the biology of chelonians. This situation can be observed in most of the European pond turtle populations studied, which show a low proportion of immatures (15-31%) (ANDREU, 1982; SERVAN, 1988; CHEYLAN, 1992; MAZZOTTI, 1995; ZUFFI & GARIBOLDI, 1995; KELLER, 1997). Despite these results our population has larger proportion of immature turtles (55.49%). This percentage indicates a growing population. On the other hand size distribution of turtles is not biased towards larger sizes. This can indicate a continuous population recruitment with high mortality

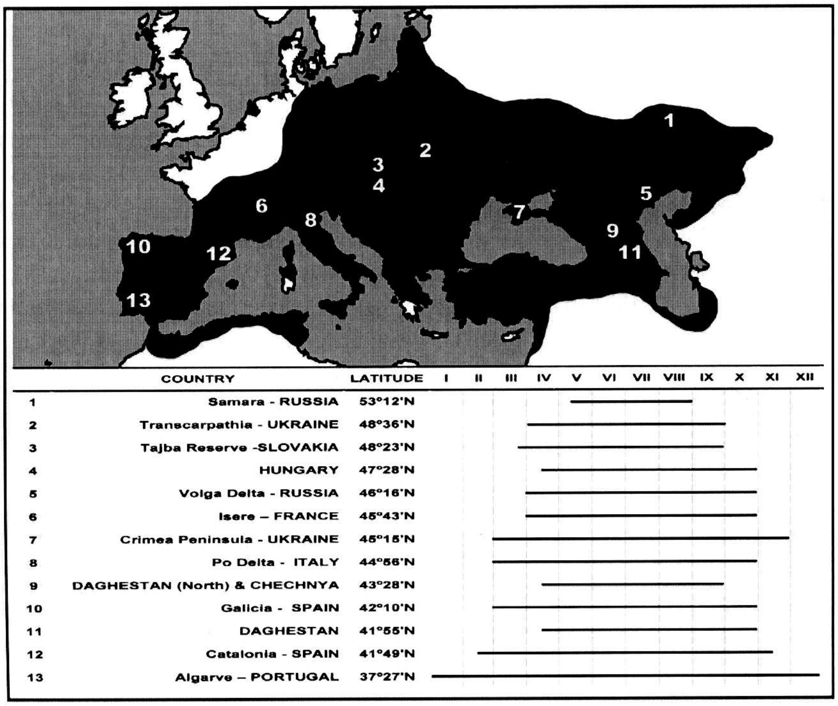

FIGURE 6. Localization and activity period in different populations of *Emys orbicularis* throughout their distribution range.
FIGURA 6. Localización y período de actividad en distintas poblaciones de *Emys orbicularis* a lo largo de su rango de distribución.

during the first period after emergence. The second juvenile peak (NCL = 80-90 mm) may correspond to an effect of population management during the 1990s. In these years some juveniles were released after their maintenance in captivity in order to reduce the new-hatchling mortality.

The main habitats of the studied population are isolated, permanent, well conserved and non-polluted ponds with an abundance of macroinvertebrates and amphibians. Although the density in these ponds can reach high levels it must be emphasised that only in five ponds are more than 10 individuals present and we estimate that the total population does not exceed 300 individuals.

The average density estimation is 1030.60 ind./ha ± 745.7, which is similar to the results obtained by NAULLEAU (1991) who estimated densities of 1222 and 1077 ind./ha in two ponds of small surface area. Lower densities were found by MAZZOTTI (1995) (5.52-9.94 ind./ha in the Po Delta), BOZHANSKY & ORLOVA (1998) (75-125 ind./ha in Russia), and BARON & DUGUY (2000) (53.99 ind./km in France). Our data reflect an unreal situation because the small and fragmented area occupied by European pond turtles in our study area involves high levels of density when the total population estimated is very small (see above).

Biological cycle is similar to other temperate areas (ROVERO, 1995; GALÁN & FERNÁNDEZ ARIAS, 1993). In the northern area of distribution of this species the biological cycle is characterized by a short activity period. We have detected a latitudinal cline between activity period and latitude (Fig. 6). A significant correlation between activity period and latitude ($R = 0.81$, $p < 0.001$), longitude ($R = 0.60$, $p = 0.0301$) and mean temperature of coldest month (January) ($r = 0.92$, $p < 0.001$) has been detected (Fig. 7),

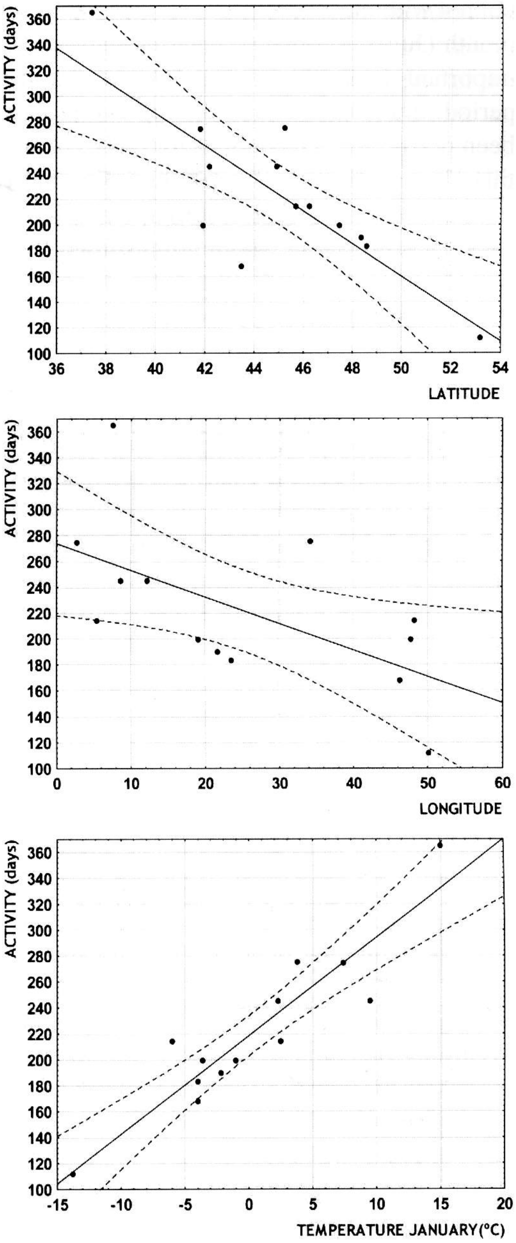

FIGURE 7. Regression and 95% confidence intervals between activity period and latitude, longitude and mean temperature of coldest month of European populations considered in the analysis.

FIGURA 7. Regresión e intervalos de confianza al 95% entre la duración del período de actividad y la latitud, la longitud y la temperatura media del mes más frío en las poblaciones europeas consideradas en el análisis.

but not with mean temperature of the warmest month (July). In order to determine the most important factor implicated in this activity period gradient, a multiple regression has been performed. The only factor that explains this cline is the mean temperature of coldest month (January) ($F_{1,9} = 10.67$, $p = 0.0097$). In conclusion, the shortening of activity period is determined by the duration of cold period in these areas.

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