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Body size and clutch size in the European pond turtle (*Emys orbicularis*) from central Italy

M. A. L. Zuffi[1], F. Odetti[1] and P. Meozzi[2]

[1] Museo di Storia Naturale e del Territorio-Università di Pisa, via Roma 79, 56011 Calci (Pisa), Italy
[2] Istituto di Patologia Speciale e Clinica Chirurgica Veterinaria-Università di Pisa, viale delle Piagge, 2, 56100 Pisa, Italy

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Abstract

The reproductive biology and ecology of the European pond turtle *Emys orbicularis* is little known, and available information was previously mainly anecdotal. Morphological data on *E. orbicularis* are now well documented in most of the known distribution area. Body size in northern populations tends to be larger than in southern populations. There are also indications that clutch size may be larger in northern than in southern European pond turtle populations. Clutch size and reproductive status of a central Italy population were detected by radiography and palpation, respectively, and comparative data were obtained by direct count of intact nest eggs. Reproductive females averaged 13.9 cm and rarely exceeded 15.5 cm carapace length. They produced a clutch size of six, the same number found in intact nests. Nest egg-size averaged 32 mm in length. Egg number was positively associated with female body size, and especially with carapace height.

Key words: European pond turtle, *Emys orbicularis*, body size, clutch size, radiography, palpation

INTRODUCTION

The European pond turtle *Emys orbicularis* (L.) is the most widespread emydid species of the Palaearctic region (Alderton, 1988; Fritz, 1995). In recent years, studies on this species have increased knowledge regarding the use of space (Lebboroni & Chelazzi, 1991; Rovero & Chelazzi, 1996) and thermoregulation (Di Trani & Zuffi, 1997). Contributions on morphological as well as biometrical features (Fritz, 1993, 1995; Zuffi & Gariboldi, 1995*a*,*b*), population structure (Mazzotti, 1995), and genetic characters (Servan *et al*., 1989; Lenk *et al*., in press) have given us additional insight on the pond turtle. Nevertheless, reports on its reproductive biology have been mainly descriptive (Rollinat, 1934; Lanza, 1983). Some experimental designs (De Haan, 1981; Servan & Pieau, 1984) refer to egg size and development. Rovero & Chelazzi (1996) illustrated patterns of movements in nesting and post-reproductive females. Studies within populations of New World emydids have often pointed out a positive correlation between body size and clutch size or clutch mass (e.g. Gibbons, Greene & Patterson, 1982; Mitchell, 1985; Congdon & van Loben Sels, 1993; Nieuwolt-Dacanay, 1997), but this pattern is not generally common (Mitchell & Pague, 1990). Comparing different species of New World emydids, it emerged that the reproductive traits (i.e. egg mass, clutch mass) as well as body mass, were negatively associated with latitude (Iverson *et al*., 1993: untransformed data of table 2). Within the species *Chrysemys picta* (Christiansen & Moll, 1973; Moll, 1973; Iverson & Smith, 1993), body size and clutch size were positively associated with latitude. Forsman & Shine (1995) found that, across emydid species of northern and central America, sexual size dimorphism in favour of females was positively correlated with increase in the annual frequency of reproduction. Nevertheless, little is known of the adaptative and evolutionary traits of the reproductive biology of Old World emydids (see also Berry & Shine, 1980; Iverson *et al*., 1993; Forsman & Shine, 1995). A basis for inter-regional comparison of reproductive ecology in *E. orbicularis* populations (i.e. clutch-size parameters; frequency of reproduction; nest-site characteristics) is therefore needed, even if a general interest is now evident (Rovero & Chelazzi, 1996; Araujo, Segurado & Raimundo, in press; Jablonski & Jablonska, in press; Mitrus & Zemanek, in press). These contributions suggest that body size could be positively correlated with latitude, and clutch size may also increase with maternal body size, with large specimens (Farkas *et al*., in press) and large clutch sizes (Jablonski & Jablonska, in press; Mitrus & Zemanek, in press) in northern populations, and with small specimens (Zuffi & Gariboldi, 1995*a*) and small clutch sizes in southern populations (Fritz, Lenk & Lenk, 1995; Rovero & Chelazzi, 1996).

The aim of the present work is (1) to provide data on body size, clutch size and egg size of pond turtles from central Italy; and (2) to investigate the relationship between body size and clutch size, within our sample.

MATERIALS AND METHODS

The study site is at the U.S. Army Camp Darby area, about 10 km south-west of Pisa and 3 km east of the Ligurian Sea coast, in the 'Parco Naturale di Migliarino, S. Rossore, Massaciuccoli', western Tuscany, central Italy (43°39′48″N, 10°16′06″E). Terrestrial vegetation is mature Mediterranean wood (*Pinus pinea*, *P. pinaster*, *Quercus ilex*). Vegetation around canals and in water is typical of wet areas. Water bodies are rectilinear canals receiving drainage waters. Conspicuous populations of fallow deer *Dama dama* and wild boar *Sus scrofa* are present.

From mid-February to mid-October 1996, we captured 95 adult female *E. orbicularis*, identified from evident phenotypical sexual characteristics (*sensu* Lanza, 1983), with a carapace length usually >9 cm (Zuffi & Gariboldi, 1995*b*). Specimens were measured according to a standardized procedure (Zuffi & Gariboldi, 1995*a*), and marked with notches on the marginal scales (Stubbs *et al.*, 1984); graphite tallies were also put on both sides of the carapace (Lebboroni & Chelazzi, 1991). During the reproductive season, from April to July, we considered only females with oviductal eggs to be definitely reproductive. Oviductal eggs were recorded with palpation (PALP) of the inguinal region in 49 animals; 20 of them were also radiographed (RAD). We also estimated a comparative clutch size from the number of eggs in intact nests.

The X-ray procedure used by Gibbons & Greene (1979) was characterized by 200 mA, 70 kV, 0.7 min (see also Altland, Highman & Wood, 1951). Gibbons & Greene (1979) did not find any significant difference in *Sternotherus odoratus* hatching success between irradiated and non-irradiated eggs, nor did they record carapace anomalies. In our experiments, exposures were made at 30 mA, 47-50 kV, 0.1 min, with rare earth cases. In addition, *S. odoratus* had a relatively smaller body-size, from 11.4 to 12 cm (Carr, 1983) than that of *E. orbicularis*. We therefore presumed the absence of significant injuries at the gonadal level in our sampled females and in hatchlings (Hinton *et al.*, 1997).

We measured the maximum and minimum diameter of eggs and their weight from intact nests. Each egg was individually marked on the upper surface to be replaced in exactly the same position after data recording. We found a perfect correspondence between plastron length measurements taken from radiography and from data taken on living turtles (Student t-test = 0.97, $P > 0.05$). Therefore, in order to compare average RAD egg sizes to female body size, egg sizes were recorded directly from radiographs. We excluded the maximum diameter, because most of the eggs were not placed parallel on the radiographic plane. Body dimensions were measured as

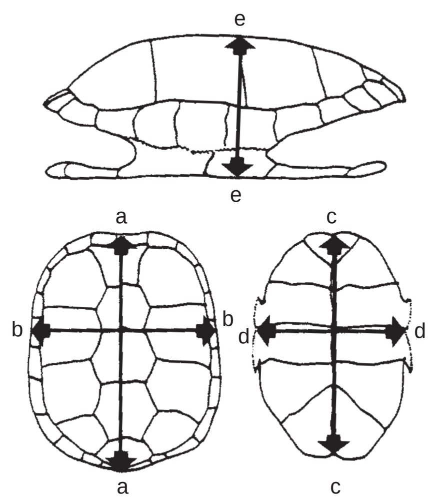

Fig. 1. Body shape of *Emys orbicularis* and measurements. Dorsal view (bottom left): a—a, carapace length; b—b, carapace width. Ventral view (bottom right): c—c, plastron length; d—d, plastron width. Lateral view (top): e—e, carapace height. Shape of carapace was redrawn from Lanza (1983) and Zuffi & Gariboldi (1995*b*), modified.

in Fig. 1 (Zuffi & Gariboldi, 1995*b*). We analysed these body size variables with correlation analysis, selecting the characteristic with the higher correlation coefficient *vs* egg number estimation (e.g. RAD and PALP eggs). This variable was used in a best-fit linear regression model to describe relationships with RAD and PALP clutch estimations. In addition, we used adjusted body size (i.e. residuals of log transformed body length *vs* log transformed body mass), to highlight the relationships with clutch size. Non-parametric tests were used in clutch size comparisons (e.g. number of PALP eggs *vs* number of RAD eggs). Each linear variable was log transformed before the analyses. Probability level was set at (alpha = 0.05, and analyses were performed with Statgraphics™ software (Statgraphics, 1986). Values were presented as mean ± 1 SE.

RESULTS

Of 49 adult females captured between 21 May and 12 July 1996, 25 were reproductive and 20 of them were radiographed. Between these dates, the relative percentage of reproductive females collected over 2-week intervals ranged from 20 to 58.3% (Table 1). For a general data set on body size and reproductive traits of the study population, see Table 2. Carapace length and body mass of reproductive females (13.85 ± 0.14 cm; 483.40 ± 14.56 g, $n = 25$) were significantly greater than that of non-reproductive females (13.03 ± 0.17 cm; 399.38 ± 14.82 g, $n = 24$) (Student's t-test for length = 3.62, $P < 0.005$; Student t-test for mass = 4.01, $P < 0.001$). Using clutch size as factor level (e.g. five groups, from 4 to 8 eggs), we did not find any significant difference between RAD female carapace length ($ANOVA_{carapace\ length}$, $F_{4,10} = 2.89$, $P > 0.05$), nor

Table 1. Seasonal occurrence of reproductive *Emys orbicularis*

Period	RF (*n*)	NRF (*n*)	RF (%)
13 May–26 May	3	12	20
27 May–9 June	7	5	58,3
10 June–23 June	9	9	50
24 June–7 July	5	5	50
8 July–21 July	5	10	33,3
22 July–4 August	0	2	0
5 August–18 August	0	1	0
19 August–1 September	0	4	0

RF = reproductive females; NRF = non-reproductive females.

between RAD female body mass values (ANOVA$_{\text{body mass}}$, $F_{4,10} = 2.27$, $P > 0.1$).

The average number of PALP eggs (3.9 ± 0.2 eggs, range 0–6, $n = 20$), calculated for the same RAD females, is significantly lower than that of RAD eggs (5.8 ± 0.3 eggs, range 4–8, $n = 15$) (Wilcoxon paired rank test = 3.264, $P < 0.002$). Carapace height was the variable most correlated to the RAD egg number (correlation analysis: 0.79, $n = 20$, $P < 0.0001$; linear model, $y = -22.42 + 16.349x$, $r = 0{,}74$, $r^2 = 54.7\%$, $F_{1,13} = 15.698$, $P < 0.002$; Fig. 2). Similar significant correlation was found using corrected body size, that is the residuals of the log carapace height *vs* log body mass in simple regression *vs* egg number (linear model, $y = 0.24 + 0.304x$, $r = 0.67$, $r^2 = 45.3\%$, $F_{1,13} = 10.76$, $P < 0.05$). Using clutch size as factor level, the carapace height was significantly different between RAD females (ANOVA$_{\text{carapace height}}$, $F_{4,10} = 5.45$, $P < 0.02$). Clutch-size evaluation from PALP eggs was in practice useless. Lack of precise judgement depended on the relative egg position inside the maternal body (Fig. 3a,b), where the innermost eggs were not detectable by the researcher. This technique, on the contrary, is costless and extremely efficient in determining the reproductive status of a female directly in the field.

Minimum RAD egg diameter was 19.46 ± 0.1 mm (17.5–21.1 mm, $n = 87$). We found significant differences between female egg sizes (ANOVA$_{\text{minimum diameter}}$, $F_{14,72} = 19.527$, $P < 0.0001$). We monitored 44 nests, 11 of them intact and 33 preyed (75% of the sample; see also Rovero & Chelazzi, 1996). To limit to a minimum any possible different hatching success due to manipulation, we handled and measured eggs of only five out of 11 intact nests. Each intact nest was protected against predators with a 1-cm side grid wire net; nests were observed twice a day until hatching. The average nest clutch size was 5.6 ± 0.3 eggs (range 4–8, $n = 11$). In 21

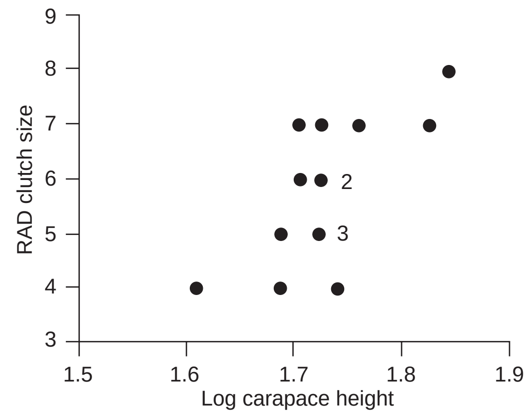

Fig. 2. Regression between natural log carapace height and egg number (X-ray technique; $n = 15$). Number close to filled circles indicates sample size greater than one.

out of 33 preyed nests we could count residual egg halves, with an average estimation of 2.4 ± 0.4 nest clutch size (range 1–5, $n = 21$). The difference in clutch estimation between preyed and intact nests was significant (Mann–Whitney rank test = −4.29, $P < 0.0001$), suggesting that predators could hide or eat some eggs far from the nest. From five intact nests, the average egg size (e.g. maximum and minimum diameter) was 32.2 ± 0.5 mm (range 27.5–37 mm, $n = 26$) and 18.5 ± 0.1 mm (range 17.5–19.3 mm, $n = 26$) respectively, and average egg mass was 6.3 ± 0.2 g (range 4.9–7.6 g, $n = 23$). We found significant differences between the egg sizes and masses of intact nests (ANOVA$_{\text{maximum diameter}}$, $F_{4,21} = 14.914$, $P < 0.0001$; ANOVA$_{\text{minimum diameter}}$, $F_{4,21} = 7.404$, $P < 0.001$; ANOVA$_{\text{mass}}$, $F_{4,18} = 11.898$, $P = 0.0001$). This was because one clutch out of the five was relatively smaller and lighter. Clutch size of intact nests (5.6 ± 0.3 eggs) was similar to RAD clutch sizes (5.8 ± 0.3 eggs) (Mann–Whitney *U*-test = 0.35, $P > 0.7$). On the contrary, sizes of RAD eggs were significantly greater with respect to intact nest eggs (minimum diameter, Student's *t*-test = −6.192, $P < 0.0001$, $n_1 = 26$, $n_2 = 87$), due to a light X-ray magnification. The average value of the RAD egg minimum diameter was not significantly correlated to carapace height (correlation matrix, 0.49, $P > 0.06$, $n = 15$).

DISCUSSION

Carapace length has been used frequently as the main predictive variable associated with clutch size and clutch

Table 2. Body size and reproductive traits of the study population

Sex	Carapace length (mm)	Plastron length (mm)	Clutch size[a]	Egg length[b] (mm)	Egg width[b] (mm)
Females ($n = 115$)	131.7 ± 0.97	122.0 ± 0.92	5.8 ± 0.3 ($n = 15$)	32.2 ± 0.5 ($n = 26$)	18.5 ± 0.1 ($n = 26$)
Males ($n = 66$)	125.9 ± 0.99	107.5 ± 0.78			

[a] Clutch size is taken from radiographs.
[b] Egg length and width are taken from five intact nests.

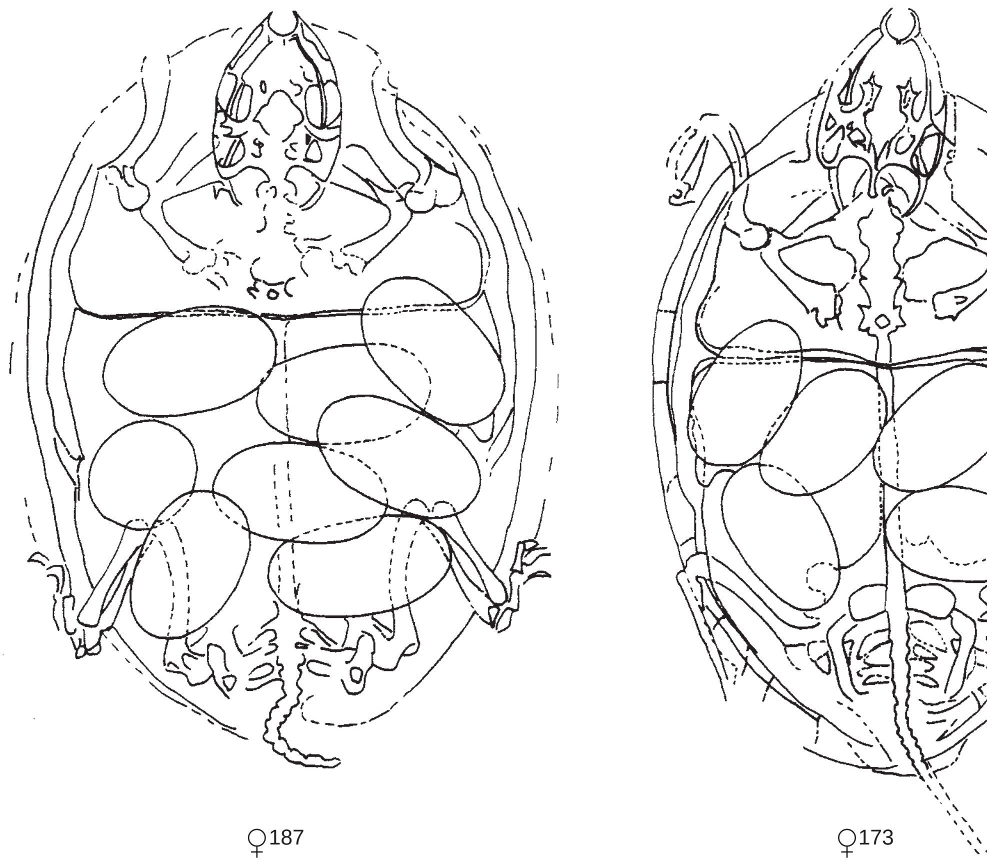

Fig. 3. Adult females 187 and 173 (drawn from radiograph).

mass variability (e.g. Gibbons *et al.*, 1982; Mitchell, 1985; Congdon & van Loben Sels, 1993; Nieuwolt-Dacanay, 1997). We also found a positive correlation between body size and clutch size, nevertheless, at least from this study, it is evident that the main factor correlated with clutch size is carapace height (see also Jackson & Walker, 1997: table 9, fig. 15). This parameter has been found to be a discriminant factor in assessing most variability of sexual dimorphism in *E. orbicularis* (Mazzotti, 1995; Zuffi & Gariboldi, 1995*a*,*b*; Zuffi *et al.*, in press.). Sexual size dimorphism of the European pond turtle should be studied in detail, with special attention to geographic variability between populations and subspecies (Fritz, 1993; Lenk *et al.*, in press; Zuffi & Ballasina, in press). The *E. orbicularis* sample in this study is comparable to those taxa that display a clutch frequency of one to two per year (Rollinat, 1934; Lanza, 1983; Forsman & Shine, 1995: fig. 1; and explanations herein). Sexual dimorphism is relatively apparent but constant, with females slightly larger than males (Zuffi & Gariboldi, 1995*b*).

Body size, clutch size and egg size of the study population were of comparable dimensions to those found in previous studies in Italian populations (Fritz *et al.*, 1995; Rovero & Chelazzi, 1996), but female body size and clutch size were smaller than those reported for some northern populations. Average female body size in eastern Poland, north-eastern Germany, and Hungarian Lowlands are 175, 173, and 152 mm, respectively (Farkas *et al.*, in press). Two different populations in Poland laid on average 13 eggs (Jablonski & Jablonska, in press) and 14–15 eggs (Mitrus & Zemanek, in press).

These preliminary comparisons suggest the hypothesis that body size and clutch size in *E. orbicularis* could increase with latitude, similarly to the trend described for *Chrysemys picta* (Christiansen & Moll, 1973; Moll, 1973; Iverson & Smith, 1993).

Further data on populations from different latitudes, as well as comparisons between populations from the same geographic area (see Congdon & Gibbons, 1983; Mitchell & Pague, 1990) will be necessary to test the influence of latitude on the interspecific variation of body size and clutch size.

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