Reproductive strategies and body shape in the European pond turtle (*Emys orbicularis*) from contrasting habitats in Italy

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Abstract

Comparisons within and among populations offer important insights into variation in life-history traits and possible adaptive patterns to environmental conditions. We present the results of observed differences in body size, body shape and patterns of reproduction in four separate populations of the European pond turtle *Emys orbicularis* in central and southern Italy – coastal ($n = 3$) and mountainous ($n = 1$) sites and pond ($n = 2$) and canal ($n = 2$) habitats – to determine whether phenotypic plasticity affects reproductive output. Although we did not find any significant latitudinal variation in body size, we observed significant differences in body shape between canal (rounded body shape) and pond (elongated body shape) systems and smaller size with rounded shape in the mountainous population. Reproductive output is similar among populations (median = 5 eggs per clutch), whereas reproductive investment (relative clutch mass to maternal body mass) is higher in the mountain population (one clutch per year) than in coastal populations (two clutches per year), suggesting differential trade-offs between geographic locality, elevation and habitat type. Turtle shell shape and geographic location together affect reproductive output in *E. orbicularis* in Italy.

Introduction

Shell structures in turtles range from complete ossification with a broadly developed carapace (i.e. genera *Testudo*, *Geochelone*) to a reduced surface of bony shell (i.e. genera *Chelydra*, *Apalone*) to a poorly ossified carapace (i.e. genus *Trionyx*; Ernst, Lovich & Barbour, 1994; Lee, 1996). In addition, clutch size is often positively related to maternal body size and to frequency of reproduction (i.e. single vs. multiple annual clutches) and inversely related to latitude (see Iverson *et al.*, 1993). Furthermore, a single annual clutch is more frequent in large species with reduced sexual size dimorphism at the adult stage, and multiple annual clutches are more frequent in small-sized species that show evident differences between sexes, and with female as the largest sex (Forsman & Shine, 1995*a*). Sperm storage occurs in marine (Pearse & Avise, 2001) and in terrestrial species (Sacchi *et al.*, 2003; Roques, Diaz-Paniagua & Andreu, 2004), a pattern positively selected in environments characterized by a low frequency of encounters between partners. In freshwater turtles, sperm storage seems, on the contrary, uncommon (Pearse, Janzen & Avise, 2001, 2002; Sarkar, Sarkar & Maiti, 2003), but scarcity of research could be one of the reasons for rarity of these data.

Comparisons of life-history traits within and among populations offer insights into the degree of variation and adaptive patterns in ecological constraints (Gibbons, Greene & Patterson, 1982; Rowe, 1994; Lomolino, 2005). Variation in life-history traits does not correlate with morphological variation within populations (Claude *et al.*, 2003), and published analyses of variation mechanisms and phenotypic plasticity are not so common in turtles (Moll, 1973; Gibbons, 1982; Mitchell & Pague, 1990; Iverson & Smith, 1993; Claude *et al.*, 2003, 2004).

Widespread species can therefore represent good models of study, and in this context several studies have been conducted in mammals (e.g. Dobson & Murie, 1987; Ebenhard, 1990) as well as in amphibians (e.g. Morrison & Hero, 2003) and reptiles (e.g. Forsman & Shine, 1995*b*).

Furthermore, because of the rarity of freshwater turtles at high elevations (Ernst *et al.*, 1994) and the scarcity of related information, comparisons between contrasting habitats (e.g. mountain vs. plain areas) may be difficult to carry out. Mountain continental climate may be particularly selective and act as directional constraints for reproductive ability in exothermic vertebrates. Mountain lower thermal profiles have been claimed as possible effects of different dimensions and of body shape variation in turtles (Capula & Luiselli, 1994) when compared with sea-level populations.

We chose the European pond turtle *Emys orbicularis* (L., 1758) as our model species, a freshwater turtle with a wide western Palearctic distribution, ranging from Portugal in the west to the area of the Aral Sea (Kazakhstan) in the east, from northern Germany and Denmark to southern Italy, Sicily and northern Africa and south-eastwards from Turkey to the eastern Caucasus Republics and the southern shore of the Caspian Sea (Podloucky, 1997; Kuzmin, 2002; Fritz, 2003). Several ecological aspects of the biology of this species have recently been studied in different parts of its range (Lebboroni & Chelazzi, 1991; Rovero & Chelazzi, 1996; Keller, Andreu & Ramo, 1998; Mitrus & Zemanek, 1998; Schneeweiss & Steinhauer, 1998; Zuffi & Odetti, 1998; Zuffi, Odetti & Meozzi, 1999; Kotenko, 2000; Zuffi, 2000; Zuffi, Di Benedetto & Foschi, 2004). Information has been focused mainly on individual populations; comparative approaches are extremely scarce (Schneeweiss, 2003; Zuffi *et al.*, 2004). Our research was aimed at answering the following questions:

(1) Do latitudinal and longitudinal gradients influence sexual size dimorphism and the age-related allometry (i.e. body size vs. body shape) of adults?

(2) Do ecological aspects of the population study sites (i.e. coastal vs. mountainous sites) and habitat frame (pond vs. canal systems; Lebboroni & Chelazzi, 1998) influence body size and body shape in reproductive females of each considered population?

(3) Do fecundity (clutch size vs. maternal body size) and reproductive traits (clutch size, relative clutch size, relative clutch mass) exhibit differential adaptive success when related to maternal body size?

Materials and methods

We studied two populations of *Emys orbicularis galloitalica* (Fritz, 2003) from Mediterranean coastal central Italy (Tuscany) and two from southern Italy (Calabria): the two from Tuscany and one from Calabria were in a Mediterranean coastal habitat and the last one was in a continental mountainous habitat. We classified our sites after Lebboroni & Chelazzi (1998), who described two primary habitat categories for Italian populations of *E. orbicularis*: pond system and canal system. We examined 239 *E. orbicularis* adults (106 males, 133 females; secondary sexual characteristics as in Zuffi *et al.*, 1999). Central Italy populations were from the San Rossore area (pond system, 41 adults: 20 males, 21 females; 9 m a.s.l., 43°42′51′N, 10°18′30′E) and from the US Army Camp Darby area (canal system, 68 adults: 29 males, 39 females; 10 m a.s.l., 43°38′03′N, 10°20′11′E), both in the Nature Park 'Migliarino San Rossore Massaciuccoli' (Pisa, Tuscany). Southern Italy populations were from the Tarsia area (canal system, 50 adults: 28 males, 22 females; 60 m a.s.l., 40 km far from the sea, 39°36′33′N, 16°16′21′E) and from the San Lorenzo Bellizzi area, on Mount Pollino (pond system, 80 adults: 29 males, 51 females; 1450 m a.s.l., 39°55′57′N, 16°17′58′E), both in the province of Cosenza (Fig. 1).
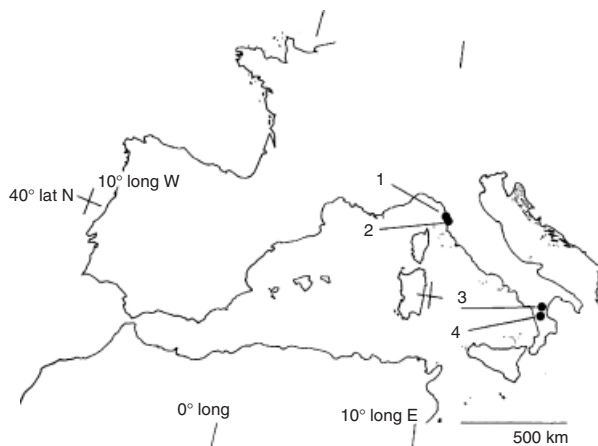

Figure 1 Distribution map of sampled localities. 1, San Rossore, province of Pisa, Tuscany; 2, Camp Darby, province of Pisa, Tuscany; 3, San Lazzaro Bellizzi, province of Cosenza, Calabria; 4, Tarsia, province of Cosenza, Calabria.

We measured turtles at capture with standard methods following Zuffi *et al.* (1999); the variables considered were carapace length and width, plastron length and width, carapace height, total tail length, cloaca opening to tail tip and body mass. Measurements were ± 1 mm and mass ± 1 g. We tested for normality and performed all analyses on log-transformed or arcsine-transformed (i.e. ratio of selected variables) values to reduce the allometry to a minimum. Interrelated variables (e.g. surface: plastron width $\times$ plastron length) and ratios [e.g. arcsine (carapace width/carapace length)] were used to test for shape variation between sexes and among populations. Two sample differences were tested with a parametric or non-parametric test according to normality or non-normality data sets. Differences among populations for a single biometric variable were tested with an ANOVA test to highlight the source of variation and then to enable the use of discriminant function analysis (DFA). DFA (Manly, 1994) was used to test for possible separation among *E. orbicularis* populations. Analyses were applied to (1) verify the extent of sexual dimorphism, in terms of selected variables, of each individual sex (i.e. adult males, adult females) among the four localities and (2) describe biometric and reproductive correlates of gravid females. Factor extraction was performed on data with eigenvalues larger than 1. DFA was then applied to verify which variable actually had a role in the discrimination (Marnell, 1998). This analysis was carried out using the stepwise method, a procedure that adds step by step any single variable, and then controls for general explained variance and, excluding each of them with eigenvalues lower than 1.0, also controls for the tolerance of each individual variable. The final model is represented by one to more significant discriminant functions that explained on average about 90% of the total variance. Additional analysis of variation among populations of non-normal data (i.e. arcsine of relative clutch mass) was performed with a Kruskal–Wallis test. Best-fit regression was selected to

analyse fecundity rate on the whole data set of egg-bearing females. Clutch size and relative clutch mass differences on single populations were tested using non-parametric statistics. The significance level was set at α = 0.05. Statistical analyses were performed with SPSS 8.0 (SPSS, Chicago, Illinois 60606, 1997).

Results

Sexual size dimorphism and body size features of adults among localities

Males in the entire sample averaged (±1 SD) 122.53 ± 12.73 mm carapace length (range 82–148 mm, $n = 97$), 106.08 ± 9.27 mm plastron length (range 74–121, $n = 97$) and 292.46 ± 74.88 g body mass (range 92–445 g, $n = 97$). Females averaged 130.56 ± 15.93 mm carapace length (range 80–174 mm, $n = 130$), 120.97 ± 14.41 mm plastron length (range 71–147 mm) and 407.81 ± 127.18 g body mass (range 84–700 g, $n = 130$). Sexual differences between all the linear carapacial measurements (log-transformed values) and body mass were statistically significant at $P < 0.0001$, total tail length was significant at $P = 0.001$, and cloaca–tail tip was only slightly significant (unpaired samples t-test, $P = 0.047$). Body shape was significantly different between sexes: the ratio of carapace width/carapace length (arcsine transformed) was slightly but significantly different (Student's t-test $= -1.979$, d.f. = 237, $P = 0.049$), whereas plastron width/plastron length was much differentiated (Student's t-test $= -2.887$, $P = 0.004$). The estimated surfaces of both dorsal and ventral view were highly different between sexes (both at $P < 0.0001$; Table 1).

Male body size was different among localities (ANOVA, P ranging from 0.004 to 0.0001; Table 2); female body size was also different among localities (ANOVA, all at $P < 0.0001$; Table 2).

Table 1 General differences in body size measurements and derived parameters in adult sexes of European pond turtles *Emys orbicularis* (males vs. females)

Variable	d.f.	t	P
ln carapace length	237	−3.605	0.0001
ln carapace width	237	−5.280	0.0001
ln plastron length	238	−8.472	0.0001
ln plastron width	238	−8.072	0.0001
ln carapace height	238	−11.167	0.0001
ln total tail length	232	3.277	0.003
ln cloaca–tail apex length	231	−1.977	0.047
ln body mass	226	−6.815	0.0001
Arcsine carapace width/length	237	−1.965	0.051
Arcsine plastron width/length	238	2.904	0.004
Carapace surface (ln carapace length/width)	237	−4.695	0.0001
Plastron surface (ln plastron length/width)	238	−8.718	0.0001
Volume (plastron surface × ln carapace height)	237	−7.932	0.0001

d.f., degrees of freedom; t, Student's t-test values; P, probability values.

DFA for males extracted one main function (natural logarithm of total tail length) that explained 46.7% of the total variance (0.711 canonical correlation coefficient); total tail length was the most correlated variable within the first discriminant function (overall Wilks' $\lambda = 0.200$, $P < 0.0001$). Central Italy males were distinguished by longer tails, whereas southern Italy males were characterized by shorter tails. DFA in adult females did not find any function with eigenvalues larger than 1, suggesting no evident differentiation at the biometrical level among localities.

Adult female body size and body shape in contrasting habitats

Carapace shape was significantly different among habitat types (arcsine carapace width/carapace length; $F_{2,109} = 14.176$, $P < 0.0001$; Fig. 2). Coastal pond females were more elongated than mountain pond females (Student's t-test $= -4.313$, d.f. = 70, $P = 0.0001$); in coastal habitat, pond females were more elongated than canal females (Student's t-test $= -2.791$, $P = 0.007$). Furthermore, coastal canal females were more elongated than mountain pond females (Student's t-test $= -2.297$, $P = 0.024$). Plastron shape was significantly different among habitat types (arcsine plastron width/plastron length; $F_{2,109} = 19.144$, $P < 0.0001$; Fig. 3); coastal pond females were more elongated than mountain pond females (Student's t-test $= -4.561$, d.f. = 70, $P = 0.0001$). In coastal habitats, pond female and canal female plastron shapes are similar (Student's t-test = 0.002, d.f. = 80, $P = 0.999$). Coastal canal females were more elongated than mountain pond females (Student's t-test $= -5.739$, d.f. = 110, $P = 0.0001$). Also the estimated volume (base surface × carapace height) had a significant geographic effect, larger in coastal pond habitat and lower in mountainous pond habitat (ANOVA, $F_{3,39} = 4.319$, $P = 0.011$).

DFA on reproductive females extracted one significant main function (natural logarithm of body mass) that explained 88.9% of the total variance (0.904, canonical correlation coefficient; Wilks' $\lambda = 0.118$, $P < 0.0001$). The among-population comparison showed that egg-bearing females were significantly lighter in the Pollino area.

Reproductive traits (fecundity, clutch size, relative clutch size, relative clutch mass) and maternal body size

Overall maternal body mass and clutch size had a significantly positive relationship ($r_{adj} = 0.127$, $F = 11.288$, d.f. = 71, $P < 0.001$), indicating an increase in fecundity rate at larger maternal body mass. Analysis among localities showed that the body mass and clutch size relationship was on average highly significantly different, especially in Pollino (SanRossore, $r_{adj} = 0.155$, $F = 5.6$, d.f. = 25, $P = 0.026$; Camp Darby, $r_{adj} = 0.274$, $F = 11.917$, d.f. = 29, $P = 0.002$; Pollino, $r_{adj} = 0.504$, $F = 12.162$, d.f. = 11, $P = 0.006$). Reproductive output, expressed as the median of clutch size for each locality, was similar among localities (median = 5, test

Table 2 Geographical differences in body size measurements and derived parameters in adults of the European pond turtle *Emys orbicularis*

Variable	Sex	cd	sr	po	ta
Carapace length	M	123.7 ± 12	130.2 ± 8.8	113 ± 10.9	127 ± 12.2
	F	132.4 ± 9.9	139.1 ± 15.7	121.1 ± 17.2	139.4 ± 12.7
Carapace width	M	91.6 ± 8.7	88.4 ± 3.3	84.9 ± 6.8	90.9 ± 7.7
	F	98.92 ± 6.4	98.7 ± 14.3	90.7 ± 12.1	99.4 ± 10.4
Plastron length	M	106.7 ± 8.9	110.5 ± 6.2	99.8 ± 9.3	109.7 ± 8.1
	F	122.7 ± 9.7	127.9 ± 10.9	112.5 ± 15.9	129.1 ± 13
Plastron width	M	68.4 ± 5.8	69.9 ± 3.3	67 ± 5.4	69.5 ± 5
	F	76.8 ± 4.4	79.5 ± 7.7	73.9 ± 10	78.9 ± 7.4
Carapace height	M	43.1 ± 3.1	47.1 ± 2.3	41.4 ± 5.2	44.6 ± 3.8
	F	53.4 ± 4.7	56.4 ± 6.1	49.1 ± 7.9	58.2 ± 6.8
Total tail length	M	83.3 ± 10.8	87.4 ± 7.2	63.3 ± 8.7	71.1 ± 12.4
	F	75.6 ± 7.2	78 ± 7.3	62.5 ± 9.5	67.1 ± 20.9
Cloaca–tail apex length	M	58.6 ± 6.6	59.4 ± 3.6	49 ± 6.1	60.3 ± 8.2
	F	64.6 ± 7	64.5 ± 5.9	54.9 ± 8.6	57.3 ± 17.7
Body mass	M	286.6 ± 74.6	344 ± 58	242.3 ± 62.1	319.3 ± 65.6
	F	414.7 ± 87.7	466 ± 119.6	328.5 ± 115	511.5 ± 127.6
Carapace width/length	M	0.74 ± 0.03	0.68 ± 0.03	0.75 ± 0.03	0.72 ± 0.04
	F	0.75 ± 0.03	0.71 ± 0.04	0.75 ± 0.03	0.71 ± 0.03
Plastron width/length	M	0.64 ± 0.02	0.63 ± 0.03	0.67 ± 0.03	0.64 ± 0.04
	F	0.63 ± 0.34	0.62 ± 0.02	0.66 ± 0.03	0.61 ± 0.03
Carapace surface	M	11 494.1 ± 121.1	11 481.7 ± 1097.9	9687 ± 1534.6	11 414.9 ± 1939.1
	F	13 209.5 ± 1768.9	13 927.6 ± 3649.2	11 169.1 ± 2847.1	13 990.2 ± 2380.4
Plastron surface	M	7398 ± 1170.1	7702.7 ± 710	6744.3 ± 1086.1	7737.7 ± 1133.9
	F	9508.9 ± 1231	10 247.8 ± 1780.8	8437.9 ± 2150.5	10 287.7 ± 1708.2
Volume	M	504 043.5 ± 123 890.7	540 790.9 ± 71 480.8	407 839.9 ± 102 823.8	515 137.8 ± 111 590.6
	F	713 644.1 ± 149 389	800 946.2 ± 271 263.8	569 396.1 ± 210 870	832 469.4 ± 202 808.1

M, male; F, female; cd, Camp Darby; sr, San Rossore; po, Pollino; ta, Tarsia [for locality, sample size and variables information (e.g. plastron surface), see Materials and methods; untransformed variables are expressed in mm or in g; carapace and plastron surface and volume are in mm^2 and mm^3, respectively, as mean ± 1 SD].
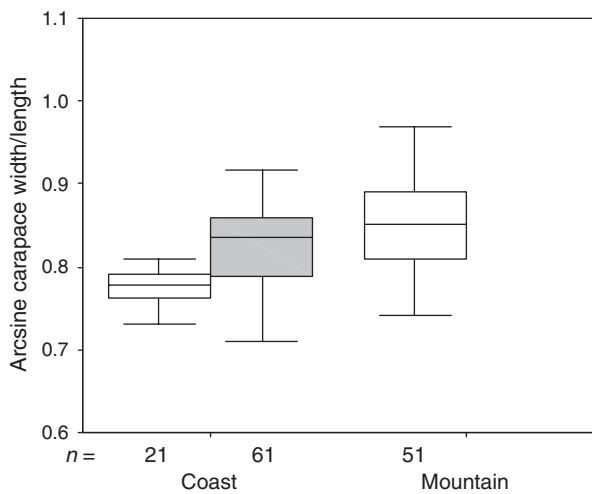

Figure 2 Shape of adult females (arcsine carapace width/length). Open boxes are the pond system and the grey box is the canal system (box plots show median, interquartile distance and extremes).
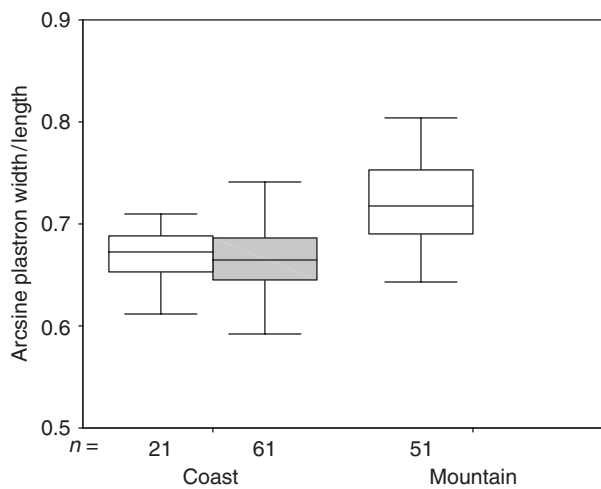

Figure 3 Shape of adult females (arcsine plastron width/length). Open boxes are the pond system and the grey box is the canal system (box plots show median, interquartile distance and extremes).

= 3.263, d.f. = 3, $P = 0.353$). Relative clutch mass {arcsine [(clutch mass/female body mass) × 100]} showed, on the contrary, a significant trend of variation, with the mountainous population characterized by a higher relative output. Excluding the Tarsia population from this analysis (Fig. 4) because of the small sample size, relative clutch mass seemed
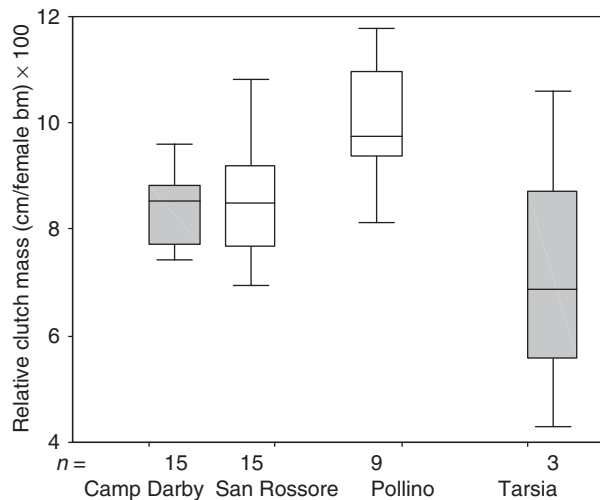
Figure 4 Relative clutch mass in female *Emys orbicularis*. Open box, canal system; grey box, pond system (box plots show median, interquartile distance and extremes). bm, body mass.

to depend on the geographic location of the population (Kruskal–Wallis, $Z = 9.249$, d.f. $= 2$, $P = 0.01$).

Discussion

General patterns of sexual dimorphism

Sexual size dimorphism is very marked and confirmed in *E. orbicularis* (Zuffi *et al.*, 1999; Fritz, 2003): males are always smaller than females. Particularly informative is the relative pattern of plastron dimensions, in which smaller plastrons could offer a wider mobility to males (see Lebboroni & Chelazzi, 1991) than to females. Our data set suggests that there was evidence of only a slight latitudinal pattern of variation in body size, decreasing from central to southern Italy, as found elsewhere in a more general context (Iverson *et al.*, 1993; Ashton & Feldman, 2003). Turtles in the southern mountain population were certainly the smallest among the other populations we studied and similar in size to those living in a medium elevation area of central Italy (Rovero & Chelazzi, 1996), suggesting that mountainous habitats might act as negative constraints in growth patterns (i.e. reducing the length of the activity period and limiting the time effort in searching for food).

Adult female body shape

Adult females that live in different habitats (canal vs. pond system) showed different body shapes (e.g. relatively elongated vs. relatively rounded carapace and plastron shape). It suggests, furthermore, that the habitat environment could select for different allometric development rates (i.e. Claude *et al.*, 2003). We can hypothesize a particularly rapid phenetic radiation of body shape between pond and canal frames in coastal habitats. Canals are relatively recent habitats derived from anthropogenic origin (Middle Age to

the early Renaissance period) and seem to have had a rapid effect on the phenotypic growth of studied populations. The characteristics of habitat systems and their effects on the reproductive traits and phenotypic plasticity of freshwater turtles have not yet been considered in depth (see Lebboroni & Chelazzi, 1998; Claude *et al.*, 2003, 2004), even if an analytical and reviewed research on 35 turtle species demonstrated the absence of any habitat effect (i.e. terrestrial, marine, freshwater habitat, but no other qualitative information) on reproductive output when removing maternal body size effect (Iverson, 1992). Furthermore, it must be noted, from other studies, how different populations of the same species may show a significantly different pattern of body size clutch size relationship, where differences in adult body size have been ascribed to a differential growth rate at the juvenile stage because of different thermal situations in the considered areas (i.e. Gibbons, 1982). Basic information on turtle carapace shape differences has been approached on a broader scale, as for instance considering aquatic versus terrestrial forms (Claude *et al.*, 2003), whereas it would deserve more attention on the small-scale variation of habitat features and their potential effects on organism structure specimen variability. As demonstrated within a wide context, differential diet and foraging habits may have an adaptive effect on skull and head morphology (Claude *et al.*, 2004). Because no one analysis on variation of body shape in contrasting habitats has yet been considered in freshwater turtles, our results could represent a suitable topic of future research.

Fecundity and reproductive traits

Fecundity pattern is only slightly related to body size, as already determined for most chelonians (Gibbons, 1982; Iverson *et al.*, 1993; Forsman & Shine, 1995a). Available data on *E. orbicularis* were mainly on single clutch size versus maternal body mass (Mitrus & Zemanek, 1998; Zuffi *et al.*, 1999; Kotenko, 2000; Rössler, 2000), whereas it is likely that much more correlation should be found considering annual clutch size, especially in populations that lay multiple clutches (Iverson, 1992; Rowe, 1994). Possible inference on the trade-off between maternal investment per single clutch, total clutch investment and hatchling survivorship may give us basic information on the whole reproductive life history (see Vanzolini, 2003), especially when taking into account several species of the same family (Iverson, 1992). Double clutch size in plain areas (Zuffi & Odetti, 1998; Kotenko, 2000) and single clutch size in mountainous areas correlate well with a lower versus a larger relative clutch mass (RCM) investment. This means that, in mountain habitats, parental investment should likely be optimized during the unique reproductive pattern (this study), whereas in plain areas parental investment is focused on a higher reproductive success (i.e. double or multiple clutch size) and could be constrained by high nest and egg predation (Zuffi *et al.*, 1999). In mountainous areas, a natural limiting factor on both adult stage and multiple clutches is almost surely the continental climate, with a short active season and

unfavourable conditions for a rapid growth rate and a second ovulation period (present study). Growth after maturity is strongly reduced in most reptiles, and resources stored are utilized mostly for reproduction, even if at sexual maturity many reptiles do not reproduce immediately, but delay first reproduction for several years (Bell, 1980; Bonnet *et al.*, 2003). A differential increase of fecundity following a further increase of body size should favour a much higher benefit for reproduction (Shine, 2000). Growth patterns and fecundity in wild *E. orbicularis* in contrasting habitats, especially in the light of its geographical variation (see Fritz, 2003), should be the focus of future studies in a much wider context.

Acknowledgements

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