Research Article

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Preliminary Data on the Population of the Sicilian Pond Turtle, *Emys trinacris* Fritz et al., 2005 (Emydidae) Inhabiting the Gorgo Tondo Basso in the "Lago Preola e Gorghi Tondi" Nature Reserve, Sicily, Italy

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Abstract: The lack of data relating to basic life-history and population dynamics is one of the gaps to be filled in order to develop a proper strategy for the conservation of the Sicilian Pond Turtle (*Emys trinacris*). In this study, we present the results of the first year of a multi-annual monitoring program focusing on a specific wetland area, located within the "Lago Preola e Gorghi Tondi" Nature Reserve (Sicily, Italy). The subpopulation size was estimated with capture-recapture method at 719 ± 47 turtles, with a mean density of 239.7 ± 15.7 ind./ha. The overall sex ratio of the captured individuals was males-biased (2.9 : 1) but also a significant differences between spring and summer was found. We discuss this finding in relation to differential reproductive strategies of the sexes, with the support of data on movements and on body condition. The importance of a multi-year monitoring approach is underlined in order to get a better understanding of the factors that affect the population ecology.

Key words: *Emys trinacris*, population abundance, sex ratio, Body Condition Index

Introduction

The Sicilian Pond Turtle *Emys trinacris* Fritz, Fattizzo, Guicking, Tripepi, Pennisi, Lenk, Joger & Wink, 2005 (Testudines: Emydidae) has recently been distinguished from the European Pond Turtle *Emys orbicularis* (L., 1758) (Testudines: Emydidae) by genetic and morphological characters (Fritz et al. 2006). Although there are still doubts about its specific status (Speybroeck et al. 2010), several authors showed a clear differentiation at the genetic level (Pedall et al. 2011, Manfredi et al. 2013, Vamberger et al. 2015) and considered it a valid cryptic species (Fritz et al. 2006, Turtle Taxonomy Working Group 2014). Endemic to Sicily, *E. trinacris* is listed in Annexes II and IV of the 43/92/CEE "Habitats" Directive and considered "Data Deficient" by IUCN (van Dijk 2009). Andreone et al. (2013) classified *E. trinacris* as "Endangered (EN) A2c" in the Italian Red List. The species seems to be mainly threatened by pollution and habitat fragmentation, release of non-native species in the wild and in some cases by illegal capture for the pet trade (Di Cerbo 2010). Prior to 2005, relatively little was known about *E. trinacris* sensu stricto other than data on distribution and biometrical pattern (Ardizzoni & Fritz 1998, Zuffi & Ballasina 1998). Even though the species was described in 2005, a lack of information still exists relating to basic life-history and only few studies were carried out on its ecology (D'Angelo et al. 2006, Lo Valvo & D'Angelo 2006, Lo Valvo et al. 2008a, b, 2015).

This information, although of fundamental importance, may not be sufficient to effectively address the management strategies of the species. For this reason we started a multi-annual capture-marking-recapture study to estimate the factors governing the population dynamics of the species in the "Lago Preola e Gorghi Tondi" Nature Reserve (SW Sicily, Italy). The development of medium and long-term monitoring program is one of the keystones to understand the effects of local (Cheylan & Poitevin 1998) or global (Paleczny et al. 2015) threats but also to evaluate concrete management (Ficheux et al. 2014) and conservation (Bertolero & Oro 2009) actions, especially in long-living species (Cadi et al. 2004). In the present study, we focused on a specific wetland site to: (i) estimate the abundance of the present sub-population, (ii) investigate some sub-population and individual parameters such as sex ratio, movements and estimate body condition index.

Materials and Methods

The Nature Reserve of the "Lago Preola e Gorghi Tondi" is a protected area of south-western Sicily established in 1998 and currently managed by the Italian Association for the World Wildlife Fund (WWF Italy Ong - ONLUS). The reserve (medium point at N37°36'36.78" E12°39'8.19"), located within the municipality of Mazara del Vallo (Trapani), has a surface of 335 hectares and is surrounded by a homogeneous agricultural landscape (vineyards and olive groves). The Nature Reserve protects four distinct wetlands (Pantano Murana, Lago Preola, Gorghi Tondi Alto-Medio and Gorgo Tondo Basso), originating in a single karstic depression. Samplings were carried out in the Gorgo Tondo Basso on three occasions (May, June and July 2015) each comprising of three trapping days. The trapping site is a lake environment with an average surface area of approximately 3 ha, an extension of 300 × 180 m and a maximum depth of approximately 12 m. The basin does not have tributaries and the groundwater table is located in the calcarenites and is fed by local meteoric recharge (Cusimano et al. 2006). The main water-body is set on a sinkhole and it is therefore immediately deep and surrounded by riparian helophytes (*Phragmites australis*, *Typha latifolia* and *Cladium mariscus*); in the west side, an area with shallow waters (1–2 m depth) of about 0.9 ha is present. The nearest water body is the Gorghi Tondi Alto-Medio complex, situated only 200 m away but separated by a street and a slight difference in elevation. Individuals were captured using double fyke nets (n = 5, length = 3 m, diameter = 50 cm, eye = 18 × 15 cm) and baited funnel traps (n = 10, length = 60 cm, diameter = 30 cm, eye = 16 × 14 cm) to avoid bias in sex and age class (Tesche & Hodges 2015), located 50 m apart of each other, during three sampling occasions (May, June and July 2015) of three trapping-days each. Date of capture, trap number, sex, age (adult/juveniles), straight carapace length (SCL, sliding callipers to the nearest 0.1 mm), width (CW), height (CH) and body mass (BM, digital balance ±1 g) were recorded, and only individuals with evident sexual characters were considered as adults (Zuffi & Gariboldi 1995). In the analysis we discarded the body mass of individuals that urinated or defecated before the measurements. All captured turtles were individually marked by unique notches on their carapace (Servan et al. 1986). The studied sub-population can be assumed to be "closed" during the experiment because sampling occurred in a short period of time and no evidence of significant migration and emigration movements were observed from and to the nearest wetland (D. Ottonello 2015, unpublished observations). Additionally, we first tested if the sub-population can be considered closed by using the Closure Test (Stanley & Burnham 1999) of software CloseTest (Fort Collins Science Center, U.S. Geological Survey 2011). Then the sub-population abundance was estimated using models for closed populations following Otis et al. (1978), which incorporate three sources of variation among capture probabilities: (i) a temporal effect (*t*), where capture probabilities vary among capture occasions; (ii) a heterogeneity effect (*h*), where capture probabilities vary among individuals; and (iii) a behavioural effect (*b*), where a previous capture influences the behaviour of individuals in the next occasions. Analyses were carried out using the Loglinear Models for Capture-Recapture Experiments "RCapture package" (version 1.4-2) for R (R Core Team 2013) and the model selection was carried out following the Akaike Information Criterion (AIC). The presence of heterogeneity was tested using a graphical approach as explained by Baillargeon & Rivest (2007). We inferred the movements of *E. trinacris* by recapturing marked individuals during the same sampling occasion. For capture and recapture traps, we plotted the GPS coordinates within QGIS 2.8.2-Wien (QGIS Development Team, Open Source Geospatial Foundation) and then the minimum linear distance was measured between these points. To estimate activity area, we used the distance between the two most distant capture points (Kornilev et al. 2010). Sexual and period differences were tested using a two-way analysis of variance (ANOVA). Data and sexual differences (males vs females) in size and body mass were firstly checked for normality with Pearson's chi-square normality test and then analysed using a t-test. Population structure was analysed using a graphical approach by dividing SCL data in size classes of 10 mm. A body condition index (BCI) was used to test differences among months of activity. The BCI was estimated as BM / SV, where SV is the shell volume calculated with a modified formula for a semi-ellipsoid as suggested by Loehr et al. (2004) and partially modified [SV = (4 / 3 × π × SCL / 2 × CW / 2 × CH) / 2]. For this analysis we used also the data collected during a previous study carried on in April, June and September 2014 to expand the temporal spectrum. An independent one-way analysis of variance (ANOVA) for males and females was used to test for difference between years, using June as month of reference. One-way ANOVA was also used to test for difference in BCI among months for males and females independently. All ANOVA tests were preceded by Pearson's chi-square normality test to verify that data were normally distributed and by a F test to verify that data had the same variance and followed by a Tukey post-hoc comparison. All statistical analyses were carried out with R (R Core Team 2013) and we considered differences significant at $p < 0.05$. Data collected in different periods from the same animal were removed from statistical analyses to avoid pseudo-replication (Hurlbert 1984).

Results

A total of 640 captures (314 in May, 141 in June, and 179 in July) of 410 turtles were made in 2015; among these 32 were already marked in previous studies. Out of the examined individuals, 263 were captured one time, 100 two times, 33 three times, seven four times, five five times, one six times and one eight times. We collected 277 males, 94 females and 39 juveniles with an overall capture sex ratio of 2.9 M : 1 F. The capture sex ratio varied significantly among periods (Chi-square = 9.82, $p < 0.01$), with the lowest values being in July (2 : 1) with respect to May (3.9 : 1) and June (4.1 : 1). The number of turtles trapped per period differed significantly both for females (Chi-square = 11.42, $p < 0.01$) and for males (Chi-square = 46.33, $p < 0.001$), with similar values in May and July; the lowest number of captures for females were in June and the highest in May, and for males – in June and July, respectively (Fig. 1).

The sampling was highly shifted toward adults reflecting the low capture rate of juveniles, which represented only the 9% of the sub-population sampled. The Closure test supports our hypothesis that the sub-population can be considered closed

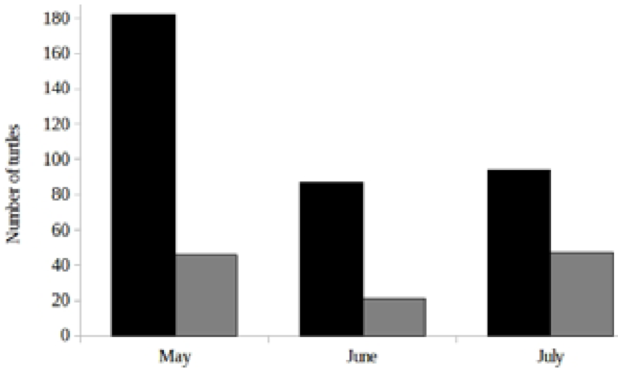

Fig. 1. Number of male (black) and female (grey) *Emys trinacris* captured per months at the Gorgo Tondo Basso

Table 1. Estimated abundances and model selection criteria for a population of *Emys trinacris*. M_0 = Equal capture model; M_t = Time variation (Schnabel) model; M_h = Heterogeneity model (Chao, Poisson, Darroch and Gamma); M_{th} = Time-heterogeneity model (Chao, Poisson, Darroch and Gamma); M_b = Behavioural response model; S. E. = Standard error; df = degree of freedom; AIC = Akaike's Information Criterion

Model	Abundance	S. E.	df	AIC
M_0	758.3	51.5	5	123.375
M_t	718.6	47.1	3	53.244
$M_{h\ Chao\ (LB)}$	779.6	61.0	4	124.738
$M_{h\ Poisson2}$	840.5	130.3	4	124.738
$M_{h\ Darroch}$	911.5	238.5	4	124.738
$M_{h\ Gamma3.5}$	995.3	385.6	4	124.738
$M_{th\ Chao\ (LB)}$	741.5	55.7	2	54.265
$M_{th\ Poisson2}$	811.1	121.8	2	54.265
$M_{th\ Darroch}$	895.3	231.0	2	54.265
$M_{th\ Gamma3.5}$	998.5	387.9	2	54.265
M_b	478.8	18.7	4	85.412

during the experiment (Chi-square = 5.46, p = 0.065). According to the AIC criterion the abundance of sub-population was estimated as 719 ± 47 individuals, selecting the time variation model M_t (Table 1), where probability of capture is different between trapping occasions. This result gives a density estimation of about 239.7 ± 15.7 ind./ha.

The sub-population was characterized by a dominance of turtles (72%) with SCL between 110 and 129.9 mm (Fig. 2). The most represented range class for males was 120–129.9 mm (46%), while for females it was 130–139.9 mm (48%), and for juveniles: 100–109.9 mm (64%).

Females were heavier (t-test = 14.72, $p < 0.001$) and bigger than males, both in terms of carapace length (t-test = 9.991, $p < 0.001$), width (t-test = 7.24, $p < 0.001$), and height (t-test = 16.95, $p < 0.001$) (Table 2).

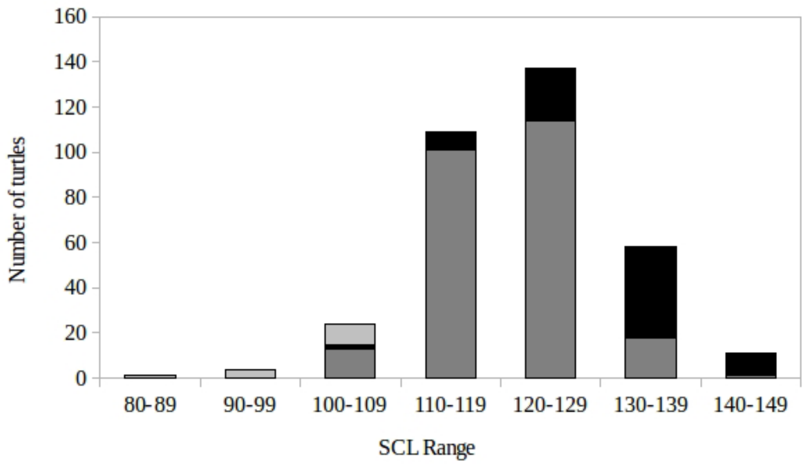

Fig. 2. Straight carapace lengths (mm) of *Emys trinacris* captured during the study at the Gorgo Tondo Basso: unsexed juveniles (light grey), males (grey), and females (black)

Table 2. Mean values of major biometric measurements (in mm and g) for *Emys trinacris*. SCL = straight carapace length; CW = carapace width; CH = carapace height; BM = body mass; N = number of individuals; S. E. = Standard Error

Biometric measurement	Sex	N	Mean	S. E.	Min	Max
SCL	♀	83	130.3	0.9	105.8	146.7
	♂	247	120.1	0.4	104.4	141.1
	J	14	102.3	1.9	87.2	108.5
CW	♀	71	102.2	0.9	77.6	121.3
	♂	189	94.8	0.5	72.8	110.0
	J	12	82.9	1.9	71.9	91.8
CH	♀	71	50.9	0.4	43.0	59.7
	♂	189	42.8	0.2	36.0	49.9
	J	12	36.1	0.8	31.1	40.7
BM	♀	81	402.6	7.5	229.0	583.0
	♂	194	283.1	3.0	192.0	414.0
	J	13	180.1	12.1	96.0	235.0

The BCI of females was lowest in May and July and highest in June (Fig. 3a), but it did not change significantly among the periods ($F_{4,108}$ = 0.186, p = 0.945). The BCI of males showed a significant variation ($F_{4,249}$= 3.797, p < 0.01) with the highest values in April and July (Fig. 3b), significantly different from May (Tukey, p < 0.05). For all periods combined, the mean BCI of females (0.072920 ± 0.00157 g/mm^3) was higher than the BCI of males (0.05085 ± 0.00607 g/mm^3), while the BCI of juveniles (0.03674 ± 0.00134 g/mm^3) was lower than that of adults.

We recaptured at least once 92 individuals, 50 in May, 22 in June, and 20 in July. The low number of recaptured juveniles (n = 6) did not allow any statistical analysis for this group. The proportion of recaptured animals did not vary among periods for males (Chi-square = 0.89, p = 0.64) and females (Chi-square = 1.95, p = 0.376). The period (F = 0.137, p = 0.872) and the sex (F = 0.984, p = 0.324) did not have a significant effect on activity area. The mean distance moved between recapture locations was 63.06 ± 21.36 m (range 0 ± 240.3 m) for females (n = 12), 46.49 ± 6.22 m (range 0 ± 275.9) for males (n = 74). We registered also a juvenile with a movement of 58.05 m; another five juveniles were recaptured in the same trap.

Discussion

These initial results are encouraging because our estimation of the sub-population inhabiting the Gorgo Tondo Basso showed a high density of individuals not reported for the species, except for a population living in a small pond (0.3 ha) near the "Rocca Busambra e Bosco della Ficuzza" Nature Reserve (Lo Valvo et al. 2008a). The population abundance of the congeneric and ecologically similar *E.*

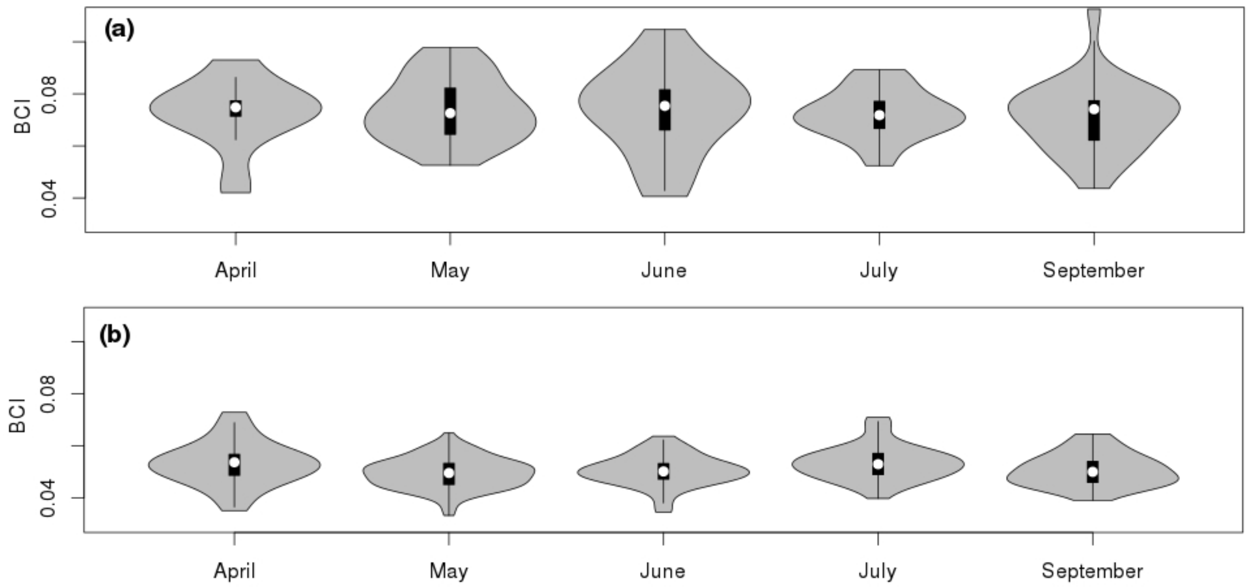

Fig. 3. Violin plot of Body Condition Index (BCI) monthly variation for female (**a**) and male (**b**) *Emys trinacris* at the Gorgo Tondo Basso; August is missing

orbicularis could vary considerably throughout its wide distribution range, depending on several factors (e.g., study methods, conservation status, habitat suitability, presence of competitors). High densities for *E. orbicularis* are reported for Hungary (128–242 ind./ha, Balázs & Györffy 2006) and Central Anatolia (242 ind./ha, Bayrakci & Ayaz 2014). In Italy, little is known about populations' abundance and density of freshwater turtles (Zuffi et al. 2010): 3–10 ind./ha of *E. orbicularis* were estimated with the linear transect census in the Bosco Mesola (Mazzotti et al. 2007) and 9.4 ind./ha in the Palude di San Genuario with the capture-recapture method (Seglie 2015). In the "Lago Preola e Gorghi Tondi" Nature Reserve, 23.8 ind./ha were estimated for *E. trinacris* with the of capture-recapture method (Lo Valvo & D'Angelo 2006). The sub-population of Gorgo Tondo Basso was apparently characterized by a size distribution that was markedly shifted towards adult age, with a bimodal distribution due to difference in size related to the sex. The low number of juveniles was not substantially different from that found by Lo Valvo & D'Angelo (2006) and could be explained by a sampling bias due to lower detectability and different habitat use (Zuffi 2000), low recruitment rates, or a combination of both (Keller et al. 2004). This is a common trait in freshwater chelonian studies due mainly to their cryptic behaviour (Pike et al. 2008). At present the absence of data on juvenile survival does not permit to formulate any hypothesis that could not be other than speculative. Relative to the previous study by Lo Valvo & D'Angelo (2006) the overall capture sex ratio of captured individuals was more shifted in favour of males, but we also detected significant differences between the periods that could be linked in our case to the different activity of males and females. This is one of the factors that can influence the sex ratio of adults as well as the sex ratio of hatchlings, differential age at maturity for males and females, differential mortality between sexes, and, obviously, sampling bias (Gibbons 1990). The analysis of the movements did not show any difference among periods and sex suggesting a similar use of the freshwater habitat by the adults, as shown with radiotelemetry both for *E. orbicularis* (Cadi et al. 2004) and for *E. trinacris* (Lo Valvo et al. 2008b, 2015). However, the different peaks of captures for the two sexes suggest varying patterns of activity during the year that are probably associated with differential reproductive strategies of the sexes, as shown for other Emydids (Morreale et al. 1984). In *E. orbicularis*, the reproductive activity starts with spring emergence, with a peak of courtship behaviour and mating between the end of March and May (e.g. Mitrus & Zemanek 2004, Servan & Roy 2004). During this period the males are very active, moving along the wetland looking for reproductive females (Lebboroni & Chelazzi 1991). This energy-expensive activity is also evident in males of Gorgo Tondo Basso, based on the significant decrease in May of the BCI, a parameter linked to nutritional and physiological status (Speakman 2001) that can correlate with fitness parameters such as survival (Shine et al. 2001) and reproduction (Dobson & Michener 1995). This is a common trait among freshwater turtles explained using a cost-benefit argument, where males disperse in search of females despite costs such as increased energy expenditure and increased predation risk (Morreale et al. 1984). On the contrary, the number of females captured reached the minimum in June, corresponding to the peak of egg-laying for Italian *E. orbicularis* (Zuffi et al. 2015) and also for this population of *E. trinacris* (D. Ottonello 2015, unpublished observations), when females can stay for several days away from the wetland (or at least in its peripheral area), or in other water bodies (Rovero & Chelazzi 1996, Meeske & Muhlenberg 2004). However, our data are consistent with published literature about *E. trinacris*, suggesting that the male-biased sex ratio seem to be common in Sicily (D'Angelo et al. 2006, Lo Valvo et al. 2006, 2008a). In our case we suspect a significant behavioural component that could influence the capture sex ratio, but in the absence of data about survival rates of females and males our considerations cannot be other than speculative. These preliminary results strengthen the importance of the multi-year monitoring approach that was chosen in order to get a better understanding of the population dynamic, essential for a proper management of the species within the protected area. Further analyses are in fact needed to explore the causes that may influence the sex ratio, the density, the population structure and the possibility of the presence of meta-population dynamics between different lakes using models for open populations.

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