

Demography and conservation of an isolated Spur-thighed tortoise Testudo graeca population in Dobrogea (Romania)

Gabriel Buică, Ruben Iosif, Dan Cogălniceanu*

University Ovidius Constanța, Faculty of Natural Sciences, Al. Universității 1, corp B, 900470,
Constanța, ROMANIA

* Corresponding author: gabriel.buica@profesor.edu.ro

Abstract. Spur-thighed tortoise is a vulnerable species. The local declines of populations led to an imperative need for conservation. *Testudo graeca* reaches its northern range limit in Dobrogea region, Romania. We studied a population from this region, which occupies an enclosed area of 32 ha within Histria Archaeological Complex. Based on a capture-mark-recapture study we estimated the population size of 221 ± 12.2 individuals. The observed density was 5.1 individuals/ha. The predicted population size suggests a relatively high density in relation to the area thus raising attention for a future conservation strategy. The population structure shows reduced sexual dimorphism and an unbiased sex ratio, implying a young population structure. We suggest correlating the future archaeological studies with conservation requirements of tortoises.

Key words: *Testudo graeca*, estimating population size, density, Romania.

Introduction

Demographic traits of animal populations offer useful information about their conservation status (WILLIAMS *et al.*, 2002) influencing their viability and persistence over time (BOYCE, 1992). The demography and population density are important as they guide the decision making process to establish if a population is suitable for conservation (AKÇAKAYA & SJÖGREN-GULVE, 2000), and help to implement the conservation measures at both local and wider geographical area (BERTOLERO *et al.*, 2007; ROZYLOWICZ & DOBRE, 2010).

Reptiles are a key component of ecosystems as predators, prey, grazers, seed dispersers and/or commensal species. Reptiles are declining worldwide, requiring extensive conservation measures (GIBBONS *et al.*, 2000). European reptiles and especially

tortoises are mostly influenced by habitat loss (COX & TEMPLE, 2009). The demographic traits differ among the species of *Testudo* genus and even between populations of the same species (WILLEMSEN & HAILEY, 2003).

The most of demographic studies concerning *Testudo graeca* report the size or the density of the studied population (e.g., KADDOUR *et al.*, 2006; RACHID *et al.*, 2007). However, density can be viewed as a measure of habitat occupancy and is the result of direct observation, dependent of field activity and study site, without offering an estimation of population size or its dynamic in time (INMAN *et al.*, 2009). Therefore, a reliable assessment of population size is required. The size of a reptile population is estimated using different methods (HILL *et al.*, 2007) because of the dissimilarities in activity patterns

(LAMBERT, 1981) and detectability of adults and juveniles (LAGARDE *et al.*, 2002). One of the widely used techniques is the capture-recapture (KENDALL & POLLOCK, 1992) since is straightforward to implement in small areas (KADDOUR *et al.*, 2006). This method is implemented in population size estimation oriented software (e.g., Mark, U-Care, POPAN; SUTHERLAND, 2006) and allows estimating the size of tortoise population, and detecting patterns of population structure (BESBEAS *et al.*, 2002) and survivability (BERTOLERO *et al.*, 2007).

The Spur-thighed tortoise, *Testudo graeca* Linnaeus, 1758, is a flagship species for conservation (WALPOLE & LEADER-WILLIAMS, 2002) due to its ability to attract public attention for conservation measures (BARUA *et al.*, 2011). It is considered a vulnerable species in Europe (COX & TEMPLE, 2009), and a species of community interest that requires the designation of special areas of conservation (HABITATS DIRECTIVE 92/43/EEC).

The populations of this tortoise are spread on a wide range around the Mediterranean Sea living in both dry and Mediterranean wet climate, reaching at altitudes up to 2000 m a.s.l. (ANADÓN *et al.*, 2012). The population densities vary between 2-6 individuals ha⁻¹ (HAILEY, 2000; DÍAZ-PANIAGUA *et al.*, 2001; KADDOUR *et al.*, 2006; ROUAG *et al.*, 2007) being reduced when compared to *T. hermanni* populations (i.e., with densities reaching 6.3 ha⁻¹ tortoises in France and 12 tortoises/ha in Romania; BERTOLERO *et al.*, 2011).

The fragmented range of *T. graeca* populations is the result of widespread agricultural activities and land use changes that led to the isolation of tortoises in island-like favourable habitats (ANADÓN *et al.*, 2007; SAUMURE *et al.*, 2007; PREDÁ *et al.*, 2009). Reduced size populations are at risk from being destroyed by vegetation fire (STUBBS *et al.*, 1985; SANZ-AGUILAR *et al.*, 2011) or from illegal collecting for pet trade (LJUBISAVLJEVIĆ *et al.*, 2001; TÜRKÖZAN *et al.*, 2008).

In Romania, *T. graeca* is found exclusively in the Dobrogea province (FUHN & VANCEA, 1961) at the northern limit of its

distribution range (COGĂLNICEANU *et al.*, 2010), with greatly dispersed populations. The largest population is in the north of the region and a second large population in the forested hills in the south (COGĂLNICEANU *et al.*, 2007, 2008).

This study is assessing the situation of an isolated population of *T. graeca* from Dobrogea. The objectives of this study were (1) to estimate the population structure, size and density, and (2) to provide adequate conservation measures for this isolated tortoise population.

Material and methods

Site description. The studied population is located in an enclosed 32 ha perimeter within the Histria Archaeological Complex (HAC; 44°32'56" N and 28°45'56" E), an area of grasslands surrounded by wetlands in the south of the Danube Delta Biosphere Reserve (Fig. 1). The HAC includes an active archaeological and touristic area with exposed ruins, and an archaeological site without touristic activity. The past archaeological activities have resulted in numerous pits, slopes and exposed ruins which in conjunction with tumuli and vegetation provide a variety of habitats for tortoises.

Estimation of demographic parameters. Monitoring and inventory were carried out during 2010-2012, throughout the active period of the tortoises (i.e., 29 visits from April to October, between 8 AM and 3 PM) using a capture-mark-recapture design. Animals were captured by active search along transects and marked on a marginal, posterior scute with a small indentation (STUBBS *et al.*, 1984). The animals were sexed based on external morphological characters (CARRETERO *et al.*, 2005), measured for straight carapace length (SCL) and curved carapace length (CCL), weighed and photographed for later identification (TICHÝ & KINTROVÁ, 2010). We used a threshold of 10 cm in SCL for separating juveniles/subadults from adults (STUBBS *et al.*, 1984). Individuals with underdeveloped sexual characters were considered subadults (WILLEMSSEN & HAILEY, 2003). We transformed the obtained data in a binary

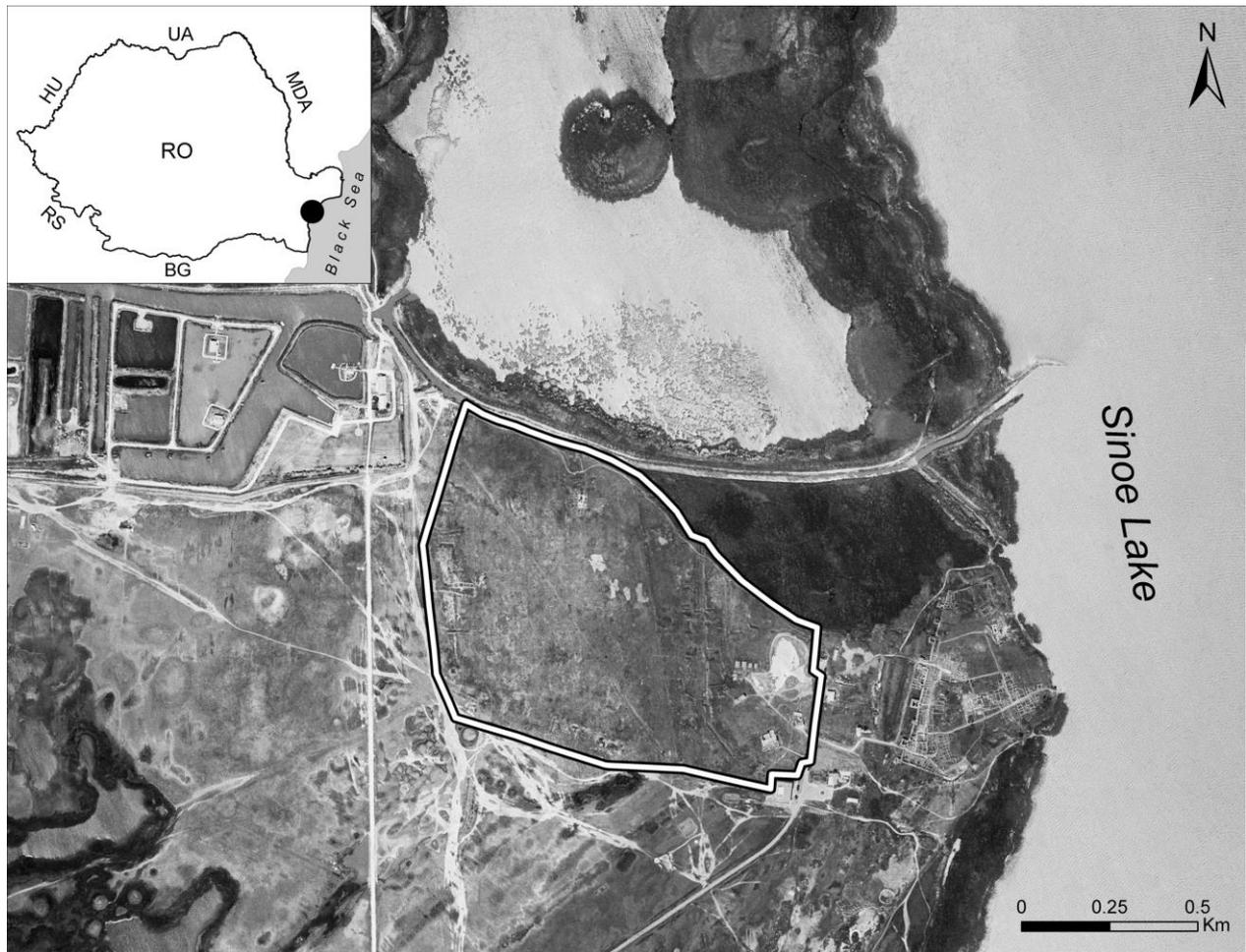


Fig. 1. Location of the study area in Romania (black dot in the upper left map) and the enclosure of Histria Archaeological Complex (white line contour).

string for each tortoise, forming the succession of capture/recapture (1) and the lack of capture (0) events. The number of events is equivalent to the number of occasions of capture-recapture.

The adult population structure was analysed using three biometric parameters (i.e., SCL, CCL and weight). The age (i.e., estimated from scutes growth ring counting; BERTOLERO *et al.*, 2005) was excluded from analyses because good estimation can be obtained only for juveniles and subadults, under 15 years (GERMANO, 1988). We tested for gender specific differences in SCL, CCL and weight using non-parametric Mann-Whitney U test.

We used the capture-mark-recapture design to estimate the population size (WHITE *et al.*, 1982) and analysed the data using Mark 6.2 software (WHITE & BURNHAM, 1999) and Capture module. The

population was assumed closed during the study due to: (i) unsuitable habitat surrounding the HAC which limits the emigration or immigration of tortoises, (ii) no dead tortoises observed during the study and (iii) low detectability for juveniles makes the assessment of population growth difficult (GUYOT & CLOBERT, 1997).

We used the models for the estimation of N included in Mark (POLLOCK *et al.*, 1990), the application being able to point to the more appropriate one (SUTHERLAND, 2006). Three estimators were selected to estimate N: a null model Mo, Daroch Mt, and Jackknife Mh. Each of these models is characterised by different features for the estimation of N (OTIS *et al.*, 1978): (1) Null model Mo requires constant probability of capturing for the entire period of the survey, (2) Mt Daroch model assumes variation of captures with time during the survey

period, (3) Jackknife Mh implies the variation of captures as function dependent on the individual, other than the capture probability for the entire population. We used these models to estimate the total population size, adult population size and separately for each sex, thus taking into consideration the gender specific differences in behaviour and different capture opportunities of adults and juveniles. Also, for this species there is no positively or negatively subsequent response to capture (PIKE *et al.*, 2005).

We also estimated the population size using temperature at the time of capture as an environmental covariate (HUGGINS, 1989). Only the adult population was estimated, using Mt model and the Huggins

Closed Captures data type in the covariation model.

Density estimation was computed using the number of individual captures and the estimated population size obtained from the Mark application in respect to the area being studied.

Results

We inventoried 164 tortoises in the study area and had 72 recaptures. Males had a higher recapture rate than females (males=58.11%, females=34.21%), which instead, attained a higher individual recapture rate. Only 14 juveniles were observed during the survey (Table 1).

Table 1. Number of captured and recaptured *T. graeca* from Histria Archaeological Complex.

	Males	%	Females	%	Juveniles	%	Total	%
Unique captures	74	45.12 ^a	76	46.34 ^a	14	8.54 ^a	164	***
Recaptures (n)	43	58.11 ^b	26	34.21 ^b	3	21.43 ^b	72	43.90 ^a
Recaptures n=1	25	58.14 ^c	13	50.00 ^c	3	1.83 ^c	41	56.94 ^c
Recaptures n=2	14	32.56 ^c	4	15.38 ^c	0	0.00 ^c	18	25.00 ^c
Recaptures n=3	3	6.98 ^c	5	19.23 ^c	0	0.00 ^c	8	11.11 ^c
Recaptures n=4	1	2.33 ^c	3	11.54 ^c	0	0.00 ^c	4	5.56 ^c
Recaptures n=5	0	0.00 ^c	1	3.85 ^c	0	0.00 ^c	1	1.39 ^c

^a - % of total captures; ^b - % of all captures for each sex; ^c - % of all recaptures for each sex.

221 ± 12.2 (Table 2).

Individuals with body weight between 1500-2000 g dominated the population (37.8% of all females and 63.3% of all males; Fig.2). The adult tortoises showed no significant differences between sexes in both SCL (Mann-Whitney U = 2803.50, *p* = 0.97; mean_{males} = 18.95 cm, mean_{females} = 18.87 cm), CCL (Mann-Whitney U = 2418.00, *p* = 0.136; mean_{males} = 24.73 cm, mean_{females} = 25.05 cm, Fig. 2) and body weight (Mann-Whitney U = 2492.50, *p* = 0.23; mean_{males} = 1679.53 g, mean_{females} = 1763.39 g).

Mt model was the most suitable for the used data set (Table 2) and estimated the adult population of tortoises at 197 ± 10.7 individuals, close to the cumulative estimation for sexes (89 ± 5.7 males and 107 ± 9.2 females). The total population, including the juveniles, was estimated at

There were no differences in estimation, for adults and individually for males and females, when Huggins Closed Capture data type is used, with temperature as covariate (Table 3).

Using direct observations, we estimated a density of 4.8 adult individuals/ha and a total density of 5.1 tortoises/ha including juveniles. Both observed and estimated densities stands within the range observed for other populations of this species (Table 4). Direct observation revealed a balanced sex ratio of 0.97, lower than that observed in other populations of the species (e.g., 1.26 in KADDOUR *et al.*, 2006).

Discussions

Although the population is isolated in a reduced area, our results reveal a population

with a high density. The population structure displays a young population with unbiased sex.

Population density is comparable with

that found in other populations occupying a reduced area, and similar to the results from other studies from Spain, Greece (HAILEY & WILLEMSSEN, 2000) and Morocco.

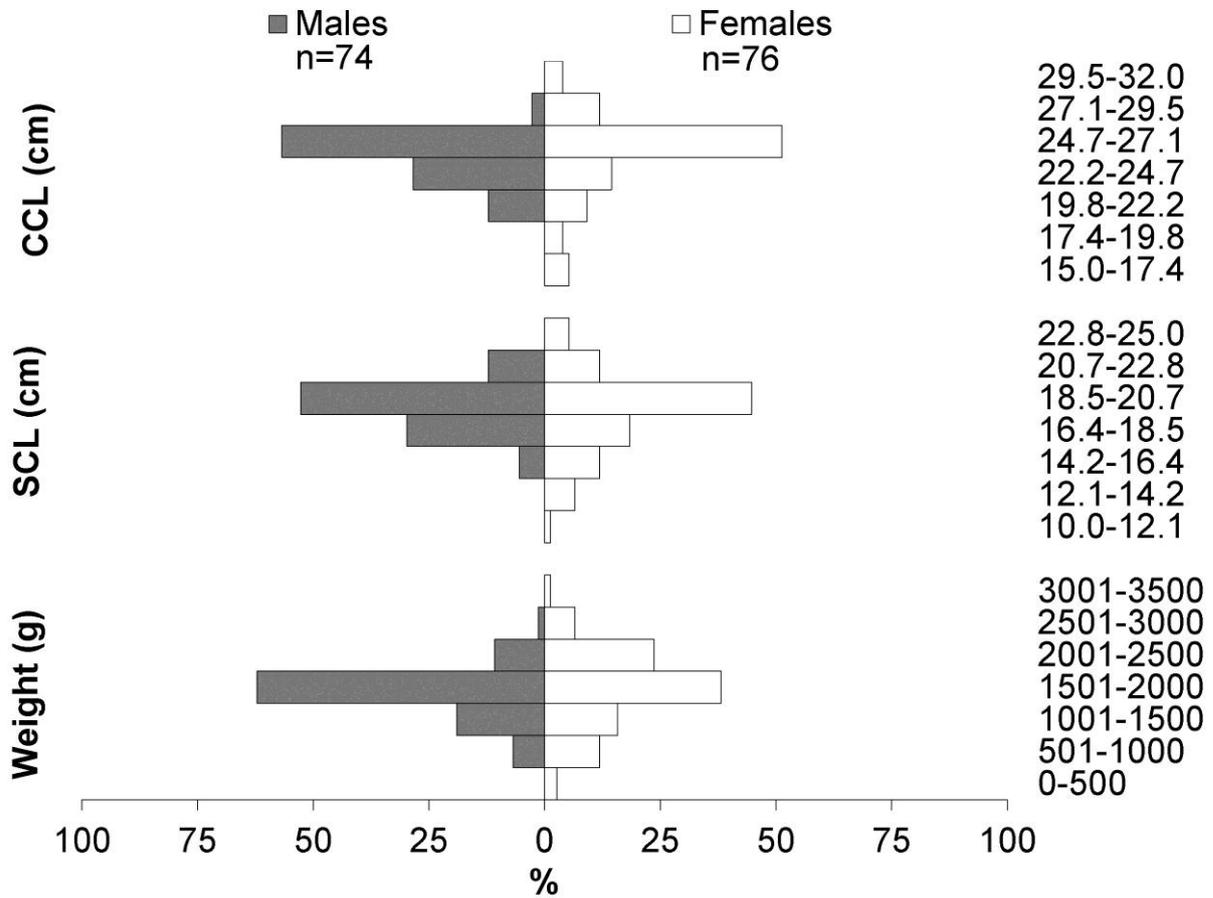


Fig. 2. The distribution of *T. graeca* adults in relation to classes of body weight, SCL and CCL.

Table 2. Estimation of *T. graeca* population size (N) (SD = standard deviation, CI = confidence interval, Mt +1 = number of unique captures and n = total number of captures, including recaptures).

	The used model	M(t+1)	n	N ± SD	CI (95%)	Maximum likelihood range
Adults and juveniles	Mo			255 ± 12.9	205-255	203-253
	Mt*	164	286	221 ± 12.2	202-250	200-249
	Mh ¹			301 ± 31.3	253-377	***
Males	Mo			92 ± 6.5	83-109	82-108
	Mt*	74	139	89 ± 5.7	82-105	80-103
	Mh ¹			100 ± 8.0	88-120	***
Females	Mo			110 ± 9.9	96-135	94-133
	Mt*	76	129	107 ± 9.2	94-131	92-130
	Mh ¹			176 ± 26.4	137-242	***
Adults	Mo			201 ± 11.4	183-228	182-226
	Mt*	150	268	197 ± 10.7	181-223	179-221
	Mh ¹			264 ± 25.6	224-326	***

* Mark-application -Capture module suggested model; ¹ interpolated estimation of N; in bold the estimation model considers most appropriate.

Table 3. Estimation of *T. graeca* population size (*N*) for adults using as estimation model of N_{M_t} and Huggins Closed Capture data type with temperature as covariate (SD = standard deviation, CI = confidence interval, $M_t + 1$ = number of unique captures and *n* = total number of captures, including recaptures).

	M (t+1)	n	N ± SD	95% CI
Adults	150	268	197.9 ± 10.9	180.7-224.6
Males	76	139	90.2 ± 6.0	81.7-106.6
Females	74	129	108.0 ± 9.5	94.2-133.1

Table 4. Density of *T. graeca* population in the study area compared with observed and estimated densities of other populations (individuals/ha).

Location	Method for estimation	Observed density	Estimated density (range)	Source
HAC (Romania)	Directly observed	5.1		This study
	Estimated - M_t model (including juveniles)	6.9	6.2-7.7	
	Estimated - M_t model (without juveniles)	6.1	5.5-6.9	
Spain	Directly observed	4.2-12	--	ANDREU <i>et al.</i> , 2000; BALLESTAR <i>et al.</i> , 2004
Greece	Directly observed	6.2	--	HAILEY, 2000
Morocco	Directly observed	5-7	--	SLIMANI <i>et al.</i> , 2002; KADDOUR <i>et al.</i> , 2006

The population sex ratio of 0.97 is unbiased than the reported value in other studies (KADDOUR *et al.*, 2006) and lower compared to similar studies on *T. hermanni* reporting values of 1.5 (HAILEY & WILLEMSSEN, 2000).

The Histria Archaeological Complex population showed no significant sexual size dimorphism (SSD). A lack of SSD was also observed in Mardin Province, Turkey (TÜRKOZAN *et al.*, 2003), while other studies showed a significant SSD (e.g., DÍAZ-PANIAGUA *et al.*, 2001; KADDOUR *et al.*, 2008).

The data shows a young tortoises population and the differences observed in this study from other population of this species should be correlated with history of the area inhabited by the tortoises. The isolated area offers favourable habitat and protection against human impact. Before this area was part of Danube Delta Biosphere Reserve the human impact was

greater with negative impact on the survival of tortoises because of on-going industrial development and agricultural practices limiting their habitat (DOROFTEI *et al.*, 2011; GIOSAN *et al.*, 2012).

The sandy habitats surrounding the study area are overgrazed and covered partly by bare soil are unfavourable for tortoise, providing little or no hiding places. This limits the dispersal of tortoises and contributes to its isolation.

The small number of juveniles captured and the lack of juveniles under age of three is the result of numerous factors and a situation encountered in other studies. The survivability rate for juveniles of tortoises is reduced (GARCIA *et al.*, 2003; DÍAZ-PANIAGUA & ANDREU, 2009) and they exhibit a more reduced activity pattern. In addition, their dimensions and the camouflage colour of carapace makes them hard to be observed (LAGARDE *et al.*, 2002).

The method for population size estimation used in this study is adversely affected by reduced recapture rate, under 50%, and especially by the low number of juveniles. This pattern is not unusual and is determined by age, activity periods (DÍAZ-PANIAGUA *et al.*, 1995) and sex of adults (DÍAZ-PANIAGUA *et al.*, 1996), environmental conditions, vegetation, temperature and precipitations (KADDOUR *et al.*, 2006).

The isolation of the studied population and its relatively high density requires specific conservation measures in the future for its survival. The favourable habitat may be reduced by archaeological activities or touristic development. The collecting of individuals and vegetation fires are risks that may lead to high mortality (STUBBS *et al.*, 1985) and ultimately to the loss of an isolated population.

We consider as conservation measure: (1) the strictly delimitation of the grazing areas closed to HAC to prevent the sheep from accidentally entering inside the tortoises' perimeter, (2) the controlled burning of vegetation (STRICKLAND, 2012) in winter month to reduce the risk of fire, of natural origin or human-made, and (3) a correlation of future archaeological studies with conservation requirements of tortoises. Stray dogs may pose a high risk to juveniles and hatchlings and limiting their access in the area is desirable.

The persistence of a population living in an enclosed area may be altered by the disturbance of a single landscape factor (e.g., changings in land use; RUGIERO & LUISELLI, 2006). Similar, the persistence and viability of our isolated population may depend on the presence of abundant vegetation that offer shelter in midsummer or of the exposed ruins that offer crevices for wintering.

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