OVIDIUS UNIVERSITY OF CONSTANȚA

FACULTY OF NATURAL SCIENCES AND AGRICULTURAL SCIENCES

SUMMARY OF THE DOCTORAL THESIS

THE STUDY OF AN ISOLATED POPULATION OF *TESTUDO GRAECA* IN DOBROGEA

Scientific Coordinator:
Prof. Dan Cogălniceanu

Doctoral student:
Buică Gabriel

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TABLE OF CONTENTS

INTRODUCTION

Testudo graeca (Spur-thighed tortoise) is a species considered vulnerable according to the IUCN Red List (www.iucnredlist.org) due to habitat reduction (Cox and Temple, 2009) following the expansion of agricultural land (Gibbons et al., 2000), which determines the overall difficulties for protecting reptiles (Anadón et al., 2006a). *T. graeca* is considered "species of interest" according to OUG 57/2007 and EC Habitats Directive 92/43/EEC. In Romania, this species reaches the northern boundary of its areal, being found only in Dobrogea, with a fragmented habitat (Cogălniceanu et al., 2007; Tudor, 2010). Long life, easiness of capture and handling, and general public attractiveness make *T. graeca* a priority species for conservation (Walpole and Leader-Williams, 2002).

The study underlying this PhD aims to present the current state of the population of *T. graeca* from Histria Archaeological Complex (Histria Citadel) in Dobrogea. The thesis is structured in two parts. A first, general part includes three chapters which present taxonomic problems of the genus *Testudo*, aspects of biology and ecology of *T. graeca*. Is performed and a brief overview of essential studies on this species in its distribution range in Europe, Africa, and the studies carried out in Romania. The second part presents personal results, consisting of a chapter describing the area where the study was conducted and three chapters for materials and methods used in this study, results, discussion and conclusions that emerged from the analysis of the obtained results.

The study was conducted with the permission of the National Museum of History and Archaeology of Constanța, Ministry of Environment and Forests (exemption no. 1173/03.08.2010), Danube Delta Biosphere Reserve Authority (authorization 21/09.04.2010 and 24/01.04.2011) and Măcin Mountains National Park Administration.

I am grateful to Professor Dan Cogălniceanu, Ph. D., for support, patience and suggestions that made possible this thesis. I thanks to associate professor Marius Skolka, Ph. D., and Marian Tudor, Ph. D., for advice given. Also I thank the guidance committee, consisting of Professor Dragomir Coprean, Ph.D., associate professor Marius Skolka, Ph. D., and Paul Szekely, Ph. D., whose contribution has improved the thesis.

Keywords: Testudo graeca, priority species, Histria, isolated population.

SCOPE AND OBJECTIVES OF THE THESIS

The study was conducted in Histria Archaeological Complex on an isolated population of spur-thighed tortoise (*T. graeca*) in order to characterize its structural parameters and identify, by comparison with other populations of tortoises, the factors responsible for specific adaptations.

Assumptions

The population of *Testudo graeca* from Histria Archaeological Complex area is isolated, vulnerable to anthropogenic activities and therefore requires specific protection measures.

Objectives

1. Assessment of the isolation degree for the *T. graeca* population from Histria Archaeological Complex.

2. Characterization of the structural parameters in the *T. graeca* population.

3. Evaluation of detectability and spatial distribution.

4. Assessment of the population’s health.

5. Development of a set for measures of protection and management.

1. TAXONOMIC CONSIDERATIONS ON GENUS *TESTUDO*

The genus *Testudo* includes species of land tortoises, with wide distribution in Europe, North Africa and the Middle East (Fig. 1). Habitats occupied by the species of this genus are diverse and strongly influenced by socio-economic activities. Most species are in decline, being included in the IUCN Red List (Groombridge, 1982) and the European reptiles Red List (Cox and Temple, 2009).

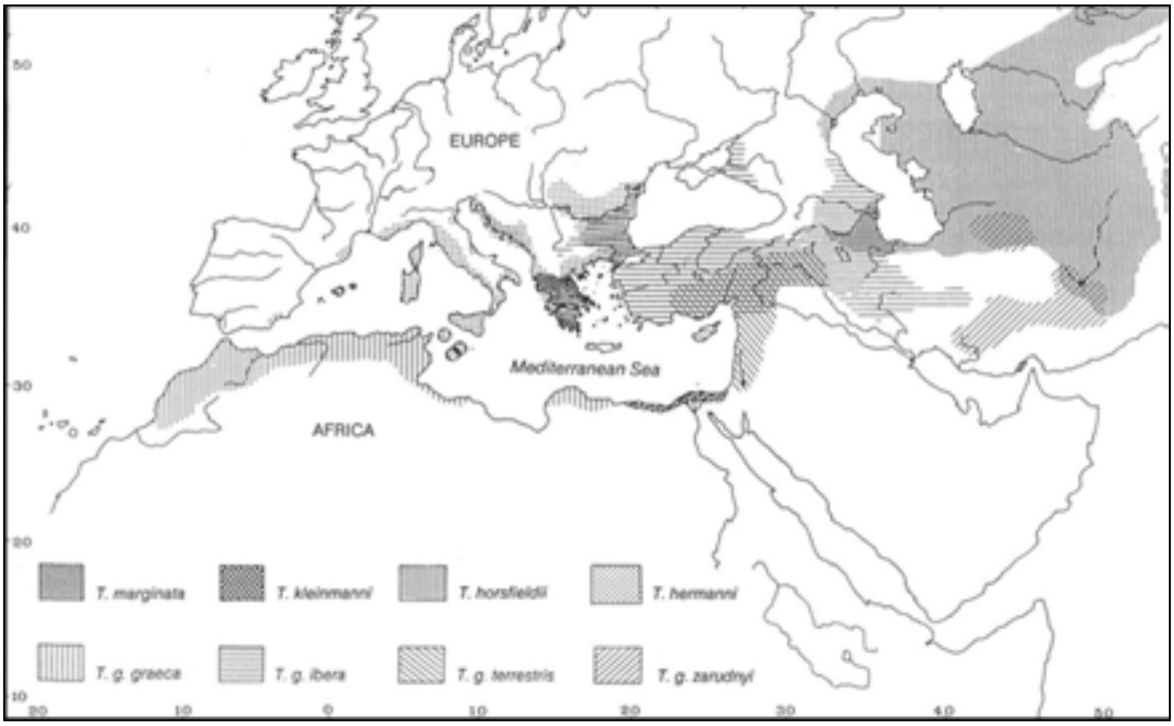


Fig. 1. The geographical distribution of the main species and subspecies belonging to the genus *Testudo* (after Stubbs, 1989).

Clarification of the taxonomic position of each species and the genus as a whole is desirable to promote a stable legislation concerning the protection of these tortoises (Mace, 2004). Most of the taxonomy of this genus is based on external and internal morphology characteristics. Highlighting the evolution of species and genus Testudo, and determining taxonomic relationships, as well as the validity of the assignment of names of species or subspecies requires molecular genetic studies, studies based on nuclear DNA and mitochondrial DNA (Le et al., 2006). Within this work, due to the controversial situation of the *Testudo* genus and uncertain taxonomic position for *T. graeca*, including subspecies *T. g. ibera*, all references to spur-thighed tortoise will be made using the scientific name of *Testudo graeca*.

2. CHARACTERIZATION OF THE STUDIED AREA

The study was conducted in the Histria Archaeological Complex (HAC), Constanța County, located in north of Săele Sandbar, in the Danube Delta Biosphere Reserve (DDBR). The base for this study is the data collected in HAC and were used for comparison with data from studies in Măcin Mountains National Park (MMNP) and Dumbrăveni Forest Nature Reserve (Fig. 2, Table 1)

Fig. 2. Localisation on Dobrogea's map of the three protected areas investigated in this study.

Table 1. Characteristics of the studied areas

Studied are	Area (ha)	Geographical position Lat N	Geographical position Long E	Elevation (m a.s.l.)	Human population density (inhabitants/km^2)*	Status
MMNP	11321	45,20065°	28,31762°	0-467	0,41	National Park
HAC	32	44,54877°	28,76603°	0-8	0,16	Scientific Reserve included in RBDD
Dumbrăveni Forest Nature Reserve	315,5	43,96504°	27,98030°	0-120	0,16	Nature Reserve

*population density estimated using data from nearby localities in the three areas (Source: County Council www.cjc.ro Constanta, Tulcea County Council www.cjtulcea.ro).

2.1. THE HISTRIA ARCHAEOLOGICAL COMPLEX

Histria Archaeological Complex integrates the ruins Histria Citadel, being known to the public as Histria Citadel (Fig. 3). According to the Romanian legislation, this area is part of the Danube Delta Biosphere Reserve, the strictly protected area Istria-Sinoe, (Law No. 82 of 20 November 1993, the law for the establishment of the "Danube Delta" Biosphere Reserve, with subsequent amendments and additions; Government decision No. 248 of 27 May 1994, for the adoption of measures to ensure the application of Law No. 82/1993 on the establishment of the "Danube Delta" Biosphere Reserve, with subsequent amendments and additions) being located in Central Dobrogea (Popovici et. al, 1984) and part of the Northern unit of the coastal zone (Malciu, 2000; O.G. 202/18 12 2002, approved by Law 280/2003 with subsequent amendments and additions). The area is located in the site of Community importance, ROSCI0065 Danube Delta (OMMDD 776/05.05.2007) and the area of Histria Citadel is included in the administrative territory of Istria village.

Fig. 3. The location of the Histria Archaeological Complex (Source: Google Earth).

The study area is about 32 ha, is not open for tourist and archaeological activities are reduced. Archaeological activities in the past have created a mosaic of microhabitats, with many bumps, favourable for tortoises, in contrast to the surrounding areas, relatively flat and monotonous which provide optimum wintering areas, shelter and oviposition (Fig. 4).

Fig. 4. Panorama of the western perimeter of the study area (Photo: Buică Gabriel).

An important aspect of HAC for this study is metal fencing on the south and west limit of the studied perimeter (Fig. 5). From the information obtained from employees HAC, the fence was completed in 2001. Initially the fence was surrounding the ruins area and about a third of the study area (Fig. 6).

Fig. 5. Overview from outside of the study area (Photo: Buică Gabriel).

Fig. 6. Fenced perimeter of the study area in HAC, in present (white dotted line) and in miniature the previously existing fencing (red dotted line and red arrow 2). Arrows mark the features of the two images. (Source:- www.cimec.ro thumbnail picture, dated 1999 and Google Earth image from 2003).

Flora and fauna include many species with protected status, both through national legislation and European legislation (Management Plan for Istria-Sinoe area, source DDBRA; GEO 57), and in the study area was encountered *Ephedra distachya*, a rare species in Romania.

2.2. ASPECTS OF SOCIOECONOMIC DEVELOPMENT

Histria Archaeological Complex is a major tourist attraction, especially in the summer. In the vicinity there is one company specialized in catering, which affords accommodation for a small number of people. Risks to flora and fauna in this area come from breeders of livestock, especially sheep. Also the risk of a fire is high, and for the study area this danger is accentuated by dry vegetation gathered over many years. Stress caused by herds of animals and fire can deeply impacts the tortoises (Stubbs and Swingland, 1985a).

3. MATERIAL AND METHOD

3.1. STUDY AREA BOUNDARY

The study was conducted in HAC, in an area of 32 hectares, 2.3 km perimeter, bounded on the south and west by a metal fence and a connecting channel between Lakes Istria and Sinoe (Fig. 7). This area was chosen for lack of tourism and extremely reduced archaeological activity. Also, the metal fence prevents livestock, locals and tourists from entering. Delimitation from the area included in the tourist route is done arbitrarily, the central alley of HAC and a wide area, about 200 m; with deep excavations and trenches that sometimes accumulate a substantial amount of water from rainfall.

Fig. 7. The study area from HAC (boundary- yellow outline, orange outline - administrative buildings, blue outline - temporary lake, green outline - depressions with depths of over 1 m, white - high areas or tumulus with heights over 1.5 m).

Given the peculiarities of the area a field assessment was made on the ground to determine the appropriate methods for inventory and monitoring. Also, were searched areas that offer opportunities for sun exposure to tortoises and areas that would enable wintering and oviposition. The integrity of the metal fence was checked. Searches have been carried out both in the touristic areaperimeter, with ruins, and the outside HAC to determine if there are specimens of *T. graeca* in surrounding areas.

3.2. THE STUDY PERIOD

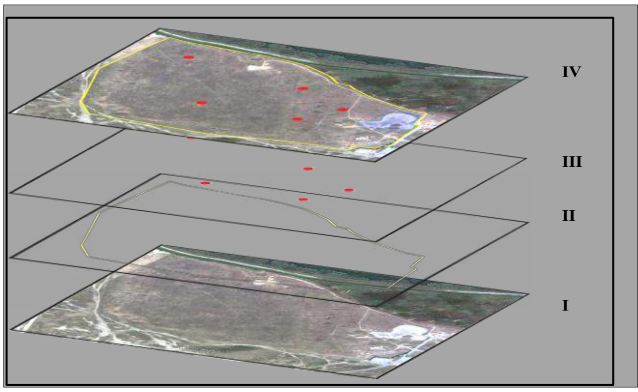
The study of *T. graeca* population in HAC was done between 2010-2012, during the months of April-June (in all three years) and September-October (not in 2012). During this period a total of 29 field trips were made, with an average duration of six hours. No field activities were performed on days with adverse weather conditions (Crosswhite et al., 1999) and in July-August due to high temperature. The time interval varied according to the season and weather conditions, generally debuting at around 9 a.m. (Hill et al., 2007). On days with high temperatures in field work activity began at around 8 a.m. and ceased when the air temperature reached 30^{o}C.

Data on tortoises from Măcin Mountains National Park were collected during 2006-2011 and for Dumbrăveni Forest in 2008, and are the results of studies conducted by members of the

Documentation Centre for the Biodiversity Studies.

3.3. SPATIAL DATA ANALYSIS

Global Positioning System (GPS) was used to record the trail every field trips, to obtain the position of each turtle captured and parts for panoramic photos. Acquired data were downloaded in the formats supported by the Google Earth (Google Inc., V. 6). Later, he realized the integration of GPS positioning biometric and meteorological (Fig. 8). Global positioning system (GPS) was used to record the route covered in every field activity, to obtain the position of caught tortoises and panoramic photos origin. The acquired data were downloaded in formats supported by the Google Earth application (Google Inc., v. 6). Subsequently, the integration of GPS positioning data with biometric and meteorological data was made (Fig. 8).



ig. 8. The steps in generating the map with the tortoises aptured positions (I. Standard Google Earth Map of the tudy area, II. Perimeter of the study area, III. GPS positions f captured tortoises, IV. Integrated map) (Source: Google Earth).

To visualize the distribution of captured tortoises based on GPS positions a grid with squares of side 50 m was created. By quantifying the catch from each square and placing data into SigmaPlot 11 application (2011 Systat Software) a distribution graph of tortoises was obtained, which was later superimposed over a map of HAC

3.4. ACQUISITION AND DATA ANALYSIS

Inventory and monitoring methods used were visual transects and active search, their use being adapted to field conditions. Mainly active search was used (Zuffi and Plaitano, 2007), a method considered effective in the case of a single observer in a complex environment with abundant vegetation and diverse microhabitate. The transect method (Mazerolle et al., 2007) was used in the central area, with reduced irregularities, especially during spring (Ivanchev, 2007). Outside HAC were conducted four search paths for *T. graeca*, up to a distance of 1.5 km south of the complex. Search was performed using visual transects with 100 m distance between.

Tortoises immobilization was accomplished manually positioning them with the shells on the ground. Data collection was performed at the capture site. The behavior of the tortoise at the moment of capture was described using a number of attributes for each activity type (Table 2) (Douglas and Niblick, 1994). The existence of external parasites was visually verified. We used a form sheet in the field foreach capture/recapture event (Table 3). We used as threshold for separating juveniles/subadults from adults a carapace length of 10 cm (Stubbs et al., 1984). The 10 cm threshold for the delimitation of adult and juveniles (Stubbs et al., 1984; Slimani et al., 2002; Ben Kaddour et al., 2006) established for this study did not allowed the inclusion of four tortoises with linear carapace length between 10-13 cm, with underdeveloped sexual characters for clear differentiation of juveniles, being considered subadults (Willemsen and Hailey, 2003). Differentiation between the sexes was based on external morphological characters (Carretero et al., 2005; Hailey, 2000).

Weather data were obtained using three dataloggers Lascar EL-USB-2 supplemented with data from the weather station at Mihail Kogălniceanu, Constanța, taken from www.tutiempo.net site. All captured tortoises were digitally photographed using a digital camera Nikon P100, 10 MPixel. We used as size standard a metal coin of 10 bani, with a diameter of 2.05 cm.

Young tortoise age was estimated using photos, based on counting of growth rings. Growth rings from at least three carapace scutes were counted (Bertolero et al., 2005). This method provides a good estimate of age for juvenile and subadults (Germano et al., 1998), but the method is not recommended for adult tortoises.

The recognition of captured tortoises in case of recapture was done in two ways. For short periods a black permanent marker was used for numbering the tortoises on the plastron (Stubbs et al., 1984). Each tortoise had a permanent marking made in the form of an indentation at one of the marginal posterior carapace scutes. Final identification was based on comparison of recaptured tortoises' photos with previous images. Analysis of the biometric characteristics of tortoises and their behaviour was achieved for all tortoises captured in both the study area and for those caught in the touristic area.

3.5. EVALUATION OF DETECTABILITY

Monitoring and inventory activity of *T. graeca* covered the whole period of activity of this reptile (Hill et al., 2007). A set of factors were used for detectability assessment: sex of individuals, age and the type of tortoises' activity, environmental factors and vegetation. The evolution of catches in the spring period, the period of reproduction, was compared with the evolution of catches in other months. Evolution of captured juveniles is also important, the latter having a much lower detectability. Catches of juveniles may indicate possible areas for submission of clutch mass. Vegetation development was monitored throughout the study by conducting a fotomonitoring activity (Proulx and Parrott, 2008). The purpose of this activity was to correlate seasonal captures of tortoises with vegetation dynamics.

Table 2. Attributes used to describe the activity for *T. graeca* observed at HAC.

Attribute	Description of tortoise activity
Repose	Is alert, with limbs close to the carapace, ready to move
Exposure to Sun	Limb position away from body
Activ	In motion
Hiding	Hiding under vegetation or digging into the ground
Hided	Completely hidden or partially visible from under vegetation or soil
Feeding	
Reproduction	

Table 3. Activity sheet for data recording during the study.

No. crt.	Date	Hour	No. recording	GPS position		Sex		Recapture		SCL (cm)	CCL (cm)	CH (cm)	Weight (g)	Air temperature (°C)	Air humidity (%rh)	Dew point (°C)	Details	Activity type							Cloud cover			Sun			Wind		
				Lat N	Long E	F	M	YES	NO									Rest	Exposure to Sun	Activ	Hiding	Hided	Feeding	Reproduction	Weak	Medium	Complete	Weak	Medium	Strong	Weak	Medium	Strong

SCL- straight carapace length
CCL- curve carapace length
CH- carapace height

3.6. POPULATION SIZE ESTIMATION

Estimating population size of *T. graeca* in the study area of the HAC was based on capture-mark-recapture method. Monitoring and inventory activity of tortoises was designed to ensure equal opportunities for tortoises to be captured, by carrying out activities throughout the tortoises' period of activity in a year. Several sets of population estimates were computed using the full set of capture / recapture, or just recaptured adults to reduce the error caused by reduced detectability for juveniles. For adults a separate estimation for males and females was conducted. Estimates were made considering the studied population as a closed population during the study (Pollock et al., 1990), which allows the estimation of population size (N) from the capture (p) and recapture (c) probabilities. Juveniles under age of three years were excluded.

The program Mark 6.2 (Mark in subsequent references) (White and Burnham, 1999) and Module Capture (White et al., 1982) included in this application was used to perform population size estimations. Input data were capture / recapture by selecting Closed Captures workflow and specify the number of occasions of capture / recapture. The estimation was detailed only for adults, using temperature at time of capture as covariate (Huggins, 1989). The data accepted by Mark are in binary format (1/0), where 1 corresponds to (re) capture and 0 its absence. The number of events is equal to the number of occasions, capture-recapture activities.

Population size estimation models were chosen based on how the data were acquired and the species studied. Capturing this species does not result in behaviour that positively or negatively influence the subsequently recapture (Pike et al., 2005), thus excluding models for estimating population size that used behaviour as variable. Given these features we selected three models to estimate N: *Null* M_o, *Daroch* M_t and M_h *Jacknife*. These models were used for all four sets of estimates, specifying the valid model suggested by application. For Huggins Closed Captures data type the selected estimation model was M_t.

The *T. graeca* population occupies a reduced territory in the HAC area and density estimation provides limited information, but useful for comparisons with other populations of this species. Density was calculated only for the area where the study was conducted within the HAC. An estimation of density was obtained for inventoried tortoises and for estimations provided by Mark relative to the area of surveyed territory. Density was estimated for the entire population and separately for adults. Application Distance 6 was also used to estimate density (Thomas et al., 2009). Population densities obtained for *T. graeca* of HAC were compared with data for population densities of this species in Spain (Andreu et al., 2000; Ballestar et al., 2004), Greece (Hailey, 2000) and Morocco (Slimani et al., 2002; Ben Kaddour et al., 2006).

3.7. MORPHOMETRIC ANALYSIS OF PLASTRON

Digital image analysis morphometry of plastron requires images with reduced spherical aberrations, reduced plastron inclination and a standard for subsequent measurements. We studied the possibility of using photographs for making complex measurements on the plastron. Thus, in addition to the usual measurements of length, height, by analyzing digital data the scutes' area may be obtained. For carapace, such an analysis is difficult due to the shape and the inclined position of scutes.

3.8. ASSESSMENT OF ANTHROPOGENIC IMPACT ON THE POPULATION OF *TESTUDO GRAECA*

Testudo graeca suffers from direct and indirect human impact. The direct impact is achieved through deliberate blows and collection as pets (Türkozan et al., 2008). An indirect impact is due to habitat destruction, fragmentation due to agricultural practices and flicks by vehicles or agricultural machinery. Given the characteristics of the pressure of human activity on the populations on *T. graeca* we evaluated the extent of damage of the carapace and plastron as a result of human activity and the frequency and type of malformations in the population (Velo-Antón et al., 2011). Juveniles were not excluded from the analysis because they allow an estimation of the present anthropogenic impact and provide a basis for future comparison. Thus, we can highlight the effects of changes in the socioeconomic climate, the effect of environmental education and the conservation activities for this species. Scars analysis was performed on a total of 750 specimens of *T. graeca*, adults and juveniles, from two populations: HAC and MMNP. For the population of MMNP we used photos and measurements made by Cogălniceanu et al. (unpublished) from 2006-2011.

Anthropogenic impact assessment based on observation of scars resulting from wounds caused by blows to the carapace and plastron (Tuegel, Weise, 2006) was performed using a carapace model with five zones and a plastron model with four zones (Fig. 9). The results were correlated with similar studies conducted on the tortoises of MMNP to have an insight into anthropogenic impact.

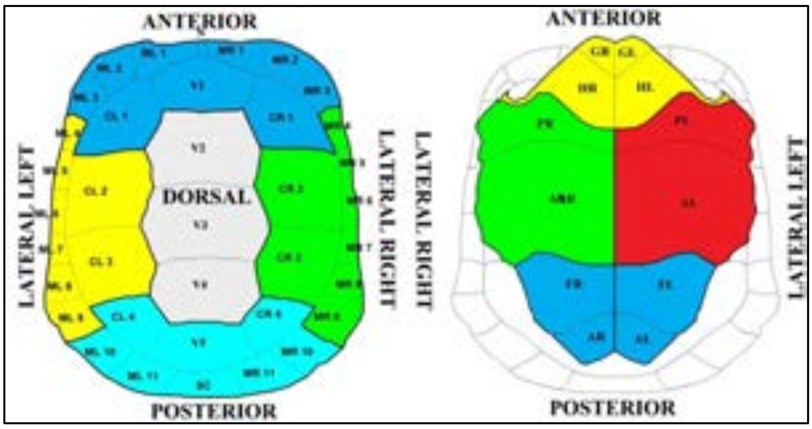


Fig. 9. Zoning pattern of the carapace and plastron for scars assessment.

Scars were classified into two categories. The first category is anthropogenic scars, which includes all of human activity assigned scars produced by motor vehicles, agricultural activities or scar resulting from wounds, such as those of the axe. The second category includes natural scars resulting from natural events such as falls, fire or digging activities (Meek, 2007) and animals' bites. The distinction between these two categories was based on the position and characteristics of the carapace and plastron wounds that caused those scars.

The zoning is useful in determining the zones of carapace and plastron with malformations. We evaluated the possibility that a reduced and isolated population will present certain malformations or malformations with higher frequency. The data were compared with those in the literature and those obtained from studies on the population of MMNP.

Fluctuating asymmetry analysis, seen as deviation from bilateral symmetry (Valen, 1962), was performed for plastron (humeral, femoral and pectoral scutes) using digital images. The area, height and width of these scutes were measured. A mean of each parameter for all three scutes on the same side of the plastron, and the differences between left and right side of the plastron were analysed for evidence of deviations from bilateral symmetry. Subadults and juveniles were excluded from analysis due to the small number of captures. The method adopted in this study was based on analysis of data obtained by digitally shape the plastron scutes (Davis and Grosse, 2008).

4. RESULTS AND DISCUSSION

4.1. THE POPULATION STRUCTURE OF THE *TESTUDO GRAECA* FROM HISTRIA ARCHAEOLOGICAL COMPLEX

In 2010-2012 we inventoried 170 tortoises in HAC, 155 adult tortoises (76 males and 79 females) and 15 juveniles. Sex ratio was lower than that observed in other populations of this species or species of this genus (Table 4). The small number of inventoried juveniles was due to their low detectability (Lagarde et al., 2002).

Table 4. Sex ratio in different populations of *Testudo*.

Species	Location	Sex ratio	Source
T. graeca	HAC (Romania)	0.96	This study
	MMNP (Romania)	1.45	Cogălniceanu et al., unpublished
	Morocco	1.26	Ben Kaddour et al., 2006
	Spain	1.66	Díaz-Paniagua and Andreu, 2009
T. hermanni	Greece	2	Stubbs and Swingland, 1985a
		1.5	Hailey and Willemsen, 2000

The distribution of adults according to the linear length of the carapace and body weight (Fig. 10) was according to a Gaussian curve, but with differences between sexes due to sexual dimorphism, females reaching larger sizes.

Age of juveniles and adults, estimated by quantifying growth rings has not been established for all individuals due to inability to identify growth rings in some adult specimens, mostly due to strong erosion of carapace scutes. These specimens were excluded from the assessment of age. Adult age class distribution (Fig. 11) is similar to the distribution of length and weight classes.

Population structure, both as size and age, was similar to the structure seen in other populations of *T. graeca* (Ben Kaddour et al., 2006) or species of this genus (Stubbs and Swingland, 1985b). Although the accuracy of the age estimation by counting growth rings is not reliable for ages older than 15 years, the data obtained with other two biometric characteristics (carapace length and weight) suggests a young population, relative to the life span of this species and the presence of juveniles indicate a reproductive population.

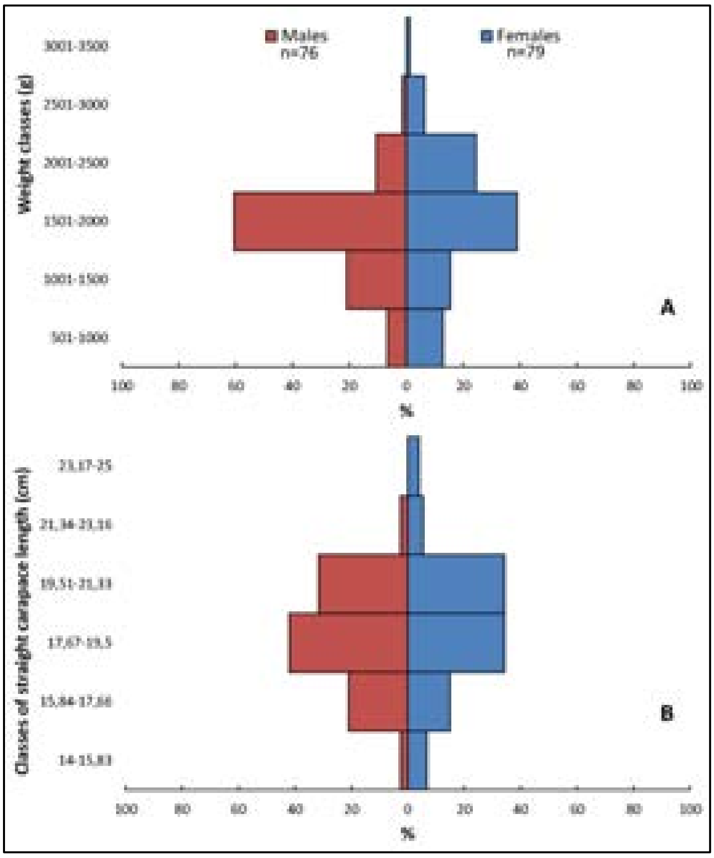


Fig. 10. The distribution of the adults of *T. graeca* in relation to body weight classes and classes of linear length of the carapace (B).

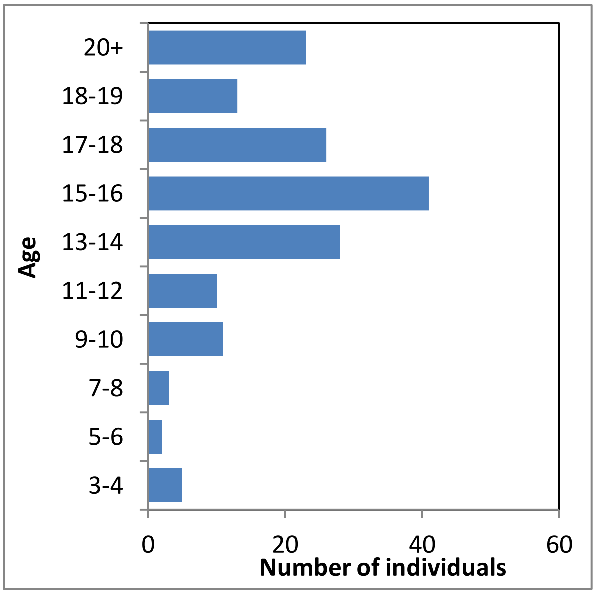


Fig. 11. Distribution of *T. graeca* age classes.

Behaviour influences the ratio males: females, males being more active, more noticeable, thus artificially increasing sex ratio.

4.2. BIOMETRIC CHARACTERISTICS

Carapace size and weight of HAC tortoises are similar to those observed in tortoises in MMNP (Cogălniceanu et al., unpublished) (Table 5). Average dimensions of carapace straight length and average curve length of adult tortoises from HAC are close to those of populations of *T. graeca* in Dobrogea, Turkey and Greece, but superior to the size of the studied tortoises' population in Spain and Morocco (Table 6). Gender differences are not statistically significant between the curve carapace length (Kruskal-Wallis P=0.123, n=79 for females and n=76 for males) and weight (P=0.203 Kruskal-Wallis, n=79 for females and n=76 for males). Morphometric parameters are significantly correlated.

Females tortoises attained the highest values of carapace curved length and weight. Analysing the biometric characteristics using the Pearson correlation coefficient (r) is observed a positive correlation between the measured parameters, highly significant for HAC tortoises and for tortoises from the MMNP (P <0.001, and r> 0.85). Therefore, I recommend that in field a single parameter should be measured, the curve carapace length because it is the easiest to measure. Photos with a standard measuring element can then be used to accurately determine the linear length of the carapace.

Table 5. Biometrics of *T. graeca* of MMNP and HAC (SD-standard deviation).

Histria Archaeological Complex						
	Juveniles n=15		Males n=76		Females n=79	
	Mean ± SD	min-Max	Mean ± SD	min-Max	Mean ± SD	min-Max
Straight carapace length (cm)	8.1 ± 2.2	4.9 – 11.5	18.8 ± 1.5	14.8 – 21.5	18.6 ± 2.6	11.9 - 25.2
Curve carapace length (cm)	10.5 ± 3.1	5.5 – 14.2	24.6 ± 1.8	20.0 - 29.0	24.8 ± 3.3	15.0 - 32.0
Carapace height (cm)	4.2 ± 1.2	2.0 – 6.0	9.7 ± 0.9	8 - 12.5	9.6 ± 1.3	4.5 - 11.7
Weight (g)	199.0 ± 141.5	26.0 – 420.0	1667.0 ± 354.9	583.0 - 2612.0	1772.0 ± 585.6	386.0 – 3332.0
Măcin Mountains National Park						
	Juveniles n=21		Males n=143		Females n=98	
	Mean ± SD	min-Max	Mean ± SD	min-Max	Mean ± SD	min-Max
Straight carapace length (cm)	8.8 ± 1.9	4.9 – 13.2	20.6 ± 2.4	13.2 - 26.5	20.6 ± 4.5	11.0 - 36.5
Curve carapace length (cm)	11.2 ± 2.5	6.2 – 17.0	25.5 ± 2.6	17.0 - 32.2	25.0 ± 4.7	14.0 - 31.6
Carapace height (cm)	5.0 ± 1.2	2.6 – 7.3	10.2 ± 1.2	6.5 - 14.0	10.5 ± 2.1	5.5 - 14.2
Weight (g)	199.0 ± 128.0	27.0 – 574.0	1771.5 ± 486.6	544.0 - 3305.0	1856.0 ± 797.0	317.0 - 3198.0

Table 6. Average straight carapace length and average curvature carapace length in different populations of *T. graeca*. (SLC-straight length of the carapace, CCL-curve carapace length).

Location	SCL		CCL		Source
	Males	Females	Males	Females	
HAC (Romania)	18.8	18.6	24.6	24.8	This study
MMNP (Romania)	20.6	20.6	25.5	25.0	Cogălniceanu et al., unpublished
Dumbrăveni Forest (Romania)	17.3	18.1	25.3	21.4	Tudor, 2010
Mardin Province Turkey	20.8	20.3	26.4	26.7	Türkozan et al., 2003
Doñana, Spain	***	***	14.0	16.9	Díaz-Paniagua et al., 2001
Jbilet, Morocco	12.5	14.7	16.3	19.2	Ben Kaddour et al., 2008
Greece *	17.8		24.8		Willemsen and Hailey, 2003

*** missing data * data is exclusively for adults, without distinction of sex.

The correlation between curve carapace length and weight for males and females tortoises from MMNP and HAC (Fig. 12) shows regression slope with similar characteristics and without are statistically significant differences (ANOVA, $P > 0.05$).

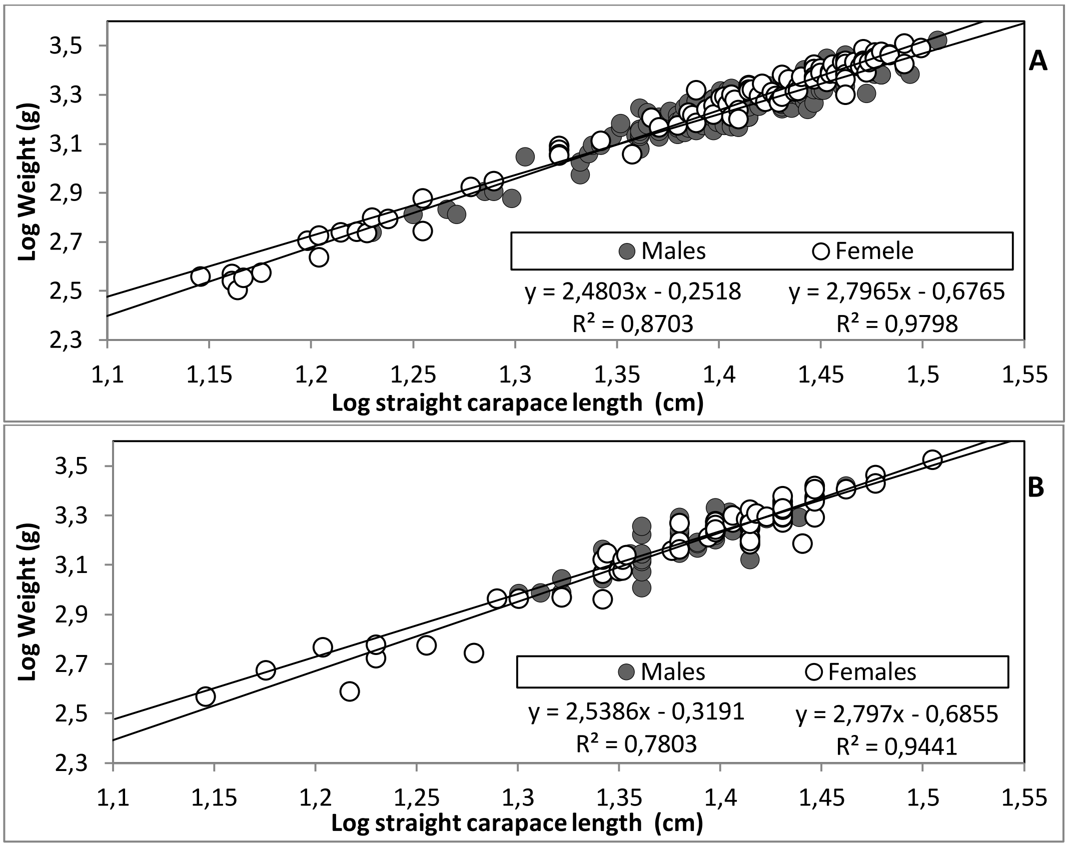


Fig. 12. Correlation between curve carapace length and weight for *T. graeca* of MMNP (A) (n = 143 for males and n = 98 for females) and HAC (B) (n = 76 for males and n = 79 for females).

4.2.1. Growth rate

Multiple captures of the same individuals in 2010-2012 allowed the estimation of growth rate for weight and straight carapace length of nine tortoises (four females and five males) (Table 7) with an average size close to observed characteristics. Considering an active period of eight months each year increases in monthly length is less than 1 mm in both sexes. Weight gain for each active month is very low, not feasible if using monthly weight increase as comparison due to its observed variability, at the level of the whole period of activity, as well as monthly.

Table 7. The average increase in weight and straight carapace length (LLC) in successional recaptured females and males in (n = 4 for females, n = 5 for males; SD is the standard deviation).

	Last capture		Difference		Average growth/ activity month
	Mean weight (g) ± SD	min-Max (g)	Mean weight (g) ± SD	min-Max (g)	Weight (g)
Females	1662.00 ± 463.26	1244.00 - 2276.00	163.36 ± 81.52	88 – 251.00	10.21
Males	1600.80 ± 225.58	1282 – 1809	105.19 ± 131.67	-34 – 284.00	6.57
	Average SCL (cm) ± SD	min-Max (cm)	Average SCL (cm) ± SD	min-Max (cm)	SCL (cm)
Females	18.71 ± 1.62	16.46 – 20.32	1.35 ± 0.95	0.24 – 2.55	0.084
Males	18.80 ± 1.20	17.07 – 20.40	1.34 ± 0.59	0.69 – 1.97	0.083

4.3. DETECTABILITY EVALUATION AND INFLUENCE OF ENVIRONMENTAL FACTORS

4.3.1. Detectability evaluation

We performed 286 observations for tortoises, including the captures and recaptures. In total we identified 164 individuals from the studied population, the remaining 122 individuals representing recaptures within the inside perimeter of HAC. Because detectability varied depending on the tortoises' size, sex, season, vegetation cover, researcher and others factors, we estimated the probability of detection for the entire study period, from April to October, except July and August, when activities were not conducted due to high temperature. A separate estimation was made for April-May and September-October in the 2011 season, excluding June when the number of activities was reduced. The probability of detection (*p*) was estimated using the application Presence 4.4. as very high (Table 8), and the ability to detect *T. graeca* during the period for which the estimation was done is also high.

Table 8. The probability of detection (*p*) calculated with application Presence 4.4 and the estimation of the minimum number of visits (n) (A) in the study area required to observe *T. graeca* compared with the probability that the species will avoid detection (B).

Time frame	*p*	AIC	2log (likelihood)	A		B	
				n; α = 0,05	*n; α* = 0,01	n=1	n=2
2010-2012	0.99	4	0	0.7	1.0	0.01	0.0001
April-May	0.99	4	0	0.7	1.0	0.01	0.0001
September-October	0.99	4	0	0.7	1.0	0.01	0.0001

Detectability was higher in the spring than during September and October (Fig. 13), with observed variation in the number of females and males captures. These monthly variations are similar to those observed in *T. graeca* in Spain (Díaz-Paniagua et al., 1995). A multiple regression using monthly average temperature and number of days with precipitation showed that male activity is influenced by temperature and precipitation to a greater extent ($R = 0.665$, n = 74) than female activity ($R = 0.386$, n = 76).

There is a significant negative correlation between the number of captures for males and monthly average temperature ($r = -0.664$, $P = 0.018$, n = 74). Higher detection of females in May and June may be related to the period in which they seek a place for oviposition and warmer temperatures also seem to favour their activity. For females no significant correlation was observed in the number of captures and the monthly average temperature and number of rainy days in each month.

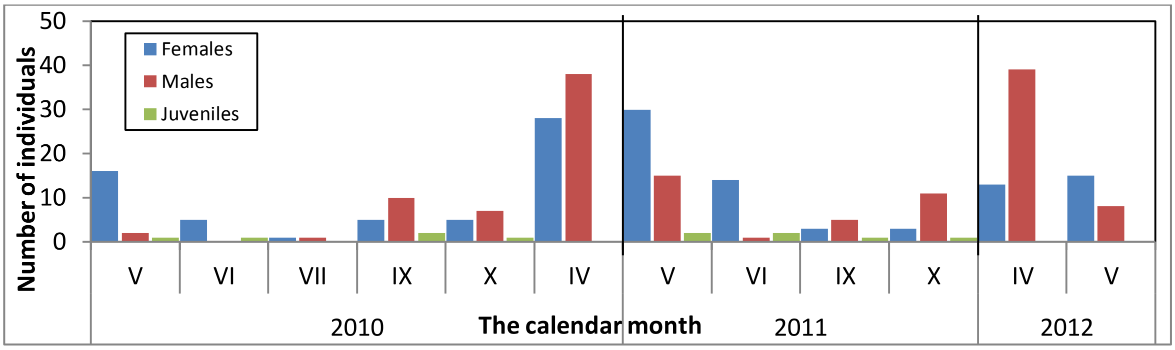


Fig. 13. Representation of captures (including recaptures) for each month of the study.

4.3.2. Influence of temperature and time on the detectability range

Capture frequency depending on air temperature indicates a high detectability for adults of *T. graeca* in the temperature range 17-21°C. Capture frequency varies hourly. Successful detection of *T. graeca* in the study area is conducted at an air temperature of 19°C and between 11-14 hours. Most tortoises are most at an average temperature of 20.8°C during April-October, measured in 2011 season in the HAC. Temperature dynamics during the activity season determines the detectability changes at different time intervals (Fig. 14). There is a significant relationship between captures of adult *T. graeca* and the hour of capture (χ^2 = 16.964, DF = 6, P = 0.009) and also with the temperature range (χ^2 = 18.760, DF = 6, P = 0.005).

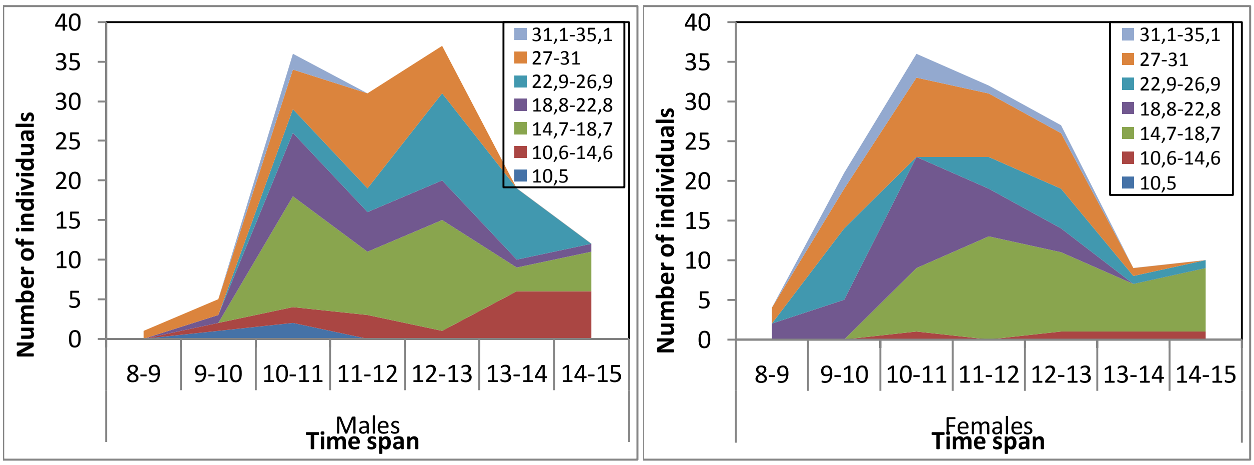


Fig. 14. The distribution of captures for *T. graeca* according to the time span and depending on the temperature (°C) at the moment of the capture.

4.3.3. The effect of spatial distribution on detectability

Spatial distribution of tortoises has a significant effect on the detectability in the study area (Fig. 15). In the spring both males and females of *T. graeca* were observed in almost the entire studied area, with a clear concentration in the east. Autumn catches were made almost entirely in the east of the studied area. To the south and west tortoises captures follow a series of tumules, walls and areas previously excavated for archaeological purposes. It is possible that these areas favour tortoises by offering high ground for thermoregulation, but also offer the possibility of finding shelter. Detectability is greatly reduced in the central area, especially for females. Juveniles can be found with higher probability in the east, but their detection is totally random, due to small size and withdrawn behaviour. The highest frequency of detection was reached in April 2011, 32 captures of *T. graeca* in 270 minutes, which corresponds to a frequency of a tortoise in about 9 minutes.

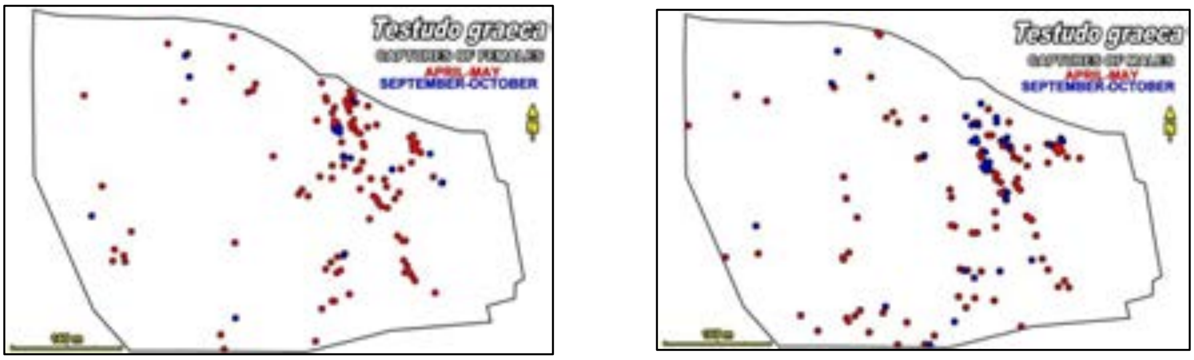


Fig. 15. Distribution of captured females (1) and males (2) in April-May and September-October at HAC.

Throughout the study we used two methods: (1) active search method and (2) transect method. This last method was applied only in the central area, with limited success in terms of tortoises' detection. To test the applicability of the transect method across the study area we use the Distance 6 with 24 virtual transects. Application Distance 6 shows that the maximum capture probability is at 12 m away from a virtual transect.

Detectability is significantly influenced by the degree of vegetation cover. Reduced height vegetation in spring favours observation from distance. With increasing vegetation height tortoises' observation are aided by the noise of their advances in vegetation or traces left in the vegetable layer.

4.4. ESTIMATION OF POPULATION SIZE

During the period of the study were captured 170 individuals of *T. graeca* throughout HAC (Table 9), of which 164 were captured in the study area, and six individuals in the touristic area.

Table 9. Gender distribution of captured *T. graeca*.

	Males	Females	Juveniles	Total unique captures	Total captures, including recaptures
HAC	76	79	15	170	293
Study area	74	76	14	164	286

Of the tortoises captured in the study area 72 were recaptured, both adults and juveniles (Table 10). Successive recapture were attained for 31 tortoises. A single female was recaptured five times, being found in the study on six occasions, while a male was recaptured four times. The evolution of tortoises at first capture was ascending in the first two years of study (2010-2011). Recaptures, including successive recapture of the same individual, exceeded captures in November 2012. The high number of captures and recapture percentage allowed the application of a model to estimate population size and density.

Table 10. Number of captured and recaptured *T. graeca* from HAC.

	Males	%	Females	%	Juveniles	%	Total	%
Unique capture	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 all captures [b]- % of all captures for each gender [c] - % of all recaptures for each gender

To estimate *N* based on the entire set of capture-recapture data, including juveniles, Mark application - module Capture suggested M_h model as the most appropriate (Table 11). Using only capture-recapture data of adults Mark recommended using the M_{tb} model (Table 12). This is a model based both on capture variation over time, but also includes animal behaviour in response to capture. Population size based on model M_t was the lowest compared to other models. Mark suggested model M_t to be used in estimation for males (Table 13) and females (Table 14). For both males and females, the number estimated by the model was the lowest very close to M_o model.

Cumulative population size estimates for males, 89 ± 5.7 individuals and female, 107 ± 9.2 individuals, are close to estimates for the entire adult population of tortoises, 196 to 197 individuals. Considering any estimate of population size for *T. graeca* to be at least undervalued M_t model appears to meet the requirements for an estimate of population size based on a capture-recapture study. This model also allows for variation in time of the number of captures. Differences from base model M_o are reduced. The model M_h estimated the largest population size, but in view of the observations in the field and that this model takes into account individual behaviour, which varies for tortoises, both under the influence of physiological factors and external factors, this model estimates can be considered as a possible upper limit of the population of tortoises.

Table 11. Estimation of *T. graeca* population size (*N*) for adults and juveniles (SD is 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
M_o			255 ± 12.9	205-255	203-253
M_t*	164	286	**221 ± 12.2**	**202-250**	**200-249**
M_h[1]			301 ± 31.3	253-377	***

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

Table 12. Estimation of *T. graeca* population size (*N*) for adults (SD is 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
M_o			201 ± 11.4	183-228	182-226
M_t	150	268	**197 ± 10.7**	**181-223**	**179-221**
M_h[1]			264 ± 25.6	224-326	***
M_{tb}*			218 ± 42.2	173-358	173-426

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

Table 13. Estimation of *T. graeca* population size (*N*) for males (SD is 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
M_o			92 ± 6.5	83-109	82-108
M_t*	74	139	**89 ± 5.7**	**82-105**	**80-103**
M_h 1			100 ± 8.0	88-120	***

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

Table 14. Estimation of *T. graeca* population size (*N*) for females (SD is 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
M_o			110 ± 9.9	96-135	94-133
M_t*	76	129	**107 ± 9.2**	**94-131**	**92-130**
M_h 1			176 ± 26.4	137-242	***

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

Considering M_t model as the most appropriate model for this population of tortoises we made an estimate of its size using the capture-recapture analysis with inclusion of temperature at the time of capture as variable. Estimates obtained using a *Huggins Closed Captures* model with Mark resulted in similar data to those obtained using the *Capture* module of Mark (Table 15).

Table 15. 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 variable (SD is standard deviation, CI confidence interval, Mt +1 = number of unique captures and n = total number of captures, including recaptures).

	M (t+1)	n	N ± SD	CI (95%)
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

The size of the adult population using estimates of the model M_t is 197 ± 10.7 individuals, with a probability range of179-221. An estimate of population size with juveniles would have a much too high error, taking into account the percentage of juveniles observed compared to the observed adults (8.53%). By extrapolating from the estimated number of adults of 197 individuals, the juveniles would be 17 individuals. This would imply a constant survival rate, independent of age. Combined, the two estimates indicate a population of 214 specimens of tortoises, which falls within the limits obtained for the model M_t, with the use of capture-recapture data for the entire population. It should be noted that, since no juvenile less than three years was captured, the juveniles of 1 and 2 years are not included in this estimate.

4.5. ESTIMATION OF POPULATION DENSITY

Population density was estimated only for the study area where the 164 tortoises inventoried allowed an estimation of density of 5.12 tortoises/ ha. Adult tortoises density is 4.81 tortoises/ ha. Directly observed density of tortoises in the study area is within the limits of densities observed in other populations of this species in Spain, Greece and North Africa (Table 16).

Table 16. Density of *T. graeca* population (individuals / ha) for the study area of HAC, observed and estimated compared to other populations of this species.

Location	Method for estimation	Density (individuals/ ha)	Estimate of the possible density range (individuals/ ha)	Source
HAC (Romania)	Directly observed	5.1		This study
	Estimated -Distance 6	5.5		
	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		4.2-12		Andreu et al., 2000; Ballestar et al., 2004
Greece		6.2		Hailey, 2000
Morocco		7		Slimani et al., 2002; Ben Kaddour et al., 2006

4.6. SPATIAL DISTRIBUTION

The distribution *T. graeca* individuals inventoried in the study area is not uniform, with a concentration in the eastern area (Fig. 16), rugged area with many depressions (green outline) and around tumules (white outline).

Fig. 16. The distribution of captures and recaptures of *T. graeca* from HAC in 2010-2012 (each tortoise is represented by a circle, circles may overlap in case of close locations) (GPS data overlaid on Google Earth image).

In the period 2010-2012 no major differences were observed in the distribution of captured or recaptured tortoises. In all three years, tortoises have been concentrated in the east, near the defence wall and near a series of trenches closed to a temporary pond. In the centre of the study area only in season 2012, April-May was recorded an increased number of tortoises. It is possible that distribution is influenced by the increased mobility associated with reproduction.

The captures of juveniles in the first two years did not differ; they were captured only in the eastern steep higher areas. Here were observed fragments of eggs. Nests were dug mainly on the southern slopes in soft ground. Concentration of juveniles in this area may be related to the tendency of females to oviposition here, considering that after hatching, in early life, juveniles do not move for long distances and the steep slopes and exposed ruins can reduce their dispersion.

Given the variable mobility of tortoises, any representation as a point or cumulative representation of captures and recaptures in a time frame is only an estimation of the spatial distribution of tortoises. Using a grid of squares with sides of 50 m, superimposed on the tortoises distribution map we obtained a more accurate map of the spatial distribution of tortoises, indicating a concentration and preference for certain areas (Fig. 17).

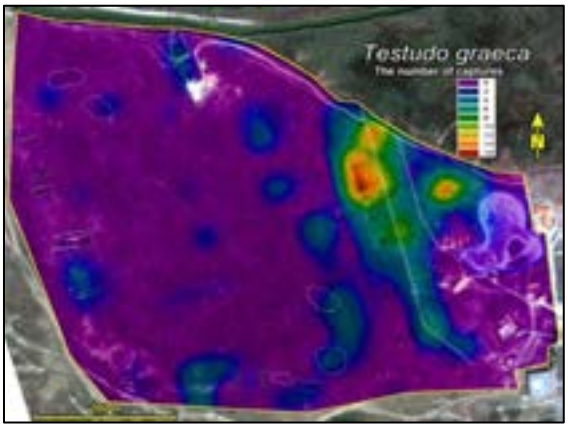


Fig. 17. *T. graeca* density in the study area of HAC (data are superimposed on Google Earth image, for easy observation the grid was removed).

The reason for this concentration in the eastern part of the study area is the need for thermoregulation, determining the tortoises to prefer higher areas, with hillocks and steep slopes, in this habitat flat. This area is also favourable for hibernation and nesting; all juveniles were captured in the maximum concentration area (represented by red). It is also possible that this region provides a favourable food resource. Tortoises cover entirely the study area, with concentration dictated by favourable areas.

Successive recaptures for several *T graeca* males and females allowed a territorial delimitation of these tortoises. This is only an estimate of the area that can be covered by tortoises, without being considered strictly as an individual territory. Territory estimation was achieved only for tortoises that were recaptured at least four times, regardless of when the capture occurred in 2010-2012. Area of territory was estimated by measuring the surface of a convex polygon whose vertices are represented by the position of each individual capture (Fig. 18; Table 17), but also including a buffer zone in the form of a circle with a radius of 50 m, for each catch, considering this distance as possible to be covered in the day of the capture (Hailey et al., 1984; Díaz-Paniagua et al., 1995).

Females tortoises (n=8) were successively recaptured in the eastern area and their estimated territories overlap. The map includes four individual estimated territories for better visibility. A single female tortoise has a defined territory with a higher area compared with the rest of females. In this case, the tortoise was captured in May and June near the defence wall, while in April and October at a distance of over 300 m in the extreme south. Trenches with southern slopes showing numerous holes and horizontal grooves can be used as hibernacula and it is possible that this tortoise hibernates in this area, moving in the summer months on the opposite side of the territory. Another female tortoise was caught six times in a small area with a diameter of about 90 m. Map with successional recaptured males (n=4) shows that they are not as localized and tend to move around the study area. In all four cases, male territory radiated from the estimated maximum density area of the tortoises.

Estimated average territory areas in this study for males (2.18 ha and 4.30 ha with additional buffer) and females (0.65 ha to 1.90 ha with additional buffer) are close to those estimated by radiotracking studies on *T. graeca*, for males (2.56 ha) and females (1.15 ha) (Anadón et al., 2006b). Average territories for adults (1.16 ha and 2.70 ha with additional buffer) are close to those observed in Spain: 3 ha/ individual (Andreu et al., 2000) and 1.17 ha / individual (Giménez et al., 2004), but above the observations from Morocco 0.2 ha / individual (Slimani et al., 2002).

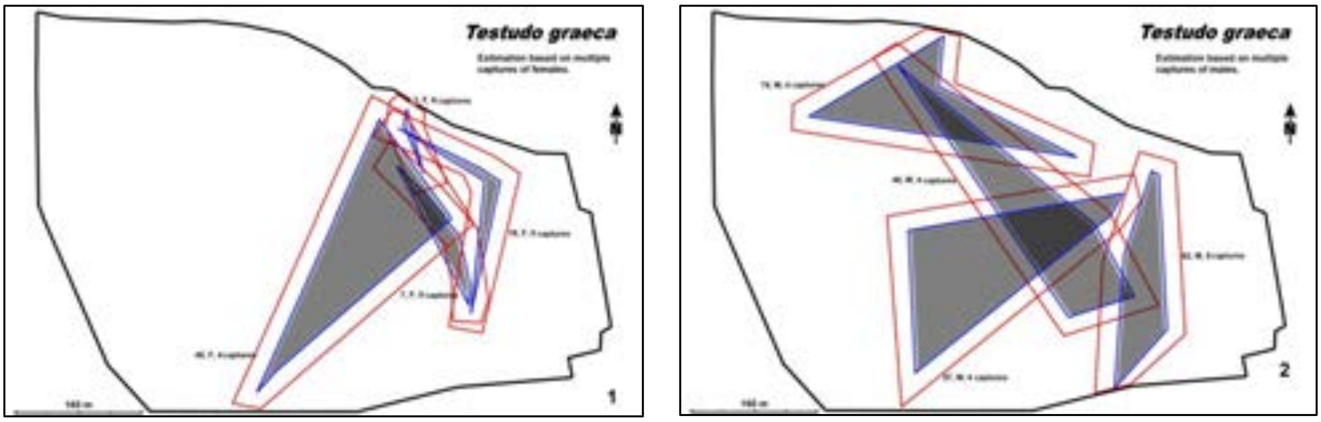


Fig. 18. Individual territory estimated for females (1) and male (2) based on multiple catches (red line polygonal contours is the estimated territory with additional buffer and the blue line contours represents the estimated territory mapped from joining the position of each capture).

Table 17. Estimated area of the individual territory for specimens of *T. graeca* with successive captures at HAC.

Specimen	Sex	Number of captures	Estimated area of the individual territory (ha)	Estimated area of the individual territory with 50 m buffer (ha)
2	F	6	0.04	0.62
7	F	5	0.39	1.27
19	F	5	0.31	1.88
24	F	4	0.30	1.24
45	F	4	2.92	5.31
56	F	5	0.76	2.0
71	F	4	0.10	1.37
121	F	4	0.43	1.51
Average for females (n=8)			**0.65 ± 0.94**	**1.90 ± 1.44**
40	M	4	2.63	4.96
57	M	4	3.08	5.55
62	M	5	1.2	2.87
74	M	4	1.83	3.82
Average for males (n=4)			**2.18 ± 0.83**	**4.30 ± 1.19**
Average for adults (n=12)			**1.16 ± 1.14**	**2.70 ± 1.76**

Distance travelled by tortoises captured in a seven days interval shows that females can travel a distance of 70 m and males, in the spring can travel between 250 and 450 m. Distances travelled by the HAC tortoises are closed to observed distances in other studies on *T. graeca* (Diaz-Paniagua et al., 1995). Average travel distance of tortoises was estimated for a total of 40 individuals (Table 18). Body size (estimated by curve carapace length) is not correlated with the average travelled distance by females (n=19, *r*=0.174, *P*=0.452) and males (n=21, *r*=-0.098, *P*=0.671).

Table 18. Average speed of travel per day estimated for 40 individuals of *T. graeca*, male and female, through multiple recaptures in the study area of HAC.

	n	Days between captures		Distance travelled (m)		Speed of travel (m/day)	
		Average ± SD	min-Max	Average ± SD	min-Max	Average ± SD	min-Max
Females	19	16.28 ± 6.92	6-29	90.12 ± 123	3-500	5.43 ± 5.53	0.25-18.51
Males	21	12.09 ± 6.75	1-28	249.76 ± 125	78-455	29.31 ± 26.09	5.85 - 108

4.7. ANTHROPOGENIC IMPACT ASSESSMENT

The population of *T. graeca* from HAC is healthy, young, and during the study were not observed individuals parasitized by ticks compared with MMNP where ticks parasitism is frequently observed.

4.7.1. Anthropogenic impact assessment by studying plastron and carapace scars

The results show that of the total analysed tortoises 35.46% showed anthropogenic or natural origin scars (Table 19), in MMNP% and 41.72 and HAC 14.11%. Juveniles with scars were observed only in MMNP (10.71%).

Anthropogenic scars (19.91%) and natural (15.86%) are present in similar proportions in studied tortoises. Only 2.93% of the examined tortoises (adults) showed both types of scars. HAC differ significantly from MMNP in human (χ^2=4.786, *P*=0.029) and natural (χ^2=3.984, *P*=0.046) impact. Incidence of anthropogenic scars in MMNP is higher in males than in females (χ^2=8.312, *P*=0.004) and for HAC the differences are not significant between gender (χ^2=1.667, *P*=0.197). There are no significant differences for natural scars between females and males from MMNP (χ^2=0.925, *P*=0.336), but there are difference for HAC (χ^2=5.444, *P*=0.002).

Table 19. Scars frequency in the studied *T. graeca* populations.

Studied area	Sample size (n)			Sex ratio	Scars frequency by origin (%)		
					Anthropogenic	Natural	Total (for each category)
MMNP	Males	300	580	1,19*	27.33	19.66	47
	Females	252			19.44	19.44	38.8
	Juveniles	28			3.57	7.14	10.71
	Total (for population)				22.75	18.96	
HAC	Males	76	170	0,96	6.57	10.52	17.10
	Females	79			12.65	1.26	13.92
	Juveniles	15			0	0	0
	Total (for population)				8.82	6.64	

*Sex ratio valid only for data from MMNP used in assessing the anthropogenic impact.

The carapace and plastron had different frequency of natural and anthropogenic scars (Table 20). Of all tortoises, 20.93% had scar only on carapace, 14.53% only on plastron and 5.46% had scars on carapace and plastron. Anthropogenic scars are more common to tortoises from MMNP instead of HAC (χ^2=4.831, *DF*=3, *P*=0.185), but differences between the two populations are lower for natural scars (χ^2=8.183, *DF*=3, *P*= 0.042).

Table 20. Frequency in percentage of anthropogenic and natural scars for tortoises from MMNP and HAC.

	MMNP				HAC			
	Females		Males		Females		Males	
	Carapace	Plastron	Carapace	Plastron	Carapace	Plastron	Carapace	Plastron
Anthropogenic scar	15.07	4.36	21.67	5.66	10.12	2.53	5.26	1.31
Natural scar	5.55	13.88	8.00	11.66	1.26	0	1.31	9.21

4.7.2. Anthropogenic impact analyses of *Testudo graeca* from Histria Archaeological Complex

The population of *T. graeca* from HAC has a low incidence of anthropogenic and natural scars, suggesting a low anthropogenic impact and less hostile natural environment, that doesn't leave a strong imprint on the carapace or plastron. Compared with populations of *T. graeca* from MMNP and Dumbrăveni Forest Nature Reserve the tortoises from HAC are healthier (Buică et al., in press).

Natural scars are present mainly on the frontal and side zones of the carapace (Fig. 19). Affected areas are correlated with behaviour of tortoises. In periods of distress the tortoises force to cover under vegetation or soil with the carapace. The human impact is visible across the carapace, the dorsal and anterior zones showing a higher percentage of lesions (Fig. 20). Plastron is much more affected by natural scar, anthropogenic scars being in lower percentage (Fig. 21). We observed individuals of *T. graeca* with anthropogenic scars, which affected both carapace and plastron (Fig. 22), on the same side of the body. Scars, both on the carapace and on the plastron, do not appear to be recent since the tissue around scar is restored, sometimes filling the wound and the edges are eroded by friction with the substrate or vegetation.

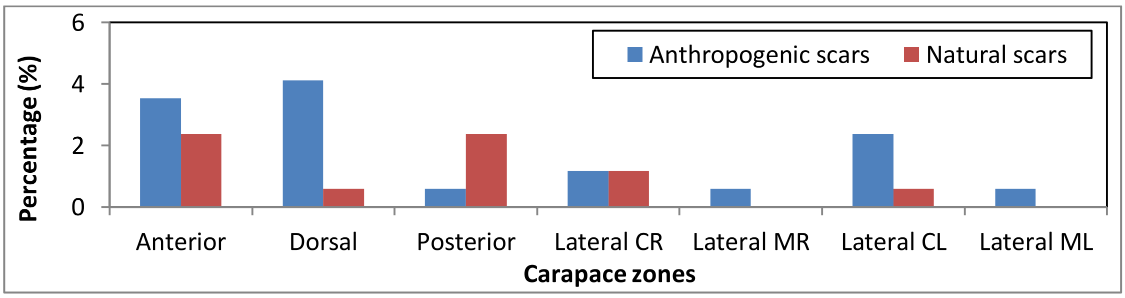


Fig. 19. Distribution of the carapace scars on *T. graeca* of HAC (C-costal, M-marginal, R-right, L-left).

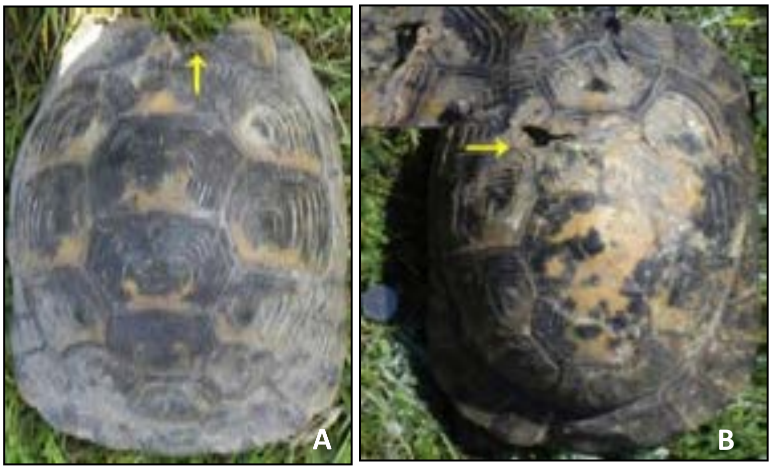


Fig. 20. *T. graeca* from HAC with significant destruction of the anterior carapace scutes (A) and dorsal (B) (Photo: Buică Gabriel).

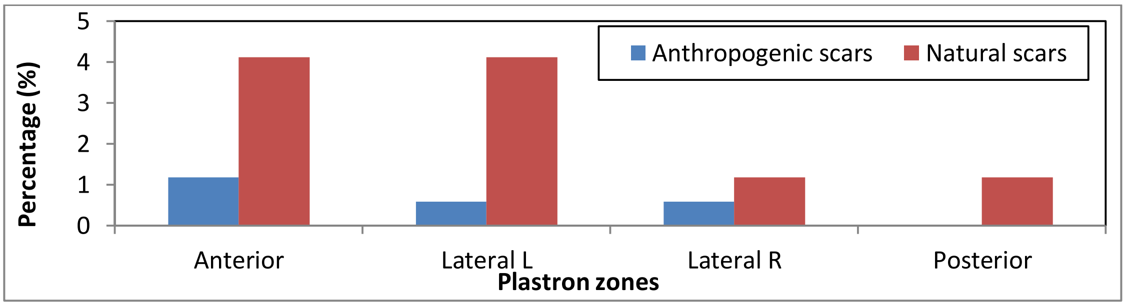


Fig. 21. Distribution of scars on the plastron of T. graeca from HAC (R-right, L-left).

Fig. 22. Anthropogenic scar affecting the dorsal and left side of the carapace of a *T. graeca* individual from HAC, the scar continues onto plastron (Photo: Buică Gabriel).

The body size, the size of carapace and plastron is related to anthropogenic impact, estimated by the frequency of scars. Thus, we observed a direct relationship between curve carapace length and frequency of anthropogenic scars (χ^2= 15.50, *DF*=4, *P*=0.004) and frequency of natural scars (χ^2=20.44, *DF*=4, *P* <0.001). The frequency of natural scars on plastron is correlated with curve carapace length (χ^2= 21.33, *DF*= 4, *P*<0.001), but not correlated for the frequency of anthropogenic scars (χ^2=5.33, *DF*=4, *P*=0.255). The anthropogenic scars frequency observed on the carapace of females and males show an increase with body size, or curve carapace length. On plastron natural scars are observe only at males and in much higher percentage than anthropogenic scars. There were no females with scars on plastron eloquent enough to be placed in the category of natural scar. Erosion and clogging with debris may be an explanation for the lack of observation of natural scar in the female plastron.

Natural and anthropogenic scars are present on the females and males carapace of similar size. Taking into account the age of tortoises with large carapace is likely that anthropogenic scars are the result of blows suffered more than 10 years ago. The lack of scars on young tortoises is due to low detectability which limits the exposure to anthropogenic factor.

4.7.3. Malformations in *Testudo graeca* from Histria Archaeological Complex

Malformations distribution was analysed using the same zoning model for carapace and plastron (Fig. 22) and were observed several types of malformations (Table 21), with variable distribution on the carapace or plastron. The carapace malformations were observed in all areas, with a higher percentage in the anterior zone. The plastron malformations are present only on the anterior zone as missing or supranumerale scutes and in very low percentage in the posterior zone.

Two tortoises were observed with malformations affecting all scutes of carapace but without affecting the plastron. Supranumeral scutes in pectoral zone of plastron were observed in four tortoises, two females and two males. Females had these supranumerale scutes on the same side of the body, while males on opposite sides. All four tortoises had different size, age and general forms of plastron.

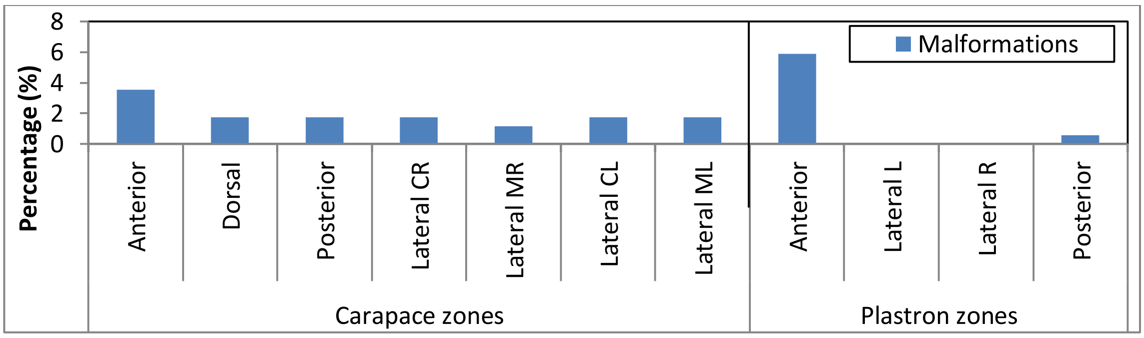


Fig. 23. Distribution of malformations on carapace and plastron of T. graeca from HAC (C-costal, M-marginal, R-right, L-left).

Table 21. Summary of observed malformations in *T. graeca* from HAC (M-males, F-females, CL –costal left, Photo: Buică Gabriel).

Malformation	The affected zone	Sex		Details	Image
		M	F		
Supranumeral scutes	Plastron, humeral	Yes	Yes	Females and males with the left side affected	
Scutes with abnormal appearance	Carapace	Yes	No	Al scutes	
Abnormal growth	Carapace, CL4 Plastron, gular scutes	No	Yes	CL4 associated with overgrowth of abnormal tissue deposit on plastron Gular associated with an excrescence on carapace	
Missing scutes	Plastron, gular scutes	No	Yes	With an excrescence on carapace	
Excrescence as result of abnormal scutes growth	Carapace, ML3	Yes	Yes	At males in association with malformation of gular scutes At females without any association	
Abnormal tissue growth	Plastron, anterior	Yes	Yes	In one male associated with an abnormal growth in a posterior, costal left, scute of carapace	

Malformations observed in tortoises from HAC were correlated with tortoise size (curve carapace length). Most malformations are observed in mature tortoises. Compared to MMNP the frequency of malformations is lower in the adult population of tortoises from the HAC (Table 22). There are no significant differences between the two populations in the frequency of malformations on the carapace or plastron of adult tortoises (Kruskal-Wallis *P*=0.333, n=155 for HAC, n=246 for MMNP).

HAC-MMNP comparative data show a small effect of inbreeding as a possible factor in the occurrence of malformations, but it is likely to be due to anthropogenic impact by reducing the gene pool of the population, stress or pollution. It is difficult to say which the limit is after a population is strongly affected by malformation. Study of European pond turtle populations *Emys orbicularis* in Spain (Velo-Anton et al., 2011), showed a wide variation in the malformation frequency (3% -69%), suggesting the possibility of similar variation in *T. graeca*.

Table 22. The frequency of malformations as a percentage in the population of *T. graeca* from HAC and MMNP.

	HAC (females n=79, males n=76)		MMNP (females n=104, males n=142)	
	Carapace	Plastron	Carapace	Plastron
Females	3.79	6.32	21.15	10.12
Males	7.89	7.89	10.56	10.66
Total	5.80	7.09	15.04	9.41

The study of scars and malformations allows tracking the evolution of anthropic and natural impact on *T. graeca* populations of different sizes, across this species range and is useful in implementing conservation policies for this species.

4.7.4. Fluctuating asymmetry assessment based on plastron scutes morphometry

I studied how the asymmetry of the plastron evolves during the development of tortoises depending on the curve carapace length (CCL) (Fig. 24). The results show no significant differences between left and right sides of the plastron. Observed deviations from symmetry are very small, resulting in lack of significant relationship with body size. Evident asymmetry was observed only for a reduced number of tortoises. Besides the deviation from symmetry found in normal tortoises there are also cases where the asymmetry is caused by malformations like supranumerale or missing scutes affecting the overall plastron dimensions. Lack of correlation among plastron asymmetry and carapace size and implicitly the tortoise's age was also observed in other studies of fluctuating asymmetry in other populations of *T. graeca* (Băncilă et al., 2012).

It is evident the tendency of deviation from bilateral symmetry with the aging of tortoises, in males and females. Females have higher levels of asymmetry, probably due to a faster growth for a longer period than males. In males, there is an increase in asymmetry in the class from 17 to 26.9 cm CCL and a reduced asymmetry at sizes over 27 cm CCL. The explanation for this can be given by rapid growth, causing scutes asymmetry and hence the plastron, followed by a slowing in growth rate that would allow a reduction in asymmetry without regaining the observed symmetry in low CCL.

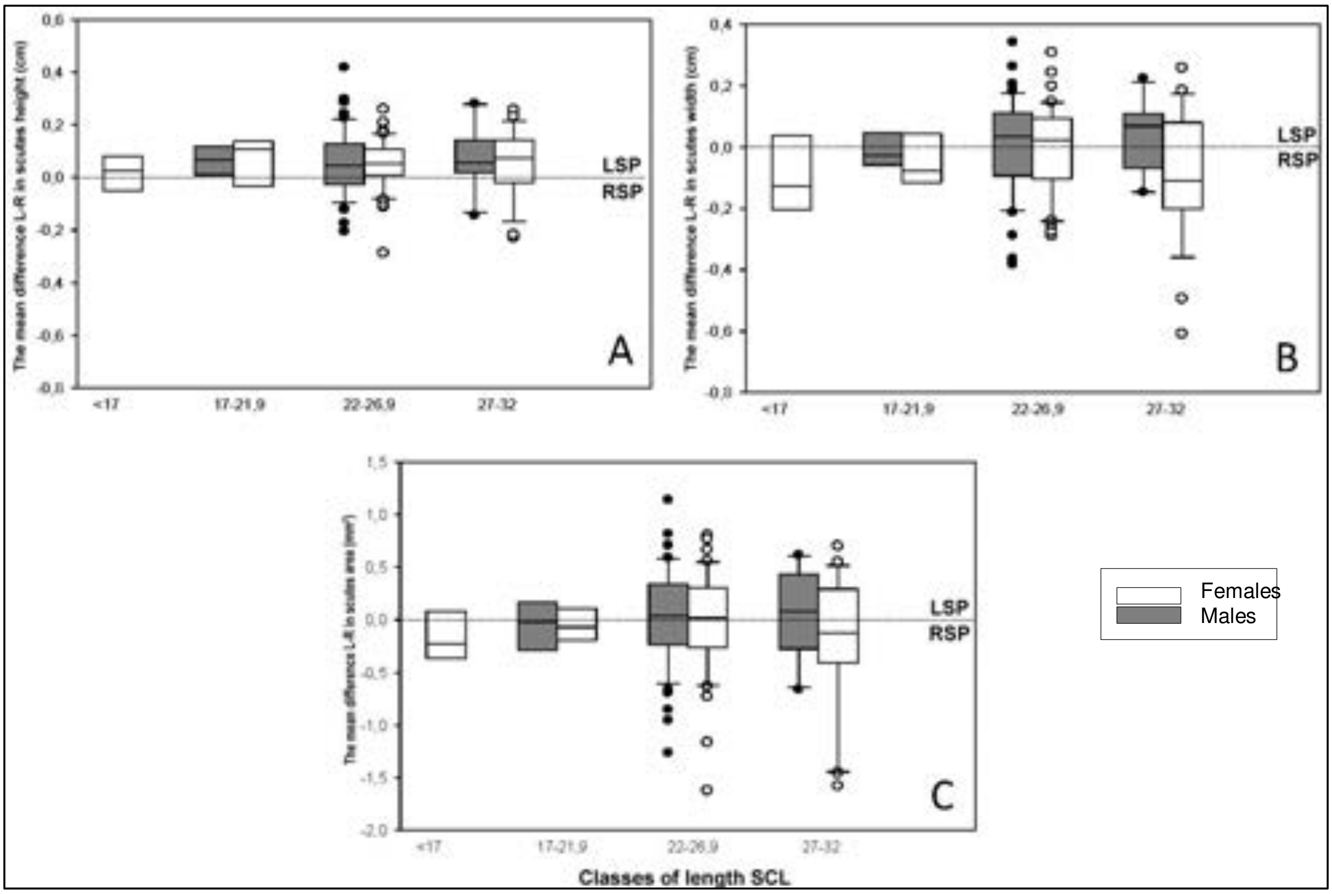


Fig. 24. The distribution of the differences between the average values for the left (LSP) and right (RSP) side of plastron for measured biometric characteristics in relative to classes of curve carapace length (CCL) and the left-right difference in mean (LR) height (A), width (B) and area (C) of *T. graeca* scutes from HAC (females n = 79, male n = 76).

These data show the current population status currently, which can evolve, requiring long-term studies. The size and structure of the analysed population, the evolution of the anthropogenic changes in the population's habitat and environmental changes as potential stressors factors should be considered in future studies. It is possible that the mechanism that causes the formation of growth rings to determine the random deformation of scutes as a result of compression of the growth rings of the same scute and between neighbouring scutes. It is also possible that fluctuating asymmetry is merely an artefact, an event generated during the growth of tortoises without consequences on the quality of life or no impact at population level. Measured differences between left and right sides are reduced and to avoid influencing the final result the data collection and analysis is important. Against the method established in the literature, using landmark sites to measure deviations from symmetry of the plastron or to obtain a numerical scale to describe its general form (Băncilă et al., 2012) we used automatic measurement of the scutes using Photoshop, for three parameters.

This method reduces the subjectivity, the researcher setting just the outline of the scutes for measuring and not the landmarks for measures. This is important because of the very varied morphology of plastron and the choice and the placement of a landmark in a large number of tortoises can cause errors, the landmark not being always placed in the same spot. Measuring the distance in a straight line between two landmarks at the same level is in many cases difficult to achieve and understand due to the form of scutes and the deviation of the midline of the plastron. Similar obtained results compared to the "classic" method shows that this approach is viable and requires further studies to be validated.

4.8. ETHOLOGY ASPECTS

During the study were observed several behavioural activities with seasonal variation. The behaviour of *T. graeca* is influenced primarily by environmental temperature and activity periods of tortoises. Any other observed behaviour is secondary to the thermoregulatory behaviour. Behaviour and detectability of the tortoises are interrelated.

The study was not design for ethological studies, but the obtained data show the sun exposure activities, normal for reptiles, and repose as dominant behavioural activities. Active behaviour, when the tortoises are moving, feeding and reproductive behaviours have been observed with reduced frequency and at certain times of the year.

Behavioural differences between females and males of *T. graeca* for the period in which observations were made (Fig. 24) are reduced. Observation throughout the day of field activity doesn’t show major differences in behavioural observations between males and females (Fig. 25). Active periods are similar to those seen in *T. graeca* in Spain (Andreu, 1987, Pérez et al., 2002).

Behavioural activities are influenced by air temperature; with a temperature of 10-20^{o}C seemingly favourable for thermoregulation of tortoises. The active tortoises are seen increasingly more as temperature rises, with a maximum at a temperature between 25-35^{o}C. Observations on feeding tortoises follow the trend of the active behaviour. No tortoises were observed feeding at temperatures below 15^{o}C, which is normal considering that tortoises require first the achieving of the optimal body temperature for normal physiological processes to take place. Reproduction was observed in the temperature range of 15-20^{o}C.

A correlation was found applying a χ^2 test on the number of sightings of tortoises displaying two types of behaviour (sun exposure activity and active movement activity) and air temperature at the time of observations ($\chi2 = 18.142$, GL = 4, P = 0.001).No correlation was observed for males or females (Fig. 26).

The thick vegetation in HAC and the soil coated with consistent vegetal leftovers allow tortoises to hide beneath the vegetation, and in some cases to be totally covered by vegetation. Tortoises exhibit this behaviour to protect themselves from excessive temperatures, using soil for thermoregulation (Tudor, 2010). This activity can be a defensive behaviour, being observed when tortoises senses that are observed or after being handled for measurements or photography. In autumn, especially in October, tortoises were observed concentrating in areas with trenches and are possible to capture multiple individuals of *T. graeca* at very close distances.

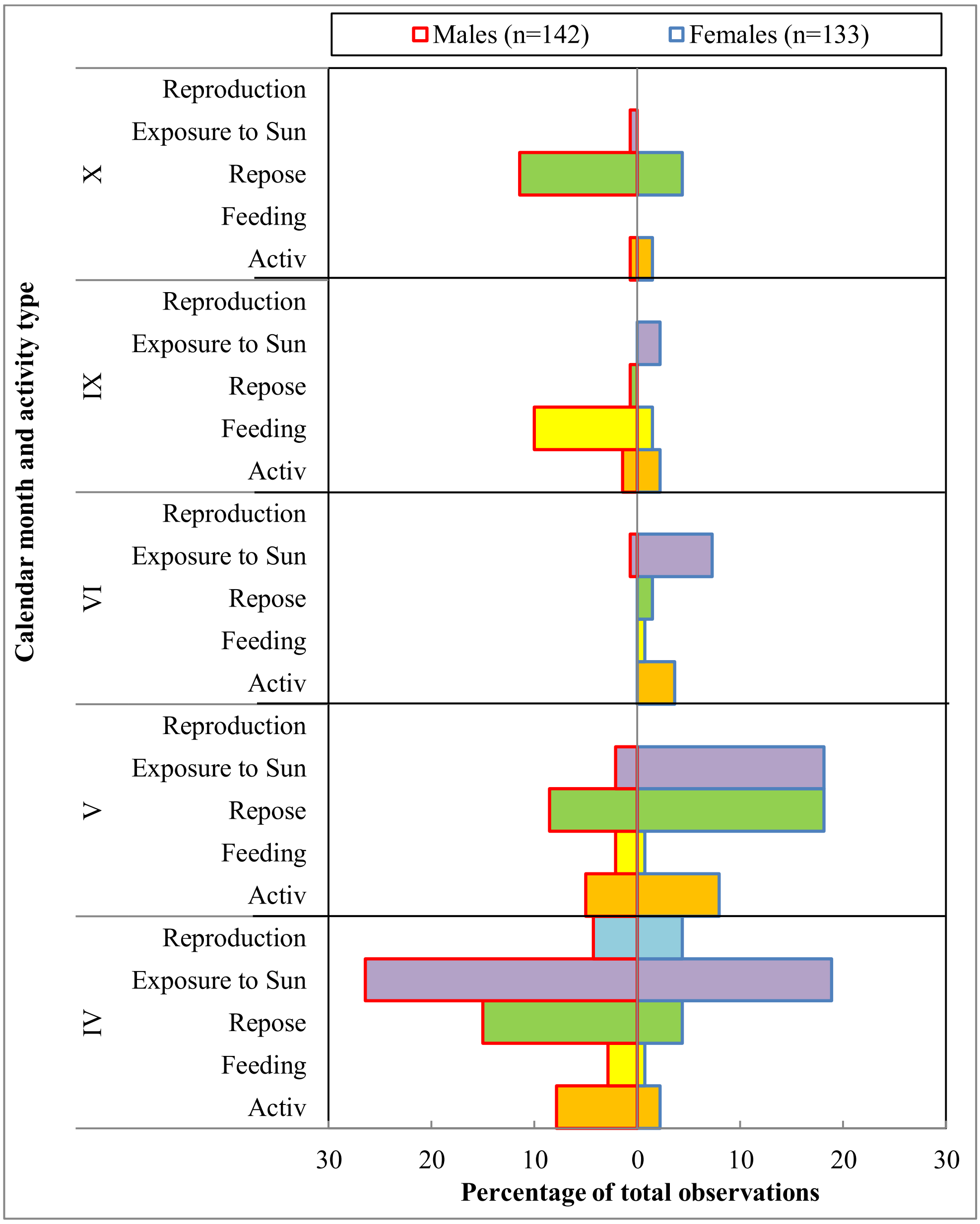


Fig. 25. Behaviours observed in *T. graeca* adults from HAC based on month calendar (n = 133 observations for females to males n = 142).

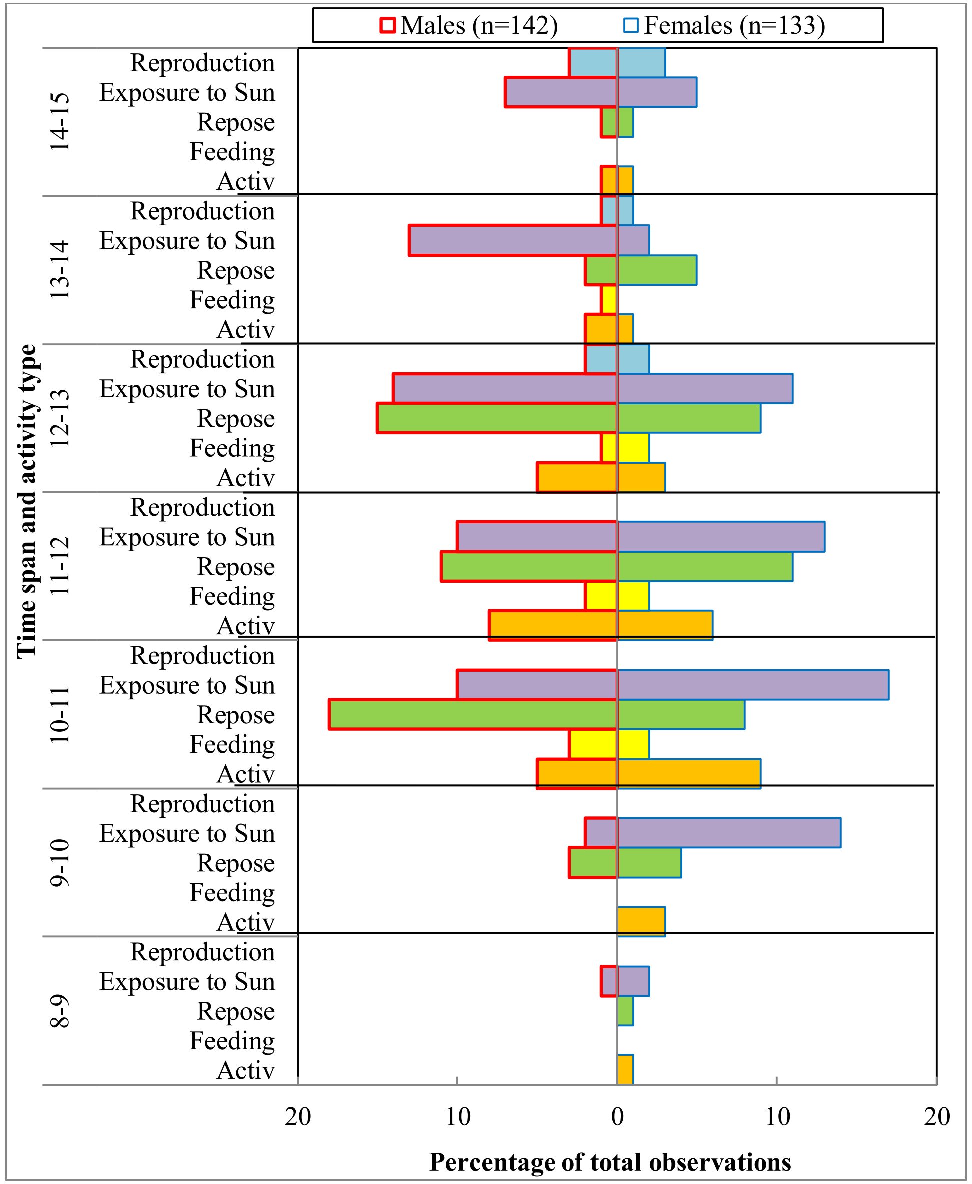


Fig. 26. Behaviours observed in *T. graeca* adults from HAC depending on the time of observations.

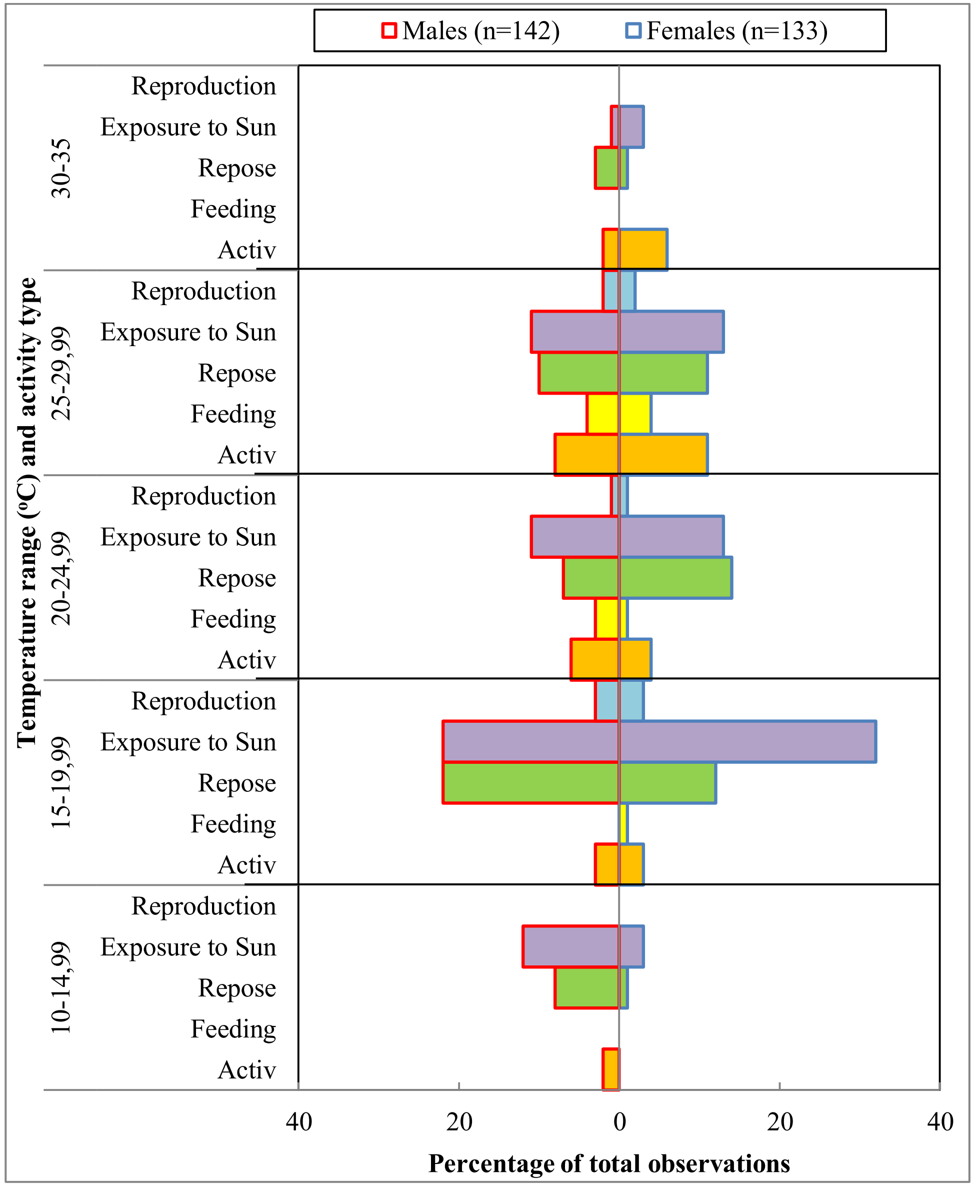


Fig. 27. Behaviours observed in *T. graeca* adults from HAC based on the temperature range of observations.

A comparison of the behaviours observed in *T. graeca* from HAC and MMNP show a roughly similar percentage of the active behaviour and reproductive behaviour observations in adults (Fig. 27). Differences occur in sun exposure behaviour, more predominantly in HAC and feeding behaviour more predominantly in MMNP. Considering the behaviour of sun exposure and repose as a behaviour in which tortoises are stationary, there is dominance of this type of behaviour in both populations of tortoises. Behavioural differences between these two populations are most likely due investigation methods being used.

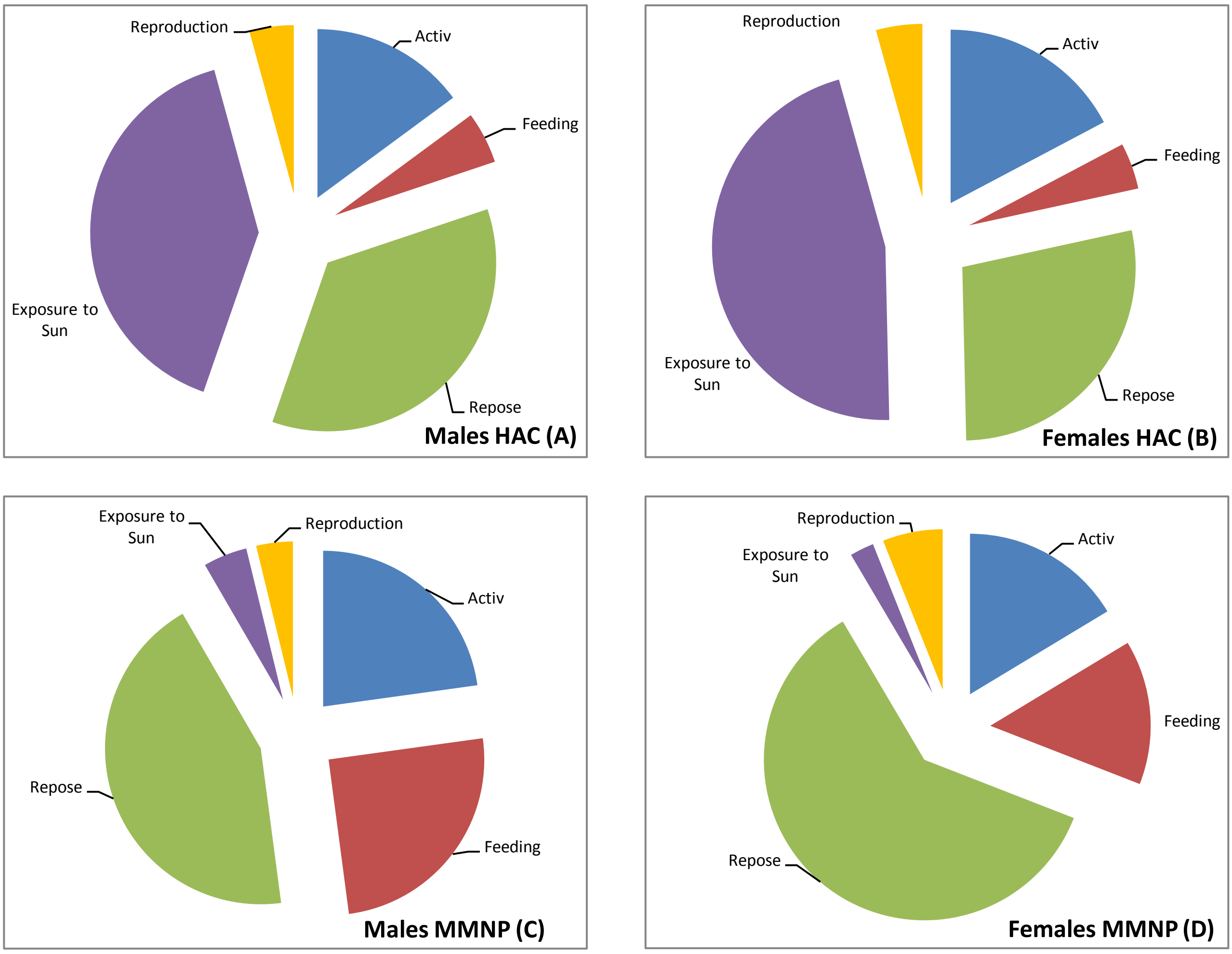


Fig. 28. Comparative distribution of observed behaviours in the adults of *T. graeca* from HAC and MMNP (n = 275 observations for HAC, n = 480 for MMNP).

4.9. RECOMANDATION FOR THE MANAGEMENT OF THE *TESTUDO GRAECA* POPULATION FROM HISTRIA ARCHAEOLOGICAL COMPLEX

HAC location in the Danube Delta Biosphere Reserve regulates a limitation of human activity in this area. However, the future of this population of tortoises is uncertain, with numerous risks of natural and anthropogenic origin (Table 23).

Table 23. SWOT analysis of the *T. graeca* status in HAC.

Strengths	Weaknesses
- Priority species for conservation - Elevated population size - Good health, no recent anthropogenic injuries or external parasites (ticks) - Successfully reproducing population - Protection offered by the existence of a metal fence around the perimeter of the HAC and the northern side channel - Reduced populations exchange with *T. graeca* populations in Vadu-Chituc area - Reduced mortality at least in adults (no carapace or carapace debris identified were identified) - The study area is not included in the touristic route - Archaeological activity is currently reduced - Diverse microhabitat due to past archaeological excavations	- HAC has a low security level outside the museum building - Degradation of the metal fence - The absence of a metal fence in the north, to prevent tourists or fishermen access - Dry vegetation accumulated each year increases the risk of fire - Failure by locals and tourists to endorse this species, including legislation that protects it - Lack of tradition for sustainable use of natural resources for the residents of the area - The existence of a grazing area near the HAC
Opportunities	**Threats**
- Possibility for studies on the biology and ecology of a large population *T. graeca* in a small area - Opportunity for testing ecology study methods and the use of modern techniques for remote data collection Possibility to attract funding for research, protection and publicity of *T. graeca* separately or together with other protected species in the area - Ability to easily promote the need to protect this species, through the tourists of HAC, both Romanian and foreign - Double status of the area, nature reserve and archaeological site	- Collection of specimens of *T. graeca* for economic benefits - Expansion of wide-ranging excavations in the study area, which would modify the existing microhabitat - Fire hazards, with natural or human origin - Touristic development in the area - Nearby constructions with touristic destination - Changes in the land use in HAC or in the perimeter of the protected area - Climate change

The turtle population management must take into account the concentration of tortoises in the eastern area of HAC, which can be consequence of the evolution of this area during the last 70 years. The protected area status is poorly signposted. There are no references to the priority species in this area included in the EU Birds and Habitats Directives, except for Dalmatian pelican (*Pelecanus crispus*). No reference is made to how the tourists should protect these species and what are for collecting or destroying the protected flora and fauna.

The presence of a large number of people can become a risk for the tortoises' population. The risk of collecting the tortoises is increased especially for juveniles, which are easy to carry, *T. graeca* being appreciated as a pet (Ljubisavljević et al., 2011).

The highest risk for the tortoises' population is represented by fire and sheep (Saumur et al., 2006).

Sheep entering in large numbers in the study area of HAC would cause competition for food resources and facilitate the entry of ticks, in present the population of tortoises is free of parasites. Droughts increase the risk of fire caused from natural or anthropogenic causes. A fire while the tortoises are active is devastating for this population. The impact is particularly strong in periods of high temperature and drought in the summer months when tortoises are not very active and take shelter under vegetal cover to protect them from heat. In autumn, any fire would affect the newly hatched tortoises.

Given the socio-economic situation and future possibilities of development for this area a number of directions, recommendations to protect the population of *T. graeca* are to be considered:

1. Improvement in the signalling of protected area status and for the protected species, specifying consequences for collection or destruction;

2. The expansion of the Istria-Sinoe integral protection regime area to cover the entire area occupied by tortoises in perimeter from HAC and to the south, between east shore of Lake Istria and 226A road.

3. Promoting among the employees of HAC the importance of protecting this species;

4. The protective fence of HAC requires maintenance to reduce the possibility for the entry of tourists, wildlife and possibly sheep;

5. Delimitation with strict enforcement by local authorities of pasture perimeters for sheep and cattle from neighbouring areas of HAC and strictly protected area Istria-Sinoe;

6. Prohibition of entry without authorization from HAC of tourists in the study area;

7. Achieving cooperation agreements between the National Museum of Archaeology of Constanța - HAC, DDBRA and "Ovidius" University-Faculty of Natural and Agricultural Sciences to conduct intensive long term studies on the *T. graeca* population;

8. The launch of archaeological studies in the study area that could affect the tortoises only after the DDBRA is notified and the measures for protecting the *T. graeca* have been taken in collaboration with stakeholders.

9. The diminution in the fire risk by controlling the accumulation of dried vegetation in the HAC and fitting of panels signalling the risk of fire.

Considering the economic difficulties and from personal observations, the low level of understanding of the need to protect the nature in general within the inhabitants of this area, the best protection for the population of *T. graeca* comes, paradoxically, from the ignorance of its existence in the area (Fig. 29).

Fig. 29. Herd of sheep in close proximity to the HAC fence and with an individual of *T. graeca* less than 5 m away from the fence (Photo: Buică Gabriel)

CONCLUSIONS

The study of the *T. graeca* population from Histria Archaeological Complex highlighted its importance for long term studies due to: (1) isolation, the population occupying a reduced area, (2) the possibility for testing and validation of monitoring and inventory methods, (3) models for estimating population size, and (4) because of its vulnerability this population requires strong measures of protection.

Objective 1. Assessment of the isolation degree for the *T. graeca* population from Histria Archaeological Complex.

No tortoise was observed beyond the boundaries of the study area, which confirms the isolation hypothesis. This hypothesis is supported by unsuitable habitats near the HAC strongly affected by human activities.

Objective 2. Characterization of the structural parameters in the *T. graeca* population.

In the period 2010-2012 a total of 170 individuals have been identified, of which 79 females, 76 males and 15 juveniles. The males: females ratio observed for the population of tortoises from HAC of 0.96, is lower than that observed in MMNP (1.45).

The estimation of the population size based on mark-recapture data was made only for the study area, excluding the catches in the touristic area of HAC. Using the capture-recapture data, respectively 164 unique captures and 286 recaptures, population size estimates were carried out for (i) adults and juveniles, (ii) adults and (iii) separately for females and males. The estimated size of the adult population ranges between 180-224 individuals and with juveniles included varies between 200-249 individuals. Density of tortoises reported in the study area, based on captures (5.1 individuals / ha), and estimated (adults = 6.9; adults and juveniles = 6.1 individuals / ha), is close to the density of other populations reported in the literature, ranging from 4.2 to 12 individuals/ ha.

The studied population is young; most adults are under 20 years of age estimated from the quantification of growth rings on the scutes of carapace. The observed number of juveniles is reduced (8.2%), a defining feature of a tortoises population. No significant differences between the studied population and the adult tortoises' population of MMNP were observed in the body biometrics.

Objective 3. Evaluation of detectability and spatial distribution.

The detectability in *T. graeca* is influenced by several parameters such as weather, vegetation structure, habitat type, age and sex. Detectability varies seasonally being highest in April, when the vegetal cover is less developed. High temperatures in July and August correlated with abundant vegetation prevented any in field study activity.

Males were more active during reproductive season and are therefore more noticeable. The temperature favourable for tortoises observation is between 17-21^{o}C and between 11-14 hours. In this study we showed the influence of air temperature on the behaviour of males, which are mainly observed at lower air temperatures than females.

The spatial distribution of tortoises varies seasonally. In spring they tend to disperse throughout the territory and in fall are observed a concentration for the approaching hibernation. The dispersion varies according to gender, being higher in males.

We observed a high density of tortoises in the east, in an area dominated by an ancient defensive wall and numerous tranches, former archaeological excavations, which give a rough layout to this area. This creates a microhabitat that offers the possibility of exposure to sunlight for thermoregulation, but also many possible hibernacula in the cavities of the trenches' slopes. This area seems favourable for nesting; remnants of egg shells being seen around the nests and all of juveniles were observed here.

The estimated area of the individual territory for an adult tortoise, recaptured in at least four successive occasions is close to the data from literature. The female tortoises are having a smaller individual territory area compared to the males (1.9 ha compared to 4.3 ha). Much larger territory of males is correlated with their increased mobility.

Objective 4. Assessment of the population's health.

Health evaluation was performed using the scutes of carapace and plastron as an indicator of human impact through characterization of scars and malformations. The zoning model of carapace and plastron was used for grouping and analysing affected zones, without quantifying the number or size of the scars. Scars frequency in the population, both naturally occurring and of anthropogenic origin suggests that the study population is healthy. Based on these data it can be estimated that the population of tortoises in HAC is less affected by anthropogenic (8.82%) and natural (6.64%) factors compared to the population of MMNP (anthropogenic scars = 22.75%; natural scars = 18.96%). Juveniles of HAC showed no anthropogenic or natural scars. The reduced age of the tortoises and the protection offered by HAC, which reduces the human impact are the likely causes of low human impact. The natural environment of HAC, lacking the steep slopes and areas of rocks and hard substrate of MMNP, is favourable for tortoises.

Malformations were analysed using the zoning pattern of the carapace and plastron, which proves to be effective in standardizing the health assessment. The degree of tortoises affected by malformations is reduced for the tortoises' population from HAC but also reduced when compared with the population of tortoises from MMNP. Adults from the HAC showed malformation on 5.80% of analysed carapaces and 7.09% of analysed plastrons and adults from MMNP showed malformations on 15.04% of their carapaces and 9.41% of their plastrons.

Health, analysed using fluctuating asymmetry, does not show differences in the results comparing to other studies in the literature. The method discussed in this thesis, using surface, width and height of three plastron scutes does not show a clear deviations from bilateral symmetry. This approach to the analysis of fluctuating asymmetry can be time consuming, but provides more information and combined with the classical method can increase the reliability of the fluctuating asymmetry analysis.

During the study were not observed external parasites (ticks) in the individuals of the studied population of HAC, unlike MMNP population, strongly parasited. Likely causes are the absence of domestic animals within HAC. Results on the health of the population of tortoises from HAC, obtained in 2010-2012, invalidates the hypothesis set at the beginning of the study, that the population is strongly affected by anthropogenic factor.

Objective 5. Development of a set of measures for protection and management.

In relation to the provisions specified in the legislation a set of measures for protection and management of this *T. graeca* population have been proposed: (1) establish a framework for cooperation between decision makers involved in archaeological activities, protection and conservation of biodiversity and biological and ecology research and (2) increase the awareness of tourists and livestock farmers in the area to the importance of this species.

The collaboration between DDBRA, the National Museum of History and Archaeology of Constanța, HAC and "Ovidius" University-Faculty of Natural and Agricultural Sciences for protection measures during excavation and long-term studies would be in the benefit of the scientific activity and for tortoises' protection.

To reduce the anthropogenic pressure in the HAC a future expansion of strictly protected areas Istria towards the eastern shore of Lake Sinoe Istria is desirable. This will cover the whole habitat of tortoises and protect other faunal elements. One such measure is for the future and for short term is necessary to prohibit the access of domestic animals, especially herds of sheep or cattle and stray dogs.

The risk of fire is increased with the accumulation of dry vegetation. Controlled burning of vegetation in winter can be tested in the HAC; the method is applied successfully in some U.S. nature reserves.

Such a measure would greatly reduce future risk of fire occurrence from natural or artificial sources with devastating effects for *T. graeca*, but also for other animals seen in the area.

Protection of juveniles and nests require special attention due to their low survival. This can be accomplished by: (1) preventing the entry of domestic and wild animals (wild boar), which may destroy the nests or damage the carapace and plastron of juveniles and (2) reducing the risk of fire, with devastating effects for juveniles. Collection of protected fauna for commercial purposes or as a pet, in this case the juveniles of *T. graeca*, although it is clearly stated in the legislation as strictly forbidden, this statement is to be brought to the attention of any tourist of HAC, to the neighbouring communities and to the public.

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