Density and sex-ratio of wild populations of three *Testudo* species in Italy

Almost half of the European species of reptiles show declining populations, and approximately one fifth of the latter are listed among the threatened species in the IUCN European Red List of reptiles (Cox & Temple 2009). The adverse impact of human activities can be particularly severe for long-lived reptiles, like tortoises (Testudinidae), which generally show delayed sexual maturity, low reproduction rates (Congdon & Van Loben Sels 1991; Congdon et al. 1993), limited dispersal ability and, often, restricted ecological tolerance (Grammont & Cuaron 2006). Tortoises are heavily endangered worldwide, including Europe, where three species live in the Mediterranean and southeast European countries: *Testudo graeca* Linnaeus, 1758, *Testudo hermanni* Gmelin, 1789, and *Testudo marginata* Schoepff, 1792. All three *Testudo* species are present in Italy with viable but threatened populations. *Testudo graeca* and *T. marginata*, probably allochthonous, occurring in Sardinia and some circum-Sardinian islands, are classified Near Threatened in the IUCN Red List assessment for Italy, while *T. hermanni*, found throughout the coastal regions of peninsular Italy and on the Islands of Sardinia and Sicily, is considered Endangered (Rondinini et al. 2013).

The assessment of the conservation status of a given taxon in a specific area requires basic ecological information, above all, on population size, density and distribution (e.g., IUCN 2012). Such data is of key importance to plan effective management actions and assess the response to the applied conservation measures (Pollock et al. 2002). In particular, long-term monitoring of such population characteristics can provide early warning signals of changes in population trends, the detection of which can be critical for habitat and species management (Thompson et al. 1998; Nussear & Tracy 2007). The distribution of *Testudo* in Italy was recently updated by Corti et al. (2013), providing new record localities for the three species, particularly *T. hermanni* in southern Italy, whereas updated information on population size and density is scarce, and long-term studies are almost lacking. Luiselli et al. (2014) provided data of this kind, inferring negative trends in three *T. hermanni* populations in Central Italy from sighting frequencies over twenty years. The aim of the present study was to provide basic information on the population ecology of the three *Testudo* species occurring in Italy assessing the parameters “population size”, “population density”, “population size stability” and “operational sex ratio” for adult individuals of selected populations.

Overall, five *Testudo* populations were surveyed: one of *T. graeca*, one of *T. marginata*, and three of *T. hermanni*. The *T. graeca* population was studied in a coastal area in central-western Sardinia (Province of Oristano, Fig. 1, Site 1), characterized by an open uncultivated area surrounded by Mediterranean maquis (dominated by *Pistacia lentiscus*, *Cistus* spp., *Phillyrea angustifolia*); *T. marginata* in a coastal area in NE Sardinia, dominated by oak forest (*Quercus ilex*) and Mediterranean maquis with patches of open garrigue (Province of Olbia-Tempio, Fig. 1, Site 5). As for *T. hermanni*, the authors surveyed one population in NW Sardinia (Province of Sassari, Fig. 1, Site 4), in open grassland patches amid a diversity of Mediterranean maquis and broad-leaved woods, a few hundred meters from the coast; one in Tuscany, along the Tyrrhenian coast and one in Abruzzi, not far from the Adriatic coast. These latter two Central Italian sites were both characterized by the presence of agricultural land; in the Tuscan site (Province of Grosseto, Fig. 1, Site 2), however, the open areas adjoined an extended zone of natural vegetation dominated by Mediterranean maquis and oak forest (*Quercus ilex*), while in Abruzzi (Province of Chieti, Fig. 1, Site 3) they were part of a thickset mosaic, along with small-sized, seminatural habitats.

Each study site (Table 1) was delimited by marked natural (i.e., changes in vegetation) or anthropogenic (i.e., roads) boundaries and searched for individuals using "visual encounter survey" technique by random walking (Crump & Scott 1994). Surveys of the five sites took place in spring 2012 (Table 1), at a mean air temperature of 25 °C (24.9 °C ± 3.4 °C, N = 206), between 8:00 h and 18:00 h (winter time), with com-

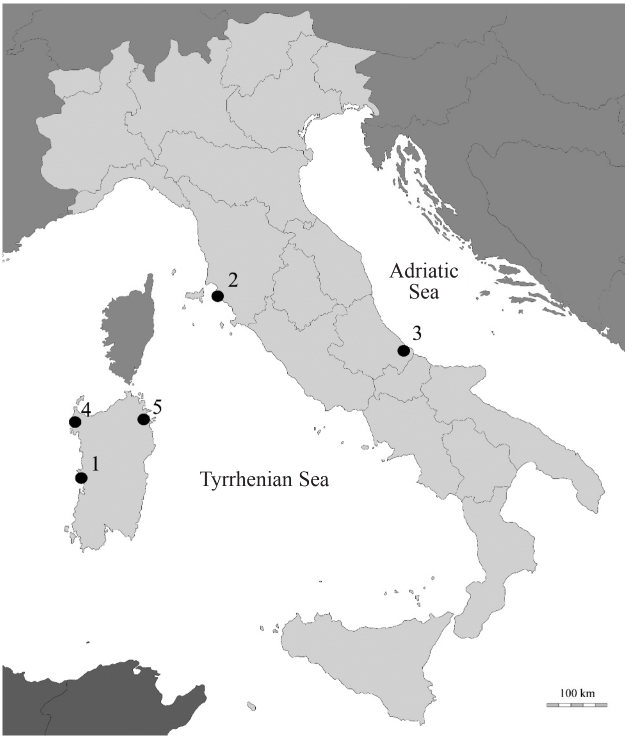

Fig. 1: Location of the five sampling sites studied in peninsular Italy and the Italian island of Sardinia in spring 2012. *Testudo graeca*: site 1 (central-western Sardinia); *T. hermanni*: site 2 (Tuscany), site 3 (Abruzzi), site 4 (NW Sardinia); *T. marginata*: site 5 (NE Sardinia).

parable sampling effort (Table 1). The capture-mark-recapture method (STUBBS et al. 1984; FILIPPI et al. 2010) was used during four sampling days per site. Individuals were temporarily marked with consecutive numbers written on the posterior region of their carapace, using non-toxic dyes. This technique guaranteed minimal disturbance to the animals and their identification from a distance. In this study, only mature individuals (carapace length > 10 cm, STUBBS et al. 1984) were considered because of the particularly secretive behavior and, consequently, lower detectability of juveniles. The authors used the program CAPTURE (OTIS et al. 1978; https://www.mbr-pwrc.usgs.gov/software/capture.shtml) to estimate population sizes, and calculated densities from this data. CAPTURE requires “closed” populations and performs closure tests to verify if the data fulfill such an assumption (Table 1).

Population size was estimated as the total number of adult individuals per study area, population density as the number of adult individuals per hectare and population size stability as the ratio between the effective population size and the total number of observed adults (N_e/N_{tot} following NUNNEY 1995). In this ratio, N_e indicates the number of breeders (fertile adults) in an idealized population sensu HARDY-WEINBERG, calculated as $N_e = (4*N_m*N_f)/(N_m+N_f)$ (WRIGHT 1938), where N_m and N_f are the numbers of adult males and females, respectively. The operational sex-ratio was calculated as the proportion of N_m on N_{tot} (WILSON & HARDY 2002).

The results of the present study are summarized in Table 1. Focusing on *T. hermanni*, in peninsular Italy, similar population densities, i.e., about six individuals per hectare (n/ha), were recorded in Tuscany,

Table 1: Characteristics of the five sampling sites and their adult populations of *Testudo hermanni* GMELIN, 1789, *T. graeca* LINNAEUS, 1758, and *T. marginata* SCHOEPFF, 1792 in Italy (comp. Fig. 1) including sampling dates; size of the sampling area [ha]; sampling effort [person hours] per sampling event; number of individuals newly marked [N_1] + number of individuals found already marked [N_2]; Closure Test results [z- and P-values]; population size [estimated number of individuals per sampling area ± Standard Error of the Mean (SE)]; 95 % Confidence Interval [CI]; population density [estimated number of individuals per ha]; operational sex ratio [proportion of number of adult males on the total number of adult individuals captured; (N_m/N_{tot})]; population size stabilty [ratio between the effective population size and the total number of observed adults (N_e/N_{tot})]. The significance level of the closure test was ≥ 0.05 in all cases, so closure was not rejected for the studied populations (OTIS et al. 1978).

Species	Site		Sampling		Sample	Population					
	Number	Area (ha)	Dates in 2012	Effort (person hours)	N_1+N_2	Closure test (z value P value)	Size (±SE)	CI 95 %	Density (N/ha)	Operational sex ratio (N_m/N_{tot})	Size stability (N_e/N_{tot})
T. graeca	1	1.833	April 27	24	22+0	-1.588	95±11.06	79-123	51.83	0.57	0.98
			April 28	24	17+8	0.056					
			April 30	18	10+13						
			May 4	24	7+15						
T. hermanni	2	3.759	April 20	20	3+0	-1.095	22±0.00	22-22	5.85	0.50	1
			May 10	24	8+2	0.137					
			May 12	24	3+3						
			May 14	24	8+3						
T. hermanni	3	3.302	June 14	20	4+0	-0.894	10±0.28	10-11	3.03	0.50	1
			June 15	24	4+1	0.186					
			June 17	20	2+1						
			June 25	20	4+0						
T. hermanni	4	2.173	May 14	24	8+0	-1.265	86±11.27	68-112	31.29	0.31	0.85
			May 15	24	11+1	0.103					
			May 16	18	8+1						
			May 24	24	9+4						
T. marginata	5	2.693	May 7	24	5+0	-1.265	50±8.63	43-78	18.57	0.72	0.93
			May 8	24	7+2	0.103					
			May 9	24	6+1						
			May 11	24	4+1						

Tyrrhenian side of Central Italy (Fig. 1, Site 2) and three in Abruzzi, Adriatic side of Central Italy (Fig. 1, Site 3). Both populations lived in environments which included cultivated areas and semi-natural habitats. As to the Tyrrhenian side of Central Italy, the present data falls into the range of densities reported in past and recent studies for the regions of Tuscany and Latium: 3.4 and 0.6 n/ha (about 15 years later) in the "Parco Regionale della Maremma" (CARBONE & PAGLIONE 1991; ZUFFI & FOSCHI 2007, unpublished), 17 n/ha in Capalbio (CHELAZZI & FRANCISCI 1979) and 2.86 n/ha in the "Parco Regionale di Veio" (FILIPPI & LUISELLI 2008). As to the Adriatic side, the few data available for *T. h. hermanni* indicate higher densities than observed in the present study. LOY et al. (2007) estimated 16.60 and 25.08 n/ha in the region of Molise, where environmental conditions included natural habitats in a mesic inland area. In the present study, such high densities of about 31 n/ha were recorded in Sardinia (Fig. 1, site 4), for which the only reference data available (5 n/ha, Asinara Island, NW Sardinia) refers to a population inhabiting mainly garrigue, maquis and pastureland (CORTI & ZUFFI 2003). Densities from 3 to about 13 n/ha were reported from south Corsica and mainland southern France (CHEYLAN 1981; STUBBS & SWINGLAND 1985; NOUGARÈDE 1998). High densities comparable to those in Sardinia were indicated for some populations of the subspecies *T. hermanni boettgeri* MOJSISOVICS, 1889, in the Balkan area, more than 20 years ago (CRUCE 1978; MEEK & INSKEEP 1981; MEEK 1984; STUBBS et al. 1984; HAILEY & WILLEMSEN 2000).

For *T. graeca* in Sardinia (Fig. 1, Site 1), the high population density of about 52 n/ha estimated by the authors, exceeds the only data available for the species in Italy, ranging from 10 to 22 n/ha on the Island of Mal di Ventre (off Central-western Sardinia) (CORTI et al. 2007), where unique habitat features (a flat, small island characterized by high habitat homogeneity and marked resource seasonality; presence of just grasses, that dry in late spring, and depressed shrubs, due to constant, strong winds) and ecological constraints come into effect. Comparative estimates from other countries indicate the following values: 3.73 n/ha in NE Algeria (ROUAG et al. 2007), 6 in Marocco (KADDOUR et al. 2006), from less than 0.05 to 17 in SE Spain (ANADÓN et al. 2009) for the subspecies *T*. g. *graeca*; 7-21 n/ha (HAILEY et al. 1988) in Greece, from 1.68 to 5.1 in Romania (BUICĂ 2011; BUICĂ et al. 2013) for the subspecies *T*. *g*. *ibera*.

An estimate of the population density for *T. marginata* (19 n/ha) in Sardinia (Fig. 1, Site 5) is provided here for the first time. The only comparative information derives from an extensive study by HAILEY & WILLEMSEN (2003), indicating very low densities (less than 2 n/ha) for 21 populations in Greece.

Overall, the operational sex-ratios were found to be balanced in all *Testudo* populations studied (Table 1). Peninsular *T. hermanni* populations showed values equal to 0.5 and the one in Sardinia was slightly female biased. This seems to contradict HAILEY & WILLEMSEN (2000) who observed male biased sex ratios, along with reduced female longevity, in the densest Greek populations and hypothesized that under such circumstances males could cause enhanced mortality among the females due to aggressive courtship behavior. On the other hand, combats among males can lead to enhanced male mortality in some *T. hermanni* populations (CORTI & ZUFFI 2003). Balanced sex-ratios were found in other Italian *T. hermanni* populations (CORTI & ZUFFI 2003; LOY et al. 2007; FILIPPI et al. 2010) and can be considered as typical of stable populations while female-biased sex ratios occur in declining *T. hermanni* populations (CHEYLAN et al. 2011). In the studied *T. graeca* population the sex ratio was even too, while it was slightly male-biased in the *T. marginata* population. The population size stability (N_e/N_{tot}) was very close or equal to 1 for all the populations analyzed (Table. 1), thus suggesting they were substantially stable in size. Indeed, N_e/N_{tot} ranges between 0.5 and 1 in stable populations (NUNNEY & ELAM 1994; NUNNEY 1995), while lower values are typical of fluctuating populations (FRANKHAM 1995; KALINOWSKI & WAPLES 2002). As generally reported for *Testudo* (LOY et al. 2007 and references therein), observations of juveniles were very scarce in the present study, representing 1 for *T. marginata* and 2 for each population of *T. hermanni* in Tuscany and Abruzzi.

In Italy, tortoises are mostly threatened by loss and alteration of their habitats (natural and semi-natural areas), due to urbanization, intensification of agricultural practices along with the abandonment of the traditional ones. In addition, illegal collection of individuals linked to pet trade is still dramatically widespread, threatening wild populations by direct reduction of population size and leading to dissemination of infectious diseases. Moreover, pet trade implies the risk of hybridization *sensu latu* (referred to mating by individuals of distinct species, but also by individuals of distinct subspecies and even populations that are genetically different, even if not taxonomically distinguished), due to release of translocated individuals (RHYMER & SIMBERLOFF 1996 and references therein; CHEYLAN et al. 2011). The present population ecology data adds, in particular, to fill an information gap for Sardinia (main island) where the highest population densities were reported among the three *Testudo* species. This could identify Sardinia as a potentially important reservoir for these species (VAMBERGER et al. 2011) although high population density does not necessarily indicate suitable habitats (VAN HORNE 1983; KAREIVA 1990).

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