

Weight and Condition Index seasonal variation of *Testudo hermanni* (Reptilia, Testudinidae) at the “Bosco della Mesola” reserve (Po River Delta, Northern Italy)

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ABSTRACT

Testudo hermanni Gmelin, 1789, commonly known as Hermann's tortoise, is present in Northern Italy, being the northernmost part of its areal. However, the original distribution of this species in Italy has been greatly fragmented, and a few disjunct populations are known. Here we present a database comprised by more than 3,600 observations of weight, morphometry and behaviour, which were recorded in nearly 25 years of research (1992-2016) monitoring a *T. hermanni* population in a Mediterranean wooded biotope, the “Bosco della Mesola” reserve. The database was used to investigate the health status of the population: the good status of specimens from the focal population was confirmed and changes experienced during the year are described in detail, with special reference to female individuals. The use of Condition Index and the availability of data for such a large timespan allowed us to depict a fine-grained picture of life history traits and physiology of this poikilotherm species in this particular habitat; the importance of large, curated database like the one that is presented here should not be underestimated when trying to characterize and predict ecological changes in warming environments.

Key words: Hermann's tortoise, *Testudo hermanni*, weight, carapace length, Condition Index, behaviour, longitudinal dataset

RIASSUNTO

Variazioni stagionali del peso e del Condition Index di *Testudo hermanni* (Reptilia, Testudinidae) al Bosco della Mesola (Delta del Po)

La testuggine di Hermann *Testudo hermanni* Gmelin, 1789 è presente nella parte più settentrionale del suo areale nel Nord Italia. La distribuzione originale di questa specie in Italia è stata notevolmente frammentata e sono note alcune popolazioni disgiunte. Presentiamo qui un database composto da oltre 3'600 osservazioni su peso, morfometria e comportamento, che sono state registrate in quasi 25 anni di ricerche (1992-2016) che monitorano una popolazione di *T. hermanni* in un biotopo boschivo mediterraneo, la Riserva di “Bosco della Mesola” nel Delta del Po. Il database è stato utilizzato per studiare lo stato di salute della popolazione: è stato confermato il buono stato degli esemplari e sono stati descritti in dettaglio le variazioni di peso stagionali subiti durante l'anno. L'uso del Condition Index e la disponibilità di dati per un arco temporale così ampio ci ha permesso di rappresentare un quadro dettagliato della storia naturale e della fisiologia di questa specie peciloterma in questo particolare habitat. Grazie alla disponibilità di questo database si possono caratterizzare e prevedere eventuali influenze di cambiamenti ecologici determinati dai mutamenti climatici in atto sulla popolazione di questa specie.

Parole chiave: Testuggine di Hermann, *Testudo hermanni*, peso, lunghezza del carapace, Condition Index, comportamento, banca dati.

INTRODUCTION

Reptiles are significantly conditioned by environmental factors, with special reference to temperature, that influences their metabolism and activity (DERICKSON, 1976; SWINGLAND & FRAIZER, 1980; MEEK & JAYES, 1982; MEEK & AVERY, 1988; PARMENTER & AVERY, 1990; DÍAZ PANIAGUA *et al.*, 1995), although most species may also control their body temperature through behavioural and physiological mechanisms (HUEY,

1982; STURBAUM, 1982; GAVAUD, 1987). Several studies have shown that thermal relations strongly influence the behaviour and ecology of Hermann's tortoise, *Testudo hermanni* Gmelin, 1789 (HAILEY *et al.*, 1984; MEEK, 1984; 1988; PULFORD *et al.*, 1984; CHELAZZI & CALZOLAI, 1986; CARRETERO *et al.*, 1995; HUOT-DAUBREMONT *et al.*, 1996; HUOT-DAUBREMONT & GRENOT, 1997; MAZZOTTI & VALLINI, 1996). Long-term research on the movement patterns and homing behaviour of Hermann's tortoise (CHELAZZI & FRANCISCI, 1979) have shown

that these tortoises stay within a stable home range, whose size varies seasonally (CALZOLAI & CHELAZZI, 1991). Moreover, environmental factors like air temperature and photoperiod may trigger metabolic and physiological changes affecting seasonal variation of tortoises' weight (GILLES-BAILLIEN, 1973).

In this paper, we describe the weight seasonal variation in a population of Hermann's tortoises. Our study was conducted in the "Bosco della Mesola" Reserve (BMR), in one of the northern sectors of its European range, and in a Mediterranean wooded biotope that is not common for this species. The climate of this study area is characterized by rainy and hot summers, as well as dry and cold winters. During the study period, annual mean air temperature was 13.4°C, with a mean of the maximum temperatures of 18.2°C and of the minimum temperatures of 9.2°C. The warmest month was August, averaging 23.5°C (17.7°C mean of the minimum temperatures; 29.6°C mean of the maximum temperatures), and the coldest month was December, averaging 4.0°C (-0.7°C mean of the minimum temperatures; 7.6°C mean of the maximum temperatures) (MASSETTI *et al.*, 2013). Annual rainfall averaged 699.5 mm; the highest rainfall occurred in October (82 mm), while the lowest one occurred in January and February (40 mm) (MASSETTI & MAZZOTTI, 2013).

In recent years, the population of Hermann's tortoises living in the BMR was repeatedly studied for what concerns ecology and genetics. The home range was found by MAZZOTTI *et al.* (2002) to be wider than other studied Hermann's tortoise populations. Furthermore, the population structure of BMR is dominated by adults over 20 years; moreover, the sex ratio is close to 1:1, and population density was estimated to about 1 specimen-hectare⁻¹, which means a population size of about 1,000 Hermann's tortoises over the entire Reserve (MAZZOTTI *et al.*, 2007).

The original distribution of Hermann's tortoise in Italy has been greatly reduced, particularly in the North-West, where a few disjunct populations remain (MAZZOTTI, 2004). This population is presently isolated from other neighbour populations, and it is presumably autochthonous, although during the last century specimens from other sites have been released there, a condition common to several Hermann's tortoise populations in Italy. Genetical analyses of this population showed that it is characterized by an eastern *T. h. boettgeri* subspecies profile, showing weak presence (9%) of hybrids and migrants from *T. h. hermanni* subspecies populations (MIRIMIN *et al.*, 2004; PEREZ *et al.*, 2014; ZENBOUDJI *et al.*, 2016). The clear genetic affiliation of BMR population with the eastern subspecies, as well as the large genetic variation at both investigated markers and the significant divergence with other Balkan samples, supports the view that this isolated population represents the westernmost part of the area occupied by *T. h. boettgeri*.

In the present work, we built up a database of Hermann's tortoise morphometrics using data gathered in more than 20 years of observations, which allows us to detail the development cycle and seasonal weight variation of these animals. This is a mandatory step in order to make this Italian Hermann's tortoise population a wider reference model over the whole species areal.

MATERIALS AND METHODS

Study area

This study was carried out in the BMR, located within the delta of the River Po, in North-Eastern Italy. This reserve protects a residual coastal forest, of about 1,000 ha in surface, growing on ancient coastal dunes. It is a dense holm-oak coppiced wood, with mostly *Quercus ilex* and *Quercus robur* in the upper layer. Open patches of grassland, scattered throughout the wood, and ranging in size from very small to a few hectares, cover about 5% of the surface (CORBETTA & PETTENER, 1976; PICCOLI *et al.*, 1983; CORBETTA *et al.*, 1984).

Methods

Field work was carried out from July 1992 to October 2016. Tortoises were methodically searched for and captured/recaptured in the two major open patches, named "Balanzetta" (44°50'50" N - 12°14'28" E) and "Elciola" (44°49'38" N - 12°15'34" E), with a surface of 37.8 ha and 37.5 ha, respectively (Fig. 1).



Fig. 1. Study area. The map shows the location of BMR with respect to peninsular Italy, as well as sampling open patches within the forest.

Each captured/recaptured tortoise was sexed and marked according to Stubbs *et al.* (1984). Following data were recorded: date, time (European Standard Time, one hour after Greenwich), weight (WGT), linear carapace length (LCL), carapace width (CW), plastron length (PL), maximum plastron length (MPL), plastron width (PW), shield height (SH), and behaviour when first sighted and undisturbed, using tags modified from HUOT-DAUBREMONT *et al.* (1996).

Analyses

Following analyses focused on length (LCL) and weight (WGT) and were carried out using custom R scripts (available from FP upon request). Normality of length and weight distribution was tested using the Shapiro and Wilk test (ROYSTON, 1982a, b); because of non-normality of both distributions, length and weight were overall compared between

female and male individuals using a Mann and Whitney two-tailed test (BAUER, 1972; HOLLANDER AND WOLFE, 1973).

To investigate weight trends through a complete solar year, we selected out 23 females and 25 males that were sampled at least 15 times during the observation timespan. Every observation of these individuals was scored under the respective month: eventually, weights of females and males were separately compared for each month to those of the following month using a Mann and Whitney two-tailed test.

Condition Index (CI) was explored using custom R scripts (available from FP upon request). CI is defined as the ratio M/M' , where M is the weight of an individual and M' is the weight predicted by the allometric equation $M = aL^b$, being L the length of the animal (HAILEY & LOUMBOURDIS, 1990; HAILEY, 2000). The allometric equation parameters were estimated for each month and for each sex by linear regression of log-transformed values, i.e. from the linear relation $\log_{10}M = \log_{10}a + b \cdot \log_{10}L$. To this purpose, only the first observation of each specimen was used for each month, in order to reduce sampling noise and biases related to oversampled individuals. The expected weight (M') was computed for each sex using the respective parameters from July (HAILEY, 2000; HAILEY & LOUMBOURDIS, 1990), as this is the period after eggs have been laid, but before the drop in rainfall, where activity may vary (HAILEY, 1989).

CI was expressed both as M/M' and $\log_{10}(M/M')$: distributions were explored using the `descdist` function of the `fitdistrplus` R package (DELIGNETTE-MULLER & DUTANG, 2015). Either variable is approximately normally distributed; furthermore, the use of log ratio outweighs the drawbacks of a slight skewness for the analysis of interaction effects in ANOVA (HAILEY, 2000); therefore, we will use $\log_{10}(M/M')$ as CI from this point onwards. Eventually, Two-Way ANOVA was carried out in R to investigate possible differences in CI between months and sexes.

RESULTS

A total of 3,619 observations was recorded in more than 24 years of research (1992–2016) monitoring the BMR Hermann's tortoise population (supplementary dataset S1), for a total of 485 specimens: 217 female, 238 males and 30 juvenile specimens that were not sexed. Those specimens were captured, marked, and released, averaging 7.46 ± 16.26 capture for each specimen (7.30 ± 15.82 for females, 8.41 ± 17.55 for males). Furthermore, the behaviour of the sampled specimen was recorded: the proportion of different behaviours varies throughout the year, with active behaviours increasing their frequency in the central months of the year (Fig. 2). Aiming to avoid noise and biases due to missing data, the subsequent analyses were carried out retaining only complete or almost complete tortoise observations.

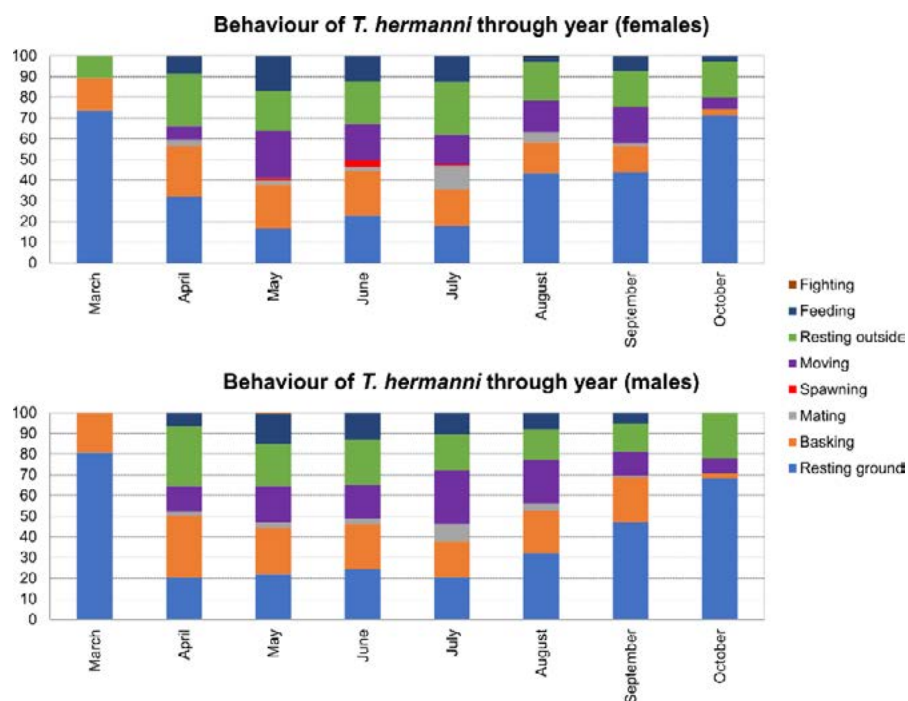


Fig. 2. Behaviours of female and male tortoises of BMR through year. Percentages of specimens caught while acting a given behaviour are shown.

As expected, the population ($N = 1,285$) is sexually dimorphic. Median length (LCL) is 181.10 mm for females and 162.50 mm for males (Tab. 1); median weight (WGT) is 1,129.50 g for females and 825.00 g for males (Tab. 2).

An exponential growth correlation between length and weight is shown in figure 3.

The weight of the 23 females and 25 males which were regularly sampled in 24 years of study shows a sharp seasonal variation (Tab. 3, Fig. 4)

	Mean	Median	SD	Lower 95% CI	Upper 95% CI
Females (N=539)	173.96	181.10	26.80	85.18	205.00
Males (N=746)	160.39	162.50	14.32	124.47	186.51

Tab. 1. Summary of linear carapace length (mm).

	Mean	Median	SD	Lower 95% CI	Upper 95% CI
Females (N=539)	1057.31	1129.50	326.04	140.47	1465.49
Males (N=746)	831.06	825.00	181.23	430.77	1198.57

Tab. 2. Summary of weight (g).

	Female (N=23)	Male (N=25)
January/February	1019.00	806.50
March	1104.50	802.75
April	1025.00	776.15
May	1083.15	783.25
June	1138.00	797.20
July	1069.00	798.00
August	1063.00	789.75
September	1088.00	802.25
October	1056.00	756.00
November/December	1034.50	791.00
overall	1081.00	791.00

Tab. 3. Female and male weight medians (g) through year.

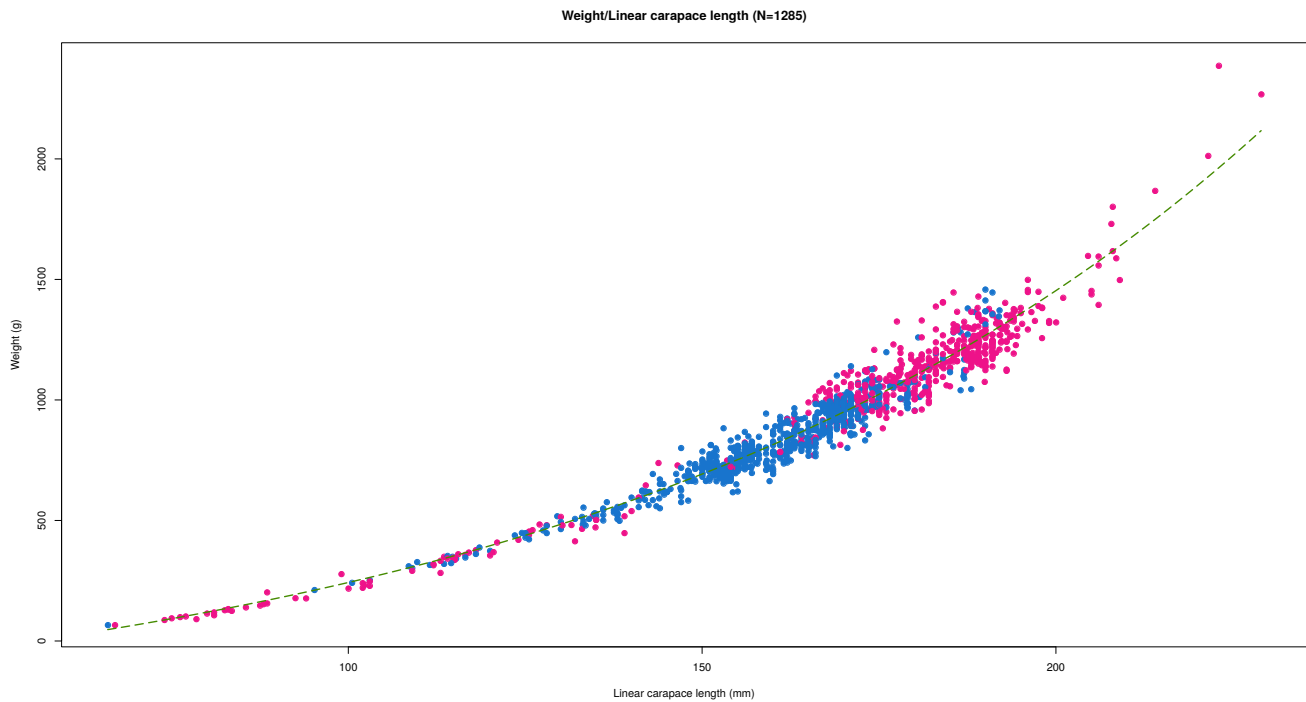


Fig. 3. Exponential relation between linear carapace length (LCL) and weight (WGT) of 539 captured females and 746 captured males from BMR. The best fitting formula was found to be $WGT = a + e^{b + c \times LCL}$.

The median weight of female specimens was 1,081 g over the whole year, ranging from 1,019 g in January to 1,138 g in June. Conversely, the median weight of male specimens was 791 g over the whole year, ranging from 756 g in October to 806.5 g in January/February. During the activity period (March–October), the weight variable is quite stable for males, while being fluctuating for females.

This is further highlighted when considering the per cent change in weight between a given month and the following one (Fig. 5). The weight per cent change is significant ($P <$

0.05) across the spring/summer boundary for females: weight increases by 5.67% between April and May and by 5.06% between May and June, and then decreases by 6.06% between June and July. On the other hand, the only significant variation in male weight is at the end of the active phase.

Patterns of the CI from 1,228 female and male individuals is shown in figure 4. In a nutshell, CI is quite stable during summer, while increasing sharply in females between April and May and mildly in males between April and June; both sexes experience a drop between June and July (Fig. 6).

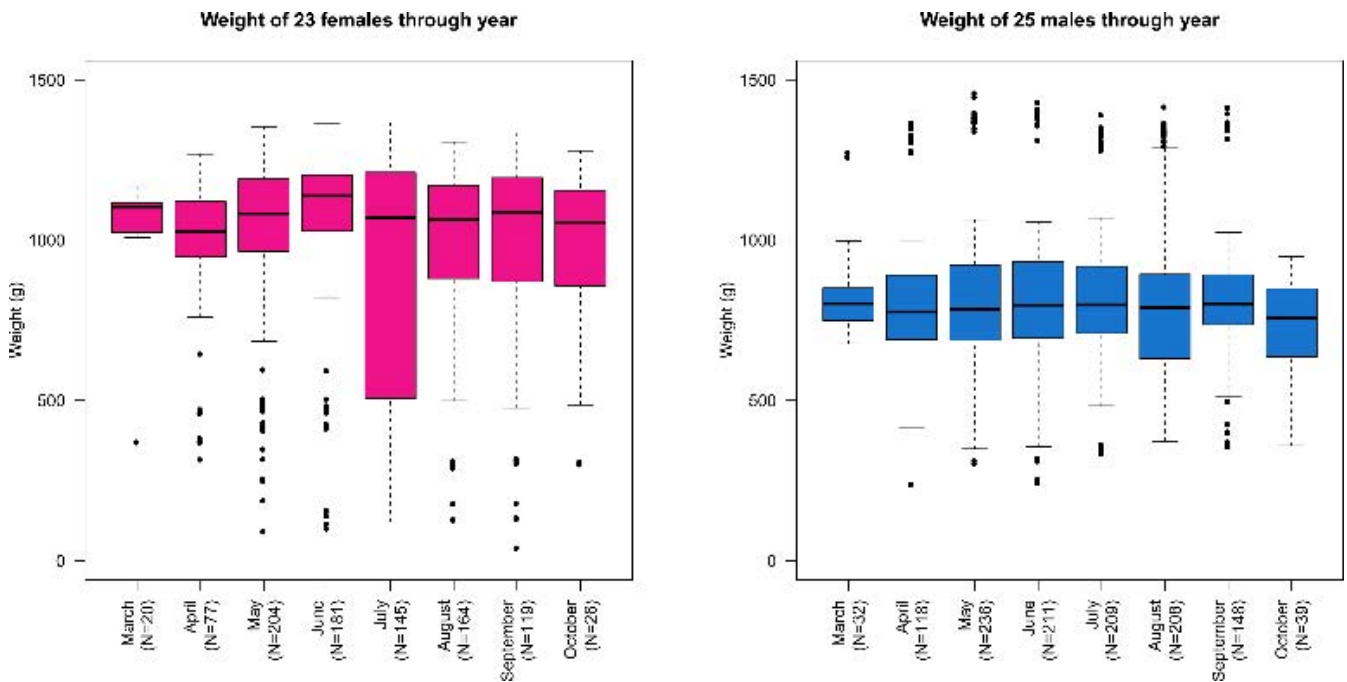


Fig. 4. Boxplots of weight (g) of 23 females and 25 males through year. January, February, November and December were discarded due to very low activity in these months and, therefore, to very low sampling rates. The black line is the median; the two hinges of the box approximate the first and the third quartile; whiskers extend to a roughly 95% confidence interval.

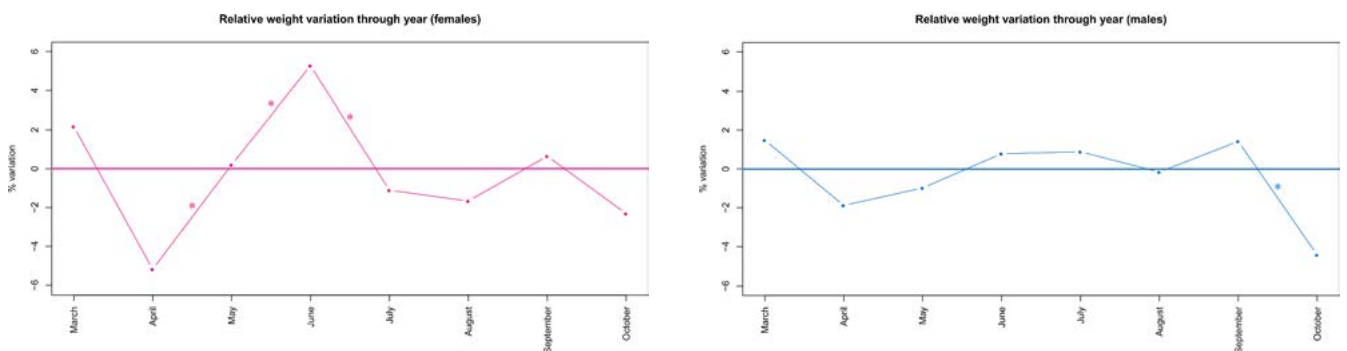


Fig. 5. Relative weight variation (%) of 23 females and 25 males through year with respect to the median value computed across the whole year. January, February, November and December were discarded due to very low activity in these months and, therefore, to very low sampling rates.

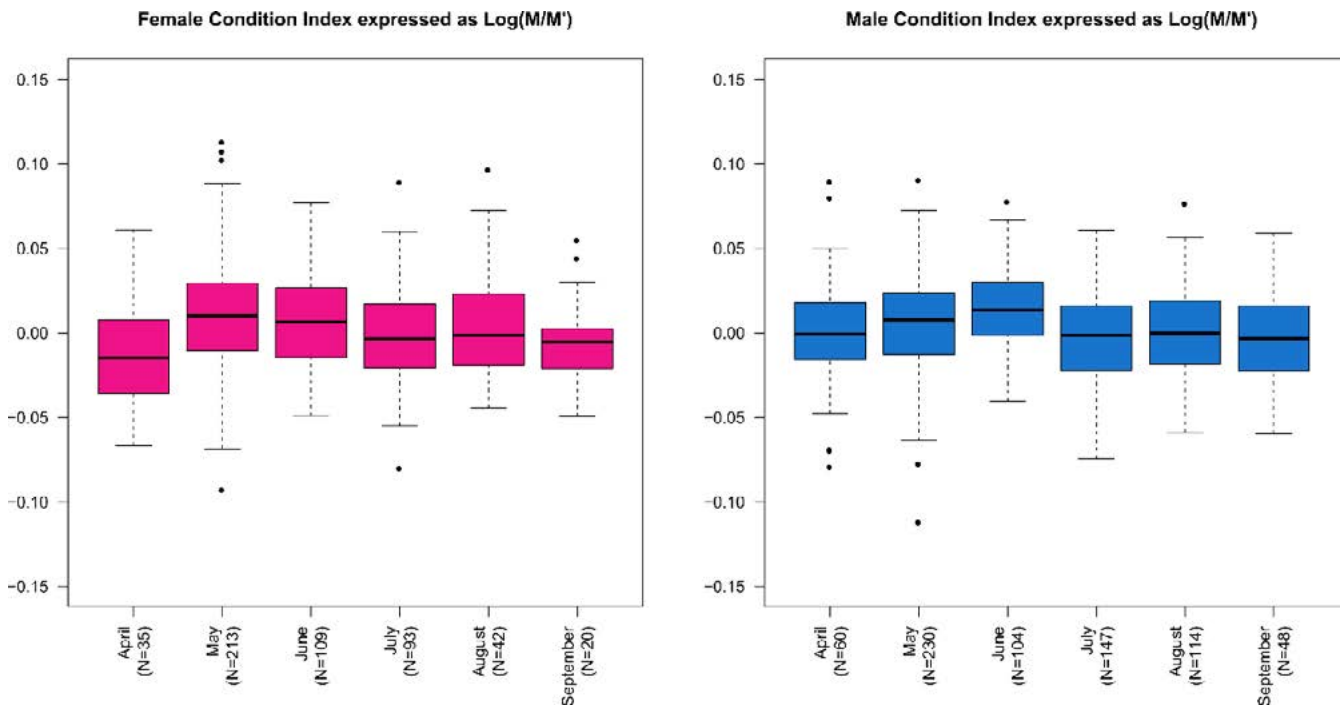


Fig. 6. Condition Index expressed as $\log_{10}(M/M')$ throughout the activity period. The black line is the median; the two hinges of the box approximate the first and the third quartile; whiskers extend to a roughly 95% confidence interval.

The variance in CI is highly structured between months (Fisher's $F = 7.1753$, $P = 1.9673 \times 10^{-8}$) and the month: sex interaction variance is structured as well (Fisher's $F = 2.3054$,

$P = 3.2234 \times 10^{-2}$), while no structure is evident between sexes alone (Tab. 4).

	Df	Sum Sq	Mean Sq	F value	p-value ¹	
Sex	1	1.3650×10^{-3}	1.3650×10^{-3}	1.6904	1.9380×10^{-1}	n/s
Month	7	4.0559×10^{-2}	5.7941×10^{-3}	7.1753	1.9673×10^{-8}	***
Sex:Month	6	1.1170×10^{-2}	1.8616×10^{-3}	2.3054	3.2234×10^{-2}	*
Residuals	1213	9.7950×10^{-1}	8.0750×10^{-4}			

¹ n/s, not significant; *, p-value < 0.05; **, p-value < 0.01; ***, p-value < 0.005.

Tab. 4. Two-Way ANOVA results. ANOVA was carried out on 1,228 CI values whose variation throughout the central part of the year is shown in figure 6. Months are from March to October

DISCUSSION

As expected, male and female individuals from the BMR show a pronounced sexual dimorphism, with males being typically smaller in terms of length and mass. However, both sexes reach greater sizes (+15-20%) with respect to populations coming from Sicily (TOMASETTI, 1997), Tuscany (PAGLIONE & CARBONE, 1990), France, and Greece (SWINGLAND & STUBBS, 1985). Recall that the BMR population is among the northernmost *Testudo hermanni* populations, colder seasonal

climates at higher latitudes may be involved in the observed difference. Moreover, previous data show that BMR tortoises exploit larger home ranges with respect to different European *T. hermanni* populations (MAZZOTTI *et al.*, 2002).

Male weight is relatively stable throughout the year, with the exception of the pre-hibernation phase; conversely, a sharp increase in female weight is observed from April to June, followed by a significant decrease from June to July (Fig. 4 and 5). Most likely, these fluctuations in female weight are connected to the seasonal reproductive cycle of eggs matu-

ration and laying (WILLEMSEN & HAILEY, 2001; BIAGGINI *et al.*, 2017). Non-significant decrease in weight was observed for both sexes in either post-hibernation and pre-hibernation phase (with the exception of the September–October drop in male weights), which may be connected to mating activity in Spring and the low level of feeding activity in Autumn (figure 2 and dataset S1; $\chi^2_{27}=59.0686$, $P=2.3157 \times 10^{-10}$). Conversely, the significant decrease in male weight which was observed in Autumn can be explained with the decrease in active (mating, moving, feeding, fighting) vs. inactive (resting, basking) behaviours which was observed in September and October (Fig. 2) and dataset S1; $\chi^2_{27}=80.4685$, $P=1.1054 \times 10^{-14}$).

The use of CI to explore body mass vagaries throughout the year allowed to retrieve a more detailed scenario, where it is clear that significant variations are connected to year periodicity rather than sex: therefore, seasonal activity cycles are the major drivers of physiological conditions of tortoises. Being the CI close to zero in most cases (Fig. 6), the measured mass of the specimens is quite close to the expected one, thus reflecting a healthy status of the population. Finally, our results about CI are in strong agreement with previously reported data (HAILEY & LOUMBOURDIS, 1990; HAILEY, 2000; WILLEMSEN & HAILEY, 2001; BIAGGINI *et al.*, 2017).

The Condition Index turned out to be a more effective tool to assess the seasonal cycle of *T. hermanni* with respect to standard measurements, like weight and carapace length. CI allowed us to confirm the good status of specimens from the focal population, as well as to explore changes experienced during the year, with special reference to female individuals. Future works, including data from colder months, and adding more precise climate variable data, will help to better understand mechanisms driving the body condition variability in *T. hermanni* in Italy. Given the current concern about environments warming around the world, a 24-year spanning database like the one which is presented here will hopefully be of outstanding value in evaluating the effects of climate change on life history traits and physiology of poikilotherm vertebrates.

The main physiological trait which is captured by the use of weight is the storage of lipids. It is well-known that reptiles often store lipids in association with reproduction rather than winter dormancy (e.g., LEHR BRISBIN JR, 1972), and thus the increase in body mass is normally observed in warmer months. Generally speaking, different groups of reptiles seem to store, mobilize, and utilize lipids under different patterns (DERICKSON, 1976), from no cycling at all to a seasonality connected to the reproductive period (typically summer) and/or the pre-dormancy period (typically fall). However, the ultimate factor connected with the cycling of lipids and, therefore, with body mass is the food availability (DERICKSON, 1976), which in turn is strongly connected to the seasonality of precipitation and temperature. In the focal case of *T. hermanni* BMR population, we can conclude that the seasonality of the Northern Italy climate entails a lipid cycling with an increase of weight in summer, connected with the reproductive period; other data are somewhat elusive, coarse-grained, or non-conclusive for non-avian reptiles (see, f.i., LEHR BRISBIN jr, 1972; DER-

ICKSON, 1976; LECQ *et al.*, 2014). Data presented here and available in our database allowed a fine-scale characterization of body mass and condition fluctuation throughout the year: further analyses will confirm whether these data can be taken as paradigmatic for tortoises, or temperate climates, or both.

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