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Inside the shell: body composition of free-ranging tortoises (*Testudo hermanni*)

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Abstract

Body condition indices (BCI – mass scaled by size) are widely used in ecological studies. They presumably reflect variations of endogenous fat reserves in free-ranging animals. In the field, however, accurately quantifying internal body reserves is a difficult task. This is especially true in armoured animals where convenient clues that may guide BCI assessment (e.g. visible subcutaneous fat deposits) remain inaccessible. Alternatively, inclusive dissections may provide anatomical abacuses to estimate body reserves in living individuals. Sacrificing animals for this purpose is not acceptable. We opportunistically tested the ability of BCI to estimate body reserves in 13 free-ranging Hermann's tortoises (Gmelin, 1789) dissected soon after they died from natural causes. On average, BCI values were lower in dissected tortoises relative to living individuals ($N > 10,000$ measurements), but they remained within the range of variation of the studied populations. Shell mass relative to body mass was high and showed considerable inter-individual variation (33.5% to 52.3%). Stomach and digestive tract content represented another important and variable part of total body mass (4.4% to 14.5%). The contribution of fat bodies was negligible (0.0% to 0.5%). Overall, in the studied tortoises, variations of body condition are weakly determined by variations of fat stores. Other endogenous (e.g. muscles, visceral tissues, liver) and “exogenous” (e.g. digestive tract content, clutch) elements should be considered to better understand age and sex specific life-history trade-offs faced by chelonians.

Key words: Body condition index; fat reserves; dissection; Chelonians

1. Introduction

Direct access to the internal elements that compose an animal, for example reproductive organs, body reserves or digestive tract content, is essential to examine a wide range of questions in ecology, evolutionary ecology and toxicology (Schmidt-Nielsen, 1975; Sakai et al., 2000; Zera and Harshman, 2001; Polilov, 2015). But in Vertebrates, most studies have focused on a single segment. Typically, diet has been assessed by scrutinizing stomach contents while other organs were overlooked (Evans and Lampo, 1996; Paltridge et al., 1997). Similarly, during inspection of ovarian structures, liver or muscles were generally ignored (Sica et al., 2001; Pizzatto et al., 2008; Gherissi et al., 2018). Whole-body composition based on dissection has been examined in only a handful of studies (Sakai et al., 2000; Landgraf et al., 2006).

Thus, for the vast majority of living Vertebrates, we cannot quantify the respective input of each main body components to overall body mass (e.g. relative mass of the skin or locomotor muscles). This limits the possibility to address ecological issues where body condition indices (BCI, mass scaled by size) are used to evaluate lipidic reserves (Stephens et al., 2009). For example, calculation and interpretation of BCI can be misleading since total body mass includes unknown shares of fat stores, muscles, partly digested food, and ovulated follicles. In most cases, measuring all main internal body components is not feasible in the field, even when using sophisticated techniques (Speakman, 2001; Cojean et al., 2018; Hofmeyr et al., 2020). Currently, only comprehensive dissections allow isolating and measuring organs (mass, dimensions), extracting and weighing whole content of the stomach or of the intestine, to exactly assess body reserves, etc., and thus to eventually obtain a comprehensive picture of the elements that constitute an animal as a whole.

But performing inclusive dissections relies on the availability of dead animals and on the willingness to devote a considerable amount of time to the partitioning and measuring of internal elements (Sakai et al., 2000). Although sacrificing free ranging animals should be avoided, accidentally or naturally dead animals can be collected without raising major ethical concern. Picking and fully dissecting randomly encountered cadavers is not in the culture of most field ecologists.

The lack of convenient technique to assess an individual's body composition might prove particularly taxing when dealing with animals protected by an external armour (e.g. shell, large scales, spines or very thick skin). First, the mass of external protection may well be substantial (e.g. ~30% of total body mass in pangolins; Wilson and Mittermeier 2011). Second, the mass of all internal components that do not belong to the tissues of the individual *sensu stricto*, like digestive tract content (e.g. faeces) or developing embryos for example, is inaccessible via palpation (ultrasound and radiography enable the visualization of follicles and eggs, however – Hofmeyr et al., 2020). Third, convenient clues to assess BCI, like visible fat deposits, cannot be used (e.g. camel hump; Gherissi et al., 2018). Overall, the relationship between body condition and actual endogenous body reserves is obscured using mass/size relationships, since exogenous matter and reproductive items also contribute to overall body mass. These complications are almost systematically ignored in the ecological literature where body condition is considered as a reliable estimator of fat reserves (Schulte-Hostedde et al., 2005; Peig and Green, 2009). Yet, the paucity of information also implies that we cannot gauge the severity and relevance of this issue.

In this study we dissected fresh, naturally dead tortoises in the course of capture-mark-recaptures studies. This offered the opportunity to examine to what extent the use of a classical BCI may have tarnished our ecological interpretations during previous studies (Bonnet et al., 2001; Djordjević et al., 2011; Lecq et al., 2014). Finally, we expected that a better knowledge of tortoise body composition may improve further ecological studies. To the best of our knowledge, this study provides the first precise data on the whole-body composition of terrestrial Chelonians, and provides exemplary points of prudence when interpreting BCI.

2. Material and methods

Study species and study sites

The Hermann's tortoise (*Testudo hermanni*) can grow up to 25 cm, attain 50 years, and displays delayed maturity and low fecundity (Bertolero et al., 2011). Estimates for

body size at maturity range between 13 cm and 15 cm of straight carapace length (SCL); we classified individuals larger than 13 cm as adults (Willemssen and Hailey, 1999).

From 2008 until 2019, we studied the ecology and morphology of tortoises in two dense populations (> 20 adults / ha) of *T. hermanni* in two nearby sites (~ 4.5 km away from each other) of the Prespa Lake region in North Macedonia: Golem Grad Island (GG, ~ 18 ha; N $40^{\circ}52'$; E $20^{\circ}59'$), and Konjsko village (KV, ~ 20 ha, N $40^{\circ}54'$; E $20^{\circ}99'$) (Djordjević et al., 2011; Arsovski et al., 2018a; Golubović et al., 2013, 2017, 2018).

Data collection

Each individual was sexed when possible (small individuals cannot always be sexed – Arsovski et al., 2018b; Golubović et al., 2018) and marked using a notch-code on the marginal-scutes (Stubbs et al., 1984). Overall, we encountered tortoises more than 18,500 times in the field (2,798 captures plus 15,789 recaptures). Straight carapace length (SCL) and body mass (BM) were recorded, and individuals were rapidly released at the exact place of capture. To limit unnecessary handling during frequent recaptures, SCL and BM were not systematically recorded. We obtained 9,680 and 1,432 measurements of both SCL and BM from Golem Grad and Konjsko populations, respectively.

During field work, we examined all dead (or dying) tortoises. In total, we found 315 dead tortoises on Golem Grad (223 males, 41 females and 51 immatures; this proportion of dead males reflects the highly biased sex-ratio in this site; Bonnet et al. 2016) and 35 in Konjsko (20 males, 14 females and 1 immature). Most of them were partly rotten or fully skeletonised (i.e. empty shells) and could not be used for dissection. We retained only 13 very fresh individuals for dissection (12 from Golem Grad and one from Konjsko; Table 1). Most of them had big cracks on the shell, revealing that they fell from cliffs, or serious injuries on the cloaca suggesting they had died from sexual harassment (Golubović et al., 2013, 2018). Animals in poor condition (e.g. severely wounded) but still alive were kept in outdoor enclosures,

wounds cleaned, and provided with water and food until they recovered or not. One recently wounded female could not be cured in the field (very deep hole in the back), thus she was euthanized.

Prior to dissection, we recorded SCL and BM. Then we opened the shell ventrally using a hacksaw to remove the plastron. The main organs of the tortoise were separated (using scissors and pliers), carefully examined and weighed using electronic scales. The mass of the shell was recorded to the nearest 0.1 g. Most components could be actually separated and weighed with precision of 0.1 g: heart, liver, lungs, stomach, intestine, gonads, growing follicles, eggs or digestive tract content for example. Several parts of the body could not be easily dissected. For example, we did not tease apart the skin, muscles and bones for the legs, head, neck and tail; thus, we only recorded the total mass of the abovementioned elements. Fluids and blood lost during dissection, along with parts which were not easily identified or assigned, like mesenteric tissues and blood vessels for example, were classified as miscellaneous material for conciseness.

Analyses

Body condition index (BCI) was calculated using residuals from a linear regression (living and dissected individuals pooled, dead but not-dissected tortoises discarded) of \ln -BM against \ln -SCL (Bonnet et al., 2001). This allowed us to evaluate to what extent the BCI of freshly dissected tortoises was representative of the values measured in the studied populations. The small proportion of dissected tortoises (0.1%) limited their contribution in the calculation of the parameters of the regression. BCI values were normally distributed (Shapiro-Wilk test, $p > 0.05$). Many living individuals were represented more than once (recaptured over time), therefore, individual identity was included as a random factor. Not-dissected dead tortoises (e.g. partly rotten, empty shells) were not included in the body condition analyses, but they were plotted on Figure 1 for comparison. Generalized linear mixed models were built in R Version 1.2.5001 (R Core Team, 2019) using the

package nlme (Pinheiro et al., 2019). Competitive models were ranked using the Akaike information criterion (AIC, Burnham and Anderson, 2002).

The masses of certain organs were highly correlated (e.g. left *versus* right legs). Furthermore, for the purpose of the current study, considering morpho-functional components instead of each separate element improved the conciseness of analyses. Therefore, we assigned all dissected elements into 11 simple units (henceforth designated as components, Table 2) that were used in most analyses. The relationship between the total body mass and the respective mass of these components was examined with a backward stepwise regression. We first verified that the mass of these components, used as the dependent variables, was correlated ($0.57 < r < 0.98$; all $p < 0.05$) with the body mass (the independent variable). Because the sum of the components represented total body mass, this stepwise analysis enabled us to identify potential major contributors of BCI.

Sex difference in body composition was tested using ANCOVA with the main component as the dependent variable, sex as the factor and body mass as the covariable. The strong correlation between the mass of each body component and body mass facilitated this analysis. However, due to the small sample size of dissected tortoises, we also used non-parametric Kruskal-Wallis ANOVA to compare the body composition (e.g. shell, somatic and visceral organs mass relative to the body mass, ratios were arcsine transformed prior to the analyses) of females ($N = 4$) relative to males ($N = 9$).

3. Results

Body condition: effect of sex, age and population

The data obtained enabled us to encompass the range of variations of body mass as a function of body size in both populations (Figure 1). Model selection revealed that interaction of locality (Golem Grad Island or Konjsko village), state (living *vs.* dissected) and sex (male, female or immature) best explained variation of BCI (Table 3). Tortoises from Konjsko exhibited higher body condition in general, regardless of

state and sex ($t = 9.88$, $p < 0.001$, Figure 1), whereas males exhibited lower body condition than females in both localities ($t = -3.33$, $p < 0.001$).

Body condition of living and dissected individuals

Dissected tortoises exhibited lower body condition compared to living individuals ($t = 4.0$, $p < 0.001$), but Figure 1 shows that the body mass relative to body size of the dissected tortoises remained within the range of variation exhibited by living ones, especially in Golem Grad where most dissected tortoises were found (Figure 1A). But considering the Konjsko population, most dissected tortoises could be classified as lean (Figure 1B).

Body composition of dissected tortoises

Body composition was highly variable among dissected individuals (Table 1). For example, focusing on adult males, the relative mass of the shell ranged from 33.5% to 51.8%. This strong variability was also observed in other body components (see Supplementary Material – Table I).

The final step of the backward regression (adjusted $R^2 = 0.999$, $F_{9, 3} = 4894.2$, $p < 0.0001$) retained nine components among eleven, that significantly contributed to total body mass (Table 4). The masses of somatic organs (legs, head, neck and tail) and shell mass were the most important contributors (standardized coefficients > 0.30). Miscellaneous material and digestive tract (net) were also substantial contributors (standardized coefficients > 0.10). Finally, the masses of kidneys, digestive tract content (i.e. partly digested material), and fat bodies, were retained in the model, but the standardized coefficients were small (~ 0.05). The masses of the heart and respiratory system provided almost null standardized coefficients (~ -0.08). The masses of the liver and reproductive organs were not retained.

Regarding sexual dimorphism, ANCOVA and Kruskal-Wallis ANOVA provided similar trends (although some non-parametric tests failed to reach statistical significance). We found sex differences for the most important components: females exhibited relatively heavier shells, while males showed higher relative somatic

organs mass – (Table 5; Figure 2). A closer inspection of the somatic organs showed that males had relatively heavier limbs and heads (Table 5). They also had higher relative values of some visceral organs, e.g. hearts and livers compared to females (Table 5). We found no other significant sex differences.

4. Discussion

Dissections of free-ranging tortoises provided new insight on the significance of body mass, and by extension of body condition in terrestrial chelonians. Several results support previous studies (e.g. about sexual dimorphism), but others were unexpected (e.g. regarding the relative contribution of digestive tract content *versus* fat bodies).

Dissected versus living tortoises

On average, dissected individuals were in the lower part of the range of variation of BCI exhibited by living tortoises (Figure 1). Dead tortoises may dehydrate rapidly in the field, especially those with large cracks in the shell. Some tortoises may have lost significant amounts of blood. Rapid loss of fluids may reduce body mass and thus body condition. One female harassed by nine males exhibited low body condition and may have died from exhaustion. On the other side of the spectrum, our sample also included one large female with a high BCI.

Overall, our dissection data encompass most of the variation in adult body size, but are not fully illustrative of the whole range of body condition observed in the populations monitored (e.g. heaviest males and small individuals were lacking). However, comparisons with other studies provide means to assess to what extent our dissection results are representative of what is currently known in chelonians.

Shell mass

The protective role of the armour is crucial in Chelonians (Polly et al., 2016), but it also imposes major morpho-functional and energetic constraints. The shell can obstruct movements in dense vegetation and limits tortoise agility (Bonnet et al.,

2001; Golubović et al., 2014, 2015; Chiari et al., 2017). Abundant resources and time are needed to build an effective shell; this shapes the growth trajectory during the first decade of life in the Hermann's tortoise (Arsovski et al., 2018a, b). Likely, crucial structural materials of the shell are not easily mobilised; we thus speculate that they play no (or limited) role as body reserves.

The fresh mass of the shell represents the main component of a tortoise, averaging 41.5% of total body mass (39.6% when removing two extreme values in very lean individuals). Dissections of captive tortoises indicate that the mean dry mass of the shell relative to the dry mass of the carcass represents 31%, and 35% in adults (Table 18 in Kopsch, 2006). Despite differences in methodologies (fresh *vs.* dry masses, relative to total body *vs.* carcass mass), previous and current studies show that the mass of the shell is considerable in tortoises (suggested by Iverson 1984). This proportion is approximately 30% (27% in adults) in terrapins (Miller and Birchard, 2005; Kopsch, 2006), and is markedly lower (17%) in adult sea turtles (Sakai et al., 2000).

We observed very strong variations among individuals in the relative fresh mass of the shell (34% to 52%). Dry masses measured in different species support our results (e.g. 23% to 80%; Kopsch, 2006). The mass of the shell correlates with body size, yet not perfectly ($r^2 = 0.81$, $N = 13$) due to variations in shell shape. Part of the variation in total body mass (~19%) is caused by interindividual variations in relative shell mass. Thus, BCI includes an unknown albeit substantial shell-component independent of body reserves.

Body reserves

Fat bodies were virtually absent in all dissected individuals, representing only 0.0% to 0.5% of the total body mass. Kopsch (2006) did not provide information regarding fat bodies in the dissections of 92 captive individuals (3 *Testudo*, 2 terrapins, and *Geochelone pardalis*); suggesting that adipose tissues were negligible. Sporadic cases of obesity have been reported in overfed captive tortoises, with pads protruding from the openings of the shell (Burchfield et al., 1980; Lamberski, 2013). But this

situation may not occur in natural conditions: we never found an obese individual in our large datasets (> 5,000 individuals) collected in different tortoise species (Bonnet et al., 2001; El Mouden et al., 2006; Djordjević et al., 2011; Lecq et al., 2014). Noticeably, in the current study, dissected individuals exhibited average to low body condition (except one very large female). Thus, we cannot ascertain that fat bodies are always negligible, especially in heaviest individuals. It would be informative to assess if fat bodies develop above a BCI threshold (this was not the case in the high-BCI dissected female, 0.3% fat). Abundant fat deposits, however, have been observed in sea turtles (Kwan, 1994), reaching 12% of the total body mass in female loggerhead turtles (calculated from Table 1 in Sakai et al., 2000). Lipidic reserves may well be more developed in aquatic than in terrestrial chelonians.

The liver is an important source of endogenous reserves (e.g. lipids and proteins) mobilized during reproduction (Telford, 1970; Bonnet et al., 1994; Heck et al., 1997; Finkler, 2013). This organ also stores reserves beyond reproduction (Collins and Anderson, 1995). Dissections showed that the liver represents an important proportion of body mass in Chelonians, 2.7% to 7.2% in the current study, 2.6% to 3.5% in female sea turtles (Sakai et al., 2001) and 1.5% to 5.0% in tortoises and terrapins (Table 32 in Kopsch, 2006).

Fat bodies, the most “classical” proxy for BCI, did not contribute significantly to variation of body mass in tortoises. Somatic organs (e.g. locomotor muscles) and the digestive tract were better contributors. Tortoises may first allocate resources in their locomotor and digestive structures, and build up fat bodies only later. Future investigations focusing on the relationships between body condition and dynamic aspects of mass variation of the main components are needed.

Sexual dimorphism in body plan

In our study, the relative mass of the shell was greater in females (38.7% – 52.3% *vs.* 33.5% – 51.8% in males; Supplementary Material, Table I). This supports the notion of a more developed shell in females to maximize protective functions and to enhance abdominal volume to hold the clutch, compared to a relatively lighter shell

with larger openings to favor mobility in males (Bonnet et al., 2001; Djordjević et al., 2011). The relative mass of organs involved in locomotor performance (e. g. limbs, heart) was greater in males compared to females (21.5% – 30.0% *versus* 15.8% – 24.3% of the total body mass; Supplementary Material, Table I). These result fit well with previous studies on sexual dimorphism of body plan in chelonians (Bonnet et al., 2010), and more generally regarding sexual dimorphism of body composition in snakes and fishes (Bonnet et al., 1998; Casselman and Schulte-Hostedde, 2004).

Exogenous components inside the body

In all dissected individuals, dissections revealed substantial amounts (4.4% – 10.2% of the total body mass in adults) of exogenous and partly digested materials in the digestive tract. Incidentally, the highest proportion (14.5%) was observed in the immature male; perhaps small and rapidly growing individuals need to feed a lot. Regardless, partly processed food contributed to total body mass more than fat bodies.

The resources invested into large follicles and ovulated eggs cannot be considered as endogenous reserves (Bonnet et al. 1994). One female carried abundant and well-developed follicles and eggs (Supplementary Material), but in the others, the ovaries and oviducts were almost empty, only small follicles being visible. This considerable variation (0.1% – 10.0% of the total body mass) explains the significant contribution of the reproductive organs + follicles and eggs in total body mass variations.

Body condition versus body composition

Body condition integrates the mass of endogenous body reserves (fat and protein stores) along with exogenous material. Fluctuations of the relative mass of the shell cannot be factored out. Further, hydration status influences body mass (Packard and Packard, 2001), as suggested by the importance of the fluids lost during dissections. Thus, the meaning of body condition should be considered with caution and certainly not reduced to lipidic stores. Yet, BCI is a useful index to assess health status in tortoises; for example, it strongly correlates with the mass of crucial

locomotor structures. Additionally, in females it may well be a good indicator of reproductive status. The mass of the reproductive items of the dissected female with well-developed eggs and follicles was 5 to 100 times greater than in non-reproductive females (Supplementary Material – Table I).

Consequences for ecological studies

The two populations we studied differ considerably in terms of body condition; this contrast requires explanation(s). Only one individual from Konjsko was dissected, precluding direct comparisons of body composition between sites however. In Golem Grad fat bodies were negligible, but this might not be true for the heaviest Konjsko tortoises. Yet, using BCI as a mere indicator of fat stores may lead to invalid conclusions. For example, on Golem Grad, tortoises with a high BCI might be incorrectly considered as fat compared to those with a low BCI - a classical shortcut in ecological studies. Instead, our results suggest that somatic organs were more important. This information may help to address specific issues. For instance, we may hypothesize that Golem Grad tortoises are locally adapted to extremely uneven topography where lightness and agility are advantageous (Golubović et al., 2017). Alternatively, does low food availability explain why Golem Grad tortoises are lean compared to Konjsko? The ecological and evolutionary processes that underpin these questions differ considerably. Well-informed BCI values would be then crucial.

In conclusion, field dissections provided support to previous studies on sexual body shape dimorphism. More importantly, they enabled us to broaden the meaning of body condition in free-ranging tortoises, notably to shift away from a fat store focus toward a more complex vision that includes various body components. This might be useful for future studies of sexual dimorphism, feeding ecology, reproductive investment, demography, and other life-history traits.

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Table 1. Summary data of dissected tortoises from Golem Grad Island (GG) and Konjsko village (KV). M (I) – immature male, M – adult male, F – adult female. SCL stands for straight carapace length (mm), BM1 and BM2 respectively for body mass measured when the animal was found (dead or alive, see text) or just before dissection. BCI means Body Condition Index (mass scaled by size, residuals). The most important components of total body mass are provided (shell, soma and viscera). Soma includes front and hind legs, head, neck and tail; Viscera includes liver, heart, thymus, spleen, respiratory organs, kidneys, reproductive organs, digestive system (stomach + intestine + food + faeces), fat bodies, miscellaneous material (blood. etc.).

Sex, ID and location	Biometry			Mass at dissection (g)			
	SCL	BM1	BCI	BM2	Shell	Soma	Viscera
M (I) 994 (GG)	95.9	211.0	0.08945	203.0	81.0 (39.9)	56.7 (27.9)	65.3 (32.2)
M 843 (GG)	182.2	1199.0	0.00065	1016.5	397.7 (39.1)	387.0 (38.1)	231.8 (22.8)
M 234 (GG)	188.0	1113.0	-0.16300	1062.0	393.9 (37.1)	418.3 (39.4)	249.8 (23.5)
M 722 (GG)	148.0	426.0	-0.44304	423.0	219.0 (51.8)	133.3 (31.5)	70.7 (16.7)
M 303 (GG)	139.2	556.0	-0.00222	555.0	224.0 (40.4)	179.2 (32.3)	151.8 (27.3)
M 1296 (GG)	187.0	1151.0	-0.11485	1078.0	473.0 (43.9)	360.5 (33.4)	244.5 (22.7)
M 1206 (GG)	174.0	968.0	-0.08291	964.0	323.0 (33.5)	381.3 (39.6)	259.7 (26.9)
M 924 (GG)	186.0	1286.0	0.01132	1220.0	450.0 (36.9)	453.6 (37.2)	316.4 (25.9)
M 338 (GG)	178.0	1076.0	-0.04183	1063.6	395.0 (37.1)	361.6 (34.0)	306.4 (28.9)
F 1776 (GG)	152.0	672.0	-0.06313	651.0	278.0 (42.7)	187.7 (28.8)	185.3 (28.5)
F 1058 (GG)	178.0	911.0	-0.20830	914.0	424.0 (46.4)	276.6 (30.3)	213.4 (23.3)
F 1712 (GG)	163.0	766.0	-0.13108	771.0	403.0 (52.3)	161.5 (20.9)	206.5 (26.8)
F NEW (KV)	225.0	2348.0	0.07152	2348.0	908.0 (38.7)	629.0 (26.8)	811.0 (34.5)

Table 2. List of the main components used to describe the body composition of the dissected tortoises. They were implemented in the stepwise backward regression analysis. Element pooled explain which organs or elements were pooled in certain components.

Component (g)	Elements pooled
Shell	-
Somatic organs	See Supplementary Material – Table I
Liver	-
Heart	-
Respiratory system	-
Kidneys	-
Reproductive organs	Both gonads, epididymis, oviducts plus follicles and ovulated eggs
Digestive tract	Gullet, stomach and intestine (net)
Fat bodies	-
Digestive tract content	Stomach and intestine content
Miscellaneous material	Blood, lymph, several mesenteric elements and blood vessels

Table 3. Model ranking of generalized linear mixed models of Hermann's tortoise body condition variation conditional to univariate, additive (+) and interactive (*) effects of sex and state (immature, male or female) and locality (Golem Grad Island and Konjsko village). One model built to evaluate whether dissected tortoises' body condition differed from that of living tortoises (dissected *vs.* alive) had a low AIC (likely due to small sample size of dissected tortoises). Models were ranked according to their respective Akaike Information Criteria (AIC) values, Δ AIC and AIC weights.

Model	AIC	ΔAIC	AIC weight
Dissected <i>vs.</i> alive	-28.734,8	479.6	0.0
Sex and state	-28.905,9	308.5	0.0
Locality	-29.204,2	10.2	0.0
Sex + Locality	-29.214,2	0.2	0.5
Sex * Locality	-29.214,4	0.0	0.5

Table 4. Final selection from the stepwise backward regression analysis (following 7 steps). Nine components among 11 (see Table 2) were retained. Standardized coefficient and coefficient are expressed \pm SE; t-test t with degree of freedom in brackets.

Component	Standardized coefficient	Coefficient	t (6)	p
Somatic organs	0.448 \pm 0.027	1.458 \pm 0.087	16.781	<0.001
Shell	0.314 \pm 0.020	0.836 \pm 0.052	15.969	<0.001
Miscellaneous material	0.208 \pm 0.014	1.975 \pm 0.129	15.223	<0.001
Digestive tract	0.124 \pm 0.014	2.609 \pm 0.282	9.280	<0.003
Kidneys	0.060 \pm 0.014	9.362 \pm 2.196	4.265	0.025
Digestive tract content	0.058 \pm 0.013	1.014 \pm 0.219	4.631	0.019
Fat bodies	0.047 \pm 0.013	11.218 \pm 3.233	3.470	0.040
Heart	-0.083 \pm 0.018	-15.591 \pm 3.360	-4.641	0.018
Respiratory system	-0.076 \pm 0.018	-5.499 \pm 1.315	-4.183	0.025

Table 5. Results from GLM (ANCOVA, columns 2 to 4) and Kruskal-Wallis ANOVA (columns 6 and 7) to compare the relative mass of different components or organs between the sexes (4 females and 9 males). SD (column 5) indicates the direction of sexual dimorphism. Components where only non-significant results were found are not reported.

Component	SC	F	P	SD	H	P
Shell	9089.3	7.466	0.021	F	2.381	0.122
Somatic organs	21476.3	12.192	0.006	M	6.095	0.013
Legs	9732.0	12.472	0.005	M		
Head	424.8	8.385	0.016	M		
Heart	29.3	12.510	0.005	M		
Liver	1508.9	10.633	0.009	M	5.357	0.020

Figure captions:

Figure 1. Relationship between body mass (g) and body size (straight carapace length, mm) of live tortoises from Golem Grad Island (Top panel A) and Konjsko village (Lower panel B). Dissected (black circles), and not dissected but dead (grey crosses) tortoises are displayed on both panels for population comparison (scales identical). One large dissected female was in very good body condition and one male was particularly lean (grey arrows). This large female was found in Konjsko, all the other dissected tortoises were found in Golem Grad.

Figure 2. Comparison of the body composition of male and female Hermann's tortoises. Mean values (\pm SD) provide the proportions (% relative to body mass) of the main body components: shell, somatic organs, visceral organs, fat bodies and faeces (see table 2 and text for details and statistics).

Figure 1.

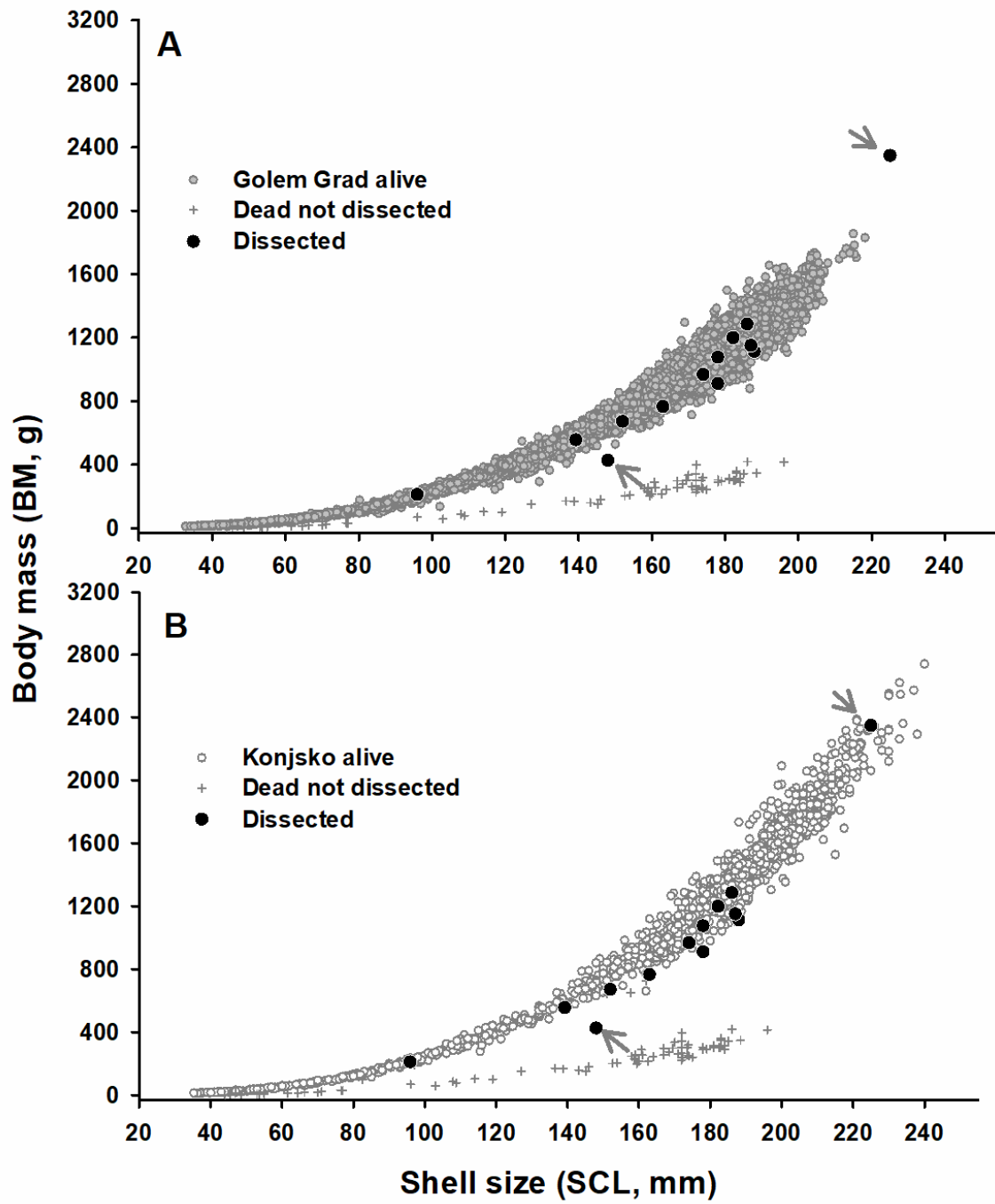


Figure 2.

