BODY MASS CONDITION IN GREEK TORTOISES: REGIONAL AND INTERSPECIFIC VARIATION

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Body mass and length data from large samples of wild *Testudo graeca*, *T. hermanni* and *T. marginata* in Greece were used to assess body mass condition. Mass-length relationships differed significantly between the sexes (females being heavier) and among the species (*T. marginata* being least heavy). Mass-length relationships for each species and sex were used to calculate the condition index (CI) log (M/M'), where M is observed mass and M' is mass predicted from length, which is equal to residuals from the regression of log M on log length. It was possible to use the empirical mass-length relationships from one population of *T. hermanni* to calculate CI in other populations of substantially different adult size. The seasonal pattern of the CI varied with latitude, with a sharper and later peak further north, and habitat, declining more in summer at a xeric coastal site. The seasonal patterns of CI in *T. graeca* and *T. marginata* were similar, with sharper and later peaks compared to *T. hermanni*. These seasonal patterns of CI were related to differences in activity and food availability among species and sites. The variability of the CI was similar in all three species, with most values between -0.1 and +0.1; seasonal variation was of relatively low amplitude, with a range of about 0.05 between the highest and lowest monthly means.

Key words: condition index, season, *Testudo graeca*, *Testudo hermanni*, *Testudo marginata*

INTRODUCTION

Many different parameters may be used to quantify the condition of an animal. Physiological variables are probably the most directly related to health but are often relatively difficult to measure, especially in chelonians which are capable of withdrawing within the margins of the shell when threatened (Jacobson, Behler & Jarchow, 1999). Blood, for example, may be examined for many variables related to health (Bonnet, 1979, Jacobson, 1987) and the results compared with normative values (Raphael *et al.*, 1994; Klemens *et al.*, 1997). Christopher *et al.* (1997) found that urea nitrogen content was a good measure of the hydration state of desert tortoises (*Gopherus agassizii*), and plasma iron, glucose and total protein were good indicators of their nutritional state. Blood may be sampled from the heart, jugular vein, brachial vein, ventral coccygeal vein, orbital sinus, or short-clipped toenails (Avery & Vitt, 1984; Jacobson, 1988, 1993; McDonald, 1976), but the procedure may be difficult and dangerous to a tortoise (Jacobson, Schumacher & Green, 1992), especially in the field. Other physiological parameters present even greater technical difficulties than collection and analysis of blood. For example, Henen (1991, 1997) measured the lipid content of live desert tortoises, but this required equilibration in a cyclopropane atmosphere for eight hours, analysis by gas chromatograph and calibration against total lipid extractions of dead tortoises.

A much simpler alternative is the mass of the tortoise in relation to its size (Jackson, 1980, 1991). Mass relative to size may be described simply as condition (for example, Blood & Henderson (1968) define normal bodily condition compared to obese, thin or emaciated animals), but this is better termed body mass condition to differentiate it from indexes based on other parameters. A further advantage of specifying body mass condition, rather than just condition or body condition, is that mass may not be linearly related to health. Very high body mass condition is likely to be an indication of poor health if an animal is obese or has egg peritonitis or fluid retention from renal or hepatic disease (Jackson, 1980; Lawrence, 1985; McArthur, 1996). As a related point, the word condition should be restricted to variables that reflect the health or physiological state of an individual, even if the relationship is not known in detail, rather than used as a short-hand description of morphological differences. The excellent study of Bonnet *et al.* (2001), for example, documented sexual dimorphism of carapace shape in *Testudo horsfieldii*, females being significantly wider and higher than males at the same length and having more bellied plastrons. There was also a significant sexual difference in mean mass adjusted for length, females being heavier. This was described as a difference in body condition and body condition index between the sexes, but only reflects the sexual shape dimorphism. Such differences in mass due to morphology have nothing to do with condition as such (Hailey, 2000: Fig. 3b) and should simply be described in terms of relative mass, not body condition.

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Body mass condition has been used with varying success in studies of chelonians. Jackson's results on *Testudo hermanni* and *T. graeca* have been widely used (Divers, 1996) and compared with other species of tortoise (Spratt, 1990). Nevertheless, the graphical study of McArthur (1996) showed no obvious differences between body mass of healthy and ill tortoises. Jacobson *et al.* (1993) did find a significant difference between healthy desert tortoises and those with respiratory disease, but identified six factors that limit the usefulness of body mass condition. These factors were related to potential differences in mass-length relationships (1) between the sexes; (2) in females before and after oviposition; (3) between the activity season and just after hibernation; (4) among populations due to differences in shape or dermal bone thickness; and the effects of (5) weight gain after drinking or (6) weight loss as faeces or urine on handling.

Nevertheless, equivalent difficulties occur with other parameters. Blood composition, for example, is known to vary with size, sex and season in chelonians (Hutton & Goodnight, 1957; Gilles-Baillien & Schoffeniels, 1965; Seidel, 1974; Frair & Shah, 1982; Taylor & Jacobson, 1982; Lawrence & Hawkey, 1986; Kim, Cho & Koh, 1987). Raphael & Jacobson (1997) also note that blood composition may vary among collection sites on the body due to differential contamination with lymph. Most of the difficulties with body mass condition can be overcome with the use of an appropriate mass-length relationship as reference. A previous study (Hailey, 2000) examined factors (1) and (3) of Jacobson *et al.* (1993), showing sexual and seasonal variation of body mass in *T. hermanni* at Alyki, a coastal site in northern Greece. There is also some information on factor (2) in this species; the presence of shelled eggs had no significant effect on body mass condition (Hailey & Loumbourdis, 1990), presumably because eggs reduce the space available in the abdomen and thus the volume and mass of gut contents (Meienberger, Wallis & Nagy, 1993). The carapace apparently limits relative clutch mass (mass of eggs/mass of body without eggs) in tortoises to about 5-10% (Hailey & Loumbourdis, 1988), in contrast to lizards where mean values greater than 50% are found in several species (Vitt & Congdon, 1978).

Testudo hermanni shows wide variation in adult body size in Greece (Willemsen & Hailey, 1999a) and it is unclear whether the empirical mass-length equations from Alyki are applicable to *T. hermanni* generally; for example, tortoises described by Meek (1985) were apparently much heavier at the same length. The first aim of this paper was to test the use of equations derived from tortoises at Alyki to calculate body mass condition in other populations of *T. hermanni*, and to examine the patterns of seasonal variation in different regions. The second aim was to derive mass-length equations for the other two species of tortoise in Greece, *T. graeca* and *T. marginata*, and then examine the seasonal variation of their body mass condition. These species differ in shape from *T. hermanni*, especially *T. marginata* which is much narrower (Bringsøe, Buskirk & Willemsen, 2001), and are thus likely to require different reference equations. They also occupy contrasting habitats and are active with different body temperatures (Wright, Steer & Hailey, 1988; Willemsen, 1991), and are thus likely to be affected by season in different ways.

METHODS

Tortoises were found by walking through the habitat and were measured in the field (Stubbs *et al.*, 1984) and released immediately afterwards at the point of capture. Straight carapace length was measured to the nearest 1 mm; this is the horizontal straight distance between the front and rear of the carapace with the plastron flat on the substrate, as shown by Stubbs *et al.* (1984), McArthur (1996) and Bonnet *et al.* (2001). The mass of most tortoises was measured to the nearest 5 g with 2 kg or 3 kg Soehnle spring balances. Small individuals were measured to 1 g with a 250 g Soehnle spring balance. Sex was determined by plastral concavity and larger tails in males; only animals larger than 10 cm carapace length are considered here. *Testudo hermanni* may be sexed from 10 cm; the size at maturity varied substantially among sites (Willemsen & Hailey, 1999a) so the data are grouped into males and females here, rather than subadults, adult males and adult females as used previously (Hailey, 2000). Sex could not usually be estimated from external appearance in *T. graeca* <13 cm or *T. marginata* <17 cm. In these species one category was identifiable males, and all other tortoises larger than 10 cm formed the other category (females + subadults). The shape of subadult tortoises is generally similar to that of females (Stubbs *et al.*, 1984), and these could not be distinguished in *T. graeca* and *T. marginata* except by size. Each individual was permanently marked with a unique code by notching the marginal scutes with a file.

Populations of *T. hermanni* were grouped to obtain sufficient data for analysis of regional trends; maps of the locations and descriptions of the habitats of these sites have been given previously (Willemsen & Hailey, 1989, 1999a,b). Three regions were examined here: (1) the south, including low altitude sites in the Peloponnese (with mean mass of adult males at each site from Willemsen & Hailey, 1999a); Kalamata (0.47 kg), Sparta (0.51 kg) and Olympia (0.61 kg); (2) Meteora, a mid-latitude and mid-altitude site in central Greece with intermediate-sized tortoises (0.70 kg), where many individuals have been marked; (3) the north, including Deskati (1.36 kg), Kastoria (1.13 kg), Agios Dimitrios (0.90 kg), Mikri Volvi (0.89 kg) and Litochoron (0.76 kg). Data for *T. graeca* and *T. marginata* were from all sites where they were observed (Willemsen & Hailey, 1989: Table 2), excluding the single *T. graeca* at Olympia that was probably an introduction. Rainfall data were from meteorological stations at Sparta for the south, Kalabaka near Meteora, and Ptolemaïdos for the north (Willemsen & Hailey, 1999a: Fig. 2). Rainfall data for Alyki were from Trikala, near Eginion (this is a

different place from Trikkala, in central Greece, described previously by Willemsen & Hailey, 1999a).

Body mass condition was calculated from the body mass (M) of a tortoise compared to that predicted (M') from the relationship between mass and length (L) (after Le Cren, 1951). The mass-length relations which were used to calculate M' included each individual tortoise only once. These allometric equations were of the form $\log M' = \log a + b \log L$, which corresponds to $M'=aL^b$ in exponential form. The simpler condition factor K calculated using $b=3$ is unsuitable where shape or density changes with size, when the allometric equation is preferable (Le Cren, 1951). Mass-length relationships of different species or sexes were compared using analysis of covariance (ANCOVA) with $\log M$ as the dependent variable, species or sex as a fixed factor, and $\log L$ as covariate.

A previous study (Hailey, 2000) found that $\log (M/M')$ was the best the condition index (CI) based on body mass; this is equal to residuals from the regression of $\log M$ on $\log L$. Log (M/M') is normally distributed, and allows analysis of interaction effects in analysis of variance (ANOVA). Values of $\log (M/M')$ were calculated with SPSS; the CI was also converted to a relative mass (M/M') for ease of interpretation, often expressed as a percentage. A tortoise with observed mass equal to predicted mass thus had $M/M'=1.0$ or 100%, and CI=0. Sexes or species were also compared using a relative mass expressed as a percentage; the ratio of their predicted (M'_a/M'_b) masses where a and b are the two groups. In this case the relative mass depends on morphological differences, not on condition as such, and should not be converted into a CI as noted in the introduction. Seasonal patterns of the CI were compared with two-way or three-way ANOVA with month and sex, region or species. These analyses used only one value of CI for each individual tortoise in each month (not replicated measures within the same month). The resulting F values are shown with main effects and residual degrees of freedom. Frequency histograms of CI values used these monthly data (Hailey, 2000).

RESULTS

TESTUDO HERMANNI

Observations of *T. hermanni* were made between April and October, although there was low activity in the north in September and October and no mass measurements were made then. Initial analyses used mass-length equations for male and female *T. hermanni* from Alyki in July (Hailey, 2000: Table 2) to calculate M' and the CI. The mean relative mass in each of the three regions was 102-103% (Fig. 1), only 1-2% different from values at Alyki calculated in the same way (i.e. using data from April to October with the July reference equations, giving a mean of 101%). The south and north had similar relative mass, and were only 1% different from that at Meteora. There was thus no evidence of major differences in mass-length relationships in *T. hermanni* among regions, despite the large difference of
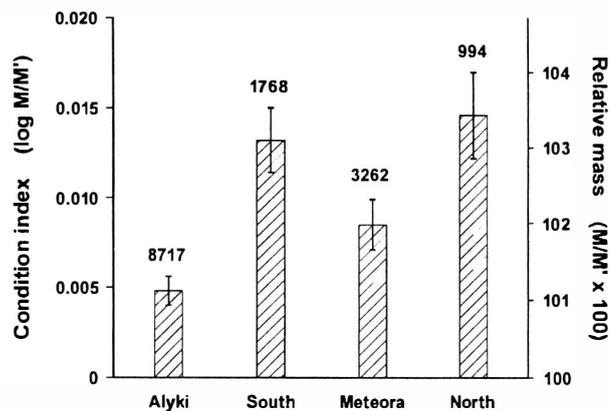

FIG. 1. Mean condition index of *T. hermanni* measured from April to October in different areas, using M' calculated from the mass-length equations for males and females from Alyki in July. The right y axis shows the CI converted to relative mass (%). Vertical lines show 95% confidence intervals, and numbers above bars show sample sizes.

mean adult size from south to north in Greece. The similarity of the CI values among sites is indeed remarkable given the independent observers and different equipment. A 1% difference in relative mass is similar to the level of precision of the field measurements; mass was recorded to the nearest 5-10 g for a 0.5-1 kg tortoise.

Three-way ANOVA of CI with month, sex and region used data from April to August only, because September and October data were not available for the north. There was a significant interaction of month × sex ($F_{4,5511}$=6.93, $P<0.001$) showing different seasonal patterns of males and females, and of month × region ($F_{8,5511}$=3.33, $P=0.001$) showing different seasonal pat-
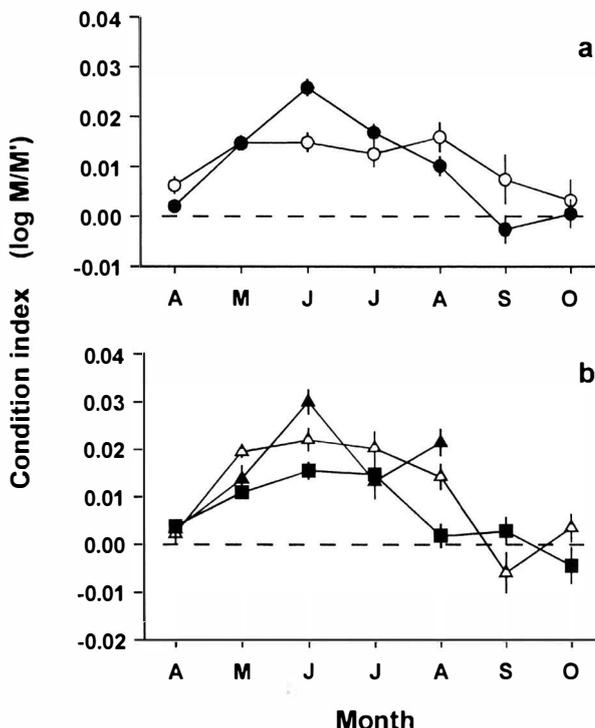

FIG. 2. The seasonal variation of condition index in *T. hermanni*. (a) Sexual differences; females (open circles), males (filled circles). (b) Regional differences; the south (open triangles), the north (filled triangles), Meteora (filled squares). Bars show ± SE.

TABLE 1. Mass-length regression equations for *Testudo* species in Greece, and example predicted masses for comparison. Values are shown ± SE; log *a* and *b* are the intercept and slope, respectively of the regression of log *M* (g) on log *L* (mm); *n* is the number of individuals; and *r²* is shown as a %. Separate equations are given for females (f) and males (m) >10 cm; for *T. graeca* and *T. marginata* the categories are females and subadults (f+s, which cannot be distinguished by morphology in these species), and males.

Species	*log a*	*b*	*n*	*r²*	Predicted mass at *L*		
					100	150	200
T. hermanni (f)	-3.188±0.030	2.774±0.014	1156	97.2	229	705	1567
T. hermanni (m)	-3.180±0.030	2.760±0.014	1948	95.3	219	670	1482
T. graeca (f+s)	-3.307±0.052	2.846±0.024	276	98.1	243	769	1745
T. graeca (m)	-2.549±0.110	2.498±0.049	239	91.5	-	771	1581
T. marginata (f+s)	-2.724±0.119	2.531±0.051	101	96.0	218	608	1259
T. marginata (m)	-2.476±0.160	2.420±0.068	116	91.7	-	617	1237

terns in the three regions. There was no significant interaction of month × sex × region ($F_{8,5511}=1.86$, $P=0.061$), showing that the difference between the sexes followed a similar seasonal pattern in all regions. The seasonal patterns of males and females are shown in Fig. 2a. The CI of males increased from April to reach a peak in June, then declined to a minimum in September. The CI of females showed less seasonal variation, with high values

from May to August. The relative condition of females ($CI_{females} - CI_{males}$) was lowest in June, and highest in September.

The seasonal patterns in the three regions (excluding Alyki) are shown in Fig. 2b. Although these patterns were significantly different, they showed the same general trends, with initially low CI in April, high values in spring and summer, and a decline in autumn (where data were available). There were no data for March when the CI was lowest at Alyki following emergence from hibernation (Hailey, 2000). There was no clear geographic trend of the seasonal pattern, apart from a tendency for the peak of the CI to become narrower and to occur later in the year from south to north. There was thus a plateau lasting from May to August in the south, a peak from May or June to July at Meteora, and a sharp peak in June, and possibly August, in the north.

TESTUDO GRAECA

There were fewer data available for *T. graeca* (or *T. marginata*) than for *T. hermanni*, and so the reference mass-length equations were derived using data from all months combined (but each individual only once). These equations are shown in Table 1, together with comparable equations for *T. hermanni* calculated in the same way (i.e. using the three regions, months combined but each individual included only once). ANCOVA of log *M* by sex with log *L* as covariate showed that the mass-length relationship differed significantly between the sexes in *T. graeca* ($F_{1,512}=9.72$, $P<0.001$) and in *T. hermanni* ($F_{1,3101}=232.9$, $P<0.001$). *Testudo graeca* were heavier than *T. hermanni* of the same length, in both females and subadults (Fig. 3a) and males. ANCOVA showed that the differences in mass-length relationships between these species were significant in both females and subadults ($F_{1,1429}=236.7$, $P<0.001$) and males ($F_{1,1392}=346.0$, $P<0.001$).

Seasonal variation of the CI in *T. graeca* is shown in Fig. 3b, compared to that for *T. hermanni* (the three regions combined). Two-way ANOVA of CI with month and species had a significant month x species interaction ($F_{6,6691}=14.37$, $P<0.001$) showing that the seasonal pattern of CI differed between these species. The major difference was more pronounced seasonal
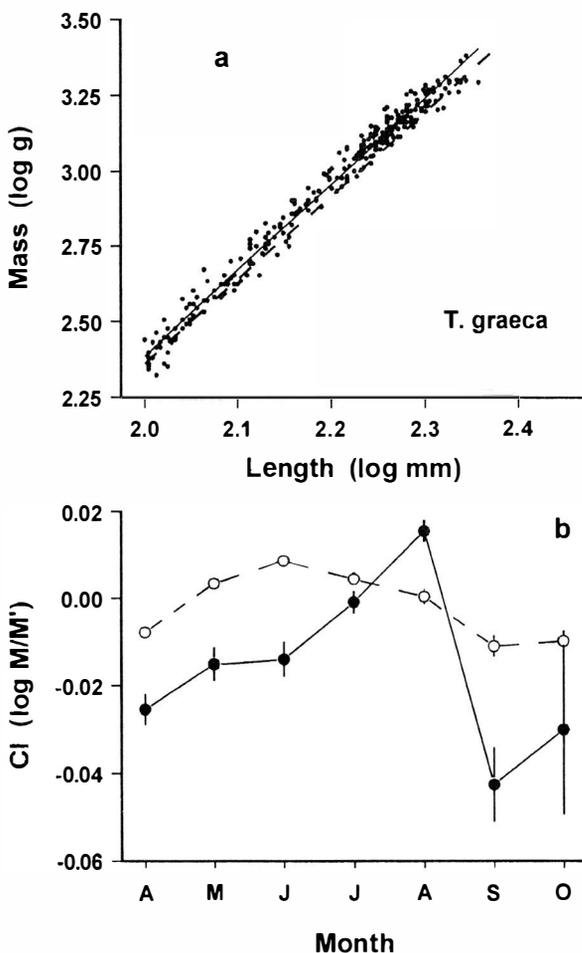
FIG. 3. (a) The relationship between mass and length in female and subadult *T. graeca*. The regression equation is given in Table 1; the dashed line shows the corresponding relationship for *T. hermanni*. (b) Seasonal variation of condition index in *T. graeca* (solid circles) and *T. hermanni* (open circles, all three regions pooled). Bars show ± SE.
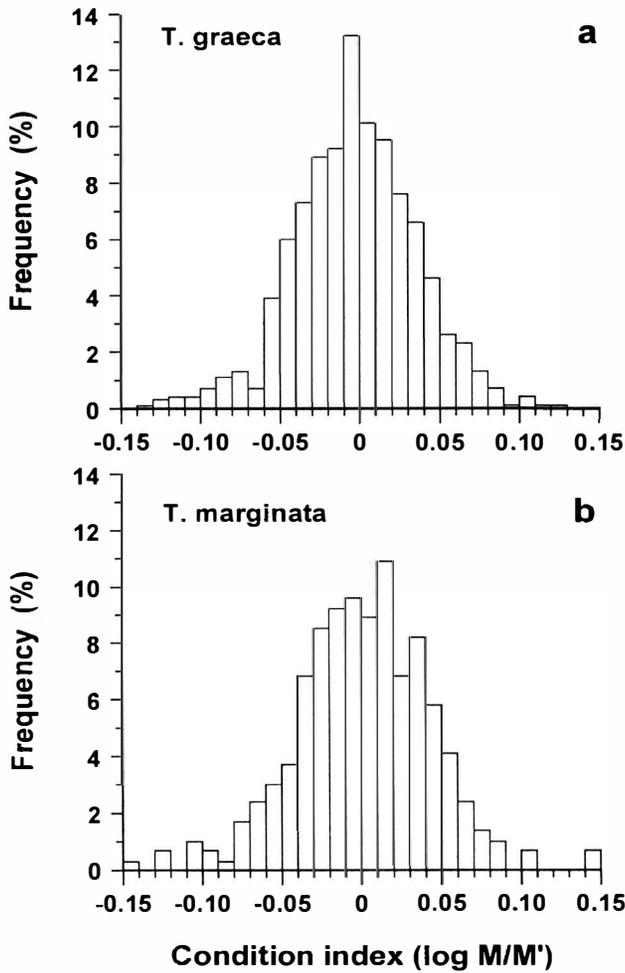
FIG. 4. Frequency distributions of condition index in (a) *T. graeca* and (b) *T. marginata* in the wild.

variation in *T. graeca*, the peak of the CI being sharper and occurring later in the year (in August, compared to June in *T. hermanni*). The standard deviation of CI in *T. graeca* was 0.039 (n=695), compared to 0.038 (n=6024) for *T. hermanni* (the three regions combined); the variability of CI was thus similar in the two species. The frequency distribution of CI values in *T. graeca* is shown in Fig 4a; most values were between -0.1 and +0.1.

TESTUDO MARGINATA

Sexual dimorphism of the mass-length relationship was only marginally significant in *T. marginata*, which is probably partly due to the small sample size for this species. The ANCOVA of log M with log L as covariate was not significant ($F_{1,214}$=2.64, P=0.106), but that of log L with log M as covariate was significant ($F_{1,214}$=7.19, P=0.008). The latter is perhaps better when comparing animals of different shape, which will be equivalent at the same body mass rather than the same length. Analyses of mass on length are generally used here, however, because the CI requires prediction of mass and this should thus be the dependent variable. *Testudo marginata* had lower mass than *T. hermanni* of the same length, in both females and subadults (Fig. 5a)
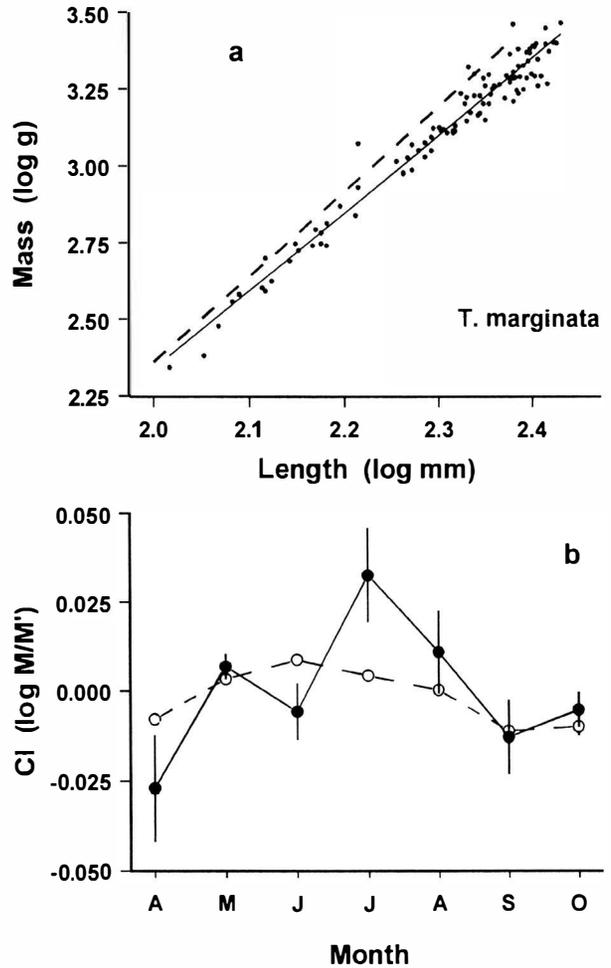

FIG. 5. (a) The relationship between mass and length in female and subadult *T. marginata*. The regression equation is given in Table 1; the dashed line shows the corresponding relationship for *T. hermanni*. (b) Seasonal variation of condition index in *T. marginata* (solid circles) and *T. hermanni* (open circles). Bars show ± SE.

and males. ANCOVA showed that the differences in mass-length relationships between these species were significant in both females and subadults ($F_{1,1254}$=445.9, P<0.001) and males ($F_{1,2061}$=454.1, P<0.001).

The mass-length relationships (Table 1) are as expected from the morphology of the three species. Quantitative comparisons are shown most clearly by predicted mass at three representative lengths. Subadults of the three species differed rather little at 100 mm, with a range of predicted mass of only 10% from about 220-240 g. Juvenile and subadult *Testudo* of all species generally have a similar shape (Bringsøe *et al.*, 2001) which only diverges with growth. Female *T. hermanni* were about 5% heavier than males of the same length, as reported previously (Hailey, 2000). The other two species may only be sexed from external morphology at larger sizes. Male *T. graeca* had similar mass to females at 150 mm but became progressively lighter than females, with a lower value of *b*. The narrow *T. marginata* diverges most from the standard tortoise shape (Bringsøe *et al.*, 2001), with the lowest values of *b*, but also showed low sexual dimorphism. In contrast
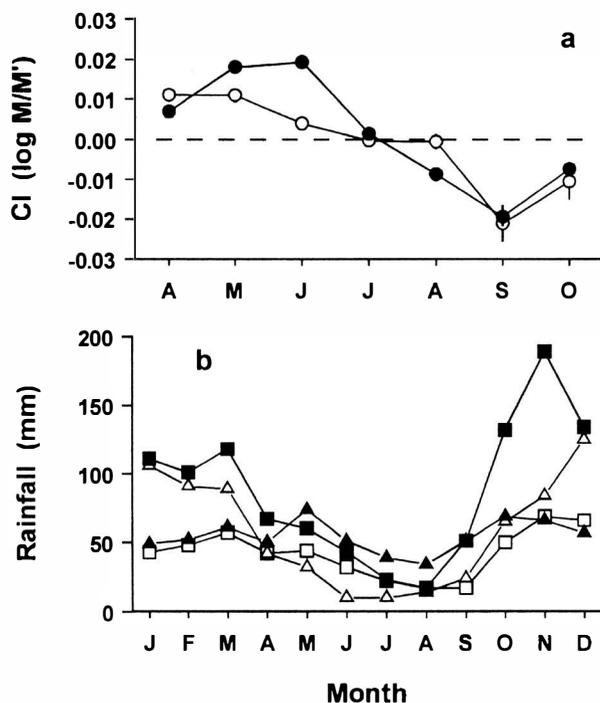
FIG. 6. (a) Seasonal variation of condition index of *T. hermanni* at Alyki; females (open circles), males (filled circles), bars show ± SE. (b) Seasonal variation of rainfall in different areas; the south (open triangles), the north (filled triangles), Meteora (filled squares), Alyki (open squares).

to their similarity at 100 mm, predicted mass of the three species differed substantially at 200 mm (Table 1). The relative mass using M' showed *T. graeca* to be 9% heavier than *T. hermanni* (average of females and males), and *T. hermanni* 22% heavier than *T. marginata*, at 200 mm.

The seasonal pattern of CI in *T. marginata* is compared with that of *T. hermanni* in Fig. 5b. Two-way ANOVA of CI with month and species showed a significant month × species interaction ($F_{6,6303}=2.48$, $P=0.021$), with *T. marginata* having more pronounced seasonal variation and a sharper and later (in July) peak of CI. The seasonal pattern of CI was thus similar in *T. marginata* and *T. graeca*, and two-way ANOVA of CI with month and species showed no significant month × species interaction between these two species

($F_{6,960}=1.79$, $P=0.099$). The standard deviation of CI in *T. marginata* was 0.046 ($n=293$); the CI was thus slightly more variable than in *T. graeca* or *T. hermanni* but most values were still between -0.1 and +0.1 (Fig. 4b).

DISCUSSION

REGIONAL VARIATION

The first question in *T. hermanni* was whether a single mass-length relationship could be used for populations from different regions with substantially different adult body size. Differences in relative mass among regions were only in the order of 1-2%. Mass-length relationships are thus very consistent provided that length is measured in a standard way. Other straight length measurements are possible, such as along the midline (shorter than the straight carapace length) or inclined downwards (longer than a horizontal measurement). These differ by up to about 5% in an individual *T. hermanni*, but would lead to greater differences in the CI; a 5% difference in L would lead to a 12-15% difference in M' (with the range of b in Table 1) and thus change the CI by 0.05-0.06. Differences in measuring L can thus affect the CI as much as disease (Willemsen *et al.*, 2002) or seasonal variation.

Body mass condition could reflect the level of hydration of a tortoise, the fullness of its gut, or the composition of body tissues, particularly changes in the mass of fat and the shell. The relative density (compared to water =1.0) is about 0.90 for body fat, 1.10 for fat-free body tissues, and 3.0 for bone mineral (Blaxter, 1989). The volume of a tortoise is relatively constant, determined by the shell, with little room for expansion around the limb openings. Increased fat content may therefore be at the expense of other tissues of higher relative density, which would reduce the CI, unlike most animals in which increased fat content is associated with higher body mass. The Quetelet index in man for example, of mass : height², is directly related to fat content (Blaxter, 1989). Increased bone mineral content would increase the CI. The fat content of chelonians is, however, rather constant (Brisbin, 1972) and there is little information on seasonal mineral cycles. Most seasonal variation of the CI observed here is therefore attributed

TABLE 2. Seasonal mass-length regression equations for *T. hermanni* >10 cm at inland sites in Greece. Values are shown ± SE; log a and b are the intercept and slope, respectively of the regression of log M (g) on log L (mm), n is the number of individuals, and r^2 is shown as a %.

	Females				Males			
	log a	b	n	r^2	log a	b	n	r^2
April	-3.106±0.050	2.734±0.023	565	96.3	-3.056±0.042	2.699±0.019	1180	94.2
May	-3.132±0.044	2.750±0.020	730	96.2	-3.042±0.044	2.698±0.021	1072	94.1
June	-3.293±0.053	2.822±0.024	395	97.3	-3.330±0.065	2.836±0.030	441	95.4
July	-3.154±0.075	2.758±0.034	206	97.0	-2.881±0.085	2.626±0.039	446	91.1
August	-3.253±0.086	2.805±0.039	121	97.8	-3.219±0.084	2.778±0.039	385	93.1
September	-3.148±0.155	2.753±0.071	55	96.5	-3.288±0.140	2.804±0.064	167	91.9
October	-3.117±0.139	2.737±0.063	67	96.6	-3.110±0.134	2.723±0.062	194	90.9

to the level of body hydration and the fullness of the gut - and these two factors will be correlated where most water comes from the food. Gilles-Baillien & Schoffeniels (1965) found that the osmotic pressure of the blood of *T. hermanni* reached a minimum in June and July, and was maximal at the end of hibernation. This is opposite to the pattern of body mass condition, which suggests that at least part of the variation of the CI was due to changes in hydration state (high body water content causing low osmotic pressure and *vice versa*).

There were significant differences in the seasonal pattern of CI among the three regions that could be due to two sets of variables; habitat and food availability, or activity and thermoregulation. Rainfall is highest in winter in all parts of Greece (Fig. 6), so spring vegetation is lush and food availability is high in all regions and habitats. The delayed peak of CI further north is thus likely to be due to differences in activity/thermoregulation rather than habitat/food availability. In particular, thermoregulation by basking in spring becomes increasingly important in *T. hermanni* from south to north in Greece (Willemsen & Hailey, 1999*b*). Time spent basking may limit early feeding activity in the north and delay the timing of maximum CI there.

One reason for the slightly lower annual mean CI at Alyki (Fig. 1) is the different seasonal cycle, shown in Fig. 6a in the same form as for the three regions described here. The CI at Alyki was similar to that in the other three areas from April to June, ranging from 0 to 0.02. The CI decreased after June at Alyki, but remained high until July or August in other areas. The different pattern at Alyki is unlikely to be due to activity/thermoregulation (which was similar to the mid-latitude site at Meteora) or climate (since all areas had lowest rainfall from June to September; Fig. 6b). The difference is more likely to be due to habitat/food availability. Alyki was a relatively xeric habitat with sandy soil and no surface water after spring, so food availability declined in summer in most parts of the site. Although the same mass-length equations may be used to calculate a CI for *T. hermanni* from different regions, those from Alyki are not the best for detailed studies because of this atypical seasonal pattern. Monthly regression equations for the pooled data for the south, Meteora and north are provided in Table 2 to allow calculation of a seasonally adjusted condition index (CI_s) for comparisons among sites or years sampled in different months. These equations have been found to be more suitable for measuring the body mass condition of *T. hermanni* at other sites (Willemsen & Hailey, 2001).

Figure 6a also shows that the seasonal pattern of the difference between the sexes at Alyki was similar to that in other areas; the CI of females relative to males was lowest in June during the nesting season. The relatively low CI of females in June might simply be due to recent oviposition, but could also reflect different activity of the sexes. Females move further (Hailey, 1989; Longepierre, Hailey & Grenot, 2001) and are sighted more frequently (Hailey & Willemsen, 2000) than males

in June, and this activity may occur at the expense of feeding. Food consumption of females is in any case likely to be low in the nesting season as eggs reduce the volume available for gut contents (Meienberger *et al.*, 1993), although this will not affect the CI unless the densities of eggs and gut contents differ substantially.

INTERSPECIFIC COMPARISONS

The mass-length relationships of the species were as expected from their shapes (Bringsøe *et al.*, 2001), with *T. marginata* being the lightest at a given length. The equations in Table 1 may be used to calculate the CI of captive European tortoises (Willemsen *et al.*, 2002). That paper describes the slight difference (of 3% relative mass) in mass-length relationships between the two subspecies of *T. hermanni*. Lambert (1982: Fig. 2) showed that there were no obvious differences between mass-length relationships of *T. graeca* from North Africa (*T. g. graeca*) and the Eastern Mediterranean (*T. g. ibera*). There are no subspecies of *T. marginata*, although the dwarf species described by Bour (1996) in the Peloponnese could be regarded as such (Bringsøe *et al.*, 2001).

Meek (1985: Fig. 6) also examined the mass-length relationships of these species (sexes combined) and reported that *T. hermanni* from Yugoslavia were substantially heavier than *T. graeca* or *T. marginata*. The equations for *T. graeca* (from Lambert, 1982) and *T. marginata* (from a reanalysis of data from Hine, 1982) give similar body masses to those found here; for example, 1501 g and 1257 g, respectively, at 200 mm, whereas the equation for *T. hermanni* gives a substantially higher body mass of 2390 g at 200 mm. The latter is 57% higher than the mass of 1525 g predicted from the average of males and females from Greece in this study (Table 1). In contrast, populations of different body size in Greece, and wild tortoises from Italy and France (Willemsen *et al.*, 2002), differed in relative mass by only up to 3%, and the seasonal peak of CI was only about 0.03 in any region or species. The discrepancy between the length-specific mass of *T. hermanni* in Yugoslavia and elsewhere cannot now be resolved (R. Meek, personal communication), but it is potentially important.

The annual cycle of CI in *T. graeca* and *T. marginata* differed from that in *T. hermanni*, with peaks occurring in summer rather than in spring. This pattern corresponds well with the activity of *T. graeca* at Alyki, the only site in Greece where this species has been studied throughout the year. *Testudo graeca* emerged later from hibernation, in April compared to March in *T. hermanni*, and occupied open coastal vegetation that had relatively little food in early spring. Food availability was greater in coastal vegetation than other habitats in summer, when some *T. hermanni* moved into coastal areas (Wright *et al.*, 1988). *Testudo graeca* also had higher body temperatures (Wright *et al.*, 1988) and larger body size (Hailey & Loumbourdis, 1988) and

thus greater thermal inertia than *T. hermanni*. Both of these factors would increase the need to bask in spring (Lambert, 1981) and limit the time available for other activities. The low CI of *T. graeca* in spring in Greece may be explained by both activity and food availability being low, but increasing in summer to give a late peak of CI. This species usually does not aestivate in Greece (although summer activity may be low in some years), unlike arid regions of Iran (Pritchard, 1966) or southern Spain (Diaz-Paniagua, Keller & Andreu, 1995).

The seasonal pattern of CI in *T. marginata* is less easy to understand. In many respects, *T. marginata* is similar to *T. graeca*, using more xeric habitats than *T. hermanni* and having higher body temperatures in the wild (Willemsen, 1991). Studies of wild tortoises suggest that thermoregulation of *T. marginata* is similar to that of *T. graeca*, so a summer peak of CI might be expected, owing to constraints of cool conditions in spring. Studies of *T. marginata* in captivity, however, show that this species is able to be active with low body temperatures in spring, in contrast to *T. graeca*. Panagiota & Valakos (1992) found that *T. marginata* in an outdoor enclosure in southern Greece did not seek refuges in winter but were inactive above ground, becoming active on warm days, while *T. hermanni* hibernated buried in soil. They found no significant differences between the body temperatures of the two species, but the range was greater in *T. marginata* (8.0-34.7 °C) than *T. hermanni* (14.7-33.3 °C). Captive *T. marginata* in Italy were also often active in early spring with low body temperatures (R. E. Willemsen, personal observations). The low CI of *T. marginata* in spring is therefore surprising in view of this capacity for low-temperature activity. Further study of the ecology of this interesting species in the wild is clearly required.

The presence of significant seasonal variation of the CI shows that body mass condition is of interest in ecological studies, especially as it is simple to measure with minimal disturbance to the tortoise. The CI may also be valuable for management of captive tortoises (Willemsen *et al.*, 2002). The six factors considered by Jacobson *et al.* (1993) to limit the usefulness of body mass condition may now be evaluated for the species studied here. Sexual differences in mass-length relationships (1) have been confirmed in all three species of *Testudo*, but only cause a problem if the CI is calculated from regressions pooling the sexes (Hailey, 2000: Fig. 3b). The use of separate regressions for males and females to calculate M', and thus the CI, compensates for these differences. Changes in body mass before and after oviposition (2) do not cause a difference between the CI of females with and without shelled eggs (Hailey & Loumbourdis, 1990) and thus seem to be minimal in *Testudo*, offset by changes in gut contents (Meienberger *et al.*, 1993). Seasonal changes in the CI (3) have been demonstrated, but are of small amplitude compared to the overall variation of the CI; seasonal variation had a maximum range of about 0.05 between the highest and lowest months. Seasonal variation may be removed

from the CI by using mass-length relationships for different months (Table 2) where sufficient data are available, or by simply comparing with known seasonal patterns.

Changes of mass after drinking (4) or voiding large amounts of faeces or urine (5) remain a problem. The possible effect of drinking is best evaluated by considering the availability of standing water sources or recent rainfall; this would in any case affect condition estimated from blood composition. The effects of voiding faeces or urine are best minimised by handling tortoises quickly and carefully and in a standard way; mass should be measured, for example, before any more disturbing handling such as marking the shell. Methods to obtain urine-free mass are available, but the precautions and delay involved largely negate the advantage of body mass condition as a practical measure. For example, treatment with pilocarpine (Dorando, 1979) successfully eliminated stored fluids but required 20 min to be effective, plus the need for sterile technique and the possibility of disturbance to subsequent behaviour. Short-term variation in body mass would be averaged out in a large sample; the best way to minimise this in an individual tortoise would be to use the mean mass measured over a few days.

Geographic variation in mass-length relationships (6) caused by differences in shell shape or dermal bone thickness did not affect the CI of *T. hermanni* in Greece, even though mean adult size varied substantially among populations. There was also little variation between subspecies of *T. graeca* (Lambert, 1982) or *T. hermanni* or between wild and healthy captive tortoises from different areas (Willemsen *et al.*, 2002). The overall mass-length relationships of male and female *T. graeca*, *T. hermanni* and *T. marginata* given in Table 1 are thus a good basis for measuring body mass condition. A facility for calculating CI for tortoises of these species of carapace length 100 mm or larger is provided at http://www.ahailey.f9.co.uk/cond.htm. Further accuracy may be achieved by using monthly mass-length relationships or by considering the seasonal pattern of CI. In conclusion, body mass condition is a useful variable for large samples of tortoises, from field studies or large captive collections. Results for individual tortoises should be used in conjunction with other indicators of health (Jacobson *et al.*, 1993).

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