THE HERPETOLOGICAL JOURNAL

ASSESSING BODY MASS CONDITION IN THE TORTOISE *TESTUDO HERMANNI*

ADRIAN HAILEY

Department of Zoology, Aristotelian University of Thessaloniki, GR-540 06 Thessaloniki, Greece

Condition in the tortoise *Testudo hermanni* was assessed using the ratio of observed mass (M) to that predicted (M') from the allometric relationship $M=aL^b$, where L is body length. A condition index (CI) was based on regressions of log M on log L in July (taken as a standard); these regressions differed significantly between females and males. The CI log M/M' was slightly left-skewed, but was preferred to the simple ratio M/M' for ease of analysis of interaction effects. Log M/M' ranged from approximately -0.1 to +0.1 in the wild, equivalent to observed mass of 80-120% of predicted mass. Condition varied seasonally, being low after emergence from hibernation, maximal in spring (April to June), and decreasing in autumn. The detailed pattern of seasonal variation differed significantly between adult females, adult males, and subadults. Regression equations for log M on log L in different months are provided for a seasonally-adjusted condition index (CI_s), to assess the relative condition of individuals and annual samples measured in different months.

Key words: allometry, condition index, season, *Testudo*, tortoise

INTRODUCTION

The ability to measure the condition of a tortoise is of interest to both field workers and those who keep chelonians in captivity. Physiological parameters such as lipid stores (Pond & Mattacks, 1984; Kwan, 1994) offer perhaps the best criteria, but are often impractical to measure in the field or involve distressing the animal. Considerable information on condition can, however, be obtained from body mass (M) in relation to length (L). Such body mass condition may be difficult to interpret as it may vary due to gut contents or the presence of eggs rather than with the condition of the soma (i.e. the body as such, excluding gut contents and eggs), but has the advantage of minimal disturbance to the animal. Body mass condition may also be calculated retrospectively from measurements collected routinely during field studies. The ratio M/L has been found to be useful for the examination of captive tortoises (Jackson, 1978, 1980), extreme low or high values indicating poor health. Nevertheless, this ratio changes with size, as pointed out by Meek (1982), who suggested the allometric equation $L=\alpha M^\beta$, conventionally used to describe the mean mass-length relationship. The inverse relationship is even more useful - fluctuation of mass is of interest for condition, so that mass should be the dependent variable; $M=a^b$.

The measurement of condition has been neglected in studies of reptiles but has a long history in fish ecology (Weatherley & Gill, 1987). The condition factor K is based on the assumption that body shape is constant with size; that is, where $b=3$. This holds approximately for salmonid fish, which were the early focus of interest for economic reasons, but is less appropriate for other animals. If b is not equal to 3, then K will change with size, with the same problems of interpretation as M/L.

Correspondence: A. Hailey, 52 Mascotts Close, London NW2 6NS, UK. *E-mail*: ah2@ahailey.force9.co.uk

Le Cren (1951) suggested the relative condition factor $K_b=M/M'$, where M' is the predicted mass, i.e. that calculated for a given length from the allometric equation. M' will be influenced by the condition of the individuals used to determine the baseline relationship, which in practice requires a large sample size for the allometric equation to be a valid reference.

The ratio M/M' (expressed as a percentage) has been used to measure condition in female *Testudo hermanni* in relation to reproduction (Hailey & Loumbourdis, 1990). An allometric relationship is linearized by plotting log M on log L, so that log M would be expected to be normally distributed around the regression line. In that case log M/M' should be a preferable condition index (CI), since this equals residuals from the regression (i.e. log M - log M'), giving a symmetrical distribution best suited for statistical analysis. This paper uses measurements of wild *T. hermanni* to find a suitable index of body mass condition for field studies, and to assess low-condition criteria for captive animals.

METHODS

Field data were from approximately 5650 individual *T. hermanni* marked at Alyki in northern Greece from 1980 to 1999. The site has been described by Stubbs, Swingland, Hailey & Pulford (1985). Tortoises were located by walking through the habitat, measured in the field (Stubbs, Hailey, Pulford & Tyler, 1984), and released immediately afterwards at the point of capture. Length was measured to the nearest 1 mm on a flat-bed scale. The measure is thus the horizontal straight distance between the front and rear of the carapace with the plastron flat on the substrate (not the maximum straight distance, which is given by tilting the animal forwards slightly). Mass was measured with a 100 g, 1 kg or 2.5 kg Pesola spring balance, depending on size. Sex was determined by plastral concavity and larger tails in males; only sexable animals (those larger than

10 cm carapace length) are considered here. Sexual maturity was based on body size criteria; males of 13 cm and females of 15 cm or more are considered to be adults (Hailey, 1990; Hailey & Loumbourdis, 1990). Each individual was permanently marked with a unique code by notching the marginal scutes with a hacksaw blade. Tortoises were also temporarily marked with a waterproof pen on a lightly-pigmented area.These marks served to avoid disturbance of frequently encountered individuals, which were not reweighed, so that most condition values were at intervals of a few months or more.

The allometric relationship $M=aL^b$ was found by linear regression of log-transformed values, giving log $M=a+b\log L$. To minimize seasonal variability, the reference equations (for females and for males) used measurements only from July (Hailey & Loumbourdis, 1990) since this is the time after females have laid eggs but before the dry months when activity (and thus possibly condition) varies substantially with rainfall (Hailey, 1989). Only the first mass measurement for each individual in July was used in the reference curves, and those for females and males were compared with analysis of covariance (ANCOVA). Statistical analysis used MINITAB and SPSS, and values of log M/M' and M/M' for other months were calculated using FORTRAN programs. Only the first condition value for each individual in each category (month x sex) was used in analysis of variance (ANOVA) of seasonal differences.

RESULTS

The mass-length relationships for females and males larger than 10 cm measured in July are shown in Fig. 1. Females were slightly (on average about 4%) heavier than males of the same length, and ANCOVA showed that mass varied significantly between the sexes after allowing for length as a covariate ($F_{1,1462}=104.9$, $P<0.001$). Expected mass M' and thus condition was therefore calculated separately for females and males. Two features of the mass-length relationships in Fig. 1 are particularly suitable for measurement of condition: there are no changes of slope, as occur in some reptiles when shape changes at maturity (e.g. Fig. 4 of Hailey & Davies, 1987), and the deviations around the regression line are rather constant throughout the size range, so that animals of different sizes may be compared directly.
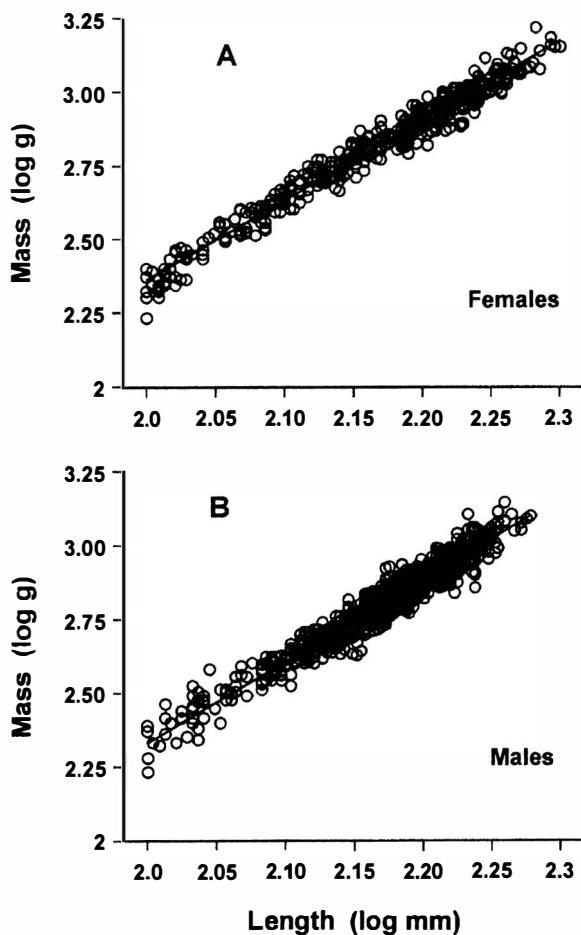

FIG. 1. The allometric relationship between mass and carapace length in *T. hermanni* larger than 100 mm, measured in July. Regression equations are given in Table 2.
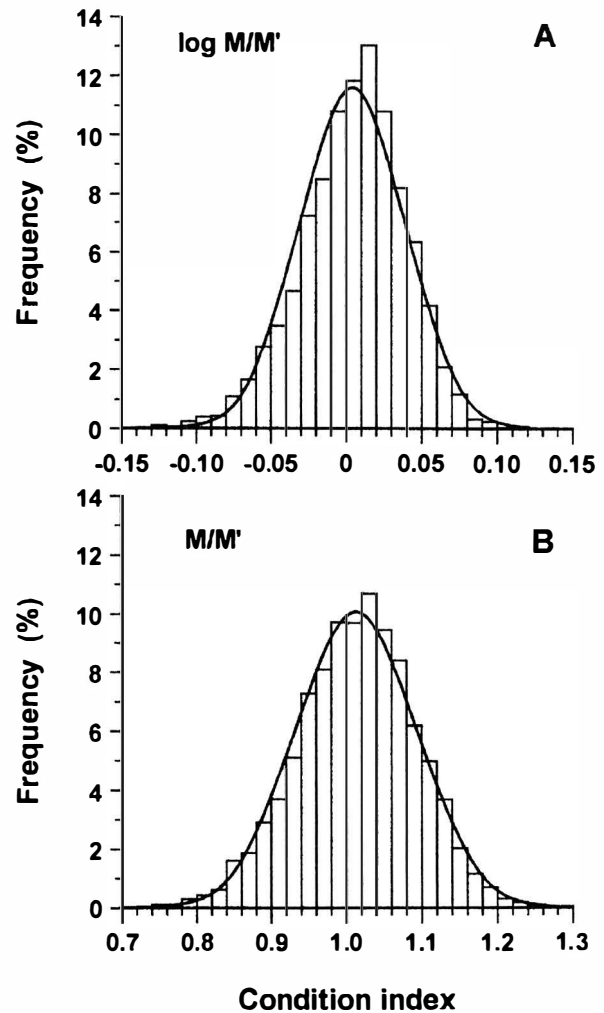

FIG. 2. Frequency distributions of condition index in male *T. hermanni*, measured throughout the year. (A) log M/M'; (B) M/M'. Fitted normal curves are also shown (parameters in Table 1).

TABLE 1. Parameters of condition in wild *T. hermanni* measured by log *M/M'* and *M/M'*. The SE of skewness is the same for both measures because this is based on the normal curve and calculated solely from sample size (P. Rothery, personal communication).

	log *M/M'*		*M/M'*	
	Females	Males	Females	Males
n	3686	7888	3686	7888
Mean	0.0048	0.0043	1.0140	1.0131
SD	0.0330	0.0346	0.0762	0.0798
Skewness	-0.376	-0.348	-0.092	-0.063
SE skewness	0.040	0.028	0.040	0.028

The condition index log *M/M'* was approximately normally distributed in both males (Fig. 2a) and females. There was a slight skewing to the left in both sexes, that is with the left tail of the distribution more drawn out and the peak shifted to the right, relative to the normal curves (Sokal & Rohlf, 1981). The degree of skewness was similar in both sexes (Table 1). The condition index *M/M'* was less skewed and fitted a normal

FIG. 3. Seasonal variation in condition index (log *M/M'*) of *T. hermanni* at Alyki. October and November combined; bars show ± SE. A, Comparison of adult females (F), adult males (M) and subadults (S). *n*=9113 measurements; no replication of individuals within groups. B, Comparison of adult females and males calculated from the pooled regression equation log *M*=-3.116+2.729 log *L* (*n*=1465, *r²*=95.0%); this spuriously shows females in better condition, as described in the Discussion.

curve even better in both males (Fig. 2b) and females (skewness in Table 1). Nevertheless, the advantage of the log ratio for analysis of interaction effects in ANOVA outweighs the potential disadvantage of the slight skewness (P. Rothery, personal communication), and log *M/M'* was therefore used as the CI here. Values of log *M/M'* fell within the approximate limits -0.1 to +0.1, equivalent to *M/M'* of 0.8 to 1.2 (Fig. 2), that is with observed mass within 20% of the predicted value. The variability of log *M/M'* was similar in both sexes, as shown by the standard deviations (Table 1).

Seasonal variation in the CI was examined in adults and subadults of both sexes using two-way ANOVA of the months March to October; a small number of captures in November were pooled with those from October. There was significant variation with month in all four groups (all *P*<0.001). The pattern of seasonal variation of CI was broadly similar in all groups (Fig. 3a), with low values following emergence from hibernation in March, highest values in spring (April to June), a decrease through summer to September and an increase before hibernation. Differences in the seasonal pattern of CI among groups were tested by the month x group interaction term of the ANOVA; there was significant variation among the four groups ($F_{21,9081}$=6.05, *P*<0.001). There was no significant difference between subadult females and subadult males ($F_{7,1798}$=1.07, *P*=0.382), which were therefore pooled. There were significant differences among ($F_{14,9089}$=8.56, *P*<0.001) and between the remaining three groups; adult females with subadults, $F_{7,3689}$=2.23, *P*=0.029; adult males with subadults, $F_{7,7206}$=8.34, *P*<0.001; adult males with adult females, $F_{7,7283}$=12.2, *P*<0.001.

DISCUSSION

CONDITION INDEX

Log *M/M'* was only slightly skewed, and is thus the preferred index of body mass condition. Other possible indices have shortcomings such as systematic variation with size (*M/L* or *K*), or difficulty of interpretation of interaction effects (*M/M'*). The difference *M-M'* has also been used successfully as a measure of body mass condition for *T. hermanni* (S. Longepierre & C. Grenot, personal communication). This CI is useful over small size ranges but makes comparisons between individu-
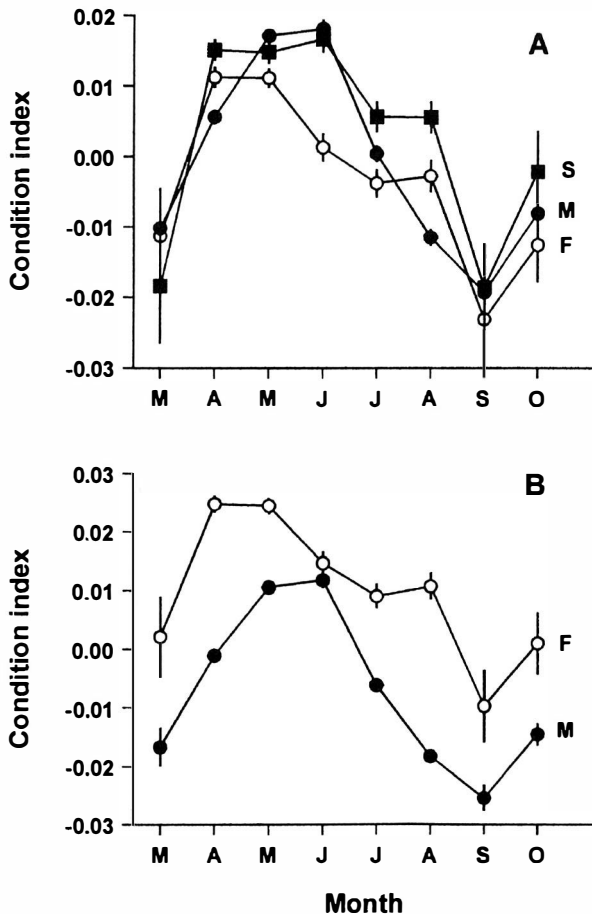
Table 2. Seasonal regression equations for *T. hermanni*. Values are shown ± SE; *a* and *b* are the intercept and slope, respectively of the regression of log *M* (g) on log *L* (mm), *n* is the number of individuals, and r^2 is the coefficient of variation (%).

	Females				Males			
	a	*b*	*n*	r^2	*a*	*b*	*n*	r^2
March	-3.420±0.177	2.868±0.081	30	97.8	-2.960±0.208	2.650±0.095	110	87.8
April	-3.091±0.039	2.729±0.018	748	96.8	-2.964±0.037	2.659±0.017	1415	94.6
May	-3.080±0.036	2.724±0.016	714	97.5	-3.138±0.037	2.744±0.017	1119	96.0
June	-2.951±0.050	2.662±0.023	385	97.2	-3.101±0.043	2.728±0.020	670	96.7
July	-3.056±0.048	2.708±0.022	468	96.9	-3.197±0.048	2.763±0.022	997	94.0
August	-3.000±0.062	2.682±0.028	379	95.9	-2.992±0.048	2.665±0.022	1004	93.5
September	-3.024±0.153	2.684±0.070	69	95.6	-3.322±0.123	2.811±0.056	280	89.9
October	-2.887±0.149	2.625±0.068	96	94.1	-3.151±0.098	2.739±0.045	372	91.0

als or populations of differing body sizes difficult, and *T. hermanni* shows a particularly large size range among populations (Willemsen & Hailey, 1999).

The seasonal variation of condition was broadly similar in subadults and adults of both sexes, with lowest condition after emergence from hibernation in March, and at the peak of the summer drought in September. Condition increased in October/November, presumably as preparation for hibernation; mass is known to decrease during hibernation in *Testudo* (Gilles-Baillien, 1974). Kirsche (1971) noted that mass of captive *T. hermanni* increased considerably immediately after hibernation, and that monthly mass increases were greatest in spring up to the end of June, the time of peak condition in the wild.

Variation among the seasonal patterns of CI in adult females, adult males, and subadults must be interpreted in relation to the activity of these groups, which will be considered in detail elsewhere. The most notable differences were the decrease in CI between May and June in adult females, and between July and August in adult males, which may be due to the end of the nesting season and a period of high courtship activity, respectively. It is notable that the nesting season (May and June) did not cause an increase in CI of adult females compared to April or to subadults and males. This pattern supports previous conclusions that the presence of eggs is not associated with increased mass in female tortoises, unlike the situation in other reptiles (for example Madsen & Shine, 1999). Females with and without shelled eggs cannot be separated on the basis of body mass during the nesting season (Hailey & Loumbourdis, 1990). Clutch size is probably limited by the volume of the carapace (Hailey & Loumbourdis, 1988), and the presence of shelled eggs may cause reduced food intake (Meienberger, Wallis & Nagy, 1993) so that the mass of eggs is offset by reduced gut contents.

Previous studies of condition in chelonians have pooled the sexes (for example Jackson, 1980). The regressions of log *M* on log *L* in July were similar in males and females, with slopes not significantly different (ANCOVA, $F_{1,1461}=3.10$, $P>0.05$), and the pooled regression could be used to calculate a rough measure of condition. It is worth using the separate regressions, however, because otherwise condition of females will appear to be higher than that of males (Fig. 3b). This result occurs because females are on average heavier than males of the same length, because of their carapace and plastron shape, and therefore appear to be in higher condition if this is calculated from a pooled regression equation. The variability of the CI was similar in the two sexes (Table 1) so that condition values may, however, be pooled for analysis after calculation from separate equations.

The lower threshold of log M/M' observed in the field was about -0.1, equivalent to M/M' of 0.8. This corresponds well with Jackson's threshold of "dangerously low" weight for length ratio, which was at about 80% of the mean (Fig. 1 in Jackson, 1985). Condition this low or lower was very rarely observed in the field (0.6% of observations), and it would seem advisable to seek veterinary attention for tortoises with such low condition unless this is due to known acute factors (such as dehydration) or unusual carapace shape (particularly narrow or flat individuals). Overweight captive tortoises, with unusually high condition values, should also be a cause of concern, because of the possible association with oedema, egg peritonitis or liver disease (Jackson, 1980; Lawrence, 1985).

SEASONALLY-ADJUSTED CONDITION

The condition index calculated from a common reference (July) varied through the year. There is little doubt that this reflects real differences in the absolute condition of the tortoises. Thus, those handled after emergence from hibernation in March, or at the height of the Mediterranean summer drought in September, are actually in poorer condition than those feeding on lush herbs in spring. This absolute CI is of limited usefulness in field studies, however, unless samples can always be made at the same time of year. Most tortoise populations occur at low population density so that sampling has to continue over long periods. A relative CI is therefore necessary to combine measurements made in different months; for example to compare year-to-year variation of condition with annual rainfall,

or to examine the correlation between condition and survival rate among individuals.

The mean CI values in Fig. 3a could be used for this purpose, by subtracting the mean for the appropriate month and group from each CI measurement. Nevertheless, the significantly different seasonal patterns in tortoises of different sizes (i.e. adults and subadults) show that the slopes of log M on log L vary among months. A better method of seasonal adjustment is therefore to calculate M' using separate regression equations for each month, rather than from the July reference. The equations in Table 2 can be used to calculate M' to give a seasonally-adjusted condition index (CI_s), to compare relative condition of tortoises independently of when they were handled. Use of separate regressions for each month will, however, obscure the seasonal change of body mass condition, for which the CI calculated from a single reference is necessary.

ACKNOWLEDGEMENTS

REFERENCES

Gilles-Baillien, M. (1974). Seasonal variations in reptiles. In *Chemical zoology, volume IX, amphibia and reptilia*: 353-376. Florkin, M. & Scheer, B. T. (Eds). New York: Academic Press.

Hailey, A. (1989). How far do animals move? Routine movements in a tortoise. *Canadian Journal of Zoology* 67, 208-215.

Hailey, A. (1990). Adult survival and recruitment and the explanation of an uneven sex ratio in a tortoise population. *Canadian Journal of Zoology* 68, 547-555.

Hailey, A. & Davies, P. M. C. (1987). Maturity, mating and age-specific reproductive effort of the snake *Natrix maura*. *Journal of Zoology, London* 211, 573-587.

Hailey, A. & Loumbourdis, N. S. (1988). Egg size and shape, clutch dynamics, and reproductive effort in European tortoises. *Canadian Journal of Zoology* 66, 1527-1536.

Hailey, A. & Loumbourdis, N. S. (1990). Population ecology and conservation of tortoises: demographic aspects of reproduction in *Testudo hermanni*. *Herpetological Journal* 1, 425-434.

Jackson, O. F. (1978). A method of assessing the health of European and North African Tortoises. *British Veterinary Zoological Society* 1978, 25-26.

Jackson, O. F. (1980). Weight and measurement data on tortoises (*Testudo graeca* and *Testudo hermanni*) and their relationship to health. *Journal of Small Animal Practice* 21, 409-416.

Jackson, O. F. (1985). The clinical examination of reptiles. In *Reptiles: breeding, behaviour and veterinary aspects*, 91-97. Townson, S. & Lawrence, K. (Eds). London: British Herpetological Society.

Kirsche, W. (1971). Metrische untersuchungen uber das wachstum der Griechischen landschildkrote (*Testudo hermanni hermanni* [L.]) in beziehung zum jahreszeitlichen rhythmus. *Der Zoologische Garten, Leipzig, Neue Folge* 40, 47-71.

Kwan, D. (1994). Fat reserves and reproduction in the green turtle, *Chelonia mydas*. *Wildlife Research* 21, 257-266.

Lawrence, K. (1985). An introduction to haematology and blood chemistry of the reptilia. In *Reptiles: breeding, behaviour and veterinary aspects*, 99-113. Townson, S. & Lawrence, K. (Eds). London: British Herpetological Society.

Le Cren, E. D. (1951). The length-weight relationship and seasonal cycle in gonad weight and condition in the perch (*Perca fluviatilis*). *Journal of Animal Ecology* 20, 201-219.

Madsen, T. & Shine, R. (1999). The adjustment of reproductive threshold to prey abundance in a capital breeder. *Journal of Animal Ecology* 68, 571-580.

Meek, R. (1982). Allometry in chelonians. *British Journal of Herpetology* 6, 198-199.

Meienberger, C., Wallis, I. R. & Nagy, K. A. (1993). Food intake rate and body mass influence transit time and digestibility in the desert tortoise (*Xerobates agassizii*). *Physiological Zoology* 66, 847-862.

Pond, C. M. & Mattacks, C. A. (1984). Anatomical organization of adipose tissue in chelonians. *British Journal of Herpetology* 6, 402-405.

Sokal, R. R. & Rohlf, F. J. (1981). *Biometry*. Second edition. San Francisco: W. H. Freeman.

Stubbs, D., Hailey, A., Pulford, E. & Tyler, W. (1984). Population ecology of European tortoises: review of field techniques. *Amphibia-Reptilia* 5, 57-68.

Stubbs, D., Swingland, I. R., Hailey, A. & Pulford, E. (1985). The ecology of the Mediterranean tortoise *Testudo hermanni* in northern Greece (The effects of a catastrophe on population structure and density). *Biological Conservation* 31, 125-152.

Weatherley, A. H. & Gill, H. S. (1987). *The biology of fish growth*. London: Academic Press.

Willemsen, R. E. & Hailey, A. (1999). Variation of adult body size of the tortoise *Testudo hermanni* in Greece: proximate and ultimate causes. *Journal of Zoology, London* 248, 379-396.