

Body size and seasonal condition of Caspian Whip Snakes, *Dolichophis caspius* (GMELIN, 1789), in southwestern Hungary (Squamata: Serpentes: Colubridae)

Körpergröße und saisonale Körperkondition der Kaspischen Pfeilnatter
Dolichophis caspius (GMELIN, 1789) in Südwestungarn
(Squamata: Serpentes: Colubridae)

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KURZFASSUNG

Die Ermittlung von Zusammenhängen zwischen phänotypischen Merkmalen und Fitness ist ein vorrangiges Ziel der Reproduktionsökologie. Da jahreszeitliche Veränderungen der Körperkondition von *Dolichophis caspius* (GMELIN, 1789) bisher nicht untersucht waren, analysierten die Autoren über einen Zeitraum von sechs Jahren Körpermaße der Kaspischen Pfeilnattern des Hügels Szársomlyó im südlichen Transdanubien, Südwestungarn. Die Körperkondition wurde mit Hilfe einer mathematischen Beziehung zwischen normalisierter Körpermasse und Kopf-Rumpflänge ("scaled mass index") quantifiziert, einem guten Indikator für Körperfettreserven.

Der größte Unterschied zwischen den Geschlechtern zeigte sich während der Paarungszeit, in welcher der Körperkonditionsindex ("scaled mass index", BCI) der Weibchen ($BCI = 384.4 \pm 25.72$ SE) wesentlich größer war als bei den männlichen Schlangen ($BCI = 324.0 \pm 19.26$ SE). Dieser Unterschied bestand zur Zeit der Einwinterung nicht mehr (Weibchen: $BCI = 397.4 \pm 16.96$ SE; Männchen: $BCI = 403.4 \pm 46.31$ SE). Die erhobenen Daten tragen zu einem besseren Verständnis der Ökologie der Kaspischen Pfeilnatter in der Nähe ihrer nordwestlichen Verbreitungsgrenze bei.

ABSTRACT

Identifying links between phenotypic attributes and fitness is a primary goal of reproductive ecology. Since seasonal patterns of body condition in Caspian Whip Snakes, *Dolichophis caspius* (GMELIN, 1789), have not been studied before, the authors analyzed body size measurements of those collected during a six year period on Szársomlyó Hill in southern Transdanubia, southwestern part of Hungary. To quantify the body condition, the "scaled mass index" was used. This measure, which correlates normalized values of body mass and snout-vent-length, is a good indicator of fatbody reserves.

The largest difference between sexes occurred during the mating period when the female body condition expressed as "scaled mass index" was substantially higher ($BCI = 384.4 \pm 25.72$ SE) than that of males ($BCI = 324.0 \pm 19.26$ SE). This difference largely declined until the onset of hibernation, when the body condition index of females ($BCI = 397.4 \pm 16.96$ SE) was similar to that of males ($BCI = 403.4 \pm 46.31$ SE). These findings contribute to better understand the ecology of the Caspian Whip Snake near the northwestern limit of its range.

KEY WORDS

Reptilia: Squamata: Serpentes: Colubridae; *Dolichophis caspius*, reproductive ecology, physiology, phenology, body size, seasonal variation of body condition, Szársomlyó Hill, Hungary

INTRODUCTION

An important aspect of physiological ecology is to understand the relationship between phenotypic and life-history traits. The body size of an organism is its most obvious characteristic, and has enormous implications. Body size plays a central role in a variety of life-history traits of snakes

(SHINE et al. 1998; BONNET et al. 2000; COATES et al. 2009). In snakes, snout-vent length is commonly used as a measure of body size (SHINE et al. 1998; GREEN 2001; FELDMAN & MEIRI 2013), as it is less affected by season, reproductive status and diet, and thus a more reliable measure of size

than body mass (BONNET et al. 2000; BROWN et al. 2002).

The body condition index has been related to reproductive potential and, consequently, fitness of the animal (NAULLEAU & BONNET 1996; BONNET et al. 2000; SCHULTE-HOSTEDDE et al. 2005; WAYE & MASON 2008; COATES et al. 2009). Generally, the body condition is determined by the relation of body mass to linear measures of body size, in reptiles usually snout-vent length. Condition indices are used to quantify individual health or well-being. A number of methods have been used to calculate body condition in animals (WEATHERHEAD & BROWN 1996; SCHULTE-HOSTEDDE et al. 2005; STEVENSON & WOODS 2006; PEIG & GREEN 2009), and there is much controversy as to which method is the most suitable (GREEN 2001; SCHULTE-HOSTEDDE et al. 2005; STEVENSON & WOODS 2006; WAYE & MASON 2008; PEIG & GREEN 2009). In snakes, body condition is commonly estimated from the residuals of the ordinary least squares (OLS) regression of mass on snout-vent length (BROWN et al. 2002; STEVENSON & WOODS 2006; WAYE & MASON 2008). The “scaled mass index” is based on the reduced major axis (RMA) method and allows errors in both variables which are not affected by scale transformations (PEIG & GREEN 2009). This index is independent of growth, sex and age of the animal. It standardizes all individuals to the same body size, and has been shown to be a better indicator of fat and protein reserves than other methods (PEIG & GREEN 2009).

The Caspian Whip Snake, *Dolichophis caspius* (GMELIN, 1789), is a large-sized colubrid species, distributed in the Balkans and adjacent west Asia. Hungarian individuals are the northernmost specimens in the western portion of the species' range, isolated from their nearest neighbors in southeastern Europe (TÓTH 2002; PUKY et al. 2005; BELLAAGH et al. 2007; NAGY et al. 2010) and restricted to small habitat patches isolated from each other (DELY 1997; TÓTH 2002; PUKY et al. 2005).

The species received some protection in Hungary since 1974, and is strictly protected since 1996 (BELLAAGH & BAKÓ 2004; PUKY et al. 2005). It is one of Hungary's rarest and most endangered snake species with the largest and only stable population in Hungary inhabiting the Szársomlyó Hill (TÓTH 2002; PUKY et al. 2005; FRANK 2011). Unfortunately, also this population shows signs of decline (MAJER 2000; BELLAAGH et al. 2007; FRANK et al. 2012), probably due to vegetation changes and anthropogenic disturbance and habitat alterations (MAJER 2000; BELLAAGH et al. 2007; ERDŐS et al. 2013).

Studying peripheral populations is important for conservation concerns, especially in isolated populations. Thus, the authors present body size and condition data of the Caspian Whip Snake from near the northernmost portion of the species' geographical range, their seasonal variation in particular.

MATERIALS AND METHODS

Study site.- Szársomlyó Hill is situated in southern Transdanubia, southwestern Hungary. It is a strictly protected nature reserve, harboring the largest *D. caspius* population in Hungary (MAJER 2000; BELLAAGH et al. 2007; FRANK et al. 2012). The bedrock consists of limestone and dolomite, which is partly covered by loess. The vegetation is quite diverse, with a mosaic of xeric shrub forests and grasslands (DÉNES 2000; BELLAAGH et al. 2007; ERDŐS et al. 2013). Mean annual temperature is 10–10.5 °C, the coldest month is January (mean: -0.9 °C), the hottest July

(mean: 23.5 °C); mean annual precipitation is 670–690 mm (ERDŐS et al. 2013).

Snake capture and measurements.- Between 1998 and 2003, members of the Baranya county section of Birdlife Hungary monitored *D. caspius* in the area under the supervision of one of the authors (G. D.). Occasional road surveys were carried out from April to September. Snakes were captured by hand during visual encounter surveys of the study areas, date and time of captures were recorded. Each snake was weighed to the nearest 1 g by a digital balance and measured for snout-vent

length (SVL) to the nearest 1 mm by stretching the animal out along a tape measure. The determination of sex was made by probing to detect hemipenes. Snakes were palpated for the presence or absence of food, eggs or faeces, and visually searched for abnormalities. After processing, snakes were released at the location of capture.

Sexual size dimorphism (SSD) was calculated as (SVL of the larger sex / SVL of the smaller sex) - 1 following SHINE (1994) and SIVAN et al. (2015) (not LOVICH & GIBBONS 1990, 1992). This index is negative, by convention, when males are the larger sex and positive when females are the larger sex.

Body condition.- The body condition was quantified using a body mass (BM) to SVL relationship, the “scaled mass index”, which is a reliable indicator of body reserves for snakes. This index is independent of growth, sex and age of the animal, it standardizes all individuals to the same body size, and has been shown to be a better predictor of variations in fat and protein reserves than other methods (PEIG & GREEN 2009; SIVAN et al. 2015). The natural logarithms of the BM and SVL values were used to estimate the parameters of a linear regression model. Calculation of the body condition index (BCI) followed equation (2) in PEIG & GREEN (2009) with the model parameters applied. Snakes which discernibly contained food items were noted in the field and not included in the analyses.

Statistical analyses.- Sex-specific analyses of variance (ANOVA) of the BCI values from April and May, when sample sizes were largest, did not reveal significant differences (all $p > 0.5$) between years; therefore, both male and female data each was pooled over years for comparisons, which were made among monthly as well as seasonally pooled BCI data. In this latter case BCI values were assigned to three time periods, a mating period in spring (April and May), a gestation/parturition period (June and July) and a post-parturition/pre-hibernation period (August and September). This comparison is simpler and more powerful than comparing among months (BROWN et al. 2002). Although some individuals' BCI values appear more than once in the sample, each observation was treated as independent, thus artificially increasing the degrees of freedom in statistical tests. Simulations showed that this kind of pseudoreplication does not compromise the conclusions from statistical tests as long as variation among individuals within a season is similar in magnitude to variation within an individual between seasons (LEGER & DIDRICHSON 1994). Body measurement values were compared using t -tests and two-way ANOVA was used to compare BCI among periods and between sexes. All statistical analyses were performed with the software PAST (HAMMER et al. 2001).

RESULTS AND DISCUSSION

Body size.- Altogether, the data consists of 76 captures and recaptures of 57 *D. caspius* individuals sampled during six years of study. Four individuals were categorized as subadults because of small body size (SVL < 300 mm, BM < 60 g), and excluded from further analyses. Food items were perceptible in one captured individual, which was subsequently excluded from further analyses. Hemipenes were detected in 14 individuals, these were categorized as males, all other specimens were treated as females. No signs of eggs were detected in female snakes, thus all specimens were categorized as non-gravid.

The SVL of adult females ($N = 39$) ranged from 506 to 1,232 mm with an average of 885.1 mm (SE 34.7 mm); the SVL of adult males ($N = 14$) ranged from 850 to 1,280 mm with an average of 1,084.8 mm (SE 38.6 mm) (Table 1). These measurements comply with previous descriptions (DELY 1978; MAJER 2000; PUKY et al. 2005). Despite the overlapping size range, adult females were significantly smaller than adult males ($t = 3.187$, $df = 2$, $p = 0.002$). The SSD index was calculated as -0.2256. Sexual size dimorphism is widespread among snake species, with females usually being larger than males. In a list of 264

Table 1: Body measurements of Caspian Whip Snakes, *Dolichophis caspius* (GMELIN, 1789), from Szársomlyó Hill, southwestern Hungary. SVL – snout-vent-length, BM – body mass, BCI – body condition indicated by the “scaled mass index” (PEIG & GREEN 2009).

Tab. 1: Körpermaße der Kaspischen Pfeilnattern *Dolichophis caspius* (GMELIN, 1789) vom Hügel Szársomlyó, SW-Ungarn. SVL – Kopf-Rumpf-Länge, BM – Körpermasse, BCI – Körperkondition angegeben als “scaled mass index” (PEIG & GREEN 2009).

	Males N = 14	Females N = 39	Test statistics	<i>p</i>
SVL (mm)			$t = 3.187$	0.002
Mean	1084.8	885.1		
SE	38.6	34.7		
Range	850-1280	506-1232		
BM (g)			$t = 2.142$	0.037
Mean	432.1	330.4		
SE	38.9	24.7		
Range	167-599	120-643		
BCI			$F = 7.396$	0.009
Mean	340.8	384.7		
SE	14.5	9.4		
Range	197.9-467.3	245.6-558.2		

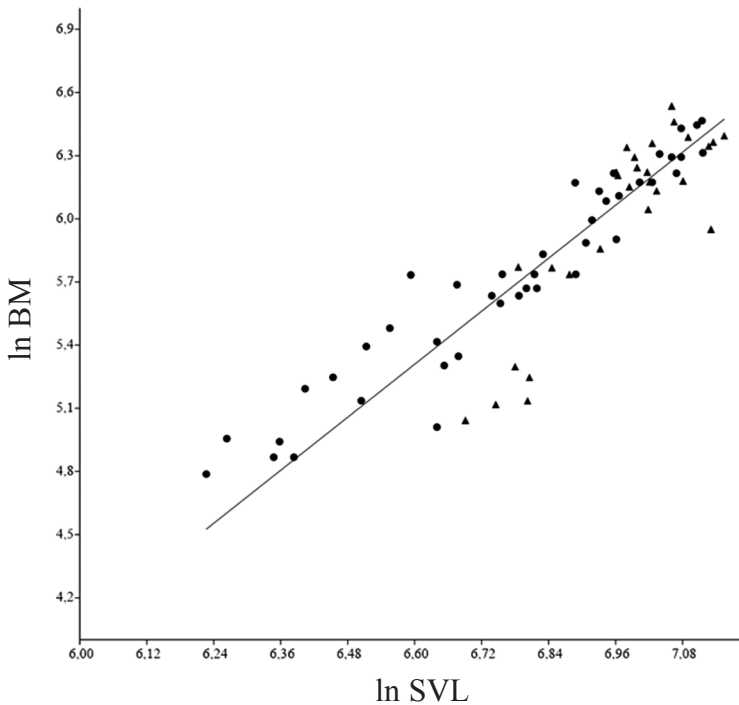


Fig. 1: The reduced major axis (RMA) regression of ln BM (g) on ln SVL (mm) in the Caspian Whip Snake, *Dolichophis caspius* (GMELIN, 1789), from Szársomlyó Hill, southwestern Hungary. BM – body mass, SVL – snout-vent-length, ▲ – males, ● – females.

Abb. 1: Die Regression mit reduzierter Hauptachse (RMA) von ln BM (g) auf ln SVL (mm) bei der Kaspischen Pfeilnatter *Dolichophis caspius* (GMELIN, 1789) vom Hügel Szársomlyó, SW-Ungarn. BM – Körpermasse, SVL – Kopf-Rumpf-Länge, ▲ – Männchen, ● – Weibchen.

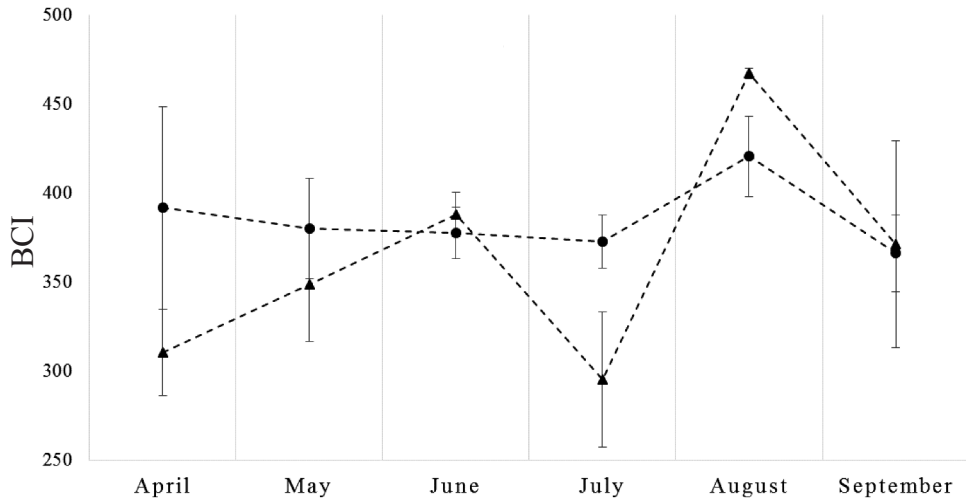


Fig. 2: Monthly mean \pm 1 SE of the body condition index (BCI, indicated by the “scaled mass index” - PEIG & GREEN 2009) in male and female Caspian Whip Snakes, *Dolichophis caspius* (GMELIN, 1789), from Szársomlyó Hill, southwestern Hungary. -- \blacktriangle -- males, -- \bullet -- females.

Abb. 2: Monatsmittel \pm 1Standardfehler des Körperkonditions-Indexes (BCI, angegeben durch den “scaled mass index” - PEIG & GREEN 2009) bei männlichen und weiblichen Kaspischen Pfeilnattern *Dolichophis caspius* (GMELIN, 1789) vom Hügel Szársomlyó, SW-Ungarn. -- \blacktriangle -- Männchen, -- \bullet -- Weibchen.

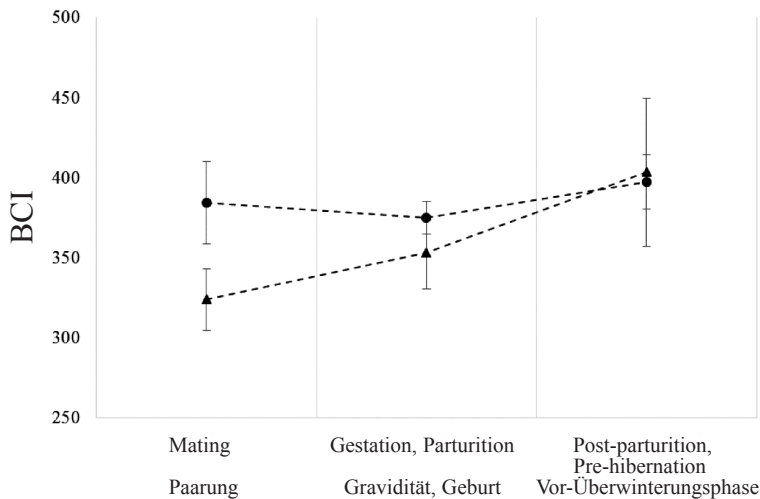


Fig. 3: Seasonal variation of the body condition index (BCI, indicated by the “scaled mass index” - PEIG & GREEN 2009) in male and female Caspian Whip Snakes, *Dolichophis caspius* (GMELIN, 1789), from Szársomlyó Hill, southwestern Hungary. -- \blacktriangle -- males, -- \bullet -- females.

Abb. 3: Saisonale Schwankungen des Körperkonditions-Indexes (BCI, angegeben durch den “scaled mass index” - PEIG & GREEN 2009) bei männlichen und weiblichen Kaspischen Pfeilnattern *Dolichophis caspius* (GMELIN, 1789) vom Hügel Szársomlyó, SW-Ungarn. -- \blacktriangle -- Männchen, -- \bullet -- Weibchen.

snake species compiled by SHINE (1994), females were the smaller sex in only 32.2 % of the species studied, and 23.1 % of the species in the family Colubridae. Larger male size has been linked to species exhibiting male-male combat in that larger males could increase their success in matings and defend a territory (SHINE 1994; RIVAS & BURGHARDT 2001; SENTER et al. 2014). This seems to fit the biology of the Caspian Whip Snake (DELY 1978; ŠČERBAK & BÖHME 1993; PUKY et al. 2005), as was also described for other colubrids (SENTER et al. 2014).

Body condition.- Based on 71 measurements, the regressions of ln BM on ln SVL was calculated as: $\ln \text{BM} = 2.099 (\pm 0.104) \times \ln \text{SVL} - 8.542 (\pm 0.510)$; $r^2 = 0.8298$; $p < 0.0001$ (Fig. 1). Slope and intercept of the regression line was similar to that previously described in snakes (WEATHERHEAD & BROWN 1996; PEIG & GREEN 2009; FELDMAN & MEIRI 2013; SIVAN et al. 2015).

There was a significant difference in the BCI between sexes and months ($F_{(1,5)} = 7.396$, $p = 0.009$; $F_{(1,5)} = 2.445$, $p = 0.044$), respectively), but no difference for the interaction of sex by month ($F_{(1,5)} = 1.488$, $p = 0.207$). In the seasonally divided data set, the difference in BCI among sexes was significant ($F_{(1,2)} = 7.104$, $p = 0.009$), but not so between periods and for the interaction of sex by period ($F_{(1,2)} = 2.965$, $p = 0.059$; $F_{(1,2)} = 1.637$, $p = 0.203$, respectively).

The BCI of females was higher than males (Table 1), except for the months June and August. The female BCI seemed to be stable over the year, with a peak in August, whereas the BCI of males showed a greater fluctuation, with an increase from April to June, a decrease in July, then a peak in August and another moderate decrease in September (Fig. 2).

A trend was more prominent in the seasonal data set (Fig. 3). The BCI of females seemed to be stable with a slight peak in the pre-hibernation period (August/September), and that of males showed a constant increase across the year. The greatest difference in BCI between males and females occurred in spring following hibernation (referred to as mating period in April and May), when the BCI of females was substantially higher than that of males

(384.4 ± 25.72 and 324.0 ± 19.26 , respectively). Female and male BCI were similar in the period before hibernation (referred to as pre-hibernation period in August/September) (397.4 ± 16.96 and 403.4 ± 46.31 , respectively).

Male Caspian Whip Snakes lost more body mass than females during hibernation, possibly caused by differences in body temperature between the sexes during hibernation (COATES et al. 2009). During the mating period, males of many snake species search intensively for mates and rely on fat reserves that help to reduce the time for energy acquisition while finding reproductive partners (RIVAS & BURGHARDT 2001; SHINE et al. 2001; COATES et al. 2009; SENTER et al. 2014). Since the body condition is strongly correlated with main body reserves and fat bodies (NAULLEAU & BONNET 1996; SCHULTE-HOSTEDDE et al. 2005; PEIG & GREEN 2009), intensive searching behavior during mating is associated with loss of body condition in male snakes. After the mating period, male body condition increases substantially, males acquire food resources to replenish energy reserves and prepare for hibernation (BROWN et al. 2002; COATES et al. 2009; SIVAN et al. 2015). Body condition (along with size) contributes positively to mating success in colubrid snake species (RIVAS & BURGHARDT 2001; COATES et al. 2009; SENTER et al. 2014; SIVAN et al. 2015), but there are too few observations to test for an association between condition and mating in *D. caspius*. However, the observed seasonal pattern of BCI suggests that during the mating period when males rely on fat reserves, a sufficient body condition was important for male Caspian Whip Snakes. In contrast to males, the BCI of the female Caspian Whip Snakes showed little fluctuations and appeared to be stable across the year. Good female body condition provides support for embryonic development, thus increasing the reproductive success (NAULLEAU & BONNET 1996; BONNET et al. 2000; COATES et al. 2009; BONNET et al. 2011; SIVAN et al. 2015). Body condition in female *D. caspius* did not differ among months or seasons, suggesting that the actual body condition at the time of mating was less important to females than males. However, this has to be considered

Table 2: Parameters of the linear regression of ln BM on ln SVL for the Caspian Whip Snake, *Dolichophis caspius* (GMELIN, 1789), from Szársomlyó Hill, southwestern Hungary, including the average SVL (SVL₀). The regression form is: ln BM (g) = b × ln SVL (mm) + a. BM – body mass, SVL – snout-vent-length.

Tab. 2: Parameter der linearen Regression von ln BM auf ln SVL für die Kaspische Pfeilnatter *Dolichophis caspius* (GMELIN, 1789) vom Hügel Szársomlyó, SW-Ungarn, einschließlich der durchschnittlichen Kopf-Rumpf-Länge (SVL₀). Die Regressionformel ist: ln BM (g) = b × ln SVL (mm) + a. BM – Körpermasse, SVL – Kopf-Rumpf-Länge.

N	a ± SE	b ± SE	r ²	p-value	SVL ₀
54	-8.542 ± 0.510	2.099 ± 0.104	0.8298	< 0.0001	967.61

with caution as no signs of eggs were detected in the female snakes, thus making impossible to compare the BCI of gravid and non-gravid females. Maybe only those female snakes were captured that did not reach a BCI threshold required for mating, so that they did not reproduce in the study period.

This is the first study in which the scaled mass index was used to estimate body condition in Caspian Whip Snakes. The method has proven to be accurate (PEIG & GREEN 2009; SIVAN et al. 2015) and can be very useful but requires a comprehensive intraspecific analysis using a large dataset.

For cases in which a comprehensive dataset is not available (SHINE et al. 1998), the two key parameters, b_{RMA} (= the scaling exponent estimated by the SMA regression of BM on SVL) and SVL₀ (= the arithmetic mean value of SVL for the study population) necessary for calculation of the scaled mass index are provided in Table 2. Generating similar parameters for other snake species would provide a tool for the study of ecological questions involving body condition without the need to compute intraspecific major analyses and the requisite to estimate comparable indexes.

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