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Comparing three body condition indices in amphibians: a case study of yellow-bellied toad *Bombina variegata*

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Abstract. Body condition is important because it is correlated with population and habitat quality parameters. Since the direct measurements are either lethal or unreliable, a wide range of non-lethal body condition indices has been proposed. The aim of our study was to apply and compare three body condition indices (Fulton's index, relative body condition mass index and residual index) using body size indicator – body mass data for 24 populations of the yellow bellied toad (*Bombina variegata*). The condition index should be independent of body size indicator, in this case snout vent length (SVL). Therefore we tested all three indices for the statistical independence of SVL and for the normality of distribution. Fulton's index violated the independence assumption, whereas the relative body condition mass index did not have a normal distribution. Residual index was found both independent of SVL and normally distributed. Moreover, the residual index highlighted biological significant differences on the basis of altitude and season. Our results recommend the residual index as a useful tool in amphibian monitoring and conservation.

Keywords: altitude, conservation, life stage, residual index, season.

The body condition was proposed as a management tool in conservation biology (Anderson and Neumann, 1996) since it is considered an indicator of environmental stress, prey availability and/or habitat quality (Sztatecsny and Schabetsberger, 2005), reproductive investment (Castellano, Cucco and Giacoma, 2004), parasite load, investment in secondary sexual characters (Green, 2001), mate choice (Uetz, Papkei and Kilincz, 2002), survival (Hoey and McCormick, 2004), vulnerability to predation (Murray, 2002; Wirsing, Steury and Murray, 2002), and/or fighting ability (Bee, Perill and Owen, 2000).

Body condition is thus a proxy of energy reserves (Schulte-Hostedde et al., 2005). The classical way of estimating it is based on the amount of fat deposits, this method being destructive for the studied individuals (Blackwell, 2002). Other alternative methods proposed like total body electrical conductivity employed for estimating lean and lipid mass in small birds and mammals did not prove very reliable (Robin et al., 2002; Snyder, Post and Finck, 2005). Considering that many amphibian populations are declining and many species have strict protective status (Stuart et al., 2004), non-destructive methods are needed to assess their body condition. A non-destructive alternative to the above-mentioned direct measurements is the use of body condition indices (BCI) based on allometric relationships between length and weight. Most frequently used body condition indices are various ratios between body mass and a linear measure of body size indicator (BSI), or the use of residuals from a linear regression of body mass against BSI (Green, 2001).

The computation of BCI is based on a series of assumptions (Blackwell, 2002; Schulte-Hostedde et al., 2005): (1) body mass increases linearly with BSI (following any data transformation); (2) the true condition (body condition index) is independent of BSI; (3) BSI is an ac-

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curate measure of structural size; (4) there is no correlation between BCI and other structural components (i.e., shape); and (5) BSI is measured without bias. The weakness of BCI is that not all of the above mentioned assumptions can be verified.

Estimating the body condition of amphibian populations is important for testing their indicator value to environmental stress. Due to their high fertility, complex life cycle with aquatic and terrestrial life stages, amphibians are sensitive to environmental quality and its change. Their permeable and moist skin makes them vulnerable to environmental pollutants (Wake, 1991; Niemi and McDonald, 2004). The decline of amphibian populations and the extinction of species were reported in many parts of the world (Houlahan et al., 2000; Stuart et al., 2004).

The yellow-bellied toad (*Bombina variegata*) is widespread in Romania (Cogălniceanu, Aioanei and Bogdan, 2000) but at European Union level is a species of community interest whose conservation requires the designation of special areas of conservation (Annex II of the Habitats Directive 92/43/EEC). Being widespread and abundant in our study sites, yellow-belied toad populations can provide baseline data on body condition and allow testing the usefulness of the body condition indices.

Our study was based on a set of body length and body mass weight data collected from 24 different populations of the yellow-bellied toad. The aim of our study was to: (1) provide baseline data of body condition for *B. variegata* populations, and (2) apply and compare the usefulness of three BCI: Fulton's index (Sztatecsny and Schabetsberger, 2005), a relative mass condition index (Hansen, 2005), and a residual index (Denoël et al., 2002).

The study was carried out between 2001 and 2005 and covered three areas in Romania: (i) Prahova Valley (mean altitude 945 m, N 45°21′-45°32′, E 25°30′-25°34′), (ii) Haţeg Geopark (mean altitude 472 m, N 45°12′-45°18′, E 22°25′-23°20′), and (iii) the middle part of the Târnava Valley (mean altitude 463 m, N 46°11′-46°23′, E 24°14′-24°57′). Specimens were captured, measured and immediately released at the site of capture. Body mass (W) was measured to the nearest 0.01 g with a portable electronic balance (AccuLab Pocket Pro). Snout-vent length (SVL) was recorded to the nearest 0.5 mm with dial-calipers. Each individual was sexed based on the presence/absence of thumb pigmentation. All individuals below 30 mm were considered juveniles.

A total of 1457 individuals were sampled. From the Prahova Valley we caught 1163 individuals from fourteen populations, of which 746 adults (316 males and 430 females), from Haţeg Geopark 121 animals from six populations, of which 109 adults (53 males and 56 females), whereas from Târnava Valley 173 animals from four populations, all adults (71 males and 102 females).

Fulton's index (K) was calculated according to the formula $K = \frac{W}{SVL^3} \times 10^5$ (Sztatecsny and Schabetsberger, 2005). The relative mass condition index (W_r) for each specimen was calculated as $W_r = 100 \times \frac{W}{W_s}$, where W_s is the body mass predicted from the linear regression of body mass on SVL, following $\log_{10}$-transformation (Hansen, 2005). The residuals of the linear regression gave the residual condition index for each specimen (Denoël et al., 2002). Each index was checked against the assumption that BCI is independent of BSI (measured as SVL), and for normality, one of the key assumptions of the linear mixed models (LMM). We used LMM to test the ability of residual index to highlight potential biological differences on the basis of sex/life stage, altitude, and season. Populations were included as a random factor in the analysis. Since there was no difference between females and males ($F_{1,1026} = 1.579$, $P = 0.209$) we analyzed life stage alone as a factor with two groups adults and juveniles. Fulton's index and relative mass condition index failed to meet one of the assumptions and were not further analysed. The residual index fulfills both the independence and normality assumptions and was therefore further tested. Least significant difference (LSD) was used for post hoc multiple comparisons. All data analyses were done using SPSS version 17.0 (SPSS Inc., 1999).

Our study is based on measurements of body mass from 24 populations for 1028 adults (out of which 588 females and 440 males) and 427 juveniles (table 1). The relationship between body mass and SVL was curvilinear. Therefore W_r and residual index were calculated using $\log_{10}$-transformed data that improved the linearity of the relationship between the two variables (fig. 1). The predicted body mass W_s was obtained from linear regression equation: $\log W = -3.94 + 2.89 \times \log SVL$.

The distribution of Fulton's index (K) was slightly positively skewed and significantly peaked (Skew ± SE $= 1.479 \pm 0.064$, $P = 0.069$; Kurtosis ± SE $= 5.16 \pm 0.128$, $P < 0.001$). The body size indicator (SVL) was significantly correlated with K ($r = 0.255$, $n = 1457$, $P < 0.001$) so larger individuals had

Table 1. Mean, SE and range (min-max) of body mass and snout-vent length of yellow-bellied toads.

	n	Mean body mass (g) ± SE (min-max)	Mean snout-vent length (mm) ± SE (min-max)
Geographic areas			
Prahova (PV)	350	4.1 ± 2.09 (0.11-12.3)	34.58 ± 0.77 (11.2-54.6)
Haţeg (HG)	121	6.67 ± 1.52 (1.2-10.7)	40.44 ± 0.53 (23.1-50.4)
Târnava (TV)	173	5.98 ± 1.33 (2.4-10.9)	40.7 ± 0.31 (31.3-50.1)
Life stage			
Adult	1028	5.52 ± 1.67 (2.4-12.3)	41.07 ± 0.14 (30.2-54.6)
Juvenile	427	0.53 ± 0.47 (0.1-2.5)	17.6 ± 0.22 (11.2-29.8)
Gender			
Female	588	5.45 ± 1.91 (2.4-12.3)	40.81 ± 0.21 (32.6-54.6)
Male	440	5.61 ± 1.28 (3.1-10.4)	41.42 ± 0.17 (30.2-50.2)

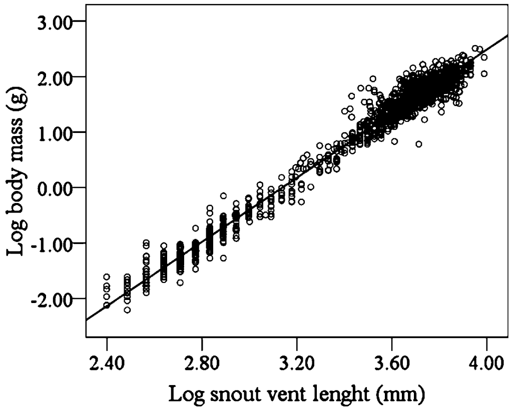

Figure 1. Relationship between $\log_{10}$ transformed body mass and snout-vent length in yellow-bellied toads (n = 1457).

higher condition scores using this index. The distribution of W_r was significantly positively skewed and leptokurtic (Skew ± SE = 8.778 ± 0.064, $P < 0.001$; Kurtosis ± SE = 332.612 ± 0.128, $P < 0.001$). The index showed no significant correlation with SVL ($r = 0.043$, $n = 1457$, $P = 0.482$). The residual index was normally distributed (Skew ± SE = 0.441 ± 0.064, $P = 0.329$; Kurtosis ± SE = 1.627 ± 0.128, $P = 0.047$) and showed no correlation with SVL ($r = 0.023$, $n = 1457$, $P = 0.49$).

Altitude and season explained significant amounts of variation in mean residual index scores (table 2). No significant difference in mean residual index scores was found between adults and juveniles ($F_{1,30.9} = 0.163$, $P = 0.689$) (fig. 2a), and between life stage and altitude ($F_{1,50.5} = 0.178$, $P = 0.675$) or season ($F_{2,204.8} = 1.015$, $P = 0.364$).

Table 2. Results from two-way linear mixed-model analyses testing the effect of altitude, season and life stage on condition measured as residual index. Populations were included as a random factor in the analysis.

Source	df	*F*	*P*
Altitude	1, 34.1	14.204	<0.001
Season	2, 35.2	4.268	<0.01
Life stage	1, 30.9	0.163	0.689
Altitude · Life stage	1, 50.5	0.178	0.675
Season · Life stage	2, 204.8	1.015	0.364

Results indicated significant differences in mean residual index scores between yellow-bellied toads from low and high altitudes ($F_{1,34.1} = 14.204$, $P < 0.001$), higher mean residual index scores being found at lower (mean ± SE = 0.090 ± 0.011) than higher altitude (mean ± SE = −0.022 ± 0.014) (fig. 2b). Season had a significant effect on residual index ($F_{2,35.2} = 4.268$, $P < 0.01$). LSD test showed that residual index scores were significantly higher in spring (mean differences = −0.062, $P < 0.05$) and summer (mean differences = −0.057, $P < 0.05$) then in autumn but no significant differences was found between summer and spring (mean differences = −0.004, $P = 0.498$) (fig. 2c).

The Fulton's index, relative mass condition index and the residual index differed in their ability to meet the assumptions tested in this study. Fulton's index was positively correlated with body size, thus violating one of the key as-

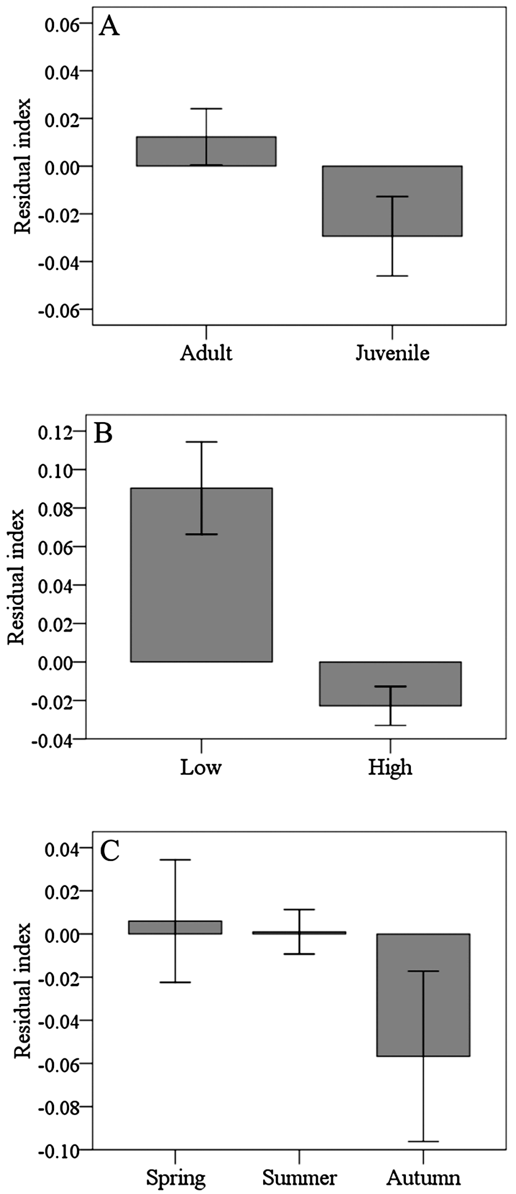

Figure 2. Influence of (a) life stage, (b) altitude, and (c) season on residual index in yellow-bellied toads. Columns represent mean residual index scores (± SE).

sumptions, the independence of body size measured as SVL. Although relative mass condition index was independent of body size the broad peak and short tails indicated a leptokurtic departure from normality. Statistical assumptions underlying ratios, their impact on data interpretation, and the limitations of the indiscriminant use of ratios to adjust data are discussed elsewhere (Allison et al., 1995). Residual index was independent of body size and normally distributed and suggested biological meaningful trends.

Residual index was not significantly correlated with life stage or gender. Juveniles may be less efficient foragers than adult toads and have a relatively shorter period of time to deposit energy reserves. Results indicated no significant interaction between life stage and season on body condition of yellow-belied toad.

The altitude was negatively correlated with body condition. The progressively poorer condition of individuals at higher altitudes probably results from longer periods of cold temperatures reducing the period of activity and therefore affecting the energy intake. It is found (Pope and Matthews, 2002) that the amount of snowfall is negatively correlated with the body condition of anurans. Body condition of yellow-bellied toads also varied seasonally. Residual index indicated that toads were in significantly better condition in spring and summer then in autumn. Body reserves are depleted during hibernation and breeding, and then they are restored during the following active period (Reading and Clarke, 1995). Winter conditions have a strong effect on body condition (Tomašević et al., 2007). Mild winter and warm pre-spawning periods have a negative effect on body condition of hibernating anurans due to an elevated metabolic rate and the increase in energy reserve utilization (Ryser, 1989; Reading and Clarke, 1995). Thus, toads are more likely to emerge from hibernation in good condition after colder winters, as was the case during our study period.

Condition indices can be easily computed and require simple field measurements, with little or no impacts on the individuals. They have been used in a wide variety of contexts in conservation and environmental biology (e.g., Stevenson and Woods, 2006). We report three positive aspects related to the use of the residual index: (i) adequate control for variation across body sizes, (ii) easy to distinguish and interpret the effects of different environmental factors and (iii) straightforward biological interpretation. These recommend the residual index as a useful tool in monitoring and conservation. We recommend that investigators use the residual index for testing differences in body condition of amphibian populations.

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