

Morphometric study on tadpoles of *Bombina variegata* (Linnaeus, 1758) (Anura; Bombinatoridae)

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Abstract. The tadpoles of Yellow-bellied toad (*Bombina variegata*) can be easily recognized from other Italian anuran species, except those of *B. pachypus* (though the two congeneric species are allopatric). In this paper we report morphometric data on *B. variegata* tadpoles from a Lombard population living near a torrent at 450 m a.s.l. On a sample of 264 tadpoles (stages 19-44, according to Gosner, 1960) we measured the following five variables: snout-vent length, tail length, maximum tail height, total length and weight. We found a slight allometric relationship between snout-vent length and tail length, while, as expected, the weight is nearly proportional to the cube of linear measures. According to literature data, our results point to highly constant proportions during the development phases up to prometamorphic stages. The ratio between snout-vent length and tail length was about 0.75 during the whole growing phase, while from stage 42 the proportion increases as the resorption of the tail starts.

Keywords. Tadpole morphology, Yellow-bellied toad, *Bombina variegata*.

Larval stages of amphibians are well studied, even if inadequate morphological descriptions are common (McDiarmid and Altig, 1999). According to morphotype classification of Orton (1957), *Bombina* tadpoles belongs to Type 3 (jaw sheaths, labial teeth, medial spiracle), and is included within the following ecomorphological categories: exotroph, lentic, benthic (McDiarmid and Altig, 1999). Differently from adults, which are considered among the most primitive anurans (Griffith, 1963; Noble, 1922), phylogeny aspects of larval morphology are not yet totally clarified (Orton, 1957; Sokol, 1975, 1977; Cannatella, 1999; Haas, 2003; Pugener et al., 2003). A description of early development stages of *B. orientalis* and a study on differentiation of cutaneous granular glands in tadpoles of *B. pachypus* were respectively reported in Prema (1981) and Delfino (1977). Besides, Bonacci et al. (2008) recently described morphology and development of the oral disc of the latter species. Regarding Yellow-bellied toad, old morphological studies on tadpoles have included both *B. variegata* and *B. pachypus* (Boulenger, 1910; Lanza, 1983), since the latter form was only

recently considered a valid species (Nascetti et al., 1982, Lanza and Vanni, 1991; Lanza and Corti, 1993). So far, we think better to reconsider the morphometric features of Yellow-bellied toad tadpoles, in order to reassess the mean measures.

The tadpoles of *Bombina variegata* can be easily recognized from other Italian anuran species, except those of *B. pachypus*. However, the two species are allopatric occurring only north and south of the Po River, respectively. Within a Lombard population living in a lotic habitat at 450 m a.s.l. (Albino, 45°45' N, 9°47' E), a total of 264 tadpoles of Yellow bellied toad were captured and measured during monthly survey (June to August) from 1994 to 2000. The study area includes a torrent section formed by a main river-bed and a series of pools more or less connected or completely isolated from the stream. It comprehends also the surrounding woody area formed by a phytocoenosis of Quercus-Carpinetum with a recent introduction of the allochthonous *Pinus strobus*. Climatically, the area is characterized by a temperate cool climate (Cfa) according to Köppen-Geiger Climate classification (Peel et al., 2007). Monthly mean air and water temperatures of the study area were reported in Di Cerbo and Biancardi (2004). However, the water temperature at pools is subject to variations due to sun exposure, pond size and connection to the stream. Overall, an increasing trend of water temperatures was observed along the season (June, mean: 19.03 °C and range: 11.4 – 32.0; July, mean: 20.4 °C and range: 13.5-34.0; August, mean: 21.3 °C; range: 15.6-30.0). Invertebrate (dragonfly nymphs, Dytiscidae, Heteroptera, Trichoptera and leeches) and vertebrate predators (*S. salamandra* larvae, *Natrix natrix*) of eggs and tadpoles, together with larval competitors (*Bufo bufo*, *Rana temporaria*) were recorded in the pools occupied by *B. variegata*.

The tadpoles were collected with a hand net and immediately put in a transparent small basin. They were dried briefly on a piece of paper towel and then separately weighed (WGT) with an electronic balance (TANITA, mod. 1479, ± 0.1 g precision). However the weights of the tadpoles up to stage 25 are missing, as well as centigrams differences in early stages (many individuals in class 0.1; 0.2 g etc.). After that, they were put on a graph paper sheet and measured using callipers (0.1 mm accuracy). The tadpoles were released just after measurement. We collected the following biometrical measures according to Grosjean (2005): total length (TL), as straight line distance measured from the tip of the snout to the tip of the tail, snout-vent length (SVL), as straight line from the tip of the snout to the opening of the vent tube, tail length (VT), as straight line from the opening of the vent tube to the absolute tail tip, maximum tail height (HT), as greatest vertical distance of the tail muscle plus both fins (Fig. 1). The statistical analyses were performed using the software SPSS ver. 17.0. Otherwise stated, reported values are means and standard deviations.

We considered the stages 19-44, according to the classical Gosner staging series (Gosner 1960). Our sample includes almost the whole development categories of amphibian larvae, and we divided them in: embryo (19-22), hatchling (23-25), tadpole (26-38), pro-metamorph (39-41) and metamorph (42-44).

The descriptive statistics of the sample are shown in Table 1. Ratio between the SVL and VT ranged between 0.40 – 1.14, but its variance was very low and the modal value was 0.75, which means that, SVL was about $\frac{3}{4}$ of VT. Previous reference values were 0.66 (Boulenger, 1910) and 0.66-0.8 (Lanza, 1983). VT/HT Ratio ranged from 1.82 to 3.25. Tail was in average about twice longer than height. Distributions of SVL and VT per development category (Fig. 2).

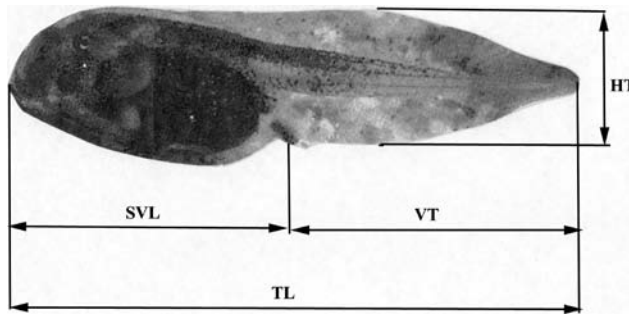


Fig. 1. Tadpole of *Bombina variegata*. Biometric measures.

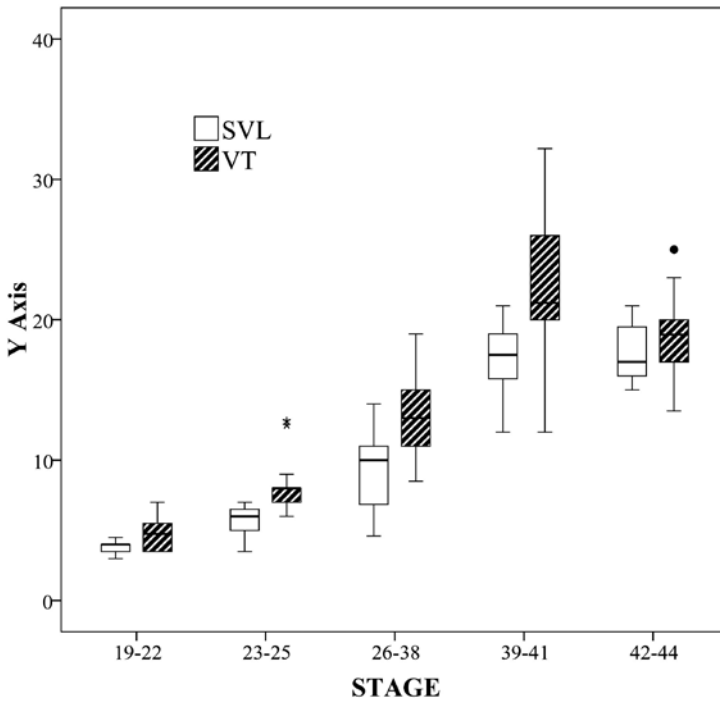


Fig. 2. Variation of SVL and VT of *B. variegata* tadpoles according to growing stage.

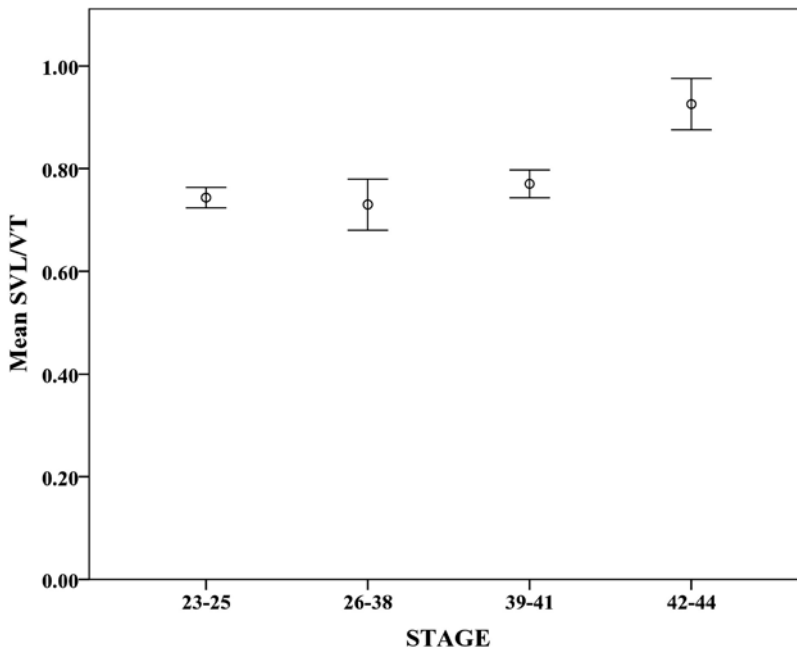
As expected, all morphological variables are highly correlated (Table 2). The body weight scales with approximately the third power of linear measures. The ratio between tail length and height (VT/HT) does not significantly change in the different stages (ANOVA: $F = 0.52$; $df = 2, 57$; $P = 0.59$). We particularly investigated the relationship between SVL, which is usually considered the best predictor of body size, and VT for its implication in the dramatically changes occurring during the metamorphic stages. The

Table 1. Descriptive statistics of the sample (variables explained in the text).

	Embryo (19-22)			Hatchling (23-25)			Tadpole (26-38)			Prometamorph (39-41)			Metamorph (42-44)		
	N	Min-Max	Mean±SD	N	Min-Max	Mean±SD	N	Min-Max	Mean±SD	N	Min-Max	Mean±SD	N	Min-Max	Mean±SD
SVL (mm)	10	3.0 - 4.5	3.8 ± 0.5	96	3.5 - 7.0	5.7 ± 0.8	75	4.6 - 14.0	9.2 ± 2.6	48	12.0 - 21.0	17.2 ± 2.2	35	15.0 - 21.0	17.4 ± 1.9
VT (mm)	10	3.5 - 7.0	4.7 ± 1.2	96	6.0 - 12.8	7.8 ± 1.0	75	8.5 - 19.0	13.0 ± 2.6	47	12.0 - 32.2	22.8 ± 4.4	34	13.5 - 25.0	19.0 ± 2.4
TL (mm)	10	7.0 - 11.0	8.5 ± 1.4	96	10.5 - 18.0	13.5 ± 1.5	75	15.5 - 32.0	22.2 ± 3.8	47	24.0 - 52.0	40.0 ± 6.3	34	32.0 - 42.0	36.3 ± 3.4
HT (mm)	-			1	9	9	32	4 - 7	5.3 ± 0.8	14	6 - 12	8.8 ± 1.8	14	6 - 11	8.7 ± 1.8
WGT (g)	-			2	0.1	0.1	68	0.1 - 0.6	0.2 ± 0.1	48	0.2 - 2.1	1.1 ± 0.4	35	0.5 - 1.3	1.0 ± 0.2
SVL/VT	10	0.57 - 1.14	0.84 ± 0.22	96	0.36 - 0.92	0.74 ± 0.10	75	0.34 - 1.09	0.73 ± 0.22	47	0.61 - 1.0	0.77 ± 0.09	34	0.64 - 1.52	0.93 ± 0.14
VT/HT	-			1	0.89	0.89	32	1.16 - 3.25	2.30 ± 0.39	14	1.91 - 3.03	2.35 ± 0.35	14	1.82 - 2.83	2.21 ± 0.33

Table 2. Pearson correlation coefficients among morphological variable of *B. variegata* tadpoles.

	TL	SVL	VT	HT	WGT
TL	1.000	.967	.977	.895	.963
SVL	.967	1.000	.890	.851	.971
VT	.977	.890	1.000	.888	.912
HT	.895	.851	.888	1.000	.863
WGT	.963	.971	.912	.863	1.000

**Fig. 3.** SVL/VT Ratio of *B. variegata* tadpoles at different growing stages.

ratio between SVL and VT is almost constant from embryo until 39-41 stages, while is significantly higher at metamorphic stages (Fig. 3): at these stages the growth stops and starts the resorption of the tail. An ANOVA confirmed the significant differences comparing the developmental category 42-44 with all the others (ANOVA: $F = 11.50$; $df = 4, 257$; $P < 0.001$), while all the comparisons among the other stages (Bonferroni post hoc test) are not significant at $\alpha = 0.5$.

Tadpole development process provides for three distinct periods (Etkin, 1968): premetamorphic (growth of feeding tadpoles; Gosner stage 25-35), prometamorphosis (growth and differentiation of hind limbs; Gosner 36-41), metamorphic climax (forelimb development and tail resorption; Gosner 42-46). All these modifications are under control of thyroid hormone (Troncale et al., 2007). Most growth of a tadpole occurs during

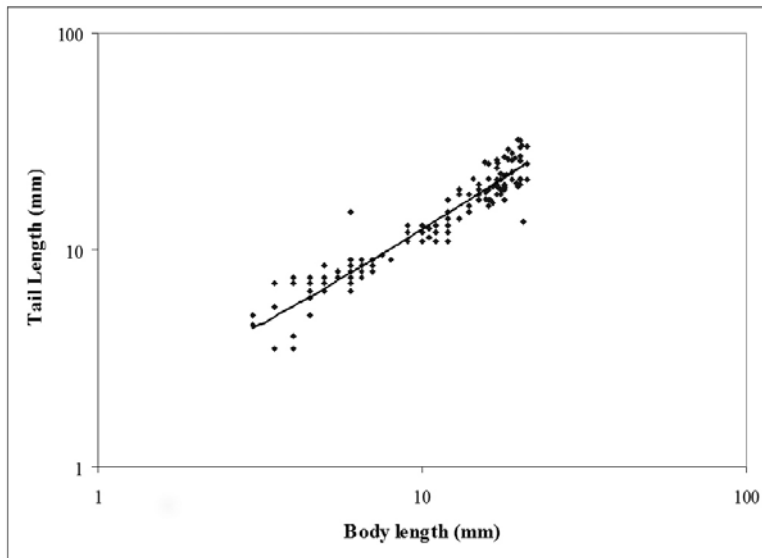


Fig. 4. Allometric relation between SVL and VT of *B. variegata* tadpoles. Both axes in logarithmic scale.

the exponential phase of a sigmoid curve and is quite isometric. This period of maximum growth and minimal development is followed by periods of significant development and little growth (Altig and McDiarmid, 1999). In fact our results show a linear relationship between snout-vent length and tail length up to the metamorphic climax ($VT = 1.03 \times SVL + 1.71$, $R^2 = 0.87$; $F = 968.74$; $df = 1, 226$; $P < 0.001$). This trend can be appreciated comparing the distribution of SVL and VT at different stages (Fig. 2: 19-22 to 39-41). When tadpoles at Gosner 42-44 are included in the regression analysis, a slight allometric relationship best fit the data. The allometric growth of body and tail, confirmed also by the presented analysis of variance, can be described by the power equation $VT = 1.59 \times SVL^{0.89}$ ($R^2 = 0.92$; $F = 2671.51$; $df = 1, 240$; $P < 0.001$) which underlines that VT is proportional to $SVL^{0.89}$. In the linear relationship between log-transformed SVL and VT, displayed in Fig. 4, the residuals of a type-II linear regression are almost homogeneously distributed. This confirms the goodness of the power relationship between body and tail lengths, while other non-linear relationships do not fit the allometric model.

Our results clearly show the growth pattern of *B. variegata* tadpoles at different stages. Being collected in different periods and years, our data reflect the growth capacity of this species during each development stage, giving reference measures for body and tail length.

Previous studies on amphibian larvae have shown that geographic and ecological parameters such as latitude, temperatures, density of population or presence/absence of predators, habitat type, water quality, food abundance and quality could influence the growth rate of tadpoles and the measures at metamorphosis (Parichy and Kaplan, 1992; Ultsch et al., 1999; Van Burkirk, 2000; Olsson and Uller, 2002; Di Cerbo and Biancardi, 2004). Besides, they could even determine intraspecific variability in anuran morphol-

ogy (Smith-Gill and Berven, 1979; Hanken and Hall, 1984; Smirnov, 1992; Strauss and Altig, 1992). For instance, recent studies emphasized the effects of the amphibian pathogen *Batrachochytrium dendrobatidis* on anuran larvae. This fungus can cause mouthpart deformities, variations in feeding kinematics, than a compromise on the feeding efficiency of tadpoles and a smaller size in infected individuals (Venesky et al., 2010). Kaplan and Phillips (2006) reported that development of *B. orientalis* tadpoles at higher temperatures increased both length and height of the tail, but was associated with decreased SVL. The tail shape can influence the swimming performance of tadpoles and their vulnerability to predators (Chovanec, 1992). Recent researches have documented predator-induced polyphenism in tadpole tail shape (Hoff and Wassersug, 2000; Van Burkirk and McCollum, 2000). In experimental conditions, Vorndran et al. (2002) observed that the presence of dragonfly nymphs (*Aeshna cyanea*) can cause a delay of growth in *B. bombina* and *B. variegata* tadpoles and led to a change in body proportions of the latter species increasing the height of its narrow tail fin. Indeed, it could be interesting to verify if morphometrical differences (and even ratios between SVL /VT and VT /HT) within single stages could be due, at least in part, to different environmental conditions, since these features are not yet investigated in *B. variegata*, until now.

Further investigations on tadpoles will be carried out in order to detect biometrical features among yellow-bellied toad populations living in permanent and in ephemeral aquatic habitats.

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