

## Life history, age and normal development of the Balkan-Anatolian crested newt (*Triturus ivanbureschi* Arntzen and Wielstra, 2013) from Sofia district

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**Abstract.** We studied the characteristics of bone growth (assessed by skeletochronology), egg characteristics (jelly and vitellus volume and jelly/vitellus ratio), sexual dimorphism and the ontogenetic development in the Balkan-Anatolian crested newt *Triturus ivanbureschi* populations from Sofia district in Western Bulgaria. The timing and patterns of bone arrested growth were observed using the phalanges of adults that were caught in spring/summer at a breeding site. Body length and age were similar in males and females. The age at maturity was 3 to 4 years and longevity was 8 and 11 years in males and females respectively. A difference in egg size characteristics between the two populations was detected and we hypothesize that it is due to a difference in habitat conditions. The ontogenetic development was traced from a fertilized egg to a fully metamorphosed individual and took approximately 4 months. We compared our results to available data on other *Triturus* species and tested for relation between climatic zone and longevity.

**Key words:** egg characteristics, normal development, skeletochronology, *Triturus*.

### Introduction

*Triturus ivanbureschi* was recently separated from the *T. karelinii* complex and as yet only molecular genetic data are available to distinguish between the taxa (Wielstra et al. 2013a, Wielstra & Arntzen 2014). Existing craniometric data did not clearly reveal differentiation among members of the *T. karelinii* complex (Ivanović et al. 2013). The range of *T. ivanbureschi* covers both Europe and Asia – it encompasses the Eastern Balkan Peninsula, including most of Bulgaria, the Eastern parts of Greece, Macedonia and Serbia, as well as North-western Turkey (along the coast of the Aegean Sea, and the western coast of the Marmara Sea, reaching up to 300 kilometers inland but not inner Anatolia). An isolated distribution pocket that is disconnected from the main range by other intervening *Triturus* species exists in Serbia (Wielstra et al. 2013a). All populations in Bulgaria, previously thought to be *T. karelinii*, are now accepted to be *T. ivanbureschi*; the species occurs across most of the country from sea level up to 1700 m elevation; it is absent around the Danube river region and the lower currents of its tributaries (Stojanov et al. 2011). It is absent from the northwesternmost part of Bulgaria, where *T. cristatus* has been established (Tzankov & Stojanov 2008, Wielstra et al. 2013b).

Amphibians are organisms with complex life cycles (Wilbur 1980) and as such they often dis-

play variations in life-history traits, including egg size, duration of larval period and size at metamorphosis. Newt eggs consist of vitellus, which provides nearly all of the requirements for larval development, and mucoïd capsule (jelly), which protects the embryo from fungal infestation, injury and predation (Duellman & Trueb 1994). Calculating the characteristics of laid eggs provides information that can be used in studying species-specific diversity patterns in *Triturus* species.

The ontogenetic development of amphibians has been a subject of various studies and it has been established that the processes of blastulation and gastrulation are similar across different species (review in Wells 2010). The genus *Triturus* sensu stricto, however, has received relatively little attention. There are development tables for *T. cristatus* (Glückson 1931) and *T. carnifex* (Horner & McGregor 1985, D'Amen et al. 2006). Horner & McGregor (1985) revealed the presence of a specific chromosomal aberration that caused the death of half of all the *Triturus* embryos, mostly between stages 26-34. There are also tables for *Ichtyosaura alpestris* (Bonacci et al. 2005) and several species from the genus *Lissotriton* (Glückson 1931, Gallien & Bidaud 1959, Barbadillo 1989, Tripepi et al. 1998), which until recently were considered part of *Triturus*. For *T. karelinii* and *T. ivanbureschi* such tables are absent, which determines the importance of the present study.

Skeletochronology is a widely used technique for estimating the animals' individual age in wild populations; it is based on the influence of climatic conditions on the metabolism of ectotherms, which is recorded in their bone growth as visible layers, called lines of arrested growth (LAG). This technique is reliable in amphibians as well as in many ectothermal vertebrates (Castanet & Smirina 1990). Newts inhabit both terrestrial and aquatic habitats and consequently have to cope with varying periods of favorable and harsh conditions, which directly influence their life cycle (e.g. life cycle in Mediterranean regions: Jakob et al. 2002, Jakob et al. 2003).

The aim of this paper is to provide an additional and detailed information on the egg characteristics, ontogenetic development and age structure for this species and compares it to existing data for congeners; it also describes how the Balkan-Anatolian crested newt reacts to local climatic conditions and attempts to analyze the role of climatic zones on longevity.

#### Materials and methods

The studied populations inhabit regions near the villages of Bistritsa (42° 35'N 23° 22'E) at 857 m above sea level and German (42° 62'N 23° 42'E) at 1015 m a.s.l.

The ponds near Bistritsa (5 in number) are relatively small (2-27 m in diameter) and are situated a short distance from one another; due to their proximity to a relatively large settlement and the easy access (dirt roads that are passable by vehicles) in some of them there is a considerable amount of everyday and industrial waste. Newts were found only in the smallest pond (3x2 m, depth of around 1 m). During the summer, there is a considerable drop in water level and by the end of August all water bodies are virtually dried up. The terrain is rugged, strewn with rocks and stones of different sizes; open vegetation is dominant, families Poaceae, Rosaceae and Cyperaceae are abundant. *Emys orbicularis*, *Pelophylax ridibundus*, *Rana temporaria*, *Lissotriton vulgaris*, *Hyla arborea* and *Natrix tessellata* were also observed.

Although the ponds near German are easily accessed by well-marked tourist routes, they are more distant from human settlements and have a lower degree of everyday and industrial pollution. The studied pond is of medium size - 45 x 30 m and 1 m in depth. After a steep climb to the ponds the terrain around them is relatively flat; forest type of vegetation predominate *Fagus sylvatica*, *Quercus* sp. (Fagaceae), *Carpinus betulus* (Betulaceae) and Cyperaceae are abundant. *Emys orbicularis*, *Lacerta viridis*, *Darevskia praticola*, *Podarcis muralis*, *Anguis fragilis*, *Bufo bufo*, *Hyla arborea*, *Pelophylax ridibundus*, *Rana dalmatina*, *Rana temporaria* and *Lissotriton vulgaris* were also observed.

All eggs were collected in situ (with the underwater plants they were deposited on) in 2009. The total number of eggs was 215 - 140 from Bistritsa and 75 from German. All eggs were at early stages of development (before early blastula, without visible cleavages) and we concluded that they have been deposited within 24 h prior to collection (see Harrison 1969). The newts were caught by dipping a net in the water during the breeding season April-May (2008-2009) and using funnel traps during the aquatic phase (2011). The Snout-Vent Length (SVL) was measured as the distance between the tip of the snout and the posterior margin of the cloaca, to the nearest mm using a ruler and the sex of the individuals was determined using secondary sexual characters. After taking measurements and samples for skeletochronology, the newts were released at the site of capture. All measured newts (32, 16 male and 16 female) were sexually mature. Sexual size dimorphism was estimated according to the index of Lovich & Gibbons (1992): mean size of larger sex/mean size of smaller sex  $\pm 1$  using +1 when males were larger and -1 when females were larger.

Eggs were photographed using a Canon PowerShot A590 IS digital camera on a 10 mm scale immediately after collection and then once per day (incl. after hatching, up to metamorphosis) in order to trace the ontogenetic development. Their dimensions (maximum length and width of jelly and maximum width of vitellus) were measured from the photographs using the computer graphics analysis program Image Tool version 3.0 (Wilcox et al. 2002). We calculated the vitellus volume (in mm<sup>3</sup>) using the equation for the volume of a sphere:  $V = \frac{4}{3}\pi r^3$ , where r is the radius of the vitellus. Because the jelly capsules have an ellipsoid shape, we calculated their volume (in mm<sup>3</sup>) using the equation for an ellipsoid volume:  $V = \frac{4}{3}\pi(R_1/2)(R_2/2)^2$ , where R1 is the length of the long axis and R2 of the short diameter of the ellipsoid. For each of the eggs' characteristics (length, width and volume of jelly; width and volume of vitellus; correlation between the two volumes) was calculated mean, standard deviation and standard error (Table 1).

Eggs and hatched larvae were housed in aquariums filled halfway with dechlorinated tap water under natural lighting and controlled room temperature of 20°C  $\pm$  2. The larvae were fed ad libitum with *Daphnia* and with the increase in size with mosquito larvae. The stages of normal development were based on clearly visible morphological features and followed those of Harrison (1969), which have been accepted as the standard in salamanders (Duellman & Trueb 1994).

For the skeletochronology the longest finger of the hind leg (i.e. the third) of each captured specimen was cut and preserved in 95° ethanol. The skeletochronology analysis was in accordance to previous procedures (Castanet & Smirina 1990): the preserved fingers were dissected, the larger bone of the second phalange was washed in running water for 24 hrs, decalcified in 5% nitric acid for 2 hours and then washed again in running water for about 12 hrs. The cross-sections (14-16  $\mu$ m) from the diaphyseal part of the phalange were obtained using a paraffin microtome, after which they were stained with

Heidenhain hematoxylin. The sections were placed in glycerin in order to be observed with a light microscope. Estimating age from the number of LAGs in cross sections requires knowing the annual number of periods of arrested growth. We counted double lines in cross sections as one year of age. In cross sections with single lines, it is not possible to determine whether this line was deposited during summer or winter. The time of deposition of single LAGs in the populations can only be established by experimental vital bone marking or individual capture-marking-recapture, but this does not interfere with the estimation of their ages. Age was successfully determined in 16 newts (8 males, 8 females).

We used a standard t-test for analyzing the egg characteristics and a t-test for single means to compare our data to previous studies by Furtula et al. (2008, 2009). A Factor Analysis for Mixed Data (FAMD) was used to ascertain relations between the animals' age for both genders (continuous attribute), species attribution and the inhabited climatic zone (discrete attributes). All statistical analyses were performed with the computer programs Statistica 7.0 (StatSoft 2004) and Tanagra 1.4 (Rakotomalala 2005). Data were presented by standard descriptive statistics as minimum and maximum values and mean  $\pm$  standard deviation. The climatic zones were defined after Metzger et al. (2005) and are as follows (abbreviations in brackets): Alpine North (AN), Alpine South (AS), Atlantic Central (AC), Boreal (B), Continental (C), Lusitanian (L), Mediterranean Mountains (MM), Mediterranean North (MN), Mediterranean South (MS), Pannonian (P). The zone Thermo-moderate and Humid Mountains (TMHM) is not covered by the Metzger et al. (2005) classification and was defined after climate zones of the Caucasus ecoregion (GRID-Arendal 2013).

## Results

For males SVL was 67-82.3 mm ( $73.8 \pm 4.42$  mm), and for females 60.5-89.2 ( $80.4 \pm 6.73$  mm). A t-test revealed that the difference between genders was statistically significant and females were indeed larger than males ( $p=0.004$ ).

**Egg size.** In this research the main goal was to establish the presence or absence of statistically significant differences in egg vitellus and egg volume between the two studied populations. For this reason, each characteristic of the eggs (length, width and volume of the jelly capsule, diameter and volume of the vitellus, ratio between the two volumes) was presented with mean, standard deviation and standard error (Table 1). The sample from Bistritsa was larger in volume and contained both lower minimum and higher maximum values; its means were significantly higher than those of the German sample (jelly length - with 5.69%, jelly width - 13.13%, vitellus width - 3.74%, jelly volume - 30.95%, vitellus volume - 11.73%, jelly/vitellus ratio - 22.52%). A t-test comparing the key characteristics was conducted and the results are given in Table 2.

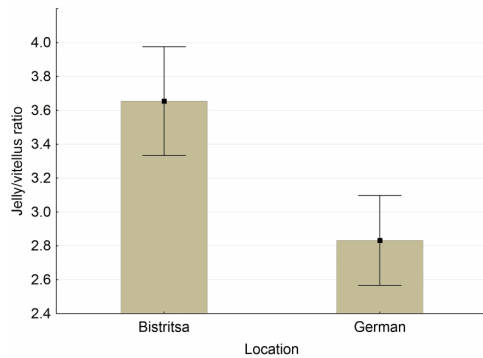
The t-test detected statistically significant differences between the two samples (Table 2). While eggs from Bistritsa had both greater jelly and vitellus volumes in absolute terms, the jelly/vitellus ratio demonstrated that eggs from German had proportionally greater vitellus volume (Fig. 1).

**Table 1.** Studied egg characteristics presented with minimum and maximum values, mean and standard deviation. The third row presents mean and standard deviation from two studies by Furtula et al. (2008; 2009); n - sample size.

	Jelly length (mm)	Jelly width (mm)	Jelly volume (mm <sup>3</sup> )	Vitellus width (mm)	Vitellus volume (mm <sup>3</sup> )	Jelly/vitellus ratio
German (n=75)	3.60-5.64 (4.44 $\pm$ 0.38)	1.82-3.20 (2.48 $\pm$ 0.24)	6.78-24.55 (14.52 $\pm$ 3.49)	1.53-2.55 (2.17 $\pm$ 0.21)	1.86-8.65 (5.48 $\pm$ 1.47)	1.35-10.10 (2.83 $\pm$ 1.15)
Bistritsa (n=140)	3.32-6.89 (4.71 $\pm$ 0.65)	1.69-4.66 (2.85 $\pm$ 0.45)	5.96-71.94 (21.03 $\pm$ 9.06)	1.44-2.94 (2.25 $\pm$ 0.25)	1.56-13.36 (6.21 $\pm$ 1.98)	1.21-13.82 (3.65 $\pm$ 1.92)
Pirot (n=450 <sup>1,2</sup> )	4.62 $\pm$ 0.32 <sup>2</sup>	-	-	2.11 $\pm$ 0.14 <sup>2</sup>	5.00 $\pm$ 1.02 <sup>1</sup>	3.51 $\pm$ 0.80 <sup>1</sup>

**Table 2.** Level of significance (p) between studied localities derived from comparison of means for egg characteristics between this study and data from Furtula et al. (2008, 2009). Statistically significant results are in bold.

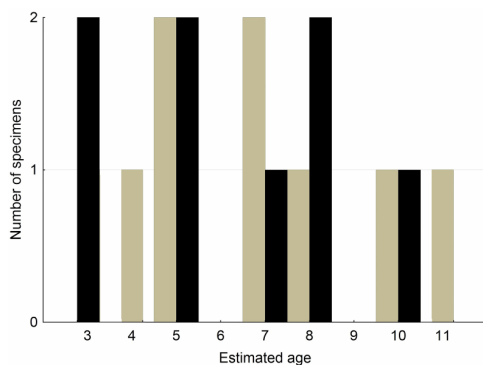
	Below diagonal vitellus volume, above jelly/ vitellus ratio (Furtula et al. 2008 n=450)			Below diagonal jelly length, above vitellus width (Furtula et al. 2009 n=450)		
	Bistritsa	German	Pirot	Bistritsa	German	Pirot
Bistritsa		<b>=0.0008</b>	=0.2153		<b>=0.0192</b>	<b>&lt;0.0001</b>
German	<b>=0.0055</b>		<b>&lt;0.0001</b>	<b>=0.0011</b>		<b>=0.0016</b>
Pirot	<b>&lt;0.0001</b>	<b>=0.0005</b>		<b>=0.0279</b>	<b>&lt;0.0001</b>	



**Figure 1.** Histogram with means and standard deviation according to jelly/vitellus ratio.

We compared our results with those of two studies by Furtula et al. (2008, 2009) on the *Triturus* species complex and particularly on a population near Pirot, which is currently assigned to *T. ivanbureschi* (Table 1). A comparison of means revealed statistically significant differences between some of the studied characteristics (Table 2). The most pronounced difference was detected in vitellus volume, with the mean from Pirot being considerably lower than those in German and Bistritsa.

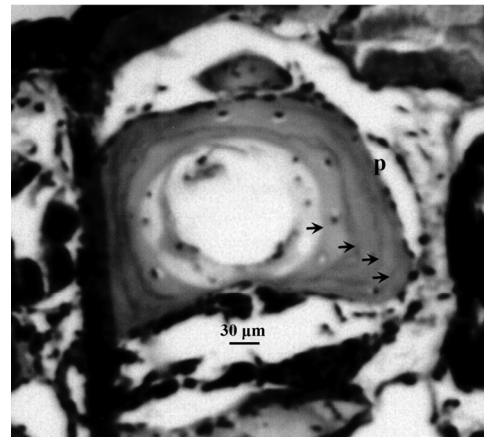
**Age.** Most individuals were between 3 and 5 years old (44%, n=7, 3 males and 4 females) but in some cases a large number of LAGs was counted, which suggests age above 8 years (19%, n=3, 2 males and 1 females). Among the females were observed the two youngest newts (3 yrs.), and among the males – the eldest (11 yrs.) (Fig. 2); the oldest female was 10 yrs., and the youngest male – 4 yr.



**Figure 2.** Individual age, estimated by skeletochronology. Gray indicate males, black – females.

In a recently metamorphosed juvenile (bred from an egg in controlled conditions) was observed a slight line near the marrow cavity; this was not interpreted as a line of metamorphosis.

In elder individuals (over 5 yrs.) we observed both single and double lines, which suggests two periods of arrested growth in the given season. Some specimens exhibited endosteal resorption; by comparing the diameter of the eroded marrow cavities of these specimens with diameter of non-eroded marrow cavities from juveniles we found that resorption destroys only (completely or partially) the first two lines. Endosteal bone deposition was not detected in any of the phalanges (Fig. 3).



**Figure 3.** Diaphyseal cross-section of the phalanx of the third finger of the hind leg in a 5-year-old male *T. ivanbureschi*. Arrows indicate lines of arrested growth (LAGs); p: periphery. The innermost LAG was eroded by endosteal resorption.

We compared our results to other available data on the size and longevity of *Triturus* species (Table 3). In all cases females were larger than males, while the sexual dimorphism index varied to small extent across taxa (0.015-0.154,  $0.073 \pm 0.035$ ; see column DI in Table 3 for details). The sexual size dimorphism index calculated from our data was very similar to studies on *T. karelinii* complex, and it differed slightly from the study of Olgun et al. (2005), which is on a population now considered to be *T. ivanbureschi*. Although a FAMD was significant only for the first axis, which explained 16.61% of the total variance, it did reveal a high correlation for age (correlation coefficients 0.850 for males and 0.822 for females), climatic zone attribution (0.897) and specific status

**Table 3.** Comparison of SVL, dimorphism index (DI), mean age, longevity and age at maturity in several species from the *Triturus* genus. Blank cells indicate missing data in the corresponding source publication. More than one source publication in a row indicates different complementary studies on the same population. For abbreviations of the climatic zones, see Materials and methods. (T.ca.- *Triturus carnifex*, T.cr.- *Triturus cristatus*, T.d.- *Triturus dobrogicus*, T.i.- *Triturus ivanbureschi*, T.k.- *Triturus karelinii*, T.ma.- *Triturus macedonicus*, T.mar.- *Triturus marmoratus*, T.p.- *Triturus pygmaeus*).

Mean SVL (mm)		DI	Mean age (yrs)		Longevity (yrs)		Age at maturity (yrs)	Altitude (m)	Climatic zone	Species	Source
F	M		F	M	F	M					
83	75	0.107	9	8	14	16	4	1282	AS	T.ca.	Maletzky et al. 2004
60.8	58.2	0.045			10	9	2		MN	T.ca.	Campolongo et al. 1989
60.8	58.2	0.045	4.4	4.6	11	9	2		MN	T.ca.	Pagano et al. 1990
84.4	80.4	0.050			18	18		1330	MM	T.ca.	Cavallotto et al. 1995
					11	11	3		C	T.cr.	Meyer 2005, Meyer & Grosse 2006, 2007
72.6	68.9	0.054			16	17	2	270	AC	T.cr.	Miaud et al. 1991, 1993
					8	10	2		AC	T.cr.	Sinsch et al. 2003
69.7	62.5	0.115			14	17	2	0	AC	T.cr.	Francillon-Viellot et al. 1990
70.8	66.6	0.063			13	16	3		C	T.cr.	Hagström 1977
						16	4		AN	T.cr.	Dolmen 1982
67.8					15	15	4		AN	T.cr.	Dolmen 1983
70.9					15		2		B	T.cr.	Dolmen 1983
68.9	65	0.060	3.5	3.2	5	4	2	2	P	T.d.	Cogălniceano & Miaud 2003
75.5	69.5	0.086	3.08	2.92	9	9		160	P	T.d.	Jehle et al. 1995, Ellinger & Jehle 1997
73.7	72	0.024	5.6	5.5	11	8	3	1200	MN	T.i.	Olgun et al. 2005
80.4	73.8	0.089	6.13	7.13	10	11	3	1015	C	T.i.	present study
			7.36	7.5	10	15	5		TMHM	T.k.	Tarkhnishvili & Gokhelashvili 1999
68.98	63.5	0.086	5	5.07	8	9	3	43	MN	T.k. complex	Üzüüm & Olgun 2009
76.25	75.1	0.015	8.3	8.8	13	14	5	400	MM	T.ma.	Kalezić et al. 1994, Cvetković et al. 1996
71.1	66.8	0.064	5	4.4	9	8	2	7	MN	T.mar.	Jakob et al. 2003
68	62.4	0.090			13	14	2	0-5	AC	T.ma.	Francillon-Viellot et al. 1990
71.8	62.2	0.154			12	11	4	550-800	L	T.ma.	Caetano et al. 1985
70.4	64.1	0.098			15	14	5	900-1500	L	T.ma.	Caetano et al. 1985
					12	11	4	550	L	T.ma.	Caetano & Castanet 1993
					15	14	5	1500	L	T.ma.	Caetano & Castanet 1993
43.9	42.3	0.038			10	8	1	5	MS	T.p.	Diaz-Paniagua et al. 1996
83	75	0.107			10	12	3	50	MS	T.p.	Caetano & Castanet 1993

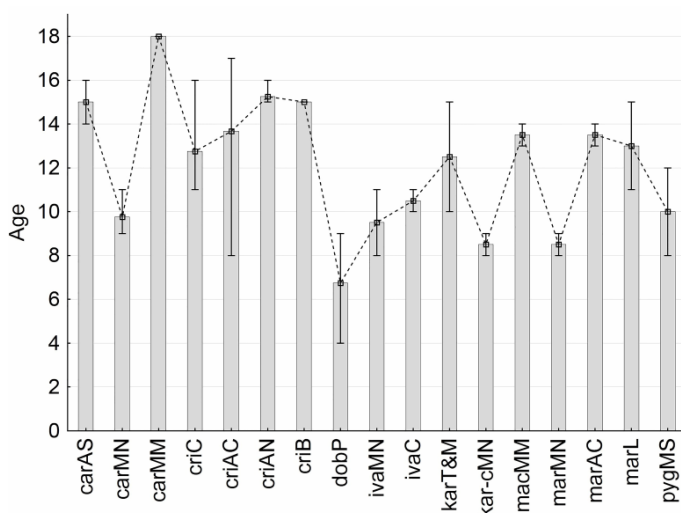
(0.752) (Fig. 4), that generally revealed a strong relationships between them. Longevity was highest in *T. carnifex* from Mediterranean Mountains and lowest in *T. dobrogicus* from the Panonian climate zone. Although in almost all sources males had greater longevity than females, the difference was not statistically significant ( $p=0.710$ ) and consequently we interpreted male and female age as one variable.

**Ontogenetic development.** Stages of the ontogenetic development of *T. ivanbureschi* were compared to other Caudata species (Table 4). Below we provide the stages of development (Fig. 5):

01. Fertilized egg – day 1.

02. Appositions of the neural folds in the spinal cord region and formation of neural tube; the embryo elongates – day 3.

03. The head becomes more distinct and the optic vesicles can be distinguished – day 4.



**Figure 4.** Histogram of age according to climatic zone and species. *Triturus* species are presented with the first three letters of their specific latin name. For abbreviations of the climatic zones, see Materials and methods. Ages for different populations/species correspond to these in Table 3.

04. Beginning of extension; head flexure approximately 100°; appearance of a tail bud – day 5.

05. Clearly visible tail bud; head flexure approximately 140° – day 7.

06. Elongation of tail bud; convexity of head with respect to trunk less pronounced, flexure over 160° – day 8.

07. Disappearance of head flexure; elongation of tail bud – day 9.

08. External gills develop as outwardly directed pockets; response of muscles to mechanical stimulus – day 10.

09. Appearance of melanophores among the somites; elongation of gill pockets – day 11.

10. Accumulation of melanophores; development of dorsal crest; branching of gills – day 13.

11. Accumulation of melanophores; spontaneous muscle movement – day 14.

12. Before hatching; cornea not transparent – day 15.

13. Cornea becomes transparent; formation of anterior limb bud – day 17.

14. Formation of two digits in forelimb; the mouth is still closed and the stomach is filled with vitellus – day 20.

15. Elongation of digits; opening of mouth and beginning of feeding – day 24.

16. Formation of third digit in forelimbs – day 42.

17. Formation of fourth digit in forelimbs; formation and elongation of hind limb buds – day 74.

18. Formation of third digit in hind limbs – day 83.

19. Formation of fifth digit in hind limbs; reduction of external gills – day 104.

## Discussion

The number and size of deposited eggs in amphibians varies widely between different taxa (Kaplan & Salthe 1979), different populations of the same species and different females within one population (Kalezić et al. 1994).

The studied populations inhabit relatively small (Bistritsa) to medium ponds (German) on a relatively high altitude under variable conditions regarding oxygen levels and temperature.

*T. ivanbureschi* occurs across various habitats, with varying conditions in terms of temperature, oxygen consumption and exposure to ultraviolet rays. The significant difference in size between eggs from the two habitats can be due to the fact that the ponds near Bistritsa run dry earlier during the summer months, while the German ponds contain water for longer periods of time. It is established that a thicker jelly protects the egg from ultraviolet rays but hinders oxygen reception (Adams et al. 2001, Palen et al. 2005). It could be suggested that because of the more unstable nature of the ponds near Bistritsa newt eggs need thicker protection against harmful rays at the expense of oxygen permeability. In contrast, the pond near German is relatively stable and increased oxygen reception may be more useful for the survival of the embryos than a heavy protection from the elements.

**Table 4.** Normal development of *T. ioanibureschi* and comparison to those of other Caudata species. Stage numbers in brackets are only rough correspondent to our description of *T. ioanibureschi*.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
<i>T. ioanibureschi</i>																				
<i>T. cristatus</i> (Gluckson 1931)	1	17	21	25	26	27-28	29-30	31	32-33	34-35	36-39	-	36-42	43-45	46	47	50-57	58-61	62-63	
<i>T. carniifex</i> (D' Amen et al. 2007)	1	17	21	25	26	27-28	29-30	31	32-33	34-35	36-39	-	-	-	-	-	-	-	-	-
<i>L. helveticus</i> (Gallien & Bidaud 1959)	0	17	21	25	26	27-28	29-30	-	31-32	33-36	37-39	-	-	-	-	-	-	-	-	-
<i>L. italicus</i> (Tripepi et al. 1998)	1	17	21	24-28	(29-30)	31-32	33	-	(34)	(35-39)	-	-	-	-	-	-	-	-	-	-
<i>L. boscai</i> (Barbadillo 1989)	1	17	21	23	24/25	(26/29)	-	-	-	-	(30-37)	-	-	-	-	-	-	-	-	-
<i>L. vulgarius</i> (Gluckson 1931)	-	-	-	-	-	-	-	-	-	-	-	-	36-42	43-44	45	46	47-55	56-58	59-61	
<i>I. alpestris</i> (Bonacci et al. 2005)	1	17	21-25		26	27-28	29	30-41	-	-	-	42	43			44				
<i>Pleurodeles waltli</i> (Shi & Bocaut 1995)	1	17	20-21	24	25	26-27	28	29	30	31-32	32-33	34	35	36-37	39	41	44	46-48		
<i>Cynops pyrrhogaster</i> (Anderson 1943)	1	17-19	20	21	-	-	-	22	-	-	-	-	-	23	24	-	25	-	-	-
<i>Paranesotriton hongkongensis</i> (Kong & Tong 1986)	1	18	23	24	25	(26)	(26)	(26)	27	(29-30)	(30-31)	-	32	36	-	38	40	42	46	
<i>Ambystoma macrodactylum</i> (Watson & Russell 2000)	-	-	-	-	-	-	-	-	-	-	-	-	1-4	5	6	7	8-12	13-15	16-19	
<i>A. maculatum</i> (Harrison 1969)	1	17	22	28-29	30	32	(34-35)	36-	(37)	(38-39)	(40)	-	(42-43)	-	-	-	-	-	-	-
<i>A. mexicanum</i> (Schreckenber & Jacobson 1975)	1	16-19	21	22-24	25	31	32-33	34	35	37	38	39-	(40)	-	-	-	-	-	-	-

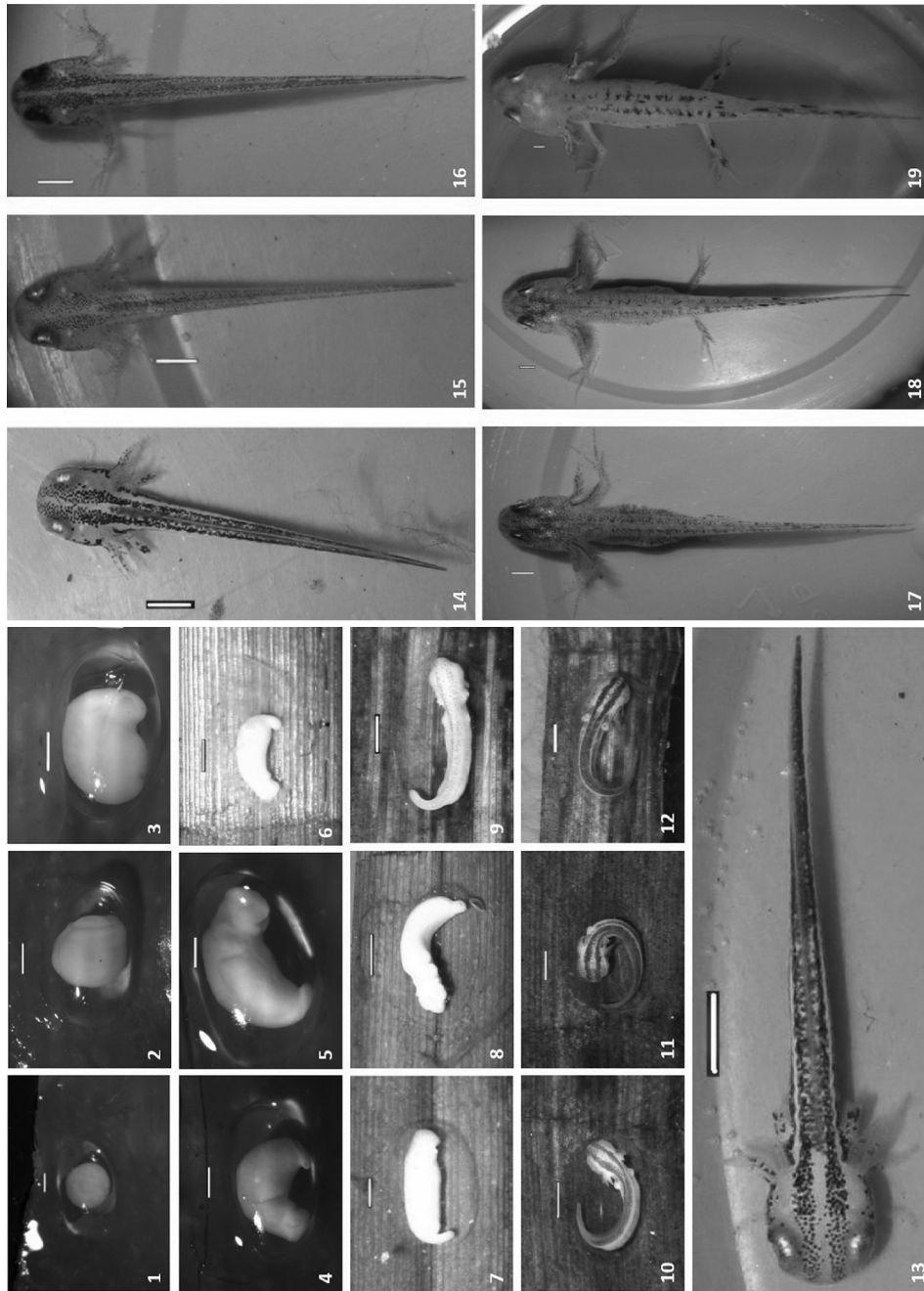


Figure 5. Developmental stages in *T. ivanbureschi*, based on morphological features. Scale bar is equal to 1 mm.

The jelly also protects the egg from mechanical injury and fungal infections while the vitellus volume reflects the amount of nutrients deposited by the female (Duellman & Trueb 1994); this explains the relatively smaller differences between vitellus

volumes and the proportionally larger amount of nutrients in the German eggs.

It has to be noted that in both studied populations egg dimensions are larger than previously reported data on this taxon (e.g. Furtula et al. 2008,



2009). This is possibly due to the fact that both populations inhabit ponds near 1000 m above sea level – the study of Furtula et al. (2008, 2009) included one population from Pirot at 367 m a.s.l.; for some salamanders it is established that egg size increases with altitude (Takahashi & Iwasawa 1990). However, there is a need for further research covering larger altitudinal range before any definitive conclusions could be made. We also have to take into account that SVLs of females (63.4–68.9 mm) from the Pirot (Furtula et al. 2008, 2009) study were significantly smaller compared to ours (60.5–89.2 mm,  $p = 0.007$ ). While female size affects mainly the number of eggs deposited (Wells 2010), it may also have some influence on egg size.

The presence or absence of a metamorphosis line could be related to leaving the water for the harsher conditions of the land in drier climate (Caetano et al. 1985, Francillon et al. 1990, Jacob et al. 2002). Since we did not detect metamorphosis lines in the studied specimens, it can be assumed that there is no clear growth cessation during this physiological transition.

The ways of depositing LAGs are influenced by environmental conditions and for various species, there is data for both hibernation and aestivation lines (Andreone 1990, Caetano et al. 1985, Francillon 1990, Jacob et al. 2002). In the present study only hibernation LAGs were observed as both sites fall in the continental climatic region.

Endosteal resorption was observed in the cross sections of adult *T. ivanbureschi*. This is a common histological process in the long bones of tetrapods, including amphibians (Castanet & Smirina 1990) and it may be linked to environmental conditions (Smirina 1972), but the data on the subject is contradictory – i.e. some authors reported less resorption for populations living in high altitudes than for lowland populations (Esteban et al. 1996, Esteban et al. 1999) and others – the opposite (Caetano & Castanet 1993).

The youngest captured specimens were 3 years of age and since all were sexually mature we concluded that age of maturity was reached no later than the third year. This result is similar to that observed in other species of the genus *Triturus*; the longevity – up to 11 years for females and up to 9 for males – was also similar (Table 3). Since age and size were strongly related to the climatic zone, it could be speculated that populations from regions with more variable climate will have smaller SVL and lower longevity. This could

partly explain the differences in the sexual dimorphism index, with populations living under more adverse conditions having less pronounced size differences between males and females. Local conditions probably also play an important role in this regard. Longevity for *T. dobrogicus*, for example, has been reported as 5 years for population from the Danubian floodplains (Cogalniceanu & Miaud 2003) and 9 years for a population living in a stable parkland habitat near Vienna (Jehle et al. 1997). Studying more populations exposed to various conditions is needed to assess interpopulational variation of these life-history traits in *T. ivanbureschi*. In their research on *Ommatotriton ophryticus* (Kutrup et al. 2005) reveal that the age at maturity was 2 years for lowland (300 m) and 4 years for highland (1300 m) populations, with the latter also having greater mean and maximum longevity – 8 (16), compared to 4 (10); individuals from the lowland population, however, had greater SVL. Whether such dependencies exist in *T. ivanbureschi* is a question that remains to be clarified, as data so far have been inconsistent. Living in permanent or temporary habitats (i.e. breeding habitat) also might influence newt age structure. Diaz-Paniagua et al. (1996) reveal that *T. pygmaeus* living in temporary ponds exhibit both a lower age at maturity (1–2 years) and shorter longevity (9–10) compared to conspecifics from regions with permanent ponds (3–4 and 10–12 years respectively).

The normal development table for *T. ivanbureschi* is consistent with classical staging tables where each stage is defined by a certain morphological state. We note the importance of using written description of each stage in addition to staging photographs. Thus the characteristics that define a certain stage set its beginning boundary and the characteristics defining the following stage set its end boundary with little chance for confusion (i.e. a larva in stage X is considered to be in that stage until the detection of characteristics for stage Y). The staging in this study was based on the appearance/disappearance of structures or easily detectable changes in existing structures – i.e. limbs, digits, balancers, pigmentation. Comparison with newts from other genera, as well as congeneric species, demonstrated that the general pattern of limb development is similar across different groups. The missing stages may highlight variations in morphological development or differences of delineation between staging tables (i.e. D'Amén et al. 2006 only describe development

until hatching, and Watson & Russel 2000 – after hatching). *Triturus karelinii* complex was stated to be with the shortest aquatic stage duration among others genus members - three months (Arntzen 2003). Species from this complex often inhabit temporary ponds, hence the necessity of shorter duration of the larval period. Under optimal conditions, however, this period has a standard duration, as demonstrated by this study.

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## References

- Adams, M., Schindler, D., Bury, R. (2001): Association of amphibians with attenuation of ultraviolet-B radiation in montane ponds. *Oecologia* 128: 519-525.
- Anderson, P. (1943): The normal development of *Triturus pyrrhogaster*. *The Anatomical Record* 86: 59-73.
- Andreone, F. (1990): Variabilità morfologica e riproduttiva in popolazioni di *Triturus alpestris* (Laurenti, 1768) (Amphibi, Urodela, Salamandridae). M.S. thesis, University of Bologna, Bologna, Italy, 331p.
- Arntzen, J. (2003): *Triturus cristatus* Superspezies – Kammolch-Artenkreis. (*Triturus cristatus* (Laurenti, 1768) – Nirdlicher kammolch, *Triturus macedonicus* (Laurenti, 1768) – Italienscher kammolch, *Triturus dobrogicus* (Kiritzescu, 1903) – Donau-Kammolch, *Triturus karelinii* (Strauch, 1870) – Sudlicher kammolch). pp. 421-514. In: Böhme, W. (ed.), *Handbuch der Reptilien und Amphibien Europas*. Band 4/IIA: Schwanzlurche (Urodela) IIA. Wiebelsheim: Aula-Verlag.
- Barbadillo, L. (1989): Descripción del desarrollo embrionario de *Triturus boscai*, Latate 1879 (Urodela, Salamandridae). *Revista Espanola de Herpetologia* 3: 209-220.
- Bonacci, A., Rizzuti, M., Tripepi, S. (2005): *Triturus alpestris inexpectatus*: normal developmental stages morphology and temperature influence. pp. 120-122. In: Ananjeva, N., Tsinenko, O. (eds.), *Herpetologia Petropolitana. Proceedings of the ordinary general meeting of the Societas Europae Herpetologica*. Saint-Petersburg.
- Caetano, M., Castanet, J., Francillon, H. (1985): Détermination de l'âge de *Triturus marmoratus marmoratus* (Latreille 1800) du Parc National de Peneda Gerês (Portugal) par squelettechronologie. *Amphibia-Reptilia* 6: 117-132.
- Caetano, M., Castanet, J. (1993): Variability and microevolutionary patterns in *Triturus marmoratus* from Portugal: age, size, longevity and individual growth. *Amphibia-Reptilia* 14: 117-129.
- Campolongo, P., Francillon-Vieillot, H., Giacoma, C., Rossi, F., Tripepi, S. (1989): Age, growth and longevity of *Triturus carnifex*. In: *Abstracts of the First World Congress of Herpetology*, University of Kent, Canterbury.
- Castanet, J., Smirina, E. (1990): Introduction to the skeletochronological method in amphibians and reptiles. *Annales des Sciences Naturelles. Zoologie et Biologie Animale*, 11: 191-196.
- Cavalloto, L., Ancona, N., Giacoma, C. (1995): La struttura di una popolazione di *Triturus carnifex* insediata nella Riserva Naturale Orientata «Moggetto». *Quaderni della Civica Stazione Idrobiologica di Milano* 19: 119-124.
- Cogălniceanu, D., Miaud, C. (2003): Population age structure and growth in four syntopic amphibian species inhabiting a large river floodplain. *Canadian Journal of Zoology* 81: 1096-1106.
- Cvetković, D., Kalezić, M., Džorović, A., Džukić, G. (1996): The crested newt (*Triturus carnifex*) in the Submediterranean: reproductive biology, body size, and age. *Italian Journal of Zoology* 63: 107-111.
- D'Amen, M., Vignoli, L., Bologna, M. (2007): The effects of temperature and pH on the embryonic development of two species of *Triturus* (Caudata: Salamandridae). *Amphibia-Reptilia* 28: 295-300.
- Diaz-Paniagua, C., Mateo, J., Andreu, A. (1996): Age and size structure of populations of small marbled newts (*Triturus marmoratus pygmaeus*) from Doñana National Park (SW Spain). *Journal of Zoology* 239: 83-92.
- Dolmen, D. (1982): Skeletal growth marks and testis lobulation as criteria for age in *Triturus* spp. (Amphibia) in central Norway. *Acta Zoologica* 63: 73-80.
- Dolmen, D. (1983): Growth and size of *Triturus vulgaris* and *T. cristatus* (Amphibia) in different parts of Norway. *Holarctic Ecology* 6: 356-371.
- Duellman, W., Trueb, L. (1994): *Biology of Amphibians*. The John Hopkins University Press, Baltimore and London.
- Ellinger, N., Jehle, R. (1997): Struktur und Dynamik einer Donaukammolch-Population (*Triturus dobrogicus*, Kiritzescu 1903) am Endelteich bei Wien: ein Überblick über neun Untersuchungsjahre. pp. 133-150. In: Hödl, W., Jehle, R., Gollmann, G. (eds.), *Populationsbiologie von Amphibien. Eine Langzeitstudie auf der Wiener Donauinsel*. Stapfia.
- Esteban, M., Garcia-Paris, M., Castanet, J. (1996): Use of bone histology in estimating the age of frogs (*Rana perezi*) from a warm temperate climate area. *Canadian Journal of Zoology* 74: 1914-1921.
- Esteban, M., Garcia-Paris, M., Castanet, J. (1999): Bone growth and age in *Rana saharica*, a water frog living in a desert environment. *Annales Zoologici Fennici* 36: 53-62.
- Francillon-Vieillot, H., Arntzen, J., Geraudie, J. (1990): Age, growth and longevity of sympatric *Triturus cristatus*, *Triturus marmoratus* and their hybrids (Amphibia, Urodela). A skeletochronological study. *Journal of Herpetology* 24: 13-22.
- Furtula, M., Ivanović, A., Džukić, G., Kalezić, M. (2008): Egg size variation in crested newts from the Western Balkans (Caudata: Salamandridae: *Triturus cristatus* superspecies). *Zoological Studies* 47: 585-590.
- Furtula, M., Todorović, B., Simić, V., Ivanović, A. (2009): Interspecific differences in early life-history traits in crested newts (*Triturus cristatus* superspecies, Caudata, Salamandridae) from the Balkan peninsula. *Journal of Natural History* 43: 469-477.
- Gallien, L., Bidaud, O. (1959): Table chronologique du développement chez *Triturus helveticus* Razoumowsky. *Bulletin de la Société Zoologique de France* 84: 22-32.
- Glücksohn, S. (1931): Äussere entwicklung der extremitäten und stadieneinteilung der larvenperiode von *Triton taeniatus* leyd. und *Triton cristatus* laur. *Wilhelm Roux Archiv. für Entwicklungsmechanik der Organismen* 125: 341-406.
- GRID-Arendal (2014): Climate zones of the Caucasus ecoregion. WWF-Caucasus, design Manana Kurtubadze. <<http://www.grida.no/graphicslib/Search?q=caucasus>>
- Hagström, T. (1977): Growth studies and ageing methods for adult *Triturus vulgaris* L. and *Triturus cristatus* LAUR. (Urodela, Salamandridae). *Zoologica Scripta* 6: 61-68.
- Harrison, R. (1969): *Organization and Development of the Embryo*. Yale University Press, New Haven, CT.
- Horner, H., Macgregor, H. (1985): Normal Development in Newts (*Triturus*) and its arrest as a consequence of an unusual chromosomal situation. *Journal of Herpetology* 19: 261-270.
- Ivanović, A., Üzümlü, N., Wielstra, B., Olgun, K., Litvinchuk, S.N., Kalezić, M.L., Arntzen, J.W. (2013): Is mitochondrial DNA

- divergence of Near Eastern crested newts (*Triturus karelinii* group) reflected by differentiation of skull shape? *Zoologischer Anzeiger* 252: 269-277.
- Jakob, C., Seitz, A., Crivelli, A., Miaud, C. (2002): Growth cycle of the marbled newt (*Triturus marmoratus*) in the Mediterranean region assessed by skeletochronology. *Amphibia-Reptilia* 23: 409-418.
- Jakob, C., Miaud, C., Crivelli, A., Veith, M. (2003). How to cope with periods of drought? Age at maturity, longevity and growth of marbled newts population (*Triturus marmoratus*) in Mediterranean temporary ponds. *Canadian Journal of Zoology* 81: 1905-1911.
- Jehle, R., Hödl, W., Thonke, A. (1995): Structure and dynamics of central European amphibian populations: a comparison between *Triturus dobrogicus* (Amphibia, Urodela) and *Pelobates fuscus* (Amphibia, Anura). *Australian Journal of Ecology* 20: 362-366.
- Kalezić, M., Cvetković, D., Djorović, A., Džukić, G. (1994): Paedomorphosis and differences in life-history traits of two neighbouring crested newt (*Triturus carnifex*) populations. *Herpetological Journal* 4: 151-159.
- Kaplan, R., Salthé, S. (1979): The allometry of reproduction: an empirical view in salamanders. *American Naturalist* 113: 671-689.
- Kong, Y., Tong, T. (1986): The developmental stages of *Paramesotriton hongkongensis* (Myers and Leviton, 1962). *Acta Herpetologica Sinica* 5: 106-118.
- Kuzmin, S. (1999): The Amphibians of the former Soviet Union. Pensoft. Sofia-Moscow. p. 538
- Kutrup, B., Bulbul, U., Yılmaz, N. (2005): Age structure in two populations of *Triturus vittatus ophryticus* at different altitudes. *Amphibia-Reptilia* 26: 49-54.
- Lovich, J., Gibbons, J. (1992): A review of techniques for quantifying sexual size dimorphism. *Growth, Development, and Aging* 56: 269-281.
- Maletzky, A., Pesta, J., Schabetsberger, R., Jehle, R., Sztatecsny, M., Goldschmid, A. (2004): Age structure and size of the syntopic populations of *Triturus carnifex* (Laurenti, 1768), *Triturus vulgaris* (Linnaeus, 1758) and *Triturus alpestris* (Laurenti, 1768) in the lake Ameisensee (1,282 m a.s.l.). *Herpetozoa* 17: 75-82.
- Metzger, M.J., Bunce, R.G., Jongman, R.H., Mücher, C.A., Watkins, J.W. (2005): A climatic stratification of the environment of Europe. *Global Ecology and Biogeography* 14: 549-563.
- Meyer, S. (2005): Untersuchung zur Überlebensstrategie der Kammolchpopulationen (*Triturus cristatus*, LAURENTI 1768) in der Kulturlandschaft Sachsen-Anhalts. Dissertation zur Erlangung des akademischen Grades doctor rerum naturalium (Dr. rer. nat.) vorgelegt an der Mathematisch-Naturwissenschaftlich-Technischen Fakultät der Martin-Luther-Universität Halle-Wittenberg verteidigt am 28.11.2005, 102 p.
- Meyer, S., Grosse, W. (2006): Zur Morphometrie und Altersstruktur des Kammolchs (*Triturus cristatus*) im Raum Halle (Saale) und Ostharz. *Hercynia N. F.* 39: 269-281.
- Meyer, S., Grosse, W. (2007): Populationsgröße, Altersstruktur und genetische Diversität einer Metapopulation des Kammolches (*Triturus cristatus*) in der Kulturlandschaft Sachsen-Anhalts. *Zeitschrift für Feldherpetologie* 14: 9-24.
- Miaud, C. (1991): Essai de synthèse sur les caractéristiques démographiques des tritons du genre *Triturus*. *Bulletin de la société herpétologique de France* 59: 1-18.
- Miaud, C., Joly, P., Castanet, J. (1993): Variation in age structures in a subdivided population of *Triturus cristatus*. *Canadian Journal of Zoology* 71: 1874-1879.
- Miaud, C., Guyétant, R., Faber, H. (2000): Age, size and growth of the Alpine newt, *Triturus alpestris* (Urodela, Salamandridae), at high altitude and a review of lifehistory trait variation throughout its range. *Herpetologica* 56: 135-144.
- Olgun, K., Üzümlü, N., Avci, A., Miaud, C. (2005): Age, size and growth of the southern crested newt *Triturus karelinii* (Strauch 1870) in a population from Bozdag (Western Turkey). *Amphibia-Reptilia* 26: 223-230.
- Pagano, M., Rossi, F., Cavallotto, P., Giacomini, C. (1990): Age determination in *Triturus carnifex*. pp. 181-184. In: Estratto da Atti del VI Convegno Nazionale dell'Associazione Alessandro Ghigi per la Biologia dei Vertebrati, Torino, Italy, 22-24 June 1989. Museo Regionale di scienze Naturali, Torino, Italy.
- Palen, W., Williamson, C., Clauser, A., Schindler, D. (2005): Impact of UV-B exposure on amphibian embryos: linking species physiology and oviposition behavior. *Proceedings of the Royal Society of London B: Biological Sciences* 272: 1227-1234.
- Rakotomalala, R. (2005): TANAGRA: un logiciel gratuit pour l'enseignement et la recherche. *Actes de EGC'2005, RNTI-E-3*. 2: 697-702.
- Takahashi, H., Iwasawa, H. (1990): Egg size variation among various breeding habitats in the salamander, *Hynobius lichenatus*. *Ecological Research* 5: 393-398.
- Tarkhnishvili, D., Gokhelasvili, R. (1999): The Amphibians of the Caucasus. *Advances in Amphibian Research in the former Soviet Union* 4: 1-239.
- Tripepi, S., Peluso, G., Rossi, F. (1998): Egg development of the newt *Triturus italicus* in relation to temperature. *Amphibia-Reptilia* 19: 345-355.
- Tzankov, N., Stojanov, A. (2008): *Triturus cristatus* (Laurenti, 1768): a new species for Bulgaria from its southernmost known localities. *Salamandra* 44: 153-161.
- Schreckenberg, G., Jacobson, A. (1975): Normal stages of development of the Axolotl, *Ambystoma mexicanum*. *Developmental Biology* 42: 391-400.
- Shi, D., Boucat, J. (1995): The chronological development of the urodele amphibian *Pleurodeles waltl* Michahelles. *International Journal of Developmental Biology* 39: 427-441.
- Sinsch, U., Lang, V., Wiemer, R. (2003): Dynamik einer Kammolch-Metapopulation (*Triturus cristatus*) auf militärischem Übungsgelände (Schmittenhöhe, Koblenz): 3. Altersstruktur. *Zeitschrift für Feldherpetologie* 10: 229-244.
- Smirina, E. (1972): Annual layers in bones of *Rana temporaria*. *Zoologicheskij Zhurnal* 51: 1529-1534.
- StatSoft, Inc. 2004. STATISTICA (data analysis software system), version 7.0. www.statsoft.com.
- Üzümlü, N., Olgun, K. (2009): Age and growth of the southern crested newt, *Triturus karelinii* (Strauch 1870) in a lowland population from Northwestern Turkey. *Acta Zoologica Academiae Scientiarum Hungaricae* 55: 55-65.
- Watson, S., Russel, A. (2000): A posthatching developmental staging table for the long-toed salamander *Ambystoma macrodactylum krausei*. *Amphibia-Reptilia* 21: 143-154.
- Wells, K. (2010): *The Ecology & Behavior of Amphibians*. Chicago, IL: The University of Chicago press.
- Wielstra, B., Litvinchuk, S., Naumov, B., Tzankov, N., Arntzen, J.W. (2013a): A revised taxonomy of crested newts in the *Triturus karelinii* group (Amphibia: Caudata: Salamandridae), with the description of a new species. *Zootaxa* 3682: 441-453.
- Wielstra, B., Crnobrnja-Isailović, J., Litvinchuk, S.N., Reijnen, B.T., Skidmore A.K., Sotiropoulos, K., Toxopeus, A.G., Tzankov, N., Vukov, T., Arntzen, J.W. (2013b): Tracing glacial refugia of *Triturus* newts based on mitochondrial DNA phylogeography and species distribution modeling. *Frontiers in Zoology* 10: art.13.
- Wielstra, B., Arntzen, J.B. (2014): Kicking *Triturus arntzeni* when it's down: large-scale nuclear genetic data confirm that newts from the type locality are genetically admixed. *Zootaxa* 3802: 381-388.
- Wilbur, H.M. (1980): Complex life cycles. *Annual Review of Ecology and Systematics* 11: 67-93.
- Wilcox, D., Dove, B., McDavid, D., Greer, D. (2002): ImageTool for Windows. Version 3.00. The University of Texas Health Science Center in San Antonio, UTHSCSA. Available at: <http://compdent.uthscsa.edu/dig/itdesc.html>