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Notes on age-related changes in body size and colorpattern in captive *Triturus dobrogicus* (KIRITZESCU, 1903) (Caudata: Salamandridae)

Altersbedingte Veränderungen in der Körpergröße und im Farbmuster bei in Gefangenschaft gehaltenen *Triturus dobrogicus* (KIRITZESCU, 1903) (Caudata: Salamandridae)

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KURZFASSUNG

An erwachsenen, in Gefangenschaft gehaltenen Donaukammolchen, *Triturus dobrogicus* (KIRITZESCU, 1903), wurden die innerhalb eines Zeitraumes von fünf Jahren aufgetretenen Veränderungen in Körpergröße und Färbung untersucht. Dabei stellten die Autoren Anzeichen eines allometrischen Körperwachstums und eine Abnahme des Wolterstorff-Indexwertes mit zunehmendem Alter fest. Die registrierte Farbmusterveränderung der Bauchseite war deutlich und drückte sich in einer Erweiterung und Zusammenlegung der schwarzen Flecken aus. Diese Beobachtung läßt Zweifel an der Verläßlichkeit von Langzeitstudien an *T. dobrogicus* aufkommen, welche individuelle Identifikationsverfahren auf Basis der ventralen Mustererkennung verwenden. Mögliche unterschiedliche Einflüsse von Freiland- und Laborbedingungen auf Wachstum und Farbmuster der Molche werden diskutiert.

ABSTRACT

Adult Danube Crested Newts, *Triturus dobrogicus* (KIRITZESCU, 1903), were analyzed for changes in body size and coloration that had developed within a period of five years living in captivity. Indications of an allometric body growth and a decrease of the Wolterstorff index value with age were found. The registered change in ventral colorpattern was conspicuous and became manifest in the expansion or merger of the black spots. This casts doubt on the effectiveness of long-term studies of *T. dobrogicus* based on ventral pattern recognition for individual identification. The potential effects of certain differences in natural and laboratory conditions on newt growth and colorpattern are discussed.

KEY WORDS

Amphibia: Caudata: Salamandridae: *Triturus dobrogicus*; growth rate, image recognition, individual pattern recognition, non-invasive marking techniques, Bulgaria

INTRODUCTION

The Danube Crested Newt *Triturus dobrogicus* (KIRITZESCU, 1903), is distributed across Central and Eastern Europe from the floodplains in the Danube river basin to the delta of the Dnieper (Dnipro) river (WIELSTRA et al. 2014). The annual life cycle of the species although not studied in detail, is generally similar to those of other members of the genus *Triturus* RAFINESQUE, 1815 (for an overview see ARNTZEN 2003). *Triturus dobrogicus* is better adapted to the aquatic life than its congeners (ARNTZEN & WALLIS 1999), among which, as a rule, *T. dobrogicus* exhibits the longest aquatic phase of adults. According to JEHLE et al. (1997) in Vienna, Austria, it enters the water in early March and exits in late July (registered during two years of the study) or in mid-October (registered during five years of the study) with the authors providing an average duration of the aquatic phase of “4 to over 6 months”. According to LITVINCHUK & BORKIN (2002) in the Zakarpatie, Ukraine, adult specimens enter the water by the end of February, beginning of March, and exit in July; in Bulgaria, which is the southernmost part of its range, a mass exit from water ponds was observed in October and November in the place Kalimok, District of Ruse (Stojanov et al. 2011).

According to studies in Ukraine, sexual maturity is reached by the age of two years in natural conditions and by the first year in captivity (Litvinchuk & Borkin 2002); Cogălniceanu & Miaud (2003) established the minimum age at maturity to be two years in males and three in females from the lower Danube floodplain in (Romania). Data on *T. dobrogicus* longevity is scarce. The maximum registered age was nine years in Vienna (Austria) (Ellinger & Jehle 1997), while it was five in the lower Danube floodplain of Romania (Cogălniceanu & Miaud 2002).

Studies on the relation between size and age in *T. dobrogicus* are few and based mainly on either one-time measurements of body length and age assessment by skeletochronology (Cogălniceanu & Miaud 2002, 2003) or on the recapture of marked specimens and their classification in age categories (Ellinger & Jehle 1997). These studies aim to clarify the dynamic of the age structure on population level, and so the individual growth rates remain virtually unknown. Lác (1957) and Litvinchuk & Borkin (2009) presented observed changes in some morphometric traits and indices in relation to body size in *Triturus* species, including *T. dobrogicus*.

Regarding colorpattern changes after attaining sexual maturity, *Triturus* newts are rarely studied, and for *T. dobrogicus* these are only mentioned by Litvinchuk & Borkin (2009). On the other hand, the ventral pattern in *Triturus* species (as well as in other amphibian species) is often used for individual recognition in capture-mark-recapture (CMR) studies (for review see Ferner 2007 and Jehle et al. 2011) which determines the need for better understanding of the changes that might occur with aging.

The aim of the current paper is to describe and analyze the changes in body size and ventral colorpattern that occurred during a five year period of captive rearing sexually mature Danube Crested Newts.

MATERIALS AND METHODS

The studied materials included five adult specimens (two males and three females) captured in May 2011 near the village of Batin, Bulgaria (locality published in Naumov & Biserkov 2013). The habitat is a canal, not permanently flown through, overgrown with abundant water and coastal vegetation throughout most of the year; the water surface is densely covered with duckweed and other plants, strongly reducing the light intensity in the central parts of the water body, where newts are usually found. Although detailed observations on the seasonal activity of the newts are lacking, the authors suggest that adult *T. dobrogicus* from this site spend the whole active period in the water and leave it only for hibernation since this locality is in the same geographic region as Kalimok (see Introduction) and the habitats in both localities are the same.

During five years, the newts were kept on the ground floor (indirect sunlight entered the laboratory through a north-facing window at ground level), in an aquarium of the size 60 cm x 30 cm x 35 cm and water temperature of 15-20 °C; they were fed two to three times per week with either live tubificid or lumbricid worms, or frozen mosquito larvae. The exact age of the specimens was not established, but considering that at the moment of capture all were sexually mature, it could be surmised that they were at least two years old when captured.

The following morphometric traits were studied: body length from the tip of the snout to the posterior end of the cloacal opening (L.corp.); tail length from the posterior end of the cloacal opening to the tip of the tail (L.cd.); distance between proximal bases of front and hind legs (D.i.p.); length of front and hind legs from the base (the axillar and inguinal region, respectively) to the tip of the longest toe (P.a. and P.p., respectively); head length from the tip of the snout to the posterior end of the lower jaw (L.cap.); and head width between the posterior ends of the lower jaw (Lt.cap.). Based on these measurements the following ratios were calculated: Wolterstorff Index (WI = P.a./D.i.p.), as well as six more indices that

Table 1: Metric features (in mm) and indices of five *Triturus dobrogicus* (Kiritzescu, 1903), assessed in 2011 and 2016. A unique number (#1 to #5) was given each specimen, derived by sorting them in ascending order according to L.corp. values from 2011. L.corp. − body length from the tip of the snout to the posterior end of the cloacal opening; L.cd. − tail length from the posterior end of the cloacal opening to the tip of the tail; D.i.p. − distance between proximal bases of front and hind legs; P.a. and P.p., respectively − length of front and hind legs from the base (the axillar and inguinal region, respectively) to the tip of the longest toe; L.cap. − head length from the tip of the snout to the posterior end of the lower jaw; Lt.cap. − head width between the posterior ends of the lower jaw; WI − Wolterstorff Index; 2011 and 2016 – values and indices in 2011 and 2016, respectively; n/a − data not available.

Tab. 1: Die 2011 und 2016 erhobenen metrischen Merkmale (in mm) und Indizes von fünf *Triturus dobrogicus* (Kiritzescu, 1903). Die Nummern #1 bis #5 wurden den Individuen nach aufsteigenden L.corp.-Werten des Jahres 2011 zugeordnet. L.corp. – Körperlänge von der Schnauzenspitze zum Hinterrand der Kloakalöffnung; L.cd. – Schwanzlänge vom Hinterrand der Kloakalöffnung zur Schwanzspitze; D.i.p. – Entfernung zwischen den proximalen Vorder- und Hinterbeinbasen; P.a. und P.p. – Länge des Vorder- bzw. Hinterbeins (von der Achsel- bzw. Leistengegend) bis zur Spitze der längsten Phalange; L.cap. – Kopflänge von der Schnauzenspitze zum Hinterrand des Unterkiefers; Lt.cap. – Kopfbreite zwischen den Hinterrändern der Unterkieferäste; WI – Wolterstorff-Index; 2011 und 2016 – Meßwerte und Indizes in den Jahren 2011 und 2016; n/a − Daten nicht verfügbar.

	Specimens - Exemplare									
Features, indices	#1		#2		#3		#4		#5	
Merkmale, Indizes	2011	2016	2011	2016	2011	2016	2011	2016	2011	2016
L.corp.	59.00	73.00	60.00	71.00	62.00	72.00	69.00	77.00	78.00	82.00
L.cd.	41.00	48.00	40.00	47.00	44.00	50.00	49.00	60.00	61.00	63.50
D.i.p.	32.80	42.05	33.15	41.60	34.90	43.20	40.30	49.80	48.10	51.25
P.a.	16.00	18.50	16.00	19.25	16.50	18.75	17.00	19.00	19.00	n/a
P.p.	17.50	21.00	17.75	21.00	18.25	20.75	18.50	20.25	20.50	21.75
L.cap.	9.80	11.20	10.20	11.00	10.00	11.00	10.50	12.00	11.80	13.00
Lt.cap.	7.50	9.00	8.00	9.50	8.00	9.00	8.20	9.20	9.30	10.00
WI (P.a./D.i.p.)	0.49	0.44	0.48	0.46	0.47	0.43	0.42	0.38	0.40	n/a
L.cd./L.corp.	0.69	0.66	0.67	0.66	0.71	0.69	0.71	0.78	0.78	0.77
D.i.p./L.corp.	0.56	0.58	0.55	0.59	0.56	0.60	0.58	0.65	0.62	0.63
P.a./L.corp.	0.27	0.25	0.27	0.27	0.27	0.26	0.25	0.25	0.24	n/a
P.p./L.corp.	0.30	0.29	0.30	0.30	0.29	0.29	0.27	0.26	0.26	0.27
L.cap./L.corp.	0.17	0.15	0.17	0.15	0.16	0.15	0.15	0.16	0.15	0.16
Lt.cap./L.corp.	0.13	0.12	0.13	0.13	0.13	0.13	0.12	0.12	0.12	0.12

represent ratios of the aforementioned traits to the body length. The measurements were taken with a calliper (accuracy of 0.05 mm) and the ventral side of the body was photographed with a digital camera (Canon PowerShot SX230 HS) from a distance of approximately 10 cm. The procedures were carried out two times: in 2011 (soon after the capture of the specimens) and in 2016. All measurements and photos were taken after the newts were sedated with a hydrous solution of benzocaine (concentration 0.3 g/L). The morphometric data are given in Table 1.

The change in body size and proportions (percent growth rate, abbreviated further in the text as PGR) which occurred during the five year period is presented as percentage towards the first measurements and was calculated separately for each trait using the formula $PGR = [(V_{2016} - V_{2011})/V_{2011}]*100$, where V_{2016} = present (2016) value; V_{2011} = past (2011) value.

The possible dependency of growth ($V_{2016} - V_{2011}$) on the initial size (V_{2011}) was tested with a Kendall τ test. For calculations and graphs, Microsoft Excel 2010 ver. 14.0 was used, the statistical tests were computed with Statistica 10 ver. 10.0 (StatSoft. Inc. 1984-2011). Because of the small number of the studied specimens, the genders were not treated separately.

The authors tested two popular image pattern recognition programs – Hotspotter ver 1.0 (Crall et al. 2013) and Wild-ID ver. 1.0 (Bolger et al. 2011) for their ability to correctly identify matching pairs of individuals from photos taken in 2011 and 2016. For this purpose, the computer programs were to scan two sets of photos, one with a general ventral view of the specimens and another set containing only the throat pattern. For each set, once the photos had been loaded into the database of the respective program, a one-by-one comparison was conducted, with the software providing a match score based on its pattern recognition algorithms. The final decision for accepting or rejecting the proposed match was made by the researchers through visual comparison of the matched photographs. Wild-ID employs the SIFT operator (Scale Invariant Feature Transform), which was specifically designed to extract distinctive image features invariant to image scale and rotation (LOWE 2004); the computed score ranges from 0.0 (for low similarity) to 1.0 (for high similarity). In order to identify an animal, Hotspotter needs to be presented with a region of interest from the photo (ROI, in the present case, the ventral or throat pattern of the newt), from which the program proceeds to extract a subimage and to compute a similarity score; the scores tend to vary according to species and size of the image set, with small image sets producing higher scores. In this manner, the authors compared all photos and recorded their respective scores.

RESULTS AND DISCUSSION

Body size and proportions

The established changes (in percent growth rate; PGR) in the morphometric traits and indices of each specimen during the period of five years are presented in Fig. 1. Among all studied traits, the greatest PGR summary value over all five specimens was registered in D.i.p. (107.60 %) and the smallest in L.cap. (56.58 %); the greatest individual value of PGR was 28.20 % (for specimen #1 regarding D.i.p.), and the smallest was 4.10 % (for specimen #5 regarding L.cd.). Regarding body length (L.corp.) the registered growth was between 14 mm (for the specimen that was the smallest initially) and 4 mm (for the specimen that was the largest initially); it has to be noted that the smallest specimen in 2011 (#1) was larger than specimens 2 and 3 in the 2016 measurement (see Table 1). The authors observed a clear negative correlation between the registered growth and the initial body size (Kendall $\tau = -1$, $p < 0.05$). In other words, the body growth rates gradually slowed down from the smallest to the largest newt during the five year study period. Hind legs growth and head width growth changed in a similar way (Kendall $\tau = -1$, $p < 0.05$ and -0.82, $p < 0.05$, respectively). In the other traits, Kendall τ varied from 0 (in L.cap.) to -0.91 (in P.a.) without being statistically significant ($p > 0.05$).

To the authors' knowledge there are virtually no literature data on the individual body growth rates of *T. dobrogicus*. Applying the von Bertalanffy model, COGĂLNICEANU & MIAUD (2002) provided a growth curve for the species based on data from southeastern Romania. As the disturbances caused by the periodic floods of the Danube determine a short life cycle of these newts (COGĂLNICEANU & MIAUD 2002), the growth curve spanned across the short range of five years in total, only three of which referred to sexually mature animals (from the second to the fourth year for males and from the third to the fifth for females). This short time period probably explains the curve demonstrating an almost constant body growth with advancing age during this time period, while the expected slowing of the growth in adults cannot be detected. A more pronounced relation to the classical hypothesis that growth decreases with age was established in a number of other newt species by applying the von Bertalanffy model, e.g., *Triturus cristatus* (LAURENTI, 1768) in France (ARNTZEN 2000), *Triturus karelinii* (STRAUCH, 1870) in Turkey (OLGUN et al. 2005) and *Ichthyosaura alpestris* (LAURENTI, 1768) in France (MIAUD et al. 2000). The character of the present data on *T. dobrogicus* does not allow the use of the von Bertalanffy model, nevertheless, the PGR and the established statistically significant correlation, clearly demonstrate that the smaller (i.e., younger) specimens grew faster than the larger (i.e., older) specimens.

Regarding the studied body proportions, the total absolute PGR value over all five specimens was largest (excluding WI and P.a./L.corp.) for L.cap./L.corp. (28.98 %), and smallest for P.p./L.corp. (7.96 %); the largest individual absolute PGR value was 10.73 % (for specimen #4 regarding D.i.p./L.corp.), and the smallest was 0.02 % (for specimen #2 regarding P.p./L.corp.). There was a general trend towards decrease of WI

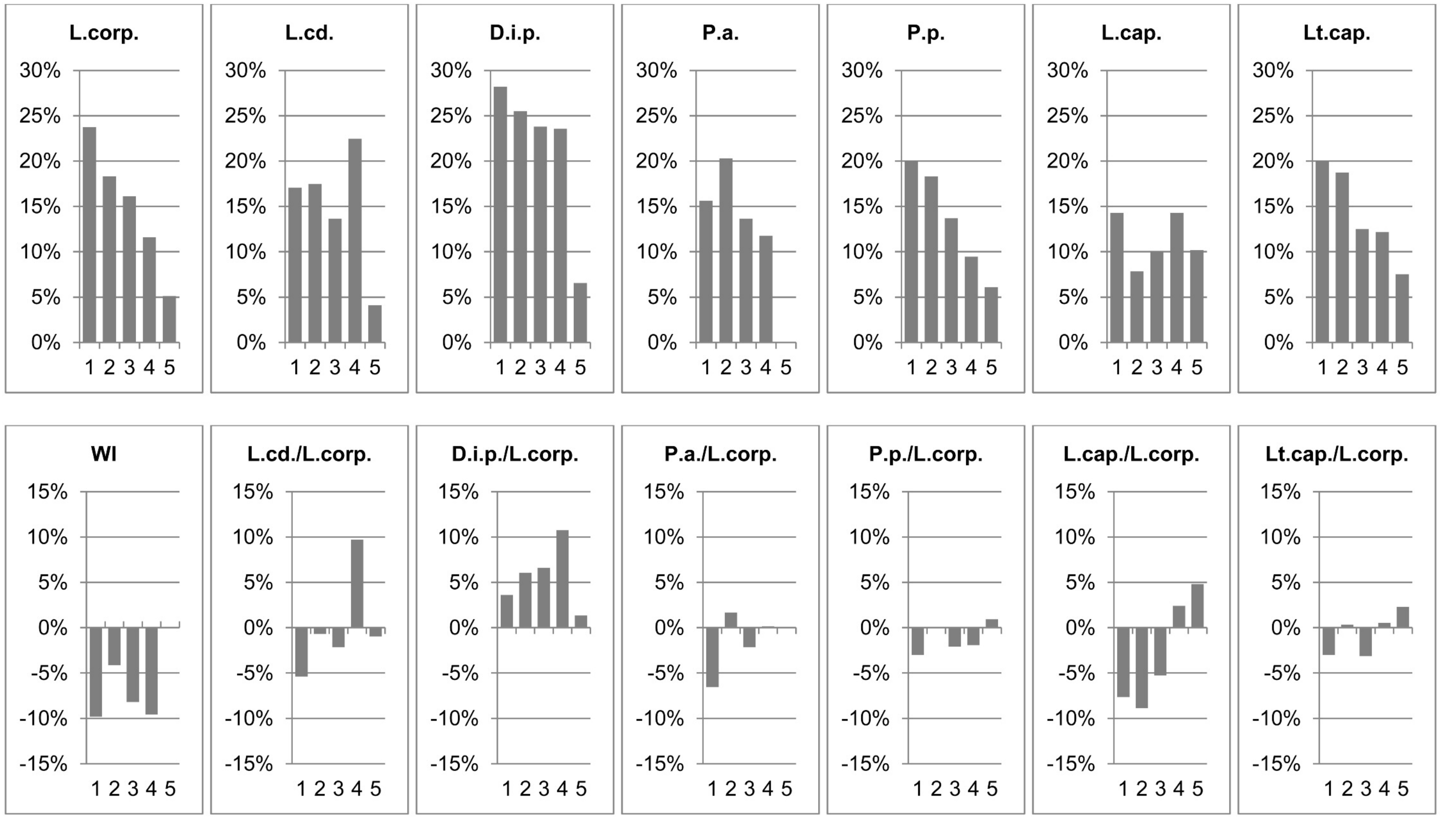

Fig. 1: Percent growth rate (PGR) of the studied traits and indices for specimens #1 to #5 of the Bulgarian sample of *Triturus dobrogicus* (KIRITZESCU, 1903). Numbers of the specimens in ascending order according to the initial value of L.corp. The abbreviations are explained in Materials and Methods and in the legend of Table 1).

Abb. 1: Prozentuale Wachstumsraten (PGR) der untersuchten Merkmale und Indizes der Bulgarischen Exemplare #1 bis #5 von *Triturus dobrogicus* (KIRITZESCU, 1903). Nummern der Proben in aufsteigender Reihenfolge nach dem Ausgangswert von L.corp. Die Abkürzungen sind in der Legende zu Tab. 1 erklärt.

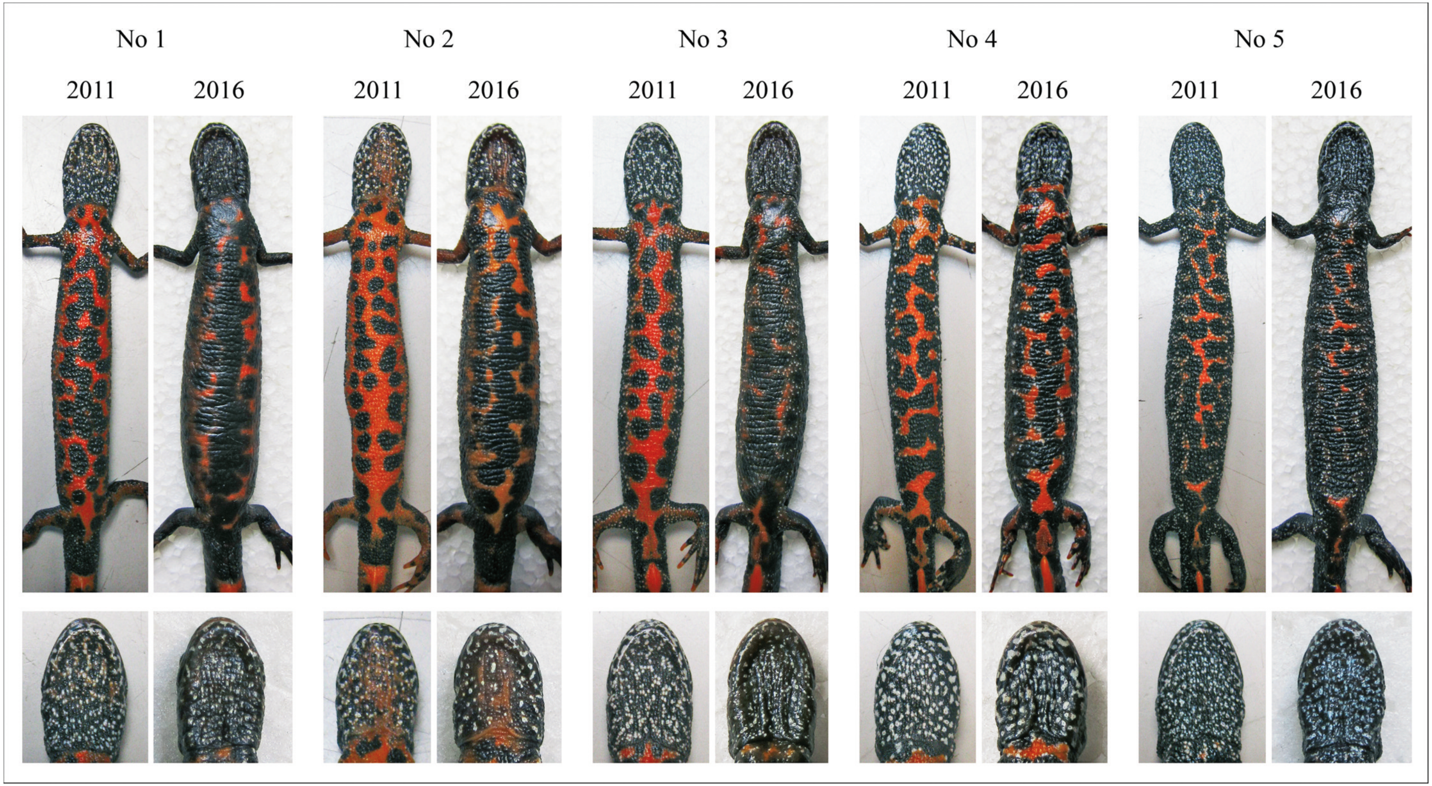

Fig. 2: Photos of belly and throat of the studied specimens #1 to #5 of *Triturus dobrogicus* (KIRITZESCU, 1903), from 2011 and 2016. Specimen numbers correspond to those in Table 1.

Abb. 2: Fotos von Bauch und Kehle der untersuchten Exemplare #1 bis #5 von *Triturus dobrogicus* (KIRITZESCU, 1903) aus den Jahren 2011 und 2016. Nummerierung der Exemplare entsprechend der in Tab. 1.

values and increase of D.i.p./L.corp. values, although in both cases the change did not correlate directly to body growth. In the other indices, the change varied between the different specimens, a common trend was not registered.

The Wolterstorff Index (WI = P.a./D.i.p.) is of significant value in species determination in the genus *Triturus* and especially the so-called "*cristatus*-group" and is mentioned in most modern identification keys (e.g., Kuzmin 1999; Arntzen 2003; Stojanov et al. 2011). According to Lác (1957) for *T. dobrogicus* from Slovakia, the WI values decrease with increasing size in females, but not in males. Analyzing literature data on some allometric variations in body proportions of species from the *cristatus*-group, Arntzen & Wallis (1994) concluded that WI decreases with increase of body size. Litvinchuk & Borkin (2009) studied a large number of specimens from the *cristatus*-group and also established a gradual decrease of WI values with increased body size. The presented data (Fig. 1) confirms the existence of a similar trend in *T. dobrogicus*. After five years, four specimens showed WI values 4 % to 10 % lower than the initial ones (in the fifth specimen the index could not be calculated because of injuries to the longest digits of the front legs). The lower WI values result from the fact that, during growth, the distance between front and hind legs increases at a faster rate than the length of the front legs.

Ventral and throat pattern

Photos of the studied specimens from 2011 and 2016 are presented in Fig. 2. Regarding the ventral pattern there was a clearly visible general trend: expansion and merger of dark spots with increase in body size (age, respectively). Litvinchuk & Borkin (2009) pointed out that in *T. dobrogicus,* with increase in body size, it is common for the dark spots to merge with each other, forming irregular black stripes, and in extreme cases the ventral side could become entirely black with no (or few) orange spots. The present observations support this claim but only after studying a larger sample from different parts of the species range can the question be answered whether this is characteristic for the species as a whole or develops only under certain conditions. Some studies on other European newts demonstrate that the ventral pattern in sexually mature specimens does not change with age. For example, Hagström (1973) who kept *Triturus cristatus* and *Lissotriton vulgaris* (Linnaeus, 1758) in an aquaterrarium for up to six years did not detect changes in the ventral pattern and provided photos of six individuals from both species captured in nature and photographed again after a two-year interval, and there are no visible changes in the ventral pattern.

Regarding the throat pattern there was a decrease of the number or size of the white specks observed in the present study. The yellow-orange pigmentation of the background was still present in specimen #2 (in the central throat region) and in specimen #1, where the traces of such pigmentation (hard to notice in 2011) had vanished; in the other specimens the background color remained invariably black.

The similarity in the ventral pattern between the specimens in 2011 and 2016 can be summarized as follows:

(1) Topography of the black spots: Corresponding elements characterized by their similarity were registered in specimens #5 (at the end of the first third of the belly, as well as near the region anterior to the cloaca), #4 (in the region anterior to the cloaca, as well as in the region between the front legs) and #3 (in the region between the front legs), while specimens #1 and #2 did not show detectable similarities in the patterns from 2011 and 2016.

(2) Topography of the white spots on the throat: Similarities were observed in specimens #2 (in the upper right and in both the lower left and right; right and left refers to the specimen's not the pictures right and left side), #3 (in the lower right) and #4 (in the upper left and lower center), while specimens #1 and #5 did not show detectable similarities in the patterns from 2011 and 2016.

The results from the image recognition programs were inconsistent (Table 2). When comparing the ventral patterns, Wild-ID managed to successfully identify two out of five image matches, while in Hotspotter it was one out of five. This low success rate, combined with the overall low scores of the

Table 2: Results received from the image recognition software products Hotspotter and Wild-ID employed in the comparison of ventral and throat patterns of five specimens (ID # 1 to #5) of *Triturus dobrogicus* (KIRITZESCU, 1903), photographed in 2011 and again in 2016. Match rank – rank of the actual match (verified by the authors) in the list of matches suggested by the program; Score – score of the match according to internal program algorithms. n/a – not in the list of matches; * – correct automatic identification.

Tab. 2: Ergebnisse der Bilderkennungssoftware-Produkte Hotspotter und Wild-ID beim Vergleich von Bauch- und Kehlmustern von fünf Exemplaren (ID #1 bis #5) von *Triturus dobrogicus* (KIRITZESCU, 1903), die zunächst 2011 und dann wieder 2016 photographiert worden waren. Reihung der Übereinstimmung − Reihung der tatsächlichen Übereinstimmung (von den Autoren überprüft) in der Liste der durch das Programm vorgeschlagenen Übereinstimmungen. Score –Punktezahl der Übereinstimmung nach dem programminternen Algorithmus; n/a − nicht in der Liste der programmseitig vorgeschlagenen Übereinstimmungen; * − korrekte automatische Identifikation.

	ID	Hotspotter Match rank / Reihung der Übereinstimmung	Hotspotter Score Punktezahl	Wild-ID Match rank / Reihung der Übereinstimmung	Wild-ID Score Punktezahl
Ventral pattern Bauchmuster					
	#1	n/a	n/a	1*	0.000003*
	#2	n/a	n/a	3	0
	#3	4	10644	5	0
	#4	5	10543	1*	0.028574*
	#5	1*	42219*	4	0.000004
Throat pattern Kehlmuster					
	#1	5	6892	1*	0.000566*
	#2	1*	8671*	1*	0.003002*
	#3	1*	15038*	3	0.002500
	#4	1*	15871*	6	0.000504
	#5	1*	10557*	3	0.001503

matches, suggest that the three cases, when auto-matching was correct, were more likely produced by chance rather than actual correct recognition by the respective algorithm. Comparison of only the throat patterns, however, revealed somewhat different results. While Wild-ID again managed to successfully identify two out of five image matches, Hotspotter correctly assigned four out of five. Maybe the multiple point-by-point system of comparison, used by Hotspotter, provides better results when comparing the less variable throat patterns, even though the scores are lower because of the smaller region of interest.

While data on the accuracy and reliability of photo-identification of amphibians and reptiles is still insufficient, there are studies that produced promising results with Wild-ID. According to BENDIK et al. (2013) pattern recognition by Wild-ID outperformed the more invasive method of visible implant of elastomers in identifying individuals of the salamander *Eurycea tonkawae* CHIPPINDALE, PRICE, WIENS & HILLIS, 2000. The study lasted from 2007 to 2010 and suggested that natural marks of the pattern could increase misidentification rates in longer-term capture-recapture studies. A 16-month study on the conspicuously patterned toad *Melanophryniscus montevidensis* (PHILIPPI, 1902) claims 90 % successful recognition using this software (ELGUE et al. 2014). Recently Wild-ID was successfully used in a three-year study on the newts *Lissotriton vulgaris* and *Ichtyosaura alpestris* from northern Greece (METTOURIS et al. 2016). These authors pointed out that the software ranked all the recaptures of the first year well within the top-ten position (most within top-five); they also stated that "the visual inspection of all within-year and between-years recaptures revealed that the spot patterns of individual newts of both species do not change over time". In their study, the lateral side of the Alpine Newts and the ventral side of the Smooth Newts were photographed. However, the accuracy of software-assisted pattern recognition can differ between species. KENYON et al. (2009) reported low efficiency of photo-identification for the treefrog *Litoria genimaculata* (HORST, 1883), probably due to the similarities in coloration of conspecific individuals or the blurred nature of their colorpattern elements. The part of the animal being photographed could also be important, since some patterns seem to be more characteristic than others. In the present study, the throat pattern produced better results than the ventral pattern. Although to the authors' knowledge Hotspotter has not been used for pattern identification in any amphibian or reptile species, the program might prove useful in capture-release-recapture studies across several years. However, a representative sample, i.e., a greater number of study specimens, is needed to test its ability to successfully recognize throat patterns after prolonged periods.

The observed considerable change in the ventral pattern during a five-years period suggests that in long-term studies of *T. dobrogicus* the method of individual recognition by ventral pattern comparison alone is not sufficiently effective. Similar observations were made for other salamanders, e.g., by BALOGOVÁ (2016) for *Salamandra salamandra* (LINNAEUS, 1758) and WAYE (2013) for *Ambystoma tigrinum* (GREEN, 1825).

In conclusion, it has to be noted that due to the lack of similar published studies on individual ontogenetic changes in *T. dobrogicus*, there is no basis for specific comparisons. It is difficult to estimate the extent to which prolonged captivity under laboratory conditions has influenced the results. Regarding the fact that the studied specimens were constantly kept in an aquatic environment one can point out that the only difference to natural conditions was the absence of hibernation periods (as mentioned above, under natural conditions these newts seem to leave the water only to hibernate). A number of publications demonstrate that, during hibernation, newts do not grow, which is the basis for age determination by skeletochronology. In the present case it can be hypothesized that the growth process was constant during the five years, but there are no reasons to suggest that the lack of hibernation periods would have influenced the growth rate of body parts relative to each other or the colorpattern. Light is another important factor that could have influenced growth and especially colorpattern development. Daylight penetrated the laboratory that housed the newts'aquarium, which, however, was located in a semi-dark installation site; on the other hand, the natural environment of the newts was most probably also ill-lit for most of the time (see Materials and Methods). In this regard, it can be assumed that the difference in ambient light between natural and laboratory conditions was not important. Since many factors such as temperature amplitude, food diversity, level of dissolved oxygen vary to a greater extent under natural, compared to laboratory conditions, the results presented here cannot provide final conclusions but should encourage further studies on this topic.

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REFERENCES

ARNTZEN, J. (2000): A growth curve for the newt *Triturus cristatus*.- Journal of Herpetology, Houston, etc.; 34 (2): 227-232.

ARNTZEN, J. (2003): *Triturus cristatus* Superspezies – Kammolch-Artenkreis (*Triturus cristatus* (LAURENTI, 1768) – Nördlicher Kammolch, *Triturus carnifex* (LAURENTI, 1768) – Italienischer Kammolch, *Triturus dobrogicus* (KIRITZESCU, 1903) – Donau-Kammolch, *Triturus karelinii* (STRAUCH, 1870) – Südlicher Kammolch); pp. 421-514. In: GROSSENBACHER, K. & THIESMEIER, B. (Eds.): Handbuch der Reptilien und Amphibien Europas. Vol. 4/IIA Schwanzlurche (Urodela) IIA; Salamandridae II: *Triturus* I; Wiebelsheim (Aula-Verlag).

ARNTZEN, J. & WALLIS, G. (1994): The 'Wolterstorff Index' and its value to the taxonomy of the Crested newt superspecies.- Abhandlungen und Berichte für Naturkunde, Magdeburg; 17: 57-66.

ARNTZEN, J. & WALLIS, G. (1999): Geographic variation and taxonomy of crested newts (*Triturus cristatus* superspecies): morphological and mitochondrial DNA data.- Contributions to Zoology, Amsterdam; 68 (3): 181-203.

BALOGOVÁ, M. (2016): Changes in dorsal spot pattern in adult *Salamandra salamandra* (LINNAEUS, 1758).- Herpetozoa, Wien; 28 (3/4): 167-171.

BENDIK, N. & MORRISON, T. & GLUESENKAMP, A. & SANDERS, M. & O'DONNELL, L. (2013): Computer-assisted photo identification outperforms visible implant elastomers in an endangered salamander, *Eurycea tonkawae*.- PLoS one [electronic resource], Lawrence; 8 (3): e59424.

BOLGER, D. & VANCE, B. & MORRISON, T. & FARID, H. (2011): Wild ID: pattern extraction and matching software for computer-assisted photographic mark-recapture analysis. WWW internet resource and software available at < http://software.dartmouth.edu/Macintosh/Academic/Wild-ID_1.0.0.zip >.

COGĂLNICEANU, D. & MIAUD, C. (2002): Age, survival and growth in *Triturus dobrogicus* (Amphibia, Urodela) from the lower Danube floodplain; pp. 777-783. In: Proceedings of the 34th International Conference of the International Association for Danube Research, August 26-30, 2002, Tulcea, Romania.

COGĂLNICEANU, D. & MIAUD, C. (2003): Population age structure and growth in four syntopic amphibian species inhabiting a large river floodplain.- Canadian Journal of Zoology, Ottawa; 81: 1096-1106.

CRALL, J. & STEWART, C. & BERGER-WOLF, T. & RUBENSTEIN, D. & SUNDARESAN, S. (2013): HotSpotter - patterned species instance recognition; pp. 230-237. In: Proceedings of the 2013 IEEE Workshop on Applications of Computer Vision (WACV 2013), January 15-17, 2013, Clearwater Beach. WWW internet resource and software available at < http://cs.rpi.edu/hotspotter/ >.

ELGUE, E. & PEREIRA, G. & ACHAVAL-COPPES, F. & MANEYRO, R. (2014): Validity of photo-identification technique to analyse natural markings in *Melanophryniscus montevidensis* (Anura: Bufonidae).- Phyllomedusa, Piracicaba; 13: 59-66.

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, Linz; 51: 133-150.

FERNER, J. (2007): A review of marking and individual recognition techniques for amphibians and reptiles.- Herpetological circular / Society for the study of amphibians and reptiles, Lawrence; 35: 1-72.

HAGSTRÖM, T. (1973): Identification of newt specimens (Urodela, *Triturus*) by recording the belly pattern and a description of photographic equipment for such registrations.- British Journal of Herpetology, London; 4: 321-326.

JEHLE, R. & PAULI-THONKE, A. & TAMNIG, J. & HÖDL W. (1997): Phänologie und Wanderaktivität des Donaukammolches (*Triturus dobrogicus*) an einem Gewässer auf der Wiener Donauinsel.- Stapfia, Linz; 51: 119-132.

JEHLE, R & THIESMEIER, B. & FOSTER J. (2011): The Crested newt. A dwindling pond-dweller; Bielefeld (Laurenti-Verlag), pp. 152.

KENYON, N. & PHILLOTT, A. & ALFORD, R. (2010): Temporal variation in dorsal patterns of juvenile green-eyed tree frogs, *Litoria genimaculata* (Anura: Hylidae).- Herpetological Conservation and Biology; 5: 126-131 [WWW online resource available at < http://www.herpconbio.org/ >].

KUZMIN, S. (1999): The amphibians of the former Soviet Union; Sofia-Moskva (Pensoft), pp. 538.

LÁC, J. (1957): Príspevok k poznaniu geografických rás mloka vel'kého (*Triturus cristatus* LAUR.) na Slovensku a poznámky k ich ekológii.- Biológia, Bratislava; 12 (10): 724-745.

LITVINCHUK, S. & BORKIN, L. (2002): Distribution, ecology and conservation status of the Danube crested newt *Triturus dobrogicus* (Amphibia, Salamandridae) in Ukraine and Moldova.- Vestnik zoologii, Kyiv (Kiev); 36 (3): 35-44. (In Russian, English summary).

LITVINCHUK, S. & BORKIN, L. (2009): Evolution, systematics and distribution of crested newts (*Triturus cristatus* complex) in Russia and adjacent countries. St. Petersburg (Evropeisky Dom Press), pp. 592 + 24 color plates. (In Russian).

LOWE, D. (2004): Distinctive image features from scale-invariant keypoints.- International Journal of Computer Vision, Dordrecht, etc.; 60 (2): 91-110.

METTOURIS, O. & MEGREMIS, G. & GIOKAS, S. (2016): A newt does not change its spots: using pattern mapping for the identification of individuals in large populations of newt species.- Ecological research, Tokyo; 31 (3): 483-489.

MIAUD, C. & GUYETANT, R. & FABER, H. (2000): Age, size, and growth of the alpine newt, *Triturus alpestris* (Urodela: Salamandridae), at high altitude and a review of life-history trait variation throughout its range.- Herpetologica, Lawrence; 56 (2): 135-144.

NAUMOV, B. & BISERKOV, V. (2013): On the distribution and subspecies affiliation of *Triturus dobrogicus* (Amphibia: Salamandridae) in Bulgaria.- Acta Zoologica Bulgarica, Sofia; 65 (3): 307-313.

OLGUN, K. & UZUM, N. & AVCI, A. & MIAUD, C. (2005): Age, size and growth of the southern crested newt *Triturus karelinii* (STRAUCH, 1870) in a population from Bozdağ (Western Turkey).- Amphibia-Reptilia, Leiden; 26: 223-230.

STOJANOV, A. & TZANKOV, N. & NAUMOV, B. (2011): Die Amphibien und Reptilien Bulgariens; Frankfurt am Main (Chimaira), pp. 582 [Frankfurt Contributions to Natural History, Vol. 35].

WAYE, H. (2013): Can a tiger change its spots? A test of the stability of spot patterns for identification of individual tiger salamanders (*Ambystoma tigrinum*).- Herpetological Conservation and Biology, Corvallis; 8 (2): 419-425.

WIELSTRA, B. & SILLERO, N. & VÖRÖS, J. & ARNTZEN, J. (2014): The distribution of the Crested and Marbled newt species (Amphibia: Salamandridae: *Triturus*) - an addition to the new atlas of amphibians and reptiles of Europe.- Amphibia-Reptilia, Leiden; 35: 376-381.

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