Body size, age and population structure of *Triturus carnifex* (Urodela: Salamandridae) in the context of facultative paedomorphosis

Giacomo Bruni[1,*], Giulia Tessa[2], Claudio Angelini[3]

[1] *Vrije Universiteit Brussel, Boulevard de la Plaine 2, 1050 Ixelles, Bruxelles, Belgium. *Corresponding author.* E-mail: giacomo.b90@gmail.com
[2] *Dipartimento di Scienze della Vita e Biologia dei Sistemi, Università degli Studi di Torino, via Accademia Albertina 13, 10123 Torino TO, Italy*
[3] *Via Guglielmo Marconi, 30, 04018 Sezze, Italy*

Abstract. Facultative paedomorphosis occurs rarely in the genus *Triturus* compared to other European newts such as *Ichthyosaura alpestris* and *Lissotriton* spp., with most of observation related to single or few individuals per site. In this paper we report about body size, age and population size of two populations of *Triturus carnifex* with paedomorphs. Since one of the populations consisted of approximately 25% of paedomorphs, this is the first study about a population of large-bodied newts with a conspicuous number of paedomorphic individuals. We found evidences for an ecological causation of paedomorphosis, as well as further support for two recent findings about paedomorphosis: the reduction of sexual size dimorphism and the female-biased sex-ratio within paedomorphs.

Keywords. Italian crested newt, heterochrony, metamorphosis, sexual-size dimorphism, population structure.

Facultative paedomorphosis is a phenotypic polymorphism occurring in several caudate species (Duellman and Trueb, 1994; Denoël et al., 2005). It consists of the retention of larval morphological features by sexually mature individuals in species which usually show a metamorphosed adult stage (Denoël, et al., 2005; Fig. 1). Paedomorphic and metamorphic phenotypes may coexist in the same population. The genetic and physiological mechanisms involved in facultative paedomorphosis are still not entirely clear (Voss et al., 2003), however it is known that this phenomenon can emerge via two different paths: progenesis and neoteny (Denoël and Joly, 2000). In the former, there is an early sexual maturation, while the latter consists of a retardation of the somatic development with a normal gonad maturation rate (Healy, 1974).

There are evolutionary advantages derived from facultative paedomorphosis which can enhance the fitness of the individual, e.g., paedomorphs do not have to bear the seasonal migration to the breeding sites and they can start to breed earlier in the season (Semlitsch and Gibbons, 1985), with subsequent increase in larvae survival chance (Ryan and Plague, 2004).

In addition, in the specific case of progenesis, the precocious sexual maturation allows paedomorphs to reproduce years before the metamorphs of the same age (Denoël and Joly, 2000). The morphological characteristics which separate the two morphs, such as the breathing mechanisms and the feeding apparatus, also entail differences in the use of aquatic habitat and food resources (Denoël et al., 2005). In fact, paedomorphs tend to stay in deep and open waters where they can prey on planktonic invertebrates, instead metamorphs prefer to stay near the surface or along the edge of the water basin, feeding on non-aquatic organisms (Denoël et al., 2005).

Facultative paedomorphosis may lead to some limitations. For instance, paedomorphosis may preclude dis-

Fig. 1. Paedomorphic individuals of *T. carnifex*. (A) Male from SF; (B) Female from AN. Scale bar refers only to picture B.

person to other breeding sites, thus preventing gene flow and giving rise to inbreeding phenomena (Whiteman, 1994). However, facultative paedomorphosis is a plastic process and paedomorphic individuals are able to metamorphose in the presence of challenging environmental conditions like desiccation (Denoël, 2003).

There is evidence that facultative paedomorphosis is promoted by ecological factors such as the depth of the water body, level of dissolved oxygen content (Denoël and Ficetola, 2014), the availability of food resources (Ryan and Semlitsch, 2003), the population density (Harris, 1987), the hydroperiod (Semlitsch et al., 1990), the temperature (Sprules, 1974) and the slope of the water basin banks (Denoël and Ficetola 2014). On the other hand, it is adversely affected by the presence of fish (Denoël et al. 2009a). Denoël and Ficetola (2014) have further pointed out that the presence of appropriate terrestrial habitats nearby does not increase the frequency of metamorphic newts in the population.

In the Salamandridae family, facultative paedomorphosis is typically revealed by the presence of feathery external gills in adult amphibians. In particular, this phenomenon appears more common in small-bodied newts like *Ichthyosaura alpestris (*Denoël et al., 2001) and various species of the genus *Lissotriton* (Denoël, 2006; Bozkurt et al., 2015).

In the large-bodied newts of the genus *Triturus,* paedomorphosis is uncommon and the few reports for *T. macedonicus*, *T. cristatus*, *T. pygmaeus* and *T. dobrogicus* generally are based on a few individuals per population (Kalezić et al., 1994; Dolmen, 1980; Ceacero et al., 2010; Mester et al., 2013). In *T. carnifex* paedomorphosis is
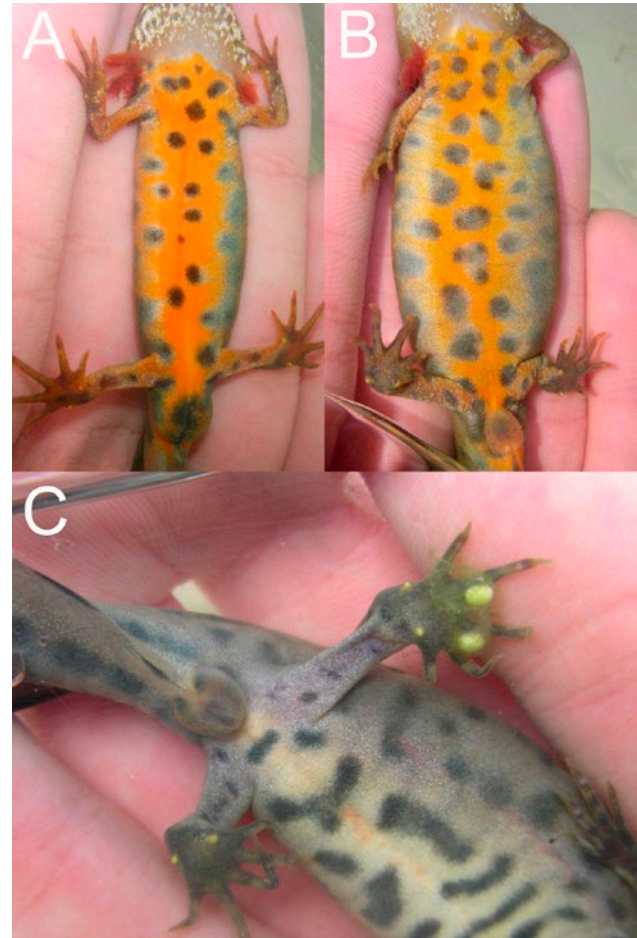

Fig. 2. Ventral view of paedomorphic individuals of *T. carnifex.* (A) Male from SF; (B) Female from SF; (C) Female from AN with two eggs stuck to the right foot.

considered rare and very little information is available about its occurrence (Piazzini et al., 2005; Vanni and Nistri, 2006).

Herein we reported new records of paedomorphosis in *T. carnifex* from two different sites in central Italy and we aim to provide population size and life-history trait of metamorphs and paedomorphs in both sites.

We studied two sites with paedomorphic individuals of *Triturus carnifex* in Tuscany, central Italy: 1) an artificial, permanent pond built in 2008, 35 m a.s.l., volume of ~390 m^3 and a depth of 0.9 m on average, inside the Natura 2000 site SCI-SPA IT51140011, in Sesto Fiorentino municipality (coded here as SF); 2) a natural permanent pool in a xerophytic wood, 655 m a.s.l., volume of about 50 m^3 and a depth of 1 m on average, in Anghiari municipality (coded here as AN). They are also breeding sites for *Lissotriton vulgaris*, neither are known to contain fish. The sites are located about 70 km from each other and

both have water levels that are approximately constant all year round. Newts were sampled by dip-netting from March 2012 to March 2013 at SF (16 capture incidents, one every 26.7 days on average,), and from July 2012 to May 2013 at AN (seven capture incidents, one every 53 days on average); these capture sessions lasted about two hours and were used for the capture-mark-recapture (CMR) study. At SF two funnel traps were also placed in the pond for the whole night before the dip-netting session. Newts were individually marked by photo of the ventral spot pattern, they were sexed and their life stage was recorded (paedomorphic or metamorphosed adult).

We captured in total 203 metamorphs (111 males and 92 females) and 143 paedomorphs (48 males and 95 females) in SF and 141 metamorphs (66 males and 75 females) and 3 paedomorphs (all females) in AN. During the last fieldwork sessions, we measured the snout-vent length (SVL) of 88 newts at SF and of 46 newts at AN (the individuals have been chosen randomly). Paedomorphic individuals were distinguished from overwintering larvae on the basis of cloaca shape (Denoël, 2016; Fig. 2) and by the presence of secondary sexual characteristics like the whitish tail band in males (dorsal crest was not fully developed in any of the observed paedomorphic males and few of them had one or two small crest teeth). Due to the very low number of paedomorphs captured in AN, they were not be included in the analyses. During the last capture session in AN and after it in SF, we surgically removed the third finger from the forelimb storing it in ethanol 70% to be used for the age assessment through scheletochronology (79 newts from SF and 46 from AN; the individuals have been chosen randomly). Newts were immediately released after the in-field procedure.

The skeletochronological analysis was successfully carried out on 35 paedomorphs and 36 metamorphs from SF and 40 metamorphs from AN, followed the standard protocol used for other large newts with some adaptations (Miaud et al., 1993). Muscles and skin were removed and the phalanx was washed in distilled water. Phalanges were decalcified in 5% nitric acid for approximately 1.5 hours, cross-sectioned at 16 mm with a cryostat and stained with Ehrlich's haematoxylin for 15 minutes. Sections were observed under a light microscope by two independent researchers and then imaged with a digital camera. We identified the lines of arrested growth (LAGs) which are caused by seasonal growth patterns (Castanet et al., 1993). Evaluation of bone resorption (Castanet and Smirina, 1990), and consequently of the resorption of inner LAGs, was carried out through osteometric analysis (Guarino et al., 2004), comparing the medium diameter of sections' external margin of younger specimens, with the medium diameter of medul-

lar cavity of older specimens. As the first LAG was partially resorbed but visible in all the samples, no additional LAGs were added to the count of the estimated age.

Age and SVL data were used to compare populations and life-stages. Due to the low number of paedomorphs we captured at AN, we ran two different analyses. Firstly, we compared metamorphs from both populations and secondly, we compared life-stages within SF. In both cases, we used a two-way ANOVA to evaluate the effect of (1) population of origin and sex and (2) life-stage and sex on age and size. Then, we ran two separate ANCOVA analyses to evaluate the effect of the same factors listed above on individual size while accounting for age. Due to significant interaction between the covariate age and the main factors, we used an independent slope design for both analyses. All the variables met the requirements of parametric statistics except age, thus we log-transformed it before of the analyses. We also compared the degree of sexual size dimorphism within the two metamorphic populations and the paedomorphs by calculating their respective size dimorphism index (SDI = [(mean SVL of females/mean SVL of males) - 1]) (Lovich and Gibbons, 1992).

The software STATISTICA 7 (StatSoft Inc.) was used for these analyses. Population size of metamorphs and paedomorphs at both sites were estimated using the capture-mark-recapture data and the superpopulation approach of the Jolly-Seber model (Wagner et al., 2011). The same data have already been used for a survival analyses in the framework of the Cormack-Jolly-Seber model (Bruni and Angelini, 2016). From this analyses it was clear that metamorphs and paedomorphs have similar survival over time in the SF populations, and that the capture probability was constant but different between the life-stages [phi(t)p(.*l)] (AICc weight = 0.998). Thus, for the current analysis, we assumed the same parametrization as above for survival and capture probability and allowed to the probability of entering the population (pent) to vary by time and to be dependent or independent from life-stage. Consequently, we obtained two candidate models to be fitted for obtaining the population sizes of metamorphs and paedomorphs split based on sex (four superpopulations). For AN population, we obtained model uncertainty in the former analysis, thus in the current analysis we ran models with the same parametrization for survival (i.e., varying by time or constant and depending or not on sex) and capture probabilities (time varying or constant), and we allowed to pent to time vary, thus obtaining eight candidate models for obtaining the population sizes of males and females (two superpopulations). Model fitting has been done via the software MARK (White and Burnham, 1999) and model selection

is based on the Akaike Information Criterion corrected for small sample size (AICc).

Information on age and SVL of the individuals belonging to SF and AN populations are summarized in Table 1, taking into account their life-stage and sex.

Table 2 reports the two-way AN(C)OVA tables for the effects "population", "sex" and their interaction, based only on metamorphs from AN and SF. Metamorphs are larger at AN, and adult females are larger than males; however, both differences depended on individual age (used as covariate), since the separate slope design did not detect any significant effects. A post-hoc test (unequal N HSD) after the separate slope design ANCOVA showed that females from SF are larger than SF males, but significantly smaller than females from AN, and they are similar to AN males. Table 3 reports the two-way AN(C)OVA tables for the effects "life stage", "sex" and their interaction, based on paedomorphs and metamorphs from SF only. At SF, metamorphs are older and larger than paedomorphs. Even after taking into account individual age, the separate slope design detected size differences between the life stages.

The SDI index was always female biased: AN metamorphs: 0.079, SF metamorphs: 0.032, SF paedomorphs: 0.018. The best JS model for SF [phi(t)p(.*l)pent(t*l), model weight = 1] estimated that during the study period there were: 407 metamorphosed males (95% C.I.: 287.9-576.2), 336 metamorphosed females (95% C.I.: 236.1-478.7), 82 paedomorphic males (95% C.I.: 65.2-103), 162 paedomorphic females (95% C.I.: 135.4-196.9). The best JS model for AN [phi(t)p(t)pent(t), model weight = 0.92] estimated that during the study period there were: 77 metamorphosed males (95% C.I.: 72.5-82.4) and 88 metamorphosed females (95% C.I.: 82.3-93.4).

Facultative paedomorphosis is a well-known phenomenon in newts and salamanders (e.g., Denoël et al., 2005), however it occurs rarely in the genus *Triturus* (Dolmen, 1980; Kaledzić et al., 1994; Denoël et al., 2007; Ceacero et al., 2010; Mester et al., 2013) and especially in *T. carnifex,* though information is mostly based on anecdotal observations (Lanza, 1983; Piazzini et al., 2005; Vanni and Nistri, 2006). This study represents the first evidence of a population of this species with a substantial number of paedomorphic individuals. SF was built in spring 2008, and soon some larvae of the year from neighbouring temporary sites were transferred into it (these neighbour sites were going to be demolished for building). Interestingly, the oldest paedomorphic newt is 6 years old, that is the same age of the pond. As LAGs are also visible in the larval period (Lima et al., 2001) and every LAG correspond to the low metabolism period (winter), some of the transferred larvae were hatched not later than Autumn 2007 (in the area where SF is located oviposition may exceptionally begin in November [Bruni, pers. obs.]), these newts at the time of sampling were in their 6th year of life. Furthermore, the presence of metamorphosed newts far older than seven years testifies to immigration from neighbour sites.

Due to the hydroperiod of the ephemeral pools, none of neighbour sites hosted paedomorphic newts (the closest site with paedomorphic *T. carnifex* was about 2 km far from SF and it was demolished in 1997 [M. Petrolo, pers. comm.]). Thus, we have strong evidence of specific ecological conditions for SF that cause paedomorphosis. Further support for the role played by ecology comes from the lower density at SF (2.5 individual/m^3, including both metamorphs and paedomorphs) compared to AN (3.3 metamorph/m^3), low density is a favourable aquatic condition for paedomorphosis (Harris, 1987; Semlitsch, 1987). We also found that a very low number of paedo-

Table 1. Age and size (SVL) of *T. carnifex* from populations SF and AN, divided by life-stage and sex.

Population	Life-stage	Sex	Age (mean ± SD; N° and range)	SVL (mean ± SD; N° and range)
SF	Metamorphs	Male	6.6 ± 0.7 n = 16, 4 – 15	6.2 ± 0.1 n = 18, 5.6 – 7.3
		Female	6.1 ± 0.4 n = 20, 3 – 11	6.4 ± 0.1 n = 26, 5.5 - 8
	Paedomorphs	Male	4.8 ± 0.1 n = 15, 4 – 6	5.6 ± 0.1 n = 15, 5.3 - 6
		Female	5.2 ± 0.1 n = 20, 4 – 6	5.7 ± 0.1 n= 29, 5 – 6.2
AN	Metamorphs	Male	5.9 ± 0.5 n = 17, 3 – 11	6.3 ± 0.1 n = 21, 5.3 – 7
		Female	7 ± 0.5 n = 23, 3 – 14	6.8 ± 0.1 n = 25, 5.3 – 7.9

Table 2. AN(C)OVA table for the effects "population", "sex" and their interaction ("population*sex") on age and size (SVL) of metamorphosed *T. carnifex* from populations SF and AN.

	Population	Sex	Population*sex
ANOVA			
Age	$F_{1,72} = 0.01$, P = 0.92	$F_{1,72} = 0.42$, P = 0.52	$F_{1,72} = 1.74$, P = 0.19
SVL	$F_{1,86} = 4.25$, P < 0.05	$F_{1,86} = 11.14$, P < 0.01	$F_{1,86} = 1.25$, P = 0.27
ANCOVA (separate slope design)			
SVL (covariate: age)	$F_{1,68} = 0.5$, P = 0.48	$F_{1,68} = 1.37$, P = 0.24	$F_{1,68} = 0.01$, P = 0.98

Table 3. AN(C)OVA table for the effects "life-stage", "sex" and their interaction ("life-stage*sex") on age and size (SVL) of *T. carnifex* from populations SF.

	Life stage	Sex	Life stage*sex
ANOVA			
Age	$F_{1,67} = 9.73$, p < 0.01	$F_{1,67} = 0.09$, p = 0.77	$F_{1,67} = 1.36$, p = 0.25
SVL	$F_{1,84} = 52.03$, p < 0.01	$F_{1,84} = 3.43$, p = 0.07	$F_{1,84} = 0.87$, p = 0.35
ANCOVA (separate slope design)			
SVL (covariate: age)	$F_{1,63} = 6$, p < 0.05	$F_{1,63} = 1.05$, p = 0.31	$F_{1,63} = 0.16$, p = 0.69

morphs has been found in AN, and, although both sites hosted populations of *L. vulgaris*, only SF hosted paedomorphic *L. vulgaris* (Caffara et al., 2014). The importance of the environment is also evident when considering the relationship between age and size of different morphs. Paedomorphic newts were younger and smaller than metamorphs of the same population; furthermore, the ANCOVA analysis showed that the size difference was not dependent on age, but likely on different growth patterns. Such patterns are consistent with the progenesis responsible for paedomorphosis (Denoël and Joly, 2000). Further, the same authors list some environmental features of SF, namely: low elevation, absence of fish and shallow water, as favourable to progenesis.

In SF, paedomorphic newts are less than half the number of metamorphs. Where paedomorphs and metamorphs of the same species coexist, the proportion between morphs may differ significantly, and it depends on the interplay between environment and genotype (Denoël et al., 2005). For example, Denoël and Ficetola (2014) found that the percentage of paedomorphs in populations of *L. helveticus* varied between 2% and 96%. Additionally, in the context of facultative paedomorphosis, the sex-ratio may vary between populations; however, it is expected that the sex ratio is male-biased within paedomorphs (continued presence in ponds increases reproductive frequencies) and female-biased within metamorphs (egg production requires higher energy income than available in permanent ponds; Whiteman, 1997; Denoël et al., 2005). Such pattern is confirmed in the genus *Triturus* for *T. macedonicus*: Kaledzić et al. (1994) found only paedomorphic males. Interestingly, in SF, metamorphs males outnumbered the female counterpart, but female paedomorphs are about twice male paedomorphs. Female-biased sex-ratios have been found in other paedomorphic populations (see Denoël et al., 2005), and also recently obtained experimental support (Mathiron et al., 2017), with paedomorphic males that metamorphose earlier in response to drying conditions.

SSD is usually female-biased in *T. carnifex* (e.g., Malmgren and Tholleson, 1999; Ficetola et al., 2010). Interestingly, the populations we studied show low SSD (see tables). We found that metamorphs from AN possessed the highest SDI index, despite AN and SF metamorphs having similar body sizes overall. The lowest SDI index was found to be that of SF paedomorphs, this is consistent with the expectation that paedomorphosis reduces SSD in female-biased SSD species (Denoël et al. 2009b). We wonder whether the low SSD of SF metamorphs depends on some ecological factors (e.g., SDI increases in cold climate (Ficetola et al., 2010) while SF is at very low altitude), or whether such low SSD is itself due to facultative paedomorphosis. That is to say, perhaps when paedomorphs undergo metamorphosis they do not develop different sizes dependant on their sex. In order to address this question we suggest two main lines of further research; one, to investigate the growth patterns of paedomorphs following metamorphosis and two, to quantify the relative population size of paedomorphic individuals that undergo metamorphosis.

REFERENCES

Bozkurt, E., Olgun, K., Wielstra, B. (2015): First record of facultative paedomorphism in the Kosswig's newt *Lissotriton* (*vulgaris*) *kosswigi* (Freytag, 1955) (Urodela; Salamandridae), endemic to northwestern Turkey. Turk. J. Zool. **39**: 976-980.

Bruni, G., Angelini, C. (2016): No short term effect of *Clinostomum complanatum* (Trematoda: Digenea: Clinostomatidae) on survival of *Triturus carnifex* (Amphibia: Urodela: Salamandridae). Acta Herpetol. **11**: 75-79.

Caffara, M., Bruni, G., Paoletti, C., Gustinelli, A., Fioravanti, M.L. (2014): Metacercariae of *Clinostomum complanatum* (Trematoda: Digenea) in European newts *Triturus carnifex* and *Lissotriton vulgaris* (Caudata: Salamandridae). J. Helminthol. **88**: 278-285.

Castanet, J., Francillon-Vieillot, H., Meunier, F.J., De Riqles, A. (1993): Bone and individual aging. In: Hall B.K., Bone: Bone growth, pp. 245-283. CRC Press, Boca Raton, FL.

Castanet, J., Smirina, E.M. (1990): Introduction to the skeletochronological method in amphibians and reptiles. Ann. Sci. Nat. Zool. Biol. Anim. **13**: 191-196

Ceacero, F., Donaire-Barroso, D., García-Muñoz, E., Beltrán, J. F., Tejedo, M. (2010): On the occurrence of facultative paedomorphosis in the three newt species of Southern Iberian Peninsula (Amphibia, Salamandridae). Amphibia-Reptilia **31**: 571-575.

Denoël, M. (2003): How do paedomorphic newts cope with lake drying? Ecography **26**: 405-410.

Denoël, M. (2006): Seasonal variation of morph ratio in facultatively paedomorphic populations of the palmate newt *Triturus helveticus*. Acta Oecol. **29**: 165-170.

Denoël, M. (2016): On the identification of paedomorphic and overwintering larval newts based on cloacal shape: review and guidelines. Curr. Zool. 63: 165-173.

Denoël, M., Duguet, R., Dzukic, G., Kalezic, M., Mazzotti, S. (2001): Biogeography and ecology of paedomorphosis in *Triturus alpestris* (Amphibia, Caudata). J. Biogeogr. **28**: 1271-1280.

Denoël, M., Ficetola, G. F. (2014): Heterochrony in a complex world: disentangling environmental processes of facultative paedomorphosis in an amphibian. J. Anim. Ecol. **83**: 606-615.

Denoël, M., Ficetola, G.F., Ćirović, R., Radović, D., Džukić, G., Kalezić, M.L., Vukov, T.D. (2009a): A multi-scale approach to facultative paedomorphosis of European newts (Salamandridae) in the Montenegrin karst: distribution pattern, environmental variables, and conservation. Biol. Conserv. **142**: 509-517.

Denoël, M., Ivanović, A., Džukić, G., Kalezić, M. L. (2009b): Sexual size dimorphism in the evolutionary context of facultative paedomorphosis: insights from European newts. BMC Evol. Bio. **9**: 278.

Denoël, M., Joly, P. (2000): Neoteny and progenesis as two heterochronic processes involved in paedomorphosis in *Triturus alpestris* (Amphibia: Caudata). P. Roy. Soc. Lond. B Bio. **267**: 1481-1485.

Denoël, M., Joly, P., Whiteman, H.H. (2005): Evolutionary ecology of facultative paedomorphosis in newts and salamanders. Biol. Rev. **80**: 663-671.

Dolmen, D. (1980): Distribution and habitat of the smooth newt, *Triturus vulgaris* (L.), and the warty newt, *T. cristatus* (Laurenti) in Norway. In: Proc. Euro. Herp. Symp., pp. 127-139. CWLP, Oxford.

Duellman, W.E., Trueb, L. (1994): Biology of Amphibians. The John Hopkins University Press, Baltimore, Maryland.

Ficetola, G.F., Scali, S., Denoël, M., Montinaro, G., Vukov, T.D., Zuffi, M.A., Padoa-Schioppa, E. (2010): Ecogeographical variation of body size in the newt *Triturus carnifex*: comparing the hypotheses using an information-theoretic approach. Global Ecol. Biogeogr. **19**: 485-495.

Guarino, F.M., Di Maio, A., Caputo, V. (2004): Age estimation by phalangeal skeletochronology of *Caretta caretta* from Mediterranean Sea. Ital. J. Zool. **71**: 175-179.

Healy, W.R. (1974): Population consequences of alternative life histories in *Notophthalmus v. viridescens*. Copeia **1974**: 221-229.

Harris, R.N. (1987): Density-dependent paedomorphosis in the salamander *Notophthalmus viridescens dorsalis*. Ecology **68**: 705-712.

Kalezić, M.L., Cvetković, D., Djorović, A., Džukić, G. (1994): Paedomorphosis and differences in the life-history traits of two neighbouring crested newt (*Triturus carnifex*) populations. Herpetol. J. **4**: 151-151.

Lanza, B. (1983): Guide per il riconoscimento delle specie animali delle acque interne italiane: Anfibi, Rettili (Amphibia, Reptilia). Collana del Progetto finalizzato "Promozione della qualità dell'ambiente", AQ/1/205. Consiglio Nazionale delle Ricerche, Roma

Lima, V., Arntzen, J.W, Ferrand, M.N. (2001): Age structure and growth pattern in two populations of the golden-striped salamander *Chioglossa lusitanica* (Caudata, Salamandridae). Amphibia-Reptilia **22**: 55-68.

Lovich, J.E., Gibbons, J.W. (1992): A review of techniques for quantifying sexual size dimorphism. Growth Develop. Aging **56**: 269-269.

Malmgren, J.C., Thollesson, M. (1999): Sexual size and shape dimorphism in two species of newts, *Triturus cristatus* and *T. vulgaris* (Caudata: Salamandridae). J. Zool. **249**: 127-136.

Mathiron, A.G., Lena, J.P., Baouch, S., Denoël, M. (2017): The 'male escape hypothesis': sex-biased metamorphosis in response to climatic drivers in a facultatively paedomorphic amphibian. P. Roy. Soc. B-Biol. Sci. **284**: 20170176.

Mester, B., Cozma, N.J., Puky, M. (2013): First observation of facultative paedomorphosis in the Danube crested newt (*Triturus dobrogicu*s Kiritzescu, 1903) and the occurrence of facultative paedomorphosis in two newt species from soda pans of the Danube-Tisza Interfluve (Kiskunság National Park, Hungary). Norw. J. Zool. **9**: 443-445.

Miaud, C., Joly, J., Castanet, J. (1993): Variation in age structures in a subdivided population of *Triturus cristatus*. Can. J. Zool. **71**: 1874-1879.

Piazzini, S., Favilli, L., Manganelli, G. (2005): L'erpetofauna del SIR 120 "Monte Penna, Bosco della Fonte e Monte Civitella" (Castell'Azzara-Sorano, Grosseto) (Toscana meridionale). Atti del Museo di Storia Naturale della Maremma **21**: 15-24.

Ryan, T.J., Plague, G.R. (2004): Hatching asynchrony, survival, and the fitness of alternative adult morphs in *Ambystoma talpoideum*. Oecologia **140**: 46-51.

Ryan, T.J., Semlitsch, R.D. (2003): Growth and the expression of alternative life cycles in the salamander *Ambystoma talpoideum* (Caudata: Ambystomatidae). Biol. J. Linn. Soc. **80**: 639-646.

Semlitsch, R.D. (1987): Paedomorphosis in *Ambystoma talpoideum*. Effects of density, food, and pond drying. Ecology **68**: 994-1002.

Semlitsch, R.D., Gibbons, J.W. (1985): Phenotypic variation in metamorphosis and paedomorphosis in the salamander *Ambystoma talpoideum*. Ecology **66**: 1123-1130.

Semlitsch, R.D., Harris, R.N., Wilbur, H.M. (1990): Paedomorphosis in *Ambystoma talpoideum*: maintenance of population variation and alternative life-history pathways. Evolution **44**: 1604-1613.

Sprules, W.G. (1974): Environmental factors and the incidence of neoteny in *Ambystoma gracile* (Baird) (Amphibia: Caudata). Can. J. Zool. **52**: 1545-1552.

Vanni S., Nistri A. (2006): Atlante degli Anfibi e dei Rettili della Toscana. Regione Toscana. Museo di Storia Naturale dell'Università degli Studi di Firenze Sezione di Zoologia "La Specola", Firenze.

Voss, S.R., Prudic, K.L., Oliver, J.C., Shaffer, H.B. (2003): Candidate gene analysis of metamorphic timing in ambystomatid salamanders. Mol. Ecol. **12**: 1217-1223.

Wagner, N., Pellet, J., Lötters, S., Schmidt, B.R., Schmitt T. (2011): The superpopulation approach for estimating the population size of 'prolonged' breeding amphibians: Examples from Europe. Amphibia-Reptilia **32**: 323-332.

White, G.C., Burnham, K.P. (1999): Program MARK: survival estimation from populations of marked animals. Bird Study **46**: 120-139.

Whiteman, H.H. (1994): Evolution of facultative paedomorphosis in salamanders. Q. Rev. Biol. **69**: 205-221.

Whiteman, H.H. (1997): Maintenance of polymorphism promoted by sex-specific fitness payoffs. Evolution **51**: 2039-2044.