

Age, size and growth of the southern crested newt *Triturus karelinii* (Strauch 1870) in a population from Bozdag (Western Turkey)

Kurtulus Olgun¹, Nazan Uzun¹, Aziz Avci¹, Claude Miaud²

Abstract. We studied the characteristics of bone growth assessed by skeletochronology in a southern crested newt *Triturus karelinii* (Urodela) population from Western Turkey. The timing and patterns of bone arrested growth were observed using the phalanges of juveniles and adults that were caught in spring at a breeding site. A metamorphosis line was found in the juveniles. In some adults, a classical succession of single lines of arrested growth was observed in about 50% of the cross sections. However, the other adults exhibited a succession of double lines of arrested growth in their phalanges. Due to the arid summer and cold winter climate in the Bozdag region (1200 m a.s.l.), we argue that in this last case, the lines of arrested growth were deposited during both the summer (aestivation) and winter (wintering). Body length, age and growth were similar in males and females. The age of maturity was 3 to 4 years old and longevity was 8 and 11 years in males and females respectively. Body length and age among taxonomically related large bodied newts of the *T. cristatus* complex were reported from populations experiencing various environmental conditions. Body length and age at maturity were similar to that observed in other newt species. However, longevity seems to be lower than expected in the *T. karelinii* studied population. We hypothesized that the arid climate of Bozdag could cause a higher mortality risk during the terrestrial phase of the life cycle. Studying more populations exposed to various conditions is clearly needed to assess interpopulational variation of these life-history traits in this newt species.

Introduction

The influence of climatic conditions on the metabolism of ectotherms is recorded in their bone growth, which provides a good tool to estimate the animals' individual age in wild populations. This technique – skeletochronology – is widely used and reliable in amphibians as well as in many ectothermal vertebrates. Castanet (2002) listed about 350 references of skeletochronological studies in amphibians and reptiles between 1906 and 2000.

Age structures of large *Triturus* newts are available (e.g. *T. cristatus*: Hagström, 1977; Francillon-Vieillot et al., 1990; Miaud et al., 1993, *T. marmoratus*: Caetano et al., 1985; Jacob et al., 2003; *T. carnifex*: Cvetkovic et al., 1996; Pagano et al., 1990; Kalezić et al., 1994; *T. dobrogicus*: Cogalniceanu and Miaud, 2003). These species were studied in one or more pop-

ulations allowing comparisons in age at maturity, body size or growth, but also on characteristics of the life cycle (i.e. existence of several periods of arrested growth: Jakob et al. 2002). Research published to date often showed intraspecific patterns and differences in life-history traits of amphibians populations living at different altitudes and latitudes (e.g. review in Morrison and Hero, 2003).

In this paper, we studied southern crested newts *T. karelinii* from Western Turkey using skeletochronology, providing the first information on the population's age structure and growth for this newt species. The altitudinal range of *T. karelinii* population is from sea level to 2100 m in Turkey (Başoğlu et al., 1994). The studied population inhabits the Gündalan at 1200 m above sea level. Inhabiting both aquatic and terrestrial habitats, newts have to cope with varying periods of harsh or favourable conditions which also influence life cycle (e.g. life cycle of the marbled newt in Mediterranean regions: Jakob et al., 2002; Jakob et al., 2003). The present paper also describes how the southern crested newt react to the local climatic conditions and we try to compare the bone

1 - Adnan Menderes Üniversitesi, Fen-Edebiyat Fakültesi, Biyoloji Bölümü 09010 Aydin, Turkey
e-mail: kolgun@adu.edu.tr

2 - UMR CNRS 5553, Laboratory of Alpine Ecology, University of Savoie, 73 376 Le Bourget du Lac, France
e-mail: claudie.miaud@univ-savoie.fr

growth characteristics (line of metamorphosis, endosteal resorption, presence-absence of aestivation line) with other populations of the *T. cristatus* complex exposed to various climatic regimes.

Materials and methods

The range of *T. karelinii* covers the south and west coast of the Black Sea region and the south and east coast of the Caspian Sea up to West Serbia (Tarkhnishvili, 1996). In Turkey, it occurs across north western Anatolia, the central Black Sea region, the eastern Black Sea, Central Anatolia, European Turkey, the Marmara region and the Aegean region (Baran et al., 1992).

The studied population inhabits the Gündalan, an extensive agricultural region with mainly potatoes fields. Climatic conditions from the closest meteorological station at similar elevation (Afyon, distant of 250 km) are presented on table 1. There are many deep wells in the fields, which are surrounded by stone walls. These wells are attractive and suitable for animals experiencing arid conditions in summer. The studied newts reproduced in two ponds (400 m² and 600 m²) about 50 metres apart, close to Bozdag village (Western Turkey; 38°20'N, 28°05'E; 1200 m elevation). The water content decreased in the summer but these ponds did not dry up. The maximum depth of both ponds, measured in May and June (when the water level was the highest), was about 70-80 cm. Several *Salix* sp. grew around the smaller pond, and numerous stones and rocks surrounded the other pond. *Rana macrocnemis*, *R. ridibunda*, *Bufo bufo*, *B. viridis*, and *Hyla arborea* were also observed in these ponds.

The newts (19 males, 22 females and 11 juveniles) were caught by dipping a net in the water during the breeding season (11 April and 25 May 2001). Four newly metamorphosed newts were also caught at these dates from under the stones around the ponds. The Snout-Vent Length (SVL) was measured as the distance between the tip of the snout and the posterior part of the vent, to the nearest mm using a rule and the sex of the individuals was determined using secondary sexual characters. The longest finger of the hind leg (i.e. second of third) was cut and preserved in 70° ethanol. All newts were released at the site of capture. The skeletochronology analysis followed previous procedures (Castanet and Smirina, 1990): the preserved fingers were

dissected, the larger bone of the 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 μm) from the diaphyseal part of the phalange were obtained using a freezing microtome, after which they were stained with Ehrlich's hematoxylin. The sections were placed in glycerin in order to be observed with a light microscope. Inferring age from the number of LAGs in cross sections requires knowing the annual number of period of arrested growth. We thus 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 in summer or during winter. Only experimental vital bone marking or individual capture-marking-recapture will allow to elucidate the timing of the deposition of single LAGs in this population, but this does not interfere with the estimation of their ages.

We used the non-parametric Mann-Whitney *U*-test to compare the age and SVL between the two sexes. The growth was estimated using von Bertalanffy's (1938) model, which has been used earlier in several studies on amphibians (e.g. Arntzen, 2000; Miaud et al., 2000). The length at metamorphosis, i.e. 25 mm, was estimated using larvae closed to metamorphosis caught in the ponds in May. The asymptotic Snout-Vent Length (SVL_{asymptotic}) corresponds to the estimated maximum average body length that can be reached. The growth coefficient *K*, the rate at which SVL_{asymptotic} is reached, defines the shape of the curve. SVL_{asymptotic} and *K* and their asymptotic confidence intervals (CI) were estimated through non-linear least-square regression with Statistica 5.0 (Statsoft Inc.). Two SVL_{asymptotic} and *K* were considered as significantly different (at 0.95 level) when their confidence intervals did not overlap.

Results

Phenology, bone histology and lines of arrested growth

As previously described in numerous newt species, the periosteal bone is deposited depending on ecological conditions, e.g. alternating favourable and unfavourable periods of bone growth. The lines of arrested growth that were observed in juvenile and adult *T. karelinii*,

Table 1. Average monthly climate variations, recorded at Afyon meteorological station (situated at 1034 m above sea level, about 250 km from the Bozdag area, SW Turkey). T: temperature in °C; RH: relative air humidity in %; P: precipitation (in mm and in days).

Months	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
T (°C)	0.2	1.6	5.0	10.4	14.9	18.8	21.9	21.8	17.5	12.1	6.9	2.3
RH (%)	79	75	68	62	60	55	49	48	53	64	73	79
P (mm)	43	40	45	46	57	40	21	10	20	33	34	47
P (days)	13	12	12	11	12	8	4	3	4	7	9	12

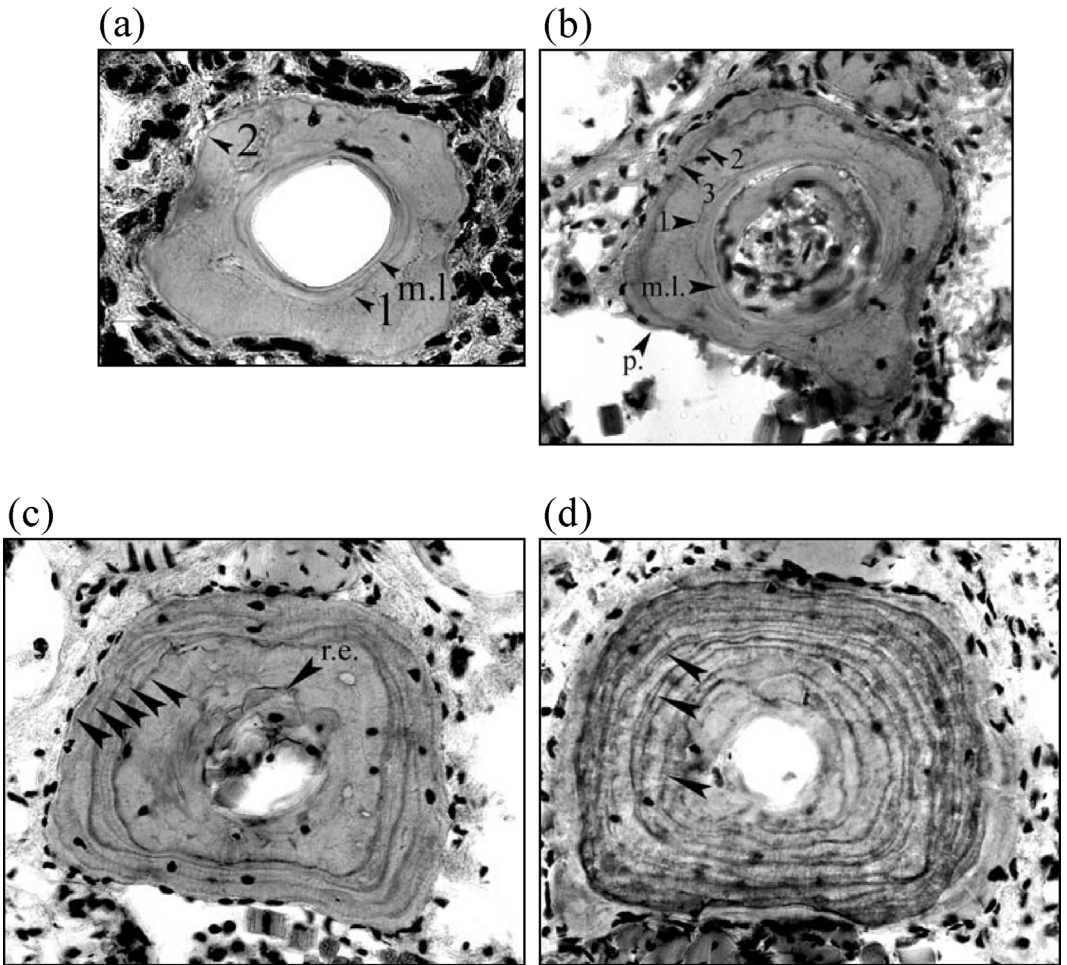


Figure 1. Cross-sections in the phalanges of the southern crested newt *Triturus karelinii* in Turkey. (a) Juvenile (SVL = 38 mm) caught in April. Note the presence of a metaphysis line and two other lines in the periosteal bone (aestivation and hibernation LAGs). Age of this juvenile was 1 year old. m.l.: metaphysis line; 1 and 2: lines of arrested growth ($\times 400$). (b) Juvenile (SVL = 46 mm). Three LAGs were observed in the periosteal bone. 1: first aestivation; 2: first hibernation; 3: second aestivation; p: periphery: second hibernation. Age of this individual caught in April was 2 years old ($\times 400$). (c) Male (SVL = 70 mm). The lines of arrested growth exhibited a classical succession of single stained lines. The innermost lines (first aestivation and hibernation) were eroded by endosteal resorption. Age of this individual caught in April was 7 years old ($\times 200$). e.r.: endosteal resorption. (d) Females (SVL = 76 mm). Several lines of arrested growth appeared as double lines, indicating two periods of arrested growth (aestivation and hibernation) per year. Age of this individual caught in May was 9-10 years old ($\times 200$).

indicated the existence of slow-down periods (fig. 1). In the studied population, the newts migrate from the land to an aquatic place for reproduction at the end of March. They stay in the water to reproduce until June and then return to terrestrial life. The adults and newly metamorphs have to face severe climatic conditions during the terrestrial phase, with high temperatures (highest temperature of June, July and Au-

gust often more than 35°C and with low precipitations: table 1 and K. Olgun, pers. obs.). These climatic conditions may lead to aestivation and can hence be responsible for bone arrested growth. The temperature and precipitation in this region are more favourable for activity (e.g. foraging) in October and November. Hibernation — i.e. another growth arrest — takes place in December-March. Metamorphosis oc-

curs in June and one year later, the smallest juveniles caught in April and May measure less than 40 mm. Cross sections were realised for juveniles between 36 and 52 mm (mean \pm standard error: 40.0 ± 3.9 , $n = 15$). Some of them ($n = 6$) exhibited a first thin line very closed to the edge of the marrow cavity (fig. 1a). We interpreted this line as a metamorphosis line. In the smallest juveniles (36 to 42 mm, $n = 12$), two lines were present in the periosteal bone (after the metamorphosis line when present, fig. 1a). We interpreted the first one as deposited during the previous summer (aestivation) and the second one as deposited during the last winter (hibernation). The first line (aestivation) was often less coloured than the second one (hibernation). The age of these small juveniles was estimated at 1 year. In larger juveniles (from 45 to 52 mm, $n = 3$), three LAGs were observed (after the metamorphosis line when present). The first inner most was deposited during the first aestivation, and the second one during the first hibernation. The third line corresponded to the second aestivation, and the periphery corresponded to the last (and second) winter (these juveniles were caught in April, and the growth since the last hibernation period was not enough to show a fourth line separated from the periphery of the cross section). Age of these juveniles was 2 years old (fig. 1b).

For both males and females and in similar proportions, the LAGs deposited in the cross sections can be divided in two main patterns: the first one (49%, $n = 20$) was a classical succession of single well-stained lines (fig. 1c). The second pattern (47%, $n = 18$), was a succession of double lines, indicating two periods of arrested growth (aestivation and hibernation) per year. Three cross sections showed both single and double LAGs (fig. 1d).

Endosteal resorption was frequently observed in the cross sections from the adults. By comparing diameter of eroded marrow cavities of adults with diameter of non-eroded marrow cavities of juveniles, we found that endosteal resorption destroy only (completely or partially)

the first aestivation line (fig. 1d). Endosteal bone deposition was also observed at certain points but was easy to distinguish from the periosteal bone because of a scalloped resting line. It was possible to estimate the age for 89% ($n = 41$) of the available phalanges.

Body length, age and growth

Males (mean \pm standard error: 72.0 ± 6.6 mm, range 60-82, $n = 18$) and females (73.7 ± 10.3 mm, range 55-90, $n = 19$) body length did not differ significantly (Mann-Whitney U -test = 154, $P = 0.60$).

The age of the male and female adults (males: 5.5 ± 1.2 years, $n = 19$; females: 5.6 ± 2.1 , $n = 22$) did not significantly differ (Mann-Whitney U -test = 460, $P = 0.96$). The youngest females and males were respectively 3 and 4 years old. As the oldest juveniles were maximum 2 years old in our sample, age at maturity was estimated at 3-4 years for this species (fig. 2a).

The growth curve of *T. karelinii* was well described by the von Bertalanffy growth model (fig. 2b). There was no significant difference between the sexes for the the growth coefficient K ($K_{\text{males}} = 0.186 \pm 0.072$ and $K_{\text{females}} = 0.200 \pm 0.08$) and the asymptotic size ($SVL_{\text{asympt-males}} = 99.9 \pm 1.75$ and $SVL_{\text{asympt-females}} = 99.0 \pm 1.71$).

Discussion

The presence of growth marks in bone cross sections has been confirmed in many amphibian populations that stop their activity due to climatic conditions (review in Smirina, 1994; Castanet, 2002). The growth patterns for amphibians exposed to a Mediterranean climate (i.e. with a large inter-annual variation in the intensity or duration of the arrested growth period) are more difficult to interpret (Esteban et al., 1996; Tejedo et al., 1997; Olgun et al., 2001). The studied *T. karelinii* population, which is exposed to an arid climate, exhibited a metamorphosis line (only visible in juveniles due to the endosteal resorption in adults) and one or two

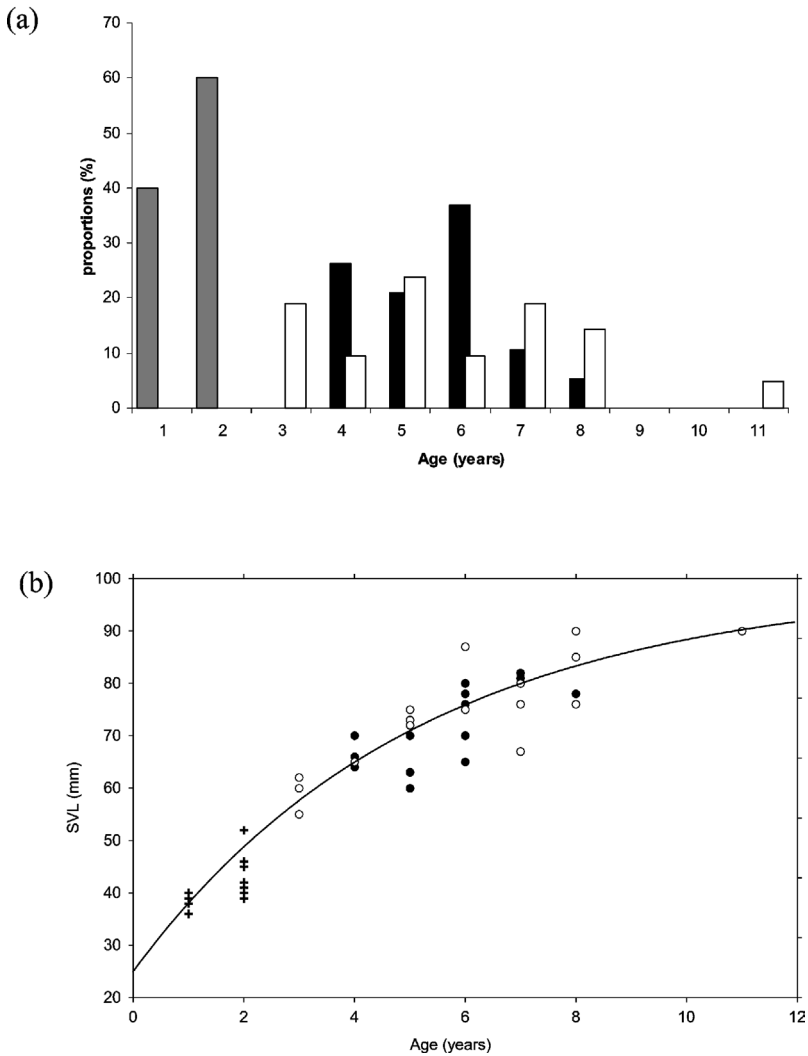


Figure 2. Age structure and growth of the southern crested newt *Triturus karelinii* in Turkey. (a) Age assessed by skeletochronology. Grey bars: juveniles ($n = 15$), open bars: females ($n = 22$), filled bars: males ($n = 19$). (b) von Bertalanffy's growth model. Growth parameters (growth coefficient and asymptotic maximum SVL) did not differ significantly (see results) between males and females. Crosses: juveniles, open circles: females, filled circles: males.

growth arrests each year in adults (aestivation and wintering).

The metamorphosis line has already been observed in salamanders (*Euproctus asper*, Montori, 1990; *Notophthalmus viridescens*, Kazmer, 1986; *Triturus marmoratus*, Jakob et al., 2002; *Hynobius nebulosus*, Ento and Matsui, 2002), toads (*Bufo bufo*, Hemelaar, 1988; *Pelodytes punctatus*, Esteban et al., 2002) and frogs (*Rana saharica*, Esteban et al., 1999; *R. nigromac-*

ulata, Khonsue et al., 2001; *R. porosa brevipoda*, Khonsue et al., 2002). Jakob et al. (2002) observed this metamorphosis line in a Mediterranean population of the marbled newt (a species closely related to *T. cristatus* complex) in the south of France, while this line was not present in populations from western France (Francillon et al., 1990), north Portugal (Caetano et al., 1985) and south Portugal (Caetano and Castanet, 1993). These authors

hypothesized that a growth arrest occurs at the metamorphosis stage in a Mediterranean climate because metamorphs leave the aquatic for the terrestrial environment which could be less favourable. The arid climate of Bozdag could similarly cause the expression of a line of metamorphosis in the bones of *T. karelinii*.

Another characteristic observed in the studied population of *T. karelinii* was the migration of juveniles to the pond. The presence of juveniles in the water is a frequent phenomenon for other large newts such as *T. marmoratus* and *T. cristatus*. The fact that the juveniles can be located in aquatic sites during winter and spring, especially in populations exposed to an arid climate, might be due to trophic resources (i.e. higher productivity or prey availability in water than on land).

The lines of arrested growth are generally believed to result from a genetically controlled growth cycle, that is synchronized and reinforced by seasonality (Castanet et al., 1992; Esteban et al., 1996; Alcobendas and Castanet, 2000). The patterns of LAG deposition can vary between populations that are exposed to different environments: in *Triturus marmoratus*, adults exhibited both winter and summer LAGs in populations from the north Portuguese highlands (Caetano et al., 1985) and from near the Mediterranean coast in France (Jakob et al., 2002). Marbled newts only showed summer LAGs in populations from south Portugal (Caetano and Castanet, 1993) and only winter LAGs in populations from the north Portuguese lowlands (Caetano et al., 1985) and from the northern part of their geographic range (e.g. western France, Francillon et al., 1990). Andreone (1990) also reported summer and winter LAGs in the Alpine newt *Triturus alpestris* living in temporary Mediterranean ponds, while only winter LAGs were observed in other European populations (Miaud et al., 2000). The population of *Mertensiella lusciani* from southwestern Turkey also exhibited both summer and winter arrested growth lines (Olgun et al., 2001).

As in others tetrapods, endosteal resorption is a common histological process in amphibian long bones (Castanet and Smirina, 1990). This phenomenon creates an erosion of the periosteal bone on the edge of the marrow cavity. Endosteal resorption was observed in the cross sections of adult *T. karelinii*. Several authors suggested that resorption may be linked to environmental conditions (Smirina, 1972), with e.g. less resorption for populations living in high altitudes than for lowland populations (Esteban et al., 1996; Esteban et al., 1999) or the opposite (Caetano and Castanet, 1993). On the other hand, Hemelaar (1988) did not detect a relationship between the intensity of endosteal resorption and the climatic conditions in any of the European populations of the Common toad *Bufo bufo* studied. This relation remains therefore to be documented in more amphibian species and, in this case, in other populations of *T. karelinii* as well.

Body length and age among the *T. cristatus* complex were reported from populations experiencing various environmental conditions (review in Cogalniceanu and Miaud, 2003). Body length in the *T. karelinii* studied population was in the range of observed lengths in other related species. Females are often larger than males in *T. cristatus*, *T. marmoratus*, *T. carnifex* and *T. dobrogicus*, but not in the *T. karelinii* studied population. Comparisons with other populations in lower and higher altitude will allow verifying if a sexual size dimorphism is also present in *T. karelinii*.

Age at maturity was reached at 3-4 years old in the *T. karelinii* studied population, which is similar to that observed in other species of the *T. cristatus* complex (Cogalniceanu and Miaud, 2003). Longevity often attained and overtook 15 years old in *T. cristatus* complex. The oldest individuals were 8 and 11 years old (males and females respectively) in the *T. karelinii* studied population. Again, studying more populations exposed to various conditions is needed to assess interpopulational variation of these life-history traits in *T. karelinii*. Age at matu-

urity and longevity are greatly influenced by local conditions (i.e. reduction of the length of the active period) and increase with altitude among populations of *T. cristatus*, *T. marmoratus* and *T. carnifex*. Living in permanent or temporary habitats (i.e. breeding habitat) also influenced newt age structure and taxonomically related large bodied newts exhibited a higher longevity in permanent habitats (Diaz-Paniagua et al., 1996). The relatively low longevity observed in *T. karelinii* could result from a higher mortality risk during the terrestrial phase caused by the arid climate of Bozdag. Such higher mortality was previously observed in floodplain and Mediterranean populations of *T. dobrogicus* (Cogalniceanu and Miaud, 2003) and *T. marmoratus* (Jakob et al., 2003).

The variation of selective regimes (e.g. climate) can result in adaptation and differentiation among the local populations (Linhardt and Grant, 1996). Amphibians — and especially species exposed to severe climatic conditions — could provide good model systems to study the possible consequences and adaptations to the environmental changes that are expected to occur in the forthcoming decades (Miaud and Merilä, 2001).

Acknowledgements. We thank J. Castanet, M. Denoël and an anonymous referee who help to improve this manuscript with useful comments. We express our gratitude to the Scientific and Technical Research Council of Turkey (TUBITAK) who provided financial support for this study.

References

- Alcobendas, M., Castanet, J. (2000): Bone growth plasticity among populations of *Salamandra salamandra*: Interactions between internal and external factors. *Herpetologica* **56**: 14-26.
- 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.
- Arntzen, J.W. (2000): A growth curve for the newt *Triturus cristatus*. *J. Herpetol.* **34**: 227-232.
- Baran, İ., Yılmaz, İ., Kete, R., Kumlucaş, Y., Durmuş, H. (1992): Batıve Orta Karadeniz Bölgesi'nin Herpetofaunası. *Turk. J. Zool.* **16**: 275-288.
- Başoğlu, M., Özeti, N., Yılmaz, İ. (1994): Türkiye Amfibileri. Ege Üniversitesi Fen Fakültesi Kitaplar Ser., İzmir.
- Bertalanffy, L. von (1938): A quantitative theory of organic growth. *Human Biology* **10**: 181-213.
- Caetano, M.H., Castanet, J. (1993): Variability and microevolutionary patterns in *Triturus marmoratus* from Portugal: age, size, longevity and individual growth. *Amphibia-Reptilia* **14**: 117-129.
- Caetano, M.H., Castanet, J., Francillon, H. (1985): Détermination de l'âge de *Triturus marmoratus* (Latreille, 1800) du Parc National de Peneda Gerês (Portugal) par squelettochronologie. *Amphibia-Reptilia* **6**: 117-132.
- Castanet, J. (2002): Amphibiens et Reptiles non aviens: un matériel de choix en squelettochronologie. *Bull. Soc. Herp. Fr.* **103**: 21-40.
- Castanet, J., Smirina, E. (1990): Introduction to the skeletochronological method in amphibians and reptiles. *Ann. Sci. Nat. Zool.* **11**: 191-196.
- Castanet, J., Francillon-Vieillot, H., Meunier, J.-F., de Riquès, A. (1992): Bone and individual aging. In: Bone, Vol. 7: Bone growth-B, pp. 245-283. Hall, B.K., Ed., CRC Press.
- Cogalniceanu, D., Miaud, C. (2003): Population age structure and growth in four syntopic amphibian species inhabiting a large river floodplain. *Can. J. Zool.* **81**: 1096-1106.
- Cvetkovic, D., Kalezić, M.L., Djorovic, A., Dzukic, G. (1996): The crested newt (*Triturus carnifex*) in Sub-mediterranean: reproductive biology, body size and age. *Ital. J. Zool.* **63**: 107-111.
- Diaz-Paniagua, C., Mateo, J.M., Andreu, A.C. (1996): Age and size structure of populations of small marbled newts (*Triturus marmoratus pygmaeus*) from Donana National Park (SW Spain). A case of dwarfism among dwarfs. *J. Zool., Lond.* **239**: 83-92.
- Ento, K., Matsui, M. (2002): Estimation of age structure by skeletochronology of a population of *Hynobius nebulosus* in a breeding season (Amphibia, Urodela). *Zool. Sci.* **19**: 241-247.
- 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. *Can. J. Zool.* **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. *Ann. Zool. Fenn.* **36**: 53-62.
- Esteban, M., Sanchez-Herraiz, M., Barbadillo, L., Castanet, J., Marquez, Z. (2002): Effects of age, size and temperature on the advertisement calls of two Spanish populations of *Pelodytes punctatus*. *Amphibia-Reptilia* **23**: 249-258.
- Francillon-Vieillot, H., Arntzen, J.W., Geraudie, J. (1990): Age, growth and longevity of sympatric *Triturus cristatus*, *Triturus marmoratus* and their hybrids (Amphibia, Urodela). A skeletochronological study. *J. Herpetol.* **24**: 13-22.
- Hagström, T. (1977): Growth studies and ageing methods for adult *Triturus vulgaris* L. and *T. cristatus* Laurenti (Urodela, Salamandridae). *Zool. Scr.* **6**: 61-68.

- Hemelaar, A. (1988): Age, growth and other population characteristics of *Bufo bufo* from different latitudes and altitudes. *J. Herpetol.* **22**: 369-388.
- Jakob, C., Seitz, A., Crivelli, A.J., Miaud, C. (2002): Growth cycle of the marbled newt (*Triturus marmoratus*) in the Mediterranean region assessed by skeletochronology. *Amphibia-Reptilia* **23**: 407-418.
- Jakob, C., Miaud, C., Crivelli, A.J., Veith, M. (2003): How to cope with periods of drought? Age at maturity, longevity and growth of marbled newts population (*Triturus m. marmoratus*) in Mediterranean temporary ponds. *Can. J. Zool.* **81**: 1905-1911.
- Kalezic, M.L., Cvetkovic, D., Djorovic, A., Dzukic, G. 1994. Paedomorphosis and differences in life-history traits of two neighbouring crested newt (*Triturus carnifex*) populations. *Herpetol. J.* **4**: 151-159.
- Kazmer, D.J. (1986): Age Determination of Urodele Amphibians by Bone Growth Annuli. MSc Thesis, Clemson Univ. N.C., USA.
- Khonsue, W., Matsui, M., Hirai, T., Misawa, Y. (2001): A comparison of age structures in two populations of the pond frog *Rana nigromaculata* (Amphibia: Anura). *Zool. Sci.* **18**: 597-603.
- Khonsue, W., Matsui, M., Misawa, Y. (2002): Age determination of Daruma pond frog, *Rana porosa brevipoda* from Japan towards its conservation (Amphibia: Anura). *Amphibia-Reptilia* **23**: 259-268.
- Linhart, Y.B., Grant, M.C. (1996): Evolutionary significance of local genetic differentiation in plants. *Ann. Rev. Ecol. Syst.* **27**: 237-277.
- Miaud, C., Joly, P., Castanet, J. (1993): Variation of age structures in a subdivided population of *Triturus cristatus*. *Can. J. Zool.* **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 life-history trait variation throughout its range. *Herpetologica* **56**: 135-144.
- Miaud, C., Merilä, J. (2001): Local adaptation or environmental induction? Causes of population differentiation in alpine amphibians. *Biota* **2**: 31-50.
- Montori, A. (1990): Skeletochronological results in the pyrenean newt *Euproctus asper* (Dugès, 1852) from one prepyrenean population. *Ann. Sci. Nat. Zool.* **11**: 209-211.
- Morrison, C., Hero, J.-M. (2003): Geographic variation in life-history characteristics of amphibians: a review. *J. Anim. Ecol.* **72**: 270-279.
- Olgun, K., Miaud, C., Gautier, P. (2001): Age, size and growth of the terrestrial Salamander *Mertensiella luschani* in an arid environment. *Can. J. Zool.* **79**: 1559-1567.
- Pagano, M., Rossi, F., Cavallotto, L., Campolongo, P., Giacomini, C. (1990): Age determination in *Triturus carnifex*. In: Atti VI Convezione Nazionale Associazione "Alessandro Ghigi", pp. 181-184. Mus. Reg. Sci. Nat. Torino.
- Smirina, E.M. (1972): Annual layers in bones of *Rana temporaria*. *Zool. Zh.* **51**: 1529-1534.
- Smirina, E.M. (1994): Age determination and longevity in amphibians. *Gerontol.* **40**: 133-146.
- Tarkhnishvili, D.N. (1996): The distribution and ecology of the amphibians of Georgia and the Caucasus: A biogeographical analysis. *Zeitschrift Feldherpetol.* **3**: 167-196.
- Tejedo, M., Requés, R., Esteban, M. (1997): Actual and osteochronological estimated age of natterjack toads (*Bufo calamita*). *Herpetol. J.* **7**: 81-82.

Received: September 15, 2003. Accepted: December 2, 2003.