Long-Term Rearing of Two Cynops Species and Fertility of Old Cynops ensicauda popei (Amphibia: Urodela)

Authors: Shiroma, Hiroki, Tokuda, Tatsuhiro, Tokuda, Ai, Kamimura, Ryo, Takenaka, Sen, et al.

Source: Current Herpetology, 42(2) : 210-218

Published By: The Herpetological Society of Japan

URL: https://doi.org/10.5358/hsj.42.210

Current Herpetology 42(2): 210–218, August 2023
doi 10.5358/hsj.42.210

Long-Term Rearing of Two *Cynops* Species and Fertility of Old *Cynops ensicauda popei* (Amphibia: Urodela)

Hiroki SHIROMA[1], Tatsuhiro TOKUDA[2], Ai TOKUDA[2], Ryo KAMIMURA[1], Sen TAKENAKA[3], and Atsushi TOMINAGA[1,4,*]

[1]*Graduate school of Engineering and Science, University of the Ryukyus, 1 Senbaru, Nishihara, Okinawa 903–0213, JAPAN*
[2]*7–4–27 Makomanaihon-cho, Minami, Sapporo, Hokkaido 005–0021, JAPAN*
[3]*6–4–3–45 Kawazoe, Minami, Sapporo, Hokkaido 005–0806, JAPAN*
[4]*Faculty of Education, University of the Ryukyus, 1 Senbaru, Nishihara, Okinawa 903–0213, JAPAN*

Abstract: Long-term rearing of *Cynops pyrrhogaster* and *Cynops ensicauda popei* confirmed that the longevities of the two species were longer than 36 years; *C. pyrrhogaster* longer than 37 years and *C. e. popei* longer than 36 years, respectively. The male *C. e. popei* that was 34 years old or older showed reproductive behavior with a conspecific young female and successfully bred. The paternal relationships between the old male and the six offspring were also genetically proven, indicating that the male *C. e. popei* retained its fertility at the age of 34 or older. Since physiological longevity and reproductive ability are important parameters for life history studies, our findings are meaningful for the conservation of the species.

Key words: *Cynops ensicauda popei*; *Cynops pyrrhogaster*; Fertility; Longevity; Long-term rearing

Introduction

An organism's longevity is closely related to many other parameters of its life history, such as lifetime reproductive success, age of sexual maturation, and survivorship. Thus, longevity is one of the most important parameters for considering the life history characteristics of different species (de Magalhães and Costa, 2009; Niemiller et al., 2016). The longevity of amphibians in the wild is often estimated by skeletochronology (Wells, 2010). However, it is difficult to estimate the exact age of long-lived species because lines of arrested growth (LAGs) can be compressed, resulting in under-estimated ages (Staub, 2016). Therefore, the records of longevity based on reliable rearing and those on recapturing of individually marked ones should be considered.

The aging process is known as a factor that determines the longevity of organisms. Aging leads to a decline in physiological functions and is associated with increased mortality and decreased fertility (Jones and Vaupel, 2017). Although aging has hitherto been considered a common feature in organisms, increasing

* Corresponding author.
E-mail address: tominaga@edu.u-ryukyu.ac.jp

empirical data indicate that some organisms have substantial resistance to aging process (Schaible et al., 2015). In salamanders, three species of Salamandridae (*Lyciasalamandra fazilae*, *Salamandra salamandra*, and *S. perspicillata*) are known to show only negligible aging (Cayuela et al., 2019).

The longevity of animals is strongly correlated with their body sizes, but some salamanders have longer longevities than those expected from their body sizes (Yun, 2021) (e.g., *Proteus anguinus* are: 100 years [Voituron et al., 2011; Tacutu et al., 2018], *S. salamandra*: 50 years [Böhme, 1979], *Andrias japonicus*: 55 years, *Ambystoma maculatum*: 25 years, *Am. tigrinum*: 25 years, *Amphiuma means*: 27 years, *Cryptobranchus alleganiensis*: 25 years, *Siren lacertina*: 25 years [Carey and Judge, 2000; de Magalhāes and Costa, 2009; Wells, 2010]).

The genus *Cynops* is a group of modern newts, occurring in Japan (Frost, 2023). The sword-tailed newt, *Cynops ensicauda* is a newt endemic to the Amami Islands and Okinawa Islands of the Ryukyu Archipelago (Tominaga et al., 2010). There are two subspecies of this species, *C. e. ensicauda* and *C. e. popei* (Inger, 1947). The red-bellied newt *C. pyrrhogaster* occupies mainland Japan and is a sister species of *C. ensicauda*.

The longevity of *C. pyrrhogaster* have been reported to be 25 years (Sato, 1943). In captivity, an individual of *C. pyrrhogaster* has been recorded as surviving to about 30 years of age (Eguchi et al., 2011). In other cases, two individuals also reported to have survived for at least 33 and 42 years, one individual survived more than 34 years, and four individuals have been kept in captivity for more than 47 years, two of which are likely to have reached more than 50 years old (Köhler et al., 2023).

The age structure of wild populations of *C. pyrrhogaster* was studied by Marunouchi et al. (2000) based on skeletochronology, and the age of mature individuals was estimated to be 3–23 years. The maximum age of *C. pyrrhogaster* was also estimated to be 31 years for males and 23 for females based on the 27-year-long field study by mark and recapture method (Kusano, 2020). However, there have been no reports on the age and longevity of *C. ensicauda*. In this paper, we report on the longevity of the two species, *C. pyrrhogaster* and *C. ensicauda*, and the maximum reproductive ages of *C. ensicauda*, based on long-term rearing records.

Materials and Methods

Procurement and rearing conditions of the newts

The female of *C. pyrrhogaster* (hereafter referred to as CpF) was purchased by the third author at an aquarium fish shop in Saitama Prefecture in the summer of 1987 (Fig. 1). It already had a mature body size at the time of purchase. The individual of *C. ensicauda* is a male (hereafter referred to as CeM) purchased by the third author from the same aquarium fish shop in the summer of 1988 (Fig. 1). At the time of purchase, the body size had already reached a mature size. Four other *C. ensicauda* were purchased at the same time and reared, but CeM was easily distinguishable from these four as the male with most white mottles. CeM and CpF were kept in the same aquarium.

In October 2020, a captive bred female of *C. ensicauda*, which was born in November 2018 (hereafter referred to as CeF), was introduced and kept with CeM and CpF from November 2020.

Snout vent length (SVL) and body weight of CeM and CeF were measured on February 18, 2023. The SVL of CpF was measured from the photograph taken on March 9, 2022, using ImageJ software (version 1.53a). SVL and body weight of these individuals were not measured at the time of purchase.

Breeding conditions in captivity

Between 1987 and 2015, the newts were kept in Saitama Prefecture in a 60×30×36 cm fish tank with a 2 cm layer of gravel on the bottom and a 5 cm depth of water. One loach (*Misgurnus anguillicaudatus*) was cohabitated during 1987–2015. They were kept in an indoor environment at room temperature. The

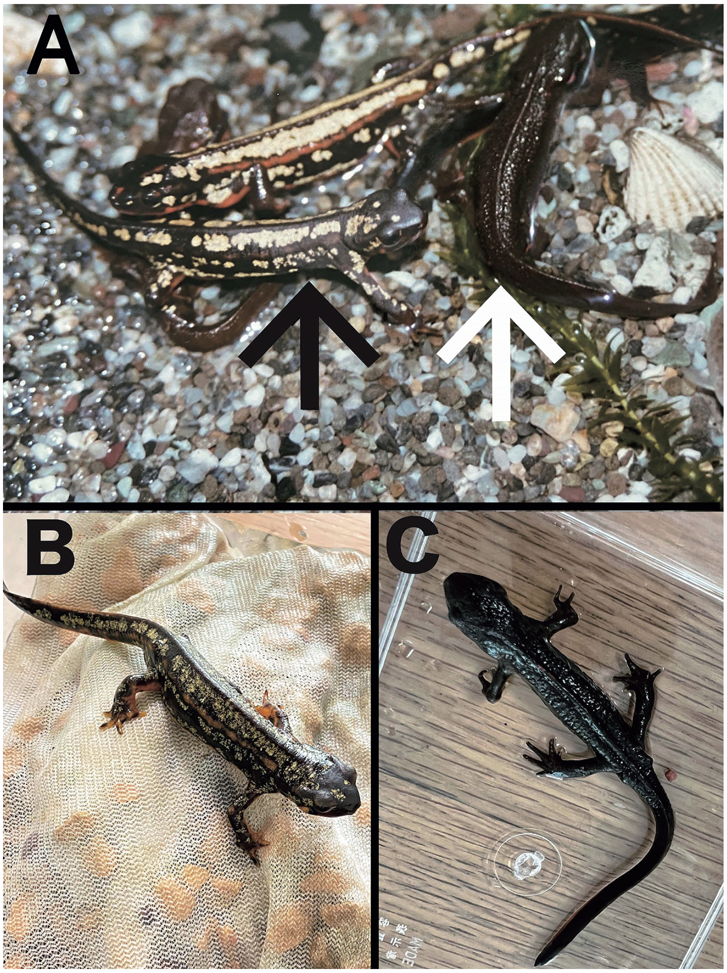

Fig. 1. A: A photograph of CpF and CeM taken on November 5, 1991. Black arrow indicates CeM. White arrow indicates CpF. B: CeM on September 7, 2021. C: CpF on March 16, 2023.

temperature exceeded 30°C on several days in summer. In winter, rearing temperatures often dropped to below 10°C. The water supplied from a well (pumping depth 180 m) was changed approximately once a week. Live larvae of nonbiting midges (Chironomidae) were fed mainly as food.

In 2015, the rearing environment has been changed because of moving the house to Hokkaido. They were kept indoors in the same fish tank as mentioned above, but aged tap water for more than one day was used as rearing water. The rearing temperature was warmer than 20°C, even in winter, and in summer was about 23°C–28°C. Commercial feed for turtles (Kamepros: Kyorin) and supplementary thawed frozen larvae of nonbiting midges have been fed since 2015. CeM and CeF were reared in the same tank since November 2020 and CeF bred thereafter.

Confirmation of parent–child relationship between offspring and CeM

Since CeF was reared with other *C. ensicauda* before introduction, it could have retained sperm received from other individuals in the seminal vesicles before introduction to the aquarium tank. In order to clarify the paternity, the parent–child relationship between juveniles obtained and CeM was investigated using microsatellite markers.

The tail tips of CeM, CeF, and six juveniles were cut under MS222 anesthesia and placed in 99% ethanol for cryopreservation. Tissue from each individual was added to a mixed solution of 660 μL of 1×STE Buffer, 60 μL of 10% SDS solution, and 10 μL of proteinase K (23 ng/μL) and incubated at 55°C overnight. Then, total DNA was extracted according to the protocol of the Promega Wizard Genomic DNA Purification Kit. Finally, it was dissolved in 50 μL of 1×TE Buffer and stored in a refrigerator.

For microsatellite analysis, 15 nuclear microsatellite loci (CP_NGS21, CP_EST30, CP_EST10, CP_NGS36, CP_NGS19, CP_NGS26, CP_EST05, CP_NGS11, CP_NGS35, CP_NGS24, CP_EST03, CP_EST21, CP_NGS43, CP_EST02, and CP_NGS46) developed by Tominaga et al. (2014) were used. Microsatellite analysis experiments followed the method of Tominaga et al. (2021). The amplified microsatellite samples were run through a sequencer (ABI3130) for fragment analysis, and fragment lengths were determined for each locus in every individual using Geneious 9.0.2 software for genetic analysis.

Paternal relationships between CeM and six offspring were confirmed by the fragment length of each locus. In all amplified loci, if both the allele of CeM and the allele of CeF were shared by a juvenile for all loci, CeM was assumed to be the father of the juvenile.

Identification of subspecies of C. ensicauda

To determine the subspecies of CeM and CeF, mitochondrial DNA (mtDNA) sequencing was performed. Part of the cytochrome b (cytb) gene of mtDNA was amplified by PCR. PCR was performed using primers SLD_cytb_L1 (5'TGACCTGAAAATCAAYGTTGTA 3') and SLD_cytb_H 2 (5'TAGCRAATARRAARTAYCAYTCTGG 3') (Tominaga et al., 2010) according to the standard protocol of GoTaq Green Master Mix (Promega). Cycle-sequencing reactions were conducted using a BigDye Terminator v. 3.1 (Thermo Fisher Scientific), and DNA sequencing was performed using a DNA sequencer (ABI3130). The sequence of each individual was searched for homology in the DNA sequence database of the DNA Data Bank of Japan (DDBJ) to identify the subspecies.

Results

Long-term rearing of C. pyrrhogaster *and* C. ensicauda

In July 2022, CpF escaped from the cage and died accidentally. CeM is still alive as of October 2022. All *C. ensicauda* except CeM, reared since 1988, died by 2015. CpF was an adult size when purchased in 1987 and thus was considered to be at least two years old (Marunouchi et al., 2000). It survived 35 years until the escape, making it at least 37 years old. CeM had also reached the mature size of this species when purchased in 1988 and was assumed to be at least two years old at that time (Tanaka, 2005). CeM is surviving until the end of 2022 and has been in captivity for 34 years, which, combined with the previous two years, makes it at least 36 years old.

Fig. 1A is a photograph of the newts taken in 1991 (four years after rearing). A comparative photograph of CeM was taken in September 2021 (Fig. 1B). Some mottles (yellowish-white spots) have faded over time, while new ones have appeared in other areas. However, the lateral red line on the right side of the body, the areas without spots on the dorsum, and the patterns of spotting defects on the right abdomen and spotting defects on the right caudal indicate that this is the same individual (Figs. 1A and B). Except for the CeF, which was introduced in 2020, all of *C. ensicauda* involved in this study were purchased in 1988. Since we have not kept any other *C. pyrrhogatser* except for CpF, there is no possibility that it was replaced by other individuals.

The SVL of CeM and CeF were 65 and 62 mm, respectively, on February 18, 2023.

The body weight of CeM and CeF were 10.0 and 8.0 g, respectively. The SVL of CpF on March 9, 2022 was estimated to be 67 mm from a photograph.

Reproduction of long-reared C. ensicauda

When CeF was introduced to CeM in November 2020 (=the beginning of the breeding season of this newt in wild), CeM showed courtship behavior toward CeF. CeF responded to this behavior, taking in spermatophores deposited by CeM. Some spermatophores that failed to be received were also observed during rearing. From December 2020 to January 2021, CeF laid eggs on the Brazilian waterweed *Egeria densa*, eventually yielding 70 eggs. In December 2020, when oviposition began, CeM and CeF were separated from each other.

Of the 70 eggs, 65 hatched (92.9% survival to hatching). Of the 65 larvae, one had cervical spine curvature, and another had thoracic spine curvature malformations (malformation rate 3.1%). Five individuals died during the larval stage, including that with thoracic spine curvature. Sixty individuals metamorphosed from mid-May to June 2021 (85.7% survival to metamorphosis). The individuals with cervical spine curvature also metamorphosed. Fifty-five individuals were alive until the end of September 2021 (survival rate 78.5%), and then they were transferred to acquaintances at the beginning of October 2021. Six individuals, including the one with cervical curvature, have remained in possession of the original owner and were alive as of the end of 2021.

Subspecies identification by mtDNA analysis

Sequence data for the 824 bp cytb gene of mtDNA for CeM and CeF were obtained. A homology search revealed that the CeM sequence showed 100% homology with the registered sequence of *C. e. popei* (DDBJ registration number: AB754750). The sequence of CeF also showed 100% homology with the registered sequence of *C. e. popei* (DDBJ registration number: AB754747). Thus, both CeM and CeF were confirmed to be *C. e. popei*.

Parent–child determination by microsatellite analysis

The paternity of CeM were confirmed by microsatellite analysis for the six offspring born from CeF. Fourteen loci as previously listed, except for CP_NGS35, were successfully genotyped for CeM and the six offspring. Thirteen loci, except for CP_NGS35 and CP_NGS43, were successfully genotyped for CeF. Of these, four loci were not informative in determining paternity because of the lack of allelic diversity. The analysis confirmed that all six offspring had one allele of CeM origin and one allele of CeF origin at all loci, indicating that the paternity of all six offspring of CeF was contributed by CeM (Table 1).

Discussion

Most estimates of amphibian longevity have been made using skeletochronology (Wells, 2010). However, in skeletochronology, lines of arrested growth (LAGs) seem to be compressed when the growth rate becomes slow in the adult stage. Therefore, it has been pointed out that it is hard to discriminate LAGs in old individuals, and that such old individuals in which LAGs could not be reliably counted may be excluded from the data (Staub, 2016). Thus, the estimates of longevity based on skeletochronology may be underestimated.

The longevity of *C. pyrrhogaster* in captive conditions has been reported to be 25 to 50 years old (Sato, 1943; Eguchi et al., 2011; Köhler et al., 2023). The longevity of the wild individuals has been reported to be 23 years as estimated by skeletochronology (Marunouchi et al., 2000) and 31 and 23 years old for males and females, respectively, based on the mark and recapture study (Kusano, 2020). In this study, we confirmed that a female *C. pyrrhogaster* survived for at least 37 years and that a male *C. e. popei* did for more than 36 years. Thus, the very long lifespan of the species in this genus is not exceptional, as Köhler et al. (2023) pointed out. These long longevity records of the genus *Cynops* are particularly long compared with the longevities of most

TABLE 1. Genotyping data from 14 microsatellite loci for the eight individuals (CeM, CeF, and six offspring) used in this study. URE: Faculty of Education, University of the Ryukyus. –9: Missing data. The alleles derived from CeM were in bold letters.

Individual name		CeM	CeF	Offspring-1	Offspring-2	Offspring-3	Offspring-4	Offspring-5	Offspring-6
Tissue number		URE6262	URE6263	URE6264	URE6265	URE6266	URE6267	URE6268	URE6269
Locus name	CP_NGS21	**178**	174	182	174	174	174	182	174
		214	182	**214**	**214**	**178**	**214**	**214**	**214**
	CP_EST30	**172**	172	172	172	172	172	172	172
		172	172	**172**	**172**	**172**	**172**	**172**	**172**
	CP_EST10	**284**	281	281	284	281	284	281	284
		284	284	**284**	**284**	**284**	**284**	**284**	**284**
	CP_NGS36	**383**	387	**383**	403	**383**	**383**	**383**	403
		411	403	403	**411**	403	387	387	**411**
	CP_NGS19	**262**	262	262	262	262	262	262	262
		262	262	**262**	**262**	**262**	**262**	**262**	**262**
	CP_NGS26	**287**	287	**287**	**287**	**295**	**295**	**287**	**287**
		295	319	287	319	319	319	319	287
	CP_EST05	**410**	410	410	410	410	410	410	410
		410	410	**410**	**410**	**410**	**410**	**410**	**410**
	CP_NGS11	**238**	238	238	238	238	238	238	238
		238	238	**238**	**238**	**238**	**238**	**238**	**238**
	CP_NGS24	**239**	239	239	239	239	239	239	239
		239	239	**239**	**239**	**239**	**239**	**239**	**239**
	CP_EST03	**402**	398	402	402	398	398	402	402
		406	402	**406**	**402**	**402**	**402**	**402**	**402**
	CP_EST21	**235**	235	**238**	**238**	235	**235**	**238**	**235**
		238	241	241	241	**238**	241	241	241
	CP_NGS43	**380**	–9	**380**	**380**	**384**	**384**	**384**	**380**
		384	–9	–9	–9	–9	–9	–9	–9
	CP_EST02	**254**	254	**254**	**254**	**254**	**254**	**254**	**254**
		254	282	282	254	254	282	282	254
	CP_NGS46	**427**	427	427	427	427	427	427	427
		427	427	**427**	**427**	**427**	**427**	**427**	**427**

other salamanders, although *P. anguinus* is known to survive longer than this, at 100 years (Voituron et al., 2011; Tacutu et al., 2018), Japanese giant salamander, *A. japonicus*, has survived more than 55 years (Goin et al., 1978) and *S. salamandra* has been known to live for more than 50 years (Böhme, 1979).

It has also been reported that female newts close to 50 years old stopped laying eggs many years ago, while similarly aged males have exhibited courtship behavior toward females (Köhler et al., 2023). We confirmed that a male of *C. e. popei* older than 34 years retained its fertility. Thus, at least the males of this genus may remain reproductive in old age.

The longevities of animals correlate with their body size, but some salamanders have longer longevities relative to body sizes

(Sousounis et al., 2014). A factor contributing to their relatively long longevities might be their resistance to aging process (Yun, 2021). A study examining the aging pattern of three species of Salamandridae (*L. fazilae*, *S. salamandra*, and *S. perspicillata*) found that their survival rates declined slowly with age, and their mortality rate remained constant regardless of age (Cayuela et al., 2019). Therefore, an age-related physiological decline commonly observed among mammals occurs only slightly in these species (Cayuela et al., 2019). It is possible that the resistance to senescence is also retained in many other salamanders. The two species of the genus *Cynops* observed in this study might also have strong resistance to senescence.

The aging of cells in organisms is induced by various types of cellular stresses, including DNA damage, telomere shortening, oxidative attack, and activation of oncogenes (Gorgoulis et al., 2019). The mechanism of resistance to senescence in salamanders is still not well understood, but the high regenerative capacity of this animal group is thought to be a factor (Seifert and Voss, 2013). Salamanders have a very high regenerative capacity compared to other animal groups (Yun et al., 2015). *C. pyrrhogaster* is also known to have an extremely high regenerative capacity, even in old age (Eguchi et al., 2011; Sousounis et al., 2015). A strong macrophage-dependent immune surveillance mechanism is significant for eliminating senescent cells as a factor in maintaining the regenerative capacity of salamanders (Yun et al., 2015). It has also been suggested that the newts and axolotls are highly resistant to cancer (Ingram, 1971; Tsonis, 1983), which may also be a factor in their resistance to aging (Yun, 2021). Future research is expected to confirm whether these factors are related to the resistance to aging and long longevity of salamanders.

Our result indicates that *C. pyrrhogaster* and *C. e. popei* are long-lived species capable of living for over 34 years. It also showed that the male of *C. e. popei* remains its fertility at that age at least. Long-lived species are generally less vulnerable than short-lived species to temporary deterioration in reproductive success caused by climate change and other factors (Morris et al., 2008). Even if there are several years with low reproductive success, long-lived species can reproduce in other years as long as adults survive. Therefore, *C. pyrrhogaster* and *C. e. popei* might be able to maintain their populations even in an environment where they are not able to reproduce in some years by poor environmental conditions (e.g., insufficient water at the breeding site, temporary disturbance of the breeding environment), as long as adults can survive. On the other hand, it has been noted that long-lived species tend to have optimistic estimates of population extinction, as there is a considerable time lag between the decline in new recruitment and the extinction of the population (Jachowski and Hopkins, 2018). Both two *Cynops* species are collected in significant numbers for the domestic and international pet trade (IUCN, 2021a, b). There are also disappearances of populations in both species due to habitat destruction. It is necessary to focus on multiple age classes and assess population status when designing conservation strategies for *C. pyrrhogaster* and *C. e. popei*.

Acknowledgments

The genetic analysis of this study was approved by the University of the Ryukyus Animal Experimentation Committee (approval number 2021055).

Literature Cited

Böhme, W. 1979. Zum höchstalter des feuersalamanders, *Salamandra salamandra* (Linnaeus, 1758). *Salamandra* 15: 176–179.

Carey, J. A. and Judge, D. S. 2000. *Longevity Records: Life Spans of Mammals, Birds, Amphibians, Reptiles, and Fish.* University Press of Southern Denmark, Odense.

Cayuela, H., Olgun, K., Angelini, C., Üzüm, N., Peyronel, O., Miaud, C., Avci, A., Lemaitre, J. F., and Schmidt, B. R. 2019. Slow life-history strategies are associated with negligible actuarial senescence in western Palearctic salamanders. *Proceedings of the Royal Society B: Biological Sciences* 286: 20191498.

de Magalhães, J. P. and Costa, J. 2009. A database of vertebrate longevity records and their relation to other life-history traits. *Journal of Evolutionary Biology* 22: 1770–1774.

Eguchi, G., Eguchi, Y., Nakamura, K., Yadav, M. C., Millan, J. L., and Tsonis, P. A. 2011. Regenerative capacity in newts is not altered by repeated regeneration and ageing. *Nature Communications* 2, 384.

Frost, D. R. 2023. *Amphibian Species of the World: an Online Reference. Version 6.1*. http://research.amnh.org/herpetology/amphibia/index/html (accessed 22 April, 2023)

Goin, C. J., Goin, O. B., and Zug, G. R. 1978. *Introduction to Herpetology, 3rd ed.* W. H. Freernan and Company, SanFrancisco.

Gorgoulis, V., Adams, P. D., Alimonti, A., Bennett, D. C., Bischof, O., Bishop, C., Campisi, J., Collado, M., Evangelou, K., Ferbeyre, G., Gil, J., Hara, E., Krizhanovsky, V., Jurk, D., Maier, A. B., Narita, M., Niedernhofer, L., Passos, J. F., Robbins, P. D., Schmitt, C. A., Sedivy, J., Vougas, K., von Zglinicki, T., Zhou, D., Serrano, M., and Demaria, M. 2019. Cellular senescence: defining a path forward. *Cell* 179: 813–827.

Inger, R. F. 1947. Preliminary survey of the amphibians of the Riukiu islands. *Fieldiana: Zoology* 32: 296–352.

Ingram, A. J. 1971. The reactions to carcinogens in the axolotl (*Ambystoma mexicanum*) in relation to the “regeneration field control” hypothesis. *Journal of Embryology and Experimental Morphology* 26: 425–441.

IUCN. 2021a. *Cynops pyrrhogaster. The IUCN Red List of Threatened Species 2021: e.T59444A177224976.* https://www.iucnredlist.org/ja/species/59444/177224976. (accessed 20 April 2023).

IUCN. 2021b. *Cynops ensicauda. The IUCN Red List of Threatened Species 2021: e.T59441A177225544.* https://www.iucnredlist.org/species/59441/177225544 (accessed 20 April 2023).

Jachowski, C. M. B. and Hopkins, W. A. 2018. Loss of catchment-wide riparian forest cover is associated with reduced recruitment in a long-lived amphibian. *Biological Conservation* 220: 215–227.

Jones, O. R. and Vaupel, J. W. 2017. Senescence is not inevitable. *Biogerontology* 18: 965–971.

Köhler, J., Gage, M., Janssen, H., Rauhaus, A., and Ziegler, T. 2023. Longevity in salamandrid newts—a rule, not an exception? Verified cases of Japanese fire-bellied newts (*Cynops pyrrhogaster*) reaching a lifespan of more than 40 years. *Revue suisse de Zoologie* 130: 121–124.

Kusano, T. 2020. Population abundance of the Japanese newts, *Cynops pyrrhogaster*, clarified by a long-term monitoring study. *Caudata* 4: 13–17.

Marunouchi, J., Ueda, H., and Ochi, O. 2000. Variation in age and size among breeding population at different altitudes in the Japanese newts, *Cynops pyrrhogaster*. *Amphibia-Reptilia* 21: 381–396.

Morris, W. F., Pfister, C. A., Tuljapurkar, S., Haridas, C. V., Boggs, C. L., Boyce, M. S., Bruna, E. M., Church, D. R., Coulson, T., Doak, D. F., Forsyth, S., Gaillard, J. M., Horvitz, C. C., Kalisz, S., Kendall, B. E., Knight, T. M., Lee, C. T., and Menges, E. S. 2008. Longevity can buffer plant and animal populations against changing climatic variability. *Ecology*. 89: 19–25.

Niemiller, M. L., Glorioso, B. M., Fenolio, D. B., Reynolds, R. G., Taylor, S. J., and Miller, B. T. 2016. Growth, survival, longevity, and population size of the big mouth cave salamander (*Gyrinophilus palleucus necturoides*) from the type locality in Grundy County, Tennessee, USA. *Copeia* 104: 35–41.

Sato, I. 1943. *A Monograph of the Tailed Batrachians of Japan.* Nippon Shuppan-sha, Osaka.

Schaible, R., Scheuerlein, A., Danko, M. J., Gampe, J., Martinez, D. E., and Vaupel, J. W. 2015. Constant mortality and fertility over age in *Hydra*. *Proceedings of the National Academy of Sciences* 112: 15701–15706.

Seifert, A. W. and Voss, S. R. 2013. Revisiting the relationship between regenerative ability and aging. *BMC Biology* 11: 2.

Sousounis, K., Baddour, J. A., and Tsonis, P. A. 2014. Aging and regeneration in vertebrates. *Current Topics in Developmental Biology* 108: 217–

246.

Sousounis, K., Qi, F., Yadav, M. C., Millan, J. L., Toyama, F., Chiba, C., Eguchi, Y., Eguchi, G., and Tsonis, A. P. 2015. A robust transcriptional program in newts undergoing multiple events of lens regeneration throughout their lifespan. *Elife* 4: e09594.

Staub, L. N. 2016. The age of plethodontid salamanders: a short review on longevity. *Copeia* 104: 118–123.

Tacutu, R., Thornton, D., Johnson, E., Budovsky, A., Barardo, D., Craig, T., Diana, E., Lehmann, G., Toren, D., Wang, J., Fraifeld, V. E., and de Magalhães, J. P. 2018. Human ageing genomic resources: new and updated databases. *Nucleic Acids Research* 46: D1083–D1090.

Tanaka, S. 2005. (4) Amphibians p. 133–143. *In*: Nature Conservation Division, Department of Environmental Affairs, Okinawa Prefectural Government (eds.), *Threatened Wildlife in Okinawa, 2nd Ed. (Animals): Red Data Okinawa*. Nature Conservation Division, Department of Environmental Affairs, Okinawa Prefecture, Naha.

Tominaga, A., Ota, H., and Matsui, M. 2010. Phylogeny and phylogeography of the sword-tailed newt, *Cynops ensicauda* (Amphibia: Caudata), as revealed by nucleotide sequences of mitochondrial DNA. *Molecular Phylogenetics and Evolution* 54: 910–921.

Tominaga, A., Matsui, M., and Matsui, M. 2021. Structure and movement of the hybrid zone between two divergent lineages of the Japanese newt *Cynops pyrrhogaster* (Amphibia: Urodela) in central Japan. *Journal of Zoological Systematics and Evolutionary Research* 59: 1097–1112.

Tominaga, A., Matsui, M., Yoshikawa, N., Hayano, A., and Inoue-Murayama, M. 2014. Characterization of fifteen microsatellite markers for the Japanese fire-bellied newt, *Cynops pyrrhogaster*. *Conservation Genetics Resources* 6: 405–407.

Tsonis, P. A. 1983. Effects of carcinogens on regenerating and non-regenerating limbs in amphibia (review). *Anticancer Research* 3: 195–202.

Voituron, Y., de Fraipont, M., Issartel, J., Guillaume, O., and Clobert, J. 2011. Extreme lifespan of the human fish (*Proteus anguinus*): a challenge for ageing mechanisms. *Biology Letters* 7: 105–107.

Wells, K. D. 2010. *The Ecology and Behavior of Amphibians.* University of Chicago Press, Chicago.

Yun, M. H., Davaapil, H., and Brockes, J. P. 2015. Recurrent turnover of senescent cells during regeneration of a complex structure. *eLife* 4: e05505.

Yun, M. H. 2021. Salamander insights into ageing and rejuvenation. *Frontiers in Cell and Developmental Biology* 9: 689062.

Accepted: 22 April 2023