

# Extreme lifespan of the human fish (*Proteus anguinus*): a challenge for ageing mechanisms

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**Theories of extreme lifespan evolution in vertebrates commonly implicate large size and predator-free environments together with physiological characteristics like low metabolism and high protection against oxidative damages. Here, we show that the 'human fish' (olm, *Proteus anguinus*), a small cave salamander (weighing 15–20 g), has evolved an extreme life-history strategy with a predicted maximum lifespan of over 100 years, an adult average lifespan of 68.5 years, an age at sexual maturity of 15.6 years and lays, on average, 35 eggs every 12.5 years. Surprisingly, neither its basal metabolism nor antioxidant activities explain why this animal sits as an outlier in the amphibian size/longevity relationship. This species thus raises questions regarding ageing processes and constitutes a promising model for discovering mechanisms preventing senescence in vertebrates.**

**Keywords:** amphibian; longevity; metabolism; subterranean environment

## 1. INTRODUCTION

Extreme lifespan among animals has generated substantial research and has been documented in many taxa, such as reptiles, birds and mammals that occupy various aquatic and terrestrial habitats. One of the major factors proposed for explaining long lifespan is the positive correlation between size and longevity [1]. While this appears to be true for most vertebrate taxa, there are exceptions, such as bats and hummingbirds, whose lifespans are high in relation to their size [2]. Protection against predators or stable habitats have often been presented as alternative explanations for the evolution of extreme lifespan [3,4].

Many cave-dwelling species live in both stable and predator-free environments, and it has often been

suggested that these species should have evolved long lifespans [5]. For centuries, many zoologists have been intrigued by the extremely long lifespans displayed by the cave-dwelling 'human fish' (or olm), *Proteus anguinus* (over 70 years in zoos). This aquatic salamander (25–30 cm) is the only cave-adapted vertebrate in Europe, exhibiting troglomorphic characteristics such as skin depigmentation, eye degeneration and neoteny [6]. This species is mainly found in Slovenia and Croatia, and colonized caves approximately 20 Myr ago [6].

In 1952, a cave (Moulis, Saint-Girons, France) was equipped with riverbed-like basins faithfully reproducing the olm's natural habitat. At the end of the 1950s, a breeding programme was started in order to produce individuals without the need to collect them in the wild. Out of all the breeding trials worldwide, it was the only successful programme. Now, nearly 60 years later, there are more than 400 individuals of various ages maintained in this cave. Breeding and deaths have been weekly recorded since 1958 [7]. Based on this information, we were able to calculate age-dependent mortalities and fecundities. Thanks to this unique collection of data on the survival and reproduction of *P. anguinus*, we have investigated different demographic traits and physiological mechanisms generally involved in slow ageing and extreme lifespan, in order to decipher the strategies allowing this species to live so long.

## 2. MATERIAL AND METHODS

To calculate the growth rate and generation time, we built a Leslie matrix according to the life cycle described in the electronic supplementary material, with  $f$  as fecundity,  $\gamma$  the probability to reproduce within a given year,  $s_e$  survival from egg to eclosion,  $s_o$  from eclosion to age 1, and  $s_i$  from age  $i$  to  $i+1$ .  $S$  is the age-invariant survival from age 1. The growth rate is calculated as the dominant eigenvalue of the matrix associated with this graph and the generation time is mean generation length or  $T_{bar}$  [8,9]. Calculations were performed with the computer program ULM [10]. The survival probability with age was calculated with the program SURVIV [11], with capture probability set to 1.

Standard metabolic rates (expressed as  $\text{ml O}_2 \text{ h}^{-1}$  converted to  $25^\circ\text{C}$  using  $Q_{10} = 2.21$ ) were derived from Makarieva *et al.* [12]. Two species were added: *Calotriton asper* and *P. anguinus*. These values have been corrected for a mass of 7.75 g.

A comparative study of lifespans was purchased using data from the AnAge database (<http://genomics.senescence.info/species/>) and body masses from [13].

## 3. RESULTS AND DISCUSSION

The overall growth rate of the population in captivity is 1.05, meaning that the population is slowly increasing—that is, that the actual values of mortality and fecundity are probably close to those displayed in natural (unfed) conditions. If anything, the estimated population turnover rate should be an overestimate with respect to the rate found in nature, since we provide food on a more regular basis and probably in higher quantities than in the wild.

The oldest olms are now at least 48 years old (most probably around 58 years old since they were collected as immature individuals of unknown age) and they do not show any signs of senescence (no sign of a drop in survival after 40:  $\chi^2 = 0.3$ ,  $p < 0.1$ ). The annual survival rate of adults is 0.984 ( $\pm 0.0027$  s.e., 2130 individual years), with no apparent decline with age ( $\chi^2 = 1.1$ ,  $p < 0.1$ ). Juvenile survival (from hatching

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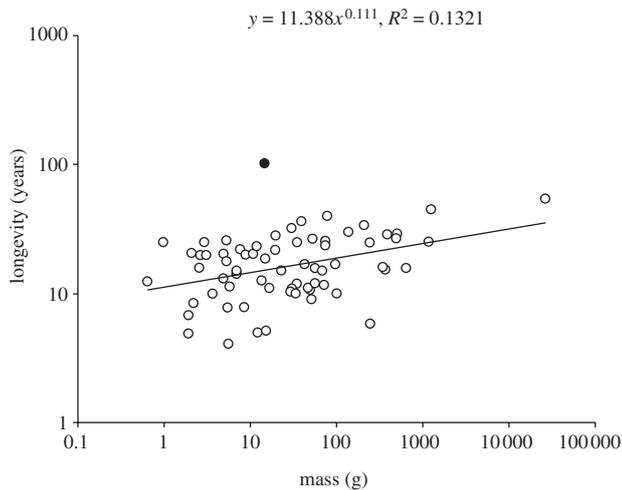


Figure 1. Longevity as a function of body mass in amphibians. Data were derived from AnAge database (<http://genomics.senescence.info/species/>) and from [13].

to age 1) is comparatively poor ( $0.5, \pm 0.015$  s.e., 1103 individuals) and slowly increases with age up to 6 years (see electronic supplementary material). Females are mature at an average age of 15.6 years ( $\pm 3.10$  s.e.) and reproduce once every 12.5 years (probability of an adult laying an egg:  $0.0734 \text{ y}^{-1}$ ,  $\pm 0.0058$  s.e., 146 clutches). Mean clutch size is 35.15 eggs ( $\pm 1.764$  s.e., 123 clutches) and the hatching rate is 0.4058 ( $\pm 0.00942$  s.e.). It follows that the generation time is 36.5 years and adult average lifespan is 68.5 years at 6 years of age (calculated as  $1/(1-s)$ ) [14]. Based on their average survival, 25 per cent of olms aged 6 should still be alive after the age of 85 (calculated as  $0.25 = 0.984^x$ , where  $x$  is the age at which only 25% of a cohort is still alive). This prediction is of course derived under the hypothesis of no senescence until the age of 85. In order to assess maximum lifespan, we use data collected in different taxonomic groups focused on species exhibiting age-independent mortality [1,15]. It appears that the average lifespan ranges from 10 to 67 per cent of the potential maximum [15]. Therefore, and being as conservative as possible (using the 67% value), the maximum longevity of the olm was predicted to be around 102 years (data used for figure 1).

Are these newly known 'human fish' life-history traits in accordance with three mainstream longevity evolution theories? While the 'mutation accumulation' theory suggests a weaker selection counteracting the accumulation of germ-line mutations late in life, the 'antagonistic pleiotropy' theory posits a trade-off between early fecundity or survival and late mortality, and the 'disposable soma' theory predicts an optimal resource partitioning between somatic maintenance and reproduction [16]. Each of these theories predict that a low level of extrinsic mortality reduces the selective premium on early versus late survival, and hence the evolution of a lower rate of senescence [3]. In that way, *P. anguinus* does not challenge longevity theories, as it typically shows *K*-selected traits (i.e. slow development, infrequent reproduction episodes and a high

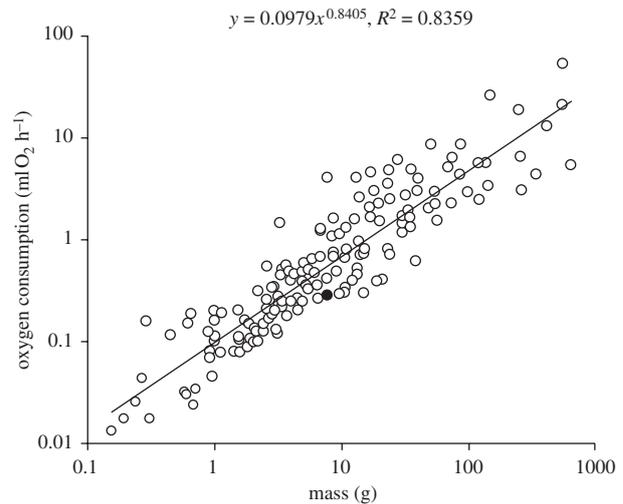


Figure 2. Standard oxygen consumption as a function of body mass for amphibians. Black dot, *Proteus anguinus*.

longevity, putatively explained by a maximization of soma maintenance).

However, a distinction must be drawn between the evolutionary (ultimate) and physiological (proximal) approaches associated with ageing [17]. Physiological constraints associated with body size have long been presented as the main explanation for variation in lifespan [1]. In vertebrate ectotherms, maximum longevity is known to be positively correlated with body size [18], but the data presented in figure 1 clearly demonstrate that the olm has evolved an especially long lifespan with respect to its weight (not exceeding 20 g). In comparison, the closest long-lived amphibian is *Andrias japonicus*, weighing over 25 kg. Within amphibians, species in the family Salamandridae display a low metabolic rate with respect to all other families within the same order [19], yet the olm shows a three times higher longevity when compared with its most closely related species and is clearly an outlier in the regression of amphibian lifespan versus body mass (see figure 1; Cook's distance: 0.29, i.e. more than 10 times the value for the nearest species).

Reducing energy expenditure by reducing basal metabolic rate has also been proposed as a way to increase longevity [20,21], body size and longevity being negatively correlated with basal metabolic rate in endotherms even if there are quite significant differences between vertebrate classes ([12]; see electronic supplementary material). Compared with other vertebrates, salamanders have reduced activity and lower metabolism [22], which might have facilitated their colonization of subterranean habitats. However, when compared with other amphibians, the olm's basal metabolic rate is not markedly different and there is no support for it as an outlier with regard to weight or lifespan (figure 2).

A more recent theory suggests that the energy expenditure *per se* is not the problem, but the fact that some reactive by-products of metabolic activity generate oxidative damages to the organism [23]. Reactive oxygen species (ROS), which are by-products of the respiratory chain, have been pointed out as being particularly noxious for the organism, especially to

DNA, proteins and lipids. As a result, species have developed antioxidant mechanisms devoted to either scavenging ROS or repairing the damage. It is therefore predicted that species with different longevity also differ in their efficiency of such antioxidant mechanisms [24,25]. However, the olm displays neither remarkable antioxidant activity when compared with other species, nor high cellular damage at an age of 28 years ([26]; see electronic supplementary material, table S1). In other words, the olm presents a paradox, since neither its basal metabolic rate nor its antioxidant activity, the two most cited mechanisms that should be involved in enhancing lifespan, differ from species with a more reduced lifespan. Even if there is currently no clear understanding of the links between mitochondrial coupling, ROS production and lifespan in ectotherms [27], we can hypothesize a high mitochondrial coupling providing high efficiency to produce ATP with low energy use and low ROS production. In this scenario, limiting activity and adjusting physiology is a way to reduce ROS production without the necessity to acquire improved physiological antioxidant capacity or to evolve a reduced basal metabolic rate.

Nevertheless, with regard to the data presented here, it is clear that *P. anguinus* raises questions regarding ageing processes and appears as a promising model to describe mechanisms preventing senescence in vertebrates.

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- 1 Calder III, W. A. 1982 The relationship of the Gompertz constant and maximum potential lifespan to body mass. *Exp. Gerontol.* **17**, 383–385.
- 2 Bonsall, M. B. & Mangel, M. 2004 Life-history trade-offs and ecological dynamics in the evolution of longevity. *Proc. Biol. Sci.* **1544**, 1143–1150.
- 3 Rose, M. R. 1991 *Evolutionary biology of aging*. Oxford, UK: Oxford University Press.
- 4 Wilkinson, G. S. & South, M. S. 2002 Life history, ecology and longevity in bats. *Ageing Cell* **1**, 124–131. (doi:10.1046/j.1474-9728.2002.00020.x)
- 5 Culver, D. C. 1982 *Cave life: evolution and ecology*. Cambridge, MA: Harvard University Press.
- 6 Bulog, B. 1994 Two decades of functional-morphological studies of *Proteus anguinus* (Amphibia, Caudata). *Acta Carsol.* **19**, 247–263.
- 7 Juberthie, C., Durand, J. & Dupuy, M. 1996 La reproduction des Protées (*Proteus anguinus*): bilan de 35 ans d'élevage dans les grottes-laboratoires de Moulis et d'Aulignac. *Mém. Biospéol.* **23**, 53–56.
- 8 Caswell, H. 2000 Prospective and retrospective perturbation analyses: their roles in conservation biology. *Ecology* **81**, 619–627. (doi:10.1890/0012-9658(2000)081[0619:PARPAT]2.0.CO;2)
- 9 Caswell, H. 2001 *Matrix population models*, 2nd edn. Sunderland, MA: Sinauer Associates.
- 10 Legendre, S. & Clobert, J. 1995 ULM, a software for conservation and evolutionary biologists. *J. Appl. Stat.* **22**, 817–834.
- 11 White, G. C. 1992 *PC SURVIV user's manual*. Fort Collins, CO: Colorado State University.
- 12 Makarieva, A. M., Gorshkov, V. G., Li, B.-L., Chown, S. L., Reich, P. B. & Gavrillov, V. M. 2008 Mean mass-specific metabolic rates are strikingly similar across life's major domains: evidence for life's metabolic optimum. *Proc. Natl Acad. Sci.* **105**, 16 994–16 999. (doi:10.1073/pnas.0802148105)
- 13 Altman, P. L. & Dittmer, D. S. 1972–1974 *Metabolism. Biology data book*, vol. 3, part IX. Bethesda, MD: Federation of American Societies for Experimental Biology.
- 14 Krebs, C. J. 2001 *Ecology: the experimental analysis of distribution and abundance* (ed. B. Cummings), pp. 801, 5th edn. Menlo Park, CA: Benjamin-Cummings Publishing Company.
- 15 Calder III, W. A. 1984 *Size, function and life history*. Cambridge, MA: Harvard University Press.
- 16 Kirkwood, T. B. L. & Austad, S. N. 2000 Why do we age? *Nature* **408**, 233–238. (doi:10.1038/35041682)
- 17 Bonsall, M. B. 2006 Longevity and ageing: appraising the evolutionary consequences of growing old. *Phil. Trans. R. Soc. B* **361**, 119–135. (doi:10.1098/rstb.2005.1738)
- 18 Blanco, M. A. & Sherman, P. W. 2005 Maximum longevity of chemically protected and non-protected fishes, reptiles, and amphibians support evolutionary hypotheses of aging. *Mech. Ageing Dev.* **126**, 794–803. (doi:10.1016/j.mad.2005.02.006)
- 19 Jorgensen, C. B. 1992 Growth and reproduction. In *Environmental physiology of the amphibian* (eds M. E. Feder & W. W. Burggren), pp. 439–466. Chicago, IL: University of Chicago Press.
- 20 McCoy, M. W. & Gillooly, J. F. 2008 Predicting natural mortality rates of plants and animals. *Ecol. Lett.* **11**, 710–716. (doi:10.1111/j.1461-0248.2008.01190.x)
- 21 Pearl, R. 1928 *The rate of living, being an account of some experimental studies on the biology of life duration*. New York, NY: Knopf.
- 22 Feder, M. E. 1976 Oxygen consumption and body temperature in neotropical and temperate zone lungless salamanders (Amphibia: Plethodontidae). *J. Comp. Physiol. B* **110**, 197–208. (doi:10.1007/BF00689308)
- 23 Harman, D. 1956 Aging: a theory based on free radical and radiation chemistry. *J. Gerontol.* **11**, 298–300.
- 24 Balaban, R. S., Nemoto, S. & Finkel, T. 2005 Mitochondria, oxidants, and aging. *Cell* **120**, 483–495. (doi:10.1016/j.cell.2005.02.001)
- 25 Halliwell, B. & Gutteridge, J. M. 1999 *Free radicals in biology and medicine*. Oxford, UK: Oxford Science Publications.
- 26 Issartel, J., Hervant, F., de Fraipont, M., Clobert, J. & Voituron, Y. 2009 High anoxia tolerance in the subterranean salamander *Proteus anguinus* without oxidative stress nor activation of antioxidant defenses during reoxygenation. *J. Comp. Physiol. B* **179**, 543–551. (doi:10.1007/s00360-008-0338-9)
- 27 Hulbert, A. J. 2008 The links between membrane composition, metabolic rate and lifespan. *Comp. Biochem. Physiol. A* **150**, 196–203.