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

Yann Voiturou, Michelle de Fraipont, Julien Issartel, Olivier Guillaume and Jean Clobert  

1 Écologie des Hydrosystèmes Fluviatifs, UMR CNRS 5023, Université Claude Bernard Lyon 1, Université de Lyon, 69622 Villeurbanne cedex, France  
2 Station d’Écologie Expérimentale du CNRS à Moulis, USR 2936, Moulis, 09200 Saint-Girons, France  
3 Université Montpellier 2, UMR 5119-UM2-CNRS-IFREMER Écolog, Equipe Adaptation Écophysiologique et Ontogenèse, case courrier 92, Montpellier Cedex 05 34095, France  
*Author for correspondence (voiturou@univ-lyon1.fr).  
These authors contributed equally to this work.*

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 65.5 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 2005). 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 My 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, $\nu$, survival from egg to eclosion, $\sigma$, from eclosion to age 1, and $\iota$, from age 1 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 ml O$_2$ h$^{-1}$ converted to 25°C using Q$_{10} = 2.21$) were derived from Makarevich 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
to age 1) is comparatively poor (0.5, ±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 (±3.10 s.e.) and reproduce once every 12.5 years (probability of an adult laying an egg: 0.0734 y⁻¹, ±0.0058 s.e., 146 clutches). Mean clutch size is 35.15 eggs (±1.764 s.e., 123 clutches) and the hatching rate is 0.4058 (±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 = 0.984⁴, 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 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 japonicas*, 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 longevities 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 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 Proteus anguinus raises questions regarding ageing processes and appears as a promising model to describe mechanisms preventing senescence in vertebrates.

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