Extremely short lifespan in the annual fish *Nothobranchius furzeri*

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Evolutionary theories of senescence postulate that lifespan is determined by the age-dependent decrease in the effects of natural selection. Factors that influence survival and reproduction at early life stages have a larger impact on fitness than factors that influence later life stages. According to these views, selection for rapid sexual maturation and a steep age-dependent decrease in fitness drive the evolution of short lifespans. Here, we report on the survival trajectory of *Nothobranchius furzeri* (Pisces: Ciprinodontidae): a member of a group of annual species found in temporary bodies of water whose life expectancy in the wild is limited to a few months. We find that maximum survival of *N. furzeri* in the laboratory is less than 12 weeks. The temporal trajectory of survival shows an age-dependent increase in the mortality rate that is typical of organisms with defined lifespans. The lifespan of *N. furzeri* is exceptionally short for a vertebrate: owing to its small size and the possibility of propagation in captivity, *N. furzeri* could be used as a convenient model for ageing research.

1. INTRODUCTION

Most animal species present a defined lifespan and a progressive decay in their survival capability with age. This age-dependent increase in the intrinsic mortality rate can be interpreted as the progressive failure of the physiological mechanisms that maintain homeostasis and repair damage, and is defined as senescence. Evolutionary theories of ageing explain senescence as a consequence of the age-dependent decrease in the effects of natural selection (Rose 1991; Holliday 1995; Kirkwood 2002). In particular, three theories have been proposed: ageing is explained as (i) the result of either pleiotropic negative effects in late life of genes that drive early maturation and growth (Williams 1957); (ii) the quasi-neutral accumulation of mutation with a phenotypic expression during old age (Hamilton 1966); or (iii) the trade-off between the resources devoted to reproduction and maintenance of somatic cells (Kirkwood & Holliday 1979).

Fishes of the genus *Nothobranchius* are adapted to the unstable and unpredictable ecological conditions of temporary bodies of waters that form during the rainy season in equatorial and subequatorial Africa (Scheel 1990).

Before their seasonal habitat disappears, adults spawn and lay eggs in the muddy bottom. Embryos survive encased in the dry mud in a dormant state and hatch at the onset of the next rainy season to repeat the life cycle that is typical of this taxon. Small-sized fishes that inhabit temporary ponds are adapted to fast growth to rapidly reach sexual maturity (Liu & Walford 1966; Markofsky & Perlmutter 1972, 1973). Evolutionary theories of ageing postulate that an increase in the extrinsic mortality rate will result in an increase in the intrinsic mortality rate and therefore shorter lifespans (for an experimental test of this theory, see Stearns *et al.* (2000)). As predicted by evolutionary theories of senescence (Rose 1991; Holliday 1995; Kirkwood 2002), these organisms show limited lifespans even when reared under controlled laboratory conditions (Liu & Walford 1966; Markofsky & Perlmutter 1972, 1973).

We have studied a captive population of *Nothobranchius furzeri* (Jubb 1971). This species was selected because it originates from a region with a temporally restricted rainy season (data from the Worldwide Agroclimatic Database, FAO, Rome) that presumably generates a selective pressure to rapid growth and sexual maturation and defined its survival curve in the laboratory.

2. MATERIAL AND METHODS

Dormant eggs of *N. furzeri* were obtained by the Associazione Italiana Killifish, Canossa, Italy. Founders of this line were collected in 1970 in Zimbabwe (Jubb 1971). Eggs were hatched in oxygenated tap water (pH 7.5, conditions 450 µS cm^{-1}) at 18 °C and the larvae were fed with newly hatched *nauplii* from *Artemia salina* for the first two weeks. Starting from the third week, fishes were fed a mixture of commercial frozen food. Fishes were housed in groups of 8 to 10 individuals in bare tanks (40 l in capacity) filled with tap water. Each tank was heated using an aquarium heater. Fishes were counted every day in the evening to record any deaths. Breeders were separated in groups of one male with several females and peat moss was used as a spawning substratum using standard techniques for cultures of killifish (Scheel 1990). The peat moss containing the fertilized eggs was dried and stored in sealed bags at room temperature and was inspected at regular intervals for the presence of fully developed embryos. After three months of incubation, most eggs contained a fully developed embryo and the hatching procedure was repeated.

3. RESULTS

Dormant eggs of *N. furzeri* were hatched ($n = 30$), the larvae fed *ad libitum* and their survival rate recorded. In a first experiment, the larvae were kept at room temperature during the months of June–August in Canossa, Italy with natural illumination and daily oscillations of temperature in the range 23–30 °C to mimic the daily temperature oscillations typical of their habitat. At four weeks of age, the largest males showed their nuptial coloration and started courting females. Some males were separated and bred with clutch-mate females in the presence of spawning substratum. At six weeks of age, the mortality rate increased steeply and the last fish died after 10 weeks (figure 1*a*). Fertilized eggs were collected, stored in dry spawning substratum for three months and then hatched ($n = 68$). The temperature was kept constant at 25 °C. The temporal profile of survival was recorded and was basically identical to the previous generation (figure 1*a*). The instantaneous mortality rate was plotted as a function of age (figure 1*b*). In a large variety of organisms, a linear relationship between the logarithm of mortality rate and age, known as the Gompertz function, is observed and is considered a hallmark of senescence and a defined lifespan
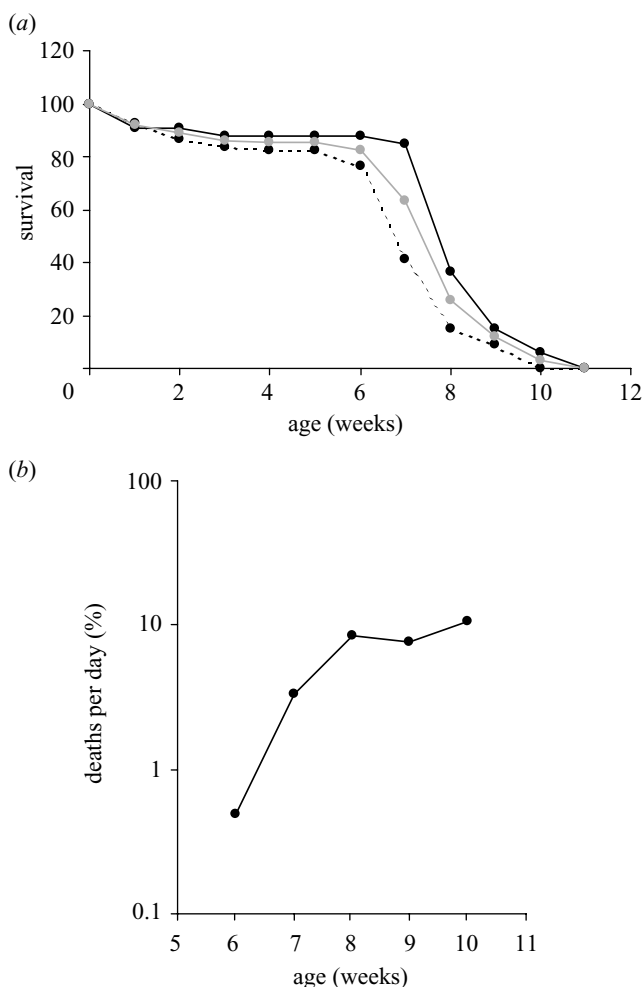
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Figure 1. (*a*) Survival trajectory of *Nothobranchius furzeri* in the laboratory. Survival is expressed as a percentage of maximum survival. The 25 °C curve (dashed line) plots the survival rate of 68 fishes kept at a constant temperature of 25 °C. The 'Var' curve (black line) plots the survival rate of 33 fishes kept at room temperature during the months June–August in Canossa, Italy. Tot (grey line) is the average of the two curves. (*b*) Mortality rate as a function of age in *N. furzeri*. The curve represents the average of the two experiments. Instantaneous mortality rate is expressed as the percentage of deaths per day. Note the logarithmic scale on the *y*-axis.

(Rose 1991; Holliday 1995; Vaupel *et al.* 1998). The increase in the mortality rate often decelerates at late ages, and the function assumes a concave shape (Vaupel *et al.* 1998). These general kinetic properties of survival are also observed in *N. furzeri* (figure 1*b*).

4. DISCUSSION

In the present paper we report the shortest lifespan ever, to our knowledge, recorded for a vertebrate. The annual fish *N. furzeri* has a maximum lifespan of only 12 weeks when cultured at 25 °C under laboratory conditions. This lifespan is comparable to that of *Drosophila* cultured at the same temperature (Kang *et al.* 2002) and is extremely short even when compared with that of other species of annual fishes that have been studied experimentally (lifespan is supposed to be 12 months; Liu & Walford 1966; Markofsky & Perlmutter 1972) and that of the

shrews (lifespan is also supposed to be *ca.* 12 months; Smith 1995). However, the kinetic properties of life trajectories, with an exponential increase of mortality probability with increasing age and a late deceleration, is consistent with the pattern observed in many species (Rose 1991; Holliday 1995; Vaupel *et al.* 1998) and can be considered to be the hallmark of a genetically limited lifespan. Two explanations can be invoked to explain the extremely short lifespan of *N. furzeri*. The captive population that we have been studying originates from fishes that were imported to the USA in 1970 (Jubb 1971) and the lifespan of the extant population might be the result of a spontaneous mutation that occurred in captivity. However, reports from the first people who kept *N. furzeri* in captivity in the early 1970s give a lifespan of a few months (Foersch 1975). Moreover, all fishes of the genus *Nothobranchius* are known for being fast growing and short lived, often living for less than 1 year (Scheel 1990). These fishes can be found in temporary bodies of waters that form during the rainy season in equatorial and subequatorial Africa (Scheel 1990). Thus, members of this taxon have to complete the reproductive cycle before their seasonal habitat disappears and it is highly likely that their short lifespan is determined by genetic factors and represents the result of pleiotropic effects of genes that drive very fast sexual maturation, or the diversion of energy from somatic cell maintenance to growth and reproduction (Kirkwood 2002). *Nothobranchius furzeri* would then represent the lower end of the lifespan spectrum in a taxon of organisms adapted for fast growth.

The extremely short lifespan of *N. furzeri* is not only interesting from an evolutionary perspective. The past decade has witnessed a monumental effort in establishing zebrafish (*Danio rerio*) as a new model system for developmental biology leading to the establishment of techniques for large-scale screening of mutants (Driever *et al.* 1996) and the production of transgenic animals (Amsterdam *et al.* 1996; Nasevicius & Ekker 2000). However, the lifespan of zebrafish in the laboratory is close to 5 years (Gerhard *et al.* 2002), and model animals that combine the advantages of small fishes with shorter lifespans would be desirable. Owing to its small size and the possibility of being propagated in laboratory, maybe *N. furzeri* could become a unique animal model for ageing research.

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