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Review Article

Nothobranchius as a model for aging studies. A review

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ABSTRACT: In recent decades, the increase in human longevity has made it increasingly important to expand our knowledge on aging. To accomplish this, the use of animal models is essential, with the most common being mouse (phylogenetically similar to humans, and a model with a long life expectancy) and *Caenorhabditis elegans* (an invertebrate with a short life span, but quite removed from us in evolutionary terms). However, some sort of model is needed to bridge the differences between those mentioned above, achieving a balance between phylogenetic distance and life span. Fish of the genus *Nothobranchius* were suggested 10 years ago as a possible alternative for the study of the aging process. In the meantime, numerous studies have been conducted at different levels: behavioral (including the study of the rest-activity rhythm), populational, histochemical, biochemical and genetic, among others, with very positive results. This review compiles what we know about *Nothobranchius* to date, and examines its future prospects as a true alternative to the classic models for studies on aging.

Key words: Aging, Fish, killifish, *Nothobranchius*

Aging is a multifactorial process that involves numerous mechanisms that operate during the normal life cycle of an organism, triggering a progressive, irreversible deterioration that ultimately leads to its death. Because of the sheer complexity of the aging process, it remains one of our great scientific challenges even today. The work invested in studying aging (a search for "aging" on Web of Knowledge[SM] returns more than 3 million results after 2003) has made it possible to identify some of the processes that take place with aging, but we are still far from being able to offer a theory that links them all together in a solid chain of cause-effect relationships. Some of these studies have been carried out on humans, but this type of research is very limited for both ethical and technical reasons, which makes it necessary (as in many other fields of science) to resort to models in which experimental designs can be used that would be impossible to apply to human beings, and which permit us to adequately study aging.

To be considered a model for aging, an animal must have a number of features. Firstly, the candidate must show signs of age-related deterioration that can be used as biomarkers. Secondly, the phylogenetic distance between the model and the human race must be as short as possible, in order to facilitate the extrapolation of the study results. Finally, the animal's life expectancy must be as short as possible, to minimize the time needed to conduct the experiments and make observations. Historically, the mouse has been the aging model par excellence, since its biology is well known and it is phylogenetically close to humans. Nevertheless, the life expectancy of these animals is at least 24 months, which is a considerable amount of time if we consider the high cost of maintaining them in laboratories over long periods of time. In addition, mice typically exhibit a nocturnal behavior pattern, which complicates their use during their activity phase in a laboratory setting. Mammals in general also give birth to relatively few offspring at a time, which increases the genetic variability of the subjects in a particular study.

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Another commonly used model in aging studies is *Caenorhabditis elegans*, a nematode measuring 1 mm in length, with a maximum life expectancy of 2 to 3 weeks. The cost of maintenance is very low and its entire life cycle can be covered by short experimental periods. The problem associated with this small worm is its great evolutionary distance from mammals, which complicates the extrapolation of the data obtained to humans. In terms of life expectancy and phylogenetic distance from humans, halfway between the mouse and *C. elegans* we find a group of vertebrates with the potential to be used as models in aging studies: fish.

What do fish have to offer?

Fish constitute the largest group of vertebrates [1], representing approximately one-half of all species. Within this vast group, we find a wide variety of species that differ greatly in terms of their longevity and versatility as experimental models [2]. As early as 1978 [3], work with guppies demonstrated that fish have great potential for use as valid models in aging studies, due to certain characteristics that have proven very interesting. The main advantage of fish is their great power of adaptation to a wide range of environmental conditions. Fish are particularly sensitive to variations in environmental temperature, which makes it relatively easy to manipulate their rate of aging. Other external factors, such as diet, space available in the environment and reproductive opportunities are also determining factors. One property of fish to consider is the generally large number of eggs laid at a time. In most cases, an experiment can be conducted with eggs laid altogether at the same time, which enables us to know with certainty the origin of the animals, all of which have the same genetic background, facilitating the monitoring of the animals throughout their entire life cycle. Finally, maintenance and rearing costs for large numbers of fish throughout their life cycle are lower than for mammals.

Fish as models for aging studies

It was in the 1960s when the scientific community began to consider fish from a gerontological perspective. The first systematic gerontological studies were carried out with a type of guppy, *Lebistes reticularis* (Peters, 1859), and described how the aging process could be modulated through calorie restriction and temperature manipulation [4-6]. Observations were also made with regard to how the guppy's reproductive capacity was affected over time [6, 7]. This work continued in the 1980s, when Woodhead *et al.* characterized the aging of guppies at the histological level [8, 9]. The guppy was later used for aging studies conducted on natural populations. Guppy populations have demonstrated different mortality rates depending on factors such as geographic location and co-existence with predators, with sexual maturity being a key factor in determining their life expectancy [10].

However, it is another species, the zebrafish, *Danio rerio* (Hamilton-Buchanan, 1822), the one that has undergone exponential growth in terms of its use as an experimental model in recent decades. These small tropical water cyprinids were used mainly in developmental biology because of the transparency of their embryos, their rapid development, their high proliferation capacity (laying as many as 200 eggs at a time) and low maintenance cost in a laboratory setting [11]. These characteristics and its tremendous versatility have gradually led to the use of the zebrafish as an experimental model in many fields of research, to the extent that it is currently one of the best characterized animals at the genetic level [12-15]. In spite of its undeniable potential as a model, the use of the zebrafish in aging studies has been somewhat less than satisfactory. The main reason is its life expectancy, which is at least 2 years [16]. It is true that this life expectancy is no greater than that of mice, and that a very high-resolution map of its genetic material has been developed, making it possible to generate mutant zebrafish with shorter life cycles or expressing aging markers earlier than normal [17]. Nevertheless, they would still be genetically manipulated individuals, and the data obtained from this type of experiments must be interpreted with caution.

The genus *Nothobranchius*. Characteristics that make it unique.

There is another very interesting group of fish in the order Cyprinodontiformes, the so-called *killifish* (from the Danish word "kilde", meaning small stream or puddle), which are quite valuable as gerontological models for experimentation. In the 1960s scientists began to use fish from the genus *Cynolebias* to determine the capacity of temperature as a modulator of the aging process [18, 19]. In the 1970s, histopathological changes were documented in the liver and kidney with age in the genus *Nothobranchius* [20, 21]. But it was around 2004 when the work by Herrera and Jagadeeswaran [22] pointed to killifish, primarily those belonging to the genus *Nothobranchius,* as potential aging models.

The genus *Nothobranchius* is formed by annual fish from Africa that live in puddles produced by seasonal rains. These fish have adapted to environmental rain cycles by developing eggs that are resistant to desiccation. Before the puddles disappear, the males stimulate the females to lay eggs, and the fertilized eggs are then buried in the substrate. Once the puddle dries up, the eggs enter into a state of diapause, and their development shows

down until the new rainy season arrives and ponds are formed once again. The juvenile fish then hatch and a new cycle begins [23]. Fish from the genus *Nothobranchius* have a short life cycle, which is obviously conditioned by the extreme environment in which they live. Depending on the species, maximum life expectancies have been reported that range from 3 (*N. furzeri*) to 28 months (*N. guentheri*) [24, 25]. This peculiar characteristic is what makes them especially attractive for aging studies, combined with the fact that they are small vertebrates, thus requiring no large laboratory equipment. They are also tolerant in terms of water quality. It is simple to establish a colony in a laboratory setting, since egg laying can be naturally induced in the laboratory. Furthermore, the eggs can be easily collected by placing a container with coconut fibre or fine sand in the bottom of the aquarium. This substrate must be allowed to air dry until it is only slightly damp before being stored in small containers; this reduces the required space and facilitates the maintenance of a continuous stock.

What do we know about *Nothobranchius?*

The first studies with *Nothobranchius* of which we have record were carried out on *N. guentheri* by Markofsky *et al.* [24, 26], characterizing the growth of isolated groups of males and describing the first aging markers in this genus: deterioration of the liver with age [21], the appearance of small malignant tumors on the kidneys [20] and the deterioration of the immune system, accompanied by generalized tumor development [27]. Balmer, in turn, related the aging process in *N. guentheri* to a drop in the energy content in the animal as a whole [28]. In the following years, researchers began to use other species from the genus *Nothobranchius*, such as *N. korthausae,* in which hyperplasia of the thyroid gland and the anterior pituitary gland were described in association with age [29], indicating the potential of the genus for use as a model for the study of tumors.

In 2003, an exceptionally short life cycle was described for another species in this genus: *Nothobranchius furzeri*, with a maximum life expectancy of 12 weeks, the average being 9 weeks, which is the shortest so far described for a vertebrate [25]. This work, along with that by Herrera and Jagadeeswaran in 2004 [22], which suggested the genus *Nothobranchius* as a potential genetic model for studying development and aging, represented the true start of the push to establish the genus *Nothobranchius* as a model for gerontological studies. From that moment on, different research groups began efforts to study aging in *Nothobranchius*. Even though today *N. furzeri* continues to be the most commonly used species, new species continue to be characterized, such as *N. rachovii* [30], each of which having distinctive properties that make them ideal for certain methodologies. This enables researchers to approach the study of aging from different angles, thereby enriching the information that we have about this process in the genus *Nothobranchius*.

1. Growth, reproduction and longevity

The different studies carried out with species from the genus *Nothobranchius* describe the existence of explosive growth in all of them. This growth is noticeable to the naked eye and can be observed from one day to the next. In this case, the growth is constant from the time the eggs hatch until the animals reach adulthood, with a body length of around 5 cm. Sexual maturity is reached 3-4 weeks after the eggs hatch. At this time, the growth rate of males exceeds that of females and the typical coloring of each species begins to become evident [25, 31]. Sexual dimorphism in *Nothobranchius* is very noticeable; males are larger than females and have bright colors that make them very attractive for hobby aquarists [22, 30-33]. It is interesting to note that the elimination of males from a population results in larger female fish that produce fewer eggs [34]. It would seem that the disappearance of sexual stimuli reduces the number of eggs the females lay, which then use this energy to grow. This seems to reflect the disposable soma theory of Kirkwood [35], which considers aging to be the result of competition for an organism's available energy between maintaining the soma and reproduction.

Once they have reached adulthood, the animals begin to display courting behaviors and, from that point on, they will lay eggs on a regular basis for the rest of their lives. However, it has been observed that the number of eggs laid each time and their viability decrease once the fish stops growing [31]. This would be supported by the work of Di Cicco *et al.* [36], who found that the gonads of *Nothobranchius furzeri* show changes associated with age, and by numerous observations made by amateur breeders of the genus *Nothobranchius* [37] and another genus of killifish, *Austrofundulus limnaeus* [38]. The reproductive senescence observed in *Nothobranchius* differs from that observed in fish species with longer life cycles, whose reproductive capacity remains intact with age [39].

One interesting aspect is the difference in life expectancy observed between different species of the genus *Nothobranchius*, although intraspecies disparity also exists. The longest life expectancy described for the genus corresponds to *N. guentheri*, with an average life span of 64 weeks and a maximum of 112 weeks [24]. However, a more recent study recorded an average life span of 43 weeks and a maximum of 64 weeks [33]. Something similar occurs with *N. rachovii*: an average life

expectancy of 34 weeks and a maximum of 37 weeks were reported [22], but a later publication described a greater life expectancy, with an average of 58 weeks and a maximum of 64 weeks [40]. These differences could be attributed to intraspecies variations (between different varieties) or differences in rearing and maintenance conditions, such as the amount of food supplied or the dietary composition, parameters that are involved in the metabolic rate, oxidative stress, disease and aging [41, 42]. For this reason, it would be important to standardize the protocols for maintaining colonies of *Nothobranchius*, taking into account aspects such as temperature, the type and frequency of feeding, photoperiod, population density, sex ratio and reproduction. This would prevent the emergence of apparently contradictory data and would go a long way to promoting the expansion of the model. Another species studied is *N. korthausae*, with an average life expectancy of 57 weeks and a maximum of 81 weeks [31]. Finally, there is the odd case of *N. furzeri*, for which different varieties are known (captured at different geographic locations), each with a specific life expectancy, ranging from the lowest average of 9 weeks and a maximum of 12-13 weeks (GRZ variety) to the longest average life expectancy of 24 weeks and a maximum of 32 weeks (MZM04/03 variety) [25, 32, 43].

The most plausible explanation for these interspecies (or intervariety) differences in life expectancy for the genus *Nothobranchius* lies in the characteristics of the habitat where each species originates, primarily the rainfall pattern in the area, a determining factor in how long puddles exist before drying up [31, 37, 43]. The habitat of *N. guentheri* (Zanzibar) is characterized by the presence of two rain cycles, and it is possible that the puddles where the fish live never dry up at any time during the year (www.worldweather.org). Something similar occurs with the habitat of *N. korthausae* (Mafia Island, Tanzania), where the climate can be considered tropical. However, the habitat of *N. furzeri* (plains of Zimbabwe) is much drier, and the puddles remain only for short periods of time. The potential correlation between the ratio of precipitation/evaporation where the fish lives and the life expectancy of each variety has been studied in the case of *N. furzeri*, observing a greater life expectancy as the ratio increases, i.e. where the precipitation is greater and/or less evaporation occurs [43]. Evolutionary pressure favoring a very short life cycle would be much greater in a habitat that literally evaporates in a short period of time. Some authors have administered treatments intended to increase the life expectancy of different species of *Nothobranchius,* such as calorie restriction [32], lowering the water temperature [44, 45] or using exogenous substances such as resveratrol [33, 46, 47], which have been suggested as possible treatments against aging [48].

2. Morphological and histopathological changes

The different species of *Nothobranchius* studied have shown numerous morphological changes associated with age, making it relatively easy to identify individuals with a senescent phenotype. The most evident morphological change is the loss of color that is observed in fish with age, which is much more noticeable in males, due to the characteristic coloring they have throughout adulthood. Both males and females also suffer a noticeable loss of body mass, which is reflected by a decrease in weight, in addition to the curvature of the dorsal spine [37] and deterioration of the fins, especially the caudal fin [31]. These changes have also been described in other genera of fish, such as the zebrafish [16] and the Japanese rice fish, or medaka (*Oryzias latipes*) [49]. In addition, in *Nothobranchius korthausae* the development of cataracts has been described in some of the individuals studied [31].

Age-related changes have also been described at a microscopic level that may be used as markers of aging. Accordingly, progressive increases in granules of lipofuscin, -galactosidase and fluoro-Jade B in tissues in organs such as the liver [32], different areas of the brain [32, 37, 43], skin [30, 44] and gills [30, 45], which is related to the cellular and tissue deterioration associated with aging. Furthermore, it has been observed that the time for the onset of these markers increases with the application of the aforementioned treatments, such as a decrease in water temperature, calorie restriction or the use of anti-aging molecules like resveratrol [32, 33, 44-47].

Another interesting observation in *Nothobranchius* is the high incidence of tumors that develop with aging. The post-mortem analysis of *N. guentheri* suffering spontaneous deaths frequently revealed neoplasms in the liver, and to a lesser extent, in the kidneys [20, 21]. Cooper *et al.* [27] also described the development of widespread tumors with age in *Nothobranchius guentheri* and *Cynolebias (Austrolebias) adloffi,* associated with a weakening of the animal's immune system. Besides tumors, a higher frequency of the onset of degenerative disorders has also been described in the liver, particularly steatosis, and the kidneys of *Nothobranchius*, something which has also been described in other fish species, such as *Poecilia reticulata* and *Austrolebias adloffi* [9, 50]. Other symptoms were found in the heart (fibrosis and aggregations of lymphocytes around myocardial fibres) and the gonads (atrophy and fibrosis). It is interesting to note that the severity of the disorders is normally greater in males than in females [36]. In this regard, *Nothobranchius* has been suggested as an accurate model to study tumorigenesis.

3. Cognitive and behavioral changes. The circadian system.

Like what has been described in humans and other animal models, the species belonging to the genus *Nothobranchius* suffer cognitive degeneration as they age. This deterioration has been quantified by different conditioned learning tests, which analyze, for example, the animal's capacity to avoid a certain type of stressor (for example, a weak electrical shock) within a certain time frame. This test is referred to as an Active avoidance task [43, 44], and it is a modification of the so-called shuttlebox originally designed for goldfish and zebrafish [51, 52]. This test showed an increase in the response time of *Nothobranchius* to the conditioning stimulus with age, accompanied by significantly fewer correct responses [43]. Calorie restriction [32], the decrease in temperature [44] and the administration of resveratrol [33, 46] produced a delay in cognitive decline.

One line of research that has shown great potential in the study of an animal's overall functional state and its deterioration with age is that of biological rhythms and, among them, the rest-activity rhythm, which is determined by the circadian system. The circadian system (CS), present at all levels in living beings, is the biological response to an environment that is constantly, yet cyclically changing. Most physiological processes in an organism are perfectly coordinated or synchronized with changes in the environment in which it lives; this is thanks to the existence of a biological clock capable of receiving changing signals from the environment, integrating them and issuing synchronized, cyclical responses (what we call biological rhythms) [53]. The quintessential environmental signal synchronizing the biological clock is the 24-hour light-dark cycle caused by the Earth's rotation. Most of the biological rhythms described have a period that lasts approximately 24 hours, in other words, that is circadian (from the Latin word *circa,* meaning near or close and *die*, meaning day).

The biological clock consists of a central pacemaker located in the central nervous system, equipped with informational input channels or afferent pathways (e.g. photoreceptors in the retina) and output channels or efferent pathways, which would drive the overt rhythms. Although not as much is known about the CS in fish as in mammals, it is believed that the function of the central pacemaker is performed by the pineal gland, which receives light information directly and through photoreceptors in the retina, and produces melatonin, a hormone secreted during the night, and which serves as a chemical signal of darkness for the organism. The presence of melatonin signals to the organism that it is in the nocturnal phase, while the higher or lower peak amplitude of this hormone release will indicate the duration of the phase, which depends on the season of the year [54]. The animal's capacity to synchronize with its environment is also affected by aging [55]. This process can be characterized by analyzing the animal's overt rhythms, and used as a marker of senescence. One of the best studied rhythms is the locomotor activity rhythm, which indicates periods of rest and activity in a species [56]. Accordingly, it has been observed that *Nothobranchius* species are diurnal, with stable, regular locomotor rhythms during adulthood that suffer a clear deterioration with age, losing a good share of their regularity, becoming fragmented and decreasing in amplitude [31]. By analyzing an animal's locomotor activity, it is possible to characterize a species' sleep-wake cycle. *Nothobranchius* evidence periods of sleep-like states [57], described as nocturnal periods of inactivity, during which time the animals float on the water with their heads pointing slightly upwards, normally near the surface, with no movement of the pectoral fins [58]. In *Nothobranchius*, these periods of sleep-like states suffer an age-related deterioration, characterized by an increase in sleep interruptions during the night [58]. In mammals, this deterioration has been associated with an imbalance in the production of melatonin by the pineal gland [59]. In *N. korthausae*, temporary exogenous administration of melatonin to aged animals produces a significant improvement in the animal's locomotor activity rhythm, with increased regularity and decreased rhythm fragmentation. This treatment also improves the quality of the sleep period, in particular due to the effect of sleepiness that occurs, as has also been described for mammals and zebrafish [60, 61], and reduces the number of night-time sleep interruptions and their duration. This effect is lost as soon as the treatment ends [58]. The benefits of administering melatonin have been widely reported in both humans and animals [62-65]. For this reason, *Nothobranchius* might be an excellent model for studies on the aging of the circadian system.

4. Biochemical changes

Many studies carried out with cell cultures and animal models support the fact that reactive oxygen species (ROS), particularly those produced by the mitochondria, play a key role in the aging process [66-68]. ROS are continuously produced as the result of the normal functioning of the electron transport chain (ETC) and oxidative phosphorylation, and they cause a cumulative and irreversible damage to the tissues, leading to cellular dysfunction with age [69, 70]. It would appear that the mitochondria are not only the main source of ROS, they are also the first target of the oxidative attack. According to the mitochondrial theory of aging, the ROS produced

by the mitochondrial ETC under normal physiological conditions cause damage to proteins, lipids and mitochondrial DNA (mtDNA) [71-73]. Some authors believe that the first target of oxidative damage in the mitochondria is the mtDNA and that as more and more mutations accumulate in critical DNA regions, the complexes that make up the ETC become less efficient or are rendered inactive, leading to mitochondrial dysfunction [74]. In recent years, many diseases associated with senescence in mammals and humans have been related to the accumulation of mutations in the mtDNA [75, 76]. This process has also been described in fish, specifically in species of the genus *Nothobranchius*. In a study conducted on *N. furzeri*, a decrease in the number of copies of mtDNA was observed in different tissues with age, as well as a reduction in the expression of peptides encoded by the mtDNA, all of which are components of the complexes that make up the ETC [77]. The explanation of the high rate of mutations observed in the mtDNA (much higher than for nuclear DNA) may have something to do with the fact that the mtDNA is greatly exposed to ROS: 1) it is located very near the internal mitochondrial membrane where ROS are produced, 2) it is not overly condensed or protected by histones and 3) it has limited repair activity [73]. However, even though all mitochondria molecules are attacked by ROS, it should be pointed out that lipid peroxidation is the most important oxidative process, primarily due to the extreme sensitivity to oxidation of polyunsaturated fatty acids (PUFA), the constituent molecules of cell membrane phospholipids [78]. Lipid peroxidation also gives way to a whole series of highly reactive derivatives that propagate the oxidation reaction throughout the mitochondrial membrane and to other molecules [68]. One recent theory on aging, that of the membrane pacemaker, suggests that the membrane lipids are the first target of ROS, and damage to these molecules causes an alteration in the membrane fluidity, resulting in a mismatch between the ETC and the oxidative phosphorylation, which in turn increases the rate at which ROS are produced [41]. Finally, the cell's repair and defense mechanisms would be overwhelmed and the oxidative damage would spread, particularly to the mtDNA. In cross-sectional studies on different animal species, including mammals, birds and reptiles, it has been observed that species with lipid membranes that are highly unsaturated, and therefore more fluid, have a high level of metabolic activity, due to the greater mobility of the membrane proteins, which are responsible for a large part of the cell's metabolism. A higher metabolic rate is associated with greater oxidative stress, which according to this theory, would primarily affect the PUFA in the membrane in a vicious circle, which would lead to a high rate of aging and a low life expectancy. There is evidence of oxidative damage associated with aging in the species of the genus *Nothobranchius*. Hsu *et al.* [30] identified an increase in oxidative stress in several tissues of *N. rachovii* with age. The authors observed and increase in lipid peroxidation and oxidative damage to proteins, along with a drop in antioxidant activity with age. Considering that mitochondrial membranes have a distinctive composition of lipid types, phospholipids, glycolipids and cholesterol related to the role of this organelle in energy metabolism and oxygen consumption [79, 80], it is easy to understand that any change, either in terms of the membrane content of the different lipid types or the fatty acid composition of the phospholipids, would affect their functions and, ultimately, the functionality of the mitochondria.

Our group has found lipid composition changes throughout the life cycle of *N. korthausae* [31], as well as in the composition of mitochondrial membranes in *N. rachovii* as a whole [40]. In the first case, a high level of unsaturation was observed in adult *N. korthausae*, which subsequently decreased in senescence. In *N. rachovii*, mitochondrial membranes are highly unsaturated, which, as in the case of *N. korthausae*, would be related to its fast metabolism, explosive growth and short life expectancy. Over the passage of time, the composition of the mitochondrial membrane undergoes significant changes. On one hand, the proportion of the different phospholipids changes. There is a decrease in the amount of cardiolipin, a phospholipid specific to the mitochondria, as well as an increase in the proportion of sphingomyelin with age [40]. This is important since cardiolipin is a key molecule for mitochondrial function, and its oxidation can lead to cellular apoptosis associated to mitochondrial dysfunction [73, 81]. Sphingomyelin, in turn, has saturated fatty acids in its molecule, for which it is credited with having stiffening properties. Membranes with a high sphingomyelin content are more rigid than the rest, and the propagation of free radicals slows down within them [82]. Sphingomyelin is also the precursor of signaling molecules associated with the process of apoptosis [83]. An alteration in the content of these phospholipids alone could explain the mitochondrial dysfunction associated with age.

Furthermore, the fatty acid composition of the phospholipids present in mitochondrial membranes is affected with age. Most of the phospholipids became more saturated with age [40]. A significant reduction in PUFA content occurred, especially in the docosahexaenoic acid (22:6n3, DHA), the fatty acid that is most susceptible to peroxidation. Taking into account that most of the changes observed in *N. rachovii* occurred after growth was completed, this may be the result of, or be related to, the effects of explosive growth and the speed with which adult size is reached by this species, which has been

associated with numerous negative effects, among them a reduction in life expectancy [84].

Species belonging to the genus *Nothobranchius* thus constitute a good model for studying the role mitochondria play in the aging process. Although we know some of the mechanisms that take place in the organelle with age, a solid cause-effect chain still remains to be determined that would connect them all and enable us to identify the origin of each.

5. Genetics

The genomic characterization of *Nothobranchius* constitutes an essential step forward towards understanding the mechanisms that determine life expectancy in this genus. Studies conducted to date have been carried out on *N. furzeri*. In 2009, two published works [85] laid the groundwork to begin to analyze the species at a genomic and genetic level [86]. From this moment on, knowledge about the genome of *N. furzeri* has increased considerably. We already know the complete mitochondrial genome sequence [77] and the nuclear genome of *N. furzeri* [87], as well as the genes that determine the differences in life expectancy among the different varieties. Furthermore, all *N. furzeri* transcripts sequenced to date have been collected into databases to facilitate the search and use of age-related genes [88]. The use of genome databases for the different species will help group and identify the genes involved in the aging process.

Even so, a great deal of work remains. Work is currently underway on the creation of stable lines of transgenic fish of the genus *Nothobranchius* [89, 90] as well as inducible expression genes [91], which will undoubtedly become a valuable tool for gerontological studies.

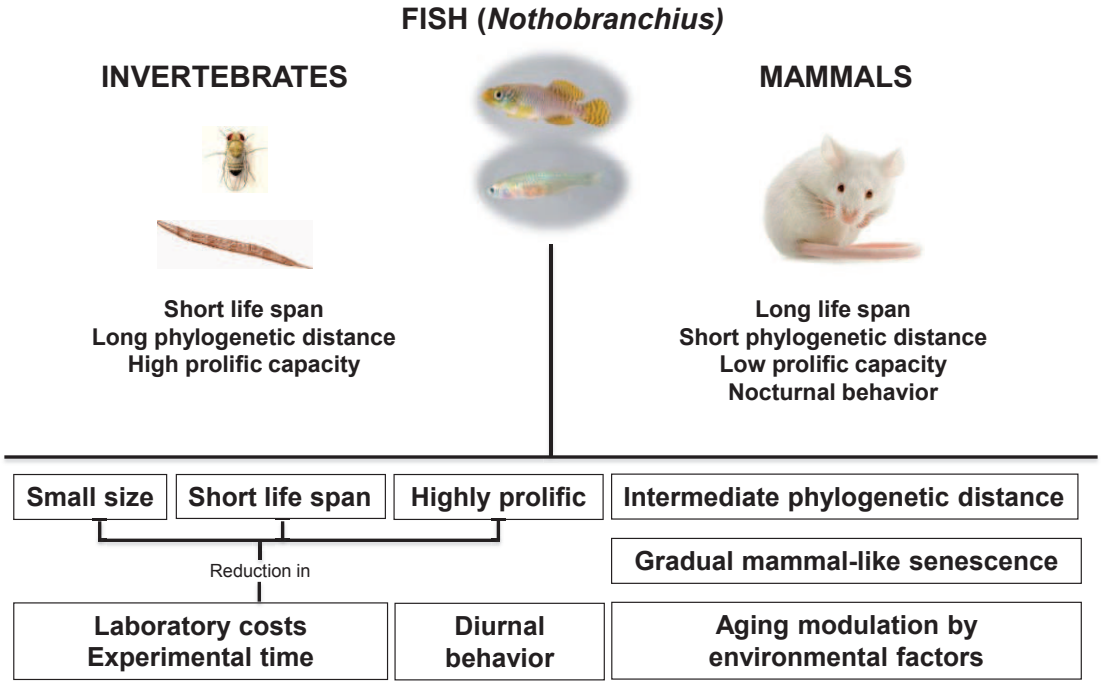

Figure 1. Advantages of *Nothobranchius* genus as a model for aging studies

Conclusions

While greater efforts are needed to standardize the maintenance protocols of this genus species in order to prevent disparities in the results obtained by different work groups, the genus *Nothobranchius* has proved to be a competitive option for studies conducted at very different levels, including populational, behavioral, histopathological and biochemical studies, and in particular, those examining circadian rhythms and genetics, thanks to the interesting characteristics described above (Fig. 1). This makes it a viable alternative for all types of age-related studies.

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