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Abstract

Ageing is the greatest risk factor for a multitude of age-related diseases including sarcopenia - the loss of skeletal muscle mass and strength - which occurs at remarkable rates each year. There is an unmet need not only to understand the mechanisms that drive sarcopenia, but also to identify novel therapeutic strategies. Given the ease and affordability of husbandry, along with advances in genomics, genome editing technologies and imaging capabilities, teleost models are increasingly used for ageing and sarcopenia research. Here, we explain how teleost species such as zebrafish, African turquoise killifish and medaka recapitulate many of the classical hallmarks of sarcopenia, and discuss the various dietary, pharmacological and genetic approaches that have been used in teleosts to understand the mechanistic basis of sarcopenia.

1. Introduction

Ageing is a naturally occurring and universal phenomenon resulting in a progressive decline in the ability of organs to perform their physiological functions. One such organ is skeletal muscle, which despite being indispensable for movement and a critical metabolic and storage organ (1), undergoes striking atrophy and a reduction in strength – known as sarcopenia - at 1% and 3% in humans each year respectively (2,3). While there is a lack of consensus on the clinical definition of sarcopenia, with no clear established diagnostic criteria for it, what is well appreciated is that sarcopenia is a complex and multifaceted neuromuscular condition. It is characterised by numerous cellular and molecular changes including muscle fibre atrophy, stem cell dysfunction, telomere attrition, epigenetic alterations, mitochondrial dysfunction, and metabolic alterations among others (reviewed in (4)). Despite being extensively characterised at a cellular level, pharmacotherapies to treat sarcopenia have thus far not been identified, with the most promising management strategies limited to exercise and healthy nutrition, which although beneficial, have their own limitations - primarily in a form of limited compliance and tolerance (reviewed in (5)). One of the major challenges that has hindered the identification of suitable pharmacotherapies is the limited understanding of the molecular, physiological and metabolic mechanisms underlying muscle wasting. While a multitude of hallmarks of sarcopenia have been identified, we currently do not understand which of these are pathogenic and directly contribute to muscle wasting, and those that are simply a consequence of the ageing process having no impact on muscle function. Further to that, it is currently unknown if these pathogenic hallmarks can be pharmacologically attenuated or reversed to ameliorate sarcopenia. Research addressing the mechanistic basis of sarcopenia is therefore needed to inform more targeted development of therapies. This is particularly important given the ever-growing aged population worldwide, which has resulted in an increased prevalence of

sarcopenia, subsequently placing a considerable social and economic burden on the health care system.

To study the mechanistic basis of sarcopenia, several animal models have been routinely used including nematodes, flies, and rodents such as rats, mice and guinea pigs. While the invertebrate models have short lifespans and are remarkably easy and cheap to maintain, they do not fully recapitulate the human ageing and sarcopenic pathologies (6). In contrast, rodent models are more commonly used given that they share several physiological characteristics with humans and therefore recapitulate not only sarcopenic pathology but also other diseases. However, due to their longer lifespan, differences in metabolic stability, cost and labour intensive husbandry requirements, alternative models that can address these experimental challenges are required (7,8). Although teleosts do not address all these limitations, they have relatively short lifespans and are easier and more affordable to maintain and use for research. This, coupled with advances in genomics, genome editing technologies and improved imaging capabilities, has made teleosts a popular model for sarcopenia and ageing research. This review will highlight the sarcopenic pathologies observed in teleost species, with a primary focus on zebrafish, African turquoise killifish and medaka; discuss how they have contributed to our understanding of the fundamental biology of sarcopenia; and highlight the implications of these findings for the identification of suitable pharmacotherapies.

2. Fish models most commonly used for sarcopenia research

Zebrafish (*Danio rerio)* are a tropical freshwater fish native to South Asia and a prominent model for understanding vertebrate biology and disease (Figure 1). While they have successfully been used to model myopathies and muscular dystrophies, they have recently attracted attention as a model for sarcopenia research. In line with the alterations observed in aged mammals, aged zebrafish display skeletal muscle degeneration and reduced muscle mass,

accompanied with increased senescence-associated β-galactosidase activity (SA-β-Gal), mitochondrial dysfunction, oxidized protein accumulation, spinal curvature and display reduced swimming performance with increasing age, indicative of a decline in muscle function (9–12). However, given that the average lifespan of zebrafish is 2 to 5 years (10,13), the use of wildtype strains is not highly feasible for ageing studies, and instead mutant models that display ageing phenotypes are commonly used to study the biology of sarcopenia.

The **African turquoise killifish** (*Nothobranchius furzeri*) are the shortest-lived vertebrates that can be bred in captivity and are an emerging model for the investigation of ageing (14). The African turquoise killifish inhabit ephemeral ponds in Zimbabwe and Mozambique. During the dry season, African turquoise killifish eggs deposited in the muddy substrate are arrested in a dormant state, and at the start of the rainy season, they exit this arrested state and undergo rapid maturation reaching sexual maturity within 4 to 5 weeks (15). The median lifespan of these fish is 4 months to 1 year depending on the strain (Figure 1). During their lifespan, African turquoise killifish not only undergo remarkable levels of growth, but they also exhibit hallmarks of ageing such as appearance of neoplastic lesions in the liver, kidney, heart, and gonads (16), reduced regenerative capacity of the fin (17), decreased mitochondrial DNA copy number and function (18), and shortening of telomeres (19). In the context of muscle, aged African turquoise killifish display moderate reduction in fibre cross-sectional area, a reduced number of muscle stem cells, muscle denervation associated with neuromuscular junction degeneration and striking alterations in lipid metabolism (20). Further to this, aged African turquoise killifish show reduced locomotion highlighting a reduction in muscle function (21). Given that African turquoise killifish display many of the typical signs of muscle ageing and have a lifespan that is comparable to many invertebrate models, they provide a unique system to study the biology of sarcopenia and muscle ageing. Notably, while *Nothobranchius furzeri*

is the most commonly used killifish species for research, several additional species including *Nothobranchius kadleci*, *Nothobranchius rachovii* and *Nothobranchius guentheri* have also been studied and are discussed in this review.

Medaka *(Oryzias latipes)*, commonly known as Japanese rice fish, have been used as a model species for research since the early 1900s (22). Medaka inhabit both brackish and freshwater environments in areas of East Asia (Figure 1) (23). While they are similar in size, and have similar anatomy and physiology to zebrafish, they are highly tolerant to inbreeding making them an optimal laboratory model. Aged medaka have been shown to display age-associated degeneration of multiple tissues including skin, liver and heart but no age-associated skeletal muscle alterations were observed, although this could be due to the limited assays (lipofuscin and BrDU) performed (22). The sarcopenic pathologies of aged medaka therefore remain to be characterised.

Multiple other fish species have been embraced for biomedical research and while we do not discuss them in any detail, they have been studied in the context of telomere biology and, as such, have been briefly introduced in Table 1.

3. Molecular hallmarks of sarcopenia and insights from teleosts

In this section, we will discuss the significant contributions made using teleost models to understand six of the main hallmarks of sarcopenia – muscle fibre atrophy and fibre composition, muscle stem cell function in growth and regeneration, telomere attrition, epigenetic alterations, mitochondrial dysfunction, and metabolic changes (Figure 2). Notably, several genetic mutants have been created as models of premature ageing (24,25), particularly

in zebrafish, and while they have contributed to our understanding of sarcopenia, they are not the focus of this review. Instead, we have focused on insights gained from the natural ageing process in teleosts.

3.1. Muscle fibre atrophy and fibre composition

The formation of skeletal muscle in fish is a highly conserved and well-characterised process of myogenesis, which has been reviewed several times (26,27). Briefly, during segmentation paraxial mesodermal cells form a monolayer against the notochord, termed adaxial cells. Upon activation of Hedgehog signalling these cells migrate to the lateral end of the myotome to form the superficial slow muscle layer, with the remaining non-adaxial cells of the posterior somite differentiating to form the fast muscle. Notably, a small number of adaxial cells remain at the notochord to form a teleost specific slow muscle cell type known as the muscle pioneer cell (28). This process of muscle development results in two distinct compartments of muscle fibres, with the oxidative, type I slow fibres located superficially, and the rest of the myotome composed of glycolytic, type II fast muscle fibres. While the compartmentalisation of slow and fast muscle cells is unique to, and typical of teleosts, not all of them follow the same profile. For example, in tuna, as an adaptation to their high performance and pelagic lifestyle, slow muscle is medially located adjacent to the spinal cord (29).

In humans, sarcopenia is characterised by the reduction in size (atrophy), primarily of the fast, type 2, muscle fibres and to a lesser extent, a reduction in number (hypoplasia) of both slow and fast muscle cells (reviewed in (30)). As in humans, aged zebrafish show reduced fibre cross-sectional area, although it is unknown if this is fibre type specific (11,12). Aged African turquoise killifish also show muscle fibre atrophy, specifically of the medium sized fast muscle fibres localised deeper within the myotome, with no evidence of hypoplasia (20). Importantly,

in humans, sarcopenia is often associated with a shift from type II, fast muscle fibres to a type I, slow phenotype (31,32). While this has not been thoroughly investigated in fish, this phenomenon of fibre type switching does not appear to occur in the African turquoise killifish (20), suggesting that fish muscle may display distinct changes in metabolic profiles during ageing.

3.2. Muscle stem cell function in growth and regeneration

A unique feature of fish muscle is its remarkable ability to expand and grow several fold as the animal approaches its adult size. Unlike in mammals whereby postnatal muscle growth occurs only via hypertrophy, defined as the increase in size of existing cells, muscle growth in fish also occurs via hyperplasia - the addition of new fibres, which is particularly prevalent in the embryonic and larval stages. In adults however, there is a plateau in hyperplastic growth and hypertrophy becomes the primary muscle growth strategy (33).

The ability to undergo hyperplasia is attributed to the presence of a unique muscle stem cell population, marked by the transcription factors *pax3* and *meox1*, found within a superficially located, extracellular matrix rich region termed the external cell layer (ECL) (33–35). Here, the stem cells are arrested in the G2 phase of the cell cycle, which has been hypothesised to prime them for growth stimuli (33). Lineage tracing experiments have been used to examine how these stem cells are deployed for hyperplastic muscle growth. These experiments have revealed that while multiple independent stem cells randomly contribute to myofiber generation during the early growth phase, a single stem cell clone comes to dominate growth in adult muscle over successive self-renewal events (33). This phenomenon, termed clonal drift, is characterised by the stochastic loss and reciprocal expansion of the progeny of individual stem cells. This ultimately leads to a pool of clonally related stem cells responsible

for replenishing the entire stem cell population, which in turn generates clonally related differentiated tissues. Importantly, while this loss in muscle stem cell clonality has been observed in adult zebrafish muscle, it is uncertain if the same occurs in aged skeletal muscle. In mice, muscle stem cells have been shown to maintain clonal complexity with homeostatic ageing, although reduced functional heterogeneity is observed (36). The differences in clonal complexity in zebrafish and mice may be explained by the difference in stem cell types, with the zebrafish experiments focussing on heterogeneity of ECL localized stem cell, and the mouse experiments focussed on the classical satellite cell population (discussed below); and the differences in growth strategies used – with zebrafish utilising hyperplastic and hypertrophic strategies and mice displaying only hypertrophic growth. In any case, although the mechanisms regulating clonal drift and the implications of it remain unknown, it can be postulated that drift may be a consequence of the selection of the most optimal stem cell clone required for growth. If this is the case, an understanding of what makes this stem cell clone the most optimal, and the processes that mediate its selection will be highly invaluable for managing sarcopenia, whereby inducing the formation of new muscle cells is desirable.

In addition to the ECL localised stem cells, teleosts also have the classical muscle stem cell population termed the satellite cells, which are dispersed in the myotome. As in amniotes, satellite cells in teleosts are marked by *pax3*, *pax7*, and *met* and are required for muscle regeneration. While the role of satellite cells in muscle regeneration in the larval setting is well-characterised (reviewed in (37)), their role in adult muscle is not well-studied. It is known that in adult zebrafish, satellite cells proliferate and form new muscle following injury, with *pax3/pax7* double mutants displaying an impairment in this process (38). However, how this process is altered during ageing, and the role of muscle stem cells in regenerating aged muscle tissue remains to be investigated. A recent study in African turquoise killifish has revealed a

significant decline in muscle stem cell number in extremely old African turquoise killifish with normal stem cell number in the aged cohort (20). Interestingly, this decrease in stem cell number was associated with increased expression of myogenic genes. It is therefore possible that the reduced stem cell number could be due to increased fusion to muscle cells to enable the maintenance of muscle size, suggesting that alterations in cell number may be a consequence of the ageing process, rather than a cause. The role of stem cells in the pathogenesis of sarcopenia has also been questioned in mammalian models, and further research in this area is warranted.

3.3. Telomere Attrition

Telomeres are repetitive DNA sequences ($TTAGGG_n$) that protect the ends of chromosomes from fusion events (39). As linear chromosomes cannot be fully replicated by replicative DNA polymerases, the presence of telomerase, a specialised reverse transcriptase enzyme that is able to elongate chromosomes is necessary (40). However, telomerase has limited expression in most human somatic cells, including muscle cells, such that telomere attrition occurs with increasing age (41,42). When telomeres are shortened to a critical length, DNA damage response pathways are activated, leading to cellular senescence (reviewed in (43)).

Several studies have investigated the length of telomeres in teleosts and shown similarities to those seen in humans (Table 1). In muscle, telomere length varies between teleosts with zebrafish muscle telomeres being the largest at around 17 kb (44,45), and medaka and African turquoise killifish muscle telomeres ranging from 8.7-13.9 kb (46–48) and 6.2-6.6 kb (19) respectively. Despite the differences in telomere length, telomeres have been shown to shorten with age in each of the three species (19,45,46). Notably, unlike in humans whereby telomere shortening has been shown to occur in multiple tissues regardless of proliferative capacity (49),

in zebrafish, telomeres shorten only in the gut and muscle, and this has been shown to systemically drive the ageing process (50). Consistent with this, muscle cells also undergo telomere shortening in African turquoise killifish, although this is strain specific. That is, while the longer-lived African turquoise killifish strain (MZM-0403) displays telomere shortening in muscle with increasing age, the shorter-lived strain (GRZ) shows no significant telomere attrition, despite the high conservation in telomerase gene expression between the two strains (19). This suggests that, at least in the short-lived strain, telomere attrition does not contribute to the pathogenesis of sarcopenia. Notably, while the sarcopenic pathologies in the long-lived strain have been characterised (20), the same has not been done for the short-lived strain, and therefore it is not possible to draw conclusions on the role of telomere attrition in sarcopenia pathogenesis in teleosts. In any case, the differences in telomere biology may provide a valuable tool to tease out the role of telomere attrition in sarcopenia and ageing.

While the functional domains of the enzyme telomerase are highly conserved between humans and teleosts (51–53), the activity profile is significantly different. That is, in humans, telomerase is active in germline cells but not in somatic cells (54) whereas in teleosts, telomerase activity is detected in most tissues, with moderate levels in muscle (19,52,53,55). The relationship between telomerase expression in muscle cells and age has been investigated in both zebrafish and African turquoise killifish. In zebrafish (45) and the short-lived African turquoise killifish strain (19), telomerase expression and activity was not altered with age, whereas in the long-lived African turquoise killifish strain telomerase expression and activity increased with age (19). While the functional consequences of this variation in telomerase expression remains to be determined, the impact of telomerase deficiency has been examined. Telomerase deficient zebrafish (*tert*$^{-/-}$) have shorter telomeres than their wild-type counterparts and display p53-dependent age-associated decay including a reduction in body mass, cachexia,

and sarcopenia which resembles the sarcopenic pathology seen in humans (44,56). Interestingly, rescue of telomerase expression in the gut of telomerase deficient zebrafish was shown to not only rescue gut senescence and restore gut integrity, but also to reduce the degeneration of various tissues including muscle, and increase the lifespan of the fish (55). This highlights the systemic nature of ageing and sarcopenia, and the importance of studying tissues as a collection rather than as individual modalities.

3.4. Epigenetic Alterations

Epigenetics is the study of heritable and reversible alterations to DNA, without altering its sequence, with the two most common forms being DNA methylation and histone modification. Within the human genome, approximately 70-80% of methylation occurs at the C5 position of cytosine (5mC) in CpG dinucleotides, catalysed by DNA methyltransferases (DNMTs), whereas demethylation is mediated by the ten-eleven translocation methylcytosine dioxygenase (TET) family of enzymes. Phylogenetic analyses have revealed a high degree of conservation of both DNMT and TET genes in teleosts, although teleosts have multiple paralogues that emerged from genome duplication events. Importantly, studies have shown clear tissue and age specific expression and function of DNMT and TET genes (57,58). In the context of sarcopenia, muscle from aged African turquoise killifish displayed age-dependent decrease in *DNMT1* and *DNMT3*, with no significant alterations in expression of the TET family of genes (58). Consistent with this reduction in methylation enzymes, analysis of global DNA methylation in skeletal muscle from African turquoise killifish showed a significant negative correlation of 5mC with age (58). While the impact of this hypomethylation on gene expression and sarcopenia remains to be examined, studies in other fish models have identified differentially methylated positions associated with muscle atrophy and quality (59) and with muscle fibre type (60). It would therefore be interesting to determine if any of these known

markers are hypomethylated in aged muscle as this may reveal novel mechanisms driving atrophy in sarcopenia.

The development of human epigenetic clocks and their strong association with chronological age in muscle, and ageing-related phenotypes is exciting and holds great potential for identifying novel mechanisms regulating sarcopenia, ageing and longevity. Epigenetic clocks utilise machine learning algorithms to determine DNA methylation sites that correlate with age (61). Epigenetic clocks have been developed for two fish species - European seabass and zebrafish – with their main purpose being age determination without the requirement for lethal sampling (62,63). Notably, both clocks were developed using a small number of CpG sites - with the European seabass clock based upon 48 CpG sites from four genes in muscle samples (62), and the zebrafish clock utilising 26 sites in caudal fin tissue (63). Although both epigenetic clocks are associated with chronological age, the functional impact of the DNA methylation changes identified and their potential role in sarcopenia have thus far not been examined. That is, are the observed DNA methylation changes a consequence of the ageing process, or do they directly cause sarcopenia? Further research in this field is required.

In addition to DNA methylation, changes in histone modification (methylation and acetylation) also contribute to sarcopenia. Histone acetylation is regulated by the enzymes histone acetyltransferases (HATs) and histone deacetylases (HDACs). Consistent with the broad expression patterns of HDAC genes in mammals, African turquoise killifish also show ubiquitous expression in embryonic stages (64). However, during ageing, there is a significant downregulation of *HDAC1* expression, and an inverse upregulation of *HDAC3* in aged skeletal muscle (64). Although the implications of this change on histone acetylation was not determined, Cencioni et al. (2019) have since characterised the epigenetic profile of skeletal

muscle from aged African turquoise killifish tissue (65). Aged African turquoise killifish muscles displayed an increase in histone marks associated with heterochromatin (H3K27me3, H3K9me3, and H4K20me3), and a reduction in euchromatin histone marks (H3K9ac and H4K16ac) which is consistent with alterations in expression of the histone acetyltransferase enzymes. ChIP-seq analysis for H3K27me3 and H3K9ac in combination with RNA-seq was subsequently used to identify promoters that may be epigenetically regulated during ageing. These studies revealed a downregulation of genes involved in cell cycle, differentiation, and DNA repair and an upregulation of inflammation and senescence genes (65). Although functional studies have not been performed to validate the involvement of these pathways in sarcopenia, the use of such epigenetic profiles can lead to the identification of novel mechanisms driving sarcopenia.

3.5. Mitochondrial dysfunction

Mitochondria are the powerhouse of the cell and while they are primarily involved in synthesising energy in the form of ATP through oxidative phosphorylation, they also have important roles in ion homeostasis, cellular metabolism, programmed cell death and the production and consumption of reactive oxygen species (ROS). Ageing and sarcopenia are characterised by an impairment in mitochondrial integrity, reduced mitochondrial biogenesis and an overall reduction in mitochondrial function subsequently resulting in excessive ROS production which induces further mitochondrial and cellular deterioration (66). Consistent with these hallmarks of sarcopenia, aged zebrafish display a reduction in mitochondrial number, an increase in mitochondrial rupture and lysis of the matrix, increased vacuolisation, and a loss of cristae (12). Additionally, aged zebrafish displayed increased ROS production and striking alterations in mitochondrial dynamics as evidenced by the reduction in mitochondrial biogenesis and fusion-related genes and upregulation in mitochondrial fission-related factors –

which may collectively result in impaired mitochondrial function, although this has not been tested (12). Remarkably, exercise is sufficient to activate the AMPK/SIRT1/PGC-1α pathway, subsequently reversing many of the defects in mitochondrial dynamics, and also ameliorating age-related muscle atrophy (12). These results not only highlight the beneficial effects of exercise in sarcopenia, but also demonstrate that the biological processes regulating mitochondrial dysfunction in aged zebrafish are highly conserved with those identified in humans.

In agreement with the zebrafish findings, aged African turquoise killifish also display a reduction in mitochondrial number and function, including reduced abundance of OXPHOS complexes, and an accumulation in mitochondrial DNA damage in skeletal muscle (18,20). Notably, while reduced OXPHOS complexes are generally thought to be detrimental, in several organisms including African turquoise killifish, it has been shown to be beneficial by triggering ROS-mediated mitohormesis and extending life span (67–70). Consistent with this idea, the reduction in OXPHOS complexes in skeletal muscle of aged African turquoise killifish has been proposed to trigger ROS-mediated mitohormesis and induce the expression of SIRT1 and PGC-1α in extremely old animals culminating in their increased life span and overall reduction in mortality rates (20). The regulation of mitochondrial biology during ageing is complex, and there may be a fine balance between mitochondrial dysfunction contributing to muscle wasting, and triggering stress responsive pathways subsequently improving muscle health and lifespan. Given that teleosts accurately model many of the mitochondrial changes seen in ageing humans, they may provide a useful system to investigate these processes to better understand the role of mitochondria in sarcopenia pathogenesis.

3.6. Metabolic Alterations

In addition to being indispensable for locomotion, skeletal muscle is an essential metabolic organ mediating systemic metabolism and influencing energy homeostasis (71). Recent studies have utilised teleost models to identify metabolic pathways associated with sarcopenia. Using a comparative approach, Maslov et al (2019) (72) examined skeletal muscle metabolites in fish with differing rates of ageing, which included muscle samples from the negligible senescent species pike and sterlet, biosamples of gradually senescent species (zander and perch), and muscle samples from rapidly senescent species (chum salmon and pink salmon). Untargeted metabolomics, which enables the unbiased identification of metabolites present in a sample, was performed on muscle from each of these three groups, following which the most overrepresented metabolites were characterised in each group (72). Using this strategy, it was reported that muscle from negligible senescent species are associated with high levels of amino acids and biogenic amines (72). Although the functional implications of these increased levels were not examined, elevated amounts of amino acids are hypothesised to improve muscle growth and energy metabolism, provide antioxidant protection, enhance protein synthesis and inhibit proteolysis, which collectively prevent the presentation of sarcopenia. The rapidly ageing salmonoid species on the other hand, were associated with an accumulation of lipid metabolites including medium and long-chain acylcarnitines, intermediates of the citric acid cycle and intermediates of sugar metabolism (72). Given the lack of available literature on the role of lipids in regulating sarcopenia, the enrichment of lipid metabolites in the rapidly ageing salmonoid species was thought to be a result of external factors independent of the ageing process. Collectively, while this work reveals a potential role of protein and lipid metabolism in the regulation of sarcopenia, the differences in the biology of each of the species including factors such as age, environment, diet and feeding regime present as significant confounding factors. Functional evidence is therefore required to determine if the metabolic pathways

identified are an inconsequential read-out of the biology of the animal or are true regulators of sarcopenia.

More recently, untargeted metabolomics experiments were performed in the African turquoise killifish, to characterise the metabolome in skeletal muscle from young, aged, and extremely old animals. Using a systems biology approach, Ruparelia et al (2023) revealed that although most metabolites are unaffected during the ageing process, of those that are significantly altered, there is an enrichment of protein and lipid metabolites (20). With regards to the alterations in protein metabolism, Ruparelia et al (2023) report an increase in peptide species in the aged cohort, which are downregulated in the extremely old animals (20). Peptide species are associated with proteolysis, and their increased abundance in the aged cohort suggests increased protein breakdown. Consistent with this argument, aged muscle was found to express high levels of *atrogin-1*, an E3 ubiquitin ligase known to be upregulated in numerous muscle wasting conditions including sarcopenia, where it ubiquitinates substrate proteins and targets them for degradation, subsequently resulting in atrophy (20). In extremely old animals on the other hand, reduced abundance of peptide species was associated with reduced expression of *atrogin-1* and stabilization of muscle fibre size, supporting the hypothesis that protein metabolic changes identified are most likely a reflection of changes in proteolysis (20). Studies in zebrafish have also reported similar changes in protein metabolism, whereby aged zebrafish display increased expression of the atrogenes *atrogin1* and *murf1*, and a downregulation of genes of the IGF-1/PI3K/Akt/mTOR signalling pathway known to regulate protein synthesis, subsequently leading to muscle atrophy (12). Remarkably, an 8-week swimming exercise regime in zebrafish was sufficient to not only restore the expression of both protein synthesis and degradation genes back to levels seen in younger animals, but also restore muscle mass (12), highlighting the importance of protein metabolism in sarcopenia.

In addition to protein metabolism, lipid metabolism has also been implicated in regulating muscle ageing and longevity in African turquoise killifish. Ruparelia et al (2023) revealed that during ageing, increased lipolysis results in the depletion of triglycerides, an important energy reserve in the cell (20). While the mechanisms for this reduction and the consequence on muscle wasting were not investigated, the authors suggest that the reduction in triglycerides during ageing mimics a state of calorie restriction. This triggers increased ROS production, subsequently activating the stress responsive pathway mitohormesis in extremely old animals, evident by the increased expression of *sirt1* and *PGC1α* (20). One proposed beneficial effect of this is the restoration of triglyceride levels to those seen in young animals, and alteration in lipid profile dynamics to enable more efficient utilisation of energy (20). Consistent with this hypothesis, fat maintenance has been shown to predict longevity in wildtype mice strains (73), and increased consumption of specific types of dietary fats extends lifespan and improves mitochondrial dynamics in aged murine skeletal muscle (74), collectively highlighting the role of adiposity in sarcopenia and longevity. While there is correlative evidence that the same occurs in teleosts, functional studies are needed to better understand this process and leverage this knowledge for developing improved nutritional and pharmacological strategies to manage sarcopenia.

4. Lifespan and health span extension strategies, and its impact on muscle health

In recent years, several dietary, pharmacological and genetic approaches have been used in teleosts to identify novel mechanisms regulating muscle health and lifespan, and strategies to improve and extend them respectively. These have been summarised in Tables 2 and 3 and are discussed below.

4.1. Dietary interventions

The most well studied dietary intervention is dietary/calorie restriction (DR), defined as an overall reduction in total caloric intake without targeting a specific dietary component, which has been shown to increase maximum lifespan in multiple teleost models (Table 2). In the African turquoise killifish however, strain differences have been reported. While DR increases the maximum lifespan in both the shorter-lived, inbred strain and the longer lived, outbred strain, in the latter, increased baseline mortality was also observed (75). These strain-specific responses to DR may be attributed to genetic variation, in that the DR regime is detrimental to some individuals and beneficial to others (75). Although such variants have thus far not been identified, recent work has implicated differences in gene function between individuals in explaining these distinct responses to DR. African turquoise killifish males have previously been shown to display increased median lifespan following DR, with the lifespan of females being unchanged (76), suggesting that DR acts in a sexually dimorphic manner. In line with this, a reduction in the AMP biosynthesis gene *APRT* has been shown to increase nutrient sensitivity specifically in males (77). These results implicate AMP biosynthesis in regulating longevity in males and highlight the potential use of teleosts to uncover mechanisms by which genes or genetic variants regulate interspecies, intraspecies, and/or sex differences in longevity.

In addition to genetic variation, differences in responses to DR may also be explained by the idea that DR may act as a stressor, and while most individuals die as result of the stress, some trigger stress-defence mechanisms which increases resistance to age-induced damage subsequently selecting for their survival. Indeed, a similar mechanism has recently been proposed in fish that naturally live longer. Although based on descriptive data, it has recently been suggested that the depletion of triglycerides, the main energy reserves in the cell mimics a state of calorie restriction resulting in increased production of reactive oxygen species. In

some animals, this triggers mitohormesis, which results in the maintenance of nutrient homeostasis, subsequently leading to the deceleration of mortality rates and the manifestation of the "extremely-old, late-life" phase. Further studies that functionally validate this mechanism and determine why certain animals selectively respond to stress, and subsequently live longer are needed.

Notably, the timepoint at which dietary interventions are initiated is particularly important in determining its impact on health and longevity. Using the short lived African turquoise killifish, it was recently shown that fasting results in reduced expression of the AMPK γ-subunit Prkag1 and a suppression of energy metabolism, protein synthesis and cellular proliferation in several tissues including muscle, which is reversed following refeeding (78). In young animals, the fasting-refeeding cycles results in oscillatory expression of Prkag1, and potentially other targets, leading to improved energy metabolism, metabolic health and a subsequent extension of lifespan (78). Aged animals however, despite being fed normal diets, show a transcriptional profile that is similar to fasted animals, including a reduction in Prkag1 expression (78). This constitutive state of fasting in aged animals prevents the restoration of Prkag1 levels, and this has been suggested to contribute to sarcopenic pathologies and ageing. The introduction of fasting and other dietary interventions in aged animals may therefore have no impact in improving muscle health or longevity and may in fact have deleterious effects. Teleosts provide a model to not only explore the mechanisms by which dietary interventions function, but also to identify the optimal timepoints in which maximum therapeutic benefit can be achieved.

4.2. Pharmacological interventions

The two most well studied lifespan/healthspan modulating pharmacotherapies that have been examined in the context of muscle in teleosts are resveratrol and metformin. Additional compounds such as rapamycin, melatonin and diosgenin have also been shown to modify lifespan in teleosts but given their impact on muscle has not been explicitly explored, we have limited their discussion to Table 3. Metformin treatment of sexually mature killifish *N. guentheri* has been shown to not only prolong lifespan, but also reduce the presentation of typical ageing pathologies including reduced lipofuscin in the liver, lower SA-β-gal activity in the skin, inhibition of inflammatory response, and improved motor, learning and memory skills – highlighting a conserved mechanism of action of metformin in teleosts lifespan (79). In line with this, resveratrol treatment of multiple additional killifish (*N. furzeri* (80) and *N. guentheri* (81–83)) has also been shown to extend both mean and maximum lifespan and improve locomotor capacity, evident by an increase in average time active and average velocity of swimming (80,81). While this could be attributed to the reduced neurodegeneration following resveratrol treatment, it could also be due to improved muscle innervation and pathology, although this was not examined. More recently, a 4-week resveratrol treatment regime on aged African turquoise killifish was shown to not only increase *sirt1* levels, but also increase skeletal muscle triglyceride levels and improve lipid droplet dynamics in muscle fibres, which is hypothesised to contribute to the extension of lifespan (20). Overall, these results demonstrate that therapeutic interventions can extend lifespan in teleosts, although further studies that examine the impact on muscle health are required.

4.3. Genetic approaches to study sarcopenia

The availability of annotated reference genomes for zebrafish (84,85), medaka (86), and African turquoise killifish (87,88), along with development of genome editing tools has enabled the generation of genetic models to study sarcopenia. Many of these have been reviewed elsewhere (24,25) and as such, here we limit our discussion to mutants that displayed muscle phenotypes. As part of a forward genetic screen in zebrafish, which used SA-β-Gal accumulation in early life stage as an outcome measure for premature senescence, several key regulators of ageing sarcopenia were identified (89). This includes identification and characterisation of the *terf1a* mutant, which showed enlarged telomere speckles and abnormal nuclear shapes, along with a reduction in mean lifespan as expected due to the role of this gene in telomere protection (89), and the *nrs* mutant line had increased SA-β-Gal staining in the skin and trunk, increased lipofuscin in the skeletal muscle along with a reduction in lifespan. Additional mutants which showed reduced SA-β-Gal staining and increased lifespan were subsequently described using similar genetic screens (90). Multiple additional screens have been performed (91) and they collectively demonstrate the capacity of forward genetic screens in fish models to identify novel genes and mechanisms regulating longevity, ageing and sarcopenia.

While many mutations in fish have been characterised, there are relatively few that show premature signs of ageing outside the context of known diseases. One mutant that has attracted attention in the field of sarcopenia and ageing is the *αklotho* mutant. αKlotho, plays an essential role in discharging excess phosphate from blood to prevent unwanted ectopic calcification and mice deficient in it show signs of ageing at about 4 weeks of age and die prematurely at 8-9 weeks of age (92). In line with the mouse mutant phenotypes, zebrafish deficient in *αklotho* display premature ageing from about 5 months of age including emaciation, protruding eyes, a

reduction in locomotor activity and premature death (93,94). At a cellular level, widespread calcification of blood vessels in skeletal muscle, as well as degeneration and fibrosis of skeletal muscle is evident in the αklotho zebrafish mutant (94). Interestingly, in zebrafish (95) and the African turquoise killifish (96), *αklotho* has been shown to be primarily expressed in the liver and kidney, with low/no expression evident in other tissues such as skeletal muscle. Despite this, the loss of αKlotho results in striking skeletal muscle phenotypes. These results highlight a role of non-autonomous mechanisms in driving sarcopenia and reiterate the need to consider these in the quest for therapeutics.

Transgenesis techniques have also been employed in teleosts and have been especially invaluable in the development of tools to enable *in vivo* imaging and lineage tracing. Examples that are particularly relevant for sarcopenia research in teleosts include: Musclebow and Killibow systems for lineage tracing (97,98), a transparent African turquoise killifish line *klara* which enables observation of processes such as regeneration (99), and FUCCI lines for investigating cell cycle dynamics (100). The application of these tools to sarcopenia and ageing studies will provide further understanding of the molecular mechanisms underlying these processes.

Finally, it would be remiss not to mention the advances in multi-omic technologies including single cell -omics and spatial transcriptomics. The recent release of a comprehensive RNA-sequencing dataset for muscle (among other tissues) of the shorter-lived strain of the African turquoise killifish at varying life stages (101) can be examined to understand tissue-specific alterations linked to the ageing process. Single cell resolution transcriptomics atlases in zebrafish are also available, although they currently do not include aged cohorts (102,103). Finally, while spatial transcriptomic approaches offer great promise to uncover cell types and

pathways regulating sarcopenia and ageing and have been used to examine muscle biology in teleosts, the resolution is limited (104). Therefore, improved spatial transcriptomics resources along with availability of more muscle targeted scRNA-seq (and other -omics based) datasets in aged teleosts will allow a more comprehensive understanding of alterations in muscle tissue and provide insight into the molecular mechanisms of sarcopenia.

5. Concluding remarks

In this review we have highlighted the sarcopenic pathologies observed in the commonly used teleost species and discussed how they contribute to our understanding of the mechanistic basis of sarcopenia. While teleosts recapitulate many of the hallmarks of sarcopenia, making them invaluable for sarcopenia research, it is important to acknowledge some of their limitations. There are obvious biological and physiological differences between fish and mammalian models that need to be considered. For example, teleosts are ectothermic, which means that their body temperature and therefore biological processes are regulated by external stimuli. Alterations in husbandry protocols including diet (105), temperature (21,106), reproductivity (107) and exposure to stressors (108) can significantly impact their development, growth, and ageing trajectories, and as such it is imperative to record and report husbandry protocols to promote reproducibility and standardisation efforts of the field. Towards this end, several husbandry protocols have recently been published, at least for the African turquoise killifish, including use of an automated feeding system (76,109). Further to this, there is substantial evidence suggesting that there are differences in the biology of captive and wild strains. For example, while male and female African turquoise killifish bred in captivity have comparable lifespans, male counterparts in the wild have been shown to die sooner (110). Consistent with this idea, at the molecular level, wild and captive *Nile tilapia* strains have been shown to display

significant differences in global DNA methylation levels and expression of immune, metabolic and muscle development genes (111). Given these differences, cross-species comparative approaches will be required to confirm the translatability of findings to human ageing. Despite these limitations, teleosts are increasingly used for sarcopenia and ageing research, and given their unique advantages, they have the potential to drive remarkable discoveries in the sarcopenia and ageing fields.

Conflict of interest

The authors have declared that no conflict of interest exists.

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Tables

Table 1: Telomere length in human, mouse and teleosts

Species	**Common Name**	**Average Lifespan**	**Telomere Length (kb)**	**Tissue(s) Examined**	**References**
Homo sapiens	Humans	70-80 years	5-15	Blood, sperm, kidney and placental DNA	(112,113)
Mus musculus	Mouse	2-3 years	20-150	Liver, bone marrow cells, skin fibroblasts, spleen and kidney	(114–116)
Danio rerio	Zebrafish	2-5 years	15-20	Whole animal cell suspension	(44,45)
Nothobranchius furzeri, *Nothobranchius kadleci*, *Nothobranchius rachovii*	Annual killifish	4-12 months	5-10 6-8 (GRZ strain)	Muscle, skin, caudal fin, whole animal cell suspension	(19,117,118)

Oryzias melastigma	Marine medaka	2–3 months	0.5-12	Whole fry cell suspension, liver	(47,119)
Oryzias latipes	Japanese rice fish (medaka)	2-5 years	7-15 kb	Whole embryo cell suspension, whole fry cell suspension, liver, kidney, intestine, muscle, gonad, heart, brain, spleen and gill	(46–48)
Oncorhynchus mykiss	Rainbow Trout	11 years	17-20	Head kidney	(120,121)
Xiphophorus maculatus, X. helleri and X. couchianus	Platyfish	1-4 years	2-6	Liver, gill, brain, eyes, testis and ovaries	(122)
Menidia menidia	Atlantic Silverside	2 years	Relative telomere length reported	Larvae, muscle and brain	(123)

Gadus morhua	Atlantic Cod	25 years	Relative telomere length reported	Liver	(124)
Gambusia holbrooki	Eastern Mosquito fish	1-2 years	Relative telomere length reported	Tail muscle	(125)
Merluccius merluccius	European Hake	15-30 years	Relative telomere length reported	Brain, kidney, liver and muscle	(126)
Cyprinus carpio	Common Carp	47 years	0.6-0.8	Muscle tissue and fin clips	(127)

Table 2: Outcomes from dietary interventions in teleosts

Intervention	**Species**	**Concentration**	**Period of treatment**	**Observed alterations**	**Number of fish**	**References**
Dietary Restriction	*N. furzeri*	DR fish were fed twice a day, every alternate day so that no uneaten food was left after 30 minutes	From 4 weeks	Increased maximum lifespan in long-lived and short-lived strains, however, increased baseline mortality in long-lived strain. DR ameliorated age-related impairment in memory and learning. DR reduced	n=10 for assays	(75)

				expression of neurodegeneration markers **(brain)** and lipofuscin **(liver).**		
Dietary Restriction	*N. furzeri*	15 mg per day, in three feedings of 5 mg over 2 hr	From 4 weeks	Increase in median lifespan for males only.	n=21-25 per group	(76)
Dietary Restriction	*D. rerio*	90 mg of dry food every alternate day and artemia once per week.	12 week period of DR in 9 month and 20 month old fish	Reduction in BMI. Old DR animals had increased protein expression of PSD95 **(brain).** Young DR animals had increased protein expression of	n=12-13 per group	(128)

				GluR2/3 **(brain).** Overall these results show DR increases elements of synaptic integrity and excitatory neurotransmission.		
Dietary Restriction	*D. rerio*	90 mg of dry food every alternate day and artemia once a week	10 week period of DR in 8-8.5 month and 26-32.5 month old fish	Reduction in weight. Shortening of telomeres **(brain).** No change in telomere length **(skeletal muscle, spleen and tail).**	n=27-34 per group	(129)

Dietary Restriction	*G. aculeatus*	2% bodyweight of bloodworms daily	300 days old until death	No change in median lifespan. Produced significantly fewer clutches.	n=32-44	(130)
Calorie restriction	*D. rerio*	HF-LD (overfed): 3x 100 mL paramecia from 5–13 days post fertilization (dpf), and 3x 10 drops of artemia from 14 dpf onwards LF-HD (CR): daily feeding of 3x 100 ml paramecia	2 weeks-18 months	When compared to overfed fish: Reduced body length. Slower mineralisation of **scales**. Reduced number and size of **adipocytes**. Reduced **oocyte** growth rate.	n=2-3 per experiment	(131)

		from 5–13 dpf, and 3x 10 drops of artemia from 14 dpf onwards		When compared to control feeding: No change in BMI. Elevated cholesterol. No change in triglycerides. No change in **ovarian** weight.		
Calorie Restriction	*D. rerio*	100 mg of dry food every alternate day and artemia once a week	12 week period of CR in 5 month and 25 month old fish	Reduced BMI. Reduction in RNA expression of stem cell marker SOX2 in calorie restricted fish when compared to overfed fish **(brain).**	n=6 for each age group	(132)

Intermittent Food Restriction	*N. guenther i*	2 consecutive days per week fed 10 mg dried worms/g fish weight/day while the other 5 days there was no restriction (40 mg dried worms/g fish weight/day	9 month old male fish	Significant reduction in body weight in 38–50-week-old fish. Increase in mean and maximum lifespan. Reduced lipofuscin staining **(gills).** Reduced protein oxidation and lipid peroxidation **(muscle).** Increased antioxidant enzyme activity **(muscle).**	n=50 for lifespan observations, n=3 for assays	(133)

Intermittent Fasting	*G. aculeatus*	Starvation for 5 consecutive days then 15% bodyweight of bloodworms for 9 days	300 days old until death	Reduction in median and maximum lifespan. Same investment in reproduction as control fish.	n=32-44	(130)
Intermittent Fasting	*D. rerio*	90 mg of dry food every alternate day and artemia once a week	8 week period of IF in 6-10 month and 26-31 month old fish	Reduction in body weight, BMI and glucose levels **(whole body).** Suppressed mTOR activity **(brain).** Increase in neurogenic marker DCAMKL1 **(brain).**	n=4-16	(134)

Table 3: Outcomes from pharmacological interventions in teleosts

Intervention	**Species**	**Concentration**	**Period of treatment**	**Observed alterations**	**Number of fish**	**References**
Resveratrol	*N. furzeri*	600 µg/g/food 120 µg/g/food 24 µg/g/food	From 4 weeks old (sexual maturity) until death	Dose-dependent lifespan extension. Retarded onset of age-dependent cognitive and locomotive deficit. Prevented neurofibrillary degeneration **(brain).**	n=20, n=60 and n=30, respectively	(80)
Resveratrol	*N. furzeri*	96 µg/g/food	4 week treatment from 33-37	A shift in metabolic profile with resveratrol	n=6	(20)

			weeks of age	treated males displaying significant increase in triglyceride levels and changes in lipid localization **(muscle).**		
Resveratrol	*N. guentheri*	12 µg resveratrol/fish /day	From 12 weeks old (sexual maturity) until death	Extension of median and maximum lifespan. Prevention of loss of radial glia **(brain).**	n=72	(81)
Resveratrol	*N. guentheri*	200 µg/g/food	From 16 weeks old until death	Extension of mean and maximum lifespan. Reduction in ROS **(skin, bone and**	n=76	(82)

				muscle). Increase in antioxidant enzyme activity **(liver)** and reduced protein oxidation **(skin, bone and muscle).** Reduction in lipofuscin accumulation in gill epithelia.		
Resveratrol	*N. guentheri*	200 µg/g/food	From 16 weeks old until death	Extension of mean and maximum lifespan. No alteration in body size Enhanced cognitive performance	n=38	(83)

				and locomotor activity. Reduction in neurodegeneration **(brain),** lipofuscin staining **(liver)** and SA-β-Gal staining **(skin).**		
Resveratrol	*N. guentheri*	200 µg/g/food	From 16 weeks old until sacrifice at either 6, 9, or 12 months of age	Reduction in the number of SA-β-gal positive cells **(thymus and kidney).** Reduced protein expression of SIRT1 and SIRT3, and increased protein	n=5	(135)

				expression of NF-κB **(thymus and kidney).**		
Resveratrol	*N. guentheri*	200 µg/g/food	From 16 weeks old until sacrifice at either 6, 9, or 12 months of age	Reduced SA-β-Gal staining **(gut).** Amelioration of the SASP phenotype **(gut).** Resveratrol treatment prevented as much reduction in intestinal stem cells and epithelial cells over lifespan.	n=3 for staining, ELISA, but n=20-30 for qPCR	(136)
Resveratrol	*N. guentheri*	600 µg/g/food	From 10-15 weeks old for	Increased embryo production and increased	n=8 per group	(137)

			20 weeks	NAMPT protein expression suggesting a positive effect on female fertility.		
Resveratrol	*N, guenther i*	200 µg/g/food	From 16 weeks old until sacrifice at either 6, 9, or 12 months of age	Reduced SA-β-Gal staining **(ovary)**. Reduced lipofuscin staining **(ovary).** Improved cell proliferation and ovarian reserve **(ovary).** Reduced inflammation and ER stress **(ovary).**	n=3	(138)

Metformin	*N. guentheri*	2 mg/g/food	From 16 weeks old (sexual maturity)	Increase in the mean survival in male fish. Significant reduction in body weight and body length. Significant reduction in cholesterol and triglyceride **(serum).** Reduction in SA-β-gal and lipofuscin staining **(skin).** Enhanced locomotor and cognitive ability. Reduced	n=48	(79)

				neurodegeneration and inflammation **(brain).**		
Metformin	*N. guentheri*	2 mg/g/food	From 16 weeks	Extension in mean and maximum lifespan. Reduction in SA-β-gal staining **(gut).** Reduced inflammation **(gut).**	n=6	(139)
Rapamycin	*D. rerio*	0.1 nM final concentration in the tank	8 week period of treatment in 6-10 month and 26-31	No difference in BMI, body weight or glucose levels **(whole body).** Inhibitory effect on mTOR pathway in	n=4-16	(134)

			month old fish	young animals **(brain).** Decreased proliferation (PCNA), astrocyte activation (GFAP), postsynaptic transmission (GEP) and autophagy (LC3-II/LC3-I ratio) seen in young animals only **(brain).**		
Melatonin	*D. rerio*	100 nM final concentration in the tank	30 minute treatment in 1 and 4 year old fish.	Counteracted the sleep alterations, reduced intrinsic circadian rhythm of	n=8-10	(140)

				activity and reduction in cognitive performance in aged zebrafish.		
Melatonin	*N. korthausae*	5 mg melatonin/kg body weight/day	72 week old male fish	Improved the regularity, fragmentation and amplitude of the rest-activity-rhythm, and sleep efficiency.	n=10	(141)
Diosgenin	*N. guenther i*	13.8 µg/g body weight/day	9 month old male fish.	Extension of mean and maximum lifespan in male fish. Reduced accumulation of lipofuscin **(gills).**	n=50 for lifespan observations, n=3 for assays	(142)

				Reduced accumulation of SA-β-gal **(caudal fin).** Lowered protein oxidation, lipid peroxidation and ROS accumulation **(muscle).**		

Figures

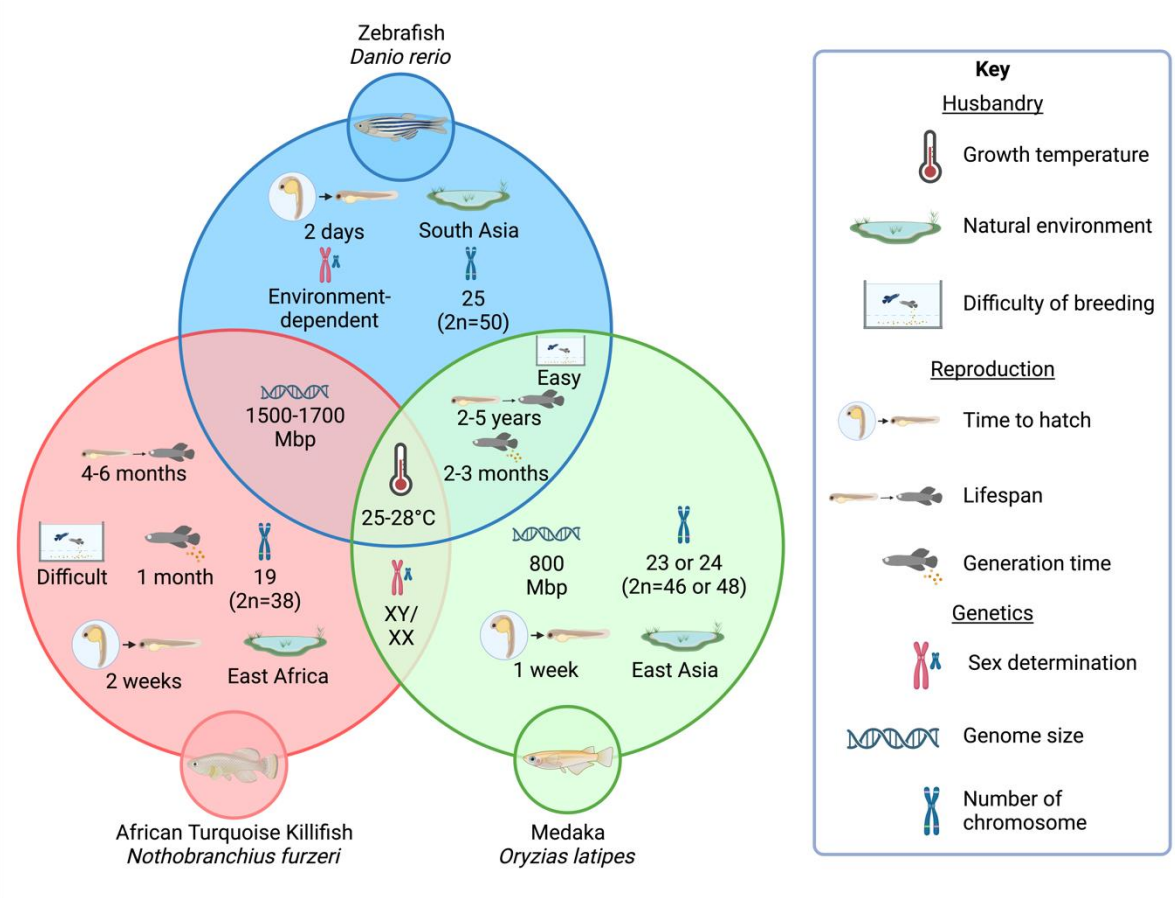

Figure 1: Characteristics of the zebrafish, African turquoise killifish and medaka.

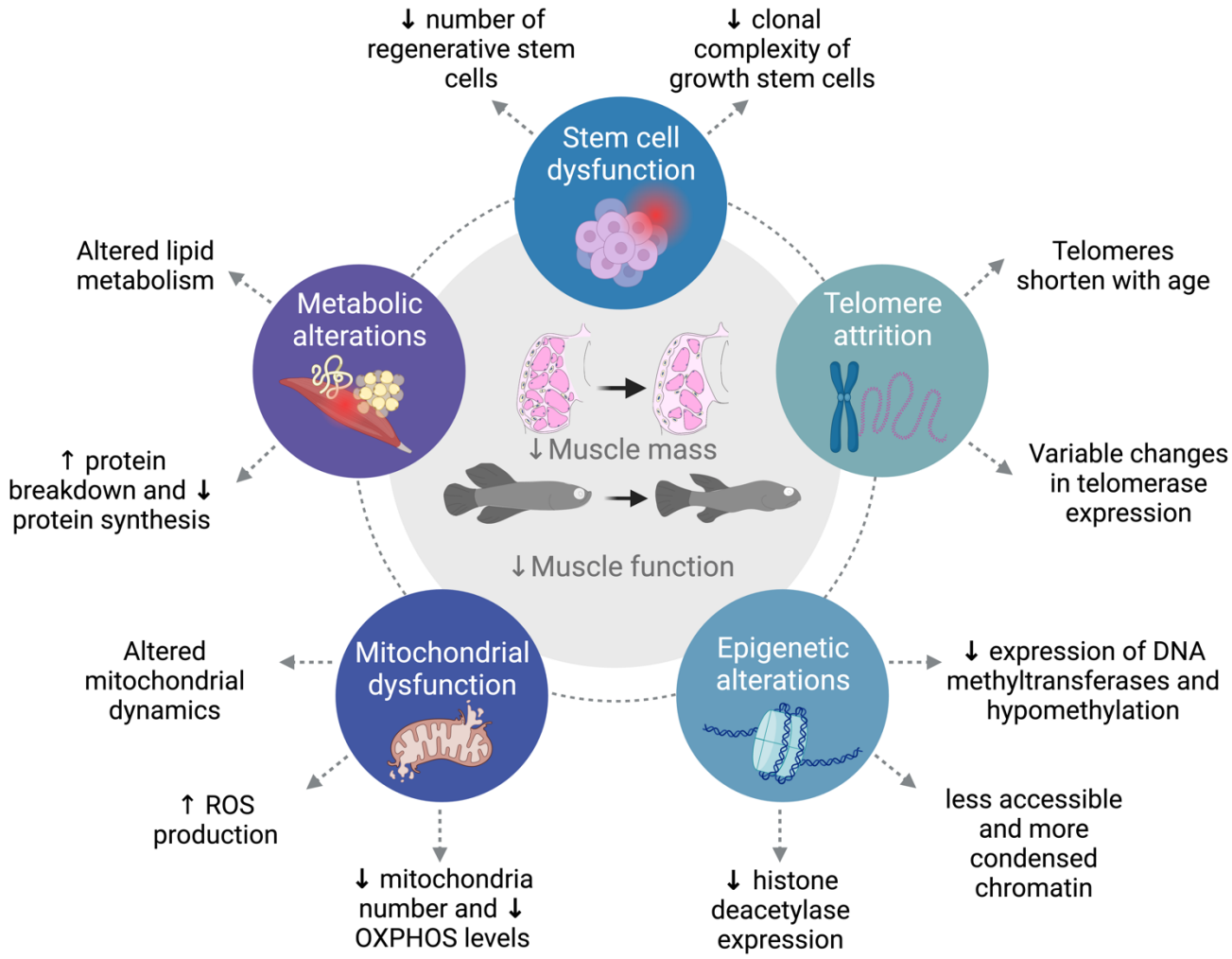

Figure 2: Summary of the hallmarks of sarcopenia pathologies in teleosts.