**Nothobranchius furzeri**, the Turquoise Killifish: A Model of Age-Related Osteoporosis?

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**Keywords**  
*Nothobranchius furzeri* · Killifish · Accelerated aging · Osteoporosis · Osteoblast · Osteoclast · Micro-computed tomography · Bone histomorphometry

**Abstract**

**Introduction:** Osteoporosis is a frequent age-related disease, which affects millions of people worldwide. Despite significant progress in the treatment of the disease, a high number of patients still are underdiagnosed and undertreated. Therefore, novel animal models for the investigation of the disease are necessary. *Nothobranchius furzeri* is the shortest-lived vertebrate (with a lifespan of 3–7 months) that can be kept in captivity. Although it is an established model for aging research, studies on bone are lacking. The aim of this study was therefore to characterize *N. furzeri* as a potential model for age-related osteoporosis. **Materials and Methods:** Bone properties of aging *N. furzeri* were investigated in male and female fish of the Gona Re Zhou strain, which were between 8 and 20 weeks old. Micro-computed tomography (Scanco Medical \(\mu\)CT35) was performed to determine the bone properties of the vertebral bodies. Bone structure and remodeling were investigated by different histological staining techniques and histomorphometry. The chemical composition of fish vertebrae and intervertebral discs was analyzed by Raman microspectroscopy. **Results:** Osteoblasts, mono- and multinucleated osteoclasts but no osteocytes could be observed in the vertebral area of *N. furzeri*. Histomorphometric evaluations revealed a significant decrease of the number of osteoblasts/bone perimeter and for osteoid volume/bone volume (BV) a trend toward a decrease in old male *N. furzeri*. Comparing male and female fish, males showed higher BV densities and cortical thickness. The relative values of the bone volume density of 20-week-old male *N. furzeri* were significantly lower than 10-week-old ones. The mineral to matrix ratio increased with age in male and female fish. In the intervertebral discs, proteoglycans in relation to the organic matrix were significantly lower in older female fish. **Conclusion:** Our finding of a lack of osteocytes is in agreement with the fact that *N. furzeri* belongs to the evolutionarily advanced teleost fish. Furthermore, not only age-specific but also sex-specific differences were visible in the bone properties of *N. furzeri*, which can be taken into consideration for the study of gender aspects of age-related musculoskeletal diseases.

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Introduction

Osteoporosis is a frequent age-related disorder "characterized by a systemic impairment of bone mass and microarchitecture that results in fragility fractures" [1]. In the European Union, each year 3.5 million fragility fractures occur; it was estimated that the economic burden of these fractures amounts to 37 billion Euro [2]. Thus, osteoporosis is associated with an enormous economic burden that – due to the aging of our society – will markedly increase in the years to come. Despite the fact that effective treatment is available, only a small proportion of patients with osteoporosis are treated adequately [2, 3].

The pathogenesis of bone fragility in osteoporosis is multifactorial; determinants of bone strength include bone mineral density (BMD), bone geometry, microstructure of bone, bone mineralization, and properties of bone matrix. From the view of cellular pathophysiology, osteoporosis results from a preponderance of the activity of osteoclasts over that of osteoblasts [1, 4]. Despite this common pathogenic mechanism, osteoporosis is a heterogeneous disease with contributions of endocrine, genetic, nutritional, lifestyle, and immunological factors [5–7].

Animal models are inevitable for the study of biology and pathophysiology of bone, the identification of drug targets, and preclinical drug testing. For osteoporosis research, traditionally rodent models such as ovariectomized rats and mice, spontaneously aged rats, or mouse models of accelerated aging are used [8–11]. As an alternative to rodent models, large animal models (pig, sheep) and fish models (Danio rerio, zebrafish) were described [12–14].

Nothobranchius furzeri

The turquoise killifish Nothobranchius furzeri inhabits seasonal freshwater pools in Southeastern Africa [15, 16]. Due to an adaptation to the conditions of their natural habitat, N. furzeri exhibit a very short life cycle; in fact, this species has been nominated as “the shortest-lived vertebrate that can be kept in captivity” [15]. As an alternative to rodent models, large animal models (pig, sheep) and fish models (Danio rerio, zebrafish) were described [12–14].

Nothobranchius furzeri

As described above, N. furzeri can be regarded as an interesting model for aging research. Nevertheless, the utility of this model for bone research, in particular for studies on the pathogenesis and treatment of age-related osteoporosis, has not been established. We hypothesize that the bones of N. furzeri show similar characteristics as bones of aged mammals. The aim of this study is to characterize N. furzeri as a model of age-related musculoskeletal diseases.

Materials and Methods

Animals

Female and male N. furzeri of the Gona Re Zhou strain used in this study were kindly provided by the Nothobranchius fish facility (founded by Oliver Pusch and Gordin Zupkovitz) of the Center of Anatomy and Cell Biology, Medical University of Vienna. Maintenance, mating, and breeding were performed as previously described [17]. Briefly, fish were grown in an overflow custom made system maintained at 27°C on a 12 h light/dark cycle. Female and male fish were kept in separate tanks. The animals were fed twice daily with commercially available frozen blood worms (Chironomus larvae). Selected fish were euthanized between 5 and 20 weeks of age by the usage of tricaine methanesulfonate (MS-222) buffered with sodium bicarbonate to a neutral pH, fixated for 48 h at room temperature with 4% paraformaldehyde, and afterward stored in 70% ethanol at 4°C. The total population investigated comprised 32 female and 35 male fish. Additionally, two male and one female killfish at 15 weeks of age with visible vertebral deformation were provided. All killfish were kept and handled according to institutional and national guidelines and laws.

Paraffin and Methyl Methacrylate Embedding

For paraffin embedding, N. furzeri were decalcified with trisaminomethane-ethylene diaminetetraacetic acid overnight at room temperature (RT) and afterward dehydrated with increasing alcohol concentrations (80%, 96%, and 100%) at RT for 1 h each. Before paraffin-embedding samples were transferred to xylol for 30 min to 1 h at RT. For methyl methacrylate embedding decalcification is not necessary. The samples were infiltrated, polymerized, and embedded as described previously [13]. Sectioning was performed with the Micromet Microm HM355S (Thermo Scientific) using specific knives for hard materials like bones.

Hematoxylin and Eosin Staining

Formaldehyde-fixated and paraffin-embedded N. furzeri were stained with hematoxylin and eosin (HE) (ROTH) to obtain the anatomical characteristics of N. furzeri.

Toluidine Blue Staining

Formaldehyde-fixated and paraffin-embedded N. furzeri were stained with toluidine blue (Fluka Analytical) to visualize osteoblasts in the vertebral area of N. furzeri. One percentage toluidine blue solution (pH = 4.5) was 1:50 diluted and applied for 2 min.

Von Kossa/Toluidine Blue Staining

Formaldehyde-fixated and methyl methacrylate-embedded N. furzeri were stained with von Kossa/toluidine blue. This staining was performed to verify the presence of osteoid in the vertebral area of N. furzeri as it allows to differentiate between mineralized
bone and unmineralized osteoid. The same toluidine blue solution, as described above, was used. Additionally, von Kossa staining (Sigma-Aldrich) was performed to demonstrate the presence of calcium deposits.

**Tartrate-Resistant Acid Phosphatase Staining**

To visualize the present osteoclasts in the vertebral area of *N. furzeri*, tartrate-resistant acid phosphatase staining was performed (Sigma-Aldrich, Serva). For this staining, formaldehyde-fixated and paraffin-embedded *N. furzeri* were used.

**Bone Micro-Computed Tomography**

Skeletal morphology and bone microstructure of vertebral bodies were assessed by micro-computed tomography (micro/µ-CT) (µCT35, Scanco Medical, Brüttisellen, Switzerland). The whole-body scans of male and female killifish were acquired at voxel size dimensions of 6 µm, and the used FOV/diameter was 12.3 mm. The energy/intensity settings were 70 kVp, 114 µA, and 8 W. An integration time of 200 ms was used during the first few measurements and later raised by the factor 1.14. For the evaluation, the Xming™ program was used. Every third vertebral body was manually delineated and afterward evaluated with a threshold value of 200 hydroxyapatite/cm³ (HA/cm³) to separate bone from nonbone tissue. The neural arch and neural spine were excluded from the evaluation. The parameters such as cortical thickness (Ct.Th), density of bone volume (BV), and the height of the vertebrae were taken into consideration and were automatically calculated by the used program. As 10-week female fish were too small and not measurable by the µ-CT device available for this study, they were excluded from the evaluation.

**Bone Histomorphometry**

Histomorphometric analysis was performed using an Osteomeasure™ system (OsteoMetrics, Decatur, GA, USA). The standardized nomenclature of the ASBMR Histomorphometry Nomenclature Committee was applied [18]. For the determination of osteoblast parameters, entire vertebral body sections were stained with toluidine blue as described above. Additionally, for the verification of the presence of osteoid in the vertebral area of *N. furzeri*, von Kossa/toluidine blue-stained sections were analyzed. Parameters assessed were number of osteoblasts (N.Ob), BV/total volume, osteoid volume (OV)/BV, and N.Ob/bone perimeter (B.Pm).

**Raman Microspectroscopic Analysis**

Raman microspectroscopic analysis utilized a Senterra (Bruker Optik GmbH) instrument, operated in a temperature-controlled room (constant temperature of 20°C), to minimize any potential performance variability due to ambient temperature fluctuations. It employs SureCAL™ technology to optimize short- and long-term precision, allowing spectral collection which is independent from typical and unforeseen instrument instabilities (SureCAL, n.d.). A continuous laser beam was focused onto the sample through a Raman fluorescence microscope (Olympus BX51, objective ×20) with an excitation of 785 nm (100 mW). The technical characteristics of the instrument have been published elsewhere [19, 20]. All Raman spectra were obtained in confocal mode (1 µm below the specimen surface, FlexFocus, Bruker Optics). For the Raman measurements, the integration time was 3 s and co-additions were 5 to improve the signal-to-noise ratio (the minimum signal-to-noise ratio for a peak to be considered acceptable was 3 [21]). The spectra were acquired from the surface of the fish vertebrae and the intervertebral ligament, using a thermoelectric-cooled charge-coupled device (Bruker Optik GmbH). All data analysis was done with the Opus I dent software package (OPUS 8.5, Bruker Optik GmbH). Raman spectra were cut (350–1,800 cm⁻¹) and baseline corrected (5-point rubber band) to account for fluorescence background. The following parameters were calculated:

- The mineral/matrix ratio from the integrated areas of the ν₂PO₄ (410–460 cm⁻¹) and the amide III (1,215–1,300 cm⁻¹) bands, which is independent of tissue organization/orientation [22], unlike the most commonly used ν₁PO₄/amide I ratio. Different from mineral content measures such as BMD, this ratio corrects mineral content for the amount of organic matrix content in the microvolume analyzed. A spectroscopically determined mineral/matrix ratio has been validated against ash weight measurements [23], and is directly proportional to bending stiffness and failure moment, as well as a superior predictor of bone-bending stiffness compared to BMD alone [24].

![Fig. 1. a 15-week-old *N. furzeri* (top: male, bottom: female). The female is smaller and displays less coloration in contrast to the male one. b Two male *N. furzeri*, 15 weeks old with vertebral deformities.](image-url)
• The maturity/crystallinity of the bone mineral apatite crystallites (crystallite chemistry and size) was approximated from the full width at half height of the \( v_1 \text{PO}_4 \) (930–980 cm\(^{-1}\)) band, validated against x-ray diffraction and small angle x-ray spectroscopy [25, 26]. Size and shape are important mineral crystallite attributes in determining bone strength. Healthy bone has a range of crystallite sizes, which are dependent on both patient- and tissue-age [27].

• The glycosaminoglycan (GAG) content was expressed as the GAG/matrix ratio (the ratio of the integrated areas of the proteoglycan/CH\(_3\) [1,365–1,390 cm\(^{-1}\)] band [representative of mucopolysaccharides] to the amide III [1,215–1,300 cm\(^{-1}\)] band) [28] and validated against a series of standard proteoglycans as well as model tissues. GAGs are part of proteoglycans, present in both cartilage and bone. In bone, they fulfill several roles involving the organic matrix assembly and modulate both organic matrix mineralization and remodeling rates [29–31]. They undergo posttranslational modifications, some of which are both age- and tissue-age dependent [32], including size, sulfation, and charge density, all critical for their specific role [29].

**Statistical Evaluation**

Unless stated differently, data are presented as mean values. For statistical evaluations, the Kruskal-Wallis test and two-way ANOVA were used. For the post hoc analysis, the Dunn’s and Bonferroni test were chosen. Furthermore, Wilcoxon, Mann-Whitney, and chi-squared tests were performed. \( p \) values <0.05 were considered as statistically significant. The GraphPad Prism software was used.
Results

Macroscopic Evaluation of *N. furzeri*

Figure 1a shows two 15-week-old *N. furzeri*. Comparing them with each other, it is visible that the female one is smaller and displays less coloration in contrast to the male one. Macroscopic evaluation revealed in some cases vertebral deformities among the studied killifish population. Figure 1b shows two 15-week-old male killifish with such vertebral deformities.

Skeletal Morphology and Whole-Bone Structure

The vertebral column of fish can be divided into precaudal and caudal vertebrae. The latter ones are responsible for supporting the caudal fin. µ-CT and our stainings showed cylindric-shaped vertebral bodies, which are connected with each other by an intervertebral ligament (Fig. 2). A neural arch and a neural spine were also found in all vertebrae. Furthermore, trabecular structures within vertebral bodies were identifiable (Fig. 2).

HE staining of the vertebral area of *N. furzeri* showed osteoblasts (Fig. 2), which are cuboidal cells located along the bone surface and are responsible for bone formation, but no osteocytes. Since the bones of *N. furzeri* lack osteocytes, they are considered as acellular. We further investigated the jaw and ribs of *N. furzeri*, where also no osteocytes could be detected (Fig. 3).

Osteoblasts are often located next to the ventral and dorsal intervertebral ligaments. In addition to HE-stained sections, osteoblasts could be visualized in von Kossa/toluidine blue-stained sections (Fig. 2).

With tartrate-resistant acid phosphatase staining, we identified mono- and multinucleated osteoclasts (Fig. 2). Although the differences were not statistically significant, the proportion of fish where osteoclasts were present...
tended to be higher in old-aged than young-aged *N. furzeri* (data not shown).

**Microstructural Analysis (µ-CT)**

Vertebral bodies within individual fish differed with respect to Ct. Th and density of BV (Fig. 4). Both parameters were significantly higher in precaudal versus caudal vertebrae. No differences were seen for vertebral height.

Values for Ct. Th, bone volume density, and height of vertebral bodies of 15- and 20-week-old *N. furzeri* were higher in male than in female fish, with the exception of bone volume density in 15-week-old fish, showing no statistically significant difference. The same parameters increased from 15- to 20-week-old fish, with the exception of no statistically significant difference for vertebral height in female *N. furzeri* (Fig. 5).

Values for the relative Ct. Th, bone volume density, and height of the vertebral bodies (in relation to the body weight) showed a different pattern (Fig. 6) with a decrease from 15 weeks to 20 weeks in male *N. furzeri*. In female *N. furzeri*, Ct. Th increased from 15 weeks to 20 weeks of age and no difference was seen for cortical density and vertebral height. Sex-related differences with higher values for male than for female *N. furzeri* were seen for all parameters, except for Ct. Th at 15 weeks of age.

Additionally, male and female *N. furzeri* with visible vertebral deformities were compared with a healthy population of the same age (Fig. 7). It was evident that the
vertebral bodies in the healthy group were denser than those exhibiting vertebral deformities. It needs to be mentioned that not all vertebral bodies of those fish exhibiting vertebral deformities could be evaluated.

**Histomorphometrical Analysis**

Age-related differences were seen for the N.Ob/B.Pm between 12- and 18-week-old male and female *N. furzeri*. For OV/BV, a trend toward a decrease was seen in old male *N. furzeri* (Fig. 8).

**Raman Microspectroscopic Analysis**

Significant age-related differences were seen in the mineral to matrix ratio in female and male fish. Significant differences were seen between 5- and 20-week-old females, as well as 10- and 20-week-old females. Males...
showed a significant increase of the mineral to matrix ratio between ages of 5 and 20 weeks. In the intervertebral discs, cartilage proteoglycans in relation to the organic matrix showed significantly lower values in 5 weeks than 10- and 20-week-old female fish (Fig. 9).

**Discussion**

With the aim to characterize *N. furzeri* as a model of age-related musculoskeletal diseases, we assessed basic bone characteristics and age- and sex-related differences. We describe for the first time that the jaws, the vertebral bodies, and the ribs of *N. furzeri* lack osteocytes. Others described the occurrence of cellular or acellular bone in different fish species (for review see [33]). In general, cellular bone is, apart from a few exceptions, universally found in nonteleost ray finned fish and within teleost fish in the group of noneuteleost fish. In contrast, bone of euteleost fish with only a few exceptions is acellular [33]. Among teleost fish Medaka (*Oryzias latipes*) and zebrafish (*Danio rerio*) are frequently used as models to study human bone diseases. Medaka belongs to euteleost fish and shows acellular bone in the vertebral bodies, whereas the bones of the euteleost zebrafish show a pattern of mixed bone types with cellular bone found in most parts.
of the skeleton and acellular bone found in small bones such as the dermato-, chondro-, and splanchnocranium [34–36]. The occurrence of acellularity in zebrafish is thought to be associated with the relatively small adult body size. The bone matrix within thin bones of the cranium might simply be too small to entrap osteoblasts for the osteocyte generation [33]. In general, comparative studies and literature suggest that the whole skeleton of fish is either cellular or acellular [33]. In the present study, only one type of bone, the vertebral bodies, was investigated in detail. However, as in mixed bone types, cellular bone is found in large rather than small bones, *N. furzeri* more likely belongs to the group with acellular bone and not with mixed bone types. In a phylogenetic context, *N. furzeri* is closer to medaka than zebrafish as both fish belong to euteleost fish. Cellular bone within the group of euteleost fish is conclusively found only in Salmoniformes and in two relatively species-poor lineages, the “true” tunas (*Tunnini*) and the opah Lampris sp. [33]. Reacquisition of osteocytes in Salmoniformes is thought to be connected to an anadromous lifestyle. Sexually mature individuals migrate from the sea up into freshwater. In “true” tunas and Lampris, an endothermic physiology based on heat production by red muscle cells is observed.

**Fig. 9.** a Mineral to matrix ratio (*v*₂PO₄/amide III), (b) proteoglycan in relation to organic matrix (GAG/amide III), (c) mineral maturity/crystallinity, and (d) cartilages proteoglycans in relation to organic matrix (GAG/amide III) in vertebrae of 5- (female *n* = 3, male *n* = 3), 10- (female *n* = 3, male *n* = 3), and 20–week (female *n* = 3, male *n* = 3)-old male and female *N. furzeri*. Age-related and sex-related differences were assessed by Bonferroni post hoc test. **p < 0.01, *p < 0.05, (f) = significant differences in females, (m) = significant differences in males.
Both an anadromous lifestyle and “red-muscle endothermy” are associated with an increased muscle activity and a greater need for calcium and phosphorus. Secondarily acquired osteocytes, by performing osteocytic osteolysis, are thought to help satisfy this greater need for minerals [33]. Neither an anadromous lifestyle nor “red-muscle endothermy” is seen in N. furzeri. Acellularity in bones of N. furzeri is therefore in line with the current understanding of the presence of osteocytes in fish bone in a phylogenetic context.

Furthermore, we describe for the first time the occurrence of mono- and multinucleated osteoclasts in the vertebral area of N. furzeri. The absence of osteocytes in fish bone is typically seen in conjunction with mononucleated osteoclasts. A possible explanation for this could be the absence of stimuli from osteocytes promoting the growth of multinucleated osteoclasts [37]. The occurrence of mono- and multinucleated osteoclasts in conjunction with acellularity is therefore not in line with previous observations in other fish species and is a unique feature in N. furzeri.

Structural parameters of vertebral bodies were assessed by µ-CT, which showed sex-related differences for almost all examined parameters at 15 and 20 weeks of age with higher values in male compared to female fish. These sex-related differences in vertebral cortical structural parameters were not seen in the first and second vertebral bodies of zebrafish [38] and are typically also not seen in humans [39–41]. Cortical thickness and density of vertebral bodies were higher in old (20 weeks) compared to younger (15 weeks) female and male N. furzeri. Also in zebrafish, age-related increases of cortical BMD were seen in caudal vertebrae [42]. In another study by Hayes et al. [43], no changes of cortical BMD were seen in the vertebrae of old zebrafish. In humans, cortical BMD decreases with age [44, 45]. Age-related structural changes in the cortical compartment of zebrafish and N. furzeri are therefore not directly comparable to human conditions. However, in males a decrease in values for relative cortical parameters in relation to body weight was observed. Taken the increase in body weight of nearly 50% seen in aging male fish, cortical parameters in humans are better than relative cortical parameters in N. furzeri. Additionally, parameters reflecting bone remodeling, like N.Ob/B.Pm, decreased in old male fish. N. furzeri might therefore be used to model changes in the cortical compartment of male human bone. The utility of our µ-CT approach was also confirmed by the demonstration of decreased density of BV in the fish that exhibited vertebral deformities. When compared with nonscoliotic patients, scoliotic patients revealed a significantly lower BMD than the control group [46]. The present finding of decreased density of BV in N. furzeri exhibiting vertebral deformities is therefore consistent with a scoliosis-related decrease of BMD.

Evaluation of trabecular structures was not possible with the µ-CT device available for this study. Given the limitation of a voxel size and therefore a maximal resolution of 6 µm, assessment excludes smaller structures such as trabeculae. Monma et al. [42] only recently published data on trabecular parameters in vertebral bodies of zebrafish assessed by µ-CT analysis. These data were obtained with a voxel size of 10 µm and the mean values for trabecular thickness in this study range from about 7 to 18 µm. The methodological approach and results obtained in this study therefore have to be questioned critically and additional evaluations are required. By means of histological methods, we were able to visualize trabecular structures within vertebral bodies. Trabecular parameters such as trabecular thickness and trabecular number will be assessed in a next step.

Bone histomorphometric evaluations revealed age-related differences for the N.Ob/B.Pm between 12- and 18-week-old male N. furzeri. For OV/BV, a trend toward a decrease was seen in old male N. furzeri. These results can be explained by a decreased bone formation. When compared with younger sheep, old sheep showed a marginal reduction of BMD and a highly decreased OV/BV [47]. Aging men and women revealed a decreasing OV, and the highest fall was seen in women between 40 and 60 years [48]. Comparing osteoporotic patients and healthy controls, osteoporotic men and women showed decreased bone formation parameters, including OV/BV [49]. Data from our laboratory provided molecular evidence of osteoblast dysfunction in elderly women and men with osteoporotic fractures [50, 51]. The present finding of decreased osteoblast numbers in 18-week-old male N. furzeri thus is consistent with an age-related decline of bone formation.

Bone mineral and organic matrix properties are dependent on factors such as health, subject age (mainly due to variable bone turnover rates), and tissue age [27, 52]. In the present study, the tissue composition analysis revealed that the mineral to matrix ratio was significantly dependent on age and sex, the former in agreement with what holds for females [27]. Comparing these results with our obtained µ-CT results, similarities regarding the bone density in male fish could be detected, as the increased mineral to matrix ratio is associated with more densely mineralized bones. Our mineral to matrix ratio data ob-
tained from *N. furzeri*, thus, suggest an increase of tissue age. Analyzing intervertebral discs, we observed that cartilage proteoglycans in relation to the organic matrix showed a decrease in old female fish, in agreement with published data in humans, which show that at birth, articular cartilage GAG content is ~50% of the cartilage dry weight, subsequently decreasing to ~15% in adult cartilage [53, 54]. Moreover, during the development of human osteoarthritis, cartilage proteoglycan content decreases [55, 56]. GAGs, part of proteoglycans, are generally composed of repeating disaccharides possessing sulfated sugars except for hyaluronic acid [57]. In aqueous environments, the polysaccharide chains of GAGs electrostatically repel each other, filling any space available [57]. Hormonal changes also affect the glycosaminoglycan composition in females [58, 59].

In our study, we detected many sex-specific differences in *N. furzeri*. We are not able to give a clear explanation for this phenomenon, since we are not aware of publications that describe different aging mechanisms in female and male *N. furzeri*. In accordance with the data published by Graf et al. [60], sex differences in the length and weight of *N. furzeri* were seen. Nevertheless, the possibility exists that due to the smaller size of the female fish, in the µ-CT analyses differences might have been harder to be captured. In contrast, by Raman microspectroscopic analysis, we were able to detect differences also within the female population.

**Conclusion**

In conclusion, the characterization of the turquoise killifish *N. furzeri* revealed osteoblasts, mono- and multinucleated osteoclasts but no osteocytes in the vertebral bodies. To assess the utility of *N. furzeri* as an animal model for bone pathologies related to human aging bone, age-related and sex-related differences were evaluated in this study. Absolute cortical structural parameters differed between male and female fish and increased in older compared to younger ones. In contrast, OV/BV and relative cortical parameters (related to body weight) decreased in older male *N. furzeri*. Absolute structural sex- and age-related differences in the cortical compartment are not comparable to conditions in human bone. However, changes of the relative structural cortical parameters and of bone remodeling might be used to model age-related changes in male human bone. Furthermore, vertebral deformities in the context of decreased BMD were observed in *N. furzeri*. Taken together, this study provides valuable insights into bone and cartilage properties and suggests that *N. furzeri* can be considered as a promising novel model for the study of age-related musculoskeletal diseases.

**Statement of Ethics**

All killifish were kept and handled according to institutional and national guidelines and laws. Protocols were approved by the Austrian Federal Ministry of Education, Science and Research under License No. BMBWF 66.009/0130-Y/3b/2018.

**Conflict of Interest Statement**

The authors declare that there is no conflict of interest.

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**Author Contributions**

Conception of the work: P.P., U.F.-S., and O.P.; acquisition and/or analysis of data: M.B., K.W.-F., M.K., S.G., and E.P.P.; and drafting of the work: M.B., U.F.-S., and P.P. All authors made a substantial contribution in the interpretation of data for the work, revised it critically for important intellectual content, and approved the final version to be published.

**Data Availability Statement**

All data generated or analyzed during this study are available from the corresponding author upon reasonable request.
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