



Deposited via The University of Sheffield.

White Rose Research Online URL for this paper:

<https://eprints.whiterose.ac.uk/id/eprint/239662/>

Version: Published Version

Article:

Hyde, A.C., Ellis, P., El-Khamisy, S. et al. (2026) Assessing frailty in aged zebrafish using a quick pseudo-frailty index. *Biology Open*. bio.062345. ISSN: 2046-6390

<https://doi.org/10.1242/bio.062345>

Reuse

This article is distributed under the terms of the Creative Commons Attribution (CC BY) licence. This licence allows you to distribute, remix, tweak, and build upon the work, even commercially, as long as you credit the authors for the original work. More information and the full terms of the licence here:

<https://creativecommons.org/licenses/>

Takedown

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.

Assessing frailty in aged zebrafish using a quick pseudo-frailty index

Anne Cathrine Hyde^{1,‡}, Pamela Ellis², Sherif El-Khamisy^{1,4}, Claire Allen³
Catarina M. Henriques², Fredericus J. van Eeden^{1,*}

¹The University of Sheffield, School of Bioscience, Bateson Centre, Healthy Lifespan Institute Sheffield, S10 2TN, England, UK

²The University of Sheffield, School of Medicine and Population Health, Bateson Centre, Healthy Lifespan Institute, Sheffield, S10 2TN, England, UK

³The University of Sheffield, Biological Services Aquarium, Sheffield, S10 2TN, England, UK

⁴Institute of Cancer Therapeutics, Faculty of Life Sciences, University of Bradford, Bradford, UK

* Author for correspondence: f.j.vaneeden@sheffield.ac.uk

‡ Present address: Faculty of Biology, Medicine and Health, University of Manchester, Manchester, M13 9PT, UK

Abstract

While biomedical advancements have significantly extended human longevity, increased lifespan is frequently decoupled from healthspan, as these additional years are often accompanied by frailty and chronic morbidity. Frailty is characterized by a multisystem decline in physiological reserve, and renders individuals disproportionately vulnerable to adverse outcomes -including mortality- when faced with health stressors. Consequently, research into the biological mechanisms linking ageing to the onset of frailty is needed.

The zebrafish is increasingly utilized as a model for human aging and frailty due to its high degree of genetic homology. With a short lifespan of approximately three years, zebrafish show several conserved senescent phenotypes, including cataracts, sarcopenia, spinal curvature, and motor decline. Crucially, as in humans, ageing in zebrafish is not strictly chronological; apparent biological age often diverges from calendar age, with physical condition indicating frailty status more accurately.

We propose a frailty index designed to evaluate the divergence between successful ageing and frailty in zebrafish. By utilizing easily quantifiable phenotypic markers such as spinal

curvature and Body Mass Index, this index provides a score that predicts frailty status as validated against expert assessment. Implementing this standardized metric will facilitate cross-laboratory comparisons and enhance the reproducibility of future zebrafish-based ageing research.

Summary statement

We present a simple scoring system for frailty zebrafish; it was optimised to best match scores from experts and allows standardisation of frailty measurements in fish ageing research across laboratories.

Introduction

The definition of what conditions make a person frail has long been a debate. Whether it means that a person should have an age when frailty can occur, or whether it means that the person is reliant on services for help. Here, we refer to frailty as a change in physiological health which increases the chance of adverse outcomes after disease (Kane and Howlett, 2021, Rockwood et al., 1994, Rockwood et al., 2000). What is clear, however, is that people with the same chronological age can vary greatly in frailty, and that chronological age may not reflect biological ageing (Franceschi et al., 2018, Mitnitski et al., 2002, Mitnitski et al., 2001). Therefore, when studying biological ageing, a consistent way to identify frail individuals is needed. This can be provided by a frailty index or a frailty phenotype, which identifies deficits in health, and the more deficits an individual accumulate, the greater frailty they have (Heinze-Milne et al., 2019, Rockwood and Mitnitski, 2011, Searle et al., 2008). One widely used score in humans is the Fried frailty score. It assesses unexpected weight loss, poor handgrip, slow speed gait, feelings of exhaustion and low physical activity. If an individual, usually over 65 years of age, scores on three or more of the five criteria, they are classed as frail and have an increased risk of disease, and death within the next three years (Franceschi et al., 2018, Fried et al., 2001). Frailty scores have been created for model organisms. In mice, frailty is usually measured by activity levels, body composition, heart conditions, and metabolic status. However, a non-invasive measurement with eight conditions has also been shown to predict frailty (Parks et al., 2012, Rockwood et al., 2017, Whitehead et al., 2014).

Zebrafish ageing has several features that resemble human ageing, and are increasingly adopted as models to study the ageing process due to their short lifespan and low cost (Gilbert et al., 2014, Khor et al., 2019, Abou-Dahech and Williams, 2024, Hudock and Kenney, 2024). However, like humans, zebrafish chronological age does not reflect their biological age, and there is no one-way to determine what fish is frail and have an increased chance of death when faced with adversity. Several physiological features have been associated with increasing age in zebrafish: Gross morphological changes in spinal curvature and loss of muscle mass, with severity increasing over time (Gerhard and Cheng, 2002, Hayes et al., 2013). Further, zebrafish have an ability to regenerate damaged tissue, however, this ability decreases with age (Gilbert et al., 2014). An increase in cancer occurrence is also associated with increasing age in zebrafish (Cayuela et al., 2018, Kishi et al., 2003, Laconi et al., 2020). Other features that also change with zebrafish age include cataract, reduced swimming speed, cognitive decline and cardiovascular dysfunction (Gilbert et al., 2014, Khor et al., 2019).

Flueck-Giraud recently published a score sheet to assess the welfare of experimental fish. There they assess several external signs of welfare such as body condition as well as behavioural signs (Flueck-Giraud et al., 2024). However, assessing general welfare of the animal may tell you more about the state of the fish at that moment, rather than their frailty status linked to ageing. The Henriques lab published promising evidence for using width/length ratios as a way to assess frailty as a frailty index. They have shown that in prematurely ageing *tert*^{-/-} fish there is a decrease in the width and length ratio of the fish (Ellis et al., 2024, Henriques et al., 2013). However, it is unclear whether there might be other markers in fish that can be used to optimise the assessment of frailty.

In this study, we aimed to create a quick way of assessing the frailty status of zebrafish starting with only five conditions. We took inspiration from other organisms and humans, and selected markers for frailty that include body mass index (BMI) which changes in humans with age with greater weight loss in frail humans (Crow et al., 2020), spinal height, presence of tumours and the presence of fin degeneration. Frailty is difficult to define and is mainly interpreted as an increased chance of dying after an insult, however, ethical considerations make it impossible to verify the “correctness” of our score. As an alternative, we asked zebrafish experts to score fish on frailty and modified the relative weights of our factors to get the optimal match to their average scores. We propose that either the original or the

optimised score can be used to allow standardisation of frailty measures across different laboratories.

Results

Frailty phenotypes increase with age

We initially explored if the aforementioned five frailty phenotypes did increase in a population of fish with age. The age of the fish in the aquarium that visually had any of the five criteria were noted and plotted against the age of the total number of fish in the aquarium. This density plot (Figure 1A) shows that the number of fish that can be classed as frail according to our frailty system increases with age, peaking at 33 months.

Creating cut-offs for the frailty index

To create a preliminary zebrafish frailty index we measured 6 robust and 6 frail female fish that were identified as robust or frail visually according to the five frailty criteria. We then created cut-offs to distinguish between robust and frail values (Figure 1B-D). By using the cut-offs identified by these (Table 1), we scored subsequent fish and four groups of adult fish were created, robust young (3-6 months) (score 0), robust old (3 years) (score 0), pre-frail old (3 years) (score 0.2) and frail old (3 years) (score 0.4 +) (Figure 1E). *The male cut offs were created in a similar fashion to the female cut offs.* We also performed qPCRs on RNA from fin clips of two frailty-associated genes *il-6* and *p21* (Figure 1F-I). We used *ribosomal protein 29 (rps29)* as our housekeeping gene as it is commonly being used in zebrafish research (Bower et al., 2017, Fargher et al., 2025, Coxam et al., 2014, Kim et al., 2020).

Scoring of old fish from proposed criteria limits

Using the criteria from Table 1, females and males scored differently at the different criteria (Table 2). It was more difficult to score males as their characteristics are less obvious. Whereas the females scored on criteria that are very visual, such as the decreased BMI and increase in spinal curvatures, males mostly scored on a decrease in width/length ratio with a decrease in BMI in second place.

Frailty scoring by zebrafish experts

To validate our frailty index, we had seven zebrafish experts, each with 15 years or more experience in using and caring for zebrafish, score images of 80 fish (10 each from our categories young, robust old, pre-frail old, and frail old; both sexes). For both females and males, the expert scores generally increased with each frailty classification (Figure 2A). However, there were some variations among the scores. Female fish that were thin uniformly scored highly, whilst fish with spinal curvatures that had varying degree of width had a variety of scores from 0.2 to 1. Regression confirmed that BMI and width/length ratio significantly decrease with higher frailty, however, the spinal height does not (Figure 2B-D). For the males, again thin fish uniformly scored high, however, fish that were not obviously thin by eye were given scores between 0.6 and 1, meaning that male fish that may have only scored on the width/length ratio and be classed as pre-frail in the scoring system might visually be frail even though they had an average BMI. Further, when plotting the density for tumours and fin degeneration against the expert scores, we saw that both criteria were mostly seen in fish that scored higher on the frailty score (Figure 2E and F). Overall, this suggests that phenotypic scoring of frailty appears more straightforward for females than for males.

Optimising frailty index with weighted criteria

We used linear regression to refine the frailty score, aiming to align it more closely with the judgment of our experts. The expert scores were used as a dependent variable whereas the scores of each individual criterion (Table 1) acted as the independent variables. The resulting slope coefficients were used to create weights which were then normalised to get a total score of 1 if a fish scored on all criteria. For the female and the male fish the weight for the fin degradation were negative, suggesting that the experts considered it unimportant and therefore this criteria was effectively removed from the score as the weight was set to 0. The equations for the optimised index is shown in Figure 3A and Figure 3B and confirms strong correlation with expert scores.

We used Root Mean Square Error (RMSE) to confirm that our new score was a better fit for the expert data. Using the indexed expert scores as the predicted values, we calculated the RMSE against two sets of actual values: the original total score and our newly optimized total score. The original RMSEs were 0.335 for females and 0.385 for males, improving to 0.326 for females and 0.358 for males after weighting. When only looking at the width and length ratios as used by the Henriques lab, again using the indexed expert scores as the

benchmark and the width/length ratio as the actual score the RMSE for the females was 0.405 and for the males 0.410.

Discussion

Ageing research is becoming increasingly important, but aged animals are difficult to obtain. Large zebrafish facilities often generate such fish as part of standard husbandry procedures, and these could be a valuable resource to study ageing. Like humans, zebrafish appear to age differentially, with some becoming frail earlier than others. In order to classify such animals consistently we propose a quick pseudo-frailty index to allow consistent scoring of fish. We use the term “pseudo-index” because it has not used death as the frailty outcome, due to ethical considerations and UK legislation.

Initially, we used five criteria, BMI, width/length ratio, spinal curvature, tumours and fin degeneration. Fish that score on none of the criteria are classed as robust, one criterion are classed as pre-frail and two or more we class as frail fish. To make the index more accurate we used linear regression analysis to assign different weights to criteria, trying to best match the expert scores. Due to physiological differences between the female and male fish, we created gender-specific thresholds and weights.

Our males scored less on the unmodified frailty index, possibly due to bias; we have found that in older stocks females appear overrepresented, suggesting that males may have to be removed earlier for health reasons. Controlled raising experiments would be required to verify this. Similar trends occur in the human population, where females tend to become frailer than men, even though they also live longer (Kane and Howlett, 2021, Park and Ko, 2021). Female wild-type mice reportedly have an increased frailty index (Whitehead et al., 2014), however, other studies contest this (Parks et al., 2012). Furthermore, more frail aged guide dogs were female (Hua et al., 2016).

While all organisms experience frailty uniquely, we observed significant intra-sex variation but also clearly distinct patterns of frailty between male and female zebrafish. We generally see that females most often scored based on a low Body Mass Index (BMI), likely because

they lose eggs as they age, causing them to thin. For males, the dominant criterion is the width/length ratio. Tumours are seen frequently in both genders, and have a prevalence of increasing with age (Laconi et al., 2020). Further, tumours in zebrafish can be fairly easy to recognise and use as a quick score. An argument for adding fin defects is that this may point to defective fin regeneration (Pfefferli and Jazwinska, 2015), which is a sign of ageing (Gilbert et al., 2014, Kishi, 2004). Interestingly, the loss of fin regeneration was not seen as important to score frailty by the experts and this criterion was therefore removed from the optimised index.

We considered and ultimately excluded several other criteria for our index. Cataracts, for example, are associated with age and frailty (Mencucci et al., 2023). However, robustly identifying cataract in aged zebrafish was too difficult to do reliably. Similarly, skin ulcers or scale damage was considered but these were rarely observed in the aged fish, and can also occur in young adults. They could be a general welfare marker rather than an ageing feature (Flueck-Giraud et al., 2024). We chose not to include reduced movement despite linkage to frailty, and use in several frailty scoring systems (Billot et al., 2020, Franceschi et al., 2018, Fried et al., 2001); the need for specialised equipment and time needed made this less desirable. Width-length ratio and spinal curvature may indirectly reflect movement as it will be affected by loss of muscle mass (Gilbert et al., 2014). We also chose to exclude genetic and molecular markers from the index as we wanted to create a rapid index that required little equipment and that would create a general index that was not limited towards one aspect of ageing, such as tying the index down to inflammation or nutrient sensing. Of course, we hope that future experiments could now use this index to examine correlations between the index and molecular and behavioural parameters.

We analysed the expression of two established frailty markers, *il-6* and *p21* in combination with our frailty index (Figure 1F-H) (Marcozzi et al., 2023, Puts et al., 2005). An orthologue of *il-6* has been identified in zebrafish and has previously been reported in the adult caudal fin after injury. An increase in *il-6* has also been observed in *sert1*^{-/-} prematurely ageing zebrafish larvae and internal organs (Bohaud et al., 2021, Mastrogiovanni et al., 2024, Abou-Dahech and Williams, 2024, Kim et al., 2019). Expression of *p21* has also been observed in zebrafish larval fins after amputation, and is recognised as a key senescence and ageing marker in zebrafish (Morsli et al., 2023, Da Silva-Álvarez et al., 2020). We found that in female fish, the expression of both markers steadily increases with frailty. Whilst there is a significant change in *il-6* between robust and frail old fish in females, there is only a trend for

this observation for $p21$. This could suggest that for frailty, inflammation is a more sensitive marker than senescence. Further, for the male fish, we only saw significant changes between young and old fish, not between the old groups. This could be a result of identifying the three different classes of old fish being more difficult in males than females. The results from the qPCR therefore, might suggest that the frailty index works well to separate out the different frailty groups of female zebrafish, but, it might not be as sensitive for male zebrafish. Alternatively, it has also been seen in mice that females tend to show a stronger correlation between inflammation and frailty than males (Kane et al., 2019). Which could also be a reason why the markers are not significant in male fish, and perhaps other frailty markers such as endocrine markers could be used to further assess the molecular patterns within the frailty groups.

To validate our frailty index further, we had seven zebrafish experts visually assess and score 80 fish, which included young, robust, pre-frail, and frail individuals. The experts' scores showed a strong positive correlation with our frailty index (Figure 2A). We noted that some criteria seem less important for frailty. Both, BMI and width/length ratio showed significant negative regression with increasing frailty scores (Figure 2B and C), however, this was not the case for the spinal curvature as it was not significant with increasing frailty, although there was a trend with the females but not the males (Figure 2D). Additionally, whilst tumours and fin degeneration were not as important for high frailty, it appeared that fish with either of these criteria scored pre-frail or higher (Figure 2E and F).

By using linear regression, we optimized our frailty index to better reflect expert judgments. The resulting slope coefficients were used as normalized weights, as seen in Figure 3A, to determine the importance of each criterion. This process revealed that fin degeneration was not a significant marker of frailty and was therefore removed from the index. Interestingly, the width-to-length ratio proved to be the most critical criteria for the females whilst BMI was the most critical for males. This observation is opposed to the percentages calculated for which observations were more common in each gender seen in Table 2. Applying this new, four-parameter index to the 60 fish scored by experts, we found a stronger correlation with the expert scores than with the original index (Figure 3B). A RMSE analysis confirmed that our optimized system provided a more accurate assessment of frailty. Additionally, our optimized index performed better than a previously used method that relies solely on the width/length ratio to estimate frailty (Ellis et al., 2024, Henriques et al., 2013), as indicated by a less favourable RMSE for that approach.

This study introduces an optimised, four-criteria index designed to quantify frailty in zebrafish through easily accessible parameters. We acknowledge that this index is currently calibrated against subjective expert assessment rather than longitudinal survival data, which remains a limitation. Furthermore, as our subjects were sourced from diverse genetic backgrounds, potential heterozygosity may have exerted subtle influences on morphology. Nevertheless, this index provides a vital framework for the standardisation of frailty metrics across laboratories. It serves as a valuable tool for distinguishing between robust and frail aging trajectories and offers a practical application for monitoring animal welfare within aquarium facilities

Methods

Zebrafish husbandry

Zebrafish were maintained at the Bateson Centre aquarium at the University of Sheffield under site licence *X57506C3D*, complying with UK laws . They were kept in freshwater at 28°C with a 14:10 light:dark cycle at a density of 5 animals per 1L or less. They received either dry food (*ZEBRAFEED*) or live artemia according to Supplementary Data 1. A health screen report and water quality parameters are also available in Supplementary Data 1. No fish were bred for the purpose of this study, only surplus fish from the aquarium were used to make this scoring system. Animals may have had wild-type or heterozygous backgrounds that do not produce an adult phenotype (Supplementary Data 2).

Culling and measurements of zebrafish

Adult zebrafish (aged 4-9 months and 35-36 months for females, 4-9 months and 23-36 months for males) were culled using tricaine (MS-222) followed by destruction of the brain (UK schedule 1 procedure) and imaged on a scale with a ruler. Their weight, gender, tumours, and frayed fins were recorded. The tail fin was clipped for RNA extraction. Images were analysed using Fiji/ImageJ. Fish length (cm) was measured from the tip of the mouth, in a straight line to the start of the tail fin (Figure 4A). The height of the spinal curve was measured from the top of the spine to a line drawn from the mouth of the fish to the middle of the fin (Figure 4C). The fish width was measured from the top of the spine to the bottom of the stomach (Figure 4A). A width-to-length ratio was calculated, and the BMI of the fish was determined by taking the weight in grams of the fish and dividing it by the square root of the

length of the fish. Tumours were identified as lumps in the fish (Figure 4B), while fin degeneration was identified by split or frayed fins (Figure 4D). We followed existing scoring systems such as the Fried index, who use a step function (Fried et al., 2001), rather than a continuous scoring system.

qPCR

The qPCR experiments followed the MIQE standards presented in Bustin et al (Bustin et al., 2009). Tail fin clips from culled adult young, robust, pre-frail and frail old fish were snap frozen on dry ice, then either stored at -70°C or processed immediately by crushing the fin clip using a micropestle rotor, and RNA extracted using Trizol and chloroform. RNA concentration and purity was measured using a nanodrop (ND-100 v3.8.1), and stored at -70°C. Young fish were used as the control group. 470ng/μl RNA was converted to cDNA using the LunarScript RT SuperMix (M3010L NEB) kit in a 10μl reaction following the kit instructions. cDNA was stored at -20°C. qPCRs performed with HOT FIREPol EvaGreen qPCR Mix Plus (no Rox) (Solis Biodyne) in a 20 μl reaction with 0.5μM of primers for *il-6* Fw: 5'TCAACTTCTCCAGCGTGATG3' Rev: 5'TCTTTCCCTCTTTTCCTCCTG3' (Safari et al., 2016), *p21* Fw: 5'AGGAAAAGCAGCAGAAACG3' Rev: 5'TGTTGGTCTGTTTGCGCTT3' (Morsli et al., 2023) and *rps29* Fw: 5'TTTGCTCAAACCGTCACGGA3' Rev: 5'ACTCGTTTAATCCAGCTTGACG3' (Bower et al., 2017), in a BioRad (HSP9601) 96 well qPCR plate. The following cycling conditions were used with wild type cDNA on 0, -1, -2, -3 times dilutions to confirm that the primers had an amplification efficiency of between 90-110% and in the experiments with cDNA diluted -1: 95°C 10 minutes, then 45 cycles of 95°C 15 seconds, 55°C 15 seconds, 72°C 30 seconds. The fluorescent intensity in the reaction was read at the end of each cycle. The amplification efficiency was calculated using the equation for primer efficiency in the BioRad's Application guide for Real-Time qPCR (2006) $((10^{-1/\text{Slope}})-1) \times 100\%$, where the slope was calculated from the average ct value from the three replicates of the diluted wild type cDNA which was used to construct a standard curve by plotting the log of the starting quantity of the template (0, -1, -2, -3) against the average ct. The specificity of the primers were further evaluated by looking at their melting curve of the amplified product. The threshold for Cq value was set automatically by the BioRad (CFX96™ Real time system) as the beginning of the exponential phase during the 45 cycles, and the Cq value was identified by the machine when the fluorescent intensity within a sample reached that threshold. Three replicates of each sample were run, as well as three replicates of water that were used as contamination control for each primer set for every qPCR. A qPCR run was deemed not contaminated if the Cq value of the water samples

were NA or higher than 45 cycles. The Ct values for the samples were calculated by subtracting the Cq value of rps29 (Housekeeping) from the Cq values of each corresponding target gene for each replicate creating a Δct value. A $\Delta\Delta\text{ct}$ value was calculated by subtracting the average young Δct value for the gene from the Δct of the individual replicates. A fold change value for each replicate was calculated by taking the $2^{-\Delta\Delta\text{ct}}$. The average fold change which was the plotted value was calculated by averaging the fold change value for the three replicates. Statistical analysis was performed in R and R-studio version 4.1.1 using one-way ANOVA with a TukeyHSD (Honesty Significant Difference) post-hoc test.

Frailty scoring by zebrafish experts

Ten images each of culled female and male young, robust old, pre-frail old and frail old fish (total =80) (Supplementary Data 4) as classed by the proposed system were shown to experienced Named Aquarium Care and Welfare Officer (NACWO)-trained aquarium staff and other zebrafish ageing researchers (n=7), all have more than 10 years of experience caring and recognising old and frail fish, and are trained by using various body scoring criteria (Clark et al., 2018). They scored these by eye from 0-5 where 0 is robust and 5 is the frailest/sickest fish they have seen. The scores for each fish were averaged and indexed and plotted within each group. The raw expert data can be found in Supplementary Data 3.

Linear regression

To identify optimal weights for our criteria, we focused on the old fish, and did linear regression where the fish experts indexed values were used as the dependent variable and each five criteria indexed were used as the independent variables. For the criteria, this means that if one of the old fish scored according to the threshold that criteria would have the value of 0.2 for that individual fish, or 0 when negative. The slope coefficients obtained from the linear regression were used as the optimal weights. If any of the slope coefficients were negative, their weight was set to 0 as it would indicate that this criterion was not important for the experts. As the index needed to be a total value from 0-1, the weights were normalised so that if a fish scored on all the criteria, they would score 1 by dividing the weights by the sum of all the weights. R code: See Supplementary Data 5.

Use of AI

AI was used to advise on efficient use of English in order to hit word limits for abstracts. No generative AI was used.

Acknowledgements

We would like to thank the NACWO's in the Sheffield Aquarium Facility for help with zebrafish frailty scoring.

Competing interests

No competing interests declared for all authors

Funding

This work was supported by BBSRC grant Biotechnology and Biological Sciences Research Council grant BB/R015457/1 to FvE, ACH was supported by a Vivensa PhD studentship, ACH and SEK were supported by Medical Research Council funding MR/Y000021/1 PE and CMH were supported by a Sir Henry Dale Fellowship, by the Wellcome Trust and The Royal Society (206224/Z/17/Z), and a Vivensa Foundation grant AISRPG2305\32

Data and resource availability

All relevant data and details of resources can be found within the article and its supplementary information.'

References

- ABOU-DAHECH, M. S. & WILLIAMS, F. E. 2024.** Aging, Age-Related Diseases, and the Zebrafish Model. *Journal of Dementia and Alzheimer's Disease* [Online], **1**.
- BILLOT, M., CALVANI, R., URTAMO, A., SANCHEZ-SANCHEZ, J. L., CICCOLARI-MICALDI, C., CHANG, M., ROLLER-WIRNSBERGER, R., WIRNSBERGER, G., SINCLAIR, A., VAQUERO-PINTO, N., et al. 2020.**

Preserving Mobility in Older Adults with Physical Frailty and Sarcopenia: Opportunities, Challenges, and Recommendations for Physical Activity Interventions. *Clin Interv Aging*, **15**, 1675-1690.

BOHAUD, C., JOHANSEN, M. D., JORGENSEN, C., IPSEIZ, N., KREMER, L. & DJOUAD, F. 2021. The Role of Macrophages During Zebrafish Injury and Tissue Regeneration Under Infectious and Non-Infectious Conditions. *Front Immunol*, **12**, 707824.

BOWER, N. I., VOGGRIN, A. J., LE GUEN, L., CHEN, H., STACKER, S. A., ACHEN, M. G. & HOGAN, B. M. 2017. Vegfd modulates both angiogenesis and lymphangiogenesis during zebrafish embryonic development. *Development*, **144**, 507-518.

BUSTIN, S. A., BENES, V., GARSON, J. A., HELLEMANS, J., HUGGETT, J., KUBISTA, M., MUELLER, R., NOLAN, T., PFAFFL, M. W., SHIPLEY, G. L., et al. 2009. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clin Chem*, **55**, 611-22.

CAYUELA, M. L., CLAES, K. B. M., FERREIRA, M. G., HENRIQUES, C. M., VAN EEDEN, F., VARGA, M., VIERSTRAETE, J. & MIONE, M. C. 2018. The Zebrafish as an Emerging Model to Study DNA Damage in Aging, Cancer and Other Diseases. *Front Cell Dev Biol*, **6**, 178.

CLARK, T. S., PANDOLFO, L. M., MARSHALL, C. M., MITRA, A. K. & SCHECH, J. M. 2018. Body Condition Scoring for Adult Zebrafish (*Danio rerio*). *J Am Assoc Lab Anim Sci*, **57**, 698-702.

COXAM, B., SABINE, A., BOWER, N. I., SMITH, K. A., PICHOL-THIEVEND, C., SKOCZYLAS, R., ASTIN, J. W., FRAMPTON, E., JAQUET, M., CROSIER, P. S., et al. 2014. Pkd1 Regulates Lymphatic Vascular Morphogenesis during Development. *Cell Reports*, **7**, 623-633.

CROW, R. S., PETERSEN, C. L., COOK, S. B., STEVENS, C. J., TITUS, A. J., MACKENZIE, T. A. & BATSIS, J. A. 2020. Reported Weight Change in Older Adults and Presence of Frailty. *J Frailty Aging*, **9**, 74-81.

DA SILVA-ÁLVAREZ, S., GUERRA-VARELA, J., SOBRIDO-CAMEÁN, D., QUELLE, A., BARREIRO-IGLESIAS, A., SÁNCHEZ, L. & COLLADO, M. 2020. Cell senescence contributes to tissue regeneration in zebrafish. *Aging Cell*, **19**, e13052.

ELLIS, P. S., MARTINS, R. R. & HENRIQUES, C. M. 2024. Adult zebrafish as a vertebrate model of ageing. In: **ALLEN, C. E. & MOCHO, J.-P.** (eds.) *Zebrafish : a practical guide to husbandry, welfare and research methodology*. CAB International.159–181.

FARGHER, E., KEATINGE, M., PEARCE, O., PIEPPONEN, P., PANULA, P., VAN EEDEN, F. J. M., MACDONALD, R. B. & BANDMANN, O. 2025. A zebrafish model of acmsd deficiency does not support a prominent role for ACMSD in Parkinson's disease. *NPJ Parkinsons Dis*, **11**, 118.

FLUECK-GIRAUD, M., SCHMIDT-POSTHAUS, H., BERGADANO, A. & ADRIAN-KALCHHAUSER, I. 2024. An adaptable, user-friendly score sheet to monitor welfare in experimental fish. *Laboratory Animals*, **0**, 00236772241271013.

- FRANCESCHI, C., GARAGNANI, P., MORSIANI, C., CONTE, M., SANTORO, A., GRIGNOLIO, A., MONTI, D., CAPRI, M. & SALVIOLI, S.** 2018. The Continuum of Aging and Age-Related Diseases: Common Mechanisms but Different Rates. *Front Med (Lausanne)*, **5**, 61.
- FRIED, L. P., TANGEN, C. M., WALSTON, J., NEWMAN, A. B., HIRSCH, C., GOTTDIENER, J., SEEMAN, T., TRACY, R., KOP, W. J., BURKE, G., et al.** 2001. Frailty in older adults: evidence for a phenotype. *J Gerontol A Biol Sci Med Sci*, **56**, M146-56.
- GERHARD, G. S. & CHENG, K. C.** 2002. A call to fins! Zebrafish as a gerontological model. *Aging Cell*, **1**, 104-11.
- GILBERT, M. J., ZERULLA, T. C. & TIERNEY, K. B.** 2014. Zebrafish (*Danio rerio*) as a model for the study of aging and exercise: physical ability and trainability decrease with age. *Exp Gerontol*, **50**, 106-13.
- HAYES, A. J., REYNOLDS, S., NOWELL, M. A., MEAKIN, L. B., HABICHER, J., LEDIN, J., BASHFORD, A., CATERSON, B. & HAMMOND, C. L.** 2013. Spinal deformity in aged zebrafish is accompanied by degenerative changes to their vertebrae that resemble osteoarthritis. *PLoS One*, **8**, e75787.
- HEINZE-MILNE, S. D., BANGA, S. & HOWLETT, S. E.** 2019. Frailty Assessment in Animal Models. *Gerontology*, **65**, 610-619.
- HENRIQUES, C. M., CARNEIRO, M. C., TENENTE, I. M., JACINTO, A. & FERREIRA, M. G.** 2013. Telomerase is required for zebrafish lifespan. *PLoS Genet*, **9**, e1003214.
- HUA, J., HOUMMADY, S., MULLER, C., POUCHELON, J. L., BLONDOT, M., GILBERT, C. & DESQUILBET, L.** 2016. Assessment of frailty in aged dogs. *Am J Vet Res*, **77**, 1357-1365.
- HUDOCK, J. & KENNEY, J. W.** 2024. Aging in zebrafish is associated with reduced locomotor activity and strain dependent changes in bottom dwelling and thigmotaxis. *PLoS One*, **19**, e0300227.
- KANE, A. E. & HOWLETT, S. E.** 2021. Sex differences in frailty: Comparisons between humans and preclinical models. *Mech Ageing Dev*, **198**, 111546.
- KANE, A. E., KELLER, K. M., HEINZE-MILNE, S., GRANDY, S. A. & HOWLETT, S. E.** 2019. A Murine Frailty Index Based on Clinical and Laboratory Measurements: Links Between Frailty and Pro-inflammatory Cytokines Differ in a Sex-Specific Manner. *J Gerontol A Biol Sci Med Sci*, **74**, 275-282.
- KHOR, E. S., NOOR, S. M. & WONG, P. F.** 2019. Understanding the Role of ztor in Aging-related Diseases Using the Zebrafish Model. *In Vivo*, **33**, 1713-1720.
- KIM, D. H., JUNG, I. H., KIM, D. H. & PARK, S. W.** 2019. Knockout of longevity gene Sirt1 in zebrafish leads to oxidative injury, chronic inflammation, and reduced life span. *PLOS ONE*, **14**, e0220581.
- KIM, H. R., SANTHAKUMAR, K., MARKHAM, E., BALDERA, D., GREENALD, D., BRYANT, H. E., EL-KHAMISY, S. F. & VAN EEDEN, F. J.** 2020. Investigation of the role of VHL-HIF signaling in DNA repair and apoptosis in zebrafish. *Oncotarget*, **11**, 1109-1130.

KISHI, S. 2004. Functional aging and gradual senescence in zebrafish. *Ann N Y Acad Sci*, 1019, 521-6.

KISHI, S., UCHIYAMA, J., BAUGHMAN, A. M., GOTO, T., LIN, M. C. & TSAI, S. B. 2003. The zebrafish as a vertebrate model of functional aging and very gradual senescence. *Exp Gerontol*, **38**, 777-86.

LACONI, E., MARONGIU, F. & DEGREGORI, J. 2020. Cancer as a disease of old age: changing mutational and microenvironmental landscapes. *Br J Cancer*, **122**, 943-952.

MARCOZZI, S., BIGOSSI, G., GIULIANI, M. E., GIACCONI, R., PIACENZA, F., CARDELLI, M., BRUNETTI, D., SEGALA, A., VALERIO, A., NISOLI, E., et al. 2023. Cellular senescence and frailty: a comprehensive insight into the causal links. *Geroscience*, **45**, 3267-3305.

MASTROGIOVANNI, M., MARTÍNEZ-NAVARRO, F. J., BOWMAN, T. V. & CAYUELA, M. L. 2024. Inflammation in Development and Aging: Insights from the Zebrafish Model. *International Journal of Molecular Sciences*, **25**, 2145.

MENCUCCI, R., STEFANINI, S., FAVUZZA, E., CENNAMO, M., DE VITTO, C. & MOSSELLO, E. 2023. Beyond vision: Cataract and health status in old age, a narrative review. *Front Med (Lausanne)*, **10**, 1110383.

MITNITSKI, A. B., GRAHAM, J. E., MOGILNER, A. J. & ROCKWOOD, K. 2002. Frailty, fitness and late-life mortality in relation to chronological and biological age. *BMC Geriatr*, **2**, 1.

MITNITSKI, A. B., MOGILNER, A. J. & ROCKWOOD, K. 2001. Accumulation of deficits as a proxy measure of aging. *ScientificWorldJournal*, **1**, 323-36.

MORSLI, S., HENRIQUES, C. M., ELLIS, P. S., MORTIBOYS, H., BAXENDALE, S., LOYNES, C. A., RENSHAW, S. A. & BELLANTUONO, I. 2023. A p21-GFP zebrafish model of senescence for rapid testing of senolytics in vivo. *Aging Cell*, **22**, e13835.

PARK, C. & KO, F. C. 2021. The Science of Frailty: Sex Differences. *Clin Geriatr Med*, **37**, 625-638.

PARKS, R. J., FARES, E., MACDONALD, J. K., ERNST, M. C., SINAL, C. J., ROCKWOOD, K. & HOWLETT, S. E. 2012. A procedure for creating a frailty index based on deficit accumulation in aging mice. *J Gerontol A Biol Sci Med Sci*, **67**, 217-27.

PFEFFERLI, C. & JAZWINSKA, A. 2015. The art of fin regeneration in zebrafish. *Regeneration (Oxf)*, **2**, 72-83.

PUTS, M. T., VISSER, M., TWISK, J. W., DEEG, D. J. & LIPS, P. 2005. Endocrine and inflammatory markers as predictors of frailty. *Clin Endocrinol (Oxf)*, **63**, 403-11.

ROCKWOOD, K., BLODGETT, J. M., THEOU, O., SUN, M. H., FERIDOONI, H. A., MITNITSKI, A., ROSE, R. A., GODIN, J., GREGSON, E. & HOWLETT, S. E. 2017. A Frailty Index Based On Deficit Accumulation Quantifies Mortality Risk in Humans and in Mice. *Sci Rep*, **7**, 43068.

ROCKWOOD, K., FOX, R. A., STOLEE, P., ROBERTSON, D. & BEATTIE, B. L. 1994. Frailty in elderly people: an evolving concept. *CMAJ*, **150**, 489-95.

ROCKWOOD, K., HOGAN, D. B. & MACKNIGHT, C. 2000. Conceptualisation and measurement of frailty in elderly people. *Drugs Aging*, **17**, 295-302.

ROCKWOOD, K. & MITNITSKI, A. 2011. Frailty defined by deficit accumulation and geriatric medicine defined by frailty. *Clin Geriatr Med*, **27**, 17-26.

SAFARI, R., HOSEINIFAR, S. H. & KAVANDI, M. 2016. Modulation of antioxidant defense and immune response in zebra fish (*Danio rerio*) using dietary sodium propionate. *Fish Physiol Biochem*, **42**, 1733-1739.

SEARLE, S. D., MITNITSKI, A., GAHBAUER, E. A., GILL, T. M. & ROCKWOOD, K. 2008. A standard procedure for creating a frailty index. *BMC Geriatr*, **8**, 24.

WHITEHEAD, J. C., HILDEBRAND, B. A., SUN, M., ROCKWOOD, M. R., ROSE, R. A., ROCKWOOD, K. & HOWLETT, S. E. 2014. A clinical frailty index in aging mice: comparisons with frailty index data in humans. *J Gerontol A Biol Sci Med Sci*, **69**, 621-32.

Figures and Tables

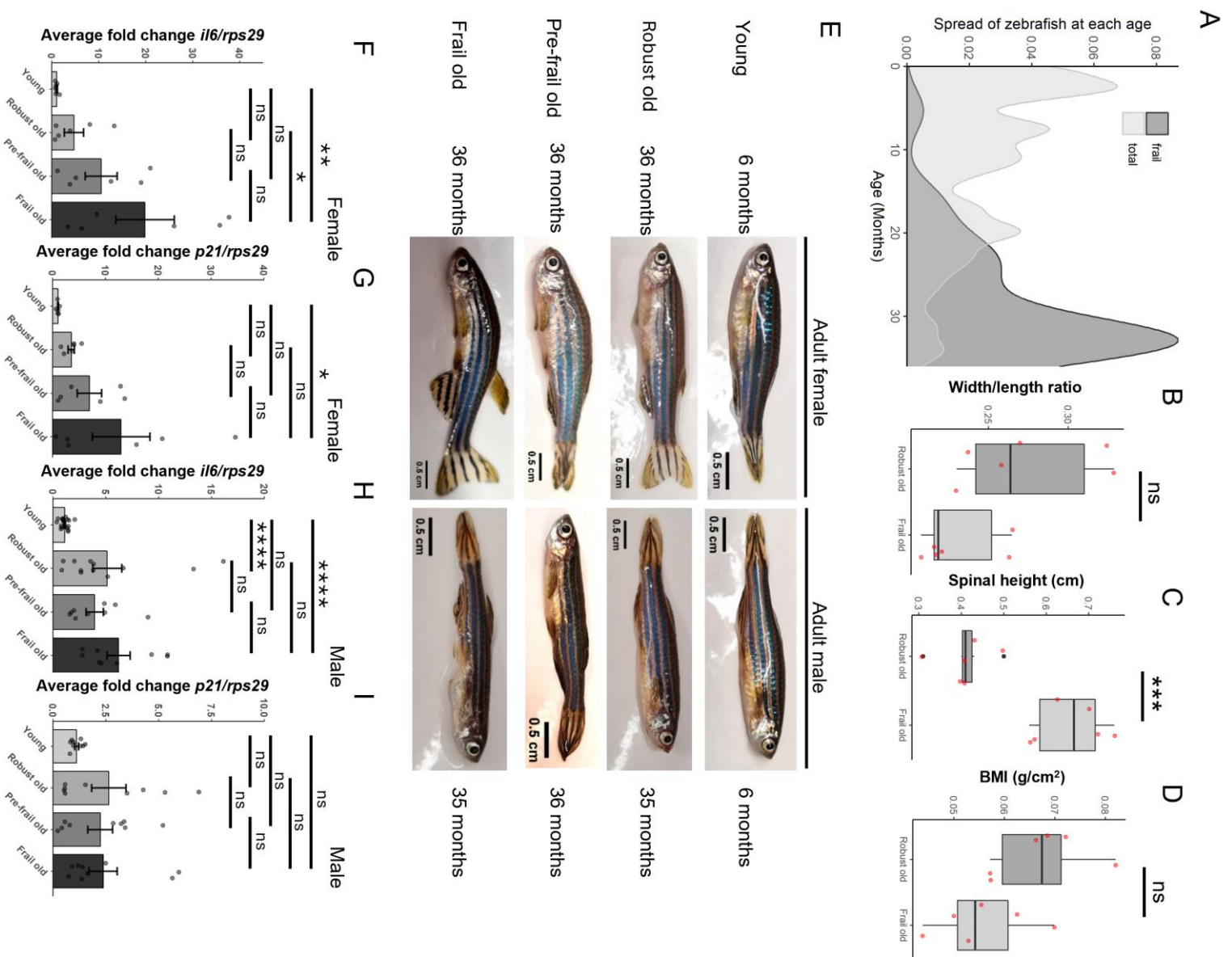


Fig. 1. Creating the pseudo-frailty index for ageing zebrafish. A: Density plot showing the ages of the total number of fish in the aquarium (light grey) against the spread of the ages the fish in the aquarium can be classed as frail based on the frailty index (dark grey). B-D: Initial width/length ratio, BMI (g/cm^2) and Spinal height (cm) values from 6 visually robust and 6 visually frail female fish that created the frailty index. Unpaired t-test, $n=6$. E: Images of female and male zebrafish in the four different frailty groups, robust young, robust old, pre-frail old, frail old. The female robust old and frail old fish are siblings, as are the males. F: qPCR of female tail fins for *il-6*, $n=6$. G: qPCR of female tail fins for *p21*, $n=6$. H: qPCR of male tail fins for *il-6*, old $n=9$, pre-frail $n=9$, robust $n=15$, young $n=18$. I: qPCR of male tail fins for *p21*, old $n=9$, pre-frail $n=9$, robust $n=9$, young $n=9$. ANOVA.

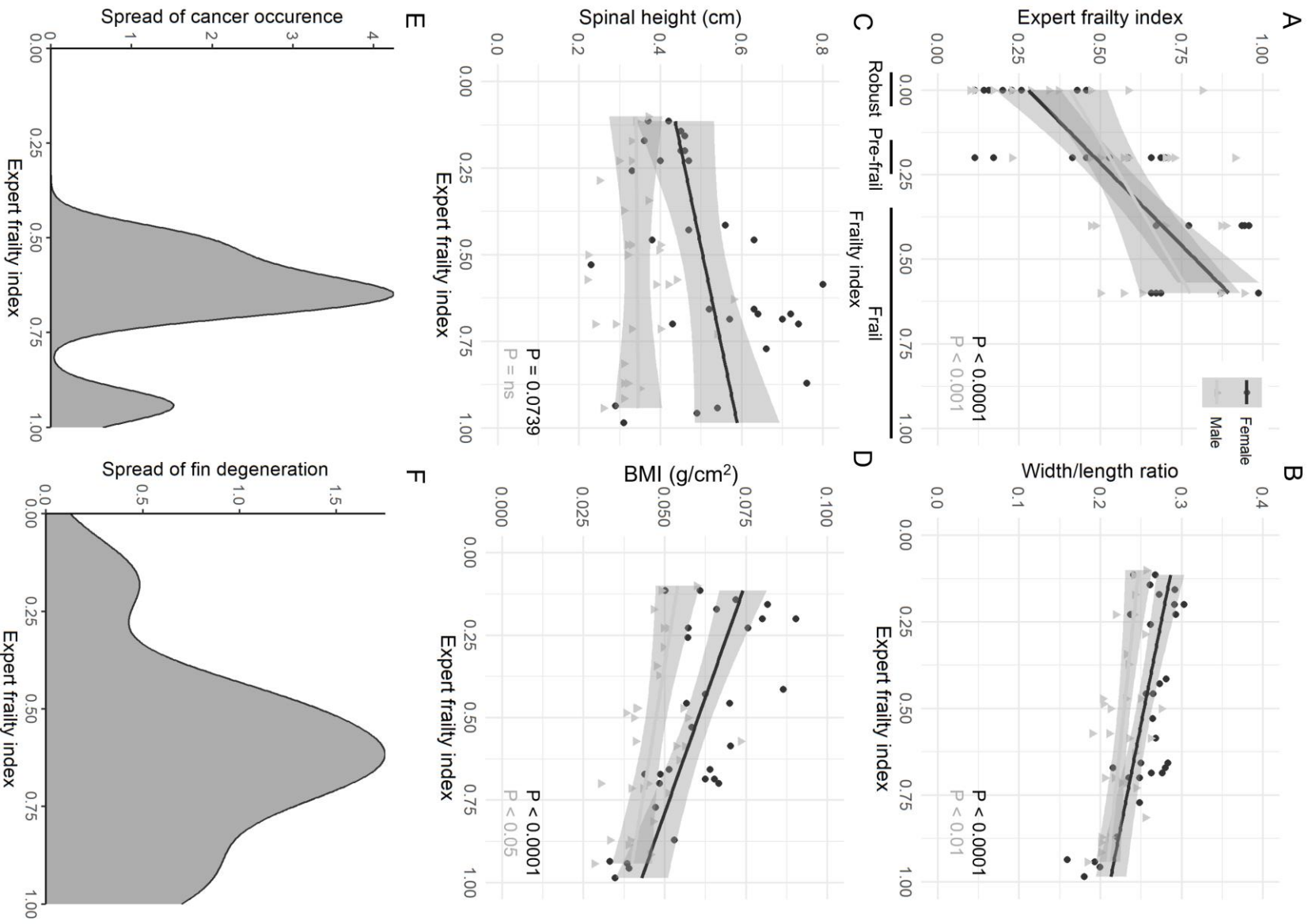


Fig. 2. Indexed frailty scoring of young, old robust, pre-frail and frail old female and male zebrafish by zebrafish experts. A: The average score per fish by the experts increases significantly with the frailty index. B: The width/length ratio decreases significantly with higher scores given by the experts. C: No significant increase in spinal height with higher scores given by experts. D: The fish BMI decreases significantly the higher the score the experts gave the fish. A, n=40. B-D n=30. Simple linear regression. E: Density plot of tumor occurrence amongst the 60 old fish against the experts' scores. n=6. F: Density plot of fin degeneration amongst the 60 old fish against the experts' scores. n=8.

A

Optimised female index

$$(0.176 \times BMI \text{ val}) + (0.192 \times Spinal \text{ val}) + (0.348 \times W / L \text{ val}) + (0.284 \times x \text{ Tumour val}) + (0 \times Fin \text{ val}) \\ = \text{frailty index score}$$

B

Optimised male index

$$(0.358 \times BMI \text{ val}) + (0.264 \times Spinal \text{ val}) + (0.258 \times W / L \text{ val}) + (0.120 \times x \text{ Tumour val}) + (0 \times Fin \text{ val}) \\ = \text{frailty index score}$$

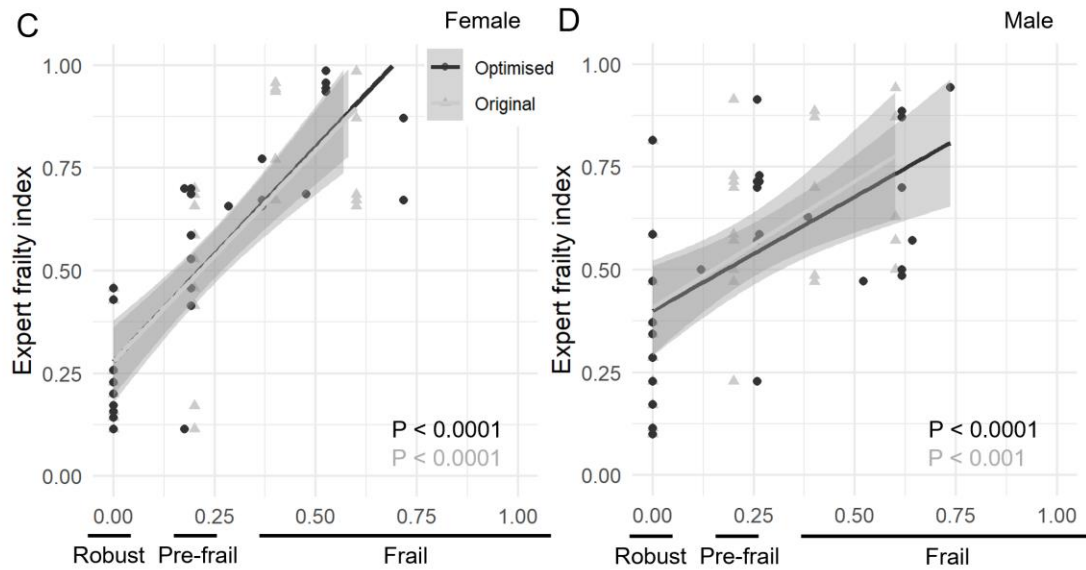


Fig. 3. Optimisation of the zebrafish frailty index. A-B: Female and male equations for optimised frailty index with weights based on expert scores. The values (val) relate to the 1 or 0 given to the fish from table 1, as to whether they have the frailty feature or not. C-D: Frailty scoring of 60 old robust, pre-frail and frail old female and male zebrafish by zebrafish experts compared to the optimised frailty index with weighting, and the original frailty index proposed in table 2. Simple linear regression, n=30.

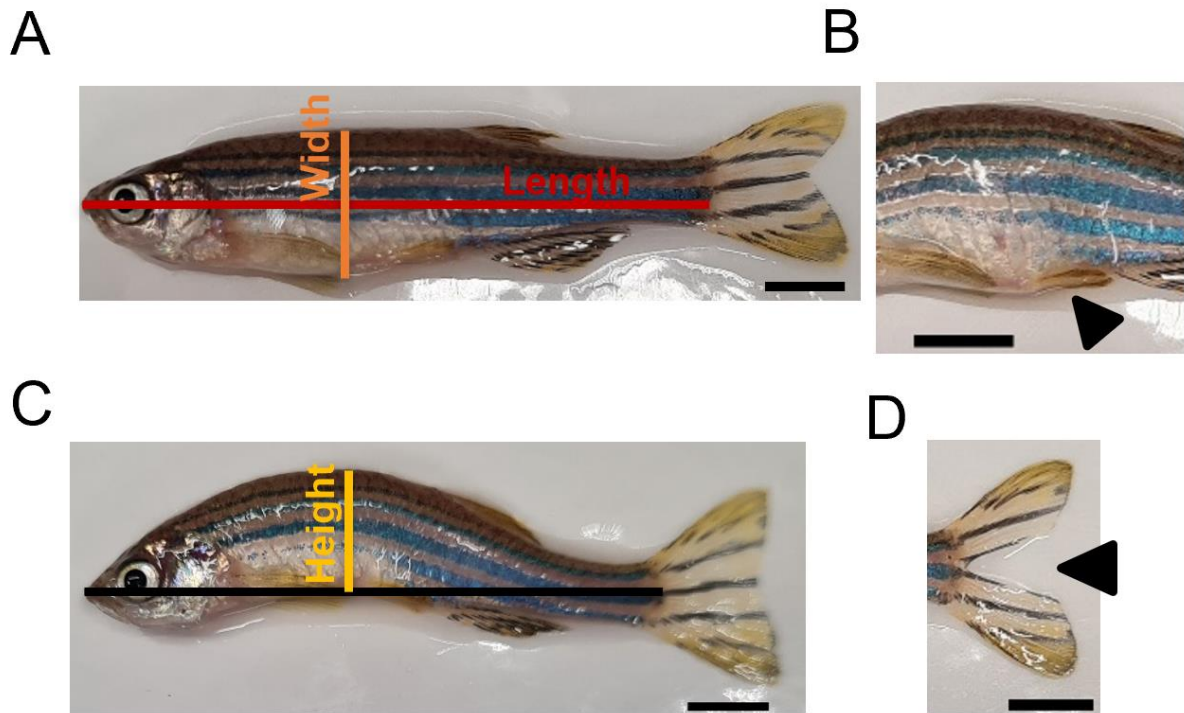


Fig. 4. Examples of how measurements were taken for the frailty index. A: Length (Red) was measured from tip of the nose to the start of the fin in a straight line. Width (Orange) was measured from the top of the spine to the bottom of the stomach in a straight line. B: Example of a tumour in the stomach region. C: The spinal height (Yellow) was measured from the line used to measure length to the top of the spine. D: Example of a split fin. Scale = 0.5cm.

Table 1. Scoring system for pseudo frailty scale for zebrafish.

Criteria	Female threshold	Male threshold	Scoring
Width/length ratio	0.23	0.22	< = 1 > = 0
BMI (g/cm ²)	0.056	0.041	< = 1 > = 0
Spinal curvature	0.55	0.4	> = 1 < = 0
Fin degeneration	Yes/No	Yes/No	Yes = 1 No = 0
Tumours	Yes/No	Yes/No	Yes = 1 No = 0

Table 2. Percentage of total and frail scoring female and male that scored on each criterion.

Criteria	Female		Male	
	Total (n=69)	Frail (34.5%)	Total (n=44)	Frail (25%)
Width/length ratio	18.8%	52%	36.4%	72.7%
BMI (g/cm ²)	42%	96%	15.9%	54.5%
Spinal curvature	39.2%	79%	18.2%	45.5%
Fin degeneration	8.7%	20%	9.1%	18.2%
Tumours	7.2%	12%	11.4%	36.4%

Dataset 1.

Available for download at

<https://journals.biologists.com/bio/article-lookup/doi/10.1242/bio.062345#supplementary-data>

Dataset 2.

Available for download at

<https://journals.biologists.com/bio/article-lookup/doi/10.1242/bio.062345#supplementary-data>

Dataset 3.

Available for download at



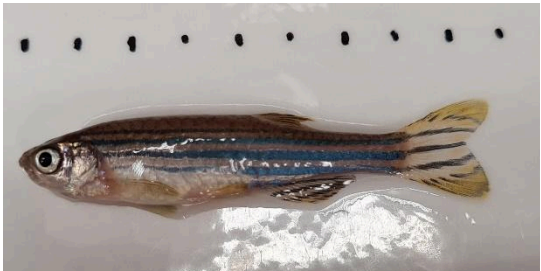


<https://journals.biologists.com/bio/article-lookup/doi/10.1242/bio.062345#supplementary-data>








Below are 40 female and 40 male fish. Some are young and some are old. Please score the fish on a scale from 0 to 5 on how frail you think the fish look. 0 is healthy, 5 is sickly frail and likely to die. If you feel the need, you can use half points.








When I think about frailty in this project, I think about what a frail human would look like.







Important note: we rarely/do not encounter extremely frail fish which I would score a 5, this is because such fish are removed from the aquarium beforehand, nevertheless you may have seen some in your career. Therefore, you should not feel obliged to use the entire range from 0-5, rather score them using your memory of “what is the frailest fish I have ever seen”.








Females







Fish id	Image	Your score 0-5
253		
15		
258		
241		
16		



263		
245		
6		
278		
279		
303		
17		

28		
305		
246		
292		
309		
336		
264		

29	 A lateral view of a fish specimen with a dark dorsal side and a lighter ventral side. It has a prominent dark stripe running along its body. The tail is slightly forked. Above the fish, there is a horizontal row of ten black dots.	
30	 A lateral view of a fish specimen, similar to specimen 29, with a dark dorsal side and a lighter ventral side. It has a prominent dark stripe running along its body. The tail is slightly forked. Above the fish, there is a horizontal row of ten black dots.	
251	 A lateral view of a fish specimen with a dark dorsal side and a lighter ventral side. It has a prominent dark stripe running along its body. The tail is deeply forked. Above the fish, there is a horizontal row of ten black dots.	
275	 A lateral view of a fish specimen with a dark dorsal side and a lighter ventral side. It has a prominent dark stripe running along its body. The tail is deeply forked. Above the fish, there is a horizontal row of ten black dots.	
42	 A lateral view of a fish specimen that is entirely orange. It has a rounded body and a slightly forked tail. Above the fish, there is a horizontal row of ten black dots.	
277	 A lateral view of a fish specimen with a dark dorsal side and a lighter ventral side. It has a prominent dark stripe running along its body. The tail is deeply forked. Above the fish, there is a horizontal row of ten black dots.	








296		
302		
44		
304		
301		
300		
289		


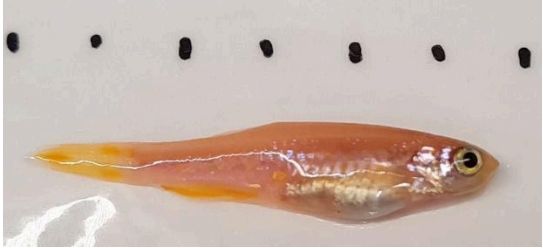





337		
131		
306		
307		
290		
308		








293		
291		







Males







1		
202		
11		
199		


4		
38		
12		
272		
25		
3		
37		

198		
49		
123		
343		
205		
102		
2		

40	 A lateral view of a fish specimen with a yellowish-orange body and a darker dorsal region. It is positioned against a white background with a row of black dots above it.	
13	 A lateral view of a fish specimen with a blue and silver striped pattern along its body. It is positioned against a white background with a row of black dots above it.	
206	 A lateral view of a fish specimen with a blue and silver striped pattern along its body. It is positioned against a white background with a row of black dots above it.	
41	 A lateral view of a fish specimen with a blue and silver striped pattern along its body. It is positioned against a white background with a row of black dots above it.	
103	 A lateral view of a fish specimen with a blue and silver striped pattern along its body. It is positioned against a white background with a row of black dots above it.	
47	 A lateral view of a fish specimen with a yellowish-orange body. It is positioned against a white background with a row of black dots above it.	
344	 A lateral view of a fish specimen with a blue and silver striped pattern along its body. It is positioned against a white background with a row of black dots above it.	

26		
338		
339		
348		
97		
48		

204		
197		
340		
349		
350		
98		

260		
112		
27		

R script to create weights for the frailty index

```
# Import library
```

```
library(readxl)
```

```
# Import your data
```

```
data <- read_excel("C:/_PATHWAY_.xlsx")
```

```
# Perform Linear Regression
```

```
regression_model <- lm(Expert ~ BMI + Spine + WL + Tumour + Fin, data = data)
```

```
# Summarize the model to see the coefficients
```

```
summary(regression_model)
```

```
# Extract the raw coefficients
```

```
raw_weights <- coef(regression_model)
```

```
print("Raw Coefficients (from regression):")
```

```
print(raw_weights)
```

```
# Extract only the criterion weights (excluding the intercept)
```

```
criterion_weights_raw <- raw_weights[names(raw_weights) != "(Intercept)"]
```

```
# --- Handle Negative Weights ---
```

```
# Set any negative weights to zero
```

```
positive_weights <- pmax(0, criterion_weights_raw)
```

```
print("Weights after setting negatives to zero:")
```

```
print(positive_weights)
```

```
# --- Normalize Weights to Sum to 1 ---
```

```
# This ensures your final optimized frailty index is also on a similar scale (e.g., 0-1)
```

```
optimized_weights <- positive_weights / sum(positive_weights)
```

```
print("Optimized and Normalized Weights:")
```

```
print(optimized_weights)
```

```
# Verify they sum to 1
```

```
sum(optimized_weights)
```