

Race Against Time: Circadian Rhythm in Fast- and Slow-Aging Fish

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Introduction

- **Circadian rhythms** regulate key physiological processes like sleep-wake cycles, metabolism, hormone production, and immunity (Bell-Pedersen et al., 2005).
- Core **clock genes** drive these rhythms through 24-hour oscillatory expression patterns and are highly conserved among vertebrates (Bell-Pedersen et al., 2005).
- **Aging** weakens circadian regulation, disrupting clock gene expression and contributing to diseases such as metabolic disorders and neurodegeneration (Hood & Amir, 2017).
- We investigate this using:
 - **Zebrafish** (*Danio rerio*): long-lived, gradual aging over 3–5 years (Zhdanova et al., 2007).
 - **Turquoise killifish** (*Nothobranchius furzeri*): short-lived, rapid aging over 4–12 months (Lee et al., 2020).

- **Gap:** It's unclear how aging affects circadian gene expression across tissues and how conserved these changes are across species.

Table 1: Crucial genes responsible for circadian rhythm regulation in fish, conserved in humans (Reppert & Weaver, 2002)

Gene	Function
BMAL	Transcription factor, the only clock gene without which the circadian clock fails
CLOCK	Circadian locomotor output cycles kaput, affecting the persistence and period of circadian rhythms
CRY	Act as negative regulators in a feedback loop that controls the expression of other clock genes
PER	Period gene, inhibitory element
RORA	Transcription factor, involved in the regulation of immune signaling pathways and immune cell development

Research questions & predictions

Research questions

- How do age-related changes in the expression of core circadian clock genes differ across tissues (liver, brain, skin) in zebrafish (*Danio rerio*) and turquoise killifish (*Nothobranchius furzeri*)?
- Are these changes conserved between species?

Hypotheses

- If core circadian clock genes undergo age-related expression changes that are evolutionarily conserved, then both zebrafish and killifish will exhibit similar directionality in these changes across comparable tissues, but the magnitude of these changes will differ due to species-specific aging rates.
- If tissue-specific circadian regulation influences gene expression, then the liver will exhibit the most pronounced age-related expression changes, followed by the brain, and then the skin.

Predictions

- Core circadian genes will show conserved age-related expression patterns, likely downregulation with age in both species.
- The magnitude of expression change will be greater in killifish than in zebrafish due to the faster aging trajectory of killifish.
- Liver and brain samples will exhibit stronger age-related differential expression than skin samples.

Methods

Data Collection

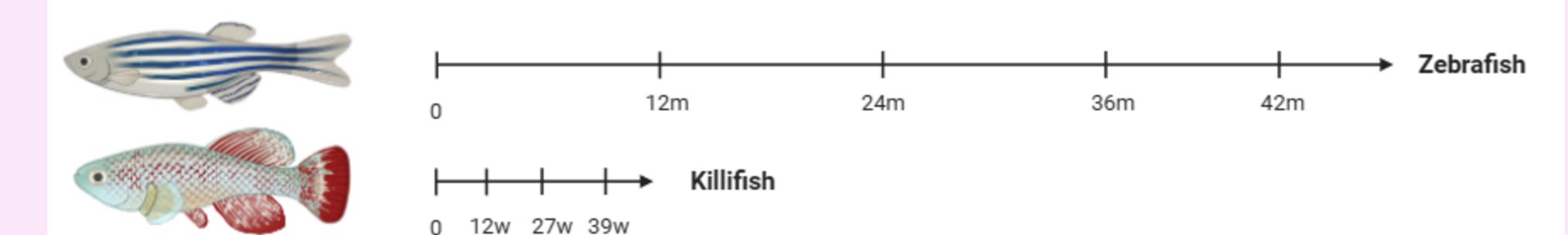


Figure 1: Schematic overview of timeline of sample collection from Zebrafish and Killifish.

Tissues

Genome Mapping

Liver, Brain, Skin

Zebrafish: Ensembl ID
Killifish: NFU Gene ID

Data Analysis in R

Zebrafish: n = 60; 26890 genes
Killifish: n = 57; 31953 genes

Preprocessing

1. Gene IDs mapped to Gene Codes
2. Count Matrix and Metadata Table
3. Normalize Ages

Count Matrix: 16417 genes

1. Gene counts < 10 filtered (n = 1)
2. DEA run with package DESeq2

Differential Expression Analysis (DEA)

1. Log2 Fold Change extracted from DEA
2. Pearson's & Spearman's correlation tests

Statistical Tests

Packages Used: dplyr, tidyverse

Principal Component Analysis (PCA)

Packages Used: dplyr, ggplot2

Results

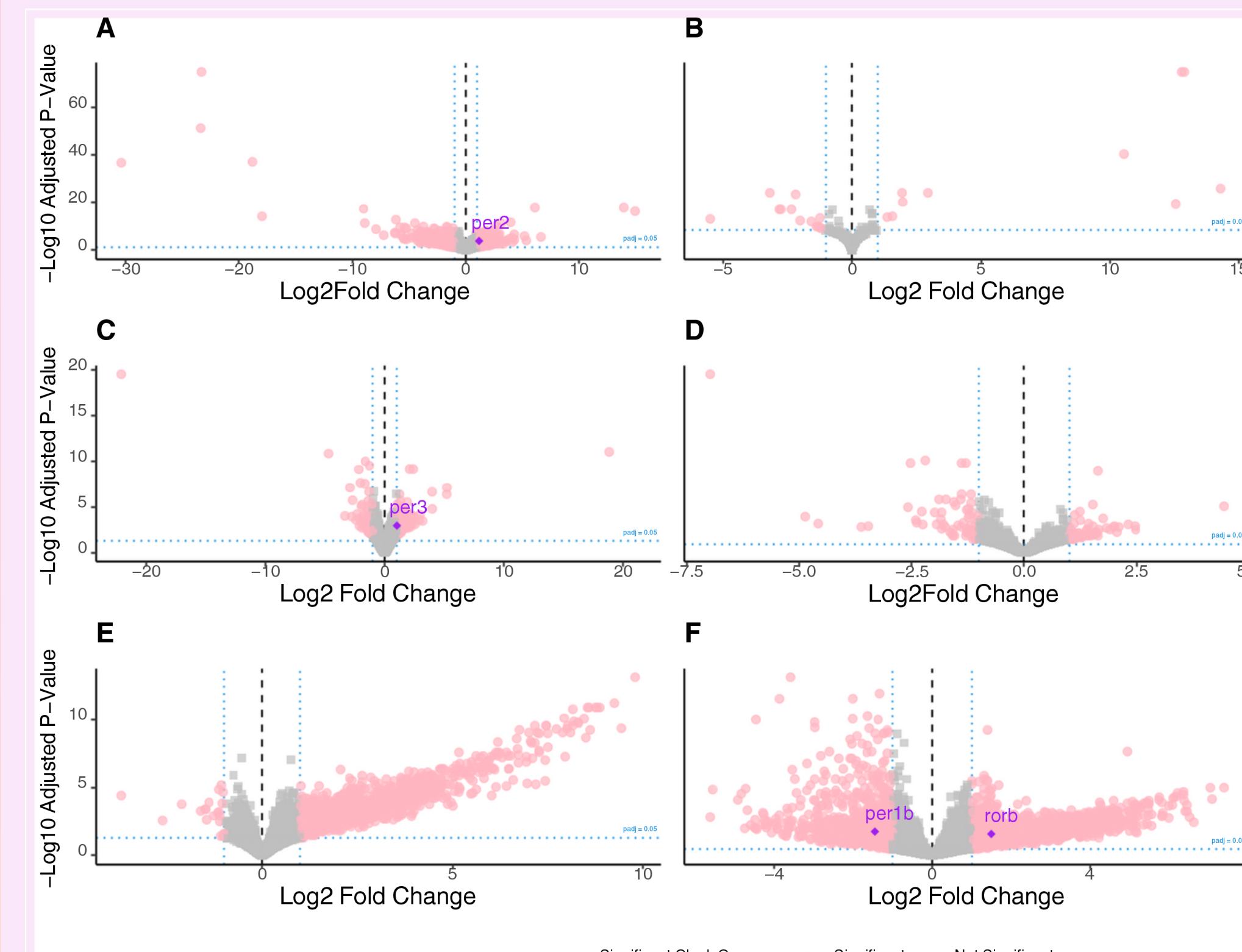


Figure 2: Volcano plots showing differential expression analysis (DEA) with Log2 Fold Change (L2FC) plotted against adjusted p-values for each gene (n = 16416 genes). Significant clock genes are labelled. L2FC < 0 indicates gene is upregulated in young fish samples. L2FC > 0 indicates gene is upregulated in old fish samples. Significance determined at padj < 0.05 and Log2FC > |1|. **A.** Zebrafish liver (n = 20 samples). Per2 is upregulated in old fish. **B.** Zebrafish brain (n = 20 samples). **C.** Zebrafish skin (n = 20 samples). Per3 is upregulated in old fish. **D.** Killifish liver (n = 15 samples). **E.** Killifish brain (n = 20 samples). **F.** Killifish skin (n = 21 samples). Per1b is upregulated in young killifish and rorb is upregulated in old killifish.

Table 2: Correlation of aging related clock gene (n = 8) expression changes between zebrafish and killifish in liver, brain and skin. Correlation coefficients are negative, suggesting no conservation, p-values are not significant.

Tissue	Correlation Coefficient (Rho)	p-value
Liver	-0.120	0.778
Brain	-0.214	0.619
Skin	-0.566	0.144

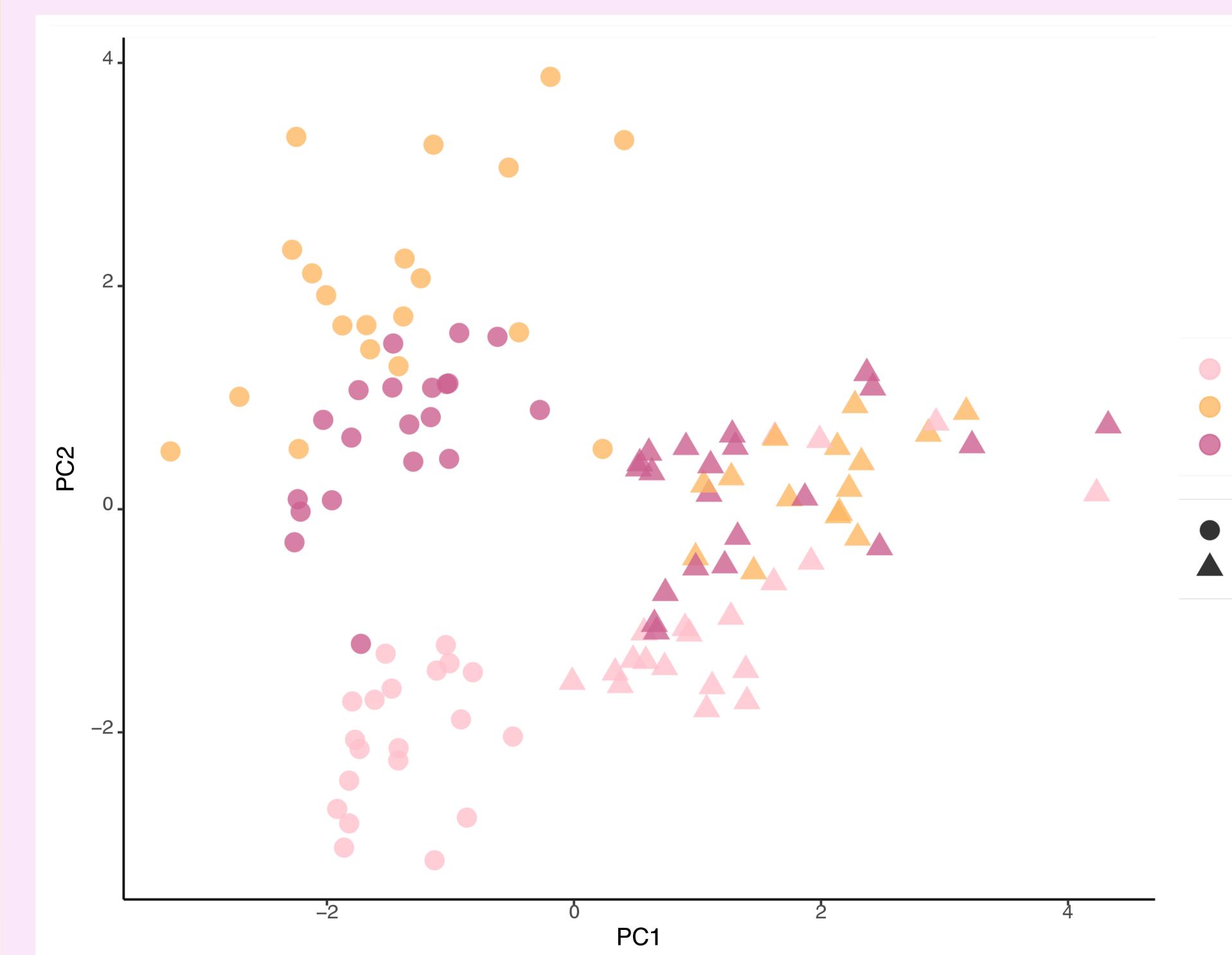


Figure 3: Principal component analysis (PCA) of variance-stabilized expression of core circadian clock genes (n = 8). dre = zebrafish, nfu = killifish. Clustering is seen within species. Clock gene expression is not conserved by species. Zebrafish are also clustering according to tissue.

Key findings

- Liver shows the biggest changes in gene expression with age (Fig 2A, D.). Liver plays a crucial role in processes tightly controlled by circadian rhythms, like **metabolism**, explaining this finding (Hood & Amir, 2017).
- Killifish has broader transcriptional shifts overall, consistent with its faster aging (Fig. 2D,E,F) (Hood & Amir, 2017).
- Among the core circadian rhythm genes, **per** and **rora** show statistically significant changes in transcription with age. However, these changes are not consistent across species or tissue types (Fig. 2A,C,F). This indicates the changes are **not conserved**.
- Figure 3 triangles (killifish) and circles (zebrafish) form distinct clusters along PC1, suggesting:
 - **species-specific** clock gene expression patterns
 - **tissue-specific** clock gene expression patterns

Research Question Results

- Both fast- and slow- aging fish show age-related tissue specific gene expression patterns in core circadian rhythm genes
- Ageing-related changes are not conserved between species however the magnitude of changes is bigger in faster aging killifish.

Limitations & future directions

Limitations

- We don't know what time of day each sample was collected or if they were collected at the same time.
- Limited sample size.
- Limited representative species.

Future directions

- Analysis of the genes affected by circadian rhythms that are not core clock genes.
- Focusing on changes in transcription in *per* gene group.

References

- Bell-Pedersen, D., Cassone, V. M., Ernest, D. J., Golden, S. S., Hardin, P. E., Thomas, T. L., & Zoran, M. J. (2005). Circadian rhythms from multiple oscillators: lessons from diverse organisms. *Nature Reviews Genetics*, 6(7), 544–556. <https://doi.org/10.1038/nrg1633>
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- Lee, S., Nam, H. G., & Kim, Y. (2020). The core circadian component, Bmal1, is maintained in the pineal gland of old killifish brain. *iScience*, 24(1), 101905. <https://doi.org/10.1016/j.isci.2020.101905>
- Reppert, S. M., & Weaver, D. R. (2002). Coordination of circadian timing in mammals. *Nature*, 418(6901), 935–941. <https://doi.org/10.1038/nature00965>
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