Nile Tilapia: Retina Gene Expression

Group 2 Oophaga: Expression between Developmental Stages

01

Introduction

Background, Research Question

Background: Nile Tilapia Developmental Shift in Photokinesis

I. Nile Tilapia exhibit phototaxis behavior in the form of kinesis

II. Developmental switch in phototaxis

   A. 12 days post fertilization (dpf): Increased kinesis in light

   B. 17 dpf: Increased kinesis in darkness

III. Underlying genetic basis of shift?

   A. Differential retina gene expression between developmental stages/age

IV. Data Collected

   A. Our samples include fish offspring from one father and 2 mothers—one with red (R) and one with blue (B) markings.

   B. 3′Tag-seq sequencing data from RNA extracted from 12 dpf and 17 dpf fish samples

How do expression values change across developmental stages?

1. How does retina gene expression change between 12 days post fertilization (dph) and 17 days post fertilization (dph)?

1. Do maternal factors play a role in retina gene expression?

Motivation: By studying how retina gene expression for phototaxis varies, we can gain insight into the development of visual systems for aquatic organisms and other species that exhibit phototaxis behavior.

Analysis Method

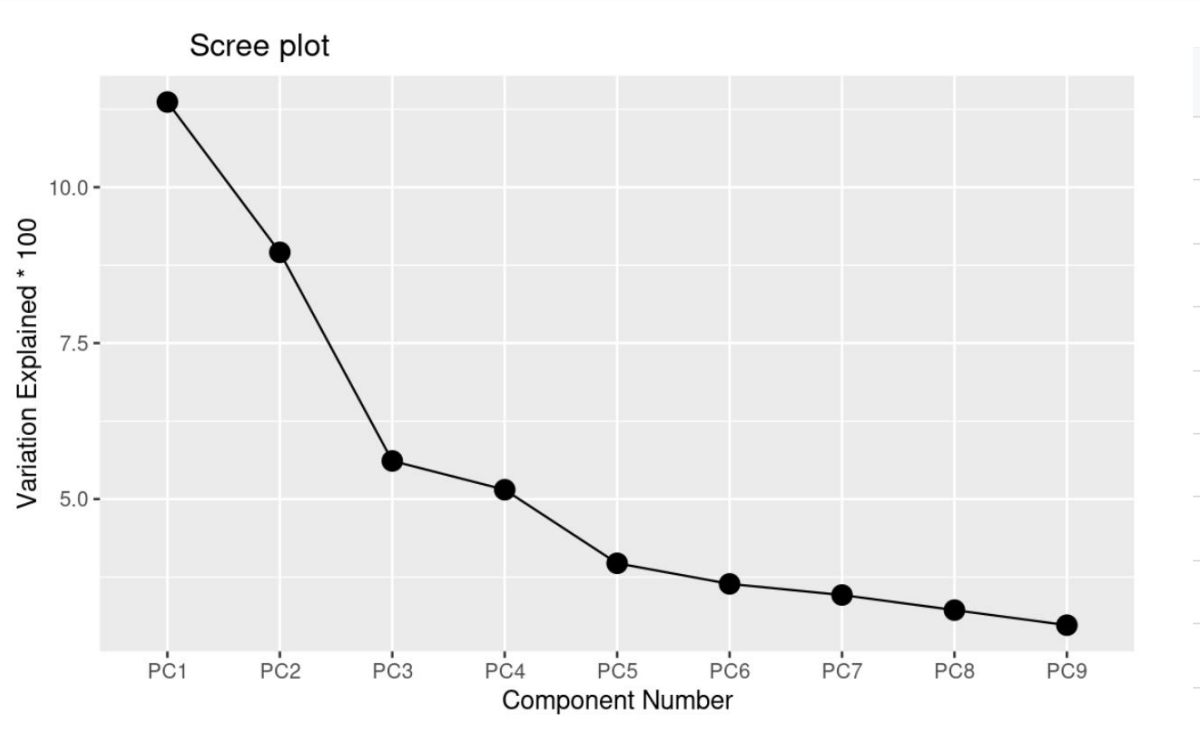
1. Clean datasets for Analysis
2. Normalized raw retina read counts using TPM
    a. Log normalization, removing 0’s & infinite values
3. Ran and Visualize PCA
4. Explore sources of variation
    a. ANOVA
    b. Further PCA
    c. DEseq2

02

Part 1:

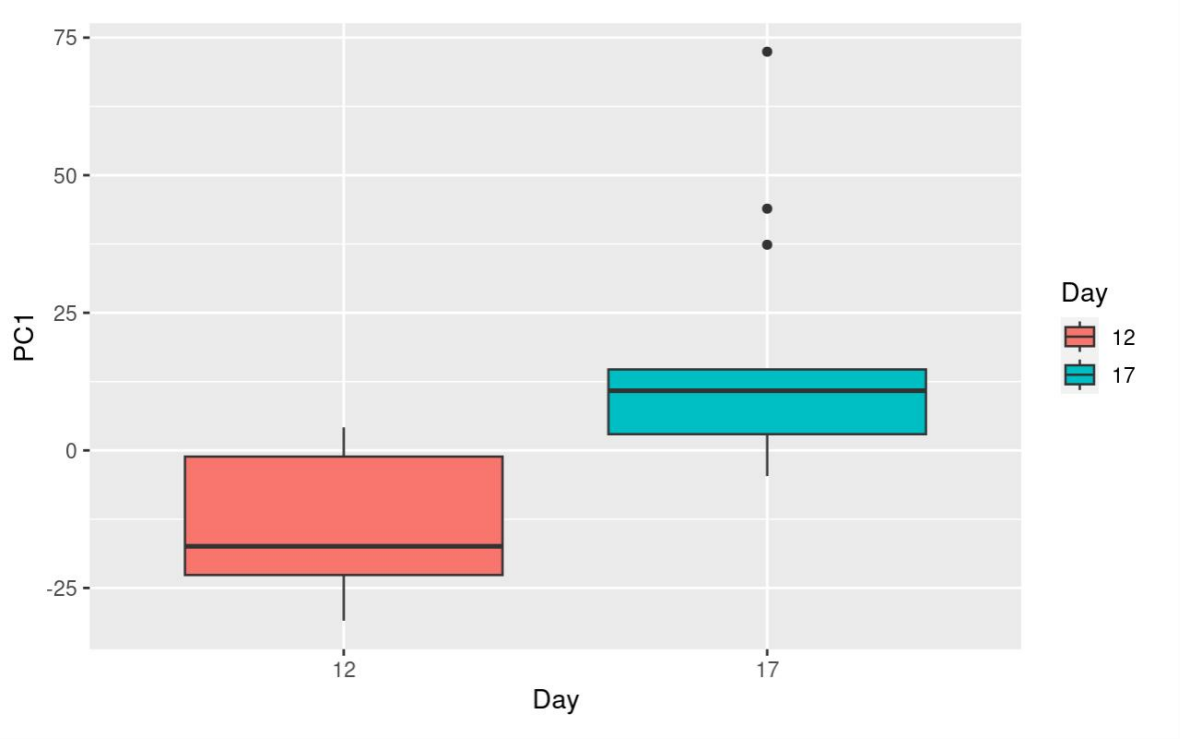
How does retina gene expression change between 12 days post fertilization (dph) and 17 days post fertilization (dph)?

Phototaxis Variation Explained by PCA



PC	var_explained
PC1	0.11365235
PC2	0.08955472
PC3	0.05611321
PC4	0.05149670
PC5	0.03970535
PC6	0.03640119
PC7	0.03461753
PC8	0.03219968
PC9	0.02978300

PC1 Score Boxplot by Day



Feature Chosen by PC1:

- Day accounts for **~11%** of the total variation of the samples

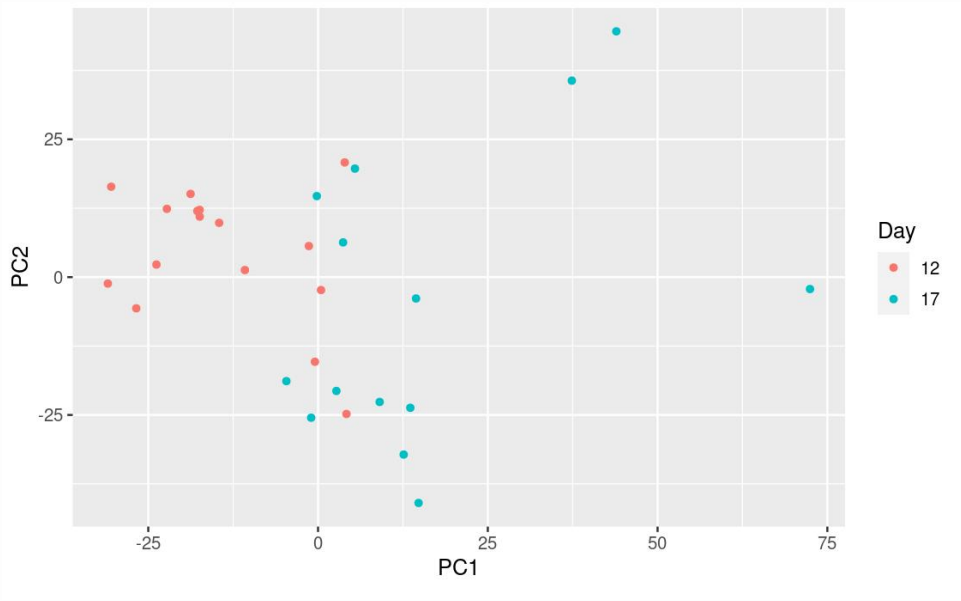
Analysis

- More Variation in Day 12 than 17
- More Outliers in Day 17

Further Questions

- Why does the day account for such a small amount of the variation?

PC	var_explained
PC8	0.03219968
PC7	0.03461753
PC6	0.03640119
PC5	0.03970535
PC4	0.05149670
PC3	0.05611321
PC2	0.08955472
PC1	0.11365235

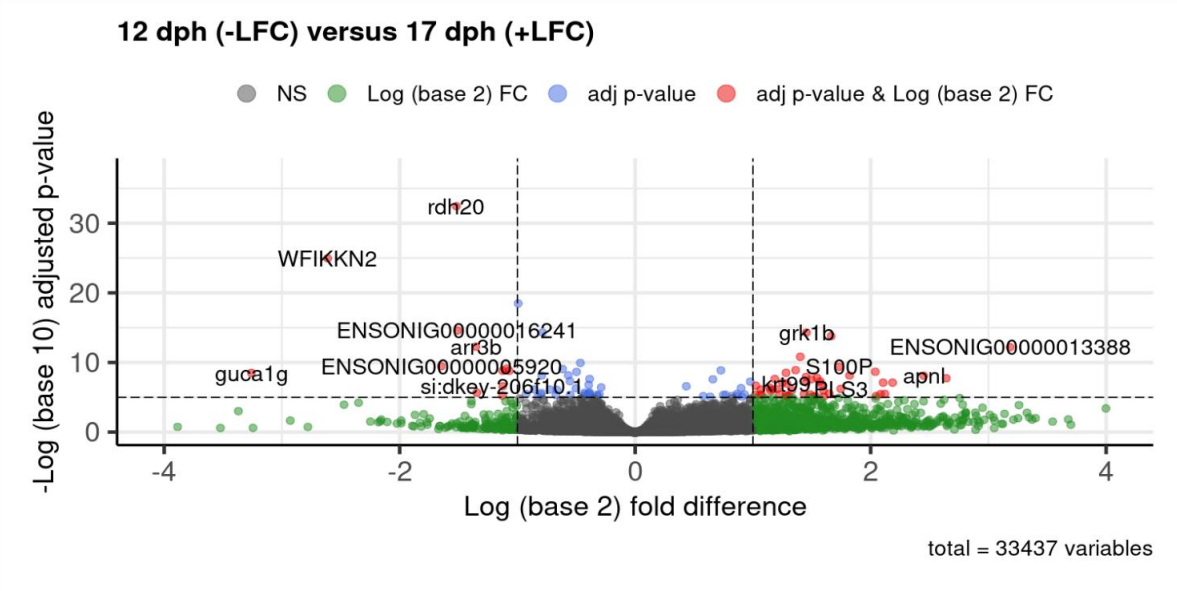


Significance: Day17>Day12

DeSeq by Stage for 17/12:

```
out of 24208 with nonzero total read count
adjusted p-value < 0.1
LFC > 0 (up)        : 1546, 6.4%
LFC < 0 (down)      : 973, 4%
outliers [1]        : 0, 0%
low counts [2]      : 7359, 30%
(mean count < 1)
[1] see 'cooksCutoff' argument of ?results
[2] see 'independentFiltering' argument of
```

Day 17 more differentially expressed than Day 12



Reason Guess:

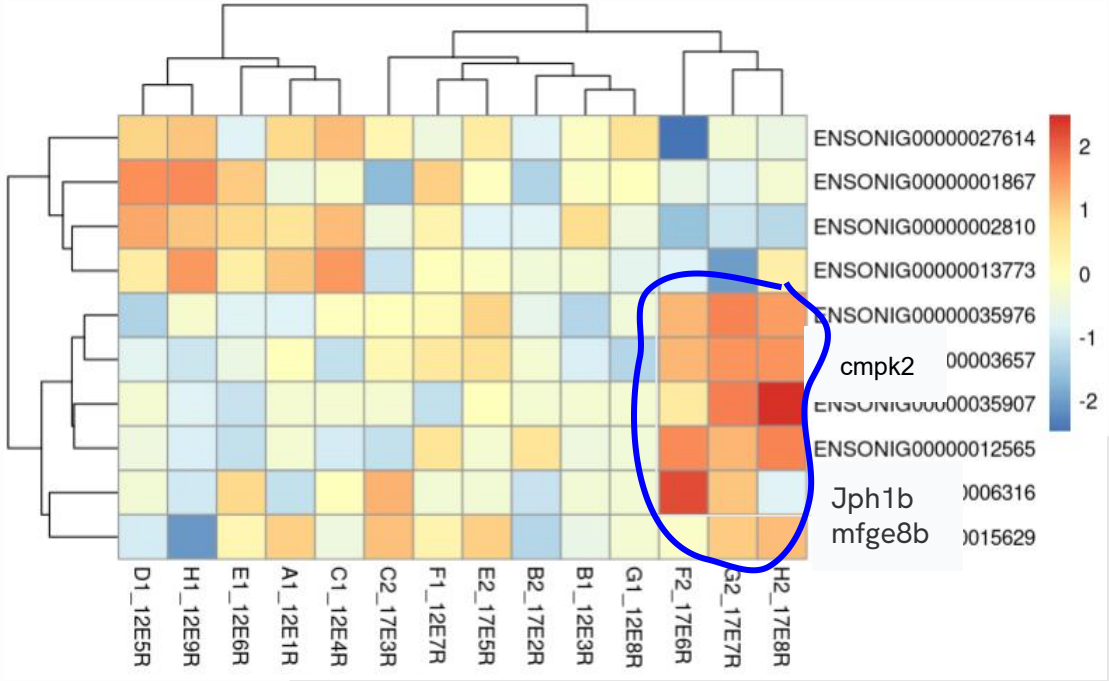
- Biological processes more actively
- Certain cellular processes responses might undergo significant changes later

Significant genes

Rdh20: protein_coding_gene
Taxon: Danio rerio(tropical fish)

WFIKKN2: human protein
Taxon: Pan troglodytes

Day 17 more significant than Day 12

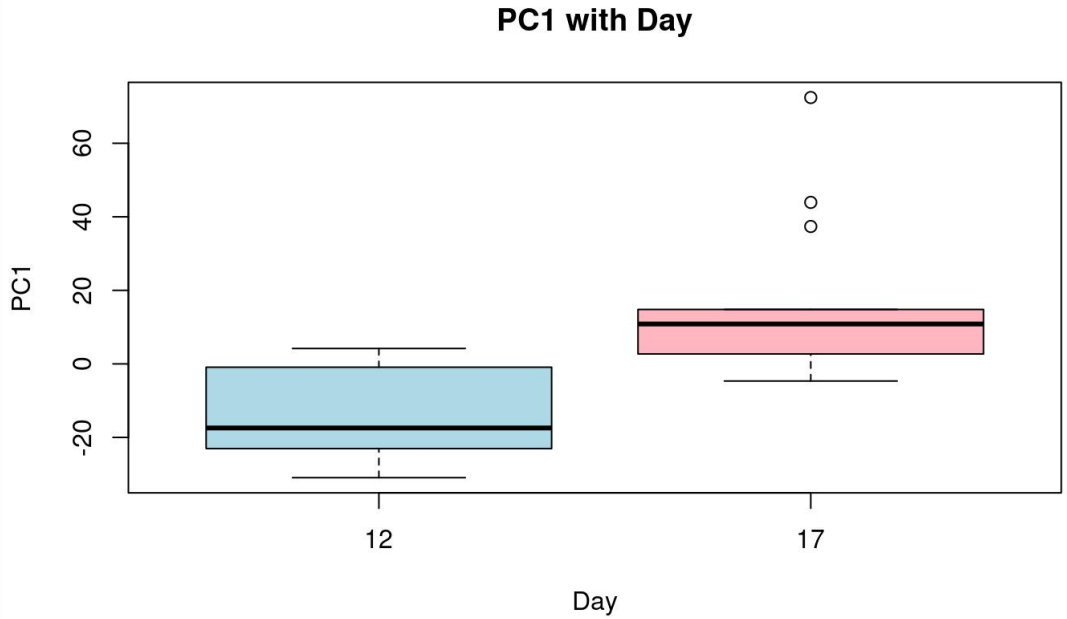


Analysis:

- Patterns and Clusters
  - Taxon: Danio rerio (jph1b&mfge8b)
  - Pan troglodytes (cmpk2)
- Intensity of Color

Prediction:

- regulatory mechanisms?
- environmental cues?
- cellular responses?
- Sample distribution?

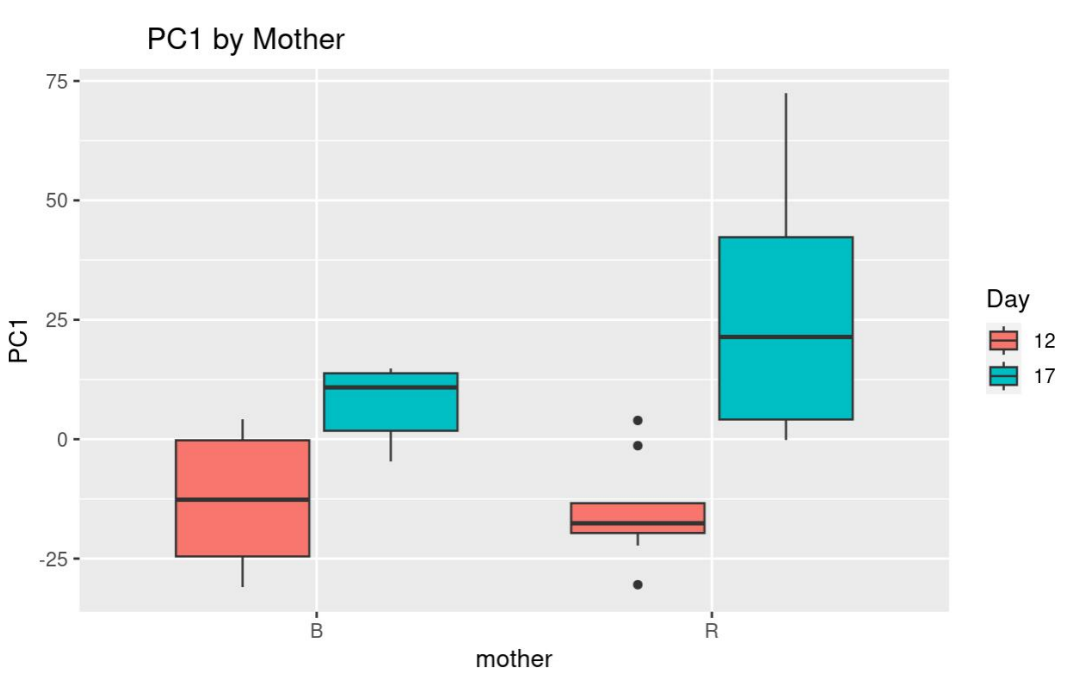

PC1 with Day
PC1
-20
0
20
40
60
12
17
Day

03

Part 2:

Do maternal factors play a role in retina gene expression?

PC1 Score Boxplot by Mother



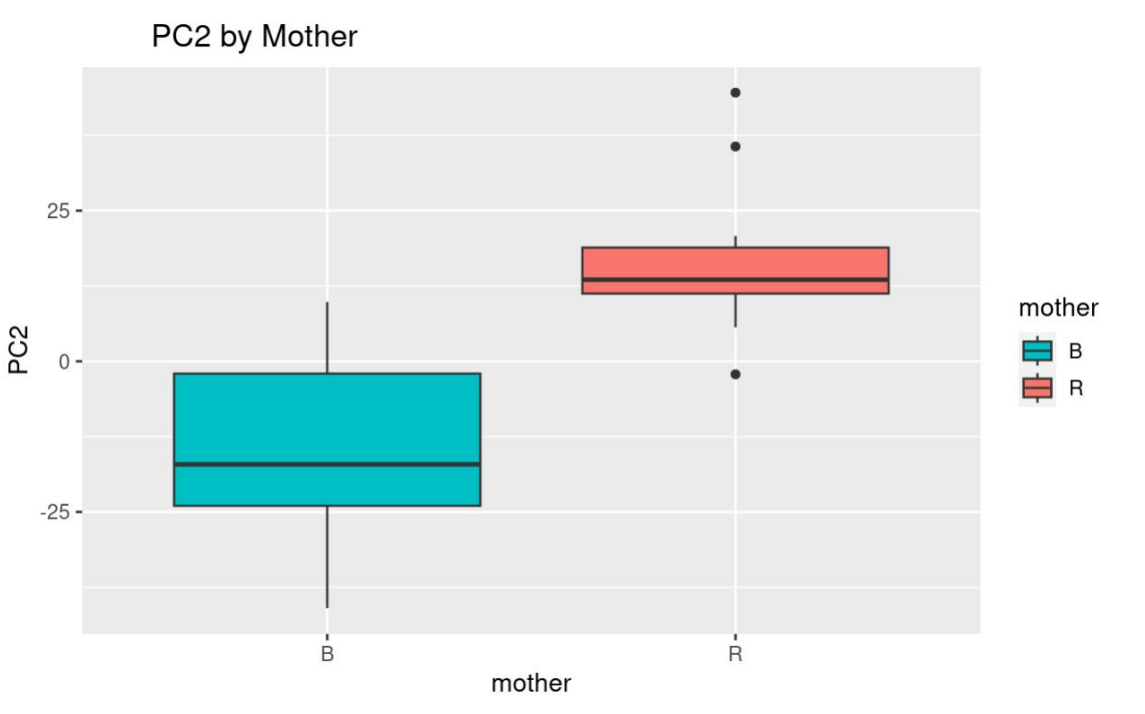
Analysis

- For both mothers, fish in 12 dph have a lower score than fish in 17 dph.
- **Variation differences in PC1 scores**
  - Significant variation when comparing days to respective mothers

Further Questions

- Why is there variation between the PC1 scores for the dph, depending on the mother?

PC2 Score Boxplot by Mother



Feature Chosen by PC2

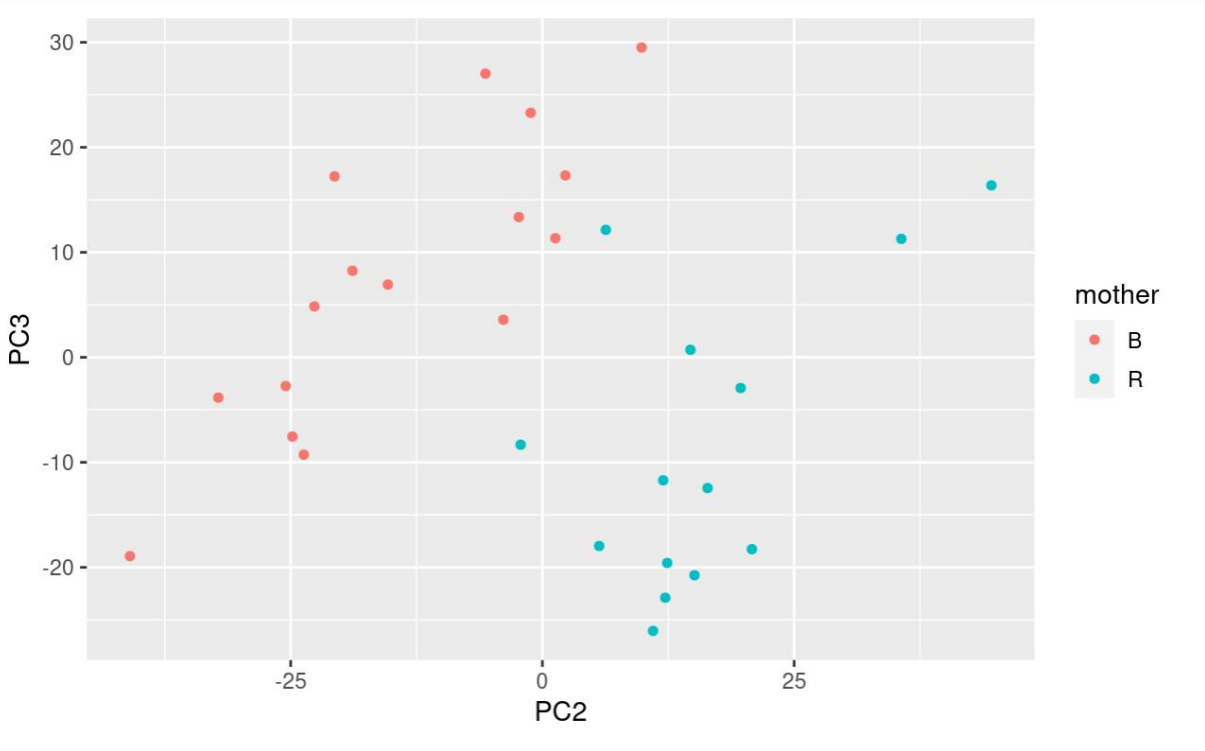
- The mother accounts for ~9% of the total variation

Analysis

- More Variation in the children of B than R
- More Outliers in mother R
- PCA Results after filtering by mother

Further Questions

- Differences in genes expressed?


30
20
10
0
-10
-20
PC3
-25
0
25
PC2
mother
B
R

Our Findings

- Identified genetic differences in Nile Tilapia based off of days post fertilization account for a significant amount of variance, with increase and less varied expression in 17 dph.
- More overall variation in offspring of Mother B.
- GO annotations to cross evaluate with highly annotated organisms
  - Identify specific molecular functional differences
  - Overlay on heatmap for better visualization

Potential Implications and Final Thoughts

- Aligns with exploration of how visual adaptations allow for mobile aquatic organisms to thrive.
- Initial concern over low counts
  - Accounted for by p value difference in DESeq versus volcano plot
  - Still significant in broader picture
- Exploring more questions
  - Why was there so much variation between PC1 & PC2 compared to rest of values?
  - Relationship between Danio rerio & Pan troglodytes & Nile Tilapia
  - How do these functions relate to the observed expression patterns and potential interactions with other genes?

Thank you!
Questions?