# Multiomics Analysis Reveals the Relationship between Muscle Cell Area and Weight in Rainbow Trout

Akash Raghu, Guglielmo Raymo, Ridwan Ahmed, Youssef Ali, Mohamed Salem

Department of Animal and Avian Science University of Maryland, College Park, MD

## Abstract

Muscle yield in Rainbow Trout is an important and marketable trait. Cell size and count have been proven to influence body size, a process regulated in party by genetic variants linked to growth and nutritional factors. **We hypothesized that phenotypic divergence between USDA's National Center for Cool and Cold Water Aquaculture (NCCCWA) selectively bred high and low fillet yield genetic lines can be explained in part by increased muscle cell size and count.** Our investigation found that there was a significant correlation between both cellular filled area and several economically relevant traits, including body weight, muscle weight, visceral weight and body length. A multi-omics approach including transcriptome interrogation and genome wide association study allows us to assess gene expression signatures, and single nucleotide polymorphisms, (SNPs), explaining phenotypic variability.

Transcriptome interrogation revealed associations of autophagic and catabolic mechanisms to increased cell size. Homeostatic and developmental pathways were also noted as upregulated in high filled area individuals. In contrast, muscle contractile pathways were noted as downregulated, a phenomenon that may be explained by the interplay of environmental conditions.

A total of 728 SNPs 728 surpassed our stringent cutoff for significance when performing genome wide association analysis. The majority of these SNPs cluster on chromosome 2 (n=713) and overlap with notable muscle regulatory genes such as ribosomal RNA processing 15 homolog, Cornichon homolog 4 and fasciculation and elongation protein zeta-2.

This work sheds light on the complex genetic architecture underpinning muscle mass accretion in genetically enhanced rainbow trout mediated by enhanced contractile machinery and metabolic support mechanisms

## Introduction

The growth of skeletal muscle is discussed heavily in literature. Skeletal muscle growth is a commercially relevant trait and a key phenotype of economic interest in aquaculture because it directly affects fillet yield and overall meat production, which are critical for profitability. Enhanced muscle growth leads to more efficient feed conversion and higher market value per fish, making it a primary target in selective breeding programs (Ali et al., 2018). Selective breeding programs conducted by the USDA's NCCCWA have been improved for years to increase muscle yield in rainbow trout populations.

Our novel approach was designed to leverage microscopy for detailed visualization of muscle samples, while also being able to connect these results with general phenotypic characteristics (i.e. weight, length). The overall process plays a critical role by allowing us to observe the composition of muscle tissue at the microscopic level, and categorize muscle samples into high and low phenotypes based on both cellular structure and the outward phenotypic composition of the fish. This strategy can be altered to fit other studies, such as connecting meat quality or taste to microscopic characteristics of muscle samples. This novel phenotyping strategy is now being used to categorize chicken samples, and has been seen in bovine research in the past (Coles et al., 2015).

## Objectives

1) **Histological Phenotyping:** Quantify skeletal muscle cell count and filled area in rainbow trout to determine their correlation with economically important traits such as body weight, muscle mass, and length.
2) **Transcriptomic Analysis (RNA Sequencing):** Identify differentially expressed genes between high and low muscle yield phenotypes to uncover biological pathways (e.g., autophagy, contractility, metabolism) associated with muscle hypertrophy.
3) **Genome-Wide Association Study (GWAS):** Map significant SNPs linked to muscle cell filled area, highlighting genetic loci that may underlie variation in muscle growth for selective breeding improvement.

## Methods

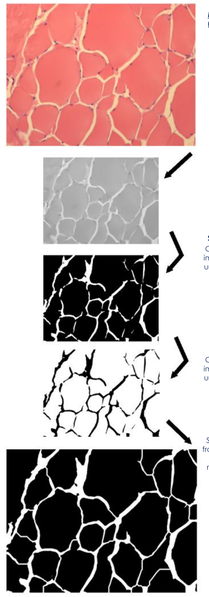


***Figure 1.*** *Description of MIPAR Image Processing (v4.3.0) workflow recipe.*

**1) Histological Phenotyping**
White muscle tissue from 86 rainbow trout was collected, fixed in Bouin's solution, and embedded in paraffin. Sections were cut at 200 µm intervals for analysis.

**2) Image Acquisition & Statistical Analysis**
Slides were imaged at 20× magnification (3 images per slide, 2 slides per fish). MIPAR v4.3.0 software was used to quantify muscle cell count and filled area. Cells <1700 px² were excluded. Mean values per fish were compared to phenotypic traits (e.g., weight, length) using two-way Student's t-tests.

**3) RNA Extraction**
Muscle samples from 8 fish (high and low phenotype) were flash-frozen and stored at –80°C. RNA was extracted using RNAzol manufacturer protocol and assessed for quality using NanoDrop and Agilent ScreenTape. Only samples with RIN ≥ 7 were used.

**4) Library Preparation & Sequencing**
RNA was processed using the xGen RNA kit with polyA selection. Libraries underwent fragmentation, cDNA synthesis, adapter ligation, and PCR amplification. Sequencing was performed on an Illumina NovaSeq S4 (paired-end, 150 bp).

**5) Differential Gene Expression Analyses**
Reads were quality-filtered and mapped to the Arlee trout genome. DEGs were identified using DESeq2 v1.49.2 (padj < 0.05) with sequencing batch correction. KEGG and GO enrichment analyses were performed using ShinyGO with FDR < 0.05.

**6) GWAS & SNP Annotation**
SNPs were identified using a proprietary pipeline. GWAS was performed in PLINK v1.9 using PCA covariates and multiple testing correction (FDR ≤ 0.05). Significant SNPs were annotated with SnpEff v5.2f and visualized in IGV aligned to chromatin state tracks from Salem et al., 2024.

## Histological Phenotyping Analysis

Histological phenotyping of muscle cell area and count correlated to fish characteristics including muscle weight or body weight can be instrumental in developing the next line of selectively bred rainbow trout by the USDA's National Center for Cool and Cold Water Aquaculture (NCCCWA). Data we have collected from histology samples of USDA provided fish have shown clear results in the relationship between the muscle cell density/size and fish characteristics. Transcriptome comparison between the areas of the population was conducted, using the upper and loweer quartiles of samples.

| Table 3a. | Filled Area | | Cell Count | |
|---|---|---|---|---|
| **Fish Characteristic** | **$R^2$** | **P-Value** | **$R^2$** | **P-Value** |
| **Body Weight** | 0.087 | 0.007* | 0.016 | 0.252 |
| **Muscle Weight** | 0.079 | 0.010* | 0.008 | 0.436 |
| **Visceral Weight** | 0.071 | 0.017* | 0.05 | 0.044* |
| **Muscle Yield** | 0.005 | 0.421 | 0.02 | 0.216 |
| **Body Length** | 0.078 | 0.012* | 0.003 | 0.668 |

**Statistically Significant*

***Table 1.*** *Student's T-test values comparing phenotypic characteristics with filled area data. Phenotypic characteristics of body weight, muscle weight, visceral weight, and body length were all found to have statistically significant (p<0.05) correlations to larger cell size.*

It was evident that further investigation was warranted to examine the underlying genotypic patterns that may shed light on why certain traits were correlated to the high cell filled area phenotype as seen above. RNA-sequencing was used to explore specific genes, either upregulated or downregulated in the high filled area population

***Table 2.*** *Noteworthy differentially expressed genes involved in high and low filled area fish.*

***Upregulated***

| Adjusted P-value | Annotation |
|---|---|
| 0.000655731 | myosin heavy chain, fast skeletal muscle-like |
| 0.032315216 | electron transfer flavoprotein regulatory factor 1 |
| 0.048667089 | CCR4-NOT transcription complex subunit 10 |
| 0.020683807 | PDZ and LIM domain 5a |
| 0.046493319 | regulator of cell cycle RGCC |
| 0.008896106 | NLR family CARD domain-containing protein 3-like |
| 0.007883539 | forkhead box C1-A |
| 1.06878-06 | Y+L amino acid transporter 2 |
| 0.020683807 | calcium-binding protein 2-like |

***Downregulated***

| Adjusted P-value | Annotation |
|---|---|
| 0.004101885 | microfibril-associated glycoprotein 4-like |
| 0.004551522 | lysozyme g |
| 0.042682566 | nascent polypeptide-associated complex subunit alpha, muscle-specific form |
| 0.000903427 | zymogen granule membrane protein 16 |
| 0.018601934 | Ig kappa chain C region, B allele-like |
| 0.004575407 | SH3 domain-containing kinase-binding protein 1 |
| 0.001893271 | interferon inducible protein |
| 0.003988274 | GTPase IMAP family member 7 |
| 0.03317847 | dispanin subfamily A member 2b |

## RNA-Seq Results

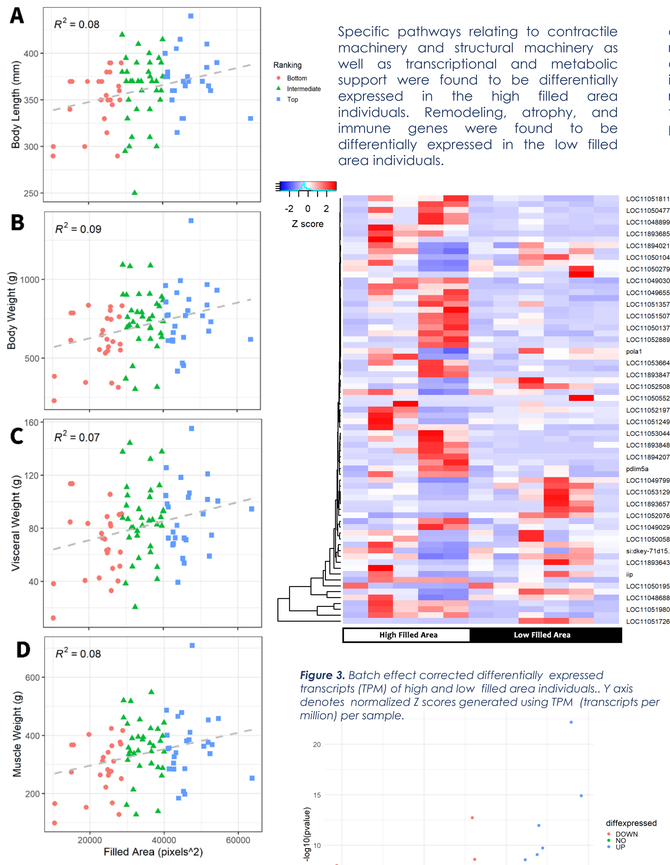


***Figure 2****. Spread of filled area groups across phenotypic characteristics shown on the Y-axis as follows; (A) Body Length in millimeters (B) Body Weight in grams (C) Visceral Weight in grams (D) Muscle Weight in grams. Highlighted data constituting the top and bottom 25% of filled area individuals show variability of cell size in relation to phenotypic characteristics. The X axis denotes filled area for each sample (px2).*

Specific pathways relating to contractile machinery and structural machinery as well as transcriptional and metabolic support were found to be differentially expressed in the high filled area individuals. Remodeling, atrophy, and immune genes were found to be differentially expressed in the low filled area individuals.




***Figure 3.*** *Batch effect corrected differentially expressed transcripts (TPM) of high and low filled area individuals.. Y axis denotes normalized Z scores generated using TPM (transcripts per million) per sample.*

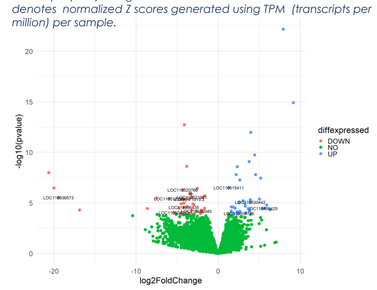


***Figure 4.*** *Volcano Plot for noteworthy differentially expressed genes involved in high and low filled area fish.*

***1) Enhanced Contractile and Structural Machinery in High-Filled Area Regions***

**Fast Myosin Heavy Chain (MHC):** Strongly upregulated, shifting fibers toward fast-twitch, high-ATPase Type II units and boosting sarcomere assembly for powerful contractions and hypertrophy. **PDZ-LIM Domain 5 (PDLIM5):** Up in high-yield lines, acting as a scaffold that links signaling proteins to the actin cytoskeleton, promoting myogenesis, satellite-cell proliferation (via p38-MAPK), and structural integrity. **Calcium-Binding Protein 2 (CABP2):** Elevated to fine-tune calcium homeostasis and signaling (via calmodulin kinase II and calcineurin), optimizing excitation-contraction coupling.

***2) Transcriptional and Metabolic Support for Growth***

**Forkhead Box C1-A (FOXC1a):** Upregulated, implicating enhanced somite development and myoblast proliferation through Notch/Fgf pathways and circRNA–miRNA regulatory networks. **Electron Transfer Flavoprotein Regulator 1 (ETFR1):** Increased expression to bolster mitochondrial electron transport and ATP supply, underpinning energy-intensive hypertrophic processes. **CCR4-NOT Subunit 10:** Up in high-filled fish, suggesting accelerated mRNA turnover and translation efficiency to favor net protein synthesis. **Y+L Amino Acid Transporter 2 (SLC7A6):** Elevated to enhance uptake of cationic/neutral amino acids (e.g., L-arginine for nitric oxide and L-leucine for mTOR activation), supporting nutrient sensing and muscle anabolism.

***3) Markers of Remodeling and Atrophy in Low-Filled Area Regions***

**Microfibril-Associated Glycoprotein 1 (MAGP-1):** Suppressed in high-filled fish (i.e. relatively higher in low-filled), consistent with looser extracellular scaffolding and reduced muscle mass. **skNAC (Muscle-Specific NAC):** Paradoxically up in low-filled areas, indicating elevated turnover and regeneration (myogenic differentiation), as smaller fibers undergo more frequent repair. **Immune/Inflammatory Genes:** A suite of cytokines, lysozyme, Ig chains, etc., are elevated in low-filled tissue, pointing to chronic inflammation and stress responses that divert energy away from hypertrophy and may drive atrophy.

## GWAS Results

Interestingly, of all 728 SNPs, the vast majority of were found to be located on chromosome 2 (n=713) in a 103.8Mb window and overlap with notable muscle regulatory genes such as ribosomal RNA processing 15 homolog, Cornichon homolog 4 and fasciculation and elongation protein zeta-2 (table 3). Of those, 25 SNPs were found in coding exons,10 led to missense mutations, and 62 were located in the 3'-untranslated region (UTR) of certain transcripts. This is especially noteworthy since MicroRNAs (miRNAs) typically bind to the 3'UTR to regulate gene expression, often inhibiting translation or promoting mRNA degradation.

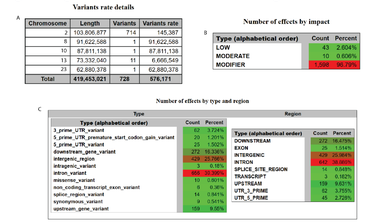


***Table 3.*** *SnpEff output tables showing variant rate details (a), number of effects by impact (b), and number of effects by type and region (c)*

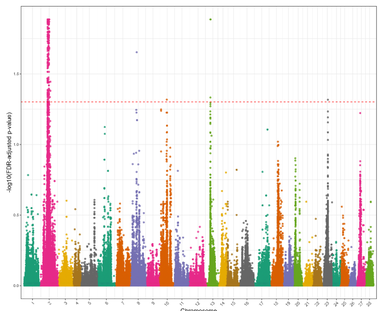


***Figure 5.*** *Manhattan plot depicting SNPs distributed across autosomes. Horizontal dashed line denotes statistical significance (FDR ≤ 0.05, -log10FDR > 1.3) when assessing filled area as a linear variable.*

Chip-seq resolved chromatin states produced by Salem et al., 2024 indicate tissue specific transcription of TGF2B, specifically this gene harboring 54 SNPs shows open chromatin only in white muscle.

***Figure 6.*** *Integrated Genome Viewer (IGV) with open chromatin states for muscle at Transforming Growth Factor Beta-2 (tgfb2)*

## Conclusions

Outcomes of this study indicate that changes in muscle cell size or filled area of rainbow trout have positive effects on body and muscle weight. Rainbow trout provided by the NCCWA have generations of selective breeding behind them, and understanding why certain families respond with higher muscle weight is important to the future of selective breeding programs. In high muscle weight fish, effects of myosin heavy chain, mRNA translation, and other pathways show increased levels of filled area of muscle, possibly leading to this increased muscle weight. On the other hand, low muscle weight fish show upregulation of immune and assembly pathways.

## Future Directions

1) Conduct functional studies to validate the role of candidate genes (e.g., myosin heavy chain, calcium-binding proteins, FOXC1) in muscle development.
2) Integrate cellular and transcriptomic markers to refine selective breeding strategies. Additional gene expression screening using qPCR, as well as sanger sequencing for chromosome 2 interesting region.
3) Investigate if noteworthy SNPs are present in other trout populations and phenotypes.

## References & Acknowledgements

Ali, A., Al-Tobasei, R., Kenney, B., Leeds, T. D., & Salem, M. (2018, August 14). *Integrated analysis of lncRNA and mrna expression in rainbow trout families showing variation in muscle growth and fillet quality traits*. Nature News. https://www.nature.com/articles/s41598-018-30655-8

Chapagain, P., Arivett, B., Cleveland, B. M., Walker, D. M., & Salem, M. (2019, October 29). *Analysis of the fecal microbiota of fast- and slow-growing rainbow trout (Oncorhynchus mykiss) - BMC genomics*. BioMed Central. https://bmcgenomics.biomedcentral.com/articles/10.1186/s12864-019-6175-2

Coles CA, Wadeson J, Leyton CP, Siddell JP, Greenwood PL, White JD, McDonagh MB. Proliferation rates of bovine primary muscle cells relate to liveweight and carcase weight in cattle. PLoS One. 2015 Apr 15;10(4):e0124468. doi: https://pmc.ncbi.nlm.nih.gov/articles/PMC4398453/

Mohamed Salem, Rafet Al-Tobasei, Ali Ali, Liqi An, Ying Wang, Xuechen Bai, Ye Bi, Huajun Zhou, Functional annotation of regulatory elements in rainbow trout uncovers roles of the epigenome in genetic selection and genome evolution, GigaScience, Volume 13, 2024, giae092, https://doi.org/10.1093/gigascience/giae092

**Special thanks to the Department of Animal and Avian Sciences, Biological Sciences Departmental Honors Program, and USDA's National Center for Cool and Cold Water Aquaculture**