

Developing critical knowledge of intestinal microbiota and mucosal immune system influence on early trout health



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Introduction

- A 16-year (8 generation) collaborative breeding program between Univ. of Idaho and USDA-ARS has developed a line of rainbow trout (CX), which thrive on a sustainable all plant protein diet and show superior growth and disease resistance
- The genetic and/or physiologic mechanisms responsible for the greater performance of the selected trout strain are still somewhat unknown
- Previous research by our group suggests the gut microbiota of the CX strain is different from other commercial lines (HC) of rainbow trout (Fig. 1)
- Preliminary microbiota results were obtained from market-size fish fed an all plant protein diet and may be confounded by the development of enteritis in the commercial strain

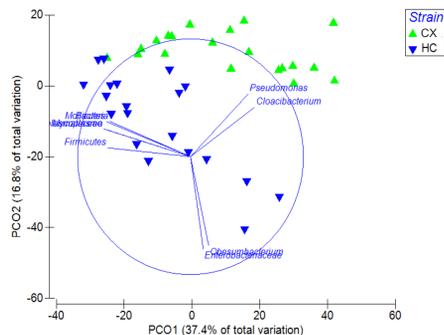


Figure 1. Principle components analysis of the gut microbiota communities present in our selected strain of rainbow trout (CX) and a commercial control strain (HC).

Objectives

- Compare the homeostatic intestinal microbiome (IM) and intestinal gene expression of the selected trout strain (CX) to that of a commercial reference strain (TL), during critical early life stages
- Compare overall survival, IM, intestinal gene expression, and peripheral immune performance of the select trout strain (CX) to that of a commercial reference strain (TL) during exposure to virulent virus or bacterium

Methods

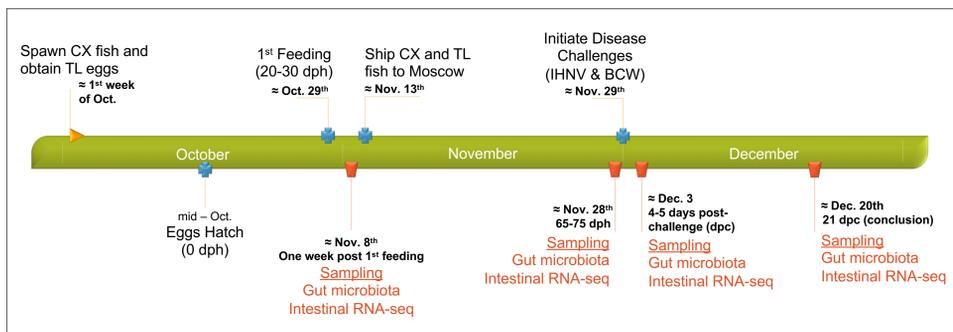
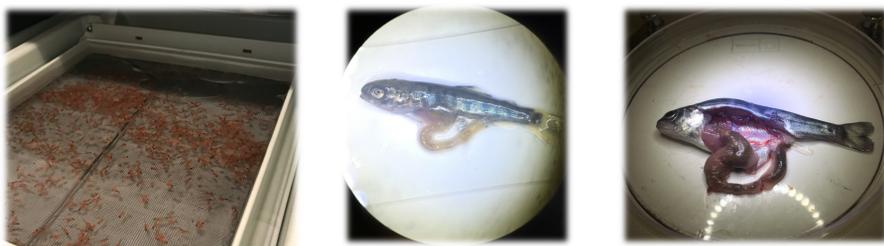


Figure 2. Timeline schematic of the study design.

- An all female cohort of our selected rainbow trout (CX) strain, as well as a common commercial strain (TL) were reared alongside one another starting from eggs, and later replicated across two rearing sites, to access the impact of host genetics while controlling for confounding variables (i.e. sex, diet, environment, age, pathology)
- Samples were collected at critical early life stages under homeostatic conditions:
 - 20 days post-hatch (dph) – initiation of exogenous feeding
 - 74 dph – critical period in survival and start of alternative diet formulations
- Bacterial (*F. psychrophilum* “Bacterial Coldwater Disease” CSF 259-93 – IM delivery) and viral (Infectious Hematopoietic Necrosis Virus 220-90 (IHNV) – static bath delivery) disease challenges were then conducted separately to assess disease susceptibility and the concurrent response of host (intestinal gene expression) and microbial ecology (gut microbiota) to infection
- Both strains of trout were challenged in triplicate tanks ($n = 45$) with a single mock control for each pathogen – 21 day challenge period
- Sampling: 0 days post-challenge (dpc), 4 dpc, and 21 dpc (survivors)



Results

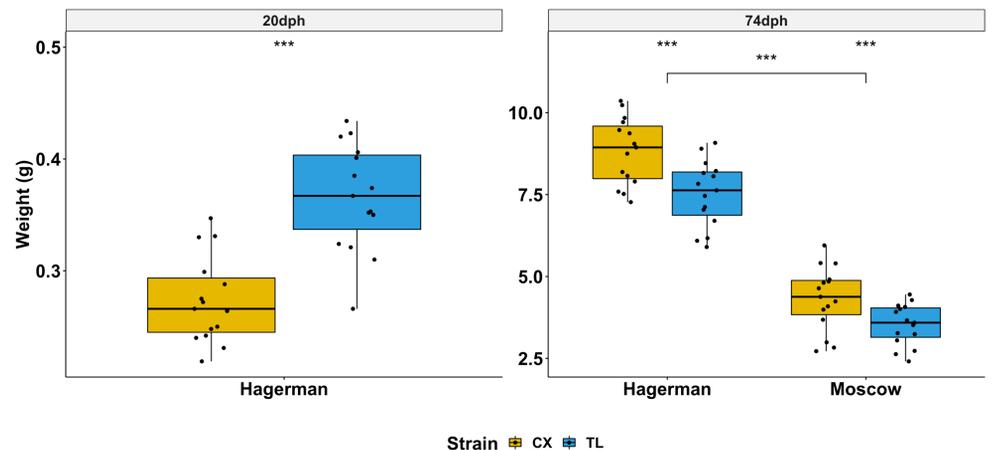


Figure 3. Fish weights by strain (CX and TL), timepoint (20 dph and 74 dph), and rearing location (Hagerman and Moscow). Fish weights at 20 dph were tested for significance by strain using a t-test ($p \leq 0.0001$). At 74 dph, fish weights were tested for significance using a two-way ANOVA, indicating a highly significant difference ($p \leq 0.0001$) by fish strain and rearing location, but no significant interaction effects (Strain*Location; $p = 0.296$).



Flavobacterium psychrophilum



Infectious Hematopoietic Necrosis Virus

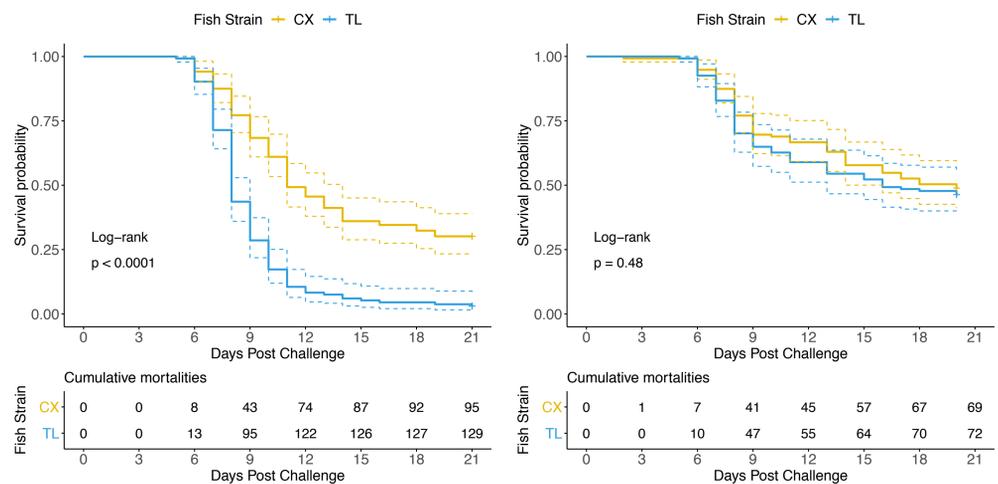


Figure 4. Kaplan-Meier curves (\pm 95% CI) resulting from bacterial (*F. psychrophilum* – Bacterial Coldwater Disease) and viral (IHNV) disease challenges conducted on our selected line of rainbow trout (CX) and a common commercial strain (TL). Photos above display mortalities exhibiting the classical disease phenotypes for both pathogens. Statistical significance ($p \leq 0.05$) was determined by log-rank test.

Ongoing Work

- Gut microbiome characterization and comparison (all timepoints)
 - 16S rRNA V3V4 gene amplicon libraries – Illumina MiSeq
- Gut transcriptomic characterization and comparison (all timepoints)
 - Stranded mRNA libraries $\geq 10M$ reads sample⁻¹ – Illumina NextSeq
- Serum lysozyme, complement, and pathogen specific titers (challenges only)
 - EnzCheck Lysozyme Assay (Molecular Probes Inc.)
 - Rabbit red blood cell lysis assay
 - ELISA

Expected Outcomes and Conclusions

The superior performance of our select strain of rainbow trout was confirmed in terms of growth and resistance to a common bacterial pathogen. Further work is underway to elucidate the mechanisms responsible for the observed phenotypes using molecular and traditional immunological assays.

- Evaluation of host-microbiome interactions in rainbow trout strains with disparity in growth performance and disease resistance
- Characterization and comparison of the effects of bacterial and/or viral pathogens on the gut microbiota and host gene expression profile