1. **Diet differentially influences physiology and gut microbiome**

Despite Zebrafish’s long-established importance as a model organism and their increasing use in microbiome targeted studies, key knowledge gaps remain about how diet influences their microbiome and body condition, a general measure of physiology. In contrast to mice, zebrafish do not have a standard reference diet. Differences in husbandry choices involving diet induce variation in study outcomes and challenge efforts to compare results across studies. Zebrafish fed different commercial and laboratory diets resulted in different body condition outcomes. Moreover, fish fed a high versus low-fat diet manifested distinct gut microbiome communities. However, what is not known is if zebrafish gut microbiome communities differ between commonly used laboratory diets. Here, we assessed whether different common laboratory diets influenced zebrafish’s gut microbiomes and physiology.

To assess the impact that diet has on physiology, we compared body condition scores between fish at 3 months post fertilization (mpf). Body condition score is a length normalized weight metric (for equation, see Methods). ZIRC fed fish are heavier and have higher body condition scores to both Gemma and Watts. Gemma and Watts fed fish did not differ from one another. As expected, female fish in each diet were heavier and had higher body condition scores to their male counterparts.   
  
Next, we asked if diet associated with gut microbiome structure. To assess microbiome diversity, we used generalized linear mixed effects models (GLMs) to identify if diet associated with variation in Simpson’s and Shannon Indices of diversity. We find that alpha diversity associated with diet. ZIRC fed fish had highest alpha diversity, followed by Gemma and Watts diets. Both indices found that ZIRC and Watts fish diversities differed significantly, but only in Simpson’s did ZIRC and Gemma differ significantly. Shannon diversity index was weakly significant. Watts and Gemma differed significantly in Shannon, but not Simpson’s indices. Moreover, we used the Bray-Curtis and Canberra dissimilarity metrics to compare pairs of microbial community composition. A PERMANOVA test using both metrics revealed that gut microbial communities between the diets were significantly different in their composition. Additionally, we measured the relative abundance of gut microbiome using ANCOM-BC. We found that 28 genera were significantly differentially abundant between at least two groups across the three diets. Chitinibacter was significantly abundant in Gemma.

We found that diet differentially influences physiology and the gut microbiome. Fish fed ZIRC diet are heavier and have higher body condition scores compared to the Watts and the Gemma diets. These results align with previous research investigating the effects of diet, physiology and the microbiome. In zebrafish, previous studies have found that different laboratory, commercial and experimental diets manifest inconsistent gut physiology, growth, health and reproductive outcomes (Leigh 2018, Fowler 2019). Leigh et al. found that in addition to nutritional composition, digestive enzyme activity played a role in shaping the physiological structure of the gut. Wong et al found a link between high-fat, high-protein diets and the microbiome diversity and composition. Unlike prior work, our study compared microbiomes of fish fed commonly used laboratory diets, which have more consistent nutritional profiles to those in previous studies interrogating the physiology, microbiome and diet. Therefore, the strong associations we see here between diet and the microbiome demonstrates that husbandry practices involving diet play a role in structuring the gut microbiome. Moreover, researchers using zebrafish as a model system should consider diet as a factor in their studies.

1. **Diet impacts the successional development of the zebrafish gut microbiome**

Zebrafish are developmentally considered adults by 3 months of age, but they continue to grow in weight and length. Additionally, zebrafish microbiomes continue to develop as they age. Prior to adulthood, zebrafish microbiome assembly is more susceptible to environmental influences of drift and dispersal, but with age these effects decline. During adulthood, zebrafish microbiomes continue to diversify, but their community compositions stabilize. To better understand the role of diet on the successional development of zebrafish, we compared body condition scores and gut microbiomes of 3 and 6 month old zebrafish.

We find that development plays a role in gut microbiome diversity and composition. Regardless of diet, gut microbiome diversity increases with time. In particular, ZIRC fed fish displayed increased diversity at 3 months of age compared to the other diets, as well as an increase in diversity between 3 months and 6. Watts fed fish diversity did not differ between time points. The microbial community composition varies over time, but the temporal sensitivity of the abundant taxa in the microbiome is less than the sensitivity of these taxa to different in. Rare microbiota, however, appear to vary more as a function of time than diet. These patterns occur regardless of the specific diet being considered.