Diet Infection Study – Section Summaries

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.

We found 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. ZIRC fed fish had higher microbiome diversity, indicating higher numbers of unique taxa. Diet also resulted in distinct microbial communities. Together, these results demonstrate that husbandry practices involving diet play a role in the structure of the gut microbiome, and suggest that 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 will 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 found that diet impacted the successional development of zebrafish gut microbiomes. We observed gut microbiome diversity increases and microbial community composition varies across time, and these observations differed by diet. Notably, 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 months. 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 diet. Rare microbiota, however, appear to vary more as a function of development than diet. These patterns occur regardless of the specific diet being considered. Time alone did not explain differences in body condition score, but ZIRC fed fish experienced an increase on body condition score. Interestingly, increased body condition score induced by ZIRC diet results in lower gut microbiome diversity. Furthermore, at 3 months ZIRC fed fish microbiome’s did not differ compositionally, but at 6 months they displayed distinct communities that stratified by high and low body condition scores. Together, these results build upon our previous results, and demonstrate that diet plays a continued role across the development of zebrafish physiology and gut microbiome.

1. **Diet influences gut microbiome’s sensitivity to pathogen exposure**

Zebrafish facilities are known to host many pathogens which can introduce non-protocol induced inconsistencies in study outcomes. One pathogen that is found in 40% of zebrafish facilities is *Mycobacterium chelonae*, and is hypothesized to be introduce through diet. *M. chelonae* causes gut inflammation in zebrafish. Previous work of ours has shown that pathogen exposure disrupted the gut microbiomes of zebrafish, but the joint effects of diet and pathogen exposure on zebrafish gut microbiomes and physiology remains unclear. Elucidating these relationships could offer microbiome-targeted treatments for preventing or minimizing the impacts of pathogen exposure on zebrafish health and study outcomes. Here, we exposed zebrafish fed different diets to *M. chelonae* to and measured the effects on zebrafish physiology and microbiome across their development at 3 and 6 months.

We found that the impact of *M. chelonae* exposure on the gut microbiome depended on diet. The gut microbiome diversity of ZIRC fed fish are uniquely sensitive to pathogen exposure, while Gemma and Watts fed fish are resistant to the effects of pathogen exposure. Post-exposure ZIRC fed fish had lower microbiome diversity, whereas microbiome diversity of fish fed Gemma and Watts diets were not different between exposure groups. However, the microbial community composition’s sensitivity to pathogen exposure is overwhelmed by the effects of diet. Microbiome compositions stratified into unique communities based on exposure type, these effects were dwarfed by the effects of diet. These results show that while pathogen exposure can impact the diversification of the gut microbiome across their development, diet plays a more influential role in the gut microbiome’s overall composition. Taken together, these results demonstrate that choice in husbandry practices involving diet can potentially mask or exacerbate the effects of pathogen exposure if not properly taken into consideration.