Zebrafish Diet and Infection 2020 – Brain dump

Title ideas

* **Common laboratory diets influence zebrafish gut microbiome’s successional development and sensitivity to pathogen exposure**
* Zebrafish fed different common laboratory diets experience differential developmental effects and sensitivity to pathogen exposure
* Adult zebrafish gut microbiome development and sensitivity to pathogen exposure differs across three common laboratory diets
* Gut microbiome development and its sensitivity to pathogen exposure differs across common laboratory diets in zebrafish
* Adult zebrafish fed different commonly used laboratory diets experience inconsistent microbiome and physiological outcomes across development and pathogen exposure
* The development and sensitivity of to pathogen exposure of zebrafish gut microbiomes differs across commonly used laboratory diets
* Gut microbiome development and sensitivity to pathogen exposure differs across fish fed different commonly used laboratory diets
* Common zebrafish laboratory diets differentially impacts their the gut microbiome’s development and sensitivity to pathogen exposure
* Zebrafish physiology and gut microbiome successional development differ across common laboratory diets and pathogen exposure
* Zebrafish gut microbiome’s sensitivity to pathogen exposure and successional development differs between common laboratory diets

Introduction

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. In contrast to mice, zebrafish do not have a standard reference diet. Differences in husbandry choices involving diet can induce variation in study outcomes and challenge efforts to compare results across studies. Zebrafish fed different experimental, commercial and laboratory diets result in different physiological and health outcomes (Watts, Leigh). Moreover, fish fed high-fat, high-protein diets manifested distinct gut microbiome communities. However, what is not known is if zebrafish gut microbiome communities differ between commonly used laboratory diets.

In contrast to diets used in previous studies investing the role of diet on zebrafish microbiome and physiology, the diets we used here differ minimally in nutritional content (Gemma, Watts and ZIRC, see Table # in supplementary material). Gemma is a commercial diet, the ZIRC diet is a mixture of commercial diets, and the Watts diet differs from these two in that it is a defined diet with known nutritional and ingredient compositions. Fish were fed the same, nursery diet up to 1 month old then fish were separated into one of three diet groups and fed a juvenile formulation for that diet until 4 months old, where they were transitioned to their respective adult diet formulations.

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 (Stephens2016). During adulthood, zebrafish microbiomes continue to diversify, but their community compositions stabilize.

Zebrafish facilities are known to host many pathogens which can introduce non-protocol induced inconsistencies in study outcomes (Kent). One pathogen that is found in 40% of zebrafish facilities is *Mycobacterium chelonae*, and is hypothesized to be introduce through diet (Stephens, Kent). *M. chelonae* causes gut inflammation in zebrafish (Kent). Previous work of ours has shown that pathogen exposure disrupted the gut microbiomes of zebrafish (Gaulke), 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.

Previous research in zebrafish and other organisms has linked microbiome diversity with obesity (citation). There is a link between diet and obesity, and diet and the microbiome. However, the link between diet and the microbiome is not fully understood. Some studies find that lower microbiome diversity, community composition and microbial metabolic function contribute to obesity (schwiertz2010, vallianou2019, oliphant2019). <studies in zebrafish> found <certain taxa> associated with higher weight and body condition score when fed a high-fat, high-protein diet

Here, we assessed whether different common laboratory diets influenced zebrafish’s gut microbiomes and physiology. We investigated the role of diet across zebrafish’s development, we compared body condition scores and gut microbiomes of 3 and 6 month old zebrafish. Finally, we exposed zebrafish to *M. chelonae* to see whether diet interacted with pathogen exposure to impact the gut microbiome. Our study clarifies how common laboratory diets differentially impact the microbiome over zebrafishs’ development, and these effects may play a crucial role in their gut microbiome’s sensitivity to pathogen exposure.

Discussion template

* Primary Finding
  + We found that…
  + What does the data mean?
  + Are the findings novel?
* Key Secondary Finding…
* Context
  + Give possible mechanisms or pathways
  + Compare your results with other people's results
  + Discuss how your findings support or challenge the paradigm
* Strengths and limitations
  + Anticipate readers questions/criticisms
  + Why results are robust
* What’s next?
  + Recommend confirmatory studies
  + Point out unanswered questions
* So what?
  + Implicate
  + Speculate
  + Recommend
* Strong conclusion
  + Restate main finding
  + Give final take-home message

Section 2

We found that the gut microbiome varies over time, but the temporal sensitivity of the abundant taxa in the microbiome is less than the sensitivity of these taxa to 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.

<Diets also differed in the amount of beta-dispersion observed within each diet across development. Variation of microbial communities changed most in abundant taxa in Gemma fed fish, while ZIRC communities variation in both abundant and rare taxa.>

We also found that gut microbiome diversity increased with development, but varied between diets. Prior work by Stephens and Burns saw similar increases in gut microbiome diversity across zebrafish development. Xiao et al 2021 investigated role environment plays on successional development in zebrafish gut microbiomes and found that succession was governed primarily by host developmental stages. Xiao and Stephens note in their studies that changes in diversity could be in part caused by changes in diet, which could explain why ZIRC fed fish had uniquely higher levels of diversity between 3 and 6 months. Of the three diets, the transition between juvenile to adult formulation is the least different in Gemma, followed by Watts and then ZIRC. Gemma adult diet is nutritionally the equivalent to the juvenile diet, but the feed size increases. Watts adult diet contains similar ingredients but has 9% less lipid content compared to the juvenile diet. ZIRC nutritional content varies and two additional diets are added to the ZIRC adult diet. In previous work investigating the link between gut microbiome structure to zebrafish development, husbandry changes related to diet impacted the ability of certain taxa to establish and thrive (Burns 2016). Another study observed a large increase in diversity following a dietary change (Xiao 2022). While the nutritional and ingredient changes may explain the increase in diversity of ZIRC fed fish, it does not fully explain why Gemma increased in diversity and Watts did not.

Moreover, we observed changes in abundance of taxa we have identified as core, keystone taxa in previous work (Sharpton). Prior work has shown that keystone taxa contribute to the stability of the gut microbiome (Xiao). Keystone taxa can also contribute to the health of their host via the gut through a variety functions, including digestion and nutrient absorption, protection from infection and communication with nervous, endocrine, and immune systems (citation). Stable states concept asserts that absent a major perturbation the gut microbiome structure remains consistent (Shaw 2019). Thus, if certain diets are disproportionately enriching for or against keystone taxa early in zebrafish development it could have long-term implications for health outcomes.

To see if the microbiome interacted with health of the zebrafish across development, we compared the body condition scores of fish fed different diets and time points. Body condition score is a generalized indicator of physiological health in fish. Unique to ZIRC fed fish, we found a link between body condition score and gut microbiome diversity. ZIRC fed fish saw a body condition score association with gut microbiome diversity, where body condition score decreased with higher microbiome diversity. Differences in taxa abundance could explain the decrease in body condition score in ZIRC fed fish between 3 and 6 months. Prior research found that keystone taxa are important to maintaining host health (Xiao). However, variation in body condition score was not explained by taxon abundance or community composition. Another possible explanation is ZIRC fed fish microbiomes with low diversity experience less competition for nutrients and habitat space, allowing them to digest nutrients more efficiently for the host. Finally, ZIRC fed fish experience a rather large change in feeds at 4 months, where they switch from juvenile to adult formulation. These new feeds could introduce new consortium of microbes and nutrient availability. Since we do not have samples of the diets from the time of the study, we cannot rule out the possibility of a feed microbiome effect confounding our results. More research is needed to clarify the ZIRC diet’s unique impact on zebrafish physiology and microbiome diversity.

Taken together, these results demonstrate that the diet-associated differences in the microbiome we see at 3 months persist across development at 6 months of age, and could have long-term implications for fish health and gut microbiome structure, and interpretations for microbiome-targeted research results.

<Limitations>

<sex>

We found a relationship between body condition score and alpha and beta diversity that suggests that body condition score and gut microbiome structure are linked to one another, but we can't exclude the possibility that sex plays a role in defining these differences in body condition score and alpha and beta diversity as well.

Section 3

We find that pathogen exposure inhibited diversification of gut microbiomes, and microbial community composition was driven primarily by diet rather than pathogen exposure. Unique to ZIRC fed fish, pathogen exposed fish were less diverse and unexposed fish were more diverse relative to pre-exposed fish. These results suggest that ZIRC fed fish are uniquely sensitive to pathogen exposure compared to Gemma and Watts diet. Interestingly, ZIRC fed fish had lower abundance of mycobacterium taxa in exposed fish compared to unexposed fish. This was also the case for Gemma fed fish. However, exposed Watts fed fish had the higher relative abundances of mycobacterium compared to unexposed controls, while Gemma and ZIRC saw decreased abundances relative to unexposed controls. One explanation for this result is the difference in gut microbial diversity between the diets. Prior work has shown higher gut microbiome diversity links to higher stability (Xiao). Watts fed fish had the lowest gut microbial diversity, which could be indicative of lower microbiome stability. Mycobacterium taxa might be uniquely situated to take advantage of the low stability to gain habitat space. This is supported by the fact that mycobacterium abundance was less abundant in exposed groups relative to the unexposed groups in the Gemma and ZIRC fed fish.

Moreover, a PERMANOVA analysis of dissimilarity of the gut microbiome community composition found that diet better explained the variance rather than pathogen exposure. In contrast to previous work investigating parasites impact on zebrafish gut microbiome, we saw a decrease in beta-dispersion of exposed groups (Gaulke2019). This suggests microbiome communities exposed to mycobacterium become more similar to one another. Could timing of infection be a reason? Route of transmission?

One limitation to this study is that fish were injected with mycobacterium, which is not the natural route of transmission. Zebrafish naturally acquire mycobacterium from their environment, and it is hypothesized to be derived from their feeds early in life (citation). Future research should expose zebrafish through a natural route. The implications of these results are diet can magnify or mask the effects of pathogen exposure in microbiome-targeted zebrafish studies. It’s unclear whether these effects exacerbate or protect zebrafish following pathogen exposure, but it illuminates the need for researchers to include diet as a confounding factor. Otherwise, husbandry practices involving choice of diet could impact the outcomes and interpretations of the study.

<dispersion?>

Stable state concept suggests that absent a major perturbation, the gut microbiome is relatively stable and does not change in diversity or composition (Shaw 2019).

Stephens et al found Mycobacterium taxa in all samples as well.

Gaulke2019 saw an increase in beta dispersion in infected fish.

<Methods>

Because presence or absence of infection relies on visual inspection of pathogens, we proceeded with our analysis looking at pathogen exposure instead of presence of infection or severity score.

<Supp>

Of the 44 exposed zebrafish, 18 of the 33 males and 10 of the 11 females were infected.

Methods

Gemma is a commercial diet, the ZIRC diet is a mixture of commercial diets, and the Watts diet differs from these two in that it is a defined diet with known nutritional and ingredient compositions. Fish were fed the same, nursery diet up to 1 month old then fish were separated into one of three diet groups and fed a juvenile formulation for that diet until 4 months old, where they were transitioned to their respective adult diet formulations.