**ZF Diet Infection Outline**

Diet differentially influences physiology and gut microbiome

* Diet -> physiology
* Diet -> gut microbiome

Diet impacts the successional development of the zebrafish gut microbiome

* Time -> physiology
* Time -> gut microbiome
* Diet + time -> physiology
* Diet + time -> gut microbiome
* Diet + physiology -> gut microbiome

Diet influences gut microbiome’s sensitivity to pathogen exposure

* Pathogen exposure -> gut microbiome
* Path exp + diet -> gut microbiome

**Diet differentially influences physiology and gut microbiome**

* Notes:
  + Not many studies looking at 3mpf+
    - Adaptive immunity, changes in diet could impact results
* Intro:
  + ZF Diets vary considerably in nutritional and ingredient composition
  + No standard reference diet
  + Diet can influence zebrafish physiology
  + Diet shown to influence microbiota (High vs low fat, not common lab diets)
  + Unclear the impacts of commonly used laboratory diets on adult zebrafish microbiomes
* Summary:
  + A
* Papers to cite here:
  + Wong 2015 – Diet (fat) and dev and env [ZF]
  + Arias-Jayo – Diet, microbiome, dysbiosis/inflamm [ZF]
  + Xiao 2021 – Host dev vs. Environment [ZF]
  + Xiao 2022 – Host dev, immunity, gut nutrient niches [ZF]
  + Liu 2022 – Diet (fiber) [Mice]
  + Fowler – Influence of commercial and lab diets ZF physiology [ZF]

Despite Zebrafish’s long-established importance as a model organism and their increasing use in microbiome targeted studies, there remains some key gaps in knowledge about them compared to other model systems like mice. One of these gaps and main motivations for the study we conducted is that currently there is no standard reference diet used in zebrafish studies. Standard reference diets are defined diets that contain consistent ingredients and nutrient compositions to allow for cross-comparisons of lab results. Instead, a variety of diets are used across zebrafish studies that include live feed and a mixture of defined/undefined ingredients. These diets can vary widely in terms of ingredients, quality and of composition of macro- and micro-nutrient profiles. This is in stark contrast to other study organisms, such as mice, who have had a standard refence diet since 1980. Given what we know about the influence diet has on host physiology and the microbiome’s important role in mediating host health, it raises important questions about whether or not we need to consider diet in microbiome-targeted zebrafish studies.

There has been some work in the zebrafish model that indicates that diet does matter. Studies have shown that type of diet can impact growth, body condition and impair reproductive outcomes in juvenile to adult zebrafish. Nutritional differences found across zebrafish diets can be responsible for undefined and uncontrolled variation in zebrafish studies. Some of these inconsistencies could be due to potential differences in how diets impact the development of zebrafish gut microbiomes. Prior research in zebrafish has shown that high and low fat diets can differentially affect microbiome development, as well as disrupt the microbiome in ways that impose negative health outcomes in the host. Similar results, investigating the role of high vs. low fat diets, have been found across other model systems and in humans. Given the results of this prior research, we sought to address gaps in knowledge regarding how different commonly used zebrafish laboratory diets may impact the gut microbiome and potentially influence their physiology.

1. Fish weight and body condition score differs between diets. Weight and body condition score of fish differed by diet. ZIRC fed fish had the highest weight and body condition scores compared to Gemma and Watts.
2. ZIRC fed fish have higher gut microbiome diversity at 6mpf, followed by Gemma and Watts fed fish, respectively.
3. Fish gut microbiome fed different diets had distinct community compositions
4. 31 genera significantly associated with diet in final control fish. In Gemma, Aeromonas and Cloacibacterium were significantly less abundant, while Plesiomonas, Cetobacterium and Chitinibacter were significantly more abundant compared to other diets. In Watts diet, Crenobacter and Shewanella were significantly less abundant, while Vibrio and ZOR0006 were significantly more abundant compared to other diets. In ZIRC, Acinetobacter was significantly more abundant compared to other diets.

**Diet impacts the physiological and successional gut microbiome development**

* Intro:
  + a
* Summary:
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Prior research in zebrafish shows that diet in tandem with host development play an influential role in shaping zebrafish gut microbiomes, and overwhelms the influence of environmental factors. However, these studies are limited in that they did not compare common laboratory diets, nor did they explore the effects of diet and host development beyond 100 days post fertilization. We collected stool samples at 3 and 6 mpf to better understand the influence of diet and time on gut microbiome succession in adult zebrafish.

1. **Time alone does not influence fish body condition score.** We did not observe a difference in body condition score between fish at 3 and 6 mpf.
2. **Fish gut microbiome diversity increases with time.**
3. **Rarer taxa are more sensitive to the effects of time, than abundant taxa.** 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 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.
4. **ZIRC diet promotes physiological growth in weight and body condition score throughout fish development**. ZIRC diet fed fish experience a change in body condition score over time, whereas Gemma and Watts diet fed fish do not. In particular, ZIRC fed fish manifest a significantly higher body score at 3 mpf as compared to 6 mpf. There was no significant difference between the body condition scores of fish fed Gemma and Watts diets over time. This temporal change in the body condition score of ZIRC fed fish appears to be driven by sex differences in body length that impact the body condition score.
5. **ZIRC diet promotes developmental gut microbiome diversification.** Microbiome diversity uniquely increased over time in ZIRC fed fish and not change over time in the Gemma and Watts fed fish. Moreover, the ZIRC fed fish manifested alpha-diversity measures at 3 mpf that were not statistically different from the corresponding measures of Watts and Gemma fed fish at 3 mpf. These observations indicate that ZIRC fed fish uniquely experience a relative increase in their microbiome biodiversity over time. (except Gemma in Simpsons index)
6. **Increased body condition score induced by ZIRC diet results in lower gut microbiome diversity.** Gut microbiome diversity uniquely increases as body condition score decreases in ZIRC fed fish, while Gemma and Watts diets remained stable across time. These observations suggest that there may be a physiological connection between ZIRC fed fish and the gut microbiome.
7. **ZIRC diet exhibits unique developmental impacts on fish physiology that linked in distinct gut microbiome communities at 6 mpf, but not at 3 mpf.** The composition of the gut microbiome of ZIRC fed fish were not distinct at 3 mpf, but at 6 mpf the gut microbiomes stratified by high and low body condition score. This pattern was not seen in Gemma and Watts fed fish. These observations suggest that over time, ZIRC fed fish uniquely select for distinct compositions of gut microbiome communities that correlate with body condition score.
8. **ZIRC diet differentially selects for certain taxa.** 33 genera were found to have a statistically significant relationship between body condition score in ZIRC fed fish. In particular, the genera Comamonadaceae, Bacteroides, Bosea and Paucibacter abundance decreased with increased body condition score, while Cetobacterium abundance increased with increased body condition score. Only Bacteroides was found to have an association with body condition score independent of diet. This observation suggests that the ZIRC diet is uniquely selecting for these taxa.

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

* Intro:
  + Zebrafish are exposed to a variety of pathogens
  + Pathogen exposure could be a source of uncontrolled variation in study outcomes
  + Pathogens hypothesized transmitted through diet
  + M. chelonae present in 40%
  + We injected Mc to ensure exposure
  + Previous research showing microbiome-pathogen effect on host health
* Summary:
  + A

Zebrafish facilities are known to host many pathogens, which can introduce non-protocol induced inconsistency in study outcomes. One pathogen that is found in 40% of zebrafish facilities is M. Chelonae, and is hypothesized to be introduce through diet. M. chelonae causes gut inflammation in zebrafish. We hypothesized that there is a gut microbiome relationship with pathogen exposure. Furthermore, given the impact of diet on zebrafish gut microbiomes, we hypothesized there could also be a relationship between diet, pathogen exposure and microbiome. If such an association exists, it could offer zebrafish facilities microbiome-targeted treatments for preventing or minimizing the impacts of pathogen exposure.

1. **Pathogen exposure inhibits diversification of the gut microbiome**. The gut microbiomes of exposed fish have lower levels of gut microbiome diversity to that of unexposed fish. Microbiome diversity of pre-exposed and exposed fish did not differ. These patterns occur regardless of the specific diet being considered. Together, these observations suggest that exposure to pathogens inhibits diversification of the gut microbiome. Furthermore, pathogen exposure may be preventing diversification of gut microbiomes.
2. **ZIRC fed fish are uniquely sensitive to the effects of pathogen exposure, while Gemma and Watts are resistant.** 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. 6 mpf unexposed ZIRC fed fish had significantly greater microbiome diversity compared to 3 mpf pre-exposed ZIRC fed fish, while 6 mpf exposed fish were significantly less diverse. Moreover, microbiome diversity of fish fed Gemma and Watts diets were not different between exposure groups. These observations suggest that fish fed the ZIRC diet are uniquely sensitive to pathogen exposure.
   1. Physiological differences? Did not see an exposure group by diet effect on body condition score, which suggests that exposure group did not have an effect on physiology depending on diet. Of the exposed fish, ZIRC had a higher overall body condition score, while Gemma and Watts did not differ from each other.
3. **The effects of diet on the gut microbiome composition overwhelms microbiome’s sensitivity to pathogen exposure.** The gut microbiome community composition stratifies by exposure group, regardless of diet. Dispersion did not differ between exposure groups at 6 mpf, but do differ when compared to 3 mpf pre-exposed fish. When diet is taken into consideration, the effect of exposure group is secondary to diet. Suggesting that community composition is sensitive to pathogen exposure, but the primary driver of composition is diet. Dispersion differed between fish at 3 mpf and 6 mpf, but these differences were not significantly different between exposure groups. Together, these observations suggest that diet masks/overwhelms the effects of pathogen exposure on gut microbiome community composition.
4. **Pathogen exposure differentially selects for certain taxa**. 54 genera are differentially abundant across exposure groups. In unexposed fish, Bacteroides and Vibrio were significantly more abundant, while Plesiomonas, Fluviicola, Flavobacterium and Shewanella were significantly less abundant in unexposed fish compared to pre-exposure and exposed fish. In exposed fish, Paucibacter, Cerasicoccus and Gemmobacter were significantly less abundant. These observations suggest that exposure has differential impacts on genera abundance.