**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**

* Intro:
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* Summary:
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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:
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* Summary:
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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 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)
5. **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.~~
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:
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* Summary:
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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.