

Common laboratory diets differentially influence zebrafish gut microbiome's successional development and sensitivity to pathogen exposure

Michael Sieler, Colleen Al-Samarrie, Kristin Kasschau, Michael Kent, Thomas J. Sharpton

Abstract

Despite the long-established importance of zebrafish as a model organism and their increasing use in microbiome-targeted studies, relatively little is known about how husbandry practices involving diet impact the zebrafish gut microbiome. Given, the microbiome's important role in mediating host physiology and the potential for diet to drive variation in microbiome composition, we sought to clarify how three different dietary formulations that are commonly used in zebrafish facilities impacts the gut microbiome. We reared approximately 60 fish on each diet throughout their lifespan and compared the composition of their microbiomes at both 4- and 7-months-old. Our analysis finds that diet has a substantial impact on the composition of the gut microbiome at both 4- and 7-months-old. Moreover, the developmental dynamics of the microbiome differ as a function of diet. We further evaluated whether the 7-month-old fish microbiome compositions that result from dietary variation are differentially sensitive to infection by a common laboratory pathogen: *Mycobacterium chelonae*. Our analysis finds that the impact of *M. chelonae* infection on the gut microbiome differs as a function of diet, especially for moderate and low abundance taxa. Overall, our results indicate that diet drives the successional development of the gut microbiome as well as its sensitivity to exogenous exposure. Consequently, investigators should carefully consider the role of diet in their microbiome zebrafish investigations, especially when integrate results across studies that vary by diet.

Introduction

The gut microbiome plays an important role in supporting the health of its host through nutrient metabolism, supporting the immune system and protecting against pathogens. To better understand the gut microbiome, zebrafish (*Danio rerio*) have emerged as an important model organism due to their extensive homology to early human development, high-throughput experimental methods and ability to directly manipulate their gut microbiomes. Despite zebrafish's 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 (Watts). Instead, zebrafish are fed a variety of diets, which impact zebrafish physiological and reproductive outcomes (Fowler, Watts). Moreover, diets consisting of varying ratios of protein, lipids and fiber manifest distinct gut microbiome compositions in zebrafish (Leigh, Wong). Despite the insights of these previous studies, it is not clear if what impact commonly used laboratory diets have on the gut microbiome of zebrafish. Clarifying the effect of diet on the zebrafish gut microbiome will support researchers seeking to compare results across microbiome-targeted zebrafish studies.

Zebrafish have a core microbiome, but there is much inter-individual variation that could be diet-related. Early in life, larval zebrafish are fed a "life feed" diet, which can consist of rotifers, ciliates, artemia and/or dry feeds (Westerfield). These live organisms are believed to be a major contributors of microbes to young zebrafish ([sources](#)), and might explain the high degree of variation observed in young zebrafish (Stephens, Burns, Xiao). As zebrafish transition from larval to juvenile and adult stages, they transition to a dry food diet. Transitioning to an adult diet is also marked by a stabilization in the microbiome (Stephens, Burns, Xiao). In the present study, we investigated three of these commonly used dry food diets, which include and will subsequently be referred to as the Gemma, Watts, and ZIRC diets (see materials and methods). While these diets are relatively similar in nutritional content, they differ in which ingredients are used, where ingredients are sourced, methods of preparation and exact nutritional content. Source of ingredients can be an inadvertent contributor of microbial transmission and intestinal inflammation (Uran, Watts2016). Given, that the microbiome mediates host health and is relatively stable once established, any diet-related influences to early-life microbiome assembly could have lasting impacts to the successional development of the gut microbiome and consequently health of the zebrafish. Therefore, it is important for zebrafish researchers targeting the gut microbiome to understand how husbandry choices involving diet may affect the gut microbiome.

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27. Asok A, et al.. Reducing Vibrio load in Artemia nauplii using antimicrobial photodynamic therapy: a promising strategy to reduce antibiotic application in shrimp larviculture. *Microb Biotechnol* 2012;5:59–68 [[PMC free article](#)] [[PubMed](#)] [[Google Scholar](#)]
28. Hoj L, Bourne DG, Hall MR. Localization, abundance and community structure of bacteria associated with Artemia: Effects of nauplii enrichment and antimicrobial treatment. *Aquaculture* 2009;293:278–285 [[Google Scholar](#)]
29. Gomez-Gil B, Thompson FL, Thompson CC, Swings J. Vibrio roiferianus sp. nov., isolated from cultures of the rotifer *Brachionus plicatilis*. *Int J Syst Evol Microbiol* 2003;53:239–243 [[PubMed](#)] [[Google Scholar](#)]
30. McIntosh D, et al.. Culture-independent characterization of the bacterial populations associated with cod (*Gadus morhua* L.) and live feed at an experimental hatchery facility using denaturing gradient gel electrophoresis. *Aquaculture* 2008;275:42–50 [[Google Scholar](#)]

Zebrafish facilities are known to host many pathogens, which can introduce non-protocol induced inconsistencies in study outcomes (Kent). The intestinal pathogen *Mycobacterium chelonae* is found in 40% of zebrafish facilities, and is hypothesized to be introduced through diet early in life (Stephens, Kent2012, Chang2019). *M. chelonae* forms granulomas in the gut intestine, which can cause gut inflammation, decreased fecundity and lifespan (Whipps2016, Varela). Previous work of ours has shown that an intestinal parasite *Pseudocapillaria tomentosa* disrupts and restructures the gut microbiome (Gaulke). However, little is known about the effects of *M. chelonae* infection on zebrafish gut microbiomes. Some studies suggest that having a highly diverse microbiome can protect against pathogen infection (citation). Given the effect of diet shaping the zebrafish gut microbiome, there could be an interaction between diet, pathogenic infection and the gut microbiome in zebrafish. Elucidating the relationships of diet, pathogen exposure and the gut microbiome could offer microbiome-targeted treatments for preventing or mitigating the impacts of *M. chelonae* and other intestinal pathogens on zebrafish health and study outcomes, but also more broadly to other animal systems and humans.

Here, we assessed how different common laboratory diets influence gut microbiome diversity and composition, as well as impact physiological development of 4- and 7-months-old zebrafish. At 30-days-old, we assigned zebrafish one of three commonly used laboratory diets, that will be subsequently referred to as the Gemma, Watts and ZIRC diets. We found that the gut microbiome associates with diet, but also time. Additionally, we investigated the diet-associated sensitivity of zebrafish to the pathogenic species *Mycobacterium chelonae*. We found that the gut microbiomes of fish fed certain diets were more resistant to the effects of pathogen exposure than others. Our study clarifies how common laboratory diets differentially impacts the successional development of zebrafish gut microbiome and sensitivity to pathogen exposure.

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Results

1. Experimental design and data collection

Treatments	Pathogen Exposure	Pre-exposure		Unexposed	
		Exposed	Unexposed	Exposed	Unexposed
Diets	Nursery diet	Juvenile diet	Adult diet (Gemma)	Adult diet (Watts)	Adult diet (ZIRC)
		Juvenile diet	Adult diet	(Watts)	(ZIRC)
Fish age (days)	0	30	129		214
# Fish sampled			89		87
Fecal sampling		X		X	
<i>M. Chelonae</i> injection		X			
Histopathology check				X	

Figure 1: Experimental design showing treatments and husbandry events during the course of the study. An "X" indicates when an event occurred (e.g., fecal sampling took place when fish were age 129 and 214).

In this study, our main goals were to reveal the influence different commonly used laboratory diets have on the zebrafish physiology, the successional development of zebrafish gut microbiome and its sensitivity to pathogen exposure. To that end, we reared 176 zebrafish that were assigned one of three diets from 1- to 7-months-old (Figure 1; see Methods and Materials). Additionally, at 4-months-old, we exposed a cohort of fish to the intestinal pathogen: *Mycobacterium chelonae*. The diets include a commercial diet (e.g., Gemma), a defined laboratory diet (e.g., Watts), and a combination of commercial diets (e.g., ZIRC). These diets will be subsequently referred to as the Gemma, Watts and ZIRC. From 1- to 4-months-old, fish were fed the juvenile formulations of their respective diets. At 4-months-old, they were switched to adult formulations of their respective diets. Fish were housed 15 fish per tank according to their assigned diet (e.g. Gemma, Watts and ZIRC) and exposure group (e.g. exposed and unexposed) throughout the duration of the experiment. We collected 89 fecal samples at 4-months-old, and then injected half the fish (e.g. exposed) to *Mycobacterium chelonae* and the other half remained unexposed. Approximately 3 months later when fish were 7-months-old, we collected 87 fecal samples from each diet and exposure group and performed a histopathology check to assess infection burden of exposed fish. Our questions focused on the effect of diet on physiology, microbial gut diversity, community composition and bacterial abundance, as well as the gut microbiome's sensitivity to *M. chelonae* exposure.

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2. Diet differentially influences physiology and gut microbiome

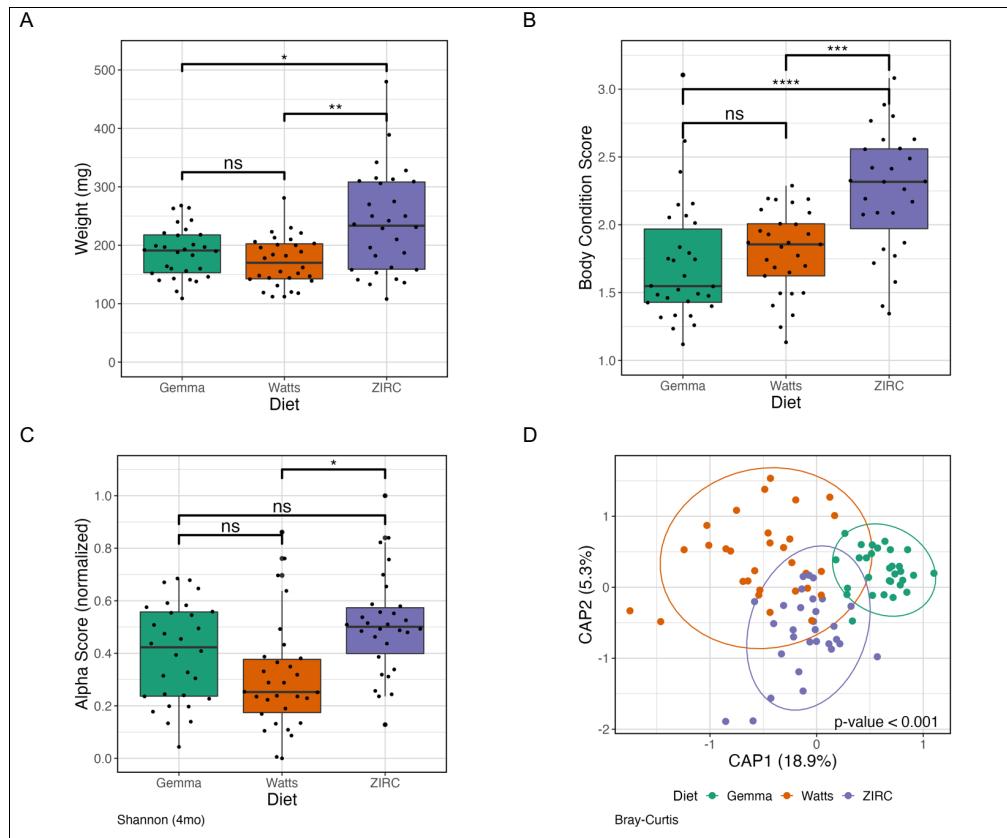


Figure 2: Effects of fish fed one of three diets (Gemma, Watts, or ZIRC) on physiology and microbiomes of zebrafish. **(A)** Weight of ZIRC significantly differs from Watts and Gemma. Gemma and Watts do not differ from each other. **(B)** Body condition score is a length normalized measure of weight. Fish fed the ZIRC Diet have significantly higher body condition scores from Gemma and Watts diets. **(C)** Simpson's Index of diversity shows that gut microbiome diversity significantly differs between Gemma and Watts, ZIRC and Watts, but not between Gemma and ZIRC. **(D)** Capscale ordination based on the Bray-Curtis dissimilarity of gut microbiome composition. The analysis shows that physiology and gut microbiome composition significantly differs between the diets. “ns” indicates not significantly different, *, **, *** indicates significant differences below the 0.05, 0.01, and 0.001 levels, respectively.

Zebrafish physiology and gut microbiome has been previously associated with diet, but these studies considered diets that differed greatly in nutritional content (e.g., high fat and protein vs. low fat and protein; citations). Our study is unique in that fish were fed three commonly used laboratory diets similar in nutritional composition. Moreover, fish were fed the same diet from 30 to 214 days (7 months) old. Here, we investigated how commonly used laboratory diets may impact the zebrafish physiology and gut microbiome diversity, composition, and relative abundance at 4 months old.

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We first determined if physiology, represented here by weight and body condition score, differed between the three diets. Wilcoxon Signed-Rank Tests found that diet and sex significantly associated with weight and body condition. Female fish had higher weight ($Z = 1,530$, $P < 0.001$; Table S1.1.2) and body condition scores ($Z = 1,631$, $P < 0.001$; Table S1.1.4) compared to males. ZIRC-diet fed fish had the highest mean body condition score compared to fish fed Gemma- ($Z = 150$, $P < 0.001$) and Watts-diet ($Z = 197$, $P < 0.001$, Table S1.1.3). Gemma- and Watts-diet fed fish did not significantly differ from one another in terms of weight and body condition scores. These results indicate that ZIRC-diet contributes to heavier fish compared to Gemma- and Watts-diet fed fish.

Next, we asked if diet associated with gut microbiome diversity. First, we built generalized linear models (GLM) to determine if diet associated with variation in one of three measures of microbiome alpha-diversity: richness, Simpson's Index, and Shannon Entropy. An ANOVA test of these GLMs revealed that alpha-diversity varies as a function of diet for all three measures of diversity we assessed ($P < 0.05$; Fig 1C; Table S1.2.2). A post hoc Tukey test clarified that ZIRC- and Watts-diet fed fish exhibited significant differences in alpha-diversity as measured by richness and Shannon Entropy ($P < 0.001$, Table S1.2.3). Moreover, we observed significant differences in diversity between Gemma- and Watts-diet fed fish in terms of richness ($P < 0.001$; Table S1.2.3), and between Gemma- and ZIRC-diet fed fish when considering the Simpson's Index ($P < 0.001$; Table S1.2.3). These results indicate that diet associates with fish gut microbiome diversity, and that diet may differentially impact rare and abundant microbial members of the gut depending on diet.

To evaluate how diet associates with microbiome community composition, we used the Bray-Curtis and Canberra dissimilarity metrics. We detected a significant clustering of microbial gut community composition based on diet (PERMANOVA, $P < 0.05$; Figure 2C, Table S1.3.1). Additionally, we assessed beta-dispersion, a measure of variance, in the gut microbiome community compositions for each diet group. We find the beta-dispersion were significant between the diet groups as measured by Bray-Curtis and Canberra metrics (Table S1.4.1). Beta-dispersion levels were significantly reduced in Gemma-diet fed fish compared to Watts-diet fed fish when measured by Bray-Curtis metric, as well as significantly reduced compared to Watts- and ZIRC-diet fed fish when measured by Canberra metric (Table S1.4.1). These results indicate that Gemma-diet fed fish are more consistent in community composition than Watts- and ZIRC-diet fed fish at 4 months old. Collectively, these results indicate that at 4 months old fish gut microbiome communities stratify by diet and fish fed different diets vary in consistency of community composition.

Finally, to better understand the interactions between the diets and the members of the gut microbiome, we quantified differential abundance using ANCOM-BC2. We observed 24 taxa at the genus level were significantly abundant in at least one of the three diets (Figure S1.5.1). Of these, Gemma-diet fed fish enriched for Chitinibacter, Watts-diet fed fish enriched for Flavobacterium. These results indicate that diets select for different taxa when fish are 4 months old.

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3. Diet impacts the successional development of the zebrafish gut microbiome

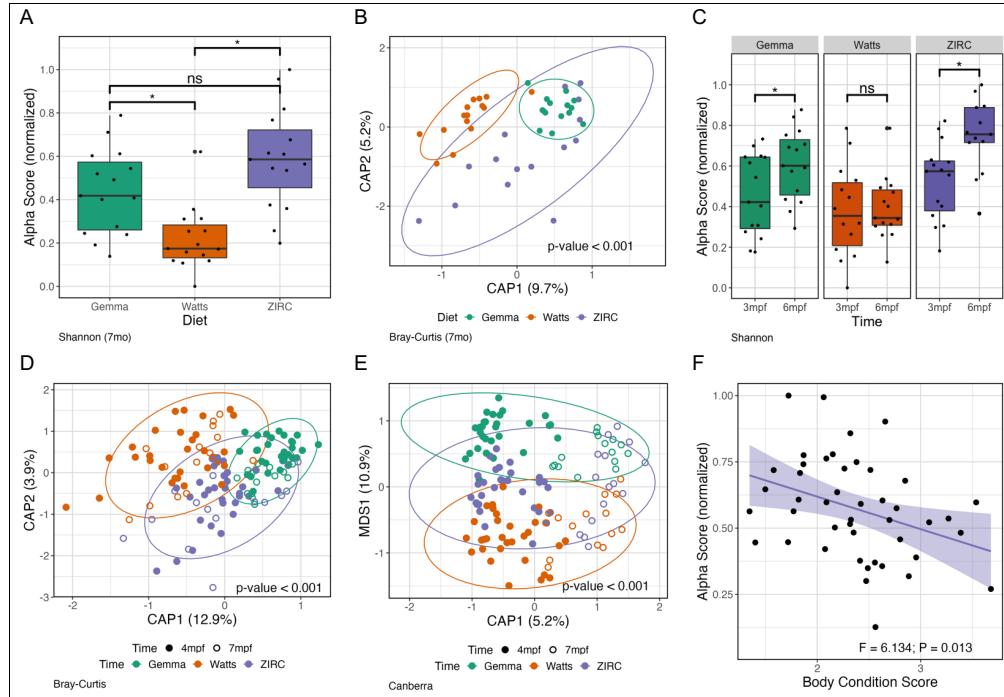


Figure 3: Development is associated with altered microbiome composition. **(A)** Shannon Entropy of diversity shows that gut microbiome diversity significantly differs between Watts-diet fed fish to fish fed the Gemma- and ZIRC-diets in 7-month-old zebrafish. **(B)** Capscale ordination based on the Bray-Curtis dissimilarity of gut microbiome composition in 7-month-old zebrafish. **(C)** Shannon Entropy for diversity shows microbial gut diversity increases with development in 4- to 7-month-old zebrafish fed the Gemma- and ZIRC-diets, but not Watts-diet fed fish. Capscale ordination of gut microbiome composition based on the **(D)** Bray-Curtis dissimilarity by diet and **(E)** Canberra measure by time. **(F)** Body condition score negatively associates with gut microbiome diversity as measured by Simpson's Index across 4- and 7-month-old zebrafish fed the ZIRC diet. The analysis shows that physiology and gut microbiome composition significantly differs between the diets across development, and there may be diet-dependent link with physiology. A "ns" indicates not significantly different, ** indicates significant differences below the 0.05 level.

Given the associations we observed above between diet, the gut microbiome and physiology at 4-month-old fish, we investigated physiology and the gut microbiome diversity and composition of fish fed the three diets at 7 months of age. We found similar significant associations between diet and physiology of 7-month-old fish as we did of 4-month-old fish ($P < 0.05$; Table S2.7.1). Additionally, linear regression analysis revealed statistically significant main effects of diet on microbial gut diversity in zebrafish for all alpha- and beta- metrics ($P < 0.05$; Fig 3A&B, Table S2.7.2). These results indicate that diet influences physiology and the gut microbiome in fish.

We next sought to determine how diet impacts the successional development of the gut microbiome by comparing 4- and 7-month-old zebrafish. Linear regression revealed microbial gut diversity was significantly associated with the main effect of time ($P < 0.05$; Table S2.2.2) for each alpha-diversity metric, but the

interaction effect between diet and time did not meet our threshold for significance for any alpha-diversity metric we assessed ($P > 0.05$; Table S2.2.2.2). A post hoc Tukey test clarified microbiome diversity was significantly different between 4- and 7-month-old Gemma- and ZIRC-diet fed fish as measured by the Shannon and Simpson's alpha-diversity metrics ($P < 0.05$; Figure 3C, Table S2.2.2.3). We did not find a statistically significant association between 4- and 7-month-old Watts-diet fed fish and any alpha-diversity metric ($P > 0.05$; Table S2.2.2.3). These results indicate that the microbial gut diversity of Watts-diet fed fish were temporally stable, while Gemma- and ZIRC-diet fed fish diversified.

A PERMANOVA test using the Bray-Curtis dissimilarity metric revealed that community composition was best explained by diet ($P < 0.05$; Figure 2C, Table S2.4.1), but an analysis using the Canberra measure found that variation in microbiome composition was best explained by time ($P < 0.05$; Fig 2D, Table S2.4.2). Given how these metrics weight the importance of abundant and rarer taxa between communities, these results indicate that abundant members of the microbiome community are more sensitive to the effects of diet, while rarer community members are sensitive to the effects of time. Moreover, we found beta-dispersion levels were significantly elevated between 4- and 7-month-old Gemma-diet fish and between 4- and 7-month-old ZIRC-diet fed fish when considering the Bray-Curtis and Sorensen metrics ($P < 0.05$; Table S2.5.3). Conversely, beta-dispersion levels were only significantly elevated between 4- and 7-month-old of Watts-diet fed fish when considering the Sorensen metric ($P < 0.05$; Table S2.5.3). These results indicate that the microbiome communities of Watts-diet fed fish are consistent between each other across the development of zebrafish, while the communities of Gemma- and ZIRC-diet fed fish become more varied across development.

Development was associated with 20 taxa at the genus level in at least one of the diets (Table S2.6.1). Of these taxa, *Fluviicola*, *Macellibacteroides*, *Bacteroides* and an unnamed genus in the *Barnesiellaceae* family were some that were enriched, while *Phreatobacter* and *Flavobacterium* were depleted. Unique to each diet only a few taxa were identified as being significant. The Gemma-diet fed fish were uniquely enriched for *Exiguobacterium* (Table S2.6.2.1) (citation). The Watts-diet fed fish were uniquely depleted of *Gemmobacter* (Table S2.6.2.2) (Source). The ZIRC-diet fed fish were uniquely enriched for *Pseudomonas* and *Haliscomenobacter* (Table S2.6.2.3) (source). Together, these results indicate that particular members of the gut microbiome associate with diet and development.

To determine if physiology associated between diet across development, we used Wilcoxon Signed-Ranks Tests to identify parameters that best explained the variation in body condition score between fish 4- and 7-month-old. Within each diet, linear regression did not observe a significant association of body condition score between diet and time ($P > 0.05$; Fig 2E, Table S2.1.1). This result indicates that while fish differ in body condition between diets at 7 months old, they grow at a similar rate between 4 and 7 months of age. Interestingly, we observed a significant negative association of body condition score and microbial gut diversity uniquely in fish fed the ZIRC diet as measured by Shannon Entropy and Simpson's Index ($P < 0.05$; Fig 2F, Table S2.2.1). This result indicates that fish gut microbiomes with higher body masses are lower in diversity compared to fish with lower body mass. For Canberra and Sorensen beta-diversity metrics, there were significant main effects of body condition score, and significant interaction effects between body condition score and diet ($P < 0.05$; Table S2.2.1.3). However, the model coefficient for the effect of body condition score and its interaction with diet is far smaller than the coefficient for the effect of diet (Table S2.2.2). We did not find a significant association between body condition score and specific taxon abundance (Table S2.2.2). Collectively, these results indicate that the gut microbiome may be sensitive to body condition of fish, but the influence of diet is much stronger.

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4. Diet influences gut microbiome's sensitivity to pathogen exposure

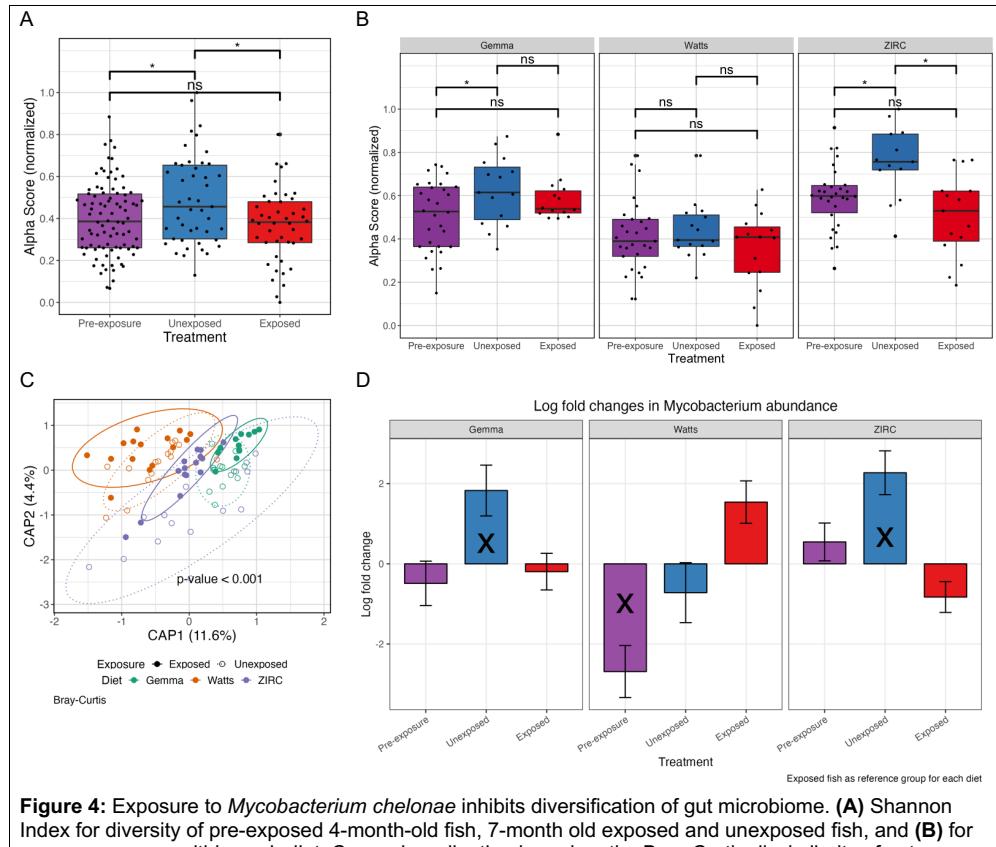


Figure 4: Exposure to *Mycobacterium chelonae* inhibits diversification of gut microbiome. **(A)** Shannon Index for diversity of pre-exposed 4-month-old fish, 7-month old exposed and unexposed fish, and **(B)** for exposure groups within each diet. Capscale ordination based on the Bray-Curtis dissimilarity of gut microbiome composition of fish by **(C)** diet. **(E)** Log fold change of *Mycobacterium* of pre-exposed, exposed and unexposed fish within each diet as calculated by ANCOM-BC. Values are in reference to exposed fish within each diet. The analysis shows gut microbiome's sensitivity to pathogen exposure is linked to diet, but *Mycobacterium*'s abundance is diet-dependent. A "ns" indicates not significantly different, and * indicates significant differences below the 0.05. An "X" indicates a group is significantly differentially abundant compared to the exposed treatment reference group.

Lastly, we sought to elucidate the potential interactions between the gut microbiome, commonly laboratory diets and exposure to the intestinal pathogen *Mycobacterium chelonae*. Briefly, after collecting fecal samples of 4-month-old fish, we injected *Mycobacterium chelonae* into the coelomic cavity of fish in the exposed treatment group. At 7 months old, we collected fecal samples to measure microbial gut diversity, composition, and taxon abundance, performed a histopathology check to assess infection counts and severity, and collected physiological measurements. Fecal samples collected prior to *M. chelonae* exposure are labeled as "pre-exposure", and samples collected after exposure are labeled as either "exposed" or "unexposed".

We first assessed whether there was an association between diet and infection status (positive or negative presence of the pathogen). A pairwise Fisher Test did not find the proportion of infections differed between diets ($P > 0.05$; Table S3.5.1). This result indicates that diet does not appear to influence whether a fish is infected or not. Next, we assessed infection status on the physiological endpoint body condition score and measures of gut microbiome diversity and composition ($P > 0.05$; Table S3.5.2). We did not find significant associations between infection status and body condition score or any of the gut microbiome diversity and composition measures ($P > 0.05$; Table S3.5.4&S3.5.5). Collectively, infection status was not predictive of physiological or microbiome analysis endpoints. However, using linear regression, we find that microbial gut diversity significantly differs between exposure groups as measured by richness and Shannon Entropy alpha-diversity metrics ($P < 0.05$; Figure 4A, Table S3.1.2.2). We did not find a statistically significant interaction effect between diet and exposure groups and any metric of alpha-diversity ($P > 0.05$; Table S3.1.2.2). Furthermore, we used a post hoc Tukey test to clarify whether microbial gut diversity of fish differed between exposure groups within each diet group. Unique to ZIRC-diet fed fish, we observed microbiome diversity differed in unexposed controls compared to pre-exposed and exposed fish as measured by all alpha-diversity metrics ($P < 0.05$, Table S3.1.2.3). However, unexposed Watts-diet fed fish significantly differed in alpha-diversity compared to exposed fish as measured by Shannon Entropy and richness metrics. Unexposed Gemma-diet fed fish differed in alpha-diversity from pre-exposure fish only in terms of Shannon Entropy. These results indicate that ZIRC-diet fed fish appear more sensitive to the effects of *M. chelonae* exposure than fish fed the Gemma or Watts diets. Moreover, pre-exposed ZIRC-diet fed fish did not differ in microbial gut diversity to exposed ZIRC-diet fed fish ($P < 0.05$; Fig 4B, Table S3.1.2.3). These results indicate that fish fed commonly used laboratory diets are differentially sensitive to *M. chelonae* exposure in terms of microbiome diversity.

Next, we evaluated how pathogen exposure influenced microbial community composition across fish fed each diet. PERMANOVA tests as measured by each beta-diversity metric found the main effects of diet and pathogen exposure were statistically significant, but the main effect of diet was greatest ($P < 0.05$; Fig 4C, Table S3.2.3). Furthermore, a PERMANOVA test found the model coefficient effect for the interaction of diet and pathogen exposure was statistically significant as measured by Canberra and Sorenson beta-diversity metrics, but the effect was less than the main effects of diet and pathogen exposure. Moreover, an analysis of beta-dispersion levels showed significant levels of dispersion across exposure and diets ($P < 0.05$; Table S3.3.2.1), but levels did not differ between exposed and unexposed groups ($P > 0.05$; Table S3.3.2.2). These results indicate that dispersion is likely driven by diet, rather than exposure. Collectively, these results indicate that the gut microbiome is sensitive to pathogen exposure, diet has a greater influence on microbiome community composition.

An analysis of differential abundance found several taxa associated with pathogen exposure. Unexposed Gemma-diet fed fish enriched for *Macellibacteroides* and *Aurantisolimonas* (Table S3.4.2), unexposed Watts-diet fed fish enriched for an unnamed genus of *Barnesiellaceae*, *Fluviicola*, *Paucibacter*, and *Brevibacterium* (Table S3.4.3), and unexposed ZIRC-diet fed fish enriched for *Macellibacteroides*, *Bacteroides*, *Mycobacterium* and unnamed genera of *Barnesiellaceae* and *Sutterelaceae* (Table S3.4.4). Across all the diets, unexposed fish enriched for *Macellibacteroides*, *Fluviicola*, *Bacteroides*, *Aurantisolimonas*, *Cerasicoccus*, and three unnamed genera of *Barnesiellaceae*, *Commonadaceae*, and *Sutterellaceae*. Exposed fish enriched for *Plesiomonas* (Table S3.4.5). These results indicate that pathogen exposure impacts the abundance of certain taxa within and across the diets. Next, to see if *Mycobacterium* species abundance differed from background, pre-exposure levels we compared *Mycobacterium* abundance between pre-exposure and unexposed control fish to that of exposed fish within each diet. Unexposed Gemma- and ZIRC-diet fed fish had significantly higher abundances of *Mycobacterium* to exposed (Figure 4D, Table S3.4.6). Pre-exposed Watts-diet fed fish had significantly more *Mycobacterium* compared to pre-exposed fish, but they did not differ significantly from unexposed control fish. These results indicate that *Mycobacterium* species abundance changes in response to exposure to a pathogenic species, but these changes depend on diet.

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Discussion

Zebrafish are an important emerging model organism for understanding the microbiome. Yet, there is little consistency across microbiome-targeted studies in husbandry practices involving diet. Given the microbiome's important role in supporting host health and diet major role in shaping the gut microbiome, we sought to better understand how three commonly used laboratory diets impacted the assembly and successional development of adult zebrafish gut microbiomes, as well as the microbiome's sensitivity following exposure to a common zebrafish intestinal pathogen, *M. chelonae*. This study represents, to our knowledge, the first to assess diets of similar nutritional composition on the development of the zebrafish gut microbiome and their physiology. Furthermore, we investigated the interaction of diet and pathogen exposure on the gut microbiome. Our results indicate that diet drives the successional development of the gut microbiome as well as its sensitivity to exogenous pathogen exposure. These results have important implications for microbiome targeted zebrafish studies as well as conservation biology.

We find that commonly used laboratory diets with relatively similar nutritional compositions stratify the gut microbiome of adult zebrafish. Previous research has found that diets with varying compositions of key macronutrients: protein, lipids and fiber impacts zebrafish physiology and gut microbiome (Leigh, Wong, Fowler). However, the nutritional compositions used in these studies targeting the gut microbiome differ from what is typically used in zebrafish husbandry and research. Our results show that even minor differences in nutritional composition can have profound impacts on the gut microbiome assembly in 4-month-old zebrafish. The differences we observe in the gut microbiome assembly between these diets could be explained by the exact ingredient formulations, where the ingredients were sourced, and the methods of preparation. Studies in Atlantic salmon and the common carp found that not all protein sources in diet are equal. Soy bean meal is a common protein source for hatchery-reared fish, but studies show it is implicated in causing intestinal inflammation. Moreover, variation in ingredient sources caused by inconsistent commodity sources in the case of commercial diets may inadvertently introduce contaminants, such as phytoestrogens, or pathogenic microbes, such as *M. chelonae*, to zebrafish (Watts/D'ambro, Kent). Furthermore, studies using the same diets may not in fact be the same due to differences between batch production (Watts/D'ambro). Therefore, zebrafish researchers seeking to target the microbiome in their studies should consider using a standard reference diet. A standard reference diet would have the benefit of transparently disclosing ingredient sources, nutritional compositions, and methods of preparation. However, in some instances variability in diet may be beneficial to researchers seeking to model the variability of diets found in human populations.

We also observed variation in the successional dynamics of gut microbiomes of fish fed different diets across their development in adulthood. We find the composition of abundant taxa are driven primarily by diet, but rarer taxa are sensitive to the effects of time. We also saw interaction effect of diet and development on the gut microbiome. For instance, gut microbiome diversity and community variation increased across development in fish fed the Gemma and ZIRC diets, but remained stable in Watts-diet fed fish. One explanation for this stability is that the Watts diet is a laboratory designed and produced diet, which might offer more consistency in ingredient sources, nutritional composition and methods of preparation to the other two commercially-derived diets. Another important point of consideration is the transition of fish from juvenile to adult diet formulations at 4 months of age. Again, we find more consistency between the Watts juvenile and adult diets compared to the Gemma and ZIRC juvenile and adults diets. In particular, the ZIRC adult diet formulation is a combination of four different diets that differ in nutritional composition and ingredient sources, whereas the Watts adult diet changes only in lipid content. The consistency in the Watts diet formulations could be linked to the consistency of the Watts-diet fed fish gut microbiome diversities across development. Previous research investigating the successional development of the gut microbiome in zebrafish finds zebrafish gut microbiome diversity is higher in juvenile fish but declines as they age. Our results contrast this overall trend, but when we compare similar time points between studies, we do find a similar increase in diversity between 4 and 7 months of age. A

limitation of these past studies is inconsistency in diet and tank environment which could explain the variability of gut microbiomes of juvenile zebrafish. Future studies should seek methodological consistency across diet and microbiome sampling to aid efforts in cross-study comparisons. A unique strength of our study is the consistency in diet and tank environment. We clearly demonstrate the effects of diet, development, and their interaction on the successional development of zebrafish gut microbiome.

Differences in early-life assembly of the gut microbiome caused by diet could have long-term impacts on the health of the host. For instance, diets that select for gut microbiomes that more efficiently metabolize nutrients early in life could provide a fitness advantage to the host and improve their reproductive success, longevity, and ability to resist disease. Previous studies find diet-related impacts to physiology and reproduction (Fowler), as well as implicated certain taxa to physiological outcomes in zebrafish (Rawls, Leigh). Indeed, in the case of ZIRC-diet fed fish we find physiological effects linked to the gut microbiome, where body condition score and microbiome diversity are negatively associated. However, we did not find specific taxa that associated with physiological measurements of body condition score across any of the diets, even within ZIRC-diet fed fish. Therefore, the ZIRC diet may not be enriching for particular taxa that influence body condition score. Instead, the intestinal environment found within high body condition score ZIRC-diet fed fish may be inhospitable to cultivating a diverse microbiome. Compared to previous studies linking specific taxa to physiological outcomes in zebrafish fed different diets, the diets we used differed minimally which may explain why we do not find similar taxa-associated effects. Another explanation could be that body condition score is not the optimal metric for identifying physiologically important. For instance, previously mentioned studies measured more specific physiological measurements such as fat tissue, intestinal length, and gut enzymatic activity.

Finally, we find that exposure to the intestinal pathogen *Mycobacterium chelonae* inhibited diversification of gut microbiomes, and microbiome community composition was driven primarily by diet rather than pathogen exposure. Additionally, *Mycobacterium*'s abundance differed between exposure groups within each diet. Compared to control fish, the exposed Watts-diet fed fish had more *Mycobacterium*, but Exposed Gemma and ZIRC had fewer relative to control fish. It's important to note that nonpathogenic *Mycobacterium* species are a common member of the zebrafish gut microbiome community. Due to the limitations of 16S analysis it's not possible to disentangle whether the *Mycobacterium* abundance we observed is the injected pathogenic strain or non-pathogenic species naturally present in the fish. Despite this limitation, we can see pathogen exposure effects on the gut microbiome across the diets. The gut microbiome diversity of ZIRC fed fish is uniquely sensitive to pathogen exposure, while Gemma- and Watts-diet fed fish were more stable. Higher gut microbiome diversity is linked to higher stability and greater ability to resist pathogens because of competition for habitat space and nutrient availability (Xiao, Gaulke?, Other?). Thus, it is possible *Mycobacterium* taxa might have taken advantage of the low diversity environment of Watts-diet fed fish to gain habitat space or utilize nutrients to increase in abundance. When assessing effects of pathogen exposure on microbiome community composition, we find they were secondary to diet and might explain why our results differ from previous microbiome-pathogen studies that saw increased microbiome community variation following pathogen exposure (Gaulke). Specifically, Gaulke et al found microbiome diversity and community composition increased in variation within pathogen exposed fish, while we find the opposite effect of exposed fish microbiome communities becoming more similar to one another and decreased diversity compared to controls. These differences could be due to the differences in pathogens, where Gaulke et al exposed fish to an intestinal helminth and we used a bacterial pathogen. Therefore, the gut microbiome may respond differently to pathogenesis. Moreover, these results demonstrate the importance of diet to the structuring of the gut microbiome. While we did not find an effect of infection on the gut microbiome, there could be other ways that pathogen exposure exerts influence on the gut microbiome. For instance, the presence of pathogenic bacteria may induce an immunological inflammatory response that affects the gut microbiome. Additionally, to ensure exposure to *M. chelonae* we injected fish with the pathogen, but this is not the natural route of transmission.

Commented [TS25]: You did? What result shows this?

Commented [SJMJ26R25]: Right. I'm being lazy here saying "pathogen exposure", but what I mean is exposure to *Mycobacterium*. It would be more precise to say *Mycobacterium* exposure inhibited diversification of the gut microbiome (Fig 3a)

Commented [TS27]: Probably not wise to use Myco as a representative for pathogen exposure here. I recommend being specific so that you aren't accused of over generalizing your results.

Commented [TS28R27]: (or anywhere else in the MS)

Commented [SJMJ29R27]: Got it.

Commented [TS30]: Personally, I think this is hard to interpret, but worth discussing regardless so long as we note that 16S can't provide insight into what types of Myco are present.

Commented [TS31]: compared to what? Remember, readers won't have read gaulke et al.

Future studies should include immunological endpoints as well as expose zebrafish using a natural route of transmission to clarify the effect of *M. chelonae* on the gut microbiome and host's health.

Beyond zebrafish husbandry, our results have important implications to the field of conservation biology for wildlife management and rehabilitation, particularly for fish species such as salmonids. The differences in nutritional composition found across the diets we investigated here can be seen as analogous to the variability in nutritional or resource availability caused by habitat fragmentation driven by the expansion of human urbanization (e.g., damming of rivers preventing salmon migration and spawning). These challenges to wildlife's ability to gather necessary resources to survive and reproduce, negatively impact their fitness. Moreover, previous research finds gut microbiomes of wildlife in their natural environments differ from those in captivity. Two proposed reasons for the variation in wild and captive animal microbiomes are the differences in diet and immune system development between their natural and captive environments. Furthermore, these differences are suspected as playing a role in the success or failure of wildlife reintroduction given the microbiomes role in digesting nutrients and supporting the immune system. However, more research is needed to clarify the microbiome's impact on successful reintroduction of wildlife. Our characterization of zebrafish gut microbiome dynamics across their development provides a useful resource for researchers and wildlife managers seeking to integrate the microbiome in their conservation efforts.

In conclusion, this study represents, to our knowledge, the first assessment to date of common laboratory diets' long-term impact on the successional development of the zebrafish gut microbiome and its sensitivity to pathogen exposure. In particular, we find diet is one of the most important factors driving variation in the zebrafish gut microbiome. Unlike prior studies, including the extensive research conducted in mammalian models, that have evaluated dietary effects on the gut microbiome using diets that fundamentally differ in macronutrient composition, our study reveals that even relatively consistent diets that are commonly selected as normal husbandry practices elicit these large impacts on microbiome composition. It may be worth establishing a standard reference diet for microbiome-targeted zebrafish studies to improve our understanding of zebrafish health and nutrition, advance knowledge of how the diet and microbiome interact, and support efforts towards reproducibility and interpretability of results across studies. Although, zebrafish diets may benefit from a variety of diets to model the variation in diets and microbiomes we see in human populations. One important challenge to establishing a standard reference diet is its ability to be made germ-free and nutritionally equivalent to conventional diets (Rawls). Significant progress is being made on this front, which supports efforts to better understand the connection between diet and the microbiome in zebrafish (Rawls, Watts). Collectively, our results indicate that researchers should carefully consider the role of diet in zebrafish microbiome studies, and the microbiome should be considered an important factor in wildlife management and rehabilitation efforts.

Methods

Fish Husbandry

A total of 270 30 day-old AB line zebrafish were randomly divided into eighteen 2.8 L tanks (15 fish/ tank). During the experiment, temperature was recorded daily and ranged from 25.5–28.3°C, with the exception of two isolated overnight temperature drops below that range due to two separate power loss events that affected the source water sump heater. All other water conditions were monitored weekly, pH ranged from 7.0–7.6, total ammonia ranged from 0–0.25 ppm (measured with pH and ammonia API test kits; Mars Fishcare North America Inc. Chalfont, PA), and conductivity ranged from 109 –166 microsiemens. Light in the vivarium was provided for 14 hours/day. One plastic aquatic plant piece approximately 6 inch in length was added to each tank for enrichment when fish were 214 days old. A stock of similarly aged Casper line fish were maintained for the duration of the experiment, with a third of the stock being maintained on each of the diet regimens matching the AB line zebrafish. These fish served as filler fish and were added to the tanks after each histological sampling time point to maintain the 15 fish/tank ratio required to maintain the prescribed diet volumes per feeding.

Diets

Fish were all fed the same nursery diet until 30 days old, a combination of paramecia, brine shrimp, and the ZIRC Nursery Mix: Zeigler AP Larval Diet (Ziegler Bros Inc., Gardners, PA) and freeze dried rotifers. Fish were then transferred to the OSU facility and assigned randomly to one of three juvenile diets: Gemma Micro 150/300 (Skretting, Fontaine-les-Vervins, France), Watts High-Fat Juvenile Mix, or ZIRC Juvenile Mix, twice daily (9 AM and 3 PM local time) until 60 days old. From 60 days of age onward, OSU fish were not fed on weekends and 1-day holidays as per the facility institutional animal care and use protocol. The total quantity fed daily was 3% fish body weight. This continued until fish were 214 days old and then they were transitioned to the adult version of their previously assigned juvenile diet: Gemma Micro 500 (Skretting, Fontaine-les-Vervins, France), Watts Low-Fat Adult Mix, or ZIRC Adult Mix, twice daily (9 AM and 3 PM local time), except weekends and 1-day holidays. The total quantity fed daily was 3% fish body weight. The prescribed amounts of each diet regimen, for both the juvenile and adult diets were delivered by 3D printed spoons specific to the diet and stage of life. These spoons were paired with conical tubes retrofitted with leveling wires to ensure consistent feeding volumes as prescribed. All fish were only fed once, in the afternoons, on sampling days.

Diet and Pathogen Exposure

Each of the eighteen tanks was assigned one of the three diet regimens: Gemma, Watts, or ZIRC. There were three tank replicates per diet regimens for a total of nine tanks that were exposed to *M. chelonae* via intraperitoneal injection. The remaining nine tanks were similarly assigned to diet regimens and were exposed to a sterile 1X-phosphate buffered saline (PBS) solution via intraperitoneal injection. Each fish was injected with 10 μ L of either the *M. chelonae* inoculum or saline solution. The injections were completed over the course of two days and the *M. chelonae* inoculum was prepared as a 0.5 McFarland each day. Day 1 *M. chelonae* inoculum was afterwards determined by plating to be 3.1×10^3 dose per fish. Day 2 *M. chelonae* inoculum was determined by plating to be 1.0×10^5 dose per fish.

Growth Parameters and Sex Determination

Growth and sex parameters were collected when fish were 101–102, 129–130, 213–214 days old for interfacility comparison. Additionally these parameters were also collected at 164–165 days old which was 5 weeks post exposure that were evaluated in comparison to the 213–214 days old measurements which were 15 weeks post exposure for evaluation of disease effects.

Sex was determined by gross differences in morphology and confirmed by histology for all samples collected for disease severity evaluation. Following overnight fecal collection, individual fish would be placed in a pre-anesthetic solution of 50 ppm MS-222 prepared with Tricaine-S (Western Chemical Inc., Ferndale, WA; a subsidiary of Aquatic Life Sciences Inc.) briefly before being transferred to a 150 ppm MS-222 anesthetic solution in a petri dish on centimeter grid paper to be photographed. Standard length and width were evaluated

Commented [SJM32]: Need Mike to review zebrafish methods

via photographs taken with an iPhone (Apple Inc., Cupertino, CA) and analyzed with ImageJ software (<https://imagej.net>).

Body condition score (BCS) was calculated using the following equation: BCS = Weight/Length³. Body condition score is a length normalized metric of weight (for equation, see Methods) and serves as a general indicator of health in zebrafish.

Commented [ST33]: Might be methods

Weight was taken while the fish was still under the effects of anesthesia by transferring them from the photography petri dish to the petri dish on a scale with a volume of tared fish water. Excess water was removed

Histopathology

Fish were preserved in Dietrich's solution, processed, and slides stained with Kinyoun's acid-fast. Severity was scored by counting total numbers of granulomas containing acid fast bacteria in the coelomic cavity, ovaries, and kidney. Score of 1 was 1–2 granulomas, 2 = multiple granulomas observed, 3 = prominent infections with granulomatous lesions occupying a large amount of the coelom or gonad. In addition, an overall severity of infection score was assigned based on the average scores of the individual structures evaluated (cite previous Kent lab paper).

Fecal Collection

Fecal material was collected from individual fish at the same sample intervals as outlined for the growth parameters. Fecal collection was set up the day before growth parameter sampling. Fish were transferred to 1.4 L tanks (1 fish/tank) containing ~0.4 L of fish water at least 30 minutes after the last feeding of the day. Fish were left to defecate overnight and all feces present were collected from each tank the following morning. Fecal samples were immediately snap frozen on dry ice and stored at -80 °C until processing.

16S Sequencing

Microbial DNA was extracted from zebrafish fecal samples and 16S rRNA gene sequence libraries were produced and analyzed following established approaches (Kundu et al., 2021). Briefly, the DNeasy PowerSoil Pro DNA kits (Qiagen) were used to extract and purify DNA. The V4 region of the 16S rRNA gene was PCR amplified using the Earth Microbiome Project 16S index primers and protocols (Walters et al., 2016). PCR products were visualized on a 1.5% agarose gel and quantified on a Qubit 2.0 (Thermofisher Scientific) using the Qubit dsDNA HS Assay. One hundred ng of each PCR sample was pooled, cleaned using the QIAquick PCR Purification Kit (Qiagen), and quality was verified on the Agilent TapeStation 4200. The prepared library was submitted to the Oregon State University Center for Quantitative Life Sciences (CQLS) for 300 bp paired-end sequencing on an Illumina MiSeq System (RRID:SCR_016379).

Commented [SJMJ34]: Ask Kristin to review

Commented [SJMJ35]: Correct?

Commented [SJMJ36]: Correct?

Commented [SJMJ37]: What is the code?

Commented [SJMJ38]: Need to review versions, citations, and packages.

Analysis

All microbiome DNA sequence analyses and visualizations were conducted in R (v 4.2.1). Fastq files were processed in using the DADA2 R package (v 1.18.0). Briefly, forward and reverse reads were trimmed at 280 and 230 bp, respectively, subsequently merged into contigs, and subject to amplicon sequence variant (ASV) identification. ASVs unannotated at the Phylum level were removed to result in 292 remaining detected ASVs. We used Wilcoxon Signed-Ranks Tests to identify parameters that best explained the variation in weight and body condition scores. Alpha-diversity was calculated using the estimate_richness function (Phyloseq v 1.38.0) and transformed using Tukey's Ladder of Powers. After transformation, scores were normalized from 0 to 1 by dividing each score by the maximum value, which allowed us to compare results across alpha-diversity metrics using general linear models (GLMs). Two-way ANOVA assess these GLMs. Beta-diversity models were generated using methods described previously (Kundu et al., 2021). Briefly, we evaluated three beta-diversity metrics—Bray-Curtis, Canberra, and Sorenson and resolved the relationship between experimental parameters and beta-diversity by applying a step-wise model selection approach as implemented in the capscale function (vegan package v2.5). Optimal models were subsequently subject to PERMANOVA analysis to determine if

Commented [ST39]: Methods.

the selected model parameters significantly explained the variation in microbiome composition across samples. Differential abundance was measured using ANCOM-BC (v).

Supplementary Tables and Figures

1) Diet

1.1) Physiology

1.1.1)

Wilcoxon Test. p. adj: BH. Weight ~ Diet

.y.	group1	group2	n1	n2	statistic	p	p.adj	p.adj.signif
Weight	Gemma	Watts	30	30	537.500	0.198	0.198	ns
	Gemma	ZIRC	30	30	301.500	0.029	0.044	*
	Watts	ZIRC	30	30	238.500	0.002	0.006	**

1.1.2)

Wilcoxon Test. p. adj: BH. Weight ~ Sex

.y.	group1	group2	n1	n2	statistic	p	p.adj	p.adj.signif
Weight	F	M	50	40	1,530.500	<0.001	<0.001	****

1.1.3)

Wilcoxon Test. p. adj: BH. Body.Condition.Score ~ Diet

.y.	group1	group2	n1	n2	statistic	p	p.adj	p.adj.signif
Body.Condition.Score	Gemma	Watts	30	30	327.000	0.070	0.070	ns
	Gemma	ZIRC	30	30	150.000	<0.001	<0.001	****
	Watts	ZIRC	30	30	197.000	<0.001	<0.001	***

1.1.4)

Wilcoxon Test. p. adj: BH. Body.Condition.Score ~ Sex

.y.	group1	group2	n1	n2	statistic	p	p.adj	p.adj.signif
Body.Condition.Score	F	M	50	40	1,631.000	<0.001	<0.001	****

1.2) Alpha-diversity

1.2.1)

`glm(Alpha.Score ~ Diet), family = quasibinomial)`

metric	term	estimate	std.error	statistic	p.value	sig
Observed	(Intercept)	-0.256	0.136	-1.886	0.063	
	DietWatts	-0.833	0.207	-4.033	<0.001	*
	DietZIRC	0.127	0.192	0.663	0.509	
Shannon	(Intercept)	-0.399	0.152	-2.622	0.010	*
	DietWatts	-0.418	0.222	-1.881	0.063	
	DietZIRC	0.426	0.213	1.999	0.049	*
Simpson	(Intercept)	-0.344	0.157	-2.198	0.031	*
	DietWatts	0.288	0.220	1.309	0.194	
	DietZIRC	0.782	0.223	3.511	<0.001	*

1.2.2)

`ANOVA(glm(Alpha.Score ~ Diet), family = quasibinomial)`

metric	term	statistic	df	p.value	sig
Observed	Diet	26.112	2	<0.001	*
Shannon	Diet	15.072	2	<0.001	*
Simpson	Diet	12.847	2	0.002	*

1.2.3)

Pairwise Tukey's HSD, p.adj: Dunnett. $\text{glm}(\text{Alpha.Score} \sim \text{Diet})$, family = quasibinomial)

metric	term	.y.	group1	group2	estimate	std.error	statistic	p.adj	p.adj.signif
Observed	Diet	Alpha.Score	Watts	Gemma	-0.833	0.207	-4.033	0.000	*
	Diet	Alpha.Score	ZIRC	Gemma	0.127	0.192	0.663	0.785	ns
	Diet	Alpha.Score	ZIRC	Watts	0.960	0.206	4.661	0.000	*
Shannon	Diet	Alpha.Score	Watts	Gemma	-0.418	0.222	-1.881	0.144	ns
	Diet	Alpha.Score	ZIRC	Gemma	0.426	0.213	1.999	0.112	ns
	Diet	Alpha.Score	ZIRC	Watts	0.845	0.220	3.833	0.000	*
Simpson	Diet	Alpha.Score	Watts	Gemma	0.288	0.220	1.309	0.390	ns
	Diet	Alpha.Score	ZIRC	Gemma	0.782	0.223	3.511	0.001	*
	Diet	Alpha.Score	ZIRC	Watts	0.494	0.221	2.232	0.066	ns

1.3) Beta-diversity

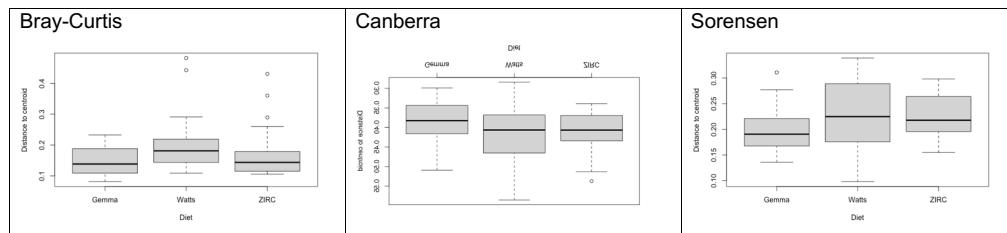
1.3.1)

Distance-based redundancy analysis (dbRDA) ordination. $\text{Beta.Score} \sim \text{Diet}$

metric	term	Df	SumOfSqs	statistic	p.value	sig
Bray-Curtis	Diet	2.00	1.100887	13.948	0.001	*
	Residual	87.00	3.433377			
Canberra	Diet	2.00	3.240437	9.342	0.001	*
	Residual	87.00	15.088729			
Sørensen	Diet	2.00	1.463523	13.198	0.001	*
	Residual	87.00	4.823751			

1.4) Beta-Dispersion

1.4.1) Diet



DF	Sum Sq	Mean Sq	F	N.Perm	Pr(>F)
2	0.03790416	0.018952081	3.807483	999	0.027
87	0.43305015	0.004977588			

Names	p-value
Gemma-Watts	0.003
Gemma-ZIRC	0.136
Watts-ZIRC	0.213

DF	Sum Sq	Mean Sq	F	N.Perm	Pr(>F)
2	0.02612888	0.013064439	3.70263	999	0.029
87	0.30697268	0.003528422			

Names	p-value
Gemma-Watts	0.014
Gemma-ZIRC	0.044
Watts-ZIRC	0.435

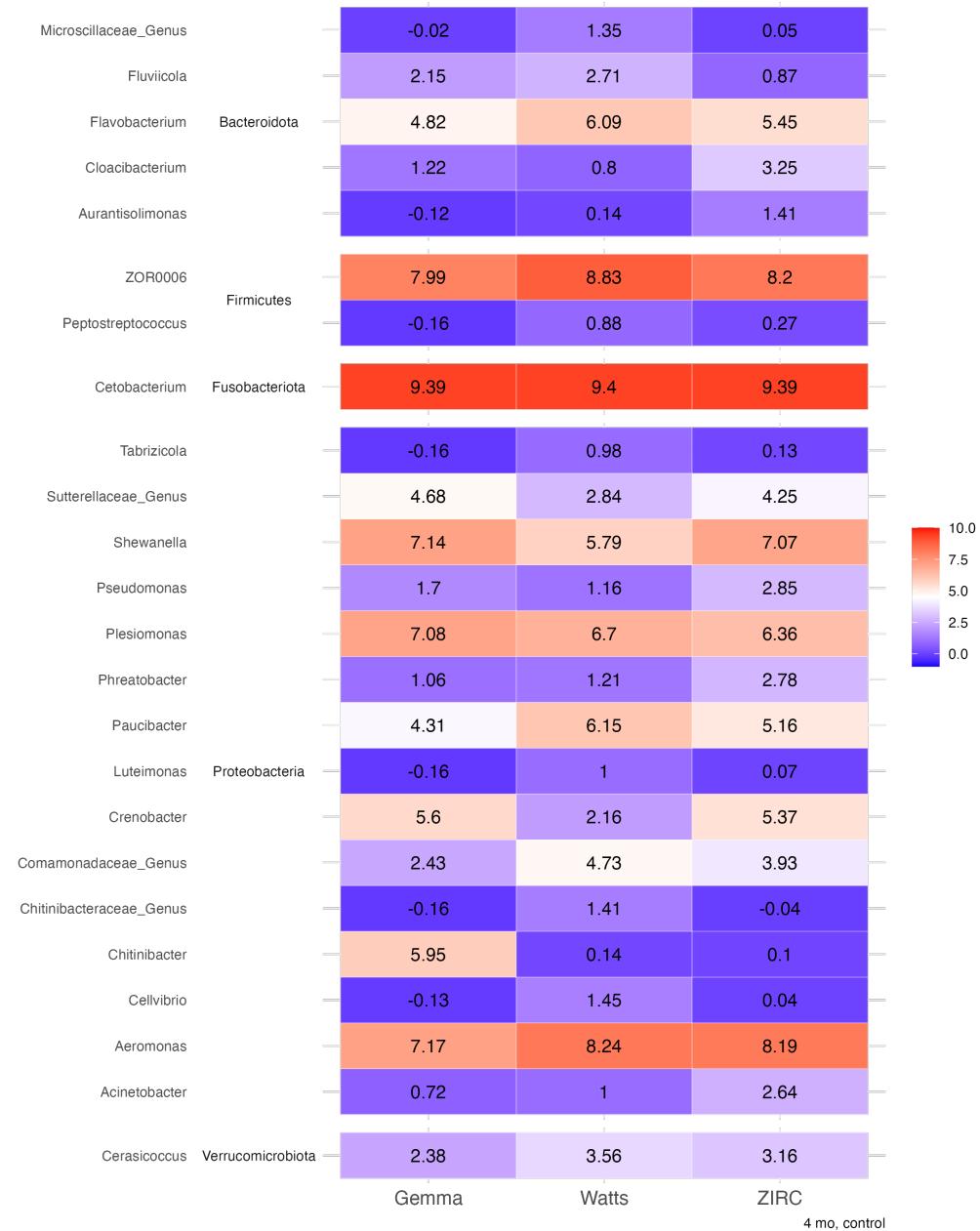
DF	Sum Sq	Mean Sq	F	N.Perm	Pr(>F)
2	0.01501706	0.007508530	2.714407	999	0.079
87	0.24065738	0.002766177			

Names	p-value
Gemma-Watts	0.104
Gemma-ZIRC	0.016
Watts-ZIRC	0.815

1.5) Differential Abundance

1.5.1)

Heat map of bias-corrected log observed abundances (Genus)



ANCOM-BC2: Summary table of abundant taxa (4mo, controls), sig taxa = 24

Taxon	W	p_val	q_val	diff_abn
Acinetobacter	15.558	0.001	0.002	TRUE
Aeromonas	68.458	0.000	0.000	TRUE
Aurantisolimonas	28.988	0.000	0.000	TRUE
Cellvibrio	31.866	0.000	0.000	TRUE
Cerasicoccus	9.516	0.017	0.027	TRUE
Cetobacterium	0.007	0.007	0.013	TRUE
Chitinibacter	1,781.599	0.000	0.000	TRUE
Chitinibacteraceae_Genus	23.392	0.000	0.000	TRUE
Cloacibacterium	29.732	0.000	0.000	TRUE
Comamonadaceae_Genus	28.866	0.000	0.000	TRUE
Crenobacter	118.313	0.000	0.000	TRUE
Flavobacterium	18.694	0.000	0.000	TRUE
Fluviicola	11.012	0.008	0.013	TRUE
Luteimonas	24.265	0.000	0.000	TRUE
Microscillaceae_Genus	24.075	0.000	0.000	TRUE
Paucibacter	26.178	0.000	0.000	TRUE
Peptostreptococcus	21.470	0.000	0.000	TRUE
Phreatobacter	21.495	0.000	0.000	TRUE
Plesiomonas	12.187	0.005	0.009	TRUE
Pseudomonas	11.073	0.008	0.013	TRUE
Shewanella	46.586	0.000	0.000	TRUE
Sutterellaceae_Genus	22.323	0.000	0.000	TRUE
Tabrizicola	16.971	0.000	0.001	TRUE
ZOR0006	32.244	0.000	0.000	TRUE

1.5.2)



ANOMOCAT: Summary table of pairwise comparison of abundant taxa (4ms, controls)																		
Taxon	Ic_DewHats	Ic_Dew2ZRC	Ic_DewZRC_DewHats	ie_DewHats	ie_Dew2ZRC	ie_DewZRC_DewHats	W_DewHats	W_Dew2ZRC	W_DewZRC_DewHats	p_DewHats	p_Dew2ZRC	p_DewZRC_DewHats	q_DewHats	q_Dew2ZRC	q_DewZRC_DewHats	df_DewHats	df_Dew2ZRC	df_DewZRC_DewHats
Cetobacterium	0.012 - 0.000	-0.012	0.142 - 0.145	0.190	0.084 - 0.086	-0.064	0.933	0.999	0.949	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000
ZOR0006	0.837 - 0.208	-0.629	0.148 - 0.151	0.196	5.651 - 1.379	-3.215	0.000	0.168	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.005	0.000
Aeromonas	1.013 - 1.018	-0.055	0.162 - 0.165	0.208	6.610 - 3.197	-0.264	0.000	0.000	0.000	0.791	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000
Pseudomonas	0.379 - 0.725	-0.346	0.211 - 0.214	0.252	-1.796 - 1.374	-0.374	0.000	0.000	0.000	0.170	0.272	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Sphaerotilus	0.000 - 0.000	-0.000	0.000 - 0.000	0.000	0.000 - 0.000	-0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Psychobacter	1.062 - 0.869	-0.693	0.963 - 0.965	0.595	5.109 - 2.363	-2.514	0.000	0.019	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.046	0.046	0.000
Comammocystis_Genus	2.203 - 1.408	-0.805	0.441 - 0.443	0.471	5.228 - 3.381	-1.711	0.000	0.001	0.087	0.000	0.003	0.000	0.000	0.000	0.000	0.240	0.240	0.000
Crenobacter	-3.452 - 0.227	0.225 - 0.338	0.341 - 0.341	0.371	-10.213 - 0.656	8.682	0.000	0.505	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Flavobacterium	1.276 - 0.838	-0.641	0.297 - 0.300	0.333	4.292 - 2.116	-1.307	0.000	0.034	0.054	0.000	0.139	0.000	0.000	0.000	0.000	0.148	0.148	0.000
Suttermicrobacter_Genus	1.849 - 0.434	1.415 - 0.400	0.403 - 0.403	0.431	-4.624 - 1.078	3.283	0.000	0.281	0.001	0.000	0.072	0.004	0.000	0.000	0.000	0.000	0.000	0.000
Gemmibacter	-0.597 - 0.403	0.193 - 0.297	0.300 - 0.309	0.587	1.000 - 1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000
Anelerobacter	3.132 - 1.931	1.618 - 0.511	0.513 - 0.513	0.539	6.612 - 3.761	3.002	0.541	0.000	0.003	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000
Chitinibacter	-3.807 - 0.860	-0.060	0.176 - 0.178	0.190	-33.107 - 0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Fusobacter	0.000 - 0.296	-0.296	0.300 - 0.301	0.569	1.127 - 2.556	-3.200	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Classobacterium	0.408 - 2.001	2.406 - 0.447	0.450 - 0.450	0.477	-0.908 - 4.448	5.046	0.344	0.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000
Crenuscoccus	1.198 - 0.780	-0.014	0.400 - 0.403	0.431	2.992 - 1.945	-0.960	0.000	0.003	0.037	0.013	0.194	0.000	0.000	0.000	0.000	0.026	0.026	0.000
Chitinibacteraceae_Genus	1.349 - 0.125	-1.425	0.340 - 0.342	0.373	4.563 - 0.365	-3.820	0.000	0.715	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.001	0.001	0.000
Pseudomicrobium	-0.506 - 1.166	1.673 - 0.488	0.491 - 0.491	0.517	-1.037 - 2.377	3.236	0.000	0.017	0.001	0.000	0.004	0.000	0.000	0.000	0.000	0.000	0.000	
Myobacterium	-0.039 - 0.357	-0.318	0.395 - 0.397	0.426	-0.099 - 0.808	-0.746	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000
Exiguobacterium	0.081 - 0.107	-0.174	0.430 - 0.433	0.460	2.049 - 2.049	-1.681	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000
Pireobacter	1.134 - 1.703	1.569 - 0.390	0.392 - 0.392	0.421	0.343 - 4.340	3.726	0.792 - 0.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000
Rhizobacteriales_Genus	0.138 - 0.131	-0.007	0.007 - 0.007	0.027	0.000 - 0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000
Autumnibacter	0.000 - 1.506	1.240 - 0.283	0.295 - 0.296	0.319	0.041 - 2.573	-3.307	0.000	0.000	0.000	0.000	0.053	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Celvibacter	1.553 - 0.171	-1.382	0.298 - 0.299	0.322	5.430 - 0.562	-4.293	0.000	0.054	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	
Hallihomocystobacter	0.095 - 0.120	-0.176	0.232 - 0.235	0.271	0.243 - 0.509	-0.648	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	
Dinghabacter	0.191 - 0.157	-0.348	0.430 - 0.433	0.460	0.445 - 0.363	-0.756	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	
Phytobacteraceae_Genus	0.119 - 0.029	-0.148	0.374 - 0.377	0.406	0.317 - 0.077	-0.364	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	
Fluviobacter	-0.356 - 0.274	0.000	0.274 - 0.277	0.310	-1.302 - 0.869	-0.267	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	
Microscutaceae_Genus	1.376 - 0.115	-1.261	0.294 - 0.297	0.330	4.675 - 0.368	-3.823	0.000	0.698	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	
Defluvimonas	0.022 - 0.476	-0.498	0.312 - 0.315	0.347	0.069 - 0.152	-1.435	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	
Iceobacter	0.239 - 0.233	-0.000	0.000 - 0.000	0.020	0.000 - 0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	
Talpibacter	0.000 - 0.294	-0.655	0.079 - 0.280	0.151	0.486 - 0.160	-2.712	0.000	0.319	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
Lutimonas	1.103 - 0.215	-0.668	0.206 - 0.229	0.266	4.884 - 0.940	-3.342	0.000	0.047	0.001	0.000	0.055	0.000	0.000	0.000	0.000	0.000	0.000	
Noxardiaeace_Genus	0.079 - 0.239	-0.318	0.235 - 0.238	0.275	0.335 - 1.004	-1.159	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	
Noxardia	0.030 - 0.028	-0.001	0.237 - 0.240	0.276	2.655 - 0.118	-2.177	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	
Longirivista	-0.147 - 0.137	0.010	0.207 - 0.210	0.249	-0.712 - 0.655	0.040	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	
Pleurobacteraceae	0.973 - 0.371	-0.602	0.211 - 0.214	0.252	4.617 - 1.737	-2.389	0.000	0.082	0.017	0.000	0.026	0.003	0.000	0.000	0.000	0.000	0.000	
Iamia	-0.225 - 0.366	-0.141	0.184 - 0.187	0.228	-1.223 - 1.960	-0.622	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	

2) Development

2.1) Physiology

2.1.1)

ANOVA. p. adj: BH. Body.Condition.Score ~ Diet*Time

term	df	sumsq	meansq	statistic	p.value	sig
Timepoint	1	0.058	0.058	0.227	0.634	
Diet	2	9.680	4.840	18.906	<0.001	*
Timepoint:Diet	2	0.020	0.010	0.040	0.961	
Residuals	129	33.023	0.256			

2.2) Physiology ~ Microbiome

2.2.1)

glm(Alpha.Score ~ Body.Condition.Score), family = quasibinomial)

metric	-y-	term	statistic	df	p.value	sig
Observed	Alpha.Score	Body Condition Score (Gemma)	0.788	1	0.375	
	Alpha.Score	Body Condition Score (Watts)	3.913	1	0.048	*
	Alpha.Score	Body Condition Score (ZIRC)	1.802	1	0.179	
Shannon	Alpha.Score	Body Condition Score (Gemma)	0.341	1	0.559	
	Alpha.Score	Body Condition Score (Watts)	3.631	1	0.057	
	Alpha.Score	Body Condition Score (ZIRC)	3.979	1	0.046	*
Simpson	Alpha.Score	Body Condition Score (Gemma)	0.289	1	0.591	
	Alpha.Score	Body Condition Score (Watts)	3.337	1	0.068	
	Alpha.Score	Body Condition Score (ZIRC)	6.134	1	0.013	*

2.2.2)

Distance-based redundancy analysis (dbRDA) ordination. Beta.Score ~ Body.Condition.Score*Diet

metric	term	Df	SumOfSqs	statistic	p.value	sig
Bray-Curtis	Diet	2.00	1.35911866	13.877	0.001	*
	Body.Condition.Score	1.00	0.06101171	1.246	0.234	
	Body.Condition.Score:Diet	2.00	0.10901377	1.113	0.302	
	Residual	129.00	6.31711356			
Canberra	Diet	2.00	3.41322719	8.491	0.001	*
	Body.Condition.Score	1.00	0.35999108	1.791	0.009	*
	Body.Condition.Score:Diet	2.00	0.58256642	1.449	0.010	*
	Residual	129.00	25.92826696			
Sørensen	Diet	2.00	1.72484640	11.533	0.001	*
	Body.Condition.Score:Diet	2.00	0.26241045	1.755	0.012	*
	Body.Condition.Score	1.00	0.12991495	1.737	0.035	*
	Residual	129.00	9.64624896			

2.3) Alpha-diversity

2.3.1) Time

2.3.1.1)

glm(Alpha.Score ~ Time), family = quasibinomial)

metric	term	estimate	std.error	statistic	p.value	sig
Observed	(Intercept)	-0.043	0.084	-0.504	0.615	
	Timepoint7mpf	0.332	0.147	2.256	0.026	*
Shannon	(Intercept)	-0.146	0.089	-1.638	0.104	
	Timepoint7mpf	0.400	0.155	2.591	0.011	*
Simpson	(Intercept)	-0.243	0.087	-2.782	0.006	*
	Timepoint7mpf	0.314	0.151	2.083	0.039	*

2.3.1.2)

ANOVA(`glm(Alpha.Score ~ Time)`, family = quasibinomial))

metric	term	statistic	df	p.value	sig
Observed	Timepoint	5.120	1	0.024	*
Shannon	Timepoint	6.754	1	0.009	*
Simpson	Timepoint	4.345	1	0.037	*

2.2.1.3)

Pairwise Tukey's HSD, p.adj: Dunnett. `glm(Alpha.Score ~ Timepoint)`, family = quasibinomial)

metric	term	.y.	group1	group2	estimate	std.error	statistic	p.adj	p.adj.signif
Observed	Timepoint	Alpha.Score	7mpf	4mpf	0.332	0.147	2.256	0.024	*
Shannon	Timepoint	Alpha.Score	7mpf	4mpf	0.400	0.155	2.591	0.010	*
Simpson	Timepoint	Alpha.Score	7mpf	4mpf	0.314	0.151	2.083	0.037	*

2.2.2) Time:Diet

2.2.2.1)

glm(Alpha.Score ~ Diet*Time), family = quasibinomial)

metric	term	estimate	std.error	statistic	p.value	sig
Observed	(Intercept)	0.187	0.128	1.458	0.147	
	DietWatts	-0.803	0.186	-4.323	<0.001	*
	DietZIRC	0.097	0.182	0.532	0.596	
	Timepoint7mpf	0.221	0.225	0.983	0.327	
	DietWatts:Timepoint7mpf	0.018	0.320	0.057	0.954	
	DietZIRC:Timepoint7mpf	0.377	0.327	1.154	0.251	
Shannon	(Intercept)	-0.142	0.138	-1.025	0.307	
	DietWatts	-0.422	0.199	-2.117	0.036	*
	DietZIRC	0.398	0.196	2.028	0.045	*
	Timepoint7mpf	0.524	0.242	2.164	0.032	*
	DietWatts:Timepoint7mpf	-0.460	0.346	-1.329	0.186	
	DietZIRC:Timepoint7mpf	0.156	0.353	0.440	0.661	
Simpson	(Intercept)	-0.564	0.147	-3.837	<0.001	*
	DietWatts	0.261	0.205	1.273	0.205	
	DietZIRC	0.689	0.204	3.375	<0.001	*
	Timepoint7mpf	0.541	0.248	2.181	0.031	*
	DietWatts:Timepoint7mpf	-0.647	0.352	-1.839	0.068	
	DietZIRC:Timepoint7mpf	-0.005	0.355	-0.014	0.989	

2.2.2.2)

ANOVA($\text{glm}(\text{Alpha.Score} \sim \text{Diet} * \text{Time})$, family = quasibinomial)

metric	term	statistic	df	p.value	sig
Observed	Diet	50.425	2	<0.001	*
	Timepoint	6.981	1	0.008	*
	Diet:Timepoint	1.677	2	0.432	
Shannon	Diet	39.641	2	<0.001	*
	Timepoint	8.736	1	0.003	*
	Diet:Timepoint	3.307	2	0.191	
Simpson	Diet	21.457	2	<0.001	*
	Timepoint	5.026	1	0.025	*
	Diet:Timepoint	4.466	2	0.107	

2.2.2.3)

Pairwise Tukey's HSD, p.adj: Dunnett. $\text{glm}(\text{Alpha.Score} \sim \text{Diet:Timepoint})$, family = quasibinomial)

metric	Diet	.y.	term	group1	group2	estimate	std.error	statistic	p.adj	p.adj.signif
Observed	Gemma	Alpha.Score	Timepoint	7mpf	4mpf	0.221	0.202	1.098	0.272146936	ns
	Watts	Alpha.Score	Timepoint	7mpf	4mpf	0.240	0.235	1.021	0.307235782	ns
	ZIRC	Alpha.Score	Timepoint	7mpf	4mpf	0.599	0.253	2.368	0.017880750	*
Shannon	Gemma	Alpha.Score	Timepoint	7mpf	4mpf	0.524	0.237	2.207	0.027347628	*
	Watts	Alpha.Score	Timepoint	7mpf	4mpf	0.064	0.261	0.244	0.807112385	ns
	ZIRC	Alpha.Score	Timepoint	7mpf	4mpf	0.679	0.247	2.751	0.005942612	*
Simpson	Gemma	Alpha.Score	Timepoint	7mpf	4mpf	0.541	0.266	2.038	0.041511288	*
	Watts	Alpha.Score	Timepoint	7mpf	4mpf	-0.106	0.240	-0.440	0.659997952	ns
	ZIRC	Alpha.Score	Timepoint	7mpf	4mpf	0.536	0.245	2.190	0.028493826	*

2.4) Beta-diversity

2.4.1)

Distance-based redundancy analysis (dbRDA) ordination. Beta.Score ~ Diet

metric	term	Df	SumOfSqs	statistic	p.value	sig
Bray-Curtis	Diet	2.00	1.322325	13.377	0.001	*
	Residual	132.00	6.523933			
Canberra	Diet	2.00	3.518408	8.676	0.001	*
	Residual	132.00	26.765643			
Sørensen	Diet	2.00	1.794303	11.879	0.001	*
	Residual	132.00	9.969118			

2.4.2)

Distance-based redundancy analysis (dbRDA) ordination. Beta.Score ~ Time

metric	term	Df	SumOfSqs	statistic	p.value	sig
Bray-Curtis	Timepoint	1.00	0.3456869	6.130	0.001	*
	Residual	133.00	7.5005708			
Canberra	Timepoint	1.00	1.5652766	7.249	0.001	*
	Residual	133.00	28.7187751			
Sørensen	Timepoint	1.00	1.0030415	12.398	0.001	*
	Residual	133.00	10.7603792			

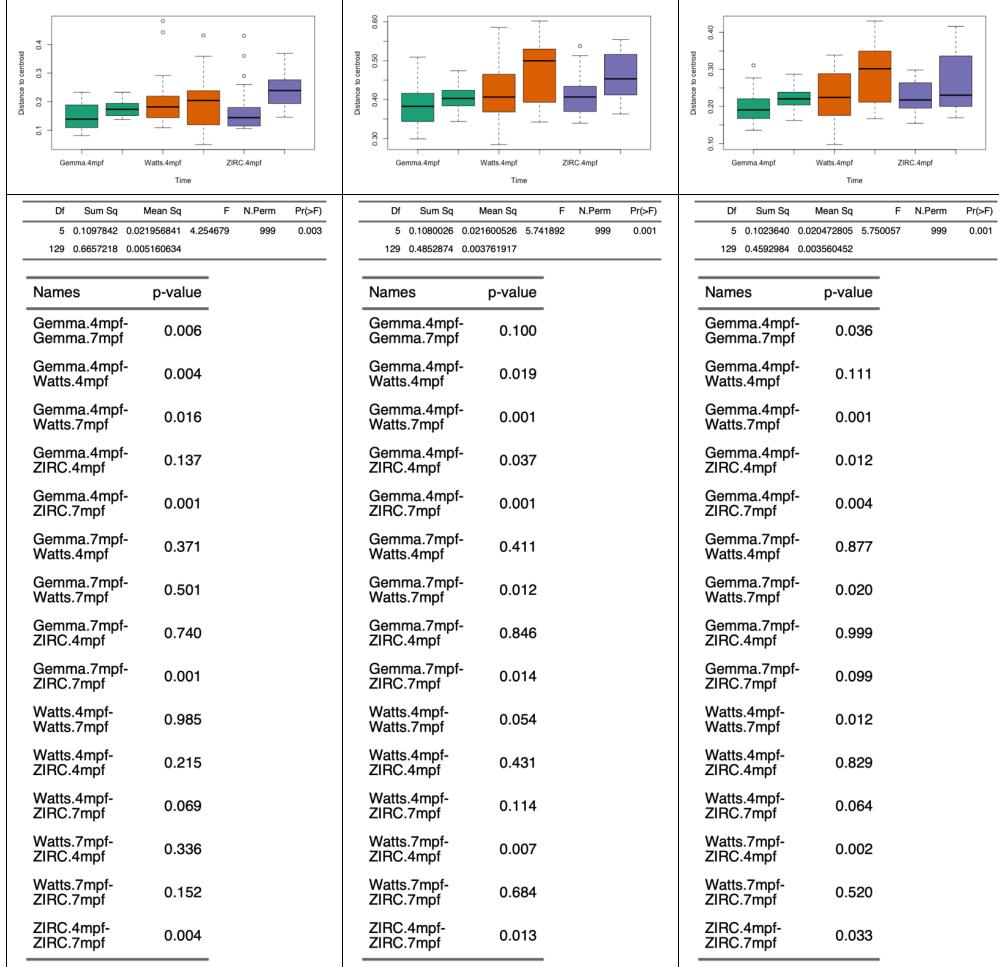
2.4.3)

2.5) Beta-Dispersion

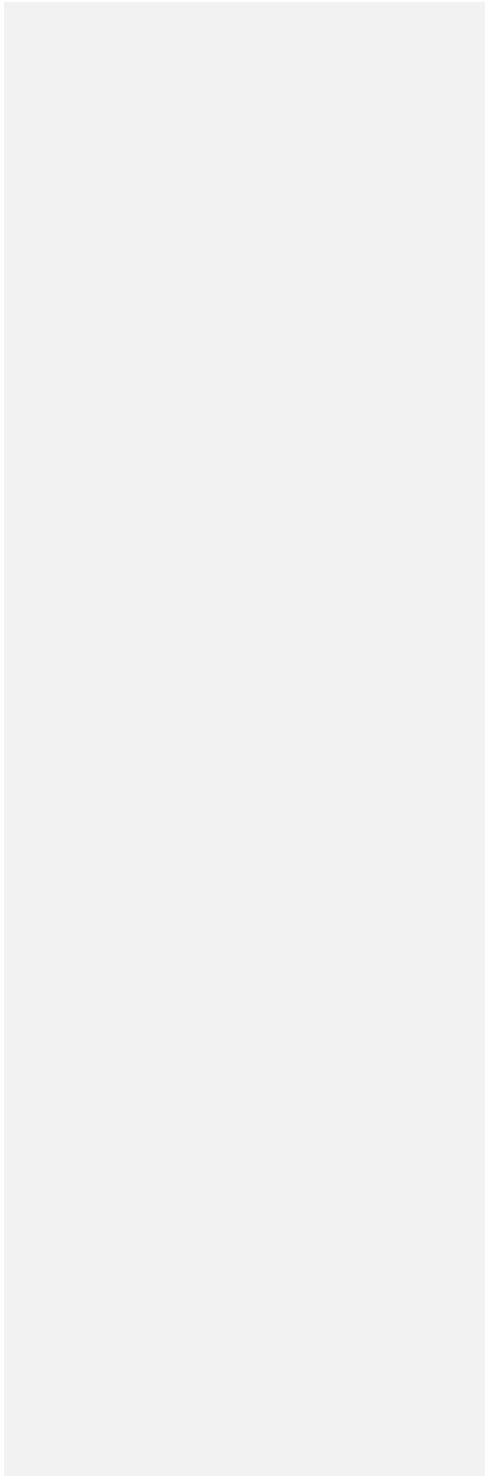
2.5.1) Diet

2.5.2) Time

2.5.3) Diet:Time

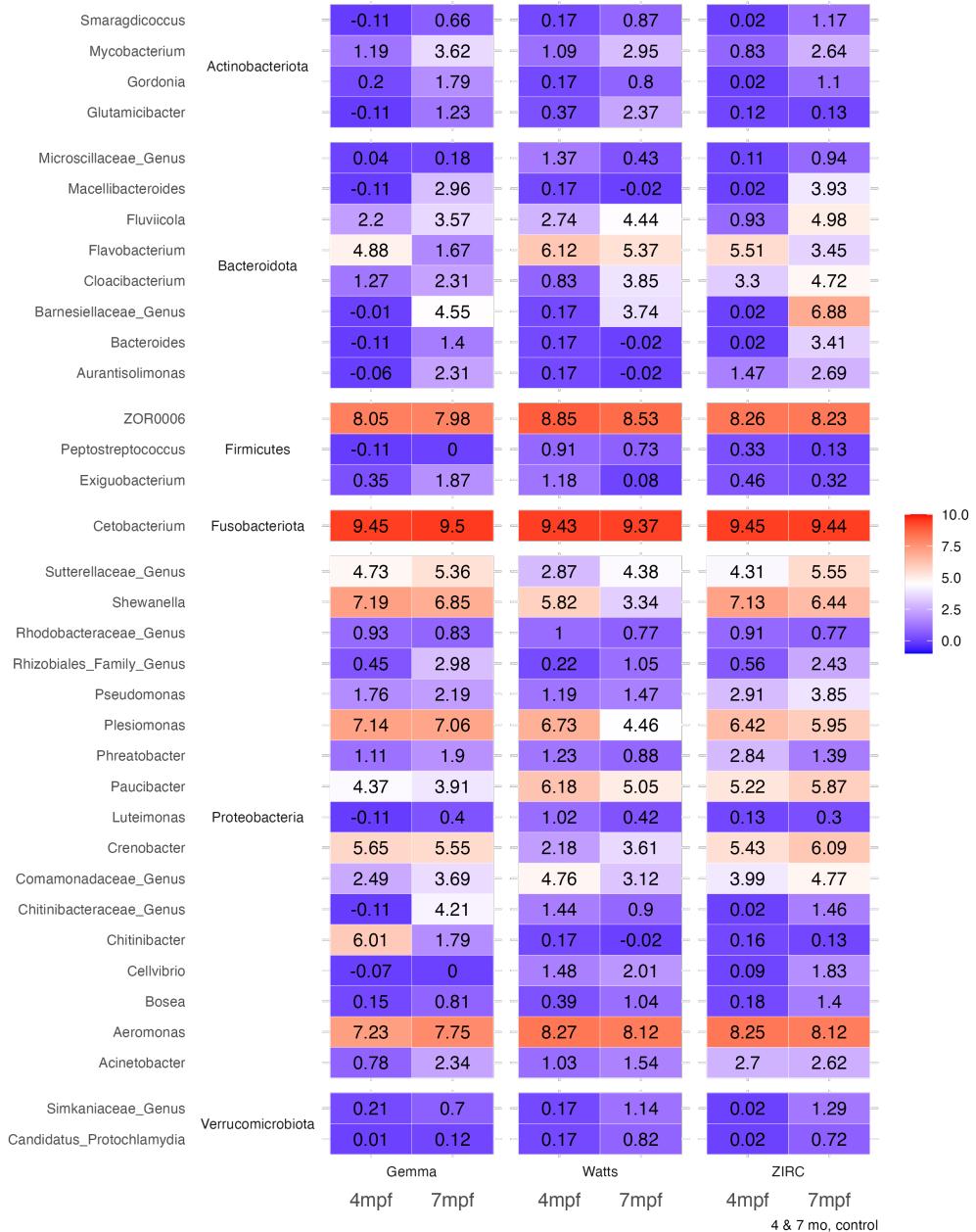


2.6 Differential Abundance



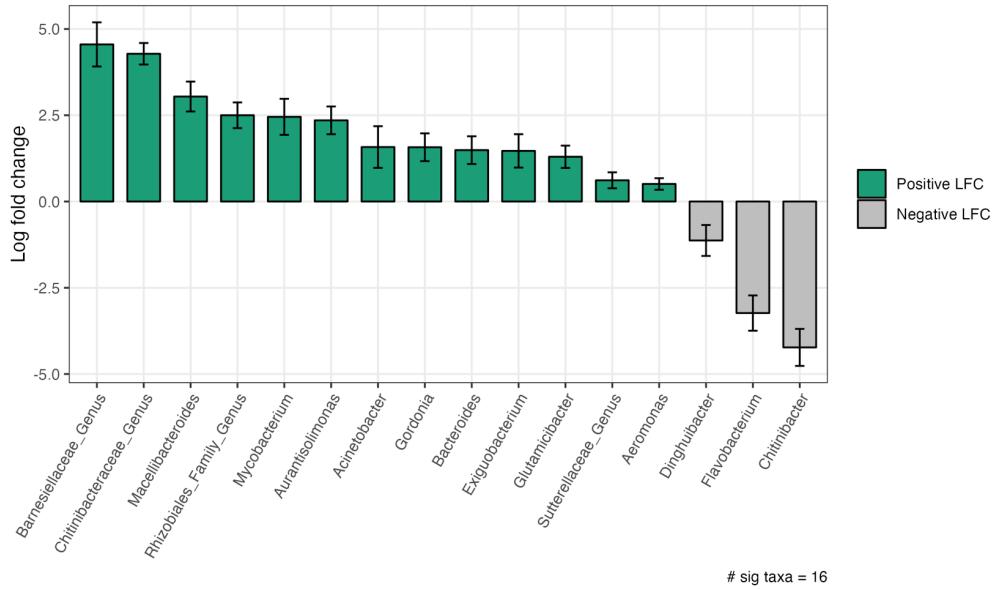
2.6.1)

Heat map of bias-corrected log observed abundances (Genus)



2.6.2)
2.6.2.1)

Log fold changes between 4 and 7 months in Gemma diet

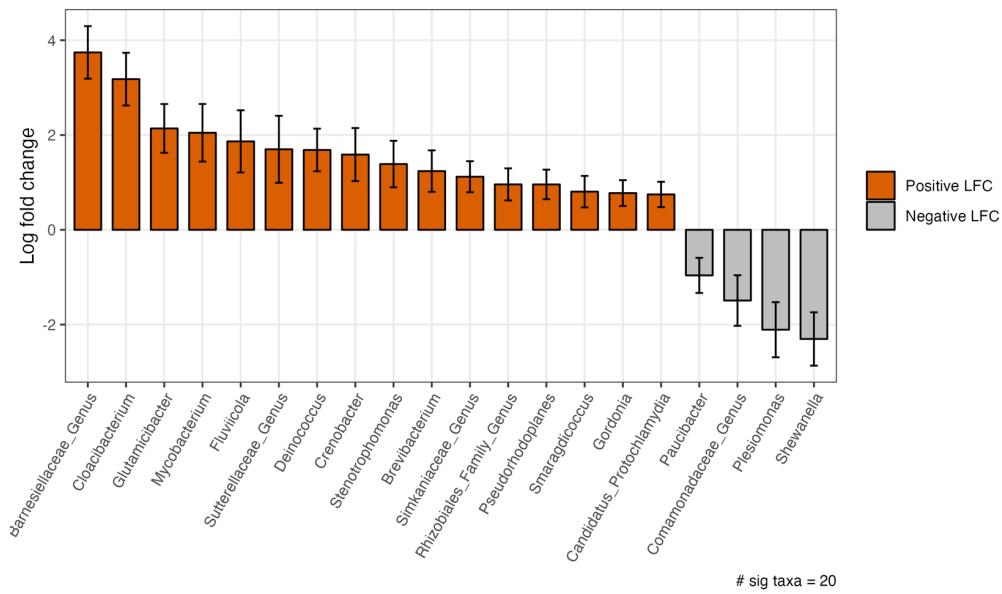


ANCOM-BC2: Log fold change in abundance. # Sig taxa = 16

Taxon	lfc_Timepoint7mpf	diff_Timepoint7mpf	direct
Barnesiellaceae_Genus	4.553613	TRUE	Positive LFC
Chitinibacteraceae_Genus	4.282578	TRUE	Positive LFC
Macellibacteroides	3.042763	TRUE	Positive LFC
Rhizobiales_Family_Genus	2.500217	TRUE	Positive LFC
Mycobacterium	2.454195	TRUE	Positive LFC
Aurantisolimonas	2.352514	TRUE	Positive LFC
Acinetobacter	1.578923	TRUE	Positive LFC
Gordonia	1.573270	TRUE	Positive LFC
Bacteroides	1.489166	TRUE	Positive LFC
Exiguobacterium	1.467458	TRUE	Positive LFC
Glutamicibacter	1.296455	TRUE	Positive LFC
Sutterellaceae_Genus	0.614859	TRUE	Positive LFC
Aeromonas	0.508558	TRUE	Positive LFC
Dinghuibacter	-1.129201	TRUE	Negative LFC
Flavobacterium	-3.233255	TRUE	Negative LFC
Chitinibacter	-4.228535	TRUE	Negative LFC

2.6.2.2)

Log fold changes between 4 and 7 months in Watts diet

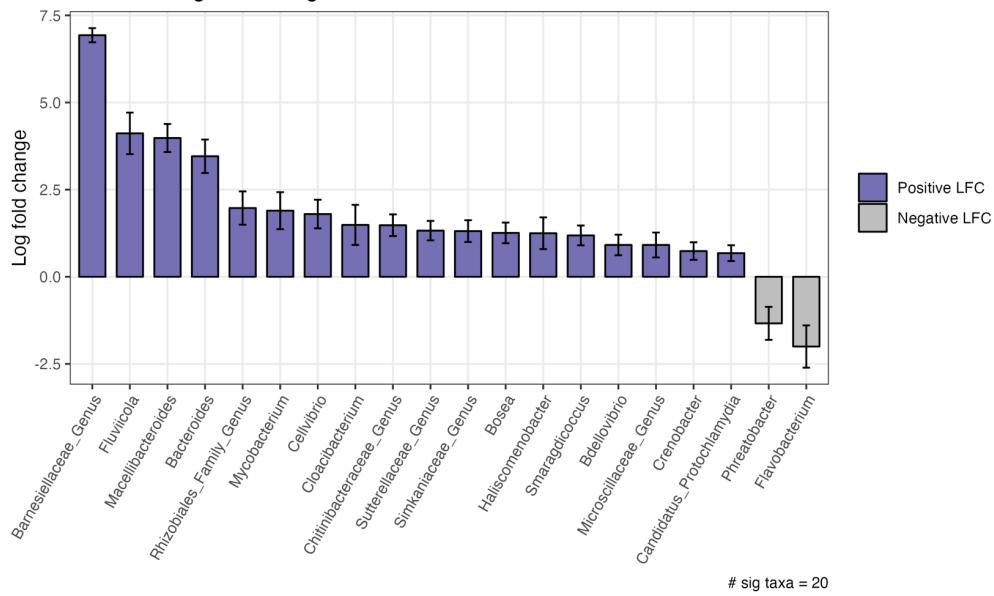


ANCOM-BC2: Log fold change in abundance. # Sig taxa = 20

Taxon	lfc_Timepoint7mpf	diff_Timepoint7mpf	direct
Barnesiellaceae_Genus	3.743419	TRUE	Positive LFC
Cloacibacterium	3.179109	TRUE	Positive LFC
Glutamicibacter	2.139606	TRUE	Positive LFC
Mycobacterium	2.047308	TRUE	Positive LFC
Fluviicola	1.865743	TRUE	Positive LFC
Sutterellaceae_Genus	1.699197	TRUE	Positive LFC
Deinococcus	1.684537	TRUE	Positive LFC
Crenobacter	1.587856	TRUE	Positive LFC
Stenotrophomonas	1.387593	TRUE	Positive LFC
Brevibacterium	1.238637	TRUE	Positive LFC
Simkaniaceae_Genus	1.120585	TRUE	Positive LFC
Rhizobiales_Family_Genus	0.958539	TRUE	Positive LFC
Pseudorhodoplanes	0.957715	TRUE	Positive LFC
Smaragdicoccus	0.805331	TRUE	Positive LFC
Gordonia	0.775047	TRUE	Positive LFC
Candidatus_Protochlamydia	0.746518	TRUE	Positive LFC
Paucibacter	-0.961683	TRUE	Negative LFC
Comamonadaceae_Genus	-1.492398	TRUE	Negative LFC
Plesiomonas	-2.108167	TRUE	Negative LFC
Shewanella	-2.303035	TRUE	Negative LFC

2.6.2.3)

Log fold changes between 4 and 7 months in ZIRC diet

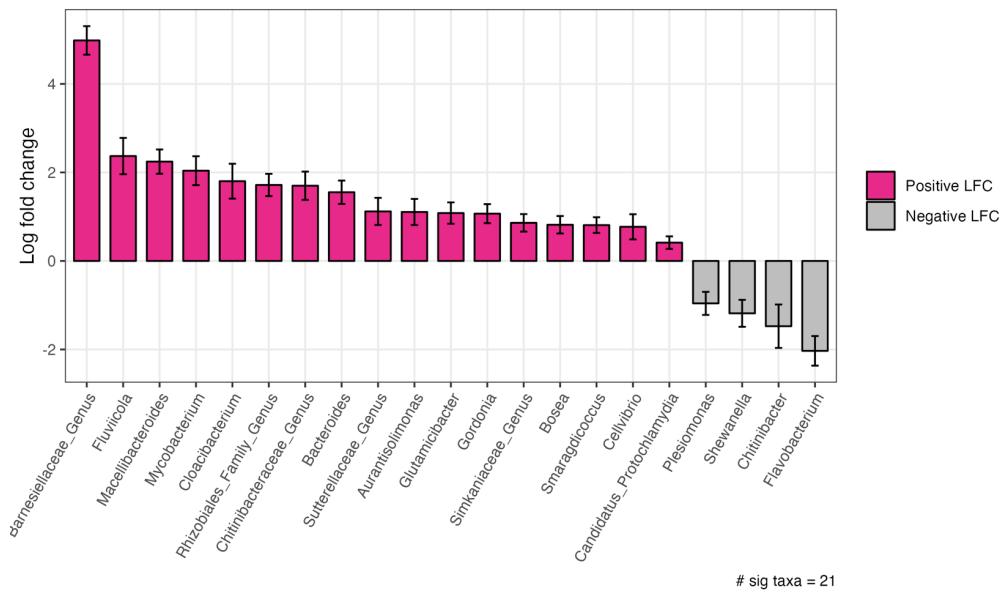


ANCOM-BC2: Log fold change in abundance. # Sig taxa = 20

Taxon	lfc_Timepoint7mpf	diff_Timepoint7mpf	direct
Barnesiellaceae_Genus	6.931000	TRUE	Positive LFC
Fluviicola	4.114707	TRUE	Positive LFC
Macellibacteroides	3.981390	TRUE	Positive LFC
Bacteroides	3.457592	TRUE	Positive LFC
Rhizobiales_Family_Genus	1.971942	TRUE	Positive LFC
Mycobacterium	1.896726	TRUE	Positive LFC
Cellvibrio	1.799895	TRUE	Positive LFC
Cloacibacterium	1.488282	TRUE	Positive LFC
Chitinibacteraceae_Genus	1.479288	TRUE	Positive LFC
Sutterellaceae_Genus	1.324464	TRUE	Positive LFC
Simkaniaceae_Genus	1.310535	TRUE	Positive LFC
Bosea	1.259080	TRUE	Positive LFC
Haliscomenobacter	1.249192	TRUE	Positive LFC
Smaragdicoccus	1.186623	TRUE	Positive LFC
Bdellovibrio	0.912548	TRUE	Positive LFC
Microscillaceae_Genus	0.912548	TRUE	Positive LFC
Crenobacter	0.737747	TRUE	Positive LFC
Candidatus_Protochlamydia	0.678023	TRUE	Positive LFC
Phreatobacter	-1.336283	TRUE	Negative LFC
Flavobacterium	-2.000803	TRUE	Negative LFC

2.6.2.4)

Log fold changes between 4 and 7 months across all diets



ANCOM-BC2: Log fold change in abundance. # Sig taxa = 21

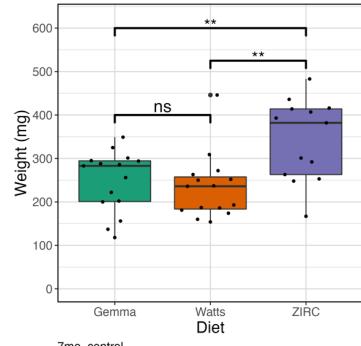
Taxon	lfc_Timepoint7mpf	diff_Timepoint7mpf	direct
Barnesiellaceae_Genus	4.982655	TRUE	Positive LFC
Fluviicola	2.370006	TRUE	Positive LFC
Macellibacteroides	2.244773	TRUE	Positive LFC
Mycobacterium	2.039387	TRUE	Positive LFC
Cloacibacterium	1.802020	TRUE	Positive LFC
Rhizobiales_Family_Genus	1.716877	TRUE	Positive LFC
Chitinibacteraceae_Genus	1.699741	TRUE	Positive LFC
Bacteroides	1.552308	TRUE	Positive LFC
Sutterellaceae_Genus	1.119485	TRUE	Positive LFC
Aurantisolimonas	1.106538	TRUE	Positive LFC
Glutamicibacter	1.081902	TRUE	Positive LFC
Gordonia	1.068741	TRUE	Positive LFC
Simkaniaceae_Genus	0.860878	TRUE	Positive LFC
Bosea	0.817625	TRUE	Positive LFC
Smaragdiloccus	0.810308	TRUE	Positive LFC
Cellvibrio	0.771431	TRUE	Positive LFC
Candidatus_Protochlamydia	0.413585	TRUE	Positive LFC
Plesiomonas	-0.958759	TRUE	Negative LFC
Shewanella	-1.183472	TRUE	Negative LFC
Chitinibacter	-1.474310	TRUE	Negative LFC
Flavobacterium	-2.031275	TRUE	Negative LFC

2.6.3)

2.7) 7 Month Analysis

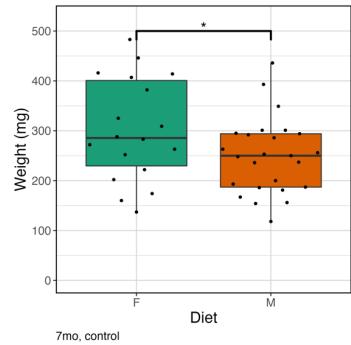
2.7.1) Physiology

2.7.1.1) Weight ~ Diet



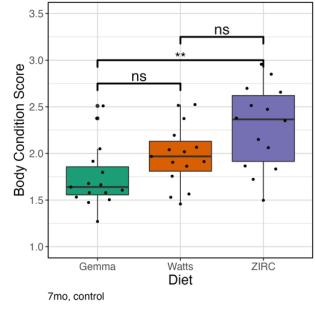
Wilcoxon Test. p. adj: BH. Weight ~ Diet								
.y.	group1	group2	n1	n2	statistic	p	p.adj	p.adj.signif
Weight	Gemma	Watts	15	15	139.000	0.285	0.285	ns
	Gemma	ZIRC	15	15	45.500	0.006	0.009	**
	Watts	ZIRC	15	15	36.500	0.002	0.006	**

2.7.1.2) Weight ~ Sex



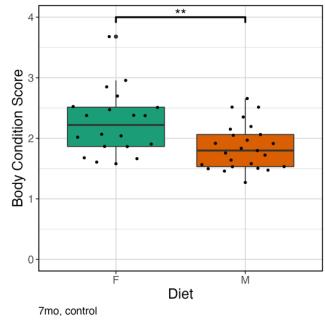
Wilcoxon Test. p. adj: BH. Weight ~ Sex								
.y.	group1	group2	n1	n2	statistic	p	p.adj	p.adj.signif
Weight	F	M	20	25	342.500	0.036	0.036	*

2.7.1.3) Body Condition Score ~ Diet



Wilcoxon Test. p. adj: BH. Body.Condition.Score ~ Diet								
.y.	group1	group2	n1	n2	statistic	p	p.adj	p.adj.signif
Body.Condition.Score	Gemma	Watts	15	15	69.000	0.074	0.074	ns
	Gemma	ZIRC	15	15	34.000	<0.001	0.002	**
	Watts	ZIRC	15	15	65.000	0.050	0.074	ns

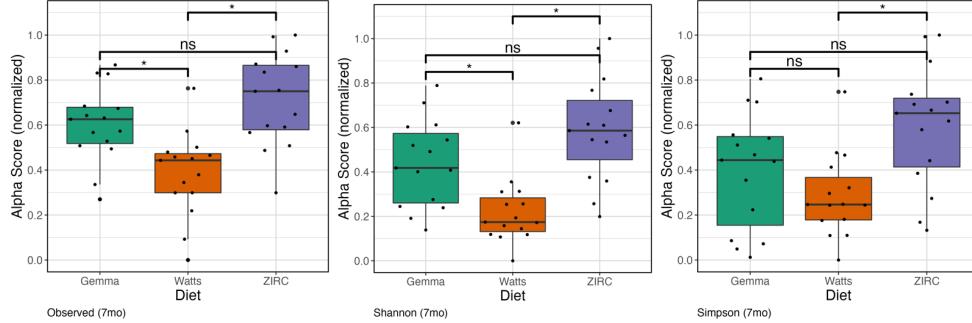
2.7.1.4) Body Condition Score ~ Sex



Wilcoxon Test, p. adj: BH. Body.Condition.Score ~ Sex						
y.	group1	group2	n1	n2	statistic	p
Body.Condition.Score	F	M	20	25	366.000	0.007

p.adj 0.007 **

2.7.2) Alpha-Diversity



`glm(Alpha.Score ~ Diet, family = quasibinomial)`

metric	term	estimate	std.error	statistic	p.value	sig
Observed	(Intercept)	0.422	0.210	2.010	0.051	
	DietWatts	-0.893	0.298	-2.997	0.005	*
	DietZIRC	0.486	0.309	1.571	0.124	
Shannon	(Intercept)	-0.245	0.213	-1.152	0.256	
	DietWatts	-1.022	0.332	-3.074	0.004	*
	DietZIRC	0.613	0.303	2.026	0.049	*
Simpson	(Intercept)	-0.412	0.263	-1.568	0.124	
	DietWatts	-0.506	0.388	-1.304	0.199	
	DietZIRC	0.797	0.372	2.145	0.038	*

`ANOVA(glm(Alpha.Score ~ Diet), family = quasibinomial)`

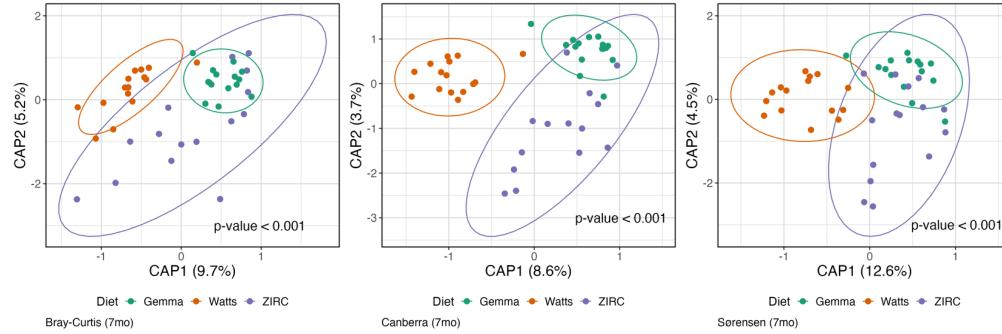
metric	term	statistic	df	p.value	sig
Observed	Diet	21.789	2	<0.001	*
Shannon	Diet	26.612	2	<0.001	*
Simpson	Diet	12.230	2	0.002	*

`Pairwise Tukey's HSD, p.adj: Dunnett. glm(Alpha.Score ~ Diet), family = quasibinomial)`

metric	term	.y.	group1	group2	estimate	std.error	statistic	p.adj	p.adj.signif
Observed	Diet	Alpha.Score	Watts	Gemma	-0.893	0.298	-2.997	0.008	*
	Diet	Alpha.Score	ZIRC	Gemma	0.486	0.309	1.571	0.258	ns
	Diet	Alpha.Score	ZIRC	Watts	1.378	0.310	4.446	0.000	*
Shannon	Diet	Alpha.Score	Watts	Gemma	-1.022	0.332	-3.074	0.006	*
	Diet	Alpha.Score	ZIRC	Gemma	0.613	0.303	2.026	0.106	ns
	Diet	Alpha.Score	ZIRC	Watts	1.635	0.334	4.899	0.000	*
Simpson	Diet	Alpha.Score	Watts	Gemma	-0.506	0.388	-1.304	0.393	ns
	Diet	Alpha.Score	ZIRC	Gemma	0.797	0.372	2.145	0.081	ns
	Diet	Alpha.Score	ZIRC	Watts	1.303	0.387	3.362	0.002	*

2.7.3) Beta-Diversity

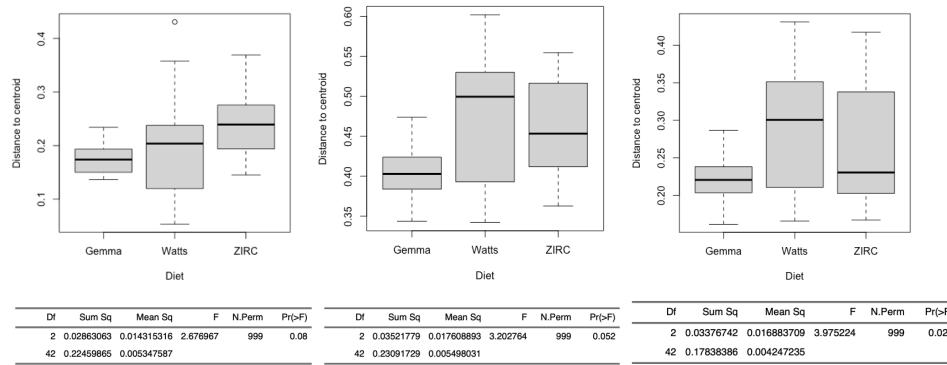
2.7.3.1)



Distance-based redundancy analysis (dbRDA) ordination. Beta.Score ~ Diet

metric	term	Df	SumOfSqs	statistic	p.value	sig
Bray-Curtis	Diet	2.00	0.3910404	3.676	0.001	*
	Residual	42.00	2.2337863			
Canberra	Diet	2.00	1.2821935	2.957	0.001	*
	Residual	42.00	9.1054866			
Sørensen	Diet	2.00	0.6800481	4.334	0.001	*
	Residual	42.00	3.2953340			

2.7.3.2) Beta-dispersion (Bray, Canberra, Sørensen)



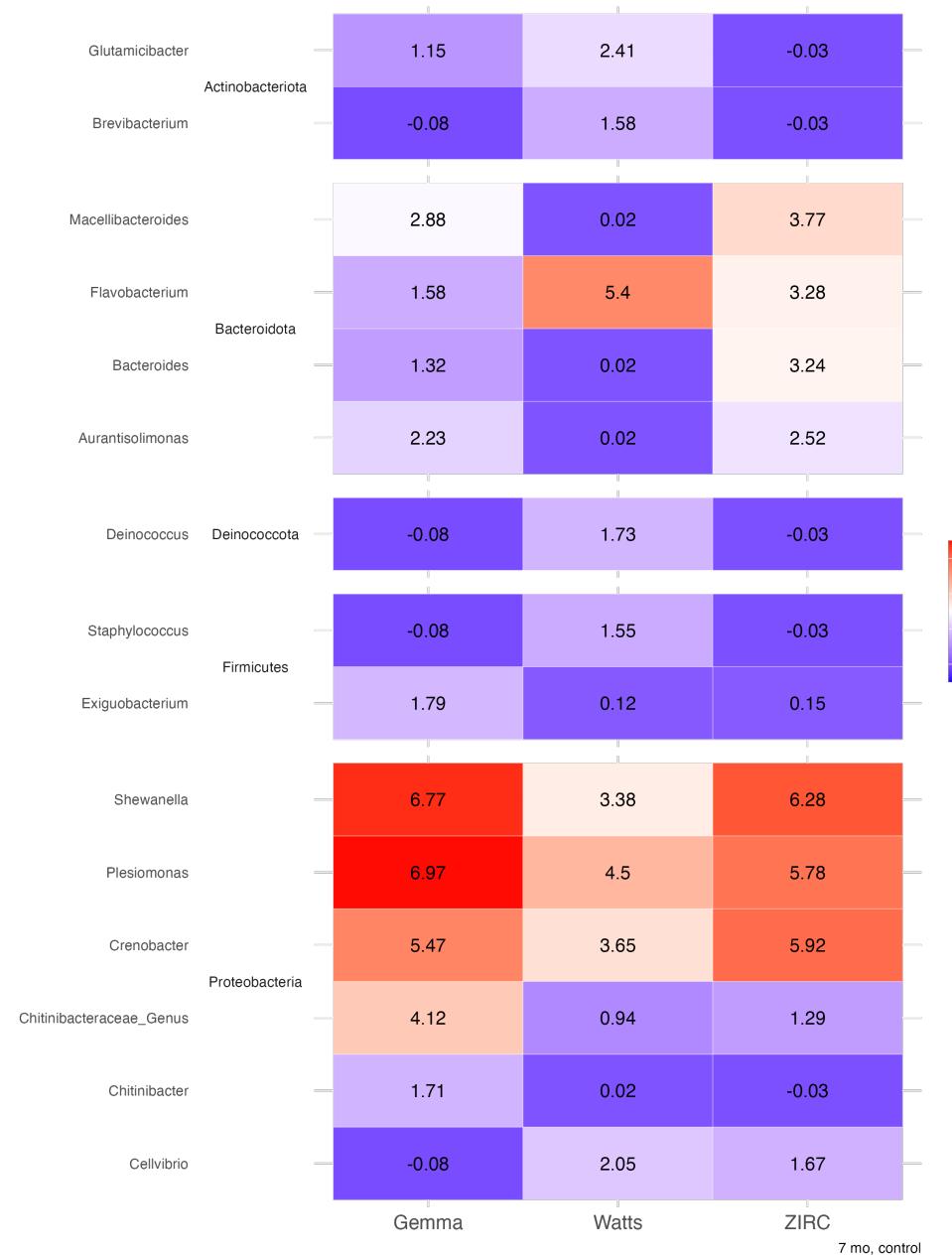
Names	p-value
Gemma-Watts	0.022
Gemma-ZIRC	0.103
Watts-ZIRC	0.535

Names	p-value
Gemma-Watts	0.484
Gemma-ZIRC	0.001
Watts-ZIRC	0.149

Names	p-value
Gemma-Watts	0.017
Gemma-ZIRC	0.017
Watts-ZIRC	0.701

2.7.4) Differential Abundance

Heat map of bias-corrected log observed abundances (Genus)



ANCOM-BC2: Summary statistics of abundant taxa (4mo, controls), sig taxa = 15

Taxon	W	p_val	q_val	diff_abn
Aurantisolimonas	15.001	0.001	0.007	TRUE
Bacteroides	17.071	0.000	0.004	TRUE
Brevibacterium	14.582	0.001	0.008	TRUE
Cellvibrio	11.925	0.005	0.024	TRUE
Chitinibacter	11.271	0.007	0.029	TRUE
Chitinibacteraceae_Genus	36.215	0.000	0.000	TRUE
Crenobacter	35.860	0.000	0.000	TRUE
Deinococcus	12.846	0.003	0.016	TRUE
Exiguobacterium	15.719	0.001	0.007	TRUE
Flavobacterium	23.363	0.000	0.000	TRUE
Glutamicibacter	11.384	0.007	0.029	TRUE
Macellibacteroides	27.900	0.000	0.000	TRUE
Plesiomonas	14.047	0.002	0.010	TRUE
Shewanella	27.209	0.000	0.000	TRUE
Staphylococcus	15.177	0.001	0.007	TRUE

3) Exposure

3.1) Alpha-diversity

3.1.1) Exposure

3.1.1.1)

`glm(Alpha.Score ~ Exposure), family = quasibinomial)`

metric	term	estimate	std.error	statistic	p.value	sig
Observed	(Intercept)	0.058	0.078	0.739	0.461	
	PrePostExpExposed	-0.184	0.136	-1.351	0.178	
Shannon	PrePostExpUnexposed	0.302	0.136	2.218	0.028	*
	(Intercept)	-0.415	0.081	-5.140	<0.001	*
Simpson	PrePostExpExposed	-0.129	0.142	-0.907	0.366	
	PrePostExpUnexposed	0.383	0.138	2.780	0.006	*
Simpson	(Intercept)	-0.243	0.085	-2.852	0.005	*
	PrePostExpExposed	-0.152	0.150	-1.013	0.313	
	PrePostExpUnexposed	0.314	0.147	2.135	0.034	*

3.1.1.2)

`ANOVA(glm(Alpha.Score ~ Exposure), family = quasibinomial)`

metric	term	statistic	df	p.value	sig
Observed	PrePostExp	9.833	2	0.007	*
Shannon	PrePostExp	11.495	2	0.003	*
Simpson	PrePostExp	7.893	2	0.019	*

3.1.1.3)

Pairwise Tukey's HSD, p.adj: Dunnett, glm(Alpha.Score ~ Exposure), family = quasibinomial)

metric	term	.y.	group1	group2	estimate	std.error	statistic	p.adj	p.adj.signif
Observed	PrePostExp	Alpha.Score	Exposed	Pre-exposure	-0.184	0.136	-1.351	0.365	ns
	PrePostExp	Alpha.Score	Unexposed	Pre-exposure	0.302	0.136	2.218	0.068	ns
	PrePostExp	Alpha.Score	Unexposed	Exposed	0.486	0.158	3.077	0.006	*
Shannon	PrePostExp	Alpha.Score	Exposed	Pre-exposure	-0.129	0.142	-0.907	0.634	ns
	PrePostExp	Alpha.Score	Unexposed	Pre-exposure	0.383	0.138	2.780	0.015	*
	PrePostExp	Alpha.Score	Unexposed	Exposed	0.512	0.162	3.163	0.004	*
Simpson	PrePostExp	Alpha.Score	Exposed	Pre-exposure	-0.152	0.150	-1.013	0.567	ns
	PrePostExp	Alpha.Score	Unexposed	Pre-exposure	0.314	0.147	2.135	0.082	ns
	PrePostExp	Alpha.Score	Unexposed	Exposed	0.466	0.172	2.708	0.018	*

3.1.2) Diet:Exposure

3.1.2.1)

`glm(Alpha.Score ~ Diet*Exposure), family = quasibinomial)`

metric	term	estimate	std.error	statistic	p.value	sig
Observed	(Intercept)	0.281	0.119	2.360	0.019	*
	DietWatts	-1.029	0.172	-5.977	<0.001	*
	DietZIRC	0.003	0.167	0.020	0.984	
	ExposureUnexposed	0.133	0.168	0.791	0.430	
	DietWatts:ExposureUnexposed	0.365	0.240	1.525	0.129	
Shannon	DietZIRC:ExposureUnexposed	0.118	0.238	0.496	0.620	
	(Intercept)	-0.255	0.130	-1.962	0.051	
	DietWatts	-0.746	0.193	-3.856	<0.001	*
	DietZIRC	0.073	0.182	0.399	0.690	
	ExposureUnexposed	0.046	0.182	0.254	0.800	
Simpson	DietWatts:ExposureUnexposed	0.268	0.268	1.000	0.319	
	DietZIRC:ExposureUnexposed	0.306	0.256	1.196	0.233	
	(Intercept)	-0.337	0.149	-2.259	0.025	*
	DietWatts	-0.104	0.210	-0.497	0.620	
	DietZIRC	0.282	0.208	1.359	0.176	
	ExposureUnexposed	-0.019	0.209	-0.092	0.927	
	DietWatts:ExposureUnexposed	0.140	0.295	0.475	0.635	
	DietZIRC:ExposureUnexposed	0.365	0.293	1.245	0.215	

3.1.2.2)

ANOVA(`glm(Alpha.Score ~ Diet*Exposure, family = quasibinomial)`
)

metric	term	statistic	df	p.value	sig
Observed	Diet	73.335	2	<0.001	*
	Exposure	9.052	1	0.003	*
	Diet:Exposure	2.422	2	0.298	
Shannon	Diet	42.879	2	<0.001	*
	Exposure	4.822	1	0.028	*
	Diet:Exposure	1.663	2	0.435	
Simpson	Diet	14.704	2	<0.001	*
	Exposure	1.604	1	0.205	
	Diet:Exposure	1.584	2	0.453	

3.1.2.3)

Pairwise Tukey's HSD, p.adj: Dunnett, glm(Alpha.Score ~ Diet*Exposure), family = quasibinomial)

metric	Diet	.y.	term	group1	group2	estimate	std.error	statistic	p.adj	p.adj.signif
Observed	Gemma	Alpha.Score	PrePostExp	Exposed	Pre-exposure	0.186	0.182	1.024	0.559706995	ns
	Gemma	Alpha.Score	PrePostExp	Unexposed	Pre-exposure	0.202	0.178	1.132	0.492192001	ns
	Gemma	Alpha.Score	PrePostExp	Unexposed	Exposed	0.015	0.210	0.072	0.997134904	ns
	Watts	Alpha.Score	PrePostExp	Exposed	Pre-exposure	-0.478	0.207	-2.306	0.054533215	ns
	Watts	Alpha.Score	PrePostExp	Unexposed	Pre-exposure	0.192	0.195	0.985	0.584424162	ns
	Watts	Alpha.Score	PrePostExp	Unexposed	Exposed	0.669	0.234	2.854	0.011635396	*
	ZIRC	Alpha.Score	PrePostExp	Exposed	Pre-exposure	-0.270	0.225	-1.201	0.450517738	ns
	ZIRC	Alpha.Score	PrePostExp	Unexposed	Pre-exposure	0.569	0.241	2.358	0.047995118	*
	ZIRC	Alpha.Score	PrePostExp	Unexposed	Exposed	0.839	0.273	3.075	0.005999463	*
	Gemma	Alpha.Score	PrePostExp	Exposed	Pre-exposure	0.304	0.198	1.534	0.273479468	ns
Shannon	Gemma	Alpha.Score	PrePostExp	Unexposed	Pre-exposure	0.473	0.193	2.445	0.037980767	*
	Gemma	Alpha.Score	PrePostExp	Unexposed	Exposed	0.169	0.226	0.749	0.732997489	ns
	Watts	Alpha.Score	PrePostExp	Exposed	Pre-exposure	-0.323	0.242	-1.334	0.373916255	ns
	Watts	Alpha.Score	PrePostExp	Unexposed	Pre-exposure	0.016	0.230	0.071	0.997231607	ns
	Watts	Alpha.Score	PrePostExp	Unexposed	Exposed	0.339	0.276	1.230	0.433509025	ns
	ZIRC	Alpha.Score	PrePostExp	Exposed	Pre-exposure	-0.381	0.232	-1.642	0.226260505	ns
	ZIRC	Alpha.Score	PrePostExp	Unexposed	Pre-exposure	0.668	0.235	2.843	0.012642149	*
	ZIRC	Alpha.Score	PrePostExp	Unexposed	Exposed	1.049	0.273	3.848	0.000315111	*
	Gemma	Alpha.Score	PrePostExp	Exposed	Pre-exposure	0.320	0.252	1.271	0.410191951	ns
	Gemma	Alpha.Score	PrePostExp	Unexposed	Pre-exposure	0.541	0.245	2.209	0.069115575	ns
Simpson	Gemma	Alpha.Score	PrePostExp	Unexposed	Exposed	0.221	0.285	0.776	0.716834746	ns
	Watts	Alpha.Score	PrePostExp	Exposed	Pre-exposure	-0.206	0.245	-0.840	0.676434035	ns
	Watts	Alpha.Score	PrePostExp	Unexposed	Pre-exposure	-0.106	0.244	-0.434	0.901028218	ns
	Watts	Alpha.Score	PrePostExp	Unexposed	Exposed	0.101	0.284	0.355	0.932604171	ns
	ZIRC	Alpha.Score	PrePostExp	Exposed	Pre-exposure	-0.550	0.252	-2.182	0.073672736	ns
	ZIRC	Alpha.Score	PrePostExp	Unexposed	Pre-exposure	0.536	0.257	2.085	0.092138722	ns
	ZIRC	Alpha.Score	PrePostExp	Unexposed	Exposed	1.086	0.297	3.651	0.000739211	*

3.2) Beta-diversity

Distance-based redundancy analysis (dbRDA) ordination. Beta.Score ~ Exposure

metric	term	Df	SumOfSqs	statistic	p.value	sig
Bray-Curtis	PrePostExp	1.00	0.1424569	2.285	0.029	*
	Residual	85.00	5.2981891			
Canberra	PrePostExp	1.00	0.5430310	2.236	0.001	*
	Residual	85.00	20.6384862			
Sørensen	PrePostExp	1.00	0.3442625	3.562	0.001	*
	Residual	85.00	8.2148177			

3.2.1)

Distance-based redundancy analysis (dbRDA) ordination. Beta.Score ~ Diets

metric	term	Df	SumOfSqs	statistic	p.value	sig
Bray-Curtis	Diet	2.00	0.8277048	7.536	0.001	*
	Residual	84.00	4.6129411			
Canberra	Diet	2.00	2.2980939	5.111	0.001	*
	Residual	84.00	18.8834233			
Sørensen	Diet	2.00	1.2544358	7.213	0.001	*
	Residual	84.00	7.3046444			

3.2.2)

Distance-based redundancy analysis (dbRDA) ordination. Beta.Score ~ Diets

metric	term	Df	SumOfSqs	statistic	p.value	sig
Bray-Curtis	Diet	2.00	0.8277048	7.770	0.001	*
	PrePostExp	1.00	0.1487798	2.793	0.012	*
	Diet:PrePostExp	2.00	0.1496117	1.404	0.150	
	Residual	81.00	4.3145496			
Canberra	Diet	2.00	2.2980939	5.302	0.001	*
	PrePostExp	1.00	0.5498407	2.537	0.001	*
	Diet:PrePostExp	2.00	0.7777731	1.794	0.001	*
	Residual	81.00	17.5558095			
Sørensen	Diet	2.00	1.2544358	7.676	0.001	*
	PrePostExp	1.00	0.3486593	4.267	0.001	*
	Diet:PrePostExp	2.00	0.3376534	2.066	0.001	*
	Residual	81.00	6.6183317			

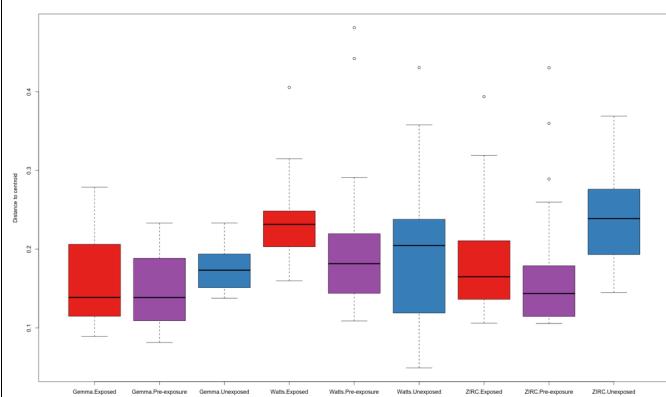
3.2.3

3.3) Beta-Dispersion

3.3.1) Exposure

3.3.2) Diet:Exposure

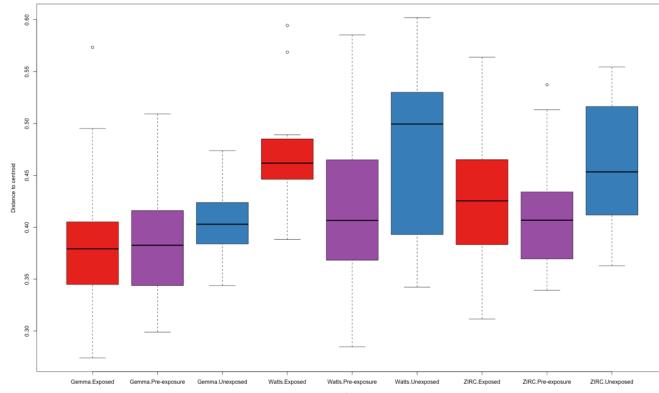
Bray-Curtis



Df	Sum Sq	Mean Sq	F	N.Perm	Pr(>F)
8	0.1676731	0.020959131	4.194491	999	0.001
170	0.8494599	0.004996823			

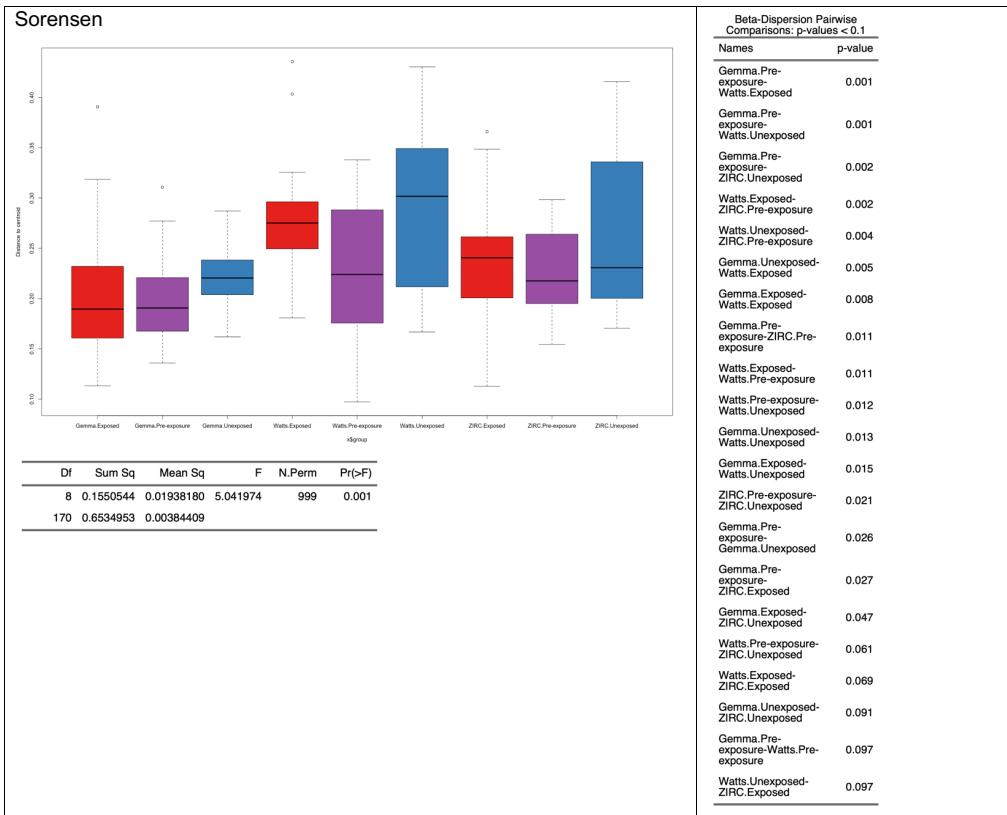
Beta-Dispersion Pairwise Comparisons: p-values < 0.1	
Names	p-value
Gemma.Exposed-ZIRC.Unexposed	0.001
Gemma.Pre-exposure-Watts.Exposed	0.001
Gemma.Pre-exposure-ZIRC.Unexposed	0.001
Gemma.Exposed-Watts.Exposed	0.002
Gemma.Unexposed-Watts.Exposed	0.002
Gemma.Unexposed-ZIRC.Unexposed	0.002
ZIRC.Pre-exposure-ZIRC.Unexposed	0.005
Gemma.Pre-exposure-Watts.Pre-exposure	0.006
Watts.Exposed-ZIRC.Pre-exposure	0.008
Gemma.Pre-exposure-Gemma.Unexposed	0.014
Gemma.Pre-exposure-ZIRC.Exposed	0.021
Gemma.Pre-exposure-Watts.Unexposed	0.028
ZIRC.Exposed-ZIRC.Unexposed	0.049
Watts.Pre-exposure-ZIRC.Unexposed	0.061
Watts.Exposed-ZIRC.Exposed	0.065
Watts.Exposed-Watts.Pre-exposure	0.091

Canberra



Df	Sum Sq	Mean Sq	F	N.Perm	Pr(>F)
8	0.1648724	0.020609053	5.179553	999	0.001
170	0.6764172	0.003978925			

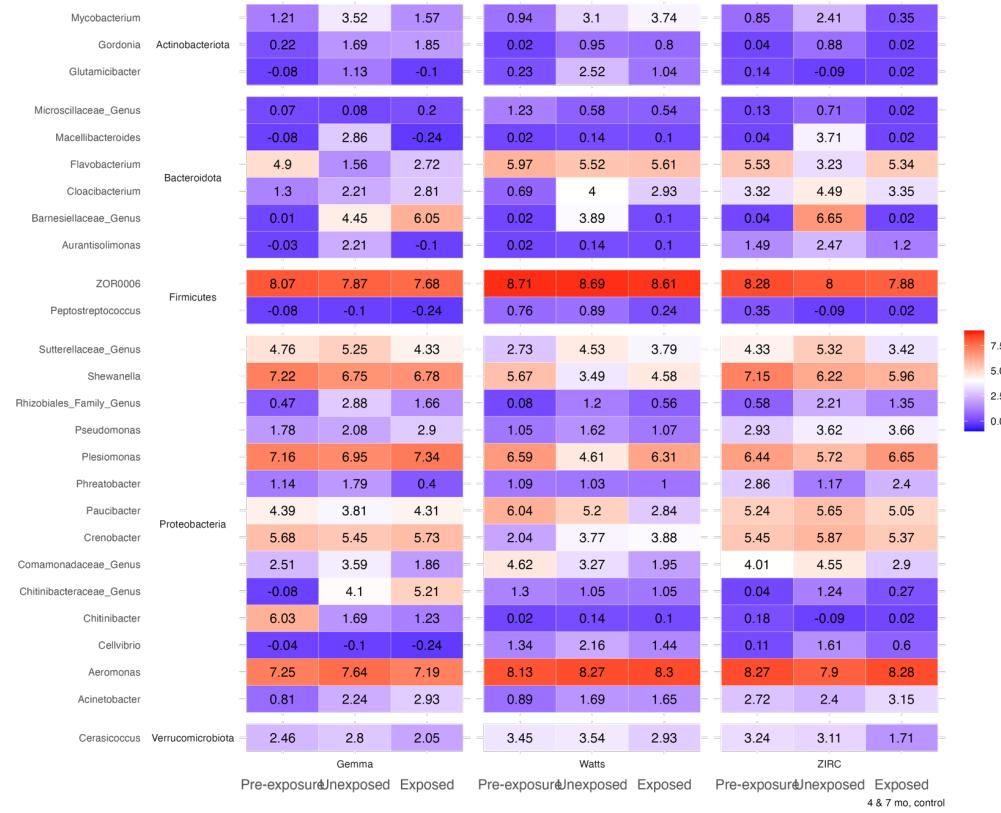
Beta-Dispersion Pairwise Comparisons: p-values < 0.1	
Names	p-value
Gemma.Pre-exposure-Watts.Exposed	0.001
Gemma.Pre-exposure-Watts.Unexposed	0.001
Gemma.Pre-exposure-ZIRC.Unexposed	0.001
Gemma.Unexposed-Watts.Exposed	0.001
Watts.Exposed-ZIRC.Pre-exposure	0.001
Gemma.Exposed-Watts.Exposed	0.002
Gemma.Exposed-ZIRC.Unexposed	0.008
Watts.Unexposed-ZIRC.Pre-exposure	0.009
Gemma.Unexposed-ZIRC.Unexposed	0.010
Gemma.Exposed-Watts.Unexposed	0.011
ZIRC.Pre-exposure-ZIRC.Unexposed	0.011
Gemma.Unexposed-Watts.Unexposed	0.012
Gemma.Pre-exposure-ZIRC.Exposed	0.015
Gemma.Pre-exposure-Watts.Pre-exposure	0.016
Watts.Exposed-Watts.Pre-exposure	0.028
Gemma.Pre-exposure-ZIRC.Pre-exposure	0.041
Watts.Exposed-ZIRC.Exposed	0.066
Watts.Pre-exposure-Watts.Unexposed	0.066
Gemma.Pre-exposure-Gemma.Unexposed	0.097



3.4) Differential Abundance

3.4.1)

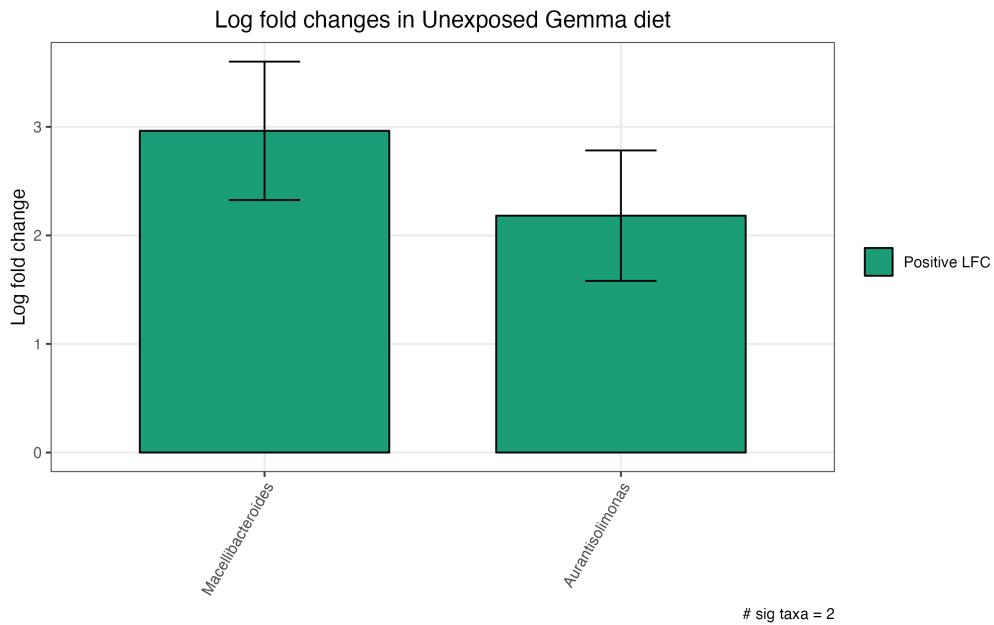
Heat map of bias-corrected log observed abundances (Genus)



ANCOM-BC2: Summary statistics of abundant taxa (4-7mo, all), sig taxa = 26

Taxon	W	p_val	q_val	diff_abn
Acinetobacter	11.669	0.006	0.012	TRUE
Aeromonas	64.347	0.000	0.000	TRUE
Aurantisolimonas	32.189	0.000	0.000	TRUE
Barnesiellaceae_Genus	17.755	0.000	0.001	TRUE
Cellvibrio	41.371	0.000	0.000	TRUE
Cerasicoccus	9.730	0.015	0.028	TRUE
Chitinibacter	230.706	0.000	0.000	TRUE
Chitinibacteraceae_Genus	32.812	0.000	0.000	TRUE
Cloacibacterium	23.288	0.000	0.000	TRUE
Comamonadaceae_Genus	12.719	0.003	0.008	TRUE
Crenobacter	161.437	0.000	0.000	TRUE
Flavobacterium	55.270	0.000	0.000	TRUE
Glutamicibacter	16.061	0.001	0.002	TRUE
Gordonia	9.599	0.016	0.028	TRUE
Macellibacteroides	11.700	0.006	0.012	TRUE
Microscillaceae_Genus	12.504	0.004	0.009	TRUE
Mycobacterium	9.465	0.018	0.029	TRUE
Paucibacter	10.715	0.009	0.018	TRUE
Peptostreptococcus	25.357	0.000	0.000	TRUE
Phreatobacter	19.865	0.000	0.000	TRUE
Plesiomonas	27.146	0.000	0.000	TRUE
Pseudomonas	29.319	0.000	0.000	TRUE
Rhizobiales_Family_Genus	10.694	0.010	0.018	TRUE
Shewanella	57.362	0.000	0.000	TRUE
Sutterellaceae_Genus	22.248	0.000	0.000	TRUE
ZOR0006	36.131	0.000	0.000	TRUE

3.4.2)

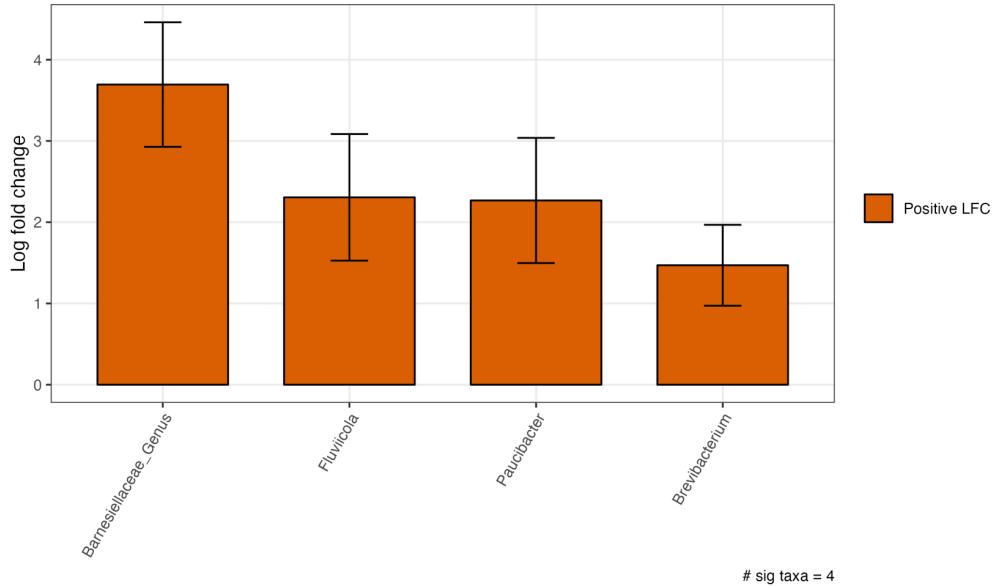


ANCOM-BC2: Log fold change in abundance. # Sig taxa = 2

Taxon	lfc_ExposureUnexposed	diff_ExposureUnexposed	direct
Macellibacteroides	2.963445	TRUE	Positive LFC
Aurantisolimonas	2.181833	TRUE	Positive LFC

3.4.3)

Log fold changes in Unexposed Watts diet

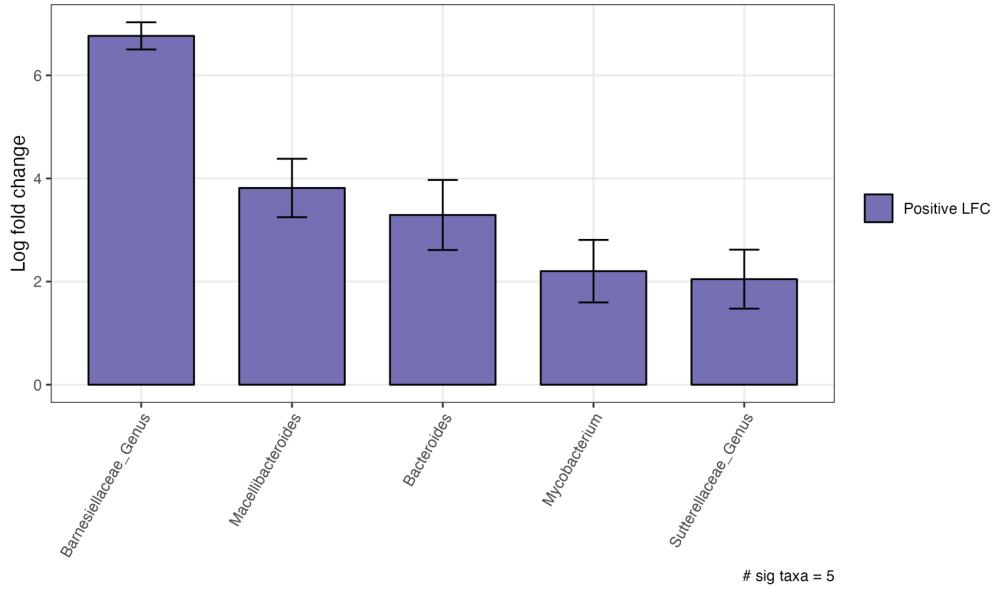


ANCOM-BC2: Log fold change in abundance. # Sig taxa = 4

Taxon	Ifc_ExposureUnexposed	diff_ExposureUnexposed	direct
Barnesiellaceae_Genus	3.694207	TRUE	Positive LFC
Fluvicola	2.306118	TRUE	Positive LFC
Paucibacter	2.267663	TRUE	Positive LFC
Brevibacterium	1.470217	TRUE	Positive LFC

3.4.4)

Log fold changes in Unexposed ZIRC diet

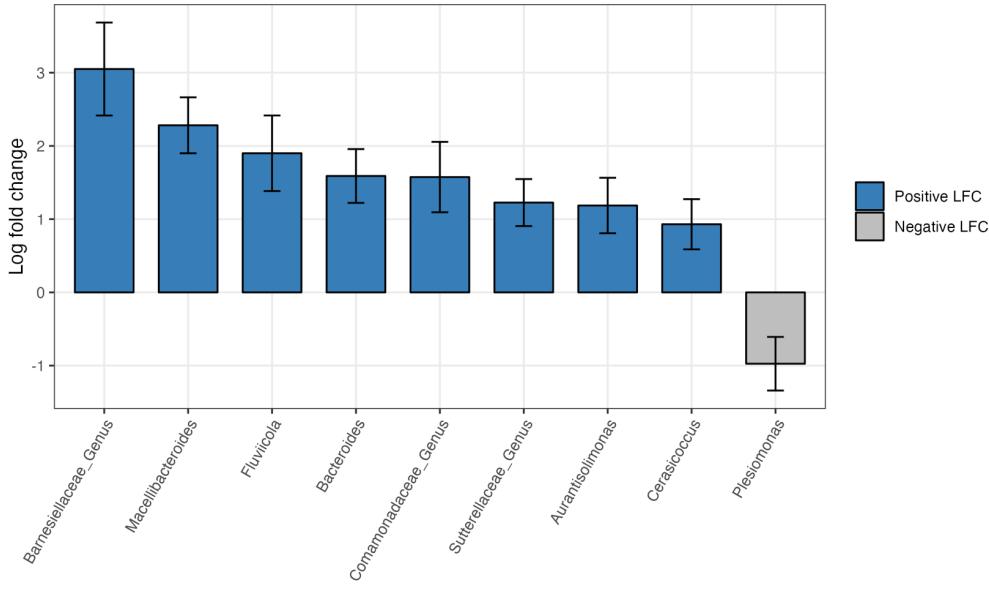


ANCOM-BC2: Log fold change in abundance. # Sig taxa = 5

Taxon	Ifc_ExposureUnexposed	diff_ExposureUnexposed	direct
Barnesiellaceae_Genus	6.765048	TRUE	Positive LFC
Macellibacteroides	3.815438	TRUE	Positive LFC
Bacteroides	3.291639	TRUE	Positive LFC
Mycobacterium	2.202108	TRUE	Positive LFC
Sutterellaceae_Genus	2.046730	TRUE	Positive LFC

3.4.5)

Log fold changes in unexposed across all diets



ANCOM-BC2: Log fold change in abundance. # Sig taxa = 9

Taxon	Ifc_ExposureUnexposed	diff_ExposureUnexposed	direct
Barnesiellaceae_Genus	3.049992	TRUE	Positive LFC
Macellibacteroides	2.281608	TRUE	Positive LFC
Fluvicola	1.899652	TRUE	Positive LFC
Bacteroides	1.589143	TRUE	Positive LFC
Comamonadaceae_Genus	1.574745	TRUE	Positive LFC
Sutterellaceae_Genus	1.226355	TRUE	Positive LFC
Aurantisolimonas	1.186200	TRUE	Positive LFC
Cerasiococcus	0.930219	TRUE	Positive LFC
Plesiomonas	-0.974312	TRUE	Negative LFC

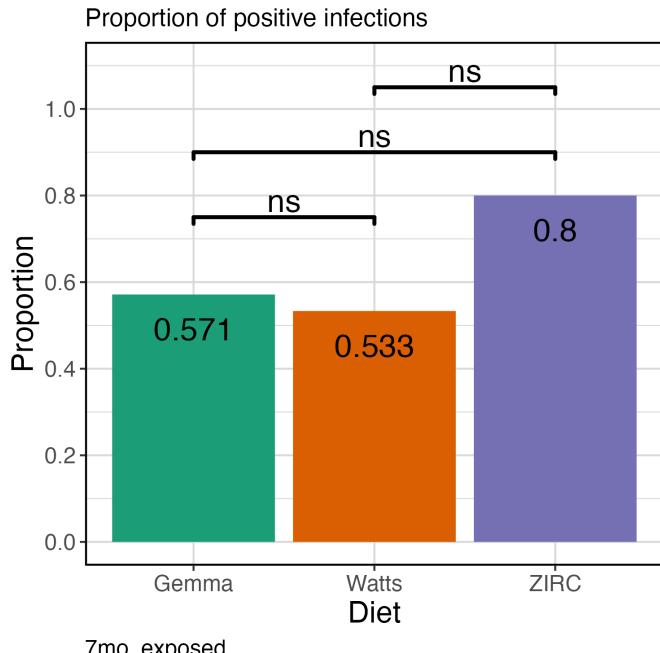
3.4.6)

ANCOM-BC2: Summary statistics of Mycobacterium abundance

Diet	PrePostExp	lfc	se	W	p	q	diff	direct
Gemma	Exposed	-0.195	0.457	-0.426	0.670	0.830	FALSE	Negative LFC
	Pre-exposure	-0.487	0.554	-0.879	0.379	0.694	FALSE	Negative LFC
	Unexposed	1.829	0.634	2.886	0.004	0.036	TRUE	Positive LFC
Watts	Exposed	1.539	0.529	2.909	0.004	0.058	FALSE	Positive LFC
	Pre-exposure	-2.687	0.649	-4.140	0.000	0.001	TRUE	Negative LFC
	Unexposed	-0.719	0.748	-0.961	0.337	0.738	FALSE	Negative LFC
ZIRC	Exposed	-0.828	0.385	-2.152	0.031	0.150	FALSE	Negative LFC
	Pre-exposure	0.546	0.471	1.158	0.247	0.686	FALSE	Positive LFC
	Unexposed	2.272	0.546	4.160	0.000	0.000	TRUE	Positive LFC

3.5) Infection

3.5.1)



Pairwise Fisher Test. p. adj: BH. Pathology.Results ~ Diet

group1	group2	n	estimate	p	conf.low	conf.high	method	alternative	p.adj	p.adj.signif
Gemma	Watts	29	1.160	>0.999	0.214	6.422	Fisher's Exact test	two.sided	>0.999	ns
Gemma	ZIRC	29	0.347	0.245	0.043	2.214	Fisher's Exact test	two.sided	0.368	ns
Watts	ZIRC	30	0.298	0.245	0.038	1.809	Fisher's Exact test	two.sided	0.368	ns

3.5.2)

Wilcoxon Test. p. adj: BH. Body.Condition.Score ~ Diet*Pathology.Results

term	df	sumsq	meansq	statistic	p.value	sig
Pathology.Results	1	0.259	0.259	1.481	0.228	
Diet	2	5.909	2.954	16.871	<0.001	*
Pathology.Results:Diet	2	0.217	0.108	0.619	0.542	
Residuals	60	10.506	0.175			

3.5.3)

glm(Alpha.Score ~ Diet*Pathology), family = quasibinomial)

metric	term	estimate	std.error	statistic	p.value	sig
Observed	(Intercept)	0.556	0.272	2.046	0.048	*
	Pathology.ResultsPositive	-0.200	0.356	-0.562	0.577	
	DietWatts	-1.623	0.388	-4.179	<0.001	*
	DietZIRC	-0.015	0.470	-0.032	0.974	
	Pathology.ResultsPositive:DietWatts	0.495	0.513	0.966	0.340	
	Pathology.ResultsPositive:DietZIRC	-0.380	0.555	-0.684	0.498	
Shannon	(Intercept)	-0.006	0.267	-0.024	0.981	
	Pathology.ResultsPositive	-0.203	0.354	-0.575	0.569	
	DietWatts	-1.228	0.398	-3.082	0.004	*
	DietZIRC	-0.017	0.462	-0.036	0.971	
	Pathology.ResultsPositive:DietWatts	0.467	0.529	0.883	0.383	
	Pathology.ResultsPositive:DietZIRC	-0.344	0.554	-0.621	0.539	
Simpson	(Intercept)	-0.150	0.314	-0.478	0.635	
	Pathology.ResultsPositive	-0.166	0.417	-0.397	0.694	
	DietWatts	-0.414	0.435	-0.952	0.347	
	DietZIRC	0.176	0.543	0.324	0.748	
	Pathology.ResultsPositive:DietWatts	0.268	0.585	0.458	0.650	
	Pathology.ResultsPositive:DietZIRC	-0.402	0.650	-0.619	0.540	

3.5.4)

ANOVA(glm(Alpha.Score ~ Diet*Pathology), family = quasibinomial))

metric	term	statistic	df	p.value	sig
Observed	Pathology.Results	0.348	1	0.555	
	Diet	32.445	2	<0.001	*
	Pathology.Results:Diet	2.517	2	0.284	
Shannon	Pathology.Results	0.419	1	0.518	
	Diet	14.795	2	<0.001	*
	Pathology.Results:Diet	2.008	2	0.366	
Simpson	Pathology.Results	0.436	1	0.509	
	Diet	0.876	2	0.645	
	Pathology.Results:Diet	1.077	2	0.584	

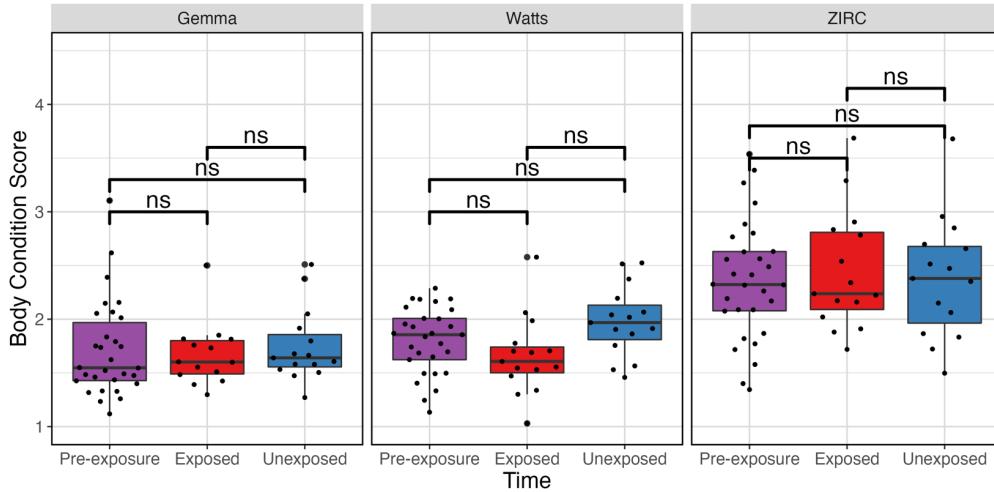
3.5.5)

Distance-based redundancy analysis (dbRDA) ordination. Beta.Score ~ Pathology*Diet

metric	term	Df	SumOfSqs	statistic	p.value	sig
Bray-Curtis	Diet	2.00	0.53156924	5.320	0.001	*
	Pathology.Results	2.00	0.08777829	0.879	0.538	
	Pathology.Results:Diet	4.00	0.15087747	0.755	0.798	
Canberra	Residual	35.00	1.74856431			
	Diet	2.00	1.72790036	4.254	0.001	*
	Pathology.Results	2.00	0.48794416	1.201	0.137	
Sørensen	Pathology.Results:Diet	4.00	0.84016409	1.034	0.392	
	Residual	35.00	7.10790377			
	Diet	2.00	0.83117951	5.999	0.001	*
	Pathology.Results	2.00	0.18102326	1.306	0.151	
	Pathology.Results:Diet	4.00	0.32213414	1.162	0.236	
	Residual	35.00	2.42474918			

3.6) Physiology

3.6.1)



3.6.2)

Wilcoxon Test. p. adj: BH. Body.Condition.Score ~ Diet:Exposure

Diet	.y.	group1	group2	n1	n2	statistic	p	p.adj	p.adj.signif
Gemma	Body.Condition.Score	Pre-exposure	Exposed	30	14	203.000	0.871	0.896	ns
	Body.Condition.Score	Pre-exposure	Unexposed	30	15	190.000	0.410	0.896	ns
	Body.Condition.Score	Exposed	Unexposed	14	15	91.000	0.561	0.896	ns
Watts	Body.Condition.Score	Pre-exposure	Exposed	30	15	297.000	0.085	0.382	ns
	Body.Condition.Score	Pre-exposure	Unexposed	30	15	170.000	0.192	0.576	ns
	Body.Condition.Score	Exposed	Unexposed	15	15	56.000	0.019	0.171	ns
ZIRC	Body.Condition.Score	Pre-exposure	Exposed	30	15	212.000	0.766	0.896	ns
	Body.Condition.Score	Pre-exposure	Unexposed	30	15	219.000	0.896	0.896	ns
	Body.Condition.Score	Exposed	Unexposed	15	15	119.000	0.806	0.896	ns