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

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9

0 **Abstract**

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1
2 Despite the long-established importance of zebrafish as a model organism and their increasing use in
3 microbiome-targeted studies, relatively little is known about how husbandry practices involving diet impact the
4 zebrafish gut microbiome. Given, the microbiome's important role in mediating host physiology and the
5 potential for diet to drive variation in microbiome composition, we sought to clarify how three different dietary
6 formulations that are commonly used in zebrafish facilities impacts the gut microbiome. We reared
7 approximately 60 fish on each diet throughout their lifespan and compared the composition of their
8 microbiomes at both 4- and 7-months-old. Our analysis finds that diet has a substantial impact on the
9 composition of the gut microbiome at both 4- and 7-months-old. Moreover, the developmental dynamics of the
0 microbiome differ as a function of diet. We further evaluated whether the 7-month-old fish microbiome
1 compositions that result from dietary variation are differentially sensitive to infection by a common laboratory
2 pathogen: *Mycobacterium chelonae*. Our analysis finds that the impact of *M. chelonae* infection on the gut
3 microbiome differs as a function of diet, especially for moderate and low abundance taxa. Overall, our results
4 indicate that diet drives the successional development of the gut microbiome as well as its sensitivity to
5 exogenous exposure. Consequently, investigators should carefully consider the role of diet in their microbiome
6 zebrafish investigations, especially when integrating results across studies that vary by diet.
7

8 **Introduction**

9
0 The gut microbiome plays an important role in supporting the health of its host through nutrient metabolism,
1 supporting the immune system, and protecting against pathogens. To better understand the gut microbiome,
2 zebrafish (*Danio rerio*) have emerged as an important model organism due to their extensive homology to early
3 human development, high-throughput experimental methods and ability to directly manipulate their gut
4 microbiomes¹. Despite zebrafish's increasing use in microbiome-targeted studies, key knowledge gaps
5 remain about how diet influences their microbiome and their health². In contrast to mice, zebrafish do not have
6 a standard reference diet³. Instead, zebrafish are fed a variety of diets, which impact zebrafish physiological
7 and reproductive outcomes⁴⁻⁶. Moreover, diet has been linked to shaping the gut microbiome as well as a
8 variety of health outcomes in other systems, such as humans and mice (citation). In zebrafish, diet's impact on
9 the microbiome has been investigated, but these diets differ vastly in nutritional content from diets commonly
0 used in zebrafish laboratory studies⁷⁻⁹. Given the role of the microbiome supporting host health and diet's
1 influence on the gut microbiome, common zebrafish diets may have differentially impact their gut microbiomes
2 and consequently their health.
3

4 Zebrafish have a core microbiome, but there is much inter-individual variation that could be explained by
5 diet^{10,11}. Early in life, larval zebrafish are often fed a live feed diet, consisting of rotifers, ciliates, and artemia¹².
6 In addition to microbes found in the surrounding tank water, these live organisms are believed to be
7 contributors of microbiome assembly in young zebrafish^{13,14}. As zebrafish transition from larval to juvenile and
8 adult life stages, they also transition through a variety of live and dry food diets to support their growth^{5,12}. At 3
9 months of age, zebrafish are considered adults and are fed exclusively dry food diets³. Here, we chose three
0 diets as representative of the variety of commonly used dry food diets across zebrafish facilities. These diets
1 will subsequently be referred to as the Gemma (a commercial diet), Watts (a laboratory, defined diet), and
2 ZIRC (combination of multiple commercial diets) diets. While these diets are relatively similar in nutritional

3 content, they differ in ingredients are used, ingredient sources, methods of production and exact nutritional
4 content (see methods and materials). These factors can be an inadvertent contributors nonprotocol induced
5 variation, intestinal inflammation, and microbial transmission^{2,15}. Given, that the microbiome mediates host
6 health and is relatively stable once established, any diet-related influences to early-life microbiome assembly
7 could have lasting impacts to the successional development of the gut microbiome and consequently health of
8 the zebrafish, for instance sensitivity to pathogens¹⁶.

9
0 Zebrafish facilities are known to host many pathogens, which can introduce inconsistencies in study
1 outcomes¹⁷. *Mycobacterium chelonae* is a common intestinal pathogen found in 40% of zebrafish facilities, and
2 is hypothesized to be introduced through diet early in life^{17–19}. *M. chelonae* forms granulomas in the gut
3 intestine, which can cause gut inflammation, decreased fecundity and lifespan¹⁷. Previous work of ours has
4 shown that an intestinal parasite *Pseudocapillaria tormentosa* disrupts and restructures the gut microbiome²⁰.
5 However, little is known about the effects of *M. chelonae* infection on zebrafish gut microbiomes. Some studies
6 suggest that having a diverse microbiome can protect against pathogen infection^{21,22}, while others show
7 diverse or highly varied microbiomes could be indicative of pathogenesis^{20,23}. Given the influence of diet on the
8 gut microbiome, there could be an interaction between diet, pathogenesis and the gut microbiome in zebrafish.
9 Elucidating these relationships could offer microbiome-targeted tools for diagnosing, preventing, or mitigating
0 the impacts of *M. chelonae* and other intestinal pathogens on zebrafish and other host organisms.

1
2 In this study we show how three different dietary formulations that are commonly used in zebrafish facilities
3 impacts the gut microbiome. Zebrafish manifest distinct gut microbiomes at 4 months of age, which persist
4 across their development at 7 months old. Additionally, we show that the gut microbiomes of some diets are
5 more stable to the effects of development, while others are more impacted by diet. Moreover, we find that gut
6 microbiome diversity may be linked to body mass of zebrafish fed certain diets. Furthermore, we show that gut
7 microbiomes of fish fed different diets vary in their sensitivity to pathogen exposure of *M. chelonae*, but diet's
8 overall impact is more substantial. Collectively, our study clarifies the role of diet on successional development
9 of adult zebrafish gut microbiomes, and the microbiome's sensitivity to pathogen exposure. Consequently,
0 investigators should carefully consider the role of diet in their microbiome zebrafish investigations, especially
1 when integrating results across studies that vary by diet. Moreover, microbiome zebrafish research may benefit
2 from applying consistent husbandry choices involving diet.

3

4

5 **Results**6 **1. Experimental design and data collection**

7

8

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 129 and 214).

In this study, our main goals were to reveal the influence of different commonly used laboratory diets on the zebrafish physiology, the successional development of the 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). At 4-months-old, we exposed a cohort of fish to the intestinal pathogen: *Mycobacterium chelonae*. Approximately 3 months later when fish were 7-months-old, we collected 87 fecal samples from each diet and exposure group and performed histopathology checks on exposed fish to assess infection burden. 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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9 **2. Diet differentially influences physiology and gut microbiome**

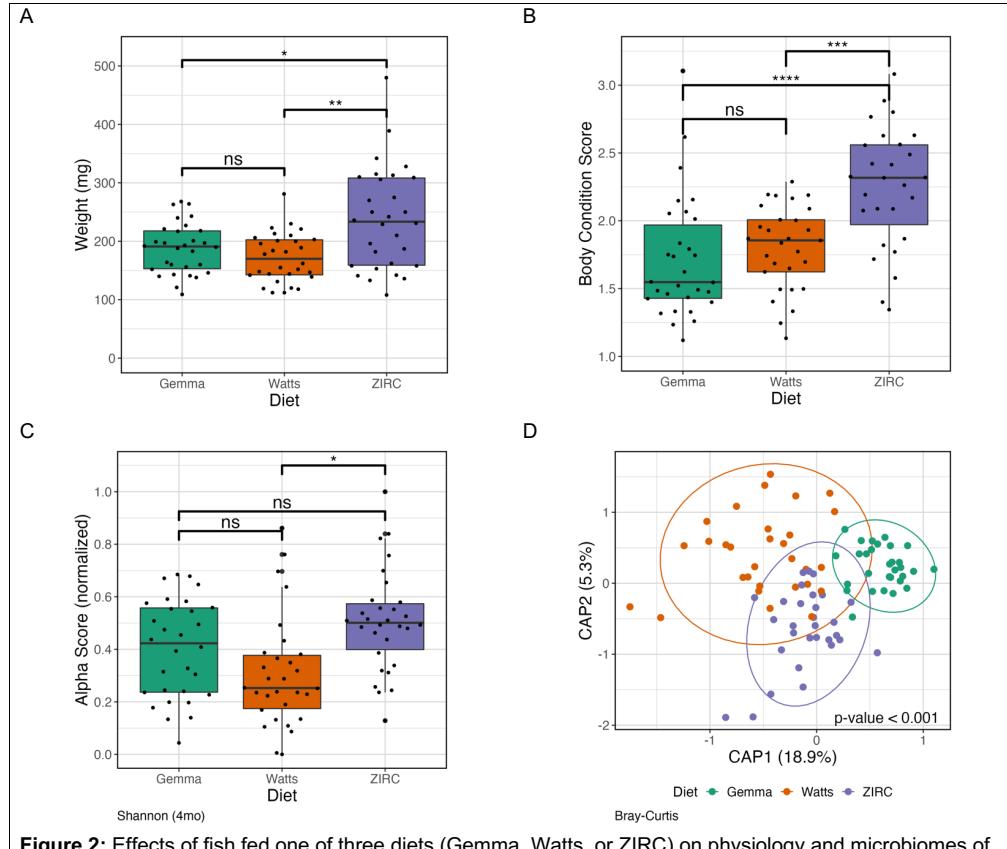


Figure 2: Effects of fish fed one of three diets (Gemma, Watts, or ZIRC) on physiology and microbiomes of zebrafish. **(A)** Weight of ZIRC significantly differs from Watts and Gemma. Gemma and Watts do not differ from each other. **(B)** Body condition score is a length normalized measure of weight. 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.

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0
1 Zebrafish physiology and gut microbiome has been previously associated with diet, but these studies
2 considered diets that differed greatly in nutritional content (e.g., high fat and protein vs. low fat and protein)⁷⁻⁹.
3 Our study is unique in that fish were fed three commonly used laboratory diets similar in nutritional
4 composition. Moreover, fish were fed the same diet from 30 to 214 days (7 months) old. Here, we investigated
5 how commonly used laboratory diets may impact the zebrafish physiology and gut microbiome diversity,
6 composition, and relative abundance at 4 months old.
7

8 To determine if physiology was differentially impacted by diet we compared the weight and body condition
9 scores of fish fed the three diets. Wilcoxon Signed-Rank Tests found that diet and sex significantly associated
0 with weight and body condition. Female fish had higher weight ($Z = 1,530$, $P < 0.001$; Table S1.1.2) and body
1 condition scores ($Z = 1,631$, $P < 0.001$; Table S1.1.4) compared to males. Between the three diets, ZIRC-diet
2 fed fish had the highest mean body condition score compared to fish fed Gemma- ($Z = 150$, $P < 0.001$) and
3 Watts-diet ($Z = 197$, $P < 0.001$, Table S1.1.3). Gemma- and Watts-diet fed fish did not significantly differ from
4 one another in terms of weight and body condition scores. These results indicate that ZIRC-diet contributes to
5 heavier fish compared to Gemma- and Watts-diet fed fish.

6
7 Next, we asked if diet associated with gut microbiome diversity. First, we built generalized linear models (GLM)
8 to determine if diet associated with variation in one of three measures of microbiome alpha-diversity: richness,
9 Simpson's Index, and Shannon Entropy. An ANOVA test of these GLMs revealed that alpha-diversity varies as
0 a function of diet for all three measures of diversity we assessed ($P < 0.05$; Fig 1C; Table S1.2.2). A post hoc
1 Tukey test clarified that ZIRC- and Watts-diet fed fish exhibited significant differences in alpha-diversity as
2 measured by richness and Shannon Entropy ($P < 0.001$, Table S1.2.3). Moreover, we observed significant
3 differences in diversity between Gemma- and Watts-diet fed fish in terms of richness ($P < 0.001$; Table S1.2.3),
4 and between Gemma- and ZIRC-diet fed fish when considering the Simpson's Index ($P < 0.001$; Table S1.2.3).
5 These results indicate that diet associates with fish gut microbiome diversity, and that diet may differentially
6 impact rare and abundant microbial members of the gut.

7
8 To evaluate how diet associates with microbiome community composition, we used the Bray-Curtis, Canberra
9 and Sorenson dissimilarity metrics. We detected a significant clustering of microbial gut community
0 composition based on diet as measured by all beta-diversity metrics (PERMANOVA, $P < 0.05$; Figure 2C,
1 Table S1.3.1). These results indicate that microbial communities of fish fed the same diet are more consistent
2 in composition to one another than to fish fed other diets. Additionally, we assessed beta-dispersion, a
3 measure of variance, in the gut microbiome community compositions for each diet group. We find the beta-
4 dispersion levels were significantly different between the diet groups as measured by Bray-Curtis and
5 Canberra metrics ($P < 0.05$; Table S1.4.1). Beta-dispersion levels were significantly reduced in Gemma-diet
6 fed fish compared to Watts-diet fed fish when measured by Bray-Curtis metric, as well as significantly reduced
7 compared to Watts- and ZIRC-diet fed fish when measured by Canberra metric (Table S1.4.1). These results
8 indicate that Gemma-diet fed fish are more consistent in community composition than Watts- and ZIRC-diet fed
9 fish at 4 months old. Collectively, these results indicate that 4-month-old fish gut microbiome communities
0 stratify by diet, but the composition of these microbial communities differ in consistency depending on diet.

1
2 Finally, to better understand the interactions between the diet and the members of the gut microbiome
3 community, we quantified differential abundance using ANCOM-BC2. We observed 24 significantly abundant
4 taxa at the genus level in at least one of the three diets (Figure S1.5.1 and Table S1.5.1). Gemma-diet fed fish
5 enriched for *Chitinibacter*, and were depleted of *Aeromonas* and *Flavobacterium*. Watts-diet fed fish enriched
6 for *Flavobacterium*, *ZOR0006*, *Peptostreptococcus*, *Cetobacterium*, *Tabrizicola*, *Cellvibrio*, and unnamed
7 genera of *Microscillaceae* and *Chitinibacteraceae*, and depleted of *Crenobacter* and a *Sutterellaceae* genus.
8 ZIRC-diet fed fish enriched for *Cloacibacterium* and *Acinetobacter*, and depleted of *Fluviicola*. Many of these
9 taxa are identified as common members of the zebrafish gut microbiome^{11,24}. These results indicate that diet
0 differentially supports particular members of the zebrafish microbiome community.

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

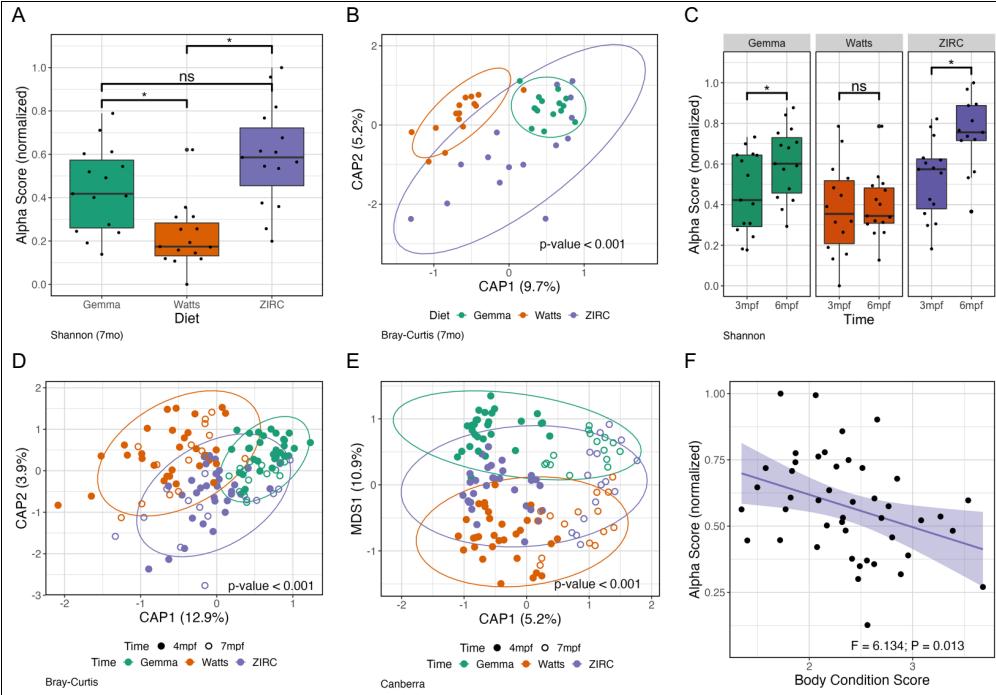


Figure 3: Development is associated with altered microbiome composition. **(A)** Shannon Entropy of diversity shows that gut microbiome diversity significantly differs between Watts-diet fed fish to fish fed the Gemma- and ZIRC-diets in 7-month-old zebrafish. **(B)** Capscale ordination based on the 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.

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-diversity metrics ($P < 0.05$; Fig 3A&B, Table S2.7.2). These results demonstrate that diet has impacts the physiology and gut microbiome of 7-month-old fish.

Next, we compared our results between the 4- and 7-month-old fish to determine how diet impacts the successional development of the gut microbiome. 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-

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

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

7
8 Development was significantly associated with 20 taxa at the genus level in at least one of the diets (Table
9 S2.6.1). Of these taxa, *Fluviicola*, *Macellibacteroides*, *Bacteroides* and an unnamed genus in the
0 *Barnesiellaceae* family were some that were enriched, while *Phreatobacter* and *Flavobacterium* were depleted.
1 Unique to each diet only a few taxa were identified as being significant (Figure S2.6.2.5). The Gemma-diet fed
2 fish were uniquely enriched for *Exiguobacterium* (Table S2.6.2.1). *Exiguobacterium* are gram-positive facultate
3 anaerobes in the phylum Bacillota, and are found across a variety of organisms and extreme environments²⁵.
4 Interestingly, they are found to increase lipid droplet number in zebrafish²⁶. The Watts-diet fed fish were
5 uniquely depleted of *Gemmobacter* (Table S2.6.2.2). *Gemmobacter* is a potential pathobiont in zebrafish due
6 to its positive association with parasite exposure and infected zebrafish^{20,27}. The ZIRC-diet fed fish were
7 uniquely enriched for *Pseudomonas* and *Haliscomenobacter* (Table S2.6.2.3). *Pseudomonas* is a common
8 member of the gut microbiome and found to increase lipid droplet growth in zebrafish²⁶. Less is known about
9 the *Haliscomenobacter* genus, but an analysis of its genome revealed presence of denitrifying genes²⁸.
0 Together, these results indicate that particular members of the gut microbiome associate with diet and
1 development.

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

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5 a significant association between body condition score and specific taxon abundance (Table S2.2.2).
6 Collectively, these results indicate that the gut microbiome may be sensitive to body condition of fish, but the
7 influence of diet is much stronger.

8

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

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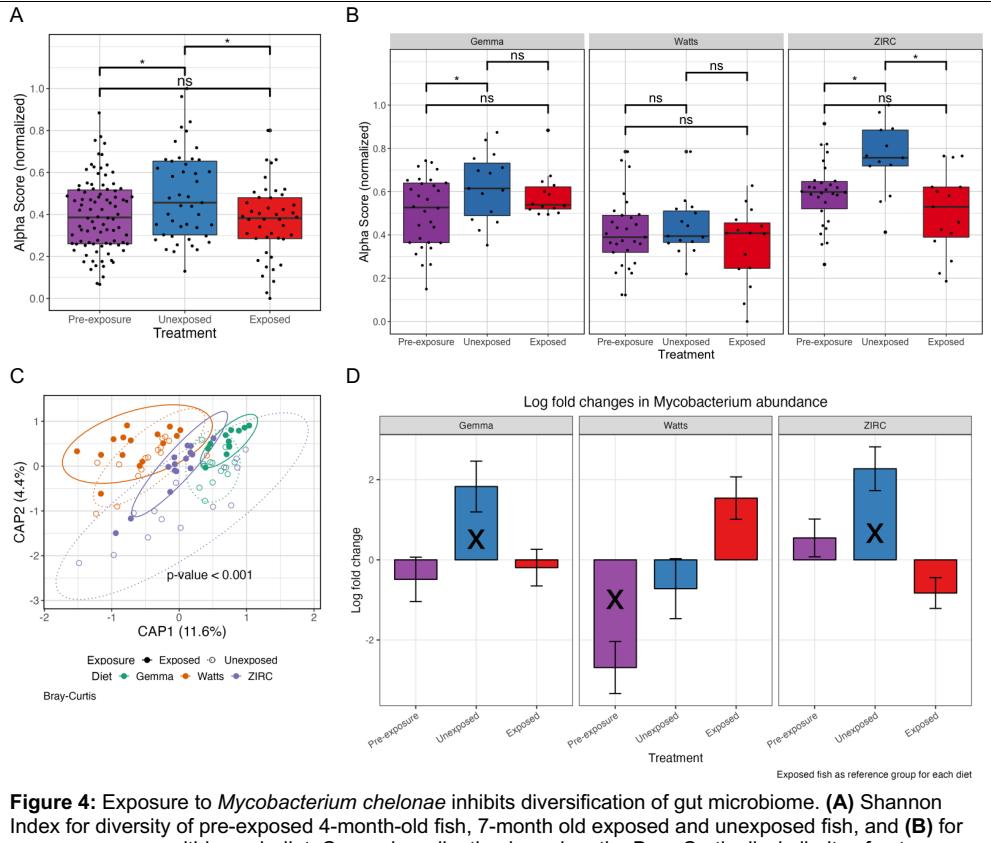


Figure 4: Exposure to *Mycobacterium chelonae* inhibits diversification of gut microbiome. **(A)** Shannon Index for diversity of pre-exposed 4-month-old fish, 7-month old exposed and unexposed fish, and **(B)** for exposure groups within each diet. Capscale ordination based on the Bray-Curtis dissimilarity 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.

0

1 Lastly, we sought to elucidate the potential interactions between the gut microbiome, diet and exposure to the
 2 intestinal pathogen *Mycobacterium chelonae*. Briefly, after collecting fecal samples of 4-month-old fish, we
 3 injected *M. chelonae* into the coelomic cavity of fish in the exposed treatment group. At 7 months old, we
 4 collected fecal samples to measure microbial gut diversity, composition, and taxon abundance, performed a
 5 histopathology check to assess infection counts and severity, and collected physiological measurements. Fecal
 6 samples collected prior to *M. chelonae* exposure are labeled as "pre-exposure", and samples collected after
 7 exposure are labeled as either "exposed" or "unexposed".
 8

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. We did not find significant associations between infection status and body condition score ($P > 0.05$; Table S3.5.2) or any of the gut microbiome diversity and composition measures ($P > 0.05$; Table S3.5.4&S3.5.5). Together, these results indicate that infection status was not predictive of physiological or microbiome analysis endpoints. However, 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). These results indicate that the gut microbiome is sensitive to pathogen exposure, but its sensitivity is not diet-dependent. Furthermore, we used a post hoc Tukey test to clarify whether microbial gut diversity of fish differed between exposure groups by diet. Unique to ZIRC-diet fed fish, we observed microbiome diversity differed in unexposed controls compared to exposed fish as measured by all alpha-diversity metrics ($P < 0.05$, Table S3.1.2.3). Watts-diet fed fish differed in unexposed controls compared to exposed fish in terms of richness ($P < 0.05$, Table S3.1.2.3). These results suggest that the ZIRC-diet, and to some extent Watts-diet fed fish, are sensitive to the effects of *M. chelonae* exposure. Collectively, these results indicate that gut microbiome diversity is sensitive to *M. chelonae* exposure, but the sensitivity to diet is greater.

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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6 **Discussion**

7
8 Zebrafish are an important emerging model organism for understanding the microbiome. Yet, there is little
9 consistency across microbiome-targeted studies in husbandry practices involving diet. Given, the microbiome's
0 important role in mediating host physiology and the potential for diet to drive variation in microbiome
1 composition, choice of diet could introduce variation in study outcomes. This study represents, to our
2 knowledge, the first to assess how different dietary formulations that are commonly used in zebrafish facilities
3 impacts the development of the zebrafish gut microbiome and their physiology, as well as sensitivity to
4 pathogen exposure. We show that diet drives the successional development of the gut microbiome, as well as
5 its sensitivity to pathogen exposure.

6
7 We find that commonly used laboratory diets with relatively similar nutritional compositions stratify the gut
8 microbiome of adult 4-month-old zebrafish. Previous research has found that diets with varying compositions of
9 key macronutrients: protein, lipids and fiber impacts zebrafish physiology and gut microbiome^{4,7-9}. However,
0 the nutritional compositions used in these studies targeting the gut microbiome vastly differ from what are
1 commonly used in zebrafish husbandry and research. A unique strength of our study is that we demonstrate
2 that minor differences in a diet's nutritional composition can have profound impacts on the gut microbiome in
3 adult zebrafish. Despite the diets used in this study having similar nutritional compositions and ingredients,
4 there can be variation between diets (e.g., ingredient sources, exact nutritional composition of macro- and
5 micronutrients, methods of production). Studies in common carp and zebrafish larvae found soybean meal, a
6 common protein source for hatchery-reared fish, induces intestinal inflammation^{15,29}. Moreover, variation in
7 sources of ingredients may inadvertently introduce contaminants, such as phytoestrogens, or pathogenic
8 microbes, such as *M. chiloniae*, to zebrafish^{3,17}. Even if the same diets are used between studies, variation
9 could exist due to differences between production batches³. Taken together, these factors could contribute
0 variation seen in zebrafish gut microbiomes across studies and facilities. One way of minimizing the
1 confounding factor of diet is to utilize a standard reference diet, as has been suggested by Watts *et al*³.
2 Benefits of a standard reference diet would be transparent disclosure of ingredient sources, nutritional
3 compositions, and methods of preparation. However, in some instances variability in diet may be beneficial to
4 researchers seeking to model the variability of diets found in human and wildlife populations. Additionally, a
5 challenge to creating a standard reference diet are developing sterile diets that are commiserate with their non-
6 sterile counterparts for the gnotobiotic study of zebrafish³⁰.

7
8 We also observed variation in the successional dynamics of gut microbiomes of fish fed different diets across
9 their development from 4 to 7 months old. We find the composition of abundant taxa are driven primarily by
0 diet, but rarer taxa are sensitive to the effects of time. Additionally, we found an interaction effect of diet and
1 development on the gut microbiome, where microbial diversity and composition increased across development
2 in fish fed the Gemma and ZIRC diets, but remained stable in Watts-diet fed fish. In line with previous
3 microbiome-targeted zebrafish studies, we find similar overall increases to microbial diversity with age^{20,31,32}.
4 However, these studies investigated fish less than 4 months old, making it difficult to directly compare our
5 results^{14,31}. Stephens *et al.* investigated the successional development of the gut microbiome in zebrafish finds
6 zebrafish gut microbiome diversity is higher in juvenile fish but declines as they age¹³. Our results contrast
7 these results, but when we compare similar time points between studies, we do find a similar increase in
8 diversity between 4 and 7 months of age. Moreover, Stephens *et al.* used a combination of live and dry food
9 diets and had several dietary and environmental changes prior to adulthood, which could have contributed to
0 the high levels of variability in the juvenile zebrafish. Other studies using defined diets observed more
1 consistency in the microbiome and might explain the stability of the gut microbiomes of Watts-diet fed fish^{9,31}.
2 Another important point of consideration is the transition of fish from juvenile to adult diet formulations at 4
3 months of age. The Gemma diet juvenile formulation differs in feed size from the adult diet, but the nutritional
4 content is similar. Due to the Gemma diet being a commercial diet, the exact ingredients used are not known

5 and might be a source of variability between formulations. The adult Watts diet includes more lipid content from
6 the juvenile formulation, but otherwise the ingredients remain consistent. The adult ZIRC diet is a combination
7 for four commercial diets that differ in ingredients and nutritional content. The introduction of new diets into the
8 ZIRC adult diet might explain the variability in the gut microbiomes of ZIRC-diet fed fish compared to the
9 stability of Watts-diet fed fish. Taken together, the minor differences in formulations between these diets and
0 the substantial differences observed in the gut microbiomes between fish fed these diets highlights the
1 importance of diet's influence on shaping the gut microbiome and challenging efforts for cross study
2 comparisons.

3 Our analysis of differential abundance identified abundance of particular bacterial genera associated with diet
4 and development. Many of these bacteria have been previously identified as common members of the
5 zebrafish gut microbiome^{11,33}. Of those, *Fluviicola*, *Cloacibacterium*, *Bacteroides*, *Bosea*, and *Cellvibrio*
6 increased in abundance across development, while others such as *Shewanella*, *Plesiomonas*, *Chitinibacter*
7 and *Flavobacterium* decreased. We also found taxa uniquely positively and negatively associated with diet
8 across development, suggesting that each diet may have differential impacts to the zebrafish gut microbiome
9 community and could explain the variation we see across microbiome zebrafish studies. These differences in
0 early-life assembly of the gut microbiome influenced by diet could have long-term impacts on the health of the
1 host¹⁶. For instance, diets that select for microbes that more efficiently metabolize nutrients early in life could
2 provide a fitness advantage to the host and improve their reproductive success, longevity, and ability resist
3 disease^{34,35}. Previous studies showed diet-related impacts to physiology and reproduction⁴, as well as
4 implicated certain taxa to physiological outcomes in zebrafish^{8,9}. Indeed, in the case of ZIRC-diet fed fish we
5 found body condition score linked to the gut microbiome, where body condition score and microbiome diversity
6 are negatively associated. However, we did not find specific taxa that associated with physiological
7 measurements of body condition score across any of the diets, even within ZIRC-diet fed fish. This result
8 suggests that ZIRC diet may not be enriching for particular taxa that influence body condition score.
9 Alternatively, body condition score may not be an ideal metric for identifying physiologically important bacteria.
0 Previous studies measuring more specific physiological measurements such as fat tissue, intestinal length, and
1 gut enzymatic activity where able to identify bacteria that associated with physiology^{8,9,36}. Future studies could
2 integrate a variety of physiological metrics in conjunction with mono-associated gut microbiomes to better
3 identify physiologically important microbes.

5 We find that exposure to the intestinal pathogen *Mycobacterium chelonae* inhibited diversification of gut
6 microbiomes, and microbiome community composition was sensitive to *M. chelonae* exposure it was driven
7 primarily by diet. The gut microbiome diversity of ZIRC-diet fed fish is uniquely sensitive to pathogen exposure,
8 while Gemma- and Watts-diet fed fish were more stable. Higher gut microbiome diversity is linked to higher
9 stability and greater ability to resist pathogens because of competition for habitat space and nutrient
0 availability²¹. When assessing effects of pathogen exposure on microbiome community composition, we find
1 they were secondary to diet and might explain why our results differ from previous microbiome-pathogen
2 studies that saw increased microbiome community variation following pathogen exposure²⁰. Specifically,
3 Gaulke *et al.* found microbiome diversity and community composition increased in variation within pathogen
4 exposed fish, while we find the opposite effect of exposed fish microbiome communities becoming more similar
5 to one another and decreased diversity compared to controls. Additionally, differences could be due to the
6 differences in pathogens, where Gaulke *et al.* exposed fish to an intestinal helminth and we used a bacterial
7 pathogen. We were interested in identifying if specific taxa abundance was linked to *M. chelonae* exposure.
8 Compared to unexposed fish, *Plesiomonas* was depleted in exposed fish regardless of diet. *Plesiomonas* has
9 been shown to reduce fat tissues in zebrafish³⁷. Each diet had specific associations between pathogen
0 exposure and taxon abundance. Interestingly, *Mycobacterium*'s abundance differed between exposure groups
1 within each diet. Compared to control fish, the exposed Watts-diet fed fish had more *Mycobacterium*, but
2 Exposed Gemma- and ZIRC-diet fed fish had fewer relative to control fish. *Mycobacterium* taxa might have

Commented [TS14]: 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.

4 taken advantage of the low diversity environment of Watts-diet fed fish to gain habitat space or utilize nutrients
5 to increase in abundance. It's important to note that nonpathogenic *Mycobacterium* species are a common
6 member of the zebrafish gut microbiome community. Due to the limitations of 16S analysis it's not possible to
7 disentangle whether the *Mycobacterium* abundance we observed is the injected pathogenic strain or non-
8 pathogenic species naturally present in the fish. Additionally, to ensure exposure to *M. chelonae* we injected
9 fish with the pathogen, but this is not the natural route of transmission. Furthermore, priority effects may have
0 inhibited *M. chelonae* from successfully colonizing zebrafish guts due to there being an already established
1 microbiome community. These factors might explain why we did not find an effect of infection on the gut
2 microbiome. However, there could have been other indicators of *M. chelonae*'s effect on the gut microbiome.
3 For instance, presence of *M. chelonae* may induce an immunological inflammatory response that affects the
4 gut microbiome and explain the inhibition of diversification in the pathogen exposed fish. Collectively, our
5 results demonstrate that the gut microbiome is sensitive to *M. chelonae* exposure, but diet plays a significantly
6 greater role in shaping the gut microbiome. Future studies should include additional immunological endpoints,
7 expose zebrafish using a natural route of transmission, and expose fish to a variety of pathogens to clarify the
8 effect of pathogen exposure on the gut microbiome and host's health.
9

0 Beyond zebrafish husbandry, our results have important implications to the field of conservation biology for
1 wildlife management and rehabilitation, particularly for threatened fish species such as salmonids. Salmon are
2 a keystone species in many aquatic systems in North America and are threatened by anthropogenic impacts to
3 their environment, such as expansion of human urbanization disrupting migration, spawning and nutrient
4 acquisition^{38,39}. The differences in nutritional composition found across the diets we investigated here can be
5 seen as analogous to the variability in nutritional or resource availability caused by habitat fragmentation⁴⁰.
6 These challenges to wild salmons' ability to acquire necessary nutrition could have negative downstream
7 impacts on the development of their gut microbiome⁴¹. Moreover, previous research finds gut microbiomes of
8 wildlife in their natural environments differ from those in captivity⁴². Two proposed reasons for the variation in
9 wild and captive animal microbiomes are the differences in diet and immune system development between
0 their natural and captive environments. These differences are suspected as playing a role in the success or
1 failure of wildlife reintroduction given the microbiomes role in digesting nutrients and supporting the immune
2 system. However, more research is needed to clarify the microbiome's impact on successful reintroduction of
3 wildlife. Our insights into the influence of diet on zebrafish gut microbiome across their development provides a
4 useful resource for researchers and wildlife managers seeking to integrate the microbiome in their
5 conservation efforts.
6

7 In conclusion, we find diet is one of the most important factors driving variation in the zebrafish gut microbiome.
8 Unlike prior studies, including the extensive research conducted in mammalian models, that have evaluated
9 dietary effects on the gut microbiome using diets that fundamentally differ in macronutrient composition, our
0 study reveals that even relatively consistent diets that are commonly selected as normal husbandry practices
1 elicit these large impacts on microbiome composition. It may be worth establishing a standard reference diet
2 for microbiome-targeted zebrafish studies to improve our understanding of zebrafish health and nutrition,
3 advance knowledge of how the diet and microbiome interact, and support efforts towards reproducibility and
4 interpretability of results across studies. Although, zebrafish diets may benefit from a variety of diets to model
5 the variation in diets and microbiomes we see in human populations. One important challenge to establishing a
6 standard reference diet is its ability to be made germ-free and nutritionally equivalent to conventional diets³⁰.
7 Significant progress is being made on this front, which supports efforts to better understand the connection
8 between diet and the microbiome in zebrafish. Collectively, our results indicate that researchers should
9 carefully consider the role of diet in zebrafish microbiome studies, and the microbiome should be considered
0 an important factor to support wildlife management and rehabilitation efforts.
1

2 Methods

3 Fish Husbandry

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

6 Diets

7 Fish were all fed the same nursery diet until 30 days old, a combination of paramecia, brine shrimp, and the
8 ZIRC Nursery Mix: Zeigler AP Larval Diet (Ziegler Bros Inc., Gardners, PA) and freeze dried rotifers. Fish were
9 then transferred to the OSU facility and assigned randomly to one of three juvenile diets: Gemma Micro
0 150/300 (Skretting, Fontaine-les-Vervins, France), Watts High-Fat Juvenile Mix, or ZIRC Juvenile Mix, twice
1 daily (9 AM and 3 PM local time) until 60 days old. From 60 days of age onward, OSU fish were not fed on
2 weekends and 1-day holidays as per the facility institutional animal care and use protocol. The total quantity
3 fed daily was 3% fish body weight. This continued until fish were 214 days old and then they were transitioned
4 to the adult version of their previously assigned juvenile diet: Gemma Micro 500 (Skretting, Fontaine-les-
5 Vervins, France), Watts Low-Fat Adult Mix, or ZIRC Adult Mix, twice daily (9 AM and 3 PM local time), except
6 weekends and 1-day holidays. The total quantity fed daily was 3% fish body weight. The prescribed amounts of
7 each diet regimen, for both the juvenile and adult diets were delivered by 3D printed spoons specific to the diet
8 and stage of life. These spoons were paired with conical tubes retrofitted with leveling wires to ensure
9 consistent feeding volumes as prescribed. All fish were only fed once, in the afternoons, on sampling days.

4 Diet and Pathogen Exposure

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

3 Growth Parameters and Sex Determination

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

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

3 **Commented [SJM15]:** Need Mike to review zebrafish
4 methods

5 **Commented [SJM16R15]:** Some of these will need
6 updating since we subset fish from the larger experiment

8 via photographs taken with an iPhone (Apple Inc., Cupertino, CA) and analyzed with ImageJ software
9 (<https://imagej.net>).
0

1 Body condition score (BCS) was calculated using the following equation: BCS = Weight/Length³. Body
2 condition score is a length normalized metric of weight (for equation, see Methods) and serves as a general
3 indicator of health in zebrafish.
4

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5 Weight was taken while the fish was still under the effects of anesthesia by transferring them from the
6 photography petri dish to the petri dish on a scale with a volume of tared fish water. Excess water was
7 removed
8

Histopathology

1 Fish were preserved in Dietrich's solution, processed, and slides stained with Kinyoun's acid-fast. Severity was
2 scored by counting total numbers of granulomas containing acid fast bacteria in the coelomic cavity, ovaries,
3 and kidney. Score of 1 was 1–2 granulomas, 2 = multiple granulomas observed, 3 = prominent infections with
4 granulomatous lesions occupying a large amount of the coelom or gonad. In addition, an overall severity of
5 infection score was assigned based on the average scores of the individual structures evaluated (cite previous
6 Kent lab paper).
7

Fecal Collection

0 Fecal material was collected from individual fish at the same sample intervals as outlined for the growth
1 parameters. Fecal collection was set up the day before growth parameter sampling. Fish were transferred to
2 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.
3 Fish were left to defecate overnight and all feces present were collected from each tank the following morning.
4 Fecal samples were immediately snap frozen on dry ice and stored at -80 °C until processing.
5

16S Sequencing

0 Microbial DNA was extracted from zebrafish fecal samples and 16S rRNA gene sequence libraries were
1 produced and analyzed following established approaches (Kundu et al., 2021). Briefly, the DNeasy PowerSoil
2 Pro DNA kits (Qiagen) were used to extract and purify DNA. The V4 region of the 16S rRNA gene was PCR
3 amplified using the Earth Microbiome Project 16S index primers and protocols (Walters et al., 2016). PCR
4 products were visualized on a 1.5% agarose gel and quantified on a Qubit 2.0 (Thermofisher Scientific) using
5 the Qubit dsDNA HS Assay. One hundred ng of each PCR sample was pooled, cleaned using the QIAquick
6 PCR Purification Kit (Qiagen), and quality was verified on the Agilent TapeStation 4200. The prepared library
7 was submitted to the Oregon State University Center for Quantitative Life Sciences (CQLS) for 300 bp paired-
8 end sequencing on an Illumina MiSeq System (RRID:SCR_016379).
9

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Analysis

0 All microbiome DNA sequence analyses and visualizations were conducted in R (v 4.2.1). Fastq files were
1 processed in using the DADA2 R package (v 1.18.0). Briefly, forward and reverse reads were trimmed at 280
2 and 230 bp, respectively, subsequently merged into contigs, and subject to amplicon sequence variant (ASV)
3 identification. ASVs unannotated at the Phylum level were removed to result in 292 remaining detected ASVs.
4 We used Wilcoxon Signed-Ranks Tests to identify parameters that best explained the variation in weight and
5 body condition scores. Alpha-diversity was calculated using the estimate_richness function (Phyloseq v 1.38.0)
6 and transformed using Tukey's Ladder of Powers. After transformation, scores were normalized from 0 to 1 by
7 dividing each score by the maximum value, which allowed us to compare results across alpha-diversity metrics
8 using general linear models (GLMs). Two-way ANOVA assess these GLMs. Beta-diversity models were
9 generated using methods described previously (Kundu et al., 2021). Briefly, we evaluated three beta-diversity
0 metrics—Bray-Curtis, Canberra, and Sorenson and resolved the relationship between experimental parameters
1 and beta-diversity by applying a step-wise model selection approach as implemented in the capscale function
2 (vegan package v2.5). Optimal models were subsequently subject to PERMANOVA analysis to determine if
3

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Commented [ST20]: Methods.

3 the selected model parameters significantly explained the variation in microbiome composition across samples.

4 Differential abundance was measured using ANCOM-BC (ν).

5

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2

3 **Supplementary Tables and Figures**

4 **1) Diet**

5 **1.1) Physiology**

6 **1.1.1)**

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

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

9 **1.1.2)**

0 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 **1.1.3)**

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

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

3 **1.1.4)**

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	****

5 **1.2) Alpha-diversity**

6

7

8

9 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	*

0
1
2 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	*

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4 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

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7 1.3) Beta-diversity

8 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			

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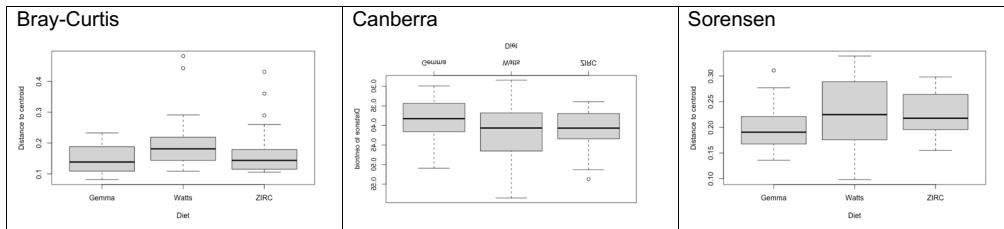
0 1.4) Beta-Dispersion

1 1.4.1) Diet

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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			

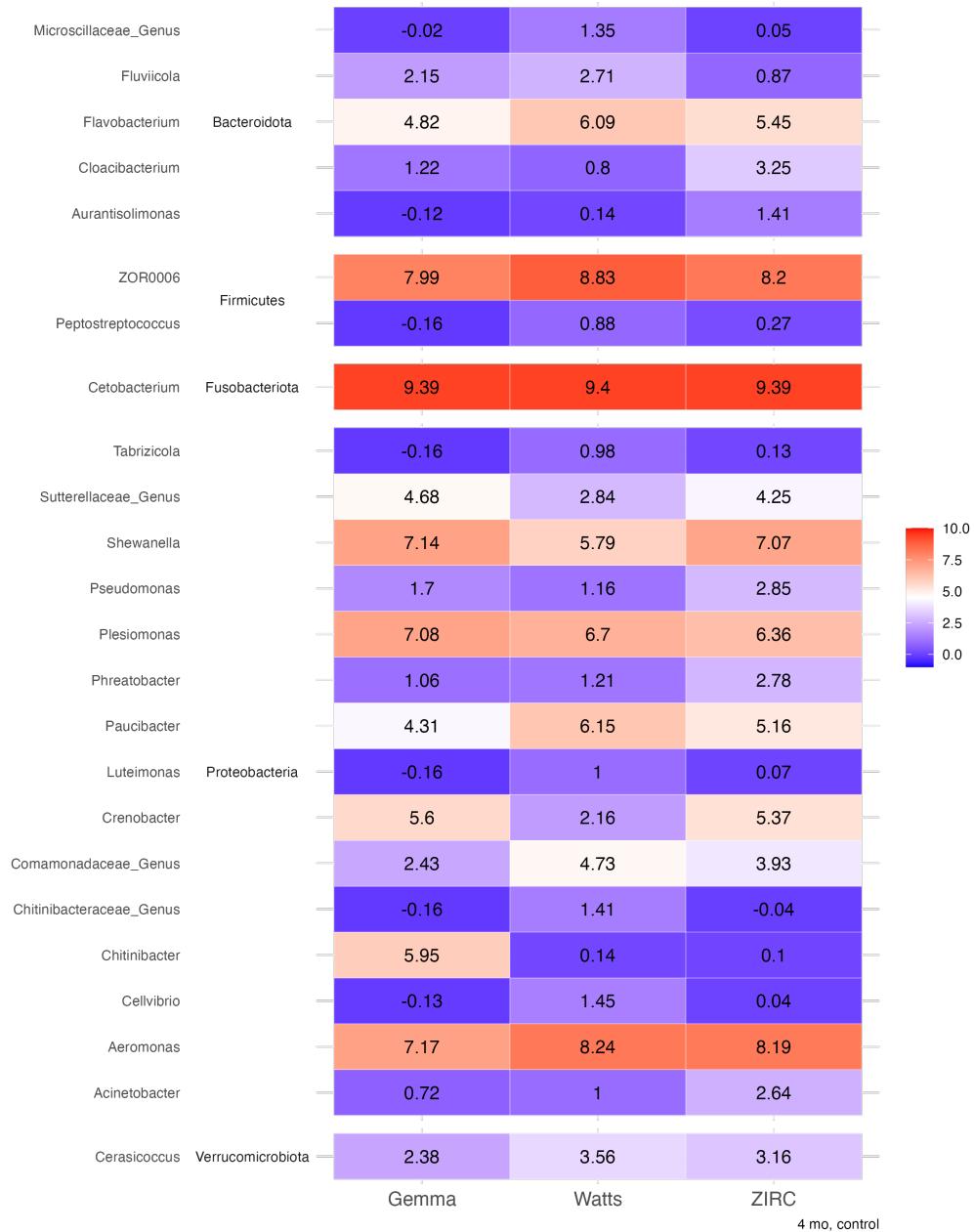
Names	p-value
Gemma-Watts	0.014
Gemma-ZIRC	0.044
Watts-ZIRC	0.435

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

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8 1.5) Differential Abundance
9

0 1.5.1)

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



4 mo, control

1

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

3 1.5.2)
4



Taxon	Rc_DewWatts	Rc_DewZIRC	Rc_DewZIRC_DewWatts	sc_DewWatts	sc_DewZIRC	sc_DewZIRC_DewWatts	W_DewWatts	W_DewZIRC	W_DewZIRC_DewWatts	p_RevRate	p_DewWatts	p_DewZIRC	p_DewZIRC_DewWatts	d_DewWatts	d_DewZIRC	d_DewZIRC_DewWatts	dr_DewWatts	dr_DewZIRC	dr_DewZIRC_DewWatts
ANCOM-B2: Summary table of pairwise comparisons of abundance bias (Rrc, rcrc)																			
Cetobacterium	0.019	-0.009	-0.019	0.149	0.149	0.196	0.094	-0.003	-0.094	0.903	0.999	0.949	1.000	1.000	1.000	1.000	1.000	1.000	1.000
ZO90006	0.837	0.208	-0.829	0.148	0.151	0.196	5.661	1.379	-3.215	0.000	0.168	0.001	0.000	0.460	0.005	0.005	0.005	0.005	0.005
Aeromonas	1.073	1.018	-0.055	0.162	0.169	0.208	6.610	6.157	-0.264	0.000	0.000	0.791	0.000	0.000	1.000	1.000	1.000	1.000	1.000
Plesiomonas	-0.379	-0.725	-0.348	0.211	0.214	0.252	-1.796	-3.391	-1.374	0.072	0.001	0.170	0.272	0.003	0.466	0.005	0.005	0.005	0.005
Shewanella	-1.350	-0.065	1.285	0.204	0.207	0.245	-6.626	-0.313	5.235	0.000	0.754	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000
Paenibacillus	1.852	0.859	-0.993	0.363	0.365	0.395	5.109	2.353	-2.514	0.000	0.119	0.012	0.000	0.051	0.045	0.045	0.045	0.045	0.045
Comamonadaceae_Genus	2.303	1.498	-0.805	0.441	0.443	0.471	5.228	3.381	-1.711	0.000	0.001	0.087	0.000	0.003	0.240	0.000	0.000	0.000	0.000
Crenobacter	-3.445	-0.237	3.285	0.381	0.381	0.371	-15.213	0.000	8.045	0.000	0.050	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Bacillus	0.796	0.408	-0.841	0.297	0.304	0.323	5.333	2.118	-1.997	0.000	0.024	0.004	0.109	0.148	0.000	0.000	0.000	0.000	0.000
Sutcliffiaceae_Genus	-1.849	-0.434	1.115	0.400	0.409	0.411	-4.624	-1.079	3.393	0.000	0.281	0.001	0.000	0.775	0.004	0.004	0.004	0.004	0.004
Gemmibacter	-0.597	-0.403	0.193	0.294	0.297	0.330	-2.029	-1.308	0.587	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Acinetobacter	0.312	1.931	1.618	0.511	0.513	0.539	6.812	3.761	3.003	0.541	0.000	0.000	1.000	0.001	0.010	0.010	0.010	0.010	0.010
Chitinibacter	-5.807	-5.893	-0.088	0.175	0.178	0.220	-33.107	-33.033	-0.390	0.000	0.000	0.697	0.000	0.000	1.000	1.000	1.000	1.000	1.000
Flavilota	0.603	-1.236	-1.840	0.538	0.541	0.566	1.121	-2.286	-3.250	0.262	0.022	0.001	0.722	0.083	0.005	0.005	0.005	0.005	0.005
Coelococcum	-0.408	2.001	2.406	0.447	0.450	0.477	-2.908	4.448	5.046	0.000	0.364	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Cercozoicus	1.198	0.763	-0.414	0.400	0.403	0.431	2.992	1.945	-0.960	0.000	0.052	0.037	0.013	0.194	0.928	0.000	0.000	0.000	0.000
Chitinibacteraceae_Genus	0.407	-1.423	-0.420	0.442	0.442	0.473	-0.570	0.605	-0.845	0.000	0.075	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Pseudomonas	0.509	-0.169	1.673	0.488	0.491	0.517	-1.037	-2.377	3.298	0.200	0.017	0.001	0.024	0.069	0.008	0.008	0.008	0.008	0.008
Mycobacterium	0.039	-0.357	-0.318	0.395	0.397	0.426	-0.096	-0.898	0.746	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Exiguobacterium	0.881	0.107	-0.774	0.430	0.433	0.460	2.049	0.248	-1.681	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Prinsebacter	0.134	1.703	1.569	0.390	0.392	0.421	0.343	4.340	3.728	0.752	0.000	0.000	1.000	0.000	0.001	0.001	0.001	0.001	0.001
Rhizobiales_Family_Genus	-0.135	0.131	0.266	0.224	0.227	0.264	-0.605	0.577	1.009	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Aurantioolomas	0.266	1.508	1.240	0.283	0.286	0.319	0.941	5.273	3.887	0.347	0.000	0.000	0.953	0.000	0.000	0.000	0.000	0.000	0.000
Celvibrio	1.553	0.171	-1.382	0.286	0.289	0.322	5.430	0.592	-4.293	0.000	0.054	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000
Hallangonibacter	0.056	-0.120	0.178	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	1.000
Dugifimbriae	0.157	-0.348	-0.348	0.442	0.442	0.460	-0.480	-0.480	4.264	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Rhodobacteraceae_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	1.000
Flavivirgus	-0.396	-0.274	0.083	0.274	0.277	0.310	-1.302	-0.989	0.267	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Microstaphylococcus_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
Defluvimonas	0.022	-0.476	-0.498	0.312	0.315	0.347	0.069	-1.812	-1.438	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Bosse	0.277	0.033	-0.244	0.199	0.202	0.241	1.392	0.165	-1.011	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Turbicloacis	1.139	0.284	-0.855	0.279	0.282	0.315	4.085	1.010	-2.712	0.000	0.313	0.007	0.000	0.860	0.025	0.025	0.025	0.025	0.025
Luteimonas	1.103	0.215	-0.888	0.226	0.229	0.266	4.884	0.940	-3.342	0.000	0.347	0.001	0.000	0.955	0.003	0.003	0.003	0.003	0.003
Nocardioides_Genus	0.079	-0.259	-0.188	0.235	0.238	0.275	0.043	-1.049	-1.049	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Leptothrix	0.809	0.009	-0.861	0.240	0.245	0.405	6.605	0.118	-0.177	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Lengjia	-0.147	-0.137	0.010	0.207	0.210	0.149	-0.713	-0.655	0.049	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Peptostreptococcus	0.973	0.371	-0.802	0.211	0.214	0.252	4.617	1.737	-2.389	0.000	0.082	0.017	0.000	0.226	0.063	0.063	0.063	0.063	0.063
Iemia	-0.225	-0.366	-0.141	0.184	0.187	0.228	-1.223	-1.960	-0.822	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

7
8 **2) Development**
9
0 **2.1) Physiology**
1 **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			

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3 **2.2) Physiology ~ Microbiome**
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5
6 **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	*

9 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	
Canberra	Residual	129.00	6.31711356			
	Diet	2.00	3.41322719	8.491	0.001	*
	Body.Condition.Score	1.00	0.35999108	1.791	0.009	*
Sørensen	Body.Condition.Score:Diet	2.00	0.58256642	1.449	0.010	*
	Residual	129.00	25.92826696			
	Diet	2.00	1.72484640	11.533	0.001	*
0 1 2 3 4 5 6	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-diversity5 2.3.1) Time
6 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	*

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9 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	*

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2 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	*

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5 2.2.2) Time:Diet
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7 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	

0 2.2.2.2)

ANOVA(glm(Alpha.Score ~ Diet*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	

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3 2.2.2.3)
Pairwise Tukey's HSD, p.adj: Dunnett.glm(Alpha.Score ~ 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	*

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6 2.4) Beta-diversity

7 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			

8
9 2.4.2)

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

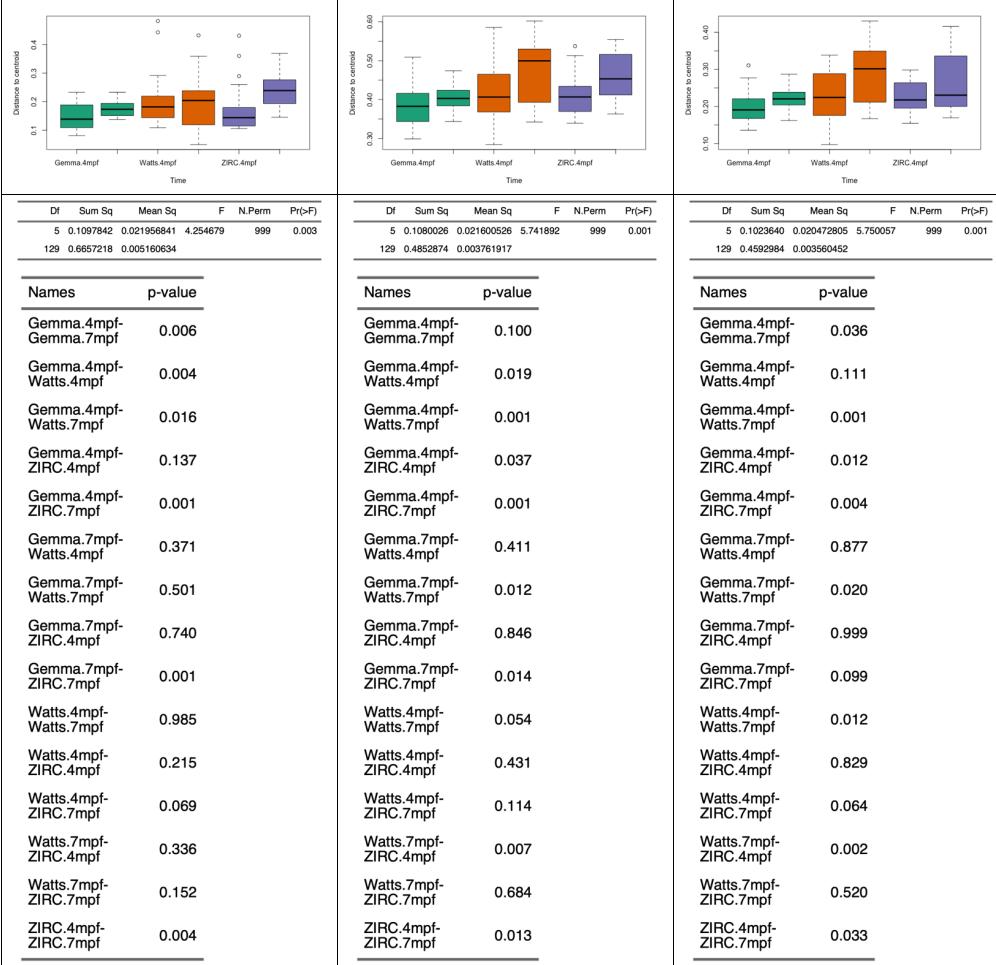
metric	term	Df	SumOfSqs	statistic	p.value	sig
Bray-Curtis	Timepoint	1.00	0.3456869	6.130	0.001	*
	Residual	133.00	7.5005708			
Canberra	Timepoint	1.00	1.5652766	7.249	0.001	*
	Residual	133.00	28.7187751			
Sørensen	Timepoint	1.00	1.0030415	12.398	0.001	*
	Residual	133.00	10.7603792			

0
1 2.4.3)
2

3 **2.5) Beta-Dispersion**
4
5 **2.5.1) Diet**
6
7

8 **2.5.2) Time**
9
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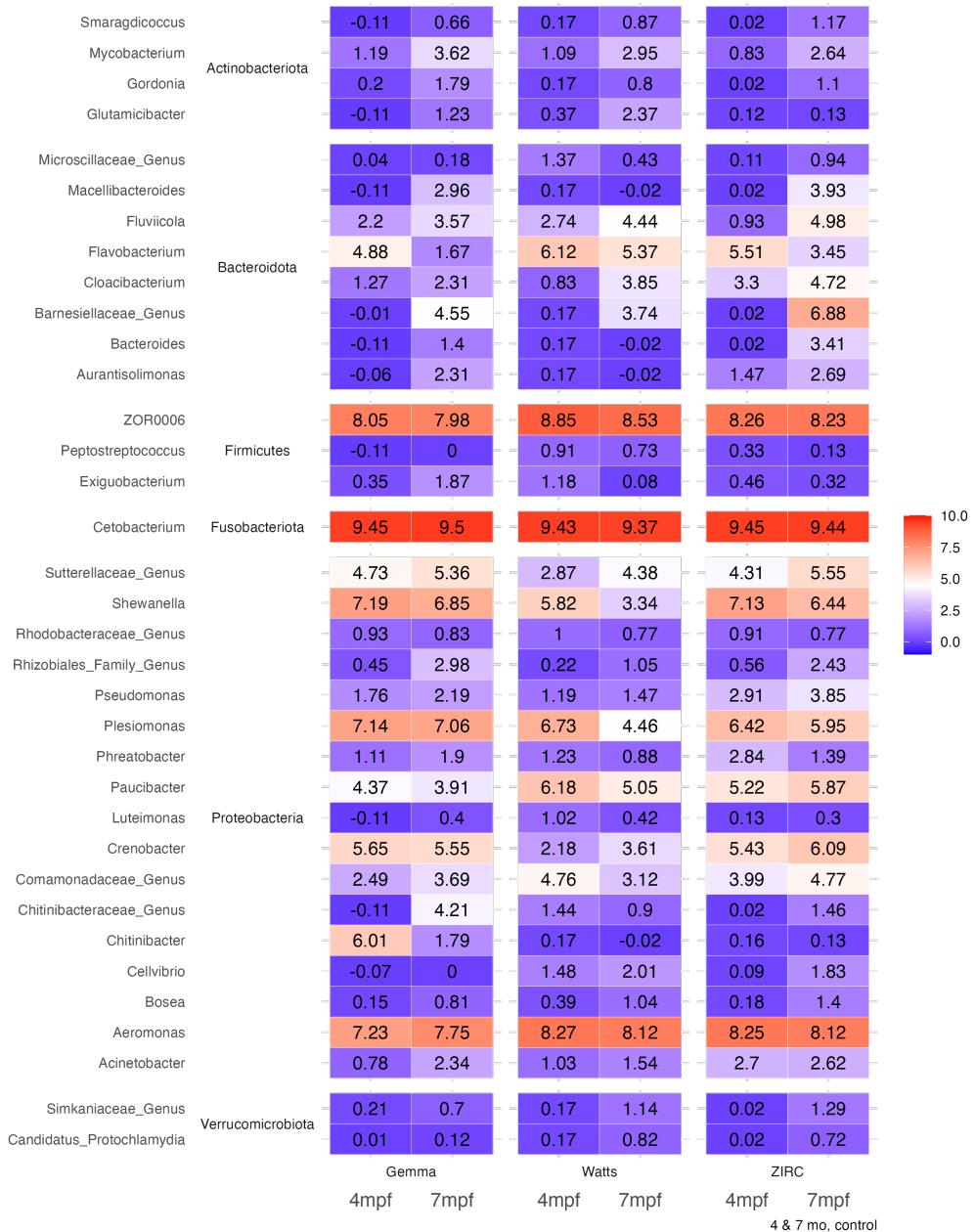
1 2.5.3) Diet:Time



2
3

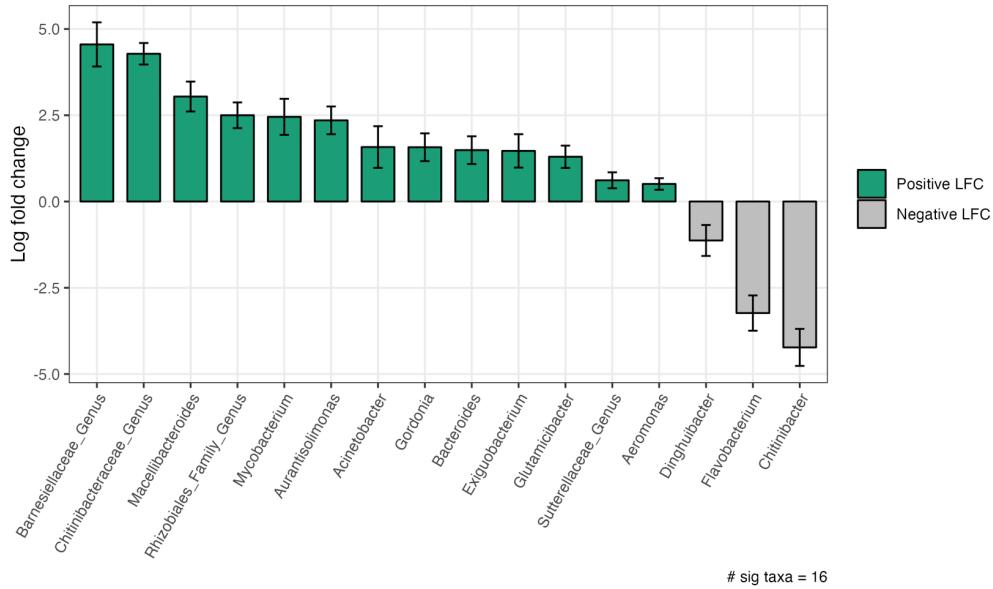
5 2.6.1)

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



7 2.6.2)
8 2.6.2.1)

Log fold changes between 4 and 7 months in Gemma diet

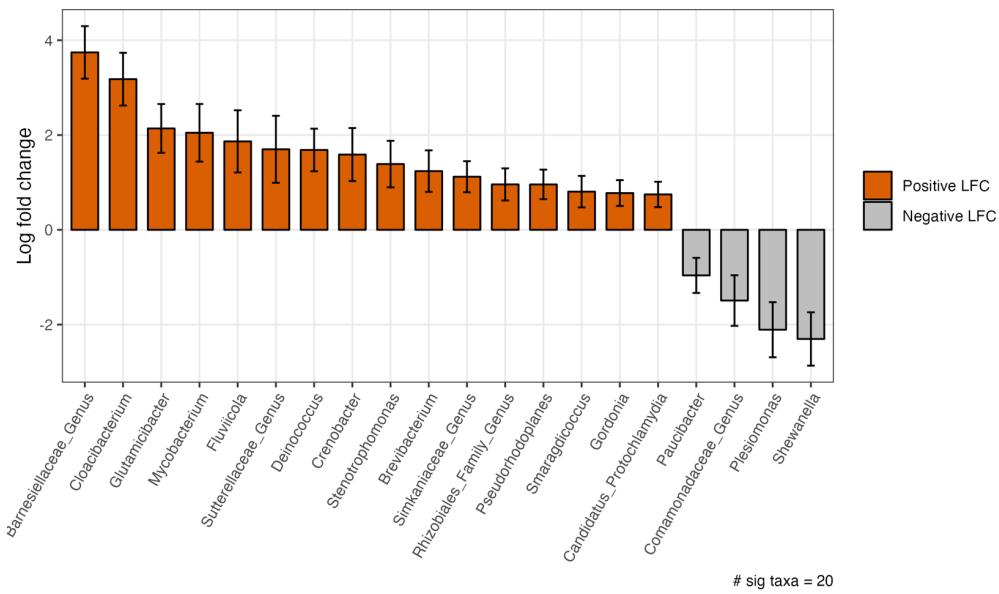


ANCOM-BC2: Log fold change in abundance. # Sig taxa = 16

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

1 2.6.2.2)

Log fold changes between 4 and 7 months in Watts diet



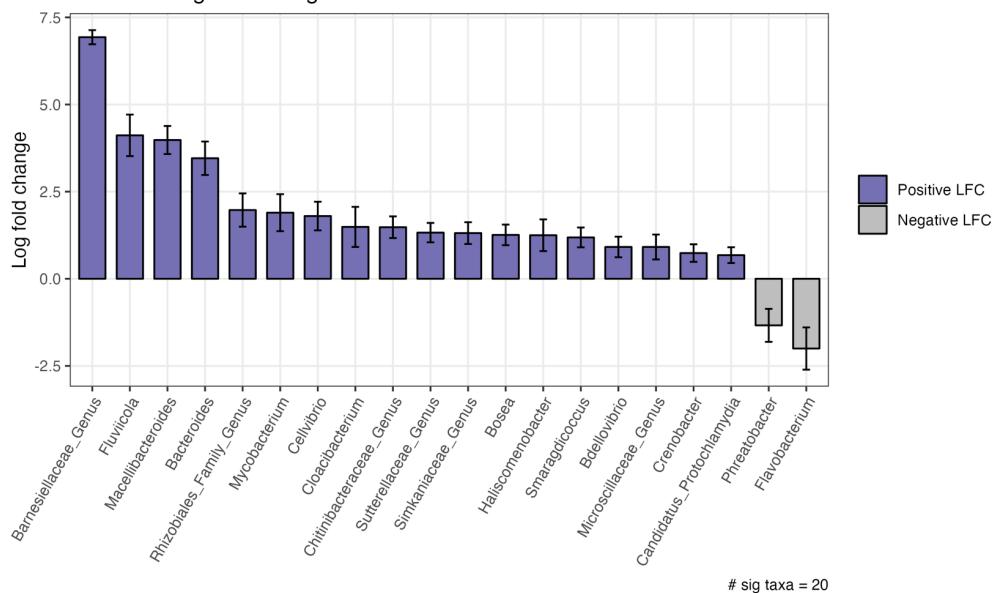
2

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

4 2.6.2.3)

Log fold changes between 4 and 7 months in ZIRC diet

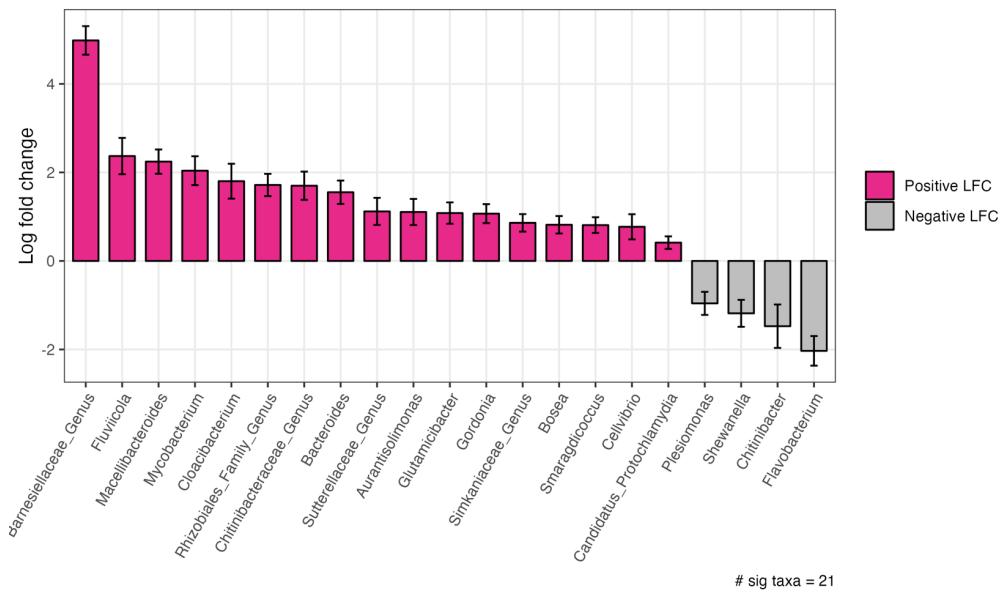


ANCOM-BC2: Log fold change in abundance. # Sig taxa = 20

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

7 2.6.2.4)

Log fold changes between 4 and 7 months across all diets



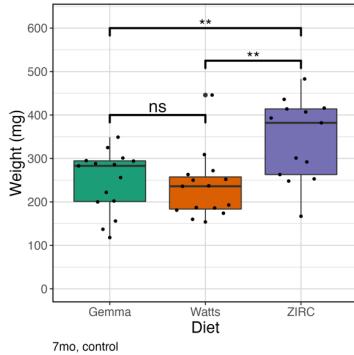
ANCOM-BC2: Log fold change in abundance. # Sig taxa = 21

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

Log fold changes of uniquely abundant taxa across diets at 7mpf

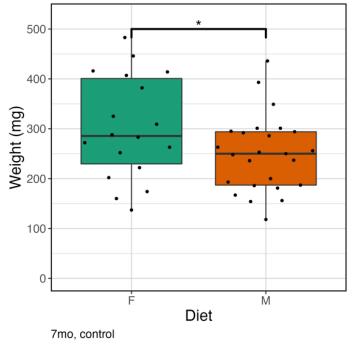


2 2.7) 7 Month Analysis
 3 2.7.1) Physiology
 4 2.7.1.1) Weight ~ Diet



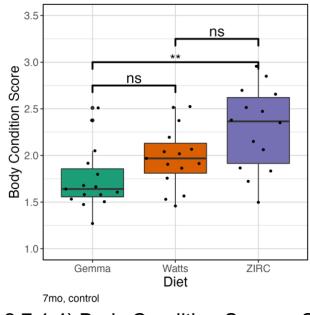
Wilcoxon Test. p. adj: BH. Weight ~ Diet								
.y.	group1	group2	n1	n2	statistic	p	p.adj	p.adj.signif
Weight	Gemma	Watts	15	15	139.000	0.285	0.285	ns
	Gemma	ZIRC	15	15	45.500	0.006	0.009	**
	Watts	ZIRC	15	15	36.500	0.002	0.006	**

6 2.7.1.2) Weight ~ Sex



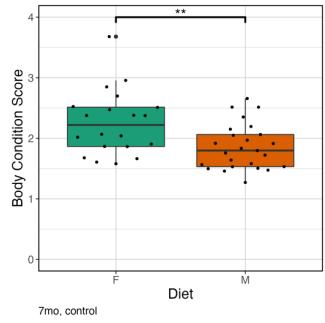
Wilcoxon Test. p. adj: BH. Weight ~ Sex								
.y.	group1	group2	n1	n2	statistic	p	p.adj	p.adj.signif
Weight	F	M	20	25	342.500	0.036	0.036	*

8 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

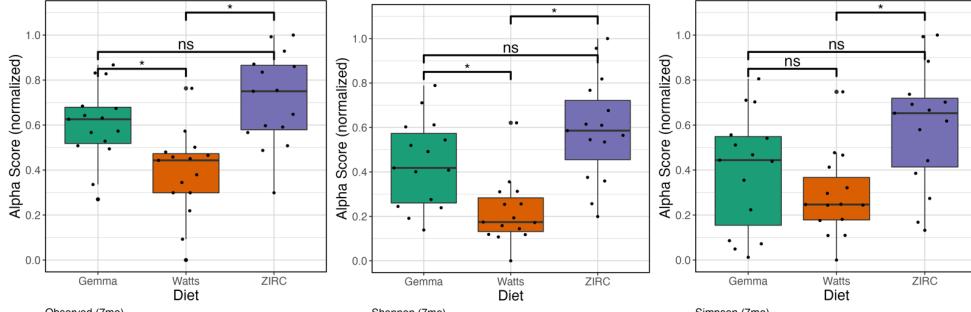
1 2.7.1.4) Body Condition Score ~ Sex



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 **

5 2.7.2) Alpha-Diversity



6

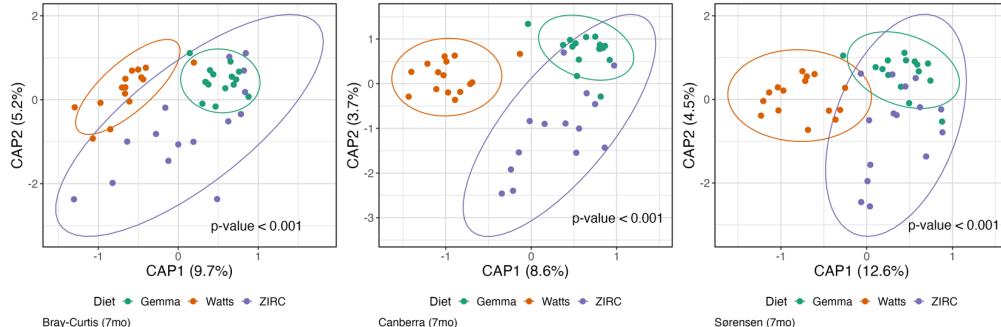
glm(Alpha.Score ~ Diet, family = quasibinomial)						
metric	term	estimate	std.error	statistic	p.value	sig
Observed	(Intercept)	0.422	0.210	2.010	0.051	
	DietWatts	-0.893	0.298	-2.997	0.005 *	
Shannon	DietZIRC	0.486	0.309	1.571	0.124	
	(Intercept)	-0.245	0.213	-1.152	0.256	
Simpson	DietWatts	-1.022	0.332	-3.074	0.004 *	
	DietZIRC	0.613	0.303	2.026	0.049 *	

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	*	

7 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	*

9 2.7.3) Beta-Diversity
 0 2.7.3.1)

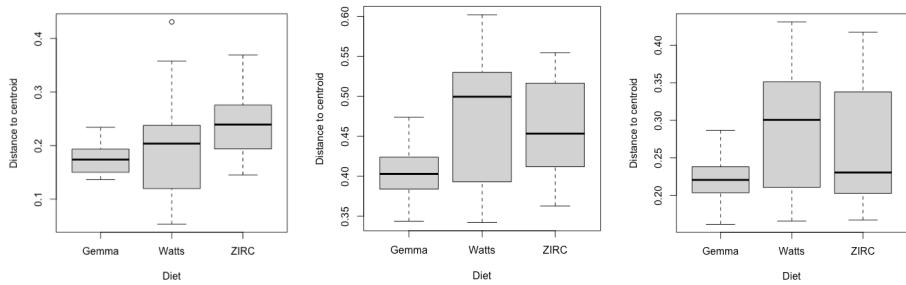


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4 Distance-based redundancy analysis (dbRDA) ordination. Beta.Score ~ Diet

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			

5 2.7.3.2) Beta-dispersion (Bray, Canberra, Sørensen)



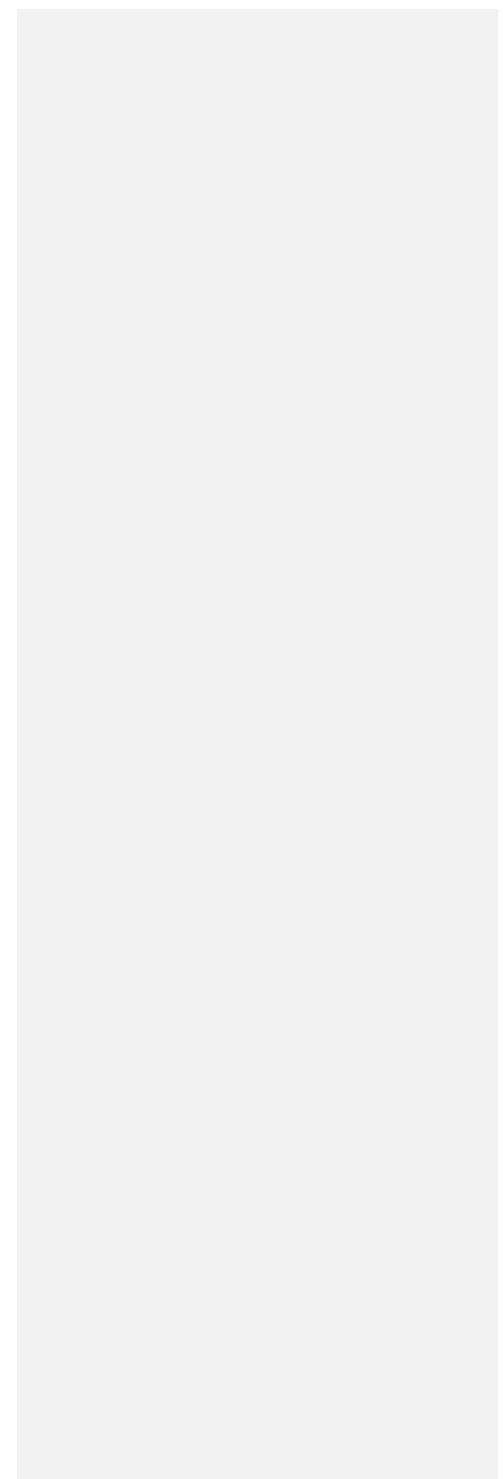
6

Df	Sum Sq	Mean Sq	F	N.Perm	Pr(>F)
2	0.02865063	0.014315316	2.676967	999	0.08
42	0.22459665	0.005347587			

Df	Sum Sq	Mean Sq	F	N.Perm	Pr(>F)
2	0.03521779	0.017608893	3.202764	999	0.052
42	0.23091729	0.005498031			

Df	Sum Sq	Mean Sq	F	N.Perm	Pr(>F)
2	0.03376742	0.016883709	3.975224	999	0.022

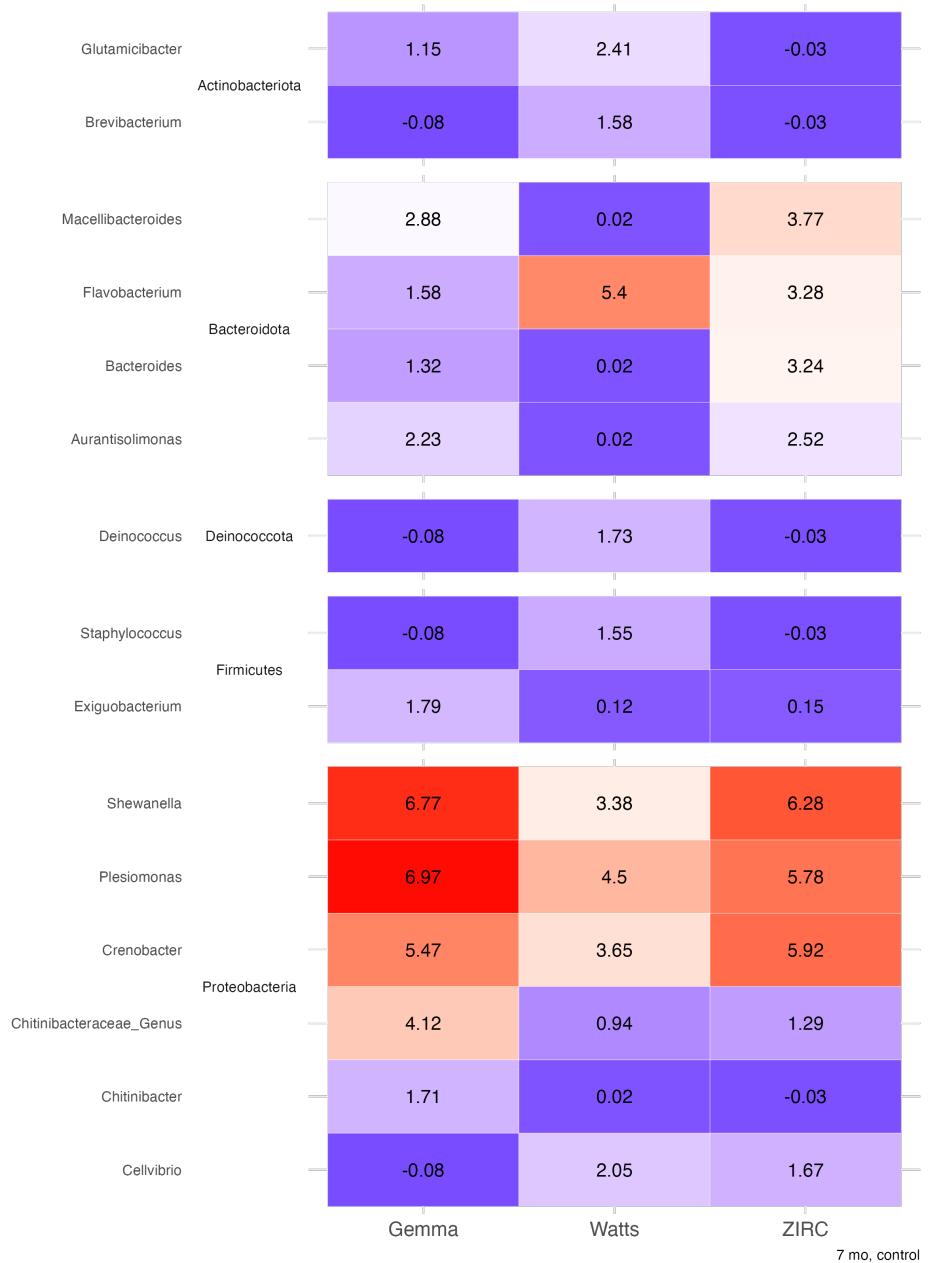
7



Names	p-value	Names	p-value	Names	p-value
Gemma-Watts	0.022	Gemma-Watts	0.484	Gemma-Watts	0.017
Gemma-ZIRC	0.103	Gemma-ZIRC	0.001	Gemma-ZIRC	0.017
Watts-ZIRC	0.535	Watts-ZIRC	0.149	Watts-ZIRC	0.701

0 2.7.4) Differential Abundance

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



7 mo, control

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

5
6 **3) Exposure**
7
8 **3.1) Alpha-diversity**
9
0 **3.1.1) Exposure**
1 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	*

2
3
4 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	*

5
6

7 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	*

8

9

0 3.1.2) Diet:Exposure

1

2 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	
3	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	

4 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	

5

6 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	*

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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			

1 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			

2 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 3.2.3

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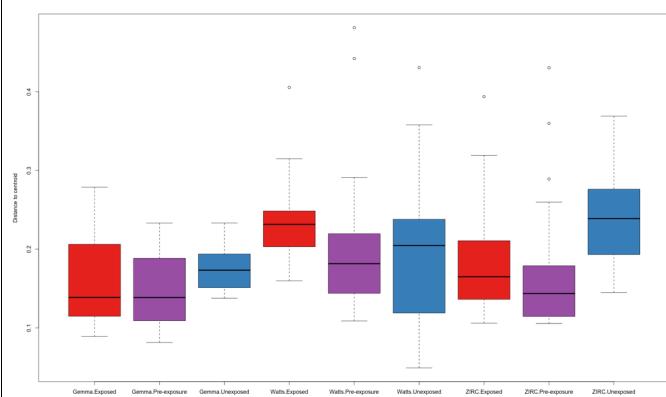
7 3.3) Beta-Dispersion

8

9 3.3.1) Exposure

0 3.3.2) Diet:Exposure

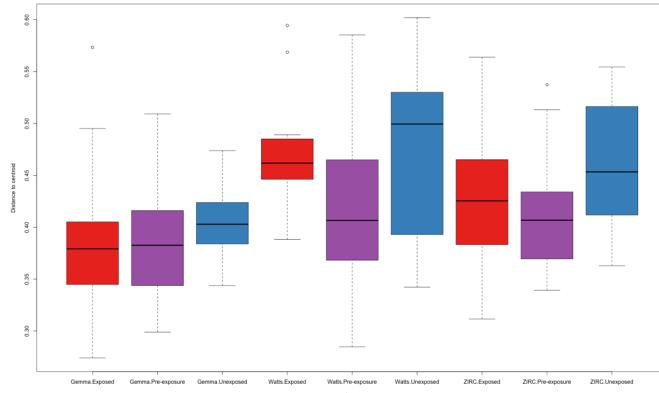
Bray-Curtis



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

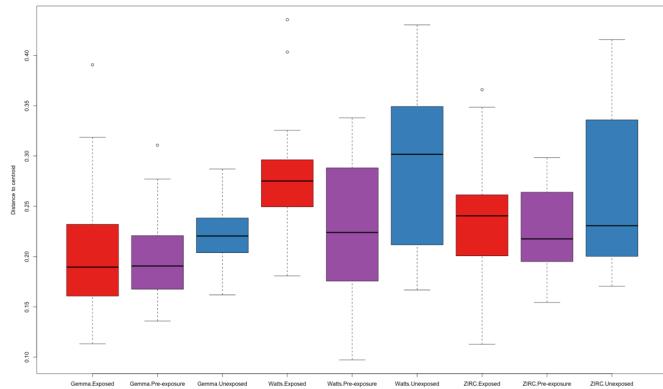
Canberra



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

Sorensen



Df	Sum Sq	Mean Sq	F	N.Perm	Pr(>F)
8	0.1550544	0.01938180	5.041974	999	0.001
170	0.6534953	0.00384409			

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	0.002
Watts.Exposed-ZIRC.Pre-exposure	0.002
Watts.Unexposed-ZIRC.Pre-exposure	0.004
Gemma.Unexposed-Watts.Exposed	0.005
Gemma.Exposed-Watts.Exposed	0.008
Gemma.Pre-exposure-ZIRC.Pre-exposure	0.011
Watts.Exposed-Watts.Pre-exposure	0.011
Watts.Pre-exposure-Watts.Unexposed	0.012
Gemma.Unexposed-Watts.Unexposed	0.013
Gemma.Exposed-Watts.Unexposed	0.015
ZIRC.Pre-exposure-ZIRC.Unexposed	0.021
Gemma.Pre-exposure-Gemma.Unexposed	0.026
Gemma.Pre-exposure-ZIRC.Exposed	0.027
Gemma.Exposed-ZIRC.Unexposed	0.047
Watts.Pre-exposure-ZIRC.Unexposed	0.061
Watts.Exposed-ZIRC.Exposed	0.069
Gemma.Unexposed-ZIRC.Unexposed	0.091
Gemma.Pre-exposure-Watts.Pre-exposure	0.097
Watts.Unexposed-ZIRC.Exposed	0.097

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4 **3.4) Differential Abundance**

5 3.4.1)

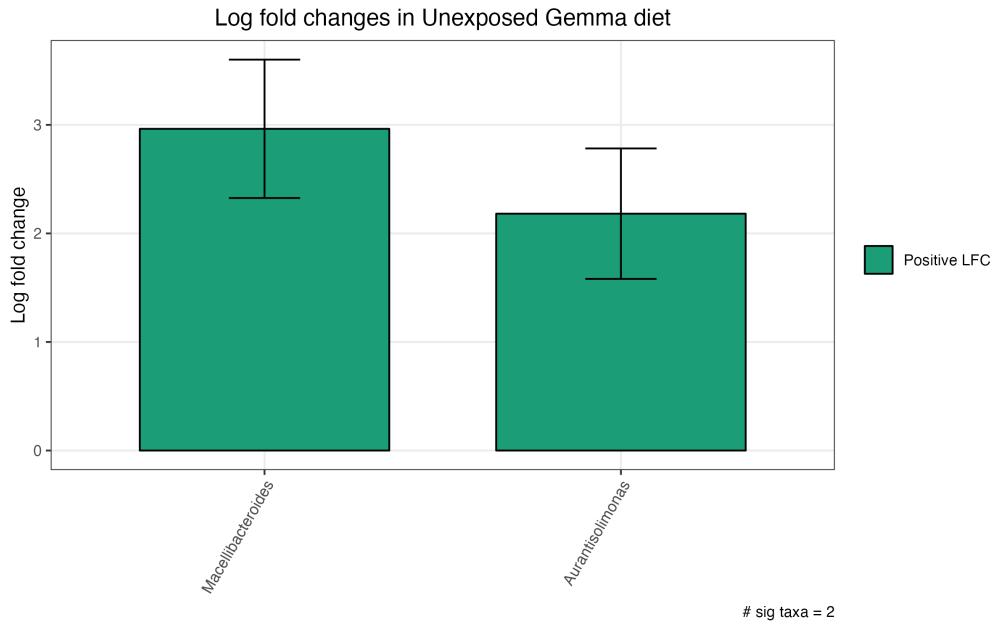
Heat map of bias-corrected log observed abundances (Genus)



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

8 3.4.2)



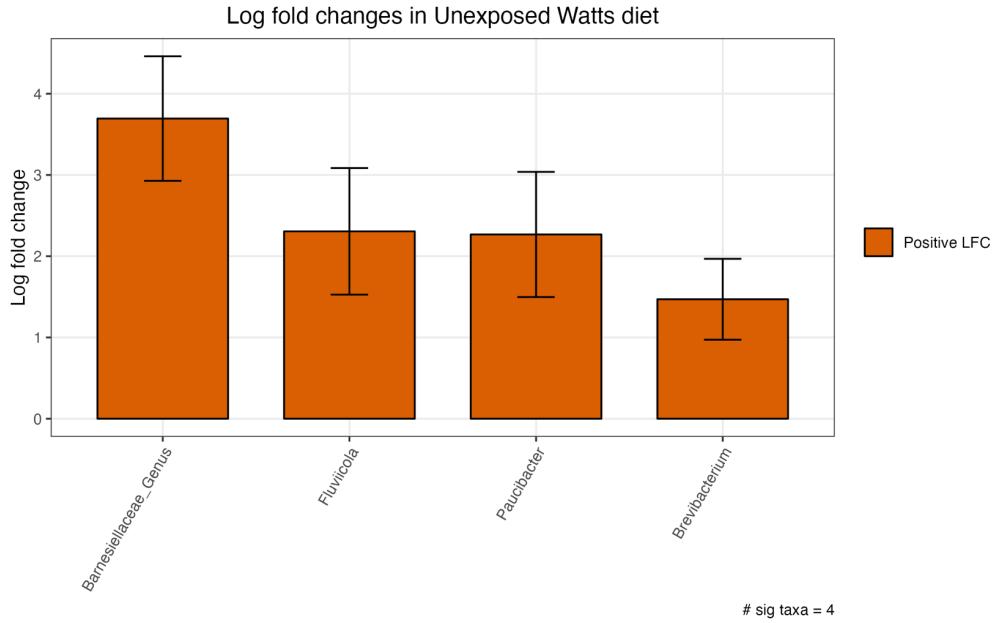
9

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

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2 3.4.3)



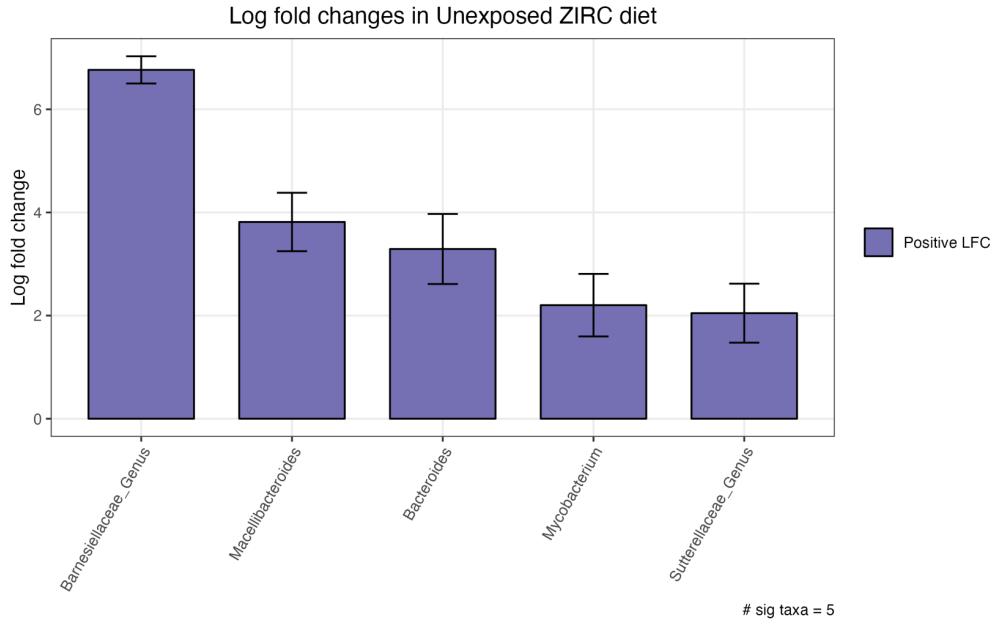
3

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

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5 3.4.4)



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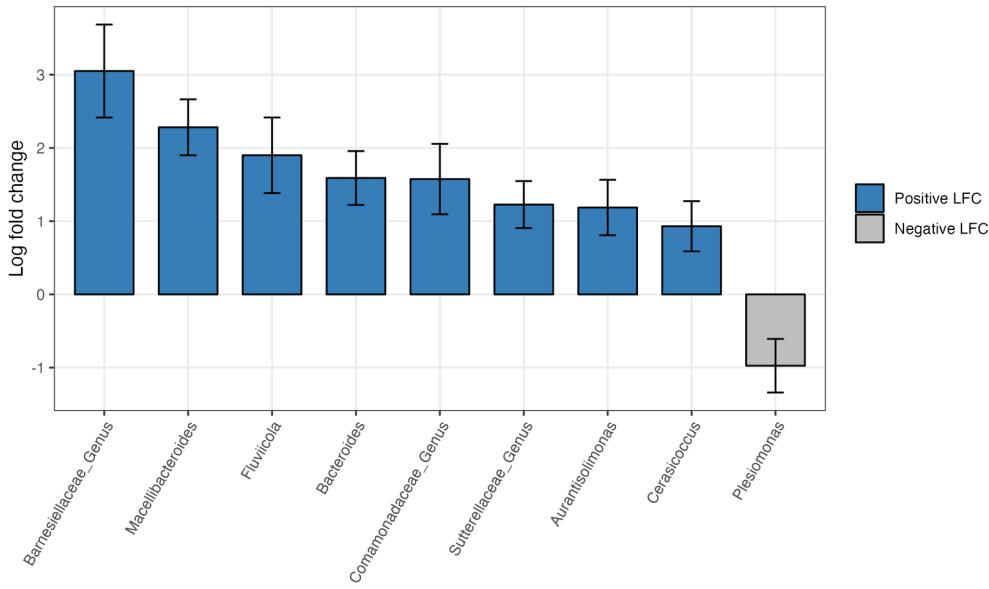
ANCOM-BC2: Log fold change in abundance. # Sig taxa = 5

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

7

8 3.4.5)

Log fold changes in unexposed across all diets



9

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

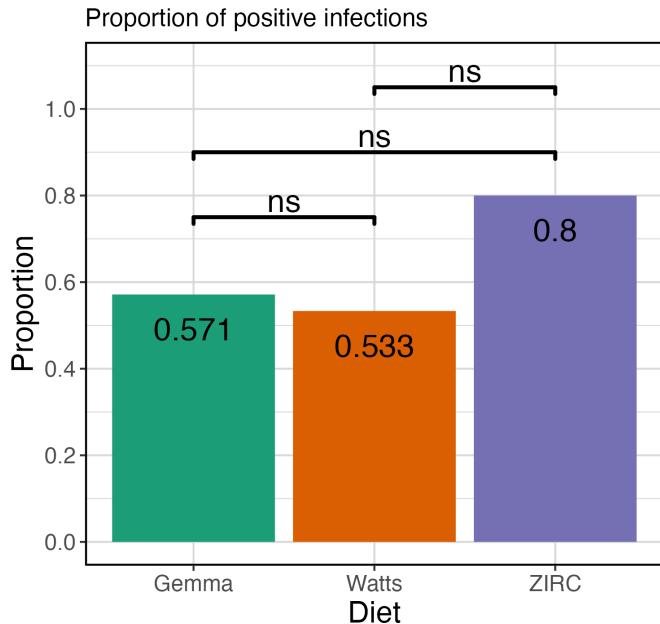
0

1 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

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4 3.5) Infection
5 3.5.1)



6 7mo, exposed

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

7
8 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			

9

0 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	

2 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	

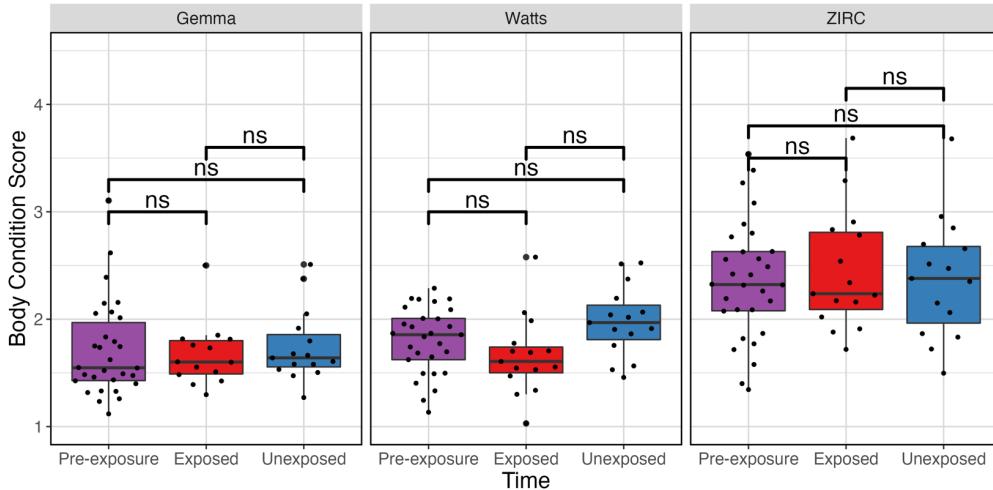
3
4 3.5.5)

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			

5

8 3.6) Physiology

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0 3.6.1)1
2 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