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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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10      **Abstract**

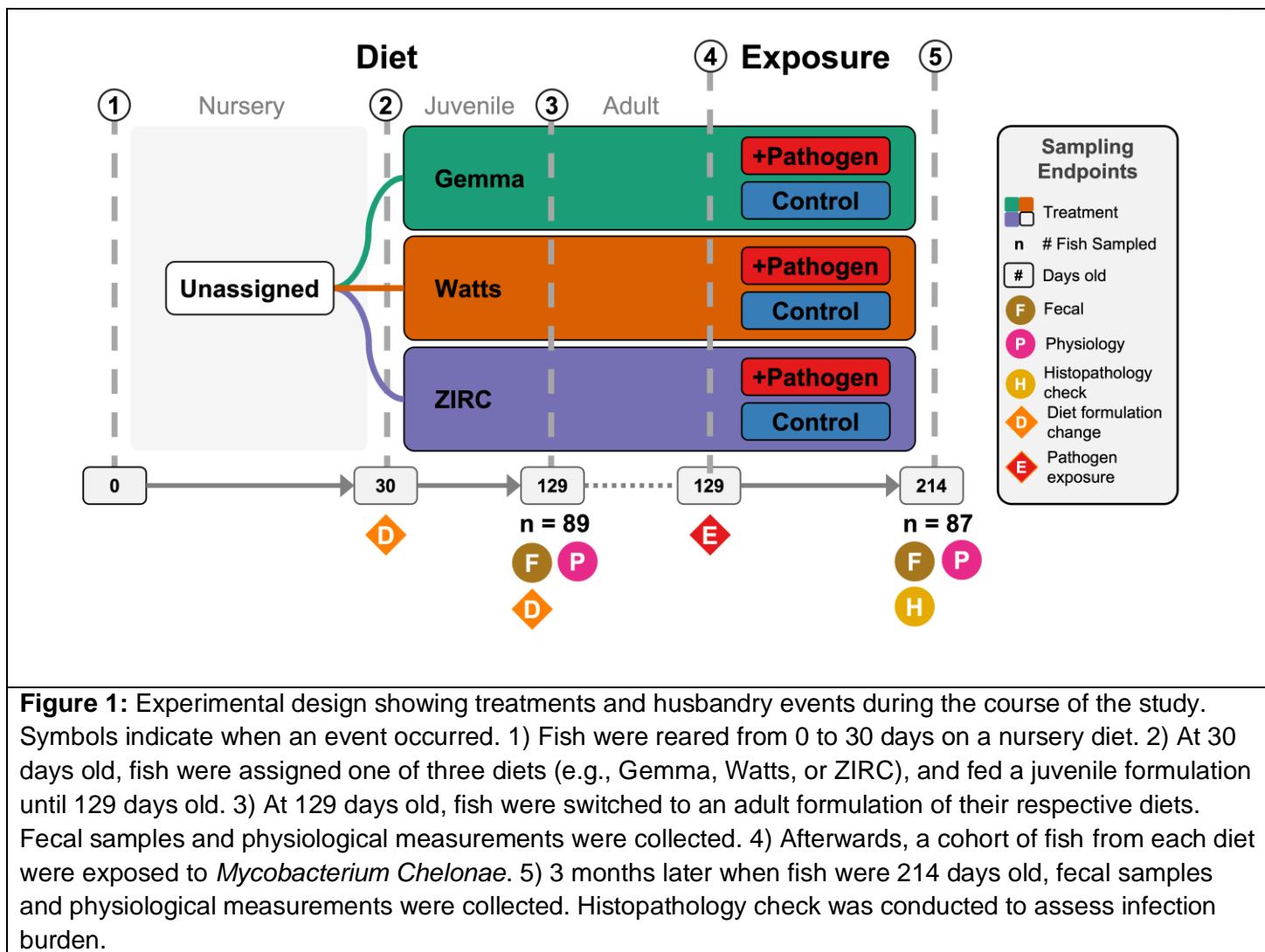
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12     Despite the long-established importance of zebrafish as a model organism and their increasing use in  
13 microbiome-targeted studies, relatively little is known about how husbandry practices involving diet impact the  
14 zebrafish gut microbiome.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

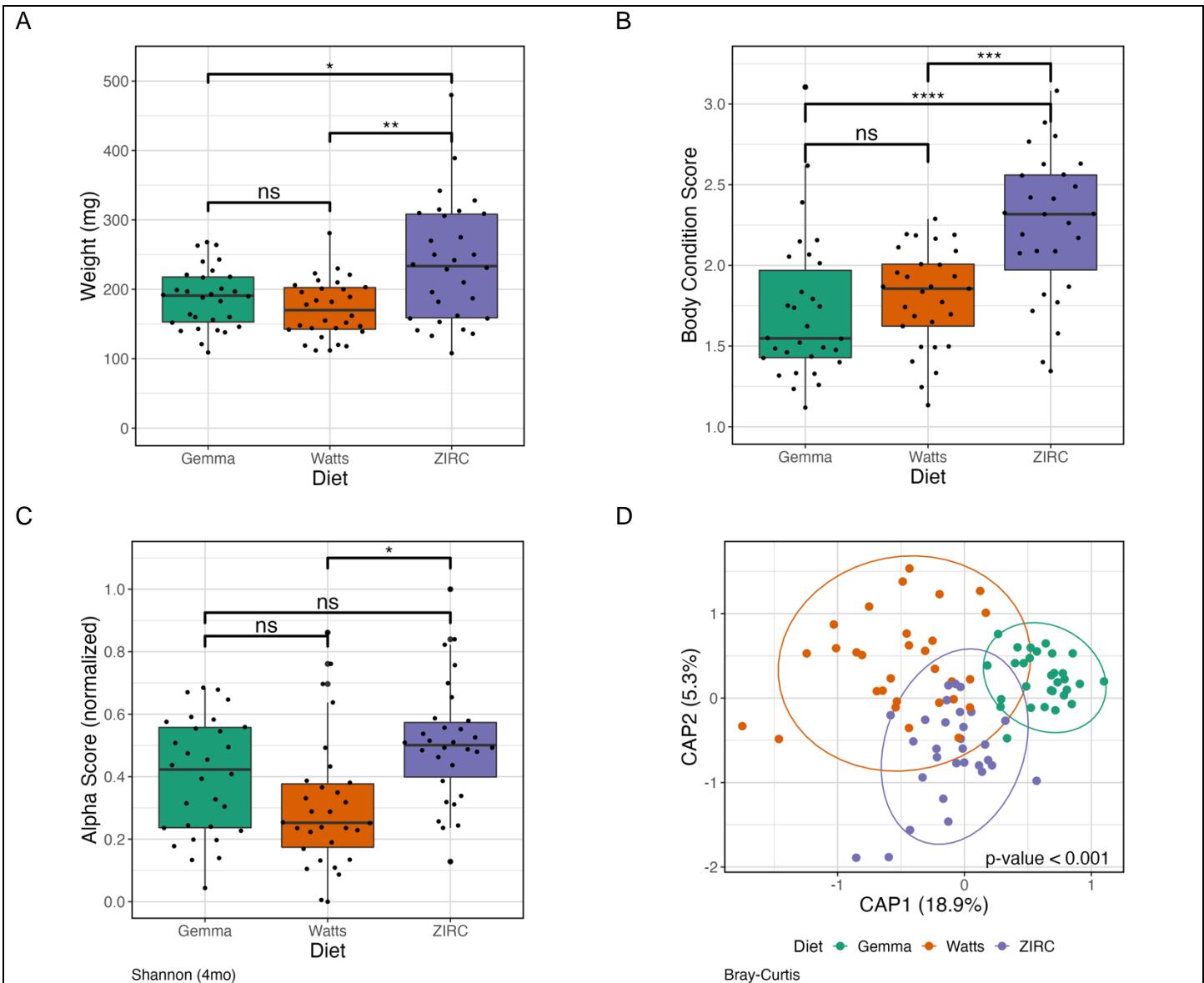
27  
28      **Introduction**

29  
30     In the effort to understand how the gut microbiome mediates vertebrate health, zebrafish (*Danio rerio*) have  
31 emerged as an important microbiome experimental model organism<sup>1</sup>. Despite the increasing use of zebrafish  
32 in microbiome research, key knowledge gaps remain about how different zebrafish husbandry practices,  
33 especially diet, influences microbiome composition<sup>2</sup>. For example, in contrast to mice, zebrafish do not have a  
34 standard reference diet<sup>3</sup>. Instead, zebrafish research facilities vary by dietary husbandry practice, which can  
35 impact physiological and reproductive outcomes<sup>4–6</sup>. Given that diet plays an important role in shaping the  
36 composition of the gut microbiome in other animal models, including mice {citation}, we hypothesize that  
37 variation in dietary husbandry practice also impacts the composition of the zebrafish gut microbiome.  
38 Quantifying this association is important because it could explain why, despite the existence of a core gut  
39 microbiome, gut microbiome composition differs across research facilities, improve efforts to integrate data  
40 across investigations, and clarify how dietary variation manifests as physiological variation.

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42     Relatively little is known about how variation in dietary husbandry practice impacts the zebrafish gut  
43 microbiome. Prior studies that measured the impact of diet on the zebrafish gut microbiome has largely  
44 considered how substantial variation in specific macronutrients impacts the gut microbiome (e.g., high fat  
45 versus low fat diets)<sup>7–9</sup>. This variation is not typically representative of the variation in nutrient content  
46 observed across standard dietary husbandry practices. Additionally, these studies have typically reared fish on  
47 a singular diet up to the point of experimentation, at which point fish are exposed to alternative diets. While  
48 insightful about acute effects, such experimental designs do not model the chronic dietary exposure that fish  
49 experience through husbandry. This prior work also does not typically consider how diet impacts the  
50 microbiome at different fish developmental periods, or whether dietary variation affects other characteristics of  
51 the gut microbiome, such as its sensitivity to exogenous agents (e.g., pathogens).

53 In this study, we sought to define the impact of rearing fish of different common facility diets on the gut  
54 microbiome of early adult (4-mo old) and fully mature (7-mo old) zebrafish. To do so, we reared fish throughout  
55 their lifespan on one of three different dietary husbandry practices: fish were fed either (1) the Gemma  
56 (Skittering) diet, which is a commercial feed widely used in zebrafish research facilities, (2) the Ziegler diet  
57 adopted by the Zebrafish International Research Center (ZIRC), which is one of the largest zebrafish stock  
58 centers in the world, or (3) a precisely defined laboratory grade diet developed by Watts {Fowler2019}. Overall,  
59 these diets are relatively similar in nutritional content, though they differ in ingredients used, ingredient  
60 sources, methods of production and exact nutritional content. In particular, we evaluated how the microbiome  
61 differed across these groups of fish as well as over development. We also determined if these differences link  
62 to variation in fish growth, as well as variation in how the microbiome responds to infection by one of the most  
63 prolific intestinal infectious agents of zebrafish research facilities, *Mycobacterium cheloneae*.  
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74 **2. Diet differentially influences physiology and gut microbiome at 4 months old**

**Figure 2:** Effects of 4-month-old fish fed one of three diets (Gemma, Watts, or ZIRC) on physiology and microbiomes of zebrafish. **(A)** Weight of ZIRC-diet fed fish significantly differs from Watts- and Gemma-diet fed fish. Gemma- and Watts-diet fed fish 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 than fish fed the Gemma and Watts diets. **(C)** Simpson's Index of diversity shows that gut microbiome diversity significantly differs between Gemma- and Watts-diet fed fish, ZIRC- and Watts -diet fed fish, but not between Gemma- and ZIRC -diet fed fish. **(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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To determine how common zebrafish diets differently impact fish physiology and the gut microbiome, we reared 176 zebrafish that were assigned one of three diets from 1- to 4-months-post fertilization (mpf) (Figure 1): Gemma, Watts and ZIRC. Prior to diet assignment, fish were fed a nursery diet (see methods). At 4 mpf, we selected 89 individuals across these three cohorts and collected fecal samples from each fish for microbiome profiling prior to measuring their weight and body condition score. Wilcoxon Signed-Rank Tests found that diet and sex significantly associated with weight and body condition. Female fish had higher weight ( $Z = 1,530$ ,  $P <$

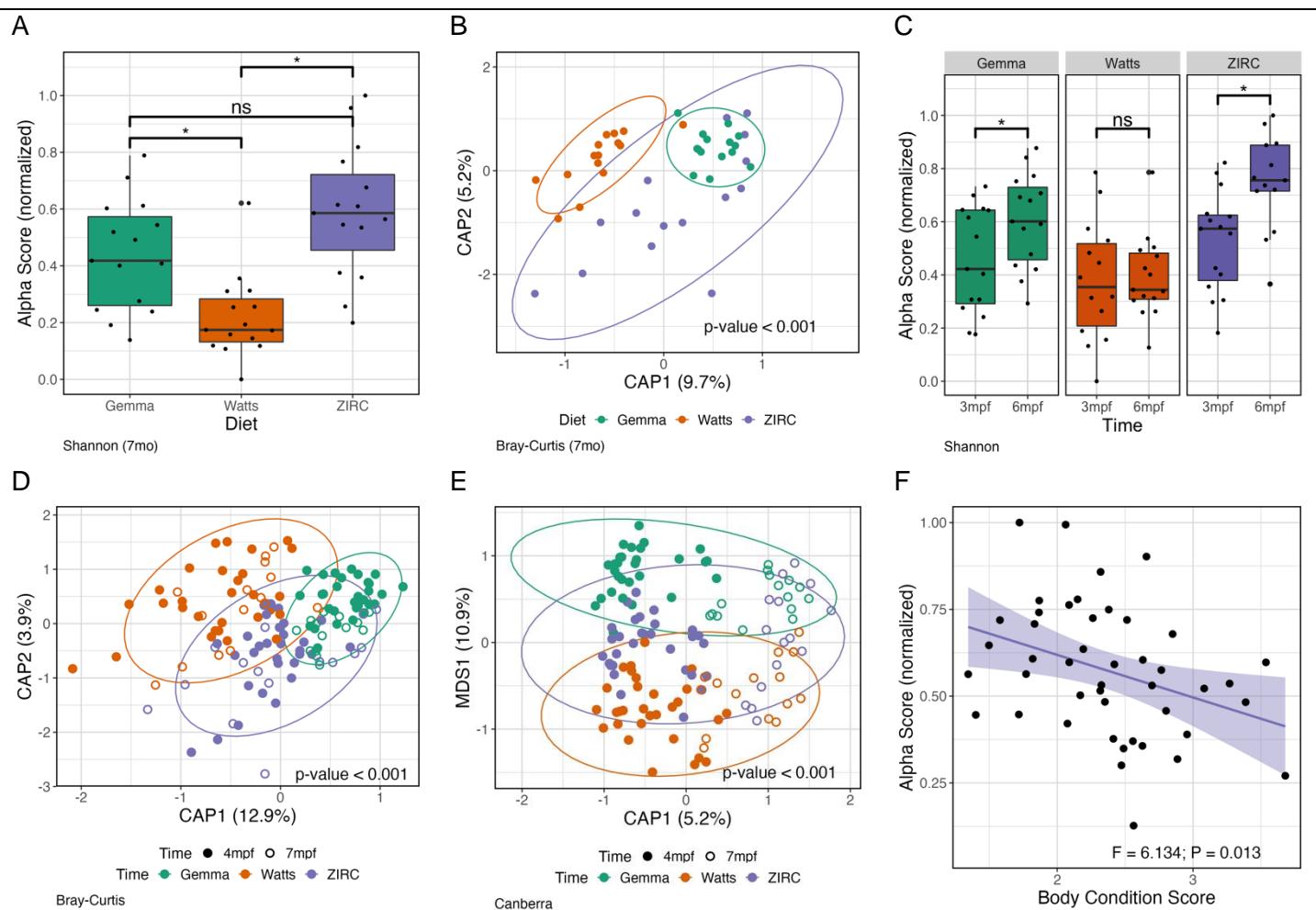
83 0.001; Table S1.1.2) and body condition scores ( $Z = 1,631$ ,  $P < 0.001$ ; Table S1.1.4) compared to males.  
84 Between the three diets, ZIRC-diet fed fish had the highest mean body condition score compared to fish fed  
85 Gemma- ( $Z = 150$ ,  $P < 0.001$ ) and Watts-diet ( $Z = 197$ ,  $P < 0.001$ , Table S1.1.3). Gemma- and Watts-diet fed  
86 fish did not significantly differ from one another in terms of weight and body condition scores. These results  
87 indicate that ZIRC-diet contributes to heavier fish compared to Gemma- and Watts-diet fed fish.

88 We next built generalized linear models (GLM) to determine if diet associated with variation in one of three  
89 measures of microbiome alpha-diversity: richness, Simpson's Index, and Shannon Entropy. An ANOVA test of  
90 these GLMs revealed that alpha-diversity varies as a function of diet for all three measures of diversity we  
91 assessed ( $P < 0.05$ ; Fig 1C; Table S1.2.2). A post hoc Tukey test clarified that ZIRC- and Watts-diet fed fish  
92 exhibited significant differences in alpha-diversity as measured by richness and Shannon Entropy ( $P < 0.001$ ,  
93 Table S1.2.3). Moreover, we observed significant differences in diversity between Gemma- and Watts-diet fed  
94 fish in terms of richness ( $P < 0.001$ ; Table S1.2.3), and between Gemma- and ZIRC-diet fed fish when  
95 considering the Simpson's Index ( $P < 0.001$ ; Table S1.2.3). These results indicate that diet associates with fish  
96 gut microbiome diversity, and that diet may differentially impact rare and abundant microbial members of the  
97 gut.  
98

100 To evaluate how diet associates with microbiome community composition, we quantified the Bray-Curtis,  
101 Canberra and Sorensen dissimilarity amongst all sample. We detected a significant clustering of microbial gut  
102 community composition based on diet as measured by all beta-diversity metrics (PERMANOVA,  $P < 0.05$ ;  
103 Figure 2C, Table S1.3.1). These results indicate that microbial communities of fish fed the same diet are more  
104 consistent in composition to one another than to fish fed other diets. Additionally, we assessed beta-dispersion,  
105 a measure of variance, in the gut microbiome community compositions for each diet group. We find the beta-  
106 dispersion levels were significantly different between the diet groups as measured by Bray-Curtis and  
107 Canberra metrics ( $P < 0.05$ ; Table S1.4.1). Beta-dispersion levels were significantly reduced in Gemma-diet  
108 fed fish compared to Watts-diet fed fish when measured by Bray-Curtis metric, as well as significantly reduced  
109 compared to Watts- and ZIRC-diet fed fish when measured by Canberra metric (Table S1.4.1). These results  
110 indicate that Gemma-diet fed fish are more consistent in community composition than Watts- and ZIRC-diet fed  
111 fish at 4 months old. Collectively, these results indicate that 4-month-old fish gut microbiome communities  
112 stratify by diet, but the composition of these microbial communities differ in consistency depending on diet.  
113

114 Finally, to better understand the interactions between the diet and the members of the gut microbiome  
115 community, we quantified differential abundance using ANCOM-BC2. We observed 24 significantly abundant  
116 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  
117 were enriched for *Chitinibacter* and were depleted of *Aeromonas* and *Flavobacterium*. Watts-diet fed fish  
118 enriched for *Flavobacterium*, *ZOR0006*, *Peptostreptococcus*, *Cetobacterium*, *Tabrizicola*, *Cellvibrio*, and  
119 unnamed genera of *Microscillaceae* and *Chitinibacteraceae*, and depleted of *Crenobacter* and a *Sutterellaceae*  
120 genus. ZIRC-diet fed fish enriched for *Cloacibacterium* and *Acinetobacter*, and depleted of *Fluviicola*. Many of  
121 these taxa are identified as common members of the zebrafish gut microbiome<sup>11,24</sup>. These results indicate that  
122 diet differentially supports particular members of the zebrafish microbiome community.  
123

124  
125 3. Diet impacts the successional development of the zebrafish gut microbiome



126  
127 **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.

128 To determine how maintaining fish on different diets impacts the development of the gut microbiome, we  
129 continued to grow fish from the same diet cohorts until 7 months post fertilization (mpf; Figure 1). Microbiome  
130 samples were collected from cohort members prior to quantification of fish weight and body condition score.  
131 To determine the effect of diet on the body condition score and the gut microbiome of 7mpf fish, we conducted  
132 the same analyses as we applied to the 4-mpf fish. At 7mpf, we find body condition score is significantly  
133 associated with diet ( $P < 0.05$ ; Table S2.7.1). Additionally, linear regression analyses revealed statistically  
134 significant main effects of diet on gut microbiome alpha- and beta-diversity and beta-dispersion for all metrics  
135 we considered ( $P < 0.05$ ; Fig 3A&B, Table S2.7.2). These results demonstrate that diet impacts the physiology  
136 and gut microbiome of 7mpf fish.

137 Next, we compared our results between the 4- and 7-month-old fish to determine how diet impacts the  
138 successional development of the gut microbiome. Linear regression revealed microbial gut alpha-diversity was  
139 significantly associated with the main effect of time ( $P < 0.05$ ; Table S2.2.2.2) for each diversity metric.  
140 However, we did not find a diet dependent effect on time for any alpha-diversity metric we assessed ( $P > 0.05$ ;  
141 Table S2.2.2.2). A post hoc Tukey test clarified that microbiome diversity was significantly different between 4-  
142 and 7-month-old Gemma- and ZIRC-diet fed fish as measured by the Shannon and Simpson's alpha-diversity  
143 metrics ( $P < 0.05$ ; Figure 3C, Table S2.2.2.3), but we did not find a statistically significant association between  
144 4- and 7-month-old Watts-diet fed fish with any alpha-diversity metric ( $P > 0.05$ ; Table S2.2.2.3). These results  
145 indicate that the alpha-diversity of the gut microbiome of Watts-diet fed fish were temporally stable, while  
146 Gemma- and ZIRC-diet fed fish diversified over time in diet-consistent ways.

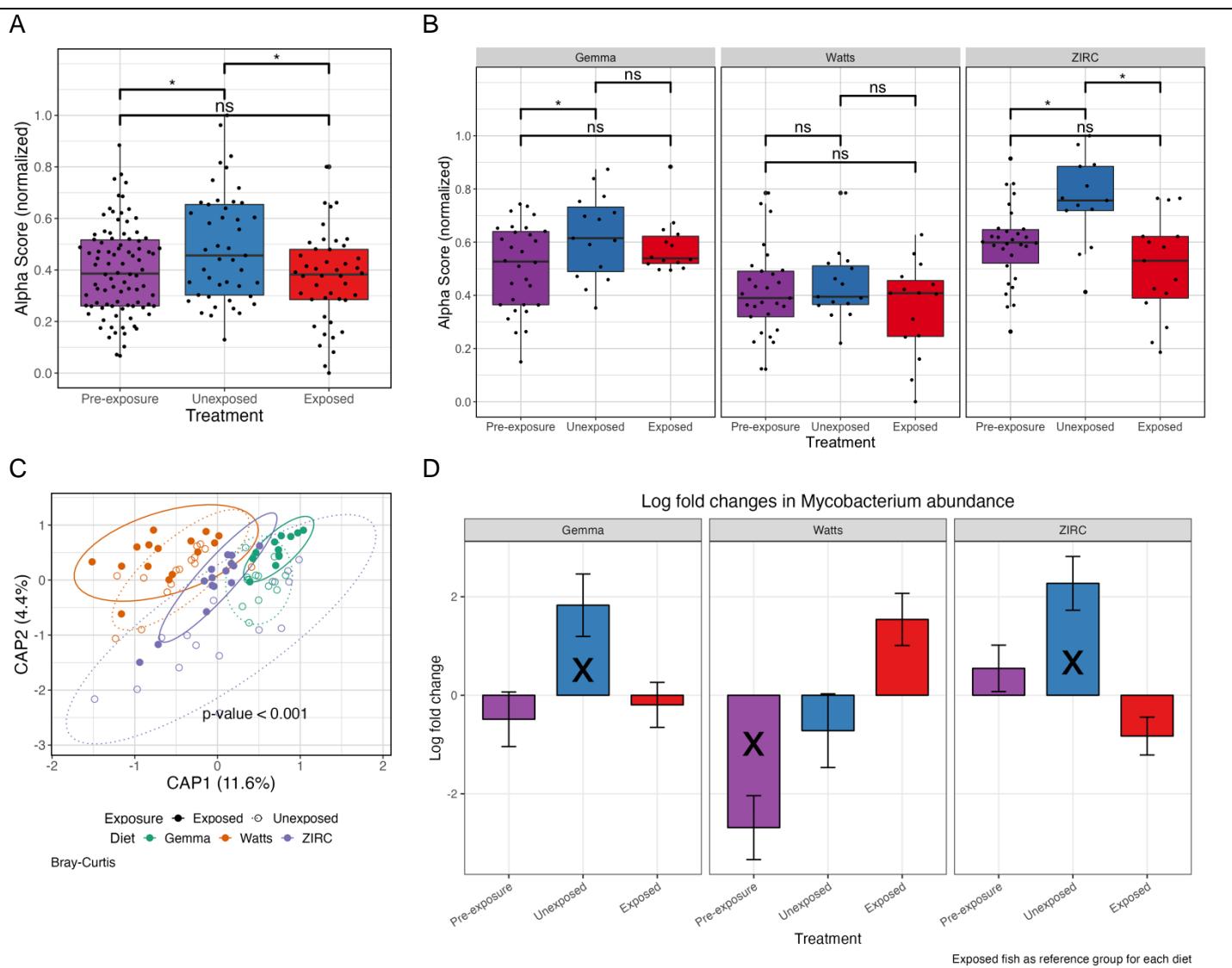
147  
148 A PERMANOVA test of the 4- and 7-mpf samples using the Bray-Curtis dissimilarity metric revealed that  
149 community composition was best explained by diet ( $P < 0.05$ ; Figure 2C, Table S2.4.1), but an analysis using  
150 the Canberra measure found that variation in microbiome composition was best explained by time ( $P < 0.05$ ;  
151 Fig 2D, Table S2.4.2). Given how these metrics weight the importance of abundant versus rarer taxa,  
152 respectively, these results indicate that abundant members of the microbiome community are more sensitive to  
153 the effects of diet, while rarer community members are sensitive to the effects of time. Moreover, we found  
154 beta-dispersion levels were significantly elevated between 4- and 7-month-old Gemma-diet fish and between  
155 4- and 7-month-old ZIRC-diet fed fish when considering the Bray-Curtis and Sorensen metrics ( $P < 0.05$ ; Table  
156 S2.5.3). Conversely, beta-dispersion levels were only significantly elevated between 4- and 7-month-old of  
157 Watts-diet fed fish when considering the Sorensen metric ( $P < 0.05$ ; Table S2.5.3). These results indicate that  
158 the microbiome communities of Watts-diet fed fish are generally more consistent between each other across  
159 the development of zebrafish, while the communities of Gemma- and ZIRC-diet fed fish become more varied  
160 across development. Collectively, these results indicate that diet can have a substantial impact on how the gut  
161 microbiome successively develops in zebrafish.

162  
163 Differential abundance analysis revealed taxa that were significantly associated with the effects of time and  
164 diet. one of the diets (Table S2.6.1). Across all three diets, the taxa that were more abundant included  
165 *Fluviicola*, *Macellibacteroides*, *Bacteroides* and an unnamed genus in the *Barnesiellaceae* family were , while  
166 taxa that were less abundant included *Phreatobacter* and *Flavobacterium*. These results indicate that  
167 irrespective of diet, the abundances of taxa change over the course of zebrafish development. We also  
168 measured how taxon abundance changed over time within each diet (Figure S2.6.2.5). The Gemma-diet fed  
169 fish were uniquely enriched for *Exiguobacterium* (Table S2.6.2.1). *Exiguobacterium* are gram-positive  
170 facultative anaerobes in the phylum Bacillota, and are linked to fatty acid metabolism in zebrafish<sup>26</sup>. The  
171 Watts-diet fed fish were uniquely depleted of *Gemmobacter* (Table S2.6.2.2). Previous work has found that  
172 *Gemmobacter* has a positive association with parasite exposure in infected zebrafish<sup>20,27</sup>. The ZIRC-diet fed  
173 fish were uniquely enriched for *Pseudomonas* and *Haliscomenobacter* (Table S2.6.2.3). *Pseudomonas* is a  
174 common member of the gut microbiome and associated with fatty acid metabolism in zebrafish<sup>26</sup>. Less is  
175 known about the *Haliscomenobacter* genus, but an analysis of its genome revealed it is an aerobic  
176 chemoorganotroph found in aquatic systems<sup>28</sup>. Together, these results indicate that particular members of the  
177 gut microbiome associate with diet and zebrafish development.

178  
179 To determine if physiology associated with diet across zebrafish development, we used Wilcoxon Signed-  
180 Ranks Tests to identify parameters that best explained the variation in body condition score between 4- and 7-  
181 mpf fish. At 7mpf, the body condition scores significantly differed between fish fed different diets. However, we  
182 did not find that body condition scores of fish were impacted by time ( $P > 0.05$ ; Fig 2E, Table S2.1.1). These  
183 results indicate that while fish differ in body condition score between diets at 7 months old, their weight and  
184 length grow proportionally at a similar rate from 4 to 7 months of age. Interestingly, we observed a significant  
185 negative association of body condition score and microbial gut diversity uniquely in fish fed the ZIRC diet as

186 measured by Shannon Entropy and Simpson's Index ( $P < 0.05$ ; Fig 2F, Table S2.2.1). This result indicates that  
187 fish gut microbiomes with higher body masses are lower in diversity compared to fish with lower body mass.  
188 For Canberra and Sorensen beta-diversity metrics, there were significant main effects of body condition score,  
189 and significant interaction effects between body condition score and diet ( $P < 0.05$ ; Table S2.2.1.3). However,  
190 the model coefficient for the effect of body condition score and its interaction with diet is far smaller than the  
191 coefficient for the effect of diet (Table S2.2.2). We did not find a significant association between body condition  
192 score and specific taxon abundance (Table S2.2.2). Collectively, these results indicate that while the gut  
193 microbiome's composition associates with body condition score, the effect of diet on the gut microbiome is  
194 much stronger

195 **4. Diet influences gut microbiome's sensitivity to pathogen exposure**  
 196



**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.

197  
 198 Lastly, we sought to determine how diet impacts the gut microbiome's sensitivity to exogenous stressors, in  
 199 particular exposure to the common intestinal pathogen, *Mycobacterium chelonae*. *Mycobacterium chelonae* is  
 200 a common intestinal pathogen found in 40% of zebrafish facilities, and is hypothesized to be introduced  
 201 through diet early in life<sup>17–19</sup>. *M. chelonae* forms granulomas in the gut intestine, which can cause gut  
 202 inflammation, decreased fecundity and lifespan. These can introduce inconsistencies in study outcomes, but  
 203 the impacts on the gut microbiome are not known<sup>17</sup>. To clarify the diet-specific sensitivities of the zebrafish gut  
 204 microbiome to *M. chelonae* exposure, we injected *M. chelonae* into the coelomic cavities of fish from each diet  
 205 cohort at 4 mpf following fecal collection. These *M. chelonae* injected fish comprised the pathogen exposure  
 206 cohort for this experiment, which we compared to the remaining, unexposed cohort of fish. At 7 mpf, we

207 collected fecal samples from exposed and unexposed fish to measure microbial gut diversity, composition, and  
208 taxon abundance, performed a histopathological analysis of intestinal tissue to assess infection severity, and  
209 measured body condition score.

210  
211 We first evaluated whether diet impacted infection outcomes, as determined by histological confirmation of  
212 infection 3 months following pathogen injection. A pairwise Fisher Test did not find that the proportion of  
213 individuals with signs of infection 3 months following exposure differed between diets ( $P > 0.05$ ; Table S3.5.1).  
214 This result indicates that the diets considered in our study do not appear to dictate the progression of infection  
215 of *M. chelonae*. Next, we assessed whether infection status links to body condition score as well as measures  
216 of gut microbiome diversity and composition. We did not observe significant associations between infection  
217 status and body condition score based on [name test here] ( $P > 0.05$ ; Table S3.5.2) or any of the gut  
218 microbiome diversity and composition measures ( $P > 0.05$ ; Table S3.5.4&S3.5.5). Together, these results  
219 indicate that infection endpoints are not linked to diet, body condition score, or the gut microbiome.  
220

221 We next considered that exposure to the pathogen could impact the gut microbiome, despite the fact that  
222 ultimate infection outcomes among exposed individuals may not. Comparing exposed to unexposed fish found  
223 that microbial gut diversity significantly differs between exposure groups as measured by richness and  
224 Shannon Entropy alpha-diversity metrics ( $P < 0.05$ ; Figure 4A, Table S3.1.2.2). That said, based on linear  
225 regression, the impact of exposure on the gut microbiome alpha-diversity does not appear to differ as a  
226 function of diet, as the interaction term for these covariates did not yield a significant effect ( $P > 0.05$ ; Table  
227 S3.1.2.2). Furthermore, we used a post hoc Tukey test to clarify whether microbial gut diversity of fish differed  
228 between exposure groups by diet. Unique to ZIRC-diet fed fish, we observed microbiome diversity differed in  
229 unexposed controls compared to exposed fish as measured by all alpha-diversity metrics ( $P < 0.05$ , Table  
230 S3.1.2.3). Watts-diet fed fish differed in unexposed controls compared to exposed fish in terms of richness ( $P <$   
231  $0.05$ , Table S3.1.2.3). These results suggest that the gut microbiome diversity of ZIRC-diet fed fish, and to  
232 some extent Watts-diet fed fish, are sensitive to the effects of *M. chelonae* exposure, but Gemma-diet fed fish  
233 are resistant to pathogen exposure. While the gut microbiomes are sensitive to the effects of pathogen  
234 exposure, we find the statistical effect of diet shaping the gut microbiome is an order of magnitude greater  
235 across all alpha-diversity metrics ( $P < 0.05$ , Table S3.1.2.2). Collectively, these results indicate that gut  
236 microbiome diversity is sensitive to *M. chelonae* exposure, but diet is the primary driver of gut microbiome  
237 diversity.  
238

239 Next, we evaluated how pathogen exposure influenced microbial community composition across fish fed each  
240 diet. For each beta-diversity metric considered, PERMANOVA tests found that the main effects of diet and  
241 pathogen exposure significantly explained the variation in microbiome composition, but that the main effect of  
242 diet was consistently larger than the effect of exposure ( $P < 0.05$ ; Fig 4C, Table S3.2.3). Furthermore, a  
243 PERMANOVA test found that the model coefficient effect for the interaction of diet and pathogen exposure was  
244 statistically significant when considering Canberra and Sorenson beta-diversity metrics, however this effect  
245 was marginal as compared to the aforementioned main effects. Moreover, an analysis of beta-dispersion  
246 revealed significant levels of dispersion across exposure and diets as measured by all three beta diversity  
247 metrics ( $P < 0.05$ ; Table S3.3.2). These results indicate that dispersion is likely driven by diet, rather than  
248 exposure. Collectively, these results indicate that the gut microbiome is sensitive to pathogen exposure, but  
249 that dietary effects tend to overwhelm evidence of this sensitivity.  
250

251 We also observed several microbiota that stratified exposed and unexposed groups of fish in both diet-robust  
252 and diet-dependent manners. Unexposed Gemma-diet fed fish were enriched for *Macellibacteroides* and  
253 *Aurantisolimonas* (Table S3.4.2), unexposed Watts-diet fed fish were enriched for an unnamed genus of  
254 *Barnesiellaceae*, *Fluviicola*, *Paucibacter*, and *Brevibacterium* (Table S3.4.3), and unexposed ZIRC-diet fed fish  
255 were enriched for *Macellibacteroides*, *Bacteroides*, *Mycobacterium* and unnamed genera of *Barnesiellaceae*

256 and *Sutterelaceae* (Table S3.4.4). Across all the diets, that taxa that were more abundant in unexposed,  
257 control fish included *Macellibacteroides*, *Fluviicola*, *Bacteroides*, *Aurantisolimonas*, *Cerasicoccus*, and three  
258 unnamed genera of *Barnesiellaceae*, *Commonadaceae*, and *Sutterellaceae*. *Plesiomonas* were more  
259 abundant in exposed fish compared to controls (Table S3.4.5). These results indicate that pathogen exposure  
260 impacts the abundance of certain taxa within and across the diets. Next, to see if *Mycobacterium* species  
261 abundance differed from background, pre-exposure levels we compared *Mycobacterium* abundance between  
262 pre-exposure and unexposed control fish to that of exposed fish within each diet. Unexposed Gemma- and  
263 ZIRC-diet fed fish had significantly higher abundances of *Mycobacterium* to exposed (Figure 4D, Table  
264 S3.4.6). Pre-exposed Watts-diet fed fish had significantly more *Mycobacterium* compared to pre-exposed fish,  
265 but they did not differ significantly from unexposed control fish. These results indicate that the abundance of  
266 taxa from the genus *Mycobacterium* changes in response to exposure to a pathogenic species in a diet-  
267 dependent manner.

268 **Discussion**

269  
270 Zebrafish are an important emerging model organism for understanding the microbiome. Yet, there is little  
271 consistency across microbiome-targeted studies in husbandry practices involving diet. Given the potential for  
272 diet to drive variation in the microbiome composition and the microbiome's role in mediating host physiology  
273 and health, choice of diet could introduce variation in study outcomes. Our study represents, to our knowledge,  
274 the first to define how different dietary formulations that are commonly used in zebrafish facilities impacts the  
275 development of the zebrafish gut microbiome and their physiology, as well as sensitivity of the microbiome to  
276 pathogen exposure. Our analysis reveals that the zebrafish gut microbiome's development and response to  
277 pathogen exposure is sensitive to diet, which holds important implications for the design and interpretation of  
278 zebrafish microbiome research studies.

279  
280 We found that diet had a substantial impact on the structure of the gut microbiome in adult zebrafish. Previous  
281 research has found that diets with varying compositions of key macronutrients (e.g.: protein, lipids and fiber  
282 content) impacts zebrafish physiology and the gut microbiome. Moreover, diet's effect on restructuring the  
283 host's gut microbiome has been observed across an evolutionarily diverse array of vertebrate and invertebrate  
284 animal hosts. However, the nutritional compositions used in these prior studies investigating the gut  
285 microbiome tend to vary considerably. In particular, feeds that are commonly used to rear zebrafish in research  
286 facilities differ vastly in nutritional composition and consistency from those used in studies investigating the  
287 effect of diet on the zebrafish gut microbiome. A unique strength of our study is that fish were fed the same diet  
288 starting at 30 to 214 days old, and the different diets varied minimally to one another in nutritional composition.  
289 Therefore, the striking differences we observed between the gut microbiomes of adult zebrafish fed commonly  
290 used diets demonstrates the profound impact that diet has on structuring the gut microbiome. Consequently,  
291 microbiome-targeted studies that use different diets will come to different conclusions.

292  
293 We also found that diet impacts the developmental variation in the gut microbiome. Prior work investigating the  
294 successional development of zebrafish gut microbiome has had inconsistent results that may be attributable to  
295 diet. For instance, Stephens et al. used a variety of live feed and dry food diets and found that juvenile  
296 zebrafish gut microbiomes were highly diverse but declined with age, while Wong et al. found opposite results  
297 for juvenile zebrafish that were fed defined diets. Furthermore, Burns et al. and Xiao et al. noted that the  
298 observed early life variability of the gut microbiome could be a result of husbandry choices involving diet. While  
299 our study differed in exact length and sampling time points to these prior studies, we do find congruent trends  
300 in gut microbiome diversity within similar sampling time points. However, it is difficult to directly compare  
301 results from prior studies to ours because sampled at different time points, they used a variety of diets  
302 throughout their study, diets differed substantially to ours, or did not disclose which diets they fed fish. It is  
303 worth noting that while our fish were fed the same diet from 30 days onward, at 114 days old our fish were  
304 switched from a juvenile formulation to an adult formulation of their respective diets. These formulations  
305 differed slightly in some diets, but in others more substantially, which may contribute to the variability in the gut  
306 microbiome between diets across zebrafish development. Despite these limitations, we found that subtle  
307 formulation changes of diets can alter the successional trajectories of adult zebrafish gut microbiomes. Future  
308 work should seek consistency in diet formulations and increase sampling time points throughout zebrafish  
309 development to further clarify diet's role in shaping the successional development of zebrafish gut  
310 microbiomes.

311  
312 We observed that the gut microbiome of zebrafish were sensitive to pathogen exposure, but diet was the main  
313 driver. Specifically, we found that gut microbiome diversification was inhibited by exposure to the common  
314 intestinal pathogen *Mycobacterium chelonae* in ZIRC-diet fed fish relative to unexposed controls, while  
315 exposed fish fed the other diets did not differ from controls. Our results contrast prior studies that found  
316 intestinal pathogen exposure was associated with an increase in microbiome diversity. One possible  
317 explanation for the discrepancy between prior studies and ours is *Mycobacterium* is hypothesized to be  
318 introduced early in life through diet, while fish in our study were exposed intravenously in their coelomic  
319 cavities at adulthood when their gut microbiomes have been firmly established. Because we found that the  
320 effect of diet was far greater than pathogen exposure on shaping the gut microbiome, future studies must  
321 consider diet effects, as they may overwhelm experimental (e.g., infection) effects. Furthermore, future work  
322 should consider using a natural mode of infection and exposing fish to a variety of pathogens to elucidate diets  
323 influence on the gut microbiome's role in mediating pathogen exposure.

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In conclusion, we found diet is one of the most important factors driving variation in the zebrafish gut microbiome. Unlike prior studies, including the extensive research conducted in mammalian models, that have evaluated dietary effects on the gut microbiome using diets that fundamentally differ in macronutrient composition, our work reveals that even relatively consistent diets that are commonly selected as normal husbandry practices elicit these large impacts on microbiome composition. While the zebrafish gut microbiome differs taxonomically from other animal systems, there is a substantial amount of shared functional capacity between zebrafish and mammal gut microbiomes. Consequently, the taxa-specific associations we found here may not translate to other animal systems, but the interactions between the microbiome, diet and pathogen exposure may be similar. Future work will illuminate the underlying mechanisms of diet's influence on structuring the zebrafish gut microbiome. Collectively, our study demonstrates that investigators should carefully consider the role of diet in their microbiome zebrafish investigations, especially when integrating results across studies that vary by diet.

340 **Methods**

341 **Fish Husbandry**

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

356 **Diets**

357 Fish were all fed the same nursery diet until 30 days old, a combination of paramecia, brine shrimp, and the  
358 ZIRC Nursery Mix: Zeigler AP Larval Diet (Ziegler Bros Inc., Gardners, PA) and freeze dried rotifers. Fish were  
359 then transferred to the OSU facility and assigned randomly to one of three juvenile diets: Gemma Micro  
360 150/300 (Skretting, Fontaine-les-Vervins, France), Watts High-Fat Juvenile Mix, or ZIRC Juvenile Mix, twice  
361 daily (9 AM and 3 PM local time) until 60 days old. From 60 days of age onward, OSU fish were not fed on  
362 weekends and 1-day holidays as per the facility institutional animal care and use protocol. The total quantity  
363 fed daily was 3% fish body weight. This continued until fish were 214 days old and then they were transitioned  
364 to the adult version of their previously assigned juvenile diet: Gemma Micro 500 (Skretting, Fontaine-les-  
365 Vervins, France), Watts Low-Fat Adult Mix, or ZIRC Adult Mix, twice daily (9 AM and 3 PM local time), except  
366 weekends and 1-day holidays. The total quantity fed daily was 3% fish body weight. The prescribed amounts of  
367 each diet regimen, for both the juvenile and adult diets were delivered by 3D printed spoons specific to the diet  
368 and stage of life. These spoons were paired with conical tubes retrofitted with leveling wires to ensure  
369 consistent feeding volumes as prescribed. All fish were only fed once, in the afternoons, on sampling days.  
370

371 **Diet and Pathogen Exposure**

372 Each of the eighteen tanks was assigned one of the three diet regimens: Gemma, Watts, or ZIRC. There were  
373 three tank replicates per diet regimens for a total of nine tanks that were exposed to *M. chelonae* via  
374 intraperitoneal injection. The remaining nine tanks were similarly assigned to diet regimens and were exposed  
375 to a sterile 1X-phosphate buffered saline (PBS) solution via intraperitoneal injection. Each fish was injected  
376 with 10 uL of either the *M. chelonae* inoculum or saline solution. The injections were completed over the  
377 course of two days and the *M. chelonae* inoculum was prepared as a 0.5 McFarland each day. Day 1 *M.*  
378 *chelonae* inoculum was afterwards determined by plating to be  $3.1 \times 10^3$  dose per fish. Day 2 *M. chelonae*  
379 inoculum was determined by plating to be  $1.0 \times 10^5$  dose per fish.  
380

381 **Growth Parameters and Sex Determination**

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

386 Sex was determined by gross differences in morphology and confirmed by histology for all samples collected  
387 for disease severity evaluation. Following overnight fecal collection, individual fish would be placed in a pre-  
388 anesthetic solution of 50 ppm MS-222 prepared with Tricaine-S (Western Chemical Inc., Ferndale, WA; a  
389 subsidiary of Aquatic Life Sciences Inc.) briefly before being transferred to a 150 ppm MS-222 anesthetic  
390 solution in a petri dish on centimeter grid paper to be photographed. Standard length and width were evaluated  
391

396 via photographs taken with an iPhone (Apple Inc., Cupertino, CA) and analyzed with ImageJ software  
397 (<https://imagej.net>).  
398

399 Body condition score (BCS) was calculated using the following equation: BCS = Weight (mg)/Length (mm)<sup>3</sup> x  
400 100. Body condition score is a length normalized metric of weight (for equation, see Methods) and serves as a  
401 general indicator of health in zebrafish.  
402

403 Weight was taken while the fish was still under the effects of anesthesia by transferring them from the  
404 photography petri dish to the petri dish on a scale with a volume of tared fish water. Excess water was  
405 removed  
406

## 407 **Histopathology**

### 408

409 Fish were preserved in Dietrich's solution, processed, and slides stained with Kinyoun's acid-fast. Severity was  
410 scored by counting total numbers of granulomas containing acid fast bacteria in the coelomic cavity, ovaries,  
411 and kidney. Score of 1 was 1–2 granulomas, 2 = multiple granulomas observed, 3 = prominent infections with  
412 granulomatous lesions occupying a large amount of the coelom or gonad. In addition, an overall severity of  
413 infection score was assigned based on the average scores of the individual structures evaluated (cite previous  
414 Kent lab paper).  
415

## 416 **Fecal Collection**

### 417

418 Fecal material was collected from individual fish at the same sample intervals as outlined for the growth  
419 parameters. Fecal collection was set up the day before growth parameter sampling. Fish were transferred to  
420 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.  
421 Fish were left to defecate overnight and all feces present were collected from each tank the following morning.  
422 Fecal samples were immediately snap frozen on dry ice and stored at -80 °C until processing.  
423

## 424 **16S Sequencing**

### 425

426 Microbial DNA was extracted from zebrafish fecal samples and 16S rRNA gene sequence libraries were  
427 produced and analyzed following established approaches (Kundu et al., 2021). Briefly, the DNeasy PowerSoil  
428 Pro DNA kits (Qiagen) were used to extract and purify DNA. The V4 region of the 16S rRNA gene was PCR  
429 amplified using the Earth Microbiome Project 16S index primers and protocols (Walters et al., 2016). PCR  
430 products were visualized on a 1.5% agarose gel and quantified on a Qubit 2.0 (Thermofisher Scientific) using  
431 the Qubit dsDNA HS Assay. One hundred ng of each PCR sample was pooled, cleaned using the QIAquick  
432 PCR Purification Kit (Qiagen), and quality was verified on the Agilent TapeStation 4200. The prepared library  
433 was submitted to the Oregon State University Center for Quantitative Life Sciences (CQLS) for 300 bp paired-  
434 end sequencing on an Illumina MiSeq System (RRID:SCR\_016379).  
435

## 436 **Analysis**

### 437

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

451 the selected model parameters significantly explained the variation in microbiome composition across samples.  
452 Differential abundance was measured using ANCOM-BC (v ).  
453

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541 **Supplementary Tables and Figures**542 **1) Diet**543 **1.1) Physiology**544 **1.1.1)**545 **Wilcoxon Test. p. adj: BH. Weight ~ Diet**

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

547 **1.1.2)**548 **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	****

549 **1.1.3)**550 **Wilcoxon Test. p. adj: BH. Body.Condition.Score ~ Diet**

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

551 **1.1.4)**552 **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	****

553 **1.2) Alpha-diversity**

## 554

## 555

## 556

557 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	*
Shannon	DietZIRC	0.127	0.192	0.663	0.509	
	(Intercept)	-0.399	0.152	-2.622	0.010	*
Simpson	DietWatts	-0.418	0.222	-1.881	0.063	
	DietZIRC	0.426	0.213	1.999	0.049	*
	(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	*

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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	*

561

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

563

564

## 565 1.3) Beta-diversity

566 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			

567

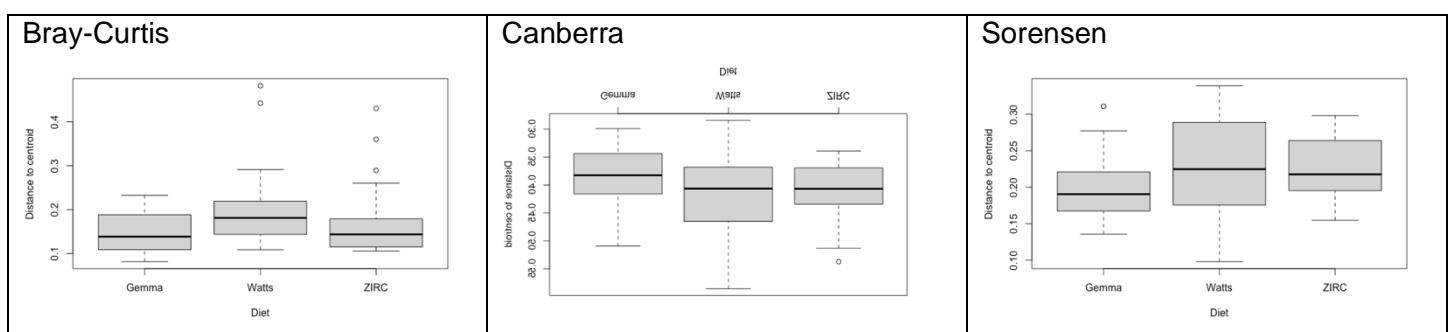
568

## 569 1.4) Beta-Dispersion

570

571 1.4.1) Diet

572



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

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

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

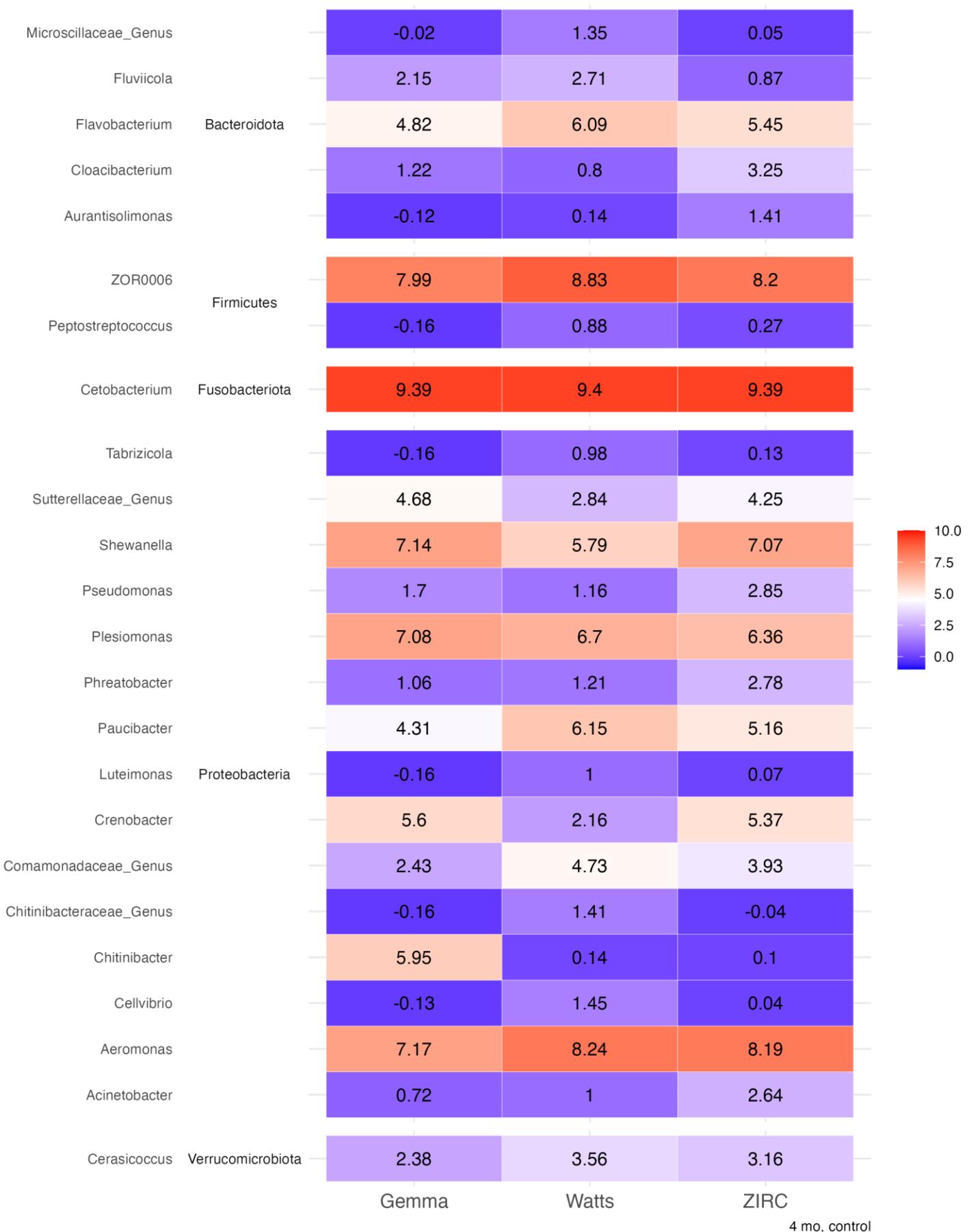
Names	p-value
Gemma-Watts	0.014
Gemma-ZIRC	0.044
Watts-ZIRC	0.435

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

573  
574  
575  
576 1.5) Differential Abundance  
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Heat map of bias-corrected log observed abundances (Genus)



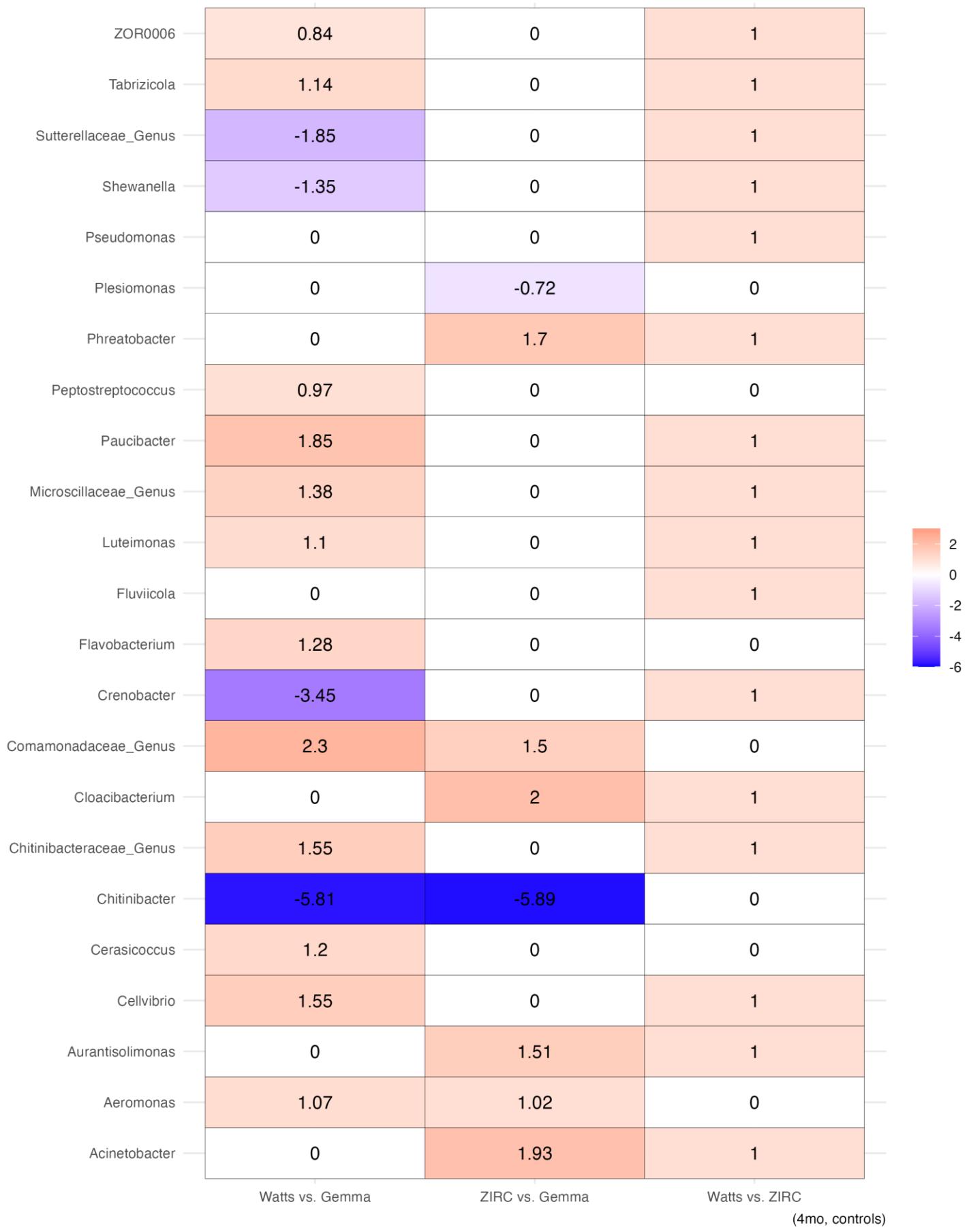
4 mo, control

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

581 1.5.2)  
582

Log fold change of pairwise comparisons



Taxon	lc_DietWatts	lc_DieZIRC	lc_DielZIRC_DietWatts	se_DietWatts	se_DielZIRC	se_DielZIRC_DietWatts	W_DietWatts	W_DielZIRC	W_W_DielZIRC_DietWatts	p_DietWatts	p_DielZIRC	q_DietWatts	q_DielZIRC	q_DielZIRC_DietWatts	diff_DietWatts	diff_DielZIRC	diff_DielZIRC_DietWatts	
Cetobacterium	0.012	-0.000	-0.012	0.142	0.145	0.190	0.084	-0.002	-0.064	0.933	0.999	0.949	1.000	1.000	1.000	FALSE	FALSE	FALSE
ZOR0006	0.837	0.208	-0.629	0.148	0.151	0.196	5.651	1.379	-3.215	0.000	0.168	0.001	0.000	0.462	0.005	TRUE	FALSE	TRUE
Aeromonas	1.073	1.018	-0.055	0.162	0.165	0.208	6.610	6.157	-0.264	0.000	0.000	0.791	0.000	0.000	1.000	TRUE	TRUE	FALSE
Plesiomonas	-0.379	-0.725	-0.346	0.211	0.214	0.252	-1.796	-3.391	-1.374	0.072	0.001	0.170	0.272	0.003	0.466	FALSE	TRUE	FALSE
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	TRUE	FALSE	TRUE
Paucibacter	1.852	0.859	-0.993	0.363	0.365	0.395	5.109	2.353	-2.514	0.000	0.019	0.012	0.000	0.051	0.045	TRUE	FALSE	TRUE
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	TRUE	TRUE	FALSE
Crenobacter	-3.452	-0.227	3.225	0.338	0.341	0.371	-10.213	-0.666	8.682	0.000	0.505	0.000	0.000	1.000	0.000	TRUE	FALSE	TRUE
Flavobacterium	1.276	0.835	-0.641	0.297	0.300	0.333	4.292	2.116	-1.927	0.000	0.034	0.054	0.000	0.129	0.148	TRUE	FALSE	FALSE
Sutterellaceae_Genus	-1.849	-0.434	1.415	0.400	0.403	0.431	-4.624	-1.078	3.283	0.000	0.281	0.001	0.000	0.772	0.004	TRUE	FALSE	TRUE
Gemmobacter	-0.597	-0.403	0.193	0.294	0.297	0.330	-2.029	-1.358	0.587	1.000	1.000	1.000	1.000	1.000	1.000	FALSE	FALSE	FALSE
Acinetobacter	0.312	1.931	1.818	0.511	0.513	0.539	0.612	3.761	3.002	0.541	0.000	0.003	1.000	0.001	0.010	FALSE	TRUE	TRUE
Chitinibacter	-5.807	-5.893	-0.086	0.175	0.178	0.220	-33.107	-33.033	-0.390	0.000	0.000	0.697	0.000	0.000	1.000	TRUE	TRUE	FALSE
Fluvicola	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	FALSE	FALSE	TRUE
Closobacterium	-0.406	2.001	2.406	0.447	0.450	0.477	-0.908	4.448	5.046	0.364	0.000	0.000	1.000	0.000	0.000	FALSE	TRUE	TRUE
Cerasiococcus	1.198	0.783	-0.414	0.400	0.403	0.431	2.992	1.945	-0.960	0.003	0.052	0.337	0.013	0.194	0.926	TRUE	FALSE	FALSE
Chitinibacteraceae_Genus	1.549	0.125	-1.425	0.340	0.342	0.373	4.563	0.365	-3.820	0.000	0.715	0.000	0.000	1.000	0.001	TRUE	FALSE	TRUE
Pseudomonas	-0.506	1.166	1.673	0.486	0.491	0.517	-1.037	2.377	3.236	0.300	0.017	0.001	0.824	0.065	0.006	FALSE	FALSE	TRUE
Mycobacter	-0.039	-0.357	-0.318	0.395	0.397	0.426	-0.099	-0.898	-0.746	1.000	1.000	1.000	1.000	1.000	1.000	FALSE	FALSE	FALSE
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	FALSE	FALSE	FALSE
Phreatobacter	0.134	1.703	1.569	0.390	0.392	0.421	0.343	4.340	3.726	0.732	0.000	0.000	1.000	0.000	0.001	FALSE	TRUE	TRUE
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	FALSE	FALSE	FALSE
Aurantisimonas	0.266	1.506	1.240	0.283	0.288	0.319	0.941	5.273	3.887	0.347	0.000	0.000	0.953	0.000	0.000	FALSE	TRUE	TRUE
Cellvibrion	1.553	0.171	-1.382	0.286	0.289	0.322	5.430	0.592	-4.293	0.000	0.554	0.000	0.000	1.000	0.000	TRUE	FALSE	TRUE
Hallstromobacter	0.056	-0.120	-0.176	0.232	0.235	0.271	0.243	-0.509	-0.648	1.000	1.000	1.000	1.000	1.000	1.000	FALSE	FALSE	FALSE
Dinghubacter	0.191	-0.157	-0.348	0.430	0.433	0.460	0.445	-0.363	-0.756	1.000	1.000	1.000	1.000	1.000	1.000	FALSE	FALSE	FALSE
Phodobacteraceae_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	FALSE	FALSE	FALSE
Fluvilicoccus	-0.356	-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	FALSE	FALSE	FALSE
Microscillaceae_Genus	1.376	0.115	-1.261	0.294	0.297	0.330	4.675	0.388	-3.823	0.000	0.698	0.000	0.000	1.000	0.000	TRUE	FALSE	TRUE
Defluvimonas	0.022	-0.476	-0.498	0.312	0.315	0.347	0.069	-1.512	-1.435	1.000	1.000	1.000	1.000	1.000	1.000	FALSE	FALSE	FALSE
Bosea	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	FALSE	FALSE	FALSE
Tabrizicola	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	TRUE	FALSE	TRUE
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	TRUE	FALSE	TRUE
Nocardiaceae_Genus	0.079	-0.239	-0.318	0.235	0.238	0.275	0.335	-1.004	-1.159	1.000	1.000	1.000	1.000	1.000	1.000	FALSE	FALSE	FALSE
Nocardiia	0.630	0.028	-0.601	0.237	0.240	0.276	2.655	0.118	-2.177	1.000	1.000	1.000	1.000	1.000	1.000	FALSE	FALSE	FALSE
Longivirga	-0.147	-0.137	0.010	0.207	0.210	0.249	-0.712	-0.655	0.040	1.000	1.000	1.000	1.000	1.000	1.000	FALSE	FALSE	FALSE
Peptostreptococcus	0.973	0.371	-0.602	0.211	0.214	0.252	4.617	1.737	-2.389	0.000	0.088	0.017	0.000	0.226	0.063	TRUE	FALSE	FALSE
lamia	-0.225	-0.366	-0.141	0.184	0.187	0.228	-1.223	-1.960	-0.622	1.000	1.000	1.000	1.000	1.000	1.000	FALSE	FALSE	FALSE

585  
586 **2) Development**  
587  
588588 **2.1) Physiology**  
589 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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592 **2.2) Physiology ~ Microbiome**  
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594 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	*

597 2.2.2)

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

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## 600 2.3) Alpha-diversity

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### 603 2.3.1) Time

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#### 2.3.1.1)

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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	*

607 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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2.2.1.3)

#### 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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613 2.2.2) Time:Diet  
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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	

618 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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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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## 2.4) Beta-diversity

625 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			

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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			

628

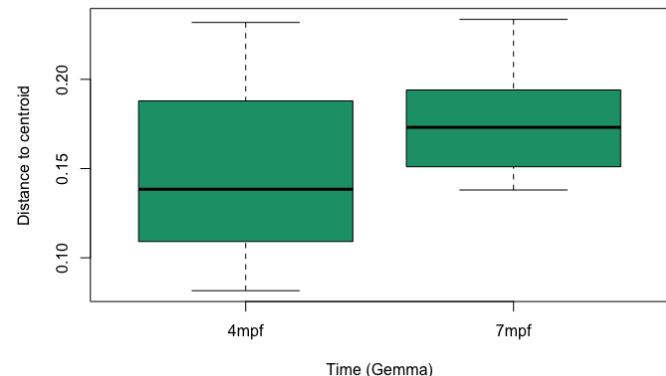
629

2.4.3)

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631 **2.5) Beta-Dispersion**632 **2.5.1) Diet**633 **2.5.1.1) Gemma**

634 Bray-Curtis



Df	Sum Sq	Mean Sq	F	N.Perm	Pr(>F)
1	0.00928085	0.009280850	6.398178	999	0.016
43	0.06237347	0.001450546			

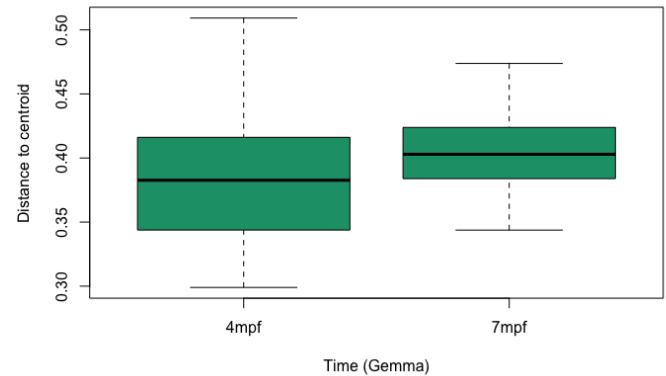
Names p-value

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4mpf- 0.01  
7mpf

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635 Canberra



Df	Sum Sq	Mean Sq	F	N.Perm	Pr(>F)
1	0.006110984	0.006110984	2.799462	999	0.096
43	0.093865283	0.002182914			

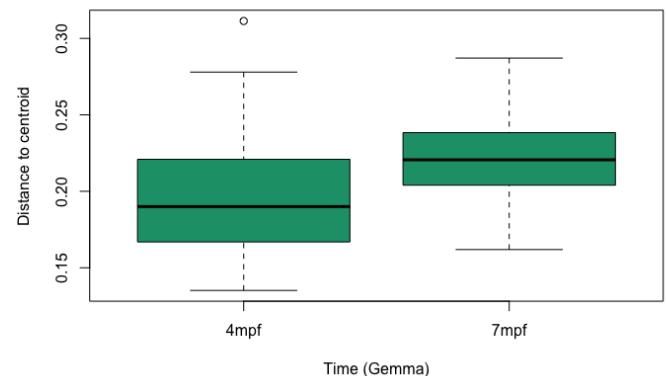
Names p-value

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4mpf- 0.096  
7mpf

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636 Sorenson



Df	Sum Sq	Mean Sq	F	N.Perm	Pr(>F)
1	0.008309949	0.008309949	4.832295	999	0.028
43	0.073945774	0.001719669			

Names p-value

---

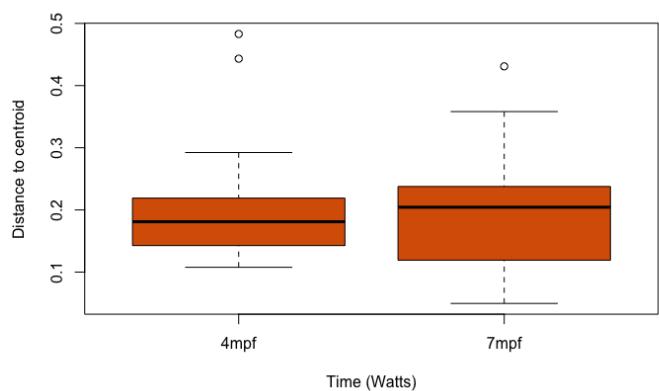
4mpf- 0.027  
7mpf

---

637

2.5.1.2) Watts

### Bray-Curtis

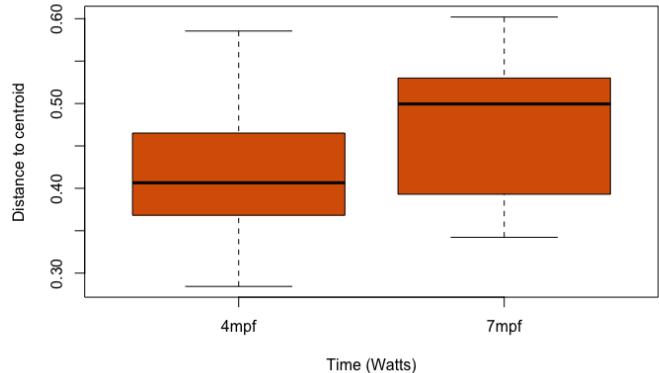


Df	Sum Sq	Mean Sq	F	N.Perm	Pr(>F)
1	0.000003599901	0.000003599901	0.0004248152	999	0.988
43	0.364383776329	0.008474041310			

Names p-value

4mpf- 7mpf 0.986

### Canberra

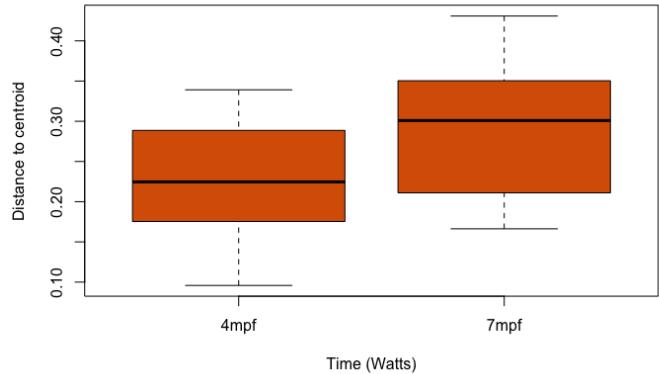


Df	Sum Sq	Mean Sq	F	N.Perm	Pr(>F)
1	0.02179502	0.021795017	3.663629	999	0.049
43	0.25580800	0.005949023			

Names p-value

4mpf- 7mpf 0.047

### Sorensen

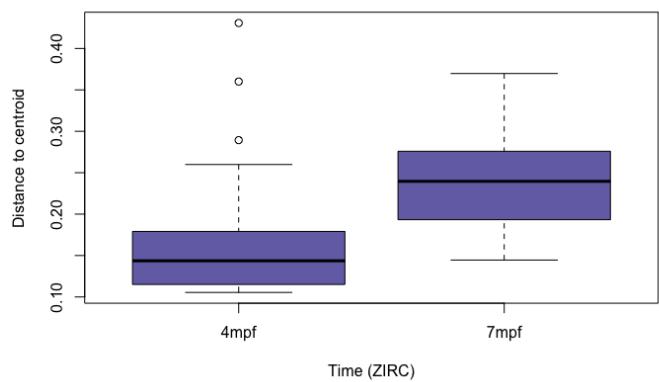


Df	Sum Sq	Mean Sq	F	N.Perm	Pr(>F)
1	0.04106955	0.041069553	7.128783	999	0.008
43	0.24772682	0.005761089			

Names p-value

4mpf- 7mpf 0.006

### Bray-Curtis

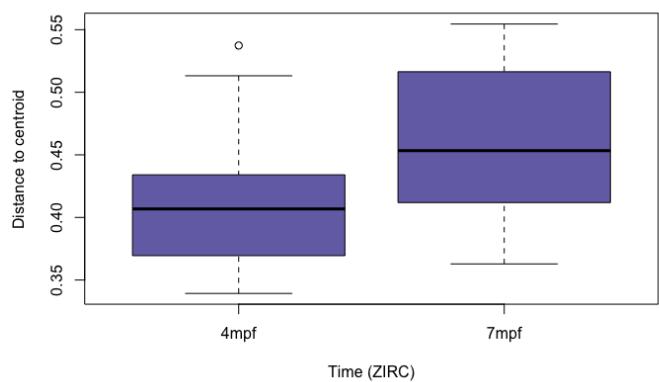


Df	Sum Sq	Mean Sq	F	N.Perm	Pr(>F)
1	0.05445603	0.054456025	9.78485	999	0.001
43	0.23930965	0.005565341			

Names p-value

4mpf- 7mpf 0.002

### Canberra

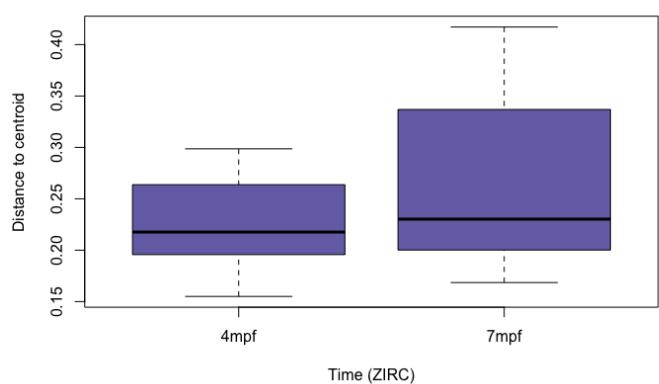


Df	Sum Sq	Mean Sq	F	N.Perm	Pr(>F)
1	0.02374327	0.023743268	7.523946	999	0.009
43	0.13569481	0.003155693			

Names p-value

4mpf- 7mpf 0.013

### Sorensen

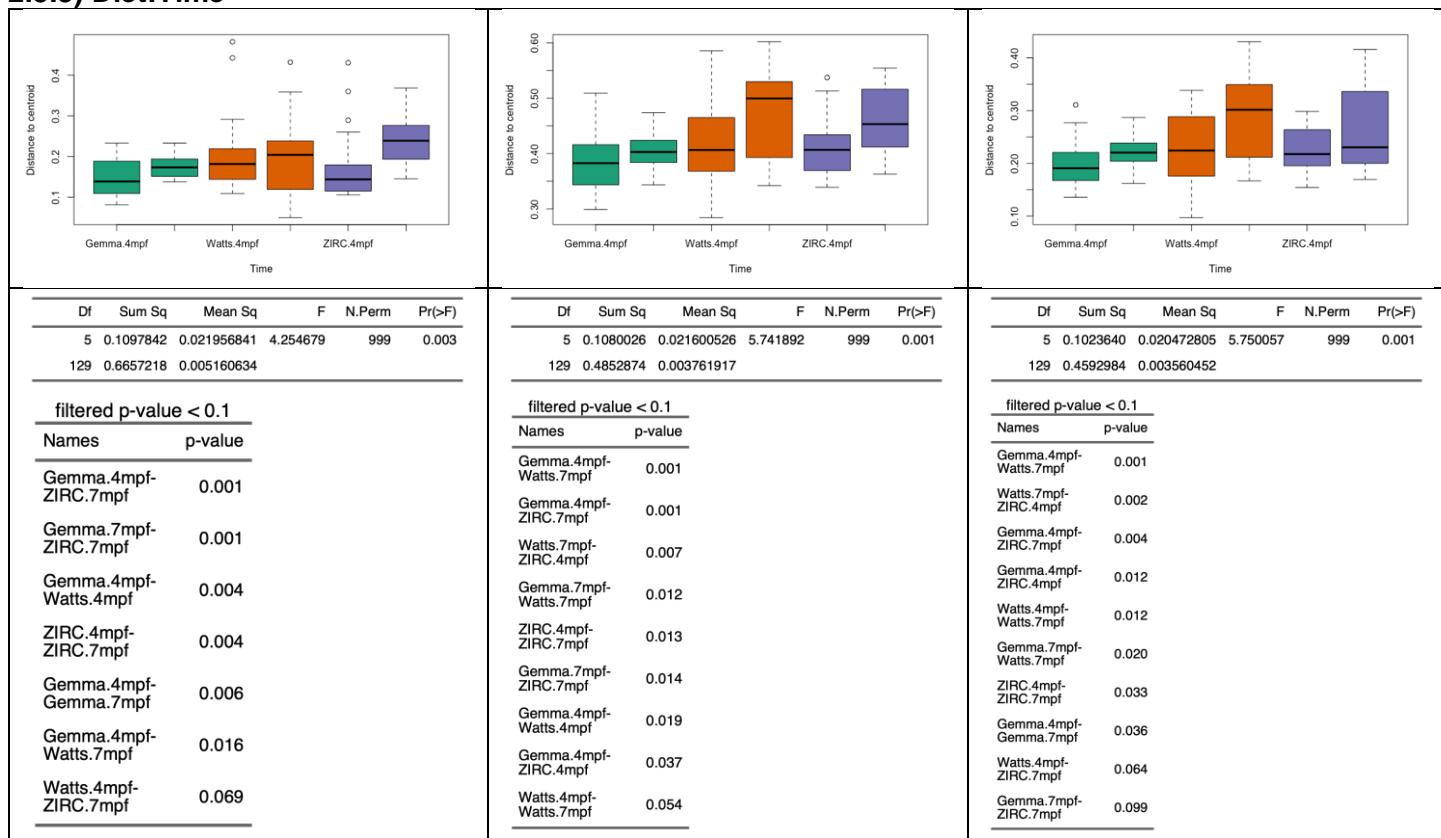


Df	Sum Sq	Mean Sq	F	N.Perm	Pr(>F)
1	0.01603226	0.016032263	4.825086	999	0.042
43	0.14287564	0.003322689			

Names p-value

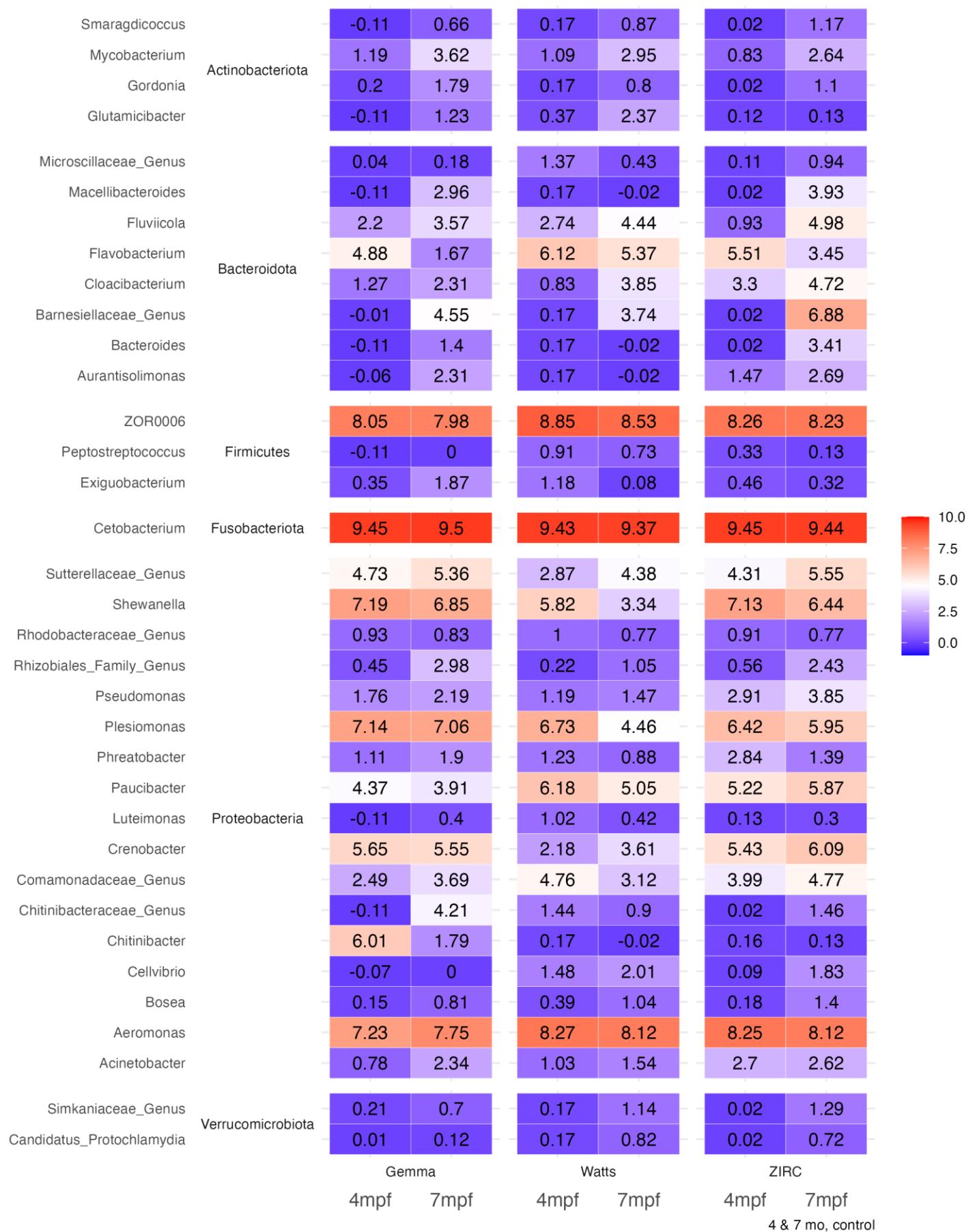
4mpf- 7mpf 0.046

### 2.5.3) Diet:Time



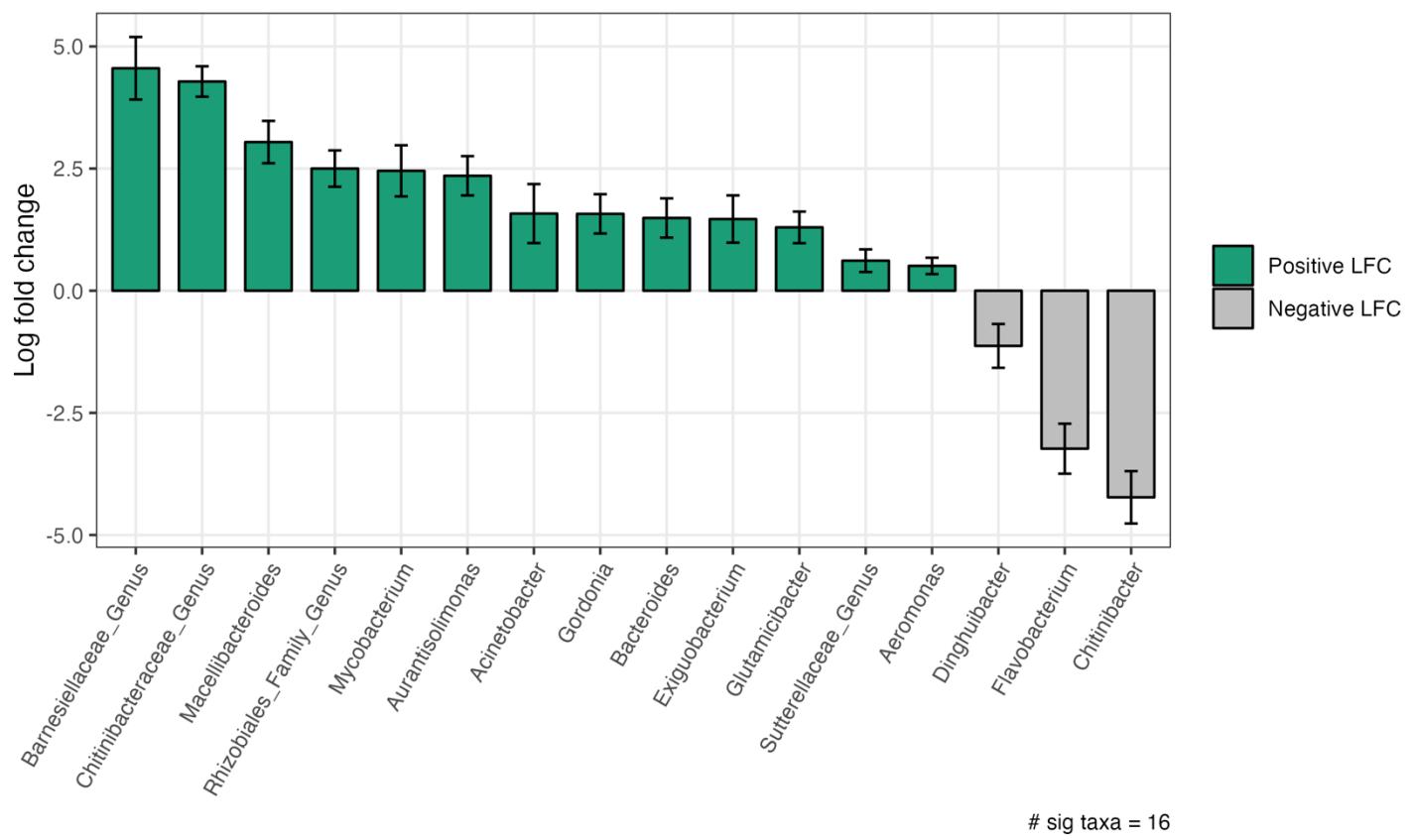


Heat map of bias-corrected log observed abundances (Genus)



649 2.6.2)  
650 2.6.2.1)

### Log fold changes between 4 and 7 months in Gemma diet



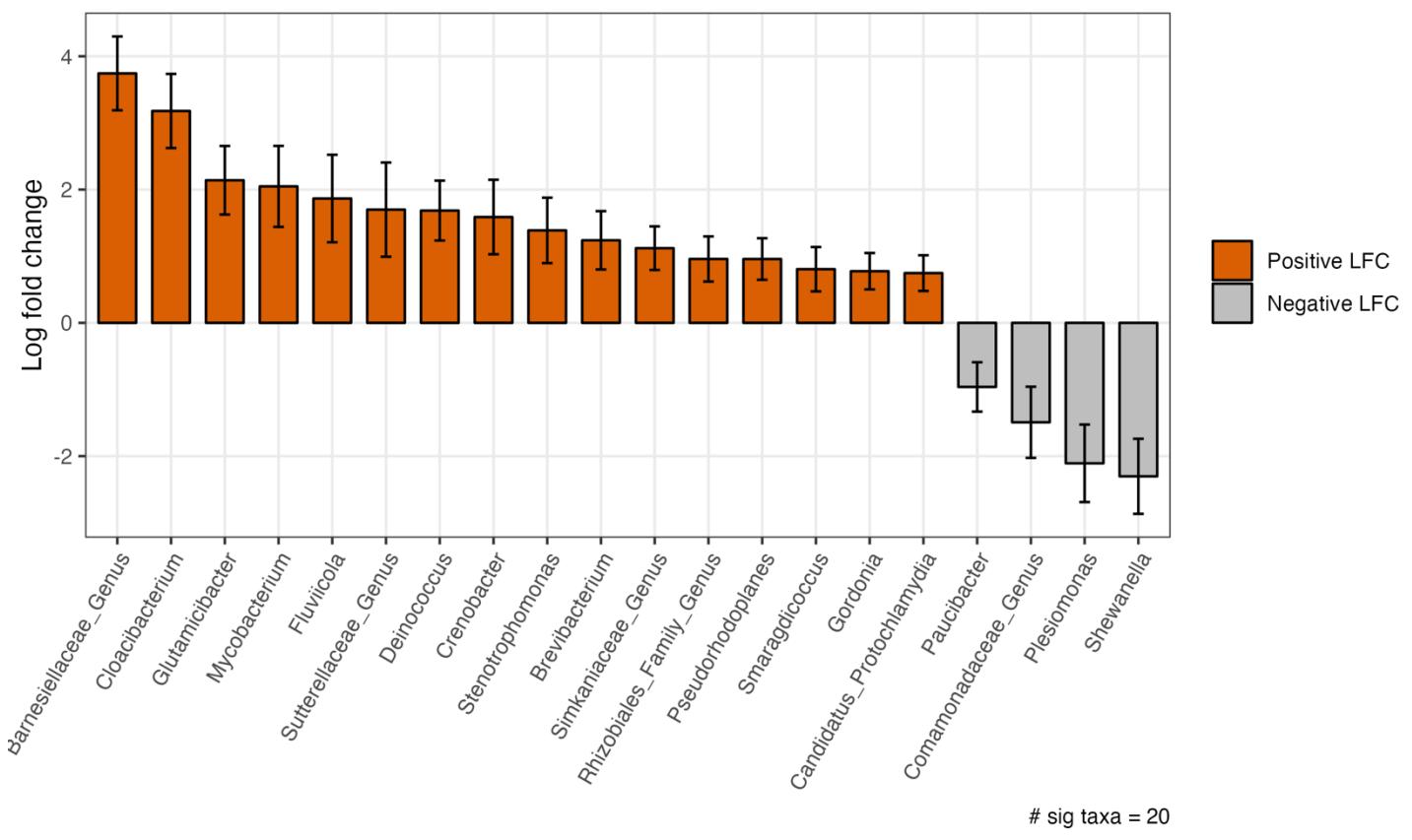
# sig taxa = 16

651

## 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

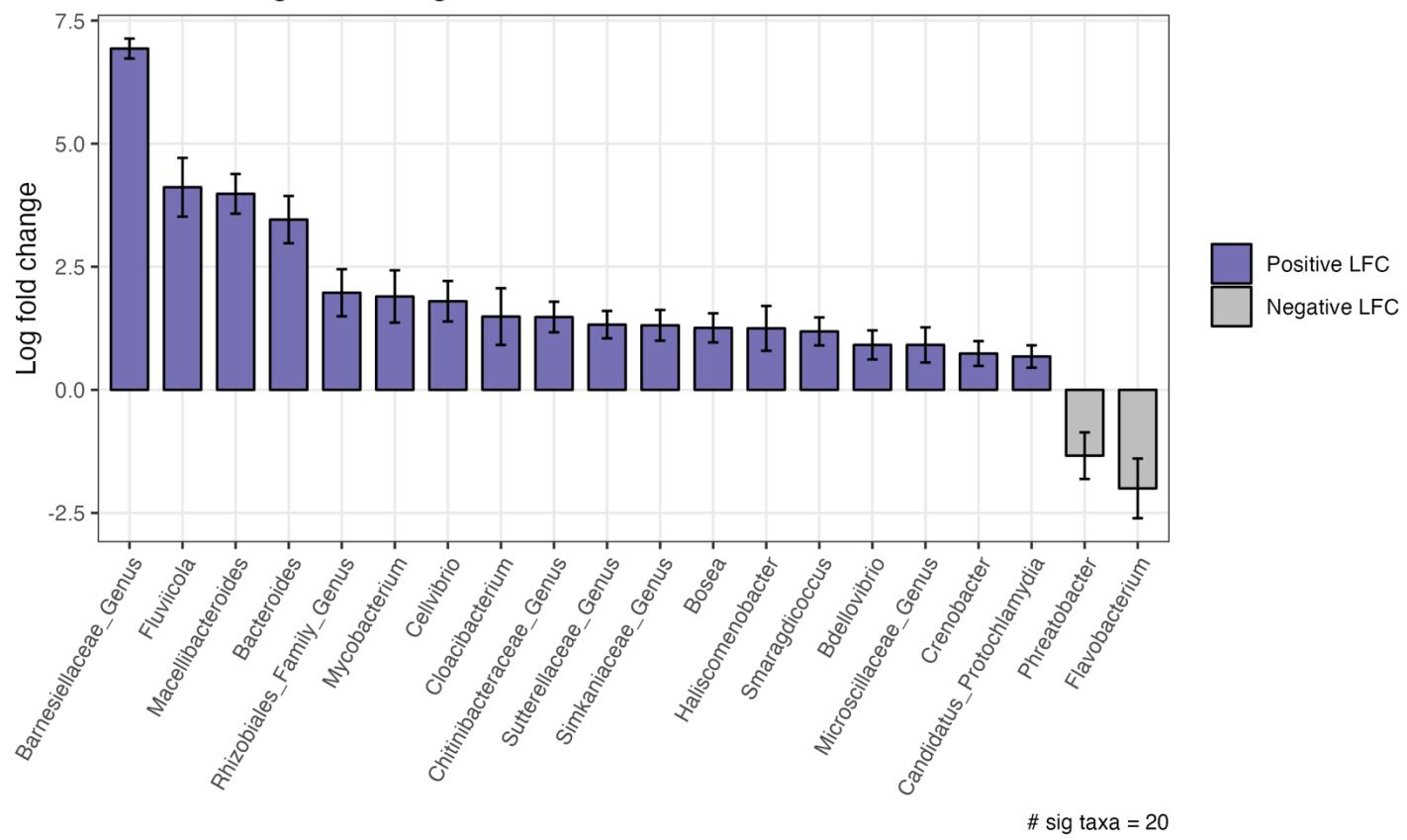
## Log fold changes between 4 and 7 months in Watts diet



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

Taxon	lfc_Timepoint7mpf	diff_Timepoint7mpf	direct
Barnesiellaceae_Genus	3.743419	TRUE	Positive LFC
Cloacibacterium	3.179109	TRUE	Positive LFC
Glutamicibacter	2.139606	TRUE	Positive LFC
Mycobacterium	2.047308	TRUE	Positive LFC
Fluviicola	1.865743	TRUE	Positive LFC
Sutterellaceae_Genus	1.699197	TRUE	Positive LFC
Deinococcus	1.684537	TRUE	Positive LFC
Crenobacter	1.587856	TRUE	Positive LFC
Stenotrophomonas	1.387593	TRUE	Positive LFC
Brevibacterium	1.238637	TRUE	Positive LFC
Simkaniaceae_Genus	1.120585	TRUE	Positive LFC
Rhizobiales_Family_Genus	0.958539	TRUE	Positive LFC
Pseudorhodoplanes	0.957715	TRUE	Positive LFC
Smaragdiccoccus	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

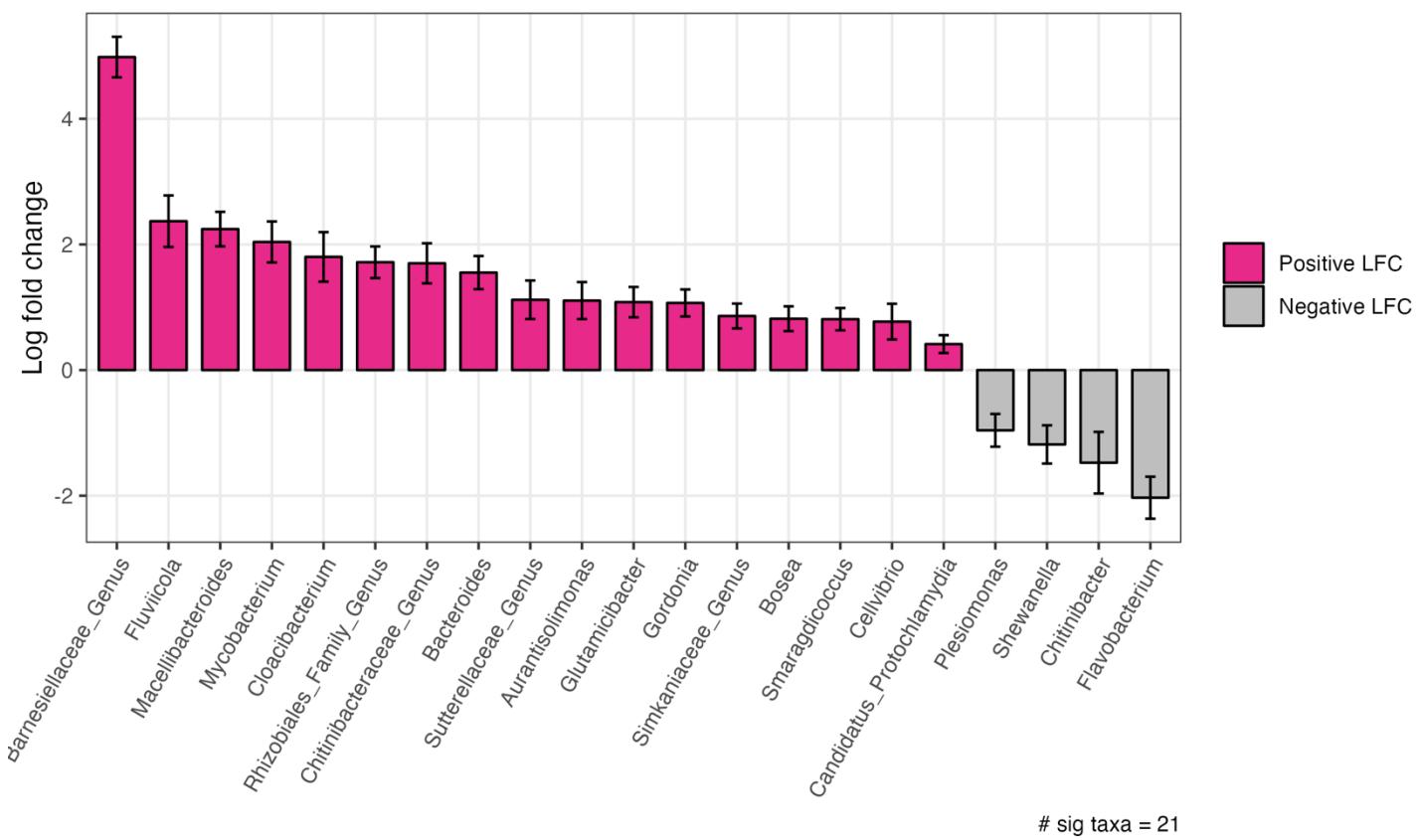
## Log fold changes between 4 and 7 months in ZIRC diet



## 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
Smaragdiccoccus	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

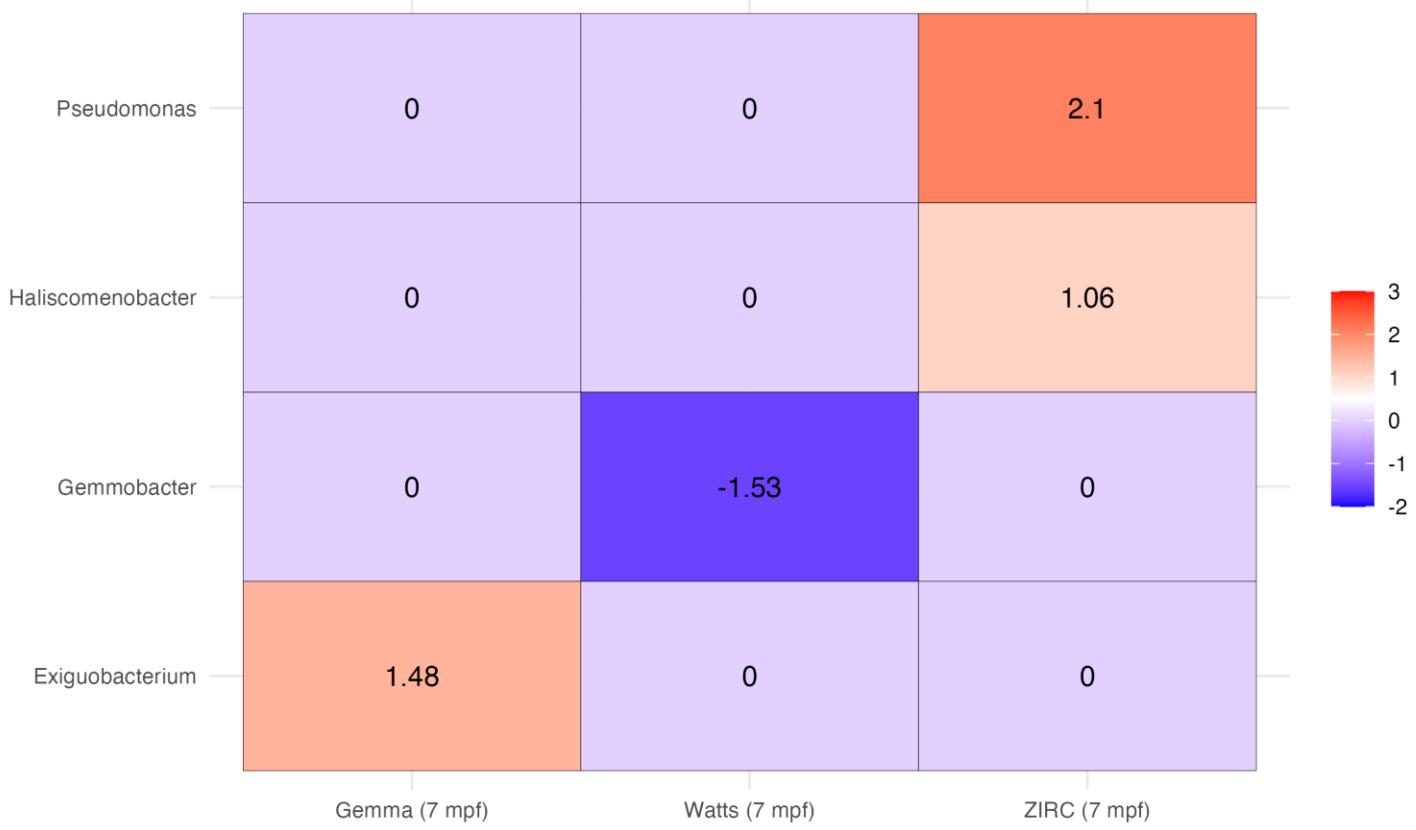
## Log fold changes between 4 and 7 months across all diets



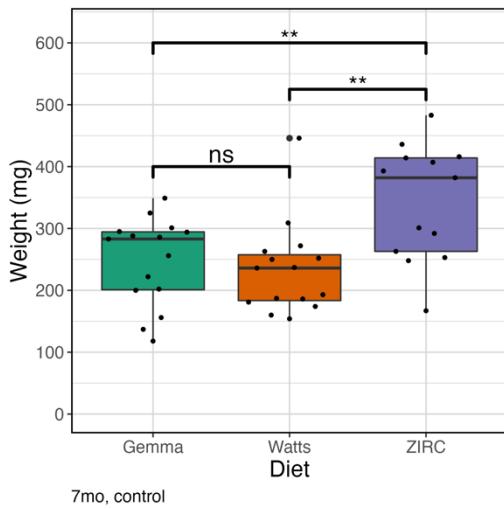
## ANCOM-BC2: Log fold change in abundance. # Sig taxa = 21

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
Smaragdicoccus	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

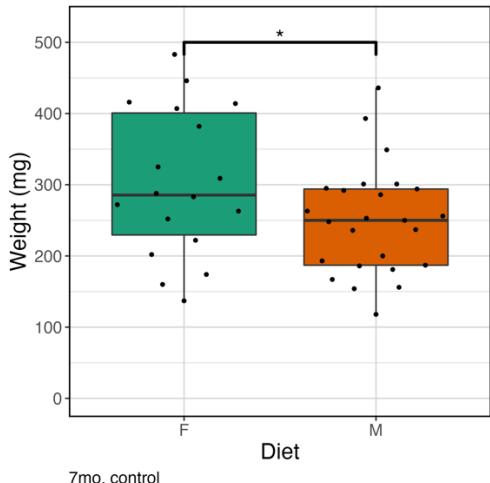


664 2.7) 7 Month Analysis  
 665 2.7.1) Physiology  
 666 2.7.1.1) Weight ~ Diet



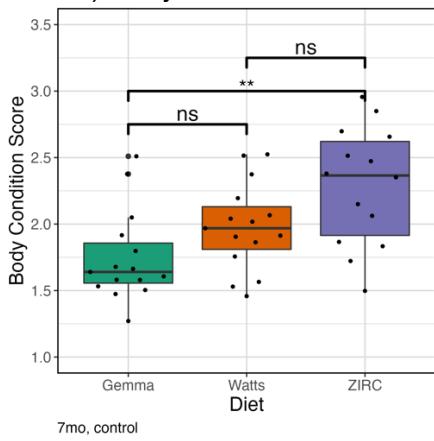
Wilcoxon Test. p. adj: BH. Weight ~ Diet								
.y.	group1	group2	n1	n2	statistic	p	p.adj	p.adj.signif
Weight	Gemma	Watts	15	15	139.000	0.285	0.285	ns
	Gemma	ZIRC	15	15	45.500	0.006	0.009	**
	Watts	ZIRC	15	15	36.500	0.002	0.006	**

668 669 2.7.1.2) Weight ~ Sex



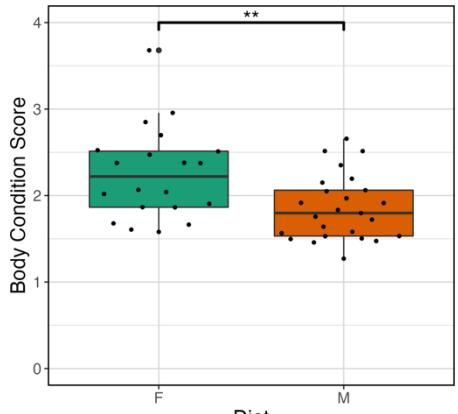
Wilcoxon Test. p. adj: BH. Weight ~ Sex								
.y.	group1	group2	n1	n2	statistic	p	p.adj	p.adj.signif
Weight	F	M	20	25	342.500	0.036	0.036	*

670 671 672 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

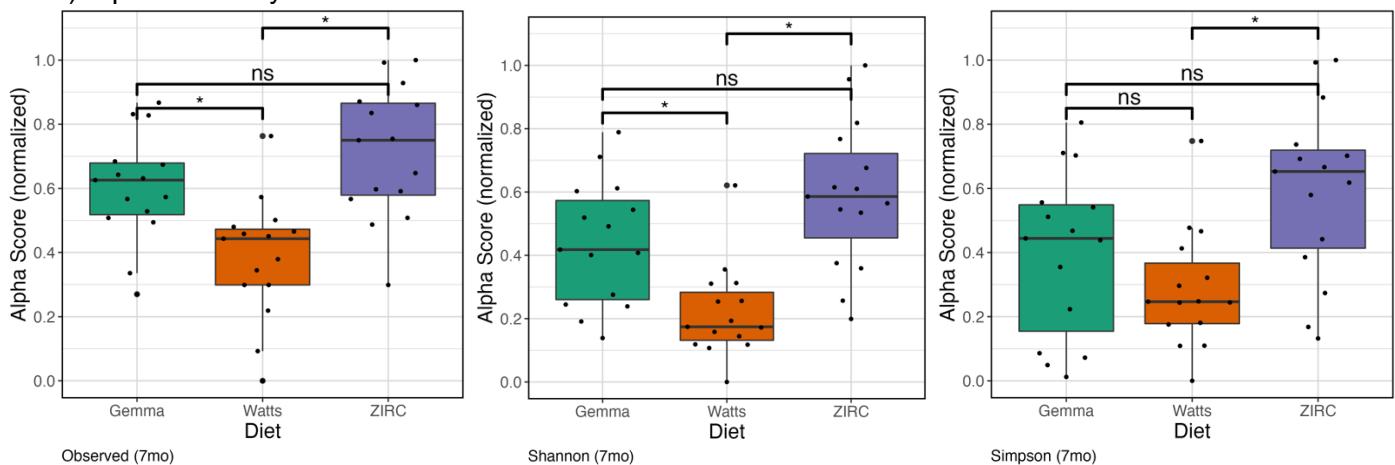
673 674 2.7.1.4) Body Condition Score ~ Sex



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	20	25	366.000	0.007	0.007	**

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## 677 2.7.2) Alpha-Diversity



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

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

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

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

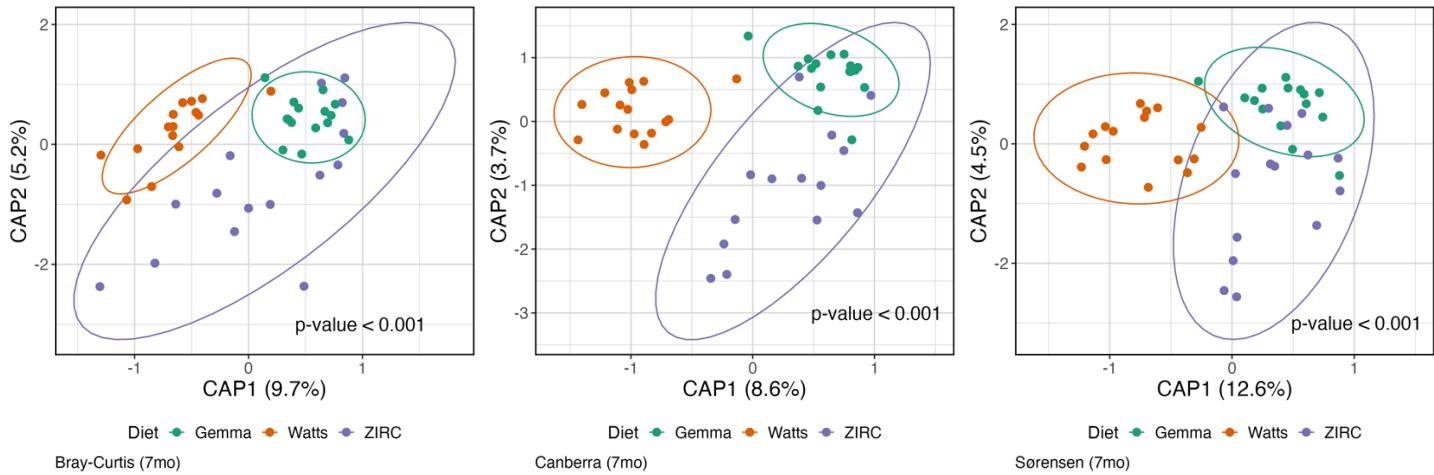
679

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	*

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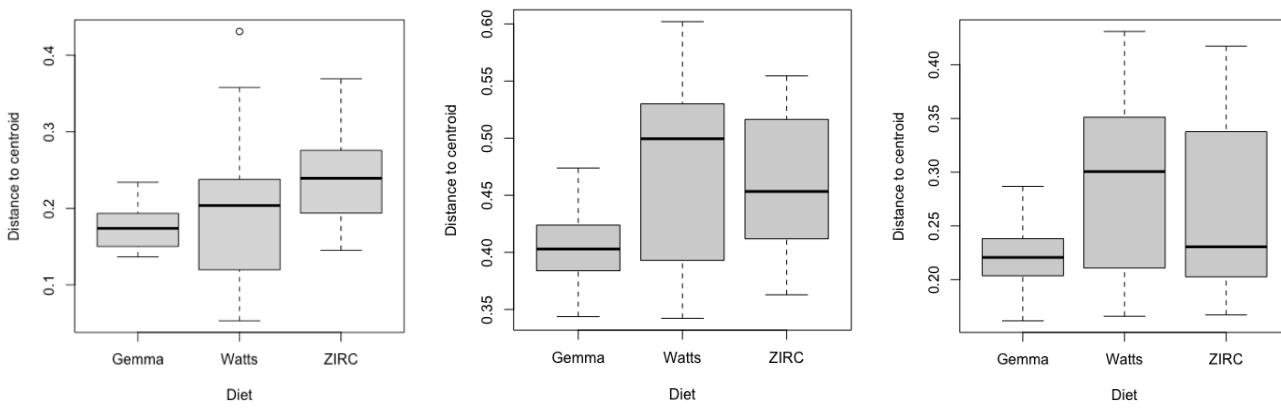
681 2.7.3) Beta-Diversity  
682 2.7.3.1)



683 684 685 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			

686 687 2.7.3.2) Beta-dispersion (Bray, Canberra, Sørensen)



Df	Sum Sq	Mean Sq	F	N.Perm	Pr(>F)
2	0.02863063	0.014315316	2.676967	999	0.08
42	0.22459865	0.005347587			

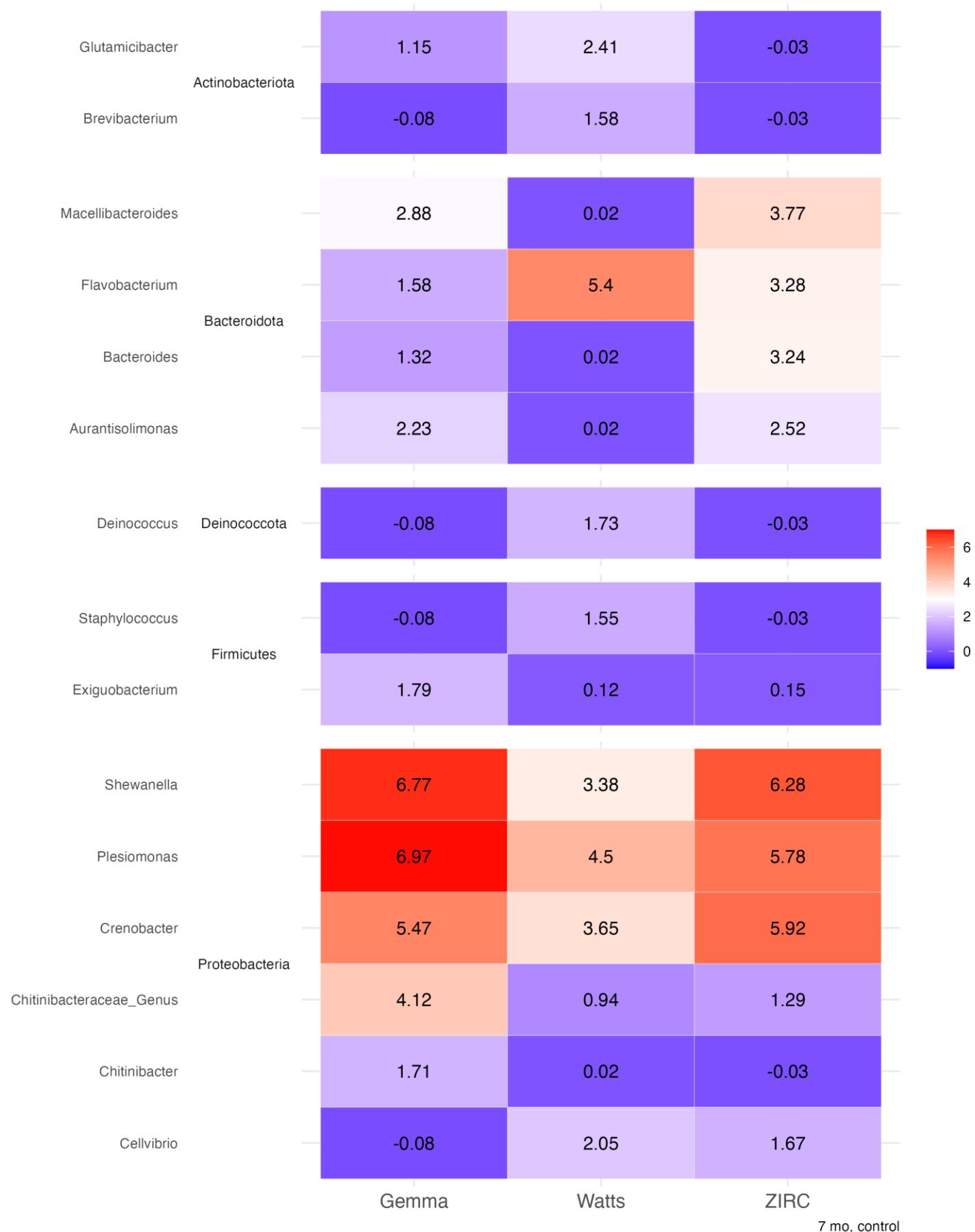
Df	Sum Sq	Mean Sq	F	N.Perm	Pr(>F)
2	0.03521779	0.017608893	3.202764	999	0.052
42	0.23091729	0.005498031			

Df	Sum Sq	Mean Sq	F	N.Perm	Pr(>F)
2	0.03376742	0.016883709	3.975224	999	0.022
42	0.17838386	0.004247235			

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

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



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

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

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## 698 3) Exposure

## 700 3.1) Alpha-diversity

## 702 3.1.1) Exposure

703 3.1.1.1)

---

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

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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	
	PrePostExpUnexposed	0.302	0.136	2.218	0.028	*
Shannon	(Intercept)	-0.415	0.081	-5.140	<0.001	*
	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	*

---

704 705 706 3.1.1.2)

---

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

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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	*

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709 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	*

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712 3.1.2) Diet:Exposure  
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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	
	DietZIRC:ExposureUnexposed	0.118	0.238	0.496	0.620	
Shannon	(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	
	DietWatts:ExposureUnexposed	0.268	0.268	1.000	0.319	
	DietZIRC:ExposureUnexposed	0.306	0.256	1.196	0.233	
Simpson	(Intercept)	-0.337	0.149	-2.259	0.025	*
	DietWatts	-0.104	0.210	-0.497	0.620	
	DietZIRC	0.282	0.208	1.359	0.176	
	ExposureUnexposed	-0.019	0.209	-0.092	0.927	
	DietWatts:ExposureUnexposed	0.140	0.295	0.475	0.635	
	DietZIRC:ExposureUnexposed	0.365	0.293	1.245	0.215	

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	

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	*

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			

723 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			

724 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			

725 3.2.3

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### 729 3.3) Beta-Dispersion

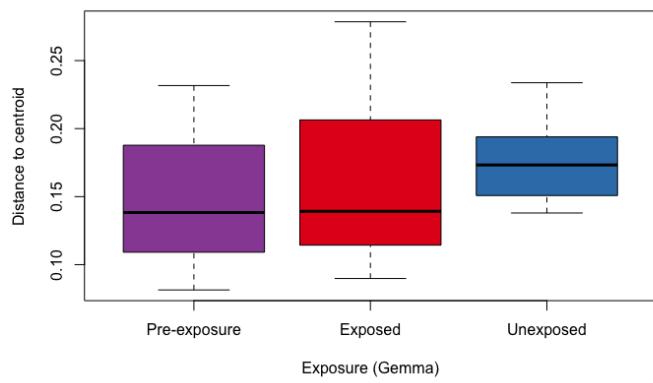
730

731 3.3.1) Exposure per diet

732

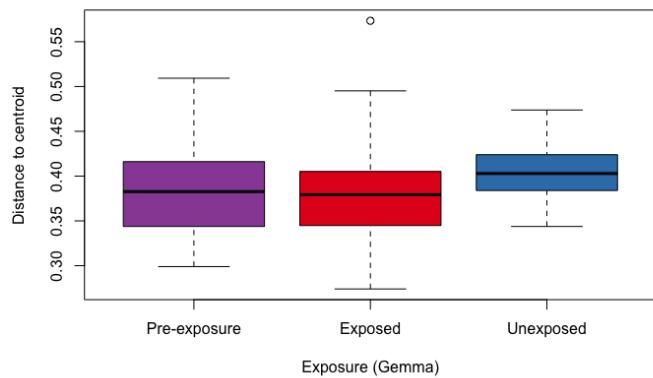
733 3.3.1.1) Gemma

### Bray-Curtis



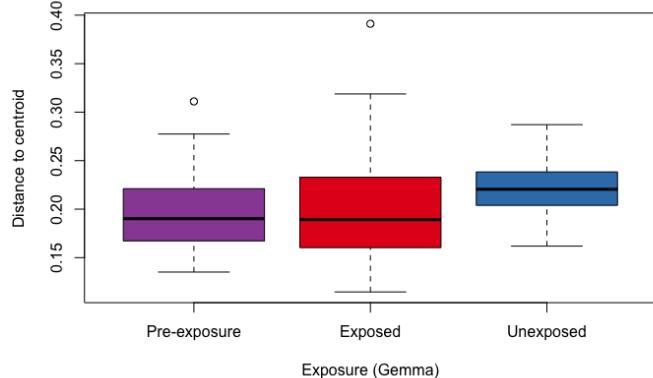
Df	Sum Sq	Mean Sq	F	N.Perm	Pr(>F)
2	0.009302604	0.004651302	2.563631	999	0.081
56	0.101603114	0.001814341			
Names p-value					
Pre-exposure-Exposed	0.565				
Pre-exposure-Unexposed	0.013				
Exposed-Unexposed	0.190				

### Canberra



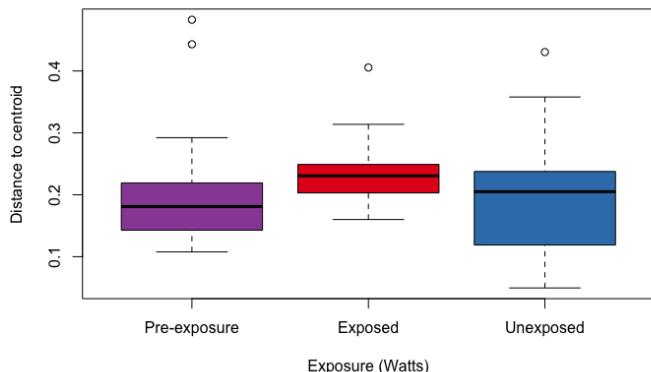
Df	Sum Sq	Mean Sq	F	N.Perm	Pr(>F)
2	0.006179761	0.003089881	0.9862029	999	0.372
56	0.175454066	0.003133108			
Names p-value					
Pre-exposure-Exposed	0.759				
Pre-exposure-Unexposed	0.093				
Exposed-Unexposed	0.398				

### Sorensen



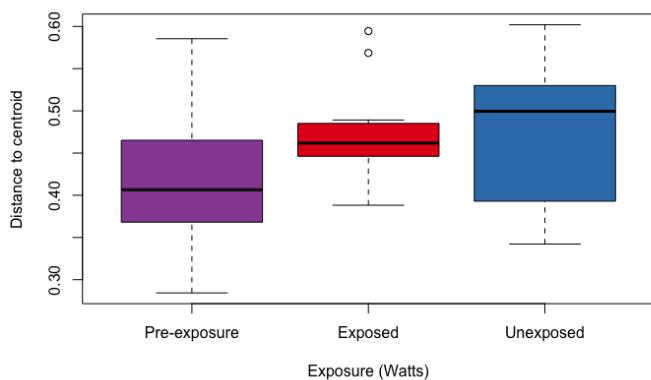
Df	Sum Sq	Mean Sq	F	N.Perm	Pr(>F)
2	0.008320015	0.004160008	1.592959	999	0.208
56	0.146243788	0.0026111496			
Names p-value					
Pre-exposure-Exposed	0.582				
Pre-exposure-Unexposed	0.028				
Exposed-Unexposed	0.391				

### Bray-Curtis



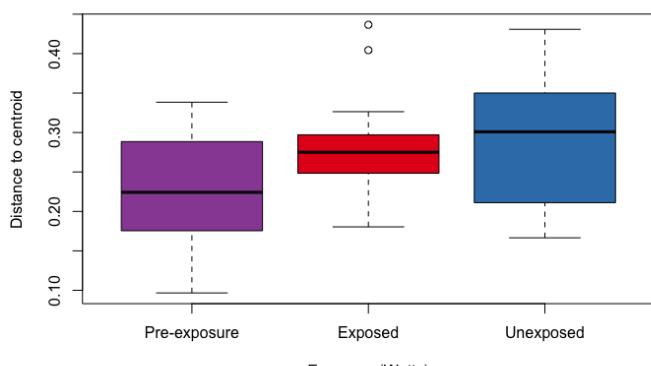
Df	Sum Sq	Mean Sq	F	N.Perm	Pr(>F)
2	0.02048418	0.010242091	1.400077	999	0.24
57	0.41697655	0.007315378			
Names					p-value
Pre-exposure-Exposed					0.074
Pre-exposure-Unexposed					0.982
Exposed-Unexposed					0.194

### Canberra



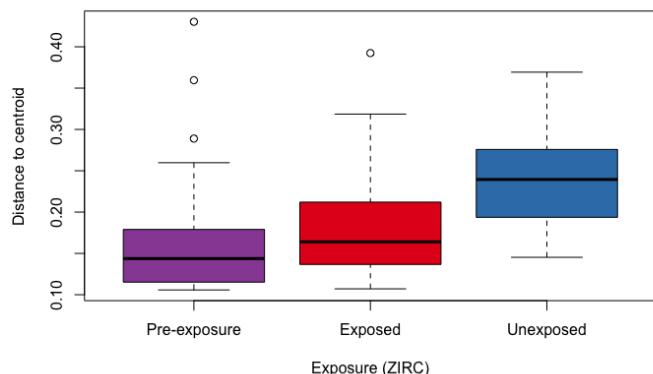
Df	Sum Sq	Mean Sq	F	N.Perm	Pr(>F)
2	0.03477851	0.017389256	3.380925	999	0.039
57	0.29317057	0.005143343			
Names					p-value
Pre-exposure-Exposed					0.020
Pre-exposure-Unexposed					0.059
Exposed-Unexposed					0.911

### Sorensen



Df	Sum Sq	Mean Sq	F	N.Perm	Pr(>F)
2	0.06056003	0.030280015	5.639522	999	0.007
57	0.30604738	0.005369252			
Names					p-value
Pre-exposure-Exposed					0.004
Pre-exposure-Unexposed					0.014
Exposed-Unexposed					0.960

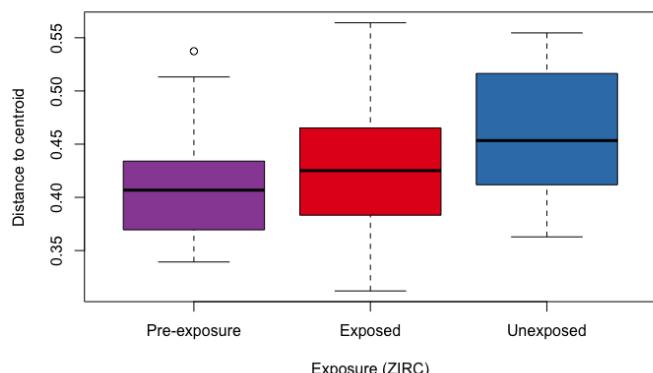
### Bray-Curtis



Df	Sum Sq	Mean Sq	F	N.Perm	Pr(>F)
2	0.05474422	0.027372111	4.719002	999	0.015
57	0.33062294	0.005800402			

Names	p-value
Pre-exposure-Exposed	0.428
Pre-exposure-Unexposed	0.008
Exposed-Unexposed	0.073

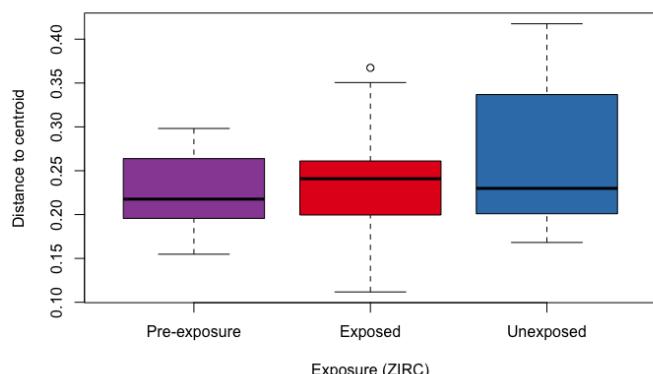
### Canberra



Df	Sum Sq	Mean Sq	F	N.Perm	Pr(>F)
2	0.02378452	0.011892259	3.250362	999	0.051
57	0.20854869	0.00365658749			

Names	p-value
Pre-exposure-Exposed	0.310
Pre-exposure-Unexposed	0.015
Exposed-Unexposed	0.258

### Sorensen



Df	Sum Sq	Mean Sq	F	N.Perm	Pr(>F)
2	0.01606932	0.008034658	2.192393	999	0.132
57	0.208889297	0.0036664789			

Names	p-value
Pre-exposure-Exposed	0.434
Pre-exposure-Unexposed	0.030
Exposed-Unexposed	0.334

738

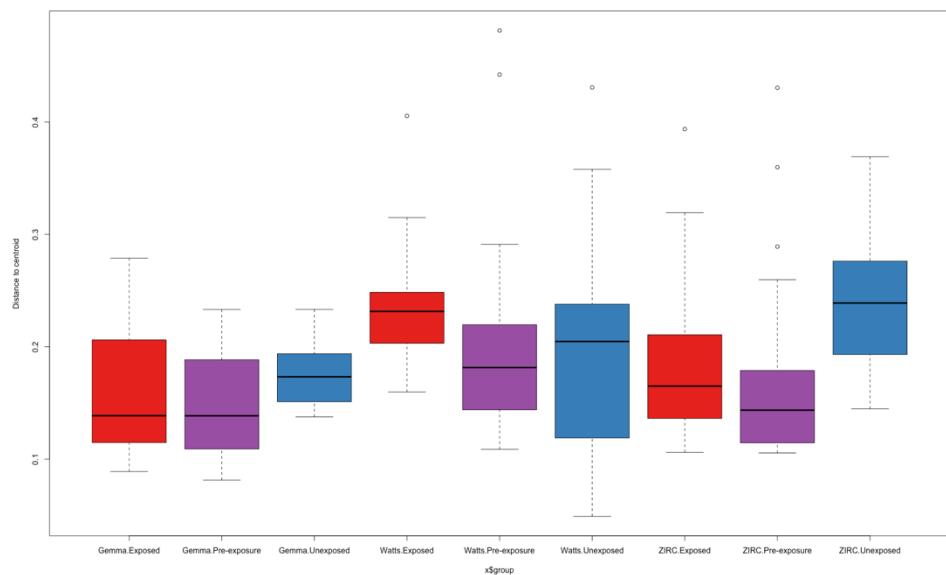
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3.3.2) Diet:Exposure

## Bray-Curtis

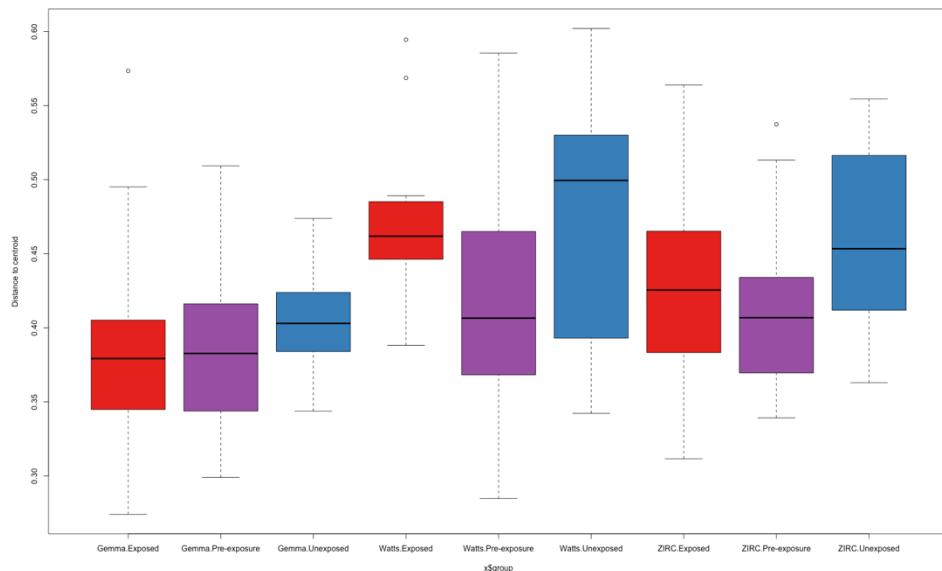


Df	Sum Sq	Mean Sq	F	N.Perm	Pr(>F)
8	0.1676731	0.020959131	4.194491	999	0.001
170	0.8494599	0.004996823			

## 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

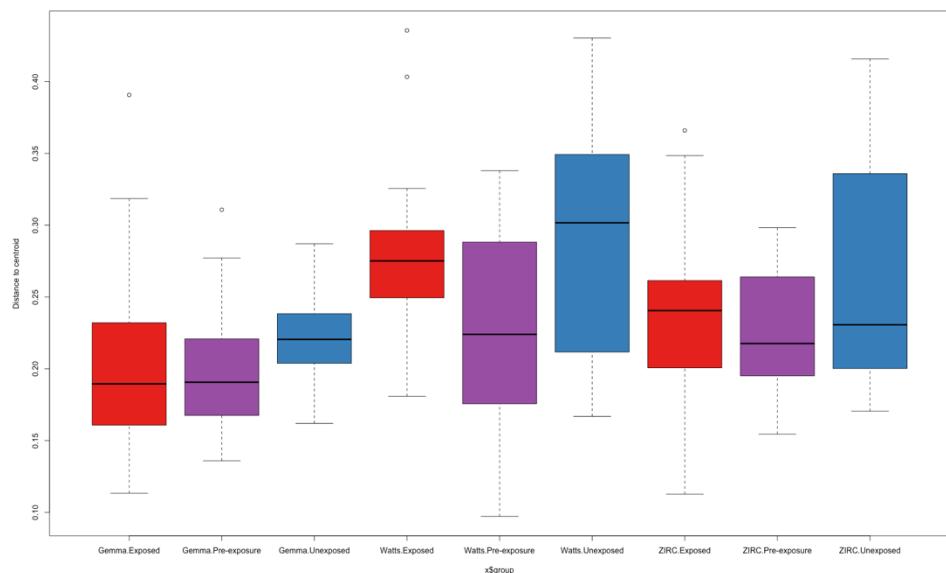
## Canberra



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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### 3.4) Differential Abundance

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

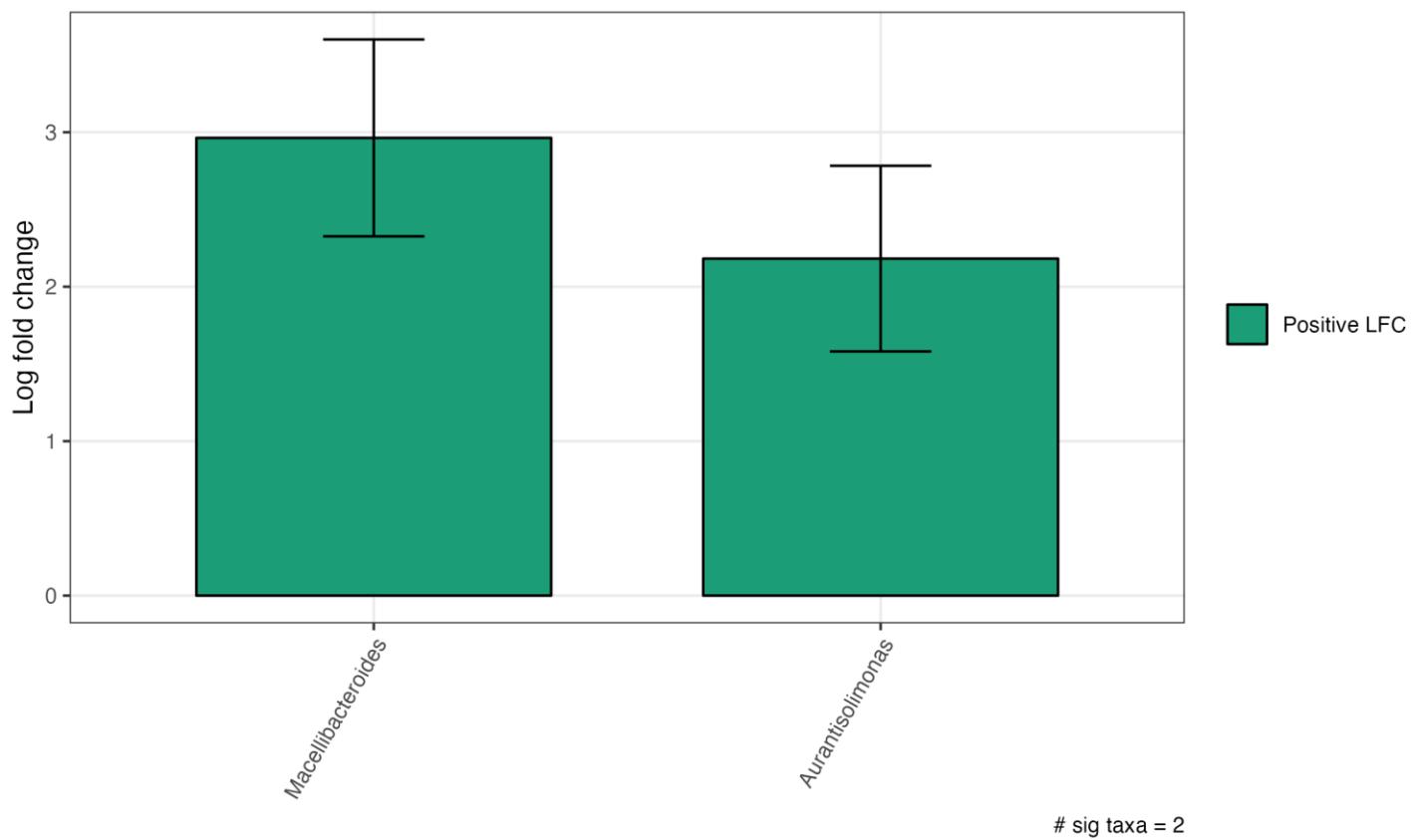


ANCOM-BC2: Summary statistics of abundant taxa (4-7mo, all), sig taxa = 26

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

748 3.4.2)

## Log fold changes in Unexposed Gemma diet



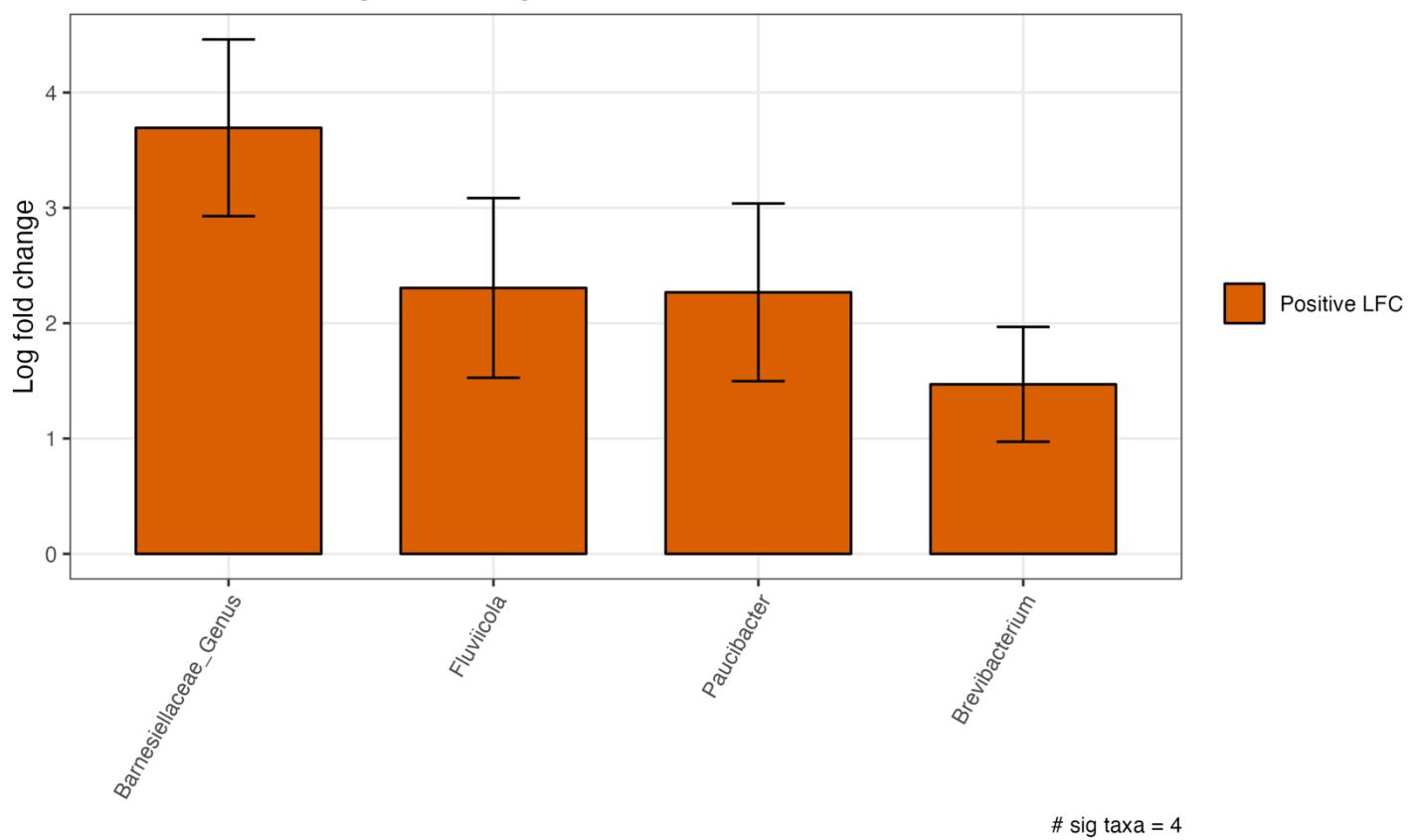
749

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

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

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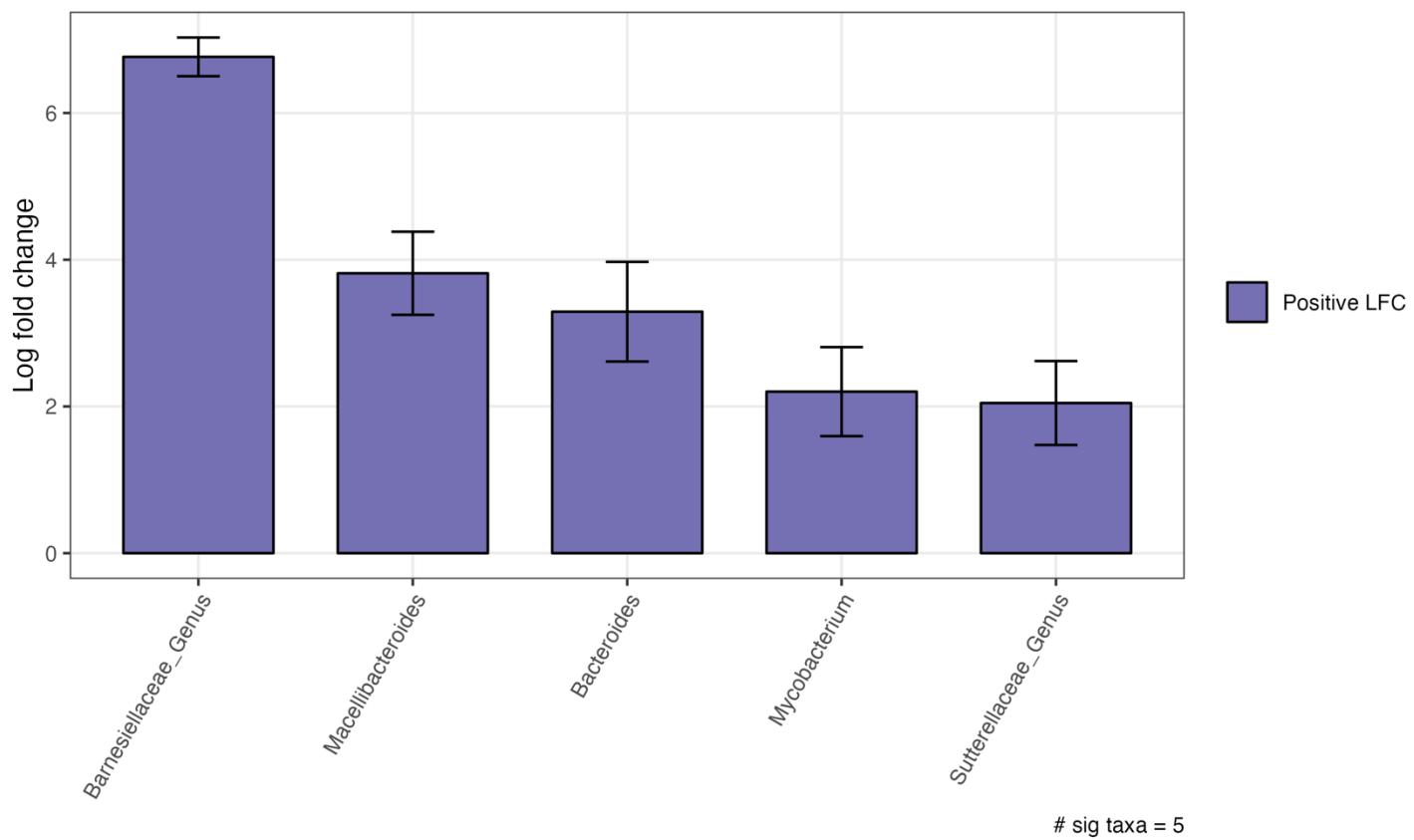
## Log fold changes in Unexposed Watts diet



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

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

## Log fold changes in Unexposed ZIRC diet

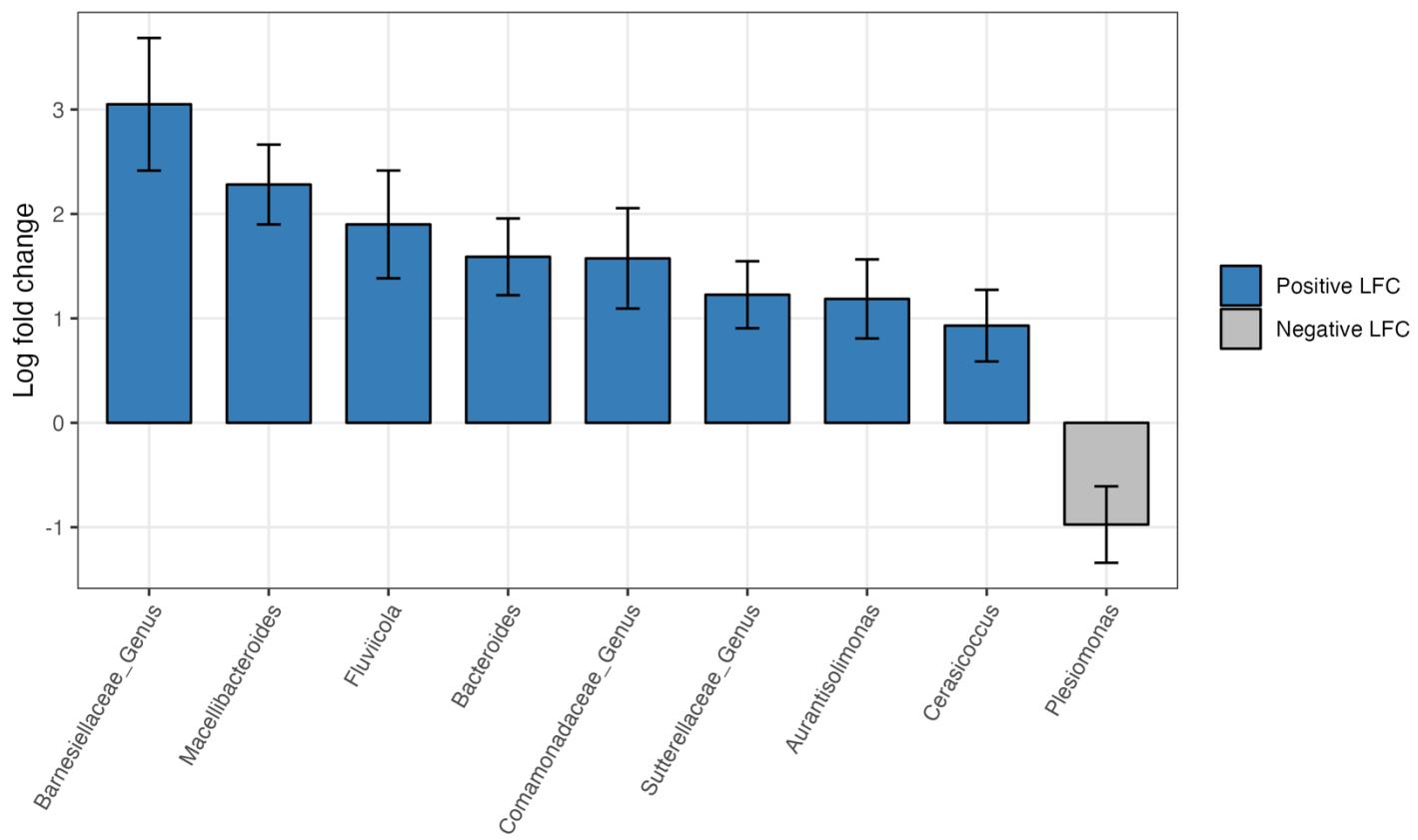


# sig taxa = 5

## 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

## Log fold changes in unexposed across all diets



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

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

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

762

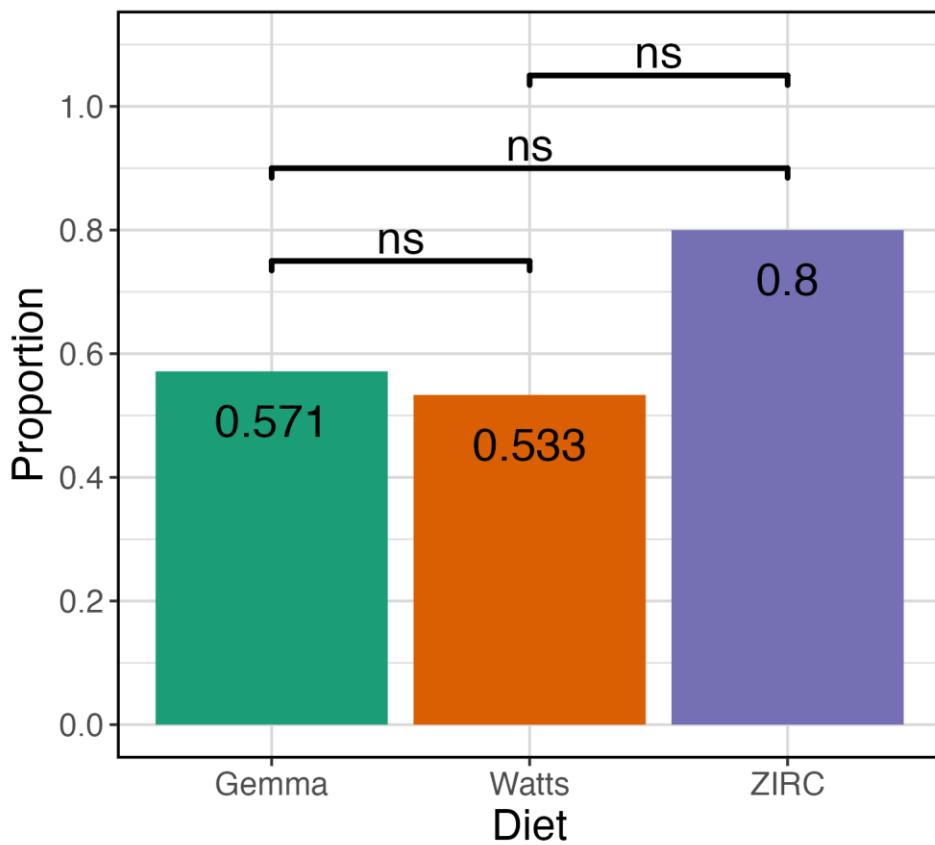
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## 3.5) Infection

## 3.5.1)

Proportion of positive infections



7mo, exposed

766

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

767  
768 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			

769

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	

772 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	

773

774 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	
	Residual	35.00	1.74856431			
Canberra	Diet	2.00	1.72790036	4.254	0.001	*
	Pathology.Results	2.00	0.48794416	1.201	0.137	
	Pathology.Results:Diet	4.00	0.84016409	1.034	0.392	
	Residual	35.00	7.10790377			
Sørensen	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			

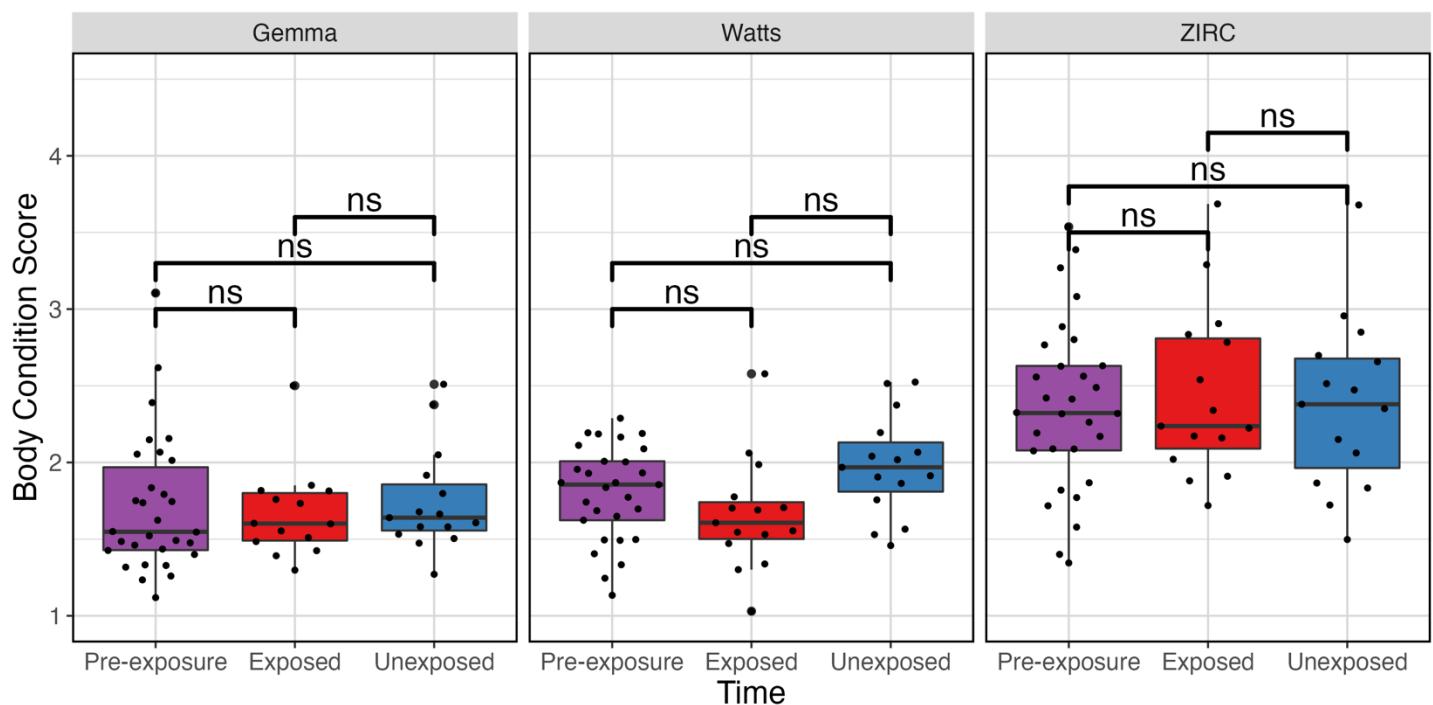
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778 3.6) Physiology

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780 3.6.1)



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782 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

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