

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

Abstract

Despite zebrafish's long-established importance as a model organism and their increasing use in microbiome-targeted studies, there is a lack of consensus regarding husbandry practices involving diet. Diet is known to influence the structure of the gut microbiome and physiology of zebrafish. Given, the microbiome's important role in maintaining host health through digestion of nutrients and fighting pathogens, diet-associated differences in the microbiome could impact host health and study outcomes. Key knowledge gaps remain about whether commonly used laboratory diets influence zebrafish microbiomes across their development and when exposed to a pathogen. Here we show that diet drives gut microbiome successional development and sensitivities to pathogen exposure. We found that at 3 months fish gut microbiomes stratified by diet, and these effects accumulated across development that resulted in diet-dependent differences in the microbiome and physiology at 6 months of age. Furthermore, we found that sensitivity to pathogen exposure depended on diet. Our results demonstrate that variation in husbandry practices around diet impacts the composition of the gut microbiome. Collectively, our results indicate that researchers should carefully consider the role of diet in their zebrafish microbiome studies and that diet should be controlled for when integrating microbiome data across studies.

Introduction

Despite zebrafish's long-established importance as a model organism and their increasing use in microbiome-targeted studies, key knowledge gaps remain about how diet influences their microbiome. In contrast to mice, zebrafish do not have a standard reference diet (Watts). Prior research has found husbandry choices involving diet can induce variation in study outcomes and challenge efforts to compare results across studies (Fowler, Watts). Moreover, experimental, commercial and laboratory diets result in different microbiome and health outcomes (Fowler, Leigh, Rawls, Others?). However, what is not known is if zebrafish gut microbiome communities differ between commonly used laboratory diets, and if these differences persist throughout development.

By 3 months of age Zebrafish are developmentally considered adults. Their immune systems have finished developing, they are sexually mature, and have reached full body size (citation). However, zebrafish microbiomes continue to develop as they age, becoming increasingly diverse and stable (Xiao). Prior to adulthood, zebrafish microbiome assembly is more susceptible to environmental influences of drift and dispersal, but with age these effects decline until senescence (Stephens2016). Additionally, the microbiome has been linked to an array of health outcomes involving obesity, X, Y and Z across an array of organisms, including zebrafish (citations). Generally, microbiomes are stable once established. Therefore, early-life assembly of the gut microbiome could have long-term implications on host health, such as resistance to infection (citation).

Pathogen exposure is known to impact the gut microbiome of zebrafish, and the microbiome could mediate these effects, either protecting, exacerbating, or having a neutral influence (citation). Zebrafish facilities are known to host many pathogens, which can introduce non-protocol induced

inconsistencies in study outcomes (Kent). One pathogen that is found in 40% of zebrafish facilities is *Mycobacterium chelonae*, and is hypothesized to be introduced through diet early in life (Stephens, Kent2012, Chang2019). *M. chelonae* forms granulomas in the gut intestine, which can cause gut inflammation, decreased fecundity and lifespan (Whipps2016, Varela). Previous work of ours has shown that pathogen exposure disrupted the gut microbiomes of zebrafish (Gaulke), but the joint effects of diet and pathogen exposure on zebrafish gut microbiomes and physiology remains unclear. Elucidating these relationships could offer microbiome-targeted treatments for preventing or minimizing the impacts of pathogen exposure on zebrafish health and study outcomes.

Here, we assessed whether different common laboratory diets influenced gut microbiomes and physiology of 3-month-old zebrafish. Next, we investigated the role of diet on zebrafish's development between 3 and 6-month-old zebrafish. Finally, we measured the diet-associated sensitivity of zebrafish to the pathogenic species *Mycobacterium chelonae*. Our study clarifies how common laboratory diets differentially impacts the successional development of zebrafish gut microbiome and sensitivity to pathogen exposure.

1. Diet differentially influences physiology and gut microbiome

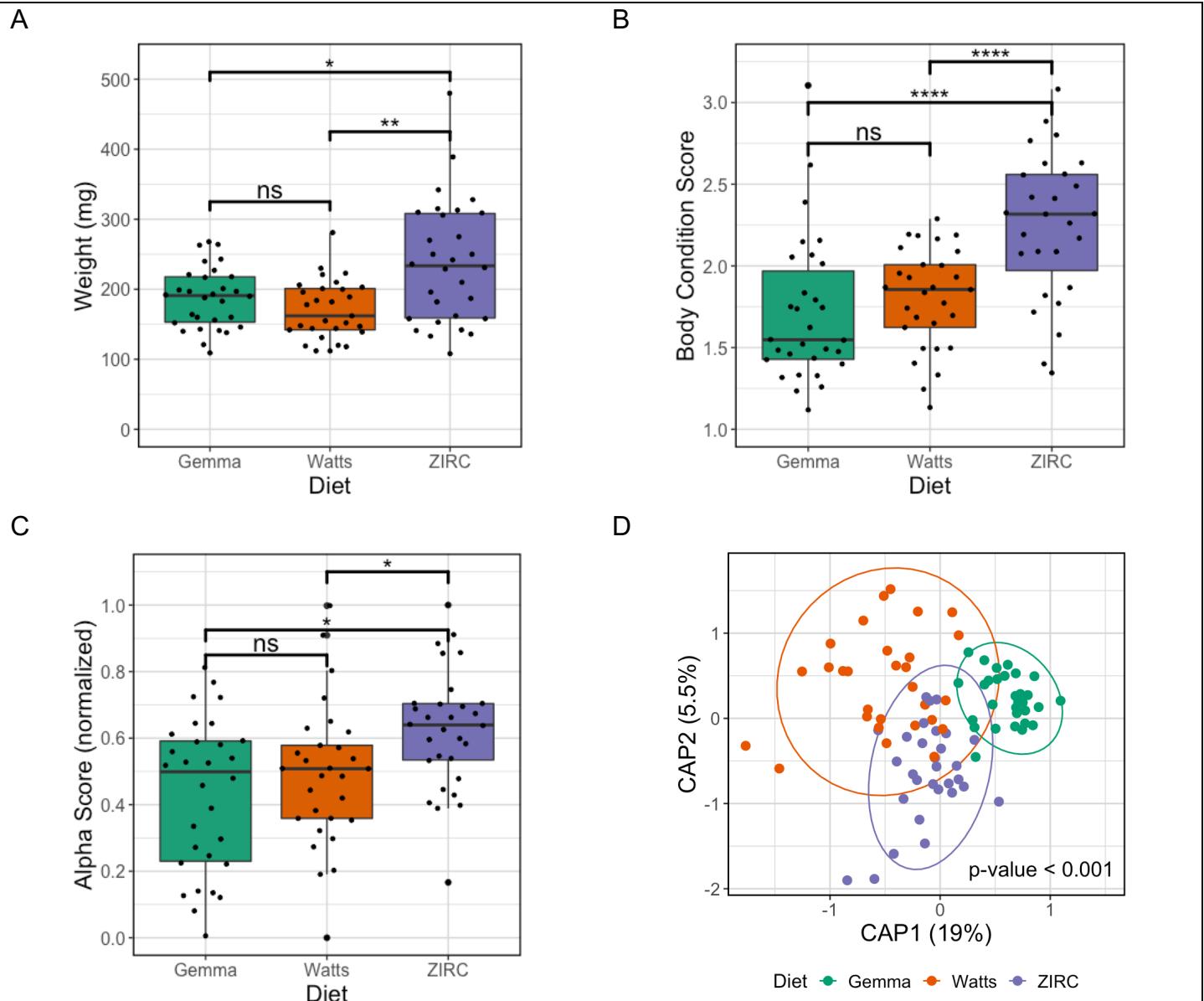


Figure 1: Effects of fish fed one of three diets (Gemma, Watts, or ZIRC) on physiology and microbiomes of zebrafish. **(A)** Weight of ZIRC significantly differs from Watts and Gemma. Gemma and Watts do not differ from each other. **(B)** Body condition score is a length normalized measure of weight. ZIRC fed fish have significantly higher body condition scores from Gemma and Watts diets. **(C)** Simpson's Index of diversity shows that gut microbiome diversity significantly differs between Gemma and Watts, ZIRC and Watts, but not between Gemma and ZIRC. **(D)** Capscale ordination based on the Bray-Curtis dissimilarity of gut microbiome composition. The analysis shows that physiology and gut microbiome composition significantly differs between the diets. “ns” indicates not significantly different, *, **, *** indicates significant differences below the 0.05, 0.01, and 0.001 levels, respectively.

<Results>

To investigate how diet may impact the zebrafish gut microbiome diversity, composition, and relative abundance, we fed zebrafish one of three commonly used laboratory diets (Gemma, Watts, and

ZIRC; see Table in supplementary material). At 3 months of age, we collected fecal samples and used 16S rRNA gene sequencing to identify microbial taxa. Additionally, we measured weight, length, and body condition score to assess how these diets may impact zebrafish physiology. Body condition score is a length normalized metric of weight (for equation, see Methods), and a general indicator of health in zebrafish.

Briefly, to determine if physiology differed between diets, we used Wilcoxon Signed-Ranks Tests to identify parameters that best explained the variation in weight and body condition score. We find that diet and sex significantly associated with weight and body condition. Female fish had higher weight and body condition scores compared to males ($Z = 1,505$, $P < 0.001$; Table S1.1). ZIRC had the highest mean body condition score compared to Gemma and Watts ($Z = 301$ and 225 , $P = 0.44$ and 0.006 , respectively; Table S1.1.1). Gemma and Watts fed fish weight and body condition scores did not significantly differ from each other. We did not observe a significant interaction between diet and sex on weight and body condition score. Collectively, results indicate that diet has an effect on physiology.

Next, we asked if diet associated with gut microbiome diversity and composition. To assess microbiome diversity, we built generalized linear models (GLM) to identify if diet associated with variation in Observed, Simpson's and Shannon Indices of diversity. An ANOVA test of these GLMs revealed that alpha diversity associated with diet across all three diversity indices ($p < 0.05$; Fig 1C; Table S1.2.2). A post hoc Tukey test showed that ZIRC fed fish diversity was significantly different to Watts fed fish across all diversity indices, and differed from Gemma only in Simpson's index ($p < 0.05$; Table S1.2.3). Gemma and Watts only differed significantly in Observed diversity index, and ZIRC and Gemma only differed in the Shannon diversity index. To assess microbiome composition, we used the Bray-Curtis and Canberra dissimilarity metrics to compare pairs of microbiome community composition. A PERMANOVA test revealed that gut microbiome communities fed different diets are significantly different from one another in their composition. Additionally, we assessed beta-dispersion, a measure of variation of microbiome communities, by calculating each gut microbiome community's distance from their respective centroid. Beta-dispersion levels for Bray-Curtis differed significantly, where Watts had higher dispersion and differed from the other two diets, but ZIRC and Gemma did not differ from each other. For the Canberra measure, Gemma fed fish had the least dispersion and was significantly different from ZIRC and Watts, but ZIRC and Watts did not differ from each other. Finally, to better understand the interactions between the diets and the gut microbiome, we quantified differential abundance using ANCOM-BC. We observed 24 taxa were significantly abundant in at least one of the three diets. Collectively, these results indicate that commonly used zebrafish laboratory diets have a differential effect on microbiome structure at 3 months of age.

<Discussion>

Here, we compared microbiomes of fish fed commonly used laboratory diets, which have more consistent nutritional profiles to those in previous studies interrogating the physiology, microbiome and diet in zebrafish. We found that diet differentially influences physiology and the gut microbiome of 3 month old zebrafish. Fish fed ZIRC diet are heavier and have higher body condition scores compared to fish fed the Watts and the Gemma diets. These results align with previous research investigating the effects of diet on zebrafish physiology (Watts, Fowler). Previous studies have found that different laboratory, commercial and experimental diets manifest different gut physiology, growth, health and reproductive outcomes (Leigh 2018, Fowler 2019). Leigh et al. found that in addition to nutritional composition, digestive enzyme activity played a role in shaping the physiological structure of the gut, and noted this could impact the gut microbiome of fish. We found that gut microbiome diversity differed by diet, and fish microbiome communities were more similar to fish fed the same diet. A recent study by Karlsen et al. has drawn attention to a “feed microbiome” effect potentially impacting fish microbiome studies (Karlsen). We cannot rule out the possibility that variance in gut microbiome diversity seen could be an artifact of microbial DNA present in their digesta collected during sampling, and may not necessarily be representative of the gut mucosa-associated microbes (Karlsen 2022). Therefore, future zebrafish microbiome-targeted research should include gut intestinal and feed samples alongside fecal samples to account for a potential feed microbiome effect. It is important to note that while each of these diets have slightly different nutrient profiles to each other, they are far more consistent in composition to one another than the diets used in the previously mentioned analyses conducted around physiology, diet and the microbiome. Where previous studies tested more extreme ranges (e.g., high-fat diets), our study differs in that the three diets used are more consistent to one another. Together, we our results demonstrate that the gut microbiomes of 3-month-old zebrafish differ by diet, and highlights the importance of minor nutritional differences ability to affect the microbiome and physiology of zebrafish.

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

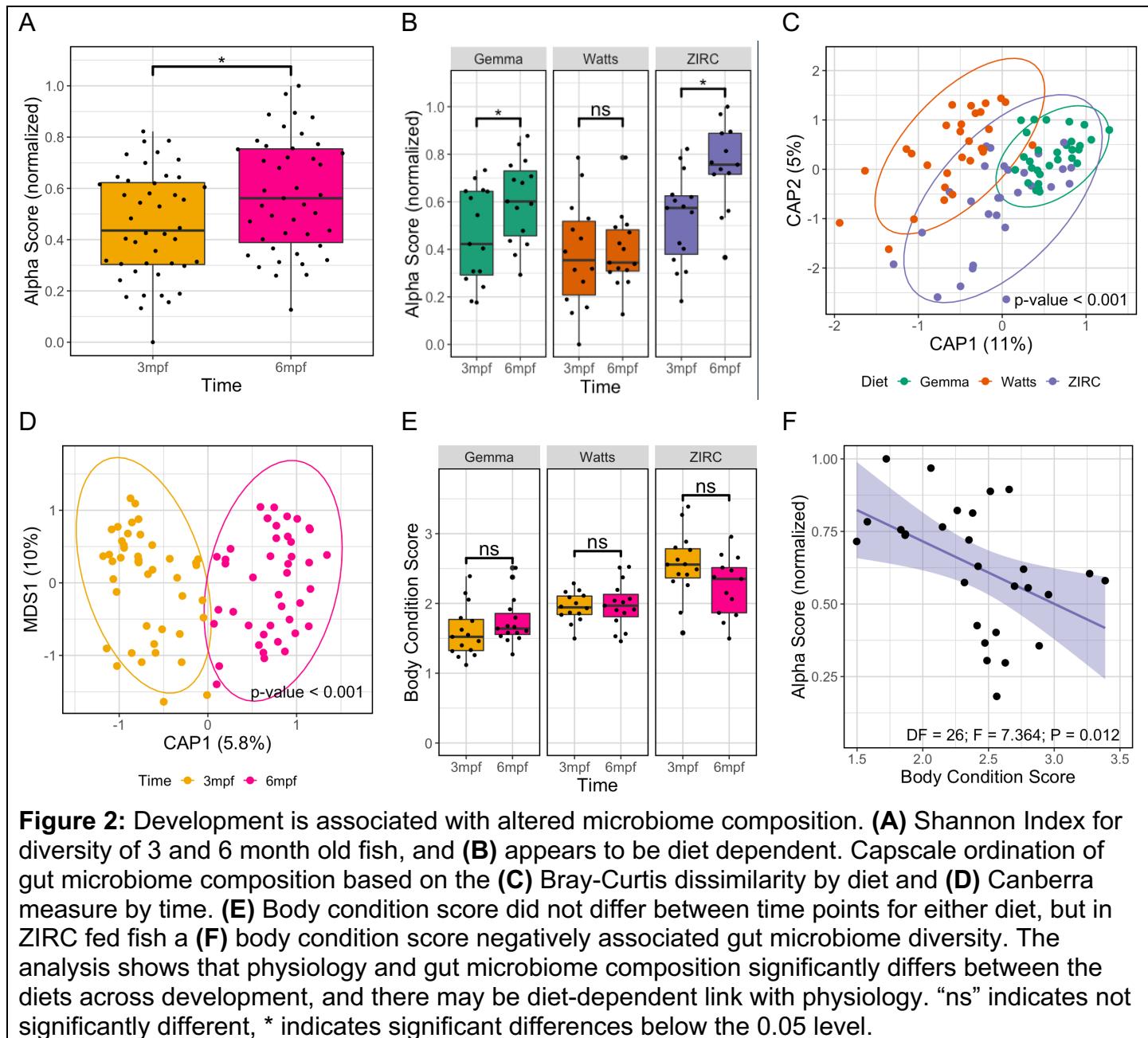


Figure 2: Development is associated with altered microbiome composition. **(A)** Shannon Index for diversity of 3 and 6 month old fish, and **(B)** appears to be diet dependent. Capscale ordination of gut microbiome composition based on the **(C)** Bray-Curtis dissimilarity by diet and **(D)** Canberra measure by time. **(E)** Body condition score did not differ between time points for either diet, but in ZIRC fed fish a **(F)** body condition score negatively associated gut microbiome diversity. 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. “ns” indicates not significantly different, * indicates significant differences below the 0.05 level.

<Results>

Given the associations we observed above between diet, the gut microbiome and physiology at 3 months of age, we next asked how microbiome structure and physiology differs between the diets across development at 6 months of age. Based on linear regression, we observed a statistically significant main effect of diet, time and an interaction effect between diet and time on gut microbiome diversity across all diversity indices ($p < 0.05$; Fig A&B, Table S2.2.2.1). A post hoc Tukey test showed microbiome diversity was significantly different between 3 and 6 months in Gemma and ZIRC fed fish in Shannon and Simpson’s Indices ($p < 0.05$; Table S2.2.2.3), but Watts microbiome diversity was not significantly different between 3 and 6 months. We next sought to determine if diet influences

microbiome composition across development. We find the microbiome community composition varies over time, but the temporal sensitivity of the abundant taxa in the microbiome is less than the sensitivity to diet. A PERMANOVA test using Bray-Curtis dissimilarity metric revealed that community composition was best explained by diet ($p < 0.05$; Fig 2C, Table S2.4.3), but Canberra measure found variation was best explained by time ($p < 0.05$; Fig 2D, Table S2.4.3). Within each diet, beta-dispersion significantly differed between 3 and 6 months in Gemma and ZIRC diets ($p < 0.05$; Fig S2.5.3), while Watts remained consistent between 3 and 6 months. An ANOVA test revealed significant beta-dispersion in metrics that emphasize abundant taxa (e.g., Bray-Curtis) and metrics rare taxa (e.g., Canberra) of ZIRC fed fish ($p < 0.05$; Fig S2.5.3), while Gemma had significant beta-dispersion among abundant taxa ($p < 0.05$; Fig S2.5.3). Finally, we used ANCOM-BC to determine if the abundance of taxa associated with development for each diet. We found 33 taxa that were significantly abundant at the genus levels in at least one diet between 3 and 6 months ($p < 0.05$; Table S2.6.1-2). Collectively, our results indicate that development differentially impacts fish gut microbiome structure depending on diet.

To determine if physiology differed between diets across development, we used Wilcoxon Signed-Ranks Tests to identify parameters that best explained the variation in body condition score. Body condition score did not significantly differ between time points across all diets ($p < 0.05$; Fig 2E, Table S2.1.1). We observed a significant interaction uniquely in ZIRC fed fish between gut microbiome diversity and body conditions score ($p < 0.05$; Fig 2F, Table S2.2.1). In ZIRC fed fish, body condition score negatively associates with an increase in microbiome diversity across development. A PERMANOVA test did not find a significant interaction effect of body condition score and diet (Table S2.2.2). Moreover, body condition score did not explain variation in taxa abundance. These results indicate that in the ZIRC diet there is a link between alpha diversity and body condition score across development.

<Discussion>

Prior work has shown that zebrafish gut microbiome is influenced early in life by stochastic processes (e.g., drift and dispersal from environment), but succession is increasingly governed by host development stages as fish age. Additionally, prior research has shown that high-fat and high-protein diets impact gut microbiome structure, but little is known how different commonly used zebrafish diets influence the successional development throughout adulthood. Here, we measured gut microbiome diversity, composition, and abundance at 3 and 6 months of age between fish fed one of three diets, and measured body condition score to assess impact to physiology.

We found the gut microbiome diversity increases and composition varies over time. Notably, in ZIRC and Gemma fed fish rare taxa appear more temporarily sensitive, and abundant taxa are sensitive to diet. Watts fed fish experienced less variation over time. At four months of age, all fish were switched from juvenile to adult diets. ZIRC adult feed incorporates several new feeds and minor changes to nutritional composition. Gemma adult feed changes in size but not nutrition. Watts feed decreases in lipid content, but the ingredients remain the same. Changes in nutritional composition can alter the microbiome (Rawls). Xiao and Stephens observed similar increases in gut microbiome diversity that they note may a result of dietary changes. Moreover, previously identified core, keystone taxa had diet dependent changes in abundance over time (Sharpton). Keystone taxa are believed to play important roles in maintaining gut microbiome homeostasis by digesting nutrients, fighting pathogens and communicating with the immune system (Xiao, Others?). Thus, if certain diets are disproportionately enriching for or against keystone taxa early in zebrafish development it could have long-term implications on microbiome succession, and possibly physiological outcomes. Indeed, we observed a link between physiology and microbiome diversity, where fish fed the ZIRC diet had higher gut diversity and lower body condition scores, and vice versa. One explanation is that higher diversity could drive more competition for resources and habitat space, which may prevent taxa from gaining a foothold and efficiently metabolizing nutrients. Additionally, we can't exclude the possibility that sex plays a role in defining these differences in body condition score and microbiome structure (Ma 2018). Taken together, these results demonstrate that diet's influence on early-life gut microbiome assembly can accumulate across development to impact gut microbiome succession, which may manifest differential physiological outcomes.

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

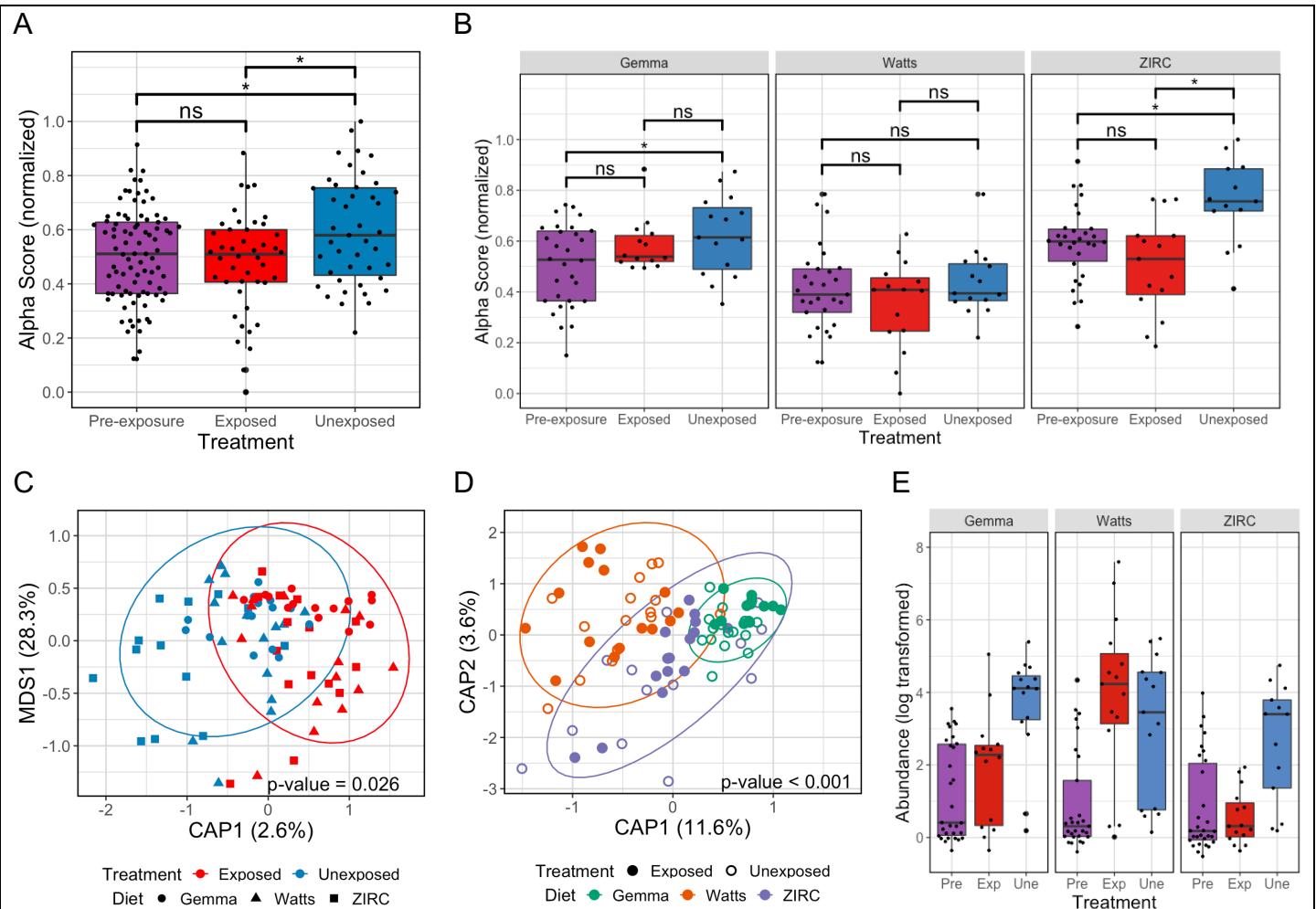


Figure 3: Exposure to *Mycobacterium chelonae* inhibits diversification of gut microbiome. **(A)** Shannon Index for diversity of pre-exposed 3-month-old fish, 6-month old exposed and unexposed fish, and **(B)** for exposure groups within each diet. Capscale ordination based on the Bray-Curtis dissimilarity of gut microbiome composition of 6-month-old fish by **(C)** exposure groups (exposed versus unexposed) and **(D)** diet. **(E)** Log observed abundances of *Mycobacterium* of pre-exposed, exposed and unexposed fish within each diet as calculated by ANCOM-BC. The analysis shows gut microbiome's sensitivity to pathogen exposure is linked to diet, but *Mycobacterium*'s abundance is diet-dependent. "ns" indicates not significantly different, and * indicates significant differences below the 0.05.

<Results>

Lastly, we sought to elucidate the potential interactions between the intestinal pathogen *Mycobacterium chelonae*, common laboratory diets and the gut microbiome. Briefly, after collecting fecal samples at 3 months old, we injected *Mycobacterium chelonae* into the coelomic cavity the fish in the exposed treatment group. Using linear regression, we find that microbiome diversity differs between exposure groups in Observed and Shannon indices ($P < 0.05$; Table S3.1.2.2), but we did not find a significant interaction effect between diet and exposure. The statistical effect of diet was far greatest across all diversity indices (Table S3.1.2.2). Furthermore, a post hoc Tukey test showed

microbiome diversity was significantly different in unexposed ZIRC fed fish between pre-exposed and exposed groups across all diversity metrics; and unexposed Gemma fish were significantly different to pre-exposed fish in Shannon index, and Unexposed watts fish were significantly different to exposed fish in Observed index ($P < 0.05$; Fig 3B, Table S3.1.2.3). Moreover, we assessed how pathogen exposure influenced microbiome composition across the diets. For all beta-diversity metrics, we find significant main effects of diet (Fig 3C) and pathogen exposure (Fig 3D); and we find interaction effects of diet and exposure group in Canberra ($P < 0.05$; Table S3.2.3). In all beta metrics, diet's statistical effect was greatest (Table S3.2.3). Finally, to determine if diet impacted *Mycobacterium* abundance we used ANCOM-BC. We find *Mycobacterium* taxa were significantly abundant in at least one group across the diet and exposure groups ($W = 26.6$, $Q < 0.001$; Fig 3 E, Table S3.4.1). *Mycobacterium* was present in pre-exposed groups at 3 months, and abundance increased in unexposed fish at 6 months ($W = 19$, $Q = 0.003$; Table S2.6). Relative to unexposed fish, we find that *Mycobacterium* had significantly decreased abundance in exposed fish in Gemma and ZIRC fed fish ($P < 0.05$; Table S3.4.2). We did not see a pathogen exposure effect on physiology. We also do not find a diet by exposure interaction with body condition score. Collectively, these results indicate that gut microbiomes of fish fed different diets vary in their sensitivity following pathogen exposure.

<Discussion>

We find that pathogen exposure inhibited diversification of gut microbiomes, and microbiome community composition was driven primarily by diet rather than pathogen exposure. The gut microbiome diversity of ZIRC fed fish is uniquely sensitive to pathogen exposure, while Gemma and Watts diet were more resistant. Interestingly, Mycobacterium's abundance differed between the diets. Exposed Watts fed fish had more Mycobacterium, but Exposed Gemma and ZIRC had fewer relative to controls. Higher gut microbiome diversity is linked to higher stability and greater ability to resist pathogens (Xiao, Gaulke?, Other?). Thus, it is possible Mycobacterium taxa might have been uniquely situated in Watts fed fish to take advantage of lower stability to gain habitat space. However, the effects of pathogen exposure on microbiome community composition were secondary to diet, and this might explain why our results differ from previous microbiome-pathogen studies that saw increased microbiome community variation following pathogen exposure (Gaulke, others?). We also saw the opposite effect of microbiome communities becoming more similar after exposure to Mycobacterium relative to controls. Three limitations to this study are 1) fish were injected with mycobacterium, which is not the natural route of transmission; 2) prior to injection all fish had Mycobacterium species present; and 3) we do not know the strain abundance of the Mycobacterium present in our samples. Thus, these factors could have hindered *M. chelonae*'s ability to successfully colonize exposed fish. Future research should attempt to expose zebrafish free of Mycobacterium using a natural route of transmission. Taken together, these data suggest that the microbiome might contribute to *M. chelonae* success depending on diet. It is unclear whether the microbiome exacerbates or protects zebrafish from pathogen exposure, but it illuminates the need for researchers to consider diet as a confounding factor that could alter the outcomes and interpretations of their study outcomes.

Conclusion

This study represents, to our knowledge, the first assessment to date of common laboratory diets long-term impact on host-pathogen-microbiome dynamics. We find that at 3 months old, fish fed different diets experience a difference in physiology and gut microbiome structure. These diet-associated differences accumulate through development at 6 months of age. We also find diet-dependent sensitivities of the gut microbiome to pathogen exposure. Together, these results demonstrate that diet and host health are intertwined with their microbiome's development and sensitivity to pathogen exposure.

It may be worth establishing a standard reference diet for microbiome-targeted zebrafish studies to improve our understanding of zebrafish health and nutrition, advance knowledge of how the diet and microbiome interact, and support efforts towards reproducibility and interpretability of results across studies. However, we do not suggest that one diet here is preferred for microbiome-targeted studies. Rather, zebrafish diets may benefit from a variety of diets to model the variation in diets and microbiomes we see in human populations. One important challenge to establishing a standard reference diet is its ability to be made germ-free and nutritionally equivalent to conventional diets (Rawls). Significant progress is being made on this front and supports efforts to better understand the connection between diet and the microbiome in zebrafish (Rawls, Watts).

Collectively, our results indicate that researchers should carefully consider the role of diet in their zebrafish microbiome studies and that diet should be controlled for when integrating microbiome data across studies.

Supplementary Tables and Figures

1) Diet

1.1) Physiology

Wilcoxon Testp. adj: BH. Weight ~ Diet

.y.	group1	group2	n1	n2	statistic	p	p.adj	p.adj.signif
Weight	Gemma	Watts	30	29	528.500	0.158	0.158	ns
	Gemma	ZIRC	30	30	301.500	0.029	0.044	*
	Watts	ZIRC	29	30	225.500	0.002	0.006	**

1.1.1)

Wilcoxon Testp. adj: BH. Weight ~ Sex

.y.	group1	group2	n1	n2	statistic	p	p.adj	p.adj.signif
Weight	F	M	50	39	1,505.500	<0.001	<0.001	****

1.1.2)

Wilcoxon Testp. adj: BH. Body.Condition.Score ~ Diet

.y.	group1	group2	n1	n2	statistic	p	p.adj	p.adj.signif
Body.Condition.Score	Gemma	Watts	30	29	327.000	0.103	0.103	ns
	Gemma	ZIRC	30	30	150.000	<0.001	<0.001	****
	Watts	ZIRC	29	30	167.000	<0.001	<0.001	****

1.1.3)

Wilcoxon Testp. adj: BH. Body.Condition.Score ~ Sex

.y.	group1	group2	n1	n2	statistic	p	p.adj	p.adj.signif
Body.Condition.Score	F	M	50	39	1,631.000	<0.001	<0.001	****

1.1.4)

1.2) Alpha Diversity

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

metric	term	estimate	std.error	statistic	p.value	sig
Observed	(Intercept)	-0.293	0.134	-2.191	0.031	*
	DietWatts	-0.917	0.209	-4.390	<0.001	*
	DietZIRC	0.143	0.189	0.760	0.449	
Shannon	(Intercept)	-0.413	0.149	-2.773	0.007	*
	DietWatts	-0.492	0.221	-2.224	0.029	*
	DietZIRC	0.429	0.208	2.058	0.043	*
Simpson	(Intercept)	-0.283	0.155	-1.821	0.072	
	DietWatts	0.243	0.220	1.101	0.274	
	DietZIRC	0.795	0.222	3.579	<0.001	*

1.2.1)

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

metric	term	statistic	df	p.value	sig
Observed	Diet	31.207	2	<0.001	*
Shannon	Diet	18.217	2	<0.001	*
Simpson	Diet	13.692	2	0.001	*

1.2.2)

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	-0.917	0.209	-4.390	0.000	*
	Diet	Alpha.Score	ZIRC	Gemma	0.143	0.189	0.760	0.727	ns
	Diet	Alpha.Score	ZIRC	Watts	1.060	0.208	5.093	0.000	*
Shannon	Diet	Alpha.Score	Watts	Gemma	-0.492	0.221	-2.224	0.067	ns
	Diet	Alpha.Score	ZIRC	Gemma	0.429	0.208	2.058	0.099	ns
	Diet	Alpha.Score	ZIRC	Watts	0.920	0.219	4.202	0.000	*
Simpson	Diet	Alpha.Score	Watts	Gemma	0.243	0.220	1.101	0.513	ns
	Diet	Alpha.Score	ZIRC	Gemma	0.795	0.222	3.579	0.001	*
	Diet	Alpha.Score	ZIRC	Watts	0.552	0.223	2.478	0.035	*

1.2.3)

1.3) Beta Diversity

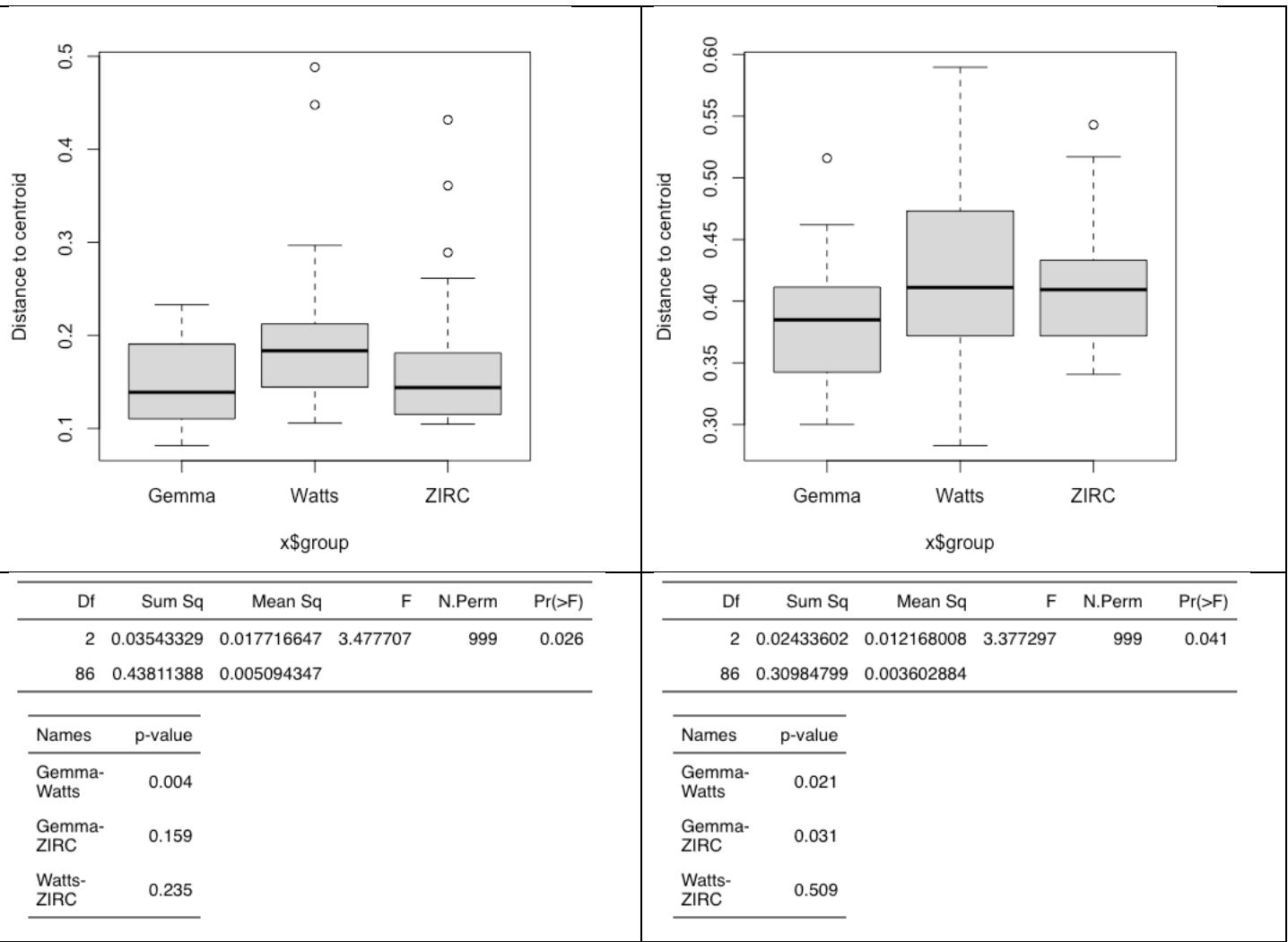
metric	term	Df	SumOfSqs	statistic	p.value	sig
Bray-Curtis	Diet	2.00	1.099108	13.949	0.001	*
	Residual	86.00	3.388087			
Canberra	Diet	2.00	3.207838	9.126	0.001	*
	Residual	86.00	15.115341			
Sørensen	Diet	2.00	1.459833	12.970	0.001	*
	Residual	86.00	4.839717			

1.3.1)

1.4) Beta-Dispersion

1.4.1) Diet

Bray-Curtis	Canberra
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2) Development

2.1) Physiology

Wilcoxon Testp. adj: BH. Body.Condition.Score ~ Diet:Time

Diet	.y.	group1	group2	n1	n2	statistic	p	p.adj	p.adj.signif
Gemma	Body.Condition.Score	3mpf	6mpf	15	15	78.000	0.161	0.242	ns
Watts	Body.Condition.Score	3mpf	6mpf	14	15	99.000	0.813	0.813	ns
ZIRC	Body.Condition.Score	3mpf	6mpf	15	13	134.000	0.098	0.242	ns

2.1.1)

2.2) Physiology ~ Microbiome

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

metric	.y.	term	statistic	df	p.value
Observed	Alpha.Score	Body Condition Score (ZIRC)	3.846	1	0.050
Shannon	Alpha.Score	Body Condition Score (ZIRC)	7.372	1	0.007
Simpson	Alpha.Score	Body Condition Score (ZIRC)	9.918	1	0.002

2.2.1)

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	0.8945429	8.698	0.001	*
	Body.Condition.Score	1.00	0.0688714	1.339	0.205	
	Body.Condition.Score:Diet	2.00	0.1227086	1.193	0.251	
	Residual	81.00	4.1651051			
Canberra	Diet	2.00	2.1839598	5.004	0.001	*
	Body.Condition.Score	1.00	0.3546246	1.625	0.019	*
	Body.Condition.Score:Diet	2.00	0.5359131	1.228	0.092	
	Residual	81.00	17.6756651			
Sørensen	Diet	2.00	1.1954593	7.229	0.001	*
	Body.Condition.Score:Diet	2.00	0.2402242	1.453	0.064	
	Body.Condition.Score	1.00	0.1100171	1.331	0.168	
	Residual	81.00	6.6975226			

2.2.2)

2.3) Alpha Diversity

2.3.1) Time

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

metric	term	estimate	std.error	statistic	p.value	sig
Observed	(Intercept)	0.018	0.120	0.147	0.883	
	Timepoint6mpf	0.337	0.172	1.954	0.054	
Shannon	(Intercept)	-0.181	0.131	-1.375	0.173	
	Timepoint6mpf	0.480	0.188	2.560	0.012	*
Simpson	(Intercept)	-0.370	0.132	-2.812	0.006	*
	Timepoint6mpf	0.440	0.186	2.370	0.020	*

2.3.1.1)

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

metric	term	statistic	df	p.value	sig
Observed	Timepoint	3.834	1	0.050	
Shannon	Timepoint	6.603	1	0.010	*
Simpson	Timepoint	5.651	1	0.017	*

2.3.1.2)

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	6mpf	3mpf	0.337	0.172	1.954	0.051	ns
Shannon	Timepoint	Alpha.Score	6mpf	3mpf	0.480	0.188	2.560	0.010	*
Simpson	Timepoint	Alpha.Score	6mpf	3mpf	0.440	0.186	2.370	0.018	*

2.2.2) Time:Diet

2.2.1.3)

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

metric	term	estimate	std.error	statistic	p.value	sig
Observed	(Intercept)	0.344	0.184	1.872	0.065	
	DietWatts	-0.827	0.266	-3.109	0.003	*
	DietZIRC	-0.189	0.258	-0.731	0.467	
	Timepoint6mpf	0.115	0.261	0.440	0.661	
	DietWatts:Timepoint6mpf	0.079	0.372	0.213	0.832	
	DietZIRC:Timepoint6mpf	0.792	0.388	2.042	0.044	*
Shannon	(Intercept)	-0.175	0.203	-0.864	0.390	
	DietWatts	-0.322	0.296	-1.090	0.279	
	DietZIRC	0.276	0.286	0.966	0.337	
	Timepoint6mpf	0.606	0.289	2.096	0.039	*
	DietWatts:Timepoint6mpf	-0.546	0.416	-1.314	0.193	
	DietZIRC:Timepoint6mpf	0.378	0.432	0.874	0.384	
Simpson	(Intercept)	-0.718	0.222	-3.234	0.002	*
	DietWatts	0.387	0.312	1.243	0.218	
	DietZIRC	0.640	0.305	2.100	0.039	*
	Timepoint6mpf	0.693	0.305	2.277	0.025	*
	DietWatts:Timepoint6mpf	-0.770	0.431	-1.786	0.078	
	DietZIRC:Timepoint6mpf	0.142	0.440	0.323	0.748	

2.2.2.1)

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

metric	term	statistic	df	p.value	sig
Observed	Diet	29.866	2	<0.001	*
	Timepoint	6.137	1	0.013	*
	Diet:Timepoint	4.972	2	0.083	
Shannon	Diet	24.198	2	<0.001	*
	Timepoint	9.728	1	0.002	*
	Diet:Timepoint	4.582	2	0.101	
Simpson	Diet	13.541	2	0.001	*
	Timepoint	7.342	1	0.007	*
	Diet:Timepoint	5.077	2	0.079	

2.2.2.2)

2.2.2.3)

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

metric	Diet	.y.	term	group1	group2	estimate	std.error	statistic	p.adj	p.adj.signif
Observed	Gemma	Alpha.Score	Timepoint	6mpf	3mpf	0.115	0.224	0.512	0.608310918	ns
	Watts	Alpha.Score	Timepoint	6mpf	3mpf	0.194	0.274	0.710	0.477616760	ns
	ZIRC	Alpha.Score	Timepoint	6mpf	3mpf	0.907	0.317	2.863	0.004202217	*
Shannon	Gemma	Alpha.Score	Timepoint	6mpf	3mpf	0.606	0.282	2.151	0.031444735	*
	Watts	Alpha.Score	Timepoint	6mpf	3mpf	0.060	0.302	0.199	0.841967493	ns
	ZIRC	Alpha.Score	Timepoint	6mpf	3mpf	0.984	0.325	3.024	0.002490914	*
Simpson	Gemma	Alpha.Score	Timepoint	6mpf	3mpf	0.693	0.324	2.143	0.032142661	*
	Watts	Alpha.Score	Timepoint	6mpf	3mpf	-0.077	0.280	-0.273	0.784806376	ns
	ZIRC	Alpha.Score	Timepoint	6mpf	3mpf	0.836	0.322	2.598	0.009388431	*

2.4) Beta Diversity

metric	term	Df	SumOfSqs	statistic	p.value	sig
Bray-Curtis	Diet	2.00	0.8395202	7.992	0.001	*
	Residual	84.00	4.4117078			
Canberra	Diet	2.00	2.2323578	5.063	0.001	*
	Residual	84.00	18.5178049			
Sørensen	Diet	2.00	1.2197670	7.294	0.001	*
	Residual	84.00	7.0234562			

2.4.1)

metric	term	Df	SumOfSqs	statistic	p.value	sig
Bray-Curtis	Timepoint	1.00	0.2568805	4.372	0.001	*
	Residual	85.00	4.9943475			
Canberra	Timepoint	1.00	1.2060007	5.245	0.001	*
	Residual	85.00	19.5441621			
Sørensen	Timepoint	1.00	0.8039921	9.186	0.001	*
	Residual	85.00	7.4392311			

2.4.2)

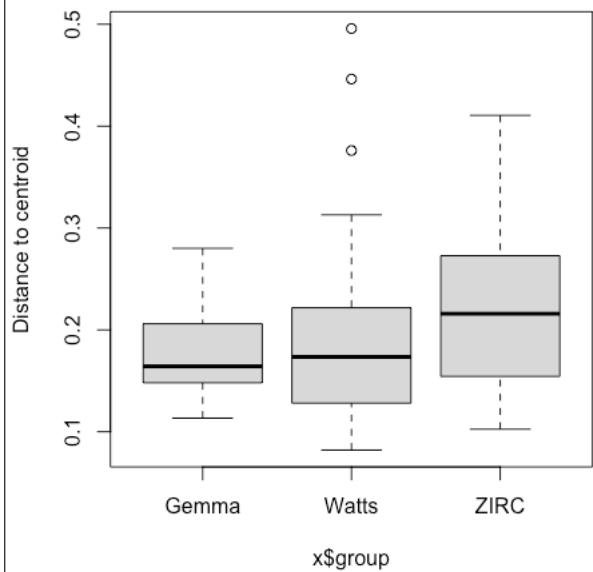
metric	term	Df	SumOfSqs	statistic	p.value	sig
Bray-Curtis	Diet	2.00	0.8395202	8.564	0.001	*
	Timepoint	1.00	0.2635537	5.377	0.001	*
	Diet:Timepoint	2.00	0.1781783	1.818	0.039	*
	Residual	81.00	3.9699758			
Canberra	Timepoint	1.00	1.2121772	6.004	0.001	*
	Diet	2.00	2.2323578	5.528	0.001	*
	Diet:Timepoint	2.00	0.9517175	2.357	0.001	*
	Residual	81.00	16.3539102			
Sørensen	Timepoint	1.00	0.8051774	11.176	0.001	*
	Diet	2.00	1.2197670	8.465	0.001	*
	Diet:Timepoint	2.00	0.3823789	2.654	0.001	*
	Residual	81.00	5.8359000			

2.4.3)

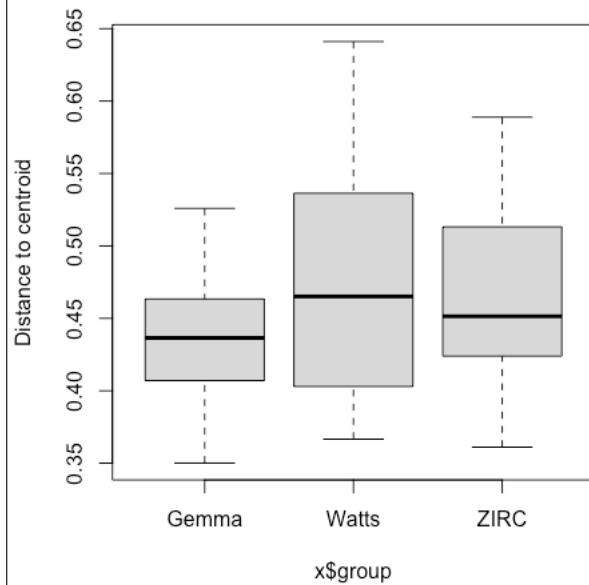
2.5) Beta-Dispersion

2.5.1) Diet

Bray-Curtis



Canberra



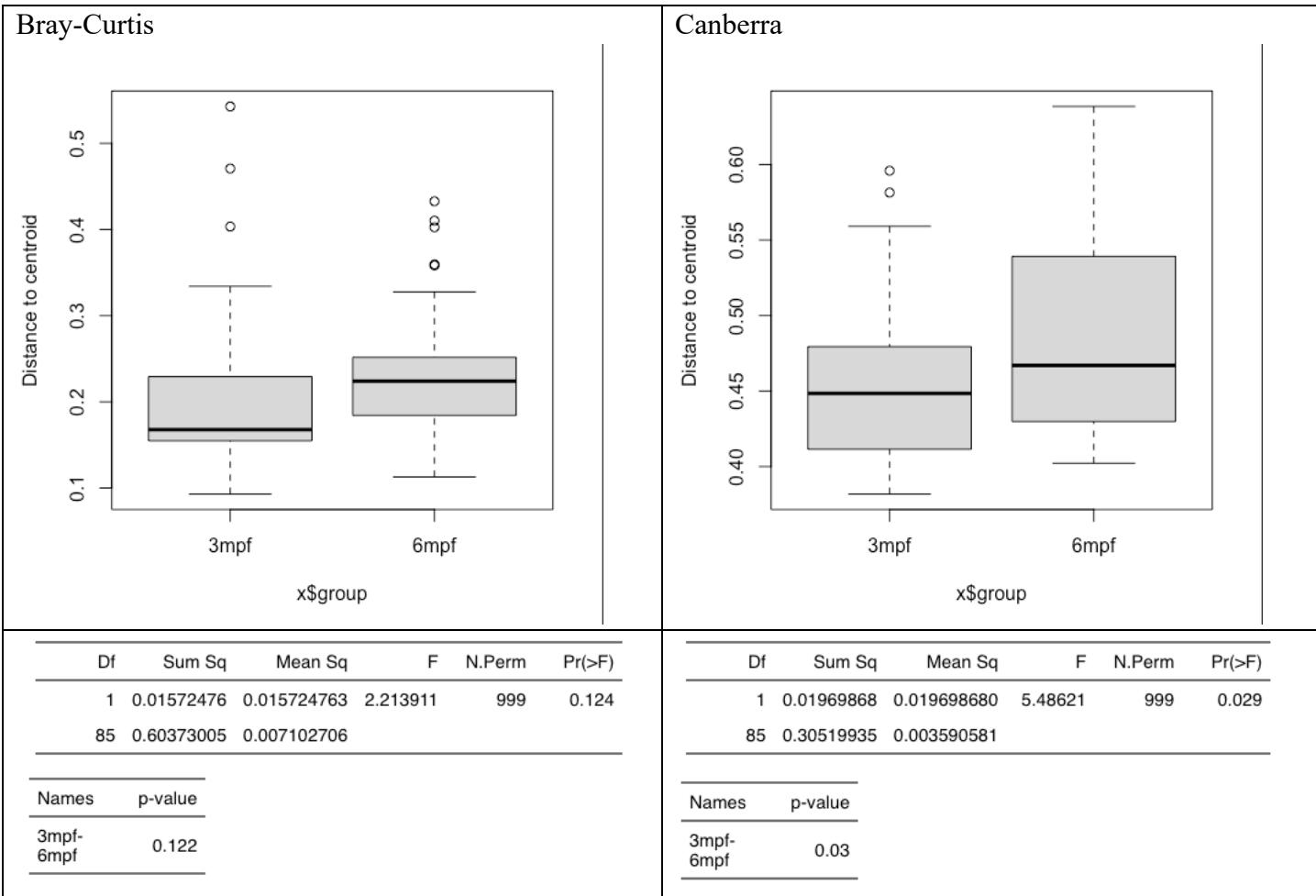
Df	Sum Sq	Mean Sq	F	N.Perm	Pr(>F)
2	0.03382856	0.016914278	2.612413	999	0.084
84	0.54386480	0.006474581			

Df	Sum Sq	Mean Sq	F	N.Perm	Pr(>F)
2	0.02089333	0.010446664	2.800836	999	0.051
84	0.31330632	0.003729837			

Names	p-value
Gemma-Watts	0.229
Gemma-ZIRC	0.012
Watts-ZIRC	0.374

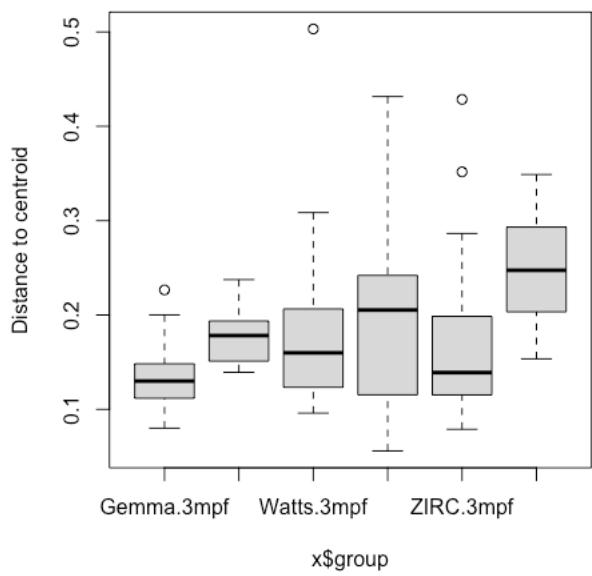
Names	p-value
Gemma-Watts	0.035
Gemma-ZIRC	0.019
Watts-ZIRC	0.886

2.5.2) Time

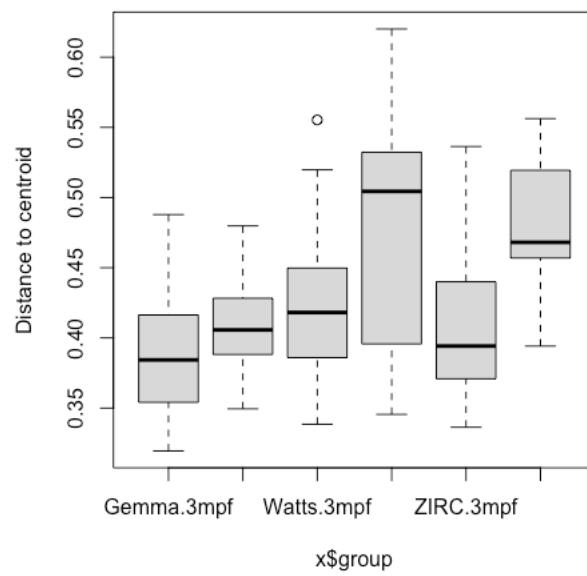


2.5.3) Diet:Time

Bray-Curtis



Canberra



Df	Sum Sq	Mean Sq	F	N.Perm	Pr(>F)
5	0.09043205	0.018086411	2.778924	999	0.016
81	0.52718214	0.006508421			

Names	p-value
Gemma.3mpf-ZIRC.6mpf	0.001
Gemma.6mpf-ZIRC.6mpf	0.001
Gemma.3mpf-Gemma.6mpf	0.004
ZIRC.3mpf-ZIRC.6mpf	0.028
Gemma.3mpf-Watts.6mpf	0.061
Watts.3mpf-ZIRC.6mpf	0.077
Gemma.3mpf-Watts.3mpf	0.115
Watts.6mpf-ZIRC.6mpf	0.136
Gemma.3mpf-ZIRC.3mpf	0.146

Df	Sum Sq	Mean Sq	F	N.Perm	Pr(>F)
5	0.09326318	0.018652636	5.333197	999	0.001
81	0.28329414	0.003497458			

Names	p-value
Gemma.3mpf-ZIRC.6mpf	0.001
Gemma.6mpf-ZIRC.6mpf	0.002
ZIRC.3mpf-ZIRC.6mpf	0.002
Gemma.3mpf-Watts.6mpf	0.006
Watts.6mpf-ZIRC.3mpf	0.015
Gemma.6mpf-Watts.6mpf	0.016
Watts.3mpf-ZIRC.6mpf	0.020
Watts.3mpf-Watts.6mpf	0.078
Gemma.3mpf-Watts.3mpf	0.154

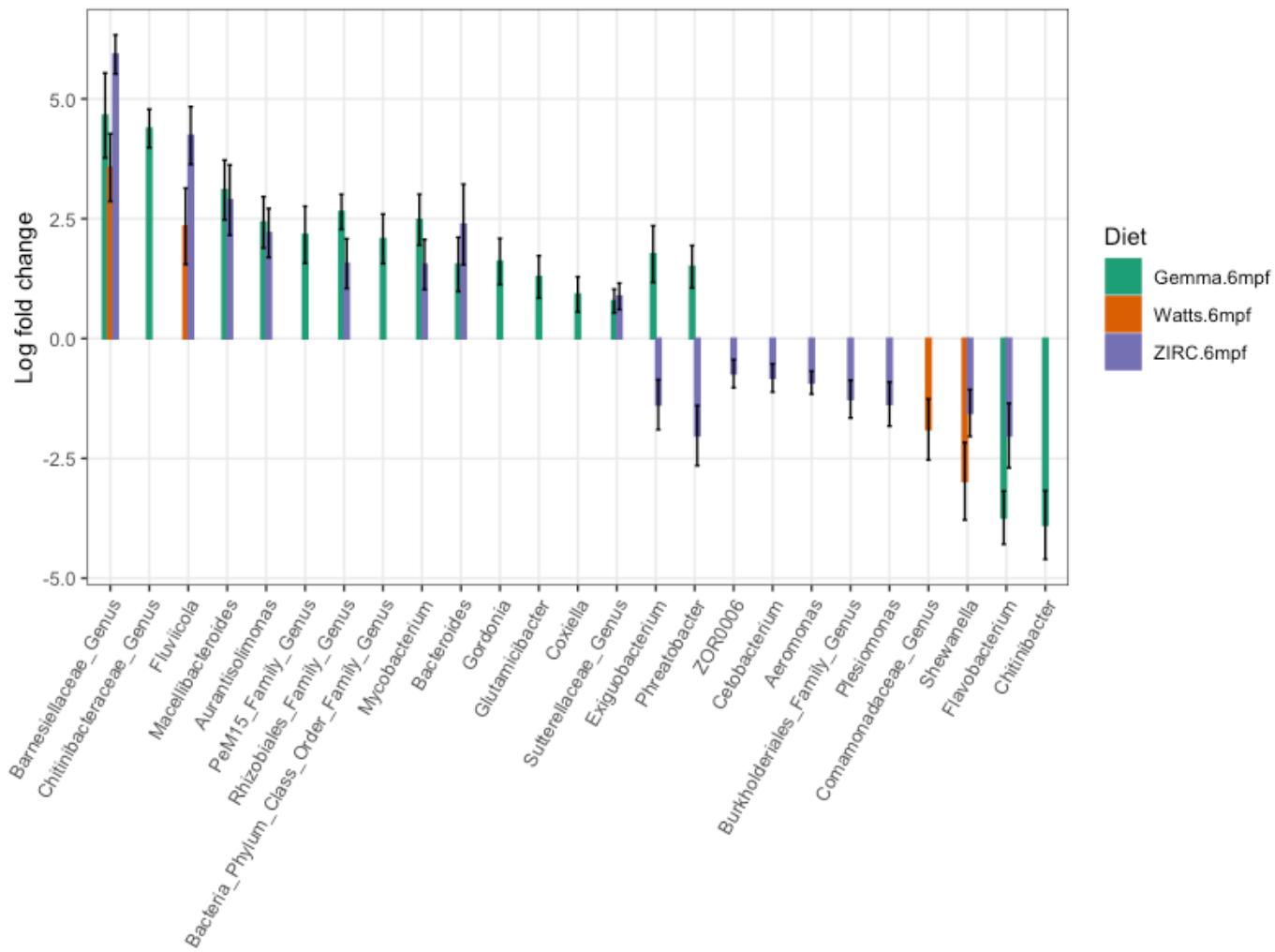
2.6 Differential Abundance

Taxon		W	p_val	q_val	diff_abn
	Mycobacterium	19.054	0.002	0.003	TRUE
	Longivirga	50.592	<0.001	<0.001	TRUE
	Glutamicibacter	48.228	<0.001	<0.001	TRUE
Bacteria_Phylum_Class_Order_Family_Genus		22.139	<0.001	<0.001	TRUE
	Bacteroides	16.514	0.005	0.008	TRUE
	Macellibacteroides	42.534	<0.001	<0.001	TRUE
	Aurantisolimonas	44.844	<0.001	<0.001	TRUE
	Fluviicola	54.593	<0.001	<0.001	TRUE
	Flavobacterium	66.064	<0.001	<0.001	TRUE
	Cloacibacterium	19.805	0.001	0.002	TRUE
	ZOR0006	61.705	<0.001	<0.001	TRUE
	Exiguobacterium	29.965	<0.001	<0.001	TRUE
	Peptostreptococcus	41.515	<0.001	<0.001	TRUE
	Cetobacterium	24.718	<0.001	<0.001	TRUE
	Rhizobiaceae_Genus	15.964	0.006	0.010	TRUE
	Phreatobacter	32.393	<0.001	<0.001	TRUE
Rhizobiales_Family_Genus		39.652	<0.001	<0.001	TRUE
	Gemmobacter	15.396	0.008	0.013	TRUE
	Aeromonas	41.677	<0.001	<0.001	TRUE
	Shewanella	64.916	<0.001	<0.001	TRUE
	Chitinibacter	711.743	<0.001	<0.001	TRUE
	Chitinibacteraceae_Genus	73.999	<0.001	<0.001	TRUE
	Crenobacter	129.621	<0.001	<0.001	TRUE
Comamonadaceae_Genus		25.220	<0.001	<0.001	TRUE
	Paucibacter	22.701	<0.001	<0.001	TRUE
	Sutterellaceae_Genus	36.712	<0.001	<0.001	TRUE
	Cellvibrio	35.572	<0.001	<0.001	TRUE
	Plesiomonas	63.616	<0.001	<0.001	TRUE
Gammaproteobacteria_Order_Family_Genus		14.404	0.012	0.019	TRUE
	Acinetobacter	30.056	<0.001	<0.001	TRUE
	Pseudomonas	13.527	0.018	0.026	TRUE
Parachlamydiaceae_Genus		41.188	<0.001	<0.001	TRUE
	Cerasicoccus	13.539	0.018	0.026	TRUE

2.6.1)

2.6.2)

Waterfall Plot of Diet:Time (Genus)



3) Exposure

3.1) Alpha Diversity

3.1.1) Exposure

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

metric	term	estimate	std.error	statistic	p.value	sig
	(Intercept)	0.055	0.076	0.724	0.470	
Observed	PrePostExpExposed	-0.152	0.133	-1.140	0.256	
	PrePostExpUnexposed	0.347	0.136	2.558	0.011	*
	(Intercept)	0.012	0.078	0.157	0.875	
Shannon	PrePostExpExposed	-0.098	0.135	-0.726	0.469	
	PrePostExpUnexposed	0.385	0.138	2.789	0.006	*
	(Intercept)	-0.271	0.083	-3.264	0.001	*
Simpson	PrePostExpExposed	-0.113	0.145	-0.779	0.437	
	PrePostExpUnexposed	0.341	0.145	2.357	0.020	*

3.1.1.1)

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

metric	term	statistic	df	p.value	sig
Observed	PrePostExp	10.997	2	0.004	*
Shannon	PrePostExp	10.878	2	0.004	*
Simpson	PrePostExp	8.278	2	0.016	*

3.1.1.2)

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	-0.152	0.133	-1.140	0.487	ns
	PrePostExp	Alpha.Score	Unexposed	Pre-exposure	0.347	0.136	2.558	0.028	*
	PrePostExp	Alpha.Score	Unexposed	Exposed	0.499	0.156	3.192	0.004	*
Shannon	PrePostExp	Alpha.Score	Exposed	Pre-exposure	-0.098	0.135	-0.726	0.747	ns
	PrePostExp	Alpha.Score	Unexposed	Pre-exposure	0.385	0.138	2.789	0.014	*
	PrePostExp	Alpha.Score	Unexposed	Exposed	0.484	0.159	3.041	0.007	*
Simpson	PrePostExp	Alpha.Score	Exposed	Pre-exposure	-0.113	0.145	-0.779	0.715	ns
	PrePostExp	Alpha.Score	Unexposed	Pre-exposure	0.341	0.145	2.357	0.048	*
	PrePostExp	Alpha.Score	Unexposed	Exposed	0.454	0.168	2.703	0.019	*

3.1.1.3)

3.1.2) Diet:Exposure

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

metric	term	estimate	std.error	statistic	p.value	sig
Observed	(Intercept)	0.283	0.116	2.439	0.016	*
	DietWatts	-0.992	0.167	-5.936	<0.001	*
	DietZIRC	0.014	0.163	0.083	0.934	
	ExposureUnexposed	0.140	0.164	0.855	0.394	
	DietWatts:ExposureUnexposed	0.326	0.234	1.393	0.165	
	DietZIRC:ExposureUnexposed	0.140	0.234	0.597	0.551	
Shannon	(Intercept)	0.198	0.126	1.573	0.118	
	DietWatts	-0.721	0.179	-4.019	<0.001	*
	DietZIRC	0.075	0.177	0.423	0.673	
	ExposureUnexposed	0.036	0.177	0.206	0.837	
	DietWatts:ExposureUnexposed	0.218	0.252	0.862	0.390	
	DietZIRC:ExposureUnexposed	0.292	0.254	1.148	0.253	
Simpson	(Intercept)	-0.362	0.144	-2.513	0.013	*
	DietWatts	-0.073	0.203	-0.358	0.720	
	DietZIRC	0.315	0.200	1.573	0.118	
	ExposureUnexposed	0.001	0.202	0.007	0.995	
	DietWatts:ExposureUnexposed	0.063	0.286	0.220	0.826	
	DietZIRC:ExposureUnexposed	0.341	0.285	1.196	0.233	

3.1.2.1)

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

metric	term	statistic	df	p.value	sig
Observed	Diet	75.452	2	<0.001	*
	Exposure	9.434	1	0.002	*
	Diet:Exposure	1.953	2	0.377	
Shannon	Diet	46.450	2	<0.001	*
	Exposure	3.852	1	0.050	*
	Diet:Exposure	1.438	2	0.487	
Simpson	Diet	16.772	2	<0.001	*
	Exposure	1.377	1	0.241	
	Diet:Exposure	1.623	2	0.444	

3.1.2.2)

3.1.2.3)

Pairwise Tukey's HSD, p.adj: Dunnett. $\text{glm}(\text{Alpha.Score} \sim \text{Diet} * \text{Exposure})$, 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	0.197	0.177	1.108	0.5067172005	ns
	Gemma	Alpha.Score	PrePostExp	Unexposed	Pre-exposure	0.225	0.174	1.296	0.3951699726	ns
	Gemma	Alpha.Score	PrePostExp	Unexposed	Exposed	0.028	0.205	0.139	0.9893749979	ns
	Watts	Alpha.Score	PrePostExp	Exposed	Pre-exposure	-0.397	0.198	-2.006	0.1097629119	ns
	Watts	Alpha.Score	PrePostExp	Unexposed	Pre-exposure	0.284	0.187	1.518	0.2809402190	ns
	Watts	Alpha.Score	PrePostExp	Unexposed	Exposed	0.681	0.223	3.057	0.0061217113	*
	ZIRC	Alpha.Score	PrePostExp	Exposed	Pre-exposure	-0.239	0.218	-1.097	0.5128253758	ns
	ZIRC	Alpha.Score	PrePostExp	Unexposed	Pre-exposure	0.710	0.252	2.822	0.0129985294	*
	ZIRC	Alpha.Score	PrePostExp	Unexposed	Exposed	0.949	0.280	3.386	0.0020551850	*
	Gemma	Alpha.Score	PrePostExp	Exposed	Pre-exposure	0.313	0.197	1.584	0.2507345213	ns
Shannon	Gemma	Alpha.Score	PrePostExp	Unexposed	Pre-exposure	0.482	0.195	2.470	0.0358379651	*
	Gemma	Alpha.Score	PrePostExp	Unexposed	Exposed	0.169	0.230	0.737	0.7396581311	ns
	Watts	Alpha.Score	PrePostExp	Exposed	Pre-exposure	-0.237	0.217	-1.092	0.5172164942	ns
	Watts	Alpha.Score	PrePostExp	Unexposed	Pre-exposure	0.116	0.212	0.550	0.8456466801	ns
	Watts	Alpha.Score	PrePostExp	Unexposed	Exposed	0.353	0.247	1.431	0.3231294133	ns
	ZIRC	Alpha.Score	PrePostExp	Exposed	Pre-exposure	-0.345	0.217	-1.589	0.2476774623	ns
	ZIRC	Alpha.Score	PrePostExp	Unexposed	Pre-exposure	0.751	0.254	2.963	0.0084755914	*
	ZIRC	Alpha.Score	PrePostExp	Unexposed	Exposed	1.096	0.282	3.892	0.0003129239	*
	Gemma	Alpha.Score	PrePostExp	Exposed	Pre-exposure	0.284	0.249	1.138	0.4890374427	ns
	Gemma	Alpha.Score	PrePostExp	Unexposed	Pre-exposure	0.547	0.242	2.261	0.0610152077	ns
Simpson	Gemma	Alpha.Score	PrePostExp	Unexposed	Exposed	0.264	0.282	0.934	0.6170860681	ns
	Watts	Alpha.Score	PrePostExp	Exposed	Pre-exposure	-0.127	0.230	-0.550	0.8456815725	ns
	Watts	Alpha.Score	PrePostExp	Unexposed	Pre-exposure	-0.049	0.229	-0.214	0.9750433810	ns
	Watts	Alpha.Score	PrePostExp	Unexposed	Exposed	0.078	0.264	0.294	0.9531833954	ns
	ZIRC	Alpha.Score	PrePostExp	Exposed	Pre-exposure	-0.476	0.241	-1.974	0.1172492161	ns
	ZIRC	Alpha.Score	PrePostExp	Unexposed	Pre-exposure	0.657	0.263	2.502	0.0324305223	*
	ZIRC	Alpha.Score	PrePostExp	Unexposed	Exposed	1.133	0.299	3.794	0.0004999882	*

3.2) Beta Diversity

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

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

3.2.1)

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

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

3.2.2)

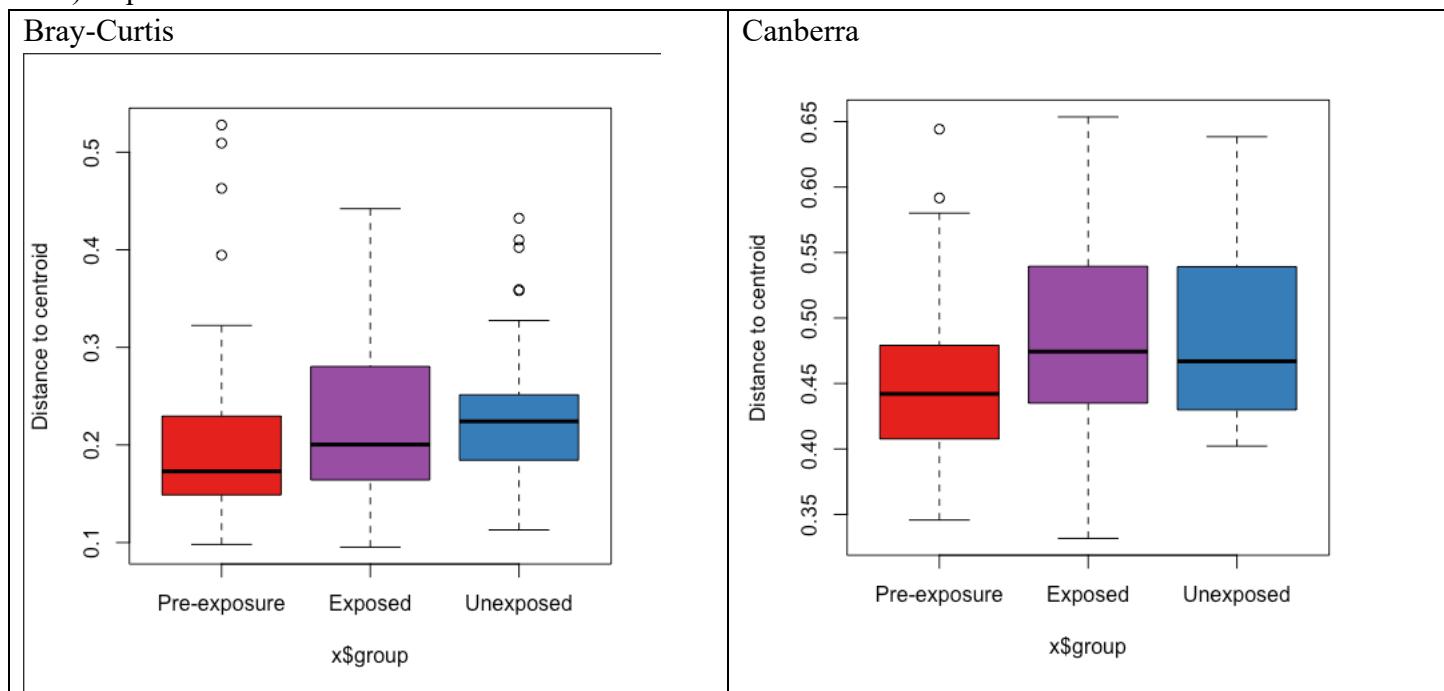
Distance-based redundancy analysis (dbRDA) ordination. Beta.Score ~ Diets

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

3.2.3

3.3) Beta-Dispersion

3.3.1) Exposure

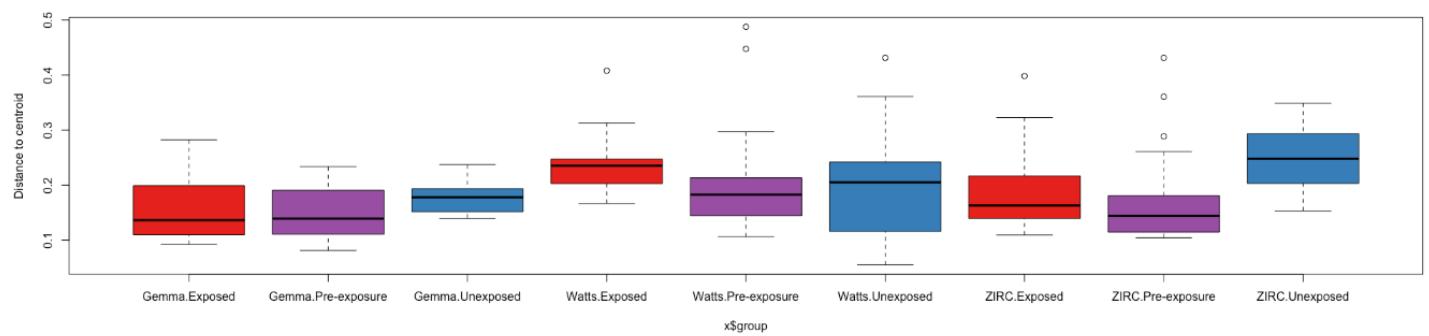


Df	Sum Sq	Mean Sq	F	N.Perm	Pr(>F)
2	0.03124468	0.015622342	2.464166	999	0.088
173	1.09678687	0.006339808			
<hr/>					
Names	p-value				
Pre-exposure-Exposed	0.128				
Pre-exposure-Unexposed	0.051				
Exposed-Unexposed	0.799				

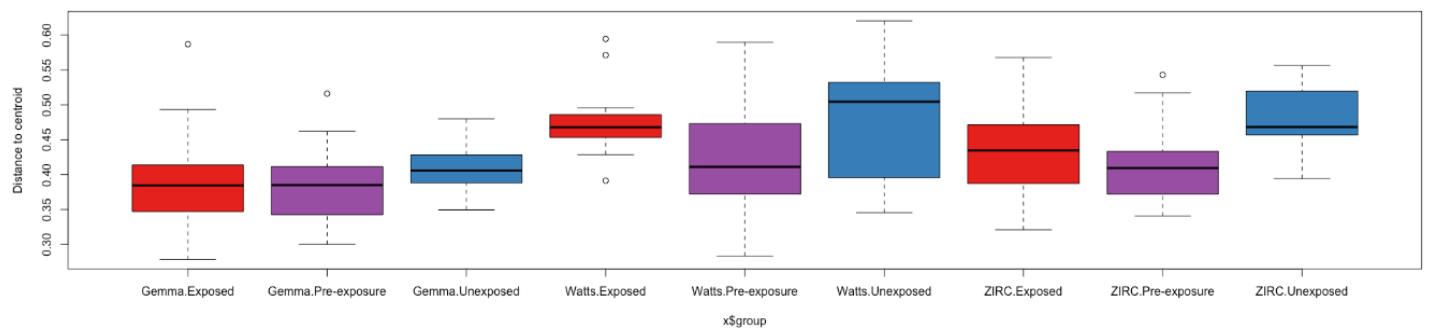
Df	Sum Sq	Mean Sq	F	N.Perm	Pr(>F)
2	0.04742029	0.023710144	6.02015	999	0.003
173	0.68135428	0.003938464			
<hr/>					
Names	p-value				
Pre-exposure-Exposed	0.012				
Pre-exposure-Unexposed	0.002				
Exposed-Unexposed	0.741				

3.3.2) Diet:Exposure

Bray-Curtis



Canberra



Bray-Curtis

Df	Sum Sq	Mean Sq	F	N.Perm	Pr(>F)
8	0.1710853	0.021385661	4.247249	999	0.001
167	0.8408750	0.005035179			
<hr/>					

Canberra

Df	Sum Sq	Mean Sq	F	N.Perm	Pr(>F)
8	0.1775982	0.022199773	5.662318	999	0.001
167	0.6547429	0.003920616			
<hr/>					

Names	p-value	sig	Names	p-value	sig
Gemma.Exposed-ZIRC.Unexposed	0.001	*	Gemma.Pre-exposure-Watts.Exposed	0.001	*
Gemma.Pre-exposure-Watts.Exposed	0.001	*	Gemma.Pre-exposure-Watts.Unexposed	0.001	*
Gemma.Pre-exposure-ZIRC.Unexposed	0.001	*	Gemma.Pre-exposure-ZIRC.Unexposed	0.001	*
Gemma.Unexposed-ZIRC.Unexposed	0.001	*	Gemma.Unexposed-Watts.Exposed	0.001	*
Gemma.Exposed-Watts.Exposed	0.002	*	Watts.Exposed-ZIRC.Pre-exposure	0.001	*
Gemma.Unexposed-Watts.Exposed	0.003	*	Gemma.Unexposed-ZIRC.Unexposed	0.002	*
ZIRC.Pre-exposure-ZIRC.Unexposed	0.004	*	Gemma.Exposed-Watts.Exposed	0.003	*
Watts.Exposed-ZIRC.Pre-exposure	0.005	*	ZIRC.Pre-exposure-ZIRC.Unexposed	0.003	*
Gemma.Pre-exposure-Watts.Pre-exposure	0.008	*	Gemma.Exposed-ZIRC.Unexposed	0.006	*
Gemma.Pre-exposure-ZIRC.Exposed	0.014	*	Watts.Unexposed-ZIRC.Pre-exposure	0.007	*
Gemma.Pre-exposure-Watts.Unexposed	0.016	*	Gemma.Pre-exposure-ZIRC.Exposed	0.015	*
Gemma.Pre-exposure-Gemma.Unexposed	0.019	*	Gemma.Unexposed-Watts.Unexposed	0.015	*
Watts.Pre-exposure-ZIRC.Unexposed	0.046	*	Gemma.Pre-exposure-Watts.Pre-exposure	0.018	*
ZIRC.Exposed-ZIRC.Unexposed	0.059		Gemma.Exposed-Watts.Unexposed	0.019	*
Watts.Exposed-ZIRC.Exposed	0.075		Watts.Exposed-Watts.Pre-exposure	0.019	*
Watts.Exposed-Watts.Pre-exposure	0.094		Watts.Pre-exposure-ZIRC.Unexposed	0.030	*
Gemma.Exposed-Watts.Pre-exposure	0.102		Gemma.Pre-exposure-ZIRC.Pre-exposure	0.031	*
Watts.Unexposed-ZIRC.Unexposed	0.138		Watts.Pre-exposure-Watts.Unexposed	0.049	*
Gemma.Exposed-ZIRC.Exposed	0.144		Gemma.Pre-exposure-Gemma.Unexposed	0.067	
			Watts.Exposed-ZIRC.Exposed	0.074	
			ZIRC.Exposed-ZIRC.Unexposed	0.100	

3.4) Differential Abundance

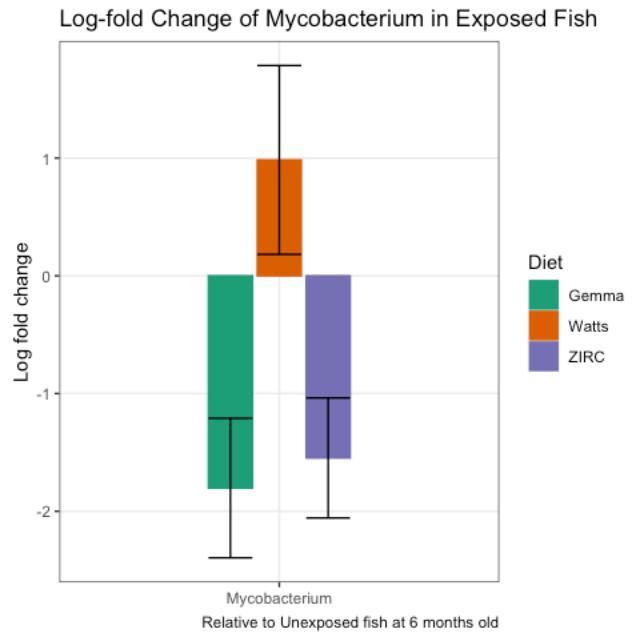
3.4.1)

Taxon	W	p_val	q_val	diff_abn
Mycobacterium	26.596	<0.001	<0.001	TRUE

ANCOM-BC: Log Fold Change Abundance

Diet	Taxon	LFC	SE	P.value	direct	sig
Gemma	Mycobacterium	-1.802717	0.5926287	0.002350897	Negative LFC	*
Watts	Mycobacterium	0.984404	0.8020263	0.219673719	Positive LFC	
ZIRC	Mycobacterium	-1.547301	0.5099201	0.002410155	Negative LFC	*

3.4.2)



3.4.3)