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Effects of diet on growth and the microbiome

Michael Sieler
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No standard reference diet currently exists for zebrafish studies

Zebrafish studies do not have a standard reference diet

- Live feed
- Commercial diets
- Laboratory diets

These diets can contain different macro- and micro-nutrient profiles

- Protein: 31 to 60%
- Lipids: 5 to 34%
- Carbohydrates: 1 to 30%
- Varying amounts of minerals and vitamins

Diet can influence zebrafish physiology

- Diet can influence weight, body composition and reproductive outcomes
- Nutritional differences can introduce undefined variation across studies
- Uncontrolled variance could be linked to diet induced inconsistencies of the microbiome

Influence of Commercial and Laboratory Diets on Growth, Body Composition, and Reproduction in the Zebrafish *Danio rerio*

L. Adele Fowler,^{1,2} Michael B. Williams,² Lacey N. Dennis-Cornelius,² Susan Farmer,³ R. Jeff Barry,² Mickie L. Powell,^{1,2} and Stephen A. Watts^{1,2}

Annual Review of Nutrition

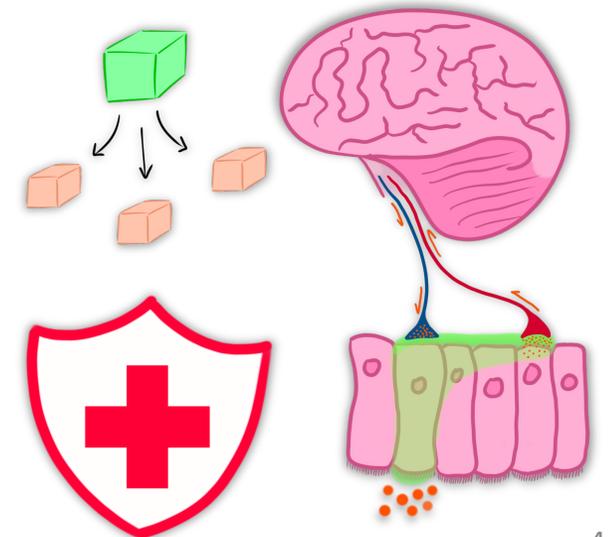
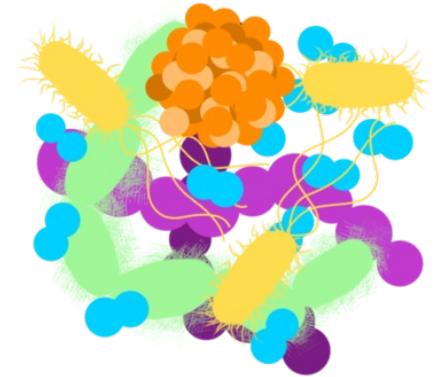
Standardized Reference Diets for Zebrafish: Addressing Nutritional Control in Experimental Methodology

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What is the microbiome?

- The microbiome is the community of microbes and their products associated with a host or environment
- Microbes include bacteria, fungi, archaea and viruses
- The microbiome plays an important role in host health
 - Digesting food
 - Metabolizing exogenous chemicals (drugs, toxicants, etc.)
 - Immune system support (fighting pathogens)
 - Interfacing with enteric nervous system

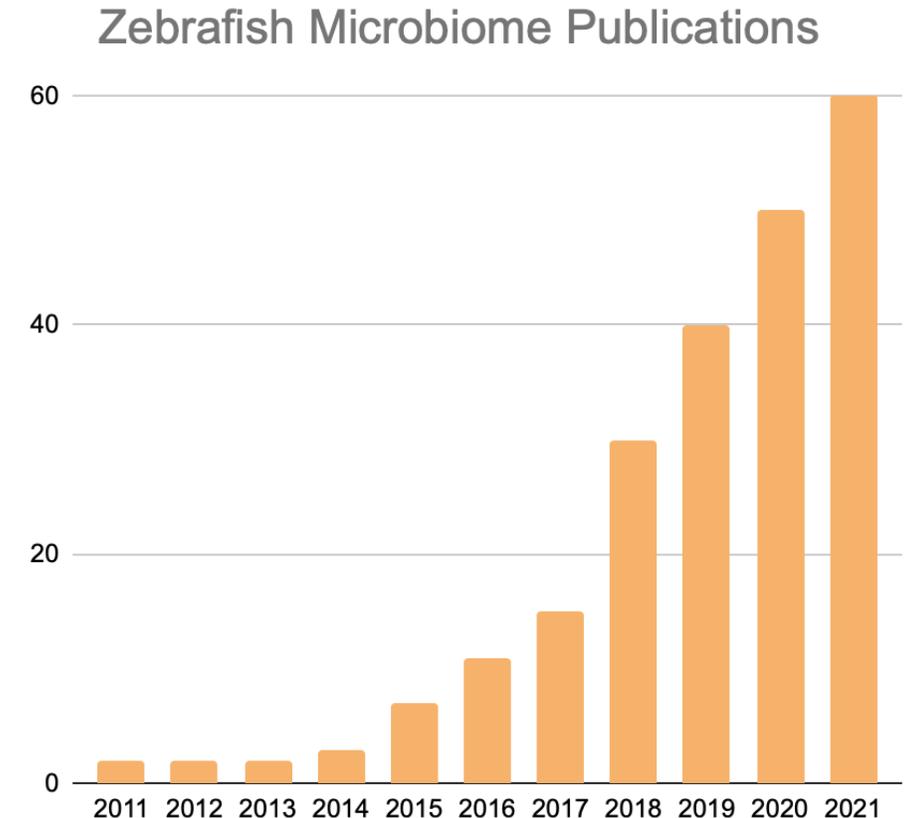


Zebrafish are an emerging fundamental microbiome research model

Benefits of using zebrafish:

- Extensive homology to early human development, genetics, and biochemistry
- Established, high-throughput methods
- Large sample sizes
- Ease of directly manipulate microbiome

Do we need to consider diet in microbiome-targeted zebrafish studies?



Source: Web of Science

Diet influences zebrafish microbiome

- Microbiomes assemble differently in high vs. low fat diets
- High fat diets can disrupt the microbiome and cause inflammation
- Diet influences gut microbiomes of other organisms as well

Ontogenetic Differences in Dietary Fat Influence Microbiota Assembly in the Zebrafish Gut

Sandi Wong,^{a,b}  W. Zac Stephens,^c Adam R. Burns,^d Keaton Stagaman,^d Lawrence A. David,^a Brendan J. M. Bohannon,^d Karen Guillemin,^c John F. Rawls^{a,e}

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High-Fat Diet Consumption Induces Microbiota Dysbiosis and Intestinal Inflammation in Zebrafish

Nerea Arias-Jayo¹  · Leticia Abecia² · Laura Alonso-Sáez³ · Andoni Ramirez-Garcia⁴ · Alfonso Rodriguez⁵ · Miguel A. Pardo¹

REVIEW

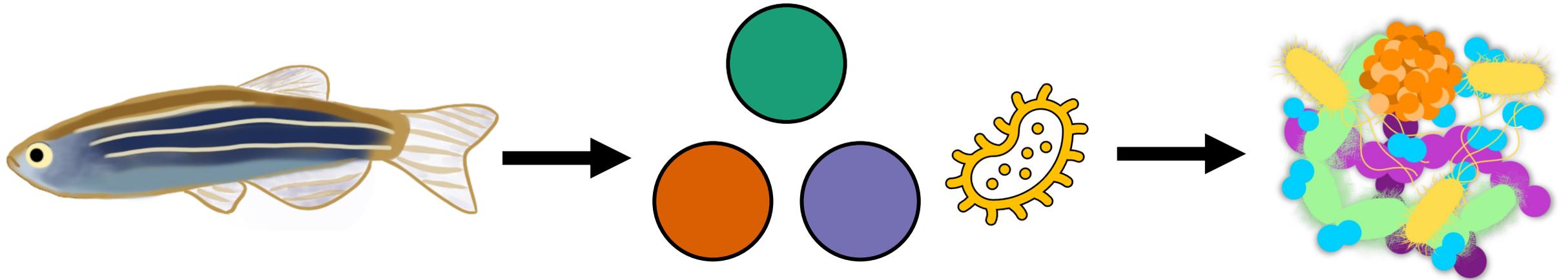
Open Access



Influence of diet on the gut microbiome and implications for human health

Rasnik K. Singh¹, Hsin-Wen Chang², Di Yan², Kristina M. Lee², Derya Ucmak², Kirsten Wong², Michael Abrouk³, Benjamin Farahnik⁴, Mio Nakamura², Tian Hao Zhu⁵, Tina Bhutani² and Wilson Liao^{2*} 

Are there diet dependent differences in zebrafish gut microbiomes?



Common laboratory zebrafish diets

Gemma

- Skretting Inc., commercial

Watts

- Defined, laboratory

ZIRC

- Mixture of diets
- Zeigler, Spirulina

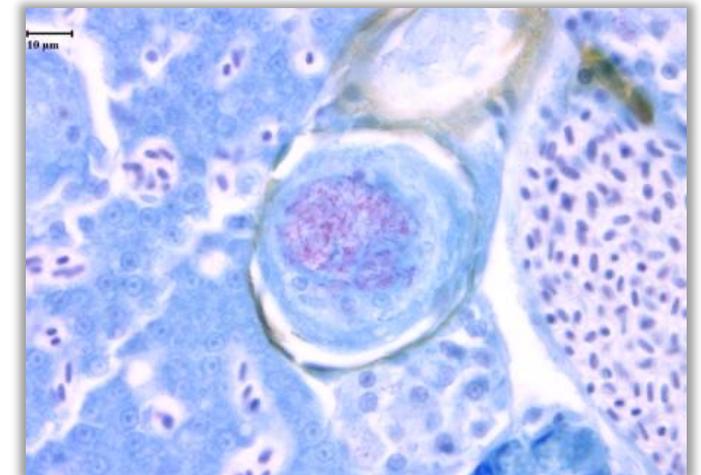
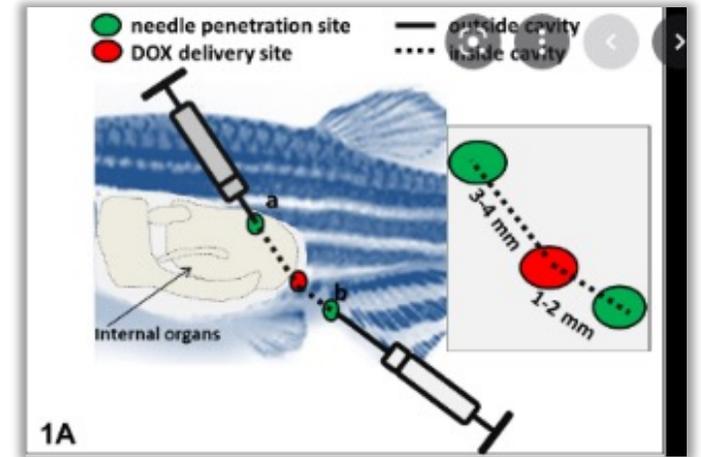
These diets are more consistent to one another than diets of previous studies

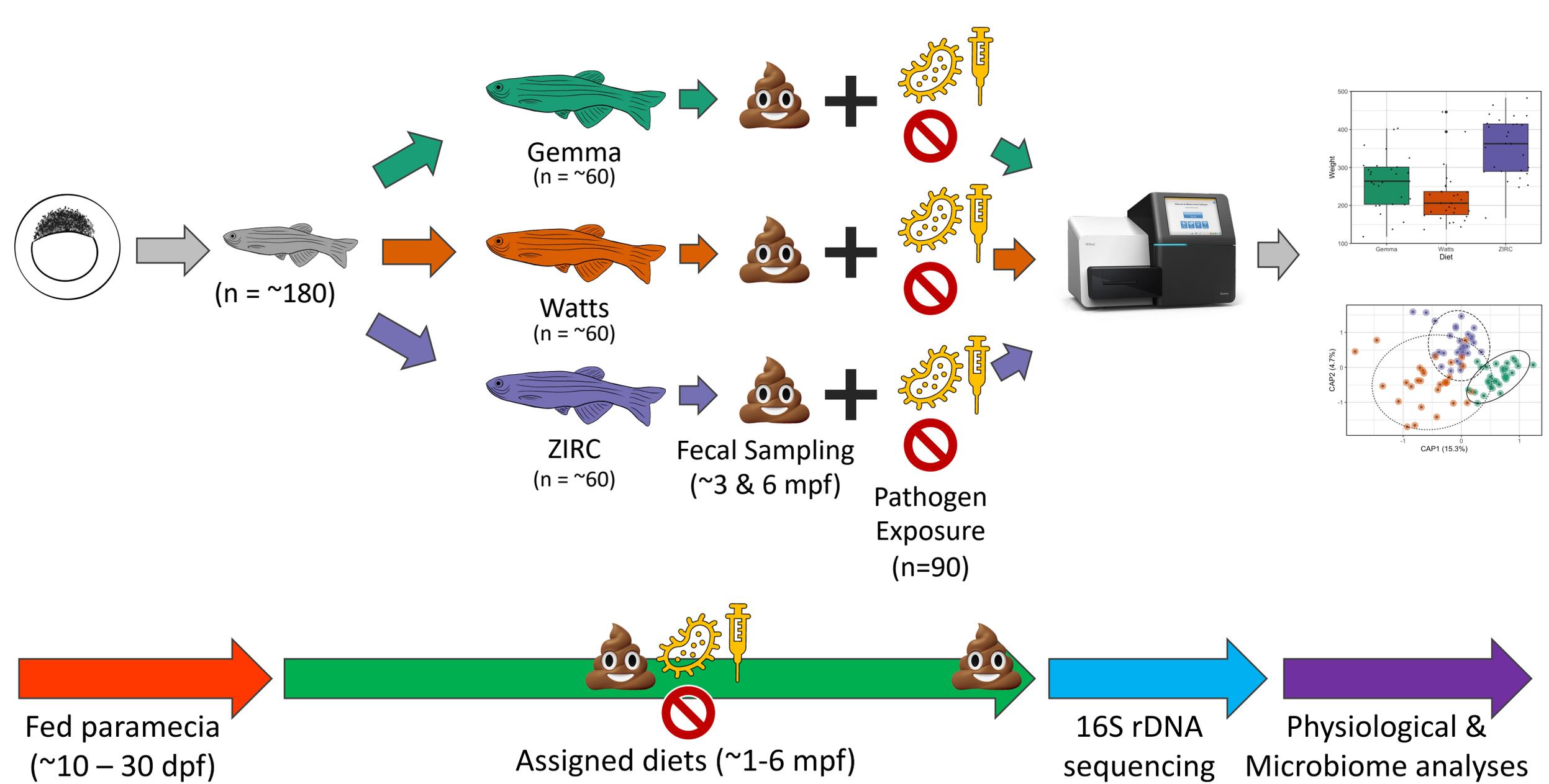
Approximate Compositions (%)			
	Gemma	Watts	ZIRC*
Protein	60	47	54
Lipids	20	11	14
Carbs	2	22	12
Fiber	0.4	2.1	1
Other	17.6	17.9	19

*Estimated

Mycobacterium chelonae

- *M. chelonae* is a common zebrafish pathogen
- Source of uncontrolled, inconsistency in results
- Transmitted through diet
- Injected fish with *M. chelonae* in coelomic cavity at 3mpf
- Infection was assessed visually

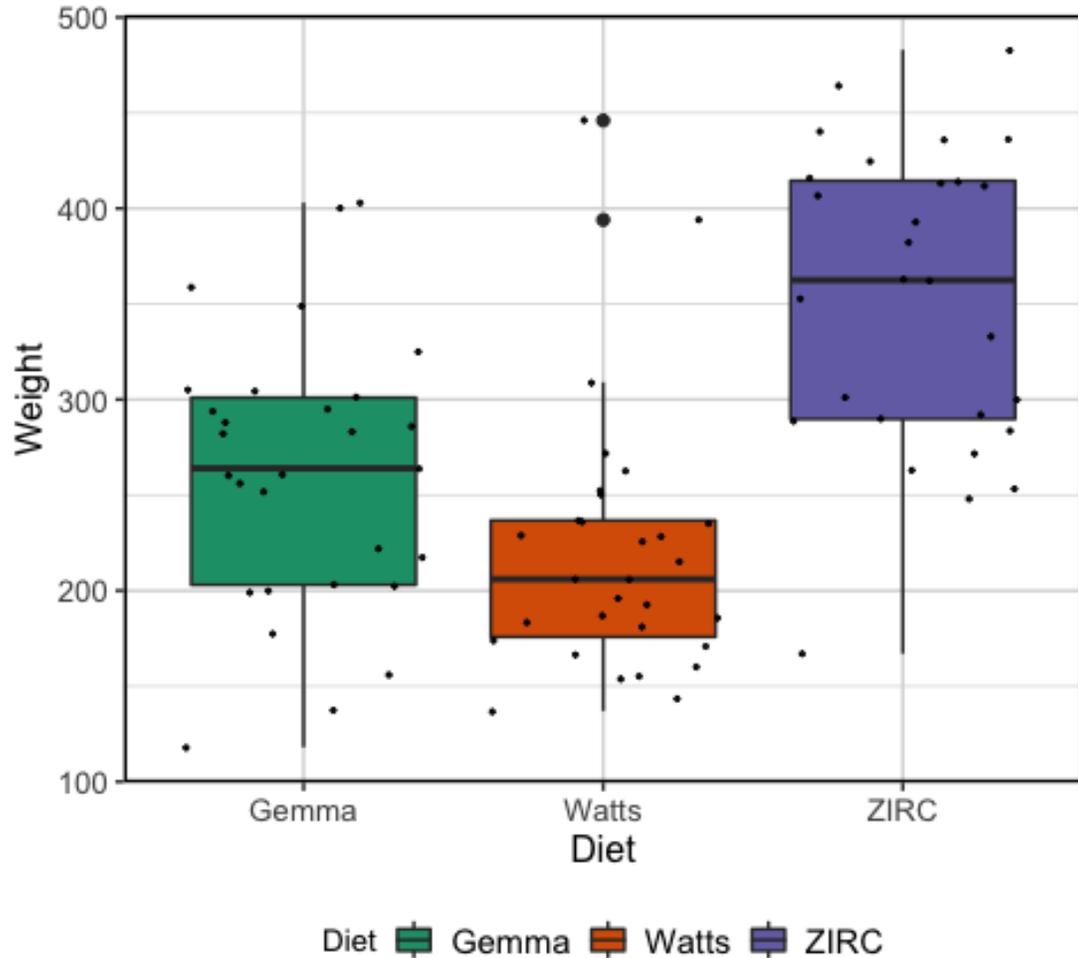




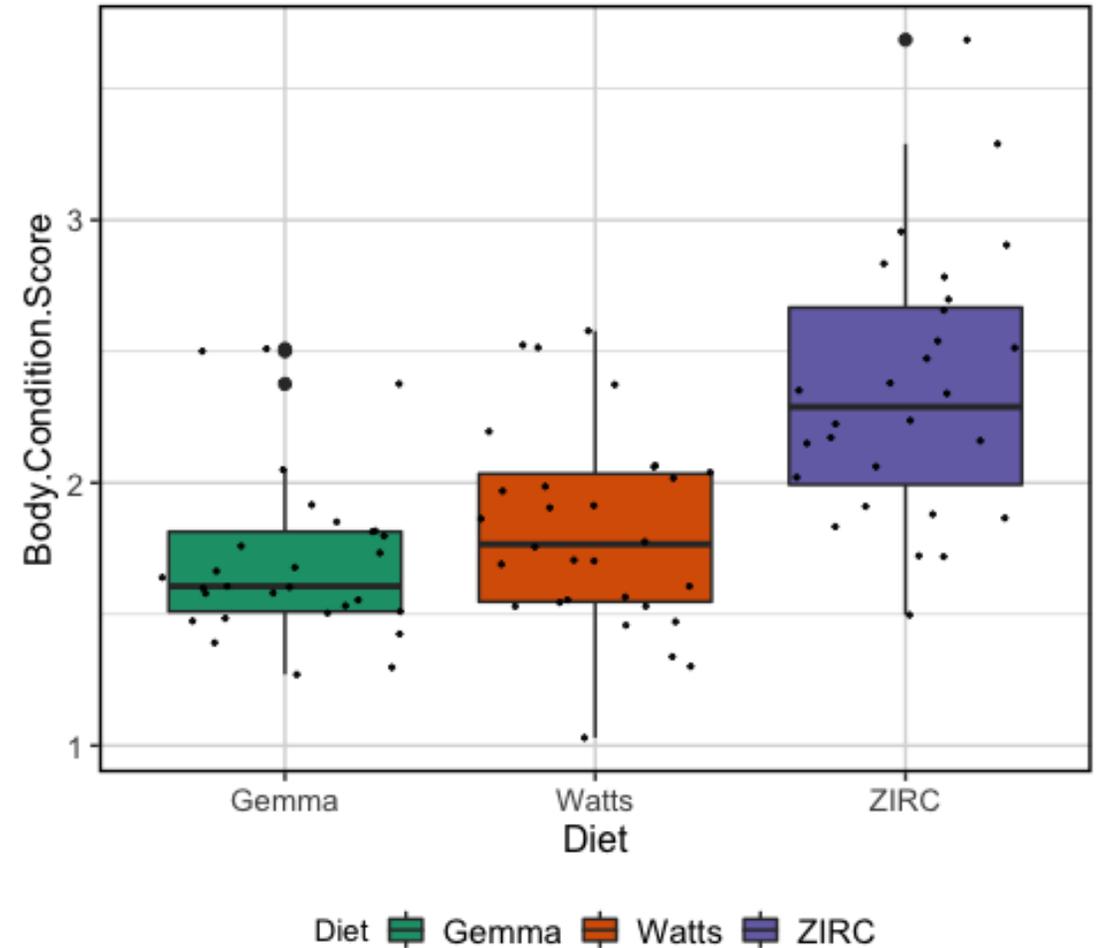
dpf = days post fertilization, mpf = months post fertilization

Weight and body condition score

ZIRC fed fish tend to weigh more and have a higher body condition score



Weight in mg

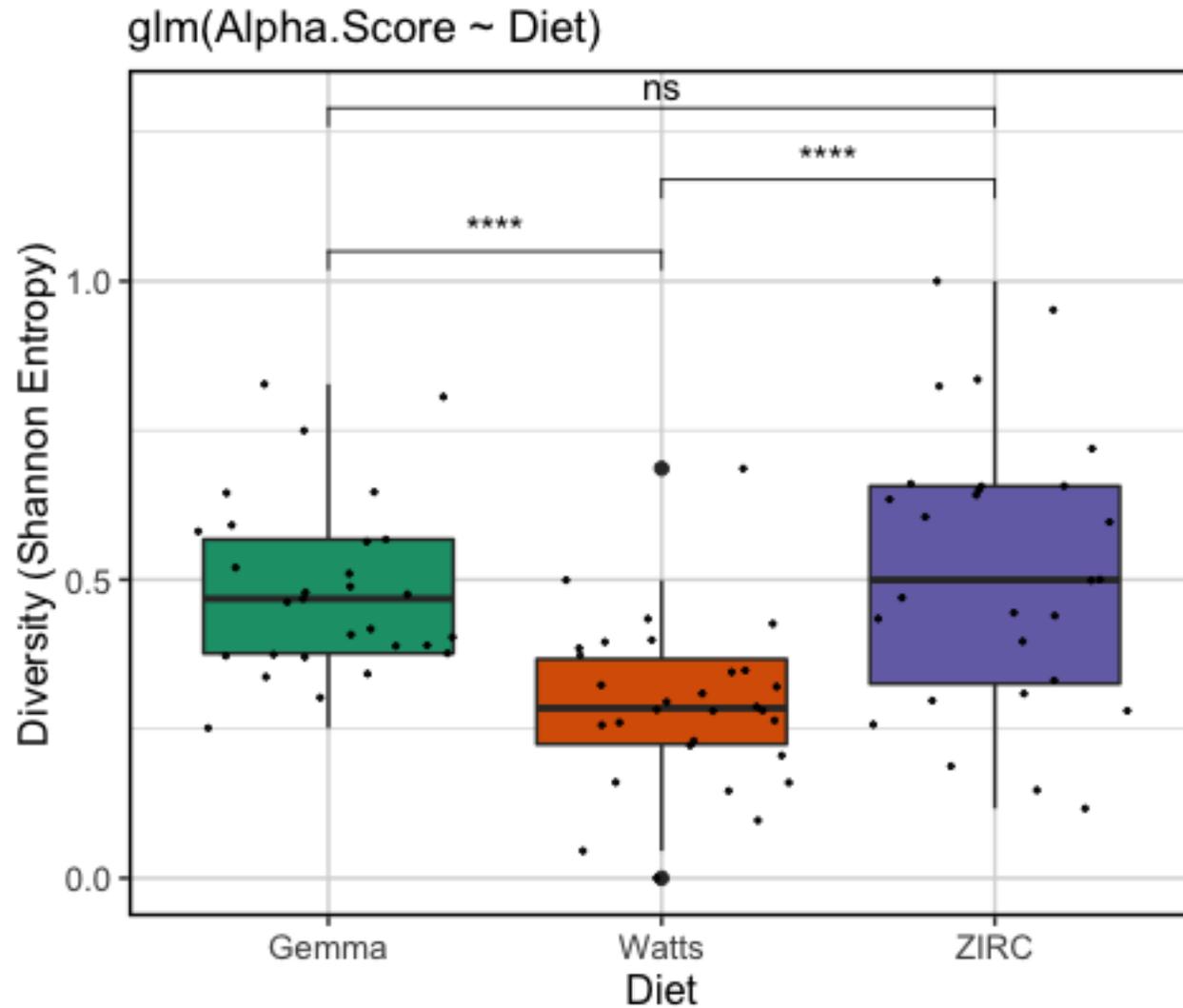


Body condition score (BCS) = $(\text{weight} / \text{length}^3) \times 100$

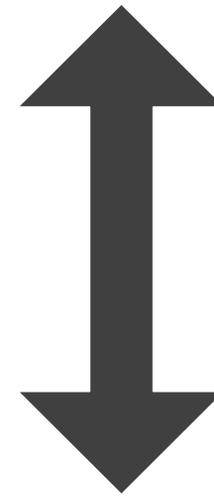
Is the microbiome intertwined with these diet by endpoint associations?

- Diversity
 - How many unique taxa are found?
- Composition
 - How similar are the types of microbes that comprise microbiome communities between hosts?
- Abundance
 - Are some taxa found more abundantly in one treatment group compared to another?

Watts fed fish have fewer taxa in their gut microbiomes



more taxa

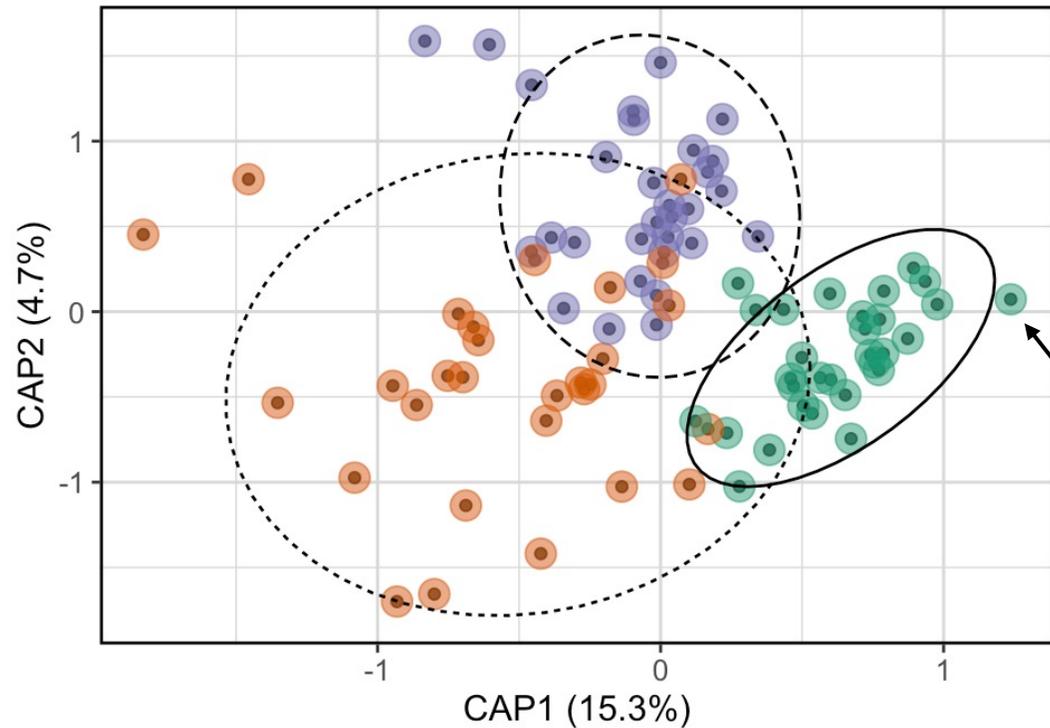


Diversity

fewer taxa

Diet ■ Gemma ■ Watts ■ ZIRC

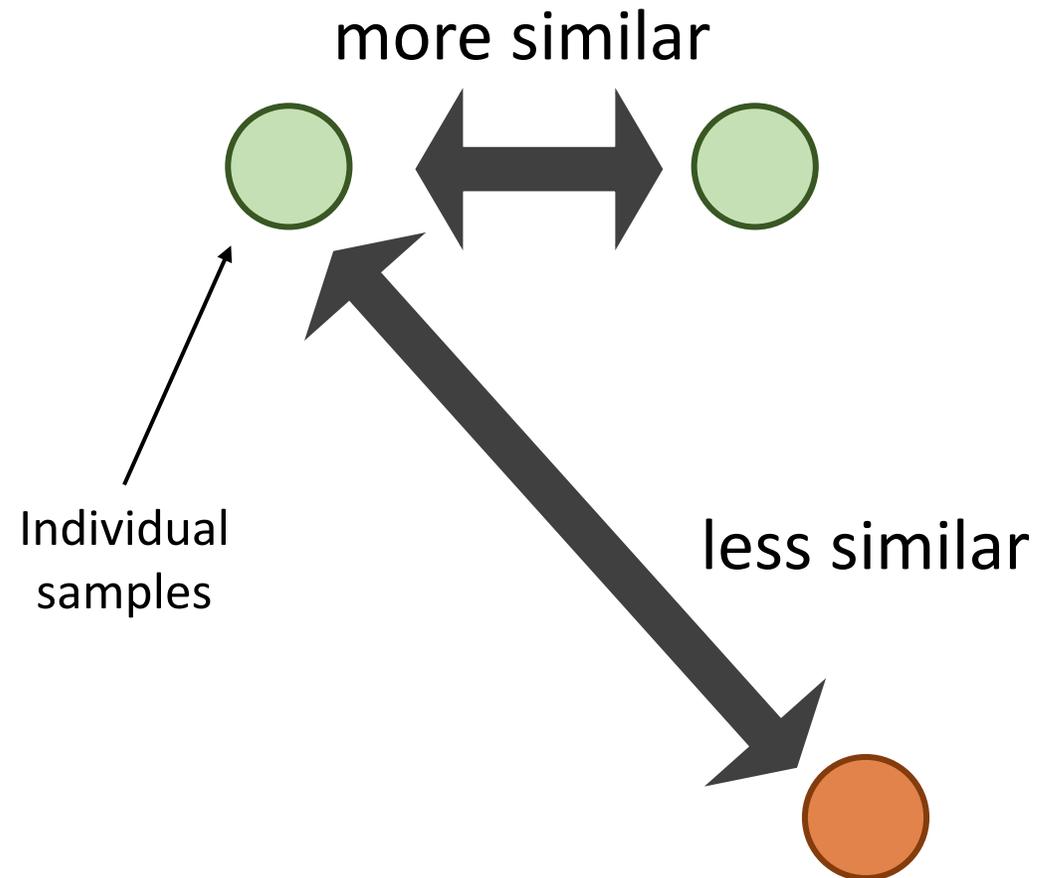
Fish fed the same diet tend to have similar microbiome compositions than to other diets



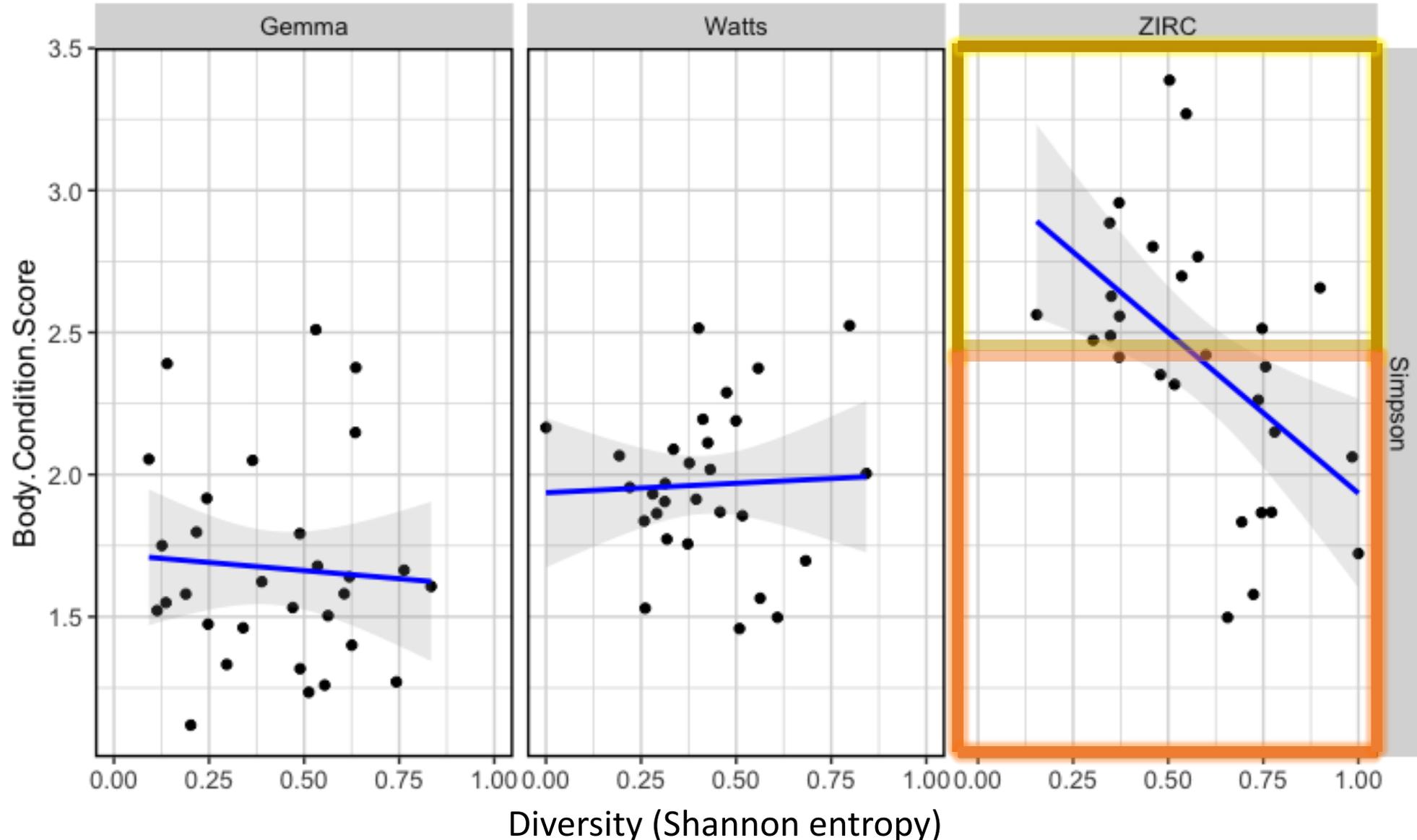
Diet ● Gemma ● Watts ● Zirc

Ellipses (95% CI) — Gemma --- Watts -- Zirc

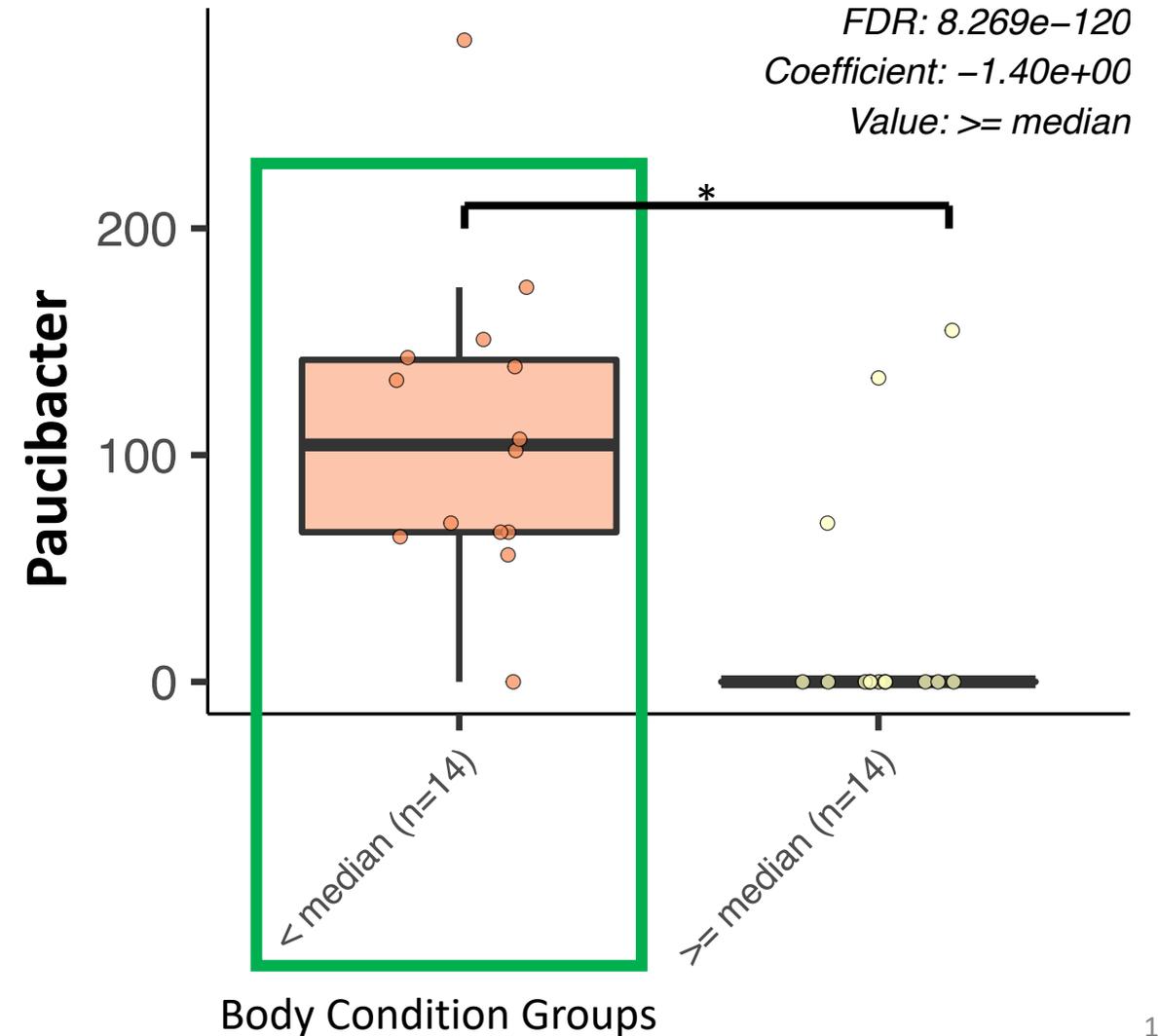
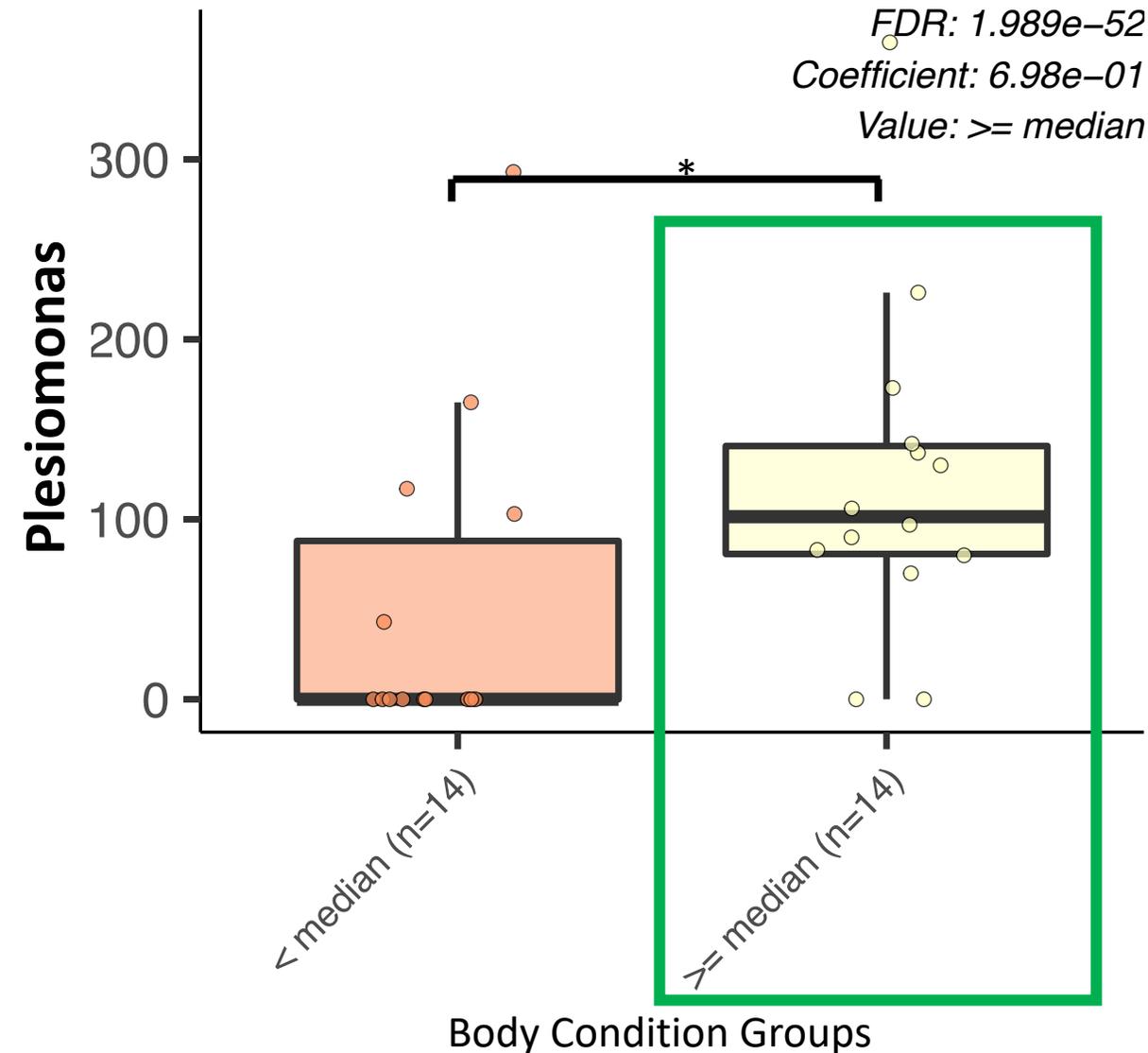
Statistic = 10.778, P-value = 0.001, capscale(), Bray-Curtis



ZIRC fed fish with less diverse microbiomes appear to have higher body condition scores

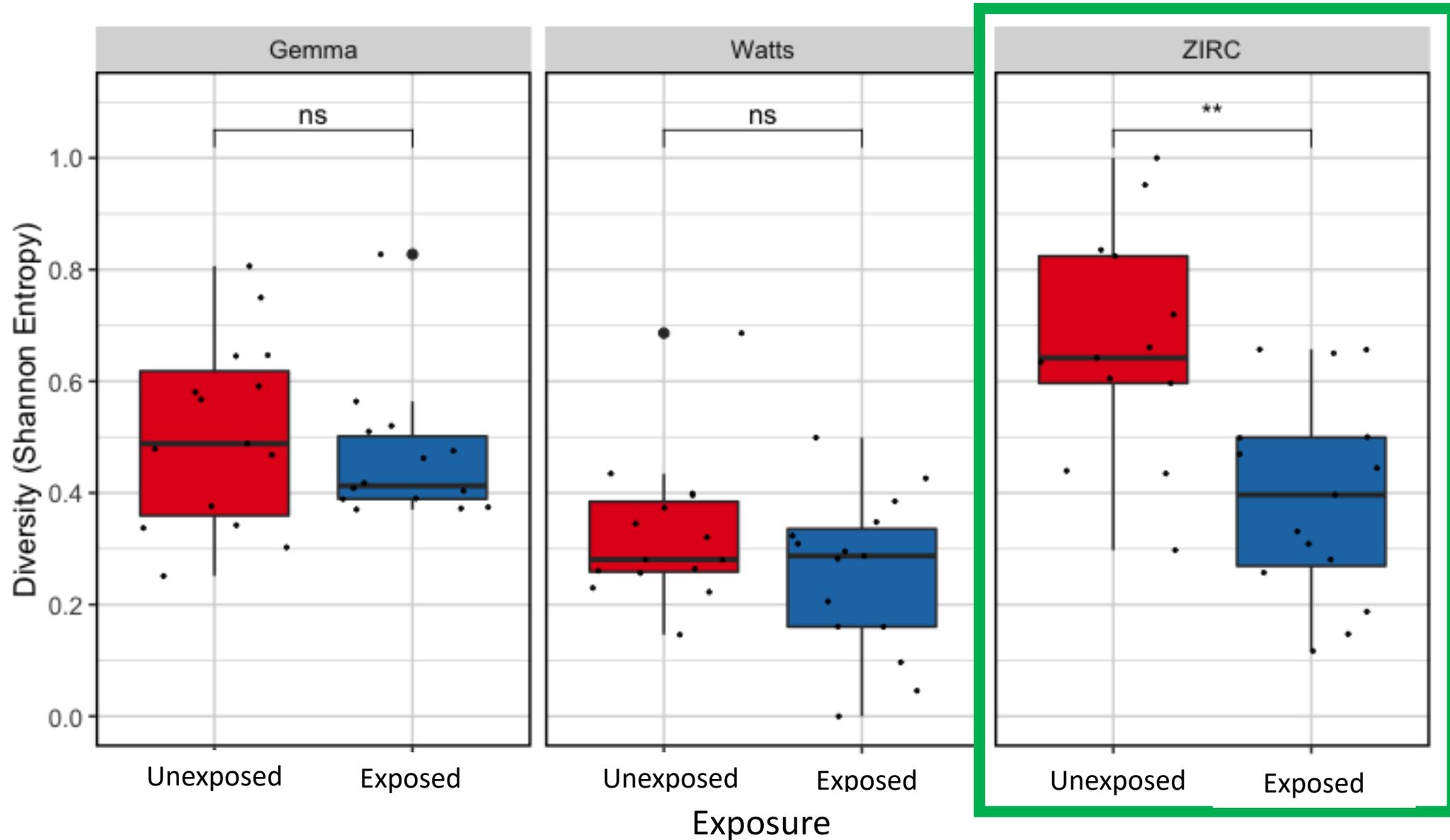


These taxa were differentially abundant in 6mpf ZIRC fed fish

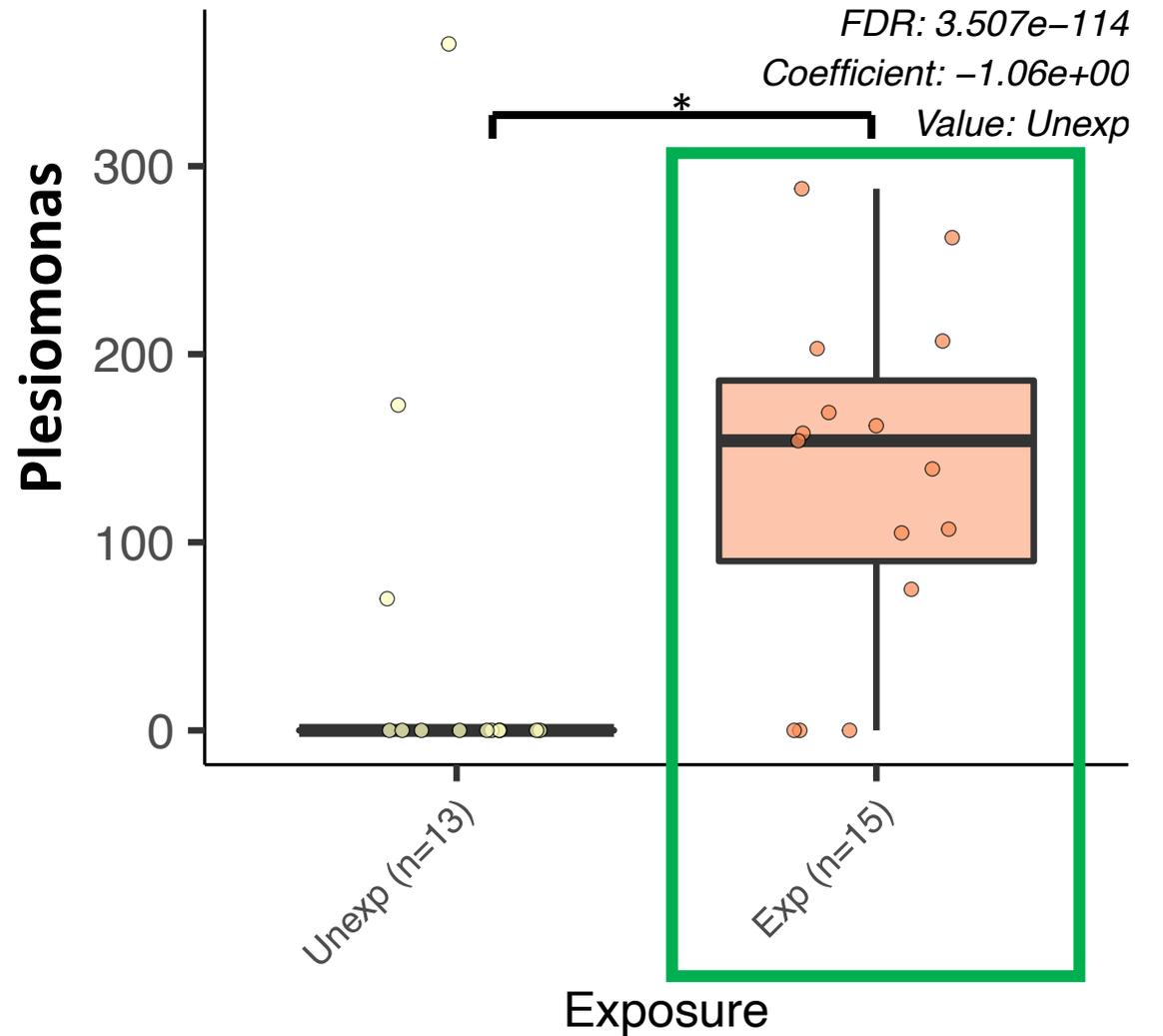
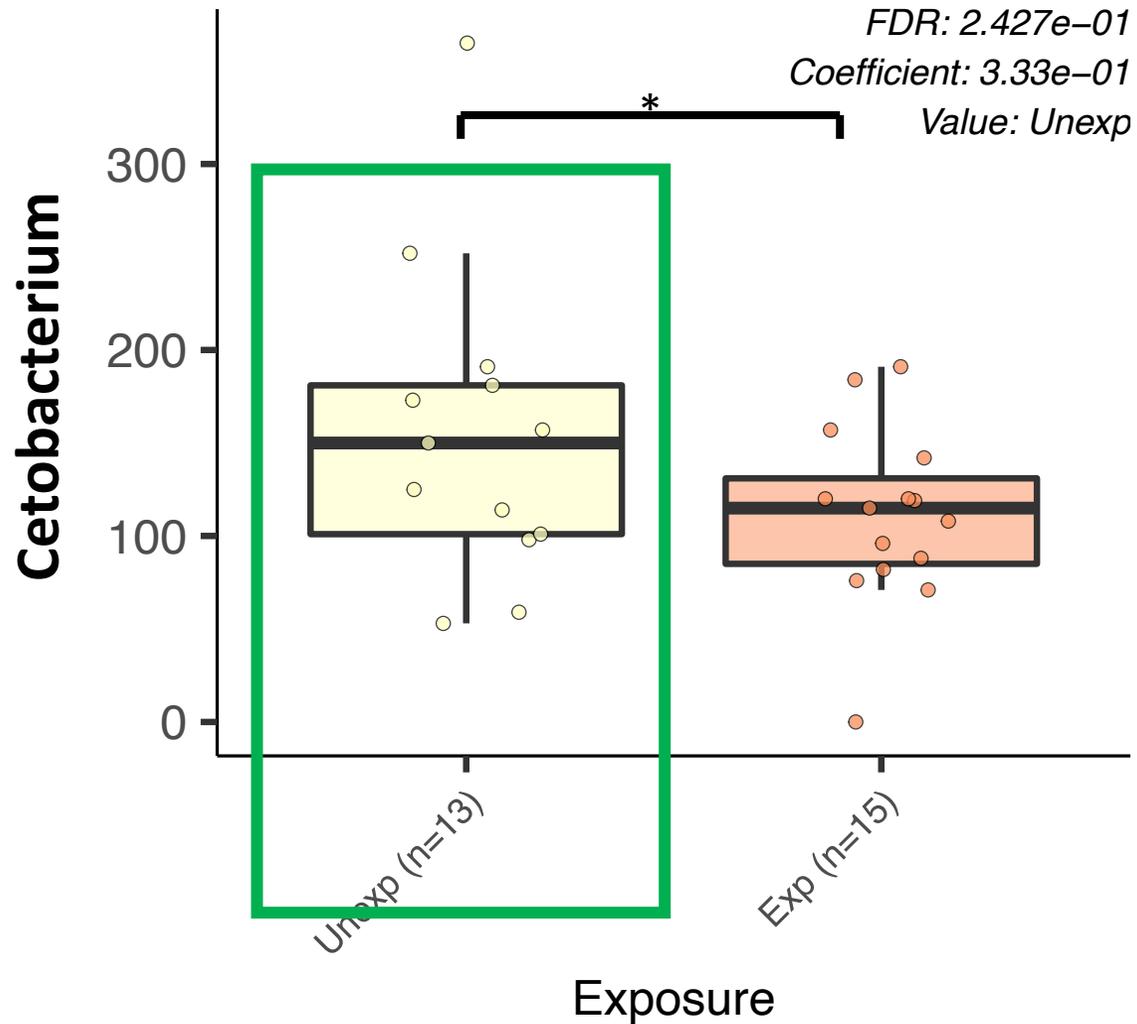


Could these differences in the microbiome affect how fish respond to pathogen exposure?

ZIRC fed fish appear more sensitive to pathogen exposure



These taxa were differentially abundant in 6mpf ZIRC fed fish



Diet differentially impacts physiology and gut microbiome development in zebrafish

Main observations:

- ZIRC heavier compared to Watts and Gemma
- Gemma and ZIRC had higher diversity of taxa to Watts
- Fish fed the same diet have similar microbiome compositions
- Watts have less consistent microbiome communities compared to Gemma and ZIRC
- Exposed ZIRC fish have lower microbiome diversity

Together, these results demonstrate that diet and host health are intertwined with their microbiome's development and sensitivity to pathogen exposure

Diet should be carefully considered in microbiome-targeted zebrafish studies

May be worth establishing a standard reference diet:

- Improve understanding of zebrafish health and nutrition
- Advance knowledge of how the diet and microbiome interact
- Support reproducibility and interpretability of studies

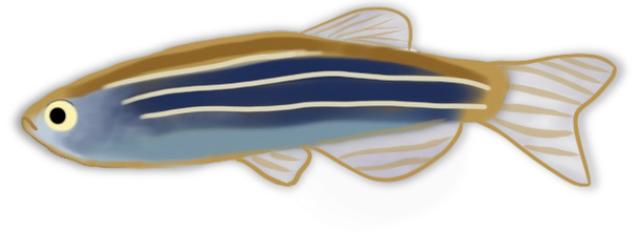
Different diets may serve different roles

- More variation could better mimic human populations
- Less variation could minimize influence of diet on results

Challenges: development of a germ-free diet that is nutritionally equivalent

Future directions

- Clarify how nutrient profile of diets associates with microbiome composition and health outcomes
- Repeat experiment
 - Natural (oral) exposure
 - Immunologic endpoints
 - Other common pathogens
- Investigate differentially abundant taxa



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ZIRC

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