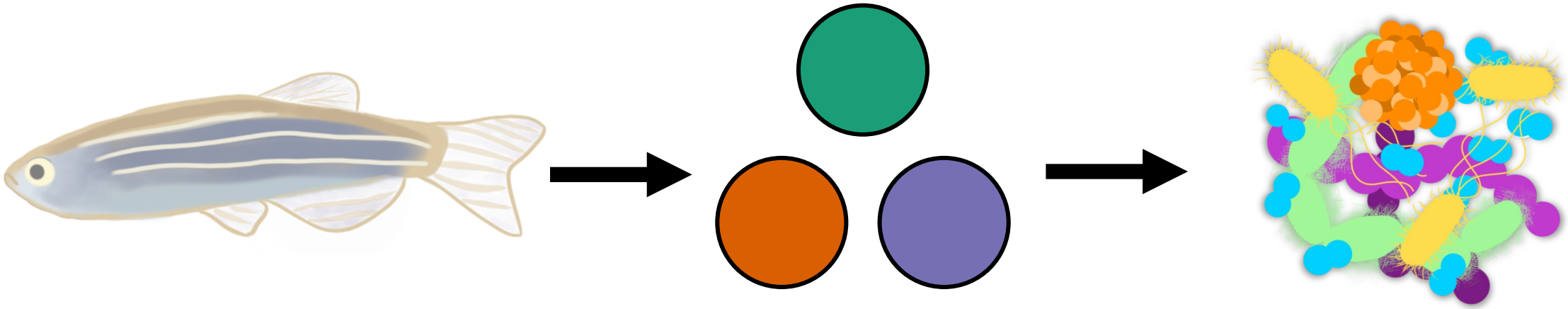


Zebrafish laboratory diets differentially alter gut microbiota composition

Michael Sieler

3rd International Fish Microbiota Workshop

23 September, 2021

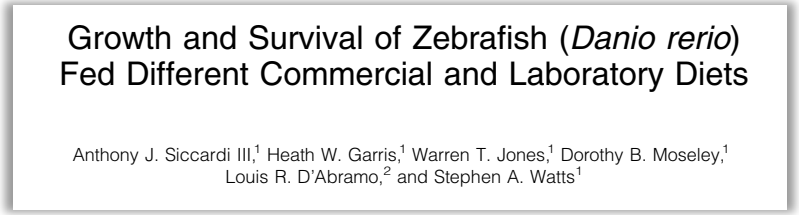
No standard reference diet currently exists for zebrafish studies

- Live feed, a variety of commercial and laboratory diets used in studies

- They contain differing 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 the physiology of zebrafish

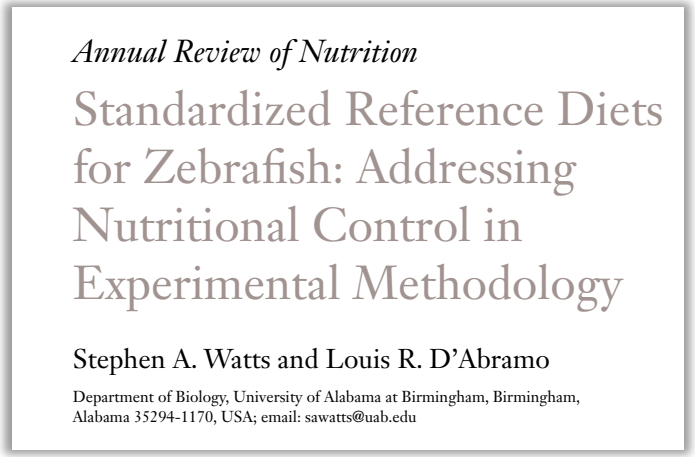
- Siccardi *et al.* 2009: Differences in weight and length but not body condition
- Fowler *et al.* 2019: Live feed, commercial and laboratory diets influence physiological differences
- Nutritional differences can influence undefined variation in studies


Growth and Survival of Zebrafish (*Danio rerio*) Fed Different Commercial and Laboratory Diets

Anthony J. Siccardi III,[1] Heath W. Garris,[1] Warren T. Jones,[1] Dorothy B. Moseley,[1] Louis R. D'Abramo,[2] and Stephen A. Watts[1]


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

Stephen A. Watts and Louis R. D'Abramo

Department of Biology, University of Alabama at Birmingham, Birmingham, Alabama 35294-1170, USA; email: sawatts@uab.edu

Diet can influence zebrafish microbiota

- Rawls *et al. 2015:* high- and low-fat diets manifested differential microbiota compositions

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. Bohannan,[d] Karen Guillemin,[c] John F. Rawls[a,e]

Department of Molecular Genetics and Microbiology, Duke University, Durham, North Carolina, USA[a]; Department of Microbiology and Immunology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA[b]; Institute of Molecular Biology, University of Oregon, Eugene, Oregon, USA[c]; Institute of Ecology and Evolution, University of Oregon, Eugene, Oregon, USA[d]; Department of Cell Biology and Physiology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA[e]

- Arias-Jayo *et al.* 2018: high-fat diet induced dysbiosis and intestinal inflammation of 30 dpf zebrafish

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

Nerea Arias-Jayo[1] · Leticia Abecia[2] · Laura Alonso-Sáez[3] · Andoni Ramirez-Garcia[4] · Alfonso Rodriguez[5] · Miguel A. Pardo[1]

- Diet influence gut microbiomes of other organisms as well

REVIEW Open Access

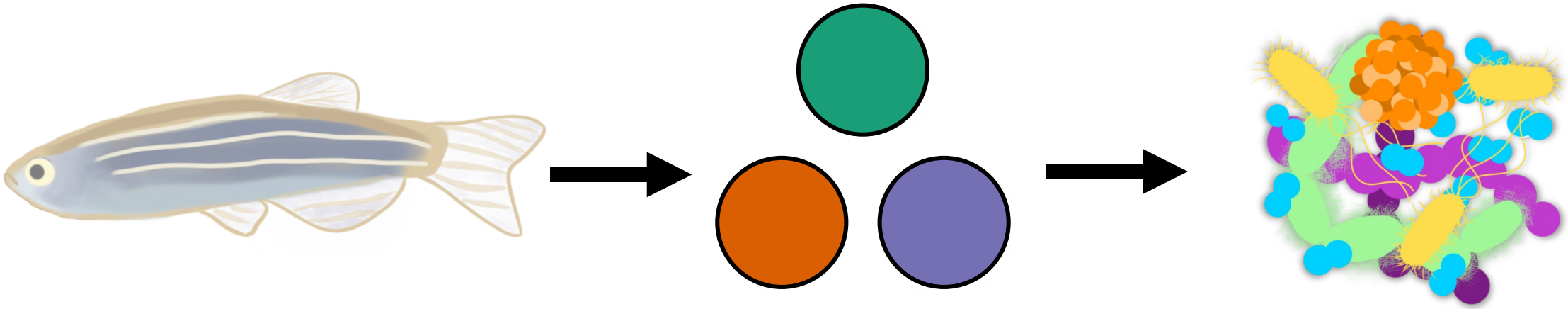
Influence of diet on the gut microbiome and implications for human health

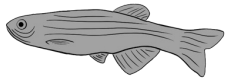
Rasnik K. Singh[1], Hsin-Wen Chang[2], Di Yan[2], Kristina M. Lee[2], Derya Ucmak[2], Kirsten Wong[2], Michael Abrouk[3], Benjamin Farahnik[4], Mio Nakamura[2], Tian Hao Zhu[5], Tina Bhutani[2] and Wilson Liao[2*]

The microbiome plays an important role in mediating host health

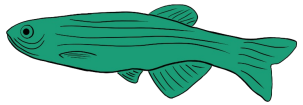
- Immune system, metabolism, nervous system
- Alterations in gut microbiota composition can negatively impact health
- Microbiome-targeted interventions could promote health
- Reproducibility across microbiome-targeted zebrafish studies

Are there diet dependent differences in zebrafish gut microbiomes?

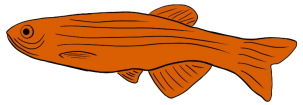




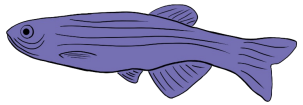
(n = 90)



Gemma
(n = 30)



Watts
(n = 30)

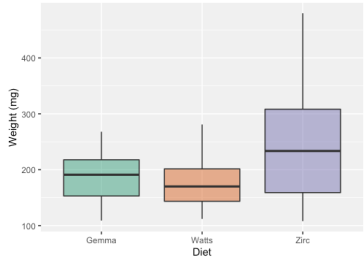


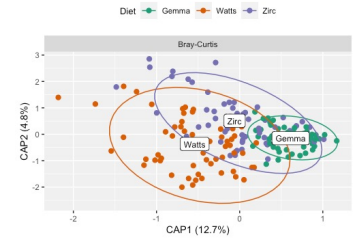
ZIRC
(n = 30)

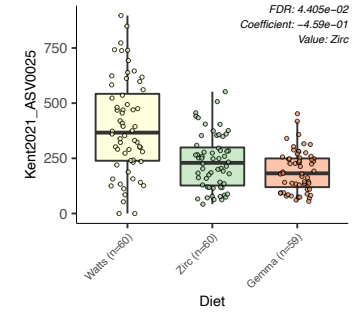
Approximate Compositions (%)

	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







Fed paramecia
(~10 – 30 dpf)

Assigned diets
(~30-130 dpf)

Fecal
Sampling

16S
sequencing

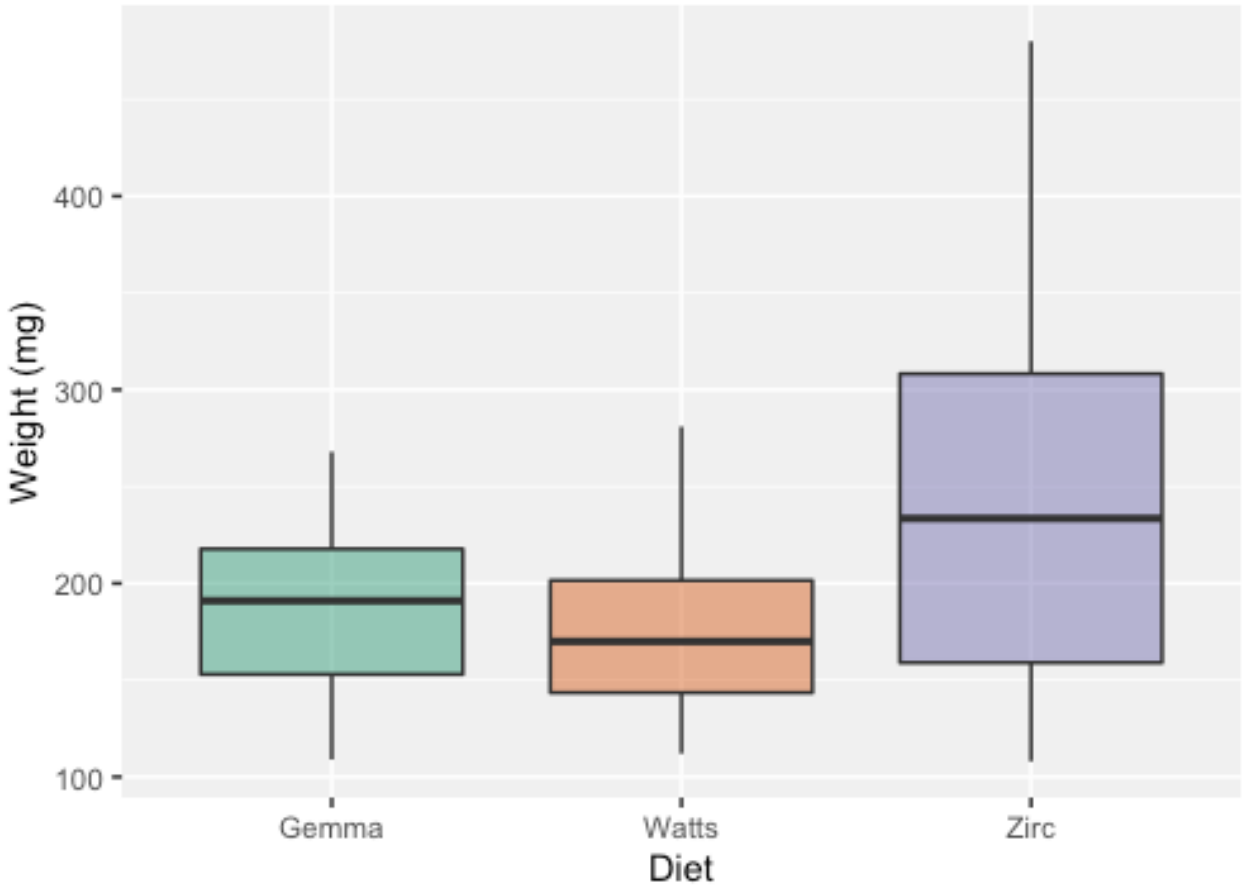
Microbiome
analysis

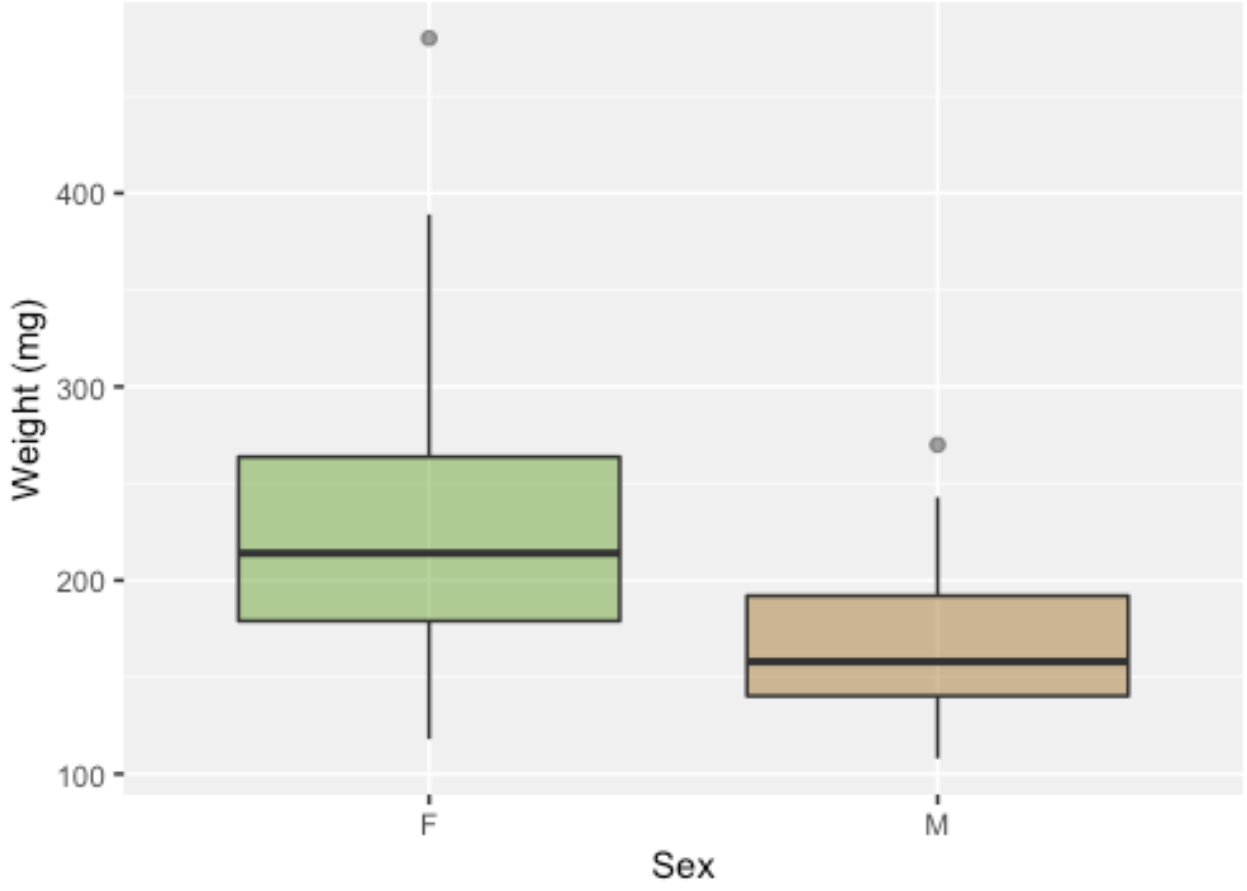
dpf = days post fertilization

Physiological Results

- Weight
- Body condition score

Diet and sex associate with weight

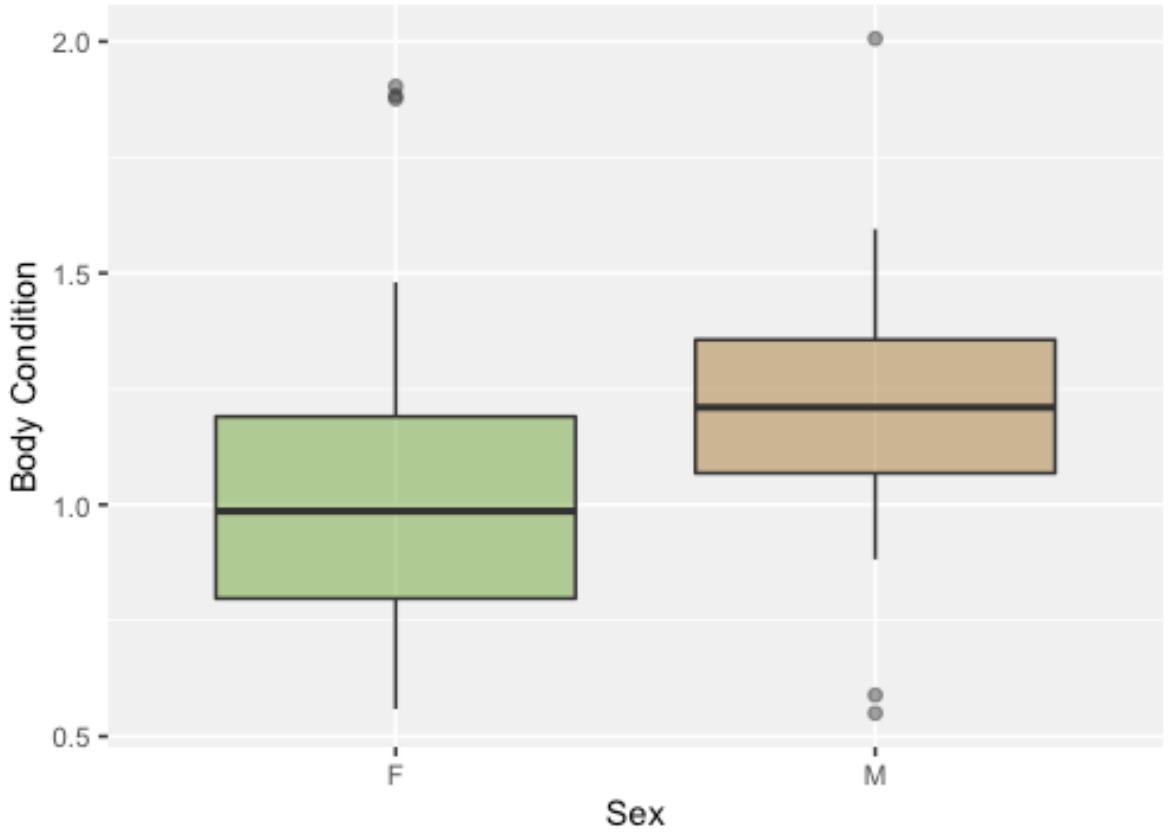




GLM(weight ~ Diet + Sex + Diet:Sex)

Term	Statistic	P-value
Diet	19.05	<0.001

Only sex associate with body condition score



GLM(Body Condition Score ~ Diet + Sex + Diet:Sex)

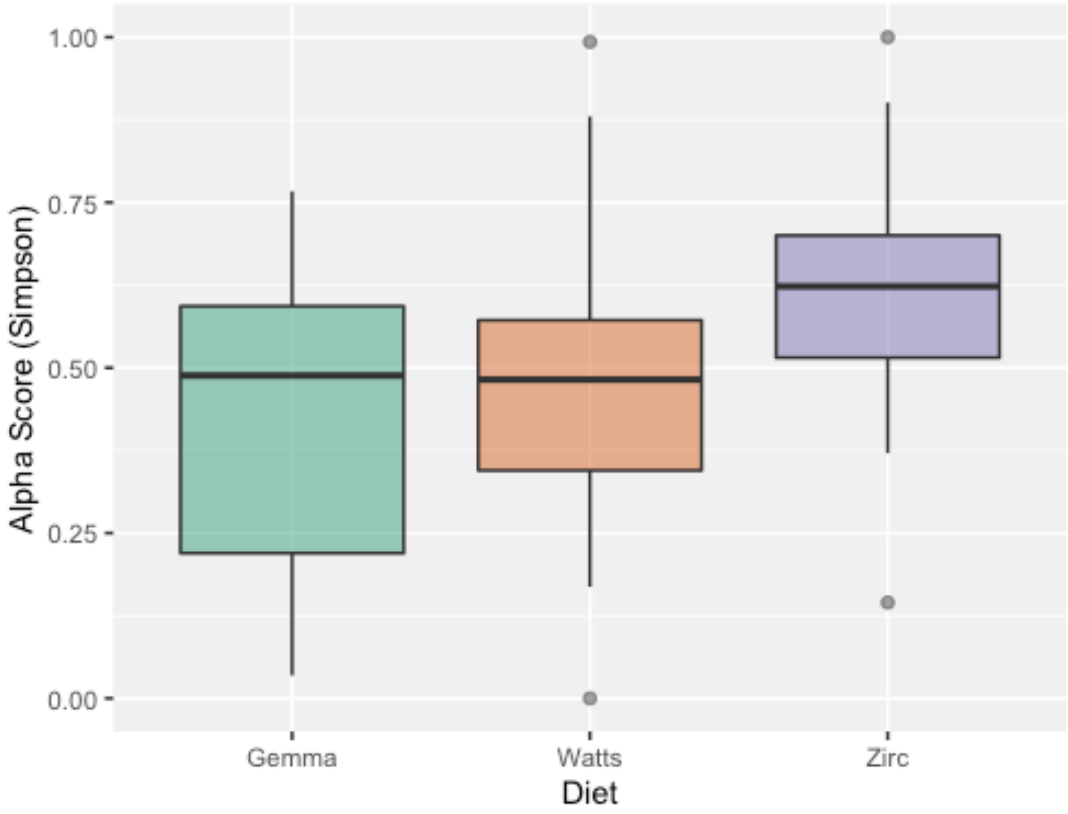
Term	Statistic	P-value
Sex	5.78	0.016

Body Condition Score =
(Weight / Length*Width^2)

Microbiota Results

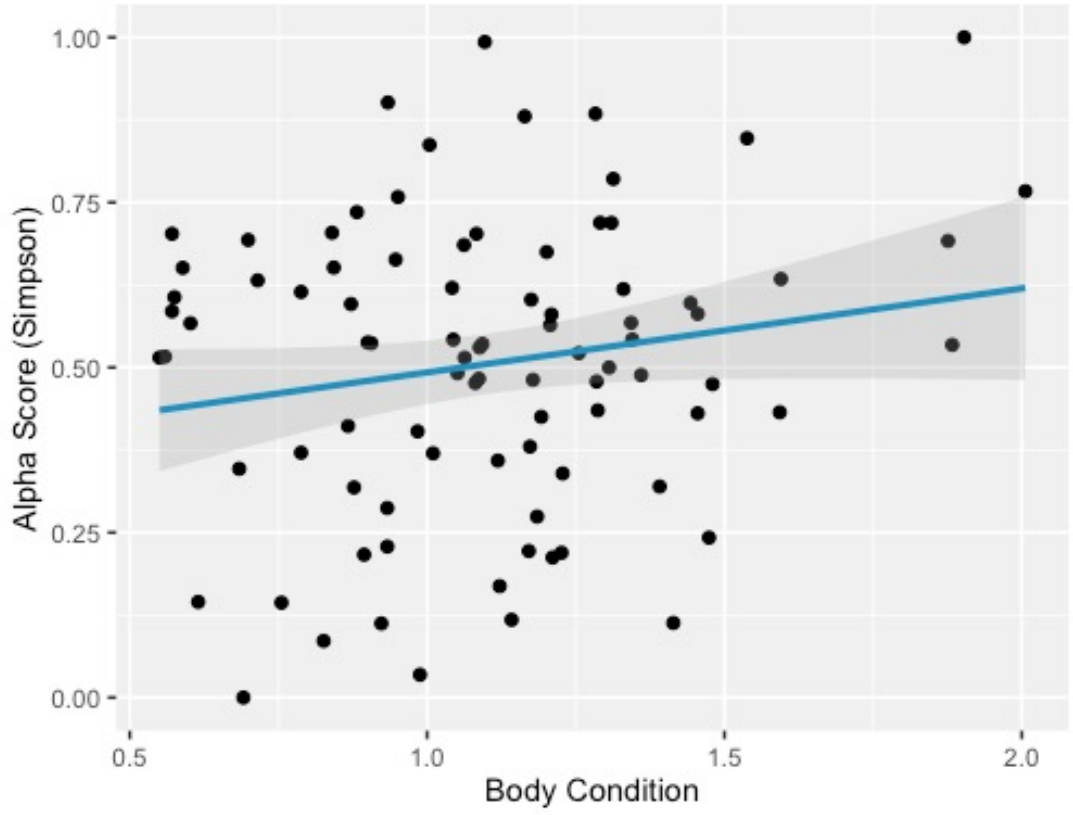
- Alpha diversity
- Beta diversity
- Differential Abundance

The ZIRC diet had the highest alpha-diversity score



Term	Statistic	P-value
Diet	21.42	<0.001

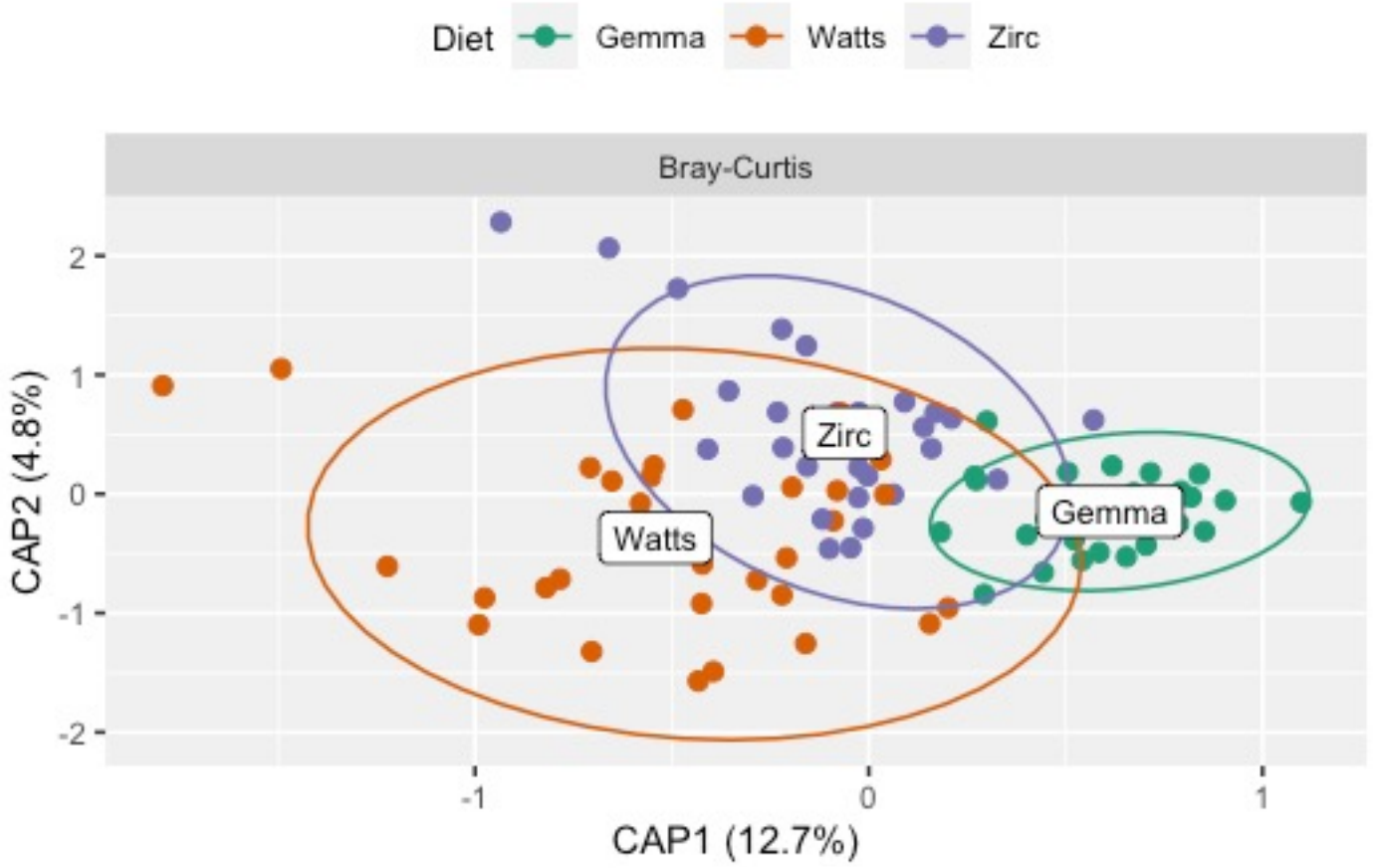
Body condition score positively associated with alpha-diversity score



Body Condition Score =
(Weight / Length*Width^2)

Term	Statistic	P-value
Body Condition	8.10	0.004

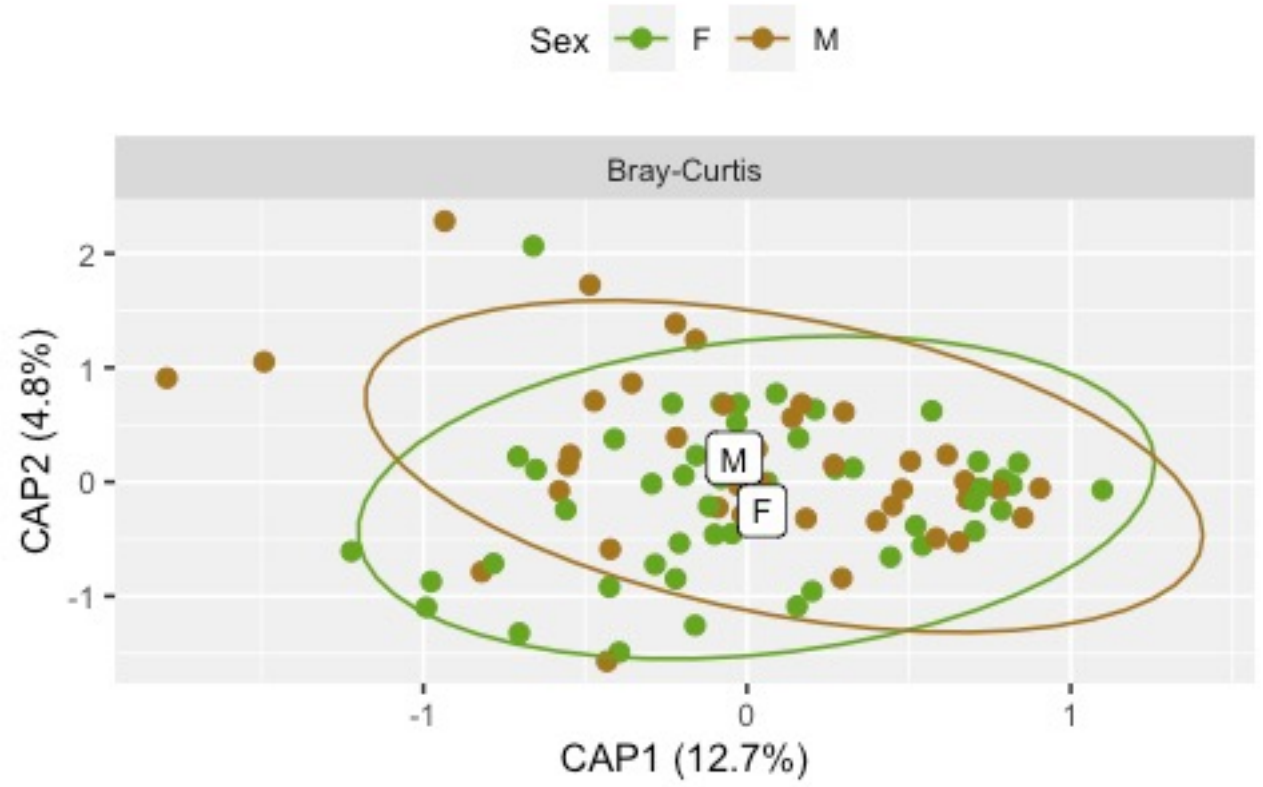
Interindividual variation associates with diet



PERMANOVA:

Term	Statistic	P-value
Diet	16.73	0.001

Interindividual variation associates with sex



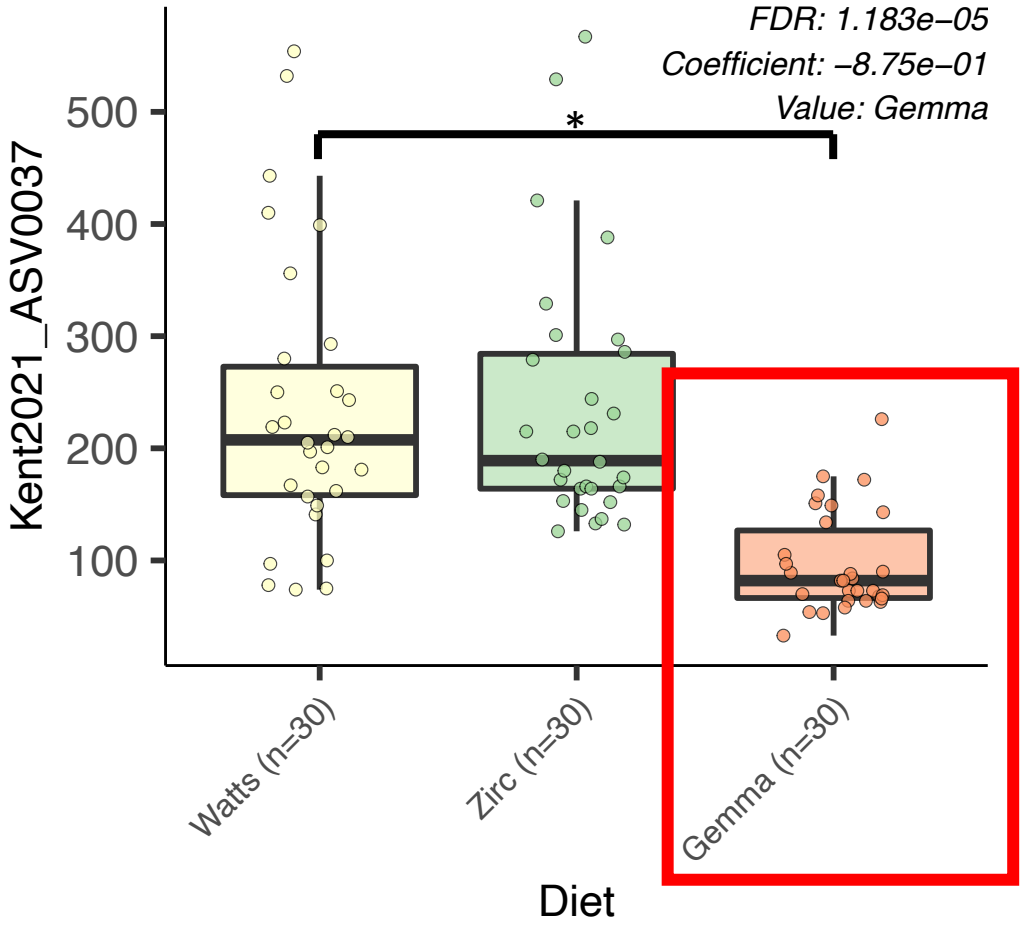
PERMANOVA:

Term	Statistic	P-value
Sex	5.13	0.001

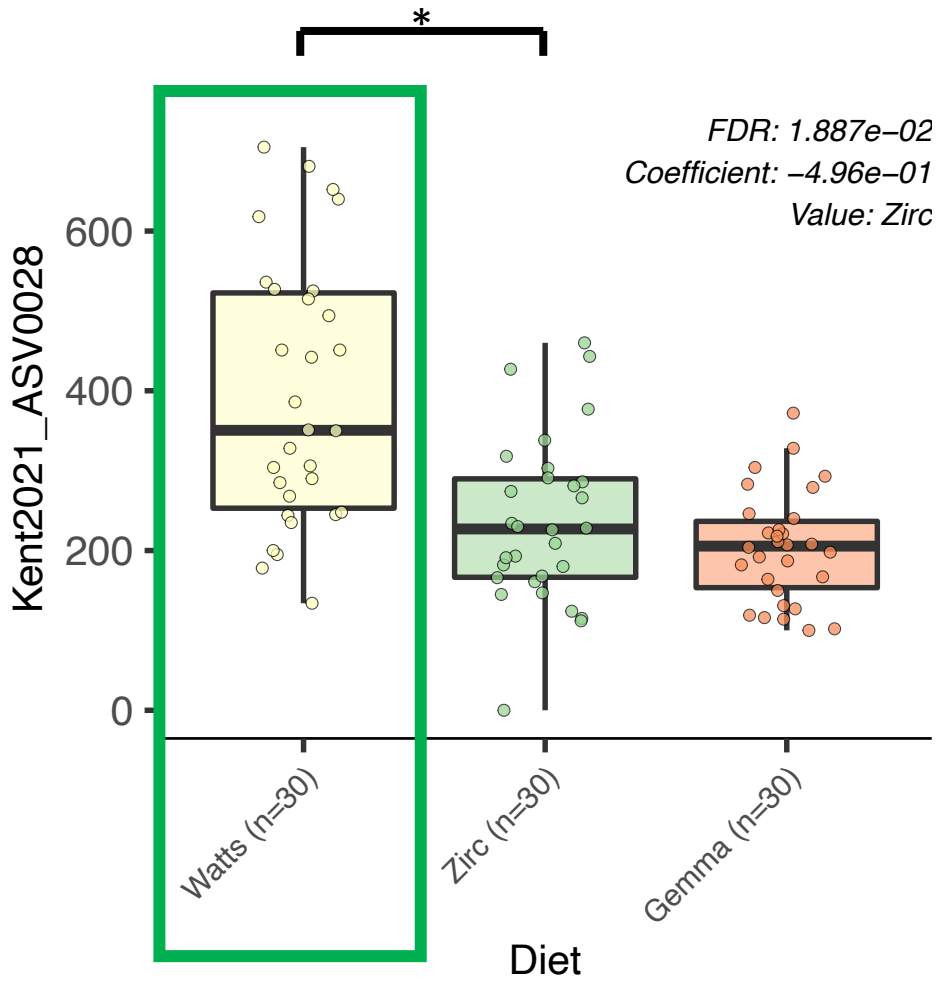
Differential Abundance

- Which ASVs are significantly more or less abundant in a diet
- Negative binomial general linear model
- After quality filtering, 175 of 2015 ASVs significantly associated with a diet

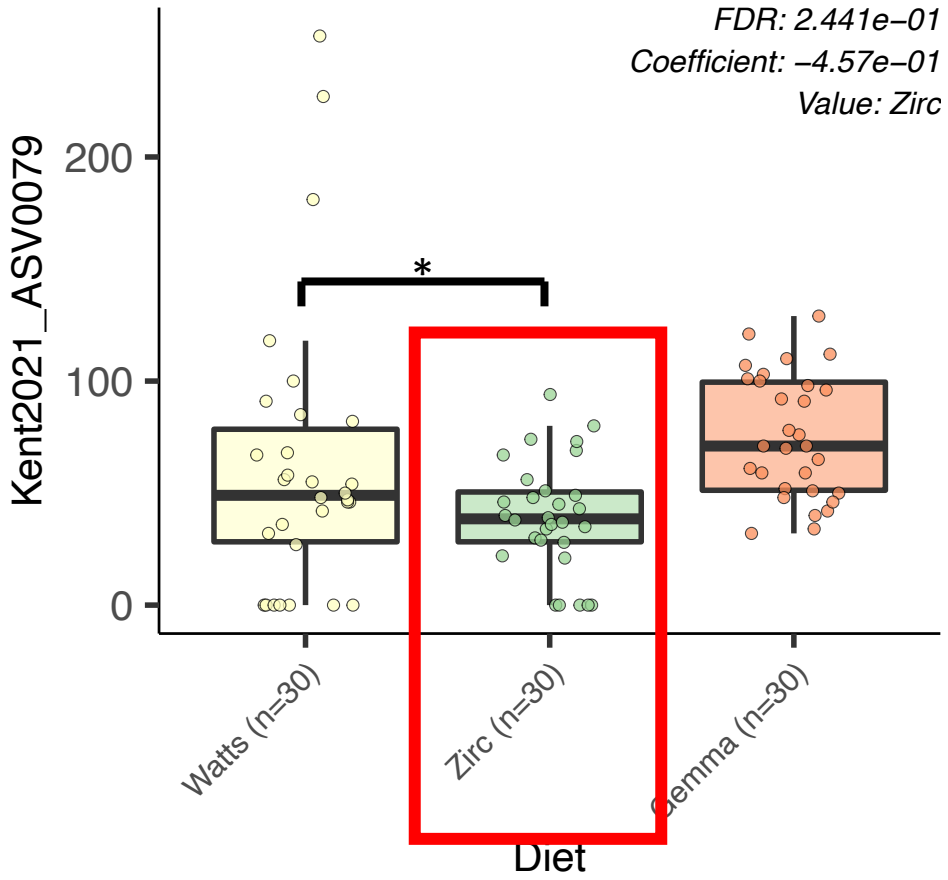
Aeromonas ASVs less abundant in Gemma diet



ZOR0006 was more abundant in Watts diet



Plesiomonas less abundant in ZIRC diet



Conclusion: Common laboratory diets differentially impact composition of the gut microbiome in zebrafish

Alpha-diversity:

- Alpha-diversity impacted by diet, higher in the ZIRC diet

Beta-diversity:

- Compositional variation differed between all three diets
- Gemma had the least beta dispersion compared to Watts and ZIRC diets

Differential abundance:

- 175 ASVs were positively or negatively associated with one or more diets

Establishing a standard reference diet is important for microbiome-targeted zebrafish studies

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

- Unclear which diet is “best” to study microbiome

- 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

- Investigate how microbiome, diet and intestinal infection interact

Acknowledgements

Sharpton Lab:

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- Colleen Al-Samarrie

ZIRC

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Oregon State University

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