

Pilot_study_results

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Contents

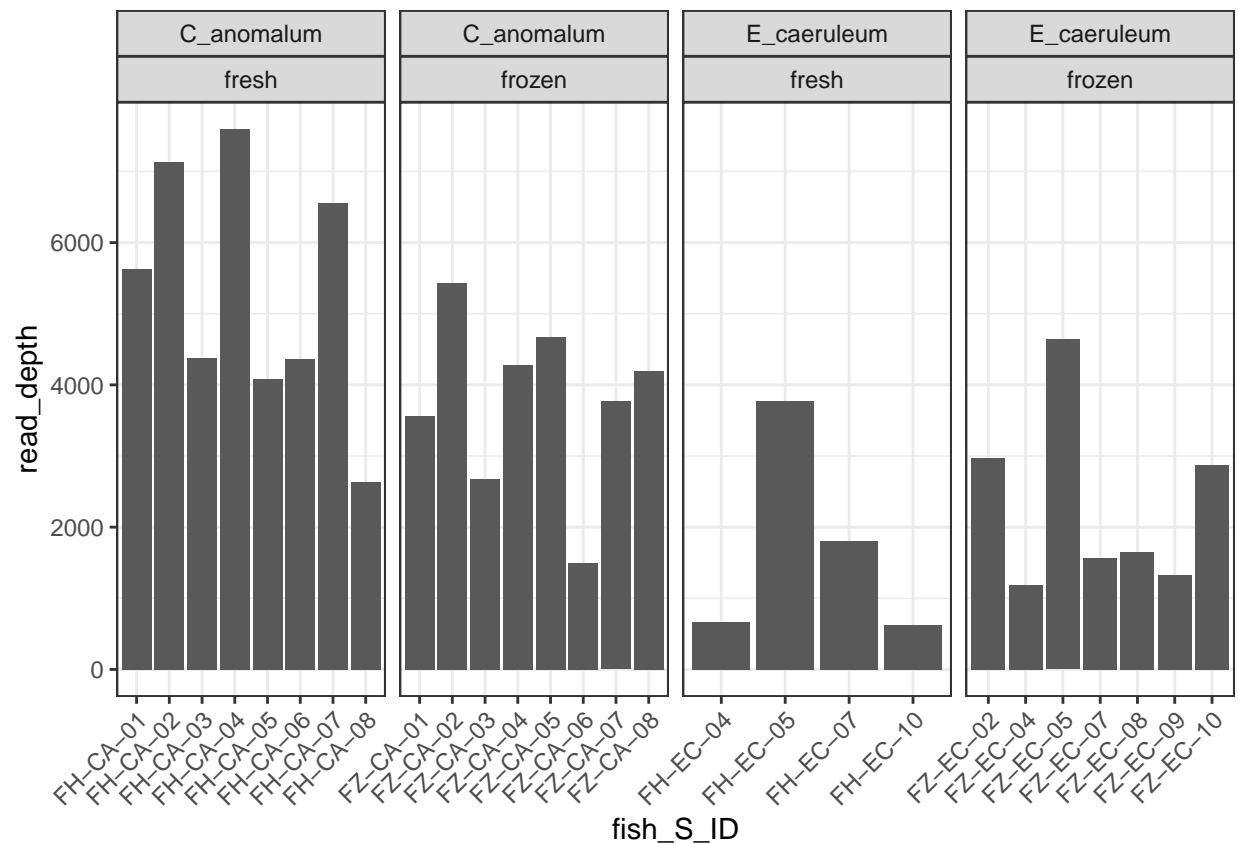
Overview	1
Read counts per sample	2
Phylum-level breakdown after rarifying	3
Alpha diversity	4
Beta diversity based on Bray-Curtis dissimilarities	6

Overview

This report is a summary of the data analysis results from the pilot study. The primary objective of the pilot study was to test whether freezing the fish prior to gut content removal would impact the resulting microbiome data. To test this, I collected 29 Rainbow darters (*Etheostoma caeruleum*; primarily insectivorous) and 16 Central stonerollers (*Campostoma anomalum*; primarily herbivorous). I immediately removed the gut contents of half of the individuals from each species, froze the other half, and removed gut contents from the frozen fishes 2 weeks later. I also did a small test of directly extracting the gut contents of some of the *Etheostoma caeruleum* individuals rather than washing with PBS, but there washing the gut contents prior to DNA extraction did not make a difference so I only show the washed results here.

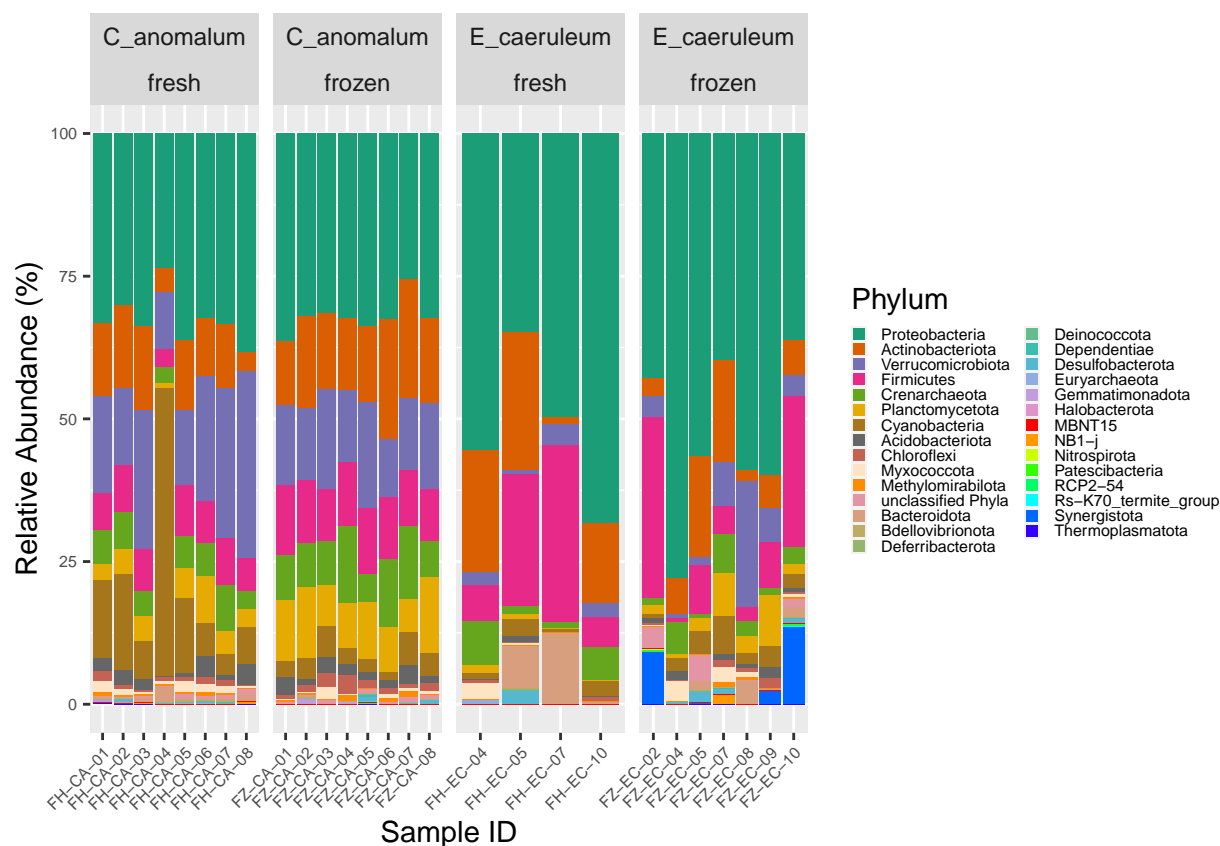
Read counts per sample

I found that the read counts per sample of *C. anomalum* were much higher than *E. caeruleum*. This could be because there was a lot more gut contents to work with in the *C. anomalum* individuals – they were on average a bit larger and their digestive tract is much more extensive. There could also be some sort of inhibition from the insects in *E. caeruleum* guts – I think I'll pass the gut contents through a sieve to remove large chunks of diet items in my future work. Following this, I rarefied all samples to an equal sequencing depth of 365 reads.



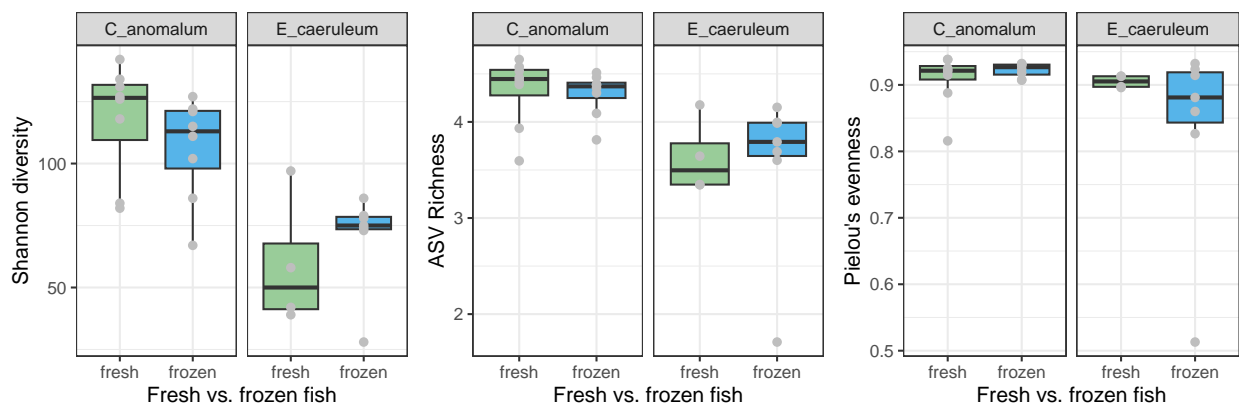
Phylum-level breakdown after rarifying

The major phyla observed in the gut microbiome of these individuals is consistent with other freshwater fish microbiome research. The proportions of different phyla were much more consistent in *C. anomalum* individuals, but this could be a result of higher read counts in these individuals (low read counts = more stochasticity).



Alpha diversity

Richness, Shannon diversity, and Pielou's evenness for fresh vs. frozen samples



ANOVA to test differences in alpha diversity metrics among samples

There were no significant differences in any of the alpha diversity metrics for fresh vs. frozen individuals after controlling for species. Note: The variable “fh_fz” denotes fresh or frozen fish.

ASV richness

Table 1: Analysis of Variance Model

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
fh_fz	1	508.5	508.5	1.04	0.318
fish_species	1	13280	13280	27.16	2.437e-05
Residuals	24	11736	489	NA	NA

Shannon diversity

Table 2: Analysis of Variance Model

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
fh_fz	1	0.1324	0.1324	0.5312	0.4731
fish_species	1	3.253	3.253	13.05	0.001393
Residuals	24	5.981	0.2492	NA	NA

Pielou's evenness

Table 3: Analysis of Variance Model

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
fh_fz	1	0.004098	0.004098	0.6411	0.4312
fish_species	1	0.01718	0.01718	2.687	0.1142

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Residuals	24	0.1534	0.006392	NA	NA

Beta diversity based on Bray-Curtis dissimilarities

There was high overlap in the microbe communities between fresh and frozen individuals of the same species. The two species were quite different from one another.

