We used one species of fish (Yellow Perch *Perca flavescens*) in our mesocosm study to investigate potential differences in those housed in benthic versus pelagic environments. We conducted an 8-week experiment where we collected fish from one pond (source pond) and moved them into treatments in a different experimental pond. We weighed, measured size, and sexed fish from the source pond at the beginning of the experiment, and then did the same in the experimental pond for all fish remaining at the termination of the experiment. We had two primary treatments, pelagic and benthic. Pelagic fish were housed in floating cages near the center of the pond whereas benthic fish were housed in cages anchored to the bottom around the outside “nearshore” of the pond. We hypothesized that fish from either treatment would have access to different prey, and therefore would result in differences in their microbiomes. To analyze microbiome, from the source pond, and at the beginning, middle and end of the experiment, we collected environmental samples that included sediment, water, potential prey (zooplankton and other invertebrates from plankton tows and Ponar sediment grabs), and macroalgae. From the fish, we collected intestinal contents. Separately, we collected stomach contents from each fish for a diet analysis. We also analyzed muscle tissue for stable isotopes (d15N, d13C).

In the attached dataset, there are columns for (1) sample type: fish guts and then multiple environmental samples (invertebrates, macroalgae, sediment, water, zooplankton). (2) treatment type: control (source pond), benthic and pelagic treatments. (3) Treatment ID: Source Pond and Experimental Pond, B1-B3 (3 replicate benthic cages where fish were housed throughout the experiment), P1-P3 (3 replicate pelagic cages). For this, we might look at variability among replicates, but most importantly we want to test differences between the control source pond, and benthic and pelagic treatments. (4) Sex (male, female). (5) stable isotope values (d15N, d13C). (6) various size measurements: standard length, max width, and total weight. (7) Sampling date, which represents the beginning (7/17/2019) and end (9/10/2019) of the experiment. For environmental samples, we also collected at the middle of the experiment (8/8/2019). (8) multiple OTU columns for stomach contents: unidentified cladocera, bosmina, daphnia, ostracod, copepod, trobidiformes, chironomid, and Ephemeroptera.

What we wish to investigate for this study are the following:

1. Evaluate whether host diet diversity and isotopic signatures (d15N, d13C) affect gut microbial diversity. For this, we should consider the interaction with sex and size (See Bolnick et al. 2014)
2. Evaluate if gut microbial diversity is sex- and size- (weight, standard length, max width) dependent?
3. Evaluate among-individual microbial variation (beta diversity and taxon composition) and microbial alpha diversity (between pelagic and benthic treatments, beginning and end dates (7/16/2019) to 9/10/2019) of the experiment, source vs experimental pond)
4. Test whether fish have microbiota characteristic of microbiota characterized for environmental sediment, water, zooplankton, other invertebrates, and macroalgae.

Reference:

Bolnick, D. I., Snowberg, L. K., Hirsch, P. E., Lauber, C. L., Knight, R., Caporaso, J. G., & Svanbäck, R. (2014). Individuals' diet diversity influences gut microbial diversity in two freshwater fish (threespine stickleback and Eurasian perch). *Ecology letters*, *17*(8), 979-987.