Monitoring Recirculating Aquaculture System (RAS) Microbiomes through Shallow Sequencing Metagenomics

Emily Olesin Denny, Marie Montjouridès, Susanna Röblitz, Håkon Dahle
Computational Biology Unit, University of Bergen, Norway

Study Motivation

RAS microbiomes play vital roles in water quality and fish health. Monitoring the composition of microbial diversity in a system can inform RAS users about key players and potential harmful species. We applied the concept of shallow sequencing and metagenomic analysis of DNA biocarrier samples across two modules within a RAS experiment to test the effectiveness of the method for monitoring.

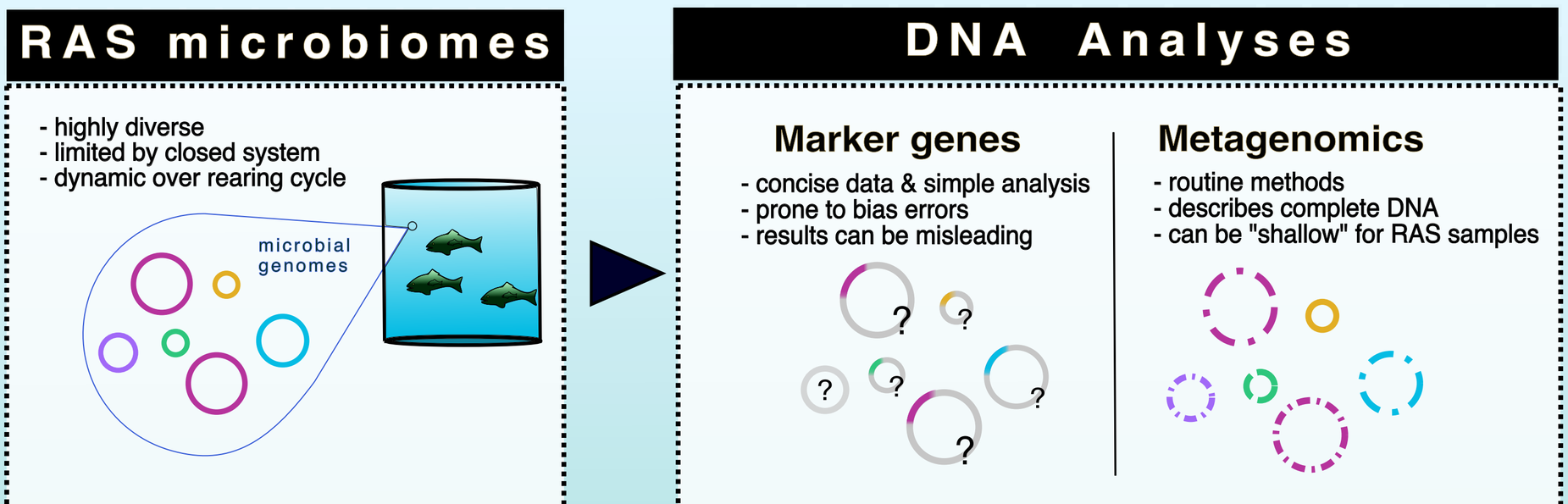
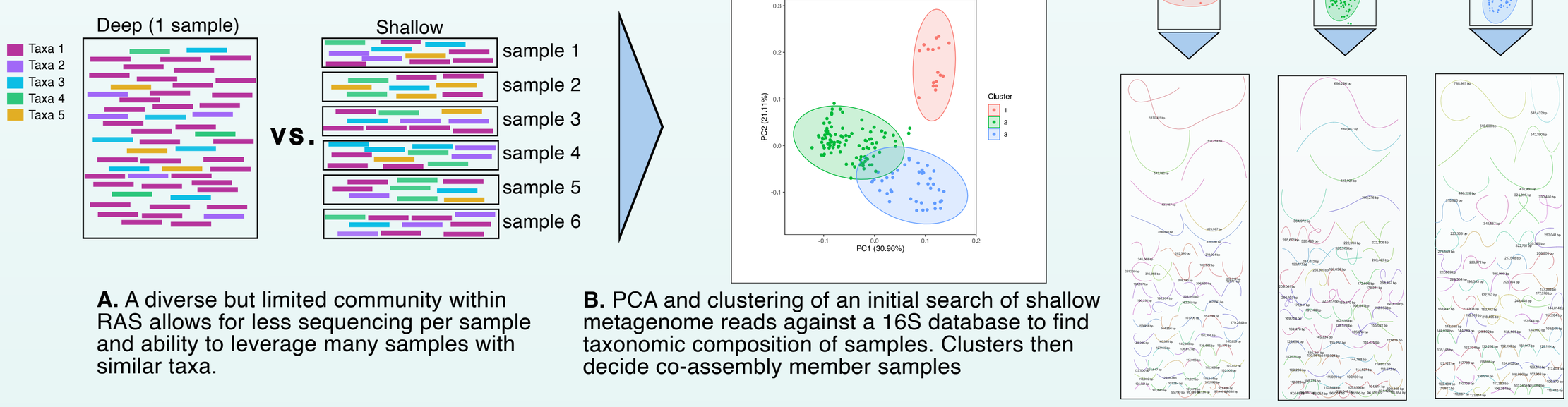


Figure 1. Extracted whole-sample DNA from RAS microbiomes can be analysed further for diversity either by lower resolution amplification-based methods (marker genes), or metagenomics. Using "shallow" metagenomics by combining data from many similar samples can provide efficiency and power for detecting microbial diversity throughout rearing cycles.

Methods



A. A diverse but limited community within RAS allows for less sequencing per sample and ability to leverage many samples with similar taxa.

B. PCA and clustering of an initial search of shallow metagenome reads against a 16S database to find taxonomic composition of samples. Clusters then decide co-assembly member samples

Figure 2. Schematic of the early pipeline steps which differ from typical metagenomic processing in the use of shallow sequencing data that allow for maximizing analysis power. Through qualifying the decision to combine multiple shallow samples by first analysing the community composition similarity, this reduces the chance for mis-assignment of fragments to genomes.

Results

Assembled Genomes

high/medium quality MAGs: **235**
Average completeness: **93%**
Average contamination: **2%**

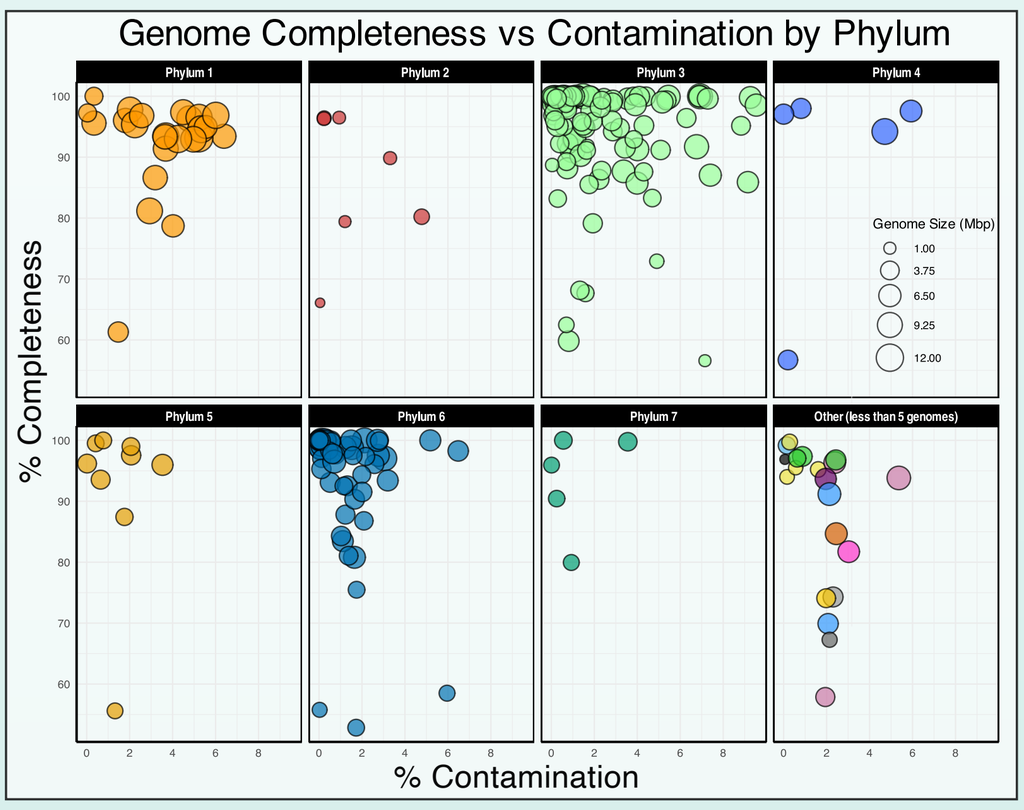


Figure 4. Scatter plot of metagenomically-assembled genome (MAG) quality-related statistics. On the x axis is the percent DNA likely orginating from other taxa in MAGs, and the y-axis is the completeness estimation of MAGs. .

Phylum-level

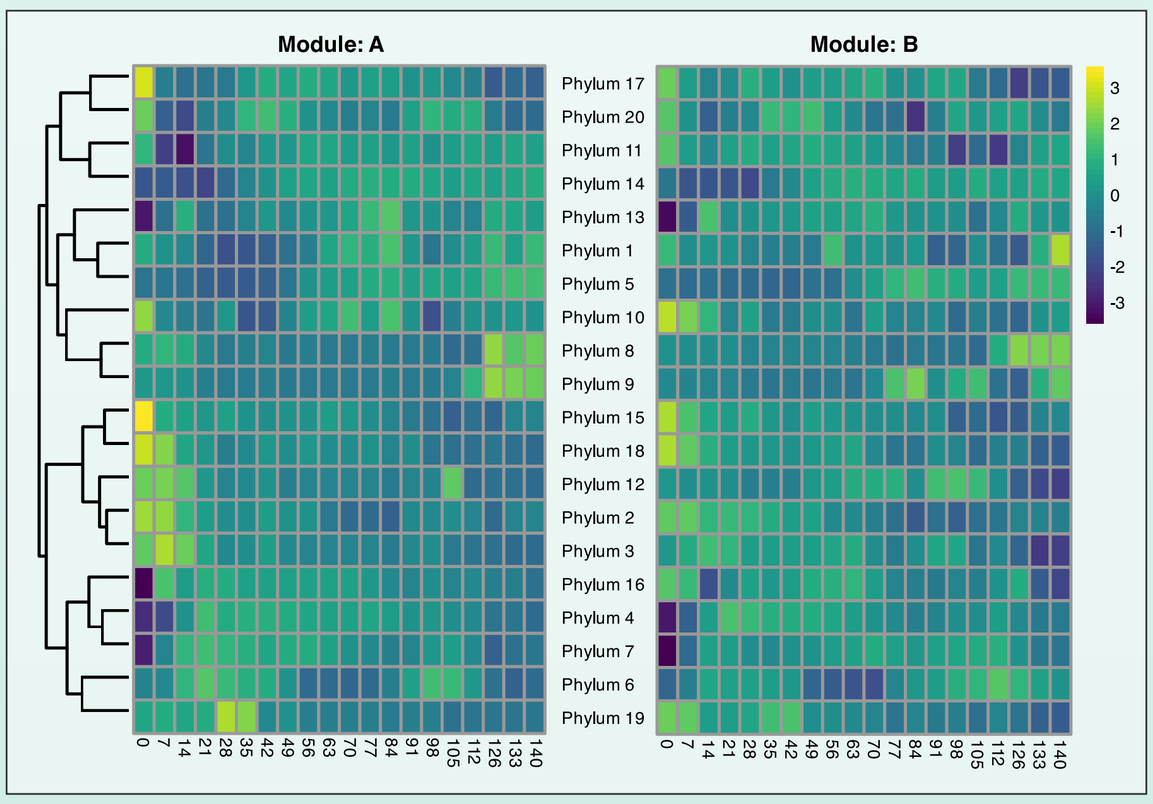


Figure 5. Heatmaps of log-ratio transformed MAG abundances over time grouped by phylum for Module A (left) and Module B(right) of the experiment. An increase or decrease in the log ratio value between days for a taxon can be interpreted as the change in the relative abundance of that taxon. For instance, a log-ratio of 1.5 for a phylum on Day 7 compared to Day 0 suggests that this group's abundance has increased by approximately 2.8 times from Day 0 to Day 7.

Genome-level

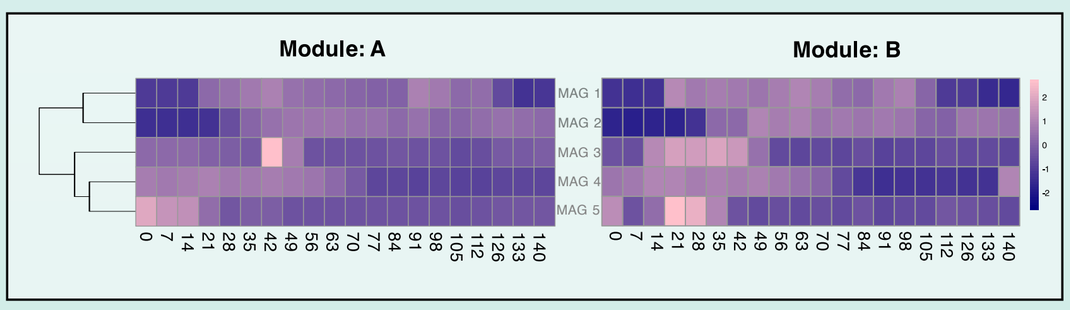


Figure 6. Because the data is binned by genome, we have the ability to track the prevalence of particular genomes over time. Above is an example of genomes of the same genus assembled from the shallow metagenomic dataset.