Questions

1. How can the DNA extraction method affect your metabarcoding results?
2. If you want to investigate either the full prokaryotic community, a fungal clade or eDNA from salmon, which markers would you choose? How does marker selection (18S, 16S, ITS, COI) affects your results?
3. When would you choose to use a short “standard” marker for Illumina sequencing and when would you choose to do long-read metabarcoding?
4. In what type of study or using which markers would you use different error correction and clustering methods (dada2, vsearch, swarm, etc.)?
5. What is the effect of multiple copies of the marker (e.g. in 16S or 18S) in genomes in downstream metabarcoding analyses? What would happen if one species has 10K copies of the 18S rRNA-gene per genome while another species has 100 copies? And if intragenomic variability is present across your marker – how will this affect your results?
6. Which effects have chimeric sequences on your results? What are the characteristics of a chimeric sequence?
7. How does the quality and completeness of the reference databases affect your results?
8. Are phylogenies based on short sequences used in metabarcoding robust? If these have limitations, which are those?
9. In what type of studies may network analyses be useful? What are the limitations of networks?
10. How can extracellular DNA (“dead DNA”) affect your ecological inferences?