



Conference Abstract

Extraction kits could affect the interpretation of metabarcoding results of sediment samples taken around salmon farms

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Abstract

Differences in PCR strategies and errors, sequencing errors and methods used for extractions affect sequence data and potentially its interpretation. These effects could vary based on the target fragments, which are also influenced by limitations of incomplete databases. In this study, we tested the effects of two different proprietary DNA extraction kits on sediment samples, for the purposes of benthic monitoring of salmon farms. The levels of organic enrichment at farms show a gradient from cage edge to more distant locations. The effects of enrichment on benthic communities can be established with metabarcoding.

We collected samples at three salmon farms in Scotland, at varying distances from cage edge. The sediments underneath two of the farms was fine while the sediment under the other was coarse, with a larger mean particle size.

We extracted the samples with two different kits, each using a different mass of sediment – Qiagen DNeasy PowerSoil Pro (0.5 g) and Qiagen DNeasy PowerMax (5 g). We then subjected each extract to three independent PCRs targeting 16S (bacterial) and CO1 (eukaryotic) fragments. The PCR products of samples and blanks were sequenced with an Illumina MiSeq instrument on a single run.

We denoised the sequenced data using DADA2 and rarefied it before analysis (Callahan et al. 2016). The 16S data was annotated against the seven-level SILVA database (Quast 2012). We collated this data at 'Family' level. The CO1 data was filtered to remove Amplicon Sequence Variants (ASVs) present in only one sample and ASVs with a frequency of less than ten reads across all samples. The read count data of family level 16S and ASVs of CO1 were transformed and converted to Bray-Curtis dissimilarity matrices (Bray and Curtis 1957). A permutational multivariate analysis of variance was carried out. We also ordinated these data with non-metric multi-dimensional scaling.

474 bacterial families and 3380 eukaryotic ASVs were included in the analysis. The samples extracted with both kits demonstrated a gradient based on distance from cage edge. This gradient in sampling stations was observed with both the 16S and CO1 markers. Data from both markers and kits showed a greater distinction between cage edge stations and more distant stations in the farms characterised by fine sediment. The two extraction kits showed similar trends but differed in their results.

The 16S data showed a separation of samples by extraction kit along the y-axis. PowerMax extractions were associated with higher values on the y-axis (Fig. 1). The multivariate analysis of variance of the 16S data showed that extraction kit contributes to approximately 7% ($p < 0.001$) of variation in data.

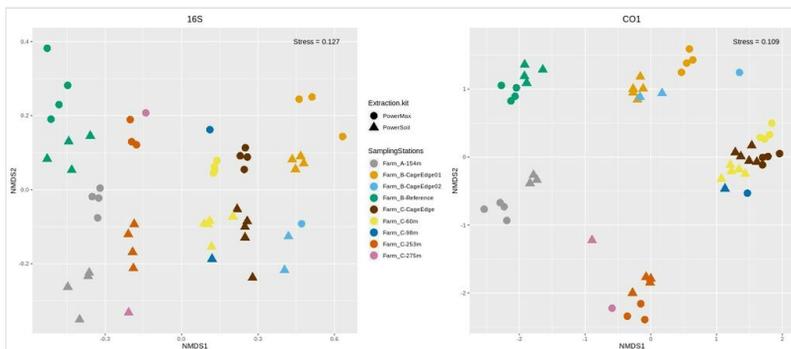


Figure 1. [doi](#)

Ordinations of 16S and CO1 data of sediment samples from farms A, B (fine sediments) and C (coarse sediment) collected at varying distances from cage edge and extracted with two different kits

The CO1 ASV data also showed a grouping of samples of both kits along the x-axis on the basis of distance from the farm (Fig. 1). The CO1 data showed that extraction kits contribute to about 5% ($p < 0.001$) of the variation. The results of the two extraction kits were more similar to each other with the CO1 marker than with 16S. The greater axes values and grouping in the CO1 ordination, indicate that it is able to split farms and distances better than 16S.

We show that both extraction kits demonstrated a gradient according to distance from the cage edge. However, there was a systematic difference between the extraction kits.

Variability due to kit was greater with the 16S marker despite it including fewer bacterial families than CO1 ASVs. We recommend that the same extraction kit be used to develop protocols for monitoring of fish farms with metabarcoding. Though both kits demonstrate the same major trend, subtle differences may not be distinguished. These variations between the kits could influence the results and interpretation of metabarcoding.

Keywords

Extraction kit, metabarcoding, 16S, CO1, salmon farms

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