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High-throughput eDNA surveys for benthic monitoring of salmon farms in Norway: a validation study (FHF grant 901092)

Draft report of scientific panel

Content:

- Presentation of the method and its scientific background
- Discussion of advantages and main challenges of the method
- Recommendations for its implementation in routine monitoring
- Annexes
- References

Introduction

Environmental (e)DNA metabarcoding uses genomic (g)DNA obtained from sediment samples as a source of information for the composition of benthic communities and its response to anthropogenic impacts. The eDNA is extracted from surface sediment samples and amplified using genetic markers that target the whole benthic diversity. The amplified markers are sequenced using high-throughput sequencing (HTS) technologies such as Illumina. The obtained DNA sequences (= eDNA metabarcodes) are analyzed using a set of bioinformatic tools to infer or predict biotic indices, and to provide an inventory of species present in the original sediment samples.

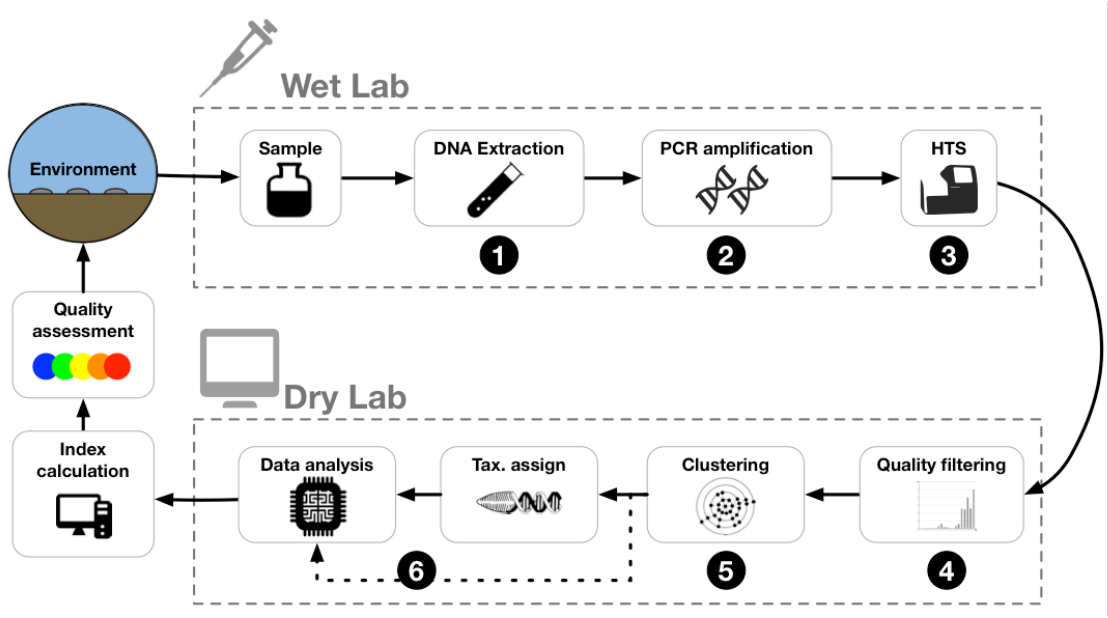

Figure 1. Schema of key steps in DNA metabarcoding applied to bioassessment (Pawlowski et al. 2018)

The method is currently applied in different fields of environmental impact assessment and biomonitoring, such as (1) detection of invasive alien species (Kelly et al. 2014, Herder et al. 2014), (2) assessment of water quality (Visco et al. 2015, Zimmermann 2015, Apothéloz-Perret-Gentil et al. 2017), or (3) biodiversity surveys of threatened aquatic ecosystems (Valentini et al. 2016, Taberlet et al. 2018). The development and testing of new eDNA-based tools are subject to intense research involving ecologists, marine biologists, limnologists, taxonomists and molecular biologists. These efforts are coordinated at European level, through COST Action DNAqua-net (http://dnaqua.net), which aims are to promote the development and implementation of eDNA methods for biomonitoring of aquatic ecosystems (Leese et al. 2018, Hering et al. 2018, Pawlowski et al. 2018).

The application of eDNA metabarcoding to benthic monitoring of salmon farms has been evaluated by several research projects conducted in Scotland, Norway, New Zealand and Canada during the last five years. These studies aimed at assessing the impact of organic enrichment associated with salmon farms activities by analyzing the diversity and composition of different benthic taxa, including bacteria (Dowle et al. 2015, Stoeck et al. 2018a), foraminifera (Pawlowski et al. 2014, 2016, Cordier et al. 2017), ciliates (Stoeck et al. 2018b, Forster et al. 2018), and metazoans (Lejzerowicz et al. 2015). Some studies proposed multitaxon approach to infer a new metabarcoding index (Keeley et al. 2018). Other studies used supervised machine learning technique to predict biotic indices from sequence data (Cordier et al. 2017, 2018). In brief, three different metabarcoding approaches have been proposed to infer/predict biotic indices:

- **Macrofauna eDNA:** Inferring traditional biotic indices based on DNA sequences assigned to known benthic macro-invertebrate species;
- **Multi-taxa index:** Development of biotic indices based on the identification of new bioindicator taxa (foraminifera, ciliates, meiofauna, bacteria, multi-taxa);
- **Machine learning:** Predicting biotic indices using a supervised machine learning (SML) approach and macrofauna data as training datasets

All studies conducted and published thus far agree that eDNA metabarcoding represents an accurate and reliable tool to assess ecological quality of sediments. The obtained results show strong congruence and pattern-matching between biotic indices inferred from molecular data on one hand and morphological data on the other hand. Metabarcoding studies also provide semi-quantitative information about benthic community, which assemblage is dominated by meiofauna and microbial species.

Advantages and challenges

The main advantage of using eDNA metabarcoding compared to traditional morphology-based approach consists in reduced time and costs of sample collection and analyses. The process of sampling is much faster and easier, reducing the time spent on sieving the samples on board. The average time for samples processing using molecular approach is less than one month. The preservation and transportation of small DNA samples do not require large volumes of toxic fixatives or any other particular precautions. All steps of molecular protocols can be standardized and automatized, reducing probability of errors. The identification of species is done based on public reference libraries and does not depend on

personal taxonomic expertise. Moreover, compared to traditional studies that assess the ecological status based exclusively on benthic macro-invertebrates, the metabarcoding can provide information about global biodiversity changes, including various taxa composing benthic community. The method could also be used in the sites where macrofauna is limited (hard-bottom or high-energy sites) and where small volumes of sediments would be sufficient for microbial analyses.

Main challenges of the method, regarding its implementation, are related to the specificity of metabarcoding data, that comes from molecular protocols. While the laboratory techniques are relatively well advanced, and the data analyses methods are widely available, the interpretation of metabarcoding data may present some major issues. The DNA sequences provide mostly information about microbial organisms that cannot be seen or genetic types that cannot be morphologically distinguished. Our knowledge about ecology and relative sensitivities of these inconspicuous organisms or genotypes is relatively limited in comparison to the traditional macrofauna species. However, the sensitivity of some groups to environmental changes is widely recognized, and there are no scientific reasons to consider them as of lower value as bioindicators compared to benthic macrofauna. On the contrary, the information provided by metabarcoding data is complementary to the traditional macrofaunal surveys and could reach the same accuracy for impact assessment, if it is correctly calibrated on reference biological or geochemical data.

Other challenges concern the reproducibility and standardization of methods used for acquisition and analysis of metabarcoding data. In general, the published studies described in detail the protocols that have been used and provide access to data deposited in public repositories. Although some studies used in-house developed pipelines for data analysis, the components of these bioinformatic pipelines are usually publicly available. There exists a variety of tools used for the analysis of metabarcoding data, especially for clustering and taxonomic assignment, yet these tools are well known, and their reliability can be tested. Similarly, the algorithm used for machine learning prediction of biotic indices is public and well-established. The reference database of benthic macrofauna DNA barcodes is available on BOLD and GenBank. Further standardization of protocols is possible, as it is currently done in the case of monitoring fish populations and benthic diatoms (proposals submitted to the European Committee for Standardization). However, such standard conditions have to be relatively flexible, because of rapid development of DNA sequencing technologies.

Recommendations

Currently, the method can be used for ASC surveys of salmon farms in Norway, based on a variance request submitted by Marine Harvest. To comply with ASC regulations, the analyses of eDNA should provide evidence that the faunal/biotic indices scores (Shannon, AMBI, BQI, or ITI) are indicating good to high ecological quality in sediment outside the AZE (indicator 2.1.2) and should demonstrate the presence of DNA sequences assigned to non-pollution macrofauna indicators among the abundant species (indicator 2.1.3).

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Based on large number of scientific publications, we see potential of the eDNA metabarcoding approach to be used as an alternative tool for benthic monitoring required by MOM-C surveys. The majority of biotic indices required by MOM-C can be inferred or predicted from eDNA multi-taxa data, as in the case of ASC surveys. Moreover, the list of macro- and meiofauna indicator species can be provided based on DNA barcodes (COI) identification.

The method can be considered as sufficiently mature to be implemented at staged manner. To ensure high quality of results, we suggest that the conditions to conduct metabarcoding surveys be clearly defined. The recommended lab protocols and analytic tools should be described in the guidelines to be prepared as part of the FHF project. The members of the panel shall be involved in guidelines preparation. The guidelines shall also indicate the best practices for preservation of DNA samples and repository of metabarcoding data. We recommend interlaboratory comparison to demonstrate reproducibility of results. We recognize that genetic biomonitoring is rapidly evolving technology and new sequencing platforms could be available in the future. We recommend that any substantial changes in technology should be evaluated with reference to existing technology and traditional taxonomic approaches.

Moreover, we propose that each farm, or a representative farm of a given geographic location, should be tested at least once in parallel for molecular and morphological analyses. This would allow to properly calibrate metabarcoding methods in their accuracy with reference to geochemical and biological (macrofaunal) data. Additionally, this would constitute a benchmark dataset against which future methods could be tested, and could help developing a set of new bioindicator taxa. We suggest, that the new method should be introduced gradually, and in the introductory/transition period, morphotaxonomic analyses shall be retained in proportion of stations until sufficient confidence is reached. Further periodic or random comparison between macrofaunal inventories and eDNA metabarcoding data could also be considered.

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Annex. 1. The list of publications dealing with eDNA benthic monitoring of salmon farms

Reference	Taxonomic group	Marker	DNA/RNA	Scope
Cordier et al. 2017	Foraminifera	18S 37F	DNA	Machine learning to predict biotic indices
Cordier et al. 2018	Multi-taxon	16S V3V4 18S V1V2,V4, V9, 37F	DNA	Machine learning vs taxonomic assignment
Cordier et al. 2018	N/A	N/A	N/A	Biotic index calculation from species table
Dowle et al. 2015	Bacteria	16S	DNA/RNA	Correlation between bacterial community and redox and TOM
Keeley et al. 2018	Multi-taxon	16S V3V4, 18S V4, 37F	DNA/RNA	Multi-trophic index
Lejzerowicz et al. 2015	Metazoa	18S V4	DNA/RNA	Correlation with AMBI and ITI
Pawlowski et al. 2014	Foraminifera	18S 37F	DNA/RNA	Forams diversity vs distance and redox
Pawlowski et al. 2016	Foraminifera	18S 37F	DNA/RNA	Forams diversity vs macrofauna indices
Pochon et al. 2015	Foraminifera	18S 37F	DNA/RNA	Forams bioindicators
Stoeck et al. 2018	Bacteria	16S V3V4	DNA	Bacterial diversity vs macrofauna indices
Stoeck et al. 2018	Ciliates	18S V9	RNA	Ciliates diversity vs macrofauna indices
Forster et al. 2018	Ciliates	18S V4, 18S V9, 28S D1, 28S D2	DNA/RNA	Evaluating best genetic regions and molecule type for monitoring compared to macrofauna indices

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