Environmental monitoring through next-generation sequencing metabarcoding: assessing the impact of fish farming on benthic macro- and meiofauna communities

Speaker: Tomas Cedhagen

JAN PAWLOWSKI,* PHILIPPE ESLING,*† FRANCK LEJZEROWICZ,* TOMAS CEDHAGEN‡ and THOMAS A. WILDING §
*Department of Genetics and Evolution, University of Geneva, Sciences 3, 30, Quai Ernest Ansermet, CH-1211, Geneva 4, Switzerland,
†IRCAM, UMR 9912, Université Pierre et Marie Curie, Paris, France,
‡Department of Biological Sciences, Aquatic Biology, Aarhus University, Aarhus, Denmark,
§Ecology Department, SAMS, Scottish Marine Institute, Oban, Argyll, UK
**What is the NGS eDNA survey?**

1. Detecting selected species (PCR, Sanger sequencing)

2. Inventory of global diversity using next generation sequencing (NGS)

*Identifying particular species or community of species present in environmental samples*
The problem

Current benthic monitoring is exclusively based on morpho-taxonomic species identification, i.e.:

- it requires an excellent taxonomic expertise,
- it overlooks the morphologically indistinguishable juvenile and life-cycle stages of macrofauna and small-sized organisms (meiofauna, protists)
- it is time consuming, and
- it is expensive
- it is today a limiting factor for certification.
The objective

Replacing the morphotaxonomic inventories by NGS eDNA surveys!
Application of NGS eDNA surveys for benthic monitoring of salmon farms

The NGS eDNA tests were based the genetic inventory of:

- Foraminifera (18S rDNA 37f region)
- Metazoa: macro- and meiofauna (18S rDNA V4 region)
Sampling

In most of the studies the eDNA and eRNA samples were taken in parallel to traditional macrofauna samples.
Sampling

- 2 salmon farms near Oban, Scotland
- 4 salmon farms in Malborough Sounds, New Zealand
Objective: Evaluate the use of benthic foraminifera as indicators of fish-farming impact
Foraminiferal species richness increases with distance from cages and sediment oxygenation.
Forams results

Some foram species seem to be good indicators of organic enrichment associated with fish-farming.
Forams results: *Psammophaga n.sp.*

![Graph showing the number of sequence reads for DNA and RNA classes at different distances from cages (m)].

- DNA Class
- RNA Class

Distance from cages (m):
- < 10
- < 20
- < 100
- < 200
- < 1000
- > 1000
Most of common foraminiferal species identified morphologically were recovered by eDNA/RNA approach.

The foraminiferal OTUs/species richness shows correlation to distance to cages and redox values (especially in RNA).

Some foraminiferal species are potentially useful bioindicators of enrichment stage.

Forams Community Index correlates with macrofaunal indices.
High-throughput sequencing and morphology perform equally well for benthic monitoring of marine ecosystems

Franck Lejzerowicz\textsuperscript{1,}\textsuperscript{2}, Philippe Esling\textsuperscript{1,}\textsuperscript{2}, Loïc Pillet\textsuperscript{1,}\textsuperscript{3}, Thomas A. Wilding\textsuperscript{4}, Kenneth D. Black\textsuperscript{4} & Jan Pawlowski\textsuperscript{1}
Metazoan results

Taxonomic composition of samples with taxa identified morphologically (middle panel), and genetically by RNA (upper panel) and DNA (lower panel)
Quantitative analysis of NGS data provides some information about the abundance of common metazoan taxa.
The ITI and AMBI indices inferred from NGS and morpho-taxonomic data provides very similar bioassessment values.
A broad range of metazoan taxa can be identified in eDNA/RNA NGS data. There is a good correlation between morphological and eDNA data for common species (especially Annelida). The values of biotic indices inferred from NGS data are very similar to the values recovered by benthic macrofauna surveys.
## Advantages of NGS eDNA surveys

<table>
<thead>
<tr>
<th></th>
<th>eDNA/RNA</th>
<th>Morphotaxonomy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>time</strong></td>
<td>fast (1-2 weeks)</td>
<td>slow (&gt; 3 months)</td>
</tr>
<tr>
<td><strong>material</strong></td>
<td>2-5 g samples</td>
<td>grab sample</td>
</tr>
<tr>
<td><strong>coverage</strong></td>
<td>high (many replicates)</td>
<td>limited</td>
</tr>
<tr>
<td><strong>biodiversity</strong></td>
<td>macro-, meiofauna, protists, bacteria</td>
<td>macrofauna</td>
</tr>
<tr>
<td><strong>identification accuracy</strong></td>
<td>depend on reference database</td>
<td>depend on taxonomic expertise</td>
</tr>
<tr>
<td><strong>sensitivity</strong></td>
<td>higher</td>
<td>lower</td>
</tr>
<tr>
<td><strong>workflow</strong></td>
<td>automatized</td>
<td>manual</td>
</tr>
</tbody>
</table>
Main goals:
• to adapt the sampling procedure to enhance the presence of macrofauna in NGS data (by working on sieved samples)
• to develop a new category of bio-indicators (meiofauna, forams), which will fit better to the NGS eDNA surveys (sediment samples),
• to improve the accuracy of NGS data analysis
Thank you very much