

METAMON

High-throughput metabarcoding of
eukaryotic diversity for environmental
monitoring of marine sediments

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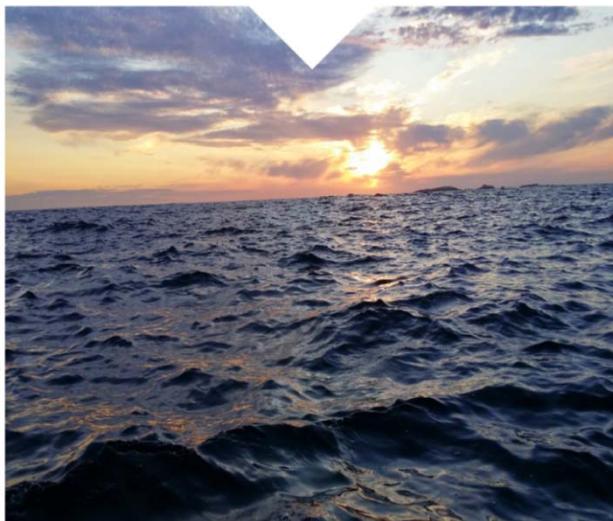
14. FEBRUAR 2019



Regulatory framework



Miljøovervåking av petroleumsvirksomheten til havs



Veileder 02:2018

Klassifisering av miljøtilstand i vann

Økologisk og kjemisk klassifiseringssystem
for kystvann, grunnvann, innsjøer og elver

www.vannportalen.no



INTERNATIONAL
STANDARD

ISO
16665

Second edition
2014-01-15

Water quality — Guidelines for
quantitative sampling and sample
processing of marine soft-bottom
macrofauna

*Qualité de l'eau — Lignes directrices pour l'échantillonnage
quantitatif et le traitement d'échantillons de la macrofaune marine
des fonds meubles*



Reference number
ISO 16665:2014(E)

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Background

- Parameters for assessing environmental status.
- Biological indicator: taxonomical ID of mesh fraction of benthic biota.
- Species lists form basis for calculating diversity and sensitivity indices.
- Work intensive, and relies on skilled taxonomists.



METAMON objectives



- Compare metabarcoding to morphological taxonomic analysis for biodiversity assessment
- Explore data for taxa as possible bioindicators
- Develop robust protocols and guidelines for metabarcoding methodology
- Calculate proportion of metazoan taxa missing in public sequence databases
- Increase number of COI barcodes for common metazoan taxa

Funding and partners



- NFR Petromaks2 program
- Equinor
- Total
- Timeline: 2018-2021

Sampling

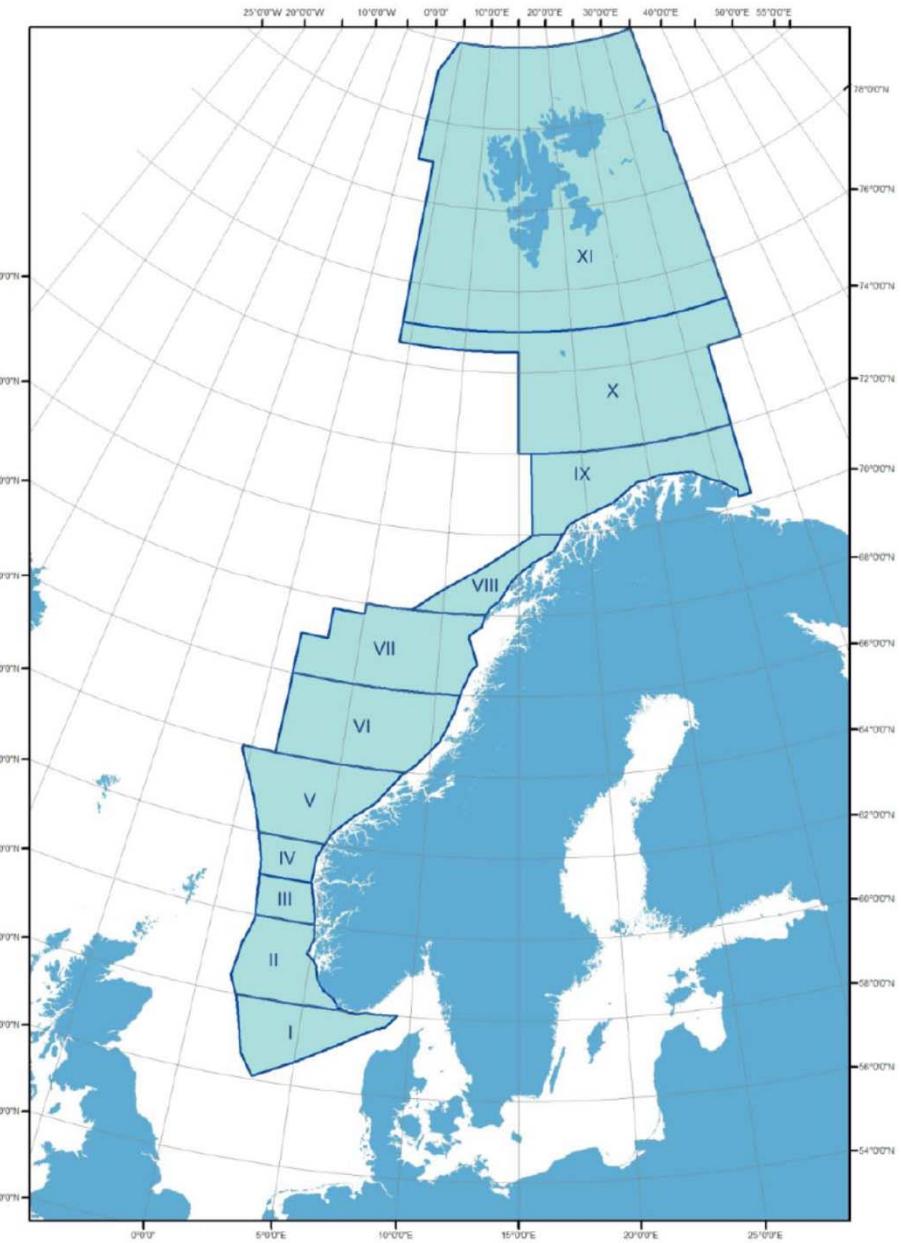


- Additional sediment samples from grab/box corer (top 2 cm) collected as part of environmental surveys in the North and Barents Seas.
- Allows comparison of metabarcoding results with other parameters in the current monitoring programs, including historical data (DNV GL MOD database).



Sampling

- North Sea region II (2018, 144 samples), Barents Sea region IX-X (2019) etc.



Methodology



- Frozen sediment samples
- DNA extraction
- 18S V1-V2 and COI sequence markers
 - Eukaryote, comparing metazoan data (COI) with data including unicellular eukaryotes (18S)
- HTS Illumina MiSeq amplicon libraries
- Filtering, OTU clustering, taxonomic assignment, statistical analysis of results
- Quantitative analysis of bioindicators (ddPCR)



Pilot study



- Extent to which metabarcoding captures diversity in morphological dataset
 - 18S V7 region partial overlap at higher levels, but complementary rather than a replacement
 - Suggests dual barcoding markers
 - Sample ethanol
 - Suggested alternative to sediment extraction, no good results

- Technical tests
 - Lab protocols, reagents and conditions
 - Number of replicates, amount of sediment, DNA extraction method
- Database coverage
 - Updated species lists (WoRMS) run against GenBank, BOLD, Silva for COI and 18S.. Identify percentage of species with markers present, and candidates for Sanger sequencing
- Ongoing
 - Use results from analysis of replicates and extraction method to sequence larger dataset
 - Comparison of COI and 18S V1-V2 results with each other and correlation to morphological taxonomy, chemical and sediment parameters

