Unattended eDNA sampling on a FerryBox

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What is an automated filtration system and why do we need it?

- Many important Essential Ocean Variables cannot yet be measured by sensors - technology development is needed to be able to collect bulk seawater and particulate (*mainly phytoplankton*) samples for shore-based analyses

- This was accomplished by starting with a FerryBox observing platform coupled to a modified Mclane PPS with reagent addition or freezing (automated filtration)

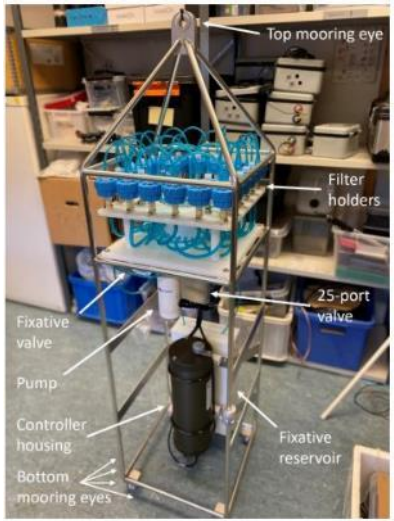
- Developed through national infrastructure NorSOOP project and H2020 NAUTILOS and JERICO-S3 projects

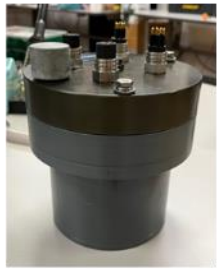
GOOS Essential Ocean Variables

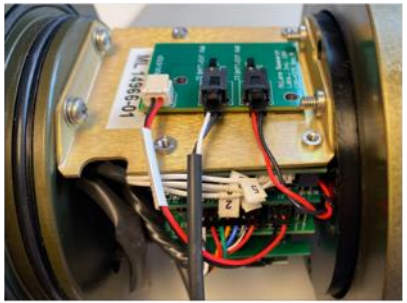
Physics	Biochemistry	Biology and Ecosystems
Sea state		
Ocean surface stress		
Sea ice		
Sea surface height	Oxygen	Phytoplankton biomass and diversity
Sea surface temperature	Nutrients	Zooplankton biomass and diversity
Subsurface temperature	Inorganic carbon	Fish abundance and distribution
Surface currents	Transient tracers	Marine turtles, birds, mammals abundance and distribution
Subsurface currents	Particulate matter	Hard coral cover and composition
Sea surface salinity	Nitrous oxide	Seagrass cover and composition
Subsurface salinity	Stable carbon isotopes	Macroalgal canopy cover and composition
Ocean surface heat flux	Dissolved organic carbon	Mangrove cover and composition
Ocean bottom pressure		Microbe biomass and diversity (*pilot)
Turbulent diapycnal fluxes (*pilot)		Invertebrate abundance and distribution (*pilot)

An automated sample filtration system can be used to collect any suspended material - any phytoplankton variable (POC, PON, POP, BSi, HPLC pigments), C/N isotopes, eDNA/eRNA, particulate metals, etc.

Modifications of the off-the-shelf Mclane PPS (other options available, but PPS is most versatile)







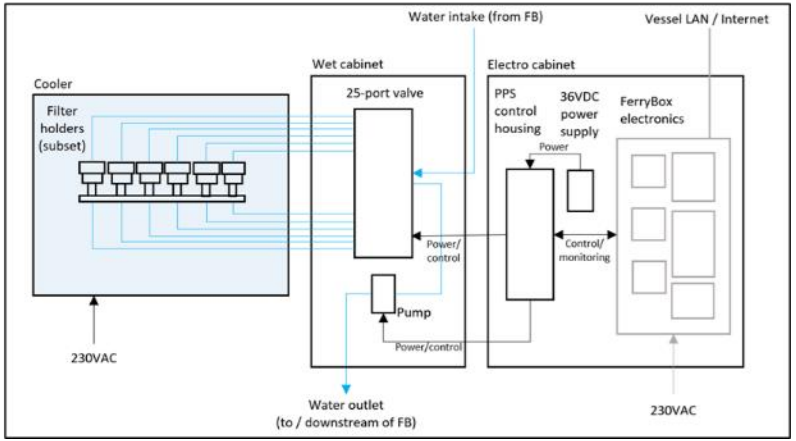
- Detached from mooring frame
- Battery holder & pressure bottle removed
- New bulkhead connector for comms + power
- Dometic CFX3 100 +20 to -20 ºC cooler
  - Wi-fi/Bluetooth monitoring/control
  - Filter holder assembly to be compacted to fit
- Lab tests externally controlled through MATLAB

Pilot studies to test/validate the filtration system

- Modified Mclane PPS deployed on NIVA's MS Color Fantasy FerryBox
- Sampling campaigns carried out for eDNA sampling in December 2023 and April 2024 with ~20 samples/campaign (also PPS x IFCB sampling in June 2024 on Bergen-Kirkenes coastal route)
- Supporting environmental data from the FerryBox also available (S, T, chl, O_2, pCO_2, pH…) and conventional/manual filtration for eDNA as reference
- Eukaryotic phytoplankton DNA analysed at NIVA (metabarcoding [Illumina MiSeq v3] and qPCR)

FerryBox integration

- Control/monitoring by LabVIEW in Windows IPC over RS-232 serial
- Power supply: 230 VAC/18-36 VDC
- Sample triggering based on
  - Vessel position
  - Data from other sensors
  - Remote control
- Water from/to FB manifold
- Sampling steps:
  - Intake flush
  - Sampling
  - Fixation
  - Drying
  - (Cooling)
  - Filter retrieval



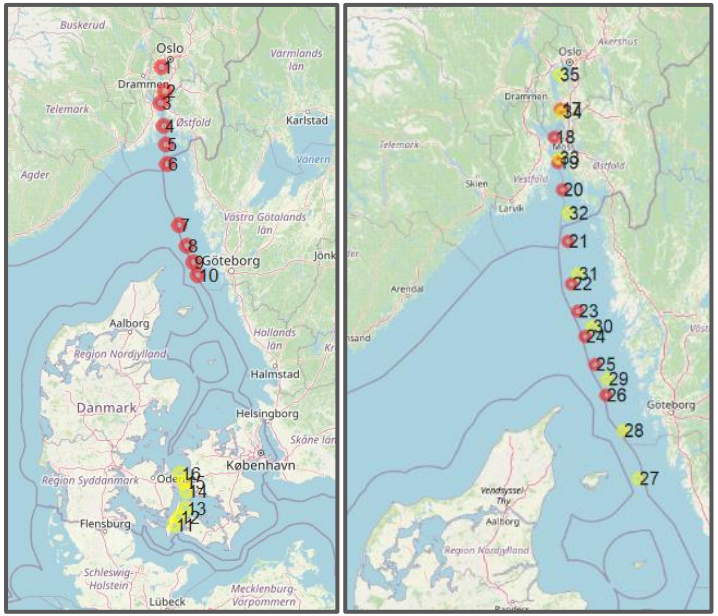
Environmental DNA - benchmarking & demonstration

Methods

- Sampling on two FerryBox cruises Oslo-Kiel (Dec. ‘23 and April ‘24)
- Filtration with benchtop setup done concurrently with PPS filtration (~1 L per sample, ~100 ml min^{-1}, <~10 mm Hg pressure)
- DNA preserved using EtOH (good recovery, low cost, practical) and stored at -20 C
- DNA isolated with commercial kit (Qiagen DNeasy PowerSoil)
- Metabarcoding with Illumina MiSeq v3

Endpoints

- Quantity of eDNA (NanoDrop absorbance)
- Taxonomic composition (metabarcoding)



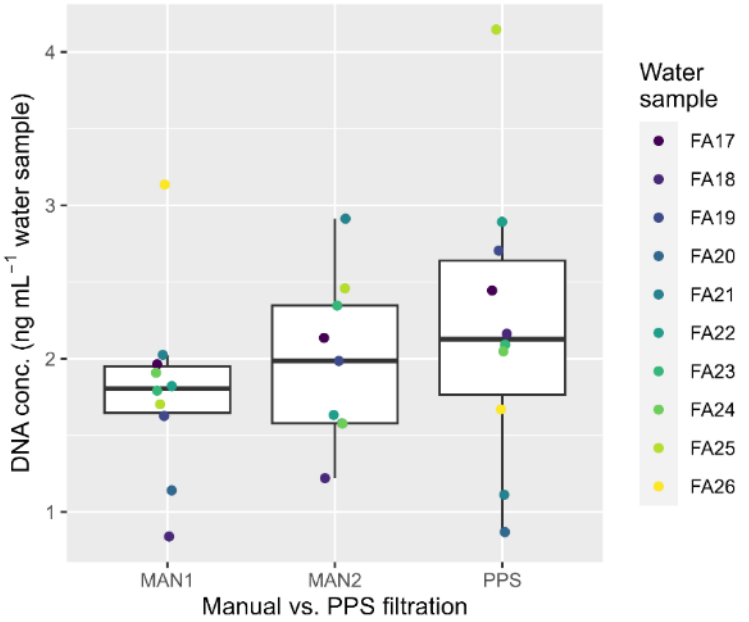
December transects:
red: 5Dec2023,
yellow: 6Dec2023

April transects:
red: 19Apr2024
yellow: 21Apr2024

Environmental DNA - benchmarking

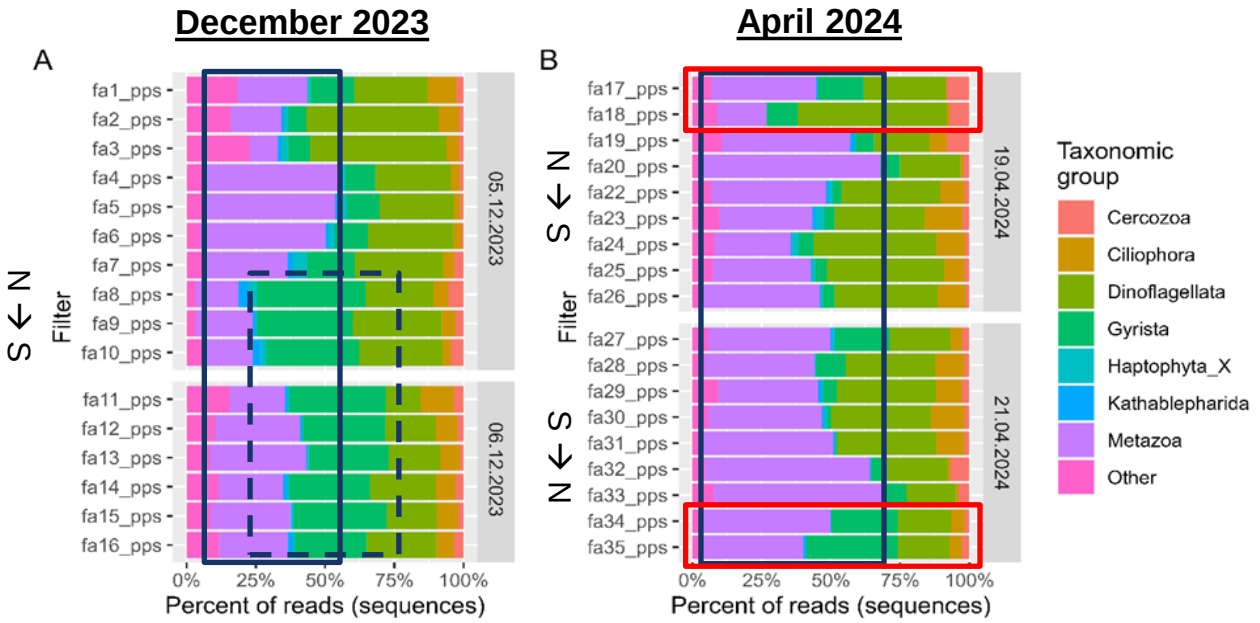
eDNA quantity

- EtOH may dissolve cell membranes → **will the DNA be flushed out of the PPS filter holder?**
- April samples measured by NanoDrop after DNA-extraction, adjusted for sample volume
- No significant difference between manual or PPS filtration or impact of EtOH flushing



Environmental DNA - demonstration

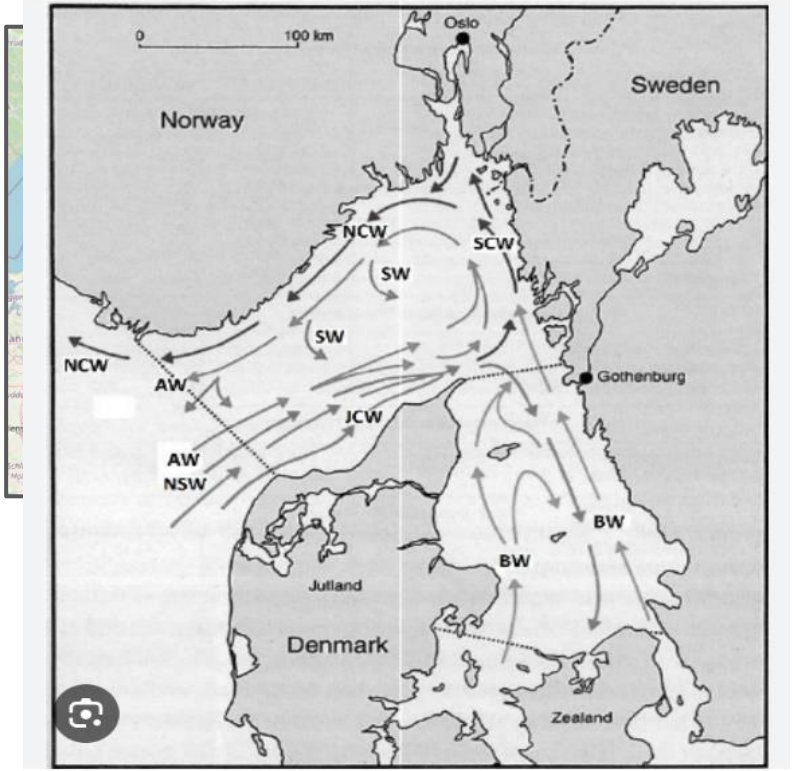
Metabarcoding (Illumina Miseq) - community composition of eukaryotes (aggregated at Division level)

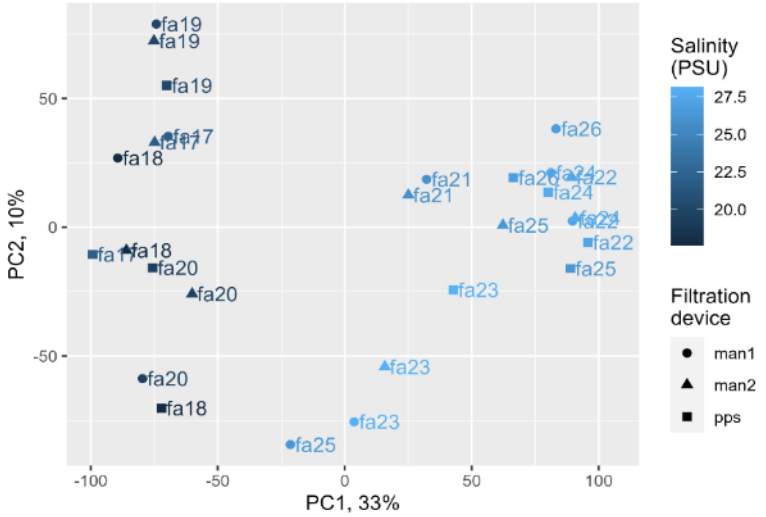


- High proportion (~50-70%) of animal sequences (Metazoa); water not pre-filtered
- December 2023: higher proportion of centric diatoms (Gyrista) reads in southern region
- April: low proportion of diatoms, difference in proportion of dinoflagellates+ciliates vs. metazoa (zooplankton) across the salinity/current boundary

Environmental DNA - demonstration

Metabarcoding - taxonomic composition PCA

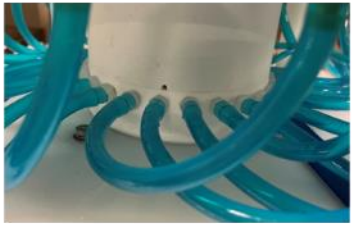


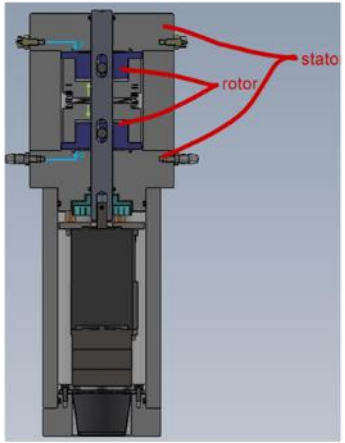


April 2024 transect

Technical challenges/lessons learned

- Careful priming necessary to avoid air in flow path
- Sensitive to intake/outlet pressure
  - Negative ΔP: Air sucked in
  - Positive ΔP: Water leaks
- Switch to piston/overflow at intake
  - Reduces leaks, improves resolution
- Particles unevenly distributed on filters
- TSM challenging, filter rims damaged
- Logistics: Priming and filter retrieval relatively time consuming in FerryBox operational context
- Neutral port filter routine replacement





Concluding remarks

- Automated particulate matter filtration developed, integrated, and tested with a FerryBox platform (modifying/adapting PPS for filtering and preserving samples)
- Field demonstrations in December 2023 and April 2024 for collection of eDNA; will be compared to microscopy samples; June 2024 PPS x IFCB sampling being analysed
- PPS eDNA sampling show spatial and seasonal differences in metabarcoding-based taxonomic composition, similar to benchtop filtration
- Future use for collecting and preserving filters for other EOVs (chl a, particulate organic C/N/P, pigments, phyto/microbe biomass, etc.)

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