Norwegian Environment Agency

eDNA as monitoring method in Freshwater in Norway

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Use of DNA in monitoring in Norway

- The main activity on e-DNA in NEA has been to test the possibility of detecting genetic material from water samples, and compare with metabarcoding of bulk samples collected by traditional sampling.
- A terrestrial insect monitoring project uses metabarcoding as the main taxonomic method.
- A project in regulated rivers tries out the water sample method to evaluate the effect of hydropower on invertebrates

eDNA in water

The last ten years:

Developing and tested methods for monitoring in water:

- Sampling- and lab-methods (water volume, filtering procedures, preparations for lab-analyses, development of primers to select specific animal groups).
- When and where to take samples
- Representativity of samples
- Compare genetic methods with traditional sampling and species determination based on morphological characters

Less money or better quality

Will DNA-based methods save money in monitoring?

- No, not at the moment ……, but probably in the future?
- Rather than talking about reduced costs, I want to focus on the possibility of improving the quality of biological monitoring in water by using DNA-based methods.

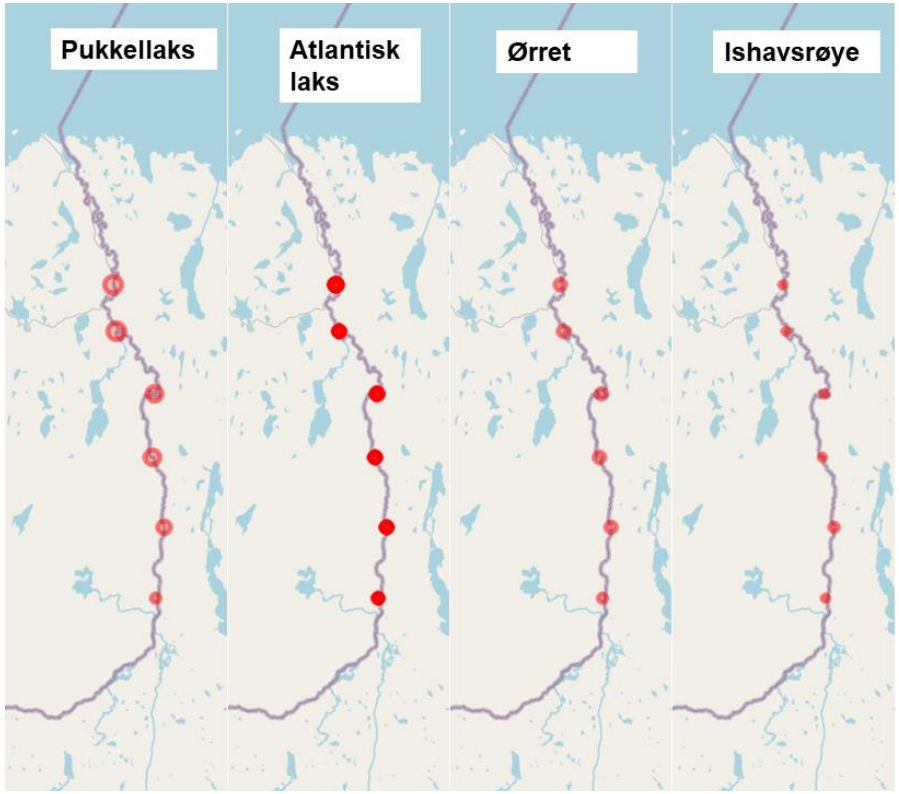
eDNA from Fish

- A project reported in 2021 (NIVA).
- Objective: develop test system for using eDNA as a tool for monitoring and mapping invasive freshwater fish.
- Primers available for most relevant fish-species (salmonids, cyprinids, perch)
- Fish species are well represented in the reference library.
- Fish-DNA are easily detected in water samples, even with very few specimen in the site/lake

eDNA fish

- eDNA do not (yet) give quantitative results,
- the method therefore is most useful to demonstrate presence of a fish species,
- a promising method for mapping invasive species.
- Successfully used in Norway to show the presence of pike upstream the natural pike-distribution and
- to map the distribution of pink salmon (escaped Pacific salmon from Russia) in rivers in Finnmark and Svalbard.

Fish in Grense Jakobselv (Finnmark)



- 1e-8 ng/mL filtrert prøve
- 1e-6 ng/mL filtrert prøve
- 1e-5 ng/mL filtrert prøve
- 1e-3 ng/mL filtrert prøve

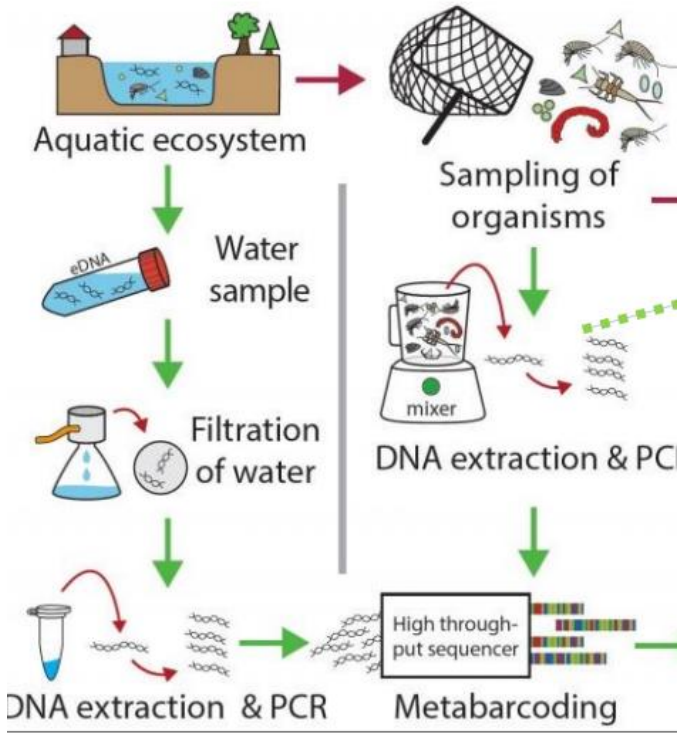
Invertebrates (Crustaceans, Insects, Molluscs)

Report from 2021 (NINA):

- Microcrustaceans, pelagic and litoral in lakes
  - Fewer species were detected with DNA from both water samples and metabarcoding of bulk samples, than detected with traditional sampling and morphological species determination.
  - Many Norwegian species are not represented in the reference library
- For bottom living animals (insects, annelids, molluscs etc) from the litoral of lakes, 67% of taxa were identified to species from water samples and > 80% identified to genus or family.

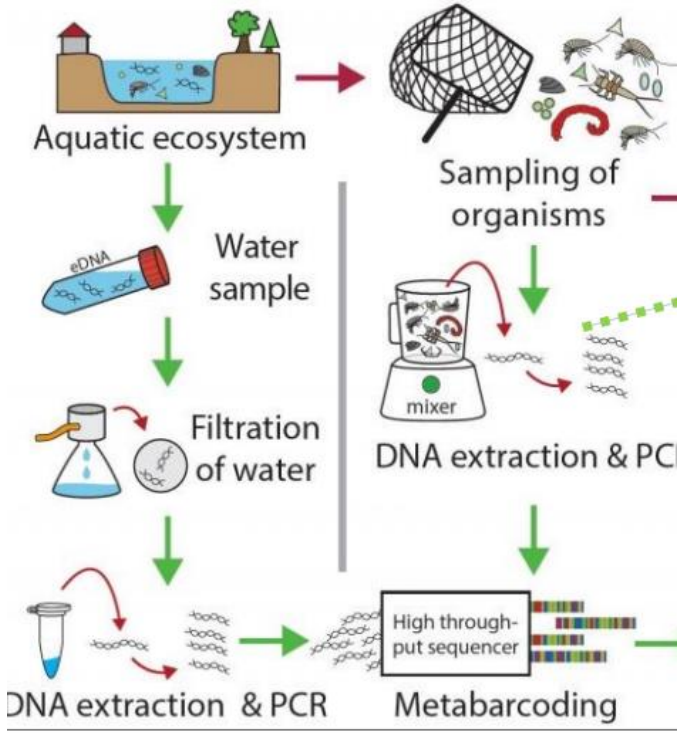
Invertebrates

- Metabarcoding based on bulk samples generally gave good results, 86% of species of bottom animals were recognized, compared to determination based on morphology.
- Water samples gave the highest number of taxa, probably because they represented a larger area of the site than the conventionel samples.



Invertebrates

- Extraction/filtering of DNA from the ethanol used to preserve invertebrate samples also gave a good representation of the species, comparable with metabarcoding of bulk samples.
- This study reported lower representation of species/groups with hard exosceleton, as crustacaens and molluscs.



Classification and DNA-methods

- Status classification using the ASPT-index gave different results using species list based on eDNA from water samples compared to species lists from conventional sampling and determined by morphological characters.
- DNA based methods require revision of biological indeces user in classification.

Invertebrates - Chironomids

- In a study which is not yet reported, the goal was to focus more on chironomids.
- This is a species-rich group of insects with several indicator species.
- A very complex taxonomy, with challenging morphological characters and we have few taxonomic experts. Chironomids are normally determined only to family-level.
- Therefore, this is an insect group which is not included in traditional monitoring or in classification indices of lakes and rivers.

Chironomids

- In spite of their compexity, Chironomids have a good representation in the NorBOL reference library.
- Could eDNA or metabarcoding solve the taxonomic complexity and make possible a use of chironomids in ordinary monitoring?
- 85% (564 of 660) of Norwegian species have a barcode in Barcode of Life Data Systems (BOLD). Most of them have larvae-stages in freshwater.

Chironomids in the Arctic

- Chironomids are common in Arcic areas (70 known species in Svalbard). The ‘monitoring groups’ ephemeroptera, trichoptera and plecoptera, commonly used i classification, have few species i Arctic regions.
- A majority of the Svalbard species have a barcode i the NorBOL reference library.

Chironomids in lakes - results

- 156 species of chironomids were recognised in water samples from Selbusjøen in 2023. 64 species are known from lakes.
- Several species known from running water and terrestrial environments were also detected in the samples from the lake.
- Transport of DNA in rivers is a challenge for the method. Lower parts of rivers have a mixture of DNA-fragments from upstream parts and from tributaries.
- Water samples from a lake will contain DNA transported to the lake from the inlet rivers.

Chironomids in lakes

- Metabarcoding of the bulk samples from the litoral in Selbusjøen detected numerous chironomid species, but low comparability between parallell samples. Explanation: there might still be a challenge to find the appropriate primers for chironomids in the PCR-analyses.
- We are still waiting for the results from Svalbard (two watersheds) and 2023-samples from Selbusjøen will be reanalysed
- We hope to get money to sample another large lake in 2025.

Summary

- Species-specific DNA from fish can be detected i a lake with very few specimen present.
- Most Norwegian fish species are represented in the NorBOL DNA-library.
- Since eDNA do not give quantitative results, the method so far is restricted to detection of the presence of fish species in a site and mapping of invasive fish species.

Summary

- For invertebrates, both barcoding of bulk samples from traditional sampling and eDNA from water samples, are very promising for mapping diversity of species (not quantity).
- Transport of DNA-fragments in running water is a challenge for representativity of samples from specific river stretches, and
- in downstream lakes, because ‘river species’ are transported to the lakes by inlet rivers.

Summary - chironomids

- eDNA and barcoding are promising methods for using the species rich insect group chironomids in monitoring programs.
- Chironomids have a good representativity in the reference library NorBOL (85% of the known species)
- DNA barcoding may ‘solve’ the major taxonomic challenges wich have omitted this indicator-rich group of insects from monitoring and status classification.