Metabarcoding of eDNA from Suspended Particulate Matter (SPM) for fish population monitoring

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1. Introduction

The evaluation of fish populations and biodiversity in aquatic environments is usually performed by time- and cost-intensive studies, and often presents only a snap-shot of the actual fish population. To date, the conventional method used for this purpose is electro fishing. However, monitoring of rare species is often unsuccessful with this method. Furthermore, this method results in increased stress levels for fish and is thus also critical with respect to animal welfare reasons.

Sampling of so called environmental DNA (eDNA) allows for non-invasive species determination and measurement of their DNA abundance. Cellular material carrying the genomic information of a large number of species might be present in the aquatic environment as fish continuously release bodily components like for example excrements, mucus, scales, or even eggs to the water.

Exclusive to the presented study is the sampling of eDNA from suspended particulate matter (SPM), which was sampled at different riverine sampling points across Germany, for a period of more than 10 years, and stored deep-frozen at the Environmental Specimen Bank at the Fraunhofer IME.

Metabarcoding of eDNA samples from SPM was performed by Next Generation Sequencing (NGS), using an amplified fragment of the COI region as barcode.

2. Workflow

Preparation of positive control
- Mix of equal DNA amounts of five fish species: Roach (*Rutilus rutilus*), perch (*Perca fluviatilis*), chub (*Squalis cephalus*), bream (*Abramis brama*), and pike (*Esox lucius*)

DNA extraction from positive control and SPM samples:
- PowerSoil extraction kit (MoBio; modified)

PCR optimization
Primer design:
- Different conserved regions (e.g. COI, Cytb, 12S)

PCR optimization:
- Development of a PCR protocol for amplification of the COI region for application in NGS with the IonTorrent technology
- Fragment size: approx. 150 bp

Sequencing on the IonTorrent platform
- Generation of a NGS library based on purified PCR products
- NGS performance

Data processing and analysis
- Generation of a Fish COI reference database (lead species and species mentioned in the European water framework directive), sequences of approx. 95 different species
- Data generation with the IonTorrent system (.fastq format)
- Development of a processing pipeline

Preliminary results
- NGS of a positive control (DNA from five different species; identical DNA amounts)
- NGS of three SPM samples (Ulm, Koblenz, Güdingen)

Figure 1: Workflow of sample preparation and analysis.

4. Conclusion and outlook

The study for the first time demonstrated the applicability of a metabarcoding approach, using the COI region of fish as barcode, for identification of fish species from eDNA isolated from SPM samples. The methodology allows identification of fishes at least to the level of the subfamily. However, based on results of the positive control, the approach generated a primer bias, with primers preferably binding to sequences of specific fish. Single fish species furthermore remained undetected. In order to improve the approach, new primer sets will be tested. The new approach should reduce the primer bias, allow identification of fish species missed so far, and ideally result in identification down to the species level.

The optimized approach should address the following questions:
- Plausibility (comparison to conventional monitoring approaches)
- Retrospective consideration of the development of fish populations
- Estimation of the abundances based on eDNA
- Detection of non-fish species

Acknowledgements

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3. Results

NGS analysis of the positive control

Table 1: NGS results of the positive control. Of the five introduced fish species, four were detected at the species level, and one at the family level. The read count demonstrate a PCR bias based on different sensitivities of primer binding.

	Introduced fish species	Identified fish species	Reads
1	*Rutilus rutilus*	*Rutilus rutilus*	8220
2	*Perca fluviatilis*	*Perca fluviatilis*	5255
3	*Squalius cephalus*	*Ballerus sapa*	2077
4	*Abramis brama*	*Abramis brama*	464
5	*Esox lucius*	*Esox lucius*	34

NGS analysis of SPM samples

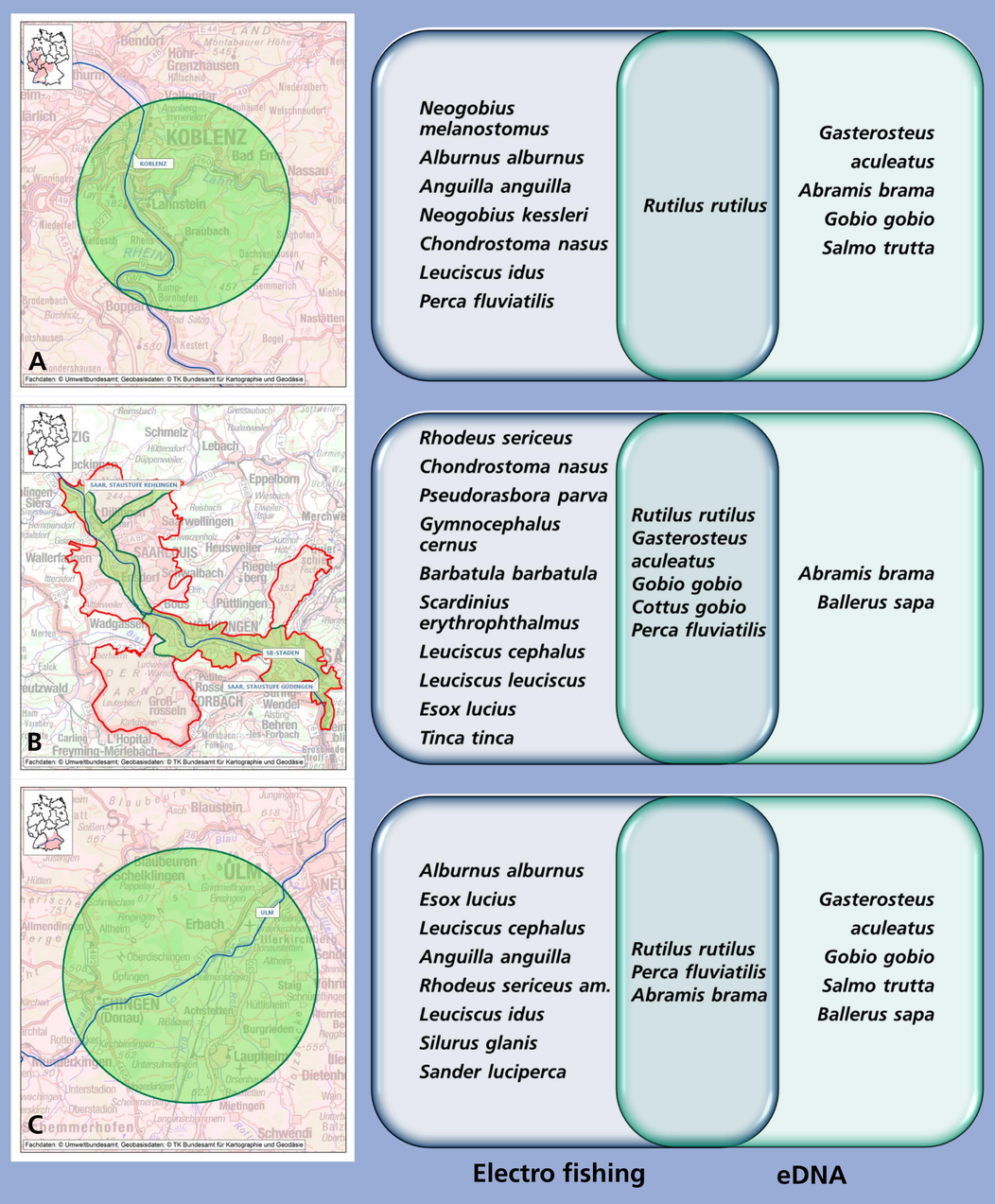

Figure 2: NGS results of the SPM samples (green), compared to results of electro fishing (blue), at the sampling sites (A) Koblenz, Rhine, (B) Güdingen, Saar, and (C) Ulm, Danube. Electro fishing results are from 2015 (Rhine), and 2003 (Saar, Danube). Note that more fish species are detected by conventional methods. However, few species are solely detected by the NGS approach. The number of species detected by both approaches differ between the three sampling sites.

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In this study we used metabarcoding as technique to analyse the whole species community. DNA was extracted from suspended particulate matter (SPM), which was sampled at different riverine sampling points in Germany. SPM samples were retrieved from the German Environmental Specimen Bank (ESB; Umweltprobenbank) which archives annually sampled SPM material since 2005 at temperatures below < 150°C. We assume that the material contains plenty of yet unused information on fish populations and possible temporal developments.

For extraction, a method was developed which resulted in a good DNA yield and purity. We tested different PCR primer pairs, which all bind to conserved regions of the genome. These regions for example encode genes for CytB, 12S, and COI. Primer pairs described in the literature as well as newly designed primers were tested. The final approach, resulting in reliable and repeatable results on diverse SPM samples, was a nested PCR approach with newly designed PCR primers, amplifying a fragment of the COI gene.

The PCR products were analyzed by Next Generation Sequencing (NGS). The results allowed the identification and discrimination of fish species. The data obtained were used for comparisons with fish monitoring data from electro fishing.

With the described approach, we developed a method to reliably identify and discriminate fish species, which allows fish monitoring in a non-invasive way. By using archived SPM samples of the German ESB as starting material, this method allows for retrospective monitoring of fish populations.