

NL Fish Population Scan: characterising fish populations quickly and efficiently using eDNA metabarcoding

The NL Fish Population Scan has recently been developed. This method makes it possible to identify a complete fish population with a single analysis of DNA traces in the surface water. The first results from samples from the river Roer and a comparison with catch data from a fish trap are very promising.

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eDNA: an alternative to traditional fish monitoring

Under the Water Framework Directive (WFD) water managers are obliged to periodically monitor the fish population. In addition, fish surveys are also carried out to meet Natura 2000 legislation. It is not easy to detect rare or hard-to-catch species using traditional survey methods such as electro-fishing and fishing with seines and trawl nets (types of dragnet). These techniques are also labour-intensive (and therefore expensive) and disrupt the fish and their habitat. Detecting fish on the basis of "DNA traces" (environmental DNA or eDNA) in the water (environment) is an alternative. This was investigated in a collaboration project involving KWR, the Brabantse Delta, Limburg and Aa en Maas district water boards, ATKB, Witteveen+Bos and Baseclear.

eDNA and metabarcoding

The eDNA methodology is based on identifying DNA traces left behind in the environment by living organisms. The eDNA in the water particularly comes from excrement, slime and skin or scales. These DNA traces spread in the water and are slowly broken down in around two weeks, depending on factors including the water temperature. DNA will not spread very much in still water, but in flowing water it will spread and therefore dilute further. A good sampling strategy is necessary in order to "catch" the eDNA of all fish species in a water sample. Such a strategy takes account of all ecologically varied habitats in a body of water or river section.

Sampling must take place at the right depth, the right distance from the bank and at multiple locations in the water.

Identical	1 base pair difference	2 base pairs difference	3 base pairs difference
Powan-Houting	Gibel carp- Goldfish	Pike perch- Perch	Rudd-Bleak
Brown/Sea/Brook trout		European Bullhead species	Bleak- White bream
		Salmon- Powan	Silver carp - Bighead carp
		Idé-Dace	Crucian carp- Gibel carp
			Crucian carp- Goldfish

Table 1: Differences in DNA composition of the analysed “barcode” between the most closely related Dutch fish species.

The metabarcoding analysis developed in this study identifies the fish species present on the basis of their unique DNA code. A short DNA fragment of around 110 building blocks of the 16S rRNA gene was selected for this (region 42’ to 45’ of the 16S rRNA gene of fish (from Satoh et al. BMC Genomics (2016) 17:719)). This gene is present in the mitochondria of fish. The composition of this piece of DNA is unique for virtually every fresh water fish species in the Netherlands, and can therefore act as a unique “barcode”. Only with very closely related fish species are the differences small or absent, such as with Trout and Lavaret/Houting (table 1). Using specific DNA techniques, only these pieces of DNA in all samples are selectively multiplied and then analysed using next-generation sequencing analysis (NGS). The DNA sequence of all these fragments was compared with DNA “barcodes” for Dutch freshwater fish in collaboration with Baseclear. The study described here is the first result from an extensive study.

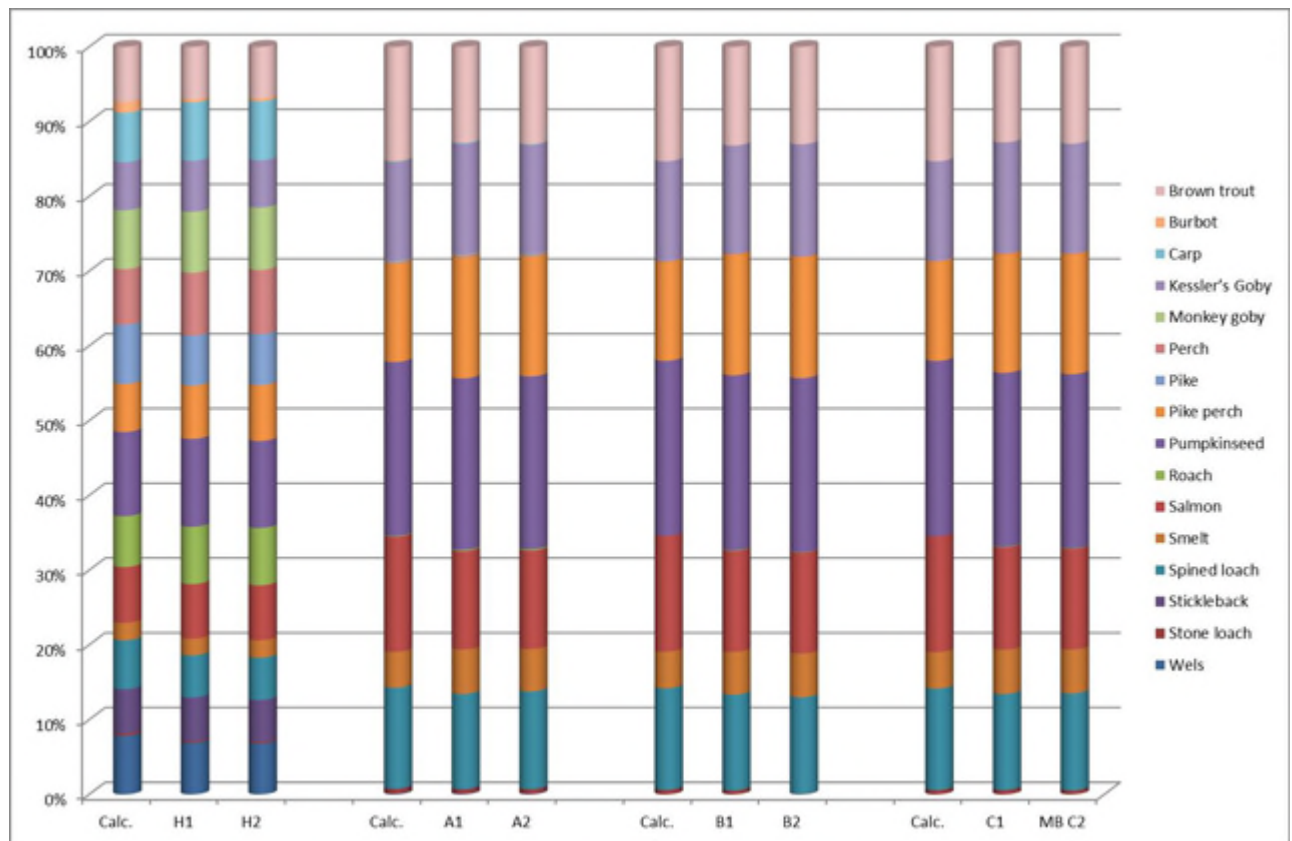


Figure 1: Percentage of the identified DNA fragments in the various metabarcoding analyses of the Mock samples (series H, A, B and C). These series differ in the number of DNA fragments added from each fish species. Series H is made up of DNA from 16 fish species. Series A, B and C included 100 (A), 10 (B) en 5 (C) DNA copies respectively from the fish species Perch, Roach, Carp, Monkey Goby, Three-spine Stickleback, Pike, Burbot and Wels Catfish. The number of DNA fragments for the other fish species in series A, B and C is the same as in series H. The "Calc." column shows the percentage of the DNA of the fish species added in the Mock concerned, and in the two subsequent columns (1 and 2 respectively) the percentage identified barcodes in duplicate analysed with the metabarcoding.

Quality control of the metabarcoding

The quality of each run of the NL Fish Population Scan has been "verified" with a special water sample, a "Mock community". This is an artificial sample containing the DNA of 16 different fish species. This sample is included in every analysis in order to verify the procedure from DNA multiplication through to identification. The sensitivity of the method has also been analysed. The DNA of some of the fish species in the Mock test has been added in very low concentrations. The results from the Mock samples show that all the selected fish species have been detected. The quantities of the identified DNA fragments correspond very well with the initial quantities (see figure 1). "Traces" of 5 to 10 DNA copies were also detected on all Mock samples. The metabarcoding analysis therefore passed this Mock test with flying colours.

Species	Upstream →							DNA	ECI
	OROER905	OROER715	OROER550	OROER520	OROER440	OROER312	OROER195	tot	2009-2014
Asp									81
Barbel									754
Bitterling									6
Bleak									3016
Bream									11019
Brown/Sea/Brook trout									192+406+374 ^b
Carp									33
Chub									272
Dace									1125
Eel									3793
European Bullhead (<i>Cottus peripretum</i>)									542 ^a
European Bullhead (<i>Cottus rhenanus</i>)									542 ^a
Gibel carp									91
Goldfish									1
Grayling									6
Gudgeon									135
Idc									116
Kessler's Goby									6
Lampert ^c									140
Minnow									36
Nase									116
Perch									72574
Pike									96
Pike perch									816
Powan									1
Pumpkinseed									120
Roach									15195
Round goby									2408
Rudd									362
Ruffe									3353
Salmon									8235
Schneider									
Sea lamprey ^c									64
Siberian sturgeon ^c									12
Spined loach									1
Stickleback									34
Stone loach									6
Sunbleak									9
Tench									85
Ten-spined stickleback									3
Topmouth gudgeon									79
Tubenosed goby									382
Wels									48
White bream									192
White-eye bream									7
Number of species	22	26	21	26	24	26	23	34	45

Figure 2: Identified fish species at various locations along the Roer at Limburg district water board compared with the number of individuals caught by species in the period 2009 to 2014 at the ECI weir in Roermond

a. The ECI study makes no distinction between the two hard-to-differentiate Sculpins;

b. The various Trout belong to one species;

c. These species have not been analysed in the metabarcoding analysis.

Practical results

The NL Fish Population Scan was carried out at the three participating district water boards at various locations, particularly in flowing water. A procedure was drawn up for the sampling for slow-flowing and fast-flowing water courses. The initial results from samples from the Roer are now available. We will present the results from the other locations and the comparison with the survey using the traditional, standardised KRW method in a follow-up article (in a subsequent edition of Water Matters). For Limburg District Water Board samples were taken on one day at seven locations in the Roer (see figure 2). A total of 33 different fish species were detected in the seven eDNA analyses. The identified fish species were then compared with the catch data from the fish trap at the ECI power station in the Roer at Roermond. A total of 45 fish species were

found here over a period of 5 years. This one-day metabarcoding sampling detected only eight fish species fewer than in the five-year research period at the ECI fish trap (NB: sturgeon and lamprey have not been included in the eDNA analysis). The metabarcoding detected the Schneider in one water sample. That fish has not been caught at the ECI, but has been detected in KRW fish population sampling in the Roer. For fish species such as the Common Bleak, Perch, Roach, Bream, Eel, Ruffe, Dace and Round Goby the picture from the catches at the ECI (>1000 individuals) corresponds to the picture in the metabarcoding analysis. There was a significant difference between the catches of Salmon and the DNA analysis. This may be because of the migratory behaviour of this fish species, as a result of which it is only present during certain periods of the year. In order to gain better insight into the completeness and reliability of the metabarcoding analysis, the results will be extensively compared at a later stage with the fish species found using the KRW method.

Conclusions

- A good procedure for the sampling of watercourses for eDNA analysis is essential;
- The 16S barcode of the NL Fish Population Scan has a very high differentiating ability for virtually all freshwater fishes that occur in the Netherlands;
- The next-generation sequential analysis (NGS) has been validated: the methodology is reproducible and highly sensitive (≥ 5 DNA copies per sample);
- A Mock test is a valuable addition with which the reliability of the analyses can be established.
- Seven water samples were taken in the Roer in one day and analysed using the metabarcoding methodology. 33 of the 45 fish species caught in five years of research at the ECI weir were detected in the samples. One species, the Schneider, was detected with the metabarcoding analysis and not caught at the ECI weir;
- A comparison of the results of the metabarcoding analysis and the fish species present according to traditional KRW sampling still needs to be carried out. A report on this will follow later.
- The NL Fish Population Scan is an advanced methodology which enables to characterise the species composition of the fish population relatively quickly and cost-effectively. For routine application a standard would have to be drafted which specifies the criteria which a metabarcoding method must meet. A Mock test must be included in every analysis.

This project was a collaboration between the Limburg, Aa en Maas and Brabantse Delta district water boards, ATKB environmental consultancy, consulting engineers Witteveen+Bos, Genomic services Baseclear and KWR Watercycle Research Institute. Funding came partly from the Surcharge for Top Consortia for Knowledge and Innovation (Toeslag voor Topconsortia voor Kennis en Innovatie - TKIs) from the Ministry of

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Summary

Water managers must periodically assess the state of their fish population e.g. for the Water Framework Directive (WFD). Depending on the water type, they measure quantities or biomass data alongside the species composition. New methods that focus on the presence of eDNA (traces) are a cheap and animal-friendly alternative compared to traditional fish population sampling, and possibly also more reliable thanks to a higher chance of detection. A new eDNA metabarcoding has been developed to monitor the species composition of a fish population: the NL Fish Population Scan. The method has been validated, and a water sampling protocol has been drawn up. The initial results show that a wide diversity of fish species has been detected in the analysed waters using this metabarcoding methodology.