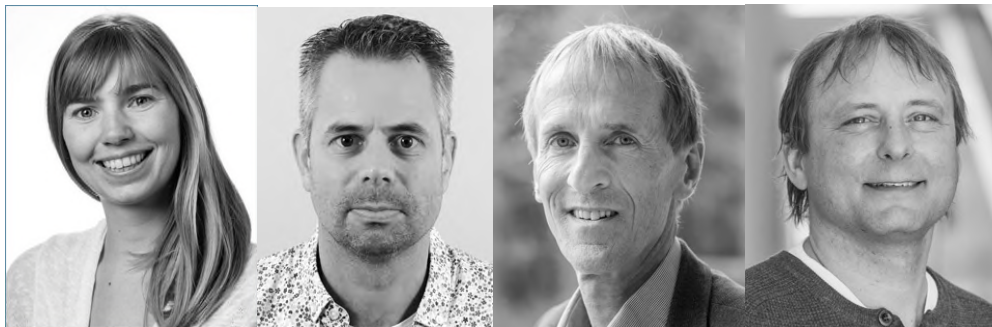


The NL fish population scan: monitoring fish populations using an eDNA approach

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The Water Framework Directive (WFD) obliges water authorities to carry out periodic monitoring of fish populations. New techniques on the basis of environmental DNA (eDNA) potentially form good and more cost efficient alternatives for the monitoring of the fish species composition. In this article results of a newly developed application 'The NL Fish Population Scan (NL Vispopulatiescan)' are presented.

Water authorities are obliged to monitor fish populations. Conventional fish inventory methods are known to have drawbacks: e.g. species are missing, the techniques are highly labour- and cost intensive and the techniques are rather invasive as it disturbs the fish and destroys the habitat.

New methods focussing on the presence of eDNA (traces) are potentially animal-friendlier, easier to standardize, more reliable and a cheaper alternative for determining the species composition of fish populations. In 2017, KWR, Water authority Limburg, Water authority Aa en Maas, Water authority Brabantse Delta, ATKb, BaseClear and Witteveen+Bos developed a new eDNA metabarcoding method, the NL Fish Population Scan. The method and the validation of the method in the laboratory was introduced in the 2nd edition of Water Matters 2017 (Wullings et al. 2017). In this follow-up article, the results of the NL Fish Population Scan are compared with conventional fish population samplings in the waterbodies

Roer, Dieze, Stadse Aa, and Aa of Weerijs.

An important comment regarding this comparison is that both methods are subject to biases due to catch and detection efficiency and the distribution of fish.

Research set-up

In the summer of 2017, in the Roer, Stadse Aa, Dieze, and Aa of Weerijs eDNA and conventional sampling took place simultaneously at 7, 2, 2 and 5 locations respectively. Both the conventional and eDNA sampling took place along a transect of 250 m. One DNA sample consisted of 10-20 subsamples. Furthermore, a control sample (i.e. no DNA present) for the determination of any possible contamination was included. The conventional sampling was carried out according to the guidelines in the national manual (Handboek Hydrobiologie, Bijkerk, 2014). At one location in the Dieze no fish were caught during conventional WFD sampling. At two locations in the Aa of Weerijs, inadequate DNA could be extracted. These locations were therefore excluded from this study. The available results were merged and compared on the level of the waterbodies.

eDNA and metabarcoding method

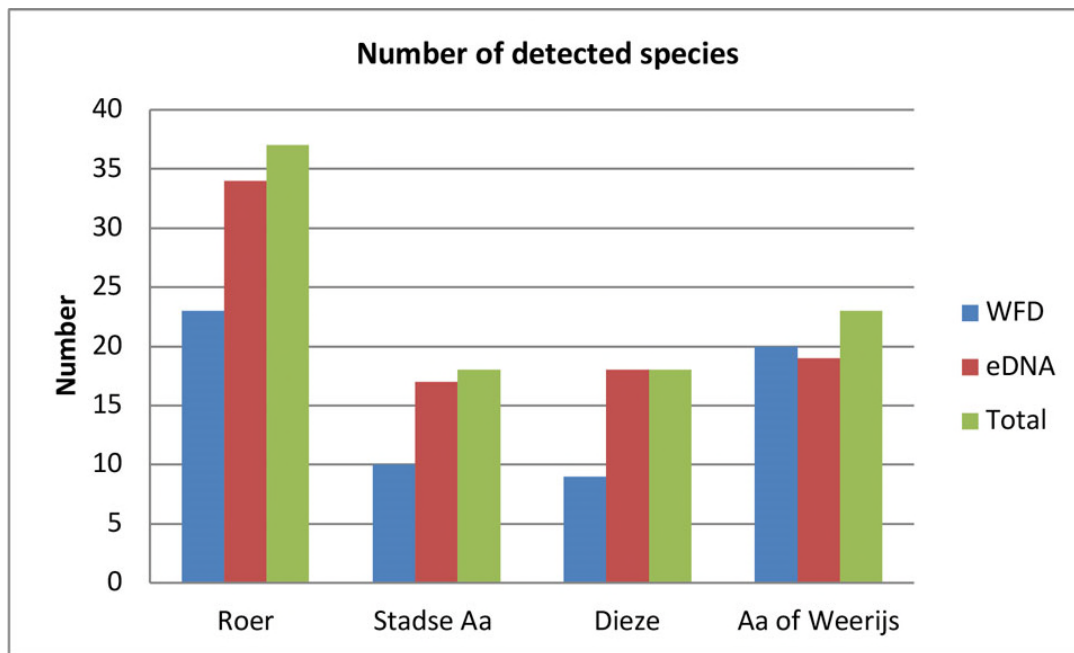
The eDNA methodology of the NL Fish Population Scan is based on identifying DNA traces that fish leave behind in the environment. This concerns traces of excrement, slime, skin or scales.

Each fish species is identified on the basis of their unique DNA code. For this purpose, a short mitochondrial DNA fragment from approximately 110 building blocks is selected. This DNA fragment is selectively multiplied (100,000 times), analysed using metabarcoding and matched with a reference database.

Results

The results of the comparison indicated that in three out of four waterbodies (Roer, Stadse Aa and Dieze) more species were detected using eDNA sampling (Figure 1). In the Aa of Weerijs one more species was detected using conventional sampling.

Both sampling methods overlap in approximately 60% of the species. Differences apply for species which are detected in very low densities (eDNA or kg/h). For instance, in the Aa of Weerijs, the species Eel, Round goby and *Cottus rhenanus* were found only with eDNA, whereas with conventional sampling only Pike-perch, Asp, Topmouth gudgeon and Ide were detected. These differences can be explained by the sampling location, sample size and the behaviour of species; which all affect the chance of detection.



Figure

1. Number of species detected per investigated body of water

The species that were additionally detected in the eDNA samples correspond with species which could be expected in these types of water systems. For three out of four water systems this concerns species such as Carp, Pike, Prussian carp/Goldfish and Sunbleak (Figure 2). Tench was missed from detection twice. In all these cases, the missed species concerned a low share (<10%) in the total number of eDNA measured sequences. A previous study by Herder & Kranenbarg (2016) using a comparable eDNA metabarcoding approach, confirm our observations. The authors also describe missing observations for species such as Carp, Prussian carp/Goldfish and Sunbleak.

A possible explanation could be that Carp-like species concern adult fish which generally appear in lower densities and are better at escaping from the conventional sampling method.

Sunbleak and Tench are phytophilic (fish which spawn on and live between plants) species which can hide effectively in high strains of vegetation. Other not detected species are only missed in one of the researched systems. Coincidence could also be an important factor in detecting species, as some species only have low DNA densities, and therefor might coexist of a very small population.

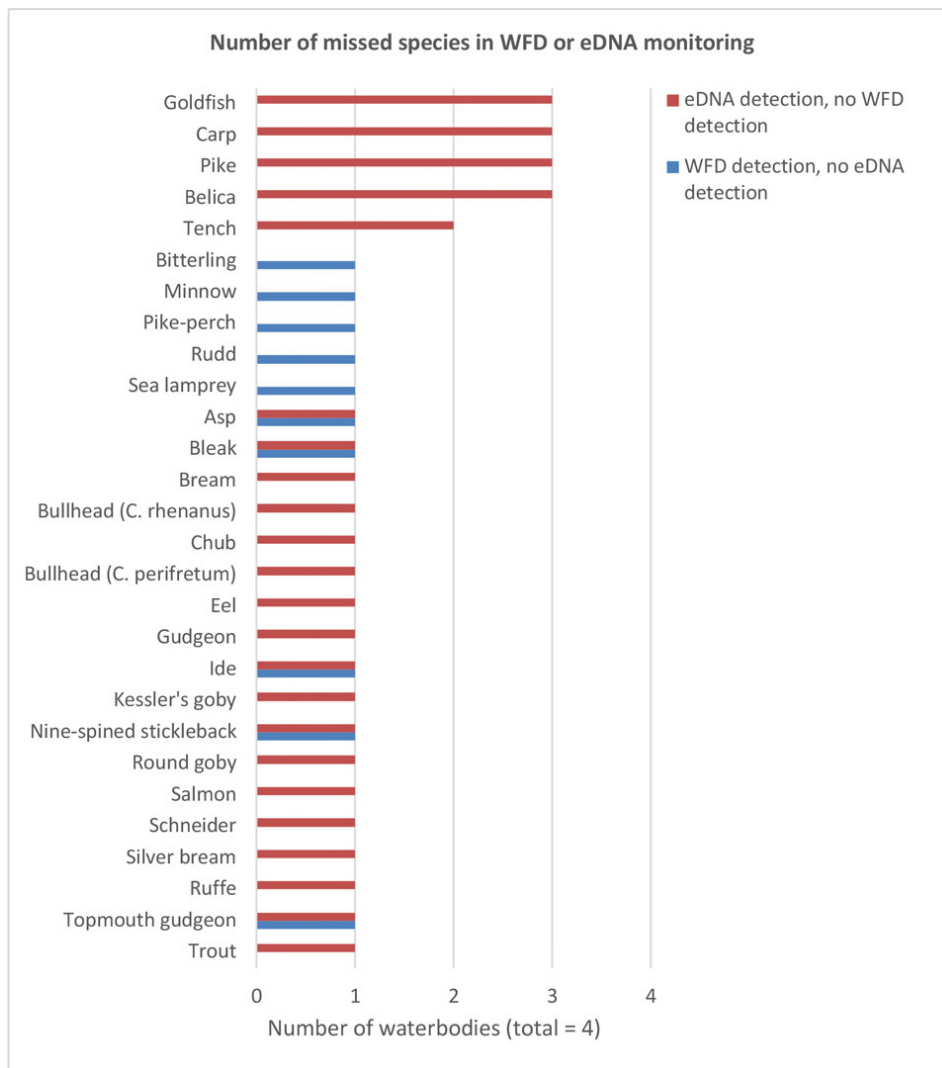


Figure 2. Per species, the number of water bodies water (maximum of 4) in which the species has indeed been shown with eDNA, but was not caught in the case of the WFD fish population sampling, and vice versa. Note: Sea lamprey was not in the DNA database and could therefore not be detected with eDNA.

With the eDNA sampling, one or sometimes a few species could not be detected (Figure 2). Usually, this concerned species which were found in relatively low densities (<0.2 kg/ha). In the Aa of Weerijis, this also concerned a relatively large population of Ide (approx. 35 kg/ha) and Pike-perch approx. 10 kg/ha). Ide and Pike-perch accounted for approximately 20 and 12% of the biomass of the populations found (at sampling location). For Pike-perch in particular this concerned large fish. From literature it is known that eDNA densities are lower by large fish. Despite the high detection sensitivity of the eDNA sampling approach, species may still be missed. For example, in water bodies with low fish densities, low eDNA concentrations can be expected. In such situations sampling needs to take place in the direct proximity of a particular

individual in order to obtain eDNA at all. Just as with every sampling method, coincidence plays an important role in the eDNA sampling approach.

Conclusions

The results of the present study show that:

- The NL Fish population scan is an advanced application that allows quick and effective determination of species composition within fish populations, in both stagnant and flowing waters.
- The NL Fish population scan gives an important additional overview of the diversity of species present in relation to the conventional fish sampling. With this approach, in three out of four systems, more and additional species were detected which could be expected in these water systems. These results are in line with previous findings by Herder & Kranenbarg, 2016 and are probably the result of the sensitivity of the developed method at which also small amounts of eDNA are detected. Due to this high sensitivity, there is also an additional risk of false-positive observations. This was excluded from this research by using a blank control sample. The current method appears to be more reliable.
- For unknown reasons the used eDNA extraction method (precipitation in the field with isopropanol) appears to be sensitive to disturbances in some of the sampling locations. At present, work is being carried out on a second extraction method on the basis of lab filtration. The current method looks promising but requires further optimization.
- Species can also be missed with the NL Fish Population Scan due to (very) low fish densities at which only small amounts of eDNA will be present and catch coincidence. Additional research is required in order to determine whether the reliability of the method will increase in the case of a higher sampling effort.

Future

The deployment of eDNA for the monitoring of fish populations potentially forms a good alternative for determining the species composition. However, in order to make eDNA a full-fledged alternative to the conventional monitoring, a number of development directions are proposed:

1. Comparison of several eDNA metabarcoding methods which are being developed, in order to reach a national or European standard to be applied. This includes for instance the standardization and/or harmonization of sampling, extraction and bioinformatics. National/European attunement and carrying out ring tests and inclusion in the national manual (Handboek Hydrobiologie) appear to be appropriate. NEN certification could also be considered.
2. Adjustment of the WFD metrics for the assessment of the fish population to the possibilities of eDNA. The current WFD assessment takes place on the basis of the species composition and relations between species in numbers and biomasses. An exploratory analysis shows that the information required about numbers and/or biomasses for the current WFD monitoring cannot (yet) be determined sufficiently reliably with

eDNA. Due to the added value of the technology and the cost effectiveness, a possible adjustment for the assessment is already being discussed. Depending on the objective, both methods can be deployed parallel to each other.

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Summary

Water authorities are obliged to monitor the fish population. However, the current conventional fish inventory methods are known to have drawbacks. New methods on the basis of eDNA appear to be a good alternative for determining the species composition of a fish population. In 2017, KWR, Water authority Limburg, Water authority Aa en Maas, Water authority Brabantse Delta, ATKB, BaseClear and Witteveen+Bos developed a new eDNA metabarcoding technique, the NL Fish population scan. The NL Fish population scan was applied in the Roer, Stadse Aa, Dieze and Aa of Weerijs. The results were compared to the results from conventional fish population samplings and show that the NL Fish population scan in the study area gives a good insight in the species composition. With the NL Fish population scan, in almost all cases more species are detected. The species which were found additionally with the NL Fish population scan could be expected in the study area. The NL Fish population scan potentially forms a good alternative for determining the species composition within the current WFD monitoring.

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