Sampling rivers for genes rather than organisms

Effective environmental management depends on a detailed knowledge of the distribution of species. But taxonomists are in short supply, and some species can be difficult to identify, even for experts. Analysis of environmental DNA offers a promising new approach for biomonitoring. *By Andres Jordi*

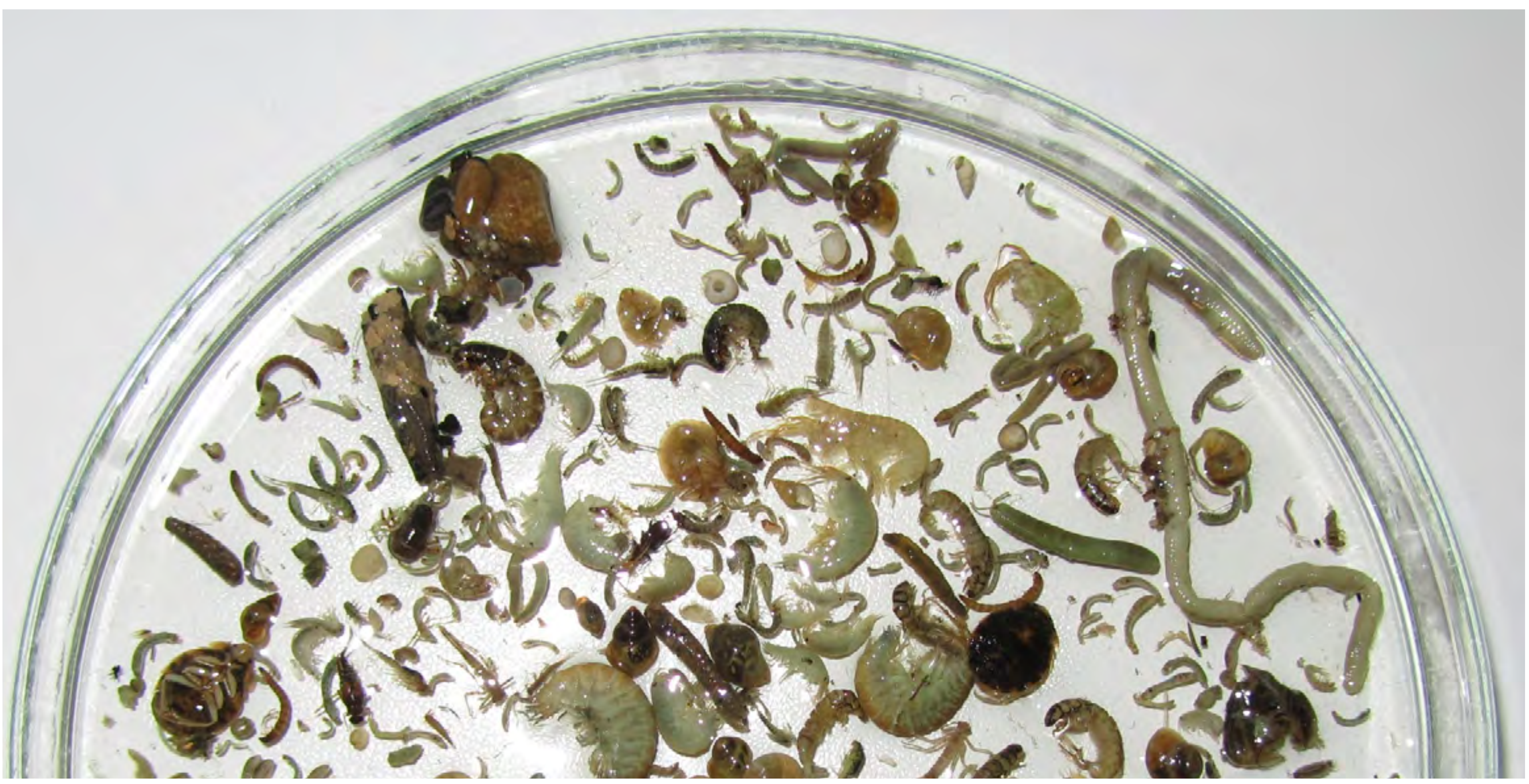

Elvira Mächler

Fig. 1: Conventional methods used to inventory macroinvertebrates are time-consuming and lethal.

If amphipods are detected in a river, are they indicator organisms providing evidence of good water quality? Or the first arrivals of an invasive species? Or perhaps a threatened species? Conservation and environmental management call for an in-depth knowledge of where species occur. However, taxonomists are themselves an increasingly endangered breed and, for the experts who remain, identifying species on the basis of morphological characteristics is frequently challenging, time-consuming and lethal for many organisms.

Laboratory-based identification

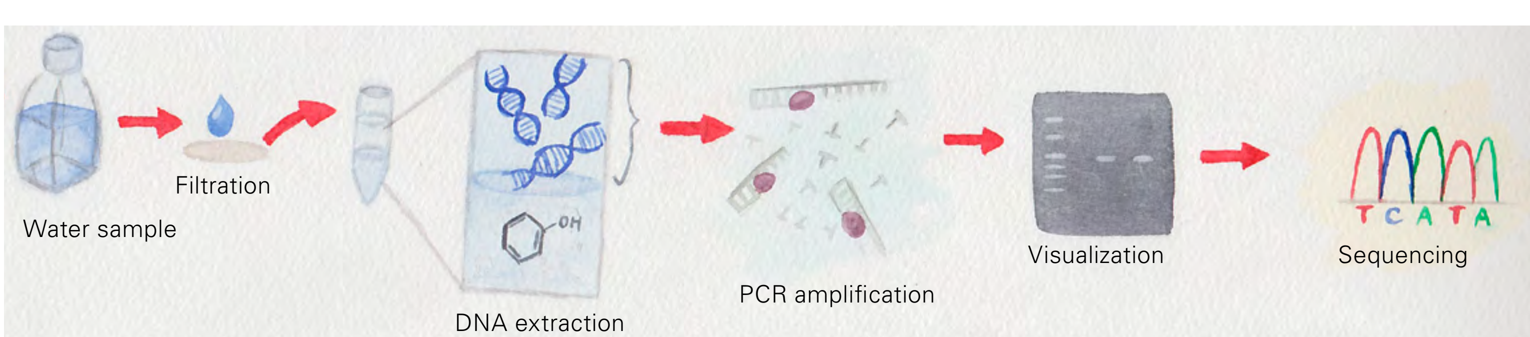
A new approach to species detection is being pursued by biologist Florian Altermatt and his colleagues Kristy Deiner and Elvira Mächler of the Aquatic Ecology department at Eawag. The method involves the use of environmental DNA (eDNA) – genetic material continuously released into the medium where organisms live in the form of faeces, hair or skin cells. Water samples collected from a river or lake can contain innumerable fragments of DNA shed by their resident organisms. As Altermatt explains, "With the latest molecular biological techniques, we can assign these segments of DNA to particular species, as long as we know the relevant genetic code." In cooperation with staff from the Canton Zurich Office of Waste, Water, Energy and Air (AWEL), the researchers investigated whether this method is suitable for the detection of macroinvertebrates (Fig. 1). Organisms such as mayflies, amphipods, mussels or snails are important bioindicators, used in the assessment of water quality and ecotoxicity. Altermatt points out: "Many of these organisms are tiny, occur at low densities or have complex life cycles, which makes them difficult to inventory with conventional methods."

As part of her Master's thesis, Elvira Mächler collected water samples from 14 lake and river habitats in Canton Zurich. These DNA cocktails were then studied for the presence of six macroinvertebrate species from various taxa – four ecological indicator species, one non native and one vulnerable species. In addition, macroinvertebrates were collected by kicknet sampling at the same sites, and species were determined by an expert in the conventional manner. The findings of the two methods could thus be directly compared.

Genetic analysis in a DNA-clean laboratory

To detect the target species via DNA, the researchers analysed the sequence of bases in a mitochondrial gene known as COI (the mitochondria are responsible for energy production in cells). Among different species, the same genes differ to a certain extent in the sequence of bases, and these differences can be used for species identification. Mächler explains: "As there are far more copies of the COI gene per cell than of other genes, it's also more likely to be detected in the environment. In addition, with COI, the sequence of bases shows low divergence between individuals of the same species and high divergence between different species, which makes it easier to identify species."

To permit genetic analysis, the water samples had to be processed in the laboratory (Fig. 2). The samples were first passed through a glass microfibre filter, and eDNA was then chemically extracted from the filter. To facilitate detection, additional copies of the DNA fragments were produced using the polymerase chain reaction (PCR) amplification method. Gene sequencing was then used to determine the base pairs in the COI genes of individual DNA segments. By comparing the sequences with genetic profiles in a database, it was then possible to establish whether water samples contained DNA from one or more of the six target species. For the laboratory



Elvira Mächler

Fig. 2: Decoding environmental DNA requires extensive processing of water samples.

studies, stringent protocols were followed to ensure that the material to be analysed was not accidentally contaminated with extraneous DNA (e.g. from the scientists themselves) (Fig. 3).

Early detection of invasive species

Mächler comments: "The eDNA method is effective across a wide taxonomic range of macroinvertebrate species. Five of the six target species were reliably detected." However, the method did not always deliver the same results as the conventional procedure involving kicknet sampling and identification via a taxonomic key. Thus, at some sites, a species was detected with one method but not with the other. The eDNA method was more effective for certain species, and kicknet sampling for others (Fig. 4). Overall, agreement between the two methods in positive species detection ranged from 43 to 100 per cent. "There is a potential for false-negative detection error with both methods", says Altermatt.

In the case of organisms occurring in small populations, however, the eDNA method appears to be more sensitive. Using this approach, the biologists detected the rare mayfly *Baetis buceratus* not only at all the sites where it was found with the conventional method, but also at two sites where no *Baetis* specimens where detected by kicknet sampling. Accordingly, the researchers believe that the new method may also be suitable for the detection of invasive species at an early stage of colonization. In fact it is already incorporated by governments for monitoring invasive carp species (US) and the highly endangered Great Crested Newt (UK).

Florian Altermatt

Fig. 3: Special precautions are taken to avoid contamination of samples with extraneous DNA.

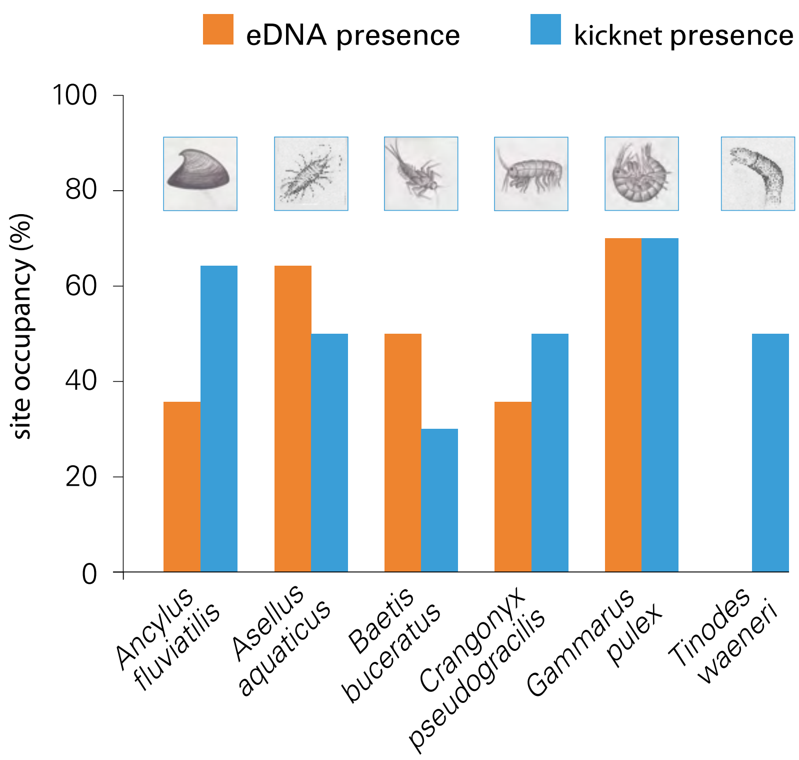


Fig. 4: The eDNA method effectively detects a wide range of macroinvertebrate species; in some cases, the results are better than with the conventional method.

Environmental DNA: present all year round

The eDNA method offers additional advantages. As eDNA is ubiquitous in freshwater and continuously produced throughout the year it offers a surveillance method that is less time-critical and non-lethal for organisms. By contrast, kicknet sampling merely provides a snapshot, and for many species it can only be carried out at certain stages of the life cycle and certain times of the year. For eDNA analysis, organisms do not have to be removed from a river or lake. Finally, as the researchers point out, the method – in theory – allows hundreds of species to be detected at the same time.

This, however, is still some way off: apart from the need for further refinements, the method is still costly and time-consuming. The cantons currently lack the necessary infrastructure and expertise. But, as Altermatt notes, the technology is developing rapidly, and he believes it will not take too long for technical standards to be established, permitting efficient operation: "eDNA analysis of entire communities will then cost a few hundred Swiss francs and will be cheaper than conventional surveys." The researchers do not believe that the new method will replace the conventional method in the near future; rather, they argue that the benefits of both approaches should be exploited. Nonetheless, a vision for the future remains: "Based on the eDNA method, continuous monitoring of freshwater biodiversity could one day be routinely performed, just as chemical parameters are monitored today", says Altermatt. "The technology exists." But even then, he admits, taxonomists would still be required – not least to validate the results of the new procedures.

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