Strategy for Successful Integration of eDNA-based Methods in Aquatic Monitoring

Estelle Lefrançois, Philippe Blancher, Frédéric Rimet, Agnès Bouchez

Abstract

Recent developments in the use of environmental DNA are opening up new horizons for the assessment of the quality of aquatic environments. These rapid and cost-effective methods make it possible to identify all the taxa present in an environmental sample (water or biota). The produced inventories can then be used for biodiversity assessment as well as for bio-indication. However, the inclusion of these new DNA-based methods in monitoring practices is not straightforward and requires harmonised actions in the coming years at national and international levels. In order to foresee and stimulate such a harmonised implementation, the European network DNAqua-Net (COST Action CA15219) brought together members of DNAqua-Net, members of ECOSTAT and other environmental biomonitoring stakeholders from different European countries. Through workshops, bringing together 51 participants in 7 sub-groups in April 2020, an implementation road map was designed. The coordinated actions to be taken in the different countries, and the possible collaboration and steps to be taken at the EU level were identified. The resulting document gives an overview of all discussions, reflecting the diversity of situations in Europe, as well as common views, and it highlights important actions required for a successful implementation of DNA-based biomonitoring of aquatic ecosystems at the horizon 2030.

Citation

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Introduction

The EU biomonitoring context

The European Union (EU) Environmental Implementation Review (2019) calls on Member States to step up efforts to improve water quality. According to the most recent statistics, only 40% of surface waters in Europe have achieved good or high ecological status as required by the WFD (Water Framework Directive)[1].

Faced with the loss of biodiversity, the degradation of aquatic ecosystems and the services provided by them, actions must be taken. To ensure the best possible restoration and preservation of these natural environments it is necessary to have high-performance diagnostic and monitoring tools (biomonitoring), implemented in an effective operational framework that ensures high quality and cost-efficient control.

Recent developments in the use of environmental DNA (eDNA or (e)DNA) are opening up new horizons for the assessment of the quality of aquatic environments. These rapid and cost-effective methods make it possible to identify all the taxa present in an environmental sample (water or biota). The produced inventories can then be used for biodiversity assessment as well as for bio-indication. The current methodology, based on morphological identification, requires great expertise and time on the part of operators for the production of inventories that are often dependent on the operator and subjected to bias. DNA-based biomonitoring may be implemented as complementary or as an alternative to the morphology-based identification. However, the inclusion of these new DNA-based methods in monitoring practices is not instant and requires harmonised actions in the coming years at national and international levels.

COST Action DNAqua-Net

Launched in 2016, DNAqua-Net (http://dnaqua.net/, CA15219) is a COST (Cooperation in Science and Technology) Action funded by the EU. Organised in 5 working groups, DNAqua-Net aims to develop and implement new genetic tools using environmental DNA for the benefit of aquatic environments. It brings together researchers from different disciplines to identify new genomic tools and new eco-genomic indices for biodiversity assessment and biomonitoring of aquatic ecosystems in Europe. Within DNAqua-Net, researchers and other actors involved in the management of the aquatic environment work together towards the implementation of these tools at the European level. DNAqua-Net members recently presented the latest developments and discussed the potential use for routine biomonitoring in Europe together with ECOSTAT[2] members (Vienna, 6 May 2019).

SYNAQUA and its prospective workshops

From 2017 to 2019, INRAE (French National Research Institute for Agriculture, Food and Environment) and Geneva University led the Interreg France-Switzerland SYNAQUA programme (https://www6.inrae.fr/synaqua/). The aim was to develop, implement and validate genomic tools for biomonitoring using diatoms and oligochaetes, to be applied in the waters and sediments of Lake Geneva and transboundary rivers. The development, testing and

[1] European Environment Agency 2018 *European waters — Assessment of status and pressures*

[2] ECOSTAT: EU working group, including experts from all Member States and key stakeholder organisations, for a common implementation strategy of WFD.

awareness-raising actions have made it possible to move towards the routine deployment of these tools, thus meeting the objectives of DNAqua-Net.

In particular, during the first half of 2019, three prospective workshops were organized in France, bringing together a panel of stakeholders involved in the biomonitoring of freshwater ecosystems. They made it possible to define an "image of the future" of biomonitoring integrating genomic tools that was considered as "desirable", and to draw up an action programme and a roadmap to move collectively in this direction by 2030.

The DNAqua-Net prospective workshops

Objectives

INRAE and DNAqua-Net wanted to extend these reflections to the European level. To this end, they organised a new prospective workshop involving members of DNAqua-Net, members of ECOSTAT and other environmental biomonitoring stakeholders from different European countries.

This seminar, organized by Estelle Lefrançois (Eco in'Eau), Philippe Blancher (Consultant), Frédéric Rimet (INRAE) and Agnès Bouchez (INRAE, Vice-Chair of DNAqua-Net), aimed to deepen and complete the action programme and the road map established at the end of the SYNAQUA workshops, by identifying:

- The actions to be taken in the different countries to undertake a similar approach;
- The possible collaboration/mutualisation and the steps to be taken at the EU level.

The reflection was organised by taking up the 6 fields of action identified by the previous SYNAQUA workshops (Figure 1).

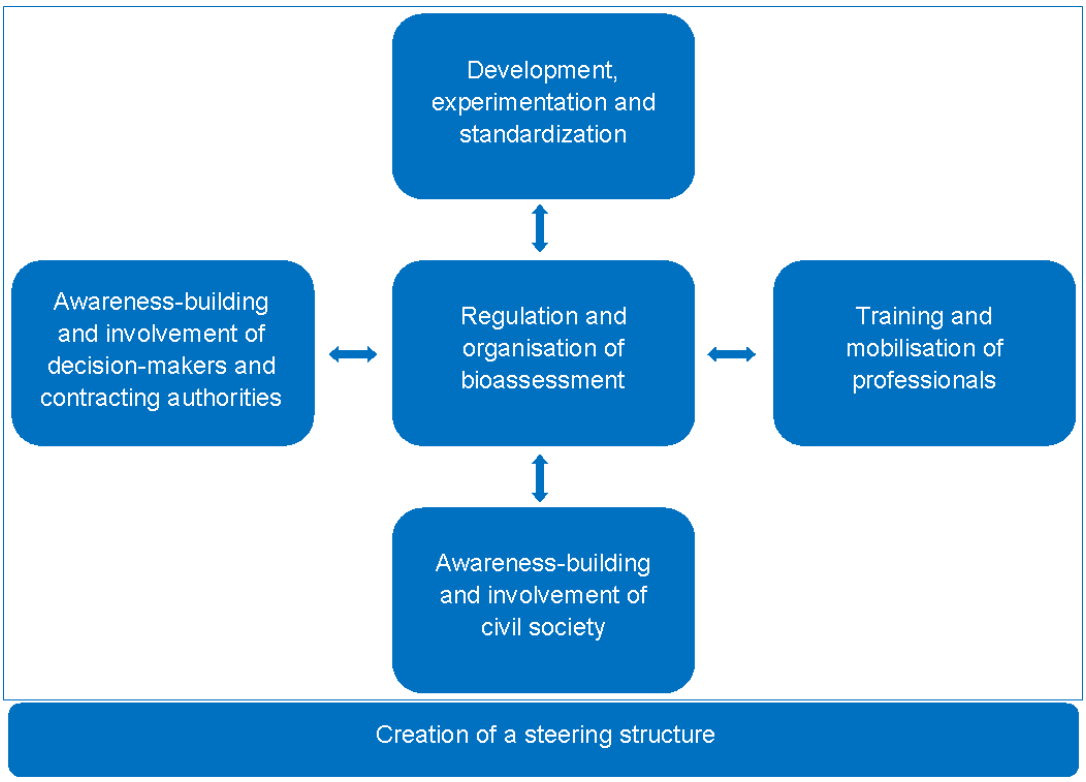

Figure 1 : Fields of actions for the implementation of DNA-based methods

Organisation

The workshop was to take place in Paris in the premises of the Ministry for Ecological Transition and Solidarity on March 10th, 2020, in conjunction with an ECOSTAT meeting. Unfortunately cancelled due to the COVID-19 pandemic, it has been changed to online sub-group conferences from April 20th to 30th, 2020.

Webinars presenting DNAqua-Net, ECOSTAT and introducing the prospective workshops were made available to participants prior to the online conferences[3]. The prospective reflection was then organised in seven different sub-groups, each of them having a moderator and a secretary among the four organizers. For each field of action, a synthesis slide based on the "desirable image of the future" drawn during the SYNAQUA workshops was proposed as a starting point for discussion. The output of all sub-group discussions is presented here for each of the six fields of action (Figure 1).

Participants

For each country participating in DNAqua-Net, were invited:

- One (or two) scientist(s) from DNAqua-Net, members of the Working Group 5 "Implementation Strategy & Legal Issues";
- One (or two) representative(s) of the competent authorities/agencies. Initially, the workshop was planned in Paris, side-to-side to an ECOSTAT meeting, in order to invite ECOSTAT members and facilitate their participation. After cancellation of both physical meetings, due to COVID-19 crisis, the invitation was enlarged to representatives of competent authorities/agencies linked to DNAqua-Net scientists.

Finally, 51 persons (Annexe 1) representing 18 countries (Figure 2) participated. Among these participants, 20 were members of DNAqua-Net and 15 members of ECOSTAT, the other participants being among national stakeholders.

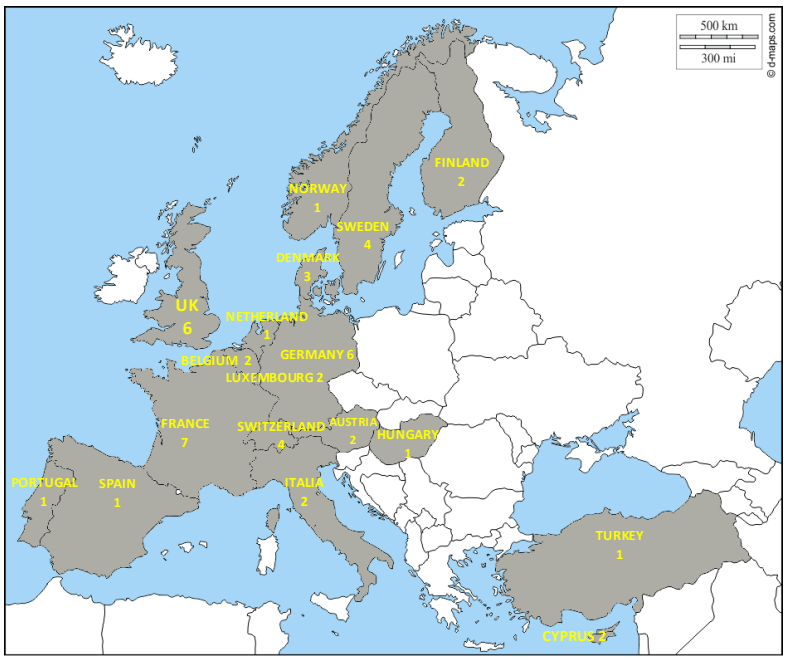

Figure 2 : Number of participants per countries

[3] https://dnaqua.net/webinar/

Preamble

The discussions from all 7 sub-groups are synthesised below, following the 6 field of actions (Figure 1). The introductory slides are presented as boxes at the beginning of each part and followed by a synthesis reflecting all discussions and the various opinions of the participants, which could in some cases be contradictory.

In the following, environmental DNA is considered *sensus lato*, including both eDNA (DNA extracted from an environmental matrix such as water or sediment) and (e)DNA (DNA extracted from environmental biota such as macroinvertebrate bulk or environmental biofilm).

The discussions were about the inclusion of DNA-based methods in biomonitoring of freshwater ecosystems, be it regulatory or not. However, the large number of participants from ECOSTAT has often driven discussions on the regulatory one.

DNA-based methods that could be included in biomonitoring ranged from more mature ones (i.e. DNA metabarcoding to produce taxa lists), to those that are under development (i.e. taxonomy-free approaches to calculate indices, functional approaches, etc.). The discussions revolved more broadly around the more mature methods, without excluding the latter.

The main orientations are presented as conclusion and should be the basis for a future community paper.

Development, experimentation and standardisation of DNA-based methods

Webinar slide / proposed orientation

The proof of concept and the demonstration of the methods are already established, but developments are still required to use them routinely. For example, uncertainty needs to be evaluated and reduced. The way we collect data from DNA-based analyses also needs to be adapted and improved.

Standardisation of the methods is also a crucial point and work is already in progress.

Research must still go on, focusing, for example, on the calculation methods of new ecological status indices.

To achieve all these tasks, it's necessary to find out how to fund the research and scientific work at a European level.

Testing methods and comparing traditional and DNA-based methods

In order to move forward, a comparison between traditional and DNA-based methods is essential. Many pilot studies in Europe and more widely have been or are being implemented focusing on the comparison between traditional and DNA-based taxa identification methods. For example, tests using diatoms at the scale of WFD river networks were done in the French

oversea territory of Mayotte and in mainland France[4], but also in Spain[5] and Portugal[6]. Pilot studies were also conducted in the Netherlands[7] and in Portugal to test DNA-based methods for zoobenthos, and in the United Kingdom for diatoms[8] and fish[9]. The project SCANDNAnet[10] is a large project involving the Scandinavian countries, that focuses on aquatic invertebrates and the comparison to morphological identifications.

Many other projects are ongoing at European level: SYNAQUA[11], Eco-AlpsWater[12], etc. One test is being carried out within the framework of the 6th Joint Danube Survey on different biological quality elements (BQEs). WAT-DIMON (Eurostar project) is a recent project that targets at increasing knowledge on molecular techniques able to be used for bioassessment of diatoms in Spain, Portugal and Cyprus. There is also some interest in large-scale tests in the United States and Canada, opening up opportunities for collaboration (see studies on diatoms conducted by the US Environmental Protection Agency).

Some tests go further than the comparison of taxa lists, aiming to develop new indices like the Swiss Diatom Molecular Index[13] in Switzerland or new metrics[14] in the United Kingdom. For example, in Switzerland, there is a study aiming to further develop the Swiss Diatom Molecular Index and to compare it with the "traditional" DI-CH (Swiss Diatom Index)[15] is conducted (with ID-Gene, Jan Pawlowski and supported by FOEN).

In the United Kingdom, comparative testing of diatom methodologies considered broad spatial and temporal coverage. Generally, there was good agreement between the two methods but differences were evident at a site level. The inability to determine if a change in class was due to a genuine environmental change, or due to a change of method, raised concerns with stakeholders. However, this work has been hugely valuable for stakeholders in driving DNA methods and research forward.

Each comparative test conducted has shown that traditional and DNA-based methods do not give the same results, but it remains crucial to study and evaluate the comparability between the two methods in order to find and demonstrate evidence of ecological equivalence or significance.

Many participants agree on the value of pilot studies to test comparability and to show the added value of DNA-based methods in different contexts and for different purposes, as well as to demonstrate their compliance with the WFD. The main pitfall remains their funding. In many cases, authorities limit their involvement in these comparison studies to the provision of samples.

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[4] Vasselon et al. 2017; Vasselon et al. 2019
[5] Perez et al. 2020
[6] Mortagua et al. 2019
[7] Beentjes et al. 2018
[8] Kelly et al. 2020
[9] Haenfling et al. 2016 ; Lawson-Handley et al. 2019
[10] https://www.syke.fi/en-US/Research__Development/Research_and_development_projects/Projects/SCANDANnet/
[11] https://www6.inrae.fr/synaqua/
[12] https://www.alpine-space.eu/projects/eco-alpswater/en/
[13] Apothéloz-Perret-Gentil et al. 2017
[14] Kelly et al. 2020
[15] conducted by ID-Gene and PR Pawlowski and supported by FOEN

Intercalibration of DNA-based methods

The intercalibration of new methods, i.e. comparing the ecological status on the basis of traditional and DNA-based identification methods, is foremost a European rather than a national issue. This implies and requires a European initiative.

The Europe-wide intercalibration of traditional sampling and WFD assessment methods, which began in 2006, is coming to an end and has been a very complex and challenging task characterized by many compromises. Some participants questioned whether an intercalibration of DNA-based methods is absolutely necessary, as it may be a major obstacle to their implementation. However, according to WFD requirements all methods have to be intercalibrated. The challenge is therefore to find the appropriate and efficient way to do so. Although several guidelines for intercalibration are already developed and successfully applied (EC, 2010, 2016)[16], they may require to be adapted to the intercalibration of novel molecular taxa identification methods. A guidance on how to carry out this intercalibration should therefore be produced before anything else.

ECOSTAT always recognised a member state's right to participate to the European intercalibration with its own method. In the United Kingdom, the diatom DNA-based method was developed, making sure that the status class boundaries for the metabarcoding approach were equivalent to those set using light microscopy. However, because the two methods were not equivalent, the new DNA-based method did not meet the criterion for simple intercalibration and further work would be needed to complete the intercalibration process.

A pan-European agreement on minimum standards for the most mature methods, such as (e)DNA metabarcoding of fish, invertebrates or diatoms, is considered as a pragmatic and eventually cost-effective first step. To oversee the implementation of this methodology, the Geographical Intercalibration Groups (GIG)[17] could be used even if it has already happened that all countries together intercalibrated their methods. Experiences of the intercalibration of traditional methodology in GIGs were inspiring and could function as a guideline. The GIGs were able, by using real data, involving people from different European countries, with different expertise and in-depth knowledge of methods and monitoring, to discuss and harmonize results obtained from many different approaches in a very pragmatic and efficient way. However, some participants highlighted that it would be clearly beneficial if such a multitude of different DNA-based methods would not emerge but that rather agreements on few standardised methods emerge to avoid tedious *a posteriori* intercalibration.

Similar processes are also underway in the COST Action DNAqua-Net where a group of diatomists is setting up a ring test on the wet-lab process, and a group of macroinvertebrate specialists is doing the same on the range of variation. Further, a newly established working group within the European standards organization, CEN, i.e. CEN TC230/WG28, is reviewing suggestions for new working items on methods and best practices within the CEN standardisation process. This work will take time, mainly because of missing funding which limits e.g. the availability of motivated experts to engage in the standardisation process. As for other types of ecological work, infrastructure is needed for DNA-based work, e.g. reference laboratories. Especially involving national accredited reference laboratories could speed up the standardisation, uptake and further development of these novel methodologies and also engage in training people, implementing the intercalibration process and testing of new methods.

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[16] https://circabc.europa.eu/sd/a/a091506c-6fc8-45a8-a588-5c6397ed2aa4/Guidance%20No%206%20-%20intercalibration%20(WG%202.5).pdf

Standardisation of DNA-based methods

Is standardisation of molecular taxa identification methods worth considering and advisable at the European level? This is a point of divergence between the participants. Some are in favour of standardisation and even consider it a priority, while some others think that we should find a good combination between guidelines and limited standardisation.

Despite these differences in opinions, standardisation of methods is already under way in CEN TC230/WG28. Here, focus is on water sampling, diatoms, and aims to address sediments in the future. Two technical reports have already been written on the diatom barcode library and diatom sampling[17] and a suggestion for guidance on sampling eDNA from water undergoes voting. Further, a paper focusing on the management of barcode libraries at EU level and concerning many BQEs is under consideration and could later give rise to a standard.

It is important to distinguish the standardisation of the sampling and biochemical analysis from the scoring or index system that will provide the assessment of the ecological status which is likely not on a pan-European scale.

As many methods have already been developed and are in use, it seems illusory to imagine using a common method on a European scale, which does not prevent the results from being compared. Because there are already so many methods in the field, in each country and sometimes even at a regional scale, standardisation will be a challenging task. Moreover, methods, especially bioinformatics, evolve rapidly. Some respondents view standardisation at EU level as an unsurmountable task. Actually, standardisation of every single workflow step in detail is regarded impossible and counter-productive. However, standardisation of key processes and steps (i.e. sampling methods, preservation of samples (short and long time), extraction of DNA, use of negative/positive controls, data reporting, and participation in a proficiency test…) seems often straightforward.

However, it is possible and desirable that sampling and other steps in the process follow the same standard protocol. It is important to have and keep a high quality in monitoring concerning the whole chain from design, sampling, the various steps from extraction and analyses of DNA up to where to store the results e.g. in databases. Databases – quality of data, where shall data be stored, in which format, which data should be available, who shall be responsible for different databases. Thus, although the results (taxa lists) may not be comparable, the intercalibration must allow the same conclusions in terms of ecological status to be obtained with traditional and DNA-based methods. What should be avoided is the situation that currently prevails in different countries, including the Netherlands: five companies use DNA for fish inventories, but they do not use the same method (primers...) and generating results that are not comparable. On the subject of the operator bias, it was pointed out that this issue already exists with the traditional methods, but that it is generally underestimated, even if it is responsible for discrepancies in the results that raise questions.

Completion of reference databases is also a topic where it would be interesting to collaborate at EU level in order to correctly characterize the biodiversity of BQEs and make them open-access. Indeed, it is important to have references from different geographic sites for a given taxon in order to consider intraspecific variations and correctly assess genetic biodiversity and ecological status.

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[17] CEN 2018a. Water quality - CEN/TR 17244 - Technical report for the management of diatom barcodes. CEN 2018b. Water quality - CEN/TR 17245 - Technical report for the routine sampling of benthic diatoms from rivers and lakes adapted for metabarcoding analyses.

For some participants, from an overall strategic and technical point of view, DNA-based methods are well suited to a harmonization at European level. Indeed, these methods are actually closer to laboratory methods for chemistry (sampling, laboratory processing, sequencing techniques) than traditional WFD biomonitoring methods. Moreover, a steering group such as WFD Common Implementation Strategy (CIS) or an ECOSTAT working group is probably an adequate level to provide guidance to Member States to develop the DNA-based methods. Likewise, European standardisation under CEN or even international standardisation at ISO is required to develop common technical standards. Method standards could therefore provide a common framework for Member States within which to develop their own indices and national surveillance strategy.

How do we cope with rapid scientific evolution while standardizing? Should we accept to block technological evolution by imposing the prevailing technology at a time *t*? For example, reference databases linking DNA barcodes to a taxonomic assignation are evolving rapidly, being larger and larger, more and more accurate, thus allowing better identification. As a reminder, CEN standards are under review every 5 years in order to follow technical evolution, and can be modified even at shorter intervals if necessary. Primary works that are on-going at CEN have favoured standards that specify minimum requirements and can adapt to the many methods already developed in Europe, as well as to technological developments.

Indeed, standards are vital and standardisation is an open process where even individual researchers can comment on standards for free. The fact that standardisation has its own set of rules and procedures should not be confused with and portrayed such that it equates with the inability of individuals to participate in the process. Rather, it is important to get involved with the process even if the use of this process requires some learning from the individual researcher's side.

Standards are important, especially for national monitoring programs for which there better be rules and strict protocols to follow. The very point of standards is to improve the comparability and to do so one needs to adopt the standard fully. While standardizing seems essential in regulatory contexts, methods for other applications are simply not constant enough to necessitate such efforts.

However, some participants expressed the need for workable standards for end-users, with acceptable effort and cost. From an end-user perspective there may need to be some balance between what is absolutely the best approach versus what is logistically possible when trying to integrate these methods into existing monitoring infrastructure. Without this pragmatic thinking, operators may only half comply by simplifying procedures.

In Switzerland, in order to homogenized practices at the national level, "Environmental DNA applications in biomonitoring and bioassessment of aquatic ecosystems-Guidelines" will be published by the end of 2020. These guidelines will provide an overview of the DNA-based methods/technics available for Swiss stakeholders and beyond, and will also give normative suggestions on best practices and recommend routine standards.

This pragmatism can be applied into the first CEN standard propositions, not focusing on a single best approach (e.g. one set of primers, one tag polymerase). In CEN, different documents can be produced, for instance: technical reports, technical specifications and standards[18]. These documents differ in their objective, but also in their methods of development, approval processes and implementation, offering flexible means to meet different market needs for requirements and information.

[18] For more information: https://www.cen.eu/work/products/Pages/default.aspx

- Technical reports are informative documents that involve no obligation at national level.
- Technical specifications are adapted when the method is still under development and when national standards already exist (both documents can co-exist)
- European standards must therefore withdraw any conflicting national standards

The way these documents are written can be binding, or only contoured depending on the strategy chosen by the authors:

- Some may only define which metadata must be reported (e.g. where sampled, by whom, how much, which filters; which primers and tags; which software for clustering, taxonomic assignment…).
- Some may be binding, defining what must absolutely be implemented (negative control requirements; decontamination measures; minimum number of replicates, QA/QC measures for DNA quality, sequencing depths; reference library that needs to be used for taxonomic assignment)
- Performance standards (e.g. round robin tests for accreditation of labs). All accredited labs may then use any approach but have to prove their performance recurrently by implementing hard standards.

All this needs to be accompanied with good practice guidance documents that can be used for decision-making. For example, DNAqua-Net WG3 published a validation study for target species survey that allows users to verify the level of credibility of the implemented assay[19]. Depending on the objective, different levels of credibility can be required: e.g. at least "level 4" for regulatory purposes.

Regulation and organisation of bioassessment for an optimal implementation

Webinar slide / proposed orientation

The priority seems to be the recognition of the DNA-based methods, whatever their applications, by the administrative authorities and managers.

Thus, for regulatory biomonitoring, that means that all the actions must be implemented in compliance with the administration schedule and the WFD management plan.

We also need to maintain both taxonomic and ecological skills of operators.

As the implementation of these methods should save money, it's important to make sure that the money-savings are ultimately assigned to favour the ecological quality of natural environment.

Finally, communication about the new strategy and process is also a priority to favour professionals' adaptation

Implementation strategy

Two overall approaches can be adopted: wait until the method is fully tested, evaluated and calibrated before implementing it, or adopt an adaptive approach as it has been done in England.

[19] https://edna-validation.com

In the first approach, because optimisation of a method is an endless task, stakeholders will have to agree on a point in time when development is deemed sufficient to move to the operational phase, even if questions remain. In the context of international regulatory monitoring, any method used needs to be agreed to be sufficiently mature to be implemented in the first place. Moreover, national agencies are often reluctant to start using methods that have not yet been approved or standardized.

In the second approach, harmonisation and comparability of the methods will be the challenge. However, even with methods undergoing fast development one can still agree to standardize some aspects even though they may be subject to subsequent changes (minimum requirements, reporting standards, proficiency tests).

Recent (e)DNA metabarcoding studies show that from individual samples very different BQEs can be assessed (e.g. fish, invertebrates and diatoms from water samples). Thus, genetic data offer options to provide information to various bioassessment and monitoring frameworks. At the same time, however, it is essential to distinguish between application in the context of the WFD and others (environmental impact assessment, biodiversity assessment, alien species monitoring, habitat directive, etc). This is because, e.g. within the WFD traditional, intercalibrated methods developed for this purpose are requested by regulation. Nevertheless, it is possible to implement a method in the WFD context with particular specifications and other methods for other applications (i.e. tracking invasive species), which can evolve more freely.

Implementation could also be done sequentially. It may be more efficient to focus on management issues not yet addressed by regulatory monitoring (monitoring the impact of restoration, biodiversity, invasive or endangered species), rather than seeking to replace the effective traditional regulatory method that is sufficiently effective, especially if the new one is more stringent in terms of ecological assessment. Demonstrating the use and added value of the new method in non-regulatory contexts would thus attract the interest of regulators who could adopt it as a second step.

In the context of the WFD, the two main points of vigilance are the compliance of the DNA-based methods to the WFD normative definitions and the ecological evaluation that should be comparable (in particular not yield systematically worse EQR results for BQE assessments than with traditional methods).

Articulation with the traditional methods

It has been shown that it is impossible to obtain compliance with the WFD normative definitions with both traditional and DNA-based methods. Besides, it is now commonly accepted that traditional and DNA-based methods are giving complementary visions of BQEs. That means that it is not relevant to implement them in parallel but rather to identify the more appropriate method according to the context, objectives and means.

The limitations of DNA-based methods need to be explained and managers need to deal with them. The major critics and encountered difficulties refer to abundance data, age structure for fish species, separating dead organisms from living ones (e.g. “Is the salmon wild or from the nearby restaurant?”, or “Were these alien species already dead by ballast water treatments?”), transport and degradation of the signal, comparison of the results with previous monitoring results. For example, the traditional method can provide many parameters, such as abundance or biovolume, which the DNA-based method cannot provide, at least for all BQEs. Relative abundance is widely used for ecological assessment and often WFD-compliant. For diatoms, it is possible to estimate it with DNA-based methods, while the question is still uncertain for

fish and macroinvertebrates. However, a recent study[20] suggests that presence/absence data can yield assessment results similar to those for abundance-based data for benthic invertebrates.

Where traditional methods are satisfactory, even more if they are required by regulation, the implementation of DNA-based methods, which will be accompanied by challenges and uncertainties, is not a priority. In such a context, DNA-based method implementation is not urgent and can be proceeded smoothly, taking time to test them, demonstrate their added-value and find their better applications. In Germany, GeDNA[21], a pioneering project, which will be completed in two years' time, should make it possible to assess the different methods pros and cons and to understand where it makes sense to complement traditional methods. Within SCANDNAnet project, the viability of the molecular approach over all Nordic countries for macroinvertebrate monitoring is currently assessed. The results will be ready before the end of this year.

Moreover, all traditional methods are inter-calibrated and included in the legislation, which was time consuming, tedious and expensive. Even if the operator biases are well known, it will then be difficult to replace them, in particular with new, non-calibrated methods that also are in the process of ongoing development and lack formal standards. It can also be linked with the current economic strategy of several countries that are reducing their bioassessment efforts (the Flemish Environment Agency recently reduced the samples by 60%). In this context, we can fear that two different methods may not be implemented even though they are complementary and beneficial to the ecological quality assessment.

To limit this risk, it could be preferable to present a single method in which traditional taxonomy and DNA-based approaches are two options of a single consolidated method.

There is a need to develop a 'biomonitoring culture' that promotes solutions leading to optimal biomonitoring: goal-oriented, i.e. aimed at understanding environmental changes, proposing targeted measures[22], integrating at the same time cost-benefit analysis and a concern for practicability, increasing the number of sampling sites, thereby enabling the assessment of sites that never would be investigated today.

Different levels of implementation

Currently, there is no European strategy to implement DNA-based methods. In many countries, implementation is even not harmonized at national level. In Spain, for example, the operational implementation of DNA-based methods would depend on the authorities at river basin level.

In practice, the national level seems to be the appropriate level to integrate these new methods into monitoring programs. The EU could provide general requirements, but they would necessarily be adapted by each Member State according to its own policy. However, if there is a pan-European consensus agreement, member states can still use a different one but they will have to prove that it is equally efficient. Such a strategy would be accompanied by intercalibration and validation of each country's assessment methods by the EU, prior to reporting. Consequently, the creation and animation of an operational working group, organizing the implementation of DNA-based methods in monitoring policies and programs (including regulation, quality management, and the method for deriving the assessment of ecological status from this type of data) is a national issue. At EU level, a steering or working group would be relevant for the overall guidance and supervision of the methodology under

[20] Buchner et al. 2019
[21] https://www.gedna.de/
[22] Poikane et al. 2019

the aegis of ECOSTAT and CEN respectively for the monitoring strategy and the technical aspects (Figure 3).

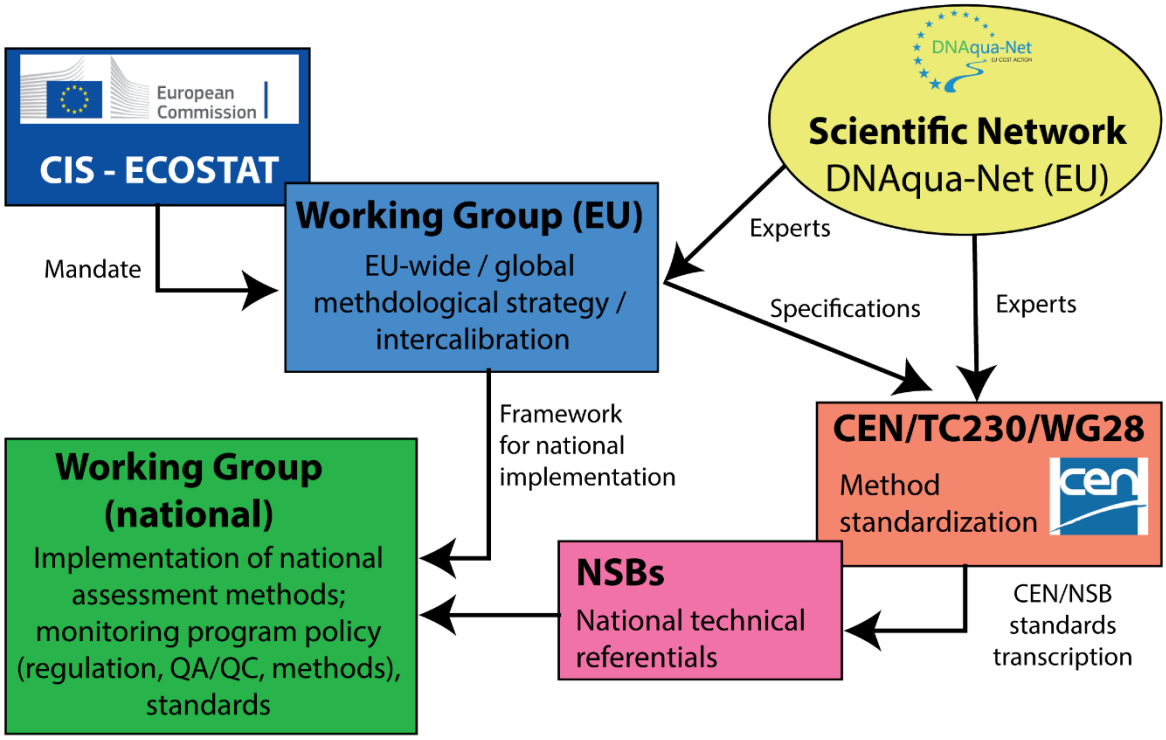

Figure 3: Diagram of the different groups and organizations involved in the implementation of DNA-based methods (©Christian Chauvin). Abbreviations: CIS: Common implementation strategy; CEN: European Committee for standardisation; NSB: National standard bodies (e.g. DIN, AFNOR, etc.)

Circumstances facilitating the implementation of the DNA/Opportunities

In a region for which there is no previous method available, the implementation of a DNA-based methods can be the simplest and most successful strategy. For example, for some of the French outermost regions of the Union, some BQEs still lack an assessment method due to the particular ecological context of these territories needing a different assessment method that the one used in the mainland. In these cases, DNA-based methods appear very promising. In Switzerland, where a method for sediments was lacking, the Water Quality Section in the Federal Office for the Environment (FOEN) and the Ecotox Center are working on the development of an oligochaete DNA-based method, and additionally methods using bacteria and microorganisms might be developed in the near future. More generally, in countries that do not comply with the minimum requirements of the WFD, whatever the reasons (political, financial…), DNA-based methods can help to reduce gaps, for instance by reducing costs, uncertainties, and increasing the frequency of controls. On the other hand, where time, energy and money have been spent to develop the traditional methods, the implementation of DNA-based methods that can replace them is much more delicate and slower. Worse still, if a new method is not efficient in terms of time and cost, for example, if tedious sorting is still necessary, its implementation is possible, yet not conducive.

Today, almost all WFD tools focus on multiple pressures[23] while there is a need of efficient tools and new metrics that can help to measure the impacts of single pressure on the environment in order to evaluate the ecological diagnosis. This could be a great opportunity for DNA-based methods.

Demonstrating the effectiveness of management and restoration measures is also a very topical issue and opportunity for DNA-based methods. To avoid criticism of their actions and despondency, managers want indicators that respond to improvements and therefore show that restoration or other measures are effective.

The dynamics of development of new methods in Cyprus as well as in Switzerland lead to the belief that it is easier to implement innovations in countries with short decision chains and small or highly decentralised territories. But other criteria may come into play, like the EU membership, the availability of money or, on the contrary, the need to make savings. Political will is probably a crucial parameter in the implementation process. For example, the Swiss Water Quality Section of FOEN supports several projects.

On the one hand, there is general enthusiasm for DNA-based methods. Water agencies, regional bodies and hydro-ecological laboratories want to move forward. Many organizations are using DNA-based methods as soon as they answer their needs even if they are not yet standardized and without waiting for the decision/validation of their government. On the other hand, some stakeholders are not in hurry to adopt DNA-based methods because they are not confident in them or simply because they are used to and satisfied with the traditional methods, that some of them have been involved in developing and calibrating.

Critical issues in the implementation process

The implementation of methods, from scientific experimentation to routine biomonitoring is a great challenge that requires coordinated actions. Many believe that standardisation is a prerequisite for routine implementation. Simple and accessible guidelines, especially for bioinformatic pipelines which appears as “black box” to most of current stakeholders, explaining all the steps involved in implementing the DNA-based method, are also strongly recommended. Usually, the bioinformatic pipelines analysed the DNA sequences defining OTUs (Operational Taxonomic Units, sequences grouped under a certain similarity threshold) or more recently ASVs or ESVs (Amplicon Sequence Variants or Exact Sequence Variants, unique sequences). The move from OTUs to ESVs is a step ahead as ESVs are a comparable unit between projects in space and time, while OTUs (as a result of the clustering of the sequences) are not. Using ESVs contributes partly to diminish the “black box” feeling.

It is also essential to strengthen the confidence of the managers in DNA-based methods. The example of Germany shows that this requires time. In this country, DNA-based methods applied on phytoplankton, phytobenthos and benthic macroinvertebrates have been tested for bioassessment. Given the lack of a clear mature, standardized method and some identified deviations compared to traditional assessments, managers (have to) focus on established traditional methods for WFD assessment but increasingly encourage and support pilot studies on the potential of genetic methods for regulatory WFD biomonitoring [24]. This is pretty much the same in the Netherlands where many pilots have been done on vertebrates, macrofauna, bacteria, and some on diatoms and phytoplankton. On the one hand, stakeholders are

[23] Kelly et al. 2016
[24] e.g. GeDNA project funded by Federal Environmental Agency: https://gedna.de

enthusiastic. On the other hand, the process is not as straightforward for them as conventional monitoring.

With traditional methods, the same operator can control the processing chain from start to finish. In France, it's mandatory that sampling, analysis and interpretation of the results are done by the same operators. But to make DNA analyses efficient and economical, many samples need to be processed at the same time and companies have to be specialized, sequencing/producing data for some of them, sampling/interpretation of data for others. Both contracting authorities and operators who outsource part of the DNA-based method tasks need to be able to control quality of contractors and traceability of results. Then, information about repeatability, uncertainty and control points of the methods are needed. More globally, DNA-based methods imply a complete reorganisation of the ecological quality and biodiversity assessment sector.

Data interpretation and ecological conclusions based on DNA-based methods, as well as those based on traditional identification, must be done by ecologists. Cooperation between different partners, openness, open-access resources and constructive critics, must be promoted as we need to avoid "black boxes", or "one-fix-all" solutions.

Under the WFD, it not possible to change the method during a management cycle i.e. before 2027. However, in order to be able to change the method at that time, testing, demonstration of compliance and validation of the method must be carried out beforehand. The fact that the situation after 2027 is currently unknown makes things even more complicated.

It is expected that DNA-based methods will not only work, but will also provide added-value in terms of increasing precision and reducing uncertainties, while at the same time reducing monitoring costs. DNA-based methods may also be very useful for exploring new taxonomic groups, like chironomids (there is already a project to barcode this biological group), developing more sensitive bioindicators (e.g. oligochaetes, nematodes, bacteria) or monitoring other usually neglected habitats like small streams. DNA-based methods could probably contribute to more effective monitoring due to a better taxonomic resolution for these promising groups.

Since DNA-based methods are high throughput methods, it becomes possible to increase the number of samples, thus obtaining more complete information on biodiversity. For example, in Cyprus, DNA-based methods applied in an ongoing national project have shown that they will definitely improve the knowledge of the distribution of fish and eel in rivers and lakes by identifying unknown biological reservoirs. There is a need to convince donors that spatial and temporal series are of interest, particularly in relation to climate change. It is important to demonstrate that the implementation of DNA-based methods is not only a cost-effective solution, but that it can be used to improve the biomonitoring and provide more information.

To achieve this goal, the development of new indices, adapted to the data acquired by DNA-based methods, is necessary and would improve the quality of ecological evaluation. Keeping the same index for fish and probably for other BQEs is not a good strategy in the long term. Projects are being undertaken using little or no reference to the taxonomy. Instead samples are analysed using OTUs (Operational Taxonomic Units) or ASVs (Amplicon Sequence Variants), and linked to environmental data, large data sets are compiled using this information and artificial intelligence techniques are then used to discern patterns in the biota that arise from single or multiple pressures[25].

The production of a database system adapted to DNA data is also a big and important issue.

[25] Graham et al. 2018 ; Cordier et al. 2018 ; Feio et al. 2020

Funding is a key issue everywhere. It is likely that working at the European level would be useful to share costs and benefits from different experiences. It is possible to highlight some very good experiences of European collaboration in this area such as DNAqua-Net.

In the next parts of this document, the following three areas of action are related to the mobilisation of different types of stakeholders:

- *Professionals, i.e. operators who will be in charge of the operational implementation of DNA-based methods for biomonitoring;*
- *Managers and policy-makers who translate the legislative context (WFD) into national monitoring routines (incl. administrative and financial context) or who will take the decisions to use these techniques;*
- *Civil society as a whole.*

Some people, by virtue of their function(s), belong to the first two categories.

Training and mobilisation of professionals

Webinar slide / Proposed orientation

On the one hand, training in new professions that are dedicated to technology (lab technicians, sequencing experts, bioinformaticians) must be developed, while guaranteeing these new operators are trained in the field of hydrobiology as well.

On the other hand, training in these new tools (eDNA, bioinformatics, etc.) must be integrated into all ecologists' curricula so that they could be able to apply them in the best possible way and interpret the results (knowledge of biases, false positives, etc.).

In-service training must ensure the upgrading or re-training of currently employed operators. Good visibility on development and implementation deadlines must encourage the transformation of the professional sector (training, recruitment, evolution of the economic model, etc.).

At the same time, with the technical and financial support of the public authorities, companies in the sector must:

- continue to be engaged in development, experimentation, standardisation and data sharing;
- train their staff and hire people with the new required skills; and
- develop new business models including the sharing of some resources that are essential to DNA-based methods.

We will first deal with the mobilisation of the operators, taking care to clarify who they are. Then, we will deal with information and training, while considering that information and training are a means of mobilisation, once the operators have been convinced of their needs.

Mobilisation of the operators

Who are the current operators?

The situation differs from country to country. In some countries, biomonitoring is carried out entirely by the public administration, which must be trained for this purpose. In others,

operations are implemented by the private sector under the supervision of the public administration. Therefore, in these countries, the first questions are: are there operators who offer the DNA-based methods, are they sufficiently competent, can they really deliver what they promise, are they well identified by the competent administrations? In all countries, there are so far few and, as seen above, each proposes a different protocol. Then, it is necessary to find a way to control the work carried out by these companies and to ensure that they adopt common methods and at the same time to train new operators, if possible among existing suppliers who are familiar with taxonomical and ecological issues.

Whatever the organization, civil servants in public administrations must be mobilized and trained to perform the functions expected of them.

To what extent are operators using traditional methods able to adopt DNA technologies?

It is a widely shared observation that it is a big gamble for private companies to invest and get involved in these rapidly evolving technologies/approaches. Moreover, as there is no regulatory demand, there is no market at the moment. In medicine, there are also huge changes in methods with the shift to molecular techniques. Are there any lessons to be learned from this?

In several countries, many see molecular technologies as a threat and express doubts about their reliability, without always weighing the drawbacks of traditional methods. DNA-based methods have pitfalls that are poorly accepted. Here again, the situation differs from country to country, for reasons coming from both the administrations and the private sector. Without being exhaustive, here are a few examples of situations encountered.

In Norway, most traditional suppliers are non-profit organisations. It is these same groups/individuals working with traditional methods that are the pioneers of DNA-based methods. Therefore, the transition seems to be quite easy. The laboratories have been able to evolve thanks to government funding. Scientists specialised in traditional methods are involved in DNA-based method development projects, as well.

In Sweden, there is currently only research, no operational biomonitoring based on DNA. Researchers send their samples for DNA analysis for example to a large central Swedish laboratory specialised in the analysis of medical and environmental samples (they are currently mobilised on the COVID-19 crisis). It should be noted that researchers in Sweden and Norway also send their samples abroad, for example to Canada, France and Germany.

In Denmark, traditional laboratories are adopting DNA-based methods.

In Finland, all analyses are outsourced and there is at present no regulatory biomonitoring with DNA. However, when it will be introduced, it will create a new market and operators will be encouraged to be trained. Companies will hire people who have the required skills.

In Germany, the situation differs for individual BQEs. Sufficient well-trained experts for fish and macroinvertebrate assessments exist either as part of small environmental bureaus or environmental agencies. For diatoms, Germany lacks experts and for some states not all monitoring samples can be analysed today. DNA-based methods are regarded as a potential method to increase the capacity and allow more/all samples to be processed. There are already private companies offering DNA-based approaches for bioassessment etc., but it is regarded as a challenge to involve the small enterprises that are financially dependent on the implementation of traditional methods - a concern shared by other countries requiring efficient training and collaboration.

In Italy, conventional biomonitoring is directly implemented by public bodies. However, the situation is different in different regions and some regions are really struggling to handle it.

They do not have the necessary funds for biomonitoring. Though, it is difficult to imagine a single approach at the national level for the transition.

Another difficulty comes from the division of labour in the processing of samples, as in public bodies in Sweden, where the tasks (sampling / laboratory analysis / reporting) are compartmentalised. Private companies can do everything or not, thus being exposed to a different economic risk. For example, a person/company performing microscopic analysis will be unemployed if DNA-based methods are implemented, whereas a company that carries out all the analyses will be able to recruit a person with molecular skills.

The situations in France and Switzerland were compared during the SYNAQUA project. In these two countries, the monitoring of aquatic environments involves private operators that carry out a large part of the tasks. The implementation of DNA-based methods will therefore impact this sector and require deep adaptations while preserving taxonomic and ecologic skills of operators. The main difference between the two countries concerns the WFD that applies in France and not in Switzerland. This results for France in the regulatory prescription of tools and methods for biomonitoring and assessment of the ecological quality of waterbodies. Any methodological evolution must therefore be consistent with the common WFD implementation schedule, which imposes an additional constraint on the private sector.

What are the current knowledge about DNA-based tools, the motivation and reluctance of operators and other stakeholders towards method implementation?

While for some participants, the demand for information/training on DNA-based methods seems low so far, others note a change of mentality among operators who are increasingly seeking to know about DNA-based methods and to understand their advantages and disadvantages.

It was apparent to many of the stakeholder participants that DNA-based methods seem to be a "black box" and they will not trust or use them until they understand them properly. A problem raised is in particular the limited time for employees to learn about the new techniques due to many other obligations. Different approaches can be considered to address this situation: appropriate and simple info material, pilot studies, demonstrations, experiments, etc. A better level of understanding of DNA-based methods needs to be achieved: identifying the precise situations (taxa, habitats) where the two approaches are complementary (even if the overall costs are increased), where they are substitutable, where one is better than the other is. These different contexts of use should be listed, and SWOT analyses should be carried out for each method. In each case, the type of sample (eDNA vs. (e)DNA for example) and the approach taken should be explained. Comparisons should also be made between e.g. tests of communities versus tests of individual species. Reliability is also a key-point regarding invasive or rare species monitoring, where DNA-based methods are not always considered reliable enough to be used alone. As resources are limited, choice remains oriented towards traditional methods.

Indeed, the notion of added-value compared to existing methods is essential. If funds are available, decision-makers and those who run monitoring programs can be ready to finance DNA-based programs either because the method would provide real additional information compared to existing programs, or because the method provides the same information as the existing program but is more cost-effective.

It was also suggested that the acceptability of these methods by the target public could be questioned in greater depth through approaches from the human and social sciences, which could also provide clues on the transferability of these methods.

Information and training of the operators

Different targets and objectives

From the previous reflections, it emerges that the training of professionals in molecular methods is very important, and that it must go hand in hand with the implementation of biomonitoring based on these methods.

However, a clear distinction must be made between the different training objectives and target audiences:

- basic knowledge for all types of stakeholders to enable them to discuss the topic, to dialogue with other actors;
- a deeper and more precise knowledge for the staff of the organisations that will have to supervise and manage the deployment of these methods: administrations, but also standardisation bodies, etc.;
- a full mastery of these techniques for the service providers and laboratories that will implement them directly.

Regarding students that are training in the field of biology and ecology, they are already supposed to learn both the traditional and the molecular approach, but with a greater emphasis for the molecular one. The key challenge is to combine taxonomic and molecular skills properly and to convey information that ecological and taxonomical expertise are important and necessary in order to be able to work properly with molecular methods in the field of environmental monitoring.

The content of the training

First, it is essential to develop a general understanding of molecular methods, how they work, their advantages and disadvantages, their pitfalls. This allows both to provide essential knowledge and to remove technical and psychological obstacles.

The molecular analysis and bioinformatics processing part must no longer be a “black box” for ecologists, who would otherwise find it difficult to interpret the results. They need to be able to understand what happens during these various procedures and be trained to interpret the data.

Then, there is a need to train operators on more specific technical aspects (e.g. sampling). The specific skills required in bioinformatics are high and few people have them today.

Finally, operators also need to know how to communicate and get the message across to policy-makers and funders.

The modalities

The transfer of knowledge is particularly effective in joint training sessions between stakeholders of different statuses, joint discussion groups, tandem teams, steering committees, etc. Virtual online training is often seen as a very effective solution. The publication of a guide is a requirement. Moreover, the training content might evolve over time due to new technologies etc. and trainings should therefore be implemented as regular activities.

In England, training is a problem, when the few taxonomists currently involved disappear, nobody else will have those skills and the English Environment Agency will not pay to keep the taxonomists. Moreover, agencies do not promise training, but rather try to save money.

In Cyprus, the implemented approach has been very positive. The promoters of molecular techniques (Administration and University) started with a workshop with selected stakeholders and contributors. It was really an introduction: what is done and where. During this workshop, organisers were very attentive to the stakeholders' questions in order to focus their inputs and streamline their efforts accordingly. In this way, they were able to create a win-win situation with the taxonomy experts who helped them a lot, and who in turn could see the benefits they could gain from the development of DNA-based methods. They explained that the methods, traditional and DNA-based, are complementary and jointly developed (in Cyprus most species are neither known nor barcoded). Therefore, there is time before total replacement, so operators were not afraid of these new methods. But it is true that Cyprus is a small island, scientists are close to stakeholders, decision chains are short and procedures can change rapidly.

Awareness-building and involvement of decision-makers and contracting authorities

Webinar slide / proposed orientation

Above all, it is essential to inform and raise the awareness of decision-makers and water managers about the challenges of high-quality biomonitoring and the potential of DNA-based methods. Then:

At the European and national level, it is essential to:

- maintain the level of ambition of the water policy, especially its human and financial means;
- promote and strengthen collaborative research networks;
- strengthen the access to national and European environmental funding;
- develop efficient biomonitoring strategies and economic models;
- evaluate the benefits from sample to global level; and
- establish a strategy to use those gains to improve the quality of aquatic environments.

At the territorial level and towards the territories, it is necessary to:

- disseminate information by means of syntheses and guides, including decision-support keys (in what situations are DNA-based methods technically and economically relevant and which methods);
- explain the possible changes in the classification of water bodies that can be related to the change of method, and support the contracting authorities to restore degraded situations with specific technical and financial means; and
- encourage local and regional authorities to commit themselves to strong initiatives in favour of biodiversity thanks to DNA-based methods and make it a criterion for granting aid.

At the company level, it is necessary to:

- promote knowledge of DNA-based methods, their advantages and limitations;
- build on the gaps of current methods (e.g. to show that a better knowledge of the environment allows a better understanding of the impacts of industrial activities and of possible persistent non-improvement); and

- build on ‘social and environmental responsibility’ approaches and aim for an overall positive impact on biodiversity.

Water managers and policy-makers see the buzz around DNA-based methods, but they need a better understanding of what these methods can achieve, without having to know all the technical details, and want to be convinced of their benefits.

What are the valuable arguments?

The participants developed differentiated analyses, sometimes slightly different, sometimes partly contradictory. In the following paragraphs, we have chosen to present the diversity of these expressions, without seeking a synthesis at all costs.

There are diverse stakeholders involved at different parts of regulatory biomonitoring and therefore target-specific arguments to inform about the advantages of DNA-based methods are needed. The different groups are:

- those who see no necessity in incorporating DNA-based methods in current biomonitoring (hardest to convince);
- those motivated by the potentially lower costs allowing to save money or expand the monitoring;
- those who are willing to pay more for more or better information require more elaborate arguments.

In any case, a form of lobbying is necessary, but you need to be able to have the right arguments.

Managers and decision-makers can be convinced as soon as we can prove that, in a specific context, the method gives good quality results, provide highly valuable information that we don’t have with other methods, that it saves money (in most cases) or is cost-effective, and last, that it can be applied routinely. For example, for species detection it works because DNA-based methods have more results, more guidelines.

This was particularly true in the United Kingdom where the involvement of the government was not an issue, from the outset it was convinced of the value of moving to DNA-based methods to save money. But in fact, it is difficult to compare the costs, to understand which method is the best, because it's not just a question of replacing one method with another. It should be noted that today it is important to keep the surveillance system up and running, despite the post-Brexit budget cuts.

Can these methods be adopted if they do not save money, at least initially (due to investment needs), but provide more and/or more useful information? With DNA-based methods, could we get a better indication of what is going on (especially in multi-pressure contexts)? This question was much debated among the participants. In any case, beforehand, it is necessary to understand what the managers' real information needs are.

For WFD applications, it would be necessary to know what it will become after 2027. Perhaps the timing will be more flexible, the approach more flexible, which could be a good opportunity to develop new methods. However, if the requirements are reduced, there is an even greater risk of moving towards a less qualitative bioindication.

We need to improve DNA-based methods and be able to show that they are far better than traditional methods in many respects. Currently, determining ecological status is a huge task

of sampling, analysis, all this to end up with a score on a 5-point scale! We know very little about the spatial distribution of species of conservation concern! We can provide what is currently requested and go further so as not to waste any more data. We need to prove that DNA-based methods can provide ecological status, but also much more.

However, in Belgium, for example, managers have to reduce the number of samples for budgetary reasons. They take their time to understand the added value of DNA-based methods in terms of results, including impact on water body classification and savings. Nevertheless, this reflexion is not primary based on scientific criteria.

In France, many Water Agencies have raw data that they do not currently valorise. Acquiring more data is not a good argument; making the most of the available data can be one.

Many managers think that they do not have problems because they are already able to do biomonitoring in compliance with the WFD. They wonder what the real added-value of a new method is. In the Netherlands, the main problem with conventional monitoring is related to fish inventories, as they are not possible or unsatisfactory in some rivers. For this reason, methods based on eDNA are now widely explored as an alternative for fish monitoring, and not for macroinvertebrates, as it addresses here the main problem encountered by managers.

For the EU, if DNA-based methods can be the key to reducing the differences between regions and countries, this will be a very strong argument. In addition, the implementation of these methods could be relevant for groundwater, and more generally where there are no restrictions in the EU framework or where biological assessment methods are lacking, e.g. transitional waters.

For one participant, the impetus may come from legislation, given the ecological impact of current methods. There is a growing interest in ethical issues and animal welfare, leading to a preference for non-invasive methods that do not destroy habitats. DNA-based methods could be of interest to policy makers, as the overall environmental impact of the method could be lower (e.g. collection, sampling and killing of animals is not necessary, less travel for operators). But we also need to look at the impacts of the laboratory molecular analysis: consumables, waste, discharges... Everything must be considered and weighed against each other.

How to ensure that the implementation of DNA-based methods does not lead to a loss of ecological assessment quality and human expertise in this field?

During the SYNAQUA project workshops in France and Switzerland, the taxonomists and ecologists who were present expressed their fear of losing much quality in an effort to save money at all costs. For this reason, the project recommended that the two methods be maintained in parallel, at least as a transition. Moreover, applying both methods is a way to compare both and better know what eDNA can measure, before deciding which method is most appropriate to which monitoring. However, for some central administration, eventually a choice will have to be made between the two methods, as there is no means to maintain both. For example, in the United Kingdom, the Environment Agency no longer applies the light microscopy and DNA methods in parallel for diatoms. The department selects the most cost-effective method based on its environmental objectives. The DNA method is not suitable for WFD reporting but can be used for investigative analysis. In the same spirit, one can fear that the administration is prepared not to retain or to reassign individuals whose skills at first sight are no longer considered as useful, even if they have considerable expertise in taxonomy and ecology. Here, careful considerations and measure are required to maintain this expertise in the field. How to go ahead

Some key-points

As DNA-based methods are constantly evolving, the regular adaptation of the actors (managers, operators...) will be necessary. Communication will have to be exemplary and very effective. This means that the transfer of knowledge is of great importance at all levels: between scientists at European level, but also from managers to scientists (to express their needs, what they can organise and finance...) and from scientists to managers (to express the methods developed, their advantages and limitations, their potential applications...).

At first, it is important to set up good pilot projects that produce compelling information showing how biomonitoring can be improved and money saved. This is essentially what can help to start convincing stakeholders. These pilot projects need to consider the cost dimension in their analysis. Such convincing applications have been implemented by the International Commission for the Protection of the Rhine (ICPR) as well as the Moselle and Saar Commission (IKSMS-CIPMS). It is very important to share the results of these international and national pilot projects widely.

These good pilot projects help to convince that DNA-based methods are valid ones. They must also generate global reflections, whether technical or more fundamental, on the usefulness and added value of these new methods for the sake of the environment and the society.

ECOSTAT sub-group

To mobilise public authorities, we need to be clear on how DNA-based methods can be used. There are many different options at the moment. Are DNA-based methods complementary or do they replace traditional methods? What methods are currently used in some countries? What about intercalibration? What are the advantages? Before applying it, we need to share knowledge and experience from other countries, and also develop protocols and standards. Therefore, we need to discuss these issues with ECOSTAT.

We are at a stage where the development of DNA-based methods for biomonitoring is advanced, as is the interest of stakeholders in the different countries. This is the right time to create an ECOSTAT sub-group, i.e. a platform for collaboration between ECOSTAT and scientists working on DNA-based methods, to discuss and advance their implementation.

European projects

The DNAqua-Net project will be completed soon. All participants would like to see a new COST Action dedicated to implementation.

However, this will hardly be enough. To be really efficient, this COST Action dedicated to transfer to stakeholders should be associated with an applied research project (a transboundary joint research survey), that specifically involves and favours better interaction between different countries who would share their samples and methods. This applied research project could be financed by the EU. Influencing the next Horizon Europe program or the thematic priorities for the future LIFE calls would help to get European funding. In this perspective it is important to collaborate regarding the mission to regenerate our oceans and waters by 2030 proposed by the *Mission Board on Healthy Oceans, Seas, Coastal and Inland Waters*, within Horizon Europe[26].

[26] See the interim report: *Regenerating our ocean and waters by 2030 : Interim report of the mission board healthy oceans, seas, coastal and inland waters – Study* (https://op.europa.eu/en/web/eu-law-and-publications/publication-detail/-/publication/d0246783-b68a-11ea-bb7a-01aa75ed71a1)

Awareness-building and involvement of civil society

Webinar slide / proposed orientation

Foster and rely on citizen mobilisation to convince decision-makers

The use of DNA-based methods in biomonitoring should be included in awareness-raising initiatives related to water and biodiversity implemented by specialized organizations (environmental protection associations, conservatories of natural areas...), which must be integrated into actions to mobilise and train professionals

Besides, interactions between scientists and civil society, including citizen science initiatives, should be developed

This policy area was addressed by only 3 of the 7 sub-groups.

Rely on citizen mobilisation to convince decision-makers

For some of the participants, seeking to involve citizens is very relevant. They have played an important role in water quality issues and they are always looking for information on this subject. The better understanding of the quality of aquatic environments provided by DNA-based methods is in line with citizens' concerns. Of course, the first step is to explain to people what environmental biomonitoring is before addressing DNA-based methods.

Several participants experienced that when they were doing DNA-based biomonitoring, people were curious to understand. The Cypriot participants had a small project with a hotel that was interested in learning about the biodiversity nearby and funded it. To their opinion, there is a lot to do in tourist areas and with businesses in this sector.

Involve citizens in biomonitoring operations

The participation of civil society in biomonitoring offers real potential. In Cyprus, a University project was carried out on the coast, with the participation of citizens, and it was a positive experience. The United Kingdom also has positive experiences in this area. However, there may be contamination problems in samples taken by citizens, especially children. It is therefore not possible to involve citizens in regulatory biomonitoring or more broadly, when the quality criteria are very strict. On the other hand, such initiatives can be particularly valuable in the early detection of invasive species thereby allowing timely measures. Further, participative science can also help to create vocations. The collaboration of fishermen is sometimes requested to collect specific species.

Democratize biomonitoring

More broadly, participants see DNA-based methods as an opportunity to democratize biomonitoring, providing an opportunity for public or private organizations to easily monitor the biodiversity present in their environment (for example, obtain a list of species by themselves).

Creation of a steering structure

Webinar slide / proposed orientation

A steering system should ensure coherence and integration of the areas of all activities. At a National level, this system could bring together the various experts/stakeholders, and would have the following missions:

- Disseminate and promote DNA-based methods among managers;
- Identify / formulate / map needs for DNA-based biomonitoring;
- Support research actions responding to the identified needs, in particular through assistance in mobilising funding;
- Implement the transfer of methods to operators and managers (guides/protocols, accreditation, standards, training...); and
- Be positioned as an advisory and support body for public policies.

The implementation of new methods is a slow process, generally slower than expected due to the gap between science and practice. Some participants fear that it will take another 5 years, to discuss, demonstrate through pilot projects, while exchanging with colleagues (advice, tools...), before seeing the implementation of DNA-based methods. It is, in this context, all the more important to set up coordination mechanisms to keep the course and pace.

The creation of a sub-group within ECOSTAT and the launching of a new COST Action dedicated to implementation could ensure this coordination of actors complementarily.

A project like DNAqua-Net involves a large number of participants (about 600) from all over the world (49 countries), whereas an ECOSTAT sub-group involves only 20 experts. The COST Action could mobilize and contribute to the development of scientific expertise worldwide, while ECOSTAT could focus on implementation and standardisation. This organisation corresponds perfectly to the usual functioning of ECOSTAT, which relies on one or more scientific bodies. The COST Action can provide scientific advice and training, but will surf mainly in academic waters.

Some participants would have liked to involve all stakeholders at the international level in a kind of commission for integrated water management. Such an implementation-oriented initiative has to come from water managers rather than scientists. On the other hand, one of the challenges is to involve all stakeholders while maintaining a light and efficient structure. A strong and motivated core-group is needed as a starting point, which could be expanded at a later stage.

ECOSTAT sub-group

ECOSTAT operates with 3-year mandates. The current mandate covers the period 2019-2021. A new sub-group could be part of the programme for the next mandate. The “DNA sub-group” proposal should be presented in a coming ECOSTAT general meeting. Member States would then be invited to participate with designated experts. However, in order for this sub-group to be created, Member-States must express an interest in this issue. We must therefore continue our efforts to raise the interest of decision-makers.

Each ECOSTAT sub-group has its own objectives, its own timetable, its own work plan, its own national experts and so on. ECOSTAT is really the place where the choice of bio-assessment methods is discussed; all EU countries having a representative. The challenge is to really include DNA-based methods in the EU monitoring toolbox!

Furthermore, as DNA-based methods are in their infancy, the creation of an ECOSTAT sub-group now would be a great opportunity to start with an approach and methods that have well-

established and to have something coherent at EU level. In many countries, the testing is starting now, and all the information and lessons that will be produced should be able to be collated and analysed centrally.

This sub-group within ECOSTAT should have a clear mandate, e.g. to share knowledge, produce recommendations, explain what we can do with DNA-based methods, how they will improve the implementation of the WFD? For some participants, the production of a realistic implementation scenario seems to be more a priority than standardisation.

The European DG Env, with its working groups in charge of the WFD and innovation, must be involved as it is in charge of the strategic vision and decides on the creation of a new structure. In addition, the relevant existing structures at national (see below) and European level should be taken into consideration.

A new "DNAqua-Net" project

The DNAqua-Net Steering Committee is planning to set up a new COST Action aligned to the first one and dedicated to implementation. The participants are very interested in this idea considering that it is difficult to influence the European level, it is a long-term objective, whereas from a technological point of view it is possible to move faster and keep pace with technological developments. In this way, it will be possible to keep up the momentum in the EU, to demonstrate the feasibility of DNA-based methods and to advance the subject as a whole.

The COST Action Management Committee, with one representative from each country, would be appropriate to create a dynamic between countries. It would ensure the transition to the creation of a permanent sub-working group within ECOSTAT.

It could, among other tasks, collect good practices and results from pilot projects, prepare step-by-step guidelines and transmit them to practitioners, show and communicate the different approaches, advantages and benefits of the methods. Setting up an online platform where people could share their experiences, results, needs, provide samples... could eventually pool research efforts and generate economies of scale[27].

For most participants, this COST network has an important role to play in several aspects related to the implementation of DNA-based methods, but it must remain a scientific reference network.

It is recalled that for DNAqua-Net it was not possible to fully achieve the planned objectives; the technical issues proved to be more complicated than imagined a priori. If the new project focuses on implementation, its objectives will have to be carefully calibrated.

Finally, COST actions have funding only for networking and need to be supported by research programs funded by other sources. This network must be in a position to help obtain national and European funding to finance pilot projects. The European Green Deal[28] could create opportunities for an ambitious project.

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[27] The Corona situation has taught us how to do meetings/conferences without having to travel by plane: https://www.nature.com/articles/s41559-020-1190-x.pdf (from members and colleagues of DNAqua-Net)

[28] https://eur-lex.europa.eu/resource.html?uri=cellar:b828d165-1c22-11ea-8c1f-01aa75ed71a1.0002.02/DOC_1&format=PDF
https://eur-lex.europa.eu/resource.html?uri=cellar:b828d165-1c22-11ea-8c1f-01aa75ed71a1.0002.02/DOC_2&format=PDF

Development and coordination of national initiatives

For some participants, a European steering structure is not a priority. National action is necessary in the first instance. ECOSTAT is perfectly adapted to take orientations, but is not operational. Then, coordination and steering seem to them simpler and more relevant at national level, even if ECOSTAT is involved. In Austria, for example, a national initiative to involve the government in matters relating to the DNA-based methods is essential before coordinated action at European level can be considered. It must be remembered that Member States must show their interest in the ECOSTAT sub-group.

However, the two levels should not be opposed, but thought out in a coordinated way. All the more so as there is great heterogeneity between national situations. The European Open Sciences Cloud[29] (EOSC) has established various working groups at the national level. It would be good if, in connection with DNAqua-Net, in the same way, national groups could be set up or continue to function in each country as an advisory body, and meet from time to time together and with ECOSTAT. A European emulation would be beneficial, while relying on the most advanced countries and the resources they have developed.

In the United Kingdom, English and Scottish governments have established initiatives such as the Department for Environment, Food and Rural Affairs (DEFRA) DNA Centre of Excellence and the Scottish Hub respectively. These groups cover a wide range of topics and aim to speed up the implementation of DNA-based methods into operational use. They work closely with the UK DNA Working Group30, an initiative set up by the Environment Agency in 2014 to provide a platform for engagement between stakeholders and the research community. This group is supported by a scientific advisory group consisting of end-users, selected academics and some small and medium-sized enterprises (SMEs).

This example shows that at both national and European levels it can be useful to involve businesses. In the face of declining public funding, partnerships with businesses can help mobilise new resources (the Carnot Institutes scheme, the equivalent of the Fraunhofer Institutes in Germany, has been mentioned for France).

[29] https://www.eoscsecretariat.eu/eosc-working-groups

[30] http://www.ukeof.org.uk/our-work/ukdna

Conclusion

Most of DNA-based methods are now mature enough to be implemented into biomonitoring and meet their end-users needs. Whatever the innovation concerned, this transition phase from the laboratory to the field is very delicate and deserves to be well thought out. Two overall approaches can be adopted: wait until the method is fully tested, evaluated and calibrated before implementing it, or adopt an adaptive approach as already adopted in England. In the first approach, because optimisation of a method is an endless task, stakeholders will need to agree on when development will be deemed sufficient to move to the operational phase, even if questions remain. In the second approach, harmonisation and comparability of the methods will be the challenge. Although no prior choices had been made, this workshop was more concerned with the second approach.

Demonstrate effectiveness and benefit of DNA-based methods

Despite the popularity of DNA-based methods, their effectiveness and added value compared to traditional methods for different uses, their compliance with the WFD as well as their cost still deserve to be demonstrated to all stakeholders before routine use. Beforehand, it is necessary to understand what the managers' real information needs are.

That is the reason why many projects of comparison between traditional and DNA-based methods, in Europe and more widely, have been or are being implemented. The English experience showed that the comparison of methods must be organized over a wide spatial coverage but also over a sufficiently long period of overlap. Thanks to pilot projects throughout Europe, it is now commonly accepted that traditional and DNA-based methods are complementary.

It appears essential to clearly distinguish applications within WFD framework from others. Where traditional methods are satisfactory, all the more if they are required by regulation, the implementation of DNA-based methods, which will be accompanied by challenges and uncertainties, is not a priority, especially if the new one is more stringent in terms of ecological assessment. It may be more efficient to focus on topical management issues not yet addressed by regulatory monitoring: monitoring the impact of restoration, biodiversity, invasive or endangered species, developing new metrics that can help to measure the impacts of multiple pressures on the environment in order to deliver ecological diagnosis, providing tools where biological assessment methods are lacking (e.g. groundwater, transitional waters) or monitoring usually neglected waterbodies like small streams. More globally, the development of new indices adapted to the data acquired by DNA-based methods is necessary and would further improve the quality of ecological evaluation.

Furthermore, the DNA-based methods implementation could be the opportunity of reducing the differences between regions and European countries.

Provide best practice guidelines and standards

In the fields of applications and in countries where DNA-based methods are beginning to be applied, difficulties appear to be linked to the heterogeneity of practices and, as a result, the difficulty of comparing results, some methods being not optimal. A need for standardisation and inter-calibration (mandatory for the WFD) at national and European levels was therefore expressed.

However, standardisation may come up against several problems:

- The impossibility of responding satisfactorily to the heterogeneity of national or even regional situations;
- The complexity and the delays of such a process, without being sure to reach the end;
- The risk of developing standards that are unsuited to the operational context, in terms of technical and/or financial feasibility, and therefore not followed;
- The risk of freezing the situation without being able to benefit from the very rapid scientific and technological progress in this field.

It is therefore recommended to develop a flexible approach consisting of producing guidelines and guides to good methods and practices for a general approach, and to go further in imposing methods and standardisation for certain key stages such as sampling. This is the approach adopted by the CEN TC230/WG28.

A guide on how to perform intercalibration of DNA-based methods should be produced.

At the national level, as it already exists in some countries, a reference laboratory or body could be the guardian of quality, responsible for training people, implementing intercalibration, testing new methods. These national bodies could coordinate at European level.

It would be interesting to collaborate at European level on reference databases, at least in order to have species from different geographical locations and then to assess the genetic biodiversity of each species.

With the rapid development of methods, it is important to initiate these efforts without delay.

Transfer knowledge to all stakeholders

Transfer of knowledge and close collaboration is of great importance at all levels: between scientists at European level, but also from managers to scientists (to express their needs, what they can organise and finance...) and from scientists to managers (to express the methods developed, their advantages and limitations, their potential applications...).

Research in this field should focus on meeting the needs of the target audiences and, to this end, these needs should be well understood and stakeholders should be further taken into account in the research. This would allow, on the one hand, adapting the methods to the needs of the target groups, on the other hand, to communicate, discuss, and inform stakeholders about existing methods. Besides, researchers often tend to think about how to transmit their knowledge to managers (through training, etc.), and forget to ask themselves how, and at what stage, to involve managers in their research, even if they are not the funders.

The question of the gap between the development of methods by scientists and their use by managers is an issue that has been raised in several fields (water, soil, landscape, etc.). Frequently, many methods developed by scientists are in the end never used by managers, and it is not just a question of knowledge transfer. It is really the general approach to the development of indicators and methods that needs to be rethought. Several studies have shown that the knowledge of managers and scientists tends to be complementary and that both are necessary in order to co-create environmental quality assessment methods.

Therefore, communication and training will have to be exemplary and very effective. They must go hand in hand with the implementation of biomonitoring based on these methods.

This transfer of knowledge is particularly effective in joint training sessions between stakeholders of different statuses, joint discussion groups, tandem teams, steering committees, etc. However, a distinction must be made between the different training objectives and target audiences:

- basic knowledge for all types of stakeholders to discuss the topic, to dialogue with other actors;
- a deeper and more precise knowledge for the staff of organizations that will have to supervise and manage the deployment of these methods: administrations, but also standardisation bodies, etc.;
- a full mastery of these techniques for the service providers and laboratories that will implement them directly.

Accompany the profound change in the environmental biomonitoring sector

All target groups should gain confidence in DNA-based methods. However, they seem to be a "black box" for stakeholders, especially the bioinformatic pipelines, and they will not trust or use them until they understand them. Different approaches can be used to address this situation: pilot studies, demonstrations, experiments...

For those involved in implementation, a better level of understanding of DNA-based methods' added-value needs to be achieved: identifying the precise situations (taxa, habitats) where the two approaches are complementary (even if the overall costs are increased), where they are substitutable, where one is better than the other.

Both contracting authorities and operators who outsource part of the DNA-based method tasks need to be able to control quality of contractors and result traceability. Then, information about repeatability, uncertainty and control points of the methods are needed.

Data interpretation and ecological conclusions based on DNA-based methods, as well as those based on traditional identification, must be done by ecologists. But there is a fear that the administration is prepared not to retain or to reassign individuals whose skills it no longer considers useful, even if they have considerable expertise in taxonomy and ecology. How to keep them?

More globally, DNA-based methods imply a complete reorganisation of the ecological quality and biodiversity assessment sector. It is a widely shared observation that it is a big gamble for private companies to invest and get involved in these rapidly evolving technologies/approaches. Moreover, as there is no regulatory demand, there is no market at the moment.

Behind these questions, funding is a key issue everywhere. Because of the current economic strategy of several countries that are reducing their bioassessment efforts, there is a fear that DNA-based methods will be introduced with the aim of saving money at all costs, losing much quality and professional expertise. It is likely that working together at the European level would be useful to share costs and benefits from different experiences, but it may not be enough.

It is important to demonstrate that the implementation of DNA-based methods is not only a cost-effective solution, but that it can be used to improve the biomonitoring and provide more information. In addition, it is important to demonstrate that this extra-information is useful for decision-makers, particularly in relation to climate change.

There is a need to develop a culture of bioindication that promotes solutions leading to optimal biomonitoring: goal-oriented, i.e. aimed at understanding environmental changes, proposing targeted measures, integrating at the same time cost-benefit analysis and a concern for practicability. Operators also need to know how to communicate and get the message across to policy makers and funders.

An impetus may come from new legislations related to the overall ecological impact of current methods. In addition, the better understanding of the quality of aquatic environments provided

by DNA-based methods is in line with citizens‘ concerns. Involve citizens in biomonitoring operations. Participative science can also help to create vocations.

In practice, the national level seems to be appropriate to integrate these new methods into monitoring programs. The EU could provide general requirements, but they would necessarily be declined by each Member State according to its own policy.

Under the WFD, it is not recommended to change the method during a management cycle i.e. before 2027. In order to consider the uptake of genetic methods after 2027, testing, demonstration of compliance and validation must be carried out beforehand.

Harmonise efforts at European level

We are at a stage where the development of DNA-based methods for biomonitoring is advanced, as is the interest of stakeholders in the different countries. This is the right time to create a steering group like an ECOSTAT sub-group, i.e. a platform for collaboration between ECOSTAT and scientists working on DNA-based methods, to discuss and advance their implementation, provide guidance and encourage, if necessary, Member States to develop the DNA-based methods. Likewise, CEN (or even ISO, but it is more complicated to be quickly operational at this level) is the appropriate level to develop technical standards.

This sub-group within ECOSTAT should have a clear mandate, e.g. to share knowledge, produce recommendations, explain what can be done with DNA-based methods, how the implementation of the WFD will be improved… For some participants, the production of a realistic implementation scenario seems to be more a priority than standardisation.

The work of the ECOSTAT sub-group could therefore provide a common framework for Member States to develop their own indices and national surveillance strategy, which is their own responsibility. National action is necessary in the first instance. However, the two levels should not be opposed, but thought out in a coordinated way, all the more so as there is great heterogeneity between national situations.

ECOSTAT has a three-year mandate. The current mandate covers the period 2019-2021. The sub-group could be part of the programme for the next mandate. The next ECOSTAT meeting in October 2020 could be the place to present this proposal. Member States would then be invited to participate with designated experts. But, in order for this sub-group to be created, Member-States must express an interest in this issue.

Furthermore, this network must be in a position to help obtaining national and European funding to finance an applied research project (e.g. a transboundary joint research survey), that involves and favours better interaction between different countries who will share their samples and methods. Influencing the next Horizon Europe program or the thematic priorities for the future LIFE calls would allow getting European funding. The European Green Deal could also create opportunities for such an ambitious project.

The DNAqua-Net project will soon be completed. All participants would like to see a new European network fully dedicated to implementation. The DNAqua-Net Steering Committee is planning to set up a new COST Action aligned to the first one and dedicated to implementation. It must remain a scientific reference network whose objectives would be to prepare step-by-step guidelines and transmit them to practitioners, show and communicate the different approaches, advantages and benefits of the methods, etc. Setting up an online platform where people could share their experiences, results, needs, provide samples, etc., could eventually pool research efforts and generate economies of scale.

Annexe 1: Participants to DNAqua-Net prospective workshops

Participants	Nationality
Didier Pont	Austria
Frank Narendja	Austria
Pat Mergen	Belgium
Pieter Boets	Belgium
Iakovos Tziortzis	Cyprus
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Sandra Poikane	Europe
Kristian Meissner	Finland
Marko Järvinen	Finland
Benjamin Jeannot	France
Christian Chauvin	France
Christine Argillier	France
Nicolas Hette-Tronquart	France
Olivier Monnier	France
Remy Marcel	France
Valentin Vasselon	France
Daniel Hering	Germany
Florian Leese	Germany
Helmut Fischer	Germany
Jens Arle	Germany
Julia Kleinteich	Germany
Sascha Krenek	Germany

Participants	Nationality
Andrea Zagyva Tünde	Hungary
Stefania Erba	Italia
Stefania Marcheggiani	Italia
Alexander Weigand	Luxembourg
Diane Neu	Luxembourg
Berry van der Hoorn	Netherland
Steinar Sandoy	Norway
Pedro Beja	Portugal
Verónica Onofre Pinto	Portugal
Rosa Trobajo	Spain
Gunilla Ejdung	Sweden
Jonas Svensson	Sweden
Maria Kahlert	Sweden
Michael Haldin	Sweden
Alina Pawlowska	Switzerland
Benoit Ferrari	Switzerland
Jan Pawlowski	Switzerland
Marie-Sophie Renevier	Switzerland
Serdar Koyun	Turkey
Alice Hiley	UK
Bernd Haenfling	UK
David Mann	UK
Kerry Walsh	UK
Martyn Kelly	UK
Willie Duncan	UK