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# Freshwater biomonitoring in the Information Age

François Keck<sup>1,2\*</sup>, Valentin Vasselon<sup>1</sup>, Kálmán Tapolczai<sup>1</sup>, Frédéric Rimet<sup>1</sup>, and Agnès Bouchez<sup>1</sup>

Freshwaters worldwide face serious threats, making their protection increasingly important. Freshwater monitoring has historically produced valuable data and continues to develop. Rapid improvements to biomolecular techniques are revolutionizing the way scientists describe biological communities and are bringing about major changes in biomonitoring. Combined with high-throughput sequencing, DNA metabarcoding is fast and cost-effective, generating massive amounts of data. In a world with numerous ecological threats, “big data” constitute a tremendous opportunity to improve the efficiency of biological monitoring. These fundamental changes in biomonitoring will require freshwater ecologists and environmental managers to reconsider how they handle large amounts of data.

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Human activities have broadly affected freshwater ecosystems, especially since the Industrial Revolution. Over the past 50 years, however, policy makers and citizens have become more attuned to environmental issues. This has led to the development of important governmental programs to assess and limit ecological impacts of human activities (Figure 1). In this context, one objective of environmental managers is to evaluate how water quality changes over time. Bioindicator organisms are commonly used for this purpose, based on the premise that the presence or absence of certain biological communities at a given site reflects its environmental quality.

Freshwater biomonitoring has a long tradition in the field of ecology. A century of research has led to substantial improvements in understanding how human disturbances can shape biological communities. Based on this knowledge, many approaches have been developed to estimate environmental quality from the richness, diversity, structure, and functioning of these communities

(Jørgensen *et al.* 2010). These widely used methods are based on solid theoretical grounds and are known to perform quite well. Most of them commonly require a taxonomical description of the community. Hence, freshwater biomonitoring essentially consists of collecting individual organisms, performing taxonomic identification, and using inventories to estimate the environmental condition of a given site. However, traditional biomonitoring also faces recurrent criticisms, mainly related to taxonomic identification relying on morphological criteria, a process that is time-consuming, complex, and technically demanding (Mandelik *et al.* 2010). These limits inevitably restrict the number of sites that can be monitored and the frequency of controls.

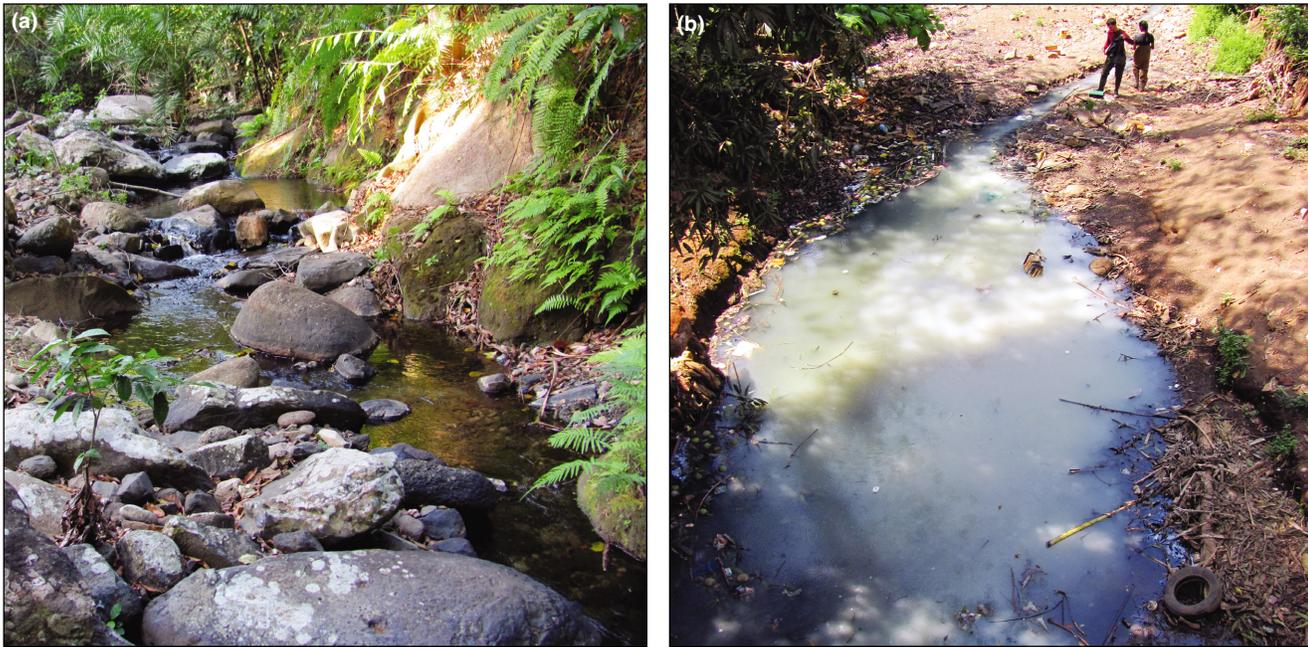
During the past decade, the idea arose that DNA analyses (Figure 2) could advantageously replace morphological methods to identify species (Hebert *et al.* 2003). Metabarcoding was developed as a set of techniques to identify multiple taxa simultaneously from an environmental sample with standard genetic markers (Taberlet *et al.* 2012; Panel 1 and Figure 2). This has led to the idea of “Biomonitoring 2.0”, which offers novel perspectives for monitoring environmental communities (Baird and Hajibabaei 2012). In this paper, we explain why and how metabarcoding will profoundly change the nature of data produced by biomonitoring. We examine these changes in the general context of massive data production – so-called “big data”, a topic that is the subject of increasing interest in biology (Marx 2013). We show why this big data revolution holds promise for ecological assessment purposes. Finally, we highlight three challenges posed by big data for metabarcoding and propose a framework that takes them into account. We illustrate our point with examples taken from freshwater monitoring, where metabarcoding is developing rapidly (Hajibabaei *et al.* 2011; Kermarrec *et al.* 2014). Nevertheless, the ideas discussed could be extended and applied to a broader context.

## In a nutshell:

- DNA metabarcoding and high-throughput sequencing methods produce massive quantities of data and will markedly change freshwater biomonitoring
- Molecular methods propel biomonitoring into the Information Age and bring exciting new opportunities to make ecological monitoring more effective and relevant
- Genetic “big data” challenge scientists to think differently about the way that biological monitoring information is analyzed; we propose and discuss alternatives to the classical taxonomic affiliation approach to process bioassessment metabarcoding data

<sup>1</sup>UMR CARTELE, Institut National de la Recherche Agronomique, Université Savoie Mont Blanc, Thonon-Les-Bains, France;

<sup>2</sup>Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences, Uppsala, Sweden \* (francois.keck@gmail.com)



**Figure 1.** Two streams included in the river monitoring network of Mayotte Island, France. (a) A pristine upstream site (Longoni River) and (b) a polluted site located downstream of village waste (Majimbini River). The biological assessment of Mayotte's rivers currently relies on benthic diatom communities studied using both classical morpho-taxonomical and metabarcoding approaches.

### ■ Biomonitoring as a source of massive data

Characterizing ecological quality from biological entities has produced important sources of data since the first attempts to do so at the beginning of the 20th century. This is because biomonitoring largely consists of sampling, identifying, enumerating, and reporting biological organisms. The saprobic system for organic pollution assessment developed by Kolkwitz and Marsson (1908, 1909) is often cited as the first bioassessment tool in freshwaters and uses 298 plant species and 527 animal species as indicator organisms. Methods soon diversified thereafter, and specific biological groups (fishes, macroinvertebrates, algae) have been employed. Increasing stringency in precision requirements has led to more powerful and sophisticated tools, based on hundreds of families and thousands of species.

The amount of data produced has increased rapidly because biomonitoring is rarely done in isolation, but instead is replicated across space (through a network of sites; eg along a river, within a watershed) and over time (long-term monitoring). Since the 1970s, general awareness of ecological issues has grown, and biomonitoring has been increasingly implemented and incorporated into legal frameworks for fresh waters, such as the Clean Water Act (CWA, 1972) in the US and the Water Framework Directive (WFD, 2000) in Europe. This guarantees the abundant production of data with respect to recognized standards.

However, biomonitoring methods are expected to change considerably in coming years. After a century of classifying taxa based on morphological criteria, species

can now be identified through the use of DNA barcodes (Hebert *et al.* 2003); for definitions of selected specialist terms used throughout, see Panel 1. The introduction of high-throughput sequencing (HTS; Shokralla *et al.* 2012) coupled with the development of extended reference databases (Ratnasingham and Hebert 2007; Benson *et al.* 2008) and efficient bioinformatics tools (eg Schloss *et al.* 2009) have enabled the production of reliable and cost-effective community inventories from environmental DNA (Chariton *et al.* 2015; Gibson *et al.* 2015; Pawlowski *et al.* 2016). While numerous issues and technical limitations remain (DNA spatial transfer and persistence over time, polymerase chain reaction [PCR] amplification biases, sequencing errors, chimeras, quantification; see also Coissac *et al.* 2012 and Shokralla *et al.* 2012), methods are improving quickly and metabarcoding is expected to be an increasingly important component of biomonitoring in the future.

The progressive adoption of metabarcoding for taxonomical identification will substantially increase the volume of data produced by biomonitoring activities and modify the characteristics of these data (Dafforn *et al.* 2016). It is often stated that characteristics of big data fulfill five “Vs”: volume, velocity, variety, variability, and value (Fan and Bifet 2013). Biomonitoring data will likely meet these five criteria in unprecedented ways in the coming years.

### Volume

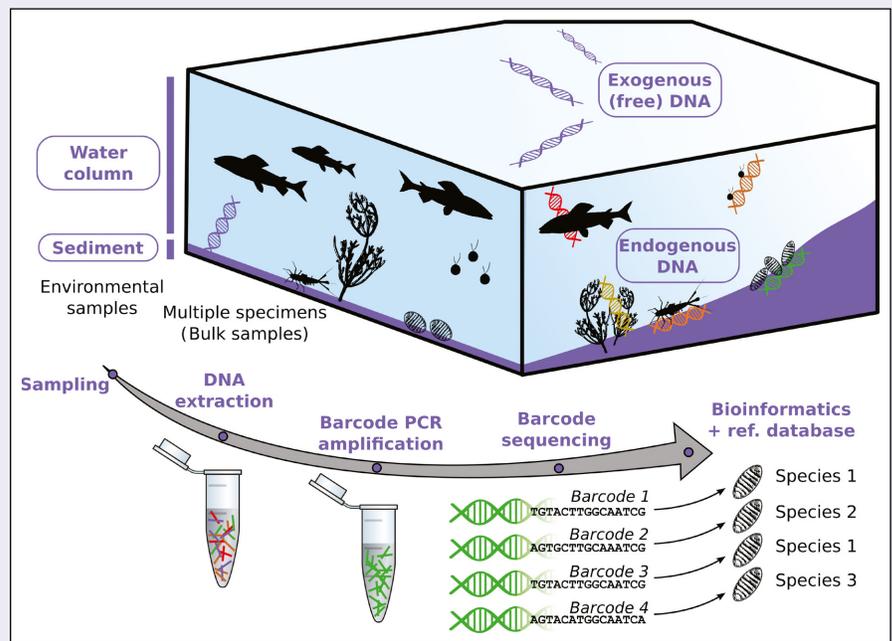
The amount of data acquired from biomonitoring is expected to increase very quickly. HTS techniques are

### Panel 1. Biomonitoring and metabarcoding

The biological monitoring of freshwater systems is traditionally based on the morphological identification of indicator species, which provides information on the ecological status of their environment. Instead of relying on morphological features (eg size, shape) to perform species identification, which requires specialized knowledge of taxonomic groups, small DNA fragments – about 300 base pairs in length, known as DNA barcodes – can be used (Hebert et al. 2003). This identification approach is termed DNA barcoding. Existing DNA barcode reference databases are based on different genes (including *COI*, *18S*, and *rbcL*) and link species taxonomy to DNA barcodes. While DNA barcoding is useful for identifying individual specimens, its application to community-level samples (ie multiple species) was difficult because it required sorted samples or even isolating and cultivating individuals. This challenge was overcome through a metagenomic method called metabarcoding, which allows for the detection of all species found in one sample directly from their DNA barcode sequences using a single workflow. The DNA is extracted directly from the sample, followed by the amplification and sequencing of the targeted DNA

barcode (Figure 2). Using bioinformatics tools, DNA barcodes are compared to those contained in a reference database to identify the species composition within the sample.

Environmental DNA was defined by Taberlet et al. (2012) as the “DNA that can be extracted from environmental samples (such as soil, water, or air), without first isolating any target organisms”. This includes DNA from microorganisms and free DNA. The free part of environmental DNA may be used to detect the presence of invasive species (Ficetola et al. 2008) or to monitor rare and indicator species (Mächler et al. 2014). Microorganisms present in environmental samples (eg bacteria, fungi, and diatoms) enable the use of longer DNA barcodes



**Figure 2.** Several steps are required to perform DNA metabarcoding: (i) the sampling of environmental samples (eg sediment, biofilm, water) or the creation of bulk samples (mix of individual specimens); (ii) the extraction of the DNA; (iii) the amplification of a DNA barcode specific to the targeted community using polymerase chain reaction (PCR) techniques; (iv) the sequencing of the amplicons (amplified DNA barcodes); and (v) the taxonomical assignment of the DNA reads (amplicon sequences) using bioinformatics and a reference database (database connecting DNA barcode sequences to their taxonomic identity). Total environmental DNA comprises “endogenous” DNA from living organisms and “exogenous” free DNA.

(Taberlet et al. 2012) and facilitate access to uncultured taxa. For example, diatom molecular inventories can be used to calculate a quality index that indicates the ecological status of the sampled river (Kermarrec et al. 2014; Visco et al. 2015). Precision and reliability of the species list obtained from DNA metabarcoding depend on the completeness and reliability of the reference database.

The development of high-throughput sequencing (HTS) enables the rapid and inexpensive sequencing of hundreds of environmental samples at a time, making the incorporation of the DNA metabarcoding into biomonitoring programs possible.

developing rapidly and have extremely high-throughput (Figure 3d). With the development of standardized protocols, the processing rate will also probably increase considerably and allow more sites to be surveyed and with greater frequency. Finally, assessments that rely on morphological criteria alone tend to underestimate species diversity, whereas the level of diversity detected by genetic methods tends to be much higher, especially for microbial communities (Caron et al. 2009), leading to larger inventory tables.

### Velocity

Traditional monitoring requires experts to undertake a long and laborious process of taxonomically identifying collected biota. Consequently, one site is typically monitored seasonally or yearly. With metabarcoding and HTS techniques, however, the identification process is automated and faster. This will allow sites to be monitored at a finer time scale and to approach real-time monitoring.

## Variety

Biomonitoring elicits multiple types of data. Community inventories generally come in the form of presence-absence or count data tables. Environmental managers often prefer to rely on multiple biological indicators (eg fishes and macroinvertebrates) to monitor multiple sources of impairment. Moreover, assessment methods commonly integrate physical and chemical data, which may also constitute big data, especially when recorded with remote sensors and with high frequency. Metabarcoding will also make it possible to work with genetic data and phylogenies (Hajibabaei *et al.* 2007).

## Variability

Biomonitoring data are valuable when there is variability in community structures between reference and impacted sites (Jørgensen *et al.* 2010). With the use of DNA, finer-scale taxonomic characterization of communities can be achieved. Thus, with appropriate analyses, it will be possible to differentiate communities in a subtler way (Stein *et al.* 2014a) and to gain capacity in distinguishing the effects of various pressures.

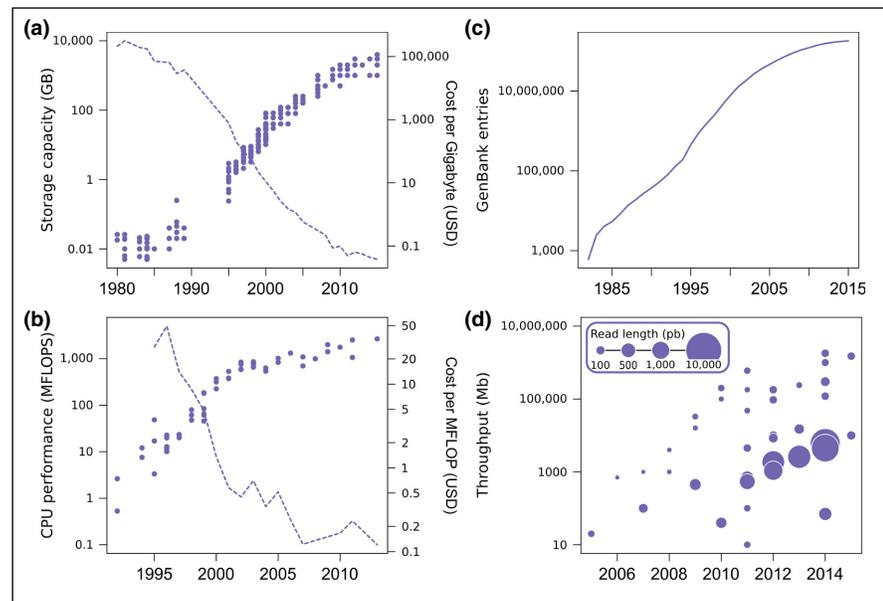
## Value

Data produced by biomonitoring are used to assess environmental quality. Many applications could be enhanced with big data, including monitoring over space and time; examining multi-trophic food web structure; and assessing the effects of pollution, environmental restoration, and invasive species. Moreover, biomonitoring data are often exploited by ecologists for purposes other than environmental assessment, such as studying biodiversity patterns or validating theoretical models (Lovett *et al.* 2007; Lindenmayer and Likens 2010).

## Modern techniques and big data

### Increasing the number of indicators

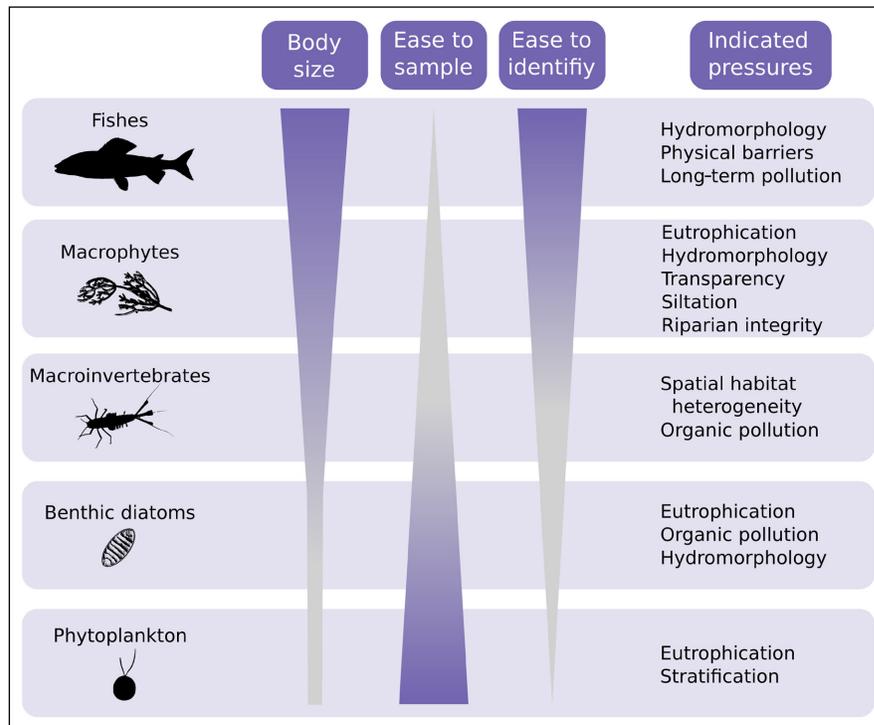
The modern concept of biomonitoring – as implemented in the WFD and CWA – is to use biological indicators accompanied by hydromorphological and physicochemical measurements (Ibáñez *et al.* 2010). For example, the WFD's bioindicators (or biological quality elements [BQEs]) are fishes, macrophytes, macroinvertebrates, benthic diatoms, and phytoplankton. Each of these indicators



**Figure 3.** The Information Age is characterized by rapid technological developments exponentially increasing scientists' capacity to produce, store, and process data. (a) Storage capacity of commercialized computer hard drives in gigabytes (dots) and average price of a gigabyte (dashed line). (b) Microprocessor performance (dots) in millions of floating-point operations per second (MFLOPS) and average price of MFLOPS (dashed line). (c) Number of entries in the open-access nucleotide sequence database GenBank. (d) The throughput and read length evolution of high-throughput sequencing technologies.

presents advantages (eg diversity, ubiquity, ecological importance) and disadvantages (sampling difficulties, lack of metrics) (Resh 2008). Each BQE can indicate different pressures and provide complementary information (Passy *et al.* 2004; Figure 4). Thus, the overall quality assessment of an aquatic ecosystem is based on the results of all BQEs. In the WFD, the “one out all out” (OOAO) rule states that the worst status of the BQEs used in the assessment determines the final status of the ecosystem. However, in practice, using all BQEs for a sampled site is seldom or only partly achieved because of both financial and logistical constraints (Birk *et al.* 2012).

There is a trade-off between the ease of sampling and the ease of identifying organisms with respect to the average size of different BQEs (Figure 4). Groups of organisms with larger individual body size (typically fishes) are more difficult to sample representatively and collect, whereas smaller or microscopic organisms such as macroinvertebrates or benthic diatoms are relatively easy to collect by sampling the substrate directly. On the other hand, larger organisms are easier to manipulate and identify. For fishes and macrophytes, identification is performed in situ, whereas macroinvertebrates, benthic diatoms, and phytoplankton require arduous laboratory-based work (chemical treatment, microscopy). Modern molecular techniques appear to offer a promising solution to the trade-off between the ease of sampling and identifying organisms.



**Figure 4.** Gradients, trade-offs, and complementarity between body size, ease of sampling, ease of identification, and the indicated pressures of the five indicators included in the Water Framework Directive.

### Covering a larger diversity

In traditional biomonitoring, taxonomical identification is rarely performed at the most precise levels of specificity because doing so is cost-prohibitive. DNA metabarcoding, however, could reveal diversity at the finest level for a fraction of that cost. With appropriate libraries, DNA barcodes can be linked to a Linnaean taxonomic name. The precision of taxonomic affiliation depends on the selected barcode and the availability of data in the reference libraries. By using correctly populated libraries, it is possible to reach the species level (eg Hajibabaei *et al.* 2011; Kermaec *et al.* 2014) with less ambiguity and discrepancy than with classical microscopy, where species-level identification is often extremely laborious and even impossible at some development stages. However, data derived from DNA carry much more information than taxonomic names alone. Baird and Hajibabaei (2012) emphasized that genetic techniques have far more potential for identifying taxa than the traditional approach of relying on morphological characteristics. DNA-based techniques should facilitate working at the infra-species level and ultimately at the nucleotide level. It will therefore be possible to disentangle cryptic species complexes and to perform population-level analyses. Having the capacity to monitor diversity at so many levels should also promote the development of very sensitive tools to monitor the effects of specific types of pollution on various biota.

### Enforcing and extending monitoring networks

High-throughput sequencing and the evolution of laboratory methods have made metabarcoding much more cost-effective (Stein *et al.* 2014b), and prices continue to decrease as technologies develop (van Dijk *et al.* 2014). DNA-based methods are also much faster than traditional methods. Sample processing can be serialized and automated with the aid of robots (Chapman 2003). Reductions in cost and processing time should boost sampling efforts by making it possible to increase the number of sites being monitored and the sampling frequency. This is an advantageous consequence of using metabarcoding, because biomonitoring often lacks spatial and temporal representativeness.

One specific site will poorly represent an entire ecosystem, particularly when habitats therein are heterogeneous and when bioindicators are micro-habitat dependent. To obtain an improved and integrated view of environmental quality, researchers must augment the number of sampling sites to account for the spatial heterogeneity of the broader area. This increases the resolution of the grid of sampled sites and enables better interpolations among the nodes of the monitored network. For a given site, the frequency of sampling is also important. A more frequent sampling protocol gives a more reliable picture of the temporal evolution of the site's environmental quality. This is especially relevant for microscopic communities, which change extremely quickly with changes in the environment. Thus, sampling plans with higher spatial and temporal resolution should enable the development of more complex spatiotemporal models and increase the capacity to detect the effects of local and diffuse pollution.

### ■ Taking advantage of the data deluge: a proposed framework

#### *From morphology to genetics: beyond the classical concept of species*

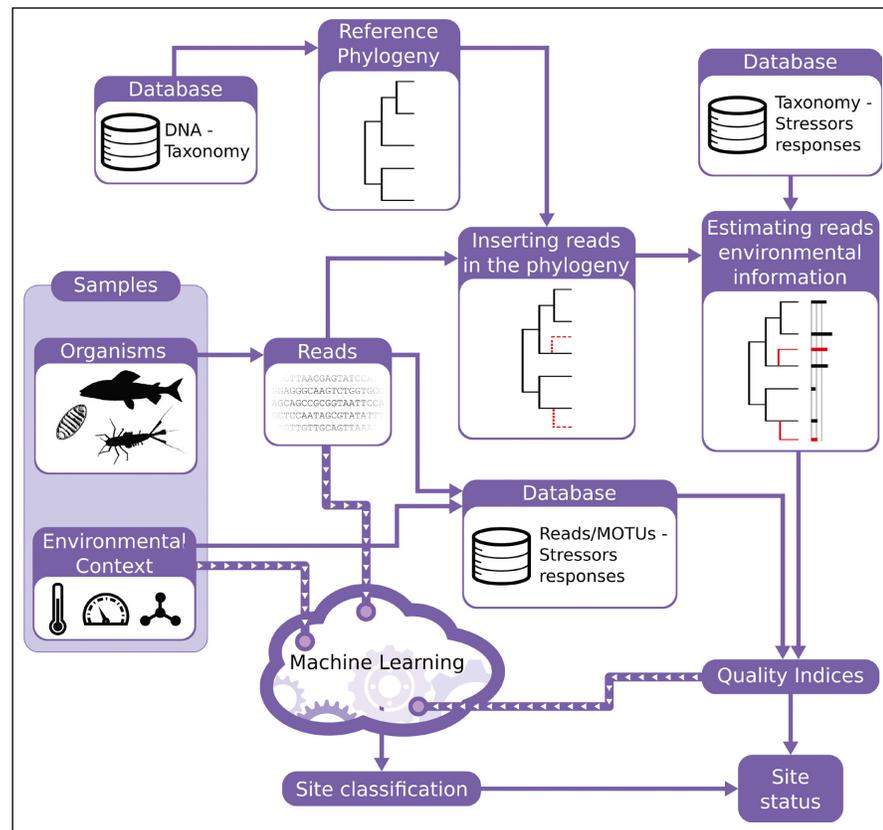
Conventional taxonomy aims to classify biological organisms in different groups based on shared traits. These groups correspond to the different taxonomic levels, with the species level as a central unit. Even if still under debate (De Queiroz 2007), the concept and definition of species provides scientists with a unit of reference for ecological studies. With the rise of molecular methods,

the DNA sequence has appeared as a promising alternative unit. Scientists have tried to integrate genetic sequences in the classical taxonomy, with varying degrees of success (Padial *et al.* 2010). However, in the context of biomonitoring, the question remains, whether the traditional Linnaean binomial species name affiliation still makes sense within a full molecular approach.

Typically, DNA reads provided by HTS are clustered into molecular operational taxonomic units (MOTUs), which are in turn converted to species units through the use of a bioinformatic workflow and a DNA reference database. The conversion from DNA reads to species units is not without drawbacks: for instance, selected barcodes may be associated with incorrect taxonomic affiliations, genetic information may be lost (unaffiliated reads are discarded), and rare species are often insufficiently studied. This approach is suitable if the reference database is sufficiently comprehensive, but this is rarely the case because of the high species diversity and the time and effort required to sequence organisms' barcodes. Previously undescribed species are also frequently detected from genetic data, while formal taxonomic description can be a very long process (Goldstein and DeSalle 2011). Moving to full molecular biomonitoring will allow for much more data to be used, beyond that limited strictly to taxonomic assignments. The greatest challenge is to develop new, high-quality indices based on DNA reads and environmental information. Three alternative but complementary approaches are described below and are represented in Figure 5.

### Developing MOTU-based indices

Biomonitoring assumes that the presence or absence of particular taxa at a site of interest is indicative of distinct environmental conditions at that site. Thus, in traditional biological assessments, an ecological profile associated with each taxon is required. Pawlowski *et al.* (2016) suggested calibrating MOTU-based indices with traditional indices computed from simultaneously conducted morphology-based identifications. However, the traditional indices could be easily adapted to the new molecular approach by computing the indices directly from the reads clustered in MOTUs (Steele *et al.* 2011). This approach would require databases associating reads, MOTUs, and their



**Figure 5.** Flowchart introducing a new framework to process bioassessment metabarcoding data. Genetic reads can be interpreted without taxonomic affiliation using reads/MOTUs-based indices, phylogenetic modeling, or machine learning.

responses to environmental stressors (Figure 5). Thus, the MOTU-based indices approach is expected to be fully functional when ecological profiles for clusters of reads are estimated directly from previous molecular inventories; this will require substantial work in addition to data compilation and sharing. As a first step, known ecological profiles for taxa can be transferred to MOTUs.

### Using phylogeny to include rare species

DNA metabarcoding can reveal a wealth of diversity, but the lack of taxon–stressor response libraries is problematic. Given that ecological profiles are usually estimated from in situ observations of general disturbances or from laboratory bioassays for specific substances, such libraries are restricted to common species and to a few types of disturbances. Rare species are often ignored (Guénard *et al.* 2011), and the effects of specific compounds remain poorly understood (Schwarzenbach *et al.* 2006).

One elegant way to solve these problems could involve phylogenetic methods harnessing the principle that species' tolerances are the legacy of evolution (Keck *et al.* 2016). The increasing availability of DNA sequences and computational power (Figure 3) should allow for the establishment of large and robust phylogenies. Then, if adequately long and informative (thereby excluding short

fragments and degraded DNA), reads can be inserted in the reference phylogeny using a posteriori replacement algorithms (Matsen *et al.* 2010; Berger *et al.* 2011). Finally, recent approaches to predict species' tolerances based on information available from other species and their respective phylogenetic positions (Guénard *et al.* 2013) could be used to estimate an ecological profile for a given read (Figure 5). Routine inclusion of such phylogenetic-based methods in biomonitoring would help to account for the immense diversity uncovered by DNA barcoding and the thousands of toxicants in the environment.

### **Machine learning techniques for ecological assessment**

Analyzing and extracting valuable information from massive datasets can be extremely challenging. This has encouraged the development of machine learning methods, which use a set of statistical algorithms designed to recognize complex patterns in vast quantities of data. These methods include modern algorithms for classification, such as random forest, gradient boosting, support vector machines, and neural networks (Hastie *et al.* 2009). Machine learning approaches are fully data-driven and do not rely on any theoretical models (Breiman 2001). This system fits particularly well with the goals of biomonitoring, where the first aim is not necessarily to understand and explain the ecological processes leading to a given observation. In an applied context, correlation approaches are interesting because the final aim is to assess the state of the environment. This does not imply that machine learning should be used indiscriminately, but that these techniques are fully compatible with the ecological monitoring philosophy.

Machine learning methods have a broad range of applications. In biomonitoring, they may be used with different kinds of inputs for site classification, analyses of spatial networks of sites, and time-series forecasting. However, the most anticipated application of machine learning for biomonitoring is the processing of genetic

data. The ultimate aim is for algorithms to classify a new site directly from the bulk of DNA reads just by identifying genetic patterns learned from previous experience.

The same data can be interpreted in various ways if analyzed by different algorithms programmed with different training for different purposes (eg detection of eutrophication, effects of toxicants, or changes in flow regime). A set of sophisticated algorithms should enable scientists to monitor the effects of complex combinations of stressors on the environment. Such approaches are needed in view of multiple global threats (Vörösmarty *et al.* 2010). Furthermore, these methods should be implemented for massive datasets and communicate with holistic and integrative algorithms for automated and autonomous monitoring systems. In contrast to other more established fields in biology (Marx 2013), bioassessment is just beginning to face the problems associated with massive datasets. Scientists will need to begin collaborating more closely with experts in computer science and applied mathematics to benefit from big data, and to develop new ways to communicate results to managers (Panel 2).

### **Conclusions**

With the development of DNA metabarcoding, traditional environmental monitoring is experiencing a period of transformation, one outcome of which will be the need to deal with unprecedented amounts of data. Ascertaining the technical requirements to obtain and analyze data is just a part of the challenge. In contrast to scientists from other disciplines, ecologists have a relatively poor culture of data sharing, despite opportunities for making big data more accessible (Reichman *et al.* 2011; Hampton *et al.* 2013). However, there are signs that this is starting to change. Making biomonitoring big data freely available will potentially allow a range of new applications such as meta-analyses and large-scale analyses of biodiversity. Metabarcoding data are particularly relevant in this case because genetic data are highly comparable. Scientists and resource

#### **Panel 2. Communication with managers**

Molecular methods constitute a new paradigm in freshwater ecosystem assessment. Environmental managers who are accustomed to traditional biological assessments and who are not familiar with genetics and molecular methods may be initially reluctant to adopt these approaches or may need training in order to do so. The widespread use of metabarcoding in biomonitoring depends on how these new tools will be implemented in future environmental assessment programs. Thus, new ways to communicate with resource managers must be developed. Communication should emphasize the benefits of metabarcoding, as well as explain the basics of genetics and the vocabulary of metabarcoding and HTS to managers in order to empower them to understand, interpret, communicate,

and benefit from the results of metabarcoding. However, we must also acknowledge difficulties, such as the challenges associated with machine learning. Although it is important that biomonitoring tools are derived from sound theoretical concepts in ecology, because machine learning often operates as a black box (ie the user does not understand how the algorithm works), it might be hard to relate results to environmental health and key stressors. The implementation of such new environmental assessment frameworks will therefore take time and require a close collaboration between scientists and managers. Knowledge and experience gained over many years must not be lost and traditional approaches should continue to be used, at least for the purposes of comparison and discussion.

managers must work together to create effective networks and to develop dedicated sharing platforms. Indeed, the technical solutions discussed in this paper require substantial quantities of data and supporting infrastructures. Sharing platforms should be accessible to citizens and ecologists and would provide both raw and processed data as well as metadata. Raw data can be re-used with new bioinformatic workflows and statistical methods, while processed data are important for non-specialists and to help inform citizens (Soranno et al. 2015). If we can make public – and make sense of – the terabytes of data that ecological assessments will produce in the foreseeable future, the entry of biomonitoring into the Information Age will be a genuine success.

### Acknowledgements

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