

Biomonitoring for the 21st Century: new perspectives in an age of globalisation and emerging environmental threats

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ABSTRACT

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As we move deeper into the Anthropocene, the scale and magnitude of existing and emerging anthropogenic threats to freshwater ecosystems become evermore apparent, yet we are still surprisingly poorly equipped to diagnose causes of adverse change in freshwater ecosystems. Our main aim in this perspectives and opinion piece is to suggest some new approaches to biomonitoring that could improve on the currently limited capabilities of existing schemes. We consider how biomonitoring might develop in the future as “Big Data” and next generation sequencing (NGS) approaches continue to revolutionize all branches of ecology, with a particular emphasis on the need to consider not just nodes in the food web, but their interactions too, and also to look beyond our current reliance on the Latin binomial system of describing biological entities as “species”, when this concept is largely meaningless for many branches of the tree of life. We highlight the possible scope for enriching existing datasets to start assembling reasonable facsimiles of food webs, the need to collect and share more data more widely, and the value of metagenomics and metagenetics approaches to characterizing biodiversity *in situ* in a far more complete way than has been possible previously. Finally, we explore how these new approaches could provide a better marriage between structure and functioning than we have at present, but which is demanded increasingly by environmental legislation.

Key words: Biomonitoring, metagenomics, food webs, next generation sequencing, big data, functional genes, operational taxonomic units, multiple stressors, anthropogenic stress.

RESUMEN

Biomonitoreo para el siglo 21: nuevas perspectivas en la era de la globalización y las amenazas ambientales emergentes

A medida que avanzamos más en la Antropocena, la escala y la magnitud de las amenazas antrópicas actuales y futuras para los ecosistemas de agua dulce son más evidentes, sin embargo, seguimos estando sorprendentemente mal preparados para diagnosticar las causas de los impactos en estos ecosistemas. Nuestro principal objetivo en esta perspectiva y artículo de opinión es sugerir algunos nuevos enfoques para el control de la calidad biológica que podrían mejorar la limitada capacidad de los esquemas existentes. Consideramos como el biomonitoreo podría desarrollarse en el futuro como lo ha hecho el "Big Data" o la secuenciación de nueva generación (NGS), enfoques que continúan revolucionando todas las ramas de la ecología, con especial énfasis en la necesidad de tener en cuenta no sólo los nodos de la red trófica, sino también sus interacciones, y también para mirar más allá de nuestra actual dependencia de la nomenclatura binomial del latín para describir entidades biológicas como “especies”, a pesar de que este concepto carece en gran parte de sentido para muchas ramas del árbol de la vida. Destacamos el posible alcance que tendría, para enriquecer las bases de datos existentes, comenzar a ensamblar facsímiles razonables de redes tróficas, reunir y compartir datos de forma más amplia, y el valor de la metagenómica y metagenética para la caracterización de la biodiversidad *in situ* de una manera mucho más completa de lo que ha sido posible hasta ahora. Por último, se explora cómo estos nuevos enfoques podrían proporcionar una mejor conexión entre estructura y función de la que tenemos actualmente, y que cada vez más es exigida por la legislación ambiental.

Palabras clave: Biomonitoreo, metagenómica, redes tróficas, secuenciación de nueva generación, “big data”, genes funcionales, unidades taxonómicas operacionales, estresores múltiples, estrés antrópico.

INTRODUCTION

Bioassessment and monitoring of freshwaters has never been more important, as we move further into the so-called “Anthropocene” and its associated degradation of biological systems (Steffen *et al.*, 2011). Freshwaters are especially vulnerable to a host of anthropogenic stressors, some of which have been present for centuries (e.g., organic pollution), whereas others are more recent (e.g., acidification) or are only now appearing on the horizon (e.g., nanoparticles). Despite this evermore complex environmental context, ecosystem biomonitoring still relies on techniques developed decades ago and remains focussed on a narrow range of stressors (see reviews by Bonada *et al.*, 2006, Friberg *et al.*, 2011) which are typically considered in isolation (e.g. Extence, 1981, Bengtsson *et al.*, 1986, Pestana *et al.*, 2010), rather than recognising that many may be operating simultaneously and, potentially, synergistically (Ormerod *et al.*, 2010). It is no surprise then that ecosystem degradation is associated with multiple stressors, although it is often difficult or impossible to gauge the degree to which each is responsible because complex responses are possible – as has been shown repeatedly in field studies and experiments (e.g. Hughes & Connell, 1999, Culp *et al.*, 2005, Culp & Baird, 2006). This generally unacknowledged fact challenges biomonitoring scientists to develop solutions which can deal with complex stressor situations, yet the standard biomonitoring schema applied in most countries remains wedded to an historical solution to a “single-stressor” phenomenon: point-source sewage effluent. In addition, increasing globalisation of trade and mass tourism has increased the connectedness of natural ecosystems to a level unprecedented in the post-glacial era (Hulme, 2009), further complicating the task of assessing, prioritising, and linking emerging threats at local, regional and global scales. The current set of assessment tools and techniques used in freshwater biomonitoring are inadequate for these increasingly complex tasks, and it is our view that we must start to look to other approaches and emerging technologies to help fill the gaps.

Biomonitoring is a deeply conservative area of applied science, which has been slow to embrace new theories and emerging technologies. It is often based on obsolete ecological notions: the widespread reliance on co-called community typologies, which are anachronistic relics of the long-abandoned superorganism concept (Clements, 1936), is one such case in point. It is also supported by a bewildering array of sampling and analytical methods (Friberg *et al.*, 2011, Demars *et al.*, 2012), most of which are essentially footnotes to the ‘saprobic index’ developed over 100 years ago to classify organic pollution in central European rivers. Just as a mere four base pairs form the template for the entire planet’s genetic biodiversity, the same basic biomonitoring template has been endlessly trawled to produce hundreds of biotic indices, all aimed at answering the question: “is a test site in a natural state?”. There are now over 300 such indices in use, many of which have appeared in the last decade (Bonada *et al.*, 2006). Almost all of these are derived from changes in relative abundances of taxa within assemblages and largely, it is asserted, in response to ‘organic pollution’ and/or eutrophication (e.g. Trophic Diatom Index, Kelly & Whitton, 1995; BMWP scores, Hawkes, 1998).

Within this general biomonitoring framework there are two dominant approaches, the “RIVPACS-style” (Wright, 2000) and “typology-based” methods (Forbes & Richardson, 1913, Thienemann, 1920, Thienemann, 1959), with the latter underpinned by ideas that were jettisoned by mainstream ecology many decades ago. The former is arguably more flexible and more grounded in modern ecological theory, using continuous responses to assess community structure in response to environmental gradients, rather than fixed categories designated *a priori* by “expert knowledge” (Friberg *et al.*, 2011). In the last decade both approaches have been extended beyond their original biogeographical context, and are now being used to assess stressors other than organic pollution, although such extrapolations are often applied without fully validating whether or not they work in their new contexts. Indeed in a recent comparative

analysis, a typology-based approach performed only marginally better than a null model (Armanini *et al.*, in press).

The general, almost universal, schema in current use has been described recently as “Biomonitoring 1.0”, with calls for a radical, rather than incremental, rethink to develop “Biomonitoring 2.0” to deal with the realities of pollution in a 21st Century environment (Baird & Hajibabaei, 2012). Here, we describe how emerging techniques in ecology and genomics can be harnessed to reboot biomonitoring science, as we enter a new age of ecoinformatics, metagenomics, and other molecular-based “omics” approaches. In doing this, we build on previous critiques (e.g. Woodward *et al.*, 2010a, Friberg *et al.*, 2011, Demars *et al.*, 2012), identifying current gaps in our knowledge, and pointing the way towards how biomonitoring might look in the future.

Key areas of biomonitoring science that are ripe for development include quantifying the roles of multiple stressors and their potential synergies with one another, and considering true higher-level responses that link community structure to ecosystem functioning more explicitly than is currently the case. In particular, there is a need to integrate these approaches with molecular microbial ecology to assess both the true biodiversity and biogeochemistry of ecosystem functioning, rather than relying on the more proxy measures involving a narrow range of taxa and single processes which have become increasingly popular in recent years, such as leaf-litter decomposition assays (e.g., Hladyz *et al.*, 2011a, Woodward *et al.*, 2012b). “Biomonitoring 1.0” has so far failed to deal with these issues and, given its cumbersome and rigid framework, we argue that it is better to start afresh, rather than attempt to modify the existing system with innumerable minor adjustments (e.g. Czerniawska-Kusza, 2005). New approaches, unencumbered with the traditions and methodological and philosophical baggage of the past, offer far greater scope for the future.

A brief summary of the major failings of Biomonitoring 1.0 include a bias towards certain taxa, necessitated by the practicalities of

organism identification, and patchy, inconsistent resolution of those that are included. Moreover, current routine biomonitoring programs often lack true functional measures and integration with structural measures (but see also Environment Canada/Govt of Alberta, 2012), despite the ready availability of suitable approaches. All biomonitoring schemes employ arbitrarily defined subsets of the community (e.g., diatoms, macroinvertebrate families, chironomid exuviae, rooted macrophytes), which require highly-skilled taxonomists to derive the basic data, often in a painstakingly slow manner. All such schemes also apply the taxonomy of their chosen assemblage in a haphazard fashion, with the more easily identified taxa (often the larger, more charismatic taxa) being identified to a higher level of resolution (e.g., Ephemeroptera / Plecoptera / Trichoptera [EPT] taxa described to species, genus or family) than the more obscure or challenging taxa (e.g. chironomids and many “other Diptera” lumped into a single category). In this way, significant amounts of data and useful information about the ecosystem are either discarded or neglected, for practical rather than scientific reasons. This is especially true for the more speciose groups of freshwater organisms (e.g. bacteria, fungi, protists) at the base of the food web, where most of the biodiversity and, by extension the greatest potential range of responses to stressors are located. Even lumping chironomids together, as is done routinely because they are difficult to identify using microscopy, can reduce the power and sensitivity of biomonitoring schemes considerably as species within this group can have markedly different responses to stressors (e.g. Ruse, 2010). These problems with biases are further compounded when samples are compared through time or across systems, as information is lost when taxa have to be aggregated due to inconsistencies in identification and/or to avoid the introduction of duplicated taxa (Friberg *et al.*, 2011).

Another particular drawback is the biogeographical constraint inherent in each scheme, which is region-specific. Generality is therefore lacking at large spatial scales, necessitating the use of complex intercalibration measures. The

resulting “metrics” that emerge after convoluted rounds of stratified sampling, subsampling, data transformations, downweighting and other statistical adjustments are so far removed from the raw data as to be largely unintelligible as ecological descriptors, difficult to communicate to stakeholders, and render the highly-derived metrics largely unusable for other purposes. Sample properties are also constrained to a lowest common denominator (e.g. samples containing larvae which are too small to identify reliably to the same resolution as in the other samples; 1-minute kick samples versus 3-minute kick samples etc), leading to yet more information loss during attempts to standardise the data across studies/regions (Friberg *et al.*, 2011). Building a scientific framework on such weak foundations compromises the integrity of “Biomonitoring 1.0”, until it is eventually no longer fit-for-purpose for gauging the emerging suites of threats faced by freshwaters in the 21st Century. The question, then, is what might the alternative(s) look like?

We put forward some suggestions here as to how we might develop new approaches by moving beyond the traditional scales and levels of organisation under investigation to develop a more holistic perspective (Table 1). We emphasise, however, that we are not claiming these to be either entirely exclusive or exhaustive alternatives to all other possible methods: rather, our suggestion is they simply provide novel ways of assessing stressors that go beyond the capabilities of current schemes. Some of these suggestions involve overhauling existing large biomonitoring databases by enriching them with additional (often pre-existing) information on functional traits and species interactions, which could be done relatively easily in the short-term, which we could call “Biomonitoring 1.5”. We also take a longer-term and more radical view in which powerful new molecular techniques could develop an entirely new way of assessing and monitoring the biota: i.e. “Biomonitoring 2.0”. We will start with the former, shorter-term view and then extend our horizons by considering the latter.

BIOMONITORING 1.5: INCORPORATION OF BIOGEOGRAPHY, GEOMATICS, FUNCTIONAL TRAITS AND SPECIES INTERACTIONS INTO BIOMONITORING SCIENCE

One way to resolve problems associated with biogeographical and methodological inconsistencies in data collection is through intercalibration, which is essentially back-calculating from existing methods to a supposedly lowest common denominator. This is far less justifiable or statistically robust than using a common approach in the first place, and reflects more an appeasement of different and often deeply-entrenched local historical traditions than it does a logical and scientific optimisation of effort (Woodward *et al.*, 2010b). Unfortunately, because so many human and financial resources have already been devoted to achieve the current state-of-play in biomonitoring, there is huge inertia against changing the status quo, but this is a challenge that cannot just be delayed *ad infinitum* as the problems outlined above are not going to disappear by simply ignoring them (Friberg *et al.*, 2011).

Several important steps that can be made to help standardise approaches include consideration of alternative descriptors of biological community structure, such as biological traits rather than Linnean taxonomy (e.g. Townsend & Hildrew, 1994, Bonada *et al.*, 2006, Menezes *et al.*, 2010, Culp *et al.*, 2011). Focusing on traits offers two key advantages: first, it removes much of the biogeographic constraints on data aggregation, allowing a more universal approach to be developed in different parts of the world and, second, it facilitates future linkage to functional measures. Biomonitoring in running waters is still dominated by structural rather than functional measures, as it has been for over a century. The question, though, is: which functional traits do we need to measure? Part of this requires at least some ecological insight as to what might be important in relation to the stressor or interest (e.g. respiratory mode in systems suffering from oxygen sags).

Table 1. Typical pros and cons of current and emerging biomonitoring approaches. These categories are represented with if such data are either typically collected or if not, whilst recognising there are exceptions. Where there is less of a clear distinction “?” is used to denote this. Community completeness refers to the majority of (microbial + non-microbial) taxa being included, across trophic levels, rather than focusing on assemblages or other groupings. *Pros y contras de los diferentes sistemas actuales y emergentes de biomonitorio. Estas categorías están representadas con si esos datos están recogidos o con si no lo están, reconociendo al mismo tiempo que hay excepciones. Cuando no hay una certeza clara, se utiliza “?”. Community completeness se refiere a que están incluidos la mayoría de taxones (microbianos y no microbianos), a través de los niveles tróficos, en lugar de centrarse en comunidades u otras agrupaciones.*

	Structure	Function	A priori predictive	Resolved to species	Population abundances	Community completeness	Example references
Typologies approaches	<input checked="" type="checkbox"/>	Forbes & Richardson, 1913; Thienemann, 1920; Thienemann, 1959.					
RIVPACS-style approaches	<input checked="" type="checkbox"/>	Oberdorff <i>et al.</i> , 2001; Oberdorff <i>et al.</i> , 2002; Pont <i>et al.</i> , 2006.					
Litter breakdown	<input checked="" type="checkbox"/>	Dangles <i>et al.</i> , 2004; Riipinen <i>et al.</i> , 2010; Boyero <i>et al.</i> , 2011; Demars <i>et al.</i> , 2011; Hladyz <i>et al.</i> , 2011a; Hladyz <i>et al.</i> , 2011b; Woodward <i>et al.</i> , 2012b					
Ecosystem metabolism	<input checked="" type="checkbox"/>	Bott <i>et al.</i> , 1985; Bunn <i>et al.</i> , 1999; Young & Huryn, 1999; Uzarski <i>et al.</i> , 2001; Glud, 2008, Young <i>et al.</i> , 2008, Yvon-Durocher <i>et al.</i> , 2010a, 2010b, Demars <i>et al.</i> , 2011.					
Trait-based approaches	<input checked="" type="checkbox"/>	Doledec <i>et al.</i> , 1999, Doledec <i>et al.</i> , 2000, Gayraud <i>et al.</i> , 2003, Bonada <i>et al.</i> , 2007, Menezes <i>et al.</i> , 2010.					
Food webs	<input checked="" type="checkbox"/>	Townsend <i>et al.</i> , 1998; Rosi-Marshall & Wallace, 2002, Layer <i>et al.</i> , 2010a, Cross <i>et al.</i> , 2011, Friberg <i>et al.</i> , 2011, Layer <i>et al.</i> , 2011, Woodward <i>et al.</i> , 2012a.					
Metagenomics / metagenetics	<input checked="" type="checkbox"/>	Handelsman, 2004, Edwards & Rohwer, 2005; Tringe <i>et al.</i> , 2005; Baird & Sweeney, 2011; Hajibabaei, 2012; Yu <i>et al.</i> , 2012.					
Metatranscriptomics	<input checked="" type="checkbox"/>	Frias-Lopez <i>et al.</i> , 2008; Vila-Costa <i>et al.</i> , 2010; Morales & Holben. 2011.					

One obvious property of species and individuals is their body size, which acts as a useful proxy for many other very important ecological traits and attributes, such as longevity, fecundity, biomass production, trophic status and nutrient cycling rates (Woodward *et al.*, 2005a). It is also sensitive to many forms of disturbance, as larger species tend to be most strongly negatively impacted by a wide range of stressors (Raffaelli, 2004). Examples of stressors in which the loss of larger organisms are widely reported include: drought, warming, acidification, and habitat loss and fragmentation (e.g. Layer *et al.*, 2010b, Layer *et al.*, 2011, Yvon-Durocher *et al.*, 2011, Dossena *et al.*, 2012, Ledger *et al.*, 2012, Woodward *et al.*, 2012a). Organic pollution often appears to have more subtle non-linear effects, yet these still appear to be predictable: the largest species often predominate in moderately-enriched conditions, with smaller taxa at either extreme where nutrient limitation or toxic effects come into play (Woodward *et al.*, 2012b).

Body size is not only a useful proxy for many autecological traits, but it is also a key determinant of the synecology of a species within the food web, in terms of its number and strength of interactions and, by extension, its role in the transfer of biomass, energy and key nutrients. The inclusion of data on size and trophic interactions therefore allows us to take a more whole-system approach to understanding stressors and ecological responses to environmental change (e.g. Layer *et al.*, 2010a, Ledger *et al.*, 2012). Since it moves beyond characterising the nodes to include more nuanced food-web properties of relevance to bioassessment, additional high-level emergent phenomena and functional attributes can be explored, such as the dynamic stability of the community and biomass flux through the ecosystem as a whole (Layer *et al.*, 2010a, Mulder & Elser, 2010, Layer *et al.*, 2011, Ledger *et al.*, 2012, Mulder *et al.*, 2012). The patterning and strength of interactions are known from experiments and modelling to be key determinants of community stability and the propensity of a system to lose species when exposed to perturbations (e.g. Emmerson *et al.*, 2005, Layer *et al.*, 2010a, Layer *et al.*, 2011),

as well as the fluxes of biomass and nutrients through the web (e.g. Reuman & Cohen, 2005). The food web therefore forms a logical bridge that connects biodiversity to the ecosystem processes, goods, and services that are increasingly the focus of attention in applied ecology, in both aquatic and terrestrial systems (Reiss *et al.*, 2009, Mulder *et al.*, 2012). Many of these are system-level attributes: for instance, community resilience, the ability of an ecosystem to sequester carbon, and the maintenance of viable fisheries are just three examples of key services provided via the food web.

Many existing biomonitoring databases could be enriched easily with additional data, by inferring typical body masses and diets from the literature, to construct relatively realistic food webs (or subwebs of assemblages) from the data already available. Although these would not be as highly resolved as some of the more quantitative food webs that have been described from repeated and intensive studies conducted in a few model systems (e.g. Cohen *et al.*, 2003, Woodward *et al.*, 2005b, Gilljam *et al.*, 2011), these potential shortcomings would be counterbalanced by the huge volume of data that would be generated. This would allow broad macroecological responses to environmental gradients to be discerned across many hundreds or thousands of sites, adding a whole new dimension to existing biomonitoring data.

Developing such an approach would represent a considerable advance over the current methods that ignore trophic interactions, despite the fact that we know they are key to many higher-level responses to perturbations, such as trophic cascades and catastrophic secondary extinctions. For instance, alternative stable states in shallow lakes represent a textbook example of how the food web can mould the community, via trophic feedbacks, even under otherwise identical environmental conditions (Jones & Sayer, 2003), and there are plenty of analogous examples from running waters (e.g. Power, 1990). Resilience, resistance and persistence of communities are all functional attributes of ecosystems that emerge from the coupling of pattern and process in the food web, and therefore they cannot be fully understood

from relative abundance data without information on trophic interactions. Since the food web explicitly connects structure to function, it can provide a new focus for the development of potentially more sensitive and relevant monitoring metrics.

In simple operational terms, one way in which this step towards a network-based approach could be achieved is to build a regional database of pairwise trophic interactions recorded in the literature, which could then be used to assign trophic interactions between species in local food webs where the nodes have been identified. Since it would be impossible to construct directly-observed food webs in their entirety for each individual system, due to the enormous amount of sampling effort required (Ings *et al.*, 2009), a sensible balance should be struck between resolution and volume of data. Links could be assigned by a combination of direct observation, extrapolation from other systems and/or based on theoretical predictions (e.g. via body size scaling relationships between putative consumers and resources). It would therefore be desirable to “ground truth” these synthetic food webs by comparing them with a subset of those that have already been constructed directly and solely from exhaustive gut contents analysis, in which yield-effort curves for links and species are close to their respective asymptotes (e.g. Ings *et al.*, 2009, Woodward *et al.*, 2010a). Further, the quality of the data could be further enriched and refined iteratively by ground-truthing new subsets of the database, for instance by randomly selecting particular species and characterising their diet directly to compare with the inferred data. This would not only help validate the accuracy of inferred dietary data but could feed back into the global database on pairwise trophic interactions to improve the next round of inference, essentially applying a systems biology approach to network-level biomonitoring. Care would obviously need to be taken to avoid introducing circularities in the database, such that different sets are used for testing and training, but this could easily be avoided by employing algorithms to screen for such scenarios. The construction of comprehensive interdigitating species and links databases,

as envisaged here, could also provide important new insights by moving beyond the reliance on coarse functional feeding groups (FFGs) which are often used as dietary or trophic proxies, but which are based on foraging modes rather than diet *per se* (Woodward, 2009, Lauridsen *et al.*, 2012, Layer *et al.*, 2013).

In addition to using empirical data from the literature to assign interactions in order to create inferred food webs, ecological theory and mathematical models could be used where there are gaps in the data. Some of these approaches have been used to predict food web structure from first principles with a high degree of accuracy (e.g. > 90 % of links assigned correctly) in freshwaters (e.g. the Allometric Diet Breadth Model, *sensu* Petchey *et al.* (2008), Woodward *et al.* (2010a)). Finally, the use of advanced computing techniques, such as machine-learning, which have recently been developed in disciplines as disparate as the social sciences and molecular biology, could also be applied to ecological data to construct food webs *in silico* (Bohan *et al.*, 2011). Such artificial networks could be challenged and validated repeatedly with real data, as is being done in studies on networks of gene regulation (Walhout, 2011). This iterative feedback between data and models, each of which is revised and refined as the cycles proceed, could offer a powerful and efficient new way of generating network data from simple species lists, thereby adding entirely new dimensions to existing biomonitoring databases.

BIOMONITORING 2.0: GENOMICS TO THE RESCUE?

Taxonomic identification is the *sine qua non* of biomonitoring, and yet ironically has always been a rate-limiting step, as taxonomists remain in short supply (e.g. McClain, 2011). Moreover the procedure of separating and identifying specimens is expensive, and, coupled with time constraints, can hamper the scope of biomonitoring programs. This reliance on microscopy and detailed taxonomic expertise has not changed for decades, yet the recent advances in molecular

ecology seem certain to challenge the current hegemony. These “big data” approaches originally emerged from microbial ecology as a means of describing the massively diverse and cryptic assemblages found in soils, but they are increasingly being applied to macrofauna (e.g. Hajibabaei *et al.*, 2011). The field of metagenomics (aka environmental genomics, ecogenomics, community genomics), which seeks to characterise the species complement from environmental samples, is revolutionising molecular microbial ecology and its potential in other fields is now starting to be recognised, including the characterisation of food webs (Purdy *et al.*, 2010, Hajibabaei, 2012). Pyrosequencing and associated next-generation DNA sequencing (NGS) technologies can generate colossal quantities of data at a rapidly reducing cost – even to the stage that the Human Genome Project (Collins *et al.*, 2004) could now be replicated in a modest sized laboratory in a matter of weeks.

Another big step forward would be to measure gene expression in the metatranscriptome, enabling us to quantify genetic responses to stressors across assemblages or functional groupings of species within the food web. Characterising both the metagenome and the metatranscriptome simultaneously could also be used to measure redundancy, and hence the potential for resilience, in the system. Comparing RNA- versus DNA-based metrics in response to stressors should tell us both what is present and what is active within the metagenome (e.g. Mason *et al.*, 2012). Some of these capabilities are already with us, whereas others are on the horizon – but given the rapid and accelerating pace of development in the field they seem sure to revolutionise our view of the natural environment and how we monitor it.

We should always be wary of excessive evangelising, but we have already entered a new era of unprecedented data generation in ecology, and if even a small portion of the full potential of NGS is realised we will have made huge advances. We are close to having the capacity to carry out true community-level analysis by including all trophic levels, from single-celled prokaryotes at the base of the food web to the largest metazoan top predators. By allying metagenomics to DNA

library-building across many taxonomic groups (e.g. Webb *et al.*, 2012), the value of ecoinformatics data collected today will inevitably increase over time, as we become better able to assign taxonomic identities to currently cryptic “operational taxonomic units” (OTUs). This will allow us to return repeatedly to the same datasets to identify new potential indicator species and responses to a wider array of stressors than may be apparent at present. In the interim these OTUs can still act as useful indicators even if we cannot yet put a species name to them, so long as they have recognisable and characteristic signatures and respond to environmental gradients. In fact, it seems likely that metagenomics will force us to question our over-reliance on the Latin binomial as a principal building block in community ecology (Raffaelli, 2007), as the species concept itself starts to break down in certain branches of the tree of life: for instance, many aquatic macrophytes hybridise readily and have different chromosome numbers even within the same “species” (Lansdown, 2009). Remaining wedded to these more traditional views of how we aggregate biological entities based primarily on morphology rather than molecular data may be slowing our progress in general ecology (Raffaelli, 2007). Indeed, there is an intriguing tension developing between different ways of perceiving the biota, with taxonomic-morphological, functional, and molecular techniques all in current biomonitoring usage, albeit with the strongest emphasis still placed on the former.

A major advantage of NGS is that biodiversity and functional diversity can be measured simultaneously in the same sample, by characterising both the community metagenome whilst also targeting key genes of functional importance that, in turn, can be mapped onto ecosystem functioning (e.g., linking certain respiratory genes to whole system metabolism, or particular enzymes to carbon metabolism as could be revealed in phenotype microarray plates to measure substrate utilisation). This could provide a far better match between structural and functional measures than can be achieved using current methods, which often rely on proxies for the latter (e.g. leaf-litter breakdown, BOD₅ [Woodward *et al.*, 2012b]).

Indeed, recent research has suggested that many of the current functional approaches do not necessarily translate in a simple linear fashion to a given environmental stressor, or to structural measures of “ecological integrity”. For example, in a study of 100 European streams across a 1,000-fold gradient in nutrient concentrations a complex three-dimensional space-filling relationship was evident between two nutrients and leaf-litter decomposition rates (Woodward *et al.*, 2012b): rates were always low at the extremes of SRP and DIN concentrations, but low-to-high rates were manifested in moderately enriched systems. In a subset of 10 % of these streams a unimodal relationship between breakdown and a classic BMWP (Biological Monitoring Working Party score) gradient in community structure in response to organic pollution was evident, with the abundance of large shredders explaining most of the variance (i.e. once again highlighting the structural-functional role of body size). This shows that functional measures on their own can be extremely difficult or impossible to interpret (e.g. pristine low nutrient waters had identical breakdown rates to those that were grossly polluted), and that they provide complementary information to that supplied by structural measures (Woodward *et al.*, 2012b). Another similarly large-scale study in a second set of 100 European streams, this time comparing litter breakdown in streams with native riparian vegetation with those that had human-altered riparian zones, found idiosyncratic responses to the perturbation: rates in the impacted sites could range from being slower, to equivalent, to faster than in the reference sites (Hladyz *et al.*, 2011a). The direction and magnitude of the response appeared to be largely contingent on the type of alteration imposed and the biogeographical setting. One of the more consistent effects that emerged, however, was that the contribution to total breakdown rates by macroinvertebrates relative to microbes was generally lower in impacted systems – but what those microbes might be remains unknown, and in need of molecular characterisation.

Molecular identification of biological species could usher in a new golden age of ecological

research, and its application in biomonitoring could drastically reduce costs while increasing the speed and coverage of bioassessment. However, its misuse or premature application could create an ecological Tower of Babel (Caterino *et al.*, 2000), and thus we must explore this promising technique in the field of biomonitoring with some caution. What is clear is that the characteristics of the data generated through NGS discovery of molecular taxonomic composition do not always align directly with traditional biomonitoring methods (e.g. Hajibabaei *et al.*, 2011) and the genes employed may yet under-report biodiversity in a sample (e.g. Tang *et al.*, 2012). Nevertheless, it is our view that these challenges – inherent in any radically new technique – can be overcome, and that in years to come, molecular identification of biodiversity from environmental samples will be routine in the near future.

CONCLUSIONS

While ‘Biomonitoring 1.0’ is practised in many countries of the world, it is often viewed as a luxury rather than a necessary component of ecosystem monitoring and assessment. Despite its clear advantage over other assessment approaches because it directly measures biotic responses, it remains stuck in the starting blocks, such that the patterns observed are often difficult to interpret, and at best are nuanced versions of simple binary outcomes (i.e. impacted/unimpacted). While multimetric approaches claim to be a step beyond simple binary outcomes, these can be difficult to communicate to stakeholders, and in many cases, illusory, and their apparent complexity often masks an inability to truly capture diverse ecosystem outcomes in the face of multiple stressors. This said, there are a number of significant new ‘Biomonitoring 1.5’ innovations which offer hope for the immediate future: the application of null models, the development of stressor-specific metrics, the use of geomatics information in habitat description and the development of data interoperability standards all can contribute towards the development of more robust, scalable,

broadly-applicable biomonitoring models with the possibility of diagnosis beyond pass/fail. However, it is towards the 'Biomonitoring 2.0' approaches that we must look for the development of truly diagnostic tools in the future (Table 1). While Biomonitoring 2.0 is a leap into the unknown in the best possible sense, we concede that it is unlikely that these techniques can be mainstreamed in ecosystem assessment in the short term (i.e. next 5 years) as they will require a longer lead-in time to achieve widespread acceptance (5-10 years). The application of food web theory, coupled with high-throughput genomics and high performance computing systems for analysis of 'Big Data' has the potential to generate high-resolution biodiversity information for monitoring purposes on an unprecedented scale. Such data offer an opportunity for ecosystem monitoring scientists to become engaged in a rapidly developing field, with Bio2 studies generating data with broad value beyond local monitoring concerns, and could foster new collaborations between applied and basic research and between academic, government, industry and other environmental stakeholders. Also, the scale and extent of observations will allow scientists to align them more closely with other observation and prediction systems (i.e. remote sensing; satellite observation, climate models), particularly in the light of new automated robotic sampling systems (e.g. Harvey *et al.*, 2012).

We are aware that advocating a move away from the reassuring traditional bedrock of Linnean taxonomy based on individual type-specimens towards a gene sequence-based unit of observation is not without some risks. However, we believe that the potential benefits offered by this new paradigm could completely revolutionise the monitoring and management of environmental risks within just a few years. The ability to monitor the ecosystem *in toto* rather than as a series of isolated components, and to do this in much greater detail, with improved precision and accuracy, will allow us to move towards a truly generic approach in ecosystem science. This will greatly facilitate globally-applicable and compatible observation, modelling and prediction of natural ecosystems.

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