Chapter 10

Freshwater Conservation and Biomonitoring of Structure and Function: Genes to Ecosystems

Clare Gray^{1,2}, Iliana Bista³, Simon Creer³, Benoit O.L. Demars⁴, Francesco Falciani⁵, Don T. Monteith⁶, Xiaoliang Sun⁷ and Guy Woodward²

¹School of Biological and Chemical Sciences, Queen Mary University of London, London, UK; ²Department of Life Sciences, Imperial College London, Ascot, Berkshire, UK; ³Molecular Ecology and Fisheries Genetics Laboratory, School of Biological Sciences, Environment Centre Wales, Bangor University, Gwynedd, UK; ⁴The James Hutton Institute, Aberdeen, Scotland, UK; ⁵Institute of Integrative Biology, University of Liverpool, Liverpool, UK; ⁶Centre for Ecology & Hydrology, Lancaster Environment Centre, Lancaster, UK; ⁷Department of Molecular Systems Biology, University of Vienna, Vienna, Austria

INTRODUCTION

Current Focus of Aquatic Biomonitoring and Conservation

Freshwater biomonitoring—i.e., the repeated, quantitative assessment of surface waters using the presence and/or abundance of groups of organisms of known environmental sensitivity—currently provides a staple tool in aquatic management and conservation and underpins wide-reaching environmental legislation including the European Union Water Framework Directive (EU WFD), Environmental Quality Standards for Surface Water in China (GB 3838-2002), and the Clean Water Act in the United States of America. Its scientific origins can be traced back to societal changes during the industrialization of the developed world and simultaneous scientific developments in epidemiology and biological taxonomy—the impacts of rising human populations on the chemical and microbiological quality of urban water supplies necessitated the development of rapid and robust methods to assess risks to public health.

The history of aquatic biomonitoring is extensively reviewed elsewhere (e.g., Metcalfe, 1989; Rosenberg and Resh, 1993; Friberg et al., 2011) and so will not be discussed in detail here, but essentially biomonitoring hinges on two basic concepts: first that aquatic organisms tend to be unevenly distributed

across environmental gradients and should therefore have value as indicators of ecosystem state, and second, that biota provide a more temporally integrated indication of ecosystem quality than many abiotic measurements, such as spot-sampled water chemistry.

Three key developments over the course of the twentieth century had major impacts on routine environmental assessment by regulatory authorities (Metcalfe, 1989). First, Kolkwitz and Marsson (1902, 1909) introduced what became the "saprobien system" in which groups of organisms were directly linked with perceived discrete levels of organic contamination, and by inference oxygen availability of waters. Second, biological diversity indices became popular around the middle of the century based largely on the premise that species richness and evenness is reduced with increasing environmental disturbance. Finally, biotic indices that combined these methodologies (such as the Trent Biotic Index, Chandler's Score System, and the Biological Monitoring Working Party) were developed. Despite the widespread adoption of these indices (in particular the average score per taxon (ASPT) approach), many surface waters are more likely to be compromised by other anthropogenic stressors such as acidification, toxins, climate change, atmospheric deposition of reactive nitrogen, and habitat modification.

During the 1980s, the need to understand the causes behind surface water acidification stimulated investigation of diatoms as paleobiological assessment tools (Renberg and Hellberg, 1982; Battarbee and Charles, 1986). These ubiquitous and chemically sensitive unicellular algae preserve well in lake sediments, thus enabling paleoecologists to reconstruct the environmental history of a water body from sediment cores. Statistical approaches based on weighted averaging procedures were developed to predict (or hindcast) lake chemistry on the basis of spatially derived "training sets" describing the chemical "optima" and tolerances of individual species (e.g., Birks et al., 1990). This approach has proved highly effective in the reconstruction of lake pH and has been applied to infer historical change in other environmental parameters with more mixed success. More recently, various community-based multivariate regression approaches have been developed to interpret the environmental significance of trends in contemporarily monitored biota including diatoms and macroinvertebrates (Monteith et al., 2005; Murphy et al., 2012), and to specifically address the extent to which biological trends can be explained by changes in water quality with time (Halvorsen et al., 2003).

In recent years, more effective water treatment regimes and environmental regulations have improved surface water quality with respect to both organic pollution and water acidity in much of the developed world. The focus of biomonitoring has consequently begun to shift from basic quantification of environmental damage to consideration of how much surface water quality, with respect to these key drivers, still deviates from a desired "reference" condition relative to a "pristine" state. The bioassessment tool RIVPACS (River Invertebrate Prediction and Classification System) pioneered this field, by quantifying the differences in the macroinvertebrate assemblage between a site under investigation relative to its "expected" assemblage at unimpacted but otherwise comparable sites. This approach and its derivatives now underpin most freshwater biomonitoring schemes across Europe (e.g., Simpson et al., 2005; Murphy et al., 2013) and other parts of the world (Simpson and Norris, 2000).

Unfortunately, despite these advances, assigning appropriate reference conditions and current status is still problematic, as preindustrial (i.e., pre-1800) target conditions are very difficult to model with confidence (Battarbee et al., 2005), as there are rarely useful paleoecological data from running waters because their sediments are well mixed, and there are mismatches between paleo and contemporary data in standing waters (as the two rarely overlap in time), so ground truthing is difficult. A notable exception is from some of the longer-term biomonitoring schemes such as the United Kingdom Acid Waters Monitoring Network (Monteith et al., 2005; Battarbee et al., 2014), where after several decades of lake biomonitoring using sediment traps, we are now finally able to compare contemporary data directly with paleoecological data (Figure 1). This has raised intriguing questions about stressor impacts: for instance, in the Acid Waters Monitoring Network (AWMN) data, the lack of evidence of clear recovery among diatom communities along the acidification trajectory evident in the sediment core records (despite improvements in water chemistry) points to hystereses in these ecosystems, and to the potential ecological importance of other factors that could be setting new environmental states that may not be reversed in the foreseeable future (Battarbee et al., 2013). The growing realization that a return to a historical preimpacted state may be unrealistic is now forcing us to consider shifting environmental baselines when assessing conservation, restoration, and the determination of when an alternative state is acceptable with respect to its function, biodiversity, and the ecosystem services it provides (UK National Ecosystem Assessment, 2011; Millenium Ecosystem Assessment, 2005). While this paleoecological reference approach to aquatic monitoring is limited to lake ecosystems (as running water sediments are turned over), there is considerable potential to extend it to other biological proxies and biogeochemical indicators such as pigments and stable isotopes, and pressures other than acidification (e.g., Smol, 2009).

All these approaches focus on linking attributes of biological assemblages to a system's chemical or physical state, and they have made important contributions to environmental assessment, policy, and legislation across ecological and evolutionary timescales. The power of these methodologies can be largely attributed to the wide variation between taxa in tolerance to specific pressures, in particular the bioavailability of oxygen, hydrogen, and aluminum ions. Newly emerging environmental threats, such as the many facets of climate change, contamination from organic micropollutants and nanoparticles etc., may not be quite so readily assessed by similar direct environment—taxa



FIGURE 1 Linking a site's contemporary biomonitoring data to its historical reference condition (*redrawn from data presented in Battarbee et al. (2014)*). Percentage relative abundances of diatom species found in sediments of a UK upland lake (Round Loch of Glenhead). Species abundances in historical sediment core samples (left) shift from left to right reflecting increased water acidity during the industrial revolution. Abundances of the same species in contemporary sediment trap assemblages (right) indicate some recent reversal (decline) of some particularly acid-loving species—e.g., *Tabellaria quadriseptata*—as acidity has declined. However, other species that increased during acidification are continuing to increase in abundance while others that were common prior to acidification show little indication of recovery.

calibration-based approaches (Figure 2) (Friberg et al., 2011). In some cases, ecosystem metrics other than the relative abundance of taxa may yield clearer insights into significant environmental shifts (e.g., Layer et al., 2011). There is therefore a growing need to determine how best to assess the impact of these emerging stressors both in isolation and in combination. Also, the structural biodiversity-centric focus of these traditional methods now needs to be augmented with more explicitly functional measures, to provide complementary insights into the impacts of stressors in freshwater ecosystems (e.g., Woodward et al., 2012).

In addition to largely lacking these explicitly functional ecosystem-level metrics, another common limitation of current taxonomic-based biomonitoring schemes is that although there is an implicit evolutionary signal embedded within them (i.e., in terms of the phylogenetic relatedness of the various indicator taxa, which constrains their functional traits), there is still no explicit recognition of the role of adaptation to new stressors and the potential

244



FIGURE 2 A hypothetical ordination to show the changes in the main drivers of habitat degradation in freshwaters in the developed world over time. Increasing temperature and habitat modification have become the significant drivers of change in the principal components (axes I-II) of community composition, replacing the more historical stressors of organic pollution and pH change. However, these historical stressors are still the major causes of habitat degradation in developing countries.

for evolutionary rescue from stressors within species populations, and evolutionary responses can occur surprisingly quickly in many freshwater taxa (e.g., Melián et al., 2011). This could cause mismatches between the reference and impacted conditions, if species are able to adapt to new conditions rather than acting as passive ciphers that are simply overlain on an environmental template (e.g., Bell and Gonzalez, 2011). This has resulted in a paradox of biomonitoring in which speciation is the mechanism that produces the response variables we measure but is then ignored when relating species distributions to environmental conditions. Although research is beginning to fill this gap in understanding (e.g., Thuiller et al., 2011; Vonlanthen et al., 2012) that currently exists in biomonitoring, this "inconvenient truth" is either ignored or obfuscated through attempted circumvention by removing the phylogenetic signal from the data (e.g., via trait-based approaches).

State of the Art in the Science of Biomonitoring: From Species Traits to Community Structure and Ecosystem Functioning

The earliest attempts to combine ecological and evolutionary approaches to biomonitoring included the use of additional measures of biodiversity including phylogenetic diversity (or taxonomic distinctness) and functional diversity conditioned by evolution (e.g., May 1990; Paradis et al., 2004; Webb et al., 2011), though most of the emphasis has been on the former, not the latter. A problem with focusing solely on taxonomy is that if species redundancy is high, as appears to be the case in many freshwaters (e.g., McKie et al., 2008; Perkins et al., 2010; Reiss et al., 2010, 2011), then species loss is likely to only have strong effects when entire functional guilds are lost; but it is these that we still have limited understanding of due to the longstanding reliance on more traditional measures of biodiversity (e.g., species richness). The realized species trait (or gene) profile at a local scale provides the means to link the potential effects of anthropogenic pressures on species (population) distribution and dynamics: i.e., the trait profile itself may therefore be used for diagnostic purposes (Statzner and Bêche, 2010). It is possible, however, that noncausal relationships between individual species traits and contemporary environmental conditions exist (e.g., Poff et al., 2006; Horrigan and Baird, 2008) because some traits may represent an evolutionary legacy rather than current adaptation (Gould and Lewontin, 1979). Empirical studies have confirmed the large role played by phylogeny or taxonomic distinctness in freshwater ecosystems (Willby et al., 2000; Poff et al., 2006; Demars et al., 2012) from the structural perspective, but their functional attributes remain far less well understood.

To interpret biomonitoring results (patterns in species composition), it is crucial to unravel biomonitoring's underlying mechanistic basis (processes that determine this pattern, both anthropogenically mediated or not). Species are not randomly distributed in time (e.g., Lyell and Deshayes, 1830) or space (e.g., Humboldt, 1849), and Demars and Edwards (2009) recently pointed out that even as far back as the nineteenth century, Darwin (1872) argued that environmental variables played only a subordinate role in the determination of species distribution. He offered a mechanistic explanation (pp. 318–319): immigration of individuals from a species (individuals) pool controlled by dispersal barriers and descent with modification regulated through natural selection, with competition being the most important pressure. He attributed the wide distribution of freshwater organisms to favorable means of dispersal (Darwin, 1872, pp. 323-330, 343-347, e.g., Pollux and Santamaria et al., 2005) and lessened competition (Darwin, 1872, pp. 346, e.g., Greulich and Bornette, 2003) in aquatic habitats. This debate of whether species distribution is more controlled by niche assembly (resource heterogeneity) or dispersal assembly is still ongoing (Demars and Harper, 2005; Heino, 2013). Moreover, numerous null models have reproduced biomonitoring patterns of species assembly: e.g., random (Tokeshi, 1990), niche (Tokeshi, 1993), neutral (Bell, 2001; Hubbell, 2001), metabolic scaling (Allen et al., 2002), fractal (Lennon et al., 2007), and MaxEnt (Harte, 2011).

The general consensus is that patterns in species composition and community structure emerge from the interactions of chance, dispersal, and resource heterogeneity in evolving metacommunities (Venail et al., 2008). This is supported by empirical studies using autocorrelation, spatial distances/ isolation, and dispersal abilities to infer proportion of resource (niche) versus dispersal community assembly (Moilanen and Hanski, 2001; Demars and Harper, 2005; Moilanen et al., 2005, 2008; Bonada et al., 2012). Essentially, this is explicitly adding the otherwise overlooked dynamical component to biomonitoring data, which are often seen as static snapshots whereby species simply map onto the environmental template. It also starts to recognize the inherent role of dispersal and selection for particular functional traits, rather than simply focusing on the phylogenetic tree in isolation.

Every species can be characterized by not only its taxonomic identity but also its biological (response) functional traits, which may be translated into functional (effect) traits (Engelhardt, 2006; Kerkhoff and Enquist, 2006; López-Urrutia et al., 2006; Enquist et al., 2007) and eventually into ecosystem services (e.g., García-Llorente et al., 2011). Mapping traits onto the tree of life reveals a convergence (independent appearance of a trait in separate clades) or divergence (appearance of a trait in a single clade) in evolution. This is highly relevant in the context of the insurance hypothesis or portfolio effect, whereby high species (or genetic) richness maintains high and constant ecosystem (or population) productivity and services in a stochastic environment (Yachi and Loreau, 1999; Schindler et al., 2010).

The ecology of a species sets the scene in which evolution operates, while evolution may influence ecological dynamics by altering the frequency of phenotypes that are available to interact: thus, there are potentially important eco-evolutionary feedbacks, which are only now starting to be recognized (e.g., Melián et al., 2011; Moya-Larano et al., 2012). The ability of a species to adapt to a changing environment is key to how it responds to stressors: species are not simply present or absent if environmental conditions are favorable or unfavorable (Box 1). According to the old adage, there are three options—"adapt, perish, or move"—that a species is faced with in a changing environment, yet biomonitoring and conservation schemes have largely ignored the first.

An important issue here is that neither ecological nor evolutionary responses occur solely at the population level of organization: no species is an island, and its interactions with those around it will determine both species-specific and the wider community's responses to changing conditions (e.g., Rybicki and Landwehr, 2007). This explains why models derived from bioclimate envelopes and extrapolations from traditional biomonitoring techniques often fail to predict species responses in the real world, because their synecology (the ecology of communities of interacting organisms) is ignored (Woodward et al., 2010; Friberg et al., 2011). The use of trait-based approaches helps to grapple with issues related to functional biodiversity at the autecological level, but it fails to embrace the more complex, higher-level synecological functional roles that species play within multispecies systems

BOX 1 Categorizing Continuous Variables in Biomonitoring

Figure 3 maps an example of a continuous ecological variable (habitat quality) onto discrete human-made categories. This human need to categorize complexity can be seen in many aspects of ecology, not just in the biomonitoring and conservation fields. Whether it's the difficulties encountered when classifying all of life on earth into discrete species (e.g., Mayden, 1997) or the questionable practice of assigning "typologies" to a given lake or river (e.g., Friberg et al., 2011), the motivation comes from our historically poor ability to process large amounts of complex information. However, this process of classification and simplification has allowed us to make some informed generalizations and useful interpretations that otherwise would not be possible. Nevertheless, with the advent of rapidly accelerating computing power, the challenge has now shifted away from our previous inability to process complex information, to the interpretation of complex information into simple messages. With expanding analytical ability comes the need to preserve as much ecological information as possible, which will allow a deeper understanding and more informed interpretations to develop the next necessary steps forward in biomonitoring science-the shift of focus away from the simple monitoring of species composition toward the monitoring of ecosystem functions and services.



FIGURE 3 Hypothetical graph showing fluctuations in four species abundances across a habitat quality gradient, alongside the discrete criteria of habitat quality (good, moderate, poor) that these continuous variables are categorized into. The dashed line shows species loss, whereas the solid black arrow shows sublethal effects to a particular species population.

such as food webs, which may have seemingly unpredictable emergent properties (Woodward, 2009). This can be exemplified by mismatches between real-time or experimental data that track transient dynamics, versus space-for-time substitutions where the different communities across the

environmental gradient may already be at equilibrium (e.g., Layer et al., 2010, 2011). Unfortunately, such data are still rare, but where they are available there is compelling evidence that the functional role of species within the food web can have important indirect and direct consequences that would be missed by relying on static data: a classic example is the seeming paradox of invertebrate abundance declining over several decades of deacidification, yet this response makes sense when the top-down effects of predators on the prey assemblage are included (Layer et al., 2011).

Figure 4 synthesizes current thinking in the role of ecology and evolution of species distribution in which taxonomic, functional, and phylogenetic diversity determine the dynamics of ecosystem functioning and services, and highlights how they can be integrated into future biomonitoring approaches.

Functional diversity provides a more direct link between species richness and ecosystem functioning, and ultimately the provision of goods and services (Naeem, 2002; Woodward, 2009). Two essential functions are primary production and decomposition, which provide the two key energy inputs into any food web, thus ultimately driving the whole system's trophic dynamics, stability, and productivity. Production and decomposition thereby provide a variety of services including the production of fish in fisheries and for recreational angling, and the processing of pollutants and waste products to produce clean water. These vital ecosystem processes, however, are not routinely measured in current biomonitoring techniques. Decomposition rates have been measured in some large-scale studies, but these too are still largely ignored in routine biomonitoring, and the responses remain complex and poorly understood (Woodward et al., 2012). Some functional measures, such as organic matter decomposition, have been the focus of attention (e.g., Young et al.,



colonisation, extinction, speciation

FIGURE 4 Ecology and evolution of species distribution generates diversity patterns in species (grids), species traits (symbols), and phylogeny (trees). From a hypothetical null model (e.g., random assemblage) and species pool at regional scale, species are sorted through the effects of niche assembly (heterogeneity of resources) and species dispersal into patterns of local species distribution. Over time, local extinction, colonization, and speciation alter the regional species pool and associated phylogeny and trait diversity. The dimensions of diversity—taxonomic, abundance, functional, and phylogenetic—determine the dynamics of ecosystem functions and services.

2008), and methods for standardizing this measure across ecosystems have been developed (e.g., Kampfraath et al., 2012), crucially allowing comparisons between studies, but these methods have yet to be adopted into biomonitoring schemes.

Functional indicators, and especially direct measures of ecosystem processes, should also play a larger role in quantifying ecosystem services (Millenium Ecosystem Assessment, 2005) and are being advocated increasingly for economic valuations of conservation, management, and restoration projects (Costanza et al., 1997; Everard and McInnes, 2013). Many ecosystem processes either are services in their own right (e.g., carbon sequestration and nutrient cycling) or underpin them (e.g., invertebrate production supporting fisheries), and include hydraulic retention (water transient storage), sedimentation rate, and greenhouse gas transfer. The magnitude and rate of many of these processes are sensitive to anthropogenic pressures, highlighting the scope to use functional indicators as diagnostic tools (Odum, 1969; Schindler, 1987; Sweeney et al., 2004; Mulholland et al., 2008; Yvon-Durocher et al., 2010; Demars et al., 2011).

Important insights into ecological and evolutionary responses to stressors, as well as their functional consequences, could be inferred from the large number of georeferenced and dated lists of taxa currently filling a multitude of databases in local regulatory and conservation agencies as well as natural history and conservation societies. Many databases are now being assembled that contain some or all of these elements (e.g., FishBase (Frose and Pauly, 2010) and Freshwater Life—http://www.freshwaterlife.org—supported by the Freshwater Biological Association). Scientists are collating decades of research to assemble species traits (and genes) in a phylogenetic context. Combining this with environmental data available from a wide range of government agencies and research bodies, and organizing this information into user-friendly databases (e.g., the Global Biotraits Database http://biotraits.ucla.edu/index.php)—connecting them to infer processes from patterns—offers great potential for future research (e.g., Demars and Harper, 2005; Demars and Trémolières, 2009).

The success of the next generation of biomonitoring will not come solely from assembling and interrogating these vast new databases to obtain new response variables, but also from explicitly testing ecological hypotheses and synthesizing different branches of science—e.g., eco-enzymatic stoichiometry that allows us to link the elemental composition of microbial communities to their nutrient content and biomass production (Sinsabaugh et al., 2009; Hill et al., 2012). Integrating biomonitoring schemes with experimental and modeling approaches will be crucial: combining whole ecosystem experiments with long-term monitoring can reveal spectacular responses to environmental change, although such large-scale, long-term studies are still very much in the minority. Classic examples include the work of Likens et al. (1977) at the Hubbard Brook Experimental Forest, Schindler (1990), Carpenter et al. (2001) at the

Experimental Lakes Area (ELA) in Canada (http://www.experimentallakesarea. ca), and Slavik et al. (2004) at the Kuparuk River station of the Long-Term Ecological Research (LTER) network. Other work has made use of these long-term data to develop new dynamical models to link biodiversity change to ecosystem functioning, such as Petchey et al.'s (2004) study based on the extensive time series data from the UK's Environmental Change Network. Recently, the American LTER network has been complemented by the National Ecological Observatory Network, NEON (http://www.neoninc.org/news/ Iterandneon), and the STReam Experimental Observatory Network (STREON, part of NEON) is now one of the most ambitious long-term biomonitoring schemes. It combines comparative surveys across the USA with experimental design (nutrient enrichment and removal of large consumers) that extends previous LYNX programs (Mulholland et al., 2008). In the United Kingdom, the AWMN has also been very effective in providing scientific insights and in influencing policy (Hildrew, 2009; Layer et al., 2010, 2013; Friberg et al., 2011). Moreover, the value of AWMN has increased progressively over the three decades since its inception, as more subtle long-term trends such as responses to climate change can now be detected. The challenge now is to establish international networks with global coverage to tackle planet-scale issues (e.g., the Global Lakes Ecological Observatory Network) that are also integrated with regional and local monitoring. Long-term monitoring can enable us to detect early warning signals of ecosystem shifts (Scheffer et al., 2009), but it is often difficult to extract research funding for such strategic research, which often appears to fail to meet the "novelty" criteria of many research councils' remits.

Future Advances and New Perspectives—Genes to Ecosystems

Over the last 20 years huge progress has been made in understanding biodiversity-ecosystem functioning (B-EF) relationships, with an increasing emphasis on freshwater systems over the last decade in particular (Loreau et al., 2002; Woodward, 2009; Loreau, 2010; Reiss et al., 2010). While biomonitoring and conservation have tended to focus on the biodiversity end of the relationship, the functioning part of the equation as well as its relationship with biodiversity has been largely ignored in the more applied fields of freshwater ecology (but see Dangles et al., 2004; Cardinale, 2011). However, the lack of functional insights is changing, and many emerging legislative and regulatory frameworks are recognizing the need for more functional approaches (e.g., the Water Framework Directive). The main finding of B-EF research to date has been the prevalence of high levels of redundancy. Species loss may initially have little impact, but once a critical threshold is passed when entire functional groups are lost, the impacts can be extremely powerful, and sensitive to further species loss (Cardinale et al., 2006). These experiments have also revealed evidence that idiosyncratic species responses are important,

harking back to earlier ideas about keystone species, where they have both strong and unique influences on a process. Despite these advances, there are still some glaring gaps in our knowledge: few studies have included more than one trophic level; most have measured just one process rather than functioning as a whole; and they have been conducted primarily in small experimental arenas over short timescales (Woodward, 2009). As such, many B–EF experiments lack the complexity of natural systems, though attempts are now being made to address these shortcomings (Reiss et al., 2010). In the context of moving from an understanding of B–EF to B–ES (biodiversity–ecosystem services) relationships, there is a huge gap to be bridged in terms of the spatiotemporal scales that are important for the latter, as ecosystem services tend to be manifested at much larger landscape scales, where source–sink, metacommunity and food web dynamics, and eco-evolutionary processes (e.g., Melián et al., 2011) are likely to be important.

The application of network-based approaches can be especially powerful here, as there is a strong food web context to where ecosystem services are located, as well as a clear trophic gradient in the scope for insurance and adaptation, which increases down the web's food chains (Figure 5). Certain stressors are associated with particular nodes in the web (e.g., bio-magnification of organochlorine pesticides in apex predators; and antibiotics with the microbial loop at the base of the web), as well as different organizational levels (e.g., food web modules; functional groups; and the network as



FIGURE 5 Mapping services onto the food web. When monitoring services we need to monitor the appropriate level of scale. The effects of stressors upon services would not show at all levels of the food web, although may magnify through the food web, or cause trophic cascades. AP = apex predator, F = fish, C = carnivore, O = omnivore, D = detrivore, HD = herbivore/detritivore, H = herbivore, AH = aquatic hyphomycete, L = leaf-litter, P = plant, A = algae.

a whole) acting as multiple biosensors. For instance, allometries in food web properties from the level of pairwise links, to tritrophic food chains, to the system's entire constraint space have been used recently to evaluate responses of experimental stream food webs to drought (Woodward et al., 2012; Ledger et al., 2013): these revealed that many of the more commonly used network metrics (such as connectance) were relatively robust to perturbations, whereas others were much more sensitive (e.g., allometric scaling of pairwise links and food chains). The food web provides an intuitive prism through which to view both the lower and the higher levels of organization and how they respond to stressors, as it makes the interactions between species explicit in the response variables, whereas most biomonitoring and conservation approaches focus solely on (a few) nodes and not the links between them at the system scale (Woodward et al., 2013). Considerable work has been done in freshwaters in terms of understanding how food webs respond to stressors, including acidification (e.g., Ledger and Hildrew, 2005; Layer et al., 2010, 2011), eutrophication (e.g., Rawcliffe et al., 2010), and hydrological change (e.g., Ledger et al., 2012, 2013). Such combinations of studies illustrate effectively that studying the feedbacks between the environment and the functioning of the whole system that are mediated by the food web can be extremely powerful, and may even induce regime shifts (Jones and Sayer, 2003; Scheffer and Carpenter, 2003).

Eco-evolutionary dynamics and feedbacks within the food web can be much faster than previously thought (e.g., Melián et al., 2011), and impacts on the epigenome can lead to quicker adaptation than traditional adaptation of the genome, via genetic plasticity (Johnson and Tricker, 2010). Consequently, we are starting to perceive how species evolve in the context of both the biotic and the abiotic environment, and how feedbacks and newly discovered mechanisms can accelerate evolutionary responses (Moya-Larano et al., 2012). In addition to the discovery of these ecological and evolutionary interactions, in recent years there have been rapid technological advances in next-generation sequencing (NGS, Box 2) and associated molecular techniques (Hajibabaei et al., 2011; Hajibabaei, 2012). This has allowed for significant advances in broadening the coverage of the tree of life and for adopting an ecoevolutionary approach to biomonitoring in freshwaters: emerging NGS approaches include new generations of molecular markers and the ability to characterize microbes in situ, allowing them to be used to monitor the functioning of ecosystems as well as determining the functions of microbes, metazoans, and macrofaunal communities directly (Purdy et al., 2010).

Novel Molecular and Microbial Approaches

An organism's molecular state results from its interaction with the environment, so measuring specific molecular machinery components can provide clues as to which stressors are present in the environment. The first generation

BOX 2 What is Next Generation Sequencing/-Omics?

The terms "next-generation" sequencing (NGS) or -omic technologies have been in use since a landmark paper (Margulies et al., 2005) detailed the use of 454 massively parallel pyrosequencings. Since then, the development of NGS platforms, accompanied by exponential increases in throughput and decreasing costs, has completely transformed the field of DNA sequencing.

For investigating functional diversity, the NGS "-omic" approaches can conveniently be broken down into discrete categories of relevance to different levels of biological organization. At the individual level, transcriptomic analyses measure differential gene expression via the analysis of expressed total RNA from specific tissues. At the community level, metagenetic and metabarcoding (Fonseca et al., 2010b; Bik et al., 2012; Taberlet et al., 2012) studies estimate environmental taxonomic richness by the en masse sequencing of environmental DNA samples (Sun et al., 2012). Shotgun metagenomic studies instead randomly sequence fragments of the total genomes present in an environmental DNA extraction (Knight et al., 2012), providing insights into both the functional and taxonomic capability of a given environment. Finally, metatranscriptomics enables researchers to investigate the actively transcribed mRNA from a community, giving an insight into the total gene expression from a local ecosystem (Filiatrault, 2011; Gilbert and Hughes, 2011).

As with microarray studies, gene expression is likely to change significantly at both short (Gilbert and Hughes, 2011) and large spatial and temporal scales, so transcriptomic analyses need to be designed around carefully and explicitly framed questions that account for environmental gene expression and short half-life of mRNA (i.e., transcript analyses are often not associated with protein composition) (Moran et al., 2013). These broad -omic categories are summarized in Figure 7.

For ecological studies, a potential disadvantage of these approaches lies in the fact that most platforms incorporate various forms of clonal amplification in the sequencing approaches, thereby introducing potential quantitative biases into datasets. New "third-generation" sequencers and technologies (Ribeiro et al., 2012; Schneider and Dekker, 2012; GridION[™] and MinION[™]) that use single molecule sequencing approaches and therefore lack any clonal amplification step prior to sequencing could produce truly quantitative data, although these are currently tailored to analyzing shorter numbers of very long reads, and many had not reached market maturity at the time of writing.

of molecular markers (Figure 6) was developed from hypothesis-driven research and based on biochemical, histological, morphological, and physiological changes in nucleic acids and proteins measured with conventional techniques (Ryan and Hightower, 1996). The number of such biomarkers is relatively small but they include some very effective examples such as the general xenobiotic response marker CYP1A (Celander, 2011), the endocrine disruption marker vitellogenin (Celander, 2011), and the metal stress marker metallothionein (Amiard et al., 2006). However, the hypothesis-driven



FIGURE 6 The evolution of biomarker discovery from the first generation approaches that use single genes whose expression is modulated by specific stressors, to the most recent advances that allow the discovery of multicomponent molecular signatures.

approach to biomarker discovery suffers from an important conceptual flaw, at least in this implementation: single genes whose expression is modulated in a highly specific manner are extremely rare.

In the last 10 years, new functional genomics technologies have provided a potential solution to this issue. Since these technologies allow the measurement of the expression of tens of thousands of genes, proteins, and metabolites in a single experiment, they provide the means to develop multigene signatures from the unbiased screening of genome-wide expression data (Van Aggelen et al., 2010; Figure 6).

The challenge of identifying specific molecular signatures hidden within hundreds of thousands of noisy variables has driven the development of statistical methods for the identification of molecular components that are differentially expressed in two or more sample groups (i.e., stressed versus controls). Although effective, this approach has limitations: in particular, it cannot identify synergistic effects between variables, it has a relatively low statistical power, and biological interpretation is challenging. The introduction of more complex modeling techniques that can assess the predictive power of combinations of biomarkers (Li et al., 2010) has been a significant step forward, particularly when applied to linking phenotypic responses (e.g., physiology) to molecular responses, especially in a network context. Ultimately this has allowed the identification of more effective and ecologically relevant biomarkers (Ankley et al., 2010).

Despite the potential of these approaches, the vast number of possible combinations of individual measurements drastically limits their ability to explore a large portion of the solution space and therefore makes it extremely difficult to capture biologically relevant pathways that respond specifically to particular stressors. One way to address this challenge is reverse engineering, a branch of systems biology that aims to reconstruct the underlying structure of a biological pathway from experimental data. This has been tremendously effective in biomedical research for identifying pathways predictive of clinical response, drug resistance, and novel therapeutic targets (Perkins et al., 2011). Again the biomedical-biomonitoring analogy can be used here to extend such approaches to environmental assessment. Because of the complexity of the datasets acquired using *-omics* technologies, any reverse-engineering approach must start from the identification of the high-level structure of the underlying biological networks and then progress to identifying more refined subnetworks associated with important phenotypic responses such as changes in reproductive ability following stress. Although in its infancy, this approach has already been applied by a number of groups for identifying novel stress pathways (Williams et al., 2011).

Overall, the use of these approaches allows the identification of more effective biomarkers than the ones based on differential expression and has opened up the possibility to develop specific multicomponent molecular signatures that are truly representative of a large number of stressors, with high specificity.

The use of biomarkers as a biomonitoring tool relies on inferences from molecular analyses. Returning to the more traditional approach of biomonitoring by using taxa themselves, and given that NGS technologies have finally enabled us to identify microbes in field conditions, these taxa represent ideal candidates for assessing how stressors alter community structure and ecosystem functioning. The pioneering "everything is everywhere, but the environment selects" theory proposed by Baas Becking (1934) suggests that the presence of all microorganisms is ubiquitous, but our ability to detect them via direct observation is limited by varying densities: i.e., rare microbes may be present but unobserved in ecological samples (de Wit and Bouvier, 2006). Consequently, the presence of different microbial species should be dictated by differences in environmental conditions rather than distance and biogeography (Zarraonaindia et al., 2013). If this is true, it could provide a truly global comparable framework for bioassessment and monitoring. Opposing theories exist, however, suggesting that microbial diversity is shaped by geography as well as the environment (Martiny et al., 2006; O'Malley, 2008). The key question is whether the environment enhances the presence of certain microorganisms in different locations, thus allowing us to compare components of the microbial community for the monitoring of ecosystems. High-throughput technologies with increased detection capabilities can assist here and there is huge potential for these to be exploited by ecologists for monitoring purposes (Green et al., 2008; Purdy et al., 2010; Poisot et al., 2013; Woodward et al., 2013).

Microorganisms play important functional roles in the major biogeochemical cycles at local to global scales, as well as in the recycling of nutrients and overall ecosystem functioning (Cotner and Biddanda, 2002; Nemergut et al., 2011), and many of these are also either ecosystem services in their own right or key processes that support important services (e.g., carbon seques-tration). Moreover, microbial communities are themselves influenced by environmental conditions. Accordingly, bacteria have been suggested as good indicators of environmental change due to some of their attractive bio-monitoring properties such as high diversity (thus broad range of environmental susceptibility), potential ubiquity, short life cycles, and minimal disturbance of the site during sampling (Lear et al., 2009; see Figure 7).

However, until recently their use was hindered by the inability to study them *in situ*, as only 5% of species are considered to be cultivable with standard techniques (Amann et al., 1995; Curtis et al., 2002), thus leading to narrowly focused approaches of single species analysis such as the targeting of specific ecotypes of pathogens, rather than whole-community detection (Hellawell, 1986; Port et al., 2012). High-throughput sequencing is already replacing historical fingerprinting approaches (Box 2) and has been used for the characterization of whole communities from a large variety of sources, from both terrestrial and aquatic systems (Roesch et al., 2007; Cole et al., 2010; Gilbert and Dupont, 2011; Foote et al., 2012; Port et al., 2012). Following sequence-based approaches, specific and identifiable microorganisms can be linked to environmental status and used as sensors for the



FIGURE 7 The many -omics approaches to sequencing life, from individuals to whole community techniques that can be adapted to each scenario. Methods applicable to a variety of scales are presented with their respective advantages and disadvantages. assessment of anthropogenic threats such as eutrophication, acidification, climate change, and land use changes (Port et al., 2012; Yergeau et al., 2012; Heino, 2013). In aquatic ecosystems, whole bacterial cell analysis can also be used for the assessment of pollution effects (Lear et al., 2009) and detection of antibiotics in the water (Port et al., 2012).

Recent studies from terrestrial and marine systems (Pommier et al., 2012; Sun et al., 2012) suggest that bacterial communities are sensitive indicators of contaminant stress, and also support the theory that the presence of microorganisms is more related to environmental conditions than to dispersal or geography. However, a freshwater study by Lear et al. (2012) found that microbial communities did not differ among different environmental pressures, whereas invertebrate sampling was the more effective monitoring tool, suggesting that either the studied microbial communities were unaffected by contaminants, or the discriminatory power of the molecular fingerprinting approaches used was insufficient.

Yergeau et al. (2012) used NGS of the 16S rRNA gene to determine the effect of pollution related to oil sands mining on nearby aquatic microbial community structure. Their findings suggest that the microbial community structure was significantly altered by distance from mining sites and support the potential use of bacteria and archaea as bioindicators of pollution. Furthermore, Kisand et al. (2012) were able to compare the microbial community composition of a highly impacted area, like the port of Genoa, with that of a protected area (low anthropogenic impact) through metagenomic analysis of the microbial communities from water samples. Distinct microbial diversity and abundance counts were detected among the different sites that can be related to the differences of environmental conditions, again demonstrating the potential for the use of metagenomics for monitoring of aquatic ecosystems.

The Functional Analysis of Microbes, Metazoans, and Macrofaunal Communities

Ecologists are increasingly striving to improve predictive power not only by identifying what organisms are present, but also by asking, "What are they doing?" The majority of functional ecological studies use organismal trait information (Tilman et al., 1997; Petchey and Gaston, 2006; Hagen et al., 2012) to provide a metric for quantitative analysis, but these cannot accurately reflect all of the functional attributes of individuals and species in complex ecological communities. In theory, the -omic toolbox can be employed to address this and to understand functional diversity in ways that have not been previously possible, although synergies with traditional ecology and taxonomy are essential if we are to fully understand the connections between biodiversity and ecosystem functioning and how they respond to stressors (Loreau et al., 2001).

If we consider a hypothetical freshwater ecosystem with both benthic and aquatic habitats, these habitats can be studied first independently but then in combination by investigating both the taxonomic and the functional diversity of the entire community using the -omic toolbox (see Box 2) tailored to organismal genome size and complemented by biogeochemical and nutrient cycling analyses. Starting with the microbial fraction, taxonomy marker genes such as 16S (Caporaso et al., 2011), ITS (Nilsson et al., 2008), and 18S (Fonseca et al., 2010a; Pawlowski et al., 2012) can be used for the highthroughput assessment of bacteria, archaea, fungi, and meiobiota, respectively, from multiple samples. Phylogenetic diversity can then be used throughout all gene marker schemes as a proxy for functional diversity by employing algorithms such as UniFrac (Lozupone and Knight, 2005; Caporaso et al., 2010; Fierer et al., 2012). Metagenomic and metatranscriptomic analyses can be employed to investigate the functional capability and specific functioning of the prokaryotic size fraction characterized by organisms with small genomes (e.g., 2-4 Mb) and their relatively small transcriptomes. Metatranscriptomic analyses are likely to be robust in simple communities of eukaryotic organisms where just a few species dominate (Durkin et al., 2012), but given the current limits of sequencing power, achieving effective coverage of replicated samples of complex eukaryotic communities (Bailly et al., 2007), whose transcriptomes can be very large (e.g., 20 Mb), is still limited. Similarly, metagenomic sequencing of eukaryotic communities is unlikely to reach the appropriate depth of coverage for ecological synthesis simply because eukaryotic genomes can be very large (the human genome alone is over 3 Gb in size).

Within prokaryotic communities, a new approach (PiCrust) (Langille et al., 2013) has emerged that links marker gene 16S studies to functional diversity maps and environmental 16S reads to their closest ancestors with full genome sequences, and predicts ancestral states of functional gene ontologies. Initial analyses suggest that this outperforms low-coverage shotgun metagenomic analyses in well-characterized communities, but further testing and examples will undoubtedly provide further insight. Nevertheless, the model provides a route between high-throughput studies and full-genome capability that may also eventually feature in the eukaryotic biosphere as more genomes are sequenced.

Advances that are likely to be provided by the -omic toolbox regarding the functional diversity of eukaryotic communities (e.g., protists, fungi, meiobiota, and macrofauna) are likely to be achieved by linking genotype phenotype data with the analysis of food webs and networks (Barberan et al., 2012; Rodriguez-Lanetty et al., 2013). The Barcode of Life Project (Ratnasingham and Hebert, 2007) strives for the provision of standardized and carefully curated DNA barcode data for organisms based on official barcode markers. So far, over 200,000 species have been barcoded. Importantly, this endeavor provides a link between a standardized genotype and the taxonomy and ecology of the barcoded species. At the start of the barcoding movement,

sequencing technologies were not mature enough to consider assessing multiple communities of organisms, but recently a multitude of "metabarcoding" studies (Epp et al., 2012; Taberlet et al., 2012) have shown that approaches used for microbial communities can be conveniently transferred to macrofaunal communities. If the featured species in the metabarcoding datasets have barcode reference data, these can provide very powerful links to the functional attributes of the organisms comprising the sequenced communities. The maturation of the field of metabarcoding not only provides a huge boost for our ability to assess large numbers of macrofaunal samples simultaneously (Ji et al., 2013), but also reasserts the need for generation of reference barcode libraries to provide the necessary links between -omic technologies and functional ecology. Moreover, since gene marker-based studies do not respect the boundaries between the living and the recently deceased or even ingested species, dietary and food web analyses can be conveniently performed using either individual or species-based sequencing of gut contents to investigate trophic interactions (Pompanon et al., 2012).

Overlying these possibilities is the further opportunity to deduct functional relationships using the analysis of ecological networks at multispecies levels of organization (Ings et al., 2009; Hagen et al., 2012). Following marker-based approaches and even metagenomic analyses, the resulting data are represented by a familiar taxon-by-sample frequency matrix of genotype occurrence (Ji et al., 2013) that can be related back to phenotype occurrence (i.e., species). The quantitative nature of the associations can be estimated on the basis of the mode of evolution and genomic content of the markers used (while acknowledging potential PCR bias), but the co-occurrence incidence matrices will reflect the distribution of species in space and time. Such power potentially enables us to delimit co-occurring ecological networks (in space and/or time) and how individual networks respond to external drivers. Moreover, some components of the sequence data matrices will be annotated to a high degree of accuracy (e.g., species level for barcoded metabarcoding data) and for all other groups potentially genus, order, family, etc., but at least phylum, enabling the researcher to characterize biological interactions (parasitism, predation, commensalism, mutualism, competition, etc.) and ecological processes (Faust and Raes, 2012). The additional strength of -omic highthroughput marker-based approaches is that with the now routine analysis of ca. 50 complex samples simultaneously, a high degree of replication and sample coverage can be achieved on scales that are simply not possible using traditional approaches for either microbial or macrofaunal samples. The combination of these emerging technologies and approaches promises a possible means of truly integrating ecological and evolutionary perspectives to responses to stressors across all the major domains of life in aquatic (and terrestrial) ecosystems.

CONCLUDING REMARKS

With an ever-increasing human population, the need to monitor and predict our effects on the natural world has never been more important. In the developed world the predominant stressors have changed, presenting new challenges to biomonitoring science (Figure 2), while developing nations such as India and China are facing the same stressors the Western world was exposed to in the twentieth century, but on a far greater scale (Abate, 1995; Yagishita, 1995; Aggarwal et al., 2001). An eco-evolutionary approach to biomonitoring will allow us to better understand the dynamics between the selective forces of evolution and the ecology of species. The ability of a community to adapt to change is key to its response to a particular stressor (Woodward et al., 2010; Moya-Larano et al., 2012), and this needs to be considered alongside biomonitoring results. With new technologies such as the rise of new molecular markers (e.g., Van Aggelen et al., 2010; Williams et al., 2011), the use of microbes (e.g., Lear et al., 2009), and advances in NGS techniques (Box 2), there is a great variety in approaches now available to monitor the functional response of aquatic communities to environmental stress.

A shift in the culture surrounding legislative biomonitoring, governance, and stakeholder implementation will be required before these advanced and promising approaches can be integrated into current protocols. There will likely be far fewer "traditional" taxonomists as NGS technologies take over, but many more bioinformaticians will be needed to process and analyze the NGS samples. The rate-limiting step in biomonitoring will shift from the slow and laborious process of identifying individuals through microscopy (data acquisition) to limitations in the efficiency with which large volumes of data can be processed. It is not impossible to imagine a future where remote sensing stations monitor environmental DNA or RNA and send sequence data back to the laboratory via telemetry as weather stations do now-unmanned and automated transmitting of results back to a central point. As bioinformatics solutions to data analysis and synthesis continue to develop over time as well as developing bioinformatics' huge potential to the biomonitoring world, it is likely to be simply a matter of "when" and not "if" this revolution will take place on a truly global scale.

ACKNOWLEDGMENTS

Thanks go to Rick Battarbee, University College London, and the Upland Waters Monitoring Network for allowing us to reproduce their data for Figure 1. CG was supported by Queen Mary University of London and the Freshwater Biology Association. BD was funded by the Scottish Government Rural and Environment Science and Analytical Services (RESAS). The project was partly supported by the Grand Challenges in Ecosystems and the Environment initiative at Imperial College London.

REFERENCES

- Abate, T., 1995. Swedish scientists take acid-rain research to developing-nations. Bioscience 45, 738–740.
- Aggarwal, S.G., Chandrawanshi, C.K., Patel, R.M., Agarwal, S., Kamavisdar, A., Mundhara, G.L., 2001. Acidification of surface water in central India. Water Air Soil Pollut. 130, 855–862.
- Allen, A.P., Brown, J.H., Gillooly, J.F., 2002. Global biodiversity, biochemical kinetics, and the energetic-equivalence rule. Science 297, 1545–1548.
- Amann, R.I., Ludwig, W., Schleifer, K.H., 1995. Phylogenetic identification and *in-situ* detection of individual microbial-cells without cultivation. Microbiol. Rev. 59, 143–169.
- Amiard, J.-C., Amiard-Triquet, C., Barka, S., Pellerin, J., Rainbow, P., 2006. Metallothioneins in aquatic invertebrates: their role in metal detoxification and their use as biomarkers. Aquat. Toxicol. 76, 160–202.
- Ankley, G.T., Bennett, R.S., Erickson, R.J., Hoff, D.J., Hornung, M.W., Johnson, R.D., Mount, D.R., Nichols, J.W., Russom, C.L., Schmieder, P.K., Serrrano, J.A., Tietge, J.E., Villeneuve, D.L., 2010. Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. Environ. Toxicol. Chem. 29, 730–741.
- Baas Becking, L.G.M., 1934. Geobiologie of inleiding tot de milieukunde. WP Van Stockum & Zoon.
- Bailly, J., Fraissinet-Tachet, L., Verner, M.C., Debaud, J.C., Lemaire, M., Wesolowski-Louvel, M., Marmeisse, R., 2007. Soil eukaryotic functional diversity, a metatranscriptomic approach. Isme J. 1, 632–642.
- Barberan, A., Bates, S.T., Casamayor, E.O., Fierer, N., 2012. Using network analysis to explore co-occurrence patterns in soil microbial communities. Isme J. 6, 343–351.
- Battarbee, R., Shilland, E., Simpson, G., Salgado, J., Goldsmith, B., Gray, W., Turner, S., 2013. Surface-water Acidity in the River Dart Area. Report for the West Country Rivers Trust.
- Battarbee, R.W., Charles, D.F., 1986. Diatom-based pH reconstruction studies of acid lakes in Europe and North America: a synthesis. Water, Air, Soil Pollut. 30, 347–354.
- Battarbee, R.W., Monteith, D.T., Juggins, S., Evans, C.D., Jenkins, A., Simpson, G.L., 2005. Reconstructing pre-acidification pH for an acidified Scottish loch: a comparison of palaeolimnological and modelling approaches. Environ. Pollut. 137, 135–149.
- Battarbee, R.W., Simpson, G.L., Shilland, E.M., Flower, R.J., Kreiser, A., Yang, H., Clarke, G., 2014. Recovery of UK lakes from acidification: an assessment using combined palaeoecological and contemporary diatom assemblage data. Ecol. Indic. 37 (Part B), 365–380.
- Bell, G., 2001. Neutral macroecology. Science 293, 2413-2418.
- Bell, G., Gonzalez, A., 2011. Adaptation and evolutionary Rescue in metapopulations experiencing environmental deterioration. Science 332, 1327–1330.
- Bik, H.M., Porazinska, D.L., Creer, S., Caporaso, J.G., Knight, R., Thomas, W.K., 2012. Sequencing our way towards understanding global eukaryotic biodiversity. Trends Ecol. Evol. 27, 233–243.
- Birks, H.J.B., Line, J.M., Juggins, S., Stevenson, A.C., Terbraak, C.J.F., 1990. Diatoms and Ph reconstruction. Philos. Trans. R. Soc. B-Biol. Sci. 327, 263–278.
- Bonada, N., Dolédec, S., Statzner, B., 2012. Spatial autocorrelation patterns of stream invertebrates: exogenous and endogenous factors. J. Biogeogr. 39, 56–68.
- Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Pena, A.G., Goodrich, J.K., Gordon, J.I., 2010. QIIME allows analysis of highthroughput community sequencing data. Nat. Methods 7, 335–336.

- Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J., Fierer, N., Knight, R., 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc. Natl. Acad. Sci. 108, 4516–4522.
- Cardinale, B.J., 2011. Biodiversity improves water quality through niche partitioning. Nature 472, 86–89.
- Cardinale, B.J., Srivastava, D.S., Duffy, J.E., Wright, J.P., Downing, A.L., Sankaran, M., Jouseau, C., 2006. Effects of biodiversity on the functioning of trophic groups and ecosystems. Nature 443, 989–992.
- Carpenter, S.R., Cole, J.J., Hodgson, J.R., Kitchell, J.F., Pace, M.L., Bade, D., Cottingham, K.L., Essington, T.E., Houser, J.N., Schindler, D.E., 2001. Trophic cascades, nutrients, and lake productivity: whole-lake experiments. Ecol. Monogr. 71, 163–186.
- Celander, M.C., 2011. Cocktail effects on biomarker responses in fish. Aquat. Toxicol. 105, 72–77.
- Cole, J.R., Konstandinidis, K., Farris, R.J., 2010. Microbial Diversity and Phylogeny: Extending from RRNAs to Genomes. In: Environmental Molecular Microbiology. Caister Academic Press, Norfolk, UK, pp. 1–21.
- Costanza, R., d'Arge, R., De Groot, R., Farber, S., Grasso, M., Hannon, B., Limburg, K., Naeem, S., O'neill, R.V., Paruelo, J., 1997. The value of the world's ecosystem services and natural capital. Nature 387, 253–260.
- Cotner, J.B., Biddanda, B.A., 2002. Small players, large role: microbial influence on biogeochemical processes in pelagic aquatic ecosystems. Ecosystems 5, 105–121.
- Curtis, T.P., Sloan, W.T., Scannell, J.W., 2002. Estimating prokaryotic diversity and its limits. Proc. Natl. Acad. Sci. USA 99, 10494–10499.
- Dangles, O., Gessner, M.O., Guerold, F., Chauvet, E., 2004. Impacts of stream acidification on litter breakdown: implications for assessing ecosystem functioning. J. Appl. Ecol. 41, 365–378.
- Darwin, C., 1872. On the Origin of Species by Means of Natural Selection. John Murray, London.
- Demars, B.O., Trémolières, M., 2009. Aquatic macrophytes as bioindicators of carbon dioxide in groundwater fed rivers. Sci. Total Environ. 407, 4752–4763.
- Demars, B.O.L., Edwards, A.C., 2009. Distribution of aquatic macrophytes in contrasting river systems: a critique of compositional-based assessment of water quality. Sci. Total Environ. 407, 975–990.
- Demars, B.O.L., Harper, D.M., 2005. Distribution of aquatic vascular plants in lowland rivers: separating the effects of local environmental conditions, longitudinal connectivity and river basin isolation. Freshwater Biol. 50, 418–437.
- Demars, B.O.L., Kemp, J.L., Friberg, N., Usseglio-Polatera, P., Harper, D.M., 2012. Linking biotopes to invertebrates in rivers: biological traits, taxonomic composition and diversity. Ecol. Indic. 23, 301–311.
- Demars, B.O.L., Manson, J.R., Olafsson, J.S., Gislason, G.M., Gudmundsdottir, R., Woodward, G., Reiss, J., Pichler, D.E., Rasmussen, J.J., Friberg, N., 2011. Temperature and the metabolic balance of streams. Freshwater Biol. 56, 1106–1121.
- Durkin, C.A., Marchetti, A., Bender, S.J., Truong, T., Morales, R., Mock, T., Armbrust, E.V., 2012. Frustule-related gene transcription and the influence of diatom community composition on silica precipitation in an iron-limited environment. Limnol. Oceanogr. 57, 1619–1633.
- Engelhardt, K.A., 2006. Relating effect and response traits in submersed aquatic macrophytes. Ecol. Appl. 16, 1808–1820.
- Enquist, B.J., Kerkhoff, A.J., Stark, S.C., Swenson, N.G., McCarthy, M.C., Price, C.A., 2007. A general integrative model for scaling plant growth, carbon flux, and functional trait spectra. Nature 449, 218–222.

- Epp, L.S., Boessenkool, S., Bellemain, E.P., Haile, J., Esposito, A., Riaz, T., Erseus, C., Gusarov, V.I., Edwards, M.E., Johnsen, A., Stenoien, H.K., Hassel, K., Kauserud, H., Yoccoz, N.G., Brathen, K., Willerslev, E., Taberlet, P., Coissac, E., Brochmann, C., 2012. New environmental metabarcodes for analysing soil DNA: potential for studying past and present ecosystems. Mol. Ecol. 21, 1821–1833.
- Everard, M., McInnes, R., 2013. Systemic solutions for multi-benefit water and environmental management. Sci. Total Environ. 461, 170–179.
- Faust, K., Raes, J., 2012. Microbial interactions: from networks to models. Nat. Rev. Microbiol. 10, 538–550.
- Fierer, N., Leff, J.W., Adams, B.J., Nielsen, U.N., Bates, S.T., Lauber, C.L., Owens, S., Gilbert, J.A., Wall, D.H., Caporaso, J.G., 2012. Cross-biome metagenomic analyses of soil microbial communities and their functional attributes. Proc. Natl. Acad. Sci. USA 109, 21390–21395.
- Filiatrault, M.J., 2011. Progress in prokaryotic transcriptomics. Curr. Opin. Microbiol. 14, 579–586.
- Fonseca, V.G., Carvalho, G.R., Sung, W., Johnson, H.F., Power, D.M., Neill, S.P., Packer, M., Blaxter, M.L., Lambshead, P.J.D., Thomas, W.K., 2010a. Second-generation environmental sequencing unmasks marine metazoan biodiversity. Nat. Commun. 1, 98.
- Fonseca, V.G., Carvalho, G.R., Sung, W., Johnson, H.F., Power, D.M., Neill, S.P., Packer, M., Blaxter, M.L., Lambshead, P.J.D., Thomas, W.K., Creer, S., 2010b. Second-generation environmental sequencing unmasks marine metazoan biodiversity. Nat. Commun. 1.
- Foote, A.D., Thomsen, P.F., Sveegaard, S., Wahlberg, M., Kielgast, J., Kyhn, L.A., Salling, A.B., Galatius, A., Orlando, L., Gilbert, M.T.P., 2012. Investigating the potential use of environmental DNA (eDNA) for genetic monitoring of marine mammals. Plos One 7.
- Friberg, N., Bonada, N., Bradley, D.C., Dunbar, M.J., Edwards, F.K., Grey, J., Hayes, R.B., Hildrew, A.G., Lamouroux, N., Trimmer, M., Woodward, G., 2011. Biomonitoring of human impacts in freshwater ecosystems: the good, the bad and the ugly. Adv. Ecol. Res. 44, 1–68.
- Froese, R., Pauly, D., 2010. FishBase. International Center for Living Aquatic Resources Management.
- García-Llorente, M., Martín-López, B., Díaz, S., Montes, C., 2011. Can ecosystem properties be fully translated into service values? an economic valuation of aquatic plant services. Ecol. Appl. 21, 3083–3103.
- Gilbert, J.A., Dupont, C.L., 2011. Microbial metagenomics: beyond the genome. Annu. Rev. Mar. Sci. 3, 347–371.
- Gilbert, J.A., Hughes, M., 2011. Gene Expression Profiling: Metatranscriptomics. High-throughput Next Generation Sequencing. Springer, pp. 195–205.
- Gould, S.J., Lewontin, R.C., 1979. Spandrels of San-Marco and the Panglossian paradigm a critique of the adaptationist program. Proc. R. Soc. Ser. B-Biol. Sci. 205, 581–598.
- Green, J.L., Bohannan, B.J.M., Whitaker, R.J., 2008. Microbial biogeography: from taxonomy to traits. Science 320, 1039–1043.
- Greulich, S., Bornette, G., 2003. Being evergreen in an aquatic habitat with attenuated seasonal contrasts a major competitive advantage? Plant Ecol. 167, 9–18.
- Hagen, M., Kissling, W.D., Rasmussen, C., De Aguiar, M.A.M., Brown, L.E., Carstensen, D.W., Alves-Dos-Santos, I., Dupont, Y.L., Edwards, F.K., Genini, J., Guimaraes, P.R., Jenkins, G.B., Jordano, P., Kaiser-Bunbury, C.N., Ledger, M.E., Maia, K.P., Marquitti, F.M.D., Mclaughlin, O., Morellato, L.P.C., O'Gorman, E.J., Trojelsgaard, K., Tylianakis, J.M., Vidal, M.M., Woodward, G., Olesen, J.M., 2012. Biodiversity, species interactions and ecological networks in a fragmented world. Adv. Ecol. Res. 46 (Pt 1), 189–210.

Hajibabaei, M., 2012. The golden age of DNA metasystematics. Trends Genet.: TIG 28, 535-537.

- Hajibabaei, M., Shokralla, S., Zhou, X., Singer, G.A.C., Baird, D.J., 2011. Environmental barcoding: a next-generation sequencing approach for biomonitoring applications using river benthos. Plos One 6.
- Halvorsen, G.A., Heegaard, E., Fjellheim, A., Raddum, G.G., 2003. Tracing recovery from acidification in the western Norwegian Nausta watershed. Ambio 32, 235–239.
- Harte, J., 2011. Maximum Entropy and Ecology: A Theory of Abundance, Distribution, and Energetics. Oxford University Press.
- Heino, J., 2013. The importance of metacommunity ecology for environmental assessment research in the freshwater realm. Biol. Rev. 88, 166–178.
- Hellawell, J.M., 1986. Biological Indicators of Freshwater Pollution and Environmental Management.
- Hildrew, A.G., 2009. Sustained research on stream communities: a model system and the comparative approach. Adv. Ecol. Res. 41, 175–312.
- Hill, B.H., Elonen, C.M., Seifert, L.R., May, A.A., Tarquinio, E., 2012. Microbial enzyme stoichiometry and nutrient limitation in US streams and rivers. Ecol. Indic. 18, 540–551.
- Horrigan, N., Baird, D.J., 2008. Trait patterns of aquatic insects across gradients of flow-related factors: a multivariate analysis of Canadian national data. Can. J. Fish. Aquat. Sci. 65, 670–680.
- Hubbell, S.P., 2001. The Unified Neutral Theory of Biodiversity and Biogeography. Princeton University Press.
- Humboldt, A., 1849. Cosmos: A Sketch of a Physical Description of the Universe. EC Otté, I. Henry G. Bohn, London.
- Ings, T.C., Montoya, J.M., Bascompte, J., Bluthgen, N., Brown, L., Dormann, C.F., Edwards, F., Figueroa, D., Jacob, U., Jones, J.I., Lauridsen, R.B., Ledger, M.E., Lewis, H.M., Olesen, J.M., van Veen, F.J.F., Warren, P.H., Woodward, G., 2009. Ecological networks - beyond food webs. J. Anim. Ecol. 78, 253–269.
- Ji, Y., Ashton, L., Pedley, S.M., Edwards, D.P., Tang, Y., Nakamura, A., Kitching, R., Dolman, P.M., Woodcock, P., Edwards, F.A., 2013. Reliable, verifiable and efficient monitoring of biodiversity via metabarcoding. Ecol. Lett. 16, 1245–1257.
- Johnson, L.J., Tricker, P.J., 2010. Epigenomic plasticity within populations: its evolutionary significance and potential. Heredity 105, 113–121.
- Jones, J.I., Sayer, C.D., 2003. Does the fish-invertebrate-periphyton cascade precipitate plant loss in shallow lakes? Ecology 84, 2155–2167.
- Kampfraath, A.A., Hunting, E.R., Mulder, C., Breure, A.M., Gessner, M.O., Kraak, M.H.S., Admiraal, W., 2012. DECOTAB: a multipurpose standard substrate to assess effects of litter quality on microbial decomposition and invertebrate consumption. Freshwater Sci. 31, 1156–1162.
- Kerkhoff, A.J., Enquist, B.J., 2006. Ecosystem allometry: the scaling of nutrient stocks and primary productivity across plant communities. Ecol. Lett. 9, 419–427.
- Kisand, V., Valente, A., Lahm, A., Tanet, G., Lettieri, T., 2012. Phylogenetic and functional metagenomic profiling for assessing microbial biodiversity in environmental monitoring. Plos One 7.
- Knight, R., Jansson, J., Field, D., Fierer, N., Desai, N., Fuhrman, J.A., Hugenholtz, P., van der Lelie, D., Meyer, F., Stevens, R., Bailey, M.J., Gordon, J.I., Kowalchuk, G.A., Gilbert, J.A., 2012. Unlocking the potential of metagenomics through replicated experimental design. Nat. Biotechnol. 30, 513–520.
- Kolkwitz, R., Marsson, M., 1902. Grundsätze für die biologische Beurteilung des Wassers nach seiner Flora und Fauna. Mitt. Pr
 üfungsanst. Wasserversorg. Abwasserbeseit. 1, 3–72.

- Kolkwitz, R., Marsson, M., 1909. Ökologie der tierischen Saprobien. Beiträge zur Lehre von der biologischen Gewässerbeurteilung. Int. Rev. Gesamten Hydrobiol. Hydrogr. 2, 126–152.
- Langille, M.G.I., Zaneveld, J., Caporaso, J.G., McDonald, D., Knights, D., Reyes, J.A., Clemente, J.C., Burkepile, D.E., Thurber, R.L.V., Knight, R., Beiko, R.G., Huttenhower, C., 2013. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nat. Biotechnol. 31, 814–821.
- Layer, K., Hildrew, A., Monteith, D., Woodward, G., 2010. Long-term variation in the littoral food web of an acidified mountain lake. Global Change Biol. 16, 3133–3143.
- Layer, K., Hildrew, A.G., Jenkins, G.B., Riede, J.O., Rossiter, S.J., Townsend, C.R., Woodward, G., 2011. Long-term dynamics of a well-characterised food web: four decades of acidification and recovery in the Broadstone stream model system. Adv. Ecol. Res. 44, 69–117.
- Layer, K., Hildrew, A.G., Woodward, G., 2013. Grazing and detritivory in 20 stream food webs across a broad pH gradient. Oecologia 171, 459–471.
- Lear, G., Ancion, P.Y., Harding, J., Lewis, G.D., 2012. Use of bacterial communities to assess the ecological health of a recently restored stream. N. Z. J. Mar. Freshwater Res. 46, 291–301.
- Lear, G., Boothroyd, I.K.G., Turner, S.J., Roberts, K., Lewis, G.D., 2009. A comparison of bacteria and benthic invertebrates as indicators of ecological health in streams. Freshwater Biol. 54, 1532–1543.
- Ledger, M.E., Brown, L.E., Edwards, F., Milner, A.M., Woodward, G., 2012. Drought alters the structure and functioning of complex food webs. Nat. Clim. Change.
- Ledger, M.E., Hildrew, A.G., 2005. The ecology of acidification and recovery: changes in herbivore-algal food web linkages across a stream pH gradient. Environ. Pollut. 137, 103–118.
- Ledger, M.E., Milner, A., Brown, L., Edwards, F., Hudson, L., Woodward, G., 2013. Extreme climatic events alter aquatic food webs. A synthesis of evidence from a Mesocosm drought Experiment. Adv. Ecol. Res. 48, 343–395.
- Lennon, J.J., Kunin, W.E., Hartley, S., Gaston, K.J., 2007. In: Storch, D., Marquet, P.A., Brown, J.H. (Eds.), Species Distribution Patterns, Diversity Scaling and Testing for Fractals in Southern African Birds. Scaling Biodiversity. Cambridge University Press, Cambridge, UK.
- Li, Y., Wang, N., Perkins, E.J., Zhang, C., Gong, P., 2010. Identification and optimization of classifier genes from multi-class earthworm microarray dataset. Plos One 5, e13715.
- Likens, G.E., Bormann, F.H., Pierce, R.S., Eaton, J.S., Johnson, N.M., 1977. Biogeo-chemistry of a Forested Ecosystem. Springer-Verlag, New York.
- López-Urrutia, Á., San Martin, E., Harris, R.P., Irigoien, X., 2006. Scaling the metabolic balance of the oceans. Proc. Natl. Acad. Sci. 103, 8739–8744.
- Loreau, M., 2010. From Populations to Ecosystems: Theoretical Foundations for a New Ecological Synthesis (MPB-46). Princeton University Press.
- Loreau, M., Naeem, S., Inchausti, P., 2002. Biodiversity and Ecosystem Functioning: Synthesis and Perspectives. Oxford University Press.
- Loreau, M., Naeem, S., Inchausti, P., Bengtsson, J., Grime, J.P., Hector, A., Hooper, D.U., Huston, M.A., Raffaelli, D., Schmid, B., Tilman, D., Wardle, D.A., 2001. Ecology biodiversity and ecosystem functioning: current knowledge and future challenges. Science 294, 804–808.
- Lozupone, C., Knight, R., 2005. UniFrac: a new phylogenetic method for comparing microbial communities. Appl. Environ. Microbiol. 71, 8228–8235.
- Lyell, C., Deshayes, G.P., 1830. Principles of Geology: Being an Attempt to Explain the Former Changes of the Earth's Surface, by Reference to Causes Now in Operation. John Murray.

- Margulies, M., Egholm, M., Altman, W.E., Attiya, S., Bader, J.S., Bemben, L.A., Berka, J., Braverman, M.S., Chen, Y.-J., Chen, Z., 2005. Genome sequencing in microfabricated highdensity picolitre reactors. Nature 437, 376–380.
- Martiny, J.B.H., Bohannan, B.J.M., Brown, J.H., Colwell, R.K., Fuhrman, J.A., Green, J.L., Horner-Devine, M.C., Kane, M., Krumins, J.A., Kuske, C.R., Morin, P.J., Naeem, S., Ovreas, L., Reysenbach, A.L., Smith, V.H., Staley, J.T., 2006. Microbial biogeography: putting microorganisms on the map. Nat. Rev. Microbiol. 4, 102–112.
- May, R.M., 1990. Taxonomy as destiny. Nature 347, 129-130.
- Mayden, R.L., 1997. A hierarchy of species concepts: the denouement in the saga of the species problem. In: Claridge, M.F., Dawah, H.A., Wilson, M.R. (Eds.), Systematics Association Special Volume Series; Species: The Units of Biodiversity, pp. 381–424.
- McKie, B.G., Woodward, G., Hladyz, S., Nistorescu, M., Preda, E., Popescu, C., Giller, P.S., Malmqvist, B., 2008. Ecosystem functioning in stream assemblages from different regions: contrasting responses to variation in detritivore richness, evenness and density. J. Anim. Ecol. 77, 495–504.
- Melián, C., Vilas, C., Baldo, F., González-Ortegón, E., Drake, P., Williams, R.J., 2011. Eco-evolutionary dynamics of individual-based food webs. Adv. Ecol. Res. 45, 225–268.
- Metcalfe, J.L., 1989. Biological water-quality assessment of running waters based on macroinvertebrate communities - history and present status in europe. Environ. Pollut. 60, 101–139.
- Millenium Ecosystem Assessment, 2005. Ecosystems and Human Well-being: Current State and Trends, vol. 1. Washington.
- Moilanen, A., Franco, A.M., Early, R.I., Fox, R., Wintle, B., Thomas, C.D., 2005. Prioritizing multiple-use landscapes for conservation: methods for large multi-species planning problems. Proc. R. Soc. B: Biol. Sci. 272, 1885–1891.
- Moilanen, A., Hanski, I., 2001. On the use of connectivity measures in spatial ecology. Oikos 95, 147–151.
- Moilanen, A., Leathwick, J., Elith, J., 2008. A method for spatial freshwater conservation prioritization. Freshwater Biol. 53, 577–592.
- Monteith, D.T., Hildrew, A.G., Flower, R.J., Raven, P.J., Beaumont, W.R.B., Collen, P., Kreiser, A.M., Shilland, E.M., Winterbottom, J.H., 2005. Biological responses to the chemical recovery of acidified fresh waters in the UK. Environ. Pollut. 137, 83–101.
- Moran, M.A., Satinsky, B., Gifford, S.M., Luo, H.W., Rivers, A., Chan, L.K., Meng, J., Durham, B.P., Shen, C., Varaljay, V.A., Smith, C.B., Yager, P.L., Hopkinson, B.M., 2013. Sizing up metatranscriptomics. Isme J. 7, 237–243.
- Moya-Larano, J., Verdeny-Vilalta, O., Rowntree, J., Melguizo-Ruiz, N., Montserrat, M., Laiolo, P., 2012. Climate change and eco-evolutionary dynamics in food webs. Adv. Ecol. Res. 47, 1–80.
- Mulholland, P.J., Helton, A.M., Poole, G.C., Hall, R.O., Hamilton, S.K., Peterson, B.J., Tank, J.L., Ashkenas, L.R., Cooper, L.W., Dahm, C.N., 2008. Stream denitrification across biomes and its response to anthropogenic nitrate loading. Nature 452, 202–205.
- Murphy, J.F., Davy-Bowker, J., McFarland, B., Ormerod, S.J., 2013. A diagnostic biotic index for assessing acidity in sensitive streams in Britain. Ecol. Indic. 24, 562–572.
- Murphy, J.F., Winterbottom, J.H., Orton, S., Simpson, G.L., Shilland, E.M., Hildrew, A.G., 2012. Evidence of recovery from acidification in the macroinvertebrate assemblages of UK fresh waters: a 20-year time series. Ecol. Indic.
- Naeem, S., 2002. Disentangling the impacts of diversity on ecosystem functioning in combinatorial experiments. Ecology 83, 2925–2935.

- Nemergut, D.R., Costello, E.K., Hamady, M., Lozupone, C., Jiang, L., Schmidt, S.K., Fierer, N., Townsend, A.R., Cleveland, C.C., Stanish, L., Knight, R., 2011. Global patterns in the biogeography of bacterial taxa. Environ. Microbiol. 13, 135–144.
- Nilsson, R.H., Kristiansson, E., Ryberg, M., Hallenberg, N., Larsson, K.-H., 2008. Intraspecific ITS variability in the kingdom fungi as expressed in the international sequence databases and its implications for molecular species identification. Evol. Bioinf. Online 4, 193.
- O'Malley, M.A., 2008. Everything is everywhere but the environment selects: ubiquitous distribution and ecological determinism in microbial biogeography. Stud. Hist. Philos. Sci. Part C: Stud. Hist. Philos. Biol. Biomed. Sci. 39, 314–325.
- Odum, E.P., 1969. The strategy of ecosystem development. An understanding of ecological succession provides a basis for resolving man's conflict with nature. Science 164, 262–269.
- Paradis, E., Claude, J., Strimmer, K., 2004. APE: analyses of phylogenetics and evolution in R language. Bioinformatics 20, 289–290.
- Pawlowski, J., Audic, S., Adl, S., Bass, D., Belbahri, L., Berney, C., Bowser, S.S., Cepicka, I., Decelle, J., Dunthorn, M., 2012. CBOL protist working group: barcoding eukaryotic richness beyond the animal, plant, and fungal kingdoms. PLoS Biol. 10, e1001419.
- Perkins, D.M., McKie, B.G., Malmqvist, B., Gilmour, S.G., Reiss, J., Woodward, G., 2010. Environmental warming and biodiversity-ecosystem functioning in freshwater Microcosms: Partitioning the effects of species identity, richness and metabolism. Adv. Ecol. Res. 43, 177–209.
- Perkins, E.J., Chipman, J.K., Edwards, S., Habib, T., Falciani, F., Taylor, R., Van Aggelen, G., Vulpe, C., Antczak, P., Loguinov, A., 2011. Reverse engineering adverse outcome pathways. Environ. Toxicol. Chem. 30, 22–38.
- Petchey, O.L., Downing, A.L., Mittelbach, G.G., Persson, L., Steiner, C.F., Warren, P.H., Woodward, G., 2004. Species loss and the structure and functioning of multitrophic aquatic systems. Oikos 104, 467–478.
- Petchey, O.L., Gaston, K.J., 2006. Functional diversity: back to basics and looking forward. Ecol. Lett. 9, 741–758.
- Poff, N.L., Olden, J.D., Vieira, N.K.M., Finn, D.S., Simmons, M.P., Kondratieff, B.C., 2006. Functional trait niches of North American lotic insects: traits-based ecological applications in light of phylogenetic relationships. J. North Am. Benthol. Soc. 25, 730–755.
- Poisot, T., Pequin, B., Gravel, D., 2013. High-throughput sequencing: a roadmap toward community ecology. Ecol. Evol. 3, 1125–1139.
- Pollux, B.J.A., Santamaria, L., Ouborg, N.J., 2005. Differences in endozoochorous dispersal between aquatic plant species, with reference to plant population persistence in rivers. Freshwater Biol. 50, 232–242.
- Pommier, T., Douzery, E.J.P., Mouillot, D., 2012. Environment drives high phylogenetic turnover among oceanic bacterial communities. Biol. Lett. 8, 562–566.
- Pompanon, F., Deagle, B.E., Symondson, W.O., Brown, D.S., Jarman, S.N., Taberlet, P., 2012. Who is eating what: diet assessment using next generation sequencing. Mol. Ecol. 21, 1931–1950.
- Port, J.A., Wallace, J.C., Griffith, W.C., Faustman, E.M., 2012. Metagenomic profiling of microbial composition and antibiotic resistance determinants in Puget Sound. Plos One 7.
- Purdy, K.J., Hurd, P.J., Moya-Larano, J., Trimmer, M., Oakley, B.B., Woodward, G., 2010. Systems biology for ecology: from molecules to ecosystems. Adv. Ecol. Res. 43, 87–149.
- Ratnasingham, S., Hebert, P.D.N., 2007. BOLD: the barcode of life data system. Mol. Ecol. Notes 7, 355–364. www.barcodinglife.org.

- Rawcliffe, R., Sayer, C.D., Woodward, G., Grey, J., Davidson, T.A., Jones, J.I., 2010. Back to the future: using palaeolimnology to infer long-term changes in shallow lake food webs. Freshwater Biol. 55, 600–613.
- Reiss, J., Bailey, R.A., Cassio, F., Woodward, G., Pascoal, C., 2010. Assessing the contribution of micro-organisms and macrofauna to biodiversity-ecosystem functioning relationships in freshwater microcosms. Adv. Ecol. Res. 43, 151–176.
- Reiss, J., Bailey, R.A., Perkins, D.M., Pluchinotta, A., Woodward, G., 2011. Testing effects of consumer richness, evenness and body size on ecosystem functioning. J. Anim. Ecol. 80, 1145–1154.
- Renberg, I., Hellberg, T., 1982. The Ph history of lakes in southwestern Sweden, as calculated from the subfossil diatom flora of the sediments. Ambio 11, 30–33.
- Ribeiro, F.J., Przybylski, D., Yin, S.Y., Sharpe, T., Gnerre, S., Abouelleil, A., Berlin, A.M., Montmayeur, A., Shea, T.P., Walker, B.J., Young, S.K., Russ, C., Nusbaum, C., MacCallum, I., Jaffe, D.B., 2012. Finished bacterial genomes from shotgun sequence data. Genome Res. 22, 2270–2277.
- Rodriguez-Lanetty, M., Granados-Cifuentes, C., Barberan, A., Bellantuono, A.J., Bastidas, C., 2013. Ecological inferences from a deep screening of the Complex Bacterial Consortia associated with the coral, Porites astreoides. Mol. Ecol. 22, 4349–4362.
- Roesch, L.F., Fulthorpe, R.R., Riva, A., Casella, G., Hadwin, A.K.M., Kent, A.D., Daroub, S.H., Camargo, F.A.O., Farmerie, W.G., Triplett, E.W., 2007. Pyrosequencing enumerates and contrasts soil microbial diversity. Isme J. 1, 283–290.
- Rosenberg, D.M., Resh, V.H., 1993. Freshwater Biomonitoring and Benthic Macroinvertebrates. Chapman and Hall, New York.
- Ryan, J.A., Hightower, L.E., 1996. Stress proteins as molecular biomarkers for environmental toxicology. Exs 77, 411–424.
- Rybicki, N.B., Landwehr, J.M., 2007. Long-term changes in abundance and diversity of macrophyte and waterfowl populations in an estuary with exotic macrophytes and improving water quality. Limnol. Oceanogr. 52, 1195–1207.
- Scheffer, M., Bascompte, J., Brock, W.A., Brovkin, V., Carpenter, S.R., Dakos, V., Held, H., Van Nes, E.H., Rietkerk, M., Sugihara, G., 2009. Early-warning signals for critical transitions. Nature 461, 53–59.
- Scheffer, M., Carpenter, S.R., 2003. Catastrophic regime shifts in ecosystems: linking theory to observation. Trends Ecol. Evol. 18, 648–656.
- Schindler, D.E., Hilborn, R., Chasco, B., Boatright, C.P., Quinn, T.P., Rogers, L.A., Webster, M.S., 2010. Population diversity and the portfolio effect in an exploited species. Nature 465, 609–612.
- Schindler, D.W., 1987. Detecting ecosystem responses to anthropogenic stress. Can. J. Fish. Aquat. Sci. 44, s6–s25.
- Schindler, D.W., 1990. Experimental perturbations of whole lakes as tests of hypotheses concerning ecosystem structure and function. Oikos 25–41.
- Schneider, G.F., Dekker, C., 2012. DNA sequencing with nanopores. Nat. Biotechnol. 30, 326–328.
- Simpson, G.L., Shilland, E.M., Winterbottom, J.M., Keay, J., 2005. Defining reference conditions for acidified waters using a modern analogue approach. Environ. Pollut. 137, 119–133.
- Sinsabaugh, R.L., Hill, B.H., Shah, J.J.F., 2009. Ecoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. Nature 462, 795–798.

- Simpson, J.C., Norris, R.H., 2000. Biological Assessment of River Quality: Development of AUSRIVAS Models and Outputs. Freshwater Biological Association, The Ferry House, Far Sawrey, Ambleside, Cumbria, LA22 0LP, UK.
- Slavik, K., Peterson, B., Deegan, L., Bowden, W., Hershey, A., Hobbie, J., 2004. Long-term responses of the Kuparuk River ecosystem to phosphorus fertilization. Ecology 85, 939–954.
- Smol, J.P., 2009. Pollution of Lakes and Rivers: A Paleoenvironmental Perspective. John Wiley & Sons.
- Statzner, B., Bêche, L.A., 2010. Can biological invertebrate traits resolve effects of multiple stressors on running water ecosystems? Freshwater Biol. 55, 80–119.
- Sun, M.Y., Dafforn, K.A., Brown, M.V., Johnston, E.L., 2012. Bacterial communities are sensitive indicators of contaminant stress. Mar. Pollut. Bull. 64, 1029–1038.
- Sweeney, B.W., Bott, T.L., Jackson, J.K., Kaplan, L.A., Newbold, J.D., Standley, L.J., Hession, W.C., Horwitz, R.J., 2004. Riparian deforestation, stream narrowing, and loss of stream ecosystem services. Proc. Natl. Acad. Sci. USA 101, 14132–14137.
- Taberlet, P., Coissac, E., Pompanon, F., Brochmann, C., Willerslev, E., 2012. Towards nextgeneration biodiversity assessment using DNA metabarcoding. Mol. Ecol. 21, 2045–2050.
- Thuiller, W., Lavergne, S., Roquet, C., Boulangeat, I., Lafourcade, B., Araujo, M.B., 2011. Consequences of climate change on the tree of life in Europe. Nature 470, 531–534.
- Tilman, D., Knops, J., Wedin, D., Reich, P., Ritchie, M., Siemann, E., 1997. The influence of functional diversity and composition on ecosystem processes. Science 277, 1300–1302.
- Tokeshi, M., 1990. Niche apportionment or random assortment species abundance patterns revisited. J. Anim. Ecol. 59, 1129–1146.
- Tokeshi, M., 1993. Species abundance patterns and community. Adv. Ecol. Res. 24, 111.
- UK National Ecosystem Assessment, 2011. The UK National Ecosystem Assessment: Synthesis of the Key Findings. Cambridge.
- Van Aggelen, G., Ankley, G.T., Baldwin, W.S., Bearden, D.W., Benson, W.H., Chipman, J.K., Collette, T.W., Craft, J.A., Denslow, N.D., Embry, M.R., 2010. Integrating omic technologies into aquatic ecological risk assessment and environmental monitoring: hurdles, achievements, and future outlook. Environ. Health Perspect. 118, 1.
- Venail, P., MacLean, R., Bouvier, T., Brockhurst, M., Hochberg, M., Mouquet, N., 2008. Diversity and productivity peak at intermediate dispersal rate in evolving metacommunities. Nature 452, 210–214.
- Vonlanthen, P., Bittner, D., Hudson, A.G., Young, K.A., Muller, R., Lundsgaard-Hansen, B., Roy, D., Di Piazza, S., Largiader, C.R., Seehausen, O., 2012. Eutrophication causes speciation reversal in whitefish adaptive radiations. Nature 482, 357–U1500.
- Webb, C., Ackerly, D., Kembel, S., 2011. Software for the Analysis of Phylogenetic Comunity Structure and Character Evolution (With Phylomatic and Ecovolve) User's Manual Version 4.2. Arnold Arboretum of Harvard University.
- Willby, N.J., Abernethy, V.J., Demars, B.O.L., 2000. Attribute-based classification of European hydrophytes and its relationship to habitat utilization. Freshwater Biol. 43, 43–74.
- Williams, T.D., Turan, N., Diab, A.M., Wu, H.F., Mackenzie, C., Bartie, K.L., Hrydziuszko, O., Lyons, B.P., Stentiford, G.D., Herbert, J.M., Abraham, J.K., Katsiadaki, I., Leaver, M.J., Taggart, J.B., George, S.G., Viant, M.R., Chipman, K.J., Falciani, F., 2011. Towards a system level understanding of non-model organisms sampled from the environment: a network biology approach. Plos Comput. Biol. 7.
- de Wit, R., Bouvier, T., 2006. 'Everything is everywhere, but, the environment selects'; what did Baas Becking and Beijerinck really say? Environ. Microbiol. 8, 755–758.

- Woodward, G., 2009. Biodiversity, ecosystem functioning and food webs in fresh waters: assembling the jigsaw puzzle. Freshwater Biol. 54, 2171–2187.
- Woodward, G., Benstead, J.P., Beveridge, O.S., Blanchard, J., Brey, T., Brown, L.E., Cross, W.F., Friberg, N., Ings, T.C., Jacob, U., Jennings, S., Ledger, M.E., Milner, A.M., Montoya, J.M., O'Gorman, E.J., Olesen, J.M., Petchey, O.L., Pichler, D.E., Reuman, D.C., Thompson, M.S.A., Van Veen, F.J.F., Yvon-Durocher, G., 2010. Ecological networks in a changing climate. Adv. Ecol. Res. 42, 71–138.
- Woodward, G., Brown, L.E., Edwards, F.K., Hudson, L.N., Milner, A.M., Reuman, D.C., Ledger, M.E., 2012. Climate change impacts in multispecies systems: drought alters food web size structure in a field experiment. Philos. Trans. R. Soc. B-Biol. Sci. 367, 2990–2997.
- Woodward, G., Gray, C., Baird, D.J., 2013. A critique of biomonitoring in an age of globalisation and emerging environmental threats. Limnetica.
- Yachi, S., Loreau, M., 1999. Biodiversity and ecosystem productivity in a fluctuating environment: the insurance hypothesis. Proc. Natl. Acad. Sci. 96, 1463–1468.
- Yagishita, M., 1995. Establishing an acid deposition monitoring network in East Asia. Water, Air, Soil Pollut. 85, 273–278.
- Yergeau, E., Lawrence, J.R., Sanschagrin, S., Waiser, M.J., Korber, D.R., Greer, C.W., 2012. Nextgeneration sequencing of microbial communities in the Athabasca river and its tributaries in relation to oil sands mining activities. Appl. Environ. Microbiol. 78, 7626–7637.
- Young, R.G., Matthaei, C.D., Townsend, C.R., 2008. Organic matter breakdown and ecosystem metabolism: functional indicators for assessing river ecosystem health. J. North Am. Benthol. Soc. 27, 605–625.
- Yvon-Durocher, G., Jones, J.I., Trimmer, M., Woodward, G., Montoya, J.M., 2010. Warming alters the metabolic balance of ecosystems. Philos. Trans. R. Soc. B-Biol. Sci. 365, 2117–2126.
- Zarraonaindia, I., Smith, D.P., Gilbert, J.A., 2013. Beyond the genome: community-level analysis of the microbial world. Biol. Philos. 1–22.