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DEVELOPMENTS IN AQUATIC INSECT BIOMONITORING: A Comparative Analysis of Recent Approaches

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■ **Abstract** Aquatic insects and other benthic invertebrates are the most widely used organisms in freshwater biomonitoring of human impact. Because of the high monetary investment in freshwater management, decisions are often based on biomonitoring results, and a critical and comparative review of different approaches is required. We used 12 criteria that should be fulfilled by an "ideal" biomonitoring tool, addressing the rationale, implementation, and performance of a method. After illustrating how the century-old but still widely used Saprobian system does not meet these criteria, we apply them to nine recent approaches that range from the suborganismal to the ecosystem level. Although significant progress has been made in the field, no recent approach meets all 12 criteria. Given that the use of biomonitoring information has important financial consequences, we suggest that societies and governments prioritize how these criteria should be ranked.

OVERVIEW AND PERSPECTIVES

The linkage between the management and biomonitoring of freshwater systems reflects the changing needs and demands of human societies in industrialized regions. In the latter half of the nineteenth century, industrialization in many parts of Europe led to growing human populations, and the increasing amount of waste produced created severe health problems (e.g., cholera, dysentery, typhoid fever) (72, 108). Thus, the first management measures, applied primarily to running waters, attempted to solve these public health problems and, correspondingly, early biomonitoring of running waters focused on bacteriological aspects (72, 80). Because of other needs in freshwater management (e.g., sewage treatment), additional microorganisms (algae, fungi, and protozoa not causing human health problems) were subsequently included in freshwater biomonitoring (80).

By 1900, the hygienic and economic importance of freshwater systems in Germany had risen to a high level, such that a research approach was prepared to use entire aquatic communities (called biocoenoses, which included macrophytes, macroinvertebrates, and fish) as indicators of pollution status (80). The first list of aquatic insects that indicated the degree of organic pollution and, in particular, the self-purification potential of fresh waters soon followed (81).

The rationale to use aquatic insects (and other benthic invertebrates) as indicators of pollution or self-purification potential was based on their capacity to reveal a source of pollution even if this source does not discharge pollutants, a feat impossible by chemical analysis alone (80). Thus, aquatic invertebrates served as indicators of pollution for the management of fisheries (aiming primarily at food production), for identifying quantities of waste that could be self-purified and thus discharged to fresh waters, or for identifying sources of water use for irrigation and industrial processes (108, 140).

Beginning in the 1970s, "ecosystem health" per se was seen increasingly as a valuable objective in many human societies. With growing public involvement, politicians (e.g., the "green" parties in Europe) entered a process that had until then been guided by experts in freshwater ecology and engineering. As a result, public demand pressured water authorities to restore the ecological health of freshwater systems, such that huge budgets are now spent on such projects. For example, the cost for investments into the restoration of the Emscher river system (starting in 1990 in a German industrial area) has been estimated at US$ 5.5 billion (http://www.emscherumbau.de/), which corresponds to ∼170,000 mean annual German gross salaries (in the 1990s; http://www.bfa.de/). Ironically, the successful restoration of the health of humans suffering from dysentery, malaria, and typhoid fever that were living in the Emscher catchment through channel regulations and sewage treatment in the 1900s required estimated investments of only ∼34,000 mean annual German gross salaries (in the 1910s; http://www.bfa.de/) (108). Today, the worldwide annual budget for water resource management exceeds US$ 100 billion (131). These figures illustrate (*a*) the importance that ecosystem health has currently reached in developed parts of the world, (*b*) the responsibility ecologists have when they develop tools for aquatic insect biomonitoring, and (*c*) that the solution to a problem affecting human health may require less money than the restoration of ecosystem health. Therefore, because the improvement and development of existing and new biomonitoring tools using aquatic insects are a major effort among aquatic entomologists (28), this review provides a comparative analysis of recent approaches in the field.

INTRODUCTION

Biomonitoring is the use of biological variables to survey the environment (53). The primary task in biomonitoring is the search for the ideal indicator (or bioindicator) whose presence, abundance, and/or behavior reflects a stressor's effect on biota. An indicator may be used for biomonitoring at many levels of organization, ranging from suborganismal (i.e., gene, cell, tissue) and organismal to population, community, and even ecosystem levels (96).

Aquatic insect biomonitoring has a long tradition (see historical overviews in References 26, 62) that developed from a few initial approaches and expanded to a large number of different methods used in various countries or regions of the world (124a). In addition, diversification of methods was related to (*a*) the type of information that different methods provide for various types of human disturbances; (*b*) variation of priorities among freshwater ecologists; (*c*) the spatial and temporal complexity of freshwater systems, which required the adaptation of existing methods to different areas; and (*d*) differences in the required precision for types of impact assessment (96, 120). Finally, developments in aquatic insect biomonitoring were often fostered by new regulatory laws and mandates (e.g., U.S. Clean Water Act, the Canadian Protection Act, or the European Water Framework Directive) (96).

In this review, we use a consistent, comparative analysis of various approaches that have been recently developed in different parts of the world. Given that the biomonitoring of running waters is the most advanced of current freshwater programs, we focus on the biomonitoring of rivers and streams and on the insects and other invertebrates that occur in these habitats.

OBJECTIVES AND CRITERIA USED IN THE COMPARATIVE ANALYSIS OF BIOMONITORING APPROACHES

In 1993, Rosenberg & Resh (120) summarized developments in freshwater biomonitoring using aquatic insects and other invertebrates. We examine the developments spanning the subsequent decade, which has been a period of rapid diversification of methods and approaches. Here, we introduce the criteria used in our comparative analysis and illustrate their application using the century-old Saprobian system as an example, which is perhaps the best-known freshwater biomonitoring tool.

That the ideal biomonitoring tool would be "a freeze-dried, talking fish on a stick" (25, p. 8) is an often-repeated description of the holy grail of biomonitoring. Although aquatic invertebrates are far from this ideal, they do offer distinct advantages for biomonitoring (12, 62, 99, 120), including (*a*) their ubiquitous occurrence; (*b*) their huge species richness, which offers a spectrum of environmental responses; (*c*) their basic sedentary nature, which facilitates spatial analysis of pollution effects; (*d*) the propensity of certain species to enter the water column (i.e., drift), which may indicate the presence of a pollutant; (*e*) the long life cycles of some species, which can be used to trace pollution effects over longer periods; (*f*) their compatibility with inexpensive sampling equipment; (*g*) the well-described taxonomy for genera and families; (*h*) the sensitivities of many common species, which have been established for different types of pollution; and (*i*) the suitability of many species for experimental studies of pollution effects. Although these advantages do not apply to many developing countries of the world (112), aquatic invertebrates are undoubtedly the most widely used organisms in freshwater biomonitoring.

The "Ideal" Biomonitoring Tool Using Aquatic Invertebrates

Depending on the organisms used and the purpose of the monitoring, an ideal biomonitoring tool would have different criteria. For example, microbial indicators of public health threats from sewage contamination would differ from those of overall environmental integrity (5). For aquatic invertebrates, we assembled 12 criteria (I–XII) that an ideal biomonitoring tool should meet (Table 1). These criteria fall into three general categories: rationale, implementation, and performance. We deliberately chose stringent criteria (from References 49, 87, 96, 98) for

TABLE 1 Twelve criteria for defining an ideal biomonitoring tool used in the comparative analysis of biomonitoring approaches relying on aquatic insects and other invertebrates[a]

Rationale
(I) Derived from sound theoretical concepts in ecology
(II) A priori predictive
(III) Potential to assess ecological functions
(IV) Potential to discriminate overall human impact (i.e., to identify anthropogenic disturbance)
(V) Potential to discriminate different types of human impact (i.e., to identify specific types of anthropogenic disturbance)
Implementation
(VI) Low costs for sampling and sorting (field approaches) or for standardized experimentation (laboratory approaches)
(VII) Simple sampling protocol
(VIII) Low cost for taxa identifications (no specialists in taxonomy required)
Performance
(IX) Large-scale applicability (across ecoregions or biogeographic provinces)
(X) Reliable indication of changes in overall human impact
(XI) Reliable indication of changes in different types of human impact
(XII) Human impact indication on linear scale

[a]Modified after References 49, 87, 96, 98.

this comparison, but ones that would be attainable by an ideal biomonitoring tool (although in practice this may be difficult). In this context, these criteria were often not addressed by the proponents of the various approaches analyzed by us. In our assessments, we did not prioritize these criteria in terms of their importance, although in practice they do differ in this regard (e.g., the ability to indicate changes in human impact is more important than low cost for sampling and sorting). To illustrate how we use these criteria in our comparative analysis, we discuss them using the Saprobian system as an example. The Saprobian system is a biotic index that has been used in Germany and other European countries since 1902 and has been modified many times even into the last decade (119, 124a). It is the progenitor of other biotic indices, which were developed before the 1990s (see Reference 120 for reviews of these less recent approaches).

RATIONALE OF THE IDEAL BIOMONITORING TOOL Testing predictions (or hypotheses) that are a priori derived from a sound conceptual framework is a well-established practice in theoretical ecology and has been the key to progress made in this discipline. Correspondingly, applied ecology could progress faster if biomonitoring tools were derived from sound and, if possible, robust theoretical concepts enabling a priori predictions (I, II). For example, the intermediate disturbance hypothesis predicts the response of species richness patterns along a gradient of natural disturbance varying in intensity or frequency (35); therefore, this concept provides a valid framework for predictions of richness patterns (e.g., a hump-shaped response curve) along gradients of human disturbances.

An ideal biomonitoring tool also should have the potential to assess ecological functions (III), because the debate about the relationship among biodiversity, ecological functions, and conservation and restoration management (92, 144) has caused reallocations of environmental budgets to enhance ecological functions (37, 128). It is obvious that a biomonitoring tool should have the potential to discriminate overall human impact (IV). However, its potential to discriminate different types of human impacts (V) is also important because managers typically have multiple restoration options and need support regarding decisions about the investment for a particular restoration measure (96, 131).

IMPLEMENTATION OF THE IDEAL BIOMONITORING TOOL The multiplicity of sampling devices and approaches to collect aquatic invertebrates and the cost of sorting procedures have been discussed extensively (28). Thus, in field approaches that use benthic macroinvertebrates, low costs for sampling and sorting (VI) are of critical importance; cost is also an important consideration for laboratory experiments. In addition, biomonitoring data must often be collected over the long term (sometimes over decades) by numerous investigators, and operator inconsistencies are a problem in long-term surveys (28). Thus, a tool that could use a simple sampling protocol (i.e., least as possible standardized techniques, such as 10 min of random kick sampling, collected once at any time of the year with a 0.5-mm mesh-sized net) would be advantageous for practical reasons in routine biomonitoring programs (VII).

Finally, the ideal biomonitoring tool should operate at low costs for taxon identifications (VIII). Because trained specialists in aquatic invertebrate taxonomy are rarely available for identifications in routine biomonitoring, most investigators gain experience through on-the-job training. Different levels of taxonomy have been used in aquatic invertebrate biomonitoring (family, genus, or species), a fact that has been widely discussed because costs and the precision of the information provided differ among taxonomic levels (10, 84, 95). Typically, the use of a particular taxonomical level depends on the purpose of a study, the budget provided, the study area, and its taxon richness (84).

PERFORMANCE OF THE IDEAL BIOMONITORING TOOL Current budgets for environmental management are used to execute policy and legislation across large geographic units (37, 96, 101). To support this practice, large organizations, such as the European Union, foster research on biomonitoring tools that should enable better assessment of freshwater systems at the European scale (37). Correspondingly, the ideal biomonitoring tool should be applicable across large spatial scales (e.g., across ecoregions) with as few regional adaptations as possible (IX).

Furthermore, current budgets for the environmental management of freshwater systems are huge and likely to increase. In Europe, about 80% of the total environmental European Union budget is for water-related expenditures (128). Therefore, it is particularly important that biomonitoring tools reliably indicate changes of overall and specific types of human impact (X, XI) without interference by natural variability patterns in control conditions (96).

Finally, the ideal biomonitoring tool should straightforwardly indicate human impact on a linear scale (XII). If costs for management measures and ecological improvements of fresh waters are not linearly related, major waste in investments in freshwater management can occur (131).

The Case of the Saprobian System

The Saprobian system is used primarily to indicate oxygen deficits caused by biologically decomposable, organic pollution in running waters. For example, the German Deutsches Institut für Normung-Saprobian system uses Saprobic values of aquatic invertebrates (mainly insect species) that score between 1.0 at the cleanest side (e.g., the stonefly *Diura bicaudata*) and 4.0 at the most polluted side (e.g., the rat-tailed maggots of the Eristalinae, Syrphidae). Depending on the specificity of a particular taxon for its Saprobic value, these scores are then weighted on a scale from 1 (no indicator value) to 16 (very good indicator value) (124).

RATIONALE The Saprobian system is based on sound ecological theory (I) because it is derived from the niche concept [originating from ideas published in the 1800s; (133)], using niche optimum (Saprobic value) and niche breadth (through the weighting) of one physiological niche dimension (oxygen requirements) to indicate organic pollution. However, the response curve of an indicator taxon along one dimension of its niche is not a priori predictable (II), i.e., the system was derived from observations on the physiological tolerance for oxygen stress; thus, for example, the occurrence of red-tailed maggots under sewage outfalls was observed and not predicted by the niche concept. Focused on the oxygen requirements of macroinvertebrates, the system does not have the potential to assess ecological functions or overall or specific types of human impact (III, IV, and V).

IMPLEMENTATION The Saprobic values of the indicator taxa are typically weighted with their quantitative or semiquantitative abundances, which should be assessed with complicated sampling protocols (17, 63). Thus, costs for sampling and sorting are high, and highly standardized field sampling [e.g., sampling a site addressing the relative abundance of 19 microhabitat types (63)] is required (VI, VII). Finally, the Saprobian index is typically assessed using species-level identifications (VIII) because higher identification levels (genus or family) decrease the indicative power of the approach (63, 124).

PERFORMANCE The Saprobian system is not applicable across large geographic areas and different impact types and is often not consistent in indicating specific forms of organic pollution (IX, X, and XI). For example, because the Saprobian system relies on only one niche dimension (oxygen) of the indicator taxa, other niche dimensions may interfere with the tool's ability to indicate organic pollution. Because temperature and flow conditions interfere with the oxygen requirements of aquatic invertebrates (73), the Saprobian index per se increases significantly with temperature and decreases significantly with flow conditions for a given level of organic pollution (136), i.e., the index is biased toward the clean end of the indicator scale for northern high-mountain streams (cool, rapid flow) compared with southern lowland streams (warm, slow flow). The need for species-level identifications (see above) is a further limit for the large-scale applicability of the approach, as macroinvertebrate identification keys to species are not consistently available across Europe. Thus, it is too difficult to apply one Saprobian system across Europe (122), and different systems adapted to the conditions of smaller European regions (typically a country) are used instead (63, 119). However, even if the system applied to smaller European regions, the interferences of temperature and flow conditions persist.

The Saprobian system does not indicate impact on a linear scale (XII). For example, the oxygen consumption of the macroinvertebrate indicator species decreases by five orders of magnitude with their Saprobic value (136), i.e., an invertebrate indicating the cleanest condition consumes ~1000 times more oxygen than an invertebrate indicating the worst possible organic pollution. Unfortunately, the cost of wastewater treatment increases by three orders of magnitude with the efficiency of the wastewater treatment, i.e., the reduction of the final 5 mg of biologically decomposable compounds in a wastewater treatment plant is ~35 times more expensive than the reduction of the first 5 mg (136). As a consequence, it is inexpensive to improve the Saprobian index through restoration in highly polluted rivers, whereas it is more expensive in moderately polluted ones. Therefore, modest investments in sewage treatments achieved obvious environmental improvement (as indicated by the Saprobian index before 1980), but further capital expenditure of approximately US$ 88 billion for wastewater treatment over the last decade of the former Federal Republic of Germany resulted in almost no environmental improvements, as indicated by the Saprobian index (131). This example underlines the importance of the goal that an ideal biomonitoring tool should indicate human impacts on a linear scale. In summary, the Saprobian system meets only 1 of our 12 criteria.

COMPARATIVE ANALYSIS OF RECENT BIOMONITORING APPROACHES

Biomonitoring approaches using aquatic invertebrates address different organizational levels (suborganismal, organismal, population, community, and ecosystem). Patterns observed at one level may be caused by changing patterns at other levels, and the understanding of ecological consequences of a human disturbance for an entire freshwater system typically requires the understanding of the underlying processes involved (6). Although community-level approaches are currently the most widely used, other organizational levels are being developed because different levels provide complementary information about disturbance effects on entire freshwater systems (1, 6, 55).

A solid knowledge of unaffected control conditions is essential in biomonitoring. Control conditions can be defined more easily in laboratory approaches than in field approaches. For the latter, "the reference condition approach" was developed (11, 116), which compares test sites with "the condition that is representative of a group of minimally disturbed sites organized by selected physical, chemical, and biological characteristics" (116, p. 834). Thus, several reference sites act as replicates to assess natural variability, enabling a more reliable biomonitoring of human impact on other sites. However, it can be difficult to select reference sites (118). Expert opinion is often used for reference site selection, which usually is insufficient for large and highly variable regions (19). In addition, reference sites are not always available (e.g., for industrial areas), which constrains the reliability of pollution indication (19, 100).

Using our 12 criteria (Table 1), we evaluate several recently developed or expanded biomonitoring approaches that cover five levels of organization: (*a*) biomarkers at the suborganismal level; (*b*) bioassays at the organismal level; (*c*) fluctuating asymmetry at the population level; (*d*) multimetric and multivariate approaches, functional feeding groups, and multiple biological traits at the community level; and (*e*) benthic secondary production and leaf-litter decay at the ecosystem level.

Biomarkers

Biomarkers are biochemical measures indicating sublethal responses to toxicants at the molecular, cellular, or tissue level (71, 83). For example, heavy metals in sediments increase hsp70, a stress protein that alters lysosomal membrane stability (148). Widely used with fish (1), new biomarkers are becoming increasingly available for use with aquatic invertebrates, such as mixed-function oxidases, acetylcholinesterase, cellulase/carbohydrase, genotoxicity, ion regulation, stress proteins, or oxidative stress responses (71, 75, 86, 94). However, because of compensatory mechanisms of organisms, biomarker responses do not automatically imply responses at higher organizational levels, such as communities or ecosystems (46, 71).

RATIONALE Biomarkers have been developed on the basis of correlations and experimental tests and are thus not a priori predictive using theoretical concepts (I, II). They currently cannot indicate ecological functions (III). Although few biomarkers have a broader sensitivity [e.g., stress proteins (75), oxidative stress responses (86)], they usually have strong specificity for one or a few stressors (71). Therefore, single biomarkers have a relatively low potential to detect overall human impact (IV). However, this strong specificity does allow detection of specific stressor-effect relationships and thus the discrimination among different types of human impact (V) (71).

IMPLEMENTATION Sampling costs are low and collecting test invertebrates requires no standardized sampling (VI, VII). However, costs for laboratory analyses are currently relatively high (24); the number of biomarkers to be selected and validated depends on the available ecological information of the test site (VI) (2). Identification costs vary, depending on whether field-collected (148) or laboratory-cultured (75) invertebrates are used, but overall, identification costs should be relatively low (VIII).

PERFORMANCE Laboratory assessments of biomarker responses at different toxic concentrations should be possible across ecoregions, but this topic has been insufficiently studied (IX). However, if biomarker responses are measured on field-collected invertebrates, these responses may depend on natural environmental factors, and genetic differences among populations may limit the large-scale applicability of the approach. For example, the synthesis of stress proteins increased in the midge *Chironomus tentans* with increasing temperature (75). Intraspecific variability of biomarkers can be high (46), which raises uncertainties about the ability of biomarkers to reliably indicate stressors. Because few studies have analyzed how biomarkers respond to abiotic [e.g., seasonality (75)] or biological [e.g., developmental stage or sex (36)] factors, the reliability of biomarker indication is currently unknown (X, XI). When clear biomarker responses have been found, linear and nonlinear relationships were observed between pollutant concentration and the biochemical parameter measured (XII) (43, 56). In summary, biomarkers at least partially meet 6 of our 12 criteria.

Bioassays

Bioassays, or toxicity tests, assess the effect of a toxicant on an organism's survival, growth, feeding rate, pupation, emergence, behavior, morphology, or reproduction (23). They often provide a dose-dependent response to acute (short-term tests) or chronic (long-term tests) exposures. Researchers employ different bioassay approaches depending on what is being tested (e.g., water toxicity, sediment toxicity, bioaccumulation), what kinds of aquatic invertebrates are used (e.g., indigenous species, standard test species), how many species are used, and where the test is conducted (e.g., laboratory, field). Currently, in situ responses of field-exposed test organisms combined with higher organizational level measures (e.g., communities) are a major emphasis (32, 90). Bioassays are now included even in some multimetric approaches to provide a broader view of cause-effect relationships at several organizational levels in risk assessments (54).

RATIONALE The use of bioassays is derived from the niche concept (I), because bioassays assess how toxicants displace aquatic invertebrates to suboptimal physiological niche conditions or displace them from their physiological niche. Although the response curves to toxic exposure of different invertebrate taxa are not a priori predictable (they are established through observations) (II), predictions about the direction of the responses are possible (e.g., increasing concentrations of a toxicant should slow growth). Assessments of ecological functions using bioassay responses are unlikely (III) because extrapolations from the organism to higher hierarchical levels are difficult, especially from single-species laboratory tests (78). In some cases, however, bioassays can provide insights into ecological functions, such as a decreasing feeding rate, which could affect a system's ability to process detritus (90). Bioassay approaches appear to be sensitive to a range of toxicants and thus may be an appropriate indicator of overall toxic pollution (IV) (38, 89). However, bioassays have not been used to indicate overall nontoxic impairment. Researchers could discriminate among different types of pollution if a battery of different species were included in the bioassays, because sensitivity to a given pollution type is taxon dependent (V) (64).

IMPLEMENTATION Bioassays are similar to biomarkers (139) because they also require low costs for sampling, need no standardized sampling, and have variable experimental costs, which depend on the test design (in the laboratory or in the field; with organisms from standard cultures or from field sites) and the knowledge of the aquatic invertebrates' sensitivity to a toxicant (VI, VII). The latter is especially important because sensitivity depends on the type of response measured and the taxon used (e.g., behavioral responses can be more sensitive than other measures) (54). Identification costs are similarly low as in biomarker studies (VIII).

PERFORMANCE Large-scale applicability and the ability to reliably indicate different human impacts depend on how the bioassay is conducted (IX), whereas the ability to indicate overall impact is not known because nontoxic impairment has not been tested (X). For laboratory bioassays, standards exist (http://www.astm.org/) that enable bioassays to be used across ecoregions, and these standards generally provide reliable indications of different types of human impacts (IX, XI) (23). However, large-scale applicability and reliable indication of specific human impact are limited when bioassays are carried out in situ because invertebrate responses may depend on other environmental factors, such as intrinsic invertebrate properties (e.g., life cycle) or extrinsic physical, chemical, and biological factors (e.g., temperature, conductivity, other taxa) (IX, XI) (23, 89). Finally, relationships between bioassay responses and toxic concentrations are often linear (XII) (9). In summary, bioassays at least partially meet 10 of our 12 criteria.

Fluctuating Asymmetry

Environmental disturbance during the development of a bilateral morphological trait may cause small, random alterations in perfect symmetry, which can be assessed with fluctuating asymmetry measurements (107). Fluctuating asymmetry of aquatic invertebrates, using Ephemeroptera (47), Odonata (59), Hemiptera (50), Diptera (57), and Trichoptera (20), has shown potential as a biomonitoring tool of human impact.

RATIONALE Fluctuating asymmetry has a theoretical base (developmental instability concept) (107) (I) that enables a priori predictions on the direction of the response (i.e., fluctuating asymmetry increases with human impact) but not on the exact response curve (II). Focused on morphological characteristics, fluctuating asymmetry cannot assess ecological functions (III). Fluctuating asymmetry is viewed to be related primarily to overall human impact (IV) (33), but the approach does not allow for the discrimination among different impact types (V). However, the latter could perhaps be accomplished if different species or body features have different response curves under different human impacts.

IMPLEMENTATION Low costs for sampling and sorting (requiring no standardization), taxon identifications, and measurements are characteristic (VI, VII, and VIII) (33), because species that are typically abundant and well-known are used, collected in the field, and measured with simple equipment. Higher costs associated with this method (VI) are caused primarily by the search for the most suitable fluctuating asymmetry characters and the need for relatively large sample sizes (20, 33).

PERFORMANCE It is currently unknown whether fluctuating asymmetry can be applied across ecoregions (IX). Although not yet demonstrated, fluctuating asymmetry may vary across species at similar environmental conditions, implying that its large-scale applicability would be limited. Fluctuating asymmetry should be an unreliable indicator of overall or specific human impact (X, XI) for several reasons. First, environmental stress affecting invertebrate development may be caused by human impact but also by natural variables [e.g., temperature (123)], including biological interactions (3). Second, fluctuating asymmetry patterns may be influenced by genetic heterozygosity levels that vary among populations (67). Third, many studies on aquatic invertebrates (see above) have indicated that there are still contradictions about the suitability of fluctuating asymmetry for freshwater biomonitoring. It is currently unknown whether relationships between fluctuating asymmetry and human impact are linear (XII). In summary, fluctuating asymmetry at least partially meets 7 of our 12 criteria.

Multimetric Approaches

Metrics are calculated measures that represent some aspects of the structure, function, or other characteristics of the biological assemblage, and these metrics change with increased human impact (13, 14). Multimetric indices are combinations of individual metrics that, together, are presumed to represent a range of assemblage responses to human impact (14, 113). As such, multimetric indices are akin to economic indices (e.g., the index of leading economic indicators) that combine various financial measures to assess the state of an economy (76).

Multimetric approaches for benthic macroinvertebrates are the most widely used approach for water-quality assessments among the aquatic resource agencies in the United States (14) and recently have been used in other parts of the world as well (126, 141, 146). The multimetric approach involves two stages. The first stage is selection and calibration of metrics and subsequent aggregation of these metrics into a combination index that is applicable to homogeneous sites (12). The second stage is assessment of the biological condition at a particular site, which involves determination of threshold levels of impairment (12).

RATIONALE Some metrics are based on sound concepts of ecology and are a priori predictive (I, II). For example, functional feeding groups measures are derived from the river continuum concept (145) (see below). In contrast, most structural metrics are based on generalizations drawn from empirical observations (e.g., a decrease in the proportion of taxa and individuals in the generally pollution-intolerant orders Ephemeroptera, Plecoptera, and Trichoptera, typically not taking into account taxa identity) rather than from sound theoretical concepts and are not a priori predictive (I, II). For example, multimetric approaches include taxon richness as a metric, but the intermediate disturbance hypothesis (35) is not considered in the predictions (e.g., richness is always presumed to decrease with human disturbance). Except for functional feeding groups (and other, yet unused functional traits that could be incorporated into a multimetric index; see below), the structural metrics do not have the potential to assess ecological functions (III). It is generally acknowledged that multimetric indices do have the potential to discriminate overall human impact

(IV) (146). Finally, multimetric approaches, and certainly the use of individual metrics (63, 93, 114), have the potential to discriminate among different kinds of human impact (V).

IMPLEMENTATION Multimetric approaches purport to have low costs for sampling and sorting (VI), at least with most recommended protocols (12). However, if replicates are taken and laboratory sorting instead of field sorting is done, costs increase (28). Highly standardized sampling is required for some multimetric indices (VII) (e.g., AQEM system, http://www.aqem.de/), whereas others have simpler sampling protocols [e.g., all habitats or specific habitats (28)]. Costs for taxonomic identifications reflect hierarchical levels used (VIII); sometimes family level but mainly genus level is used (28).

PERFORMANCE Multimetric approaches and their use of homogeneous site groupings do not have large-scale applicability across ecoregions (IX) (117, 146). The case histories described by the proponents of this method (12) have suggested that they are reliable indicators of changes in overall human impact (X), although the natural variability inherent in many metrics (110) or regional variation in the indication reliability among multimetric indices (51) may limit their spatial and temporal applicability (114). However, when applied to several river types that were affected by different human impacts, different individual metrics reliably responded to specific human impacts on each river type (XI) (63). Finally, with the multimetric approach, human impact indications are not on a linear scale because metrics tend to have threshold responses (XII) (4, 39). In summary, multimetric approaches at least partially meet 10 of our 12 criteria.

Multivariate Approaches

Similar to the multimetric approaches, multivariate approaches assess human impact using comparisons between patterns observed at a test site and patterns expected in the absence of human impact (the reference condition approach, see above). In contrast to multimetric approaches, multivariate approaches rely on statistical analyses to predict the expected patterns (e.g., typically taxon identity and thus taxon richness or biotic indices) instead of using the range of patterns observed at reference sites (96, 98). The River InVertebrate Prediction And Classification System [RIVPACS (149)], the AUStralian RIVer Assessment Scheme [AUSRIVAS (125)], the BEnthic Assessment SedimenT [BEAST (115, 121)], or the recent Assessment by Nearest Neighbor Analysis [ANNA (85)] are widely used multivariate biomonitoring approaches. Neural networks are also a multivariate approach (104, 147) but are insufficiently developed to be included in this analysis.

RATIONALE Multivariate approaches are based on predictive, correlative models that compare communities observed in test sites (which may be impaired) with communities expected in these sites in the absence of human impacts (149). Models are built using reference sites that are classified in groups or ordinated [using nonmetric multidimensional scaling (85)] according to similarities in invertebrate community composition and then correlated with several natural environmental variables. Thus, depending on correlations between invertebrate composition and multiple site characteristics, these approaches are conceptually based on the niche concept (i.e., predicting taxa occurrence from environmental conditions; I). They are typically not a priori predictive (II), rather they are a posteriori predictive. However, a priori predictions occasionally address the intermediate disturbance hypothesis (35), because an observed taxon richness that is greater than the expected one could indicate human impact (II) (125). Multivariate approaches are designed to assess changes in overall community composition caused by human impact and are currently not assessing ecological functions (III), although the latter option could be developed (114). These approaches are considered to have a high potential to discriminate overall human impact (IV) (121, 149), but they were not designed to discriminate different types of human impact (V), although it could be an option if indices detecting specific stress are incorporated (149).

IMPLEMENTATION Multivariate approaches have relatively low costs for sampling and sorting (VI) because field sampling uses kicknets for a fixed time or space and typically only subsamples are sorted following standardized sorting protocols (125, 149). Sampling is moderately standardized in all methods (VII) and includes either diverse microhabitats or only well-defined habitat types (102). The identification level varies from species to families among the approaches used (VIII) (60). The optimization between multivariate model precision and costs for sampling, sorting, and taxon identifications depends on (*a*) the environmental characteristics of a study region (102), (*b*) the species richness of invertebrate families in a study area (60), and (*c*) the possible exclusion of taxa that have high identification costs (e.g., midges of the Chironomidae) (60).

PERFORMANCE Multivariate approaches rely on homogeneous reference sites with similar biota that differ among regions. Therefore, multivariate models have no large-scale applicability (IX) because they have to be adapted to individual regions [e.g., various regions or stream types in the United Kingdom (149)]. The reliability of overall human impact indication (i.e., misclassification in a wrong quality class) using multivariate approaches varies across a range from 0 to 66% (X) (34, 125). This variation is caused by (*a*) inadequate modeling of the expected values because interfering environmental variables were not included (34); (*b*) insufficient replication of test sites, which obscures whether deviations from reference conditions result from natural variability or from real human impact (68); and (*c*) unavailability of a sufficient number of reference sites (34). Whether the multivariate approaches can reliably indicate different types of human impact is currently unknown (XI). The linear-scale indication of multivariate approaches (XII) has been found for observed versus expected ratios of taxon richness and pollutants (91, 127). However, as in multimetric approaches, nonlinear relationships were also found if species had a tolerance threshold when faced with increasing pollution (4, 39). In summary, multivariate approaches at least partially meet 9 of our 12 criteria.

Functional Feeding Groups

Assignment of aquatic invertebrates to functional feeding groups indicates the type of food source used and the feeding mechanism involved (41). In freshwater biomonitoring, single feeding groups (as absolute or relative abundance), ratios between two groups, or an index that includes several trophic aspects [e.g., the index of trophic completeness (106)] has been used. Functional feeding groups have also been combined with other metrics (see Multimetric Approaches, above, and Multiple Biological Traits, below).

RATIONALE This approach is based on the river continuum concept (I) (145), which predicts feeding group changes along river systems in relation to food availability. For example, elevated relative abundances of leaf-consuming shredders in headwaters, grazers in intermediate river sites, and collectors in lower sites should result from downstream gradients of leaf inputs from the riparian vegetation, solar radiation reaching the stream bottom, and particle sizes of organic matter. Thus, effects of human impacts on invertebrate food availability are a priori predictable from the river continuum concept (II), and obviously the approach has the potential to assess ecological functions (III). This approach is not designed to discriminate overall human impact (IV), but rather impact types that have effects on invertebrate food availability (V).

IMPLEMENTATION Functional feeding group measures have low costs for sampling and sorting (VI) because rapid bioassessment protocols can be applied (12). Sampling is not standardized (VII) and different sampling methods can be used (106, 109), but it is currently unknown how variation in sampling affects these measures. The taxonomic level used to assign aquatic invertebrates to functional feeding groups varies from species to family (VIII), but lower taxonomic levels should preferably be used (111). Major constraints are associated with the feeding group assignments of the aquatic invertebrates. The proper feeding group assignment can be difficult if larvae shift their diet among instars (7). Such assignments should be obtained by analyzing mouthpart morphology (41), but typically they are obtained from the literature and these are usually based on gut contents (111).

PERFORMANCE Although it is difficult to apply functional feeding group assignments from one area to another (79), a proper assignment across regions would enable the application of the approach across ecoregions (IX). However, reliable large-scale applicability depends on the metric used. Functional feeding group composition may naturally vary across ecoregions (61), but the index of trophic completeness appears to be independent of distance from source, geographical characteristics, or seasonality (18). Reliability for discriminating overall human impact should be limited because this approach indicates only human impacts that change food availability (see above) (X). But, for example, the approach did not indicate overall impairment in a South African river despite its poor chemical water quality caused by industrial and urban pollution (103). A review of multimetric approaches developed in the 1990s concluded that responses of functional feeding groups of aquatic invertebrates were generally poor indicators of human disturbances of lotic systems because aquatic invertebrates probably do not always feed according to their assumed group (76). Concerning the reliable discrimination of different impact types using the approach, we found no support for the index of trophic completeness (18) and only weak support for single feeding groups [e.g., scrapers discriminate acidification from other impact types (109)] (XI). Likewise, the prevalence of generalist feeders among aquatic invertebrates suggests that caution is needed in using the approach when assessing changes in system-level trophic dynamics caused by dams (29). Finally, analyses of linear-scale indications using this approach are insufficient to evaluate this criterion (XII), but metrics based on functional feeding groups may respond nonlinearly across pollution gradients (97). In summary, functional feeding groups at least partially meet 8 of our 12 criteria.

Multiple Biological Traits

Because examination of multiple biological traits of aquatic and terrestrial organisms (e.g., size, body form, life cycle, food and feeding habits, reproductive and other traits) in the context of environmental constraints constitutes a substantial portion of theoretical population and community ecology (133), this approach has been recently developed for freshwater biomonitoring. Current research on the use of multiple biological traits of aquatic invertebrates in biomonitoring focuses on running waters of Europe and a multitude of traits that are weighted by the abundance or occurrence of the taxa. The advantage of this approach relates to its large-scale applicability (e.g., across continents) because aquatic invertebrates worldwide can be described and compared on the same scale for a given trait (illustrated for aquatic insects in Reference 134). The disadvantage of this approach is the problem of consistently describing the traits of invertebrate taxa on the same scale, because this information is currently lacking for many parts of the world.

RATIONALE The multiple trait approach meets all five rationale criteria (I–V). The use of species traits originated from a test of the habitat template concept (135), which predicts that a multitude of biological traits that confer either resistance or resilience to organisms should respond to natural environmental disturbance in rivers (I) (142). Thus, these a priori habitat template predictions on trait responses to natural disturbances of rivers (142) provide a framework for discriminating overall human impact (II, IV). Using the multi-probe idea of the approach (multiple traits may respond differently to various types of human impact), one can also a priori predict (e.g., a human-induced change in flow should affect the relative occurrence of streamlined or swimming forms) and discriminate among various types of human impact (II, V) (49). Some of the biological traits used do relate directly or indirectly to ecological functions (III) [e.g., food and feeding habits, size, and the correlated ratios of production/biomass and of production/respiration (16, 129)].

IMPLEMENTATION On the basis of the presence-absence data in the taxon-weighting of the traits, few sample replicates in space or time (e.g., across seasons or subsequent years) and identifications at the genus level (using the mean trait profile of species of each genus and excluding taxonomically difficult oligochaetes and dipteran families) reliably describe the trait composition of lotic invertebrate communities (8, 48, 52). This creates low costs for sampling and sorting (VI), requires no standardized sampling (VII) and creates relatively low costs for identifications (occurrence of easily identifiable genera instead of abundance of species) (VIII). What is further important for its implementation is that a European trait profile of multiple biological traits is now available for most of the aquatic invertebrate genera of Europe (138).

PERFORMANCE Analyses of lotic systems using the European trait profiles of invertebrate genera demonstrated that many of the invertebrate traits remain relatively stable across seminatural stream sites of France (31) and natural or seminatural regional stream types or sites of Europe (130, 132). Thus, the reference state in terms of the trait composition of lotic invertebrate communities varies little across large geographic regions, so that regional adaptations of a biomonitoring tool using this approach should be unnecessary (IX). Existing studies illustrate that the trait composition of benthic invertebrate communities reliably discriminates overall human impact (either a mixture of impact types or single, but different, impact types) (X) on the scale of a stream site (30), along rivers flowing through different ecoregions (49, 143), and across Europe (52, 130). Individual trait categories also consistently respond to a given type of human impact (e.g., sewage, regulation) (130), but the replicates of affected sites are too low to allow safe conclusions on this criterion (XI). Finally, the scale of indication has not yet been tested (XII). In summary, multiple biological traits meet 10 of our 12 ideal criteria.

Benthic Secondary Production

Secondary production, the accumulation of produced animal biomass over time (70), is a functional measure that is considered a biomonitoring approach (22). Although assessed at the population or community level, it links populations and communities to ecosystem-level processes (16).

RATIONALE If secondary production is assessed for functional feeding groups (16), the same criteria apply as mentioned above (I–V). However, if secondary production is assessed specifically, this approach addresses the niche concept (linking production to species identity and performance) and again, a priori predictions of the exact response curves cannot be made (I, II). Finally, if overall production is used, this approach is not derived from an ecological theory and it is not a priori predictive (I, II). Secondary production provides good assessments of ecological functions (III) [e.g., it generates food resources for predators (44)], especially if combined with information on trophic interactions (70). In addition, secondary production should enable assessments of overall human impact on lotic ecosystems (IV) (15, 22) and perhaps of different impact types (V) because it is assumed that secondary production is affected in different ways by the latter [e.g., increasing or decreasing overall secondary production or production by individual species (15)].

IMPLEMENTATION Costs for sampling and sorting are relatively high (VI) because (*a*) repeated (typically monthly) samples are taken typically over one year (15) and (*b*) sorting quantitative samples to obtain invertebrate densities requires more time than other methods [e.g., quantitative versus qualitative sampling (137)]. Sampling is not standardized (VII) because different studies use different quantitative sampling techniques with different mesh sizes (58, 77), but it is currently unknown how this would interfere in biomonitoring. Costs for taxon identifications are variable because secondary production has been determined for species, genera, or families (VIII) (22).

PERFORMANCE Although unconstrained by taxonomic variation, the large-scale applicability of secondary production has not been tested (IX). However, it should be limited because secondary production depends on many naturally varying factors such as alkalinity (82), temperature (74), habitat (69), or stream order (58). In the context of the high natural variability of secondary invertebrate production, there is not enough evidence to determine whether this approach reliably indicates changes in overall human impact or different impact types on linear scales (X, XI, XII). Sewage effluents (16, 45) and catchment logging (77) increase overall secondary production, whereas heavy metal pollution decreases shredder production (27), and chemical contaminants of sediments in the Rhine-Meuse decrease the production of gastropods but not of Chironomidae or Oligochaeta (44). In summary, benthic secondary production at least partially meets 6 of our 12 criteria.

Leaf-Litter Decay

Measuring in situ leaf-litter decay rates is an approach that assesses the functional integrity of lotic ecosystems (55). Because a significant percentage of the annual leaf-litter decay is caused by invertebrate activities [>25% by shredders (40)], this approach includes aquatic invertebrates.

RATIONALE The leaf-litter decay approach relates to food web theory, which predicts that human disturbances make food webs less complex and thus less efficient in the processing of organic matter (I) (66). Therefore, the direction of changes in decay rates with increasing human impacts can be a priori predicted, although the exact response curve has to be obtained from observations (II). Undoubtedly, this approach has a higher potential to assess ecological functions than do the previously reviewed approaches (III) because it integrates different aspects of lotic ecosystems [i.e., abiotic processes and activities of microorganisms and invertebrates (65, 88)]. With two response possibilities (faster or slower decay rates), the approach may indicate overall human impact, but a priori should be limited in the indication of different impact types (IV, V). The latter could be achieved with the quantification of decay rates of different leaf species available in a region (similar to the use of multiple taxa in the bioassays; see above).

IMPLEMENTATION The approach relies on leaf decay over time assessed using litterbag or litter-pack exposure in streams (21). Thus, costs for sampling and sorting are relatively low (VI) (150) but sampling requires high standardization (VII) [e.g., leaf species, mesh size of the litterbags, period of exposure, location of litterbags (150)]. Costs for taxon identifications (55) are negligible if compared with all previously reviewed approaches (VIII).

PERFORMANCE Leaf-litter decay rates can be measured across ecoregions, but natural properties can modify these rates [e.g., temperature, calcium, flow fluctuations, or presence of particular species (88, 150)], suggesting that a consistent large-scale applicability is impossible (IX), i.e., the approach has to be adapted to river types and ecoregions (55). The ability to consistently indicate overall human impact is a priori high but limited by natural variability (X) (55). Compared with reference conditions, leaf-decay rates respond differently to various human impacts, although these responses are not always consistent for a given type of human impact (XI) (55). Linear relationships between leaf-decay rates and human impact have been found (XII) (42, 105). In summary, leaf-litter decay at least partially meets 10 of our 12 criteria.

CONCLUSIONS

Illustrating the method we used in this comparative analysis, the century-old freshwater biomonitoring approach, the Saprobian system, meets only 1 of our 12 criteria of an ideal biomonitoring tool (Table 2). In comparison, however, the recently developed biomonitoring approaches using aquatic invertebrates meet these criteria much better (Table 2), and so we conclude that progress has been made in this field. However, none of these recent approaches completely meets all the criteria, and there are four potential explanations. First, some of the approaches considered here (e.g., biomarkers) are too recent and more research is needed to provide solid evidence about their performance (note the abundance of "?" for performance

TABLE 2 Summary of results of our comparative analysis of the oldest (Saprobian system) and nine more recent biomonitoring approaches that use aquatic insects and other invertebrates[a]

	Criterion number												
	Rationale					Implementation			Performance				
Biomonitoring approach	**I**	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**	**XI**	**XII**	**Σ[b]**
Saprobian system	+	−	−	−	−	−	−	−	−	−	−	−	1
Biomarkers	−	−	−	±	+	±	+	+	?	?	?	±	6
Bioassays	+	±	±	?	+	±	+	+	±	?	±	±	10
Fluctuating asymmetry	+	±	−	+	±	±	+	+	?	−	−	?	7
Multimetric approaches	±	±	±	+	+	±	±	±	−	±	+	−	10
Multivariate approaches	+	±	?	+	±	+	+	±	−	±	?	±	9
Functional feeding groups	+	+	+	−	±	+	?	±	±	−	±	?	8
Multiple biological traits	+	+	+	+	+	+	+	+	+	+	?	?	10
Benthic secondary production	±	±	+	+	±	−	?	±	?	?	?	?	6
Leaf-litter decay	+	±	+	+	±	+	−	+	−	±	±	+	10

[a]Correspondence of each approach with the criteria for an ideal biomonitoring tool (see Table 1) is indicated in terms of matches (+), mismatches (−), matches/mismatches (±), or unknown (?).

[b]Number of criteria with + or ±. Note this is a limited rating because the criteria differ in importance (see The "Ideal" Biomonitoring Tool Using Aquatic Invertebrates, above).

criteria in Table 2). Second, the criteria for our ideal biomonitoring tool are too stringent to be met completely by any practical biomonitoring approach, and it was apparent that meeting the rationale criteria was far easier than meeting the performance criteria (Table 2). However, we derived these criteria from myriad scientific, logistic, economic, and political factors that define the framework of aquatic invertebrate biomonitoring in the real world (49, 87, 96, 98). Thus, mismatches of biomonitoring approaches with these criteria suggest there may be insufficient communication between those who create biomonitoring tools (i.e., freshwater ecologists) and those who use them to allocate funds for the ecological improvement of freshwater systems (i.e., managers) or have unrealistic expectations (i.e., legislators). Third, the typical end products of aquatic invertebrate biomonitoring available to decision makers and the public are generally summary scores or even maps defined by colors indicating levels of "ecological integrity" (e.g., blue is the best, red the worst). The need to reduce the diverse information that aquatic invertebrate biomonitoring can provide to such simple categories is frustrating for those developing biomonitoring approaches, which perhaps reduces the ambition to develop an ideal biomonitoring tool. Finally, aquatic invertebrate biomonitoring research is very competitive, as the implementation of a new tool in routine biomonitoring provides numerous advantages for the research group(s) that developed it. This favors the formation of "schools" or "camps" and the resulting rigid stances that ultimately hamper scientific progress.

In conclusion, the financial consequences of how information from aquatic invertebrate biomonitoring is used in setting restoration goals are enormous. Societies and governments should prioritize which groups of criteria or which individual criterion that we propose (Tables 1 and 2) should be given the highest ranking in deciding whether to continue with existing approaches, modify them, or develop new ones. As seen with the Saprobian system, a long history of use is not a sufficient reason to continue with a biomonitoring tool that is far from ideal.

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Contents

Errata

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