



*Review*

## **Assemblage-based biomonitoring of freshwater ecosystem health via multimetric indices: A critical review and suggestions for improving their applicability**

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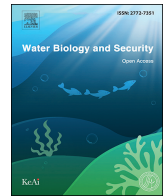
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# Assemblage-based biomonitoring of freshwater ecosystem health via multimetric indices: A critical review and suggestions for improving their applicability

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## ABSTRACT

Freshwater biota are more comprehensive and direct indicators of biological impacts, and more meaningful to the public than water quality or physical habitat surrogates. Freshwater biotic data and the multiple biological indicators developed from them offer a much richer array of data for assessing the impacts of pollution controls than a limited set of physical or chemical measures. In recent decades, assemblage-based assessments by ecologists, environmental scientists, and water quality agencies have been employed globally for determining the condition of, and threats to, freshwater ecosystems. A key step in this advance has been the development of multimetric indices (MMIs) or indices of biotic integrity (IBIs) based on quantitative assessments of algae, macrophyte, macroinvertebrate, fish or riparian bird assemblages. In Europe, where biological assemblages are mandated for assessing freshwater ecosystem health, many indices are multimetric. However, the proliferation of MMIs globally has not always occurred through the application of rigorous study designs and monitoring protocols, nor have they always effectively incorporated functional metrics, stressor assessments, and statistical analyses. Therefore, in this review, we discuss eleven major concerns with the development and application (including logistical limitations) of multimetric indicators based on freshwater biota to encourage more rigorous and widely applicable (transferable) MMI use and implementation. Specifically, our concerns focus on reference conditions; sampling effort, methods, and season; trophic guild definition; metric comprehensiveness, options, screening and scoring; and MMI validation. MMIs could also benefit from increased attention to ecological mechanisms and metric development, to further improve our understanding of anthropogenic impacts as well as rehabilitation effects on freshwater ecosystems globally. Paying closer attention to study designs, ecological mechanisms and metric development should further improve our understanding of anthropogenic impacts and better facilitate rehabilitation of degraded freshwater ecosystems, as well as aiding in the conservation of healthy freshwater ecosystems globally.

## 1. Introduction

Advances in the biotic assessment of freshwater ecosystems can better address the cumulative and synergistic impacts of water pollution, hydrological alteration, and physical habitat degradation in these systems, beyond what physicochemical (abiotic) sampling alone can provide (Karr, 1981; Karr and Chu, 1999; Karr et al., 2022; Suter, 1993; Yoder and Rankin, 1998). Traditionally, government agencies focus on measuring parameters such as dissolved oxygen, nutrients, chlorophyll, or fish abundance while ignoring their natural regional differences (Larsen et al., 1988; Rohm et al., 1987; Whittier et al., 1988). What constitutes good habitat structure and water quality varies by region (Kaufmann et al., 2022a) and season (Fierro et al., 2021). Freshwater biota are more sensitive and more directly detect biological effects than traditional physicochemical water quality parameters, flow regime, or physical habitat surrogates (Kaufmann et al., 2022b). Nonetheless, it is useful for freshwater impacts to be assessed with a combination of such data and biotic abundance, biodiversity, and biotic-index approaches (Karr and Dudley, 1981; Rankin, 1995; Storch et al., 2022; Suter et al., 1995; USEPA, 2016a, 2016b, 2016c, 2020; Vadas, 1992c, 1997, 1998, 2000). Some biotic parameters, physical habitat structure, and water quality measures are naturally highly variable, and this limits our ability to assess human and natural impacts on freshwater biota using those parameters alone (Allan, 1984; Landres et al., 1988; Suter et al., 1995; Vadas, 1998). Similarly, population abundances and physiological conditions vary naturally with habitat, season, life cycle, epigenetics, and the ages of individual organisms (Hynes, 1970; Khatun et al., 2020; Schreck, 2010; Stevenson et al., 2013; Vadas, 1991, 1992b; Warren, 1971). Rigorously developed and diverse biological indicators based on clearly defined concepts can help us understand the sources of biological variability. In addition, ecologically comprehensive multimetric indices (MMIs) can reduce indicator variability. That is, they act similarly to diversified investment portfolios, which help protect investors from the variability of single assets. MMIs also behave like species-rich fish assemblages and life-history rich fish populations that respectively limit assemblage and species variability (Karr et al., 1986; Schindler et al., 2010). Therefore, Karr (1981), Davies and Jackson (2006) and Hughes and Noss (1992) argued that biological condition is best assessed by evaluating multiple structural and functional metrics.

Hence, MMIs, multivariate analyses, and observed/expected (O/E) taxa richness have become important assemblage-based approaches to

enhance bioassessment sensitivity. This is a shift from the narrow, abiotic, single-factor focus that (1) characterized historical freshwater impact assessments and (2) largely ignored the breadth of freshwater ecosystem stressors (Karr, 1981; Karr and Dudley, 1981; Karr and Chu, 1999; Ruaro et al., 2020; Yoder, 1995; Yoder and Rankin, 1998, Fig. 1). We focus this paper on MMIs because of their global popularity (Ruaro and Gubiani, 2013; Ruaro et al., 2020), and have reserved comparisons with other approaches to the Discussion. MMIs are useful for determining both temporal and spatial trends, and for identifying which remediation actions have provided beneficial biotic effects (Moncayo-Estrada et al., 2012; Pyron et al., 2008; Stainbrook et al., 2006; Yoder et al., 2005, 2019). Such information can save societies money by relating costs to benefits, prioritizing ecosystems at greatest biological risk, and providing warnings of impending ecosystem collapse (Callisto et al., 2019; Diaz et al., 2019; Hughes et al., 2021b; Martins et al., 2021c).

However, Suter (1993) argued that MMIs 1) lack grounding in ecological theory, 2) have no meaning because scores may result from one low-scoring metric or multiple moderately scoring metrics, 3) are unpredictable and inapplicable to regulatory problems, 4) lack diagnostic power, 5) effects on one metric may be eclipsed by another, and 6) reasons for various index scores are unknown. Here, we briefly counter his arguments in order. (1) MMIs contain critically important

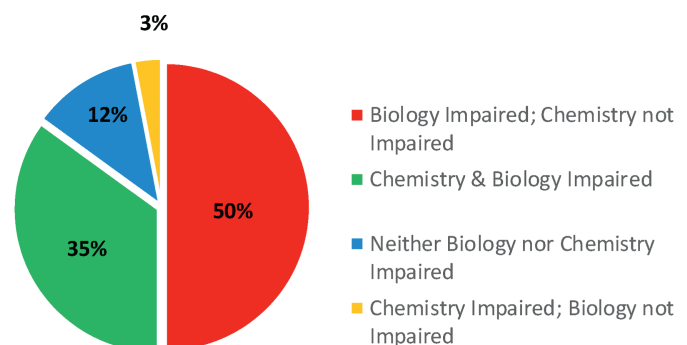


Fig. 1. Example of the disconnects between condition assessments based on water chemistry versus those based on fish and macroinvertebrate MMI scores (from Hughes and Noss, 1992). Based on four years of original data collected at 1030 sites (Ohio EPA, 1990). Results such as these led to widespread use of MMI and biological criteria in the USA (Davis and Simon, 1995).

components of biological and ecological condition (e.g., taxa richness, taxa generally sensitive to anthropogenic disturbances, taxa generally tolerant to anthropogenic disturbances, representation of multiple trophic and life history guilds or traits, anomalies) (Karr, 1981; Ruaro et al., 2020; Stoddard et al., 2008). Thus, we argue that MMIs are grounded in ecological theory. (2) MMI scores result from averaging the scores of all the metrics in the index, so a single low-scoring metric is unlikely to shift a good MMI score to a poor MMI score (Karr et al., 1986; Stoddard et al., 2008). Multiple moderately scoring metrics will necessarily yield a moderate MMI score, as intended. (3) MMI scores are somewhat unpredictable because of the many interacting pressures and stressors affecting them, and those pressures and stressors vary regionally (Herlihy et al., 2020; Martins et al., 2021a; Silva et al., 2018), but MMI scores are certainly appropriate in regulatory applications. The best examples come from Ohio EPA, where fish and macroinvertebrate MMIs have been used to inform reductions of point and diffuse source discharges for over 30 years (Yoder and Barbour, 2009; Yoder et al., 2005; Yoder et al., 2019). (4) The differing MMI metrics have diagnostic power. The percent anomalies metric (% deformities, eroded fins, lesions, tumors) is diagnostic for toxics and disease in fish (Hughes and Gammon, 1987; Mebane et al., 2003). Migratory fish metrics are a diagnostic for how instream barriers affect the number and percent of migratory fish taxa (Herlihy et al., 2020; Hughes et al., 2022). Shredder macroinvertebrate metrics are a diagnostic of the condition of riparian vegetation (Oliveira et al., 2011; Stoddard et al., 2008). Ephemeroptera (mayfly) relative abundance and clinger richness were most affected by electrical conductivity, and collector/filterer relative abundance was mostly affected by arsenic concentration (Vander Laan et al., 2013). (5) A relatively low score for one metric might lower the overall MMI score slightly, but it will not overwhelm the total MMI score. For example, an otherwise high-quality stream may be dominated by invasive non-native trout species and would, therefore not be considered as being in as good an ecological condition as a stream supporting only native fish species (Davies and Jackson, 2006; Hughes et al., 2004; Lomnický et al., 2021; Mebane et al., 2003). (6) Major reasons for poor MMI scores are known. In the Brazilian Atlantic Rainforest (Mata Atlântica), the most important relative risks for poor macroinvertebrate MMI scores were physical habitat quality, riparian vegetation condition, and catchment condition (Jimenez-Valencia et al., 2014). For Brazilian neotropical savanna (Cerrado) streams, the major relative risk factors for poor macroinvertebrate MMI scores were turbidity, percent fine sediments, and percent catchment agriculture (Silva et al., 2018), or percent catchment pasture and low streambed stability in the drier northern savanna (Martins et al., 2021c). Nationally in conterminous USA streams, the major attributable risk factors for poor macroinvertebrate and fish MMI scores were excess macronutrients (nitrogen and phosphorus); but for fish in the western USA, it was poor riparian vegetation cover (USEPA, 2016b). Total N and P were also the major attributable risk factors for poor macroinvertebrate MMI scores in conterminous USA lakes (USEPA, 2016c). The major attributable risk factors for poor wetland-vegetation MMI scores were vegetation removal, soil compaction, and ditching (USEPA, 2016a). At smaller spatial extents, excess total N and turbidity were the best predictors of poor macroinvertebrate MMI scores in five of nine USA ecoregions, whereas relative streambed stability and dams predicted best in three ecoregions (Herlihy et al., 2020). The best predictors of poor fish MMI scores in five USA ecoregions were percent fines, but in three ecoregions they were total-N, chloride, turbidity, and dams (Herlihy et al., 2020). Therefore, as recommended by Suter (1993), an array of biological responses are assessed by MMIs, they are being used for diagnosing causes of impaired condition and are employed for regulatory and management mitigative measures.

As a result of their ecological and regulatory applicability, freshwater MMI assessments based on algae, macrophyte, macroinvertebrate, fish, and riparian-bird and vegetation assemblages are becoming more widely adopted (Charles et al., 2021; Hughes and Vadas, 2021; Karr and Chu, 1999; Ruaro et al., 2020; Ticiani et al., 2018; Yoder and Kulik, 2003). Karr and Chu (1999) described sampling techniques, useful metrics for

assessing various anthropogenic impacts, and the superiority of MMIs for assessing anthropogenic impacts on North American streams and other freshwater ecosystems. MMI metrics usually fall into three categories: species-composition (richness, diversity, dominance, tolerance), trophic and trait composition (life histories and feeding, habitat, flow, mobility, and thermal guilds), and abundance/disease/hybridization metrics, comprising a hierarchically diverse approach that enhances detection of anthropogenic impacts (An et al., 2006; Karr and Chu, 1999; Moyle et al., 1998; Ruaro et al., 2020; Ticiani et al., 2017; Yoder et al., 2008; 2015; 2016). An entire MMI score is considered more reliable than any one metric score for assessing the degree of impact across biogeographical regions because the individual metrics were designed to vary in their sensitivity to multiple anthropogenic pressures and stressors (Fausch et al., 1990; Karr et al., 1986; McCormick and Peck, 2000; Miller et al., 1988). Also, metric aggregation reduces inaccuracy compared to any individual metric (Loeb and Spacie, 1994; Davis and Simon, 1995). The main strengths of an MMI over past impact-assessment approaches is that it integrates environmental damage (including diffuse and point source pollutants and cumulative impacts of multiple anthropogenic disturbances), reduces bioassessment subjectivity, is more conservative than strictly physicochemical methods, and facilitates protection of other freshwater biota (Harris, 1995; Karr and Chu, 1999; Winter and Hughes, 1996). Rigorous MMIs can be developed by using statistical relationships with stressors at regional and trans-regional levels. Such an MMI is holistic in scope, relevant to assemblage-environmental relations, and indicates how good conditions are for freshwater biota (Karr, 1981), much like analogous indices do for assessing economic or human health in layperson-friendly formats (Karr and Chu, 1999).

Karr and Chu (1999) and Karr et al. (2022) addressed MMI criticisms since the index was first formulated by Karr (1981) and Karr et al. (1986) as an index of biotic integrity (IBI) and critiqued alternative methods for assessing freshwater ecosystem impairment. Although MMIs have been applied to streams of various sizes, as well as lake, wetland, and estuarine systems worldwide, many MMIs suffer from some fundamental environmental biology challenges and misapplications that are not always adequately addressed. Indeed, Karr's (1981) original concern that the index would experience mindless band-wagoning is a very real problem, i.e., developing unique MMIs for every different stream, lake, estuary, or wetland rather than developing fewer MMIs that can be widely implemented (e.g., Martins et al., 2020, 2021a; Moya et al., 2011; Pont et al., 2006; Riato et al., In Press; Stoddard et al., 2008). Although regional or local MMIs can more accurately assess ecological conditions in the areas in which they were developed, they are frequently inapplicable elsewhere (Martins et al., 2020; Suter, 1993) which can hinder pluralistic research and scientific progress (Vadas, 1994). Therefore, our objectives here are to encourage freshwater scientists and managers to seriously consider the concerns raised below to develop more robust MMIs, apply them to improve watershed and water body management, and to consider tradeoffs between widespread MMI applicability and loss of local specificity. We believe that doing so would lead to a better understanding of assemblage responses to anthropogenic pressures and stressors. This could help identify the dominant pressures and stressors by region and waterbody type and help determine the sensitivity differences of different assemblages to those pressures and stressors. Additionally, this would provide greater insights into the mechanisms driving impairment and indicate where societies can most cost-effectively focus their limited natural-resource rehabilitation and protection efforts to meet sustainable development goals (e.g., MBI, 2015a, 2015b; Miltner, 2021; USEPA, 2016a, 2016b, 2016c, 2020). Consequently, we have identified eleven major concerns with MMI development and application (Hughes, 2012).



## 2. Concerns

### 2.1. Appropriate reference conditions

Having a range of reference (control) and degraded (treatment) sites is critical to adequately assess metrics and MMI changes resulting from human activities (Karr and Chu, 1999). Reference sites are those with minimally disturbed conditions, based on historical, *in situ* data (before human activities became intense) and/or data from nearby minimally disturbed watersheds or wetlands having similar natural characteristics (Hughes et al., 1986; Karr and Chu, 1999; Stoddard et al., 2006; Vadas, 1998). A widely unresolved problem is exactly what constitutes minimally disturbed sites or catchments. Indeed, such sites are often absent in highly developed regions (Hughes et al., 1986; Roset et al., 2007; Stoddard et al., 2006). Karr and Chu (1999) acknowledged this issue and emphasized the need to look for biophysical thresholds that separate water bodies with different levels of anthropogenic impact. They recommended using status designations based on whether sites were above, near, or below average conditions. Such an equal-proportion approach assumes that environmental responses are relatively linear and leads to a shifting baselines dilemma (Hughes et al., 2022; Pauly, 1995; Thururow et al., 2020). Suter et al. (1995) instead suggested that freshwater ecosystems should be considered disturbed if they show at least 20% difference in metric scores relative to pristine conditions. But one could argue that truly pristine ecosystems do not exist because of global climate change, atmospheric deposition and invasion by non-native species (Callisto et al., 2019; Landers et al., 2010; Vadas et al., 2016). USEPA (2020) used the 75th percentile of least-disturbed sites in ecoregions (Herlihy et al., 2008) to classify test sites as being in good, fair, or poor condition. Similarly, Hughes et al. (1986), Ligeiro et al. (2013b), and Roset et al. (2007) recommended using sites in the least-disturbed condition, in regions that are extensively disturbed. Such ecoregional reference sites, although not pristine, have been successfully used by the Ohio EPA to improve biological conditions statewide and at selected discharge sites (USEPA, 2013; Yoder, 1995; Yoder and Rankin, 1995, 1998; Yoder et al., 2005, 2019). In addition, the criteria used for classifying sites as either reference or impaired, may vary between the approaches used (Ruaro et al., 2020), which may also affect the MMI responses to environmental disturbance (Hawkins, 2006; Hawkins et al., 2010). Furthermore, it is essential for MMI development to have a substantial disturbance gradient, and numerous sites displaying it, so that statistical inference is sufficiently robust for assessing cause-effect relationships between a range of stressors and using different metrics.

We also acknowledge the importance of accounting for natural regional differences (i.e., physiographic regions or ecoregions) when selecting reference sites, developing MMIs, and comparing disturbed sites (Agra et al., 2019; Bailey et al., 2004; Buss et al., 2015; Hughes et al., 1986; Kaufmann et al., 2022a; Martins et al., 2018; Yoder and Rankin, 1995). Reference conditions must be regionally appropriate. Failing to make them so will lead to biased measurements of deviation (Feio et al., 2014; Hawkins et al., 2010). In addition, Davies and Jackson (2006) emphasized characterizing reference condition quality by using a biological condition gradient (BCG). The BCG provides a step toward a standardized scale for assessing biological condition. Clearly, when constructing new MMIs and when making biological assessments based on them, it is important to select meaningful, relatively large numbers of minimally or least-disturbed reference sites for setting attainable regulatory benchmarks (Yoder and Rankin, 1995).

Four confounding problems are commonly associated with defining reference sites: nutrient enrichment, climate change, invasive non-native species, and loss of keystone taxa.

#### 2.1.1. Nutrient enrichment

Nutrient enrichment resulting from both point and diffuse sources (USEPA, 2020), including atmospheric deposition (Stoddard et al., 2016), is a globally pervasive stressor affecting most of Earth's

ecosystems to varying degrees. Freshwater plant and animal productivity often increases with mild nutrient enrichment (Davies and Jackson, 2006; Esselman et al., 2015; Magurran, 1988; Nichols et al., 2000; Rising et al., 2004; Vadas, 1998; Warren, 1971; Washington, 1984), as does total-taxa richness (Brito et al., 2020; Davies and Jackson, 2006; Gee et al., 1985; Hughes et al., 2004; Johnson and Angeler, 2014; Mebane et al., 2003). However, sensitive and rare taxa typically decline or even disappear with minimal enrichment (Davies and Jackson, 2006; Leitão et al., 2016; Stevenson et al., 2008). For example, nutrient enrichment adversely affects coldwater fish species but benefits cool and warmwater fishes (Hooper, 1999; Jacobson et al., 2019; Kitchell et al., 1977; Lee and Jones, 1991). This is because salmonids and other cold-stenothermic fishes generally require more highly oxygenated water (Davis, 1975) as well as colder temperatures, but enriched and warmer waters hold less oxygen (Cole, 1979; Jacobson et al., 2019).

#### 2.1.2. Climate change

Reference sites should include those minimally affected by changing climate and largely free of other anthropogenic impacts — if they can be found (Isaak et al., 2018; Jacobson et al., 2019; Vadas et al., 2016). As with nutrient enrichment, taxonomic diversity may often increase, and sensitive taxa will be reduced or even lost as waters become warmer and streams and lakes are increasingly subjected to extreme events like floods and droughts (Colvin et al., 2019; Jacobson et al., 2019; Leitão et al., 2016; Robinson et al., 2019; Vadas, 2000; Winfield et al., 2016). Because climate change is becoming increasingly problematic, reference sites should be resampled periodically to account for and document such changes (e.g., Pollock et al., 2022). Resampling also helps ensure ecologically reasonable expectations for MMI test sites and provides time-series data for future climate-impact analyses.

#### 2.1.3. Invasive non-native species

Invasive non-native species (artificial diversity) can adversely affect biological condition through biotic interactions and/or habitat modifications that degrade ecosystem processes and native biodiversity (Barbour et al., 1999; Gehrke et al., 1995; Hermoso et al., 2010; Hossain et al., 2018; Hughes and Herlihy, 2012; Karr and Chu, 1999; Lassuy et al., 1994; Lomnický et al., 2007; Moyle, 1994; Ruaro et al., 2018; Stanford et al., 1996; Whittier et al., 2002, 2006; Yoder et al., 2008). Su et al. (2021) reported an increasing trend for local phylogenetic, taxonomic, and functional richness in over half the world's fish assemblages, which was explained primarily by non-native species introductions that compensate for — or exceed — extinctions of native species in most rivers. In other words, increased diversity may not be ecologically desirable.

Non-native species are sometimes included as an MMI metric because their abundances are considered biological pollution and they are linked to degraded freshwater ecosystems (Hughes et al., 2004; Hughes and Gammon, 1987; Mebane et al., 2003; Whittier et al., 2007). However, they also occur in western USA mountain sites and Chilean streams that are otherwise minimally disturbed (Habit et al., 2010; Lomnický et al., 2007; Mebane et al., 2003; Whittier et al., 2007). The limited presence of non-native species is common in many European and Brazilian reference sites, and reference conditions there should be based on admissible ecological limits for their presence (Casarim et al., 2020; Segurado et al., 2014). However, non-native species are often ignored when determining reference sites (Ruaro et al., 2021). Information on the occurrence, abundance, and autecologies of non-native species may be insufficient to determine the difference between pre- and post-invaded ecosystems (Ruaro et al., 2021), or even to set abundance thresholds of non-native species for impact assessments on native assemblages (Ruaro et al., 2018, 2021). This can occur for various reasons. For instance, it is analytically circular to use non-native species data to classify sites as reference or impaired and then to calculate, select, and score non-native metrics from them (Kanno and Vokoun, 2008; Kanno et al., 2010; Parker et al., 1999; Ruaro et al., 2018; Stoddard et al., 2006). Therefore, we recommend considering non-native species as biological pollution

(Davies and Jackson, 2006), avoiding the inclusion of such sites as reference sites, and using metrics based on native species (e.g., native-taxa richness vs. total-taxa richness; e.g., Mebane et al., 2003; Whittier et al., 2007).

#### 2.1.4. Loss of key species

Overfishing and construction of migration barriers have reduced fish migration and the upstream transport of nutrients and taxa (Storch et al., 2022). Reductions in marine nutrients from overfishing and impassable dams can result in oligotrophication of some headwater streams because of losses of anadromous species (Gende et al., 2002; Vadas, 1998). Similarly, impassable dams and stream-road crossings have eliminated potamodromous and catadromous species from streams and rivers (Hughes et al., 2022; Leitão et al., 2018; Oberdorff and Hughes, 1992). Depending on their ecological functions, these losses can alter the structure and functions of otherwise high-quality reference sites. A similar case can be made for loss of native beavers (*Castor* spp.), given their roles in water storage and flow and thermal modulation (Naiman et al., 1986; Vadas et al., 2016). Therefore, where possible, it is important to consider once naturally occurring key species abundances when selecting reference sites.

#### 2.2. Incorporating variability when estimating taxa richness

Karr and Chu (1999) and Karr et al. (1996) described the benefits of various MMI metrics that quantify different components of taxonomic richness, noting that those metrics are more reliable than taxa-diversity indices. However, taxa-richness metrics (like taxa-diversity indices) are sample-size dependent until an asymptote (plateau of diminishing returns) is reached (Hughes et al., 2021a; Magurran, 1988; Peet, 1974; Pompeu et al., 2021; Vadas, 1998; Washington, 1984) because of rarely occurring species (Hughes et al., 2021a; Kanno et al., 2009) that also often represent unique traits (Leitão et al., 2016). We agree with Karr and Chu (1999) that small sample sizes, e.g., the criterion of 100 individuals often used for macroinvertebrate assessments, are tenuous for reaching asymptotes (Cao et al., 2002; Chen et al., 2015; Hughes and Vadas, 2021; Hughes et al., 2012; Li et al., 2003, 2014; Ligeiro et al., 2013a; Segurado et al., 2014; Silva et al., 2017; Vadas, 1998). We recommend determining sample-size vs. taxa richness relationships for biotic assemblages to ensure that taxa-richness metrics are reliable (Cao et al., 2001; Flotemersch et al., 2011; Hughes and Herlihy, 2007; Hughes et al., 2002, 2012, 2021a; Kanno et al., 2009; LaVigne et al., 2008; Pompeu et al., 2021; Reynolds et al., 2003; Terra et al., 2013a).

Natural environmental gradients markedly affect the taxa richness of fish (Chen et al., 2017; de Carvalho et al., 2017, 2020; Fausch et al., 1984; Macedo et al., 2014; McCormick et al., 2001; Oberdorff et al., 2002; Pont et al., 2006, 2009; Terra et al., 2013b) and macroinvertebrates (Bello-González et al., 2022; Chen et al., 2014, 2017; Ferreira et al., 2014; Hawkins, 2006; Hawkins et al., 2000; Klemm et al., 2003; Macedo et al., 2014; Moya et al., 2011; Silva et al., 2016; Stoddard et al., 2008). Natural gradients are incorporated in the apparent deviation of assemblages from reference conditions if reference conditions co-vary with natural gradients (e.g., Reynoldson et al., 1997). Therefore, especially when MMIs are developed for large areas (ecoregions, river basins), some calibration is required to separate natural variability from the co-occurring effects of anthropogenic disturbance on taxa richness.

Furthermore, as Cao et al. (2007), Karr and Chu (1999) and Ligeiro et al. (2013a) have noted, standardization of taxonomic richness by sample size, spatial unit area or rarefaction is necessary but somewhat controversial given inadequate testing and site-based variability. Similar concerns exist for eDNA, which has substantial potential for more accurate and cost-effective determinations of local species richness for algae (Keck et al., 2022), macroinvertebrates (Fernández et al., 2019; Keck

et al., 2022; Suh et al., 2019; Uchida et al., 2020) and fish (Blabolil et al., 2021; Cantera et al., 2022; Keck et al., 2022; Lavergne et al., 2021; Pont et al., 2019). We believe that taxa-richness metrics must be standardized by consistent sampling and sample processing efforts and be calibrated for natural gradients when making comparisons among sites.

#### 2.3. Trophic metric robustness

Trophic metrics may require modification despite Karr and Chu's (1999) optimism for vertebrate versus macroinvertebrate applications, given that many taxa are generalist or opportunistic feeders (Ferreira et al., 2015; Vadas, 1990, 1997). For example, the omnivorous fish metric, which has been useful as an indicator of poor freshwater condition in temperate waters, is compromised by naturally common omnivory — especially in minimally altered temperate (Peters, 1977; Vadas, 1990, 1992a, 1998) and tropical (Hossain et al., 2018; Ibañez et al., 2009) freshwater ecosystems. Hence, Karr and Chu's (1999) contention that fish trophic metrics behave more predictably than those for zoobenthos, given better ecological knowledge and less dietary opportunism, is questionable. Moreover, because apex-predator fish species eat only fish, other vertebrates, and crayfish when large, MMI biologists should divide such predatory species into large (apex predator) versus small (insectivorous) size classes (Esselman et al., 2013; Peters, 1977; Vadas, 1990). For example, a nursery stream with inadequate flows for adult game fishes (Schlosser, 1982; Van Deusen, 1953) would be classified as having high piscivory, even though juvenile game fish feed mostly on freshwater and/or terrestrial invertebrates (Kandem Toham and Teugels, 1997). Hence, the caveat that freshwater ecological dynamics need further study to improve trophic metrics (Davis and Simon, 1995; Karr and Chu, 1999) should be heeded. This is also the case when considering natural movements of different life stages of fish into and out of temporary freshwater habitats like headwaters, non-perennial (ephemeral, intermittent) streams and rivers, and off-channel refuges (Colvin et al., 2009; Pompeu et al., 2012; Vadas, 1992b). Although trophic guild metrics certainly have merit and offer insights into ecosystem mechanisms and processes, they could be improved by increased knowledge of the feeding ecologies and life histories at genus and species levels for taxa used in MMIs. Notably, the river continuum concept stresses longitudinal shifts in energy sources, particularly herbivory in unimpaired, mid-sized, temperate rivers for freshwater macroinvertebrates (Resh, 1995; Vannote, 1981; Vannote et al., 1980; Webber et al., 1992), but this logic needs further extension to fish (e.g., Ibañez et al., 2009; McGarvey and Hughes, 2008). MMI researchers have not usually considered longitudinal changes in trophic composition when developing metrics (McCormick and Peck, 2000; Plafkin et al., 1989; Simon and Lyons, 1995). However, Crumby et al. (1990) and Pont et al. (2009) used higher piscivory criteria for downstream areas or other co-variables of stream size, whereas Oberdorff et al. (2002) used higher omnivory and lower invertivory criteria for downstream sites.

#### 2.4. Single-taxon metrics

The original IBI and some other MMIs have used metrics based on a single fish species or family, rather than on all relevant species having a particular habitat, tolerance, life-history, reproductive or trophic trait (e.g., Chen et al., 2019b). Indeed, confamilial fish species can vary in feeding habits (Vadas, 1990) and habitat uses (Vadas and Orth, 2000; Vadas, 1991, 1992b), thus forming ecological guilds with species from other fish families. Stable isotopes offer a cost-effective mechanism for determining trophic guilds without having to identify individual food items (de Carvalho et al., 2020), as can ecomorphological analyses to some extent (Gatz, 1979, 1981; Vadas, 1988). Ecomorphologies are also useful for determining habitat and behavioral guilds (Alvarenga et al.,

2021; Chan, 2001; Chen et al., 2019b; Leitão et al., 2018; Silva et al., 2021a).

As Karr (1981), Karr et al. (1999), and Vadas (1992a) emphasized, not all lotic fish species in a family share the same sensitivities to disturbance. This is certainly true for major fish families in tropical (Martins et al., 2021c, 2022; Pinto et al., 2006; Terra et al., 2013b) and temperate regions (Hughes et al., 2022; Mebane et al., 2003; Whittier and Hughes, 1998; Whittier et al., 2007; Zaroban et al., 1999). Moreover, a given fish species (e.g., the generally tolerant Green Sunfish, *Lepomis cyanellus*) may be more sensitive to some pollutants than others (Suter, 1993). Fortunately, Karr and Chu (1999) highlighted more-generalized metrics that have improved the ability to apply MMIs across continental (Pont et al., 2006, 2009; Stoddard et al., 2008; USEPA, 2020) and national (Moya et al., 2011; Oberdorff et al., 2002) spatial extents.

Similarly, taxa within many macroinvertebrate taxonomic groups vary in their sensitivities to disturbance. Within the tropical Odonata, most Zygoptera (damselflies) tend to be more sensitive than Anisoptera (dragonflies) (Oliveira-Junior et al., 2015, 2017; Silva et al., 2021a, 2021b), but not always (Oliveira-Junior et al., 2015). Some Chironomidae (midge) genera are more sensitive than others (Deshon, 1995; Martins et al., 2021b; Yoder and Rankin, 1995). Several Trichoptera (caddisflies), Heteroptera (true bugs), Ephemeroptera (mayflies), and Plecoptera (stoneflies) genera differ in the directions of their responses to anthropogenic disturbances (Vadas, 1997, 1998; Linares et al., In Press; Martins et al., 2021c, 2022). Like fishes, specific macroinvertebrate taxa may be more sensitive to some stressors (e.g., sedimentation and/or heavy metals) than others (Karr and Chu, 1999; Rosenberg and Resh, 1993; Vander Laan et al., 2013). This may explain why the relative abundance of EPT (combined Ephemeroptera, Plecoptera and Trichoptera) metrics are less sensitive to riparian impacts than they potentially could be if the tolerant EPT were removed (Vadas, 1997, 1998).

Compared to single-taxon metrics, more generalized metrics have reduced the need for *ad hoc* modifications that are common when new regions are studied (Davis and Simon, 1995). Nonetheless, we recommend caution if assuming all species in a family share the same habitat use, tolerance, life-history, reproductive or trophic traits. Further work on ecological guilds is warranted, albeit intra-guild members may not always respond similarly to disturbances (Orth, 1980), likely a reflection of guild differences on other environmental axes. Also, because different biotic assemblages respond differently to environmental disturbances (Herlihy et al., 2020; Martins et al., In Press; USEPA, 2016b, 2016c; Vadas, 1998), it is usually best to restrict an MMI to one assemblage at a time, to enhance ecological interpretation and diagnosis. Furthermore, because different assemblages and metrics are more sensitive to some anthropogenic disturbances than others, this could provide a basis for more strategic development of metrics for diagnosing limiting factors.

## 2.5. Functional guilds & traits

Karr's original MMI included trophic guilds based on taxonomy. Compared to taxonomic metrics, a broader set of functional traits such as reproductive strategies, habitat preferences, or other life-history characteristics sometimes offer greater insights into the biotic and ecological mechanisms relating anthropogenic disturbances to assemblage responses (Alvarenga et al., 2021; Chen et al., 2019b; Leitão et al., 2018; Moya et al., 2011; Silva et al., 2021a, 2021b; Vadas, 1997; Yoder et al., 2008). However, accurate assessments of those traits also require sufficient monitoring to detect taxa that may be rare or patchily distributed because functional richness is correlated with taxa richness (Alvarenga et al., 2021; Leitão et al., 2016). In addition, accurate determination of functional traits requires accurate taxonomic identifications and sufficient biological knowledge of those taxa, which calls for additional research, especially in bio-diverse tropical regions (Ferreira et al., 2015; Leitão et al., 2018; Silva et al., 2021b). But ecologists still lack such information for many freshwater faunas, especially the megadiverse tropical faunas, or where taxa can only be identified to family or higher

taxonomic levels. Nonetheless, we recommend employing more metrics based on habitat, tolerance, life-history, reproductive, and trophic traits, and fewer based on single taxa that may not be ubiquitous (Bryce et al., 2002; Bryce, 2006; Chen et al., 2019b; Esselman et al., 2013; McCormick et al., 2001; Riato et al., In Press; Stevenson et al., 2013; Stoddard et al., 2008).

## 2.6. Robust sampling methodology

Many MMI metrics are selected without the benefit of a robust data set or survey design. MMIs based on fewer than 40 sites per region or water body class, those of limited geographic scope, and those lacking clear disturbance gradients are likely to suffer from a lack of generality, which greatly restricts their usefulness, assessment accuracy, and precision. Likewise, metrics that are based on few study sites cannot be rigorously evaluated statistically. Similarly, metrics that are based on neighboring sites on the same stream or in the same lake suffer from autocorrelation or pseudoreplication (Griffith, 1987; Hurlbert, 1984; Hurlbert and Lombardi, 2015; Vadas, 1998; Whittier et al., 2002). Therefore, it is important to employ enough sites selected across an entire ecoregion or river basin to ensure wide gradients of natural conditions, as well as with different levels of anthropogenic disturbance, to develop reliable MMIs (Feio et al., 2014). Probability surveys are especially useful for this purpose (Bryce, 2006; Bryce et al., 2002; Hughes et al., 2000, 2004; Klemm et al., 2003; Macedo et al., 2016; McCormick et al., 2001; Olsen and Peck, 2008; Pont et al., 2009; Riato et al., In Press; Silva et al., 2017; Stevenson et al., 2013; Stoddard et al., 2008; Tang et al., 2016; Whittier et al., 2007). But if minimally disturbed reference and highly disturbed sites are uncommon across the study area, they should be added via stratified-random (de Carvalho et al., 2017; Silva et al., 2017) or pollution-survey (MBI, 2015b; Yoder et al., 2008) designs.

## 2.7. Metric screening

Candidate metrics should be screened for their range in values, redundancy, repeatability, natural gradients, and sensitivity (Hughes et al., 1998; Jun et al., 2012; Klemm et al., 2003; McCormick et al., 2001; Riato et al., In Press; Stoddard et al., 2008, Fig. 2).

### 2.7.1. Metric range & redundancy

Metrics based on few taxa or having similar values (low range) at most sites will not vary sufficiently to discriminate different conditions. Although metric redundancy may increase the MMI environmental sensitivity via enhanced weight of evidence (Karr and Chu, 1999), total scores will be biased if certain metrics are overrepresented via such high redundancy (Millsap et al., 1990). Metrics that are highly correlated ( $r > [0.7]$ ) are redundant and therefore, add little new information and can inflate MMI variance, thus reducing statistical power. In fact, many potential metrics may be highly correlated to the point that avoiding redundancy can be difficult (Logez et al., 2013). However, non-redundant and heterogeneous metrics respond to different facets of assemblage responses, to differing anthropogenic disturbances (Chen et al., 2019b; Vander Laan et al., 2013), and to differing degrees of the same disturbance (Karr et al., 1986). This commonly occurs with metrics that are sensitive to a disturbance compared to those that are tolerant. The former, by definition, respond earlier and negatively along the disturbance gradient, whereas the latter respond later and positively along that gradient direction. If that disturbance has been sufficiently great, the biota representing the sensitive metric have already disappeared. For example, neotropical Anisoptera (dragonflies, positive response) and Zygoptera (damselflies, negative response), both in the Odonata order, generally respond in different directions to riparian vegetation removal (Oliveira-Junior et al., 2015, 2017; Silva et al., 2021a, 2021b). The same is true for several other freshwater insect taxa regarding low levels of both catchment and riparian revegetation (Brito et al., 2020; Martins et al., 2021c). Van Sickle (2010) found that as the



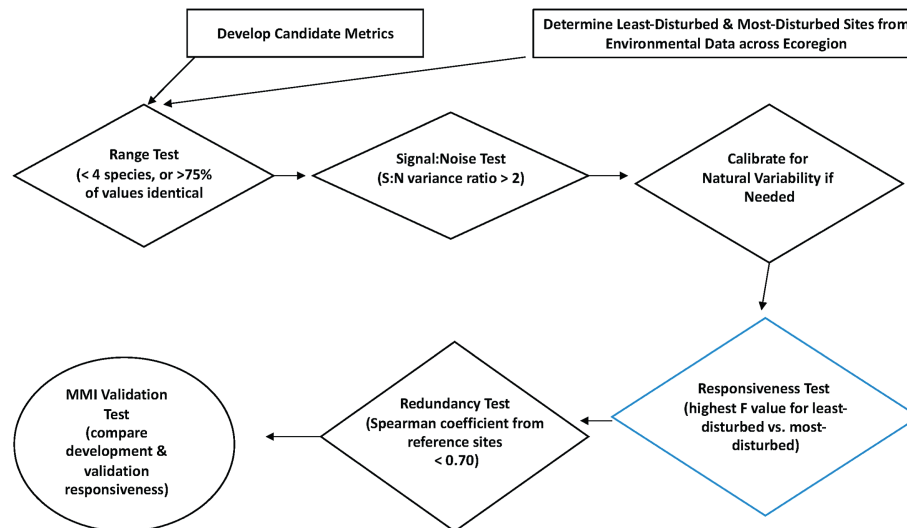


Fig. 2. Suggested MMI metric screening process (adapted from Stoddard et al., 2008 and Whittier et al., 2007).

average correlation among metrics increased, the MMI precision and capacity to distinguish differences among sites decreased.

#### 2.7.2. Metric repeatability

Metrics with low repeatability, i.e., those having high sampling variation between repeat visits at reference sites (noise variance, N) relative to the variation among test sites (signal variance, S) have questionable assessment value (Kaufmann et al., 1999; 2022a). Metrics with an  $S/N < 1$  should be rejected in favor of those with an  $S/N > 2$ .

#### 2.7.3. Metric natural variability

Metrics that are strongly correlated with natural gradients cannot separate anthropogenic effects from natural effects on metric scores (Kaufmann et al., 2022a; Oberdorff et al., 2002; Pereira et al., 2016; Pont et al., 2006, 2009). Typical gradients include topological (water body size, catchment area, elevation, stream slope), climatic (air temperature, precipitation), and geological (lithology, groundwater input) variables (e.g., Esselman et al., 2013; Pereira et al., 2016; Pont et al., 2009; Silva et al., 2017; Stevenson et al., 2013). To correct for those influences, raw metrics having natural-gradient regression correlations  $>0.1$  – or random forest correlations  $>10\%$  – should have their scores substituted by their regression-residual scores (Chen et al., 2019a; Macedo et al., 2016; Pont et al., 2009; Silva et al., 2017; Stoddard et al., 2008; Terra et al., 2013). For example, regression residuals in reference sites have been used to separate stressor effects from natural variation in stream size, channel slope, elevation, and air temperature for fish assemblage metrics (Chen et al., 2017; de Carvalho et al., 2017; Esselman et al., 2013; Oberdorff et al., 2002; Pont et al., 2006, 2009). Similarly, macroinvertebrate metrics have been calibrated for pH, conductivity, stream size, latitude, elevation, catchment area, channel slope, temperature, and precipitation (Chen et al., 2017, 2019a, 2019b; Fierro et al., 2018; Macedo et al., 2016; Martins et al., 2020; Moya et al., 2011). Lotic diatom metrics have been calibrated by latitude, longitude, elevation, temperature, catchment soils, flow, catchment area, and channel slope (Tang et al., 2016). Such site-scale corrections for natural variability have improved the performance of metrics and the MMIs that they comprise. However, we recommend caution when attempting to distinguish natural and anthropogenic effects on metrics, because anthropogenic disturbance covaries with stream size, soil fertility, and many other natural environmental variables (Tang et al., 2020).

At continental and state extents, MMIs or metrics have been calibrated by ecoregions (Ohio EPA, 1988; Stoddard et al., 2008; Yoder and Rankin, 1995) and assemblage typologies (Schmutz et al., 2007; Tang et al., 2016). But within large heterogeneous ecoregions or river basins,

meaningful differences exist for macroinvertebrate (Agra et al., 2019; Hawkins, 2006; Hawkins et al., 2010; Martins et al., 2018) and diatom (Tang et al., 2016; Theroux et al., 2020) metrics, so site-specific metric modeling is usually warranted.

Importantly, the effects of water-quality and physical-habitat degradation may be confounded by the natural responses of biotic assemblages to both natural (Soria et al., 2020; Vadas et al., 2016) and anthropogenic flow intermittence (Colvin et al., 2019; Falke et al., 2011; Riato et al. In Press; Vadas, 2000). For example, Crabot et al. (2021) showed that current French macroinvertebrate-based bioindicators were unable to detect organic contamination when annual-flow intermittence occurred 30–60% of the time. Munne et al. (2021) demonstrated that current MMIs could not be applied for much of the year in non-perennial Spanish rivers. Over half of the world's river network is prone to flow intermittence (Messenger et al., 2021), and such episodicity is likely to increase because of changes in global climate and land and water uses (Colvin et al., 2019; Falke et al., 2011; Vadas, 2000; Vadas et al., 2016). Therefore, alternatives are needed to integrate non-perennial rivers into current biomonitoring programs (Crabot et al., 2021).

Also, proximity to mainstem rivers (Davis and Simon, 1995; Garman et al., 1982; Hitt and Angermeier, 2008; Osborne and Wiley, 1992; Osborne et al., 1992; Whiteside and McNatt, 1972), and lentic ecosystems and irrigation canals (Alofs, 2019; Courtney, 1995, 1997; Hossain et al., 2018; Vadas, 1998) may affect fish species composition and richness through entirely natural dispersion processes that may not necessarily indicate site degradation issues directly.

#### 2.7.4. Metric sensitivity

Lastly, metrics should clearly discriminate between reference and disturbed sites. This is often determined via box plots that display no or little overlap between the quartiles of reference and disturbed sites (Pont et al., 2009; Stoddard et al., 2008). The preceding metric screening process markedly improves the capacity of MMIs to reliably assess anthropogenic disturbances by reducing the influences of natural environmental gradients and sampling variability. Notably, future MMI applications could benefit from examining divergences among metric sensitivities to better diagnose problems, rather than simply focusing on total MMI scores.

### 2.8. Metric scoring & quality classes

Although Karr (1981) and Karr et al. (1986) scored metrics as 1 (poor), 3 (fair), or 5 (good), subsequent MMI metrics have been scored continuously from 0 to 1 or 0–10 (Ganasan and Hughes, 1998; Magee

et al., 2019; Mebane et al., 2003; Minns et al., 1994; Stoddard et al., 2008; Tang et al., 2016), similar to habitat-suitability metrics (Vadas and Orth, 2001). Stepped (1, 3, or 5) scoring increases MMI variability and widens confidence intervals (Blocksom, 2003) because it scores metrics the same at the bottom or top of the step range (Stoddard et al., 2008). Continuous metric scoring eliminates step functions in metric scoring as well as an unnecessary step in metric-condition classification that propagates error (Reash, 1995), leaving site condition classification to the final MMI score itself. However, European multimetric indices use five quality classes, but often the disturbance gradient is not wide enough to reliably define class boundaries (Segurado et al., 2014). Similarly, only three of potentially six quality classes were classified in an assessment of Rio Grande sites for both fish and macroinvertebrate assemblages (Hughes et al., 2022). This may be why the USEPA uses only three classes in its national reports (USEPA, 2016a, 2016b, 2016c, 2020).

### 2.9. Temporal variability

As Karr (1981) and Karr et al. (1986) warned, data collected from the same sites during different seasons should be used with caution when making assessments of anthropogenic impacts, because of substantial natural variation among seasons in stream flows, water quality, and biotic phenologies and life-histories (Callisto et al., 2021; Chen et al., 2014; Fierro et al., 2021; Vadas, 1992a). We strongly recommend sampling when flows are most stable, habitat types are most distinct, access is easiest, and conditions are safest for field crews (usually the dry, low-flow season) for permanent waters (Callisto et al., 2021; Eriksen et al., 2021; Fierro et al., 2021; Hughes and Peck, 2008; Vadas and Orth, 2000; Yoder and Smith, 1999). Wet-season sampling is necessary for non-perennial streams and rivers (Colvin et al., 2009; Dieterich and Anderson, 2000; Hartman and Brown, 1988), although disconnected pools occurring in the dry season (Faustino and Terra, 2020) or on the floodplain (Hartman and Brown, 1988; Vadas, 1992b) must also be considered. Nonetheless, it is useful to understand how varying flows affect metric scoring, as shown by Fierro et al. (2021). Longer-term weather fluctuations associated with ocean conditions (i.e., the El Niño-Southern Oscillation, Indian Ocean Dipole, and Pacific Decadal Oscillation) can also alter freshwater conditions, especially via the abundances of anadromous species (Storch et al., 2022). Thus, both seasonal and annual weather conditions must be acknowledged as natural factors altering biological performances.

### 2.10. Sampling variability

Karr (1981) and Karr et al. (1986) also warned about comparing data collected with different sampling methods. Different site-scale sampling designs, especially focusing on different habitat types, will naturally yield divergent numbers of individuals and taxa (Gerth and Herlihy, 2006; LaVigne et al., 2008; Li et al., 2014; Ligeiro et al., 2020; Mercado-Silva and Escandon-Sandoval, 2008; Vadas and Orth, 1993). The same is true when comparing data collected with different sampling gear (Callisto et al., 2021; Dunn and Paukert, 2020; Robinson et al., 2019; Vadas, 1992a; Yoder and Smith, 1999). Europe-wide intercalibration of MMIs was implemented in the last decade to ensure comparability of methods and results (Poikane et al., 2014). Therefore, MMI development and testing, as well as subsequent monitoring programs, should use standardized sampling methods that are cost effective and easily employed (Bonar et al., 2009; Hughes and Peck, 2008; Pont et al., 2006; Vadas and Orth, 1993).

### 2.11. MMI validation

MMI development without validation is an inappropriate practice. This problem is best resolved by applying the developed MMI to an independent dataset (Riatio et al., In Press; Vadas and Orth, 2001; Van Sickle, 2010). The most common approach for doing so is randomly

selecting separate sets of sites for MMI development from those used for validation, and then assessing the performance of the new MMI on the independent sites (de Carvalho et al., 2017; Klemm et al., 2003; Mercado-Silva et al., 2002; Oliveira et al., 2011; Pereira et al., 2016). Alternatively, MMI researchers have validated MMIs on data from other river basins (Mebane et al., 2003; Martins et al., 2020, 2021; Moya et al., 2011). Such hypothesis testing of model transferability (Martins et al., 2020, 2021; Vadas and Orth, 2001) should improve our understanding of ecological mechanisms, and thus the predictive science and efficacy of environmental remediation (Suter, 1993; Vadas, 1994).

### 3. Caveats

Despite our plea for developing more rigorous and spatially extensive (national, continental, large regional) MMIs, we realize that individual researchers and less developed nations commonly lack the resources to create MMIs following all our recommendations. In many cases, there is deliberate political opposition towards rigorous bioassessment programs, apparently because they threaten entrenched political and economic interests (Hughes, 2014; Hughes et al., 2021b; Zhang et al., 2021). Furthermore, high degrees of endemism and biodiversity together with limited taxonomic functional knowledge and poorly developed institutional infrastructure may hinder creating rigorous MMIs, especially in the tropics (Feio et al., 2021). Unlike the USA, the European Union, South Africa, and South Korea (where a Clean Water Act, Water Framework Directive, National Water Act, or National Aquatic Ecological Health Monitoring Program have been established), there are no legal, governmental mandates or funding for implementing continental- or national-extent biological assessments across most political jurisdictions. Finally, the very human tendencies to prefer one's own sampling methods over others, and to avoid sharing data, limit aggregating data sets collected via standard methods. How might some of these barriers be minimized?

We believe that those caveats can be addressed by common guidance, quality control, data monitoring, and close collaboration. Case studies from Oregon, Brazil, and South Africa provide useful examples. Rather than attempt to develop MMIs for the entire nation, South African government scientists have focused on applying fish and macroinvertebrate MMIs in individual river basins (Kleynhans, 1999, 2008; Roux et al., 1999; Thirion, 2008). Basin-focused MMIs were also developed for fish assemblages in the nearby Okavango River, Namibia (Hay et al., 1996; Hocutt et al., 1994). In Brazil, a consortium of collaborating scientists from four universities funded by a hydropower company developed MMIs after sampling 195 Wadeable stream sites in five large hydrologic units in the Cerrado biome of Minas Gerais. From that database, they developed both fish and macroinvertebrate MMIs (de Carvalho et al., 2017; Macedo et al., 2016; Silva et al., 2017) and conducted probability assessments of the major freshwater stressors in the biome (Martins et al., 2021a; Silva et al., 2018). In Oregon's Willamette basin, data from a watershed council, multiple university researchers, two city governments, and state and federal agencies (total of 689 sites) were shared to determine the biological condition and key stressors and pressures of both Wadeable streams and Boatable rivers (Mulvey et al., 2009). That assessment was facilitated by the widespread use of standard sampling methods, a probability survey design, and fish and macroinvertebrate MMIs. The current trend by many scientific journals and funding agencies to publish data online provides additional hope.

We are not arguing that MMIs are necessarily superior to other statistical approaches or analytical methods, but we quantitatively compared MMI results with alternatives where both have been conducted from the same data. Ganasan and Hughes (1998) assessed fish assemblage condition at 13 sites sampled in the Khan and Kshipra Rivers, India, through use of both an MMI and Bray-Curtis Similarity Analysis. They found that axis-2 of the similarity scores produced anomalous results, but axis-1 separated the three most-disturbed sites from the five least-disturbed sites. However, their MMI indicated that there were six



impaired sites, three intermediate sites, and only three sites in acceptable condition (i.e., a finer, more-powerful partitioning of site condition). Hughes and Gammon (1987) sampled fish assemblages from 26 systematically selected sites in the Willamette River, Oregon (USA) and examined the data patterns through use of an MMI and Detrended Correspondence Analysis (DCA). The DCA separated the sites into upper river (7 sites), middle river (9 sites), Newberg Pool (5 sites), and Portland Metro (5 sites) reaches (i.e., a longitudinal delineation). On the other hand, their MMI detected seven sites with point-source impacts that the DCA missed. Hawkins (2006) compared data sets from Ohio and the Mid-Atlantic Highlands (USA) and determined that regional assessments of biological condition were similar for those based on MMIs versus those based on O/E taxa richness indices. However, the O/E indicated greater numbers of sites in non-reference condition, suggesting greater sensitivity to disturbance. The USEPA sampled macroinvertebrate assemblages from 1,392 randomly selected wadeable stream sites across the conterminous USA and analyzed the data using both an MMI (Stoddard et al., 2008) and an O/E index (Hawkins et al., 2000). The MMI results indicated that 28% of the stream length was in good condition and 41% was in poor condition (Paulsen et al., 2008). Conversely, the O/E index indicated that 52% of the stream length experienced <20% taxa loss (good condition) and 10% of the stream length experienced >50% taxa loss (poor condition; Paulsen et al., 2008), i.e., the MMI was more sensitive than the O/E. Based on simulations of data from 394 stream sites in four western USA states (Idaho, Montana, Oregon, Washington), Hawkins et al. (2010) determined that O/E indices and MMIs had similar precision, but O/E had slightly greater sensitivity. Vander Laan et al. (2013) applied a macroinvertebrate O/E and an MMI to 401 Nevada (USA) sites and found that the O/E index was more sensitive than the MMI to increased electrical conductivity. However, based on 379 test sites, their MMI was more precise, sensitive, and responsive to overall degradation than their O/E index (Vander Laan and Hawkins, 2014). Terra et al. (2015) used Redundancy Analysis (RDA) for assessing the effects of environmental variables on fish taxonomic and functional species at 46 randomly selected Atlantic Forest sites in Brazil. They found that four of ten predictor variables were natural (altitude, % bedrock, % boulder, % root cover), but did not assess assemblage condition or causation. Using an MMI, they determined which sites were least-disturbed, intermediate, and most-disturbed by anthropogenic activities (Terra et al., 2013b).

One might also ask, why not use diversity indices or taxa richness for making biological assessments? Hughes and Noss (1992) and Noss (1990) stressed the multifaceted aspects of biological diversity, incorporating the structural, compositional, and process aspects of life from genes to landscapes. However, diversity is commonly assessed simply as assemblage taxa richness or a diversity index. Neither of those indicators relate well to the usefulness of biological integrity as a policy directive (Angermeier and Karr, 1994; Hughes, 2019), nor to Frey's (1977) definition of biological integrity as the capacity to support an adaptive biotic system incorporating the components and processes occurring in natural habitats. Furthermore, total taxa richness and biological diversity may increase with anthropogenic disturbance as native species are replaced by invasive non-native species, and generally intolerant or sensitive species are replaced by tolerant species (Davies and Jackson, 2006; Hughes and Gammon, 1987; LaVigne et al., 2008; Moncayo-Estrada et al., 2012; Stainbrook et al., 2006). Such invasive processes are key considerations of most MMIs.

We believe that the above differences in MMI and alternative statistical tools result from the latter evaluating all species equally, whereas MMIs discriminate among sensitive, tolerant, native, and invasive non-native taxa. Also, similarity analyses (DCA and RDA) are at least partly driven by natural environmental conditions that are modeled out in O/E and by more advanced MMIs and multivariate analyses (e.g., Reynoldson et al., 1997). Indeed, multivariate clustering of aquatic taxa among months can show notable variation associated with natural thermal and/or flow differences (Vadas, 1991, 1992b, c). On the other hand, an

MMI detected site differences but showed no significant temporal effect from two summer sampling events over 25 y (Pyron et al., 2008). The capacity to separate sampling and natural variability from anthropogenic disturbance is a critical component of useful indicators (Kaufmann et al., 1999; In Press a).

Others might ask, why are MMIs preferable to measures of fish health or population trends (e.g., Arciszewski and Munkittrick, 2015; Munkittrick and Dixon, 1989)? Certainly, if one is concerned with specific fish species or populations, such measures are appropriate (e.g., Nehlsen et al., 1991; Storch et al., 2022) and their results often attract considerable public and management support. However, indicator species condition does not necessarily equate with assemblage condition or biological diversity (Hughes and Noss, 1992; Landres et al., 1988). The same is true of fishing quality indices versus fish assemblage MMIs (Lomnický et al., 2021; Oliveira et al., 2009).

#### 4. Summary

The proliferation of freshwater MMIs may represent dissatisfaction with existing indicators (Rosenberg and Resh, 1993), as well as enthusiasm regarding potentially powerful indicators of ecosystem condition (Ruaro et al., 2020; Ticiani et al., 2018). Also, MMIs and sampling effort have been standardized and have improved bioassessment at continental and national spatial extents as Karr and Chu (1999) and Karr et al. (1986) had hoped they would (Herlihy et al., 2020; Magee et al., 2019; Martins et al., 2020; Moya et al., 2011; Oberdorff et al., 2002; Pont et al., 2006, 2009; Riato et al., In Press; Stevenson et al., 2013; Stoddard et al., 2008; Tang et al., 2016; USEPA, 2016a, 2016b, 2016c, 2020). Nonetheless, MMIs could benefit from increased attention to ecological mechanisms and better metric development to further improve our understanding of anthropogenic impacts, as well as rehabilitation effects on freshwater ecosystems globally (Feio et al., 2021; Suter, 1993). Doing so will increase our understanding of basic ecology, better separate assemblage responses to anthropogenic disturbance versus natural gradients, quantitatively document the dominant anthropogenic disturbance that most affect freshwater biota regionally, and focus our limited rehabilitation resources more cost effectively (e.g., USEPA, 2016a, 2016b, 2016c, 2020). Although local MMIs often more accurately and precisely assess local conditions, we as scientists and managers should continuously be seeking scientific generalizations, including MMIs, that are widely applicable to improve our science and our resource management. After all, Karr (1981) based his initial IBI on stream data collected from hundreds of sites located across two midwestern USA states and generated biological metrics (without the aid of computer analyses) that remain useful 40 y later.

Clearly, there needs to be a balance between statistical rigor and biological wisdom, because statistical significance does not necessarily imply biological significance if low p-values result merely from large sample sizes (Karr and Chu, 1999; Rosenberger and Angermeier, 2003; Ross et al., 1980; Williams, 2010). Our aim in this paper was to review both MMI studies and basic ecology to offer better guidance for improving future MMI applications. We must also remember that MMIs are not ends in themselves. Rather, they (1) are means to important ends — the more practical, accurate, and robust assessment of freshwater condition within management frameworks — and (2) make those frameworks and their outcomes more accurate, complete, and protective of freshwater ecosystems.

#### Author contributions

RLV: Conceptualization, Writing-Original Draft, Writing-Review & Editing; RMH: Conceptualization, Writing-Original Draft, Writing-Review & Editing; YJB: Writing-Review & Editing; MJB: Writing-Review & Editing; MC: Writing-Review & Editing; DRdC: Writing-Review & Editing; KC: Writing-Review & Editing; MTF: Writing-Review & Editing; PF: Writing-Review & Editing; OB-G: Writing-Review &

Editing; JSH: Writing-Review & Editing; DI: Writing-Review & Editing; CJK: Writing-Review & Editing; DRM: Writing-Review & Editing; IM: Writing-Review & Editing; NM-S: Writing-Review & Editing; NM: Writing-Review & Editing; SJN: Writing-Review & Editing; PSP: Writing-Review & Editing; RR: Writing-Review & Editing; DROS: Writing-Review & Editing; RJS: Writing-Review & Editing; BdFT: Writing-Review & Editing; CT: Writing-Review & Editing; DT: Writing-Review & Editing; LW: Writing-Review & Editing; COY: Writing-Review & Editing.

## Declaration of competing interests

We declare no known, financially competing interests or personal relationships that could potentially influence the work reported in this paper.

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