Ecological Indicators 34 (2013) 460–477

Contents lists available at SciVerse ScienceDirect

Ecological Indicators

journal homepage: www.elsevier.com/locate/ecolind

Original article

# Tools for bioindicator assessment in rivers: The importance of spatial scale, land use patterns and biotic integration

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ARTICLE INFO

*Article history:*
Received 30 October 2012
Received in revised form 5 June 2013
Accepted 10 June 2013

*Keywords:*
Bioindicator
Macroinvertebrate metrics
Traits
Land use metrics
Environmental predictors

ABSTRACT

This study outlines an alternative, versatile and flexible procedure to the Assessment System for the Ecological Quality of Streams and Rivers throughout Europe using Benthic Macroinvertebrates (AQEM) protocol for selecting and assessing candidate bioindicators for Water Framework Directive (WFD) compliant monitoring programmes. Based on different forms of benthic macroinvertebrate data using relative abundance of family level taxonomic groups, metrics and traits collected during spring 2010 at 96 lotic sites across northern Portugal, the procedure employs components of top down and bottom up analytical processes and introduces the concept of niche breadth to assess biological quality element response to environmental and stressor parameters across different spatial levels. Random Forest classification revealed that fractal and non-fractal land use metrics at basin and local level were extremely important determinants of Water Framework Directive determination of "Good" ecological quality, particularly at the local scale. The amount of urbanization at the lower spatial level was a particularly important determinant of ecological quality, while the extent and type of forest (especially coniferous) was more important at higher, river basin scale. Distance-based linear models (DISTLM) and distance-based redundancy analysis (dbRDA) were used to determine associations between invertebrate data and non-redundant environmental predictors selected using the Akaike Information Criterion (AIC). Results revealed a stronger association between invertebrate traits and selected environmental predictors compared to the other types of invertebrate data, although some association between invertebrate relative abundance and eutrophication was detected. Principal Components Analyses (PCA) were run for the non-redundant sets of predictors for each macroinvertebrate data set to extract an environmental quality gradient along the first axis. Niche breadth, calculated for candidate indicators to avoid bias resulting from expert judgement, was distributed by rank along its respective PCA gradient. Five candidate indicators for each data type were selected for their preference for the most pristine sites and five were selected due to their close link with the most degraded streams. Candidate bioindicators for impacted sites tended to be stenobiotic in character, due to the impoverished structural and functional diversity associated with such conditions. Finally Partial Least Squares Regression was used to refine and validate selected candidate metrics, to produce a comprehensive final list of macroinvertebrate measures of ecological quality.



## 1. Introduction

EU Member States must continually strive to improve the translation of biological information into measurable classes of ecological quality for effective implementation of Water Framework Directive (WFD) compliant ecological assessment system. This constant refinement assesses and selects metrics that reliably translate the response to environmental change across a range of selected biological quality elements (BQE). Numerous papers outline methodologies for establishing metrics and developing multimetric systems (Buffagni et al., 2004; Hering et al., 2004; Karr and Chu, 1999; Karr and Kerans, 1992). Overall WFD status for aquatic resources and associated ecosystems derives from classification of chemical, hydromorphological and ecological quality, the latter being based on BQE responses to pressures via measurable change in composition and abundance compared to minimally disturbed or reference conditions. The importance of analytically robust bioindicator selection and evaluation processes is absolutely vital, given the fundamental role of BQE in reporting overall surface

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http://dx.doi.org/10.1016/j.ecolind.2013.06.004

water status and the highly dynamic interaction of both natural and anthropogenic factors that influence these systems and the communities that occupy them. However, output from any selection and evaluation process will only be as reliable as the quality and provenance of the data it is built upon.

An ideal candidate metric should respond predictably to a particular stressor or a known range of stressors, have low natural variability, exhibit a typologically independent response and give ecologically interpretable indication of environmental conditions (Hering et al., 2006b). Normally, the sensitivity of candidate metrics is validated according to given stressor gradients using available environmental data. Candidate metrics exhibiting measurable, quantitative impact-response variation across a given stressor gradient can be considered as reliable, interpretable and unaffected by natural variation. Selected metrics should also be spatiotemporally robust, displaying similar responses to a given stressor in other geographical regions or exhibiting low dependence on effects related to seasonality or inter-annual variability.

Traits based metrics are an increasingly popular alternative to taxonomically based metrics because of their spatiotemporal robustness, which ensures adequate response patterns to particular stressors (Dolèdec et al., 1999; Feio and Dolédec, 2012; Friberg et al., 2010; Menezes et al., 2010; Pollard and Yuan, 2010). Macroinvertebrate based monitoring tends to be highly skewed towards oxygen sensitivity/tolerance based systems, excluding other types of perturbation such as habitat degradation. Most metrics display a "wedge-shaped" response to single pressures with weak correlative power once the effect of the pressure becomes moderate or low (Friberg et al., 2010). However, the performance of multimetric indices will differ along gradients of environmental alteration due to the different responses of the integrated component metrics (Hawkins et al., 2011). Multimetric indices, which originated in the USA (Karr and Chu, 1999) but have been widely adopted in Europe (Hering et al., 2006a), include a range of biological measures or metrics that provide an integrated overview of the biological community at a given sampling site.

Many bioindicator selection studies tend to be biased towards the assessment of local impact responses, since the complex interaction of multi-scale environmental variables influencing the physical habitat and the lack of standardization between river habitat assessment methodologies make results difficult to interpret (Cortes et al., 2011; LeCraw and Mackereth, 2010). The effect of such limitations can be reduced by assessing a sufficient number of complementary metrics that compensate for such effects and by discriminating between natural and human sources of variation.

This study outlines a sequential methodology developed to overcome the limitations listed above and obtain a versatile set of indicators (metrics) of disturbance. Based on benthic macroinvertebrate fauna collected from rivers in North Portugal, this study comprises two sets of objectives. The first set of objectives outlines methods for metric selection, taking into account the following factors: (a) the assessment system should integrate a wide range of human impacts; (b) indicators should reflect human impacts at both local and regional scales; (c) the set of indicators and the derived classification system must be flexible and open to improvement by incorporating data from new surveys; (d) formulation of the assessment system should follow WFD principles and lend itself to large scale ecological monitoring.

We assessed candidate metrics against pressure gradients using a sequential approach similar to that used in the WISER project, which assessed and developed systems to assess ecological status in European aquatic systems (Hering et al., 2012). WISER based assessments include (i) the definition of explanatory pressure variables that reflect stress intensity; (ii) grouping of stressed and unstressed sites; (iii) selection and calculation of metrics; (iv) assessment of quantitative and qualitative relationships between biological metrics and stress variables, and between disturbed and non-disturbed sites, using non inferential statistics, parametric and non-parametric tests.

Reliable ecological assessment cannot depend solely on the selection of appropriate biological metrics but also on the use of appropriate environmental data. Thus, the second group of objectives focuses on the identification of pressure gradients or environmental descriptors that exert a stronger influence on the macroinvertebrate community, thereby affecting determination of ecological status. We include descriptors of land use, land cover and fragmentation at river basin and local scale, to assess the effects of land use gradients on stream health and on the aquatic biota (see Clapcott et al., 2010).

Thus, this study aims to obtain a subset of the principal environmental variables influencing aquatic systems, from catchment to local level, focusing on large scale variables such as landscape metrics that reflect human stressors. The final aim of this study is to develop a versatile, analytical tool to improve biological assessment by selecting candidate indicators that incorporate multiple scales of disturbance and to define environmental characterization criteria by assessing the appropriate spatial scale of disturbance gradients.

## 2. Methodology

### 2.1. Study area

Data were collected across northern Portugal, from the Portuguese section of the transnational Minho catchment to the Douro (Fig. 1). Other large river basins included the Lima, Cávado, Ave and Leça. These extensively regulated systems flow westward towards the Atlantic Ocean. Tributaries are mostly small, flowing through deep, narrow canyons while the lower littoral reaches of these rivers systems are intensely urbanized and degraded by human activity (Cortes et al., 2009). Remaining areas are dominated by agroforestry activities with low human population density levels. The sampled sites, belonging to three WFD compliant River Basin Districts (RBD1 – Minho and Lima basins, RBD2 – Cávado, Ave and Leça basins and RBD3 – Douro basin), were distributed across a wide range of perceived environmental conditions, ranging from minimally disturbed to highly degraded. Artificial or highly modified systems (reservoirs or river segments immediately downstream of large dams) were not included in this study. For WFD monitoring purposes, biological and environmental data were collected during spring 2010 at 171 sites, of which 96 were retained for analyses since all three WFD compliant quality elements (biological, physicochemical and hydromorphological) were sampled at them, thereby providing a full suite of data for analyses.

### 2.2. Biological indicators and ecological assessment

BQE comprised benthic macroinvertebrate assemblages which were collected, sorted and identified to family level following a WFD compliant protocol established by the National Water Institute (INAG, 2008). The taxonomic level of family was deliberately chosen due to the lack of information on many endemic invertebrate species (morphological, life history and species-environmental relations in Portugal). The macroinvertebrate data were organized into 3 different types of data sets (Fig. 2, step 1) summarized below:

(a) Relative abundance of family level taxa.
(b) Metrics calculated using the ASTERISCS software (version 3.1.1.), developed as part of the EU funded AQEM project (Hering et al., 2004) (http://www.aqem.de). AQEM calculates