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A multiple index integrating different levels of organization

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ABSTRACT

Many methods in freshwater biomonitoring tend to be restricted to a few levels of biological organization, limiting the potential spectrum of measurable of cause-effect responses to different anthropogenic impacts. We combined distinct organisational levels, covering biological biomarkers (histopathological and biochemical reactions in liver and fish gills), community based bioindicators (fish guilds, invertebrate metrics/traits and chironomid pupal exuviae) and ecosystem functional indicators (decomposition rates) to assess ecological status at designated Water Framework Directive monitoring sites, covering a gradient of human impact across several rivers in northern Portugal. We used Random Forest to rank the variables that contributed more significantly to successfully predict the different classes of ecological status and also to provide specific cut levels to discriminate each WFD class based on reference condition. A total of 59 Biological Quality Elements and functional indicators were determined using this procedure and subsequently applied to develop the integrated Multiple Ecological Level Index (MELI Index), a potentially powerful bioassessment tool.

1. Introduction

Aquatic ecosystems are extremely complex, shaped by multiple dynamic biotic and abiotic interactions. This complexity includes the assessment of pressures which often have synergistic, additive or antagonistic effects in the aquatic environment (Solimini et al., 2009). Quantification of these effects can only be properly addressed using an integrated "weight-of-evidence" approach, designed around the assessment of several complementary parameters, measured at different levels of biological organization (Sanchez and Porcher, 2009). Such an approach provides valuable data, improving the link between biological responses to change in the surrounding environment, thereby improving accuracy in reporting ecological status to prevent environmental degradation. Lagadic et al. (1997) warn about the limitations of single-level assessment approaches and some studies have combined community metrics and bioassays to quantify a spectrum of impacts (Gerhardt et al., 2004). The fish fauna has been extensively used either as biomarkers or as bioindicators since it integrate disturbance responses over several spatial scales (see Trautwein et al., 2013, for a pan-European bio-ecological trait based approach and Maceda-Beiga and De Sostoa (2011), who developed a rapid bioassessment protocol of fish species to stressors expressed at the micro and meso-habitat level). In our study we considered four levels of organization, namely the sub organism, the organism, the community level and ecosystem function, using two groups of aquatic organisms (benthic macroinvertebrates and fish) and a facet of ecosystem function (Fig. S1). We apply and assess a suite of analytical approaches to assess anthropogenic impacts across different organization levels that cover the use of biomarkers, histopathological analyses, community based metrics and traits and alder leaf decomposition rates (Fig. S1).

The aim of this study is to assess the combined Biological Quality Elements (BQE) situated across different levels of biological organization as a means to improve the actual Water Framework Directive (WFD) assessment methods, which currently overlooks biomarker and functional indicator based assessment methods (Friberg et al., 2011). Similar to Sanchez and Porcher (2009), we promote an integrated approach to improve the links between biological and ecological responses to anthropogenic

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impacts. The goal is to combine biomarkers, bioindicators and functional indicators, which provide readily interpretable variables and avoid complex multi-scale combinations to produce an integrative index based on the WFD reference condition concept.

The study (see Fig. S1) uses fish fauna at the sub-organism and organism levels for biomarkers of effect (biochemical/histopathological) and morphological parameters. Community level bioindicators include taxonomic composition, metrics and traits, included benthic macroinvertebrates *sensu strictu*, chironomid pupal exuviae and fish. Finally, at the highest level, leaf litter processing was used as functional indicator, since litter decomposition has been proposed as an integrative indicator of stream functioning (Gessner and Chauvet, 2002; Graça et al., 2015).

Given the potential costs and labour intensive constraints of this holistic approach, we applied our study to a selected number of sites, covering a known quality gradient. The approach is designed to be flexible, allowing different data sets to be used independently or as proxies of other methods to provide information on ecosystem condition. This also allows select combinations of methods, describing response at different levels of organization, to be screened and used according to the type and complexity of sources of disturbance under assessment or availability of technical expertise.

2. Methods

2.1. Study area and sampling design

The selected survey sites were part of established WFD operational and survey networks situated in two River Basins Districts (RBD2 and RBD3) situated in NW Portugal. The 9 selected sites (Caldas Vizela–CVZ, Graça–GRA, Pinhão–PIN, Junqueira–PJU, Pingue–PPI, Trofa–PTR, Prado–PRA, Tejão–TEJ and Vizela–VSA; Fig. 1) were all of similar order number (Strahler 3–4) and hydromorphological condition to maximise comparability. Based on the results of the WFD 2010 sampling programme, the sites covered all 5 WFD quality classes and a wide gradient of types of disturbance including organic, toxic pollution and hydromorphological degradation. Human impacts are particular intense in the lower part of the Ave catchment (RBD2) because of high concentration of industrial activities, where textiles and metallurgies units are dominant, coupled with organic effluents from cattle breeding. There is also a contribution of urban areas, since the NW of the country exhibits the highest population density. On the contrary, tributaries belonging to the Douro catchment (in RBD3) offer reference conditions, since the soil use is mainly forest, shrub and extensive agriculture with low fertilizer loads. More information about these River Basin Districts, can be find at http://www.apambiente.pt/?ref=16&subref=7&sub2ref=9&sub3ref=834.

Benthic invertebrates, chironomid pupal exuviae and fish were sampled seasonally (a total of four times) in the summer and winter of 2011–12, the summer of 2012 and the spring of 2013. Exceptionally high and prolonged precipitation during the winter of 2012 resulted in prolonged spate flows which delayed the last sampling campaign until spring 2013. The ecological status of these sites was determined based on national indexes, for BQEs and support elements. Because of the low number of sites in each quality category (reference sites: PPI, PIN, TEJ; degraded sites: PRA, VSA, GRA; highly degraded: CVZ, PTR, PJU) we only considered 3 ecological quality classes, namely "Good" (comprising sites above good status) "Moderate" and "Poor" (comprising Bad and Poor sites).

2.2. Sampling and processing of biological and ecological indicators

A brief summary of the biological and functional indicators and biomarkers tested in this study is provided in Table 1.

Macroinvertebrate assemblages were collected following a

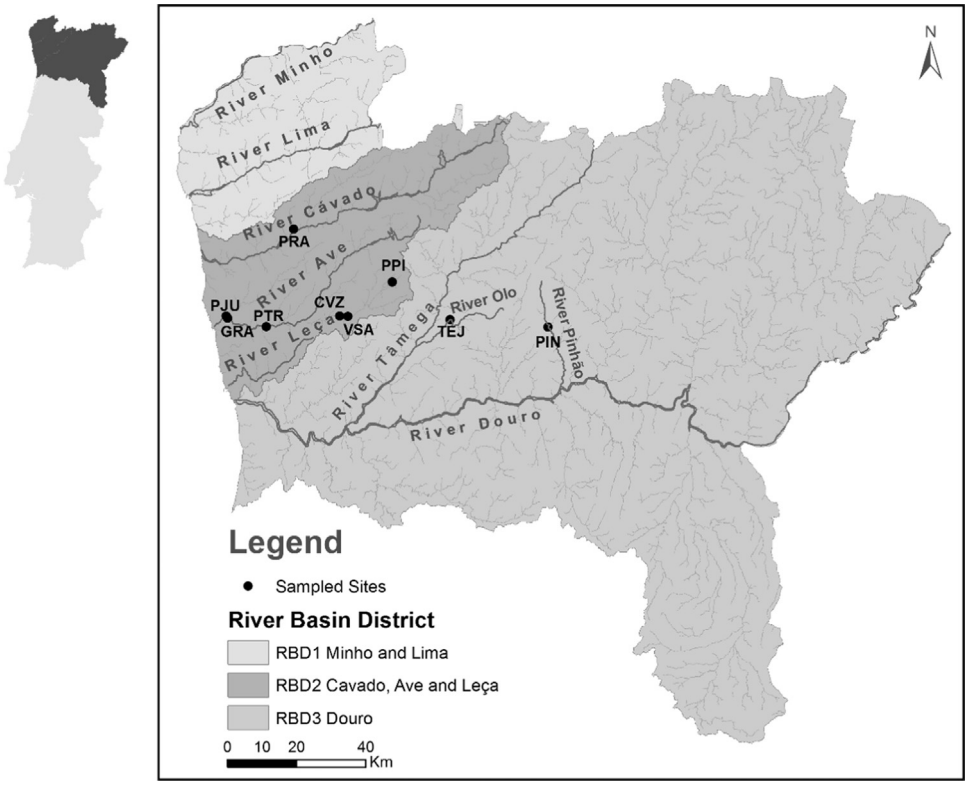

Fig. 1. Locations of all sites sampled in 2011–2013.