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A review on the ecological quality status assessment in aquatic systems using community based indicators and ecotoxicological tools: what might be the added value of their combination?

Monica Martinez-Haro[a,*], Ricardo Beiras[b], Juan Bellas[c], Ricardo Capela[d], João Pedro Coelho[e], Isabel Lopes[f], Matilde Moreira-Santos[a], Armanda Maria Reis-Henriques[d], Rui Ribeiro[a], M. Miguel Santos[d,g], João Carlos Marques[a]

[a] IMAR—Institute of Marine Research, Marine and Environmental Research Centre, Department of Life Sciences, University of Coimbra, 3004-517 Coimbra, Portugal
[b] ECIMAT—Universidade de Vigo, Illa de Toralla s/n, Coruxo-Vigo, Galicia 36331, Spain
[c] Centro Oceanográfico de Vigo, Instituto Español de Oceanografía, IEO, Subida a Radio Faro, 50, 36390 Vigo, Spain
[d] CIMAR/CIIMAR—Interdisciplinary Centre for Marine and Environmental Research, University of Porto, Rua dos Bragas, 289, 4050-123 Porto, Portugal
[e] CESAM (Centre for Environmental and Marine Studies) & Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal
[f] Department of Biology & CESAM, University of Aveiro, 3810-193 Aveiro, Portugal
[g] FCUP—Department of Biology, Faculty of Sciences, University of Porto, Rua do Campo Alegre, 4169-007 Porto, Portugal

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ABSTRACT

The European Water Framework Directive (WFD) represents a transformation of the guidelines for water quality assessment and monitoring across all EU Member States. At present, it is widely accepted that the WFD requires holistic and multidisciplinary ecological approaches by integrating multiple lines of evidence. Within the scope of the WFD, the scientific community identified clear opportunities to take advantage of an ecotoxicological line of evidence. In this context, ecotoxicological tools, namely biomarkers and bioassays, were proposed to contribute to the integration of the chemical and biological indicators, and thus to provide an overall insight into the quality of a water body. More than one decade after the publication of the WFD, we reviewed the studies that have attempted to integrate ecotoxicological tools in the assessment of surface water bodies. For this purpose, we reviewed studies providing an ecological water status assessment through more conventional community based approaches, in which biomarkers and/or bioassays were also applied to complement the evaluation. Overall, from our review emerges that studies at community level appear suitable for assessing the ecological quality of water bodies, whereas the bioassays/biomarkers are especially useful as early warning systems and to investigate the causes of ecological impairment, allowing a better understanding of the cause–effect-relationships. In this sense, community level responses and biomarkers/bioassays seem to be clearly complementary, reinforcing the need of combining the approaches of different disciplines to achieve the best evaluation of ecosystem communities' health.

1. Introduction

The European Water Framework Directive (2000/60/EC, hereafter WFD; European Commission, 2000) constitutes one of the most important European Union (EU) pieces of environmental legislation in the water field. It represents a transformation of the guidelines for water quality assessment and monitoring across all EU Member States in terms of protection and management of inland surface, transitional, coastal and ground waters. The inherent aim of the WFD is to protect and prevent deterioration of European waters on the basis of their ecological community structures and, therefore, it implicitly relies on a good knowledge of the ecosystem functioning under specific environmental conditions, an ambitious assumption considering the complexity and heterogeneity of aquatic ecosystems. This 'Ecosystem Approach' (not included in the previous directives on water quality assessment) is a

* Corresponding author. Tel.: +351 239 836386; fax: +351 239 823603.
E-mail address: monica.martinezharo@gmail.com (M. Martinez-Haro).

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reflection of Europe's increasing efforts to improve, protect and conserve aquatic ecosystems and it is in line with the aims of other European Directives (such as the Habitats and Species Directive—92/43/EEC, Marine Strategy Framework Directive—2008/56/EC, Environmental Impact Assessment Directive—2011/92/EU).

Under the WFD, monitoring both the chemical and ecological status is seen as an extremely important tool to evaluate progress towards the established environmental objectives and to achieve, by 2015, the main aim of 'good water status' for all EU waters. To best obtain an assessment of the ecological quality status (EcoQS), the WFD goes further by making a distinction among three modes of monitoring surface waters: (1) surveillance monitoring to assess long-term water quality changes, while providing data to design and implement future monitoring programmes; (2) operational monitoring to establish the status of those water bodies at risk of failing environmental objectives; and (3) investigative monitoring to ascertain the causes of a water body failing to achieve the environmental objectives.

Currently, while each type of monitoring programme is required to cover the status of water bodies through a number of quality elements (biological and physico-chemical elements together with chemical pollutants), the techniques, guidelines, protocols and assessment tools to be used are not fully specified being still under different degrees of discussion and development (Allan et al., 2006). Thus, the successful implementation of the WFD created – and is currently raising – new challenges for the scientific community. There is a need to integrate chemical and ecological information to better address the quality of individual water bodies (Dworak et al., 2005; Graveline et al., 2010; Mostert, 2003). At present, it is widely accepted that new ecological perspectives for the WFD require holistic and multidisciplinary approaches by integrating multiple lines of evidence (Burton et al., 2002; Chapman et al., 2002; de Jonge et al., 2006).

Within the scope of the WFD, the scientific community identified clear opportunities to take advantage of an ecotoxicological line of evidence within the ecological approach (Brack et al., 2005; Sanchez and Porcher, 2009; Triebskorn et al., 2001, 2003). Although the WFD monitoring programme involved the use of both chemical and biological parameters, the use of biological effects methods, namely biomarkers and bioassays, were proposed to contribute to the smooth integration of the chemical and biological information, and thus to provide an overall insight into the quality of a water body (Allan et al., 2006; Hagger et al., 2006). Biomarkers and bioassays are recognized as potentially important lines of evidence to establish cause–effect relationships in ecological quality assessment within the WFD (European Commission, 2009, 2010). More specifically, they improve the capability to ascertain the causes of a failing ecological status in a water body and whether pollutants are the cause for not achieving a 'good status', closing thus the gap between ecology and chemistry (ICES (International Council for the Exploration of the Sea), 2007; Maas and van den Heuvel-Greve, 2004). Consequently, there are clear opportunities for the integration of biological effects into the three types of monitoring programmes for surface water, especially in investigative monitoring, in order to provide a more realistic assessment of impacts and exposure of aquatic organisms to contaminants and to unravel the underlying mechanisms of disruption (Allan et al., 2006; Collins et al., 2012; de Jonge et al., 2006; Dworak et al., 2005; ICES (International Council for the Exploration of the Sea), 2011; Keddy et al., 1995).

More than one decade after the publication of the WFD, we reviewed the studies that have attempted to integrate biological effects methods in the assessment of surface water bodies. For this purpose, we examined studies providing an ecological water status assessment through a traditional approach, based on the status of biological quality elements, and in which ecotoxicological tools, namely biomarkers and/or bioassays, were also applied to complement the assessment.

1.1. Community based approaches to assess the ecological quality status under the WFD: Ecological indices

Under the ecological approach of the WFD the assessment of the quality of the biological elements is based upon community level measures that represent key community aspects of one or more different biological compartments of the ecosystem (i.e. phytoplankton, other aquatic flora, benthic invertebrate and fish). Among the various approaches available for assessing the quality of the biological elements, the most commonly used in many European countries are those based on ecological indices (Birk et al., 2012; Pinto et al., 2009).

Overall, ecological indices are numerical adimensional values expressing the general status of ecosystems through the description of different aspects of the structure and the sensitivity of communities (diversity, abundance, tolerance and/or its combination). These metrics are commonly based on taxonomic identification of organisms, from family to species level. In this sense, we follow the definition by Hyatt (2001), see also Pinto et al. (2009) of ecological indices, which are used as: "quantitative tools in simplifying, through discrete and rigorous methodologies, the attributes and weights of multiple indicators with the intention of providing broader indication of a resource, or the resource attribute(s), being assessed".

Following the publication of the WFD many efforts have been done to develop ecological indices and improve previously described ones to holistically assess the ecological status of water bodies. Nowadays a large number of indices have been developed and fine-tuned for this purpose (Borja and Dauer, 2008; Borja et al., 2009; Dauvin et al., 2010, 2012; Diaz et al., 2004; ICES (International Council for the Exploration of the Sea), 2004; Lyche-Solheim et al., 2013; Pérez-Domínguez et al., 2012; Pinto et al., 2009; Vačkář et al., 2012).

These indices can be classified as univariate (based on individual-species data or community structure measures, such as species diversity, richness, abundance) or multimetric (based on the combination of several metrics of community response to stress, and can be complemented with multivariate analysis methods (based on ordination or correlation analyses) to describe the assemblage patterns.

1.1.1. Reference conditions

A key point of the community approach consists in establishing specific reference conditions. Furthermore, the use of appropriate methods for setting reference conditions appears to be key in order to be able to detect pressures to assess the EcoQS with precision (Borja et al., 2012; van Hoey et al., 2013). These reference conditions must be specifically established not only for the general categories of surface water defined by the WFD (rivers, lakes, transitional waters, coastal waters and heavily modified or artificial water bodies) but also for the different types of water bodies within each category, in order to obtain a best approach for different geographical and habitat conditions.

To establish reference conditions, EU Member States are required to make decisions about what constitutes a minor human disturbance, which brings some technical and conceptual difficulties. On one hand, there is an enormous natural temporal variation (e.g., seasonal changes) in the physicochemical and biological characteristics within each water body (Beiras and Durán, 2013). On the other hand, there are ecological questions about the real meaning of a high status that can only be addressed under the perspective of societal values and other practical considerations (Pollard and

Huxham, 1998). The reference conditions became the basis for the classification schemes, with inherent consequences for all subsequent operational aspects of the WFD implementation (Borja, 2005; van Hoey et al., 2010).

Under the WFD, the type-specific reference conditions have been derived from a hierarchical approach using various procedures as follow: (1) a spatially based network of high status sites was applied, after this (2) modelling approaches using historical, palaeoecological and other data, and then (3) a combination of the spatial network and modelling approaches or (4) expert judgement, where the other methods cannot establish the reference conditions, were carried out. In fact, a combination of these procedures to set reference conditions was proposed as an adequate way to obtain final quality assessments related to the pressures (Borja et al., 2012).

1.1.2. Methodologies intercalibration—Harmonizing status classifications

Prior to the implementation of the WFD, the methodologies must be intercalibrated among the EU Member States within each eco-region to ensure consistency of classifications across the communities by establishing the boundaries between the different quality classes. The intercalibration aims at harmonising the water quality assessment across the large variety of water habitats in Europe (European Commission, 2008). The habitat heterogeneity of the water bodies covered by the EU Member States made the intercalibration of EcoQS between the different national monitoring programmes, a feasible but difficult exercise (Birk et al., 2013; Borja et al., 2007, 2009; von der Ohe et al., 2007), which is still unavailable for several/many types of water bodies.

For intercalibration purposes the EU area is divided into six ecoregions regarding to transitional and coastal waters, and into twenty-five for rivers and lakes (European Commission, 2000; Annex XI). Furthermore, once a surface water body (either rivers, lakes, transitional waters, coastal waters and heavily modified or artificial water bodies) has been identified as failing under the WFD, this must be defined according to two alternative typological systems (*system A or B*). These systems are described in Annex II of the WFD for each of the water body categories. In this sense, *system A* differentiates a surface water body first by the ecoregion to which it belongs and later, by surface water body types according to defined descriptors as altitude, geology or salinity, among others. On the other hand, *system B* differentiates a water body into types using the values of obligatory factors based on the location and boundaries descriptors of the water body; together with other optional factors including numerous physical descriptors (European Commission, 2000).

1.2. Ecotoxicological approaches—Biological cause–effect techniques

Changes in the structure of biological communities are not useful as a preventive tool, since they reflect the responses of the organisms once the alteration of the ecosystem already took place. Therefore, it is necessary to combine ecologically relevant indicators of anthropogenic disturbance with ecotoxicological tools able to provide early-warning signals that allow taking preventive measures before the ecological damage occurs. Within the present review the ecotoxicological approach is focused on the use of two types of biological assessment tools, namely biomarkers and bioassays. In the context of pollution monitoring and control, we understand biomarkers as “quantitative measurements of changes occurring at cellular, biochemical, molecular, or physiological levels, that can be measured in cells, body fluids, tissues or organs within an organism and that may be indicative of xenobiotic exposure and/or effect” (Vidal-Liñán and Bellas, 2013). While, the term ‘bioassay’ is here understood as *a procedure that uses living material to establish the relationship between the levels of chemicals and their adverse effects on populations, communities, and ecosystems, and to identify biological resources at risk* (e.g., Cairns and Pratt, 1989). Biomarkers and bioassays are intended to be sensitive (early-warning), rapid and cost-effective, compared to the monitoring of community level responses, but it must be kept in mind that both biomarkers and bioassays are conceived as monitoring tools to detect the potential risk of damage to the ecosystem by contaminants present in a given environmental compartment.

Response to environmental stress within a biological system may initially involve changes at the molecular level that may eventually lead to ecosystem scale impacts (Depledge and Fossi, 1994). By definition, metrics recorded using population abundances are sensitive to environmental stress only when deleterious effects altering the community structure have already taken place, and metrics based on occurrence are expected to be less sensitive. Carson (1962) warned us with a paradigmatic example of how the effects of persistent chemical pollutants impairing reproductive traits manifest only after long exposure periods, at a stage where the destructive process may have gone beyond the point where it can be easily reversed (see also Soares et al., 2009; Sumpter, 2005). But the toxicity of those pollutants can be easily identified and quantified measuring the appropriate biological responses (e.g., egg shell thickness, calcium metabolism) in environmentally or laboratory exposed organisms. Moreover, although responses at the community or population level are directly relevant in terms of ecological effects, it cannot always be proven that differences among sites are due to contaminants or to natural factors; i.e., as we ascend in the biological organization level we obtain ecological relevance but we lose specificity, speed and reproducibility as a routine technique for environmental monitoring. In this context, it is clear that biomarkers and bioassays offer, contrary to the ecological approach, early-warning signals reflecting the adverse biological responses towards anthropogenic environmental pollutants. In this case, responses measured at the lower levels of biological organization are usually sensitive and specific responses, indicative of effect and/or exposure to toxicants. For example disruption on feeding activity at the individual level can be related to ecosystem impairment directly by having an immediate effect at the functional level (e.g., organic matter decomposition), and also indirectly as effects on life history traits at the individual level (e.g., growth, reproduction, survival) that may be propagated to successively higher levels of biological organization (Amiard-Triquet, 2009; Baird et al., 2007; Forrow and Maltby, 2000).

1.2.1. Biomarkers

The development of biomarkers as ecotoxicological tools was motivated by the need for more sensitive early-warning indicators of sub-lethal ecological effects. Nowadays, a wide range of biomarkers is available which can reveal if a studied organism/population has been exposed to or affected by environmental pollutants and/or environmental stress. In comparison with chemical analyses, the biomarker approach has the advantage of providing information on the exposure and the effects of chemicals (even short-lived chemicals) on living organisms, while chemical analyses provide information about the presence and/or concentrations of the substances, which may not always be relate with a toxic effect. Moreover, aquatic organisms are usually exposed simultaneously to a wide range of chemicals rather than to individual substances. Also, current methods of chemical analyses are not adequate for detecting all possible pollutants and products of their transformation entering the marine environment. For this reason, biomarkers are frequently advocated to complement chemical analyses. Overall, biomarkers used in environmental monitoring are classified into two main categories: biomarker of exposure and effect.

Definition of these categories were provided by the WHO (World Health Organization) (1993): a biomarker of exposure is "an exogenous substance or its metabolite or the product of an interaction between a xenobiotic agent and some target molecule or cell that is measured in a compartment within an organism"; and a biomarker of effect is "a measurable biochemical, physiological, behavioural or other alteration within an organism that, depending upon the magnitude, can be recognized as associated with an established or possible health impairment or disease".

Briefly, examples of well-established biomarkers of exposure are the induction of proteins as metallothioneins, which respond following exposure to certain metal species, or cytochrome P450 monooxygenase system induced following exposure to organic pollutants such as aromatic hydrocarbons, polychlorinated biphenyls or dioxins. Biomarkers of effect are for example: the enzyme delta-aminolevulinic acid dehydratase (ALAD), inhibited even at small levels of lead; the cholinesterase enzymes (ChE), inhibited following exposure to organophosphates and carbamates pesticides and also to some non-essential metals; the comet assay or micronucleus assay to evaluate DNA or chromosomal damage, respectively, due to genotoxins; or imposex phenomenon (imposition of male secondary sexual characteristics on gastropods females) due to organotin compounds. Several reviews can be found regarding the different categories of biomarkers from different perspectives focusing, for example, on the target species (e.g., Depledge and Fossi, 1994; Monserrat et al., 2007; Valavanidis et al., 2006; van der Oost et al., 2003), the relationships at population and community levels (e.g., Boudou and Ribeyre, 1997; Cajaraville et al., 2000; Clements, 2000, Hyne and Maher, 2003; Lagadic et al., 1994), or the utility in environmental impact assessment (e.g., Depledge and Galloway, 2005; Galloway, 2006; Hagger et al., 2006; Schettino et al., 2012).

1.2.2. Bioassays

A bioassay is a biological method, alternative or complementary to a chemical analysis, intended to detect and measure the presence and/or effect of a substance. In general toxicological studies aim at quantifying the toxicity of individual chemicals or mixtures of known composition by exposing whole living organisms under standardized conditions over a certain period of time (Cairns and Pratt, 1989; Rand et al., 1995), the so called dose–response experiments. In environmental studies, ecotoxicological bioassays consist of the exposure of test organisms in controlled conditions to environmental matrices (water, sediment) whose toxicity is intended to assess, either in controlled conditions in the laboratory or under field conditions (in situ bioassays), and the measurement of ecologically-relevant quantitative responses. In order to obtain results relevant at the ecosystem level, the measured responses should have implications on the biological fitness of the individual (e.g., mortality, growth, reproduction, feeding rates; see e.g., Rand et al., 1995). For example, here, early life stages provide advantages related to sensitivity and ecological relevance (reviewed by His et al., 2000). The observed biological effect in a bioassay is generally the result of the bioavailability of a complex mixture of pollutants that may be present in a sample of water/sediment, but is also dependent on physicochemical parameters of the environment (Keddy et al., 1995). Hence, because the environmental conditions of an ecosystem are difficult to replicate in the laboratory, in situ bioassays, provide a more realistic exposure scenario than conducted under laboratory-controlled conditions, by integrating major natural fluctuating environmental variables, which is particularly relevant for ecosystems where environmental conditions are highly variable (Allan et al., 2006; Crane et al., 2007; Wharfe et al., 2007).

The use of liquid and solid-phase bioassays in environmental risk assessment and management has a long international history particularly in North America (e.g., Environmental Protection Agency—USEPA), but also in marine environmental monitoring in many other countries (Bougis et al., 1979; Kobayashi, 1991; Vashchenko and Zhadan, 1993), and over the past two decades has also been established within some European programs (e.g., OSPAR Coordinated Environmental Monitoring Programme, UK National Marine Monitoring Program).

Adverse effects include both lethal and sub-lethal effects, being the latter, currently the most common effects measured in aquatic organisms. Chronic toxic effects may occur when the chemical produces deleterious sub-lethal effects as a result of a single exposure, but more often they are a consequence of repeated or long-term exposures to low levels of persistent chemicals, alone or in combination. In this case, the most common sub-lethal effects in aquatic organisms are behavioural (e.g., swimming and feeding) and physiological (e.g., growth, embryo and larval development, reproduction). Some sub-lethal effects may have little or no effect on the organism because they are rapidly reversible or diminish or cease with time, or in contrast, they may indirectly result in mortality, e.g., changes in swimming behaviour may diminish the ability of aquatic organisms to find food or to escape from predators.

2. Comparing methodologies

A literature search was conducted on articles published to 2014 through the online database SCOPUS, by using the following keywords: 'WFD, ecological indices, biomarker, bioassay, ecological status and quality status'. In addition to the database search, we screened the reference lists of the retained papers to identify additional relevant studies. Fifteen studies addressed the EcoQS assessment under the WFD using an ecological (based namely on ecological indices classified under the different ecological quality classes defined in the Directive) and, concomitantly, an ecotoxicological approach (namely through the use of biomarker and/or bioassays techniques). Table 1 summarizes these studies on which we developed this section. Additionally, an interesting work that we thought has to be highlighted was that developed by Hagger et al. (2008). Although in the latter study the ecological quality assessment was not based on biological quality elements, the authors considered point-source pollution, alien species and hydromorphological factors. Thus, this study has been included due to its complete approach based on the assessment of biological-effects endpoints (at different biological organization levels) to classify the ecological health of aquatic ecosystems.

Most studies based the EcoQS assessment on one of the biological elements recommended by the WFD for the corresponding water body type. Among the different biological elements, those based on benthic invertebrate fauna were the most frequently used, being represented in 14 of the revised works. In fact, benthic invertebrates are one of the key biological components considered for the assessment of benthic integrity in the context of the WFD, being one of the most prevalent functional groups used in aquatic assessment (Birk et al., 2012). Regarding the use of biomarkers and bioassays, none of the studies included both types. Furthermore, we found a balanced use of both types of tools in the revised studies: seven studies addressed the use of biomarkers and eight that of bioassays. In the studies addressing biomarkers, invertebrates (molluscs and arthropods) represented the target species in 4 of the 7 studies. Biomarkers in vertebrates (namely fish) were applied in 3 of the 7 studies. In all studies addressing biomarkers, the set of responses evaluated intended to capture and establish cause–effect relationships. Among the most used biomarkers are those related to oxidative stress (e.g., enzymes related to GSH; Mayon et al., 2006; Prat et al., 2013) and tissue damage (e.g., both at lipid and DNA levels; Damásio et al., 2011). Additionally, some studies included

Table 1
Studies assessing the ecological quality status of water bodies based simultaneously on ecological indices and ecotoxicological assays (namely biomarkers and/or bioassays).

System*	Country	Community	Biological elements**	Bioassay	Biomarker	Species	Comparison	References
R	Germany	IBI	BMI, F		Set	Fish	Multivariate statistics	Böhmer et al. (2001), Dietze et al. (2001), Triebskorn et al. (2001), (2003)—VALIMAR
R	UK	Shannon index–H′, ASPT, BMWP, EQI N-taxa, EQI ASPT	BMI	In situ feeding rate		Amphipods	Pearson, least-squares regression	Maltby et al. (2002)
CW	Spain	AMBI, BENTIX, RBI, ABC	BIM	Embryo toxicity test		Sea urchins	Pearson correlation	Marín-Guirao et al. (2005)
R	Belgium	IBI	F		Set	Fish	Simple comparison	Mayon et al. (2006)
R	Spain	IPS, IBMWP	BMI		Set	Fish	Simple comparison	Damásio et al. (2007)
CW	Spain	Previous works (M-AMBI)	All	Microtox 10-d mortality		Bacterium, Amphipods	Simple comparison	Borja et al. (2008)
CW	Spain	H′, Margalef's d, RBI, EBI	BMI	Embryo toxicity test		Sea urchins	Simple comparison	Cesar et al. (2009)
R	Germany, Belgium, Spain	AQEM assessment system	BMI	LC50		Cladoceran, Algae, Fish	Correlation	von der Ohe et al. (2009)—MODELKEY
R	Germany	Multimetric index	BMI	Embryo toxicity test		Fish	Simple comparison	Bartzke et al. (2010)
R	Spain	QBR, IHF, IBMWP, IASPT	BMI		Set	Trichoptera	PCA	Damásio et al. (2011)
TW	Italy	H′, Evenness index, Simpson index, Margalef index—d, R-MaQI	A, BMI, F	*Vibrio fischeri Corophium orientale Parancentrotus lividus*			Weight-of-evidence	Micheletti et al. (2011)
TW	Portugal	AMBI, M-AMBI	BMI		Set	Decapod	PCA	Pereira et al. (2012)
TW	France	IFREMER* (AMBI, BENTIX, BOPA, BQI)	BMI		Set	Bivalve	Simple comparison	Tankoua et al. (2012) Dauvin (2007)
TW	Portugal	M-AMBI-EDI	BMI, F	ToxScreen		Bacterium	PCA, scaled value index	Azevedo et al. (2013)
R	Spain	IBMWP, IMMiT	BMI		Set	Trichoptera	Simple comparison	Prat et al. (2013)
Other related study								
TW	UK	Environment Agency of England and Wales	O		Set	Bivalve	Simple comparison	Hagger et al. (2008)

* Water body type: R—river, L—lakes, TW—transitional waters, CW—coastal water.
** P—phytoplankton, OF—other flora, BMI—benthic macroinvertebrates, F—fish, All—all the biological elements, O—other (point-source pollution, alien species and hydro-morphological).

a wide range of biomarkers covering different levels of organization or biological response, below cell level (Dietze et al., 2001; Triebskorn et al., 2001, 2003). Regarding bioassays, embryo toxicity tests (sea urchin and fish) were the most used (e.g., Bartzke et al., 2010; Marín-Guirao et al., 2005), being represented in four of the 8 studies, followed by bacterium tests represented in three of the studies (e.g., Azevedo et al., 2013; Borja et al., 2008).

Overall, most studies were carried out in rivers and transitional waters (8 out of 15 and 4 out of 15, respectively) and only three in coastal waters (Table 1). Studies on lakes, however, were not found. The number of studies assessing the status of transitional waters was high despite that estuarine science has been considered as the "poor relation" of the aquatic research for many years (Elliott and Whitfield, 2011). This could be due to the fact that transitional waters are natural environments difficult to classify and assess due to their inherent variability (Dauvin, 2007; Elliott and Quintino, 2007). In fact, we found the first studies addressing rivers in 2001 (Böhmer et al., 2001—VALIMAR project), and was not until 2008 when the first works for transitional waters appeared (Borja et al., 2008). Furthermore, Borja (2005) pointed out the little attention that has been put into coastal and transitional waters in relation to the WFD: since 1999, the number of scientific papers published in peer-review journals on WFD in marine ecosystems (including transitional waters), only represented 10% of the total papers published over the period 1998–2004. Currently this percentage increased to 17% (SCOPUS—March 2014—key words: "Water Framework Directive" and "transitional waters" or "estuaries" or "coastal" or "marine", over the period 1998–2014).

To address both approaches together, there are methodologies from simple comparisons based on positive/negative results (e.g., Bartzke et al., 2010; Damásio et al., 2007; Mayon et al., 2006), to complex methodologies based on multivariate statistics (Dietze et al., 2001), principal component analyses (PCA; Azevedo et al., 2013; Damásio et al., 2011; Pereira et al., 2012), or the integrated weigh-of-evidence methodology (Micheletti et al., 2011). The different methodologies used to integrate the assessment from ecological and ecotoxicological approaches, makes difficult the comparison among studies and the establishment of standardized analytical protocols to carry out the integration. In the studies in which more complex statistical analyses were performed, the use of ecotoxicological tools was in many cases useful to distinguish more clearly the most environmentally disturbed sites (Pereira et al., 2012). In the studies developed by Maltby et al. (2002) and/or Marín-Guirao et al. (2005), a formal comparison of results obtained under both approaches was performed using Pearson correlations and/or least squares regression analysis. The analysis of raw results, in different ways, is the most common procedure used in the revised studies to treat data from ecotoxicological tools. For example, in the study developed by Cesar et al. (2009), data from both approaches were integrated through PCA to characterize the ecological quality of the study area. Despite this, a formal comparison (i.e. namely using statistic methodologies) between community level responses and ecotoxicological results was not performed. Similarly, a formal comparison was not provided in Borja et al. (2008), Damásio et al. (2007), or Mayon et al. (2006).

Other studies classify the ecotoxicological values into the recommended categories to assess the EcoQS of waters under the WFD (High, Good, Moderate, Poor and Bad) as is applied for ecological indices. For example, for the sea urchin embryo toxicity test, Marín-Guirao et al. (2005) associated the results obtained from the toxicity bioassays to the five EcoQS dividing by five the percentage of normally developed embryos (from 0 to 100%). After this, the established threshold values were tested against several sediment quality attributes. Then, the results from ecological indices and ecotoxicological analyses were analysed through Pearson correlations. Similarly, Bartzke et al. (2010) developed an index based on fish embryo toxicity test, the fish teratogenicity index. Observed effects were weighted according to the effect intensity and mathematically combined in an index. Finally, after checking for statistical differences between the sample and the reference values, five quality thresholds for the values of the index were determined according to the WFD. Under a similar approach, Hagger et al. (2008) constructed a biomarker response index to simplify the complex biological alterations measured by multiple biomarkers into a single predefined quality class. Their index was composed by biomarkers at different levels of biological organization, weighted accordingly and ranked based on the biological responses determined from previous laboratory and field studies. The values obtained were then classified into four categories of biological impact to assess the degree of alteration from normal/reference responses, as recommended under the WFD for ecological status.

From the studies outlined above, it emerges the wide heterogeneity between the revised studies, makes difficult obtaining comparable information. We could not find a standardized approach to carry out a formal review, although it was possible to extract several points that are discussed below.

3. Ecological status combining community and ecotoxicological based approaches

Community and ecotoxicological approaches were congruent in 60% of the studies revised. First evidences came from the integrated, multi-level approach of the VALIMAR project (Triebskorn et al., 2001, 2003). Active and passive monitoring experiments along with the study of indicators at different levels of organization, i.e. biochemical, cellular, individual and population-level, were addressed. Results of the VALIMAR project showed that the difference in ecological quality between the two selected streams can be detected by most of the biomarker responses in fish (Böhmer et al., 2001; Dietze et al., 2001; Triebskorn et al., 2001, 2003). Furthermore, although macrozoobenthos communities indicated much clearer long-term differences between the sites than biomarkers, which showed much more temporal variation (Böhmer et al., 2001), the studied biomarker responses were related to effects in fish populations as well as in macrozoobenthic communities (Triebskorn et al., 2001, 2003). Beyond this project, similar results were described by Maltby et al. (2002) through a simpler approach. A single short-term bioassay (amphipod in situ feeding assay) was applied in different rivers in England and Scotland. The correlation detected between the selected bioassay and the biological quality assessment of the studied rivers, suggested its utility as indicative of community and ecosystem level responses over longer time periods in rivers.

A clear correlation between the assessment of biological communities and ecotoxicological indicators was also detected in historically polluted coastal areas where metal concentrations exceed background levels for the corresponding area (Borja et al., 2008; Cesar et al., 2009; Marín-Guirao et al., 2005). In these studies, the response of bioassays based on invertebrate (namely sea urchin embryo bioassay, amphipod bioassay) and bacterial responses (Microtox® assay), fitted satisfactorily with the ecological assessment performed with ecological indices. More specifically, bioassays supported the findings of the ecological indices, being able to quantify and estimate the cumulative effects of multiple stressors on benthic biota between the low-and high-impacted zones (Cesar et al., 2009; Marín-Guirao et al., 2005). In contrast, indices based on pollution resulting from organic enrichment might not be as successful as in the case of purely toxic pollution (Pinto et al., 2009).

Similarly, Damásio et al. (2011) and Pereira et al. (2012), found some relationship between the ecological status based on invertebrate and biomarker responses in moderately contaminated sites. Through the factor analysis applied to all data sets, biomarkers were able to detect different responses at some of the sites, which allowed to discriminate between contaminated and uncontaminated sites. In addition, the inclusion of more markers belonging to different metabolic pathways increased substantially the discrimination power among sites (Damásio et al., 2011; Pereira et al., 2012). Hence, an integrated approach appears to be particularly promising in monitoring programmes designed for specific descriptors used in the WFD, by proving the possibility to discriminate the environmental quality among sites of moderate degradation.

The environmental quality assessment based on macrobenthic communities alone, sometimes led to unclear results in some studies such as, for instance, Cesar et al. (2009), Marín-Guirao et al. (2005), and Pereira et al. (2012). These authors report that despite some signs of poorer benthic quality have been detected in the respective study areas, differences among sites were not significant, and therefore a biotic index can inform about a good environmental status of the affected sites. Thus, the selection of an appropriate ecological index with the ability to correctly characterize the study area, is one of the most difficult tasks. Although ecological indices may provide a good assessment of the status of the biological environments, a universal index efficient in all systems (or at least in systems of the same ecological type), appears difficult to achieve (Pinto et al., 2009). This may be related with the complexity of biological communities are the fact that not all organisms are equally sensitive to the same types of anthropogenic disturbance, and therefore might respond differently to diverse types of stressors (Birk et al., 2012; van Hoey et al., 2013), their assessment can be readily accommodated in modified community assessments for some pollutants (Peters et al., 2014a,b). In this sense, the combination of different approaches considering several components (such as chemical analyses, toxicity assessments, and biological elements) at different levels of organization can provide an integrated view of the environmental stresses affecting an ecosystem.

Conversely, Damásio et al. (2007), Prat et al. (2013), Tankoua et al. (2012), and von der Ohe et al. (2009), did not find consistent results between both approaches. Damásio et al. (2007) did not detect effects based on two ecological indices (one based on benthic diatoms and other in benthic macroinvetebrates), after an accidental fuel oil spill in a river, but a clear evidence of exposure to the spilled fuel oil was observed using fish biomarkers. Furthermore, one of the biomarkers affected (EROD activity) has been suggested to precede effects at various levels of biological organization (Whyte et al., 2000), from which an additional ecological relevant adverse effect can be also suspected. The lack of a similar response between both approaches could be related to sensitivity differences among trophic levels (see e.g., Comber et al., 2011). Although population and community parameters are more ecologically relevant and reflect integrated conditions over a long time period, the ecological community approach often lacks specificity since it may be affected by environmental factors (other than pollution) related to the variability within and between ecosystems (such as river morphology; Triebskorn et al., 2001), especially for metrics which rely upon predicted reference conditions. Thus, in the case of Damásio et al. (2007), it is possible a few weeks after the spill the biological communities did not had time to react but the responses on fish biomarkers were evident even a few months after the spill. Their study highlights some of the advantages of incorporating ecotoxicological tools in environmental quality assessment monitoring programs.

Similarly, the study carried out by Prat et al. (2013), aiming to evaluate the effect of introducing reclaimed water from a wastewater treatment plant on the river quality in a polluted river basin, showed that most of the metrics selected to assess the biological quality indicated a slight biological impairment after the introduction of the treated water, even though the ecological status remained poor. However, indicators of additional stress to the populations were found using several biomarkers, suggesting a potential of further deterioration of the ecological status of the river. Hence, the authors concluded that structural indicators are unable to indicate further impairment in polluted rivers, for which biomarkers may be a useful tool to detect environmental impoverishment. This suggests that through appropriate sampling/analyses, biomarkers could response to a selection of stressors being strongly related with ecologically relevant endpoints.

Finally, in the study developed by Tankoua et al. (2012) intersex (i.e. a variation in sex characteristics including chromosomes, gonads, and/or genitals that do not allow an individual to be distinctly identified as male or female) in a bivalve was detected in all estuaries studied. Although no causal relationship was demonstrated between intersex and population effects, intersex was detected at all studied estuaries, even in those estuaries where the chemical and ecological status was considered "good" according to the criteria of the WFD. Since not all substances suspected to affect the endocrine system were analysed at each site, it could be not ruled out the hypothesis that, even in the systems classified as "good ecological status", some chemicals could be present at concentrations high enough to disrupt gonad development. Hence, the subtle effects of endocrine disrupting chemicals was pointed out, which can be active at very low doses, often in the absence of any other sign of toxicity. Therefore the study suggested the need to incorporate in environmental monitoring, early-warning systems that can anticipate the impact at higher level of biological organization.

Based on the revision detailed above, it emerges that both approaches (community and ecotoxicological tools) appear to relate well in aquatic systems under a constant source of pollution, such as historically polluted systems (e.g., Borja et al., 2008; Marín-Guirao et al., 2005). However, this congruence is absent in those studies in which a point source of pollution was addressed, such as uncontrolled spills or leaks (e.g., Damásio et al., 2007; Prat et al., 2013). In this sense, biomarkers and bioassays seem to be more sensitive than ecological indices as early-warming system of stressors that may potentially impact ecosystem function. Given these characteristics, ecotoxicological tools appear to be useful within the three different monitoring programmes outlined by the Directive, particularly in operational and investigative monitoring.

The progress made in standardization of biomarker and bioassays provides us with a broad set of monitoring tools adequate to evaluate the wide diversity of pollutants encountered under the WFD (e.g., biological active substances, metals, pesticides or nutrients among others; European Commission, 2000; Annex VIII). Nevertheless, the numbers of ecotoxicological tools are not the same for different stressors (e.g., there are more ecotoxicological tools developed to screen the effect of priority pollutants such as heavy metals, PAH, PCBs, than emerging pollutants), and therefore further research is needed to develop and validate ecotoxicological tools as early-warming systems of emerging pollutants of concern.

Overall, we believe that ecotoxicological and community based indicators should be utilized in a combined way. The ecotoxicological tools seems to be more valuable as early-warming systems and to identify the nature of the stressor since they are mostly based on the measurements of a biological responses. Although community based approaches cannot be effective to detect a specific stressor they are able to assess a general trend in ecological relevant endpoints. Moreover, the community based approaches are essential to evaluate environmental factors other than chemical pollution that can affect the ecosystems, such as habitat loss, in which other drivers than pollutants are usually involved.

4. Conclusion

From the literature review it emerges that investigations at community level appear suitable for the assessment of ecological quality, whereas bioassays/biomarkers appear to be specially useful to investigate the causes of ecological impairment, allowing a better understanding of the cause–effect-relationships and to work as early warning, rapid evaluation and cost effectiveness systems of ecosystem disturbance. In this sense, community level responses and ecotoxicological approaches seem to be complementary reinforcing the need to use combined approaches of different disciplines in order to achieve the best evaluation of the ecosystem community health.

The European regulatory authorities are presently in the phase of implementing the WFD all over Europe, and results will be assessed in the next few years, which will eventually lead to the introduction of improvements and changes in the Directive. It might then become pertinent to stand for the combined/complementary use of ecological indices, biomarkers, and bioassays in assessing ecological quality status. Further research will improve current knowledge and allow methodological and data analysis harmonization, aiming at generalization to other assessment and classification schemes worldwide.

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