Hydrobiologia (2009) 633:181–196
DOI 10.1007/s10750-009-9881-y

EUROPEAN SURFACE WATERS

Opinion Paper

Current status of macroinvertebrate methods used for assessing the quality of European marine waters: implementing the Water Framework Directive

Angel Borja · Alison Miles · Anna Occhipinti-Ambrogi · Torsten Berg

Published online: 29 July 2009

Abstract This contribution reviews the current status of the macroinvertebrate methodologies proposed for European coastal and transitional waters under the Water Framework Directive (WFD), showing the weaknesses and strengths of the WFD implementation process and proposing future research topics and challenges. In total, 12 different methodologies have been officially accepted by European Member States (MSs). Most of these methods are multimetric, i.e. including several metrics into an equation, others are multivariate and some others are univariate. The methodologies vary in their use of the parameters included in the WFD (e.g. disturbance-sensitive species composition, richness, diversity, density, etc.), and they are described in this contribution. The results from the intercalibration undertaken by MSs are shown, including the boundaries between the quality classes, for each European eco-region and type. Finally, four areas in which scientific agreement is needed to satisfy future macroinvertebrate quality management are identified and discussed: (i) reduction of the present bewildering array of available indices by identifying the index approaches, components and formulations that are most widely successful; (ii) establishing minimum criteria for index validation processes that demonstrate index accuracy and reliability; (iii) comparing and intercalibrating methods to achieve uniform assessment scales across geographies and habitats and (iv) integrating indices across media and ecosystem elements.

Keywords Water Framework Directive · Ecological status · Macroinvertebrates · Methodologies · Intercalibration · Europe

Guest editors: P. Nõges, W. van de Bund, A. C. Cardoso, A. Solimini & A.-S. Heiskanen
Assessment of the Ecological Status of European Surface Waters

A. Borja (✉)
AZTI-Tecnalia, Marine Research Division, Herrera Kaia, Portualdea s/n, 20110 Pasaia, Spain
e-mail: aborja@pas.azti.es

A. Miles
Environment Agency (EA), Kingfisher House, Goldhay Way, Orton Goldhay, Peterborough PE2 5ZR, UK

A. Occhipinti-Ambrogi
DET - Dip. di Ecologia del Territorio, Sezione di Ecologia, Universitá degli Studi di Pavia, Via S. Epifanio 14, 27100 Pavia, Italy

T. Berg
MariLim Gewässeruntersuchung, Heinrich-Wöhlk-Straße 14, 24232 Schönkirchen, Germany

Introduction

The European Water Framework Directive (WFD; 2000/60/EC) establishes a basis for the protection and improvement of transitional (i.e. estuarine) and coastal waters, amongst other systems. Its final objective is to achieve not less than 'good ecological status' for all waters, by 2015 (Directive, 2000; Borja, 2005). Ecological assessment is based upon the status of biological, hydromorphological and physico-chemical quality elements. The biological elements to be considered are phytoplankton, macroalgae, angiosperms, benthic macroinvertebrates and, in transitional waters only, fish.

Ecological status (ES) of a water body is determined by comparing data obtained from monitoring networks (Ferreira et al., 2007) with reference (undisturbed) conditions, thus deriving an ecological quality ratio (EQR). The EQR is expressed as a numerical value lying between 0 and 1; 'High status' is represented by values close to 1, whilst 'Bad status' values lie close to 0. The range is divided into five ES classes, 'High', 'Good', 'Moderate', 'Poor' and 'Bad'. This legislative demand presents substantial challenges for scientists, who are asked to deal with 'non-scientific' concepts. 'Good' or 'Poor' ES is immediately and clearly understandable by everyone, yet at the same time absolutely vague when they have to be translated into a set of rules or quantified into a numerical value. Therefore, different experts may have a different definition of them.

The normative definitions (Annex V) of the WFD describe the parameters of the biological quality elements that must be included in the ES assessment of a water body. For the marine macroinvertebrate community, these include composition and abundance of invertebrate taxa and the proportion of disturbance-sensitive and tolerant taxa. Following these criteria, several indices and approaches have been published to assess the benthic invertebrate ES (e.g. Borja et al., 2000, 2004a, 2007; Simboura & Zenetos, 2002, Rosenberg et al., 2004; Muxika et al., 2007; Perus et al., 2007; Meyer et al., 2008).

Proposed methodologies should be applicable to the range of types into which the main European eco-regions are divided (see CIS, 2003; Borja et al., 2004a; Heiskanen et al., 2004; European Commission, 2008; GIG, 2008). The purpose of defining these types is to enable type-specific reference conditions to be established, making it possible to assess the ES for different geographical and habitat conditions. These type-specific reference conditions are the basis of the classification schemes, and, as such, impact on all subsequent aspects of the implementation of the WFD (including intercalibration of the quality class boundaries assessed by different methodologies, assessment of the quality status of each of the biological elements, and monitoring, assessment and reporting of the water body status).

The objective of this contribution is to review the current status of the macroinvertebrate methodologies proposed for European waters under the WFD, showing the weaknesses and strengths of the WFD implementation process and proposing future research topics and challenges.

Some of the information in this contribution have been obtained from the European Commission (2008) and the Geographical Intercalibration Group (GIG) reports (2008), available at http://circa.europa.eu/Public/irc/jrc/jrc_eewai/library.

European types

The European maritime area has been divided into four eco-regions (Fig. 1): (i) the Atlantic/North Sea Eco-region Complex comprising the Northeast Atlantic Ocean, North Sea, Norwegian Sea and the Barents Sea; (ii) the Baltic Sea Eco-region; (iii) the Mediterranean Sea Eco-region and (iv) the Black Sea (CIS, 2003; Casazza et al., 2003; Borja, 2005). These eco-regions have been used as the basis for the intercalibration groups (GIG, 2008), with the objective of harmonising the process of water quality assessment across the large variety of marine habitats in Europe.

Under the WFD, each eco-region must be divided into types, which can be defined according to two alternative typology systems. System A uses eco-region, salinity and mean depth as determination factors. System B uses latitude, longitude, tidal range and salinity as obligatory factors; together with current velocity, wave exposure, mean water temperature, mixing characteristics, turbidity, retention time (in the case of enclosed bays), mean substratum composition and water temperature range as optional factors.

As stated by Borja (2005), when scientists are confronted with establishing types, their first reaction is often to account for a high number of very specific types (resulting from the combination of the different geomorphological and hydrodynamic characteristics included in the definition). However, this approach produces an unmanageable situation in subsequent

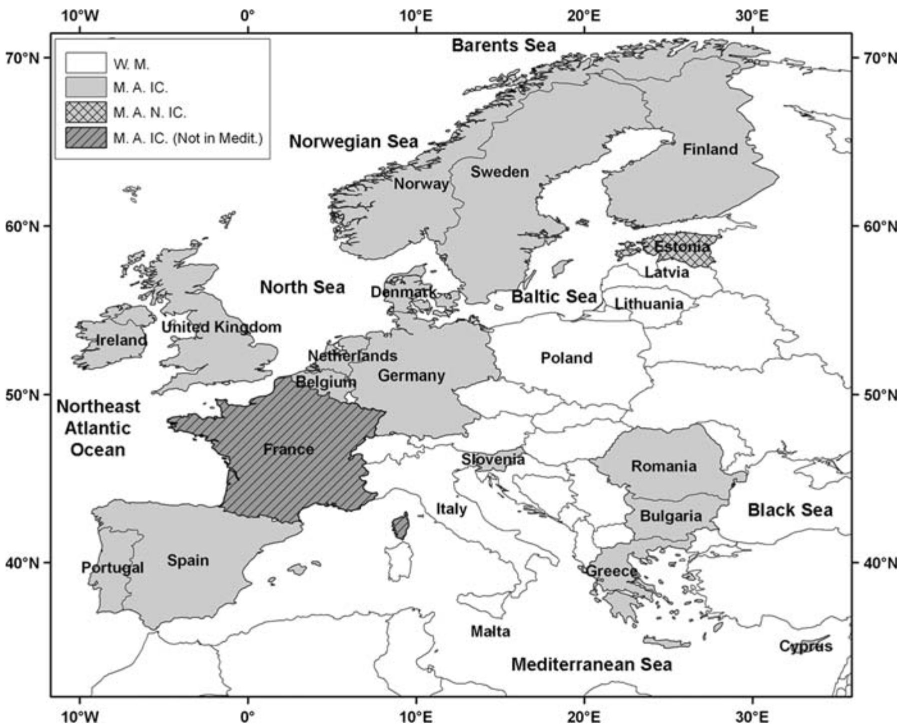

Fig. 1 Map of Europe showing eco-regions and countries applying the WFD (those with names) and those which have approved methodologies for macroinvertebrate quality assessment. Key: W.M.—without method agreed; M.A.IC.—with method agreed and intercalibrated (France has a method agreed and intercalibrated within the Atlantic, but not in the Mediterranean); M.A.N.IC.—with method agreed, but not intercalibrated

steps of the WFD, e.g. the establishment of the monitoring and management plans for each of the water bodies and types.

One of the most common criticisms of WFD typology implementation arises from the over-simplicity of the approach, from a scientific point of view, because of the heterogeneity of habitats within the different types and water bodies (see Borja, 2005). From the dozens (even hundreds) of potential types across Europe, only 18 coastal types have been intercalibrated (Table 1). These are divided into 6 types in the Baltic Sea; 10 types in the Atlantic Sea; 1 type in the Mediterranean Sea; 1 type in the Black Sea (European Commission, 2008; GIG, 2008).

These types reflect, at a certain level, the main eco-region characteristics. Hence, (i) Baltic Sea types present generally different salinity values, ranging from marine waters to nearly freshwater, are sheltered coasts, and experience some ice cover in winter; (ii) Atlantic Sea types reflect the wide latitudinal range of this eco-region, with very different characteristics and (iii) the Black Sea type reflects the main influence of Danube River upon the coastal salinity.

In turn, within the Mediterranean Sea, the data available did not allow a complete intercalibration among the Member States (MSs) that participated in the IC exercise; only one type (representing shallow sedimentary bottoms, not the prevailing one, among the four initially selected) could be intercalibrated.

Currently, there has been no intercalibration of ES assessment systems for transitional water bodies, as typifying and intercalibration of these waters on a European scale represent huge challenges, due to the heterogeneity of waters which include both estuaries of very different sizes and coastal lagoons with a large variety of connections to the open sea and to continental waters.

On the other hand, hard bottom communities have not yet been intercalibrated, mainly because sampling methods, significance of presence/absence and density of species, and methods for quality assessment are profoundly different from those in use with mobile sedimentary bottoms.

Methodologies in assessing benthic quality status

The benthic invertebrates are well-established components in environmental quality status assessments, with various studies having demonstrated that the benthic macroinvertebrates respond relatively rapidly to anthropogenic and natural stress (Pearson &

Table 1 Types intercalibrated in Europe, at each of the eco-regions (seas), characterised using different variables, such as salinity, wave exposure, water depth, tidal range, current velocity, ice cover, water mixing and residence time

Seas	Type	Characterisation	Salinity (PSU)	Exposure	Depth (m)	Tidal range (m)	Current velocity(knots)	Ice cover (days)	Mixing	Residence time
Baltic	B0	Bothnian Bay (Northern Quark)	0.5–3	Sheltered	<30			>150		
	B2	Bothnian Sea	3–6	Sheltered	<30			>150		
	B3 a	Southern Bothnian Sea, Archipelago Sea, western Gulf of Finland	3–6	Sheltered	<30			90–150		
	B3 b	Southern Bothnian Sea, Archipelago Sea, western Gulf of Finland	3–6	Exposed	<30			90–150		
	B12 a	Eastern Baltic Sea	5–8	Sheltered	<30					
	B12 b	Western Baltic Sea	8–22	Sheltered	<30					
Atlantic	NEA1/26a	Open oceanic	>30	Exposed or Sheltered	<30	1–5	1–3		Fully mixed	Days
	NEA1/26b	Enclosed seas	>30	Exposed or Sheltered	<30	1–5	1–3		Fully mixed	Days
	NEA1/26c	Enclosed seas	>30	Exposed or Sheltered	<30	<1–5	1–3		Partly stratified	Days to weeks
	NEA1/26d	Scandinavian coast	>30	Exposed or moderately exposed	<30	<1	<1		Partly stratified	Days to weeks
	NEA1/26e	Areas of upwelling	>30	Exposed or Sheltered	<30	1–5	1–3		Fully mixed	Days
	NEA3/4	Wadden Sea type	18–30	Exposed or moderately exposed	<30	1–5	1–3		Fully mixed	Days
	NEA7	Deep fjordic and sea loch systems	>30	Sheltered	>30	1–5	<1		Fully mixed	Days
	NEA8	Skagerrak Inner Arc Type	18–30	Sheltered	<30	<1	<1		Partly stratified	Days to weeks
	NEA9	Fjord with a shallow sill at the mouth	18–30	Sheltered	>30	<1	<1		Partly stratified	Weeks
	NEA10	Skagerrak Outer Arc Type	18–30	Exposed	>30	<1	<1		Partly stratified	Days
Mediterranean	M3	Sedimentary shallow coast	>30		<40					
Black Sea	BL1	Black Sea	18–30	Moderately exposed	<30	<1				

Data extracted from European Commission (2008) and GIG (2008)

Rosenberg, 1978; Dauer, 1993). Several authors have reviewed the use of indices for assessing the benthic 'health' of a system (Díaz et al., 2004; Pinto et al., 2009). Some (e. g. Washington, 1984) contend that an index is unlikely to be universally applicable, as organisms are not equally sensitive to all types of anthropogenic disturbance and are likely to respond differently to various types of perturbation. Several methods have been published to assess the benthic macroinvertebrates ES in European marine waters (see complete names in Table 2): AMBI (AZTI's Marine Biotic Index) and M-AMBI (Borja et al., 2000, 2004a; Muxika et al., 2007); Bentix (Simboura & Zenetos, 2002); BQI (Rosenberg et al., 2004). Moreover, many others have been proposed during the WFD intercalibration exercise (Borja et al., 2007), but are only published as annexes to the WFD reports (GIG, 2008) and by the European Commission (2008).

In total, 12 different methodologies have been officially accepted by European MSs (Table 2). Most of these methods are multimetric, i.e. combining several metrics into an equation (e.g. BQI, DKI, IQI, MarBIT, NQI, etc.), others are multivariate (e.g. M-AMBI and P-BAT) and some are univariate (e.g. MEDOCC and Bentix). Some countries use different methodologies within different eco-regions (e.g. Germany, in Northeast Atlantic and Baltic).

The methodologies vary in their use of the parameters outlined in the WFD normative definitions; some of them utilising all metrics prescribed by the Directive, some only parts thereof and some incorporating different parameters. Hence, for (i) the proportion of disturbance-sensitive taxa, 13 countries (methods) use AMBI as the indicator, 3 the BQI, 2 the Bentix and 2 other methods; (ii) the level of diversity, 11 countries use Shannon's index, 2 Simpson's index and 1 ES_{100} (Hurlbert, 1971) and (iii) abundance of invertebrate taxa, 6 incorporate density, 14 richness, 1 Margalef index, 1 abundance distribution and 2 incorporate biomass (Table 2). In addition, two countries incorporate similarity and one uses feeding guilds.

The selection, from a list of ecologically relevant candidate metrics to be used within any methodology, should emphasise metrics that are sensitive (respond to anthropogenic action—both degradative and restorative) and representative (able to measure status and trends relative to policy decisions and management actions) (after Borja & Dauer (2008)). In general, the metrics used in the intercalibrated European methodologies are based upon community level characters that represent key community aspects and these characteristics are related to the normative definitions required by the WFD.

Although most of the benthic methods have only been applied to coastal waters, some of them are also being used or are being further developed for use, within transitional waters, such as M-AMBI (Muxika et al., 2007; Borja et al., 2009c), IQI (Prior et al., 2004) or Bentix (Simboura & Reizopoulou, 2008). At the same time, a plethora of new methodologies is being proposed across Europe for water categories (lagoons and coastal and transitional waters), types or habitats; some of which are mentioned below.

(i) Multimetric indices based on macroinvertebrates and aquatic flora, for lagoons: the Ecofunctional Quality Index (EQI) (Fano et al., 2003) and the Fuzzy Index of Ecosystem Integrity (FINE) (Mistri et al., 2007, 2008).
(ii) Daphne, a multimetric index for coastal waters (Forni & Occhipinti-Ambrogi, 2007).
(iii) Brackish Water Benthic Index (BBI), for Baltic Sea (Perus et al., 2007).
(iv) Benthic Opportunistic Polychaetes Amphipod index (BOPA) (Gómez Gesteira & Dauvin, 2000; Dauvin & Ruellet, 2007; Dauvin et al., 2007), for coastal and transitional waters.
(v) Methods or indices based on taxonomic-free descriptors, such as biological-functional traits or body size distributions, for lagoons (Mouillot et al., 2006; Reizopoulou & Nicolaidou, 2007; Marchini et al., 2008).
(vi) The Benthic Index based on Taxonomic Sufficiency (BITS) for non-tidal lagoons (Mistri & Munari, 2008).

Many of the 'new' indices or methods have been developed for Mediterranean coastal waters and lagoons in particular, where the peculiar environmental conditions make it difficult to use 'generalised' methods, and have stimulated scientists to create ad hoc methods. As a matter of fact, the application of indices mainly developed for the Atlantic coastal waters has achieved controversial results in Mediterranean lagoons or enclosed basins (Ponti & Abbiati, 2004; Marín-Guirao et al., 2005, Labrune et al., 2006, Pranovi et al., 2007; Simboura & Reizopoulou, 2008). On the other hand, the

Table 2 Benthic quality assessment methods proposed and/or approved by each Member State of the European Union, including the metrics used in the methodology

Sea	Member State	Assessment method	AMBI	BQI	Bentix	ISI	Sensitive/ opportunistic	ES100	Shannon's diversity	Abundance distribution	Density	Richness	Margalef index	Similatity	Simpson index	Biomass	Feeding guilds
Baltic	Estonia	ZKI—Index of zoobenthos community														X	X
	Finland	BBI- Finnish Brackish Water Benthic Index		X					X		X	X					
	Sweden	BQI-Swedish multimetric biological quality index		X													
	Denmark	DKI—Danish multimetric quality index	X						X		X	X					
	Germany	MarBIT—Marine Biotic Index Tool					X			X	X	X					
Atlantic	Norway	NQI—Norwegian Quality Index				X		X				X					
	Sweden	BQI—Swedish multimetric biological quality index		X													
	Denmark	DKI—Danish multimetric quality index	X						X		X	X					
	Germany	M-AMBI—Multivariate Factorial Analysis	X						X			X					
	Netherlands	BEQI—Benthic Ecosystem Quality Index									X			X		X	
	Belgium	BEQI—Benthic Ecosystem Quality Index									X			X		X	
	UK	IQI—Infaunal Quality Index	X									X			X		
	Ireland	IQI—Infaunal Quality Index	X									X			X		
	France	M-AMBI—Multivariate Factorial Analysis	X						X			X					
	Spain	M-AMBI—Multivariate Factorial Analysis	X						X			X					
	Portugal	P-BAT—Portuguese Benthic Assessment Tool	X						X				X				
Mediterranean	Spain	MEDOCC	X														
	France	Under development															
	Italy	M-AMBI—Multivariate Factorial Analysis	X						X			X					
	Slovenia	M-AMBI—Multivariate Factorial Analysis	X						X			X					
	Greece	Bentix			X												
	Cyprus	Bentix			X												

Table 2 continued

Sea	Member State	Assessment method	AMBI	BQI	Bentix	ISI	Sensitive/opportunistic	ES100	Shannon's diversity	Abundance distribution	Density	Richness	Margalef index	Similatity	Simpson index	Biomass	Feeding guilds
Black Sea	Bulgaria	M-AMBI—Multivariate Factorial Analysis	X						X			X					
	Romania	M-AMBI—Multivariate Factorial Analysis	X						X			X					

Data extracted and adapted from European Commission (2008) and GIG (2008)

ISI Indicator Species Index (Rygg, 2002); for other acronyms, see text

accuracy of methods for evaluating ES is expected to decrease when they are applied at larger spatial scales, because no model (or method or index) can be simple, general and accurate at the same time (Scardi et al., 2008).

The increasing number of proposed methods makes it difficult to assess and compare their suitability for quality assessment. Only in very few cases (see Borja & Muxika, 2005; Borja et al., 2008b) do the authors of the methodologies give guidance in the application of the method, indicate the comparability of their results and discuss the strengths and/or weaknesses of the indices used. This guidance is important for further quality assurance and harmonisation of the methods at the European level and is essential for the implementation of management decisions based on the assessment, which may involve significant economic consequences.

The recommendation of Díaz et al. (2004), on placing greater emphasis on evaluating the suitability of indices that already exist prior to the development of new ones, has not been followed in Europe. Although some authors (e.g. Borja et al., 2004b) have argued for consensus in the use of these new methodologies, it no longer seems possible. This is probably due to the large ecogeographical differences across Europe. However, the selection of multiple methods in assessing benthic quality makes any further validation and intercalibration of the methodologies used in the WFD more difficult. Moreover, the presence of the many, often local, types, to which the methods are applied, requires the definition of the type-specific reference conditions to be used, making more complicate the comparison.

Reference conditions

The reference condition for a biological quality element in a water body type is a description of the biological element relating to undisturbed (=pristine) conditions, i.e. with no, or with only a very minor, impact from human activities (Directive, 2000; Borja et al., 2004a; Muxika et al., 2007). The purpose of setting reference condition standards is to enable the assessment of the biological quality relative to these standards. Also by agreeing on reference conditions for a shared ecosystem type, MSs are giving a first step towards comparability of their assessment methods. Type-specific reference conditions must summarise the range of possibilities and values for the biological quality element, over periods of time and across the geographical extent of the type (CIS, 2003).

The WFD identifies four options for deriving reference conditions: (i) comparison with an existing 'pristine'/undisturbed site (or a site with very minor disturbance); (ii) using historical data and information; (iii) using models or (iv) using expert judgement. Borja et al. (2004a) have stated that one of the problems in deriving reference conditions for some types arises from the absence of un-impacted areas or lack of pre-industrial historical data. The use of 'virtual' reference locations as an 'expert judgement' approach has therefore been defined and proposed by Borja et al. (2004a) and Bald et al. (2005) and has been used successfully in macroinvertebrate status assessment (Rosenberg et al., 2004; Muxika et al., 2007; Borja et al., 2009a). Modelling the reference conditions using autecological data has also been used (Meyer et al., 2008). In some eco-regions (e.g. the Mediterranean Sea), marine reserves have been proposed as possible reference areas, as they provide the best ecological conditions within the eco-region (Casazza et al., 2004).

The methods used to determine reference conditions varies greatly between MSs: (i) using data from marine reserves (Greece), (ii) deriving them from the autecology of all macrozoobenthos species reported (Germany); (iii) using the highest (or lowest, depending on the metric) values of each of the metrics found in the data material classified to be at least in good status (Denmark); (iv) using the median of the 10% highest values of the method applied (Finland) or (v) using a mixture of expert judgment, historical data and modelling (Spain).

However, it is very difficult to confirm the values used as reference conditions, and, to our knowledge, complete reference conditions have only been published for the Spanish method (Muxika et al., 2007; Borja et al., 2009b) and, partially, for the British, Irish, Danish and Norwegian methods (Borja et al., 2007). More information, although still only partial, can be found in GIG (2008). This lack of detailed, yet essential, information makes comparison of some methods extremely difficult. Proposers of new indices should provide all the necessary details.

Validation of methodologies

Following Borja & Dauer (2008), validation of the methodologies should ideally include: (1) testing of the index using an independent data set different to the index development data set (calibration data set), (2) setting a priori correct classification criteria and/or (3) presentation of a strong a posteriori justification for use, based upon best professional judgment (see also Duel et al., 2007). Moreover, it should be demonstrated that the methodologies respond to anthropogenic pressures. Independent validation, by scientists other than those proposing the methodology, should be done. However, this is not the case for several of the European methodologies.

Some degree of validation has been undertaken for some of the indices, as shown below.

- AMBI and M-AMBI have been validated using many different anthropogenic pressures, on a worldwide basis (e.g. Borja et al., 2003; Solís-Weiss et al., 2004; Chenery & Mudge, 2005; Muniz et al., 2005; Muxika et al., 2005; Carvalho et al., 2006; Quintino et al., 2006; Dauvin et al., 2007; Fleischer et al., 2007; Cheung et al., 2008). Following these investigations, AMBI has been found to respond to different drivers and pressures, such as: hypoxia and eutrophication processes; oil platform discharges; engineering works; dredging; fish aquaculture; etc.; however, not to some hydro-morphological pressures, such as sand extraction (Muxika et al., 2005). M-AMBI has also been validated against several pressures, including hydromorphological ones (Muxika et al., 2007; Bigot et al., 2008; Borja et al., 2009a).
- Bentix has been checked using anthropogenic pressures, such as those coming from aquaculture (Aguado-Giménez et al., 2007), mining debris (Simboura et al., 2007) or eutrophication (Simboura et al., 2005).
- Several different pressures, sometimes unspecific, have been used in the validation of BQI (e.g. Rosenberg et al., 2004; Reiss & Kröncke, 2005; Labrune et al., 2006; Dauvin et al., 2007; Zettler et al., 2007).
- For other methods, the literature is less clear (see Prior et al., 2004; Josefson et al., 2008).

In a very few cases, methods used in Europe have been compared (even intercalibrated) with others used in the USA (Borja et al., 2008a; Chainho et al., 2008). The results from these exercises indicate that, although the approaches in both geographical areas are different, the conclusions regarding the benthic health can be very similar.

Intercalibration of methodologies

Prior to the implementation of WFD assessment, any proposed methodology must be intercalibrated between the MSs within an eco-region (Directive, 2000; Borja et al., 2007). Each MS shall divide the EQR scale for their monitoring system into the abovementioned five ES classes, by assigning a numerical value to each of the class boundaries. The value for the 'High/Good' and the 'Good/Moderate' class boundaries should be established through the intercalibration exercise. This is to ensure that the established class boundaries are consistent with the normative definitions of the WFD, and the different methodologies used are comparable between MSs.

Currently, there are very few peer-reviewed publications concerning the intercalibration of macroinvertebrates (Borja et al., 2007; Simboura & Reizopoulou, 2008; Occhipinti-Ambrogi et al., accepted), although many of the coastal types have been already intercalibrated within the WFD intercalibration groups (European Commission, 2008; GIG, 2008).

From the Baltic Sea, only Finland, Sweden, Germany and Denmark have intercalibrated their methodologies within five of the European types (Table 3, Fig. 1). The other Baltic countries (Estonia, Latvia, Lithuania and Poland) are at different stages of developing assessment methods and have only been able to contribute partially to the intercalibration exercise (Fig. 1). It is interesting to note the important differences in the boundaries across and within the types, indicating that the biogeographical and hydrographical characteristics (mainly salinity) of the types requires type-specific boundaries to make the results from different methods comparable. Hence, the boundaries for high-good classes range between 0.69 and 0.99 of the EQR, whilst the boundaries for good-moderate range between 0.31 and 0.60 (Table 3).

In the case of the North East Atlantic Sea, all countries have participated in the intercalibration, assigning boundaries for 12 types or sub-types (Table 3, Fig. 1). In this eco-region, the ranges of the boundaries are narrower (even it encompasses a much broader latitudinal range than previous geographic group), with EQRs extending from 0.67 to 0.92, in the case of high-good boundary, and 0.53 to 0.81, in the good-moderate boundary (Table 3).

Within the Mediterranean Sea, only four out of six MSs (Cyprus, Greece, Slovenia, and Spain) have agreed their boundaries, within one type (Table 3, Fig. 1). The EQR values of the boundaries range between 0.73 and 0.83 for high-good and from 0.47 to 0.62 for good-moderate classes (Table 3). Italy and France have not yet completed the process. Italy is working towards evaluating actual reference sites, which take into account the Italian coastal variability.

Within the Black Sea, Bulgaria and Romania have agreed EQR boundaries for each of the metrics or methods they use (Fig. 1), ranging between 0.83 and 0.89 for high-good and from 0.53 to 0.69 for good-moderate classes (Table 3).

When comparing median, mean and standard deviation values for the boundaries between status classes, it can be seen that mean high-good boundary lies around 0.8 in all eco-regions, whilst good-moderate boundary lies around 0.6, except in the Baltic Sea where the mean value is 0.41 (Fig. 2). Median values are close to mean values ($\leq$0.02 EQR), excepting in the Baltic Sea, in which the differences are of 0.05 EQR, within both quality classes (Fig. 2). As mentioned above, this is due to the special characteristics of this sea, where the strong salinity gradient, together with a general dominance of euryoecious and tolerant species, may make it difficult to distinguish between natural stress and anthropogenic pressures. This problem has been discussed elsewhere (Dauvin, 2007; Elliott & Quintino, 2007), and can cause difficulties in implementing the WFD within transitional waters, especially in oligohaline and tidal freshwater stretches of the estuaries.

Future research

Indices used to evaluate ES are often perceived as an objective procedure. In reality, they involve many steps that are based on subjective expert judgement,

Table 3 Types intercalibrated between different Member States, setting the boundaries between High-Good and Good-Moderate macroinvertebrate quality status

Seas and types	Member State	Ecological quality ratios boundaries	
		High-good	Good-moderate
Baltic			
CW B0	Finland	0.99	0.59
	Sweden	0.77	0.31
CW B2	Finland	0.95	0.57
	Sweden	0.77	0.31
CW B3 a	Finland	0.89	0.53
	Sweden	0.77	0.31
CW B3 b	Finland	0.90	0.54
	Sweden	0.77	0.31
CW B 12 b	Denmark	0.69	0.37
	Germany	0.80	0.60
	Sweden	0.78	0.35
Atlantic			
NEA1/26a,b,c,d,e; NEA 7	Denmark	0.67	0.53
	France	0.77	0.53
	Germany	0.85	0.70
	Ireland	0.75	0.64
	Norway	0.92	0.81
	Portugal	0.79	0.58
	Spain	0.77	0.53
	United Kingdom	0.75	0.64
NEA1/26b; NEA3/4	Belgium	0.80	0.60
	Netherlands	0.80	0.60
NEA8/9/10	Denmark	0.82	0.63
	Norway	0.92	0.81
	Sweden	0.89	0.68
Mediterranean			
	Cyprus	0.75	0.58
	Greece	0.75	0.58
	Slovenia	0.83	0.62
	Spain	0.73	0.47
Black Sea			
Shannon diversity	Bulgaria/Romania	0.89	0.69
AMBI	Bulgaria/Romania	0.83	0.53
M-AMBI	Bulgaria/Romania	0.85	0.55

Data extracted from European Commission (2008) and GIG (2008)

which is the key for any environmental assessment, evaluation or diagnosis (Scardi et al., 2008). All the proposed macroinvertebrate indices are based on some subjective choices, such as: metrics selection, classification of species into ecological groups, identification of coefficients in the mathematical formulas, definition of thresholds, etc. The use of expert judgement has not always been acknowledged and made evident by authors proposing the indices, thus leaving room for discussion and further refinement (Weisberg et al., 2008). The reduction of subjective steps, towards a more objective methodology, is necessary to reach the required scientific agreement for the translation of benthic indices into national legislation. Advanced computational techniques, such as fuzzy logic or neural networks, could

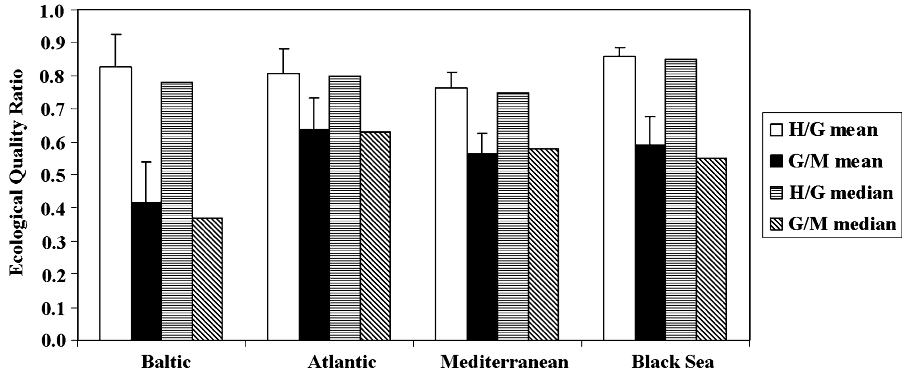

Fig. 2 Median, mean and standard deviation values for the boundaries between High-Good and Good-Moderate status, calculated within each European eco-region, using data in Table 3

provide help in dealing with the sources of uncertainty that affect the process of index development (Scardi et al., 2008; Marchini et al., 2009).

In a special issue of *Marine Pollution Bulletin*, focussing on marine integrated assessment Borja et al. (2009c) identified four areas from which scientific agreement was needed to satisfy future macroinvertebrate quality management: (i) reduction of the present bewildering array of available indices by identifying the index approaches, components and formulations that are most widely successful (Index Format); (ii) establish minimum criteria for index validation processes that demonstrate index accuracy and reliability (Index Validation); (iii) compare and intercalibrate methods to achieve uniform assessment scales across sites and habitats (Index Intercalibration) and (iv) integrate indices across media and ecosystem elements (Index Integration). In relation to Index Format, the challenge for the next decade is to accomplish sufficient index performance comparisons to reach scientific consensus on preferred index approaches for macroinvertebrates. The influence of local and regional natural variabilities (Bonsdorff et al., 2003), and the nature of the habitat or ecosystem element on index accuracy and stability must also be investigated (de Paz et al., 2008).

Index validation is the part of index development that critically evaluates the accuracy and precision of an index (Borja et al., 2009c). The credibility of an index or index combination, among scientists and managers, and its acceptability for evaluating condition at the geographic scale and level of habitat heterogeneity of interest depend on the demonstration of index reliability in a meaningful validation process (see Borja & Dauer, 2008). Any new methodology or index without a reliable and replicable validation, using independent sources, should be discarded or used with extreme caution (Borja et al., 2009c).

When validating an index for the WFD, the use of clear gradients of disturbance and/or recovery, both spatial and temporal, is essential (see Teixeira et al., 2008b; Borja et al., 2009a). Any mismatch between the biological and physical–chemical monitoring results, which may be an indication that the biological methods used are not sensitive to the effects of anthropogenic changes, should also be investigated (Chainho et al., 2008). In this way and prior to any intercalibration exercise, clear and reliable reference conditions for each of the types (even habitat: Blanchet et al., 2008) should be determined. Until now, some interesting approaches have been published (Nielsen et al., 2003; Borja et al., 2004a; Muxika et al., 2007; Teixeira et al., 2008b), but more investigation is needed.

Although many indices were successfully validated during the last decade, the challenge for the coming years is the intercalibration of methods within transitional water types (some attempt to compare indices have been already undertaken, such as in Pinto et al. (2009)). Scientific agreement regarding an approach in transitional waters, which takes into account the high number of natural patterns responsible for general trends regarding benthic diversity variation, is urgently needed (Teixeira et al., 2008a).

Other issues, related to the integration of indices at the water body level, have been undertaken in some of the published studies (Borja et al., 2008c, 2009a, b); however, more research in this topic is needed.

As stated by Borja et al. (2009c), the proliferation of indices adds an element of confusion back into what environmental managers had hoped to simplify. Some of the confusion arises as a result of the different processes used for developing, calibrating and validating indices in different regions. This in turn leads to inconsistencies in assessment across regions. Whereas, the last decade was characterised by an explosion of indices, the coming years should be those of consolidation and agreement (Borja et al., 2009c).

Some of these challenges, together with others such as the influence of climate change on reference conditions, will be undertaken in a new European research project named 'Water bodies in Europe: Integrative Systems to assess Ecological status and Recovery' (WISER), coordinated by Daniel Hering (University of Duisburg-Essen, Germany). One of the modules, focusing on marine and transitional waters, will provide (i) new validation of some indicators; (ii) investigation of the response of indicators to different pressures, such as hydromorphological pressures, eutrophication, pollution (metals and organic compounds), etc.; (iii) investigation of macroinvertebrate good ecological potential assessment, within Heavily Modified Water Bodies (see Borja & Elliott, 2007); (iv) an error estimation exercise of the uncertainty in assessing the ES, including a wide range of geographical regions and types; (v) different ways of combining single metrics into multimetrics or holistic assessment; (vi) a sensitivity analysis indicating which parts of the metrics are redundant, if there is duplication or double-counting, and which proportion of the end result is dependent on the individual metrics; (vii) correlations between the sensitive metrics/indicators developed above and (viii) data generated using low-cost monitoring methods will be tested.

Acknowledgements This is contribution number 468 of the Marine Research Division (AZTI-Tecnalia). Ibon Galparsoro (AZTI-Tecnalia) assisted us in preparing Fig. 1 and two anonymous reviewers provided us interesting comments.

References

Aguado-Giménez, F., A. Marín, S. Montoya, L. Marín-Guirao, A. Piedecausa & B. García-García, 2007. Comparison between some procedures for monitoring offshore cage culture in western Mediterranean Sea: sampling methods and impact indicators in soft substrata. Aquaculture 271: 357–370.

Bald, J., A. Borja, I. Muxika, J. Franco & V. Valencia, 2005. Assessing reference conditions and physico-chemical status according to the European Water Framework Directive: a case-study from the Basque Country (Northern Spain). Marine Pollution Bulletin 50: 1508–1522.

Bigot, L., A. Gremare, J.-M. Amouroux, P. Frouin, O. Maire & J. C. Gaertner, 2008. Assessment of the ecological quality status of soft-bottoms in Reunion Island (tropical Southwest Indian Ocean) using AZTI marine biotic indices. Marine Pollution Bulletin 56: 704–722.

Blanchet, H., N. Lavesque, T. Ruellet, J. C. Dauvin, P. G. Sauriau, N. Desroy, C. Desclaux, M. Leconte, G. Bachelet, A.-L. Janson, C. Bessineton, S. Duhamel, J. Jourde, S. Mayot, S. Simon & X. de Montaudouin, 2008. Use of biotic indices in semi-enclosed coastal ecosystems and transitional waters habitats – implications for the implementation of the European Water Framework Directive. Ecological Indicators 8: 360–372.

Bonsdorff, E., A. O. Laine, J. Hänninen, I. Vuorinen & A. Norkko, 2003. Zoobenthos of the outer archipelago waters (N. Baltic Sea) – the importance of local conditions for spatial distribution patterns. Boreal Environment Research 8: 135–145.

Borja, A., 2005. The European Water Framework Directive: a challenge for nearshore, coastal and continental shelf research. Continental Shelf Research 25: 1768–1783.

Borja, A. & D. M. Dauer, 2008. Assessing the environmental quality status in estuarine and coastal systems: comparing methodologies and indices. Ecological Indicators 8: 331–337.

Borja, A. & M. Elliott, 2007. What does 'good ecological potential' mean, within the European Water Framework Directive? Marine Pollution Bulletin 54: 1559–1564.

Borja, A. & I. Muxika, 2005. Guidelines for the use of AMBI (AZTI's marine biotic index) in the assessment of the benthic ecological quality. Marine Pollution Bulletin 50: 787–789.

Borja, A., J. Franco & V. Pérez, 2000. A Marine Biotic Index to establish the ecological quality of soft-bottom benthos within European estuarine and coastal environments. Marine Pollution Bulletin 40: 1100–1114.

Borja, A., I. Muxika & J. Franco, 2003. The application of a Marine Biotic Index to different impact sources affecting soft-bottom benthic communities along European coasts. Marine Pollution Bulletin 46: 835–845.

Borja, A., J. Franco, V. Valencia, J. Bald, I. Muxika, M. J. Belzunce & O. Solaun, 2004a. Implementation of the European Water Framework Directive from the Basque Country (northern Spain): a methodological approach. Marine Pollution Bulletin 48: 209–218.

Borja, A., J. Franco & I. Muxika, 2004b. The Biotic Indices and the Water Framework Directive: the required consensus in the new benthic monitoring tools. Marine Pollution Bulletin 48: 405–408.

Borja, A., A. B. Josefson, A. Miles, I. Muxika, F. Olsgard, G. Phillips, J. G. Rodríguez & B. Rygg, 2007. An approach to the intercalibration of benthic ecological status assessment in the North Atlantic ecoregion, according to the European Water Framework Directive. Marine Pollution Bulletin 55: 42–52.

Borja, A., D. Dauer, R. Díaz, R. J. Llansó, I. Muxika, J. G. Rodríguez & L. Schaffner, 2008a. Assessing estuarine benthic quality conditions in Chesapeake Bay: a comparison of three indices. Ecological Indicators 8: 395–403.

Borja, A., J. Mader, I. Muxika, J. G. Rodríguez & J. Bald, 2008b. Using M-AMBI in assessing benthic quality within the Water Framework Directive: some remarks and recommendations. Marine Pollution Bulletin 56: 1377–1379.

Borja, A., S. B. Bricker, D. M. Dauer, N. T. Demetriades, J. G. Ferreira, A. T. Forbes, P. Hutchings, X. Jia, R. Kenchington, J. C. Marques & C. Zhu, 2008c. Overview of integrative tools and methods in assessing ecological integrity in estuarine and coastal systems worldwide. Marine Pollution Bulletin 56: 1519–1537.

Borja, A., I. Muxika & J. G. Rodríguez, 2009a. Paradigmatic responses of marine benthic communities to different anthropogenic pressures, using M-AMBI, within the European Water Framework Directive. Marine Ecology 30: 214–227.

Borja, A., J. Bald, J. Franco, J. Larreta, I. Muxika, M. Revilla, J. G. Rodríguez, O. Solaun, A. Uriarte & V. Valencia, 2009b. Using multiple ecosystem components in assessing ecological status in Spanish (Basque Country) Atlantic marine waters. Marine Pollution Bulletin 59(1–3): 54–64.

Borja, A., A. Ranasinghe & S. B. Weisberg, 2009c. Assessing ecological integrity in marine waters, using multiple indices and ecosystem components: challenges for the future. Marine Pollution Bulletin 59(1–3): 1–4.

Carvalho, S., M. B. Gaspar, A. Moura, C. Vale, P. Antunes, O. Gil, L. Cancela da Fonseca & M. Falcao, 2006. The use of the marine biotic index AMBI in the assessment of the ecological status of the Obidos lagoon (Portugal). Marine Pollution Bulletin 52: 1414–1424.

Casazza, G., C. Silvestri & E. Spada, 2003. Classification of coastal waters according to the new Italian water legislation and comparison with the European Water Directive. Journal of Coastal Conservation 9: 65–72.

Casazza, G., C. López y Royo & C. Silvestri, 2004. Implementation of the 2000/60/EC Directive, for coastal waters, in the Mediterranean ecoregion. The importance of biological elements and of an ecoregional co-shared application. Biologia Marina Mediterranea 11: 12–24.

Chainho, P., M. L. Chaves, J. L. Costa, M. J. Costa & D. M. Dauer, 2008. Use of multimetric indices to classify estuaries with different hydromorphological characteristics and different levels of human pressure. Marine Pollution Bulletin 56: 1128–1137.

Chenery, A. M. & S. M. Mudge, 2005. Detecting anthropogenic stress in an ecosystem: 3. Mesoscale variability and biotic indices. Environmental Forensics 6: 371–384.

Cheung, S. G., N. W. Y. Lam, R. S. S. Wu & P. K. S. Shin, 2008. Spatio-temporal changes of marine macrobenthic community in sub-tropical waters upon recovery from eutrophication. II. Life-history traits and feeding guilds of polychaete community. Marine Pollution Bulletin 56: 297–307.

CIS, 2003. Transitional and Coastal Waters – Typology, Reference Conditions and Classification Systems. Common Implementation Strategy for the Water Framework Directive (2000/60/EC), Guidance Document 5, Working Group 2.4, COAST, European Commission: 119 pp.

Dauer, D. M., 1993. Biological criteria, environmental health and estuarine macrobenthic community structure. Marine Pollution Bulletin 26: 249–257.

Dauvin, J. C., 2007. Paradox of estuarine quality: benthic indicators and indices, consensus or debate for the future. Marine Pollution Bulletin 55: 271–281.

Dauvin, J. C. & T. Ruellet, 2007. Polychaete/amphipod ratio revisited. Marine Pollution Bulletin 55: 215–224.

Dauvin, J. C., T. Ruellet, N. Desroy & A. L. Janson, 2007. The ecological quality status of the Bay of Seine and the Seine estuary: use of biotic indices. Marine Pollution Bulletin 55: 241–257.

de Paz, L., J. Patrício, J. C. Marques, A. Borja & A. J. Laborda, 2008. Ecological status assessment in the lower Eo estuary (Spain). The challenge of habitat heterogeneity integration: a benthic perspective. Marine Pollution Bulletin 56: 1275–1283.

Díaz, R. J., M. Solan & R. M. Valente, 2004. A review of approaches for classifying benthic habitats and evaluating habitat quality. Journal of Environmental Management 73: 165–181.

Directive, 2000. Directive 2000/60/EC of the European Parliament and of the council of 23 October 2000 establishing a framework for community action in the field of water policy. Official Journal of the European Communities L327: 1–72.

Duel, H., S. Groot & D. Boorman, 2007. Relationships between biological quality elements and physico-chemical pressures: validation and practical use of relationships. Synthesis Report D18, REBECCA Project. http://www.rbm-toolbox.net/docstore/docs/3.1713.D18.pdf.

Elliott, M. & V. Quintino, 2007. The Estuarine Quality Paradox, environmental homeostasis and the difficulty of detecting anthropogenic stress in naturally stressed areas. Marine Pollution Bulletin 54: 640–645.

European Commission, 2008. Commission Decision of 30 October 2008, establishing, pursuant to Directive 2000/60/EC of the European Parliament and of the Council, the values of the Member State monitoring system classifications as a result of the intercalibration exercise (notified under document number C(2008) 6016) (2008/915/EC). Official Journal of the European Union L332: 20–44.

Fano, E. A., M. Mistri & R. Rossi, 2003. The ecofunctional quality index (EQI): a new tool for assessing lagoonal ecosystem impairment. Estuarine, Coastal and Shelf Science 56: 709–716.

Ferreira, J., C. Vale, C. Soares, F. Salas, P. Stacey, S. Bricker, M. Silva & J. Marques, 2007. Monitoring of coastal and transitional waters under the E.U. Water Framework Directive. Environmental Monitoring and Assessment 135: 195–216.

Fleischer, D., A. Gremare, C. Labrune, H. Rumohr, E. vanden Berghe & M. L. Zettler, 2007. Performance comparison of two biotic indices measuring the ecological status of water bodies in the Southern Baltic and Gulf of Lions. Marine Pollution Bulletin 54: 1598–1606.

Forni, G. & A. Occhipinti-Ambrogi, 2007. Daphne: a new multimetric benthic index for the quality assessment of marine coastal environment in the Northern Adriatic Sea. Chemistry and Ecology 23: 427–442.

GIG, 2008. WFD intercalibration technical report. Part 3 – Coastal and Transitional Waters. Sect. 2 – Benthic invertebrates. Four parts: Mediterranean GIG; Black Sea GIG; North East Atlantic GIG; and Baltic GIG. http://circa.europa.eu/Public/irc/jrc/jrc_eewai/library.

Gómez Gesteira, J. L. & J. C. Dauvin, 2000. Amphipods are good bioindicators of the impact of oil spills on soft-bottom macrobenthic communities. Marine Pollution Bulletin 40: 1017–1027.

Heiskanen, A. S., W. van de Bund, A. C. Cardoso & P. Nõges, 2004. Towards good ecological status of surface waters in Europe – interpretation and harmonisation of the concept. Water Science and Technology 49: 169–177.

Hurlbert, S. H., 1971. The nonconcept of species diversity: a critique and alternative parameters. Ecology 52: 577–586.

Josefson, A. B., J. L. S. Hansen, G. Asmund & P. Johansen, 2008. Threshold response of benthic macrofauna integrity to metal contamination in West Greenland. Marine Pollution Bulletin 56: 1265–1274.

Labrune, C., J. M. Amouroux, R. Sarda, E. Dutrieux, S. Thorin, R. Rosenberg & A. Gremare, 2006. Characterization of the ecological quality of the coastal Gulf of Lions (NW Mediterranean). A comparative approach based on three biotic indices. Marine Pollution Bulletin 52: 34–47.

Marchini, A., C. Munari & M. Mistri, 2008. Functions and ecological status of eight Italian lagoons examined using biological traits analysis (BTA). Marine Pollution Bulletin 56: 1076–1085.

Marchini, A., T. Facchinetti & M. Mistri, 2009. F-IND: a framework to design fuzzy indices of environmental conditions. Ecological Indicators 9: 485–496.

Marín-Guirao, L., A. César, A. Marín, J. Lloret & R. Vita, 2005. Establishing the ecological quality status of soft-bottom mining-impacted coastal water bodies in the scope of the Water Framework Directive. Marine Pollution Bulletin 50: 374–387.

Meyer, Th., T. Berg & K. Fürhaupter, 2008. Ostsee Makrozoobenthos Klassifizierungssystem für die Wasserrahmenrichtlinie. Referenz-Artenlisten, Bewertungsmodell und Monitoring. MARILIM Gewässeruntersuchung: 131 pp. http://www.marilim.de/marbit/download/files/MarBIT.pdf.

Mistri, M. & C. Munari, 2008. BITS: a SMART indicator for soft-bottom, non-tidal lagoons. Marine Pollution Bulletin 56: 587–599.

Mistri, M., C. Munari, R. Rossi & A. Marchini, 2007. Validation of a Fuzzy Index of Ecosystem Integrity, FINE, in northern Adriatic lagoons. Chemistry & Ecology 23: 443–454.

Mistri, M., C. Munari & A. Marchini, 2008. The fuzzy index of ecosystem integrity (FINE): a new index of environmental integrity for transitional ecosystems. Hydrobiologia 611: 81–90.

Mouillot, D., S. Spatharis, S. Reizopoulou, T. Laugier, L. Sabetta, A. Basset & T. Do Chi, 2006. Alternatives to taxonomic-based approaches to assess changes in transitional water communities. Aquatic Conservation: Marine and Freshwater Ecosystems 16: 469–482.

Muniz, P., N. Venturini, A. M. S. Pires-Vanin, L. R. Tommasi & A. Borja, 2005. Testing the applicability of a Marine Biotic Index (AMBI) to assessing the ecological quality of soft-bottom benthic communities, in the South America Atlantic region. Marine Pollution Bulletin 50: 624–637.

Muxika, I., A. Borja & W. Bonne, 2005. The suitability of the marine biotic index (AMBI) to new impact sources along European coasts. Ecological Indicators 5: 19–31.

Muxika, I., A. Borja & J. Bald, 2007. Using historical data, expert judgement and multivariate analysis in assessing reference conditions and benthic ecological status, according to the European Water Framework Directive. Marine Pollution Bulletin 55: 16–29.

Nielsen, K., B. Somod, C. Ellegaard & D. Krause-Jensen, 2003. Assessing reference conditions according to the European Water Framework Directive using modelling and analysis of historical data: an example from Randers Fjord, Denmark. Ambio 32: 287–294.

Occhipinti-Ambrogi, A., G. Forni & C. Silvestri, accepted. The Mediterranean intercalibration exercise on benthic invertebrates with special emphasis on Italian situation. Marine Ecology.

Pearson, T. & R. Rosenberg, 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. Oceanography and Marine Biology Annual Review 16: 229–311.

Perus, J., E. Bonsdorff, S. Bäck, H. G. Lax, A. Villnäs & V. Westberg, 2007. Zoobenthos as indicators of ecological status in Coastal Brackish Waters: a comparative study from the Baltic Sea. Ambio 36: 250–256.

Pinto, R., J. Patricio, A. Baeta, B. D. Fath, J. M. Neto & J. C. Marques, 2009. Review and evaluation of estuarine biotic indices to assess benthic condition. Ecological Indicators 9: 1–25.

Ponti, M. & M. Abbiati, 2004. Quality assessment of transitional waters using a benthic biotic index: the case study of the Pialassa Baiona (northern Adriatic Sea). Aquatic Conservation: Marine and Freshwater Ecosystems 14: 31–41.

Pranovi, F., F. Da Ponte & P. Torricelli, 2007. Application of biotic indices and relationship with structural and functional features of macrobenthic community in the lagoon of Venice: an example over a long time series of data. Marine Pollution Bulletin 54: 1607–1618.

Prior, A., A. C. Miles, A. J. Sparrow & N. Price, 2004. Development of a classification scheme for the marine benthic invertebrate component, Water Framework Directive. Phase I & II – transitional and coastal waters. Environment Agency (UK), R&D Interim Technical Report, E1-116, E1-132: 1–103 (+appendix).

Quintino, V., M. Elliott & A. M. Rodrigues, 2006. The derivation, performance and role of univariate and multivariate indicators of benthic change: case studies at differing spatial scales. Journal of Experimental Marine Biology and Ecology 330: 368–382.

Reiss, H. & I. Kröncke, 2005. Seasonal variability of benthic indices: an approach to test the applicability of different indices for ecosystem quality assessment. Marine Pollution Bulletin 50: 1490–1499.

Reizopoulou, S. & A. Nicolaidou, 2007. Index of size distribution (ISD): a method of quality assessment for coastal lagoons. Hydrobiologia 577: 141–149.

Rosenberg, R., M. Blomqvist, H. C. Nilsson, H. Cederwall & A. Dimming, 2004. Marine quality assessment by use of benthic species-abundance distributions: a proposed new protocol within the European Union Water Framework Directive. Marine Pollution Bulletin 49: 728–739.

Rygg, B., 2002. Indicator species index for assessing benthic ecological quality in marine waters of Norway. Norwegian Institute for Water Research, Report No. 40114: 1–32.

Scardi, M., S. Cataudella, P. Di Dato, E. Fresi & L. Tancioni, 2008. An expert system based on fish assemblages for evaluating the ecological quality of streams and rivers. Ecological Informatics 3: 55–63.

Simboura, N. & S. Reizopoulou, 2008. An intercalibration of classification metrics of benthic macroinvertebrates in coastal and transitional ecosystems of the Eastern Mediterranean ecoregion (Greece). Marine Pollution Bulletin 56: 116–126.

Simboura, N. & A. Zenetos, 2002. Benthic indicators to use in ecological quality classification of Mediterranean soft bottom marine ecosystems, including a new biotic index. Mediterranean Marine Science 3: 77–111.

Simboura, N., P. Panayotidis & E. Papathanassiou, 2005. A synthesis of the biological quality elements for the implementation of the European Water Framework Directive in the Mediterranean ecoregion: the case of Saronikos Gulf. Ecological Indicators 5: 253–266.

Simboura, N., E. Papathanassiou & D. Sakellariou, 2007. The use of a biotic index (Bentix) in assessing long-term effects of dumping coarse metalliferous waste on soft bottom benthic communities. Ecological Indicators 7: 164–180.

Solís-Weiss, V., F. Aleffi, N. Bettoso, P. Rossin, G. Orel & S. Fonda Umani, 2004. Effects of industrial and urban pollution on the benthic macrofauna in the Bay of Muggia (industrial port of Trieste, Italy). Science of the Total Environment 328: 247–263.

Teixeira, H., F. Salas, A. Borja, J. M. Neto & J. C. Marques, 2008a. A benthic perspective in assessing the ecological status of estuaries: the case of the Mondego estuary (Portugal). Ecological Indicators 8: 404–416.

Teixeira, H., F. Salas, J. M. Neto, J. Patrício, R. Pinto, H. Veríssimo, J. A. García-Charton, C. Marcos, A. Pérez-Ruzafa & J. C. Marques, 2008b. Ecological indices tracking distinct impacts along disturbance-recovery gradients in a temperate NE Atlantic Estuary – guidance on reference values. Estuarine, Coastal and Shelf Science 80: 130–140.

Washington, H. G., 1984. Diversity, biotic and similarity indices. A review with special relevance to aquatic ecosystems. Water Research 18: 653–694.

Weisberg, S. B., B. Thompson, J. A. Ranasinghe, D. E. Montagne, D. B. Cadien, D. M. Dauer, D. Diener, J. Oliver, D. J. Reish, R. G. Velarde & J. Q. Word, 2008. The level of agreement among experts applying best professional judgment to assess the condition of benthic infaunal communities. Ecological Indicators 8: 389–394.

Zettler, M. L., D. Schiedek & B. Bobertz, 2007. Benthic biodiversity indices versus salinity gradient in the southern Baltic Sea. Marine Pollution Bulletin 55: 258–270.

Author Biographies

Angel Borja (b. 1959), biologist, graduated at the University of the Basque Country, Spain (1981; PhD: 1984). He is head of projects at AZTI-Tecnalia's Marine Research Division. He has been dealing with benthic community ecology, and his main current interest is integrative assessment of marine waters. He is Editor-in-Chief of the *Open Marine Biology Journal* and the *Revista de Investigación Marina*; member of the editorial boards of *Marine Pollution Bulletin*, *Ecological Indicators* and *Continental Shelf Research*, and guest editor of five special volumes of different journals.

Alison Miles (b. 1968) graduated at the University of Bangor, Wales (1989 BSc in Marine Biology and Chemical Oceanography; PhD: 1995, Bristol University, England). She is currently a technical advisor (marine) in Monitoring and Modelling Policy for the Environment Agency of England and Wales. She has been working on the development and implementation of ecological status assessment for the EU Water Framework Directive for the United Kingdom.

Anna Occhipinti-Ambrogi (b. 1953), biologist, graduated at the University of Pavia, Italy (1976). She is full professor of Ecology at the University of Pavia, where she teaches Ecology, Applied Ecology and Hydrobiology. She has been working in the ecology of marine and brackish water environments, focusing on structure and dynamics of macrobenthos, with emphasis on introduced species and their relationship with the environment. She is currently working on the development of ecological indicators in the framework of the EU Water Quality Water Directive. She is member of the editorial board of Transitional Water Bulletin and co-editor of a special volume of Marine Pollution Bulletin (55).

Torsten Berg (b. 1967), biologist, graduated at the University of Kiel, Germany (1994 BSc in physical oceanography, 1998 MSc in marine biology). He is head of the zoobenthos group at MariLim aquatic research. He deals with macrozoobenthos communities from brackish and marine waters and especially with assessment methods for the implementation of the Water Framework Directive in the Baltic Sea. He is coordinating the macrofauna group of the Baltic Geographical Intercalibration Group within the WFD and is a member of the quality assurance working group for the German federal monitoring programme.