

# Meta-analysis of a large data set with Water Framework Directive indicators and calibration of a Benthic Quality Index at the family level

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## ABSTRACT

A large data set of marine benthic samples from the Eastern Mediterranean was used to develop Benthic Quality Index (BQI)-family, a new indicator based on the BQI index, which was calibrated by maximizing the consensus with other existing indicators namely BQI, Shannon diversity H', AZTI Marine Biotic Index (AMBI) and BENTIX. The values calculated for the BQI-family indicator are significantly and highly correlated ( $p < 0.0001$ ) to those calculated for all the aforementioned indicators and it provides judgment on ecological status close to their average. Furthermore, it combines the strong points of all these methods with the increased reliability, speed and low cost of the identification at higher taxonomic levels.

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## 1. Introduction

Benthic macrofauna is an excellent mirror reflecting the ecological status of the marine environment and therefore, it has become a standard component of marine environmental monitoring (Bilyard, 1987). In the context of the European Water Framework Directive (WFD, 2000/60/EC), a series of indicators have been proposed as a means of assessing the ecological quality of the benthic environment; for example AZTI Marine Biotic Index (AMBI) (Borja et al., 2000), BENTIX (Simboura and Zenetos, 2002) and Benthic Quality Index (BQI) (Rosenberg et al., 2004, as modified in Leonardsson et al., 2009) as well as the Shannon Diversity index H' (Shannon and Weaver, 1949), the values of which have been separated into intervals corresponding to different ecological status or environmental quality (Labruno et al., 2006). The ability of these indices to detect environmental change in benthic communities, their inter-correlation as well as their convergence or divergence in describing the environmental quality has been discussed in numerous papers during the past decade (e.g. Borja et al., 2003, 2009a,b, 2011, 2012; Muxika et al., 2007; Simboura, 2004; Simboura and Reizopoulou, 2007, 2008; Simboura and Argyrou, 2010; Occhipinti et al., 2009;

Salas et al., 2004; Grémare et al., 2009; Kröncke and Reiss, 2010; Carletti and Heiskanen, 2009 and references therein) in different disturbance/pollution contexts, geographical regions and benthic habitats.

Although it could be argued that the ecological quality limits are largely arbitrary (as in the Shannon H') or based on changes in the contribution of different ecological groups in the fauna (as in AMBI and BENTIX), it has been shown using Best Professional Judgment (BPJ) techniques (Teixeira et al., 2010) that a large percentage of authors, although from different geographic areas and using different approaches, tend to provide a similar characterization of the ecological quality of the samples. In this context it is, therefore, worth trying to maximize consensus among proposed methods particularly since they are all based on the same principles, i.e. the balance between r- and K-strategy species which changes along a disturbance gradient as described by Pearson and Rosenberg (1978). According to Diaz et al. (2004) there are already too many indicators so the current trend is to test and to optimize rather than introduce new ones and Labruno et al. (2006) suggest that there is a need for standardization of the protocols and the procedures in order to achieve useful indicators, i.e. easy to compute, efficient in detecting disturbances and usable for most European coasts.

During the past 30 years there has been a spectacular increase in the use of multivariate techniques in benthic ecology using

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the strategy proposed by Field et al. (1982). These powerful techniques are capable of readily detecting environmental change due to anthropogenic or natural disturbance. Warwick (1986) and several authors after him (Ferraro and Cole, 1995; Olsford et al., 1998; Karakassis and Hatzilyanni, 2000; Dauvin et al., 2003) have shown that there is little information loss when analyzing data at higher taxonomic levels. This approach is widely known as the “*taxonomic sufficiency*” and it is one of the rapid assessment techniques (RATs) used in the biodiversity and ecosystem health assessment. It aims at being the best choice between time and costs of an environmental study and accuracy of the information pattern derived by the taxonomic level used. The concept has been proven to be highly beneficial in the cases of well described gradients in that the environmental variables associated are highly correlated with the patterns derived by the higher taxonomic categories (e.g. Olsford and Somerfield, 2000; Olsford et al., 1997, 1998). The latter concept is known as the “*hierarchical-response-to-stress*” hypothesis. Lampadariou et al. (2005) used a cost/benefit ratio analysis and showed that the analysis at the family level gives the best balance between precision of the results and decrease in taxonomic effort. The taxonomic sufficiency concept was also used in biotic indices as in the case of the Benthic Opportunistic Polychaetes Amphipods index (BOPA/BOPA2A) indices that use the Amphipoda and Clitellata at phylum level and only opportunistic Polychaete species (Dauvin and Ruellet, 2007, 2009). However, so far the analysis at higher taxonomic levels has not been used in the context of the WFD, except again in the case of the BOPA/BOPA2A index (de-la-Ossa-Carretero, 2009; Pinedo et al., *in press*) despite the advantages it may give in terms of cost and time. Partly this is due to the WFD definition of high quality in coastal and transitional waters, implying that “All the disturbance-sensitive taxa associated with undisturbed conditions are present” and that “The level of diversity and abundance of invertebrate taxa should also be reported”. On the other hand, practically all the metrics proposed and used for the implementation of the WFD have used scores rather than the presence/absence of taxa.

The aim of the present study was to investigate the suitability of an indicator operating with data at higher taxonomic levels as a means for assessing the ecological quality in the context of the WFD. To this end, we developed the BQI-family indicator which combines the objectivity of the characterization of families as *r*- or *K*-selection taxa with the convenience and the low cost of the identification of specimens at higher taxonomic levels. This indicator is calibrated with a large database of benthic macrofaunal samples from the Eastern Mediterranean and its limits of ecological quality are derived from a procedure maximizing consensus with the aforementioned proposed indicators for the WFD monitoring.

## 2. Methods

A large database of macrofaunal samples was compiled by assembling various data sets from the Eastern Mediterranean (Table 1). Those included sublittoral/continental shelf surveys of various types, such as mapping of benthic communities in oligotrophic waters, the effects of organic enrichment, and other types of anthropogenic or natural stressors, published (Karakassis and Eleftheriou, 1997; Karakassis et al., 2000; Arvanitidis et al., 2005a,b, 2006, 2009; Apostolaki et al., 2007; Faulwetter et al., 2008; Papageorgiou et al., 2009; Lampadariou et al., 2005; Simbora, 2004; Simbora and Reizopoulou, 2007, 2008; Simbora et al., 2007; Simbora and Argyrou, 2010) or unpublished data from the University of Crete, or the Hellenic Center of Marine Research (HCMR). The sampling covered parts of the Adriatic Sea, Ionian Sea, Aegean Sea, the shelf of Crete and Cyprus. The data sets were checked for synonyms against online tools, such as the TaxonMatch in the World Register of Marine Species

(<http://www.marinespecies.org/aphia.php?p=match>) and all stations with fewer than 50 individuals taken from depths of <20 m were excluded because the BQI definition involves different limits of ecological status for shallow (<20 m) benthic communities. This procedure left in total 1010 samples and 449,891 specimens.

The BQI at the species level was calculated following the formula provided by Rosenberg et al. (2004) as revised in Leonardsson et al. (2009). The sensitivity values ( $ES_{50,0.05}$ ) were calculated with all the 1010 samples but we excluded very rare species, i.e. present in fewer than 5 stations or with fewer than 30 total individuals in the total data set. For BQI as well as for AMBI and BENTIX we retained only the “safe” samples, i.e. those that had scores for species covering at least 80% of the specimens.

The Shannon  $H'$  ( $\log_2$ ) was calculated using the PRIMER software (Clarke and Warwick, 1994). AMBI was calculated using the calculation software provided by the authors (Borja et al., 2000) in their website (<http://ambi.azti.es/>) using the species inventory provided (February 2010). BENTIX (Simbora and Zenetos, 2002) was also calculated using the software (<http://bentix.ath.hcmr.gr>) and the species inventory provided by the authors (February 2010). We used the latest species inventory for both indices.

The BQI at the family level (BQI-family) was calculated using the revised methodology described in Leonardsson et al. (2009) but the sensitivity values ( $ES_{50,0.05}$ , which could be considered as  $EF_{50,0.05}$  in this case) were calculated for each family using aggregated data at the family level. In order to assign ecological quality boundaries for the BQI-family we followed the following steps, which are described step by step in Section 3: we used the samples where 3 out of 4 indices agree on the ecological status and assigned this as the ecological status of the sample. We then sorted all these samples in ascending BQI-family value order and consequently after identifying the zones of overlapping we divided each one of them into two parts with range proportional to the number of samples belonging to each one of the two successive groups.

We used two different methods to validate the results given by the BQI family index. In the first method we used a number of samples not used in the calculation of the ecological quality boundaries as independent data set to compare and validate the results of the BQI family with the other four. There were two groups of samples: (i) a group of 20 samples in which all four WFD indices had given the same ecological status and (ii) a group of 417 samples where details in ecological status (Bad to High) were ignored and they were only characterized as Acceptable (High or Good), Unacceptable (Bad to Moderate) according to the majority of the 4 WFD indices, or Unclear (i.e. two indices Acceptable and two Unacceptable).

The second validation method was to compare the performance of the BQI family index to a well described pressure gradient, in this case Total Organic Carbon (TOC). TOC data were available for 160 samples in which the result of the BQI family index was “safe” as well. The average BQI family was calculated for TOC ranges  $\leq 10 \text{ mg g}^{-1}$ ,  $10\text{--}35 \text{ mg g}^{-1}$  and  $\geq 35 \text{ mg g}^{-1}$  as in Hyland et al. (2005).

## 3. Results

The sensitivity values ( $ES_{50,0.05}$ ) calculated for all the families in the data set of 1010 samples are shown in Appendix I. Among the 260 families found in this data set, 110 were represented by a single species, 15 were identified only to the family level, and 23 had very low variance (<20%) between the sensitivity values of their species-members. The remaining 112 families had 2–30 species-members showing, in most cases, sensitivity values close to the most tolerant species of the family. In Appendix II, a list of the sensitivity values ( $ES_{50,0.05}$ ) of all the 873 species of the present study are shown.

**Table 1**

Data sets used in the present study.

Serial number	Location	Sampler	Sediment type	Disturbance source	Sampling year
1–99	Continental shelf of Crete	van Veen	Mud/Sand/Silt	Mostly none/trawling	1986–1987
100–135	Malia Bay, Crete	van Veen	Mud/Sand/Silt	Domestic wastes	1992–1993
135–173	Fish farms, Aegean & Ionian Seas	van V/Corrers	Mud/Sand/Silt	Fish feed and faeces	1995–1997
174–249	Cyprus, Sicily, Aegean Sea	van V/Corrers	Sand/seagrass	Fish feed and faeces	2002–2004
250–346	Cretan, Aegean, Ionian Seas	Corrers	Sand/Mud	Fish feed and faeces	2001–2002
347–375	Sounio, Siteia, Astakos, Kefalonia	Corrers	Sand/Mud	Fish feed and faeces	2006–2007
376–395	Libyan Sea S. Crete	van Veen	Sand	Trawling	2006–2007
396–420	N. Adriatic	van Veen	Silt	Mostly undisturbed	1996–2002
421–448	N. Adriatic	van Veen	Silt	Domestic wastes	1995
449–576	N. Adriatic	van Veen	Silt	Gulf	1985–2004
577–687	Ionian Sea	van Veen	Mixed	Gulf	1995–1996
688–753	Aegean Sea, Lesbos	Ponar grab	Sand/Mud	Gulf	1986–1988
754–774	Ionian Sea	van Veen	Sand	Near coast	1990–1991
775–841	Aegean Sea Saronikos gulf	Ponar grab	Mud/Sand/Silt	Gulf	1989–1990
842–890	Aegean Sea Crete	van Veen	Mud/Sand/Silt	Coastal vs offshore	1990–1991
891–941	Aegean Sea/Ionian Sea	van Veen	Mud/Sand/Silt	Lagoons	1989–1990
942–955	Aegean Sea Evoikos gulf	van Veen	Mud	Industrial wastes	2003–2004
956–960	Aegean Sea Messinan gulf	Ponar grab	Sand/Mud	Gulf monitoring	2006
961–968	Aegean Sea Santorini Island	van Veen	Silt/Mud	Sea diamond shipwreck	2007
969–1010	Aegean Sea Saronikos gulf	van Veen	Mud/Sand/Silt	Gulf monitoring	2004

For three major groups (Polychaeta, Crustacea and Mollusca) we calculated the coefficient of variation ( $CV = \text{std. deviation/average}$ ) of the sensitivity values ( $ES_{50,0.05}$ ) at the species and family levels. The CV for POL, CRU and MOL was 0.41, 0.45 and 0.51 at the species level and 0.49, 0.46 and 0.53 at the family level, respectively, indicating that the variability changed very little from the species to family level.

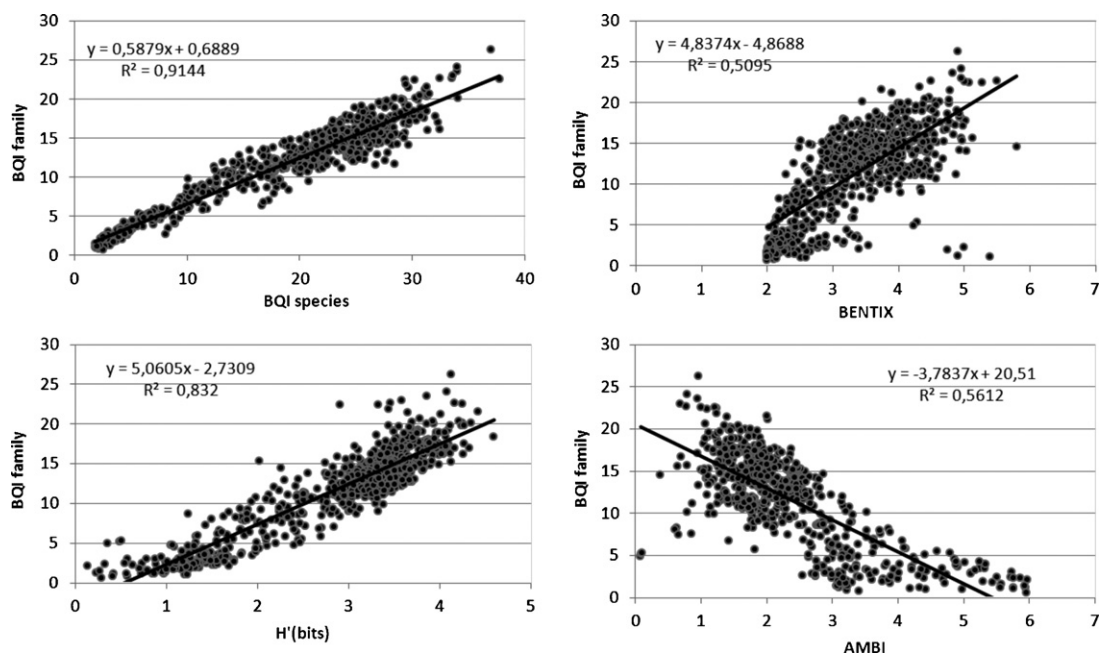
The values of the ecological quality indicators, BQI (at the species level), Shannon  $H'$ , BENTIX and AMBI were calculated (Table 2) using only the “safe” samples, i.e. those that were at least 80% of the abundance were included in the calculation of the indicator. As may be seen in Table 2, all the 1010 samples were “safe” for Shannon  $H'$  and BENTIX, whereas BQI and AMBI had 713 and 902, respectively. It may also be seen that Shannon  $H'$  and BQI identified 64 and 87 samples as “BAD” (7% and 12% of the samples, respectively) whereas BENTIX and AMBI had no sample in this category. On the other hand, Shannon  $H'$  and BENTIX classified 58% and 46% of the samples, respectively, as acceptable (i.e. Good or High status) whereas BQI and AMBI classified 75% and 86%.

**Table 2**

Number of “safe” samples per ecological status found with each particular indicator.

Ecol. status	BQI	Shannon $H'$	BENTIX	AMBI
Bad	80	64	0	0
Poor	79	183	130	50
Moderate	55	212	316	87
Good	69	501	394	684
High	430	50	170	81
Total	713	1010	1010	902

Using the sensitivity values for the families in the Table of Appendix I, we calculated the BQI at the family level which was significantly correlated to the other indicators in a total of 640 samples, i.e. those that were “safe” for all the indicators (Fig. 1). BQI-family was correlated to BQI-species ( $r = 0.91$ ,  $p < 0.0001$ ), Shannon  $H'$  ( $r = 0.83$ ,  $p < 0.0001$ ), BENTIX ( $r = 0.51$ ,  $p < 0.0001$ ) and AMBI ( $r = -0.55$ ,  $p < 0.0001$ ). All indices are positively correlated to BQI-family except AMBI which is negatively correlated since it uses a reverse scale.

**Fig. 1.** Linear regression between BQI-family and BQI-species, Shannon ( $H'$ ), BENTIX, AMBI in 640 samples.

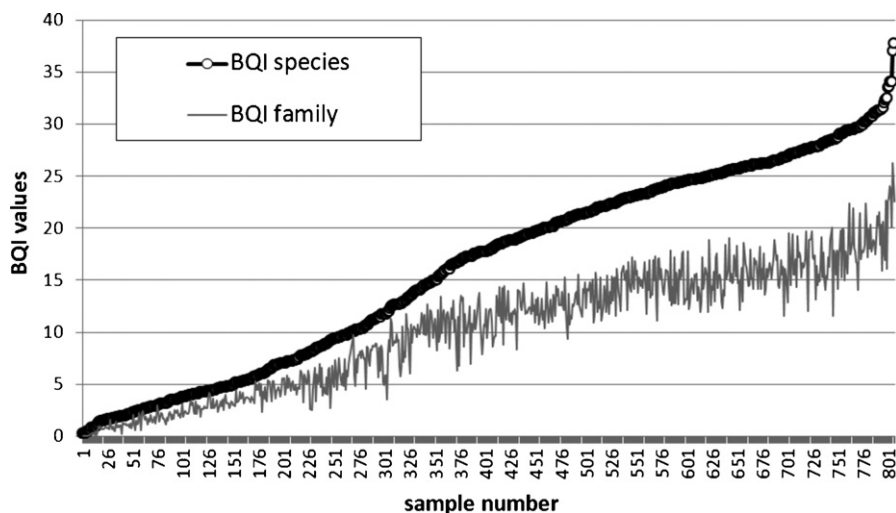


Fig. 2. Comparison of BQI-family and BQI-species values in a data set of 713 samples arranged in increasing order of BQI-species values.

Although the correlation coefficient between BQI-family and BQI-species is high, the values are not identical (Fig. 2). It may be seen that in highly disturbed situations (low BQI), both indices show similar values, probably because, in this case, both curves are driven by the strong presence of a small number of opportunistic species. But as ecosystems become less disturbed, the BQI family tends to have smaller values, resulting in a significant deviation in undisturbed ecosystems. As a result it was necessary to calculate new ecological status boundaries for the BQI-family indicator. To this end, we assigned the ecological status agreed between at least 3 out of 4 indicators to each one of the 640 “safe” samples. These are the 281 samples in Fig. 3. Then we identified the zones of overlapping, i.e. the intervals where we have samples belonging to two different (successive in all cases) ecological status groups. Each one of these zones was divided into two parts which were proportional to the number of samples belonging to each one of the two successive groups. The results of this procedure are shown in Table 3.

Using the above ecological quality boundaries, we assigned the BQI family status to all the 640 “safe” samples. In comparison to the other 4 indicators (Fig. 4), BQI-family attributed 72% to acceptable

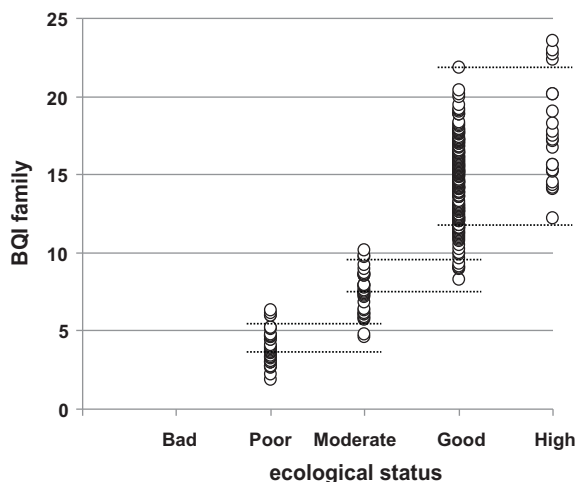


Fig. 3. Calibration of the BQI-family indicator using 286 “safe” samples assigned with an ecological status depending on the maximum consensus among BQI-species, H', BENTIX and AMBI. The horizontal lines indicate the overlapping zones between consecutive ecological status groups, which were then divided based on the weighted number of samples.

Table 3

Ecological status limits for BQI-species (from Rosenberg et al., 2004) and BQI-family (present study).

Ecol. status	BQI-species	BQI family
High	$\geq 16.0$	$\geq 20.8$
Good	12.0 to $\leq 16.0$	9.2 to $\leq 20.8$
Moderate	8.0 to $\leq 12.0$	5.7 to $\leq 9.2$
Poor	4.0 to $\leq 8.0$	1.9 to $\leq 5.7$
Bad	0 to $\leq 4.0$	0 to $\leq 1.9$

ecological status (i.e. High or Good) being less stringent than BENTIX (46%) and Shannon H' (58%) and stricter than BQI-species (74%) or AMBI (86%).

Regarding the validation, with the first data set of 20 samples which had a certain ecological status (i.e. all WFD indices agreed) we found that BQI family index assigned the same ecological status in all cases (100% agreement). The second data set, with 417 samples (Table 4) it was found that in all cases where at least 3 out of 4 indicators assigned an Acceptable or Unacceptable status the agreement with the outcome of BQI family was 100% whereas when the characterization was unclear (1 pair of indices on each side), the BQI family index spitted them into Acceptable (74%) and Unacceptable (26%).

The BQI family was highly correlated ( $r=0.63$ ,  $p<0.0001$ ) to TOC values and the average BQI family for three different TOC ranges ( $\leq 10 \text{ mg g}^{-1}$ ,  $10\text{--}35 \text{ mg g}^{-1}$  and  $\geq 35 \text{ mg g}^{-1}$ ) was 15.33, 9.15

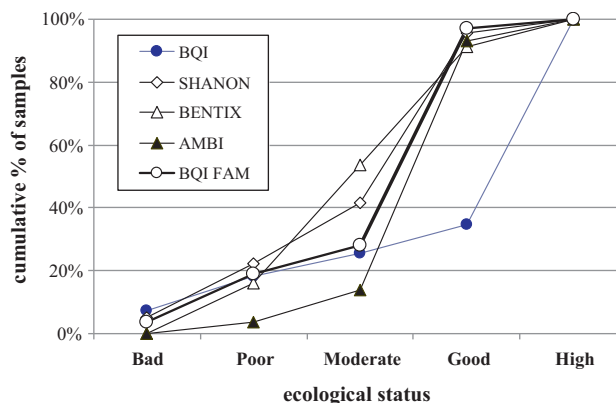


Fig. 4. Ecological status of 640 samples which was “safe” for all the indicators. The BQI-family ecological status limits were those in Table 3.



**Table 4**  
Validation of the BQI family boundaries with 417 independent samples.

4 WFD indices		BQI family	
		Acceptable	Unacceptable
Acceptable	217	217	0
Unclear	72	53	19
Unacceptable	128	0	128
Total	417	270	147

and 4.33, respectively, following the trend of ES10 as described in Hyland et al. (2005).

#### 4. Discussion

Warwick (1986) stated that the analysis at higher taxonomic levels could be sufficient for the detection of biological impacts in well-defined benthic community gradients, when analyzing macrofaunal data with multivariate numerical techniques. This concept has subsequently been termed “*taxonomic sufficiency*” and was also confirmed by research carried out in several parts of the world (Vanderklift et al., 1996; Murugesan et al., 2007; Włodarska-Kowalczyk and Kędra, 2007; Del-Pilar-Ruso et al., 2008; Puente and Juanes, 2008, etc.) and using different types of disturbance (Hall et al., 1996; Gaston et al., 1998; Rumohr and Karakassis, 1999; Dauvin et al., 2003; Huff, 2011); all concluded that there is little information loss when analyzing samples with specimens identified at higher taxonomic levels (Olsgard et al., 1997, 1998). Cost-benefit analyses (Karakassis and Hatzilyanni, 2000; Lampadariou et al., 2005) had shown that the identification at the level of family provides the best balance between the time and effort required and the accuracy obtained. However, there was some reservation in using lower taxonomic resolution in the context of WFD most probably because in this particular case the output expected should not be a multivariate pattern to be used in ordination plots but a single value summarizing the overall environmental quality. This single value should be clear and reliable so that it could be used as a basis for managerial action and intuitively one would rather trust data involving identification at the species level rather than data identified at higher taxonomic levels. However, one should note that variability is higher at the species level primarily because of species substitutions (Warwick, 1986). In the present paper, however, we showed that the analysis of fauna and the use of BQI at the family level could provide values highly correlated to those derived from all the indices calculated with species-abundance data and that the overall result could be very close to the results of all the other proposed indices.

It has been stated (Rosenberg et al., 2004) that BQI assigns sensitivity values (scores) which are somehow objectively obtained through the  $ES50_{0.05}$ , whereas AMBI and BENTIX are calculated based on a subjective literature-based classification of species into groups with different types of response to the disturbance. However, since all these indicators are theoretically based on the benthic community succession paradigm of Pearson and Rosenberg (1978), they are all inter-correlated, probably grasping different aspects of the qualitative changes induced by the anthropogenic disturbance of benthic habitats.

On the other hand, the calculation of  $ES50_{0.05}$  is not straightforward since it requires a substantially large data set often exceeding the capacity of a small or medium-size monitoring program. The BQI-family indicator presented here probably combines the advantages of all the aforementioned indicators: (a) it allows the build up of a database of sensitivity values for families (as in the Appendix) which are more likely to be present in larger geographical areas than individual species, (b) the reliability of the identification of specimens increases as we identify animals at higher taxonomic

levels and (c) the ecological quality boundaries are not set arbitrarily but by incorporating those set for AMBI, BENTIX, BQI and H' and maximizing consensus.

As was mentioned above, stations with depth <20 m and with <50 individuals, or when <20% of the fauna had indicator scores, were excluded from the analysis and sensitivity values were not calculated for taxa with <30 individuals in the data set, or were present in <5 stations. Despite all these exclusion filters, we had a quite large data set to work with and derive our results from so we are fairly confident that, at least for the Eastern Mediterranean, the ecological quality limits proposed for the BQI-family will not be noticeably different if the above exercise is repeated with another equally large data set.

Many studies have shown that very little information is lost by working at a taxonomic level higher than species (e.g. family). In addition, studies investigating the relationship among species diversity, functional diversity and ecosystem function in marine ecosystems showed a strong relation between species diversity and functional diversity (Bell, 2007; Bremner et al., 2003; Hewitt et al., 2008; Micheli and Halpern, 2005; Papageorgiou et al., 2009). In this context we can assume that there is a functional coherence of species belonging to the same family and that the morphological characteristics associated with family identifications are more aligned with functional properties of an organism than are finer morphological distinctions at the species level (Warwick, 1993; Terlizzi et al., 2009). However, it is true that there are cases where species belonging to the same family show remarkable diversity in terms of r- or K-selection strategies. For instance, in our data *Capitella* spp. have a sensitivity value ( $ES50_{0.05}$ ) of 1.83, whereas another capitellid, *Leiocapitella glabra* has 24.21. Therefore, one could argue that the proposed indicator in such cases will not work satisfactorily and may give questionable results. We believe there are some fundamental mathematical reasons why this would not happen. Families with no opportunistic species will have a high sensitivity value whereas families even with a few opportunistic species will tend to have sensitivity values close to that of their “opportunistic” members because these species will be very abundant in the disturbed stations of the data set thus reaching easily the 5% or the total abundance of the family. However, when using the indicator BQI-family, the results of this inconsistency between r and K selection members of the family have little effect on the overall performance of the indicator. For a station with only one or two individuals from the family of Capitellidae, the overall score will be high since the sensitivity value is weighted with their (low) percentage in the abundance of the particular station, whereas a very high number of capitellids in another sample (which will affect the score of the sample) is more likely to be observed in the presence of *Capitella* sp. rather than in the case of other, less opportunistic species such as *Leiocapitella glabra*. So opportunistic species will affect the score of a sample only when in excessive abundance, which is something that opportunistic species do anyway.

Setting limits of ecological quality status particularly in a 5-levels scale inevitably involves a certain level of variability which at least for biotic indices is related to the model of the benthic community reaction in terms of ecological group composition along a pressure gradient in the specific region each index was developed. Therefore, it has been seen that the same sample may be characterized with different quality status depending on the indicator used (Labruno et al., 2006; Borja et al., 2004; Simboura, 2004; Simboura et al., 2005; Simboura and Reizopoulou, 2008). On the other hand, it is true that for a large number of samples, experts from different parts of the world tend to agree on the ecological characterization although there are some disagreements between intermediate classes (Teixeira et al., 2010; Borja et al., 2007, 2009a,b). In the present study we tried not to introduce a new arbitrary scale but to calibrate our scale with the already proposed scales of AMBI,

BENTIX, BQI and Shannon H' which have been tested in various parts of the world and have gained the support of various authors (Borja et al., 2003, 2009a,b, 2011; Labruno et al., 2006; Salas et al., 2004; Simboura, 2004; Marín-Guirao et al., 2005; Grémare et al., 2009; Occhipinti et al., 2009; Dauvin et al., 2010, 2012). In doing this we tried to maximize consensus among the indicators on the limits of ecological quality using relatively objective mathematical procedures. As a result the proposed method gives closer classification results to all the indicators already proposed (than the four indices between them) despite the fact that it uses less information input than any of them. In this context the BQI-family may be seen as a "consensus indicator" because it has been calibrated with the existing ones and it performs more moderately than some of the proposed indicators so far.

The validation tests showed that the BQI family index, with the boundaries proposed here, characterized the samples in remarkable agreement with the existing WFD indicators: when all 4 of them agreed, BQI family assigned the same ecological status and when the majority pointed at Acceptable or Unacceptable status so did BQI family as well. On the other hand, the comparison with the Hyland et al. (2005) paper showed that samples from the three TOC ranges corresponded to noticeable changes in the values of the BQI family index as well, despite the fact that TOC enrichment was not the only source of disturbance in the overall data set.

Of course, it would be advisable to check/validate the BQI-family method against the existing/proposed ones in various sets of local conditions or different types of disturbance gradients, and using BPJ techniques (Teixeira et al., 2010) to improve the reference conditions. Still, we predict that in a large data set the values of different indicators will be significantly correlated with the BQI-family and for most of the stations the ecological quality assigned to BQI-family and any other of the above indices will be identical.

It should be stressed that the most critical boundary for WFD is the boundary between good and moderate status which sets the line for management measures and restoration. In this context, intercalibration activity processes for indices adopted by EU member states are aiming at reaching an agreement on each index boundary value or Ecological Quality ratio in the Good to High and especially in the Good to Moderate boundary, or else harmonize boundaries when necessary according to the intercalibration guidance (EC, 2003). In that sense, the BQI-family performance should be also checked especially in the area of the Good to Moderate boundary with other indices adopted by Mediterranean countries for the WFD as the BOPA, MEDOCC (MEDiterranean OCCidental index) and M-AMBI (Occhipinti et al., 2009; Subida et al., in press).

The implementation of the WFD is expected to increase the demand for monitoring and consequently for scientific labor and high expertise in invertebrate taxonomy in Europe. Sooner or later this will impose dilemmas regarding the spatial coverage, the sampling intensity and the sampling frequency in order to obtain reliable results, particularly since the information obtained is expected to trigger management action to achieve Good or High ecological status when and where this is not found. The proposed methodology using the Taxonomic Sufficiency concept allows at least for the use of a rapid assessment of the ecological quality which could allow focusing perhaps only on cases where the gray zone between Moderate and Good ecological status is located and where management action is mostly needed.

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## Appendix A. Supplementary data

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