Ecological Indicators 29 (2013) 26–33

Contents lists available at SciVerse ScienceDirect

Ecological Indicators

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

Methodological considerations on the coastal and transitional benthic indicators proposed for the Water Framework Directive

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ARTICLE INFO

Article history:
Received 3 July 2012
Received in revised form
14 December 2012
Accepted 24 December 2012

Keywords:
Benthic indicators
Diversity
Mesh size
Samplers
Seasons
Taxonomic sufficiency

ABSTRACT

A large data set from the coastal environment of the Mediterranean across benthic enrichment gradients was used to test the performance of various indices as indicators of the seabed environmental conditions. Comparisons involved samples sieved through different mesh sizes, taken in different seasons and taken with different samplers. Most indices were found not to be affected by sieve mesh size, they showed some variations with sampling season and they varied considerably with sampler (sampling size) used, but also they were found to be significantly intercorrelated. Among the indicators tested, the Benthic Quality Index at the family level (BQI-family) was found to be the least sensitive in changes in the sampling configuration; it is highly correlated with all the other indicators and needs less time and taxonomic expertise. Our results indicate that there is a need for standardization of the methodology used during sampling for the Water Framework Directive, since the sampling configuration (and the indicator used) may to a large extend determine the results of the Ecological Status (ES) analysis.

1. Introduction

A number of ecological indicators have been proposed in the past 12 years as a means for assessing disturbance of the benthic environment. Among these, M-AMBI (Muxika et al., 2007a) which is a combination of the AMBI index (AZTI's marine biotic index) (Borja et al., 2000) and Shannon's diversity index, BENTIX (Simboura and Zenetos, 2002), Benthic Quality Indicator (BQI) (Rosenberg et al., 2004) as modified in Leonardsson et al. (2009), as well as the Shannon Diversity index H' (Shannon and Weaver, 1949), have been widely discussed as potential tools for the implementation of the Water Framework Directive (WFD, 2000). More recently some indicators based on higher taxonomic levels were introduced such as the Benthic Opportunistic Polychaetes Amphipods index (BOPA, Dauvin and Ruellet, 2007), the BQI-family index (Dimitriou et al., 2012) and the $\text{BENTIX}_{\text{family}}$ index (de-la-Ossa-Carretero et al., 2012) aiming at reducing the taxonomic effort and expertise needed and the associated cost of identifying macrofaunal specimens in the framework of environmental monitoring. All these indices are based on the well-known paradigm of benthic succession along gradients of organic enrichment (Pearson and Rosenberg, 1978). Besides those indices, new sample-size independent diversity metrics based on taxonomic distinctness have been used as a means for assessing effects of disturbance on biodiversity (Warwick and Clarke, 1995; Clarke and Warwick, 1998) and it has been suggested (Somerfield and Clarke, 2003) that they can detect impacts not identified through other diversity metrics.

Attempts to compare the performance of the ecological indices at a given site suggest discrepancies in ecological classification depending on the index used (Pranovi et al., 2007; Afli et al., 2008). Comparative analysis of benthic data with various indicators has been the subject of numerous papers (Borja et al., 2003, 2009a,b, 2011, 2012; Muxika et al., 2007a; Simboura, 2004; Simboura et al., 2007; Simboura and Reizopoulou, 2008; Simboura and Argyrou, 2010; Labrune et al., 2006; Occhipinti et al., 2009; Salas et al., 2004; Grémare et al., 2009; Kröncke and Reiss, 2010) and it has been shown that although the values of different indicators are usually significantly inter-correlated, they often diverge in the description of environmental quality. This discrepancy reflects the way that the quality class boundaries were set and probably would be solved to a certain extent by intercalibration and subsequent modification of boundaries in order to achieve consensus (Borja et al., 2009a).

The analysis of benthic enrichment gradients on the seabed beneath and in the close vicinity of fish farm cages in different

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1470-160X/$ – see front matter
http://dx.doi.org/10.1016/j.ecolind.2012.12.020

geographic areas is particularly suitable for testing the performance of the above-mentioned indices. Data sets of this type usually involve strong enrichment gradients over relatively short distances thereby reducing the probability of having simultaneous effects of various stressors or crossing multiple gradients that could result in confounding of the results. These data sets have in general shown results (Brown et al., 1987; Weston, 1990; Findlay et al., 1995; Karakassis et al., 2000; Pohle et al., 2001; Nickell et al., 2003) similar to those predicted by Pearson and Rosenberg (1978). Usually, total organic carbon (TOC) concentration in the sediment beneath the fish cages increases due to the deposition of fish farm effluents (Holmer et al., 2008; Cromey et al., 2012), resulting in significant negative correlation with Shannon's diversity H' (Papageorgiou et al., 2010). Indeed, Hyland et al. (2005) have shown that there is a quantitative relationship between TOC and species diversity metrics, such as Hurlbert $E(S_{10})$ and Shannon-Wiener diversity H', and therefore concluded that TOC could be used as an indicator for the preliminary assessment of the state of the marine benthos.

A series of studies have used one or more of the above indicators to assess their performance in cases of organic enrichment of the seabed beneath fish farms. It has been found that macrofaunal change with distance from the farms may be sufficiently described by AMBI (Borja et al., 2009b), or AMBI and BENTIX but not H' (Aguado-Giménez et al., 2007). Some studies have found AMBI and H' showed similar patterns (Borja et al., 2009b) whereas others did not (Carvalho et al., 2006; Callier et al., 2008). One study (Aguado-Giménez et al., 2007) showed that H', AMBI and BENTIX did not show significant differences with sampler used (van Veen or SCUBA-diving equipment, i.e. manual grabs and cores). Two studies addressing the stability of the results among different seasons gave conflicting results (Chainho et al., 2007; Reiss and Kröncke, 2005). Couto et al. (2010) have assessed the changes with sieve mesh size in AMBI as well as in Shannon, Margalef and Pielou indices in a small number of samples from estuarine environment concluding that using 1.0 mm sieve is probably enough for such environments but also recommending further validation and additional tests. Results from estuaries Muxika et al. (2007b) indicated that sample size may significant affect the classification of samples into ecological status classes.

All of the above implies that there is a need to clarify how the specification of the sampling configuration (such as sieve mesh size, sampling season and sampler size) might affect the results of these indices and also to determine whether their values may be correlated to other more easy-to-measure geochemical variables. The aim of the present paper was to assess the performance of these indices (including also those using taxonomic sufficiency) on a relatively large data set from the Eastern Mediterranean comprising samples taken at various sites along organic enrichment gradients. In particular the aims of the study were to test a series of related hypotheses:

- their values are not affected by sieve mesh size,
- their values are not affected by season,
- their values are not affected by the size of the sampler used,
- their values are not correlated to geochemical variables, nor to conventional descriptors of community diversity.

The testing of these hypotheses should be taken into account before decisions are made on the standard use of the above indices for monitoring environmental quality. Finally, the issue of the potential use of these indices in aquaculture is discussed as an example of the enforcement of the WFD in coastal waters.

2. Materials and methods

2.1. Data-set description

To test the above mentioned hypotheses, a series of data sets were used, using samples collected in different parts of the Mediterranean in the framework of the projects MERAMED (Modelling environmental response to Aquaculture in the Mediterranean http://meramed.akvaplan.com/metadata/) and MedVeg (Effects of nutrient release from Mediterranean fish farms on benthic vegetation in coastal ecosystems, http://www.euraquaculture.info/files/MEDVEG_web.pdf) and IBIS (Fish farming effects on benthic biogeochemical processes). A total of 15 sites were sampled, 9 of which were located in the Aegean Sea, 3 in the eastern Ionian Sea, 1 in Cyprus, 1 in Sicily and 1 in the Mediterranean coast of Spain (Table 1). The fish farms varied between the sites in terms of production size, bathymetry, topography and sediment type. To protect commercially sensitive information, a confidentiality agreement was signed with the farmers that the results of individual farms will not be disclosed, therefore, maps together with other detailed geographic information of the sites are not presented here.

In sites 1–7 and 12–15, six sampling stations were selected at increasing distance from the farm (0, 5, 10, 25 and 50 m from the edge of the cages) and a control station in similar depth and sediment type, which was situated approximately 1 km upstream the main current direction and not subject to any other apparent source of pressure. In sites 8–11, two different sediment types were selected; bare and vegetated with the seagrass *Posidonia oceanica* (L.) Delile. At each sediment type 0, 25 m and control stations were sampled.

Three to five replicate samples were collected from each site and station either by divers, using plexiglas core tubes (9.5 cm internal diameter) or a suction device ($0.2\,m^2$) in the case of the vegetated stations, or by means of a van Veen grab ($0.1\,m^2$). Three sampling cruises were conducted during two different periods: two during the warm period of the year (July and October), i.e. during maximal food supply and maximal loss of wastes to the environment, and one during the cool period (March). The samples were sieved through 0.5 and/or 1.0 mm mesh sieves and preserved in 10% formalin with the addition of Rose Bengal to facilitate sorting. All specimens were identified to species level, where possible.

The data sets used were the following:

2.2. Data set A. Mesh size

Samples were collected from each sampling event, site and station and used in order to study if the benthic indices are affected by the mesh size of the sieve (total sample count was 201). The samples were sieved through 0.5 and 1.0 mm mesh sizes. Fauna retained on the 1.0 mm mesh is referred to as the large fraction whereas fauna retained on the 0.5 mm sieve after passing through the 1.0 mm sieve is referred to as the small fraction. Consequently, the total macrofaunal assemblage consists of the sum of the small and the large fraction, which is referred to as the combined fraction.

2.3. Data set B. Season

A series of benthic samples were taken from each sampling station at 3 sites in the Aegean Sea (site 1–3) during 3 different sampling events (July 2001, March 2002, October 2002), in order to study if the benthic indices are affected by season (3 seasons with 18 samples each). All samples were taken by divers using core tubes and sieved through 0.5 mm mesh sieve, as described above.