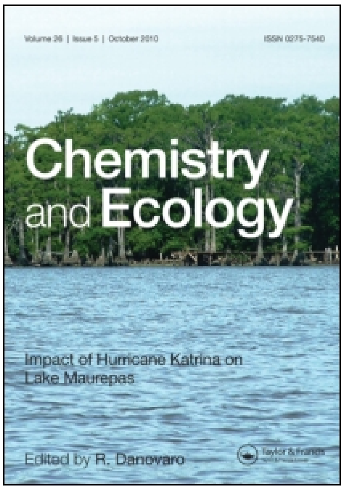

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Daphne: a new multimetric benthic index for the quality assessment of marine coastal environment in the Northern Adriatic Sea

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A new benthic index, named *Daphne*, is proposed for the Northern Adriatic coastal area, near the Po river delta. It is based on six characteristics of the community that do not require in-depth taxonomic expertise: number of mollusc species, % of bivalves, % of polychaetes, abundance of the opportunistic species *Corbula gibba*, % of amphipods and number of 'typical mollusc species' that are individuated by multivariate analysis. The application of the index in selected stations along a gradient of decreasing disturbance shows that it is simple to use in regular monitoring campaigns and that the results are consistent with environmental quality data in the special conditions of this area subjected to considerable river runoff. The index can be used in addition and as a complement to more widespread indices (such as M-AMBI); a comparison of the two indices performance is discussed.

Keywords: Soft bottom macrobenthos; Biotic index; Coastal environment; Ecological Status; AMBI; Italy

1. Introduction

The European Water Framework Directive (WFD 2000/60/CE) introduced the concept of Ecological Quality Status (ES) for the assessment of the ecological quality of water bodies. The ecological status should represent the complexity of the aquatic ecosystem and must therefore incorporate the physical-chemical conditions of the water and sediment, the water flux characteristics and the water body physical properties with particular attention to the ecosystem biotic elements [1]. Within the European Union all the Member States are required to achieve at least a 'good ecological status' for all water bodies, by the year 2015.

In coastal waters, considered by the Directive to be among the 'significant' water bodies (rivers, lakes and ground water, transitional and coastal waters), benthic invertebrate fauna is one of the biological quality elements required for the classification. The usefulness and the advantages of soft bottom benthos as bioindicator have been widely recognized [2] and benthos has been frequently used in marine monitoring programs. Many benthic indices have been developed for the quality assessment of marine coastal environments: some are based on the

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presence or absence of species characteristic of particular environmental conditions [3]; others consider changes in the community structure or biomass [4]; others combine physical-chemical and biological analyses [5]. Some authors have provided an overview of the main benthic indices proposed in recent years [6, 7]. Often the common disadvantage to the majority of these indices is their limited practicality and difficulty in application, while fast and easily-applicable methods are needed.

Thus in recent years, developing new biotic indices, or evaluating the suitability of those already existing, has become an important and urgent goal for the scientific and technical community for the management of marine coastal environment. Since 2000, the journal *Marine Pollution Bulletin* has published more than 20 papers presenting new benthic indices [8, 9] or testing and discussing those already known [10] and it has hosted a series of debates [11, 12] arising from the difficulty in finding indices of wide validity and general application. Very recently a whole special volume has been dedicated to the implementation of the Water Framework Directive (*Marine Pollution Bulletin*, Volume 55, 2007).

At present, the most frequently used indices also tested within the Mediterranean Geographic Intercalibration Group (MED-GIG) (the working group created for the implementation of the European Directive within the Mediterranean Countries) are AMBI (AZTI Marine Biotic Index) [8], Bentix [13] and BQI (Benthic Quality Index) [9]. The first two indices are based on the Pearson and Rosenberg paradigm [14], which states that sensitive species tend to disappear as the level of disturbance increases, while tolerant species thrive. Each benthic species is assigned to different ecological groups according to its sensitivity to pollution. The AMBI and Bentix are calculated considering the percentage of individuals belonging to each ecological group; the two indices differ in the number of ecological groups and in the formula used to calculate them. AMBI has been widely tested both for different environments and disturbance sources [15–17] and in general it has proved to be satisfactory.

The critical point of these indices is the fact that, besides requiring the identification of all organisms in the sample to species level, the species are not assigned to the different ecological groups objectively, but rather on the basis of literature data and the authors' experience.

The BQI attempts to address this issue by proposing a method to determine the species tolerance values through an objective analysis applied to the study area data-set. It considers that tolerant species are predominantly found in disturbed environments where the diversity is low, whereas sensitive species are restricted to undisturbed environments with high diversity. BQI has been tested in the North Sea [18], along the Mediterranean French coast [10] and more recently in the Baltic Sea [19] and in the English Channel [20]. In these studies the main critical point is that the species tolerance value can be obtained only from a very large data set including both impaired and unimpaired sites (the authors used their own data from 257 stations sampled on 1114 occasions over a 40-year period).

Among the WFD requirements, another important step, which has to be undertaken in the next few years, is the development of Reference Conditions (RC). RC are defined as a description of the biological quality elements that exist, or would exist, at high status, that is, with no, or very minor disturbance from human activities [21]. The objective of setting standard RC is to enable the assessment of ecological quality against these standards. The main problem in establishing RC in many European regions derives from the huge difficulty in finding unimpaired sites and the lack of historical pre-industrial data. For this reason, some authors [22] have proposed considering 'virtual reference conditions', which do not actually exist but rather are based upon experience gained from the area and conceived as the potential component that should be present.

In this context we have developed a new index, straightforward and easy to compute. It is proposed for the Northern Adriatic Sea, a very peculiar marine region, already considered as a vulnerable area by the Italian legislation (D.L. 152 11 May 1999). This semi-enclosed shallow area of the Adriatic shelf is strongly influenced by meteorological conditions and river runoffs. The Po is the most important river of the Northwest Adriatic basin, largely influencing haline stratification, sediment texture patterns and nutrient load of the coastal zone [23]. The macrobenthic community shows a high capacity to thrive and adapt to these peculiar environmental conditions; it is characterized by the presence of a few dominant species, highly abundant and subjected to occasional demographic blooms, and of many species with low abundance [24, 25]. Due to these particular but natural characteristics of the Northern Adriatic benthic community, the methods for assessing the environmental conditions which are based only on structural community variations run the risk of underestimating the quality status.

Taking all this into account, we tried to develop an index that constitutes a rapid tool to be routinely used by the technical personnel of the Regional Environmental Agency, appointed to the monitoring of this coastal area.

2. Materials and methods

2.1 *Data collection*

The index was developed using soft bottom macrobenthos data collected in 5 stations at about 10 m depth (see figure 1). The first data set comprises three stations in Porto Tolle, sampled in summer and autumn 1993 and in spring, summer and autumn 1994 (total number of samples equalled 24). The second data set refers to two stations in Porto Garibaldi and Cesenatico, sampled seasonally from July 1996 to July 2002 (total number of samples equalled 50). These stations are placed at an increasing distance from the Po delta and therefore they are differently influenced by the trophic load carried by the river [26]. The index was then validated on a third data set collected, in the framework of the 'NITIDA'[†] project, in May, July and October 2004, at about 10 m depth, in Porto Garibaldi, Ravenna, Cesenatico and Cattolica. These stations are located, parallel to the coastline, along the North-South gradient of decreasing disturbance caused by the riverine input.

The first data set (Porto Tolle stations) was collected by the ENEL Environment Research Centre in connection with ecological monitoring near a thermal power plant site; the data were published in a series of detailed ENEL reports and had been summarized in several publications [27, 28].

The second and third data sets were collected by our Institute by sampling surveys carried out in the course of the oceanographic vessel 'Daphne II' belonging to the Emilia Romagna Regional Agency for Environmental Protection (ARPA). At each station, four faunal replicates were collected together with a sample of sediment for grain size analysis using a 0.06 m^2 van Veen grab. The collected material was sieved through 1 mm size mesh and preserved in a formaldehyde seawater solution (10%). Physical-chemical data were measured by 'Daphne II' on an approximately weekly basis. Sediment texture was assessed following the Buchanan method [29].

2.2 *Development of the Daphne index*

The index, named after the oceanographic vessel 'Daphne II' used for sampling, has been developed considering an array of measures (metrics) that individually provide limited information on biological condition but when integrated, function as an overall indicator [30, 31].

[†]The study was carried out as a part of the MURST PRIN 2003 Project, entitled "Nuovi Indicatori di stato Trofico e d'Integrità ecologica Di Ambienti marini costieri e ambienti di transizione (NITIDA)".

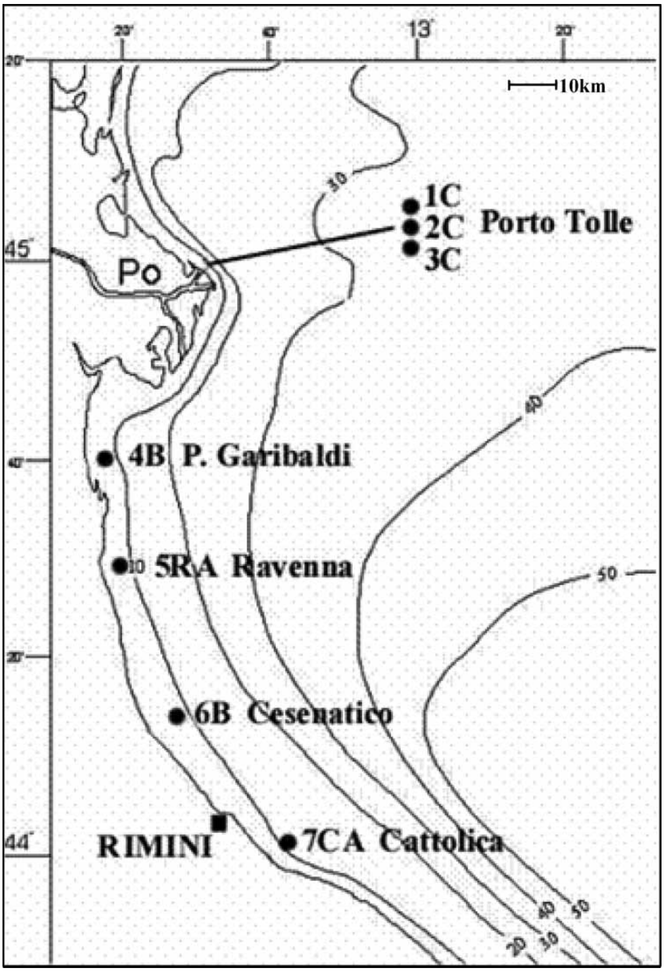

Figure 1. Sampling area. Data from stations 1C, 2C, 3C (sampling period 1993–1994) and 4B, 6B (sampling period 1996–2002) were used to develop the Daphne index. Data from stations 4B, 5RA, 6B, 7CA (sampling period 2004) were used to validate the index.

In the first step, the metrics were selected on the basis of bibliographic researches and taking into consideration our own data. Characteristics such as simplicity and rapidity in the computation of the metric, both during collection and analysis of data, were considered as fundamental.

As the ecology of many marine species is known, specific identification allows more accurate information on the macrobenthic community but, on the other hand, it is time consuming and requires good systematic expertise. This is particularly true for the phylum of polychaetes. In some cases characteristics which are essential for the identification at species level are extremely difficult to recognize; furthermore these organisms are often quite small, fragile and therefore easily damaged during sampling operations, thus hampering the detection of the taxonomic characteristics.

For this reason, metrics such as community richness or diversity, which require the identification of all organisms in the sample to species level, were not considered. We also rejected those metrics based on the classification of the macrobenthic community in feeding guilds. Besides requiring the identification to species level, the assignment of each species to a feeding group is an even more difficult task [32] for the lack of available literature data and different authors' often contrasting classification.

For all of the above-mentioned reasons, we decided to use metrics that require: the identification to species level only for the molluscs, which are important ecological indicators and easier to identify; the identification to class level for polychaetes, regarded as difficult to identify and frequently as the most labour-intensive of the main taxonomic group in the study of soft bottom macrobenthos [33]; the identification to order level for crustacean; only the amphipoda are considered, whose value as bioindicators is widely recognized [34].

Six metrics (see table 1) were selected on the basis of their efficiency in discriminating between more (Northern) and less (Southern) disturbed stations and on the basis of their relation with structural and physical-chemical parameters. The first four metrics are based upon the assumption that molluscs, considered both as number of total species and percentage of bivalves, and crustacean amphipods decrease when the disturbance increases, whereas polychaetes, generally more tolerant, show an inverse trend. All these biological attributes are commonly considered as potential metrics in developing multimetric benthic indices and their indicator value is known [35, 36]. Bivalves as a group are not always indicative of healthy environments, and many species are considered as pollution or stressed tolerant species second to polychaetes. Yet in estuarine environments or areas influenced by riverine runoff they are consistently assuming high percentages and are considered as the natural dominant group. Moreover, the abundance of the bivalve *Corbula gibba* (not counted in '% of bivalves') is considered itself as a metric, as this species, typical of unstable sediment [37] with a high rate of sedimentation, is widely spread in this area of the Northern Adriatic, near the Po delta. It is also considered an indicator of organic enrichment and anoxic conditions [38], therefore its abundance is related to disturbed conditions. The last metric refers to the presence of those ten mollusc species (both bivalves and gastropods), that, found at high abundances in most samples, are considered as 'typical' of the Northern Adriatic benthic community by the application of multivariate analyses SIMPER test [39]. The species are: *Abra alba*, *Abra prismatica*, *Chamelea gallina*, *Mysella bidentata*, *Nucula nitidosa*, *Pharus legumen*, *Spisula subtruncata*, *Tellina fabula*, *Tellina nitida*, *Cylichna cylindracea*. The higher the number of typical species in a sample, the more its community is similar to the expected one. In table 1, metrics calculated for the Cesenatico station are reported as an example.

The second step in the development of the Daphne index required the assignment of a numerical value to each metric. This was a particularly difficult task since no pristine coastal areas exist anymore in the Northern Adriatic, making it impossible to find real reference conditions and assign an absolute score to each metric. For this reason the scores were defined on the basis of the maximum or minimum value recorded for each metric in the sampling stations over the entire study period. The highest score, equal to 1, was assigned to:

- the maximum value recorded for those metrics that increase in good environmental conditions (number of molluscs species, % of bivalves (excluding *C. gibba*), % of amphipods, number of typical species);
- the minimum value recorded for those metrics that increase in bad environmental conditions (% of polychaetes and abundance of *C. gibba*).

In all other cases, the score for each metric was computed as a ratio of the maximum or minimum value (see table 2). In this way the problem of having values expressed with different measurement units (number of species, percentage of bivalves, abundance of *C. gibba* etc.) has been solved.

The reference value for '% of bivalves (excluding *C. gibba*)', '% of amphipods' and '% of polychaetes' was adjusted in order to add up to 85%, given that the average percentage of the remaining taxonomic groups is 15%.

Table 1. Values of the selected metrics (first column) at Cesenatico station samples (6B), from July 1996 to July 2002. In the second column, the reference value obtained from the data-base for each metric is shown.

Metrics	Reference value	7/96	10/96	2/97	5/97	7/97	10/97	3/98	5/98	7/98	10/98	3/99	5/99	7/99	10/99	2/00	5/00	7/00	10/00	3/01	5/01	7/01	10/01	3/02	5/02	7/02
Mollusca spp. (n°)	23	20	16	7	16	9	22	16	13	17	18	13	7	17	17	8	6	13	17	15	13	18	16	21	17	21
Bivalvia (%)	43.3	1.4	5.5	12.3	3.0	8.0	54.97	6.9	9.3	35.5	28.8	5.8	4.4	10.2	11.7	7.0	1.0	1.4	31.6	11.9	22.6	14.6	22.4	35.2	11.2	3.7
Polychaeta (%)	9.9	75.3	75.6	65.8	45.7	70.5	12.6	91.1	78.6	45.5	43.7	90.8	85.6	22.5	73.0	84.8	90.5	83.3	63.4	70.9	68.8	44.9	63.0	53.3	85.1	92.0
Amphipoda (%)	31.8	0	1.0	3.4	13.7	3.9	0.0	0.4	1.1	7.5	17.1	3.0	8.6	40.32	5.5	7.0	6.1	10.9	3.6	16.3	6.9	30.7	10.2	2.6	0.4	0.3
C. gibba (ind. m^{-2})	0	4129	1150	21	642	129	1392	50	4	183	192	4	0	1008	213	4	175	333	196	13	13	233	33	8	254	292
Typical spp. (n°)	9	5	9	3	9	6	8	9	8	8	7	8	5	9	8	2	4	6	7	5	6	8	7	8	9	8

Table 2. Score assigned to each metric at Cesenatico station samples (6B), from July 1996 to July 2002. In the last two rows, the Daphne Index value and the related Ecological Status (ES) are reported. P = poor, M = moderate, G = good, H = high.

Metrics	Reference value	7/96	10/96	2/97	5/97	7/97	10/97	3/98	5/98	7/98	10/98	3/99	5/99	7/99	10/99	2/00	5/00	7/00	10/00	3/01	5/01	7/01	10/01	3/02	5/02	7/02
Mollusca spp.	1	0.87	0.70	0.30	0.70	0.39	0.96	0.70	0.57	0.74	0.78	0.57	0.30	0.74	0.74	0.35	0.26	0.57	0.74	0.65	0.57	0.78	0.70	0.91	0.74	0.91
% Bivalvia	1	0.03	0.13	0.28	0.07	0.19	1.00	0.16	0.22	0.82	0.67	0.13	0.10	0.24	0.27	0.16	0.02	0.03	0.73	0.27	0.52	0.34	0.52	0.81	0.26	0.09
% Polychaeta	1	0.27	0.27	0.38	0.60	0.33	0.97	0.10	0.24	0.61	0.63	0.10	0.16	0.86	0.30	0.17	0.11	0.19	0.41	0.32	0.35	0.61	0.41	0.52	0.17	0.09
% Amphipoda	1	0.00	0.03	0.11	0.43	0.12	0.00	0.01	0.04	0.24	0.54	0.10	0.27	1.00	0.17	0.22	0.19	0.34	0.11	0.51	0.22	0.97	0.32	0.08	0.01	0.01
C. gibba	1	0.00	0.72	0.99	0.84	0.97	0.66	0.99	1.00	0.96	0.95	1.00	1.00	0.76	0.95	1.00	0.96	0.92	0.95	1.00	1.00	0.94	0.99	1.00	0.94	0.93
Typical spp.	1	0.56	1.00	0.33	1.00	0.67	0.89	1.00	0.89	0.89	0.78	0.89	0.56	1.00	0.89	0.22	0.44	0.67	0.78	0.56	0.67	0.89	0.78	0.89	1.00	0.89
Daphne	1	0.29	0.47	0.40	0.61	0.44	0.75	0.49	0.49	0.71	0.72	0.46	0.40	0.76	0.55	0.35	0.33	0.45	0.62	0.55	0.55	0.75	0.62	0.70	0.52	0.49
ES	H	P	M	M	G	M	G	M	M	G	G	M	P	G	M	P	P	M	G	M	M	G	G	G	M	M

In the third step, the scores of each individual metric were combined into a single value. For each sample the scores were summed and divided by the total number of metrics. The final value ranges between 0 and 1, the closer the value is to 1 the better the environmental conditions are.

In the fourth step, the interval between 0 and 1 was divided into five equal levels corresponding to the five different classes of ecological status required by the Water Framework Directive: Bad status, Daphne $\leq$ 0.20; Poor status, 0.20 $<$ Daphne $\leq$ 0.40; Moderate status, 0.40 $<$ Daphne $\leq$ 0.60; Good status, 0.60 $<$ Daphne $\leq$ 0.80; High status, Daphne $>$ 80.

2.3 *Data analysis*

The results obtained by the application of the Daphne index to macrobenthic data from stations sampled in 2004, were compared to those obtained by the application of Multimetric AMBI method (M-AMBI) to the same data. This method, more recently proposed [22], considers the use of AMBI index as a part of a set of measures, such as Shannon diversity (H) and richness (S), in a multimetric approach. It allows us to directly obtain the EQR, comparing the value of the three metrics (AMBI, H, S) measured in a sample with those corresponding to the RC. The AMBI index considers the percentages of abundance of five ecological groups, within each sample, according to the following formula:

$$\mathrm{AMBI} = \{(0 \times \%\mathrm{GI}) + (1.5 \times \%\mathrm{GII}) + (3 \times \%\mathrm{GIII}) + (4.5 \times \%\mathrm{GIV}) + (6 \times \%\mathrm{GV})\}/100,$$

where % GI is the relative abundance of sensitive species, % GII of indifferent species, % GIII of tolerant species, % GIV of II order opportunistic species and % GV of I order opportunistic species. AMBI ranges between 0 and 6, 6 being when all the individuals belong to group V and the environmental conditions are heavily disturbed.

In this work M-AMBI was calculated for the whole data-base using the software AMBI 4.0, freely available on AZTI's web page (www.azti.es); RC and quality class boundaries provided as default by the software were used. The default RC are: for Bad ES, Ambi $=$ 6, H and S $=$ 0; for High ES the software select the lowest AMBI value and the highest H and R values in the dataset. The quality class boundaries given as default are those derived by the European benthic intercalibration exercise [40]: Bad status, M-AMBI $<$ 0.20; Poor status, 0.20 $\leq$ M-AMBI $<$ 0.39; Moderate status, 0.39 $\leq$ M-AMBI $<$ 0.55; Good status, 0.55 $\leq$ M-AMBI $<$ 0.85; High status, M-AMBI $\geq$ 0.85.

Macrobenthos community structure was investigated by univariate and multivariate analysis using the software package PRIMER [41]. Statistical analysis (non parametric Spearman correlation and Cohen's Kappa test [42]) was performed using software SPSS 11.0.

3. Results

The Oceanographic Structure of the ARPA has been monitoring the Emilia Romagna coast since 1977 by collecting physical-chemical data, therefore detailed information about the environmental conditions in the Northern Adriatic is available [43]. In the study area a North-South gradient of improvement in the environmental conditions exists.

The Daphne index has rated 15% of the stations used to develop the index to be in good ecological condition, 51% to be in moderate ecological condition and 34% to be in poor ecological condition. In keeping with the environmental conditions known for the area, the Northern stations, Porto Tolle and Porto Garibaldi, were found to be more disturbed; the 36% of the samples from the Cesenatico southern station were found to be in good ecological condition.

Starting from these observations, we tested the index on some stations located at about the same depth along a transect parallel to the coastline, for which the physical-chemical parameters were available and the environmental conditions were a-priori known. The box-plots in figure 2, show the trend of some physical-chemical parameters in Porto Garibaldi (4B), Ravenna (5RA), Cesenatico (6B) and Cattolica (7CA) during 2004 (outliers and extremes are not shown). An improvement in the water trophic conditions moving from the North towards Cattolica is evident.

Bottom concentration of dissolved oxygen in Porto Garibaldi falls under critical values (minimum value 0.5 mg.l^{-1}), on more than one occasion. Although the median is similar in all the stations, the interquartile range, which contains 50% of the values, decreases, indicating that bottom oxygen concentration is less variable in Cesenatico and Cattolica. The chlorophyll 'a' concentration in the water column decreases (median = 5.5 μg.l^{-1} in PG, and 1.5 μg.l^{-1} in CA) following the expected gradient as does nitrate concentration (median = 381.5 μg.l^{-1} in PG and 75.8 μg.l^{-1} in CA). From the box-plot representing the phosphate concentration,

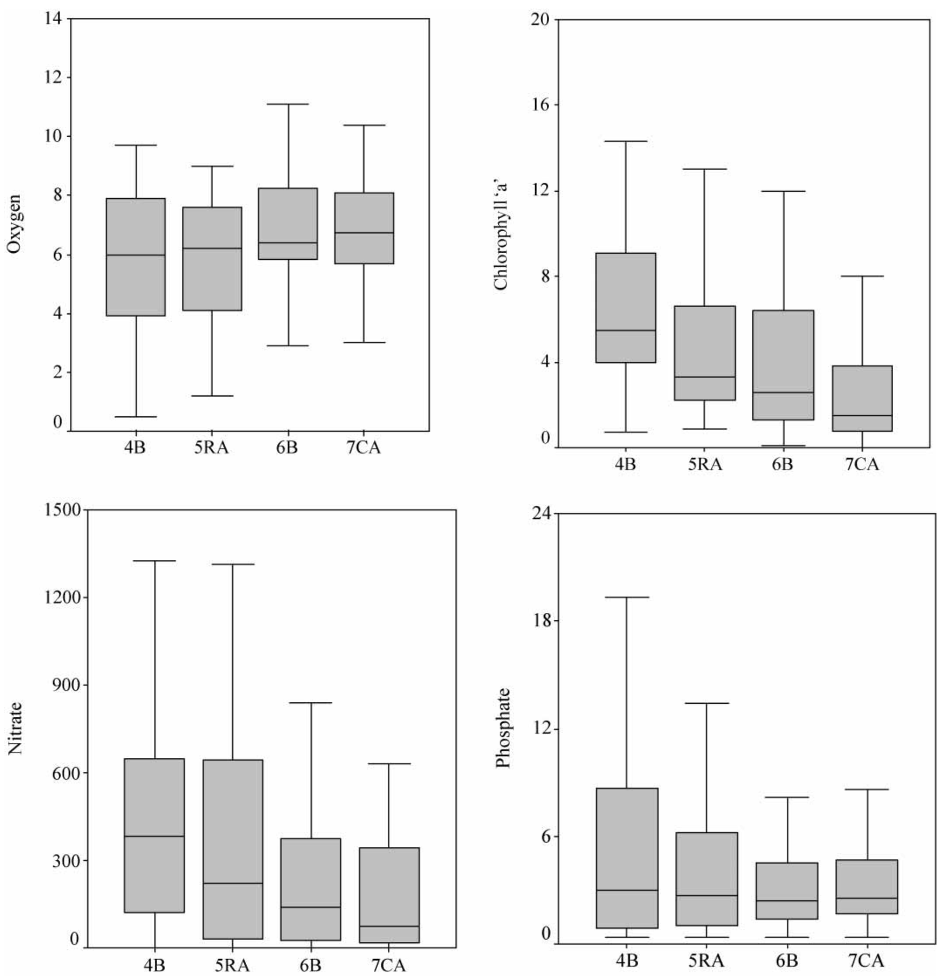

Figure 2. Box plot of bottom dissolved oxygen (mg.l^{-1}), chlorophyll 'a' (μg.l^{-1}), nitrate (μg.l^{-1}) and phosphate (μg.l^{-1}) measured weekly in the sampling stations (4B = Porto Garibaldi, 5RA = Ravenna, 6B = Cesenatico, 7CA = Cattolica) during 2004.

a clear reduction of the median value along the transect is not evident, but in Cesenatico and Cattolica a minor variability exists (interquartile range = 8.1 in PG, 3.3 in CA).

The North-South improvement gradient is confirmed by the average values of main structural parameters that describe the macrobenthic community (see richness and diversity in table 3) with minimal differences between Porto Garibaldi and Ravenna. The most variable parameter is the richness of species: the average annual value in Cattolica (59.7) is nearly twice as that recorded in Porto Garibaldi and Ravenna.

The Daphne index values are consistent with the general trend in the environmental conditions (see figure 3). The average annual values increase gradually from Porto Garibaldi (Daphne = 0.49) and Ravenna (Daphne = 0.54) to Cesenatico (Daphne = 0.62) and Cattolica (0.68). The metrics mainly responsible for the improvement are: number of mollusc species (the average score increases from 0.46 in Porto Garibaldi to 0.90 in Cattolica); the % of amphipods, which is less than 2% of the community in Porto Garibaldi and Ravenna (average score close to 0.05), and reaches high percentages in Cesenatico and Cattolica with an average score over 0.57; *C. gibba* has the lowest abundances in Cesenatico and Cattolica, where the average score is 0.97; the average score assigned to the metric 'typical species' reaches 0.81 in Cesenatico and Cattolica. The other 2 metrics, the '% of bivalves' and the '% of polychaetes' do not change too much and do not contribute to the improvement of the index along the transect.

The differences in the community structure, resulted by the application of Daphne, cannot be due to differences in the sediment texture; with the exception of Porto Garibaldi, which

Table 3. In the first two columns reference values of AMBI, diversity and richness in low and high ecological status and corresponding M-AMBI value (last row) are reported; in the other columns the annual average values of the three metrics and M-AMBI in the sampling stations in 2004 (4B = Porto Garibaldi, 5RA = Ravenna, 6B = Cesenatico, 7CA = Cattolica).

	Reference values		Annual average values along N–S transect			
	Bad	High	4B	5RA	6B	7CA
AMBI	6	1.22	2.81	2.89	1.87	1.42
Diversity	0	4.14	3.24	3.23	3.56	3.80
Richness	0	67	33.67	32.67	39.67	59.67
M-AMBI	0	1	0.65	0.63	0.77	0.92

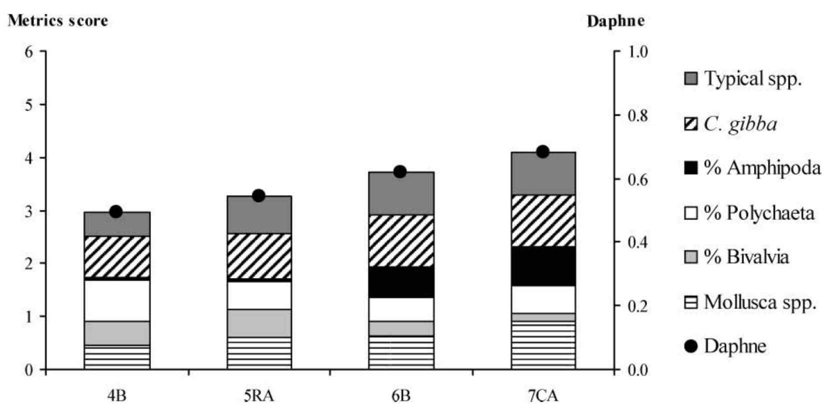

Figure 3. Annual average values of Daphne index (black point) at the sampling stations (4B = Porto Garibaldi, 5RA = Ravenna, 6B = Cesenatico, 7CA = Cattolica) in 2004 (right axis) and contribution of each metric (histograms, left axis).

has an average percentage of silt and clay over 50%, in all the other stations sand is always the highest fraction (>65%).

Non-parametric Spearman correlation between the Daphne index and some physical-chemical and community structural parameters was tested (see table 4) considering all the sample for which physical-chemical data are available in the first case all the data collected. In the second the index is negative correlated with chemical factors indicative of the trophic conditions, and positively with transparency. Among structural parameters it is positively correlated with specific richness, Shannon diversity and evenness, and negatively correlated with the Simpson dominance index.

The M-AMBI roughly follows the physical-chemical environmental conditions too (see table 3), reaching a value higher than 0.9 in Cattolica.

Considering the AMBI index, the highest values (worst ecological conditions) were recorded in Porto Garibaldi and Ravenna, respectively AMBI = 2.8 and 2.9. In these stations, the dominant ecological group is GIV (see figure 4). The high percentage in the GIV, is due to the molluscs *C. gibba* and *Anadara demiri* and the polychaetes *Heteromastus filiformis*, belonging to the Capitellidae family, and *Prionospio caspersi* mainly in Ravenna. Because of the higher abundance of sensitive and indifferent species (GI and GII) in Cesenatico and Cattolica, the

Table 4. Non parametric Spearman correlation between Daphne index and the main physical-chemical and structural parameters. Only significant correlation is shown, $^{*}p = 0.05$, $^{**}p = 0.01$.

		Richness	Diversity	Dominance	Evenness
Daphne value	Rho	0.637**	0.582**	−0.527**	0.382**
	Significance level	0.000	0.000	0.000	0.000
	N	86	86	86	86
		Chlorophyll ‘a’	Nitrate	Phosphate	Transparency
	Rho	−0.475**	−0.423**	−0.287*	0.493**
	Significance level	0.000	0.001	0.032	0.000
	N	56	56	56	56

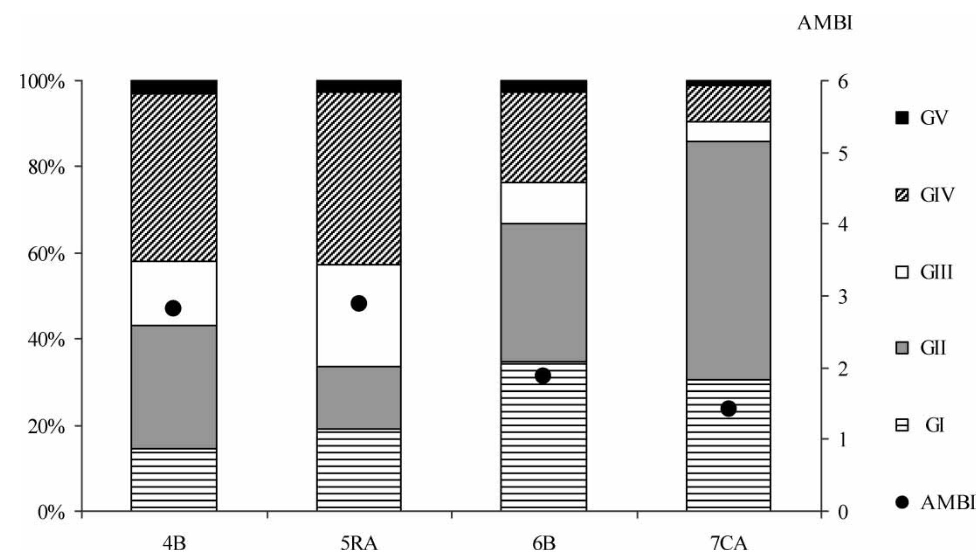

Figure 4. Annual average values of AMBI (black point) at the sampling stations (4B = Porto Garibaldi, 5RA = Ravenna, 6B = Cesenatico, 7CA = Cattolica) in 2004 (right axis) and percentage of individuals in each ecological group (GI, GII, GIII, GIV, GV) (histograms, left axis).

Table 5. Annual average values of the indices Daphne and M-AMBI and related Ecological Status (ES) recorded in the sampling stations in 2004 (4B = Porto Garibaldi, 5RA = Ravenna, 6B = Cesenatico, 7CA = Cattolica). M = moderate, G = good, H = high.

	4B	5RA	6B	7CA
Daphne value	0.49	0.54	0.62	0.68
ES classification	M	M	G	G
M-AMBI value	0.65	0.63	0.77	0.92
ES classification	G	G	G	H

AMBI decreases (AMBI = 1.9 and 1.4 respectively). More abundant here are species assigned to GI, such as the molluscs *Chamelea gallina*, *Hyala vitrea* and *Spisula subtruncata*, the polychaete *Aricidea assimilis* and the crustacean belonging to *Iphinoe* genus; whereas the polychaete *Owenia fusiformis* and the crustacean *Ampelisca diadema* are assigned to GII.

As already analysed the other two metrics considered by M-AMBI follow the same AMBI trend.

Non parametric Spearman correlation between the Daphne index and the M-AMBI has been tested too, taking into account all the data: a significant positive correlation is found (rho = 0.714, $p < 0.01$).

The values of Daphne and M-AMBI along the North-South transect show a similar trend of improvement. Nevertheless, the classification in Ecological Status following the two indices not always coincides (table 5). The Daphne index rates the Northern stations to be in moderate status, while Cesenatico and Cattolica are in good ecological status; the M-AMBI rates Porto Garibaldi, Ravenna and Cesenatico to be in good ecological status and Cattolica in high ecological status. Kappa analysis, applied to all the data, shows no agreement between the classification obtained by the two indices.

4. Discussion and conclusions

In the Northern Adriatic Sea, where the Po river run-off influences a large area, macrozoobenthic communities experience the effects both of physical conditions and eutrophication.

The gradient of sediment grain size depending on the instability of terrigenous deposits carried by the Po river [44], is paralleled by different benthic community patterns established at different depths that can be referred to the benthic community classification by Pérès and Picard [37]: near the coast the community corresponds to the ‘Fine sand biocoenosis in very shallow waters’ (SFHN); at intermediate depth to the ‘Well sorted fine sand biocoenosis’ (SFBC); moving off-shore, community composition corresponds to that of the ‘Coastal Terrigenous Muds’ (VTC) [25, 45].

The influx of riverine discharges causes a peculiar composition of benthic communities (*e.g.* the presence of euryhaline species such as *Heteromastus filiformis*, *Lentidium mediterraneum*, *Cyclope neritea* etc.), moreover, the nutrient load from river output causes different trophic conditions, from mesotrophic to hypertrophic. High nutrient load, coupled with algal bloom and good meteorological and hydrodynamic conditions can then generate hypoxia, or even anoxia, which strongly affects the benthic community [44, 46]. In fact, when organic loading increases species richness decreases and the benthic community assemblage shifts toward higher densities and total abundances of few opportunistic species [47]. In the Northern Adriatic Sea the eutrophication conditions are less extreme than in other eutrophicated coastal areas and the structure and the composition of the benthic community is the result of an intermittent recovery from periodic disturbances, such as dystrophic events, that maintain transitory successional stage [48].

For the aforementioned natural properties of the Adriatic benthic community, a new index specific for the Northern Adriatic area was developed, in order to consider its particular characteristics and consequently to avoid misleading information on the environmental quality.

The Daphne index was developed along the lines of multimetric indices. The multimetric approach was initially developed for streams but the method has increasingly been applied to the macrobenthos of some North American estuarine areas [49, 50]. These indices have the advantage of being able to integrate and summarize in a single value a lot of different information about organisms, referring both to the presence of indicator taxa and to community structure. Related to the metrics selected '% of bivalves' and '% of polychaetes' do not always show a clear trend following the disturbance gradient. Their usefulness will have to be tested and validated on more data from the same study area. Nevertheless, if individually some metrics do not always reflect the environmental conditions, from their combination into a single index a more reliable indicator of the effect of environmental disturbance on the natural communities is derived and thus misleading or ambiguous results are avoided [31].

The Daphne index can be presented as a fast and easy method for essentially two reasons: first, although all the organisms in the sample have to be counted, identification to species level is required only for the molluscs; second, it does not entail long and laborious phases of data collection and analysis, as with biomass and production measures, or complicated statistical processing. The scores assigned to each metric are combined into a single value by means of a simple mathematical operation (arithmetic mean).

The Daphne index provided results that are in accordance with the environmental condition pattern in the area and closely correlated with the main chemical and structural parameters, calculated on the whole set of species of the benthic community.

However the method does have some limitations. For example, each group of metrics works on distinct biocenotic units. The index was developed from data on the macrobenthic community existing at the same depth, with similar and known features such as the presence of specific typical species, or the presence of *C. gibba*. Also, the scores are assigned to different metrics by calculating the ratio between the measured value of a metric and the reference value (maximum or minimum value) recorded for the corresponding data base; therefore this procedure has to be fitted to each community present in the area.

Thus, the use of the Daphne index is limited to neighbouring areas, colonized by similar benthic communities. Nevertheless, in the Northern Adriatic Sea, this procedure is indispensable due to both the important differences in macrobenthic communities at different depths and to the difficulty in finding non-impacted sites to use as reference sites in an area strongly influenced by human activities. Moreover, the reference values for each metric have been recorded in different samples, while the index is equal to 1 (maximum value) when all the metrics reach the maximum values in the same sample. In this way, there is a risk of establishing elevated reference conditions and consequently underestimating the ecological conditions that actually exist. In order to improve the reliability of the method, the number and the variability of data used to develop the index should be increased and it should be tested in as many different situations as possible.

While it is clear that the trend of the index reflects the environmental conditions, it is difficult to find the exact correspondence between the index value and the ecological conditions assessed. Setting values that correspond to the boundaries of the five quality classes required by the Directive is very difficult. We adopted the simple procedure as other authors did [9, 51]: the distance between the minimum and maximum theoretical index value (0–1) has been divided into five classes of equal width.

Results obtained by Daphne have been compared to those obtained by M-AMBI, one of the more widespread methods at European level. A comparison of the performance of AMBI (alone) and Bentix in the Northern Adriatic Sea was previously made [52]. AMBI was found to correlate better than Bentix both with the community structure and the physical-chemical parameters and was found to be more suited to describe the Adriatic environmental conditions. AMBI was first developed for the Atlantic coastal and estuarine ecosystems, therefore it is more suitable than the Bentix index in assessing those benthic communities, which host a low biodiversity with few species and high densities: conditions similar to those existing in the Northern Adriatic. On the contrary Bentix index fits best to high biodiversity and evenly distributed benthic community [11]. Moreover, in a community dominated by few species, the results obtained by these kind of indices are strongly influenced by the assignment into ecological groups of the dominant species; this problem is even more pronounced if the choice is between only two ecological groups [12], as in the Bentix index.

M-AMBI reflects the trend of the results obtained by Daphne in the sampling stations and it correlates with it, but M-AMBI rates all the stations to be in better ecological condition than Daphne rates, underlying that more data and more work are needed to establish the boundaries between the quality levels.

In conclusion, the Daphne Index appears to be a practical tool for analysing the environmental quality in the Northern Adriatic area, offering concise statements which are easy to understand and communicate. It provides results according to the environmental conditions in the area and it differently classifies stations that are located along a gradient of decreasing eutrophication. Nevertheless the index needs to be tested on more data to try to better identify the relation between index value and quality level especially in known sites of extremely high or bad ecological conditions.

It could be used along with other indices in a kind of two-stage procedure: the first stage using a 'universal' index such as M-AMBI or Bentix, widely applied within Member States and necessary for the application of the WFD; the second stage using a 'local' index, in some way comparable to the universal one, and more useful for frequent monitoring and evaluation of local factors of variation in order to better indicate the direction of change in environmental quality responding to peculiar aspects of the community. This kind of second stage approach can also undergo a kind of generalization on its principles, if not in its practical constituents and calculations, and might be adapted also in other 'peripheral' situations on the basis of the knowledge of the local benthic community.

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