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**Evaluation of the applicability of a fuzzy index of ecosystem integrity (FINE) to characterize the status of Tyrrhenian lagoons.**

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**Abstract**

The new index FINE, a multimetric, fuzzy-based index for the evaluation of environmental quality for Mediterranean transitional waters, was calculated using biotic data gathered between 2000 and 2006 at 15 stations in 4 Tyrrhenian transitional water ecosystems (Orbetello Lagoon, Padrongiano Delta, Stagno di S. Teodoro, and Stagno di Tortoli), i.e. in a different biogeographic sub-province respect to the one in which the index was developed and validated. The rationale of FINE is that certain attributes, selected on the basis of established principles of benthic ecology, are fundamental for lagoon ecosystem function. A set of other indices (Simpson's  $1-\lambda'$ , the W-statistics, AMBI, and BOPA) was also calculated and compared to FINE outputs. FINE,  $1-\lambda'$ , and the W-statistics were significantly correlated with sedimentary organic matter content. Some stations were unequivocally assigned to the same ecological status, independently from the index used; for others, some discrepancies were evident. Mediterranean transitional waters probably share ecosystem complexities which are not yet fully captured by indices developed and validated elsewhere.

*Keywords:* Benthos; Environmental quality; Multimetric index; Fuzzy logics; Tyrrhenian Sea

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## Introduction

The European Water Framework Directive (WFD, 2000/60/EC) recommends the development of biotic indices which, if they constitute an extreme in information reduction, are most straightforward and easy to present to potential end users. Although the absolute values of an individual biotic index may be specific to a given ecoregion, theories and methods used in development should be applicable across systems. A universal index that works in all systems or even in systems of the same ecological type, however, is unrealistic because communities are complex and geographically diverse. Most indices so far proposed for the implementation of the WFD are based on the Pearson-Rosenberg paradigm (Pearson and Rosenberg, 1978): the AMBI / M-AMBI (Borja et al., 2000; Muxica et al., 2007), the BENTIX (Simboura and Zenetos, 2002), the BQI (Rosenberg et al., 2004), and the BOPA (Dauvin and Ruellet, 2007) are the most recently proposed biotic indices. AMBI, as well as the related BENTIX, accounts for the transition towards the dominance of mostly sensitive species during secondary succession. BQI is the product of two terms, one accounting for the relative dominance of tolerant versus sensitive species, and the second one for species richness. BOPA considers the total number of individuals, and the frequency of opportunistic polychaetes and amphipods.

Despite their obvious ecological and economical interests, transitional water bodies have received little attention about development of ecological status indicators. Recently, a multimetric index for transitional waters, the Fuzzy Index of Ecosystem Integrity (FINE: Mistri and Marchini, 2007), was proposed. In the development of FINE, a different approach from the sensitivity/tolerance one was chosen, because a) the classification of the different species or taxa are subjectively made and may vary between scientists and geographical areas (Rosenberg et al., 2004), b) there are problems with making this operational due to our current lack of understanding of the links between the effects of human activities and changes in

populations of such species (ICES, 2004), and c) identification errors can lead to incorrect classifications and false interpretations of the indices (Dauvin, 2005). FINE takes into account 7 ecosystem attributes (variables or metrics), each having ecological relevance for lagoon ecosystems: 1) biomass of seaweeds, 2) presence of seagrass, 3) biomass of macrobenthos, 4) macrobenthic diversity (as the classical Shannon's  $H'$ ), 5) macrobenthic functional diversity (as Shannon's  $H'$  on functional groups), 6) abundance of macrobenthos, 7) number of macrobenthic taxa. The 7 variables are combined in a system of 768 logic rules, and the result is a number, ranging from 0 to 100, which expresses the ecological quality of the considered sample.  $FINE_{100}$  corresponds to the optimal conditions for all variables, whereas  $FINE_0$  indicates that all the variables are displaying their worst modality; therefore,  $FINE_{100}$  and  $FINE_0$  can be regarded as reference for HIGH and BAD ecological quality (WFD, 2000/60/EC), respectively. All the algorithms for FINE calculation are based on the fuzzy set theory, which has repeatedly been proposed as a useful method to develop ecological models and indices of environmental conditions (Salski et al., 1996; Silvert, 1997; 2000; Lu and Lo, 2002; Liuo et al., 2003; Adriaenssens et al., 2004). The online version of the index is freely available at: <http://web.unife.it/progetti/FINE/>.

Recently, the FINE index was successfully tested in two Adriatic lagoons (Mistri et al., 2007). Aim of this study was to evaluate its applicability and effectiveness in transitional waters from the Tyrrhenian Sea (Figure 1), i.e. a different biogeographic sub-province respect to the one in which FINE was developed and validated. A set of indices was also calculated and compared to FINE outputs: Simpson's  $1-\lambda'$ , the W-statistics, AMBI, and BOPA. BENTIX and BQI were not used since the former is closely related to AMBI, and the latter is not applicable to very shallow transitional environments, where depth rarely exceed 1.5 m even at high tide.

## Materials and Methods

### *Study areas*

The Orbetello lagoon (southern Tuscany, eastern Tyrrhenian Sea) has a surface area of 27 km<sup>2</sup>, and is embraced within two sandbars. A third incomplete spit, on which the town of Orbetello lies, is connected with Mount Argentario by a cause-way, which partially divides the lagoon into two basins. The western basin is linked to the sea by the shorter Nassa Channel, and to the mouth of the Albegna river by the longer Fibbia Channel. It is a shallow, non-tidal environment with weak hydrodynamics, which reduces the dilution potential of organic matter and nutrients discharged from urban areas, aquaculture facilities, and agriculture waste waters. Three stations representative of different areas in the western basin were sampled in March and July 2003: stations ORBE-F and ORBE-N were close to the Fibbia and Nassa channels respectively, while ORBE-C was in the centre of the lagoon.

The Rio Padrongiano Delta (2.5 km<sup>2</sup>) lies in the north-eastern coast of Sardinia (western Tyrrhenian Sea), and has depth of about 0.8 m. It receives marine waters from the Gulf of Olbia, and freshwater from the Rio Padrongiano, whose flow ranges from torrent-like to almost dry behaviour in summer. It also receives waters from the adjacent Olbia harbour, the most important industrial and tourist port of Western Sardinia. The area is characterized by coarse and poorly vegetated (*Cymodocea nodosa*, *Z. noltii*) sediments. Padrongiano deltaic area hosts fisheries and mollusc (clams and mussels) aquaculture activities. Three stations were sampled in March, July and October 2003. At stations PADR-L and PADR-S, beds of the bivalve *Musculista senhousia* were present, while PADR-C was on bare sediments.

The Stagno di San Teodoro (22 km<sup>2</sup>) is a heavily eutrophicated, shallow (average depth: 0.7 m) coastal pond in northeastern Sardinia (western Tyrrhenian Sea). It is characterized by sandy bottoms with granitic outcrops, and connects to the sea through a narrow (20 m wide) and shallow (0.3 m deep) mouth, which is often impounded by sand and

*Posidonia oceanica* debris. The Stagno, which is scarcely exploited for fishing, receives municipal wastewaters from the town of San Teodoro, the most important tourist centre in northern Sardinia, and nutrient rich freshwater from the Rio San Teodoro and Rio Filicani. Massive algal blooms due to the discharge of untreated wastewaters caused recently dramatic mortalities to the biota, resulting in legal actions against the top management of the treatment plant (Info Regioni 2007: <http://www.edipol.it/images/news/94-108.pdf>; page 15 of the document). Three stations were sampled in June 2006, just before the mortality event. Station STEO-D was close to the outflow of wastewaters, in the southernmost area of the Stagno, station STEO-BM was in the centre of the Stagno, and station STEO-C was close to the sea mouth.

The Stagno di Tortolì is a shallow (average depth: 1.0 m) coastal pond (25 km<sup>2</sup>) in central-eastern Sardinia (western Tyrrhenian Sea). It is connected to the sea by two channels, and receives freshwater inputs from the Rio Mannu. Bottoms are sandy-muddy, and large areas are covered with seagrasses (mainly *Z. noltii*). The Stagno hosts a flourishing finfish and shellfish traditional fishery. Six stations were sampled in June 2000 and January 2001: stations TORT-1 (south-eastern basin), TORT-4 (central basin) and TORT-5 (north-western basin) were located on seagrass prairies, TORT-2 (southern basin) was close to a sea inflow, TORT-6 (northern basin) to the Rio Mannu outflow, and TORT-3 was in the easternmost, confined area of the Stagno.

Main pressures (*sensu* EC, 2002) on the 4 transitional systems considered are summarized as: a) >25% agricultural land use according to first level CORINE (COoRdination of INformation on Environment; [http://reports.eea.europa.eu/COR0-landcover/en/tab\\_content\\_RLR](http://reports.eea.europa.eu/COR0-landcover/en/tab_content_RLR)) categories (Orbetello, S. Teodoro); b) fishing and aquaculture operations (Orbetello, Padrongiano, S. Teodoro, Tortolì); c) discharge of eutrophicated waters from the catchment basin (Orbetello, Padrongiano, S. Teodoro); d) wide

water level variations (S. Teodoro); e) boating (Orbetello, Padrongiano); and f) introduction of alien species (Padrongiano).

#### *Environmental and biotic data collection*

At each station, water parameters (salinity, temperature, dissolved oxygen) were collected with an OxyGuard® Mk III probe and an ATAGO S/Mill-E refractometer. Sediment cores (4.5 cm i.d.) were also taken for total organic matter content (weight loss after drying and incineration at 450°C). Three replicate benthic samples were collected for the analysis of the macrofaunal community using a Van Veen grab (sampling area: 0.027 m<sup>2</sup>); fauna retained on a 0.5 mm screen were identified to the species level and counted. The biomass of fauna was obtained by oven-drying to constant weight, and incineration (ash-free dry weight, gAFDW m<sup>-2</sup>). Seaweeds and seagrass, when present, were also collected and weighed to obtain wet weight.

#### *Data analysis*

FINE was calculated using the FINE software (<http://web.unife.it/progetti/FINE/>). Besides FINE, the following indices were also applied to macrofaunal data: Simpson's 1- $\lambda'$ , the W-statistics (Warwick, 1986; Clarke, 1990), the marine biotic index AMBI (Borja et al., 2000), and the benthic opportunistic polychaetes/amphipods index, BOPA (Dauvin and Ruellet, 2007). The W-statistics condenses the information embedded in the ABC method (Abundance-Biomass Comparison, Warwick, 1986) reflecting the degree of overlapping of k-dominance curves for biomass and abundance of benthos. The theoretical basis of AMBI is that of the ecological strategies of the *r*, *K* and *T* (Pianka, 1970), and the progressive steps in stressed environments (Pearson and Rosenberg, 1978). BOPA considers the total number of individuals collected in the samples, the frequency of opportunistic polychaetes, and the frequency of amphipods. An increase in the values of FINE, 1- $\lambda'$  and W-statistics is understood as an improvement of environmental quality, while it means exactly the contrary



in the case of AMBI and BOPA.  $1-\lambda'$  and the W-statistics were calculated using the PRIMER<sup>®</sup> package, AMBI using the AMBI<sup>®</sup> software (<http://www.azti.es>), while BOPA was calculated as:

$$\text{BOPA} = \log [(f_P / f_A + 1) + 1]$$

where  $f_P$  and  $f_A$  are the opportunistic polychaetes and amphipods (excluding *Jassa*, but this opportunistic amphipod was not present at our study sites) frequencies, respectively. The purpose was to compare the performance of the different indices in distinguishing between different environmental scenarios. The response of each index as a function of different environmental variables was assessed through regression analyses, and regression ANOVAs.

Using multivariate analysis, we tried to assess which index was able to capture most of the information embedded in the macrofaunal species/abundance matrices. We used only AMBI, BOPA and FINE because of their 5-category (High, Good, Moderate, Poor, Bad) quality classification system. For each index, a matrix was constructed assigning to each study station its ecological quality (calculated with that index) in numerical form (5 = High, 4 = Good, 3 = Moderate, 2 = Poor, 1 = Bad). A second-stage MDS (Clarke and Warwick, 2001) was then performed on these 3 one-row matrices (AMBI, BOPA, FINE) and the matrix based on abundance of species from all stations (SPECIES), using Euclidean distance to measure similarities. The idea was that “classification subsets” (i.e. AMBI, BOPA and FINE classifications) whose multivariate pattern links well to the information given by the biotic data will be represented by points on the second-stage MDS which lie close to the SPECIES point. Paired comparisons between indices and the SPECIES matrix were then performed through RELATE analysis (Clarke and Gorley, 2006).

## Results

In Table 1, physico-chemical characteristics of water and sediments at the various stations are shown. Table 2 summarizes the biotic data collected. Ecological conditions among stations varied greatly, as shown by the values of the input variables: 1) biomass of seaweeds varied from 0 to 25000 gWW m<sup>-2</sup>, 2) biomass of seagrass from 0 to 890 gWW m<sup>-2</sup>, 3) biomass of macrobenthos between 0.8 and 606.5 gAFDW m<sup>-2</sup>, 4) macrobenthic alpha-diversity between 0.42 and 2.69, 5) macrobenthic functional diversity between 0.39 and 1.49, 6) abundance of macrobenthos between 2002 and 48901 ind m<sup>-2</sup>, 7) number of macrobenthic taxa between 5 and 62. The gradient of impact represented by the data set ranged from very impacted (e.g., STEO-BMJun and STEO-DJun, characterized by extremely depauperated benthic communities and huge amount of seaweeds), to high environmental quality (e.g. many stations at Tortoli, characterized by luxuriant seagrass prairies). Table 3 shows FINE outputs, together with the different grades of membership associated to each ecological status class. Table 4 shows the values of the other indices estimated at the sampling stations. While some stations resulted unequivocally assigned to the same ecological status, independently from the index used (e.g. PADR-SJul), for others some discrepancies were evident. For example, the ecological quality at station PADR-LMar should be considered disturbed from 1-λ', moderately disturbed from the W-statistics, good from AMBI, and high from BOPA. In Table 5 regression ANOVAs are summarized. Significant negative correlations were found between sediment organic matter content and FINE (r=-0.57; p<0.01), 1-λ' (r=-0.55; p<0.01), and W-statistics (r=-0.49; p<0.01).

Figure 2 shows the second-stage MDS plot. The AMBI, BOPA and FINE points were segregated in different zones of the plot, while the SPECIES point was grossly in its centre. It seemed that the FINE point lies closer to the SPECIES point compared to the BOPA and AMBI points. Table 6 shows the quantitative results of paired comparisons, through RELATE

analysis, between indices (AMBI, BOPA and FINE) and SPECIES matrix: the highest correlation (Spearman's  $Rho=0.263$ ) was found with the FINE classification.

## Discussion

The successful development of a system capable of assessing the quantity, quality and functional value of aquatic systems is not a trivial task. It will require a holistic ecosystem-based approach that at the same time focuses on individual species and habitats as well as accounting for those factors that affect these components at various temporal and spatial scales (Diaz et al., 2004). A number of indices have been developed in the few past years; Diaz et al. (2004), reviewing the peer-reviewed literature, summarized the structure of 64 commonly used benthic habitat quality indices (Table 2, *op. cit.*), and reproached the scientific community for a “tautological development of new indices”. The WFD bears a part of the responsibility for this phenomenon, since many of the new indices (*e.g.* AMBI / M-AMBI, BENTIX and BQI) were developed specifically with the WFD in mind and yet all are based on the Pearson and Rosenberg (1978) model for organic enrichment. Moreover, all the recently developed indices require a species identification that is as precise as possible, while the problems of species identification for research consultancies conducting environmental assessments has been examined by Dauvin (2005). From this viewpoint, FINE (as well as BOPA; Dauvin and Ruellet, 2007) has some advantages over other methods: there is no need for a deep taxonomic expertise or the painstakingly detailed analysis of species, since, instead of identifying and counting, say, *Gammarus aequicauda*, *G. insensibilis*, *Echinogammarus veneris* and *Neogammarus adriaticus*, one can count as Gammaridae sp. 1, sp. 2, sp. 3 and sp. 4, simply on the basis of morphological differences, since for FINE calculation, it is the number of species and not their names which is important. This is also true for assigning animals to broad functional categories, since, with a few exceptions (*e.g.*, the detritivorous

amphipod *Corophium insidiosum*), species belonging to the same lower taxonomic level (e. g. family) usually feed in the same manner.

Many of the proposed recent indices provide information about the relative abundances of sensitive species faced with increasing organic matter in the sediment and those of the species that are resistant or indifferent to such increases, or even favored by such conditions. Recently Dauvin (2007) highlighted that all the so far proposed indices, which aim to determine anthropogenic stress, relate to abundances of stress tolerant species (i.e. species tolerant of natural stressors likely to occur in transitional waters). Moreover, such indices relate to anthropogenically organic-rich systems whereas transitional waters are naturally organic rich systems. These complex systems are characterized by low benthic diversity, and, given such natural impoverishment, certain indices might lose their effectiveness. The usefulness of AMBI in detecting impact gradients has been clearly demonstrated in the north-eastern and south-western Atlantic coastal area (Borja et al., 2003; Muniz et al., 2005; Muxica et al., 2005). In the Mediterranean ecoregion, and in its transitional waters (Ponti and Abbiati, 2004; Occhipinti-Ambrogi and Forni, 2004), the usefulness of AMBI is still uncertain: a clear impact gradient due to cage culturing of fish was detected at 20-30 m water depth in Western Greece (Muxica et al., 2005), while it showed discrepancies in detecting trawling impacts at 16-30 m water depth in the Gulf of Lion (Labruno et al., 2006), and failed to detect the spatial differentiation of ecological quality along a gradient of anthropogenic disturbance in the Sea of Marmara (Albayrak et al., 2006). The BOPA proved its effectiveness in coastal marine and polyhaline systems in the English Channel (Dauvin and Ruellet, 2007), but to a lesser extent in Tyrrhenian lagoons. Dauvin and Ruellet (2007), however, stressed the need for future adaptations of BOPA for mesohaline and oligohaline estuarine zones. We do not dispute the effectiveness of AMBI and BOPA for coastal and estuarine waters. Mediterranean transitional waters, however, probably share ecosystem complexities which are not yet fully captured by

these indices, as shown by the second-stage MDS and by the RELATE analysis. As pointed out by Cognetti (1992), the detection of impact gradients in transitional waters on the basis of opportunistic and tolerant organisms is an extremely difficult task, since the functional phenotypical flexibility of genetic nature which is characteristic of alolymnobic species gives them a wider margin of response to environmental alterations (Cognetti and Maltagliati, 2000). The finding that FINE, which is based on benthos data plus vegetation data, was closer to the SPECIES point (Figure 2) than other indices exclusively based on benthos data should not be a surprise. Aquatic vegetation (i.e. seagrass and seaweeds) is known as a key structural component and regulator in shallow water ecosystems (Carpenter and Lodge, 1986; Jaynes and Carpenter, 1986), and a key factor in driving pattern and structure of faunal benthic assemblages (Edgar, 1990; Bachelet et al., 2000; Yamamuro et al., 2006).

Environmental managers and policymakers require tools capable of distinguishing the degree of degradation to the biotic community. While the WFD asks for a 5-category quality classification system (“high”, “good”, etc.), to environmental managers and policymakers the only important boundary is that between “moderate” and “good”, since if the environmental quality of an area is “moderate” they have to spend (a lot of) money to make it “good”. We feel that an advantage of FINE is that areas of intermediate quality can be finely identified through its membership grades (i.e. the outputs resulting from “fuzzification” and processing across the 768 model rules by means of logic operators, such as “and”, “if...then” and “or”, of the input data; Mistri and Marchini, 2007) Let us consider, for example, the case of sites PADR-SMar and PADR-SJul (Table 2). FINE values obtained for these sites were quite similar (61 and 75), and both fell into the “good” (FINE range: 60-80) quality class. However, PADR- SMar had membership grade 0.563 to “moderate”, and 0.437 to “good”, (and it was slightly inclined towards the condition “moderate”), while PADR-SJul had 0.005 to “moderate” and 0.995 to “good” (and it was more inclined towards the condition “good”). A

comparison of FINE values (and its membership grades) through time, should be able to reveal if the environmental quality of an area is improving or worsening.

In this paper, the effectiveness of FINE has been tested in 4 Tyrrhenian lagoons, i.e. in a different biogeographic sub-province respect to the one in which the index was developed and validated, and succeeded in producing an ecologically relevant classification of the considered areas. Notwithstanding the fuzzy-based FINE has proven its effectiveness, at least in some Italian transitional waters, it does not conform to the WFD. In fact, the FINE model includes seaweeds, seagrass and benthos, while the WFD states the need to evaluate each element separately, in order to determine the impacts from different pressures over each of the elements, and this cannot be assessed when all of them are evaluated together. Finally, although FINE does not require a precise identification of species, it requires the measurement of biomass for benthic fauna and seaweeds, thus a notably larger sampling effort. These are certainly some of the limitations of FINE.

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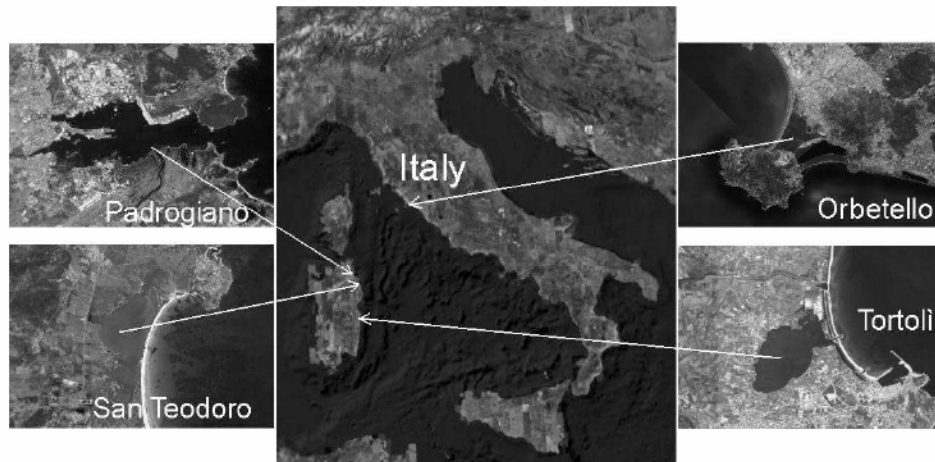
**Figure legends**

Figure 1. Map with the location of the considered lagoons.

Figure 2. Second-stage MDS ordination of inter-matrix rank correlations. Underlying similarity matrices derived from abundances of species (SPECIES), and ecological quality values (i.e. classifications through the indices FINE, BOPA and AMBI).

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Figure 1



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Figure 2



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Table 1.

Environmental characteristics at the study sites (DO: dissolved oxygen; Sal: salinity; OM: organic matter). Station name based on lagoon name, location within lagoon and month of sampling (e.g. ORBE-CJul: Orbetello lagoon, site C, July).

Station	Depth (m)	Temp (°C)	DO (mg L <sup>-1</sup> )	Sal psu	OM %
ORBE-CJul	0.8	29	8.5	33	4.8
ORBE-FJul	0.7	28	7.6	28	5.3
ORBE-NJul	1.1	29	8.1	30	4
ORBE-CMar	0.9	11	7.7	28	2.8
ORBE-FMar	0.8	11	9.5	27	2.1
ORBE-NMar	1.2	12	10.1	28	2
PADR-LMar	0.7	13	10.2	42	5.3
PADR-SMar	0.8	12	11.3	42	4.5
PADR-CMar	0.9	13	12	42	2.3
PADR-LJul	0.8	21	9.9	42	5
PADR-SJul	0.9	20	8.5	43	4.9
PADR-CJul	1	21	9.5	42	1.3
PADR-LOct	0.7	12	10.1	28	5.1
PADR-SOct	0.7	12	9.6	39	5.1
PADR-COct	0.9	11	11.2	37	2
TORT-1Jun	0.9	27	9.4	38	2
TORT-2Jun	1.2	27	10.7	38	3.2
TORT-3Jun	2	27	6.8	33	4.9
TORT-4Jun	0.9	28	9.8	38	4.1
TORT-5Jun	0.8	28	9.9	38	3.3
TORT-6Jun	1.9	29	5.9	34	3.9
TORT1-Jan	1.3	13	11.8	31	2.6
TORT-2Jan	1.6	13	13.7	32	1.6
TORT-3Jan	2.2	12	11.1	35	4
TORT-4Jan	1.3	13	12.3	32	3.9
TORT-5Jan	1.2	13	13.3	33	3.2
TORT-6Jan	2.6	14	10.9	35	4.5
STEO-CJun	0.7	25	8.4	34	2.9
STEO-BMJun	1.2	24	12.9	23	6.8
STEO-DJun	1.5	24	9.0	25	7.7

Table 2

Biotic data. S: number of macrobenthic species; H': benthic alpha-diversity; H'f: benthic functional diversity; BB: biomass of benthic fauna; SwB: biomass of seaweeds; SgW: biomass of seagrass.

Station	S	Abundance (ind m <sup>-2</sup> )	H'	H'f	BB (gAFDW m <sup>-2</sup> )	SwB (gWW m <sup>-2</sup> )	SgB (gWW m <sup>-2</sup> )
ORBE-CJul	25	29563	2.53	1.07	173.3	0	0
ORBE-FJul	23	75134	1.41	0.88	113.8	0	0
ORBE-NJul	32	37592	2.25	1.29	134.1	1000	0
ORBE-CMar	34	29797	2.57	1.19	145	0	0
ORBE-FMar	17	13270	2.18	1.07	29.6	0	0
ORBE-NMar	25	48901	1.85	1.08	77.7	0	0
PADR-LMar	34	22107	0.65	0.39	158.3	0	0
PADR-SMar	44	6752	1.76	0.86	48.9	0	0
PADR-CMar	37	6410	2.2	1.12	4.1	0	0
PADR-LJul	34	23060	1.79	1.19	606.5	0	0
PADR-SJul	36	5966	2.61	1.33	110.9	0	0
PADR-CJul	36	15466	2.61	1.43	10.7	104	0
PADR-LOct	54	31218	2.31	1.42	205.1	200	0
PADR-SOct	62	26732	2.69	1.48	57.3	0	0
PADR-COct	42	10767	2.44	1.46	23.9	50	0
TORT-1Jun	29	10754	2.12	1.33	45.8	0	320
TORT-2Jun	32	6925	2.77	1.49	247.9	0	0
TORT-3Jun	13	5426	1.33	0.85	12	0	0
TORT-4Jun	22	21188	1.33	1.02	243	0	570
TORT-5Jun	19	27984	1.52	1.11	86.2	0	890
TORT-6Jun	22	7868	2.27	1.46	56.8	290	0
TORT1-Jan	42	18056	2.53	1.31	345.3	0	325
TORT-2Jan	34	8596	2.65	1.44	42.4	0	0
TORT-3Jan	26	2503	2.63	1.25	25.3	0	0
TORT-4Jan	14	5377	1.36	0.86	208.8	0	173
TORT-5Jan	23	11901	1.99	1.4	97.5	0	127
TORT-6Jan	23	2836	2.33	1.05	40.6	0	0
STEO-CJun	26	3774	2.14	0.98	3.27	50	0
STEO-BMJun	14	12444	1.48	1.19	3.77	15000	0
STEO-DJun	5	2002	0.52	0.42	0.8	25000	0



Table 3

FINE outputs with the different grades of membership (i.e. the result of fuzzification, inference through *if...then* rules and defuzzification) associated to each ecological status class.

Station	Bad	Poor	Moderate	Good	High	FINE	EcoQ
ORBE-CJul	0	0	0.32	0.68	0	67	Good
ORBE-FJul	0	0	0.793	0.207	0	55	Moderate
ORBE-NJul	0	0	0.12	0.88	0	72	Good
ORBE-CMar	0	0	0.09	0.91	0	73	Good
ORBE-FMar	0	0	0.445	0.555	0	64	Good
ORBE-NMar	0	0	0.273	0.727	0	68	Good
PADR-LMar	0	0	1	0	0	50	Moderate
PADR-SMar	0	0	0.563	0.437	0	61	Good
PADR-CMar	0	0	0.709	0.291	0	57	Moderate
PADR-LJul	0	0	0.095	0.905	0	73	Good
PADR-SJul	0	0	0.005	0.995	0	75	Good
PADR-CJul	0	0	0.071	0.929	0	73	Good
PADR-LOct	0	0	0	1	0	75	Good
PADR-SOct	0	0	0.068	0.932	0	73	Good
PADR-COct	0	0	0.053	0.947	0	74	Good
TORT-1Jun	0	0	0	0	1	100	High
TORT-2Jun	0	0	0.02	0.98	0	75	Good
TORT-3Jun	0	0.004	0.819	0.177	0	54	Moderate
TORT-4Jun	0	0	0	0.24	0.76	94	High
TORT-5Jun	0	0	0	0.076	0.924	98	High
TORT-6Jun	0	0	0.002	0.998	0	75	Good
TORT1-Jan	0	0	0	0	1	100	High
TORT-2Jan	0	0	0.057	0.943	0	74	Good
TORT-3Jan	0	0	0.075	0.925	0	73	Good
TORT-4Jan	0	0	0	0.305	0.695	92	High
TORT-5Jan	0	0	0	0	1	100	High
TORT-6Jan	0	0	0.223	0.777	0	69	Good
STEO-CJun	0	0	0.792	0.208	0	55	Moderate
STEO-BMJun	0.001	0.371	0.628	0	0	41	Moderate
STEO-DJun	0.877	0.123	0	0	0	3	Bad

Table 4

Values of the different indices estimated at the sampling stations.

Station	$1-\lambda$	W-stat	AMBI	BOPA
ORBE-CJul	0.9	0.21	3.28	0.33
ORBE-FJul	0.55	-0.08	3.83	0.63
ORBE-NJul	0.79	0.11	2.98	0.12
ORBE-CMar	0.89	0.17	3.24	0.28
ORBE-FMar	0.83	0.18	3.73	0.4
ORBE-NMar	0.77	0.07	3.24	0.1
PADR-LMar	0.2	0.02	2.09	0.02
PADR-SMar	0.64	0.08	4.63	0.05
PADR-CMar	0.82	0.02	1.28	0.28
PADR-LJul	0.69	0.13	2.68	0.07
PADR-SJul	0.89	0.25	1.36	0.16
PADR-CJul	0.89	0.19	1.23	0.35
PADR-LOct	0.86	0.1	4.39	0.41
PADR-SOct	0.88	0.12	3.71	0.53
PADR-COct	0.85	0.14	3.14	0.3
TORT-1Jun	0.77	0.07	3.35	0.46
TORT-2Jun	0.92	0.37	2.88	0.21
TORT-3Jun	0.6	-0.11	3.7	0.67
TORT-4Jun	0.52	0.31	2.75	0.07
TORT-5Jun	0.68	0.04	1.81	0.05
TORT-6Jun	0.88	0.16	1.94	0.04
TORT1-Jan	0.85	0.2	2.71	0.3
TORT-2Jan	0.89	0.2	2.95	0.42
TORT-3Jan	0.88	0.23	2.75	0.37
TORT-4Jan	0.54	0.16	1.72	0.05
TORT-5Jan	0.79	0.16	1.37	0.01
TORT-6Jan	0.85	0.25	2.94	0.42
STEO-CJun	0.77	0.08	4.58	0.22
STEO-BMJun	0.68	-0.06	1.4	0.01
STEO-DJun	0.24	-0.29	3	0

Table 5

Significancy of regression ANOVAs (p-values) between different indices and environmental variables DO: dissolved oxygen; OM: organic matter).

	FINE	1- $\lambda'$	W-stat	AMBI	BOPA
Salinity	0.104	0.708	0.052	0.205	0.717
DO	0.276	0.803	0.325	0.108	0.237
OM	<b>0.001</b>	<b>0.0015</b>	<b>0.006</b>	0.759	0.29

Table 6  
RELATE analysis between indices  
and SPECIES matrix.

Paired comparisons	Rho
SPECIES vs FINE	0.263
SPECIES vs BOPA	0.183
SPECIES vs AMBI	0.004
FINE vs BOPA	0.123
FINE vs AMBI	-0.023
BOPA vs AMBI	-0.019