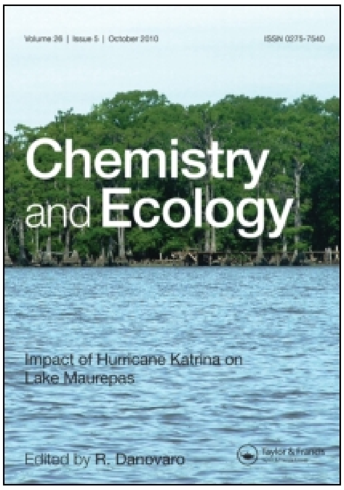


## Chemistry and Ecology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713455114

# *TWIN*: a TWo-stage INdex to evaluate ecological quality status in lagoon environments by means of hard bottom zoobenthos

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**To cite this Article** Marchini, A. and Occhipinti-Ambrogi, A.(2007) '*TWIN*: a TWo-stage INdex to evaluate ecological quality status in lagoon environments by means of hard bottom zoobenthos', Chemistry and Ecology, 23: 6, 455 — 469
**To link to this Article: DOI:** 10.1080/02757540701702751
**URL:** http://dx.doi.org/10.1080/02757540701702751

*Chemistry and Ecology*
Vol. 23, No. 6, December 2007, 455–469

# *TWIN*: a TWo-stage INdex to evaluate ecological quality status in lagoon environments by means of hard bottom zoobenthos

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*(Received 31 July 2006; in final form 19 December 2006)*



The macrobenthos colonizing the artificial hard bottoms in lagoons has been used in biomonitoring programs as it requires easy sampling procedures and provides a variety of responses to different environmental pressures, like marine or continental influence, eutrophication, and urban pollution. In this paper we present the development and application of a new TWo-stage INdex (*TWIN*), aimed to assess water quality of lagoon environments using the hard bottom macrobenthos community. *TWIN* is calculated in two separate stages. First, the presence and abundance of macrobenthic species are used to define, through a fuzzy model, a station's membership grade in six pre-defined ecological sectors (Lagoon Mouth, Vivified Lagoon, Rough Eutrophic, Calm Eutrophic, Urban, Estuarine), each corresponding to characteristic communities. Then a formula links the membership grades to the five ecological status classes ranging from high to bad quality, as requested by the European Water Framework Directive. This method was tested in four Adriatic lagoons: the results are consistent with literature information and expert judgement, therefore we propose its use in the definition of water quality in lagoons.

*Keywords*: Adriatic lagoons; Ecological quality status; WFD; Zoobenthos; Hard bottoms; Fuzzy logic

## 1. Introduction

The quality elements required for the classification of transitional waters by the European Water Framework Directive are grouped into three types: biological elements, hydromorphological elements and physical-chemical ones (Directive 2000/60/EC; Annex V, 1.1.3). The Directive does not indicate a methodology for combining the quality elements to achieve a classification scheme: nevertheless it states that all types of quality elements should be taken into account, with priority being assigned to the biological ones [1]. Therefore, the ecological status of the water body is defined as primarily based on biological elements, namely the composition and abundance of phytoplankton, other aquatic flora, benthic invertebrate fauna and fish fauna. For the benthic macroinvertebrates, the presence of all the disturbance-sensitive taxa associated with undisturbed conditions corresponds to high ecological status (Directive 2000/60/EC; Annex V, 1.2.3).

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*Chemistry and Ecology*
ISSN 0275-7540 print/ISSN 1029-0370 online © 2007 Taylor & Francis
http://www.tandf.co.uk/journals
DOI: 10.1080/02757540701702751

In the lagoons bordering the Adriatic Sea, and in particular along its northern coast, several studies have focused on the benthic fauna colonizing the wooden poles, which are largely used in those lagoons to mark the path of navigable canals or to arrange fishing nets [2–8]. Monitoring of biological associations on hard substrates, such as wooden poles, has the obvious practical advantage of simple and replicable sampling. Moreover, the fouling organisms belong to a large variety of taxa, showing different responses to disturbance, null or scarce mobility and relatively long life cycles, and are considered good bioindicators of environmental conditions [8, 9]. However, studies on human impact on the hard bottoms subtidal community are scarce [9], compared with those on the soft bottom community. Therefore, the development of new tools for hard-bottom substrata is considered an important challenge for benthologists [10].

Recently developed biotic indices are mainly suitable for soft bottom habitats in marine waters [11, 12], but do not provide satisfactory quality assessments in coastal lagoons [12, 13]. Environmental quality classes are defined with reference to habitats unaltered by human disturbance, but a community of sensitive taxa associated with unaltered conditions is not likely to be found in lagoons for two reasons: (i) all lagoon species are tolerant towards the large natural variability of this ecosystem (i.e. fluctuations in water current velocity, temperature, salinity and dissolved oxygen concentration [14, 15]); (ii) pristine conditions no longer exist in the majority of European coastal lagoons [16]. A lagoon classification based on hard-bottom sessile invertebrates was proposed by Occhipinti-Ambrogi et al. [17]; six ecological sectors were identified in the Venice lagoon: 'Lagoon Mouth' (M), 'Vivified Lagoon' (V), 'Rough Eutrophic' (RE), 'Calm Eutrophic' (CE), 'Urban' (U) and 'Estuarine' (E). This classification scheme, based on field observations, multivariate analyses and expert knowledge, is an attempt to organize in a clear framework the distribution of macrobenthic communities and to reduce the complexity of the Venice lagoon to a few prevailing environmental pressures: marine and continental influence, eutrophication, sea water dilution, urban pollution. It is important to point out that the six ecological sectors do not correspond to topographical regions of the lagoon, but rather to characteristic benthic communities associated with specific environmental conditions. The benthic community at sector M is composed of typical marine species and is represented by the few individuals able to withstand high current velocity; sector V, where current velocity is weaker, also presents a community dominated by marine organisms, but is slightly richer in quantity and species; in sector RE euryecious marine species appear together with typical lagoon species, the latter becoming dominant in sector CE, which represents the inner lagoon environment, with poor water renewal, high nutrients content and considerable biomasses. Simplified benthic communities inhabit the remaining two sectors: in sector U, because of high pollution, only a few species can adapt to the extremely disturbed conditions; in sector E, which is governed by strong desalination, only a small number of oligohaline species can thrive (table 1).

Sconfietti et al. [6] applied the same classification scheme to another large northern-Adriatic basin, the Grado-Marano lagoon, obtaining a satisfactory description of the environment. The main constraint in the application of the original classification by Occhipinti-Ambrogi *et al.* [17] is the absence of a detailed operational protocol, so that the applicability of the method depends on the expert's personal knowledge. On the other hand, the design of a standard deterministic model is prevented by three kinds of problems [18]: (i) the macrobenthos of the wooden poles is not suitable for precise density estimates, due to the colonial nature of most organisms; therefore the most commonly used approaches are qualitative or semi-quantitative, for example abundance descriptors such as 'scarce', 'abundant', 'very abundant' [19]; (ii) the relation between biocenosis and ecological sector is not defined by an analytical formula, but based on statements like 'if species A are abundant and species B are absent then the ecological sector is C', expressed with a linguistic description, typical of observed natural behaviours

Table 1. Relationship between ecological sectors and macrobenthic community composition, as resulting from the classification scheme worked out after a multi-annual series of field surveys in the Lagoon of Venice [17]. Scale of abundance: 1 = presence of isolated individuals or colonies, 2 = numerous individuals or colonies, 3 = very numerous individuals or colonies, 4 = overwhelming abundance.

| | Macrobenthos variables | | | |
|---|---|---|---|---|
| Ecological sector | Marine species abundance | Lagoon species abundance | Oligohaline species abundance | Total abundance |
| Lagoon mouths(M) | high | low | low | low |
| Vivified lagoon(V) | high | low | low | medium |
| Rough Eutrophic(RE) | high | high | low | high |
| Calm Eutrophic(CE) | low | high | low | high |
| Urban(U) | low | high | low | low |
| Estuarine(E) | low | low | high | low |

[20]; (iii) the six ecological sectors represent an output of a qualitative and non-ordinal nature, which summarizes the information of multi-dimensional ecological conditions.

For this reason a non-conventional approach was required to translate the qualitative scheme of Occhipinti-Ambrogi et al. [17] into a formal model. The method proposed by Marchini and Marchini [18] uses fuzzy logic to tackle the above-mentioned problems, as in case (i) it can easily deal with imprecise data, in case (ii) it allows linguistic reasoning, and in case (iii) it manages qualitative information. In fact, the fuzzy theory introduced in 1965 by Zadeh [21] as a possible way to handle uncertainty is particularly useful for processing imprecise data and expert knowledge, when it is difficult to model the underlying system, due to its high complexity. In fuzzy systems, a variable is not represented by a numerical value, but rather by adjectives that express properties of a variable, e.g. *low*, *high*. A suitable function needs to be designed to represent a variable value's membership grade in a given property, and this grade can vary from 0 (null membership) to 1 (full membership). Intermediate values can assume partial membership grades in both the properties *low* and *high*, thus transforming the uncertainty contained in the data into a source of additional information: that variable will not be represented by a single numerical value, but rather by two adjectives that describe its properties, with relative membership grade. The fuzzy model developed by Marchini and Marchini [18] uses benthic species abundance as input and generates as output a membership grade in each of the six ecological sectors (example in figure 1). The sector which obtains the maximum membership grade represents the final result ('Rough Eutrophic' in the case of figure 1), which can be used for comparisons and statistics.

As mentioned before, the model was created for the lagoon of Venice [18], and was then tested in the northern-Adriatic lagoons of Grado-Marano and Sacca di Goro [22], in all

| Species | Abundance |
|---|---|
| *Mytilus galloprovincialis* | 3 |
| *Ostrea edulis* | 1 |
| *Pomatoceros triqueter* | 1 |
| *Balanus amphitrite* | 1 |
| *Balanus improvisus* | 1 |
| *Nolella gigantea* | 1 |
| *Schizoporella errata* | 3 |
| *Tricellaria inopinata* | 3 |

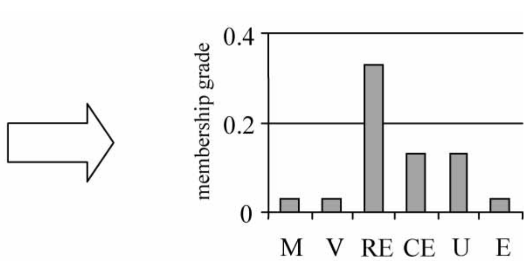


Figure 1. Example of species abundance input data (on the left) and output of the fuzzy logic model designed by Marchini and Marchini [18], expressed as the sampling station's membership grade in each of the six ecological sectors (on the right).

cases providing results which are consistent with literature information and known temporal trends. This classification scheme, although useful in distinguishing different communities in the lagoon ecosystem, each dominated by different driving forces, does not lend itself to environmental quality judgement, which is required for management purposes.

In this paper we present an advancement of the fuzzy model in order to define the ecological quality status of a station, as required by the European Directive 2000/60/EC. The proposed procedure involves two separate stages: first, the presence and abundance of zoobenthic species is used to define a station's membership grade in the six ecological sectors; then a formula links the membership grades to the five ecological status classes. Finally, we show the application of the method in four Adriatic lagoons (eastern Italy), using both literature data and new unpublished data collected in recent surveys.

## 2. Materials and methods

### 2.1 *First stage: from the zoobenthos to the ecological sectors*



As the sessile zoobenthos includes both colonial and solitary organisms of different sizes, a practical way to evaluate species abundance is a semi-quantitative scale of abundance [23]: 1 = presence of isolated individuals or colonies, 2 = numerous individuals or colonies, 3 = very numerous individuals or colonies, 4 = overwhelming abundance. The abundance indices are used as input data for the fuzzy model [18]. On the basis of relevant literature and personal experience, the species are divided into four groups, depending on their tolerance to salinity, hydrodynamism and trophism: marine, lagoon, oligohaline and opportunistic. The relationships among the abundance of marine, lagoon and oligohaline species and the total abundance of the zoobenthos are used to define the main logic rules of the fuzzy model (table 1). An example of the rules is as follows: 'if marine and lagoon species are abundant and other species are absent or scarce and the total abundance of the community is high, then the station has a community typical of sector RE'. These assumptions were used to manage a larger set of rules, deriving from the combination of all the four variables (marine, lagoon, oligohaline species abundance and total abundance) according to three different levels of abundance, and evaluated for all the six ecological sectors. The assortment of variables and abundance levels creates 81 combinations: the few that correspond to the logic rules presented in table 1 were assigned full membership grade in the ecological sector they accurately describe, and were assigned partial or null membership grade in the other sectors. Similarly, the combinations that could not be unequivocally related to any sector were assigned partial or null membership grade in all the six sectors. These 81 combinations represent the full range of relationships among marine, lagoon and oligohaline species that can be observed in a lagoon; therefore, any final result will certainly fall within the 81 theoretical outputs.

The output of the fuzzy model is in fuzzy form: six membership grades, each ranging from 0 to 1, in the six ecological sectors. The fuzzy output provides a lot of information, but in order to achieve a greater synthesis it has to be 'defuzzified'. Defuzzification is the process of combining several partial memberships to produce a single result, compatible to non-fuzzy approaches [24]. Several defuzzification methods have been proposed in the literature [25–27], but the majority of them are related to ordinal types of output. Since the ecological sectors are of non-ordinal nature, a simple and effective defuzzification strategy was chosen, i.e. the maximum operator: the final result is the ecological sector obtaining the highest membership grade.

The detailed aspects of the fuzzy model and the complete rule-base are described in [18].

### 2.2 *Second stage: from the ecological sectors to the quality status*

The attribution of quality status to the six ecological sectors represents the practical management usage, as required by the European Directive. It is evident that an ecological sector, as results from the application of the fuzzy model [18] on a lagoon station, cannot be directly converted into a class of ecological status, because the criteria that define the six sectors are only partially dependent on water quality. Nevertheless, the ecological sectors can indirectly be related to ecological status if we consider that the benthic communities involved in marine vivification processes (e.g. V and RE) are more resilient to potential impacts than those living in confined conditions (e.g. CE and U). Resilience is intended here as the extent of disturbance that a community is able to absorb before changing its structure. As the Adriatic lagoons are permanently subjected to anthropogenic pressures resulting from agriculture, industry and urban waste, navigation and aquaculture, a high or low resilience can be reasonably associated with good or bad quality, respectively. Therefore, some ecological sectors are more associated with good ecological status than others.

Moreover, it must be taken into account that important differences might exist among communities that are classified in the same ecological sector. For instance, consider the two stations A and B, both classified as CE, but A having high membership grade in sectors RE and V and B high membership grade in sector U: the resilience of their communities is clearly not the same and thus the ecological status in A and B is likely to be different.

These considerations led us to create a system for the conversion of ecological sectors into ecological status classes, by means of a different defuzzification strategy, which takes into account the resilience of each ecological sector to anthropogenic impacts. For this purpose, we adopted the same defuzzification method proposed by Angel *et al.* [28] in a paper about the impacts of fish farming on the benthos: a linear combination of the membership grades. The choice of coefficients, or weights, of the linear combination must reflect the different performances of the ecological sectors, for example higher weights could be assigned to sectors with bad ecological status, and lower weights to sectors with high ecological status.

In this framework, sector M was not considered for the conversion as it describes a peculiar situation of a community depleted by the physical disturbance (the high current velocity prevents the attachment of organisms to the hard substrate), which does not depend on the environmental quality, thus providing misleading information. Moreover, sector M represents a boundary condition between lagoon and sea, and its quality assessment should be based on marine criteria rather than on transitional waters criteria. Therefore, sector M remains among the possible outputs of the fuzzy model, but is not included in the evaluation of the ecological status, and is not considered in the assignment of weights: this reduces the number of ecological sectors to 5.

Because the sum of the membership grades of sectors V, RE, CE, U, E is different for every fuzzy output, a preliminary normalization is necessary in order to make the ratios between ecological sectors comparable to several model outputs: supposing $\mu_i$ is the membership grade of the $i^{th}$ sector as resulting from a fuzzy model output; then the normalized membership grade $\bar{\mu}_i$ is:

$$\bar{\mu}_i = \mu_i / \Sigma\mu_i, \quad \text{with } i \in \{\text{V, RE, CE, U, E}\}.$$

Subsequently, the linear combination can easily be calculated as follows: each normalized membership grade has to be multiplied by its relative weight, $w_i$, to obtain the final value of *TWIN*.

$$\begin{aligned} TWIN &= \sum w_i \times \bar{\mu}_i \\ &= (w_V \times \bar{\mu}_V) + (w_{RE} \times \bar{\mu}_{RE}) + (w_{CE} \times \bar{\mu}_{CE}) + (w_U \times \bar{\mu}_U) + (w_E \times \bar{\mu}_E). \end{aligned}$$

To associate the value of *TWIN* with an ecological status, a reference framework is needed for the five classes proposed by the European Directive 2000/60/EC, i.e. bad, poor, moderate, good, high. As the fuzzy model contains in its rule-base all the possible output combinations, the reference conditions can be identified from among its 81 theoretical outputs, in particular the 'best' and 'worst' combinations which mark the extremes of the range of values that *TWIN* can have.

All the 81 combinations of the fuzzy model have to be weighted in order to calculate the extremes. Supposing $M^{m\times n}$ is the set of matrices with $m$ rows and $n$ columns, $A \in M^{81\times 5}$ is the matrix of the 81 theoretical outputs of the model, after a row-by-row normalization, and $W \in M^{5\times 1}$ is the vector containing the weights assigned to the five ecological sectors; then the multiplication of $A$ by $W$ produces the vector $T \in M^{81\times 1}$, whose 81 rows are the sums of the weighted membership grades, i.e. the 81 theoretical *TWIN* values.

$$A = \begin{pmatrix} \bar{\mu}_{V(1)} & \bar{\mu}_{RE(1)} & \bar{\mu}_{CE(1)} & \bar{\mu}_{U(1)} & \bar{\mu}_{E(1)} \\ \bar{\mu}_{V(2)} & \bar{\mu}_{RE(2)} & \bar{\mu}_{CE(2)} & \bar{\mu}_{U(2)} & \bar{\mu}_{E(2)} \\ \cdots & \cdots & \cdots & \cdots & \cdots \\ \cdots & \cdots & \cdots & \cdots & \cdots \\ \bar{\mu}_{V(81)} & \bar{\mu}_{RE(81)} & \bar{\mu}_{CE(81)} & \bar{\mu}_{U(81)} & \bar{\mu}_{E(81)} \end{pmatrix}; \quad W = \begin{pmatrix} w_V \\ w_{RE} \\ w_{CE} \\ w_U \\ w_E \end{pmatrix}.$$

$$A \times W = \begin{pmatrix} w_V \times \bar{\mu}_{V(1)} + w_{RE} \times \bar{\mu}_{RE(1)} + w_{CE} \times \bar{\mu}_{CE(1)} + w_U \times \bar{\mu}_{U(1)} + w_E \times \bar{\mu}_{E(1)} \\ w_V \times \bar{\mu}_{V(2)} + w_{RE} \times \bar{\mu}_{RE(2)} + w_{CE} \times \bar{\mu}_{CE(2)} + w_U \times \bar{\mu}_{U(2)} + w_E \times \bar{\mu}_{E(2)} \\ \cdots \quad \cdots \quad \cdots \quad \cdots \quad \cdots \\ \cdots \quad \cdots \quad \cdots \quad \cdots \quad \cdots \\ w_V \times \bar{\mu}_{V(81)} + w_{RE} \times \bar{\mu}_{RE(81)} + w_{CE} \times \bar{\mu}_{CE(81)} + w_U \times \bar{\mu}_{U(81)} + w_E \times \bar{\mu}_{E(81)} \end{pmatrix} = \begin{pmatrix} TWIN_{(1)} \\ TWIN_{(2)} \\ \cdots \\ \cdots \\ TWIN_{(81)} \end{pmatrix} = T.$$

The minimum and maximum values of vector $T$ represent the extremes that correspond to best and worst ecological status. In particular, given that the weights assigned to those sectors representing the 'best' ecological conditions are lower than the weights assigned to the 'bad' sectors, the 'reference condition' value for high ecological status is represented by $TWIN_{\min} = \min_x(TWIN_x)$, with $X = \{1, 2, \ldots, 81\}$, whereas $TWIN_{\max} = \max_x(TWIN_x)$ represents the worst possible condition, i.e. 'bad' ecological status. The range $[TWIN_{\min}, TWIN_{\max}]$, divided into five equal parts, identifies the five classes of ecological quality status (figure 2).

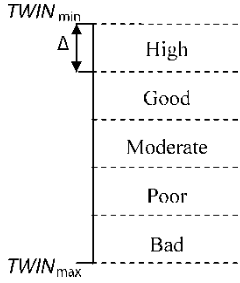


Figure 2. Ecological status classes obtained from the subdivision of the range between $TWIN_{\min}$ and $TWIN_{\max}$ in five equal parts: $\Delta = (TWIN_{\max} - TWIN_{\min})/5$.

This system works with any weight assigned to the ecological sectors, provided that sectors representing 'good' ecological conditions have lower weights than sectors representing 'bad' conditions. Determining weights in fuzzy rule-based systems can be obtained by hybrid techniques, such as artificial neural networks and genetic algorithms. However, while the former require huge amounts of data, the latter require cost functions which can be derived from reference situations. In our case, neither requirement is available, thus making both solutions unfeasible. Therefore, the criteria to decide weights in *TWIN* were not data-driven, but knowledge-driven, and set up by means of the trial and error method, which is a common approach to optimize fuzzy models [27]. Several combinations of weights were tested on the 81 outputs of the model by taking into account two criteria: (i) they had to be in agreement with expert judgement, and (ii) able to discriminate among the 81 theoretical cases. Specifically, they had to provide an even distribution of the five ecological status classes, corresponding to the complete combination (=81 outputs) of ecological conditions. In particular, the first point (i) was addressed by choosing the ranking of the weights assessed to each ecological sector and the second one (ii) by applying different sets of weights in the linear combination.

The selected ranking of the ecological sectors from best to worse is: V-RE-E-CE-U and the weights are: $w_V = 1; w_{RE} = 2; w_E = 3; w_{CE} = 4; w_U = 5$. Sector U unequivocally represents the worst condition, as it describes a benthic community which is very impoverished due to strong anthropogenic disturbance. CE also describes a situation of confinement and poor water renewal, thus particularly vulnerable to alteration or disturbance. On the other hand, sectors RE and principally V represent lagoon communities periodically 'washed' by the sea and thus more tolerant towards events of disturbance. Sector E has been ranked in an intermediate position as its naturally impoverished community cannot display high resilience like V and RE, but neither should be associated *a priori* to high disturbance like U and CE. The linear combination using these weights as coefficients defines the extremes of *TWIN*: $TWIN_{\min} = 1.42$ and $TWIN_{\max} = 4.29$; the five ecological status classes can be then identified at the following intervals:

| | |
|---|---|
| high: | [1.42, 2.00]; |
| good: | [2.01, 2.57]; |
| moderate: | [2.58, 3.15]; |
| poor: | [3.16, 3.72]; |
| bad: | [3.73, 4.29]. |

### 2.3 *Study area*

The proposed two-stage procedure has been tested on hard-bottom data from four Adriatic lagoons of the Italian coast (figure 3), from north to south: Grado-Marano and Venice, the second largest and largest Italian brackish water basins respectively, Goro in the Po river delta and Lesina in the Garganic peninsula.

As regards Goro and Lesina, we used unpublished data, which we collected during two sampling surveys carried out in May and July 2004 as part of the national project NITIDA, whereas for the lagoons of Grado-Marano and Venice we considered a subset of the data already used for the classification of the ecological sectors in the two lagoons [6, 17, 18]. In particular, for the assessment of ecological quality status in Grado-Marano we used data from 15 stations collected in July 2000 and 2001, and for Venice we used data from 32 stations collected in July 1993 and 2001. The hard-bottom zoobenthos sampling procedures are described in [6].

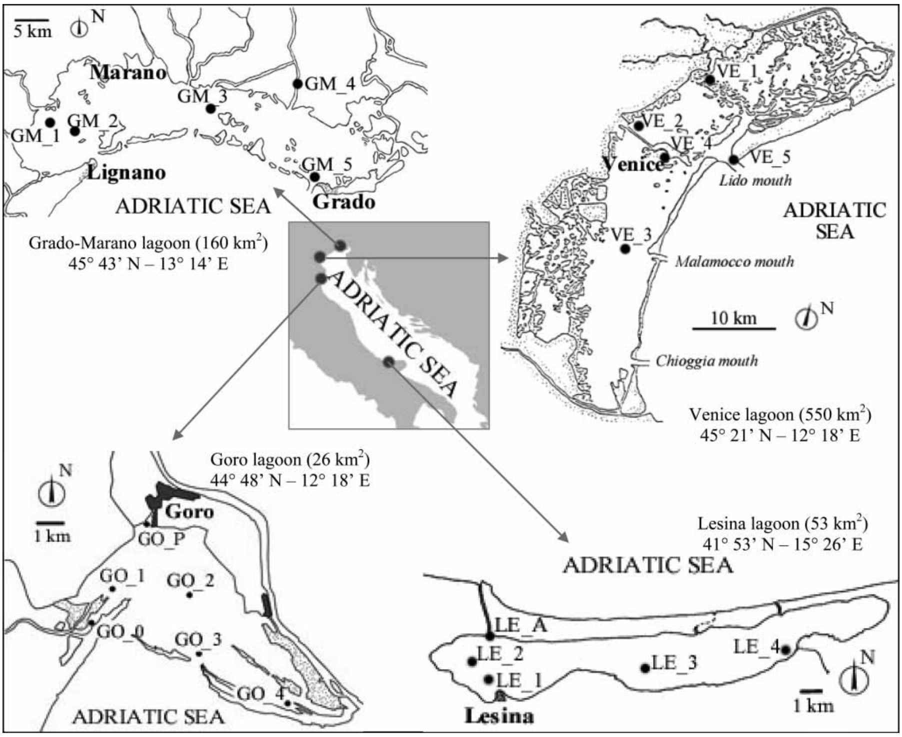


Figure 3. Location of the four lagoons in the Adriatic Sea considered for *TWIN* application.

A Wilcoxon test, recommended for non-normally distributed data, was performed in order to compare the *TWIN* values obtained on the different sampling dates for each one of the considered lagoons.

## 3. Results

Five selected stations from each of the Adriatic lagoons of Grado-Marano, Venice, Goro and Lesina were chosen to show the results of the *TWIN* index calculation. The output of the fuzzy model (first stage), i.e. the ecological sectors that have the maximum membership grade in each station, and the classification of ecological status (obtained by the combination of weighted membership grades) are shown in table 2. In this table, the Grado-Marano and Venice data are those of July 2001 and the Goro and Lesina data were taken in July 2004.

None of the selected stations belongs to sector U, and none of the stations rates bad ecological status, even though sector U obtains relatively high membership grades at stations GM_3 (Grado-Marano), VE_4 (Venice) and LE_A (Lesina). Grado-Marano displays poor ecological status at two stations located in confined areas (*sensu* Guelorget and Perthuisot, [29]): GM_1, where the freshwater inflow is strong, and GM_3, at the hydrographical boundary of two sub-basins, in an area with scarce water renewal [30]. Station GM_4, located in a typical estuarine area, is classified as moderate status; whereas stations GM_2 and GM_5, near the lagoon mouths of Lignano and Grado, display good and high status respectively. Similarly, the estuarine station VE_1, representing the area of freshwater influence in the northern sub-basin of the Venice lagoon [6], is assigned poor ecological status, together with station VE_2, located in a calm eutrophic area near the bridge connecting the island of Venice to the mainland. High

Table 2. Results of the *TWIN* index application on 20 selected stations in the Adriatic lagoons: Grado-Marano (GM), Venice (VE), Goro (GO) and Lesina (LE). The first stage of the calculation yields the prevailing ecological sector, the second stage classifies the ecological status according to five water quality classes.

| Lagoon | Station | $\xrightarrow[stage]{first}$ | Fuzzy model output | $\xrightarrow[stage]{second}$ | Ecological status |
|---|---|---|---|---|---|
| Grado-Marano (July 2001) | GM_1 | | E | | poor |
| | GM_2 | | RE | | good |
| | GM_3 | | CE | | poor |
| | GM_4 | | E | | moderate |
| | GM_5 | | RE | | high |
| Venice (July 2001) | VE_1 | | E | | poor |
| | VE_2 | | CE | | poor |
| | VE_3 | | RE | | high |
| | VE_4 | | CE | | moderate |
| | VE_5 | | V | | good |
| Goro (July 2004) | GO_3 | | CE | | poor |
| | GO_2 | | CE | | poor |
| | GO_3 | | CE | | poor |
| | GO_4 | | E | | moderate |
| | GO_P | | E | | moderate |
| Lesina (July 2004) | LE_1 | | RE | | moderate |
| | LE_2 | | E | | moderate |
| | LE_3 | | RE | | good |
| | LE_4 | | RE | | high |
| | LE_A | | RE | | moderate |



and good ecological status are displayed by two stations influenced by tidal currents to and from the lagoon mouths of Malamocco (VE_3) and Lido (VE_5). Station VE_4, at the southern entrance of the urban centre of Venice, is assigned moderate status. The stations in the lagoon of Goro, notoriously affected by hyperautotrophic and dystrophic events of anthropic origin [31], are classified with moderate and poor ecological status, whereas Lesina, which is less exploited and has a smaller nutrient load, displays stations in good and high ecological status.

It is important to note (table 2) that stations classified in the same ecological sector can be assigned different ecological status classes: for example GM_1 and GM_4 at Grado-Marano, both classified as estuarine (E), have poor and moderate ecological status respectively; the same applies to LE_3, LE_4, LE_A at Lesina, all in the RE sector, but assigned good, high and moderate status respectively. This proves that the second stage of *TWIN* does not directly convert an ecological sector, as it results from the fuzzy model [18], to an ecological status class. The differences among the various combinations of membership grades that lead to the same ecological sector are indeed taken into account, producing a result which reflects the real ecological conditions of the community investigated.

More detailed results of the *TWIN* application in Goro and Lesina (figure 4) show the seasonal differences between the samples of May and July in both lagoons. As regards Goro, the ecological status in May is mainly moderate or poor, with only one occurrence of good status at station GO_3, near the lagoon mouth. The summer season and its related problems of eutrophication affected the hard-bottom zoobenthos, resulting in poor ecological status of most stations in July. The difference, however, is not statistically significant (*TWIN* values in May are lower than in July in 4 out of 6 stations; Wilcoxon test: $p$-value $> 0.05$). In the lagoon of Lesina the stations mainly belong to moderate and good ecological status, with only one case of poor status in May, at station LE_1, near the town of Lesina, and one case of high status in July, in the area covered by seagrass beds (LE_4). Despite a large variation of the *TWIN* value between May and July observed in station LE_3, in the entire lagoon no

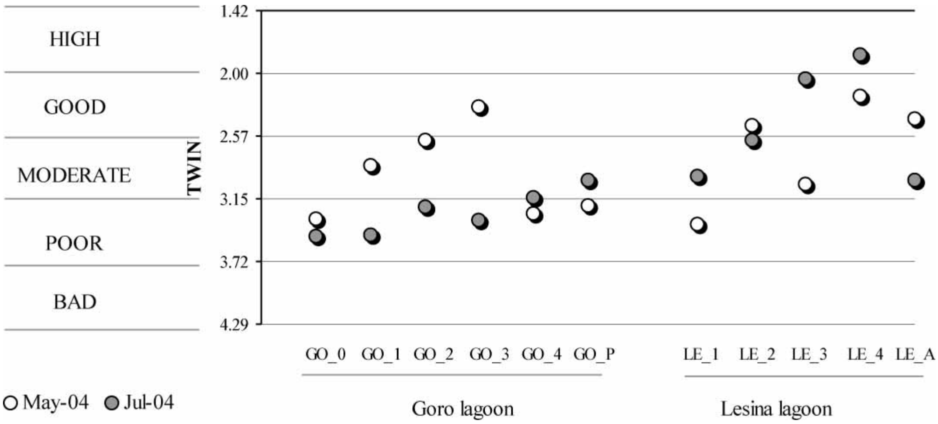


Figure 4. Results of the *TWIN* application in the lagoons of Goro and Lesina, in May and July 2004. The corresponding five classes of ecological status are reported on the left.



significant seasonal difference is demonstrated (*TWIN* values in July are lower than in May in 3 out of 5 stations; Wilcoxon test: $p$-value $> 0.05$).

Finally, the *TWIN* index was applied on the larger datasets from the lagoons of Grado-Marano and Venice (figure 5). Grado-Marano, investigated in 15 stations in July 2000 and 2001, shows a rather even distribution of the four classes from poor to high, with prevalence of the 'moderate' class (40% and 53% of occurrence in 2000 and 2001 respectively). The difference in *TWIN* values between the years 2000 and 2001 is not significant (*TWIN* values in 2000 are lower than in 2001 in 9 out of 15 stations; Wilcoxon test: $p$-value $> 0.05$).

The available data-sets for 32 stations in the lagoon of Venice allow us to compare the results of July 1993 to those of July 2001. Moderate and poor ecological status is predominant in 1993, with one occurrence of bad status at the urban centre of Venice and none of high status, while in 2001 good and high status occur in 40% and 25% of stations respectively, with poor ecological status assigned only to a few stations in the inner part of the lagoon (*TWIN* values in 2001 are lower than in 1993 in 28 out of 32 stations; Wilcoxon test: $p$-value $< 0.001$).

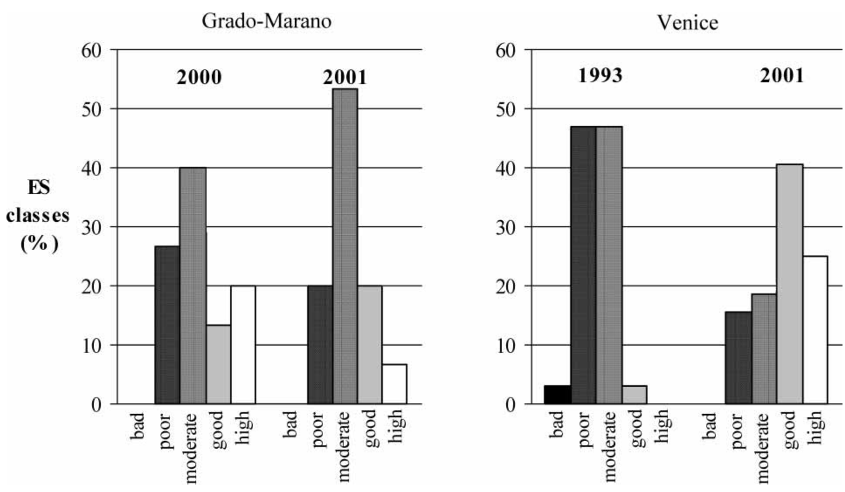


Figure 5. Percent of stations belonging to the five ecological status (ES) classes in the lagoons of Grado-Marano (left) and Venice (right), as resulting from the application of *TWIN*.

## 4. Discussion

### 4.1 *Why a two-stage index?*

The relevance of the biological elements for the assessment of coastal water quality represents one of the main innovative concepts introduced by the WFD. However, this legal requirement has highlighted serious gaps in our knowledge of the structure and function of biological communities [16]. In the case of zoobenthos of lagoons and transitional waters, the species colonizing these habitats are selected by their ability to withstand a highly variable environment, so it is particularly difficult to investigate the responses of single species to any additional stress factor. For this reason, our method uses the biological elements in an indirect way only and the ecological status is defined through a two-stage procedure: first, the species ecology is used to identify the membership of the community in ecological sectors, and second these sectors are associated to the ecological status.

Two separate stages and the use of an uncommon approach such as fuzzy logic involve increasing complexity and technical/scientific terms that might hinder communication with administrators and end-users. This is a major concern in the development of classification methods; the use of clear language and tools is strongly advocated for effective cooperation between environmental protection agencies and the scientific research community [32]. However, the methodological complexity of the *TWIN* index does not imply operative difficulty: the end-user has only to produce a list of benthic species with relative abundance indication, as the model has been programmed to calculate the ecological sector and ecological status class. On the other hand, multidimensional ecosystems such as lagoons cannot be fully understood or represented by simplistic models, which would not be able to cope with the complex, non-linear interactions (physical, chemical, biological, economical, social) that affect these environments.



Using the linear combination as a defuzzification method still implies the reduction of the output from the n-dimensional space of the ecological sectors to the one-dimensional space of the ecological status [25], but presents the advantage of taking into account all the information from the fuzzy output. Even though this approach gives us an approximation of the system complexity, a linear output in the form of an ecological status class is actually much more suitable for monitoring and quality assessment than a n-dimensional output, and can be easily understood without references to the fuzzy set theory [24]. The output framework is defined by choosing linear combination coefficients (weights) which are related to the fuzzy output sets (ecological sectors) but are independent from the input variables, in this case marine, lagoon, oligohaline species abundance and total abundance. Therefore, the defuzzification strategy is strictly related to the structure of the underlying framework and to the meaning and interpretation of the membership values [26].

### 4.2 *Reference conditions*

Reference conditions must represent the ‘best’ ecological status of a system, that is, conditions with minimal disturbance from human activities; this is essential for the subsequent classification of the water body. EU member states have planned to identify sites for each ecoregion which represent this reference (i.e. high ecological status) [32]. However, in most of the European coastal regions, areas without any anthropogenic impact are absent [16]. Lagoons, in particular, have witnessed centuries of human influence and exploitation, thus unaltered examples are very unlikely to be found. Moreover, areas with strong salinity gradients add more difficulty in the definition of what a ‘high’ ecological status is [33], because of their naturally impoverished biological communities. Where easily recognizable undisturbed sites are not present, the WFD identifies three other options for deriving reference conditions: historical data and information, expert judgement and models (Directive 2000/60/EC; Annex II, 1.3). *TWIN* addresses the third option: the fuzzy logic model designed for the Venice lagoon [18] is comprehensive of all the possible combinations of its variables and therefore it can be used to identify the theoretical reference condition for 'high' ecological status within its own rules. Similarly, the model allows us to identify the theoretical condition of 'bad' status, so that no real data can produce results outside of these two extremes.

This procedure for identifying reference conditions is simple and automatic, and does not require external interventions of a subjective nature. A change in the weights assigned to the ecological sectors produces a change in the minimum and maximum of the weighted sum, as the new best and worst combinations of the fuzzy model reflect the new idea of environmental quality related to the new weights assigned to the ecological sectors. Of course the reference condition identified by this procedure can only work within *TWIN* application and not in a more general framework, that is, it does not aim to represent the 'best' ecological status in all the European transitional waters, or even on the ecoregional scale.

The assessment of weights represents the critical point of the whole procedure, as it is performed as a subjective decision. The weights assigned to the ecological sectors, defined for the lagoon of Venice, may not be valid in other environments where the best ecological status could be linked to the estuarine sector rather than the area influenced by the sea. Most likely, the estuarine sector represents the bad ecological status in the case of basins receiving rivers of poor water quality, whereas in the case of running waters of high ecological status, the estuarine area of the corresponding lagoon should be considered good quality. Hence, the possibility of varying the weights according to the characteristics of each lagoon basin should be taken into account: on the one hand, it would make the method open to variations based on subjective knowledge, on the other hand, it would be flexible and adaptable to complex environments such as lagoons.



### 4.3 *TWIN in the Adriatic lagoons*

The application of *TWIN* in the four Adriatic lagoons of Grado-Marano, Venice, Goro and Lesina generally produced satisfactory results, assigning poor or moderate ecological status to the stations mostly influenced by urban waste, e.g. VE_4 (classified as 'bad' in 1993), GO_P and LE_1 as well as to stations impacted by nutrient and contaminant loads from the nearby river inlets [34–36], e.g. VE_1, GO_0 and GO_1. Conversely, *TWIN* assigned good or high ecological status to stations that are 'washed' by marine waters and therefore less subject to disturbance, e.g. GM_5, VE_3, VE_5 and GO_3 in May (table 2, figure 4). Generally, the lagoons of Grado-Marano and Venice presented a wider range of ecological sectors and ecological status classes, due to the fact that in these two big lagoons the interaction of continental and marine influences create a notable spatial heterogeneity [6, 30, 37, 38], whereas the lagoons of Lesina and, in particular, Goro, showed a consistent distribution of ecological sectors (table 2), due to the biocenotic homogeneity of these basins [7, 39].

*TWIN* also correctly represented some short- and long-term temporal trends. The most interesting result is the very highly significant improvement of the overall ecological status in the Venice lagoon from 1993 to 2001, as a consequence of the considerable decrease of nutrient loads [40]. The comparison of Grado-Marano data, obtained from the same stations during the summer of 2000 and 2001, suggests a relative stability from year to year. As regards the seasonal comparisons carried out at the Goro and Lesina stations, *TWIN* highlighted the deteriorating ecological status of Goro from May to July, related to the eutrophication effects occurring during the summer in this shallow lagoon [31], which are always associated with ecosystem stress [41–43]. On the other hand, Lesina displayed a higher overall quality classification, with no evident deterioration during the summer season, thus giving additional support to indications of a better state of health of this basin, if compared with Goro [44].

Some results of the index, however, might be difficult to explain, for instance the large *TWIN* variation between May and July of the GO_3 station at Goro and LE_3 station at Lesina (figure 4). Such results could be due to local disturbance events, for example enhanced turbidity caused by trawling activities near station GO_3, located in an area of clam farming [45], but an intrinsic instability of the *TWIN* index cannot be excluded and further tests should be performed to obtain clearer results.

## 5. Conclusions

The European Water Framework Directive requires the assessment of several biological elements, but these elements may not react in the same ways to disturbance [46]. Phytoplankton, other aquatic flora, zoobenthos and fish fauna may have different responses to different types of disturbance, and within the class of zoobenthos, hard-bottom and soft-bottom communities may present considerable differences. As they are independent from the characteristics of the sediment, the macroinvertebrates of the wooden poles directly respond to the quality of the water column [7] but can be influenced by physical stress such as high current or turbidity [47]. For this reason, the hard-bottom zoobenthos itself cannot precisely identify a specific type of impact, but gives a comprehensive response to different sources of disturbance and should be carefully considered in order to achieve an integrated evaluation of quality status.

To the best of our knowledge, *TWIN* at present is the only index based on the hard-bottom macrobenthos, and one of the few indices specifically designed for lagoons. Unlike most common biotic indices based on the marine benthos [11, 12], *TWIN* indirectly assesses the ecological status of the benthic community. The ecology of the species is used to identify the membership grades of a community in different lagoon sectors, which are then interpreted in terms of ecological status. Reference conditions and ecological status classes are derived from the fuzzy model on which *TWIN* itself is based.

While it is relatively easy to design an index capable of differentiating between impacted and reference sites, the distinction among levels of degradation, as requested by the European Water Framework Directive, is by far a more complicated issue, and is still under debate within the European Member States [10, 48]. Moreover, there are few tools available at present to validate the results of a new index in terms of ecological quality status: a possible strategy might be the comparison with indices based on other biological components in the same lagoon. *TWIN* is one of the indices developed within the NITIDA project, and before being proposed to administrators, its results will have to be compared with those obtained by the application of other indices in the Goro and Lesina lagoons.

## Acknowledgements

This work is a part of the National Project NITIDA (Nuovi Indicatori di stato Trofico e d'Integrità ecologica Di Ambienti marini costieri e ambienti di transizione), supported by the Italian Ministry for University and Scientific Research (MIUR - Cofin 2003).

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