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VIEW POINT

Water quality evaluation in Mediterranean lagoons using the Multimetric Phytoplankton Index (MPI): Study cases from Sardinia

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Abstract

- 1 Water quality in four Sardinian lagoons (western Mediterranean Sea) was assessed using the Multimetric Phytoplankton Index (MPI), which is consistent with the EU Water Framework Directive. The index was developed using data on phytoplankton abundances, species structure and chlorophyll *a* concentrations in Venice Lagoon, Italy.
- 2 The aim of this study was to test the MPI on a larger geographical scale and across a range of lagoon types. Therefore, it was applied to assess water quality in the Cabras, S'Ena Arrubia, Santa Giusta and Calich lagoons in Sardinia. These lagoons are all "choked", but exhibit a range of sizes and morphometric features. They are directly affected by human activity within the lagoons themselves, such as fisheries, aquaculture and the construction of dams and canals, and are indirectly affected by anthropogenic activities in their catchments, including intensive agriculture, industrial activity and urban development.
- 3 The data used in the present study were collected monthly over a period of 4 years (Calich, Santa Giusta and S'Ena Arrubia) to 7 years (Cabras). Samples were collected at three stations at each of the Cabras, Santa Giusta and Calich lagoons, and at two stations at the S'Ena Arrubia Lagoon, providing a total of 220 samples.
- 4 The water quality in three of the four lagoons investigated (Cabras, S'Ena Arrubia and Calich) was classified as *bad* using the MPI. Among these three, water in Cabras Lagoon exhibited the worst condition. Water quality in Santa Giusta Lagoon was classified as *poor* using the MPI.
- 5 Although we present preliminary results that require further verification, the index appears to be a useful tool for assessing the ecological status of typical Mediterranean lagoons.

Keywords: Biological index, Phytoplankton, Water Framework Directive, Mediterranean lagoons, Sardinia.

Introduction

The EU Water Framework Directive (WFD; 2000/60/EC) is the legal instrument aimed at maintaining and improving the ecological quality of fresh and coastal waters

throughout the European Community. It requires assessment of the quality of water bodies based on biological elements in comparison with reference conditions. For each ecosystem type, the requirements for a

given ecological status under the WFD vary on regional, national and European scales. This has resulted in the implementation of a multitude of methods and multimetric indices for ecological evaluation (Birk *et al.*, 2012). In Italy, the sampling protocol for transitional waters is described in the El-Pr-TW-Monitoring Protocol-03.06 (Italian Ministerial Decree 56/2009; ISPRA, 2011). However, only the use of the Macrophyte Quality Index (MaQI; Sfriso *et al.*, 2009; ISPRA, 2010) and Multivariate Marine Biotic Index (M-AMBI; Muxika *et al.*, 2007) for macrozoobenthos are currently mandatory under national law (Italian Ministerial Decree 260/2010). The use of the Benthic Index based on Taxonomic Sufficiency (BITS; Mistri and Munari, 2008) for assessing macroinvertebrates is optional. The Italian Ministerial Decree 260/2010 does not provide at the moment any indices assessing phytoplankton and fishes, even if they are among the biological quality elements required by the WFD to the ecological classification of transitional waters.

In Mediterranean lagoons, macrophytes often surpass phytoplankton as the most important primary producers (Giordani *et al.*, 2005; Souchu *et al.*, 2010). In these cases, the role of phytoplankton only becomes relevant in periods when macrophytes are absent. Nevertheless, in a number of lagoons, phytoplankton is the sole or prevalent primary producer throughout the year. In these cases, phytoplankton is a powerful indicator of trophic conditions due to their ability to respond to environmental changes, particularly nutrient enrichment. Consequently, phytoplankton is considered to be one of the most useful biotic elements for assessing the environmental quality of water bodies in which eutrophy presents a major environmental issue (Tremel, 1996; Brettum and Andersen, 2005).

Several methods for classification of

transitional waters based on phytoplankton data have been proposed (Facca and Sfriso, 2009; Borja *et al.*, 2013; Lugoli *et al.*, 2012). However, these require metrics that are not routinely collected by local authorities (e.g., biovolumes or cell sizes), and are therefore unable to be used to determine an official classification (Italian Ministerial Decree 260/2010). To resolve this issue and to meet the WFD requirements, the Multimetric Phytoplankton Index (MPI; Facca *et al.*, 2011) was developed for classification of the environmental quality of transitional water bodies in Italy. The index was developed using data on phytoplankton abundance, species structure and chlorophyll *a* concentration in Venice Lagoon, Italy. The MPI is easy to calculate, and it is based on approved methods and parameters often regularly collected by most of local agencies and marine scientific institutions. It derives from the complete description of phytoplankton community (diversity, abundance, dominance) and so it can give indications on both trophic and ecological conditions. The main drawback is the high level of taxonomic definition required; this needs both a great experience of the operators in the correct identification of species and additional efforts in intercalibration process, within and between laboratories.

Validation of this wide-ranging index is still in progress, but results obtained from other lagoons suggest that it is a useful indicator of water quality (Bazzoni *et al.*, 2012; Facca *et al.*, 2012).

With the aim to verify the reliability of MPI and to test the index on a larger geographical scale, considering a major number of study cases and typology of lagoons, it was applied to four Sardinian lagoons: Cabras, S'Ena Arrubia, Santa Giusta and Calich. These lagoons belong to the Mediterranean climate typology (Basset *et al.*, 2006; Tagliapietra and Volpi Ghirardini, 2006) and are characterized by remarkable differences in morphology,

environmental conditions and human impacts and, thanks to the availability of long-term data, were considered interesting study cases for the application of the MPI.

Materials and Methods

Study sites

Sardinia is the second largest Mediterranean island. It contains many transitional ecosystems, which cover a total area of approximately 10,000 ha, and constitute 2.6% of all lagoons in Italy (Cottiglia, 1981). Fishing and aquaculture in the Sardinian lagoons make them important to the local and regional economies. In the last century, Sardinian lagoons and wetlands were extensively reclaimed for a variety of human uses. These actions have led to profound changes in the hydrological conditions of the

lagoons. Furthermore, increased industrial activity, agriculture and urban discharge have deeply modified the natural equilibrium of these ecosystems. Cannas *et al.* (1998) reported that approximately 50% of Sardinian lagoons are eutrophic.

The four lagoons considered here are all located on the western coast of Sardinia. Calich (CL) is situated on the northern part of the coast, whereas Cabras (CB), Santa Giusta (SG) and S'Ena Arrubia (SA) are located in the central part, connected to the Gulf of Oristano (Fig. 1). The latter three sites are research stations included in the 'Marine Ecosystems of Sardinia', part of the Italian Network of Long-Term Ecological Research (LTER-Italia; http://www.lteritalia.it).

The lagoons in this study are all 'choked' sensu Kjerfve (1986), but display significant

Figure 1. Map showing the locations of the four lagoons considered here.

variation in several morphological features. For example, CL and SA are among the smaller Sardinian lagoons, whereas CB and SG are among the larger. Mean water depth also varies markedly among the four lagoons (Table 1). The characteristics of water in the lagoons are defined by the extent of water exchange with the sea, and the amount of freshwater inflow from catchments. As a result, CL and CB are meso-polyhaline, compared with SG and SA, which are marine lagoons.

All the ecosystems in this study range from eutrophic to hypereutrophic, and show large seasonal variations in nutrient concentration. This is a result of polluted inputs from the respective watersheds, which is largely a consequence of inadequate purification of urban and agricultural wastewater. Dystrophic crises, frequently associated with microalgal blooms, can occur during summer, leading to extensive fish mortality (e.g., CB in 1999, SG in 1989 and 2010, and several episodes in SA from 1960 onwards; Sechi *et al.*, 2001; Trebini *et al.*, 2005). CB is typically dominated by phytoplankton, whereas SG, SA and CL also support macrophyte growth during spring–early summer (Trebini *et al.*, 2005; Ceccherelli *et al.*, 2009). The lagoons are protected by several national and international agreements due to their value as natural resources (e.g., the Ramsar Convention, European SCI and SPA, and local and regional parks).

Data sources

We derived datasets from data collected monthly over a range of 4 years (CL, SG and SA) to 7 years (CB, Table 2). These data were collected at two stations in SA, and at three stations in CL, SG and CB.

Water samples, collected from the superficial water layer (30-50 cm depth), were considered as representative of the entire water column

Table 1 - Morphological features of the four lagoons studied.

due to the shallowness and the well mixed regime of the lagoons. Temperature, salinity, dissolved oxygen and pH were measured *in situ* with a multi-parameter probe (Idronaut and YSI 6600V2). Samples were preserved in cold and dark conditions for laboratory analyses of nutrients (ammonia, nitrate, nitrite, reactive silica, orthophosphates and total phosphorus) following Strickland and Parsons (1972), and for chlorophyll *a* (SCOR-UNESCO, 1997). Dissolved inorganic nitrogen (DIN) was obtained as the sum of ammonia, nitrate and nitrite.

Phytoplankton samples were fixed with Lugol's solution or buffered formaline solution at a final concentration of 4% and analysed according to the Utermöhl technique (1958).

Water Quality Index

The complete procedure for applying the MPI was previously described by Facca *et al.* (2011, submitted) and Bazzoni *et al.* (2012). Shortly, calculation of the index requires the

following four metrics to be calculated:

- 100-Hulburt index: the dominance of the two most abundant species (Hulburt's index; Hulburt, 1963);

- 100-Bloom Frequency: the frequency at which the most abundant species accounted for >50% of organisms at each station (bloom frequency);

- Menhinick's diversity index (Menhinick, 1964): this index evaluates environmental stress, as reduction of species diversity within a community is generally indicative of natural or anthropogenic alteration;

- the geometric mean of chlorophyll *a*, calculated with log-transformed chlorophyll values. Antilog transformations are then required to calculate the MPI.

To calculate each metric, we only considered microalgae at genus or specie level. We averaged values at each station over a 1-year period to reduce seasonal variability.

Here, the ecological quality ratio (EQR) is defined as the ratio between the metrics calculated for the four Sardinian lagoons and the reference conditions (Facca and Sfriso, 2009; Facca *et al.*, 2011), individuated in an area of the LTER site Venice Lagoon and characterized by a low anthropogenic pressure and by a few direct inputs of pollutants.

The EQR calculated varied between 0 and 1. Values used in the MPI correspond to the mean of the four EQRs. To attribute each station and each lagoon to a defined ecological quality class, five equal intervals of the MPI were used, following the WFD: 0.00–0.20 (*bad*), 0.21–0.40 (*poor*), 0.41–0.60 (*moderate*), 0.61–0.80 (*good*) and 0.81–1.00 (*high*).

Statistical analyses

A Pearson correlation matrix was generated using the mean annual values for each lagoon to assess the relationship between MPI scores and nutrient concentrations; p-values ≤ 0.05 were considered significant. Principal component analysis (PCA) was performed

to highlight the system variance in relation to environmental variables and the MPI. For this calculation, our datasets were integrated with the data collected at the reference site (Facca and Sfriso, 2009). This ensured that a range of nutrient concentrations and all quality classes were represented. Salinity (S), DIN and orthophosphates (P-PO4) were considered for each of the lagoons. In addition, dissolved oxygen (Ox) and reactive silica (Si) were used for CL and CB. Consequently, we performed two different calculations to demonstrate the correlation between the MPI and environmental variables. All statistical analyses were carried out using Statistica 7.1 (Statsoft Italia srl).

Results

Nutrients and phytoplankton

Nutrient values were high in all the lagoons in the annual cycles (Table 2). Mean annual DIN values were $3.7-15.9$ μ M in SG, 6.2-22.6 µM in SA, 3.9–171.7 µM in CB, and 55.4–88.0 µM in CL. Mean annual P-PO4 values were 0.7–2.7 µM in CB, 1.6–3.1 µM in CL, 1.9–3.5 µM in SG, and 4.4–5.1 µM in SA.

The complete dataset comprised 233 phytoplankton taxa. Despite the different number of annual cycles considered at each location, the lagoons all supported a similar number of taxa, with the minimum (96) in SA and the maximum (114) in CL.

The mean annual value of phytoplankton cell density varied from 2×10^6 cells 1^{-1} in SG to approximately 5×10^9 cells l^{-1} in CB (Table 3). In general, phytoplankton abundance was lowest in SG, moderate in SA and CL, and highest in CB. A clear dominance of Bacillariophyceae and Cyanobacteria was observed in CB (Fig. 2). The relative importance of phytoplankton classes varied widely in SA and SG, with Chlorophyceae and Bacillariophyceae much more abundant than other classes in certain annual cycles (Fig. 2). In CL, Bacillariophyceae were the

Site	Annual cycles	Cell Densities $(10^6 \text{ cells } l^{-1})$				Chlorophyll a (µg l^{-1})			
		St.1	St.2	St.3	Mean	St.1	St.2	St.3	Mean
CL	\mathbf{I}	11	5	τ	8	11	10	14	12
	\mathbf{I}	47	46	62	52	21	23	23	$22\,$
	$\rm III$	51	56	47	51	13	13	11	12
	IV	30	32	27	29	$\overline{7}$	9	11	9
$\mathbf C\mathbf B$	$\mathbf I$	56	80	28	55	130	148	146	141
	\mathbf{I}	27	34	26	29	55	48	53	52
	$\rm III$	36	53	34	41	37	38	42	39
	IV	3603	4838	6547	4996	32	36	63	44
	V	1186	1195	1357	1246	23	18	22	21
	VI	2519	2496	2839	2618	12	14	16	14
	VII	526	663	683	624	112	112	122	115
SG	\bf{I}	86	87	84	85	18	15	24	19
	\mathbf{I}	7	$\mathbf{1}$	8	5	14	5	12	$10\,$
	$\rm III$	$\overline{2}$	$\overline{\mathbf{3}}$	6	$\overline{4}$	6	$\,8\,$	10	$\,8\,$
	IV	$\mathbf{1}$	$\overline{\mathcal{L}}$	$\overline{2}$	$\overline{2}$	9	6	12	9
SA	\mathbf{I}	16	17	n.a.	17	14	11	n.a	12
	\mathbf{I}	17	22	n.a.	20	6	9	n.a	$\overline{7}$
	Ш	17	20	n.a.	18	35	61	n.a	48
	${\rm IV}$	15	$\,$ $\,$	n.a.	12	23	$18\,$	n.a	20

Table 3 -Annual means of cell densities and chlorophyll *a* concentration in the four studied lagoons.

dominant class in all annual cycles, with Dinophyceae and other classes (mainly Cryptophyceae) also contributing (Fig. 2). Mean annual values of chlorophyll *a* were 8–19 μ g l⁻¹ in SG, 9–22 μ g l⁻¹ in CL, 7–48 μ g l⁻¹ in SA, and 14–141 μ g l⁻¹ in CB (Table 3). Overall, the highest chlorophyll *a* values were consistently recorded in CB, with levels up to five times higher than the other lagoons.

Application of the MPI

The data regarding phytoplankton assemblage composition and cell abundance allowed us to apply the MPI by calculating the four necessary metrics for each lagoon (Table 4). The mean value of 100-Hulburt index for the lagoons was lowest in SA and CB (6.36 and 6.76, respectively), moderate in CL (9.44), and highest in SG (15.65). The widest range among stations within one lagoon was observed in CL (6.47–11.26), whereas index

values were relatively consistent between stations in the other lagoons.

The mean of 100-Bloom Frequency varied from a minimum of 1.39 in SA, to higher values in CB (10.35) and CL (13.56) , and reached a maximum of 32.79 in SG (Table 4). Similarly, Menhinick's diversity index was lowest in CB (0.001) and highest in SG (0.015; Table 4). The geometric mean of chlorophyll *a* varied from 8.51 to 65.7 μ g l⁻¹, measured in SG and CB, respectively (Table 4). To accurately assess the ecological status of transitional water, comparison with a nearpristine reference site is necessary. The MPI showed a generally homogeneous situation within each lagoon (Fig. 3). The MPI value indicated a *bad* condition in CL and CB (Table 5), and a *poor* condition in SG. SA was the only lagoon that demonstrated a difference in water quality across stations: *bad* at Station 1, closer to the sea and *poor*

Figure 2. Mean annual density of phytoplankton classes in the four lagoons studied.

at Station 2 (Fig. 3). However, the average condition of SA remains *bad* (MPI = 0.18), similar to CL and CB.

Statistical analyses

The statistical analyses supported the conclusion that the MPI is suitable for assessing ecological water quality, as there was a significant inverse correlation (p-value < 0.05) between the MPI and P-PO4 content. Moreover, the MPI was positively correlated with salinity. Such relationships are better depicted by the PCA in Fig. 4a. The first two components account for almost 80% of the system variance, and are therefore strong indicators of environmental conditions.

In CL and CB, data regarding oxygen saturation and silicate concentration were also available. It was therefore possible to verify that MPI was directly correlated with conditions oversaturated with oxygen (Fig. 4b). Where such conditions existed, the MPI also showed a significant correlation with DIN, probably because CL was particularly rich in nitrogen compounds.

Discussion

It has already been observed that phytoplankton can provide important information on the ecosystem status (Facca and Sfriso, 2009). In our study, the application of an index based on phytoplankton variables, the MPI, to four

Mediterranean lagoons indicated for all a critical state of their water quality.

It was supported by the results of the statistical analysis, and was coherent with that reported in previous studies (Lugliè *et al.*, 2001; Sechi *et al.*, 2001; Trebini *et al.*, 2005; Pulina *et al.*, 2011, 2012; Padedda *et al.*, 2012).

CL showed the highest DIN concentration, except in 1999 when a strong dystrophic event occurred in CB, whereas SA showed highest P-PO4 values. CL and SA were the smaller lagoons considered in the present study, but the former has a drainage basin of similar area to CB, the largest lagoon, whereas a large part of the catchment area of SA is used for intensive arable agriculture and cattle breeding. Regarding SA, it is important to point out that the bad condition likely occurred because the data were collected before 2000, when an important 'reframe' widened the mouth of the canal. This widening was implemented with the aim of reducing the trophic level of the lagoon by improving tidal flushing and lagoon hydrodynamics (Trebini *et al.*, 2005). Moreover, the MPI was positively correlated with salinity, indicating that improved conditions existed in areas located farthest from sites of freshwater input and, in the case of SA, it reflected on the different water quality across stations, expressed by MPI .

In any case, all the four lagoons showed nutrients values several times higher than the legal thresholds for both DIN (25 µM) and P-PO4 (0.8 µM; Italian decree n. 152/2006, ex. 152/1999). This highlights the significant and persistent nutrient enrichment occurring in the lagoons, especially for P-PO4 in each lagoon and for DIN only in CL. In particular, the high trophic condition in CB resulted by the application of other ecological indexes, too: the TSI index and the TRIX index (Padedda *et al.*, 2010).

Overall, the studied lagoons were dominated by phytoplankton communities with high

13.20 14.27 15.56 14.34 57.08 58.82 65.71 60.54 19.21 24.44 21.83 11.69 8.51 14.29 11.50

 $\overline{71}$ 65.

82 58.

57.08

14.34

15.56

14.27

 \overline{c} $13.$

60.54

11.50

29 $\frac{1}{4}$

8.51

11.69

83 $\overline{21}$

 $\overline{4}$ 24.

 $\overline{21}$ $\overline{19}$

Table 4. Multiannual means of phytoplankton metrics calculated according to Facca

able 4. Multiannual means of phytoplankton metrics calculated according to Facca et al. (2011) and Bazzoni et al. (2012).

et al. (2011) and Bazzoni *et al*. (2012).

Chlorophyll *a* (µg I^{-1} Chlorophyll a (μ g I⁻¹)

○ high ● good ● moderate ● poor ● bad

Figure 3. Classification of water quality at the sampling stations, based on the MPI.

biomass and high frequency of blooms. The less affected one was SG, in which all metrics indicated higher biodiversity and lower biomass. This indicates that phytoplankton dominance was generally high across the four lagoons, but there is still a general gradient among them. However, annual cycles showed important changes in phytoplankton in all four lagoons, both in abundance and class composition.

CB, where the most high values of cell density and chlorophyll *a* were detected, was also the only lagoon exclusively dominated by phytoplankton, particularly Cyanobacteria. This condition is considered the final stage of eutrophication in coastal lagoons (Viaroli *et al.*, 2010).

In sum, this work support the use of the MPI as an efficient tool that is able to assess the ecological status of transitional waters, in accordance with the requirements of the WFD, and to plan modulated management actions. Despite this success, the following critical points should be noted:

Table 5. MPI values calculated in relation to reference site in each station and as average between stations.

Site	St. 1	St.2	St.3	MPI
CL	0.17	0.15	0.10	0.14
C _B	0.06	0.06	0.07	0.07
SG	0.32	0.31	0.32	0.32
SА	0.13	0.23	n.a.	0.13

- in Sardinian lagoons, and other transitional waters, the phytoplankton abundance can reach thousands of cells and dominant taxa are often small organisms, usually belonging to the nanoplankton and picoplankton. The MPI protocol requires classification at least at the genus level, which can be difficult in the case of small species. This may result in the exclusion of unidentified organisms, which could be a large percentage of the total abundance, and this would render the MPI value unreliable. Moreover, we have to consider that MPI is based on cell density, but high abundances of very small species may represent only a modest aliquot of the total phytoplankton biovolume;

- most Sardinian lagoons are characterized by marked anthropogenic pressures and, for this reason, a system with good water quality (i.e., a reference site) cannot be found easily. The results of this study appear to confirm that the MPI provided a reliable assessment of water conditions; however, to thoroughly evaluate the validity of the MPI, it is necessary to apply it to near-pristine sites in the same region;

- the rich dataset used for the MPI calculation was obtained primarily through long-term monitoring programs carried out for various scientific purposes in the four Sardinian lagoons. As a result, the sampling design and

Figure 4. a) PCA1, comparing the four Sardinian lagoons and the reference site. b) PCA2, comparing CL, CB and the reference site. The variance of each component is reported in the axis title.

parameters analysed varied depending on the purpose of the research. In this case, the data available were always sufficient for the requirements of the MPI, but this is commonly not the case, making it difficult to verify the MPI at other sites.

Conclusions

Although we have presented preliminary results that require further investigation, it appears that the MPI is a useful, simple and rapid tool for assessing the water quality in Mediterranean lagoons where suitable data are available. Further research should be aimed at extending the application of the MPI, and verifying its use by resolving some of the issues outline above.

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