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Macrophytes and ecological status assessment in the Po delta transitional systems, Adriatic Sea (Italy). Application of Macrophyte Quality Index (MaQI)

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The paper provides the first checklist and information on the macrophytes of the Po Delta (Italy), a complex of small lagoons and ponds among the largest in the Mediterranean Sea, until now little studied in spite of the water surface of approx. 200 km². The abundance and assemblage composition of macrophytes that colonize these environments and their ecological status have been studied taking into account the most common physico-chemical parameters and the concentration of nutrients in the water column and in the surface sediment.

Sampling was carried out in 17 sites, placed at Marinetta, Vallona, Caleri, Canarin, Barbamarco and Scardovari lagoons, during two surveys on 13-15 May and 13-15 October 2008. The ecological status was assessed by applying the Macrophyte Quality Index (MaQI) which was adopted by the Italian Ministry of the Environment for the ecological classification of the Italian transitional environments, in agreement with the Water Framework Directive (2000/60/EC) requirements.

Key words: algae, checklist, indicator species, parameters, Po Delta lagoons, North- western Adriatic Sea

INTRODUCTION

The European Water Framework Directive (WFD, 2000/60/EC) (EUROPEAN UNION, 2000; BORJA, 2005) invited the scientific community to undertake specific studies for the assessment of the Ecological Status of marine coastal waters and transitional environments.

The definition of Ecological Status is based mainly on the monitoring of Biological Quality Elements (BQEs): Coastal waters (CW) base the quality assessment by a) phytoplankton, b) macroalgae and angiosperms, c) benthic invertebrate fauna. Transitional waters (TW) base the assessment by a) phytoplankton, b) macroalgae, c) angiosperms, d) benthic invertebrate fauna and e) fish fauna. Macroalgae and angiosperms (namely aquatic macrophytes) in TW should produce a quality assessment result by different BQEs, however, during the intercalibration process macroalgae and aquatic angiosperms were considered together because these elements live closely associated with each other and respond univocally to environmental stressors.

Macroalgae and aquatic angiosperms, show a different tolerance to environmental stressors, which can affect both their structure and the composition of the taxa (SFRISO *et al.*, 2009). Since aquatic benthic macrophytes are mainly sessile organisms, they respond directly to the abiotic and biotic factors of aquatic environments and thus represent sensitive indicators of their changes (ORFANIDIS *et al.*, 2003). When nutrient loads increase, the species composition shifts from the dominance of angiosperms and sensitive macroalgal species to blooms of opportunistic and thionitrophilous macroalgae (SFRISO *et al.*, 1988; VIAROLI *et al.*, 2008). However, when waters are very turbid and light cannot penetrate to the bottom, macroalgae slow down their growth and phytoplankton and cyanobacteria become the only primary producers. For these reasons the succession of the benthic vegetation from k-selected/sensitive species to r-selected/opportunistic ones, has been used to evaluate the Ecological Status in transitional systems (ORFANIDIS *et al.*, 2003, 2011; SFRISO *et al.*, 2007, 2009, 2014). In these environments, macrophytes - and especially aquatic angiosperms - play a key role in the primary production and constitute the basic structure of the ecosystems. They regulate the nutrient cycles, determine the oxygen concentration, supply food and nursery for the marine organisms and prevent sediment resuspension and erosive processes (SFRISO *et al.*, 2005a, b). Therefore, the integrity of angiosperm beds, or at least their presence, is of primary importance to maintain pristinely or achieve good environmental conditions. On the contrary, the replacement of angiosperm beds by free-floating opportunistic macroalgae is a symptom of eutrophication and degradation of the environment (VIAROLI *et al.*, 2008).

Notwithstanding the ecological and socio-economic importance the Po Delta, a heterogeneous and dynamic complex of lagoons and ponds, originated from the sediment deposition of the Po River, has been poorly studied (AMBROGI *et al.*, 1989 and references therein; FERRARI & MAZZOCCHI, 1985; RELINI *et al.*, 1985) and lacks information on the macrophytes growing in its lagoon system. Recently, CECERE *et al.*, (2009) carried out a study to retrieve information on the Italian transitional systems and reported an inventory of what was known of their flora (species number) and vegetation (species assemblages). Of the examined transitional areas only the lagoon of Venice and the Mar Piccolo of Taranto show abundant literature, on the contrary for other sites, it is poor if not missing. That is precisely the case of the Po Delta.

The paper aims at filling this gap, investigating the flora and vegetation of that wide area in relation to some physico-chemical parameters and the concentrations of nutrients in the water column and surface sediments. The project was born from the need of the Italian Environmental Agencies (ARPA) to assess the ecological quality status (EQS) of these environments by applying the index MaQI (Macrophyte Quality Index) as required by Italian law in accordance with the regulations of the WFD (2000/60/EC).

MATERIAL AND METHODS

Study area

The Po Delta (44°57’N, 12°23’E) includes a wide area of the Veneto Region (north-western Adriatic Sea, Italy) with a water surface of approx. 200 km², of which only approx. 100 km² are affected by the tidal expansion. The main river course (Po di Venezia) is divided into 7 main branches (from North to South): Po di Levante, Po di Maistra, Po di Pila, Po di Tolle, Po di Gnocca or di Donzella, Po di Goro and Po di Volano, which identify transitional areas of different geomorphological and environmental features, including those taken into account in this research: Caleri, Marinetta, Vallona, Barbamarco, Canarin, Scardovari (Fig. 1). The main differences concern salinity, in relation to the river inflows, seawater exchange and depth, which ranges from 0.5-1m to 1.5-2.5m, according to the basin. The lagoons are almost deprived of hard substrata with the exception of any levees, and the many natural oyster beds scattered on the bottoms.

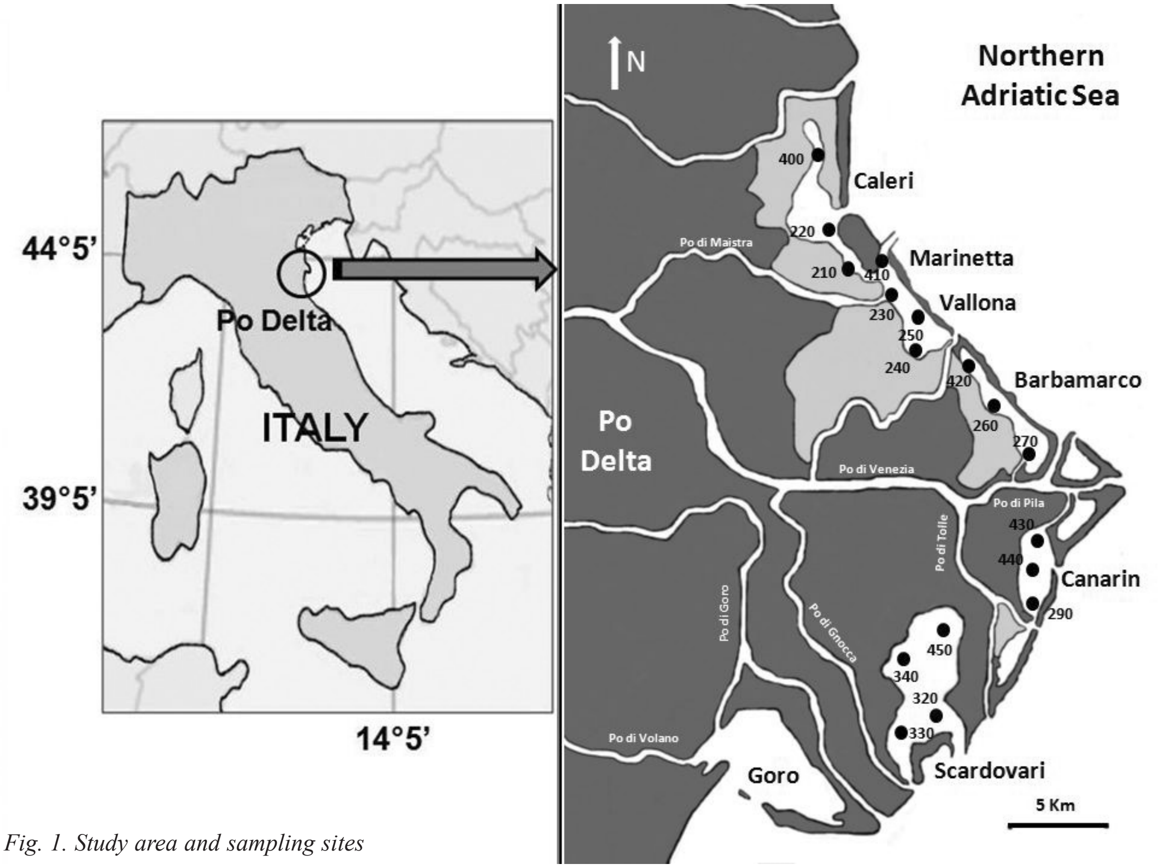

Fig. 1. Study area and sampling sites

Sampling was carried out on the natural soft substrata and oyster beds on May 13-15 and October 13-15, 2008, in 17 sites: Marinetta (2), Vallona (2), Caleri (3), Canarin (3), Barbamarco (3), Scardovari (4, Fig. 1), according to the Italian monitoring protocol "El-Pr-TW-Protocolli Monitoraggio-03.05" (ISPRA, 2011).

Physico-chemical parameters and nutrient concentrations

In each sampling station, water temperature and the percentage of dissolved oxygen saturation (% DO) were obtained by a portable oximeter, whereas water transparency was measured by means of a Secchi disk. Data were normalized both at one-meter depth (100% of transparency when the Secchi disk was >1 m) and to the total depth (percentage of disk visibility with respect to the total depth).

Six surface water samples were recorded by a home-made bottle (i.d. 4 cm, height 150 cm) repeatedly plunged in the water column and mixed in a tank in order to obtain a representative water sample. Water aliquots ranging from 100 to 1000 ml were filtered through GF/F Whatman glass fiber filters (porosity: 0.7 µm) and stored frozen till nutrient analyses (ammonium, nitrites, nitrates, reactive phosphorus) according to the spectrophotometric procedures by STRIKLAND & PARSONS (1984). The filters were folded and stored at -20 °C until Chlorophyll-*a* (Chl-*a*) analysis to the procedure by LORENZEN (1967). Salinity was determined in the laboratory by chlorine titration according to OXNER (1962). The 5 cm sediment surface top layer was collected by a Plexiglas corer (i.d. 10 cm). In each station, three corers were put together and carefully mixed samples were retained for nutrient analyses (i.e. inorganic, organic and total carbon; inorganic, organic and total phosphorus; total nitrogen) according to the procedures reported in SFRISO & MARCOMINI (1996). Fines (sediment fraction <63 µm) were obtained by wet sieving approx. 50 g of dry sediment by means of an "Endecotts" sieve (net light: 63 µm). The amount of dry sediment per volume unit (dry density) was obtained by drying overnight approx. 30 g of wet sediment at 110 °C.

Macrophyte sampling

In soft substrata of each station, the total macroalgal biomass was determined by 3-6 replicates with a square aluminum box of 71*71 cm (0.5 m^2) in an area of 15-30 m according to the procedure of SFRISO *et al.* (1991). Samples were dripped by a centrifuge for salad and wet weighed on board by an electronic balance (precision ± 1g). The mean value of three-six replicates, depending on the biomass level, assures a result accuracy ≥ 95%.

Total macrophyte cover was assessed in the same area by touching 20 times the soft bottom by a rake in order to detect the presence/absence of macroalgae. Results are reported as a percentage of the cover according to the monitoring protocols by ISPRA (2011). One touch with the rake accounts for a biomass cover of 5%. That is the limit considered in MaQI to discriminate areas where macrophytes are able to bloom from areas where the growth is hampered by disturbance factors (SFRISO *et al.*, 2014). The Rhodophyta/Chlorophyta biomass ratio, another metric considered in MaQI to discriminate between score 0.25 and 0.35 in the "Poor" class, was obtained by sorting and wet weighting (precision ± 1 g) the macroalgae collected in 6 additional random samples by scraping the bottom for approx. 1 m with the rake all around the boat. Macrophyte subsamples representative of the collected biomass were preserved in 4% formaldehyde seawater and determined at specific and intra-specific level by means of a stereo-zoom microscope and a light microscope. Some samples of doubtful identification were also kept fresh for molecular analyses.

Ecological status determination

The Ecological Quality Status (EQS) of the studied areas was assessed by applying the Macrophyte Quality Index (MaQI) (SFRISO *et al.*, 2014) obtained by the integration of an Expert method E-MaQI (SFRISO *et al.*, 2009) and a Rapid method R-MaQI (SFRISO *et al.,* 2007). The two indices are highly correlated to each other: $r = 0.962$, $p < 1.5 * 10^{-11}$ in 20 stations (SFRISO *et al.*, 2009), $r = 0.979$, $p < 8.1 * 10^{-7}$ in 5 stations (SFRISO & FACCA, 2011).

The index, set up by correlating all the macrophytes and the main pollutants and stressors recorded in many lagoons has a categorical structure which allows the environment assessment also in the presence of a very low cover (<5%) or a low number of taxa which cannot be obtained with a continuous 0 to 1 scale. In fact, the EQS of many environments almost deprived of vegetation may be assessed by the identification of small epiphytes that are attached to shells or stones on the bottom or by fragments of free-floating taxa regardless of their cover. Historical records, when available, are also of great help, especially if the examined environment in the past had very different ecological conditions. A classic example is the "Valli di Comacchio", a choked Italian basin that in the '70s had a rich vegetation of aquatic angiosperms and macroalgae of high ecological value (GIACCONE, 1974) while now, as a result of organic discharges from intensive farming of eels, the basin is almost completely free of macrophytes.

The EQS assessment by MaQI is based on several metrics: the total number of species, number and percentage of sensitive macroalgal taxa (score = 2) (SFRISO *et al.*, 2009), the total percentage of macroalgal cover, Rhodophyta/Chlorophyta biomass ratio and percentage of aquatic angiosperm cover. The EQS calculation is obtained by two entries, one for macroalgae and the other for angiosperms, if are present (Fig. 2) (SFRISO *et al.*, 2014). Sampling campaigns were carried out twice a year, in spring and in autumn, so as to collect both cold and warm taxa.

Statistical analyses

The correlations between environmental parameters and the macrophyte metrics were obtained by the non-parametric Spearman's

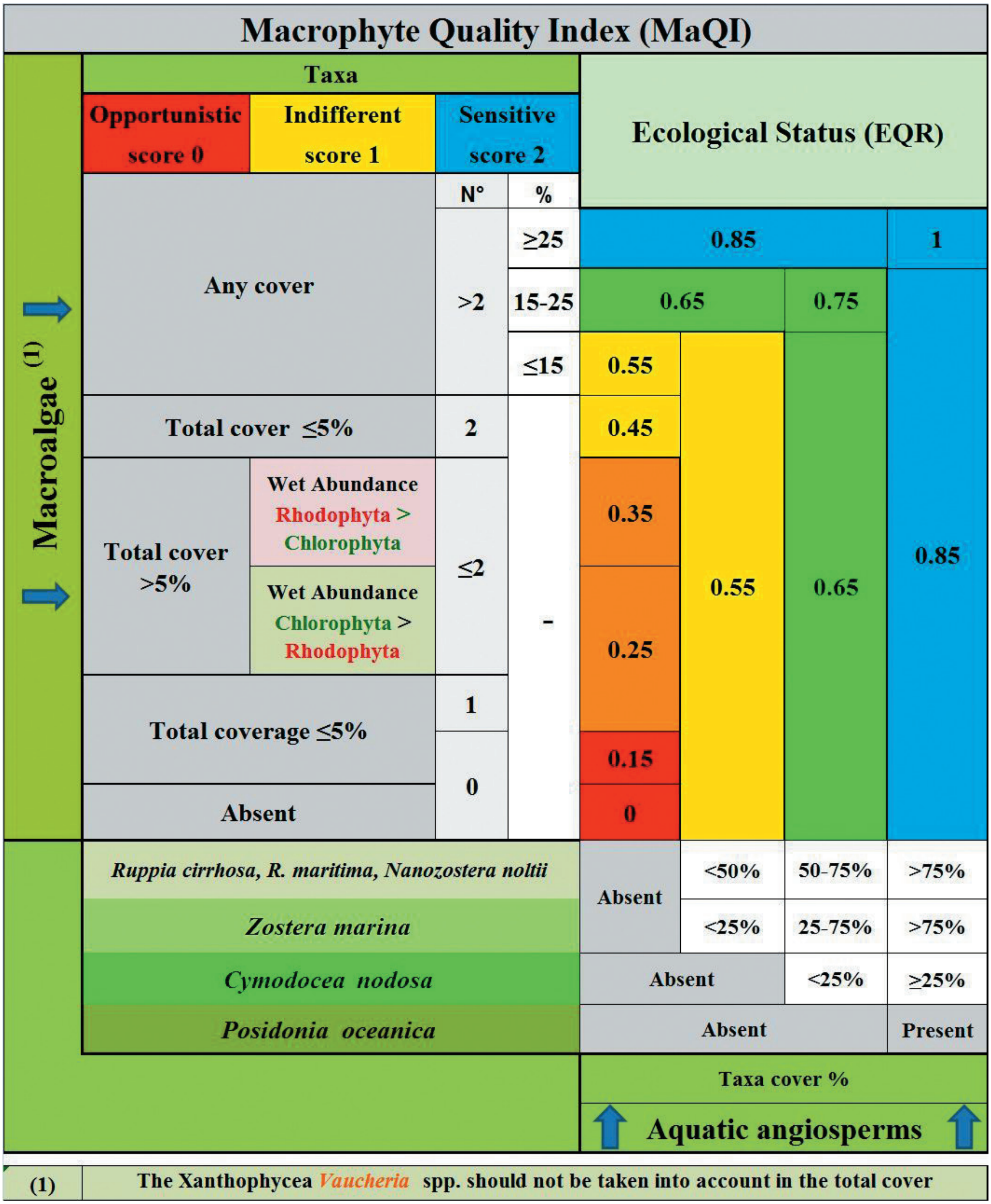

Fig. 2. Macrophyte Quality Index (MaQI) from Sfriso et al. *(2014)*

coefficients ($p<0.05$). The principal component analysis (PCA) has allowed discovering the significant loading between the considered parameters and macrophyte metrics in the variance of the system. Moreover, the plotting of the results of the first two components shows their relationships in the different quadrants. Both analyses were processed using Statistica 10 (StatSoft, Tulsa, U.S.A) software.

RESULTS

Environmental parameters and nutrients concentrations

The lagoons, ranging from 0.5 to 3.0 m, showed a mean±std depth of 1.40±0.62 m and an average transparency of 83% with respect to depth (94% with respect to 1 m), without significant fluctuations comparing the two surveys (Table 1).

Water temperature ranged between 21.0 and 25.2 °C in May and between 18.1 and 19.9 °C in October. Salinity was much lower (19.4±3.81) and variable in May than in October (27.8±2.71). The %DO saturation was much higher in May (241±87%) than in October (87±8%), when all the considered basins presented homogenous under-saturation conditions. Similarly, Chl-*a* was ca. twice as high in May (9.54±6.37 µg dm^{-3}) as in October (5.53±4.27 µg dm^{-3}) with a peak value (27.7 µg dm^{-3}) in the Marinetta lagoon. Dissolved nutrients showed strong seasonal variations for reactive phosphorus (RP) and in a minor extent for DIN with higher values in October. In particular, RP displayed very high values (max: 24.9 µM), never found in any other Italian transitional environment. Surface sediment (Table 1) on average exhibited 62.4±36.8 percent of fine sediments (Fines) and a dry density of 0.89±0.31. Organic carbon and total nitrogen in the 5 cm top layer were high and similar to the highest values recorded in the Venice Lagoon (SFRISO *et al.*, 2003). The concentrations showed the maximum values in May when macroalgae were more abundant.

Macrophytes and ecological assessment

Overall, 74 taxa were recorded (38 Chlorophyta, 30 Rhodophyta and 6 Ochrophyta, Table 2). In May, the number of taxa was 58, in October it decreased to 48. No aquatic angiosperms were found. The total number of taxa collected in the two surveys (Fig. 3a) ranged from 3 at st. 290 (Canarin) to 39 at st. 270 (Barbamarco) near the Pila inlet that connects the lagoon with the northern Adriatic Sea. The mean number

Table 1. Mean, standard deviation (Std), minimum (Min) and maximum (Max) values of the parameters and macrophyte metrics recorded in the two seasons.

	Water column												Surface sediments							Macrophytes					
	Temp	Salinity	DO	depth	Transparency		Chl-a	RP	NH4+	NO2-	NO3-	DIN	Density	Fines	Corg	Cinorg	Ntot	Porg	Pinorg	Cover	Biom	Chlor	Rhod	Ochr	Taxa
	°C		Sat %	cm	normalized to			µM							mg g-1			µg g-1		%	g m fwt	N°			
					1 m	bottom																			
May																									
Mean	22.9	19.4	241	136	95	83	9.54	0.38	6.29	0.45	9.13	15.9	0.89	62.1	3.04	29.4	1.79	110	498	56	2071	5.2	4.1	0.5	9.7
Std	1.30	3.81	52	66	11	19	6.37	0.23	2.58	0.25	6.83	9.7	0.31	36.8	3.07	7.57	1.07	58	83	37	2254	4.1	3.2	0.9	7.5
Min	21.0	14.9	157	50	60	50	1.68	0.15	2.24	0.19	1.67	4.1	0.45	1.79	0.55	15.4	0.13	12	326	0	0	1	1	0	2
Max	25.2	29.2	334	300	100	100	27.7	1.00	14.3	1.06	23.1	38.5	1.38	99.6	11.6	39.8	3.80	193	664	100	8190	16	10	3	28
October																									
Mean	19.3	27.8	87	144	93	80	5.53	6.95	6.10	1.09	22.6	29.8	0.89	62.8	2.64	27.3	1.17	101	452	28	679	5.4	4.6	0.1	10.2
Std	0.47	2.71	8	59	16	19	4.27	9.64	3.04	0.52	10.3	13.9	0.32	37.8	1.58	6.24	0.65	56	66	36	1471	3.6	3.4	0.3	6.7
Min	18.1	21.3	75	50	45	42	0.91	0.05	1.70	0.48	8.22	10.4	0.45	3.4	0.20	15.5	0.21	20	341	0	0	1	1	0	2
Max	19.9	32.2	109	240	100	100	13.2	24.9	12.8	2.15	47.0	61.9	1.47	99.4	5.20	34.7	2.62	221	569	100	4500	12	13	1	25
(Total) May-October																									
Mean	21.1	23.6	164	140	94	82	7.54	3.67	6.20	0.77	15.9	22.8	0.89	62.4	2.84	28.3	1.48	106	475	42	1449	5.3	4.4	0.3	9.9
Std	2.07	5.37	86	62	13	19	5.71	7.50	2.78	0.52	11.0	14.3	0.31	36.8	2.41	6.92	0.93	56	77	39	1997	3.8	3.2	0.7	7.0
Min	18.1	14.9	75	50	45	42	0.91	0.05	1.70	0.19	1.67	4.1	0.45	1.79	0.20	15.4	0.13	12	326	0	0	1	1	0	2
Max	25.2	32.2	334	300	100	100	27.7	24.9	14.3	2.15	47.0	61.9	1.47	99.6	11.6	39.8	3.80	221	664	100	8190	16	13	3	28

Captions: Temp = temperature; DO = dissolved Oxygen; Chl-a = chlorophyll-a; RP = reactive phosphorus; DIN= dissolved inorganic nitrogen; Biom = biomass; Chlor = Chlorophyta; Rhod = Rhodophyta; Ochr = Ochrophyta; Sat = saturation; fwt = fresh weight; N° = number; std = standard deviation; Min = minimum value; Max = maximum value.

of taxa per stations was 10 both in May and October; the integration of the records of May and October was 17 (Table 2). On average, the number of Chlorophyta exceeded that of Rhodophyta whereas the presence of Ochrophyta was negligible showing a maximum of 6 taxa in May. Out of them some small thalli of Sargassum *muticum* (Yendo) Fensholt were only found at the Pila inlet attached to oyster shells. Except for the perennial holdfast of *Sargassum*, all the others Ochrophyta had disappeared in the warm season because of their life cycle and in October were missing. In addition to Ulvaceae, the most abundant taxa were some opportunistic non-native species: *Solieria filiformis* (Kützing) P. W. Gabrielson, *Agardhiella subulata* (C. Agardh) Kraft *et* M. J. Wynne and *Gracilaria vermiculophylla* (Ohmi) Papenfuss that was identified for the first time in the Mediterranean Sea by molecular analyses just in those lagoons. *G. vermiculophylla* was found almost everywhere in the Po Delta lagoons and, in spring 2008, it was also recorded in some confined areas of the Venice Lagoon where the ecological conditions are low (SFRISO *et al.,* 2012a). Small epiphytic sensitive taxa reported in the list for MaQI application (SFRISO *et al.*, 2007, 2009) were only occasionally recorded at Caleri: *Centroceras clavulatum* (C. Agardh) Montagne, *Gayliella flaccida* (Harvey *ex* Kutzing) T. O. Cho *et* L. McIvor, *Pneophyllum fragile* Kützing and *Sphacelaria cirrosa* (Roth) C. Agardh. All the other macroalgae were opportunistic or indifferent species.

In Table 2 the full taxonomic list of the recorded species is reported together with their score and MaQI assessment. The complete absence of aquatic angiosperms in all the water bodies and the almost complete absence of sensitive species indicate that the ecological conditions of the entire study area were severely compromises. Two sites (one in Marinetta lagoon and the other in Sacca Scardovari) displayed a negligible biomass cover (<5%) of opportunistic taxa (EQR: 0.15 = Bad), 9 stations were dominated by free-floating Ulvaceae (EQR: 0.25 = Poor), the remaining sites were characterized by free-floating Gracilariaceae and/or Solieriaceae (EQR: 0.35 = Poor, Table 2).

The mean biomass was more than three times as high in May (2071±2254 g fwt m^{-2}) as in October (679±1471 g fwt m^{-2}) with an *Ulva* peak (8190 fwt m^{-2}) at st. 330 (Scardovari), whereas some sites were completely deprived of biomass during one or both the sampling periods (Fig. 3b). In addition, st. 330 that in May showed the highest biomass, in October was almost depleted of macroalgae. Other areas such as sts. 430 and 440 were covered by dense populations of *S. filiformis* or Gracilariaceae. Similarly, macrophyte cover was twice as high in May (56±37%) as in October (28±36%) with a high range of variability within the stations (Fig. 3c).

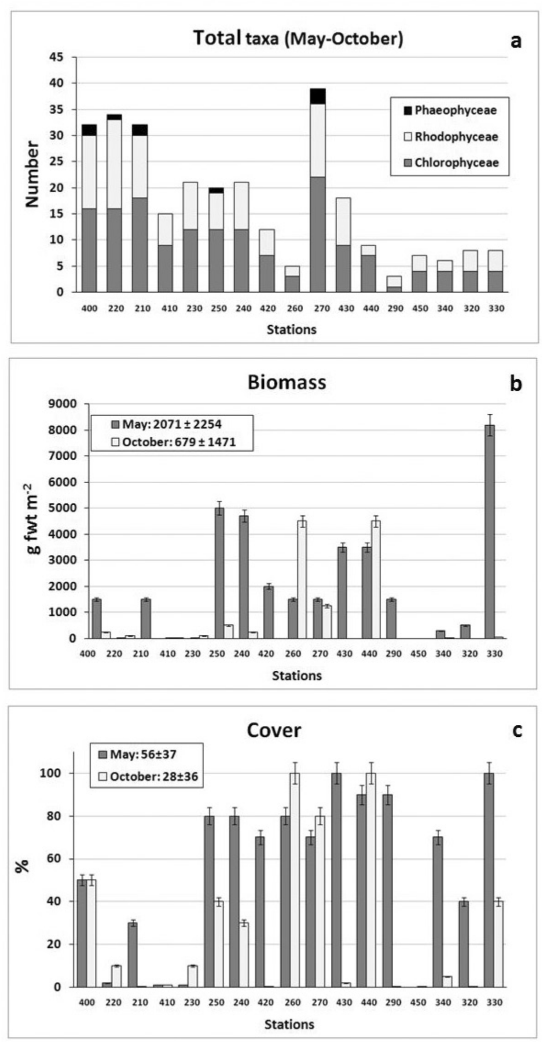

Fig. 3. Number of total taxa: a) total biomass, b) and total cover, c) of the macrophytes recovered in the study areas (mean ± std)

Table 2 - Total check-list of the macroalgae sampled in May and October 2008 in the Po Delta lagoons and ecological status assessment by applying the index MaQI. The numbers: 0, 1 and 2 are the scores of each taxon according to Sfriso et al. (2009)

				Caleri			Marinetta		Vallona		Barbamarco			Canarin			Scardovari			
				Station																
				400	220	210	410	230	250	240	420	260	270	430	440	290	450	340	320	330
		CHLOROPHYCEAE	Taxon Score																	
1	1	*Blidingia minima* (Nägeli ex Kützing) Kylin	0	0	0	0	0	0		0	0		0	0	0				0	
2	2	*Blidingia marginata* (J. Agardh) P. J. L. Dangeard ex Bliding	0			0	0	0												
3	3	*Blidingia ramifera* (Bliding) Garbary et Barkhouse	0		0			0					0							
4	4	*Blidingia subsalsa* (Kjellman) Kornmann et Sahling ex Scagel et al.	0	0							0									
5	5	*Bryopsis cupressina* J. V. Lamouroux var. adriatica J. Agardh	0					0					0							
6	6	*Bryopsis hypnoides* J. V. Lamouroux	1	1	1	1				1			1							
7	7	*Bryopsis plumosa* (Hudson) C. Agardh	1										1							
8	8	*Chaetomorpha aerea* (Dillwyn) Kützing	0	0	0	0	0		0	0	0									
9	9	*Chaetomorpha ligustica* (Kützing) Kützing	0		0	0		0	0			0								
10	10	*Cladophora aegagropila* (Linnaeus) Rabenhorst	0			0		0	0											
11	11	*Cladophora albida* (Nees) Kützing	1			1							1				1	1		
12	12	*Cladophora laetevirens* (Dillwyn) Kützing	0		0	0	0						0							
13	13	*Cladophora sericea* (Hudson) Kützing	0							0										
14	14	*Cladophora vagabunda* (Linnaeus) C. Hoek	0										0							
15	15	*Codium fragile* (Suringar) Hariot subsp. tomentosoides (Goor) P. C. Silva	1	1																
16	16	*Gayralia oxysperma* (Kützing) K. L. Vinogradova ex Scagel et al.	0	0		0		0		0										
17	17	*Neostromatella monostromatica* M.J.Wynne, G.Furnari & R.Nielsen	0							0										
18	18	*Rhizoclonium tortuosum* (Dillwyn) Kützing	0										0							
19	19	*Ulothrix flacca* (Dilllwyn) Thuret	0	0	0	0		0	0			0	0				0			
20	20	*Ulothrix implexa* (Kützing) Kützing	0		0	0							0	0			0			0
21	21	*Ulva clathrata* (Roth) C. Agardh	0					0					0				0			
22	22	*Ulva compressa* Linnaeus	0	0	0	0	0	0	0	0			0	0	0			0		
23	23	*Ulva curvata* (Kützing) De Toni	0	0				0						0	0					

24	24	*Ulva fasciata* Delile	0			0														
25	25	*Ulva flexuosa* Wulfen	0			0				0			0	0	0					
26	26	*Ulva flexuosa* Wulfen subsp. pilifera (Kützing) Wynne	0										0							0
27	27	*Ulva intestinalis* Linnaeus	0	0			0		0		0		0	0						
28	28	*Ulva laetevirens* Areschoug	0		0	0		0	0	0			0							0
29	29	*Ulva linza* Linnaeus	0						0											
30	30	*Ulva multiramosa* Taskin	0	0	0				0				0					0	0	
31	31	*Ulva prolifera* O. F. Müller	0		0	0	0		0		0		0	0	0					
32	32	*Ulva prolifera* O. F. Müller subsp. gullmariensis (Bliding) Sfriso & Curiel	0	0						0	0									
33	33	*Ulva rigida* C. Agardh	0	0	0		0		0	0	0	0	0	0	0	0		0	0	0
34	34	*Ulva rotundata* Bliding	0	0	0	0							0	0	0					
35	35	*Ulvella leptochaete* (Huber) R.Nielsen, C.J.O'Kelly & B.Wysor	0		0															
36	36	*Ulvella scutata* (Reinke) R.Nielsen, C.J.O'Kelly & B.Wysor	0	0																
37	37	*Ulvella viridis* (Reinke) R.Nielsen, C.J.O'Kelly & B.Wysor	0	0		0	0		0	0			0						0	
38	38	*Urospora penicilliformis* (Roth) J. E. Areschoug	0		0															
	38	Total		16	16	18	9	12	12	12	7	3	22	9	7	1	4	4	4	4
		RHODOPHYCEAE																		
39	1	*Acrochaetium savianum* (Meneghini) Nägeli	1	1	1		1	1						1					1	
40	2	*Agardhiella subulata* (C. Agardh) Kraft et M. J. Wynne	1	1	1	1			1	1	1	1		1		1	1	1		1
41	3	*Aglaothamnion tenuissimum* (Bonnemaison) Feldmann-Mazoyer	1	1	1	1			1	1			1						1	
42	4	*Audouinella sp*.	1										1							
43	5	*Bangia fuscopurpurea* (Dillwyn) Lyngbye	1		1															
44	6	*Callithamnion corymbosum* (J. E. Smith) Lyngbye	1		1	1											1			
45	7	*Caulacanthus ustulatus* (Turner) Kützing	1	1		1		1		1										
46	8	*Centroceras clavulatum* (C. Agardh) Montagne	2	2																
47	9	*Ceramium siliquosum* (Kützing) Maggs et Hommersand var. siliquosum	1				1													
48	10	*Ceramium virgatum* Roth	1	1	1								1							
49	11	*Erythropeltis discigera* (Berthold) F. Schmitz	1		1		1													
50	12	*Erythrotrichia bertholdii* Batters	1		1															
51	13	*Gayliella flaccida* (Harvey ex Kützing) T.O. Cho et L. McIvor	2			2														
52	14	*Erythrotrichia carnea* (Dillwyn) J. Agardh	1	1	1	1		1		1			1	1						
53	15	*Gracilaria bursa-pastoris* (S.G. Gmelin) P.C. Silva	1	1							1									
54	16	*Gracilaria gracilis* (Stackhouse) Steentoft et al.	0	0	0	0		0	0		0		0	0	0					

55	17	*Gracilaria vermiculophylla* (Ohmi) Papenfuss	0				0	0	0	0	0		0	0						0
56	18	*Gracilariopsis longissima* (S. G. Gmelin) Steentoft et al.	0	0	0		0	0	0	0			0	0	0	0		0	0	0
57	19	*Gymnogongrus griffithsiae* (Turner) Martius	0	0																
58	20	*Neosiphonia elongella* (Harvey) M.S. Kim et I.K. Lee	0		0		0						0							0
59	21	*Pneophyllum fragile* Kützing	2		2															
60	22	*Polysiphonia arachnoidea* (C. Agardh) Zanardini	1			1														
61	23	*Polysiphonia denudata* (Dillwyn) Greville ex Harvey	1		1	1		1	1	1			1							
62	24	*Polysiphonia sanguinea* (C. Agardh) Zanardini	0			0		0												
63	25	*Polysiphonia cfr. stricta* (Dillwyn) Greville	0	0		0				0			0							
64	26	*Polysiphonia sertularioides* (Grateloup) J. Agardh	0	0	0	0			0	0	0		0	0						
65	27	*Pyropia olivii* (Orfanidis, Neefus et T.L. Bray) J. Brodie et Neefus	1					1					1							
66	28	*Sahlingia subintegra* (Rosenvinge) Kornmann	1		1															
67	29	*Solieria filiformis* (Kützing) P. W. Gabrielson	0	0	0							0	0	0			0		0	
68	30	*Stylonema alsidii* (Zanardini) K. M. Drew	1										1	1						
	30	Total		14	17	12	6	9	7	9	5	2	14	9	2	2	3	2	4	4
		PHAEOPHYCEAE																		
69	1	*Dictyota dichotoma* (Hudson) J. V. Lamoroux var. intricata (C. Agardh) Greville	1		1															
70	2	*Elachista sp*.	1										1							
71	3	*Hincksia mitchelliae* (Harvey) P. C. Silva	0	0		0			0											
72	4	*Myrionema sp*.	1										1							
73	5	*Sargassum muticum* (Yendo) Fensholt	1	1									1							
74	6	*Sphacelaria cirrosa* (Roth) C. Agardh	2			2														
	6	Total		2	1	2	0	0	1	0	0	0	3	0	0	0	0	0	0	0
TOTAL TAXA			74	32	34	32	15	21	20	21	12	5	39	18	9	3	7	6	8	8
				Total Biomass (g fwt m-2)																
				2500	10	1500	10	1500	5000	4695	2000	1500	1500	3500	3500	1500	1	300	500	8190
				% Total cover																
				50	6	15	1	5.5	60	55	35	90	75	51	95	45	0.01	38	20	70
				% Abundance of the main taxa																
Chlorophyceae				65	30	35	45	40	68	53	25	+	38	45	73	35	+	35	35	94
Rhodophyceae				29	58	13	53	55	31	42	25	97	56	60	28	15	1	63	15	6
others				4	13	3	3	5	2	4	+	+	5	+	+	+	+	1	+	+
number of sensitive taxa (score 2)				1	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MaQI score				0.25	0.35	0.25	0.15	0.35	0.25	0.25	0.25	0.35	0.35	0.35	0.25	0.25	0.15	0.35	0.25	0.25
ASSESSMENT				P	P	P	B	P	P	P	P	P	P	P	P	P	B	P	P	P

Legenda: 0 = opportunistic taxa, 1 = indifferent taxa, 2 = sensitive taxa, P = Poor, B = Bad, + = cover < 1%.

Statistical analyses

The non-parametric Spearman's coefficients (Tale 3) between the recorded environmental parameters and the macrophyte metrics showed some significant ($p<0.05$) correlations. Aside from the obvious correlations with temperature, macroalgal biomass and cover macrophytes showed significant correlations with water transparency and %DO saturation and an inverse correlation with salinity. Dissolved nutrients, due to their pulsing concentrations and the analysis of only two seasonal surveys do not show significant correlations with macrophytes, except for nitrites that were inversely correlated with macroalgal cover and biomass. On the contrary, macrophytes exhibited significant correlations with total nitrogen, organic phosphorus and inorganic phosphorus in surface sediment, showing the key role of these nutrients in governing the eutrophication processes of these lagoons. In particular, the total number of taxa, the number of Rhodophyta and the number of Chlorophyta were inversely correlated with total nitrogen and organic phosphorus whose accumulation in surface sediments was favored by the bloom of single species (*Ulva rigida*, *Gracilaria vermiculophylla* or *Solieria filiformis*). In contrast, a positive correlation was recorded between the inorganic phosphorus, the number of Chlorophyta, the number of Ochrophyta, the macroalgal biomass and MaQI.

PCA extraction (Fig. 4) summarizes the loading of the single environmental parameters and macrophyte variables in the total variance of the system. Three factors explained 52% of the total variance. The 1st factor (23.1%) displayed the significant loading of some environmental parameters: temperature, dissolved oxygen, inorganic phosphorus and salinity. The 2nd one (16.5%) showed the significant contribution of three macrophyte variables: the total number of taxa, the number of Chlorophyta and the number of Rhodophyta. Finally, the 3rd factor (12.4%) highlighted the loading of fines. The plotting of factors 1 and 2 on a plane showed that the

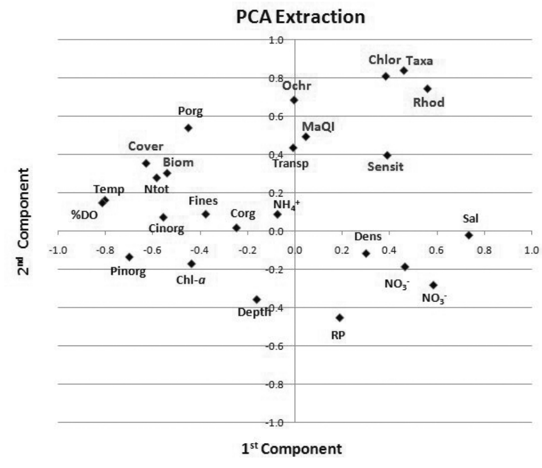

Fig. 4. Principal Component Analysis. Captions: Temp. Temperature; DO = dissolved oxygen; Sal = salinity; Transp = water transparency; Dens = sediment density; Porg = organic phosphorus; Pinorg = inorganic phosphorus; Ntot = total nitrogen; Corg = organic carbon; Cinorg = inorganic carbon; Biom = biomass; Chlor = Chlorophyta; Rhod = Rhodophyta; Ochr = Ochrophyta

Table 3. Non-parametric Spearman's coefficients between some environmental parameters and macrophyte variables. The parameters showing significant correlations were only reported. In bold significant values: p<0.05 per r> |35|

	Water Column							Surface Sediment		
	Temp	Salinity	%DO	Depth	Secchi disc	Chl-a	NO2-	Ptot	Porg	Pinorg
N° Taxa	-0.22	0.32	-0.29	**-0.36**	0.28	**-0.37**	0.16	**-0.35**	0.30	**-0.46**
N° Chlorophyta	-0.21	0.29	-0.25	-0.32	0.24	-0.31	0.16	-0.24	0.35	**-0.36**
N° Rhodophyta	-0.26	0.32	-0.31	**-0.35**	0.29	**-0.40**	0.14	**-0.46**	0.21	**-0.55**
N° Ochrophyta	0.02	0.10	0.02	0.00	0.15	-0.21	-0.16	-0.02	**0.42**	-0.05
Cover	**0.40**	-0.29	**0.45**	-0.06	**0.42**	-0.00	**-0.39**	-0.09	0.33	0.17
Biomass	**0.45**	**-0.45**	**0.54**	-0.16	**0.51**	-0.02	**-0.39**	-0.06	**0.44**	0.15
MaQI	0.00	0.16	-0.07	-0.01	0.04	-0.02	-0.09	-0.26	**0.37**	-0.12

Captions: Temp = temperature; DO = Dissolved Oxygen; Chl-a = Chlorophyll-a; N° = number; Cinorg = Inorganic Carbon; Ptot = Total Phosphorus; Porg = Organic Phosphorus; Pinorg = Inorganic Phosphorus number of taxa, the number of Rhodophyta and the number of Chlorophyta are placed in a quadrant in opposition to that of macrophyte cover and macrophyte biomass and the great part of the environmental parameters, especially those of surface sediments. MaQI, that in environments where all the 5 ecological classes are present (SFRISO *et al.,* 2012b, 2014) is plotted at the edge of the quadrant displaying the best environmental conditions, in these basins of poor quality, affected by the effluents of the Po Delta Valley, has a loading of little significance.

DISCUSSION

Macrophytes and environmental parameters

The Po River, driving the salinity changes and the inputs of suspended sediments, nutrients and pollutants drained from the Po Valley, strongly affects the ecological conditions of these deltaic environments. Despite the high number of basins which are present in the Delta, macrophytes showed high homogeneity, with the dominance of a low number of free-floating opportunistic taxa, such as Ulvaceae, Gracilariaceae and Solieriaceae, well adapted to the salinity variation (range: 14.9-32.2 psu). The high phosphorus and nitrogen availability, especially in the surface sediments, favored a luxuriant growth that in late spring can cause dystrophic crises, loss of biomass and death of benthic organisms and fish. As a consequence, the biodiversity and the ecological value of the sampled macrophytes was very poor and no aquatic angiosperms were recorded, even though fishermen remember that in the past *Ruppia cirrhosa* (Petagna) Grande was present, as it was reported with reference to Sacca di Goro (PELLIZZARI *et al.*, 2009), the southern lagoon of the Po Delta in the Emilia-Romagna Region, which was not investigated during these surveys.

Salinity has a key role in decreasing the number of taxa (WALLENTINUS, 1991; FLEISCHER & ZETTLER, 2009; SCHUBERT *et al.*, 2011; JANOUSEK & FOLGER, 2012) that starts to decline in mesohaline environments and is minimum in oligohaline conditions, but has not influence on their ecological value. At low salinities, in the presence of pristine-oligotrophic (undisturbed) conditions, the number of taxa is very low but macrophytes are represented by aquatic angiosperms or macroalgae of high ecological value (SFRISO *et al.,* 2012b; ORFANIDIS *et al.*, 2003). In contrast, in the Po Delta basins, with the exception of small specimens of only four sensitive taxa (*C. clavulatum*, *G. flaccida*, *P. fragile* and *S. cirrosa*) recorded at sts. 210, 220 and 240 in the lagoon of Caleri, which is the basin less affected by the Po river inputs, the dominant taxa were laminar and filamentous Ulvaceae and some cylindrical-filamentous or slightly compressed allochthonous Rhodophyta such as *S. filiformis*, *A. subulata* and *G. vermiculophylla.* The latter species is native from Japan and Korea and was firstly recorded in the Po Delta lagoons in 2008 (SFRISO *et al.*, 2010) during these surveys. In the northern Europe, this species has colonized eutrophic and turbid environments showing high tolerance of salinity variation, turbidity, sedimentation, desiccation, grazing and nutrient enrichment (THOMSEN & MCGLATHERY, 2007; WEINBERGER *et al.*, 2008; NEJRUP *et al.*, 2012). It is a stress-tolerant species that can even replace *Ulva* due to its high resistance to high water temperatures and grazing pressures. These traits explain its invasive success in transitional environments and estuaries, and also its ability to replace Ulvaceae, although the latter exhibits higher growth rates. In fact, the grazing pressure of crustaceans and gastropods that affect Ulvaceae (SFRISO, 1995; BALDUCCI *et al.*, 2001), reducing their biomass during the warm season and overcoming the species growth rates, is not present in this species (NEJRUP *et al.*, 2012). Grazers usually prefer native species with low structural complexity and higher nitrogen content such as Ulvaceae (GEERTZ-HANSEN *et al.*, 1993; CEBRIÁN & DUARTE, 1994). *Gracilaria vermiculophylla* has also a high resistance to temperature and sedimentation rates (THOMSEN & MCGLATHERY, 2007) growing even in conditions prohibitive for *Ulva* which, at high temperatures (>28-30 °C), rapidly collapses. *Agardhiella subulata* that in Venice Lagoon coexists in the same environment has very similar ecological characteristics. However, the last species lives attached to the bottom and is not able to grow free-floating. In spring 2014, *G. vermiculophylla* and *Agardhiella subulata* have replaced Ulvaceae in the area at the north of the translagoon bridge (Ponte della Libertà) where the abundant May-June biomass production triggered a complete collapse in July 2013 (BASTIANINI *et al.*, 2013). A rapid increase in temperature above 30 °C caused a dystrophic crisis with the complete degradation of ca. 10,000 tons (fresh biomass) of macroalgae in a few days and the death of fish and benthic fauna, causing the concern of the Venice people. In early June 2014, water temperature increased up to 32.5 °C but *G. vermiculophylla* and *A. subulata* have not suffered from these extreme temperatures (personal communication).

By analyzing the data collected, it was observed that the Po River, draining the whole Po Valley conveys high nutrient loads and suspended sediments and favors the presence and the dominance of opportunistic species. In addition, the intense fishing activities to catch the Manila clam *Ruditapes philippinarum* Adams & Reeve occurring in many of these areas, destroy the natural sediment texture and resuspend high amounts of fine sediments which dramatically reduce light availability favoring cylindrical and filamentous thalli as observed in Venice Lagoon (SFRISO *et al.*, 2005a,b). Where hydrodynamics is low, a layer of sediment covers macroalgae and reduces or hampers their growth. Also, the laminar thalli of *Ulva,* which usually dominate in eutrophic environments, in the presence of high water turbidity and a depth higher than 1-1.5 m are replaced by cylindrical-filamentous to broadly flattened macroalgae such as *Gracilaria*, *Solieria* and *Agardhiella* or are almost deprived of vegetation. Among these opportunistic species, particularly abundant was the invasive *G. vermiculophylla,* a species that in the northern Europe (THOMSEN *et al.*, 2007; THOMSEN & MCGLATHERY, 2007) and in Venice Lagoon has colonized only eutrophic and confined areas (SFRISO *et al.*, 2012a).

Ecological status assessment

The requirement of the Italian Regions to implement the WFD (2000/60/EC) to TW environments was an opportunity to study the flora and vegetation of these deltaic transitional water systems that are governed by the Po river outflow. The degraded ecological conditions of these environments are evident even without the application of indices of ecological status. MaQI validated in a set of stations of some Italian lagoons, ranging all the 5 ecological classes, from "Bad" to "High" (SFRISO *et al.*, 2009), and calibrated with similar situations of Greek and French transitional basins (ORFANIDIS *et al.*, 2012; EUROPEAN COMMISSION, 2010, 2013) confirmed the low quality of these systems by assessing all the Po Delta lagoons in "Poor/Bad" conditions (Table 2). Almost all the sampling sites, characterized by high macroalgal biomass and cover, especially in late spring, were assessed as "Poor". The other ones, where macroalgae were not able to grow (cover <5% in both sampling seasons and in the presence of only opportunistic taxa), received the "Bad" status: st. 410 (Marinetta), placed close to the Po di Levante outflow, and st. 450 (Scardovari) located in the innermost part of the lagoon where even the species belonging to the genus *Blidingia* Kylin or *Vaucheria* A.P. de Candolle, which usually colonize the most degraded environments (SFRISO *et al.*, 2007, 2009), were completely missing, suggesting the presence of some toxicants. Data to prove the presence of substances toxic to macrophytes are not available but the mass media constantly report discharges of large quantities of Atrazine or other herbicides prohibited by law just in the major cities of the Po Valley.

In these environments is interesting to note that MaQI shows a positive correlation with the macroalgal biomass and cover (Table 3, Fig. 4). It is because all the stations were only assessed as "Poor" or "Bad" and by applying the MaQI (Fig. 2) we can infer the worst conditions in the presence of a negligible or null cover in contrast to the "Poor" conditions where macroalgae are abundant and able to bloom. In the environments where all the 5 quality classes are present MaQI lies on the opposite side of the macroalgal cover and biomass (SFRISO *et al.*, 2012b). This negative classification is in agreement with the "Poor–Bad" assessment recorded in many meso-polyhaline choked areas of the Venice Lagoon. However, meso-polyhaline choked areas, showing high ecological conditions, are also present both in the Venice Lagoon (SFRISO *et al.*, 2012b) and in the lagoon of Lesina (SFRISO *et al.*, 2007, 2009). In these environments the dominant macrophytes are aquatic angiosperms and some macrophytes of high ecological value such as *Lamprothamnium papulosum* (K.Wallroth) J. Groves, *Polysiphonia spinosa* (C. Agardh) J. Agardh, *Valonia aegagropila* C. Agardh and many small calcareous epiphytes such as *Hydrolithon boreale* (Foslie) Y.M. Chamberlain, *Hydrolithon cruciatum* (Bressan) Y.M. Chamberlain and *Pneophyllum fragile* Kützing.

Unfortunately, the lagoons of the Po Delta, as the great part of the choked areas of the Venice Lagoon, are affected mainly by two anthropogenic pressures: the high nutrient and pollutant inputs and the high water turbidity. Nutrient concentrations are continuously pulsing with the change of the river outflows and/or remain permanently trapped in these basins whereas water turbidity is due both to the erosion of the riverbanks, always deprived of vegetation, and the destruction of the bottom by the fishing of the Manila clams. In addition, many anthropogenic interventions to dig canals or to rebuild salt marshes and the lagoon embankments contribute greatly to the destruction of natural vegetation and further highlight the sediment resuspension that hampers and prevents both the presence and the growth of macrophytes, especially the aquatic angiosperms and the sensitive macroalgae.

CONCLUSIONS

The study on the Po Delta can be considered an achievement to gain knowledge on the flora and vegetation of the Italian transitional systems and their ecological status since it fills the lack of information on a system of lagoons and ponds among the largest in the Mediterranean Sea. A complete checklist of the species recorded in spring and autumn in 17 sampling sites spread over the whole Po Delta has highlighted the presence and dominance of thionitrophilous and opportunistic species, mostly Ulvaceae, Gracilariaceae and Soleriaceae, which adapt well to the eutrophic and turbid conditions of those basins. In particular, *Gracilaria vermiculophylla*, *Solieria filiformis* and *Agardhiella subulata,* three allochthonous species introduced in the Mediterranean sea only recently (ZENETOS *et al.*, 2010), have found in these transitional areas of the Po Delta the most suitable conditions for their growth, favored by high nutrient availability and water turbidity which enhance their spreading and reduce the growth of the Ulvaceae which prefer eutrophic but less turbid environments.

The dominance of these opportunistic taxa, their cover and the application of the MaQI show that the surveyed lagoons and ponds have ecological conditions severely compromised. All stations have been classified “Poor-Bad” enhancing the necessity of a policy of interventions to improve conditions and achieve the “Good” ecological status as requested by the WFD (2000/60/EC).

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Makrofiti i procjena ekološkog stanja u prijelaznim vodama delte rijeke Po, Jadransko more (Italija). Primjena Indeksa kvalitete makrofita (MaQI)

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SAŽETAK

U radu se iznose prvi popis i informacije o makrofitima u delti rijeke Po (Italija) koja se sastoji od brojnih malih laguna i ribnjaka i koja je jedna od najvećih u Sredozemnom moru, koja je do sada slabo istraživana unatoč činjenici što obuhvaća površinu vode od cca. 200 km². Istraživan je ekološki status, brojnost i skupni sastav makrofita koji naseljavaju ovo okruženje uzimajući u obzir najuobičajenije fizikalno-kemijske parametre i koncentraciju nutrijenata u vodenom stupcu i površinskom sloju sedimenta.

Tijekom dva ispitivanja obavljena između 13. i 15. svibnja i 13. i 15. listopada 2008. provedeno je uzorkovanje na 17 mjesta raspoređenih u lagunama Marinetta, Vallona, Caleri, Canarin, Barbamarco i Scardovari. Ekološko stanje procijenjeno je primjenom Indeksa kvalitete makrofita (MaQI) kojeg je usvojilo Ministarstvo zaštite okoliša za ekološku klasifikaciju talijanskih prijelaznih područja u skladu sa zahtjevima Okvirne direktive o vodama (2000/60/EC).

Ključne riječi: alge, popis, indikatorske vrste, parametri, lagune delte rijeke Po, sjeverozapadni Jadran