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Environmental heterogeneity patterns and assessment of trophic levels in two Mediterranean lagoons: Orbetello and Varano, Italy

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ABSTRACT

The management of coastal lagoons is of particular interest due to their high economical importance. In spite of their great productivity, coastal lagoons are often impacted by human pressure which produces water eutrophication. The aim of this paper is to assess the trophic state of the two Mediterranean lagoons taking into account chemical–physical parameters, nutrient concentrations and biological parameters. Two Italian lagoons, Orbetello and Varano (respectively located in Tyrrhenian and Adriatic coast, Italy) were studied between May 2003 and April 2005. Both these systems receive treated urban outflows, agricultural effluents and rivers freshwater inputs. Field collected data showed that studied lagoons were characterized by different human and natural pressures. Orbetello showed the highest water eutrophication, highlighted by the trophic index values, while Varano showed lower eutrophication levels except for the summertime. The values of physical, chemical and biological parameters measured in Orbetello and Varano lagoons indicate that a wide spatial and seasonal gradient of the water characteristics was established during the study period, but in particular in winter. This gradient, typical of estuarine systems, was essentially due to the mixing of freshwater, seawater and anthropogenic inputs. Orbetello lagoon seemed much more affected by the urban impact and the fish-farming activities than Varano lagoon, but the latter showed a greater agriculture activities impact as showed by the remote sensing images.

1. Introduction

Coastal lagoons, located geographically between sea and land, are the youngest and the most complex ecosystems of all the coastal environment. Lagoon systems take up 13% of the world coasts (Carrada, 1990) and, in Italy, there are about 150,000ha of coastal lagoons, whose half part is used as nursery areas and for aquaculture and fisheries exploitations (Cataudella et al., 1995). The importance of such a system for fisheries and aquaculture at global level, and for Italy in particular, directs research towards the study of the relationships between abiotic environmental factors and the biological productivity. The ecological characteristics of all organisms living in coastal lagoon are related to environmental stress due to the alternating inputs of marine and fresh waters, in addition to the increased soluble nutrients due to human activities that impacted these systems (Nixon, 1982; Sfriso et al., 1992). Coastal lagoons are commonly

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characterized by high biological productivity due to their restricted water exchange, shallow depths and small water volume. Due to small water volume, these ecosystems present lower chemical and physical inertia comparing to near seawaters. The strong dependence from surrounding watershed makes them vulnerable to human impact and freshwater inputs (Carrada, 1990; Perez-Ruzafa et al., 2005), to nutrient inputs particularly (Taylor et al., 1995). In contrast to the situation in the open sea, in many coastal lagoons or closed coastal zones major perturbations of the trophic cycle seem to be caused by the excessive production of organic matter stimulated by nutrient inputs (EEA, 1999; Nixon, 1995).

Numerous studies have been focused on understanding spatial and temporal patterns of all parameters closely linked to trophic condition and water quality. Various classification criteria have been considered in order to assess the trophic condition and water quality of coastal ecosystems and freshwater lakes through the use of specific indexes based on environmental factors (Carlson, 1977; Vollenweider, 1976; Karydis et al., 1983; Herrera-Silveira et al., 2002; Gikas et al., 2006; Coelho et al., 2007).

As many other coastal environments (Morand and Briand, 1996), Orbetello lagoon is characterized by a high biological productivity based on intensive fish-farming plants which discharge their effluents into the lagoon water. Over time it has developed a considerable seaweed (macroalgae) proliferation (Lenzi, 1992; Bombelli and Lenzi, 1996). The phenomenon has human origins (urban, aquaculture and agriculture wastewater), enhanced from a developed tourist trade (Lenzi, 1992). The increase in eutrophication has gradually led to a qualitative and quantitative change from seagrasses (phanerogams) to macroalgae. Various species of opportunistic macroalgae have dominated (Lenzi and Mattei, 1998; Lenzi et al., 1998); macroalgal blooms began to appear in the middle of 1960s and have been periodically accompanied by microalgal blooms (Tolomio and Lenzi, 1996). The algal masses, with an uninterrupted production throughout the year, are moved by the winds and accumulated at high densities (sometimes exceeding 20kg m^{-2}; Lenzi, unpublished data). During the cold season, the seaweed biomasses decomposition and subsequent sulphate reduction processes caused a drastic dissolved oxygen decrease and the development of toxic reducing gases, which has led to mortalities of aquatic fauna (Izzo and Hull, 1991). These in turn led to a reduction in the quantity and quality of the fish caught from the lagoon in the 1980s (Lenzi, 1992), and the discoloured water outflow to the adjacent beach areas, causing economical damages.

The coastal lagoon of Varano, located on the South-Western Adriatic coast, is an ecosystem with a long tradition in aquaculture practice. Inside it mussels culture has been introducing since late '60s with an annual production of 6000–8000t of *Mytilus galloprovincialis* (Breber and Scirocco, 1998). It shows no strong anthropogenic pressure, although within its watershed, agricultural practices are evolving into intensive irrigated crops, with the result of increasing nutrients reaching the lagoon (Eltcon — Technical Reports, 1995, 1997, 1998; Villani et al., 2000). For a period of years dark red dinoflagellate blooms appeared during the summer causing general mortality in the fauna and forcing the mussel farmers to remove their stocks. In the lagoon *Dinophysis sacculus* (potential producer of diarrhoeic shellfish poisoning — DSP) have been recurrent phytoplankters (Tolomio et al., 1990; Caroppo, 2000) without any known cases of DSP from shellfish collected in the area. The seasonal abundance of this dinoflagellate, the interesting morphological variety of this species and the relationship to environmental factors were studied, but *D. sacculus* presence and proliferation were not associated with biotoxicity (Caroppo, 2001).

Despite its ecological, biological and economic importance, there is a lack of knowledge of the detailed spatial and temporal variability of environmental factors about the lagoon of Varano.

The aim of this paper is to assess the trophic state of the two Mediterranean lagoons taking into account chemical–physical parameters, nutrient concentrations and biological parameters. We studied two Italian coastal lagoons (Orbetello lagoon in the North-Western Italy and Varano lagoon in the South-Eastern Italy) used for aquaculture exploitations. Spatial and temporal distributions of environmental parameters were examined and trophic indexes were determined in order to define the trophic assessment of these lagoons and a multivariate approach was used in order to evaluate the different behaviour between the two systems. In addition, the remote sensing analysis of the surrounding areas (Landsat 7 ETM) was applied. Remote sensing analysis was useful to understand the impact of agricultural area and urban activity on the study areas.

2. Methods

2.1. Study areas

2.1.1. Orbetello lagoon

Orbetello lagoon (Fig. 1) is located in the Southern Tuscany (Italian West Coast), between 42 25 and 42 29 lat. North and between 11 10 and 11 17 long. East, and covers a total surface of 25.25 km^2. This lagoon is divided by a dam in two communicating basins known as Western (Ponente) and Eastern (Levante) with an area of 15.25 and 10.00 km^2 respectively (Travaglia and Lorenzini, 1985). Only three communicating channels (Nassa, Fibbia and Ansedonia) make possible lagoon–seawater exchanges. The average depth is about 1 m (range of 0.30–1.70 m) with maximum reported for the lagoon centre in both basins. The geomorphology of Orbetello lagoon and the presence of the dam reduce water circulation. To face the anoxia phenomena, during the summertime, seawater are pumped throughout the Nassa and Fibbia communicating channels into the Ponente basin and are forced to flow through the Levante basin and the Ansedonia channel. It is one of the largest lagoons in the western Mediterranean and its shallow brackish waters, poor circulation, low water volume with limited turnover and partial isolation from the sea drastically reduce the dilution potential of organic additions, nutrients (from urban effluent, aquaculture plants and agricultural waste water) and anthropogenic contaminants (Innamorati, 1998). It occurs that the most common wind magnitude is 4 m s^{-1} showing a seasonal variability in intensity and direction. North-Westerly and South-South-easterly wind directions are dominant during

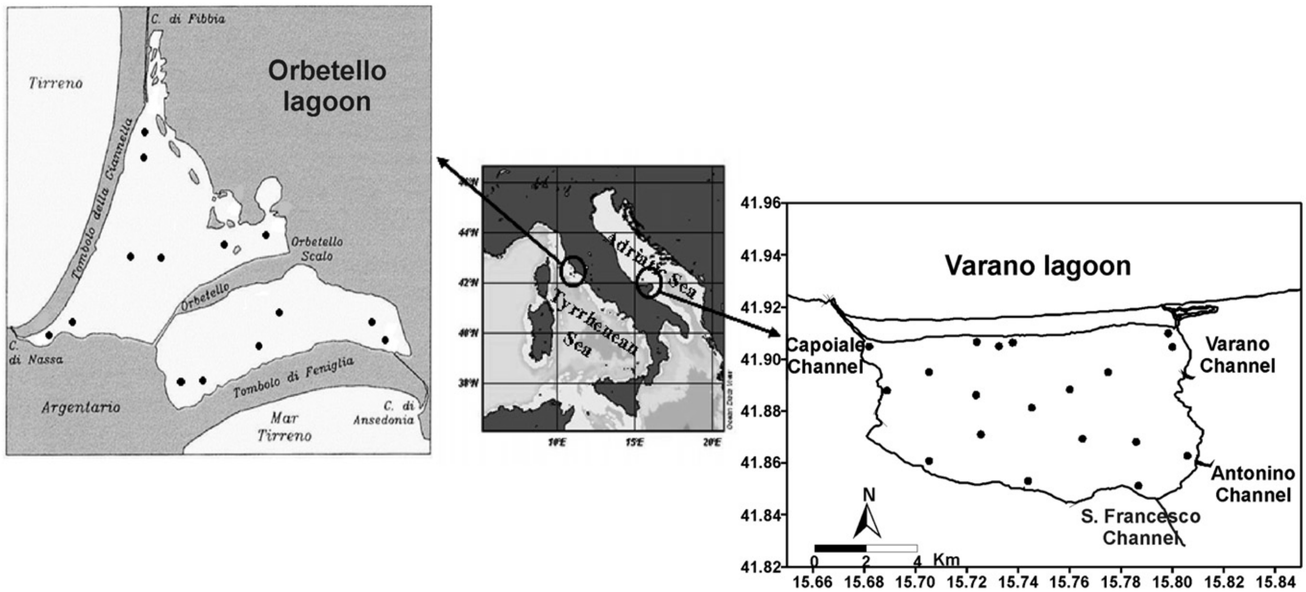

Fig. 1 – Sampling stations grid in Orbetello (Tyrrhenean Sea) and Varano (Adriatic Sea) lagoons.

the winter period. Strong winds ($15.4\ m\ s^{-1}$) are responsible for the low temperatures and the increase of water oxygenation and turbidity during the winter. Rains are usually related to the winter, in 2000–2001 from September to June, 686 mm was rained.

2.1.2. *Varano lagoon*

Varano lagoon is located on the Northern coast of the Gargano Promontory (Southern Adriatic Sea) (41.88°N; 15.75°E) (Fig. 1). It looks a real lake for its both shape and coasts sloping straight down to waters. The lagoon is partially isolated by the Southern Adriatic Sea through a coastal barrier, named “isola”, characterized in both the western and eastern sides by two channels, Capoiale and Varano respectively, that makes possible the communication with the sea through hydrodynamic balance produced by tide level, wind strength and direction and anthropogenic action. According to the climatic station located in near Cagnano Varano (urban centre), the most rainy season is winter, running from November to February, with a precipitation average of $848.9\ mm\ year^{-1}$. North-North-Western winds are very frequent in this area, above all during the winter season, helping sea water inputs into the lagoon. Hydrological investigations on the water balance of the lagoon (Villani et al., 2000; Spagnoli et al., 2002) estimated a freshwater input of approximately $87{,}000\ m^3\ d^{-1}$ with an organic content mostly originating from urban and agricultural runoff, fish-farming and zoo-technique activities. Due to the low tide excursion and reduced exchange with the adjacent coastal area, water time residence is very long and it is estimated to about 1.5 years.

2.2. *Sampling*

Water samplings were performed at 14 georeferenced stations in Orbetello lagoon from May 2003 to September 2004 (8 cruises) and at 19 fixed stations in Varano basin from February 2004 to July 2005 (11 cruises) (Fig. 1), according to the marine, groundwater and discharge channel influence. Surface water samples (at depth of 1 m) were collected using a Niskin bottle, stored in 1 L HDPE bottles and kept on ice during transport until the laboratory filtration. *In situ* measurements of temperature (°C), salinity (psu) and dissolved oxygen (mg/L and % of saturation) were performed with a Corr-Teck Hydrometria (mod. Datasonde 4a) in Orbetello lagoon and with an Idromar multiparametric probe in Varano lagoon. All sampling cruises were carried out between 08:00 and 13:00h, local time in order to reduce daily basis fluctuations of chemico-physical parameters.

2.3. *Laboratory analysis*

At the laboratory, water samples were filtered (0.5–1.0 L) using cellulose acetate fibre filters (Millipore, 0.45 μm fibre Ø) for the determination of dissolved nutrients (ammonia, $N{-}NH_4^+$, nitrite, $N{-}NO_2^-$, nitrate, $N{-}NO_3^-$ and soluble reactive phosphorus, SRP) according to APHA (1998) and Grasshoff et al. (1999) methods. Total nitrogen (TN) and total phosphorus (TP) were determined on not-filtered water samples according to Grasshoff et al. (1999). Water samples (0.5 L) for chlorophyll *a* (chl *a*) determinations were filtered through Millipore 0.45 μm glass fibre filters. Pigments were extracted in the laboratory with 90% acetone and measured according to Jeffry and Humphrey (1975). Spectrophotometric measurements were performed using a Milton Roy Spectronic (mod. 1201) and a Beckman DU-60 spectrophotometers.

Dissolved inorganic nitrogen (DIN) was obtained as sum of ammonia, nitrite and nitrate and N:P ratios were calculated on the basis of DIN and soluble reactive phosphorus (SRP).

2.4. *Statistical analysis*

For a start, a non-parametric test was used in order to explore strong correlations between the analysed variables for each system (chemical, physical and biological parameters). Spearman rank order R was calculated using raw data matrix. A k-means clustering analysis was then performed to cluster the stations with similar environmental characteristics. At this step, a matrix consisted of average data of strongly correlated variables (deduced by Spearman index, $P < 0.001$ and $P < 0.01$) for each station was used and the analysis of variance applied in each cluster and between clusters was performed.

Principal components analysis (PCA) was performed on data of both systems in order to determine which variables were correlated and identify the key variables with the greatest influence on the total multivariate distribution of data matrix. As variables had different units, all data were normalized and log transformed (Clarke and Green, 1988).

PCA is a powerful technique in multivariate statistical analysis (Chatfield and Collins, 1980) used to define correlation and similarities between variables, but it is little flexible in defining dissimilarities and its distance-preserving properties are poor (Clarke and Warwick, 1998). PCA weakness were also tested by Non-metric Multi-Dimensional-Scaling (nMDS) that have, on the other hand, a great flexibility both in the definition and conversion of dissimilarities to distances and its rationale is the preservation of these relationships in the low-dimensional ordination space (Somerfield and Clarke, 1995). nMDS represents the averages as points in low-dimensional space (2D) such that the relative distances apart of all points are in the same rank order as the relative dissimilarities (or distances) of the averages (Shepard, 1962; Kruskal, 1964). nMDS was run imposing minimum stress as 0.01 and restarting the process 1000 times; minimum values of stress obtained (0.06) occurred 586 times. The Spearman rank order and cluster analysis were performed with a computer package StatSoft version 5.5. PCA analysis was performed with the software Primer-E (Playmouth Marine Laboratory, UK) according to the methods of Clarke and Warwick (2001).

A seasonal variation at the sampling stations representative of the resulting clusters was performed. In addition, spatial distributions of environmental factors were obtained using geostatistical gridding method, the kriging (Matheron and Armstrong, 1987). Kriging produced visually appealing contour and surface plots from spaced data. Contours were constructed from data using computer package SURFER version 8.0.

2.5. *Trophic status index*

An estimate of trophic status of both Orbetello and Varano lagoons was performed through the use of suitable indicators. The classical Carlson's Trophic State Index (TSI) (Carlson, 1977), developed for freshwater lakes and proposed by US Environmental Protection Agency (USEPA, 2000) to classify lakes and reservoirs, was calculated using monthly mean values of chl a ($\mu g\ L^{-1}$) and TP (μM), according to the following equations:

$$\mathrm{TSI(Chl}\ a) = 9.81\ \ \ln\ (\mathrm{Chl}\ a) + 30.6 \tag{1}$$

$$\mathrm{TSI(TP)} = 14.42\ \ \ln\ (\mathrm{TP}) + 4.15. \tag{2}$$

TSI using Secchi depth (SD) was not determined as data were not available.

The Trophic Index TRIX (Vollenweider et al., 1998), introduced to characterize the trophic conditions of seawater and used by the Italian authorities on a routine basis to monitor the trophic state of the Adriatic Sea, was also used. The Trophic Index TRIX is a linear combination of the logarithms of four state variables that indicate pressure (DIN and TP, μM), biological response (chl a as phytoplankton biomass, $\mu g\ L^{-1}$) and environmental disturbance (the absolute percentage deviation from oxygen saturation, aDO%) in the water quality (Pettine et al., 2007). It was calculated as follows:

$$\mathrm{TRIX} = [\ \log\ (\mathrm{Chl}\ a \times \mathrm{aDO\%} \times \mathrm{DIN} \times \mathrm{SRP}) + 1.5]/1.2 \tag{3}$$

where 1.5 and 1.2 were scale factors based on an extended dataset concerning the northern Adriatic Sea.

Numerically, TSI index is ranged from 0 to 100, while TRIX index assume values between 0 and 10.

2.6. *Remote sensing analysis*

Two Landsat ETM+ images (path, row) (Figs. 2 and 3), dated June 2004, were registered and resampled to a UTM projected output image composed of 30 · 30 m pixels with an RMS error of less than 1.0 pixel. Resampling was done by the nearest neighbour method, which set the radiometric value of the output pixel equal to the nearest input pixel in the original geometry, in order to preserve the original values of the image. The two Landsat 7 TM images of June 2004 have been georeferenced, namely based on a system of geographic reference, the system UTM, with international ellipsis ED1950. Geo-referencing involved the bringing together of topographic cards and satellite images with the recognition of precise geographic coordinate points. For every image, 30 points with precise coordinates were registered; the entire image may be seen via the software Erdas Imagine 8.4. In order to

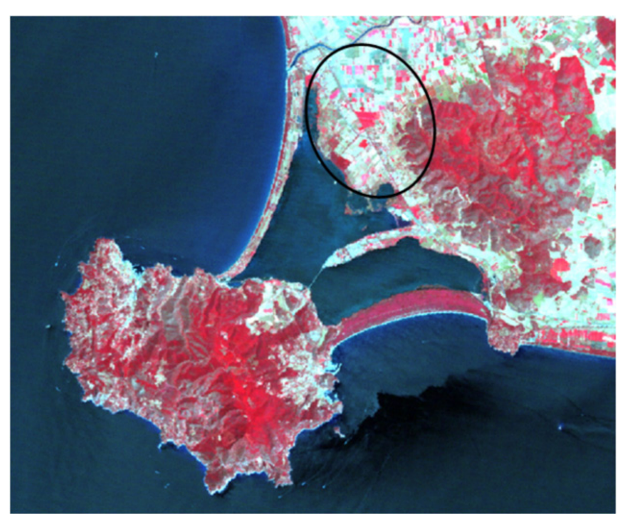

Fig. 2 – Elaboration of Landsat 7 ETM of July 2004 obtained for Orbetello lagoon; false colour composition Band 4, Band 3 and Band 2 respectively in red, green and blue. The agricultural area around the Orbetello lagoon is highlighted by a circle and the Albenga river which cross along this area by arrow.

Fig. 3 – Elaboration of Landsat 7 ETM of July 2004 obtained for Varano lagoon; false colour composition Band 4, Band 3 and Band 2 respectively in red, green and blue. In this figure the agricultural area extensive enough along the eastern side of the lagoon can be observed (highlighted by the circle).

characterize the lagoons ecosystem, we elaborated a technique based on the use of satellite measurements of reflected and emitted electromagnetic radiation in the visible (400–700 nm), near infrared (700–1100 nm) and thermal infrared (10,000–13,000 nm) wavebands. Satellite data are now widely used to examine spatial and temporal trends in the environment (Focardi et al., 2006). The availability of a historic series of data permits the analysis of environmental changes due to natural or induced factors. As satellite data is obtained nearly instantaneously over a wide area, the study of the spatial distribution of specific territorial characteristics is facilitated. The availability of information on the emission in several wavebands from the surface of a territory gives valuable information about the conditions and cover of these areas. The use of high or medium resolution data permits the creation of specific indices that can be used to make intra-territorial comparisons and decisions. Landsat Thematic Mapper (TM) images were used to map the cover of Jinja area. TM data have advantages over the other sensors that TM records an additional infrared channel at 1.55–1.75 μm. This is important for discriminating different vegetation types (Fuller et al., 1989; Townshend, 1992). Image interpretation: Vegetation is the dominant and important component in most ecosystems and useful indicator environmental conditions. Many remote sensing mechanisms operate in the green, red and near infrared regions of the electromagnetic spectrum. They can discriminate radiation absorption and reflectance of vegetation. Changes in vegetation are useful for recognizing changes in other environmental factors. Identifying vegetation in remote sensing images depends on plant characteristics: leaf shape and size, overall plant shape, water content, and associated background (e.g. soil types and spacing of the plants). For interpretation is used single band images (bands 3, and 4) and bands compositions are particularly useful.

3. Results

3.1. Spatial distribution of environmental parameters

Monthly arithmetic means and standard deviation values of physico-chemical parameters, nutrients and chlorophyll *a* for both Orbetello and Varano lagoons during the sampling periods are presented in Tables 1 and 2. Due to the peculiar geomorphology of both lagoons, which influence water circulation, winds direction and nutrients distribution, a great spatial variability of almost all variables was observed (highlighted by high standard deviation values). Although measurements of the physico-chemical parameters were not monthly performed during sampling periods, Orbetello and Varano lagoons showed a regular seasonal cycle of the temperature values, with the highest average temperatures reached in summer and the lowest means (Tables 1 and 2) in winter. In both lagoons, higher means of salinity were measured in autumn, after a period of low rainfall, which decreased in winter, during the rainy season, up to reach lower values in spring season (Tables 1 and 2). Spatial distribution of temperature and salinity values highlighted the frequent presence of cooler and saltier waters close to the communicating channels with the sea (Ansedonia and Nassa for Orbetello, Capoiale for Varano), above all in winter period when the exchanges of waters between the two lagoons and sea occurred, forced by wind action (Fig. 4).

Average DO levels (percentage of oxygen saturation) were lower in Orbetello lagoon than the ones measured in Varano lagoon. In Orbetello basin, the lowest oxygen saturation values were measured in August 2003, when high biomass decomposition rates probably occur, while in Varano lagoon concentration peaks (near to 100%) were measured during late spring–summer season (June 2005) in the middle area; in the last basin, values higher than 70% were measured most of the study period.

The two lagoons exhibit different behaviour in connection to the inorganic nutrient concentrations. For Orbetello lagoon, the average value for ammonia on yearly basis calculated for the whole lagoon was 3.9 μM but a great difference between the two basins was observed for this parameter (3.5 μM for Ponente and 4.3 μM for Levante). Station 9, close to the two municipal wastewater effluents, showed the highest ammonia values; during the summertime the increase in tourists presence produced higher N–NH_4^+ concentrations (20.3 μM in July *vs* 13.2 μM in January). Yearly average of nitrite were 2.9 μM, but two particular stations (9 and 10) showed a different behaviour compared to the whole lagoon. Nitrate average concentrations showed lower values during both spring and summer seasons and higher values, with wide spatial variability, were recorded during the rainy seasons; the highest concentrations were observed in January 2004 in the central zone of the lagoon. High concentrations of DIN were observed in the Ponente basin (winter) and in the Levante basin waters also (summer) (Fig. 5a, b). For Varano lagoon, mean ammonia concentrations showed both seasonal and yearly variability (Table 2). During the sampling performed in 2004, the lowest values (0.71–0.77 μM) were recorded in April in the central and north-eastern part of the lagoon and the highest values (23.31 μM) were measured in October at the

Table 1 – Monthly arithmetic mean and standard deviation (S.D.) of physico-chemical, chemical and biological parameters in Orbetello lagoon from 2003 to 2004 period

		2003				2004			
		May	Jun	Aug	Oct	Jan	Feb	Jul	Sep
Temperature (°C)	Mean	20.70	27.80	28.70	19.50	7.20	10.50	27.60	24.90
	S.D.	1.50	1.90	1.30	1.10	1.90	1.00	0.90	0.90
Salinity (psu)	Mean	37.30	38.80	39.40	38.90	35.30	36.00	36.10	40.70
	S.D.	1.20	1.00	0.90	1.30	1.40	1.10	2.40	1.40
DO (%)	Mean	35.20	27.20	20.10	55.70	77.30	89.60	23.30	41.80
	S.D.	5.90	7.40	9.30	22.10	30.20	9.00	9.80	11.10
Ammonia (μM)	Mean	2.56	3.29	5.23	2.67	4.26	4.90	3.48	2.94
	S.D.	0.12	0.33	0.24	0.32	0.81	0.52	0.67	0.85
Nitrite (μM)	Mean	0.78	0.34	0.21	0.56	5.64	5.04	0.40	0.28
	S.D.	0.12	0.13	0.21	0.14	0.81	0.40	0.33	0.25
Nitrate (μM)	Mean	1.22	0.56	0.67	2.21	14.50	9.30	0.62	0.42
	S.D.	0.11	0.01	0.09	0.07	1.70	0.70	0.10	0.10
SRP (μM)	Mean	0.22	0.17	0.23	0.15	0.46	0.51	0.61	0.34
	S.D.	0.05	0.09	0.26	0.12	0.84	0.92	0.31	0.32
Chl *a* (μg L^{-1})	Mean	7.50	7.60	12.30	4.20	5.70	6.50	4.30	10.50
	S.D.	0.20	0.30	1.30	0.10	2.10	1.60	4.30	3.90
DIN (μM)	Mean	4.56	4.19	6.11	5.44	24.40	19.24	4.50	3.64
	S.D.	0.91	0.23	0.45	0.33	3.22	4.34	1.19	0.22
TP (μM)	Mean	0.88	1.25	1.79	1.56	1.84	2.03	2.05	2.50
	S.D.	0.65	0.22	0.29	0.51	0.78	0.80	0.84	1.15
DIN:SRP	Mean	20.70	24.60	26.60	36.30	52.90	37.40	7.40	10.60
	S.D.	133.00	156.00	222.00	64.00	167.00	188.00	153.00	192.00

Where DO (%) = percentage of oxygen saturation; DIN= the sum of ammonia, nitrite and nitrate.

stations located in the south-eastern part of the lagoon. In 2005 mean concentrations did not exceed 5.00 μM. Nitrite mean concentrations showed lower values during both spring and summer seasons with a minimum of 0.02 μM measured in the western end. Higher mean values, with higher spatial variability, were recorded during rainy seasons; the highest concentrations (maximum of 3.20 μM) was observed in December 2004 in the central zone of the lagoon. Nitrate pattern showed a well-defined seasonal variability. The highest values were recorded in February 2004 (Table 2), after a

Table 2 – Monthly arithmetic mean and standard deviation (S.D.) of physico-chemical, chemical and biological parameters in Varano lagoon from 2004 to 2005 period

		2004							2005			
		Feb	Apr	Jul	Aug	Sep	Oct	Dec	Apr	May	Jun	Jul
Temperature (°C)	Mean						20.45	12.05	17.94	22.83	27.98	28.80
	S.D.						0.59	0.51	0.82	0.46	0.74	0.55
Salinity (psu)	Mean						29.10	26.73	23.82	24.76	25.51	27.00
	S.D.						0.72	2.19	0.29	0.47	0.38	0.45
DO (%)	Mean						78.64	77.54	77.46	88.84	91.46	95.48
	S.D.						7.55	6.41	4.40	4.56	8.08	6.39
Ammonia (μM)	Mean	4.11	1.35	3.42	11.08	15.64	14.86	11.32	5.00	3.90	3.53	3.42
	S.D.	0.94	0.63	2.30	3.60	4.08	4.08	2.68	1.67	1.53	1.79	1.34
Nitrite (μM)	Mean	1.60	1.39	0.19	0.69	1.12	1.59	2.30	0.31	0.44	0.12	0.13
	S.D.	0.32	0.39	0.09	0.22	0.32	0.42	0.52	0.10	0.18	0.07	0.07
Nitrate (μM)	Mean	33.61	23.36	0.81	7.37	9.83	14.62	17.62	10.24	4.09	2.37	2.12
	S.D.	10.24	6.10	0.82	3.81	4.01	6.61	5.33	3.53	3.03	2.70	3.34
SRP (μM)	Mean	0.13	0.19	0.20	0.20	0.15	0.12	0.11	0.16	0.14	0.10	0.17
	S.D.	0.06	0.09	0.02	0.05	0.06	0.04	0.02	0.08	0.04	0.06	0.04
Chl *a* (mg L^{-1})	Mean	1.01	3.08	2.46	3.46	2.17	0.96	1.28	4.46	3.37	3.38	4.64
	S.D.	0.16	2.37	1.19	1.00	0.44	0.09	0.36	2.60	2.30	1.40	2.52
DIN (μM)	Mean	39.33	26.10	4.49	19.13	26.59	31.06	31.24	15.55	8.42	6.01	5.67
	S.D.	10.85	6.22	2.84	6.58	7.38	6.35	6.35	4.28	3.63	3.56	3.80
TP (μM)	Mean	0.76	0.82	0.85	0.91	0.84	0.88	0.83	0.86	0.85	0.85	0.95
	S.D.	0.30	0.07	0.09	0.04	0.09	0.08	0.09	0.12	0.09	0.07	0.24
DIN:SRP	Mean	356.34	183.94	22.10	106.31	194.19	268.18	286.21	124.94	62.69	76.62	37.10
	S.D.	180.96	118.63	13.26	53.44	70.24	76.01	69.81	71.57	25.82	52.78	27.36

White area = measurements not available; DO (%) = percentage of oxygen saturation; DIN = the sum of ammonia, nitrite and nitrate.

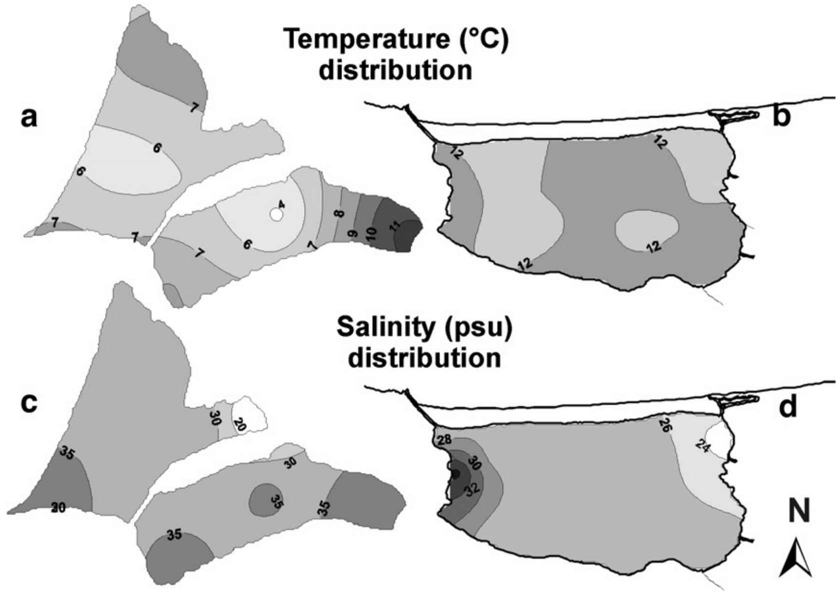

Fig. 4 – Winter spatial distribution of temperature (°C) (a, b) and salinity (psu) (b, d) in Orbetello and Varano lagoons, respectively.

rainy period, while the mean values decreased towards the summer season and the lowest concentrations (0.21 μM) were observed in July 2004. Spatial distribution of DIN in winter and summer seasons are reported in Fig. 6a and b. Orbetello lagoon waters were characterized by higher mean values of SRP than Varano waters (Tables 1 and 2). In Varano lagoon, mean phosphorus concentrations were never more than 0.20 μM, although maximum values of 0.22 μM and 0.30 μM were obtained in July 2004 and August 2004 respectively, along the southern and south-eastern coast of the lagoon. A spatial heterogeneity in both winter and summer seasons was observed for Orbetello (Fig. 5c, d), while a relatively

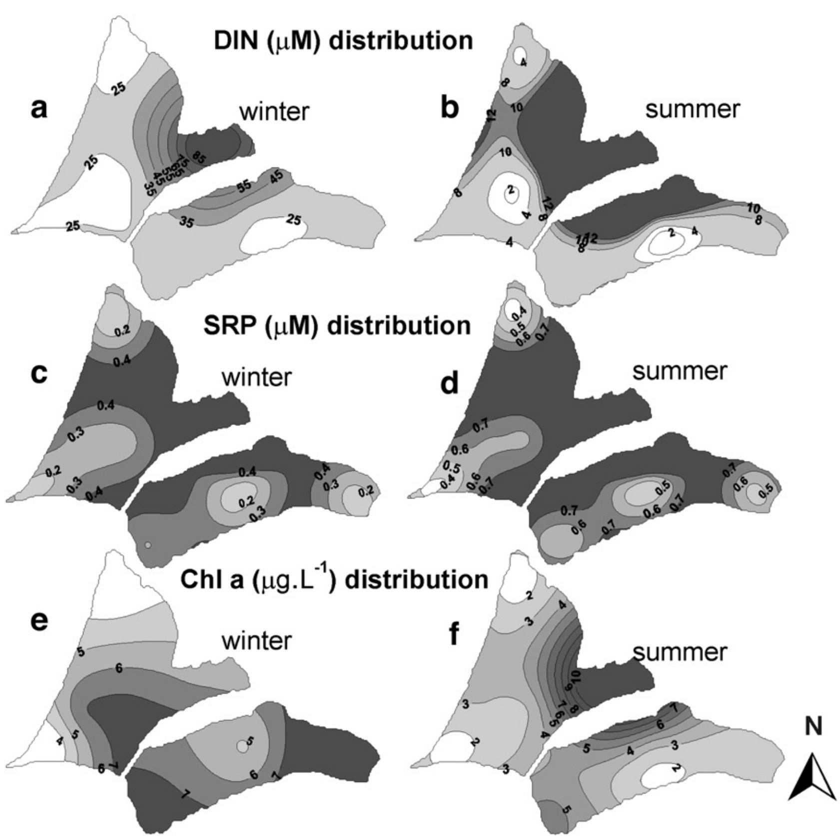

Fig. 5 – Spatial variability of DIN ($NH_4^+ + NO_2^- + NO_3^-$) (a, b), phosphate (SRP) (c, d) and chlorophyll *a* (chl *a*) (e, f) in Orbetello lagoon during winter and summer seasons, respectively.

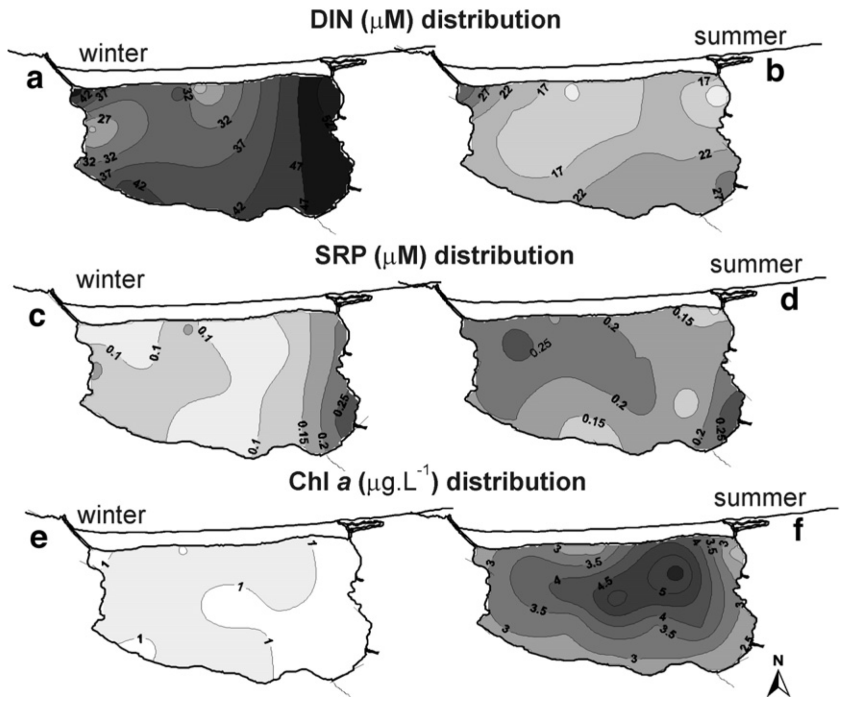

Fig. 6 – Spatial variability of DIN ($NH_4^+ + NO_2^- + NO_3^-$) (a, b), phosphate (SRP) (c, d) and chlorophyll *a* (chl *a*) (e, f) in Varano lagoon during winter and summer seasons, respectively.

homogeneous spatial distribution was observed for Varano most of the period (Table 2 and Fig. 6c, d). Total phosphorus mean concentrations were always higher than 1.5 μM in Orbetello and lower than 1 μM in Varano, but the TP variation between sampling sites in the first lagoon was high.

The highest mean values of chl *a* were measured in Orbetello lagoon, where a maximum of 15.3 μg L^{-1} was observed during the summer 2004, while in Varano lagoon chl *a* mean concentrations were under 5 μg L^{-1} most of the study period, except in April 2005 when a maximum of 10.13 μg L^{-1} was calculated. A spatial gradient was observed for phytoplankton biomass in Orbetello lagoon in winter, while homogeneity conditions characterized the Varano waters (Figs. 5e, f and 6e, f).

Table 3 – Spearman rank order correlations between all variables for surface water of Orbetello and Varano lagoons during the study period

	Nitrite	Nitrate	Ammonia	SRP	TP	Chl *a*	Temperature	Salinity	DO
Orbetello									
Nitrite	1.00	0.83***	−0.70	0.24***	0.12*	−0.59**	−0.90***	−0.86***	0.76***
Nitrate		1.00	0.24***	0.12	0.07	−0.50**	−0.78***	−0.69**	0.64***
Ammonia			1.00	0.55*	0.62***	0.05	0.12	−0.26*	−0.13
SRP				1.00	0.32***	−0.07	−0.19*	−0.52**	0.12
TP					1.00	−0.16*	−0.26**	−0.45***	0.19*
Chl *a*						1.00	0.83***	0.26*	−0.90***
Temperature							1.00	0.63***	−0.93***
Salinity								1.00	−0.45**
DO									1.00
Varano									
Nitrite	1.00	0.75***	0.42***	−0.14*	−0.55***	−0.46***	−0.2*	−0.006	−0.05
Nitrate		1.00	0.24***	−0.13	−0.48***	−0.37***	−0.39***	0.06	−0.15
Ammonia			1.00	−0.13	−0.32***	−0.3***	0.75***	0.21*	0.59***
SRP				1.00	0.44***	0.21**	0.097	−0.24*	0.018
TP					1.00	0.56***	0.21*	−0.27**	0.08
Chl *a*						1.00	0.3**	−0.31**	0.27**
Temperature							1.00	0.10	0.7***
Salinity								1.00	−0.03
DO									1.00

Where SRP = soluble reactive phosphorus; TP = total phosphorus; DO = percentage of oxygen saturation; * = $P<0.05$; ** = $P<0.01$; *** = $P<0.001$.

The DIN:SRP ratio calculated in Orbetello lagoon was lower than values obtained in Varano and values close the normal Redfield ratio (16:1) were observed during the study period except for winter season when ratios higher than 100:1 were obtained. In Varano lagoon the N:P ratio ranged from 8.95 (July 2004) to 826.50 (February 2004).

3.2. *Statistical assessment and seasonal trend*

Spearman rank order correlations between observed variables for surface water of Orbetello and Varano lagoons during the study period are shown in Table 3. In Orbetello lagoon, temperature was well correlated ($P < 0.001$) with salinity (positively) and DO (negatively). Temperature and salinity showed strong negative correlation with oxidized nitrogen, SRP and TP, while DO was positively correlated with nitrite and nitrate($P < 0.001$) and chlorophyll *a* ($P < 0.05$). Nitrate showed a good positive correlation with nitrite and ammonia, but ammonia was correlated only with nitrate. SRP was strongly correlated with nitrite and TP ($P < 0.001$). Chlorophyll *a* was correlated (negative) with nitrite, nitrate and TP, but it did not show correlation with ammonia and SRP.

In Varano lagoon the only positive correlation, concerning physico-chemical parameters, was found between temperature and DO. Temperature water showed a positive correlation with ammonia, TP and chl *a* and negative with nitrate ($P < 0.001$) and nitrite ($P < 0.05$). Good correlations (negative) were found between salinity and SRP, TP and chl *a*, while DO showed correlations with ammonia ($P < 0.001$) and chl *a* ($P < 0.01$). Dissolved nitrogen species showed significant positive correlations amongst themselves, while phosphate was well correlated (positive) with total phosphorus (TP). Chlorophyll *a* was well negatively correlated with nitrogen species ($P < 0.001$) and positively correlated with total and dissolved phosphorus.

A *k*-means clustering analysis produced four clusters for Orbetello lagoon and five clusters for Varano basin. This was the best result obtained by considering both the strongly correlated variables and the analysis of variance (Table 4). This last result shows an obvious spatial heterogeneity of both lagoons, highlighting the influence of freshwaters, seawaters and wastewaters on environmental parameters.

Table 4 – Analysis of variance of *k*-means clustering in Orbetello and Varano lagoons

Variable	Between group	*d.f.*	Within group	*d.f.*	*F*	*P*
Orbetello		Sum of square				
Ammonia	677.43	3	0.59123	10	3819.3	<0.0001
Nitrite	2.58	3	0.16883	10	51	<0.0001
Nitrate	11524	3	0.08589	10	447210	<0.0001
Chl *a*	20.76	3	7.71126	10	9	0.0035
Varano		Sum of square				
Ammonia	0.11	4	0.23	14	1.66	0.2155
Nitrite	0.098	4	0.21	14	1.59	0.231
Nitrate	0.93	4	0.42	14	7.86	0.0015
Chl *a*	3.98	4	0.76	14	18.39	<0.0001

Where *d.f.* = degrees of freedom.

Table 5 – Correlation coefficients between the environmental variables and the principal component

Variable	PC1	PC2	PC3	PC4	PC5
Temperature	0.177	0.44	−0.266	−0.118	−0.24
Salinity	0.308	−0.265	−0.296	0.049	0.242
DO	−0.307	0.084	0.448	−0.397	0.388
Ammonia	−0.21	−0.055	−0.644	−0.642	0.18
Nitrite	−0.183	−0.469	0.069	−0.059	−0.02
Nitrate	−0.361	−0.21	0.128	0.123	−0.023
TN	0.133	−0.469	−0.275	0.301	−0.035
TP	0.335	−0.233	0.168	−0.216	0.147
SRP	0.229	−0.34	0.235	−0.463	−0.533
DIN	−0.347	−0.253	−0.065	−0.007	−0.094
DIN:SRP	−0.386	−0.04	−0.175	0.209	0.135
Chl *a*	0.346	−0.081	0.103	−0.004	0.605

Where SRP = soluble reactive phosphorus; TP = total phosphorus; TN = total nitrogen; DO = percentage of oxygen saturation.

PCA analysis produced five principal components, but only the first two principal components (PC1 and PC2) accounted together for 78.5% of the total variance (51.2% for the PC1 and 27.4% for PC2). The correlation coefficients between these two principal components and the variables are reported in Table 5. PC1 showed the highest positive correlations with salinity, TP, SRP and chl *a* and the highest negative correlations with nitrate, DIN, DIN:SRP ratio and DO. On the other hand, PC2 showed the highest positive correlations with temperature and the highest negative correlations with nitrite and TN. The ordination diagram of seasonal means of

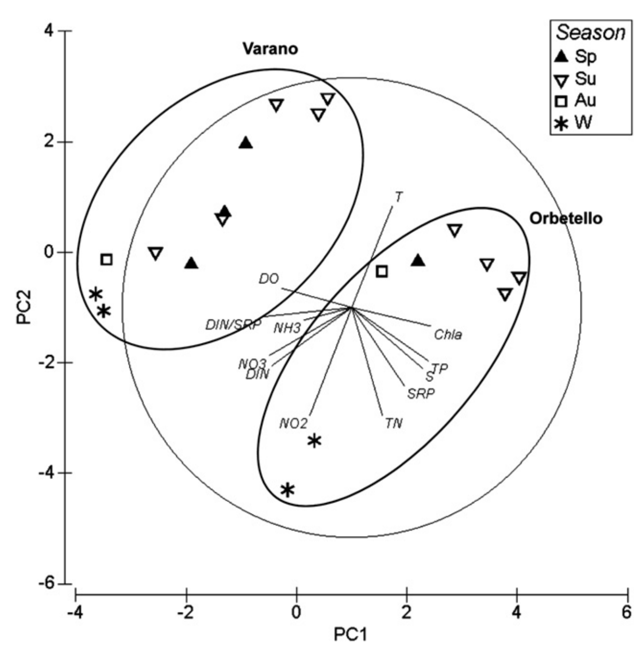

Fig. 7 – PCA ordination diagram of the seasonal mean variables. The first two principal components (PC1 and PC2) accounted together for 78.5% of the total variance. TP = total phosphorus; SRP = soluble reactive phosphorus; TN = total nitrogen; NO_2 = nitrite; T = temperature; DO = oxygen saturation; NH_3 = ammonia; NO_3 = nitrate; DIN = sum of ammonia, nitrite and nitrate.

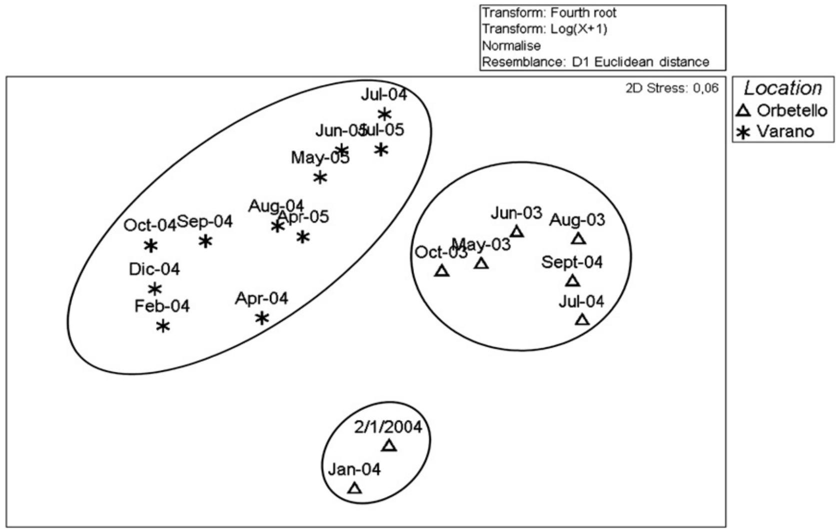

Fig. 8 – Non-metric Multi-Dimensional-Scaling applied to the seasonal average data for both Orbetello and Varano lagoons.

variables measured in both lagoons on the first two principal components is shown in Fig. 7. The symbols (triangles, squares and asterisk) represent the correlations between seasonal means and the principal components. As expected, the distribution of seasonal means highlights a different behaviour of the two systems; in particular, Varano showed a great difference between the seasonal means, while in Orbetello the seasonal pattern was more homogeneous. Fig. 8 represents the non-metric Multi-Dimensional-Scaling applied to average data of both systems and it well shows the Varano lagoon variables pattern clearly dissimilar to the one of Orbetello lagoon. Also, it can be possible to distinguish three groups: Varano lagoon, Orbetello lagoon winter and Orbetello lagoon in other seasons.

A seasonal pattern of chemical and biological parameters was performed for each station representative of the clusters obtained in both lagoons. For Orbetello, DIN pattern showed a light increase of concentrations from October 2003, especially at station 9, close to the municipal wastewater treatment plant effluents (Fig. 9a). SRP showed the highest mean values during the summertime (Fig. 9b). DIN:SRP ratio showed high values at all selected stations in July 2004, but the peak was obtained at station 4, characterized by high nitrite concentrations and low values of SRP (Fig. 9c). Chl *a* peaked in summer with a defined seasonal trend (Fig. 9d).

A well-defined seasonal pattern was observed for inorganic nitrogen species (DIN) in Varano lagoons (Fig. 10a); they showed their lowest mean values during summer seasons at all selected stations, while waters with high nitrogen values were observed from autumn to winter, during rainfalls. On the other hand, phosphate showed a random pattern with selected stations alternated between high and low concentrations; however values from 0.2 μM to 0.3 μM were observed at station 12 close to the wastewaters input (south-eastern side

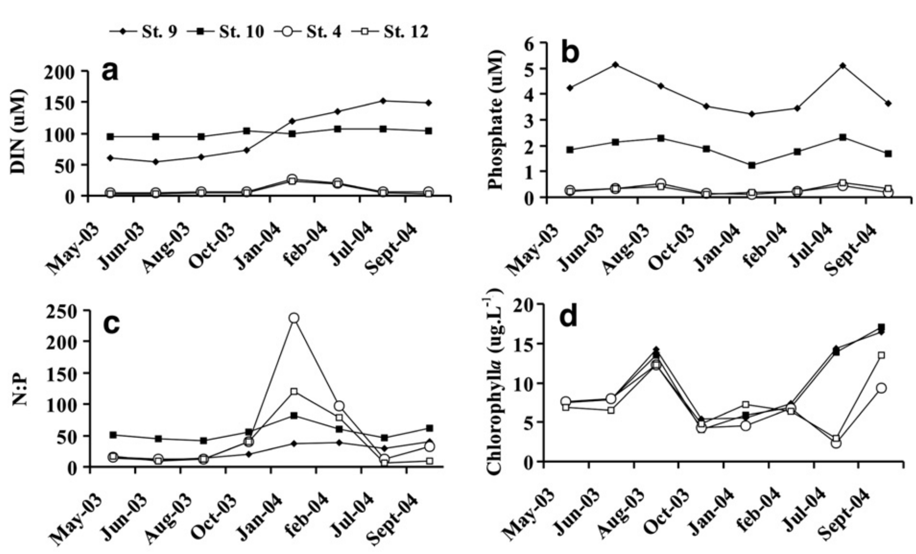

Fig. 9 – Seasonal variability in Orbetello lagoon of mean concentrations of DIN (a), phosphate (b), N:P (c) ratio and chlorophyll *a* (d) at four stations representative of the obtained clusters, from 2003 to 2004 period; the legend in shown in graph a.

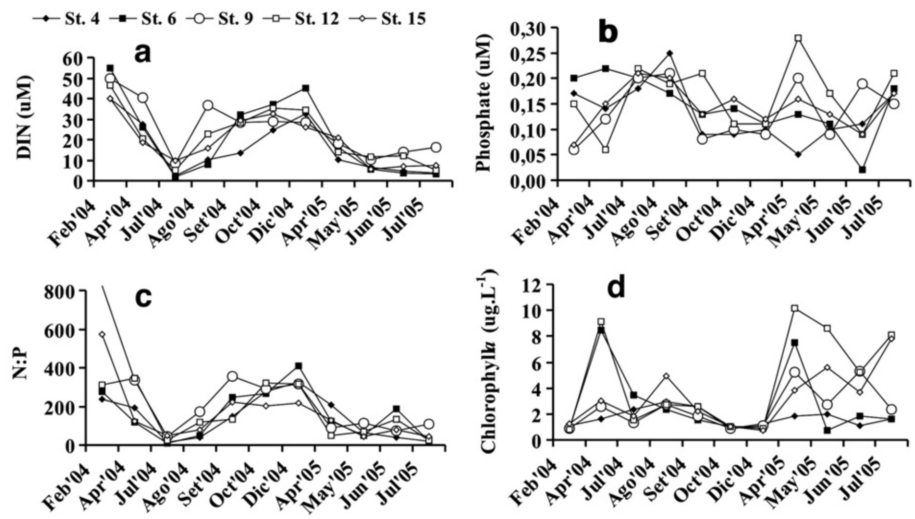

Fig. 10 – Seasonal pattern in Varano lagoon of mean concentrations of DIN (a), phosphate (b), N:P (c) ratio and chlorophyll *a* (d) at five stations representative of the obtained clusters, from 2004 to 2005 period; the legend in shown in graph a.

of the lagoon) from October 2004 to May 2005 (Fig. 10b). DIN:SRP ratio pattern was similar to nitrogen species trend (Fig. 10c) with an increasing of values during wet season. It can be observed that stations near the freshwater and wastewater inputs (stations 9 and 12) showed the highest values of DIN:SRP ratio. Ratios lower than 16 (Redfield ratio) were only recorded in July 2005 at stations 4 and 6. Chl *a* values exhibited a seasonal trend with peak concentrations observed during spring (Fig. 10d), above all at stations located near the pollution source (stations 12, 6) along the eastern coast of the lagoon.

3.3. Trophic state indexes

The classical Carlson's Trophic State Index (TSI) calculated with chl *a*, mean values in each monthly sampling for Orbetello lagoon ranged between 44.7 (October 2003) and 55.19 (August 2003), corresponding to mesotrophic and eutrophic conditions, respectively. Sampling stations located near the urban wastewater treatment plants effluents showed the highest Carlson's index values during the most of year. TSI (TP) was minimum (2.3) in May 2003 and maximum (17.36) in September 2004, corresponding to constant oligotrophy. In Varano lagoon the lowest values of TSI (chl *a*) were determined in February 2004 (30.67) and October 2004 (30.17), indicating a oligomesotrophic state during autumn and winter seasons, while the highest indexes were calculated in April 2005 (45.24) and July 2005 (45.63), indicating mesotrophy in both spring and summer. TSI (TP) lowest value was calculated in February 2004 (0.75) and the highest in July 2005 (3.41). These low values suggest oligotrophic conditions for Varano lagoon also. The TSI (chl *a*), showed higher values than the one determined with TP for both lagoons (Fig. 11a, b). From seasonal variations it is possible to observe an yearly change of the trophic status of both lagoons.

For index TRIX, the trophic scale ranges from 1 to 10, classifying the state of a system as high with low trophic conditions (2–4), good with waters moderately productive (4–5), mediocre with high trophic level (5–6) and poor with waters

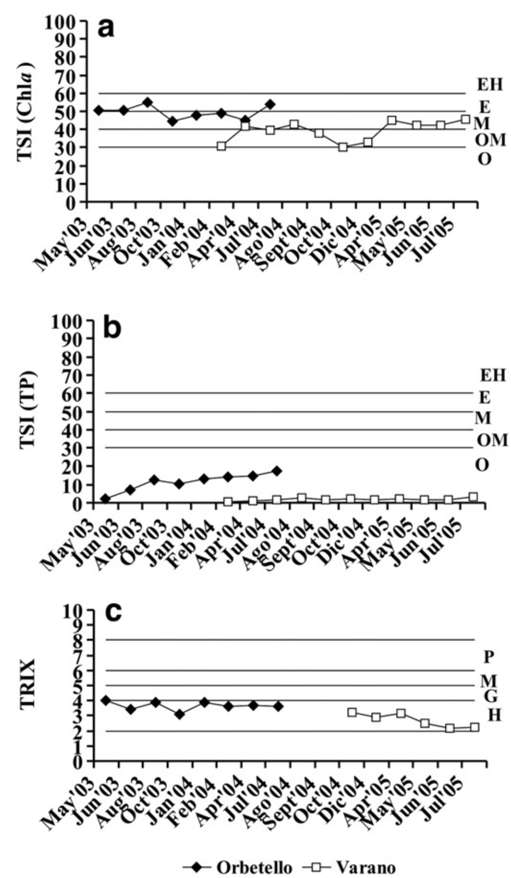

Fig. 11 – Seasonal trend of trophic state indexes in Orbetello and Varano lagoons: a) TSI determined with chlorophyll *a*; b) TSI determined with TP; c) TRIX index. On the right side of the figures the trophic status corresponding to fixed values of the index was highlighted. For TSI: O = oligotrophic; OM = oligomesotrophic; M = mesotrophic; E = eutrophic; EH = eutrophic to hypereutrophic. For TRIX: H = high water quality; G = good water quality; M = mediocre wastewater quality; P = poor water quality.

highly productive (6–8) (Penna et al., 2004). In Orbetello lagoon, the mean value of TRIX index was included between 2 and 4 (3.7 ± 0.31), typical values of a system with low trophic conditions. For Varano lagoon the TRIX index was not determined for the entire sampling period for lack of oxygen data, but from October 2004 to July 2005, the majority of the samples (90%) corresponded to high water quality, while the minority of the samples (10%) had a trophic level lower than 2. A trend of constant values was observed for most of the period (Fig. 11c).

3.4. *Remote sensing*

By the elaboration of Landsat 7 TM data of June 2004 it was possible to identify all the surrounding areas of both lagoons. It has been analysed and elaborated the two Landsat 7 TM images of June 2004 obtained on the same days as the *in situ* measurements. Remote sensing analysis showed that both lagoons were bordered by agricultural areas which are crossed by rivers (Figs. 2 and 3). Agricultural activities enhance nutrient loading to the rivers and subsequently to the lagoons with dry fall. The comparison of the two study wetlands with the surrounding water bodies showed clear differences resulting from both water optical properties and temporal variability. Particularly, the Varano lagoon seems to be much more affected by the impact of agricultural activities than Orbetello.

4. Discussion

The values of physical, chemical and biological parameters measured in Orbetello and Varano lagoons indicate that a wide spatial and seasonal gradient of the water characteristics was established during the study period, but in particular in winter. This gradient, typical of estuarine systems, was essentially due to the mixing of freshwater, seawater and anthropogenic inputs. The low water flow and the restriction of water exchange, due to the morphologic characteristics of both systems, create areas with high nutrient concentrations. This is evidenced by the clustering analysis which isolated the stations close to the anthropogenic inputs: urban wastewaters and agricultural drainage watercourses in Varano basin (along the eastern margin of the lagoon) and the municipal wastewater treatment plants effluents in Orbetello, which release high nutrient concentration and represent a source for the whole lagoon basin (Renzi and Focardi, 2000). In addition, PCA analysis highlighted a distinct behaviour of the two systems in time; the greatest difference between Orbetello and Varano was observed in winter months.

The average salinity on yearly basis showed similar trend in the two systems, but mean values measured in Orbetello lagoon are higher than in Varano lagoon, due to the influences of northern Thyrrenean sea waters which are characterized by salinity values higher than southern Adriatic sea waters. Orbetello was characterized by saltier waters located in the central zone of Levante (43psu) and Ponente (40psu) basins at the end of the summertime 2004; these data are in agreement with the water circulation produced by the pumping stations which work from spring to autumn and force the water to circulate in the following direction: sea → Ponente basin → Levante basin → sea. In addition, an increasing trend of salinity from spring to summer was related to the prevalence of a net evaporation rather than dilution with freshwaters. The Spearman rank correlations highlighted that chl *a* was positively correlated with temperature and salinity and negatively with DO in Orbetello lagoon, while the opposite happened in Varano lagoon. The peaks of phytoplankton chl *a* concentrations in Orbetello were observed in dry summer (August) when the nutrients were not transported from freshwaters to the lagoon; this suggests that an internal loading from sediments could be significant, also enhanced by the higher summer temperatures, and the decreasing of oxygen could be related to the organic matter decomposition. In Varano lagoon, phytoplankton blooms occurred during the spring months, following the rainfall period, and the inverse relationship between chl *a* and salinity, suggests that freshwater inputs are an important forcing function stimulating phytoplankton growth. In addition, the strong positive correlation between chl *a* and oxygen saturation is due to photosynthetic oxygen production. In Varano lagoon, higher concentrations of nitrogen species and lower values of SRP than results obtained in previous studies (Marolla, 1981; Villani et al., 2000; Caroppo, 2001) were observed; by contrast, in Orbetello lagoon an increase trend of SRP values were observed if compared to previous research (Innamorati and Melillo, 2004). By comparing with other works carried out in Mediterranean environment, the nutrient load in Orbetello and Varano lagoons is considerably lower than the one observed in coastal lagoons impacted by non-point source pollution (Gikas et al., 2006), but higher (for nitrogen species) than the lagoons of N.E. Greece, very important in terms of fish production (Sylaios and Theocharis, 2002). For Orbetello lagoon, the nutrient load could be principally explained by urban and fish-farming effluent discharges, while the agricultural activities could be much more significant for Varano lagoon. In fact, the presence of numerous small channels for water drainage which connect the agricultural areas (highlighted by remote sensing images) to the lagoons, facilitates the exchange of nutrients and fertilizers.

Nitrate was the most abundant nitrogen compound from autumn to winter, while ammonia contribution was higher from spring to summer, for both systems. In Varano lagoon, ammonia could be released through re-mineralization processes (Caffrey, 1995), enhanced by high temperature (as highlighted by Spearman rank correlations), while in Orbetello the excretion by benthic organisms, zooplankton and fish could be the main source for this molecule.

In relation to the indicators of trophic level, it is concluded that the eutrophication status of both lagoons is not worsening if compared to previous studies. From the trophic index TSI (chl *a*), Orbetello was moved from mesotrophy (autumn and winter) to eutrophy (summer), while Varano was classified as a system with tendency to be oligomesotrophic (autumn and winter)–mesotrophic (summer). No trophic category change was highlighted by the TSI (TP), which classify both lagoons as oligotrophic systems. According to the index TRIX, Orbetello and Varano lagoons had similar trophic conditions, characterized by high water quality with a low trophic level. It has to be verified and discussed in the framework of the EEA activities if the limits proposed and used by Vollenweider et al. (1998) are an acceptable assumption for monitoring and assessing the trophic state of all European coastal and marine waters (EEA, 2001), as the index was developed for the northern Adriatic waters. Despite these restrictions, it was used to assess the trophic status of a European coastal lagoon also (Coelho et al., 2007). Numerous models, indicators and indexes are in use to assess eutrophication in lakes, marine and coastal waters, taking into account nutrient concentrations as descriptor for the trophic status, but a classification system for coastal, brackish and saline lagoons has been never proposed. Therefore, all data describing environmental characteristics of coastal lagoons are important, because they could contribute to the development of a model for assessing the eutrophication status of these systems and, above all, its time evolution.

5. Conclusions

The management of coastal lagoons is of particular interest due to their high economical importance. In spite of their great productivity, coastal lagoons are often impacted by human pressure which could produce water eutrophication. Orbetello showed the highest water eutrophication, highlighted by the trophic index values. Only four clusters were observed and two of them were due to the presence of two spot sources of contamination represented by the urban wastewater effluents. Varano showed lower eutrophication levels except for the summertime. Orbetello lagoon seemed much more affected by the urban impact and the fish-farming activities than Varano lagoon, but the latter showed a greater agriculture activities impact as showed by the remote sensing analysis. Although no serious eutrophication events occurred during the study period, the results of this study suggest the need for the continuous control of freshwater quality entering lagoons through the drainage channels and the development of action programmes to reduce the inputs of nitrates from agricultural sources.

Protective measures and actions should be studied to improve the lagoon's water quality. Furthermore, the use of advanced monitoring tools, such as satellite remote sensing, can provide additional qualitative and quantitative information. Although the remote sensing technique is limited to the surface layer of the water column and is widely used for the coastal seawater monitoring, it could give useful complementary information to traditional *in situ* measurements to assess the state of the lagoon environment. In this context the possibility to measure the biomass of phytoplankton as chlorophyll *a* is promising. Eutrophication mapping through satellite colour data should mostly address the creation of 'time composite' products for those periods of the year when blooms may occur. The integration between the physical–chemical and biological parameters monitoring with the remote sensing analysis which illustrate the extension of surrounding areas also, could represent a valid support in coastal waters management. In fact, the proposed approach can enhance both the comprehension and the knowledge of the surrounding areas dynamics.

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