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Hydrological heterogeneity, nutrient dynamics and water quality of a non-tidal lentic ecosystem (Lesina Lagoon, Italy)

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ABSTRACT

The dynamics of the Lesina coastal lagoon (Italy) in terms of nutrients, phytoplankton and chemical–physical parameters were evaluated, together with their functional relationships with freshwater inputs, in order to identify ecosystem responses to changes in driving forces in a Mediterranean non-tidal lentic environment. Lesina Lagoon is a shallow coastal environment characterised by limited exchange with coastal waters, which favours enrichment of nutrients and organic matter and benthic fluxes within the system. Lagoon–sea exchanges are influenced by human management. There is a steep salinity gradient from East to West. High nitrogen and silica values were found close to freshwater inputs, indicating wastewater discharges and agricultural runoff, especially in winter. Dissolved oxygen was well below saturation (65%) near sewage and runoff inputs in the western part of the lagoon during summer. Classification in accordance with EEA (2001) guidelines suggests the system is of "poor" or "bad" quality in terms of nitrogen concentrations in the eastern zone during the winter rainy period. In terms of phosphate concentrations, the majority of the stations fall into the "good" category, with only two stations (close to the sewage and runoff inputs) classed as "bad". In both cases, the raw nitrogen levels make the lagoon a P-limited system, especially in the eastern part. There was wide space–time variability in chlorophyll *a* concentrations, which ranged from 0.25 to 56 $\mu g\,l^{-1}$. No relationships between chlorophyll *a* and nutrients were found, suggesting that autotrophic biomass may be controlled by a large number of internal and external forcing factors driving eutrophication processes. Water quality for this type of environment depends heavily on pressure from human activities but also on the management of sewage treatment plants, agricultural practices and the channels connecting the lagoon with the sea.

1. Introduction

Coastal lagoons are among the most dynamic and complex aquatic environments on earth, characterised by multiple interfaces, resilience, high biological productivity and high ecological potential, features that result from their limited exchanges with the adjacent sea and their ability to accumulate nutrients from the whole of the catchment basin (Carrada and Fresi, 1988; Alonghi, 1998; Tett et al., 2003). Lagoonal ecotones are structural and functional units with a marked space–time dichotomy of abiotic and biotic components that reflect exchanges of material and energy between the lagoons and their surrounding ecosystems, be they agricultural, urban or marine. Lagoonal ecotones modulate and determine the boundary conditions, which are driving forces influencing the internal structure and functional dynamics of the lagoon itself (Barnes, 1980; Kjerfve, 1994; Vadineanu, 2005). Lagoons are described as self-maintaining or life-supporting ecological systems in that they provide a broad range of natural and human-dominated resources that depend on the level of auxiliary energy and material inflow into the lagoon (Odum et al., 1995; Vadineanu, 2001). Due to their exposure to rapid nutrient enrichment, coastal lagoons are vulnerable to eutrophication. In recent years, this has caused deterioration of water quality, with consequences for the ecology of the lagoons themselves and for biological communities (Nixon, 1995; Karlson, et al., 2002). Environmental stress caused by eutrophication also has effects on public health (Belzunce et al., 2004). Eutrophication is often a consequence of human activities such as agriculture (Bell, 1991; Viaroli et al., 2005), urbanisation, industrialisation (Bock et al., 1999; Lee and Arega, 1999; White et al., 2004) and, over the last 20 years, aquaculture (Strain and Yeats, 1999; Jones et al., 2001).

The Water Framework Directive (WFD, 2000/60/EC) sets out a plan for the protection, enhancement and restoration of all water bodies in the Member States, in order to obtain good ecological

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status by 2015. In accordance with the requirements of the WFD, numerous studies have been carried out regarding its implementation in European lagoonal ecosystems, from the mesotidal lagoons of the Atlantic to the microtidal lagoons of southern Europe, with a range of physiographical and hydrological features and subject to anthropic impacts of various kinds (Basset et al., 2006). In addition, abiotic and biotic indices and indicators have been applied to the components of the system (water, phytoplankton, macrophytobenthos, zoobenthos and fish) in order to evaluate their state of ecological quality (Diaz et al., 2004; Loureiro et al., 2006; Ballesteros et al., 2007; Coelho et al., 2007). There is an intense scientific debate in this regard concerning the definition of transitional waters and ecosystems and the definition of their ecological status (Borja et al., 2004; Simboura et al., 2005; Basset et al., 2006; McLusky and Elliott, 2007).

Given the unique hydrogeomorphological nature of each lagoonal ecosystem, in the complex European context a sensible initial approach to the application of the WFD would be to select those lagoonal systems which are most representative.

Mediterranean lagoons are characterised by shallow waters and limited exchanges with the sea. In terms of climate, the typical seasons are autumn and winter characterised by low temperatures and high rates of precipitation and spring and summer characterised by higher temperatures and limited precipitation. The hydrological regime is strongly influenced by continental freshwater inputs and by local meteorological conditions (winds and rains). The salinity variability is determined by freshwater inputs, precipitation, evaporation, morphology and the exchange efficiency of the tidal channels (Kjerfve, 1994; Gamito et al., 2005). Badosa et al. (2006) suggest that hydrology is usually the main driver of nutrient supply in coastal lagoons. Depending on their hydrological and trophic conditions, these shallow coastal organic matter-enriched environments may be characterised by occasional dystrophic crises (Sorokin et al., 1996; Lardicci et al., 1997; Souchu et al., 1998). Coastal lagoons have an important functional role as nursery grounds for fish and crustacean species (Franco et al., 2006).

The objectives of the present study were to: a) illustrate the lagoon's nutrient dynamics under various environmental conditions and the functional relationships of these dynamics with freshwater inputs, in order to better understand the ecosystem's response to changes in driving forces and gain insight into how sustainable management of the lagoon may be developed; b) describe the functional relationship between hydrological variability and phytoplankton measured as chlorophyll *a* and cell abundance, in order to provide insight into how hydrological variables influence spatial and temporal phytoplankton distribution; c) assess the trophic state and quality of the waters using various classification criteria in order to expand our knowledge of the functioning of non-tidal lentic lagoonal ecosystems during the period of the implementation of the Water Framework Directive.

2. Materials and methods

2.1. Study area

The Lagoon of Lesina (41.88°N and 15.45°E; Fig. 1), situated on the southern Adriatic coast of Italy (Fig. 1), is characterised by shallow waters (0.7–1.5 m) and limited exchanges with the sea, features which it shares with many other Mediterranean lagoons. The Lagoon lies within the Gargano National Park and in 1995 was declared a Site of Community Importance (IT9110015). The lagoon is about 22 km long, with a total area of about 51 km^2 and the catchment area is about 600 km^2. The lagoon is separated from the sea by a sandbar about 18 km long, and connected to it by two tidal channels: Acquarotta to the West, about 2 km long, and Schiapparo to the East, about 1 km long. The two channels are protected on the seaward side by gabions, and their beds are lined with concrete, as are the banks at the seaward end. There are sluices and 10 mm grilles at the lagoon end, use of which has been inconsistent over the years. At the seaward end of the Acquarotta channel there has been an illegal landing stage for fishing boats for about a decade. Over the last ten years, rainfall (400–700 mm a year) has tended to be concentrated in autumn–winter. However, heavy rainfall events have also been recorded in spring. The hydrological regime is strongly influenced by continental freshwater inputs and by local meteorological conditions (winds and rains). The residence time of the waters is estimated to be about 70–100 days (Manini et al., 2002).

The lagoon's main hydrological features, temperature and salinity follow a seasonal trend, with minimum values in winter and maximum values in summer. Temperatures generally range from 3 to 32 °C and salinity from 5 to 51, the latter value having been recorded in October 1993 (Marolla, pers. comm.). The salinity gradient, which rises towards the West, is determined by freshwater inputs (distributed along the southern shore), precipitation, evaporation (high in summer) and the exchange efficiency of the tidal channels (Bullo, 1902; De Angelis, 1963; Manini et al., 2002; Fabbrocini et al., 2005).

Numerous watercourses flow into the basin, mainly along its southern edge. Two of these are year-round (the Lauro river and the Zannella channel), while others are intermittent, their flows varying in relation to precipitation. Roughly 80% of the annual freshwater budget is discharged into the eastern part of the lagoon (Francavilla, pers. comm.). The partially treated waste waters of three municipalities with a total of 30 000 inhabitants are discharged into the lagoon by the Elce channel (8000 inhabitants), the river Lauro and the Idrovora Lauro pumping station. Waters drained from nearby arable land (21 000 ha) are discharged into the lagoon by the Lauro and Zannella watercourses and the Idrovora Pilla pumping station; the area surrounding the western end of the basin is used for intensive vegetable farming and wheat; to the East, agriculture is characterised by two kinds of crop rotation which are cereal-based and vegetable-based (Franchi and Pelosi, 1998). In addition, there are three intensive aquaculture plants on the coast, waste waters from which are discharged into the lagoon by small channels on the western side and by the Zannella channel on the eastern side; the eastern plant makes use of geothermic waters. Finally there is a cattle farm (about 1500 head) situated on the western shore, near the Acquarotta channel.

The macrophytobenthos community is made of the rhizophytes *Ruppia cirrhosa* and *Nanozostera noltii*, mainly distributed in the Eastern and central parts of the basin. In the western area, variations in salinity and progressive nutrient enrichment over the last thirty years have led to a succession of uniform carpets of macroalgae, from *Gracilaria* sp. to *Cladophora* sp. and *Valonia* sp., which have occasionally caused dystrophic crises (Cozzolino, 1995; Manini et al., 2003). The lagoon of Lesina is a nursery area for numerous fish and crustacean species of commercial value (Villani, 1998). An important economic role is played by fishing and onshore aquaculture, and was played in the past by the production of the macroalga *Gracilaria* sp.

2.2. Sampling and analysis

Sampling was performed every month from September 2006 to August 2007 in 12 stations (between 9:00 and 12:00), six of which were situated along the southern shore next to the freshwater inputs (stations 1, 4, 7, 9, 10 and 12), two near the channels connecting the lagoon to the sea (stations 2 and 11) and four along

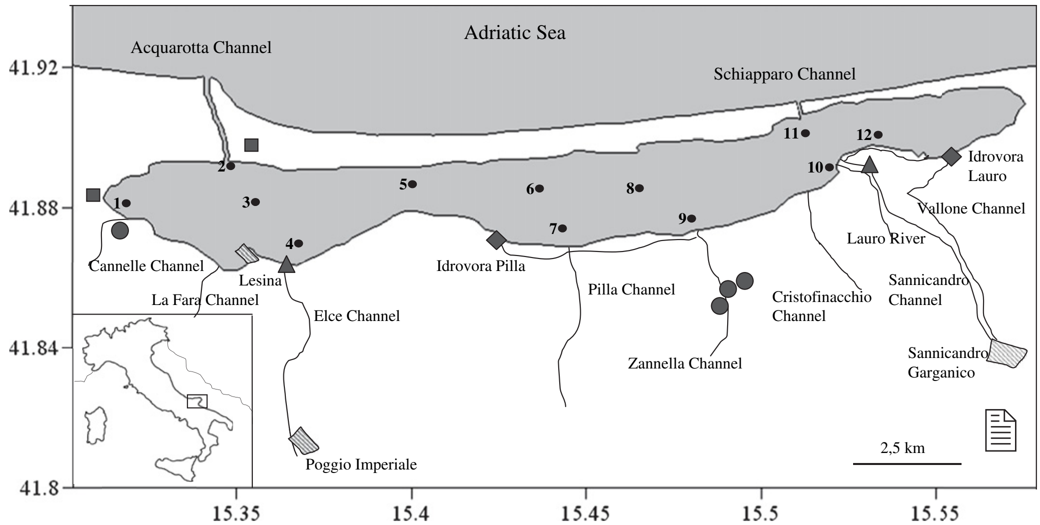

Fig. 1. The Lesina Lagoon showing the location of sampling sites, population centres (◈), drainage pumping stations (Idrovora Lauro and Idrovora Pilla, ◇), urban wastewater treatment plants (△), livestock farms (□) and fish farms (○).

a central longitudinal transect (stations 3, 5, 6 and 8) (Fig. 1), according to the results of a previous monitoring program (Fabbrocini et al., 2005). Temperature (°C), salinity, dissolved oxygen (% saturation) and pH were measured using a YSI 556 MPS multiprobe. The water samples were taken in triplicate at a depth of 50 cm, conserved in refrigerated dark bottles and transported to the laboratory for subsequent analyses of nutrients, chlorophyll *a* and suspended particulate matter. In addition, in stations 1, 3, 6 and 10, samples were taken for counting phytoplankton cells.

2.2.1. Nutrients

The analysis of nutrients – ammonium (NH_4^+), oxidised nitrogen ($NO_x = NO_2^- + NO_3^-$), nitrite (NO_2^-), soluble reactive phosphorus (SRP), soluble reactive silicate (SRSi), total nitrogen (TN) and total phosphorus (TP) – was performed using a Bran + Luebbe QuAAtro flow analyser in accordance with the methods described in Grasshoff et al. (1999).

2.2.2. Chlorophyll a and phytoplankton

The water samples were filtered using Millipore cellulose acetate filters (porosity 0.45 μm), and pigment extraction took place in 90% acetone. Pigment concentration was estimated by spectrophotometry following the method and equations of Jeffrey and Humphrey (1975). The phytoplankton cells were counted and identified by inverted microscope following Utermöhl's (1958) method. The organisms were classified and grouped into three main taxonomic components: diatoms (Bacillariophyceae), dinoflagellates (Dinophyceae) and others (Cryptophyceae, Cyanophyceae, Euglenophyceae).

2.2.3. Total suspended matter

Suspended particulate matter was concentrated using fibreglass Whatman GF/F filters, dried to determine the quantity of total suspended matter (TSM) and subsequently calcined in a muffle furnace in order to estimate the organic fraction (POM), in accordance with the methods set out in Strickland and Parsons (1968).

2.3. Data processing

2.3.1. Statistical analysis

The statistical analysis of the data was performed using JUMP 6.0. The relationships between the variables considered were obtained by means of Pearson's correlation. To describe the space–time variation of the system, one-way variance analysis was performed on the considered variables (Underwood, 1997). Subsequently the Bonferroni correction was applied to adjust the *p* value. Where the variance analysis yielded significant results, Tukey's Honestly Significant Difference test (HDS) was applied to highlight the space–time variations of each variable. In order to visualise the spatial and temporal trends, factorial analysis (FA) was performed, applying the Varimax rotation to improve the interpretation of the components (Zar, 1984; Boyer et al., 1997). Canonical Correspondence Analysis (CCA) was performed in order to understand the relationship between the environmental variables and phytoplankton (Ter Braak, 1986). The abundance of the taxonomic components considered here was $\log_{10}(x+1)$-transformed.

2.3.2. Trophic indices and water quality

The trophic state and the quality of the waters were determined by means of various indices: Carlson's (1977) Trophic State Index (TSI) for fresh waters, the Trophic Index (TRIX) proposed by Vollenweider et al. (1998) for coastal waters, and the set of criteria adopted by the European Environmental Agency (EEA, 2001) for freshwater, marine and transitional environments.

For classifying the trophic state, the TSI uses two criteria independently: algal biomass as chlorophyll *a* concentration ($\mu g\, l^{-1}$) and total phosphorus ($\mu g\, l^{-1}$). The following equations are applied (Carlson and Simpson, 1996):

$$\mathrm{TSI(CHL)} = 9.81\ \ln(\mathrm{CHL}) + 30.6$$

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

The range of the index is approximately 0–100.

The TRIX (Vollenweider et al., 1998) combines nutrients (DIN and TP expressed in $\mu g\, l^{-1}$ of N and P respectively), chlorophyll *a* concentration ($\mu g\, l^{-1}$) and oxygen (absolute deviation from saturation). The equation is the following:

$$\mathrm{TRIX} = [\log_{10}(\mathrm{Chl}a \times |\mathrm{DO\%}| \times \mathrm{N} \times \mathrm{P}) + 1.5]/1.2$$

Numerically, it varies in a range from 0 to 10. Penna et al. (2004) saw a correspondence between the TRIX and the quality of coastal waters. According to this criterion the quality of the waters varies from high, characteristic of a system with low productivity and a low trophic level (TRIX: 2–4), to poor, typical of a highly productive system with high trophic levels (TRIX: 6–8).

The relationships of the DIN ($NH_4^+ + NO_3^- + NO_2^-$) and SRP concentrations to the N/P ratio are the criteria for evaluating water quality adopted by the European Environmental Agency (EEA, 2001). The quality of the waters is expressed in various categories, from to *good* to *bad*.

3. Results

3.1. Physical and chemical water parameters

The average values of the parameters considered are shown below, together with the relative standard deviations as the spatial variation of each variable.

The water temperature followed a seasonal trend, with minimum values in December (10.3 ± 0.79 °C) and maxima in July (27.54 ± 0.99 °C) (Fig. 2a). Salinity was characterised by marked fluctuation over the period, ranging between 10.57 ± 2.47 in January and 28.40 ± 5.39 in August (Fig. 2b). The lowest value (6.01) was recorded in January at station 12, and the highest value (37.56) in August near the Acquarotta channel (station 2). Dissolved oxygen (% of saturation) was lower (85.54 ± 19.95%) in July and higher in (120 ± 24.85%) in November (Fig. 2c). The pH values ranged from 8.28 ± 0.28 in August to 8.72 ± 0.33 in April (Fig. 2d).

The highest concentrations of NH_4^+ were recorded in December (4.91 ± 6.05 μM), whereas the lowest values were seen in June (1.09 ± 0.86 μM) (Fig. 3a). The highest concentrations of NO_2^- were recorded in December (3.01 ± 7.65 μM); the lowest values were seen in August (0.33 ± 0.57 μM) (Fig. 3b). NO_x concentrations were higher in December (38.66 ± 47.05 μM) and lower in August (2.35 ± 3.54 μM) (Fig. 3c). The highest SRP values were recorded in December (0.90 ± 1.61 μM), and the lowest in May (0.08 ± 0.15 μM) (Fig. 3d). SRSi concentrations were greater in December (70.06 ± 71.72 μM) and lower in August (20.46 ± 11.91 μM) (Fig. 3e). The total nitrogen concentration in December was 56.73 ± 25.73 μM and 92.58 ± 10.40 μM in July (Fig. 3f). The total phosphorus concentration ranged from 0.36 ± 0.19 μM in September to 1.07 ± 0.40 μM in August (Fig. 3g). TSM was lowest in September (9.26 ± 2.57 mg l^{-1}); rising in summer, it reached a maximum in July of 41.78 ± 10.69. POM also peaked in July, at 13.7 ± 2.8 mg l^{-1}, and dropped to a minimum in December (3.73 ± 1.16) (Fig. 3h).

3.2. Phytoplankton

The average chlorophyll *a* concentration was lower in winter (minimum: 1.93 ± 0.65 μg l^{-1}, December) and higher in summer (maximum: 8.99 ± 15.04 μg l^{-1}, June), reaching a peak of 56.32 μg l^{-1} at station 1. The average concentration of phaeopigments was also lower in winter–spring, with the minimum in March (0.03 ± 0.01 μg l^{-1}), and higher in summer, with the maximum in June (2.56 ± 2.59 μg l^{-1}) (Fig. 4a). Phytoplankton abundance varied from 0.017 × 10^6 cell l^{-1} in September (station 10) to 11.21 × 10^6 cell l^{-1} in May (station 3) (Fig. 4b). Cryptophyceae, Cyanophyceae and Euglenophyceae were dominant at stations 1, 3 and 6 in winter and spring, whereas in summer the Dinophyceae prevailed. The phytoplankton community at station 10 was dominated by Cryptophyceae, Cyanophyceae and Euglenophyceae in September and October; by Dinophyceae in November and December; by Bacillariophyceae from January to May and by Dinophyceae in summer (Fig. 4c).

3.3. Spatio-temporal variability

Table 1 shows the results of the variance analysis and, where the variance was significant, of Tukey's test (HDS). Significant time differences were seen for temperature, salinity and dissolved oxygen ($p < 0.0001$); NO_x ($p = 0.01$); silicate ($p = 0.02$); total nitrogen, TSM and POM ($p < 0.0001$); chlorophyll *a* ($p = 0.002$) and phaeopigments ($p < 0.001$). Significant spatial differences were observed for pH, ammonium, nitrite and nitrate ($p < 0.0001$), phosphate ($p = 0.01$), silicate ($p = 0.01$), chlorophyll *a* ($p < 0.05$) and phaeopigments ($p < 0.05$). Tukey's test showed highest NH_4^+, NO_x and SRSi concentrations at the stations near the inputs and specifically in the eastern part. For chlorophyll *a* concentrations temporal variability was found in summer.

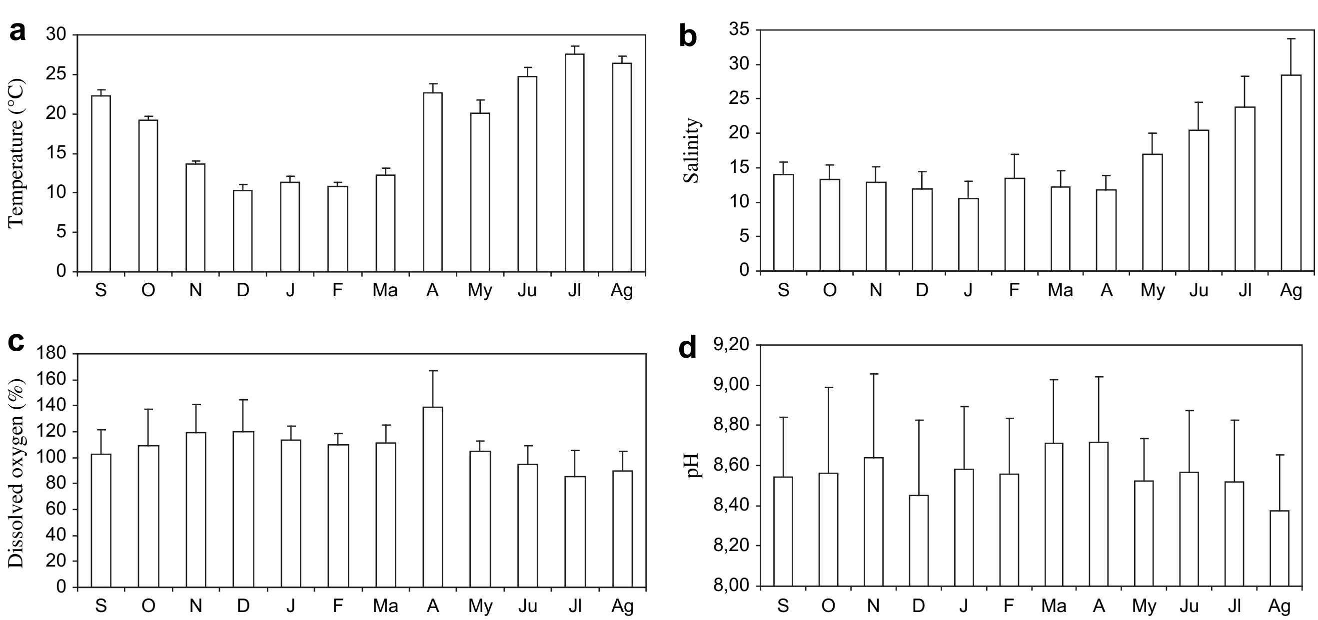

Fig. 2. Monthly variation of physical–chemical parameters in Lesina Lagoon (mean ± SD): temperature (a); salinity (b); oxygen saturation percentage (c); pH (d).

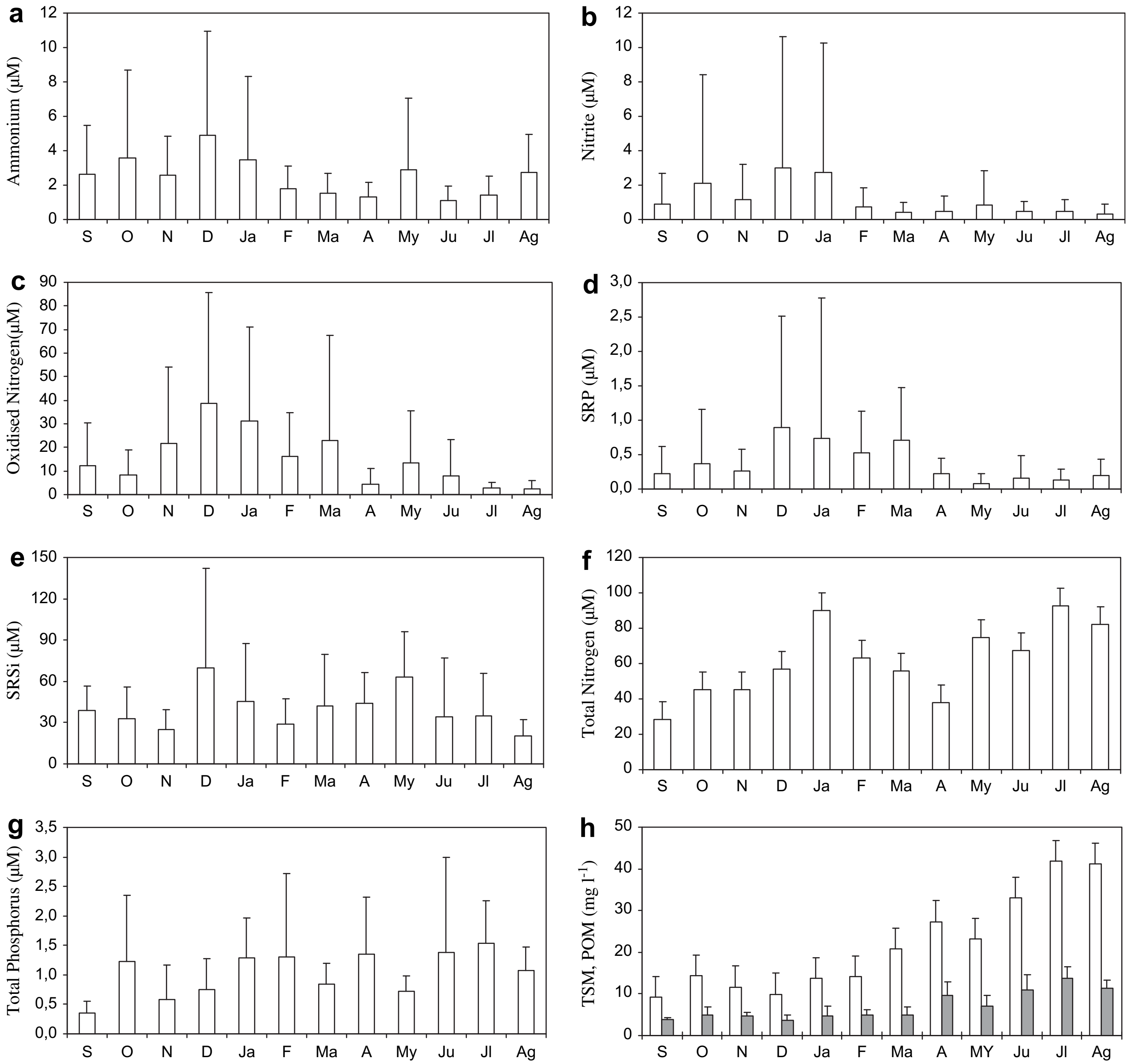

Fig. 3. Monthly variation in Lesina Lagoon (mean ± SD) of: ammonium (a); nitrite (b); oxidised nitrogen (c); soluble reactive phosphorus (d); soluble reactive silicate (e); total nitrogen (f); total phosphorus (g); total suspended matter (□) and particulate organic matter (■) (h).

Factorial correspondence analysis (FCA) of 15 parameters (T, S, DO, pH, NO_2^-, NO_x, NH_4^+, SRP, SRSi, TN, TP, chl *a*, TSM, POM, PHE) was conducted in order to describe their space–time variability. The first component (F1) explains 25.42% of the percentage of variance, the second component (F2) 23.3% and the third (F3) 12.33%. The first component is defined by the correlations between temperature, salinity, dissolved oxygen, chlorophyll *a*, TSM and POM and can be regarded as the trophic gradient of the system. Increased temperature and salinity accelerate mineralisation of organic matter and cause dissolved oxygen to fall. In summer, the dissolved nutrients lead to high levels of chlorophyll *a*.

The relative weight of nitrite and phosphate, both associated with microbial decomposition in sediments and inorganic absorption, establishes the biochemical component. Ammonium concentrations may be affected by various biogeochemical and biological processes, including uptake by phytoplankton, excretion by zooplankton and bacterial remineralisation, since both of them can be stimulated by high temperature conditions. The inverse correlation between ammonium and temperature suggests that increased ammonium is related to resuspension of sediments caused by winds or the jet of springs flowing into the lagoon as pulse events, especially during winter. High silicate concentrations are related to freshwater inputs. In fact, the positive correlation of ammonium, nitrite, oxidised nitrogen, phosphate, silicate and total nitrogen in the second factor probably indicates the importance of the freshwater inputs with their inorganic and organic load (see Fig. 5).

The third component highlights the positive correlation between total phosphorus and pheophytin, produced by the decay of phytoplankton assemblages, confirming that the availability of

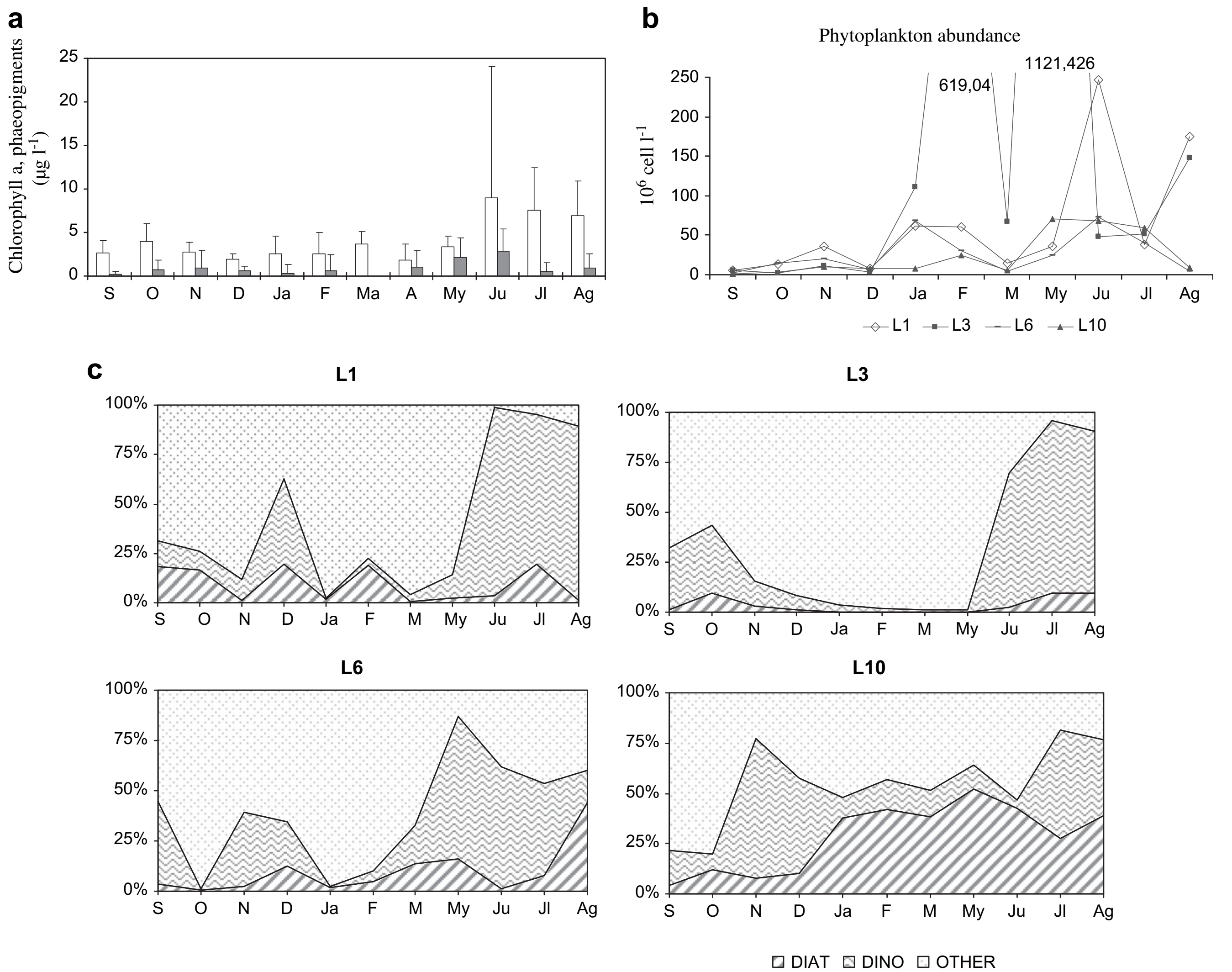

Fig. 4. Monthly variation (mean ± SD) of chlorophyll *a* (□) and phaeopigments (■) (a); evolution of total phytoplankton abundance (b); relative frequency of each phytoplankton components in the four sampling sites (c).

Table 1
Results (*F* values and *p* values according to Bonferroni's correction) of analyses of variance performed on hydrological characteristics, considering temporal and spatial factors. Results of Tukey's (HDS) test for each variable are also shown indicating spatial–time variability (characters in bold represent the highest values).

Parameters	Months		Stations		HSD (months)	HSD (stations)
df	11		11			
T	560.230	0.001	0.062	12	**Jl** > **Ag** > **Ju** > A > S > My > O > N > Ma > Ja > F > D	–
S	36.872	0.001	1.773	0.780	**Ag** > **Jl** > **Ju** > **My** > S > F > O > N > Ma > D > A > Ja	–
DO	7.011	0.001	0.379	11.544	A > D > N > Ja > Ma > F > O > My > S > Ju > Ag > Jl	–
pH	1.068	4.704	15.222	0.001	–	1 > 5 > 3 > 6 > 2 > 4 > 8 > 12 > 11 > 7 > 9 > 10
NH_4^+	1.469	1.804	6.156	0.001	–	**7** > **9** > 11 > 12 > 10 > 4 > 1 > 8 > 3 > 2 > 6 > 5
NO_2^-	0.759	8.148	7.987	0.001	–	**7** > **9**>(10 – 5)
NO_x	2.265	0.175	6.195	0.001	–	**10** > **7** > **9** > **11** > **4** > **12** > 1 > 8 > 6 > 2 > 3 > 5
SRP	1.219	3.360	2.332	0.144	–	–
SRSi	2.144	0.255	3.003	0.012	–	–
TN	6.630	0.001	2.321	0.145	Jl > Ja > Ag > My > Ju > F > D > Ma > N > O > A > S	–
TP	1.536	1.507	1.822	0.672	–	–
Chl *a*	2.874	0.024	1.948	0.468	**Ju** > **Jl** > **Ag** > O > Ma > My > N > Ja > F > S > D > A	–
PHE	3.343	0.005	1.890	0.552	**Ju** > **Jl** > **Ag** > O > Ma > My > N > Ja > F > S > D > A	–
TSM	35.951	0.001	0.953	5.904	**Jl** > **Ag** > **Ju** > A > My > Ma > O > F > Ja > N > D > S	–
POM	29.739	0.001	0.9	6.504	**Jl** > **Ag** > **Ju** > A > My > F > Ma > O > Ja > N > S > D	–
N:P	1.3125	2.688	3.174	0.012	–	**10** > **12** > **11** > **4** > **9** > **7** > 8 > 6 > 5 > 2 > 3 > 1
N:Si	1.424	2.028	1.359	2.388	–	–
TN:TP	2.428	0.104	6.138	0.001		**10** > 12 > 11 > 8 > 6 > 5 > 9 > 3 > 2 > 4 > 1 > 7

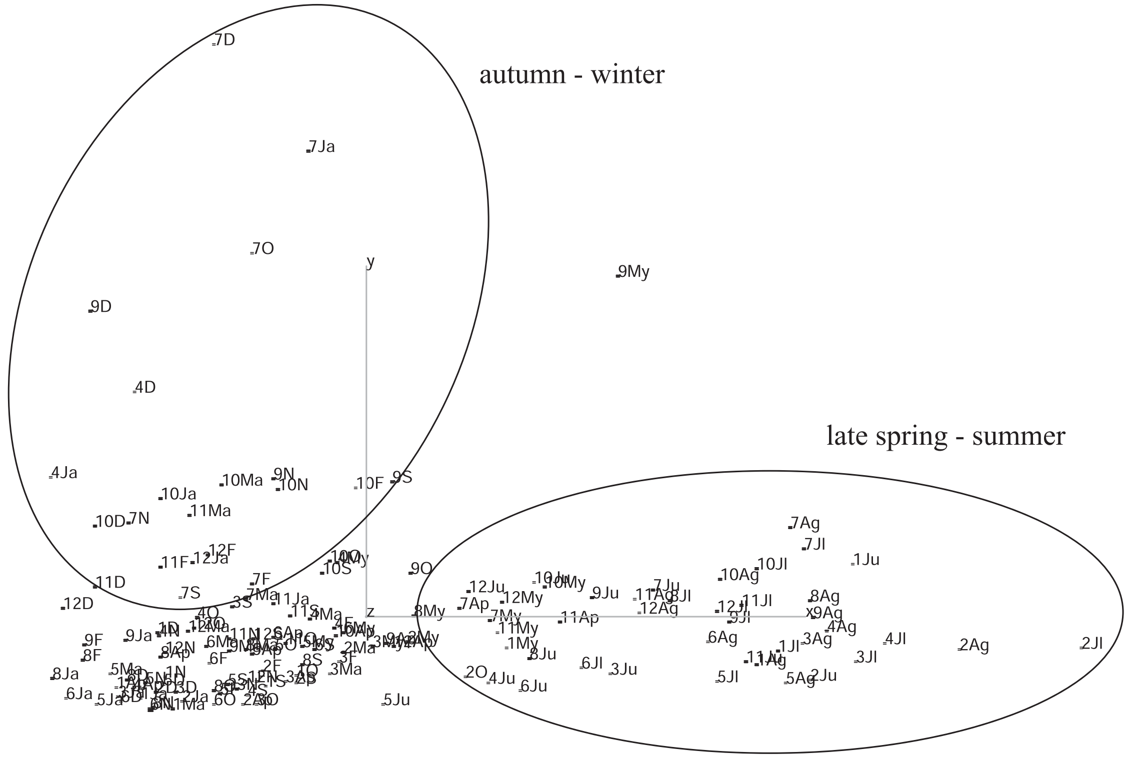

Fig. 5. Factorial component analysis performed on environmental parameters. Station codes: first character corresponds to the sampling site and subsequent ones to the month of sampling. Cumulative percentage variance on first three factors is 61%.

phosphorus in the system is also probably linked to processes of mineralisation (Table 2).

The results of the CCA are shown in Fig. 6. The projections of the environmental vectors are not very different from those derived from FCA. The dinoflagellates were correlated with high temperatures and high concentrations of TSM, TP and PHE, which coincide with the component corresponding to the mineralisation processes described in the multivariate analysis. Abundance of diatoms is correlated with high nitrate concentrations, low salinity and high Si/N ratios. The remaining phytoplankton taxa (Cryptophyceae, Cyanophyceae and Euglenophyceae) dominate the phytoplankton community in the presence of low DIN, SRSi, salinity and TP values.

Table 2
Factor weights of the multivariate factor analysis in Lesina Lagoon (numbers in bold represent significant hydrologic parameters for a specific factor).

Parameter	Factor I	Factor II	Factor III
T	**0.774**	−0.057	0.084
S	**0.865**	−0.113	0.097
DO	−0.579	0.106	0.256
pH	−0.225	−0.619	0.328
NH_4^+	−0.112	**0.852**	0.011
NO_2^-	−0.128	**0.788**	0.095
NO_x	−0.283	**0.760**	−0.125
SRP	−0.239	**0.452**	0.219
SRSi	−0.171	**0.718**	0.087
TN	0.451	**0.540**	0.141
TP	−0.002	0.079	**0.823**
Chl *a*	**0.457**	0.053	0.512
TSM	**0.875**	−0.099	0.236
POM	**0.868**	−0.113	0.209
PHE	0.185	−0.002	**0.721**
% Variance	25.423	23.300	12.336

4. Discussion

4.1. Abiotic and biotic variability

As with other lagoonal systems, the geomorphological characteristics of the Lagoon of Lesina, understood as a *filter* (*sensu* Cloern, 2001), modulate the equilibrium between energy flows (solar radiation, wind and tides); matter flows (rains, inputs from surface and subterranean waters and runoff); and mechanisms that guide processes inside the system itself (such as mineralisation of organic matter, accumulation of autotrophic biomass and sedimentation/resuspension rates, i.e., vertical flows). The combination of these factors and the internal system processes that derive from them determines space–time fluctuations in the hydrological heterogeneity of lagoonal ecosystems (Medina-Gómez and Herrera-Silveira, 2003, 2006; Coelho et al., 2007).

The space–time variability of hydrological heterogeneity in the Lagoon of Lesina is strongly influenced by meteorological and climatic conditions. Due to its shallow depth, the characteristics of the mass of water in the lagoon are mainly influenced by atmospheric events, continental inputs and tidal exchanges. Temperature was lowest in winter, as was salinity, due to the abundant rains and continental inputs. In summer, when freshwater inputs fall and higher temperatures result in faster evaporation, the highest salinity values were recorded, also due to the entry of seawater through the tidal channels. Dissolved oxygen reached its lowest values (65% of saturation) near the freshwater inputs in summer. In this period, the area near the Acquarotta channel had greater salinity and lower dissolved oxygen, probably due to the decomposition of macroalgal biomass which had accumulated above all in that part of the basin. The area near the Schiapparo channel had lower salinity due to the greater flow of fresh water into the eastern part. The higher dissolved oxygen in this part was probably due to

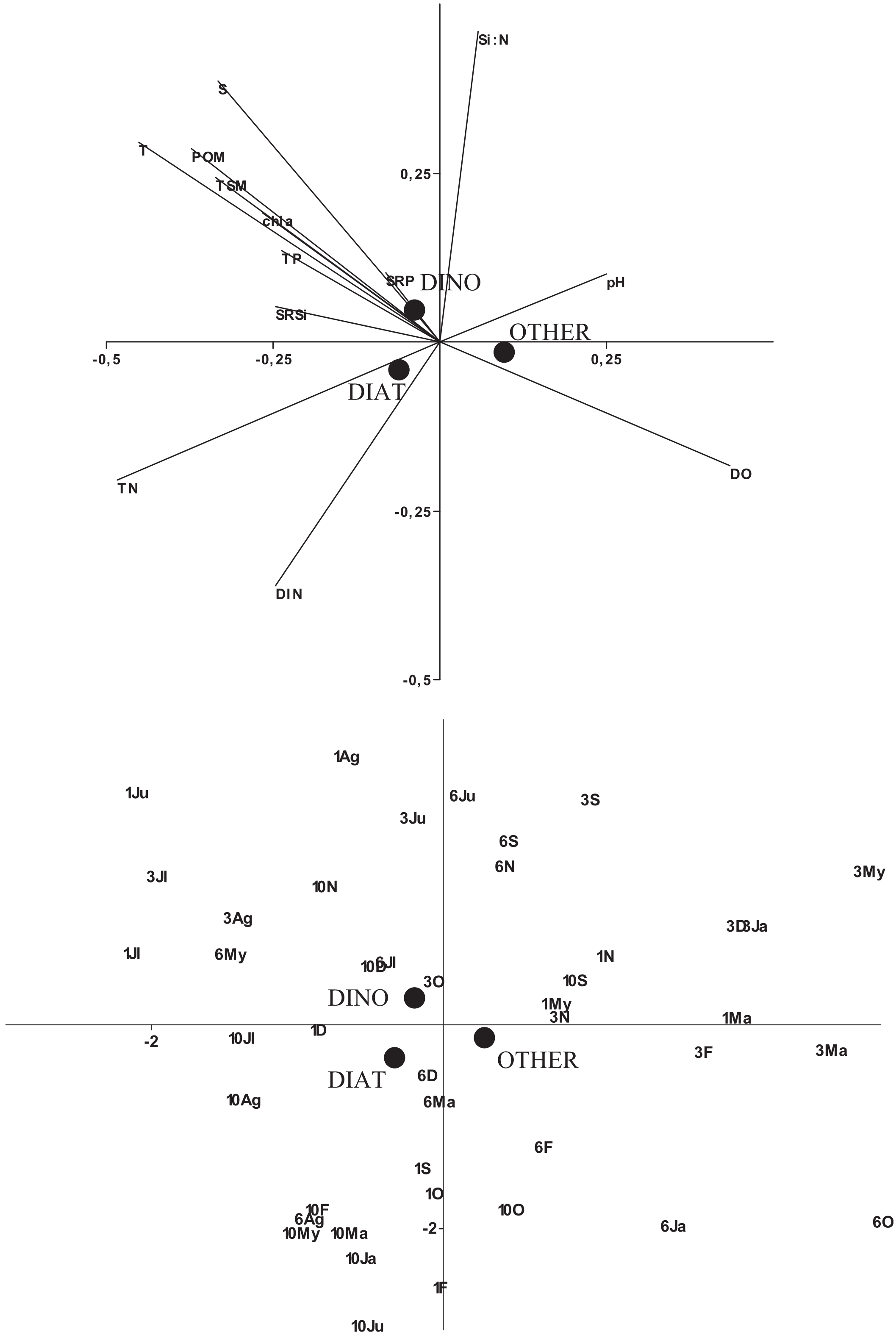

Fig. 6. Canonical Correspondence Analysis performed with the phytoplankton components. Phytoplankton: DIAT – diatoms; DINO – Dinophyceae; OTHER – Cryptophyceae, Chlorophyceae, Cyanophyceae, flagellate algae (a). Station codes: first character corresponds to the sampling site and subsequent ones to the month of sampling (b).

the efficiency of tidal exchange. The flow of water in the tidal channels is due to tides, winds and river inputs. The movement of the waters contributes to the dispersion of dissolved and suspended material, and the channels thus play a fundamental role in flushing the lagoon and maintaining the quality of the waters (Smith, 2001).

During the rainy period, the Lagoon of Lesina is characterised by intense fluctuations and broad spatial variability in both the chemical–physical parameters and the concentration of matter and nutrients. This suggests that the spatial behaviour of the system is strongly influenced by both environmental processes and the biogeochemical processes that derive from them. In the Lagoon of Lesina, the high concentrations of DIN in winter and spring may depend on agricultural runoff and/or the microbial regeneration of organic compounds of marine or terrestrial origin (Newton et al., 2003). Factorial analysis highlighted the contribution of surface fresh waters during winter and spring, especially at the stations in the south-eastern part of the basin where the main freshwater inputs are located. The greater relative weight of NH_4^+ in the second component (Fig. 5) at the stations subjected to discontinuous inputs highlights the preponderance of processes dependent on oxide-reduction. Labile organic matter transported in winter by the surface fresh water streams is deposited on the surface of the sediments and its decomposition contributes to the production of NH_4^+, as described by Brogueira and Cabeçadas (2006). Although precipitation in the sampling period was greatest in late winter–spring, the highest concentrations of dissolved inorganic nutrients were recorded in December, suggesting that the effect of agricultural runoff was particularly intense, as described by Newton and Mudge (2005) for the lagoon of Ria Formosa. Most agricultural fertilisers contain nitrogen (as ammonium, nitrate and urea) in forms that plants can absorb, but since these are completely soluble in water they are easily washed out of the soil by rainwater or irrigation (Addiscott, 1996). Indeed, the fertilisers used in this area, in which NH_4NO_3 is the dominant ingredient (Franchi and Pelosi, 1998), are usually applied at the beginning of the crops' period of growth, i.e., late summer/early autumn, and are thus carried into the lagoon following the first autumn rains. In addition, the high ammonium load recorded at stations 1 and 9 may be associated with inputs carrying inadequately treated waste waters from intensive aquaculture plants, as happens in the Lagoon of Orbetello (Porrello et al., 2003). Nitrite concentrations were low. The highest concentrations of SRP were also recorded in winter, especially on the southern side of the lagoon near the inputs carrying urban waste waters and runoff.

Nitrogen and phosphorus enrichment in the Lagoon of Lesina is strongly associated with the presence of human activities, as described in numerous systems characterised by limited exchange of waters with the sea (Newton and Mudge, 2005; Pérez-Ruzafa et al., 2005; Viaroli et al., 2005; García-Pintado et al., 2007). The south-eastern part seems to be the system's main source of exogenous nitrogen, due to the presence of the main freshwater inputs. Phosphate concentrations are also probably linked to biogeochemical sedimentary processes and the resuspension of sediments due to turbulence (Gikas et al., 2006). The DIN:SRP ratio, used to measure the relative importance of N and P as factors limiting primary production (Conley, 2000), follows a spatial gradient ($p = 0.001$), with high N/P ratios at the stations near the inputs and in the eastern part, which thus appears to be phosphorus-limited. The western end of the lagoon has ratios of about 16, due to the weaker freshwater input and high concentrations of phosphorus (Table 1; Fig. 7a), as also described by Puigserver et al. (2002) in a coastal system.

Silicate, a structural ingredient in the cells of diatoms and silicoflagellates (Valiela, 1995), fluctuated during the sampling period (Fig. 7b) and was present in high concentrations in the eastern part of the lagoon and at a central station. The negative correlation of SRSi with salinity ($p < 0.01$) and its positive correlation with dissolved inorganic nitrogen ($p < 0.001$) suggest that the silicate is of exogenous origin. The Si/N ratio, generally >1, did not exhibit significant space–time variation (Table 1) and the diatoms were able to compete with the other algal classes that require lesser quantities of Si (Officer and Ryther, 1980; Conley et al., 1993). The ratio was <1 mainly in winter at the stations near the freshwater inputs. Although the ratio was never less than 0.1, the phytoplankton community seemed to be essentially dominated by phytoflagellates and dinoflagellates (Radach et al., 1990), especially in summer in the stations where the ratio was much higher than 1. In contrast, diatoms were dominant in the eastern area, which is most influenced by freshwater inputs (Fig. 4). A high Si/N ratio was also observed in the central area, due to the presence of subterranean waters with which algae of the *Chara* genus are associated (Bornette et al., 1996).

Phytoplankton biomass (chl *a*) in the Lagoon of Lesina did not exhibit significant spatial variation, nevertheless the greatest values at the stations near the freshwater inputs were recorded, it was not found to be correlated with inorganic nutrient concentrations. This is not in agreement with traditional models that assume a direct response of phytoplankton to nutrient load (Phase I *sensu* Cloern, 2001) and at the same time indicates that phytoplankton do not control nutrient concentrations (Pérez-Ruzafa et al., 2005). In the Lagoon of Lesina, autotrophic biomass may be controlled by a number of factors including the availability/limitation of nutrients, mineralisation processes, water residence times, tidal exchanges and the macrophytobenthic component in the basin as a whole. The complex relations between the variables are an important component of the system understood as a filter, *sensu* Cloern (2001), able to modulate changes in nutrient load in the ecosystem. Chl *a* concentrations were greater in summer samplings and were positively correlated with temperature, salinity, TSM and POM (Fig. 5). Total nitrogen is correlated with chl *a* ($p < 0.001$) as documented by numerous authors (Molot and Dillon, 1991), and in the case of the Lagoon of Lesina it may be mainly correlated with the remineralisation of organic matter. TSM and POM were positively correlated with TN ($p < 0.001$), especially in summer samplings. The study highlighted significant positive correlations between chl *a* and TP ($p < 0.005$), between TP and TSM ($p < 0.01$) and between PHE and TP ($p < 0.0001$). In shallow-water systems the sediment is strongly influenced by surface turbulence, and the relationships between TP, chl *a* and PHE may be closely dependent on the presence of suspended material. The high turbidity due to solid material in suspension may reduce the production of chl *a* by limiting both the availability of light for photosynthesis and soluble phosphorus, the latter as a result of the adhesion of phosphate to solid particles (Ferris and Tyler, 1985). The correlation observed between chl *a*, TP, TN, PHE and TSM shows that TSM in the Lagoon of Lesina may be a factor that makes organic matter available for mineralisation processes.

In addition, the high TN:TP ratios (on average > 50) confirm that phosphorus may be the main nutrient controlling the development of phytoplankton biomass (Attayde and Bozelli, 1999). Furthermore, the TP dynamics depend on P sequestration by algae, followed by its release into the water column, either directly or after being deposited in the sediments (Walsh et al., 1994).

Other factors that may explain the increased phytoplankton biomass in summer samplings are water residence time and the presence of macrophytobenthos. In shallow lagoons, autotrophic benthic organisms which can regulate pelagic–benthic coupling are dominant. Microalgae and macroalgae are important in controlling flows of dissolved inorganic and organic nitrogen between the sediments and the water column (Tyler et al., 2003). Presumably, during winter when the water residence time is lower, the phytoplankton cells spend less time within the lagoon and cannot take full advantage of the high nutrient concentrations. Therefore allochthonous inputs of inorganic nutrients fertilise the lagoon and promote the production of macrophytes at the expense of phytoplankton, as described for the Lagoon of Dzilam by Medina-Gómez and Herrera-Silveira (2006). In coastal lagoons the subsequent

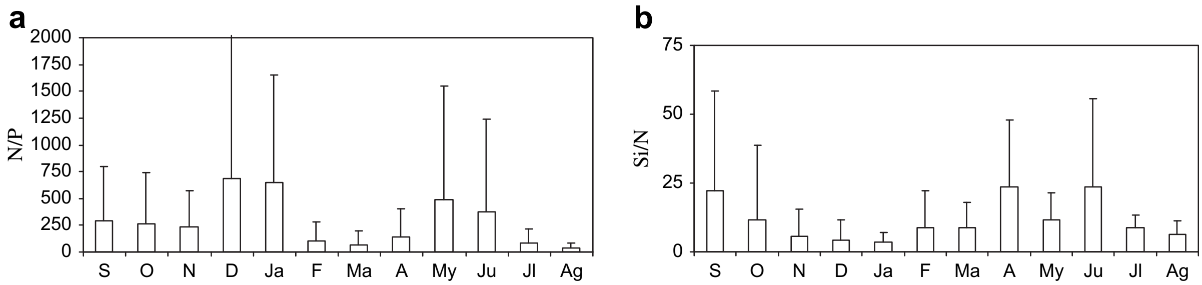

Fig. 7. Monthly variation (mean ± SD) of N/P ratio (a); Si/N ratio (b) in Lesina Lagoon.

decomposition of submerged aquatic vegetation and associated processes are believed to be an efficient mechanism for the retention of nutrients (Holmer and Olsen, 2002). In the Lagoon of Lesina the dynamics of nutrients and autotrophic biomass also seem to be influenced by the presence of macrophytobenthos, as described for the Lagoon of Venice (Sfriso and Marcomini, 1996). In the eastern area, *Ruppia cirrhosa* is associated with low chl *a* values and *Chaetomorpha* sp. is associated with low N values, particularly NH_4^+, as described by Menéndez (2005). The algae *Chara* sp. are most frequent in the central basin, where the lowest concentrations of nutrients and chl *a* are found; generally associated with more oligotrophic waters (Palma-Silva et al., 2004), they are able to inhibit phytoplankton photosynthesis (Anthoni et al., 1980). The western area of the lagoon, characterised by higher concentrations of phosphorus and chl *a*, was observed to host extensive meadows of *Gracilaria* sp. during the sampling period. The phosphorus released as a result of the decomposition of macroalgal biomass is promptly re-used by phytoplankton (Gomez et al., 1998). In this environment, phosphorus is stored and subsequently released by sediments (including those transported from the catchment area), mainly in the form of phosphates, especially in more saline environments; as a limiting factor it plays a significant role in regulating phytoplankton assemblages in the water column above (Gomez et al., 1998; Jordan et al., 2008).

Diatoms are typical of more turbulent environments (Wong et al., 2007); in the Lagoon of Lesina they are correlated with high nitrate levels, low salinity and low Si/N ratios, which indicate dependence on freshwater inputs. Dinoflagellates, characteristic of calmer waters (Wong et al., 2007), are positively correlated with the T, S, TP and TSM. The group containing Cryptophyceae, Cyanophyceae, Euglenophyceae, Chlorophyceae and flagellate algae competes with diatoms and dinoflagellates (Fig. 6). Whereas in oligotrophic conditions, smaller phytoplankton such as flagellate algae are dominant with respect to larger phytoplankton such as diatoms and dinoflagellates, as the level of nutrients increases the opposite is seen (Pérez-Ruzafa et al., 2002). In the western and central parts of the lagoon, Cryptophyceae, Cyanophyceae, Euglenophyceae, Chlorophyceae and flagellate algae were the dominant forms of phytoplankton from September 2006 to May 2007, replaced in summer by dinoflagellates. In the eastern area, a succession was observed in the phytoplankton assemblage from dinoflagellates at the beginning of winter to diatoms in late winter, lasting until the beginning of summer when the dinoflagellates become dominant again (Fig. 4). Opportunist dinoflagellates usually dominate over diatoms in periods of nutrient enrichment and low turbulence (Pérez-Ruzafa et al., 2002), as happens in summer in the Lagoon of Lesina. In addition, although other factors such as temperature or turbidity may play a part in determining the succession of dominant phytoplankton species, the inversion in the dominance of the main phytoplankton taxonomic groups is usually linked to eutrophication of anthropic origin (Balkis, 2003; Hashimoto and Nakano, 2003), as seems to be the case in the Lagoon of Lesina.

4.2. Trophic state and water quality

A number of indicators, indices and models have been developed to assess the trophic state and quality of waters in freshwater and marine–coastal systems. The most widely-used indicators are nutrient concentration, chlorophyll *a* and dissolved oxygen concentration. Complex models combine information from various sectors of the ecosystem, from chemical–physical parameters to phytoplankton, zooplankton, benthos, macroalgae and sediments (Newton et al., 2003). Since coastal lagoons are systems with complex spatio-temporal interaction, identifying descriptors as required by the WFD and determining their individual scales of variation represent a tough challenge for the scientific community (Basset and Abbiati, 2004; Basset et al., 2006).

In the Lagoon of Lesina, inorganic nitrogen concentrations do not exceed the threshold values (DIN < 15 mg l^{-1} N–1072 μM N; Nitrates < 25 mg l^{-1} N–403 μM N; Ammonium < 1 mg l^{-1} N–71 μM N) set out in the European Directives concerning water quality (74/440/EEC; 76/464/EC; 78/659/EC; 80/68/EC; 98/15/EC). Other parameters considered in the European directives include dissolved oxygen (>5 mg O_2 l^{-1}), pH (5–9), total suspended solids (<35 mg l^{-1}) and total phosphorus concentration (<1 mg l^{-1} P–32 μM P); of these, in the Lagoon of Lesina only pH and total suspended solids were higher than the required ranges. A pH of more than 9 was recorded at station 1, probably due to the discharge of domestic waste waters and the greater concentrations of phosphorus (Coelho et al., 2007).

Given that the Lagoon of Lesina is a system that oscillates between hyposaline and hypersaline conditions, the trophic state and the quality of the waters were assessed by applying various classification criteria commonly used for fresh, marine–coastal and transitional waters (Carlson, 1977; Vollenweider et al., 1998; EEA, 2001; Bricker et al., 2003; Penna et al., 2004).

Carlson's (1977) Trophic State Index (TSI), used for lakes, classifies the Lagoon of Lesina as a system tending towards oligomesotrophic–mesotrophic ($30 < \text{TSI} < 50$) in terms of chlorophyll *a* concentration, and mesotrophic–eutrophic ($40 < \text{TSI} < 60$) in terms of total phosphorus concentration. Regarding the first of these parameters, the TSI has higher values in summer samplings, probably due to the low level of water in the basin, which enables primary producers to use the nutrients available in the whole of the water column, as suggested by Newton et al. (2003). In the case of TP concentration, the system shifts towards higher trophic levels in winter samplings, due to the more abundant rains and greater organic load. The stations near the freshwater inputs have higher trophic values for both parameters. The two variables (chl *a* and TP) have also been found to yield differing TSI values in other cases (Rakocevic-Nedovic and Holler, 2005; Coelho et al., 2007), where chlorophyll *a* may be able to predict the development of algal biomass (Carlson and Simpson, 1996).

According to the United States National Estuarine Eutrophication Assessment (Bricker et al., 2003), in terms of its average DIN and TP concentrations, the Lagoon of Lesina is in the "medium eutrophication" category ($0.1\ mg\,l^{-1} \leq DIN < 1\ mg\,l^{-1}$; $0.01\ mg\,l^{-1} \leq TP < 0.1\ mg\,l^{-1}$), whereas its average annual chlorophyll *a* concentration puts it in the "low eutrophication" category ($\leq 5\ \mu g\,l^{-1}$).

According to Vollenweider et al. (1998) and Penna et al. (2004), the Lagoon of Lesina is in a state of *good* to *mediocre* water quality (with a score of about 54%), characteristic of moderate to high productivity with a high trophic level (TRIX: 4–6). The stations situated near the freshwater inputs have *poor* water quality, especially in winter months. Although TRIX was developed as a water quality index for coastal waters, and has been applied mainly to the Adriatic and Tyrrhenian (Vollenweider et al., 1998; Giovanardi and Vollenweider, 2004; Penna et al., 2004), recently it has also been used for coastal lagoons (Coelho et al., 2007). Since lagoon–sea exchanges are limited in volume and the parameters in the lagoon typically have different values from the nearby coastal waters, TRIX needs to be adapted in order to provide a more specific set of classification criteria for coastal lagoons (Newton and Mudge, 2005). In fact, the European Environmental Agency (EEA, 2001) suggests that the TRIX ranges should be calibrated for different regions or areas. It would be also be a good idea to implement the Directive by including the morphological homogeneity of the basins as a separate variable.

Using the criteria laid down by the European Environmental Agency (EEA, 2001, 2003), water quality is assessed with reference to the N/P ratio and the concentration of dissolved nitrogen and orthophosphate. Autumn and winter (monthly averages) are characterised by *poor* and *bad* water quality in terms of N concentration; N/P ratios in this period are high, confirming that the waters are affected by agricultural runoff. The summer months have *good* water quality, partly due to the fall in DIN. December and January also have *poor* water quality in terms of phosphate concentration. The rest of the samples are mostly of *good* quality (Fig. 8a and b). The application of the EEA system to the sampling sites (annual average) is shown in Fig. 8c and d. The stations in the eastern area and near the freshwater inputs are of *poor* and *bad* quality, whereas the stations in the western area, where the N/P ratios are lower, indicating a lower presence of nitrogen and a greater presence of phosphorus, are of *good* quality. Considering phosphate concentrations, only stations 7 and 4, which are affected by runoff waters from farmland and urban waste waters, were of *bad* quality, whereas all the others, even the most easterly ones and those with high N/P ratios, were of *good* quality (Fig. 8d).

Generally speaking, lagoons are heavily influenced by their freshwater inputs; in the Lagoon of Lesina, these inputs include runoff from farmland and wastewater discharges from livestock and domestic sources and vary over the course of the year. Communication between lagoon and sea, especially in Mediterranean lagoons, is thus essential for the natural maintenance of the ecosystem. Due to the artificial origin of the tidal channels of the Lagoon of Lesina, it is of a crucial importance to avoid their periodical silting up, in order to improve tidal dilution and the flow of nutrients, thereby minimising the effects of accumulation (Newton and Mudge, 2005; Coelho et al., 2007) and preserving the nursery function of these ecosystems.

In terms of trophic state and water quality, application of the various indices places the Lagoon of Lesina in a range of categories. However, these indices describe real conditions linked to the space–time variability of the system, as highlighted mainly by the stations located closest to the freshwater inputs.

Although in spatial terms the water quality passes from a good state to a bad one, the basin is in dynamic equilibrium, and this equilibrium is favoured by the exchange of matter and energy with the surrounding environment. Cases of anoxia, which occur in those points where water quality is bad, are therefore minimised by the effect exerted on the system by the surrounding environment. When one or more factors, of natural or anthropic origin, undergo a sharp variation, the equilibrium is broken, with severe effects for the biota such as anoxia or massive colonisation by opportunistic species.

Indeed, at the beginning of summer 2008, the whole of the western part of the Laguna of Lesina was affected by severe anoxia for about a month. Symptoms included white waters, hydrogen sulphide fumes, dissolved oxygen concentrations below $5\ mg\,l^{-1}$ and the death of fish. As well as high temperatures and the absence

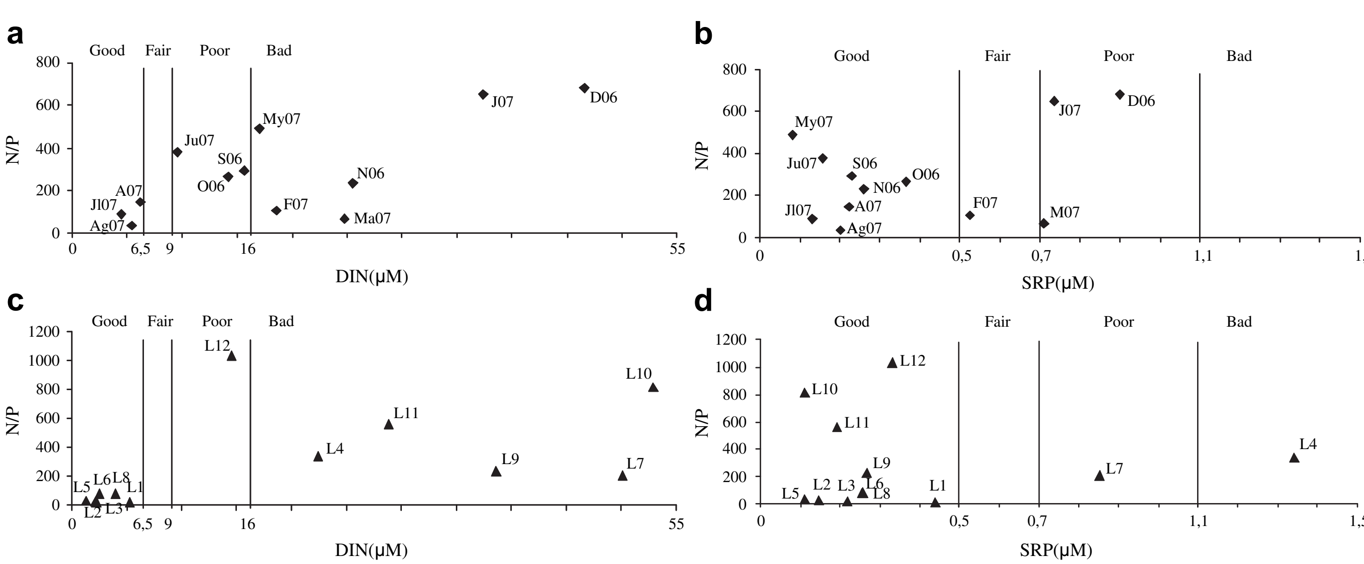

Fig. 8. The N/P ratio plotted against the total dissolved nitrogen concentration: monthly and spatial variation (a and b); and the N/P ratio against the phosphate concentration: monthly and spatial variation (c and d). In each case, key values by which the water quality can be classified according to the EEA (2001) are included.

of wind, the causes of this event may have included the microbial decomposition of macroalgal biomass, composed mainly of Gracilariaceae, which over the previous two years had formed a homogeneous carpet. Another cause was the temporary partial blockage of the Acquarotta channel, through which seawater enters the lagoon especially in summer (Ficca et al., 1995). In contrast, the eastern part of the lagoon was not affected by the dystrophic conditions since the system was maintained in equilibrium by the greater contribution of fresh waters and the efficiency of lagoon–sea exchange.

Numerous measures could be adopted to limit and/or resolve the severe eutrophication which is a potential cause of anoxia, such as physically removing the algal biomass, controlling waste water discharges and avoiding the silting up of the tidal channels (Lardicci et al., 2001; Newton and Mudge, 2005).

5. Conclusions

The fate of lagoonal environments is closely dependent on the actions of human beings, who, by affecting the main forcing factors such as the accumulation of nutrients, the introduction of opportunistic species, the protection of sea–lagoon exchanges and commercial activities such as fishing and aquaculture, determine the quality of the environment.

The role of these environments has always been seen as essential in modulating the interaction between catchment area and coastal waters. This is also the case with the Lagoon of Lesina, thanks to various qualitative and quantitative mechanisms linked to the different types of forcing factors affecting the system. In addition, the geomorphological characteristics of the lagoon modulate the equilibrium between energy flows, matter inputs and the mechanisms governing the system's internal processes.

The combination of numerous forcing factors and internal system processes determines the space–time fluctuations in the hydrobiology of this ecosystem. The space–time variability, strongly influenced by meteo-climatic conditions, is determined by exogenous factors including freshwater inputs and runoff, and by endogenous ecological processes including the mineralisation of organic matter.

In the Lagoon of Lesina, the autotrophic phytoplankton biomass depends not so much on the nutrient load as on the remineralisation of organic matter (for which the necessary vector may be suspended particulate matter), the limitation of phosphorus and the presence of macrophytes distributed throughout the basin which compete effectively for nutrients. Consequently, the complex relationships between the variables and the forcing factors constitute an important component of the system, able to modulate effects arising from the input of organic matter and nutrients and the resulting internal dynamics, and to influence its dynamic equilibrium.

Concerning the trophic state and quality of the waters, the Lagoon of Lesina is classified into different categories depending on which the various indices are applied. However, these indices all describe conditions governed by the temporal and spatial variability of the system, and highlight the distinctive properties of the areas near the freshwater inputs.

Application of trophic and water quality indices with a view to establishing patterns of environmental quality in lagoons requires a clear specification of ecological forcing factors and an experimental evaluation of their relative importance, in order to avoid over-generalisation or over-simplification of the complex space–time interactions. The approach of treating all transitional aquatic environments in the same way is too broad, and is thus unable to account for the real ecological categories (both structural and functional) observed in the various geographical contexts, especially concerning the effects of characteristic forcing factors such as tidal range and inputs from the catchment area, which control the hydrological balance of the lagoon.

However, the results obtained in this study and the observed dystrophic event – indicating a breakdown in the dynamic equilibrium of the system – demonstrate that the monitoring plan for each ecosystem, including the frequency of sampling (Carstensen, 2007) and the number and position of the stations is important in the application of the WFD. A well-thought-out monitoring plan helps to identify elements that may cause a breakdown in the equilibrium of the ecosystem; it is also fundamental to lagoon management, which must take account of nutrient load (waste waters and/or fertilisers), use of surface waters for irrigation and the functioning of tidal channels. All these assume natural evolution of lagoonal environments; however, since such environments are directly affected by anthropic activities, it is human beings who determine their trophic state and environmental quality.

The interpretation of environmental heterogeneity and relationships between environmental variables is fundamental to improve our knowledge of the dynamics of coastal lagoons and thus to the planning and implementation of management strategies designed to guarantee the conservation of the ecosystem and the sustainable exploitation of its resources.

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