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RESEARCH ARTICLE

Fluctuations of physicochemical characteristics in sediments and overlying water during an anoxic event: a case study from Lesina lagoon (SE Italy)

Antonietta Specchiulli[1*], Raffaele D'Adamo[1], Monia Renzi[2,3], Fabio Vignes[4], Adele Fabbrocini[1], Tommaso Scirocco[1], Lucrezia Cilenti[1], Marisa Florio[1], Paolo Breber[1], Alberto Basset[4].

[1]National Research Council - Institute of Marine Science, Department of Lesina (FG), Via Pola 4, 71010 Lesina (FG), Italy.
[2]Department of Environmental Science, University of Siena, Via Mattioli 4, 53100 Siena, Italy.
[3]Research Centre in Lagoon ecology, fishery and aquaculture (Ecolab), Polo Universitario Grossetano, University of Siena, Via Lungolago dei Pescatori s.n., 58015 Orbetello (GR), Italy.
[4]Department of Biological and Environmental Sciences and Technologies, University of Salento; Centro Ecotekne, sp Lecce-Monteroni - 73100 Lecce, Italy.
[*]Corresponding author: Phone +39 0882992702; Fax +39 0882991352; E-mail antonietta.specchiulli@fg.ismar.cnr.it

Abstract

1 - Coastal lagoons are vulnerable systems often affected by severe anoxic events, also as direct consequence of excessive accumulation of organic matter. During these events, also in function of their location and geomorphology, they are characterized by large temporal and spatial fluctuations of physical and chemical conditions.

2 - Results presented in this work are related to an anoxic event occurred in Lesina lagoon (SE Italy) in summer 2008. The main aim is to describe the short-term dynamics of physicochemical characteristics of both sediment and overlaying water, inside and outside the critical area of the anoxic event.

3 - Nine sampling campaigns were carried out on a weekly basis between the first week of July and the second week of September 2008. The water sampling was performed at three sites located in the western side (inside and outside area interested by crisis) and in the eastern zone of the lagoon, close to the communicating channel with the sea, while the sediment samples were collected in four stations, including two sites within crisis area and two outside. At each occasion T, S, pH, DO, nutrients and chl a were measured in water and TOC, TN, TP, TFe and ORP were performed on superficial sediments.

4 - In relation to the water, wide temporal fluctuations of physico-chemical parameters were observed for the site inside the anoxic area, which exhibited the highest concentrations of chl a at the beginning of the observation period, with a peak of biomass of 180 $\mu g.L^{-1}$ followed by saturation of oxygen higher than 100%. A strong significant difference among sampling sites was observed in relation to nutrients; in particular, the site within the anoxic area exhibited broader concentration ranges of both SRP and TP than the other sites, while TN was in general higher in the site outside. Significant differences related to the sedimentary variables were also observed between anoxic and reference area, except for TP which was homogeneously distributed during the observation period in all the sites. Disturbed sites showed the highest contents of TOC at the beginning of the observation period (July 2008), TN contents almost two times lower and mean contents of TFe higher than those obtained in control sites. All sites were characterized by highly reducing conditions despite the well oxygenated conditions of the overlying waters.

5 - These findings highlight wider time-related fluctuations of physico-chemical parameters (for both

sediments and overlying water) within anoxic area than in control area, but, at the same time, stress the significant role of the factor "space" (anoxic sites and control sites) for the observed physico-chemical diferences. Also, this integrated approach between sediments and water indicates sediment-related source of nutrients in the anoxic overlying water, suggesting that quick mineralization processes and nutrient release in mid-term happened mainly in disturbed area. The high biomass production observed along the overlying water within the anoxic area at the beginning of the observation period was related to this greater availability of nutrients in disturbed area.

Keywords: Anoxic event; Eutrophication; Mediterranean coastal lagoon; Multivariate analyses; Nutrient; Sediment.

Introduction

Transitional waters and coastal zones are very heterogeneous complex systems with different characteristics, which react in different ways to the nutrient and organic matter loadings from watersheds, as well as to global changes (Crossland *et al.*, 2005; Eisenreich, 2005). Among these, coastal lagoons are vulnerable systems, located between the land and the sea, enriched by both the marine and continental inputs and are among the most productive aquatic ecosystems (Nixon 1988), playing an important economic role. They are often nutrient rich (Cauwet 1988; Colombo 1977) as a result of input by rivers and recycling between sediment and water column (Nowicki and Nixon 1985; Schleyer and Roberts 1987). Consequently, they are affected by severe anoxic events, often as direct consequence of excessive accumulation of organic matter (Nixon, 1995; Guyoneaud *et al.*, 1997; Karakassis *et al.*, 2000; Lardicci *et al.*, 2001; De Falco *et al.*, 2004; Magni *et al.*, 2008). During these events, also in function of their location, coastal lagoons are characterized by large temporal and spatial fluctuations of physical and chemical conditions (Kjerfve, 1994). Although several studies have investigated the relationships among chemical variables of sediments and between biotic and abiotic factors in the period following the anoxic events (Lardicci *et al.*, 2001; Magni *et al.*, 2005, 2008), still little information is available on the dynamics occurring during the anoxic crisis and the development of the systems during these negative events. This study is based on a such anoxic event occurred in Lesina lagoon located within a protected area and tourist resort of the SE Italy, Gargano National Park (established by Italian law 394/91). By the way, the lagoon, especially the eastern part, is internationally known as a breeding area for many migratory bird species and it was classified as site of special protection according to the International Council for Bird Preservation (ICBP). The lagoon historically has a high economic rating due to fishery activity. This study aims to: I) describe the temporal fluctuations of physico-chemical variables of both sediment and overlying water inside and outside the critical area; II) assess differences among sites with respect to the observed parameters; III) identify the factor (location, week of sampling, month sampling) responsible of this difference; IV) assess any relationship among variables in both sediment and overlying water.

Methods

Main characteristics of the study area

Lesina lagoon is a shallow ecosystem (mean depth of 0.8 m) located in the southeastern Italy (41.88° N; 15.43° E) (Figure 1) with an area of 51 Km^2 and two narrow openings to the Adriatic sea, Acquarotta and Schiapparo. Since 1997, the flow of the both channels has been partially reduced by wooden barriers and weir systems called "lavorieri". These barriers have been preventing the water exchange and the free passage of fishes between the lagoon and sea. The lagoon is characterized by a

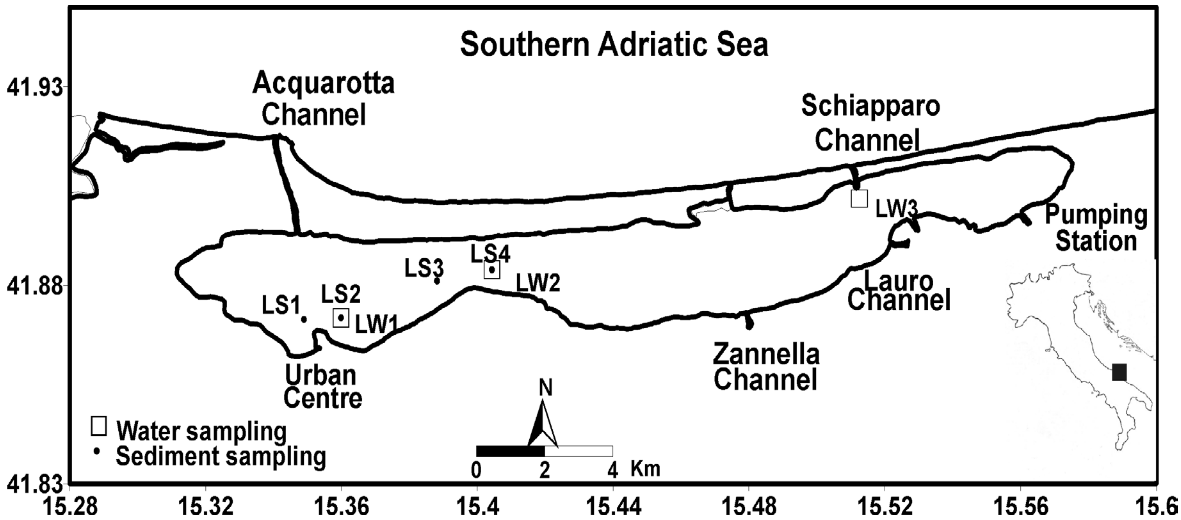

Figure 1. Study area and location of the sampling sites in Lesina lagoon (south-eastern coast of Italy).

drainage basin covering around 460 Km^2 and mainly exploited for intensive aquaculture and agricultural (vegetable farming and wheat) activities (Franchi and Pelosi, 1998). The water retention time varies in time from 30 (Autumn) to 300 (Spring) days (Manini et al., 2005; Giordani *et al*., 2008). The south-western part of the lagoon is affected by urban wastewaters and waters drained by the intensive aquaculture farms, while the south-eastern side receives freshwater inputs collecting agricultural drainage waters from two pumping stations and is mainly colonised by large meadows of *Zostera noltii* and *Ruppia* sp.. A massive growth of the macroalga *Valonia aegagropila* C. Agardh occurred in the western zone during the period 1998-1999, because of very low precipitations and elevated evaporation rates (Manini *et al*., 2005), followed up to date, by a homogeneous carpet composed mainly of Gracilariaceae. The moderate water exchange with the sea and the combination of sea water and freshwater inflows, produce a salinity gradient in the lagoon with decreasing values from west to east. Strong seasonal variations of temperature (3°C in winter and 26-32°C in summer) and salinity (from 5 to 51 psu, practical salinity unit) characterize Lesina lagoon (Marolla *et al*. 1995; Priore *et al*. 1994). The macrobenthic community assemblages reflect the environmental gradients due to marine and freshwater inputs. The high salinity fluctuations are considered as the dominant factor able to determine a spatial segregation in benthic macrofauna assessment (Specchiulli *et al*., 2008). The lagoon is generally dominated by fine-grained sediments in both the western and central sides. In particular, the sediments are mainly composed of silt and clay which account for about half of the western part component and are rich in organic matter, while the zone closed to the Schiapparo channel (eastern side), is covered by the coarsest component (51% sand) (Specchiulli *et al*., 2008).

Experimental design

Following an extensive survey performed on the whole basin in June 2008, 10 sampling campaigns were carried out on a weekly basis, between the first week of July and the second week of September 2008. These surveys and their frequency were related to the presence of a pronounced dystrophic event occurred in Lesina lagoon near the urban centre, as highlighted by high temperatures, complete absence of wind, surface waters white coloured and satellite images (Vignes *et al*.). Both sediment and overlaying water were sampled in order to investigate the main physical and chemical characteristics. The water sampling was performed at three sites located in the western side (LA1 inside and LA2 outside area interested by crisis) and in the eastern zone of the lagoon, close to the Schiapparo channel (LA3), while the sediment samples were collected in four stations, including two sites within crisis area (LS1 and LS2) and two outside (LS3 and LS4) (Figure 1). On each occasion, physico-chemical parameters (temperature, salinity, pH and dissolved oxygen) were measured along the water column using a multiparametric probe (YSI 556 MPS multiprobe). Triplicate water samples were collected to analyse both dissolved and total nutrients and phytoplanktonic biomass. Superficial sediment samples (0-5cm) were collected in triplicate, using a box-corer (15x15x15cm), for total organic carbon (TOC), total nitrogen (TN), total phosphorus (TP) and total iron (TFe) analysis. *In situ* measurements of oxidation-reduction potential (ORP) were performed using a Hanna Instrument HI 9026 portable pH/millivolt meter inserted directly into the cored sediment. All samples were transported to the laboratory under refrigerated conditions.

Analytical methods

Water samples analysis

For dissolved nutrients (nitrogen ammonium $N\text{-}NH_4^+$, nitrogen nitrous $N\text{-}NO_2^-$, nitrogen nitric $N\text{-}NO_3^-$, soluble reactive phosphorus SRP and soluble reactive silicate SRSi), the water samples were filtered through 0.45 μm cellulose acetate filters after the sampling and stored at -20°C before the analyses by a Bran+Luebbe QuAAtro flow analyser, according to the methods reported by Grasshoff (1999). Total nitrogen (TN) and total phosphorus (TP) were analysed from unfiltered water samples by the continuous-flow Autoanalyzer, after a persulfate digestion at 120°C with an initial pH of 13 and final pH of about 2, according to Grasshoff (1999) methods. Total chlorophyll *a* (chl *a*) was determined as an estimate of phytoplankton biomass and it was measured as extractable chl *a* from water samples. Aliquots of 100-300 mL were filtered through Whatman GF/F fibre filters (pore: 0.7 Am) by using a swinnex apparatus (Millipore Corporation, Bedford, USA), frozen and analysed by spectrofluorimeter (Shimadzu Mod. RF 1501) following the methods described by Yentsch and Menzel (1963).

Sediment analysis

Sediments samples collected for physicochemical analyses were pooled and further treated as one single sample. A portion of each sample was dried at 60°C in oven until constant weight and weighed. Dried sediments were analysed in order to determine: total organic carbon (TOC, %), total phosphorus (TP, $\mu g.g^{-1}$) and total nitrogen (TN, $\mu g.g^{-1}$). Samples were analysed in triplicate; average and standard deviation (SD) were calculated. Chemicals and reagents were analytical grade and glassware was carefully washed to avoid sample contamination. Determinations of TOC and TN were performed by direct total flash combustion using a CHNS Elemental Analyzer with a thermo-conductivity detector TCD (Perkin Elmer, mod. CHN/O 200) according with ICRAM methods (2001). TOC analyses were performed by preliminary acid digestion with HCl 19% and total flash combustion. TP determination was carried out according to Aspilia *et al*. (1976) methods, by acid digestion and next quantification using a spectrophotometer UV-VIS (Perkin Elemer, mod 6505) after colorimetric reaction.

Recoveries and reproducibility were checked by analysing procedural blanks and reference materials purchased from: National Institute of Standard and Technologies (NIST - NewJork waterway Sediment SRM1944), Institute of Environmental Chemistry Academy Sinica Beijing China (Tibet soil), and NIST (Estuarine Sediment SRM1646a). Analytical blanks were prepared prior to the testing samples using the same analytical procedures. A solvent blank was analysed every 15 samples to check the response of the instrument detection. Standard reference materials were analysed in statistical replicates (n=10) to calculate averages and standard deviation (SD) of recoveries. Measured average recoveries were the follows: TOC 101.4% (0.33 SD, SRM1944), TN 94.4% (0.005 SD, Tibet Soil), TP 98.9% (3.30 SD, SRM1646a). Concentrations were not recovery corrected. Limit of detection (LOD) was defined as the average blank (n=10) plus three standard deviation (SD) and were the following: 0.01% for TC, TOC, and TN while 0.001% for TP. The concentration of iron (Fe) was determined in 0.1 g of dried sediment sample using the ferrozine assay described by Lovely and Phillips (1987). Iron was extracted from sediments with a ferrozine solution in HEPES buffer (pH adjusted to 7.0) after 1 h samples were centrifuged at 2000 rpm for 10 minutes and 20 μL of the liquid fraction was sampled and mixed with 980 μL of the ferrozine reagent, and absorption was measured spectophotometrically performing lectures at 562 nm and using $FeCl_3*6H_2O$ as reference solutions for instrument calibration.

Statistical analysis

All data were analysed using univariate and multivariate methods in order to evaluate differences between sites inside and outside the area interested by the anoxic event, individuate the factor (variable) responsible of this difference and assess any relationship among variables in both sediment and overlying water. Spearman rank order R was separately performed on each raw data matrix (water and sediment) in order to explore correlations among variables. Box-and-Whisker diagrams were used to highlight the spatial and temporal fluctuations of water parameters and the significant differences between means were tested by ANOVA analysis (F test and p). Differences among sediment sites were analysed with the non parametric Mann-Whitney *U*-test. These statistics were performed using Statistica 7.0 (StatSoft Inc., Oklahoma-USA). In order to investigate correlations and similarities among variables, a multivariate exploratory data analysis technique, namely Principal Components Analysis (PCA) was used. Non metric Multi Dimensional Scaling (*nm*MDS) was applied to better explore variables dissimilarities, performing runs by the application of the Kruskal stress formula 1, imposing minimum stress of 0.01 and restarting the process 50 times. The significant differences were also tested by the Anosim (Analysis of similarities) test statistic R one-way (otherwise defined by a single factor). The system behaviour in relation to the date of sampling, the month of sampling, the location of sampling sites was used as "factor". The results obtained on sediment samples were superimposed (with the bubble techniques) to water results in order to evaluate significances among sediment observed segregation and variables concentration in water. Multivariate analyses were developed using Primer-E Software package v6.0 (Plymouth Marine Laboratory, UK) according with Clarke and Warwick (2001).

Results

Water variables

Mean values and standard deviations of the overlaying water parameters measured at the three selected stations of Lesina lagoon during the anoxic event are shown in Table 1.

Table 1 - Mean values and standard deviations of the overlaying water parameters measured at the three selected stations (LW1, LW2 and LW3) of Lesina lagoon during the anoxic event.

	n	LW1		LW2		LW3	
		mean	s.d.	mean	s.d.	mean	s.d.
T (°C)	10	26.5	3.58	25.9	3.2	25.91	3.08
S (psu)	10	25.6	3.28	22.4	1.4	23.10	1.86
DO (%)	10	81	42.66	93	11.32	106.00	12.79
pH	10	7.9	0.55	8.36	0	8.39	0.38
N-NH_4^+ (μg.L^{-1})	27	2.81	6.33	2.03	1.67	3.80	1.99
N-NO_2^- (μg.L^{-1})	27	1.1	2.22	0.41	1.16	8.96	6.73
N-NO_3^- (μg.L^{-1})	27	0.74	1.47	0.25	0.8	0.71	1.36
SRP (μg.L^{-1})	30	0.24	0.27	0.12	0.13	0.10	0.11
SRSi (μg.L^{-1})	30	18.16	17.48	53.41	41.11	47.33	28.19
TN (μg.L^{-1})	18	9.71	6.71	22.16	13.78	36.34	19.65
TP (μg.L^{-1})	18	3.04	1.07	0.74	0.63	0.53	0.70
chl *a* (μg.L^{-1})	27	79.08	52.56	2.51	2.15	1.88	1.65

For the explanation of variables see the text in the section "Methods"

Water temperature at the three sampling sites ranged within narrower intervals in the first week of July 2008 (27-28.7°C) than in the first week of August (27.3-31.2°C), reaching similar values in all the stations at the end of the observation period (18.5-18.9°C) (Figure 2a). Maximum value of the temperature was recorded at the site LW1 (31.2°C) and were slightly higher than those observed at both the sites LW2 and LW3 in the first two weeks of August. The salinity range was 20-30 psu with the lowest values (20-22 psu) registered at the beginning of the observation period and the highest values (29.98 psu) in September at the inner site (Figure 2b). Wider temporal fluctuations of salinity were observed for the site LW1 than for the others stations, as also highlighted by higher standard deviations in Table 1. In general, pH was on the alkaline side, ranging from 7.05 to 8.80. Lower values of pH were observed at the end of the observation period for all the three stations, but wider fluctuations of values characterized the site within the anoxic area, ranged from 7.05 to 8.70. The dissolved oxygen concentrations in the site LW1 varied from 29% to 153% saturation, while narrower intervals were observed in the sites LW2 (71-105%) and LW3 (85-121%) (Figure 2c). Chlorophyll *a* concentrations measured in the overlying water were extremely high (Table 1) at the inner site of the anoxic area (LW1). Moreover, the site LW1 exhibited broader concentration ranges of chlorophyll *a* than the other sites with the highest concentrations reached in the first week of July (180 μg.L^{-1}) and in the first week of September (134 μg.L^{-1}) (Figure 2d). Comparable values of chlorophyll a were obtained in the other two sites, which showed little difference between the beginning and the end of the observation period, with levels ranging from 0.5 to 4.5 μg.L^{-1}. The three sites exhibited different behaviour in connection to the inorganic nutrient concentrations. Figure 3 shows the median, the percentile 25% and 75%, maximum and minimum concentrations of ammonia, nitrite + nitrate, SRSi, SRP, total P and total N in each sampling site, during the occurrence of the anoxic event. Ammonia

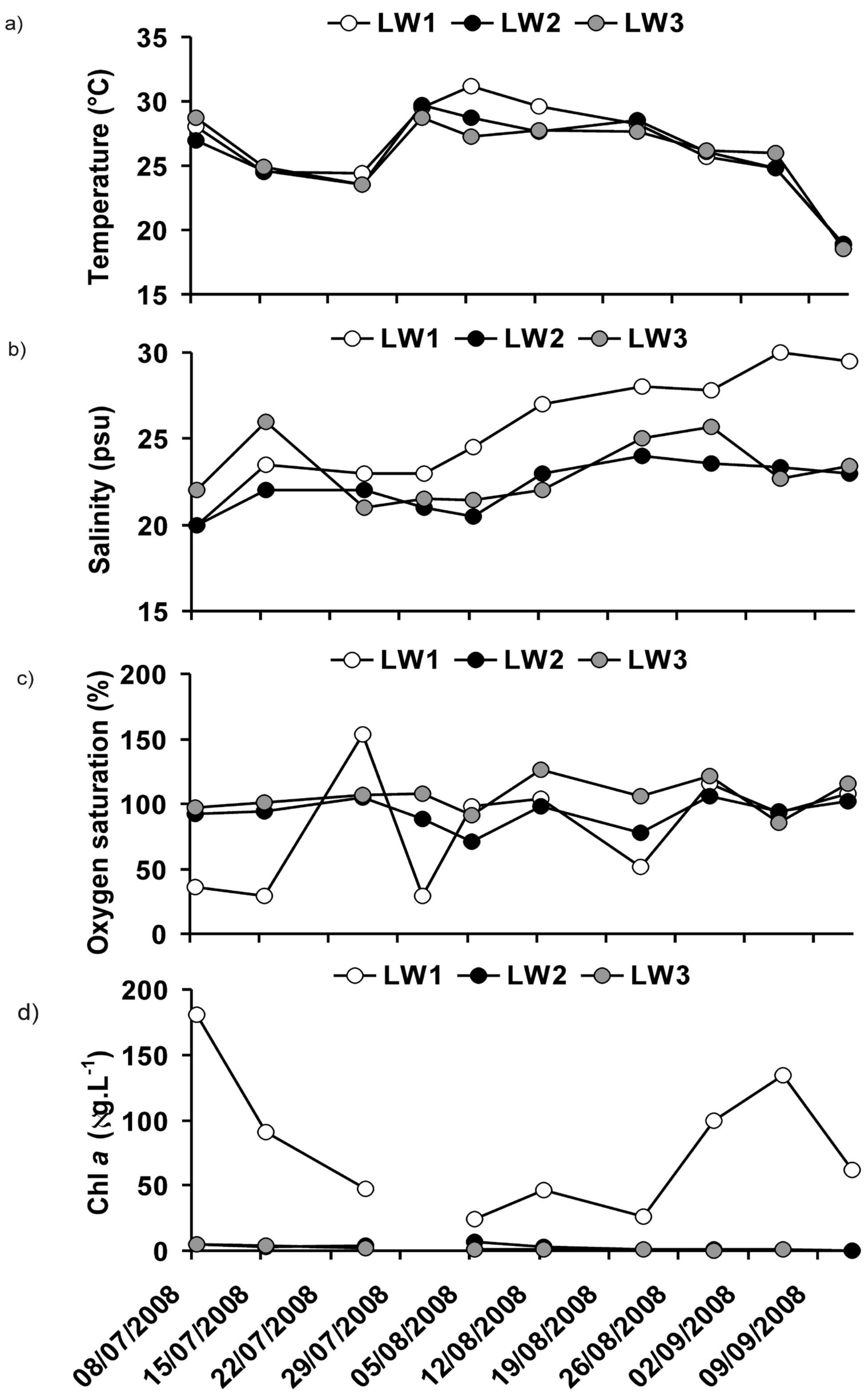

Figure 2. Fluctuations of water temperature (a), salinity (b), oxygen saturation (c) and chlorophyll *a* (d) at the three sampling sites sampled in Lesina lagoon during the anoxic event.

was in general higher in LW3 (4.17 μg.L^{-1}) and significantly ($p<0.01$) higher than in the sites LW1 and LW2 (Figure 3a).

A strong significant difference ($p<0.001$) among sampling sites was observed in relation to nitrite + nitrate which reached the highest values in LW3 (maximum of 17.57 μg.L^{-1} and an outlier value of 25.42μg.L^{-1}) and the lowest concentrations in LW2 (median of 0.31 μg.L^{-1} and an extreme value of 5.30 μg.L^{-1}) (Figure 3b). Although the site LW1 exhibited broader concentration ranges of nitrite (0.047-2.036 μg.L^{-1}) than the other sites, no significant differences were obtained among sites. SRSi concentrations reached the highest values in LW2 (maximum of 120.43 μg.L^{-1}) and although the median value in the site LW2 was comparable with that obtained in the site LW3, a significant ($p<0.05$) difference was obtained between sites in relation to SRSi values (Figure 3c).

The site LW1 (within the anoxic area) exhibited broader concentration ranges of both SRP (0.02-0.68 μg.L^{-1}) (Figure 3d) and

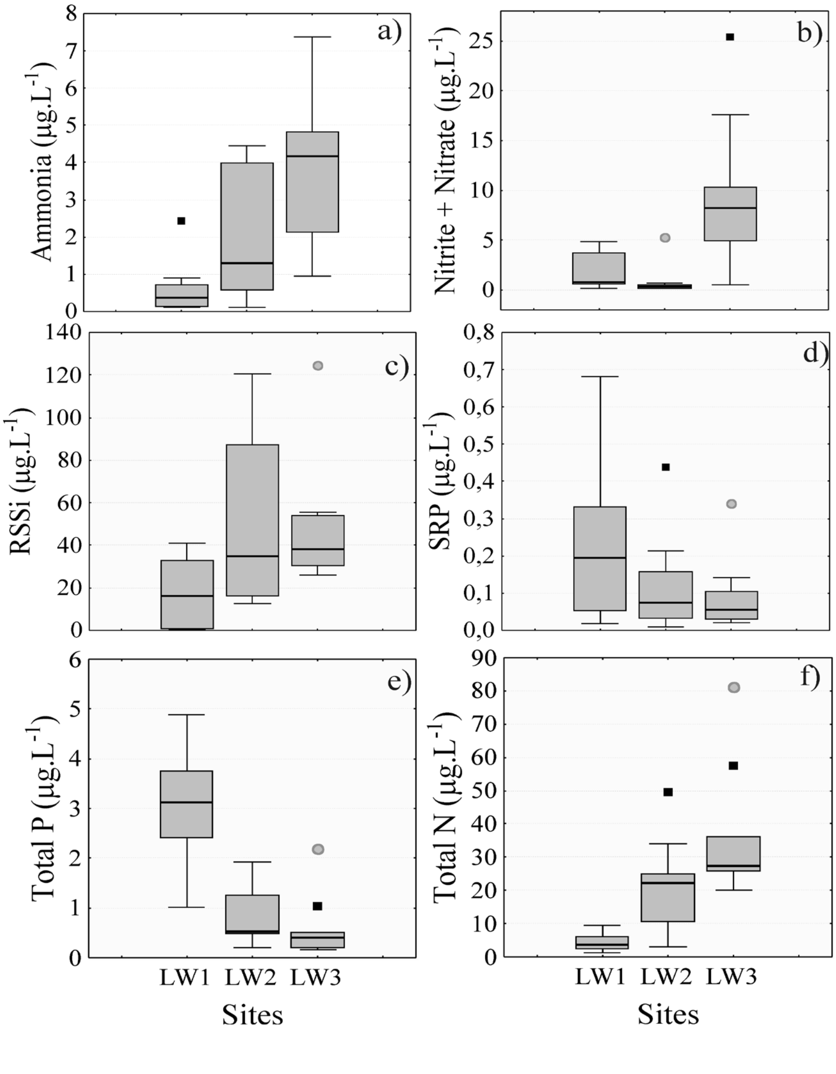

Figure 3. Median, percentile 25% and 75%, maximum and minimum, outliers (black circles) and extreme values (grey circles) of ammonia (a), nitrite + nitrate (b), RSSi (c), SRP (d), total phosphorus (e) and total nitrogen (f) in the three water sampling sites (LW1, LW2 and LW3) during the occurrence of the anoxic event in Lesina lagoon.

TP (1.01-4.88 µg.L^{-1}) (Figure 3e) than the other sites. Moreover, TP values in the site LW1 were significantly ($p<0.001$) higher than in the sites LW2 (0.17-1.92 µg.L^{-1}) and LW3 (0.14-0.51 µg.L^{-1}).
Also, significant differences ($p<0.001$) among sites were observed in relation to TN which was in general higher in the site LW3 (maximum of 36.18 µg.L^{-1} with an extreme value of 81.18 µg.L^{-1}). Spearman rank order correlations between observed variables for overlying water during the study period are shown in Table 2. Temperature showed correlation ($P<0.05$) with oxygen saturation (negative) and pH (positive), while it did not show correlations with nutrients. Salinity showed strong negative correlation with pH ($P<0.001$) and SRSi ($P<0.01$). Dissolved oxygen was only correlated ($P<0.05$) with nitrate and TN. Chlorophyll *a* was strongly correlated ($P<0.001$) with ammonia and TN (negatively) and TP (positively). Nitrogen species showed significant positive correlations amongst themselves, while SRP was well negatively correlated with SRSi ($P<0.001$) and positively with TP ($P<0.05$). A strong negative correlation ($P<0.001$) was observed between total nutrients.

Chemical characteristics of surface sediments
The characteristics of the sediment are given as means of the nine observations performed during the anoxic occurrence (Table 3).
In relation to the total organic carbon (TOC) content, fluctuations of values were observed in all the sites inside and outside anoxic area throughout the period. To better highlight these variations, LS1 and LS4 were chosen as representative of anoxic and control areas, respectively (Figure 4). In the sediment of the sites LS1 and LS2 (within anoxic area), the highest values of TOC (4-4.5%) were determined at the beginning of the observation period (July 2008) (Figure 4a), while the lowest values (2-2.5%) occurred in August, except in the last observation (4 September) when higher percentages of TOC (4% in LS2) were observed. On the contrary, in the site LS3 and LS4 (outside the anoxic area) the lowest values (2% in LS3

Table 2 - Correlation coefficients (Spearman rank order) among water variables (n = 30)

Water	T	Sal	pH	DO	Chl *a*	$N\text{-}NH_4$	$N\text{-}NO_3^-$	$N\text{-}NO_2^-$	SRSi	SRP	TN	TP
T	1.00	-0.14	0.38*	-0.36*	0.09	0.34	-0.25	-0.31	0.26	0.01	-0.3	0.14
Sal		1.00	-0.65***	0.22	0.20	-0.13	-0.13	-0.08	-0.5**	0.28	-0.21	0.13
pH			1.00	-0.09	-0.06	0.22	-0.15	-0.32	0.66***	-0.45*	0.031	-0.17
DO				1.00	-0.17	0.05	0.44*	0.25	0.13	-0.07	0.37*	-0.20
Chl *a*					1.00	-0.69**	-0.33	0.14	-0.19	0.28	-0.86***	0.87***
$N\text{-}NH_4^+$						1.00	0.39*	-0.06	0.0003	-0.15	0.64***	-0.62**
$N\text{-}NO_3^-$							1.00	0.81***	-0.12	0.04	0.62**	-0.26
$N\text{-}NO_2^-$								1.00	-0.36	0.29	0.2	0.25
SRSi									1.00	-0.65***	0.1	-0.32
SRP										1.00	-0.22	0.44*
TN											1.00	-0.83***
TP												1.00

For the explanation of variables see the text in the section “Methods”; *p<0.05, **p<0.01, ***p<0.001.

and 3.5% in LS4) were determined in the first two weeks of the study and the highest concentrations (means of 5.4% in LS3) in August. At site LS4, TOC was consistently at site LS2 in the second week of August (Figure 4b). Differences among sites were not significant for TP (Table 3). Over the whole study area, TP ranged from 0.025% (LS1, 01

Table 3 - Total organic carbon (TOC), redox potential (Eh), total nitrogen (TN), total phosphorus (TP) and total iron (TFe) contents at the superficial sediment of LS1 and LS2 (within anoxic area) and LS3 and LS4 (outside the anoxic area) in Lesina lagoon during the study period.

	TOC (%) n = 9	Eh (mV) n = 9	TN ($\mu g.g^{-1}$) n = 9	TP (%) n = 9	TFe ($\mu g.g^{-1}$) n = 9
LS1	3.62 ± 0.88	-326.11 ± 86.53	0.18 ± 0.04	0.04 ± 0.01	3397.66 ± 758.05
LS2	3.47 ± 0.67	-353.44 ± 78.64	0.19 ± 0.04	0.04 ± 0.01	3256.28 ± 743.50
LS3	3.45 ± 1.52	-338.43 ± 73.50	0.31 ± 0.07	0.05 ± 0.01	2639.27 ± 584.92
LS4	4.78 ± 0.89	-287.18 ± 51.64	0.38 ± 0.04	0.04 ± 0.007	2402.99 ± 852.69
U-test	** (LS1-LS4) (LS2-LS4) * (LS3-LS4)	*** (LS2-LS4) ** (LS3-LS4) * (LS1-LS4)	*** (LS1-LS4) (LS2-LS4) ** (LS1-LS3) (LS2-LS3) * (LS3-LS4)	n.s.	* (LS1-LS3) (LS1-LS4) (LS2-LS4)

Means and ranges (± S.D.) are reported (n = 20 for all the variables). Significant differences among the means are indicated with asterisks: *p < 0.05, **p < 0.01, ***p < 0.001, n.s. = not significant. Mann-Whitney's U-test determined significance.

higher than the other three sites (Table 3). In addition, TOC content in surface sediments varied largely among sites, with a significant difference between LS1-LS4 and LS2-LS4 (*U*-test, $p<0.01$). In relation to TN content, the concentrations were almost two times higher in the sites outside the anoxic area (LS3 and LS4) than in LS1 and LS2 (*U*-test, $p<0.001$) (Table 3, Figure 4b) and significant differences (*U*-test, $p<0.01$) were observed between LS1-LS3 and LS2-LS3, with an opposite pattern of TN values from 17 July to 04 September.

The highest values of TN were measured at LS4 (0.35-0.42 $\mu g.g^{-1}$) for almost the entire period of observation, while the lowest concentrations (0.12 $\mu g.g^{-1}$) were obtained August) to 0.076% (LS3, 11 August). The site LS3 exhibited mean values of TP relatively higher than the other sites (Table 3), but TP concentrations were homogeneously distributed during the observation period in all the sites.

The total iron content (TFe) of the surface sediments ranged between 1127 (LS4, 21 August) and 4262 (LS1, 01 August) $\mu g.g^{-1}$ (Figure 4c). Mean contents of TFe were higher in the sites within the anoxic area than in those outside the area (Table 3).

A moderately significant difference was observed between LS1-LS3 and LS2-LS4 (*U*-test, $p<0.05$), while the contents differed little between sites belonging to the same area. In relation to the ORP, the sites were

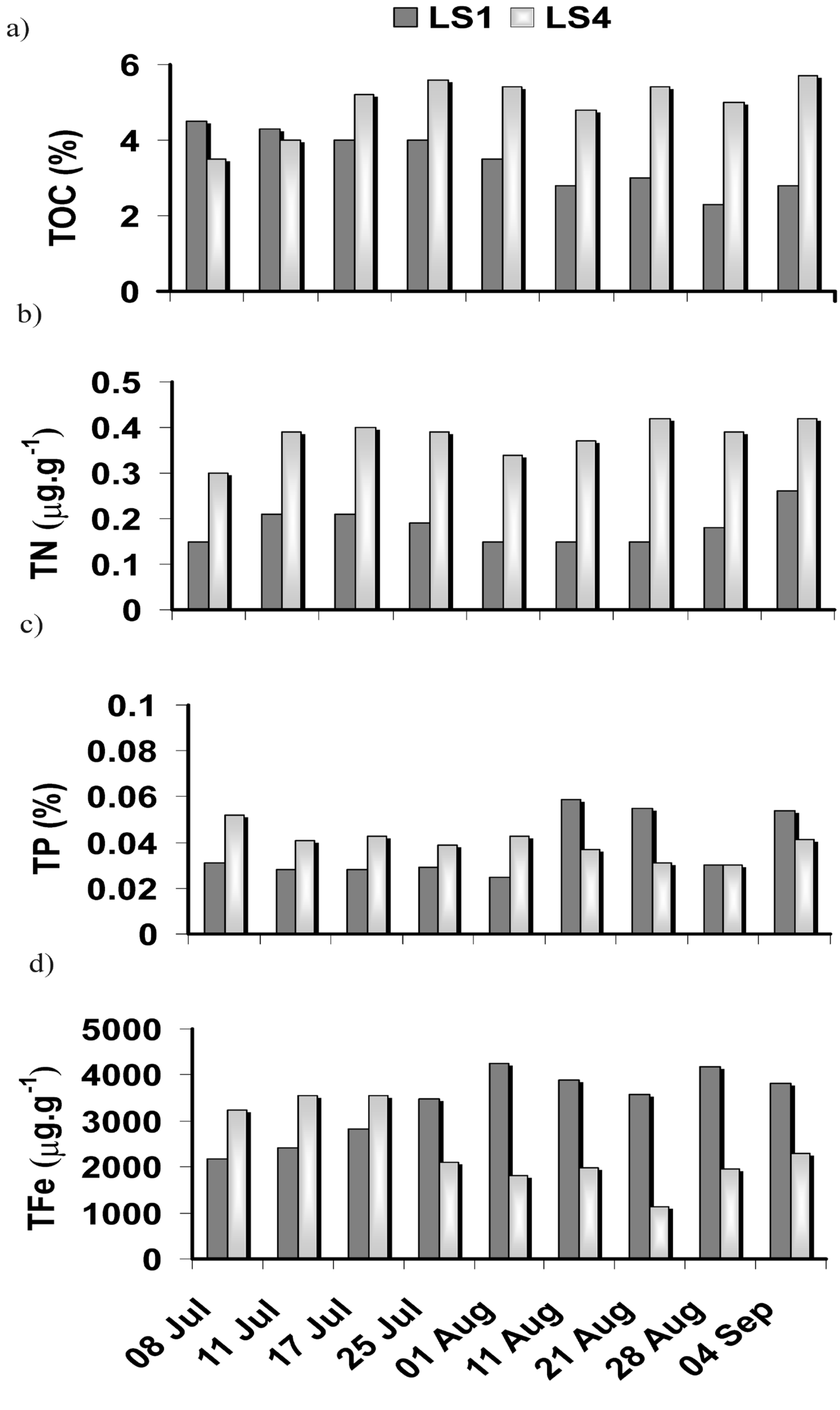

Figure 4. Fluctuations of TOC (a), TN (b), TP (c) and TFe (d) at sites LS1 and LS4, representative of anoxic and control areas, respectively.

characterized by highly reducing conditions (Table 3) despite the well oxygenated conditions of the overlying waters.

In addition, the redox potential was lower at the site LS2 than at the organic-rich site (LS4), and especially low (-407 mV) at LS2 in August. The Mann-Whitney test showed a difference between LS2-LS4 highly significant (*U*-test, $p<0.001$), but significant differences were observed between LS3-LS4 ($p<0.01$) and LS1-LS4 ($p<0.05$) (Table 3). Most sedimentary variables showed significant correlations between one another (Table 4).

It is worthwhile to note that TOC was positively correlated with TN ($P<0.001$) and ORP ($P<0.05$) and negatively with TFe ($P<0.005$), while TN was well correlated with ORP ($P<0.01$). No correlations were observed between TP and the other variables.

for PC2 and 13.6% for PC3) (Figure 5a). The first axis had a strong negative correlation with SRP (0.309), TP (0.388) and chl *a* (0.409) (SRP, TP and chl a were strongly correlated to each other by Spearman rank order) and positive correlation with SRSi (0.324), TN (0.351) and ammonia (0.272). The second axis produced the second group of variables: temperature, oxygen saturation, pH and dissolved nitrogen salts (except for ammonia). The eigenvectors were -0.432 for temperature, 0.235 for oxygen saturation, -0.430 for pH and 0.294 for nitrate and 0.368 for nitrite. SRSi was found also to be with loadings in PC2 (-0.356). Also, Figure 5a indicates a strong separation of site LW1 from the other two stations, due to its extremely high values of chl *a* and TP and low values of TN and SRSi, while the dissimilarities due to the month of water sampling, more pronounced along the component PC2, were related to T, pH, SRSi and nitrite. Differences between anoxic and control areas, also related to month and week of sampling, were tested by ANOSIM test one-way (number of permutations of 9999).

Table 4 - Correlation coefficients (Spearman rank order) among sediment variables (n = 30)

Sediment	T	pH	ORP	TOC	TN	TP	TFe
T	1.00	0.06	-0.23	-0.16	-0.33*	0.15	-0.12
pH		1.00	-0.002	0.11	-0.15	0.05	0.15
ORP			1.00	0.33*	0.52**	0.06	-0.19
TOC				1.00	0.62***	-0.17	-0.37*
TN					1.00	-0.04	-0.38*
TP						1.00	0.24
TFe							1.00

For the explanation of variables see the text in the section "Methods"; *p<0.05, **p<0.01, ***p<0.001.

Multivariate analysis and relationship between sediment and overlying water

PCA analysis produced five principal components for water variables and the first three components accounted for a cumulative variation of 70.7% (35.6% for PC1, 21.5%

Results confirmed a significant difference related to area (perturbed and control) (R=0.381, P=0.01%) and month of sampling (R=0.189, P=1.2%), while the factor "week" of sampling was not significant. PCA applied to the sedimentary variables showed that the first three components explained 67.1% of the total variance and more than half of the total system variability was due to the first two axes PC1 and PC2 (52.0%). Figure 5b displays the variables which contributed to the first two component variance. The first component (PC1, 34.5%) was positively to the time of sampling, along both PC1 and PC2, and *a* clear separation among sites along PC1 due mainly to the variables TOC and TN.

A further multivariate analysis was performed to evaluate the relationship between sediment and water variables. The rank correlation method was based on weighted Spearman rank correlations between the similarity matrices of the two data sets and the results showed that sedimentary variables were mostly correlated with chl *a* and TP. This analysis was performed on data of only two

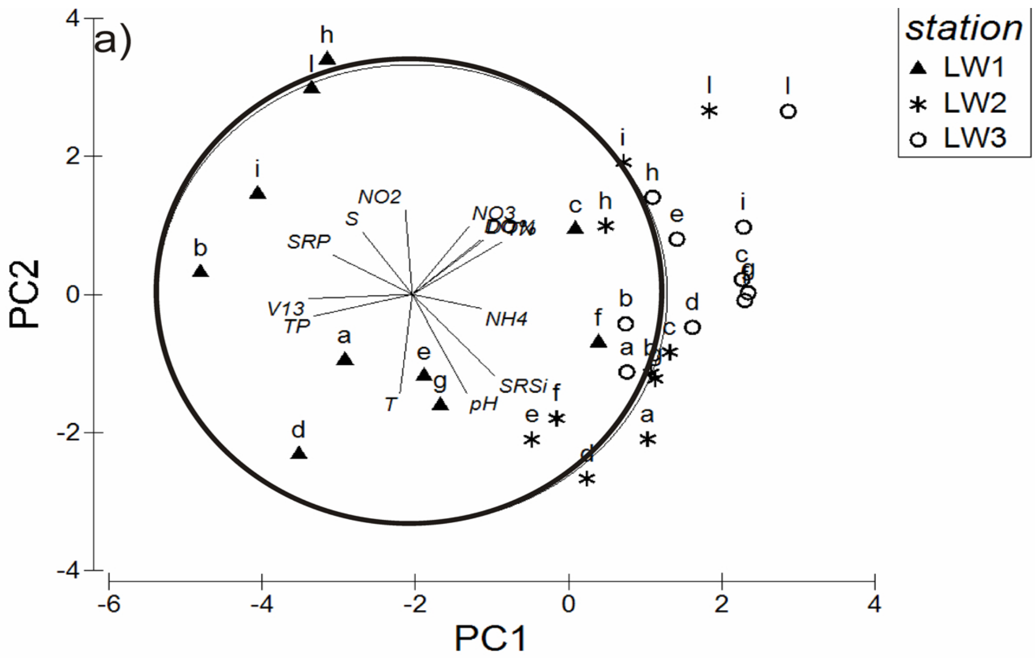

Figure 5a.

and strongly correlated with TOC (0.472) and TN (0.562), which showed significant correlations (Table 4), while the second component (PC2, 17.5%) was positively correlated with TP (0.656). The variables T, ORP and TFe were found to be with loadings in both PC1 and PC2. The eingenvectors for T were -0.228 in PC1 and -0.363 in PC2, for ORP were 0.413 and 0.436 in PC1 and PC2, respectively, and for TFe were -0.395 in PC1 and 0.430 in PC2. Figure 5b shows a heterogeneous distribution of sites related sites (LW1 and LW2 for water, LS1 and LS3 for sediments) where *a* simultaneous sampling was made.

In Figure 6, the superimposition of water variables on *nm*MDS based on correlation matrix of sedimentary variables is reported. Water variables were superimposed as bubbles whose sizes reflect the magnitude of these variables and it can be noted the relationship between the two compartments sediment-water; in fact, the sites LS1 (within anoxic area) and LS3 (control area), different from *a* sedimentary point of view, were characterized by different concentrations of chl *a* (Figure 6a) and TP (Figure 6b).

Discussion and Conclusions

The study carried out during the occurrence of the anoxic event in Lesina lagoon shows the role of the disturbance of the crisis with respect to the variables of both sediment and overlying water. LW1 was characterized by lower values of DO (overall the lowest percentages) than LW2 and LW3 during the first two weeks of observation and by a strong increase of concentrations (peak of 153%) in the next week (Figure 2c). These very low values of DO (undersaturation) could reflect high consumption due to organic matter decomposition and the presence of sediments very rich in organic matter within the anoxic area (LS1 and LS2) (Table 3) suggests that the oxidation of organic matter, enhanced by high temperatures and probably just started before the sampling, was more pronounced than in outer area. The following increase of DO and the broader interval observed in the site within the anoxic area indicated supersaturation due to phytoplanktonic biomass, reflecting the excessive photosynthesis activity. The DO measurements range obtained in this study were comparable with that typical of summer period observed in other Italian lagoons (D'Autilia *et al.*, 2004; Facchini *et al.*, 2007). In all sites the DO fluctuations were consistent with those of temperature

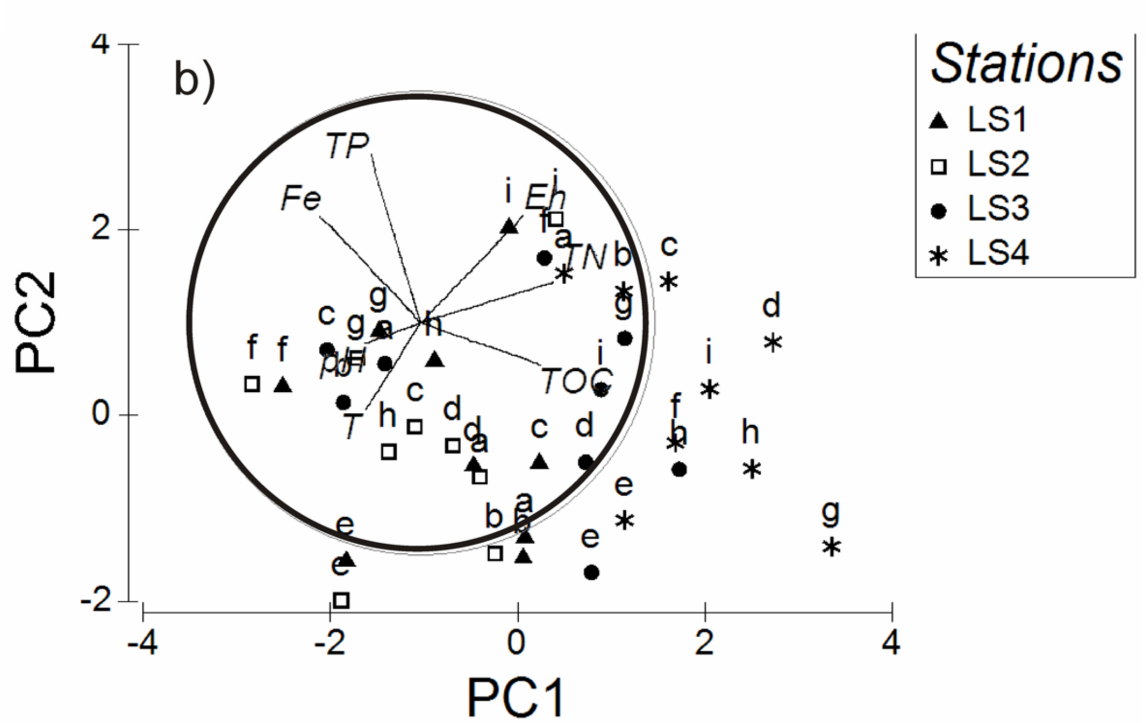

Figure 5a-b. Results of PCA analysis of the overlying water variables (a) and sediment parameters (b). The first two principal components (PC1 and PC2) accounted for 57.1% (water) and 52% (sediment) of the total variance. Letters near each site symbol indicate the different week of sampling: a = the first week of July 2008, b = the second week of July 2008, etc.

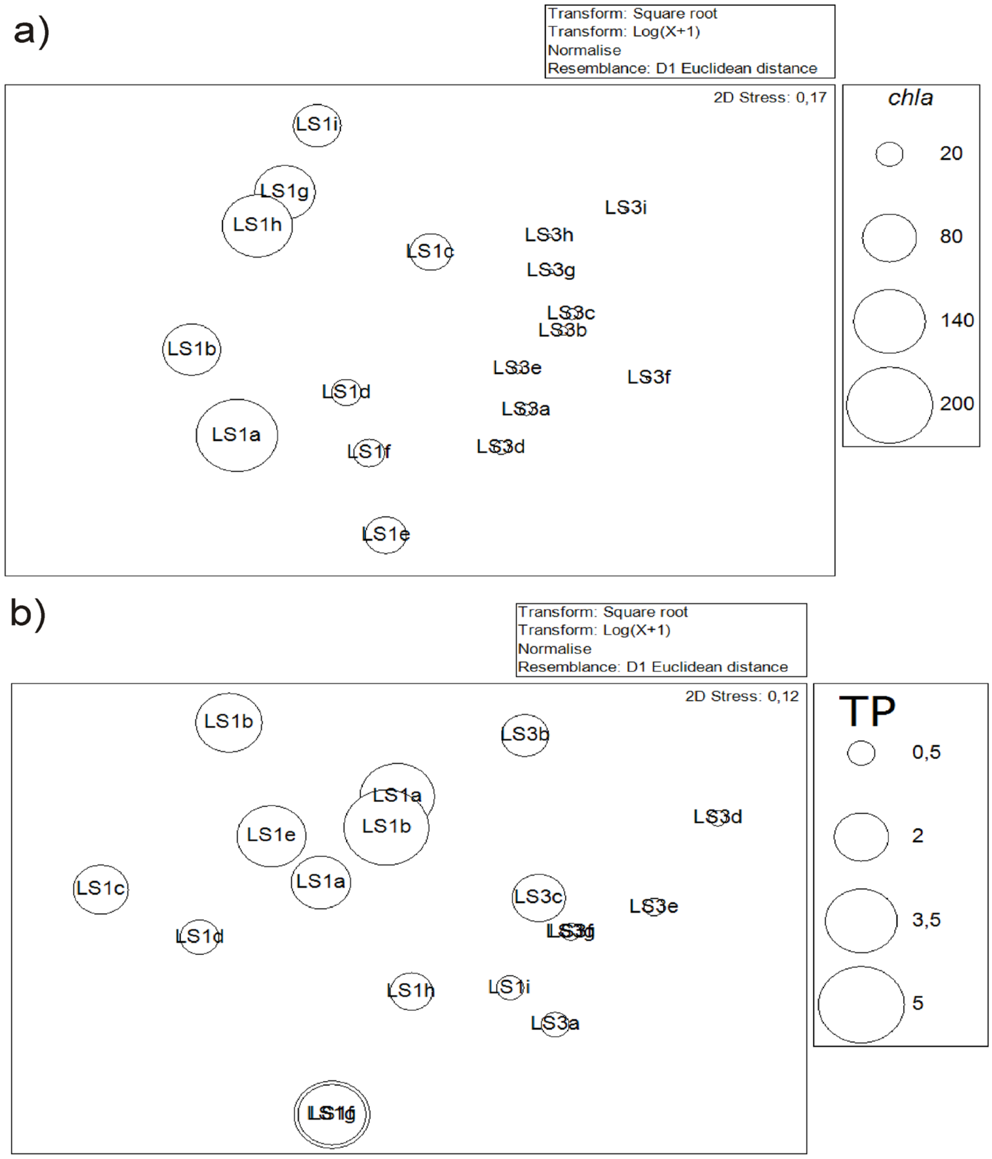

Figure 6. Ordination diagrams (nmMDS) obtained for sediment variables, superimposed on water variables: chl *a* (a) and TP (b).

(Table 2) which is a key factor affecting the benthic fluxes of nutrients (Van Raaphorst *et al*., 1992; Kristensen 1993; Wilson and Brennan 2004) and promotes a temporal variability in sediment-water nutrient exchanges in coastal environments with marked seasons (Forja *et al*., 1994; Vidal and Morguí 1995). Nevertheless, no correlation was observed between temperature and water nutrients suggesting influences of other variables besides temperature. No significant correlation between nutrients and salinity was observed by Spearman's rank, except for SRSi which showed an inverse relationship with salinity. These results show that the source of nutrients in the overlying water is mainly related to the sediments. TOC concentrations in sediments exhibited pronounced variability between perturbed and control sites with a decreasing trend of values in disturbed sites, while TN and TP contents within the anoxic area were lower than those in the outside area. These findings suggest that quick mineralization processes and nutrient release in mid-term happened mainly in disturbed area. These processes were responsible for the significant positive correlations observed between TOC concentrations and ORP values in anoxic conditions.

The high biomass production observed along the overlying water within the anoxic area at the beginning of the observation period was related to this greater availability of nutrients in disturbed area. TOC content obtained in this study were comparable or even lower than those typically found in organic enriched or eutrophic Mediterranean lagoons interested also by anoxic crises (Picone *et al*., 2008; Lardicci *et al*., 2001; Lenzi *et al*., 2005; Magni *et al*., 2005; 2008). Increased values of TP (three times higher than means) and a slight decreased of TN content at the end of August happened in disturbed area, followed by the second phytoplankton bloom (first week of September), mainly related to nitrogen species.

The organic content and the redox conditions of sediments play a significant role in shaping the distribution of phosphorus species concentrations in sediments (Lukawska-Matuszewska and Bolalek, 2008). The deterioration in redox conditions in the sediments involves the increase of concentrations of ammonia and hydrogen sulphide in the pore water, a decrease in the TP concentration, related to the desorption of phosphate ions, the dissolution of iron and phosphorus compounds as a result of Fe (III) reduction and the mineralization of organic matter. Regarding the total iron, the values increased in disturbed area, indicating a phosphorus release at the beginning of the crisis, responsible of the first peak of phytoplankton observed.

The findings obtained in this study highlight wider time-related fluctuations of physico-chemical parameters (for both sediments and overlying water) within anoxic area than in control area, but, at the same time, stress the significant role of the factor "space" (anoxic sites and control sites) for the observed physico-chemical differences. The disturbance was clear in the sites within the anoxic area in the short-terms and the response of sediments was more immediate than overlying water.

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