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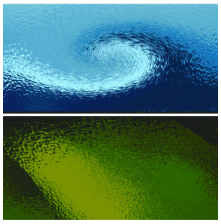

RESEARCH ARTICLE

# Resistance and resilience of ecosystem descriptors and properties to dystrophic events: a study case in a Mediterranean lagoon

A. Basset[1], E. Barbone[4], I. Rosati[1], F. Vignes[1], P. Breber[2], A. Specchiulli[2], R. D'Adamo[2], M. Renzi[1,*], S. Focardi[3], N. Ungaro[4], M. Pinna[1]

[1]Department of Biological and Environmental Sciences and Technologies, University of the Salento, S.P. Lecce-Monteroni, 73100 Lecce, Italy.
[2]National Research Council - Institute of Marine Science, Department of Lesina (FG), Via Pola 4, 71010 Lesina (FG), Italy.
[3]University of Siena, Via Mattioli 4, 53100 Siena, Italy.
[4]ARPA Puglia, Corso Trieste 27, 70100 Bari, Italy.

*Corresponding author: E-mail address: monia.renzi@unisalento.it

## Abstract

1 - Mediterranean lagoons are naturally exposed, during the dry season, to dystrophic and hypoxic events determining dis-equilibrium conditions along temporal and spatial scales, which are linked to metabolism and life cycle of the biotic components.

2 - In summer 2008, Lesina lagoon (SE Italian coastline) was interested by a geographically localized dystrophic crisis which affected up to 8% of the total lagoon surface.

3 - Temporal dynamics of principal descriptors of abiotic (water, sediment) and biotic (phytoplankton, benthic macroinvertebrate) compartments have been followed during the 2008 by collecting data inside stressed and control lagoon areas before a dystrophic event and in the six months after the dystrophic event.

4 - The aim of the study was to analyse the pathways of ecosystem responses to dystrophic stress, searching for the characteristic scales of ecosystem compartment resistance and resilience.

5 - The characteristic time-scale of abiotic and biotic component time responses varied from days, for the selected markers of the water column, to year, for the benthic ones. Short-term biotic and abiotic responses in the water column were strongly coupled while biotic and abiotic responses at the sediment level were remarkably un-coupled. Dynamics and recovery time of water column and benthic components do not match in Lesina following the dystrophic crisis, highlighting an intrinsic individualistic behavior within the lagoon community driving ecosystem processes and ecosystem level responses.

6 - Taxonomic and non-taxonomic descriptors of both phytoplankton and benthic macroinvertebrates showed different response patterns as early warning signals and overall resilience. The emphasized differences in the stability components, i.e., resistance and resilience, of water column and sediment abiotic and biotic characteristics as well as of taxonomic and non-taxonomic descriptors has key implication in planning monitoring strategies and programs for transitional waters in the Mediterranean and Black Sea EcoRegions.

**Keywords:** Dystrophic crisis; Ecosystem abiotic and biotic components; Resistance; Resilience; Lesina lagoon.

## Introduction

Almost 25 years ago a hierarchical concept of ecosystem was proposed by O'Neil *et al.*, (1986), opening the field to a deeper understanding of ecosystem organisation and dynamics. Ecosystem level properties, such as vigor, organisation and resilience on which are based current assessments of ecosystem health, were shown to be the emergent result of a hierarchical organisation of the rates of processes (Costanza, 1992; Costanza and Megeau, 1999). Decoding ecosystem properties from population and individual properties, or functional traits (sensu McGill *et al.*, 2006), understanding and quantifying scales and strengths of top-down and bottom-up interactions and cascading pathways are main issues for the ecological application of ecosystem theory concepts and valuable tools to ecosystem conservation and management. Since most process rates are someway dependent on the body size of the species involved in the process (e.g. Peters, 1983; Brown *et al.*, 2004) and spatial and temporal scales are also shown to incorporate some body size dependency, body size has already been proposed as a key individual trait in the decoding, understanding and quantifying down-scaling of ecosystem properties for applied purposes (Haskell *et al.*, 2002; Basset and De Angelis, 2007; Barbone *et al.*, 2012; Basset *et al.*, 2012). Ecological status is the ecosystem property addressed in Europe at the normative level to preserve and manage in a sustainable way aquatic ecosystems (Water Framework Directive, WFD CE2000/60). The concept of ecological status is close to that of ecosystem health, both of them describing the overall performance of the systems, the former being measured from behavior at the level of the main guilds [i.e., biological quality elements (BQEs) sensu WFD] while the latter is measured from the behavior of other ecosystem properties, such as vigor, organisation and resilience (Costanza, 1992). Attempts to decode ecosystem properties from structural community or guild components, based on species composition and sensitivity to perturbations, date back to more than 100 years ago (the saprobic system; Kolkovitz and Marsson, 1908) and received an increasing attention during the last 10 years for the requirements of WFD implementation (Orfanidis *et al.*, 2008; Ponti *et al.*, 2008; Pinna *et al.*, 2013). The two different assessment approaches has converged into similar evaluations, but the procedures defined into the normative framework of WFD has strongly pushed towards the development of guild level descriptors of structural components; approaches to ecosystem functions and properties are still too few to allow effective comparisons (Basset *et al.*, 2007). On the other hand, if guild level assessments do not manage to describe the actual behavior of ecosystem properties contributing to ecosystem health, their actual relevance in the assessment of ecological status of aquatic ecosystems might become questionable.

A lack of apparent consistency between guild level and ecosystem level assessment of ecological status of aquatic ecosystems can, also, be a simple result of the hierarchical structure of ecosystems, since small scale processes, occurring at much faster rates than ecosystem level processes, incur a number of feedback steps, which may minimise their actual influence on the ecosystem level properties (Basset *et al.*, 2008a). On the other hand, since species pertaining to guilds characterised by very different individual body sizes, such as phytoplankton, benthic macroinvertebrates and fishes, have different process rates and spatial and temporal scales of interaction, it may be possible that they give different pictures of the ecological status of a same ecosystem, only some of them being tuned with the scales of ecosystem properties (Soininen and Könönen, 2004; Vignes *et al.*, 2012). In transitional water ecosystems, some evidences of the relevance of individual body

 

size and functional traits in the assessment of ecological status have been already shown, even though a full cross-scaling comparison of individual and ecosystem properties has not yet developed (Basset *et al.*, 2004; Basset *et al.*, 2008b). The integration of assessments based on different quality elements is an important step of ecological status assessment that has not yet been fully developed by WFD (Johnson *et al.*, 2006). In fact, current assessment procedures are based on a single biological quality element (Hering *et al.*, 2006; Ponti *et al.*, 2009) and the evaluation of their comparative behavior in assessing functional properties and responses to stress are still almost completely lacking, mainly for transitional water ecosystems. Numerous factors (geomorphology, morphometry, hydrology and hydrodynamics, meteo-climatic conditions and nutrient loads) can affect productivity in aquatic ecosystems. Some of them (i.e. water exchanges, bottom depth, human effluents) could be managed affecting significantly ecosystem trophic level, primary and secondary productivity. Furthermore, during dystrophic events, changes in different descriptors in the abiotic compartments produce several possible related effects on the biota. Here, we have investigated the relationships between ecological status descriptors, at the guild level, and ecosystem properties in the Lesina lagoon (South-Eastern Italian coastline), which is a non-tidal and Mediterranean type transitional water ecosystem, based on Puglia Regional Administration's Water Protection Plan (2010). Resilience has been targeted as ecosystem property by investigating the contribution of different guilds and abiotic components to the definition of the lagoon resilience to a pulse stress event constituted by a dystrophic crisis. During summer 2008, the lagoon was affected for more than one month by a strong dystrophic crisis on a well-defined up to 4 km2 area in front of the Lesina town (Vignes *et al.*, 2009). Since Lesina lagoon was monitored over the last two years before 2008 in the framework of the Puglia Regional Monitoring Program of aquatic ecosystem health, the crisis gave us the opportunity to study the short term patterns of variation of abiotic and biotic components of the ecosystems in relation with the longer term baseline conditions, considered as reference.

The aims of this research are: i) to evaluate selected abiotic descriptors resistance and resilience towards a dystrophic stress; ii) to analyse the responses to the dystrophic crisis of phytoplankton and benthic macroinvertebrate guilds, that are characterised by different individual body size and energetic; iii) to evaluate the responses of taxonomic and non-taxonomic metrics of phytoplankton and benthic macroinvertebrate guilds; iv) to evaluate the response time-scales of different guilds and metrics in relation with the time-scale variation of the physical and chemical characteristics of water column and sediments. Response time scales refer to the short-term reaction and to resilience properties of the two considered guilds to the perturbation event. Both short-term reaction and recovery time scale are important component of every structural and functional ecosystem component when used as a descriptor of stress. In principle, fast reaction and short recovery time are two important properties of every metric used to describe pulse pollution events. Moreover, understanding the response time of metrics is essential in order to integrate their responses among different guilds.

## Methods

### *Study area*

Lesina lagoon (Fig. 1) is a shallow ecosystem (mean depth of 0.8 m) located in the South-Eastern Italy (41.88° N; 15.43° E) having a surface of 51 km$^2$ and two narrow openings to the Adriatic sea named Acquarotta and Schiapparo. It lies within the Gargano

National Park and in 1995 was declared "Site of Community Importance" (IT9110015). The lagoon is characterized by a drainage basin of 400 km$^2$ and a slow water renewal. The hydrological regime is strongly influenced by continental freshwater inputs and by local meteorological conditions (winds and rains). The water retention time varies seasonally from 30 (autumn) to 300 (spring) days (Giordani *et al.*, 2008) with average values of about 100 days (Manini *et al.*, 2002), and strong seasonal variations of temperature (7 °C in winter and 28 °C in summer) and salinity (from 5 to 34 PSU) are determined by freshwater inputs (distributed along the southern shore), precipitation, evaporation and the exchange efficiency of the tidal channels (Vignes *et al.*, 2009 and references therein).

The macrophytobenthos and benthic macroinvertebrates guilds are very similar to the other South Adriatic lagoons (Menéndez *et al.*, 2003; Sangiorgio *et al.*, 2004; Barbone *et al.*, 2007; Beqirai *et al.*,

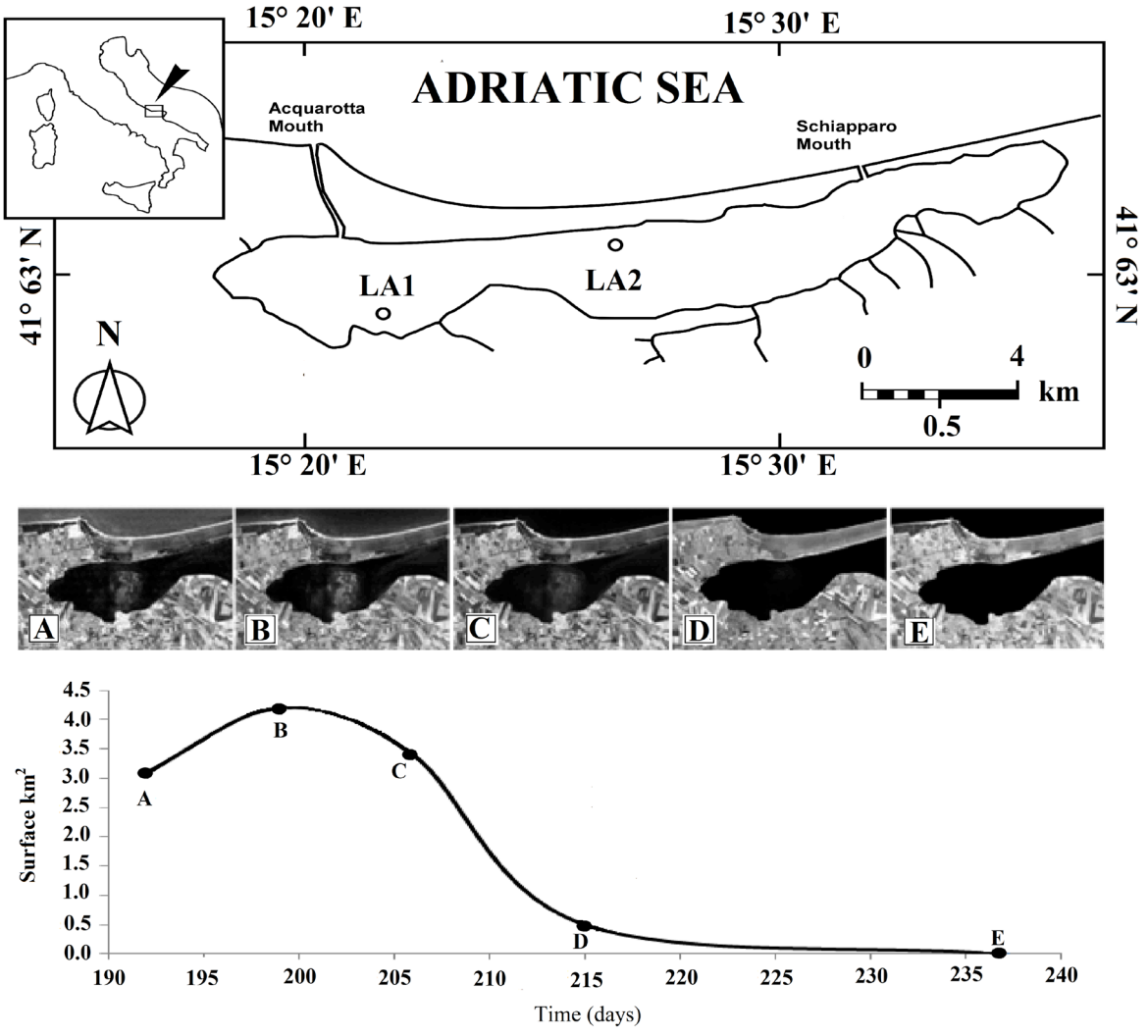


Figure 1. Study area. Lesina lagoon and geographic location of the sampling stations LA1 (inside the dystrphic area) and LA2 (outside dystrophic area). The temporal evolution and surface of dystrophic event is reported both in the satellite photos and in the graph.

2007; Galuppo *et al.*, 2007; Orfanidis *et al.*, 2007; Evagelopoulos *et al.*, 2008; Sangiorgio *et al.*, 2008). Macrophytobenthos is dominate by the rhizophytes *Ruppia cirrhosa* (Petagna) Grande and *Nanozostera noltii* (Hornemann) Tomlinson and Posluszny in the Eastern and central parts of the basin and by *Gracilaria* sp., *Cladophora* sp., and *Valonia utricularis* in the Western area, where dystrophic crises occasionally occur (Manini *et al.*, 2003). Benthic macroinvertebrates guild reflects 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.*, 2010).

*Experimental design*

The dystrophic event occurred in Lesina lagoon near the urban centre (Western basin), as highlighted by increasing temperatures, complete absence of wind, surface waters white coloured, and satellite images (Vignes *et al.*, 2009). Two sampling stations were selected inside (LA1) and outside (LA2) the lagoon area interested by the dystrophic crisis (Fig. 1). LA1 was located in the most perturbed area of Lesina lagoon (Pinna *et al.*, 2013). Physico-chemical data on water column, phytoplankton, and benthic macroinvertebrates were acquired during the whole year in twelve different dates (days: 56, 147, 189, 196, 206, 217, 224, 234, 241, 248, 255, 356) before, during and after the dystrophic crisis, while on the contrary, sediments were collected in 10 sampling campaigns carried out on a weekly basis performed from the first week of July 2008 (189 day) to the second week of September 2008 (255 day). Concerning water, physico-chemical parameters [temperature (T, °C), salinity (S, PSU), dissolved oxygen ($O_2$, mg/l), pH] were measured using a multi-parametric field probe (MPS Multiprobe, YSI 556). Superficial water samples were collected in HDPE bottles and analysed in triplicate to determine phytoplankton taxonomy and to quantify dissolved nutrients [ammonium ($NH_4^+$), nitrites ($NO_2^-$), nitrates ($NO_3^-$) soluble reactive phosphorus (SRP)], total nutrients [total nitrogen (TN), total phosphorous (TP)], soluble reactive silicates (silicates), phytoplankton biomass as total chlorophyll-*a* (Chl-*a*), and phytoplankton biomass fractions (micro-, nano-, pico). Concerning sediments, *in situ* measurements of oxidation-reduction potential (Eh), pH, and sediment temperature (T, °C) were recorded using a field probe (Hanna Instrument, HI 9026 portable pH/millivolt meter) by inserting electrodes directly inside sediment core samples superficial layers. Superficial sediment (0-5 cm) were sampled in triplicate using a box-corer (17 cm x 17 cm x 15 cm) and collected in HDPE bottles for the determination of total organic carbon (TOC), total nitrogen (TN), total phosphorus (TP) and bioavailable iron (Fe). Phytoplankton taxonomic composition and size structure were determined at each sampling site and date on three replicate of 500 ml water samples collected from 0.5 m depth using a Ruttner water sampler and immediately preserved with Lugol's solution (0.5% final concentration). All samples were soon transferred in dark and refrigerated boxes and kept refrigerated (4 °C) until the analysis. Sediment samples for the macroinvertebrate determinations were collected by an Ekman-Birge grab sampler (15 cm x 15 cm x 15 cm). Three replicates of sediment samples were sieved in the field using a 1 mm mesh size sieve and collected benthic macroinvertebrate were preserved in buffered formalin 4% until the laboratory analysis.

*Laboratory analyses*

Water abiotic descriptors

Dissolved nutrients ($NH_4^+$, $NO_2^-$, $NO_3^-$, SRP) and silicates were determined in water

samples after filtration through 0.45 µm glass microfiber filters by a Bran+Luebbe QuAAtro flow analyser, according to the methods reported by Grasshoff *et al.*, (1999). TN and TP were analysed in unfiltered water samples by the continuous-flow auto-analyser, after a persulphate digestion at 120 °C with an initial pH of 13 and final pH of about 2, according to methods reported in Grasshoff *et al.*, (1999).

Sediment descriptors

Sediments sample replicates collected for physico-chemical analyses at each sampling station were pooled and further treated as one single sample. A portion of each pool was dried at 60 °C in oven until constant weight was reached. Dried sediments were then analysed in triplicates in order to determine: TOC (%), TN (%), TP (%) and Fe (mg/kg). Averages and standard deviations (SD) were calculated and referred to dry weight. Chemicals and reagents were analytical grade and glassware was carefully washed to avoid sample crossing-over contamination. Determinations of TN were performed by direct total flash combustion using aluminium capsules and a CHNS Elemental Analyzer with a thermo-conductivity detector TCD (Perkin Elmer, CHN/O 200), according to ICRAM methods (2001). TOC was determined by a preliminary acid digestion with 19% HCl in silver capsules and the successively total flash combustion as described in literature (Specchiulli *et al.*, 2010). CHN/O Analyzer was calibrated before the analyses using an acetanilide certified standard. TP determination was carried out according to Aspila's methods (Aspila *et al.*, 1976), by acid digestion and next quantification using a spectrophotometer (Perkin Elemer, UV-VIS 6505) after the colorimetric reaction. The concentration of bioavailable iron (Fe) was determined in 0.1 g of dried sediment sample using the ferrozine assay according to Lovely and Phillips (1987). Samples were 1h-long extracted by ferrozine solution in HEPES buffer (pH adjusted to 7.0) and successively centrifuged at 2000 RPM for 10 minutes. After that, 20 µl of the supernatant were sampled and mixed with 980 µl of the ferrozine reagent. Absorptions were measured by spectrophotometry at $\lambda$ = 562 nm after calibration with $FeCl_3*6H_2O$ as reference solution. Recovery and reproducibility were checked by analysing procedural blanks and reference materials purchased from National Institute of Standard and Technologies (NIST - New York waterway Sediment SRM1944), Institute of Environmental Chemistry Academy Sinica Beijing China (Tibet soil), and NIST (Estuarine Sediment, SRM1646a). Solvent blanks were 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 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), Fe 91.3% (0.5 SD, Tibet Soil). Measured 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 TOC, TN, while 0.001% for TP and 0.5 mg/kg for Fe.

Phytoplankton descriptors

Chlorophyll-*a* (Chl-*a*) extracted in 90% acetone was determined as an estimate of phytoplankton total biomass and after differential filtration to evaluate micro-, nano- and pico- phytoplankton biomass. A know amount of water was filtered immediately after sampling on Whatman GF/F fibre filters (pore: 0.7 µm) by a Swinnex apparatus (Swinnex® Filter Holder - Millipore Corporation, Bedford, USA). For the determination of <2 µm size fraction, samples were pre-filtered on 2 µm Nucleopore

membranes whereas for the determination of <20 µm size fraction water samples were pre-filtered on 20 µm net. Filters were stored at -20 °C until analysis within one month later. Chl-*a* was extracted in 10 ml of 90% acetone, for 24 hours (4 °C, in the dark) and quantified with spectrofluorimeter (SHIMATZU, RF 1051) before and after addition of hydrochloric acid 0.5 N (Yentsh and Menzel, 1963).

Individual cell size and taxonomic identification were performed on a sub-sample of 400 cells, viewed at 400X magnification under an inverted microscope (Nikon, T300E) connected to a video-interactive image analysis system (L.U.C.I.A, Version 4.8, Laboratory Imaging Ltd, Prague) with a lower detection limit of 5 µm, following the Utermöhl's method (Zingone *et al.*, 1990). The individual cell volume ($\mu m^3$) of each cell measured was derived by approximating the cell shape to the most similar regular solid (Vadrucci *et al.*, 2007), and then converting to individual cell weight (pg C) according to Menden-Deuer and Lessard (2000). Phytoplankton nomenclature was according to Tomas (1997).

Benthic macroinvertebrate descriptors

Macroinvertebrates benthic samples were sorted out under a stereomicroscope after washing away formalin. All individuals were then identified at the lowest degree of taxonomic resolution possible, counted, measured individually (total length) to the nearest 0.01 mm by an image analysis device (Leica, Q-Win) and weighed to the nearest 1 µg after drying for 72 h at 60 °C (Pinna *et al.*, 2004). Ash content was obtained at the individual level (for large species) or on groups of co-specific individuals (for small species) after muffle furnace combustion at 450 °C for 12 h. All data regarding individual body sizes were then expressed as ash free dry weight (AFDW), subtracting ash content percentage from each specimen (Rosati *et al.*, 2012).

*Statistical analysis*

Multivariate routines were run using the Primer v6.0 software package (Primer-E Ltd., Plymouth Marine Laboratory, UK), whereas univariate ones were performed using the GraphPad Prism (GraphPad Software, San Diego California USA, www.graphpad.com) package. General univariate statistics (mean and standard error) were calculated for each considered variable separately both in LA1 (dystrophic area) and LA2 (control). According to the literature (Zuur *et al.*, 2010), data were opportunely pre-treated before running multivariate routines. In particular, concerning environmental data, Euclidean's distances resemblance matrices were calculated after square root ($\sqrt{}$) and log(x+1) functions transformation, and successive normalization (Clarke and Green, 1988), whereas statistics on the biotic descriptors were performed on Bray-Curtis's resemblance matrices after an opportune data transformation. A first screening aimed to verify on a multivariate statistical basis that the dystrophic event produced significant difference between stressed station (LA1) and control (LA2), was performed on variables selected as principal descriptors of the abiotic matrices (water and sediments) and biotic components (phytoplankton and benthic macroinvertebrate). The significance of observed ordinations were tested by the application of the ANOSIM (Analysis of Similarities) test statistic R two-ways (otherwise defined by two factors) which was performed running 9,999 permutations. This procedure allows testing hypothesis for differences between groups of samples according to *a priori* defined factors of interest, using permutation/randomisation methods on resemblance matrix. Principal Components Analyses (PCA) was applied to investigate major responsible descriptors for correlations and similarities among sampling

 

stations (Chatfield and Collins, 1980). Cluster analysis was performed applying the single-linkage cluster mode and the SIMPROF test (1,000 permutations for mean profile, 999 simulation permutations, 5% of significance level). Results were superimposed to the PCA to evaluate significance of observed segregations. Univariate statistic analyses (t-Student test and F-ratio, $p<0.05$) were performed with the aim to select descriptors significantly affected by the dystrophic event. Once selected significant descriptors for each matrix ($p<0.01$), their temporal trends were evaluated to determine their resistance and resilience (recovery time after the occurrence of the pulsed stress). This evaluation was performed using, for each descriptor, the weighted ratio between LA2 and LA1 values (n=3). The observed distances from 1.0 (total identity) were assumed significant in both directions (±) when for each descriptors the calculated ratio resulted to exceed the range 1±0.1*absolute maximum ratio observed. We assumed as completely recovered the first time after the event for which no significant differences were observable comparing levels in stressed (LA1) and not-stressed (LA2) stations.

## Results

*Physico-chemical descriptors in water and sediment*

Average values and temporal variability of key water column and sediment abiotic variables were described using the univariate statistics (Table 1). Data (±SD) are reported separately for LA1 and LA2 stations. Statistical differences were observed between the two stations comparing average values and variances of the different environmental parameter at the univariate level (Table 1). LA1 station was characterized by higher average values of salinity, TP, Chl-*a* and sediment Eh and Fe levels and a lower values of silicates, TN in water and TOC, TN in sediments than LA2 station (Table 1). Moreover, LA1 station had generally a higher variance on most water column variables than LA2 station and a lower variance in the sediment variables. Principal component analyses (Fig. 2) performed contextually on chemical descriptors measured in water and sediments from both considered sampling stations evidenced that the first three components (PC1, PC2, PC3) account for the 62.4% of the total variance (respectively 29.0%, 19.6%, and 13.8%). Coefficients in the linear combinations of variables making up PC's, which show the correlations of first three axes are, also, reported in figure 2. Eigenvectors results evidence a significant positive correlation among the first axis and TN in sediments, whereas a negative correlation is recorded among the first axis and TP and Chl-*a* in water. The second axis is positively related to T °C (both in water and sediments) and pH in water. Results obtained by the cluster analysis are superimposed to the PCA in the same figure evidencing a statistically significant difference between LA1 and LA2 which occurs at any *temporal replicates* (SIMPROF test, $P<0.05$) and among *temporal replicates* from LA1 *sampling station* along the temperature gradients. The ANOSIM test one-way performed separately on both of the factor of interest: *sampling station* (LA1 versus LA2) and *temporal replicates* (J, A, S) confirms significance related to segregations observed according to the factor *sampling station* (Global R = 0.629, with a significance level of sample statistic P = 0.01% and a number of permuted statistics greater than or equal to Global R, NPS, of 0). Plotting the ANOSIM test on the factor *temporal replicates* a minor significance (Global R = 0.145, P = 8.5%, NPS = 846) is observed. The natural occurrence of seasonal fluctuations produces a recordable effect on the PCA assessment, in fact, data collected in August (A) are characterized in both *sampling stations* by a significant increase of pH in water, $NH_4^+$,

Table 1 - Univariate statistics performed on water and sediment descriptors. Data are reported as mean ± standard error (SE). In both tables *p* values obtained by the univariate statistic (t-Student test and F-ratio) applied to evaluate significant differences between the two populations of data are reported. Significant results ($p<0.05$) are highlighted in bold. Notes: T = temperature, S = salinity, $O_2$ = dissolved oxygen, pH = pH, $NH_4^+$ = ammonium, $NO_2^-$ = nitrites, $NO_3^-$ = nitrates, SRP = soluble reactive phosphorous, TN= total nitrogen, TP= total phosphorous, Chl-*a* = total chlorophyll-*a*, Eh = redox potential, TOC = total organic carbon, Fe = total iron, n.d. = not determined

| Descriptor | Unit | Water | | | | Sediment | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | LA1 | LA2 | t-test (*p*) | F-test (*p*) | LA1 | LA2 | t-test (*p*) | F-test (*p*) |
| T | °C | 26.14±2.41 | 25.51±2.06 | 0.695 | 0.668 | 26.64±0.73 | 26.02±0.85 | 0.281 | 0.677 |
| S | PSU | 25.9±2.2 | 22.4±0.9 | **0.009** | 0.021 | n.d. | n.d. | n.d. | n.d. |
| $O_2$ | mg/l | 6.08±1.96 | 7.22±0.72 | 0.290 | **0.010** | n.d. | n.d. | n.d. | n.d. |
| pH | pH units | 7.9±0.4 | 8.3±0.3 | 0.166 | 0.704 | 7.16±0.29 | 7.29±0.22 | 0.472 | 0.461 |
| $NH_4^+$ | µg/l | 2.76±4.41 | 1.94±1.16 | 0.723 | **0.001** | n.d. | n.d. | n.d. | n.d. |
| $NO_2^-$ | µg/l | 0.75±0.49 | 0.42±0.41 | 0.307 | 0.642 | n.d. | n.d. | n.d. | n.d. |
| $NO_3^-$ | µg/l | 1.10±0.74 | 0.64±0.57 | 0.336 | 0.485 | n.d. | n.d. | n.d. | n.d. |
| SRP | µg/l | 0.26±0.15 | 0.12±0.09 | 0.118 | 0.218 | n.d. | n.d. | n.d. | n.d. |
| Silicates | µg/l | 16.8±12.4 | 51.1±29.7 | **0.048** | **0.023** | n.d. | n.d. | n.d. | n.d. |
| TN | µg/l | 9.66±4.70 | 21.98±9.19 | **0.030** | 0.075 | 0.20±0.04 | 0.38±0.03 | **<0.001** | 0.485 |
| TP | µg/l | 3.05±0.75 | 0.73±0.43 | **<0.001** | 0.140 | 0.047±0.009 | 0.040±0.004 | 0.155 | 0.056 |
| Chl-*a* | mg/m$^3$ | 79.08±35.04 | 2.51±1.44 | **<0.001** | **<0.001** | n.d. | n.d. | n.d. | n.d. |
| Eh | mV | n.d. | n.d. | n.d. | n.d. | 79.7±22.3 | 149.3±27.4 | **0.001** | 0.577 |
| TOC | % | n.d. | n.d. | n.d. | n.d. | 3.43±0.47 | 4.97±0.51 | **<0.001** | 0.822 |
| Fe | mg/kg | n.d. | n.d. | n.d. | n.d. | 3326±548.7 | 2426±564 | **0.036** | 0.940 |

and T °C in sediments levels. Univariate t-Student test and F-ratio were performed on descriptors to select those major responsible of observed differences between LA1 and LA2 (Tab. 1). Concerning water, t-Student test evidenced high significance ($p<0.01$) for S, TP, Chl-*a* and slight significance ($p<0.05$) for silicates and TN. F-ratio test evidenced high significance ($p<0.01$) for $O_2$, $NH_4^+$, Chl-*a* and slight significance ($p< 0.05$) for S and silicates. In sediments, t-Student test evidenced high significance ($p<0.01$) for Eh, TOC, TN and a slight significance ($p<0.05$) for Fe, whereas, F-ratio test do not evidenced significance for any descriptor.

*Patterns in phytoplankton descriptors*

Average values and temporal variability of key phytoplankton variables were described using the univariate statistics (Table 2). Data are reported separately for LA1 and LA2 stations. Statistical differences were observed between the two stations comparing average values and variances of the different environmental parameter at the univariate level (Table 2). Principal component analyses (Fig. 3) performed contextually for both considered sampling stations evidenced that the first three components (PC1, PC2, PC3) account for the 92.5% of the total variance (respectively 61.2%, 23.3%, and 8.0%). Results obtained by the cluster analysis are superimposed to the PCA in the same figure evidencing a statistically significant difference between LA1 and LA2 which occurs at any *temporal replicates* (SIMPROF test, $P<0.05$) and among them. Coefficients in the linear combinations of variables

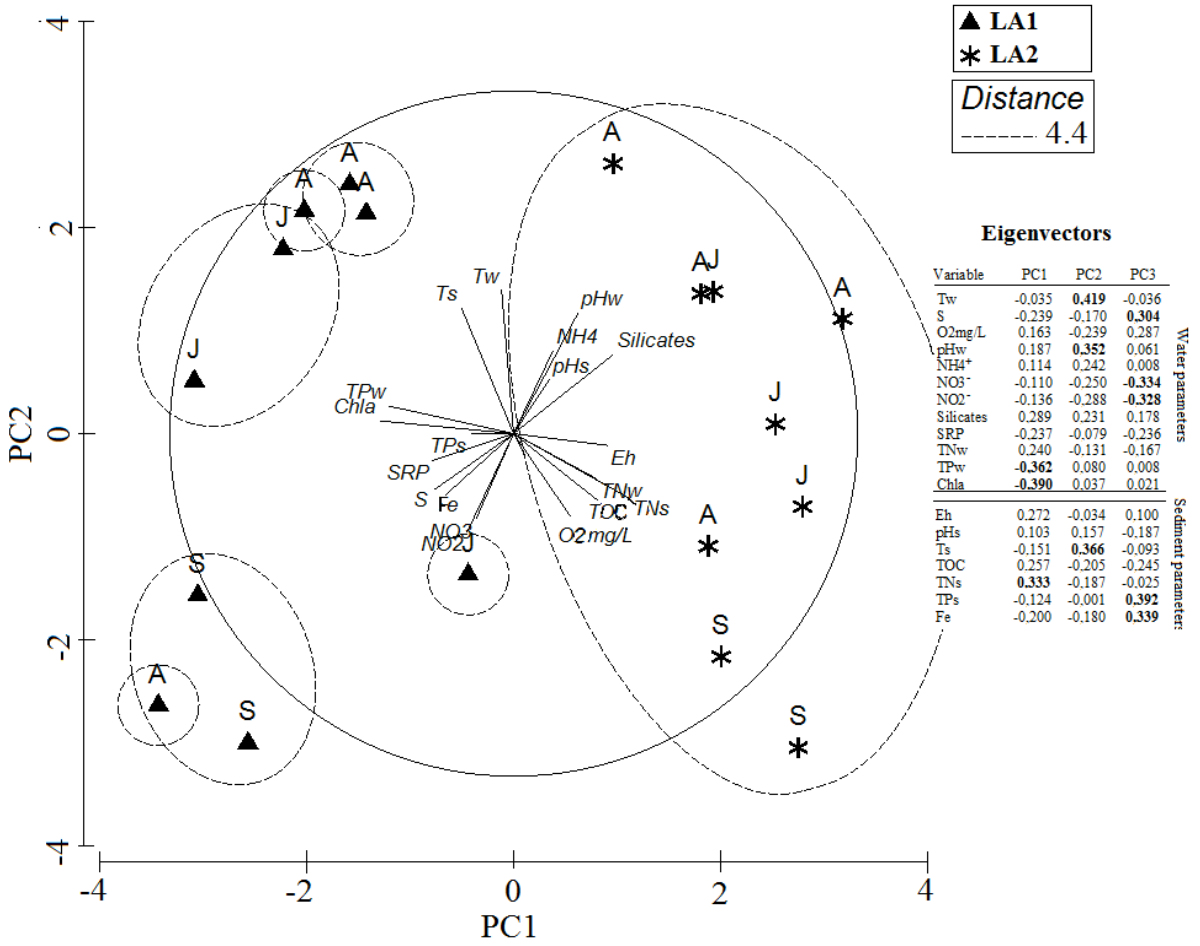


Figure 2. Principal component analysis performed on physico-chemical descriptors. Results obtained from the single-linkage ($p$<0.05) cluster analysis are overlay to the PCA. Eigenvectors calculated for each variable are, also, evidenced (in bold major significance are highlighted). J = June, A = August, S = September.

Table 2 - Univariate statistics performed for phytoplankton and benthic macroinvertebrate descriptors. Data are reported as mean ± standard error (SE). In both tables $p$ values obtained by the univariate statistic (t-Student test and F-ratio) applied to evaluate significant differences between the two populations of data are reported. Significant results ($p$<0.05) are highlighted in bold, * = density is expressed as cell/l for phytoplankton and ind/m$^2$ for benthic macroinvertebrates, n.d. = not determined.

| | | Phytoplankton | | | | Benthic Macroinvertebrates | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | **LA1** | **LA2** | t-test ($p$) | F-ratio ($p$) | **LA1** | **LA2** | t-test ($p$) | F-ratio ($p$) |
| Taxonomic Richness | number | 5.01±1.27 | 6.06±1.50 | 0.234 | 0.618 | 4.28±1.67 | 6.69±0.76 | **<0.001** | **<0.001** |
| Density | * | $2.55\ 10^7$±$1.04\ 10^7$ | $1.96\ 10^6$±$0.85\ 10^6$ | **<0.001** | **<0.001** | 2,717±2,583 | 5,316±3,212 | **<0.001** | 0.581 |
| Cell size | pgCcell$^{-1}$ | 55.3±40.4 | 156.0±139.9 | 0.124 | **<0.001** | n.d. | n.d. | n.d. | n.d. |
| Chl-*a micro* | mg/m$^3$ | 29.43±23.69 | 1.09±0.78 | **0.011** | **<0.001** | n.d. | n.d. | n.d. | n.d. |
| Shannon Index | number | n.d. | n.d. | n.d. | n.d. | 0.68±0.22 | 1.11±0.23 | **<0.001** | 0.976 |
| Distinctness | number | n.d. | n.d. | n.d. | n.d. | 85.60±12.52 | 90.30±3.13 | 0.301 | **<0.001** |
| Biomass | g/m$^2$ | n.d. | n.d. | n.d. | n.d. | 6.542±4.813 | 13.627±4.291 | **<0.001** | 0.935 |
| Body Size | mg | n.d. | n.d. | n.d. | n.d. | 1.39±0.92 | 2.07±0.92 | 0.061 | 0.876 |

making up PC's, evidenced that the first axis is negatively correlated to individual average cell dimension (-0.475), and Chl-*a* levels in micro- (-0.439), nano- (-0.492), and pico-plankton (-0.506). The second axis is significantly and negatively correlated to species (-0.615) and individual average cell dimensions (-0.704). As expected, the natural occurrence of seasonal fluctuations produces a recordable effect on the PCA assessment, for this reason, the two-way ANOSIM test performed nested on both of the factor of interest: *sampling station* (LA1 *vs* LA2) and *temporal replicates* (sampling dates) confirms significance related to segregations observed according to the factor *sampling station* (Global R = 0.422, P = 0.01%, NPS = 0). Univariate t-Student test and F-ratio were performed on descriptors to select those major responsible of observed differences between LA1 and LA2 (Tab. 2), whereas relative abundances of most representative taxa during the time in both sampling stations are reported in figure 4. The t-Student test evidenced high significance ($p$<0.01) for average cell density and slight significance ($p$<0.05) for Chl-*a* micro, whereas, F-ratio test evidenced high significance ($p$<0.01) for density, average individual cell dimensions and Chl-*a* micro.

*Patterns in benthic macroinvertebrate descriptors*

Average values and temporal variability of key benthic macroinvertebrates variables were described using the univariate statistics (Table 2). Data are reported separately for LA1 and LA2 stations. Statistical differences

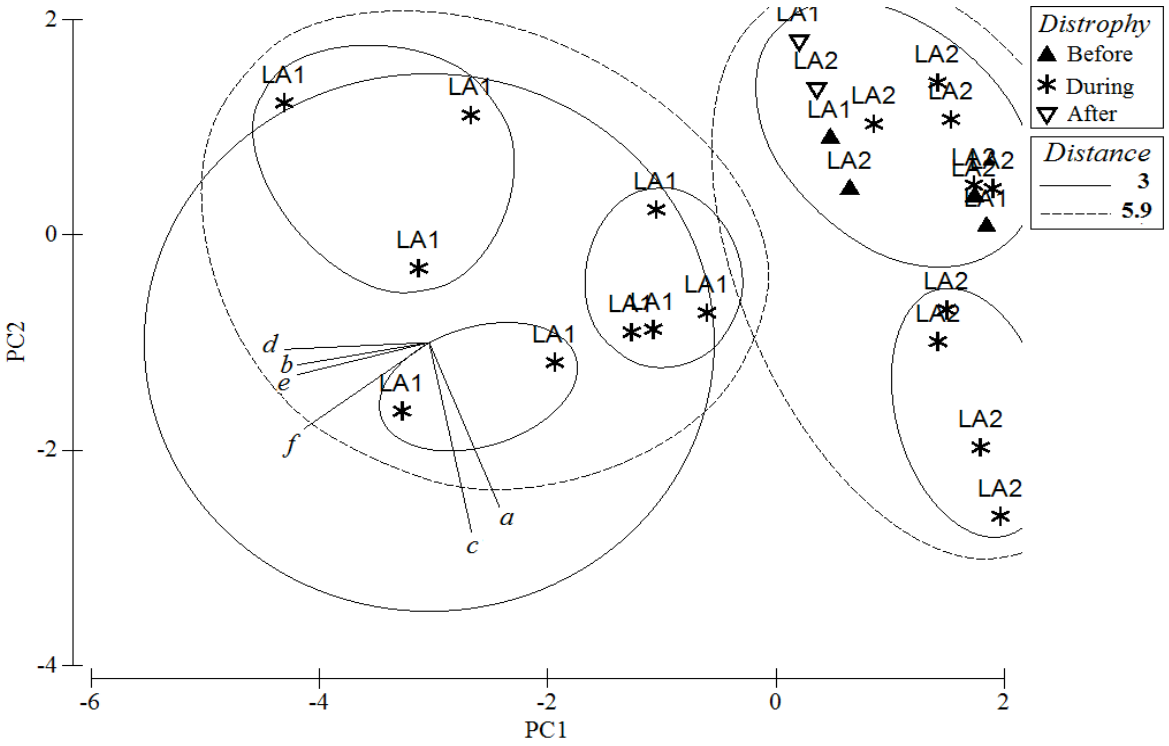


Figure 3. Principal component analysis performed on phytoplankton descriptors. Two dimensional projection of phytoplankton descriptors evidencing two factors of statistical interest: *sampling station* (LA1 *vs* LA2) and *temporal replicates* (before, during, after crisis). Results obtained from the single-linkage ($p$<0.05) cluster analysis are overlay to the PCA. Notes: *a* = n. taxa; *b* = average cell density; *c* = Cell size; *d* = Chl-*a* picoplankton; *e* = Chl-*a* nanophytoplankton; *f* = Chl-*a* microphytoplankton.

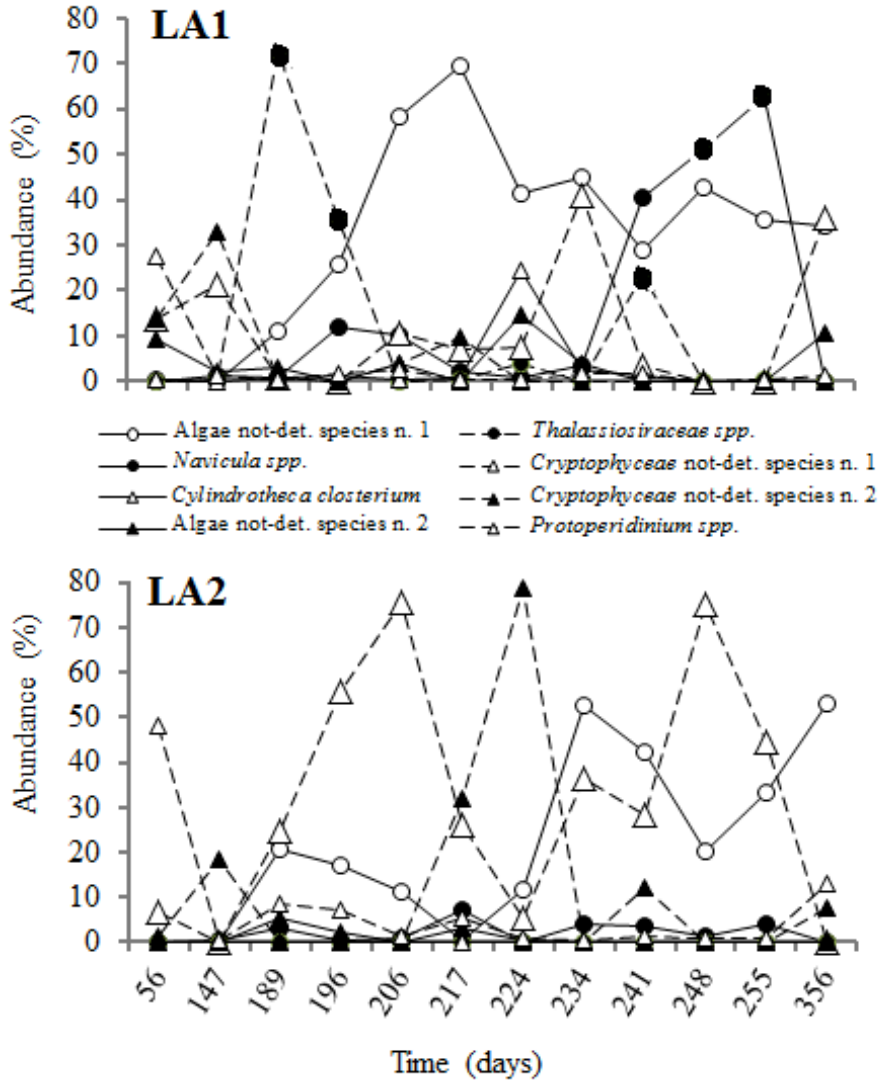


Figure 4. Relative abundances of most representative phytoplankton taxa during the time in stressed (LA1) and not stressed (LA2) sampling stations.

were observed between the two stations comparing average values and variances of the different environmental parameter at the univariate level (Table 2). Principal component analyses performed on benthic macroinvertebrate descriptors (Fig. 5) contextually for both considered sampling stations evidenced that the first three components (PC1, PC2, PC3) account for the 97.8% of the total variance (respectively 67.2%, 21.7%, and 8.9%). Results obtained by the cluster analysis are superimposed to the PCA in the same figure evidencing a statistically significant difference between LA1 and LA2 which occurs at any *temporal replicates* (SIMPROF test, $P<0.05$) and among them. Coefficients in the linear combinations of variables making up PC's, evidenced that the first axe is negatively correlated to number of species (-0.491), biomass (-0.525), and individual body size (-0.521). The second axis is significantly and negatively correlated to Shannon Index (-0.553) and positively related to density (0.646). Even in this case, the natural occurrence of seasonal fluctuations produces

a recordable effect on the PCA assessment (Global R = 0.268, P = 0.01%, NPS = 0), for this reason, the ANOSIM test two-way performed nested on both of the factor of interest: *sampling station* (LA1 *vs* LA2) and *temporal replicates* (sampling dates) confirms significance related to segregations observed according to the factor *sampling station* (Global R = 0.266, P = 0.01%, NPS = 0). Univariate t-Student test and F-ratio were performed on descriptors to select those major responsible of observed differences between LA1 and LA2 (Tab. 2). The t-Student test evidenced high significance *(*$p<0.01$) for density, species number, Shannon index, Biomass, while, F-ratio test evidenced high significance ($p<0.01$) for species number and Distinctness. In Table 3, total abundances of identified taxa in Lesina lagoon are reported evidencing significant differences (* = $p<0.05$; ** = $p<0.01$) between disturbed and not-disturbed sampling station. In Figure 6 temporal evolution of density and species richness in LA1 and LA2 are reported evidencing both high resistance and resilience time of both descriptors to dystrophic stress.

*Comparison of the rate of change of ecosystem components and descriptors*

Results obtained from the statistic analysis performed to evaluate the temporal duration of the difference between the LA1 and LA2 stations are summarized at any sampling times (number of days of the year) in Table 4 only

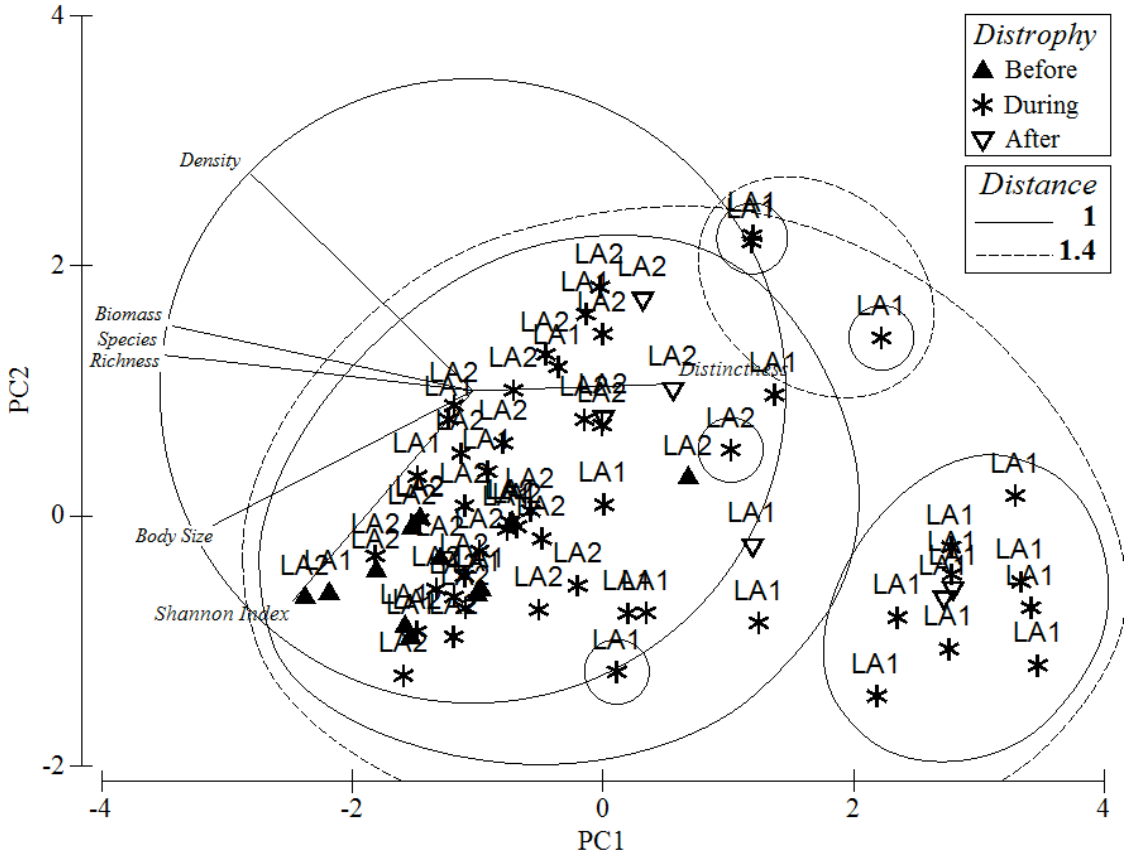


Figure 5. Principal component analyses performed on benthic macroinvertebrate descriptors. Two dimensional projection of phytoplankton descriptors is reported evidencing two factors of statistical interest: *sampling station* (LA1 *vs* LA2) and *temporal replicates* (before, during, after crisis). Results obtained from the single-linkage ($p<0.05$) cluster analysis are overlay to the PCA.

for descriptors evidencing high significant ($p$<0.01) results from the t-Student test and F-ratio analyses.

In Table 5 a synthesis of different recovery time is reported for each significant stressed descriptor in different considered environmental compartments to scale responses and evaluate resilience patterns. Recovery times differ among components and component features. Water descriptors show a fast alteration as stress response but a quick and quite complete recovery, on the contrary sediments evidence slowing alterations but difference are also evidenced at the end of the study period. Phytoplankton descriptors showed a quick response to dystrophic stress but a slow and complete recovery, on the contrary, benthic macroinvertebrates evidenced a delayed response (higher resistance) and very long time of recovery (high resilience).

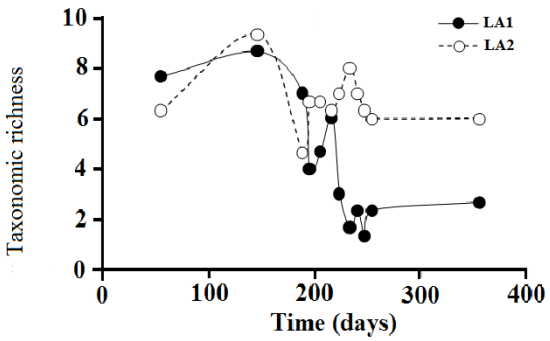


Figure 6. Taxonomic richness in sampling stations related to the evolution before, during and after dystrophic event.

## Discussion

Dystrophy is defined by Valiela (1984) as a reversible event of an aquatic ecosystem characterized by well-identifiable morphological features as well as the production of white water due to the diffusion

Table 3 - Relative abundance (%) of benthic macroinvertebrate taxa and significant differences between stressed and not-stressed stations. The presence of taxon in the stations LA1 and/or LA2 is reported as 1, the absence as 0. Significant difference between stations of taxon abundance is reported for $p$<0.05; not significant difference is reported as n.s.; n.c. indicates that the comparison was not made.

| Class | Taxon | Relative Abundance (%) | Presence/Absence | | |
|---|---|---|---|---|---|
| | | | LA1 | LA2 | *p* |
| Gastropoda | *Ecrobia ventrosa* | 56.70 | 1 | 1 | <0.05 |
| Bivalvia | *Abra segmentum* | 13.96 | 1 | 1 | n.s. |
| Insecta | *Chironomus salinarius* | 11.64 | 1 | 1 | <0.05 |
| Mollusca | *Mytilaster minimus* | 7.35 | 1 | 1 | <0.01 |
| Polychaeta | *Neanthes succinea* | 4.00 | 1 | 1 | <0.01 |
| Crustacea | *Lekanesphaera hookeri* | 2.32 | 1 | 1 | <0.05 |
| Crustacea | *Gammarus aequicauda* | 1.13 | 1 | 1 | <0.05 |
| Crustacea | *Cyathura carinata* | 0.90 | 1 | 1 | n.s. |
| Mollusca | *Cerastoderma glaucum* | 0.90 | 1 | 1 | n.s. |
| Polychaeta | *Ficopomatus aenigmaticus* | 0.42 | 1 | 0 | n.c. |
| Crustacea | *Idotea balthica* | 0.21 | 1 | 1 | n.s. |
| Polychaeta | *Neanthes sp.* | 0.16 | 1 | 1 | n.s. |
| Mollusca | *Musculista senhousia* | 0.10 | 1 | 0 | n.c. |
| Crustacea | *Sphaeroma serratum* | 0.07 | 1 | 1 | n.s. |
| Polychaeta | *Hediste diversicolor* | 0.05 | 1 | 1 | n.s. |
| Polychaeta | *Polidora ciliata* | 0.04 | 1 | 0 | n.c. |
| Polychaeta | *Harmotoe sp.* | 0.01 | 0 | 1 | n.c. |
| Oligochaeta | *Oligochaeta* | 0.01 | 0 | 1 | n.c. |
| Polychaeta | *Heteronereis sp.* | 0.01 | 1 | 0 | n.c. |

 

of sulphides from sediments. Ecological effects of dystrophy involve the whole ecosystem inducing severe changes in abiotic physico-chemical characteristics (Vignes *et al.*, 2009) and species assessment (Phil *et al.*, 1991). In particular, changes on biota include specie-specific shifts concerning dominances but, also, changes of relationships at a functional level. Economic impacts induced by the occurrence of dystrophic crisis are

Table 4 - Temporal trend of descriptors. This evaluation was performed using, for each descriptor, the pure ratio between LA2 and LA1 values ($n$ = 3). The observed distances from 1.0 (total identity) were assumed significant in both directions (±) when for each descriptors the calculated ratio resulted to exceed the range 1±0.1*absolute maximum ratio observed.

Legend of symbols adopted: - means no data acquired for this sampling time; n.s. means $H_0$ hypothesis of "not observed significant differences between LA1 and LA2" is accepted; $p<0.01$ means that $H_0$ hypothesis is rejected.

| | **Water** | | | | | **Sediment** | | | | **Phytoplankton** | | | **Benthic macroinvertebrates** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Day* | S | TP | Chl-***a*** | $O_2$ | $NH_4^+$ | Eh | TOC | TN | Fe | Density | Cell size | Chl-*a micro* | Density | Taxon. richness | Shannon Index | Dist. | Biom. |
| 56 | - | - | n.s. | n.s. | - | - | - | - | - | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
| 147 | - | - | n.s. | n.s. | - | - | - | - | - | n.s. | *p<0.01* | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
| 189 | n.s. | n.s. | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | n.s. | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | n.s. | n.s. | n.s. | ***p<0.01*** | n.s. |
| 196 | n.s. | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | n.s. | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | n.s. | n.s. | n.s. | ***p<0.01*** | n.s. |
| 206 | n.s. | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | n.s. | n.s. | n.s. | ***p<0.01*** | ***p<0.01*** | n.s. |
| 217 | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | n.s. | n.s. | ***p<0.01*** | ***p<0.01*** | n.s. |
| 224 | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | n.s. | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** |
| 234 | ***p<0.01*** | n.s. | ***p<0.01*** | n.s. | n.s. | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | n.s. | n.s. | *p<0.01* | *p<0.01* | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** |
| 241 | ***p<0.01*** | n.s. | ***p<0.01*** | n.s. | n.s. | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | n.s. | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** |
| 248 | ***p<0.01*** | n.s. | ***p<0.01*** | n.s. | n.s. | n.s. | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | n.s. | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** |
| 255 | ***p<0.01*** | n.s. | ***p<0.01*** | n.s. | n.s. | n.s. | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | n.s. | ***p<0.01*** | ***p<0.01*** | ***p<0.01*** | n.s. | ***p<0.01*** | ***p<0.01*** |
| 356 | - | - | n.s. | - | - | - | - | - | - | n.s. | n.s. | n.s. | ***p<0.01*** | ***p<0.01*** | n.s. | n.s. | ***p<0.01*** |

Table 5 - Temporal scaling responses: resistance and resilience pathways.

| **Descriptor** | **Resistance** | **Resilience** |
|---|---|---|
| *Water column* | | |
| S | Medium (7 weeks - 6 months) | High (<1 month) |
| TP | Low (<1 month) | Medium (7 weeks - 6 months) |
| Chl-*a* | Low (<1 month) | High (<1 month) |
| $O_2$ | Low (<1 month) | Medium (7 weeks - 6 months) |
| $NH_4^+$ | Low (<1 month) | Medium (7 weeks - 6 months) |
| *Sediment* | | |
| Eh | Low (<1 month) | Medium (7 weeks - 6 months) |
| TOC | Medium (7 weeks - 6 months) | High (<1 month) |
| TN | Low (<1 month) | High (<1 month) |
| Fe | Low (<1 month) | High (<1 month) |
| *Phytoplankton* | | |
| Av. cell density | Medium (7 weeks - 6 months) | High (<1 month) |
| Cell size | Low (<1 month) | Medium (7 weeks - 6 months) |
| Chl-*a* micro | Low (<1 month) | Low (> 6 months) |
| *Benthic macroinvertebrates* | | |
| Density | Medium (7 weeks - 6 months) | High (<1 month) |
| Taxonomic richness | Medium (7 weeks - 6 months) | High (<1 month) |
| Shannon Index | Medium (7 weeks - 6 months) | High (<1 month) |
| Distinctness | Low (<1 month) | High (<1 month) |
| Biomass | Medium (7 weeks - 6 months) | High (<1 month) |

 

remarkably including ecological and economic exploitation losses of resources. Aquaculture and fishery activities reduce significantly their productivity when a dystrophic event occurs. Several geomorphological and meteorological features as well as wind intensity, rains (Lapointe and Matzie, 1996), cloud covering (D'Avanzo *et al.*, 1996) irradiance, precipitation (Beck and Bruland, 2000), efficiency of marine-lagoon water exchanges both human- and tidal- driven, balance between freshwater inflow and evaporation rates (Haas, 1977; Edwards *et al.*, 2004) are able to affect the occurrence of dystrophy in a coastal lagoon ecosystem. Due to the great importance of these aspects conditioning the occurrence of such a critical event, management strategies in these ecosystems are often engineering-based and finalized to improve water circulation, marine-lagoon water exchanges and bottom depth.

Lesina is an eutrophic ecosystem (Roselli *et al.*, 2009) which in May 2008 was interested by a complete interruption of marine-lagoon water exchanges due to dredging operations and structural engineering works along the Acquarotta canal. At the same time these management actions, winds reduced significantly till June 2008 inducing the dystrophic crisis which severely stressed a geographically confined lagoon area localized closed to the Acquarotta canal (Vignes *et al.*, 2009). The analysis of satellite images evidenced that visible effects of dystrophy in Lesina water (area named LA1) started from June and were recognizable till August (Vignes *et al.*, 2009). Higher salinity and temperature values are observed in water sampled in LA1 dystrophy-stressed lagoon area compared to the internal control ones (LA2). This occurrence induced the formation of a dense, low-circulating water mass inside the dystrophic area consequently to the forced interruption of natural sea-lagoon exchanges. The absence of both water mixture and wind have produced a severe lack of oxygen inside water from LA1 area enhancing dystrophy.

Winds are principal responsible of steady soft sediments oxygenation in undisturbed lagoon areas throughout re-suspension phenomena (Hopkinson, 1985) which activate bacterial oxidative mineralization (Fanning *et al.*, 1982) and increase the re-mineralisation rates (Wainright, 1987; Wainright, 1990) enhancing processes by a factor between 2 and 5 (Stahlberg *et al.*, 2006). Natural oxygenation phenomena produce low levels of phosphates in water column increasing silicates, nitrites and nitrates concentrations (Tengberget *et al.*, 2003). Furthermore, wind-mediated sediment oxygenation activate nutrient release (Wainright and Hopkinson, 1997) from bottoms towards interstitial water, increase oxygenation levels, pH and redox potential in sediments. On the other hand, an increase in nitrate concentrations which occurs in anoxic microhabitats (Fenchel, 1992) produces high denitrification rates (Herbert and Nedwell, 1990), resulting in a loss of part of sediment nitrogen as $N_2$ or $N_2O$. When these phenomena occur, the general eutrophy of the system lowers (Novicki *et al.*, 1997). The occurrence of dystrophy is characterized by severe reduction of oxygen concentration in water which induces the prevalence of anaerobic metabolic pathway for the decomposition of sedimentary organic substance throughout sulphate respiration bacterial processes (Jørgensen, 1983) and the production of acidifying ($CO_2$) and toxic ($H_2S$) gases. As a consequence, pH values and redox-potential (Eh) in sediments decreases leading to a build-up of both reduced and reducing chemical components also inducing ammonium production and nitrite increase by ammonification of organic matter (Marty *et al.*, 1990; Torres-Beristain *et al.*, 2006).

As a consequence of exposed dynamics, measured pH, $O_2$, and silicates are lower in LA1 water than LA2 whereas TP, $NH_4^+$, $NO_2^-$

and $NO_3^-$ levels are higher. In sediments, Eh and TOC are lower in LA1 than LA2. In oxidized sediments, orthophosphates (SRP) are strongly bound to ferric oxides-hydroxides (Golterman, 1995; 2001), carbonates and clays (Dodge *et al.*, 1984; De Jonge and Villerius, 1989) becoming unavailable to macroalgae. In the case of nitrogen compounds, the oxidation accelerates the nitrification processes producing a nitrates predominance against the presence of reduced forms represented by nitrite and ammonium (Revsbech *et al.*, 1980).

The buffering property of sediments due to the SRP adsorption from the column water onto the oxidized iron forms (Golterman, 1995; 2001) as sulphide by ferrous iron, as ferrous sulphide and then as pyrite (Berner, 1984; Luther, 1991; Richard and Luther, 1997; Theberge and Luther, 1997; Rozan *et al.*, 2002) notably decrease in anoxic conditions due to the reduction of oxidized iron. The iron reduction and its blockade by $H_2S$ releases SRP which previously were bound to ferric oxides-hydroxides (Gunnars and Blomqvist, 1997; Golterman, 2001; Rozan *et al.*, 2002). Therefore SRP are mobilized into the water column where become available to the algae growth. The occurrence of this phenomenon is the reason for which even if LA1 sediments showed significantly higher Fe values than LA2, SRP in LA1 water are notably higher than LA2.

Physico-chemical changes of selected descriptors for abiotic compartments were widely described and discussed by previously published researches (Specchiulli *et al.*, 2009; Vignes *et al.*, 2009). Results obtained in this research evidence that selected descriptors for water and sediments account for the great part of variance between dystrophy stressed areas and controls in an eutrophic lagoon ecosystem and are able to significantly discriminate stressed by non-stressed stations. Furthermore, concerning sampled matrices S, $O_2$, $NH_4^+$, silicates, TN, TP, Chl-*a* micro in water and Eh, TOC, TN, Fe in sediments differ significantly in stressed and non-stressed stations independently by seasonal fluctuations.

Changes occurring for phytoplankton descriptors during dystrophy are well described by a previous research paper (Vadrucci *et al.*, 2009). The principal contribute to observed statistical differences between LA1 and LA2 stations are mainly due to density and Chl-a micro components both higher in dystrophy stressed lagoon area than in control.

Concerning benthic macroinvertebrates, descriptors that are major responsible of observed differences between sampling stations are: taxonomic richness, Density, Shannon index and Biomass which all decrease in dystrophic stressed station indicating a notable biodiversity reduction occurring during critical episodes. The tendency to maintain during the time a certain values of a descriptor could express the resistance towards alteration of the descriptor itself. Furthermore, the time which occur to a certain descriptor after the end of the dystrophy occurrence (as defined by Valiela, 1984) to recovery previous levels could represent an expression of its resilience.

Concerning resistance/resilience times of considered water descriptors, results obtained in this paper evidence that observed responses to the stress of all of them are very fast (days/week) whereas recovery times are descriptors-dependent. In particular in water, recovery time of dissolved oxygen, TP and $NH_4^+$ levels are fast (weeks) and complete (absence of significant differences at the end of the monitoring period between stressed and not-stressed areas), whereas, recovery time of Chl-*a* is very slow (months) but complete. Concerning these two descriptors, reasons related to the observed behavior could be substantially different. The absence of recovery of salinity could be due to a

 

scarce water dilution occurred till the end of monitoring period. In fact, observations ended in December 2008, few and not rainy months after the dystrophic episode. This consideration is supported by a long average renewal time (70-100 days) reported for this ecosystem during normal water circulation conditions (Manini *et al.*, 2002).

Chl-*a* micro represents an estimation of the phytoplankton standing-crop. Results obtained in this paper evidence significant differences between LA1/LA2 occurring fast (days 189, 196) and till September (day 255) with wide fluctuations that are, also, well described by other researches (Vadrucci *et al.*, 2009; Vignes *et al.*, 2009). Secondary increases of this descriptor observed between days 217-224 and days 241-255 in LA1 seem to be probably due to ecological phenomena (reduction of local competitors after the crisis) rather than to a direct effects of nutrient increases on phytoplankton standing-crop. These hypotheses are also confirmed by results obtained on phytoplankton studies (Vadrucci *et al.*, 2009). Changes of considered descriptors (Density, individual average cell dimensions, Chl-*a* micro) were fast but recoveries were also fast only concerning individual average cell dimensions, whereas, density and Chl-*a* micro evidenced a slow (months) and complete recovery.

In water, a low resistance toward alteration of water descriptors associated to a great resilience was recorded (TP, $O_2$, $NH_4^+$). In sediments, resistance of selected descriptors was very low and changes occur within few days/week, nevertheless, recovery was very slow (months) or absent (significant differences between stations at the end of the monitoring period) evidencing low resilience. Phytoplankton descriptors evidenced a different behavior: all of considered descriptors showed low resistance but individual average dimensions showed higher resilience than others. Benthic macroinvertebrate descriptors evidences the higher resistance but resilience times are notably longer (years).

**Conclusions**

Results obtained in this study allow to select a few number of significant descriptors of dystrophic crisis applicable for coastal lagoon monitoring of different abiotic (water, sediment) and biotic (phytoplankton, benthic macroinvertebrates) matrices. Furthermore resistance and resilience of selected descriptors are evaluated in term of specific resistance and resilience expressed as recovery time after the occurrence of a severe dystrophic stress. Benthic macroinvertebrates statistically significant descriptors (density, taxonomic richness, Shannon index and Biomass) evidence higher resistance compared to others and a long time of recovery (> 6 months) after the occurrence of the dystrophic event.

Obtained results evidence that some among considered water (TP, Chl-*a*, $O_2$, $NH_4^+$), sediment (Eh, TN, TP) and phytoplankton (average cell size, Chl-*a* micro) descriptors show earlier alterations and could be useful to evaluate and describe temporal variation of ecosystem resistance. On the contrary, most of the benthic macroinvertebrates descriptors considered in this paper (density, taxonomic richness, Shannon index, Biomass) identify the occurrence of the dystrophic induced stress after longer times (7 weeks - 6 months) from the beginning of critical period and could be very useful to monitor ecosystem resilience.

Obtained results has key implications on planning monitoring strategies and programs by highlighting the need to incorporate early warning descriptors, mainly non-taxonomic ones, to address descriptor-specific typology (Basset *et al.*, 2013), uncertainty (e.g. Dromph *et al.*, 2013) and type specific conditions (e.g., Basset *et al.*, 2013). Including descriptor specific resistance and resilience offers tools

 

to reduce uncertainty in the assessment of ecological status of transitional waters as well as to detect early warning signal towards a proactive monitoring of the highly dynamic ecosystems.

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