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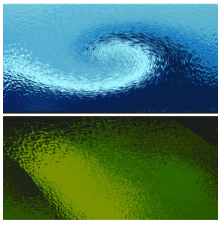

RESEARCH ARTICLE

Dynamics of phytoplankton guilds under dystrophic pressures in Lesina lagoon

Maria Rosaria Vadrucci[1], Annita Fiocca[2], Fabio Vignes[2], Adele Fabbrocini[3], Leonilde Roselli [3], Raffaele D'Adamo [3], Nicola Ungaro [4], Alberto Basset [2]

[1]Agenzia Regionale per la Protezione dell' Ambiente della Regione Puglia-Dipartimento provinciale di Lecce.
[2]Dipartimento di Scienze e Tecnologie Biologiche e Ambientali, Università del Salento.
[3]C.N.R. - I.S.MAR. Sezione Ecosistemi costieri e Lagunari Lesina
[4]Agenzia Regionale per la Protezione dell' Ambiente della RegionePuglia-Direzione Scientifica.

Abstract

1. We analyzed the dynamic of phytoplankton guild during an dystrophic crisis observed in an area of Lesina Lagoon from July to September 2008.
2. A two-weekly scale, patterns of variation of phytoplankton biomass (as Chl*a*), numerical abundance, species richness, diversity, taxonomic and size structure were compared in two stations: one within the area affected by the crisis (AT2 station) and one outside, as control (AT3 station).
3. Phytoplankton biomass and cell abundance varied significantly between sampling stations and sampling times. Biomass was on average about 100 time higher in AT2 than AT3 station, with peaks of 180,67±22,12 mgChla/m^3 (8 July) and 134,05±47,26 mgChla/m^3 (5, September). Cell density was on average about 30 times higher in AT2 station, with values always higher than 20*106 cell/l.
4. Species richness and diversity did not varied significantly between stations and not show a trend with the evolution of anoxic crisis.
5. The taxonomic composition is similar in the two stations (taxonomic similarity among stations was 76,04%), even if the relative abundance of species was significantly different.
6. As regard size structure, in the AT2 station, microphytoplankton dominates at beginning of biomass bloom (dominant taxa: *Thalassiosiraceae*, average weight (AW) 163,51 pgC), whereas from the end of July until September and in the AT3 station, nanophytoplankton was the dominant fraction (dominant taxa: until the middle of August: Algae indet AW 3,14 pgC and Navicula sp. AW 2,50 pgC).

Introduction

Due to their restricted exchange with the adjacent ocean, transitional water ecosystems are considered particularly vulnerable to eutrophication. Increasing of eutrophication phenomena occurred as a direct result of increasing population densities along transitional water ecosystems coastline and use of fertilizers for agriculture in their surrounding watershed (Cloern, 2001). Dystrophic crises represented one of the more dramatic consequence of eutrophication processes. Dystrophic crises can affect the biogeochemical cycle of nutrients: oxygen depletion in the sediments can favour the release of recycled nutrients in the water column, in particular phosphorus, that, in presence of particular meteo-climatic and hydrodynamic conditions, can persist in doing the eutrophication status (feed-back effect: Jensen *et al.*, 1995; Conley *et al* 2002). Phytoplankton shown patterns of variation of own structural and functional features that are directly related to nutrient input (Pearl 2005). As a consequence, phytoplankton can be considered as one of

the primary indicator of the variation of the trophic status of aquatic ecosystems versus eutrophic conditions (Conley *et al* 2009). Therefore the study of spatio-temporal dynamic of phytoplankton guilds during a distrophic event give the opportunity to study how phytoplankton community evolved and what are the parameters that better describe (phytoplankton biomass, cell density or diversity) temporal and spatial patterns of variation. These results are important in prospective to identify the best phytoplankton parameters by using as descriptors for the evaluation of health status of transitional water ecosystems. The Lesina lagoon is a transitional water ecosystem localized along the Southern Adriatic coast in a region of southeast of Italy. The lagoon represent an high economic good for the local population. Since it provide important ecosystem services, including fish and shellfish production and human recreational activities. It has a surface area of 51,4 km² and an average depth of 0,7m. The salinity of the lagoon ranges between 20.0 psu and 29,5 psu. Water temperature ranges from 10 °C in winter to 29 °C in summer. No stratification exists in the water column. The lagoon is isolated from the Mediterranean Sea by a 22- km long sandy bar (Bosco Isola), crossed by two very shallow channels (Acquarotta and Schiapparo) and received freshwater contribution by a series of watercourses and from two water-scooping machines that drain the surrounding grounds. During recent decades, agriculture in the watershed was increase, at the same time, increasing tourist activities have increased volumes of sewage. As a result, the waters of the lagoon have experienced an increase in nutrient levels and as a consequence an increase of eutrophication events. These events have never been documented. At the beginning of the summer 2008 a severe dystrophic crisis was observed in the lagoon. The event was observed in a localized area near the Lesina city and was characterized by phytoplankton bloom, decrease of oxygen in the sediments and dead of great number of organisms, in particular fish and macrobenthonic fauna. The distrophic event evolution in the water column and in the sediments were described in Vignes et al., and Specchiulli et al., in this issue. The aim of this work is to describe the pattern of temporal variation of phytoplankton guilds under pressures deriving from the above indicated dystrophic event. In particular, the temporal variation of phytoplankton parameters such diversity, cell density, biomass will be examined and compare in a station located within the area affect by crisis and a control station localized in an area not affected by dystrophic event. Moreover, the study was expanded for including period before and after the dystrophic crisis.

Materials and methods

Sampling strategy and Laboratory measurements

According 152/2006 Italian regulation, in 2008, the Lesina lagoon was included in a monitoring plan for the determination of health status of surface water bodies of Puglia Region. Water samples were collected in six sampling stations. The sampling was carried out from February 2008 to December 2008 with a three-months frequency. Sampling were increase during the dystrophic events, so from July 2008 to September 2008 water samples were collected at two-weekly frequency but in only two of the sampling stations including in the monitoring plan. The two selected stations were localized in the area affected by dystrophic event (AT2 station) and in an area out of dystrophic events (AT3 station) that was considered as a control (Figure 1).

For determining phytoplankton taxonomic composition and size structure, at each

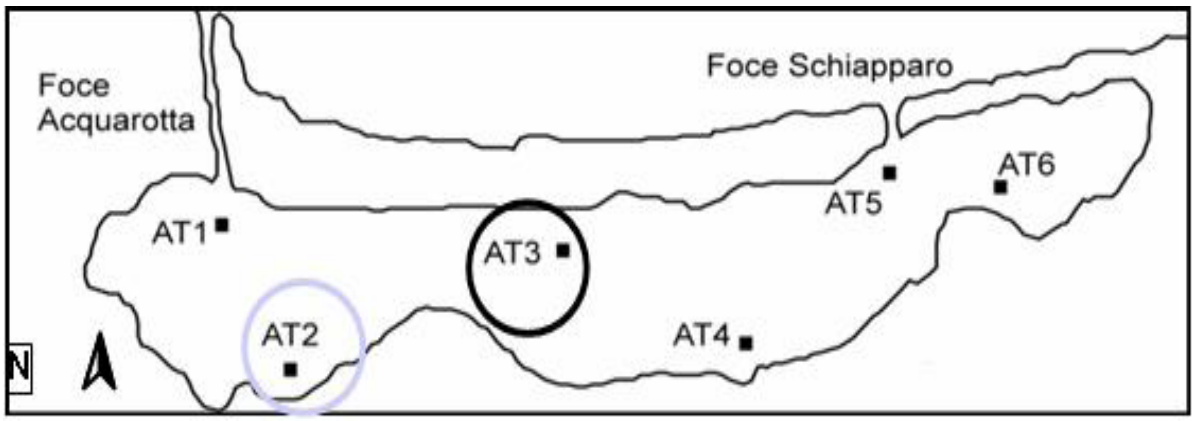

Figure 1. Localization of sampling stations in the Lesina Lagoon , in the dark circle station within the area affected by anoxic event, in the grey circle station localized out of the anoxic event and considered as control station.

sampling point, three replicate of 500ml-water samples were collected from 0.5m depth, with a Ruttner bottle, and were immediately preserved with Lugol's solution (5 ml for litre sample). Water sample were kept cold (<10oC) and in the dark until analysis. 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 Utermöhl's method (Zingone *et al*., 1990). The individual cell volume (V, µm3) 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).

For total and size-fractionated chlorophyll *a* determination, 1 litre-water samples were collected in three replicates and filtered immediately, after collection, using a GF/F (total chl*a*). For the determination of <2 µm size fraction, samples were filtered before on 2 µm Nucleopore and then on GF/F filter, whereas for the determination of <20 µm size fraction water samples were filtered on 20 µm net and then on GF/F . Filters were stored at -20°C until analysis, carried out in most cases one month later. Chl*a* was extracted in 90% acetone, for 24 hours, at 4°C, in the dark. Chl*a* was determined with a spectrofluorimeter SHIMATZU-1051 before and after acidification with hydrichloric acid 0,5 N (Yentsh and Menzel, 1963)

Results

Taxonomic structure

The list of phytoplankton taxa was shown in Table 1. In all sampling period we have identified 56 taxa; 44 in the AT2 station and 48 in the AT3 station. In the AT2 station 41 % were diatoms the 3% were dinoflagellates, 10% were criptophyceae and the reimaning 46% taxa belong to other taxa. At the AT3 station 0,14% were diatoms the 17% were dinoflagellates, 49% were criptophyceae and the reimaning 45% belong to other taxa. During dystrophic event 42 taxa were

Table 1 - List of phytoplankton taxa observed in the Lesina Lagoon during the 2008 in the AT2 and AT3 station.

List of taxa	AT2	AT3	List of taxa	AT2	AT3
Bacillariophyceae			**Other phytoplankton**		
Amphora sp.	*		Alga indet. 3	*	*
Chaetoceros sp.	*	*	Alga indet. 4	*	*
Cocconeis scutellum	*	*	Alga indet. 5	*	*
Cocconeis sp.		*	Alga indet. 7	*	
Coscinodiscus sp.		*	Alga indet. 8	*	
Cylindrotheca closterium	*	*	Alga indet. 9	*	*
Diploneis crabro	*	*	Alga indet. 11	*	
Gyrosigma sp.	*		Alga indet. 12	*	*
Licmophora flabellata	*		Alga indet. 13	*	
Melosira sp.		*	Alga indet. 14	*	
Navicula sp.	*	*	Alga indet. 16	*	*
Navicula transitans	*	*	Alga indet. 21		*
Nitzschia sp.	*	*	Phytoflagellates indet.	*	*
Pleurosigma sp.		*	*Phaeocystis pouchetii*	*	*
Pseudo-nitzschia sp.		*	*Pyramimonas sp.*	*	*
Thalassiosiraceae indet.	*	*	Cryptophyceae indet. 1	*	*
Bacillariophyceae indet.	*	*	Cryptophyceae indet. 2	*	*
Dinoflagellates			Cryptophyceae indet. 3	*	*
Amphidinium carterae	*	*	Cyanophyceae indet.	*	
Amphidinium sp.	*	*	*Euglena sp.*	*	
Diplopsalis sp.	*		*Tetraselmis sp.1*	*	*
Gymnodinium sanguineum	*	*	*Tetraselmis sp.2*		*
Gymnodinium sp.	*	*	*Tetraselmis sp.3*	*	*
Gymnodinium splendens		*			
Mesoporos sp.		*			
Oxytoxum sp.	*	*			
Prorocentrum minimum	*	*			
Prorocentrum scutellum	*	*			
Prorocentrum sp.	*	*			
Protoperidinium sp.	*	*			
Scrippsiella trochoidea	*	*			
Dinoflagellates indet.	*	*			

identified. Most of taxa (>60%) are in common among the stressed station and in the control station.

Most of taxa were abundant and frequent (25 out of 56 taxa for all sampling period and 23 out of 42 taxa during the dystrophic events), that is, contributed to 90% of total abundance and were observed at least in the 25% in either sampling stations for all sampling times analyzed.

More of the 80% of most frequent and abundant taxa are also in common among the two stations, however temporal dynamics of numerical abundance shown large differences among the AT2 and AT3 stations. This was evident in results of the nMDS ordination diagram of Bray–Curtis similarities carried out on non transformed taxa specific abundances data where five groups were identify (Figure 2). One group included data of numerical abundance taxa at AT2 station during the dystrophic event, one included taxa of numerical abundance data at AT3 station in the same period. The other three groups included the sampling carried out in period before and after the dystrophic event. The average similarity between the two stations during the anoxia period was of 5,21%, and was higher in the period before and after the dystrophic event (average Bray-Curtis Similarity 37,98%). Comparative analyses of phytoplankton guild evolution during the dystrophic event was made at two different taxonomic level: at taxon level and at class /group level. SIMPER analysis was performed in order to highlight phytoplankton differences at taxon level. This analysis identified 9 important taxa as typifying the phytoplankton guild during the dystrophic event. In any case, phytoplankton bloom was characterized by the increases of biomass of three taxa (Figure 3), two of which were present in either sampling stations and increase their density during the dystrophic event with values higher than a magnification order in the AT2 station. Specifically to temporal dynamic, a first peak was observed at the

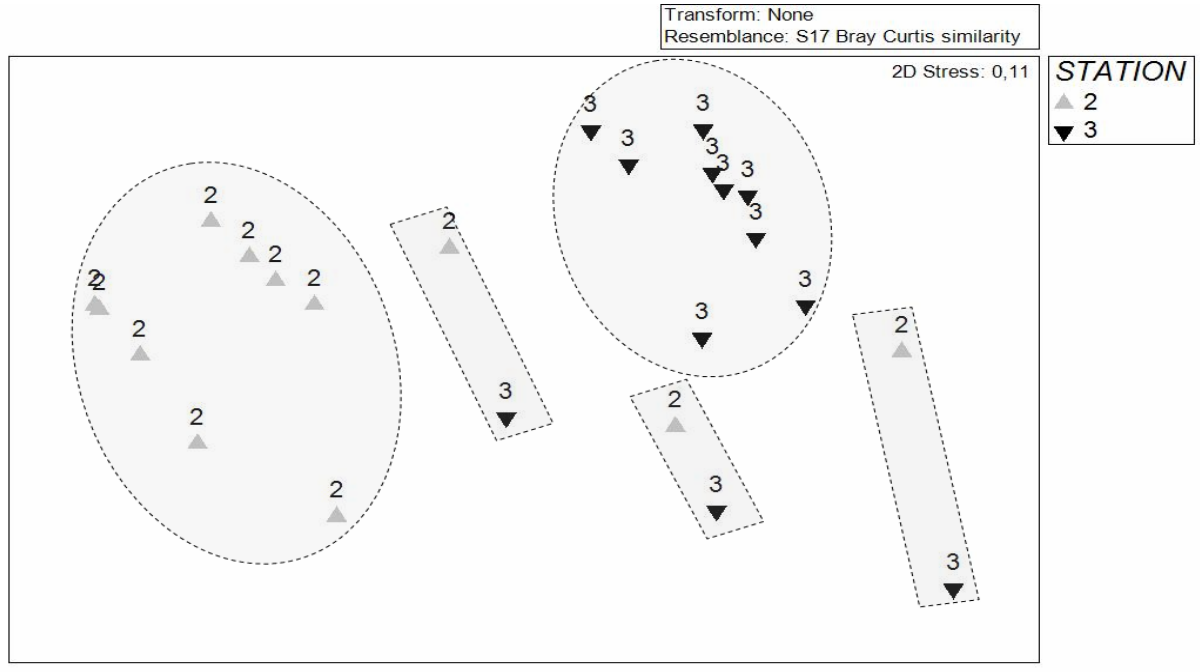

Figure 2. Ordination diagram of centroids relative to the sampling points according to the taxonomic composition and relative abundance of phytoplankton taxa in the study period considered

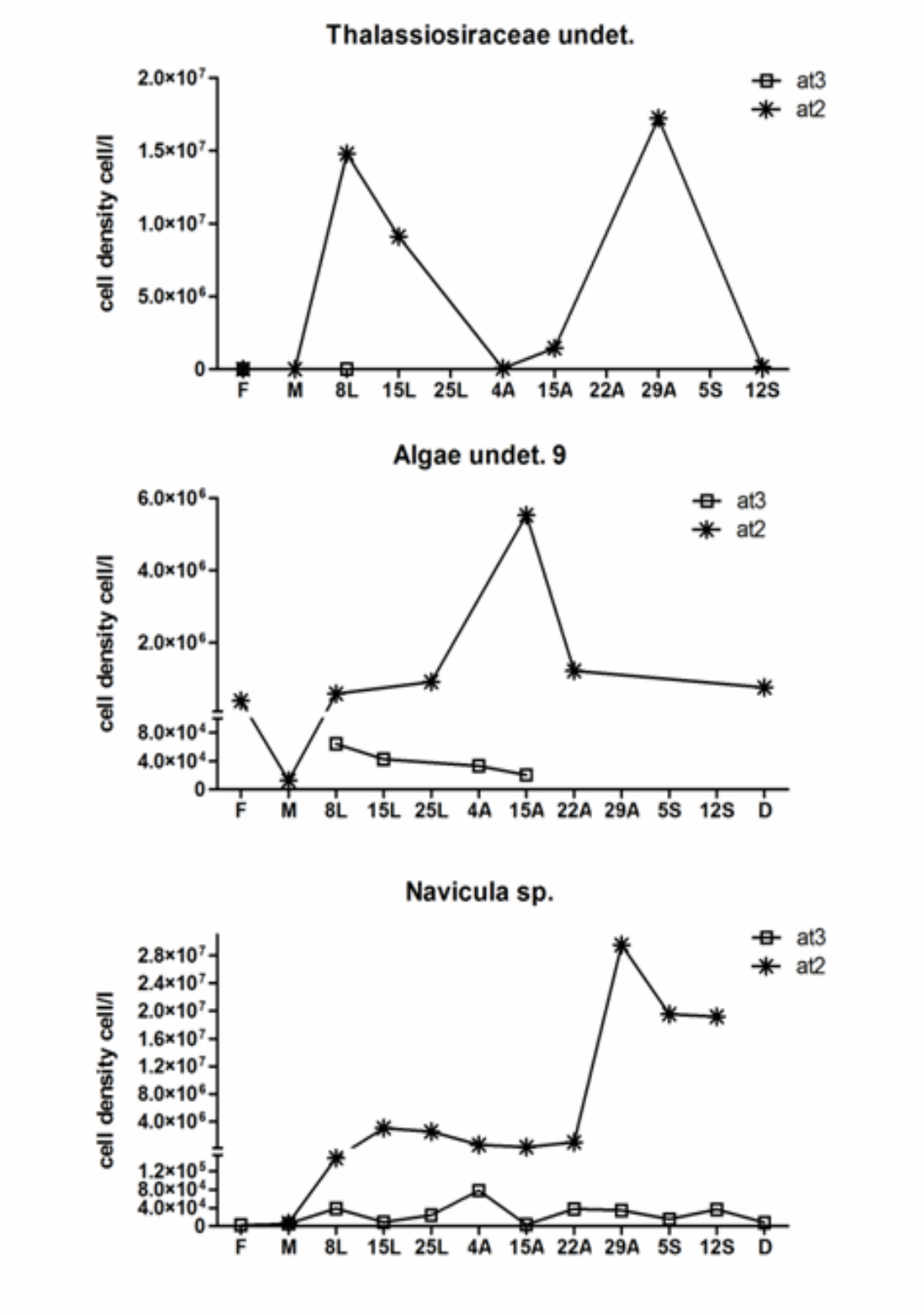

Figure 3. Temporal variation of cell density of eight of nine specie typifying the anoxic event of the Lesina Lagoon.

begin of July and was due to increasing of a specie with an average weight of 163,51 pgC, belong to *Thalassiosiraceae* taxa, that reached a maximum of $1,47*10^7$ cell/l. Then, becoming to the first week of August and until the end a bloom, an increase of undetermined phytoflagellates cell density (maximum: $2,19*10^7$ cell/l) occurred. Finally, at the end of August a new diatoms bloom was observed, due at a species belong to *Navicula* genera and Thalassiosiraceae group (maximum: $2,9*10^7$ cell/l). At class level, cryptophyceae and undetermined phytoflagellates were dominant taxonomic groups in the control station and before and after the dystrophic event in the stressed station, whereas Bacillariophyceae were the dominant class during the dystrophyc event (Figure 4).

Numerical Abundance and total and size fractionated phytoplankton biomass

As a consequence of the results shown above total phytoplankton cell density varied significantly between the two stations and among time sampling (two way anova

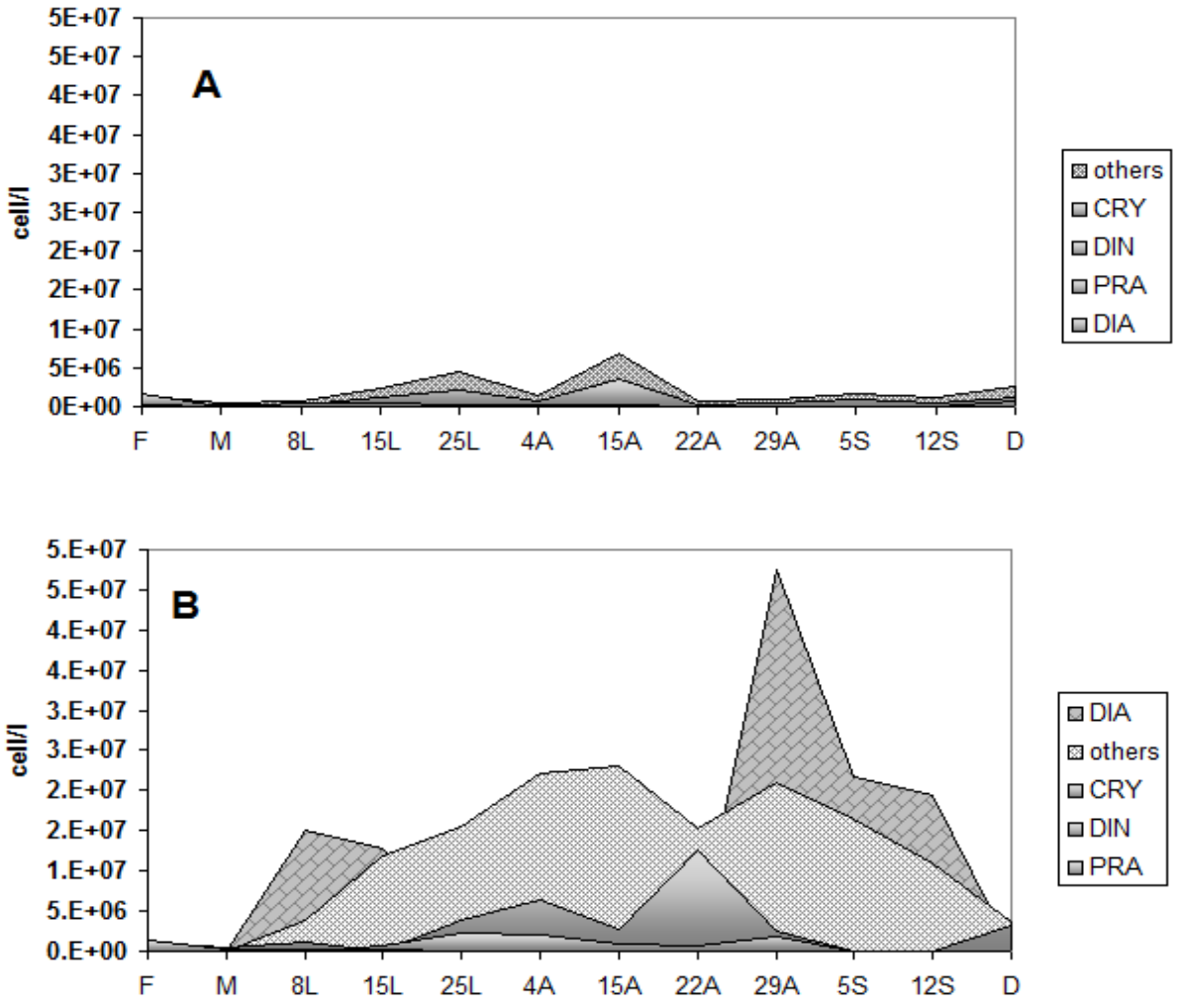

Figure 4. Temporal variation of phytoplankton taxonomic classes of group in the AT3 station (a) and in the AT2 station (b).

test $F_{stations\ 1,11}$ 10,9; P<0,001 $F_{time\ 1,11}$ 268,9 P<0,001). These variations were significant only during the eutrophication period (Bonferroni post test among stations is not significant in the sampling carried out before and after the dystrophic event). Cell density increases during the bloom period, starting from 2,07x10^7 cell/l (July 2008) to 5,48 x10^7 cell/l (29 August 2008). During the bloom the values of cell density at AT2 station were one order of magnitude higher than in the AT3 station. On the contrary, the values of cell density were comparables in the two stations before and after the dystrophic events (Figure 5).

The phytoplankton biomass (expressed as chlorophyll *a*, Figure 5) also varied significantly between the station within the area interested by dystrophic crisis and the control station and among sampling time (two-way ANOVA $F_{stations\ 1,\ 11}$ 34,87; P<0,001 $F_{time\ 1,11}$ 29,80 P<0,001). These variations were

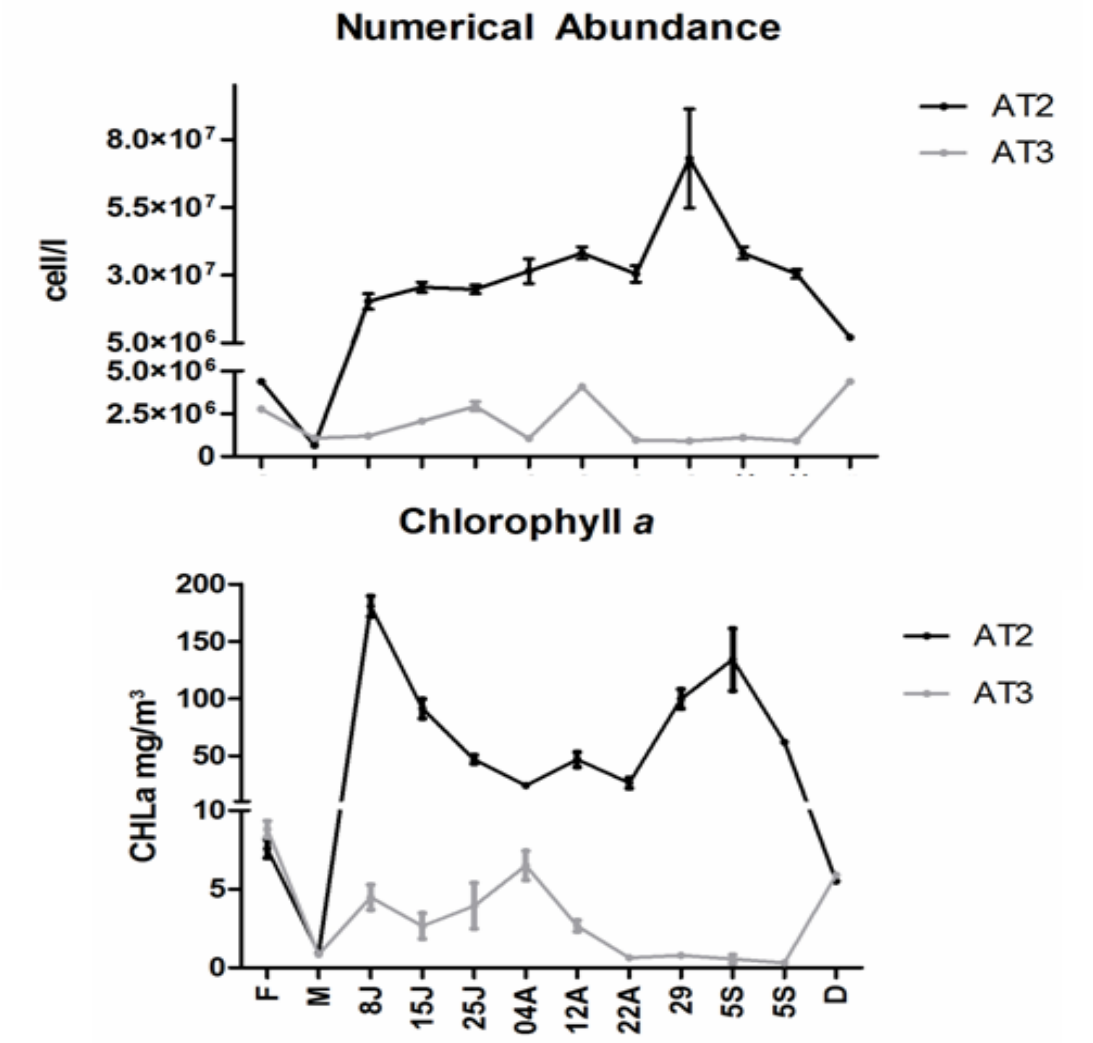

Figure 5. Temporal variation of phytoplankton cell density and biomass (expressed as chla) in the Legina Lagoon during the study period.

significant only during the eutrophication period (Bonferroni post test among stations is not significant in the sampling carried out in 4 August, 22 August and in period when dystrophic crisis do not occurred: February, May and December 2008). At AT2 stations biomass maximum (as chlorophyll *a*), observed the 8 July, was of 180,27±22,12 mg/

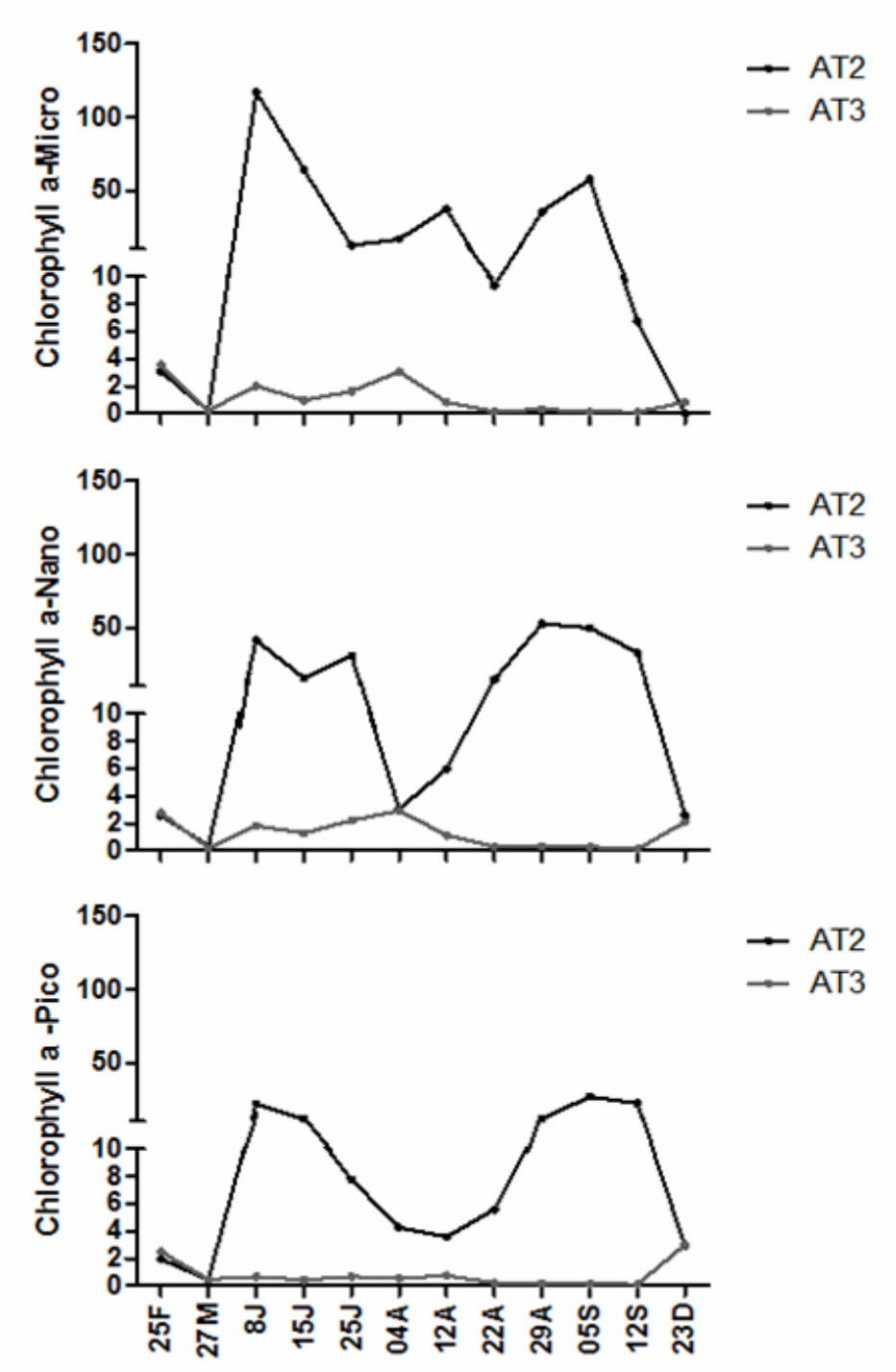

Figura 6. Temporal variation of micro-, nano-, and picophytoplancton biomass (expressed as chlorophylla) in the Lesina Lagoon during the study period considered.

m^3, followed by a second peak in September, with value of 134,05±47,26 mg/m^3. During all period where dystrophic crisis was observed, the value of chlorophyll *a* was always higher than 20 mg/m^3. In the period out of bloom the biomass values were comparable at those observed at the AT3 station. Average values at AT3 station was of 3,17±2,79 mg/m^3, higher values were observed in the first time of the bloom, then the biomass value decrease at values lower than 1 mg/m^3 .
Phytoplankton size fractions also varied significantly between station affected by dystrophic events and control station (Figure 6). All three fractions shown higher chlorophyll *a* values in the AT2 station with respect to the control station, during the dystrophic event whereas before and after the crisis the size fraction biomass concentration was comparable in the two stations. The microphytoplankton contribution increasing during the first period of dystrophic event. The relative contribution was in average the 70% of total biomass. In the second part of bloom nano-phytoplankton and micro-phytoplankton biomass contributed in average of 40% of total biomass. The contribution of picophytoplankton become more important just after the dystrophic event but in any cases its contribution do not exceed 20% of total biomass in average.

Specie Richness and Diversity

Specie richness did not varied significantly among the two stations (two-way ANOVA was not significant for station factor), but varied among sampling time. However the significance among sampling time was due for the sampling of 4 and 12 August were the number of taxa at the AT2 station was significantly lower than at the AT3 stations ($F_{time\ 1,11}$ 11,63 $P<0,001$). At the two stations the average value of species richness was of 10 ± 3 at AT2 station and 11±3 at AT3 station (Figure 7).

The diversity of phytoplankton (H di Shannon, Figure 7) did not vary significantly among stations but varied significantly among sampling time (two-way ANOVA for time F $t_{ime\ 1,11}$ = 15,85 $p<0,001$). This significance was due to the difference in the diversity index values at the four sampling time during the dystrophic event (8 July, 4 August, 12 August and 12 September) were the diversity index values were generally lower in the AT2 station with respect to AT3 station, in correspondence of the dominance peak of species responsible of bloom. The average value of index was of 0,55 ±0,13 at the AT2 Station and 0,56 ±0,18 at the AT3 station.

Discussion

In the present study, we have analyzed the evolution of some phytoplankton parameters during the anoxic event occured in the Lesina Lagoon during the summer 2008. The anoxic event was indeed considered as an stressor where the dynamic evolution of phytoplankton parameters can be analyzed and discussed in term of evaluation of the level of response of phytoplankton parameters to stress events. Phytoplankton dynamic was analyzed for two months with a two-weekly frequency. As regard methodological aspects, taxonomic classification was in some cases very difficult for the high concentration of detritus in the samples and for the presence of species with linear dimensions less than 4 µm. So, on more of 15 of the most abundant taxa was impossible to make a taxonomic characterization.
The results reach with this work can be summarized as follow:
1) The bloom was generated by phytoplankton species that were present in the lagoon in a vegetative state in bloom period and in non-bloom period. If we comparing floristic list of phytoplankton taxa in the station affected by anoxic crisis and in the control station,

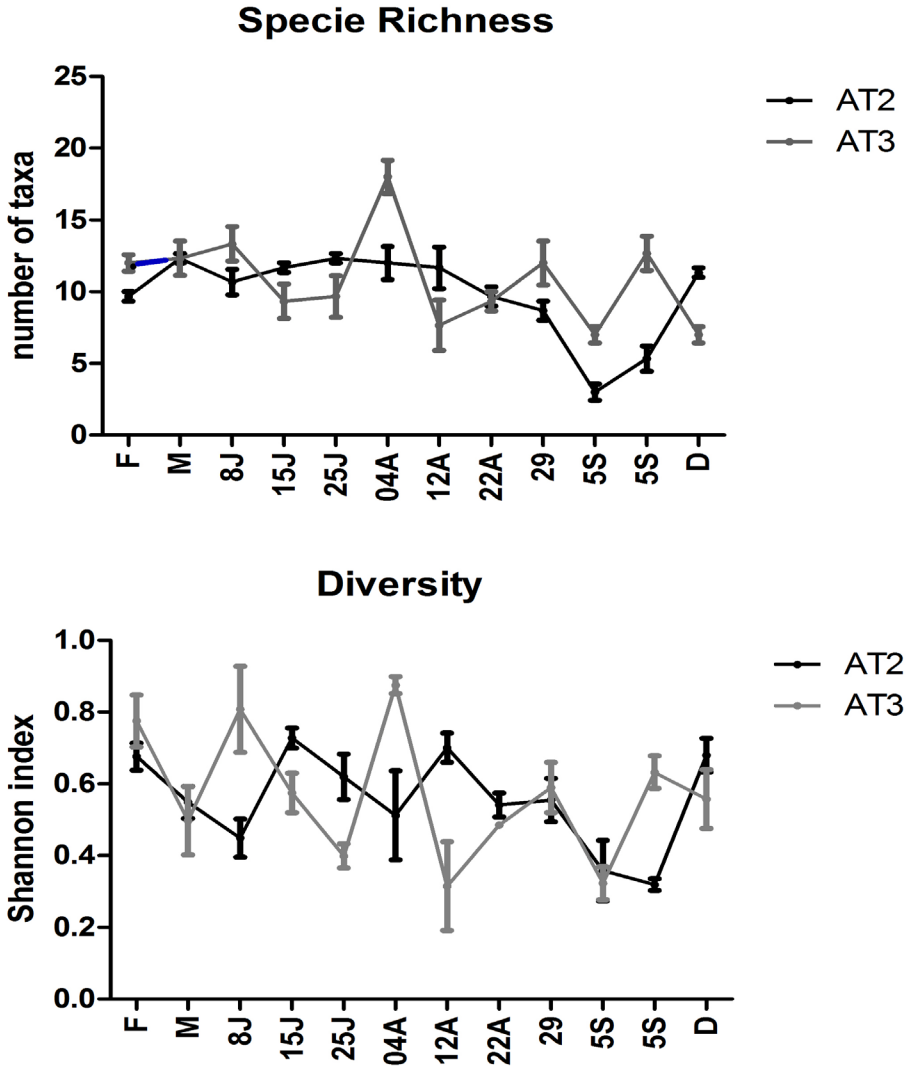

Figure 7. Temporal variation of Species richness and diversity (H di Shannon) in the Lesina Lagoon during the study period.

they had more than 80% of the most abundant species in common in the period before and after the bloom and this percentage increase to 87% during phytoplankton bloom. This is confirmed by the results of specie richness and diversity, that, didn't shown significant variation during the bloom between AT2 and AT3 stations. On the contrary, the contribution of biomass and cell density of some common species increase, so that quantitative variation of phytoplankton taxa resulted statistically different in the two stations during the dystrophic event. This result was common also in other transitional water ecosystems where phytoplankton blooms were determined by specie already present in a vegetative state in the ecosystems. In the Lesina lagoon, the taxonomic composition of the phytoplankton community did not change during a bloom, whereas single species become more dominant with increasing their biomass (Cartensen *et al*., 2004). As a consequence phytoplankton parameters that respond better to the eutrophication

events were phytoplankton biomass and cell density, that increasing their values of one order of magnitude during the bloom event. SIMPER analysis identified nine species as discriminants of phytoplankton composition, but in term of biomass and numerical abundance, only three species were responsible of develop and maintenance of bloom. A first specie was a diatom belong to Thalassiosiraceae group, it probably increase their biomass before of the first sampling time, and decrease within the two week later. Then, the bloom was maintained by an unidentified form of phytoflagellates with a small body size, that became dominant on diatoms population when the level of nutrient decrease. At the end of bloom, a new peak of *Thalassiosiraceae* and *Navicula* sp. was observed. A diatom bloom was typical, at the begin of a eutrophycation period, also in other transitional water ecosystems. For example in a study carried out in the Kattegad Bay, diatom blooms was generally observed at the begin of a summer blooms (Castersen *et al*, 2004). Moreover, also the phytoplankton succession, from a early summer diatom dominated bloom to a later summer flagellate dominated bloom, is common in temperate coastal waters (Margalef, 1958). Many authors (Officer and Ryther, 1980), suggest that this sequence from one population to the other is controlled by the silicate regeneration cycle. The silicate levels are principally determined by the land weathered, municipal water input levels, so in a first time probably in the Lesina Lagoon there is a nutrient level enough to sustain a diatom bloom. In fact, whereas the organic nitrogen and phosphorus components of the diatoms will be recycled rapidly back to an inorganic form through zooplankton grazing, respiration and decay. The silicon is largely confined in the skeletal material of the diatoms. The animals which feed on the diatoms have no use for silicate and reject it directly. We may, then, expect following the initial diatom bloom period that a new nutrient pool will be produced which is rich in inorganic nitrogen and phosphorus but depleted in silicon. The relative amount of silicon depletion in this second nutrient pool will, of course, also depend on the amounts and rate process relations between the organic nutrient cycling and input from land watershed. We now have conditions suitable for a flagellate bloom, and depending on the nutrient levels this could lead to an excessive, eutrophic, growth. In the Lesina lagoon this second pool of nutrient could be sustained by release nutrient of sediments. However, at the end of the anoxic event a new diatom bloom was observed probably due to the develop of favourable situation, such nutrient rich water from sediment and new land watershed input generated also by changes in hydrological conditions, that stimulate a re-suspension and release of nutrient input from the bottom, as supported by the presence of *Navicula* sp. that generally growth at the sediment surface layers being a phyto-benthonic species.

The values of phytoplankton biomass and cell density reach during the bloom was very high with respect to other transitional water ecosystems. In fact, it reach 180 mg/m^3 in term of chlorophyll a. In the bloom observed in the Kettegat bay maxim value of chlorophill *a* was 80 mg/l, and similar values was obtained in the Venice Lagoon during anoxic events (Sfriso *et al*., 2003).

Also, the dynamic of fractionated phytoplankton biomass varied significantly under pressures determined by dystrophic event and was significantly different in the control area with respect the area were the phytoplankton bloom occurred. In this area a dominance of microphytoplankton was evident above all at the begin of the eutrophication process, but in the middle and at the end of bloom period the nanophytoplankton together to microphytoplankton were the dominant fractions. In any case at the end of bloom

the micro-phytoplankton species that shown an high cell density had a lower body size. This represent an important result because this support the hypothesis on the cause that give origin to the bloom. The dominance of micro-phytoplankton in the first part of the bloom indicate that an external nutrient input it triggered the event. Microphytoplankton dominate on other fraction when the availability of nutrient is high. After the first phase, the bloom was maintained by nutrient derived by internal input probably released from anoxic sediments. Nanophytoplankton in fact, for its metabolic proprieties , can have a competitive advantage in nutrient pulsed release.

2) A second important result is that the bloom was develop in a localized area of the lagoon and none transport phenomen were evident. The extent of bloom was on the range of up to 5 km, which indicates that the physical conditions sustaining the summer bloom were local in nature and were not applied to the entire Lesina Lagoon. Bloom develop in the area in front of Lesina City and remained localized in this area. So the bloom was initially sustained by a nutrient external input, probably derived by the discharge of the Lesina City, then a concomitant factors could have led the persistence of bloom: such as, the absence of wind or the reduced wind speed can be prevented the collapse of bloom, the close of channels of communication of the sea can have affected the dynamic of water circulation and led to the depletion of oxygen in the water column and in the sediment favoring a release of nutrient input from sediments and the increasing of micro algae biomass in the area for a long period.

Conclusions

In conclusion, this work highlighted the phytoplankton parameters respond at pressures deriving by dystrophic events. However this was true only for parameters relatively to quantitative variation of abundance of phytoplankton organisms and do not for parameters regarding species richness and diversity. Moreover, also body size fractions shown a significant variation during the dystrophic event.

This result was very important because, in studies like these, where the taxonomic classification can be difficult, it can be supported by knowledge of the morphometric characteristics that may be useful to support the explanation of particular phenomena. This work represent a first step and want just describe how some phytoplankton parameters evolved during the anoxic event and make supposition of what are the main factors that are responsible of variation of phytoplankton dynamics during the bloom. The second step is to correlate these parameters to stressed factors. In any case, in this study we have shown that phytoplankton respond to environmental factors and for this can be considered as a good quality elements for highlighting trophic variation in transitional water ecosystems.

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