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Environmental study in Cabras Lagoon: the trophic state and the microalgal component with particular attention to Harmful Algal Species

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This work is dedicated to Laura:

"We are looking forward to the moment
when you can read it
and make your own comments"

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1. General Introduction

Coastal lagoons are inshore water bodies where marine waters, dominated by tides, and freshwaters penetrate (Bramanti, 1988). So, they are transitional ecosystems among marine and continental environments. The exchange of water masses of different origins is crucial to the natural functioning of coastal lagoons, where ecological processes are controlled by this mixing (Piccini *et al.*, 2006).

Lagoons are characterized by shallow depths and have high sediment-surface-area to water volume ratios. Therefore, processes occurring within the sediment and at the water-sediment interface can strongly influence both water quality and the biota (Castel *et al.*, 1996; Viaroli *et al.*, 2004). In these kind of environments the sediments play an important role as regulator for all ecosystem, in relation to their capability of supply and renewal for organic matter and nutrients (Golterman, 1995; JØrgensen, 1996; De Wit *et al.*, 2001).

Due to their shallow depth, usually light arrives at the sediment-water interface intensively, hydrodynamics is influenced by bottom topography and wind acts on the whole water column determining the resuspension of materials, nutrients and small organisms from the surface of the sediment. Furthermore, coastal lagoons are characterised by a lot of physical and ecological boundaries and gradients: between water and sediment, pelagic and benthic assemblages, lagoon and marine, freshwater, terrestrial systems and with the atmosphere (Pérez-Ruzafa *et al.*, 2005).

The synergy of all these physical and biological characters makes coastal lagoons highly dynamic ecosystems, considered among the most productive in the exosphere, populated by a large variety of organisms that permits their classification as environments with maximum value of biodiversity (Tomàs Vives, 1996). Moreover they are often important as bird reproduction areas and as nursery for fish and shellfish.

For a lot of these reasons, such areas are extensively exploited for fishing and aquaculture (Carafa *et al.*, 2006). They were early sites for human settlement and continue to the present day as important centres for economic, social and cultural development (Barraqué *et al.*, 1998).

Aquaculture practices are increasing in all the world due to progressive impoverishment of natural fish populations and increasing demand for fish-associated proteins (FAO, 2000). On the other hand, farming activities have a strong negative effect on natural fish populations because of the large taking of fish from natural stocks to be converted in farmed fish feed (Naylor *et al.*, 2000) and the deterioration of coastal areas as a consequence of the high loads of organic matter and nutrients introduced by fish farms (Hall *et al.*, 1992; Christensen *et al.*, 2000; Bartoli *et al.*, 2005).

Just in relation to their exploitation and because they are highly vulnerable to terrestrial inputs, coastal lagoons are considered naturally stressed environments, subject to frequent fluctuations and alterations (UNESCO, 1981; Kjerfve, 1994).

Eutrophication, the enrichment of nutrients, in particular of nitrogen and phosphorus, in the water, is one of the most important processes of degradation of lagoon environmental conditions and it is often due to the civil and industrial wastes and agricultural and zootechnical activities. It is known that the pressures of human activities on coastal systems have been dramatically increased in the last few decades and this process will continue and evolve, especially in developed countries (Viaroli *et al.*, 2007). On the Mediterranean coast, almost all of the lagoons were intensively used for centuries as fishery grounds and their subsequent use as outlets for domestic and industrial wastewater, led to their eutrophication (Vicente and Miracle, 1992; Pastres *et al.*, 2004).

As a biological consequence of nutrients enrichment, primary producers often proliferate (Menéndez and Comin, 2000), increasing the demand for oxygen needed for biological degradation processes. This may, under the concomitance of different conditions, such as the increase of temperature and the lack of wind, cause a condition of anoxia and lead to dystrophic crises with a strong loss of biodiversity for the large killing of fish and other aquatic organisms (Bachelet *et al.*, 2000; Carlier *et al.*, 2007). Therefore, dystrophic crises are the last phase, the summit of persistent conditions of intense eutrophication. When a strong enrichment of nutrients is observed, the general patterns described include the substitution of macrophytes by macroalgae at the benthic level in a first phase and then a shift to a phytoplankton based system with anoxic events (Niehnius, 1992). According to the model described by Grey (1992) the phase of nutrients enrichment determines the growth of macroalgae and phytoplankton and, as indirect effects, the growth of benthic animals and fish. The initial effect of eutrophication is a change in the species composition over these categories of organisms. The secondary effect consists in a shading depth reduction for macroalgae, in possible toxic/nuisance blooms of phytoplankton and in changes of behaviour of benthic sediment-living species and fish. Then, it reaches an extreme effect, consisting in the mass growth of macroalgae, in toxic effects of phytoplankton blooms and in mortality of benthos and fish for the decrease of oxygen concentrations or for the increase of organic material in the sediments. Finally, Grey described an ultimate effect consisting in anaerobic conditions of water and H_2S production, which being toxic, causes mass mortalities of most organisms.

So, eutrophication causes strong unbalances in the ecosystem, and can culminate in dystrophic crises just for the high demand of oxygen and for the decomposition of organic matter, produced excessively in respect to the metabolic capacity of water body (Sechi and Ulzega*,* 1990; Marchetti *et al*., 1994). Furthermore, the anoxia event, that starts in the bottom and then moves towards the superficial layers, can be determined by the high water temperature that reduce the oxygen solubility strongly. The anoxia condition, if prolonged in time or if repeated systematically, causes the escape of aquatic animals and in the worst case, it causes their death (Rensel and Whyte, 2003; Hallegraeff, 2003).

However, the reaction of ecosystems to nutrient load increase varies widely world wide because biological control mechanisms of the eutrophication process are not always the same. For example, predation can be a very efficient control mechanism removing excess biomass generated by excess nutrients (Pérez-Ruzafa *et al*., 2002).

Reduction of the light in the water column due to the increasing of phytoplankton growth resulting by extreme eutrophication, can also be a disadvantage towards less tolerant phytoplankton species and induce dramatic alterations in their composition (Chomérat *et al*., 2007). The environment degradation due to hypertrophic conditions favours the dominant development of cyanobacteria in respect to less tolerant algal species (Sorokin *et al*., 1996a; Scheffer *et al*., 1997), even if, nowadays, the link between very high trophic conditions and cyanobacteria blooms has been documented only in few Mediterranean lagoons (Abrantes et al., 2006; Chomérat et al., 2007).

Among the mechanisms used by some phytoplanktonic classes for survival in adverse environmental conditions, there is a production of dormant cysts. For example, a lot of dinoflagellates have a heteromorfic life-cycle, alternating a vegetative, motile, planktonic stage with a dormant, non-motile, benthonic stage (hypnozygote), that have a fundamental role for the survival and persistence of species (Hallegraeff, 1998; Anderson, 1998; Imai and Itakura, 1999). In fact, the development of dormant cysts involves different advantages for the species that produce them: permit the genetic recombination, being a result of sexual reproduction; pre-adapt the populations in the environments; are a source of inoculation for new blooms; are vectors of expansion in the geographical distribution of species; constitute a resistant form against adverse environmental conditions (Anderson *et al*., 1984; Harrys, 1994; Matsuoka and Fukuyo, 2000).

In low density conditions, the planktonic dynamics is very rapid, so that it cannot be detected with normal samplings patterns (Dale, 1983). Moreover, confronting the vegetative phase with the benthic one, the differences between species of the same genus are more obvious at the cysts level. So, the study of the dynamics of the cysts in the lagoon sediment is extremely important to complete the information obtained from the planktonic compartment, representing a potentiality of appearance of species in the water column, and to improve the knowledge on biodiversity of the site (Nehring, 1997).

This aspect is even more interesting and needs more careful investigations if we think that the major part of Harmful Algal Species (HAS) produce these kind of cysts.

Eutrophication, anthropic commercial exchange and the enlargement of sites that favour the different phases of life cycle of some species, in particular of the latent phase, are recognized as the most important factors contributing to the global expansion of the proliferation of Harmful Algal Blooms (HABs) (Anderson *et al.* 2002; Hallegraeff, 2003; Glibert, 2006). They are the results of interactions of physiological adaptations and biological behaviours with different environmental factors.

In particular, the affirmation of HAS can affect the lagoon biodiversity in different ways. Some algal species are possible producers of biotoxins dangerous for aquatic biota but also for human health, through direct alimentation (drinking water) or through the ingestion of aquatic organisms (shellfish, fish) that have accumulated high concentration of toxins in their tissues. The danger is not always in relation to the high densities of species, in fact some toxins are so strong that their effect can be explicated also in conditions of low cell density (Anderson *et al.*, 2002). Algal blooms can cause intense discoloration of waters, with consequences on benthic algae and on all trophic net. Then, HAS can affect other aquatic organisms with a direct action, for example they produce mucilagine or have thorns or other processes that can cause occlusion or wounds of gills. Another negative effect is due to the production of high biomasses with consequent anoxia for the degradation of organic matter.

Phytoplankton dynamics can be influenced by bottom-up and/or top-down control factors (Krebs, 1994). Bottom-up factors control species growth (e.g. light intensity, temperature, salinity, availability of nutrients), while top-down factors control its biomass (e.g. predation, competition) (Wehr and Descy, 1998). This, as the basis of the trophic chain, constitutes the biological community in which scientific attention is focused when a management plan is needed or an assessment of the ecosystem health is required (Sin *et al.*, 1999; Gameiro *et al.*, 2007). In relation to their responses to changes in nutrient concentrations, water renewal time, physical, chemical and biological parameters, phytoplankton is one of the biological quality elements to be used for assessment of the ecological status of coastal waters according to the European Water Framework Directive (WFD; 2000/60/EC). This is the legal tool for maintaining and improving the ecological quality of fresh and coastal waters (Henriksen, 2009).

Several indicators, indexes and models have been developed to assess eutrophication and water quality in freshwater, coastal and marine systems. Nevertheless, the assessment of eutrophication and water quality classification in coastal lagoons is not an easy task, due to the great variability of spatial and temporal conditions (Coelho *et al.*, 2007). The intrinsic high variability of these systems also determined the lack of generalizations for phytoplankton distributional and successional patterns in coastal lagoons (Sarno *et al.*, 1993). Then, this intrinsic complexity of planktonic system in coastal lagoons, makes the analysis of long temporal series particularly necessary. These consent to have reliable reconstructions of the seasonal cycle of phytoplankton and permit to distinguish regular and recurrent patterns from occasional and exceptional events. Furthermore, long temporal series are extremely important to define if eventual changes of trophic and biologic characteristics are determined by local scale human influence and/or by global scale climatic fluctuations (Shiganova, 1998; Ribera-D'Alcalà *et al.*, 2004).

Phytoplankton blooms are notoriously difficult to predict, and scientists in various parts of the world have been working on prediction for decades with little success to date (Martin *et al.*, 2009).

2. Objectives and contents

The implementation of the knowledge regarding the trophic state, the composition and dynamics of phytoplankton in Cabras Lagoon was the principal aim of this PhD thesis, to improve the comprehension of recurrent stressing events that have often injured the stability of the ecosystem. In fact, Cabras Lagoon, that has always been a source of great wealth for Sardinia in relation to its fishing production, during last decades suffered a strong loss in this sense because of its very high trophic state. This has become a chronic condition by now and it has often culminated in strong dystrophic crises.

This lagoon represents an important exception from an ecological point of view. In fact, despite a lot of other similar Mediterranean environments (Giordani *et al.*, 2005) and also other Sardinian lagoons, where the role of principal primary producer is carried out by macrophyte, in Cabras Lagoon this role is carried out by phytoplankton (Sechi *et al.*, 2006). Therefore, the study of this biological component, that is at the basis of trophic web, is extremely important for the comprehension of the dynamics of this ecosystem.

The following chapters investigate these thematic and show the results obtained during this PhD and those obtained during previous studies, elaborated in this occasion to compare the different series available for Cabras Lagoon.

In the complexity of this work, among phytoplankton, a key rule was carried out by the Cyanophyceae Class, that resulted the most important since 1999, when, in summer, a bloom of the cyanobacterium *Anabaena* cf. *mucosa* Komarkova-Legnerova and Eloranta was observed simultaneously with the strongest dystrophic crises that struck the lagoon. The high trophic state of the lagoon, confirmed nowadays, and the dominance of cyanobacteria seem to be the main ecological aspects characterizing this ecosystem. Moreover the study of the lagoon sediment, in terms of presences and dynamics of dinoflagellates cysts, confirmed potentially scarce and sporadic presence of dinoflagellates, already observed in the water column, and more specifically the scarce and sporadic presence of HAS, which for the most part belong to this class.

In particular:

- Chapter 1 shows which environmental and climatic parameters determined the temporal variations of the orders of cyanobacteria in the lagoon from 2007 to 2009. This study was submitted to the journal “Scientia Marina”.
- Chapter 2 shows the results relative to the seasonal samplings of lagoon sediment, carried out during the two same annual cycles, to investigate the composition and the temporal and spatial dynamics of dinoflagellate cysts.
- Chapter 3 exhibits the results regarding the trophic state of the lagoon and the composition, diversity and dynamics of phytoplankton from 2000 to 2002, just after the dystrophic event of 1999. This study was submitted to the journal “Marine Pollution Bulletin”.
- Chapter 4 exhibits the results regarding the long-term composition, diversity and dynamics of phytoplankton in relation to environmental parameters from 2000 to 2008.

In the appendix of this work, the first study “La Laguna di Cabras: stato trofico, fitoplancton e presenza di Harmful Algal Species” was presented to the meeting of PhD students in Ecology 2009 (Parma-Italy), organized by S.It.E. (Società Italiana di Ecologia) in collaboration with A.I.O.L. (Associazione Italiana di Oceanologia e Limnologia). It was submitted for the publication in a special issue of “S.It.E. atti”.

The second “Diatoms and water courses quality in North-Central Sardinia” was presented to the 28ème Colloque de l’Association des Diatomistes de Langue Française (ADLaF) (Banyuls-sur-Mer, France). It was submitted to the journal “Vie et Milieu – life and environment”.

3. Chapter I

The dominance of cyanobacteria in Mediterranean hypertrophic lagoons: a case study of Cabras Lagoon (Sardinia, Italy).

Submitted to "Scientia Marina"

The dominance of cyanobacteria in Mediterranean hypertrophic lagoons: a case study of Cabras Lagoon (Sardinia, Italy)

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SUMMARY

An intense proliferation of cyanobacteria in Cabras Lagoon was monitored over a period of two years (July 2007–June 2009). Environmental and climatic parameters in the lagoon that determine temporal variations in orders of cyanobacteria were investigated. For 18 months, Chroococcales was the only cyanobacterial order present in the lagoon, with *Cyanobium*-type cells being the most important with respect to presence and density. The importance of this taxon decreased only during the autumn–winter of the second annual cycle, when a prolonged period of intense rainfall led to a sudden drop in the lagoon's salinity, with the subsequent appearance of Oscillatoriales, specifically, *Planktothrix* sp. and *Pseudanabaena catenata*. The results provided evidence of the strict relationships between Oscillatoriales and phosphorus levels, the DIN/SRP ratio, and pH. Nutrient depletion in the late spring together with both a decrease in the DIN/SRP ratio and water of low salinity favoured Nostocales, predominantly *Aphanizomenon gracile*, *Aphanizomenon aphanizomenoides*, and *Anabaenopsis circularis*.

Keywords: cyanobacteria, phytoplankton, coastal lagoons, eutrophication, Sardinia, Mediterranean Sea

INTRODUCTION

Coastal lagoons are highly dynamic environments that show wide spatial and temporal variability in their physical and chemical characteristics in response to the influence of freshwater and marine water inputs. This exchange of water masses of different origins is crucial to the natural functioning of coastal lagoons and controls most of their basic ecological processes (Piccini *et al.*, 2006). Indeed, studies have shown that, as a consequence of the high sediment surface area to water volume ratios in Mediterranean coastal lagoons, processes occurring within the sediments and at the water-sediment interface strongly influence ecosystem metabolism, nutrient budgets, and biota (Castel *et al.*, 1996).

This process has been exacerbated by the pressures of human activities on coastal systems, which have dramatically increased in the last few decades and is predicted to continue increasing especially in developed countries (Viaroli *et al.*, 2007). Southern European lagoons, most notably those of the Mediterranean coast, are particularly vulnerable to anthropogenic pressures due to mass tourism, urbanization, industry, and intensive agriculture (Hemminga, 1998). Furthermore, during the summer, the concomitance of the increase in temperature, the lack of wind, and the degradation of organic matter can result in a reduction of oxygen availability, eventually leading to dystrophic crises (Bachelet *et al.*, 2000).

Most of the primary production in Mediterranean lagoons is carried out by macrophytes (Giordani *et al.*, 2005). When the trophic level increases, phytoplankton growth becomes more important, with a consequent reduction of light and thereby a decrease in macrophyte abundance (Austoni *et al.*, 2007). The frequent confluence of these circumstances underlines the need to study phytoplankton abundance and community structure in such environments, particularly vulnerable to anthropogenic inputs and naturally stressed by frequent disturbances or environmental fluctuations (Kjerfve, 1994). In general, the widespread availability of inorganic and organic nutrients as well as light supports a high level of primary production by species adapted to highly varying geomorphological, physical, and chemical conditions (Marinov *et al.*, 2008). Nutrient loading may have different impacts on the ecosystem at different times (Glibert *et al.*, 2007). The development of a specific algal species depends not only on the availability of nutrients, but also on its preference for specific nutrient forms, the nutritional status of the species at the time of nutrient delivery, and its physiological response to the prevailing environmental factors (Glibert and Burkholder, 2006). It is well-documented that environment degradation due to hypertrophic conditions threatens less-tolerant phytoplankton species while favoring the development of cyanobacteria (Scheffer *et al.*, 1997). Recently, a link between highly trophic conditions and cyanobacterial blooms has been documented in a number of Mediterranean lagoons (Chomérat *et al.*, 2007).

The intense proliferation of cyanobacterial cells of very small size (<2 µm) and their dominance over other phytoplankton size classes has been studied in a few coastal environments (Del Negro *et al.*, 2007). Their results have served to highlight the increasing importance of these small cells, which for decades were recognized as the main primary producer only in oceans and oligotrophic aquatic ecosystems (Anxelu and Morán, 2007). However, the increasingly important role played by small free-living cyanobacteria in Mediterranean coastal blooms and the ecological damage they induce in areas with high trophic levels have yet to be thoroughly investigated (Sorokin *et al.*, 1996a).

Here we provide a case study of Cabras Lagoon, a Mediterranean hypertrophic lagoon dominated by cyanobacteria. The lagoon is the most extended shallow-water body in Sardinia (Italy) and one of the most important in the Mediterranean, and its major primary producer is phytoplankton (Sechi *et al.*, 2006). During the last 20 years, eutrophication has become a chronic condition of Cabras Lagoon, often culminating, especially during summer, in major dystrophic crises that result in an abrupt decrease in production. Already in the early 1980s, Sechi *et al.* (1981) classified the lagoon as hypertrophic, and although subsequent data are lacking, since then the hypertrophy appears to have worsened. Indeed, in June 1999, the immensity of a dystrophic event was such that it led to the total killing off of the lagoon's fish component, which had a particularly dramatic impact on the local economies since fishery is the main source of income for local residents.

During this severe dystrophic event, a bloom of the cyanobacterium *Anabaena* cf. *mucosa* Komarkova-Legnerova and Eloranta was documented. Cyanobacerial cells in an advanced state of degradation and the presence of large amounts of mucilage were noted. Today, cyanobacteria continue to predominate in Cabras Lagoon, with extremely high cell densities. Published studies on the hypertrophic conditions in the lagoon are scarce, while a detailed study of phytoplankton community composition and dynamics has not yet been published.

The purpose of this study was therefore to investigate the spatial and temporal dynamics of cyanobacteria in Cabras Lagoon with respect to environmental conditions and climate during two year-long cycles (from July 2007 to June 2009). The larger aim of this work was to identify the parameters that determined the seasonal succession of the different cyanobacterial orders and species in the lagoon during the period considered.

MATERIALS AND METHODS

Description of study site

Cabras Lagoon is located on the west coast of Sardinia (Italy), in the Gulf of Oristano (39°56'37''N, 08°28'43''E; Fig. 1), and occupies about 23.80 km^2, with a mean water depth and maximum of 1.6 and 3 m, respectively. The watershed of the site extends over approximately 430 km^2. The input of freshwater into the lagoon is scant and irregular because of the semi-arid Mediterranean climate. Most of the freshwater comes from the small Mare 'e Foghe River, located in the north.

The predominance of agriculture in the region and the release of poorly depurated urban waste account for the high nutrient loads deposited in Cabras Lagoon. The resident population of about 38,000 inhabitants is grouped in 19 urban centers, the largest being Cabras, which is located on the southeast coastal side of the lagoon.

During the twentieth century, the lagoon and its watershed underwent several modifications as a consequence of human activities that affected the hydrology and hydraulics of the region. In addition, in the late 1970s, water exchange with the sea was altered by the dredging of a large canal, the *Scolmatore* (=spillway), which connected the lagoon with the adjacent Gulf of Oristano (Fig. 1). The canal was constructed to avoid flooding of adjacent land during the heavy rainfalls that regularly occur in winter. In addition, a cement dam was built into the *Scolmatore* to prevent further increases in the lagoon's salinity and artificial barriers were constructed to control the fish catch, thereby impeding direct communication between the lagoon and the sea. Instead, the only link to the sea is via four very narrow creeks that from the southern part of the lagoon flow into the large canal, over the barrier (Fig. 1).

The lagoon has a high economic rating due to extensive fishery activities, involving about 300 people, their families, and those involved in related enterprises.

Sampling strategy and analyses

The reported data were obtained during two yearly cycles (July 2007–June 2009), during which fortnightly samplings were carried out during the first and monthly samplings during the second. The samples were collected at three stations: station 1, near the major input of freshwater; station 2, representing intermediate conditions; and station 3, near the lagoon's connection with the sea (Fig. 1).

The cumulative monthly rainfall were provided by the meteorological station at Zeddiani (Rete Agrometereologica Nazionale property), few km from the northern part of the lagoon.

The in situ water transparency was measured with a Secchi disk, while temperature, salinity, dissolved oxygen (DO), and pH were determined with a CTD probe (YSI 6600V2). Water samples were collected with a bucket from the superficial water layer (50 cm depth) and preserved under cold, dark conditions for laboratory analyses of suspended matter (total solids in suspension) and dissolved nutrients (ammonia, nitrite, nitrate, reactive silica, orthophosphates, total phosphorus), as described in Strickland and Parsons (1972). The chlorophyll *a* content was quantified following the method of Golterman *et al.* (1978). The values of parameters measured in situ were confirmed in laboratory analyses.

Phytoplankton abundance was determined in samples fixed in situ with Lugol's, according to Uthermöhl's technique (1931). Five-ml subsamples were counted at 200, 400, and

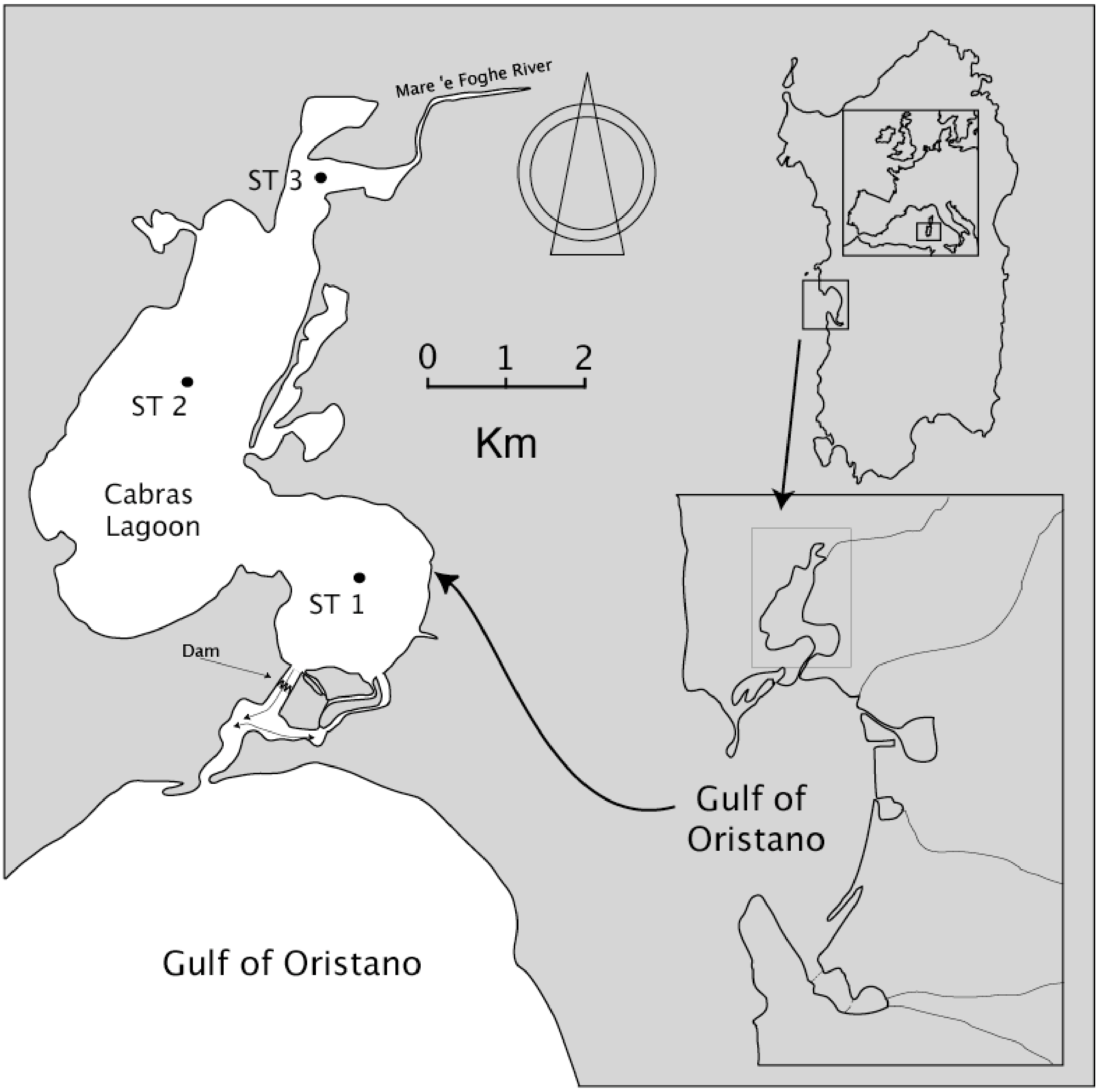

Figure 1: Map of Cabras Lagoon, showing its location and the sampling sites.

1000X magnification in an adequate number of fields. When necessary, the samples were diluted. Phytoplankton biomass was determined following Findenegg's method (1974).

Algal species were identified in non-fixed samples analyzed just after their collection by traditional optical microscopy (using an inverted microscope, Zeiss Axiovert 25) and fluorescence microscopy (using an inverted microscope, Zeiss Axiovert 100, equipped with blue excitation filter blocks, 420-480 nm) for the identification of algal species. For unlikely recognizable species, identification was determined by scanning electron microscopy (ESEM, Zeiss EVO 10).

Cyanobacteria were identified as described by Komárek and Kováčik (1989), Komárek and Anagnostidis (1998, 2005), Hindák (2000), and Komárek (2005).

Statistical analyses

Two-way ANOVA was carried out to test whether the environmental parameters among the three stations were statistically different, with $p<0.05$ considered significant. Differences in the environmental parameters during the two yearly cycles were assessed by testing data obtained in each season using Student's t test, with $p<0.05$ considered significant.

Canonical correspondence analysis (CCA) (Ter Braak, 1986) was carried out to quantify the influence of environmental variables on cyanobacterial composition with respect to order and species abundances. All canonical axes were used to evaluate the significant variables under analyses by means of a Monte Carlo test (1000 permutations). Data used to construct the environmental and order/species matrixes were square-root transformed. Statistical analyses were done using the software PRIMER (for ANOVA) and MVSP (for CCA).

RESULTS

Environmental parameters and nutrient dynamics

Figures 2 and 3 show the statistical distributions of the mean values of the analyzed parameters for the two study years. The values of nearly all of the parameters varied over a wider range in the second year (Table 1).

Temperatures in the lagoon were, as expected, highest in summer (maximum mean value 28.45°C, in July 2007) and lowest in winter (minimum mean value 9.49°C, in February 2008) (Fig. 4), with the same annual average for the two study cycles (18.2°C) (Table1; Fig. 2). Differences among the sampling stations were not significant (ANOVA, $p>0.05$).

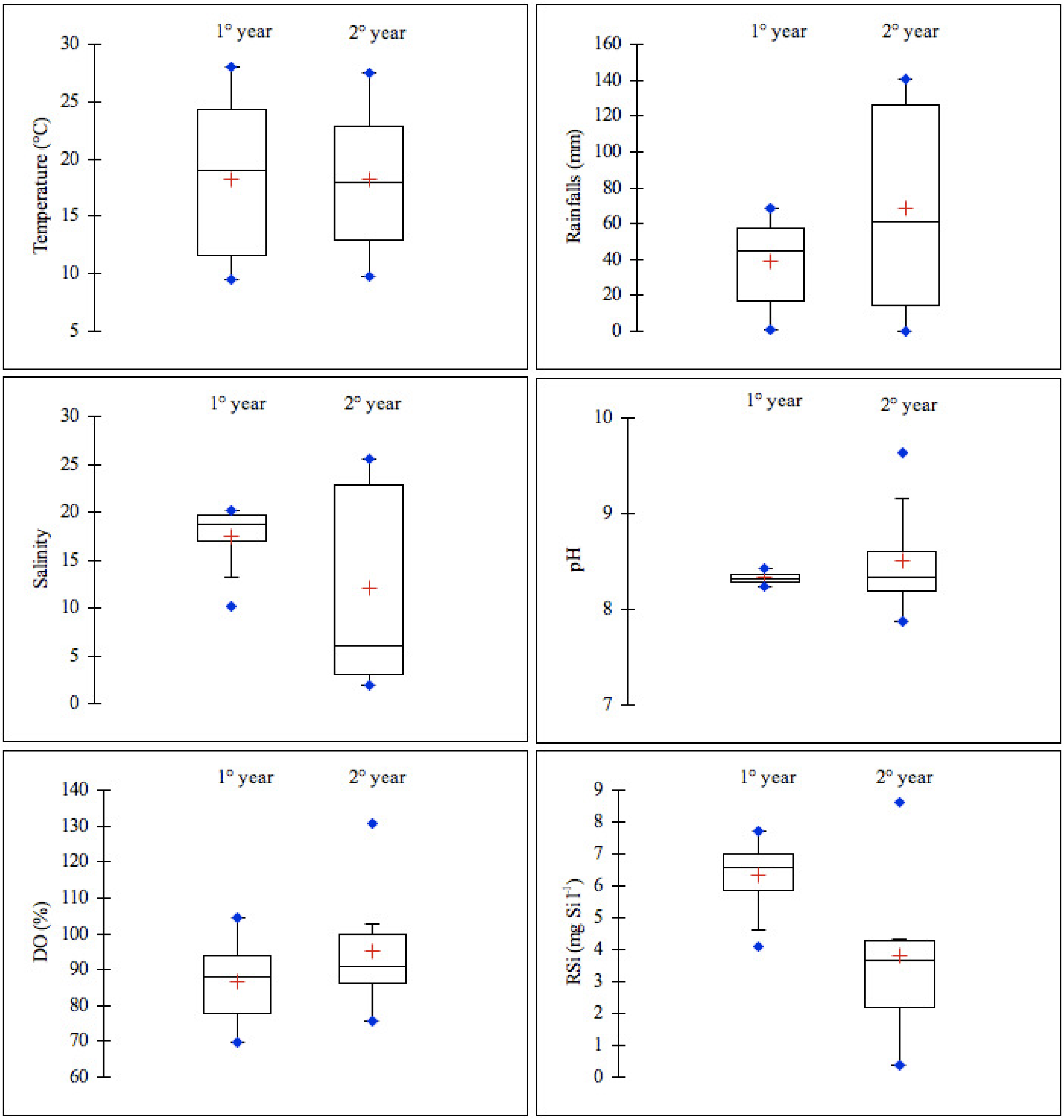

Figure 2: Statistical distribution of mean values of the environmental parameters and reactive silica in Cabras Lagoon during the two annual cycles.

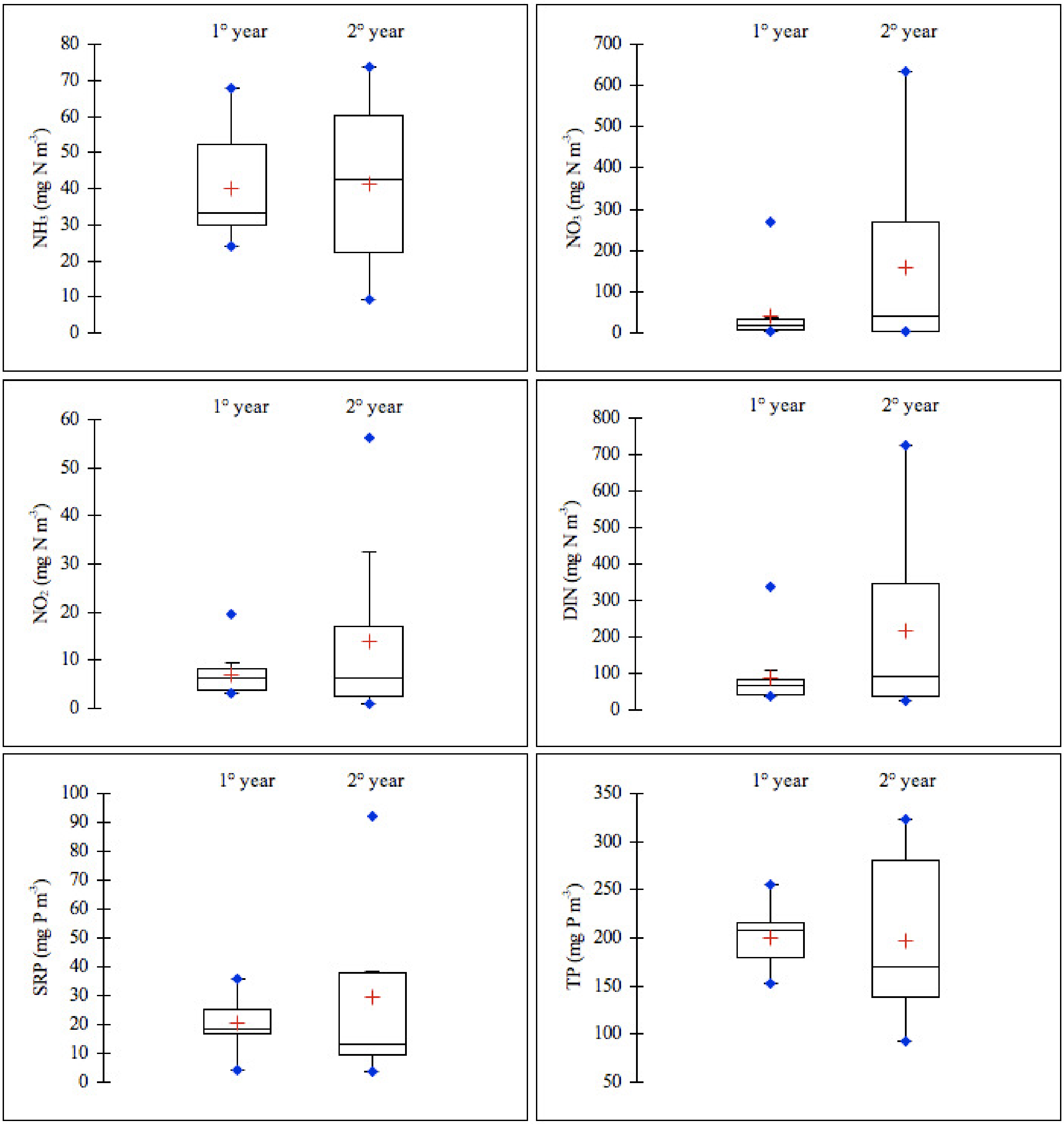

Figure 3: Statistical distribution of the mean values of nutrients in Cabras Lagoon for the two year-long cycles of this study.

Table 1: Mean and standard deviation (± SD), maximum and minimum values of the environmental parameters, and nutrients and chlorophyll *a* content in Cabras Lagoon during the two study cycles (n. d. = value was lower than the detection limit of the method).

		2007-2008			2008-2009		
	CCA Code	Mean ± SD	Max	Min	Mean ± SD	Max	Min
Temperature (°C)	Tem	18.2 ± 7.1	28.0 St. 1 - Jul, Aug; St. 2, 3 - Jul	9.4 St. 2 - Feb	18.2 ± 6.3	27.7 St. 3 - Jul	9.4 St. 1 - Dec
Salinity	Sal	17.5 ± 3.1	22.7 St. 1 - May	8.9 St. 2 - Jul	11.9 ± 10.4	27.6 St. 1- Nov	0.6 St. 3 - Mar
pH	pH	8.3 ± 0.1	8.6 St. 2 - Oct	8.1 St. 1 - May	8.5 ± 0.6	9.8 St. 2 - Apr	7.3 St. 1 - Feb
Alkalinity (meq l^{-1})	Alk	2.6 ± 0.3	2.0 St. 2 - Gen	3.2 St. 3 - Jul; St. 2 - Aug	2.6 ± 0.5	3.3 St. 3 - Jun	1.3 St. 3 - Jen
Dissolved oxigen (%)	DO	87 ± 10.1	121 St. 1 - Jun	50 St. 2 - Jen	95 ± 16.7	168 St. 3 - May	70 St. 1 - Dec
Total solids in sospension (mg l^{-1})	TSS	87 ± 44.7	400 St. 2 - Mar	40 St. 3 - Jul	68 ± 25.9	158 St. 3 - Jen	19 St. 3 - Mar
Reactive silica (mg Si l^{-1})	Rsi	6.3 ± 1.1	10.8 St. 2 - Sep	2.6 St. 2 - Apr	3.8 ± 2.5	10.4 St. 3 - Sep	0.1 St. 1- Aug
Soluble reactive phosphorus (mg P m^{-3})	SRP	20 ± 8.7	62 St. 3 - Jen	3 St. 2 - Jen	30 ± 31.0	275 St. 3 - Jen	n.d. St. 2 - Jen, Jun; St. 1 - Jun
Total phosphorus (mg P m^{-3})	TP	200 ± 29.3	302 St. 3 - May	124 St. 1 - Jul	196 ± 84.4	843 St. 3 - Jen	63 St. 1 - Sep
Nitrite (mg N m^{-3})	NO_2	7 ± 4.5	50 St. 2 - Jen	n.d. St. 2 - Nov	14 ± 17.1	78 St. 3 - Jen	n.d. St. 1, 2, 3 - Jul, Jun
Nitrate (mg N m^{-3})	NO_3	40 ± 72.8	1331 St. 3 - Jen	n.d. St. 2 - Jun	160 ± 215.3	844 St. 1 - Dec	n.d. St. 1, 2, 3 - Jun
Ammonia (mg N m^{-3})	NH_3	40 ± 14.1	113 St. 3 - Jen	16 St. 1, 2 - Aug	41 ± 22.0	459 St. 1 - Jun	4 St. 1 - Sep
Dissolved inorganic nitrogen (mg N m^{-3})	DIN	86 ± 82.5	1493 St. 3 - Jen	23 St. 1, 3 - Aug	215 ± 240.8	986 St. 3 - Jen	5 St. 2 - Jun
DIN/SRP ratio	N/P	6.3 ± 5.9	26.4 St. 3 - Jen	0.7 St. 3 - Oct	13.4 ± 13.5	89.5 St. 1 - Jen	0.4 St. 3 - Jun
Chlorophyll *a* (mg m^{-3})	-	19 ± 4.1	44 St. 3 - Oct	9 St. 1 - Aug	52 ± 54.3	299 St. 3 - Jun	3 St. 1 - Oct

Rainfall was greater during the autumn and winter seasons of both years, but was much higher during the second year (Fig. 2). In fact, in the autumn–winter period of 2008–2009, rainfall was intense and prolonged (maximum 140.2 mm, in January 2009) (Fig. 4).

Salinity increased steadily from the beginning of the study (mean value of 10.2‰) until November 2008 (mean value of 24.9‰), when contemporarily with the intense rainfall and the consequent large freshwater input into the lagoon, it decreased abruptly (mean value of 5.0‰, in December) (Fig. 4). A clear and consistent spatial salinity gradient was observed, with the values decreasing from station 1 to station 3, consistent with the proximity to seawater exchange and the input of freshwater, respectively.

Rainfall and salinity were the only two variables that differed significantly between the two cycles and did so at the seasonal level: in autumn for rainfall (Student's *t* test, $p<0.05$) and in winter, spring, and summer for salinity (Student's *t* test, $p<0.05$).

Regarding nutrient availability for phytoplankton growth, SRP and DIN ($N\text{-}NH_3 + N\text{-}NO_2 + N\text{-}NO_3$) concentrations were consistently high (Fig. 4), with annual mean values greater in the second year than in the first (Table 1, Fig. 3). The maximum peaks occurred during the autumn–winter period of 2008–2009, when the mean DIN and SRP in the lagoon was >700 mg N m^{-3} and 90 mg P m^{-3}, respectively. ANOVA revealed significant seasonal differences between the three stations: in winter, for both DIN and SRP ($p<0.05$) and in summer only for SRP ($p<0.05$). During these seasons, the highest values of the two parameters were recorded at station 3.

The mean values of the DIN/SRP ratio were very low (<6) during most of the sampling months (Fig. 4). The highest mean value recoded (maximum mean value 47) concurred with the highest peaks of DIN in the winter of the second year-long cycle.

Phytoplankton dynamics

During the investigation period, the most important phytoplankton class in the lagoon was Cyanophyceae, which, regularly detected, reached very high densities during the two years of the study (over 10^9 cell l^{-1}). This class dominated all other classes, usually accounting for >80% and only rarely <50% of total phytoplankton density throughout most of the study (Fig. 5). By contrast, due to the reduced size of these cyanobacterial cells, the biomass contribution of this class was relatively small, amounting only in a very few cases to >50% of the total phytoplankton biomass (Fig. 5).

The dynamics of chlorophyll *a* during the first year-long cycle were relatively constant, with mean values <20 mg m^{-3}, whereas during the second cycle there was a large increase

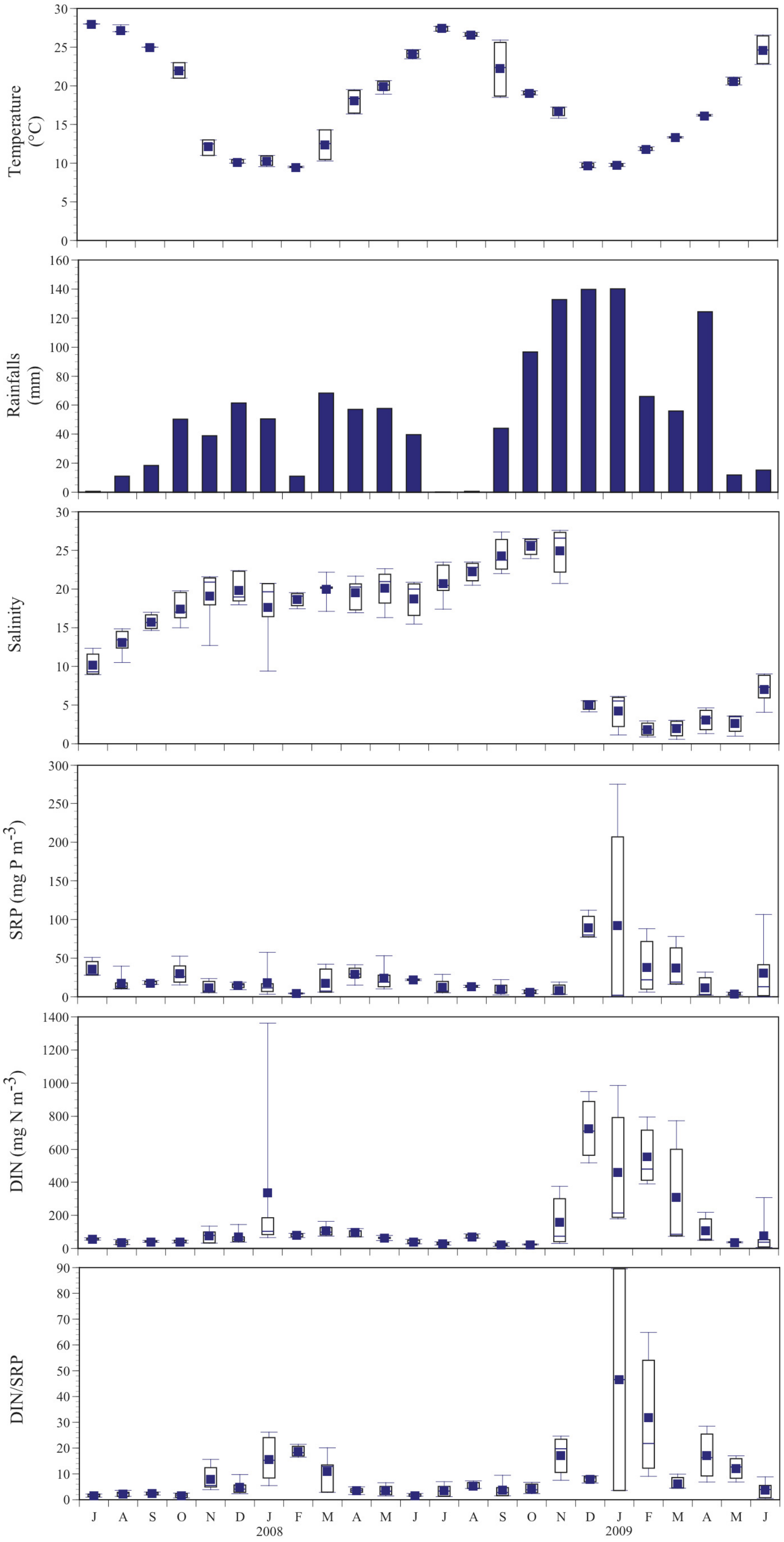

Figure 4: Seasonal variations in the main environmental parameters, nutrients, and DIN/SRP ratio during the investigation period.

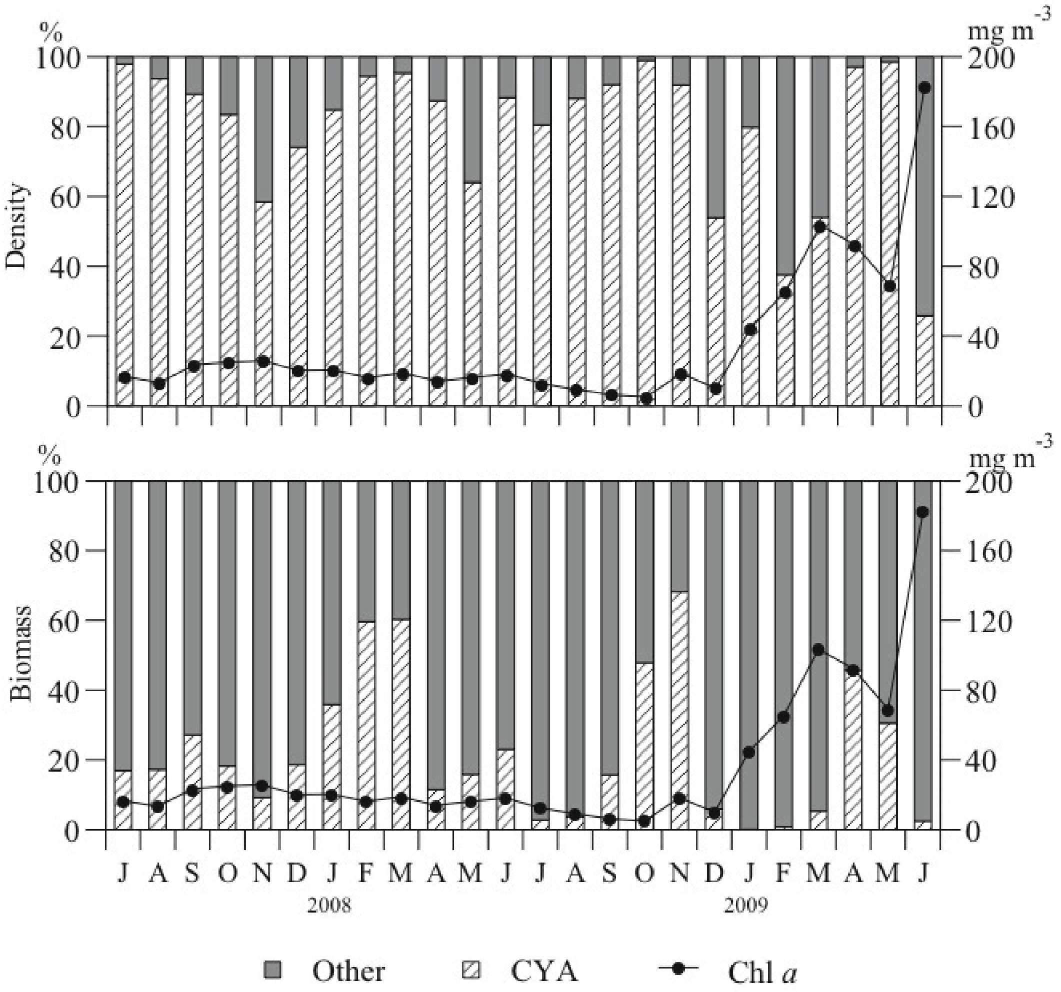

Figure 5: Seasonal dynamics of abundance (a) and biomass (b) of cyanobacteria and other phytoplankton classes in Cabras Lagoon during the investigation period respect to the dynamic of chlorophyll *a*. Data are the mean values obtained from the three sampling stations.

corresponding to the simultaneous appearance of species of large cell size (Table 1; Fig. 5).

Nine species of cyanobacteria were identified, belonging to the following orders (Table 2): Chroococcales (4), Oscillatoriales (2) and Nostocales (3). For the first 18 months, cyanobacteria were almost exclusively represented by Chroococcales (Figs. 6, 7), including a species probably belonging to the genus *Cyanobium* Rippka and Cohen-Bazire (Fig. 8). This species was always present and at all three stations was the most abundant phytoplanktonic taxon (Fig. 9). During the same period, other Chroococcales, i.e. *Merismopedia tenuissima* Lemmerman and *Rhabdoderma* sp., were observed, but their presence was very sporadic and their abundance not significant (Fig. 9). *Aphanocapsa* sp. was detected only during the last month of sampling (Fig. 9).

The phytoplankton composition in the lagoon remained the same until the end of 2008, when a strong increase in the amount of rainfall and the consequent drop in salinity coincided with a change in distribution. Specifically, while the presence of *Cyanobium*-like organisms became less important, species of the order Oscillatoriales appeared, beginning with *Plankthotrix* sp. (December 2008) and followed by *Pseudanabaena catenata* Lauterborn (February 2009) (Fig. 9). The contribution of these species increased during the last period, reaching an abundance and biomass equivalent to >90% of all cyanobacteria at all three stations (Figs. 6, 7).

During the spring of 2009, species belonging to the order Nostocales also appeared, initially only *Aphanizomenon gracile* (Lemm.) Lemm. (Fig. 8), in April and May, followed by *Aphanizomenon aphanizomenoides* (Forti) Horecká et Komárek and *Anabaenopsis circularis* (G.S. Weast) Woloszyńska et Miller (Fig. 8) in June (Fig. 9). The abundance and biomass of this order was highest (almost 20% of all cyanobacteria) at station 3 (Figs. 6, 7).

Among the other classes of phytoplankton, small Chlorophyceae, *Chlorella* sp., and *Monoraphydium minutum* (Näg.) Kom.-Legn. were the most abundant at all three stations during the first year-long cycle (maximum abundance reached in autumn and spring). Beginning in January 2009, simultaneous with a decrease of Chroococcales, the eukaryotic phytoplankton composition became more varied. In winter, small cells of Dinophyceae *Heterocapsa rotundata* (Lohmann) Hansen 1995 (formerly *Katodinium rotundatum* (Lohmann) Loeblich III 1965) and of Prasinophyceae *Pyramimonas* sp. were detected in important amounts. During the spring, the Bacillariophyceae *Cyclotella* sp. and

Table 2: Mean, maximum, and minimum of density, biomass, and cell size (length × width) of the cyanobacteria observed in Cabras Lagoon during the two annual cycles of this study (n = number of cells considered; n. o. = not observed).

		2007-2008			2008-2009		
	CCA Code	Mean	Max	Min	Mean	Max	Min
Cyanobium - type	Cya-type						
Density (cell l^{-1})		2.1 x 10^{9}	9.8 x 10^{9} St. 2 - Mar	4.5 x 10^{8} St. 1 - Nov	4.0 x 10^{8}	1.8 x 10^{9} St. 3 - Oct	1.6 x 10^{6} St. 3 - Jen
Biomass (mg l^{-1})		2	13 St. 2 - Mar	2 x 10^{-1} St. 1 - Nov	3 x 10^{-1}	2 St. 1 - Nov	2 x 10^{-3} St. 3 - Jen
Cell diameter (µm)		1.1 (n = 120)	2.0	0.5	1.3 (n = 110)	2.5	0.5
Rhabdoderma sp.	Rha sp.						
Density (cells l^{-1})		4.4 x 10^{5}	1.4 x 10^{7} St. 1 - Aug	1.6 x 10^{5} St. 1 - Jun	-	- -	- -
Biomass (mg l^{-1})		2 x 10^{-4}	7 x 10^{-3} St. 1 - Aug	8 x 10^{-5} St. 1 - Jun	-	- -	- -
Cell size (µm)		2.0 x 1.2 (n = 40)	2.5 x 1.5	1.5 x 1.0	-	-	-
Aphanocapsa sp.	Aph sp.						
Density (cell l^{-1})		-	- -	- -	7.4 x 10^{5}	5.6 x 10^{5} St. 3 - Jun	3.1 x 10^{4} St. 2 - Jun
Biomass (mg l^{-1})		-	- -	- -	4 x 10^{-4}	4 x 10^{-4}	4 x 10^{-4}
Cell diameter (µm)		-	-	-	1.0 (n = 20)	1.0	1.0
Merismopedia tenuissima	Mer ten						
Density (cells l^{-1})		1.5 x 10^{5}	5.3 x 10^{6} St. 3 - May	4.0 x 10^{6} St. 3 - Sep	6.4 x 10^{5}	6.4 x 10^{7} St. 3 - Jun	8.9 x 10^{5} St. 3 - Jul
Biomass (mg l^{-1})		1 x 10^{-4}	3 x 10^{-3} St. 3 - May	3 x 10^{-4} St. 3 - Sep	8 x 10^{-4}	3 x 10^{-2} St. 3 - Jun	5 x 10^{-4} St. 3 - Jul
Cell diameter (µm)		0.7 (n = 20)	1.0	0.5	1.0 (n = 30)	1.0	1.0
Pseudanabaena catenata	Pse cat						
Density (cells l^{-1})		-	- -	- -	1.5 x 10^{8}	1.6 x 10^{9} St. 2 - May	2.1 x 10^{5} St. 1 - Jun
Biomass (mg l^{-1})		-	- -	- -	2	17 St. 2 - May	2 x 10^{-3} St. 1 - Jun
Cell size (µm)		-	-	-	5.3 x 1.6 (n = 50)	7.0 x 2.0	4.5 x 1.5
Planktothrix sp.	Pla sp.						
Density (cells l^{-1})		-	- -	- -	2.6 x 10^{7}	3.3 x 10^{8} St. 1 - May	1.1 x 10^{5} St. 1 - Dec
Biomass (mg l^{-1})		-	- -	- -	8 x 10^{-1}	9 St. 2 - Apr	3 x 10^{-3} St. 1 - Dec
Cell size (µm)		-	-	-	3.6 x 3.1 (n = 50)	5.0 x 4.0	2.0 x 2.0
Aphanizomenon gracile	Aph gra						
Density (cells l^{-1})		-	- -	- -	1.4 x 10^{7}	5.6 x 10^{5} St. 3 - Jun	3.1 x 10^{4} St. 2 - Jun
Biomass (mg l^{-1})		-	- -	- -	3 x 10^{-1}	6 St. 2 - May	4 x 10^{-1} St. 2 - Apr
Vegetative cell size (µm)		-	-	-	5.0 x 3.2 (n = 40)	7.0 x 3.7	3.6 x 2.5
Heterocyte size (µm)		-	-	-	5.2 x 3.2 (n = 40)	6.2 x 3.7	4.9 x 2.5
Akinete size (µm)		-	-	-	17.7 x 4.7 (n = 40)	24.5 x 6.2	12.4 x 3.6
Anabaenopsis circularis	Ana cir						
Density (cells l^{-1})		-	- -	- -	2.6 x 10^{4}	5.6 x 10^{5} St. 3 - Jun	3.1 x 10^{4} St. 2 - Jun
Biomass (mg l^{-1})		-	- -	- -	1 x 10^{-3}	3 x 10^{-2} St. 3 - Jun	2 x 10^{-3} St. 2 - Jun
Vegetative cell size (µm)		-	-	-	7.3 x 4.2 (n = 20)	9.0 x 4.5	5.5 x 4.0
Heterocyte diameter (µm)		-	-	-	4.2 (n = 20)	4.5	4.0
Akinete size (µm)		-	-	-	n. o.	n. o.	n. o.
Aphanizomenon aphanizomenoides	Aph aph						
Density (cells l^{-1})		-	- -	- -	3.6 x 10^{5}	11.4 x 10^{6} St. 3 - Jun	5.8 x 10^{6} St. 3 - Jun
Biomass (mg l^{-1})		-	- -	- -	1 x 10^{-2}	5 x 10^{-1} St. 3 - Jun	2 x 10^{-1} St. 3 - Jun
Vegetative cell size (µm)		-	-	-	3.5 x 4.7 (n = 20)	3.7 x 5.0	2.5 x 3.7
Heterocyte diameter (µm)		-	-	-	5.0 (n = 20)	6.2	3.7
Akinete diameter (µm)		-	-	-	8.5 (n = 20)	11.2	7.4

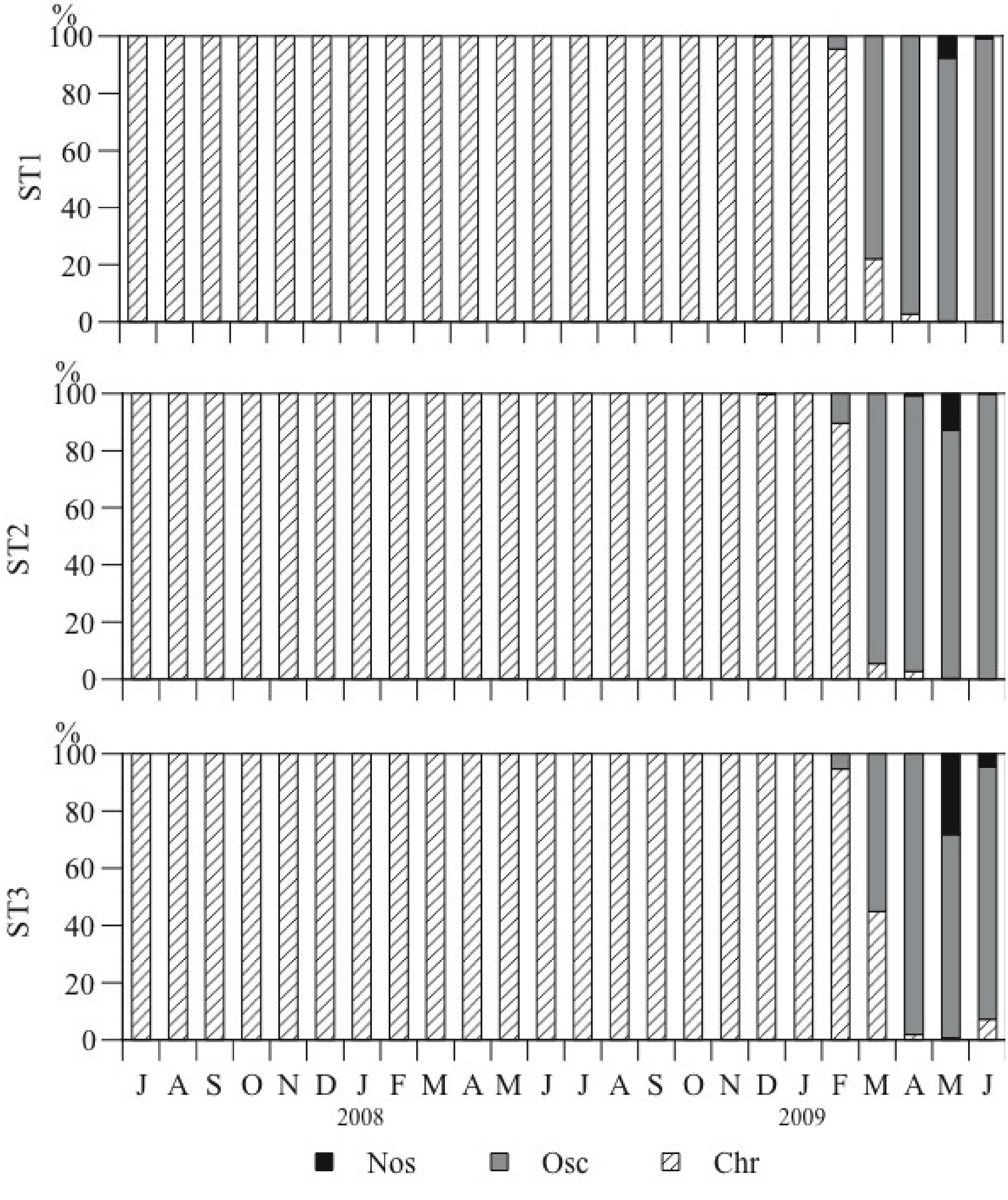

Figure 6: Seasonal dynamics of Chroococcales (Chr), Oscillatoriales (Osc), and Nostocales (Nos) abundance at the three sampling stations.

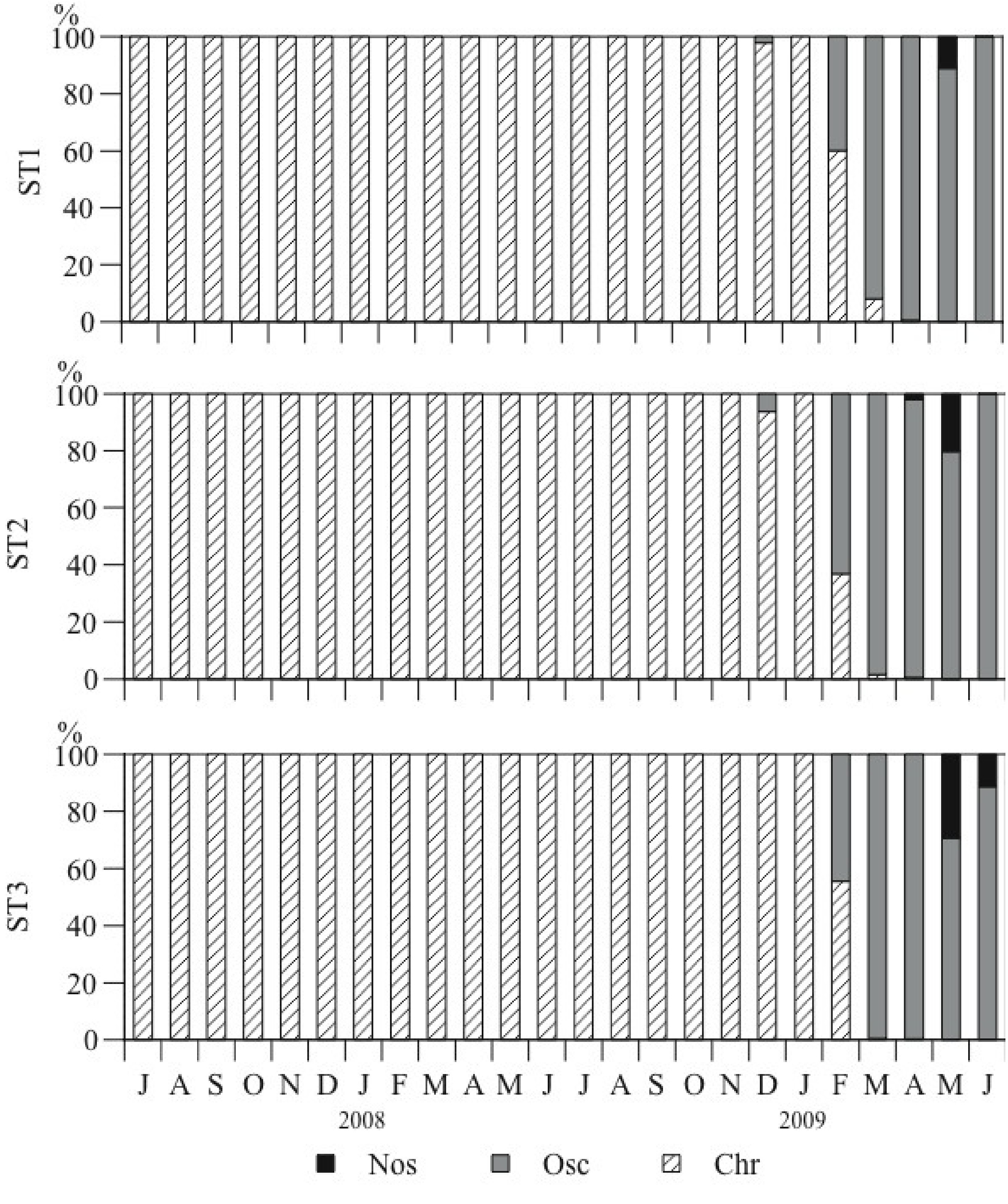

Figure 7: Seasonal dynamics of Chroococcales (Chr), Oscillatoriales (Osc), and Nostocales (Nos) biomass at the three sampling stations.

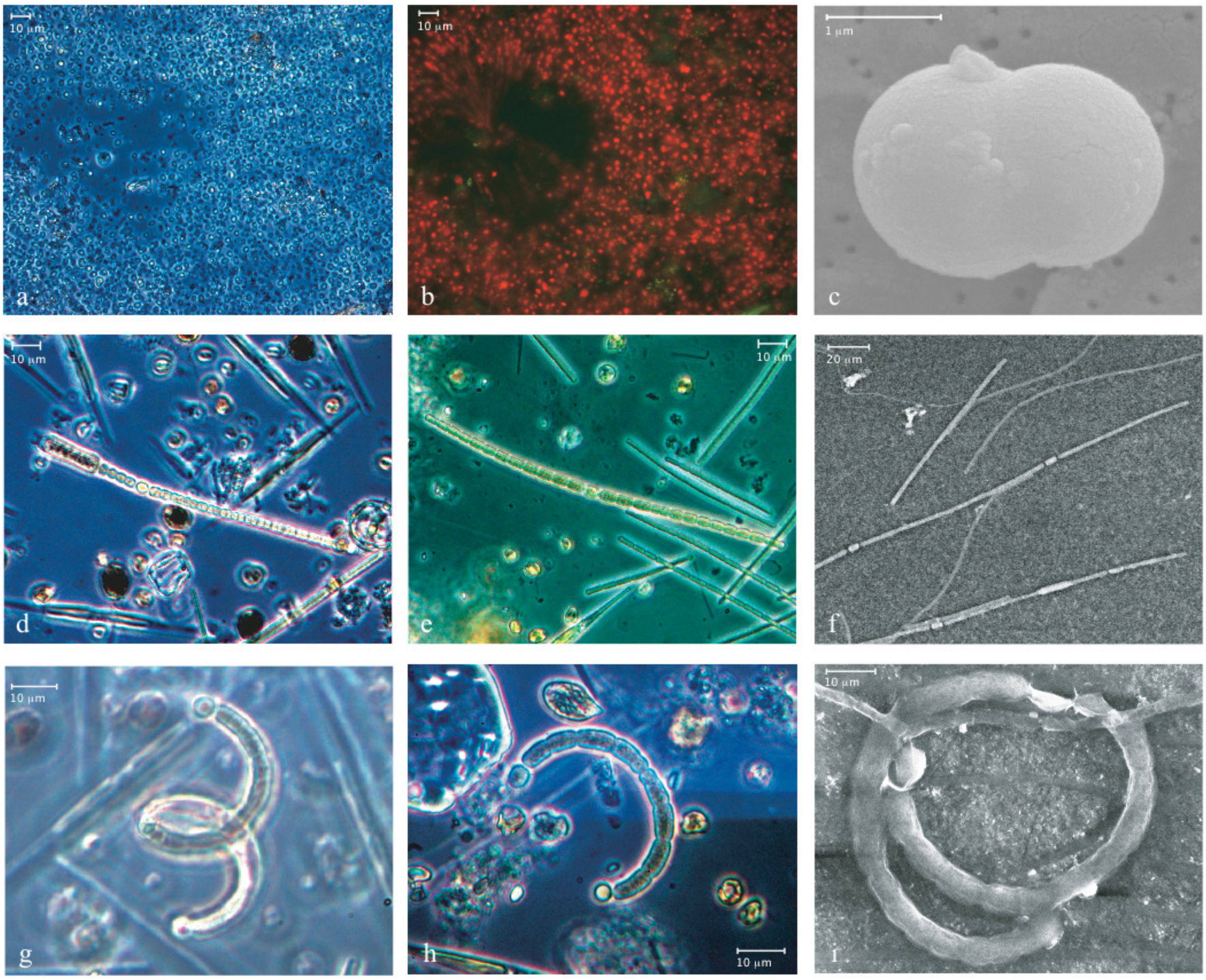

Figure 8: *a* – *c* *Cyanobium*-type cells, *a* optical microscopy, *b* fluorescence microscopy, *c* ESEM; *d* – *f* *Aphanizomenon gracile*, *d* and *e* optical microscopy, *f* ESEM; *g* – *i* *Anabaenopsis circularis*, *g* and *h* optical microscopy, *i* ESEM.

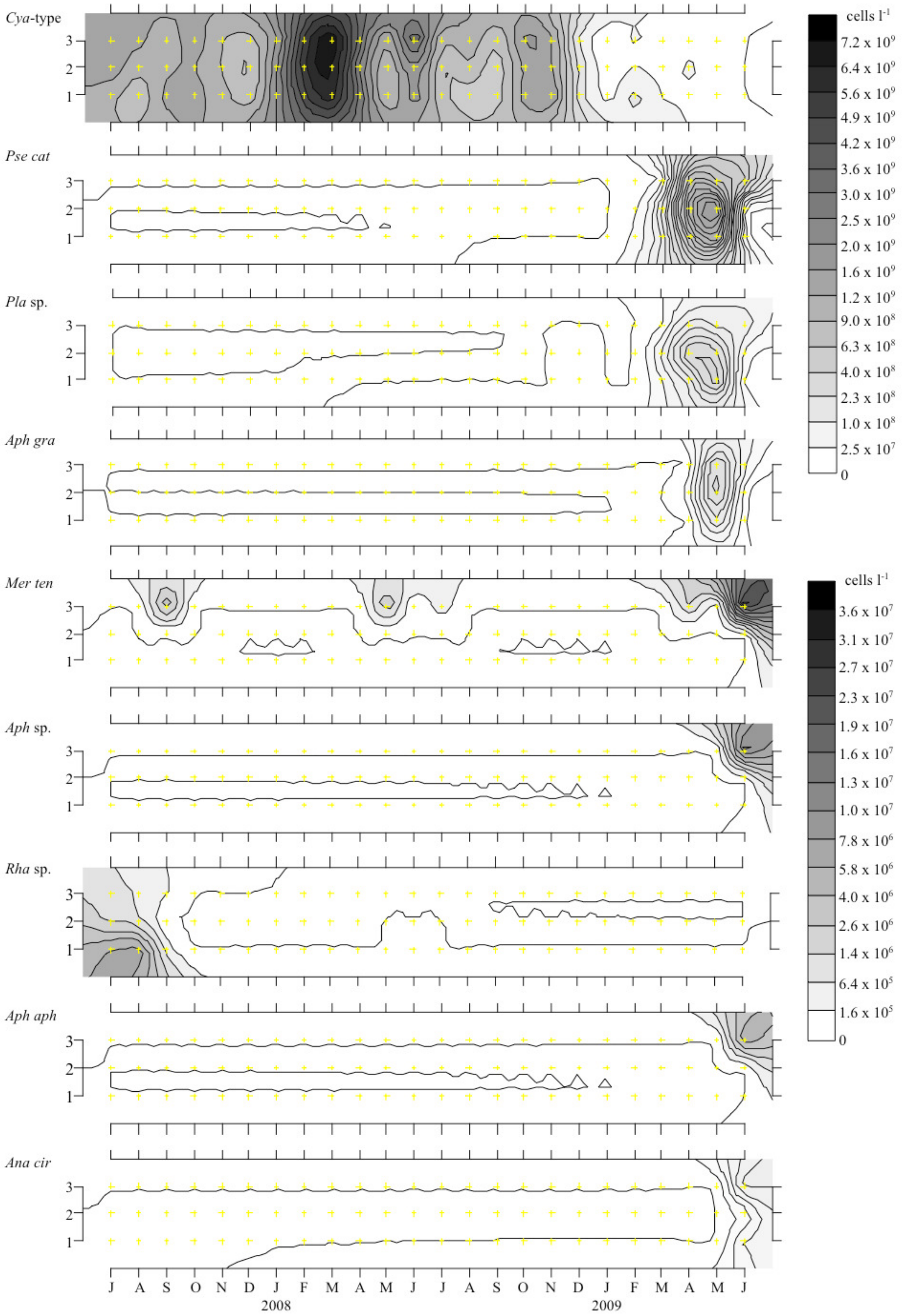

Figure 9: Seasonal variations of cyanobacterial species abundance at the three sampling stations of Cabras Lagoon (1, 2, 3) during the investigation period. For abbreviations, see Table 2.

Chaetoceros sp. were particularly abundant while in summer there was a bloom of the Prymnesiophyceae *Prymnesium* sp.

Correlation with environmental parameters

In a CCA of orders of cyanobacteria, the first two axes of the CCA accounted for 100% of the total variance (93.89% for the first axis and 6.11% for the second) in cyanobacterial orders and environmental data. The orders-environment correlation between 14 environmental variables and the three orders was 0.96 for the first axis and 0.88 for the second. Monte Carlo tests showed that all canonical axes were significant (p<0.001).

CCA was also carried out for cyanobacterial species, with the first two axes accounting for 88.36% (80.74% for the first axis and 7.62% for the second) of the total variance of species and environmental data. The species-environment correlation between the 14 environmental variables and the nine species was 0.97 for the first axis and 0.58 for the second. Monte Carlo testing again showed that all canonical axes were significant (p<0.001).

Canonical correspondence analysis confirmed the strong relationship between the order Chroococcales (almost exclusively represented by *Cyanobium*-type cells) and salinity (Fig. 10). *M. tenuissima* was observed constantly during the summer months; its presence was related to high temperatures, whereas the outlying position of *Aphanocapsa* sp. was consistent with its appearance only during a very short period (Fig. 11). The contributions of *A. aphanizomenoides*, *A. gracile*, and *A. circularis* could mainly be explained by the accompanying physical parameters, i.e. pH and DO (*A. aphanizomenoides*, *A. gracile*) and temperature and alkalinity (*A. circularis*) (Fig. 11). This tendency was clearly reflected also at the order level whereas the presence of Oscillatoriales was mainly controlled by nutrients (TP, DIN/SRP) and pH (Fig. 10). The presence of both Oscillatoriales and Nostocales was negatively correlated with salinity.

DISCUSSION

During the 2-year investigation period, the high trophic level of Cabras Lagoon was confirmed and the main representatives of its phytoplankton populations identified, specifically, cyanobacteria predominated at all three stations and in almost all of the samplings.

The intense proliferation of cyanobacteria in coastal eutrophic ecosystems at all latitudes is well documented in several studies (Kanoschina *et al.*, 2003; Sorokin *et al.*, 1996a; Gasiūnaite *et al.*, 2005). In the Mediterranean basin, different orders of cyanobacteria have

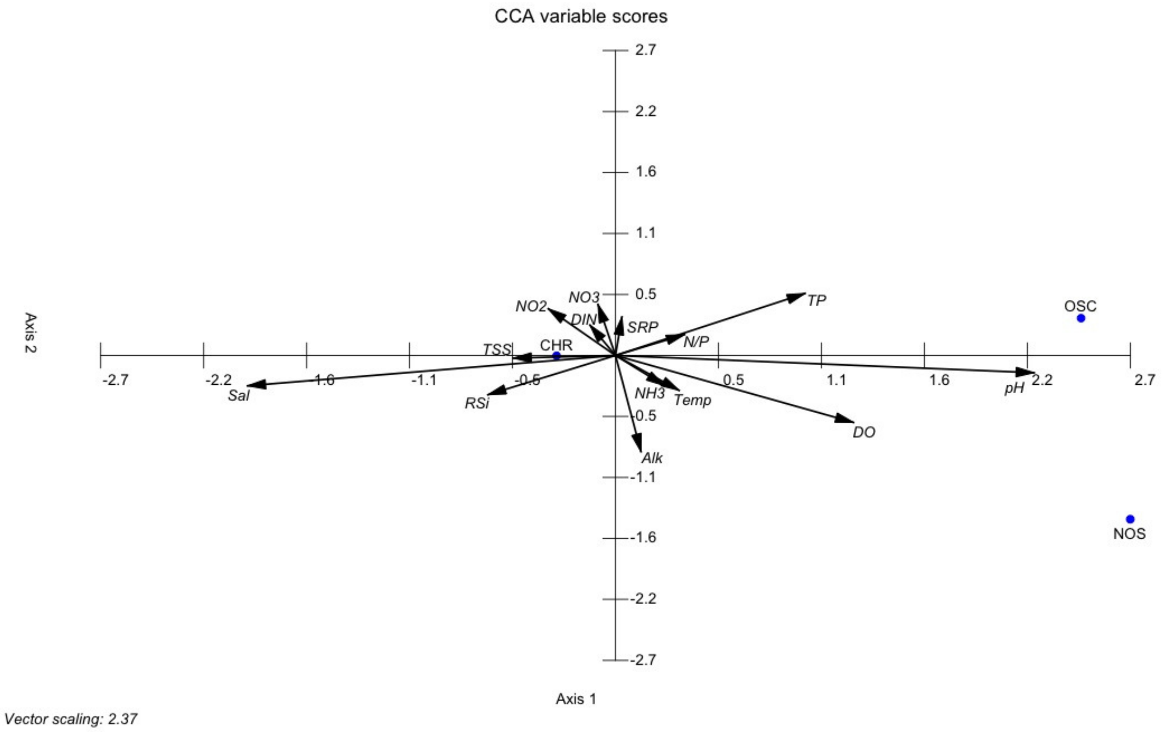

Figure 10: Canonical corresponding analysis (CCA) of the relationship orders–environment. For abbreviations, see Table 1 for environmental factors and Figure 6 or 7 for orders.

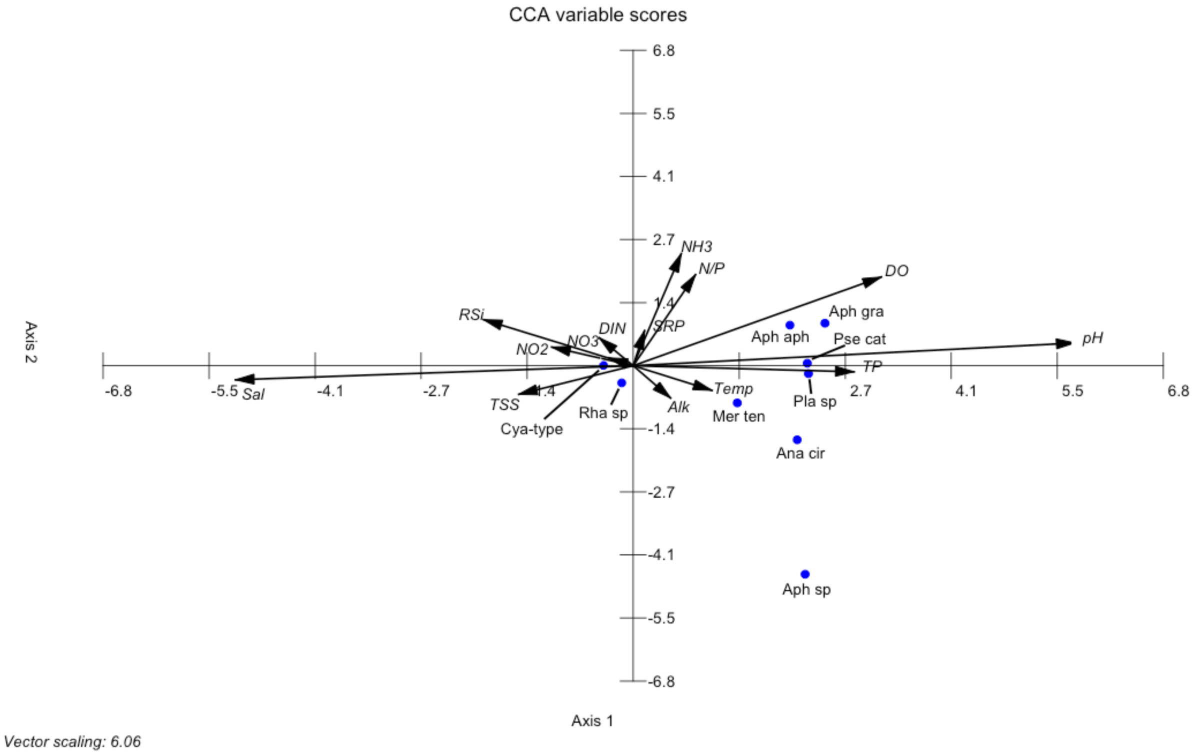

Figure 11: Canonical corresponding analysis (CCA) of the relationship species–environment. For abbreviations, see Table 1 for environmental factors and Table 2 for species.

been observed, depending on the environmental conditions and the season. Findings similar to ours have been reported by other authors in studies of lagoons with analogous conditions. In the hypertrophic Bolmon Lagoon in the south of France, a permanent dominance of cyanobacteria was observed, with a seasonal pattern characterized mainly by *Planktothrix agardhii* (Gomont) Anagnostidis et Komárek in winter, colonial Chroococcales in spring, Pseudanabaenaceae in summer, and *P. agardhii* in autumn and then in winter again (Chomérat *et al.*, 2007). A similar situation, with a persistent dominance of Oscillatoriales, was described for the hypertrophic Albufera Lagoon in Spain (Romo and Miracle, 1993).

The occurrence of small single cyanobacteria has been reported in other Mediterranean lagoons (Del Negro *et al.*, 2007). In the hypertrophic lagoon system of Comacchio (NW Adriatic Sea, Italy), an extremely intense bloom of Chroococcales that persisted for many years resulted in the collapse of that ecosystem (Sorokin *et al.*, 1996).

While picocyanobacteria have long been recognized as important and typical biological components of oceans and, in general, of oligotrophic ecosystems (Anxelu and Morán, 2007), during the last few years, they have been increasingly reported in brackish and highly trophic environments. The reasons for this expansion are largely unknown but may involve physical variables or alterations in nutrients and/or grazers. In Cabras Lagoon, Chroococcales, and especially *Cyanobium*-type cells, was the only cyanobacterial order present for a period of 18 months.

In most published scientific works, free-living cyanobacteria of small size are described under the generic name "*Synechoccocus* sp." (Komárek and Anagnostidis, 1998). The genus *Cyanobium* has been reported in coastal lagoons in a few cases, although many of the organisms described in those studies as *Synecoccocus*-type instead corresponded to the genus *Cyanobium* (Komárek and Anagnostidis, 1998). For example, in 1989, Andreoli *et al.* provided a detailed report of picocyanobacterial cells in Santa Gilla Lagoon (Sardinia, Italy); but, according to current information, the cells may well have been *Cyanobium* (Komárek and Anagnostidis, 1998). Among the described species of the genus *Cyanobium*, those most similar to the cells observed in Cabras Lagoon based on morphological and ecological characteristics are *Cyanobium bacillare* (Butcher) Komárek *et al.* and *Cyanobium planctícum* (Drews) Komárek *et al.* However, the lack of cytomorphological and/or molecular biological data have made species identification very difficult, as the infrageneric taxonomy of this genus is still incomplete (Komárek and Komárková – Legnerová, 2002).

The results obtained for the Cabras Lagoon during the two year-long cycles provided evidence of a strong relationship between the occurrence of *Cyanobium*-like cells and the salinity of the water. The presence of such cells in the lagoon decreased enormously only after the strong rainfall in the autumn and winter of 2008–2009, when the large input of freshwater into the lagoon caused a decrease in its salinity. A strict correlation between *Cyanobium*-like cells and salinity was also observed in the Ebro estuary (Spain), where an increase in these species was directly proportional to the increase in salinity (Pérez and Carrillo, 2005).

In Cabras Lagoon, the suddenly decrease in both salinity and *Cyanobium*-type abundance was accompanied by the appearance of typical freshwater species, i.e. *Planktothrix* sp. and *P. catenata*. That the occurrence of filamentous cyanobacteria is favored under conditions of high nutrient availability, in particular phosphorus, and by low-light conditions has been well documented (Scheffer *et al.*, 1997) and is in agreement with the our results. Specifically, we observed that a strong rainfall resulted in a large-scale input of nutrients and materials from rivers, as demonstrated by a maximum SRP, an increase in the turbidity of the water, and maximum values of total solids in suspension. Filamentous cyanobacteria are also very tolerant of high pH (Hasler *et al.*, 2003), condition observed in Cabras Lagoon during the period of their permanence (up to pH 9.84).

The Oscillatoriales first appeared in the lagoon in winter but reached their maximum abundance and biomass in spring, as was also the case in the above-described Bolmon Lagoon (France) and Albufera Lagoon (Spain). In the former, during the study cycle, Oscillatoriales were dominant throughout, achieving a maximum in spring and a minimum in summer. During the entire year, the salinity of this lagoon was similar to that of Cabras Lagoon during the period when non-heterocystous filamentous cyanobacteria were present. The intense proliferation of phytoplankton, in particular of *Planktothrix* sp. and *P. catenata*, in spring induced the depletion of nutrients later in the season, which favored heterocytous nitrogen-fixing species, initially *A. gracile* and then *A. aphanizomenoides* and *A. circularis*. CCA analysis showed a negative correlation between the order Nostocales and the different forms of inorganic nitrogen. It also confirmed the strict relationship between the presence of this order of cyanobacteria and specific conditions of the lagoon. In fact, the proliferation of Nostocales species was contemporaneous with maxima of pH and DO. Moreover, the presence of *A. circularis*, a species with high demands for light and warmth (Komárek, 2005), correlated with high temperatures, which accounts for its presence in the lagoon only in June.

In the succession dynamics of the three orders (Chroococcales, Oscillatoriales, and Nostocales), temporal variations in DIN/SRP ratio were clearly important. Thus, the dominance of *Cyanobium*-type cells during the first 18 months of the study was likely due to the low DIN/SRP ratio. This conclusion is supported by the well-documented capacity of the *Synecoccocus* genus to fix nitrogen (Stal *et al.*, 2008) and the strict genetic link of this genus with *Cyanobium* (Komárek and Anagnostidis, 1998), even though the latter has yet to be studied in detail. After an intense rainfall, there was a strong increase in the DIN/SRP ratio, contemporaneous with the dominance of Oscillatoriales. After the DIN/SRP ratio had reached its maximum peaks, there was a shift towards a reduction of values, coincident with the low salinity of the water. These conditions were less than optimal for *Cyanobium*-like cells but could have favored the growth of other nitrogen-fixing species, specifically, those belonging to the order Nostocales.

Another aspect concerning the intense occurrence of cyanobacteria in lagoons is related to negative effects, both direct and indirect, on fish and seafood. The high biomass produced by massive cyanobacterial proliferation results in oxygen depletion, anoxia, and ultimately the death of aquatic biota. In addition, the large quantity of mucilage produced by some cyanobacterial species obstructs the gills of aquatic animals and causes their death (Cronberg and Annadotter, 2006). As mentioned above, this scenario has been observed in recent years in Cabras Lagoon and poses a threat to it and other lagoon ecosystems where, as a rule, fishery and the harvest of shellfish are the main economic activities. The situation is exacerbated by the presence of toxin-producing cyanobacterial species even if it is very difficult to unequivocally ascribe the death of natural populations of aquatic animals, especially fish, to cyanotoxin poisoning (Chorus and Bartram, 1999). Nonetheless, it is highly likely that large populations of cyanobacteria, such as *A. gracile* (Pereira *et al.*, 2004), that could produce toxins responsible for paralytic shellfish poisoning (PSP) in humans (Steidinger, 1993) could likewise endanger lagoon environments.

In conclusion, this study confirmed the dominance of cyanobacteria, in particular the intense and prolonged occurrence of single small cells belonging to the order Chrooccocales, in a hypertrophic brackish lagoon of the Mediterranean basin. Among the factors that could explain this predominance, other studies of similar environments described the absence in the lagoon of grazers that preferentially feed on such cells (Sorokin *et al.*, 1996). Moreover, our results suggest a very strict relationship between the presence of these cyanobacteria and the dynamics of salinity, as confirmed during the second study cycle by the decrease of Chrooccocales cells concomitant with the sudden drop of salinity. The impact of this event was a disturbance in the phytoplankton composition of Cabras Lagoon, resulting in changes that persisted over a period of 18 months and which may very well be permanent.

In winter, the intense proliferation of filamentous cyanobacteria supported the hypothesis proposed in studies of the Bolmon and Albufera Lagoons. At those sites, the mild winters and hot summers typical of the Mediterranean climate modify the typical patterns observed at higher latitudes, where filamentous cyanobacteria generally dominate the phytoplankton in late summer but decline during the cold period. A comparison of our findings with those obtained in other eutrophic brackish lagoons with the same climatic conditions will provide a better understanding of cyanobacterial populations, with respect to their composition and their dynamics, in these environments.

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4. Chapter II

Composition and distribution of dinoflagellate cyst assemblages in surface sediments of the Cabras Lagoon (Sardinia, Western Mediterranean).

Composition and distribution of dinoflagellate cyst assemblages in surface sediments of the Cabras Lagoon (Sardinia, Western Mediterranean).

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ABSTRACT

Dinoflagellate cyst assemblages were studied at different times in Cabras Lagoon (Sardinia, Western Mediterranean). The number of cyst morphotypes recovered was low (14) but in according to the salinity values and the scarce presence of dinoflagellates in the water column on long term database. *Pentapharsodinium* sp. and an undetermined cyst (type A) were the widespread morphotypes. Common cosmopolitan species were signalled in the lagoon (e.g. *Scrippsiella trochoidea*, *Polykrykos schwartzii*, *Gonyaulax spinifera*), allowing new information on phytoplankton biodiversity. These species, together with *P.* cf *daleii*, *S. precaria* and the genus *Fragilidinium*, had never been recognized in the water samples.

Cyst assemblages varied significantly in the different samplings (Global R: 0.481; *p*: 0.1%) but not among stations (Global R: -0.148; *p*: 91%). The spatial surveys showed the highest values in the central- southern area of the lagoon and the morphotype distribution was quite heterogeneous.

Keywords: dinoflagellates, resting cysts, dinoflagellate cysts, Cabras Lagoon, Mediterranean basin

1. Introduction

In shallow coastal and protected areas many planktonic organisms can produce resting stages in their life cycle (Boero et al., 1996). Dinoflagellates is one of the groups that count for the major number of species producing resting cysts, and many of these species can be harmful at different levels (Anderson and Wall, 1978; Dale, 1983). The alternation of planktonic vegetative state and benthic resting cyst existence leads to a discontinuous presence of these species in the water column (Boero et al., 1996) and, consequently, at a possible overlooking and sub valuation of biodiversity (Dale, 1983). The employment of studies on benthic resting cysts can integrate records of the planktonic populations and can be very useful in describing the diversity of an area, and completing the information on the plankton composition (Boero et al., 1996; Rubino et al., 2000, 2002; Orlova et al., 2004; Bravo et al., 2006; Satta et al., 2009). Indeed, the identification of vegetative stages of dinoflagellates can be very difficult and a number of analyses are necessary for a good taxonomic placement. On the contrary, in some cases the morphological differences among cysts allow a relative more simple identification of the species (e.g *Scippsiellas* species, Lewis, 1991), also supported by molecular data (D'Onofrio et al, 1999; Montresor et al., 2003). Moreover, many new species are recently described starting by an isolated resting cyst (Montresor and Zingone, 1988; Janofske, 2000; Attaran-Fariman and Bolch, 2007; Gu et al., 2008).

In the last years, many studies on dinoflagellate cyst assemblages have been published, but these studies regard especially coastal marine areas, harbours or embayments (Bolch and Hallegraeff, 1990; Nehring, 1997; Montresor et al., 1998; Persson et al., 2000; Rubino et al., 2000, 2002; Morquecho and Lechuga-Devéze, 2003; Orlova et al., 2004; Giannakourou et al., 2005; Satta et al., 2009). In our knowledge data on assemblages of Dinoflagellate cysts in lagoons regard very few sites (Southern New England, Pospelova et al., 2004; Malaysia, Furio et al., 2006) but not the Mediterranean basin.

Coastal lagoons are, generally, natural systems consisting of shallow, saline or brackish waters isolated or semi-isolated from the open sea by coastal barriers (Healy, 1997). The connection with the adjacent sea is assured by the presence of channels or inlets, frequently humanly modified (Como et al., 2007). Lagoons are subject to extreme environmental fluctuations because they depend on the amount and frequency of exchange with the sea and, contemporarily, on the inputs from the watersheds (Chomérat et al., 2007; Pérez-Ruzafa et al., 2005). Coastal lagoons are, in many cases, eutrophic or hypereutrophic systems because of their position at the end of the drainage basins. These convoy waters enriched in nutrients, especially nitrogen and phosphorous, due to natural or anthropogenic sources (Cloern, 2001).

Lagoons are highly productive areas often exploited for fishery and aquaculture. These activities, like ecological aspects of these environments, can be damaged by the proliferation of harmful algal species, which can be also the trigger for dystrophic events.

In this paper we report data on composition and distribution of dinoflagellate cyst assemblages at different times in the Cabras Lagoon; it is the first study on this topic for this site and for the Mediterranean basin. The main purpose is to increase the information on phytoplankton diversity of the study site, at least in respect to dinoflagellates that produce resting cysts. At the same time we investigate the presence of toxic or noxious species possibly overlooked in the monitoring programs. The availability of pluriannual data for environmental variables, phytoplankton data and main nutrients is a further support to the understanding of our results.

2. Methods

2.1 Study area

The Cabras Lagoon (Fig. 1) is a shallow transitional system located near the city of Oristano and with an area of 22.28 km^2 it is the largest brackish basin in Sardinia (western Mediterranean Sea). An average depth of 1.6 m and a maximum depth of around 2.1 m characterize the lagoon. Fishery activities have a high economical importance involving about 250 fishermen, and with a yearly production of about 850 tons (Murenu et al., 2004).

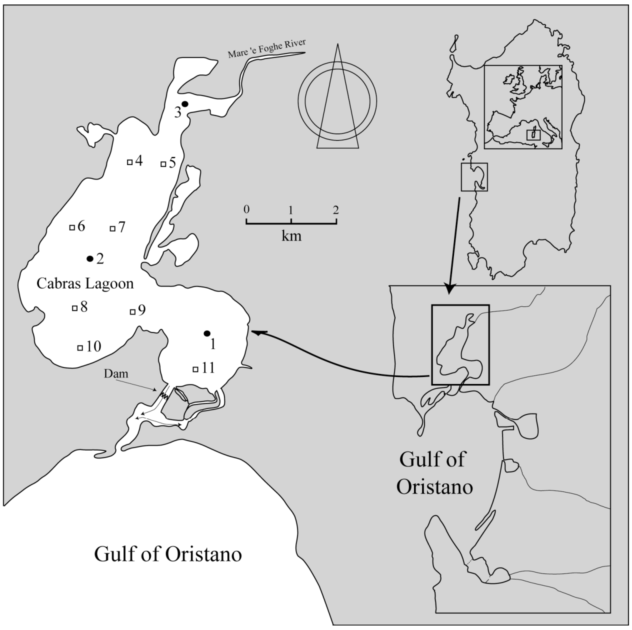

Fig. 1 Study area and sampling stations in the Cabras Lagoon. • indicates the three routine stations; ■ indicates all the other stations sampled in May 2009.

2.2 Cyst sample collection and processing

Four sampling surveys were conducted on October 2007, February and August 2008 and May 2009. Three fixed stations were sampled in all the surveys, and these stations were representative of the main sedimentary characteristics of the lagoon (De Falco et al., 2004). A spatial sampling, covering a total of 11 stations, was also conducted in May 2009 (Fig.1).

Sediment samples were taken with manual cylindrical plastic cores (40 cm long with a diameter of 5 cm). Samples were stored in the dark at 4°C and left undisturbed for 1-4 days to allow the sedimentation of all the material suspended in the water above the sediment. At the start of processing this water was carefully removed, and the sediment cores were sliced at 1 cm intervals down to 5 cm. The data of the 2-5 cm were used only for drawing up the floristic list. In May 2009 only the first cm was sectioned.

Subsamples (2–3 cm^3) of the sliced sections were suspended in filtered seawater (FSW) and sonicated for 2 min using a Bandelin Sonoplus to separate cysts from sediment particles. The obtained suspension was sieved through 10- and 100-μm mesh sieves. The fraction remaining on the 10-μm mesh sieve was washed with FSW and collected in a 50-ml tube. Subsamples (5 ml) of the slurry were centrifuged to recover cysts. The sodium polytungstate density gradient was used to separate the cyst fraction from the pellet, as proposed by Bolch (1997) and modified by Amorim et al. (2001) and Bravo et al. (2006). The resulting sample was rinsed in a 10-μm sieve and collected with 5–15 ml of FSW. Cysts were counted in sedimentation chambers with an inverted microscope (Axiovert 100) at 200× magnification. Empty cysts were not considered. Total densities were expressed in cysts gr^{-1} of dry weight (dw) and morphotype densities in relative percentage of the total (%). All morphotypes were photographed and then isolated with a glass Pasteur micropipette and transferred into IWAKI tissue-culture multi-well plates. These were filled with f/2 or f/16 medium prepared with FSW and subsequently maintained at 18–20 ± 1°C in a 12:12 h light:dark cycle. Fluorescence tubes provided illumination at a photon irradiance of 100 μmol photons m^{-2} s^{-1}. Plates were controlled every 2–4 days for germination and species identification. The works by Wall and Dale (1968), Dale (1983), and Matsuoka and Fukuyo (2000, 2003) were used to confirm cyst identification and to describe the dinoflagellate cysts with the appropriate terminology. Thecal plates of germinated cells of armored Dinoflagellates were analyzed by staining with Calcofluor White and examined under epifluorescence (Fritz and Triemer, 1985).

For the statistical treatment of the results a one-way analysis of similarity (ANOSIM) was used to test the significance of temporal and spatial variation in the morphotype assemblages in the three fixed stations among the samplings (Clarke and Warwick, 1994). Tests were calculated on the basis of the Bray-Curtis rank similarity matrix calculated using log(x+1) transformed data. This transformation was used to normalize the asymmetric distribution of data and to reduce the importance of extreme data. Ordination by non-metric Multi Dimensional Scaling (nMDS) was carried out on the basis of Bray-Curtis similarity coefficent. Also on the bases of Bray-Curtis similarities, the similarity percentage analysis (SIMPER) was applied in order to obtain the percentage contribution of each morphotype to the dissimilarity among samplings.

2.3 *Environmental data*

Fortnightly samplings were carried out from January 2000 to December 2001 and from July 2007 to June 2009, on three stations that coincided with the sediment routinely stations (Fig. 1). Physical and chemical parameters were measured in situ with a multi-parameter probe (Idronaut/YSI 6600V2). Nutrients were evaluated using the Strikland & Parsons (1972) methods. Data that we have considered for this study were relative to temperature, salinity, dissolved inorganic nitrogen (DIN; sum of N-NH4, N-NO2 and N-NO3) and orthophosphate (RP).

Phytoplankton samples were fixed with Lugol’s solution and analysed with the Utermöhl’s technique (1958), using an inverted microscope (Zeiss, Axiovert 25) after sedimentation of variable volumes of water (5-10 cc), depending on phytoplankton density. Cell counts were made at different magnifications (100x, 200x and 400x) adequately to the species size. The species were determined following Faust (2002), Germain (1981), Humm & Wicks (1980), Husted (1985), Rampi e Bernard (1978, 1980, 1981), Ricard (1987), Sournia (1986), Tomas (1997).

3. **Results**

3.1 Morphotype composition

A total of 14 morphotypes were recorded in the sediments of the Cabras Lagoon from all the surveys. Among morphotype, 5 were identified at species level, 3 at genus level and 2 at group level (Table 1). Two morphotypes (*Gonyaulax spinifera* (Claparède et Lachmann) Diesing, and *Pentapharsodinium* cf *daleii* Indelicato et Loeblich) were observed only in the 2-5 cm section. Already described cysts of harmful algal species were not detected in the lagoon sediments. Cyst types that could not be identified were reported as type A-D.

These with other morphotypes (3) not yet reported in literature have been described as following.

3.1.1 Gymnodiniales type 1 (Fig. 2 c)

This morphotype is oval (22-28 µm long and 20-25 µm wide; n=3) and surrounded by a mucilaginous layer that gives the cyst a ‘half moon’ shape. The cellular content is granular and greyish in colour. The yellowish accumulation body is not well defined. Germination of this morphotype produced a short-lived small gymnodinioid cell. A more detailed identification was therefore not possible.

3.1.2 Pentapharsodinium sp. (Fig. 2 e)

The cyst is rounded (20-26 µm in diameter; n=10) and the wall is clear, thick and sometimes covered by detrital particles. The cyst has a pale granular content and a ruby red accumulation body is present, generally closer to the cyst wall. The germination of the cyst produced a *Pentapharsodinium*-like cells. The first morphological observations of the plate tabulation revealed a similarity with *P. daleii* (Lewis, 1991). The main differences between the Cabras morphotype and this species regarded both the notable dorso-ventrally compression of the vegetative cells and the lack of the organic processes covering the cyst wall in the former. This morphotype was largely distributed in the lagoon and reached in some samples the highest density values.

3.1.3 Scrippsiella sp. (Fig. 2 m)

The cyst is ovoid (27-35 µm long and 25-33 µm wide; n=4), dark brown in colour. The cellular content is granular with a red accumulation body often not clearly visible. Calcareous crystals cover the wall. Germination experiments were unsuccessful.

3.1.4 Cyst type A (Fig. 2 n)

This cyst is spherical (10-12 µm in diameter; n=15), with clear contents and a red accumulation body. The cyst wall is thin and surrounded by very fine processes (3-4 µm long). This morphotype was, with *Pentapharsodinium* sp., widely distributed in the sediment of the lagoon. Unfortunately all the germination experiments were not successful.

3.1.5 Cyst type B (Fig. 2 o)

The cyst is subspherical to ovoid (12 µm in diameter; n=1) with a clear granular content and a red accumulation body. The cyst wall is clear, smooth and thin.

3.1.6 Cyst type C (Fig. 2 p)

This morphotype is oval (22-27 µm long and 22 µm wide; n=2) with a granular content and a ruby-red accumulation body. The double wall is thick and covered with detrital particle.

Table 1 Dinoflagellate cyst total counts, relative abundance and species richness from the sediment of Cabras Lagoon. + = presence of the morphotype in the 2-5 cm section

Sampling	Station	Sediment section (cm)	Species Richness	Total Cysts densities (cyst gr^{-1} dw)	*Fragilidinium* sp.	*Gonyaulax spinifera*	Gymnodiniales type 1	*Pentapharsodinium* cf *daleii*	*Pentapharsodinium* sp.	*Polykrykos schwartzii*	Round Brown	*Scrippsiella precaria*	*Scrippsiella trochoidea*	*Scrippsiella* sp.	Cyst type A	Cyst type B	Cyst type C	Cyst type D
Oct	1	0-1	2	326	0	0	0	0	60	0	0	0	0	0	40	0	0	0
		2-5			0	+	0	0	+	0	0	0	0	0	+	0	0	0
	2	0-1	2	633	0	0	0	0	43	0	0	0	0	0	57	0	0	0
		2-5			0	0	0	0	+	0	0	0	0	0	+	0	0	0
	3	0-1	1	41	0	0	0	0	0	0	0	0	0	0	100	0	0	0
		2-5			0	0	0	0	+	0	0	+	0	0	+	0	0	0
Feb	1	0-1	3	52	0	0	0	0	0	0	0	0	33	0	33	0	33	0
		2-5			0	0	0	0	0	+	0	0	+	+	+	0	+	0
	2	0-1	5	483	0	0	0	0	38	0	0	0	6	8	31	0	17	0
		2-5			+	+	0	+	+	0	+	0	+	+	+	0	+	0
	3	0-1	3	124	0	0	0	0	22	0	0	0	0	6	72	0	0	0
		2-5			0	0	0	0	+	0	0	0	0	0	+	0	0	0
Aug	1	0-1	4	131	0	0	0	0	58	0	17	0	0	8	17	0	0	0
		2-5			0	0	0	0	+	0	0	0	+	+	+	0	0	0
	2	0-1	4	329	0	0	0	0	54	0	14	0	0	0	27	5	0	0
		2-5			+	0	+	0	+	0	+	0	0	0	+	+	0	+
	3	0-1	2	241	0	0	0	0	50	0	50	0	0	0	0	0	0	0
		2-5			0	0	0	0	+	0	+	0	0	0	+	0	0	0
May	1	0-1	6	46	10	0	0	0	30	0	10	0	0	20	20	0	0	10
	2	0-1	5	287	6	0	0	0	41	0	0	15	0	6	0	0	0	32
	3	0-1	4	121	0	0	0	0	29	0	41	0	6	0	0	0	0	24

Sampling	Station	Sediment section (cm)	Species Richness	Total Cysts densities (cyst gr^{-1} dw)	*Fragilidinium* sp.	*Gonyaulax spinifera*	Gymnodiniales type 1	*Pentapharsodinium* cf *daleii*	*Pentapharsodinium* sp.	*Polykrykos schwartzii*	Round Brown	*Scrippsiella precaria*	*Scrippsiella trochoidea*	*Scrippsiella* sp.	Cyst type A	Cyst type B	Cyst type C	Cyst type D
May	4	0-1	5	88	0	0	0	0	42	0	0	26	11	11	11	0	0	0
	5	0-1	7	259	0	0	0	0	24	6	6	0	12	0	24	3	0	26
	6	0-1	5	136	0	0	5	0	14	0	33	0	0	0	19	0	0	29
	7	0-1	4	60	9	0	0	0	45	0	0	36	0	9	0	0	0	0
	8	0-1	7	225	0	0	0	0	18	0	15	0	18	3	27	3	0	15
	9	0-1	2	145	0	0	0	0	57	0	0	0	0	0	43	0	0	0
	10	0-1	4	163	0	0	0	0	22	0	22	0	11	0	44	0	0	0
	11	0-1	3	165	0	0	0	0	40	0	0	50	0	0	0	0	0	10

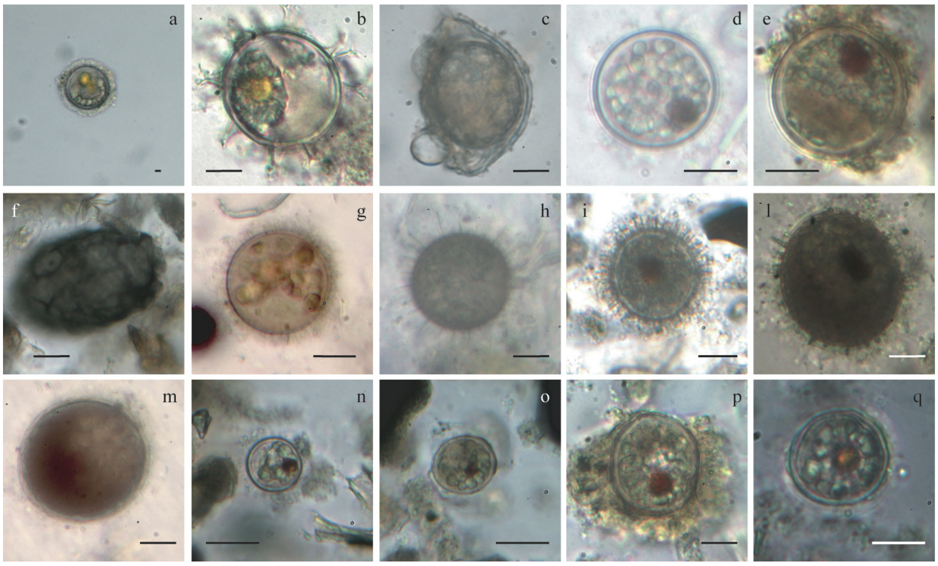

Fig. 2 Dinoflagellate cysts isolated from sediments of the Cabras lagoon: (a) *Fragilidinium* sp. (b) *Gonyaulax spinifera*. (c) Gymnodiniales type 1. (d) *Pentapharsodinium* cf *daleii*. (e) *Pentapharsodinium* sp. (f) *Polykrykos schwartzii*. (g-h) Round Brown cysts. (i) *Scrippsiella precaria*. (l) *Scrippsiella trochoidea*. (m) *Scrippsiella* sp. (n) cyst Type A. (o) cyst Type B. (p) cyst Type C. (q) cyst Type D. All scale bars 10 µm.

3.1.7 Cyst type D (Fig. 2 q)

The cyst is rounded (18 µm in diameter; n=3) very similar to the type A but bigger and presents an orange accumulation body. The germination experiments for this morphotype were unsuccessful.

3.1.8 Round Brown (Fig. 2 g-h)

This group includes a number of cysts that are brown in colour, rounded and lacking the accumulation body. The size varies substantially and the wall is or not covered by organic processes. Heterotrophic dinoflagellates belonging to the *Protoperidinium* or *Diplopsalis* often produce these kinds of cysts, but without information on germinated cells or archeopile structure determination is impossible.

3.2 Cyst assemblage abundances and compositions

Dynamics of total cyst density were different in the three stations (Fig. 3; Table 1). The maxima of station 1 and 2 were reached in October (respectively 326 cysts gr^{-1} dw and 633 cysts gr^{-1} dw), with an intense decrement of the values and without a regular trend in the following samplings at station 1 and with a smoothed and regular decrement of the densities at station 2. The maximum of station 3 was assessed in August (241 cysts gr^{-1} dw) after an increment from the first sampling and followed by a decrement in May. The highest cyst density was recorded in October at station 2 (633 cysts gr^{-1} dw) and the lowest in the same sampling at station 3 (41 cysts gr^{-1} dw). In all the samplings, station 2 showed the highest densities.

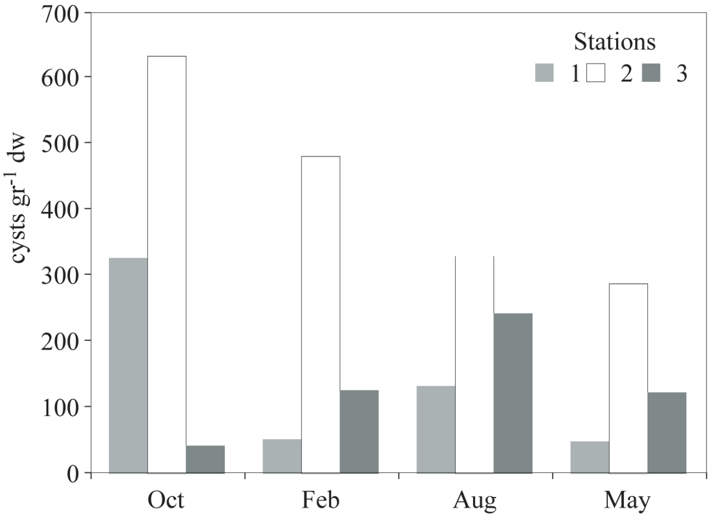

Fig. 3 Total cyst densities in the different stations and among samplings.

Although the described differences among the dynamics, the ANOSIM test did not indicate significant differences among stations as regards morphotype assemblage composition

(Global R: -0.148; *p*: 91%). On the contrary, the differences among samplings were significant (Global R: 0.481; *p*: 0.1%) and the nMDS ordination reflected this result (Fig. 4).

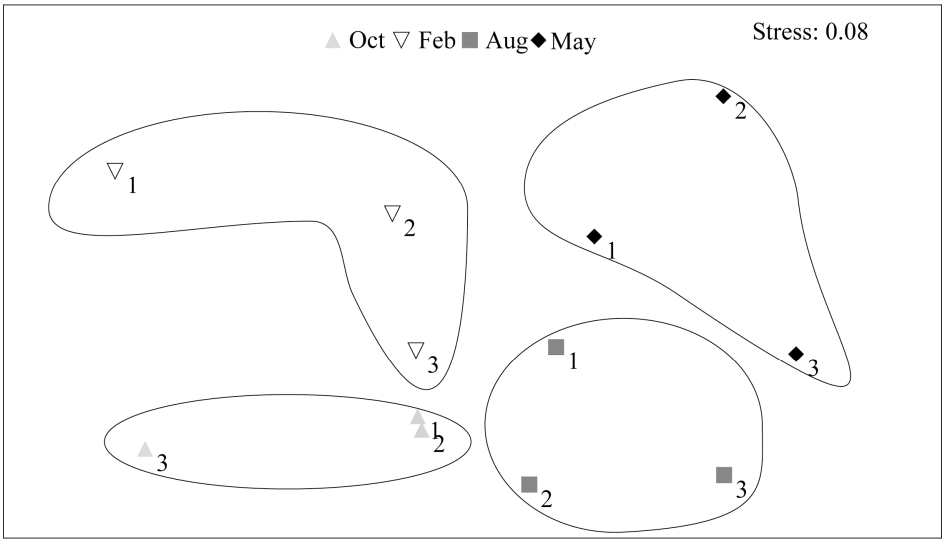

Fig. 4 nMDS ordination plots of samplings based on morphotype assemblage data.

The number of recognized morphotype for sampling was respectively 4 in October, 10 in February, 9 in August and 8 in May (considering only the three routine stations). The morphotype percentage compositions (Fig. 5) underlined the temporal variation and indicated *Pentapharsodinium* sp. as the most important in all the samplings. The SIMPER analysis (Table 2) confirmed the assemblage similarity at the different stations and revealed that only few morphotype contribute to the similarity between samplings (cyst type A, *Pentapharsodinium* sp., Round Brown and cyst type D).

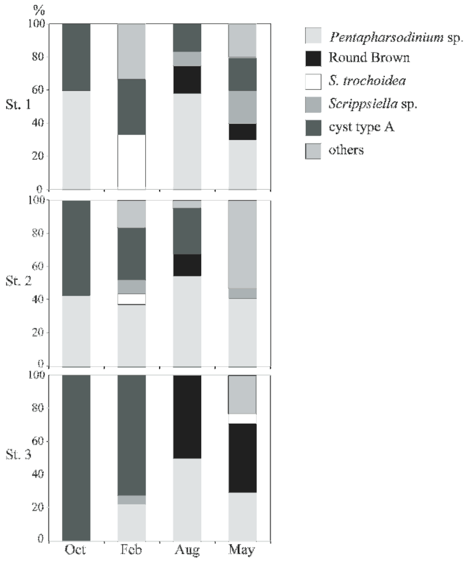

Fig. 5 Morphotype percentage contribution among samplings.

Table 2 SIMPER (similarity percentages procedure) analysis results: average similarity and most important contributory species to each sampling.

Average similarity (%)	Contributory species:	
Oct		
65.59%	cyst type A	75.21%
Feb		
50.26%	cyst type A	52.13%
	Pentapharsodinium sp.	14.01%
Aug		
68.19%	*Pentapharsodinium* sp.	58.30%
	Round Brown	38.30%
May		
48.67%	*Pentapharsodinium* sp.	41.73%
	cyst type D	31.34%

3.3 Spatial survey

The distribution of cyst abundances obtained in the spatial survey in May 2009 (Fig. 6a; Table 1) indicated highest values in the central and southern part of the lagoon. The maximum was registered at station 2 (287 cysts gr^{-1} dw). Stations 7 and 1 showed very low values (60 and 46 cysts gr^{-1} dw respectively) compared to their adjacent sampling areas (respectively station 11 and stations 2 and 6). New morphotypes were not observed even although the high number of stations sampled in this survey. Spatial cyst composition (Fig. 6b) revealed a substantial heterogeneity among the stations. *Pentapharsodinium* sp., cyst type A and cyst type D showed the wider distribution. *Pentapharsodinium* sp. reached the maximum percentage of 57% at station 9, followed by cyst type A with a percentage of 44% at station 10. The Round Brown group was quite widespread among the lagoon, and it was particularly abundant at stations 3 and 6 (41% and 33% respectively). The group of morphotypes named ‘others’, included those that were not largely distributed but reached high percentages at specific stations (e.g. *S. precaria* at station 7 and 11 with 34 and 50% respectively).

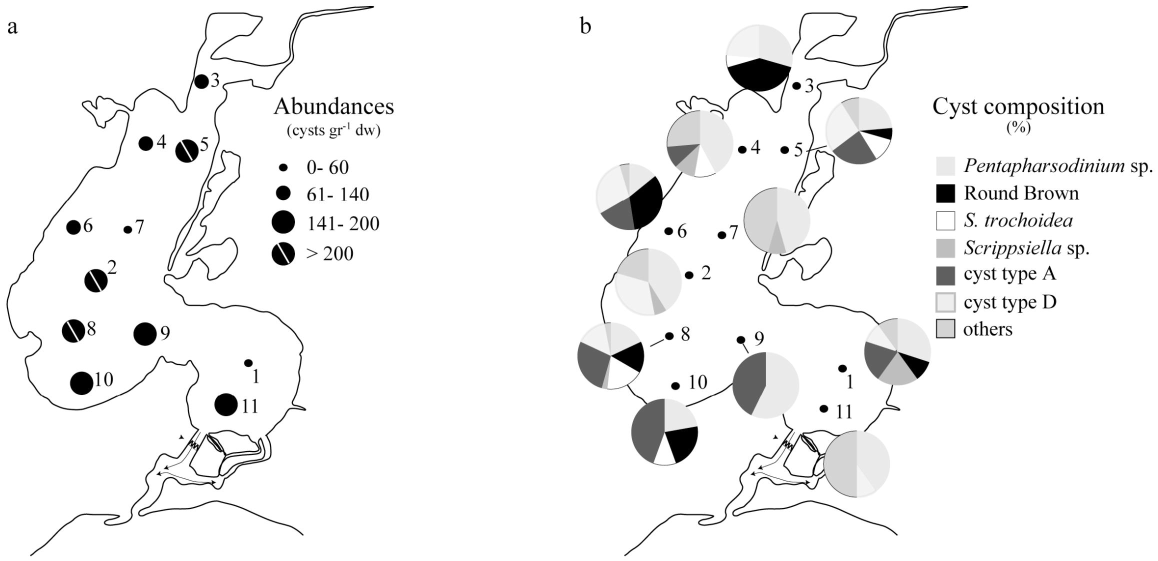

Fig. 6 Results of the spatial survey: total cyst densities (a), and morphotype percentage contribution (b).

3.4 Phytoplankton, nutrient and environmental data

The results of pluriannual investigations were reported as means of the whole lagoon. Phytoplankton class composition (expressed as relative percentage of the total density on the mean annual cycle, Fig. 7) showed the strong importance of Cyanophyceae and the relative low percentage of dinoflagellates. Considering the phytoplankton class composition inside the single annual cycles, the relative importance of dinoflagellate changed in 2000, 2001 and 2008 (Fig. 8): they reached the highest percentage in 2000 (late winter, early spring and summer months), which decreased in 2001, until it disappeared in 2008.

Temperature showed the typical seasonal cycle of the Mediterranean region with maxima in July and August (up to 27.2°C in August) and minima in December and January (10.3°C and 10.1°C respectively). Salinity showed lower values (<15‰) during the first semester of the mean annual cycle and higher in the second (up to 30.3‰ in August). In the interannual series salinity showed very different behaviour in 2000 and 2001 with the highest values (up to 41‰ in September 2000) and the lowest (5.4‰ in February 2001) of the entire series. High nutrient availability was observed in the lagoon (Fig. 7), with maxima of DIN especially in winter and autumn seasons (up to 22.2 µM N in January) and of RP in winter and summer (up to 2.1 µM P in February). DIN/RP ratio values were < 16 in all the mean annual cycle except in January (Fig. 7).

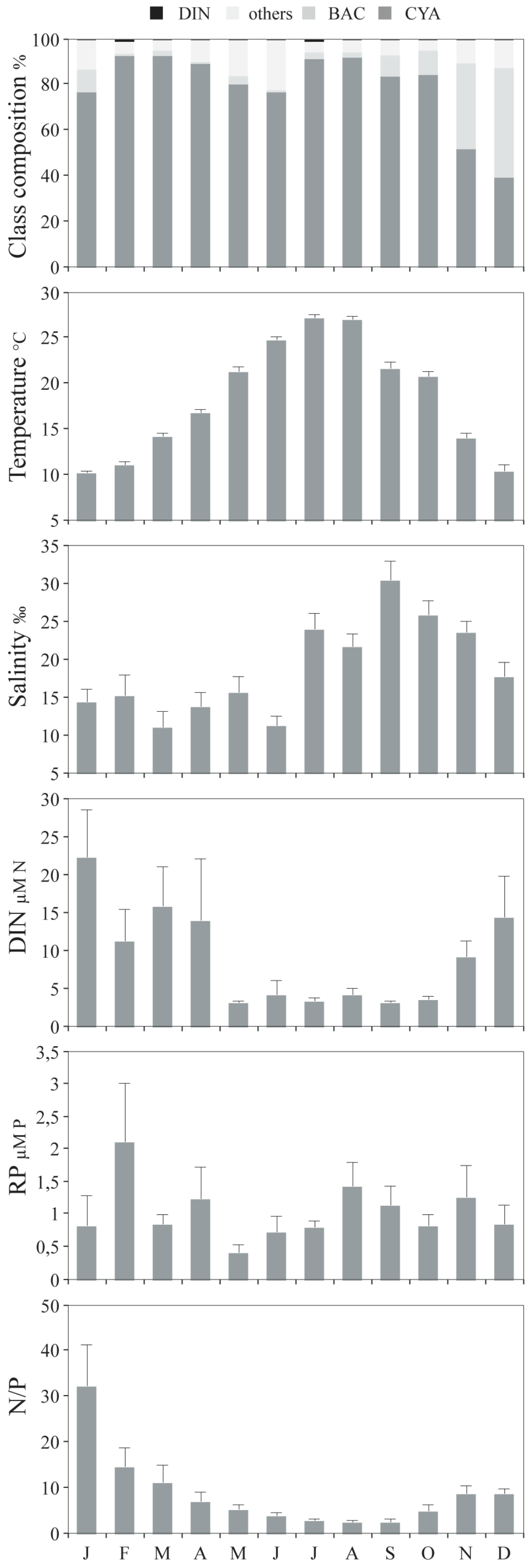

Fig. 7 Phytoplankton, environmental and nutrient data: monthly mean +S:E (N/P ratio: N = nitrates plus nitrites plus ammonia; P = orthophosphates).

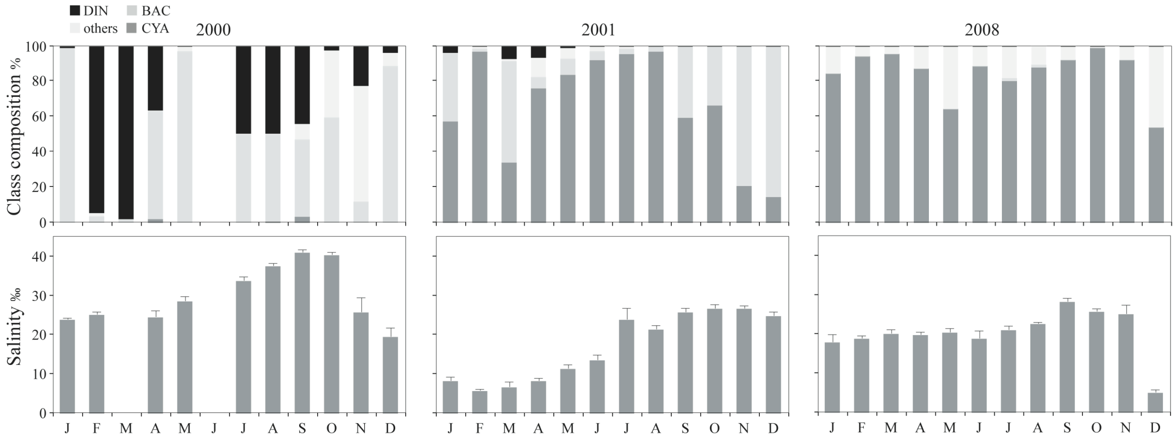

Fig. 8 Interannual variation of phytoplankton data and salinity.

4. Discussion

In the Mediterranean basin a number of studies were carried out on cyst assemblage composition in coastal marine areas (Montresor et al., 1998; Rubino et al., 2000, 2002; Giannakourou et al., 2005) and enclosed human impacted sites (Satta et al., 2009). More specific investigations were conducted on selected species (Montresor et al., 1993, 1994; Meier and Willems, 2003; Montresor et al., 2003) or harmful ones (Garcés et al., 2004; Bravo et al., 2006; Angles et al., 2009). None of these works investigated on peculiar areas such as coastal lagoons. These kinds of ecosystems are very sensible and characterized by high variability of environmental variables, particularly due to their connecting position between the continental and the marine systems (Boutière, 1974). Besides, in the last decades lagoons have suffered strong direct or indirect human impacts. Intense fishery activities as well as the over enrichment of nutrients linked to agriculture, urban and industry water waste or the modification of natural connection with the adjacent sea, are some of the factors that influence the ecological functions of these areas. Artificial structures and barriers impair the interaction with the adjacent marine waters, affecting the trophic status and function of these ecosystems worldwide (Vicente and Miracle, 1992; de Cunha and Wasserman, 2003; Pastres et al., 2004; Como et al., 2007). Moreover, some recent studies on the relationship between sedimentary records of dinoflagellate cysts and historical evolution of anthropogenic activities over the past 450 years, revealed that after a notable increase in species richness with the early urbanization and population growth, the number of cyst morphotypes reduced drastically in response to higher human pressures (Pospelova et al., 2002). The Cabras Lagoon is characterized by a high trophic level due to the intense human activities in its watershed (Sechi et al., 2006). It has also suffered in recent years, for some anthropogenic hydraulic changes. The most important was the opening of the Scolmatore canal (1970) with the consequent construction of a W-shaped dam, to prevent the periodical floods of neighbouring villages. De Falco et al. (2004) and Magni et al. (2008) hypothesized a reduction of the hydrodynamic energy of the lagoon as a consequence of this human intervention. They observed high percentages of fine sediments (particularly sortable silt fraction) and the increment in organic matter and total organic carbon. Our studies revealed notable variations across the years especially in phytoplankton class composition and salinity values. Dinoflagellates and Diatoms decreased drastically in favour of Cyanophyceae, as expected in strongly degraded ecosystems (Scheffer et al., 1997; Chomérat et al., 2007). Pluriannual salinity varied substantially through the year cycle with lower values (<15‰) between January and June and an intense increase in summer months, with the maximum in September. Interannual fluctuations revealed high ranges of variations in maxima and minima values that could have strongly affected the dinoflagellates because of only few species tolerate salinity below 20‰ (Dale, 1996). The scarce presence of dinoflagellates in the water column of the Cabras Lagoon agreed with the low cyst density values and number of cyst morphotypes. These results are consistent with those of southern New England and Malaysia lagoons (Pospelova et al., 2004; Furio et al., 2006) and some oligohaline environments (Dale, 1996; Ellegaard, 2000; Mudie et al., 2001).

In spite of the low number of morphotypes recorded, our results led to an increase on phytoplankton biodiversity information, at least as regarding dinoflagellate cyst producers, confirming the important use of benthic studies as a tool to increase this kind of information (Orlova et al., 2004; Bravo et al., 2006; Zingone et al., 2006; Satta et al., 2009). *Scrippsiella trochoidea* (Stein) Balech ex Loeblich, *Polykrikos schwartzii* Bütschli, *G. spinifera, P.* cf *daleii*, *S. precaria* Montresor et Zingone and the genus *Fragilidinium* were never signalled in pluriannual water samples. Difficulties on taxonomic placement without an exhaustive analysis of thecal plates are common for Peridiniales species (Nehring 1997; Orlova et al., 2004). In some cases it is not sufficient, because different species have the same plate tabulation but differ for cyst morphology and for the results of genetic analyses (Montresor et al., 2003; Gottschling et al., 2005; Gu et al., 2008). The *Pentapharsodinium* sp. morphotype, widespread distributed in the Cabras Lagoon, is similar to the cyst of *P. daleii* but lacks the organic processes. The small-germinated cells seem to have 5 cingular plates (as *Pentapharsodinium* genus) and the same plate tabulation of *P. daleii*, but they are much more dorso-ventrally compressed. For this, further studies are necessary for the correct taxonomic recognition of this species.

It is assumed that resting stages are influenced in their sedimentation by all the hydrodynamic factors that influenced fine sedimentary particles (Dale, 1976). The sediment grain size in the lagoon is well described, and the highest percentages are represented by the silt and clay fractions (De Falco et al., 2004; Magni et al., 2008). In the Cabras Lagoon cyst abundances showed a clear spatial pattern of distribution with maxima registered in the central and southern areas. This result agreed with the sedimentary character of the lagoon and with the internal hydrodynamic model proposed by Ferrarin and Umgiesser (2005).

This work represent the first study case on dinoflagellate cysts in a lagoon of the Mediterranean Sea and one of the few conducted in other geographical areas (Pospelova et al., 2004; Furio et al., 2006). The obtained results confirmed the importance of integrated studies between planktonic and benthic communities to the evaluation of the biodiversity of the ecosystem. Moreover it allowed confirming the role of the sediment as synthesis tool of ecological pluriannual dynamic of phytoplankton, particularly of dinoflagellates.

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5. Chapter III

The trophic condition of the Cabras Lagoon (Sardinia, Italy) in relation to nutrients and phytoplankton.

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The trophic condition of the Cabras Lagoon (Sardinia, Italy) in relation to nutrients and phytoplankton.

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ABSTRACT

Temporal variations in environmental parameters and phytoplankton were studied in the Cabras Lagoon (Sardinia, Italy) in order to evaluate the trophic state of the system after a major dystrophic event. Samples were collected at five stations from October 2000 to September 2002. The analysis found wide variations in salinity and high nutrient availability, especially for phosphorus compounds, in summer; in winter and spring, increased concentrations of dissolved inorganic nitrogen were observed, due to the watershed discharge.

Chlorophyll *a* concentrations and phytoplankton densities were very high and positively correlated with seasonal nutrient peaks. In particular, phytoplankton diversity was reduced, with the community being mainly dominated by Cyanophyceae of reduced cell size with *Cyanobium*-type cells. During the study period, some harmful algal species were observed: *Prorocentrum minimum*, *Dinophisys acuminata, Heterocapsa rotundata* and *Alexandrium* and *Chattonella* species.

Several trophic state indices and water quality indicators were applied; an overall analysis confirms the severely eutrophic character of the lagoon.

Keywords: trophic state, phytoplankton, eutrophication, coastal lagoon, Sardinia, Mediterranean Sea.

1. Introduction

Coastal lagoons and estuaries play an important role in filtering and transforming inorganic and organic materials (Conley, 2000); these areas are characterized by high primary productivity.

They are located in the part of the coastline where the effects of human activities, such as pollution, eutrophication and physical disturbances, have the most direct negative impact (Gazeau et al. 2004; Simpson and Rippeth, 1998).

Because algal growth rates are often naturally limited by the availability of nutrients, particularly nitrogen and phosphorus, the fertilization of estuarine-coastal waters stimulates the growth, biomass accumulation, and primary production of phytoplankton (Cloern, 2001). In recent decades, increased anthropogenic inputs of these elements have led to severe eutrophication problems, inducing higher phytoplankton primary production in many coastal areas (Cloern, 2001; Glè et al. 2007).

In such systems, nutrient dynamics are also greatly influenced by climatic variability (Glè et al., 2007). Climate stability is also believed to play a role in determining phytoplankton succession patterns in temperate coastal waters (Reynolds et al., 2002; Smayda and Reynolds, 2003).

Studies of phytoplankton abundance and structure are crucial to understanding ecosystem dynamics (Angsupanich and Rakkheaw, 1997), especially in cases where primary production is dominated by this biotic component.

The process of eutrophication also increases the frequency and intensity of phytoplankton growth, which can trigger dystrophic crises. These can, in turn, lead to significant changes in the structure and function of the affected ecosystems (Phlips et al., 2002). Eutrophication also promotes the development and persistence of many harmful algal blooms (HABs). It was recently assessed as one of the reasons behind the worldwide increase in HABs (Heisler et al., 2008).

Phytoplankton is now also seen as a powerful tool for describing trophic conditions. In fact, because of its peculiar ecology, phytoplankton is considered to be one of the most useful biotic elements for assessing the environmental quality of water bodies (Thunmark, 1945; Nygaard, 1949; Hörnström, 1981; Tremel, 1996; Brettum and Andersen, 2005). This point of view has assumed a central role in the last few years in the context of applied aquatic ecology, due in large part to the influence of European Union Directive 2000/60/CE (WFD, Water Framework Directive), which requires the use of numerical indices based on biological parameters when assessing the ecological quality of aquatic ecosystems.

Models for assessing the trophic state of lagoons (especially phytoplankton-based models) are not in widespread use as they are in other environments. Moreover, the differing trophic conditions, highly variable parameters and uncertain typological identification of lagoons make it very difficult to develop specific tools for evaluating water quality in these environments. Consequently, methods developed for freshwater environments are often applied to lagoons. These models are generally not well-suited to lagoons; results should therefore be considered arbitrary and interpreted with extreme caution.

In Mediterranean lagoons, macrophytes often surpass phytoplankton as the most important producers (Giordani et al., 2005). In these cases, the role of phytoplankton only becomes relevant in periods when macrophytes are absent. Less frequently, phytoplankton is the sole primary producer throughout the year.

Macrophytes are generally the dominant producers in lagoons in Sardinia (Italy, Mediterranean Sea). The Cabras Lagoon is one of the few exceptions (Fiocca et al., 1996; Lugliè et al., 2001a, 2001b; Sechi et al., 2001; Trebini et al., 2005). This extensive, shallow water body is among the largest lagoons in the Mediterranean and has limited macrophyte development (Sechi et al., 2006). It is affected by extremely high anthropogenic nutrient loads; consequently, eutrophication has been a chronic condition in recent decades.

Eutrophication is also believed to be responsible for decreased lagoon production, due to verified dystrophic crises, which can be caused by hypoxia and/or harmful algal blooms. The observed dystrophic crises have mostly occurred in summer months and have caused severe fish mortality (with losses of up to 1.5 million euro in 1999) in the Cabras Lagoon.

Because phytoplankton abundance, seasonality, distribution and species composition in the Cabras Lagoon had not yet been described, our objectives were:

(1) to valuate the temporal dynamics of phytoplankton with respect to environmental conditions, taking into account data collected over two years (from October 2000 to September 2001 and from October 2001 to September 2002);

(2) to assess the presence of harmful algal species and their importance within the phytoplankton community; and

(3) to compare the trophic levels obtained for the lagoon by applying various indices that consider different variables, including phytoplanktonic chlorophyll *a*.

The overall goal was to assess whether phytoplankton composition and dynamics could be a possible cause of dystrophic events similar to the one that occurred in 1999.

2. Materials and methods

2.1 Study area

The Cabras Lagoon is a shallow water body located on the west coast of Sardinia, in the Gulf of Oristano (western Mediterranean Sea; 39° 56’ 37’’ N, 08° 28’ 43’’ E; Fig. 1).

With an area of about 23.8 km^2 and a mean depth of 1.6 m (maximum depth of around 3 m), the Cabras Lagoon is the largest lagoon in Sardinia and one of the largest in the Mediterranean Sea (Sechi et al., 2006). Due to its environmental significance, it is protected by international agreements, including the Ramsar Convention (EU Directive Special Protection Area, Site of Community Importance, Natural Protected Area). It is also part of the Sinis-Montiferru Natural Reserve (Sardinia).

The Cabras Lagoon watershed extends over an area of approximately 430 km^2. Water flowing within the system is derived from natural rivers and from canals that drain the surrounding lowlands. Most of the lagoon's freshwater input originates from the Rio Mare e Foghe, which drains an area of 313 km^2. River discharge is, however, rather limited as a result of the low rainfall regime characteristic of the region (ca. 10–100 mm, from July to December) and the increasing demand for water, especially for agricultural purposes (Magni et al., 2005). In its current form, Rio Mare e Foghe is a canal created in the ‘60s after land was reclaimed from a natural wetland. This was one of numerous land reclamation projects carried out in Sardinia, starting in the ‘20s, to recover territories deemed unproductive and unhealthy. A minor tributary flows into the eastern part of the lagoon, near the town of Cabras.

In general, the basin consists of a flat zone, with a surface area of about 215 km^2 and an elevation not exceeding 60 m above sea level (a.s.l.) and a submountainous/mountainous zone, with elevations up to 1050 m a.s.l. The flat zone, located in the southern part of the watershed, is the site of intensive agricultural activity, which releases high nutrient loads. The fertile soils of the submountainous/mountainous zone (over 200 m a.s.l.) in the northern part of the watershed are occupied by vineyards, olive groves, pastures, and forests. This region is frequently plagued by large-scale fires, which threaten soil conservation. The total resident population inside the watershed is about 38,000 inhabitants, grouped in 19 urban centers. The largest of these is the town of Cabras (about 8,800 inhabitants), situated on the southeastern coast of the lagoon. The urban wastes of Cabras are collected separately and do not flow into the lagoon. However, no urban center within the catchment area has a sewage treatment system equipped for phosphorus and

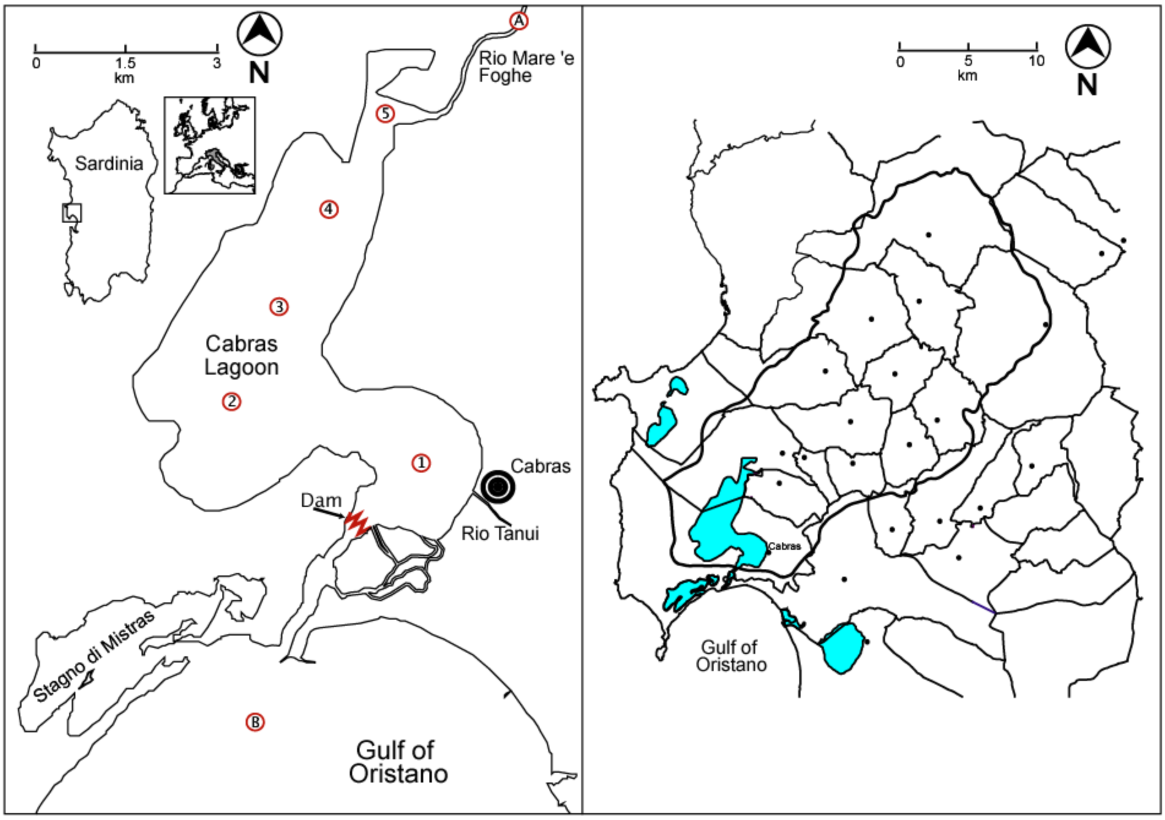

Fig. 1. Location of the Cabras Lagoon and of the sampling stations is shown in the left panel; the bold line in the right panel delimits the Cabras Lagoon watershed and adjacent urban areas.

nitrogen removal. Accordingly, based on estimates of agricultural and non-agricultural nutrient loads from the watershed, an input of 29 t phosphorus year^{-1} (Sechi et al., 2006) and 240 t nitrogen year^{-1} has been calculated (Casula et al., 1999).

The lagoon is mainly connected to the adjacent Gulf of Oristano through four narrow creeks that flow into the large, southernmost canal, the *Scolmatore* (=spillway), which was dredged in the late ‘70s. The canal was constructed to drain excess water during the heavy winter rainfalls, which regularly submerged a district of the nearby town of Cabras, called, appropriately, “Little Venice.” The *Scolmatore* was subsequently modified to include a 30-cm-high dam, built to prevent further increases in the salinity of the lagoon by stopping the inflow of seawater. In addition, artificial barriers have been constructed to control the fish catch.

The lagoon’s sediments are dominated by silt in the southern area, while clay content increases towards the central-northern section. The surface sediments contain large amounts of organic matter (10%) and total organic carbon (33 mg g^{-1}) (De Falco et al., 2004). The benthic environment of the lagoon is very poor in macrofaunal communities, with a few predominating taxa that typically occur in degraded and heavily disturbed sites (Magni et al., 2004).

Salinity in the lagoon follows a net temporal and spatial gradient, increasing from about 8–10‰ during the winter up to 30‰ in summer, with the difference (Δ) between the northern (near the main tributary) and southern (near the mouth of the lagoon) sectors normally being about 4‰ (Sechi et al., 2006). The long-term mean values for the lagoon are about 77 mg N m^{-3} for dissolved inorganic nitrogen and 42 mg P m^{-3} for dissolved inorganic phosphorus. Dissolved oxygen fluctuates greatly over time, ranging from under- to over-saturation and having a mean value of 107% (Sechi et al., 2006).

The lagoon has a high economic rating due to its numerous fisheries (e.g., *Liza ramado*, *Mugil cephalus*), which employ about 300 people and their families, and to fishing-related activities.

In 1998, fish productivity reached 40,000 kg km^{-2}, corresponding to a profit of about 3.5 million euros (Magni et al. 2005). However, dystrophic events often cause massive fish mortality (the last such event occurred in the summer of 1999).

2.2 Sampling strategy

Field work was carried out between October 2000 and September 2002. Samples were collected at approximately two-week intervals from five stations along a gradient of distance from the sea mouth to the main freshwater tributary (Fig. 1).

Temperature (Tem), salinity (Sal), dissolved oxygen (DO), pH and fluorimetric chlorophyll *a* (fChl *a*) were measured *in situ* with a multi-parameter probe (Idronaut, YSI 6600 V2).

A bucket was used to collect water samples from the superficial water layer (50 cm depth) and preserved in cold, dark conditions for laboratory analyses of alkalinity (Alk), ammonia (NH_3), nitrite (NO_2), nitrate (NO_3), reactive silica (RSi), orthophosphate (RP) and total phosphorus (TP), following Strickland and Parsons (1972), and for Chl *a* (Golterman et al., 1978). Dissolved inorganic nitrogen (D.I.N.) was obtained as the sum of NH_3, NO_2 and NO_3. Data for rainfall and solar radiation were provided by the Sistema Informativo Agricolo Nazionale (SIAN) and refer to the meteorological station of Santa Lucia, in the vicinity of the lagoon.

Phytoplankton samples were fixed with Lugol's solution and analysed according to the Utermöhl technique, using an inverted microscope (Zeiss, Axiovert 25) after the sedimentation of variable volumes of water (5-10 cc), depending on phytoplankton density. Cell counts were made at 100X on the entire bottom of the sedimentation chamber for the larger and more easily identifiable species, and replicated at 200X and 400X on an adequate number of fields for the smaller cells. Species were determined following Balech (1995), Faust and Gulledge (2002), Germain (1981), Humm and Wicks (1980), Husted (1985), Komárek and Anagnostidis (1998), Komárek (2005), Rampi and Bernard (1978, 1980, 1981), Ricard (1987), Sournia (1986) and Tomas (1997).

For difficult-to-recognize species, the identification was checked using a scanning electron microscope (Zeiss 962 DSM), after the appropriate treatments.

2.3 Data treatment

The Shannon-Wienner diversity index (Weaver and Shannon, 1949) and Evenness index (Pielou, 1966) were calculated for each sampling date and each station to evidence temporal and spatial variations in the richness and relative abundance of phytoplankton.

Statistical non-metric multidimensional scaling (nMDS) analyses of abiotic and biotic variables were carried out to assess spatial and temporal differences on monthly and seasonal scales.

Similarity matrixes were obtained using monthly and seasonal means at the five sampling stations.

The dataset of abiotic parameters was composed of 13 environmental variables (rainfall, solar radiation, temperature, pH, alkalinity, DO, salinity, NH_3, NO_2, NO_3, TP, RP, RSi) and similarity matrixes were based on the normalized Euclidean distance.

The dataset of biotic parameters was composed of 11 variables (Chl *a*, 9 phytoplankton classes and ultraplankton, a dimensional group with cell size <5µm) and similarity matrixes were based on the Bray-Curtis similarity index.

The significance of spatial and temporal differences was assessed using a two-way Analysis of Similarities (ANOSIM) test (probability percentages lower than 3% were considered significant).

A canonical correspondence analysis (CCA) was performed (Ter Braak, 1986) to quantify the influence of environmental variables (temperature, pH, alkalinity, DO, salinity, NH_3, NO_2, NO_3, D.I.N., N/P (D.I.N./RP), TP, RP, RSi) on phytoplankton (Chl *a*, 9 phytoplankton classes and ultraplankton), while considering class abundances.

All canonical axes were used to assess the significant variables through analyses by means of a Monte Carlo test (1000 permutations).

The data used to construct the environmental matrix were square-root transformed. The phytoplankton matrix was obtained via a $log(x+1)$ transformation of the total densities of algal classes (including ultraplankton).

Statistical analyses were performed using PRIMER (for the ANOSIM test and nMDS) and MVSP (for diversity indices and CCA) software.

Relationships among the considered environmental variables and fitoplancton classes abundance were tested with the Pearsons correlation.

To obtain a better description of the trophic status, the TRIX (Vollenweider et al., 1998) and TSI indices (Carlson, 1977) were calculated.

The latter index, developed for freshwater environments, is based on Chl *a*, Secchi disk depth and TP, and takes into account the values for each sample. It ranges from 0 to 100. Values between 0 and 20 are considered typical of ultra-oligotrophy, between 20 and 40 of oligotrophy, between 40 and 50 of mesotrophy, between 50 and 70 of eutrophy and over 70 of hyper-eutrophy. During this investigation, since Secchi disk depth data were not collected in Cabras Lagoon, TSI was calculated as TSI (CHL) and TSI (TP), using the following formulae, respectively:

(1) $\mathrm{TSI\ (CHL)} = 9.81 \ln (\mathrm{Chl}\ a) + 30.6$ (2) $\mathrm{TSI\ (TP)} = 14.42 \ln (\mathrm{TP}) + 4.15$

The TRIX index is proposed for coastal waters and ranges from 0 to 10, from oligotrophy to eutrophy. It considers four variables: Chl *a*, TP, D.I.N. and DO, as absolute deviation from saturation.

The index was calculated using the following formula:

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

3. Results

3.1 Chemical/physical parameters and nutrients

Table 1 reports the average values of variables analysed throughout the lagoon during the entire study period. Figure 2 displays relative temporal and spatial variations in the studied parameters.

Rainfall patterns (Table 1, Fig. 2a) differed in the two years of the study, with a distinct wet fall and dry summer in the first, and a more homogeneous dynamic all year round in the second. Major differences were detected between the two annual cycles as regards rainfall abundance, with mean monthly cumulated rainfall of 55.3 mm in the first and 33.4 mm in the second. The rainiest month was November in both years, with a cumulative rainfall of 164 mm in 2000 and 85.8 mm in 2001.

The dynamics of monthly means of daily solar radiation (Table 1, Fig. 2b) showed only very slight differences between the two annual cycles. A regular increasing trend, from lower winter values (under 5000 KJ m^{-2} in December) to higher summer values (over 21000 KJ m^{-2} in July), was detected in both years. The mean values were 12,725.9 KJ m^{-2} for the first cycle and 12,980.3 KJ m^{-2} for the second.

Temperature and salinity displayed seasonal dynamics, characterised by an increasing trend from winter to summer and a decrease from summer to winter. The temperature (Fig. 2c) reached a maximum of 31°C in August 2001 at ST2 and a minimum of 6°C in December 2001 at ST1. In general, no significant differences were observed among stations at each sampling date. The mean salinity of the lagoon (Fig. 2d) exhibited an evident spatial gradient from ST5 to ST1 (Δ of about 4‰ in terms of annual averages) depending on their relative position with respect to freshwater inputs and seawater exchanges. The greatest seasonal variations occurred in the first annual cycle, with a rapid

Table 1. Mean values and standard deviation of environmental variables and Chl *a* in the Cabras Lagoon.

		ST1	ST2	ST3	ST4	ST5	Mean
Temp.	°C	18.5 ± 6.6	18.4 ± 6.6	18.4 ± 6.5	18.7 ± 6.3	18.4 ± 6.5	18.5 ± 6.3
Sal.	‰	21.2 ± 8.5	20.4 ± 8.8	20.1 ± 8.6	18 ± 7.8	16.6 ± 8	19.3 ± 9.1
pH		8.7 ± 0.4	8.8 ± 0.4	8.8 ± 0.4	8.7 ± 0.4	8.7 ± 0.4	8.7 ± 0.4
DO	%	119.5 ± 31.7	116.4 ± 31.4	115.8 ± 29.9	118.3 ± 28.5	116.9 ± 32.4	117.4 ± 30.1
Alk	meq l^{-1}	2.7 ± 0.4	2.5 ± 0.6	2.6 ± 0.4	2.7 ± 0.4	2.7 ± 0.3	2.6 ± 0.4
RP	mg P m^{-3}	27 ± 28.6	32.6 ± 41.3	34.9 ± 52.8	38.7 ± 41.9	40.5 ± 49.9	34.7 ± 43
TP	mg P m^{-3}	276.3 ± 121.9	278 ± 124.3	285.6 ± 128.4	319.2 ± 133	329.3 ± 156.2	297.7 ± 131.9
D.I.N.	mg N m^{-3}	99.2 ± 119.4	103.5 ± 126.9	88.8 ± 113.3	140.5 ± 179	134.4 ± 191.5	113.3 ± 146.9
NH_3	mg N m^{-3}	54.4 ± 38	53.6 ± 34.9	53 ± 31.3	56.5 ± 35	57.8 ± 39.4	55.1 ± 34.9
NO_3	mg N m^{-3}	35.5 ± 86.9	43.4 ± 110.1	29.5 ± 95.7	74.7 ± 154	67.5 ± 174.8	50.1 ± 126.6
NO_2	mg N m^{-3}	9.3 ± 13	6.5 ± 8.9	6.3 ± 9.1	9.2 ± 11.6	9.1 ± 11.9	8.1 ± 10.9
RSi	mg Si l^{-1}	6.2 ± 2.9	6.4 ± 2.9	6.8 ± 2.9	7.1 ± 3.2	7.4 ± 3.3	6.8 ± 3.1
Chl *a*	mg m^{-3}	39.3 ± 32.4	37.2 ± 33.2	40.5 ± 30.3	45.7 ± 30.8	48.9 ± 29.7	42 ± 30.9

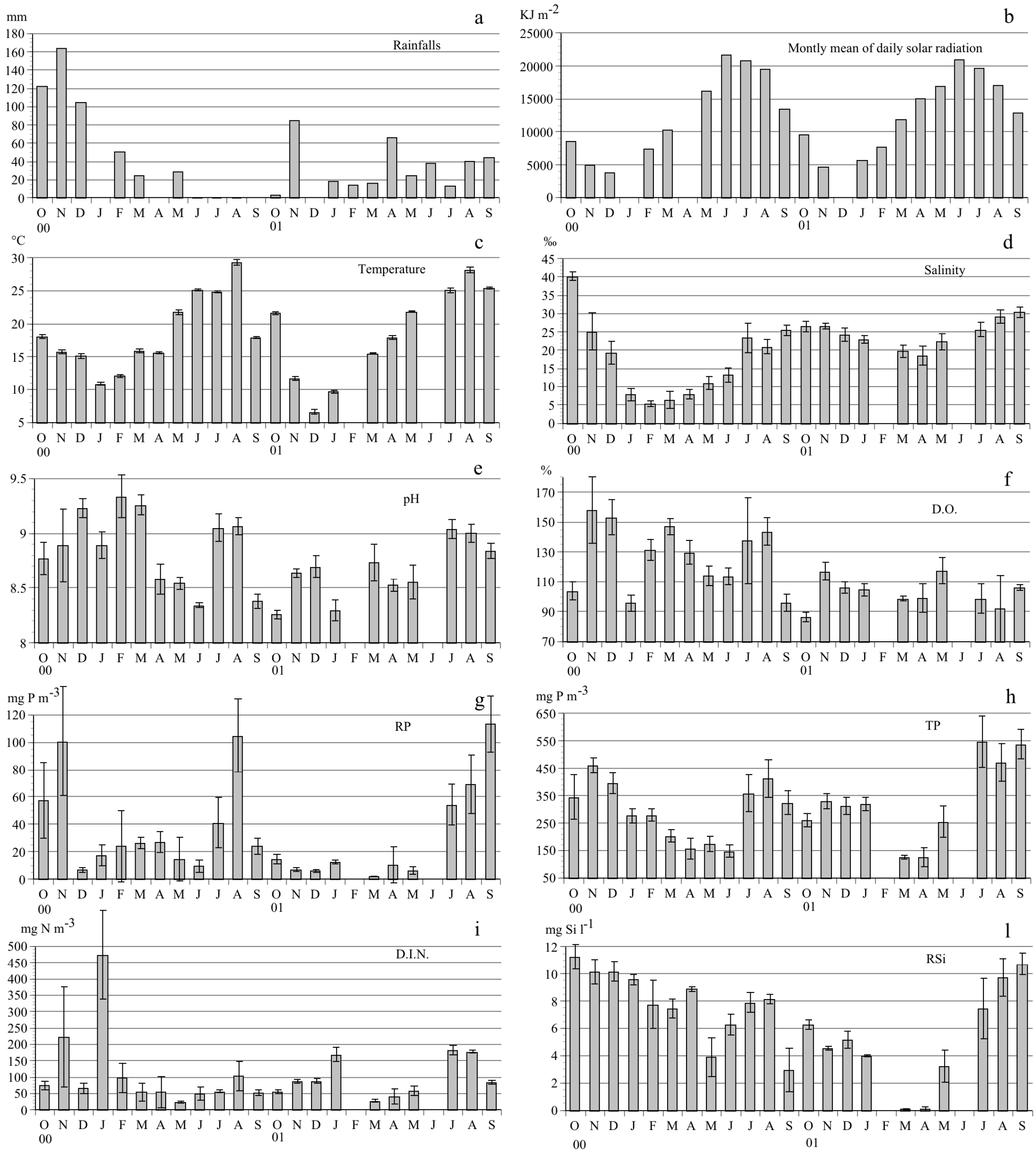

Fig. 2. Trend of abiotic parameters in the Cabras Lagoon: (a) rainfall, (b) monthly mean of daily solar radiation, (c) temperature, (d) pH, (e) salinity, (f) dissolved oxygen, (g) reactive phosphorus, (h) total phosphorus, (i) D.I.N., (l) reactive silica.

decrease from the absolute maximum (41.3‰) to the minimum (2.7‰) in the first semester, followed by an increase to about 25‰ in the second. The second annual cycle was characterised by a more restricted range of values (from 30.4‰ to 18.6‰). Consequently, the two annual mean values were clearly different, 17.2‰ and 24.6‰ respectively.

The pH (Table 1, Fig. 2e) and DO (Table 1, Fig. 2f) varied widely during the study period, but with no well-defined trends. Lower mean values were observed for both variables in the second year. Very high mean values were recorded at the beginning of the investigation, with maxima in February 2001 (monthly mean of 9.3; maximum of 9.55 at ST2) and in November 2000 (monthly mean of 158.1%; maximum of 227% at ST5), respectively. The lowest mean monthly values occurred at the beginning of the second annual cycle (October 2001); these were of 8.3 for pH (minimum of 7.81 at ST5) and 86.5% for DO. As regards individual stations, the minimum value of DO was observed at ST5 in August 2002 (54%). Differences from surface to bottom were not significant, with percentages near the bottom always >50%.

When it comes to nutrients, wide spatial and temporal variations were observed. RP (Table 1, Fig. 2g) values were fluctuant and remained under 60 mg P m^{-3} during nearly all of the study period. Concentrations were particularly low (less than 20-30 mg P m^{-3}) from October to July 2002. Peaks were observed in November 2000 (maximum of 254 mg P m^{-3} at ST3), August 2001 (maximum of 180 mg P m^{-3} at ST5) and July-September 2002 (maximum of 144 mg P m^{-3} at ST5).

TP (Table 1, Fig. 2h) showed a similar trend as regards maxima (533 mg P m^{-3} at ST5 in November 2000; over 600 mg P m^{-3} at ST4 in August 2001; 701 mg P m^{-3} at ST5 in July-September 2002).

RP represented about 10% of average TP values in the lagoon throughout the study period. It was higher during summer months (up to 25% in August 2001 and 20% in August-September 2002) and lower in winter (from 2% to 4% between October and March 2002).

In general, D.I.N. (Table 1, Fig. 2i) was lower than 100 mg N m^{-3}. Peaks were frequent throughout the study period, occurring more frequently in winter (monthly mean of 473.7 mg N m^{-3} in January 2001, with a maximum of 954 mg N m^{-3} at ST5) and late summer. The minimum value was of 13 mg N m^{-3}, registered at ST5 in April 2001 (monthly mean of 54.2 mg N m^{-3}). The mean value for the first year was 110.9 mg N m^{-3} and 97.3 mg N m^{-3} for the second. During the annual cycles, ammonia resulted as the main available component, representing about 50% of D.I.N.. A prevalence of nitrate was only observed in January 2001.

RSi dynamics (Table 1, Fig. 2l) displayed two different phases: a general progressive decrease from October 2000 (monthly mean of 11.3 mg Si l^{-1}; maximum of 13.07 mg Si l^{-1} at ST4) to March 2002 (monthly mean approximately equal to 0 mg Si l^{-1}; minimum of 0.3 mg Si l^{-1} at ST4) and a net increase from May to September 2002 (monthly mean of 10.7 mg Si l^{-1}; maximum of 11.91 mg Si l^{-1} at ST5). The mean value for the first year was 7.9 mg Si l^{-1} and 5.1 mg Si l^{-1} for the second.

An nMDS of the entire set of environmental parameters (Fig. 3a) showed significant differences among seasons (ANOSIM R=0.301 and p=0.1%). Differences among sampling stations (Fig. 3b) were not significant (ANOSIM R=-0.15 and p =98%).

3.2 Phytoplankton assemblages and chlorophyll a

Chl *a* always expressed mean lagoon concentrations higher than 10 mg m^{-3} (Table 1, Fig. 4a), varying from a monthly mean of 13.6 mg m^{-3} in May 2001 (minimum of 7.7 mg m^{-3} at ST2) to 96.2 mg m^{-3} in January 2001 (maximum of 163.7 mg m^{-3} at ST2). In both annual cycles, the seasonal dynamic showed very high mean values in the fall-winter seasons, which fell in spring and rose again in summer. The lagoon mean was 43.2 mg m^{-3} for the first year and 44 mg m^{-3} for the second.

Phytoplankton density was generally greater than 10^8 cells l^{-1} (Fig. 4b), with an annual mean of 6 x 10^8 cells l^{-1} for the first cycle and 5.9 x 10^9 cells l^{-1} for the second. Lagoon mean values increased, with wide fluctuations, from the beginning of the study until the end, varying from 5.5 x 10^7 cells l^{-1} in April 2001 (minimum of 1.1 x 10^7 cells l^{-1} at ST2) to 1.5 x 10^{10} cells l^{-1} in August 2002 (maximum of 2.2 x 10^{10} cells l^{-1} at ST5). In both annual cycles, the highest abundances were observed in winter and summer, while lower values were observed in spring.

Total phytoplankton abundance was highest at ST5 (located near the main tributary), with a mean value of 3 x 10^9 cells l^{-1} for the entire study period, and lowest at ST1 (located near the exchange with the sea), with a mean value of 2.2 x 10^9 cells l^{-1}.

48 phytoplankton taxa belonging to 9 classes were identified during the study period (Table 2), with Bacillariophyceae (15), Dinophyceae (14), Chlorophyceae (7) and Cyanophyceae (5) being more prevalent than the other classes. Unidentified cells of size <5µm were considered in the dimensional group of Ultraplankton. Most of the observed

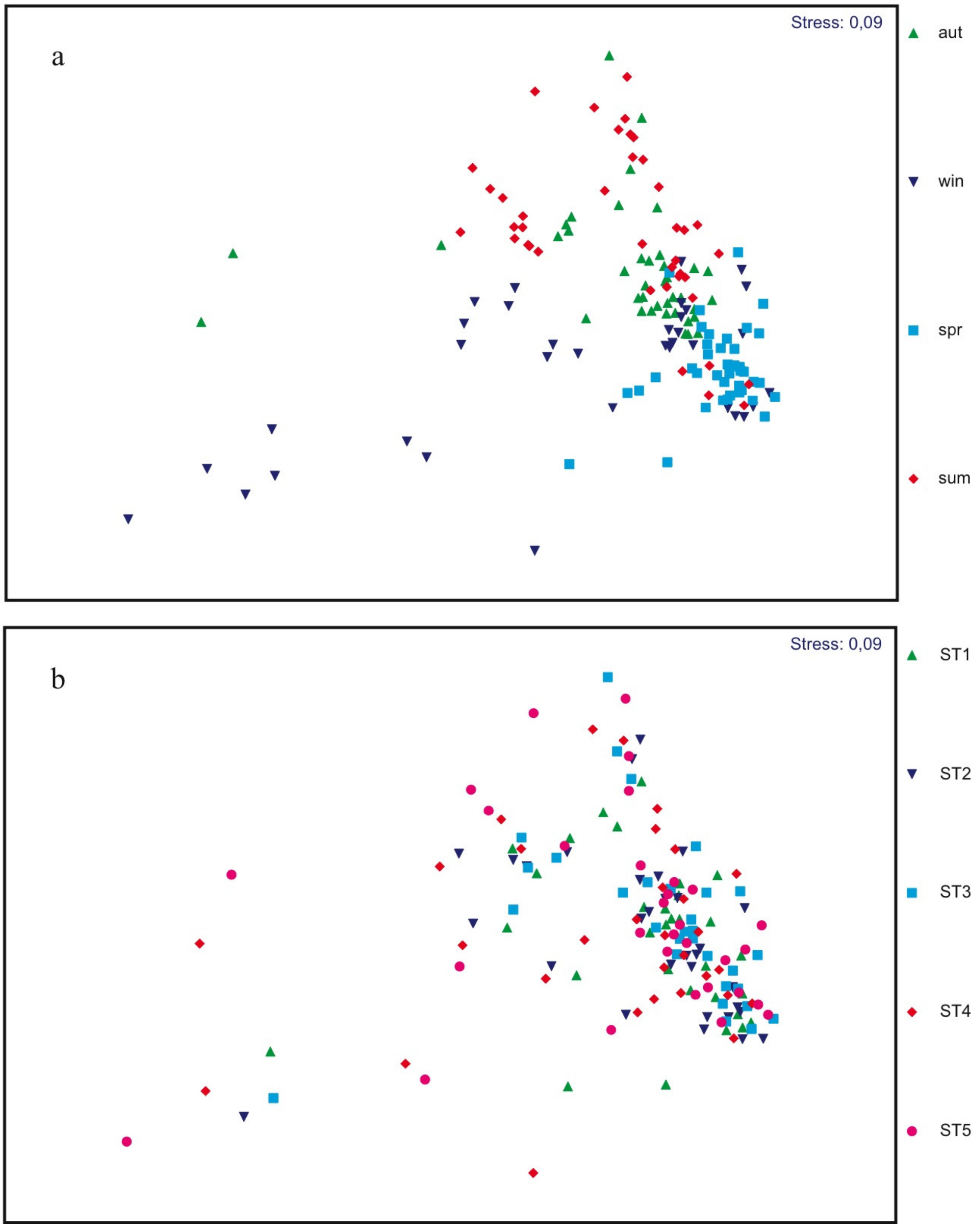

Fig. 3. nMDS of abiotic parameters in the Cabras Lagoon: (a) differences among seasons, (b) differences among sampling stations.

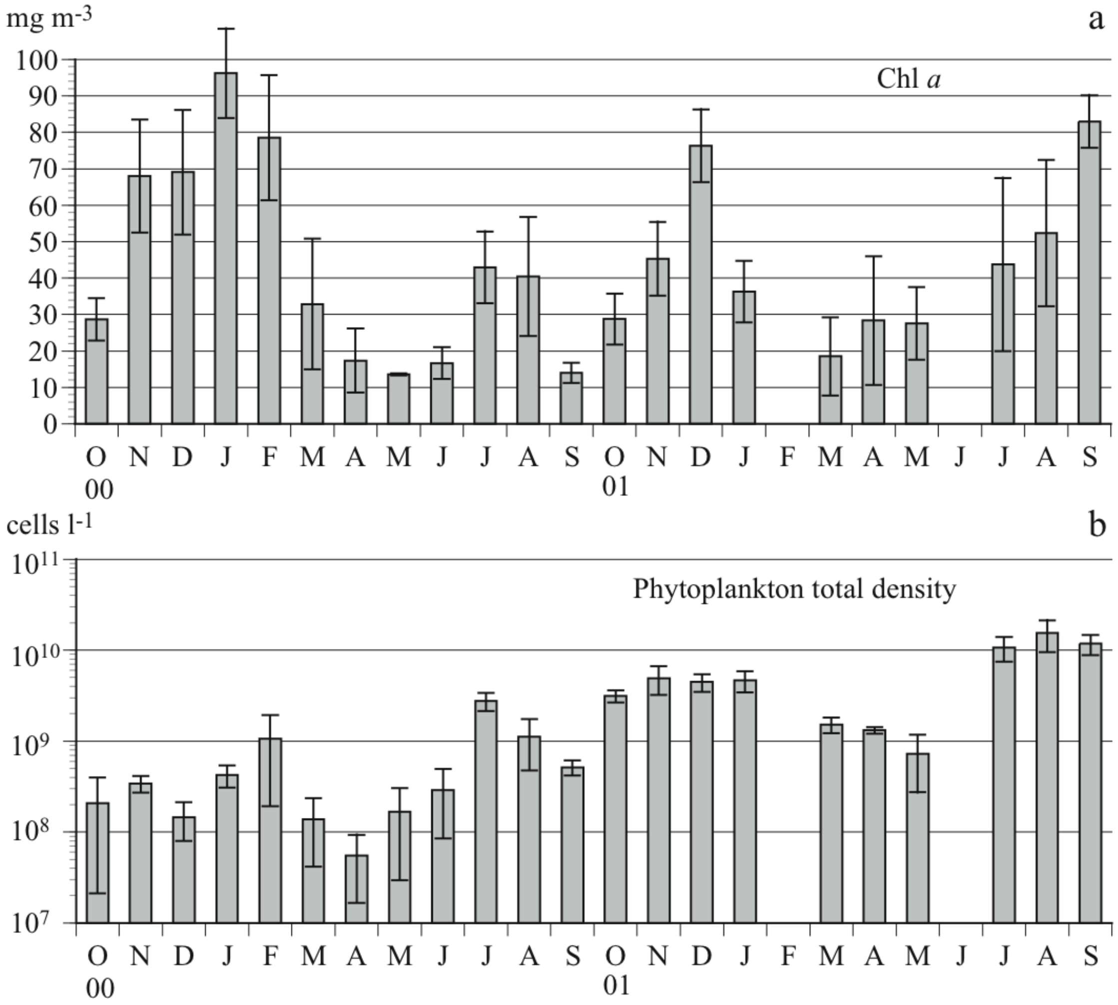

Fig. 4. Trend of chlorophyll *a* (a) and total phytoplankton density (b) in the Cabras Lagoon.

Table 2. List of phytoplankton species in the Cabras Lagoon, their relative presence at each sampling station and HAS. 1, 4 = Faust and Gulledge, 2002; 2 = Andersen et al., 1996; 3 = Verity, 2009; 5 = Hallegraeff, 2003.

Prokaryotes	**Species**	**ST1**	**ST2**	**ST3**	**ST4**	**ST5**	**HAS**
CYANOPHYCEAE	Other Cyanophyceae	x	x	x	x	x	-
	Anabaena sp.		x	x	x	x	-
	Anabaenopsis sp.	x	x				-
	Cyanobium-type cells	x	x	x	x	x	-
	Limnothrix sp.	x	x	x	x	x	-
	Rhabdoderma sp.	x	x	x	x	x	-
Eukaryotes							
BACILLARIOPHYCEAE	Other Bacillariophyceae	x	x	x	x	x	-
	Amphora sp.					x	-
	Aulacoseira distans			x			-
	Aulacoseira granulata					x	-
	Chaetoceros spp.			x	x	x	-
	Climacosphenia spp.					x	-
	Cocconeis sp.			x			-
	Cyclotella atomus	x	x	x	x	x	-
	Cylindrotheca closterium	x	x	x	x	x	-
	Fragilaria sp.	x	x	x	x	x	-
	Licmophora sp.				x		-
	Minidiscus sp.	x	x	x	x	x	-
	Navicula spp.	x	x	x	x	x	-
	Nitzschia sp.	x	x	x	x	x	-
	Skeletonema costatum	x	x	x	x	x	-
	Skeletonema potamos			x	x	x	-
	Thalassiosira sp.	x	x	x	x	x	-
CHLOROPHYCEAE	Other Chlorophyceae			x			-
	Carteria sp.	x		x	x	x	-
	Chlamydomonas sp.	x	x	x	x	x	-
	Chlorella sp.	x	x	x	x	x	-
	Monoraphydium sp.	x	x	x	x	x	-
	Oocystis sp.	x					-
	Pediastrum sp.		x				-
	Scenedesmus spp.	x	x	x	x	x	-
CHRYSOPHYCEAE	*Kephyrion* sp.				x	x	-
	Kephyrion spirale	x	x	x	x	x	-
	Ochromonas sp.	x	x	x	x	x	-
CRYPTOPHYCEAE	Other Cryptophyceae	x	x	x	x	x	-
DINOPHYCEAE	Other Dinophyceae	x	x	x	x	x	-
	Alexandrium sp.	x					1
	Dinophysis acuminata	x		x	x	x	2
	Gymnodinium sp.	x	x	x	x	x	-
	Gyrodinium sp.	x	x	x	x	x	-
	Heterocapsa rotundata	x	x				3
	Oxyphysis oxytoxoides	x	x	x	x	x	-
	Oxyrrhis marina	x	x	x	x	x	-
	Oxytoxum sp.	x			x		-
	Peridinium sp.	x	x	x	x	x	-
	Pronoctiluca sp.		x				-
	Prorocentrum micans	x	x	x	x	x	-
	Prorocentrum minimum	x	x	x	x	x	4
	Protoperidinium spp.	x	x			x	-
	Scrippsiella sp.		x	x	x		-
EUGLENOPHYCEAE	Other Euglenophyceae	x	x	x	x	x	-
	Euglena sp.	x	x	x	x	x	-
	Eutreptiella sp.	x	x				-
HAPTOPHYCEAE	Other Haptophyceae				x		-
	Calyptrosphaera sp.	x			x		-
PRASINOPHYCEAE	*Pyramimonas* sp.				x	x	-
	Tetraselmis sp.	x	x	x	x	x	-
RAPHIDOPHYCEAE	*Chattonella* sp.	x					5
Ultraplankton	Ultraplankton	x	x	x	x	x	-
Incertae sedis	*Ebria tripartita*					x	-

species were found at similar abundances in all the stations, and only a few species had localized distributions.

Cyanophyceae were the most significant class in presence and density. They dominated from late spring to early fall at all the stations, representing more than 90% of total mean abundance in both years (Fig. 5). Ultraplankton was dominant in various periods at all the stations (over 90% of total mean abundance in October-December 2000, January 2001, April 2001, October 2001-January 2002 and April 2002), showing a preference for fall-winter months. Bacillariophyceae showed a less regular seasonal pattern, prevailing in the spring months of 2001 (about 60% of total mean abundance), in summer 2001 (about 20%) and in the fall-winter period between 2001 and 2002 (about 30%). In individual samplings at all stations, all the other classes expressed percentages lower than 20% (Fig. 5).

In nearly all the samples, an intense presence of Cyanophyceae and Bacillariophyceae species of very small cell size (the smallest size lower than 2 µm) was observed, which made the analysis very difficult. Among Cyanophyceae, *Cyanobium*-type cells contributed to the highest abundances of 2001, with a density higher than 10^9 cells l^{-1}. However, the highest densities observed in July 2002 were attributable to *Rhabdoderma* sp., another representative of the Cyanophyceae (up to 21.5 x 10^9 cells l^{-1} at ST5, in August 2002). Bacillariophyceae were principally represented by a small species in the genus *Fragilaria* and *Cyclotella atomus* Hust. Their highest abundances occurred from July 2001 to January 2002, up to 2.5 x 10^9 cells l^{-1} at ST4 for the former and up to 2.7 x 10^7 cells l^{-1} at ST3 for the latter.

An nMDS (Fig. 6a) of the monthly average distributions of phytoplankton classes revealed significant differences between seasons (ANOSIM R= 0.335 and p= 0.1%). No significant differences (Fig.6b) were observed between stations (ANOSIM R= -0.02 and p= 98.5%).

3.3 Harmful Algal Species.

A total of 5 potential harmful species were observed during the study period: 4 Dinophyceae and 1 Raphidophyceae (Table 2). *Prorocentrum minimum* (Pavillard) was frequently detected at all the stations, and reached densities higher than 2 x 10^7 cells l^{-1} in spring 2001. *Alexandrium* sp. was detected on only one occasion, in summer 2001 at ST1, at a density of 2 x 10^5 cells l^{-1}. *Dinophysis acuminata* Claparéde and Lachmann was observed in the spring periods of both annual cycles, at a maximum density of 1 x 10^3 cells l^{-1} (at ST4, May 2002). *Heterocapsa rotundata* (Lohmann) Hansen was observed in the spring-summer period of 2001, reaching a maximum of 2 x 10^5 cells l^{-1} at ST1. *Chattonella*

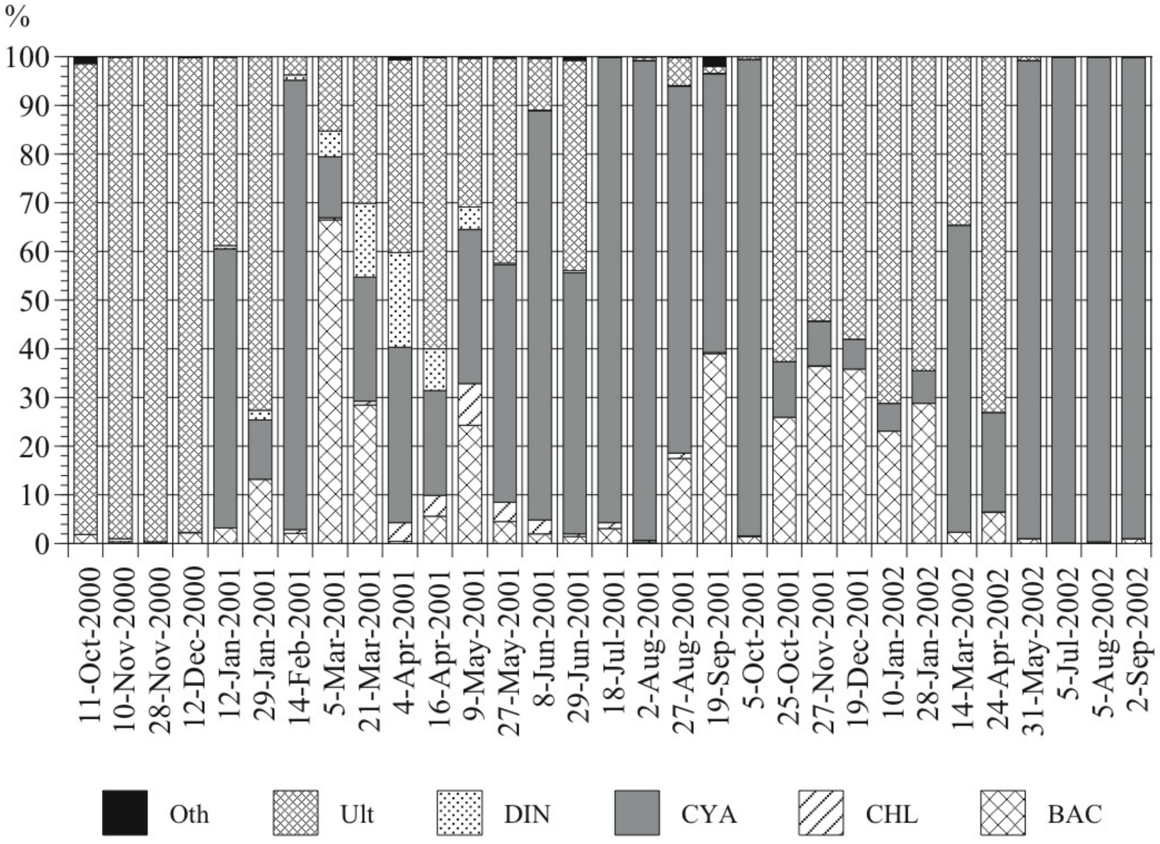

Fig. 5. Trend of phytoplankton class percentage composition in the Cabras Lagoon. (BAC-Bacillariophyceae; CHL-Chlorophyceae; CYA-Cyanophyceae; Ult-Ultraplankton; Oth = Others).

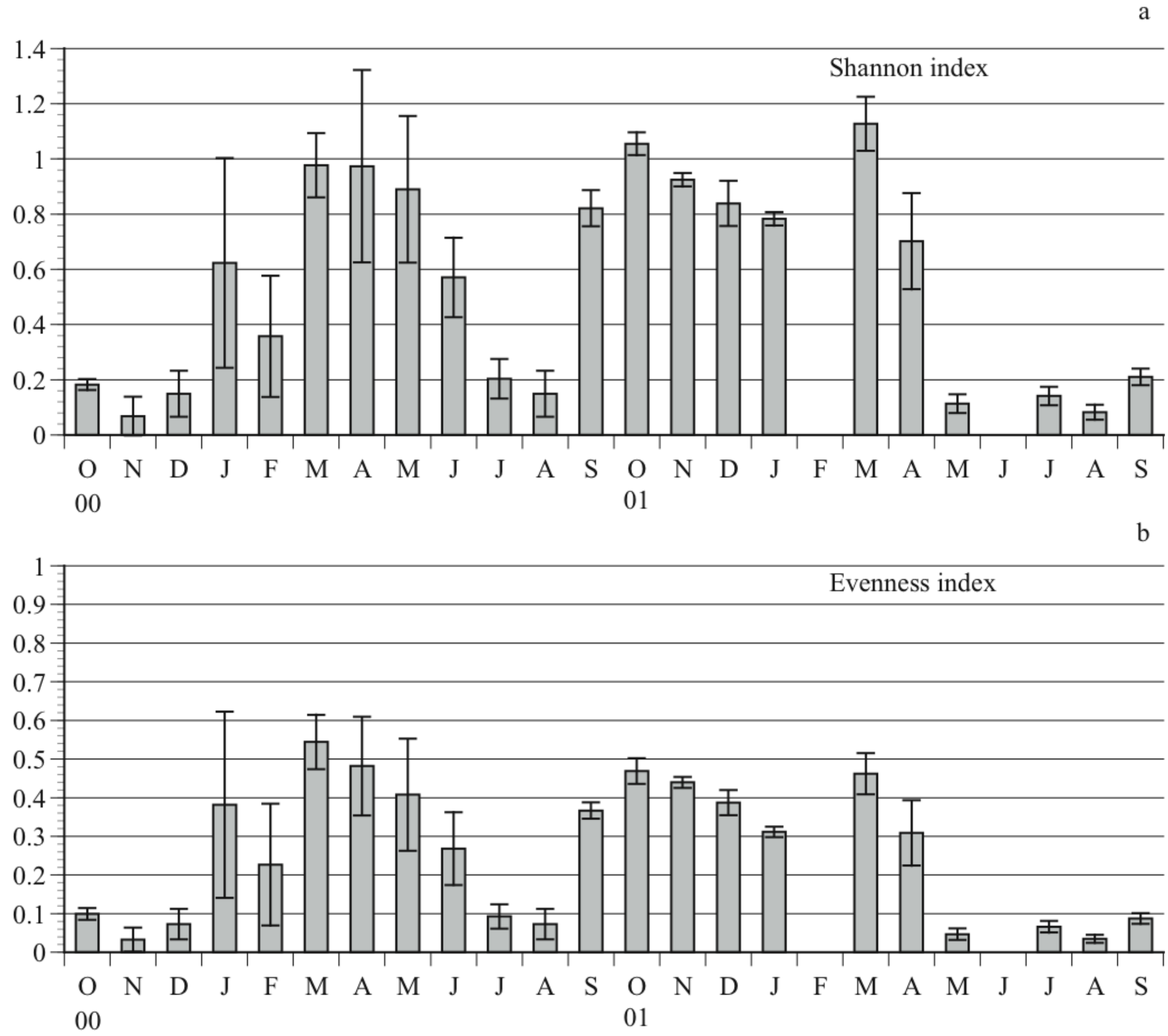

Fig. 6. Shannon (a) and Evenness (b) indices for phytoplankton assemblages in the Cabras Lagoon.

sp. was detected on only one occasion, in summer 2001 at ST1, at a density of 5 x 10^5 cells l^{-1}.

3.4 Phytoplankton diversity.

The Shannon diversity index and the Evenness index (Fig. 7a and 7b) showed large temporal and spatial fluctuations and a trend nearly opposite to the one observed for phytoplankton density. The lowest mean values were observed in November 2000 (0.068 for Shannon index and 0.033 for Evenness) and August 2002 (0.082 for Shannon index and 0.035 for Evenness), when phytoplankton densities were caused by intense proliferations of, respectively, Ultraplankton and *Rhabdoderma* sp., which represented 98-99% of the total number of phytoplankton cells. The maxima of both indices were in March-May 2001, October-November 2001 and March 2002. In the first period, phytoplankton density was at low level (mean about 10^8 cells l^{-1}) compared to general abundance in the lagoon, whereas in the other two periods, mean densities were higher (more than 10^9 cells l^{-1}). For most sampling dates, the highest Shannon diversity index values were recorded at ST4; peak Evenness index values were reached at ST4 and ST1.

3.5 Correlation between environmental variables, Chl a and phytoplankton assemblages.

RP and TP were positively correlated with NH_3, RSi and solar radiation (Table 3). TP was also strongly correlated with salinity. Of course, solar radiation and rainfall were inversely correlated, and both those variables control salinity. Chl *a* resulted positively correlated with nutrient concentrations (NH_3, NO_2, NO_3, TP and RSi), rainfall and pH. However, phytoplankton density was not significantly correlated with any of the other parameters considered. To investigate this result in greater depth, the effect of environmental conditions on phytoplankton assemblages (objects arranged in classes) was analysed by means of a CCA.

The first two axes of the CCA accounted for 52.54% of the total variance of phytoplankton classes and environmental data. The first axis accounted for 30.32% and the second for 22.22% of the total variance, respectively. The correlation between the 13 environmental variables and 11 phytoplankton ones was 0.58 for the first axis and 0.62 for the second. A Monte Carlo test showed that all canonical axes were significant ($p<0.0001$).

The results of the CCA (Fig. 8) showed that Cyanophyceae were mainly controlled by salinity, TP and alkalinity, whereas Bacillariophyceae and Ultraplankton were controlled by nitrogen nutrients such as D.I.N., NO_2, NO_3, NH_3 and by N/P ratio. Dinophyceae

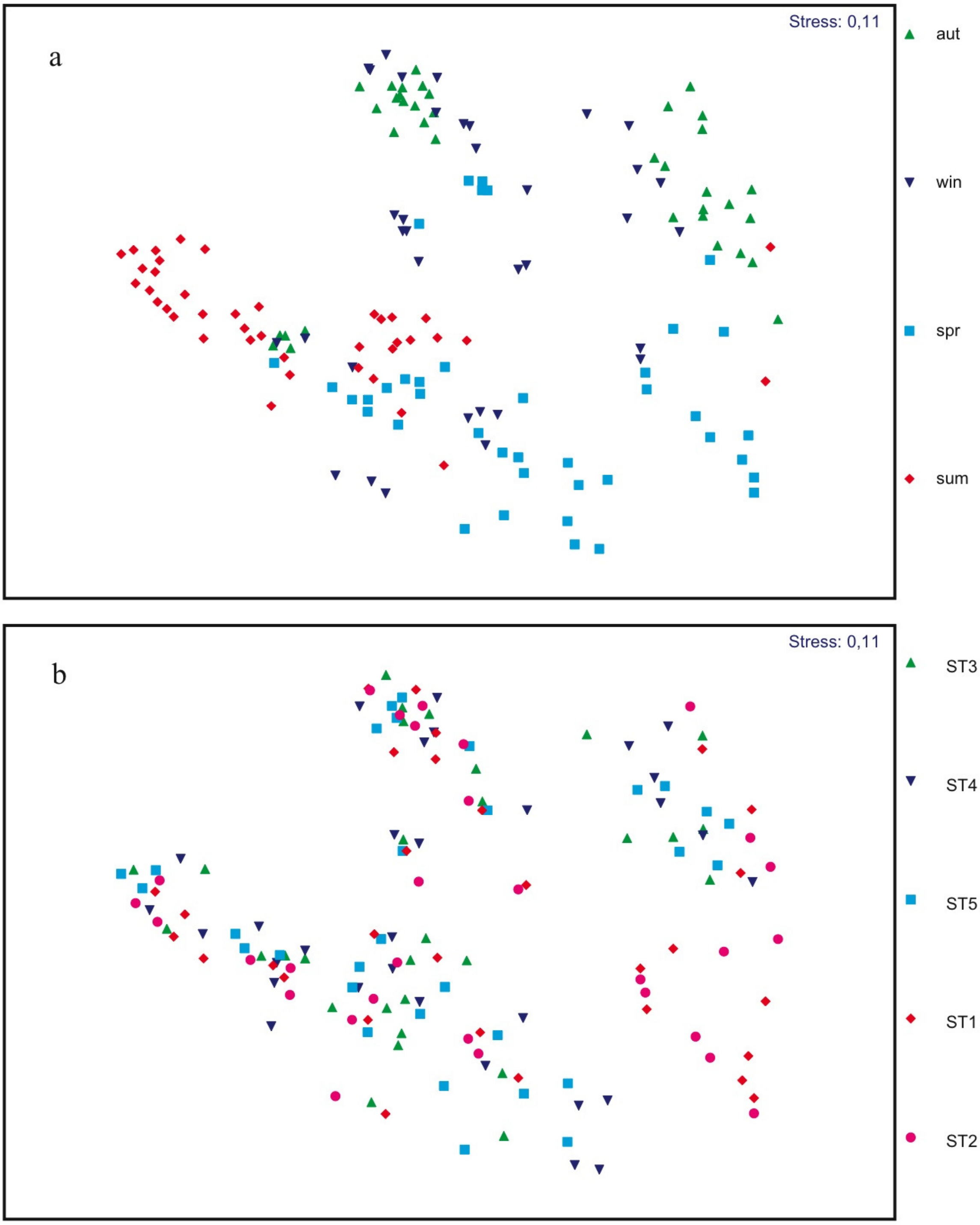

Fig. 7. nMDS of biotic parameters in the Cabras Lagoon: (a) differences among seasons, (b) differences among sampling stations.

Tab. 3. Pearson's correlation matrix. Bolt values are different from 0 at a significance level of $p<0.05$, (*) indicates $p<0.01$.

Variables	Alk	NH3	NO3	NO2	DO	RP	TP	pH	Sal	Rsi	Temp	Rainfalls	Solar Rad	Chl *a*	Phyt Dens
Alk	**1**														
NH3	0.059	**1**													
NO3	**-0.519**	0.304	**1**												
NO2	-0.249	**0.441**	***0.842**	**1**											
DO	-0.242	-0.090	-0.107	-0.154	**1**										
RP	0.191	**0.455**	0.069	0.287	0.242	**1**									
TP	0.172	***0.797**	0.123	0.343	0.097	***0.721**	**1**								
pH	-0.315	0.137	0.172	0.352	***0.558**	0.367	0.414	**1**							
Sal	0.353	**0.501**	-0.267	-0.194	-0.318	0.391	***0.576**	-0.196	**1**						
RSi	-0.050	0.403	0.305	**0.431**	0.362	***0.612**	***0.600**	**0.530**	0.133	**1**					
Temp	0.405	-0.034	-0.252	0.008	-0.040	**0.529**	0.307	0.067	0.255	0.190	**1**				
Rainfalls	-0.363	-0.126	0.384	0.464	0.199	-0.168	-0.128	***0.672**	***-0.735**	0.209	-0.287	**1**			
Solar Rad	0.391	0.436	-0.136	-0.058	-0.188	**0.541**	***0.651**	-0.169	***0.820**	0.390	0.284	**-0.575**	**1**		
Chl *a*	-0.218	***0.535**	***0.546**	**0.504**	0.169	0.313	***0.536**	***0.551**	-0.017	***0.536**	-0.288	**0.516**	0.028	**1**	
Phyt Dens	0.101	0.172	-0.233	-0.337	-0.327	-0.381	-0.119	-0.386	0.332	-0.384	-0.381	-0.213	0.173	-0.016	**1**

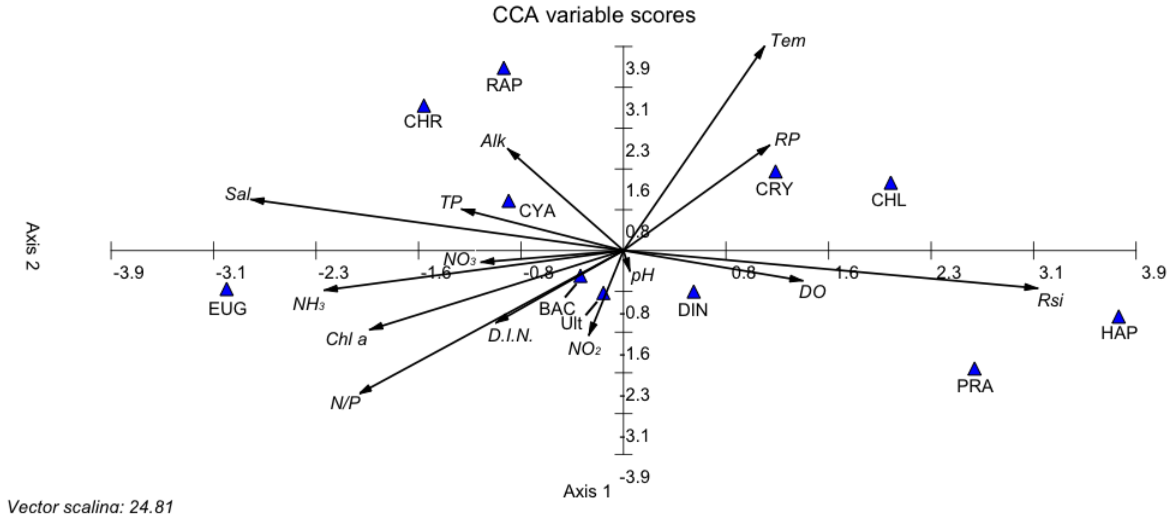

Fig. 8. CCA - Canonical correspondence analysis of phytoplankton classes (BAC-Bacillariophyceae; CHL-Chlorophyceae; CHR-Chrysophyceae; CRY-Cryptophyceae; CYA-Cyanophyceae; DIN-Dinophyceae; EUG-Euglenophycea; HAP-Haptophyceae; RAP-Raphydophyceae; Ult-Ultraplankton) and environmental variables (Alk-Alkalinity; D.I.N.-Dissolved Inorganic Nitrogen; NO_3-Nitrate; NO_2-Nitrite; NH_3-Ammonia; DO-Dissolved Oxygen; Sal-Salinity; RP-Reactive Phosphorus; RSi-Reactive Silica; Tem-Temperature; TP-Total Phosphorus; N/P).

abundance was positively correlated with RSi, DO and pH, and Cryptophyceae with RP and temperature. The outlying position of Raphidophyceae, Chrysophyceae, Euglenophyceae, Prasinophyceae, Haptophyceae and Chlorophyceae was consistent with the fact that they only appeared for very short periods.

3.6 Trophic state indices.

The trophic state index TSI calculated using Chl *a*, TSI (CHL), was sensibly lower than the TSI (TP), calculated using total phosphorus. The mean value for TSI (CHL) was 65.05, a clear indication of the eutrophic condition of the lagoon (Fig. 9a). On about 70% of the sampling dates, Cabras Lagoon could be classified as eutrophic, while on the remaining 30% it was hyper-eutrophic. TSI (TP) indicated a more eutrophic condition (Fig. 9b), with a mean value over the study period of 85.39. The lagoon's condition was classified as hyper-eutrophic on 100% of the sampling dates.

The trophic index TRIX (Fig. 9c) showed values indicative of a high trophic level. The mean value was 6.6, indicating eutrophic conditions or poor water quality. On 79% of sampling dates, the quality of the lagoon water was poor, while in the remaining 21% of cases it was moderate.

4. Discussion and conclusions

The natural and anthropogenic pressures which have influenced the Cabras Lagoon, as a result of past actions, have significantly altered its hydrological and environmental state and have likely affected its biological communities in several ways (Sechi et al., 2006). We have identified some important events that have affected the lagoon: (i) the transformation of the natural wetland of Mare ‘e Foghe into a canal in the ‘60s, with the consequent loss of its natural phyto-depuration capacity; (ii) the reconfiguration of the sea mouth during the late ‘70s; and (iii) the severe dystrophic crisis that occurred in 1999. These events produced effects on various aspects of the ecosystem, from salinity distribution to nutrient availability and phytoplankton development.

The data considered in this paper were collected about a year after the strong dystrophic crisis that occurred in June 1999 and led to a total die-off of fish in the lagoon. Around 850 tons of fish were harvested annually (450 kg ha^{-1} year^{-1}) until 1998, while after the severe dystrophic crisis the total catch fell below 80 tons (Murenu et al., 2004). Currently, total catches are known to be around 150 kg ha^{-1} year^{-1}. During the severe dystrophic event, a bloom of the cyanobacterium *Anabaena* cf. *mucosa* Komarkova-Legnerova and Eloranta was documented (unpublished data). Cyanobacterial cells in an advanced state of degradation and the presence of large amounts of mucilage were noted. For this reason,

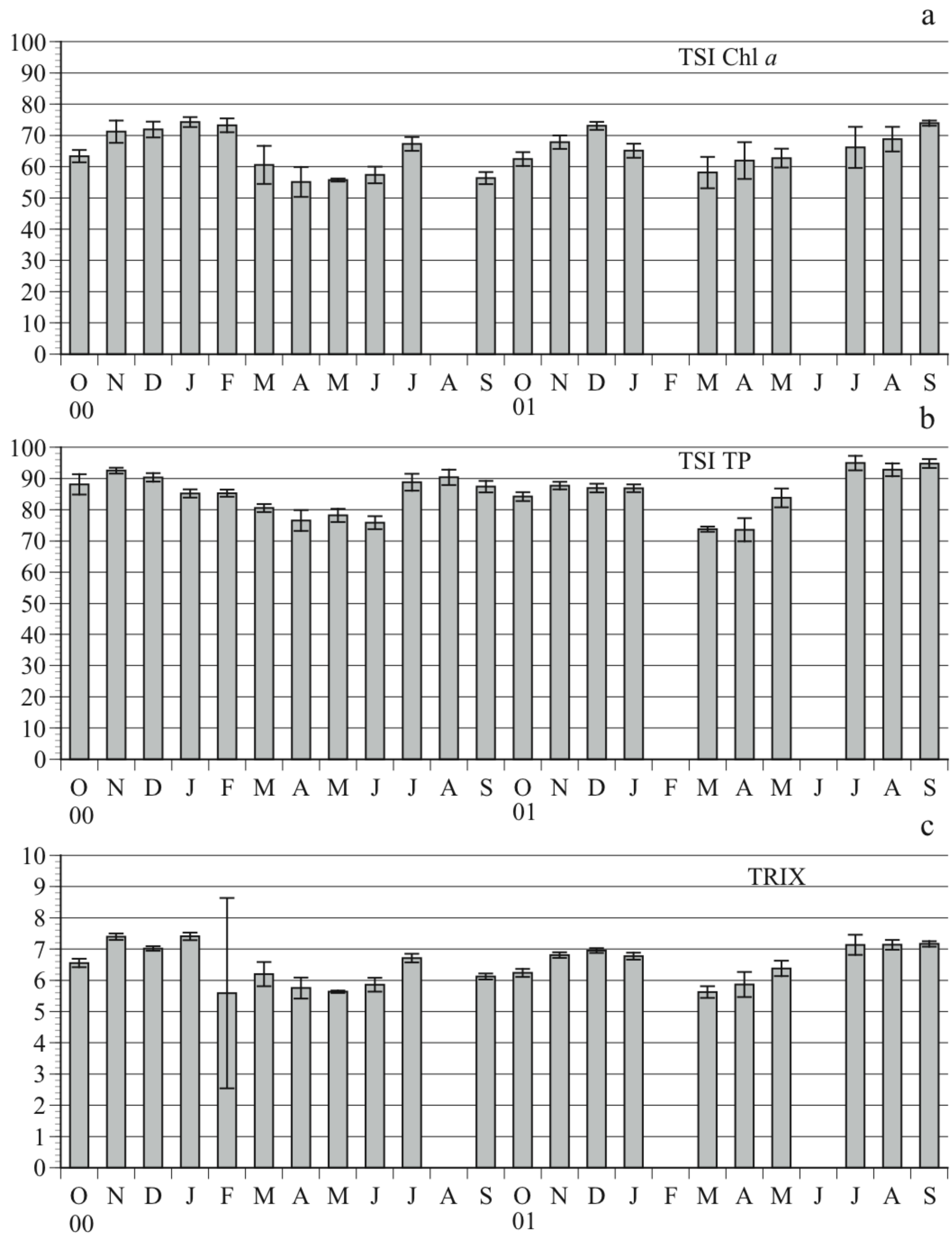

Fig. 9. Trophic state index TSI calculated using chlorophyll *a* (a), total phosphorus (b), and the TRIX formula (c) in the Cabras Lagoon.

one of our main priorities was to conduct an in-depth study of phytoplankton and its dynamics with respect to environmental variables. We need to arrive at an understanding of the lagoon's ecology so we can propose adequate recovery and management plans for both the lagoon and the watershed, to the local and regional administrations. For the same reason, the other main objectives of our study were to assess the presence of potentially harmful algal species and to evaluate the trophic state of the lagoon.

We used several trophic state indices to assess the environmental condition of the lagoon and compare it with the phytoplankton assemblages.

With regard to TSI (TP) and TSI (CHL), the Cabras Lagoon was classified as being eutrophic. This condition was as we expected, based on nutrient and Chl *a* concentrations, and as was observed by Sechi (1981). TSI (CHL) values were lower than TSI (TP) values, which was indicative of hyper-eutrophy.

A similar result was obtained from the TRIX index, whose values indicated eutrophic conditions (mean value of 6.6).

TSI and TRIX were applied by Roselli et al. (2009) to estimate the trophic state of Lesina Lagoon (Italy) and by Cohelo et al. (2007) to the Foz de Almargem coastal lagoon (Portugal). In these cases, the TSI determined using Chl *a* also presented lower values than the TSI determined using TP, and classified the first lagoon as an oligotrophic-mesotrophic system and the second as a mesotrophic-eutrophic system. Several other trophic indices were applied to both study sites, in addition to TSI and TRIX, and the lagoons were classified in different categories depending on which of the various indices were applied. The results of both studies highlighted the limitations of indices in assessing the trophic condition of lagoons.

The TSI and TRIX indices were developed for freshwater and marine coastal ecosystems, respectively, and should be applied with extreme caution in other contexts. They are used in lagoons mainly because no well-developed index has been designed to assess trophy in lagoons dominated by phytoplankton (Newton et al., 2003). In fact, many of the indices developed for lagoons require an evaluation of macrophyte composition and abundance (Austoni et al. 2004; Giordani et al., 2009); however, this biotic component is not present in the Cabras Lagoon. The European Environmental Agency considers the TRIX approach to be a useful tool in determining eutrophication trends in European marine coastal waters (EEA, 2001). This highlights the necessity of defining the index, and the kind of data that should be used for its calculation, on a regional scale. A major limitation in applying the two indices considered to lagoons is the high nutrient concentration that generally characterizes these transitional environments. This intrinsic feature tends to shift their trophic classification towards higher levels.

High nutrient concentrations and variations in salinity were the most evident abiotic characteristics of the Cabras Lagoon. The lagoon appeared generally uniform and variations, when they occurred, affected the entire system (as clearly evidenced by nMDS results) and were often dependent on adjacent systems (the watershed for nutrients and the sea for salinity).

Phosphorus peaks often occurred in late summer, while nitrogen peaks also occurred in winter. The former could be a result of internal inputs from the re-suspension of previously sedimented material caused by intense summer winds, since inputs from the watershed where very low or absent during the dry summer season. Moreover, the discrepancy between TP concentrations measured and expected TP concentrations, as calculated by Sechi et al. (2006) based on watershed loads, could be explained by such internal inputs. In general, all nutrients could also come from an internal input from the sediment. It is known that lagoons recycle large amounts of material, largely due to their very shallow depth (Mee, 1978). Clearly, an assessment of the nutrient flux for all compartments is crucial point in understanding the ecology of brackish environments. Using models to budget such assessments is an extremely simple and advantageous method, and allows for integral large-scale estimates (Simpson and Rippeth, 1998).

Winter nitrogen peaks appeared to be principally correlated with fall-winter rains and, consequently, with watershed discharge.

Salinity was another important characteristic of the lagoon. There were clear differences between stations linked to their localization in the lagoon, in respect to freshwater inputs and sea-water exchange (about 4‰ from ST1 to ST5), and a regular upward trend was noted from the beginning of the study period. A deeper valuation should be done as regards the hydrological balance of the lagoon, considering also losses due to evaporation and different freshwater inputs in the different annual cycles. DO values were generally over-saturated, but steep reductions were occasionally observed at all stations. Bottom DO is one of the variables proposed by the European Environment Agency (EEA) (2001) to identify eutrophication in coastal marine areas. This variable is also considered in the Italian law, DLgs 152/99, which calls for the use of DO near the bottom to classify lagoon waters based on the number of anoxic days per year (DO values in the range of 0 – 1.0 mg l^{-1}) that involve more than 30% of the lagoon's surface. During the study period, the water column of the lagoon was fully mixed and no anoxic phenomena were detected at the bottom. This procedure was therefore not very useful in this context.

Chl *a* and phytoplankton dynamics are two of the other variables proposed by the EEA (2001) as good indicators of water quality. These can be better interpreted if analysed together with the complex of hydrological, physical and chemical factors that interact in a coastal lagoon.

The mean lagoon value of Chl *a* always exceeded the limit of 8 mg m^{-3} used to define freshwater eutrophic systems (Vollenweider and Kerekes, 1982). At the same time, phytoplankton densities were elevated, with the highest levels frequently being observed in the summer and winter months, coinciding with TP and D.I.N. peaks, respectively.

Phytoplankton species composition in the Cabras Lagoon was characterized by significant spatial uniformity (as indicated by nMDS) and by high numbers of very small species (in the ranges of 5-2μm and <2μm).

Although picoplankton have always been described as a typical component of oceans and oligotrophic environments (Bec et al., 2005), their presence and dominance over the other size classes in brackish and hypertrophic ecosystems in the Mediterranean area in recent years has been well documented (Vanucci et al., 1994; Caroppo, 2000; Pérez and Carrillo, 2005; Del Negro et al., 2007). Further careful investigations will therefore be necessary to understand which environmental, climatic and/or biological factors could control the dynamics of picoplankton, and to identify the potentially dramatic consequences of their proliferation. In the hypertrophic lagoon system of Comacchio (NW Adriatic Sea, Italy), an extremely intense bloom of picocyanobacteria that persisted for many years resulted in the collapse of that ecosystem (Sorokin et al., 1996a).

The environmental degradation caused hypertrophic condition favours the dominance of cyanobacteria over less tolerant algal species (Sorokin et al., 1996a; Scheffer et al., 1997), although the link between very hypertrophic conditions and cyanobacteria blooms has thus far been documented only in a few Mediterranean lagoons (Abrantes et al., 2006; Chomérat et al., 2007).

Cyanobacteria were also dominant at all stations in the Cabras Lagoon for much of this study. CCA showed a strong correlation between the presence of cyanobacteria and increased salinity and TP. It is probable that, when phosphorus increases, it leads to a lower N/P ratio in the lagoon, favouring the proliferation of *Cyanobium* type-cells, since this taxon is believed to be capable of fixing nitrogen. This conclusion is supported by the well-documented capacity of the *Synechoccocus* genus to fix nitrogen (Stal et al., 2008) and the close genetic link between this genus and *Cyanobium* (Komárek and Anagnostidis, 1998), although the latter has not been studied in detail. In most published scientific works, small free-living cyanobacteria are described under the generic name "*Synechoccocus* sp." (Komárek, 1996). The genus *Cyanobium* has been reported in coastal lagoons in a few cases, although many of the organisms described in previous studies as *Synechoccocus*-type, with the current knowledge, could instead correspond to the genus *Cyanobium* (Komárek and Anagnostidis, 1998). This hypothesis was also proposed for Santa Gilla Lagoon, one of the few other Sardinian lagoons dominated by phytoplankton (Andreoli et al., 1989; Komárek and Anagnostidis, 1998).

The results also showed that increases in the various forms of nitrogen, and the consequent increase in the N/P ratio, was advantageous for Bacillariophyceae and Ultraplankton, the other two phytoplankton groups that, next to Cyanophyceae, were most significant as regards their presence and density in the Cabras Lagoon.

Another important consideration for lagoon ecosystems where the fishery and shellfish harvest constitute the main economic activities is the direct and indirect negative effects of HAS on fish, shellfish and humans. Of the HAS observed in Cabras Lagoon during the course of this study, *P. minimum*, *D. acuminata* and *Alexandrium* spp. may produce VSP toxins, DSP toxins and okadaic acid, and PSP toxins, respectively (Andersen et al., 1996; Faust and Gulledge, 2002). *Chattonella* is a well-known harmful phytoplankton genus that causes mass die-offs among farmed fish, due to its production of high biomasses (Hallegraeff, 2003) and toxic compounds (Onoue Y. and Nozawa K., 1989). Lastly, *H. rotundata* has been identified as a species that can cause "red tides" (Cohen, 1985; Verity, 2009). Among the HAS observed, none expressed dangerous characteristics during the study period, but their presence in the lagoon should be investigated in depth.

In conclusion, the results of this study showed that the Cabras Lagoon is in critical condition due to constant high phytoplankton densities, the intense presence of cyanobacteria of a very small size, the presence of various harmful algal species, and high trophic levels, considered as single environmental parameters or as trophic state indices.

Overall, salinity, nutrient concentrations and nutrient ratios appeared to be critical factors in controlling phytoplankton. They should therefore be considered when drawing up management and recovery plans for the lagoon.

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6. Chapter IV

Long-term phytoplankton abundance, structure and diversity in the eutrophic Cabras Lagoon (Italy, W Mediterranean Sea).

Long-term phytoplankton abundance, structure and diversity in the eutrophic Cabras Lagoon (Italy, W Mediterranean Sea).

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ABSTRACT

The Cabras Lagoon is an eutrophic Mediterranean type lagoon characterized by high nutrient loads and very poor sea exchanges. In this paper we describe the spatial and temporal dynamic of phytoplankton and environmental parameters from 2000 to 2008. Particularly, the mean annual cycles of phytoplankton abundance, assemblage structure and diversity are discussed.

1. Introduction

Over the last centuries, different direct and indirect human activities have modified the loads and fluxes of nutrients and sediments into surface waters, ground waters, and finally, through the hydrographic system towards the marine coastal ecosystems. The drainage basins with a wide range of human activities and the coastal waters into which they drain, are large-scale ecosystems. They have to be protected from deterioration and the understanding of the dynamics and causes of the large-scale and long-term changes of nutrient loads have been regarded as particularly important (Wassmann and Olli, 2004). Scientific results clearly indicate that monitoring programs that try to determine long term trends of basin scale nutrient fluxes require high resolution data sets (Stålnacke, 2005).

Large increases in nutrient loading associated with increases in population growth, changes in agricultural practices including the increased use of inorganic fertilisers, changes in collection and treatment of sewage, and increases in nitrate deposition from the atmosphere have occurred (Wassmann and Olli, 2004). The comprehension of ecological responses to these kinds of solicitations is not a simple task because of both the complexity of the systems and other long-term processes contemporarily acting on the considered ecosystems (Boero et al., 2008), such as climate change and the resulting warm effects of aquatic ecosystems.

The response of coastal ecosystems to nutrient loading is a crucial point in estuarine and marine coastal zone research (Nixon et al., 1996), especially the role of nitrogen, phosphorus and reactive silica in limiting the growth of phytoplankton. In the last decade, major reductions in P loading through better sewage treatment and as a consequence of new law indications, with a more or less maintained N loading have occurred (Wassmann and Olli, 2004). These changes in nutrient loadings and ratios have affected the productivity of coastal and marine waters and have changed the potential limiting nutrients in governing system production (Conley, 2000).

The major and evident consequence of river basin pollution of nutrients has been an increase of the eutrophication phenomenon, inducing also proliferations of toxic phytoplankton species in many lagoons (Glibert and Burkholder, 2006).

Coastal lagoons are highly sensible ecosystems with an ecology strongly depending on coastal and continental inputs. They are under considerable natural stress because of the great variability of a large part of basic factors such as temperature, salinity, strength of tidal and internal currents (Postma, 1994).

Smayda & Reynolds (2003) defined a ‘habitat template’ for marine environments and, even if the layout was mainly conceptual, the representation identifies habitats able to support substantial phytoplankton biomass in the near-shore habitats and coastal lagoons. These ecosystems are characterised by the least enduring or least severe constraints of energy and/or nutrient resources and by a potential for high net production and a relatively high supportive capacity for planktonic biomass.

Cloern & Jassby (2009) reported that whereas much of phytoplankton variability in the open ocean is generated by the annual cycles of solar radiation and atmospheric heat input, phytoplankton variability in estuaries and nearshore coastal waters is generated by many additional processes that propagate across their interfaces with land, ocean, atmosphere, and underlying sediments. Comparisons across the diversity of these types of ecosystems where marine waters are influenced by connectivity to land revealed a large range of variability patterns belonging to three very different scales: I) dominated by a recurrent seasonal pattern, II) dominated by annual (i.e., year-to-year) variability as trends or regime shifts, III) dominated by the residual component, which includes exceptional bloom events such as red tides.

It is known how the phytoplankton community is fundamental to the functioning of aquatic ecosystems. Phytoplankton variability is a primary driver of chemical and biological dynamics in the coastal zone because it directly affects water quality, biogeochemical cycling of reactive elements, and food supply to consumer organisms (Cloern & Jassby, 2009). Accordingly, the indications provided by monitoring the phytoplankton serve as very useful environmental sensors of environmental quality (Marchetto et al., 2009). In particular, phytoplankton, as the basis of the trophic chain, constitutes one of the most important structural component in which scientific attention is focused when a management plan is needed or an assessment of the ecosystem health is required (Monbet, 1992).

The intrinsic complexity of phytoplankton system in lagoons, makes the analysis of long temporal series particularly useful, consenting to have exploitable reconstructions of seasonal cycle of phytoplankton (Cloern, 2009). Such studies are primarily utilized to distinguish nonsystematic natural variability from trends or shifts in the ecosystem that have often been related to eutrophication or anthropogenic influences and/or on a global scale by climatic fluctuations (Ribera d'Alcalà et al., 2004; Mozetič et al., 2009).

The relevance of long-term studies in ecosystem management programs is now widely recognized, in fact, in order to measure anthropogenic influence on a certain ecosystem, a good knowledge of the natural variability of the system is a necessary requisite (Gameiro et al., 2007). In time series, data of chlorophyll *a* used as a proxy for phytoplankton biomass variability are frequently considered. It is also because standard methods are established for this biotic variable and it is routinely measured in research and monitoring programs (Cloern and Jassby, 2009; Mozetič et al., 2009). On the contrary, one of the greatest problems when maintaining a long term data set is the enumeration and identification of species (Wiltshire et al., 2008) and perhaps also for this, long term studies on phytoplankton less frequently use species composition as a measure of its variability, especially in the Mediterranean lagoons.

Cabras Lagoon is an hypertrophic lagoon sited along the western coast of Sardinia (Italy), in the western Mediterranean basin. In Cabras Lagoon, phytoplankton is the main primary producer (Sechi et al., 2006) and the anthropogenic disturbances and meteoclimatic factors appeared to have an important role in controlling phytoplankton assemblages and abundances.

Its high trophic state has often submitted the lagoon to important dystrophic crises that had determined a large decrease of its fishing productivity. Since the strong dystrophic crisis that interested Cabras Lagoon during the summer of 1999, and that caused the killing of the totality of the aquatic biota, a long time series with high frequency measurements and sampling of environmental and biological parameters (chemical-physical, nutrients, phytoplankton density and composition) has been carried out. Thanks to this, today Cabras Lagoon is one of the aquatic ecosystems listed in “n. 14 Marine ecosystems of Sardinia” which takes part in the Italian Network of Long Term Ecologic Researches (*LTER-Italia*; http://www.lter-europe.net/networks/italy).

In this paper, the temporal variation of trophic state and phytoplankton abundance, structure and diversity in Cabras Lagoon has been analysed as a first approach to assess the general features of phytoplankton succession and its long term tendencies in respect to environmental factors, mainly nutrients.

2. Material and methods

2.1 Study area

Cabras Lagoon is a shallow water body located on the west coast of Sardinia, in the Gulf of Oristano (western Mediterranean Sea; 39° 56’ 37’’ N, 08° 28’ 43’’ E; Fig. 1). With an area of about 23.8 km^2 and a mean depth of 1.6 m (maximum of about 3 m), the Cabras Lagoon is the largest in Sardinia and one of the largest in the Mediterranean Sea (Sechi et al., 2006). Due to its environmental importance, it is protected by international agreements, including the Ramsar Convention (EU Directive Special Protection Area, Site of Community Importance, Natural Protected Area), and is part of the Sinis-Montiferru Natural Reserve (Sardinia).

The watershed of Cabras Lagoon extends over an area of approximately 430 km^2. Water flowing within the system is derived from natural rivers as well as from artificial canals that drain the surrounding lowlands. Most of the freshwater input in the lagoon originates from the Rio Mare ‘e Foghe, which drains an area of 313 km^2.

The connection between the lagoon and the adjacent Gulf of Oristano mainly consists of four narrow creeks that flow into a large southernmost canal, the *Scolmatore* (=spillway), which was dredged in the late 1970s. The canal was constructed to allow the flushing of excess water during the heavy rainfalls in winter which regularly submerged a district the near town of Cabras, called, appropriately, “Little Venice.” The *Scolmatore* was subsequently closed by a 30-cm-high dam built to prevent further increases in the salinity of the lagoon by stopping the outflow of river-derived freshwater, which over time has strongly decreased due to agricultural demands. In addition, artificial barriers have been constructed to control the fish catch. Although the tidal amplitude in the Gulf of Oristano is <40 cm, the dam and barrier system limit the exchange of water between the lagoon and the Gulf. Consequently, the hydrodynamism of Cabras Lagoon is mainly governed by wind forcing, particularly with respect to water circulation, whereas the tide determines discharges through the lagoon’s inlets and modulates the circulation pattern established by the wind (Ferrarin and Umgiesser, 2005). The results of simulations have shown that the wind tends to create a circular current in the larger expanse of the lagoon, with speeds along the shore that are higher than those in the centre of the basin wind (Ferrarin and Umgiesser, 2005).

The lagoon has a high economic rating due to its numerous fisheries (e.g., *Liza ramada*, *Mugil cephalus*), which employ about 300 people and their families, and to fishing-related activities. In 1998, fish productivity reached 40,000 kg km^{-2}, corresponding to a profit of about 3.5 million Euros (Sechi et al., 2006). However, dystrophic events often cause massive fish mortality (the last such event occurred in the summer of 1999). In fact, the brackish ecosystem of Cabras Lagoon is eutrophic, such that water quality has steadily deteriorated, with a consequent decrease in the lagoon’s fish productivity (actually around 20,000 kg km^{-2}).

2.1 Samplings and data treatment

Samplings have been carried out in Cabras Lagoon over an eleven-year period, beetwen June 1999, just after the dystrophic crisis, to June 2009. Fortnightly surveys were carried out on three stations. The three stations were chosen to characterize the spatial variability of the environmental factors in the lagoon (Fig. 1). Temperature (Tem), salinity (Sal), dissolved oxygen (DO), pH and fluorimetric chlorophyll *a* (fChl *a*) were measured *in situ* with a multi-parameter probe (Idronaut/YSI 6600V2).

Water samples were collected from the superficial water layer (30 cm depth) and preserved in cold, dark conditions for laboratory analyses of ammonia (NH_3), nitrite (NO_2), nitrate (NO_3), reactive silica (RSi), orthophosphate (RP) and total phosphorus (TP) following Strikland & Parsons (1972) and for CHL*a* (Golterman et al., 1978). Dissolved inorganic nitrogen (D.I.N.) was obtained as the sum of NH_3, NO_2 and NO_3.

Data for rainfall were provided by the Sistema Informativo Agricolo Nazionale (SIAN) and refer to the meteorological station of Santa Lucia, in the vicinity of the lagoon.

Phytoplankton samples were fixed with Lugol's solution and analysed with the Utermöhl's technique (1958), using an inverted microscope (Zeiss, Axiovert 25) after sedimentation of variable volumes of water (5-10 cc), depending on phytoplankton density. Cell counts were made at 100X on the entire bottom of the sedimentation chamber for the larger and more easily identifiable species, and replicated at 200X and 400X on an adequate number of fields for the smaller cells. The species were determined following Balech (1995), Faust (2002), Germain (1981), Humm & Wicks (1980), Husted (1985), Rampi e Bernard (1978, 1980, 1981), Ricard (1987), Sournia (1986), Tomas (1997).

The Shannon-Wienner diversity index (Weaver & Shannon, 1949) was calculated in each sampling and in each station to evidence temporal and spatial variation of phytoplankton richness and relative abundances.

Relationships among the considered environmental variables and fitoplankton classes abundance were tested with the Pearsons correlation.

In this paper data on salinity, D.I.N., RP, Chl *a*, class composition of phytoplankton, density and diversity are discussed.

To calculate the mean annual cycle of the different variables, only data of five annual cycles were available, namely those from January 2000 to December 2002 (three years; 43 samplings) and from January 2007 to December 2008 (two years; 28 samplings), due to the interruptions and different patterns in the collection of data that were adopted during the pluriannual period considered.

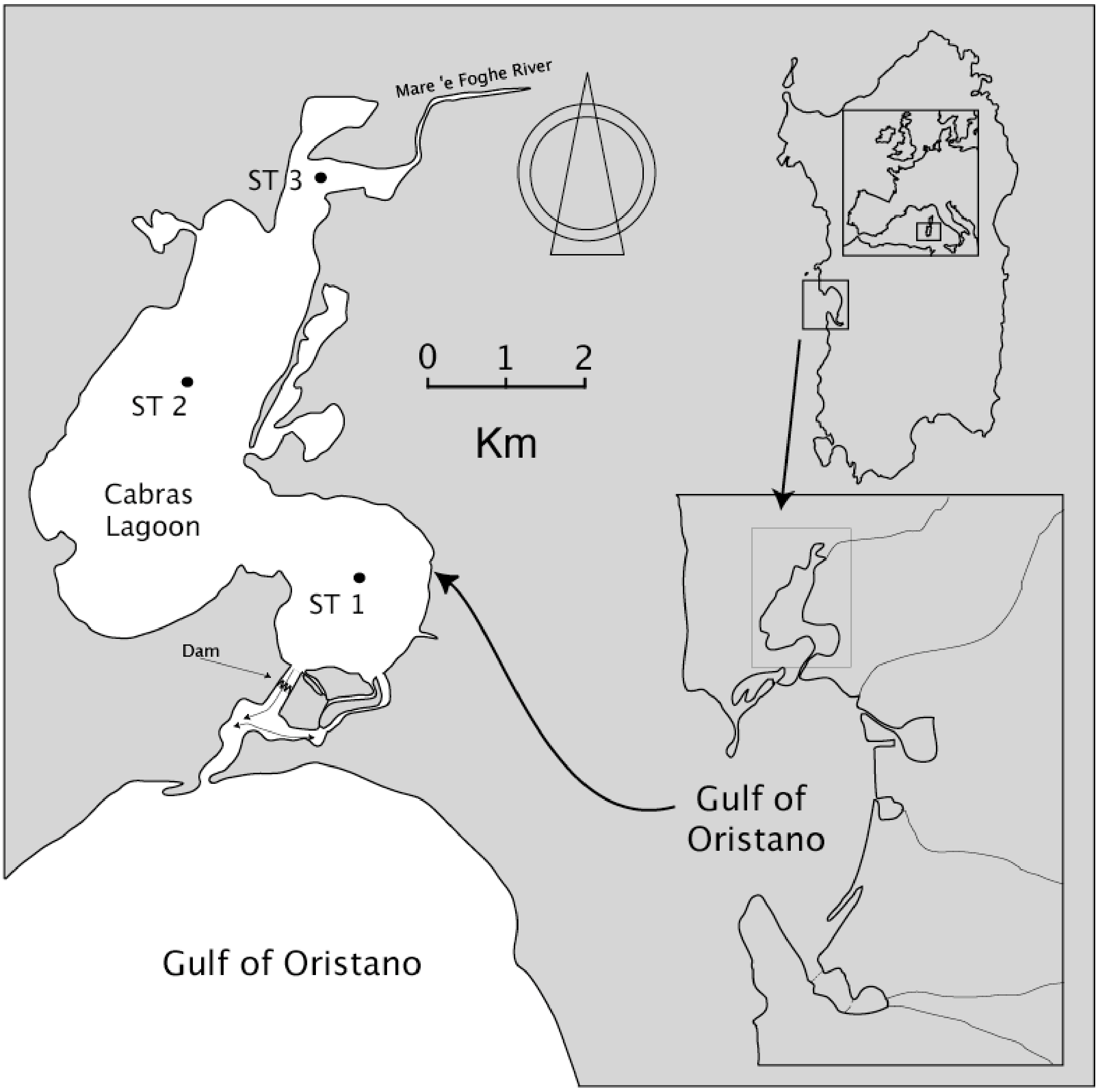

Fig. 1. Map of the Cabras Lagoon and sampling stations.

3. Results and Discussions

Regarding the dynamic of salinity, it exhibited a tendency to decrement as annual means, from 2000 to 2008 (Tab. 1). Furthermore, it was very irregular in its temporal dynamic (Fig. 2), particularly there were abrupt falls of salinity in the winter months between 2000 and 2001, and in the autumn 2008 due to strong rainfalls (Fig. 2).

D.I.N., after the maxima peaks in summer 1999 (monthly mean of 4007 mg N m^{-3} in July, when the dystrophic crisis was in progress), showed a decrement of values that stayed below 200 mg N m^{-3} during the years (Fig. 2). The major peaks were observed in January of every annual cycle considered (the peaks in April 2000 and in July and August 2002 were exceptions). The very high values detected in autumn-winter seasons of 2008-2009 (monthly mean of 725 mg N m^{-3} in December) were consequent to the period of persistent and strong rainfall already mentioned (Fig. 2). RP showed the major values in summer months for the most of the years considered, with autumn and winter peaks only in 2000 and between 2008-2009 (Fig. 2). As for D.I.N., these last peaks were associated with the event of strong rainfalls.

Consequently, the N/P ratio (D.I.N./RP) showed a pluriannual dynamic with the highest peaks in autumn and winter seasons, in general when the maxima of D.I.N. were observed (Fig. 2).

The pluriannual dynamics of total density showed an increase from the beginning of the study (mean values were of about 10^8 cells l^{-1}) until the summer of 2002, when the maximum peak was observed (monthly mean value of 154 x 10^8 cells l^{-1} in August). During these years the highest peaks occurred in the summer and autumn seasons. Then, the values were a little lower in the last two study years than in 2002, with a tendency to the decrement towards the end of the investigated period. In this case the maxima were observed in the late winter-spring period (maximum mean of 75 x 10^8 cells l^{-1} in March 2008) (Fig. 3).

During all the investigated period, 11 phytoplankton classes were assessed in Cabras Lagoon (Tab. 2).

The pluriannual dynamic of phytoplankton composition showed important changes during the study years (Fig. 3). From the beginning of the study to 2002 a major number of classes was relatively important contemporaneously, in respect to that observed in the last years considered, characterised by a high prevalence of only one class (Cyanophyceae). A clear dominance of Bacillariophyceae Class (BAC) until the end of 2000 was observed. In this interval of time also Ultraplankton (Ult) and Dinophyceae Class (DIN) were important, the

Table 1. Annual values in the years considered completed.

		Years				
Parameters	units	2000	2001	2002	2007	2008
Tem	°C	17.9 ± 1.5	18.6 ± 1.5	19.1 ± 2.4	19.6 ± 2.3	18.1 ± 1.5
Sal	‰	29.9 ± 2	15.5 ± 1.9	24 ± 1.4	16.5 ± 1.1	20.1 ± 1.1
pH		8.6 ± 0.1	8.7 ± 0.1	8.7 ± 0.1	8.3 ± 0	8.3 ± 0
DO	%	108 ± 10.3	117.4 ± 6	101.7 ± 5.1	85 ± 3.7	87.1 ± 2.9
Alk	meq l^{-1}	2.7 ± 0.1	2.7 ± 0.1	2.8 ± 0.1	2.9 ± 0.1	2.5 ± 0.1
NO_2	mg N m^{-3}	6.8 ± 3.8	8.6 ± 3	9 ± 2.4	5.1 ± 1.1	9.6 ± 2.5
NO_3	mg N m^{-3}	39.7 ± 32.4	45.8 ± 31.8	35.8 ± 13.3	19.8 ± 6.9	123.3 ± 60.3
NH_3	mg N m^{-3}	64.5 ± 20.4	46.6 ± 6.9	68.7 ± 15.5	29.3 ± 2.4	39.6 ± 5.3
D.I.N.	mg N m^{-3}	110.9 ± 54.9	101 ± 36.7	113.5 ± 28.6	54.3 ± 8.5	172.5 ± 64
RP	mg P m^{-3}	55.8 ± 20.2	27 ± 7.7	36 ± 13.8	20.2 ± 3.5	21.2 ± 4.9
TP	mg P m^{-3}	353 ± 31.5	252.1 ± 22.5	342 ± 57.7	180.6 ± 11.3	174.2 ± 12.5
N/P		6 ± 2.4	11 ± 4.8	7.5 ± 2	3.8 ± 1	9.4 ± 2.5
RSi	mg Si l^{-1}	7.4 ± 1.2	6.8 ± 0.5	5.1 ± 1.4	6.9 ± 0.3	4.3 ± 0.6
SS	mg l^{-1}	-	-	-	66 ± 6.6	103 ± 16.6
Chl *a*	mg m^{-3}	51.9 ± 10.1	38.8 ± 7.3	41.4 ± 7.1	20.9 ± 2.7	14.2 ± 1.3

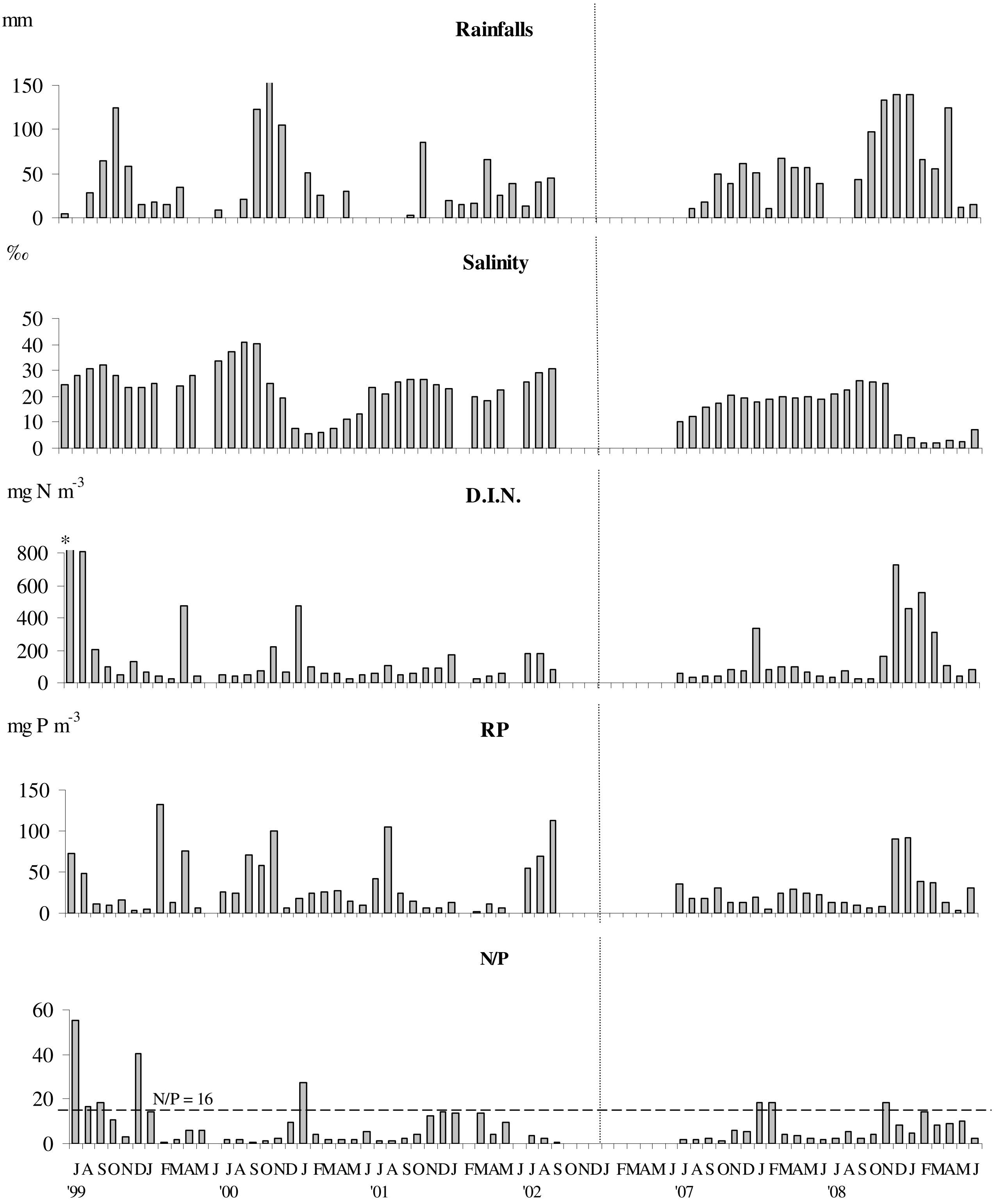

Fig. 2. Pluriannual tendencies of the considered climatic and abiotic parameters. The data are monthly mean values. * = 4007 mg N m^{-3}.

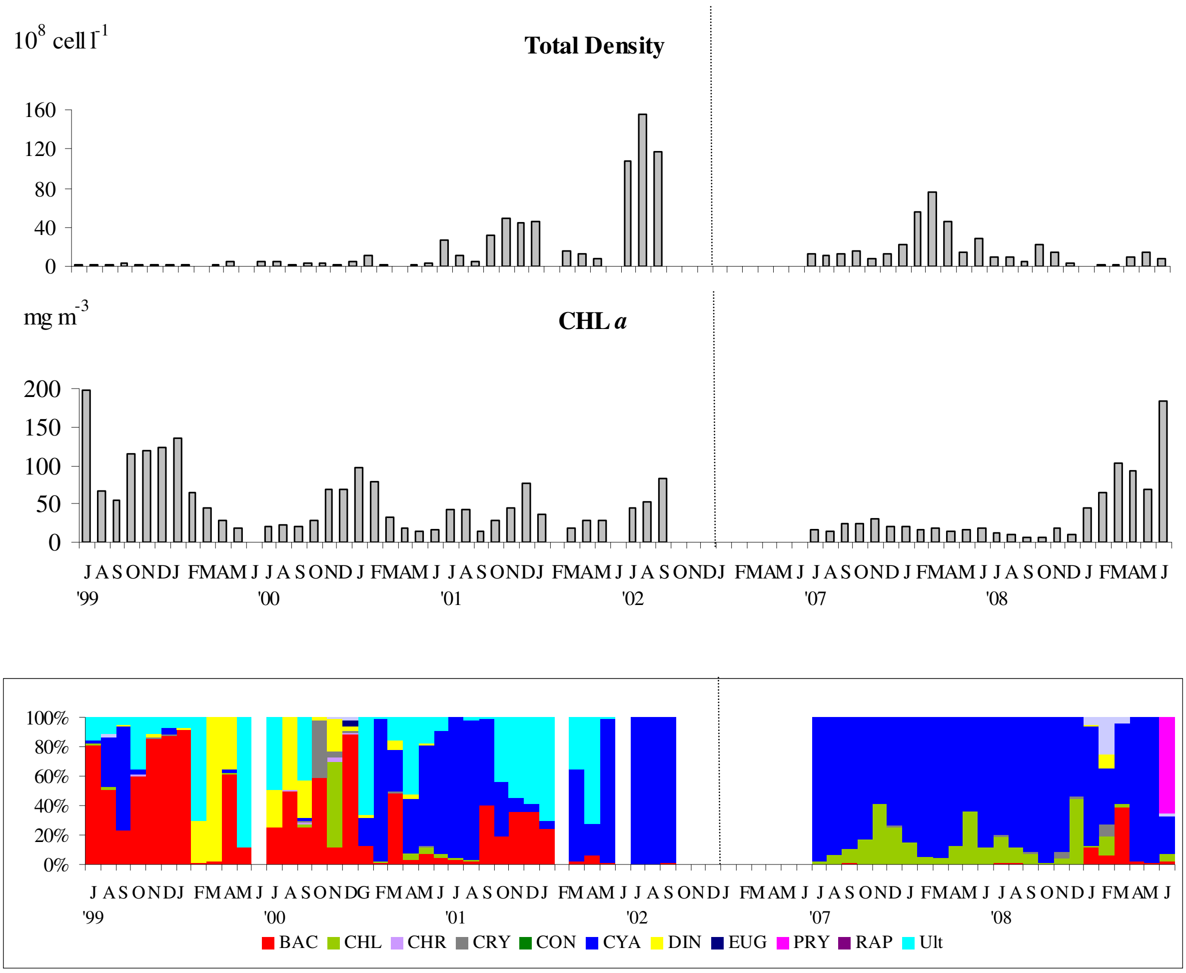

Fig. 3. Pluriannual tendencies of the considered phytoplankton parameters and percentage class composition of density. The data are monthly mean values.

Table 2. Floristic list.

Prokariotes	
CYANOPHYCEAE	
Anabaena cf. *mucosa*	Komarkova-Legnerova and Eloranta
Anabaena sp.	Bory
Anabaenopsis circularis	(G.S. Weast) Woloszyńska et Miller
Aphanizomenon aphanizomenoides	(Forti) Horecká et Komárek
Aphanizomenon gracile	(Lemm.) Lemm.
Aphanocapsa sp.	Nägeli
Cyanobium type-cells	Rippka and Cohen-Bazire
Limnothrix sp.	(Woloszynska) Meffert 1988
Merismopedia tenuissima	Lemmerman
Merismopedia sp.	Meyen
Oscillatoria sp.	Vauch.
Plankthotrix sp.	Anagnostidis et Komárek
Pseudanabaena catenata	Lauterborn
Rhabdoderma sp.	W. Schmidle and R. Lauterborn
Spirulina sp.	Turpin
Eukariotes	
BACILLARIOPHYCEAE	
Amphiprora sp.	Ehrenberg
Amphora sp.	Ehrenberg
Aulacoseira distans	(Ehrenberg) Simonsen
Aulacoseira granulata	(Ehrenberg) Simonsen
Aulacoseira sp.	Thwaites
Chaetoceros curvisetus	Cleve
Chaetoceros spp.	Ehrenberg
Climacosphenia spp.	Ehrenberg
Cocconeis sp.	Ehrenberg
Coscinodiscus sp.	Ehrenberg
Cyclotella atomus	Hust.
Cyclotella sp.	(Kützing) Brébisson
Cylindrotheca closterium	(Ehrenberg) Reimann & Lewin
Diploneis sp.	Ehrenberg ex Cleve
Fragilaria sp.	Lyngbye
Licmophora sp.	Agardh
Minidiscus sp.	Hasle
Navicula spp.	Bory
Nitzschia sp.	Hassall
Pleurosigma sp.	W. Smith
Rhizosolenia setigera	Brightwell
Skeletonema costatum	(Greville) Cleve
Skeletonema potamos	(C.I.Weber) Hasle
Skeletonema sp.	Greville
Synedra acus	Kützing
Thalassiosira sp.	Cleve
Triceratium sp.	Ehrenberg
CHLOROPHYCEAE	
Carteria sp.	Diesing
Chlamydomonas sp.	Ehrenberg
Chlorella sp.	Beÿerinck
Monoraphydium minutum	(Näg.) Kom.-Legn.
Monoraphydium sp.	Komarkova & Legnerova
Oocystis sp.	Nägeli
Pediastrum simplex var. *echinulatum*	Wittr.
Pediastrum sp.	Meyen
Scenedesmus spp.	Meyen
Tetrastrum sp.	Chodat

Table 2 (*Continued*).

CHRYSOPHYCEAE	
Kephyrion spirale	(Lackey) Conrad
Kephyrion sp.	Cienkowski
Ochromonas sp.	Wyssotzski
CONJUGATOPHYCEAE	
Closterium aciculare	T. West
Closterium sp.	Nitzsch ex Ralfs
CRYPTOPHYCEAE	
Cryptomonas sp.	Ehrenberg
Hemiselmis sp.	Parke
Plagioselmis sp.	Butcher
Rhodomonas sp.	Karsten
DINOPHYCEAE	
Akashiwo sanguinea	(Hirasaka) G. Hansen et Moestrup
Alexandrium sp.	Halim
Cochlodinium sp.	Schütt
Dinophysis acuminata	Claparède & Lachmann
Dinophysis fortii	Pavillard
Gymnodinium sp.	Stein
Gyrodinium sp.	Kofoid & Swezy
Heterocapsa rotundata	(Lohmann) Hansen
Oxyphysis oxitoxoides	Kofoid
Oxyrrhis marina	Dujardin
Oxytoxum sp.	Stein
Peridinium sp.	Ehrenberg
Polykrikos sp.	Bütschli
Pronoctiluca sp.	Fabre-Domergue
Prorocentrum micans	Ehrenberg
Prorocentrum minimum	(Pavillard) Schiller
Protoperidinium spp.	Bergh
Scrippsiella trochoidea	Stein (Loeblick) III
Scrippsiella sp.	Balech
EUGLENOPHYCEAE	
Euglena mutabilis	Shmitz
Euglena sp.	Ehrenberg
Eutreptiella marina	Da Cunha
Eutreptiella sp.	Da Cunha
PRASINOPHYCEAE	
Pyramimonas sp.	Schmarda
Tetraselmis sp.	Stein
PRYMNESIOPHYCEAE	
Calyptrosphaera sp.	Lohmann
Prymnesium sp.	Maxart ex Conrad
Rabdosphaera sp.	Haeckel
RAPHYDOPHYCEAE	
Chattonella sp.	Fabre-Domergue
Incertae sedis	
Ebria tripartita	(Shumann) Lemmermann
Other Flagellates	
Ultraplankton	

last particularly from February to September 2000. From 2001 the affirmation of Cyanophyceae (CYA) increases, and during the last study years they were the most important. Chlorophyceae Class (CHL) was observed in high densities in November 2000 and during the autumn and spring months of 2007 and 2008.

Cyanophyceae Class was principally represented by very small size species (the smallest size lower than 2µm) belonging to the genera *Rhabdoderma* and *Cyanobium*. The former dominated principally in summer months (its intensive proliferation in July 2002 determined the maximum peak of total density observed), whereas the second during the autumn seasons. *Cyanobium* was the most important taxon during the last two annual cycles, reaching its highest peaks during the spring of 2008. Bacillariophyceae were represented by more Centrales (*Cyclotella atomus* Hust., *Skeletonema potamus* (C.I.Weber) Hasle, *Skeletonema costatum* (Greville) Cleve and *Chaetoceros curvisetus* Cleve) than Pennales (*Cylindrotheca closterium* (Ehrenberg) Reimann & Lewin, *Fragilaria* sp.). Chlorophyceaea Class was principally represented by *Chlorella* sp., *Monoraphydium minutum* (Näg.) Kom.-Legn. and *Monoraphydium* sp..

In general, CHL *a* didn't show the same pluriannual dynamic of density (Fig. 3): the highest values were detected in summer or in autumn-winter seasons, depending on the annual cycles, and the maximum peaks were observed in June 1999 and June 2009 (197 mg m^{-3} and 183 mg m^{-3}, respectively). We can explain the different dynamics of density and CHL *a*, at least in part, considering the phytoplanktonic classes and the relative species detected during the years, with the exception of the samples corresponding to the dystrophic period at the beginning of the study. In fact, the data relative to counting of these samples could be affected by error due to the bad quality of the samples, very degraded and with abundant detritus. The highest concentrations of CHL *a* were observed in correspondence of the affirmation of classes (Bacillariophyceae, Dinophyceae, Chlorophyceae and Prymnesiophyceae) with species of a major size than those dominant among Cyanophyceae, represented by very small cells (size <2µm). The high peaks of CHL *a* in 2002 were detected together with the highest peaks of density. In this occasion, these values of CHL *a* were determined by the extremely high values of density of Cyanophyceae, despite the low cell size.

Considering the whole lagoon and the mean annual cycles, the mean annual concentrations of D.I.N. and RP ranged between 54.3 ± 8.5-172.5 ± 64 mg N m^{-3} and 20.2 ± 3.5-55.8 ± 20.2 mg P m^{-3}, respectively (Tab. 1). Maximum mean values of both nutrients were observed in each year in correspondence of ST3, that is sited near to the major freshwater input (Fig. 4). D.I.N. showed a similar seasonal dynamic at the three stations, with lower values from late spring to early fall. The months from July to October were also interested by lower pluriannual variability of the values. On the contrary, less temporal and spatial uniformity was assessed for highest values, that were in April at ST1, November at ST2 and in January at ST3. Moreover, these were the months characterised by the widest variability of the pluriannual data. These results support the hypothesis that in Cabras Lagoon D.I.N. could be highly affected by watershed discharge, due to rainfalls during wet months from late fall to early spring, that are typical of the climatic regime of Sardinia. Seasonal trend of RP showed peaks at all the stations in winter (February) and in early summer (June), accompanied by spring increments at the extreme stations (ST1 and ST3) and autumn at the central station (ST2) (Fig. 4). In general, also summer concentrations (August and September) were high, between 30 mg P m^{-3} and 60 mg P m^{-3}, and characterised by reduced variability of the puriannual values in respect to those observed in the months interested by the maxima. Months interested by very limited pluriannual variability were also February, May and July, which were characterised by very low RP values, too. These data can be interpreted considering the important role that sediments assume, especially during summer, as internal nutrient source in lagoons, as a consequence of organic matter decomposition and water mixing due to winds, which can re-suspend particles already settled in shallow environments. Therefore, only in part D.I.N. and RP seasonal dynamics coincided, leading to N/P dynamic with maxima in autumn-winter for all sampling sites (Fig. 5). The ratio values were low especially at ST3 and higher at ST1, even if the maximum was assessed at ST2. The observed seasonal tendency was similar to that reported for other Mediterranean lagoons, such as Orbetello on the Tuscany coast (Caroppo et al. 2002) and S'Ena Arrubia, in Sardinia, not far from Cabras Lagoon (Fiocca et al., 1996). The values were >16 only in January in all the stations. These results highlighted a prevalency of N-limitation in respect of P-limitation in Cabras Lagoon, on both spatial and temporal scale. Different causes can have led to this situation, from the different kinds and speeds of the biogeochemical cycles of the two nutrients, to the biological actions, very important in different steps of the N cycle and the consequent possible enrichment and depletion of N in the system (Viaroli et al., 2004). In any case, it was shown that low N/P ratio favours N-fixing cyanobacteria affirmation in aquatic environments (Romo and Miracle, 1993; Chomérat et al., 2007). This aspect has characterised the phytoplankton of the Cabras Lagoon in the major part of the considered period, as reported in the following.

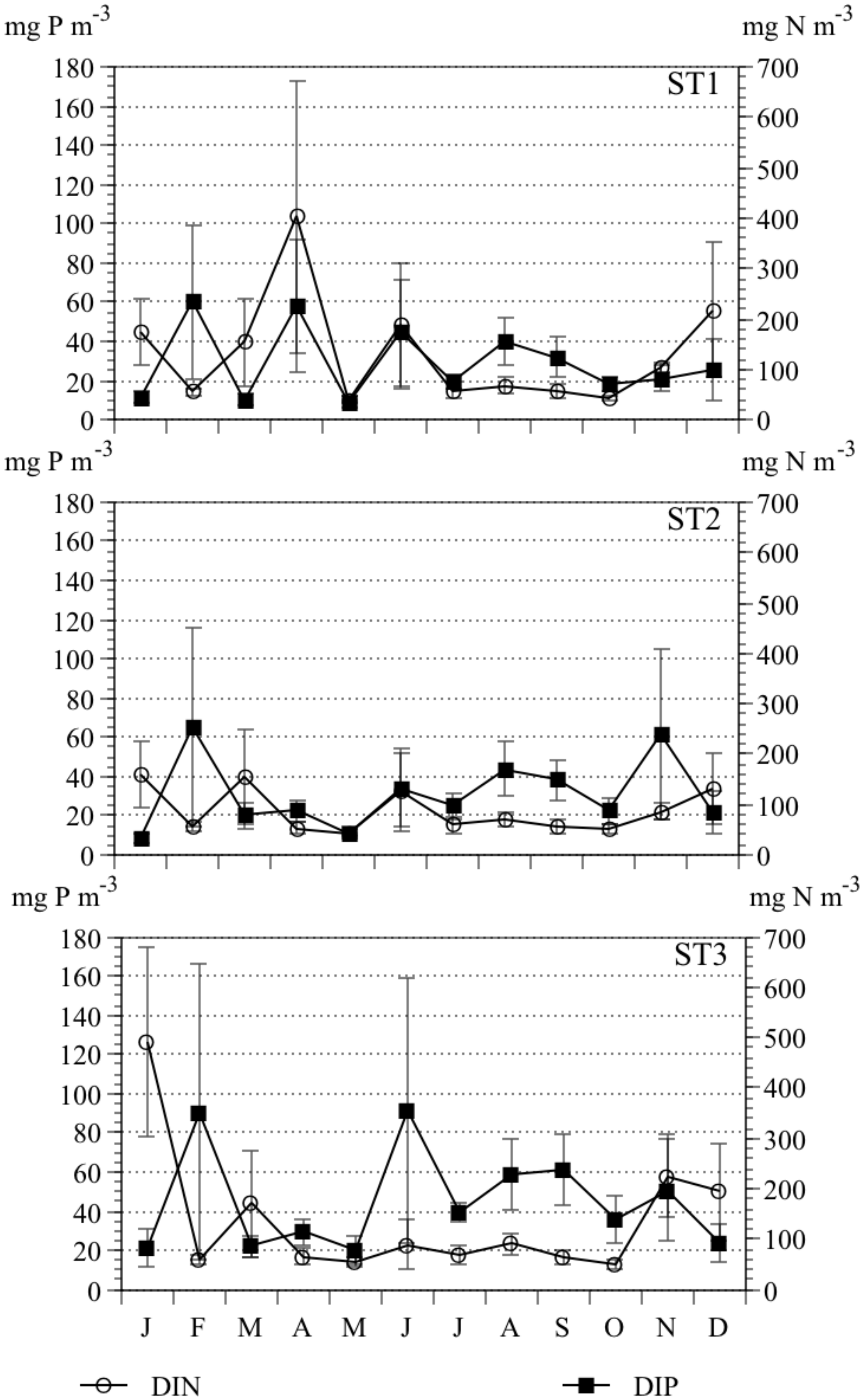

Fig. 4. D.I.N. and RP monthly mean concentrations and S.E., calculated on the pluriannual data serie, in the three stations.

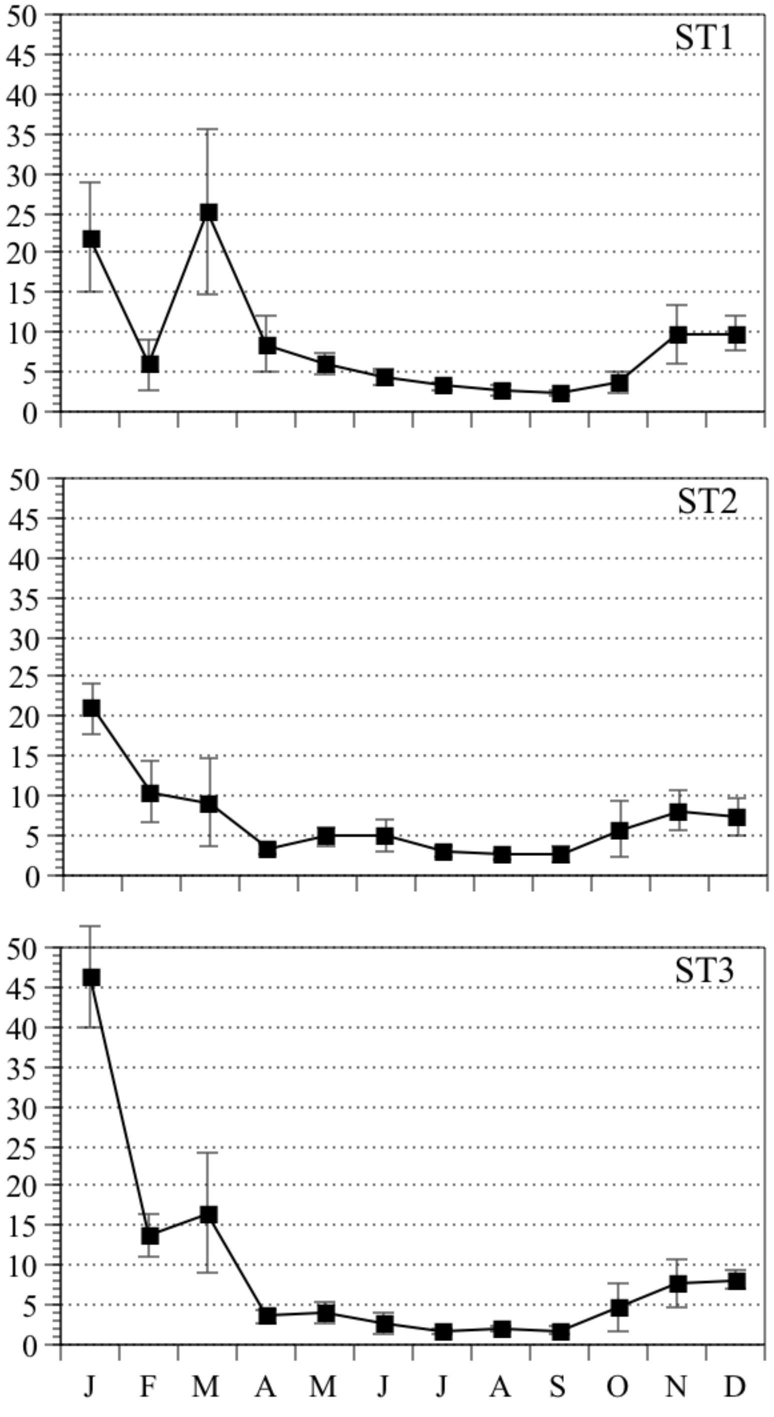

Fig. 5. N/P monthly mean values and S.E., calculated on the pluriannual data serie, in the three stations.

With regards to phytoplankton biomass, expressed as CHL *a*, the dinamic showed a clear temporal trend in each station with higher values in winter and autumn (as already seen for the analysis of pluriannual tendencies), with peaks, respectively, in January (particurlarly at ST1 and ST2), February (ST3), November and December (Fig. 6). These months were also interested by wider variability of the pluriannual data on the contrary of spring and summer months that showed lower variability. Concentrations were high all year round, only rarely less than 20 mg m^{-3} and up to about 70 mg m^{-3} in each station at least in one month. Annual mean concentrations, considering the whole lagoon, ranged between 14.2 ± 1.3 mg m^{-3} and 51.9 ± 10.1 mg m^{-3} (Tab. 1), with higher pluriannual mean value at ST3 (37 mg m^{-3} in respect to 31.9 mg m^{-3} at ST1 and 30.3 mg m^{-3} at ST2).

In the mean annual cycle, the total density of phytoplankton exhibited very high values in each station, ranging between two orders of magnitude, from more than 10^8 to more than 10^9 cells l^{-1} (Fig. 7). The dynamic at the three stations was characterised by at least three common aspects: lowest values were observed in spring (particurlarly in May), maxima occurred especially during summer (July and August) and lower pluriannual variability was assessed in May and October in respect to the other months at all the stations.

Regarding to the phytoplankton composition, Cyanophyceae was the most important one for its constant presence and because determined the highest density observed in all stations (Fig. 8). In particular, it exceeded the 90% of total density from February to March and from June to September. The Ultraplankton was relevant in all the stations during the autumn months and in January, when it reached the 50% of the total density.

Bacillariophyceae was also important (about 20% of total density) principally in autumn (from September to November) and in winter (from December to January). As already explained, the intensive proliferation of these species with a size larger than those of Cyanophyceae species and Ultraplankton could have caused the highest values of CHL *a* observed just in these months (Fig. 7).

Class composition and dominant species observed in Cabras Lagoon were notably different from those reported for other Mediterranean lagoons where other taxonomic characterisation were observed: Nanoplanktonic fraction composed by Gymnodiniaceae spp., cryptophyceans, centric diatoms, small flagellates were the most abundant in Orbetello Lagoon, where the communities were dominated by cryptophyceans and other flagellates in all the sites, with centric diatoms bloom in summer, particularly in the area where the sea-water smoothed variations of the environmental conditions, mainly temperature and salinity (Caroppo et al., 2002). In S'Ena Arrubia Lagoon, Cholorophyceae

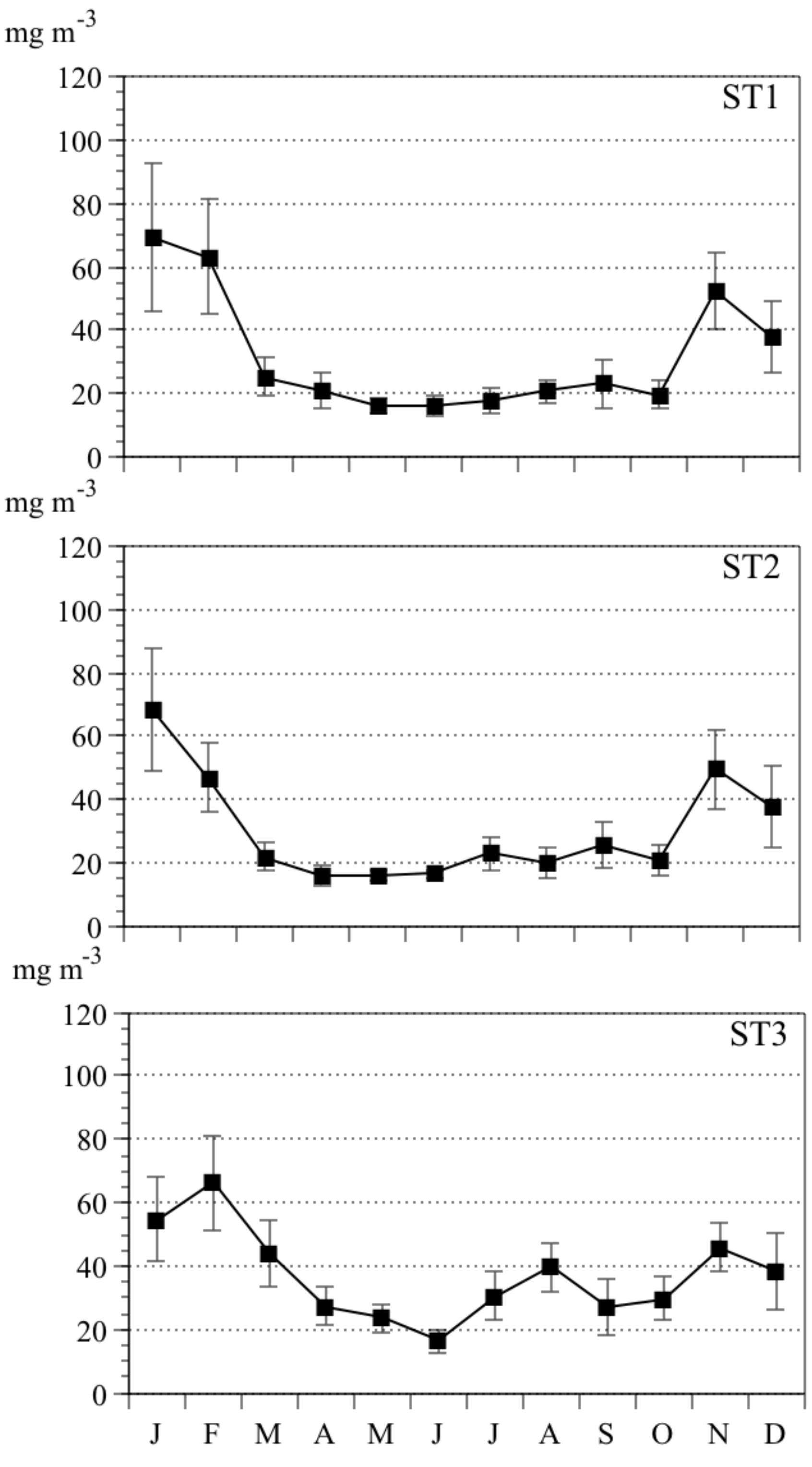

Fig. 6. Chlorophyll *a* monthly mean concentrations and S.E., calculated on the pluriannual data serie, in the three stations.

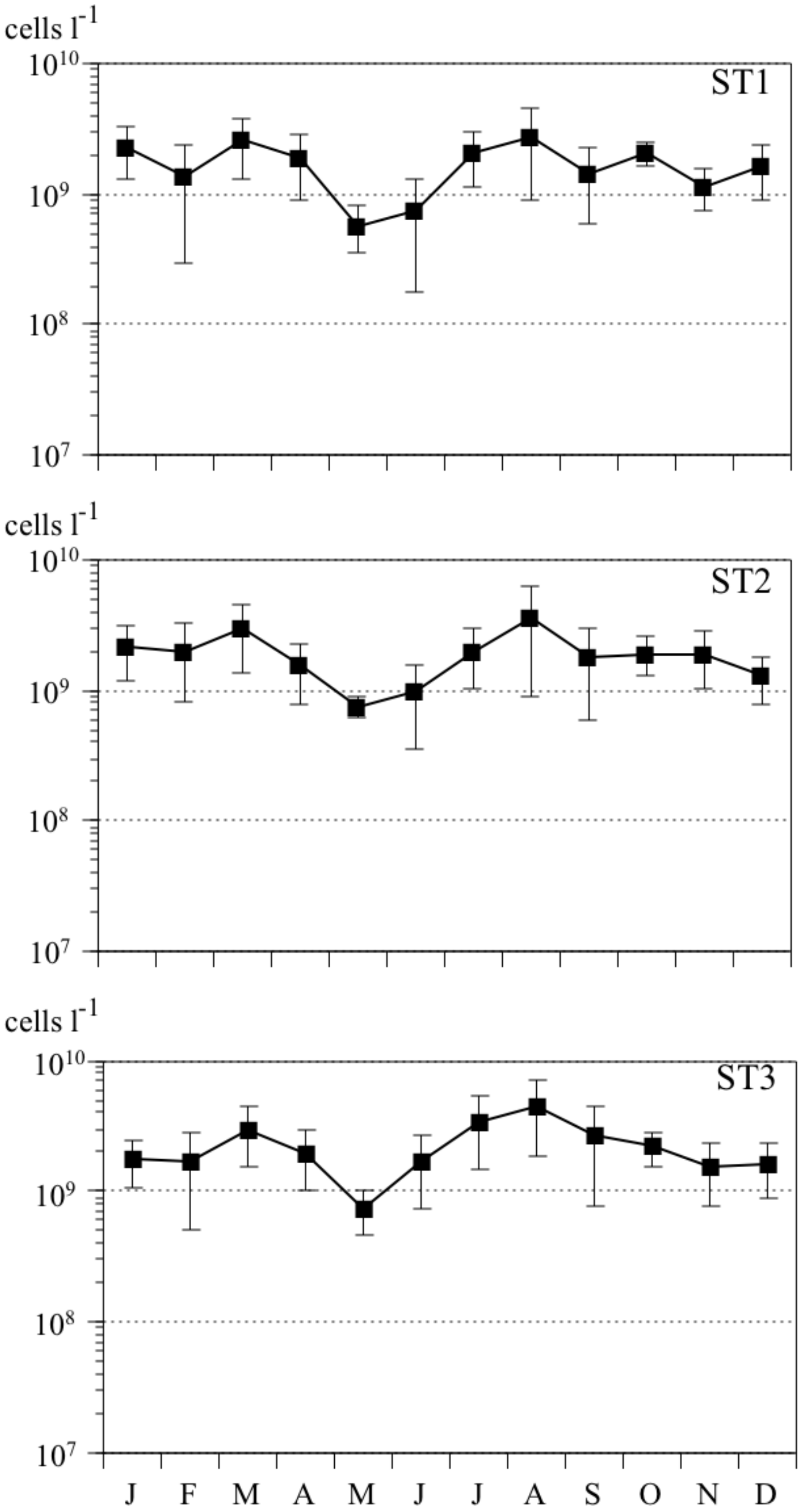

Fig. 7. Total phytoplankton monthly mean densities and S.E., calculated on the pluriannual data serie, in the three stations.

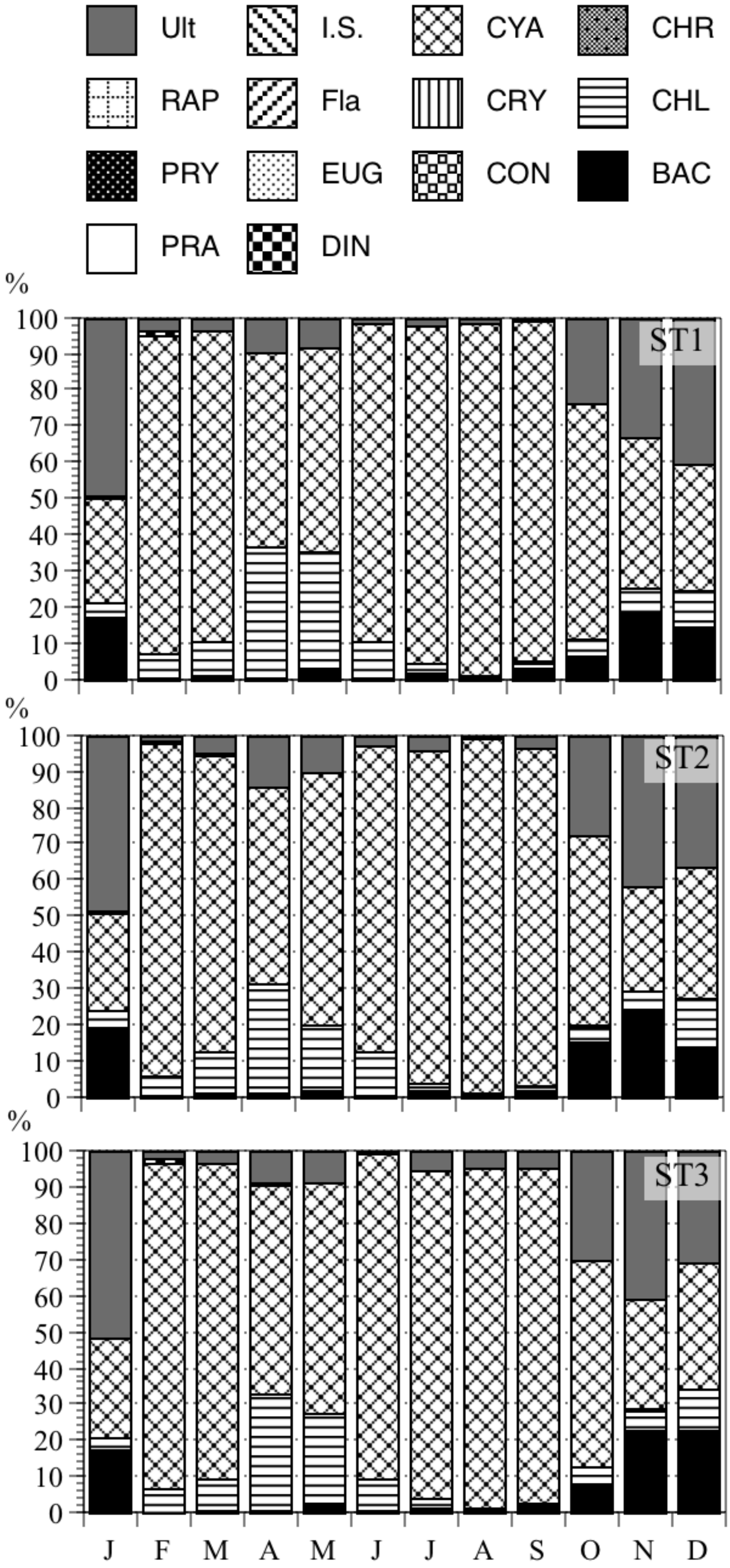

Fig. 8. Percentage class composition of phytoplankton monthly mean densities, calculated on the pluriannual data serie, in the three stations.

(principally *Chlorella* sp.) and Bacillariophyceae (principally *Cyclotella* sp.) were the dominant classes, with maximum peaks of density in summer for the former and in summer and autumn for the second (Trebini et al., 2005). In the winter period Euglenophyceae and Dinophyceae were observed in high densities. On the other hand, different orders of Cyanophyceae have been observed, depending on the environmental conditions and the season in a number of Mediterranean lagoons: in the hypertrophic Bolmon Lagoon in the south of France, a permanent dominance of Cyanophyceae was observed, with a seasonal pattern characterized mainly by *Planktothrix agardhii* (Gomont) Anagnostidis et Komárek in winter, colonial Chroococcales in spring, Pseudanabaenaceae in summer, and *P. agardhii* in autumn and then in winter again (Chomérat et al., 2007); in the hypertrophic Albufera Lagoon in Spain (Romo and Miracle, 1993) a persistent dominance of Oscillatoriales was described; in the hypertrophic lagoon system of Comacchio (NW Adriatic Sea, Italy), an extremely intense bloom of Chroococcales that persisted for many years resulted in the collapse of that ecosystem (Sorokin et al., 1996). Therefore, the environment degradation due to hypertrophic condition is indicated as favouring the development of cyanobacteria in respect to less tolerant algal species (Sorokin et al., 1996; Scheffer et al., 1997), even if, nowadays, the link between very high trophic conditions and cyanobacteria blooms has been documented only in few Mediterranean lagoons (Abrantes et al., 2006; Chomérat et al., 2007).

Consequently to the striking dominance of Cyanophyceae in late winter and summer, the Shannon diversity index clearly showed the maximum values during autumn (October and November), early winter (December and January) and spring (April and May) in all the stations, when major affirmation of Bacillariophyceae, Chlorophyceae and Ultraplankton were observed (Fig. 9).

According to the matrix of Pearson correlation coefficients (Tab. 3), Cyanophyceae abundance was significantly positively correlated with TP, RP and salinity, whereas Ultraplankton with NH_3 and N/P. Moreover, Ultraplankton was significantly inversely correlated with temperature. Bacillariophyceae were positively correlated with NH_3, TP and salinity, whereas Chlorophyceae negatively with alkalinity, DO and temperature. Dinophyceae was significantly positively correlated with pH and TP, whereas it was inversely correlated by temperature and salinity.

Cyanophyceae and Ultraplankton were negatively correlated, whereas the latter showed a highly significant positive correlation with Bacillariophyceae. Cyanophyceae class was positively correlated with Chlorophyceae.

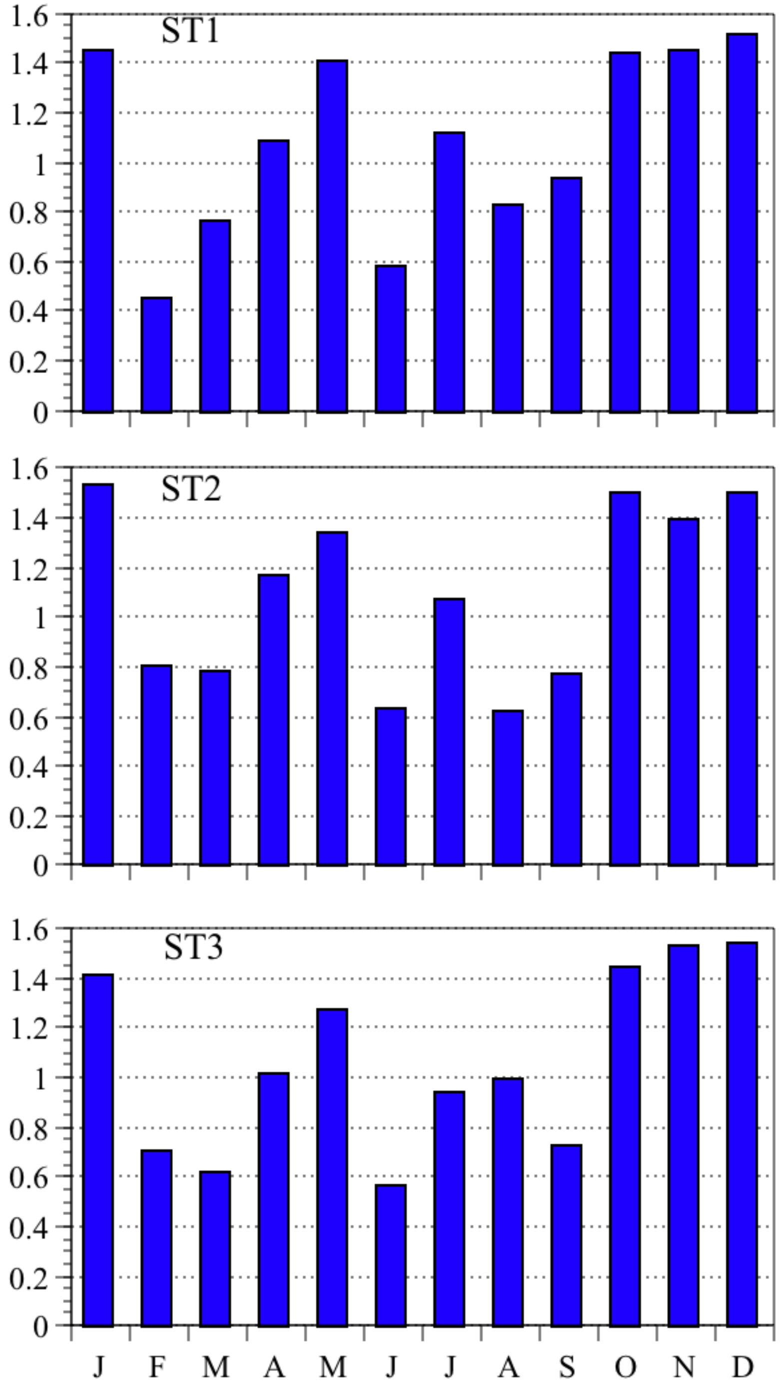

Fig. 9. Monthly mean values of the Shannon index, calculated on the pluriannual data serie, in the three stations.

Table 3. Pearson correlation coefficients between phytoplanktonic variables (CHL *a*, mg m^{-3}; BAC, Bacillariophyceae; CHL, Chlorophyceae; CHR, Chrysophyceae; CON, Conjugatophyceae; CRY, Cryptophyceae; CYA, Cyanophyceae; DIN, Dinophyceae; EUG, Euglenophyceae; Fla, Flagellate; PRY, Prymnesiophyceae; I.S., *incertae sedis*; PRA, Prasinophyceae; RAP, Raphidophyceae; Ult, Ultraplankton; all phytoplanktonic cell densities as cells l^{-1}), nutrient concentrations (RP and TP, mg P m^{-3}; D.I.N., and NH_3, NO_3, NO_2, mg N m^{-3}; N/P; RSi mg Si l^{-1}) and other values of abiotic parametres (Alk, alkalinity, meq l^{-1}; pH; DO, %; Sal, salinity, ‰; Tem, temperature, °C). Bold values are significant at a alpha=0.05.

	BAC	CHL	CHR	CON	CRY	CYA	DIN	EUG	Fla	HAP	I.S.	PRA	PRY	RAP	Ult	Alk	CHLa	DIN	NH3	NO3	NO2	N/P	DO	RP	TP	pH	Sal	Rsi	Tem
BAC	**1**																												
CHL	-0.106	**1**																											
CHR	0.195	0.239	**1**																										
CON	0.464	0.850	0.965	**1**																									
CRY	0.006	-0.075	-0.049	**1.000**	**1**																								
CYA	-0.126	**0.203**	-0.038	0.251	**-0.228**	**1**																							
DIN	-0.099	**-0.160**	-0.184	-0.011	-0.106	-0.137	**1**																						
EUG	0.032	**-0.822**	0.160		-0.529	-0.220	-0.097	**1**																					
Fla	-0.318	0.112			0.207	-0.042	0.038		**1**																				
HAP	**0.976**	-0.270	-0.234		-0.321	**0.905**	**0.881**		-0.014	**1**																			
I.S.	-0.162	-0.098			0.178	-0.446	0.125		-0.252		**1**																		
PRA	-0.103	-0.254	-0.299		0.313	-0.071	0.323	-0.387				**1**																	
PRY	-0.281	0.588			0.882	0.968	0.977						**1**																
RAP	0.270	-0.244	0.611		-0.515	-0.178	-0.057				0.918			**1**															
Ult	**0.892**	-0.041	-0.211		-0.144	**-0.197**	**-0.169**	0.008		-0.469	0.820	-0.138			**1**														
Alk	0.106	**-0.405**	0.138	0.649	0.027	-0.005	**0.162**	-0.075	-0.234	0.146	0.011	0.106	0.945	0.146	0.022	**1**													
CHLa	**0.151**	**-0.156**	**0.329**	0.412	-0.144	**0.243**	**0.203**	**0.335**	0.058	-0.149	0.089	-0.180	0.788	-0.162	-0.015	0.053	**1**												
DIN	0.009	0.075	0.198	-0.666	0.014	0.118	-0.086	-0.074	-0.004	-0.054	0.075	-0.221	0.500	-0.283	0.093	**-0.188**	0.082	**1**											
NH3	**0.223**	0.000	0.095	0.891	-0.053	**0.338**	-0.114	0.026	0.128	0.225	-0.046	-0.246	0.447	-0.176	**0.223**	0.076	**0.188**	**0.661**	**1**										
NO3	-0.061	0.093	0.215	**-1.000**	0.036	0.052	-0.063	-0.133	-0.022	-0.084	0.070	-0.166	0.866	-0.253	0.026	**-0.256**	0.033	**0.959**	**0.425**	**1**									
NO2	-0.061	0.028	0.176	-0.500	0.001	**0.185**	-0.069	-0.167	0.073	-0.265	0.331	-0.075	0.500	-0.335	-0.022	-0.105	0.053	**0.826**	**0.719**	**0.707**	**1**								
N/P	0.115	0.128	0.107	-0.969	-0.141	-0.067	0.075	0.057	0.004	-0.364	-0.061	-0.263	-0.059	-0.365	**0.140**	**-0.176**	**0.372**	**0.283**	0.071	**0.313**	**0.216**	**1**							
DO	0.007	**-0.200**	0.015	-0.500	-0.120	-0.111	0.086	**0.400**	-0.071	-0.122	0.042	-0.086	0.733	0.112	**-0.142**	**-0.147**	**0.354**	-0.036	0.098	-0.079	0.005	-0.056	**1**						
RP	**-0.145**	-0.104	-0.107	**0.999**	0.005	**0.391**	0.103	-0.065	0.131	0.232	0.027	**0.414**	0.756	0.048	**-0.179**	**0.158**	0.079	**0.269**	**0.365**	**0.188**	**0.281**	**-0.230**	0.074	**1**					
TP	**0.142**	**-0.163**	-0.040	0.869	0.020	**0.548**	**0.129**	0.101	0.188	0.360	-0.142	0.146	0.884	0.018	0.028	**0.178**	**0.472**	**0.175**	**0.451**	0.049	**0.174**	-0.072	**0.217**	**0.666**	**1**				
pH	-0.009	**-0.169**	0.051	**1.000**	-0.123	**0.259**	**0.337**	0.202	-0.023	-0.307	0.052	-0.056	-0.866	-0.149	**-0.204**	-0.043	**0.588**	-0.103	0.036	**-0.137**	-0.055	**0.147**	**0.623**	0.043	**0.345**	**1**			
Sal	**0.151**	0.043	-0.152	-0.410	-0.003	**0.335**	**-0.159**	-0.081	0.088	0.630	0.205	-0.279	-0.890	-0.415	0.127	**0.178**	-0.028	**-0.162**	**0.122**	**-0.232**	**-0.178**	**-0.220**	**-0.188**	**0.201**	**0.317**	**-0.203**	**1**		
Rsi	**-0.119**	-0.111	-0.136	0.590	-0.013	**0.298**	-0.060	0.008	0.043	-0.235	0.111	-0.130	0.854	0.176	-0.124	**-0.247**	0.064	0.083	0.084	0.066	**0.114**	-0.030	**0.222**	**0.215**	**0.270**	**0.148**	**0.142**	**1**	
Tem	**-0.344**	**-0.214**	-0.106		**-0.224**	**0.242**	**-0.259**	-0.212	-0.274	**0.725**	-0.189	-0.090		0.234	**-0.397**	**0.164**	**-0.351**	**-0.235**	-0.103	**-0.248**	**-0.142**	**-0.358**	0.004	0.098	-0.003	**-0.117**	**0.225**	**0.193**	**1**

Finally, CHL*a* was positively correlated with Euglenophyceae (EUG), Chrysophyceae (CHR), Dinophyceae and Cyanophyceae. The former results indicated that also classes which did not reach high densities, such as Euglenophyceae, Chrysophyceae and Dinophyceae could have played an important role in the lagoon.

4. Conclusions

All the variables considered showed a condition of very high trophy that it have been interested the Cabras Lagoon since 1999.

Among nutrients, D.I.N. showed annual mean similar from 2000 to 2008 (the only exception is 2007), with the maxima values observed during the autumn and winter seasons of each annual cycle. Regarding RP, after 2000, their annual means decreased and remained similar until 2008. The maxima were observed in autumn and winter seasons but also in summer, therefore, in this case is more probable a strict link with the re-suspension of the nutrient by sediments.

As regard to the phytoplankton variables, total density and CHL *a* values were always representative of the high trophic state of the lagoon. CHL *a* showed a strong decrement of annual mean concentrations from 2000 to 2008. In fact, observing the dynamic of phytoplankton composition, a shift from organisms of larger size (principally Bacillariophyceae) towards organisms of a very small size (<2μm; principally Cyanophyceae) was detected during the considered interval of time.

The results obtained demonstrated that the major phytoplankton biodiversity observed at the beginning of the study until 2002 was coincident with the major variability in the time of the values of all considered parameters. From 2007, the higher stability of these parameters for more than a year, permitted the persistent affirmation of some species of Cyanophyceae, which adapted well to live in those lagoon condition (salinity > 15‰; N/P < 16; DIN < 100 mg N m^{-3}; RP > 40 mg P m^{-3}). Only in the autumn 2008, when the strong rainfall started, the phytoplankton composition became more various, with an increase of the biodiversity, in relation to the sudden fall of salinity and the high increase of nutrients.

The repetitive character of the dynamic of some variables and the obtained correlations suggest the importance of salinity and N/P in driving of the phytoplankton seasonal composition in Cabras Lagoon, especially in the Cyanophyceae dominance. Moreover, they are also among the variables that, in the long term ecological reserches, should be observed at site level to better understand the pulses and presses of climatic changes and their interactions on natural and anthropogenical impacted systems.

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7. General Conclusions

This PhD thesis investigated the trophic state of Cabras Lagoon and the composition and the dynamics of phytoplankton in relation to the dynamics of several environmental and meteo-climatic variables. The overall objective was to assess whether in Cabras Lagoon the phytoplankton composition and its dynamics could be a possible cause of dystrophic events that have often injured the stability of the ecosystem.

Since the strong dystrophic crisis that interested the lagoon during the summer of 1999, and that caused the killing of the totality of the aquatic biota, a long time series with high frequency measurements and sampling of environmental and biological parameters (chemical-physical, nutrients, phytoplankton density and composition) has been carried out. This allowed us to observe and analyse the pluriannual tendencies of such parameters to better understand the actual environmental condition of Cabras Lagoon.

All variables considered, were representative of a very stressing situation of the lagoon, indicating a trophic level very high during all the years.

Among the nutrients, nitrogen peaks occurred in autumn-winter seasons in the majority of the annual cycles considered, due to rainfalls during wet months from late fall to early spring, that are typical of the climatic regime of Sardinia. This result is consistent with the hypothesis that nitrogen could be highly affected by watershed discharge in Cabras Lagoon. Phosphorus peaks, instead, often occurred in late summer. This could be a result of internal inputs from the decomposition and re-suspension of previously settled material caused by intense summer winds, since inputs from the watershed where very low or absent during the dry summer season. Consequently, the N/P ratio (D.I.N./RP) showed a pluriannual dynamic with the highest peaks in autumn and winter seasons, in general when the maxima of D.I.N.

Regarding phytoplankton, the pluriannual dynamics of total density has always showed very high values and a shift in the observation of the maximum peaks, from the summer and autumn seasons until 2002, towards the late winter and spring seasons from 2007. The results obtained, showed also important changes in the phytoplankton composition during the years. A major phytoplankton biodiversity was observed in the first part of the study until 2002, in respect to that observed in the last two annual cycles. Bacillariophyceae, Chlorophyceae, Cyanophyceae, Dinophyceae and Ultraplankton were dominant or co-dominant with similar importance levels until 2002. The higher variability observed at the beginning of the study was coincident with the major variability in the time of the values of the environmental parameters, namely nutrient quantities and ratios and salinity. From 2007, the higher stability of these parameters for more than a year, permitted the persistent affirmation of some species of Cyanophyceae, which adapted well to live in that lagoon condition (salinity > 15‰; N/P < 16; DIN < 100 mg N m^{-3}; RP > 40 mg P m^{-3}).

In particular from July 2007, Cyanophyceae was the most important class for presence and density reached in the lagoon and, for 18 months, it was almost exclusively represented by the order of Chroococcales, including a species probably belonging to the genus *Cyanobium* Rippka and Cohen-Bazire. This species was the most abundant phytoplanktonic taxon until the end of 2008.

The results obtained for Cabras Lagoon during these two year-long cycles provided evidence of a strong relationship between the occurrence of *Cyanobium*-like cells and the salinity of the water. The presence of such cells in the lagoon decreased enormously only after the strong rainfalls in the autumn and winter of 2008–2009, when the large input of freshwater into the lagoon caused a decrease in its salinity. In that moment, other orders of Cyanophyceae appeared, the Oscillatoriales at first and the Nostocales then. In the succession dynamics of the three orders, temporal variations in N/P ratio were also clearly important. Thus, the dominance of *Cyanobium*-type cells during this period was likely due to the low N/P ratio, in relation to its probable capacity to fix nitrogen.

In the pluriannual analysis, the repetitive character of the dynamic of some variables and the obtained correlations among them suggest the importance of salinity and N/P in driving of the phytoplankton seasonal composition in Cabras Lagoon, especially in the Cyanophyceae dominance.

As regard to the HAS, from 1999 until now, several of them were observed in the lagoon, with a scarce and sporadic presence. They principally belong to the Dinophyceae Class and none expressed dangerous characteristics during the study period.

The study of composition and density of Dynophyceae cysts in the sediments of the lagoon agreed with the pluriannual scarce presence of Dinoflagellates in the water column of the Cabras Lagoon. The results showed a strict positive correlation between this class and the salinity of the water. In fact, Dynophyceae were observed in high density only in February and March 2000, when salinity reached its maximumum values from 1999 to now. The pluriannual fluctuations, with high ranges of variations in maxima and minima values of salinity, could have strongly affected the Dinoflagellates because of only few species tolerate salinity below 20‰. In spite of the low number of cyst morphotypes recorded, our results led to an increase on phytoplankton biodiversity information, at least as regarding Dinoflagellate cyst producers, confirming the important use of benthic studies as a tool to increase this kind of information.

In conclusion, the results of this study showed that Cabras Lagoon is in critical condition due to constant high phytoplankton densities, the intense presence of Cyanophyceae of a very small size, the presence of various harmful algal species, and high trophic levels. Furthermore, this study confirms the general tendency, detected during the last years in several lagoons with a high trophy, also Mediterranean Lagoons, to observe a major affirmation of phytoplankton with small cell size on the other cell sizes. This study also confirms the strict link between the high trophic condition in lagoon environment and the dominance of Cyanophyceae.

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Appendix

Submitted for the publication in a special issue of "S.It.E. atti":

La Laguna di Cabras: stato trofico, fitoplancton e presenza di Harmful Algal Species

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Abstract

Durante gli ultimi decenni l'eutrofizzazione è stata una condizione cronica della Laguna di Cabras (Sardegna centro-occidentale) ed è stata responsabile del suo decremento produttivo in relazione all'instaurarsi di crisi distrofiche che hanno determinato pesanti morie della componente ittica. Vengono qui riportati i risultati preliminari di uno studio svolto dal luglio 2007 con la finalità di capire le dinamiche spaziali e temporali del fitoplancton, con particolare attenzione alla presenza di Harmful Algal Species (HAS), in relazione alle condizioni ambientali. La finalità è quella di comprendere le dinamiche ecosistemiche che possono determinare condizioni di innesco di crisi distrofiche. Al fine di implementare le informazioni ottenute dal comparto planctonico, sono state svolte indagini anche sulle cisti bentoniche di dinoflagellati nel sedimento, poiché a questo gruppo algale appartengono diverse specie problematiche, anche ittiotossiche. I risultati confermano la situazione di elevata trofia, con valori medi annuali del fosforo totale maggiori di 180 mg P m^{-3}. Le densità del fitoplancton sono state molto elevate e l'analisi per classi di taglia ha evidenziato una netta dominanza del picoplancton. Le classi più importanti sono state quelle delle Cyanophyceae, Bacillariophyceae e Chlorophyceae. La presenza di HAS è stata sporadica e poco abbondante, con la presenza delle sole specie *Prorocentrum minimum* (Pavillard) Schiller e *Dinophysis caudata* Saville-Kent. Lo studio delle cisti bentoniche di dinoflagellati ha portato all'individuazione di specie non osservate nel plancton, fornendo dati utili all'incremento delle informazioni sulla biodiversità del sito.

1. Introduzione

A differenza di altri ambienti simili del Mediterraneo, dove le macrofite sono il principale produttore primario, nella Laguna di Cabras tale ruolo è svolto in maniera prevalente dal fitoplancton (Sechi *et al.*, 2006). Lo studio dell'abbondanza e della struttura dei popolamenti fitoplanctonici risulta essere quindi di estrema importanza per la comprensione di una tipologia di sistema che, per definizione, è particolarmente vulnerabile agli inputs terrestri e antropici ed è considerata naturalmente stressata da frequenti disturbi e fluttuazioni ambientali (Kjerfve, 1994).

Inoltre l'intrinseca complessità del sistema planctonico in queste tipologie di ambiente, rende particolarmente necessaria l'analisi di lunghe serie temporali che consentano di fornire ricostruzioni affidabili del ciclo stagionale del fitoplancton, permettendoci di distinguere andamenti regolari e ricorrenti da eventi eccezionali e occasionali e di definire eventuali cambiamenti delle caratteristiche trofiche e biologiche determinate sia dall'influenza umana a scala locale, sia da fluttuazioni climatiche a scala globale. Per questo motivo la Laguna di Cabras è anche uno degli ecosistemi acquatici del sito "n. 14 Ecosistemi marini della Sardegna" che afferiscono alla Rete Italiana di Ricerche Ecologiche a Lungo Termine (*LTER-Italia*).

Si tratta della maggiore zona umida della Sardegna (2380 ha) ed è considerata tra le più importanti di tutto il bacino del Mediterraneo (Fig. 1). Negli ultimi anni, in relazione agli elevati apporti antropogenici di nutrienti, l'eutrofizzazione è diventata una condizione cronica che spesso è culminata, specialmente nei mesi estivi, in pesanti crisi distrofiche, che hanno comportato un forte decremento della sua produzione ittica.

Inoltre, tra le problematiche più rilevanti legate alle attività di pesca in laguna, sono comprese anche le possibili affermazioni di specie microalgali tossiche e nocive (HAS-Harmful Algal Species) che possono compromettere la componente ittica con diverse modalità: produzione di elevata biomassa e conseguenti anossie, azione meccanica diretta, produzione di ittiotossine (Hallegraeff, 1998). Molte HAS, inoltre, hanno un ciclo vitale eteromorfico, alternando stadi planctonici mobili flagellati con altri non-motili bentonici (cisti temporanee, ipnozigoti), che svolgono un ruolo fondamentale per la sopravivenza e la persistenza della specie (Anderson, 1998; Imai *et al.*, 1999). Per questo motivo diviene importante il contemporaneo studio dei sedimenti del fondo lagunare. Questi infatti possono ospitare la potenzialità di comparsa di specie nella colonna d'acqua ed i dati raccolti implementano le informazioni ottenute dal comparto planctonico anche rispetto alla biodiversità del sito.

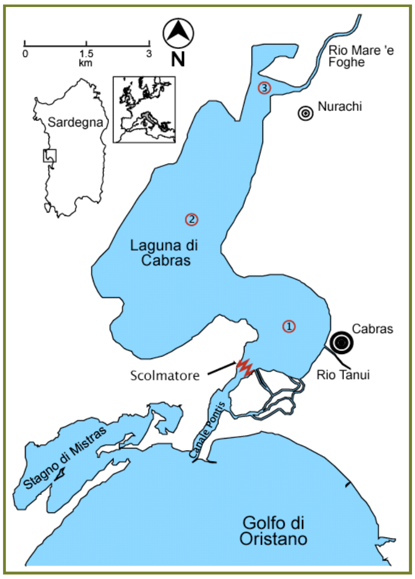

Fig. 1- Localizzazione della Laguna di Cabras e stazioni di campionamento.

2. Materiali e metodi

Dal luglio 2007 si stanno effettuando campionamenti ad intervalli quindicinali, su tre stazioni collocate in siti ritenuti rappresentativi, in base a dati pregressi, di diverse condizioni lagunari (stazione 1, in prossimità del maggior apporto di acque dolci; stazione 3, in prossimità dello scambio con il mare; stazione 2 in condizioni intermedie). *In situ* mediante il disco di Secchi è misurata la trasparenza dell'acqua e per mezzo di una sonda multiparametrica (YSI, Modello 6600-V2) è possibile rilevare la salinità, il pH, l'ossigeno disciolto e la temperatura. In laboratorio sono determinati i solidi sospesi, la silice reattiva, il fosforo totale e reattivo, l'azoto ammoniacale, nitroso e nitrico (tutti secondo Strickland e Parsons, 1972) e la clorofilla *a* (Golterman *et al.*, 1978). Per il riconoscimento delle specie algali si osservano campioni *in vivo* sia in microscopia ottica tradizionale che in epifluorescenza (Fritz & Triemer, 1985). Sono in corso approfondimenti tassonomici con l'impiego della microscopia elettronica a scansione. I conteggi per la valutazione delle densità cellulari (Utermöhl, 1931) sono effettuati su campioni fissati con soluzione di Lugol, sui quali è stata anche eseguita la stima delle biomasse (Findenegg, 1974). Per il picoplancton è adottata la metodica indicata da Maugeri *et al.* (1990). Per lo studio delle cisti sedimentarie di dinoflagellati sono state attuate tre campagne (autunnale, ottobre 2007; invernale, febbraio 2008; estiva, agosto 2008), prelevando i campioni di sedimento nelle tre stazioni già descritte, con l'uso di un campionatore autocostruito. Le carote di sedimento sono state tagliate in dieci sezioni da 1 cm di spessore. Subcampioni di ogni sezione sono stati trattati con il metodo del gradiente di densità, utilizzando il sodio politungstato (Bolch, 1997, modificato da Amorim *et al.*, 2001). La frazione finale è stata osservata al microscopio ottico invertito per la quantificazione ed il riconoscimento delle specie di appartenenza. Le cisti sono state poi isolate e messe in coltura (terreno di coltura f/2, con regime di radianza di 100 µmol fotoni m^{2}·s^{-1}, per 12:12 ore luce/buio a 18 °C) per la conferma dell'identificazione.

3. Risultati

La tabella 1 riassume i valori medi, massimi e minimi nel periodo di studio dei principali parametri considerati, per una parte dei quali viene di seguito riportato un breve commento.

Tab. 1 - Valori medi, massimi e minimi dei principali parametri considerati nella laguna.

	N	Media	Minimo	Massimo
Temperatura (°C)	90	18,14	9,4	27,69
Salinità (‰)	90	17,71	0,87	27,58
pH	90	8,28	7,61	8,83
Alcalinità (meq l^{-1})	90	2,61	1,30	3,20
Ossigeno (%)	90	87	50	130
Fosforo reattivo (mg P m^{-3})	90	23	0	275
Fosforo totale (mg P m^{-3})	90	185	53	843
Azoto nitrico (mg N m^{-3})	90	84	0	1331
Azoto nitroso (mg N m^{-3})	90	10	0	78
Azoto ammoniacale (mg N m^{-3})	90	38	4	129
Silice reattiva (mg Si l^{-1})	90	5,47	0,11	10,96
Clorofilla *a* (mg m^{-3})	90	19,15	2,81	90,51
Densità totale (10^{8} cell. l^{-1})	90	19	0,02	108
Biomassa totale (mg l^{-1})	90	6,26	0,77	26,17

Durante il periodo d'indagine la salinità (Fig. 2a) ha mostrato una notevole variabilità spaziale e temporale, con un netto gradiente dalla st.1 (media di 19,2‰) alla st.3 (media di 15,5‰) ed un incremento dei valori dall'inizio dello studio (media lagunare di 10,2‰) a novembre 2008 (media lagunare di 24,9‰), seguito da un intenso e repentino calo (media lagunare di 5,1‰), coincidente con un periodo di precipitazioni abbondanti (dicembre, gennaio e febbraio).

Sia il P totale che il DIN (N-NH$_4$ + N-NO$_2$ + N-NO$_3$) hanno mostrato durante il periodo considerato valori sempre molto elevati, con i picchi massimi negli ultimi campionamenti in relazione ai maggiori apporti d'acqua dolce nella laguna (Fig. 2b e 2c). Inoltre i valori più elevati sono stati osservati, in genere, nella st.3, cioè in quella situata in prossimità del principale apporto d'acqua dolce. Tra le diverse forme del DIN, quella ammoniacale è stata prevalente, ad eccezione che dei mesi autunnali ed invernali quando, per i maggiori apporti d'acqua dolce dal bacino imbrifero, la forma nitrica è diventata quella più importante. Il valore medio dell'ossigeno disciolto nel periodo di studio è stato prossimo alla saturazione (87%) e non sono mai stati osservati valori critici, inferiori al 50%.

La clorofilla *a* ha mostrato valori elevati nell'intero periodo di studio (media lagunare di 19,15 mg m^{-3}), con i picchi massimi negli ultimi campionamenti, coincidenti con quelli della biomassa totale (Fig. 3a e 3b). La densità totale, pur mostrando valori sempre molto alti nelle tre le stazioni (Fig. 3c), ha evidenziato quelli più bassi nell'ultimo periodo invernale e la sua dinamica è stata caratterizzata da massimi primaverili (107 x 10^{8} cell l^{-1} nella st.2 a marzo).

Particolarità del fitoplancton della Laguna di Cabras è stata quella di essere caratterizzato da specie di dimensioni talmente ridotte (0,5-1,5µm) da rendere molto difficoltoso il loro riconoscimento ed il loro conteggio. Nell'analisi per classi di taglia, considerando i valori di densità, si è osservata una netta dominanza del picoplancton in tutte le stazioni. Solo in concomitanza con la forte riduzione della salinità osservata negli ultimi mesi, si è verificata la affermazione del nanoplancton.

Gli elevati valori di densità osservati, sono stati attribuiti principalmente alle Cyanophyceae (Chroococcales), che sono state talmente importanti da aver determinato la dinamica della densità totale in tutte e tre le stazioni. Alla composizione della biomassa per classi algali hanno contribuito in maniera importante anche le Bacillariophyceae nel periodo tardo estivo e le Chlorophyceae nei mesi primaverili ed autunnali (Fig. 4).

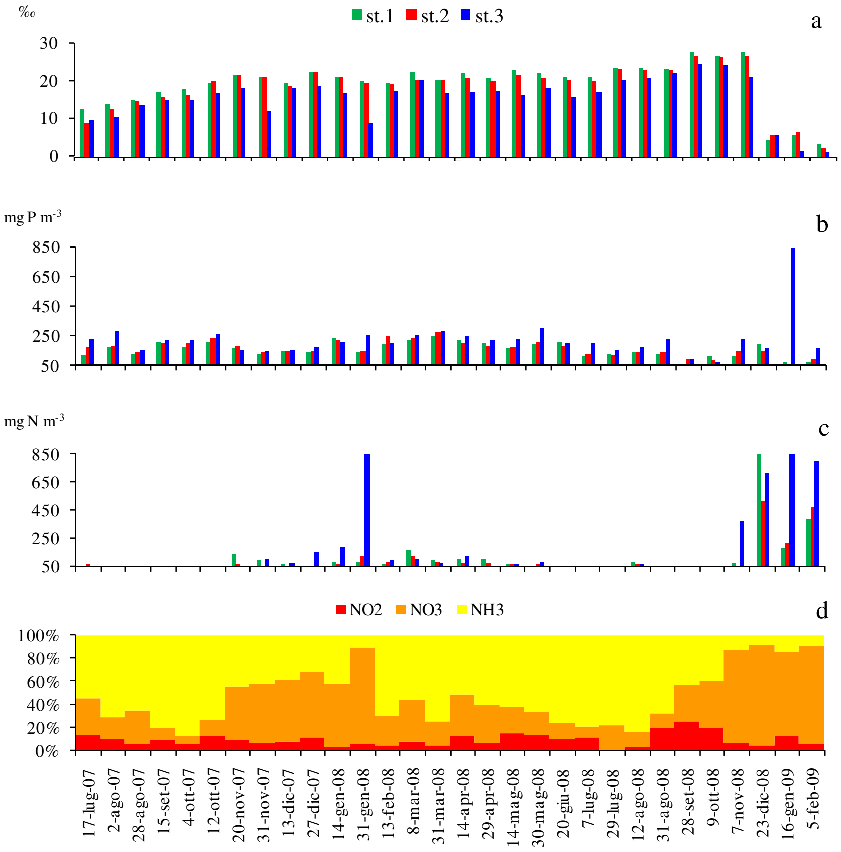

Fig. 2 - Dinamica della salinità (a), del fosforo totale (b), del DIN (c) e delle diverse forme di azoto inorganico.

Per quanto riguarda la presenza di HAS, sono state osservate *Prorocentrum minimum* (Pavillard) Schiller (VSP; ittiotossine) che, da gennaio 2009 è stato rilevato con costanza e con densità sino a 75 x 10^4 cell. l^{-1}, e *Dinophysis caudata* Saville-Kent (DSP) che è stato osservato sporadicamente e in basse densità.

L'analisi delle cisti di Dinoflagellati nel sedimento ha evidenziato maggiori densità totali nella st.2, con un incremento dei valori dalla campagna autunnale all'estiva. In generale i massimi sono stati osservati nei primi cm del profilo verticale di ciascuna carota di sedimento. In tutto sono stati osservati 18 morfotipi, tra i quali *Scrippsiella precaria* Montresor & Zingone, *Gonyaulax* complex, una Gymnodiniales e un piccolo tecato ancora in fase di riconoscimento, che non erano mai stati osservati nella colonna d'acqua durante il periodo di studio.

4. Conclusioni

L'insieme dei dati raccolti ha confermato lo stato di elevata trofia della laguna, strettamente relazionato al forte inputs di nutrienti provenienti dal bacino idrografico ed amplificato dalle particolari caratteristiche morfologiche e idrologiche, come chiaramente indicato dalla dinamica spaziale e temporale dei nutrienti e della salinità. Aspetto da non sottovalutare e ancora da indagare, è quello che riguarda il carico interno dal sedimento per il riciclo che si instaura in questi ambienti fra il fondo e le masse d'acqua (Viaroli *et al.*, 2004). L'importante affermazione del picoplancton, con una predominanza spesso osservata nel periodo di indagine, evidenzia la necessità di approfondite indagini su quali siano i fattori implicati nel controllo delle dinamiche di questo raggruppamento (nutrienti, luce, grazing). Infatti, da decenni il picoplancton è considerato il principale produttore primario degli oceani e degli ecosistemi acquatici oligotrofici (Stockner, 1988), mentre molto poco si sa ancora sulle sue dinamiche in ambienti costieri (Anxelu *et al.*, 2007) e ad elevata trofia (Carrick & Schaelske, 1997), anche se la sua importanza in questa tipologia di ecosistemi sta aumentando radicalmente (Bec *et al.*, 2005).

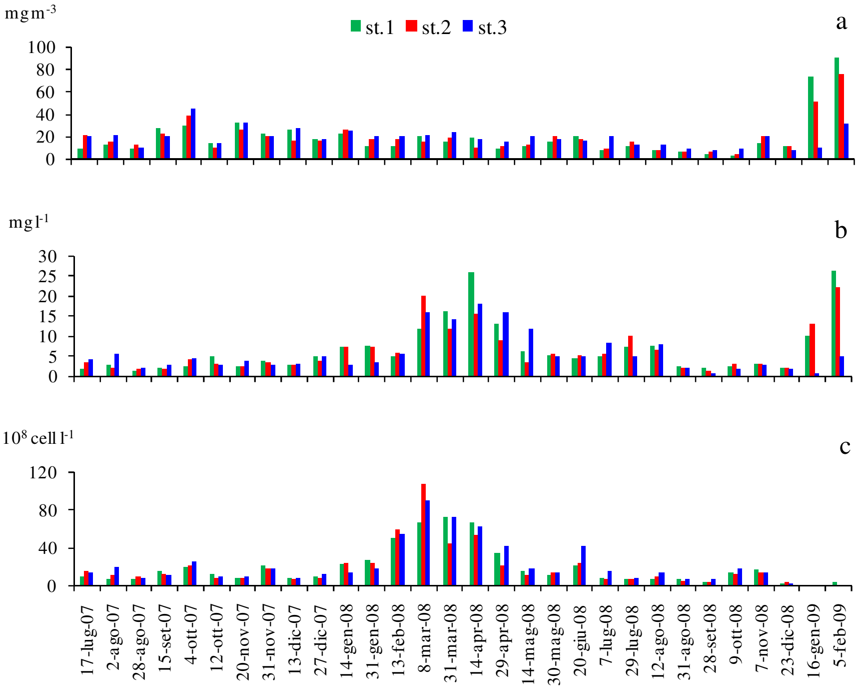

Fig. 3 - Dinamica della clorofilla *a* (a), della biomassa fitoplanctonica totale (b), della densità fitoplanctonica totale.

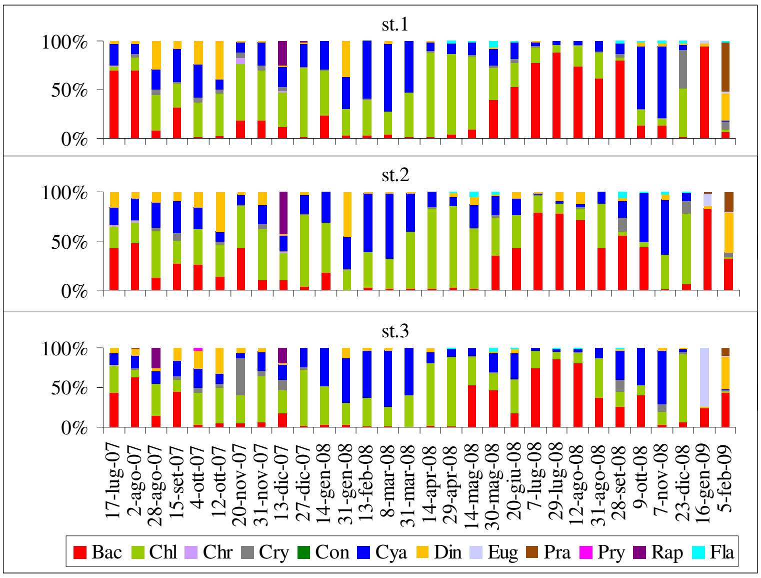

Fig. 4 - Dinamica delle composizione percentuale per classi algali della biomassa fitoplanctonica.

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Diatoms and water courses quality in North-Central Sardinia

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PERIPHYTIC ALGAE
DIATOMS
BIOLOGICAL QUALITY
DIATOM INDICES
EPI-D
IBD
WATER COURSES
SARDINIA

ABSTRACT - A study regarding eight water courses of north-central Sardinia was carried out between 2008 and 2009 to value their environmental quality and obtain the first useful indications for the management of the territory. Some significant physical-chemical and microbiological parameters were analysed on a total of 15 stations and epilithic diatoms were examined with the application of EPI-D and IBD indices. In the 21 collected samples, 152 taxa were identified belonging to 34 genera, but the diatom flora observed, was also represented by others species not yet determined with certainty. The observed and identified diatoms are common to most of the different examined sites and cosmopolitan. Among the most frequent and sometimes the most abundant taxa, *Cocconeis placentula var. pseudolineata* and *Navicula confervacea* were observed. In particular, the presence of *Achnanthes rupestoides*, that like *N. confervacea* is considered an invasive species, is noted. These species are not considered by the EPI-D method, proposed for Italian water courses. Their integration can be important to improve the method applicability in insular contexts of Mediterranean typology. EPI-D and IBD showed a good applicability with values a little different, indicating in general a mediocre and bad quality in nearly all the considered stations. The EPI-D results obtained seem still more acceptable and coherent with the delineated situation by physical-chemical and microbiological parameters and the typology of investigated environments.

INTRODUCTION

Numerous European countries regularly use benthic diatoms in the valuation of running waters quality. Some of these, to reach also the WFD2000/60/CE's objectives, introduced the methods based on diatoms in programs of annual sampling on hydrographic network (Rimet *et al.* 2005 a). For the use of these valuation systems, the right knowledge of these algal component is the fundamental presupposition. This is one of the principal motives for which the EPI-D index (Dell'Uomo 2004), especially developed and tested in the water courses of central Apenninic region, is still applied sporadically in other regions in Italy. Until now, in Sardinia diatom flora has not been studied a lot and a higher knowledge is necessary for its employment in the valuation of waters biological quality. Eight water courses in four hydrographic basins of north-central Sardinia, Mannu of Porto Torres, Padrongianu, Tirso and Mare Foghe (Fig. 1, Table I), were studied to describe the diatom assemblages and verify the applicability of the indices EPI-D and IBD. In the first three systems, possible perturbations caused by urban wastes of Sassari and Olbia and industrial wastes of Ottana were verified. On the other hand, in the Mare Foghe system, the possible impacts of intensive agricultural activities on some canals that flow into Cabras Lagoon, the largest of the island and one of the most important of Mediterranean area, were valued.

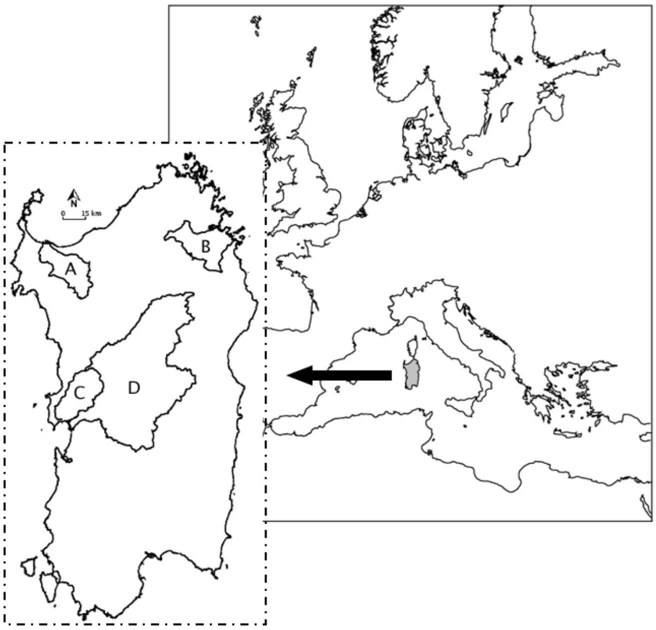

Fig. 1 Study areas and relative catchments (A = Mannu of Porto Torres; B = Padrongianu; C = Mare Foghe; D = Tirso).

Table I. Catchments, their surface, geology and water courses. * = Canals; ° = without name.

	Catchments						
	Mannu of Porto Torres		Padrongianu	Mare Foghe			Tirso
Code	A		B	C			D
Surface (km^2)	671		450	286			3400
Prevalent geological substratum	Calcareous		Granitic	Basaltic			Granitic
Nome of the water courses	Tributary of Mascari°	Mascari	Padrongianu	Pauli Gippa*	Tanui*	Iscas*	Tirso
N° stations	4	2	3	1	1	1	2

MATERIALS AND METHODS

The study was carried out on 15 stations, located in the middle-terminal reaches of four natural water courses and four canals (Table I). The collection of water and epilithic diatoms samples was effectuated in different periods between April 2008 and April 2009. In all the stations the most significant physical-chemical and microbiological parameters were measured. Temperature, pH, conductivity and dissolved oxygen were measured *in situ* with a CTD probe (YSI556), whereas alkalinity, Cl^-, BOD$_5$, soluble reactive phosphorus (SRP) and total phosphorus (TP), N-NH$_3$, N-NO$_3$, N-NO$_2$, total nitrogen (TN), reactive silica (RSi) and total suspended solids were determined in laboratory, according to Italian Standard methods (IRSA 1994). The microbiological analysis considered the research of *Escherichia coli*, *faecal* and *total coliforms* and was effectuated using the method of filtration on membrane (IRSA 1994).

Diatom samples were collected scraping the substrate, principally rocks and stones, with a small hard brush and always in full current (APAT 2005). The 21 samples totally collected were immediately fixed *in situ* with 4% neutralized formaldehyde. Contrary to other systems, in Rio Mannu of PortoTorres, subjected to heavy anthropic pressures, diatoms were collected in two samplings, respectively in winter and spring. The aim was to follow the temporal evolution of the biological water quality. The winter sampling, started in December 2008 was completed in March 2009 because of the notable flow due to elevated rainfall in three of the six stations. Diatom samples were treated in the laboratory with hydrogen peroxide (H_2O_2) and acetic acid (CH_3COOH) under heat, until the complete oxidation of the organic substance (Schrader 1973). The cleaned frustules were mounted on permanent slides using Storax resin (index of refraction 1.59). The taxonomic determination at a specific and infraspecific level was carried out using an inverted optical microscope (Zeiss, Axiovert 10) at 1000 magnification and using monographs by various authors: Bourrelly (1981), Germain (1981), Hustedt (1985), Krammer & Lange-Bertalot (1986, 1988, 1991), Lange-Bertalot *et al.* (2003). To uniform the procedure with already adopted by other European countries, the valuation of the abundance of the species was done with the count of about 400 valves and/or frustules (Ciutti *et al.* 2004). To calculate the indices EPI-D and IBD the manuals by Dell'Uomo (2004) and Prygiel & Coste (2000) were used. The results of EPI-D were converted in scale from 1 to 20 and compared with those obtained by the application of the IBD method.

RESULTS

The diatom flora of water courses

The analysis of epilithic diatoms has revealed a total presence of 152 taxa belonging to 34 genera (Table II). The diatom flora observed is nevertheless made up of other species, in some cases rare, in others infrequent and abundant, not yet identified with certainty.These taxa, even if they are not determining in the valuation of the biological water quality are of relevant interest for the biodiversity because they are part of the periphytic communities of the water courses investigated. Among the detected genera, four belong to order of Centrales and 30 to Pennales and the major number of species belong to genera *Navicula* (41), *Nitzschia* (21), *Fragilaria* (12), *Achnanthes* (11) and *Gomphonema* (10). The total number of taxa for each sample varied from a minimum of 12 in the station 4A to a maximum of 48 in the station 2D. The major part of the observed diatoms was common in several stations but some taxa resulted exclusive for only one of them. Generally, the species found are considered cosmopolitan and typical of environments with mediocre and bad quality. Among the taxa most frequent and sometimes abundant in the samples analized, *Cocconeis placentula var. pseudolineata* Geitler and *Navicula confervacea* (Kützing) Grunow in Van Heurk do not result in the list of the method EPI-D. *N. confervacea* (Fig. 2), an invasive species (Coste & Ector 2000), already observed in the north of the island (Lai *et al.* 2007), is also present in the Tirso River and in the canals of Mare Foghe system (central Sardinia). This thermophile species appeared in the spring and summer samples with a range of temperature of 11.9-22.6 °C. The presence of *Achnanthes rupestoides* Hohn (Fig. 3), another tropical taxa until now never seen in Sardinia, was highlighted. This species was present in the two stations upstream of the Rio Mascari tributary, one of the most important affluents of Rio Mannu in Porto Torres. The diatom assemblages in the canals of Mare Foghe catchment, have shown differences in the floristic composition, in respect to those of other investigated systems, with species like *Pleurosira laevis* (Ehrenberg) Compère, *Thalassiosira weissflogii* (Grunow) Fryx. and Hasle, *Achnanthes inflata* (Kützing) Grunow, *Achnanthes brevipes* Agardh, *Cymatopleura solea var.apiculata* (W.Smith) Ralfs, *Neidium dubium* (Ehrenberg) Cleve, never abundant in these stations and absent in all the other water courses investigated.

The quality of the water courses

The values of the physical-chemical and microbiological parameters are reported in Table III. Tables IV and V reports the results of the diatom indices and the range of EPI-D and

Table II. Genera and diatom taxa identified.

Genus	Taxa	Genus	Taxa
Achnanthes	11	Frustulia	1
Amphipleura	1	Gyrosigma	1
Amphora	6	Gomphonema	10
Anomoeoneis	1	Hantzschia	1
Aulacoseira	1	Melosira	1
Bacillaria	1	Meridion	1
Caloneis	3	Navicula	41
Cyclostephanos	2	Neidium	1
Cyclotella	3	Nitzschia	21
Cymatopleura	1	Opephora	1
Cymbella	6	Pinnularia	4
Cocconeis	5	Pleurosira	1
Diatoma	1	Rhoicosphenia	1
Diploneis	3	Stephanodiscus	1
Epithemia	1	Surirella	3
Eunotia	3	Synedra	2
Fragilaria	12	Thalassiosira	1
Total genera 34		**Total taxa 152**	

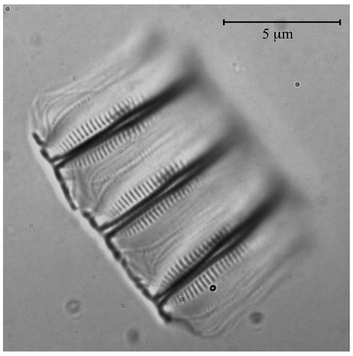

Fig. 2 Colony of Navicula confervacea. LM picture, scale bar = 5 µm.

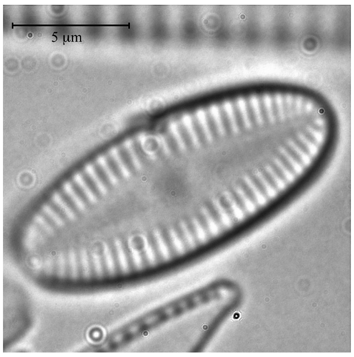

Fig. 3 Achnanthes rupestoides, valve without raphe. LM picture, scale bar = 5 µm.

IBD with the relative classes and levels of quality. Results of diatom indices are reported in the Fig. 4.

As regards the system of Rio Mannu of Porto Torres, all the investigated stations resulted with phenomena of water contamination, with significant values of algal nutrients and microbiological parameters. The diatom indices demonstrated that the most contaminated stations were the 1 and 2, those more upstream on the affluent of Rio Mascari, and station 6 on Rio Mascari, that receives urban and industrial wastes. Both the indices also indicated a decline in the quality of the waters during the spring period, coinciding with an important reduction of flows. Moreover, in the same period, a reduction of the number of taxa was observed in stations 1, 3 and 6. This sign of a probable simplification of the diatom assemblages corresponded with a decline of quality. Seasonal variation of biological quality in Mannu of Porto Torres system are reported in Fig. 5.

Table III. Results of physical, chemical and microbiological variables analysed. Results of Mannu of Porto Torres (1A-6A) system are mean values of different samplings.

Parameters		**Stations of water courses**														
		1A	**2A**	**3A**	**4A**	**5A**	**6A**	**1B**	**2B**	**3B**	**1C**	**2C**	**3C**	**4C**	**1D**	**2D**
Temperature	°C	15.6	14.0	15.4	15.4	11.2	13.2	18.0	20.3	19.2	21.9	22.4	22.6	22.4	14.1	15.5
Conductivity	$mS\ cm^{-1}$	1016	1121	1098	954	1045	1157	1008	1345	595	756	832	1227	818	480	638
pH		7.69	8.07	7.99	7.83	8.11	8.14	7.75	7.18	7.45	7.44	7.68	8.31	8.03	7.69	7.74
Alcalinity	$meq\ l^{-1}$	4.27	2.80	4.14	3.01	4.54	4.71	1.37	1.80	1.53	4.22	4.20	4.78	2.06	1.28	3.00
Dissolved Oxigen	$mg\ O_2\ l^{-1}$	8.3	9.4	8.9	9.0	9.5	8.3	9.04	8.23	7.82	4.6	6.4	11.8	10.0	9.0	8.2
Oxigen saturation	%	84	91	88	90	87	78	95	100	85	52	73	137	115	88	82
BOD_5	$mg\ l^{-1}$	2.1	2.2	2.1	7.9	2.2	3.9	2.0	5.7	1.1	1.2	3.1	5.4	3.4	3.1	3.3
Cl^-	$mg\ Cl^-\ l^{-1}$	117	144	128	125	125	129	60	142	81	99	96	177	113	-	-
SRP	$mg\ P\ l^{-1}$	0.106	0.228	0.141	0.581	0.234	0.508	0.020	0.928	0.270	0.114	0.146	1.199	0.392	0.117	0.164
TP	$mg\ P\ l^{-1}$	0.143	0.277	0.247	0.771	0.392	0.680	0.050	1.282	0.331	0.187	0.301	1.462	0.484	0.282	0.348
$N\text{-}NH_3$	$mg\ N\ l^{-1}$	0.060	0.034	0.074	0.878	0.078	0.328	0.046	0.067	0.051	0.042	0.364	0.056	0.020	0.086	0.105
$N\text{-}NO_2$	$mg\ N\ l^{-1}$	0.036	0.040	0.064	0.148	0.044	0.172	0.008	0.021	0.027	0.010	0.160	0.207	0.005	0.055	0.044
$N\text{-}NO_3$	$mg\ N\ l^{-1}$	6.324	6.405	6.501	3.101	2.113	5.721	0.460	3.320	1.356	0.342	0.366	3.810	2.086	0.492	0.629
TN	$mg\ N\ l^{-1}$	9.641	7.967	8.838	6.037	4.652	5.012	1.197	8.151	2.068	1.287	2.068	5.520	3.046	1.641	1.907
RSi	$mg\ Si\ l^{-1}$	3.62	3.47	2.30	3.30	6.91	4.55	9.24	7.76	8.91	6.04	8.03	7.66	11.02	7.41	7.83
Suspended solids	$mg\ l^{-1}$	3.5	7.9	6.7	22.7	28.6	11.0	1.8	32.2	4.2	8.5	27.0	13.0	15.5	-	-
Escherichia coli	$UFC\ l^{-1}$	17667	7333	28800	20033	1453	31500	650	10600	610	92	1700	1000	700	8000	1000
Faecal coliforms	$UFC\ l^{-1}$	17867	10489	23934	44014	9100	48350	580	14800	770	88	6700	8100	1100	12000	1000
Total coliforms	$UFC\ l^{-1}$	55467	26934	54800	73000	28534	118500	1460	40000	1760	600	26000	30700	6800	67000	24000

Table IV. Results of EPI-D e IBD in the sampling stations. Results of Mannu of Porto Torres system are mean values of different samplings.

Catchments	Water courses	Stations	Date of sampling	EPI-D	IBD
Mannu of Porto Torres	Tributary of Rio Mascari	1A	02/12/2008 - 07/04/2009	6.9	6.9
		2A	02/12/2008 - 07/04/2009	4.9	7.2
		3A	02/12/2008 - 07/04/2009	8.3	9.8
	Rio Mascari	4A	10/03/2009 - 07/04/2009	9.5	11.1
		5A	10/03/2009 - 07/04/2009	10.8	13.6
		6A	10/03/2009 - 07/04/2009	6.6	9.4
Padrongianu	Rio Padrongianu	1B	06-06-08	13.54	15.5
		2B	06-06-08	10.74	13.8
		3B	06-06-08	9.27	8.4
Mare Foghe	Rio Pauli Gippa	1C	28-08-08	10.0	11.8
	Rio Tanui	2C	28-08-08	9.36	8.6
	Rio Iscas	3C	28-08-08	7.65	5.6
	Rio Mannu	4C	28-08-08	8.27	6.5
Tirso	Tirso River	1D	23-04-09	10.83	12.2
		2D	23-04-09	10.64	13.6

Table V. Ranges of values EPI-D and IBD, classes and water quality assessment.

EPI-D	IBD	Quality	Class	Color
20 > EPI-D > 15	IBD ?17.0	Very good	I	░
15 > EPI-D > 12	17> IBD ?13.0	Good	II	▒
12 > EPI-D > 9	13.0 > IBD ? 9.0	Moderate	III	▓
9 > EPI-D > 6	9.0 > IBD ? 5.0	Bad	IV	█
6 > EPI-D > 1	IBD < 5.0	Very Bad	V	█

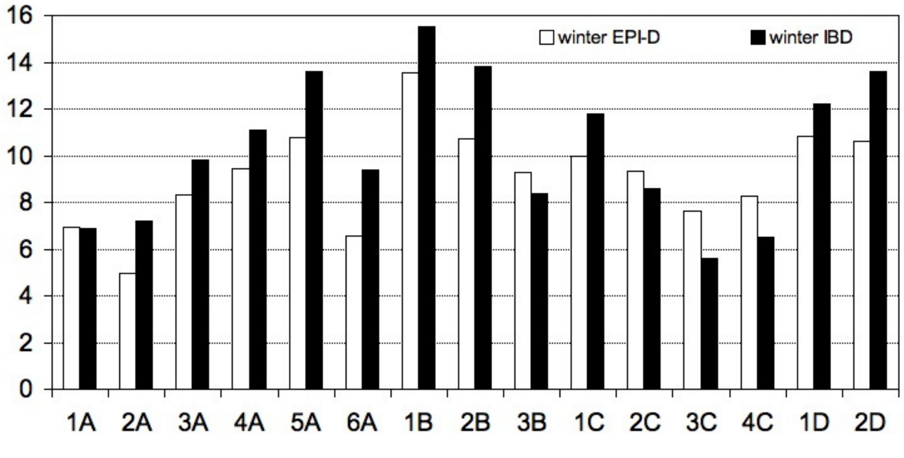

Fig. 4 EPI-D and IBD results in the different stations of the four systems (A = Mannu of Porto Torres; B = Padrongianu; C = Mare Foghe; D = Tirso).
Data of Mannu of Porto Torres system are mean values of winter and spring samplings.

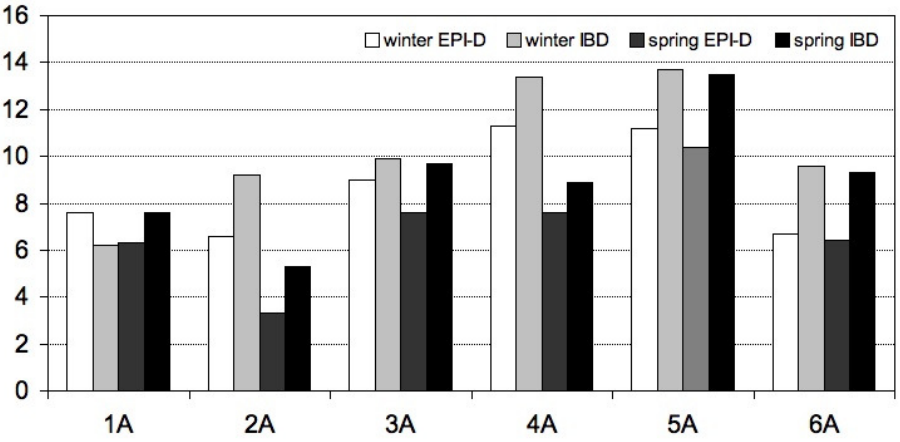

Fig. 5 Seasonal variation of EPI-D and IBD in Mannu of Porto Torres system.

For Padrongianu River the diatom indices confirmed a decline of the water quality, from upstream to downstream, highlighted also by the physical-chemical and microbiological parameters. In the highest station both indexes indicated a situation of good quality. For station 2, located in correspondence with the waste discharge of the town of Olbia, the quality in relation to EPI-D and IBD was respectively mediocre and good. The last station lowest down resulted the most polluted with a mediocre-bad quality for EPI-D and bad for IBD. The dominant species was *N. incospicua* a good indicator of meso-eutrophic environments (Dell'Uomo 2004).

In the catchment of Rio Mare Foghe, Rio Iscas and Rio Mannu were the worst in terms of quality. Both have demonstrated the highest values levels of BOD_5, $N\text{-}NO_2$, SRP, and TP. The microbiological investigation also showed a contamination of organic nature. For both water courses the EPI-D and IBD indices indicated, in agreement, a state of bad quality. The dominant species were *Nitzschia incospicua* Grunow, *Cyclotella meneghiniana* Kützing and *Bacillaria paxillifer* (Muller) Hendey. The last two taxa, in particular, are considered excellent indicators of eutrophic environments (Dell'Uomo 2004). EPI-D and IBD also indicated a mediocre-bad and bad quality for Rio Tanui, which showed higher values of nutrients and microbiological parameters in respect to Rio Pauli Gippa, its affluent. In the latter both indexes indicated a mediocre quality.

Finally, for the two stations on Tirso River, the quality was mediocre and both indexes showed a slight improvement of the biological quality of the water in station 2, downstream from the industrial area of Ottana. In this station, higher algal nutrients and lower values for the microbiological parameters were observed, too.

DISCUSSION

The study carried out offers a contribution to knowledge of diatom flora in Sardinia water courses and, at a more general level, in the Mediterranean area. The previous list of 157 taxa already obteneid with others investigations (Lai *et al*. 2007) is extended with other 50 taxa. In particular, the presence of *A. rupestoides*, that like *N. confervacea* is considered an invasive species, is noted. The observation of these tropical species maybe an important signal of the possible heating of continental freshwaters. More studies will better define the entity of the distribution of these species in waters of the island. Such considerations are true for each of the elements of the Sardinian diatom flora. To reach this result, it is necessary the knowledge of the specific composition, the distribution and the ecological needs of the taxa on different spatial and temporal scales. The gathered informations have interested until now high anthropic pressure water courses, whereas there are not indications about assemblages present in modest or absent impact conditions. For this, it is important to consider a wider number of stations along the water courses, to understand and value the dynamics on the entire considered basins. A further objective is the integration of the list of taxa actually considered by the EPI-D method, with the relevant species in the Sardinian context, to contribute to a better application of the index in the island and in general in the Mediterranean islands. The majority of the observed species is common to those indicated in the water courses in Sicily and Corse (Finocchiaro & Ferlito 2007), (Rimet *et al.* 2005 b). The lay-out of a first check-list of the observed species could result particularly useful to help future studies.

This investigation permits to confirm the utility of diatom indices also in Sardinia for the valuation of the quality of lotic waters and underlines their utility also for the management of the territory. The EPI-D and IBD methods have consented to more complete and coherent valuation in the situation of the territory, and the impacts present in respect to those given by only the traditional parameters. The results obtained were quite agreeable with those of physical-chemical and microbiological parameters and both indexes, even if with slightly different values, showed a good applicability in all lotic contexts investigated. However EPI-D values obtaneid seem more reliable. The systems of Rio Mannu of Porto Torres and of Rio Mare Foghe, resulted those with stronger phenomena of water contamination in all the stations examined. The typology of the pollution observed seemed to correspond to that of organic nature, for the most part due to agricultural and zootechnical activities carried out in the basins and to civil wastes not adequately depurated.

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