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"Phytoplankton: quality indexes, functional groups and toxicity"

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A mia nonna

E a tutti coloro che mi sono stati vicini e mi hanno sostenuta

Contents

Preface

The primary scientific centre of my doctorate is phytoplankton, especially some specific aspects of its ecology. Plankton, according to the first definition provided by Viktor Hensen in 1887, is "*all the particles of organic nature which float freely and involuntarily in open waters excluding the sediment and the littoral areas"*. The following and numerous corrections added by different authors (Reynolds, 1984; Wetzel, 2001) lead us today to define plankton as "*the community of plants and animals which live in suspension in the sea and fresh water and which is subject to passive movement created by currents and winds"* (Reynolds, 1984). Plankton occupies its substratum, the water column, in every spatial direction and its horizontal and vertical distribution is determined by the modification of environmental characteristics, such as the value of temperature, the supply of the solute, the turbulence and the intensity of the different biotic relationships. Phytoplankton, the plant component, constitutes together with phytobenthos and periphyton the photosynthetic entity of inland waters (Tonolli, 1964). In man-made lakes, because of the great variations in the water level, which can reach 10-15 meters in Sardinia (Sechi, 1986), the presence and importance of phytobenthos and periphyton is almost negligible, so that all the primary productive processes are due to phytoplankton (Sechi, 1986).

In particular, my interests were: to verify the possibility of using the approach of functional groups as proposed by Reynolds *et al*. (2002) to classify phytoplankton of Sardinian reservoirs (**thematic A**; Chapter 1); to evaluate the water quality of lotic and lentic Sardinian environments using phytoplankton and/or microalgae as bioindicators, as suggested by the WFD 2000/60/CE (**thematic B**; Chapters 2, 3 and 4); to verify the relationships between trophic states of Sardinian reservoirs and toxic Cyanobacteria blooms (**thematic C**; Chapters 5 and 6), which could invalidate the use of the waters (Bartram *et al*., 1999; Funari *et al*., 2000; Sotero-Santos *et al*., 2006).

1. General Introduction

1.1 Functional Groups

When we analyse the temporal sequence of different phytoplankton assemblages, and in particular the conditions of equilibrium or steady-state which can be seen in certain periods, we cannot fail to mention fundamental concepts, such as disturbance, diversity and competition between species.

The use of the word "succession" to describe changes in the composition of the phytoplankton comes from the term already in use by terrestrial ecologists. The definitions of the succession described by the different authors (Clements, 1916; Gleason, 1927; Margalef, 1963; Odum, 1969; 1971; Pickett *et al*., 1987; Chapman and Reiss, 1994; Colinvaux, 1995; Alard *et al*., 2005) applied to vascular plants were in accordance with the specific definitions described for the phytoplankton (Reynolds, 1980), although characterised by the different temporal scales. Owing to the frequent external disturbances, periods characterized by autogenic succession of phytoplankton, with establishing dominance and declining diversity, alternated with periods of biomass reduction and rises of diversity and photosynthetic activity (Trifonova, 1993). Successional development can thus be blocked by different environmental factors, such as, for example, intense rainfall, storms or periods of strong remixing of the water produced by the wind, which, can be interpreted as unexpected changes in the development of phytoplankton (Reynolds, 1991). Such phenomena also have a different impact, depending on their intensity, according to the moment in which they occur, and on the conditions of the lake. It is also necessary to explain the strong link which exists between disturbance and diversity (Reynolds *et al*., 1993).

According to the "Intermediate Disturbance Hypothesis, IDH" proposed by Connell in 1978, considered at first on the coral reefs and tropical rain forests, "*the disturbances are mainly events not connected to the other organisms, unpredicted, that are shown in distinct and unexpected changes in the composition and that interfere with the natural tendency of a community to reach an internal equilibrium, these events act in an intermediate period with respect to the generational phytoplankton scales*". Such a model, used universally, asserts that disturbance is a phenomenon studied and measured only as far as its effects. In fact, although the stimulus can be generally external, its effects are judged in relation to the answers that it produces within the community (Pikett *et al*., 1989).

Connell's theory (1978) states that the frequency of disturbance, induced by external forces, has an effect on the diversity of the community. Beginning from the IDH postulate, Sommer *et al*. (1993) express a series of conditions which, if satisfied, can represent the state of equilibrium: (I) 1, 2 or 3 phytoplankton species contribute more than 80% to the total biomass; (II) their existence or coexistence lasts for a rather long time; (III) during this period the total biomass does not increase significantly. Experimental conditions show that in order to reach equilibrium, a period of 35-60 days can be necessary (Sommer, 1985, 1989).

Reynolds (1997, 2001, 2002), drawing on the methods of classification previously adopted by Tuxen (1955) and Braun-Blanquet (1964) for the terrestrial environment, proposed for the first time, in 1980, an autogenic sequence of different associations or functional groups to assume as a model of the possible temporal evolution of phytoplankton in temperate lakes. At first, analysis was based only on English lakes and lead to the identification of 14 groups (Reynolds, 1980), which are now twice that number (Reynolds *et al*., 2002). At the moment 33 functional groups have been described (Reynolds *et al*., 2002; Padisák *et al*., 2003b, 2006). These regrouping are divided by using an alphanumeric code (Reynolds, 1997) though the suffix "-etum", typical of the association of terrestrial plants, is no longer in use. The assemblages of phytoplankton populations are considered on the basis of morpho-functional similarities and differences of the species which coexist in a determinate environment (Reynolds *et al*., 2002). The assemblages of species and their temporal successions are the best and most reliable indicators of the changes in environmental conditions, rather than the evolution of the presence or absence of single specific components (Reynolds *et al*., 2002). Because the dominance of the functional groups is conditioned by the occurrence of a specific spectrum of trophic conditions (Reynolds 1977; 1999), they can play the role of indicators of the ecological quality of a lake. Their distinction reflects the simultaneous reaction of individual species to environmental variables, among which are the heating or cooling of the water, mixing caused by the wind and thermal stratification (Reynolds, 1984b; 1988; 1995, 1997; Reynolds *et al*., 2002) variation of some chemical-physical parameters, or low irradiation and high quantity of nutrients, or, on the contrary, high radiation and low quantity of nutrients (Huszar and Caraco, 1998).

In the work of 2002, Reynolds proposes the use of the methodology based on the functional groups in different aquatic contexts, in order to set up the necessary basis for the evaluation of its possible application and its results.

For the Mediterranean area the proposal has been accepted by the realisation, at the moment, of not more than ten works (Albay and Alçaalan, 2003; Naselli-Flores and Barone, 2003). Man-made lakes are an important element in this climatic area of which there have been very few studies on phytoplankton ecology. Very a few studies (Naselli-Flores and Barone, 2003) were conducted to evaluate phytoplankton ecological succession by the approach of the functional groups as proposed by Reynolds *et al*. (2002), either for seasonal events or the operational regime used for human activities.

The objectives of my research on this theme were (a) the classification of phytoplankton of Sardinian reservoirs following Reynolds *et al*. (2002) via the floristic associations, the species composition during the ecological succession in the variability of the environment in which it is found; (b) this improvement of predictability, on the basis of the temporal succession, of the initiation, the growth and maintenance and the dispersal/dissipation/termination of potentially toxic Cyanobacteria blooms. These events are able to invalidate the use of the waters. The possibility to manage them provide a support to the authority of the water source, for its best use and a minor energy cost in the phase of water treatment. It was on this basis that I conducted a large part of my doctorate experimental activity. In particular my research was conducted on six reservoirs located in the centre and in the north of Sardinia: Bidighinzu, Cedrino, Cuga, Montelerno (Pattada), Sos Canales and Temo (Monteleone Roccadoria) in cooperation with l'En.A.S., Ente Acque Sardegna, the authority that manages these reservoirs.

1.2 Quality Indexes

The Directive 2000/60/CE (WFD, Water Frame Directive) of the European Parliament and the European Council 23 October 2000, has set up an account for Community action for the question of water (Water Frame Directive), to which Italy is conforming. Specificly, for the ecological quality of water it is required the evaluation on the basis of the present communities. It is because they are able to integrate the impulses coming from the abiotic component and from the biotic component, changing them into adaptation forms.

The hydromorphological, chemical and chemical-physical elements are believed to support the biological ones, which become in that sense a priority. This is the most important new element from the WFD. The phytoplankton was indicated for the lakes as one of the biotic elements available to the evaluation of environmental quality, and the benthonic Diatoms in lotic environments.

The use of phytoplankton, at different taxonomic levels, species or higher taxa, for the evaluation of water quality has a long history. Many indexes have been so far developed for lakes (Thunmark, 1945; Nygaard, 1949; Hörnström, 1981; Brettum, 1989; Tremel, 1996; Schönfelder, 1997; Salmaso *et al*., 2006; Marchetto *et al*., 2006) and flowing water (Ghetti, 1986; 1997; Siligardi *et al*., 2000; Dell'Uomo, 2004). Nevertheless none has so far been widely accepted, and moreover, few were the indexes developed for the Mediterranean lakes. My interest was directed to this aspect, in the ambit of the collaboration with I.S.E.-C.N.R. (Verbania-Pallanza) and with G.I.G. (Geographic Group of the Intercalibration). My objective was first directed to find which indexes already developed in other Mediterranean countries (Catalàn, Agència Catalana de l'Aigua, 2003, and Brettum, Dokulil *et al*., 2005) and Italy (PTI, Salmaso *et al.*, 2006), could be applied also in the Mediterranean reservoirs (Marchetto *et al.*, 2006). The second fundamental objective in this part of my thesis was to collaborate in the building of a specific index that, better than others already known, could be useful to value quality of our reservoirs, (Padedda *et al*., 2007). Still in the ambit of this aspect of my thesis, I collaborated to evaluate the quality of eleven water courses in the north of Sardinia by the application of the EPI-D (Dell'Uomo, 2004), an index based on benthonic Diatoms, worked out and successfully experimented in the central Appennin region.

1.3 Toxicity

Harmful Algal Blooms-HABs is the term used to indicate the proliferation of microscopic algae that can manifest damage connected to high cellular densities and potential production of toxins (WHO, 2002). The interest for the HABs is ingrowing expansion and it is due to increasing world-wide reports, including the Mediterranen area (Cook *et al*., 2004), and also due to acknowledgement that the impact the HABs can have on human health, the environment (including various forms of life) and the economy (Anderson *et al*., 1993; Chorus and Bartram, 1999).

In the lakes the production of toxic substances by the Cyanobacteria in the main source to which humans can be exposed if the water is used for drinking or for recreational activities (Funari *et al*., 2000). Cyanobacteria are the group of prokariot organisms, to be found in practically all of the planet. In eutrophic and ipereutrophic water bodies, Cyanobacteria often dominate the summer phytoplankton (Bartram *et al*., 1999) and they can accumulate in the surface water, sometimes forming a so-called bloom concentrating on the surface as foam, in calm or the absence of wind (Skulberg *et al*., 1984). A particular set of environmental situations promote an intense growth of Cyanobacteria: a certain stability in the water column with low turbulence and water velocity, due to light or absent wind, high insolation and favourable morphometry of the water system; a low nitrogen:phosphorus ratio, high levels of nutrients, a water temperature which must be between 15 and 30 °C, and pH values between 6 or 9 or higher (Costa and Silva, 1969; Skulberg *et al*., 1984; Carmichael, 1994; Codd, 1996; 2000; Mur *et al*., 1999; Xie *et al*., 2003). It should be noted that not all Cyanobacteria produce toxins, but the number of the species which can produce them has increased (Carmichael, 1994; Lawton *et al*., 1994).

Toxins are contained within the living cells and can be released into the water in the senescence period or at the time of the death of the cells (Falconer, 1993). The high concentrations of toxins liberated into the water could also be due to the collapse of the Cyanobacterial bloom or damage to the bacterial cells (Jurczak *et al*., 2005). The toxins present in solution sometimes pass through the normal process of treatment and are resistant to boiling (Falconer *et al*., 1989). The blooms or flowerings of Cyanobacteria can cause some of the most damaging effects on the aquatic ecosystems, and also a negative effect on man and nature

(Sotero-Santos *et al*., 2006) such as contamination of food products, diseases in fish and death of animals (Mez *et al*., 1997; Falconer, 1999). The mechanisms by which cyanotoxins act at present described and known are rather diverse and cover epatotoxicity, neurotoxicity and dermatotoxicity to a more general inhibition of the protein synthesis. These act at a neurotransmitter level (neurotoxins), of the epatic cells (hepatotoxins), of the grastroenteric system (enterotoxins) and of the epidermis (dermatotoxins) (Skulberg *et al*., 1984; Carmichael *et al*., 1985; Volterra, 1989; Carmichael, 1997). The toxins are divided into three wide molecular chemical groups: Cyclic Peptides, Alcaloids and Lipopolysaccarides (LPS) variously distributed in the different genera (Sivonen and Jones, 1999).

The World Health Organization (WHO) has confronted the problem and has published a value as a guide line to specify the quality of drinking water as 1 μg l^{-1} for the microcystin-LR (L-lisine; R-arginine) (WHO, 1998). The microcystins are more widely distributed geographically in freshwater (Bartram *et al*., 1999) compared to the other toxins which Cyanobacteria can produce.

The human populations more subjected to both the acute and chronic contaminations by the cyanotoxins, are those whose supply is due to the use of the waters with origins in eutrophic lakes or some way dominated by the Cyanobacteria. This is the case of Sardinia, whose primary drinking water and irrigation source is represented by reservoirs, a lot of them, more than 90% eutrophic (Marchetti *et al*., 1992; Sechi and Lugliè, 1992; 1996).

The objectives of my research conducted on this thematic area in my doctorate, in collaboration with Istituto Superiore di Sanità (Roma) were to evaluate (a) the frequency and intensity of the toxicity events due to Cyanobacteria in some Sardinian reservoirs whose waters are allocated to production of drinking water and (b) the relationship that link such events with the trophic levels of the lakes.

Thematic A: Functional Groups

Chapter 1:

Functional groups of freshwater phytoplankton in Mediterranean reservoirs (Sardinia, Italy) in response to disturbance events.

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Abstract

Intrannual variations of phytoplankton functional groups were studied in 6 Mediterranean reservoirs (Bidighinzu, Cedrino, Cuga, Pattada, Sos Canales and Temo), situated in the north of Sardinia (Italy). The study period (June 2006-July 2007) was distinguished in steady or non-steady state phases in each reservoir, following Sommer's *et al.* criteria (1993). The state was primary characterised in relation to the allogenic disturbance represented by the variations of the Zm:Zeu mean values, considering the influences that meteorological variables (wind velocity and solar radiation) can have on the ratio. In addiction to this disturbance other influences that can act on the selection of phytoplankton species, such as specific ecological characters of the different reservoirs and their hydrological management, were taken in account. The nutrients were evaluated also to distinguish the different seasonal phases in relation to the presence of different functional groups.

In each reservoir, annual cycle was distinguished in a number of periods, from a minimum of 4 (SCN) to maximum of 8 (CED and PAT). In general, the number of non steady-state phases was always higher in each reservoirs, with the only exception of SCN, with an equal number. Altogether, 12 steady-state phases were found considering all the reservoirs, 8 of them in condition of strong stratification and 4 in situation of complete circulation of the water column. The main functional groups found during the steady-state phases were **P**, **A** and **Lm**. Instead 27 non steady-state were found, 20 in the stratification and 7 in the mixing phases. In correspondence of non steady-state periods **Lm**, **Y, A** and **P** were the most abundant. Globally 13 functional groups were observed in all the reservoirs, the major part of them was widely distributed, on the contrary **W2** was important only in BID, **J** in CED, **R** in PAT, **C** in SCN, **G** in TEM, **F** in CED and SCN and **D** in BID and CUG.

Keywords: Phytoplankton functional groups, steady-state, meteoclimatic disturbance, phytoplankton biomass

1.1 Introduction

An assemblage (Reynolds *et al*., 2002; Rojo & Álvares-Cobelas, 2003) is a group of natural populations of similar organisms (e.g. similar taxa, such as fishes, ciliates, or microalgae, as in the case of phytoplankton) that show to coexist in similar environmental conditions. In the case of phytoplankton, this could not be the result of common ecological tolerances and sensitivities of the present taxa (Huszar *et al*., 2003) but, at least for a part of them, the consequence of accidental events which may have introducted the species by advection or entrainment, for example, from benthos or littoral area. Functional Group (Reynolds *et al*., 2002) is the term that appears more appropriated if we want to refer to sets of appropriate adaptive specialisms and the clusters of species that share them. It is a different way to approach species composition and diversity in respect to a more traditional view, which focuses on taxonomic dominance at different levels. It offers the clearest way into understanding and predicting the distributions and dynamics of natural populations of phytoplankton (Reynolds *et al*., 2002). The presence or the dominance of different functional groups is in relation to steady (or equilibrium) or non steady-states (i.e. non-equilibrium), a concept often used to explain community ecology (Komárková & Tavera, 2003; Huszar *et al*., 2003). In aquatic environments, the steady-state of the water column is considered a key factor in controlling the structure and composition of phytoplankton (Harris, 1983; Dokulil & Teubner, 2003; Morabito *et al*., 2003), whereas to explain the presence of phytoplankton species for most of their time in a non steady-state condition it is often appropriate to refer to the Hutchinson's (1961) "paradox of the plankton". In fact, in nature, non steady-state conditions are the rule rather than the exception. The steady-state is identified as the result of a dynamic equilibrium due to the absence of disturbance or in presence of an extended and continuous disturb (Connel, 1978).

Connel (1978) proposed the "Intermediate Disturbance Hypothesis" (IHD) to explain the relationship between community diversity and frequency of disturbances. In fact, on one hand frequent disturbances make the coexistence of many species difficult, on the other, infrequent disturbances allow competition. So, in both cases, the diversity remains low, whereas an intermediate disturbance permits a relevant increase of diversity.

Sommer *et al*. (1993) provide a set of conditions that, when satisfied, can help to identify a steady-state condition and corresponding periods of dominance of particular species groups:

(1) 1, 2 or 3 species of algae contribute to more than 80% of total biomass, or at least 50-80% of total abundance (Komárková & Tavera, 2003);

(2) their existence or coexistence persists for long enough (*sensu* Sommer, 1985; 1989). Experimental investigations demonstrate that a period of 35-60 days to achieve an equilibrium state is required;

(3) during this period the total biomass does not increase significantly.

It is evident that lake morphologies together with recurrent seasonal cycles of combinations of main environmental variables provide repetitive competitive "arenas" that allow the "best adapted" species dominate repetitively in certain periods of the seasonal succession (Padisák *et al*., 2006). The physical structure of the system (hydrology, temperature and light), the availability of nutrients (phosphorus, nitrogen, silica and carbon) are the most important abiotic controlling factors of phytoplankton (Reynolds, 1980). Some of these factors are also influenced by peculiar meteorological conditions, that modify the physic and chemistry of the water column (Morabito, 2001).

These are the basis for identifying phytoplankton assemblages as functional groups (Reynolds *et al*., 2002). At the moment, 33 functional groups are described (Reynolds *et al*., 2002; Padisák *et al*., 2003b). Studies on phytoplankton as functional groups have been carried out on a wide casuistry of lake typologies, such as NW England lakes (Reynolds, 1980, 1997), Alpine lakes (Morabito *et al*., 2003 Teubner *et al*., 2003; Dokulil and Teubner, 2003; Salmaso, 2003; Sommer, 1986) and Mediterranean lakes (Albay and Alçaalan, 2003; Naselli-Flores and Barone, 2003). A limited number of studies with this kind of approach concerns reservoirs, which are an important target inside ecological studies, mainly due to the allogenic instability typical of these water bodies (Thornton *et al*., 1990). For example, the operational regime produced by human activities, such as anthropogenic management, alters the hydrological cycle of the man-made lakes and these alterations may interfere with the 'natural' dynamics of phytoplankton. That is particularly true in reservoirs located in semi-arid climates, as demonstrated for Sicilian lakes (Naselli-Flores and Barone, 2000). In these cases, allogenic factors change the expected trajectory of phytoplankton, and other algae

groups adapted to those perturbations can appear (Reynolds, 1993). Marked differences have been reported between the ecology of natural lakes and reservoirs, although the growth and biomass of phytoplankton are controlled by similar parameters (Wetzel, 1990; Komárková & Hejzlar, 1996).

In this research, the abundance and distribution of Reynolds's functional groups are described in relation to steady or non steady-state conditions and to allogenic disturbances in 6 Mediterranean reservoirs (Sardinia, Italy).

For each reservoir, the chemical-physical parameters were also described to explain the intrannual phytoplankton dynamic during each period, obtained following Sommer *et al*. (1993). The wished results could be useful also to improve water management by the increase of predictability of initiation, growth, maintenance and decline of problematic assemblages, such as those comprising potentially toxic Cyanobacteria, on the base of the temporal succession.

1.2 Study area

The 6 reservoirs are located in the north-west Mediterranean, in the northern part of Sardinia (Figure 1). They are Bidighinzu (BID), Cedrino (CED), Cuga (CUG), Pattada (PAT), Sos Canales (SCN) and Temo (TEM). All these ecosystems are eutrophic (E) or ipereutrophic (IE) and only one (SCN) is mesotrophic (M), on the basis of about 30 years of observations (Sechi and Lugliè, 1992) (Table 1). Anyway, the trophic state is very variable, ranging in each reservoir from a low to a very high level, year by year. It depends mostly on the different hydrological and climatic conditions and on the water management.

Their surface area ranges from 0.3 km^2 (SCN) to 4.4 km^2 (PAT). Theoretic maximum volumes range between 4.3 10^6 m^3 (SCN) to 55.4 10^6 m^3 (TEM). The mean depths are in the range from 7.3 m (BID) to 26.5 m (CED). The water use is mainly for drinking and, subordinately, for irrigation (especially CED, CUG, PAT and TEM) (Table 1).

Due to a peculiar semi-arid climate, Sardinian reservoirs show more or less stable stratification periods from summer to autumn, while they are completely mixed during the rest of the year.

A meteorological station is located near the dam of each reservoir. This allowed to record main meteorological variables such as solar radiation, wind velocity and direction, and the amount and frequency of precipitations during the study period.

Moreover, inside each reservoir, there is an automatic system that records profile of temperature, dissolved oxygen (D.O.), pH, and fluorimetric chlorophyll *a*, daily and along the water column. Water-level fluctuation of the reservoirs is calculated about every week, with sensors positioned near the dams.

1.3 Materials and methods

Sampling and chemical analyses were carried out from June 2006 to July 2007 with a different time scale: fortnightly from June to September, monthly from October to December, bimonthly from January to May and again monthly in the following period. Reservoirs were sampled in a station located about 500 m from the dam. Samples were taken with a Niskin bottle at different depths: 0 m, 1 m, 2.5 m, 5 m, 7.5 m, 10 m, 15 m and, below this, at intervals of 10 m down to the bottom. Transparency was measured with a Secchi disk (SD).

Nutrients analysis were performed within 4 hours from the sampling to assess the concentrations of ammonium nitrogen ($N\text{-}NH_4^+$) (Fresenius 1988), nitrate ($N\text{-}NO_3^-$), reactive (SRP) and total phosphorus (TP), soluble reactive silicate (SRSi) (Strickland and Parsons, 1972). Chlorophyll *a* was analysed according to Goltermann *et al*. (1978).

Samples for phytoplankton analysis were fixed with Lugol's solution and cell density was evaluated at 200x and 400x magnifications, according to Utermöhl's method (Sournia, 1978 and Innamorati, 1990), using an inverted-microscope (Zeiss, model Axiovert 10). Species were identified using taxonomic monographs by: Bourrelly (1972, 1981), Germain (1981), Huber-Pestalozzi (1938, 1941, 1942, 1955, 1961, 1968, 1982, 1983), Hustedt (1985), Komárek & Anagnostidis, (1999, 2005), Krammer e Lange-Bertalot (1986-1991). Biomass was estimated using specific biovolumes, obtained by geometrical approximations, according to Findenegg (1974).

1.3.1 Data treatment

To highlight and describe the seasonal variations of phytoplankton, the sets of collected data were re-arranged to obtain synthetic parameters as follow.

Temperature, chlorophyll *a*, and biomass were averaged over the epilimnetic zone, whereas nutrients ($N\text{-}NH_4^+$, $N\text{-}NO_3^-$, SRP, TP, SRSi) were averaged over the entire column.

The depth of the mixing layer (Zm) was assumed to be the top of the metalimnion, estimated from temperature and dissolved oxygen (D.O.) profiles in each reservoir. The euphotic depth (Zeu) was considered equal to 2.5 the Secchi disk depth (Zeu=2.5*ZSd).

Consequently, according to Naselli-Flores (2000) and Naselli-Flores & Barone (2000), Zm:Zeu ratio (mixing depth:euphotic depth) was used to explain under-water light conditions. In particular, the variation over 1 time of the standard deviation from the mean value of the ratio was considered a physical perturbation of the water column (Rojo & Álvarez-Cobelas, 1993c).

Shannon index (H') (Shannon & Weaver, 1963) for the phytoplankton assemblages was determined according to the following formula:

$$H' = - \sum (BV_n/Bv_{tot}) \log_2 (BV_n/Bv_{tot})$$

were $\log_2$ is the base 2 logarithm, BV_n is the biomass of a given *n*-species (obtained multiplying the mean *n*-species biovolume for the correspondent density), while BV_{tot} is the total biomass of the sample and N is the total number of found species.

Results of data treatment were grouped to identify different homogeneous periods for each examined reservoir, following Sommer *et al*. (1993).

In particular, the periods were characterized also in relation to disturbance events as indicated by Rojo & Álvarez-Cobelas (1993c), considering as main discriminator factors of disturbance the variation of Zm:Zeu ratio, the water-level fluctuations, and additionally, the presence of stratification or mixing in each reservoir.

1.4 Results

1.4.1 Meteorological data

Considering data relative to 2006, the values of annual total wind velocity were always from about 70 x 10^3 km y^{-1} (BID, CUG and TEM) to about 90 x 10^3 km y^{-1} (CED and PAT). The maximum was registered in SCN (about 98 x 10^3 km y^{-1}).

During the study period (June 2006/June 2007), the maximum as absolute value was observed in PAT (894 km y^{-1} on the 13th April) and, globally considering all the reservoirs, the maxima ranged from this value to 471 km y^{-1} in BID (29th May). The number of events characterised by wind velocity equal at least two times the annual mean value of each reservoir was very high in CED (37), during winter and spring. It was followed by SCN (34) and TEM (31) whereas the minimum number was observed in CUG (16), PAT (10) and BID (5).

Precipitations were particularly abundant during the winter season. Considering all the reservoirs, the annual mean was of about 500 mm y^{-1} on about 40 rainy days. CED showed the highest annual mean of 893 mm distributed on 63 days and with an extraordinary event registered on the 30th of January (200.4 mm in a day). The minimum annual mean was observed in CUG (461.4 mm y^{-1}). The lowest number of rainy days was in TEM (31). The reservoir with the highest annual solar radiation was BID (1676.1 kwh m^2 y^{-1}), and the lowest was SCN (1582.7 kwh m^2 y^{-1}). Generally, the mean solar radiation for all the system was of about 1600 kwh m^2 y^{-1} (Table 2). The higher values were always registered during summer months (June and July) and the lower in winter (December and January).

1.4.2 Nutrients and trophic status

During the study period (June 2006/June 2007), the maximum as absolute value of TP was observed in BID (541 mg P m^{-3} in October) and globally the annual mean values ranged from 30 mg P m^{-3} (SCN) to 342 mg P m^{-3} (BID). The maximum values were distributed mainly in autumn when the thermal stratification disrupted. A similar dynamic was observed for N-NH_4^+, too. The maximum as absolute values was observed in BID (1736 mg N m^{-3} in September). The annual mean values ranged from 27 mg N m^{-3} (TEM) to 659 mg N m^{-3} (BID). The absolute maximum of N-NO_3^- was observed in CED (1436 mg N m^{-3} in March). The annual means ranged from 172 mg N m^{-3} (CUG) to 540 mg N m^{-3}

(CED). Higher values were distributed mainly from the late August to the beginning of October when there were rain events.

The absolute maximum of chlorophyll *a* in the photic zone was observed in BID (70.25 mg m^{-3} in July) when the annual means ranged from 4.4 mg m^{-3} (TEM) to 37.1 mg m^{-3} (BID). The peaks already ranged from the reported value to 12 mg m^{-3} in TEM in April. The maximum as absolute value of total biomass in the photic zone was observed in CUG (64.94 mg l^{-1} in October). The maxima ranged from this value to 2.87 mg l^{-1} in TEM in July. The biomass annual means varied from 6.55 mg l^{-1} (CUG) to 1.26 mg l^{-1} (SCN) (Table 3). For both this parameters, the events characterised by high values were registered prevalently in the summer and autumn periods.

1.4.3 Bidighinzu reservoir (BID)

Phytoplankton of BID reservoir showed the maximum phytoplankton growth in autumn. The annual dynamic was characterised by higher values in summer-autumn months than in winter-spring months, both for biomass (about 15 mg m^{-3} in October) and Chlorophyll *a,* (about 100 mg m^{-3} in October). Class and species dominance varied during the year: in winter the most abundant species were *Cryptomonas* spp. and *Trachelomonas* spp.; in summer, more species were important, such as *Stephanodiscus* sp., *Ceratium hirundinella* (O. F. Muller) Schrank, *Aulacoseira granulata* (Ehr.) Ralfs, *Anabaena flos-aquae* Bréb. ex Born. et. Flah., *Trachelomonas* spp. and *Synura* sp.; the number of important species decreased both in autumn (*Cryptomonas* spp., *Pediastrum simplex* (Meyen) and *Carteria* sp.) and in spring (*Stephanodiscus* sp., *Cyclotella* spp. and *Trachelomonas* sp.). Considering Zm (Figure 2a), BID was stratified from June 2006 to October and showed a total circulation of the water from October to May 2007; subsequently a new phase of stratification started. The water level showed a regular decrease from June 2006 to December, followed by an increase until June 2007. The most different values from the annual mean (-11.33 m), which can be considered autogenic disturbs, were between June 2006 (from -6.66 m to -8.79 m) and July and between October and December (from -14.32 m to -16.02 m).

The mean annual concentration of TP (342 mg P m^{-3}) confirmed that BID is the most eutrophic among the studied reservoirs (Lugliè and Sechi, 1993; Sechi and Lugliè, 1996; Sechi, 2000; Lugliè *et al.,* 2001). The maximum value was 541 mg

P m^{-3} in October, when biomass was also highest. N-NH_4^+ showed an annual mean of 659 mg N m^{-3}, with the maximum values between summer and autumn, during stratification. N-NO_3^- annual mean was 363 mg N m^{-3} and the maximum was achieved in winter, during circulation and in correspondence of major freshwater input from watershed. SRSi showed a similar dynamic. The maximum of 9.51 mg Si l^{-1} was observed in winter (annual mean, 5.19 mg Si l^{-1}).

According to the established criteria (Sommer *et al*., 1993) and considering the seasonal cycle, 7 periods were identified (Table 3a; Figures 2): 3 steady-state phases, one of which was during stratification (period III) and 2 during the mixing (periods V and VI); 4 non steady-state phases, all during the stratification (Periods I, II, IV and VII). Total biomass was significantly different among the period: the minimum was assessed in the steady-state period in spring (VI; mean of 1.752 mg l^{-1}), whereas the maximum was registered in the non steady-state period in autumn (IV; mean of 9.595 mg l^{-1}), with the correlated highest Shannon index value (mean of 3.3).

Period I, a non steady-state phase, was from June 2006 to 1Jul/06 and showed: a low value of biomass (mean of about 3 mg l^{-1}); the co-dominance especially of the functional groups **D** and **W2**, while the others single groups were lower than 10% (**F** and **H1**); a small variation of Zm:Zeu ratio in respect to the annual mean due to an increase of Zm; a significant variation of the wind velocity (mean value 194 km d^{-1} and direction of 207°; Table 3a); a decrease of the water level of about one meter in all the samplings (Table 3a; Figures 2a and 2b).

During the period II, a non steady-state phase in July, some of the main variations observed were: an higher biomass value (mean values about 6.155 mg l^{-1}); a changed importance of the main functional groups **W2** and **H1**, which represented more than 90% of the total biomass; a small variation of Zm:Zeu ratio in respect to the annual mean due to an increase of Zm and decrease of Zeu; a small variation of the wind velocity in respect to the precedent period (160 km d^{-1} and 180°; Table 3a); a further decrease of the water level (Table 3a; Figures 2a and 2b).

In the period III, the first steady-state phase (August), the biomass did not show wide variation in respect to the previous period but it was made up about for 80% by **Lm**, **P** and **A**. A significant increase in the wind velocity was reported during this period (mean value 234 km d^{-1} and direction of 224°; Table 3a). Moreover,

there was observed a decrease of the water level of about two meters as mean value in respect to the previous period (Table 3a; Figures 2a and 2b).

In the period IV, non steady-state phase from September to October, the number of functional group with similar importance increased with **Y** (*Cryptomonas* spp.), **J** (*Pediastrum simplex* Meyen) and **A** (*Cyclotella* spp.) as the main. The relative importance of species not yet considered in the Reynolds's list was confirmed and they are here reported as n.d. group.

The passage between period IV and V was marked by the inversion of the tendency of the water level variations. In fact, from the minimum level observed (-16.02 m), the values increased by more than one meter in the following sampling (Table 3a; Figures 2a). This period, the second steady-state phase, from December to February, was interested by a net decrease of total biomass (mean value of 4.076 mg l^{-1}) and the reduction of the number of co-dominant functional groups mainly represented by **Y**, **W2**, **A** and n.d (*Laghereimia* sp. and *Carteria* sp.).

This period corresponded to the end of the stratification and Zm:Zeu ratio was significantly different from the mean value (over 1 time the SD). This condition could be in relation to more than one factors, such as lowest mean value of the solar radiation (2 kwh m^{-2}) (Table 3a) and the decrease of transparency, due to the input from watershed.

The period VI, a subsequent steady-state phase, from April to May, was characterised by the presence of a different set of functional group but in low number also in this period: **A** and **D**. They dominated the total biomass (about 80-90%) which did not show significant changes in respect to the values of the previous period. Period VI was characterised by a wind velocity of 180 km d^{-1} and a direction of 191°.

In the last period VII, non steady-state phase, corresponding to June 2007, the sum of the dominant assemblages amounted for about 73%, with a composition very similar to that of period VI (**A** and **D**, with a decrease of group **D** in favour especially of groups **J, Y, H1** and **W2**). As summary, an increase of wind velocity was observed (288 km d^{-1} and a direction of 290°).

W2, **D**, **Y**, **A** and **Lm** were the most important functional groups in non-steady-state conditions, whereas **Lm** and **P** during steady-state phase in stratification conditions and **Y**, **D** and **A** in steady-state phases during mixing.

1.4.4 Cedrino reservoir (CED)

Phytoplankton of CED reservoir showed the biomass and chlorophyll *a* maxima in August (5.284 mg l^{-1}, and 54.25 mg l^{-1} respectively) and October (3.441 mg l^{-1} and 33.93 mg l^{-1} respectively) with the annual dynamic characterised by lower values in winter (Jan) and summer samplings. Chlorophyll *a,* had a peak in spring (21.21 mg l^{-1}). Class and species dominance varied during the year: the most abundant species were *Cyclotella* spp., *Sphaerocystis* spp. and *Staurastrum gracile* Ralfs ex Ralfs, in winter; a wider number of species was important in summer and among them *Sphaerocystis* spp., *Anabaena spiroides* Kleb., *Cosmarium* spp. and *Oocystis* spp.; *Cryptomonas* spp., *Anabaena planctonica* Brunnth. and *A. flos-aquae* dominated in autumn whereas *Cyclotella* spp., *A. granulata* and *Pediastrum borianum* (Turp.) Menegh..

Considering the depth of Zm (Figure 3a), CED was stratified from June 2006 to September and showed the vertical circulation of the water from October to June 2007, subsequently a new phase of stratification was assessed. The water level decreased regularly from June 06 (-1.23 m) to October (-8.57 m). It remained constant in the subsequent sampling (1Oct/06) but showed not regular increases in the followings. It was highest in June 2007 (-0.5 m) and remained the same in July 2007. The mean annual concentration of TP was 80 mg P m^{-3}, typical of eutrophic condition. The maximum value for $N\text{-}NH_4^+$ was registered at the beginning of autumn, at the end of stratification (mean of 349 mg N m^{-3}). The mean annual concentration was of 123 mg N m^{-3}. $N\text{-}NO_3^-$ maximum was registered in spring (mean of 1063 mg N m^{-3}), with an annual mean of 540 mg N m^{-3}. It was the most important inorganic nitrogen compound. The SRSi didn't occur at very high levels (annual mean of 2.22 mg Si l^{-1}), with the maximum concentration registered at the end of the study period (mean of 3.08 mg Si l^{-1} period VII) (Table 3a).

The seasonal cycle of phytoplankton in CED was divided in 8 periods (Table 3a; Figures 3): 2 steady-state phases, one during stratification (period I) and one during the mixing (periods VI) and the other 6 in non steady-conditions.

The maximum value of total biomass was registered during the period V, a non steady-state phase (as absolute value of 3.440 mg l^{-1}) when the minimum value of Shannon index (0.6) was observed.

Considering the functional groups, in the period I, a steady-state phase between June to July 2006, was observed the co-dominance of two main assemblages **N** and **F**, together accounting for more than 80% of total biomass. In this period water level (-1.23 m) was significantly different from the mean value (-4,31 m) (more than 1 time the SD) as reported in Figure 3a. The wind velocity was about 186 km d^{-1} and the direction was 131°.

The **F** assemblage was the most important in the period II, a non steady-state phase, in the first sampling of August. It did not exceed a percentage of 30% of the total biomass. In this phase the maximum of the variability (3.8 Shannon index) was registered and the wind had a velocity of 172 km d^{-1} and a direction of 179°.

In the periods III, a non steady-state phase in the second sampling of August, there were assessed a very important affirmation of two functional groups: (90%) **H1** and **Y**. The wind velocity increased in respect to previous period (225 km d^{-1}) and the wind direction changed too (152°).

The period IV, a non steady-state phase, showed at the beginning a net decrease of the importance of **H1** (69-74%) group which represented more than 50-60 % at the end. Other co-dominant groups, especially during the first sampling were **Y**, **F** and **A** This period was characterised by a significant negative variation of the water level with the lowest mean value (-8.47 m), an higher value of wind velocity in respect to the previous period (from 225 km d^{-1} to 297 km d^{-1}) with a different direction (from 152° to 159°) (Table 3a).

In the period V, a non steady-state phase (2Oct/06), the most important group was **Y**.

There were the higher variations of the Zm:Zeu ratio, caused by a net increase of Zm (from 16 m to 21 m) due to the intensity of wind velocity (as absolute value from 417 km d^{-1} in 1Oct/06 to 300 km d^{-1} in 2Oct/06) and a corresponding decrease of the Zeu depth (from 4 m to 1-1.7 m) (Table 3a; Figures 3a and 3b), with a decrease of solar radiation (from 5 kwh m^{-2} to 4-3 kwh m^{-2}) (Table 3a).

The following period VI, a steady-state phase from November to January, was characterized by the affirmation of the group **A** (*Cyclotella* sp.) followed by **F** (*Sphaerocystis* sp.) and **P** (*S. gracile*). The wind velocity decreased (124 km d^{-1} and a direction of 137°).

Also the beginning of the period VII was interested by the dominance of groups **A** and **P** but at the end the co-dominant groups changed: **N**, **P** and **J**. The wind velocity was of 179 Km d^{-1} and a direction of 150°.

A very low dominance by single functional groups in the species was observed in the period VIII, a non steady-state phase, which was characterised by the highest Shannon index value (3.8). The main group was **Y** but it represented not more than 20%. It was registered a wind velocity of 220 km d^{-1} and a direction of 174°.

To sum up **A**, **Y**, **H1**, **F**, **N** and **P** were the most important functional groups in the non steady-state-periods, **N**, **F** and **P** the main groups during steady-state phase in stratification conditions and **A, F** and **P** those of steady-state phases during mixing.

1.4.5 Cuga reservoir (CUG)

Phytoplankton of CUG reservoir showed the biomass and chlorophyll *a* maxima in autumn (36.045 mg l^{-1} and 21.2 mg l^{-1} respectively) even if the peaks were not coincident (64.935 mg l^{-1} in October and 30.45 mg l^{-1} in June, respectively). The annual dynamic was characterised by higher values in this season than in summer and winter-spring months. Biomass differed with isolated peaks also in winter (2.744 mg m^{-3} in November) and spring (2.755 mg m^{-3}, 3.626 mg m^{-3} in May and June, respectively). Class and species dominance varied during the year: in winter the most abundant species were *Microcystis wesembergii* (Kom.) Kom. in Kondr., *Woronichinia naegeliana* and *Cyclotella* spp.; a similar number of species were important in summer, *Oocystis* spp., *Pediastrum duplex* Meyen, *Closterium aciculare* T. West, ,*Cyclotella* spp., *C. hirundinella*, *Alaucoseira granulata*, and *A. planctonica*, autum, (*Aphanizomenon flos-aquae,* (L.) Ralfs, *M. aeruginosa*, *Microcystis* wesembergii, Ceratium *hirundinella, Cyclotella* spp. and *A. granulata*), spring (*Stephanodiscus* spp., *Cyclotella spp*., *Alaucoseira distans*, *Oocystis* spp., *M. wesembergii*, *M. flos-aquae* and *M. aeruginosa and Aphanizomenon flos-aquae*). Considering the depth of Zm (Figure 4a), CUG was stratified from June 2006 to the beginning of August and than from April to June 2007. The vertical circulation of the water started from the end of August to February 2007. The water level decreased from -4.79 m to -11.62 m in the period June - August 2006 then increased slowly up to –8.31 m in October, reaching a value lesser than in June 2006. The annual mean of TP was

86 mg P m^{-3} typical of eutrophy. The maximum was find in winter (166 mg P m^{-3} in September), on the contrary the lower values were registered during the summer months (44 mg P m^{-3} in June). $N\text{-}NH_4^+$ showed an annual mean concentration of 65 mg N m^{-3}. The maxima were found in summer during stratification. $N\text{-}NO_3^-$ maximum was in winter period (459 mg N m^{-3} in April) and its annual mean was 329 mg N m^{-3}. The SRSi, as in CED, did not occurred at very high levels (annual mean of 2.01 mg Si l^{-1}), with the maximum in April (4.71 mg Si l^{-1}).

The seasonal cycle of phytoplankton in this reservoir was divided in 6 periods (Table 3a; Figures 4). Only one steady-state phase was identified in this reservoir and it was during the mixing period (period V) whereas the other 5 non steady-state phases were found principally during stratification (periods I, II, III, IV and VI).

The non steady-state period in June 2006 (I), the dominant functional groups were **D**, **Lm**, **F**, **H1** and **P**. The wind velocity was of 232 km d^{-1} with a direction of 198°; it was observed a variation in the water level of about 2 meters (-5.37 m) in respect to the mean values.

Period II, a non steady state phase in July, was characterised by the groups **A** and **H1** which dominated for more than 90% of the total biomass. The wind velocity was lesser (167 km d^{-1}) with a direction of 150°. The water level decreased again.

In the period III, a non steady-state phase from July to September, the co-dominance was less evident with **F**, **J**, **P** and **A** as the most important groups. The percentages of the other species were high in this period. In the sampling of September chlorophyll *a* reached the maximum. The wind velocity increased up to 235 km d^{-1} as a mean with a direction of 202°. A further negative variation of about 2 m of water level was registered in the first two samplings.

In the period IV, non steady-state phase, interesting the month of October, the minimum value of Shannon index (mean of 1.2) was assessed in concidence to the maximum of biomass (64.934 mg l^{-1} in October). It was dominated by **H1**, **Lm** and **A** (*Cyclotella* spp.) functional groups. The wind velocity was lesser than in the previous period (108 km d^{-1} an the direction of 155°).

During the period V, (Jan/07 and Feb/07), there were Zm:Zeu ratio value very higher than mean value, due to an increase of Zm values (from about 14 m to 15-16 m) and a decrease of Zeu values (from about 2 m to 1 m) (Figures 4a and 4b).

In this period was also observed an increase of the wind velocity (from 108 km d^{-1} to 141 km d^{-1}) without a sensible variation in the direction (mean of 155°).
It was the only steady-state phase but it was longer than the others, from November 2006 to April 2007. It was observed the evident predominance of a restricted number of functional groups: **A**, **P**, **Lm**, and, but at a very lower level, **Y** and **H1**.
The following period VI, a non steady state phase from May to June 2007, showed a wider co-dominance between the observed functional groups, without a really prevalence of one on the others, **A**, **Lm**, **H1**, **D** and **F** were the most abundant. The wind velocity was of 158 km d^{-1} and the direction of 185°.
As general summary, the most important functional groups in the CUG reservoir were **A**, **P** and **Lm** during the only one steady-state period, observed in mixing condition, and **D**, **H1**, **A**, **Lm** and **F** an higher number during non steady-state when the reservoir was stratified and when it was in circulation **A**, **P**, **H1** and **Lm**.

1.4.6 Pattada reservoir (PAT)

Phytoplankton of PAT reservoir showed the higher biomass values in summer (4.184 mg l^{-1} in July) winter (3.061 mg l^{-1} in January) and spring periods (3.209 mg l^{-1} in March), than in late summer and autumn months. Chlorophyll *a* showed the maximum values at the beginning of summer season (10.71 mg m^{-3} in July) in coincidence with high biomass value (4.184 mg l^{-1} in July). Class and species dominance varied during the year: the most abundant species were *A. distans* and *S. gracile* in winter; *Cyclotella* spp., *Fragilaria crotonensis* and *A. distans* dominated in spring; a wider number of species was important in summer and among them *S. gracile*, *Staurastrum* spp*., C. aciculare, Fragilaria crotonensis*, *C. hirundinella* and *A. planctonica*; *C. hirundinella, M. aeruginosa, Microcystis* spp*., A. planctonica* and *Staurastrum sp.* were dominated in autumn. Considering the depth of Zm (Figure 5a), PAT was stratified from June 2006 to October and showed to the mixing from November to March 2007, subsequently a new phase of stratification was assessed. The water level decreased slowly from June (-1.85 m) to November (-7.77 m) and increased more quickly till June (-2.23 m). Mean annual concentration of TP was 38 mg P m^{-3}, with the maximum values in autumn and the minima in summer. For $N\text{-}NH_4^+$ the annual mean was 67 mg N m^{-3} and the maximum values were reported in autumn. $N\text{-}NO_3^-$ annual mean

concentration was of 236 mg N m^{-3} with the maxima in winter (444 mg N m^{-3} as absolute value). SRSi annual mean concentration was the lowest among all the studied reservoirs (annual mean of 0.52 mg Si l^{-1}).

The seasonal cycle of phytoplankton in PAT was divided in 8 periods (Table 3b; Figures 5): 3 steady-states were found during stratification (periods I, II and IV) whereas the 5 non steady-state phases were especially during mixing (with the exception of period III). Total biomass maximum was registered in the period V (3.386 mg l^{-1}), a non steady-state phase, when it was also calculated the maximum value of Shannon index (3.4). The minima Shannon index were observed in the periods I and IV (2.1 and 1.8).

In the period I, a steady-state phase in July 2006, the co-dominance of **Lm**, **P** and **H1** was observed. The sum of the percentages of these functional groups reached more than 80% of the total biomass. During the first sampling of this period, in the Jun/06, an important decrease of water level was registered. In the second sampling, a lowest Zm:Zeu ratio was also observed, due to an increase of Zm value (from 6 to 4 m) (Figures 5a and 5b). The wind velocity was of 210 km d^{-1} and the direction of 209°.

In the period II, a steady-state phase from July to August 2006, there was a strong dominance of the only one **P** functional group. It represented a percentage from 80% to 90% of the total biomass. The wind velocity was of 188 km d^{-1} and the direction of 193°.

Period III, a non steady state phase from the end of August to September 2006, was characterised by the decrease of importance of **P** group and the affirmation of **Lm** and **Y** groups. Values of Zm:Zeu ratio were higher than the mean also at the end of this period, due to an increase of Zm (from 7.9 m to 12.9 m and from 13.9 m to 14.9 m respectively) and decrease of Zeu value (from 3.5 m to 1 m and from 2.2 to 1.5 m respectively), as it was in the previous period, (Figures 5a and 5b).

The growth of **Lm** group was observed also in the following period IV, a steady-state phase in October. The co-dominance with **H1** was observed. Also this period was characterised by a net increase in the mean value of the wind velocity (from 188 km d^{-1} to 255 km d^{-1} and from 246 km d^{-1} to 306 km d^{-1}, respectively) (Table 3b).

The period V, a short non steady-state phase in November, was characterised by the major presence of **P** and **Lm** groups, but especially by the presence of species

not yet listed by Reynolds *et al*. (2002). In this period there were a negative variation of the water level (-7.77 m) and a wind velocity of 306 km d^{-1} from a direction of 156° (Table 3b).

Also the period VI and VII were non steady-state phases and regarded ,each one, only one sampling, respectively January and March, the former was interested by the increased relative importance of **P** group whereas the latter by the decrease of **P** group in favour of **A** group higher. In any case, as it was in November, the species not yet considered in the Reynold's groups were very important. The wind velocity was of 311 km d^{-1} and the direction of 303° in the period VI and of 208 km d^{-1} and a direction of 185°.

The period VIII, which was the last. Also in the period VIII the dominant group was **P** and at lower level **Y** and **R**. The wind velocity was 220 km d^{-1} from a direction of 129°. As general summary, the most important functional groups in the PAT reservoir were **P**, **Lm** and **H1** during the 3 steady-state periods, observed in stratification condition, and **P**, **Lm**, **Y**, **H1** and **R** during non steady-state when reservoir was stratified and **P**, **A**, **Lm**, **H1** and **Y** when it was in circulation.

1.4.7 Sos Canales reservoir (SCN)

Phytoplankton of SCN reservoir showed the biomass maxima in the summer and autumn months (6.089 mg l^{-1} in August and 5.334 mg l^{-1} in October). Chlorophyll *a,* showed a similar dynamic with the maximum in September (28.6 mg m^{-3}). Class and species dominance varied during the year and the seasons: the most abundant species were the Diatoms *A. distans, Asterionella formosa* Hass. and *Cyclotella* spp. in winter; there was a intense dominance of *A. planctonica* with a wider number of co-dominant species as *Anabaena sp.*, *C. hirundinella* and *Cyclotella* spp. in summer and autumn; a wider group of species was important: *Cyclotella* spp., *C. hirundinella, Anabaena* sp*., R. minuta* and *Cryptomonas* spp. in spring and at the beginning of summer. SCN was stratified from June 2006 to November and showed the mixing from January to February; subsequently a new phase of stratification was assessed. The water level decreased slowly and regularly from June (-5.87 m) to October (-12.34 m). There was an abrupt and intense increase in November (-5 m) followed by a decrease (-11.3 m) and a new increasing phase (up to –5.74 m). The mean annual concentration of TP was of 30 mg P m^{-3}, indicative of a probably mesotrophy, condition, with the maximum in

winter, while lower values were reported between June and July 2006. $N\text{-}NH_4^+$ annual mean was 48 mg N m^{-3}, with the maximum value in winter. For $N\text{-}NO_3^-$ was assessed an annual mean of 382 mg N m^{-3} and, as for $N\text{-}NH_4^+$, the maximum was in winter (mean of 500 mg N m^{-3}). The annual mean concentration of SRSi was 2.64 mg Si l^{-1}, with the maximum registered in spring (mean of 3.34 mg Si l^{-1}).

By the model of Sommer *et al.* (1993) it was possible divided the seasonal cycle of phytoplankton in 4 periods (Table 3b; Figures 6): 2 steady-states, one of which during stratification and the other during mixing (period II and III respectively), and 2 non steady-state phases, both during stratification (periods I and IV).

In period I, a non steady-state phase between June and July 2006, the main functional groups were **Lm** and **Y** and, at a lower level of importance, **P** and **A**. In this period very lower values of Zm:Zeu ratio were registered, due to high values of Zeu in respect to Zm. The wind velocity was 192 km d^{-1} from a direction of 98°.

The period II was a long steady-state phase from July to November. It was interested by the maximum values of total biomass (mean of the period, 4.280 mg l^{-1}) and minimum values of Shannon index (mean of 0.8). In this period the dominance of **H1** group was very evident (about 100%). The other groups, represented not more than 40% of the total biomass and the most abundant were **Lm**, **A**, and **P**. The period was characterised by wide water level fluctuations, at the beginning of the period, both at the end of the period, especially during the last three samplings of this period (1Oct/06, 2Oct/06 and Nov/06) and in the first sampling of the following (Jan/07) (Figure 6a). It was observed, as a mean, a wind velocity of 170 km d^{-1} by the 99° of direction, with a maximum of 332 km d^{-1} in August.

In the period III, a steady-state phase from January to February, a strong change in the functional group composition was observed. **P** group became the most important (about 90%). **A** and **C** groups achieved percentages of about 10% respectively.

As already highlighted, the first sampling of this period was interested by a wide variation of Zm:Zeu ratio due to a very important increase of Zm (from 6 m to 15 m) and a contemporaneous decrease of Zeu (from 2.2 m to 0.7 m) (Figures 6a and

6b). The wind velocity increased up to 455 km d^{-1} paralleled by the decrease of the solar radiation (from 5 Kwh m^{-2} to 3 Kwh m^{-2}) (Table 3b).

In the last period (IV), a non steady-state phase from May to June, the percentage of the values of the single dominant functional groups were similar. The most abundant were **Lm**, **A** and **H1**. The values of Zm:Zeu ratio were affected by the increase of Zeu depth (from 5 m to 7 m and 12.5 m). A strong increase of the solar radiation was observed (from 3 km d^{-1} in Feb/07 to 8 km d^{-1} in May/07) (Table 3b). The mean wind velocity was 215 km d^{-1} with a direction of 145°. Globally, the most important functional groups in SCN reservoir were **H1**, **Lm**, **P** and **C** during the two steady-state periods, in both stratification and mixing conditions, and an higher number during non steady-state phases both when the reservoir was stratified (**Lm**, **Y**, **P**, **H1, A** and **F**).

1.4.8 Temo reservoir (TEM)

Phytoplankton of TEM reservoir showed the biomass maximum in November, December, April and June (from 1.734 mg l^{-1} to 2.872 mg l^{-1}). The annual dynamic was characterised by higher values at the end of autumn and in spring months. Chlorophyll *a* highlighted a similar trend and showed the maximum in spring (8.5 mg m^{-3}, 12 mg m^{-3} in April). Class and species dominance varied during the year: the most abundant species were *Cyclotella* spp*., A. planctonica, C. aciculare* and *Anabaena* sp. in late autumn and in winter, whereas *C. hirundinella*, *Cyclotella* spp., *Staurastrum* sp., *Microcystis* spp., *Aphanizomenon flos-aquae* and *Rhodomonas* sp. at the beginnings of autumn; a wider number of species was important in summer, as *A. distans, Anabaena* sp., *Microcystis* spp., *Aph. flos-aquae, Schroederia* sp., *Volvox* sp., *Staurastrum* sp., *Cosmarium* sp., *Cyclotella* spp., *C. hirundinella* and *R. minuta*; *Cyclotella* sp., and *Cyclotella atomus* Hustedt with *Coelastrum pseudomicroporum* Kors., *C. hirundinella* and *M. aeruginosa* were important in spring. Considering the depth of Zm (Figure 7a), TEM was stratified from June 2006 to December and showed a total circulation of the water from December to June 2007, subsequently a new phase of stratification was assessed. The water level showed a slow decrease from June 2006 to August, followed by an abrupt negative variation between August and September. After a constant period until October, the level increased until June 2007. The water level variations were not very wide autogenic disturbance

could not be very important. The annual mean concentration of TP was about 59 mg P m^{-3} with the maximum values in autumn and winter months (mean value of **70** mg P m^{-3}) and lower in June 2006 (46 mg P m^{-3}). N-NH_4^+ annual mean was 27 mg N m^{-3} with the maximum values in autumn and winter. N-NO_3^- annual mean was 277 mg N m^{-3}, with the maximum of 353 mg N m^{-3} in June 2006. For SRSi was registered a mean annual concentration of 2.33 mg Si l^{-1}. The minimum was registered in June 2007 (mean of 1.56 mg Si l^{-1}).

In TEM reservoir 6 different periods were identified during the seasonal cycle of phytoplankton (Table 3b; Figures 7). The only one steady-state phase was found during stratification whereas the non steady-state phases, were in 4 cases during stratification (periods I, II, III and VI) and one during mixing (period V).

In the period I from June to August, there was the coexistence of at least three different functional groups, with similar level of importance. The major were **A, P**, **H1**, **N**, **Y, G** and n.d. (due to *Schroederia* sp.). The mean wind velocity was of 188 km d^{-1} and the direction of 212°.

In the periods II, a non steady-state phase in August, the number of dominant groups decreased, with an increased importance of **H1** and the co-dominance of **P** and **A**. This period was characterised by an increase in the wind velocity (383 km d^{-1}) with a direction of 245°.

In the period III, a non steady-state phase from September to October, at least three groups were contemporaneously relevant: **A**, **H1** and **P** during the first sampling and **Y**, **Lm**, **A** and **P** in the second. In this period the wind velocity decreased (293 km d^{-1}) whereas the direction remained about the same, 249°.

During period IV, the only one steady-state phase from October to February, the dominance of **A** and **H1** as main groups in co-dominance but at lower level with **P** and **Y** was observed. The wind velocity greatly reduced (100 km d^{-1}), the direction was 196°. There was an inversion in the water level dynamic with the increase of the level at the end of the period. For three consecutive samplings (the last two samplings of the period IV) values of Zm:Zeu ratio were high, due to the higher Zm values. It was probably caused by the increased wind velocity (from 100 km d^{-1} to 162 km d^{-1}) with coincided with the end of stratification, too (Figures 7a and 7b).

Period V, a non steady-state phase from April to May, was characterised by the co-dominance of **A** and **F** groups, with a less important contribution by **Lm**. The

wind velocity was of 141 km d^{-1} and the direction of 155°. The water level decrease from -3.42 m to -5.40 m at the end of the total considered periods. The Chlorophyll *a* and the Shannon index was very high (12.00 mg m^{-3} and 3.6 in April respectively).

In last period VI, a non steady-state phase in June 2007, the co-dominace of the two groups was observed: **Lm** and **A**. It was interested by the maximum of total biomass in correspondence to an high Shannon index value. The wind velocity was of 158 km d^{-1} and the direction of 185°.

As general summary, the most important functional groups in the TEM reservoir were in a lower number **A**, **H1**, **P** and **Y** during the only one steady-state period, observed in mixing condition, and **H1, P**, **N**, **Lm**, **G** and **Y** an higher number during non steady-state when both the reservoir was stratified and **A**, **F** and **H1** during the mixing.

1.5 Discussion and Conclusion

The reported results and the relative elaborations represent a first step of the study on functional groups, which is in course on the phytoplankton of the six considered reservoirs. The data regards a time series of a year and it will completed with those of at least a second years. It is because a very wide variability has been already descript a regard phytoplankton annual dynamic of Sardinian reservoirs (Sechi and Lugliè, 1992, 1996). Anyway, some aspects and preliminary results can be discussed and highlighted. The study is based on the assumption that the functional approach is a very reliable way to describe the ecology of phytoplankton and offers the possibility to have a verifiable quantitative method in describing community structure and its changes (Fabbro & Duivenvorden, 2000; Reynolds *et al*., 2002; Kruk *et al*., 2002). In this context, it is focused the attention on the valuation if phytoplankton assemblages descript by functional groups may be related to steady-state (SS) and non steady-state (NSS) periods in reservoirs. Considering all the reservoirs and according to criteria proposed by Sommer *et al*. (1993; already reported in the Introduction), the number of distinct periods inside the annual cycle varied from 4 (SCN) to 8 (CED and PAT). Globally, the number of NSS periods (27) prevailed on the SS periods (12). The number of NSS periods was always higher in each reservoirs, with the only exception of SCN, with an equal number. Both the SS and NSS periods were

mainly during stratification (respectively, 8 and 20 during stratification and 4 and 7 during mixing) (Figure 8). It could depend on the fact that the reservoirs were stratified for longer periods (in general, during summer, autumn and the ending of spring). So, in the present study, it was observed that the stratification wasn't the stable phase for the phytoplankton groups. Table 4 reports the assessed periods in each reservoirs and the relative main functional groups. It can be observed that the presence of 13 functional groups was assessed. They were (Reynolds *et al*., 2002): **A**, characteristic for spring diatom development in lakes typically clear, well mixed, diluted in solutes and deficient in phosphorus, mainly represented in the studied reservoirs by *Cyclotella* species; **Y**, tolerant low light and usually in small enriched lakes, represented prevalently by *Cryptomonas* and *Rhodomonas* genus, in Sardinian reservoirs; **P**, which includes the diatoms as *A. distans* and the Conjugatophyceae as *S. gracile*, with a strong dependence upon physical mixing and eutrophic epilimnion as usually habitat; **F**, species with an elevated light threshold, prefering clear water and tolerant deep mixing, represented by *C. pseudomicroporum*, *Coelastrum* sp., *Oocystis* spp. and *Sphaerocystis* sp.; **C**, diatoms subjected to the availability of silicon and dependence upon turbulence for suspension leaves them relatively sensitive to mixed depth and the seasonal onset of near-surface density stratification with *A. formosa* as the most important; **D**, diatoms of these Groups are mostly found in shallow, nutrient-enriched, well-ventilated waters, liable to be turbid, well represented by *Stephanodiscus* spp. in Sardinian reservoirs; **Lm**, species typically present in summer epilimnion in eutrophic lakes and sensible to mixing, poor stratification light, mainly represented by *C. hirundinella*; **H1** dinitrogen-fixing nostocales, as *A. planctonica*, sensible to mixing, poor light and low phosphorus; **G**, species as *Volvox* sp., responding to nutrient-rich conditions in stagnanting water columns and, therefore, most familiar in small eutrophic lakes and during very stable phases in larger river-fed and storage reservoirs; **R**, species as *Planktothrix* sp., which are prominent in the stable, optically deep-water layers in the stable gradients of small, density-stratified lakes at all latitudes; **N**, species like *Cosmarium* genus, associated to temperate lakes of lower latitudes and during summer period; **W2**, euglenoids, represented particularly by *Trachelomonas* species, are also found in the distinctive bottom-dwelling community of shallow, aerated lakes which appears in open water on occasions; **J**, of mainly non-

gelatinous, non-motile Chlorococcales is prominent in shallow, highly enriched systems represented by *Scenedesmus*, *Pediastrum* and *Coelastrum* species.

In general, the steady-state periods were characterised by a low number of co-dominant species and functional groups, with associated different levels of stability and diversity. **P**, **A** and **Lm** were the most frequent observed dominant and co-dominant groups during SS periods, in both stratification and mixing conditions. In the former, **H1** was often observed with **N** and **F** groups, even if these two less frequently. In the mixing periods **Y**, **D**, **F** and **C** groups were present but each one in only one case. Globally, during SS periods the presence of 10 functional groups were assessed, 6 of them during stratification and 7 during mixing. Among these groups, 2 were found only when the reservoirs were stratified (**H1** and **N**) and 3 when there was the mixing (**Y**, **D** and **C**) (Table 5).

Periods of non equilibrium state were characterised by higher number of dominant species and functional groups with lower relative importance. **Lm**, **Y**, **A**, **P** and **H1** groups were the most frequent reported dominant or co-dominant groups during NSS phase. The widest number of functional groups (11) was observed during stratificated periods in NSS phases with **Lm**, **Y**, **A** and **P**. A lower number of groups (6) was relieved in mixing condition during NSS periods, with **H1** and **A** as the main. These 6 groups were assessed during stratification, too, whereas 5 groups (**W2**, **D**, **J**, **R** and **G**) were observed only in this periods and not during mixing (Table 5).

The reservoirs shared a wide number of functional groups as dominant and co-dominant. Other groups had a more restricted distribution: **W2** was important only in BID, **J** in CED, **R** in PAT, **C** in SCN, **G** in TEM, **F** in CED and SCN and **D** in BID and CUG (Table 6).

According to the observations, the steady-state periods occurred mainly during strong stratification and it can be generalised that the main factor that caused and maintained the steady-state condition was the presence of stratification.

Moreover, it was observed that, when disruption of the stratification occurred, net decreases of the total biomass were observed. This could be explained according Charpin *et al*. (1998): physical factors that lead the seasonal cycles of mixing and stratification may considerably affect the photosynthetic physiology of phytoplankton. In fact, a change in the structure of the underwater light field may lead to important modification in the structure of phytoplankton compositions.

Obviously the extension of these factors is variable in relation to the different cases. Moreover water level fluctuations and summer drawdown is another factor that in reservoirs greatly interfere with the periodicity and stability of stratification, as observed in Sicilian reservoirs (Naselli-Flores, 2003), and in Sardinian reservoirs (Sechi and Lugliè, 1992, 1996).

This first part of the work was finalised to individuate the dominated functional groups in equilibrium or non-equilibrium phases in Sardinian reservoirs. It must be followed by the necessary mathematical elaborations to individuate, which, among the abiotic factors, both physics (wind velocity and direction, solar radiation and water level fluctuation) and the nutrients (NH_4^+, NO_3^-, SRP, TP and SRSi), have the primary role in the affirmation and ending of the groups. The main objective is in fact to individuate the factors that, in primary terms and in specific conditions, determine the phytoplankton successions in Sardinian reservoir

Table 1. Morphological data for the studied lakes: Abb=abbreviation,; Loc=localization, Zmed:mean depth; Zmax: maximum depth, the use: A= alimentary; I=irrigation, and their Trophic state: IE=ipertrophic; E=eutrophic; M=mesotrophic.

Lakes	Abb	Loc (European 1950)		Altitude (m a.s.l.)	Drainage basin area (m^2*10^6)	Volume (m^3*10^6)	Area (Km^2)	Zmed (m)	Use	Trophic state
Bidighinzu	BID	40° 33' 23.27" N	8° 39' 51.60" E	334	51.7	12.2	1.7	7.3	A	IE
Cedrino	CED	40° 19' 45.50" N	8° 39' 36.70" E	103	631.2	30.0	1.1	26.5	A, I	E
Cuga	CUG	40° 36' 49.20" N	8° 27' 04.90" E	114	58.4	35.0	3.1	11.3	A, I	E
Pattada	PAT	40° 35' 24.40" N	9° 09' 05.70" E	560	159.9	65.5	4.4	14.9	A, I	E
Sos Canales	SCN	40° 33' 16.70" N	9° 18' 51.90" E	714	15.9	4.3	0.3	13.2	A	M
Temo	TEM	40° 28' 44.70" N	8° 33' 53.10" E	226	142.5	55.4	3.5	15.8	A, I	E

Table 2. Principal idrological data of the studied sites reported as sum in 2006 year.

Lakes	wind velocity Km y^{-1}	rain (mm y^{-1})	total rainy days	maximum daily value (mm d^{-1})	solar radiation Kwy m^2
Bidighinzu	74419	578.4	65	42.8 (14th Sep)	1676.1
Cedrino	91836	893.4	63	**200.4** (30th Jan)	1651.9
Cuga	74314	461.4	49	43.2 (25th Sep)	1647.4
Pattada	95558	559.4	34	48.2 (11th Mar)	1615.3
Sos Canales	97795	590.6	34	**84.8** (30th Jan)	1582.7
Temo	76139	542.6	31	31.6 (12th Sep)	1589.7

Table 4. Number of periods and main functional groups in all the examined reservoirs.

reservoirs	SS		NSS		total periods	total groups
	S	**M**	**S**	**M**		
BID	2 Lm, P	1 Y, D, A	4 W2, D, Y, A, Lm	0	7	6 Lm, P, Y, D, A, W2
CED	1 N, F, P	1 A, F, P	4 Y, P, N, A, J	2 H1, F, Y	8	7 N, F, P, A, Y, J, H1
CUG	0	1 A, P, Lm	4 D, H1, A, Lm	1 A, P, H1, Lm	6	5 A, P, Lm, D, H1
PAT	3 P, Lm, H1	0	2 P, Lm, Y, H, R	3 P, A, Lm, H1, Y	8	6 P, Lm, H1, Y, R, A
SCN	1 H1, Lm, P, A	1 P, C	2 Lm, Y, P, H1, A, F	0	4	6 H1, Lm, P, A, C, Y, F,
TEM	1 A, H1, P	0	4 H1, P, N, Lm, G, Y	1 A, F, H1	6	6 A, H1, P, Lm, G, Y

Table 5. Number of total periods and main dominant and co-dominant functional groups in all the examined reservoirs.

reservoirs	SS		NSS	
	S	**M**	**S**	**M**
total periods	8	4	20	7
	12		27	
main groups	P, A, Lm		Lm, Y, A, P, H1	
	P, A, Lm, N, F, H1	P, A, Lm, Y, D, F, C	Lm, Y, A, P, H1, W2, D, J, R, F, G	Lm, Y, A, P, H1, F
	6	7	11	6
total groups	10		11	

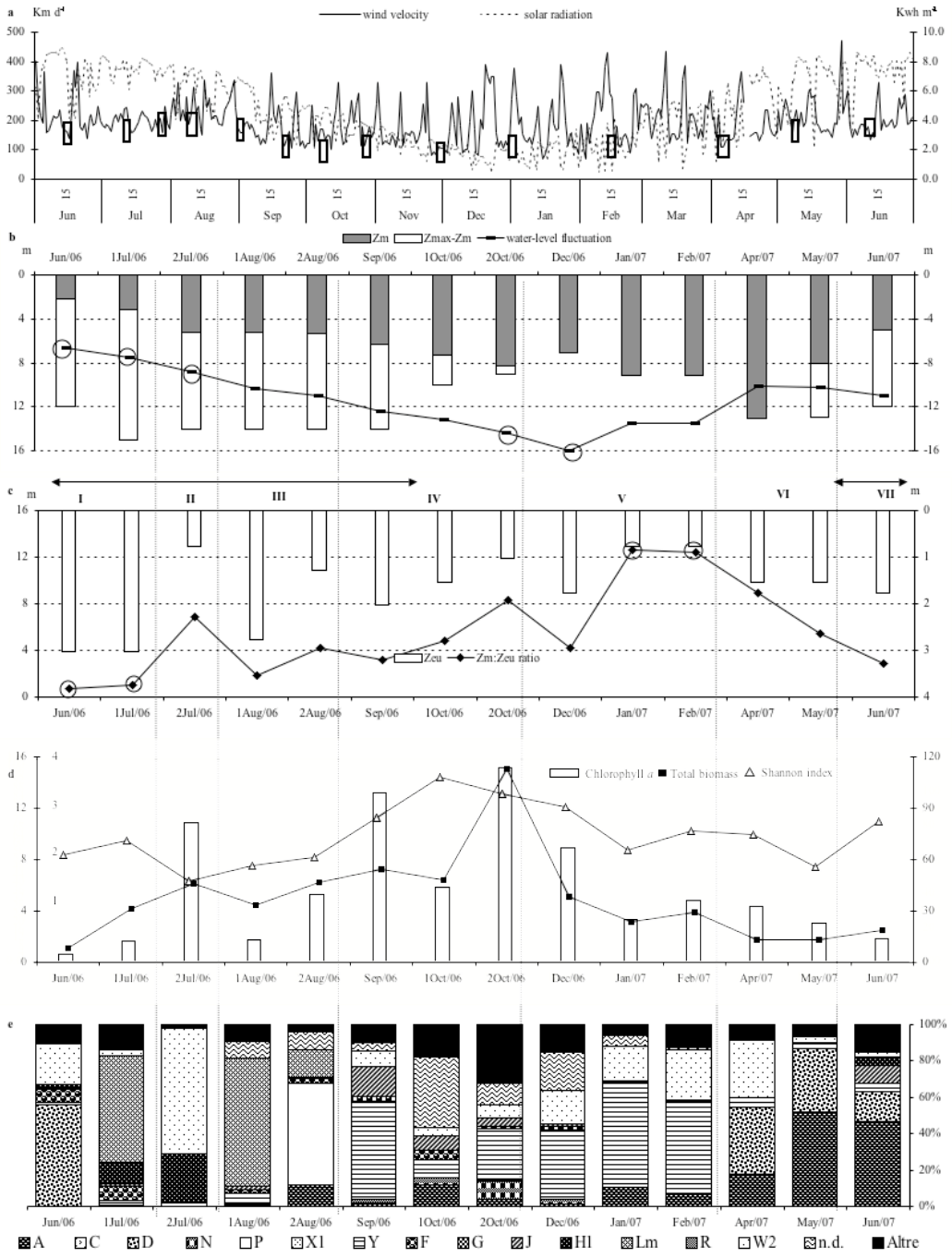
Table 3 (a). Mean (m) and standard deviation (s) of 5 nutrients 8 physical and 3 biological parameters in 2006-07 period in the studied reservoirs.

Reservoirs			Period I			Period II			Period III			Period IV			Period V			Period VI			Period VII			Period VIII			total period
			m	s	n	m	s	n	m	s	n	m	s	n	m	s	n	m	s	n	m	s	n	m	s	n	
IID			Jan/96-15ul/06			22ul/96			1Aug/06-2Aug/06			Sep/96-20ct/07			Dec/96-Feb/97			Apr/07-May/07			Jun/07						
nutrients	$N\text{-}NH_4^+$	mg N m^{-3}	678	9	2	765		1	1425	313	2	1054	616	3	215	222	3	195	217	2	56		1				659
	$N\text{-}NO_3$	mg N m^{-3}	65	61		127			38	17		86	64		1125	90		247	257		622						365
	SRP	mg P m^{-3}	111	31		132			169	4		315	62		106	32		79	86		160						162
	TP	mg P m^{-3}	366	47		380			396	35		460	73		234	45		236	26		532						342
	SRSi	mg Si l^{-1}	6.27	1.81		6.11			5.75	1.03		4.52	8.35		5.75	1.82		6.16	4.32		3.38						5.39
physical	epilimnetic temperature	°C	25.0	2.2		25.0			23.0	1.4		20.6	1.6		10.4	1.7		16.3	2.8		23.1						19.8
	Zm	m	2.6	8.7		5.2			5.2	0.1		7.3	1.8		8.7	1.1		18.8	3.8		5.0						6.8
	Zen	m	3.0	8.0		8.8			2.0	1.1		1.3	0.5		1.1	8.6		1.5	0.0		1.8						1.7
	Zm:Zen ratio		8.9	8.2		6.9			3.1	1.7		5.4	2.6		9.7	4.8		5.2	2.3		2.9						5.5
	wind velocity	Km d^{-1}	198	36		180			216	16		140	17		161	32		180		1	288						185
	wind direction	°	207	41		180			216	23		160	18		211	38		191		1	290						204
	solar radiation	Kwh m^{-2}	8	1		7			7	1		5	1		2	1		8		1	8						6
	water-level	m	-7.85	0.62		-8.79			-10.69	0.49		-13.10	8.94		-14.36	1.48		-10.17	0.11		-10.98						-11.33
biological parameters	Chlorofill a	mg m^{-3}	6.9	4.9		78.3			22.5	16.6		73.9	32.2		36.6	18.7		23.9	6.1		11.3						35.1
	Shannon diversity	H'	2.3	8.2		1.6			2.0	0.1		3.3	0.4		2.6	8.6		2.2	0.4		2.8						2.5
	total biomass	mg l^{-1}	2.876	2.176		6.136			5.216	1.243		9.595	6.778		6.076	0.994		1.752	0.007		2.523						4.942
CID			Jan/96-23ul/06			1Aug/96			2Aug/06			Sep/96-10ct/96			20ct/06			Nov/96-Jan/97			Mar/07-Jun/97			Jul/97			
nutrients	$N\text{-}NH_4^+$	mg N m^{-3}	90	94	3	123		1	131		1	349	277	2	119		1	79	9	2	30	8	2	41		1	123
	$N\text{-}NO_3$	mg N m^{-3}	510	171		187			29			130	95		628			1063	184		991	638		292			560
	SRP	mg P m^{-3}	70	31		90			5			28	8		22			54	7		38	58		13			41
	TP	mg P m^{-3}	164	35		81			51			71	7		68			95	8		77	42		52			80
	SRSi	mg Si l^{-1}	2.41	0.27		2.34			1.11			1.68	8.12		1.18			2.78	2.27		3.88	1.88		2.01			2.22
physical	epilimnetic temperature	°C	28.6	2.2		28.8			24.5			22.6	0.8		20.2			13.6	4.2		16.6	5.5		21.8			21.4
	Zm	m	3.5	8.6		7.5			8.0			15.7	0.8		20.7			18.5	5.4		25.3	3.8					13.0
	Zen	m	2.9	8.6		2.8			1.5			4.3	0.8		1.3			2.1	0.5		4.3	2.5		2.5			2.9
	Zm:Zen ratio		1.2	8.4		8.0			8.0			3.7	0.8		16.8			8.1	5.9		6.9	4.8					8.5
	wind velocity	Km d^{-1}	186	35		172			225			197	168		308			134	6		179	55		228			206
	wind direction	°	131	15		179			152			179	102		228			137	19		150	25		174			158
	solar radiation	Kwh m^{-2}	8	0		7			7			5	1		4			5	0		6	2		8			6
	water-level	m	-2.21	1.82		-6.04			-7.85			-8.47	8.15		-4.97			-5.86	0.90		-1.63	1.58		-1.08			-4.31
biological parameters	Chlorofill a	mg m^{-3}	9.2	8.9		4.5			54.2			19.8	20.8		30.8			3.5	5.5		17.1	5.8		5.6			15.6
	Shannon diversity	H'	2.1	8.5		3.8			1.7			2.8	0.2		0.8			1.6	0.4		2.6	0.8		3.8			2.5
	total biomass	mg l^{-1}	1.952	1.281		1.939			5.284			3.184	5.618		5.441			1.975	1.289		3.132	5.027		1.818			3.525
CUG			13un/06-22un/06			13ul/96			23ul/06-Sep/06			10ct/06-20ct/06			Nov/06-Apr/07			May/07-Jun/07									
nutrients	$N\text{-}NH_4^+$	mg N m^{-3}	47	44	2	189		1	145	87	4	28	17	2	28	2	4	25	2	2							65
	$N\text{-}NO_3$	mg N m^{-3}	118	16		81			77	48		151	41		329	168		167	98								172
	SRP	mg P m^{-3}	15	7		16			25	6		28	2		44	11		19	3								27
	TP	mg P m^{-3}	48	6		52			92	51		85	4		116	19		72	5								86
	SRSi	mg Si l^{-1}	0.89		1	0.93			1.35	0.41		1.79	8.07		3.53	1.29		2.86	0.18								2.81
physical	epilimnetic temperature	°C	23.8	2.4		27.7			24.6	5.6		21.3	1.8		14.7	4.1		21.6	2.9								21.2
	Zm	m	6.3	2.8		4.3			18.5	5.2		14.8	0.7		12.8	6.5		4.3	1.5								9.6
	Zen	m	2.7	8.4		3.0			1.7	0.9		1.3	0.4		1.1	8.5		1.5	1.1								1.7
	Zm:Zen ratio		2.5	1.5		1.4			8.6	5.6		12.2	2.8		12.8	9.5		4.3	4.0								7.8
	wind velocity	Km d^{-1}	232	18		167			235	101		198	8		140	39		158	29								178
	wind direction	°	198	95		150			202	85		155	53		155	61		185	82								177
	solar radiation	Kwh m^{-2}	7	3		8			5	2		4	1		4	2		8	0								5
	water-level	m	-5.37	0.82		-7.49			-10.36	1.36		-8.61	8.42		-8.28	1.22		-7.25	0.21								-8.30
biological parameters	Chlorofill a	mg m^{-3}	13.0	8.6		4.7			18.8	12.9		21.2	5.5		15.8	8.1		21.2	15.1								14.8
	Shannon diversity	H'	2.5	8.6		1.7			3.1	0.2		1.2	0.8		2.3	8.5		3.1	0.5								2.5
	total biomass	mg l^{-1}	2.526	1.544		1.615			1.586	1.145		36.046	40.855		1.777	1.086		3.191	0.636								6.546

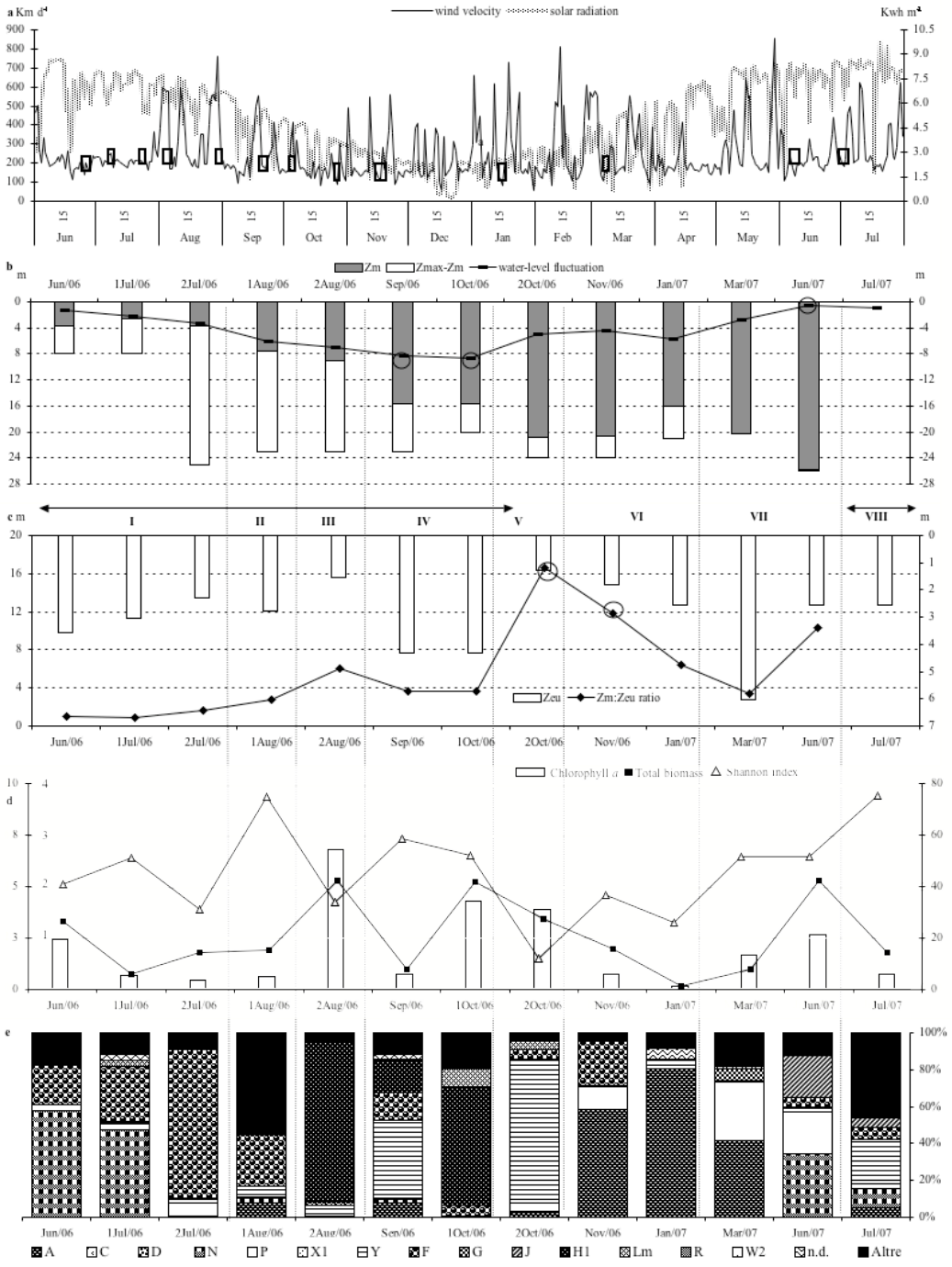
Table 3 (b). Mean (m) and standard deviation (s) of 5 nutrients 8 physical and 3 biological parameters in 2006-07 period in the studied reservoirs.

Reservoirs			Period I			Period II			Period III			Period IV			Period V			Period VI			Period VII			Period VIII			total period
			(I)	s	n	(II)	s	n	(III)	s	n	(IV)	s	n	(V)	s	n	(VI)	s	n	(VII)	s	n	(VIII)	s	n	
PAT			Jan/06-Jul/06			2Jul/06-3Aug/06			2Aug/06-Sep/06			1Oct/06-2Oct/06			Nov/06			Jan/07			Mar/07			1Jun/07-2Jun/07			
nutrients	$N\text{-}NH_4^+$	mg N m^{-3}	49	15	2	36	8	2	56	6	2	121	5	2	218	1	23	1	26	1	17	3	2	67
	$N\text{-}NO_3$	mg N m^{-3}	282	121		287	26		135	12		66	18		122		466		584		234	1		236
	SRP	mg P m^{-3}	7	2		6	1		5	6		4	8		58		7		9		5	1		7
	TP	mg P m^{-3}	41	22		38	7		36	2		34	3		78		44		51		36	1		38
	SRSi	mg Si l^{-1}	1.48	1.60		0.38	0.85		0.39	0.03		0.28	8.07		8.54		0.75		0.40		8.88	0.04		0.52
physical	epilimnetic temperature	°C	22.7	2.1		24.2	1.6		19.8	0.3		18.4	0.9		16.8		9.5		11.0		10.8	3.8		18.8
	Zm	m	5.0	1.4		6.0	2.8		13.0	0.1		14.5	0.7		19.8		25.1		25.9		11.4	4.9		5.0
	Zen	m	3.8	8.4		2.9	8.8		1.8	1.1		1.9	0.5		3.5		3.5		5.5		3.5	1.8		3.1
	Zm:Zen ratio		1.4	8.5		2.0	8.4		8.1	5.4		8.1	2.7		5.7		7.2		4.7		3.3	8.5		5.0
	wind velocity	Km d^{-1}	210	66		188	5		255	95		246	154		306		311		288		228	25		236
	wind direction	°	289	59		195	17		285	79		174	63		156		330		185		229	61		207
	solar radiation	Kwh m^{-2}	6	2		7	1		6	1		4	8		3		1		4		8	1		5
	water-level	m	-2.35	0.71		-4.49	1.80		-5.86	0.40		-6.53	8.58		-7.77		-6.45		-4.55		-2.57	0.48		-4.80
biological parameters	Chlorofill a	mg m^{-3}	8.9	2.5		8.1	1.4		3.7	1.1		4.6	2.9		4.8		7.1		14.3		28.8	27.8		8.9
	Shannon diversity	Hʹ	2.1	8.1		2.2	8.1		3.0	0.2		1.8	0.8		3.4		2.2		3.1		2.8	1.0		2.4
	total biomass	mg l^{-1}	4.177	0.930		3.930	0.851		0.665	0.196		1.595	0.663		0.567		3.961		3.230		2.304	1.635		2.518
SUN			Jan/06-Jul/06			2Jul/06-Nov/06			Jan/07-Feb/07			May/07-Jun/07															
nutrients	$N\text{-}NH_4^+$	mg N m^{-3}	30	1	2	60	48	7	74	19	2	28	4	2													48
	$N\text{-}NO_3$	mg N m^{-3}	345	149		363	32		500	50		338	26														382
	SRP	mg P m^{-3}	5	1		6	5		4	1		5	8														5
	TP	mg P m^{-3}	38	2		32	4		42	14		33	8														30
	SRSi	mg Si l^{-1}	2.59		1	2.49	0.68		2.50	0.61		3.54	8.05														2.64
physical	epilimnetic temperature	°C	18.2	7.9		17.8	4.0		7.9	0.8		18.2	2.7														17.0
	Zm	m	3.5	2.1		3.8	1.1		15.0	0.8		5.4	2.8														5.5
	Zen	m	8.0	8.4		1.5	8.8		2.9	5.8		9.8	3.8														4.0
	Zm:Zen ratio		8.5	8.1		3.2	1.5		11.5	12.8		8.4	0.4														3.4
	wind velocity	Km d^{-1}	192	6		170	82		455	188		215	8														227
	wind direction	°	98	12		99	36		199	32		145	54														118
	solar radiation	Kwh m^{-2}	8	0		5	2		5	1		8	1														6
	water-level	m	-6.45	0.85		-9.77	2.58		-10.50	1.13		-6.15	8.64														-6.87
biological parameters	Chlorofill a	mg m^{-3}	3.9	1.9		15.0	8.1		2.6	1.5		5.7	1.7														8.2
	Shannon diversity	Hʹ	3.0	8.5		8.8	8.5		1.5	0.3		2.7	0.4														1.6
	total biomass	mg l^{-1}	8.338	8.888		4.280	1.535		1.521	0.395		0.882	0.255														2.540
TEM			Jan/06-1Aug/06			2Aug/06			Sep/06-1Oct/06			2Oct/06-Feb/07			Apr/07-May/07			Jun/07									
nutrients	$N\text{-}NH_4^+$	mg N m^{-3}	17	5	5	32	1		22	11	2	47	25	4	25	1	2	29	1								27
	$N\text{-}NO_3$	mg N m^{-3}	355	41		311			216	93		258	67		254	7		232									277
	SRP	mg P m^{-3}	28	3		26			23	17		43	18		34	0		29									31
	TP	mg P m^{-3}	46	3		36			31	28		75	12		66	1		63									39
	SRSi	mg Si l^{-1}	2.36	0.26		2.86			2.26	0.61		2.48	8.19		1.74	0.88		1.56									2.33
physical	epilimnetic temperature	°C	26.2	2.5		22.6			23.0	0.0		15.7	4.8		18.8	8.6		25.5									21.7
	Zm	m	3.7	8.8		6.6			7.5	1.4		16.3	9.7		4.2		1.0	2.1									8.0
	Zen	m	3.1	8.6		4.5			3.6	0.9		2.6	0.8		4.5		1.0	2.5									3.2
	Zm:Zen ratio		1.2	8.2		1.6			2.2	0.9		6.2	2.3		0.9		1.0	8.8									2.7
	wind velocity	Km d^{-1}	188	61		183			293	176		188	34		162	36		163									186
	wind direction	°	212	28		245			249	28		196	69		198	31		146									208
	solar radiation	Kwh m^{-2}	7	1		7			5	2		2	1		7	1		7									5
	water-level	m	-3.94	0.41		-3.83			-7.21	0.16		-6.97	8.94		-3.99	0.10		-5.40									-5.26
biological parameters	Chlorofill a	mg m^{-3}	2.7	8.8		3.0			3.5	1.4		8.4	2.6		8.5	5.0		8.7									4.6
	Shannon diversity	Hʹ	3.1	8.2		2.3			3.5	0.4		2.4	0.2		3.5	8.2		2.3									2.9
	total biomass	mg l^{-1}	0.718	0.539		0.661			0.621	0.347		1.734	0.958		1.798	0.626		2.872									1.260

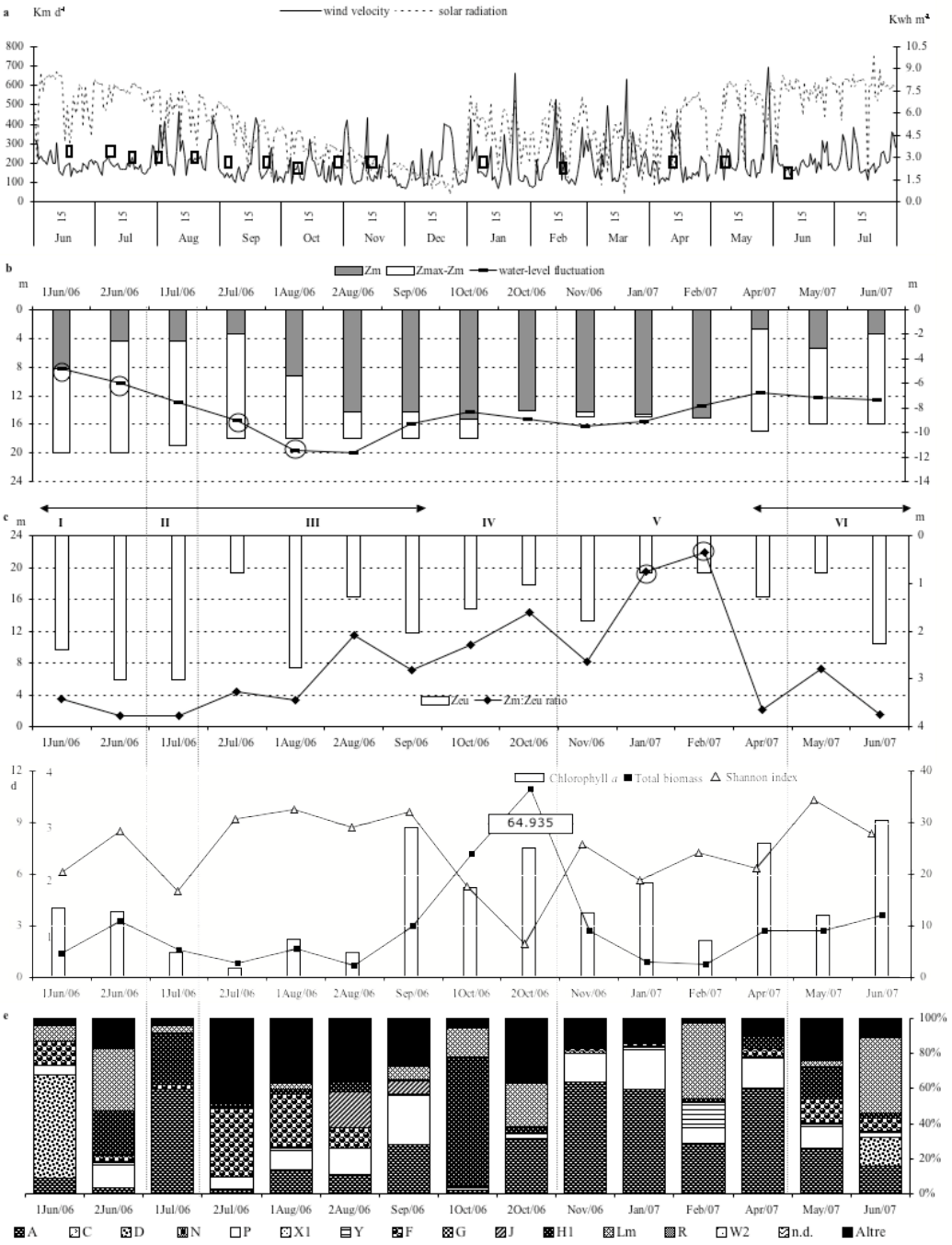
Figure 1 – Localisation of Sardinian reservoirs.



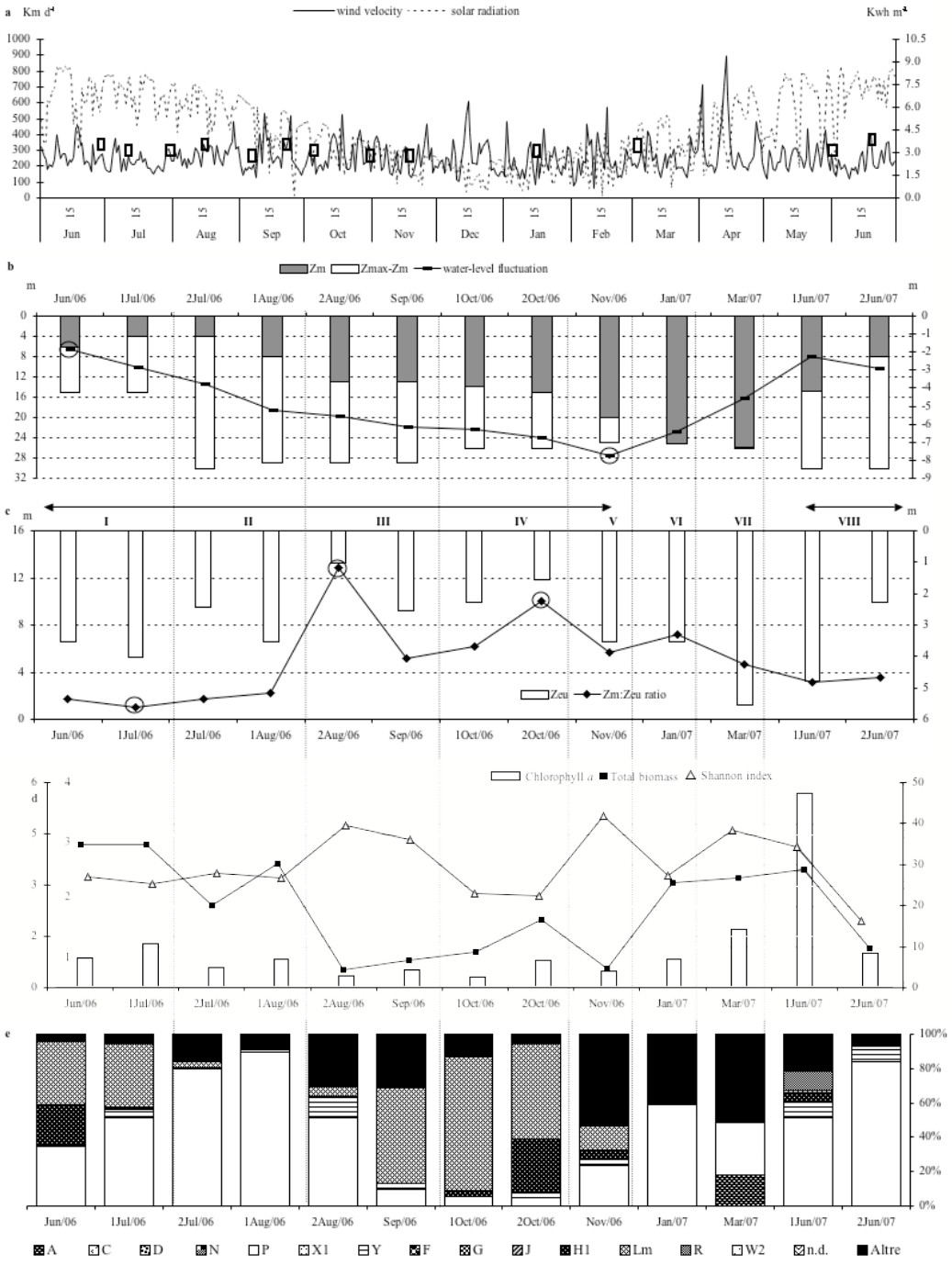
Figures 2 Bidighinzu reservoir: a) Wind velocity (left axis) and solar radiation (right axis) with the sampling data in square; b) mixing depth (Zm) and Zmax-Zmix (left axis) in percentage, water level variation (in negative, right axis), disturbance events are evidenced with the circle; c) Zm:Zeu ratio (left axis) and euphotic (Zeu) (right axis); d) seasonal dynamics of total biomass and Shannon diversity index (outside and inside on the left axis) and chlorophyll *a* (right axis); e) percentages of biomass of dominant functional groups.



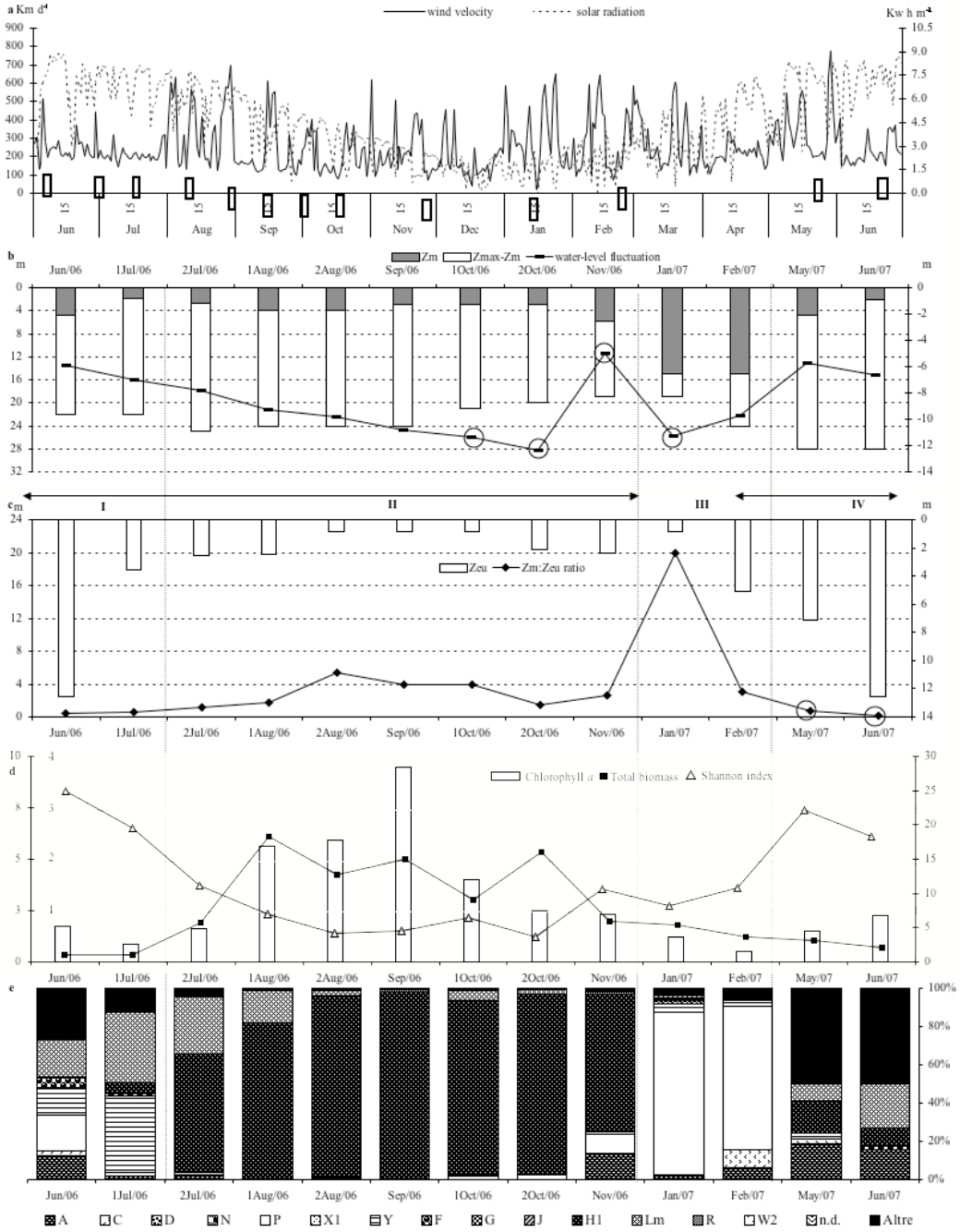
Figures 3 Cedrino reservoir: a) Wind velocity (left axis) and solar radiation (right axis) with the sampling data in square; b) mixing depth (Zm) and Zmax-Zmix (left axis) in percentage, water level variation (in negative, right axis), disturbance events are evidenced with the circle; c) Zm:Zeu ratio (left axis) and euphotic (Zeu) (right axis); d) seasonal dynamics of total biomass and Shannon diversity index (outside and inside on the left axis) and chlorophyll *a* (right axis); e) percentages of biomass of dominant functional groups.



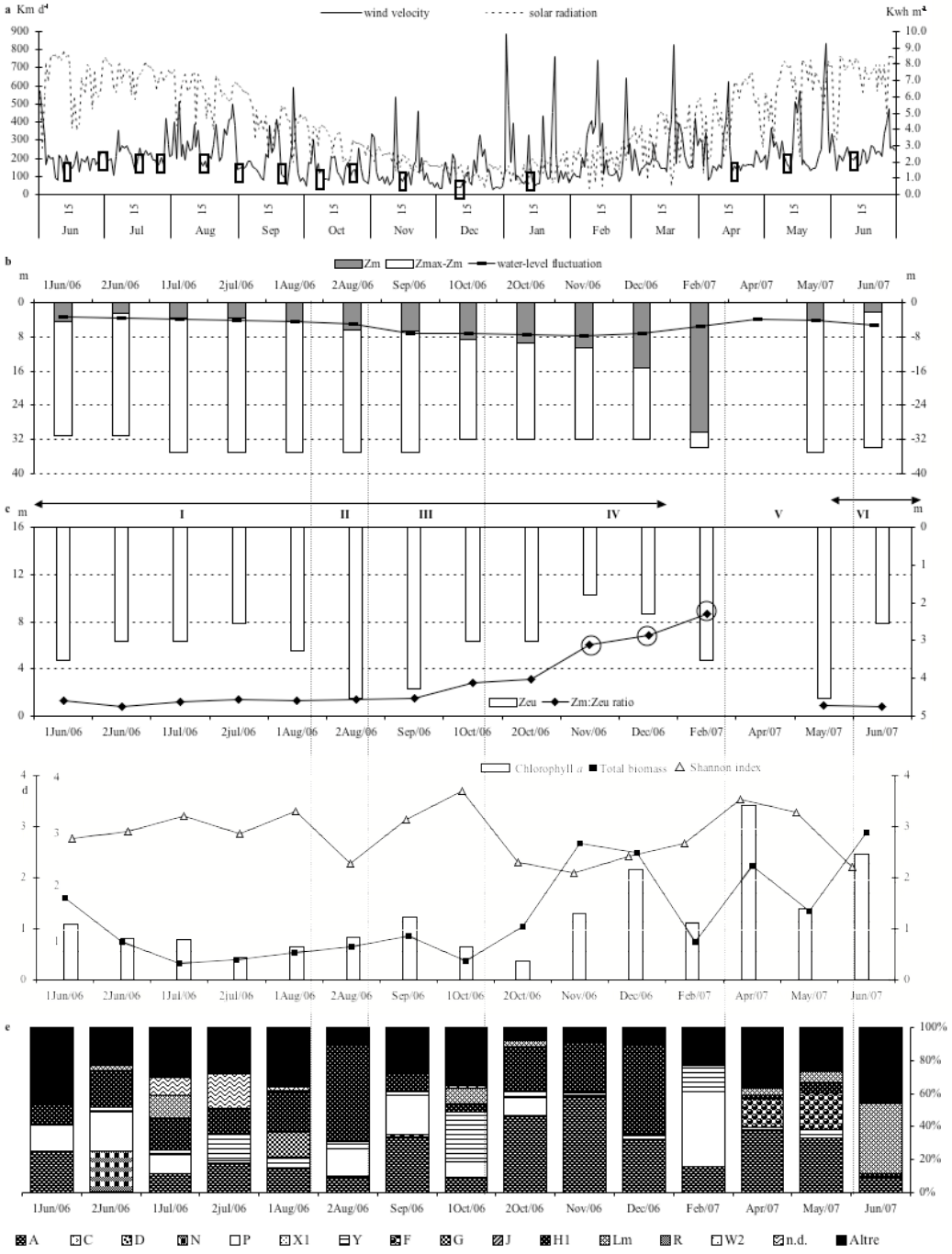
Figures 3 Cuga reservoir: a) Wind velocity (left axis) and solar radiation (right axis) with the sampling data in square; b) mixing depth (Zm) and Zmax-Zmix (left axis) in percentage, water level variation (in negative, right axis), disturbance events are evidenced with the circle; c) Zm:Zeu ratio (left axis) and euphotic (Zeu) (right axis); d) seasonal dynamics of total biomass and Shannon diversity index (outside and inside on the left axis) and chlorophyll *a* (right axis); e) percentages of biomass of dominant functional groups.



Figures 5 Pattada reservoir: a) Wind velocity (left axis) and solar radiation (right axis) with the sampling data in square; b) mixing depth (Zm) and Zmax-Zmix (left axis) in percentage, water level variation (in negative, right axis), disturbance events are evidenced with the circle; c) Zm:Zeu ratio (left axis) and euphotic (Zeu) (right axis); d) seasonal dynamics of total biomass and Shannon diversity index (outside and inside on the left axis) and chlorophyll *a* (right axis); e) percentages of biomass of dominant functional groups.



Figures 6 Sos Canales reservoir: a) Wind velocity (left axis) and solar radiation (right axis) with the sampling data in square; b) mixing depth (Zm) and Zmax-Zmix (left axis) in percentage, water level variation (in negative, right axis), disturbance events are evidenced with the circle; c) Zm:Zeu ratio (left axis) and euphotic (Zeu) (right axis); d) seasonal dynamics of total biomass and Shannon diversity index (outside and inside on the left axis) and chlorophyll *a* (right axis); e) percentages of biomass of dominant functional groups.



Figures 7 Temo reservoir: a) Wind velocity (left axis) and solar radiation (right axis) with the sampling data in square; b) mixing depth (Zm) and Zmax-Zmix (left axis) in percentage, water level variation (in negative, right axis), disturbance events are evidenced with the circle; c) Zm:Zeu ratio (left axis) and euphotic (Zeu) (right axis); d) seasonal dynamics of total biomass and Shannon diversity index (outside and inside on the left axis) and chlorophyll *a* (right axis); e) percentages of biomass of dominant functional groups.

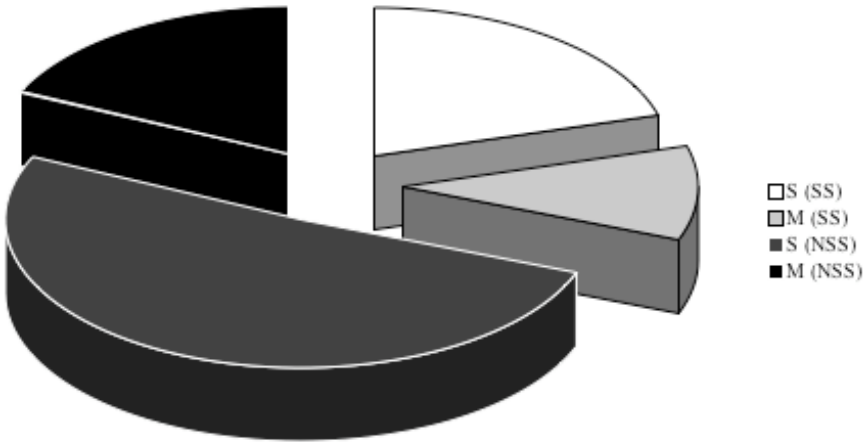


Figure 8. Steady (SS) and non steady (NSS) states in the stratification (S) and mixing (M) phases.

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Thematic B: Quality Indexes

Chapter1:

Confronto tra indici di qualità basati sul fitoplancton per l'implementazione della "WFD" Direttiva 2000/60/CE ai laghi italiani.

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Abstract

The Water Framework Directive (2000/60/CE) requires that the ecological quality of water bodies is estimated by comparing a series of biotic indicators to a reference optimum condition.

To establish which already developed indices fit best the estimate of the trophic status of Italian lakes, two indices were applied to 11 natural lakes in the sub-alpine area and 8 reservoirs in Sardinia: Catalàn Index and Brettum Index. The results show a good correspondence of the Brettum Index for the lakes of the sub-alpine ecoregion only; none of the two indices seems to be right to assess the ecological quality of Sardinian reservoirs.

Riassunto

La Direttiva 2000/60/CE richiede che la qualità ecologica dei corpi idrici sia valutata in base al confronto tra i valori di una serie di indicatori biotici rispetto ad una condizione ottimale di riferimento. Per verificare quali indici già sviluppati si adattino meglio alla stima della qualità dei laghi italiani, ne sono stati testati due a 11 laghi naturali dell'area subalpina e ad 8 bacini artificiali della Sardegna: l'indice di Catalàn e quello di Brettum. I risultati mostrano una buona corrispondenza unicamente dell'indice di Brettum per i laghi dell'ecoregione alpina mentre per quelli artificiali sardi nessuno dei due indici pare adatto alla valutazione della loro qualità ecologica.

1.1 Introduzione

La Direttiva Quadro 2000/60/CE indica le azioni che i paesi membri devono compiere in termini di qualità delle acque, richiedendo che la qualità dei corpi idrici venga definita attraverso l'uso di indicatori biotici rispetto a condizioni di riferimento specifiche per ogni tipologia di ambiente acquatico. Gli elementi idromorfologici e chimico-fisici quindi, vengono valutati solo in relazione all'influenza che questi possono esercitare sulle comunità presenti. Il fitoplancton è, nel caso specifico degli ambienti lacustri, uno degli elementi biotici considerato utile per la valutazione di qualità dell'ambiente. Questo è possibile in virtù del fatto che i valori del fosforo totale, considerato l'indicatore più importante nella valutazione dei livelli trofici lacustri (OECD, 1982), è ben correlato con due variabili che sono, a loro volta, diretta espressione dell'intensità dello sviluppo fitoplanctonico: biovolume e clorofilla *a*. L'obiettivo di questo lavoro è stato quello di verificare la risposta di due diversi indici, quello di Catalàn (Agència Catalana de l'Aigua, 2003) e quello di Brettum (Dokulil *et al*., 2005), in differenti contesti lacustri italiani, al fine di valutarne un loro possibile utilizzo. I due indici considerati sono una parte di quelli valutati nell'ambito dei Gruppi di Intercalibrazione Geografica GIG-mediterraneo e GIG-alpino.

1.2 Materiali e metodi

Sono stati considerati 19 laghi rientranti in tre delle categorie definite dal progetto "ECOSTAT" (van den Bund *et al*., 2003):

- 5 grandi laghi naturali profondi dell'ecoregione alpina (L-AL3; Z>15 m) (Lago Maggiore, Idro, Mezzola, d'Iseo e di Como);
- 6 grandi laghi naturali poco profondi dell'ecoregione alpina (L-AL4; Z<15 m) (Lago Annone, Caldaro, Pusiano, Segrino, Ganna ed Endine);
- 8 laghi artificiali mediterranei della Sardegna (I-SAR; Lago Alto Flumendosa, Bidighinzu, Liscia, Medio Flumendosa, Monteleone Roccadoria, Mulargia, Pattada e Sos Canales).

Per ogni lago si sono considerate le serie di dati pluriennali disponibili (da un minimo di una annata ad un massimo di 14 annate) per i soli mesi estivi (giugno - settembre), presumibilmente di massima produttività, prendendo in esame i valori di biovolume, clorofilla *a* e fosforo totale, ottenuti rispettivamente con le

metodiche di Findenegg (1974), Golterman *et al*. (1978) e Strickland & Parsons (1972).

1.3 Risultati

I valori di clorofilla *a* sono risultati ben correlati al fosforo totale per tutte le diverse tipologie lacustri prese in esame nelle due ecoregioni (Fig. 1a). A sua volta, il biovolume è risultato ben correlato con i valori di clorofilla *a* (Fig. 1b).

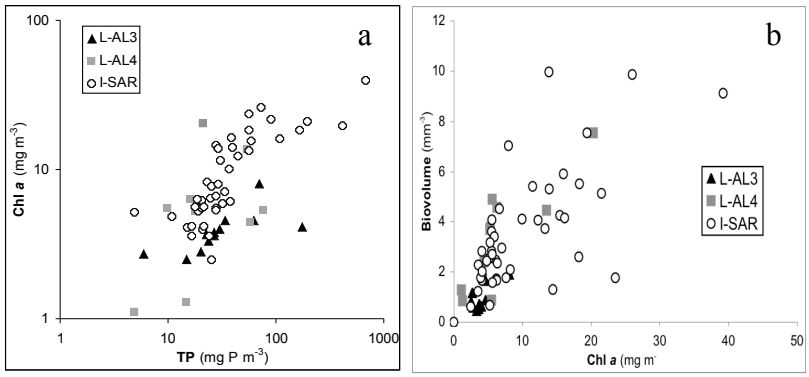

Fig. 1. Correlazione dei valori medi (a) di clorofilla a e del fosforo totale (n=66, r=0,463) e (b) del biovolume (n=65, r=0,516) rispetto a quelli della clorofilla a per l'insieme dei laghi considerati.

Dall'applicazione dei diversi indici si evince che quello di Catalàn, nonostante la buona correlazione mostrata con il fosforo totale nei laghi dell'ecoregione alpina (Fig. 2a), non descrive bene il loro stato qualitativo, in particolare per quelli a minore trofia. Inoltre, non pare assolutamente adeguato per i laghi sardi dell'ecoregione mediterranea (Fig. 2b).

Anche l'indice di Brettum, si correla in modo significativo ai dati della sola tipologia laghi alpini, descrivendone una relazione inversa (Fig. 3a), e risulta non valido nell'applicazione ai laghi della Sardegna (Fig. 3b).

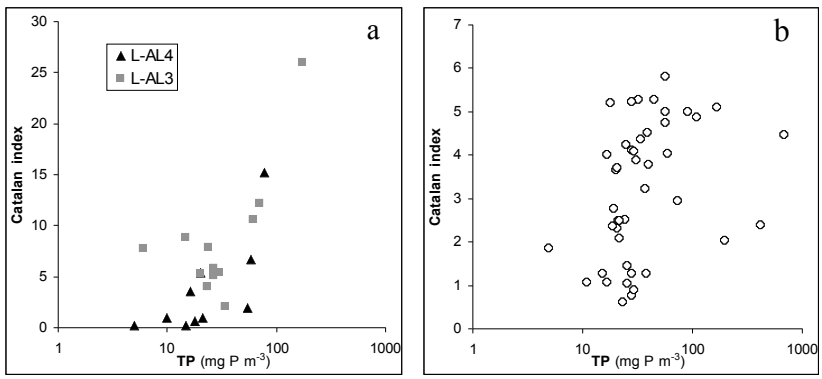

Fig. 2. Correlazione del fosforo totale con l'indice di Catalàn (a) per i laghi dell'ecoregione alpina (n=22, r=0,743) e (b) per i laghi sardi dell'ecoregione mediterranea (n=44, r=0,024).

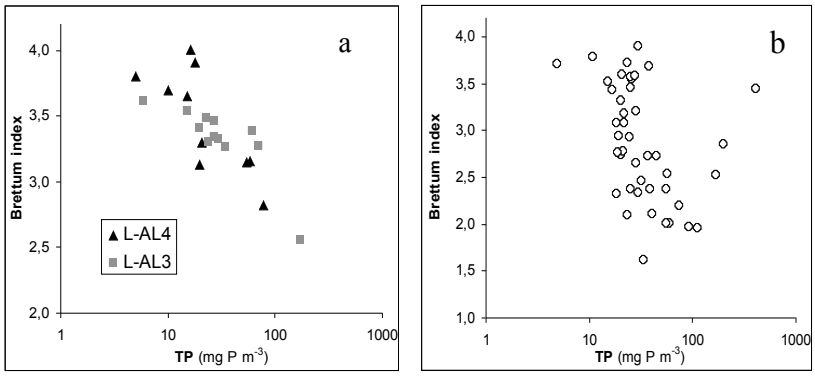

Fig. 3. Correlazione del fosforo totale con l'indice di Brettum (a) per i laghi dell'ecoregione alpina (n=22, r=0,626) e (b) per i laghi sardi dell'ecoregione mediterranea (n=44, r=0,006).

1.4 Discussioni e conclusioni

Sulla base delle elaborazioni, allo stato attuale, la qualità dei corpi idrici dell'ecoregione alpina risulta descrivibile attraverso l'applicazione dell'indice di Brettum, mentre quello di Catalàn non risulta idoneo a nessuna delle due categorie considerate di questa ecoregione (L-AL3 e L-AL4). Per i laghi artificiali della Sardegna, e quindi dell'ecoregione mediterranea, i due indici non sono in grado di valutarne la qualità delle acque.

Sia per l'indice di Brettum che di Catalàn, per aumentarne il livello di significatività, si propone, come sviluppo dell'indagine, una loro ricalibrazione, includendo alcune specie caratteristiche dei singoli contesti regionali e/o escludendo quelle che invece inficiano l'applicabilità degli indici stessi.

Emerge inoltre la necessità di estendere la valutazione dell'applicabilità di altri indici ai laghi esaminati. Per esempio il PTI, già calibrato per i laghi profondi dell'ecoregione alpina (Salmaso *et al*., 2005), può essere testato anche per le altre tipologie lacustri considerate in questo lavoro.

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Chapter 2:

Proposal of a new ecological water quality index for the Mediterranean reservoirs: MedPTI.

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Abstract

This paper reports a proposal for a new ecological index based on phytoplankton (MedPTI) as suggest by WFD 2000/60/CE. The elaborated index can be used to verify the effects of eutrophication, one of the main freshwater ecological problems, in Mediterranean reservoirs belonging to different categories of the WFD. For the MedPTI elaboration a data set from 31 Sardinian reservoirs was employed. A list of 46 selected species was obtained. For each of these species the trophic (vk) and the indicator values (ik) were calculated. MedPTI was submitted to the procedure of intercalibration for the definition of the reference conditions with the calculation of EQR and class limits. It was found a good correlation between the index and the trophic gradient, represented by the total phosphorus concentrations (OECD, 1982). Finally, the index was applied to 6 Sardinian reservoirs, considering data collected during 2006-2007 and for 3 of the 6 reservoirs also pluriannual data (from 3 to 5 years). Results were compared with those obtained by the use of the OECD probabilistic model on the same series of data. On these bases, MedPTI results a valid index to classify the ecological quality of the Mediterranean reservoirs belonging to L-M7 and L-M8 categories of the WFD categories.

Keywords: Phytoplankton, Trophic state, Eutrophication, Water Frame Directive, Mediterranean ecoregion.

2.1. Introduction

The phytoplankton, for its peculiar ecology, is considered one of the biotic elements useful to the evaluation of environmental quality of the lakes (Thunmark, 1945; Nygaard, 1949; Hörnström, 1981; Brettum, 1989; Tremel, 1996; Schönfelder, 1997; Salmaso *et al*., 2006; Marchetto *et al*., 2006). This thematic has assumed in the last years a central role in the ambit of aquatic applied ecology. It is especially due to the impulse of the Directive 2000/60/CE (WFD, Water Framework Directive), which requires for the evaluation of the ecological quality of the aquatic ecosystems the use of numerical indexes constructed on the basis of biological parameters (EU Work Group ECOSTAT/IC, 2003a,b). For lakes, biovolume and phytoplankton composition seem to be able to this purpose, also because the wide availability of data concerning them. Moreover, a number of European countries have indexes for the ecological water quality evaluation based on phytoplankton. It is especially if eutrophication is considered as the most important anthropic pressure with the acidification, but the latter only in the northern countries.

In general, two types of indexes can be identified. The first includes indexes based on trophic preferences of each species. To construct them the abundance of each species in lakes of different trophic state is evaluated, and they are given a trophic score and, in some cases, an indicator value. The latter expresses how possible it is to find the considered species in environments different from those corresponding to its trophic score. Species parameters are always obtained using field data in a relatively uniform region, to minimize the effects of biogeographic and climatic features. Lake ecological quality is then evaluated on the basis of the trophic cores and indicator values of the species found in it, generally through the use of a weighted mean. Indexes of this type are used in Norway (Brettum, 1989), Austria (revised Brettum index, Dokulil *et al*., 2005), Germany (PTSI, Riedmüller et al., 2006), Sweden (TPI, Willén, 2007) as well as in Italy, for the deep Alpine lakes (Salmaso *et al*., 2006).

All these indexes are calibrated on data coming from a number of lakes (calibration data set) used to estimate the trophic values and the indicator values of the species by weighted averaging (ter Braak, 1987) or using lake score in a constrained ordination, using the trophic gradient as explicative variable (Salmaso

et al. 2006). Brettum's index expands this concept by the use of the percent frequency of finding of each species in lakes of 5 arbitrary trophic classes (Brettum, 1989; Brettum & Anderson, 2005; Dokulil & Teubner, 2006).

Indexes belonging to the second type, on the contrary, are based on percent biovolume of a given algal group, or on the ratios between the biovolumes of two algal groups. These indexes simplify the enumeration of the algae, allowing the evaluation of the water quality using a lower level of taxonomic precision, at the expenses of more stringent assumptions on the composition of species assemblages. They need them to be used with care, because every algal groups includes species with different ecological preferences.

Indexes of this kind are used in France (ITP or Barbe index, Philippe *et al*., 2003) and Spain (Catalàn index, Agenzia Catalana de l'Aigua, 2003). In particular, the Catalàn index, calibrated on reservoirs of the Autonomous Region of Catalonia, is the first index developed for the Mediterranean sites (Agenzia Catalana de l'Aigua, 2003).

The application of some of the indexes above mentioned (properly Barbe, Brettum and Catalàn index) showed that none of them correctly evaluated the ecological quality of Mediterranean Italian lakes. It is especially if reservoirs are considered. In fact, a part from the very particular and small category of the volcanic Italian lakes, the majority of Mediterranean Italian lakes are reservoirs. It was evident that the considered indexes did not clearly reflect the response of algae to the trophic gradient in a wide casuistry of Sardinian reservoirs (Marchetto *et al*., 2006).

The aim of this study is to propose a specific index for Mediterranean lakes and reservoirs, following an approach similar to that used by Salmaso *et al*. (2006) for deep Alpine lakes.

This index was used in the pan-European intercalibration procedure carried out for the implementation of the WFD, in order to obtain index values for reference conditions and boundaries among quality classes.

2.2. Area of application

The elaborated index is calibrated and can be used to evaluate the effects of the eutrophication, in the following type of lakes:

- Reservoirs at a level less than 800 m a.s.l. in mainland and insular Italy at a latitude less than 44° north, having a mean depth superior to 15 m, and having a conductivity less than 2.5 mS cm^{-1}.

This type corresponds to types 22 and 23 of the wider Italian typology, ME-4 and ME-5 of the simplified Italian typology, and to L-M5, L-M7 and L-M8 as defined by the exercise of intercalibration in the ambit of the procedure of implementation of Directive 2000/60/CE (EU Work Group ECOSTAT/IC, 2003a,b).

This does not exclude the possibility to use the index in other freshwater sites of the Mediterranean ecoregion. It cannot be used in brackish and half-brackish sites, with conductivity higher than the marked threshold, that include different compositions of phytoplankton.

2.3. Data set

The dataset used to construct MedPTI was composed by data concerning 31 Sardinian reservoirs, belonging to L-M7 and L-M8 categories (Fig. 1; Tab. 1). Seasonal data collected in 1994 (4 samplings) were considered for all the reservoirs. For 4 lakes (Alto Flumendosa, Medio Flumendosa, Mulargia and Sos Canales) pluriannual data, from 1985 to 1999, were also considered, from a minimum of 4 years (Alto Flumendosa, Medio Flumendosa and Sos Canales) to a maximum of 15 years (Mulargia). In these cases, 4 samplings for each year and reservoir were selected, possibly the summer ones because problems due to eutrophication are more evident in this season. The pluriannual data were assumed for each year and reservoir as series in themselves. As a whole, the dataset regarded 240 samplings and data concerning biovolume of phytoplankton taxa, chlorophyll *a* (CHL *a*) and total phosphorus (Ptot) were considered. The list of species was composed by 148 elements, with a taxonomic precision ranging from the genera to the variety. This "row" list of species and the corresponding Ptot values of their findings were the basis for the mathematical elaboration of MedPTI.

Biovolume was obtained multiplying the cell density of each taxon, evaluated according to Utermöhl's method (Sournia, 1978 and Innamorati, 1990), by unitary cell biovolume of the same taxon, obtained by geometrical approximations. Total phosphorus and chlorophyll *a* were analysed following, respectively, Strickland and Parsons (1972) and Goltermann *et al*. (1978).

2.4. Mathematical data treatment

The MedPTI index was constructed applying the method of weighted means on the database above described.

The mathematical treatment was finalised to attain different goals: (1) identify the species among the 148 elements of the "row" list to be considered useful to assess water quality; (2) individuation of the samplings of the dataset useful to assess water quality, on the base of the species selected (point 1); (3) calculate the trophic and indicator values of the selected species (point 1); (4) formulation of MedPTI index and (5) definition of the limits of the quality categories and conditions for the use of MedPTI index.

These goals were reached according to the following steps:

1. Identification of the species to be used

1a. **Calculation of the mean biovolume (bk_j) of each *k*-nth of n species in each *j*-nth of m lakes.**

The data were calculated as weighted means in the photic zone (2.5 times the depth of disappearance of the Secchi Disk) in the cases of availability of discrete samples at different depths, instead of the data of integrate samples along the photic zone, if they were not available.

1b. **Calculation of the mean percentage biovolume of every *k*-nth species in each sampling of each *j*-nth of m lakes,** according to the equation:

(*Formula 1.*) $$p_{k,j} = \frac{b_{k,j}}{\sum_{k=1}^{n} b_{k,j}} \cdot 100$$

1c. **Taxa which did not account for at least 1% of the total biovolume in 3 or more annual samples were discredited, or joined together in a larger**

taxonomic unit. The selection procedure led to a shorter taxa list including 46 elements (Tab. 2). The selected taxa belong to 7 classes and 12 orders (Tab. 3). The most important groups were, respectively Cyanophyceae, Bacillariophyceae and Chlorophyceae for classes and Chlorococcales and Centrales for orders.

2. Individuation of the sampling data to be used

2a. Individuation of the samplings in which the sum of biovolume of the selected species found (Tab. 2) represented at least 70% of the total biovolume, and contemporary elimination of the samplings which did not satisfy this necessary condition (necessary condition number 2).

3. Calculation of the trophic and indicator values

Being MedPTI index based on species, two scores were calculated for each of the 46 selected species (Tab. 2): the trophic value (v) and the indicator value (i).

3a. Calculation of the trophic value of the k-nth species (vk). These values represent the frequency of finding of each species in a definite trophic level. To obtain these values it was necessary to calculate P and L as following:

3b. Calculation of mean total phosphorus concentrations (Ptot) in the water column as integrate samples or as weighted means in the cases of discrete samples at different depths (P_j) in each of the selected samplings (point 2a) of the each *j*-nth of m lakes. It seems to be more appropriate the use of averaged concentrations in the photic zone in natural lakes, stratified during the summer. On the contrary, in the case of reservoirs, whose summer stratification is often disturbed by water management and level variations, it is recommended to calculate average concentrations along the entire water column.

3c. Calculation of the logarithm (L) of such Ptot concentrations and scale linearly the obtained values multiplying them by 4 and subtracting 3.7. The trophic values are then linearly rescaled (multiplying by 4 and subtracting 3.7) to allow them to fall between 1 and 5:

(Formula 2.) $Lj = \log_{10} Pj \cdot 4 - 3.7$

The trophic value of the k-nth species (v_k) is then obtained using the following equation:

(Formula 3.) $$v_k = \frac{\sum_{j=1}^{m} p_{k,j} \cdot L_j}{\sum_{j=1}^{m} p_{k,j}}$$

3d. Calculation of indicator value of the k-nth species (i_k). The indicator value of the *k*-th taxon (i_k) represent for that taxon to be found only in trophic conditions close to those corresponding to its trophic value. To obtain these values it was necessary to calculate, for each sampling and for each species, the quadratic difference ($D_{k,j}$) between the trophic value of the species (v_k) and the logarithm value L_j:

(Formula 4.) $Dk,,j = (v_k - L_j)^2$

The indicator value i_k is the inverse of the mean of the calculated quadratic differences, weighted on the biovolumes:

(Formula 5.) $$i_k = \frac{\sum_{j=1}^{m} p_{k,j}}{\sum_{j=1}^{m} p_{k,j} \cdot D_{k,j}}$$

This formulation of the indicator value, equal to the inverse of the square of the tolerance, was chosen to simplify successive calculations for the determination of the index for every single reservoir.

4. MedPTI formulation

To obtain the value of MedPTI index in a reservoir the following formula must be employed:

(*Formula 6.*) $$MedPTI = \frac{\sum_{k=1}^{n} p_k \cdot t_k \cdot i_k}{\sum_{k=1}^{n} p_k \cdot i_k}$$

where the numerator is the sum of the product of p_k (*Formula 1.*), v_k (*Formula 3.*) and i_k, (*Formula 5.*), for each of the selected taxon found in the considerated reservoir reported in Table 2 and, and the denominator is the sum of the product of p_k (*Formula 1.*) and i_k (*Formula 3.*) for the same species.

5. Definition of class limits and ecological quality ratios

To define the class limits of MedPTI the results of the phytoplankton intercalibration exercise carried out by the Mediterranean Geographical Intercalibration Group (GIG; WFD Intercalibration Technical Report, pp. 243), were used. For details of the selection procedure and the approval of the sites see also the technical documents prepared by the Work Group ECOSTAT/IC (EU 2003a,b).

The results of the calculation of MedPTI index in each of the GIG sites are reported in Table 4.

Following the Mediterranean GIG indications, the reference for the MedPTI index was chosen in the median value of the index calculated considering reference site data. The value was equal to 3.08, whereas the limit between "good" and "moderate" classes was the 95° percentile of the values of the Mediterranean GIG sites considered as the boundary between the two classes. It resulted equal to 2.45, so that, the relative EQR value was 0.78.

For the definition of the other class limits, it was employed the technique of equal intervals. The obtained class limits and the relative EQR are summarised in Table 5.

2.5. Validation of the boundary setting protocol

Reference conditions and boundaries among quality classes were agreed among Mediterranean countries during the ECOSTAT intercomparison exercise (Mediterranean GIG, 2007). The boundary between "good" and "moderate" quality classes was also agreed, as this is the most important boundary for WFD implementation, e.g. the minimum quality that reservoirs should show at the end of their restoration. The agreed values were different for reservoirs laying in siliceous and calcareous catchments.

During the IC procedure, the reference conditions and class boundaries were set on the basis of a number of flagship sites provided by the Member States and reported to represent reference and boundary conditions. However, the WFD guidelines for setting boundary limits, states that it would be preferable to set the limits in correspondance with natural discontinuity in the quality gradient.

In fact, The most important class limit, between “good” and “moderate”, effectively separates the 31 Sardinian reservoirs into two well differentiates groups of oligo-mesotrophic and eutrophic environments. In Figure 2 is shown the correlation between the MedPTI index values and the trophic gradient, represented by the Ptot concentrations ($r=0.76$, $n=61$, $p<0.05\%$). The horizontal line represents the limit between “good” and “moderate” classes, obtained during the ECOSTAT/IC procedure (Mediterranean GIG, 2007). It separates the Sardinian reservoirs into the above mentioned trophic groups. In fact, the reservoirs which show MedPTI higher than the limit are also characterised by Ptot concentrations higher than 60 $\mu g\ P\ l^{-1}$ and are therefore considered decisively eutrophic.

2.6 Testing the MedPTI index

MedPTI was applied to 6 reservoirs of north-centre Sardinia: Bidighinzu (BID), Cedrino (CED), Cuga (CUG), Pattada (PAT), Temo (TEM) and Sos Canales (SCN), considering data not included in the dataset used to formulate it. The phytoplankton data of these reservoirs were collected during 2006-2007. Moreover, the index was applied also on pluriannual data concerning SCN (1991, 1992, 1993, 1997 and 2005), PAT (1989, 1994 and 1997) and TEM (1989, 1994 and 1997). The MedPTI results were compared to the trophic valuations obtained

by the OECD model (1982) - already well tested for the examined reservoirs (Sechi and Lugliè, 1992, 1996) - which considers Ptot, CHL a (annual mean and maximum) and Secchi Disk transparency (annual mean and maximum) as principal trophic descriptors. To simplify the informations, the probabilistic percentages of trophic state were summed in two groups ("eutrophy + hypereutrophy" and "mesotrophy + oligotrophy"), arbitrarly considered to correspond to the categories "moderate + worse" and "good + better" respectively. The distinction between the "good" and "moderate" categories is in fact very important to individuate the sites to be recovered within 2015, according to WFD (2000/60/CE).

Samples were collected during 2006-2007 fortnightly from June to September and monthly from October to May at one station in each reservoir, using a Niskin bottle at depths of 0 m, 1 m, 2.5 m, 5 m, 7.5 m, 10 m, 15 m e, and over this level, at intervals of 10 m till the bottom.

The used methods were the same already described in the "Data set" paragraph, both in 2006-2007 and in the previous years. 4 summer samplings, from June to September, were considered for the MedPTI application whereas all the year data were used for the OECD model.

It was possible apply the index on 5 reservoirs over the 6 considered using the 2006-2007 data because in BID the sum of the biovolumes of the species referred to the MedPTI list, was less than the minimum of 70%, a necessary condition for the index application (*Section 2.*).

BID, since its first filling up in 1956, has showed very variable and problematic limnetic conditions. It is due to its high chronic eutrophy, with very different composition of the dominant species year by year (Messina, 1966; Bo *et al*., 1968; Bo, 1969; Alamanni *et al*., 1968; 1971; Sechi, 1986; Marchetti *et al*., 1992; Lugliè and Sechi, 1993; Sechi and Lugliè, 1996; Sechi, 2000; Lugliè *et al*., 2001). This does not seem good for the effective application of the MedPTI index in reservoirs characterised by so much high eutrophy, highlighting the necessity to enlarge the currently formulated MedPTI also in the case of this kind of environments.

On the contrary, the sums of the species biovolumes in the other 5 reservoirs were higher than 70% (Tab. 6): 90% for CED, 99% for CUG, 92% for PAT, 99% for SCN and 94% for TEM.

The trophic levels of these reservoirs were in the range from eutrophy (CUG, CED) to mesotrophy (PAT, SCN) to oligo-mesotrophy (TEM).

Water quality indication using MedPTI index was mainly in agreement with the classification obtained with the OECD model.

As shown in Figure 3, MedPTI index results positively correlated to Ptot ($r=-0.818$, $n=16$ and $p<0.05\%$) in the tested reservoirs (BID excluded). On one hand this confirms that Ptot really represents the best abiotic indicator for eutrophication in Sardinian reservoirs, and, on the other, the good response of MedPTI in describing this environmental problem.

On the contrary, Secchi Disk transparency does not appear a good variable in valuating water quality of Mediterranean reservoirs. It could depend on a series of causes and, among them, the turbidity induced by suspended material originated by the intense runoff of the watersheds, as already observed in Sardinian reservoirs (Sechi and Cossu, 1979).

Among the reservoirs for which MedPTI values were calculated, CUG appears the worst. On the base of MedPTI, this reservoir results in the poor-bad category (1.17). This condition is confirmed by the OECD model, which indicates eutrophy as the most probably state of this reservoir (Fig. 4).

CED and SCN are classified in the moderate-poor category by MedPTI (respectively 1.98 and 1.82). This is mainly confirmed by the OECD model, which classifies them as eutrophic, even if with a higher probably for CED.

In fact, SCN is classified by the OECD model, at least for Ptot, in a better trophy condition. The good agreement between OECD evaluation based on CHL a data (both annual mean and maximum) and MedPTI is an important result because the index should reflect, as it seems it does, the biological quality of the reservoir.

Both PAT and TEM, show MedPTI values near to the boundary between the two categories (2.26 and 2.41), well pointed out also by the OECD model.

TEM appears to be more probably eutrophic on the base of Ptot (55%), but mesotrophic and oligotrophic for mean (60%) and maximum CHL a (48%).

Analysing the pluriannual series, the characteristic wide trophy variability of Mediterranean reservoirs appears very evident (Fig. 5).

MedPTI indicates a very high quality as regard SCN (Tab. 7; Fig. 5) in 1991 while is not possible to calculate it in 1992 and 1993 because the necessary condition 1 (*Section 2*.) is not satisfied. A probably eutrophic condition is express by the OECD model for both the three years. MedPTI seems more related with OECD model in 1997, 2005 and 2006.

A kind of "double trophic identity" of SCN, corresponding to mesotrophy for Ptot and to eutrophy, till ipereutrophy, for annual mean and maximum CHL a values already emerged in past studies (Lugliè *et al*., 1996). Anyway, if the species composition of phytoplankton in each of the first years (from 1991 to 1993) is taken in account, it result that one of the most important species in determining total biovolume is not included in the present MedPTI list of species. This highlights the necessity to enlarge the actual list.

TEM and PAT series show more agreements between MedPTI and OECD indications. PAT is constantly classified as more probability eutrophic by OECD model and in moderate-poor category by MedPTI index during 1989, 1994, 1997 and 2006 (Fig. 5), whereas both the most probably trophic state and quality category of belonging seem improved in 2006 (Fig. 5).

2.7 Conclusions

The elaborated and proposed MedPTI index can be considered an useful tool to the evaluation of the ecological quality of reservoirs in mainland and insular Italy, limited to types 22 and 23 (ME-4 and ME-5), in agreement to the guidelines of the WFD/2000/60/CE. In fact, the followed procedure in defining the class limits and the relative EQR was based on the dataset of the Mediterranean GIG. The obtained limits of the index actually separate the Sardinian reservoirs in quality categories in agreement with the OECD trophic classification. Among the parameters considered by this probabilistic model, the index is especially well correlated with Ptot. It is confirmed that, Secchi disk transparency is not a good

variable in valuating water quality of Mediterranean reservoirs, with a tendency to give a worst evaluation of the trophic state (Sechi and Cossu, 1979). On the contrary, Salmaso *et al.* (2006) found that the same parameter underestimates the trophic status of some deep Alpine lakes.

The proposed MedPTI list of species useful to estimate ecological quality of reservoirs is composed by 46 elements, with the predominance of taxa belonging to Cyanobacteria, Bacillariophyceae and Chlorophyceae classes and Chlorococcales and Centrales as the most important orders. The comparison with the list proposed by Salmaso *et al.* (2006) for lakes of Alpine ecoregion highlights a higher number of elements (39) but a different composition. In fact, both the number of classes (9) and orders (15) were higher in the Alpine list in respect to the Mediterranean (7 classes and 12 orders).

Some limitations in the use of MedPTI appear evident: on one hand the difficulty in applying the index in very eutrophic waters, as it was for BID, and on the other in the case of reservoirs with a not very defined trophic conditions, especially on the boundary between mesotrophy and eutrophy, as it was for SCN. Both these cases highlighted the necessity to enlarge the proposed list of species.

For this, the definition of the index is not and cannot be in anyway definitive. Other species with different ecological exigencies can be found in Mediterranean reservoirs, or be present in sites not considered in this study. For this reason the list of trophic and indicator values given in table 1 should be properly updated, by the use of other previous collected data and when further results of monitoring programmes concerning Italy and/or other Mediterranean countries will be available.

Table 1. List of the considered lakes, their main morphological data and related years used to formulate MedPTI index. (Use: AL= alimentary; EE=electric energy; IN=industrial; IR=irrigation; Trophic state: IE=hypereutrophic; E=eutrophic; M=mesotrophic).

Reservoir	Altitude (m a.s.l.)	Drainage basin area ($m^2 10^6$)	Volume (m^3 10^6)	Area (km^2)	Zmed (m)	Use	Trophic state	Study years
Alto Flumendosa	802	180	61.4	3.2	19.2	-	M	1990/97
Bau Pressiu	249	29	6.2	0.2	31.0	AL	M	1994
Benzone	150	89	1.1	0.3	4.0	EE, AL, IN	E	1994
Bidighinzu	334	52	12.2	1.7	7.3	AL	IE	1994
Bunnari Alta	287	18	1.2	0.1	15.4	AL	E	1994
Casteldoria	25	2378	8.3	0.4	20.8	IR, EE, AL, IN	E	1994
Cedrino	103	631	30.0	1.1	26.5	AL, IR	E	1994
Cixerri	40	426	25.3	4.2	6.0	IR, AL	IE	1994
Coghinas	170	1729	258.7	17.2	15.0	-	E	1994
Corongiu II	155	34	0.6	0.2	4.0	AL	E	1994
Corongiu III	201	34	4.3	0.3	16.1	AL	M	1994
Cucchinadorza	318	92	17.5	1.1	16.9	EE	E	1994
Cuga	114	58	35.0	3.1	11.3	AL, IR	E	1994
Flumineddu	275	253	1.5	0.1	12.5	AL, IR, EE, IN	E	1994
Gusana	645	191	59.5	2.6	22.9	EE	E	1994
Is Barrocus	415	95	11.9	1.1	10.9	AL	E	1994
Leni	250	75	20	1.1	17.7	AL, IR	E	1994
Liscia	180	284	105.0	1.3	25.7	AL, IR, IN	E	1994
Medio Flumendosa	270	572	300.0	4.2	23.8	-	M	1986/90, 1994
Medau Zirimilis	-	29	5.0	0.6	9.1	-	E	1994
Monte Pranu	46	435	50.0	5.3	9.4	IR, AL	E	1994
Monteleone Roccadoria	226	143	55.4	3.5	15.8	AL, IR	E	1994
Monteponi	-	8	1.0	0.1	10.1	AL, IN	E	1994
Mulargia	260	179	300.0	10.5	23.8	AL, IR, EE, IN	E	1985/99
Omodeo	118	2077	148.6	13.5	11.0	EE, IR, AL	E	1994
Posada	43	614	27.8	3.0	9.3	IR, AL	E	1994
Punta Gennarta	257	37	9.8	0.6	15.8	AL, IR	M	1994
Santa Lucia	-	49	3.7	0.4	8.6	-	E	1994
Sos Canales	714	16	4.3	0.3	13.2	AL	M	1991/94
Surigheddu	50	6	2.1	0.5	4.0	AL, IR	E	1994
Torrei	800	14	3.0	0.2	17.6	AL	E	1994

Table 2. List of selected MedPTI species and their trophic values (t) and indicator values (i).

Taxon	Authors	t	i
Anabaena flos-aquae	Bréb. ex Born. et. Flah.	1.33	6.32
Anabaena planctonica	Brunnth.	1.58	0.63
Anabaena sp.	(Bory) Bornet & Flahault	2.9	3.69
Anabaena spiroides	Kleb.	0.41	5.25
Ankistrodesmus falcatus	(Corda) Ralfs	3.14	3.64
Ankistrodesmus sp.	Corda	3.26	1.07
Aphanizomenon flos-aquae	(Linné) Ralfs ex Born. et Flah.	1.62	1.12
Aphanocapsa sp.	Nägeli	3.34	4.63
Aphanothece sp.	Nägeli	3.27	17.18
Aulacoseira ambigua	(Grunow) Simonsen	2.57	0.49
Aulacoseira distans	(Ehrenberg) Simonsen	1.42	1.64
Aulacoseira granulata	(Ehrenberg) Simonsen	3.14	1.13
Ceratium hirundinella/furcoides	(O. F. Muller) Schrank	2.83	0.74
Chlamydomonas sp.	Ehrenberg	4.14	0.75
Chlorella sp.	Beij.	3.17	1.32
Chroomonas sp.	Ehrenberg	1.1	1.69
Closterium aciculare	T. West	3.32	1.33
Closterium gracile	Bréb. ex Ralfs	1.34	5
Coelastrum sp.	Näg.	2.24	0.58
Cryptomonas sp.	Ehrenbg.	2.77	0.89
Cyclotella ocellata	Pantocksek	3.32	1.27
Cyclotella sp.	Kützing	3.03	0.74
Fragilaria crotonensis	Kitton	3.42	1.72
Fragilaria sp.	Lyngbye	3.19	4.9
Gemellicistis sp.	Teiling	3.14	1.31
Gymnodinium sp.	Stein	3.04	14.09
Melosira spp.	Agardh	2.32	0.73
Microcystis aeruginosa	(Kützing) Kützing	1.11	5.89
Microcystis flos-aquae	(Wittrock) Kirchner	1.11	3.13
Microcystis sp.	Kützing ex Lemmermann	1.06	1.85
Oocystis spp.	A. Br.	1.44	1.6
Pandorina morum	(Müller) Bory	3.68	2.32
Pediastrum simplex	Meyen	3.54	2.56
Peridinium sp.	Ehrenbger	3.13	4.73
Plankothrix sp.	Anagnostidis et Komárek	1.81	1.15
Planktothrix "agardhii-rubescens"	(Gomont) Anagnostidis et Komárek	3.24	4.99
Rhodomonas minuta	Skuja	3.2	4.51
Scenedesmus sp.	Meyen	2.6	1.1
Staurastrum gracile	Ralfs	3.1	1.2
Staurastrum sp.	Meyen	2.96	2.75
Stephanodiscus hantzschii	Grun.	1.24	25.52
Stephanodiscus sp.	Ehrenberg	1.11	1.34
Synedra sp.	Ehrenberg	3.19	1.34
Tetraedron minimum	(A. Br.) Hansg.	2.58	3.62
Trachelomonas sp.	E. em Defl.	2.22	0.51
Woronichinia sp.	Elenkin	2.85	1.38

Table 3. Main classes and orders of the selected MedPTI List.

Classes	**Orders**		**n° of taxa**
Cyanophyceae	Nostocales		5
	Chroococcales		6
	Oscillatoriales		2
		Total	*13*
Chlorophyceae	Chlorococcales		9
	Volvocales		2
		Total	*11*
Bacillariophyceae	Centrales		8
	Pennales		3
		Total	*11*
Dinophyceae	Peridiniales		3
		Total	*3*
Cryptophyceae	Cryptomonadales		3
		Total	*3*
Conjugatophyceae	Desmidiales		4
		Total	*4*
Euglenophyceae	Euglenales		1
		Total	*1*

Table 4. GIG sites and related MedPTI values.

	Lake	Country	MedPTI
reference sites	Tehniti Limni Tavropou	Grecia	3.03
	Castelo de Bode	Portugal	3.19
	Salime	Spain	3.08
	Lefkara	Cyprus	3.04
	Arenós	Spain	3.09
	Eugui	Spain	3.17
		Median value:	***3.08***
"Good" and "Moderate" boundary Med GIG sites	Agavanzal	Spain	3.05
	Agueda	Spain	2.94
	Aguieira	Portugal	3.17
	Alto Lindoso	Portugal	3.11
	Asprokremmos	Cyprus	3.06
	Bao	Spain	3.05
	Caniçada	Portugal	3.02
	El Yeguas	Spain	2.80
	Fronhas	Portugal	2.37
	Guadalest	Spain	2.91
	Guadalmellato	Spain	2.77
	Kouris	Cyprus	3.04
	Loriguilla	Spain	2.86
	Maranhão	Portugal	2.62
	Monte da Rocha	Portugal	2.66
	Mulargia	Italy	2.94
	Negratin	Spain	2.98
	Pálmaces	Spain	2.18
	Portodemouros	Spain	3.01
	San Esteban	Spain	3.07
	Sau	Spain	2.53
	Sos Canales	Italy	2.41
	Talarn	Spain	2.91
	Valparaiso	Spain	3.05
		5° percentile	***2.45***

Table 5. MedPTI class limits and the relative EQR.

Class limit	MedPTI	EQR
high – good	2.77	0.89
good – moderate	2.45	0.79
moderate – poor	2.13	0.69
poor – bad	1.81	0.59

Table 6. Percentage of biomasses of MedPTI selected species to total biomass.

Years	SCN	PAT	TEM
1989		75%	99%
1991	91%		
1992	97%		
1993	100%		
1994		75%	96%
1997	99%	91%	98%
2005	95%		
2006	99%	92%	94%

Table 7. Main trophic OECD descriptors and MedPTI values.

		mean Ptot (mg P m^{-3})	mean CHL *a* (mg m^{-3})	max CHL *a* (mg m^{-3})	mean SD (m)	MedPTI
Lakes	Bidighinzu	356.0	40.5	98.4	0.7	
	Cuga	86.1	12.8	29.8	0.77	1.17
	Cedrino	85.5	21.2	78.1	1.16	1.98
	Sos Canales	29.8	9.8	28.6	1.11	1.82
	Pattada	38.4	6.6	14.3	1.2	2.26
	Temo	57.7	3.6	7.6	1.25	2.41
SCN	1991	31.0	8.8	22.8	3.27	3.01
	1992	33.6	10.8	40.3	3.22	3.04
	1993	27.0	20.9	34.0	2.30	3.02
	1997	45.8	38.1	250.8	2.23	1.99
	2005	42.6	5.3	7.1	3.25	2.62
	2006	29.8	9.8	28.6	1.11	1.82
PAT	1988-89	60.3	18.7	36.1	1.70	2.02
	1994	34.9	17.6	25.4	1.68	2.05
	1997	51.8	16.2	37.5	1.20	1.96
	2006	38.4	6.6	14.3	1.20	2.26
TEM	1988-89	139.0	16.1	42.5	1.25	1.27
	1994	148.4	17.0	53.3	0.65	0.78
	1997	156.5	30.9	181.9	0.81	1.17
	2006	57.7	3.6	7.6	1.25	2.41

Figure 1. Localization of Sardinian reservoirs.

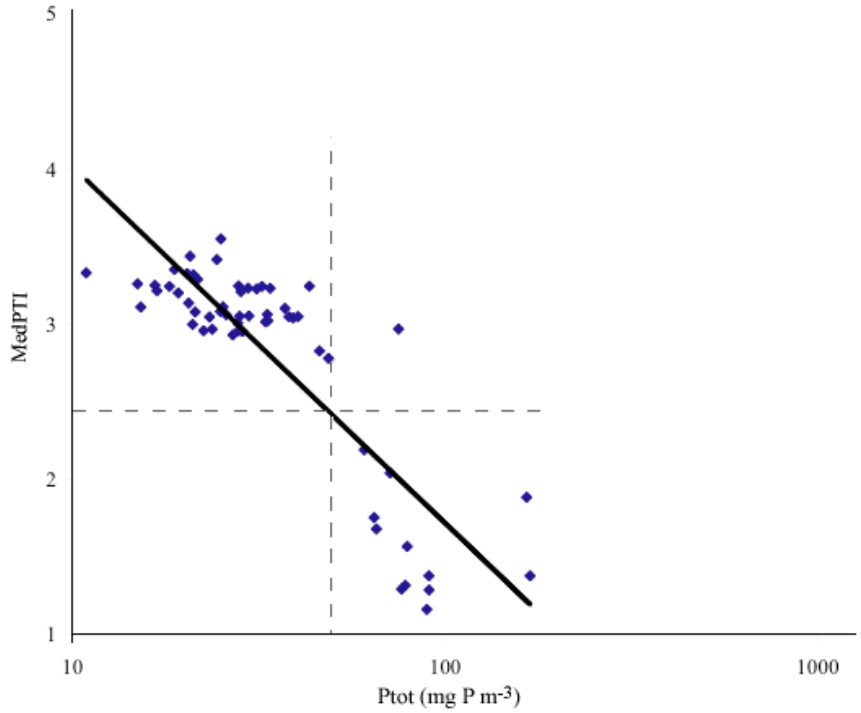

Figure 2. Relationship between MedPTI trophic index and Ptot concentration in MedPTI sites.

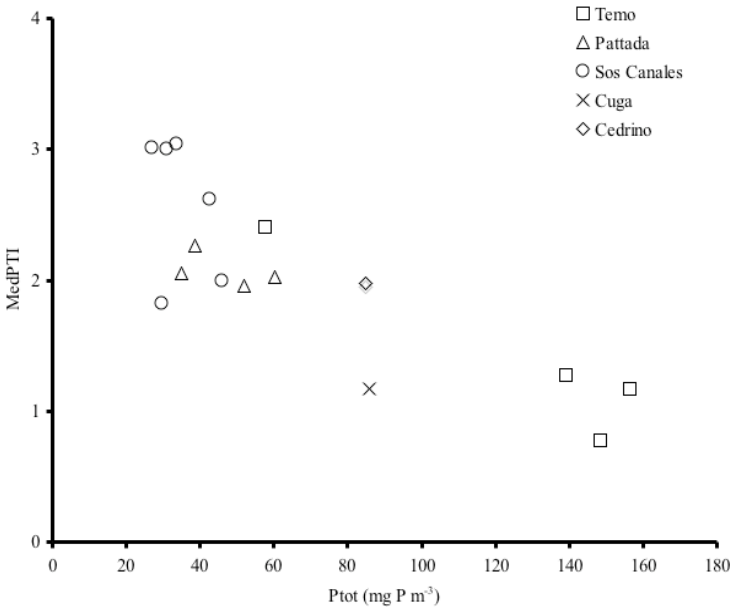

Figure 3. Relation between MedPTI index values and Ptot concentrations in the testing MedPTI Sardinian reservoirs.

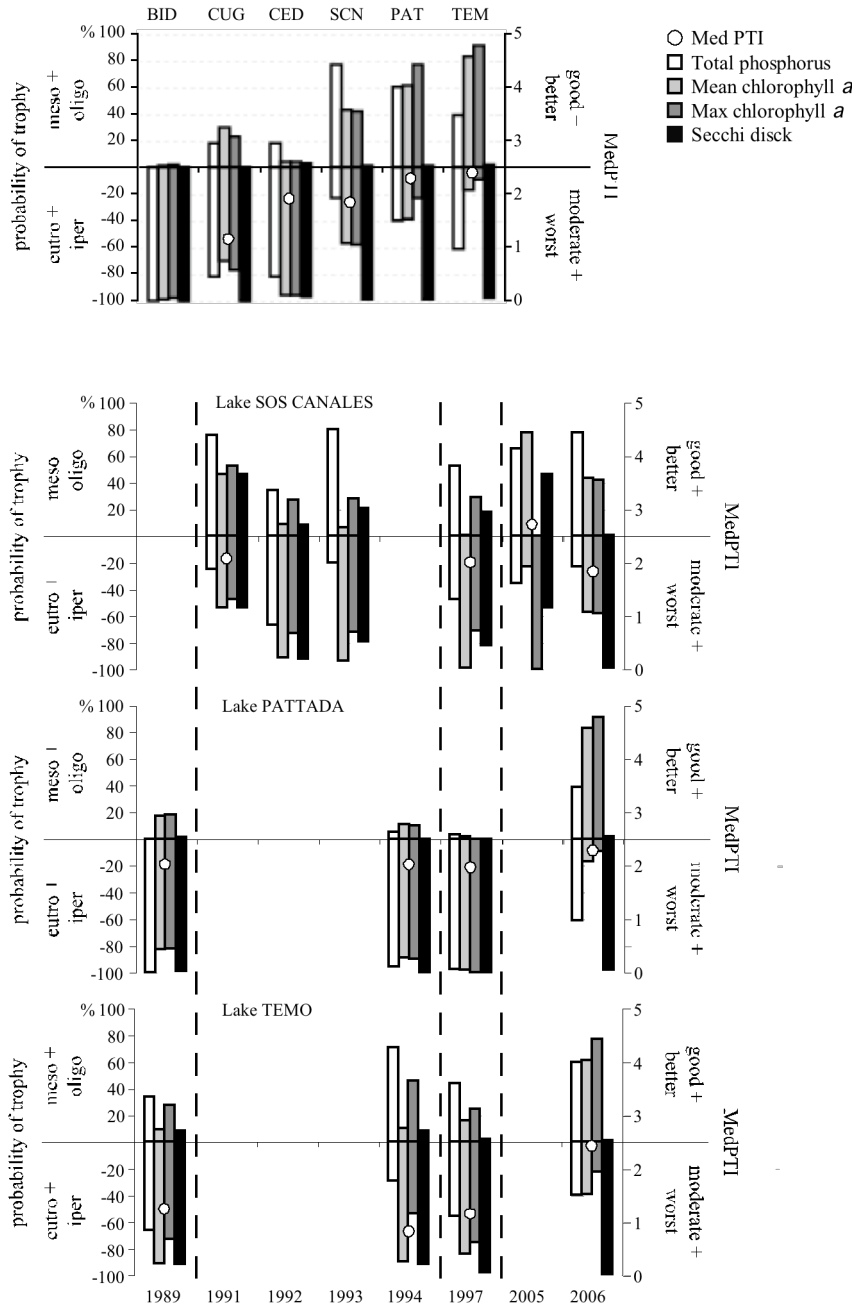

Figure 4. Comparison of water quality classification using MedPTI index and OECD probabilistic model.

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Chapter3:
Un'esperienza di approccio integrato nella valutazione della qualità ambientale di corsi d'acqua del Nord-Sardegna con applicazione dell'Indice EPI-D.

An experience of an integrated approach in valuating water quality of North-Sardinian water – courses with the application of EPI-D index.

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Sommario

Nel 2003 è stato condotto uno studio su 11 corsi d'acqua della Sardegna settentrionale utilizzando un approccio integrato che ha implementato l'indagine chimico-fisica e microbiologica con l'analisi delle Diatomee epilitiche e l'applicazione dell'Indice EPI-D. Nei campioni raccolti sono stati identificati 157 taxa (34 generi). Nei soli conteggi per il calcolo dell'Indice, applicato a 8 dei corsi d'acqua indagati, i taxa sono stati 107 (26 generi), una parte dei quali (11%) non contemplati tra quelli utilizzati da Dell'Uomo (2004). Tra questi ultimi si vuole evidenziare l'osservazione di *Navicula confervacea,* specie di origine tropicale finora non segnalata nelle acque lotiche sarde. I valori di EPI-D hanno indicato una qualità ottima e buona in due corsi d'acqua e scadente in tutti gli altri, coerentemente con i parametri chimico-fisici e microbiologici. L'inquinamento organico rilevato deriva per lo più da scarichi civili e da attività agro-zootecniche.

Summary

This paper reports the results of an integrated research carried out on 11 water- courses in North Sardinia in 2003. An integrated approach was adopted considering both chemical - physical and microbiological analysis and the study of epilithic Diatoms. 157 taxa (34 genera) were observed in the collected samples. 107 of them were also present in the counted slides for EPI-D. Some of these taxa are not in the list proposed by Dell'Uomo (2004). It is the case, for example, of *Navicula confervacea,* a tropical species, which has never been reported in the Diatom flora of Sardinian water-courses. EPI-D indicated an excellent and good quality in two water -courses and bad in all the others, according to the chemical-physical and microbiological parameters. The main kind of pollution was organic, prevalently caused by urban waste and farming and zootechnical activities.

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3.1 Introduzione

Le conoscenze relative allo stato di qualità dei corsi d'acqua della Sardegna sono complessivamente molto esigue rispetto a quelle acquisite su altre tipologie di ambienti acquatici della regione, soprattutto sugli invasi artificiali (circa 40), sia su scala annuale che pluriennale (Marchetti *et al*., 1992; Sechi e Lugliè, 1992, 1996). In particolare gli studi sui popolamenti diatomici delle acque superficiali correnti risultano finora piuttosto scarsi (Nughes *et al.,* 2005, Zanetti *et al*., 2007). D'altra parte, in particolari ambienti quali le pozze temporanee, la flora diatomica riscontrata pare di estremo interesse perchè caratterizzata da taxa non ancora segnalati in altri contesti geografici (Lange-Bertalot *et al.,* 2003). Numerosi indici biologici tra quelli sviluppati per la valutazione della qualità delle acque lotiche prendono in analisi proprio i popolamenti a Diatomee, con un'utilità ampiamente riconosciuta ed esteso utilizzo in numerosi paesi europei ed extraeuropei (Whitton *et al*.,1991; Whitton & Rott., 1996; Prygiel *et al*., 1999; Rimet *et al*., 2005). L'applicazione di indici sviluppati e sperimentati anche in altre realtà territoriali rappresenta un'importante opportunità di raffronto e contemporaneamente di acquisizione di informazioni utili sugli ambienti fluviali sardi, che presentano caratteristiche del tutto eccezionali. I numerosi e piccoli corsi d'acqua isolani hanno infatti un andamento e un regime strettamente legati alle peculiari condizioni litologiche e morfologiche del territorio e al clima tipicamente mediterraneo, contrassegnato da elementi di discontinuità e variabilità (Fadda e Pala, 1992). Il carattere torrentizio si manifesta con un lungo periodo di siccità durante la stagione estivo-autunnale e un periodo di deflussi più abbondanti in quella invernale-primaverile, in cui si concentrano le precipitazioni. La notevole variazione dei deflussi durante l'anno, con fenomeni di prosciugamento dell'alveo, aumento della temperatura o della salinità durante l'estate, piene improvvise in inverno, si ripercuote naturalmente sulla struttura e distribuzione delle comunità. A queste pressioni naturali possono aggiungersi importanti impatti di origine antropica che incidono, in misura maggiore, nel periodo in cui avviene la riduzione delle portate e la risorsa idrica è più scarsa. Nel corso del 2003 il Dipartimento di Botanica ed Ecologia Vegetale dell'Università di Sassari, in collaborazione con l'E.A.F., Ente Autonomo del Flumendosa (attualmente EN.A.S., Ente Acque della Sardegna), ha svolto una prima indagine per la valutazione della qualità ambientale di alcuni corsi d'acqua della Sardegna

settentrionale. Le analisi chimico-fisiche e microbiologiche inizialmente previste, sono state implementate con lo studio dei popolamenti diatomici e l'applicazione dell'Indice EPI-D, Indice di Eutrofizzazione/Polluzione basato sulle Diatomee, elaborato per i corsi d'acqua italiani e da diversi anni sperimentato con successo principalmente nella regione appenninica centrale (Dell'Uomo 1996, 1999; Dell'Uomo e Tantucci 1996; Grandoni e Dell'Uomo 1996; Dell'Uomo e Grandoni, 1997; Dell'Uomo *et al*., 1999; Torrisi e Dell'Uomo 2001a, 2001b; Dell'Uomo 2004). In attesa del recepimento della Direttiva Europea Quadro (WFD 2000/60/CE), diverse regioni del territorio nazionale hanno recentemente condotto esperienze di applicazione dell'Indice EPI-D, effettuando in alcuni casi il confronto con altri indici diatomici e con l'I.B.E., previsto dal D.L. 152/99, cui fa essenzialmente riferimento il controllo qualitativo delle acque. La Direttiva comunitaria infatti individua gli indicatori biologici come elementi prioritari nella valutazione dello stato di qualità dei corpi idrici. I parametri idromorfologici e chimico-fisici, tradizionalmente utilizzati, vengono considerati in relazione alla loro influenza sulle comunità, che rappresentano la memoria storica e spaziale di fenomeni naturali e perturbazioni antropiche. Di fatto, in Italia, la situazione è caratterizzata dalla scarsa applicazione di metodologie biologiche che presuppone, a sua volta, anche un'ampia conoscenza di base delle comunità presenti nei diversi ambienti del territorio, allo stato attuale piuttosto carente. Ciò assume grande rilevanza per gli ecosistemi lotici che, come primi recettori di scarichi e reflui nei bacini idrografici, sono in grado di segnalare eventuali rischi di alterazione qualitativa per le acque dei bacini lacustri.

3.2 Obiettivi

Gli obiettivi principali dello studio svolto, i cui risultati vengono qui in parte riportati, sono stati:

1. acquisire un quadro conoscitivo dello stato di qualità dei corsi d'acqua indagati ed effettuare il raffronto tra i diversi bacini idrografici;
2. contribuire ad una migliore conoscenza dei popolamenti diatomici in termini di composizione, distribuzione ed ecologia delle specie;
3. verificare l'utilità e l'applicabilità dell'Indice EPI-D nel contesto ambientale sardo, differente da quello appenninico nel quale è stato maggiormente sperimentato.

3.3 Area di studio

L'area di studio ha riguardato 7 bacini idrografici ubicati principalmente nella Sardegna settentrionale (Fig. 1, Tab. 1). Tali bacini ricadono in 4 aree tra quelle a potenziale rischio di inquinamento, individuate in maniera puramente indicativa, su scala regionale e distinte in base alle differenti tipologie di rischio:

- Area di Bosa: rischio agro-zootecnico e urbano
- Area di Alghero: rischio agro-zootecnico, industriale, urbano
- Area di Sassari-Porto Torres: rischio industriale, portuale e urbano
- Area del Golfo di Olbia: rischio industriale, portuale e urbano.

Complessivamente sono stati esaminati 11 corsi d'acqua, di cui 6 inclusi tra i corpi idrici significativi in base al D.L. 152/99 e quindi sottoposti a monitoraggio col metodo I.B.E e l'analisi dei macrodescrittori, e uno tra quelli non significativi che necessitano di essere monitorati (Tab. 1). Nella fase preliminare sono state individuate contestualmente alle suddette aree, 15 stazioni di campionamento (Tab. 1) sulla base di criteri di tipo essenzialmente morfoedafico ed ambientale.

3.4 Breve descrizione dei bacini idrografici

La Tabella 1 riporta alcune informazioni inerenti i bacini idrografici e di seguito vengono descritti i loro caratteri salienti.

Il *Fiume Temo*, si origina da numerose piccole sorgenti, in parte perenni, ai piedi del Monte Teppero (510 m.s.l.m.) e sfocia, con un ampio estuario, nella spiaggia di Bosa Marina, dopo un percorso di 60 km. Si tratta dell'unico corso d'acqua nell'isola a consentire la navigazione con piccole imbarcazioni, seppure nel tratto terminale. Il centro urbano più importante del bacino idrografico (Sardegna centro-occidentale), interessato da intenso sviluppo agricolo anche di carattere intensivo e da allevamenti, è Bosa. La stazione di campionamento è stata individuata nel tratto terminale del corso d'acqua in prossimità di Bosa, ossia a valle dei 2 invasi artificiali creati, a Monteleone Roccadoria e a Monte Crispu, mediante sbarramento dell'asta principale.

Il *Riu Barca* nasce a nord di Monte S'Unchinu (219 m.s.l.m.) e sfocia nella parte centrale dello Stagno di Calich, dopo un percorso di 25 km. Nel territorio circostante, nel quale si svolgono in maniera prevalente attività agricole anche intensive, il centro di una certa rilevanza è Alghero. L'unica stazione di

campionamento, è ubicata nel tratto terminale del corso d'acqua, in ingresso allo stagno.

Il *Riu Mannu di Porto Torres* ha origine tra il Monte Pelao (730 m.s.l.m.) e Punta Matteuzzu (540 m.s.l.m.) e sfocia nel Golfo dell'Asinara, a ovest dell'abitato di Porto Torres, dopo un percorso di 64 km. Il bacino idrografico (Sardegna nord-occidentale) è fortemente antropizzato, risultando interessato da numerosi centri urbani (Sassari e Porto Torres i più importanti), da un'intenso sviluppo agricolo e da rilevanti insediamenti industriali. Sono state individuate 3 stazioni di campionamento, rispettivamente nel tratto iniziale, medio e terminale del corso d'acqua: Riu Bidighinzu (a monte del lago artificiale), Riu Mannu di Sassari e Riu Mannu di Porto Torres.

Il *Fiume Coghinas* nasce tra i rilievi del Monte Traessu (717 m.s.l.m) e del Monte Rispisu (602 m.s.l.m.), nella catena del Marghine, e sfocia nella spiaggia di Campo Coghinas, nella parte orientale del Golfo dell'Asinara, dopo un percorso di 46 km. Il bacino è largamente interessato da attività agro-zootecniche ed il centro maggiore è Ozieri. Sono state individuate 5 stazioni di campionamento: due sull'asta principale del fiume (una sul Riu Mannu di Ozieri, a monte del Lago Coghinas a Muzzone e una a valle del Lago Coghinas a Casteldoria), mentre le altre sono state individuate sul Riu Mannu di Oschiri, Riu S'Aidolzas e Riu Giobaduras.

Il *Fiume Liscia* nasce tra Punta Balestrieri (1359 m. s.l.m.) e il Monte Giogantinu (1333 m.s.l.m.), nel massiccio del Limbara e sfocia, con un largo estuario, nella spiaggia di Porto Liscia dopo un percorso di 64 km. Il maggior centro del bacino idrografico (Sardegna orientale) è Tempio Pausania. Le attività agricole sono in gran parte concentrate nella zona circostante il lago artificiale del Liscia. Sono state monitorate 2 stazioni dell'asta principale, una sul Rio Carana e una sul Liscia, rispettivamente a monte e a valle del lago artificiale.

Il *Rio S. Giovanni* nasce dai versanti dei Monti Alturina (495 m s.l.m.) e Scupagliu (445 m s.l.m.) e ha sbocco con foce a delta nell'insenatura di Arzachena, dopo un percorso di 26 km. Il Riu De S.Nicola, appartenente al bacino del Rio S.Giovanni, nasce da Punta De Su Quadreddu (458 m s.l.m.) e ricopre una distanza di 8 km, sfociando nel Porto Romano (Golfo di Olbia). Nel bacino si individuano una parte nord-occidentale, prevalentemente con attività agricole e zootecniche e una parte sud-orientale fortemente urbanizzata per la presenza della

città di Olbia, interessata anche da attività agricole, zootecniche e industriali. Le due stazioni di campionamento sono entrambe localizzate nel tratto terminale dei due corsi d'acqua indagati.

Il *Rio Padrongianu* nasce dalle falde del Monte Niddoni (1231 m.s.l.m.), nel versante orientale della catena del Limbara, e ha sbocco, dopo un percorso di 39 km, nel Golfo di Olbia con una foce a delta a più lobi, unica tra quelle degli altri corsi d'acqua sardi. Il bacino idrografico (Sardegna orientale) è scarsamente urbanizzato e prevalentemente interessato da attività agricole e zootecniche. L'unica stazione di campionamento è localizzata sul tratto terminale del corso d'acqua.

3.5 Materiali e Metodi

Il prelievo dei campioni d'acqua per le analisi dei parametri chimico-fisici e microbiologici è stato effettuato con cadenza mensile, dal gennaio all'ottobre 2003. Alcune variabili chimico-fisiche (temperatura, pH, conducibilità, ossigeno disciolto e percentuale) sono state rilevate direttamente in campo con strumenti portatili WTW, mentre altre (alcalinità, cloruri, durezza, BOD_5, COD, fosforo reattivo e totale, azoto nitroso, nitrico, ammoniacale e totale, silice reattiva e solidi sospesi), sono state determinate in laboratorio secondo le metodiche di Mohr, (1989), Rodier, (1961), Winkler, (1971), Strickland & Parsons, (1972). L'indagine microbiologica ha riguardato la ricerca di *Escherichia coli* ed è stata effettuata mediante il metodo di filtrazione su membrana (IRSA-CNR, 1994). Il prelievo delle Diatomee epilitiche è stato programmato in due campionamenti stagionali, uno primaverile (aprile-maggio) e uno estivo (agosto). La raccolta dei campioni è stata effettuata in tutte le stazioni mediante raschiatura di massi e ciottoli in piena corrente con uno spazzolino a setole rigide. Soltanto per il Fiume Temo, data la notevole profondità delle acque nella stazione individuata, il prelievo è stato effettuato raschiando la superficie del pilone del ponte sotto cui scorre. I campioni raccolti sono stati fissati in campo con formalina neutralizzata al 4% e successivamente trattati in laboratorio mediante il metodo del perossido di idrogeno (H_2O_2) e dell'acido acetico (CH_3COOH) a caldo (Schrader, 1973), fino a completa ossidazione della sostanza organica. Per ciascun campione è stato montato un vetrino permanente mediante l'utilizzo della resina Storax. La determinazione delle Diatomee è stata effettuata utilizzando un microscopio ottico

invertito (Zeiss, mod. Axiovert 10) a 1000 ingrandimenti e l'impiego di monografie di vari autori: Bourrelly (1981), Germain (1981), Hustedt (1985), Krammer & Lange-Bertalot (1986, 1988, 1991). Per il calcolo dell'Indice EPI-D è stato conteggiato un minimo di 400 valve e/o frustuli ed è stata utilizzata la formula di Zelinka & Marvan (1961), secondo quanto previsto dal metodo di Dell'Uomo (1996).

3.6 Risultati e discussione

Le analisi dei popolamenti a Diatomee sono state condotte su 13 stazioni fluviali in 10 corsi d'acqua. Per le stazioni F27 ed F30 (Fig. 1, Tab. 1), infatti, il prelievo delle Diatomee epilitiche è stato impedito dall'inacessibilità dei siti. L'osservazione dei campioni raccolti ha permesso di rilevare complessivamente 157 taxa appartenenti a 34 generi. In generale la maggior parte delle specie rinvenute è risultata comune a diverse stazioni e soltanto in qualche caso sono state osservate specie esclusive di una di esse. La Tabella 2 e la Figura 2 riportano la composizione in base alle specie dominanti, ordinate gerarchicamente per le singole stazioni nel campionamento di morbida. La stazione F38 è stata quella con la maggiore ricchezza di specie e quindi una elevata biodiversità, con la presenza di numerose specie con importanza simile. ll fenomeno di risalita del cono salino nelle stazioni F25 ed F26, prossime alla foce, ha condizionato fortemente i popolamenti delle Diatomee presenti. In particolare l'analisi dei campioni ha mostrato per l'F25 la compresenza di specie dulciacquicole tolleranti e di altre dominanti, tipiche di ambienti salmastri, come *Achnanthes brevipes* (Agardh, 1824) e *Navicula mutica* (Kützing, 1844) e per l'F26 la dominanza di specie nettamente eurialine, quali *Navicula gregaria* (Donkin, 1861) e *Navicula phyllepta* (Kützing, 1844). In tali stazioni chiaramente non è stato applicato l'Indice EPI-D, concepito esclusivamente per le acque dolci correnti. L'indice è stato applicato soltanto in 8 stazioni, di cui 5 campionate stagionalmente e 3 nel solo periodo di morbida, date le condizioni di secca in cui sono venute a trovarsi nel periodo estivo. I campioni primaverili delle stazioni F28, F29 ed F35, hanno evidenziato uno scarso numero di frustuli, probabilmente imputabile ad un difetto di campionamento. In queste stazioni la composizione dei popolamenti, costituiti da una decina di taxa, è risultata piuttosto simile a quelle trovate nelle altre stazioni. Durante il conteggio dei campioni utilizzati per l'applicazione

dell'indice, sono stati osservati 107 taxa ripartiti tra 26 generi (Tab. 3), tra i quali quelli maggiormente rappresentati sono stati *Navicula* e *Nitzschia*, rispettivamente con 18 e 14 specie. Il numero totale di taxa per ciascun campione ha variato da un minimo di 12 nella stazione F33 a un massimo di 56 nell'F38, entrambi osservati in condizioni di morbida. Dei 107 taxa rinvenuti nel conteggio, 13 (ossia l'11%), quasi sempre presenti con un numero molto basso di esemplari, ma talvolta anche in quantità maggiori, non sono stati presi in considerazione nel calcolo dell'indice per l'assenza dei necessari valori di i (indice integrato ponderato di sensibilità della specie) ed r (affidabilità della specie come indicatore). Questi taxa non sono infatti contemplati nella lista di quelli utili per il calcolo dell'indice, basato soprattutto sui popolamenti osservati nei corsi d'acqua dell'Italia centrale (Dell'Uomo, 2004). Tra le specie escluse, in particolare, è importante citare *Navicula confervacea* (Kützing) Grunow in Van Heurk (1880) di rilevante interesse dal punto di vista ecologico e finora non segnalata in altri ambienti lotici della Sardegna. Infatti questa specie, originaria delle regioni tropicali o subtropicali, è attualmente presente anche in numerose regioni temperate, nelle quali può costituire un eccellente indicatore del riscaldamento delle acque superficiali correnti (Coste & Ector, 2000). I risultati dell'Indice diatomico, le classi e i giudizi di qualità in base ai valori EPI-D (Dell'Uomo 2004) sono riportati nella Figura.3 e Tabella 4. I valori assunti dall' EPI-D hanno evidenziato una qualità scadente (IV classe) per le stazioni FH, F32, F60 ed FL e mediocre (III classe) per l'F33 e l'F34. Soltanto le stazioni F37 ed F38 hanno presentato valori dell'indice ascrivibili, rispettivamente, ad ambienti di ottima e buona qualità (I e II classe), coerentemente con l'alto grado di naturalità dei territori drenati. Nelle stazioni per le quali è stato effettuato il campionamento stagionale si è osservato in generale un peggioramento qualitativo durante l'estate. Ciò risulta particolarmente evidente nella stazione F33, per la quale si è passati da un valore primaverile pari a 1,72, corrispondente ad una qualità buona/mediocre (II/III classe) a uno di 2,77 nel periodo estivo, corrispondente ad una cattiva qualità (IVclasse). Le analisi chimiche e microbiologiche (Tab. 5) sono in linea di massima concordanti con il quadro delineato dal metodo EPI-D. Nel complesso buona parte delle stazioni ha presentato nel periodo dell'indagine valori significativi dei nutrienti algali, di COD ed *Escherichia coli* e, in qualche caso anche di BOD_5, indicando una contaminazione delle acque prevalentemente di

natura organica. Escludendo le stazioni F25 ed F26 interessate dalla risalita del cono salino, i valori più elevati di conducibilità e cloruri, quasi certamente attribuibili sia al contenuto salino dei suoli che all'effetto dello spray marino, sono stati riscontrati nelle stazioni FH, F27 ed F30. Le stazioni F37 ed F38, le migliori dal punto di vista biologico, hanno presentato in generale valori buoni anche per le variabili chimico-fisiche, fatta eccezione per il COD, risultato sempre più elevato nell'F38. Dal confronto di alcune variabili chimico-fisiche e l'indice EPI-D, riportato a titolo di esempio nella Fig.3, emerge che l'indice sembra dipendere maggiormente da alcuni parametri rispetto ad altri con i quali la corrispondenza è apparsa meno evidente. Ciò trova spiegazione nel fatto che l'EPI-D, come Indice integrato e ponderato è, per sua natura, determinato dall'insieme dei parametri che interagiscono nei corpi idrici, ma può essere di volta in volta condizionato da quelli dominanti nel caratterizzare le condizioni ambientali dei diversi tratti fluviali.

3.7 Conclusioni

L'applicazione dell'Indice EPI-D ha consentito di ottenere una prima indicazione sulla qualità biologica di 8 degli 11 corsi d'acqua indagati, sostanzialmente in accordo con il quadro delineato dai parametri chimico-fisici e microbiologici. In particolare la tipologia di inquinamento riscontrata più frequentemente è quella di natura organica, imputabile a scarichi civili non adeguatamente depurati e alle attività agro-zootecniche, confermando quanto già in precedenza riscontrato nel Nord-Sardegna (Lugliè *et al.,* 1989). Il carattere torrentizio e le condizioni di secca degli alvei in F34, F37 ed F60 nel periodo estivo hanno impedito il campionamento stagionale limitando la valutazione dell'evoluzione temporale della qualità biologica. Degli 8 corsi d'acqua cui è stato applicato l'EPI-D, 4 sono risultati scadenti, 2 mediocri, 1 buono e 1 ottimo. Il peggioramento qualitativo durante l'estate, come atteso per la notevole riduzione delle portate, è stato chiaramente confermato dai valori dell'indice in tutte le stazioni. Lo studio, pur con tutti i limiti di una prima esperienza nell'utilizzo di tale approccio, ha fornito un importante contributo alla conoscenza delle Diatomee presenti in ambienti fluviali del territorio regionale e ha permesso l'acquisizione di una serie di informazioni utili per ulteriori e successive esperienze. Dato il notevole valore intrinseco e la peculiarità degli ecosistemi

lotici nel contesto ambientale sardo, nonché la loro importanza in termini socio-economici, si auspica l'implementazione di programmi di monitoraggio che prevedano anche l'utilizzo dell'Indice EPI-D. Ulteriori studi potranno rivelarsi molto utili per l'approfondimento delle conoscenze relative alla composizione specifica dei popolamenti osservati nell'area mediterranea e per l'integrazione dei taxa attualmente utilizzati per l'applicazione dell'Indice.

Bacino idrografico	**Superficie** *km²*	**Abitanti**	**Substrato geologico**	**Corsi d'acqua** *Fiume*	*n° stazioni*	*Sigla*
Temo	839,5	22842	basalti e trachiti	Temo*	1	F25
Barca	353,5	3553	scisti, graniti, calcari marne e arenarie	Barca*	1	F26
Mannu di P.Torres	671,3	178712	calcari	Mannu di P.Torres*	2	F28 FH
				Mannu di Sassari	1	F27
Coghinas	2561,6	59167	trachiti, graniti calcari e marne	Mannu di Oschiri**	1	F32
				Giobaduras	1	F30
				Coghinas*	2	F33 F29
Liscia	570,7	26406	graniti	Liscia*	2	F35 F34
S. Giovanni	175,7	56219	graniti	S. Giovanni	1	F37
				De S. Nicola	1	FL
Padrongianu	450,7	6284	graniti	Padrongianu*	1	F38

* Corpo idrico significativo ** Corpo idrico non significativo che necessita di essere monitorato; (D.L. 152/99)

Tab. 1 – Informazioni generali sui corsi d'acqua indagati

FH	F25	F26
Fragilaria pinnata Ehrenberg	*Fragilaria fasciculata* (Agardh) Lange-Bertalot	*Nitzschia incospicua* Grunow
Nitzschia incospicua Grunow	*Achnanthes brevipes* Agardh	*Navicula gregaria* Donkin
Navicula gregaria Donkin	*Nitzschia hungarica* Grunow	*Navicula phyllepta* Kützing
Fragilaria fasciculata (Agardh) Lange-Bertalot	*Nitzschia constricta* (Kützing) Ralfs	*Nitzschia hungarica* Grunow
Achnanthes lanceolata (Brébisson) Grunow	*Nitzschia incospicua* Grunow	*Navicula cari* Ehremberg
FL	**F33**	**F34**
Nitzschia incospicua Grunow	*Amphora pediculus* (Kützing) Grunow	*Cyclostephanos invisitatus* (Hohn & Hell.) T.S. & Hak.
Achnantes delicatula (Kützing) Grunow	*Cocconeis pediculus* Ehrenberg	*Achnanthes minutissima* Kützing
Nitschia amphibia Grunow	*Rhoicosphenia abbreviata* (Agardh) Lange-Bertalot	*Cocconeis placentula* Ehrenberg
Achnantes lanceolata (Brébisson) Grunow	*Nitzschia amphibiA* Grunow	*C. placentula* var *euglypta* Ehrenberg
Navicula lanceolata (Agardh) Ehrenberg	*Achnanthes lanceolata* (Brébisson) Grunow	*Aulacoseira granulata* (Ehrenberg) Simonsen
F37	**F38**	**F60**
Achnantes minutissima Kützing	*Achnantes minutissima* Kützing(Grunow)	*Achnanthes hungarica* Grunow
Fragilaria capucina Desmazières	*Amphora pediculus* (Kützing) Grunow	*Cocconeis placentula* Ehrenberg
Navicula cryptocephala Kützing	*Cocconeis placentula* Ehrenberg	*C. placentula* var. *lineata* (Ehrenberg) Van Heurck
Nitzschia incospicua Grunow	*Melosira lineata* (Dillwyn) Agardh	*Nitzschia incospicua* Grunow
Gomphonema parvulum Kützing	*Nitzschia amphibia* Grunow	*Nitzschia amphibia* Grunow

Tab. 2 – Specie dominanti, in ordine gerarchico, in 9 delle stazioni indagate nei campioni primaverili.

Genere e varietà	n° specie	Genere e varietà	n° specie
Achnantes	8	*Epithemia*	1
Amphipleura	1	*Eunotia*	2
Amphora	4	*Fragilaria*	12
Anomoeoneis	1	*Frustulia*	1
Aulacoseira	3	*Gomphonema*	6
Bacillaria	1	*Gyrosigma*	1
Cocconeis	5	*Melosira*	1
Cyclotella	2	*Navicula*	18
Cymbella	10	*Nitzschia*	14
Cyclostephanos	2	*Rhoicosphenia*	1
Denticula	2	*Stephanodiscus*	1
Diatoma	1	*Surirella*	3
Diploneis	3	*Synedra*	3
Tot. specie e varietà			107

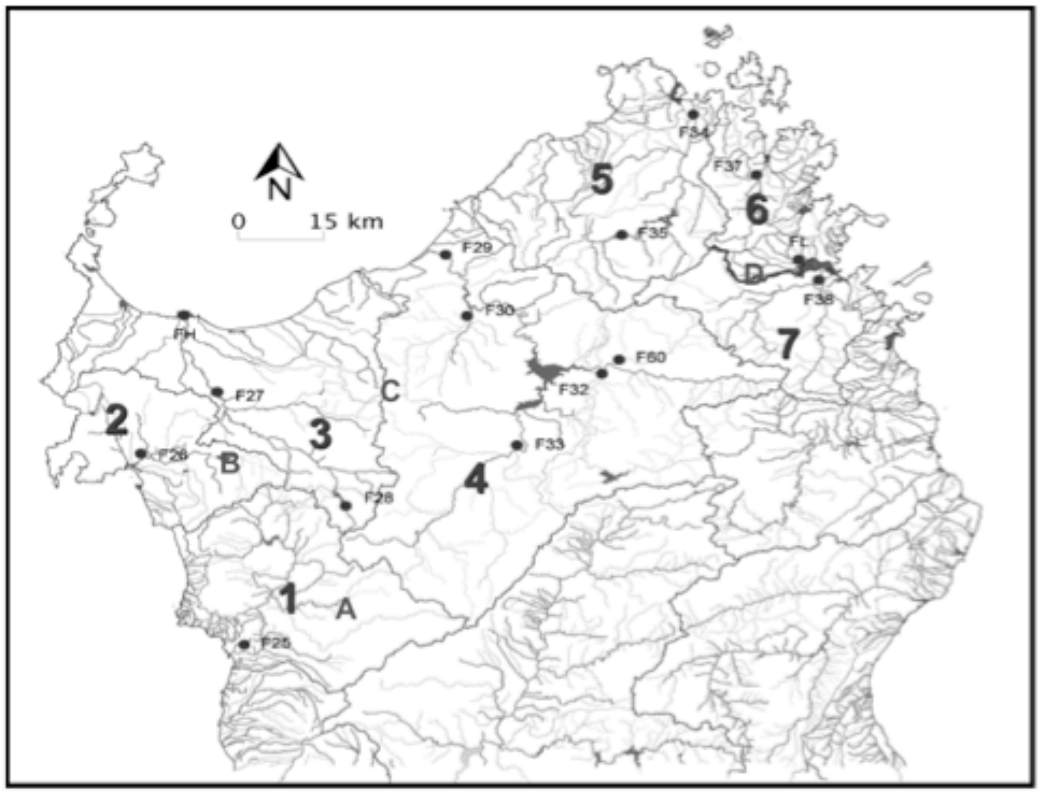
Tab. 3 – Elenco dei generi e loro ricchezza in specie.

Stazione	EPI-D morbida	EPI-D magra	EPI-D medio	Classe di qualità
FH	2.38	2.43	2.40	IV-cattiva
F32	_	2.74	2.74	IV-cattiva
F33	1.72	2.77	2.24	III-mediocre
F60	2.62	secca	2.62	IV-cattiva
F34	2.01	secca	2.01	III-mediocre
FL	2.50	2.59	2.54	IV-cattiva
F37	0.94	secca	0.94	I-ottima
F38	1.56	1.66	1.61	II-buona

Tab. 4 – Valore dell'EPI-D e classi di qualità.

		F25	F26	FH	F27	F28	F29	F30	F32	F33	F60	F34	F35	FL	F37	F38
Temperatura	*°C*	18.3	15.4	17.0	14.7	14.0	17.0	18.3	14.5	14.4	15.0	13.8	13.2	15.1	14.2	16.4
pH		8.01	8.15	7.98	7.84	8.00	7.58	8.86	8.00	7.91	7.72	7.84	7.86	7.38	7.47	7.77
Conducibilità	$\mu S\ cm^{-1}$	32399	3887	1142	1095	899	787	1554	503	724	419	559	389	659	465	445
Alcalinità	$meq\ l^{-1}$	2.65	5.69	4.76	4.85	4.93	1.48	5.07	1.23	2.88	1.16	1.50	1.06	1.84	1.02	1.40
Cloruri	$mg\ l^{-1}$	11385	1040	213	204	139	172	259	99	127	86	112	110	116	99	89
Durezza	$mg\ l^{-1}$	3332	1054	418	450	418	202	517	150	216	135	158	153	190	119	144
Ossigeno disciolto	$mg\ l^{-1}$	7.8	6.1	5.7	4.7	5.6	6.5	8.7	6.5	5.5	6.8	6.0	6.9	3.8	6.0	6.5
Ossigeno percentuale	*%*	90	63	58	48	56	68	97	65	54	65	59	67	38	62	68
BOD5	$mg\ l^{-1}$	2.2	2.5	2.2	7.3	2.3	1.9	7.0	1.8	2.6	1.9	2.4	2.3	3.2	2.2	2.7
COD	$mg\ l^{-1}$	50	26	27	30	11	27	42	22	19	29	23	26	23	25	27
Fosforo reattivo	$mg\ l^{-1}$	0.069	0.057	0.569	0.518	0.198	0.123	0.425	0.028	0.262	0.032	0.008	0.217	0.108	0.014	0.014
Fosforo totale	$mg\ l^{-1}$	0.118	0.085	0.724	0.659	0.271	0.185	0.626	0.092	0.414	0.085	0.045	0.257	0.185	0.038	0.057
Azoto ammoniacale	$mg\ l^{-1}$	0.11	0.04	0.28	1.48	0.16	0.05	0.97	0.05	0.52	0.13	0.04	0.08	0.57	0.04	0.03
Azoto nitroso	$mg\ l^{-1}$	0.011	0.010	0.140	0.335	0.094	0.020	0.103	0.027	0.193	0.031	0.016	0.030	0.103	0.004	0.009
Azoto nitrico	$mg\ l^{-1}$	0.45	3.07	3.38	2.70	0.87	0.96	1.22	1.28	0.95	0.99	0.62	1.82	1.09	0.30	0.93
Azoto totale	$mg\ l^{-1}$	3.630	4.774	6.845	6.641	3.092	2.552	4.918	2.048	2.333	1.579	1.263	2.600	2.866	0.811	1.551
Solidi sospesi	$mg\ l^{-1}$	23.2	3.9	11.1	8.3	9.9	11.8	16	18.5	8.9	5.4	4.2	4.9	4.5	2.5	4.3
Silice reattiva	$mg\ l^{-1}$	2.6	4.5	7.0	7.8	8.4	5.8	11.1	5.9	4.6	6.6	5.1	8.1	6.8	5.6	6.8
Escherichia coli	$UFC\ 100\ ml^{-1}$	19144	48	7944	2500	1775	401	83500	778	6533	438	72	435	6400	105	119

Tab. 5 – Valori medi annuali dei parametri chimico-fisici e microbiologici nelle stazioni fluviali monitorate.



Legenda:
Aree a rischio ambientale: A. Bosa; B. Alghero; C. Sassari-Porto Torres; D. Golfo di Olbia
Bacini idrografici: 1. Fiume Temo; 2. Riu Barca; 3. Riu Mannu di Porto Torres; 4. Fiume Coghinas; 5. Fiume Liscia; 6. Riu S.Giovanni; 7. Riu Padrongianu
Corsi d'acqua e stazioni fluviali: FH: Riu Mannu di Porto Torres); FL: Riu De S.Nicola; F25: Fiume Temo; F26: Riu Barca; F27: Riu Mannu di Sassari; F28: Riu Bidighinzu; F29: Fiume Coghinas ; F30: Rio Giobaduras; F32: Riu Mannu di Oschiri; F33: Riu Mannu di Ozieri; F34: Fiume Liscia; F35: Riu Carana; F37: Rio S.Giovanni; F38: Rio Padrongianu; F60: Riu S'Aidolzas

Fig. 1 – Area di studio e localizzazione delle stazioni.

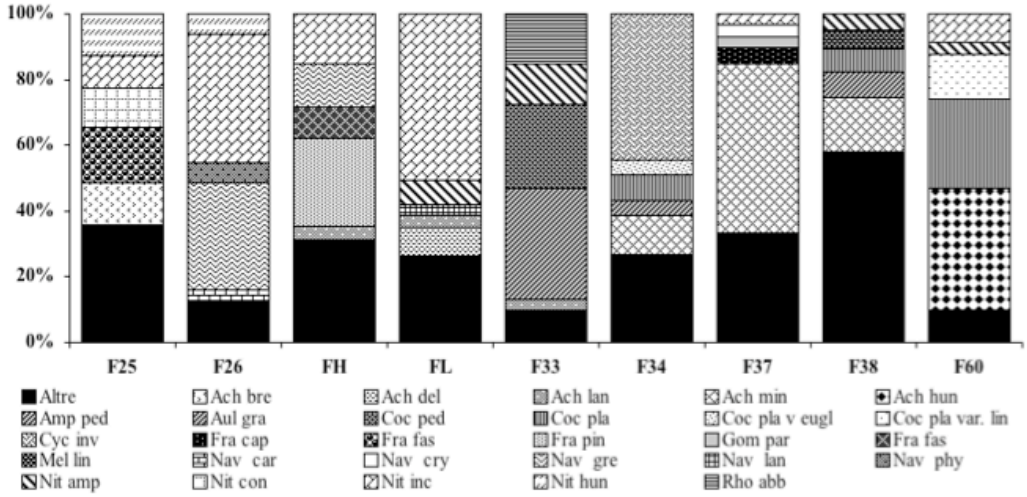


Fig. 2 – Composizione percentuale in base alle specie più importanti.

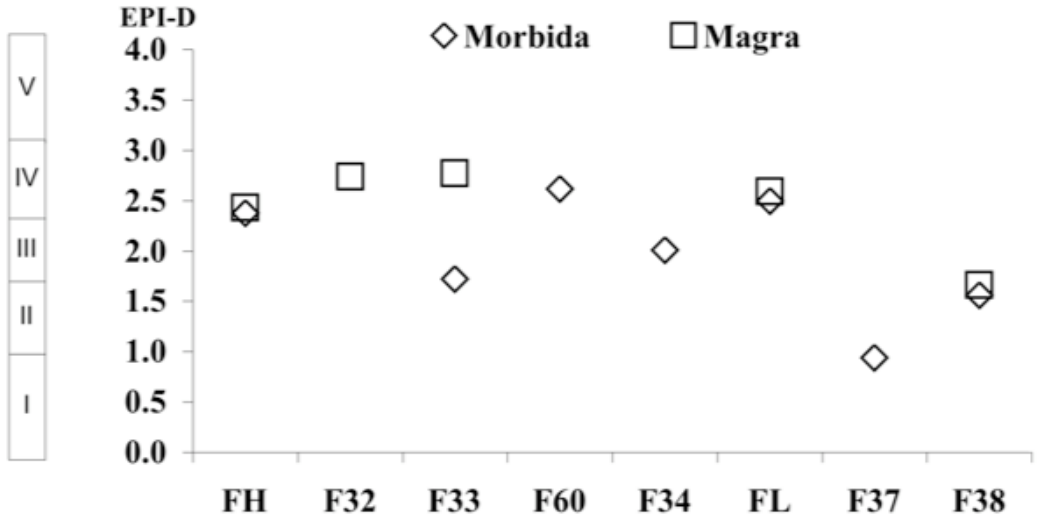


Fig. 3 – Risultati dell'indice EPI-D.

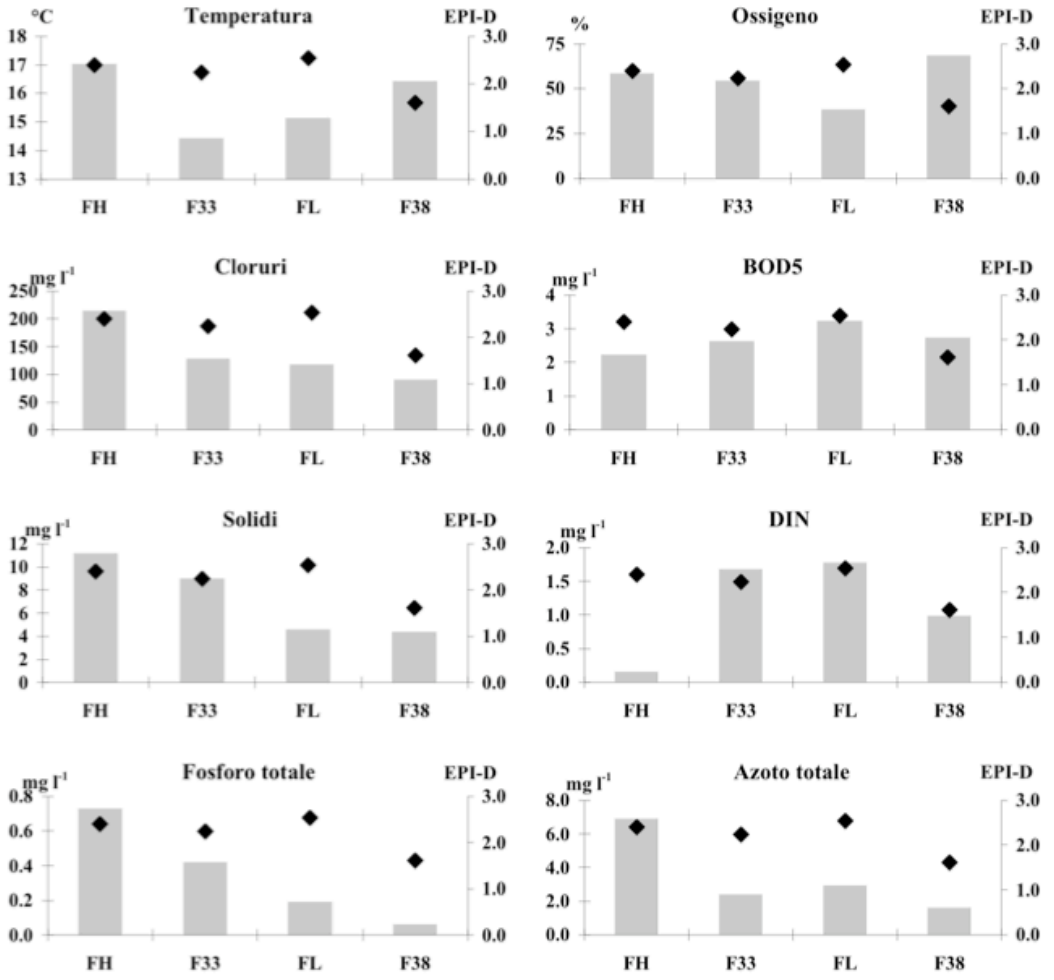


Fig. 4 – Raffronto dei valori di EPI-D medi con alcune delle variabili chimiche e fisiche indagate.

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Thematic C: Toxicity

Chapter1:

Cyanotoxins occurrence in Italian freshwaters.

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Abstract

Cyanobacterial toxic algal blooms in freshwater bodies destined to human uses are a general occurrence in the world due to increasing euthrophication and climatic changes of global warming. This study focuses on the occurrence and distribution of cyanobacterial toxins from 1989 to 2006 in several Italian lakes different for characteristics and human uses, from drinking to recreational purposes. Phytoplankton and LC/MS/MS toxin analyses were performed on surface water samples collected from 28 Italian lakes. The most widespread toxic species belonged to the genera *Microcystis*, *Planktothrix* and *Anabaena*. Total extracellular microcystin concentration varied from traces to 226.16 ng/ml, total cylindrospermopsin varied from 0.3 ng/ml to 126 ng/ml, and anatoxin-a varied from 115.1 ng/g (wet weight) to 100 μg/g (dry weight).

The water toxin contents were not always correlated with the cyanobacteria cell densities, suggesting the need of control studies including toxin detection in water together with microscopic cell valuations, in order to avoid possible toxin underestimations.

Keywords: cyanobacteria, cyanotoxins, microcystin, cylindrospermopsin, anatoxin-a, Italy.

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1.1 Introduction

Cyanobacteria are a widespread, successful group of organisms colonizing all ecosystems. They are common inhabitants of freshwater bodies throughout the world and several of them are bloom-forming species: under favourable conditions several species of cyanobacteria may become dominant in the phytoplankton of water bodies, reaching densities of many millions of cells per litre (Chorus and Bartram, 1999).

In potable and recreational waters a bloom can be defined in terms of cell density causing nuisance to humans, and in this terms a density of 20 x 106 cells/L has been proposed (Oliver and Ganf, 2000). Other authors suggested lower limits in case of blooms from toxic species (5 x 106 cells/L, Pilotto *et al*., 1997)

Cyanobacteria are known to produce several metabolites significant from the public health perspective of acute exposure: lipopolysaccharides (Stewart *et al*., 2006), and cytotoxic, tumor-promoting and enzyme-inhibiting metabolites like cyclic depsipeptides, cyclic peptides (anabaenopeptins and nostophycins), linear peptides (aeruginosins and microginins) (Bickel *et al*., 2001, Forchert *et al*., 2001). Recently, a neurotoxic non-protein aminoacid (N-methylamino-L-alanine, BMAA), widely produced among cyanobacteria (Cox *et al*., 2005), has been associated with neurodegenerative diseases such as Alzheimer's disease, amyotrophic lateral sclerosis/parkinsonism-dementia complex (Murch *et al*., 2004).

Many cyanobacterial species can produce several categories of powerful toxins that are unique to this group of organisms, with the exception of saxitoxins; the dominant species during toxic blooms belong to different genera, with *Anabaena*, *Aphanizomenon*, *Microcystis*, *Nodularia* and *Planktothrix* as the most frequent. The toxicity of cyanobacteria was brought to the attention of scientists by farmers and veterinarians, through recurrent reports of animal poisonings after consumption of water from ponds and lakes hosting cyanobacterial blooms (Chorus and Bartram, 1999).

Cyanotoxins are known to cause in humans skin irritation, gastroenteritis or death. Cyanotoxin poisoning in humans occurred through exposure to contaminated drinking water supplies (Annadotter *et al*., 2001; Falconer, 2005), recreational waters (Chorus and Bartram, 1999; Behm, 2003), medical dialysis (Azevedo *et al*., 2002).

Most poisoning by cyanobacteria involves acute hepatotoxicosis caused by a structurally similar group of small molecular weight cyclic hepta-peptides, the microcystins (MCYSTs). Their general structure is cyclo-(D-Ala1-X2-D-MeAsp3-Z4-Adda5-D-Glu6-Mdha7-) where X and Z are variable L-aminoacids, D-MeAsp3 is D-erythro-β-methylaspartic acid, Mdha is N-methyldehydroalanine and Adda (2S,3S,8S,9S-3-amino-9methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid) is the unique structural feature of these toxins which confers toxicity (Namikoshi *et al*., 1989). MCYSTs are genotoxic (Bouaicha *et al*., 2005), protein phosphatase-inhibiting (Dawson, 1998) toxins responsible for liver failure and death in humans (Azevedo *et al*., 2002), wild animals, livestock and aquatic life (Sivonen and Jones, 1999; Mwaura *et al*., 2004). Over seventy MCYSTs variants are now described (Sivonen and Jones, 1999; Brittain *et al*., 2000). MCYSTs are produced, among others, by *Microcystis aeruginosa* (Kutzing) Lemmermann*, M. viridis* (A. Braun) Lemmermann, *Anabaena flos-aquae* Brébisson ex Bornet et Flahault, *Planktothrix agardhii* ex *Oscillatoria agardhii* (Gomo.) Anagn. et Kom., *P. rubescens* ex *Oscillatoria rubescens* (D.C. ex Gomont) Komarek Anagnostidis, Nostoc sp. Vauch., *Aphanocapsa cumulus* Komárek et Cronberg, *Aphanizomenon flos-aquae* (L.) Ralfs) and *Oscillatoria tenuis* Agardh. Indirect evidence supporting tumour promotion of human cancer from MCYST exposure comes from the studies of Yu (Yu, 1989), Ueno (Ueno *et al*., 1996) and Zhou (Zhou *et al*., 2002) in China, and Fleming (Fleming *et al*., 2002) in Florida.

Poisoning episodes are also reported from a different toxin, cylindrospermopsin (CYL), which is a nephrotoxic, thymotoxic and hepatotoxic cyclic guanidine alkaloid (m.w. 415) (Terao *et al*. 1994), produced by *Cylindrospermopsis raciborski* (Woloszinska) Seenayya and Subba Raju, Umezakia natans Watanabe, *Aphanizomenon ovalisporum* Forti, *Anabaena lapponica* Borge and *Raphidiopsis curvata* Fritsch et Rich. The histological effects of CYL poisoning showed marked hepatocellular cytoplasmatic vacuolation and variable degrees of necrosis. Only recently, mutagenicity of CYL was shown in vitro and strong evidence also exists for its carcinogenicity in vivo (Humpage *et al*., 2000; Shen *et al*., 2002).

Poisoning episodes by the toxin called anatoxin-a increased in severity in recent years (Behm, 2003), and are becoming more common and more widespread: anatoxin-a is a potent neurotoxic alkaloid first detected in the cyanobacterium *A*.

flos-aquae, and it is perhaps one of the most powerful cyanobacterial toxin (Carmichael, 1994).

In 1985 suspected toxic algal blooms occurred in the Italian artificial lakes of Medio Flumendosa and Mulargia (Sardinia, western Mediterranean Sea) were reported to our laboratory. In those years MCYST standards were not available, but some analysis on this blooms of P. rubescens evidenced the presence of an hepatotoxic substance, with a LD50 of 100 mg/Kg body weight in mice and with MCYST characteristics (considerable liver damages, pulmonary and kidney haemorrhages) (Loizzo *et al*., 1988).

Since then, reports of toxic blooms continually reached our laboratory, and in 1993 a data bank concerning both marine and freshwater algal bloom episodes was set up.

The presence of cyanobacterial blooms in drinking and recreational waters required a survey on their occurrence and presence of toxins, in order to avoid sanitary risks and to provide information for successful restoration programs.

The present study focuses on the occurrence and distribution of cyanobacterial toxins from 1989 to 2006 in several Italian lakes of different characteristics and human uses, from drinkable to recreational purposes.

1.2 Materials and methods

1.2.1 Samples collection

Surface water samples for chemical, toxicological and biological analysis were collected from 28 Italian lakes, all assigned to drinking and/or recreational uses, during 17 years (1989-2006, table 1 and 2), from stations situated close to the lake shore. Sampling stations 500 m from the dams were adopted for all the Sardinian lakes. Subsamples (100 mL) were immediately fixed with Lugol's solution for the microscopic evaluations of cell densities. Samples were stored in ice chests and transported in dark and refrigerated conditions to the laboratory where toxicological analysis were performed.

1.2.2 Reagents and Chemicals

MC-RR, MC-YR, MC-LR, MC-LA, MC-LW were purchased by Calbiochem, La Jolla, CA. CYL was from Sigma-Aldrich, Milwaukee, WI. Trimethacarb (Riedel-de Haën, Seelze, Germany) is an obsolete insecticide and

was used as internal standard (IS) for quantifying MCYSTs, while quantification of CYL was carried out using 1,9-diaminononane (Sigma-Aldrich) as IS.

Individual standard solutions of the five MCYSTs and CYL were prepared by dissolving each compound in water to obtain 25 µg/mL concentrations. After preparation, these solutions were stored at -18 °C in the dark to minimize analyte degradation. They were freshly prepared every two months. A composite working standard solution of MCYSTs was prepared by mixing the above solutions and diluting with water to obtain analyte concentrations of 1 µg/mL. One mg/mL solutions of the two ISs, that is trimethacarb and 1,9-diaminononane, were separately prepared by dissolving them in acetonitrile. We obtained distinct working solutions of the two ISs at 1.5 µg/mL concentration by diluting with 10 mmol/L formic acid-acidified acetonitrile. When unused, all working standard solutions were stored at 4 °C in the dark, and renewed after one working week.

Acetonitrile RS of gradient grade was obtained from Carlo Erba, Milan, Italy. Trifluoroacetic acid (TFA) was from Fluka Bucks, Switzerland. All other solvents and chemicals were of analytical grade (Carlo Erba), and were used as supplied.

1.2.3 Phytoplankton analysis

The microscopic observations of phytoplankton were carried out on fresh and Lugol fixed samples in 25/10/5 ml sedimentation chambers, using an inverted microscope (Leitz Labovert FS; Zeiss Axiovert 100). Cell density was determined according to Utermöhl (1931) and Lund *et al*. (1958). Species were determined on both fresh and fixed samples according to Anagnostidis and Komarek (1990), Komarek and Anagnostidis (1989, 2005), Cronberg and Annadotter (2006).

1.2.4 Toxins extraction

Toxins were extracted from algae following the procedure described by Meriluoto and Eriksson (1988). Fresh algal aliquots (10–50 mg) obtained by centrifugation of water samples, were extracted two times with 2 ml portions of sterile bidistilled H2O. The solution was stirred, sonicated (5 min at 30°C–40°C) (Vibra-Cell, Sonics & Materials Inc.), then centrifuged for 10 minutes at 11.000 rpm (Beckman, LT-55 Ultracentrifuge) to eliminate debris. The supernatant was then collected and the whole process repeated twice.

Filtered water samples were analyzed for extracellular MCYST contents.

1.2.5 Water samples extraction

Water sample extraction followed by LC/MS/MS analysis was performed according to a method described by Bogialli et al. (2006).

Briefly, MCYSTs were extracted from 0.5 L of lake water passing the sample through a cartridge filled with 0,5 g Carbograph 4, while CYL was directly injected to LC/MS/MS apparatus after filtration. MCYST eluition was made in back-flushing mode with 1 mL of methanol followed by 4 ml of 10 mmol/L TFA-acidified CH2Cl2/CH3OH (80:20, v/v). After adding IS the eluate was evaporated to about 50 µl in a water bath at 50°C, 200 µL of a mobile fase (see below) was added to the residue and 50 µL of the final extract was injected into the LC column.

1.2.6 Instrumental analysis

Cyanotoxins detected up to year 2000 were analyzed by HPLC–DAD (MCYSTs) or GC-MS (anatoxin-a) (see cited literature); since then analysis were performed by LC-MS/MS (tables 1, 2). Due to higher accuracy, and to the availability of several MCYST standards, this latter group of analysis was examined for MCYST variant percentages.

LC/MS/MS analysis was performed according to the method described by Bogialli *et al*. (2006). By following the method described by Hummert et al. (2001), two demethylated varieties of MC-RR and one demethylated form of MC-LR were identified on monitoring cyanotoxins in Lake Albano. Standards of these three MCYSTs were not available to us. Thus, we quantified the above cited MCYSTs by assigning to them the same molar response factors of the respective fully methylated MCYSTs. LOQs were then calculated on the basis of a minimal accepted value of the signal-to-noise ratio (S/N) of 10. As calculated by us, LOQs of MCYSTs were between 2 (MC-RR) and 9 (MC-LW) ng/L. In spite of the fact that no enrichment step of CYL could be performed by the SPE cartridge, LOQ of this toxin was estimated to be 300 ng/L (Bogialli *et al*., 2006).

1.3 Results

Cyanobacterial hepatotoxins (MCYST and CYL) and the neurotoxin anatoxin-a were detected in 87 surface water samples from the 28 considered lakes. Almost all the positive cyanotoxin samples showed the contemporary presence of Cyanobacteria. Only in a very few cases the microscopic observation did not assess Cyanobacteria cells or species already known as toxin producers. In the cases of Sardinia lakes Benzone (December 2004), Govossai (December 2004) and Gusana (December 2004), the samples were collected just after dense and prolonged blooms (from summer to autumn) of toxic species (*Microcystis* spp.). The assessed toxicity might be in connection with these events, which were not analyzed for toxicity. Only in a case the assessed toxicity was not in presence of toxic species or just after events of this kind (Gusana, May 2005, fig. 1).

Cyanobacteria total densities ranged from less than 106 to more than 200 x 109 cells/L. The dominant species (>70% of the cyanobacterial total density of each sample) belonged to 11 genera: *Anabaena*, *Aphanizomenon*, *Aphanocapsa*, *Aphanothece*, *Cylindrospermopsis*, *Lyngbya*, *Merismopedia*, *Microcystis*, *Oscillatoria*, *Planktothrix*, *Pseudanabaena*, *Woronichinia*. The blooms were dominated by one or two species. *Planktothrix* (mainly *P. rubescens*) and *Microcystis* blooms (mainly *M. aeruginosa*) dominated in the samples from 33% of the lakes, respectively. The other lakes presented blooms caused by the assemblages of two or more co-dominating cyanobacterial genera. Figure 2 shows the different species assemblages observed as percent composition of the total densities of Cyanobacteria in some of the considered Sardinian reservoirs during 2003-2005, the respective assessed toxicity and Cyanobacteria total densities.

Six MCYST variants, desmethyl MC-RR ([Dha7] MC-RR or [D-Asp3, (E)-Dhb7]MC-RR), MC-RR, MC-YR, MC-LR, MC-LA and MC-LW were detected in the samples.

Total MCYST concentration in superficial scums varied between 107 (Iseo Lake, wet weight) and 1160 x 103 ng/g (San Puoto Lake, dry weight) (table 1).

The scums formed by *Planktothrix* blooms showed the highest MC-RR and MC-RR plus desmethyl MC-RR amounts in Simbirizzi Lake (480 x 103 ng/g MC-RR, dry weight) and Albano Lake (198.8 x 103 ng/g, wet weight) (table 1); the highest concentration of MC-YR (1160 x 103 ng/g, dry weight) in San Puoto Lake, and

the highest value of MC-LR (80.3 x 103 ng/g, wet weight) in a scum from Albano Lake.

In the scum samples from *Microcystis* blooms the highest MC-RR plus desmethyl MC-RR amount was detected in Trasimeno Lake (39 x 103 ng/g, wet weight); the highest concentrations of MC-YR (150 x 103 ng/g, wet weight) and of MC-LR (380 x 103 ng/g, dry weight) were found in Massaciuccoli Lake and Liscia Lake, respectively.

In Albano Lake, the cell density related to the analyzed surface scums led to a total intracellular MCYST concentration/L ranging from 84 µg/L to 10.1 mg/L.

The proportion of dissolved toxin compared to total toxin concentration ranged from 0.003% to 1.8% in these superficial blooms.

Extracellular MCYST concentrations in water were detected in 51 samples from 18 lakes, and ranged from traces (minor than 0.004 ng/ml) to 226.16 ng/ml (total extracellular concentration, table 2).

The three highest values of extracellular total MCYSTs in water (226.16 ng/ml, 8.32 ng/ml and 6.77 ng/ml) were found in Monteleone, Liscia and Posada lakes in November 2004 and October 2004, respectively.

Twelve out of the 51 samples (23%) exceeded the WHO provisional value of 1 µg/L (Kuiper-Goodman *et al.*, 1999).

LC-MS/MS analyses showed desmethyl MC-RR plus MC-RR (being desmethyl MC-RR the main constituent) and MC-YR as the predominant MCYST variants in the extracellular concentrations of *Planktothrix* dominated lakes (from 30% to 100% and from 5% to 50%, respectively) while MC-LR and MC-YR were the predominant toxins in *Microcystis* dominated lakes (from 8% to 100% and from 10% to 100%, respectively); in the superficial scums desmethyl MC-RR plus MC-RR and MC-YR were generally predominant (from 32% to 100% and from 2% to 100%, respectively; analyses subsequent to year 2000).

MC–LW and MC–LA extracellular concentrations in surface water were almost always under detection limits, with maxima in Massaciuccoli Lake (June 2004), where an amount of 0.23 ng/ml MC-LA was detected (June 2004), and in Monteleone Lake in which a concentration of 2.27 ng/ml MC-LW was found (November 2004).

In the analyzed scums, anatoxin-a concentrations varied from 9.8 (Canterno Lake, wet weight sample, September 2000) to 100 ng/g (Mulargia Lake, dry weight

sample, 1990), the latter value found during an *Anabaena planctonica* Brunnth. bloom (table 1). *A. planctonica* blooms in Mulargia Lake showed the presence of not identified MCYST– like peptides (Bruno *et al*., 1994), which may give reason for the slight MCYST amounts in December 2004 Posada Lake sample.

Albano Lake showed concentrations of 21.1 (September 2006) and 175.1 (September 2004) ng/mg intracellular CYL in wet weight samples from two scums of Aph. ovalisporum (table 1).

CYL concentrations in surface water ranged from 0.3 (Trasimeno Lake, September 2004) to 126 ng/ml (Albano Lake, September 2005) (table 3). CYL detection was always connected with blooms dominated or co-dominated by Aph. ovalisporum or C. raciborskii species. The two highest values were observed in Albano Lake (see above) and in Monteleone Lake (56.3 ng/ml, November 2004). Except for Trasimeno Lake, all the extracellular concentrations exceeded the recommended limit of 1 μg/L (Humpage and Falconer, 2003).

1.4 Discussion

The problem of cyanobacterial blooms in Italian freshwaters is not a new evidence, but it was almost exclusively considered under the aspect of phytoplanktonic population dynamics, and only recently for its possible toxicological implications (Pomati *et al*., 2000). Cyanobacterial dominance is widespread in reservoirs of Southern Italy such as in Sardinia (Sechi and Lugliè, 1992, 1996), Calabria and Sicily regions (Barone *et al*., 1991). It is consistent with high summer temperatures and strong water stratification of these reservoirs, despite their relative shallowness, and with the very frequent eutrophication of these freshwater environments (Marchetti *et al*., 1992).

This study showed that the most widespread toxic species in the considered Italian lakes from 1989 to 2006 were *P. rubescens*, *M. aeruginosa* and *Anabaena* spp. In the continental part of Italy, *P. rubescens* was the dominant blooming and MCYST-producing cyanobacterium, followed by *M. aeruginosa*. In the island the latter species was dominant.

Most samples analyzed contained more than three MCYSTs, being demethylated MCYSTs, MC-RR, MC-LR and MC-YR the main constituents.

Demethylated MCYSTs were in general the predominant toxins, as Fastner et al. already observed in German freshwaters (Fastner *et al*., 1999); they were always

detected in *Planktothrix* blooms (being (D-asp3, (E)-Dhb7 or Dha7) the most abundant), and in several Microcystis blooms.

MC-LA was rarely detected in the Italian samples, as it was described in natural blooms of other countries (South Africa, Scott, 1991; Morocco, Oudra *et al*., 2001; Greece, Gkelis *et al*., 2005).

The total MCYST concentration in superficial scums in Italy is comparable with other European countries (Poland, 23-1687 μg/g, Jurczak *et al*., 2004; Denmark, 11-737 μg/g, Henriksen and Moestrup, 1997) and with concentrations detected in surface waters from various other continents (from few ng/L up to 1300 μg/L; Fromme *et al*., 2000). Comparison of our data showed lower MCYST contents/g in *Microcystis* dominated lakes than in *Planktothrix* dominated lakes.

Two anatoxin-a producing blooms of *A. crassa* (Lake Canterno, Latium) and *A. planctonica* (Lake Mulargia, Sardinia) were detected. The *A. planctonica* bloom was, in our experience, the first case of a known non toxic species which blooms revealed to be toxic during the analyses (Bruno *et al*., 1994).

Finally, the detection of CYL was less frequent. It might be due to a narrow distribution and a minor intensity of the presence of the species *C. raciborskii* and *Aph. ovalisporum*, to which the toxicity appears to be connected.

The cyanobacterial densities assessed in our study surpassed in a significant percentage of samples the WHO recommended level for drinking water (1 μg/L,) and the Italian governmental limit for bathing waters (0.84 μg/L corresponding to 5 x 106 cell/L, Falconer et al., 1994) which prohibits freshwater bathing at 5000 cell of cyanobacterial toxic species/ml (Health Min. Circ. 31/7/1998 IX.400.4/13.1/3/1447). Other studies suggest lower limits (0.1 μg/L, Annadotter *et al*., 2001; 0.01 μg/L, Ueno *et al*., 1996; Hernandez *et al*., 2000).

The toxin contents detected in waters often did not correlate with the cyanobacterial cell density (e. g. CYL, table 5, and the extracellular MCYST contents, table 4).

1.5 Conclusions

This is the first study demonstrating the widespread occurrence of different MCYSTs in Italian freshwaters. The evidence that toxin contents do not correlate with the cyanobacterial cell density suggests monitoring analysis based on toxin detections in water together with microscopic cell count instead of the only

taxonomic evaluation, to avoid possible underestimations of the real toxin levels present in the waters.

Blooms of the described kind occur all over our territories sometimes with alternation of toxic species in the various seasons, in lakes and reservoirs that are often the only drinking water supply for local populations. This highlights the perspective of the risks of the direct use (examples are animal and human drinking use and human recreational uses), and suggests the possibility of others due to indirect uses, such as drinking water supply from groundwaters contaminated by percolation (Eynard *et al*., 2000; Ueno *et al*.,1996) and consumption of ichthyic products from freshwaters hosting toxic cyanobacterial blooms (Cook *et al*., 2004). This latter problem may be accentuated by recent human introductions of invasive species known to be able to accumulate MCYSTs (e.g., Procambarus clarkii) in areas where formerly they were absent (Vasconcelos *et al*., 2001). The indirect uses are more difficult to be demonstrated in respect to the direct uses, although some documentations are already available.

In fact, MCYSTs were detected in two wells for human use near Lake Albano, one of them being the main source of drinking water for a neighbouring town (Messineo *et al*., 2006), and the data collected from this lake in our research can explain these events.

Moreover, other consequences have to be investigated at environmental level: before the arising of public interest on toxicity, fish deaths associated to cyanobacterial blooms were considered in Italy as a consequence of anoxia caused by the blooms decline. Recent preliminary evidences seem instead to confirm MCYST contamination in ichthyic fauna from Italian lakes (Bogialli *et al*., 2005), pointing out the need for further studies on fish death causes in our country.

Lake	Date	Toxin	Amount (ng/g)	Extract	Dominant species	Cyanobacteria cell density (10^6 cells l^{-1})
Northern Italy						
Iseo	Dec 2000	MC-RR+ dem MC-RR	107	wet weight	*P. rubescens*	n.a.
Spino	Jan 2001 (Viaggiu et al. 2004)	Anatoxin-a	12.13*10^3	wet weight	*P. rubescens*	857
	Oct 2001	MC-RR+ dem MC-RR	24.6 *10^3	wet weight	*P. rubescens*	n.a.
		MC-LR	271.5			
		MC-YR	1551			
		Anatoxin-a	115.1			
Central Italy						
Albano	Jan 2000 (Viaggiu et al. 2003, Messineo et al. 2006)	MC-RR+ dem MC-RR	74 *10^3	wet weight	*P. rubescens*	660
	Jan 2004 (Messineo et al. 2006)	MC-RR+ dem MC-RR	115.5 *10^3	wet weight	*P. rubescens*	878
		MC-YR	2.2 *10^3			
		MC-LA	22			
	May 2004 (Messineo et al. 2006)	MC-RR+ dem MC-RR	90.3*10^3	wet weight	*P. rubescens*	48000
		MC-YR	15.3 *10^3			
	Sep 2004	Cylindrospermopsin	175.1*10^3	wet weight	*Aph. ovalisporum*	7000
	Nov 2004	MC-RR+ dem MC-RR	198.8 *10^3	wet weight	*P. rubescens*	298
		MC-YR	80.9 *10^3			
		MC-LR	80.3 *10^3			
	Apr 2005 (Messineo et al. 2006)	MC-RR+ dem MC-RR	2.9 *10^3	wet weight	*P. rubescens*	105
		MC-YR	1.9 *10^3			
		MC-LR	710			
		MC-LA	32.4			
	Sep 2006	Cylindrospermopsin	21.2*10^3	wet weight	*Aph. ovalisporum*	30
Canterno	Jul 2000	MC-YR	94 *10^3	wet weight	*M. aeruginosa*	n.a.
	Sep 2000	Anatoxin-a	9.8 *10^3	wet weight	*A. crassa*	346
Fiastrone	Jan 1998	MC-RR+ dem MC-RR	32 *10^3	wet weight	*P. rubescens*	n.a.
Gerosa	Apr 2003	MC-RR+ dem MC-RR	1*10^3	wet weight	*P. rubescens*	n.a.
Liscione	1991 (Volterra et al.1992)	MC-LR	15.8 *10^3	dry weight	*M. aeruginosa*	n.a.
	Jun 2001	MC-RR+ dem MC-RR	606	wet weight	*M. aeruginosa*	n.a.
Massaciuccoli	Jul 2000	MC-LR	20 *10^3	wet weight	*M. aeruginosa*	n.a.
		MC-YR	150 *10^3			
Nemi	Jan 2003	MC-RR+ dem MC-RR	21.9 *10^3	wet weight	*P. rubescens*	274
		MC-YR	traces			
	Feb 2003	MC-RR+ dem MC-RR	47.7 *10^3	wet weight	*P. rubescens*	150346
		MC-YR	5.1*10^3			
Polverina	Aug 1998	MC-LR	50*10^3	wet weight	*M. aeruginosa*	n.a.
S. Puoto	Mar 1990 (Bruno et al. 1992)	MC-YR	1160 *10^3	dry weight	*P. rubescens*	n.a.
Trasimeno	Sep 2000	MC-RR+ dem MC-RR	39 *10^3	wet weight	*M. aeruginosa*	n.a.
Southern Italy and Sardinia						
Bidighinzu	Jul 2003	MC-RR+ dem MC-RR	83.33	wet weight	*Anabaena spiroides* *Aphanocapsa* spp.	0.6
		MC-LA	250			
Cedrino	May 1998	MC-RR	500	wet weight	*M. aeruginosa*	20.87
Flumendosa	Sep 1989 (Bruno et al. 1992)	MC-YR	220 * 10^3	dry weight	*O. tenuis* *P. isothrix* *P. rubescens*	35.90
Liscia	1991 (Volterra et al. 1992)	MC-LR	380*10^3	dry weight	*M. aeruginosa*	n.a.
	3 Sep 2003	MC-RR+dem MC-RR	1.02*10^3	wet weight	*Microcystis* spp. *Aphanocapsa* spp.	43.97
	24 Sep 2003	MC-RR+dem MC-RR	12.66 *10^3	wet weight	*Microcystis* spp.	293.93
		MC-LR	3.05*10^3			
		MC-YR	2.2*10^3			
	Oct 2003	MC-RR+dem MC-RR	5.6*10^3	wet weight	*Microcystis* spp.	226.75
		MC-LR	692			
		MC-YR	923			
	Sep 2004	MC-RR+dem MC-RR	764	wet weight	*Microcystis* spp.	212553
		MC-LR	621			
		MC-YR	914			
		MC-LA	44			
Simbirizzi	Jan 1989 (Bruno et al. 1992)	MC-RR	480 *10^3	dry weight	*O. tenuis* *P. rubescens*	462.35
Mulargia	Jul 1990 (Bruno et al. 1994)	Anatoxin-a	100*10^3	dry weight	*A. planctonica*	34.12

n.a. not analyzed

Table 1. Cyanotoxin concentrations in superficial scums tested from 1989 to 2006.

Lake	Date	MC-RR+ dem MC-RR	MC-YR	MC-LR	MC-LA	MC-LW	TEM	Dominant species	Cyanobacteria cell density (10^6 cells l^{-1})
Northern Italy									
Iseo	Sep 2004	0.06	0.04	n.d.	n.d.	n.d.	0.10	*P. rubescens*	n.a.
Central Italy									
Albano	May 2004	0.21	0.11	n.d.	n.d.	n.d.	0.32	*P. rubescens*	839
	Jun 2004	1.17	0.12	n.d.	n.d.	n.d.	1.29	*P. rubescens*	0.5
	Oct 2004 (Messineo et al. 2006)	0.02	0.02	n.d.	n.d.	n.d.	0.04	*Aph. ovalisporum* *P. rubescens*	0.11 0.14
	Nov 2004	0.11	0.02	n.d.	n.d.	n.d.	0.13	*P. rubescens*	0.17
	Dec 2004	0.20	0.25	0.22	n.d.	n.d.	0.67	*P. rubescens*	0.012
	Jan 2005	2.07	0.13	0.25	n.d.	n.d.	2.45	*P. rubescens*	0.004
	Feb 2005	0.02	0.01	0.02	n.d.	n.d.	0.05	*P. rubescens*	2.26
	Mar 2005	0.06	0.02	0.02	n.d.	n.d.	0.10	*P. rubescens*	3.28
	Apr 2005	1.38	0.80	0.36	n.d.	n.d.	2.54	*P. rubescens*	3.4
	May 2005	n.d.	n.d.	trace	n.d.	n.d.	traces	*P. rubescens*	0.006
Massaciuccoli	Jun 2004	n.d.	0.11	0.15	0.23	n.d.	0.49	*M. aeruginosa*	n.a.
	Dec 2004	0.88	0.19	0.10	n.d.	n.d.	1.17	*M. aeruginosa*	n.a.
Fiastrone	Jun 2005	2.72	0.40	0.60	n.d.	n.d.	3.72	*P. rubescens*	n.a.
Canterno	Jul 2000	n.d.	1.12	trace	n.d.	n.d.	traces	*M. aeruginosa*	n.a.
Trasimeno	Sep 2000	4.52	n.d.	n.d.	n.d.	n.d.	4.52	*M. aeruginosa*	n.a.
	May 2005	n.d.	n.d.	0.02	n.d.	n.d.	0.02	*M. aeruginosa*	n.a.
	Jun 2005	0.19	0.46	0.33	n.d.	n.d.	0.98	*M. aeruginosa*	n.a.
	7 Aug 2005	n.d.	0.08	0.07	n.d.	n.d.	0.15	*M. aeruginosa*	n.a.
	14 Aug 2005	n.d.	0.03	0.02	n.d.	n.d.	0.05	*M. aeruginosa*	n.a.
	21 Aug 2005	n.d.	0.02	0.03	n.d.	n.d.	0.05	*M. aeruginosa*	n.a.
	29 Aug 05	n.d.	0.03	0.04	n.d.	n.d.	0.07	*M. aeruginosa*	n.a.
	4 Sep 2005	trace	0.06	0.11	0.05	n.d.	0.22	*M. aeruginosa*	n.a.
	11 Sep 2005	n.d.	0.01	0.02	0.01	n.d.	0.04	*M. aeruginosa*	n.a.
	25 Sep 2005	n.d.	0.01	0.03	n.d.	n.d.	0.04	*M. aeruginosa*	n.a.
	10 Oct 2005	n.d.	trace	0.01	trace	n.d.	0.01	*M. aeruginosa*	n.a.
	16 Oct 2005	n.d.	trace	0.01	n.d.	n.d.	0.01	*M. aeruginosa*	n.a.
Vico	Mar 2006	0.61	n.d.	0.06	n.d.	n.d.	0.67	*P. rubescens*	0.137
Southern Italy and Sardinia									
Liscia	Oct 2004	0.02	n. d.	0.08	n. d.	n. d.	0.10	*Microcystis* spp.	34.9
	Nov 2004	4.26	2.81	1.23	n.d.	0.02	8.30	*Microcystis* spp. *Aph flos-aquae*	312.3
	Dec 2004	0.86	0.31	1.55	n.d.	n.d.	2.72	*Microcystis* spp.	38.8
Pattada	Nov 2004	n.d.	n.d.	trace	n.d.	n.d.	traces	*Aph. flos aquae,* *Woronichinia* sp.	3.5
	May 2005	trace	n.d.	n.d.	n.d.	n.d.	traces	*Aphanothece* sp. *Anabaena* sp.	11.8
Posada	Oct 2004	0.35	3.04	3.38	n.d.	n.d.	6.77	*A. planctonica* *Aphanocapsa* sp.	19.9
	Dec 2004	trace	0.03	0.04	n.d.	n.d.	0.07	*Lyngbya* sp. *A. planctonica*	43.8
Monteleone	Oct 2004	0.14	0.15	0.30	n.d.	n.d.	0.59	*Aph.* cf *ovalisporum* *Merismopedia* sp. *Aph. flos-aquae*	13.4
	Nov 2004	109.22	36.79	77.88	n.d.	2.27	226.16	*Microcystis* spp. *A. planctonica*	3335.9
	Dec 2004	0.15	0.11	0.44	n.d.	n.d.	0.70	*Aphanocapsa incerta*	13.4
Torrei	Nov 2004	0.03	trace	trace	n.d.	trace	0.03	*Planktothrix* sp.	0.5
	Dec 2004	0.30	n.d.	n.d.	n.d.	n.d.	0.30	*Planktothrix* sp.	1.4
	Jan 2005	0.13	trace	0.01	n.d.	n.d.	0.14	*Planktothrix* sp.	2.2
	Feb 2005	0.01	n.d.	n.d.	n.d.	n.d.	0.01	*n.a.*	n.a.
	Mar 2005	0.1	n.d.	0.02	n.d.	n.d.	0.12	*Planktothrix* sp. *Aph. flos-aquae*	1.4
Truncu Reale	*entry Nov04	0.48	0.17	0.28	n.d.	n.d.	0.93	n.d.	n.d.
	exit Nov 04	0.35	n.d.	0.21	n.d.	n.d.	0.56	n.d.	n.d.
Govossai	Dec 2004	0.33	0.09	0.24	n.d.	n.d.	0.66	*Aphanocapsa* sp.	1.8
Cucchinadorza	Dec 2004	0.02	0.02	0.04	n.d.	n.d.	0.08	*Aphanocapsa* sp. *Lyngbya* sp.	0.6
Benzone	Dec 2004	n.d.	0.01	n.d.	n.d.	n.d.	0.01	*Aphanocapsa* sp.	1.9
Gusana	Dec 2004	0.01	0.01	n.d.	n.d.	n.d.	0.02	*Aphanocapsa* sp. *Lyngbya* sp.	1.1
	May 2005	0.09	0.03	0.02	n.d.	n.d.	0.14	*n.a.*	n.a.
Cecita	Oct 2006	n.d.	n.d.	2.35	n.d.	n.d.	2.35	*M. aeruginosa*	n.a.

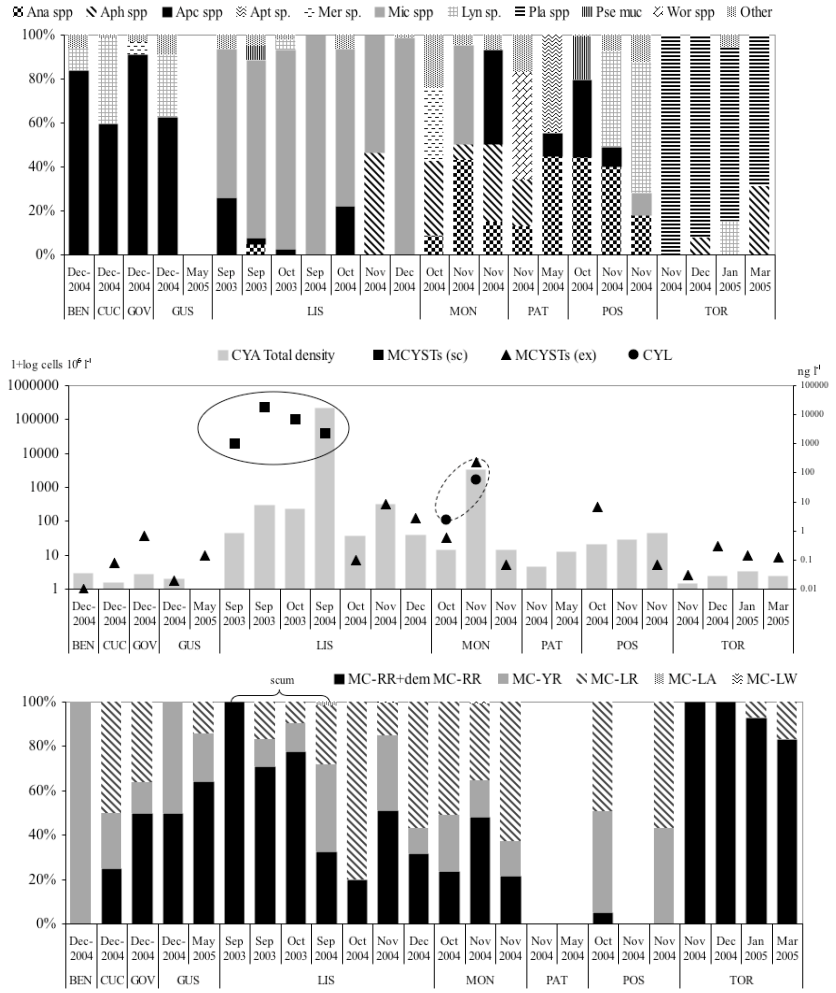
n.d. not detected trace < 0.004 ng/ml n.a. not analyzed * Truncu Reale water plant (Sardinia) TEM= total extracellular microcystins

Table 2. Extracellular microcystin concentrations in Italian freshwaters (ng ml^{-1}).

Lake	Date	Cylindrospermopsin (ng/ml)	Dominant species	Cyanobacteria cell density (10^6 cells l^{-1})
Monteleone	Oct 2004	2.40	*Aph*. cf *ovalisporum* *Merismopedia* sp. *Aph. flos-aquae*	13.4
	Nov 2004	56.31	*Microcystis* spp. *A. planctonica* *Aph*. cf *ovalisporum*	3335.9
Albano (Manti et al. 2005)	July 2004	15	*C .raciborskii*	1.1
	Oct 2004	14.90	*P. rubescens* *Aph. ovalisporum*	0.14 0.11
	Nov 2004	3.28	*P. rubescens* *Aph. ovalisporum*	0.17 0.11
	Sep 2005	126	*Aph. ovalisporum*	0.5
Trasimeno (Manti et al. 2005)	20 Sep 2004	0.30	*C. raciborskii*	9
	29 Sep 2004	0.46	*C. raciborskii*	6.5

Table 3. Cylindrospermopsin concentrations in Italian freshwaters.

Figure 1. Geographical localisation of the 28 sampled water bodies.



Lake abbreviations:
BEN=Benzone; CUC=Cucchinadorza; GOV=Govossai; GUS=Gusana; LIS=Liscia; MON=Monteleone; PAT=Pattada; POS=Posada; TOR=Torrei
Species abbreviations:
Ana spp=Anabaena planctonica, A. spiroides, Anabaena sp.; Aph spp=Aphanizomenon flos-aquae, Aph. ovalisporum; Apc spp=Aphanocapsa incerta, Aphanocapsa sp.; Apt sp.=Aphanothece sp.; Mer sp.=Merismopedia sp.; Mic spp=Microcystis aeruginosa, M. viridis, M. flos-aquae; Lyn sp.=Lyngbya sp.; Pla spp=Planktothrix ruscens, Planktothrix sp.; Pse muc=Pseudanabaena mucicola; Wor spp=Woronichinia naegeliana, Woronichinia sp.
Toxin abbreviations:
MCYTs (sc)=total microcystin concentrations in scums Different ; MCYTs (ex)=total extracellular microcystin concentrations; CYL=cylindrospermopsin concentrations

Figure 2. Percent species composition (upper figure) of the Cyanobacteria assemblages (Cyanobacteria total density and respective assessed toxicity, central figure) and percent composition of total extracellular microcystin (bottom figure) in some of the considered Sardinian reservoirs during 2003-2005.

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Chapter 2:

Cyanobacteria seasonal dynamics, microcystins in some Mediterranean reservoirs (Sardinia, Italy) and related risks to human health.

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Abstract

This article reports the results of an investigation on the presence of some microcystins in 11 Sardinian reservoirs (Italy, Western Mediterranean) characterised by different trophic status and all used for drinking.

The results of the investigation showed that *Microcystis* spp. were the most frequent and abundant Cyanobacteria found, especially in summer-autumn months, followed by *Aphanocapsa* spp. and *Aphanizomenon* spp.. Among the examined MC variants, only MC-RR and MC-LR were determined at levels up to 158.66 µg l^{-1} and 74.589 µg l^{-1}, respectively. Their concentrations varied according to the season and trophic levels of the reservoirs.

In all the examined reservoirs, 46% of the samples were MC positives. For most of them, MCs occurred at levels below the WHO drinking water guideline (1 µg l^{-1} for MC-LR). Nevertheless, in 18% of the cases, MCs reached very high levels (up to 233 µg l^{-1}) which, without any treatment, would correspond to an acute risk.

Keywords: Microcystins, Cyanobacteria, Eutrophication, *Microcystis,* Sardinia, Mediterranean

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2.1 Introduction

Harmful Algal Blooms (HABs) ascribe a heterogeneous group of events that are caused by microalgae and have negative impact on human interests and/or on ecosystems (Smayda, 1990; Anderson *et al*., 1993). One of the major effects regards the risks for human health. In freshwater environments these correspond mainly to the hazards caused by Cyanobacteria.

Several Cyanobacteria produce, as secondary metabolites, a high variety of toxins, known as cyanotoxins, that give rise to some concern for human health. Indeed, they have been included among emerging pathogenic microorganisms, even though they do not colonize and invade the host (OECD, 2005). Cyanotoxins can be classified into categories which reflect their biological effects on the systems and organs which they affect most strongly (Codd *et al*., 2005). They include hepatotoxins (microcystins and nodularines), neurotoxins (anatoxin-a, homoanatoxin-a, anatoxin a-(s), saxitoxins), cytotoxins (cilyndrospermopsin), irritants and gastrointestinal toxins (aplysiatoxin, debromoaplysiatoxin, lyngbyatoxin -produced by marine Cyanobacteria), lipopolisaccharidic (LPS) endotoxins, other cyanotoxins whose toxicological or ecotoxicological profile is still only partially known (e.g., microviridin J and β-N-methylamino-L-alanine). Microcystins (MCs) are the most frequently Cyanobacterial toxins so far reported (Chorus and Bartram, 1999; Carrasco *et al*., 2006; Sotero-Santos, 2006). They are cyclic heptapeptide hepatotoxins which include more than 70 variants (Sivonen and Jones, 1999). On the basis of acute toxicity data, MC-LR is considered among the most potent MCs and is by far the most studied. MC-LR and MC-RR are among the most commonly encountered members of this family.

Microcystin mechanism of action is associated with specific inhibition of protein serine/threonine phosphatases (PP1 and PP2A), altering phosphorilation of cellular proteins involved in signal trasduction (Gehringer, 2004). At high levels of exposure (representative of acute intoxication), MC-LR produces a cascade of events (cytoskeleton alterations, lipid peroxidation, oxidative stress, apoptosis) leading to centrolobular toxicity with intrahepatic hemorrhagic areas due to damage of sinusoidal capillaries. At low doses (typical of long term exposure), phosphatases inhibition induces cellular proliferation and hepatic hypertrophy

(Gehringer, 2004). MC-LR is able to induce oxidative stress and apoptosis in human cell lines (Botha *et al*., 2004).

Humans may be exposed to cyanotoxins through the oral route, due to consumption of contaminated drinking water or food (i.e.: aquatic organism and some diet supplements) or by ingesting water during recreational activities (Bowling and Baker, 1996; Pilotto *et al*., 1997; Carmichael, 2000; Gilroy *et al*., 2000). Dermal and inhalation exposure may also occur, associated with recreational, sport and professional activities (i.e. fishery) in waters interested by blooms, or domestic use of cyanotoxin-containing water, as in the case of showering. The parenteral route is also possible, when water from contaminated superficial water bodies is used for hemodialysis (Pouria *et al*., 1998; Jochimsen *et al*., 1998; Domingos *et al*., 1999; Carmichael *et al*., 2001).

Several studies have been published in relation to the possible effects on human health associated with short and long-term exposures to cyanotoxins, based on epidemiological and anedoctal data as well as toxicological properties, as reported in dedicated scientific publications (van Apeldoorn *et al*., 2007; Codd *et al*., 2005; Chorus and Bartram, 1999; Dittmann and Wiegand, 2006; Zurawell *et al*., 2005; Duy *et al*., 2000; Funari and Testai, 2008; WHO, 2003, 2004). Problems especially arise when abundant growth of potential toxic Cyanobacteria creates severe practical problems for water supply. In fact, Cyanobacteria are a frequent component of the communities of many freshwater and marine ecosystems and, under suitable conditions, can form blooms or surface scums (Ressom *et al*.,1994). Favorable conditions are, especially, waters rich in nutrients (Kardinaal & Visser, 2005; Sotero-Santos, 2006), exposed to sunlight and with reduced turbulence (Skulberg *et al*., 1984; Mur *et al*., 1999; Reynolds *et al*., 2002; Herry *et al*., 2007).

The occurrence of toxic Cyanobacterial blooms in northern European countries has been extensively investigated (Cook *et al*., 2004). As to the Mediterranean area, only few data are available on Cyanobacterial biodiversity and toxicity (Greece, Cook *et al.*, 2004; Spain, Carrasco *et al*., 2006; Portugal, Vasconcelos, 1994; France, Maatouk *et al*., 2002, Tunisia, Herry *et al*., 2007 and Morocco, Oudra *et al*., 2002), although potential toxic species are frequently dominant in lakes and reservoirs, especially in eutrophic and hypertrophic waters (Loizzo *et al*., 1989; Bruno *et al*., 1992, 1995; Sechi, 1992, 2000; Barone *et al*., 1991;

Barone and Naselli-Flores, 1994; Sechi and Lugliè, 1992, 1996; Naselli-Flores and Barone, 1998, 2000; Naselli-Flores, 2000; Barone, 2003; Salmaso, 1996, 2000; Morabito *et al*., 2001).

This aspect assumes relevance in the regions where, due to climatic and geographic features, very limited sources of freshwater are available and those accumulated in reservoirs represents the only source for drinking use, as in Sardinia. In fact, in the second island of the Mediterranean, 95% of its water need is supported by more than 40 reservoirs (surface area > 0.5 km^2; Marchetti *et al*., 1992; Sechi and Lugliè, 1992), mostly eutrophic or hypertrophic (Sechi, 1989; Marchetti *et al*., 1992; Sechi and Lugliè, 1992, 1996). A common feature of these reservoirs is the dominance of Cyanobacteria, especially during summer and autumn seasons, with potential toxic species as the most abundant (Sechi and Lugliè, 1992; Sechi, 2000). On the other hand, population dynamics of these harmful species in terms of seasonality and development are affected by wide variability, both at intra and interannual levels (Sechi *et al*., 1985, 1998; Sechi and Lugliè, 1987, 1989, 1992; Lugliè and Sechi, 1992; Lugliè *et al*., 2001; Sechi and Vacca, 1992; Mura *et al*., 1992; Virdis *et al*. 1998; Meregalli *et al*., 2002). Toxic blooms concerning particularly serious cases have already been reported (Volterra *et al*., 1986; Sechi and Lugliè, 1987, 1989; Loizzo *et al*., 1988, 1989; Bruno *et al*., 1994; Pellegrini *et al*., 1994; Sechi, 2000).

In spite of the numerous studies conducted on Sardinian reservoirs no researches has been carried out to assess cyanotoxins occurrence in their waters and to understand the relationships among presence of cyanotoxins, trophic levels and species assemblages of Cyanobacteria. This study was undertaken to try to overcame these knowledge gaps. Eleven Sardinian reservoirs were investigated between 2001 and 2005, during both bloom events and "normal" situations, to examine the occurrence of Cyanobacteria and MCs, considering their seasonality and trophic state.

2.2 Materials and Methods

2.2.1 Study sites and sampling

Figure 1 shows the location of the 11 studied reservoirs, all used also as drinking water supplies. Table 1 reports the morphometric characteristics of these

reservoirs, their trophic status, on the basis of published studies (Sechi, 2000; Sechi, 1989; Sechi and Lugliè, 1992; Lugliè *et al*., 2001).

Surface samples were collected in each reservoir at one station located at about 500 m from the dam, between 2001 and 2005. Samplings were performed especially in summer and autumn months, when Cyanobacteria are often dominant.

Three kinds of samplings were performed:

- in the period June 2001-March 2002 scum samples were taken from 5 reservoirs (CED, GOV, LIS, MRO and PAT);
- during July-October 2003 one reservoir (BID) was sampled with a fortnight frequency;
- in the period August 2004-February 2005, monthly samplings were carried out in 9 reservoirs (BEN, CUC, GOV, GUS, LIS, MRO, PAT, POS, TOR).

Immediately after sampling, phytoplankton samples were fixed with Lugol's solution. Water samples were transported to the laboratory in dark and refrigerated condition (-18 °C) until analysis. Samples collected in the period June 2001-March 2002 were stored at -80 °C .

2.2.2 Environmental parameters

During this investigation, the following environmental parameters were determined: temperature (using an Idromar multiparameter probe); ammonium nitrogen (Fresenius 1988); nitrate nitrogen, reactive and total phosphorus (Strickland and Parsons 1968). All the laboratory determinations were carried out within 24 h after sampling.

2.2.3 Cyanobacteria identification and counting

Cell density was valuated analysing subsamples of 5-10 ml of the fixed samples with an inverted microscope (Zeiss, Axiovert 10) at 200x and 400x magnifications, according to Utermöhl (Sournia, 1978; Innamorati, 1990).

For the taxonomic identifications both fresh and fixed samples were observed, following Anagnostidis *et al*. (1988), Baker (1981), Chang Tsang-Pi (1988), Hindák (1992), Huber-Pestalozzi (1938), Komárek (1991), Komárek and

Anagnostidis (1999, 2005), Komárek and Hindák (1988), Komarkova-Legnerova and Eloranta (1992) Pollingher (1991), Witthon and Potts (2000).

2.2.4 Analysis of MCs by LC-MS

All reagent used were of high-performance liquid chromatography grade. Acetonitrile was obtained from Lab-Scan (Dublin, Ireland), methanol from Panreac (Montcada i Reixac, Spain), glacial acetic acid from Carlo Erba (MI, Italy) and ammonium acetate from J.T. Baker (Deventer, Holland). High-purity water produced with a Milli-Q Milli-ρ system (Millipore, Bedford, MA, USA) was used.

MC-LR, MC-RR, MC-LF, MC-LW were purchased from Alexis (San Diego, CA, USA). Standard solution of MC-RR was prepared in a solution of methanol:water (80:20) while the other toxins in methanol and stored at –20 ^{o}C.

SPE cartridges, Suplclean LC-18, 500 mg in 3 ml, were purchased from Supelco (Bellefonte, USA).

MCs analyses were performed according to published procedures, with minor modifications (Lawton *et al*., 1994). Briefly, all the aqueous samples were freeze-thawed twice before analysis. The freeze-thawing operation caused cell lysis with the consequent release of intracellular cyanotoxins. The total MCs content was determined as follows: all samples were filtered through glass microfiber filters (Whatman GF/C) to remove cells debris and suspended particulate matter; the filtrate acidified (1 L) was applied to a preconditioned superclean LC-18 cartridge at a flow rate of 10 ml/min using an automatic solid phase extraction system (Aspec XL, Gilson); the cardrige was washed with 10 ml of pure water and 10 ml 20% methanol. MCs were eluted with 100% methanol and dried under N_2 flow, the residue dissolved in 500µl of mobile phase, filtered through a 0.45µm filter (Millex HV, Millipore) and stored frozen until LC/MS analysis.

Liophylized scum samples (20mg each) were extracted with 3ml of methanol for 1 h at room temperature under agitation. The extracts were centrifugated at 3000g for 10 min, to remove particulate material. The extraction procedure was repeated a further twice. The supernatant were pooled and dried by evaporation, the residue was dissolved in 500µl of mobile phase, filtered through a 0.45µm filter (Millex HV, Millipore) and stored frozen until LC/MS analysis.

MCs quantification was performed using a liquid chromatography electrospray ionization coupled mass spectrometry (LC-ESI-MS). LC was performed with a Series 200 LC Pump (Perkin Elmer, Shelton, CT, USA) fitted with a Rheodyne 7125 injection valve equipped with a 20 µl sample loop. Separation was achieved using a 250 x 1mm I.D. Kromasil 100-5C18 column (Eka Chemicals AB, Sweden). Water and acetonitrile, both containing 5mM of ammonium acetate, were used as mobile phases. Acetonitrile was held constant at 30% for the first 5 min, then it was linearly increased to 45% and held for 2 min, finally brought back to 30% and held for 10 min until the next injection. The flow rate was maintained at 1ml/min. A flow splitter was mounted after the HPLC column, thus allowing a flow-rate of only 40 µl/min to the mass-spectrometer.

MS measurement were performed using an API 150EX single quadrupole mass spectrometer (Applied biosystem, Foster City, CA, USA), equipped with an atmospheric pressure ionisation source operating in turbo-ionspray mode.

The turbo-ionspray voltage was set at 5.5 kV and the decluster potential at 70V. The desolvation gas (nitrogen) temperature and flow-rate were set at 350 ^{o}C and 300 l/h, respectively. The instrument operated in the positive ion mode.

For the set up of method's parameters, authentic toxin standard was dissolved in mobile phase and infused at 10 µl/min with a Harvard Apparatus infusion pump (St-Laurent, Quebec) into the mass spectrometer. Full scan spectra were acquired in the positive ion mode over the mass range of m/z 400-1500 at 3 s/scan.

For the analysis, the MS system was operated in Single Ion Monitoring (SIM) to give highest sensitivity and selectivity. Ions centred at m/z 995.6$[M+H]^{+}$ for MC-LR, at 1026.2 m/z $[M+H]^{+}$ for MC-LW, at 986.5 m/z $[M+H]^{+}$ for MC-LF and at 1038.0 m/z $[M+H]^{+}$ and 520.0 m/z$[M+2H]^{++}$ for MC-RR.

Spectra and chromatograms were processed with Analyst software Version 1.1.

A detection limit (LOD) of 1ng/ml and a quantification limit (LOQ) of 5 ng/ml were reached for the MC-LR standard; a LOD of 5 ng/ml and a LOQ of 10 ng/ml were achieved for the other MCs standard.

Quantitative determination of MCs was carried out by comparing peak areas and the retention times of the pure toxins with the corresponding ones in the environmental samples.

2.3 Results

2.3.1 Environmental parameters

The environmental parameters monitored during the overall period are summarised in table 2. In summer months very high temperatures (up to 32 °C) were recorded in some reservoirs. Considering to nutrient concentrations, particularly high values of total phosphorus (1400-2142 mg P m^{-3}) were found in LIS, MRO and PAT. Nitrate concentrations higher than 1000 mg N m^{-3} were determined in all the reservoirs with the exceptions of BID, CED, GOV, TOR. Ammonium nitrogen reached the highest values in LIS and BID (414 mg N m^{-3} and 635 mg N m^{-3}, respectively). The assured nutrient data are in agreement with the previous trophic classification of the reservoirs (Tab. 1).

2.3.2 Cyanobacteria identification, counting and MCs contents

Table 3 reports Cyanobacteria species and their densities in the 11 reservoirs during the studied period. Fourteen genera were identified with 12 potentially MC producers. When the species were not distinguished, their densities were calculated as sums of species of the same genus.

Among the all observed Cyanobacteria, *Aphanocapsa, Microcystis* and *Aphanizomenon* showed the widest distributions (respectively, in 11, 9 and 8 of the studied reservoirs). *Aphanizomenon* cf. *ovalisporum* (Forti), *Cianocatena* sp., *Chroococcus* sp. and *Woronichinia naegeliana* (Unger) Elenkin were observed each one in only one reservoir.

Tables 4 and 5 report analytical data on MC concentrations found in the samples. Among the tested MC variants, MC-LF and MC-LW were never found at the sensitivity of the applied method, whereas MC-LR and MC-RR were determined in all the reservoirs, even though in a very large range.

Table 4 shows the results achieved on MC concentrations in lyophilized scum samples. MC-LR and MC-RR were found only in samples from reservoirs dominted by *Microcystis* spp. blooms. The two MC variants were found in two out of four investigated lakes. The highest MC concentration (0.510 μg l^{-1}) was found in June 2001 in MRO.

In samples from BID, MC variants were never found even though this reservoir was characterized by the occurrence of Cyanobacteria at relatively high densities (up to 236 x 10^6 cells l^{-1} in July 2003 due to *Cianocatena* spp.).

Table 5 reports the MC contents in water samples from the 9 reservoirs analyzed in the period 2004-2005. As shown in this table, MC-LR and MC-RR were found in all the investigated reservoirs. The highest total MC concentration was observed in LIS (233.253 μg l^{-1} in September). PAT and MRO showed relatively high levels (7.973 and 17.011 μg l^{-1}, respectively), too. The high MC contents parallel the high densities of Cyanobacteria (213 x 10^9, 10 x 10^9 and 3 x 10^9 cells l^{-1}, respectively in LIS, PAT and MRO), most of which potentially toxic (Tab. 3).

This relationships is better explained in Figure 2, where the densities of the most important potentially toxic Cyanobacteria are compared with MC concentrations. From this figure it is possible to notice the clear season dependence of MC levels. MCs peaked in late summer and beginning of autumn, in coincidence with the highest densities of potentially toxic Cyanobacteria, prevalently *Microcystis* and *Aphanocapsa*. With respect to this pattern, a certain delay was noticed between the highest MC level and the respective highest Cyanobacterial density in GUS.

No MCYST positive samples were found in BID from July to October 2003 (sampling type B), nevertheless Cyanobacteria densities were up to 239 x10^6 cells l^{-1} in July due to *Cianocatena* sp..

2.4 Discussion and Conclusions

MC-LR and MC-RR were determined in about 80% of the tested reservoirs and in about 46% of the analysed samples. These results are similar to those reported in a study carried out on eutrophic reservoirs in central Spain (Carrasco *et al*., 2006). MCs were found at the highest levels in summer-autumn, as in other Mediterranean areas (Maatouk *et al*., 2002; Cook *et al*., 2004; Nasri *et al*., 2007; Carrasco *et al*., 2006; Herry *et al*., 2007), and were prevalently assessed in the interval temperature of 20 - 30 °C.

MC-LR and MC-RR were detected, with a wide variability during the study period. MC-LR was the most frequently occurring MC variant. MC-RR levels were higher than MC-LR during maximum MC production whereas MC-LR contents prevailed in almost all the samples when the concentrations were lower.

The analysis of the analytical data obtained in this investigation shows that MC maximum detection frequency was found at total cell densities higher than 10^7 cells l^{-1} (Fig. 3), with percentages of positive samples higher than 60% above this density. Positive direct relationships were observed between MCs concentrations and densities and biomass of Cyanobacteria potentially toxic ($r=0.996$, $p<0.01$, $n=33$ and $r=0.680$, $p<0.01$, $n=33$, respectively) (Fig. 4). This relationships are positive also considering total Cyanobacteria density and biomass ($r=0.996$, $p<0.01$, $n=33$ and $r=0.611$, $p<0.01$, $n=33$, respectively). Similar results were obtained in a study in Greece (Cook et al., 2004) .

In our study, MC-LR concentrations were above the WHO provisional guideline for drinking water (1 µg l^{-1}; WHO, 2003) in 6 cases (Fig. 5; 18%), in 4 reservoirs (36%), with an evident lower incidence than in Greek reservoirs (Cook *et al*., 2004). The results does not change if MCs data are considered.

Microcystis, *Aphanocapsa,* and *Aphanizomenon* were the genera with the widest distribution and the most abundant in Sardinian reservoirs. Except for *Aphanocapsa*, these genera are reported as dominants in toxic events also in reservoirs of Portugal (Vasconcelos, 1994, 2001), France (Maatouk *et al*., 2002), Greece (Cook *et al*., 2004), Central Spain (Carrasco *et al*., 2006), Tunisia (Herry *et al*., 2007). *Aphanocapsa delicatissima* (W. et G.S. West) Kom.-Legn. & Cronb. is the most frequently found species of the genus in Sardinian reservoirs and is probably world-wide distributed (Komárková-Legnerová and Cronberg, 1994, Cronberg and Annadotter, 2006). At the moment, there are no data on its toxicity but other species of this genus have been shown to be toxic (Domingos *et al*., 1999). *Microcystis aeruginosa* (Kg.), *M. viridis* (A. Braun in Rabenhorst) Lemmermann and *Aphanizomenon flos-aquae* (L.) Ralfs, respectively the main species of the two genera, are reported as common in eutrophic inland waters and with a world-wide distribution (Abdel-Rahman *et al*., 1993; Vasconcelos, 1994; 2001; Vezie *et al*., 1997, 1998; Maatouk, *et al*., 2002; Cook *et al*., 2004; Carrasco *et al*., 2006). This study confirms that *Microcystis* spp. are particularly important in MC productions in Mediterranean reservoirs. Furthermore, a strong relationship between *Microcystis* species and trophic status was already assessed in Sardinia (Sechi and Lugliè, 1996). The data achieved in this study show positive relationships between total phosphorus, assumed as primary descriptor of trophy,

and both Cyanobacterial densities ($r=0.691$, $p<0.01$, $n=33$) and MC concentrations ($r=0.709$, $p<0.01$, $n=33$).

The highest number of Cyanobacteria species, densities, and MC concentrations were found in LIS, PAT and MRO reservoirs, with a percentages of positive samples ranging from 50% (LIS) to 70% (MRO). These three reservoirs are really characterised by high level of eutrophication, as described in previous studies (Cossu and Sechi, 1988; Sechi, 1989; Sechi e Lugliè, 1992, 1996; Lugliè *et al*., 1992; Marchetti *et al*., 1992; Sechi, 2000; Mameli *et al*., 2002).

On the other hand, MCs were never determined in BID, in spite of its strong eutrophication, probably because of the low densities of toxic species or strains, at the sampling time (*Aphanizomenon*, *Cianocatena* and *Chroococcus* as dominants). In other years this reservoir was interested by the occurrence of higher densities of potentially toxic species, such as *M. aeruginosa*, *Microcystis flos-aquae* (Wittr.) Kirchn., *Anabaena flos-aquae* (Lyngb.) Bréb, *Aph. flos-aquae* and *Aphanocapsa* sp. (Lugliè and Sechi, 1993; Sechi and Lugliè, 1996; Sechi, 2000; Lugliè *et al.,* 2001). The extreme variability of species composition and densities of Cyanobacteria is a typical feature of Sardinian reservoirs and, in general, of Mediterranean ones (Sechi and Lugliè, 1987; Barone, 2003; Cook *et al*., 2004).

MCs were found also in mesotrophic waters (GOV, CUC, TOR), even though at a very lower frequency and concentrations. TOR was interested by the lowest Cyanobacterial densities (not exceeding 2×10^6 cells l^{-1}), a number of positive samples (43%) and MC levels not exceeding 0.095 $\mu g\ l^{-1}$. Also in these reservoirs, MC peaks were recorded in coincidence of *Microcystis* maxima, with the exception of TOR, where the maximum levels seemed to be produced by *Aphanocapsa* spp. In this reservoir, *Planktothrix* spp. did not appear to be able to produce MCs (Fig. 2), whereas *Planktothrix* strains were responsible for one of the most dangerous toxic events in Sardinia (Volterra *et al*., 1986; Sechi and Lugliè, 1987, 1989; Loizzo *et al*., 1988, 1989).

In a sample collected in GUS, high MC concentrations were determined after one month from a bloom of potentially toxic *Microcystis* and *Aphanocapsa* spp. This high MC concentration may be due to dissolved MCs or to the presence, even though at low densities, of strains particularly toxic. This point out the possibility of a delayed extracellular MC presence in the water after intense blooms and

highlights the necessity of high-frequency samplings to allow the detection of also short-duration blooms.

In all the examined reservoirs, for most of the studied period, MCs occurred at levels below the WHO guideline (WHO, 1998; Fig. 5). This means that the chronic risk associated with the consumption for drinking of these waters is negligible. Anyway, in at least one of these cases (233 μg l^{-1}, in LIS), the consumption of the water for drinking without any treatment might correspond to an intake that could cause acute effects, which is of about 150 μg/person (Fromme *et al.*, 2000). But this is the worst possible scenario. Indeed, raw waters are treated before their distribution and strong degrees of reductions of Cyanobacteria and cyanotoxins concentrations are generally achieved (Dietrich and Hoeger, 2005). Moreover, these events are extremely visible due to the very high associated cell densities and for this reason they are very difficult to be ignored. On the other hand, the global number of positive samples (46%), also with concentrations decisively below the WHO guideline, indicates the necessity to valuate better the possible chronic effect on human local populations.

With reference to the use of these waters for agricultural activities, in general the risk of accumulation of MCs in crops would be small and in case limited to the LIS. Nevertheless, it can be recommended not to use these waters for irrigation when there are Cyanobacterial blooms and scums.

2.5 Acknowledgment

The study was conducted in the framework of the national project, partially funded by the Italian Ministry of Health.

Lake	Abbreviations	Watershed m^2x10^6	Volume m^3x10^6	Area m^2x10^6	Mean depth m	Trophic status
Benzone	BEN	89.4	1.1	0.27	4.0	E
Bidighinzu	BID	51.7	12.2	1.70	7.3	IE
Cedrino	CED	631.2	30.0	1.10	26.5	E
Cucchinadorza	CUC	91.6	17.5	1.10	15.9	M
Govossai	GOV	29.7	2.8	0.27	10.4	M
Gusana	GUS	190.6	59.5	2.60	22.9	E
Liscia	LIS	284.1	33.9	1.32	25.7	IE
Monteleone Roccadoria	MRO	142.5	55.4	3.50	15.8	IE
Pattada	PAT	160.0	65.5	4.40	14.9	IE
Posada	POS	613.6	27.8	3.00	9.3	E
Torrei	TOR	14.5	3.0	0.17	17.6	OM

Legend: IE: Ipertrophic, E: Eutrophic, M: Mesotrophic, OM: Oligo-Mesotrophic

Tab. 1 - Studied reservoirs, abbreviations and main morphological characters.

	Temperature (°C)			$N\text{-}NO_3$ (mg N m^{-3})			$N\text{-}NH_3$ (mg N m^{-3})			P-Ptot (mg P m^{-3})		
	Mean	***Min.***	***Max.***	***Mean***	***Min.***	***Max.***	***Mean***	***Min.***	***Max.***	***Mean***	***Min.***	***Max.***
BEN	21.0	9.0	28.0	783	304	1559	55	10	111	35	23	54
BID	19.6	17.3	21.7	61	28	80	483	375	635	289	212	357
CED	21.8	14.0	28.0	204	5	643	23	16	27	91	39	134
CUC	17.2	7.5	25.0	1112	793	1999	37	14	84	34	21	43
GOV	12.7	3.0	23.0	408	7	879	60	14	125	22	13	29
GUS	16.4	7.5	23.0	1056	700	1529	43	7	123	35	21	56
LIS	21.2	7.0	29.5	312	0	1288	56	11	414	203	16	2142
MRO	18.5	7.0	32.0	454	34	2218	32	16	55	208	28	1400
PAT	17.0	8.0	30.0	322	14	1051	68	15	169	259	25	1734
POS	18.4	9.0	29.0	579	5	1429	52	0	133	36	30	45
TOR	14.1	4.0	23.0	189	16	389	38	21	61	24	15	30

Tab. 2 – Mean values, minima, maxima of the considered environmental parameters.

Tab. 3

	BEN	BID	CED	CUC	GOV	GUS	LIS	MRO	PAT	POS	TOR	MC producer	References
Nostocales													
Anabaena													
A. planctonica												yes	3
A. flos-aquae		+										yes	17, 1, 16, 20
A. spiroides												yes	11
Anabaena species													
Aphanizomenon													
Aph. flos-aquae												yes	21, 15, 10, 19
Aph. cf. ovalisporum													
Aphanizomenon sp.													
Oscillatoriales													
Lyngbya													
Lyngbya sp.													
Planktothrix													
P. agardhii-rubescens group												yes	18, 6, 2, 9, 19
Pseudanabaena													
P. mucicola												yes	14
Pseudanabaena sp.												yes	14
Chroococcales													
Aphanocapsa													
A. incerta													
Aphanocapsa species												yes	5
Aphanothece													
Aphanothece species													
Chroococcus													
Chroococcus sp.													
Cianocatena													
Cianocatena sp.													
Coelosphaerium													
Coelosphaerium sp.													
Merismopedia													
Merismopedia sp.													
Microcystis													
M. aeruginosa							+	+		+		yes	4, 21, 22, 20, 13, 14
M. flos-aquae								+				yes	7, 8, 15
M. viridis							+	+				yes	23, 7, 8
Microcystis sp.		+					+					yes	17, 1, 16
Microcystis species												yes	12, 17 16, 1, 6
Snowella													
Snowella sp.													
Woronichinia													
W. naegeliana													
Woronichinia sp.													
Total density (cells 10^6 l^{-1})	136	239	1740	38	56	348	212553	3336	9970	44	2		
MCs (µg l^{-1})	0.21	n.d.	n.d.	0.39	0.96	14.23	233.25	17.01	7.97	0.21	0.09		

Legenda:

1) Bateman et al., 1995; 2) Briand et al., 2002; 3) Bruno et al., 1994; 4) Carmichael et al., 1988; 5) Domingos et al., 1999;
6) Dukobu et al., 2000; 7) Harada et al., 1990a; 8) Harada et al., 1990b; 9) Herry et al., 2007; 10) Maatouk et al., 2002;
11) Health Min. Circ. 31/7/1998 IX.400.4/13.1/3/1447; 12) Namikoshi et al., 1992; 13) Oudra et al., 2001; 14) Oudra et al., 2002;
15) Quesada et al., 2000; 16) Rinehart et al., 1994; 17)Sivonen et al., 1992d; 18) Sivosen et al., 1989; 19) Torokné et al., 2007;
20) Vasconcelos, 2001; 21) Vezie et al., 1997; 22) Vezie et al., 1998; 23) Watanable et al., 1988

10^6 cells l^{-1} < density > 10^6 cells l^{-1} + = presence

Tab. 3 - List of the observed species, their presence, achieved levels of total density and MCs concentration in the different reservoirs.

		June	*August*	*September*	*October I*	*October II*	*March*
CED	*Tot-MCs*	n.d.	n.d.	n.d.			n.d.
	MC-LR	n.d.	n.d.	n.d.			n.d.
	MC-RR	n.d.	n.d.	n.d.			n.d.
GOV	*Tot-MCs*					tr	
	MC-LR					tr	
	MC-RR					tr	
LIS	*Tot-MCs*	0.022					
	MC-LR	0.010					
	MC-RR	0.012					
MRO	*Tot-MCs*	0.510			0.095	0.342	
	MC-LR	0.180			0.090	0.146	
	MC-RR	0.330			0.005	0.196	
PAT	*Tot-MCs*				tr		
	MC-LR				tr		
	MC-RR				tr		

n.d.: concentration below LOD tr: traces not quantifiable

Tab. 4. - Total MCs, MC-LR and MC-RR concentrations in scum samples (expressed in µg/l) from 4 reservoirs during 2001-2002.

		August	*September*	*October*	*November*	*December*	*January*	*February*
	Tot-MCs	0.024	0.127	0.207	0.050	n.d.	n.d.	n.d.
BEN	*MC-LR*	0.024	0.079	0.196	0.050	n.d.	n.d.	n.d.
	MC-RR	n.d.	0.048	0.011	n.d.	n.d.	n.d.	n.d.
	Tot-MCs	0.120	0.387	0.024	n.d.	n.d.	n.d.	n.d.
CUC	*MC-LR*	0.112	0.217	0.024	n.d.	n.d.	n.d.	n.d.
	MC-RR	0.008	0.170	n.d.	n.d.	n.d.	n.d.	n.d.
	Tot-MCs	0.016	0.961			n.d.	n.d.	n.d.
GOV	*MC-LR*	0.012	0.297			n.d.	n.d.	n.d.
	MC-RR	0.004	0.664			n.d.	n.d.	n.d.
	Tot-MCs	0.036	0.026	14.233		n.d.	n.d.	n.d.
GUS	*MC-LR*	0.012	0.026	4.949		n.d.	n.d.	n.d.
	MC-RR	0.024	n.d.	9.284		n.d.	n.d.	n.d.
	Tot-MCs	3.573	233.253	0.048	0.010	n.d.	n.d.	n.d.
LIS	*MC-LR*	1.121	74.589	0.029	0.010	n.d.	n.d.	n.d.
	MC-RR	2.452	158.664	0.019	n.d.	n.d.	n.d.	n.d.
	Tot-MCs	2.496	0.012	0.044	17.011	n.d.	n.d.	n.d.
MRO	*MC-LR*	1.852	0.012	0.020	12.591	n.d.	n.d.	n.d.
	MC-RR	0.644	n.d.	0.024	4.420	n.d.	n.d.	n.d.
	Tot-MCs	7.973	0.830	n.d.	n.d.	n.d.	n.d.	n.d.
PAT	*MC-LR*	3.264	0.440	n.d.	n.d.	n.d.	n.d.	n.d.
	MC-RR	4.709	0.390	n.d.	n.d.	n.d.	n.d.	n.d.
	Tot-MCs	0.205	0.037	0.077	0.213	n.d.	n.d.	n.d.
POS	*MC-LR*	0.125	0.022	0.077	0.128	n.d.	n.d.	n.d.
	MC-RR	0.080	0.015	n.d.	0.085	n.d.	n.d.	n.d.
	Tot-MCs	0.095	0.027	0.005	n.d.	n.d.	n.d.	n.d.
TOR	*MC-LR*	0.095	0.005	n.d.	n.d.	n.d.	n.d.	n.d.
	MC-RR	n.d.	0.022	0.005	n.d.	n.d.	n.d.	n.d.

n.d.: concentration below LOD tr: traces not quantifiable

Tab. 5. Total MCs, MC-LR and MC-RR concentrations in surface samples (expressed in µg/l) from 9 reservoirs during 2004-2005.Fig. 1 - Location of the studied reservoirs.

Fig 1 Location of the studied reservoirs

Fig. 2 - Dynamics of main Cyanobacteria microcystin producer species and MCs during 2004-2005.

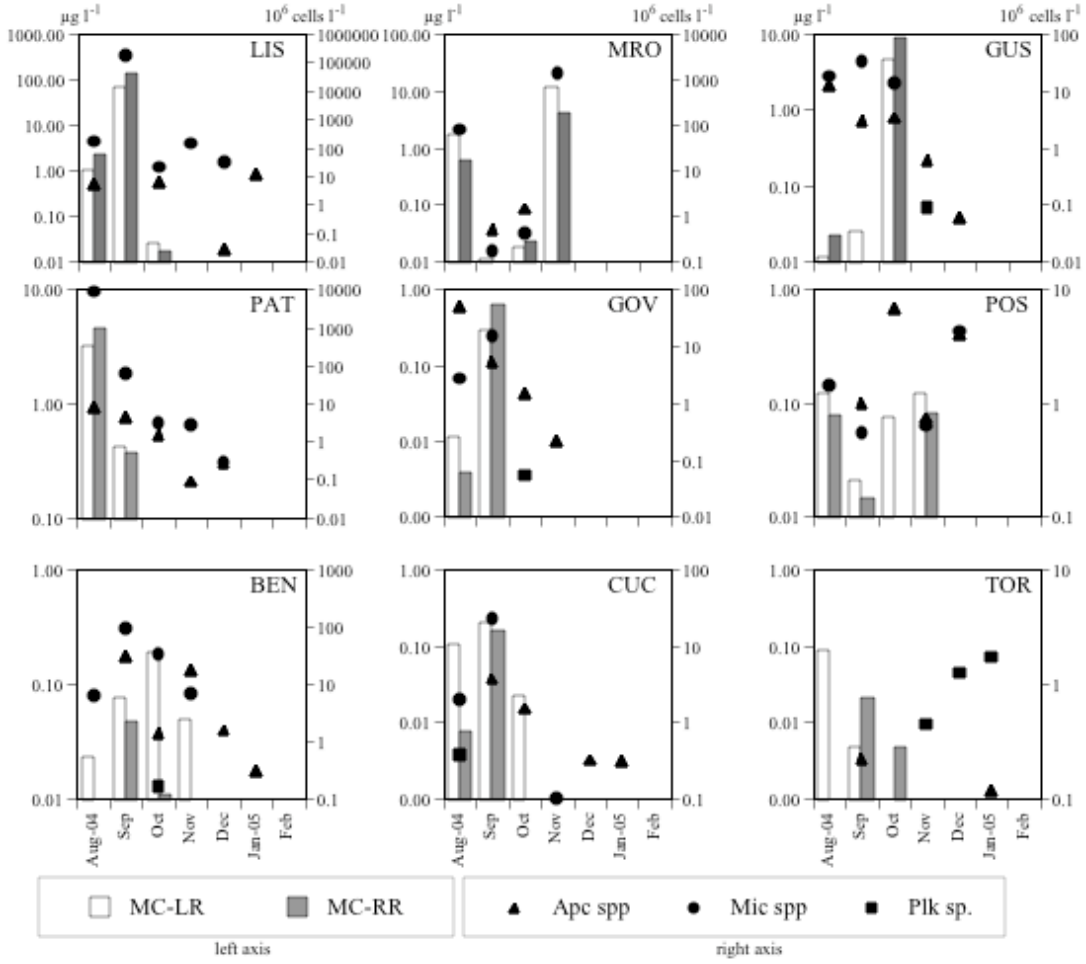


Fig. 3 - Frequency of MCs detections at the different density (d) classes.

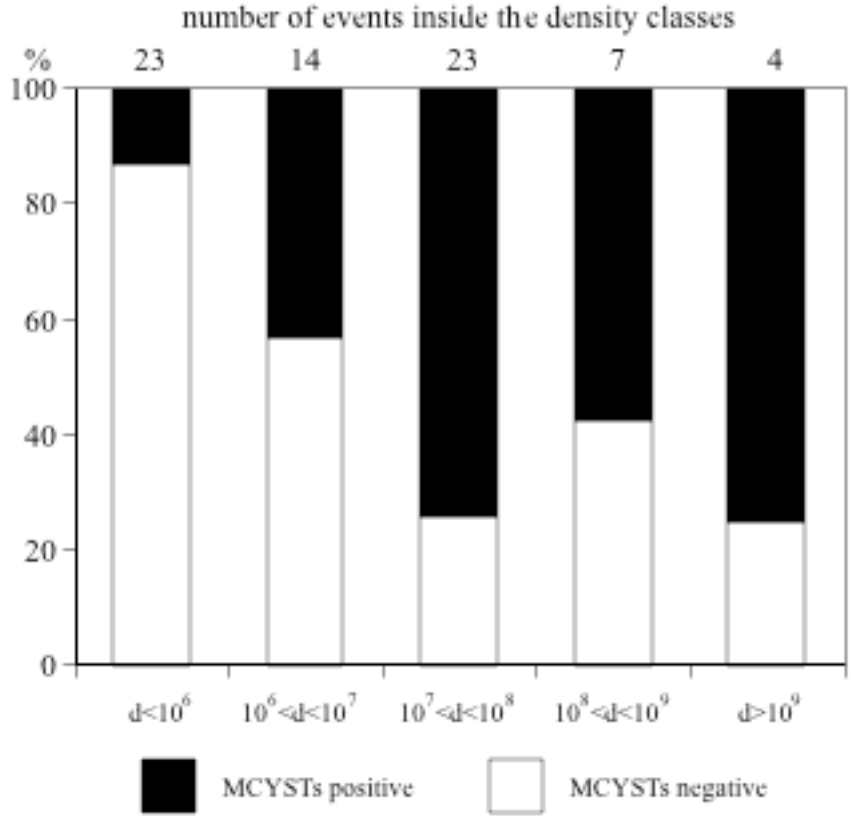


Fig. 4 - Relationships between Cyanobacteria potentially toxic density and MCs concentration (upper) and between Cyanobacteria potentially toxic biomass and MCs concentration (lower).

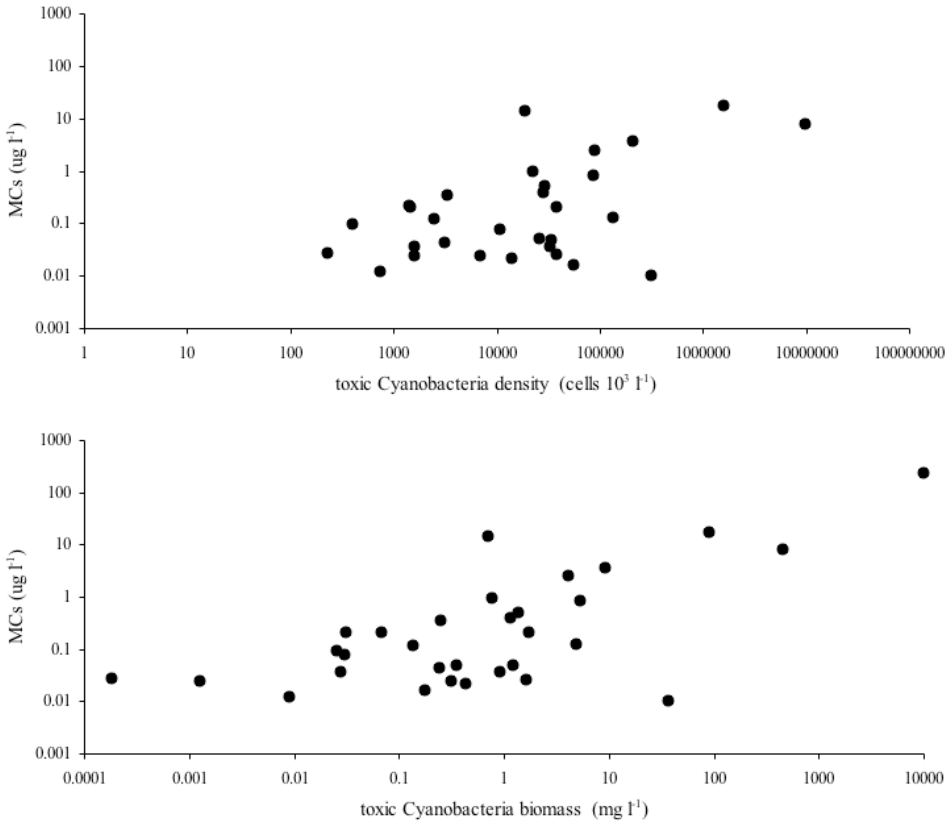
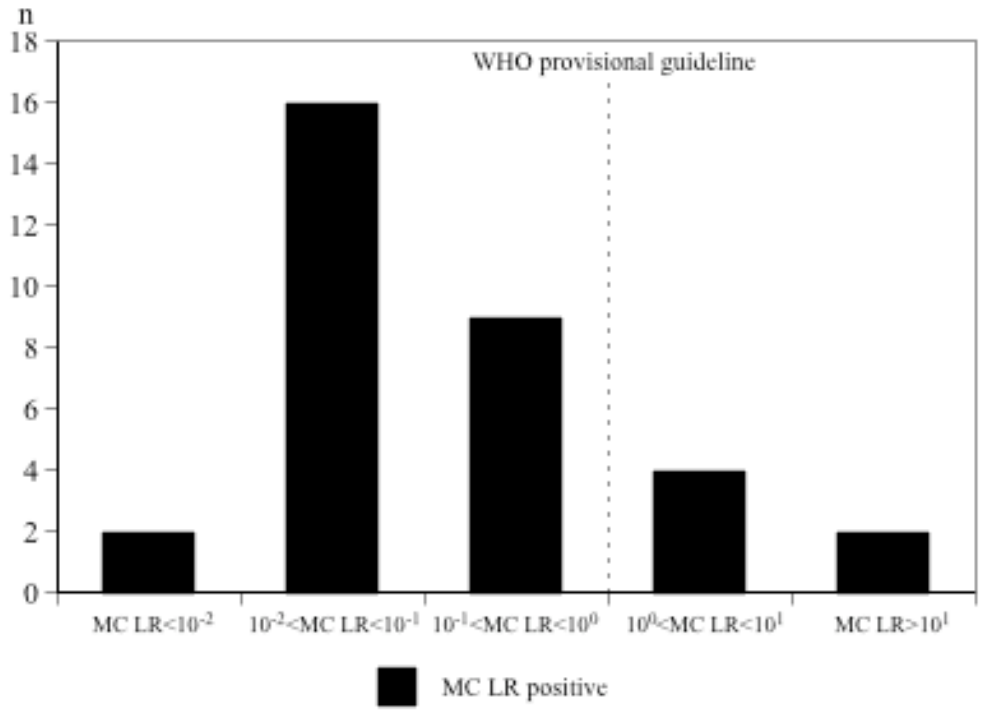


Fig. 5 Frequency of MCs detections at the different concentration classes.



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Appendix

Università degli Studi di Sassari - Dipartimento di Botanica ed Ecologia Vegetale

2nd European Ponds Workshop: *Conservation of pond biodiversity in a changing European landscape*
Toulouse (France), 23th – 25th February 2006

MICROALGAL ASSEMBLAGES AND ENVIRONMENTAL CONDITIONS IN MEDITERRANEAN TEMPORARY PONDS: FIRST DATA FROM SARDINIA (ITALY)

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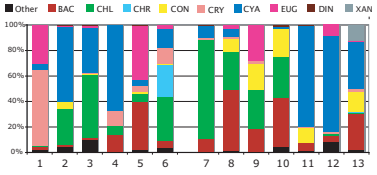
The study on macrophytes of Mediterranean temporary ponds started in Sardinia (Italy) in 2004 and extended to microalgae, physicochemical parameters and nutrients in 2005, in order to improve their knowledge and understand their ecology. In fact, no limnological data were collected in temporary ponds of the island till now, but many researches were and are still carried out on Sardinian reservoirs due to their strategic importance as water source from the last '70s (Sechi & Lugliè, 1992,1996). In Sardinian environment, as well as in the Mediterranean area as a whole, ponds are believed to be critical due to their biodiversity, which is not well known and investigated yet, especially for some of the various components of the present communities. This study describes 13 temporary ponds with regard to their limnological characters and suspended microalgal assemblages, considering the general environmental belonging scenario. The microalgal component fits with the metaphyton sensu lato: a group of microalgae aggregated in the littoral zone, which are neither strictly attached to substrata nor truly suspended (Wetzel, 2001). In this case, the possibility to find this component is extended to the entire surface area because of very low depths (<1 m) of ponds.

The ponds

Sites were selected according to their bioclimatic characters and geological substrate. According to this latter aspect, they were divided into two groups: basaltic/riolitic (6, green group) and granitic (7, yellow group) ponds. The ponds are inserted in very different vegetational and landscape conditions and the areas are affected by a wide range of human impacts at different level of intensity: from that of non-disturb or almost so to that of grazing by bred and wild animals to dumps. Morphology, morphometry and origin of studied ponds were also different: from little natural depressions to man-made channels.

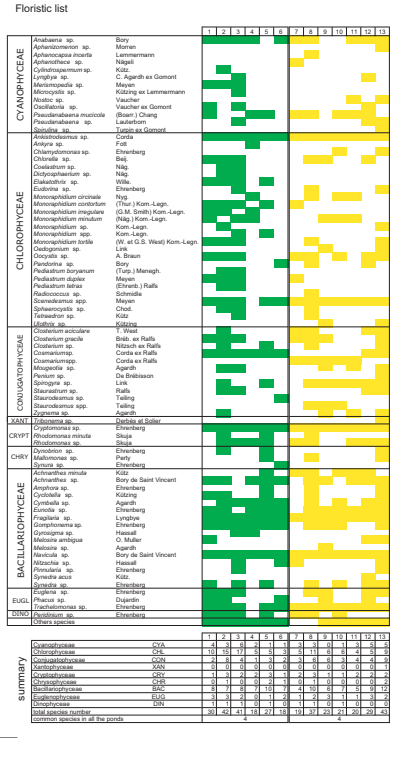
Methods

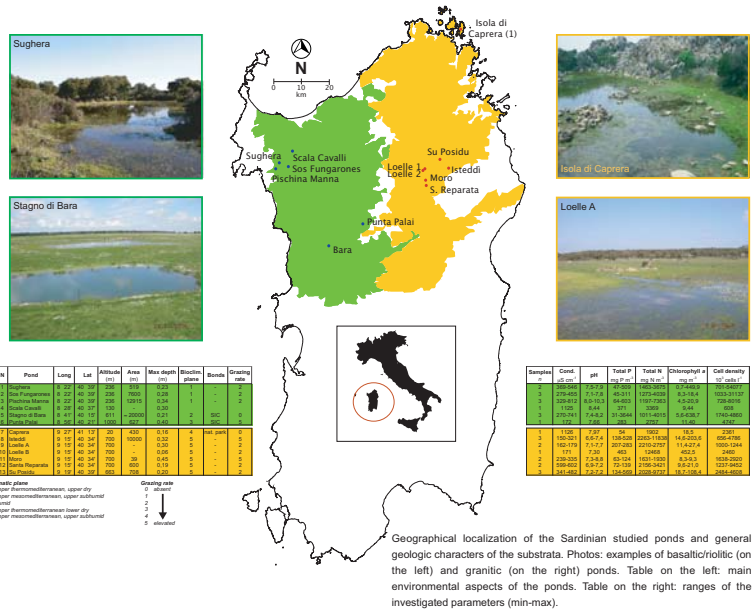
The samples were collected from the surface of the ponds monthly from April to June. Water temperature was measured using a field thermometer. A part of the collected water was fixed with Lugol's solution to study species composition and cell density of microalgae, following Utermöhl. Another part of the samples was analysed to value dissolved oxygen (Winckler method), nutrients (ammonium, nitrite and nitrate nitrogen, reported as DIN-Dissolved Inorganic Nitrogen; reactive and total phosphorus; reactive silica; according to Strickland and Parsons, 1968) and chlorophyll *a* (Golterman *et al.*, 1978). Values of conductivity and pH were also measured.
Only a part of the results are here reported.



Class percentuage composition for each temporary pond

Floristic list





	N	Pond	Long	Lat	Altitude (m)	Area (m²)	Max depth (m)	Bioclim. plane	Bonds	Grazing rate
BASALTIC-RIOLITIC	1	Sughera	E 22° 40' 39"	N 40° 39'	236	519	0.23	1	-	2
	2	Sos Fungarones	E 22° 40' 39"	N 40° 39'	236	7600	0.28	1	-	2
	3	Pischina Manna	E 22° 40' 39"	N 40° 39'	236	12915	0.34	1	-	2
	4	Scala Cavalli	E 26° 46' 37"	N 40° 37'	100	-	0.30	2	-	2
	5	Stagno di Bara	E 8° 47' 40"	N 39° 19'	611	> 20000	0.25	2	SIC	0
	6	Punta Palai	E 8° 56' 46"	N 40° 37'	1000	657	0.40	3	SIC	5
GRANITIC	7	Caprera	E 9° 27' 41"	N 41° 13'	20	430	0.15	4	not. perm.	0
	8	Isteddi	E 9° 16' 46"	N 40° 34'	700	50000	0.50	5	-	5
	9	Loelle A	E 9° 15' 46"	N 40° 34'	700	-	0.30	5	-	2
	10	Loelle B	E 9° 15' 46"	N 40° 34'	700	-	0.06	5	-	2
	11	Moro	E 9° 15' 46"	N 40° 34'	700	39	0.45	5	-	5
	12	Santa Reparata	E 9° 15' 46"	N 40° 34'	700	600	0.15	5	-	2
	13	Su Posidu	E 9° 15' 46"	N 40° 39'	660	708	0.20	5	-	2

Bioclimatic plane
1 upper thermomediterranean, upper dry
2 upper mesomediterranean, upper subhumid
3 humid
4 upper thermomediterranean lower dry
5 upper mesomediterranean, upper subhumid

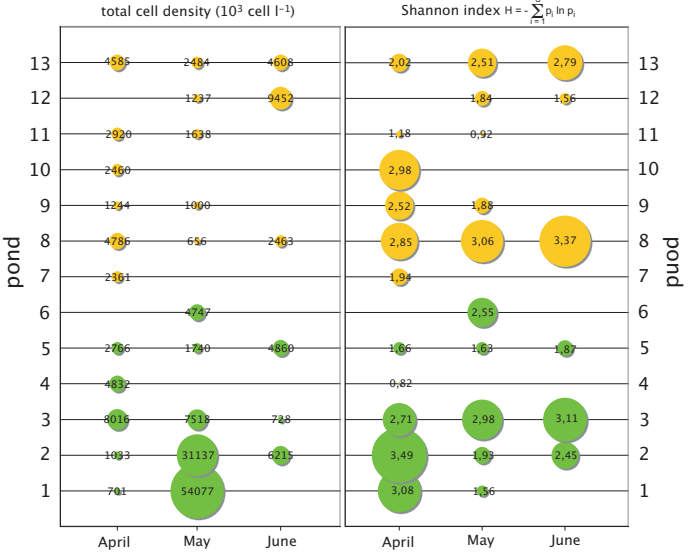
Grazing rate
0 absent
1
2
3
4
5 elevated

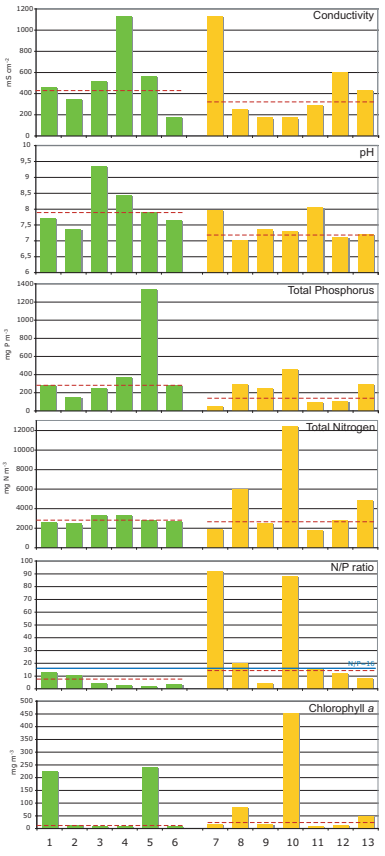
Samples n	Cond. µS cm⁻¹	pH	Total P mg P m⁻³	Total N mg N m⁻³	Chlorophyll a mg m⁻³	Cell density 10^3 cells l⁻¹
2	289-946	7.5-7.9	47-309	1463-3675	0.7-449.9	701-54077
3	279-455	7.1-7.5	45-311	1272-4538	8.3-18.4	1033-31137
3	329-812	8.0-10.3	84-603	1197-7363	4.5-20.5	728-6016
1	1126	9.44	371	3269	9.44	658
3	270-741	7.4-8.2	31-3646	1011-4515	5.6-638.7	1740-44667
1	172	7.28	283	2757	11.40	4747
1	1126	7.97	54	1902	18.5	2361
3	180-321	6.8-7.4	138-226	2253-11838	14.8-203.6	656-4786
2	162-179	7.1-7.7	207-283	2210-2757	11.4-27.4	1000-1244
1	171	7.30	463	12468	452.3	2460
2	238-335	7.3-8.8	83-124	1831-1930	8.3-9.3	1638-2920
2	599-652	6.9-7.2	72-139	2156-3421	9.6-21.3	1237-9452
3	341-492	7.2-7.7	134-509	2005-5727	18.7-108.4	2484-4608

Geographical localization of the Sardinian studied ponds and general geologic characters of the substrata. Photos: examples of basaltic/riolitic (on the left) and granitic (on the right) ponds. Table on the left: main environmental aspects of the ponds. Table on the right: ranges of the investigated parameters (min-max).

Results

The number of samplings varied from 1 (in 4 ponds) to 2 (4) to 3 (5) depending on the temporariness of the ponds. The cell densities of metaphytes varied from about 10^5 cells l^{-1} up to more than $50x10^6$ cells l^{-1}. A clear and common trend of the density dynamics was not observed. The same trend was shown by biodiversity, which ranged from 0.82 to 3.49. Cyanophyceae, Chlorophyceae and Bacillariophyceae were the main algal classes for both the expressed density values and species number. Occasionally, Cryptophyceae and Euglenophyceae were dominant. All the ponds shared only 4 taxa whereas the most of the observed species was common only in 1-2 ponds. The chlorophyll *a* ranged between <1 and >600 mg m^{-3} and pond average values were higher than 20 mg m^{-3} in 5 of the 13 ponds.
Conducibility was between 500 to 1000 µS cm^{-1} in about 60% of the samples. The general trend showed values of basaltic/riolitic group higher than those of granitic one. pH normally ranged from 7 to 8 units. It was lower in 7% of the samples (min. of 6.6) and higher in 6% (max. of 10.3), with higher median value for the basaltic/riolitic group.
Total nitrogen and phosphorus always showed high concentrations (>30 mg P m^{-3}, >1000 mg N m^{-3}), with the total nitrogen and phosphorus median values higher for the granitic group. An increasing trend in values was revealed compared to the time, probably as an effect of the reduction of water volume in the ponds.





Environmental parameters of the temporary ponds; red broken line indicates median values for the two pond groups (n=13, basaltic/riolitic group; n=14, granitic group).

Discussion

First data concerning limnology and microphytic component highlighted the absence of significant differences between the two groups of ponds by the ANOSIM and MDS analysis. This might be due to the low availability of data, especially for the temporariness of the ponds. Recent data will be a larger base for a more reliable and detailed evaluation.
High availability of nutrients, reported in many studies and consequence of the abundant presence of grazing cattle in the proximity (or even inside!) of the ponds, was confirmed (Ruggiero *et al.*, 2004). Species composition of metaphyton rapidly changed among samplings. Among the species detected, some of them are generally found in phytoplankton of Sardinian man-made lakes, but most of them seems to be typical of the ponds, as espected. The floristic list is to be considered absolutely provisional, because many species are still under determination and this lets the study open to a further evaluation of possible relationships between microphytic and macrophytic components, as well as the general limnological characteristics of the investigated ponds.

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