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Original Research Article

Consequences of eutrophication in the management of water resources in Mediterranean reservoirs: A case study of Lake Cedrino (Sardinia, Italy)



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ABSTRACT

One of the primary detrimental effects of eutrophication is the tendency of nuisance cyanobacterial species to increase in number and biomass in freshwater ecosystems. The aim of this study was to investigate possible management actions to control eutrophication and assure water use of a eutrophic deep Mediterranean climate reservoir, dominated by cyanobacteria. With this goal, we defined the trophic state of Lake Cedrino (Sardinia, Italy) and studied its phytoplankton, paying particular attention to cyanobacteria, and to seasonal variation of phytoplankton in relation to seasonal variation of environmental variables. The water samples were collected monthly from September 2010 to August 2011 at differing depths from the surface of the water to the bottom at a station located in the deeper portion of the reservoir. Physical, chemical, nutrient, qualitative and quantitative analyses of phytoplankton were performed, and the trophic state was evaluated based on the Trophic State Index and the OECD model. Abundance of nutrients and phytoplankton (cell density, biomass and chlorophyll *a*) indicated a eutrophic condition of the reservoir. In summer, phytoplankton species composition was dominated by nuisance cyanobacteria, particularly *Aphanizomenon flosaquae*, thereby requiring management plans for harmful blooms. On the base of lake features, we propose management actions at different scales and levels to resolve eutrophication and to allow water use: from nutrient load reduction in the watershed (primarily from point-sources) to deep water aeration, to immediately face an attenuation of eutrophic effects. This study is the first explorative step in planning restoration of Lake Cedrino.

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1. Introduction

Water reservoirs are man-made ecosystems of particular scientific interest, being the combination of natural phenomena and human manipulation. These ecosystems exist because of their uses and the relative goods and services that they assure to

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humans (e.g., they are sources for drinking and irrigation water, energy production, aquaculture exploitation, flood management, touristic and leisure activities). Reservoirs have rapidly increased in number and size in the last century, mainly in response to increased water demands for irrigation and energy (Jørgensen et al., 2005). Nowadays, there are more than 500,000 reservoirs in the world covering at least 0.1 km² with a highly heterogeneous geographical distribution, being more concentrated where lakes are scarce (Marcé and Armengol, 2010). In semi-arid areas, such as those of the Mediterranean climate, reservoirs represent the main source of water supply for human populations (Marcé and Armengol, 2010). In the Mediterranean climate, which dominates regions from 32° to 40° north and south of the Equator and is one of the five major climatic regions (Gulati et al., 2005), water is becoming the most limiting natural resource due to climate change. In particular, the Mediterranean basin is considered one of the most vulnerable area of the world for the impacts of warming and modification of precipitation regime (Erol and Randhir, 2012). Consequently, further water restriction is waited. While attempting to maximize economic profit from water resources exploitation, strong management conflicts are arising among the different stakeholders and institutions for management of reservoirs uses (Ribas, 2014).

Eutrophication is the most important worldwide environmental issue regarding reservoirs and many other types of aquatic ecosystems (Smith et al., 1999; Schindler, 2006), and is responsible for water quality degradation and severe restriction in water uses (Codd, 2000). In eutrophic conditions, hypolimnetic oxygen depletion can occur, due to the excessive amount of organic matter, produced by algal blooms, that cannot be completely degraded, increasing levels of toxic reduced substances, with development of bad odours and tastes (Jørgensen, 2001; von Sperling et al., 2008; Yu et al., 2016). Phytoplankton is the main primary producer in Mediterranean climate reservoirs, due to the high water level fluctuations that characterize these ecosystems (Naselli-Flores and Barone, 2005). In Mediterranean reservoirs, strong seasonal variations are found in phytoplankton growth and species composition, requiring adaptive management of water resources, especially in drinking treatment plants (Baker et al., 2006; Pahl-Wostl, 2006). Summer, in particular, is reported as the most favourable season for cyanobacteria dominance, even though their harmful blooms can also occur in autumn and winter (Mariani et al., 2015a,b).

For drinking and irrigation purposes, one of the main detrimental effects of eutrophication is the increased occurrence of harmful algal blooms (HABs), especially of cyanobacteria (Cyano-Habs) (Codd, 2000). Cyanobacteria are the most competitive and pervasive organisms among phytoplankton (Paerl and Otten, 2013). In addition to nutrient availability, their establishment is also favoured by high temperatures, intense solar radiation, a stable water column (Paerl and Huisman, 2008; Salmaso et al., 2012) and high pH (Paerl and Otten, 2013). Additional advantages are the ability to regulate their position within the water column with gas vacuoles, to overcome adverse periods by forming dormant cells (*akinetes*), to directly fix gaseous nitrogen by means of specialized cells (*heterocysts*) (Komarek and Anagnostidis, 1999, 2005).

Among the varying issues linked to cyanobacteria, the most serious is the ability of roughly forty species within ten genera to produce a wide variety of toxic compounds which seriously affect water use as well as the environment and cause human health hazards and sanitation alarms (Burch, 2008). Therefore, knowledge of the ecology of cyanobacteria is an essential step towards proper management of water resources.

Defining the environmental characteristics of reservoirs, their seasonal variations and the factors that influence phytoplankton growth and cyanobacteria proliferation are indispensable steps for planning both water resource management and, if necessary, ecological quality improvement, in compliance with the Water Framework Directive's (European Union, 2000) requirement of reservoirs being in good condition by 2027 (Poikane et al., 2014). Moreover, climate change is expected to further increase eutrophication, Cyano-HABs and their impacts worldwide, including areas at different latitudes than the Mediterranean climate, raising new challenges for their mitigation and management (Sahoo and Schladow, 2008; Paerl and Huisman, 2008). However, with the current levels of knowledge, researchers cannot comprehensively evaluate the complex interactions between eutrophication, Cyano-HABs and climate change, and filling these knowledge gaps is, nowadays, of paramount importance (Mariani et al., 2015a,b).

In this paper, we present a study carried out on Lake Cedrino, a deep Mediterranean climate reservoir located in Sardinia (Italy), used for drinking water and irrigation supplies. Since its first years of water accumulation, Lake Cedrino has exhibited very poor water quality (Padedda and Sechi, 2008). In the present work, we investigated its recent trophic state and phytoplankton abundance and composition, paying particular attention to cyanobacteria. Our hypotheses were that i) due to Mediterranean climate condition, there would be a strong seasonality in the phytoplankton of Lake Cedrino and, ii) because of the eutrophic state of the lake, cyanobacteria would dominate, especially in summer. These aspects influence water uses and require an adaptive approach in water management, particularly in the case of drinking use (Pahl-Wostl, 2006), such as in the case considered. Specifically, the main objective was to study the seasonal variation of phytoplankton in relation to seasonal variation of environmental variables to assess which of the considered environmental drivers most greatly affected phytoplankton, particularly cyanobacteria. In this way, we have been able to propose right interventions required to improve the water quality of the lake, assuring a multiple use of the resource, e.g. for drinking and agriculture. Our study represents the first experimental explorative step in planning reservoir restoration to control eutrophication since, at the moment, no actions have been taken to reduce the eutrophic process or to tackle the problem of phytoplankton and cyanobacteria growth in Lake Cedrino.

2. Study area

In Southern Italy, approximately 130 reservoirs with a volume greater than $0.5 \times 10^6 \text{ m}^3$ are present (about 75 in Sicily and Sardinia), with a total drainage capacity of about $6 \times 10^9 \text{ m}^3$. The richest region of large dams is Sardinia, the second largest island in the Mediterranean Sea, with about forty artificial lakes and a total water accumulation capacity greater than $2 \times 10^9 \text{ m}^3$.

Lake Cedrino (also called the Pedra 'e Ottoni reservoir) resulted from the damming of the Cedrino River in 1984 with a gravity dam. The reservoir is located in the eastern region of central Sardinia (Fig. 1), in the municipality of Dorgali. It covers a surface area of $1.5 \times 10^6 \text{ m}^2$ and has a volume of approximately $20 \times 10^6 \text{ m}^3$ when it is filled to maximum capacity (103 m a.s.l.), with a mean depth of 26.5 m. The reservoir has a dendritic shape and runs for roughly 8.5 km along the bed of the Cedrino River (main tributary), and for approximately 2.4 km along the Flumineddu River. The catchment covers approximately $627 \times 10^6 \text{ m}^2$ with inside of 50,000 inhabitants (Padedda and Sechi, 2008). Lake Cedrino can be considered a prototype

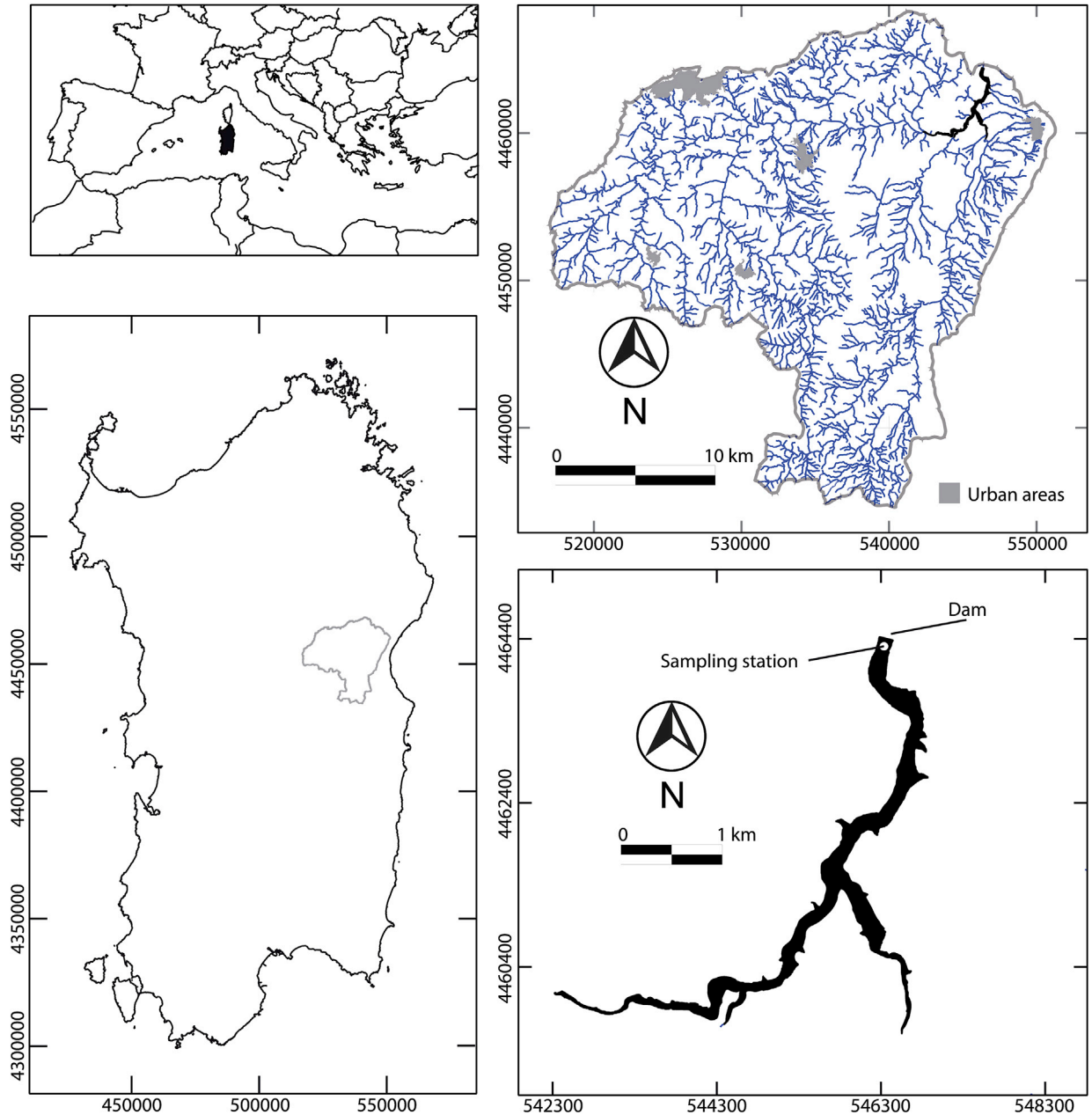


Fig. 1. Location of Lake Cedrino, of its drainage basin, dam and sampling station.

among the Sardinian reservoirs, with its main physiographic features close to the median, occupying the 52° percentile for its surface area and 62° percentile for its volume. Instead, considering its mean depth, it has an extreme position, being at the 94° percentile.

According to ECOSTAT (2004), Lake Cedrino belongs to the LM7 category (calcareous artificial lakes, altitudes <800 m a.s.l., mean depths >15 m, conductivity <2.5 mS cm⁻¹).

The lake is also a research station of the Italian, European and International Long Term Ecological Research network, with the code IT10-002-A.

3. Materials and methods

3.1. Sampling strategy and analyses

Samples were taken monthly at a station located in the deeper portion of the reservoir from September 2010 to August 2011, except for November 2011 due to adverse weather conditions. Water samples were collected at different depths (0 m, 1 m, 2.5 m, 5 m, 7.5 m, 10 m, 15 m, 20, and 30 m), using a Niskin bottle. Water temperature (Tem), conductivity (Con), dissolved oxygen (DO) and pH were measured *in situ* with a multi-parameter probe (YSI 6600 V2) while transparency was measured with a Secchi disk (SD). Euphotic zone depth was calculated using SD measures (Zeu = 2.5 times the SD depth; Poikane, 2009).

The samples were stored in a cold, dark environment for laboratory analyses of alkalinity (Alk), ammonium (NH₄), nitrate (NO₃), nitrite (NO₂), total nitrogen (TN), reactive silica (RSi), orthophosphate (RP) and total phosphorus (TP) following Strickland and Parsons (1972), and for chlorophyll *a* (Chl *a*) (Golterman et al., 1978). Dissolved inorganic nitrogen (DIN) was obtained as the sum of NH₄⁺, NO₃⁻ and NO₂⁻.

Samples for phytoplankton analysis were collected at 0 m, 1 m, 2.5 m, 5 m, 7.5 m, 10 m. Phytoplankton samples were fixed with Lugol's solution and analysed after the sedimentation of variable volumes of water (5–10 ml) depending on phytoplankton cell density, according to the Utermöhl technique (Utermöhl, 1958) using an inverted microscope (Zeiss, Axiovert 25). Cell counts were made at 200 × magnification of the entire bottom of the sedimentation chamber for the larger and more easily identifiable species, and replicated at 400 × on a selected number of fields for the smaller cells. Species were determined using several taxonomic guides as reported in Mariani et al. (2015a,b). Unidentified cells of size <5 μm were categorized to be in the dimensional group of Nanoplankton. Any other species for which precise identification was not possible due to the absence of or difficulty to observe all the necessary diagnostic characters, were grouped as Others.

Data for rainfall (Rain) was obtained from the meteorological station nearby the dam.

3.2. Data treatment

A non-metric Multidimensional Scaling (nMDS) ordination analysis was conducted to assess the differences between seasons (Sep–Nov = Autumn; Dec–Feb = Winter; Mar–Apr = Spring; May–Aug = Summer) using two different similarity matrices built from a starting set of 12 environmental variables (Tem, pH, Alk, DO, Con, NH₄, NO₃, NO₂, TN, TP, RP, RSi) and a set of 72 phytoplankton taxa identified, at minimum, to the genus level. The environmental matrix was built using a prior square root data transformation and based on Euclidean distance while the phytoplankton matrix was built using a previous log(x+1) data transformation and based on Bray–Curtis distance. The significance of differences between seasons was tested with a one way ANOSIM test; *p* values < 3% were considered significant (Clarke and Warwick, 2001). Using the same matrices, a SIMPER analysis was carried out to identify the percentage contribution of each environmental variable as well as phytoplankton taxon to the seasonal differences (Clarke and Warwick, 2001).

A multivariate analysis was performed (Ter Braak and Šmilauer, 2002; Fadda et al., 2011) to quantify the influence of environmental variables on phytoplankton species composition, taking into account the 15 species with mean abundances >5% of total density in at least one sample (Table 2). First, a detrended correspondence analysis (DCA) was conducted on the selected species, which indicated a linear distribution (with a gradient length between 2 and 3), which therefore validated the use of a direct linear methodology, such as a redundancy analysis (RDA). The dataset was composed of eight environmental variables (Rain, SD, Tem, pH, DO, NH₄, NO₃, TP) and the phytoplankton of the abovementioned selected taxa (Table 2). The data used to construct the environmental matrix and the phytoplankton matrix was obtained via a log(x+1) transformation. The significance of environmental variables explaining the variance of the selected species in the RDA was tested using Monte Carlo simulations with 499 permutations.

Statistical analyses were performed using PRIMER (for the nMDS, SIMPER and ANOSIM test) and Canoco (for RDA) software.

For identification of the trophic state of Lake Cedrino, the percentage probability of a trophic state was calculated using OECD Probability Distribution Diagrams (Vollenweider and Kerekes, 1982) and the Trophic State Index (TSI, Carlson, 1977). The OECD method evaluates the probability distribution of five trophic classes (ultra-oligotrophic, oligotrophic, mesotrophic, eutrophic or hypertrophic) considering the mean annual concentrations of TP and Chl *a*, the peak concentration of Chl *a* and the mean annual value of SD transparency. The TSI index is based on monthly data of Chl *a*, SD transparency and TP, takes into account the values from each sampling and ranges from 0 to 100. Values between 0 and 20 are considered typically ultra-

oligotrophic, between 20 and 40 oligotrophic, between 40 and 50 mesotrophic, between 50 and 70 eutrophic and over 70 hypereutrophic. TSI was calculated as TSI(CHL), TSI(TP) and TSI(SD) using the Carlson's formulae (1977), respectively:

$$\text{TSI(CHL)} = 9.81 \ln(\text{Chl } a) + 30.6 \quad (1)$$

$$\text{TSI(TP)} = 14.42 \ln(\text{TP}) + 4.15 \quad (2)$$

$$\text{TSI(SD)} = 60 - 14.41 \ln(\text{SD}) \quad (3)$$

4. Results

4.1. Environmental variables

The water temperature indicated a warm monomictic behaviour of the reservoir (Fig. 2a, Table 1), characterized by a homeothermic mixing phase, from October to February, and a phase of clear thermal stratification, from May to August. During stratification, the epilimnion extended down to approximately -7.5 m (0 – 7.5 m = surface water layer, SWL), the metalimnion between -7.5 and -10 m, and hypolimnion in the depths below, down to the bottom (minimum summer depth of 31.5 m; 7.5 m-bottom = deep water layer, DWL). The mean annual temperature was approximately 16 °C in the water column, with minimum in December (9.6 °C) and maximum in August (27.4 °C).

Conductivity was generally stable and uniform along the water column (Fig. 2b), with a mean annual value of 328 $\mu\text{S cm}^{-1}$. For pH, the mean annual value was 7.9 in the water column, with minimum values in DWL in autumn and maxima in spring in SWL (Fig. 2b, Table 1). DO, with a mean annual concentration of 75% in the water column, was variable along the water column and among the seasons (Fig. 2c, Table 1). Complete saturation was always measured in the SWL, with events of oversaturation in spring. Conversely, under saturation values were observed in the DWL in summer. Average annual alkalinity was 2.31 meq l^{-1} , with small seasonal variability and along the water column (Fig. 2d, Table 1).

Annual average TN was of 1384 mg N m^{-3} in the water column. Lowest values were in spring and highest in January, during the mixing (Fig. 2d, Table 1). It increased from surface to bottom in all months. 59% of the TN mean annual value was composed of DIN, which, in turn, was mainly due to NO_3 (70%), followed by NH_4 (28%) and NO_2 (2%).

NH_4 concentration (Fig. 2e), with an annual mean of 208 mg N m^{-3} in the water column, was higher in the DWL than in the SWL, especially during summer stratification. In the SWL, NO_3 concentration increased from September to January and decreased from February to the summer months (Fig. 2f). In the DWL, NO_3 concentration was related more to those of DO concentration and, consequently, it was extremely low in summer and autumn, when DO decreased and NH_4 increased. The NO_2 concentration was mainly under 20 mg N m^{-3} (Table 1).

Annual average TP was 99 mg P m^{-3} , with values higher in the DWL than in the SWL (Fig. 2g, Table 1). Lowest concentrations occurred in spring whereas highest were in summer. The annual mean value of RP was 47 mg P m^{-3} , with higher values in the DWL than in the SWL (Fig. 2g, Table 1), similar to TP. RP was more than 50% of TP in the DWL.

For RSi, the mean annual value was 3.8 mg Si l^{-1} , with higher values in the DWL than in the SWL (Table 1).

4.2. Chlorophyll a and phytoplankton assemblages

Annual average Chl *a* in the euphotic zone was 12 mg m^{-3} , with lower values in autumn and winter and considerably higher values in summer and spring (Fig. 3). Annual average SD transparency was approximately 2 m. Maxima of Chl *a* coincided with minima of SD transparency in summer and autumn months (Fig. 3, Table 1).

83 taxa of phytoplankton belonging to 7 classes (Cyanobacteria, Dinophyceae, Bacillariophyceae, Chlorophyceae, Cryptophyceae, Chrysophyceae and Euglenophyceae) were identified. For the phytoplankton of Lake Cedrino, the highest number of species (27 taxa) was observed within the Chlorophyceae class, followed by cyanobacteria (15 taxa) and Bacillariophyceae (13 taxa) (Table 2). Further, the number of species were highest in June (28), August (22) and September (27) and lowest in April (15) and October (16).

During the study, phytoplankton abundance, measured using cell density, had two peaks: a major peak in September (mean value in the euphotic zone of 2.3×10^8 cells l^{-1}) and the second in August (1.4×10^8 cells l^{-1}) (Fig. 3). A third lesser important peak was observed from March to April (1.5×10^6 cells l^{-1} and 4.1×10^6 cells l^{-1} , respectively) (Fig. 3).

Highest densities were concomitant with cyanobacteria dominance in summer-autumn (from September to October and from July to August). Cyanobacteria growth was mainly due to *Dolichospermum planctonicum* (Brunnthal) Wacklin, Hoffmann and Komarek, *Microcystis aeruginosa* (Kützing) Kützing and, especially, *Aphanizomenon flosaquae* (L.) Ralfs ex Bornet & Flahault, all potentially toxic species (Table 2). From winter to spring, the phytoplankton varied abundantly, with moderate dominance of different classes, rapidly replacing one with the other (Fig. 4): Cryptophyceae presence was especially due to *Plagioselmis lacustris* (Pascher & Ruttner) Javorn growth in winter, Dinophyceae by *Peridiniopsis* sp. in early spring, Bacillariophyceae by *Stephanodiscus hantzschii* Grunow and *Cyclotella* spp. and Chlorophyceae by *Coelastrum reticulatum* (P.A. Dang.) Senn, *Sphaerocystis* spp. and *Oocystis* spp. in late spring.

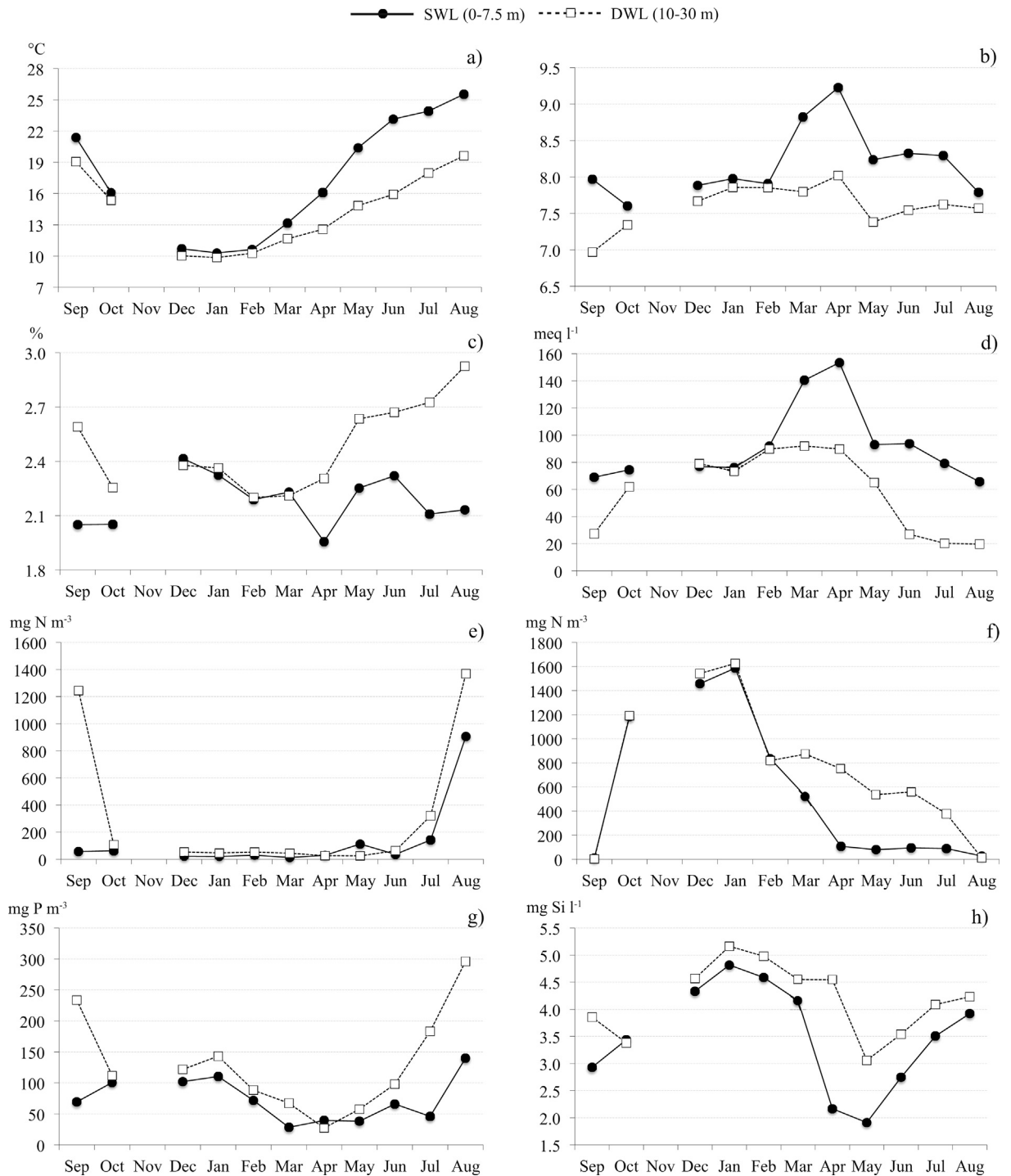


Fig. 2. Trends of temperature (a), pH (b), dissolved oxygen (c), alkalinity (d), ammonium (e), nitrate (f), total phosphorus (g) and reactive silica (h) in the surface water layer (0–7.5 m, SWL) and deep water layer (10–30 m, DWL) of Lake Cedrino.

4.3. Temporal data ordination and correlation between environmental variables and phytoplankton

Large seasonal differences for both environmental variables (Fig. 5a) and phytoplankton abundances and species composition (Fig. 5b) were identified (nMDS analysis). The nMDS data ordination revealed that samples of winter months were grouped with spring samples, while summer samples were grouped with autumn samples. This similarity among seasons was more evident for phytoplankton species ordination than for the environmental variables. Accordingly, the

Table 1

Monthly and annual mean values of main variables in the surface water layer (0–7.5 m, SWL) and deep water layer (10–30 m, DWL) of Lake Cedrino. Rain is reported as cumulate values.

Variable	Layer	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Mean
Rain (mm)		24.8	257.2	83	22.6	61.2	123.6	111.2	102.2	5.6	20.6	0.4	0	67.7
SD (m)		1.5	1.2	–	2.5	3	1.1	2.0	1.7	3.0	2.3	1.7	1.5	2.0
Tem (°C)	SWL	21.4	16.1	–	10.7	10.3	10.6	13.2	16.1	20.4	23.2	23.9	25.5	17.4
	DWL	19.1	15.3	–	10.0	9.9	10.3	11.7	12.6	14.9	15.9	18.0	19.6	14.3
pH	SWL	8.0	7.6	–	7.9	8.0	7.9	8.8	9.2	8.2	8.3	8.3	7.8	8.2
	DWL	7.0	7.3	–	7.7	7.9	7.9	7.8	8.0	7.4	7.5	7.6	7.6	7.6
DO (%)	SWL	69.1	74.5	–	76.9	76.1	92.0	140.4	153.4	93.0	93.6	79.1	65.7	92.2
	DWL	27.4	62.0	–	79.0	73.4	89.8	92.0	89.8	65.0	27.0	20.3	19.7	58.7
Alk (meq l ⁻¹)	SWL	2.1	2.1	–	2.4	2.3	2.2	2.2	2.0	2.3	2.3	2.1	2.1	2.2
	DWL	2.6	2.3	–	2.4	2.4	2.2	2.2	2.3	2.6	2.7	2.7	2.9	2.5
NH ₄ (mg N m ⁻³)	SWL	57	62	–	23	21	30	13	29	113	36	142	907	130.1
	DWL	1244	107	–	54	46	53	45	26	25	63	320	1370	304.7
NO ₃ (mg N m ⁻³)	SWL	9	1184	–	1457	1586	835	522	107	80	94	89	27	544.5
	DWL	4	1192	–	1541	1625	819	873	752	536	560	378	13	753.8
NO ₂ (mg N m ⁻³)	SWL	4	28	–	31.4	21	27	14	16	5	5	12	5	15.3
	DWL	3	30	–	31.0	33	31	19	18	15	10	38	1	20.8
TN (mg N m ⁻³)	SWL	1305	1096	–	1762	1861	1816	939	727	682	681	1192	1708	1305
	DWL	1483	1507	–	1947	2026	1883	1307	1113	880	936	1168	1514	1483
RP (mg P m ⁻³)	SWL	1	59	–	66	77	42	3	1	13	11	9	4	26.1
	DWL	128	59	–	83	93	47	36	6	39	60	140	122	73.7
TP (mg P m ⁻³)	SWL	69	101	–	102.0	110	72	28	39	38	66	46	140	73.8
	DWL	234	112	–	121.8	143	88	67	27	58	98	183	296	129.8
RSi (mg Si l ⁻¹)	SWL	2.9	3.4	–	4.3	4.8	4.6	4.2	2.2	1.9	2.7	3.5	3.9	3.5
	DWL	3.9	3.4	–	4.6	5.2	5.0	4.6	4.5	3.1	3.5	4.1	4.2	4.2

ANOSIM test revealed significant differences among seasons considering both the environmental (Global R = 0.457 p = 0.1%) and phytoplankton (Global R = 0.505 p = 0.1%) data sets.

NO₃ (19% of dissimilarity), RP (13%), NH₄ (13%), NO₂ (10%) and TP (6%) mainly explained the seasonal differences among environmental variables and *A. flosoquae* (>30%), *Aphanocapsa* sp. (13%), *Coelosphaerium* sp. and *Merismopedia* sp. (<10%) among phytoplankton species (SIMPER analysis).

The first two axes of the RDA accounted for 68.94% of the total variance of phytoplankton species and environmental data. The first axis accounted for 41.1% and the second for 26.4% of the total variance. A Monte Carlo test showed that all canonical axes were significant (p < 0.0001). The correlation between the eight environmental variables and the 14 selected phytoplankton species was 0.970 for the first axis and 0.998 for the second.

Flagellate and non-flagellate colonial taxa, which positioned on the left section of the first axis (with the only exception of *Rhodomonas* sp.), were well-differentiated from a second group of unicellular taxa, both flagellate and non-flagellate, which positioned on the right part of the same axis (Fig. 6). Colonial forms comprised all the selected Cyanobacteria species and one species of Chlorophyceae (*Sphaerocystis* spp.). Cyanobacteria, *A. flosoquae*, *Aphanothece* sp., *Chroococcales* unidentified and *Coelosphaerium* sp. were mainly correlated with Tem, TP, and NH₄. Notably, these species developed in summer and autumn months, when the reservoir was thermal stratified, Tem was high and TP and NH₄ were strongly higher than in other seasons (Fig. 2e and g). The outlying position of *Merismopedia* sp. and two colonial forms of Chlorophyceae, *C. reticulatum* and *Oocystis* spp., was consistent with their establishment for very short periods (June). The second group, positioned on the right section of the first axis, was more heterogeneous, with species belonging to Dinophyceae (*Peridiniopsis* sp.), Cryptophyceae (*Cryptomonas* sp., *P. lacustris*), Bacillariophyceae (*Cyclotella* spp., *Cyclotella atomus*) and Chlorophyceae (*Chlamydomonas* spp.). Among these species, *Peridiniopsis* sp., *Cyclotella* spp. and *C. atomus* correlated with DO, NO₃, pH and Rain. All these species established in winter and spring months, when mixing and intense watershed discharge occurred, the water was well oxygenated and concentrations of NO₃ were increased higher than in other seasons.

4.4. Trophic state

Trophic state evaluation according to OECD Probability Distribution Diagrams, indicated a probability >79.6% that Lake Cedrino would express eutrophic-hypereutrophic conditions and about a 20.4% probability for oligotrophic-mesotrophic conditions (Table 3). TSI values were 70.4 for TSI(TP), 49.6 for TSI(CHL) and 55.1 for TSI(SD), clearly indicating a eutrophic-hypereutrophic condition.

5. Discussion

5.1. Trophic state

Results obtained from applying the OECD probabilistic model clearly indicated a higher probability for Lake Cedrino to be eutrophic and hypereutrophic than mesotrophic and oligotrophic.

Table 2

Phytoplankton floristic list. Species abbreviations are reported only for those used in the RDA analysis (mean abundance >5% of total density in, at least, one sampling). Nuisance abbreviations: T = Toxic; T&O=Taste and Odour; D/S = Discoloration or Scum; P = Treatment Plant drawbacks.

Class	Species	Abbreviation	Nuisance	References
Other	Other			
BAC	<i>Achnanthes</i> sp.		P	Sechi and Lugliè, 1992
	<i>Amphora</i> sp.			
	<i>Aulacoseira ambigua</i>		P	Sechi and Lugliè, 1992
	<i>Aulacoseira distans</i>		P	Sechi and Lugliè, 1992
	<i>Aulacoseira granulata</i>		P	Sechi and Lugliè, 1992
	<i>Aulacoseira granulata</i> var. <i>angustissima</i>		P	Sechi and Lugliè, 1992
	<i>Cyclotella atomus</i>	Cyc ato		
	<i>Cyclotella</i> spp.	Cyc spp		
	<i>Fragilaria</i> sp.		P	Sechi and Lugliè, 1992
	<i>Navicula</i> spp.			
	<i>Nitzschia</i> spp.			
	Pennales unidentified			
	<i>Stephanodiscus hantzschii</i>			
	<i>Synedra</i> sp.			
CHL	<i>Ankyra</i> sp.			
	<i>Botryococcus</i> sp.			
	<i>Carteria</i> sp.			
	<i>Chlamydomonas</i> spp.	Chl spp		
	Chlorococcales unidentified			
	<i>Coelastrum</i> cf. <i>astroideum</i>			
	<i>Coelastrum</i> cf. <i>microporum</i>			
	<i>Coelastrum reticulatum</i>	Coe ret		
	<i>Crucigeniella</i> sp.			
	<i>Dictyosphaerium</i> sp.		P + D/S	Sechi and Lugliè, 1992; Paerl et al., 2001
	<i>Elakatothrix</i> sp.			
	<i>Lagerheimia</i> sp.			
	<i>Monoraphidium minutum</i>			
	<i>Monoraphidium</i> spp.			
	<i>Oocystis</i> spp.	Ooc spp	P	Sechi and Lugliè, 1992
	<i>Pandorina morum</i>		T&O	Terrel and Perfetti, 1989
	<i>Pediastrum duplex</i>			
	<i>Pediastrum simplex</i>			
	<i>Pediastrum</i> sp.			
	<i>Scenedesmus disciformis</i>		P + D/S	Sechi and Lugliè, 1992; Paerl et al., 2001
	<i>Scenedesmus obtusus</i>		P + D/S	Sechi and Lugliè, 1992; Paerl et al., 2001
	<i>Scenedesmus</i> spp.		P + D/S	Sechi and Lugliè, 1992; Paerl et al., 2001
	<i>Schroederia</i> sp.			
	<i>Sphaerocystis planctonica</i>			
	<i>Sphaerocystis</i> spp.	Sph spp	D/S	Paerl et al., 2001
	<i>Tetraedron minimum</i>			
	Volovocales unidentified			
CHR	<i>Mallomonas akrokomos</i>			
	<i>Mallomonas</i> spp.			
CON	<i>Closterium gracile</i>			
	<i>Cosmarium</i> spp.			
	<i>Staurastrum gracile</i>			
	<i>Staurastrum</i> sp.			
CRY	<i>Cryptomonas</i> spp.	Cry spp	D/S	Paerl et al., 2001
	<i>Plagioselmis lacustris</i>	Pla lac		
	<i>Rhodomonas</i> sp.	Rho sp.	D/S	Paerl et al., 2001
CYA	<i>Dolichospermum flosaquae</i>		P + T	Terrel and Perfetti, 1989; Sechi and Lugliè, 1992; Paerl et al., 2001
	<i>Dolichospermum planctonicum</i>			
	<i>Dolichospermum</i> sp.			
	<i>Aphanizomenon flosaquae</i>	Aph flo	T + T&O	Terrel and Perfetti, 1989
	<i>Aphanocapsa</i> spp.		T	Paerl et al., 2001
	<i>Aphanothece</i> sp.	Apn sp.		
	Chroococcales unidentified	Chr uni		
	<i>Chroococcus</i> sp.			
	<i>Coelosphaerium</i> sp.	Coe sp.	T	Cronberg and Annadotter, 2006
	<i>Cyanobium</i> sp.		T	Cronberg and Annadotter, 2006
	<i>Merismopedia</i> sp.	Mer sp.		

Table 2 (continued)

Class	Species	Abbreviation	Nuisance	References
	<i>Microcystis aeruginosa</i>		P + T	Sechi and Lugliè, 1992; Paerl et al., 2001
	<i>Microcystis flosaquae</i>		P + T	Sechi and Lugliè, 1992; Paerl et al., 2001
	<i>Microcystis</i> spp.		P + T	Sechi and Lugliè, 1992; Paerl et al., 2001
	<i>Snowella</i> sp.		T	Sechi and Lugliè, 1992; Paerl et al., 2001
	<i>Woronichinia</i> sp.			
DIN	<i>Ceratium hirundinella</i>		T&O	Terrel and Perfetti, 1989
	Dinophyceae unidentified			
	<i>Peridiniopsis</i> sp.	Per sp.		
EUG	Euglenophyceae unidentified			
	<i>Trachelomonas</i> sp.			

According with Carlson (1977), TSI trophic classification labels Lake Cedrino as hypereutrophic, with TSI(CHL) and TSI(SD) values closer together and lower than TSI(TP) ($TSI(TP) > TSI(CHL) = TSI(SD)$). Possible interpretations of this finding are that, although phytoplankton dominates light attenuation, some factors such as nitrogen limitation, zooplankton grazing or toxics limit algal biomass (Carlson, 1983). Unfortunately, no studies have been carried out on zooplankton and toxics in Lake Cedrino. In any case, we can affirm that phytoplankton of Lake Cedrino were mainly P limited during the study period, if we consider the ratio of TN to TP of 9:1 (Salas and Martino, 1991). In fact, TN/TP values were always >9, with the only exceptions in September 2010 (9) and August 2011 (8). Similar results were by Vollenweider and Kerekes (1982) with a more restrictive ratio > 17:1.

Consequently, we consider the reduction of nutrient loads from catchment to be mandatory in order to manage the eutrophication of Lake Cedrino. Successful eutrophication management and control is based primarily on the restriction of nutrients in the water column, accomplished by a wide variety of external and internal controls (Cooke, 2005). In the case of Lake Cedrino, on the basis of a catchment-based approach, Padedda et al. (2015) identified and estimated the theoretical external nutrient loads which originate in the drainage basin of the reservoir and which were high enough to cause eutrophication. Further, Padedda et al. (2015) also evaluated the trophic state variations related to the theoretical manipulation of nutrient inputs. The managing of wastewater treatment plants (WWTP) is the main problem to be addressed for a reduction of nutrient loadings into Lake Cedrino. In fact, WWTP of small towns (up to 10,000 inhabitants) are in most cases obsolete and without the third purification stage (ATO, 2002), unlike that of the largest city (Nuoro, approximately 40,000 inhabitants) which has a modern WWTP. Unfortunately, it is not fully operation, currently treating not more than 50% of urban wastewater (ISTAT, 2017).

Further reductions in P loading can be achieved by developing recovery and recycling strategies in agricultural plans, such as reducing the use of P-containing fertilizers and the mineralization of livestock manure in soils, thereby avoiding direct discharge into rivers (Roy, 2017). Other strategies can exploit the construction of ecological filters for organic constituents. For example, interceptor basins or ponds can reduce the rate and quantity of agricultural nutrient inflow. Phytodepuration systems directly on or near the river bed can remove nutrient pollutants with the combined effect of plant uptake (Vymazal, 1995) and rhizosphere denitrification (Lee et al., 2009). Phytoremediation is a cost-effective, and environmentally friendly technology for water quality restoration (Salt et al., 1995).

However, the reduction of external nutrient loadings alone do not necessarily result in immediate water quality improvement (Carvalho et al., 1995). In fact, the internal sources of nutrients that have accumulated in the sediments through time, can cause possible delays in a lake's response time to external reductions in P load (Jeppesen et al., 2005) and even if the removal of phosphorus-rich sediments results in a more effective response and recovery, this approach must be carefully considered due to the shallowness of the lake and issues concerning sediment re-suspension (Eggleton and Thomas, 2004). Conversely, symptomatic approaches to reduce the effects of eutrophication can be adopted. Among those, water aeration to reduce hypolimnetic anoxia conditions can improve water quality for potabilization and, concurrently, act on the water–sediment interface, influencing P-release from sediments (Beutel and Horne, 1999; Ma et al., 2015). This procedure requires careful modulation of the aeration of hypolimnetic water, avoiding summer thermal stratification disruption which, in turn, favours Cyanobacteria proliferation (Lugliè and Sechi, 1993). Other methods comprise hydraulic flushing (including hypolimnetic siphoning) that can remove both nutrients and algal biomass through forced flushing (Hickey and Gibbs, 2009). However, flushing requires other sources of pristine water, currently not available for this lake, as well as for the majority of Mediterranean climate reservoirs. In addition, impacts on the downstream environment need to be considered. Chemical treatments that use flocculation and sediment capping are helpful to reduce nutrient availability for the growth of algae (Hickey and Gibbs, 2009). Alongside traditional use of sand, gravel, clay, allophane and aluminium sulfate, new semi-natural products, such as modified zeolites and the La-modified bentonite clay Phoslock™, are taking place. Particularly interesting results were obtained in a recent study for Phoslock treatments, with significant reductions of TP, chlorophyll *a* and increased Secchi disk depth (Spears et al., 2016). These responses were most pronounced in summer, just the most critical period for Lake Cedrino. Anyway, these methods should be regarded as short-to medium-term management strategy, as the capping layers will become buried with fresh material from the catchment after few years and repeat treatment is needed. Further,

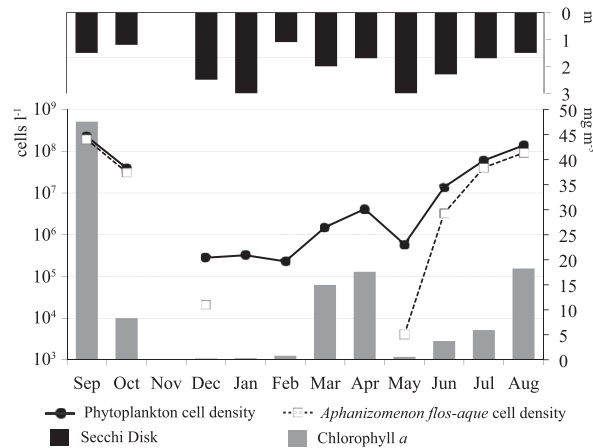


Fig. 3. Total phytoplankton and *Aphanizomenon flosaque* cell densities (left axis), chlorophyll *a* and Secchi disk transparency (right axis) values in Lake Cedrino.

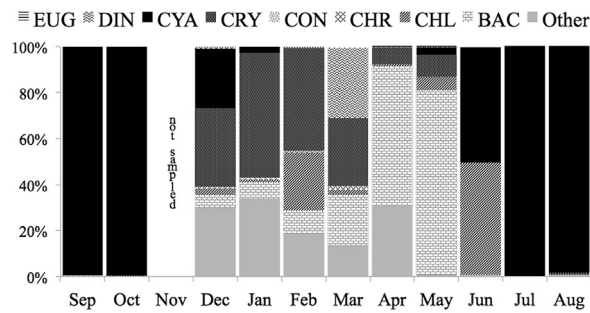


Fig. 4. Phytoplankton percentage class composition. EUG-Euglenophyceae, DIN-Dinophyceae, CYA-Cyanobacteria, BAC-Bacillariophyceae, CHL-Chlorophyceae, CRY-Cryptophyceae, CHR-Chrysophyceae.

management decisions concerning the use of chemicals for the remediation can have high implementation and monitoring costs, which warrant a critical assessment process (Hickey and Gibbs, 2009). Biomanipulation can be also considered among methods for the trophic state control (Tuzun and Mason, 2009). It acts as a top-down control of algal biomass through the trophic pyramid. Most approaches have focused on the removal of zooplanktivorous fish to stimulate zooplankton populations in order to increase grazing on phytoplankton. Effects of biomanipulation are strongly dependent upon many factors (chemical, physical and grazer-related) that modify the impact that grazers have on phytoplankton biomass. For example, as find by McQueen (1974), successful fish manipulations may only be effective when chemical and physical factors are altered to produce an algal species composition (non blue-green algae or Cyanobacteria) that permits strong top-down control of prey by predators or large-bodied grazers. In the study case, Cyanobacteria dominated phytoplankton for long time in the annual cycle (from late spring to late autumn) and data on zooplankton and fish are not available. In addition, size and depth of the lake do not assure good results using this method.

5.2. Phytoplankton

Data collected in this study clearly indicate that summer is the most important period for water quality in Lake Cedrino, similar to other reservoirs in the Mediterranean climate (Coppens et al., 2016). During summer season, fresh water inflow is reduced, the water column is thermally stratified and high temperatures and intense solar radiation allow phytoplankton proliferations, resulting in massive blooms. The excessive presence of phytoplankton in the euphotic zone coinciding with hypolimnetic anoxia conditions, and large amounts of reduced compounds (e.g., ammonia), can make it difficult to adequately purify the water for drinking purposes.

Abundance and species composition of Lake Cedrino's phytoplankton were considerably diverse in summer and autumn months compared to in the winter and spring (RDA results). Differences occurred in terms of taxonomic composition (cyanobacterial species in summer-autumn and species of dinoflagellates, cryptophytes, diatoms and chlorophytes in winter–spring) and thallus organization (colonial forms vs unicellular forms). These differences imply that the potabilization plant for processing water from Lake Cedrino should work with different protocols during the year, adequately to remove different assemblages of algae cells and their metabolites. It is very important because of the high density levels attained by

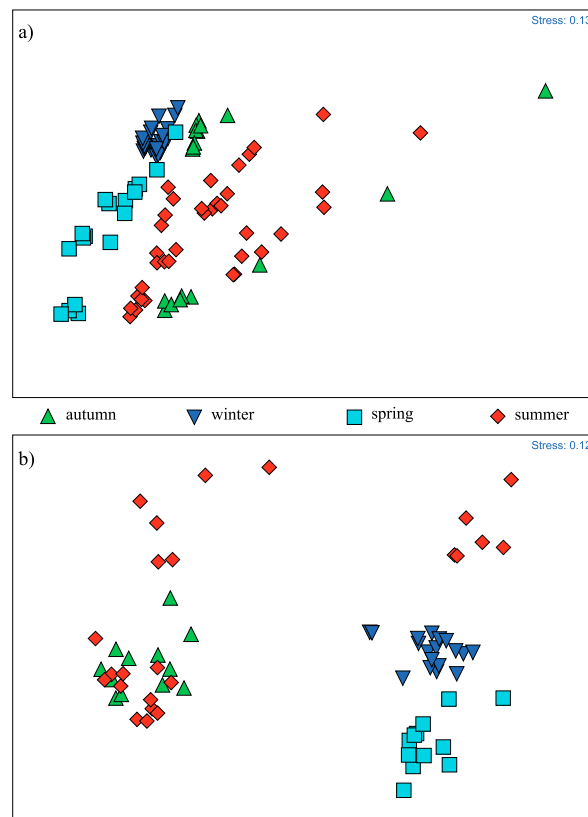


Fig. 5. nMDS ordination for environmental variables (a) and phytoplankton species (b) by season.

phytoplankton and the presence of potentially toxic species amongst Cyanobacteria (Table 2). Further, a number of species found in Lake Cedrino are unaffected in the coagulation–flocculation–sedimentation steps in drinking water treatment and their presence embues taste and odours to the water (Table 2).

While Cyanobacteria were abundant for the majority of the studied period, they strongly dominated the phytoplankton of Lake Cedrino in summer. The environmental degradation caused by the eutrophic conditions that are associated with the dominance of Cyanobacteria over less tolerant algal species, is well documented in many Mediterranean climate lakes (Barone et al., 2009; Carrasco et al., 2006; Mariani et al., 2015a,b; Moustaka-Gouni et al., 2006). In our case, blooms were almost monospecific and dominated by the potentially harmful Cyanobacteria *A. flosaquae*.

Many countries have developed programs to monitor Cyanobacteria blooms (Carmichael and Falconer, 1993). For example, Australian water authorities and U.S. Environmental Protection Agency have listed Cyanobacteria and their toxins as priority drinking water contaminants in terms of occurrence as well as health effects, water treatments methods and analytical research, since the late nineties. As a result of incidents attributed to toxic Cyanobacteria blooms, the World Health Organisation (WHO) and several national authorities around the world recommended that risk assessment plans and safety levels include Cyanobacteria as a parameter that must be monitored (Codd et al., 2005). However, in Italy, there is only a national limit for cyanotoxins and Cyanobacteria abundance in bathing water, and no limit has been established for cyanotoxins in drinking water (Messineo et al., 2009). The provisional WHO guideline of $1 \mu\text{g l}^{-1}$ microcystin-LR is used if local authorities suspect a risk to human health (Burch, 2008).

5.3. *Aphanizomenon flosaquae*

The genus *Aphanizomenon* belongs to the order Nostocales and includes 15 well known species. Most of these species are common in temperate regions, while few have a limited distribution in tropical regions. *Aphanizomenon* species were linked to PSP saxitoxin production in Greece (Gkelis and Zaoutos, 2014) and in Portugal (Ferreira et al., 2001; Pereira et al., 2000), anatoxin-a production in Finland and Germany (Sivonen and Jones, 1999) and cylindrospermopsin production in Poland (Mankiewicz-Boczec et al., 2012) and Germany (Preußel et al., 2006). Production of microcystin by *Aphanizomenon* species has not been conclusively demonstrated (Maatouk et al., 2002; Mooney et al., 2011).

A. flosaquae is a fairly common N-fixing cyanobacterial species in eutrophic lakes and is native to temperate zones. It is becoming increasingly common in the Mediterranean climate (Carrasco et al., 2006; Gkelis et al., 2014; Cirés and Ballot, 2016).

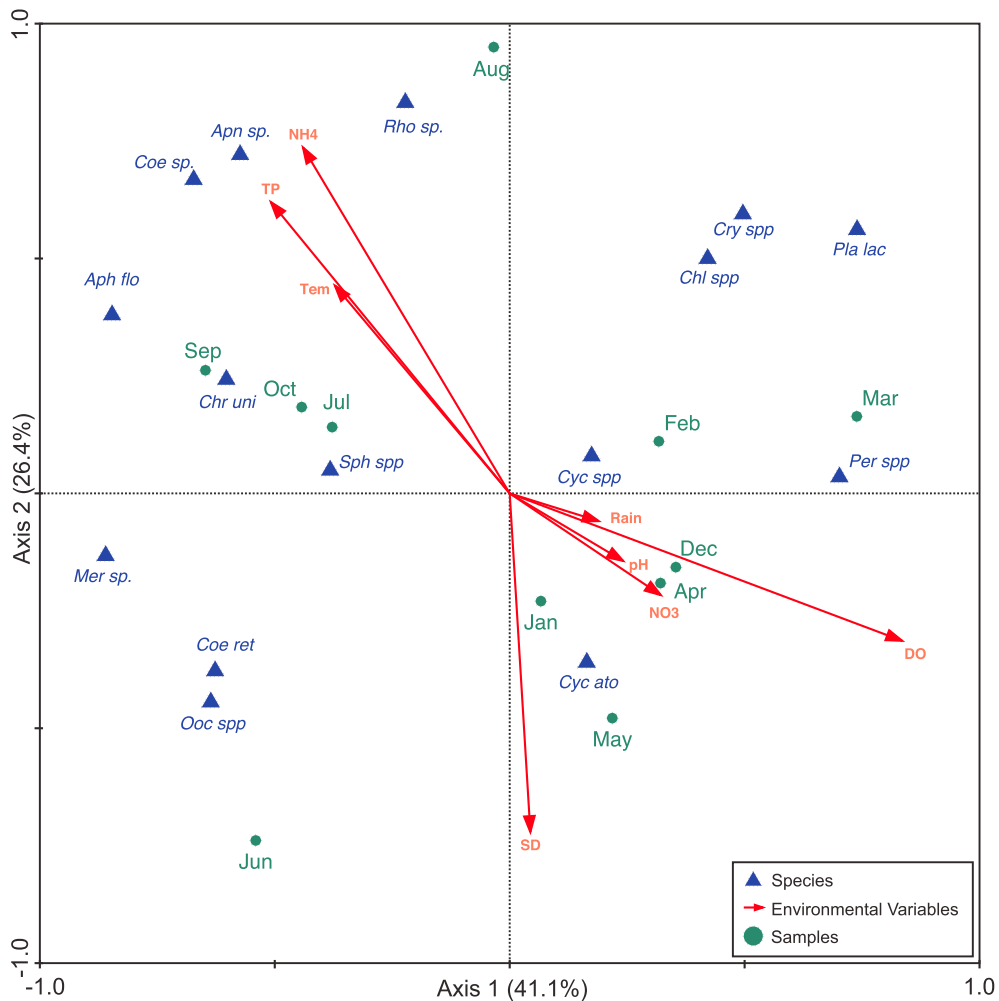


Fig. 6. Redundancy analysis (RDA) of main phytoplankton species, samplings and controlling environmental variables. (*Aph flo* = *Aphanizomenon flosaquae*; *Aprn sp.* = *Aphanothece* sp.; *Chl spp* = *Chlamydomonas* spp.; *Chr uni* = Chroococcales unidentified; *Coe ret* = *Coelastrum reticulatum*; *Coe sp.* = *Coelosphaerium* sp.; *Cry spp* = *Cryptomonas* spp.; *Cyc ato* = *Cyclotella atomus*; *Cyc spp* = *Cyclotella* spp.; *Mer sp.* = *Merismopedia* sp.; *Ooc spp* = *Oocystis* spp.; *Per spp.* = *Peridiniopsis* sp.; *Pla lac* = *Plagioselmis lacustris*; *Rho sp.* = *Rhodomonas* sp.; *Sph spp* = *Sphaerocystis* spp.).

Table 3
Results of OECD trophic classification (in bold maximum values).

	Categories	TP	Chl <i>a</i>	SD	average
		98.7 mg P m ⁻³	12.1 µg l ⁻¹	2.0 m	%
OECD Model	hypereutrophy	65%	8%	30%	34.3%
	eutrophy	23%	58%	55%	45.3%
	mesotrophy	12%	32%	15%	19.7%
	oligotrophy	0%	2%	0%	0.7%
	ultra-oligotrophy	0%	0%	0%	0%

This species survives the winter in the sediments as *akinetes*, while the blooms occur mostly from summer and early autumn, under stratification conditions similar to our observations in Lake Cedrino. In fact, temperature is considered the main controlling factor for species development: values < 14–11 °C are reported as a growth inhibitor (Yamamoto and Nakahara, 2005), while values > 28 °C double the rate of cell duplication (Ferreira et al., 2001). In Lake Cedrino, summer temperatures were over 25 °C during the study period. However, *A. flosaquae* has a remarkable ecophysiological plasticity that allows the species to achieve successful growth in either cold and low light or warm and high light environments (Üveges et al., 2012). The photoperiod seems to have an inhibitory effect even if the energy levels of solar radiation are high. Growth is limited on

day/night cycles <10:14 (Yamamoto and Nakahara, 2005). In Lake Cedrino, the species showed most development from June to October when the day length ranged from 11 to 16 h, suggesting the probable importance of this variable together with temperature to determine the seasonality of the species (Yamamoto and Nakahara, 2005). In addition to temperature and light, the most important environmental factors that control *A. flosaquae* development are pH and nutrients (Paerl et al., 2001). pH strongly affects *A. flosaquae* and values < 7.2 completely inhibit its growth because of its preference for HCO₃ rather than CO₂ as a carbon source (Yamamoto and Nakahara, 2005). In Lake Cedrino the species showed most development with pH values from 7.5 in June to 8.0 in October.

Regarding nutrients, *A. flosaquae* blooms are promoted by high availability of N and P. Further, the N/P ratio also has an important role in *A. flosaquae* establishment in mesotrophic lakes, similar to other N-fixing species (Cottingham et al., 2015). In fact, N-fixing cyanobacteria often dominate when the N/P ratio is between 10 and 16, as reported in the literature (Yamamoto and Nakahara, 2005). In Lake Cedrino, the N/P ratio fell within this range from July to October 2010 and from May to August 2011, and was higher in winter and spring months (from December to April). High values of the N/P ratio were due to high NO₃ concentrations in the water column in the lake. NO₃ originated by the intense autumn and early spring watershed discharges in connection to rainfall seasonality (Table 1). Excluding December, *A. flosaquae* observations coincided precisely to the periods with N/P < 16, confirming previous observations on its ecological preference and the potential role of N-fixing Cyanobacteria on nutrient cycling (Cottingham et al., 2015).

The average duplication time of *A. flosaquae* varies from few hours to several days (Istituto Superiore di Sanità, 2000). Under optimal conditions, the cells duplicate so rapidly that a bloom can occur in two days and may persist for five–seven days or longer. Therefore blooms can occur relatively suddenly, exacerbating the necessity of adaptive procedures at the potabilization plants. Further, blooms may be macroscopic and therefore visible with naked eye, as occurred in Lake Cedrino in both 2010 and 2011. Indeed, *A. flosaquae* developed abundantly and gave rise to the formation of clusters more or less strictly localized at the surface of the water body. *A. flosaquae* shows a filamentous organization of the thallus (tricome). Tricomes can come together in bundles. The cells are wrapped in pouches made of gelatinous mucilage secreted by the cell itself (Dean and Sigeo, 2006). These characteristics result in considerable difficulties during the potabilization treatment of raw water because the colonies can reach considerable sizes and because the presence of mucilage prevents flocculation. In our case, data from the potabilization plant that processes raw water from Lake Cedrino indicate a total removal of cells from the produced drinking water (data not shown). This result is obtained due to the ability to choose the intake depth of raw water by a modifiable system, avoiding the depths with highest cell densities. This approach requires the continuous monitoring of phytoplankton abundance and species composition along the vertical profile, particularly for potential toxic cyanobacterial species. In addition, one possible reduction strategy of phytoplankton entering in the potabilization plant could be the mixing of lake water with pristine groundwater. The use of groundwater reduces problems caused by the presence of toxic cyanobacterial blooms diluting water upon entry and during the treatment process (Jurczak et al., 2005).

Some strains of *A. flosaquae* produce neurotoxins, such as saxitoxin, neo-saxitoxin and anatoxin-a, and hepatotoxins, such as cylindrospermopsin (Sivonen and Jones, 1999; Paerl et al., 2001; Cirés and Ballot, 2016). These toxins are human health hazards via direct ingestion, bio-accumulation or by direct contact. In addition, toxins can affect the entire ecosystem causing death of aquatic organisms. Currently, *A. flosaquae*, although widespread, has so far resulted in non-toxic strains in Italy (Mattei et al., 2005). Even though been represented by non-toxic strains during the study period (data not shown), the potential for toxin gene acquisition in commonly nontoxic species via transduction or other lateral gene transfer events cannot be overlooked (Paerl and Otten, 2013). This suggests that, as a precaution, management decisions should be based at the genus level.

6. Conclusions

Currently, the eutrophication process appears to be the most widespread problem affecting the use of the waters of Mediterranean climate reservoirs (Naselli-Flores, 2011). Eutrophication affects almost 80% of the stored water (21 of 31 reservoirs) in Sardinia (Lugliè et al., 2015) and represents the main issue to be addressed since water for drinking supplies is acquired from this resource (Naselli Flores and Lugliè, 2014).

Environmental parameters and phytoplankton assemblages in Lake Cedrino indicate a status of severely poor water quality due to its eutrophic state. In this condition, a management plan for reducing eutrophication is urgently needed that acts on external loads (namely, on WWTP) and, as much as it is possible, on internal P release from sediments (e.g., improving oxygenation of water layer at the water–sediment interface). Additionally, this study highlights the need to increase the adaptability of treatment plants to process waters with different quantitative and qualitative microalgae presence. Furthermore, for potentially toxic cyanobacterial species, a precise monitoring protocol for the assessment of cyanotoxins in the reservoir, the raw water intake, and at various stages in the water treatment process must be planned to reduce the risks associated with these compounds (Codd, 2000).

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