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Multiannual decrement of nutrient concentrations and phytoplankton cell size in a Mediterranean reservoir

Silvia Pulina[1], Antonella Lugliè[1], Maria Antonietta Mariani[1], Marco Sarria[1], Nicola Sechi[1], Bachisio Mario Padedda[1]

1 *Department of Architecture, Design and Urban Planning, University of Sassari, Via Piandanna 4, 07100 Sassari, Italy*

Corresponding author: *Silvia Pulina* (pulinasi@uniss.it)

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Abstract

Reservoirs are primary water resources for many uses in the Mediterreanean region and need dedicated studies for understanding the complexity of their dynamics particularly vulnerable to local and global stressors. This study focused on phytoplankton variations in relation to seasonal environmental changes on a multiannual time scale (2006–2015) at a Mediterranean eutrophic reservoir (Bidighinzu Lake, Italy) belonging to the Italian, European and International Long Term Ecological Research networks. Phytoplankton cell density, volume and biomass and chlorophyll *a* concentrations were analysed together with meteo-climatic, hydrological, physical and chemical variables to detect trends and correlations. The period under study was also compared with previous years to assess the presence of significant differences in the environmental and planktonic compartments. Multiannual changes were more pronounced in summer than in the other seasons during the decade under study. The most conspicuous environmental changes were a significant decrease in summer nutrient concentrations in the reservoir and a simultaneous anthropic pressure reduction in the watershed. In addition, the mixing zone and euphotic zone ratio also increased. Multiannual changes in summer phytoplankton composition consisted of an increased density of smaller Bacillariophyceae and Cyanophyceae, which replaced larger species of the same phytoplankton classes. This resulted in opposite trends of total phytoplankton cell density (increasing) and mean phytoplankton cell volume (decreasing) over the study years. The nutrient decrement was statistically the strongest environmental driver of the phytoplankton changes observed in the reservoir. However, the mixing zone and the euphotic zone ratio and water temperature also significantly affected the multiannual phytoplankton variations. Therefore, we conclude that the success of small cell-sized phytoplankton in Bidighinzu Lake was most probably due to the synergic interactions of more environmental forces related to changing anthropic pressures and climate variability. Our results highlight the importance of long-term monitoring of reservoirs in the Mediterranean basin, especially in semi-arid regions where the need and scarcity of high quality water will be further exacerbated due to the global climate change.

Keywords

Phytoplankton, cell size, nutrients, climate change, reservoir, LTER

Introduction

In all aquatic ecosystems, phytoplankton growth depends on water temperature and on light and nutrient availability (Winder and Sommer 2012). These growth-limiting factors are in turn regulated by physical processes, such as water circulation, mixed-layer dynamics and upwelling, which are strongly linked to climate (Jovanović et al. 2017). Being the basis of pelagic trophic web as the primary producers, phytoplankton is a fundamental element for understanding the dynamics of the ecosystem and for predicting and managing the ecosystem responses to local and global changes (Guinder et al. 2010).

Different selective pressures, such as light availability, nutrient limitation, fluctuating nutrient supply or grazers, can influence the size structure in natural phytoplankton communities (Litchman et al. 2010). A shift to smaller cell sized phytoplankton has been predicted as a consequence of ocean warming, with profound consequences for the ecosystem's food web composition and efficiency, from the very bottom to the upper trophic levels (Polovina and Woodworth 2012, Boyce et al. 2015, Sommer et al. 2017a). Large losses of energy, nutrients and carbon throughout the food web can be expected when small phytoplankton dominates (Legendre and Rassoulzadegan 1996, Falkowski et al. 1998), with consequences on the ecosystem functioning (Kamenir and Morabito 2009).

Many studies have highlighted drastic modifications in phytoplankton cell size composition and abundance in lakes related to changes in nutrient concentrations (Salmaso 2010 and references therein). On the other hand, some physical variables, such as water mixing and underwater light availability, resulted in being the most important drivers in determining phytoplankton size and shape structure in several reservoirs (Naselli-Flores and Barone 2007).

This work examines phytoplankton variations in relation to seasonal environmental changes on a multiannual time scale in a warm monomitic eutrophic Mediterranean reservoir (Bidighinzu Lake, Sardinia, Italy), mainly used for drinking water for 20 urban areas and about 100,000 inhabitants. In the Mediterranean area, climate conditions (such as long lasting periodic droughts) seriously restrict the water availability, especially during summer and cause strong variations in the water level of reservoirs, which in turn affect the phytoplankton abundance and composition (Naselli-Flores 2003). In addition, in semi-arid areas, such as the Mediterranean region, reservoirs are the basic sources of freshwater for human uses (García-Ruiz et al. 2011). The Mediterranean region is one of the climate change hotspots in the word (Giorgi and Lionello 2008), where a substantial decrease in precipitation (around 10%) is predicted for the period 2040–2070 by most of the current forecast models (García-Ruiz et al. 2011). Consequently, an increasing pressure on stored water resources is expected which will likely also affect the overall state of the reservoir ecosystem.

Multiannual observations allow detecting meaningful ecological shifts, distinguishing significant changes from the normal patterns and the background noise and the assessment of whether ecological changes are due to human or natural causes (Pugnetti et al. 2013). Investigating changes in phytoplankton cell size on a multiannual time scale may be useful for forecasting phytoplankton variations in relation to global change and other environmental impacts, such as those of local human activities (Weithoff and Gaedke 2017).

Phytoplankton and environmental variables have been monitored since 1978 (Marchetti et al. 1992) in Bidighinzu Lake, a site that belongs to the Italian (LTER-Italy), European (LTER-Europe) and International (ILTER) Long Term Ecological Research networks (www.ilter.network). A long term decreasing trend in nutrient concentrations in this reservoir has been recently reported for the period 1988–2012 (Mariani et al. 2015), accompanied by significant increasing trends in the density and biomass of Chlorophyceae, Chrysophyceae and Dinophyceae and significant decreasing trends in the density of Cyanophyceae Nostocales. In addition, the density and biomass of Bacillariophyceae increased in summer and decreased in winter, while those of Cyanophyceae Chroococcales increased in winter and decreased in summer. The present work focused on the structure and temporal dynamics of phytoplankton in the most recent decade (2006–2015) of this monitoring programme. We hypothesised that the reduction in nutrient concentrations in Bidighinzu Lake 1) may have been accompanied by a reduction in phytoplankton cell size, a descriptor not yet investigated in the Bidighinzu Lake and in other Mediterranean reservoirs and 2) may have been linked to variation in the anthropic pressures in the watershed and to climate variability.

Material and methods

Study site

The Bidighinzu Lake is located in the northern part of Sardinia (Italy, 40°33'22"N 8°39'41"E) at an altitude of 334 m a.s.l. (Figure 1). The reservoir was constructed in 1956, it has a surface area of 1.7×10^6 m^2, a theoretical maximum volume of 12.2×10^6 m^3, a maximum depth of 39.5 m and a mean depth of 7.3 m. The watershed has a surface area of 52.18 km^2. The main use of Bidighinzu Lake is to store drinking water. Problems affecting the potability of Bidighinzu Lake's water have occurred since the lake was built, particularly hypolimnetic deoxygenation and the excessive presence of algae in the epilimnion (Lugliè et al. 2001). To address these problems, an aeration system was installed adjacent to the water intake tower in 1966. A by-pass was built in 1987 to divert urban and

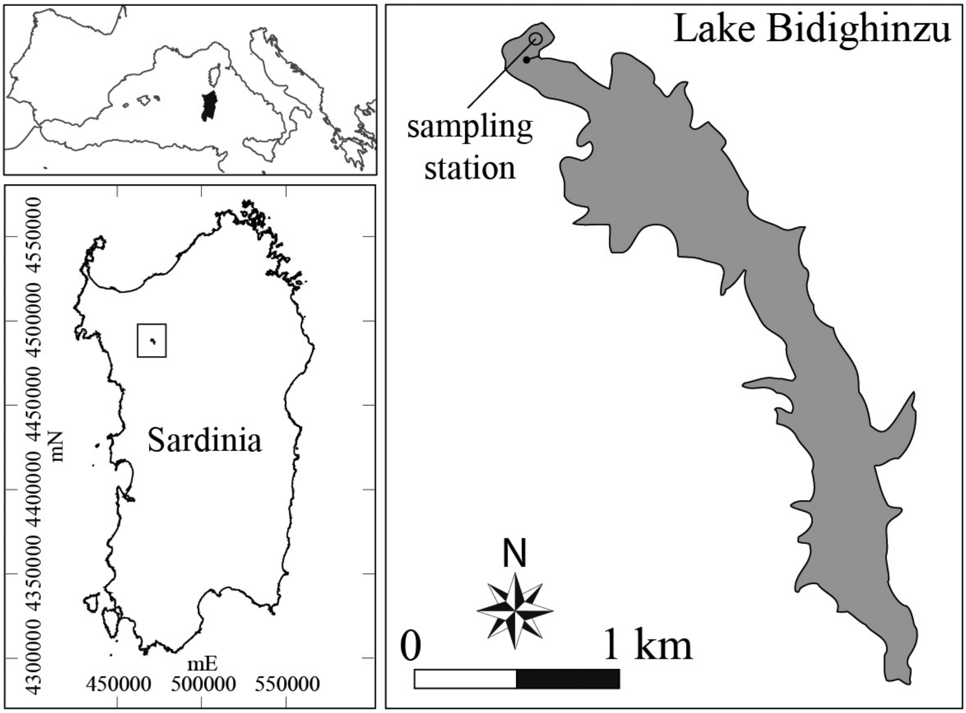

Figure 1. Location of the study site and sampling station.

industrial wastes from the town of Thiesi to downstream of the reservoir (Marchetti et al. 1992, Lugliè et al. 2001). An experimental water aeration system with micro-air bubbles was activated in the reservoir from spring 2014 to autumn 2015 (Mariani et al. 2016).

Multiannual sampling

Water sampling was conducted in the Bidighinzu Lake at monthly frequency from March 1988 to April 1989, from March 1994 to November 1997, from July to October 2003 and from March 2006 to October 2015 at a single station close to the deepest part of the reservoir (Figure 1). As part of the results of the WISER project (Water bodies in Europe: Integrative Systems to assess Ecological status and Recovery), the data analyses of phytoplankton and environmental variables collected at different stations within the reservoir demonstrated that the single sampling station, routinely considered, is representative of the entire Bidighinzu Lake (Thackeray et al. 2013). The sampling was more frequent in the summers of 2006, 2009, 2014 and 2015 (4 samples in each summer of these years), during the present study. Samples were collected at fixed depths using 2.5 l Niskin bottles at 0, 1, 2.5, 5, 7.5, 10, 15 m and subsequently at intervals of 10 m until 1 m from the bottom.

Analyses of environmental and meteo-climatic variables

The water transparency was measured with a Secchi disc and the water temperature was recorded using a multi-parametric probe (Hydrolab Datasonde 5 and YSI 6600V2). Water samples (1.5 l) from the Niskin bottles were stored in cold (4 °C) and dark conditions prior to laboratory analysis for ammonium ($N\text{-}NH_4$), nitrite ($N\text{-}NO_2$), nitrate ($N\text{-}NO_3$), total nitrogen (TN), reactive silica ($Si\text{-}SiO_4$), orthophosphate ($P\text{-}PO_4$) and total phosphorus (TP) according to Strickland and Parsons (1972). Total dissolved inorganic nitrogen (DIN) was calculated as the sum of ammonium, nitrate and nitrite. Total organic nitrogen (TON) was calculated by subtracting DIN from TN.

The Consiglio per la Ricerca in Agricoltura (CREA) provided daily meteorological data of rainfall and air temperature from a meteorological station nearby the Lake (about 15 km) from January 2006 to December 2015. Daily temperature values were monthly averaged and daily rainfall values were monthly cumulated. Monthly data on the climatic index WEMO (Western Mediterranean Oscillation) were provided by the Climatology Group of the University of Barcelona (Spain). This index measures the difference between the standardised atmospheric pressure recorded at Padua in northern Italy and San Fernando in south-western Spain (Martín-Vide and Lopez-Bustins 2006). Compared to the Mediterranean Oscillation index (Conte et al. 1989), which covers the atmospheric dynamics of the whole Mediterranean basin, WEMO is defined only within the synoptic framework of the western Mediterranean basin and its vicinities and shows a greater independence from the external dynamics of the Mediterranean basin (Martín-Vide and Lopez-Bustins 2006). WEMO is able to explain the pluviometric variability in the eastern fringe of the Iberian Peninsula, an area weakly or not related to the NAO (North Atlantic Oscillation index) pattern (Rodó et al. 1997, Martín-Vide and Lopez-Bustins 2006). Monthly data of the WEMO index were available continuously (i.e. without gaps) from 1988 to 2015.

Phytoplankton analyses

The phytoplankton samples (100 ml), taken from the Niskin bottles, were immediately fixed after collection in 2% acid Lugol's solution. Sample aliquots of 5 to 10 ml (depending on cell density) were analysed to estimate the cell density using the Utermöhl method (1958) with an inverted microscope (Zeiss, Axiovert 10). Cell counts were made at magnifications of 200× and 400× on at least 10% of the total bottom area of the sedimentation chamber. Additional non-fixed samples were observed immediately after collection to facilitate the identification of certain species. The species were identified following the taxonomic guides listed in Mariani et al. (2015). Results are shown at class level (and at order level only for the most abundant classes) in the present work.

Mean cell volume of each species was obtained by geometrical approximations from measurement of at least 30 cells in each sample according to Sun and Liu (2003). The mean volumes of all the species observed were added to obtain the total cell volume in each sample. The total cell volume was divided by the total cell number to obtain the mean cell volume of the whole phytoplankton community in each sample (abundance-weighted mean cell volume according to Sommer et al. 2017a). The biovolume in each sample was converted to biomass based on the assumption that 1 mm^3 = 1 mg of fresh-weight biomass (Weyhenmeyer et al. 2013). The cell biomass of each species was determined in each sample by multiplying its mean cell volume by its cell density.

Water samples (1.5 l) for measuring chlorophyll *a* concentrations were stored in cold (4 °C) and dark conditions before the laboratory analysis (within 24 hours), which were conducted with a spectrophotometer (50 SCAM, Varian), according to Goltermann et al. (1978).

Data and statistical analysis (2006–2015)

Environmental data were depth-averaged for the entire water column, whereas phytoplankton data were depth-averaged only for the euphotic zone assuming that these organisms live and grow mainly in this water layer.

The depth of the euphotic layer was calculated as Z_{eu} = 2.5 times the Secchi disc depth (Poikane 2009). The depth of the mixed layer (Z_{mix}) was calculated as the zone from the surface to the depth where the temperature gradient exceeded 1 °C x m^{-1} (Ruttner 1963), except in the case of absence of stratification, when mixing depth was taken equal to the maximum depth of the reservoir. The mixing depth and euphotic depth ratio (Z_{mix}/Z_{eu}) was used as a good proxy for underwater light climate since it indicates the time spent by phytoplankton in good or poor light conditions when they are in the mixed water column: the highest is the Z_{mix}/Z_{eu} value in the lake, the lowest is the lifetime spent in suitable light conditions (Naselli Flores and Barone 2007).

Seasons were considered as: summer = July–September, autumn = October–December, winter = January–March and spring = April–June.

For each separate season, the non-parametric Mann-Kendall test (Gilbert 1987) was applied to detect significant monotonic trends in environmental and phytoplankton variables. Since the Mann-Kendall test results indicated the most significant trends for the summer season and for the phytoplankton cell density and cell volume datasets, all the subsequent statistical analyses were performed only on these phytoplankton descriptors in summer. Redundancy analysis (RDA, Ter Braak and Šmilauer 1998) was used to assess which environmental variable (amongst WEMO index, air temperature, rainfall, Z_{mix}/Z_{eu}, water temperature, Si-SiO_4, P-PO_4, TP, N-NO_3, N-NO_2, N-NH_4, DIN, TN and TON) significantly affected the multiannual patterns of the cell density and cell volume of all the phytoplankton classes observed consistently throughout the analysed decade (Bacillariophyceae, Chlorophyceae, Chrysophyceae, Conjugatophyceae, Cryptophyceae, Cyanophyceae, Dinophyceae, Euglenophyceae). Before analysis, phytoplankton data were $\log_{10}(x+1)$ transformed to stabilise variance and reduce the influence of dominant taxa on the ordination.

All statistical analyses were performed using R 3.4.3 software (R Core Team 2017). An *a priori* level of significance was established at $p < 0.05$, i.e. 95% confidence interval.

Statistical analyses of the long-term dataset (1988–2015)

The non-parametric Mann-Kendall test was applied to detect significant monotonic trends in the summer WEMO index over all the historical datasets.

One-way analysis of variance (ANOVA) was performed to assess significant differences in environmental (WEMO index, air temperature, rainfall, Z_{mix}/Z_{eu}, water temperature, $Si\text{-}SiO_4$, $P\text{-}PO_4$, TP, $N\text{-}NO_3$, $N\text{-}NO_2$, $N\text{-}NH_4$, DIN, TN and TON) and phytoplankton (cell density and cell volume of each phytoplankton class observed) variables amongst the four continuous time cycles of samplings available (1988–1989, 1994–1997, 2003, 2006–2015). Prior to ANOVA, all data were $\log_{10}(x+1)$ transformed to meet ANOVA assumptions: normal distribution (Kolmogorov-Smirnov test) and homogeneity of variance (Bartlett test). When significant differences in the dependent variables based on these factors were observed, the post hoc Tukey's pairwise comparisons test was performed.

All the analyses were performed using R 3.4.3 software (R Core Team 2017). An *a priori* level of significance was established at $p < 0.05$, i.e. 95% confidence interval.

Watershed land cover and land use analysis

To explore the land cover and land use changes of the Bidighinzu Lake watershed (52.18 km^2) over time, a spatial data processing analysis with the support of a geographic information system (GIS) was performed (Padedda et al. 2015). The analysis was focused on the evaluation of land cover by using techniques of classification generating polygons by clipping the shape of the three time-series CORINE land cover maps: 1990 (EEA 2018a), 2000 (EEA 2018b) and 2012 (EEA 2018c). The automatic segmentation was carried out on the whole shape layer of the watershed of the reservoir. Each segmentation analysis was performed in order to obtain objects in accordance with the CLC system 2000 nomenclature (EEA 2000). For the purposes of this paper, the identified objects were aggregated into three homogeneous macro-classes (Urban Areas, Agricultural Areas and Natural and semi-Natural Areas) in accordance with the CLC classification at the 4th hierarchical level.

Results

The seasonal values of environmental and phytoplankton parameters considered during the decade 2006–2015 are reported in Tables 1 and 2, respectively.

Table 1. Minimum (Min), maximum (Max) and mean values ± standard deviation (SD) of environmental variables in Bidighinzu Lake considering the decade 2006–2015 (WEMOi, Western Mediterranean Oscillation index; Z_{mix}/Z_{eu}, mixing depth-euphotic depth ratio; Si-SiO_4, silicate; P-PO_4, orthophosphate; TP, total phosphorous; N-NO_3, nitrate; N-NO_2, nitrite; N-NH_4, ammonia; DIN, dissolved inorganic nitrogen; TN, total nitrogen; TON, total organic nitrogen). Number of observations are reported in Table 3.

	Winter			Spring			Summer			Autumn		
	Min	**Max**	**Mean±SD**	**Min**	**Max**	**Mean±SD**	**Min**	**Max**	**Mean±SD**	**Min**	**Max**	**Mean±SD**
WEMOi	-2.6	1.6	-0.2±1.0	-4.3	1.2	-1.4±1.4	-2.6	1.1	-1.0±0.9	-2.7	1.6	-0.6±0.9
Air temperature (°C)	4.8	10.8	8.3±1.5	12.4	22.8	17.1±3.5	19.2	26.0	23.1±2.0	7.1	18.9	12.5±3.7
Rainfall (mm)	4.0	104.6	47.8±26.8	0.8	111.0	47.6±31.0	0.0	101.2	16.4±23.4	2.6	191.6	59.5±41.9
Water temperature (°C)	6.6	11.5	9.3±1.4	12.0	26.4	16.8±3.3	17.7	24.8	20.4±1.8	8.6	19.1	15.0±2.2
Water transparency (m)	0.3	11.1	0.6±0.2	0.3	1.6	0.9±0.3	0.3	2.5	1.1±0.5	0.3	1.3	0.8±0.1
Z_{mix}/Z_{eu}	1.8	26.7	14.3±6.4	0.3	22.8	5.1±5.7	0.2	20	4.1±4.0	3.3	26.7	10.4±5.1
Si-SiO_4 (mg l^{-1})	0.7	9.5	6.1±2.0	0.0	9.2	4.8±1.8	0.2	62.0	5.1±10.0	0.1	7.1	2.6±0.8
P-PO_4 (mg m^{-3})	13.0	164.2	84.1±33.5	5.0	159.7	70.7±35.1	24.8	343.0	130.1±62.2	31.9	243.0	96.2±75.3
TP (mg m^{-3})	83.5	355.1	183.7±70.2	61.0	399.4	170.1±75.0	79.8	457.9	246.9±79.6	68.9	396.3	190.9±89.1
N-NO_3 (mg m^{-3})	328.8	1295.6	878.5±361.0	16.2	684.4	231.3±199.1	6.7	126.7	38.3±21.3	24.0	1435.3	344.3±97.5
N-NO_2 (mg m^{-3})	11.7	60.4	22.1±5.4	29.0	720.6	13.0±7.3	2.6	37.4	666.4±402.8	3.3	47.6	16.7±8.1
N-NH_4 (mg m^{-3})	25.4	648.0	111.1±180.0	2.9	37.0	171.9±171.0	40.0	1736.4	620.4±404.1	22.3	939.9	427.4±250.5
DIN (mg m^{-3})	504.9	1386.6	1011.6±295.5	63.0	803.6	416.3±182.4	73.2	1778.3	7.6±8.2	53.1	1977.0	788.4±229.0
TN (mg m^{-3})	1839.2	4049.2	2703.9±653.5	1171.7	3519.1	2114.2±596.6	1212.0	4658.7	2431.0±925.8	442.0	4131.8	2347.4±848.6
TON (mg m^{-3})	1004.0	3238.8	1692.3±584.1	858.1	3086.0	1697.9±588.6	344.5	4284.1	1766.1±985.6	146.2	3411.4	1572.2±872.8

Table 2. Minimum (Min), maximum (Max) and mean values ± standard deviation (SD) of phytoplankton variables in Bidighinzu Lake considering the decade 2006–2015. Number of observations are reported in Table 4.

	Winter			Spring			Summer			Autumn		
	Min	**Max**	**Mean±SD**	**Min**	**Max**	**Mean±SD**	**Min**	**Max**	**Mean±SD**	**Min**	**Max**	**Mean±SD**
Total cell density (10^3 cells l^{-1})	448	52970	10298±13641	3068	515428	86784±133223	2633	1570361	203256±308627	1542	803909	149885±217932
Bacillariophyceae	56	2613	720±757	14	6300	594±1259	41	15128	3224±3493	216	6926	2530±2171
Chlorophyceae	78	4026	1123±1169	759	68065	9957±12968	324	36047	5657±7989	267	32226	9736±9146
Chrysophyceae	0	383	55±94	0	70	13±20	0	705	55±137	0	1344	127±298
Conjugatophyceae	0	35	7±10	0	62	8±15	0	336	42±75	2	1429	213±422
Cryptophyceae	0	24693	3706±6394	5	4143	972±1120	9	2299	498±571	39	9884	1465±2179
Cyanophyceae	0	27783	4618±8417	0	513989	75209±133927	1020	1562687	193740±308718	696	780464	135650±216116
Dinophyceae	0	81	11±24	0	58	4±11	0	148	15±27	0	56	12±16
Euglenophyceae	0	276	57±77	0	158	27±36	0	130	23±28	0	1528	153±325
Mean cell volume (μm^3)	0.52	25.68	6.95±6.64	0.06	18.44	1.97±3.57	0.03	34.67	3.12±6.58	0.09	14.80	2.40±3.87
Bacillariophyceae	3.12	256.49	66.59±91.78	1.54	941.19	169.59±228.36	0.13	120.96	20.08±29.79	1.48	70.09	1.48±70.09
Chlorophyceae	0.14	29.04	7.43±8.48	0.04	24.06	2.12±4.79	0.11	14.98	3.05±3.33	0.22	26.35	2.87±5.58
Chrysophyceae	0.11	2590.58	219.07±659.78	3.89	1469.34	270.71±407.10	4.10	1012.90	165.82±211.08	1.14	769.60	146.41±213.10
Conjugatophyceae	4.37	3306.90	542.12±826.55	73.73	12708.87	1367.56±3076.65	4.35	4772.39	475.39±824.45	0.56	3164.18	477.99±760.72
Cryptophyceae	0.12	178.12	22.07±47.11	0.04	61.57	9.02±15.50	0.70	209.54	15.39±36.20	0.26	44.28	9.61±12.64
Cyanophyceae	0.00	5.22	0.43±1.33	0.00	0.14	0.02±0.03	0.00	0.27	0.02±0.05	0.00	0.08	0.02±0.02
Dinophyceae	9.80	3834.09	637.89±1299.79	0.68	103248.12	14703.87±28210.65	73.333	172092.97	17474.80±35443.87	31.92	32951.37	5757.98±9131.45
Euglenophyceae	28.88	2283.72	414.01±574.54	43.14	4971.75	557.40±1035.71	1.89	2870.58	471.52±658.84	3.79	338.55	117.14±11.23
Total cell biomass (mg l^{-1})	0.20	9.19	3.46±2.51	0.28	29.86	3.87±5.57	2.14	68.86	13.12±13.43	0.65	21.60	9.18±5.90
Bacillariophyceae	0.08	7.54	2.39±2.57	0.03	24.95	1.52±4.58	0.05	65.43	6.78±11.70	0.49	16.99	3.42±3.47
Chlorophyceae	0.01	0.69	0.18±0.19	0.12	10.89	1.60±2.11	0.04	3.67	1.11±1.05	0.06	9.88	2.47±2.73
Chrysophyceae	0.00	0.04	0.01±0.01	0.00	0.04	0.01±0.01	0.00	2.39	0.13±0.47	0.00	1.39	0.10±0.30
Conjugatophyceae	0.00	0.09	0.01±0.02	0.00	0.11	0.01±0.02	0.00	0.45	0.06±0.10	0.00	1.51	0.20±0.38
Cryptophyceae	0.00	2.58	0.61±0.82	0.00	0.76	0.19±0.19	0.00	2.12	0.16±0.36	0.00	4.33	0.59±0.96
Cyanophyceae	0.00	0.16	0.02±0.04	0.00	5.55	0.46±1.15	0.01	21.22	3.99±5.85	0.00	13.22	1.32±3.43
Dinophyceae	0.00	0.23	0.02±0.06	0.00	0.25	0.02±0.06	0.00	7.34	0.68±1.40	0.00	4.81	0.70±1.27
Euglenophyceae	0.00	0.14	0.20±0.31	0.00	0.57	0.07±0.11	0.00	0.20	0.04±0.04	0.00	4.79	0.38±1.02
Chlorophyll *a* (mg m^{-3})	2.16	33.90	13.86±8.85	2.15	40.70	10.52±8.64	3.40	150.54	20.50±28.88	2.81	58.61	22.04±14.13

Multiannual trends of meteo-climatic and environmental parameters (2006–2015)

The Mann-Kendall test revealed no significant multiannual trends in the meteo-climatic variables considered nor in the water temperature and transparency during the study decade (Table 3). Instead, a very significant increasing trend of Z_{mix}/Z_{eu} ratio was observed in summer (Table 3). This increment was confirmed, although not significant, when the year 2015 (breaking of stratification due to the activation of the aeration system) was excluded from the analysis (Mann Kendall test: S = 12, p = 0.828, n = 35). The multiannual patterns of Z_{mix}/Z_{eu} summer mean values showed fluctuations over the years, from 0.80 in 2012 up to 11.0 in 2015 (Figure 2). Strong decreasing trends of TP, $N\text{-}NH_4$ and DIN were detected in summer (Table 3). TP values decreased from a summer mean value of 390.8 mg m^{-3} in 2006 to 160.3 mg m^{-3} in 2015 (Figure 2). DIN values decreased strongly from a summer mean value of 1351.4 mg m^{-3} in 2006 to 391.6 mg m^{-3} in 2015, with $N\text{-}NH_4$ always representing more than 80% of total DIN (Figure 3). $Si\text{-}SiO_4$ decreased in summer too, whereas TON increased, although their trends were close to the limit of statistical significance (Table 3). $N\text{-}NH_4$ decreased significantly in winter and DIN even in spring. TON and $N\text{-}NO_2$ increased significantly in autumn and TON even in spring (Table 3).

Table 3. Results of the Mann-Kendall test for detection of long-term trends (2006–2015) in the environmental parameters (WEMOi, Western Mediterranean Oscillation index; Z_{mix}/Z_{eu}, mixing zone and euphotic zone ratio; $Si\text{-}SiO_4$, silicate; $P\text{-}PO_4$, orthophosphate; TP, total phosphorous; $N\text{-}NO_3$, nitrate; $N\text{-}NO_2$, nitrite; $N\text{-}NH_4$, ammonia; DIN, dissolved inorganic nitrogen; TN, total nitrogen; TON, total organic nitrogen) in Bidighinzu Lake. Significant trends are in bold (S = Kendall score, it indicates the trend direction; p = significance; n = number of observations).

	Winter			Spring			Summer			Autumn		
	S	p	n	S	p	n	S	p	n	S	p	n
WEMOi	33	0.568	30	40	0.486	30	-34	0.556	30	15	0.802	30
Air Temperature	27	0.566	26	24	0.649	28	-6	0.912	26	20	0.657	25
Rainfall	38	0.440	27	20	0.721	29	53	0.303	28	20	0.707	28
Water Temperature	-17	0.544	24	2	0.986	30	52	0.469	35	21	0.620	24
Water Transparency	-21	0.444	24	62	0.274	30	-19	0.797	35	45	0.271	24
Z_{mix}/Z_{eu}	9	0.715	24	2	0.985	30	**181**	**0.007**	**35**	16	0.671	24
$Si\text{-}SiO_4$	-50	0.063	24	100	0.077	30	**-141**	**0.047**	**35**	-65	0.112	24
$P\text{-}PO_4$	-10	0.773	24	17	0.775	30	-62	0.386	35	-16	0.710	24
TP	-14	0.621	24	-27	0.643	30	**-175**	**0.013**	**35**	-39	0.346	24
$N\text{-}NO_2$	-23	0.404	24	-50	0.382	30	132	0.063	35	**83**	**0.042**	**24**
$N\text{-}NO_3$	12	0.677	24	-53	0.353	30	73	0.306	35	27	0.519	24
$N\text{-}NH_4$	**-56**	**0.037**	**24**	-67	0.239	30	**-159**	**0.025**	**35**	-33	0.427	24
DIN	10	0.733	24	**-157**	**0.005**	**30**	**-161**	**0.023**	**35**	35	0.399	24
TN	-6	0.850	24	103	0.069	30	113	0.112	35	63	0.124	24
TON	32	0.163	24	**128**	**0.027**	**30**	**221**	**0.040**	**35**	**113**	**0.031**	**24**

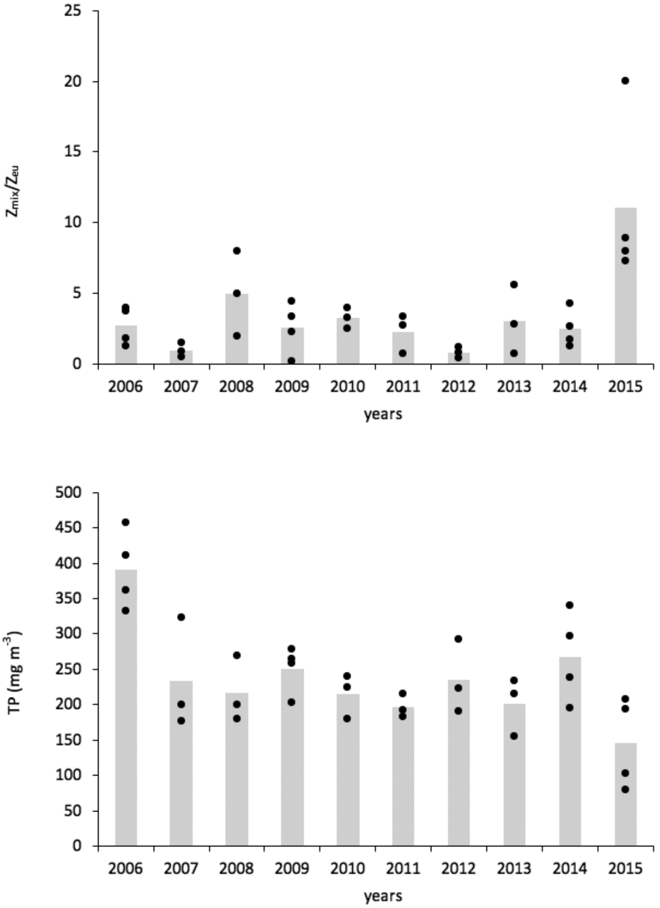

Figure 2. Multiannual (2006–2015) variation of mixing zone and euphotic zone ratio (Z_{mix}/Z_{eu}) (upper panel) and of total phosphorous (TP) (lower panel) in Bidighinzu Lake in summer. Each black dot represents a single sample and each column represents the mean value.

Multiannual phytoplankton trends (2006–2015)

A significant increasing trend resulted for total phytoplankton cell density in summer during the study decade (Table 4). This increment was confirmed also when the year 2010 (exceptional peak of the total phytoplankton density) was excluded from the analysis (Mann Kendall test: S = 190, p = 0.002, n = 35). Summer total phytoplankton cell density varied from about 20 × 10^6 cells l^{-1} in 2006 to about 126 × 10^6 cells l^{-1} in 2015, with an exceptional event (about 1 × 10^9 cells l^{-1}) in 2010, when a bloom of Cyanophyceae was observed (Figure 4). Cyanophyceae contributed most to the total phytoplankton cell density (> 60%), followed by Chlorophyceae (1–25%) and

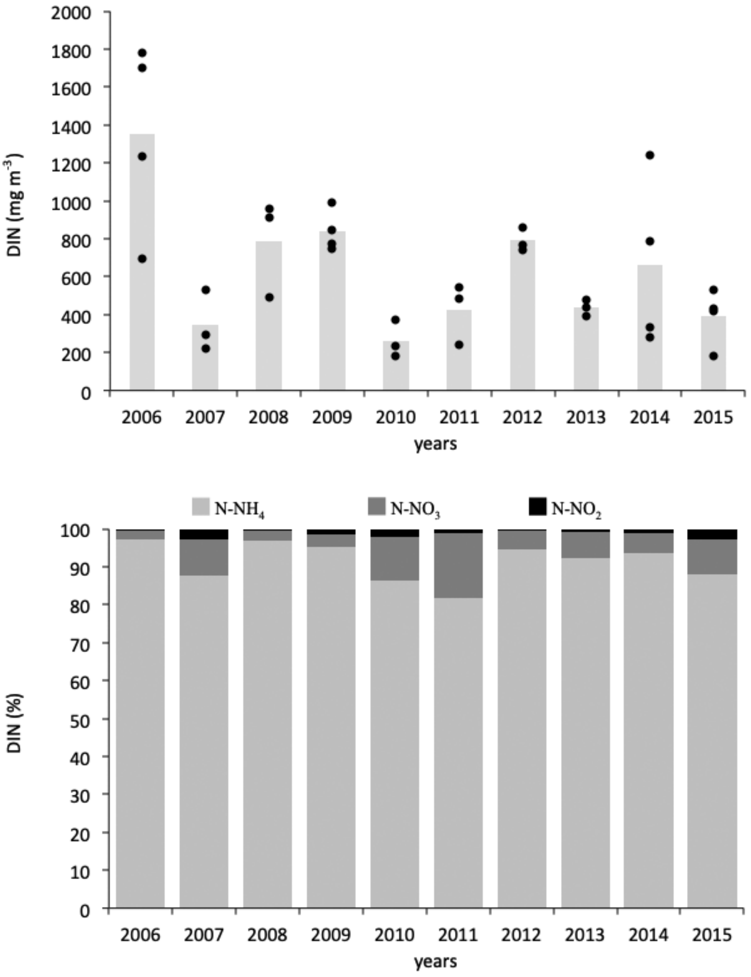

Figure 3. Multiannual (2006–2015) variation of dissolved inorganic nitrogen (DIN) (upper panel) and of the contribution of nitrate (N-NO$_3$), nitrite (N-NO$_2$) and ammonium (N-NH$_4$) to DIN (lower panel) in Bidighinzu Lake in summer. Each black dot represents a single sample and each column represents the mean value.

Bacillariophyceae (1–16%) (Figure 5). Significant increasing trends were observed for Bacillariophyceae and Cyanophyceae cell density in summer over the study period (Table 4). Bacillariophyceae cell density increased from a summer mean value of about 1 × 10^6 cells l^{-1} in 2006 to about 7 × 10^6 cells l^{-1} in 2015 (Figure 6A). Cyanophyceae cell density increased from a summer mean value of about 13 × 10^6 cells l^{-1} in 2006 to about 1 × 10^9 cells l^{-1} in 2010, decreasing to about 107 × 10^6 cells l^{-1} in 2015 (Figure 6C). Instead, no significant multiannual trend was observed for Chlorophyceae cell density (Table 4).

Table 4. Results of the Mann-Kendall test for detection of long-term trends (2006–2015) in the phytoplankton variables in Bidighinzu Lake. Significant trends are in bold (S = Kendall score, it indicates the trend direction; p = significance; n = number of observations).

	Winter			**Spring**			**Summer**			**Autumn**		
	S	**p**	**n**	**S**	**p**	**n**	**S**	**p**	**n**	**S**	**p**	**n**
Total cell density	24	0.300	24	106	0.065	30	**175**	**0.009**	**34**	43	0.236	24
Bacillariophyceae	30	0.192	24	**-142**	**0.008**	**30**	**249**	**0.000**	**34**	**81**	**0.024**	**24**
Chlorophyceae	4	0.892	24	12	0.836	30	-85	0.213	34	-65	0.071	24
Chrysophyceae	22	0.344	24	23	0.677	30	**201**	**0.003**	**34**	51	0.058	24
Cryptophyceae	-2	0.964	24	62	0.252	30	47	0.495	34	-49	0.176	24
Conjugatophyceae	-1	1	24	**-140**	**0.007**	**30**	**-212**	**0.002**	**34**	**-99**	**0.006**	**24**
Cyanophyceae	**60**	**0.008**	**24**	**130**	**0.015**	**30**	**167**	**0.014**	**34**	65	0.071	24
Dinophyceae	38	0.074	24	76	0.139	30	-68	0.318	34	12	0.744	24
Euglenophyceae	24	0.300	24	39	0.476	30	-10	0.894	34	-29	0.430	24
Mean cell volume	-18	0.444	24	-105	0.063	30	**-129**	**0.030**	**34**	-9	0.821	24
Bacillariophyceae	-18	0.444	24	**168**	**0.002**	**30**	**-197**	**0.004**	**34**	-29	0.430	24
Chlorophyceae	8	0.753	24	14	0.807	30	51	0.458	34	43	0.236	24
Chrysophyceae	7	0.766	24	5	0.910	30	**-128**	**0.049**	**34**	22	0.526	24
Cryptophyceae	17	0.428	24	34	0.536	30	-47	0.406	34	**87**	**0.015**	**24**
Conjugatophyceae	25	0.235	24	46	0.064	30	112	0.072	34	**129**	**0.000**	**24**
Cyanophyceae	-29	0.166	24	**-142**	**0.005**	**30**	**-195**	**0.004**	**34**	**-71**	**0.048**	**24**
Dinophyceae	-8	0.465	24	26	0.260	30	37	0.453	34	18	0.244	24
Euglenophyceae	-3	0.921	24	-41	0.378	30	-7	0.915	34	22	0.526	24
Total cell biomass	16	0.599	24	23	0.695	30	89	0.192	34	17	0.691	24
Bacillariophyceae	24	0.420	24	**-141**	**0.012**	**30**	**141**	**0.038**	**34**	**89**	**0.029**	**24**
Chlorophyceae	-6	0.861	24	21	0.721	30	-85	0.213	34	-53	0.197	24
Chrysophyceae	6	0.861	24	30	0.602	30	121	0.075	34	**111**	**0.006**	**24**
Cryptophyceae	-18	0.551	24	107	0.059	30	49	0.477	34	-25	0.551	24
Conjugatophyceae	2	0.972	24	**-143**	**0.009**	**30**	**-166**	**0.014**	**34**	-53	0.197	24
Cyanophyceae	20	0.505	24	67	0.239	30	13	0.859	34	25	0.551	24
Dinophyceae	28	0.323	24	89	0.101	30	-64	0.348	34	28	0.486	24
Euglenophyceae	16	0.599	24	-8	0.900	30	-19	0.790	34	-7	0.882	24
Chlorophyll *a*	4	0.892	24	15	0.803	30	9	0.909	34	-139	0.100	24

Considering the mean cell volume of the whole summer phytoplankton community, a multiannual decreasing trend was detected in the total assemblage, with a strong decrement in Bacillariophyceae and Cyanophyceae (Table 4). Summer Chrysophyceae cell volume also decreased, but to a lesser extent. The mean cell volume of the whole phytoplankton community decreased from a summer mean value of about 11 μm^3 in 2006 to about 1 μm^3 in 2015, apart from a high summer mean value of 18 μm^3 in 2012 (Figure 4). Summer Bacillariophyceae cell volume increased from a mean value of 45 μm^3 in 2006 to 58 μm^3 in 2009, then decreased to 4 μm^3 in 2015 (Figure 6B). Summer Cyanophyceae cell volume decreased from a mean value of 0.081 μm^3 in 2006 to 0.005 μm^3 in 2015 (Figure 6D). Dinophyceae was the most important class contributing to the mean cell volume of the whole phytoplankton community (19–97%), followed by Conjugatophyceae (1–67%) and Euglenophyceae (1–38%) (Figure 5).

No significant multiannual trends were observed for total phytoplankton biomass and chlorophyll *a* (Table 4).

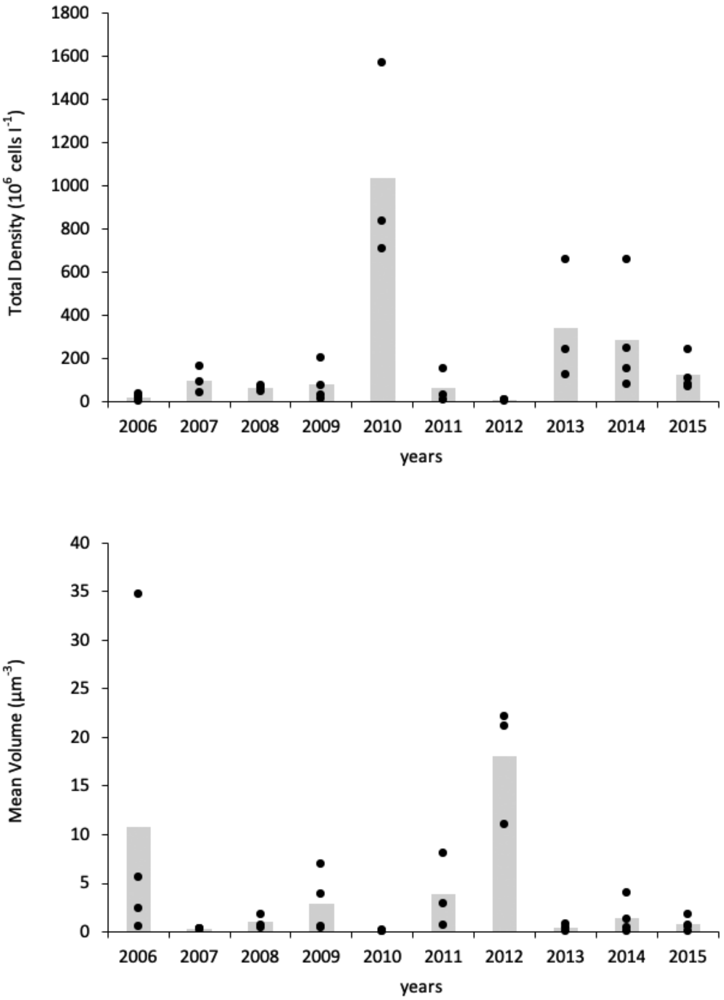

Figure 4. Multiannual (2006–2015) variation of total cell density (upper panel) and mean cell volume (lower panel) of the whole phytoplankton community in Bidighinzu Lake in summer. Each black dot represents a single sample and each column represents the mean value.

Multiannual changes in phytoplankton composition (2006–2015)

Opposite significant trends were observed for the Cyanophyceae Chroococcales and Bacillariophyceae Centrales: cell density increased (Mann-Kendall test: $S = 197$, $p = 0.004$, $n = 34$ and $S = 253$, $p < 0.001$, $n = 34$, respectively) while their cell volume decreased (Mann-Kendall test: $S = -179$, $p = 0.008$, $n = 34$ and $S = -205$, $p = 0.002$, $n = 34$, respectively). In addition, a multiannual decrement in summer was detected for the Cyanophyceae Nostocales only in cell density (Mann-Kendall test: $S = -102$, $p = 0.040$, $n = 34$; Suppl. materials 1, 2).

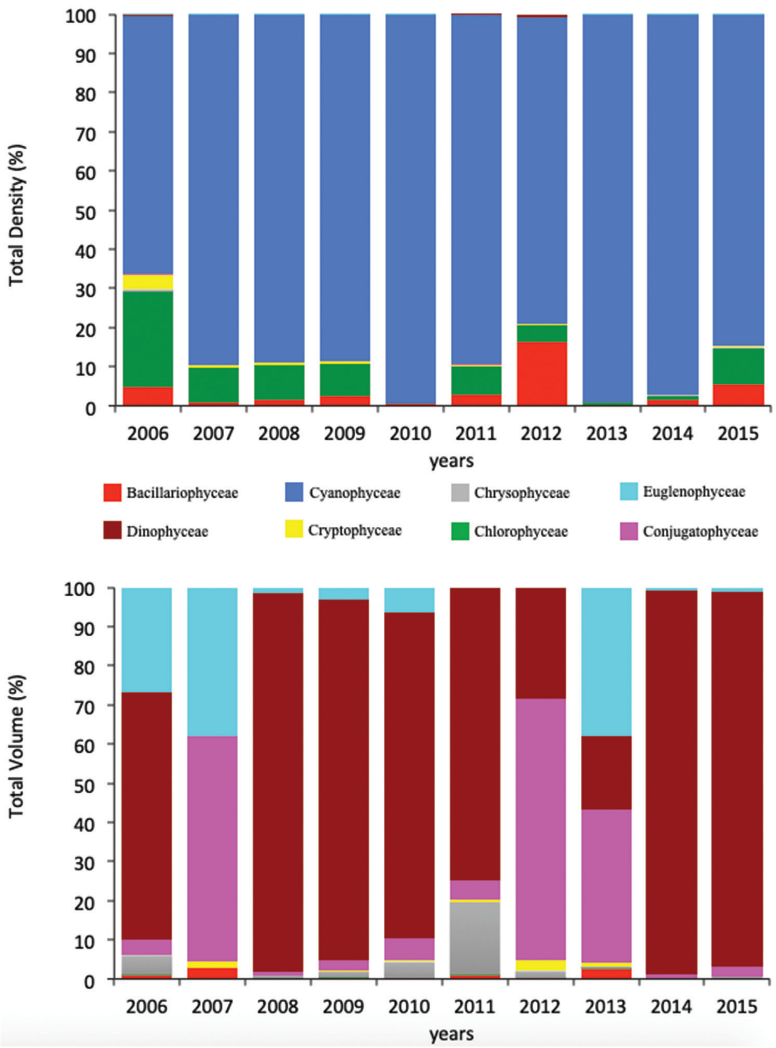

Figure 5. Multiannual (2006–2015) variation of the percentage contribution of all phytoplankton classes to the total cell density (upper panel) and mean cell volume (lower panel) of the whole phytoplankton community in Bidighinzu Lake in summer. BAC = Bacillariophyceae, CHL = Chlorophyceae, CHR = Chrysophyceae, CON = Conjugatophyceae, CRY = Cryptophyceae, CYA = Cyanophyceae, DIN = Dinophyceae, EUG = Euglenophyceae.

Chroococcales were mainly represented by *Aphanocapsa* sp., *Merismopedia* sp. and *Chroococcus* sp. (mean cell volume of 0.76 μm^3, 1.12 μm^3 and 77.15 μm^3, respectively) at the beginning of the study decade and by *Aphanothece* sp., *Aphanocapsa* spp. and *Merismopedia tenuissima* Lemmermann (mean cell volume of 1.30 μm^3, 0.44 μm^3 and 0.54 μm^3, respectively) at the end. Amongst Nostocales, *Dolichospermum flos-aquae* (Brébisson ex Bornet & Flahault) P. Wacklin, L. Hoffmann & J. Komárek (mean cell

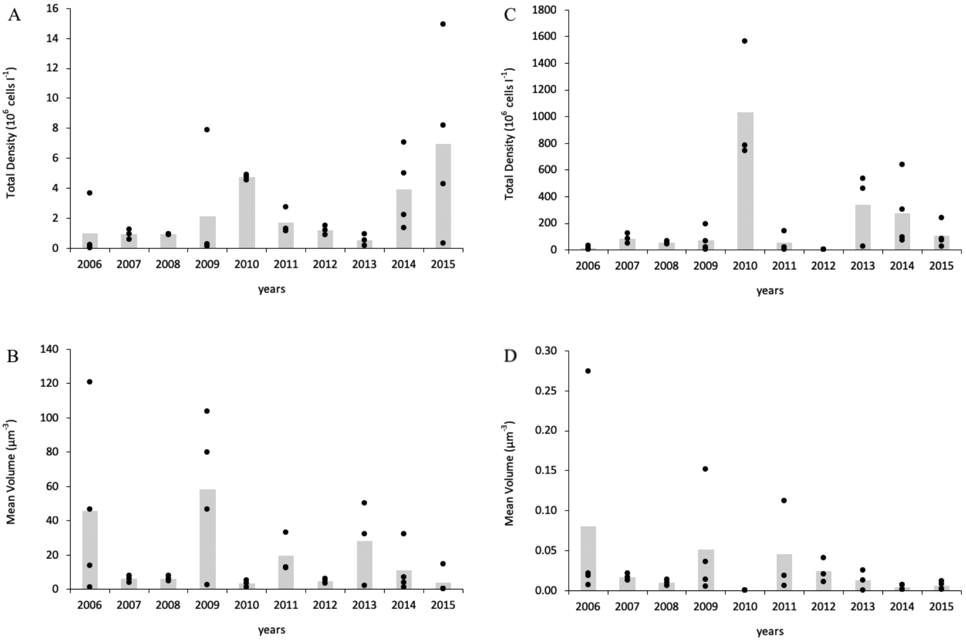

Figure 6. Multiannual (2006–2015) variation of total cell density (upper panels) and mean cell volume (lower panels) of Bacillariophyceae (A and B) and Cyanophyceae (C and D) in Bidighinzu Lake in summer. Each black dot represents a sample and each column represents the mean value.

volume of 73 μm^3) and *Dolichospermum spiroides* (Klebhan) Wacklin, L. Hoffmann & Komárek (mean cell volume of 519 μm^3) determined the highest cell density values at the beginning and at the end of the considered period, respectively. Centrales were mainly represented by *Cyclotella* spp. and *Aulacoseira granulata* (Ehrenberg) Simonsen (mean cell volume of 1035 μm^3 and 982 μm^3, respectively) at the beginning of the study and by *A. granulata* and *Aulacoseira granulata* var. *angustissima* (Otto Müller) Simonsen (mean cell volume of 608 μm^3 and 180 μm^3, respectively) during the last years.

Relationships between phytoplankton and environmental variables (2006–2015)

The results of the RDAs showed that, in summer, all environmental variables accounted for 57.8% and 53.8% of the variation in the 2006–2015 phytoplankton total cell density and volume, respectively. These results were confirmed when the years 2010 (exceptional peak of the total phytoplankton density) and 2015 (breaking of stratification due to the activation of the aeration system) were excluded from the analysis.

DIN ($F = 4.21$, $p = 0.004$), N-NH_4 ($F = 3.97$, $p = 0.002$) and TP ($F = 3.18$, $p = 0.007$) were significant environmental variables, which provided a greater explanation for the variability in summer total phytoplankton cell densitiy, followed by Z_{mix}/Z_{eu} ($F = 2.62$, $p = 0.029$) (Figure 7A). Total phytoplankton cell density and Cyanophyceae, Bacillariophyceae, Chrysophyceae and Euglenophyceae cell densities were negatively correlated

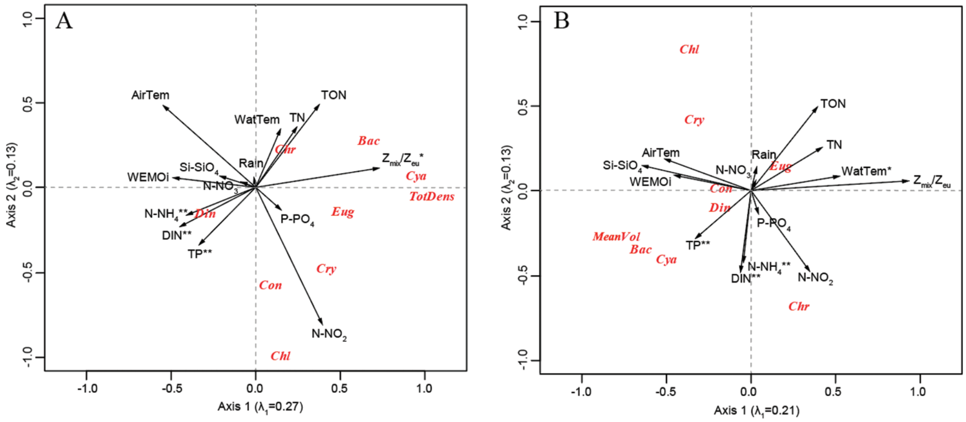

Figure 7. Results of Redundancy Analysis on the relationships between environmental explanatory variables (vectors) and phytoplankton variables (responses), considering (A) the total phytoplankton cell density and (B) the mean phytoplankton cell volume data during the decade 2006–2015 in Bidighinzu Lake in summer. Eigenvalues of the first two axes are indicated by λ_1 and λ_2. Asterisks indicate statistical significance (*p < 0.05; **p < 0.01; ***p < 0.001) of environmental variables. WEMOi, Western Mediterranean Oscillation index; AirTem, air temperature; Rain, rainfall; Z_{mix}/Z_{eu}, mixing zone and euphotic zone ratio; WatTem, water temperature; Si-SiO_4, silicate; P-PO_4, orthophosphate; TP, total phosphorous; N-NO_3, nitrate; N-NO_2, nitrite; N-NH_4, ammonia; DIN, dissolved inorganic nitrogen; TN, total nitrogen; TON, total organic nitrogen; Bac, Bacillariophyceae; Chl, Chlorophyceae; Chr, Chrysophyceae; Con, Conjugatophyceae; Cry, Cryptophyceae; Cya, Cyanophyceae; Dino, Dinophyceae; Eug, Euglenophyceae; TotDens, total phytoplankton density; MeanVol, mean cell volume of the whole phytoplankton community.

with these nutrients, but Dinophyceae was positively correlated (Figure 7A). Instead, the cell density of total phytoplankton and of the phytoplankton classes mentioned above, with the exception of Dinophyceae, were positively correlated with Z_{mix}/Z_{eu}.

TP ($F = 3.34$, $p = 0.004$), DIN ($F = 2.82$, $p = 0.007$) and N-NH_4 ($F = 3.18$, $p = 0.009$), followed by water temperature ($F = 3.52$, $p = 0.027$), provided a greater explanation for the variability in summer mean phytoplankton cell volume (Figure 7B). Mean cell volumes of the whole phytoplankton community and of Bacillariophyceae, Cyanophyceae, Dinophyceae and Conjugatophyceae were positively correlated with TP and negatively correlated with water temperature (Figure 7B). Euglenophyceae cell volume was negatively correlated with TP Chrysophyceae and Chlorophyceae cell volumes were related positively and negatively to DIN and N-$NH_{4,}$ respectively.

Long-term dynamics of meteo-climatic, environmental and phytoplankton parameters (historical dataset 1988–2015)

A significant decreasing trend (Mann-Kendall test: $S = -949$, $p < 0.001$, $n = 84$) was observed for the WEMO index in summer during the period 1988–2015. One-way ANOVA highlighted significant differences in the WEMO index, TP and P–PO_4

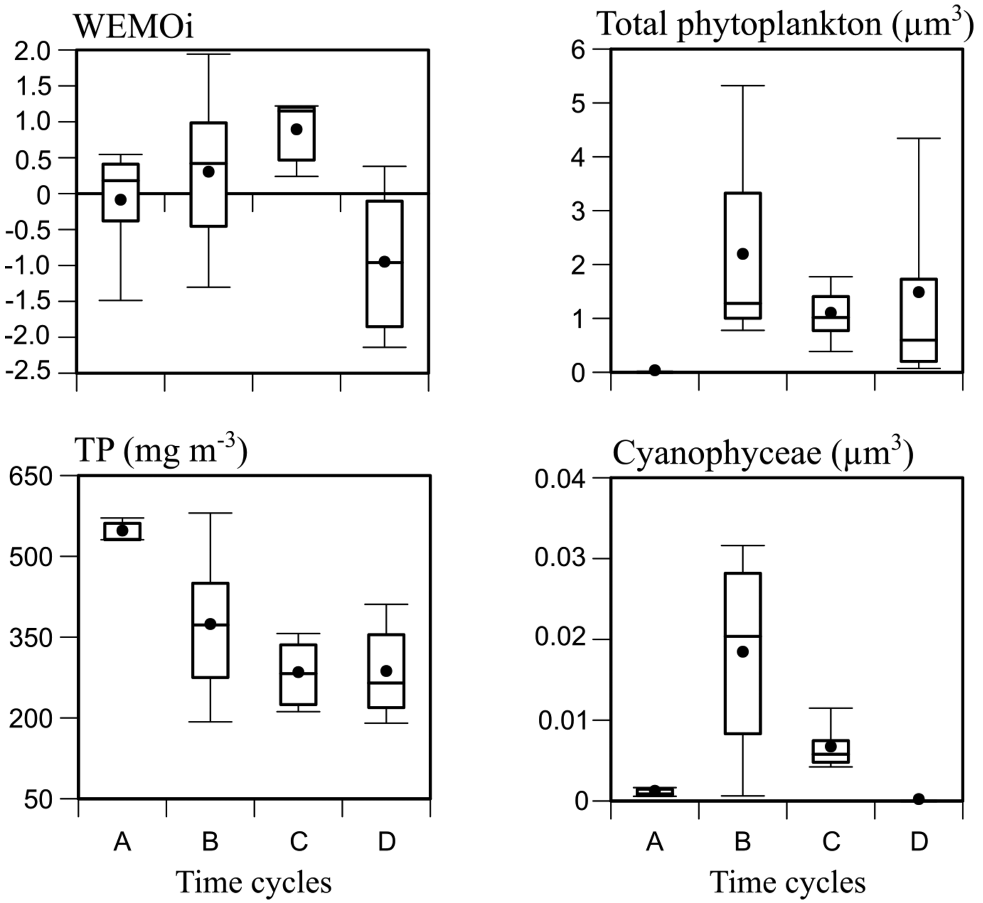

Figure 8. Variation of the Western Mediterranean Oscillation index (WEMOi), total phosphorous (TP), mean cell volume of the whole phytoplankton community (Total phytoplankton) and mean Cyanophyceae volume during the four time cycles of samplings (A = 1988–1989, B = 1994–1997, C = 2003, D = 2006–2015) in summer in Bidighinzu Lake. The lowest, second lowest, middle, second highest and highest lines in the box plots represent the 10th percentile, 25th percentile, median, 75th percentile and 90th percentile, respectively. Means are represented by black dots.

amongst the four time-cycles of samplings (i.e. 1988–1989, 1994–1997, 2003, 2006–2015; Table 5). Mean values of the summer WEMO index increased from -0.1 in the first cycle (1988–1989, A) to 0.8 in the third cycle (2003, C), then decreased strongly to -1.0 in the last period (2006–2015, D) (Tukey's test: B-D, $p = 0.011$; C-D, $p < 0.001$) (Figure 8). Mean values of summer TP decreased from 540.5 mg m^{-3} at the beginning of the time series (1988–1989, A) to 246.9 mg m^{-3} at the end (2006–2015, D) (Tukey's test: A-D, $p < 0.001$) (Figure 8). The analysis did not reveal significant differences in total phytoplankton cell density amongst the different time cycles, even if significant differences were detected in total cell density of Bacillariophyceae, Chlorophyceae, Chrysophyceae, Cryptophyceae, Conjugatophyceae, Dinophyceae and

Table 5. Results of the one-way ANOVA (F-test and p-value) to assess significant differences in selected environmental and phytoplankton variables amongst sampling year cycles (1988–1989, 1994–1997, 2003, 2006–2015) in summer in Bidighinzu Lake (WEMOi, Western Mediterranean Oscillation index; Z_{mix}/Z_{eu}, mixing zone-euphotic zone ratio; Si-SiO_4, silicate; P-PO_4, orthophosphate; TP, total phosphorous; N-NO_3, nitrate; N-NO_2, nitrite; N-NH_4, ammonia; DIN, dissolved inorganic nitrogen; TN, total nitrogen; TON, total organic nitrogen, ns = not significant).

	F	p		F	p		F	p
WEMOi	7.83	***	Total cell density	1.76	ns	Mean cell volume	3.60	*
Water Temperature	1.03	ns	Bacillariophyceae	10.92	***	Bacillariophyceae	77.56	***
Water Transparency	1.32	ns	Chlorophyceae	11.27	***	Chlorophyceae	406	***
Z_{mix}/Z_{eu}	1.24	ns	Chrysophyceae	13.35	***	Chrysophyceae	0.15	ns
Si-SiO_4	2.26	ns	Cryptophyceae	20.45	***	Cryptophyceae	46.96	***
P-PO_4	9.44	***	Conjugatophyceae	9.44	***	Conjugatophyceae	0.89	ns
TP	7.33	***	Cyanophyceae	2.01	ns	Cyanophyceae	123.3	***
N-NO_2	0.55	ns	Dinophyceae	9.47	***	Dinophyceae	9.43	***
N-NO_3	1.45	ns	Euglenophyceae	6.02	**	Euglenophyceae	3.91	*
N-NH_4	0.25	ns						
DIN	0.33	ns						
TN	2.21	ns						
TON	1.17	ns						

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

Euglenophyceae, but not Cyanophyceae (Table 5). Instead, significant differences in mean cell volume of the whole phytoplankton community and in Bacillariophyceae, Chlorophyceae, Cryptophyceae, Cyanophyceae, Dinophyceae and Euglenophyceae cell volumes were assessed (Table 5). Summer mean cell volumes of total phytoplankton and Cyanophyceae increased at the beginning of the time series, from 0.004 μm^3 and 0.001 μm^3 to 2.48 μm^3 and 0.02 μm^3, respectively, from the first (1988–1989, A) to the second sampling cycle (1994–1997, B). Subsequently, summer mean values decreased strongly to 1.45 μm^3 and to a value very close to 0 μm^3 in the last sampling cycle (2006–2015, D) (Tukey's test: B-D, $p < 0.001$ for both) (Figure 8).

Long-term variation of watershed land cover and uses (1990–2000–2008)

Significant variations in land cover and land use have been assessed in the Bidighinzu Lake's watershed during the last 20 years. The GIS analyses of CORINE maps for the time series 1990, 2000 and 2012, highlighted a decrement in Agricultural Area of about 7.2% from 1990 (32.59 km^2) to 2000 (30.25 km^2) and of a further 16.8% from 2000 to 2012 (25.17 km^2), with a total decrement of about 23% from 1990 to 2012. On the other hand, Natural and semi-Natural Areas increased by about 14.6% from 1990 (16.63 km^2) to 2000 (19.06 km^2) and by a further 25.3% from 2000 to 2012 (23.89 km^2), with a total increment of about 44% from 1990 to 2012. A noteworthy variation in Urban Area was also assessed with an increase of about 2.5% from 1990 (1.61 km^2) to 2000 (1.65 km^2) and of a further 4.8% from 2000 to 2012 (1.73 km^2), with a total increment of about 7.5% from 1990 to 2012.

Discussion

This work showed that, during the decade 2006–2015, significant multiannual changes occurred in both environmental and phytoplankton variables in Bidighizu Lake, more in summer than during the other seasons. Our results confirmed those reported from other LTER studies in Sardinian reservoirs (Mariani et al. 2015, Pulina et al. 2016, Padedda et al. 2017). In the Mediterranean climate, winters are mild and wet and summers are warm-to-hot dry, whereas spring and autumn are more variable (Lionello 2012). Winter and summer are also the most critical seasons for freshwater ecosystems and predictions of most climate models (García-Ruiz et al. 2011) indicate that major environmental changes are expected during these periods (García-Ruiz et al. 2011, Lionello 2012).

Multiannual summer decrement in nutrient concentrations

We observed strong decreases in summer concentrations of various nutrients, i.e. Si-SiO_4, TP, DIN and N-NH_4 and a significant but weaker increment of TON values during the decade 2006–2015 in Bidighizu Lake. It has been previously documented that the phosphorous reduction in summer has been on-going since 1978, immediately after the application of recovery actions for the reservoir and it was most likely favoured by the diversion of urban and industrial wastes being initiated in 1987 (Marchetti et al. 1992, Lugliè et al. 2001, Mariani et al. 2015). The ANOVA results of the present study suggest that the decrease in summer nitrogen and silicate and the increase in TON instead may have been more recent and they had not been reported so far (Mariani et al. 2015).

Explaining how the dissolved nitrogen forms vary in lakes is not straightforward as they depend upon variations in natural and pollutant sources and a variety of physical, biological and metabolic features of the lake (Quirós 2003). The strong decrement of DIN values in Bidighinzu Lake could be attributed to the concomitant decrease of N-NO_3 and N-NH_4 in the reservoir. In eutrophic lakes, such as the Bidighinzu Lake, nitrification may stop in conjunction with summer thermal stratification associated with hypolimnetic relatively low dissolved oxygen and low pH. As a consequence, denitrification may increase and lead to a complete natural depletion of nitrate. In hypoxic and anoxic conditions, N-NH_4 is mainly generated as a product of decomposition of the organic matter instead of N-NO_3 (Wetzel 2001). Consequently, N-NH_4 concentrations also depend on the quantities of settled organic matter and oxygen concentrations in the waters. Further, in hypoxic and anoxic conditions, denitrification causes the formation of volatile nitrogen compounds that are released from aquatic ecosystems, contributing to reduced concentrations of inorganic nitrogen compounds (Wetzel 2001). Unfortunately, the rate of denitrification was not measured in Bidighinzu Lake; however, based on oxygen concentrations over time (data not shown), it was likely to be relevant in summer in the majority of the considered annual cycles. N_2-fixation due to Cyanobacteria was also not measured. Finally, as summer is also the dry season in the Mediterranean climate, the reduction of external loadings in that season causes the loss of an important input of inorganic nitrogen carried by the drainage from river basin waters (Coppens et al. 2016, Padedda et al. 2010, 2017). In the present work, the GIS analysis of the Bidighinzu watershed land cover and land use variations over the last 20 years highlighted the significant decrease in agricultural areas and the opposing relevant increase in natural and semi-natural areas accompanied by a small increase in urban areas. These changes may have contributed significantly to a shift in material inputs, supporting the significant trends observed. In particular, considering the different forms of nitrogen, the significant increase of TON in the reservoir may be linked to the partially re-naturalisation of the watershed (Volungevicius et al. 2015). This latter, in turn, may also be responsible, at least in part, for the significant detriment of inorganic nitrogen compounds (DIN and $N\text{-}NH_4$) due to reduction in the use of fertilisers (Jomaa et al. 2016). This is a relevant outcome compared to other reservoirs in Sardinia, where dissolved inorganic nitrogen is generally the main component of TN. For example, Padedda et al. (2015) found a contribution of DIN around 60% of TN in Lake Cedrino, an ecosystem similar to Lake Bidighinzu.

Multiannual trends of summer phytoplankton

Relevant changes in summer multiannual phytoplankton dynamics were observed simultaneously to the summer nutrient reduction in Bidighinze Lake during the decade 2006–2015. The total phytoplankton cell density increased significantly, whereas the mean cell volume of the whole phytoplankton community decreased significantly. Consequently, it is not surprising that the total phytoplankton biomass and chlorophyll *a* concentration did not show any significant trend in the same period. The phytoplankton classes that contributed more to total density in the analysed decade remained mainly Cyanophyceae, followed by Chlorophyceae and Bacillariophyceae as observed in the previous years (Mariani et al. 2015). While significant multiannual trends were not observed for Chlorophyceae, the Cyanophyceae and Bacillariohyceae cell density increased significantly from 2006 to 2015 and their mean cell volume decreased strongly, determining the same multiannual trends for total cell density and mean cell volume of the whole phytoplankton community. According to the ANOVA results, the increment in total phytoplankton cell density was recent (i.e. concerning the period 2006–2015), whereas the reduction in mean cell volume of the whole phytoplankton community and of Cyanophyceae was found to have begun earlier, from the late 1990s. The strong decrease in mean phytoplankton cell volume can be explained by changes in the taxa composition of phytoplankton, not observed at classes' level, but present at order level and consisting of an increasing density of smaller taxa during the decade under study. In fact, the density of Cyanophyceae Chroococcales increased strongly, replacing the larger Cyanophyceae Nostocales at the end of the considered period. In addition, changes in Chroococcales species composition, with an increasing importance of smaller Chroococcales species (e.g. *Aphanothece* sp. and

Merismopedia tenuissima instead of *Aphanocapsa* sp. and *Chroococcus* sp.) caused a strong decrement of the Chrooccocales cell volume during the years. Similarly, the density of Bacillariophyceae Centrales, smaller than the Bacillariophyceae Pennales, increased significantly in the reservoir. Amongst Centrales, smaller species (e.g. *Aulacoseira granulata* var. *angustissima*) replaced the larger ones (e.g. *Cyclotella* spp.) in the last years of the study, inducing the reduction of mean Centrales cell volume during the decade. The increasing density of smaller Cyanophyceae Chroococcales and Bacillariophyceae Centrales during the long-term decrement in nutrient concentrations was also observed in Temo Lake, a reservoir located in the same geographical area of Bidighinzu Lake in Sardinia (Pulina et al. 2016), as well as in the natural subalpine Maggiore Lake (Kamenir and Morabito 2009).

Environmental drivers of multiannual phytoplankton variations

Statistical analysis revealed a strong relationship between the interannual patterns of algal nutrients and of total cell density and mean cell volume of the whole phytoplankton community at the Bidighinzu Lake in summer. Specifically, the summer TP and DIN (mainly N-NH_4) reduction affected, significantly and negatively, the summer total cell density. On the other hand, summer TP and DIN reduction significantly influenced the summer mean cell volume in a positive way. The decrement in phytoplankton size at lower nutrient concentrations was observed elsewhere, such as in another eutrophic Sardinian reservoir (Temo Lake, Pulina et al. 2016) and in different lake typology worldwide (e.g. Lehman 1991, Masson et al. 2000, Kamenir and Morabito 2009). The positive relationship between phytoplankton cell volume and nutrient concentrations was also detected experimentally in a highly eutrophic urban lake in Austria (Dokulil et al. 2007). Small-cell sized phytoplankton are expected to be favoured under lower nutrient concentration because of the high surface area to volume ratio and smaller diffusion boundary layer that enables rapid nutrient exchange through the cell surface (Harris 1994). In addition, the different strategies of nutrient utilisation can explain the distribution patterns of phytoplankton cell size classes along nutrient concentration gradients (Litchman and Klausmeier 2008). According to recent sudies on phytoplankton's nutrient uptake-related traits, low nutrient environments should favour smaller-celled species while high nutrient environments should favour larger-celled species since maximum nutrient uptake velocity (V_{max}) and half-saturation constant for nutrient uptake (K) are positively correlated with cell size (Litchman et al. 2007).

In the hypertrophic Lake Arancio (Sicily, Italy), as well as in many other Sicilian reservoirs, nutrients have never been observed to play an important role in determining the structure of the phytoplankton community (Naselli-Flores and Barone 2007). The morphological changes observed in phytoplankton of this lake were instead strongly related to water mixing and underwater light availability (Naselli-Flores and Barone 2007). Specifically, Z_{mix}/Z_{eu} was detected as one of the most important factors in determining phytoplankton structure in a study of 21 Sicilian reservoirs (Naselli-Flores

2000). In our study case, a significant increasing trend was detected for Z_{mix}/Z_{eu} values during the present study decade 2006–2015. This change might have been partially linked to the activation of the water aeration system in the reservoir only in the period 2014 and 2015. This experimental activity prevented the summer thermal stratification of Bidighinzu Lake in 2015 but not in 2014 (Mariani et al. 2016). Our data analysis showed that the summer Z_{mix}/Z_{eu} value was a significant positive driver of summer dynamics of total phytoplankton density in Bidighinzu Lake, although its role was statistically weaker than that of nutrients. Higher Z_{mix}/Z_{eu} values in Bidighinzu Lake enhanced an increment in total phytoplankton density and especially in smaller species density, though significant relationships with total phytoplankton cell volume were not observed. These findings agreed with the evidence that smaller cells have a lower sinking rate compared to the larger ones (Litchman and Klausmeier 2008) and may be more efficient at utilising low light because of the smaller packaging effect, with self-shading of light-capturing pigments (Kirk 1994, Litchman et al. 2010).

The strong size reduction of marine phytoplankton under increasing ocean warming has also been well documented, although without univocal evidence (Gardner et al. 2011). It remains controversial whether the cell size reduction under increasing temperatures is i) a direct temperature effect or ii) an indirect effect mediated by changes in size selective grazing or by enhanced nutrient limitation that alter the selection on phytoplankton's nutrient uptake-related traits, favouring smaller species over larger cells (Peter and Sommer 2013, Lewandowska et al. 2014). In the present work, no significant multiannual trends of meteo-climatic parameters were detected at the Bidighinzu Lake during the decade 2006–2015. However, summer water temperature significantly affected summer phytoplankton cell volume in the reservoir, though in a weaker way compared to the nutrients. The opposite trends observed for summer phytoplankton cell volume (decreasing) and summer water temperature (increasing), though not significant, seem to agree with the Atkinson's ecological rule, according to which the average size of individuals is inversely related to temperature (Atkinson et al. 2003), also supporting similar findings proposed by several study cases in literature (Sommer et al. 2017b and reference therein).

Conclusions

A significant multiannual decreasing trend in nutrient concentrations was detected in summer in the Bidighinzu Lake simultaneously with reduced anthropic pressure in its watershed. These environmental changes significantly affected the phytoplankton community of this Mediterranean reservoir, favouring a strong increment in the density of smaller Bacillariophyceae and Cyanophyceae taxa. This increment led to a significant multiannual increasing trend in total phytoplankton cell density and a strong multiannual decrease in mean phytoplankton cell volume. In addition, the variations of Z_{mix}/Z_{eu} and of water temperature in summer favoured smaller phytoplankton taxa, although their role in structuring phytoplankton communities was statistically weaker compared to that of nutrients. We can not rule out that the consumer pressure may also have acted on the phytoplankton community of Bidighinzu Lake, as observed in other environments (Peter and Sommer 2012, Lewandowska et al. 2014, Boyce et al. 2015), but data on grazers are not available for our study area.

This work confirms the importance of acquiring long-term ecological data in studies on phytoplankton to understand the temporal evolution of aquatic ecosystems in relation to natural and anthropogenic forces. The affirmation of smaller phytoplankton cells in Bidighinzu Lake suggests a shift of the system towards a less energy-efficient trophic web based on smaller and lower-quality prey for grazing zooplankton and planktivorous fish. The ecological changes we observed in the reservoir were most probably due to the complex and synergic interactions between the investigated environmental variables related to changing anthropic pressures and climate variability. Considering the need and scarcity of high quality water in semi-arid regions and the paucity of studies on Mediterranean reservoirs, our findings provide useful information for our understanding of these crucial ecosystems and for their management and conservation, thus adding greater value to the LTER-Italy network.

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Supplementary material 1

Figure S1

Authors: Silvia Pulina, Antonella Lugliè, Maria Antonietta Mariani, Marco Sarria, Nicola Sechi, Bachisio Mario Padedda

Data type: multimedia

Explanation note: Multiannual (2006–2015) variation of the contribution of the orders Centrales and Pennales to the Bacillariophyceae total cell density (upper panel) and mean cell volume (lower panel) in Bidighinzu Lake in summer.

Link: https://doi.org/10.3897/natureconservation.34.30116.suppl1

Supplementary material 2

Figure S2

Authors: Silvia Pulina, Antonella Lugliè, Maria Antonietta Mariani, Marco Sarria, Nicola Sechi, Bachisio Mario Padedda

Data type: multimedia

Explanation note: Multiannual (2006–2015) variation of the contribution of Cyanophyceae orders to the Cyanophyceae total cell density (upper panel) and mean cell volume (lower panel) in Bidighinzu Lake in summer (CHR = Chroococcales; NOS = Nostocales; OSC = Oscillatoriales).

Link: https://doi.org/10.3897/natureconservation.34.30116.suppl2