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Driving factors of the phytoplankton functional groups in a deep Mediterranean reservoir

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ABSTRACT

The control of phytoplankton growth is mainly related to the availability of light and nutrients. Both may select phytoplankton species, but only if they occur in limiting amounts. During the last decade, the functional groups approach, based on the physiological, morphological and ecological attributes of the species, has proved to be a more efficient way to analyze seasonal changes in phytoplankton biomass. We analysed the dynamics of the phytoplankton functional groups *sensu* Reynolds, recognising the driving forces (light, mixing regime, and nutrients) in the Sau Reservoir, based on a one–year cycle (monthly surface-water sampling). The Sau Reservoir is a Mediterranean water-supply reservoir with a canyon-shaped basin and a clear and mixed epilimnion layer. The long stratification period and high light availability led to high phytoplankton biomass (110.8 fresh-weight mg L^{-1}) in the epilimnion during summer. The reservoir showed P-limitation for phytoplankton growth in this period. All functional groups included one or more species (**X2**-*Rhodomonas* spp.; **Y**-*Cryptomonas* spp.; **F**-*Oocystis lacustris*; **K**-*Aphanocapsa* spp.) selected by resources, especially phosphorus. Species of *Cryptomonas* (group **Y**) dominated during the mixing period (winter season) in conditions of low light and relatively high availability of dissolved nutrients. Increases in water-column stability during spring stratification led to phytoplankton biomass increases due to the dominance of small flagellate functional groups (**X2** and **X3**, chrysophyceans). The colonial chlorophycean *O. lacustris* (group **F**) peaked during the mid-summer stratification, when the mixed epilimnion was clearly depleted in nutrients, especially SRP. High temperature and increases in nutrient concentration during the end-summer and mid-autumn resulted in a decrease of green algae (group **F**) and increase of *Aphanocapsa* spp. (cyanobacteria, group **K**) and dinoflagellates (group $\mathbf{L_o}$). The study also revealed the important role of physical processes in the seasonal gradient, in selecting phytoplankton functional groups, and consequently in the assessment of ecological status. The Q index (assemblage index) based on functional

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groups indicated the overall good ecological status of the Sau Reservoir, which varied as a function of the mixing regime. This is the first application of the Assemblage Index to a European water-supply reservoir.

1. Introduction

The physical structure of the environment and the availability of nutrients are not the only drivers of the periodicity of the phytoplankton assemblage. Both factors are, directly or indirectly, the most important variables likely to influence the general phytoplankton composition (Reynolds, 1980). This means that the synergy between the mixing regime and nutrients favours the phytoplankton community. Phytoplankton species in natural and man-made lakes have much in common. Both kinds of systems largely share the same species and have identical habitats, e.g., the pelagic zone (Kalff, 2002). Broadly similar algal types occur in both lakes and reservoirs, and the many species-specific responses to fluctuations in resource availability and the severity of processing constraints are similar in both kinds of habitats (Reynolds, 1999a).

Phytoplankton species have developed morphological and physiological adaptive strategies for surviving in different environments (Margalef, 1978; Reynolds, 1998). Based on Grime's (1979) seminal work on terrestrial vegetation and using morphological and physiological traits, Reynolds (1997) defined several phytoplankton functional groups that may potentially dominate or co-dominate in a given environment (Reynolds et al., 2002). These groups are often polyphyletic and share adaptive features, based on the physiological, morphological and ecological attributes of the species. At the present time, the phytoplankton functional groups approach uses 38 assemblages, identified by alpha-numeric codes according to their sensitivities and tolerances (Padisák et al., 2009). Two main ideas lie beneath the functional groups theory: i) a functionally well-adapted species is likely to tolerate the constraining conditions of factor deficiency more successfully than a less well-adapted species; ii) a habitat typically constrained by light, P, C, N, or other response variable is more likely to support species with the appropriate adaptations to function there. As a consequence, the term 'functional group' is sensitive to the sets of appropriate adaptive specializations and the clusters of species that have them. Splitting species into different functional groups requires a thorough knowledge of the autecology of single species or species groups (Padisák et al., 2009).

The phytoplankton functional groups approach applied to aquatic systems has provided important information for understanding the dynamics of species selection in the pelagic communities in temperate (Huszar et al., 2003; Leitão et al., 2003), tropical (Lopes et al., 2005; Sarmento et al., 2007), subtropical (Becker et al., 2009; Fabbro and Duivenvoorden, 2000; Kruk et al., 2002), and Mediterranean (Romo and Villena, 2005) regions. The phytoplankton functional groups approach, originally developed for lakes, is also useful for reservoirs, since both systems are generally similar considering the small spatial and temporal scales (Reynolds, 1999a).

Recently, Padisák et al. (2006) developed an index using functional groups (Q index). The index combines the weight of functional groups relative to the total biomass, with a factor number for each assemblage; which might be related to the type of water body (Padisák et al., 2006). Crossetti and Bicudo (2008) successfully applied this index in an urban tropical reservoir and Becker et al. (2009) in a subtropical water-supply reservoir; it is a promising tool in assessing the ecological status of aquatic ecosystems independently of the geographic region (Padisák et al., 2006).

In light of previous reports on this reservoir that revealed the vertical and longitudinal dynamics of the phytoplankton community (Marcé et al., 2007; Caputo et al., 2008) and the importance of the mixing regime in this system (Armengol et al., 1999; Han et al., 2000; Rueda et al., 2006), we hypothesised that the mixing regime plays an important role in phytoplankton dynamics in the pelagic zone, because of the availability of resources such as light and nutrients. To test our hypothesis, we evaluated the phytoplankton dynamics using the functional groups approach *sensu* Reynolds (Reynolds et al., 2002) in this system, identifying the driving forces, based on the autecology of the group related to their adaptations to environmental changes. We also assessed the reservoir's ecological status through the application of the assemblage index. Although it may be important in controlling species composition, we did not consider biological regulation by zooplankton grazing in this study.

2. Materials and methods

2.1. Study site

Sau Reservoir is located in northeast Spain (41°58′N; 2°22′E). It is canyon-shaped, 18.5 km long, and deep ($z_{max} = 75$ m), located on the middle stretch of the 200-km-log River Ter, at 425 m a.s.l. (Fig. 1). For some years the river received strong urban and agricultural inputs (Vidal and Om, 1993; Sabater et al., 1995), which diminished after the construction of tertiary sewage-treatment plants in the economically most important cities of the watershed. The waters of the Sau are now somewhat less eutrophic (Armengol et al., 1999; Marcé et al., 2007). Sau is one of five reservoirs that provide water for the 4.4 million inhabitants of the Barcelona region, which includes a hundred cities. The River Ter has a very irregular flow, which is a typical feature of Mediterranean rivers. The discharge of the river into the reservoir can vary from 2 $m^3 s^{-1}$ to less frequent values of 2000 $m^3 s^{-1}$, with an average of 18 $m^3 s^{-1}$ (Ordóñez, unpublished data). The largest contributions from the Ter occurred in 2003, with 562.9 hm^3 of inflow. Inflows in 1965–1990 and 1991–1998 were closer to the annual averages, 540.5 and 547.1 hm^3, respectively (Ordóñez, unpublished data).

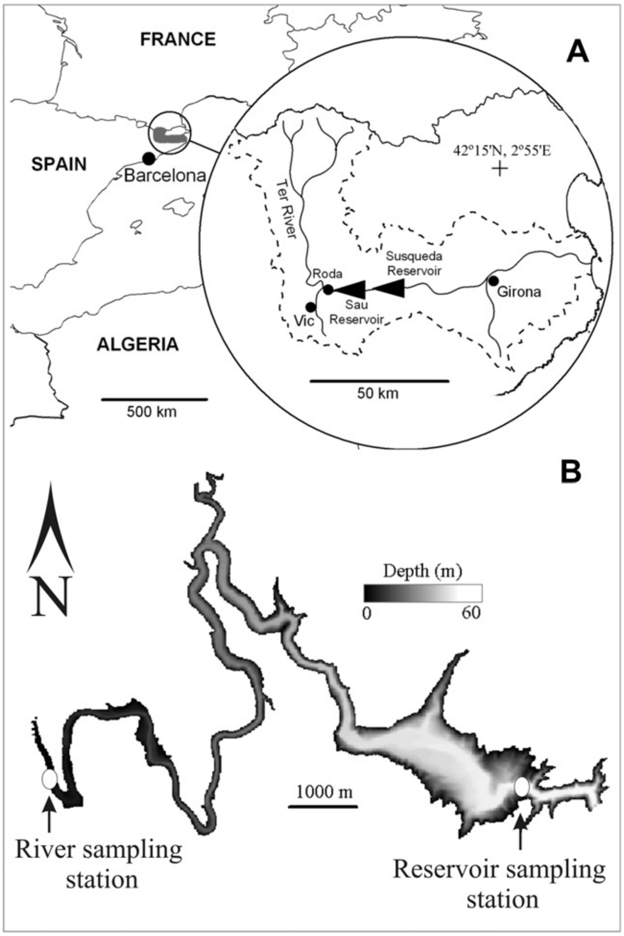

Fig. 1 – A-Location of Barcelona and the Sau Reservoir; B-Sau Reservoir showing the sampling stations and the main tributary (River Ter).

The local climate is Mediterranean (Csa type; Köppen, 1936) with humid winters and warm dry summers, an annual mean temperature of 17.5 °C, and an annual mean total precipitation of 1000 mm (753 mm for the period 2000–2008).

2.2. *Sampling*

We took water samples at monthly intervals from January 2003 through November 2003, at the sampling station located in a deep lacustrine section near the dam (Fig. 1). We selected the sampling stations and depths based on continuous monitoring of the reservoir.

We measured the depth profiles of temperature, pH, oxygen concentration, and conductivity with a high-resolution multiparametric probe SBE 19 plus (CTD). Based on these profiles, we collected a variable number of water samples with a 5 L UWITEC hydrographic bottle. For this study, we used the means of dissolved nutrients from the eplimnion layer (0.5 and 5.0 m depths). We determined the phytoplankton community from a 5.0 m integrated water sample collected with a plastic pipe. We fixed the samples with neutral Lugol's solution.

2.3. *Sample analysis*

We analysed the dissolved inorganic nutrients: soluble reactive phosphorus (SRP), nitrate ($N{-}NO_3^-$), ammonium ($N{-}NH_4^+$), and soluble reactive silicate (SRSi), in filtered samples. We measured the SRP spectrophotometrically (Murphy and Riley, 1962), $N{-}NO_3^-$ by liquid chromatography (Konik KNK 500-A), $N{-}NH_4^+$ by the indophenol spectrophotometric method (Solorzano, 1969), and SRSi by the molybdate method (Mullin and Riley, 1955). We counted the phytoplankton with an inverted microscope (Utermöhl, 1958) at 400x, noting the settling units (cells, colonies, and filaments) in random fields (Uhelinger, 1964), and counting at least 100 specimens of the most frequent species ($p < 0.05$; Lund et al., 1958).

2.4. *Data analysis*

We calculated the euphotic zone (z_{eu}) as 2.7 times the Secchi depth (Cole, 1994). We estimated the mixing zone (z_{mix}) from the dissolved-oxygen profile, assuming that the mixing depth is where dO_2/d_z achieves its maximum value (Armengol et al., 1999). We used the euphotic zone to mixed layer (z_{eu}/z_{mix}) ratio as a measure of light availability in the mixed layer (Jensen et al., 1994).

We calculated the algal biovolume using formulae for geometric shapes (Hillebrand et al., 1999), and expressed the fresh-weight unit in mass, where 1 $mm^3\ L^{-1} = 1\ mg\ L^{-1}$ (Wetzel and Likens, 2000). We combined the species contributing >5% to the total biomass into functional groups, using the criteria of Reynolds et al. (2002). The assemblage index (Q index) followed Padisák et al. (2006) and includes the relative share (p_i, where $p_i = n_i/N$; n_i biomass of the i-th functional group; N: total biomass) of functional groups in total biomass, and a factor number (F) established for the i-th functional group in the given lake type. For this, we integrated the biological data arithmetically. We assigned an F value ranging from 0 to 5, with higher values for the more-pristine assemblages on the reservoir, and lower values for assemblages typical of less-pristine conditions. We used five levels of classification for the Q index: 0–1: poor ecological status; 1–2: tolerable; 2–3: medium; 3–4: good; and 4–5: excellent.

We performed a principal components analysis (PCA) using the PC-ORD program (McCune and Mefford, 1997) to determine spatial and temporal changes of physical and chemical conditions. For the ordination analysis, we transformed the abiotic and biological data by $\log_x + 1$. In the PCA analysis we included temperature, dissolved oxygen, conductivity, pH, z_{eu}/z_{mix} ratio, nitrate ($N{-}NO_3^-$), ammonium ($N{-}NH_4^+$), soluble reactive phosphorus (SRP), soluble reactive silicon (SRSi), and chlorophyll-*a*.

3. Results

3.1. *Physical and chemical scenario*

Temperature profiles allowed us to identify only one period of total vertical mixing, which occurred during winter (January–February 2003; Fig. 2). During the summer the water column was strongly stratified (July–August 2003). The mixing zone

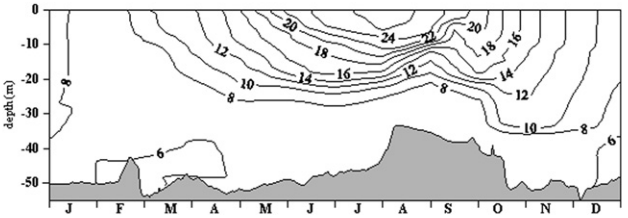

Fig. 2 – Depth-time isopleths for water temperature (°C) in the Sau Reservoir in 2003. The dark area is the bottom of the reservoir.

deepened in the beginning of the spring (March 2003) and autumn (October–November 2003) seasons.

The availability of light in the water column, expressed by the z_{eu}/z_{mix} ratio, may be an important determinant of phytoplankton selection. Considering that the entire water column was mixed during the winter, with a 20 and 17 m euphotic zone (z_{eu}) during January and February, respectively, light reached only 30–36% of the mixing zone (z_{mix}) (Fig. 3). From spring to mid-summer, the availability of light increased in the mixing zone, reaching 100% in July.

Chemical determinants were also seasonally variable. Mean alkalinity at the surface waters ranged from 0.9 mEq L^{-1} (summer) to 3.0 mEq L^{-1} (winter). Following the same pattern, pH was always higher than 8.0 (Table 1). Nutrient dynamics (Fig. 4) were mainly driven by the mixing regime. During the mixing period, dissolved inorganic N and P showed the highest values in the epilimnion. After the winter overturn, soluble reactive phosphorus (SRP) and ammonium ($N{-}NH^{+}{}_4$), but not nitrate ($N{-}NO_3^-$), decreased sharply throughout the strong end-summer stratification. Dissolved N and P concentrations increased again during the deepening of the mixing zone in autumn. Soluble reactive silicon (SRSi) showed a similar pattern to dissolved N and P, except for lower values in winter.

Total nitrogen (TN) and total phosphorus (TP) concentrations were very high during the entire cycle, with similar patterns to the dissolved nutrients (Table 1). SRP comprised 70% of the TP during the mixing period, but only 8% during stratification. DIN, however, was always a large fraction of TN (83 and 63% respectively). The TN:TP ratio (by atom) remained high throughout the year, always greater than 53 (Table 1).

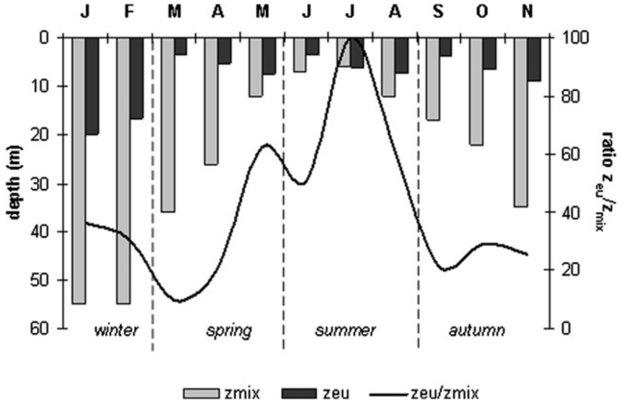

Fig. 3 – Seasonal variation of the mixing zone (z_{mix}), euphotic zone (z_{eu}), and the z_{eu}/z_{mix} ratio in the Sau Reservoir.

The principal components analysis (PCA) using nine abiotic variables explained 69.8% of data variability on the first two axes (axis 1 = 48.6%; axis 2 = 19.8%; Fig. 5). The most important variables for axis 1 ordination were temperature (−0.88), pH (−0.86), conductivity (0.74), $N{-}NH_4^+$ (0.57), SRP (0.84), SRSi (0.75), and chlorophyll *a* (−0.81). In regard to axis 2, DO (−0.50), $N{-}NO_3^-$ (0.54) and z_{eu}/z_{mix} (0.63) were the most important variable for its ordination. The PCA results indicated that the first principal component reflected the seasonal gradient and the mixing regime (Fig. 5). On the negative side of axis 1, sampling units of summer and autumn seasons correlated with strong stratification and higher values of chlorophyll-*a*, temperature, and pH. On its positive side, the sampling units of winter and spring correlated with mixing and, consequently, higher conductivity and dissolved nutrient concentrations.

3.2. *Phytoplankton dynamics*

The 98 algal species identified were members of eight major taxonomic categories and 16 functional groups. The 19 descriptor species (>5% of the total biomass) were members of 11 functional groups (Table 2). Cyanobacteria, cryptophyceans, and chlorophyceans dominated at least once in the seasonal cycle, and **F**, **K**, and **Y** were the main functional groups.

A unimodal pattern of the total phytoplankton biomass was apparent, with a maximum during mid-summer (110.8 mg L^{-1}), 3.8 times higher than the annual mean (Fig. 6). The winter mixing showed the lowest value of the average phytoplankton biomass (8.1 mg L^{-1}), while during the summer stratification the average was 64.6 mg L^{-1} (Table 1).

The phytoplankton functional groups occurred in the Sau Reservoir, with one or more species contributing to their composition (Fig. 6). During the winter mixing, functional group **Y** (crytophyceans – *Cryptomonas curvata*, *Cryptomonas* sp., and *Rhodomonas minuta*) dominated (47% of biomass). Phytoplankton biomass increased in parallel to water-column stability during spring. Small flagellates of the functional groups **X2** (*Rhodomonas* spp.) and **X3** (chrysophyceans) dominated during the beginning of the period. During mid-spring, colonial green algae (functional groups **F**, *Oocystis lacustris* and **J**, *Coelastrum microporum*, *Scenedesmus* spp.) increased (Fig. 6); the biomass of the other groups was very low due to the dominance of group **F** (91%). Two species of *Aphanocapsa* (cyanobacteria, group **K**) supplanted the green algae at the end of summer. Subsequently, with the deepening of the mixing zone, cryptomonads (group **Y**) and *Aphanocapsa* (group **K**) shared dominance in the Sau Reservoir during autumn.

3.3. *Q index*

We applied the assemblage index (Q index) using the phytoplankton functional groups approach in the Sau. The factor *F* weights for each functional group identified appear in Table 2. The water quality varied with season, changing the ecological

Table 1 – Means and ranges of limnological variables in the epilimnion of the Sau Reservoir in 2003.

	Winter		Spring		Summer		Autumn	
	Mean	interval	Mean	interval	Mean	interval	Mean	interval
T water (°C)	7.3	6.9–7.8	12.9	10.6–16.2	24.4	22.3–21.8	7,3	12.6–21.8
pH	8.2	8.1–8.4	8.4	8.2–8.6	8.9	8.3–9.3	8.5	8.5–8.6
DO (%)	78.7	60.8–96.7	112.1	99.5–124.7	108.0	75.2–135.9	103.4	86.2–116.9
Cond (mS cm^{-1})	559.7	528.0–591.3	417.4	399.8–443.2	370.4	316.0–475.7	480.0	459.2–513.8
Alkal (meq L^{-1})	3.0	2.9–3.0	2.6	2.4–2.8	1.3	0.9–1.5	1.5	1.0–2.4
TN (μM)	184.1	167.0–201.2	183.2	164.2–204.5	89.3	64.2–113.1	124.2	89.9–141.5
TP (μM)	3.1	2.7–3.5	1.5	0.7–2.5	1.2	1.0–1.2	2.2	1.7–2.7
TN/TP (by atom.)	59.8	57.2–62.5	154.5	71.4–250.2	77.8	53.9–91.6	52.8	52.8
Chl *a* (μg L^{-1})	6.0	4.9–7.2	9.0	3.9–15.9	15.9	8.6–22.9	28.3	12.7–54.2

T = temperature; DO = dissolved oxygen; Cond = conductivity; Alkal = alkalinity; TN = total nitrogen; TP = total phosphorus; Chl = chlorophyll-a.

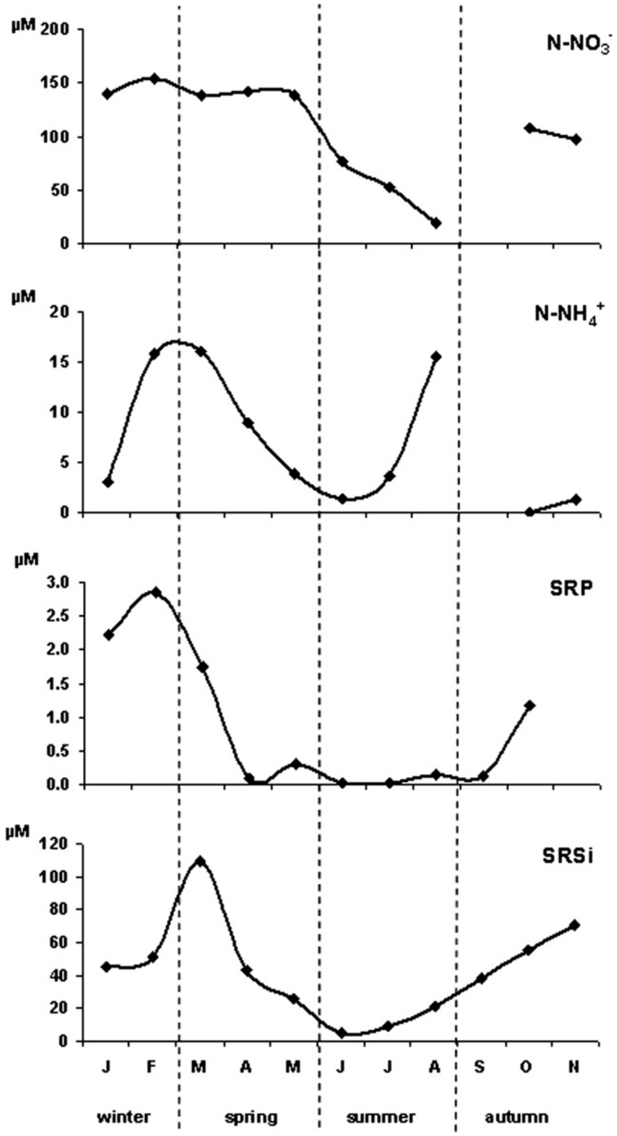

Fig. 4 – Seasonal variation of dissolved inorganic nutrients ($N–NO_3^-$, $N–NH_4^+$, SRP, and SRSi) in the epilimnion in the Sau Reservoir.

status of the Sau from *medium* to *excellent*. The highest qualification (4.7) occurred in the early spring, and the lowest evaluation (2.1) in the summer stratification (Fig. 7).

4. Discussion

Temporal changes in the water-column movements in lentic systems, related to the patterns of water circulation (mixing vs. stratification), are one of the main environmental forces that affect phytoplankton dynamics. In this respect, turbulence and the availability of growth-limiting resources for algae, such as light and nutrients, are the most important variables in determining phytoplankton assemblages (Margalef, 1978; Reynolds, 1984). The pelagic "world" of phytoplankton is a blend of deficiencies of differing intensity and frequency, especially with respect to the availability and

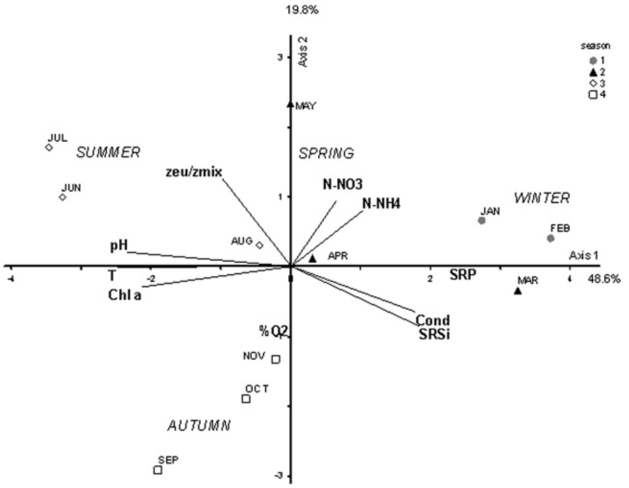

Fig. 5 – Principal components analysis (PCA) scores applied to environmental variables in the Sau Reservoir. sample units = months of the year seasons = 1 Winter; 2 Spring; 3 Summer; 4 Autumn Temp = water temperature; Cond = conductivity; z_{eu} = euphotic zone; z_{mix} = mixing zone; SRP = soluble reactive phosphorus; $N–NO_3^-$ = nitrate; $N–NH_4^+$ = ammonium; SRSi = soluble reactive silicate; Chl *a* = chlorophyll-*a*.

Table 2 – Main phytoplankton species with their taxonomic and functional groups, and respective *F* factors for the Sau Reservoir in 2003.

Functional Group	Phytoplankton Species	Taxonomic Group	F Factor
C	*Stephanodiscus* sp.	Bacillariophyceae	3
D	*Nitzschia palea* (Kütz.) W. Sm[a]	Bacillariophyceae	2
E	*Mallomonas* sp.	Chrysophyceae	5
F	*Oocystis lacustris* Chod.[a]	Chlorophyceae	2
H1	*Aphanizomenon flos-aquae* Ralfs *ex* Born.& Flah.	Cyanobacteria	0
J	*Coelastrum microporum* Näg.[a]	Chlorophyceae	2
J	*Lagerheimia subsalsa* (Lagerh.) Chod.	Chlorophyceae	2
J	*Scenedesmus acunae* Comas	Chlorophyceae	2
J	*Scenedesmus calyptratus* Comas	Chlorophyceae	2
J	*Scenedesmus disciformis* (Chod.) Foot. & Kom. [a]	Chlorophyceae	2
K	*Aphanocapsa* sp.[a]	Cyanobacteria	4
L_o	*Gymnodinium* sp.[a]	Dinophyceae	4
L_o	*Peridinium* sp.[a]	Dinophyceae	4
MP	*Pseudanabaena catenata* Lauterb[a]	Cyanobacteria	1
N	*Cosmarium laeve* Raben.[a]	Zygnemaphyceae	5
P	*Fragilaria crotonensis* Kitton	Bacillariophyceae	0
P	*Staurastrum* sp.	Zygnemaphyceae	0
S1	*Limnothrix* sp.	Cyanobacteria	0
W1	*Lepocynclis* sp.	Euglenophyceae	0
W2	*Trachelomonas volvocinopsis* Swirenko[a]	Euglenophyceae	1
X1	*Schroederia setigera* (Sch.) Lemm.	Chlorophyceae	3.5
X2	*Cryptomonas tetrapyrenoidosa* Skuja[a]	Cryptophyceae	5
X2	*Rhodomonas lacustris* Pasc. et Rutt.[a]	Cryptophyceae	5
X2	*Rhodomonas minuta* Skuja[a]	Cryptophyceae	5
X3	flagellated chrysophyceans[a]	Chrysophyceae	5
Y	*Cryptomonas* sp.[a]	Cryptophyceae	3
Y	*Cryptomonas curvata* Ehr. emend. Pen[a]	Cryptophyceae	3

[a] Descriptor species (>5% of the total biomass).

accessibility of nutrient resources and the solar energy needed to process them (Reynolds, 2006).

The Sau Reservoir is an eutrophic system, with a mixed epilimnion layer and conspicuous water-column segregation during the stratification period (Armengol et al., 1999; Caputo et al., 2008). The long stratification period and high light availability led to the development of high biomass in the epilimnion, mainly in summer.

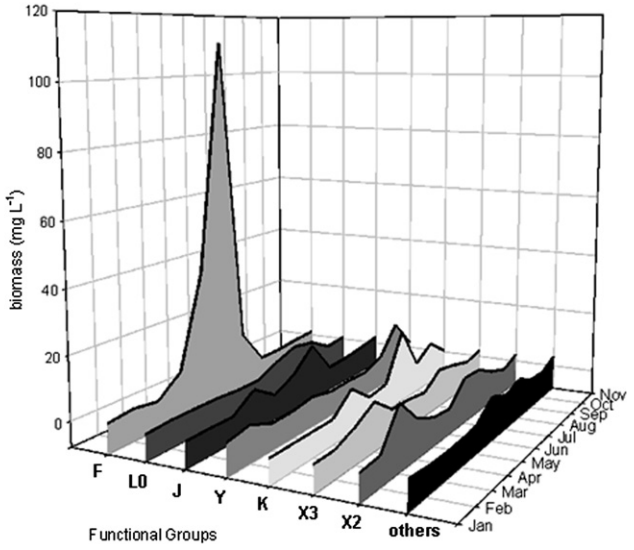

Fig. 6 – Biomass of phytoplankton functional groups (mg L^{-1}) in the epilimnion in the Sau Reservoir.

Considering that the dissolved nutrient concentrations and the algal requirements based on half-saturations for growth, phytoplankton in the Sau Reservoir are P-limited ($K_s = 0.1$ μM, Reynolds, 1997) during the period of stratification of surface waters in summer and early autumn (mean SRP = 0.1 μM). SRP is commonly the limiting nutrient for algae in freshwater reservoirs, mainly in temperate regions (Oliver and Ganf, 2000). Nedoma et al. (2006) previously described this P-limitation pattern for the Sau based on a study of alkaline–phosphatase activity. Feijoó et al. (2008) found that certain species of phytoplankton of Sau can take up excess phosphorus that can be stored to use in situations of low nutrient availability. Using an analogous criterion, we found no N limitation of phytoplankton growth, because DIN

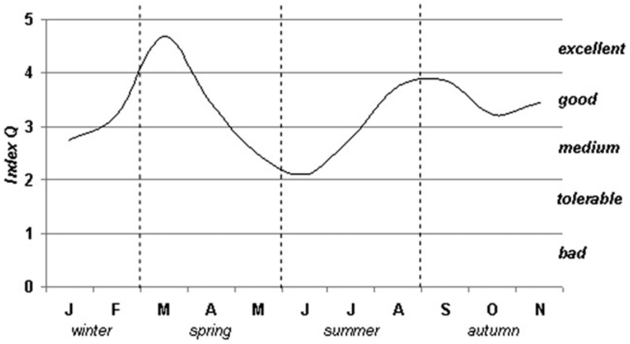

Fig. 7 – Ecological status evaluation according to the Q index in the Sau Reservoir in 2003.

concentrations were on average 20 times higher than 7 μM (Reynolds, 1997), the threshold for half-saturation for most algal species.

TN/TP ratios are also in wide use to address nutrient limitations of total phytoplankton growth (Rhee, 1978; Smith, 1983; Bulgakov and Levich, 1999). However, Reynolds (1997, 1999b) postulated that ratios are a consequence of uptake and not drivers of changes in phytoplankton composition, and more recently Klausmeier et al. (2007, 2008) discussed and modelled this concept. Although this is a controversial issue in nutrient limitation, our TN/TP results (>53) confirmed the tendency for P limitation of total biomass in Sau Reservoir, where N is limiting when TN:TP <9 and P is limiting when TN:TP > 22 (Søndergaard et al., 1999).

Similarly to total biomass, light and nutrients showed the same behaviour, driven by the mixing regime, governing the phytoplankton composition. Phytoplankton studies in the Sau Reservoir have focused on the dynamics of these organisms on a longitudinal gradient, because of the importance of hydrodynamics in this system (Comerma et al., 2001; Marcé et al., 2007; Caputo et al., 2008). Caputo et al. (2008) have studied the functional groups and their relationships to resources, light and nutrients, in four Catalonian reservoirs including the Sau, in a horizontal-gradients approach. They showed that the phytoplankton functional groups normally respond to the oligotrophication process taking place in stratified reservoirs from the river to the dam.

In this study, during the mixing period (winter) and the pre-mixing (autumn) in the Sau, light availability may have acted as a limiting factor for some phytoplankton functional groups, selecting groups adapted to low light intensity, such as group **Y** (cryptomonads) (Klaveness, 1988).

Light also contributed to the chlorophycean dominance during the summer stratification. The non-motile Chlorococcales *O. lacustris* (group **F**) embedded in mucilage is able to grow under thermal stratification and at a relatively deep optical depth (Happey-Wood, 1988). Clear epilimnia might contribute to the success of these species, since colonial green algae generally have a substantially higher light requirement than most planktonic cyanobacteria or diatoms (Padisák, 2003). In this period, the system was strongly stratified and the mixing zone was 70% illuminated on average. The low nutrient availability in the epilimnion during this period was also an important factor, leading to the dominance of functional group **F** which is tolerant to scarcity of resources (Reynolds et al., 2002). More recently, Caputo (unpublished data) described a similar pattern of colonial green algae dominance in several stratified reservoirs with contrasting trophic conditions (from meso-to eutrophic), in 19 Catalan reservoirs, coinciding with the pattern reported by Dasí et al. (1998).

The functional group **K** (non-N fixing cyanobacteria, small colonial cells) only dominated in the Sau at the end of summer, when the ammonium levels were lowest (15.5 μM). Non-N-fixing cyanobacteria, as represented in group K, may dominate the phytoplankton under conditions of low DIN concentrations, but when some ammonium is still present in the water (Blomqvist et al., 1994). We presume that the high amount of N, generally with low P concentrations, in the water of the Sau Reservoir can be prevented the occurrence of cyanobacterial blooms.

Similarly to some subtropical wetlands (O'Farrell et al., 2003) and moderately eutrophic shallow lakes (Huszar et al., 2000; Padisák et al., 2003), the groups **K** and **Y** co-dominated the phytoplankton assemblages in the Sau during autumn, and were related to the increase of nutrient availability in the water column (epilimnion layer).

Our study suggested the importance of DIN in selecting for cryptomonads, because this group dominated in periods with high $N-NO_3^-$ concentrations. However, the scarcity of information about the detailed physiological aspects of these species with respect to nitrogen (Marinho and Huszar, 2002) hinders further interpretation. This functional group indicates nutrient-rich habitats, showing sensitivity to mixing (Reynolds et al., 2002). The low light intensity and increased nutrients (SRP, $N-NO_3^-$) favoured this group. Other, related factors may regulate cryptomonad seasonality in lakes under widely different climatic conditions (Klaveness, 1988; Barone and Naselli-Flores, 2003). In Lake Kinneret, Israel, for instance, the dominant cryptomonads *Rhodomonas* and *Cryptomonas* appear in the eplimnion when autumn destratification begins (Pollinger, 1981). It seems that the only proven key factor in the success of these species is their low light requirement (Klaveness, 1988).

Our results showed a phytoplankton succession pattern in the Sau Reservoir. This behaviour has already been described in many studies of temperate eutrophic lakes, defined as the PEG Model (Sommer et al., 1986), a model of plankton seasonal succession developed for a comparative study by the Plankton Ecology Group. In this model, thermal stratification in lakes and reservoirs is the key factor controlling the dynamics of the community and, consequently, succession. In synthesis, the PEG Model suggests two phytoplankton biomass peaks: one in the spring season, with small edible algal species such as **X2** and **Y** found in this study, and the other peak during the summer with large colonial algae, resistant to zooplankton grazing, such as functional group **F**.

The tendencies revealed in this study indicated that the mixing regime is the main factor determining resource availability in the pelagic compartment, and consequently the temporal dynamics of the phytoplankton functional groups in the Sau. Although the response of phytoplankton to nutrients is one of the major concerns of aquatic ecology (Gu et al., 1997), Seip and Reynolds (1995) observed that physical factors, induced by trophic and seasonal gradients, may better explain the phytoplankton distribution and abundance. In addition, Naselli-Flores (1998) showed in Sicilian reservoirs, that both physical and chemical characteristics drove the structure of the phytoplankton functional groups, but physical factors were more important in promoting the development of a specific algal group.

The functional scheme for phytoplankton (Reynolds et al., 2002) provided valuable and complementary information on algal strategies, adaptations, and tolerances to characterise and understand phytoplankton dynamics in the Sau Reservoir. The results also confirmed that the functional groups approach can be applied to reservoirs. This indicates that even with the distinct hydrological characteristics of reservoirs and lakes, the mechanisms for phytoplankton selection respond similarly to resources availability (Reynolds, 1999a). Thus, lakes and reservoirs contain phytoplankton species or groups that share common attributes (Reynolds, 1997).

The authors of the functional groups assemblage index originally proposed it for use in assessing the ecological status of different types of lakes in the context of the Water Framework Directive (Directive, 2000) proposed by the European Community. However, according to the authors of this index, there is no geographic limitation to its application. Crossetti and Bicudo (2008) successfully applied the Q index to a tropical shallow impacted ecosystem, and Becker et al. (2009) to a subtropical reservoir, proving this to be a promising tool in monitoring processes. The index is very sensitive to species occurrence. The application of the assemblage index in the Sau Reservoir indicated an excellent ecological status during the spring, due to the contribution of the **X2** and **Y** functional groups.

However, in contrast to the behaviour expected based on trophic indices, the ecological state worsened when the phosphorus concentrations were very low, identifying phosphorus as limiting for the phytoplankton growth. During this episode, which occurred in the end of spring and early summer, large colonial green algae (group **F**) were dominant. Since the algal assemblages are defined on basis of their preferences for certain combinations of habitat properties (Padisák et al., 2006), the Q index might be suitable to show a more coherent ecological status in systems where P is naturally available; meanwhile, the general trophic status indices may possibly indicate worse conditions. Sau Reservoir presented good ecological condition in the most part of the year, varying according to the mixing regime identified, probably as a response to the oligotrophication process to which the ecosystem has been underwent to.

5. Conclusions

1. The study confirmed the potential use of the functional-groups scheme for phytoplankton, and provided valuable and complementary information on algal strategies, adaptations, and tolerances to characterise and understand phytoplankton dynamics in reservoirs.
2. The tendencies revealed in this study indicated that the mixing regime is the main factor determining resource availability in the pelagic compartment, and consequently the temporal dynamics of the phytoplankton functional groups in the Sau.
3. Phosphorus' limitation and light availability were key factors in the selection of phytoplankton species in the studied reservoir
4. According to the Assemblage Index, the Sau Reservoir showed good ecological conditions during most of the year, varying according to the mixing regime. This is the first application of the Assemblage Index to a European water-supply reservoir.

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