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Contribution of Long-Term Monitoring to the European Water Framework Directive Implementation

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ABSTRACT

Aquatic ecosystems are of critical ecological, social and economic importance. Worldwide increase in their use and anthropogenic pressures alter their health which is essential for the maintenance of water quality, biodiversity, and fishing activities. The European Parliament sets out a framework for managing and protecting water for each main catchment basin at a European level. The aim is to propose objectives to conserve or restore the state of water-bodies into a status that would be considered "good" in development and ecological terms. A key issue in the implementation of the Water Framework Directive is the classification of water-bodies into "quality status" using biological indices. Comparison and classification of water bodies throughout Europe require the improvement and development of new methodologies based upon scientific knowledge. New methods and indices have been proposed for categorizing water bodies into quality status. In this study we took advantage of the long-term data set of phytoplankton abundance and composition sampled in Lake Geneva (France-Switzerland) to test an index formally developed for Austrian lakes. Lake Geneva has suffered from eutrophication in the 80's. Comparisons between the tested and classical indices show that recovery from eutrophication is a long process that could be altered by large scale pressures such as climate change.

Keywords: ecological indicators, phytoplankton, long-term trend, climate change, indices, trophic status, water-quality index, Lake Geneva

INTRODUCTION

Water is life for living organisms and an indispensable resource for economy. For these reasons, protection and improvement of surface and ground water have become one of the cornerstones of environmental protection in Europe. These tasks should be reached through the European Water Framework Directive (WFD; 2000/60/EC) that establishes a framework to achieve at least "good water status" for all water bodies by 2015. Surface water bodies should be classified within a scale of 5 environmental status ratings - bad, poor, moderate, good and high - "High Ecological Status", being the reference condition defined as the biological, chemical and morphological conditions associated with no or very low human pressure. It differs from "Good Ecological Status" that indicates water bodies having the biological and chemical characteristics expected under sustainable conditions. The WFD has required Member States to assess the ecological status of water bodies within an intercalibration exercise to harmonise the understanding of "Good Ecological Status" in all states. In Lakes one of the biological quality elements to be considered is the phytoplankton community which is strongly influenced by human pressure and has already been suggested as indicator of ecological status (Rosén, 1981; Stoermer and Smol, 1999; Padisak *et al.*, 2006, Salmaso *et al.*, 2006).

In lakes, "Good Ecological Status" appears strongly related with eutrophication that has been considered as one of the main ecological problems of the 20th century. Indeed, eutrophication caused by excess of nutrients is recognized as the major cause of the water-quality degradation in European lakes (OCDE, 1982; Jeppesen *et al.*, 2005). Climate change is another key pressure impacting on lake ecosystems (Adrian *et al.*, 1995; Livingstone 2003, Dokulil *et al.* 2006). It may operate directly (through impact on metabolic and reproductive processes) or indirectly (through impact of prey, predator and competitors) on species, altering inter-specific relationships and ecosystem functioning (Winder and Schindler, 2004; Blenckner, 2005). Since the late 90's, the multiplying examples of climate induced changes on aquatic systems have led to an increasing concern on this confounding factor on eutrophication or reoligotrophication (Howarth *et al.*, 2000; Carvalho and Kirika, 2003; Jeppesen *et al.*, 2005; Wilby *et al.*, 2006). Understanding the combined effects of environmental drivers on species, communities and ecosystems is nowadays a key challenge for research management (Harrington *et al.*, 1999**,** Howarth *et al.*, 2000, Niemi and McDonald, 2004; Wilby *et al.*, 2006).

Frequent monitoring over a long-time period is essential to investigate the ecological impact of global climatic changes or local environmental perturbations. Such long-term monitoring make up an interesting basis that can be used in many ways to support the WFD (Wilby *et al.*, 2006). In this paper we take advantage of the data set from Lake Geneva. Using a water-quality phytoplankton index derived from Brettum (1989) and the common trophic status parameters recommended by the OECD (OCDE, 1982), we relate the trophic story and long-term changes in the ecological status of this lake. We then investigate the long-term changes in phytoplankton community to identify the forcing factors responsible for such changes. Finally, the results are discussed within the context of the sensitivity of phytoplankton community to anthropogenic and climatic forces and the role of phytoplankton species as indicators of water quality.

MATERIAL AND METHOD

Studied site: Lake Geneva is one of the largest lakes in Western Europe (lake area: 582km^2, mean depth: 152m, maximum depth: 309m, biovolume: 89km^3), situated between France and Switzerland (46°27'N, 6°32'E) at an elevation of 372m. A monitoring programme to measure the water quality began in the late 50's. In 1963, the monitoring came under the direction and founding of the CIPEL (International Commission for the protection of Lake Geneva – (www.cipel.org), which has been founded by a convention between French and Swiss governments. Data series of physical, chemical and biological parameters are available dating back to the late 50's or 70's depending on the parameters.

Table 1. Brettum's indicator species contributions (%) to the axes I (Fac1) and II (Fac2) of the PCA, months of their main occurrence (Month) and trophic quality class showing the highest score for the considered species (Max *x*). The trophic quality classes are indicated from A to F for the highest to the worst quality. Only species whose contribution is higher or equal to 0.1% for at least one of the two first axes are indicated in the table.

AbrEsp	**Fac 1**	**Fac 2**	**Month**	**Max *x***	***Esp***
CeratHi	31.43	21.07	7-8	A	*Ceratium hirundinella*
MougGr	17.07	24.17	9-10-11	E	*Mougeotia gracillima*
AphFAq	7.21	0.35	9-10-11	E	*Aphanizomenon flos aquae*
DinoSoc	6.94	0.13	7-8	C	*Dinobryon sociale* var. *americanum*
PlankRub	6.33	27.13	9-10-11	D	*Planktothrix rubescens*
ClostAci	4.02	5.87	9-10-11	F	*Closterium aciculare*
StephMin	3.85	0.79	4-5-6	E-F	*Stephanodiscus minutulus*
FragCr	3.42	4.15	7-8	B	*Fragilaria crotonensis*
StephNeo	3.25	0.96	12-1-2-3	D	*Stephanodiscus neoastraea*
AulIsl	1.63	0.01	12-1-2-3	C	*Aulacoseira islandica* subsp *helvetica*
StaurCin	1.61	3.05	7-8	E	*Staurastrum cingulum*
AphaClav	1.51	1.42	7-8	C	*Aphanothece clathrata* var. *rosea*
EudEl	1.29	1.89	7-8	F	*Eudorina elegans*
CycCy	1.28	0.69	9-10-11	A-B	*Cyclotella cyclopuncta*
PhacLen	1.23	1.25	7-8	E	*Phacotus lendneri*
StaurSebV	0.88	1.44	7-8	E	*Staurastrum sebaldii* var. *ornatum*
DictyosphPul	0.82	0	7-8	D-E	*Dictyosphaerium pulchellum*
StephAlp	0.81	0.19	12-1-2-3	C	*Stephanodiscus alpinus*
GymnHel	0.74	0.38	12-1-2-3	B	*Gymnodinium helveticum*
StauraMf	0.54	0.22	7-8	E	*Staurastrum messikomerii* f *planctonicum*
StephBin	0.43	0.03	7-8	F	*Stephanodiscus binderanus*
ChlorVul	0.34	0.05	4-5-6	F	*Chlorella vulgaris*
MalAc	0.31	0.01	9-10-11	E	*Mallomonas acaroides*
PedBor	0.31	0.26	7-8	E	*Pediastrum boryanum*
PedDup	0.27	0.42	7-8	F	*Pediastrum duplex*
AulGraV	0.22	0	7-8-9-10-11	E-F	*Aulacoseira granulata* var. *angustissima*
AphaDel	0.22	0.18	9-10-11	C-D	*Aphanocapsa delicatissima*
CoelastRet	0.21	0.02	9-10-11	C	*Coelastrum reticulatum*
GymnLan	0.18	0	12-1-2-3-4-5-6	B	*Gymnodinium lantzschii*
CycRa	0.18	0	12-1-2-3-4-5-6	E	*Cyclotella radiosa*
ClostAcV	0.18	0.2	7-8	E	*Closterium acutum* var. *variabilis*
TetraedrMin	0.15	0.24	9-10-11	E	*Tetraedron minimum*
OocyLac	0.12	0.38	7-8	E	*Oocystis lacustris*
GymnEx	0.11	0.15	7-8	B	*Gymnodinium excavatum*
OocySol	0.11	0.24	7-8	E	*Oocystis solitaria*
SphaerocSch	0.07	1	7-8	F	*Sphaerocystis schroeteri*
PandMor	0.02	0.11	7-8	F	*Pandorina morum*
AnkLan	0.01	0.22	7-8	E	*Ankyra lanceolata*
FragUlVa	0	0.12	9-10-11-12-1-2-3	C	*Fragilaria ulna* var. *angustissima*
AnkJud	0	0.37	4-5-6-7-8	E	*Ankyra judayi*

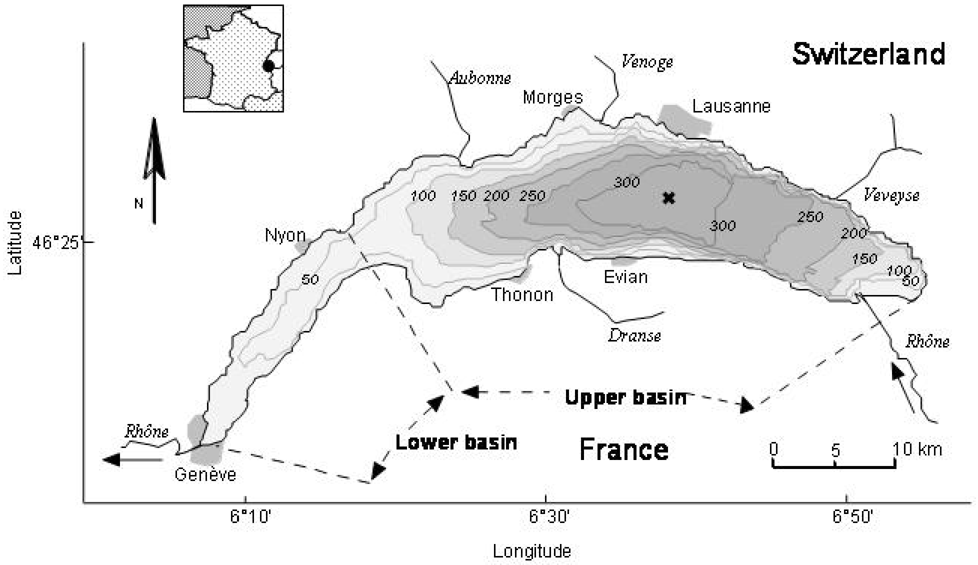

Figure 1. Bathymetric map of Lake Geneva. The sampling site is indicated by a cross.

Data collection:

The data used in this study originates from a pelagic sampling station located in the centre of the upper basin of Lake Geneva, at its deepest part (figure 1). Sampling was performed once a month till 1980 and then twice a month during the phytoplankton growing season (May to October).

Transparency was equal to the depth at which a white-disk disappears (diameter: 30 cm). Temperatures and nutrient concentrations were sampled at a series of discrete depths between the surface and the bottom of the lake until 1998, then after a multiparameter-probe was used for temperature measurements. Values used in this study correspond to the mean water temperature measured in the 0-10m layer. Total phosphorus concentrations were measured using unfiltered samples after digestion with potassiumperoxodisulfate.

Water samples for the determination of phytoplankton species composition and biomass have been collected in the 10 upper meters till 2001, then after they were collected in the upper 20 meters. Water was sampled using a custom-made integrating bell (Pelletier, INRA patent 1978). Phytoplankton identification and cell counts were carried out in sedimentation chambers under an inverted microscope (Utermöhl, 1958). Species biovolumes were derived from cell numbers and mean cell volumes using geometrical models. The total biomass was then estimated by summing the biovolumes for each species, assuming a fresh weight of 1 g·cm^{-3}.

Summarizing interannual changes in water-quality:

Annual mean concentrations of phosphorus concentrations (Lazzarotto *et al.*, 2006), annual means of transparency and the minimum transparency recorded within the year were used to assess the long-term changes in the trophic status of Lake Geneva (OCDE**,** 1982).

Phytoplankton index was computed using the Brettum index (1989) based on a set of indicator species (Dokulil and Teubner, 2006). For the computation of this index, phytoplankton species are assigned a taxon-specific score for 6 trophic classes (Wolfram *et al.*, 2007: http://circa.europa.eu). For each trophic class an index *Ij* is computed by multiplying the relative biovolume *vi* of a taxa *i* with their scores *xij* for the considered trophic class *j*:

$$I_j = \frac{\sum_{i=1}^{n} v_i x_{ij}}{\sum_{i=1}^{n} v_i}$$

$$BI = \frac{\sum_{j=1}^{6} I_j T_j}{\sum_{j=1}^{6} I_j}$$

The final index is the weighted average of the 6 class index *Ij*. The weights *Tj* go from 6 to 1 for the first class to the last one. The Brettum index (BI) ranges from 1 to 6.

Long-term changes in the abundances of Brettum's indicator species (BIndSp):

Temporal changes in the abundances of the BIndSp were investigated using multivariate methods (ADE4 software, Thioulouse *et al.*, 1997). A centred Principal Component Analysis (PCA) was performed on BIndSp abundance data in order to identify the nature of the main variability. Focus on interannual variability was performed using the Between Group Principal Component Analyses (BGPCA), a method specifically designed for studying data whose variance can have distinct causes. Mainly used to have a distinct view of the respective influence of the seasonal succession and the sample location on the variability of the measures (Dolédec and Chessel, 1989), this method has also been used to distinguish between variability generated by seasonal and interannual dynamics (Anneville *et al.*, 2005, Anneville *et al.*, 2007). The method is applied to data-matrices whose objects are gathered by groups (Dolédec and Chessel, 1989) and it consists of a PCA run on the group-weighted average values of the variables. The weight of a group is computed during the analysis and equals the number of samples pertaining to this group. In our analysis, the groups were the years. A BGPCA was computed for winter (January to March), spring (April to June), summer (July to September) and autumn (October to December) separately. Before the computation of the multivariate analyses the abundance data were log-transformed. For further analysis concerning the links between the changes in phytoplankton community and environmental variables, the temporal changes in BIndSp were represented by time series of annual trajectories scores.

Trend analysis and link between variables:

Water temperature, transparency and phytoplankton biomass data were averaged at a monthly frequency. Seasonal means were computed for the 4 seasons previously described and then standardised at zero mean and unit variation. Increasing or decreasing trends were tested using the non-parametric Kendall test. The cumulative sums method (Fromentin and Ibanez, 1994) was applied to detect changes and to extract the general trend in the series. The method consisted in subtracting a reference value, here the mean of the series, from each data. Then, the residuals were successively added, resulting in a cumulative function. The Shapiro test was used to test the normality of the data. The potential relations between the studied variables were assessed using Pearson's or Spearman correlation coefficients.

RESULTS

Between 1957 and 1976, mean annual phosphorus concentrations increased from 12.4 µg.l^{-1} to 89.6 µg.l^{-1} (figure 2A). In the same period, the trophic status of the lake shifted from oligotrophic to eutrophic. Phosphorus concentrations maintained high for 4 successive years and then started to decrease in 1980, bringing back the lake to a mesotrophic status in the early 2000's. In 2005, total phosphorus concentrations were equal to 29.4 µg.l^{-1}. Long-term changes in the minimal transparency put forward fluctuations between oligotrophic and mesotrophic status but no clear trend appeared (figure 2B). The trophic status indicated by mean annual water transparency has been oligotrophic for the entire studied period (figure 2C). Furthermore, the transparency has been decreasing since 1956 and no improvement in this parameter was noticeable.

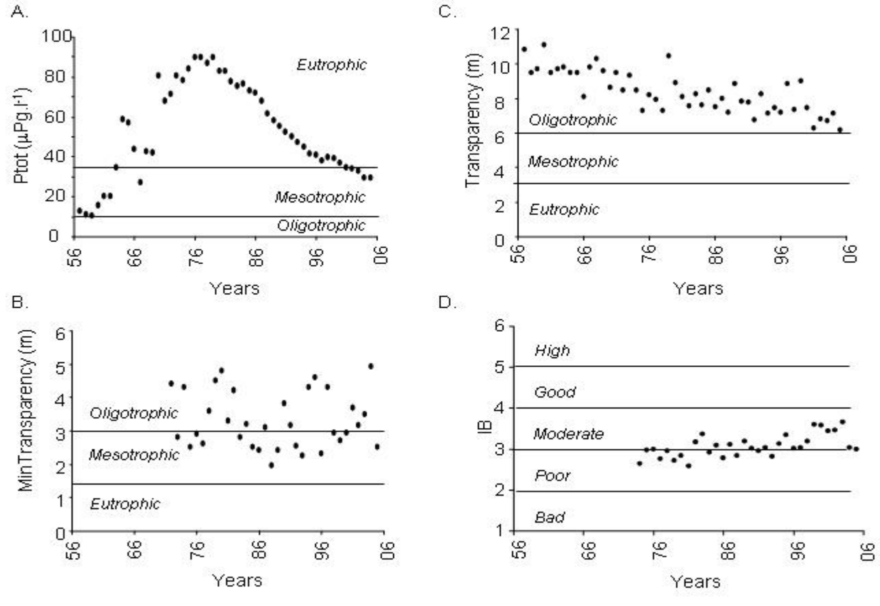

Figure 2. Long-term changes in water-quality of Lake Geneva assessed by: A) mean annual phosphorus concentration, B) minimal transparency recorded during the year, C) mean annual transparency, D) Brettum water quality index (BI).

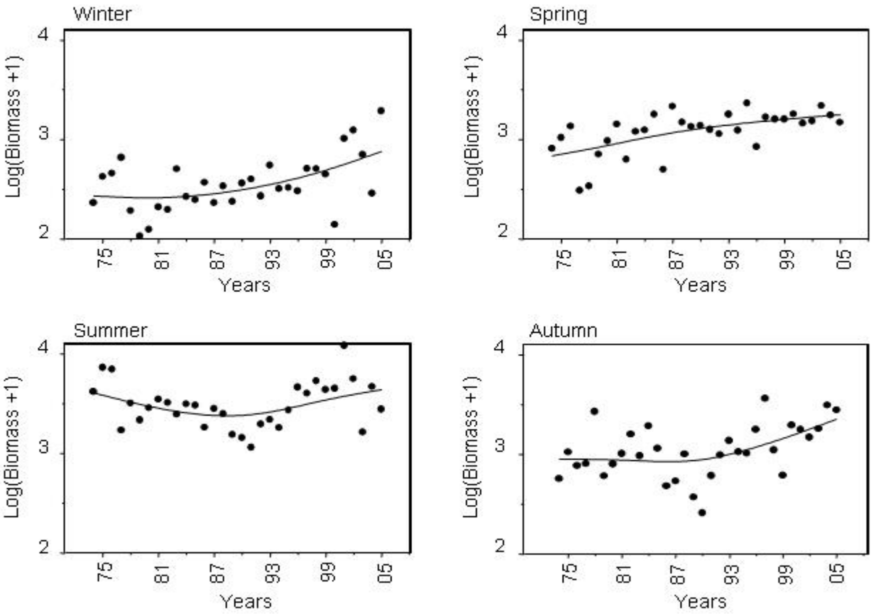

Figure 3. Long-term changes in total phytoplankton biomass. The general trends of the time series are obtained by Spline (black line).

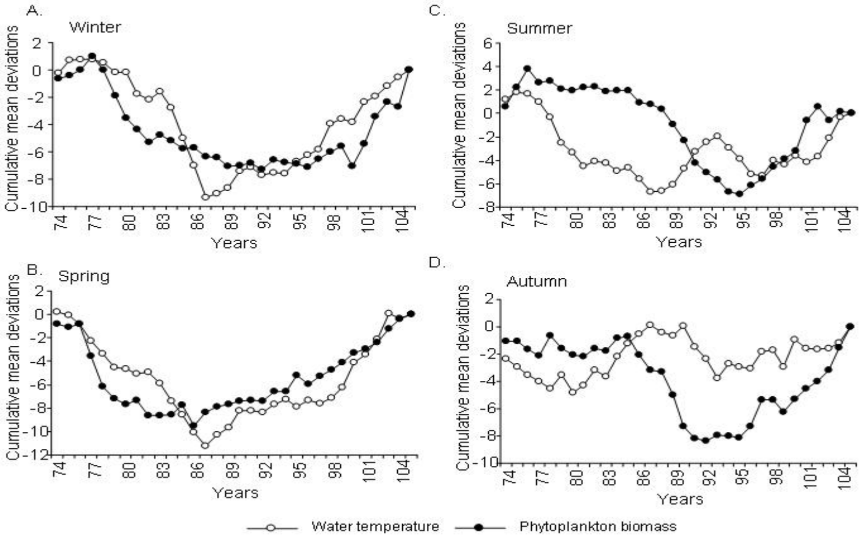

Figure 4. Cumulative sum series of mean water temperature and total phytoplankton biomass from 1974 to 2005.

This decrease in water transparency came with an increase in phytoplankton biomass. Between 1974 and 2005, long-term changes in annual transparency were related to the variability in annual phytoplankton biomass (Pearson p-value<0.05). Except in spring, phytoplankton biomass and water transparency showed significant negative correlations (Spearman p-value<0.05). During the studied period, significant increases (Kendall p-value<0.01) in phytoplankton biomass have been recorded in winter, spring and autumn. In summer the biomass decreased till the beginning of the 90's and then increased to values equal to those observed in the 70's (figure 3).

Mean seasonal phytoplankton biomass and water temperatures were significantly correlated (Spearman p-value<0.05) for winter and spring seasons. Analyses of their trends revealed strong synchronisms (figure 4A, 4B). The cumulative sum series pointed out a decreasing slope from 1974 to the mid 80's which characterized a group of values lower than the mean for both temperatures and biomass; and then an increasing slope till 2005 that characterized warm years and high phytoplankton abundances compared to the whole series. In summer and autumn no synchronisms between the time-series appeared (figure 4C, 4D).

Lake Geneva phytoplankton community was subject to changes in its species composition. Contribution of BIndSp to total phytoplankton biomass presented strong fluctuations (mean= 53 ± 26). Long-term changes in the annual mean of BindSp presented a synchronism with annual means of phytoplankton biomass (figure 5A). Interannual variability in BIndSp biomass were significantly correlated with phytoplankton biomass (figure 5B) and explained 85.7% of its interannual variability (Pearson p-value<0.001). Long-term changes in Brettum index underlined a slight improvement of the water quality (figure 2D) that is significantly correlated with phosphorus concentrations (Spearman p-value< 0.001).

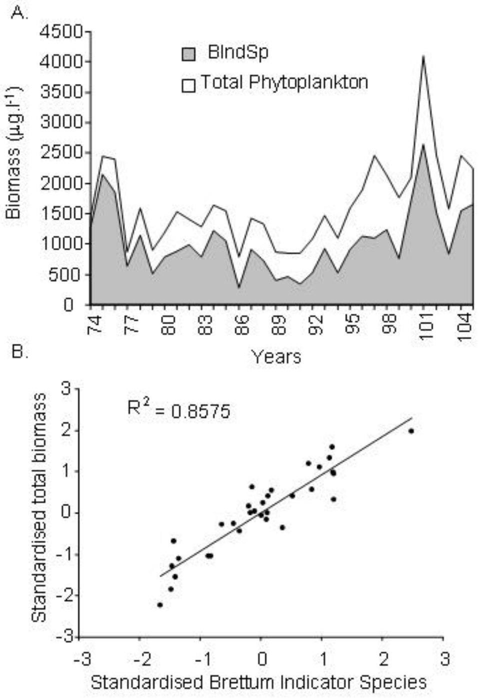

Figure 5A. Comparison of interannual changes in total phytoplankton biomass and BIndSp biomass. B) Linear relation between the variability in BIndSp biomass and total phytoplankton biomass.

Temporal changes in BIndSp composition were mainly explained by the first two axes of the PCA that accounted for 28% of the total variability. Given the high number of variables (118 taxa), the first plane provided a good summary of the temporal species organisation during the studied period. The species contributing most to the formation of the axes were shown in Table 1. The first plane described a seasonal pattern where the first axis opposed taxa that presented high biomass during the warm months to taxa that occurred during winter and spring. The second axis opposed summer to autumn species. BGPCA performed on each season underlined distinct temporal patterns (figure 6). Inter-annual changes in BIndSp composition were mainly explained by the first two axes; they accounted for 37, 43, 38 and 40% of the inter-annual variability for winter, spring, summer and autumn seasons respectively. Analysis of inter-annual changes on axis I revealed strong inter-annual fluctuations for the winter and autumn seasons. In contrast, particular temporal patterns were observed for the summer and spring seasons. In summer, the first axis opposed the 1970's, 1980's to the 1990's and the 2000's. *Mougeotia gracillima* together with *Cyclotella cyclopuncta* had a strong contribution to the formation of this axis. Both species characterized the second period, being however indicators of quite different water quality. The other indicator species related to this period were mainly indicators of medium water quality, in contrast with those characterizing the first period which were indicator of poor water quality. Finally, in accordance with the ecological feature of the BIndSp, PC1 scores presented a significant correlation with Ptot (Spearman p-value<0.001) and spring water-temperature (Spearman p-value<0.01). In spring, the inter-annual trend recorded on axis I was similar to those of Ptot and water-temperature (Spearman p-value< 0.005). However, the BIndSp contributed to the axis regardless to their trophic character. In contrast this axis opposed taxa that are characteristic of the spring seasons and taxa that dominate during summer and autumn. In recent years, summer or autumn taxa tend to appear and increase during the spring season. This trend indicates changes in the phenology of species succession.

DISCUSSION

The EU Water Framework Directive requires development of biological indicators for the assessment of the ecological water quality status. Numerous projects and workshops have been organized between the member and associate states of the EU to harmonize the ecological quality "targets" and discuss on phytoplankton attributes and indicator groups that could be used as biological indicators. National classification methods based on phytoplankton abundance and composition are available in Austria and Germany (Wolfram *et al.*, 2007: http://circa.europa.eu). These methods should be able to detect quality changes across a range of spatial and temporal scales. Ecology deals with processes that occur on different scales of space, time and organization complexity. Because the observed variability of the system is conditional on the scale of description (MacArthur, 1992), investigation of the mechanistic relationship between variation of abiotic and biotic components of the systems require that their scales of variation match. Eutrophication and recovery that lead to floristic changes in phytoplankton community operate on decadal scales (Reynolds, 1990). In most biological indicators, taxa are assigned weights reflecting their ecological preferences or sensitivity to environmental degradation. These weightings usually come from inter-site comparisons and expert knowledge (Padisak *et al.*, 2006, Howe *et al.*, 2007). Such indices can be well adapted for lake classifications but may be not sensitive enough to track temporal changes in response to local management within a lake. Additional statistical analyses on existing long-term data may lead to a better understanding of the temporal variability. Finally coupled with inter-site comparisons, long-term monitoring may be helpful to improve the species-qualification system, refine the biological indices and finally check their sensitivity to long-term changes. In this study, time-series appear as a useful tool to i) investigate the causes of deterioration of the ecological status of waters, linking them to chemical or other significant anthropic or climatic pressures and ii) raises the question on the advantages and limits of indicators based on species composition.

i) Implication of climate and anthropic pressures on long-term changes of water-quality:

In the beginning of the 1900's, an increase in human pressure in the watershed of Lake Geneva, lead to an increase in phosphorus loading and a consequent occurrence of phytoplankton blooms and decrease in the recruitment of some commercial fishes (Laurent, 1972, Anneville *et al.*, 2001). Concern about the degradation of water quality led to the creation of the CIPEL and to the edition of recommendations for the reduction in P concentrations. CIPEL's recommendations consisted in several actions such as the optimisation of fertilisers used in agriculture, the construction of waste water treatment plants, the connection of the population to the sewerage system, improvement of the waste water collection systems by elimination of parasitic clear water, the introduction of dephosphatisation in the water treatments plants, the monitoring of the efficiency of waste water treatment plants, the banning of phosphates in washing detergents in Switzerland and their reduction in France.

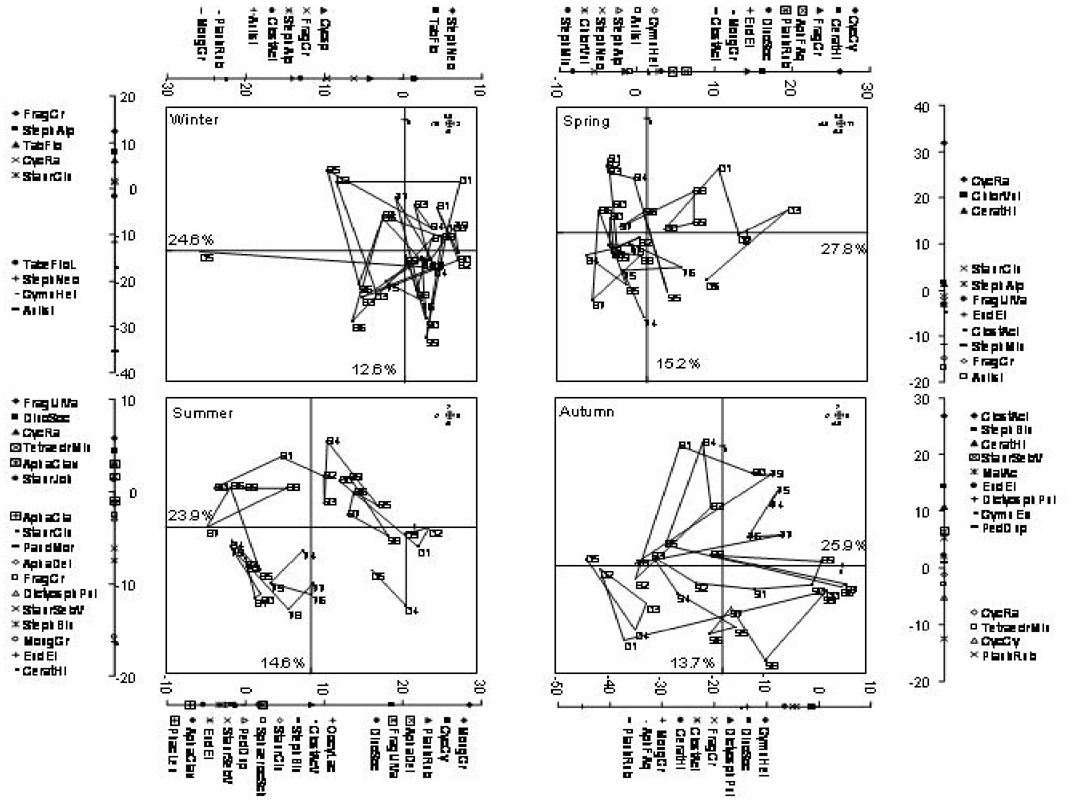

Figure 6. Graphical representation of BGPCA results showing long-term changes in BIndSP during winter, spring, summer and autumn. Projection of the barycentre of the years on the first plane made by axes I and II. Contributions of the taxa to the formation of the axis are indicated on separate axes.

Water transparency overestimates the quality of the lake and indicates an overall degradation of water quality in spite of reductions in phosphorus concentration. The observed bias in the estimated water-quality probably might come from low phytoplankton abundances relative to phosphorus when compared to other lakes (Dokulil and Teubner, 2003). Over-estimation of Lake Geneva's water-quality can also be induced by the disk used to determine water transparency. This disk is larger than the standard Secchi disk. In contrast, long-term decrease in water transparency has a biological cause. According to our results, it is linked with an increase in total phytoplankton biomass.

Behind the fact that the ecological indicators are not in agreement with the trophic status of Lake Geneva, biological indicators present the well known phenomena of hysteresis that have been observed in many lakes during reoligotrophication (Jorgensen and De Bernardi, 1998; Cooke *et al.*, 2005). In Lake Geneva this hysteresis emerges from a combination of several factors. Phosphorus was not the limiting factor of phytoplankton production till the beginning of the 90's (R. Tadonléké unpubl. data). Then after, phytoplankton biomass maintained because of readjustment in species composition and the development of large species which are resistant to zooplankton grazing and adapted to the new environmental conditions (Anneville *et al.*, 2002). In addition to these observations, the present study suggests a relationship between water temperature and phytoplankton biomass inter-annual variations during winter and spring. Water temperature in Lake Geneva is strongly influenced by large scale hemisphere circulation (Anneville *et al.*, 2002, Molinero *et al.*, 2007). The recorded changes in water-temperature may probably impact the growth of phytoplankton directly through the metabolism and indirectly through changes they create on the dynamic of water stratification. Additional monitoring of the zooplankton community has underlined changes in herbivorous species. *Daphnia*, the main zooplankton grazer in lakes, has been showing a significant decrease since the mid 80's (Anneville and Lainé, 2006). As a consequence, decrease in *Daphnia* abundance may reduce predation pressure on phytoplankton favouring the development of small species in spring. Finally, as shown by the present results, the early development of not grazable summer species during spring may contribute to the increase in spring biomass. Such a change in phytoplankton phenology might be related to the earlier occurrence of a eutrophic layer depleted in phosphorus (Anneville *et al.*, 2002), as well as warmer temperature, as suggested by the significant correlation between water temperature and BGPCA scores.

ii) Advantages and limits of indicators based on species composition:

Recovery from eutrophication is a complex process that does not involve a simple decrease in phytoplankton biomass. It can appear as a rearrangement of pre-existing taxa (Salmaso *et al.*, 2006) or as an unexpected development of large species together with less abundant accompanying species that are indicators of good trophic status (Anneville *et al.*, 2005). For this reason, indices based on species indicators are more sensitive and well adapted to detect an ecosystem response to decrease in phosphorus concentrations. The Brettum index can be improved since it only considers a few species. However, it appeared to be quite in agreement with the changes indicated by phosphorus concentration in Lake Geneva. Long-term changes recorded in this study confirm previous studies showing a change in the annual phenology coupled with a long-term readjustment of the phytoplankton community during the growing season (Anneville *et al.*, 2002). This readjustment is characterized by i) an increase of indicator species for good conditions, ii) a decrease of indicator species for poor-water quality and ii) the development of species adapted to low light intensity and better adapted to cope with the new environmental conditions characterized by a depletion in phosphorus that covers the eutrophic layer and extend below the thermocline (Anneville *et al.*, 2002). Water-quality indicator species present thus long-term dynamics which are associated to both changes in phosphorus concentrations and temperature variability.

Perceived quality should be a judgment based upon needs and expectations (Cook *et al.*, 2005). Indeed, with the increasing human utilization and pressure on lake ecosystems, long-term sustainable use of water for the benefit of both people and wildlife will require taking into account the economical point of view for the definition of water status (Moschella *et al.*, 2005). In Lake Geneva, the phosphorus concentration is still able to sustain excessive development of algae (Druart and Tadonléké, in press) and the Brettum index indicates medium quality. The quality required for the main uses is however quite correct. Lake Geneva is a freshwater reservoir whose quality of water is good enough to require only basic treatment to transform it into drinking water according to regulations (www.cipel.org). Fish communities sustain important professional and recreational fishing (Gerdeaux, 2004). Finally, the water quality allows bathing and leisure activities. In addition to eutrophication, other harmful effects related to industrial chemicals can impact the water-quality, but little is known on their effects on phytoplankton assemblages. Indices that focus on just one trophic level can thus lead to erroneous conclusions or discrepancies between the ecological and socio-economic definition of water quality. Here is the advantage of using multiple indices based on the combination of chemical and biological indicators of different trophic levels (macrophytes, fishes, invertebrates), as recommended by the WFD.

Finally, the "Ecological status" refers to the structure and functioning of the ecosystem (2000/60/EC, OJ L327 22.12.2000). Functioning of the ecosystem involves the interactions between organisms and the physical environment; it depends on the efficiency of the energy flows between the trophic levels and on the nature of carbon sources. Biological Indices proposed for the WFD give a picture of the taxonomic composition that is associated with a model of functioning, but it fails in contemplating ecosystem functioning (Moschella *et al*., 2005). The last decades are marked by the increasing use of developing methods to study food webs and energy flows. For example, stable isotopes that can be easily used as tracers of carbon fluxes, give reliable information on changes in carbon flux within ecosystems (Perga and Gerdeaux, 2003). Biological quality elements should thus include such additional indicators which could be interesting supplementary tools for the evaluation of the ecological status.

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