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Trophic history of French sub-Alpine lakes over the last ~150 years: phosphorus reconstruction and assessment of taphonomic biases

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ABSTRACT

Like many lakes worldwide, French sub-Alpine lakes (lakes Annecy, Bourget and Geneva) have suffered from eutrophication in the mid-20th century. Although restoration measures have been undertaken and resulted in significant reductions in nutrient inputs and concentrations over the last 30 years in all three lakes, the limnological monitoring does not extend back far enough to establish the reference conditions, as defined by the European Water Framework Directive. The over-arching aim of this work was to reconstruct, using a palaeolimnological approach, the pre-eutrophication levels and subsequent temporal changes in the lakes trophic status over the last century. The objectives were three-fold: i) to test whether fossil diatoms archived in deep sediment cores adequately reflect past changes in the planktonic diatom communities for these deep sub-Alpine lakes based on data from lake Geneva; ii) to investigate changes in the diatom communities over the last 150 years in the three lakes; and iii) to infer the past total phosphorus (TP) concentrations of the lakes from a diatom based transfer function. Annual palaeolimnological and limnological diatom countings for lake Geneva were strongly correlated over the last 30 years. Most notable differences essentially resulted from both taphonomic and depositional biases, as evidenced by the underestimation of thin skeleton species such as Asterionella formosa *and* Diatoma tenuis *in the palaeolimnological dataset and the presence of many benthic taxa. The fossil diatom records revealed shifts in the communities in the three lakes over time, most of which were changes typically associated with nutrient enrichment. Indeed, in all three lakes, the proportion of* Cyclotella *spp. was very high before the 1950s, but these species were then replaced by more eutrophic taxa, such as* Stephanodiscus *spp, by the mid-20th century. From the 1980s, some but not all diatom species typical of re-oligotrophicated conditions* (i.e. Cyclotella *sp.) re-appeared in all three lakes. Yet, not all changes that occurred in the diatom communities since then may be attributed to decreasing TP. Total phosphorus concentrations inferred from weighted averaging with classical deshrinking in lake Annecy and lake Geneva and weighted averaging with inverse deshrinking in lake Bourget were very close to the monitored values. Comparisons of diatom-inferred TP concentrations to other palaeo-proxies for trophic status revealed though that the transfer functions were rather insensitive to changes occurring below 10 µg* L^{-1}*, which limits the study ability to set accurate TP reference conditions below this threshold. However, current diatom community compositions arestill rather different from the pre-eutrophication ones, suggesting that reference conditions are still not achieved.*

Key words: diatoms, valves preservation, trophic state.

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INTRODUCTION

The European Water Framework Directive (WFD) (European Commission, 2000) has highlighted a crucial need for the ecological assessment of lakes. One of the key issues faced by managers concerns the establishment of reference conditions, defined by the WFD as those associated with no, or only very minor, anthropogenic impact (European Commission, 2003). These baselines are required to evaluate how much the lake under consideration has been impacted by human activities and to set the restoration objectives. The combination of spatial surveys, modelling, expert judgement and temporally based methods using historical data or palaeo-reconstruction has successfully resulted into the establishment of reference conditions that are specific to some lake typologies (Wolfram *et al.,* 2009). There are however some lakes that lack typologic analogs or for which the type-specific definition of reference conditions may not be accurate enough to assess ecological status. This is particularly true for the very large, deep European lakes, which have unique system dynamics due to their size (Loga *et al*., 2004). For these lakes, reference conditions need to be site-specific, and, in the absence of sufficiently long-term monitoring data, ecological and chemical reference conditions and deviation from the reference state may be defined through palaeoecological approaches (Bennion and Battarbee, 2007).

This study focused on three French deep sub-Alpine lakes (lakes Geneva, Annecy and Bourget) that are essential elements of social and economic activities in this region. All three lakes exhibited some symptoms of eutrophication in the mid-1970s (Anneville, 2002; Millet *et al*., 2010; Perga *et al*., 2010). Total phosphorus (TP) concentrations measured during water mixing, either routinely such as for lake Geneva or sporadically as for the two other lakes in the 1970s, confirmed that lakes Geneva and Bourget had reached a eutrophic status (with respectively 90 and 120 µg L^{-1}), while lake Annecy got, at most, oligo-mesotrophic (17 µg L^{-1}) (INRA long-term observatory database). Phosphorus (P) abatement measures were subsequently successfully undertaken, leading to substantial decrease in P concentrations in the water column over the last 30 years. The lakes Geneva and Bourget are currently mesotrophic with winter TP <20 µg L^{-1} while lake Annecy is oligotrophic (TP=6 µg L^{-1}, INRA long-term observatory database). Although all lakes are now under routine monitoring (started in 1957 for lake Geneva, 2004 for lake Bourget and 1996 for lake Annecy), these datasets do not extend back far enough to establish lake reference conditions. Previous palaeolimnological studies investigated the ecological changes these lakes have undergone over the last century (Alric, 2012; Druart and Pelletier, 1998; Giguet-Covex *et al*., 2010; Jenny *et al*., 2013; Perga *et al*., 2010) and triggered by changes in nutrient concentrations. None of them though was specifically focussed on reconstructing past TP concentrations.

This study aimed to use an existing diatom based transfer function to estimate the past TP concentration of the three French sub-Alpine lakes, in an approach similar to that of Marchetto *et al*. (2004) on lake Maggiore. Some transfer functions are now subjected to some strong criticism. In Telford and Birks (2009), different issues are raised: a good replication can be difficult to achieve, the statistical significance must be well adapted to the potential variation of the reconstruct parameter in the studied time period. Juggins *et al.* (2013) mentioned also different bias of epilimnetic TP reconstruction using diatoms: i) the impact of secondary variables such as alkalinity and depth; ii) the not significant relationship between TP concentration and many taxa in models; iii) the poor or no spatial replicability. Nevertheless, transfer functions remain a good tool to estimate TP concentration using diatom communities, and Diatom Inferred TP concentration can be compared to currently monitored TP concentration in order to evaluate reference conditions. In a prior step, we tested if the diatom fossil communities archived in the sediment mirrored adequately the changes in the pelagic diatom communities of the lakes over time. There have been, so far, only a handful of such attempts on sub-Alpine lakes. Bennion *et al.* (1995) and Wessels *et al.* (1999) performed comparisons based on long-term datasets for the lakes Mondsee and Constance, respectively, and Marchetto and Musazzi (2001) conducted a comparative study on lake Maggiore using the relative abundance of six selected planktonic species. The lake Geneva provided a unique opportunity to conduct such a calibration analysis since i) long-term limnological data, covering the last 30 years, were available, and ii) its annually laminated sediment could be dated at very high-resolution.

METHODS

Study sites

The lakes Geneva, Bourget and Annecy are all warm-monomictic lakes located on the northwest edge of the French Alps. The surface water of these lakes is never frozen over. They are the largest deep lakes in France with surface areas of 582, 42 and 27 km^2 and maximum depths of 309, 145 and 69 m for lake Geneva, Bourget and Annecy, respectively.

The increasing anthropogenic pressure due to the demographic and economical development, during the 1930s and 1940s, led to the first symptom of eutrophication of all three lakes, as evidenced, from palaeo-approaches, by increased *Daphnia* abundance (Alric 2012), increased organic matter and P content of sediment (Jenny *et al*., in press; Perga *et al.*, 2010). Maximum levels of eutrophication yet varied between lakes. P abatement measures were undertaken rather early in lake Annecy, through the set up of wastewater collector all around the lake as soon as 1967. As a result, lake Annecy eutrophication was limited to a mesotrophic state (Druart and Pelletier, 1998) at the end of the 1960s (maximum [TP] <20 µg L^{-1}). Current [TP] is <6 µg L^{-1} (INRA long-term observatory database). In contrast, restoration measures started only during the 1970s for lakes Geneva and Bourget, while these lakes were already eutrophic (maximum [TP]=130 µg L^{-1} in 1977 in lake Bourget and 90 µg L^{-1} in 1979 in lake Geneva, INRA long-term observatory database, winter mean). The settlement of water-treatment plants (early 1970s around lake Geneva and late 1970s in lake Bourget), the ban of P-containing detergents (in 1986 in Switzerland, Rapin *et al*., 1989; and 2007 in France, Lazzarotto *et al*., 2011) and the diversion, in 1981, of treated sewage waters into the Rhône river to preserve lake Bourget (Jacquet *et al*., 2005) successfully resulted in TP reductions (in 2010, TP were 17 µg L^{-1} in lake Bourget and 23 µg L^{-1} in lake Geneva).

Lake Geneva monitoring survey (managed by the International Commission for the Protection of Lake Geneva Waters and the INRA, French National Institute for Agricultural Research) started in 1957. Sampling for the long-term monitoring has been performed at the deepest point of the lake (SHL2). Until 1980, sampling occurred once a month, and biweekly during the stratified season (March-November) since 1981. Water samples dedicated to phytoplankton analysis were collected in the 0-10 m depth water layer from 1974 to 1999, which was extended down to 0-20 m depth from 2000. Microscopic analyses have been performed following the Utermohl method (Utermohl, 1958), now standardised at the European level (AFNOR, 2006). After classical Utermohl determination under inverted microscope, a part of the sample has been treated with hydrogen peroxide for diatom preparation following the European standard for diatom sample treatment and determinations with a 1000× magnification (AFNOR, 2004). The chemical concentration of TP has been measured using the acid molybdate method (AFNOR, 1982). Annual averages have been calculated for the 0-20 m depth water layer*, i.e.* phytoplankton sampling depth.

Long-term routine monitoring surveys started in 1996 for lake Annecy (managed by the Intercommunal Association of Lake Annecy and INRA) and 2004 for lake Bourget (Lake Bourget Water Agency and INRA). From the late 1960's and until the settlement of the long-term monitoring, TP had not been measured for every year (available data for lake Annecy: 1969-1981 and 1991-2007; lake Bourget: 1970, 1971 and 2004-2007). Such data were available from the French Alpine Lakes Long-Term Observatory Database (Database SOERE-INRA of Thonon-les-Bains, France). As in lake Geneva, all the data have been collected at the deepest point of these two lakes and annual averages have been calculated for the 0-20 m depth water layer.

Lake coring and sediment dating

Several short sediment cores were collected from the deepest point of each lake between 2004 and February 2009 using a quadruple gravity corer (UWITEC, Mondsee, Austria). Sediment dating was performed from one master core for each lake using radiometric methods (^{210}Pb, ^{226}Ra, ^{137}Cs and ^{241}Am activities) and counting of annual laminations, which provided accurate chronologies for high-resolution sampling in the three lakes. All working cores (those on which palaeolimnological analyses were performed) were sampled according to the annual laminations, with the same temporal precision (annual or, at most, triennial resolution) and correlated to the master core using lithological tie points and lamina counting performed on both master and working cores (Zolitschka, 2003). Such high-temporal resolution and accuracy were required in order to integrate instrumental and palaeo-data from multiple cores.

Fossil diatom preparation and counting

Diatom counts were performed on 32 (lake Annecy, core LDA09P302D2), 48 (lake Bourget, core LDB09P302D1) and 65 (lake Geneva, cores LEM10P601 and LEM11P14A) subsamples. sub-samples were weighted before and after drying (60°C during 48 h). After classical H_2O_2-HCl digestion (Renberg, 1990), diatoms were mounted in Naphrax on optical microscope slide (one slide per core sample). On each slide, at least 400 valves were counted and identified by light microscopy using phase contrast or differential interference contrast with 1000× magnification. In order to keep an homogeneous determination in the different samples, several pictures were taken for each species. The identifications and counts followed the European standard method (AFNOR, 2004). Determinations were carried out using the Krammer and Lange-Bertalot floras (1986, 1988, 1991a, 1991b) and other specialized bibliographical data when needed.

Data analyses

Diatom stratigraphy

To reveal the timing of major changes in the communities, chronological clustering analyses, computed from Bray-Curtis dissimilarity indexes, were performed (CONISS in R package rioja, Juggins, 2009). The broken-stick approach (Bennett, 2006) was used to assess the number of statistically significant biozones.

Comparison of limnological and palaeolimnological diatom records

For lake Geneva, changes over time in the taxonomic compositions (expressed as species relative abundances per year) were compared between the limnological and palaeolimnological records over a 30-year period (1977-2007). Correlations between the two datasets were detected using a non-parametric Mantel test (Mantel, 1967) performed on two Bray-Curtis distance matrices (Ginkgo software, (De Caceres *et al*., 2007). The Mantel test was performed using xlstat (http://www.xlstat.com, Addinsoft)

To explore the differences between palaeolimnological and limnological countings, the Indval Method (Dufrene and Legendre, 1997) was performed using PC-Ord 5 (McCune and Mefford, 2006) to define which species are more characteristics for the limnological or the palaeolimnological samples.

Transfer functions

Annual mean TP concentrations were reconstructed from a diatom inference model based on 86 surface sediment samples collected from sub-Alpine lakes in the Alps region (Wunsam and Schmidt ,1995). This calibration dataset includes 86 lakes in the Alpine region (Italy, Germany and Austria) spanning a large gradient in altitude (198-1763 m asl), maximum depth (2-270 m) and mean annual TP (2-266 µg L^{-1}). The model was calibrated using log-transformed TP data. The original calibration presented by Wunsam and Schmidt (1995) using tolerance weighted WA showed a bootstrapped root mean square error of the prediction (RMSEP) of 0.346 log units. Bootstrapped average and maximum bias were 0.009 and 0.86 log units, respectively. The selected model was then applied to the diatom biostratigraphy of each lake. The diatom-inferred TP reconstructions (DI-TP) reconstructions were performed using program C2 - version 1.7.2, (Juggins, 2007). Several models were tested: weighted averaging with classical deshrinking (WAcla regression), weighted averaging with inverse deshrinking (WAinv regression) (Ter Braak and van Dam, 1989), weighted averaging partial least squares regression (WAPLS) (Ter Braak and Juggins, 1993), modern analogue technique (MAT) with squared chord distance and five analogues (Overpeck *et al*., 1985); and their relative performances were estimated using r^2 and the root mean square error of prediction (RMSEP) calculated using a cross validation method (bootstrapping, 500 permutations) on the calibration dataset. The same bootstrapping procedure was used to calculate the standard error of the prediction of the core samples.

The diatom-inferred TP reconstructions were compared to TP limnological data, and also to palaeolimnologically reconstructed changes in *Daphnia* abundances (Alric, 2012) since these are known to be good indicators of TP dynamics (DeMott and Gulati, 1999).

RESULTS

Comparison of limnological and palaeolimnological data in lake Geneva

The temporal changes in the relative abundances of the most commonly occurring species over the 1977-2007 time-period in lake Geneva are presented in Fig. 1. Over the 1977-2007 time-period in lake Geneva, a total of 80 taxa were present in the limnological dataset and 114 in the palaeolimnological one. A comparison of the species relative abundances in the limnological data and palaeolimnological data highlighted some differences: *Asterionella formosa*, *Cyclotella costei* and *Diatoma tenuis* relative abundances were higher in limnological data, and, in contrast, *Stephanodiscus binderanus* and *S. minutulus* had lower relative abundances than in the palaeolimnological data (between 1989 and 1996).

The Mantel test showed that the two datasets were significantly correlated (P<0.0001) but the strength of the relationship was low (r=0.498). Here, only 52 taxa, which had a relative abundance >2%, were kept for the Indval method. Among these taxa, 38 species were typical for only one of the two datasets (Tab. 1). These 38 species were separated following their life form (benthic or planktonic) according to Berthon *et al*. (2011) and Rimet and Bouchez, 2012b. The proportion of benthic species is very low and did not exceed 9% in lake Annecy and lake Bourget and 6% in lake Geneva. This classification underlined that the majority of the species only present in the palaeolimnological dataset were benthic species (Tab. 1 - group 1). Only one benthic species (*Achnanthidium minutissimum* (Kutzing) Czarnecki) was present in both datasets (group 2) but with a higher relative abundance in the palaeolimnological data. Three benthic taxa were detected only in the limnological dataset (*Fragilaria capucina* var. *radians* which is principally recognizable in living samples using the star shape of the colonies, *Navicula* sp. and *Nitzschia* sp. - group 3). Among the planktonic taxa, again, the majority belonged to group 1, seven belonged to group 3 and only two

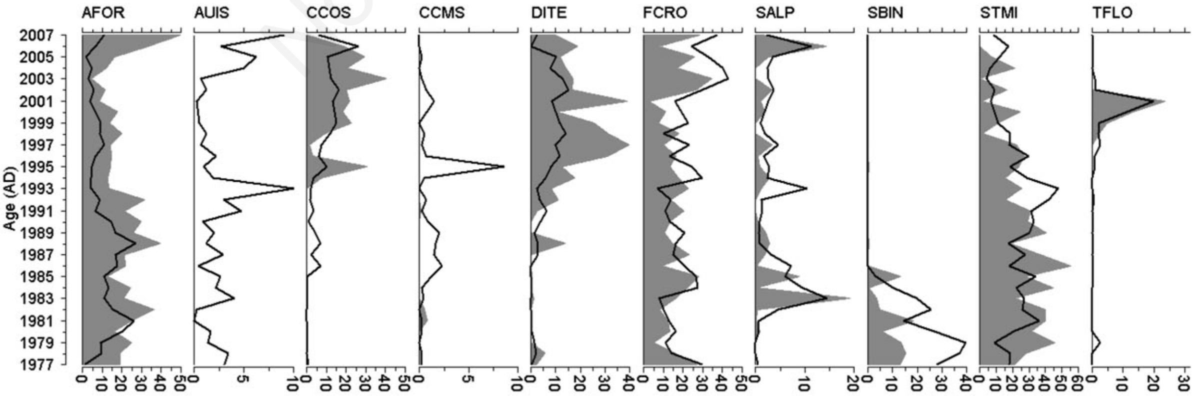

Fig. 1. Comparison of the major planktonic diatom species in the fossil diatom record and in the phytoplankton samples (relative abundances in the diatom community percentage) of lake Geneva 1977-2007. Black line, palaeolimnological data; grey silhouette, phytoplankton data; AFOR, *Asterionella formosa* Hassall; AUIS, *Aulacoseira islandica* (Muller) Simonsen; CCOS, *Cyclotella costei* Druart & Straub; CCMS, *Cyclotella comensis* Grunow in Van Heurck; DITE, *Diatoma tenuis* Agardh; FCRO, *Fragilaria crotonensis* Kitton; SALP, *Stephanodiscus alpinus* Hustedt in Huber-Pestalozzi; SBIN, *Stephanodiscus binderanus* (Kutzing) Krieger; STMI, *Stephanodiscus minutulus* (Kutzing) Cleve and Moller; TFLO, *Tabellaria flocculosa* (Roth) Kutzing.

were present in the two datasets and in higher abundance in the limnological dataset (*Asterionella formosa* and *Diatoma tenuis* - group 4).

Changes in the fossil diatom assemblages

Fossils from 145, 239 and 203 diatom species for, respectively, lakes Annecy, Bourget and Geneva, were recovered from the cores. Six species represented more than 80% of the total planktonic frustules in lake Annecy, and ten species for lake Bourget and lake Geneva (Fig. 2). The oldest core samples in the three lakes were rich in *Cyclotella* species. The relative abundance and the diversity of *Cyclotella* valves decreased over the time. This trend was partly reversed in the most recent samples. Another common feature was a generally increasing contribution of *Fragilaria crotonensis* (Kitton) over time.

No diatom valves could be detected in the lowermost part of the core of lake Annecy, corresponding to time periods prior to the early 1930s. *Cyclotella costei* (Druart & Straub) [synonym of *Cyclotella cyclopuncta* Håkansson & Carter (Houk *et al.,* 2010)] and *Fragilaria crotonensis* were the dominant species, making, on average, up to 70% of the fossil remains. Between 1937 and 2007, chronological clustering identified six biozones (a1-a6). Major shifts in the fossil diatom communities were identified in 1941-1942, 1950-1951, 1963-1964, 1967-1968

Tab. 1. IndVal method results. Only species with a significant P-value (<0.05) are presented. These species are characteristics of one of the two compared datasets. The species of the group 1 (last column) are only present in the palaeolimnological dataset, the species of group 2 are present in the two datasets but are characteristics of the palaeolimnological dataset, the species of group 3 are only present in the limnological dataset and the species of group 4 are present in the two datasets but are characteristics of the limnological dataset.

Species	P value		Group
	Limnological dataset	Palaeolimnological dataset	
Benthic species			
Achnanthidium minutissimum (Kutzing) Czarnecki		0.0284	2
Amphora pediculus (Kutzing) Grunow		0.0002	1
Cocconeis placentula Ehrenberg var. *placentula*		0.0052	1
Cymatopleura solea (Brebisson in Breb. & Godey) W.Smith var. *solea*		0.0018	1
Diatoma moniliformis Kutzing		0.0016	1
Encyonema minutum (Hilse in Rabh.) D.G. Mann		0.0002	1
Fragilaria capucina Desmazieres var. *capucina*		0.0002	1
Fragilaria capucina Desmazieres var. *radians* (Kutzing) Lange-Bertalot	0.0002		3
Fragilaria capucina Desmazieres var. *vaucheriae* (Kutzing) Lange-Bertalot		0.0234	1
Fragilaria mesolepta Rabenhorst		0.0022	1
Gomphonema pumilum (Grunow) Reichardt & Lange-Bertalot		0.049	1
Navicula cryptotenella Lange-Bertalot		0.011	1
Navicula reichardtiana Lange-Bertalot var. *reichardtiana*		0.258	1
Navicula sp.	0.0002		3
Navicula subrotundata Hustedt		0.004	1
Nitzschia dissipata (Kutzing) Grunow var. *dissipata*		0.002	1
Nitzschia fonticola Grunow in Cleve et Muller		0.0002	1
Nitzschia sp.	0.0002		3
Staurosira mutabilis (Wm Smith) Grunow		0.0006	1
Planktonic species			
Asterionella formosa Hassall	0.0002		4
Aulacoseira ambigua (Grunow) Simonsen		0.0004	1
Aulacoseira granulata (Ehr.) Simonsen var. *angustissima* (Muller) Simonsen	0.0002		3
Aulacoseira islandica (Muller) Simonsen		0.0002	1
Aulacoseira islandica (Muller) Simonsen subsp. *helvetica* (Muller) Simonsen	0.0002		3
Cyclotella comensis Grunow in Van Heurck		0.0002	1
Cyclotella delicatula Hustedt		0.0002	1
Cyclotella sp.	0.0004		3
Diatoma tenuis Agardh	0.0224		4
Discostella pseudostelligera (Hustedt) Houk and Klee		0.0086	1
Discostella stelligera (Cleve et Grun.) Houk and Klee		0.0002	1
Fragilaria ulna (Nitzsch.) Lange-Bertalot var. *acus* (Kutz.) Lange-Bertalot	0.0002		3
Fragilaria (Ulnaria) *ulna* Sippen *angustissima* (Grun.) Lange-Bertalot	0.0002		3
Nitzschia acicularis (Kutzing) W.M.Smith	0.0002		3
Stephanodiscus irregularis Druart. Reymond Pelletier and Gasse		0.0014	1
Stephanodiscus parvus Stoermer and Hakansson		0.0002	1
Stephanodiscus species		0.0106	1

and 1976-1977. Before the early 1950s (biozones a1 and a2), the fossil diatom community was the most diverse, with notable amounts of *Cyclotella distinguenda* var. *distinguenda* Hustedt, *Cyclotella comensis* Grunow in Van Heurck and *Stephanodiscus minutulus* (Kutzing) Cleve & Moller. *Cyclotella costei* and *Fragilaria crotonensis* were dominant in all counts from the 1950s (biozones a3 to a6). In the 1950s-60s (biozone a3), the relative abundance of *Cyclotella costei* exceeded 60% but the relative abundance of *Fragilaria crotonensis* increased up to 45% from the 1960s, so that the relative abundance of these two species were comparable within the biozones a4-a5. Increasing abundances of *Fragilaria crotonensis* in biozones a4-a5 occurred with increased amounts of *Stephanodicus parvus* Stoermer et Håkansson. The diatom composition in the most recent samples (biozone a6)

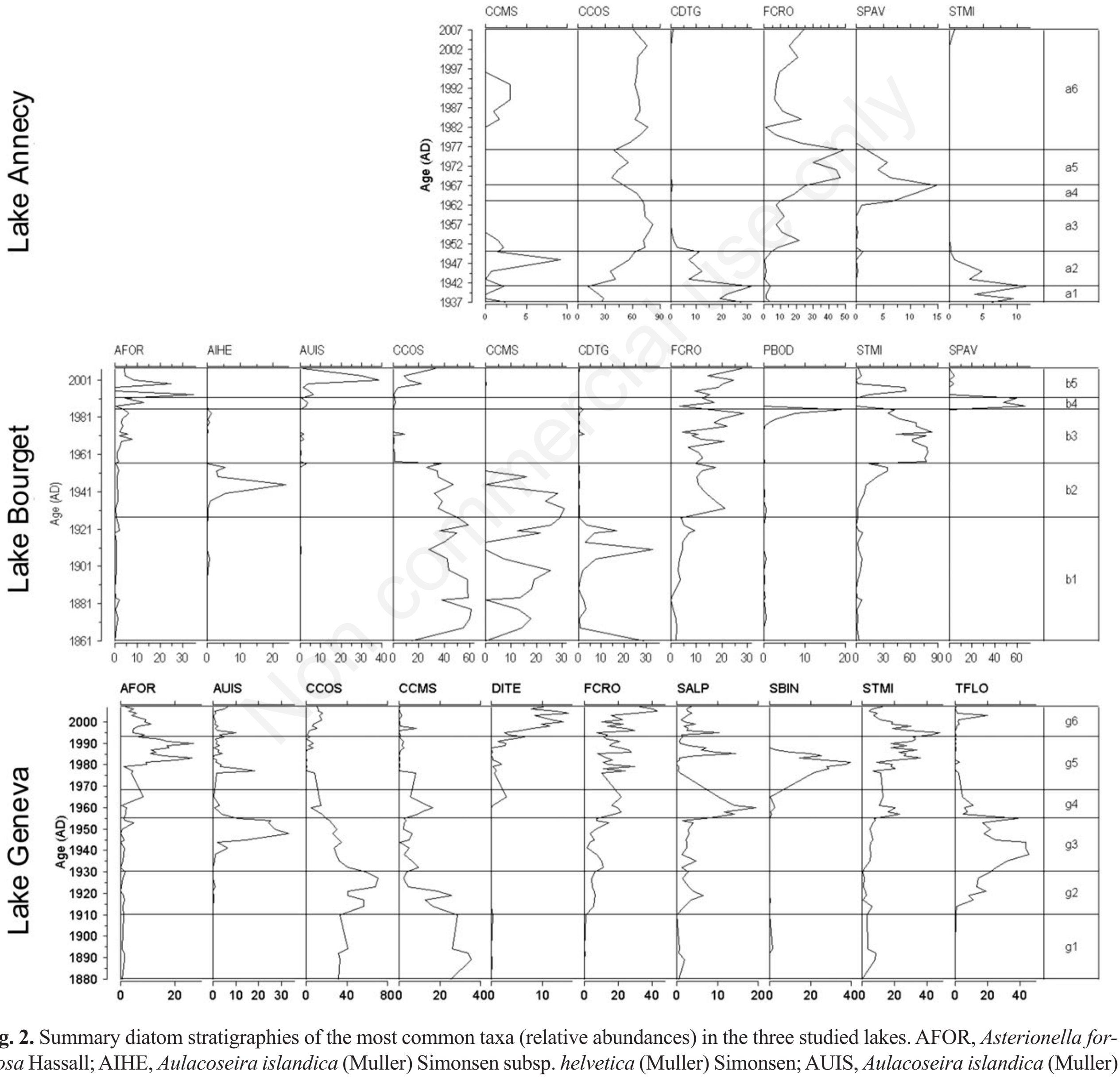

Fig. 2. Summary diatom stratigraphies of the most common taxa (relative abundances) in the three studied lakes. AFOR, *Asterionella formosa* Hassall; AIHE, *Aulacoseira islandica* (Muller) Simonsen subsp. *helvetica* (Muller) Simonsen; AUIS, *Aulacoseira islandica* (Muller) Simonsen; CCOS, *Cyclotella costei* Druart & Straub; CCMS, *Cyclotella comensis* Grunow in Van Heurck; CDTG, *Cyclotella distinguenda* var. *distinguenda* Hustedt; DITE, *Diatoma tenuis* Agardh; FCRO, *Fragilaria crotonensis* Kitton; PBOD: *Puncticulata bodanica* (Grunow in Schneider) Håkansson; SALP, *Stephanodiscus alpinus* Hustedt in Huber-Pestalozzi; SBIN, *Stephanodiscus binderanus* (Kutzing) Krieger; SPAV, *Stephanodiscus parvus* Stoermer and Håkansson; STMI, *Stephanodiscus minutulus* (Kutzing) Cleve and Moller; TFLO, *Tabellaria flocculosa* (Roth) Kutzing.

strongly resembled that of biozone a3. Changes in community structure were more striking in lake Bourget. Five biozones were identified (b1-b5), with transitions occurring in 1926, 1956, 1986, and 1991. In biozone b1, the community was dominated by *Cyclotella costei, C. comensis* and *C. distinguenda* var. *distinguenda*. In biozone b2 (1930s-1950s), *C. distinguenda* var. *distinguenda* disappeared while the contributions of *Stephanodiscus minutulus* and *Fragilaria crotonensis* increased. Biozone b3 (late 1950-late 1980s) was almost exclusively represented by these two species. Biozones b4-b5 saw the reappearance of some of the *Cyclotella species* that were previously detected in biozones b1-b2 such as the decreasing abundances of *Stephanodiscus minutulus* (except for a peak in the 1990s). However, the contributions of species that were detected only at very low levels before, *i.e. Asterionella formosa* Hassall, *Aulacoseira islandica* (O. Muller) Simonsen ssp. *helvetica* (O.M.) Simonsen and *Stephanodiscus parvus*, substantially increased in biozones b4-b5. Hence, the diatom composition in the most recent samples of b5 was not similar to any of those observed in the preceding biozones.

In lake Geneva, six biozones were identified (g1-g6), with transitions in 1910, 1930, 1955, 1968 and 1994. *Cyclotella costei* and *Cyclotella comensis* were very abundant in the biozones g1-g2. The main difference between the two biozones was due to an increased contribution of *Tabellaria flocculosa* (Roth) Kutzing, *F. crotonensis* and *S. alpinus* for the biozone g2. The community then shifted in the early 1930s to dominance by *Tabellaria floculosa*, along with the appearance of *Fragilaria crotonensis*, *Aulacoseira islandica* ssp. *helvetica* and *Stephanodiscus minutulus* (g3). The biozone g4, between the mid 1950s and the early 1970s, was characterized by dominance of *S. minutulus*, *S. alpinus* and *F. crotonensis*. *Stephanodiscus* species were very abundant by the end of the 1980s (middle of the biozone g5) making up to 60% of the total community. In biozone g6, some *Cyclotella* species (*Cyclotella costei*), which had disappeared in the previous biozones, became more abundant. The community was, however, dominated by *F. crotonensis* (40%) and *Diatoma tenuis* Kutzing (20%), which had been rare so far. *Tabellaria floculosa* represented 20% of the community in 2000. Because of their richness in the three latter species, the most recent samples of the biozone g6 were very different from the rest of the core.

Transfer functions

In comparison with the WA and WAPLS models, MAT models performed poorly. There were no relevant differences between WA and WAPLS model performances. The RMSEP of the models compared well with the standard error of the prediction (SEP) calculated on the core samples (Tab. 2). Results presented in Fig. 3 are those obtained using the WA_{cla} regression for lake Annecy and lake Geneva and using the WA_{inv} regression for lake Bourget. In the three lakes, for each sample, the proportion of taxa present in the calibration dataset was high: 90% in lake Annecy, 91% in lake Bourget, and lower in lake Geneva: 83% (average value).

In lake Annecy, before 1945, the DI-TP concentration varied between 10 and 15 µg L^{-1}. DI-TP values were transiently lower (around 7 µg L^{-1}) during the following 14 years but increased back to 11 µg L^{-1} between 1963 and 1976. The diatom-inferred TP reconstructions values decreased back after this light mesotrophic episode up to the top of the core (around 8 µg L^{-1}). The diatom-inferred TP reconstructions values were consistent with instrumental data and adequately tracked the decrease in P concentrations over the last 30 years. The temporal dynamics in *Daphnia* abundance generally matched that of DI-TP, with maxima reached between the mid 1960s and 1970s, except for the period before the 1940s for which very low *Daphnia* abundance did not support the relatively high DI-TP. In lake Bourget, before 1952, DI-TP concentrations were low (mean value=12 µg L^{-1}). The diatom-inferred TP reconstructions values dramatically increased from the early 1950s to reach 68 µg L^{-1} in 1957. The diatom-inferred TP reconstructions transiently decreased and fluctuated between 36-73 µg L^{-1} to reach a maximum of 80 µg L^{-1} in 1973. Between 1973 and 2007, DI-TP dropped to 14 µg

Tab. 2. Performance of the different tested transfer function models.

Reconstruction method	r^2	Lake Annecy		Lake Bourget		Lake Geneva	
		Model RMSEP	Deepest slices SEP	Model RMSEP	Deepest slices SEP	Model RMSEP	Deepest slices SEP
WA_{cla}	0.60	0.34	0.34	0.34	0.34	0.34	0.35
WA_{inv}	0.60	0.30	0.30	0.30	0.30	0.30	0.30
WAPLS	0.60	0.30	0.30	0.30	0.30	0.30	0.30
MAT	0.38	0.31	0.33	0.31	0.32	0.31	0.10

r^2, bootstrapped squared correlation between inferred and observed values; Model RMSEP, root mean squared error of prediction evaluated through bootstrap; deepest slices SEP, standard error of the prediction in the deepest slices of each core; WA_{cla}, weighted averaging with classical deshrinking; WA_{inv}, weighted averaging with inverse deshrinking; WAPLS, weighted averaging partial least squares regression; MAT, modern analogue technique. Unit: $\log^{10}$ total phosphorus concentration.

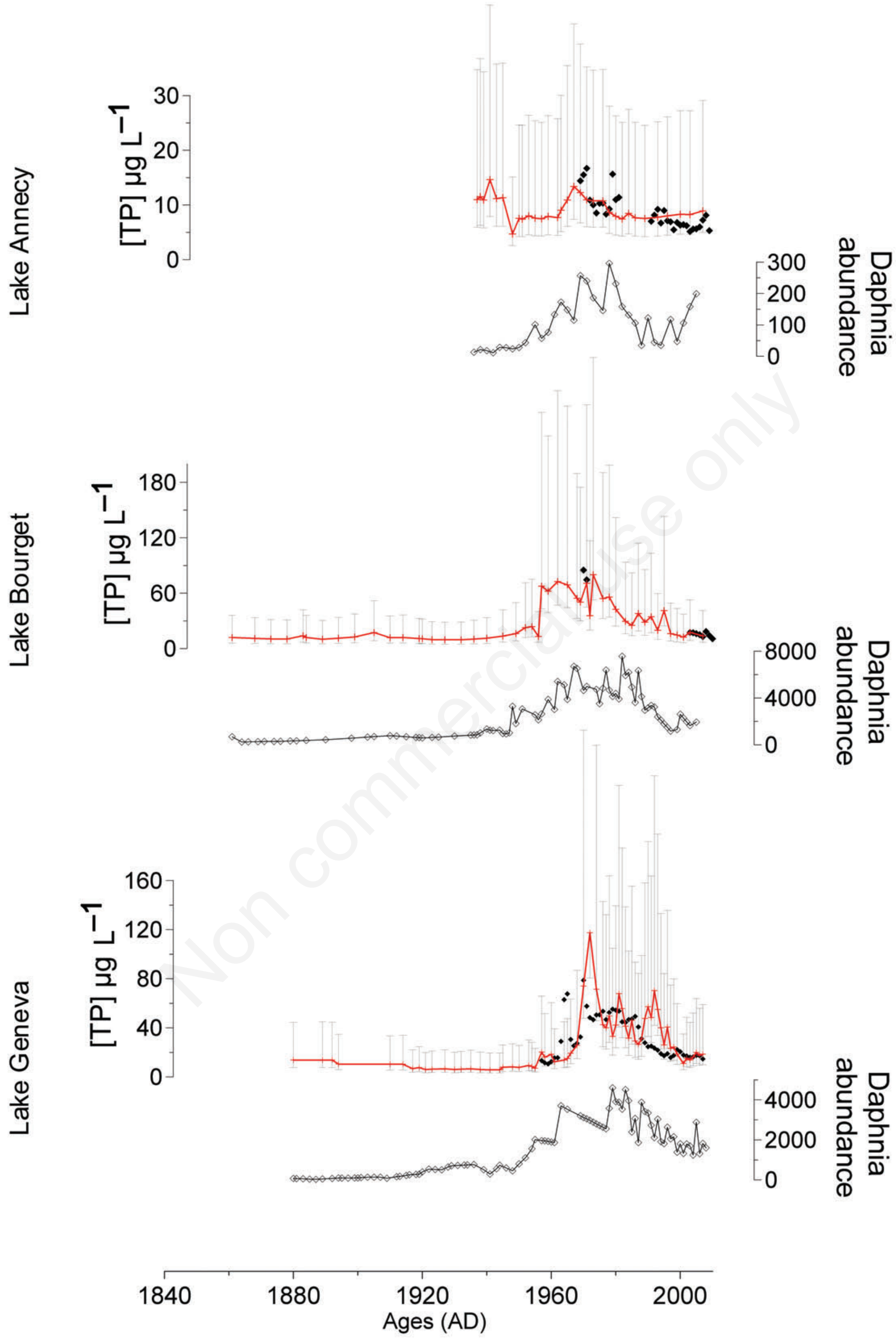

Fig. 3. Diatom-inferred total epilimnetic phosphorus (µg L^{-1}) (black line, bars indicate bootstrap estimated standard error of the prediction) compared with long-term measured epilimnetic phosphorus concentrations in the study lakes (circle, annual values) and abundance of fossil *Daphnia* per gram of dry sediment.

L^{-1} in 2007, consistent with monitored data. *Daphnia* abundance was generally consistent with that of DI-TP. *Daphnia* maxima matched those in DI-TP. However, it seems that *Daphnia* abundance increased from the 1940s: *i.e.,* a decade earlier than the first detected increase in DI-TP. In lake Geneva, DI- TP concentration was low and fluctuated between 6 and 14 μg L^{-1} before 1955. In 1957, values started to increase, to reach 120 μg L^{-1} in 1974. The general dynamics of DI-TP during eutrophication mirrored that of the monitored data, except that DI-TP tended to over-estimate the P maximum. As for lake Bourget, the general dynamics of *Daphnia* abundances were consistent with DI-TP, except that they started to increase a decade earlier. The diatom-inferred TP reconstructions values in lake Geneva decreased from this peak, to reach 19 μg L^{-1} in 2007, except for a period of very high DI-TP in the 1990s that was not corroborated by monitored TP data, nor by reconstructed changes in *Daphnia* abundance.

DISCUSSION

Fidelity of fossil records

Comparisons of palaeolimnological to limnological diatoms countings are important to evaluate the accuracy and relevancy of palaeoenvironmental inferences (Marchetto and Musazzi, 2001). These comparisons allowed an assessment of the fidelity of fossil records and the potential bias arising from taphonomic processes occurring before and after the remains are archived in the sediment. In deep and large sub-Alpine lakes, representation of living diatom communities by deep-water sedimentary diatom assemblages has been already studied by Marchetto and Musazzi (2001), who compared relative abundance of six selected planktonic species in plankton counts and sediment samples in lake Maggiore between 1981 and 1998. Besides, Wessels *et al.* (1999) used the few historical phytoplankton data present in the literature to check whether sediment data reflect planktonic diatom community in lake Constance from 1955, but the comparison performed was based on few species and on sporadic sampled years. A more powerful approach was possible herein because lake Geneva has been carefully monitored for 30 years, and also because the lake's annually-laminated sediment can be dated with very high accuracy. These kinds of diachronic comparison performed on long time periods for a single lake are rare because limnological monitoring often contains temporal gaps.

Different potential errors inherent to the used palaeolimnological and limnological methods can be observed. Sediment records can suffer from taphonomic biases and errors in the cores samples dating (Battarbee *et al.,* 2005). However, first, the temperature and pH conditions, which are considered the most important parameters for good valves conservation in sediments (Battarbee *et al*., 2002; Flower, 1993), allowed a good preservation of diatoms valves, in the three studied lakes. Yet, in lake Annecy, before 1937, no diatoms valves could be detected in the sediment samples. Druart and Pelletier (1998) encountered that same limits while conducting on a long-term palaeolimnological study on lake Annecy and gave two hypothesis: i) the very low trophic level could restrict the algal production; ii) the sediment are rich in detrital particles that could dilute the organism rests. Second, radiometric methods and counting of annual laminations provided accurate chronologies (radiometric methods: ±5 years; counting of annual laminations: ±1 year). Furthermore, cores were meticulously studied to show irregularities in sediment deposit (*e.g.,* flood events have been spotted) and we chose to work at the deepest part of the three lakes to limit the effect of phenomenon as bioturbation within the sediment column, which can provide a worse representation of the diatom communities. In limnological methods, biases can appear when the protocol is not strictly respected or when the operators change: differences in result can be observed in inter-comparison exercises [Prygiel *et al*., 2002); External Quality Assessment Trials Phytoplankton (EQAT, www.planktonforum.eu]. The participation of our limnological database operators to this kind of exercises allows a good use and respect of the standard.

The Mantel test showed that the palaeolimnological and limnological datasets were highly correlated, hence highlighting a high compositional fidelity of the sediment archive in lake Geneva. Good preservation has also been shown for Cladoceran subfossils in these deep sub-Alpine lakes and is attributed to rapid and direct sedimentation processes in an environment relatively undisturbed by sediment resuspension and transportation (Alric and Perga, 2011). Nevertheless, differences between the two datasets may arise from three non-exclusive processes. First, diversity was greater in the palaeolimnological dataset than in the limnological dataset of lake Geneva. Ecological groups enabling discrimination between benthic and planktonic species, as designed by Berthon *et al.* (2011) and Rimet and Bouchez (2012b), were used to interpret the Indval Method results (even if these study were based on river assemblages, we believe that the principle of classification, based on genera life history traits, remains valid in lakes). Results showed that the higher diversity of the palaeolimnological record could be attributed to the presence of rather numerous benthic species, that might arise from lateral transport of diatom remains from littoral areas (sediment focusing (Blais and Kalff, 1995; Likens and Davis, 1975). Overall though, this bias was kept low, since it represented <10% of the total community abundance and tends to be limited by geomorphology of these deep large lakes with restricted littoral areas, which are 7 km from the sampling site in lake Geneva. Second, some species were found only in the limnological data, which could be explained by the constant evolution of diatom taxonomy and the use of different taxonomic books over time (Rimet and Bouchez, 2012a). It could also be explained by operator determination skills in the case of *Fragilaria* and *Aulacoseira*, since both genera encompass several morphologically similar species that can be confused. Third, two dominant species (*Asterionella formosa* and *Diatoma tenuis)* were under-estimated in the palaeolimnological dataset relative to the limnological dataset. These species have a very thin siliceous skeleton and might be more sensitive to taphonomic processes during sedimentation. Their preservation might be lower than for other species with thicker frustules, which might explain why valves detected on the slides were broken. Such underestimations of the abundance of *Asterionella formosa* and *Diatoma tenuis* in profundal core samples were also observed by Marchetto and Musazzi (2001).

Lake trophic histories

As mentioned above, fossil diatoms in lake Annecy cannot be used to reconstruct the trophic history before 1937, because they were not preserved in the sediments. Lake Annecy did not reach a high level of eutrophication (maximum TP concentration 15 μg L^{-1} in 1966 and 1970 – winter mean, limnological data) but a slight decrease in *Cyclotella costei* and a replacement by more eutrophic species, namely *Stephanodiscus parvus* were yet observed. The increase in DI-TP concentration has been stronger in lake Bourget and lake Geneva according major shifts in the fossil diatom communities, most notably the replacement of *Cyclotella* species by mainly *Stephanodiscus minutulus*. These patterns were partially reversed following lake restoration. In lake Geneva the transition between the oligotrophic and eutrophic phases seemed to be slower than in lake Bourget, with periods during which mesotrophic species as *Tabellaria floculosa* and *Diatoma tenuis* developed (Rimet *et al.,* 2009). Lakes Bourget and Geneva underwent a similar trophic history and diatom species succession to that reported for lake Maggiore (Italy) and lake Constance (Germany/Switzerland boundary) while that of lake Annecy was more limited. Indeed, in the latter, the species successions has been less contrasted since the abundance of *Cyclotella costei* decreased but the species could maintain in the diatom community, in contrast to lake Bourget and lake Geneva, where they disappeared. Similarly to lake Maggiore and lake Constance (Marchetto *et al.,* 2004), the trophic status of lake Bourget and lake Geneva are still higher than before eutrophication. The composition of the diatom assemblages in these two lakes today is markedly different from that of the pre-eutrophication period with particularly lower abundance of *Cyclotella comensis* in the recent sediment and the presence of *Asterionella formosa* and *Diatoma tenuis* (mesotrophic species) in lake Geneva. Re-oligotrophication is more advanced in lake Maggiore where *Cyclotella comensis* is currently abundant (Marchetto *et al.,* 2004).

Total phosphorus reconstructions

In spite of the annual variability in TP concentration in lakes, the annual mean TP concentration measured in the three study lakes compares well with the DI-TP values. Apart some sporadic points, the difference between measured and inferred TP is markedly lower than the standard error of the prediction estimated using the bootstrapping techniques. This pattern was also found by, for example, Marchetto and Bettinetti (1995) in other deep lakes, and it is probably due to the large morphometric heterogeneity of the lakes included in the calibration data set. In fact, in the bootstrapping procedure, the error component specific to each fossil sample (v1 in Birks *et al.,* 1990) is markedly smaller than the component due to the imperfection in the calibration procedure (v2 in Birks *et al*., 1990). Indeed, there are not enough large deep lakes in Europe to build a specific data set, so that Wunsam and Schmidt (1995) enlarged their calibration data set with shallower lakes. A consequence is an inflated noise in the transfer function. Nevertheless, DI-TP values were very close to the measured TP for samples which diatom composition was very similar to those used for the calibration. In contrast, in samples rich in small *Stephanodiscus*, which have no modern analog in the calibration data set, the DI-TP values were quite different from the measured values.

WA_{cla} reconstruction models provided TP values closest to the limnological data and the most realistic in relation to the known history of the lakes Annecy and Geneva during the last century (limnological data+*Daphnia* abundance reconstruction). In contrast, the most adequate model was the WA_{inv} for lake Bourget. WAPLS did not provide satisfactory reconstructions: for instance, in lake Bourget, between 1860 and 1940, the WAPLS reconstruction lead to marked fluctuations between 12 and 90 μg L^{-1} which were not supported by others proxies such as cladocerans (Alric, 2012). Furthermore, WAPLS, as an inverse approach, seems to perform best if the fossil samples are similar in composition to samples in the central part of the calibration data (Birks, 1998), which was not the case here. A high abundance of some species in the dataset used to calculate the DI-TP concentration caused some overestimations or underestimations compared with monitored TP (Wunsam and Schmidt, 1995). The overestimated values corresponded to the time periods when the relative abundance of *Stephanodiscus* species was very high although the WA_{cla} model seemed to be less sensitive to this problem. DI-TP values were higher than expected between 1937 and 1945 in lake Annecy, and in 1972 and between 1989 and 1996 in lake Geneva. This over-estimation by the model has been observed in previous studies. *Stephanodiscus minutulus* was very abundant during the 1980s in the palaeolimnological record of lake Maggiore (Marchetto *et al.,* 2004) while *Stephanodiscus* spp. relative abundance was higher than 30% in the Esthwaite Water and the Windermere South Basin diatom community (Bennion *et al.,* 2005). Bennion (1995), in lake Mondsee, showed the same overestimation due to the same *Stephanodiscus* species and speculated it could be explained by the high abundances of these species in the most eutrophic lakes of the calibration dataset. It should also be noted that, between 1989 and 1996, in lake Geneva, taphonomic bias might cause an over-estimation of *Stephanodiscus minutulus* (Fig. 1). *Cyclotella costei*, which was very abundant in the three studied lakes, may have the opposite effect since high abundances in the sediment appear to be associated with TP underestimations. Then, assuming that mesotrophic species (*Tabellaria floculosa* and *Diatoma tenuis*) increasing abundances do not cause any progressive increase in DI-TP concentration in lakes Bourget and Geneva, the model may not account sufficiently for these species. As a result, the reconstruction using this transfer function does not detect small fluctuations of TP concentrations at low TP levels (<10 μg L^{-1}), as evidenced by the comparison with Daphnia biostratigraphies. The consequence is that the model cannot date accurately the early beginning of eutrophication in lakes Bourget and Geneva, hence partially limiting the accuracy of the TP reference conditions.

Reference conditions and present state

Reference conditions are determined as the state observed before the industrialisation and agricultural intensification (but they do not equate to the pristine state because the anthropogenic impacts started before) (Bennion *et al.,* 2005). Using the transfer function result, as we said in the 4.3 section, we cannot define precisely the references conditions for TP concentration in the three studied lakes. Furthermore, for lake Annecy, the absence of diatoms in the lowermost section of the core (before 1937 AD) limited the extent of the record. Average DI-TP concentrations values calculated over the known pre-eutrophication period (lake $\text{Annecy}_{1938\text{-}1950}$: 10 μg L^{-1}; lake $\text{Bourget}_{1861\text{-}1950}$: 11 μg L^{-1}; lake $\text{Geneva}_{1880\text{-}1950}$: 8 μg L^{-1}), are slightly higher than values observed in lake Maggiore and lake Constance (Marchetto *et al*., 2004; Wessels *et al.,* 1999) and this might arise from the model limits as specified above.

The current diatom communities compositions in the three studied lakes are still different from those observed for the defined reference conditions, with, for instance, lower abundances of *Cyclotella*. However, TP concentration is not the only parameter that can drive changes in the diatom communities. For instance, increasing abundance of *Fragilaria crotonensis* - which is typically a thermophilic species in these lakes (Rimet *et al*., 2009) - over the most recent years of the studied period may be attributed to the increasing temperature in the region, especially from the late 1980s - HISTALP data set (Auer *et al.*, 2007). The potential effect of parameters as temperature should also explain the differences between eutrophication and re-oligotrophication trajectories

CONCLUSIONS

Paleolimnological methods using diatoms can suffer from some bias due to, for example, valve preservation in the sediment, especially for thinly silicified species, or to the presence of benthic species in sediment in very deep part of lakes (Battarbee *et al.,* 2002). However, the comparison of limnological and palaeolimnological datasets for lake Geneva confirmed the faithfulness of the palaeolimnological records for these sub-Alpine lakes. The fossil diatom records are, therefore, appropriate for assessing environmental change in these systems. The comparison between DI-TP and limnological monitoring data showed good agreement, thus enabling the reconstruction trophic history of the three study lakes and the establishment of relatively accurate reference P concentrations. However, in spite of the bias inherent to the used transfer function models [like WA, which assume, for example, always an unimodal relationship between species and environmental variables even if some taxa may show a skewed unimodal or sigmoid (and not linear) response (Birks 1998; Huisman *et al.,* 1993)], transfer functions have been used in the past to infer different limnological variables. One important point in the application of transfer functions is that the inferred variable should be an important factor controlling the development of the specific community used for inference. In the case of diatoms, it is well known that species distribution is mainly dependent on salinity, pH, trophic status and habitat availability.

Part of the diatom responses observed during re-oligotrophication might also be driven by climate variability. Climate change and the lakes responses to the combination of decreasing TP concentration and climate change are still difficult to understand (Battarbee *et al.,* 2005). Perspectives to this work would be to quantify the role of the different perturbations that potentially impacted the diatom communities in these three lakes changing their compositions (eutrophication, climate change and fisheries management practices). This kind of study has been already done for Esthwaite Water (Dong *et al.,* 2011). Such an approach is required to evaluate how much the diatom composition at the TP restoration objectives might differ from those observed at temporal reference conditions.

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