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**Occurrence and excessive development of *Mougeotia*
spp. in large lakes**

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1 Introduction

Algal blooms appear when one or a few species of algae outcompete others, often causing negative effects on the affected systems and for human uses of the waterbody (Canter-Lund & Lund 1995). Although in freshwater ecosystems the most common and the most harmful blooms are related to Cyanobacteria (Havens 2008), it is not the only group that can cause mass production.

During the last decades, especially in large deep lakes in the peri-alpine region, the occurrence of green algae blooms hampering professional fishing and drinking water supply (Rimet et al., 2008) have been increasing. These blooms commonly occur in lakes which underwent eutrophication and appear during the recovery period when phosphorus concentrations had considerably decreased. The blooming genus, *Mougeotia* C. Agardh 1824 is being common in these lakes. *Mougeotia* became an important and significant member of the phytoplankton in e.g. Lake Garda (Salmaso 2000; Salmaso 2002), Lake Iseo (Garibaldi et al. 2003), Lake Lugano (Polli & Simona 1992), Lake Maggiore (Morabito et al. 2003; Morabito et al. 2002) and it contributes to the hysteresis of phytoplankton in regard to phosphorus decrease in Lake Geneva (Anneville et al. 2002).

Mougeotia is a diverse genus belonging to the family Zygnemataceae which includes many of the most common filamentous green algal species in freshwaters (Guiry 2013). Most of the studies on *Mougeotia* are physiological studies on benthic or littoral populations (Graham et al. 1996a; Graham et al. 1996b; Klug & Fischer 2000). In the last decades some studies have been published on planktic populations of the genus as a consequence of its frequent appearance in lakes, mainly in great, deep lakes in the peri-alpine region.

Recent observations show that occurrence of *Mougeotia* is related to the trophic change of the lakes. In Lake Lugano the first report of the presence of *Mougeotia* was published from the period between 1955 and 1958 when the first symptoms of eutrophication became apparent but the highest biomass value was observed in the autumn 1986, six years after the phosphorus concentration began to decrease. During the period with the highest trophic level, the species was not recorded (Polli & Simona 1992). Colonisation of the taxon in Lake Geneva started in the 1960's in parallel with the beginning of the eutrophication of the lake (Anneville & Pelletier 2000). Its abundance declined during the period of strong eutrophication but became more abundant again in the 1990's with the decreasing phosphorus concentration. Success of

Mougeotia in the mentioned lakes can be partly due to its good competition for phosphorus but also to these filaments which work as antennae that can effectively utilise light even under low light conditions (Reynolds 2006). *Mougeotia* is therefore able to develop in the metalimnion where phosphorus concentrations are higher than in the depleted epilimnion and where phosphorus supply from the deeper water layers may occur due to hydrodynamics and diffusion processes (Cuypers et al. 2011).

Another competitive advantage of the taxon lies in its morphology. The long filament form helps the organism to reach lower sinking velocity which is an important adaptive feature in the stratified period enabling the population to persist in the upper layers for longer periods (Padisák 2003). The importance of this sinking property is also confirmed by Salmaso (2003) and Morabito et al. (2003). Besides abiotic factors there are biotic factors that can play role in the development of *Mougeotia*. Long filaments of the species are inedible for most zooplankton species (Salmaso 2003).

Apart from the alpine region, planktic *Mougeotia* is also common in the subtropical region, in Lake Kinneret, Israeli (Yacobi & Zohary 2009). In Lake Kinneret the phytoplankton composition underwent substantial changes during last decades and became rather unpredictable. One of the unexpected changes was the occurrence of *Mougeotia* sp., reaching high biomass during April and May in 2005. Several observations indicate that *Mougeotia* is a permanent element of the phytoplankton flora in deep lakes even though blooms have not been observed (Padisák et al., 2010).

Studies referring to planktic *Mougeotia* commonly give a general knowledge of the taxon's ecology and discuss why its contributions to the total phytoplankton biomass have changed. However, few studies explain how the low nutrient concentrations recorded in meso- and oligotrophic lakes can sustain *Mougeotia* blooms and biomass similar to those observed in eutrophic lakes. Furthermore, the knowledge on the combination of environmental factors that induce blooms and play key role in their development has been scarce.

2 Aim of study

In this study, long-term data of *Mougeotia* biomass from four lakes presenting regular mass development of *Mougeotia* (Lake Garda, Lake Maggiore, Lake Geneva and Lake Kinneret) have been analysed in order to better understand the pattern of occurrence of this genus. Coupled statistical analyses between *Mougeotia* biomass and key environmental parameters were performed to identify combinations of abiotic factors responsible for *Mougeotia* blooms appearance. The aim of this study is to provide a description of the annual and inter-annual patterns of *Mougeotia* in the studies lakes, environmental factors associated with the occurrence of *Mougeotia* and the combination of factors associated with blooms.

3 Material and methods

3.1 Study sites

Lake Geneva

Lake Geneva, the largest deep lake in Western Europe is located on the French-Swiss border (46°26'N, 6°33'E – mean geographical position) (Figure 1. a-b). Its surface area of 580 km² shared by Switzerland (60%) and France (40%), with a maximum length of 73 km and a maximum width of 14 km. Two parts of the lake can be distinguished; “Grand Lac” on the eastern side and the smaller, narrow section to the west, the “Petit Lac”. “Grand Lac” represents the 96% of the total water volume (89 km³) with a maximum depth of 309 m. Since the lake does not freeze in winter and the water column remains stratified during a long period, Lake Geneva is considered as a warm monomictic lake, although complete mixing occurs very rarely (Perroud et al. 2009). Maximal annual mixing depth varies from 50 m to the lake bottom (Anneville et al. 2002). Last complete overturns took place in the winter of 2004-2005 and 2005-2006, 20 years after the last complete mixing (Lazzarotto and Rapin, 2007) and in 2011-2012. The “Petit Lac” with its 76 m maximal depth mixes every year, though (Perroud et al. 2009).

The main tributary of the lake is the Rhone river which originates from the Rhone Glacier at an altitude of 1763 m (Loizeau & Dominik 2000) entering the lake at the eastern side between Villeneuve and Le Bouveret before flowing slowly towards its egress at Geneva at the western side of the lake. Although River Rhone represents the main inflow of the lake, other important entering rivers are Dranse, Aubonne, Morges, Venoge, Vuachère, and Veveyse.

Lake Garda

The greatest lake of Italy is Lake Garda, located at the northern part of the Mediterranean country, just in the foothills of the Alps (45°38'N 10°40'E) at an altitude of 65 m (Figure 1. a-b). The lake's surface is 368 km² with a volume of 49 km³, and it has a catchment area of 2260 km². Lake Garda is divided into two basins that are separated by an underwater ridge connecting Punta S. Vigilio with the Sirmione Peninsula. The larger basin at the west is deeper ($z_{\max}=350$ m), whereas the east basin is smaller and represents less than the 7% of the lake's total volume. Maximum depth of the east basin is 80 m (Salmaso 2002). Compared to other deep subalpine lakes, Lake Garda has a relatively long water renewal time (*ca* 27 years) (Ambrosetti & Barbanti 1997). The main inflow river, River Sarca, can be found at the northern edge of the

lake, but similarly to Lake Geneva, beside the main inflow, there are other tributaries with minor importance. These mainly flow towards the west and north shores. The outflow is River Mincio at the southern edge of the lake with an average discharge of $58 \text{ m}^3 \cdot \text{s}^{-1}$ (Salmaso 2002).

Lake Maggiore

Lake Maggiore is another deep, sub-alpine lake in Italy. It is the second largest but the longest lake of Italy, it is located to the west from Lake Garda, at the foothills of the Alps, similarly to that one ($45^{\circ}57'N$ $8^{\circ}38'E$) (Figure 1. a-b). The main part of the lake is located in Italy, but a smaller part in the north is in Switzerland. Lake Maggiore is 68 km long, its width is 3 to 5 km except at the bay opening westward, where it is 10 km wide. Surface area of Lake Maggiore is 212.5 km^2 and its volume is 37.5 km^3 . Maximal depth of the lake is 370 m, whereas the mean depth is 176.5 m. Main tributaries are rivers Ticino, Maggia, Toce and Tresa. Beside these, rivers Verzasca, Giona, Cannobino also flow into the lake but are less important. The lake's main outflow is Ticino which then joins to river Po. On winters, ice cover on Lake Maggiore is absent and the average frequency of total mixing is 4 years, the mixing type of the lake is oligomictic.

The three peri-alpine lakes have many similar features allowing the comparability. They are all deep, warm monomictic (or oligomictic), belonging to the same geographical (peri-Alpine) and climatological (continental) region (Gallina et al. 2013). The morphometrical similarities of peri-alpine lakes are the results of the similar glacial formation processes.

Lake Kinneret

Lake Kinneret (alternative names: Sea of Galilee, Lake of Gennesaret, Lake Tiberias) is located in the northern part of Israel ($32^{\circ}50'N$, $35^{\circ}35'E$) at an altitude of -209 m (lowest freshwater lake) (Figure 1. a-c). It is a subtropical, warm monomictic lake of medium size; its surface area is 170 km^2 , mean depth is 24 m and its maximal depth is 43 m. Lake Kinneret is the largest freshwater lake in Israel with a length of 21 km and a width of 13 km. Main inflow of Lake Kinneret is the River Jordan but it is also fed by underground springs. Jordan flows through the lake from north to south.

The formation of Lake Kinneret is quite different from that of peri-alpine lakes. It is one of the remnant lakes that formed by ancient water bodies filling a tectonic depression during the Pleistocene-Holocene periods. This depression called the Kinneret basin in the northern part of the Jordan Valley (Hazan et al. 2005).

The main morphometrical features of the studied lakes are summarized in Table 1.

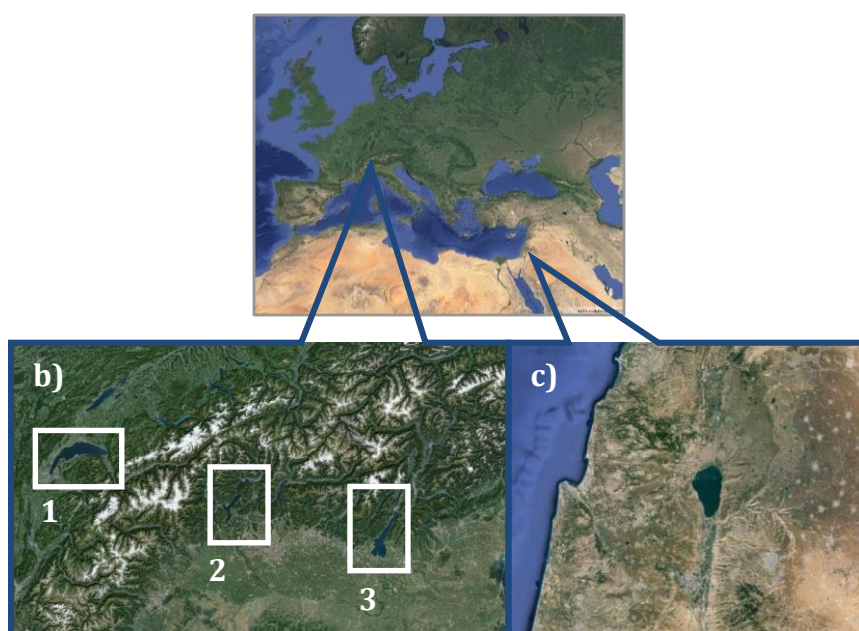


Fig. 1. Location of studied lakes on large scale (a) and a closer view of peri-alpine lakes (b); Lake Geneva (b-1), Lake Maggiore (b-2), Lake Garda (b-3) and Lake Kinneret (c)

Tab. 1. Main morphological features of the studied lakes

	Geneva	Maggiore	Garda	Kinneret
Catchment area (km²)	7975	6387	2350	2730
Area (km²)	584	212.5	370	170
Volume (km³)	88.9	37.5	49	4
Mean depth (m)	152.7	176.5	136	25.6
Maximal depth (m)	309.7	370	346	53

3.2 Sampling methods

Sample site (SHL2) on Lake Geneva for physico-chemical and phytoplankton parameters used in this study is presented on Figure 2. The sampling point is in the midway between Evian (FR) and Lausanne (CH); where the deepest point (309 m) can be found.

These parameters were sampled at a series of discrete depths between the surface and the bottom (309 m) of the lake. There was a methodological change in the sampling of phytoplankton during the study period. Before 2000, samples were collected in the upper 10 m but after this year, samples were collected in the upper 20 m. The sampling has been carrying out with a custom-made integrating bell sampler (Druart & Rimet 2008). Identification of phytoplankton and cell counts were carried out with the Utermöhl-method, using sedimentation chambers and inverted microscope (Utermöhl 1958). The analytical methods of environmental parameters are detailed in a CIPEL annual report.

The water samples from Lake Garda were collected biweekly or monthly at the station of Bardolino in the eastern basin of the lake (Figure 5). The sample collection was carried out with a 5 l and 0.5 m length Niskin bottle at integrated depths of 0-2 m, 9-11 m, and 19-21 m. The 19-21 m depth represent the euphotic layer during the most productive months.

Identification of phytoplankton was carried out on subsamples preserved in acetic Lugol's solution (Saraceni & Ruggiu 1974). Algal cells were counted on Zeiss Axiovert 135 and IM35 inverted microscopes with the method of Lund et al. (1958). Biomass was estimated from specific biovolumes listed in Salmaso & Decet (1997).

Chemical variables have been measured by the Agency for the Environment Protection of the Regione Veneto (ARPAV, District of Belluno, Italy) following standard methods (APHA, 1976). Proper analytical procedures are described by Decet & Salmaso (1997).

Water samples from Lake Maggiore for phytoplankton analysis were collected fortnightly, or monthly during January, February, November and December. Sampling was carried out at the station of Ghiffa, corresponding to the deepest point of the lake (Figure 4).

A bottle designed to take integrated sample was used to take sample in the 0-20 m water layer. Phytoplankton samples were preserved in acetic Lugol's solution and the determination and counting was carried out using a Zeiss Axiovert 10 microscope, following Lund et al. (1958). Phytoplankton biomass was estimated from the measurement of the cell volume following Hillebrand et al. (1999).

Water for chemical analysis was collected always monthly and chemical parameters have been determined at the chemical laboratory of the Istituto per lo Studio degli Ecosistemi (Pallanza, Italy), following the methods reported in Mosello & Ruggiu (1985). The temperature profiles were obtained using reversing thermometer.

Phytoplankton samples in Lake Kinneret were collected weekly or biweekly at sampling point (KI-C) indicated on Figure 3. The samples were taken with a 5 l vertical Rodhe sampler from from 0, 1, 2, 3, 5, 7, 10, 15, 20, 30 and 40 m. Phytoplankton samples were preserved in acid Lugol's solution. Phytoplankton determination and counting were carried out following the Utermöhl method (1958), using a Zeiss Axiovert M135 inverted microscope. Biomass was estimated using the specific density (1 g cm^{-3}) and biovolume estimated from the geometrical shapes of the species (Hillebrand et al. 1999). Samples for nutrient determinations (as well as all other chemical parameters) are taken from the same water samples as for the phytoplankton. Chemical analyses on samples from Lake Kinneret are made using Standard Methods (APHA 1976).

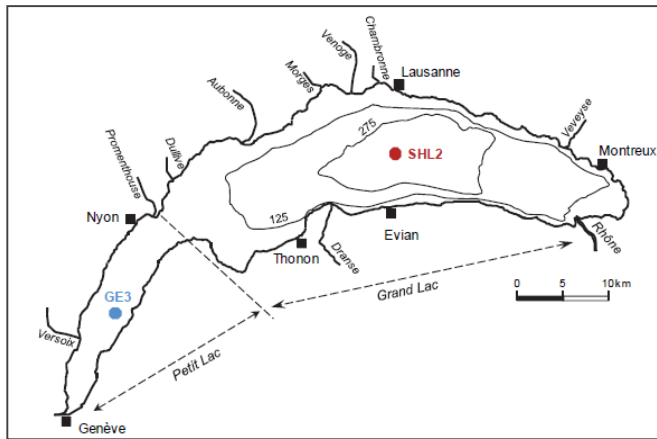


Fig. 2. Lake Geneva with the sampling points in the 'Petit Lac' (GE3) and in the 'Grand Lac' (SHL2).
Data used in this study originates from SHL2.

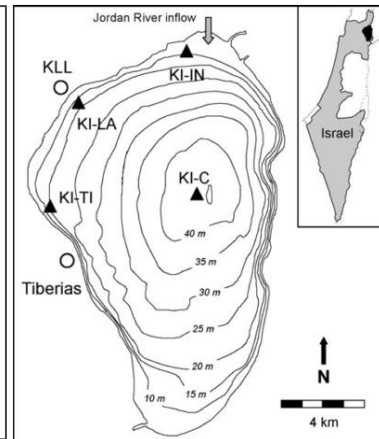


Fig. 3. Lake Kinneret with the sampling site (KI-C) at the deepest point of the lake.

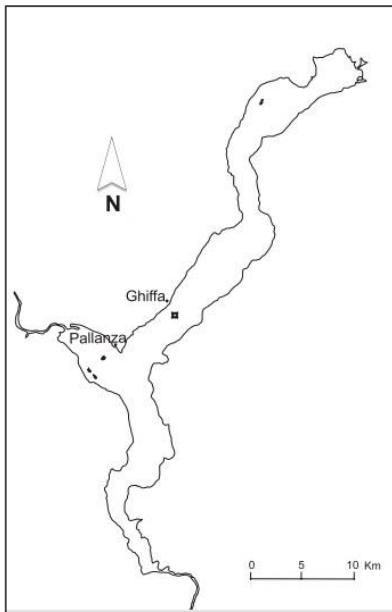


Fig. 4. Lake Maggiore with the sampling point at the station of Ghiffa.

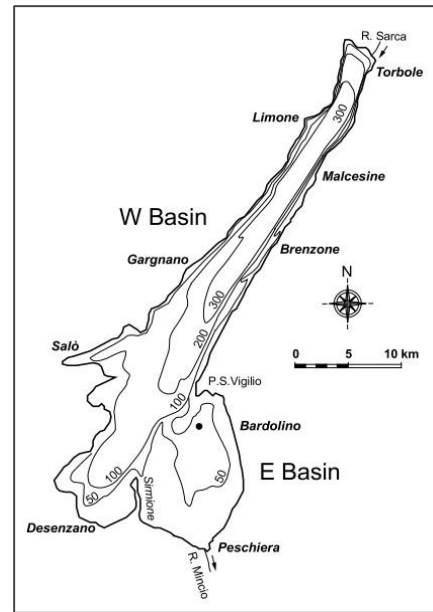


Fig. 5. Lake Garda with the sampling point in the eastern basin at the station Bardolino.

3.3 The dataset

The dataset I used in this study contains the values of *Mougeotia* sp. biomass ($\mu\text{g l}^{-1}$) and the environmental factors that I used; temperature (T [$^{\circ}\text{C}$]), Secchi-depth [m], total phosphorus (TP [$\mu\text{g l}^{-1}$]), soluble reactive phosphorus (SRP [$\mu\text{g l}^{-1}$]). Other parameters (euphotic depth, relative thermal resistance) were estimated from the above ones. Both the temporal and vertical resolution of those parameters differs in the case of each studied lake. Lake Geneva dataset covers the period between 1974 and 2011 with biweekly or monthly frequency. From 1974 to 1980 the sampled depths were 0-5-10-20-30-40-50-100-150-200-225-250-275-309 m. From 1981, the vertical resolution has been higher with the depths of 0-2.5-5-7.5-10-15-20-30-50-100-200-250-275-280-285-290-295-300-305-309 m.

Mougeotia sp. biomass data have no vertical resolution; values are from the upper 20 m strata. Chlorophyll-*a* database contains values from 1976 to 2012 with biweekly or monthly frequency. Data are from the following depths; 0-1-2-4-5-8-10-15-20-30 m. Secchi-depth values are from 1974 to 2012, also with biweekly or monthly frequency.

In Lake Garda the study period lasts from 1995 to 2008 and the sampling frequency was biweekly or monthly. Both biomass values and values of environmental factors are from three layer; the 0-2 m, 9-11 m and 19-21 m strata. For temperature, the vertical resolution is higher; values are from the 0-5-10-15-20 m depths.

Study period in Lake Maggiore is from 1984 to 2011 with monthly temporal resolution. Phytoplankton and chemistry data recorded in the 0-20 m water layer, but temperature values are recorded in six depths; 0-5-10-20-30-50 m.

Lake Kinneret database has higher temporal resolution; values recorded weekly or biweekly from 1998 to 2012. Data recorded at different depths; 0-1-2-3-5-7-10-15-20-30-40 m. Sometimes the maximal depth had been sampled is 15 m.

In this study I used the Welch's Relative Water Column Stability (RWCS) as an indicator of the thermal stability of the water column. RWCS was computed by the density difference between the surface layers and the hipolimnetic layer (20 m) compared to the density difference between 4 and 5 $^{\circ}\text{C}$ (Kalff 2002):

$$\text{RWCS} = \frac{D_b - D_s}{D_4 - D_5}, \text{ where}$$

RWCS is the relative thermal resistance, D_b and D_s are the water density at the bottom layer and on the surface, D_4 and D_5 are the density of water at 4 and 5 $^{\circ}\text{C}$

Euphotic depth was calculated by multiplying the Secchi depth measured by the Secchi-disk by 2.5 (Vollenweider & Kerekes 1982).

3.4 Statistical analysis

In order to identify the environmental conditions associated to a bloom of *Mougeotia* between blooming and non-blooming periods, linear discriminant analysis (LDA) was run by using the MASS package (Venables & Ripley 2002) of the R statistical software (R Development Core Team 2008). A matrix was created where each column corresponds to an environmental factor (variable) and each row corresponds to a year. Then, the matrix has been filled with the values of the particular variables that correspond with the date of the *Mougeotia* bloom in the given year. The variables were RWCS, temperature, TP, SRP, Zeu. Years were gathered by groups according to the density of *Mougeotia* during the annual peak. Then, three groups were identified as “years of low biomass” for biomass values between the minimum and the 1st quartile of the biomass data of annual peaks; “years of medium biomass” for biomass values between the 1st and 3rd quartile, and “high biomass years” for values between the 3rd quartile and the maximum value. These groups occupied another column in the matrix. LDA was used to identify which combinations of parameters that provide the best discrimination between these 3 groups.

To compare the vertical pattern of TP and T during bloom vs. non-bloom periods I used the same linear discriminant analysis but with a differently structured matrix. First of all, I could successfully implement this analysis only on data of Lake Geneva. Vertical data from other lakes were appropriate for LDA, either the vertical resolution was low (Lake Garda) or the depth range was not enough wide, with gappy dataset (Lake Maggiore, Lake Kinneret). In this analysis the variables were the values of the same factor at different depths (0-5-10-15-20-50-100 m) during the *Mougeotia* biomass peak in each year (objects). It means I had one LDA for temperature and one for total phosphorus.

3.5 Morphological examination

Morphological examination was carried out of *Mougeotia* cells and filaments on samples of the sample collection of INRA UMR CARRTEL from Lake Geneva. Results were compared with morphological data of *Mougeotia* sp. from the other lakes that were provided by colleagues from the lab CNR Istituto per lo Studio degli Ecosistemi (Italy), Natural Resources Department, IASMA Research Center (Italy), Y. Allon Kinneret Limnological Laboratory (Israel).

4 Results

4.1 Environmental factors

4.1.1 Temperature

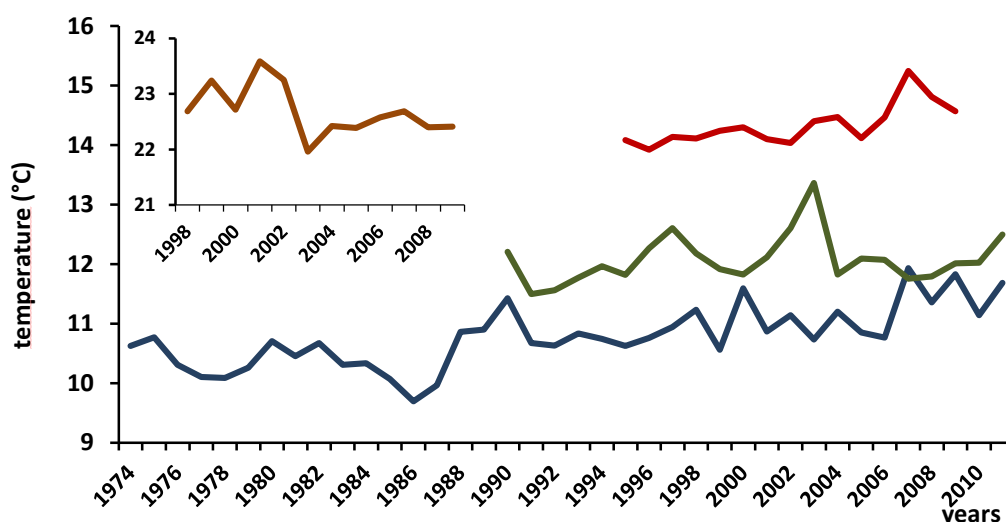


Fig. 6. Long-term change in annual mean water temperatures in the studied lakes; Lake Geneva (blue), Lake Maggiore (green), Lake Garda (red), Lake Kinneret (brown). Temperature values are annual means from the top 20 metres (Lake Geneva, Lake Garda, Lake Maggiore) or top 10 metres (Lake Kinneret).

Figure 6 shows the changes in annual mean temperatures measured in the four lakes. There is a slight increase in the annual temperature values of peri-alpine lakes. The lowest temperature values occur in Lake Geneva (10-12°C) and the warmest in Lake Garda among the peri-alpine lakes with temperature values of 14-15°C. Obviously the warmest lake among the studied ones is the subtropical Lake Kinneret (22-23.5°C). Data of Lake Kinneret do not show the increasing trend observed in the others. Moreover, data from 1998 to 2003 showed higher values than between 2003 and 2009.

The seasonal pattern of the temperature is presented on Figure 7. The three peri-alpine lakes show similar patterns; the lowest temperature values occur in February. Then warming starts in April-May reaching a temperature peak by August. After this peak, temperature decreases. Winter 0-20m water temperature is warmer in Lake Garda. Furthermore, in this lake temperature reaches 15°C between May and June while it reached this value in June in Lake Maggiore and July in Lake Geneva. In Lake Kinneret, winter water temperature varies between 15°C and 19°C while the summer temperature can reach 30°C. The time of minimal and maximal values during the year is the same as in the other lakes, but the whole pattern has a slight different shape. Warming is quicker and earlier and the maximal temperature in August

is reached more progressively. Similarly, declining of this maximum is also progressive. The achievement and decline of the maximal temperature are more sudden in the peri-alpine lakes.

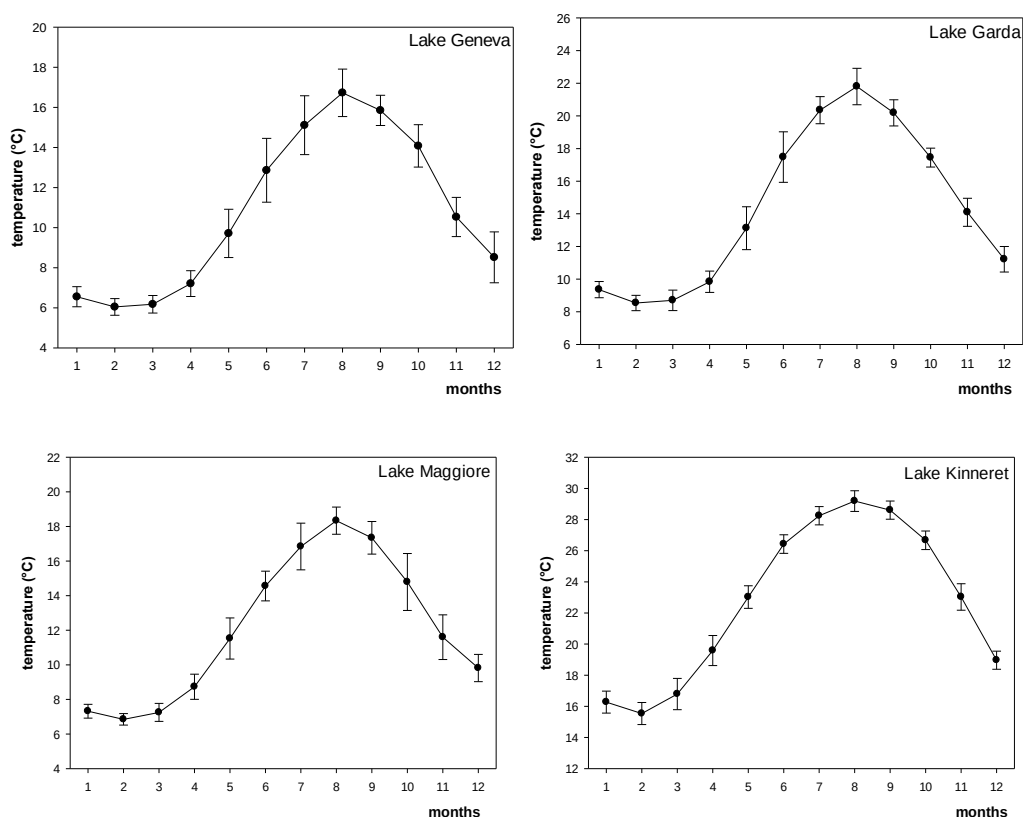


Fig. 7. Seasonal distribution of monthly average temperature values. Dots indicate the means of the long-term values, error bars are standard deviation. Values are average of 0-20 m depth.

The figure also shows that there are differences in the standard deviations between Lake Kinneret and the peri-alpine water bodies. In the latter, the inter-annual variability of temperature is higher after April and lower before. In Lake Kinneret, this tendency is not observed.

4.1.2 Mixing properties, water column stability

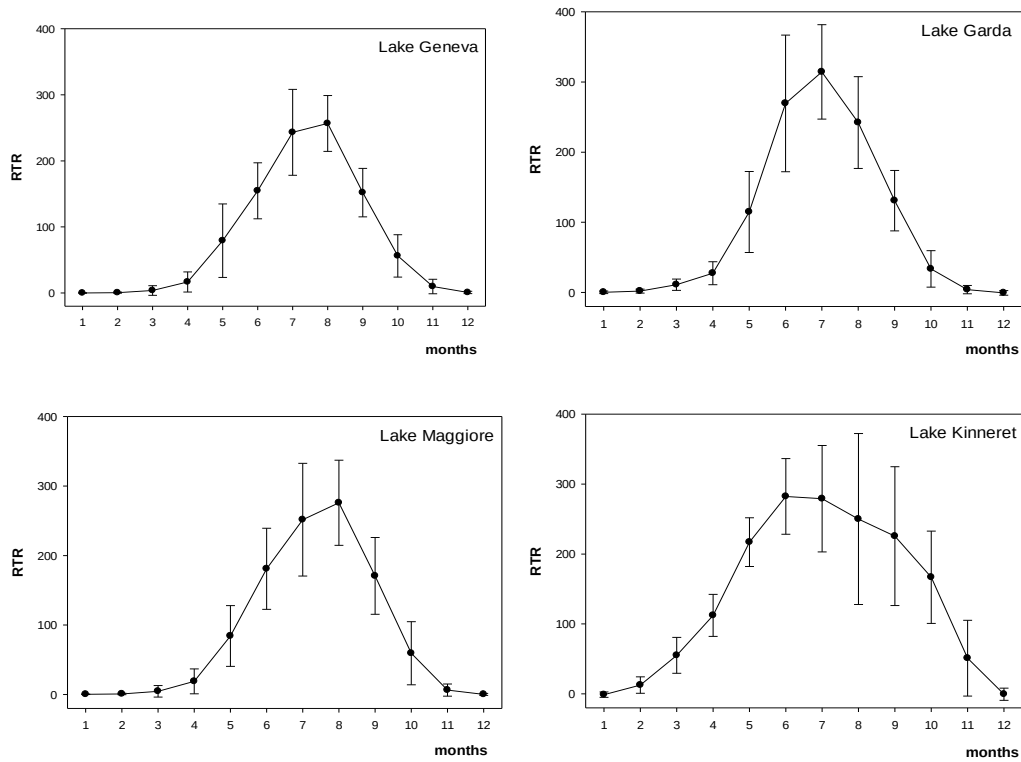


Fig. 8. Seasonal distribution of monthly average thermal resistance values. Dots indicate the means of the long-term values, error bars are standard deviation.

Figure 8 shows the relative thermal resistance of the water column based on the water density difference between surface and deep layers.

Similarly to the temperature data, the seasonal dynamics of relative thermal resistance is quite similar in the peri-alpine lakes. The water column is mixed during late autumn and winter, resulting in low RWCS values and obviously in low variance during this period. Stratification starts in late spring and the water column is the most stable in July-August. During the examined time period, thermal resistance values show high interannual variability. In Lake Kinneret, the mixing period is shorter, spring warming starts earlier and stratification lasts longer. We noticed that inter-annual variability of thermal stability is much higher during the second half of the year.

4.1.3 Light conditions

Euphotic depth is used to estimate the depth of the zone where net photosynthesis still occurs. This depth (estimated from the secchi-depth) is presented on Figure 9. All lakes have a similar pattern but values in Lake Kinneret are lower (6-10 m) than in the peri-alpine lakes (10-30 m). The euphotic zone is the deepest during the winter period and reaches its minimal value during spring.

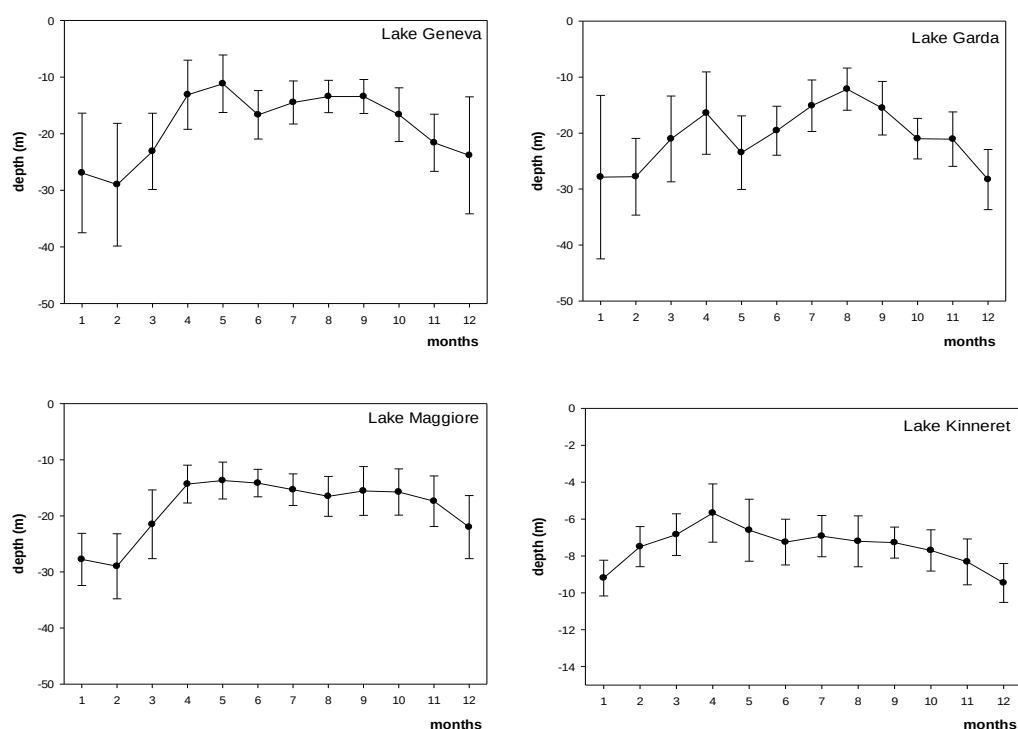


Fig. 9. Seasonal distribution of monthly average euphotic depth values. Dots indicate the means of the long-term values, error bars are standard deviation.

4.1.4 Phosphorus concentration

Long-term change in Lake Geneva annual means of total phosphorus concentrations (TP) is presented on Figure 10. A strong decrease in total phosphorus concentration has occurred between the 80's and late 90's. Since this time, the annual total phosphorus concentration has been around 15-20 $\mu\text{g l}^{-1}$. In the Italian lakes, annual TP values are lower ($\sim 10 \mu\text{g l}^{-1}$) and has been ranging around this value during the examined period. In Lake Kinneret, a decreasing trend in total phosphorus concentration can be defined but we observed outstanding values in 2002-2004 (30-35 $\mu\text{g l}^{-1}$).

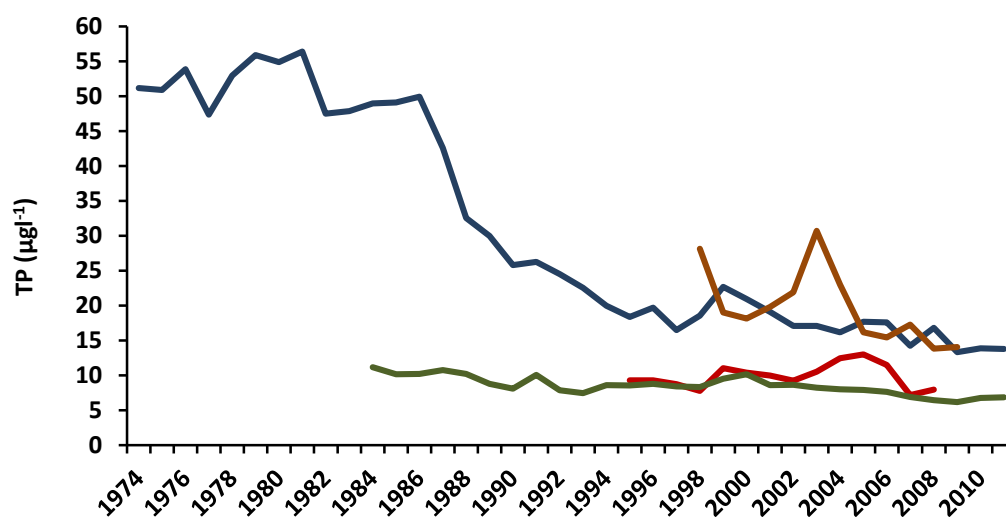


Fig. 10. Long-term pattern of annual average total phosphorus concentration in the studied lakes; Lake Geneva (blue), Lake Maggiore (green), Lake Garda (red), Lake Kinneret (brown). Values are annual averages from the top 20 metres (Lake Geneva, Lake Garda, Lake Maggiore) or top 10 metres (Lake Kinneret).

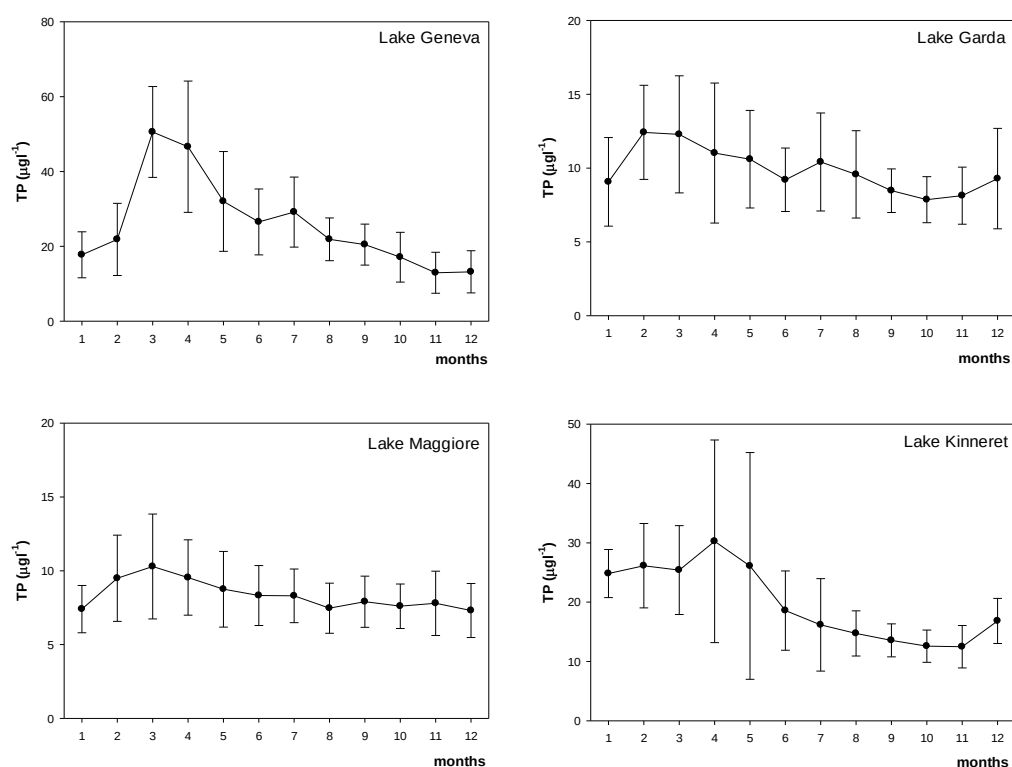


Fig. 11. Seasonal distribution of monthly average total phosphorus values. Dots indicate the means of the long-term values, error bars are standard deviation.

Figure 11 shows the average seasonal pattern of total phosphorus in each studied lake. During the studied period interannual variability was high. In each lake, the maximal concentrations in the top layers occurred in spring, and then started to decrease.

Obviously, soluble reactive phosphorus (SRP) concentrations show much lower values than total phosphorus (Figure 12). SRP values have also a strong interannual variability. The annual patterns are similar between lakes: the highest values are observed in February-March but then concentrations measured in the 0-20 (or 0-10) m reached the detection limit. Highest values are observed in Lake Geneva in early spring, but later in the year they are comparable to the other lakes.

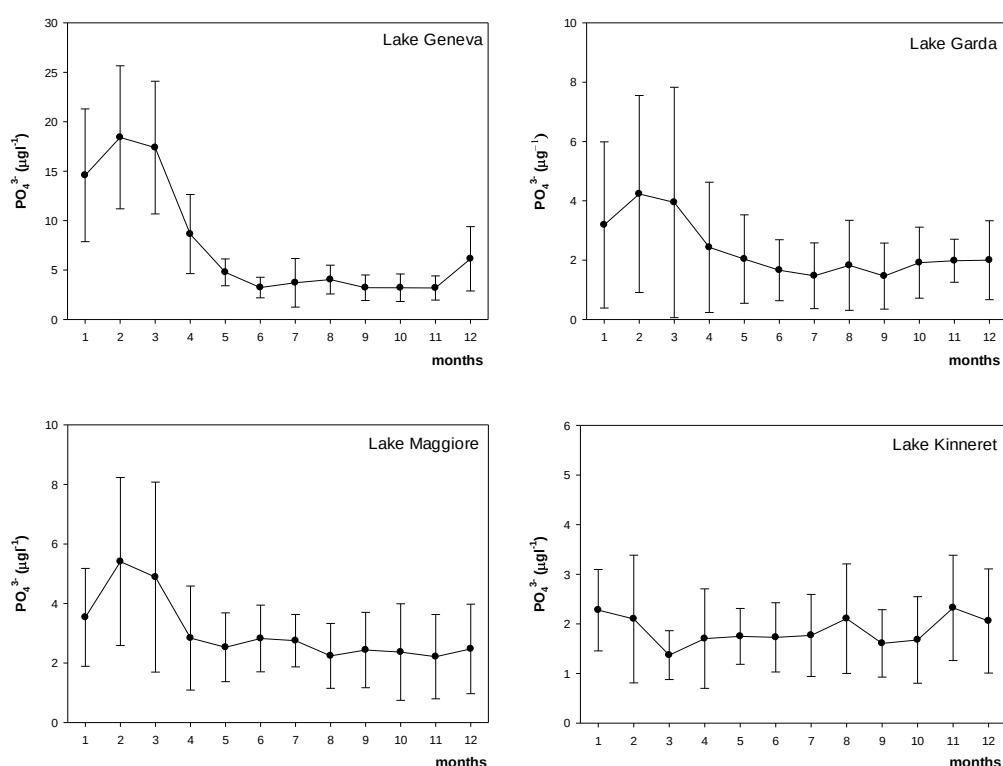


Fig. 12. Seasonal distribution of monthly average SRP values. Dots indicate the means of the long-term values, error bars are standard deviation.

4.2 Morphological examination on *Mougeotia* sp.

Due to uncertainties in species level identification of *Mougeotia* in the studied lakes, I made morphological comparison. Results of morphological examination carried out on samples from Lake Geneva are presented on Figure 13. Cell length of *Mougeotia* varied in a wide range between 30 and 80 μm during the studied period and a sudden increasing can be observed in the samples from 2001.

The width of cells varied in a range of 3 to 5 μm during the whole study period. Between the cell length and cell width there is strong correlation, shown on Figure 14.

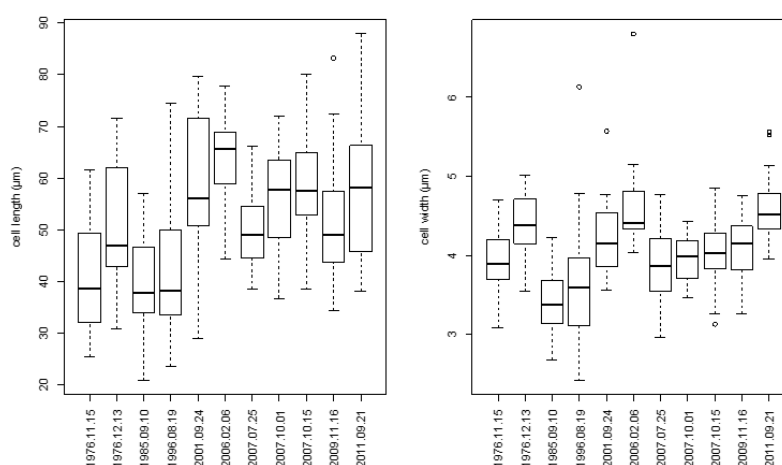


Fig. 13. Length and width of *Mougeotia* cells at different dates during the study period.

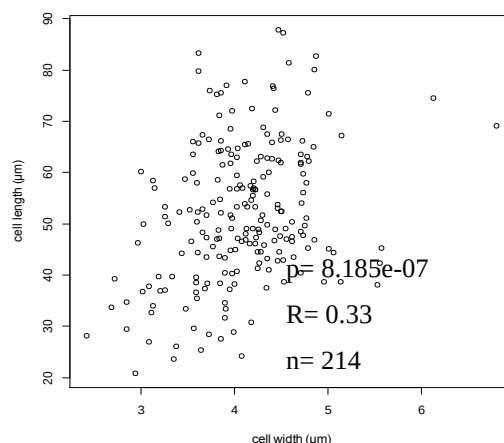


Fig. 14. Correlation between cell length and cell width

Examinations show that *Mougeotia* from Lake Garda has a width of 4 and 8 μm and length of 50-100 μm . The species's width in Lake Kinneret varies between 3.1 and 4.7 μm , while its length is between 36 and 95 μm . There is no morphological data about *Mougeotia* in Lake Maggiore.

However zygospores could be observed in the samples of Lake Garda and Lake Kinneret (Figure 16. a-b), we are lack of this identification criterion in Lake Geneva.

These morphological data are not sufficient for identification at species level.

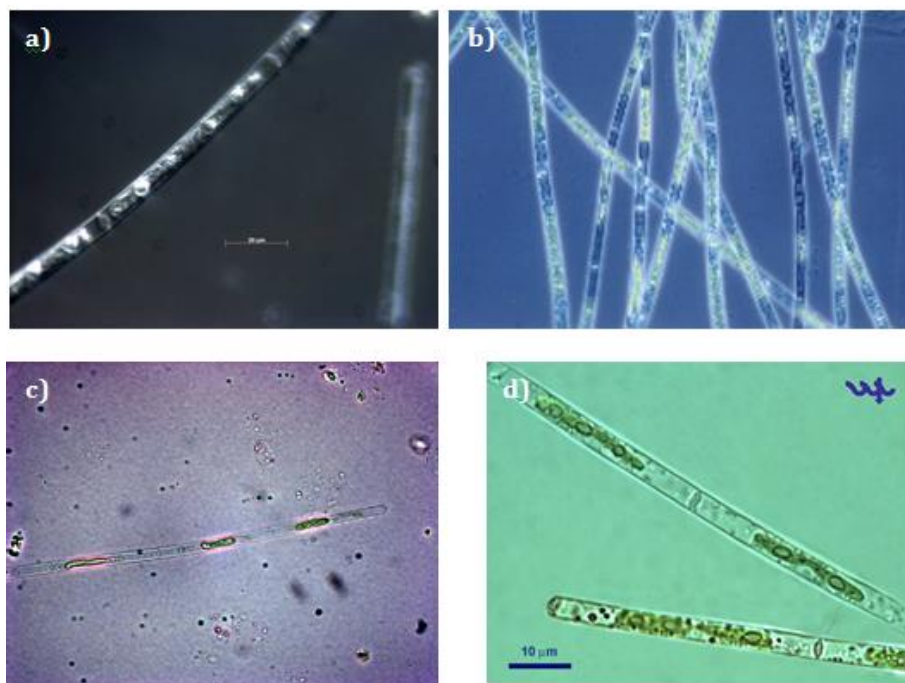


Fig. 15. Photos of *Mougeotia* sp. filament from Lake Geneva (a), Lake Garda (b), Lake Maggiore (c), Lake Kinneret (d)

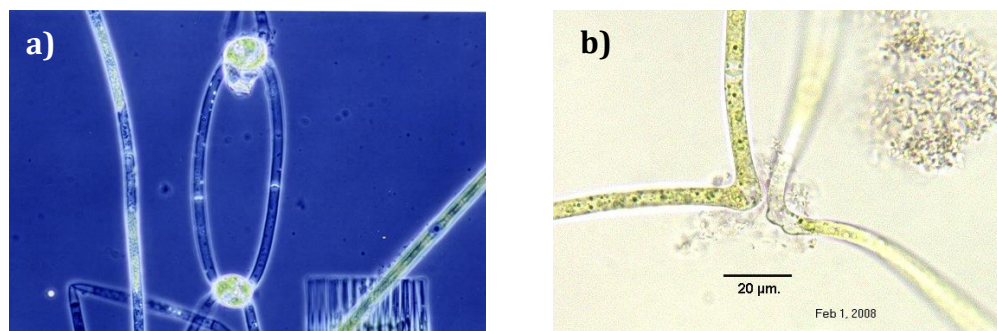


Fig. 16. Conjugation and zygospore forming of *Mougeotia* sp. from a) Lake Garda and b) Lake Kinneret

4.3 Long-term changes and seasonal patterns of *Mougeotia* sp. biomass

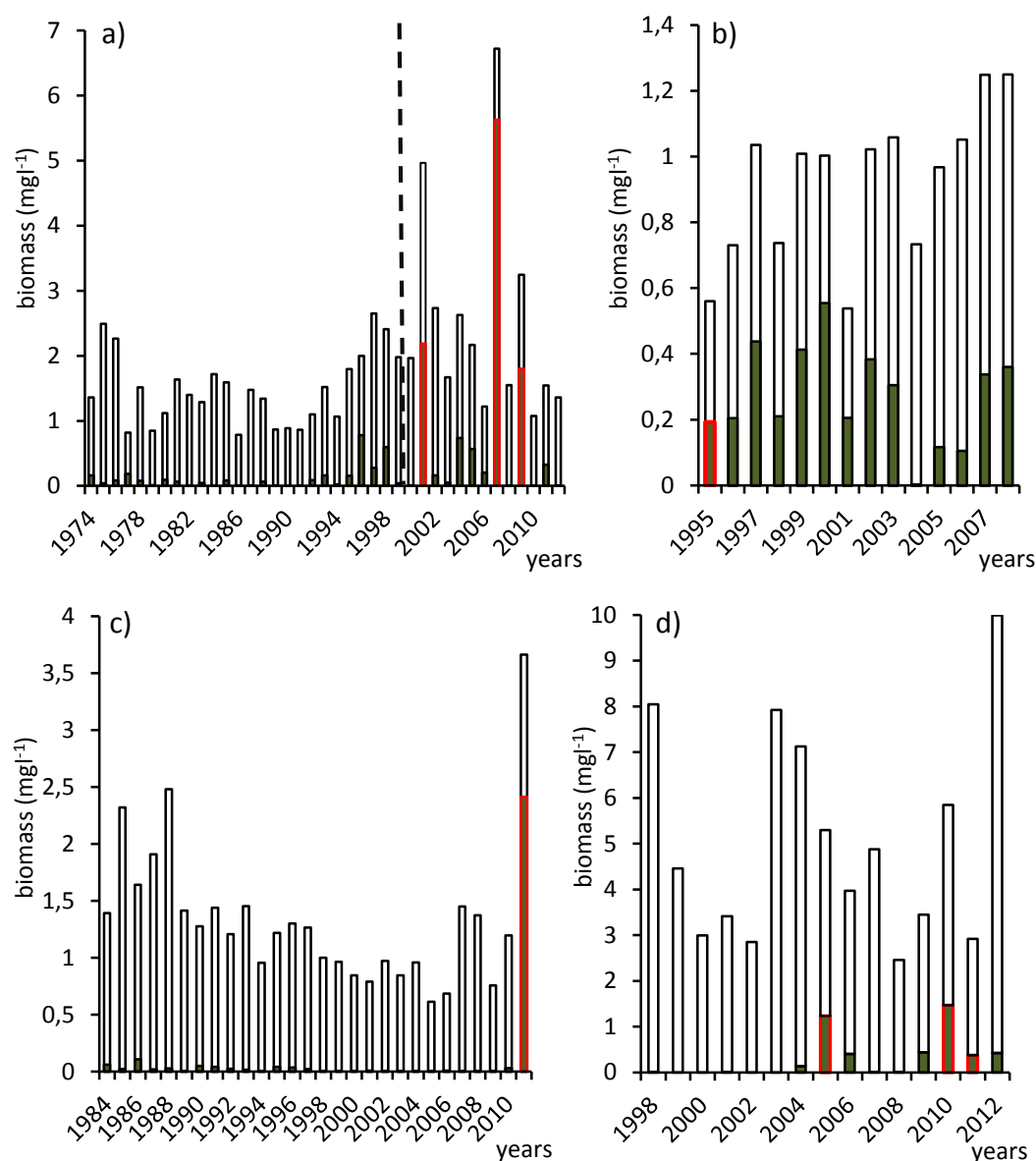


Fig. 17. Annual mean of total phytoplankton (empty bars) and *Mougeotia* sp. (green bars) biomass in Lake Geneva (a), Lake Garda (b), Lake Maggiore (c) and Lake Kinneret (d). Bars with red border indicate years when *Mougeotia* sp. had a contribution >80% to total biomass during the year. Dashed line indicates the change in sampling method from 2000 in Lake Geneva.

Figure 17 shows the interannual changes in total phytoplankton and *Mougeotia* sp. biomass. In Lake Geneva, *Mougeotia* sp. was present in each year but its biomass increased in the late 90's. The most affected years were 2001, 2007 and 2009, when *Mougeotia* showed not only high annual mean biomass but high contribution to total biomass during its blooming period in each year too. In Lake Garda, presence of the species is more constant; both the total and *Mougeotia* sp. annual mean biomass varies in a small range. Total biomass varies between 0.6-1.2 mg.l⁻¹, *Mougeotia* sp. biomass varies between 0.2-0.6 mg.l⁻¹. 1995 was the only year during the study

period when *Mougeotia* sp. reached a more than 80% contribution during a period in the year. In Lake Maggiore, *Mougeotia* sp. shows a low annual biomass in the whole study period except the last year, 2010, when it reached an annual mean biomass value of 2.4 mg l^{-1} and a high contribution during its bloom. In Lake Kinneret *Mougeotia* sp. appeared in 2004 and was observed each year, except in 2007 and 2008 with annual average biomass varying between $400 \text{ } \mu\text{g l}^{-1}$ and $1500 \text{ } \mu\text{g l}^{-1}$. Even though its annual mean biomass was never outstanding compared to total biomass, in 2005, 2010 and 2011 *Mougeotia* sp. contribution to total phytoplankton biomass occasionally reached 80%.

The seasonal dynamics of *Mougeotia* sp. is presented on Figure 18. In Lake Geneva the species is usually not present in the first half of the year and starts to establish dense populations in late summer-autumn. From 1974 to 1982 it was observed every years and its biomasses were low from August or September to almost the end of the year. Between 1983 and 1991, *Mougeotia* sp. occurred occasionally, it was nearly absent during this period. From the 90's an increasing trend in its seasonal duration can be observed. The biomass peaks occur in July-September period. The highest biomass values were observed in August, 2001 ($14\text{-}15 \text{ mg l}^{-1}$), late August, 2007 (39 mg l^{-1}) and late September - early October, 2009 ($12\text{-}13.5 \text{ mg l}^{-1}$). In some years, *Mougeotia* sp. had overwintering populations with low biomass, except the winter of 2004/2005, 2005/2006 and 2006/2007, when biomass values were 1.4 mg l^{-1} , 1.7 mg l^{-1} and 0.6 mg l^{-1} .

Seasonal distribution of *Mougeotia* sp. in Lake Garda is different from that in Lake Geneva. During the examined period, the species was present in every year, with different biomass values but the variability between years was lower than in Lake Geneva. Population starts to establish in spring and reaches a biomass peak by early summer in June, July. When column stability declines, *Mougeotia* sp. population starts to decline too. Values during the biomass peaks ranged from 1 to 3 mg l^{-1} .

Mougeotia sp. biomass in Lake Maggiore was low until the last year (2011) of the study period (1984-2011). It occurred only with low biomass values during the years with a seasonal peak in late spring-early summer, similarly to that in Lake Garda. These biomass peaks ranged between 100 and $500 \text{ } \mu\text{g l}^{-1}$. 2011 was highly affected by the species, reaching high biomass in the summer; its biomass value in July was 20 mg l^{-1} .

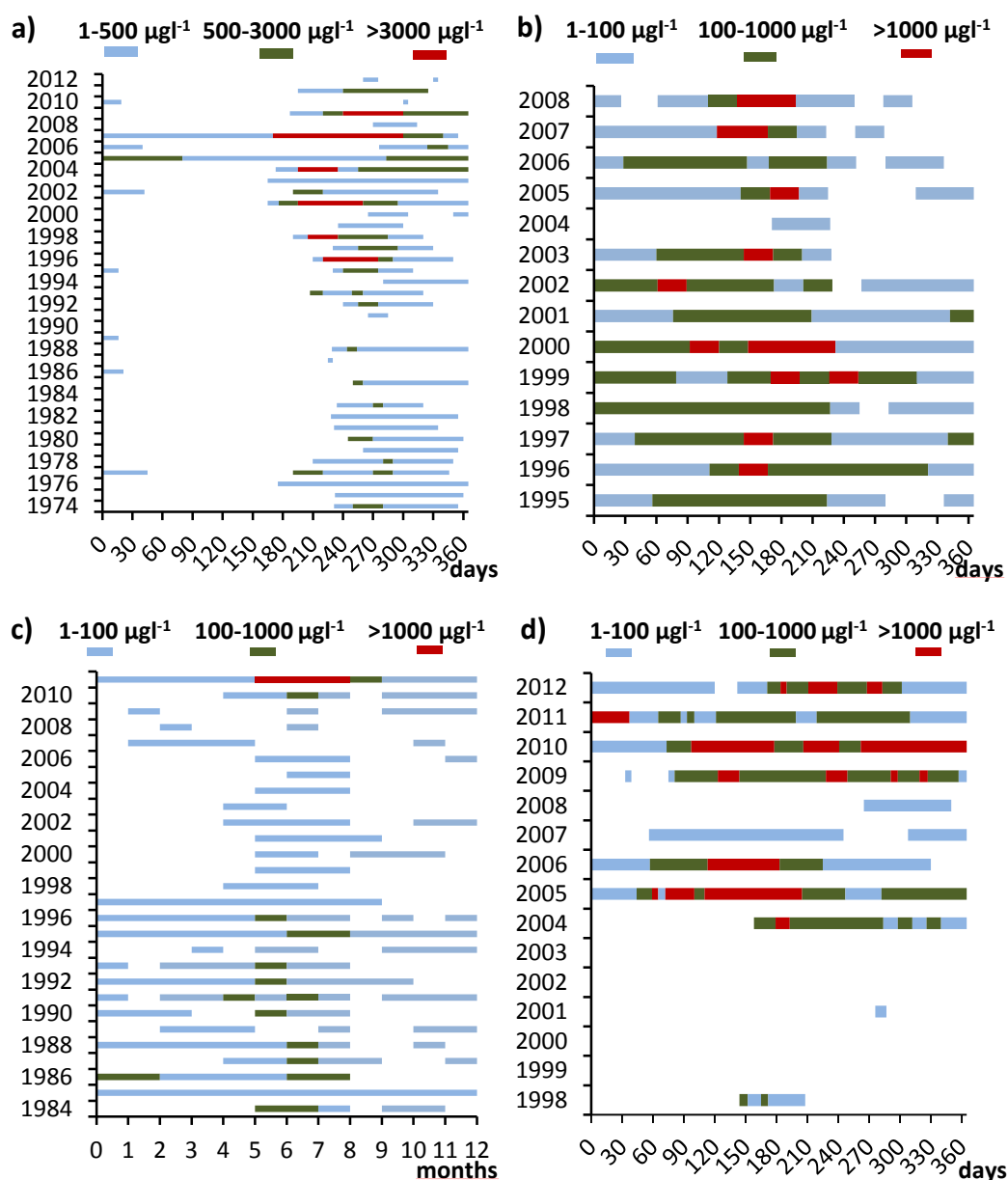


Fig. 18. Long-term change in the seasonal pattern of *Mougeotia* sp. biomass – a) Lake Geneva, b) Lake Garda, c) Lake Maggiore, d) Lake Kinneret

In Lake Kinneret, *Mougeotia* sp. became high from 2004. It commonly appears in early spring, sometimes in February and remains dominant until autumn or some cases during the whole year. In its bloom period biomass can reach 1-4 (6) mg l⁻¹ in the epilimnion. Overwintering populations were present in some years with lower biomass values than in the summer. The year 2010 was exceptional since *Mougeotia* sp. biomass started to grow in March reaching a high biomass during the whole year. The maximum biomass (6.2 mg l⁻¹) was observed in the end of December, than dropped to 2 mg l⁻¹ by February, 2011.

4.4 Environmental optima of *Mougeotia* sp.

Figure 19 shows that the total phosphorus concentration, for which high *Mougeotia* biomass is observed, are similar in the four studied lakes. High biomass are observed when TP concentration range between 5-15 $\mu\text{g l}^{-1}$. In peri-alpine lakes the highest biomass are observed when temperature range between 15-20 °C which is actually the highest temperature value that can be measured in two of the three lakes. In Lake Kinneret that is warmer, maximal biomass of *Mougeotia* sp. are observed when temperature was between 20-25 °C. These conditions are usually present during the stratified period of summer when temperature is higher and phosphorus concentration is low due to the depletion in the epilimnion.

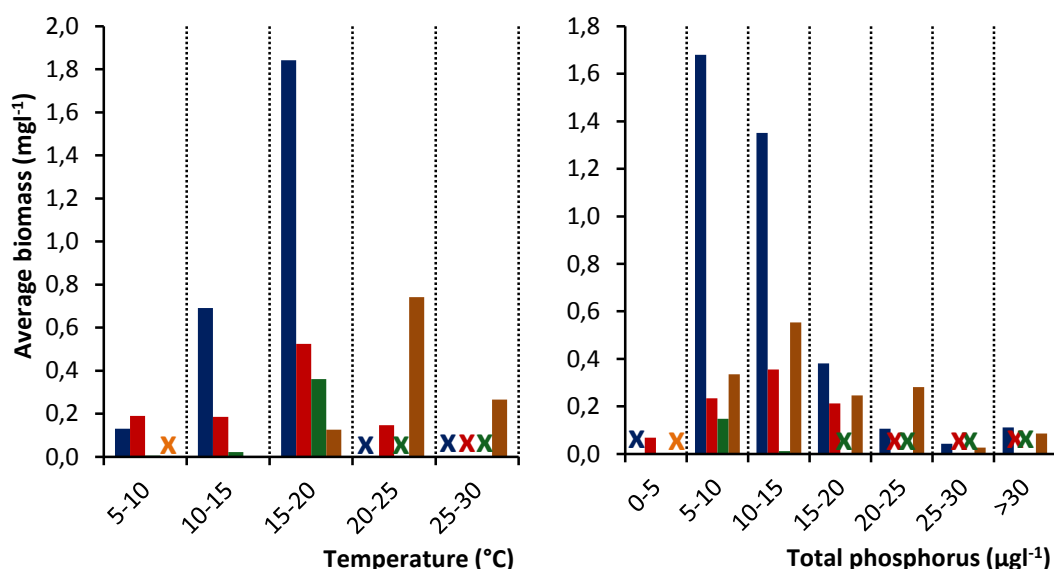


Fig. 19. Temperature and total phosphorus optima of *Mougeotia* sp. in the studied lakes; Lake Geneva (blue), Lake Garda (red), Lake Maggiore (green), Lake Kinneret (brown)

4.5 Comparison of environmental conditions during bloom and non- bloom of *Mougeotia*

We wanted to see which environmental factors can discriminate the years presenting *Mougeotia* sp. blooms to the other years. Discriminant analyses were used for this purpose. Results of the linear discriminant analysis with the values of linear discriminant coefficients are given on the scatter plots of Figures 20, 21, 22, 23. We can see that the main selected bottom up environmental factors enable to discriminate, the three groups of years based on the highest *Mougeotia* biomass of the year (low, medium, high). In each lake years with low biomass and years with high biomass are well separated along the first axis. In Lake Geneva and Lake Garda, the group ‘medium’ is also separated along the second axis, but this group is not distinct in the case of Lake Maggiore and Lake Kinneret. However the role of the different variables in the

separation is quite different in each case. In Lake Geneva, the relative thermal resistance (RWCS), as an indicator of thermal stability of the water column, and euphotic zone seems to be the most important discriminating factors. High values of these variables are associated with low biomass. In contrast, high biomass are associated with low RWCS and euphotic values and high SRP. In Lake Garda the euphotic zone (z_{eu}), total phosphorus concentration (TP) and temperature (T) are the main discriminating factors that are associated to years with lower biomass, while the higher thermal resistance and SRP values occurs when *Mougeotia* has high biomass. Thermal stability and temperature are also the most important factors in Lake Maggiore. Nevertheless, while high thermal resistance values are associated to low biomass, high temperature are associated to high *Mougeotia* biomass. In Lake Kinneret, temperature doesn't seem to play any role in triggering *Mougeotia* blooms, while total phosphorus and SRP are important factors. Low values of these parameters in Kinneret are associated to high *Mougeotia* biomass.

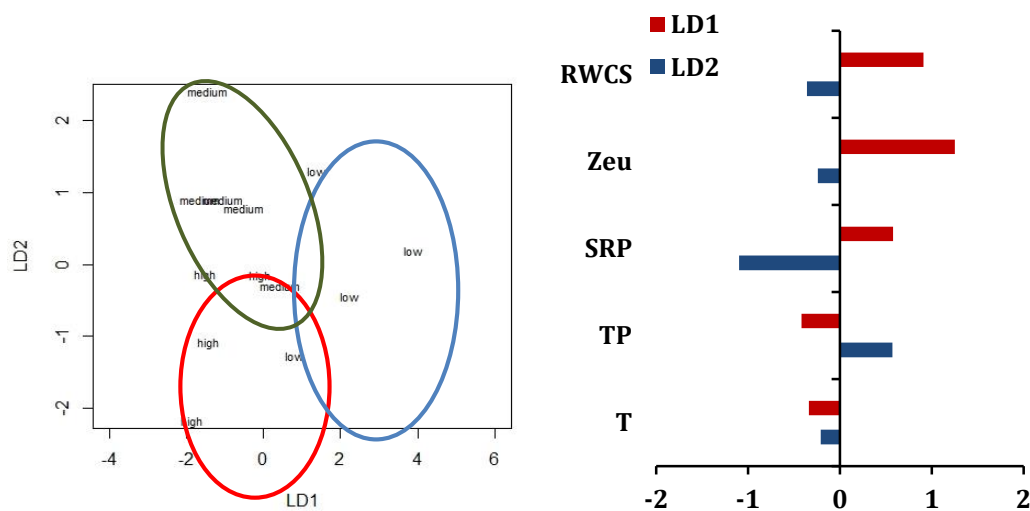


Fig 20. LDA scatter plot (left) and linear discriminant coefficients of variables (right) in Lake Geneva

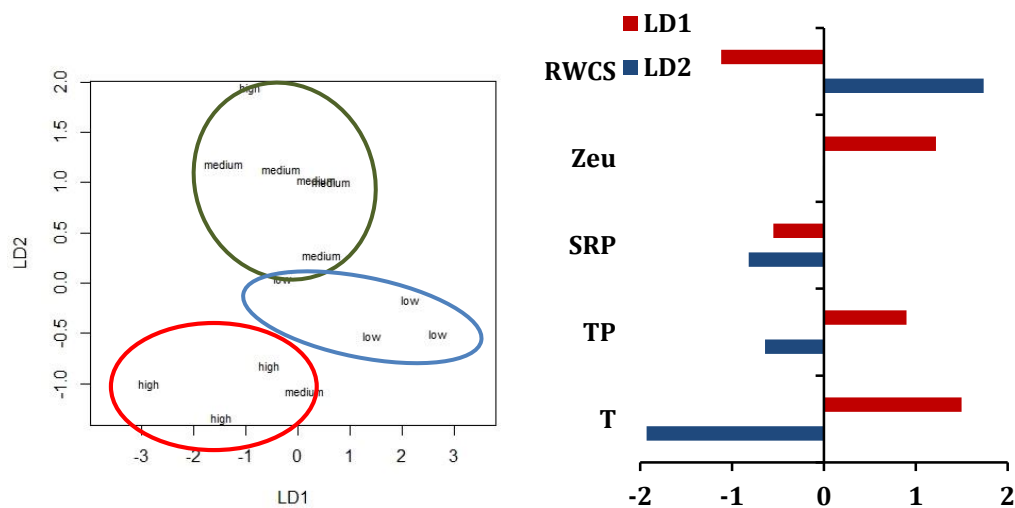


Fig. 21. LDA scatter plot (left) and linear discriminant coefficients of variables (right) in Lake Garda

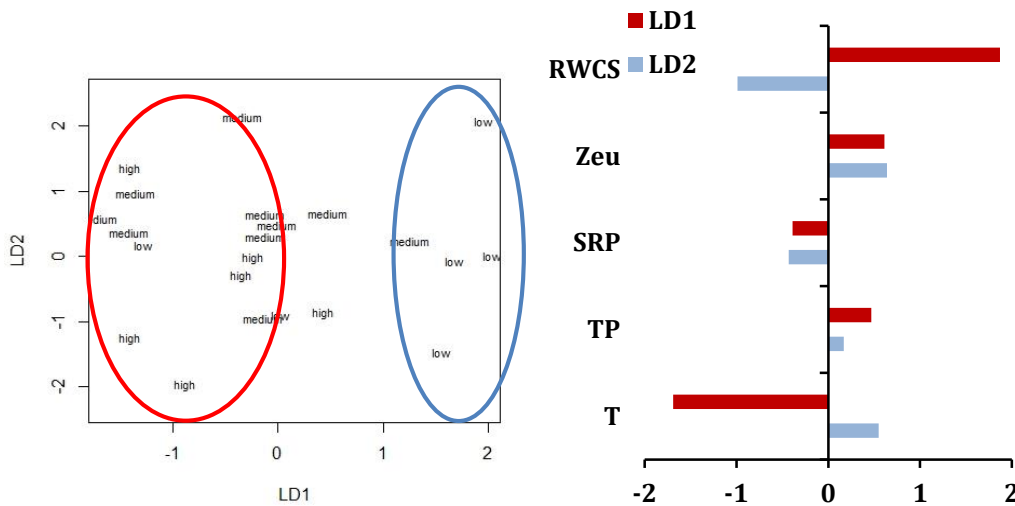


Fig. 22. LDA scatter plot (left) and linear discriminant coefficients of variables (right) in Lake Maggiore

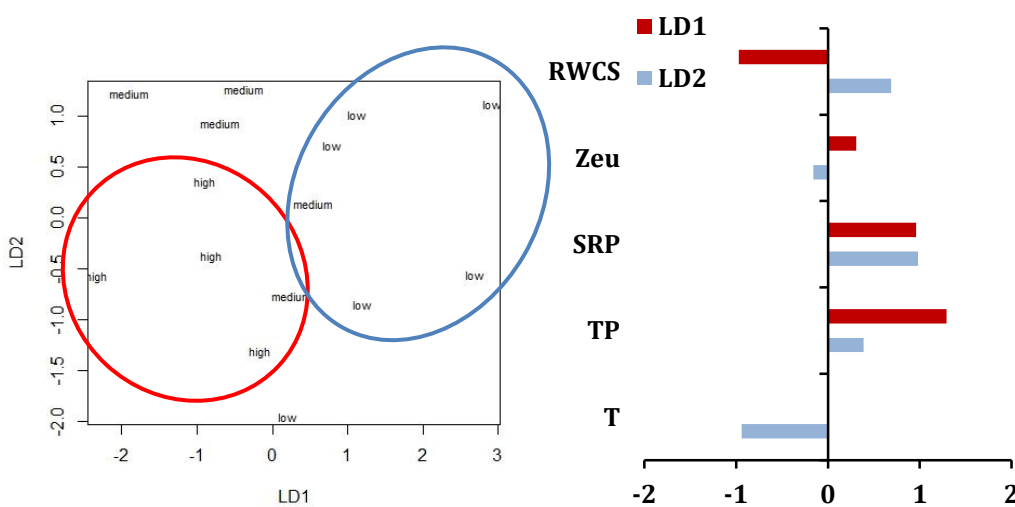


Fig. 23. LDA scatter plot (left) and linear discriminant coefficients of variables (right) in Lake Kinneret

4.6 Vertical distribution of *Mougeotia* and environmental variables

Figure 24 shows the vertical concentrations of total chlorophyll-*a* during three periods in Lake Geneva when *Mougeotia* was present with extremely high biomass. In 21/08/2001 the vertical profile of chlorophyll-*a* was not homogenous: an explicit maximum between the 10-15 m depth occurred. In 25/07/2007 chl-*a* increased from 4 to 10 metres and reached its maximum at 20 m. In 21/09/2009 chl-*a* increases till 10 m and remains high till 20 m. Vertical temperature profiles are also displayed on the graphs showing slight decrease along the vertical profile, indicating a weak thermocline during these time periods.

Figure 25 shows also three periods with high *Mougeotia* biomass at three different depths in Lake Garda. Even the resolution of the vertical profile is low, we can see that the distribution of the biomass is variable; in 03/06/1997 it was nearly homogenous, in 29/05/2007 the maximum biomass occurred at a depth of 20 m, in 27/05/2008 it was at 10 m.

In Lake Kinneret (Figure 26) *Mougeotia* sp. did not show vertical heterogeneity in its biomass during the stratified periods, it was homogeneously distributed in the epilimnion.

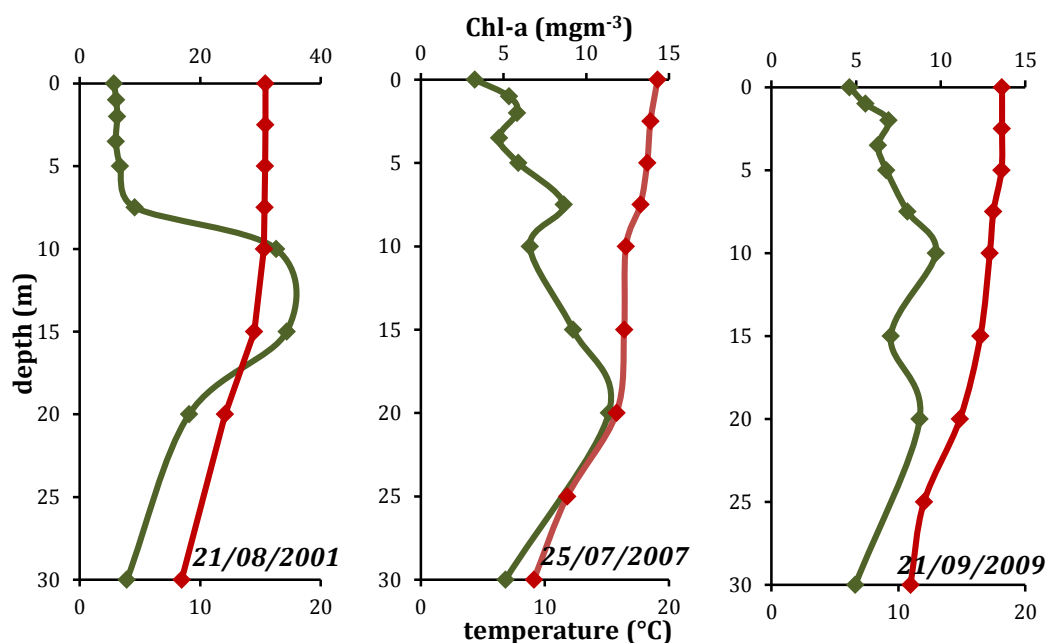


Fig. 24. Vertical profiles of chlorophyll-*a* and temperature at three dates when *Mougeotia* sp. had high contribution to total biomass in Lake Geneva; 2001.08.21 (85%), 2007.07.25 (99%), 2009.09.21 (87%)

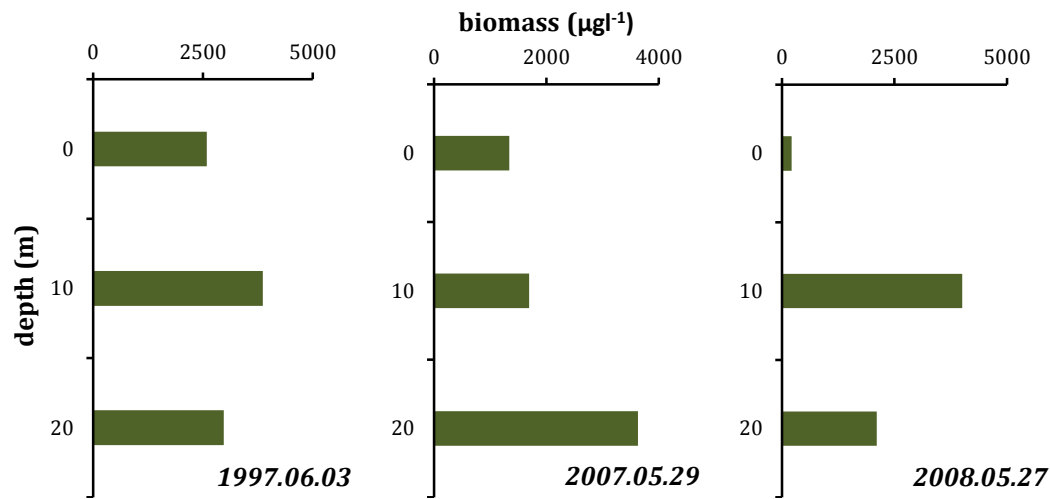


Fig. 25. *Mougeotia* biomass along the vertical profile in three depths (0 m, 10 m, 20m) in Lake Garda

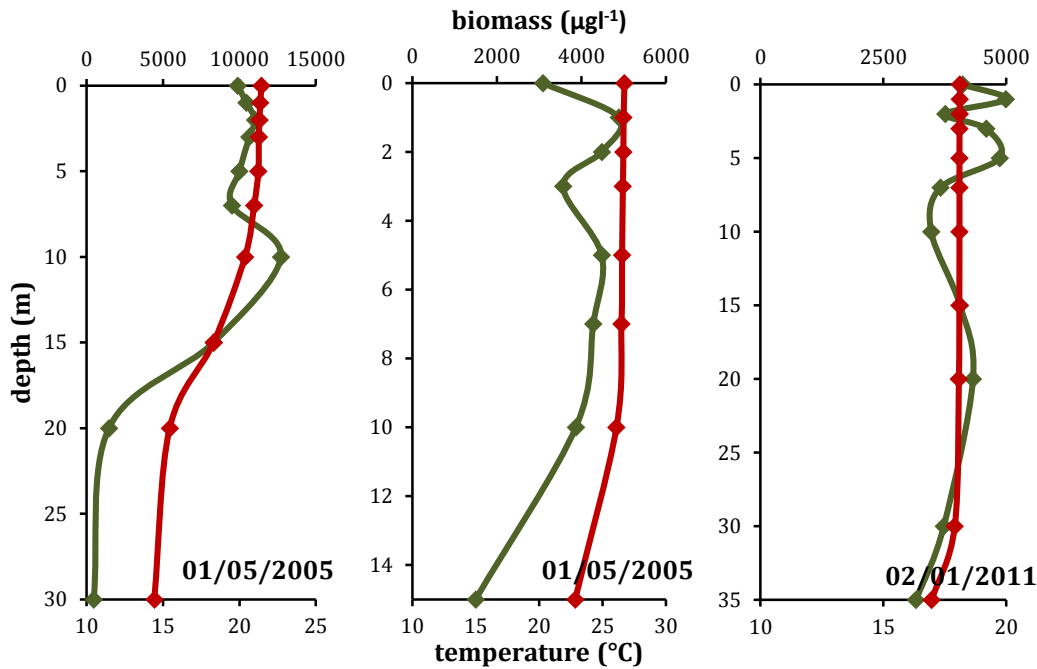


Fig. 26. Vertical distribution of *Mougeotia* biomass (green) and temperature (red) in Lake Kinneret during periods with high *Mougeotia* biomass. Note the different scale on the vertical axis.

To test the importance of the vertical profile of temperature and total phosphorus, I run discriminant analysis. The aim was to find if different years with different *Mougeotia* biomass can be separated by the values of one variable but in different depths. Figure 27 shows that the three groups are well discriminated by the temperature values at different depths. It is obvious that layers near the surface (0, 5, 10, 15 m) have more importance than temperature in the hypolimnion (50, 100m). It is remarkable that values at 0 m and 15 m have an opposite effect than values at other depths. The distribution of the three groups is also notable; group 'high' is located between 'low' and 'medium' along the long axis. Group 'medium' is well separated from group 'low' and 'high' along the first axis, group 'high' separated from 'low' along the second axis.

The groups can be also discriminated by using total phosphorus values at different depths (Figure 28). Similarly as above, group 'medium' is separated from the other groups along the first axis, and group 'high' is separated from 'low' along the second axis. We can see that total phosphorus values at different depths have different degrees of impact; the most important depths are depth at 15 m, 50 m and 100 m.

This kind of discrimination based on the values of the same factor but different depths do not work in the case of the other studied lakes.

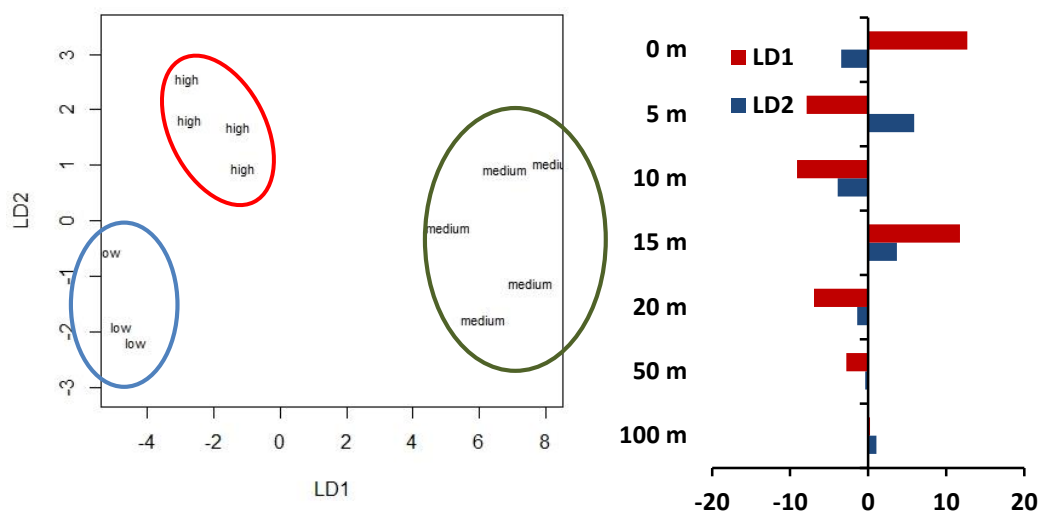


Fig. 27. LDA scatter plot (left) and linear discriminant coefficients of variables (right) in Lake Geneva. Variables are temperature values at different depths.

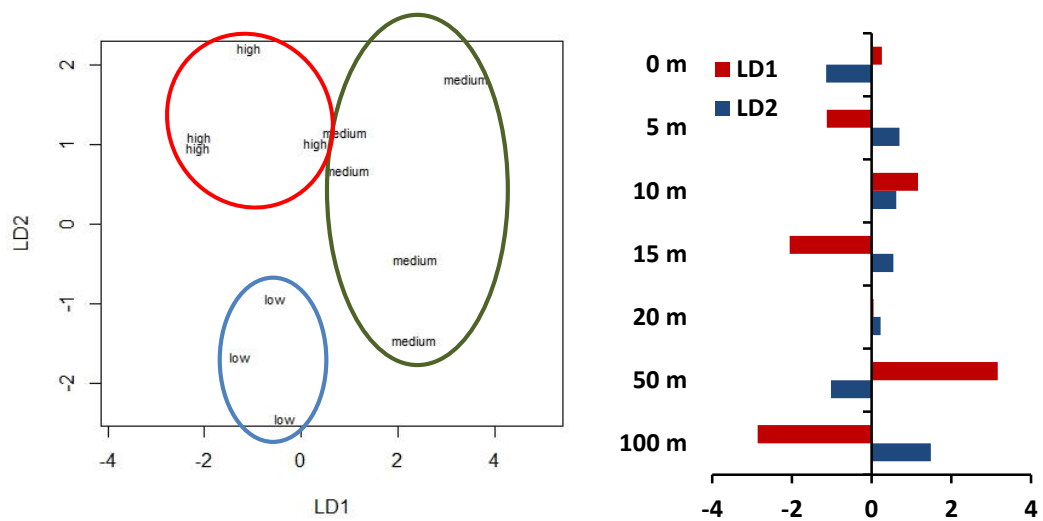


Fig. 28. LDA scatter plot (left) and linear discriminant coefficients of variables (right) in Lake Geneva. Variables are total phosphorus values at different depths.

5 Discussion

5.1. Do we have the same *Mougeotia* species in the different lakes and over time?

The genus *Mougeotia* is diverse encompassing more than 190 species (Guiry & Guiry 2013). With such a diversity, one of the first question I wanted to address, was to know if different species were present in the different lakes, or if different species could have occur in a given lake over time. Studies of phytoplankton in Lake Geneva always mention to *Mougeotia gracillima* (cf. Cipel reports since 1974). This identification was based on morphological features, and in particular cell dimensions. However in many cases *Mougeotia* can be identified at species level with certainty only when zygospores are present, simply because features to identify species level are based on zygospores morphology (Brook A.J. & Johnson L.R., 2002). Samples from Lake Geneva lacked this important identification criterion. Determinations in Lake Garda and Lake Maggiore only refer to the genus *Mougeotia*. According to Salmaso (personal discussion) the species was reported as *M. cf. calcaria* once but the identification is not reliable enough. The same taxonomical uncertainties are encountered in the case of Lake Kinneret.

A solution could be the use of genetic makers to add another tool for indentification and drawing new conclusions about the presence (or not) of different species.

The problem of the harmonization of identification results between different institutes, laboratories is sometimes complex. The incomparability can be due to the different identification literature used, technological equipments, methods or simply changes in the person who does the identification or even the changes in the scientists responsible (Straile et al. 2013). In her study Baillot (2013) showed by using detrended correspondence analysis that the samples coming from the same French region had very different phytoplankton composition and were separated into two groups. The reason explaining this separation was that two different persons made the identifications. This problem emerges especially when a study requires the cooperation, collaboration of many institutes, often from different countries. In their benthic diatom intercomparison study, Prygiel et al. (2002) carried out an exercise on sampling, slide preparation, counts and preparation and they found that 80% of the variance of the tested diatom index was due to the diatomist and only the rest was due to the sampling or the preparation. The diatom intercalibration exercise carried out by Kahlert et al. (2008) in rivers, also focus on these differences between diatomists. Kahlert et al. (2008) suggest to harmonize the identification skills of diatomists by i.e. using the same counting methods, the same literature and exchanging information about identification problems.

5.2. Does these different *Mougeotia* species have equivalent ecologies?

These uncertainties in the accurate identification of *Mougeotia* species can bother the comparability between the different lakes. But do we really need a so accurate identification (i.e. species level)? If all these species have the same ecology, can we avoid the problems of intercomparability by using a genus identification?

From a biomonitoring perspective, two conceptions are opposed. In the first conception, some authors underline the importance of accurate identification for a specific biomonitoring. In the second conception, on the contrary, authors think that for a robust biomonitoring you should on less accurate identifications, or regrouping of taxa, but which allow inter-comparability of data. In the first conception, requiring a species (or genus identification), can be found several phytoplankton indices. There are several indices using phytoplankton as indicator organisms for ecological status assessment, such as the Brettum Index (BI) for Austria and Slovenia, the PSI for Germany and the Phytoplankton Trophic Index (PTI) for Italy (Dokulil et al. 2005; Nixdorf et al. 2006; Salmaso et al. 2006). The driving parameters of these indices are species composition and abundance along the nutrient level (Kaiblinger et al. 2009). Rimet & Bouchez (2012) studied the influence of taxonomic resolution on diatom bioassessment in an ecoregional framework. They found that a precise ecoregional bioassessment requires fine taxonomic resolution.

In the second conception, authors are making regrouping of taxa. For instance, a functional classification of freshwater phytoplankton was published by Reynolds et al. (2002) and reviewed by Padisák et al. (2008). The concept of functional groups is to collect species with similar characteristics in the utilization of the resources in groups. In other words, the 'functional group' concept differentiates among phytoplankton on the basis of adaptations and requirements. The reason of the great success of this concept probably lies in the fact that it simplifies the handling of the long taxonomical lists into less numerous functional traits (Padisák et al. 2008). Although this concept has its own pitfalls, in some cases it does not require species level identification. Morpho-functional groups created by Salmaso & Padisák (2007) is based on the idea that phylogenetic classification does not reflect the ecological functions and pooling species with similar characteristics into groups might be more efficient.

Reynolds et al. (2002), in their work classified *Mougeotia* sp. to the codon **T** that characterized by species adapted to persistently mixed layers in which light is increasingly the limiting

constraint. It contains several filamentous genera, in spite of their disparate phylogenetic affinities. They mention nutrient deficiency as a factor these species are sensitive to.

This simplifying classification must be effective in some cases but we have to be aware of the diverse habitats *Mougeotia* spp. can live in. Klug & Fischer (2000) and Graham et al. (1996a, 1996b) showed the highly adaptive feature of *Mougeotia* sp. to acidified lakes, in other studies this factor did not play a key role in the occurrence and presence of *Mougeotia* sp. As I already mentioned in Chapter: “Introduction - Presence of planktic *Mougeotia* in freshwater lakes”, the species appears on a wide geographical scale and wide scale of climatic conditions. It can be present in the Amazonian tropical Lake Batata where the annual mean temperature is 27°C (Melo & Huszar 2000) or in the tropical central African Lake Malawi (Sarmiento 2006) but it is also common in e.g. the peri-alpine lakes analysed in my study (Anneville et al. 2002; Salmaso 2010; Morabito et al. 2003). My results showed that in peri-alpine lakes *Mougeotia* sp. was abundant at a temperature range of 15-20°C that is the highest values which can be observed in two of the three peri-alpine lakes. In Lake Kinneret this optimum is between 20-25°C. In each case, these optima are present during the warm, stratified summer period of the year. Even if the temperature ranges that I used in my study seem to be wide we still can define an optimum in all of the studied lakes. Notable that in few years in which *Mougeotia* sp. was present with relatively high biomass during the year, it overwintered. It is documented that filamentous species that were common during the stratified period can keep a stable population during winter (e.g. Yamamoto 2009). Studies I mentioned in the Introduction showed that planktic *Mougeotia* species favour meso- or oligotrophic conditions. Years with high *Mougeotia* sp. biomass became more frequent with the phosphorus reduction in Lake Geneva and Lake Kinneret. This fact confirms the preference of *Mougeotia* sp. to meso-, or oligotrophic conditions. Trophic state of Lake Garda and Lake Maggiore was already oligotrophic during the study period but the same relation can be suggested. My results confirm that *Mougeotia* is favoured in the nutrient-poor epilimnion where total phosphorus concentration (as the limiting potential nutrient) was between 5-15 $\mu\text{g l}^{-1}$. Preference of this low phosphorus values confirm Salmaso's (2002) results that *Mougeotia* sp. is good competitor for phosphorus. These optima of temperature and phosphorus can also explain the seasonal pattern of the species; in Lake Garda, Lake Maggiore and Lake Kinneret it starts develop its population during spring with the formation of thermocline and reach the maximum biomass by early summer. In the Italian lakes it declines with the rising of the autumn phytoplankton assemblage, In Lake Kinneret *Mougeotia* population can last until the end of the year but the population peak is still on

summer. Lake Geneva is the exception because *Mougeotia* population starts to develop on summer and reaching its peak by the autumn.

It seems that despite of the similarities; we have some differences between the *Mougeotia* populations in the studied lakes in their morphology, ecology, phenology.

5.3. Are there any differences in the environmental conditions between bloom vs. non-bloom years and do we have the same triggering factors in each lake?

Reynolds (2006) showed that water column stability (mixing properties), nutrients (mainly phosphorus), temperature, and euphotic zone are the most important factors affecting phytoplankton. When considering the early summer to autumn period, we observed an important interannual variability of these factors in all lakes. Similarly, we observed an important interannual variability in the biomass of *Mougeotia* during these seasons.

The results of DLA showed that these parameters are really important and related to the *Mougeotia* biomass. However the different parameters have different importance in each lake. In some cases the results are hardly interpretable but it is obvious that the two Italian lakes have similar results with the high importance of the thermal stability of water column and the temperature. These parameters are also important in all of the studied lakes. Indeed, this species is morphologically adapted to stay in the epilimnion (Reynolds et al., 2002; Padisák 2003; Padisák et al. 2008). It is worth to note that while temperature is an important factor in Lake Garda and Lake Maggiore, it is not in Lake Geneva and Lake Kinneret. The relevance of euphotic zone as a triggering factor is difficult to interpret since this value depends on phytoplankton biomass, and in particular of *Mougeotia* blooms; but according to Reynolds (2006), filamentous species are good light antennae that can harvest light more efficiently and have competitive advantage against non-filamentous species. Since the photic zone is deeper than the epilimnion during the stratified period, this competitive advantage can be responsible for the dominance of *Mougeotia*. Under these circumstances *Mougeotia* that develop at the border of the epi- and metalimnion, can benefit from the nutrient input from deeper layers. The efficient light harvesting feature can also come into effect to avoid self-shading and let the population to reach high biomass.

Important to know that although there are filamentous algal species (mainly among Cyanobacteria) that are adapted to the stratified summer period of deep lakes, it is not the same case as the *Mougeotia*'s. While *Planktothrix rubescens* (Padisák et al. 2003) or *Aphanizomenon flos-aquae* (Tapolczai 2011) are adapted to stay in the depth of thermocline with the help of gas vesicles (Lee 2008), *Mougeotia* cannot regulate its vertical movement. Although its filamentous

morphology helps to avoid fast sinking, *Mougeotia* population is adapted to stay in the well-mixed epilimnion (Reynolds et al. 2002). My results of the vertical distribution of *Mougeotia* show that it develops around 10 and 20m. The temporary accumulation in any depth is probably due to sinking of the filaments (Camacho 2006). However the depth profile of temperature seems to be important to distinguish bloom from non-bloom periods in Lake Geneva. This parameter is highly related to the thermal stability of the water column that, as we could see above, has a high importance in the forming of *Mougeotia* sp. population. The fact that I could not observe the importance of the vertical distribution of temperature for other lakes is likely due to the inappropriate dataset I used; in some cases the resolution was not fine enough or it was not consistent during the time period.

It is important to mention that although I was focusing on the bottom-up factors in this study, there are many other factors that can have important effect on *Mougeotia* population. Interactions with other organisms such as grazing, competition, predation or parasitism control phytoplankton population; it would worth examining these factors in later studies to better understand the mechanism of *Mougeotia* blooms.

To summarize, my study showed that *Mougeotia* sp. became more abundant during the last decades in the peri-alpine lakes but also in the subtropical Lake Kinneret. According to my results and former studies this phenomenon can be related to the phosphorus reduction (changes in trophic state) which has been occurring in these lakes. Using the main environmental factors that affect the presence of phytoplankton I could separate in each lake years presenting *Mougeotia* blooms from years without any blooms. The importance of these factors differed in the lakes but the more the geographical similarities between lakes, the more similarities observed in the importance of environmental factors. It suggests that in the case of *Mougeotia* sp. Reynold's conception of functional groups can be useful in a demarcated geomorphological range of lakes but the triggering factors can change in a lake with more different geomorphology or climate.