

Pannon Egyetem
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DIPLOMADOLGOZAT

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

Tapolczai Kálmán

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Feladatkiírás

Mougeotia blooms in large alpine lakes

Looking for triggering factors

Phytoplankton is an essential biological element to assess the ecological quality of lakes. Lake of Geneva is subject to massive development (blooms) of a filamentous alga (*Mougeotia gracillima*) in the plankton. These blooms are also observed in other peri-alpine lakes (Lago Maggiore, Lago di Garda in Italy) and subtropical deep lakes (Lake Kinneret in Israel) and cause a nuisance of professional fishing (nets clogging). Drinking water production can be also impacted by clogging filters. These blooms are observed since the beginning of years 2000 in lake of Geneva (which is in restoration phase): in the Italian lakes cited above *Mougeotia* is very common, although not regularly blooming. The reasons of these blooms are partly unknown. The managers of these lakes (e.g. CIPEL, water agencies, services cantonales in Switzerland) are waiting for scientific explanations for a better management.

The objective of this study is to identify the triggering factors controlling these blooms. For this purpose, the student will analyze data coming from the long term monitoring of Lake Geneva. In order to generalize the conclusions, data from Lago Maggiore, Lago di Garda and Lake Kinneret will also be included in the analyses. From a scientific point of view, this work will enable to understand the massive development of this micro-alga in environment where it was rarely dominant until now.

Testable hypotheses are about the influence of chemical environment in a global warming context. Global warming has been observed in these lakes since the 70ies.

Expected skills: To manage a database and to analyze data. Knowledge in bio-statistics and hydrobiology are welcomed. A final report has to be written (English or French).

Nyilatkozat

Alulírott Tapolczai Kálmán diplomázó hallgató, kijelentem, hogy a diplomadolgozatot a Pannon Egyetem Környezettudományi Intézet/ Limnológia Intézeti Tanszékében készítettem a Környezettudomány mesterszak (M. Sc. in Environmental Science) diploma megszerzése érdekében. Kijelentem, hogy a diplomadolgozatban foglaltak saját munkám eredményei, és csak a megadott forrásokat (szakirodalom, eszközök, stb.) használtam fel. Tudomásul veszem, hogy a diplomadolgozatban foglalt eredményeket a Pannon Egyetem, valamint a feladatot kiíró szervezeti egység saját céljaira szabadon felhasználhatja.

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hallgató aláírása

Alulírott Dr. Padisák Judit témavezető kijelentem, hogy a diplomadolgozatot Tapolczai Kálmán a Pannon Egyetem Környezettudományi /Limnológia Intézeti Tanszékében készítette a Környezettudomány mesterszak (M.Sc. in Environmental Science) diploma megszerzése érdekében.

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ABSTRACT

During the last decades the filamentous green alga, *Mougeotia* spp. has been having important contributions to the phytoplankton of some large peri-alpine lakes (Lake Geneva, Lake Garda, Lake Maggiore) and also in subtropical Lake Kinneret (Israel). The occurrence of algal blooms usually related to the main bottom-up factors that affect the phytoplankton. These are the temperature, light intensity, mixing properties in the lake and nutrients.

Aim of my study was to define how these factors affect the seasonal and interannual distribution of *Mougeotia* sp. in the studied lakes.

By analysing the dataset from each lake

- i) I made morphological examinations of *Mougeotia* sp. from Lake Geneva and compared it with data from the other lakes.
- ii) Then I defined the seasonal and interannual pattern of both the species's abundance and the environmental parameters.
- iii) In a third step I defined the ecological preferences for the environmental parameters.
- iv) Finally using multivariate statistical analysis (discriminant analysis) I compared the environmental parameters between years with different *Mougeotia* sp. abundance.

According to my results and former studies, the more and more frequent bloom related to the phosphorus reduction that have been occurred in these lakes. Using the main environmental factors I could separate years in each lake with *Mougeotia* sp. 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 were observed in the importance of environmental factors.

keywords: *Mougeotia*, bloom, Lake Geneva, Lake Garda, Lake Maggiore, Lake Kinneret, deep lakes, phytoplankton

TARTALMI ÖSSZEFOGLALÓ

Az utóbbi évtizedekben jelentősen megnőtt a *Mougeotia* sp. fonalas zöldalga jelentősége a planktonban egyes Alpok környéki mély tó esetében (Genfi-tó, Garda-tó, Maggiore-tó), de hasonló jelenség figyelhető meg a szubtrópusi Kinneret-tóban (Izrael) is. Az algák tömeges elszaporodásának kiváltója lehet a fitoplankton elterjedését befolyásoló főbb környezeti paraméterek kedvező interakciója. Ezek a hőmérséklet, fényintenzitás, a víztest keveredési sajátosságai és a növényi tápanyagok.

Munkám célja annak vizsgálata, hogy ezen környezeti paraméterek hogyan hatnak a *Mougeotia* sp. szezonális és hosszú távú mintázatára a vizsgált tavakban.

Munkám során:

- morfológiai vizsgálatokat végeztem a Genfi-tó fitoplankton mintáiból származó *Mougeotia* sp. sejteken és összevetettem a Garda-, Maggiore-, Kinneret-tó adataival,
- meghatároztam mind a fő környezeti paraméterek, mind a *Mougeotia* spp. szezonális és hosszú távú eloszlását,
- meghatároztam a faj ökológiai preferendumát,
- végül többváltozós statisztikai analízis (diszkriminancia analízis) segítségével összehasonlítottam a különböző *Mougeotia* spp. abundanciával jellemezhető éveket a környezeti paraméterek értékei alapján.

Eredményeim és előző tanulmányok alapján elmondható, hogy a *Mougeotia* egyre gyakoribb és intenzívebb megjelenése kapcsolatba hozható a vizsgált tavak esetében zajló vagy már lezajlott foszforterhelés intenzív csökkenésével. A vizsgált környezeti paraméterek alapján elkülöníthetők egymástól a magas *Mougeotia* abundanciával jellemezhető évek az alacsony *Mougeotia* abundanciát mutatóéktól. Az egyes környezeti faktorok jelentősége eltért az egyes tavak esetében; a geomorfológiailag hasonló tavaknál a környezeti paraméterek jelentőségében nagyobb volt a hasonlóság.

kulcsszavak: *Mougeotia*, algavirágzás, Genfi-tó, Garda-tó, Maggiore-tó, Kinneret-tó, mély tavak, fitoplankton

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1 Introduction

1.1 Algal blooms: overview of their reasons and consequences

Both in the open sea and in large lakes primary production is based on photosynthesis of pelagic community of microalgae, the phytoplankton. Taxonomically, phytoplankton is a very diverse assemblage that consists in tens to hundreds of species that can differ in their size (one to hundreds, even thousands micrometers) with a generation time of no more than a few days. Phytoplankton abundance and composition are greatly affected by floating abilities of individual species, by abiotic factors, mainly light, temperature, nutrients, water movement and also interactions with other organisms, such as grazing, competition, parasitism and symbiosis. These factors balance the rapid growth rate of the community (Wetzel 2001). In some cases, one or a few species can overcome the losses due to favourable coincidence of circumstances, causing a high abundance and dominance of the species. This excessive development of the phytoplankton is called bloom that can last for several weeks or even months (Canter-Lund & Lund 1995).

Consequences and effects of blooms can be very different. They can cause greatly adverse effects such as oxygen depletion, reduced water quality aesthetics, clogging of fish gills, or toxicity. Less direct consequences related to economy can be the losses of fisheries due to net clogging, negative effects on tourism, health care.

In freshwater lakes, the most common bloom forming algal group is Cyanobacteria (Havens 2008) and because of their success and ubiquity, one of the best studied, too (Bryant 1995). Studies on cyanobacteria blooms and cyanobacteria became important with the eutrophication problems of lakes. Cyanobacteria present a broad diversity of skills making them rather successful under this condition, i.e. higher temperature optima, low light-energy requirements, superior uptake kinetics for inorganic carbon, low TN/TP-ratio requirement, ability to fix N₂ from the atmosphere, buoyancy of some species and toxicity (Dokulil & Teubner 2000).

Beside cyanobacteria, representatives of other algae groups can also cause water blooms with great ecological or even practical consequences.

Red tide is the common name of a type of algal bloom caused by dinoflagellates. The colour of the blooms is red or brown because of the accessory pigments of this group of algae. Red tides can occur both in fresh and marine waters but are more common in the latter, in coastal areas. The phenomenon is quite common i.e. in the coastal waters of Florida (Kirkpatrick et al. 2004) or in the Gulf of Mexico caused by *Gymnodinium breve* (Tester & Steidinger 1997). However freshwater bodies rarely affected by blooms of dinoflagellates; Lake Tovel (Italy) is an exception. *Glenodinium sanguineum* regularly causes blooms in the oligo-mesotrophic alpine lake. Under nutrient-poor conditions, trigger of these blooms is not the enrichment in nutrients but the species's great efficiency of assimilation and retention of phosphorus (Cantonati et al. 2003). In 1990, the water of Lake Geneva was coloured red by the cryptophytes *Rhodomonas minuta* during a few days, without clear understanding of the reasons (Druart & Balvay 2007).

Since the end of the 90's, Lake Geneva is also facing the regular occurrence of a Zygnemataceae – *Mougeotia* sp. – blooms. This taxon has also been observed to bloom in other lakes of the peri-alpine region and in the subtropical Lake Kinneret (Israel).

1.2 Description of the genus *Mougeotia*

The filamentous chlorophyte alga, *Mougeotia* belongs to the Zygnemataceae family (Zygnematales, Chlorophyta). The three genera of the family (*Spirogyra*, *Zygnema*, and *Mougeotia*) represent the most common filamentous green algae in freshwaters. Usually in their filaments are not constricted between the cells and are always unbranched, except a few species being able to produce short rhizoids. The cell walls are thin and generally secrete conspicuous mucilage. The shape of the chloroplasts, are specific of each genera. *Spirogyra* cells contain one to 16 long and narrow bands that lying against the cell wall and spiralling along the length of the cell. Each of these bands contains a series of pyrenoids. *Zygnema* has a pair of star-shaped chloroplasts each with a central pyrenoid. *Mougeotia*'s chloroplasts are single flat or slightly twisted plate with a median series of pyrenoids.

The vegetative reproduction of filaments occurs by fragmentation of the filaments into short filaments or cells. Asexual reproduction occurs by forming akinetes or aplanospores. Both are forming thick-walled cells helping to survive under unfavourable conditions.

Sexual reproduction of Zygnemataceae is the conjugation. Conjugation can occur between adjacent cells of the same filament (lateral) or, more commonly, ladder-like between separate filaments (scalariform). During conjugation the opposite walls where filaments are joined, break down resulting the conjugation tube. This tube ensures that the gametes formed from the vegetative cells can encounter with each other, forming the zygospore. The shape of zygospore that can be spherical to ellipsoidal or quadrate to triangular is a very informative identification criterion. Lateral conjugation is very common in the genus *Spirogyra* but very rare in *Mougeotia* while scalariform conjugation is more prevalent.

Mougeotia (C. Agardh 1824) filaments are simple and unattached. The cells are cylindrical and 5 to 20 times longer than broad, having plane end walls. Chloroplast (usually 1 per cell) is a flat plate, occupying most of the cell length with a linear row of pyrenoids. Nucleus lies against the cell wall. The zygospores of the genus range from spherical to compressed-spherical, ovoid, ellipsoidal to quadrate-ellipsoidal with flattened or truncate angles (Brook A.J. & Johnson L.R., 2002).

1.3 Studies on the ecophysiology of *Mougeotia*

Most of the studies dealing with the physiology or ecology of *Mougeotia* are studies on benthic species or populations in the littoral (Graham et al. 1996). It is well documented that benthic populations of *Mougeotia* can reach quite high abundances in the shoreline of acidified lakes but the direct physiological effect of low pH has been unclear (Klug & Fischer, 2000). There are hypotheses that the higher abundance under low pH is due to the lack of grazers under these circumstances. Although it seems to be an acceptable explanation, it still cannot explain that *Mougeotia* is rarely dominant in high pH waters. Klug & Fischer (2000) showed in their mesocosm experiments on planktic *Mougeotia* that low pH has a negative effect on the growth of *Mougeotia* but the preference of low dissolved inorganic carbon (DIC) concentration compensates this negative effect.

One unique feature of the genus *Mougeotia* is the orientational movement of the chloroplast with respect to light. The complex molecular and physiological mechanism of this phenomenon is well studied by Wagner & Klein (1981). In their study, they precisely showed the exact processes from the triggering stimulus of the light to the responding mechanism of the moving chloroplast. It seems obvious that this kind of physiological adaption means important advantage in the nature. The filamentous characteristic of *Mougeotia* supplemented by the ability of chloroplast movement makes it efficient light antennae, especially when oriented correctly in the photon flux field. That would suggest the competitive advantage of *Mougeotia* species under turbid and well-mixed circumstances (Reynolds, 2006).

1.4 Presence of planktic *Mougeotia* in freshwater lakes

Even if benthic or metaphytic *Mougeotia* are more studied, several studies deal with the planktic form of this genus. According to the functional classification of phytoplankton species (Reynolds et al., 2002; Padisák et al. 2008) *Mougeotia* belongs to the codon **T**. Codon T represents a transition between the desmids-*Aulacoseira* assemblage **P** and the *Limnothrix/Planktothrix agardhii* assemblage **S**. This group is characterized by species that are common in more persistently mixed layers where light is the limiting constraint. Several filamentous algae species are well adapted under these circumstances (*Binuclearia*, *Geminella*, *Tribonema*, *Mougeotia*) (Reynolds et al., 2002) of optically deep, mixed environments such as the clear epilimnion of deep lakes in summer (Padisák et al. 2008). This group merges into the group **S** that contains photoadapting solitary filamentous cyanobacteria. The most familiar species in this group is *Planktothrix agardhii*, which is common in enriched, exposed and shallow lakes at most latitudes. Codon **T** is usually associated with a habitat of deep, well-mixed epilimnion where species of this group tolerate light deficiency well but sensitive to nutrient deficiency (Reynolds et al., 2002).

Mougeotia appears on a very wide scale of climatic conditions. Lake Batata is an Amazonian tropical (annual mean temperature of 27 °C), clear-water, flood-plain lake, located in Brazil. In the lake there is an annual cycle of water level fluctuation which depends mainly on seasonal variation of precipitation in the

catchment area. This hydrological variability drives the changes in physical, chemical parameters and also of biotic factors (Huszar & Reynolds 1997). In Lake Batata, *Mougeotia* cf. *delicata* was present during low-water level. During this period, the maximal depth is the lowest (3 m) and the whole water column is well-mixed with the euphotic depth reaching the bottom. Nutrient concentrations are the highest during this period, but it is still in the range of mesotrophic level (Melo & Huszar 2000).

Mougeotia also occurs in the phytoplankton of Lake Malawi, in tropical central Africa (Sarmiento 2006). It occurs from May to September, in the dry season when temperature start to decrease and the thermocline starts to deepen (Eccles 1974).

Occurrence of planktic *Mougeotia* in peri-alpine lakes is regularly recorded, and in some cases might cause blooms with high abundances. In Lake Garda *Mougeotia* became so important member of the phytoplankton assemblage that it has been defined as one of the ‘master species’ (Salmaso 2002). *Mougeotia* starts to develop regularly during spring months and maintains or increases its abundance in the warmest months (Salmaso 2003). Success of *Mougeotia* in Lake Garda is explained by its good competition for phosphorus and its resistance against sinking (Padisák 2003) and grazing (Salmaso 2003). According to Salmaso (2010), the species showed higher biomasses in years after cold winters and deep mixing that, since the lake is oligomictic, is accompanied by greater replenishment of phosphorus in the euphotic layers. Similar features favour *Mougeotia*’s success in other, similar lakes in the region, like Lake Iseo (Garibaldi et al. 2003). In Lake Maggiore, *Mougeotia* is also important in the phytoplankton assemblage. It starts to develop in spring and reaches its maximum development around mid-summer, the same reasons as in Lake Garda of its abundance can be given: good competitive ability for phosphorus and low sinking rates (Morabito et al. 2003; Morabito et al. 2002). In Lake Lugano, the first report of *Mougeotia* was published from the period between 1955 and 1958 when the first effects of eutrophication became apparent, but the highest biomass value was observed in the autumn 1986, six years after the phosphorus concentration began to decrease (Polli & Simona 1992). After 1988, *Mougeotia* occurred only occasionally in the lake.

A long-term study analyzing the seasonal changes in the phytoplankton of Lake Geneva between 1974-1998, also mentioned *Mougeotia gracillima* as an important member of the phytoplankton community. In this study, Anneville et al. (2002) showed that *M. gracillima* is a typical late summer species, which starts to develop in early summer and reaches its maximum abundance in late-summer or early autumn. They examined the seasonal succession of the phytoplankton and as it is documented in their study, the annual pattern fits well with the succession described by the PEG model (Sommer et al. 1986). During the first half of the year (December-May) mainly abiotic factors limit the phytoplankton composition, such as light intensity, temperature, mixing. In May, the zooplankton grazing and decreasing silicate concentration lead to a different composition by June. By June the stable stratification and nutrient depletion lead to a situation where autogenic factors become important and the environment selects for the better competitors for the limiting resource. The so called 'stress tolerant species' (S) (Reynolds 2006) become more important such as *Mougeotia gracillima* that is more efficient at exploiting the resources and conserve rather than propagating its biomass. Since *M. gracillima* is better adapted for autumnal conditions (deepening mixed layer, decreasing vertical temperature gradient and light intensity), it reaches its maximal abundance during September and October (Anneville et al. 2002). As also documented by Anneville & Pelletier (2000), *M. gracillima* started to colonise Lake Geneva in the 1960's, which was the beginning of the eutrophication of the lake. Its abundance declined during the period of strong eutrophication but became more abundant again in the 1990's with the decreasing phosphorus concentration. It suggested that changes in the pattern of phosphorus availability have made *M. gracillima* more competitive, indeed its morpho-functional traits make it better adapted to summer environmental conditions now characterized by a P-depleted euphotic zone and a metalimnion that can occasionally be enriched with phosphorus pulses coming from the P-enriched deep layers of the lake (Anneville et al. 2002).

Beside the alpine region, planktic *Mougeotia* is also common in the subtropical region, in the Israeli Lake Kinneret (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 gracillima*, reaching high biomass during April and May in 2005.

1.5 Aim of study

As I showed in the introduction, unexpected occurrence and sometimes excessive development of planktic *Mougeotia* species in freshwater lakes is increasingly observed in recent years or decades. This phenomenon is often linked to the changes in species composition of phytoplankton. These changes are usually induced by the changes in trophic level of these water bodies or probably by changes of other abiotic factors, such as temperature, light intensity, mixing pattern. Several lakes in the peri-Alpine region are affected by this problem, like Lake Garda, Lake Maggiore, Lake Iseo or Lake Geneva. A similar phenomenon occurred in lakes with a completely different morphometry or a very different climate, like Lake Kinneret (Israel), Lake Batata (Brasil) or the Ömerli reservoir in Turkey. In this study I focus on three peri-alpine lakes; Lake Geneva, Lake Garda, Lake Maggiore and the subtropical Lake Kinneret.

My hypothesis is that the more and more frequent blooms of *Mougeotia* sp. are related to changes in bottom-up factors.

To address this assumption, I used to work on long term monitoring data of 4 different lakes presenting regularly *Mougeotia* blooms: 3 are peri-alpine lakes (Lake Geneva, Lake Garda, Lake Maggiore) one is a subtropical lake (Lake Kinneret).

First I made some morphological examinations of *Mougeotia* sp. from Lake Geneva, and made comparison with data of the other studied lakes due to taxonomical uncertainties.

Then I defined the seasonal and interannual patterns of the species's biomass.

In a third step I defined the ecological preferences for the major environmental parameters controlling phytoplankton: phosphorus concentration, temperature, transparency and mixing properties.

Finally I looked for the environmental factors triggering *Mougeotia* blooms. To achieve this fourth aim, multivariate statistical analysis (discriminant analysis) was carried out.

2 Material and methods

2.1 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 $58 \text{ m}^3\text{s}^{-1}$ (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 ($45^{\circ}57'\text{N}$ $8^{\circ}38'\text{E}$) (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 ($32^{\circ}50'\text{N}$, $35^{\circ}35'\text{E}$) 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.

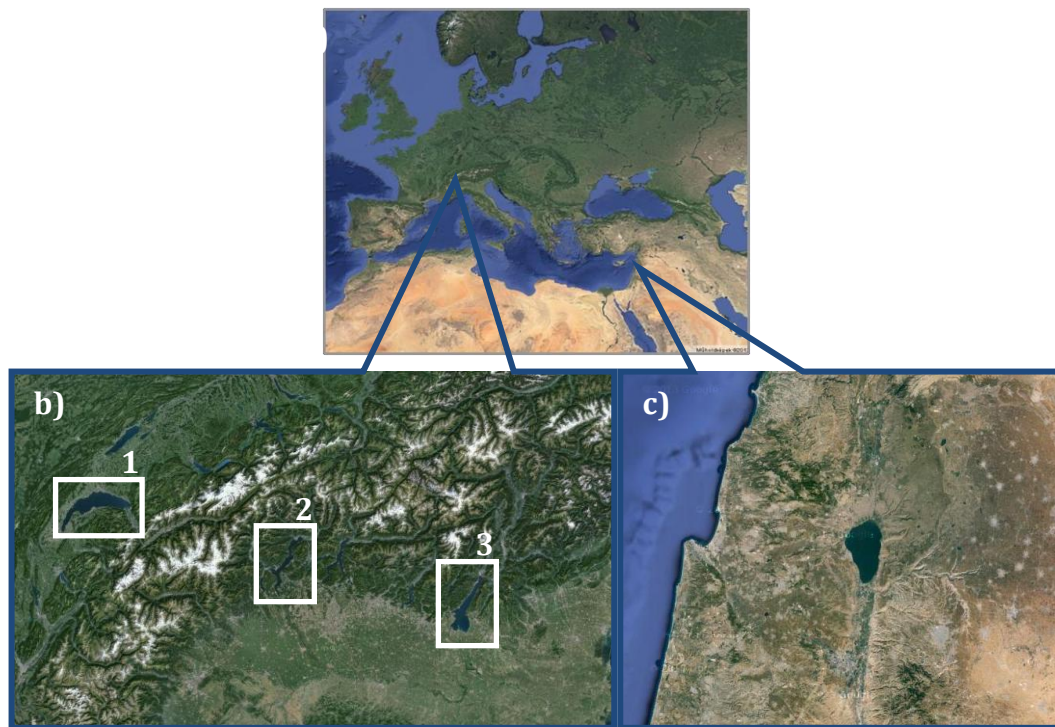


Fig. 1. Location of studied lakes on large scale (a) and a closer view of peri-alpine lakes (b); Lake Geneva (b-1), Lake Maggiore (b-2), Lake Garda (b-3) and Lake Kinneret (c)

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

2.2 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.

The 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 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 2005).

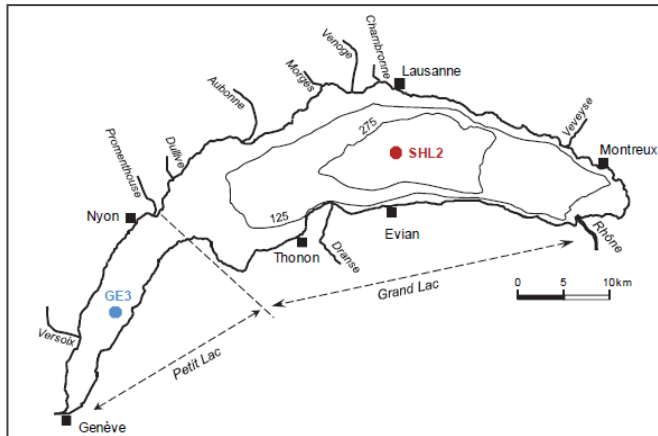


Fig. 2. Lake Geneva with the sampling points in the ‘Petit Lac’ (GE3) and in the ‘Grand Lac’ (SHL2). Data used in this study originates from SHL2.

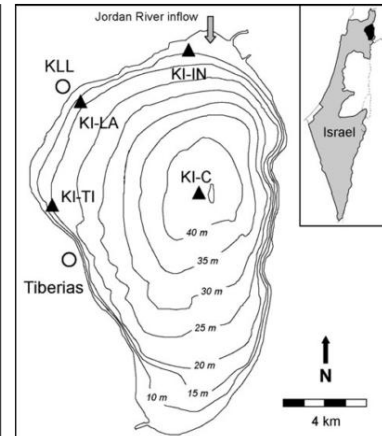


Fig. 3. Lake Kinneret with the sampling site (KI-C) at the deepest point of the lake.

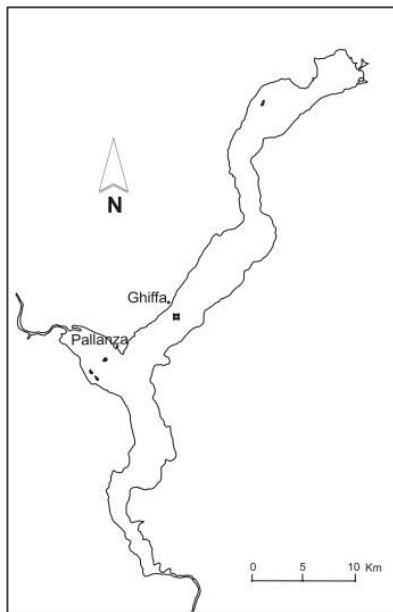


Fig. 4. Lake Maggiore with the sampling point at the station of Ghiffa.

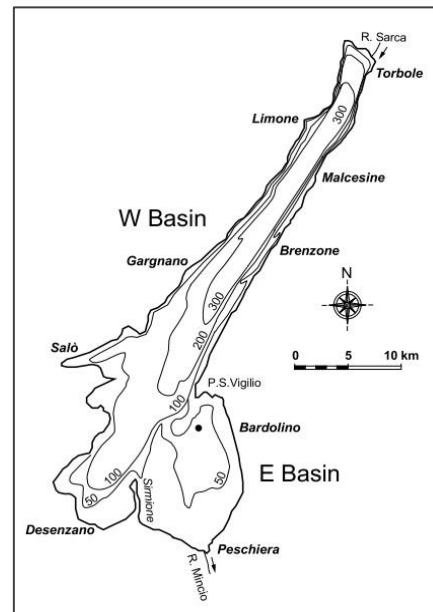


Fig. 5. Lake Garda with the sampling point in the eastern basin at the station Bardolino.

2.3 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. These are the followings: 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 the parameters differ 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 m, 5 m, 10 m, 20 m, 30 m, 40 m, 50 m, 100 m, 150 m, 200 m, 225 m, 250 m, 275 m, 309 m. From 1981, the vertical resolution has been higher with the depths of 0 m, 2.5 m, 5 m, 7.5 m, 10 m, 15 m, 20 m, 30 m, 50 m, 100 m, 200 m, 250 m, 275 m, 280 m, 285 m, 290 m, 295 m, 300 m, 305 m, 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 m, 1 m, 2 m, 4 m, 5 m, 8 m, 10 m, 15 m, 20 m, 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 layers; the 0-2 m, 9-11 m and 19-21 m strata. For temperature, the vertical resolution is higher; values are from the 0 m, 5 m, 10 m, 15 m, 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 m, 5 m, 10 m, 20 m, 30 m, 50 m.

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

In this study I used the relative thermal resistance (RTR) as an indicator of the thermal stability of the water column. RTR 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°C (Kalff 2002):

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

RTR= relative thermal resistance
 D_b = water density at bottom layer
 D_s = water density at the surface
 D_4 = density of 4°C water
 D_5 = density of 5°C water.

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

2.4 Statistical analysis

To compare the environmental conditions during blooming and non blooming periods I used a multivariate analysis: linear discriminant analysis (LDA). LDA was run by using the MASS package (Venables & Ripley 2002) of R statistical software (R Development Core Team 2008). The analysis required creating a matrix with variables (environmental factors) and object (years) (Figure 6.). The purpose of this analysis was to find the linear combinations of the original variables that give the best possible separation between the groups. In our case the groups were based on the *Mougeotia* abundance.

	RTR	Zeu	SRP	TP	T	Group
Year₁						
Year₂						
...						
Year_n						

Fig. 6. Template of the matrix used in LDA

I have run the LDA on two types of matrices to answer two questions:

1. Which environmental parameters discriminate between a bloom and non-bloom events?

I created matrices separately for each lake but the variables were the same; T, TP, SRP, z_{eu} , RTR. In each case I used the years during the study period, but study period was different in the case of each lake; 2000-2012 in Lake Geneva (the change in the sampling method required to shorten the original study period from 1974 to 2012), 1995-2008 in Lake Garda and 1984-2011 in Lake Maggiore. In each year I selected the values of environmental parameters at the time of *Mougeotia* biomass peak and used these values of variables in the matrix. The groups were determined based on the *Mougeotia* abundance during its peak. After identifying the maximal *Mougeotia* biomass value in each year getting a set of values of which I calculated the 1st and 3rd quartile. The 1st and 3rd quartiles marked the boundaries of the groups; low (<1st quartile), medium (between 1st and 3rd quartile), high (>3rd quartile). To make the interpretation of the loadings in LDA easier, I standardized the input variables so that those have mean zero and within-groups variance of 1.

2. Does the vertical profile of environmental parameters have effect on the *Mougeotia* abundance during its annual peak?

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 m, 5 m, 10 m, 15 m, 20 m, 50 m, 100 m) during the *Mougeotia* biomass peak in each year (objects). It means I had one LDA for temperature and one for total phosphorus. Figure 7. shows the template table for this analysis. Estimation of the groups was the same as above.

	V_{0m}	V_{5m}	...	V_{100m}	Group
Year ₁					
Year ₂					
...					
Year _n					

Fig. 7. Template of the matrix used in LDA

2.5 Morphological examination

For the morphological examination of *Mougeotia* cells and filaments I analyzed samples of the sample collection of INRA UMR CARRTEL from Lake Geneva. I chose 11 samples of different dates. The conditions of selection were the followings:

- chose samples that cover the whole study period, with more samples from the more affected years,
- relatively high *Mougeotia* sp. biomass and contribution to total phytoplankton biomass.

In every sample the length and width of 20 cells were measured.

Morphological data of *Mougeotia* sp. from the other lakes were given 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).

3 Results

3.1 Environmental factors

3.1.1 Temperature

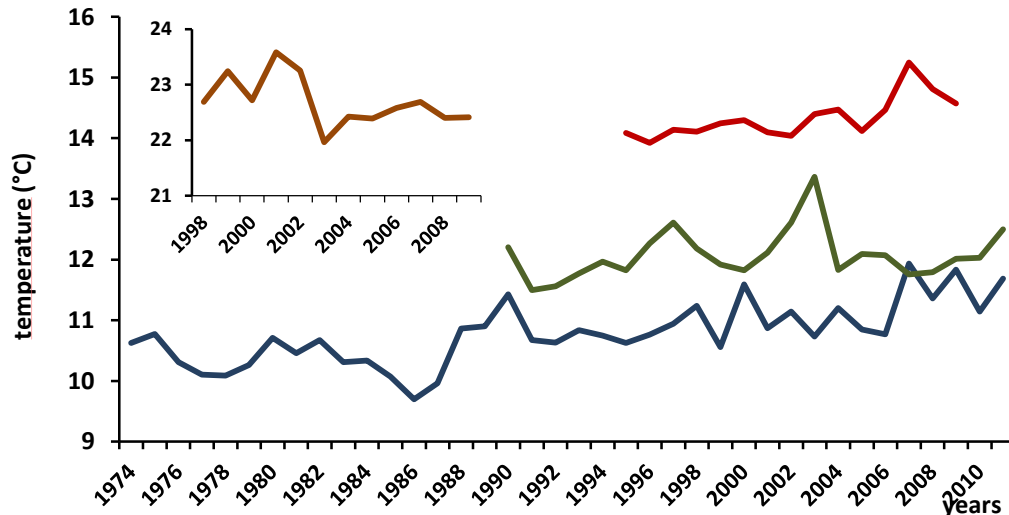


Fig. 8. Long-term change in annual mean water temperatures in the studied lakes; Lake Geneva (blue), Lake Maggiore (green), Lake Garda (red), Lake Kinneret (brown). Temperature values are annual means from the top 20 metres (Lake Geneva, Lake Garda, Lake Maggiore) or top 10 metres (Lake Kinneret).

Figure 8 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 9. 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 slightly 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.

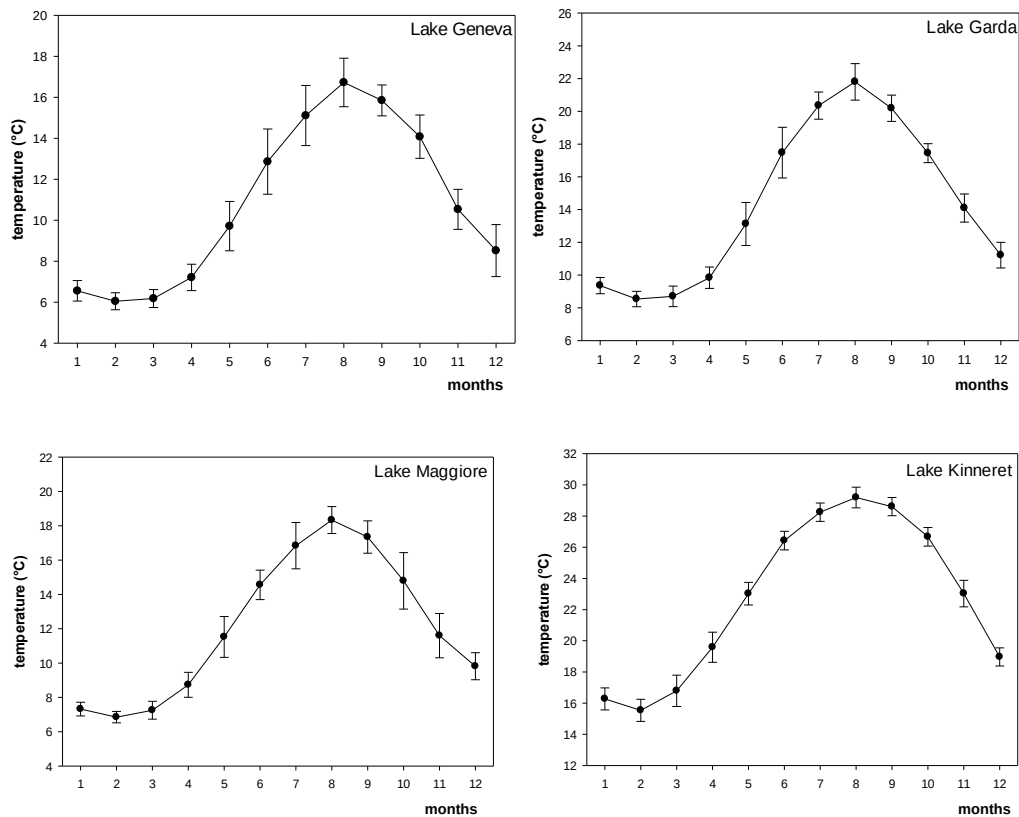


Fig. 9. Seasonal distribution of monthly average temperature values. Dots indicate the means of the long-term values, error bars are standard deviation. Values are average of 0-20 m depth.

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.

3.1.2 Mixing properties, water column stability

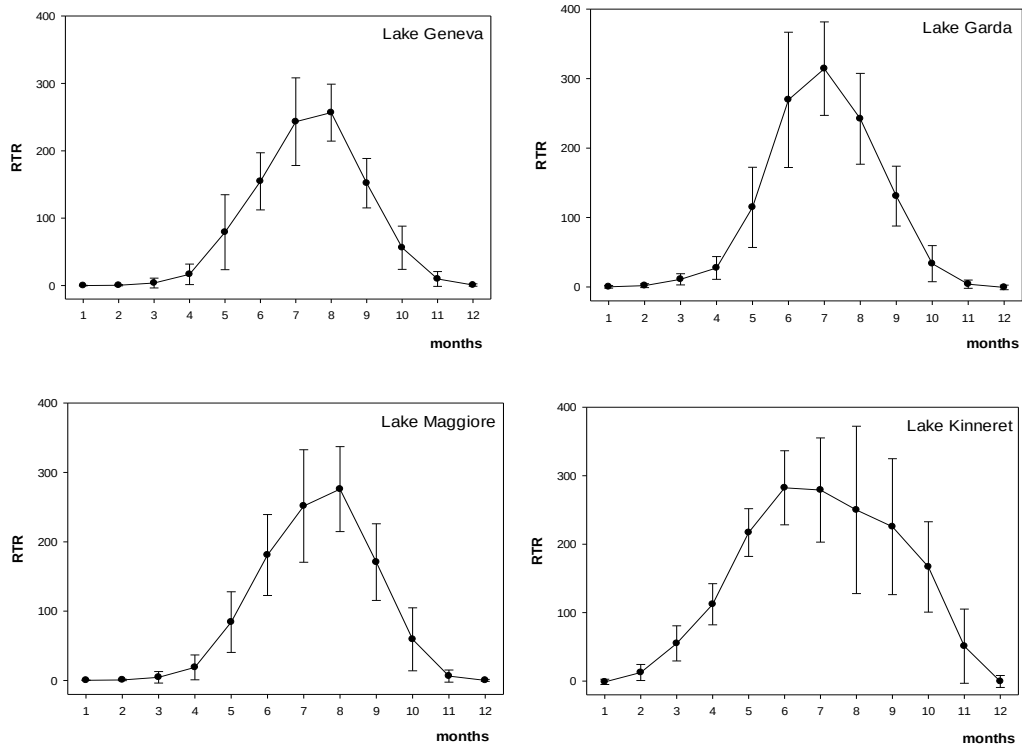


Fig. 10. Seasonal distribution of monthly average thermal resistance values. Dots indicate the means of the long-term values, error bars are standard deviation.

Figure 10 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 RTR 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 variety. 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.

3.1.3 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 11. 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.

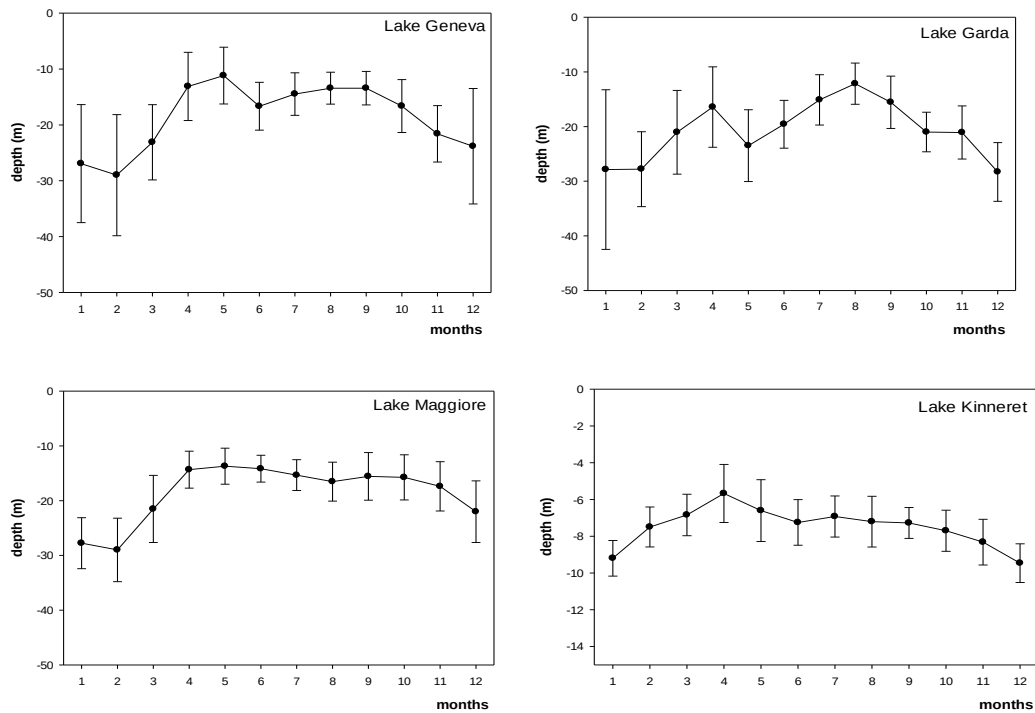


Fig. 11. Seasonal distribution of monthly average euphotic depth values. Dots indicate the means of the long-term values, error bars are standard deviation.

3.1.4 Phosphorus concentration

Long-term change in annual means of total phosphorus in Lake Geneva is presented on Figure 12. 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 $\mu\text{g l}^{-1}$).

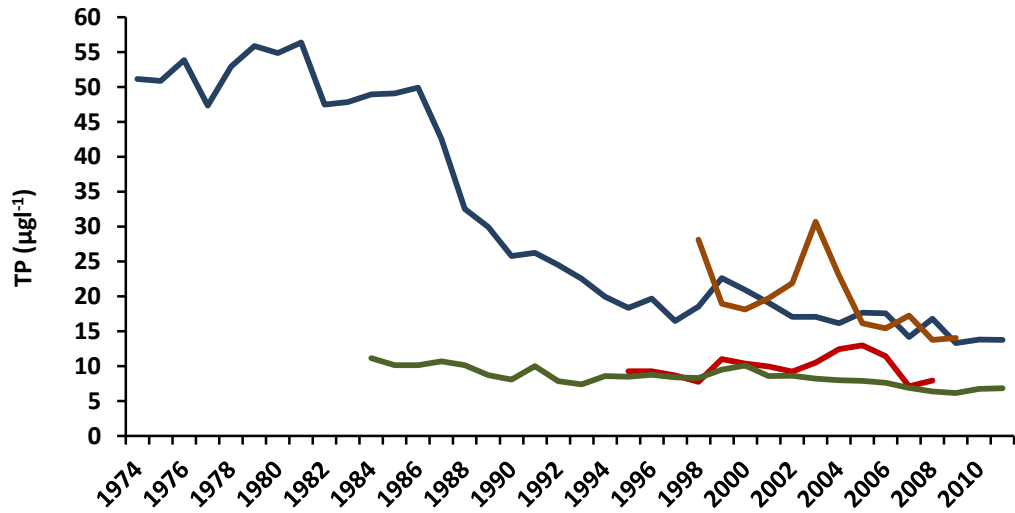


Fig. 12. Long-term pattern of annual average total phosphorus concentration in the studied lakes; Lake Geneva (blue), Lake Maggiore (green), Lake Garda (red), Lake Kinneret (brown). Values are annual averages from the top 20 metres (Lake Geneva, Lake Garda, Lake Maggiore) or top 10 metres (Lake Kinneret).

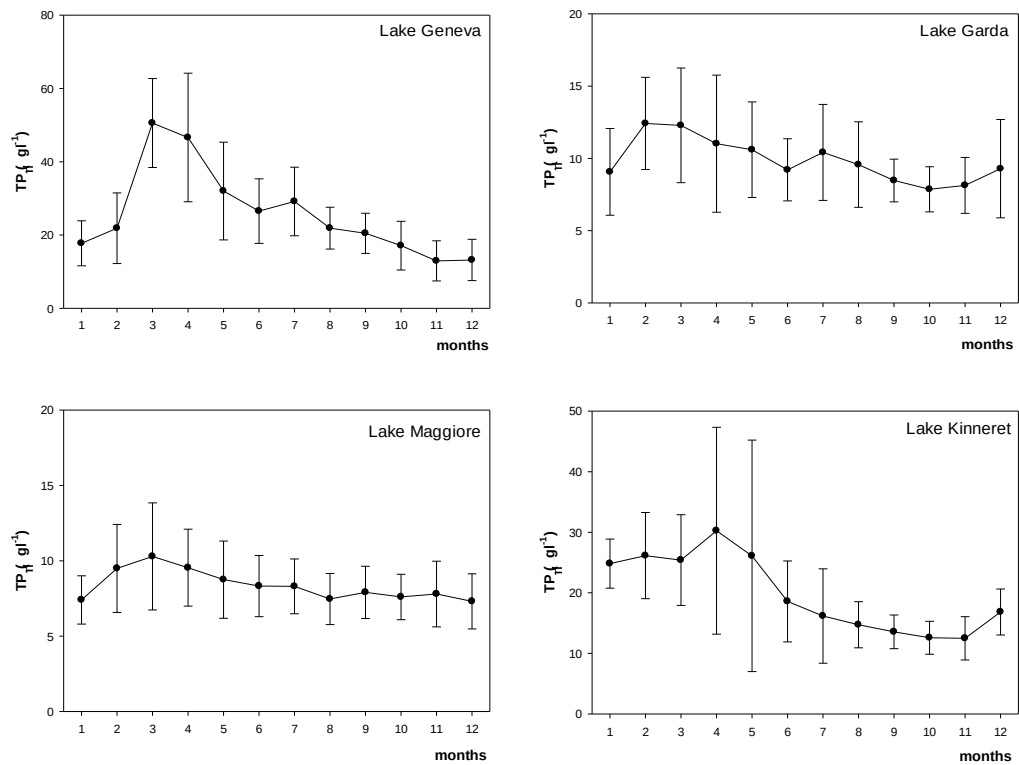


Fig. 13. Seasonal distribution of monthly average total phosphorus values. Dots indicate the means of the long-term values, error bars are standard deviation.

Figure 13 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.

SRP values have also a strong interannual variability. The annual patterns are similar among 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.

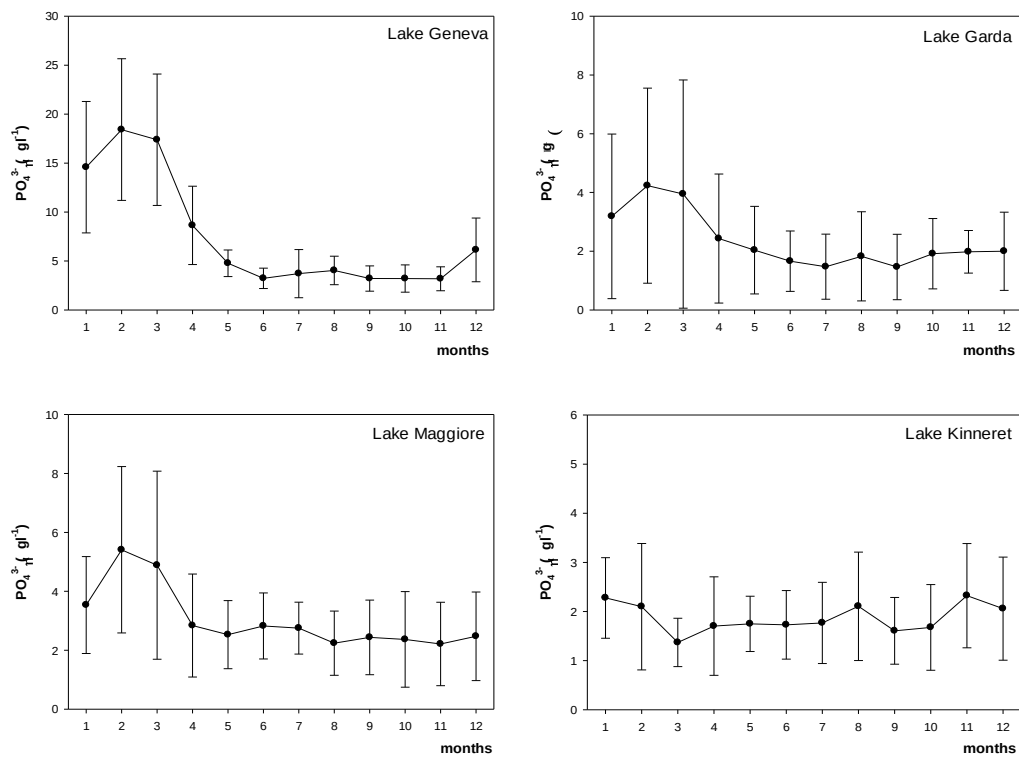


Fig. 14. Seasonal distribution of monthly average SRP values. Dots indicate the means of the long-term values, error bars are standard deviation.

3.2 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 15. Cell length of *Mougeotia* varied in a wide range between 30 and 80 μm during the studied period and a sudden increase 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 in Figure 16.

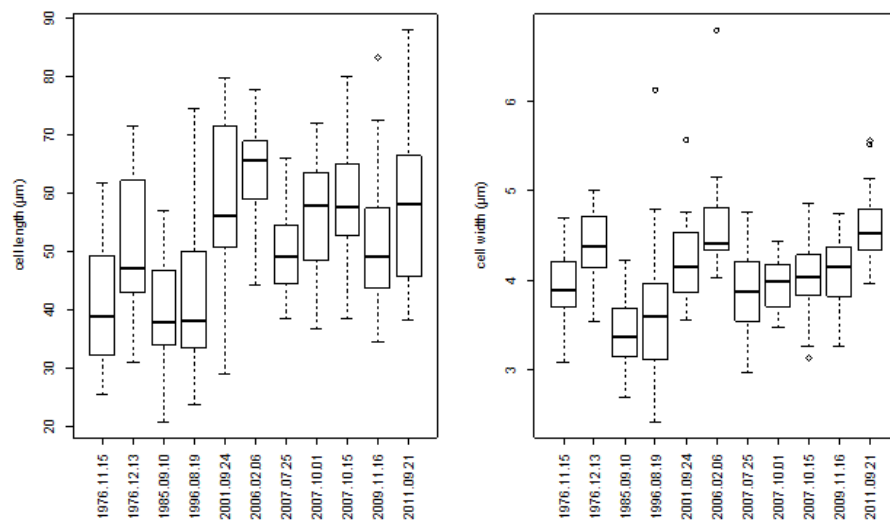


Fig. 15. Length and width of *Mougeotia* cells at different dates during the study period.

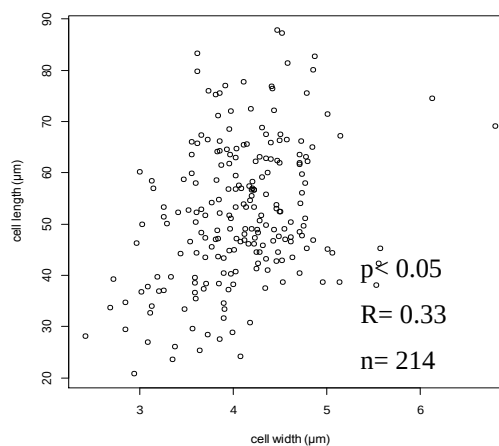


Fig. 16. Correlation between cell length and cell width

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 18. a-b), we are lack of this identification criterion in Lake Geneva.

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

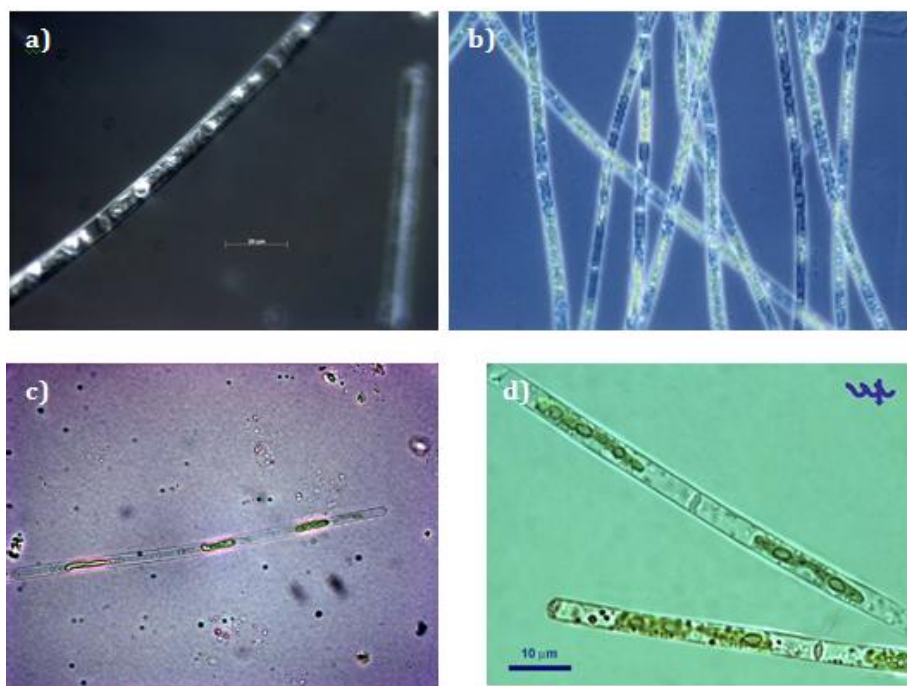


Fig. 17. Photos of *Mougeotia* sp. filament from Lake Geneva (a), Lake Garda (b), Lake Maggiore (c), Lake Kinneret (d)

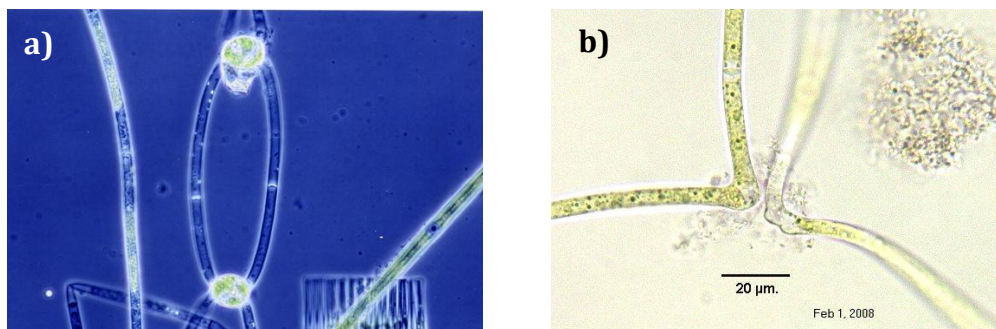


Fig. 18. Conjugation and zygospore forming of *Mougeotia* sp. from a) Lake Garda and b) Lake Kinneret

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

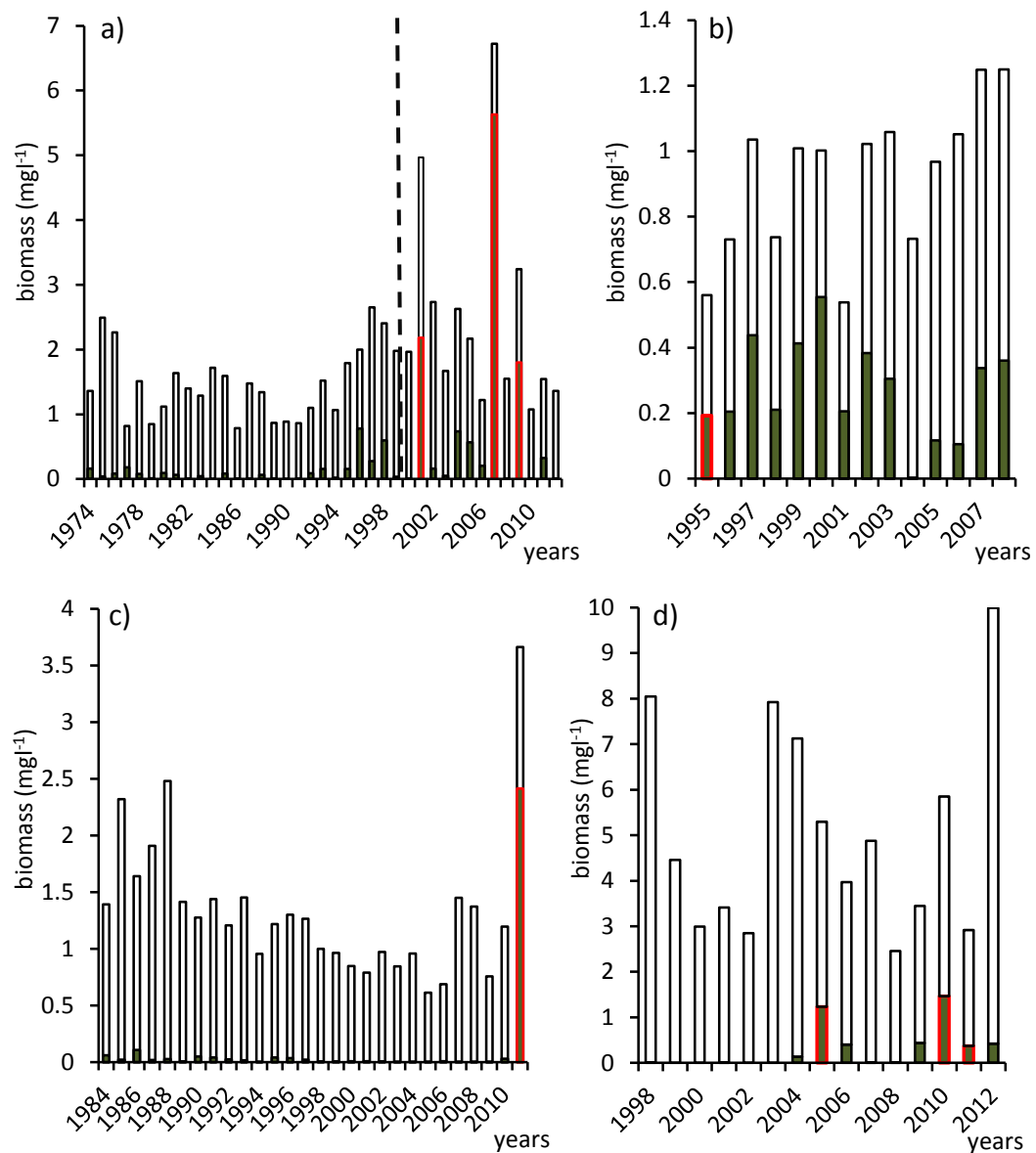


Fig. 18. Annual mean of total phytoplankton (empty bars) and *Mougeotia* sp. (green bars) biomass in Lake Geneva (a), Lake Garda (b), Lake Maggiore (c) and Lake Kinneret (d). Bars with red border indicate years when *Mougeotia* sp. had a contribution >80% to total biomass during the year. Dashed line indicates the change in sampling method from 2000 in Lake Geneva.

Figure 18 shows the interannual changes in total phytoplankton and *Mougeotia* sp. biomass. In Lake Geneva, *Mougeotia* sp. was present in each year but its abundance 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 was more constant; both the total and *Mougeotia* sp. annual mean biomass varied 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 abundance 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 0.4 mg l⁻¹ and 1.5 µg 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 19. 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 and autumn. From 1974 to 1982 it was observed every year and its abundances 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 (14-15 mg l⁻¹), late August, 2007 (39 mg l⁻¹) and late September - early October, 2009 (12-13.5 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 0.1 and 0.5 mg l⁻¹. 2011 was highly affected by the species, reaching high abundance in the summer; its biomass value in July was 20 mg l⁻¹.

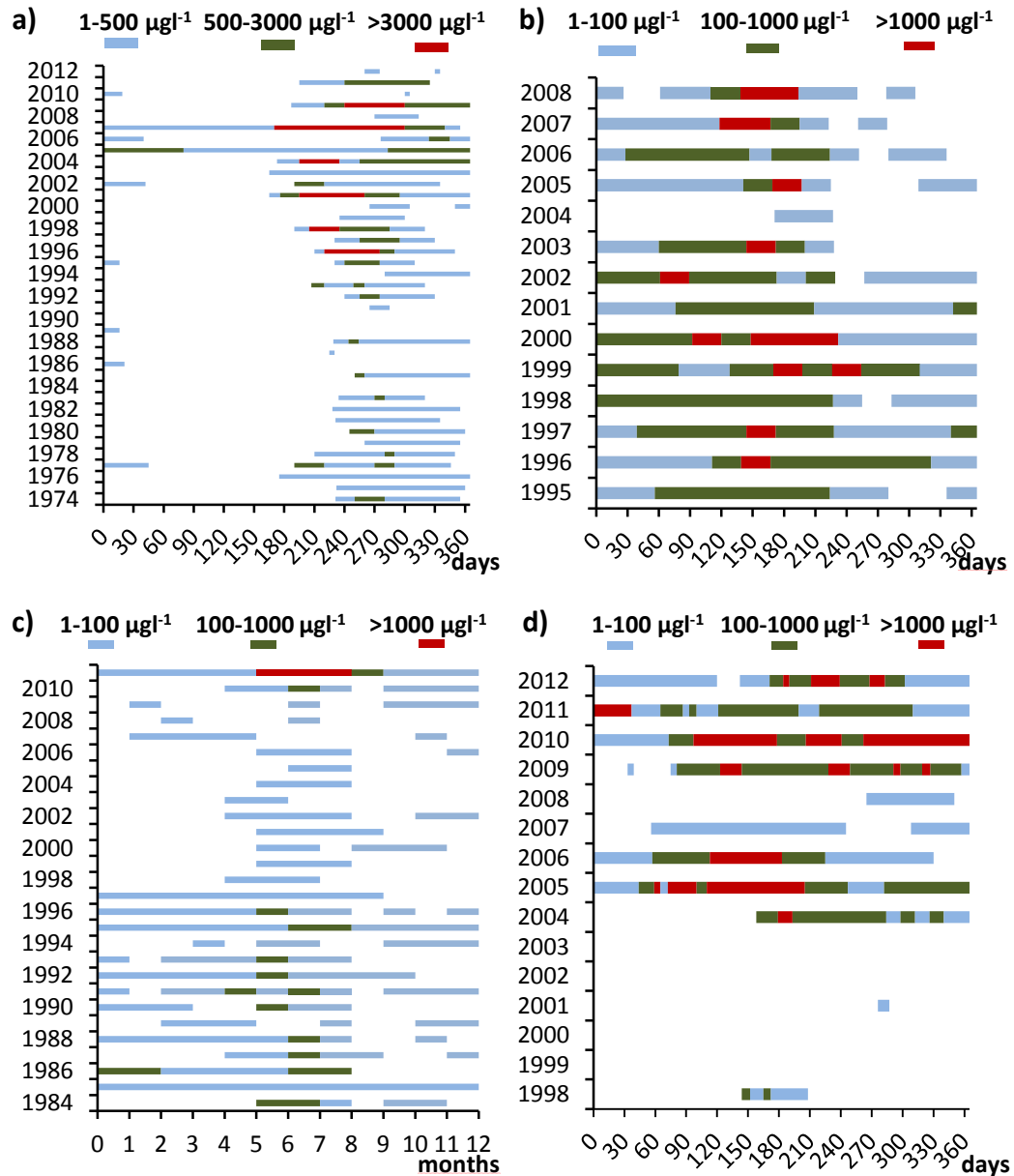


Fig. 19. Long-term change in the seasonal pattern of *Mougeotia* sp. biomass – a) Lake Geneva, b) Lake Garda, c) Lake Maggiore, d) Lake Kinneret

In Lake Kinneret, *Mougeotia* sp. became high from 2004. It commonly appears in early spring, sometimes in February and remained 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 abundance during the whole year. The maximum abundance (6.2 mg l⁻¹) was observed in the end of December, than dropped to 2 mg l⁻¹ by February, 2011.

3.4 Environmental optima of *Mougeotia* sp.

Figure 20 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 µgl⁻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.

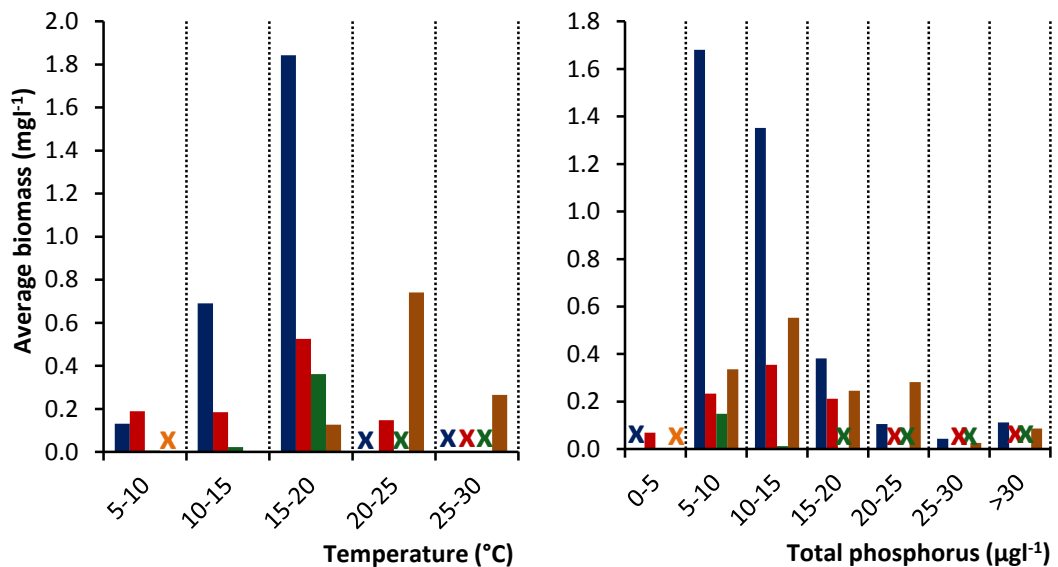


Fig. 20. Temperature and total phosphorus optima of *Mougeotia* sp. in the studied lakes; Lake Geneva (blue), Lake Garda (red), Lake Maggiore (green), Lake Kinneret (brown)

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

We studied which environmental factors can discriminate between 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 21, 22, 23, 24. 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 (RTR), as an indicator of thermal stability of the water column, and euphotic zone seemed 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 RTR 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* abundance.

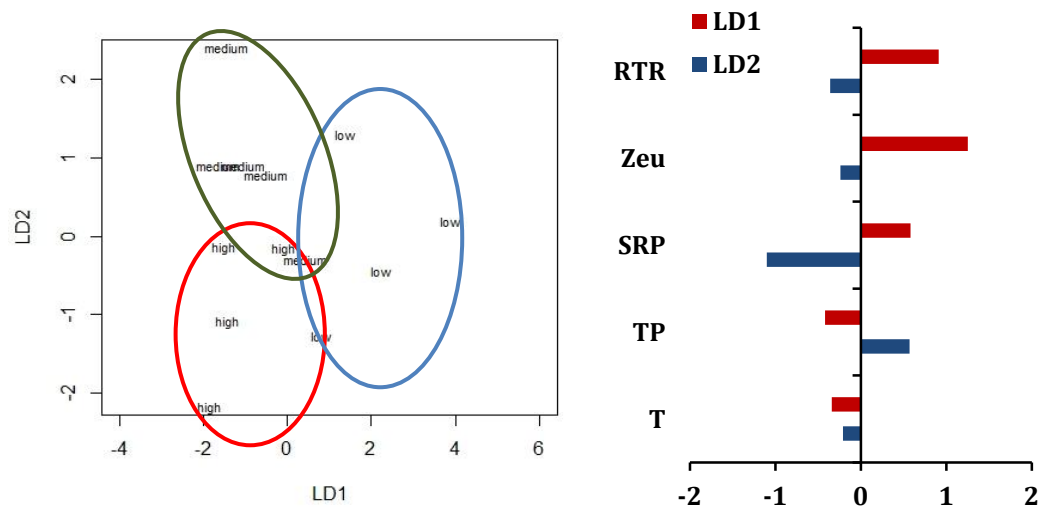


Fig 21. LDA scatter plot (left) and linear discriminant coefficients of variables (right) in Lake Geneva

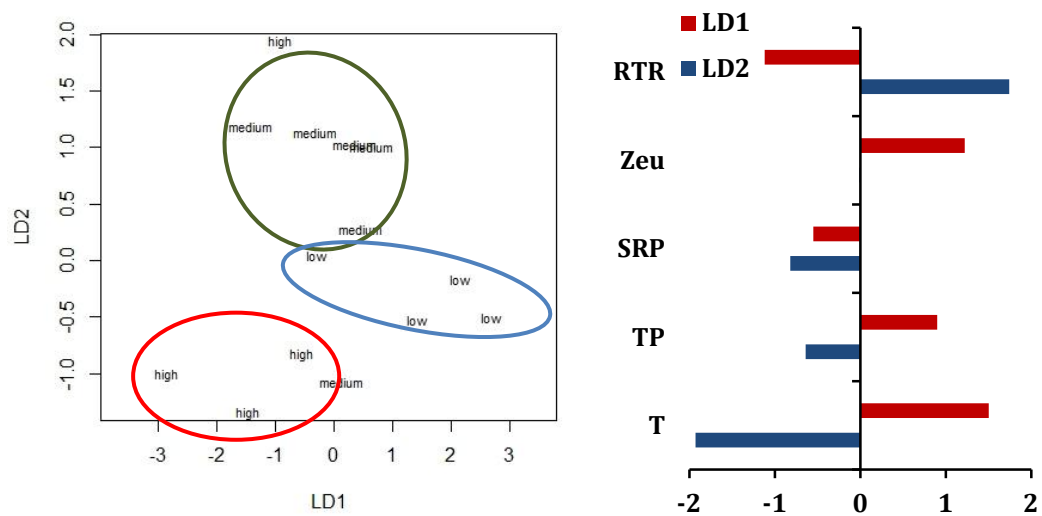


Fig. 22. LDA scatter plot (left) and linear discriminant coefficients of variables (right) in Lake Garda

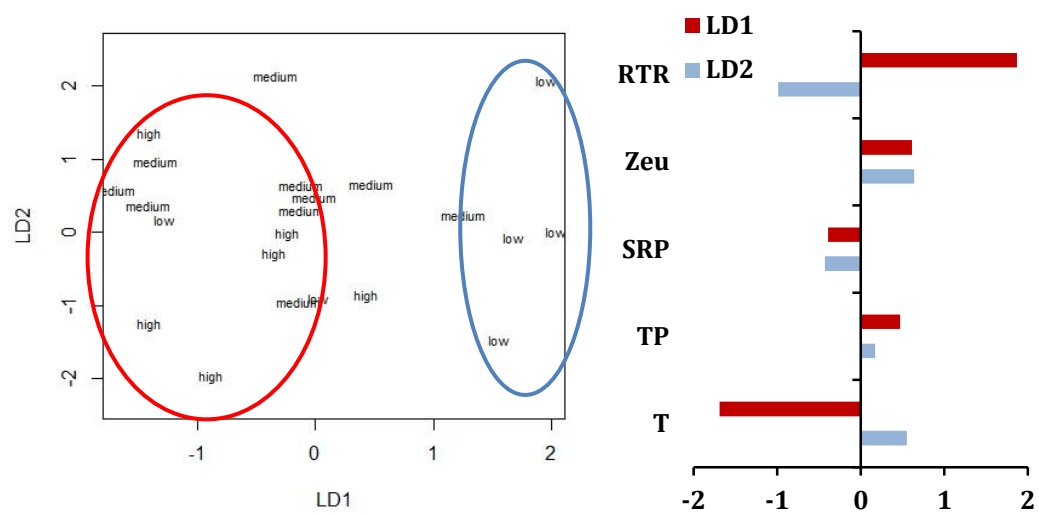


Fig. 23. LDA scatter plot (left) and linear discriminant coefficients of variables (right) in Lake Maggiore

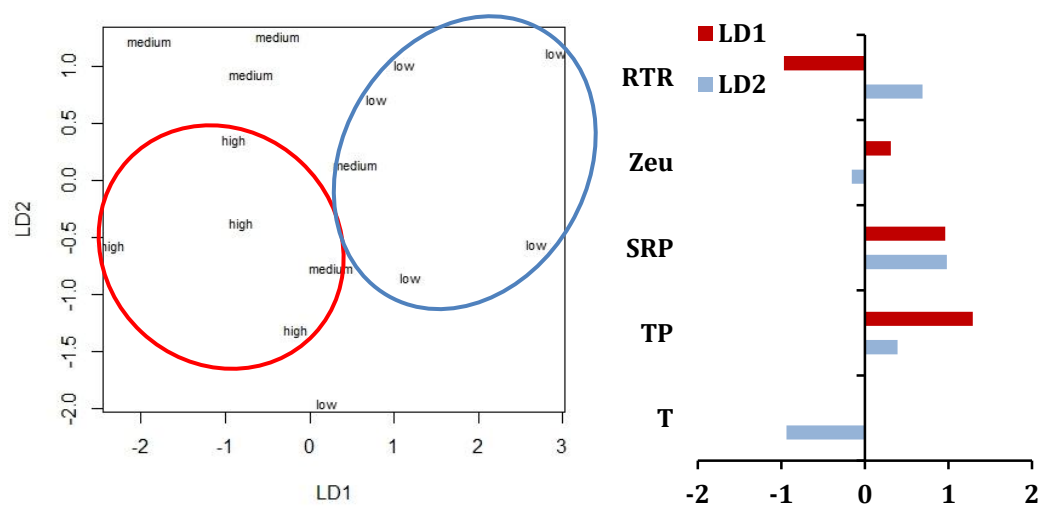


Fig. 24. LDA scatter plot (left) and linear discriminant coefficients of variables (right) in Lake Kinneret

3.6 Vertical distribution of *Mougeotia* and environmental variables

Figure 25 shows the vertical concentrations of total chlorophyll-*a* during three periods in Lake Geneva when *Mougeotia* was present with extremely high abundance. 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 26 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 27) *Mougeotia* sp. did not show vertical heterogeneity in its abundance during the stratified periods, it was homogeneously distributed in the epilimnion.

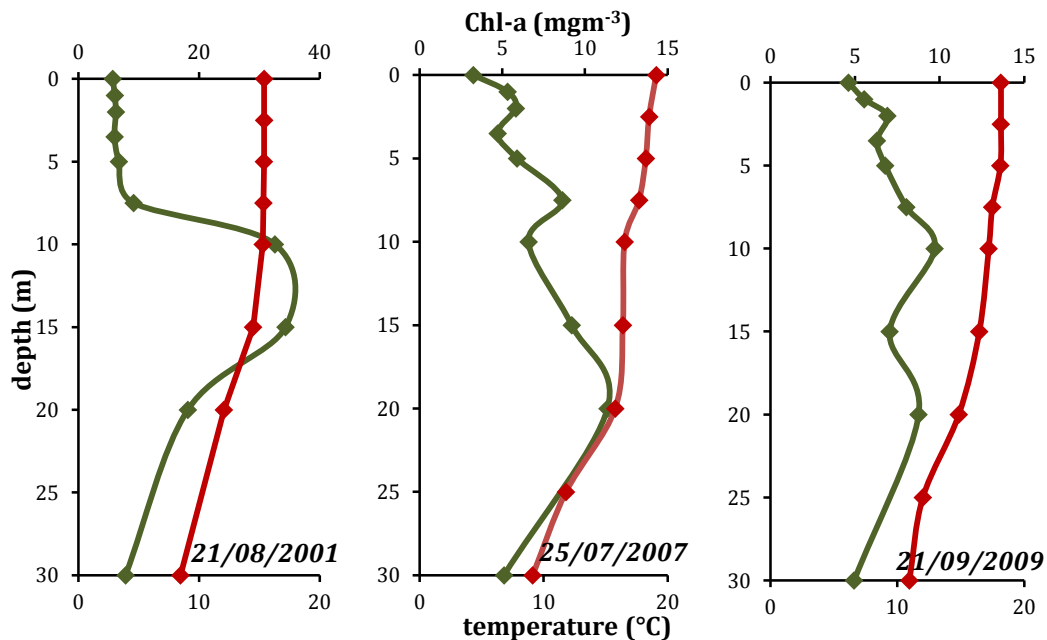


Fig. 25. Vertical profiles of chlorophyll-*a* and temperature at three dates when *Mougeotia* sp. had high contribution to total biomass in Lake Geneva; 2001.08.21 (85%), 2007.07.25 (99%), 2009.09.21 (87%)

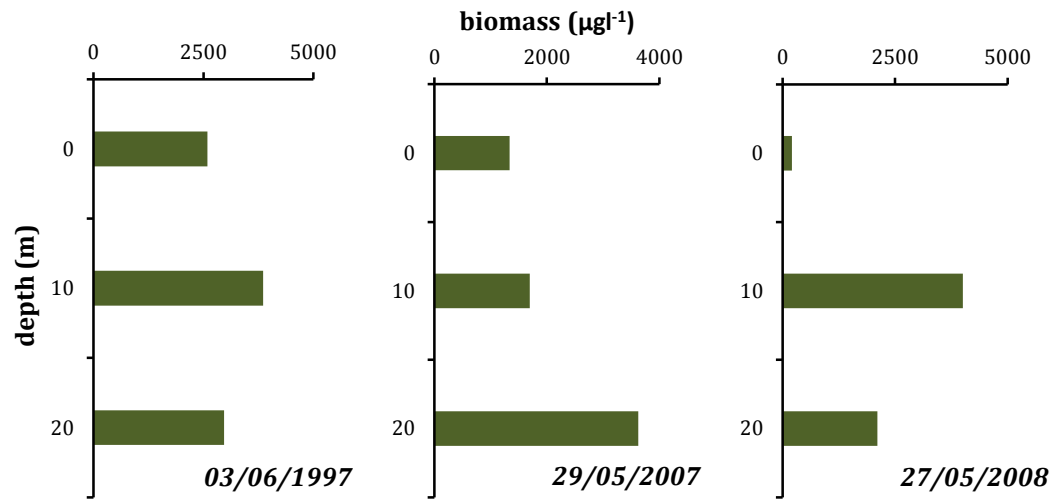


Fig. 26. *Mougeotia* biomass along the vertical profile in three depths (0 m, 10 m, 20m) in Lake Garda

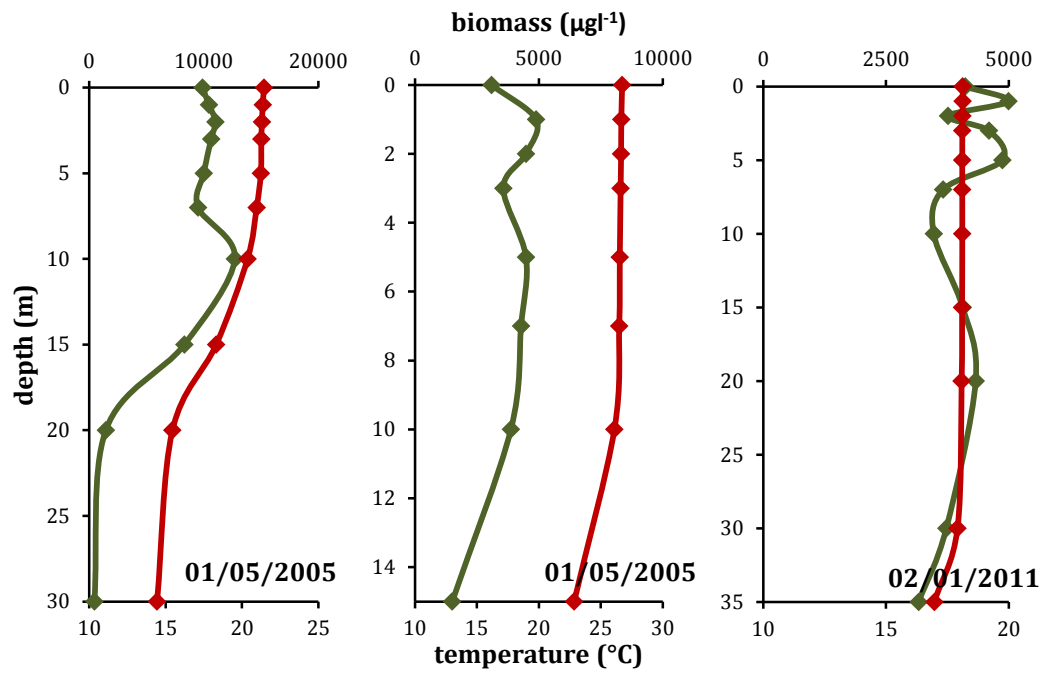


Fig. 27. Vertical distribution of *Mougeotia* biomass (green) and temperature (red) in Lake Kinneret during periods with high *Mougeotia* abundance. Note the different scale on the vertical axis.

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* abundance can be separated by the values of one variable but in different depths. Figure 28 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 29). 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.

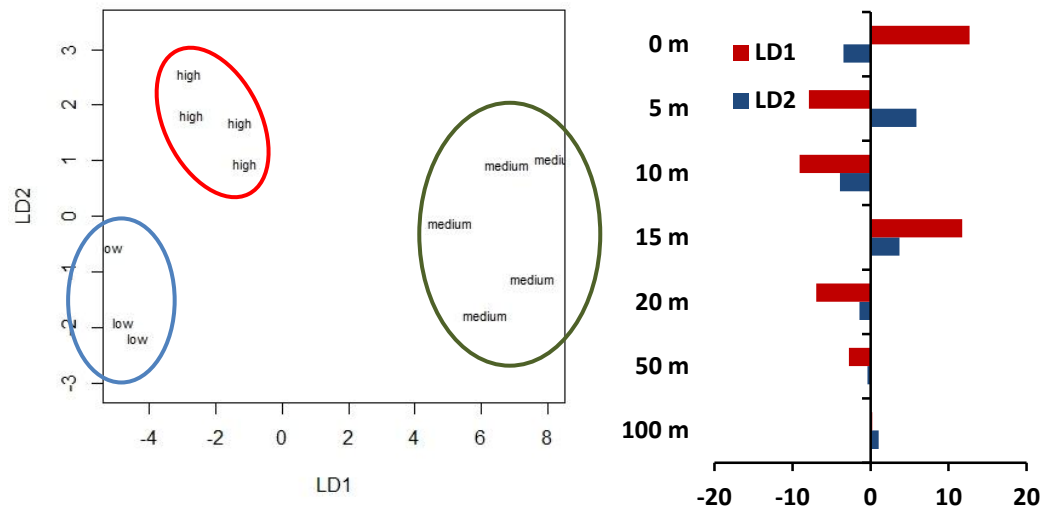


Fig. 28. LDA scatter plot (left) and linear discriminant coefficients of variables (right) in Lake Geneva. Variables are temperature values at different depths.

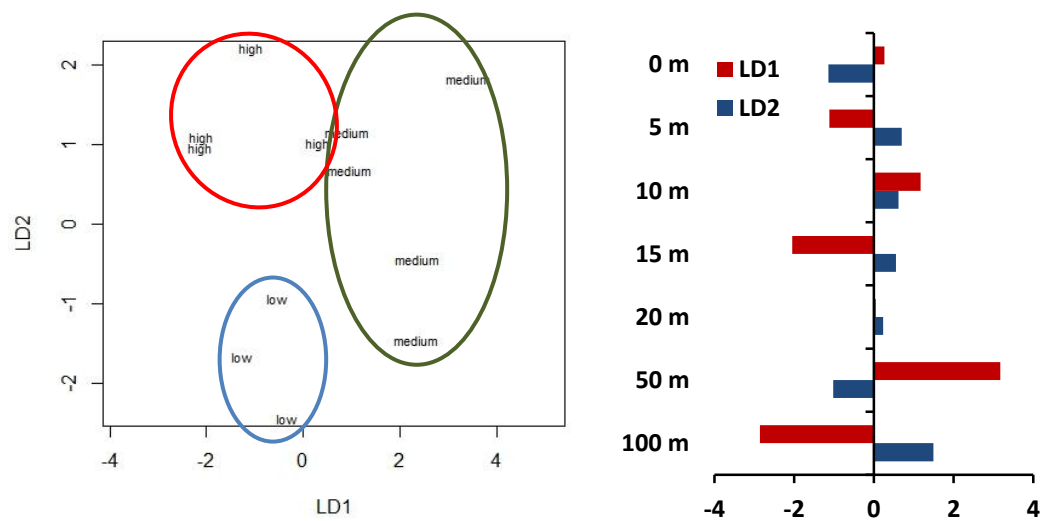


Fig. 29. LDA scatter plot (left) and linear discriminant coefficients of variables (right) in Lake Geneva. Variables are total phosphorus values at different depths.

4. Discussion

4.1 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 diversity, one of the first questions I wanted to address was to know if different species were present in the different lakes, or if different species could have occurred 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 identification 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.

4.2 Does these different *Mougeotia* species have equivalent ecologies?

These uncertainties in the accurate identification of *Mougeotia* species make the comparison difficult 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 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 a robust biomonitoring cannot be based on species level identification, instead, higher taxonomic levels or operative taxonomic units should be used, 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. (1996) 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 on 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. It is notable that in few years in which *Mougeotia* sp. was present with relatively high abundance 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. abundance 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* favours 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 abundance 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.

4.3 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* abundance. 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 abundance.

Important to know that although there are filamentous algal species (mainly among Cyanobacteria) that are adapted to the stratified summer period of deep lakes, these are physiologically different from *Mougeotia*. 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 20ms depth. 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 be 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.

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