D30: Primary Elements in Food Chain

Work package 34:	**Primary elements in foodchain**
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1 EXECUTIVE SUMMARY

The purpose of the work package was to examine the usefulness of primary elements of food chains (PEFC) for the measurement and monitoring of lake trophic status due to the original way they participate to the flow of energy and matter in pelagic areas of lakes.
After a presentation of the objectives, the report gives a brief description of the way the study has been conducted based on existing data from three lakes (Lake Geneva, Bourget Lake and Lake Constance) and on data especially achieved during the Eurolakes project (Bourget Lake, Loch Lomond).

The first part of the report (Part I) gives a definition of PEFC, which refers to all organisms that constitute lower parts of food chains from pelagic area of lakes. PEFC feature several original characteristics that make them good potential candidates as indicators of lake trophic status. PEFC represent a wide diversity of metabolisms and feeding modes and they are able to use a large diversity of compounds as source of energy and carbon (both mineral and organic nutrients). One important characteristic of PEFC is their high resilience, which means that they will quickly recover from a disturbance.
The next chapter of the report (part II) presents results from the comparison between the three studied lakes: Bourget Lake, Lake Geneva and Lake Constance, for which quite long datasets concerning PEFC have been achieved before the Eurolakes project. Because of the lack of data on PEFC on Loch Lomond, four campaigns have been conducted especially for the Eurolakes project on Loch Lomond and at the same time, on Bourget Lake. Results show that some PEFC can be well compared to non-PEFC parameters in their ability to contribute to lakes discrimination then leading to the conclusion of a true relevance of PEFC as indicators of lake trophic status.

In the two last parts of the report, the relevance of PEFC as indicators of lake quality and functioning is discussed. Lake monitoring based on PEFC presents definitive advantages by comparison to the one based on higher level of food chains. Because of their small size and high concentration, PEFC make it easy both samplings and measurements. Changes in lake trophic status could of course be assessed more accurately when it is possible to get deeper inside the analysis of both, composition and activities of biocoenosis. The improvement of techniques, especially those based on molecular biology for taxonomic determination, will bring this degree of expertise within the reach of people in charge of lake monitoring probably soon. In addition, a better understanding of PEFC dynamic and more generally of relationships between food chains functioning and lake trophic status could be gained from long term datasets. A large part of PEFC members (phytoplankton) is already included in all large lakes monitoring. However they still need to be improved to better take into account the smallest members of pigmented organisms (picoplankton).

We are convinced that there will be an increasing need to improve lake monitoring in adding regular measurements of organisms involved in the organic pathway of trophic food webs, which, for a large part, belong to PEFC.

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2 ABBREVIATIONS

ABB	active bacterial biomass
Bo	Bourget Lake
BP	bacterial production
Chloro	chlorophyll a
Co	Lake Constance (Bodensee)
CV	Coefficient of variation
Le1	Lake Geneva, 1998-1999 period
Le2	Lake Geneva, 2000-2001 period
NB	the northern basin
NO_3	nitrates
NS period	non-stratification period
OCDE	OECD organisation for economic co-operation and development
PEFC	primary elements of food chains
Phyto	phytoplankton biomass
PO_4	phosphate
PP	primary production
SB	the southern basin
S period	stratification period
TBB	total bacterial biomass
Temp	temperature

3 OBJECTIVES AND INPUT TO WORK PACKAGE

The purpose of the work package was to examine the usefulness of primary elements of food chains (PEFC) for the measurement and survey of lake trophic status due to the original way they participate to the flow of energy and matter in pelagic areas of lakes.
Indeed PEFC feature several original characteristics that make them good potential candidates as indicators of lake trophic status:

- their place within the food webs in between nutrients (bottom-up control) and zooplankton (top-down control)

- their ability to use (separately for most microbes) both mineral and organic nutrients

- due to their relation to health disorders some microbes are themselves criteria for water quality

- due to the abundance of microbes, measurements of most of their features are quite easy and cheap.

The study was intended to investigate especially the balance between top-down and bottom-up control of PEFC and to discuss its modulation by hydrodynamics (circulation, stratification and turbulence), by suspended particles concentration, by inflow-throughflow and by meteorology.

None of the other WPs of the project was supposed to be a particular input for this work package. However because the importance of physical parameters as driving forces for microbial populations, the discussion and understanding of the results will gain from conclusions of almost all of the key processes' WPs.

Results from WP34 together with those from WP31 (Microbial biodiversity) are complementary. Both will be used as inputs for WP35 (Substantiated Ecological Targets) and WP36 (Ecological criteria).

4 DESCRIPTION OF WORK

1- from a screening of literature, synthesis of knowledge on contribution of primary elements in food chain to lakes general functioning and productivity. Such human induced perturbations that lead to changes in hydrodynamic, water circulation, sedimentation and stratification will be considered in their effects on the balance between top-down and bottom-up control of primary elements in the food chain. Other effects of man-induced perturbation will also be considered.

2- from existing data on at least three lakes (Lake Geneva, Bourget Lake and Lake Constance) out of the four studied lakes in the Eurolakes project, an analysis will be made of the way their differences establish from primary elements in food chains (biomass and activity).

3- a similar work will be done from data especially achieved for the Eurolakes project on two of the lakes included in the project (Bourget Lake, Loch Lomond).

4- discussion on the way PEFC could be introduced in mathematical models and in lake survey protocols and recommendations.

Two partners only were involved in this work package:

CAR was in charge to collect data from literature about bottom-up (primary production and bacterial production) and top-down control (grazing) in lakes to assess the importance of primary elements in food chain as sink or link in food webs.
It was also in charge to collect and compare existing data on PEFC from the four Eurolakes lakes.
Experiences have also been conducted by CAR on Loch Lomond to offset the lack of data on bacterial production on this lake. Similar measurements have also been made on Bourget Lake during the same periods so as to make possible a comparison of the two systems.

ISF provided information on primary food chain from the Bodensee and contributed to the analysis and understanding of the results.

5 PART I. DEFINITION OF PRIMARY ELEMENTS IN FOOD CHAINS

5.1 COMPONENTS

In this study, "primary elements". It means that both, prokaryotic and eukaryotic as well as pigmented and non-pigmented organisms will be considered. All these organisms can be qualified as members of plankton because they could not efficiently fight against water displacements, even if some of the larger crustaceans (Leptodora, Bythotrephes) are able to swim over significant distances. However several components of the plankton are able to perform vertical migrations based on their ability to change their buoyancy.

In this study it will not be especially discussed about organisms that are attached to suspended particles. They will be considered as members of plankton all together with free suspended organisms. Most planktonic organisms are single cells while the other ones are colony-forming organisms. This remark is important with regard to their overall size and their ability to be grazed by higher trophic levels, a process of interest for both, regulation of populations and transfer of matter and energy to the top of the food webs.

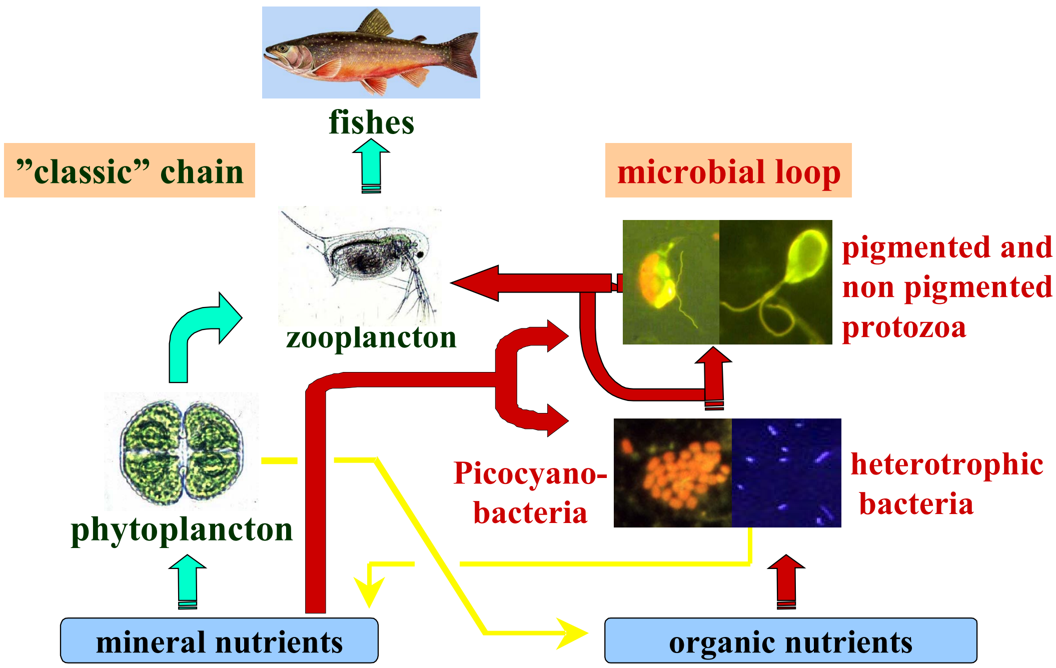


Fig. 5-1: Main components of trophic food webs in lakes

In this study, we will not refer to those members of planktonic populations that are badly known in freshwaters such as virus or which play a minor role in aquatic systems because of their particular metabolic requirements such as fungi. We will not mention either benthic communities despite their obvious participation to trophic food webs. Studies on this part of biocoenosis are still too rare.

As for the pelagic area, carbon flows basically proceed along a mineral pathway also called "classical chain" (Fenchel 1988) starting from mineral carbon (CO_2 and bicarbonates) and an organic pathway (Fig. 5-1) based on organic compounds either dissolved or particulate and originating either from the lake itself (autochthonous organic matter) or from its watershed (allochthonous organic matter). The organic pathway, which involved bacteria, flagellates and ciliates, is called microbial loop (Pomeroy 1974), (Azam *et al.* 1983).

5.1.1 Pigmented organisms

Pigmented organisms belong to the two super kingdoms, prokaryota and eukaryota.

Prokaryotic cells could be divided into two groups according the source of proton used to reduce mineral carbon into organic carbon:

- anaerobic pigmented bacteria (Chlorobacteriacea, Thiorhodacea) which photosynthesis is based on sulphide. They are bacteriochlorophyll containing bacteria and of almost no importance in large lakes because of their simultaneous requirement in both, hydrogen sulphide (generally produced from sediment rich in organic matter then close the bottom of lakes) and light (only available close to the surface of the water).

- cyanobacteria featuring mainly large filamentous forms and picocyanobacteria. Their photosynthesis is based on the water itself

Eukaryotic cells are represented by several divisions and classes: Chlorophyta, Bacillariophycaea (diatoms), Chrysophyceae, Dinophyta Cryptophyta, Xanthophyceae, Euglenophyta, Phaeophyceae, Rhodophyta. They all practice a water-based photosynthesis.

Biomass production of most pigmented organisms is based on photosynthesis associated to a consumption of CO_2 and other minerals, mainly PO_4 and NO_3. Mixotrophs are pigmented organisms that are also able to produce biomass by photosynthesis but that could also switch to heterotrophic production when conditions of their environment are changing (Bennett *et al.* 1990, Olrik 1998, Hitchman & Jones 2000).

5.1.2 Non-pigmented organisms

Here again organisms belong to the two super kingdoms of prokaryota and eukaryota. Prokaryota are represented by heterotrophic bacteria. Eukaryota divide into unicellular organisms (Protists: amoeba, flagellates, ciliates), fungi and metazoans (crustaceans: copepods, cladocera and rotifera). Biomass production of non-pigmented organisms is based on organic matter as carbon source.

Little information is available on the importance in lakes of those non-pigmented organisms that use chemosynthesis for their energy requirement and producing biomass from reduction of C-CO_2.

5.1.3 Metabolic activities and role of PEFC in biogeochemical processes and lake functioning

Lake quality and trophic state are directly related to biochemical processes. In a lake matching good quality no component, either chemical or biological, should be present in excess. This means that biogeochemical processes should be adjusted so as optimum recycling rates occur for both chemicals and organisms.
Bacteria, as member of PEFC, participate in two ways to these processes, firstly as mineralisers (converting organic matter to minerals) and secondly as preys for several organisms (flagellates, ciliates, some crustaceans) then linking together lower and higher levels of food chains. Other members of PEFC also participate to these processes as mineralisers (producing CO_2 and other minerals through their excretions) and as biomass producers, themselves representing or not, a link to the next trophic level.
Another criteria of good health for a lake is that, to some extent, its biocoenosis could resist disturbances (high resistance) and are able to recover quickly their original state (high resilience).

When considered members of PEFC as a whole, they appear to share several properties of importance with regard to their participation to biogeochemical processes and lake healthiness:

- their average production rate is higher than the one of upper trophic levels allowing for a faster recovery from a disturbance or for a faster reaction to change in nutrient supply, for example,

- they represent a wide diversity of metabolisms and feeding modes then addressing a large diversity of compounds as source of energy and carbon,

- they accommodate a large variety of environmental conditions as for the oxygen concentration or light conditions for example.

Property 1 gives PEFC a high resilience (Richardson 1980) which means that they will quickly recover from a disturbance or quickly react to an additional nutrient supply then reducing the delay a part of the corresponding amount of energy will be available for the upper levels of trophic webs. This property also means that PEFC status (concentration, metabolic activity) establishes according to the short term status of environmental conditions from a couple of days to more than one month depending on their specific reproduction rates and the season.

Property 2 can be translated in term of efficiency of PEFC in their ability to use as many as possible available sources of compounds and energy from the system. In particular, by converting dissolved organic matter (DOC) to POC, they make available to the upper levels of trophic webs amounts of quickly which otherwise will not be used.

Property 3 is also a part of the efficiency of PEFC by widening the range of environmental conditions that still allow nutriments uptake.

However these properties vary largely according to the member of PEFC, meaning that either a global or a detailed picture of the trophic status of a lake can be achieved from their analysis depending the member of PEFC considered and the aggregation level (as functional groups, for example) used in their assessment. As an example of clustering it can be distinguished between primary producers (pigmented organisms), decomposers (mainly bacteria) and predators from different levels (herbivorous, 1st order carnivorous, 2nd order carnivorous, …). A trophic pyramid can then be built where these different groups are represented either as number of individual per ml or biomass. The trophic status of the whole PEFC could then be inferred from the shape of the pyramid (steep/flat, balanced/unbalanced degrees).

Problems here are that the trophic status of members of PEFC are unequally known and that assessment of specific levels of the pyramids is neither easy nor accurate.

Then it is often more easy to consider PEFC in a more specific way as for example addressing mixotrophs. Probably because their ability to use either mineral or organic source of carbon, their proportion within the whole planktonic communities seems to increase together with the nutrient impoverishment of the system (Christaki *et al.* 1999, Dolan & Perez 2000). Then, alone, they could be used as indicators of the trophic degree of a lake.

Another example of "intermediate" degree of aggregation is given by the relative number of flagellates by comparison to bacterial concentration. It has been stated (Jugnia *et al.* 2000) that a minimal proportion of 1000 bacteria per heterotrophic flagellate characterises a microbial food web where flagellates consume preferentially bacteria than algae. It could then be concluded that the organic pathway is from a particular importance for the lake functioning. The ratio primary production / heterotrophic production, even based on some only of the pigmented (phytoplankton) and non-pigmented organisms (bacteria), could also be an indicator of an unusual imbalance between the two processes (Conan *et al.* 1999) and hence of some quantitative or qualitative changes in the nutrient supply of a lake (Tranvik 1989).

Another information on food web's functioning and hence on lake trophic status, is given by the abundance of small cells that is increasingly high either when nutrient supply is shortening (because of their high surface / volume ratio) or when pressure of predation is growing.

Of course, the taxonomic assessment of PEFC would give the most accurate picture of a lake trophic status by comparison to these aggregating approaches. However it is still needing too much expertise to be introduced easily in lake monitoring.

In summary, the way PEFC can be used as indicators of lake trophic status depends on the degree of aggregation that is made in their assessment, a high degree of aggregation (considering planktonic biomass as a whole, for example) being less informing for the understanding of the way the lake status establish.
By comparison to higher levels of food chains, the specificity of PEFC as indicators rely on several points:

1. their direct link with nutrient supply which allow a detailed picture of variations over time of lake trophic status

2. their high resilience (elasticity) which makes PEFC status representing environmental conditions over quite short periods (a fortnight on an average). In particular, their quick recovery from disturbances represents a real advantage compared with higher levels of food chains as they will be able to testimony the return to normal conditions soon after the end of disturbances.

3. their diversity in types of metabolism and in the way they accommodate a large variety of environmental conditions which give a large choice for the use of a member of PEFC to investigate details of lake functioning (or dysfunctioning) or specific parts of a lake.

5.1.4 Regulation processes: ”bottom-up” & “top-down” controls of PEFC

Bottom-up controls are all nutrients related processes from which biological production is directed. Top-down controls are processes like predation and fishing from which a part of the biological production of one level of the trophic chains either is moved up to the upper trophic level or is removed from the lake overall biomass. From the balance between these two types of controls depends the accumulation of biomass at one specific level (Billen *et al.* 1990, Psenner & Sommaruga 1992, Pace & Cole 1994).

Bottom-up control of PEFC varies according to the level considered along the food webs. Production rate of pigmented organisms is driven by concentrations of minerals like phosphates, nitrates and silica while concentration of the easily degradable part of dissolved organic matter drives the bacterial heterotrophic production. Moving up to higher levels of the PEFC webs, the bottom-up control will be the availability of preys such as bacteria for non-pigmented flagellates. Through the use of fertilisers and fishing actions, human activities may participate to both ends of the control processes. The functioning of a lake will be satisfactory when both, ”bottom-up” and “top-down” processes stay in control of each level of food webs.

5.2 DYSFUNCTIONINGS

Dysfunctioning of PEFC food webs may originate on one hand, from nutrient supply either too high or too low and on another end, from toxics. In rivers, dysfunctioning of food webs may also originate from physical processes like the frequency of discharge variations or marked change in temperature at the outlet of a nuclear power plant cooling tower. We do not know any related example of such a disturbance in the case of large lakes.

5.2.1 Nutrients

In nature the rule for all organisms is to be limited by food. In such systems where energy is rare (oligotrophic systems) it is observed that life develops number of strategies trying to collect all types of energy leading to a high biological diversity. For a part, biological diversity establishes in relation to food diversity (Fisher *et al.* 2000). When a high quantity of one type (or of a limited number of types) of nutrient is introduced in a lake, these organisms that are the most able to use this type of nutrient will be promoted often at the expense of some of the neighbouring communities because the competition for shared rare compounds (like metals, enzymatic cofactors, vitamins, …), for oxygen, for light or merely for vital space. Biodiversity will then decrease, as the biomass of privileged organisms will increase. This increase may then lead to an inflated population either because it is too high for the grazing capacity of predators or because some traits of the privileged organisms (filamentous structure, colony forming organisms) make it inedible to be grazed.

Then, excess input of nutrient in aquatic systems may lead to both, a decrease of biodiversity and the overwhelming development of the biomass of organisms that are the most able to use the nutrients (Schimel 1998, Hulot *et al.* 2000). Such sudden developments (blooms) are commonly observed in meso to eutrophic lakes for cyanobacteria.

As PEFC communities are able to address almost all types of nutrients from at least one of its members, their biodiversity and biomass distribution over their members could be used to assess trophic dysfunctionings of lake food webs.

5.2.2 Toxics

All members of PEFC are not equally sensible either as for the concentrations (deadly thresholds) or as for the types of toxics. "Primitive" forms of PEFC are neither less nor more affected than the more evolved ones or even than non-PEFC species from the high levels of food chains (Madoni *et al.* 1996). In that matter there is no general rule.
Both diversity and biomass are generally affected by toxics. However because their selective effects, and by lowering competition and regulating processes (predators) the effect of some toxics may also result in biomass imbalance and populations dominated by some species (Nsabimana *et al.* 1996, Foekema *et al.* 1998, Kusel *et al.* 2001).

PEFC do not keep memory of past toxic pollution because they do not have time to significantly accumulate in organisms having short generation time. Nevertheless analysis of microbial communities has been proposed to assess sediment restoration after an oil spill (Haines *et al.* 2002).

In summary, the way PEFC can be used to monitor toxic pollution as case sensitive, depending from a lot of parameters among them: the type of toxic, its concentration, environmental conditions (as for example the buffered capacity of the water), the way it is introduced into the system (duration, frequency). The dependency on life stage (instars) and on physiological status of the exposed organisms is not relevant for PEFC.

5.2.3 Unwanted species

Four main types of organisms could be qualified as unwanted species:

- Organisms having a high growth rate and/or that escape regulation processes because their shape or structure (filamentous or colony forming species). These organisms will behave as invaders. They often burst in blooms like cyanobacteria.
- Species that are poisonous against other members of planktonic populations. They also often behave as invaders. Cyanobacteria are good examples of such organisms as some species contain toxins that could be harmful for human health (Vasconcelos *et al.* 1996).
- Parasites
- Human health related organisms

Even if they are generally not easy to understand, changes in proportions of unwanted species can be considered as indicators of changes in lake food chains and therefore in lake overall quality. Mass developments (blooms) of cyanobacteria are well known in eutrophic systems (Christoffersen *et al.* 1990). However they could also be observed in mesotrophic lakes during re-oligotrophisation processes (as in Bourget Lake for example). At the time surface layers are depleted in phosphorous and because their ability to catch low light intensity (from low wave length radiation), cyanobacteria are able to grow under several meters where phosphorous concentrations are still high enough. Their presence in high concentration should then be considered as a criterion of both, eutrophy and one intermediate trophic stage typical of lake recovery.

Occurrence of parasites can hardly be related to trophic status of lakes. The reasons why they occasionally develop in high number are related to several ecological parameters among them several other members of biocoenosis that all are submitted to their own regulating parameters. No simple and general explanation has been reported about variations in their abundance, which remain generally badly known. This is the case for example of the agent of the human cercarial dermatitis Trichobilharzia sp, a parasite of dukes, which was increasingly present in all Alpine lakes whatever their trophic status were during the recent years. Even though they cannot be related to trophic status of lakes, of course parasites' development can be considered as criteria for lakes water quality.

Same remarks apply to other human health related organisms like pathogenic bacteria which occurrence in high concentration cannot be related to trophic status of lakes. When observed in large lakes, such concentrations generally affect local areas close to river tributaries or inputs from sewage outfalls.

6 PART II. CASE STUDIES

Case studies come here in addition to conclusions of chapter 5 to bring more specific statements on possible interest of PEFC as indicators of lake trophic status. The analysis will be based on the specific cases of four of the Eurolakes lakes (Constance, Bourget, Geneva, Lomond) as examples of large deep European lakes, despite the slight differences of their trophic status from oligo-mesotrophic (Loch Lomond) to mesotrophic (Bourget Lake).

Two types of datasets will be examined, those already achieved before the Eurolakes project started ("old datasets") and the ones ("new datasets") issued from specific experiments that have been conducted during the Eurolakes project.

Only parts of old datasets will be used here, namely those that include as much as possible shared microbial parameters. Microbial parameters are unfortunately the ones that have been the most infrequently measured in all lakes. In that matter the most recent detailed study that has been performed on Bourget Lake was during years 1995-96 and years 1998-2001 on Lake Geneva. The period we choose for Lake Constance includes years 1996-97 [1]. The absence of simultaneous measurements is not very important here because the goal was to use different trophic situations to find out which among the parameters best demonstrate the differences between these situations.

The dataset of Lake Geneva has been split between period "Le1" (1998-1999) and period "Le2" (2000-2001) in order to compare lakes over periods of same length (2 years). Comparison between Le1 and Le2 was done to study to what extent short generation time parameters like PEFC, could demonstrate a within lake evolution of water quality over a four years period.

6.1 DATA SET STRUCTURE

6.1.1 Parameters

Here is the list of all parameters that are considered in this chapter according their availability in at least two datasets. Abbreviations of parameters are given in chapter 2.

<u>Abiotic parameters:</u>

- Secchi depth,
- O_2 concentration,
- NO_3
- PO_4

[1] Datasets provided by ISF

Biomass

- Total bacterial biomass was estimated by use of epifluorescence microscopy after DAPI staining (Porter & Feig 1980), except for Lake Constance where AODC method was used (Hobbie *et al.* 1977).
- Active bacterial biomass was estimated by use of epifluorescence microscopy, according (Rodriguez *et al.* 1992).
- Phytoplankton biomass
- Chlorophyll a

Metabolic activity

- Bacterial production: 3H-thymidine method (Fuhrman & Azam 1980, Fuhrman & Azam 1982) in Bourget Lake, in Lake Geneva and in Loch Lomond and 3H-Leucine method (Kirchman *et al.* 1985, Simon & Azam 1989) in Lake Constance
- Primary production according 14C method (Steemann Nielsen 1952, Steemann Nielsen 1977)

6.1.2 Time scale of PEFC growth rate & associated sampling strategies

Because of the high turnover rate of PEFC, a fortnight represents the maximum delay that should separate samples in order to get a representative picture of their variations. Of course this goal cannot be achieved in all cases because of the cost and the time consuming analysis that have to be performed. This is generally applied in large lakes monitoring during the spring ("growth period") and summer periods. During the other seasons lake monitoring is often based on a monthly sampling strategy, assuming smaller variations of most parameters.

6.1.3 Integrated versus local measurements

In lake monitoring, some parameters, like temperature, are measured at a very precise depth. Because the need to reduce costs, most parameters are measured on so called integrated samples prepared by mixing together different proportions of waters from different depths.

Another type of parameter, represented by the Secchi depth, intrinsically integrates depths.

6.1.4 Integration of values over time and depth

Due to seasonal variations of parameters and their heterogeneity over the water column, comparison of lakes could not be done from single values that would be supposed to represent lakes at the scale of the whole water column and the whole year. At these scales, only the range of variation of the parameters could be meaningful.
This is why, in order to get inside a more detailed analysis, comparison of lakes that are performed in the following chapters will take place within as homogeneous periods and space as possible. Then, in addition to values directly coming from analysis of integrated samples, integrated values have been computed over two periods (no-stratification, NS and stratification, S periods) and four depth intervals as given in Table 6-1.

Lakes	actual sampling depths	parameters	computed integrated values
Geneva	0, 2, 5, 7,5, 10, 15, 20, 30, 50, 100, 150, 200, 250, 275, 290, 300, 305, 309	Temp, PO_4, NO_3, ABB, TBB, BP	0-20 m, 20-50m, 50-309m
	euphotic zone	Chloro, PP	0-30m -
Bourget	2, 15, 30, 50, 80, 130, 140	Temp, PO_4, ABB, TBB, BP	0-20 m, 20-50m, 50-145m
	euphotic zone	Chloro, PP	0-30m -
Constance	0, 1, 2.5, 5, 7.5, 10, 15, 20, 30, 50, 100, 150, 200, 230, 250	Temp, PO_4, NO_3	0-20 m, 20-50m, 50-250m
	1, 3, 6, 10, 20, 50	TBB, BP	0-20 m, 20-50m
	euphotic zone	Chloro, PP	0-30m

Table 6-1: Correspondence between actual sampling depths and computed integrated values. Temp: temperature, ABB: active bacterial biomass, TBB: total bacterial biomass, BP: bacterial production, Chloro: Chlorophyll a, PP: primary production.

Sub-datasets has been defined to include as much as possible of the original datasets. The parameter used to separate the two periods is the following ratio:

$$R = \frac{\text{2.5 m depth temperature - bottom temperature}}{\text{bottom temperature}}$$

Two groups of sampling dates have then been defined within each year based on the K-means algorithm clustering method. K-means clustering method splits a set of observations into a selected numbers of groups by maximizing between-cluster distance relative to within-cluster distance.

6.2 COMPARISON OF LAKE GENEVA, BOURGET LAKE AND LAKE CONSTANCE

Based on data from the study periods and according to the OECD classification (OCDE 1982), trophic status of the three lakes has been established as follows: Lake Constance and Lake Geneva, both are mesotrophic whereas Bourget Lake is meso-eutrophic. As presented before, the dataset of Lake Geneva has been divided into two periods (Le1 and Le2) in order to perform lakes comparisons on the same basis of a 2 years time frame.

The overall trophic status of Lake Geneva seems to be unchanged between the two periods (Le1 / Le2). Indeed two out of the 5 parameters give a lower status to Le1 over Le2 as opposed to total phosphorus and maximum concentration of chlorophyll a, indicating that quality of water would have improved from Le1 to Le2.

Before using PEFC values, in the following paragraph, for the comparison of lakes, let us remind that two of them are related to biomass: bacterial biomass (TBB) and chlorophyll a (Chloro), an index for phytoplankton biomass. The two other PEFC parameters are related to biomass production: bacterial production (BP) and primary production (PP).

6.2.1 Lakes characteristics based on PEFC values

Study of PEFC based on their orders of magnitude give a general description of lakes. This study constitutes a first approach to highlight differences between lakes and to characterise each lake from PEFC parameters. This comparison is based on the sub-dataset of the 0-50m integrated values, because of the lack of measures for bacterial components in Lake Constance below 50m.

As for chlorophyll a, the highest difference appears between Bourget Lake (Bo) and the two other Lakes (Fig. 6-1): Lake Geneva (Le1, Le2) and Lake Constance (Co). Values are about five times higher in Bourget Lake than in the other lakes during the NS period and ten times during the S period.

During the NS period, chlorophyll a concentration in Le1 ($2.8x10^{-3}$ $mg.l^{-1}$) is close to the one in Lake Constance whereas during the S period, the concentration is twice higher in Lake Geneva ($4x10^{-3}$ $mg.l^{-1}$). The highest difference between Le1 and Le2 appears during the NS period with an average twice higher in Le2 than in Le1.

The most significant result is that Bourget Lake is characterised by high values of chlorophyll a concentration in comparison to the other lakes.

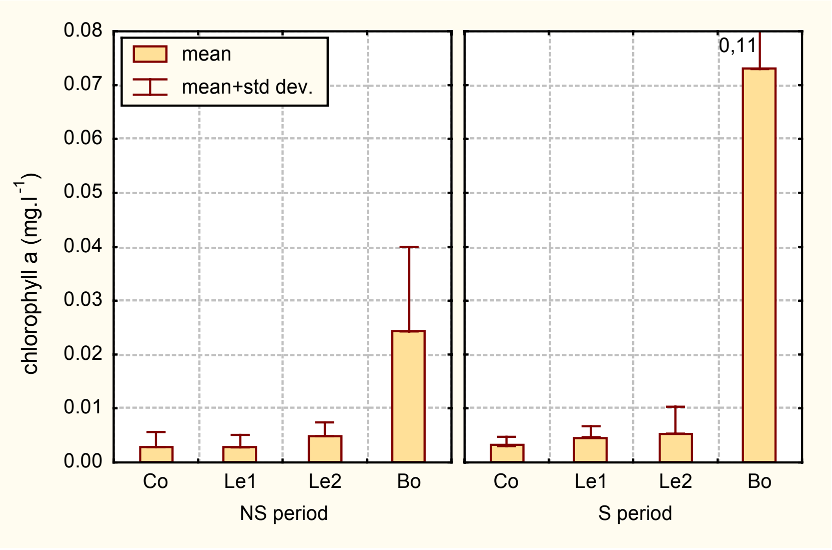


Fig. 6-1: Chlorophyll a concentration in each studied lake for the NS & S periods (std dev.: standard deviation).

As for primary production, the highest difference appears here again between Bourget Lake and the other lakes (Fig. 6-2). Primary production is on an average ten times lower in Bourget Lake (around 4×10^{-3} $mgC.l^{-1}.j^{-1}$) whatever the period than in Lake Constance or in Lake Geneva (Le1 & Le2). The comparison between Lake Geneva and Lake Constance shows that primary production is two times lower in Lake Constance than in Lake Geneva. Primary production tends to increase in Lake Geneva between Le1 and Le2.

The most significant result from primary production is that Bourget Lake is characterised by low values by comparison to the other lakes.

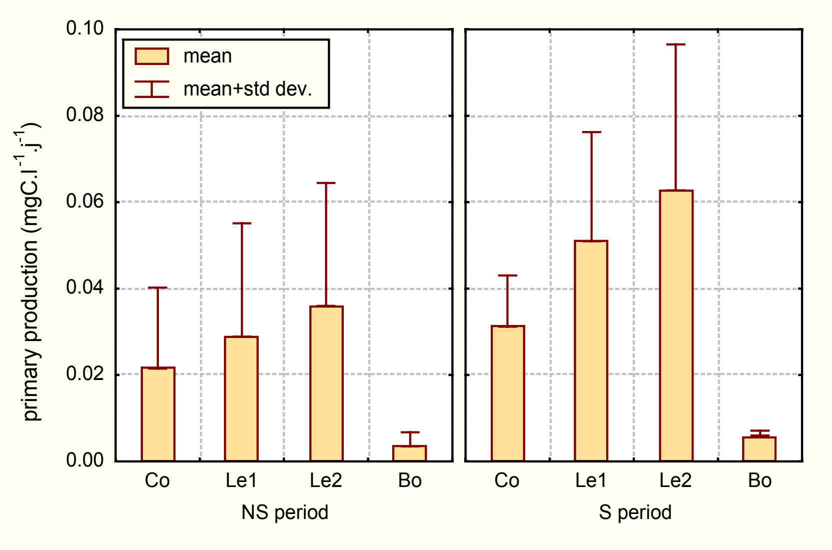


Fig. 6-2: Primary production in each studied lake for NS & S period (std dev.: standard deviation).

As for total bacterial biomass (TBB), values are lower in Lake Constance than in the other lakes, especially during the S period (Fig. 6-3). This result can be explained partly by the size of bacteria that is lower in Lake Constance. Simon (Simon 1987) reports an average biovolume of 0.054 µm^3 while our measurements give values of 0.092 µm^3 and 0.113 µm^3 respectively for Bourget Lake and Lake Geneva. The difference between the two periods (S and NS) is the highest in Lake Geneva Le1. During these two years bacterial biomass was low during the NS period (values similar to the ones from Lake Constance) and high during the S period (values higher than the ones from Bourget Lake and from Lake Geneva Le2).

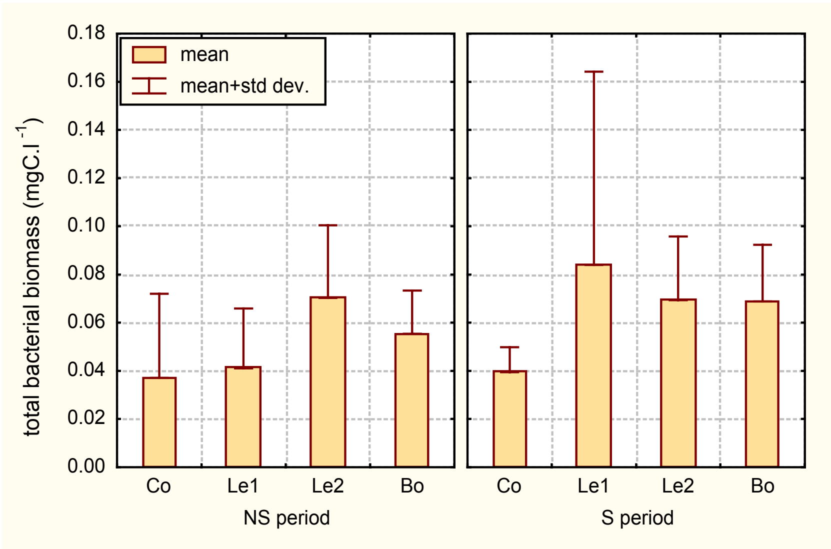


Fig. 6-3: Total bacterial biomass in each studied lake for NS & S period (std dev.: standard deviation).

As for bacterial production, the highest difference occurred between Lake Geneva Le1 and the other lakes. On average, values from Lake Le1 are respectively five times and ten times higher than the ones of Bourget Lake and Lake Constance. The most significant result is that Lake Geneva Le1 is characterised by high values of bacterial production, whatever the period, compared to the other lakes.

This first comparison based on orders of magnitude of PEFC parameters shows that significant differences occurred for Lake Geneva Le1 and Bourget Lake when compared to Lake Constance and Lake Geneva Le2. Parameters related to the mineral pathway, chlorophyll a and primary production are the ones, which display the highest between lakes differences. These differences established clearly only between Bourget Lake and the other lakes. Parameters related to the organic pathway, bacterial biomass and production, also displayed some differences between Lake Geneva Le1 and the other lakes.

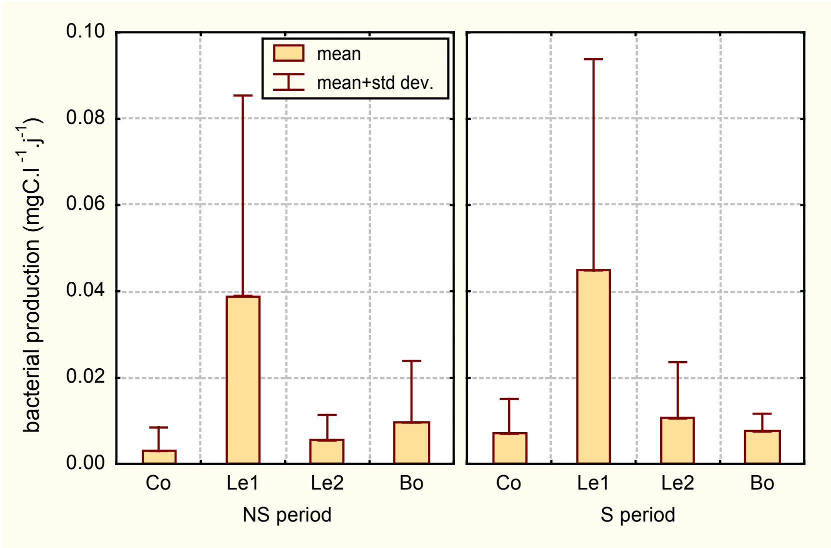


Fig. 6-4: Bacterial production in each studied lake for NS & S period (std dev.: standard deviation).

Comparison of lakes based on individual parameters is not easy to understand because of the high number of cross relationship that link them together. For example, values of biomass production are generally tightly related to the concentration of cells from which originate the production. Comparison of lakes can then be improved by considering parameters relationships (comparisons based on paired parameters) or by using standardised parameters (computing ratios between parameters, coefficients of variations…).

Two ratios (production over biomass) were computed from PEFC parameters for each pathway (mineral and organic): PB-TBB and PP-Chloro ratio. Comparison between lakes was performed on dataset, which include both NS and S period.

Figure 6-5 shows that parameters related to the mineral pathway (PP-Chloro), display a significant difference between Bourget Lake and the other lakes. Bourget Lake is characterised obviously by a low PP-Chloro ratio. As for BP-TBB ratio, values of the ratio seem to be lower in Lake Geneva Le1 than in Lake Constance and the opposite seems to appear concerning Lake Geneva Le2.

Figure 6-6 shows that the low PP-Chloro ratio, which characterises Bourget Lake, is explained both by high values of chlorophyll a and low values of PP.
This result indicates that phytoplankton cells are less efficient to produce biomass in Bourget Lake than in the other lakes. It is in agreement with former observations (Cotner & Biddanda 2002) that in lakes from high trophic status, large cells are relatively more abundant than small cells because nutrients are not or less limited. Growth efficiency is smaller in large cells because of their low surface/volume ratio.

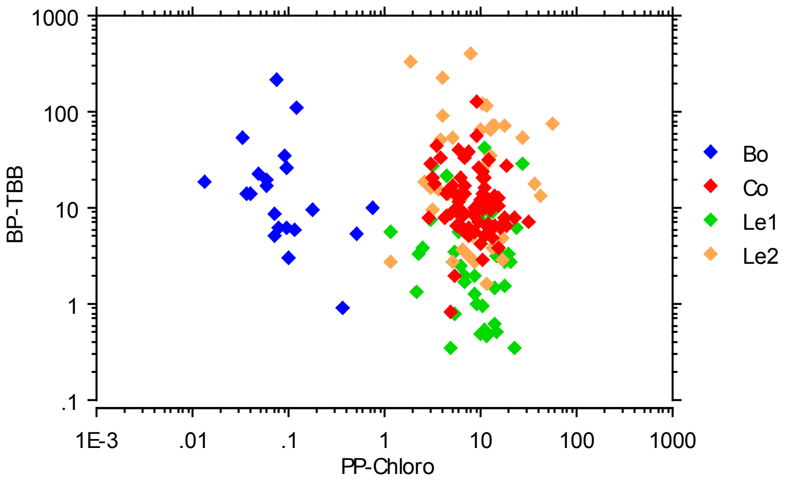


Fig. 6-5: Lakes comparison based on parameters ratio: PP-chloro versus BP-TBB (PP: primary production, Chloro: chloropyll a, BP: bacterial production, TBB: total bacterial production).

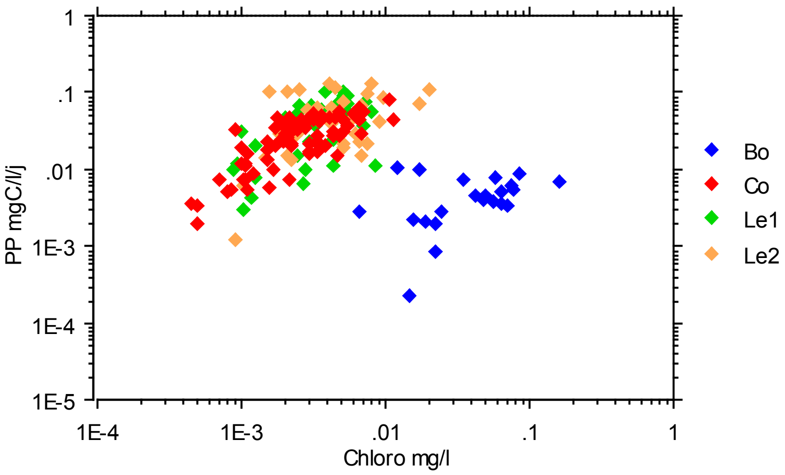


Fig. 6-6: Lakes comparison based on the relationship between chlorophyll a (Chloro) and primary production (PP).

Despite the low value of primary production in Bourget Lake, values of bacterial production (BP) are similar to the ones of Lake Constance. Primary production is the main process in lakes, from which an autochthonous organic matter easily degraded by bacteria is produced. Results then advocate for a low dependency of bacteria from autochthonous carbon, in Bourget Lake, which, in turn suggest that the high trophic level of this lake could be due to higher allochthonous carbon supply than in the other lakes.

Figure 6-7 also shows that both, PB and PP tends to be higher in Lake Geneva-Le1 than in Lake Constance.

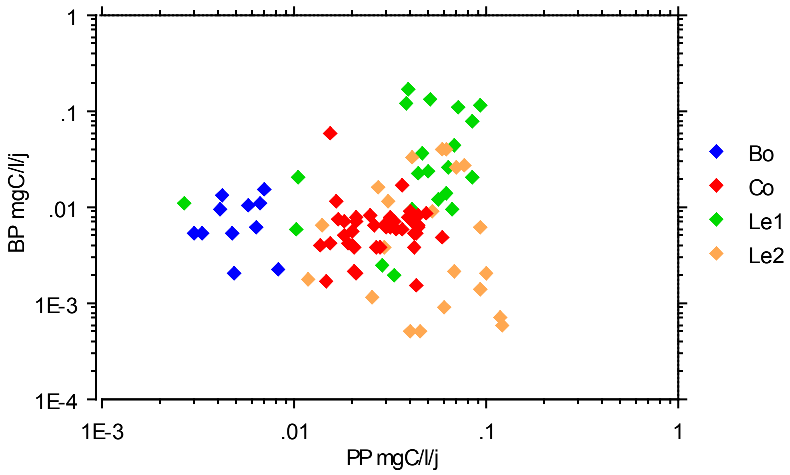


Fig. 6-7: Lakes comparison based on the relationship between primary production (PP) and bacterial production from S period.

In conclusion, the most significant results from the above comparisons based on means, ratio and parameter relationships, are that parameters related to the mineral pathway, PP and Chloro, are the ones displaying maximum differences between lakes. Bourget Lake clearly separates from the other lakes both, from its high values of chlorophyll a and its low values of PP. Parameters related to the organic pathway seem to discriminate Geneva-Le1 from its high values of bacterial production, by comparison to the other lakes. When considering all periods (NS & S), lakes are most of the time better discriminated during the S period than during the NS period.

It is necessary to remind here that the above comparisons were performed on datasets including only the 0-50m layer, because of the lack of data on bacterial compartment below 50m in Lake Constance. As heterotrophic processes extend along the whole water column while phototrophic processes are restricted to the euphotic zone, parameters related to the organic pathway (TBB and BP) have been underestimated at the scale of the whole lakes, by comparison to parameters related to the mineral pathway (chloro & PP). It must be then considered that results from the above comparisons do not apply at the scale of the whole lakes.

6.2.2 Lakes characteristics based on PEFC variability

The objective here is to analyse the range of variations of PEFC parameters in the studied lakes. Assuming that high variability of a parameter is mainly due to its high sensitivity to changes in environmental conditions (by comparison to variability due to the uncertainty of measurement), PEFC which values are highly variable will be considered to be more able to indicate changes in lake trophic status.

Variation coefficients (VC = standard deviation / mean) of each PEFC parameter has been computed from a sub-dataset based on values integrated over the 0 to 50m layer, including all periods (NS & S periods) and all lakes.

Table 6-2 shows that chlorophyll a and bacterial production (BP) are associated to the highest coefficients of variation (about 200%) whereas VC values for primary production and for total bacterial biomass are the lowest ones (around 80%).

	coefficients of variation (%)
Bacterial production (BP)	191
Total bacterial biomass (TBB)	73
Primary production (PP)	83
Chlorophyll a	209

Table 6-2: Coefficients of variation of PEFC (all depths, all periods, all lakes considered).

A more precise study is then performed by computing variations coefficients within each lake (Co, Le1,Le2, Bo), including all depths and all periods. The ranking of these coefficients indicates which PEFC best participates to the structure of the lake over time and space.

The ranking of bacterial production and chlorophyll a (Chloro) in each lake compared to the one found when all lakes are pooled (Table 6-2) shows that BP and Chloro are the most variable parameters. When considering all lakes, the highest variations are those from bacterial production (BP). It is then followed by Chlorophyll a (at the exception for Geneva-Le1) and by Primary production. Parameters with low coefficient values are related to bacterial biomass and phytoplankton production.

Then, according to the coefficient ranking for each individual lake, the parameter which could best participate to the system structure appears to be bacterial production, a parameter related to the organic pathway.

The comparison of the lakes as for the coefficients ranking shows that Lake Geneva "Le2" is close to Bourget Lake whereas Lake Constance is close to Lake Geneva "Le1" (Fig. 6-8) One reason why PEFC ranking may change according to the lake is because their different trophic status.

The differences between the lakes as for the ranking are generally due to total bacterial biomass (TBB), which variations appear to be higher in lakes from lower trophic status (Lake Constance, especially). In these lakes, bacterial biomass then seems to be an important structuring parameter. This results is in agreement with the general assumption that oligotrophic water support predominantly microbial food webs whereas in eutrophic waters, food webs consist of larger autotrophs and phagotrophic heterotrophs (Biddanda *et al.* 2001, Cotner & Biddanda 2002).

In conclusion it can be noticed that highly variable parameters that best display differences between lakes are both related to organic pathway (BP and TBB).

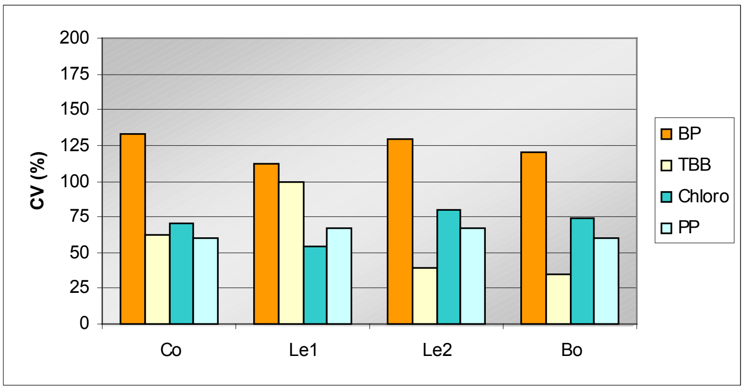


Fig. 6-8: Coefficients of variations for PEFC (all depths, all periods considered) for Lake Constance (Co), Lake Geneva (Le1, Le2) and Bourget Lake (Bo). BP: bacterial production, Chloro: chlorophyll a, TBB: total bacterial biomass, PP: primary production.

Another degree of specificity can be given to the analysis by considering variation coefficients computed for each lake and each period. Results for the S period (Fig. 6-9B) are close to the ones already given on Fig. 6-8. The S period appears to be the more sTable period compared to the NS period (Fig. 6-9A). Here again bacterial production display higher variations compared to the other parameters whatever the period.

Changes observed in ranking patterns according lakes and periods (Figure 6-9) could partly be related to lakes trophic status. Lake Constance is characterised by higher variations of bacterial biomass (TBB) during the NS period than during the S period, whereas the opposite appears for Lake Geneva Le1. Geneva Le2 displays higher between-periods variations for chlorophyll a with a higher value during the S period. In Bourget Lake, the variability of bacterial production during the S period is lower than during the NS period.

The comparison of the lakes about VC ranking here again shows that two out of the three parameters, from which differences establish, are related to the organic pathway (BB and BP).

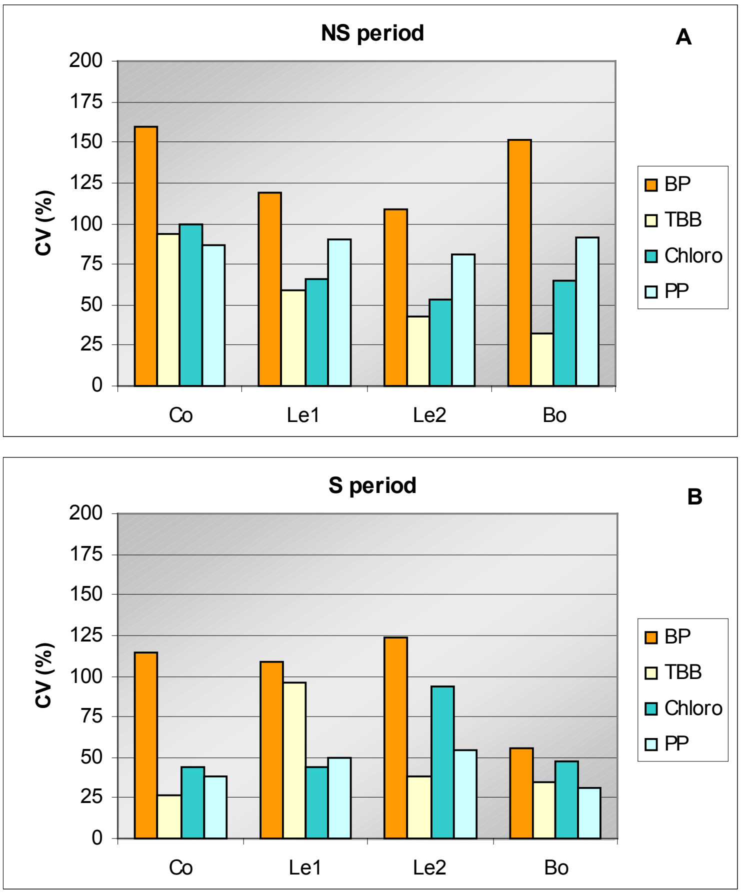


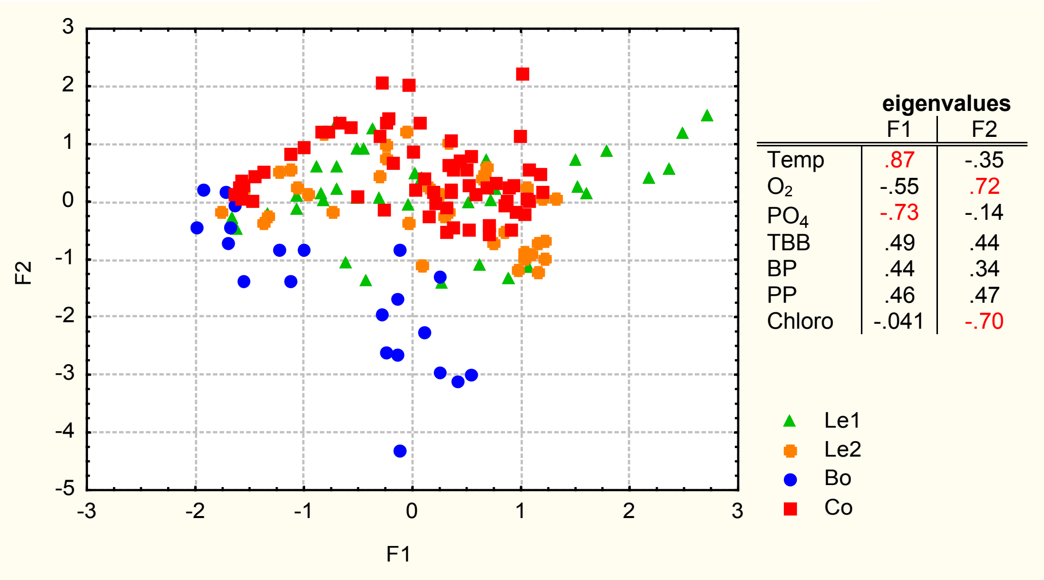
Fig. 6-9: Coefficient of variations for PEFC within 0-20m layers and for the NS period (A) and the S period (B). Lake Constance (Co), Lake Geneva (Le1, Le2), Bourget Lake (Bo), BP: bacterial production, Chloro: chlorophyll a, TBB: total bacterial biomass, PP: primary production.

6.2.3 Participation of PEFC to the overall discrimination of lakes

The objective here is to study how PEFC participate to the discrimination of lakes by comparison to some non-PEFC parameters.

Factor analysis is performed on the datasets of the 0-50m integrated values. PEFC parameters are included in the analysis together with non-PEFC parameters which play an important role within the system: temperature, oxygen, phosphates. No data was available about other important parameters like light absorption or organic matter (in this later case, data cannot be compared because of the difference in the technique from which they were achieved).

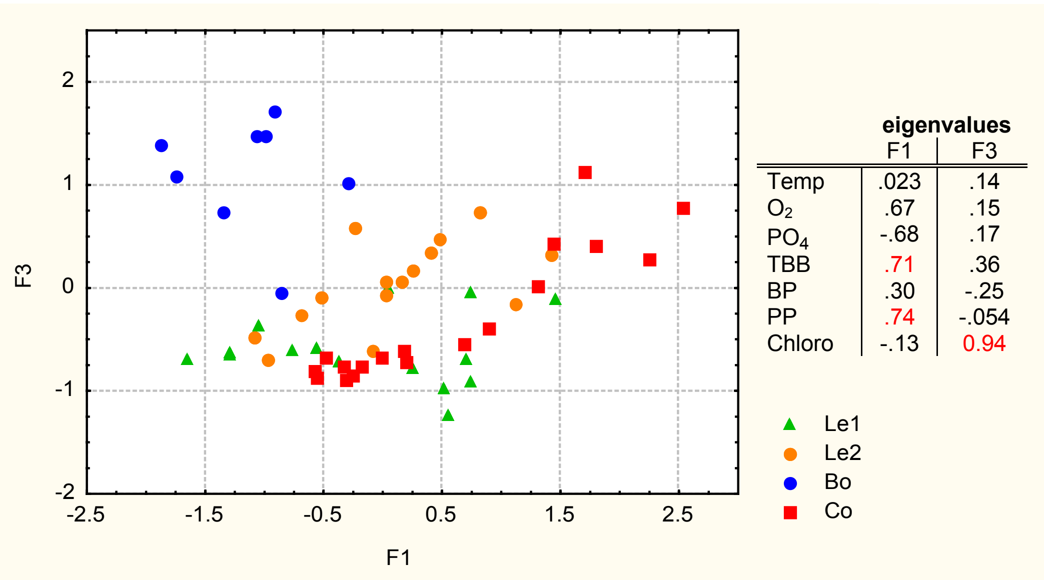
The first analysis is performed on dataset including all period (NS & S). It shows that Bourget Lake is discriminated from the other lakes by the F2 axis represented both, by chlorophyll a, a PEFC parameters and by oxygen (Figure 6-10).



eigenvalues	F1	F2
Temp	.87	-.35
O_2	-.55	.72
PO_4	-.73	-.14
TBB	.49	.44
BP	.44	.34
PP	.46	.47
Chloro	-.041	-.70

Fig. 6-10: F1-F2 map showing discrimination between lakes (NS & S periods included). Eigenvalues are presented in a Table (significant values in red).

The discrimination of Bourget Lake by chlorophyll a also appears when the analysis is restricted to the NS period as shown on Figure 6-11 (the F3 axis being significantly represented by chlorophyll a). However lakes are not discriminated along the F1 axis which is significantly explained by two PEFC parameters (TBB & PP).



eigenvalues	F1	F3
Temp	.023	.14
O_2	.67	.15
PO_4	-.68	.17
TBB	.71	.36
BP	.30	-.25
PP	.74	-.054
Chloro	-.13	0.94

Fig. 6-11: F1-F2 map showing discrimination between lakes for the NS period. Eigenvalues are presented in a Table (significant values in red).

For the S period, lakes are no more discriminated by chlorophyll a (re 6-12) Bourget Lake and Lake Constance are opposed to Lake Geneva Le1 and Le2 along the F3 axis which is significantly explained by PP. This analysis give the best separations of the lakes even though lakes from different trophic level (Bourget and Constance) surprisingly appear to be brought together according to PP while being opposed to Lake Geneva.

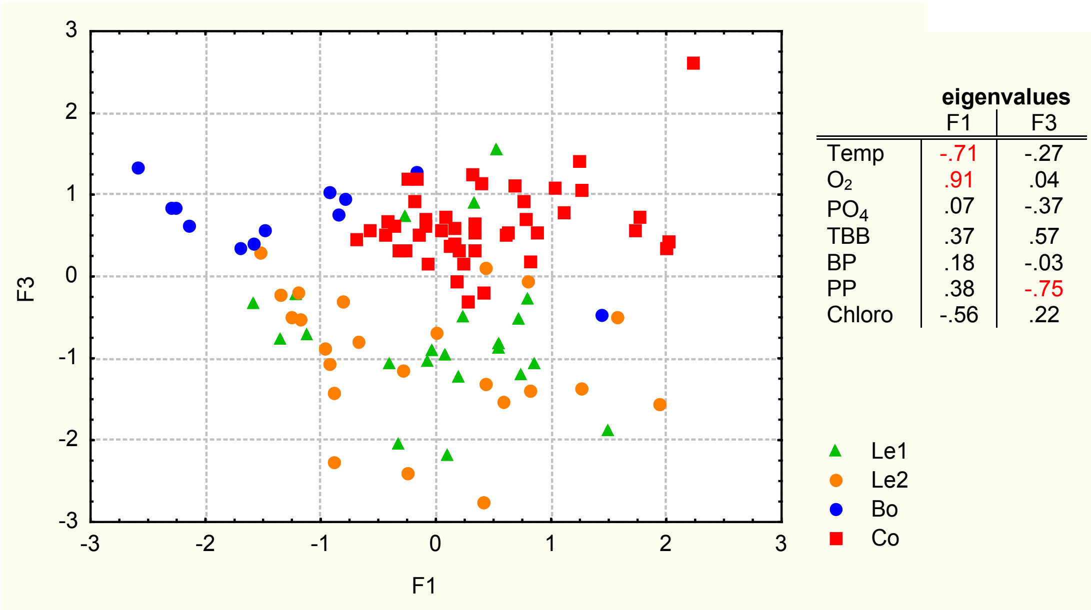


Fig. 6-12: F1-F2 map showing discrimination between lakes for the S period. Eigenvalues are presented in a Table (significant values in red).

In conclusion results from factor analysis show that PEFC significantly discriminate lakes even if main non-PEFC parameters are considered at the same time.
PEFC parameters which best discriminate lakes are related to the mineral pathway.
PP is the most significant parameter during the S period whereas chlorophyll a is the one which best discriminate lake during the NS period.

6.3 COMPARISON OF BOURGET LAKE AND LOCH LOMOND

The comparison between Bourget Lake and Loch Lomond is different from the previous comparison (Bourget Lake, Lake Constance and Lake Geneva) because of the own characteristics of Loch Lomond. The two main characteristics of Loch Lomond, which are different compared to the other studied lakes, are its humic character and its location on a crystalline bedrock. Loch Lomond is constituted by two main basins which have contrasting trophic level. The northern basin is oligotrophic whereas the south basin is mesotrophic.

Experiences has been conducted on Loch Lomond to offset the lack of data about bacterial components on this lake. Similar measurements has been carried out on Bourget Lake during the same period so as to make possible a comparison between the two systems.

Four campaigns have been conducted on Loch Lomond and on Bourget Lake over two years 2001-2002. Measurements were carried out three times per campaign (three consecutive days) and during two periods: NS and S periods (respectively non-stratified and stratified period). Two basins in each lake (northern and southern basin) were sampled at four depths according lake depth and euphotic area. Two depths were sampled in euphotic area which is defined according to the transparence (Secchi disk measurements): 1/3 secchi and 2/3 Secchi. Two depths were sampled in aphotic area: 30 and 50 meters; except in southern basin in Loch Lomond: 10 and 18m (maximum depth: 20m).

Results presented here concerned the same parameters than the ones studied for the comparison between Lake Geneva and Bourget Lake and Lake Constance, i.e. chlorophyll a, primary production (PP), total bacterial biomass (TBB), bacterial production (BP).

As for chlorophyll a, results show that values are by far higher in Bourget Lake than in Loch Lomond (Figure 6-13). The difference is more important during the NS period with values six times higher in Bourget Lake than in Loch Lomond. During the S period, difference between the two lakes is more significant considering their northern basins (NB).

Within Loch Lomond, the influence of the seasons is more important with an increase of the chlorophyll a concentration up to five times in S period compared to NS period within SB. This increase is higher in the basin from higher trophic level, in which nutrients limitation is lower.

The high influence of the seasons in Loch Lomond is probably due to a part to its humic character. Humic particles could be more abundant during the NS period because of the higher precipitations, and then significantly limit primary production.

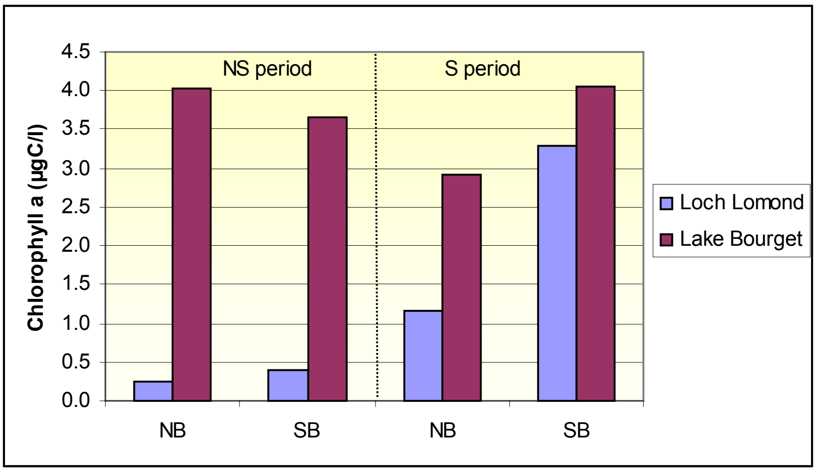


Fig. 6-13: Means of chlorophyll a concentration in Bourget Lake and in Loch Lomond for the northern basin (NB) and for the southern basin (SB) during NS and S period (respectively non-stratified and stratified period).

As for primary production (PP), differences between lakes are higher that the ones display by chlorophyll a. Values are on average ten times higher in Bourget Lake(Fig. 6-13) than in Loch Lomond (Figure 6-14) in S period while values are more than fifty times higher during NS period. As for PP, differences between lakes are more important during the NS period, certainly in relation to humic particles. In Loch Lomond, values of PP are always largely lower within NB than within SB (Figure 6-15). This result is in agreement with the lower trophic level within NB compared to the one of SB.

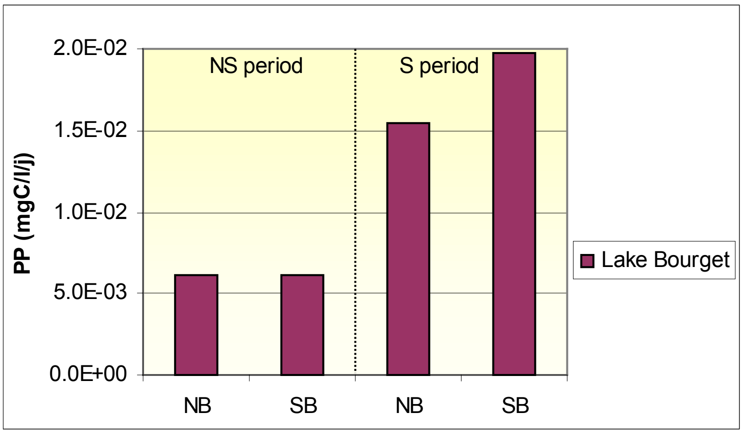


Fig. 6-14: Means of primary production (PP) in Bourget Lake for the northern basin (NB) and for the southern basin (SB) during the NS and the S period (respectively non-stratified and stratified period).

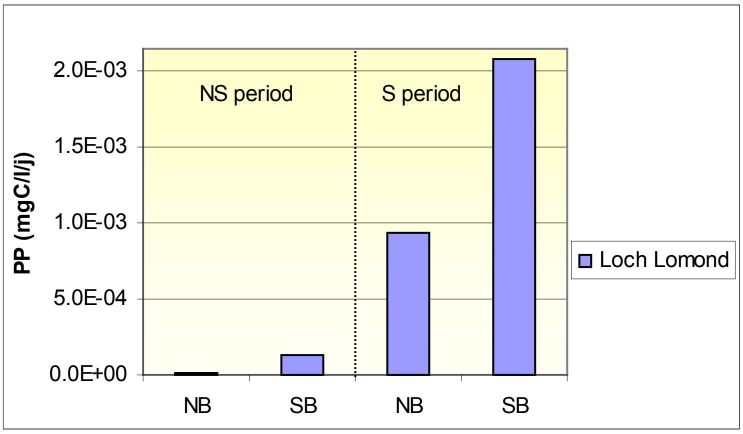


Fig. 6-15: Means of primary production (PP) in Loch Lomond for the northern basin (NB) and for the southern basin (SB) during NS and S period (respectively non-stratified and stratified period).

As for total bacterial biomass (TBB), differences between lakes are not very important. Values of TBB are always higher in the lake from higher trophic status (Bourget). The highest difference is displayed for SB during NS period, although this result is difficult to explain. Parameters related to organic pathway seem to be less affected by the humic character of Loch Lomond. TBB is in this case a bad indicator of differences between lakes.

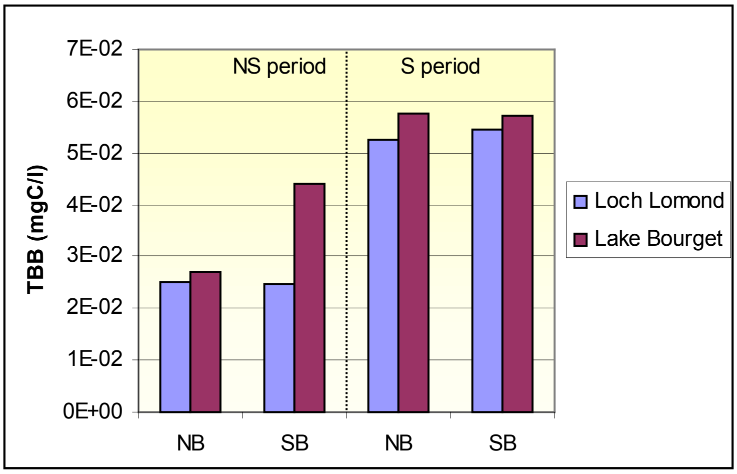


Fig. 6-16: Means of total bacterial biomass (TBB) in Bourget Lake and in Loch Lomond for the northern basin (NB) and for the southern basin (SB) during NS and S period (respectively non-stratified and stratified period).

As for bacterial production (BP), here again higher values are displayed in Bourget Lake compared to Loch Lomond and the highest difference between lakes appears within NB. The lower values of BP in Loch Lomond during NS period is probably the consequence of the lower values of PP. Lower PP values means less autochthonous carbon supply and then lead to a decrease of BP values within this basin. This decrease of autochthonous carbon seems to be not compensate by allochthonous inputs.

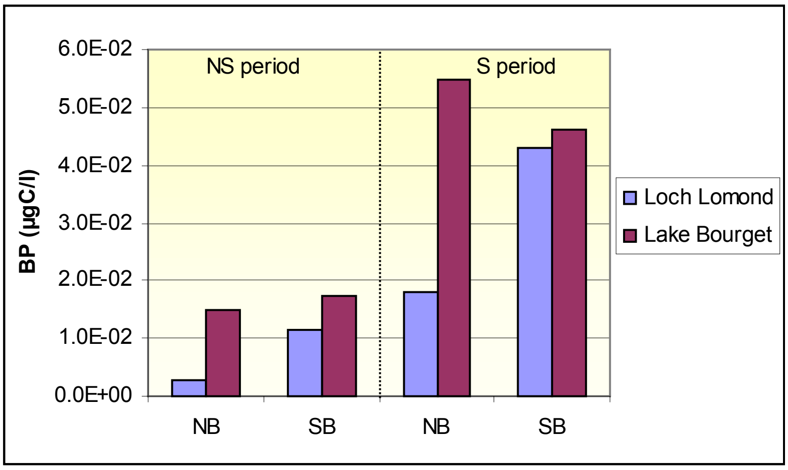


Fig. 6-17: Means of bacterial production (BP) in Bourget Lake and in Loch Lomond for the northern basin (NB) and for the southern basin (SB) during NS and S period (respectively non-stratified and stratified period).

Comparison of Bourget Lake and Loch Lomond, which have contrasting trophic level show clearly that PEFC could discriminate lakes (even not better then non PEFC ?). Results are in agreement with the ones found for the previous comparison (between Lake Geneva, Bourget Lake and Lake Constance). Parameters related to mineral pathway (PP & Chloro) are those showing the highest differences between lakes compared to the other parameters (TBB & BP).

Amongst parameters related to mineral pathway, PP is the parameter which display the highest differences between lakes whereas BP is the one, amongst parameters related to organic pathway, showing the highest differences.

Within this comparison (Bourget-Lomond), PEFC showing differences between lakes which are explain both by trophic level and by humic character of Loch Lomond. It's probably the reason why differences are more significant compared to the previous comparison (Geneva-Bourget-Lomond).

7 PART III. RELEVANCE OF PEFC AS INDICATORS OF LAKE QUALITY AND FUNCTIONING

7.1 CONCLUSIONS FROM CASE STUDIES

From factor analysis applied to our datasets concerning the three Lakes: Bourget, Geneva and Constance, it appears that some PEFC actually participate to lakes discrimination. Datasets used in this study included all periods and values integrated over a 0-50m depth range. We tried then to answer two additional questions: 1) ranking of PEFCs by comparison to each other in their ability to discriminate between different trophic status 2) when (what period) lakes mainly differ i.e. sampling should be done to highlight their differences.

PEFC ranking has been achieved from factor analysis performed on each lake. The results show that primary production is the parameter which discriminate lakes during the S period whereas chlorophyll a discriminate lakes during the NS period. Despite their different trophic level, Lake Constance and Bourget Lake are surprisingly brought together by factor analysis according the PP parameter. However the study of relationships between parameters shows that Bourget Lake is well separated from lake Constance about the PP:Chloro ratio. This ratio is low in Bourget Lake meaning that phytoplankton cells are less efficient in this lake than in Lake Constance.

Finally, our results suggest that lakes could be discriminated by the PP:Chloro ratio when their trophic level are very different (as shown above) and by the BP:PP ratio (BP: bacterial production), when their trophic levels are closer. Indeed, as shown in Figure 6-7, the BP:PP ratio seems to slightly discriminate between Le1, Le2 and Co.

Results from PEFC ranking based on coefficient of variations show that parameters related to the organic pathway are the ones which display the highest variations. Some of these parameters would then be the more able to indicate changes in trophic status (as PP and Chloro) whereas other PEFC parameters such as BP and TBB would be more able to indicate small changes in water quality. Results also show that the most discriminating period is the one when lakes are stratified (S period).

Results from the comparison between Bourget Lake and Loch Lomond are in general in agreement with results from the comparison of the three studied lakes (Geneva-Bourget-Constance).

All the above analysis lead to the conclusion of a true relevance of PEFC as indicators of lake trophic status as long as such a sampling frequency between 15 days and a month is possible. In this way the high turnover rate of PEFC appears as a constraint while it otherwise appears from a valuable help when it is necessary to estimate early stages of the recovery of the system after the end of a disturbance or early changes in lake functioning after changes have been introduced in lake management procedure or policy.

7.2 ECONOMIC AND TECHNICAL LIMITATIONS

Lake monitoring based on PEFC presents definitive advantages by comparison to the one based on higher level of food chains. Because of their small size and high concentration, PEFC makes it easy both samplings (only small volumes are needed allowing automatic sampling) and measurements. Even though they are far from being evenly distributed within the whole pelagic area, the problem of achieving representative sample is less critical than when addressing higher levels of food chains.

Fast improvement of techniques based on molecular biology let us predict that PEFC analyse will become even easier and more accurate especially as for taxonomic determinations. Techniques associated to some PEFC, like bacteria, are generally less demanding as for human labor (and then probably cheaper) than those related to higher level of food chains. This is because they are very similar to the ones used in water chemistry and can be automated.

7.3 REQUIRED EXPERTISE

It is already possible to achieve a lot of information on PEFCs without a high degree of expertise. Even though these measurements are generally the most global ones (total bacteria concentration, bacterial production, pigments concentration, primary production…) they cannot be done by any ordinary laboratory dedicated to water analysis because the need of sophisticated equipments (isotope analysis, high performance microscope, fluorescent probes, …).

In addition changes in lake trophic status is of course more accurately assessed when it is possible to get deeper inside the analyze of both, composition and activities of biocoenosis. This has been demonstrated for example about the increasing importance of mixotrophs during processes of re-oligotrohication (Domaizon *et al.* 2003). Here again, the improvement of techniques based on molecular biology will probably soon bring this degree of expertise within the reach of people in charge of lake monitoring.

7.4 RECOMMENDATIONS OF RANGES AND THRESHOLDS

Knowledge on food web functioning is far from being well understood. In addition scientists only recently experience eutrophic lake recovery or change in lake functioning induced by global change. Therefore, no range or threshold can seriously be recommended nowadays whatever the component of PEFC. However, fast progress is now being achieved for some of them (cyanobacteria, for example) that should lead soon to more precise statements in that matter.

8 PART IV. THE FUTURE OF PEFC AS PART OF LAKE MONITORING

A large part of PEFC members (phytoplankton) is already included in all large lakes monitoring. However they still need to be improved to better take into account the smallest members of pigmented organisms (picoplankton) as for both, their overall concentration and their participation to transfers of carbon up to higher levels of food chains.

In addition, we are convinced that there will be an increasing need to improve lake monitoring in adding regular measurements of organisms involved in the organic pathway of trophic food webs, which, for a large part, belong to PEFC. This need is particularly true in two fields: 1) study and monitoring of re-oligotrophication processes where the part of biomass production based on the organic pathway increased while mineral nutrients become increasingly limiting (Capblancq 1990, Biddanda *et al.* 2001) and 2) understanding and treatment of problems generated by diffuse organic pollution which is of major concern as water enrichment from human activities after most huge point source pollutions have been eradicated.

Any progress in these fields still require improvement of existing methods (fluorescent or isotopes labeling, tracers based methods, cytofluorimetry) and the emergence of new ones (about grazing measurement, for example). As stated before, methods based on molecular biology are very promising for the assessment of specific microbial biomass as well as for specific metabolic activities. They will probably be very helpful to promote the introduction of PEFC as part of lake monitoring while still requiring being very neat in the corresponding lab work.

PEFC modeling represents an invaluable tool to help understanding complex relationships that establish between structure of food webs and lake trophic status (Capblancq 1990, Hobbie *et al.* 1999). Simulation of processes is also needed, especially in the case of PEFC, because it is often hardly possible to achieve as much data as it would be necessary to take in account their high turnover rate. PEFC modeling and more generally food webs models are not new (Harris 1968, Dietz *et al.* 1977, Stefan & Riley 1986, Peduzzi *et al.* 1993) especially concerning phytoplankton (Gawler *et al.* 1988) and algal bloom (Valley *et al.* 1989, Yabunaka *et al.* 1997).

Despite recent modeling studies dedicated to transfer of organic carbon (Cajal-Medrano & Maske 1999) and to indicators (Reavie & Smol 2001), PEFC and food chains modeling are far from being introduced in regular monitoring programs. More progress could be expected from initiatives where joint studies associating specialists from the two fields (modelers and hydrobiologists) would be promoted.

A better understanding of PEFC dynamic and more generally of relationships between food chains functioning and lake trophic status could be gained from long term datasets. At the exception of phytoplankton, such records concerning PEFC are very rare. Some incentive financial programs have recently been launched in Europe.

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