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Use of marker pigments and functional groups for assessing the status of phytoplankton assemblages in lakes

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Abstract Phytoplankton is a key biological quality element for the establishment of the Water Framework Directive (WFD) ecological status in reservoirs and lakes. In freshwaters, inverted microscope examination is the traditional standard method for estimating phytoplankton and assessing taxonomic composition. Based on the enumeration of algal units and measurements for biovolume calculation, this technique is cumbersome and time-consuming. In large monitoring programmes, such as the application of the WFD in lakes and reservoirs, chemotaxonomy (HPLC pigment analysis and CHEMTAX treatment) is ideally suited as an alternative method because it allows the rapid processing of large numbers of samples from numerous locations and depths, thereby providing ideal temporal and spatial resolution. The low taxonomical detail obtained by HPLC and CHEMTAX (phytoplankton classes or phyla) can easily be overcome by a rapid inverted microscope screening with identification of the dominant species. Combining HPLC and microscopy provides a useful method for monitoring phytoplankton assemblages, which can be used to implement the WFD with respect to phytoplankton. Here, we present the application of a method combining marker pigments and microscopy to phytoplankton samples from 12 Belgian reservoirs. This method substantially reduced the workload and enabled us to assess the status of the phytoplankton assemblage in these lakes. The method complies with the WFD, as it takes into account taxonomic composition, assesses abundance and biomass of the phytoplankton taxa, and easily detects blooms. Additionally, a set of templates of probability of occurrence of phytoplankton functional groups at the maximal ecological potential for reservoirs from the Central/Baltic region is presented, based on reference conditions defined for natural lakes from other regions.

Keywords HPLC · Pigments · CHEMTAX · Chemotaxonomy · Water Framework Directive · Phytoplankton · Functional groups · Lakes · Reservoirs

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Introduction

Phytoplankton is a key component of the biocenosis in lakes, reservoirs, large rivers, estuaries and coastal waters. Accordingly, phytoplankton has been retained among the biological quality elements for the assessment of the ecological status of water bodies, as defined in the Water Framework Directive (WFD, European Union 2000). However, few methods based on the composition of "algal" assemblages are currently available, other than those designed to estimate trophic status in lakes, which are based on abundance ratios of the main algal classes (or phyla). These indices, however, do not meet the requirements of the WFD as, for instance, assessing status from the distance to reference assemblages in types of water bodies, and taking into account taxonomical composition and presence / absence of algal blooms.

The standard method for estimating phytoplankton and assessing taxonomic composition is based on examination with the inverted microscope, and involves enumerating algal units and measuring dimensions for calculating biovolume (Utermöhl 1958). Although necessary for obtaining detailed quantitative description at the genus species level, the technique is cumbersome and time-consuming. In addition, more often than not, the information is exploited mainly at

the class level, by adding up biovolumes of all taxa within each class. Moreover, interpretation of ecological conditions in freshwaters based on phytoplankton (i.e. relevant for assessing ecological status) has been based on dominant taxa, usually at the genus level (e.g. Reynolds et al. 2002; Lepistö et al. 2004, 2006; Padisák et al. 2006; Salmaso et al. 2006) or higher taxonomical levels (e.g. Moss et al. 2003; Borja et al. 2004; Sondergaard et al. 2005). So, full taxonomical detail at the lowest level achievable by inverted microscopy is not necessarily required for monitoring and assessing the status of phytoplankton assemblages.

Alternatively, phytoplankton marker pigments have been used widely for assessing biomass at the class level, with many applications in marine, estuarine (reviewed in Wright and Jeffrey 2006), and freshwater environments (e.g. Hurley and Watras 1991; Yacobi et al. 1996; Descy et al. 2000; Fietz and Nicklisch 2004; Buchaca et al. 2005; Descy et al. 2005; Fietz et al. 2005; Sarmento et al. 2006; Schlüter et al. 2006). The most delicate part of the assessment of algal abundance from pigment concentrations has been solved by development of different techniques, involving ratios of marker pigment to chlorophyll *a* (chl *a*) (Mackey et al. 1996, Descy et al. 2000, Lewitus et al. 2005, Latasa 2007). CHEMTAX (for CHEMical TAXonomy) is a computer program that allows allocation of chl *a* among different algal groups defined by a suite of pigment markers. From an initial ratio matrix (or input matrix) usually derived from pure cultures of phytoplankton, the program uses an iterative process to find the optimal pigment:chl *a* ratios and generates the fraction of the total chl *a* pool belonging to each pigment-determined group.

Chemotaxonomy is often used and is commonly accepted as a standard method in oceanographic studies and monitoring programs (Jeffrey et al. 1997). However, this approach is less widespread in the freshwater scientific community, probably because large-scale monitoring programs such as oceanographic cruises, with a large number of samples, are relatively rare in freshwaters. The fact that the large lakes are amongst the rare examples of extensive application of HPLC pigment analysis and CHEMTAX processing for phytoplankton assessment in freshwaters further exemplifies this (Fietz and Nicklisch 2004; Descy et al. 2005; Fietz et al. 2005; Sarmento et al. 2006).

A number of authors have compared assessments based on marker pigments with those from microscopy enumerations (e.g. Roy et al. 1996; Schmid et al. 1998; Schlüter et al. 2000; Henriksen et al. 2002; Rodriguez et al. 2002; Garibotti et al. 2003; Buchaca et al. 2005; Ediger et al. 2006; Muylaert et al. 2006; Schlüter et al. 2006). Most studies confirmed reliability of the "pigment method" combined with CHEMTAX processing, even if some of these works point to minor discrepancies in particular cases. Recently, Schlüter et al. (2006) stressed the utility, and once more proved the applicability of HPLC-CHEMTAX to field freshwater samples. In this latter study, the authors proposed a new pigment ratio input matrix derived from algal cultures, slightly different from that proposed by Descy et al. (2000).

Although the chl *a* distribution among algal groups can be defined with great precision and reproducibility using HPLC analysis of pigments combined with CHEMTAX processing, this technique obviously has limits when greater taxonomical detail than the class level is needed. Indeed, a typical

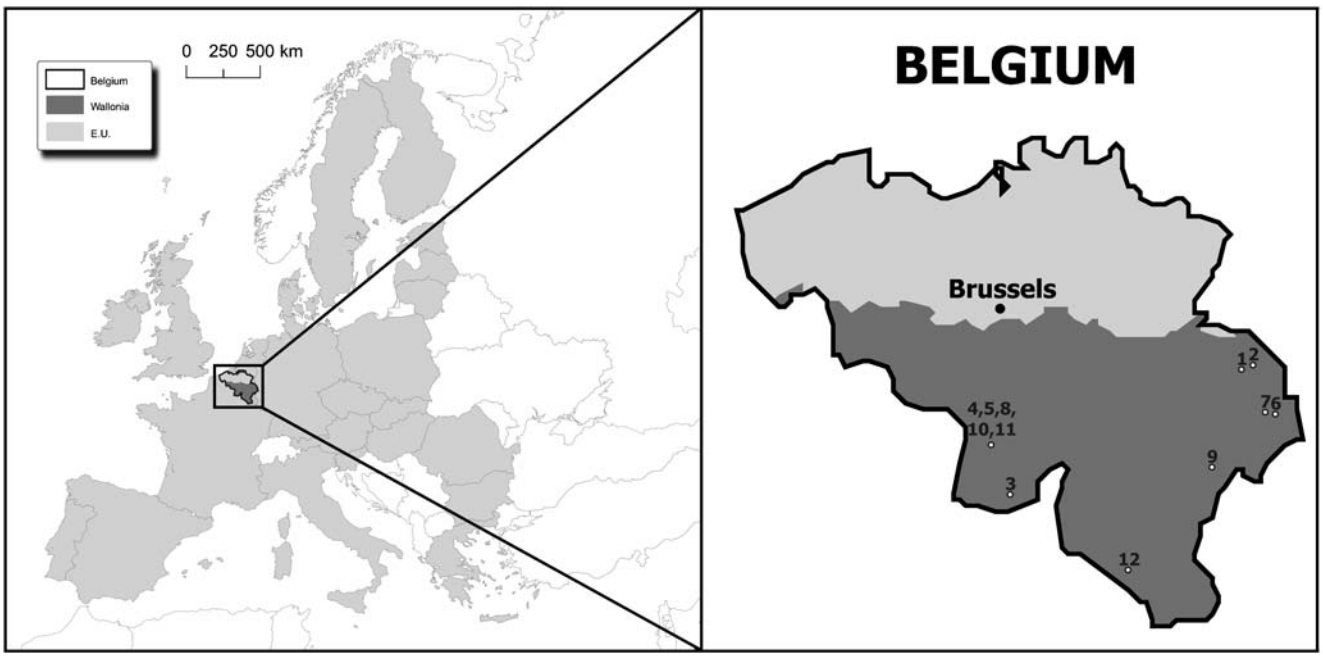

Fig. 1 Map of the European Union and the location of the 12 Belgian lake reservoirs covered in this study (see Table 1 for lake names and characteristics)

CHEMTAX output is absolute or relative contribution to total chl *a* of phytoplankton classes or phyla, i.e. the chl *a* biomass of chlorophytes, chrysophytes, cryptophytes, cyanobacteria, diatoms, dinoflagellates and euglenophytes. Differences in cell pigment contents within a group can sometimes be used to increase the chemotaxonomic resolution: for instance, separate assessment of cyanobacteria "type 1" and "type 2", can be done on the basis of the presence or absence of carotenoids, as echinenone or various glycosylated xanthophylls which are found in these prokaryotes (Descy et al. 2000). Although further research on pigment profile types within algal groups might increase the resolution of pigment-based methods, the taxonomic resolution will likely remain insufficient for assessing ecological conditions with the detail required for assessing the ecological status of water bodies. Nevertheless, quite successful attempts have been made at estimating ecological status in estuaries using pigment diversity in the phytoplankton assemblage (Paerl et al. 2003). However, it is doubtful that similar applications can be developed in freshwaters, where pigment diversity is lower than in brackish and marine waters.

In this paper, we present results from a 1-year study carried out on a series of reservoirs located in the southern part of Belgium (WFD ecoregions "Western plains" and "Western highlands") where phytoplankton was used as a biological quality element, as an example of the utility of marker pigments in the application of the WFD. Analysis of phytoplankton assemblages was based on HPLC analysis of marker pigments, combined with identification of the dominant taxa using microscopy. Using these data, we applied the functional classification of Reynolds et al. (2002) in a similar way to the application developed in the EC REBECCA project (Reynolds 2005). We demonstrate that our approach using HPLC and relatively quick microscopy screening, allowing identification of phytoplankton functional groups in numerous samples, can be a useful and reliable tool for assessing the ecological status of lakes and reservoirs.

Methods

Study sites

This study was part of a monitoring program established in collaboration with the Walloon Region water authorities (Région Wallonne—Direction Générale des Ressources Naturelles et de l'Environnement) in the WFD regional policy context: it aimed at classifying 12 reservoirs (Fig. 1, Table 1), defining their maximal ecological potential and their actual status.

According to the WFD application, freshwater reservoirs are heavily modified water bodies, whose ecological potential should be established by reference to the most similar natural lake. As there is no natural lake in the studied region, we used

Table 1 Characteristics, basic limnological parameters, typology and trophic classification (OECD 1982) of the 12 Belgian lake reservoirs covered in this study

Reservoir name		Gileppe	Eupen	Ry de Rome	Plate-Taille	Eau d'Heure	Bütgenbach	Robertville	Ry Jaune	Nisramont	Féronval	Falemprise	Vierre
Location	(see Fig. 1)	1	2	3	4	5	6	7	8	9	10	11	12
Catchment area	(km^2)	54	106	10	8	79	72	118	10	740	10	42	242
Volume	(hm^3)	26.4	25	2.2	67.8	14.7	11	7.7	1.1	3	0.8	1.2	1
Surface	(Ha)	130	126	26	351	165	120	63	31	47	21	47	35
Maximum depth	(m)	58	56	25	60	25	23	47	10	14	12	12	9
Mean depth	(m)	20.3	19.8	8.5	19.3	8.9	9.2	12.2	3.5	6.4	3.8	2.6	3
Retention time	(days)	315	122	200	44	268	97	18	163	6	114	35	5
Alkalinity	(meq L^{-1})	0.2	0.2	0.4	2.6	2.5	0.6	0.7	1.9	0.9	2.9	3.6	0.8
Secchi disc	(m)	2.0[a]	1.5[a]	3.9[a]	5.2	3.7	2.4	3	1.5	1.5	1.2	1.8	1.2
Total P	(µg L^{-1})	7.7	7.2	9.6	20.2	20.5	24.5	27.5	27.6	41.9	55	59.4	72
Mean chl *a*	(µg L^{-1})	0.8	2.2	6.3	2.4	3.4	11.2	11.5	7.9	11.7	22.1	20.5	23.5
Max chl *a*	(µg L^{-1})	1.8	5.1	9.3	8.2	13.6	27.2	33.4	26.7	31.6	116.8	129.5	60.2
Typology	L-CB3	L-CB3	L-CB3	L-CB1	L-CB1	L-CB3	L-CB3	L-CB1	L-CB3	L-CB1	L-CB2	L-CB2	L-CB2
Trophic classification	Oligotrophic	Oligotrophic	Oligo-mesotrophic	Oligo-mesotrophic	Mesotrophic	Meso-eutrophic	Meso-eutrophic	Meso-eutrophic	Eutrophic	Eutrophic	Eutrophic	Eutrophic	Eutrophic

[a] Humic lake: Secchi disc transparency was not took into account for the trophic classification

Table 2 Lake typology adapted for Belgian reservoirs

Belgian reservoirs typology (Heavily Modified Water Bodies/Lake-Central/Baltic)			
Type	L-CB1	L-CB2	L-CB3
Characterisation	Lowland, shallow, Calcareous	Lowland, very shallow, Calcareous	Lowland, shallow, small, Siliceous (moderate alkalinity)
Altitude (m)	<600	<600	<600
Mean depth (m)	3 – 25	<3	3–25
Alkalinity (meq L^{-1})	>1	>0.5	0.2–1
Residence time (years)	0.1–1	0.01–0.1	0.1–1

the *Geographical Intercalibration Groups* (GIG) and the lake typology defined by the EC-projects REBECCA (*Relations between ecological and chemical status of surface waters*) and ECOSTAT (*Common Implementation Strategy of the WFD*). We considered that the closest lake type was the Central-Baltic (L-CB) lake type; however, we had to make some adaptations of the thresholds of this classification system, to account for obvious differences in altitude, to disregard residence time, which is lower in reservoirs (which are used for water supply and energy generation) than in natural lakes (Table 2).

Field sampling

A monthly monitoring program was organised in the 12 reservoirs during 8 months (March–October) in 2006. Sampling points were located at the deepest location of the reservoirs and at least 50 m away from the dam. Samples were collected at different depths in the water column (from surface to bottom, every 2.5 m in the deep lakes and every 1 m in the shallow lakes) with a 3 L Van Dorn bottle. A set of 18 physical and chemical variables (including nutrients and pesticides) was surveyed by the regional Scientific Institute of Public Services (ISSeP—Institut Scientifique de Service Public).

Microscopic examination

Samples for examination by microscopy were immediately fixed with Lugol's solution and concentrated by settling. These concentrates were further preserved with neutral formaldehyde (2–4% final concentration) for long-term storage in the dark. Following HPLC analysis and CHEMTAX treatment (see below), at least one sample per lake and per month was selected from the vertical profile for a rapid screening with the inverted microscope (Leica DM IL with phase-contrast) and, whenever necessary, species identification with a standard microscope (Zeiss Axioskop equipped with an AxioCam digital camera). The choice of samples for identification by microscopy was made taking into account the vertical biomass profiles from the marker pigment analysis, for instance to identify taxa in phytoplankton developing at particular depths. Identifications were based on specialised taxonomic literature.

Pigment extraction, HPLC analysis and data processing

Samples for chl *a* and secondary pigment analysis were treated following a procedure described in Descy et al. (2000): water was filtered on Macherey-Nägel (Düren, Germany) GF/3 filters until filter-clogging. Pigment extraction was carried out in 8 mL 90 % HPLC grade acetone. After two 15 min sonications separated by an overnight period at 4°C in the dark, HPLC analysis was carried out using the gradient elution method of Wright et al. (1991), with a Waters system comprising a Waters 996 PDA detector and a Waters 470 fluorescence detector. Calibration was made using commercial external standards (DHI, Denmark). Carotenoids not present in the standard were quantified against fucoxanthin, using the ratio of the specific absorbance coefficients at 440 nm in methanol as relative response (Jeffrey et al. 1997). Identification of pigments was checked against a library of pigment spectra obtained by diode array acquisition of chromatograms from pure pigment solutions and from acetone extracts of pure cultures of algae. Chromatogram data were processed using Waters Millennium software.

Abundances of algal taxa were determined from HPLC algal pigment measurements using CHEMTAX, a matrix factorisation program that estimates the contribution of each specified phytoplankton pigment class to the total chl *a* concentration in a water sample, (Mackey et al. 1996). A unique initial ratio matrix was used for all lakes (Table 3), and CHEMTAX processing was run until the pigment ratios in the output ratio matrix became stable.

Functional group grid templates

A template of distribution of the phytoplankton functional groups (Reynolds et al. 2002) can be represented on a grid (Fig. 2), where horizontal lines correspond to seasons and vertical lines represent a gradient of increasing nutrient

Table 3 Generic input pigment ratio matrix [normalised to chlorophyll *a* (chl *a*)] used in this study

	Peridinin	Fucoxanthin	Neoxanthin	Violaxanthin	Diadino- + Diato-xanthin	Alloxanthin	Lutein	Zeaxanthin	Echine none	β-carotene	Chlorophyll *b*
Chlorophytes			0.018	0.024			0.117	0.018			0.153
Chrysophytes		0.220		0.088							
Cryptophytes						0.212				0.016	
Cyanobacteria-T1								0.109			
Cyanobacteria-T2								0.036	0.085		
Diatoms		0.563			0.129						
Dinoflagellates	0.629				0.250						
Euglenophytes			0.010		0.250						0.300

enrichment, for different types and trophic categories of natural lakes, as in Reynolds (2005). This presentation form allows easier and simultaneous visualisation of quantitative and qualitative aspects of the seasonal succession of phytoplankton functional groups. The relevance of each functional group in a certain period of time is given by its relative abundance, expressed by a colour fill (as described in Fig. 3) in the corresponding functional group grid cell. In this study, we used the algal group contribution to chl *a* obtained from the marker pigment analysis for filling in the relative abundance of the functional group grids. This approach is discussed further below.

Results

The application of the typology adapted for Belgian reservoirs (Table 2) based on mean depth, alkalinity and residence time, resulted in four lakes in type L-CB1 (lowland, shallow, calcareous), two in type L-CB2 (lowland, very shallow, calcareous) and six in type L-CB3 (lowland, shallow, siliceous–moderate alkalinity) as shown in Table 1. This distribution reflects, beside the average depth, the geological contrast between the Ardennes region and the rest of the country.

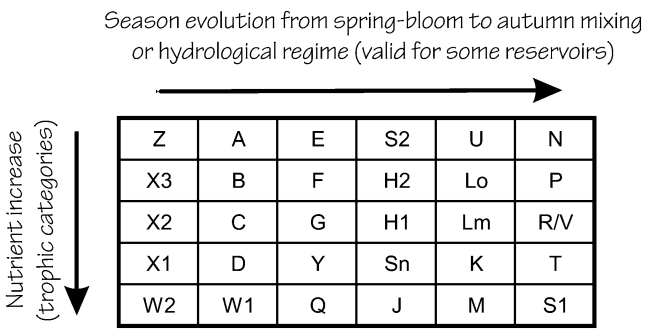

Fig. 2 Template grid of phytoplankton functional groups (adapted from Reynolds 2005)

A total of 480 samples were treated by HPLC and CHEMTAX in this study. Two examples of results obtained by HPLC analysis and CHEMTAX treatment, incremented with a microscopic photography of some of the dominant taxa, are illustrated in Figs. 4 and 5. The complete results of phytoplankton (and the major physico-chemical features) from the 12 Belgian reservoirs can be found at http://tinyurl.com/3coazz. A preliminary analysis of this type of plot resulted in selection of 97 samples for rapid screening of dominant taxa by inverted microscopy.

The plots presented in Figs. 4 and 5 represent the results from pooled epilimnion samples. However, in some cases, the detailed vertical profiles revealed different populations at different depths, as in the case of a deep chlorophyll maximum (DCM) dominated by *Gymnodinium* sp. in the Ry de Rome reservoir (July and September curves in Fig. 6). These particular features were taken into account in making sample selection for inverted microscope observation.

The combined results from HPLC-CHEMTAX and inverted microscopy allowed us to fill the functional group grids with observation values (data available at http://tinyurl.com/3coazz). The results from this survey showed that the epilimnia of meso- and eutrophic Belgian lakes in summer (generally corresponding to the annual maxima of chl *a*) were usually dominated by Y assemblages (*Cryptomonas*), tolerant to low light conditions. In some lakes, colonial green algae (assemblage J, typical taxa: *Pediastrum*, *Scenedesmus*, *Coelastrum*…) and small-celled colonies of cyanobacteria (assemblage K, typical taxa: *Aphanocapsa*, *Aphanothece*…) were also prominent during summer. The

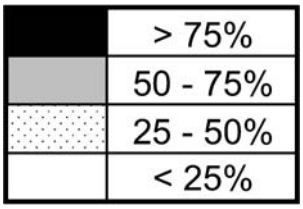

Fig. 3 Scheme of colouring template grid cells to show probability that the functional group will be represented in the phytoplankton (Figs. 7, 8 and 9) (adapted from Reynolds 2005)

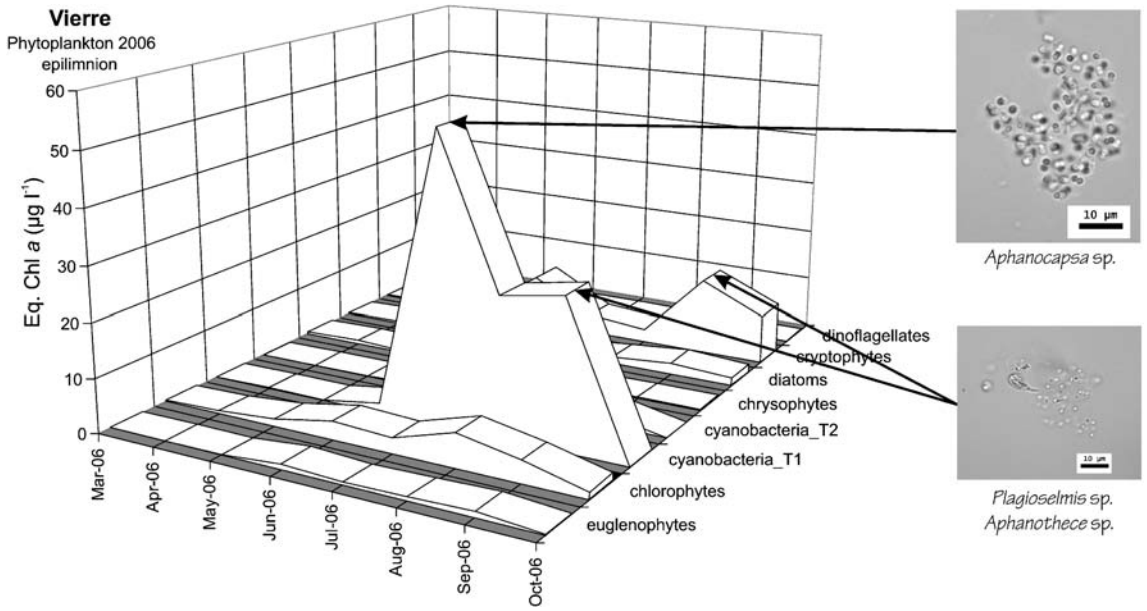

Fig. 4 Example of results obtained from HPLC pigment analysis and CHEMTAX processing in a Belgian eutrophic shallow reservoir (Vierre, epilimnion pooled sample). Indication of some genera identified by microscopy

dominant assemblages of the oligotrophic lakes were more variable, but flagellated taxa were often well represented: in some cases smaller taxa such as *Plagioselmis* or eukariotic picoplankton (assemblages X2 and X3, respectively) were observed, in others larger organisms such as *Gymnodinium* (assemblage Lo) or *Uroglena* (assemblage U).

These observations were used as a starting point to develop a template of probability of occurrence of

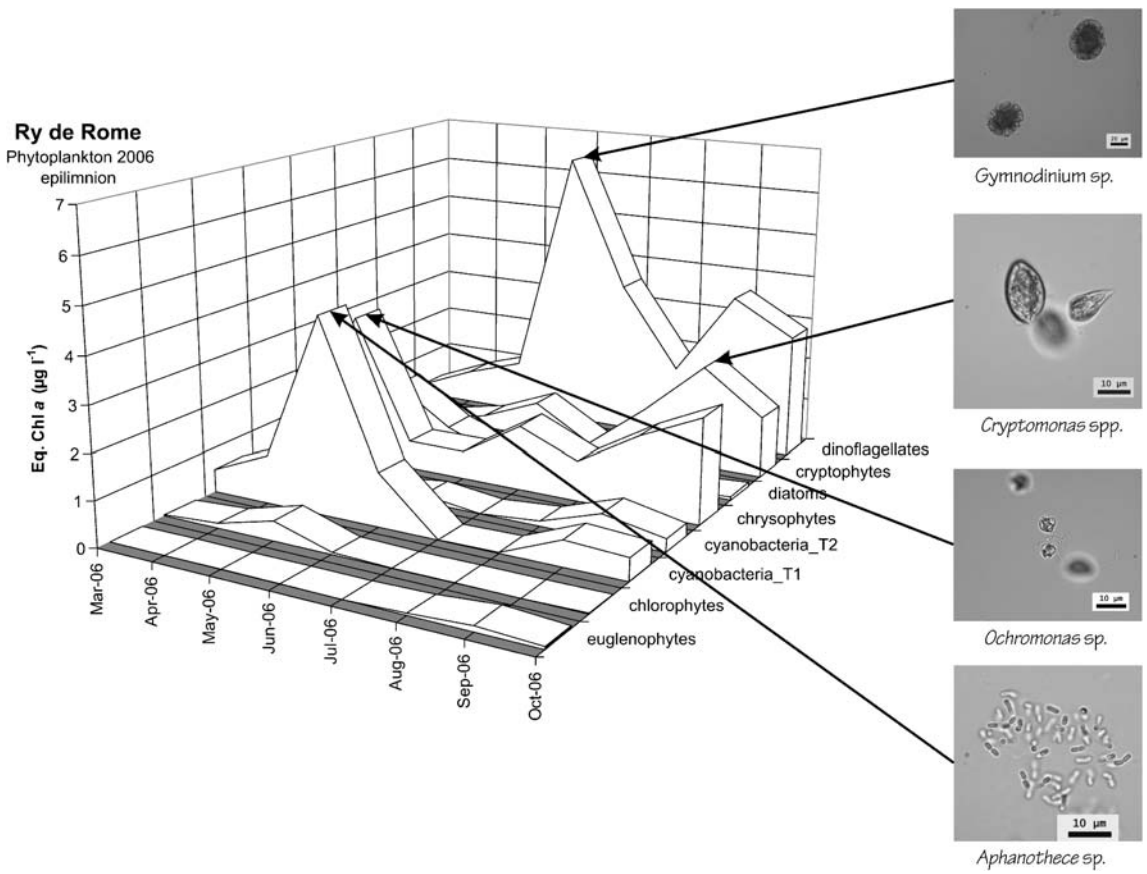

Fig. 5 Example of results obtained with HPLC pigment analysis and CHEMTAX processing in a Belgian oligo-mesotrophic reservoir (Ry de Rome, epilimnion pooled sample). Indication of some genera identified by microscopy

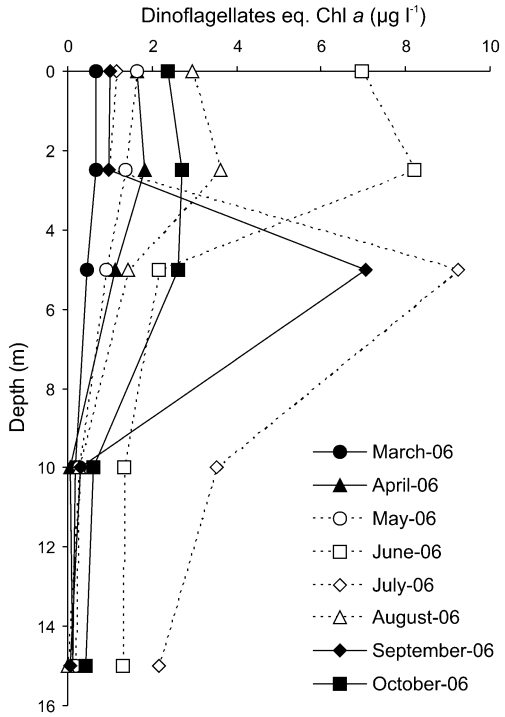

Fig. 6 Profile of the dinoflagellate population (from HPLC pigment analysis and CHEMTAX processing) in the 2006 monitoring of a Belgian oligo-mesotrophic reservoir (Ry de Rome)

phytoplankton functional groups in the different types of Belgian reservoirs at the maximal ecological potential, following the reference conditions defined by Reynolds (2005) for natural lakes from other regions (Figs. 7, 8 and 9), according to the physical and chemical characteristics of each lake type.

Discussion

In the application of the WFD to reservoirs and lakes, phytoplankton is a key biological quality element for the establishment of ecological status. An HPLC-CHEMTAX survey is ideally suited for large-scale monitoring because it allows the processing of large numbers of samples from numerous locations and depths relatively quickly; additional taxonomical detail can be provided by identification with an inverted or standard microscope. In marine systems, for routine monitoring of phytoplankton Havskum et al. (2004) recommend the use of the CHEMTAX program based on HPLC pigment analyses accompanied by a screening for the dominating species by microscopy, and by flow-cytometry for quantification of picocyanobacteria. In this study we tested the feasibility of using this combination of techniques for monitoring freshwater phytoplankton in lakes of different trophic status and hydraulic regime.

It is interesting to stress that all the work presented in this paper, from the field sampling to the ecological interpretation of the functional groups, including laboratory and microscopy work, was carried in 1 year by one full-time scientist and one full-time technician. Surely, most studies involving 12 lakes, with complete phytoplankton monitoring, would have involved a larger work force, unless samples from different depths were pooled, at the expense of ecological information.

The increasing interest for chemotaxonomy can be demonstrated by the steady increase in the number of peer-reviewed publications containing the word CHEMTAX (Fig. 10) within article titles, keywords and abstracts (ISI web science search). The great majority of such publications concern methodological advances or adaptations to new applications (estuarine or freshwater systems, for example). As the search was carried out on article titles, keywords and abstracts, these numbers may underestimate the total number of scientific studies in which CHEMTAX was applied.

HPLC systems are now widespread in laboratories, especially in those involved in water quality control, and, given the commercial availability of pigment standards, pigment analysis in numerous samples is no longer a problem. However, processing of the pigment data to

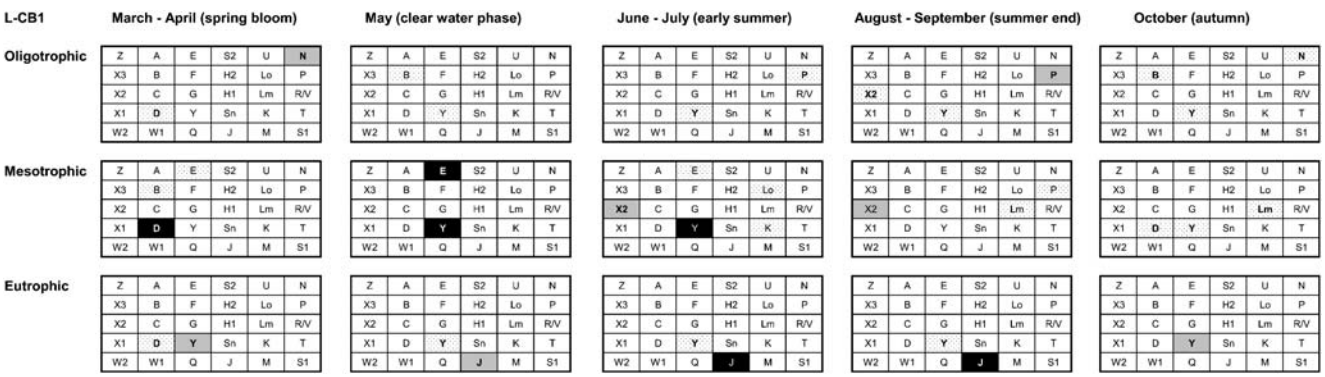

Fig. 7 Template of probability of occurrence of phytoplankton functional groups in heavily modified water bodies type L-CB1 (Central/Baltic type 1) at the maximal ecological potential, following the reference conditions defined by Reynolds (2005) for natural lakes from other regions (see Figs. 2 and 3 for legends)

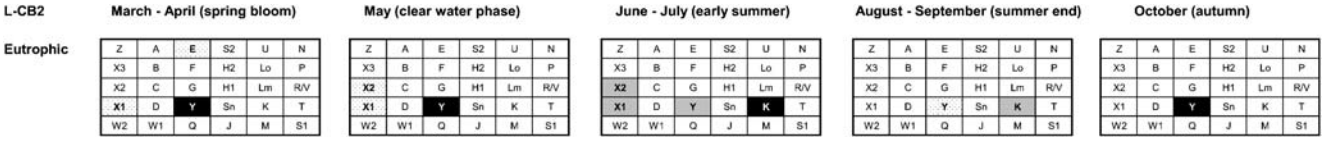

Fig. 8 Template of probability of occurrence of phytoplankton functional groups in heavily modified water bodies type L-CB2 (Central/Baltic type 2) at the maximal ecological potential, following the reference conditions defined by Reynolds (2005) for natural lakes from other regions (see Figs. 2 and 3 for legends)

estimate the contribution of phytoplankton groups to chl *a* still requires some expertise and access to adequate software, e.g. CHEMTAX. Although one may think that choosing values for the pigment ratios needed in the calculations could be a major difficulty, several studies have provided useful references (e.g. Descy et al. 2000; Schlüter et al. 2006). In our study, despite the large intrinsic diversity among the 12 studied reservoirs (illustrated by their assignment to three different lake types, Table 1), CHEMTAX treatment with a single generic input pigment ratio matrix (Table 3) was acceptable, with no major pigment ratio variation in the output matrix. In fact, a method to reduce processing errors related to wrong estimates of initial ratios is available: if the user repeats CHEMTAX processing in successive runs, using the output from each run as the input for the next one, the pigment ratios generally adjust automatically towards the true value, greatly improving initial pigment ratio values and, therefore, biomass estimates (Latasa 2007). In other words, even if initial ratios values are uncertain, repeated CHEMTAX runs as described above ensure that pigment ratios eventually converge towards their true value, and hence towards a good biomass estimation.

Several authors have used the functional group approach based on phytoplankton assemblage for classification of the ecological status of lakes (e.g. Reynolds 2005; Padisák et al. 2006). One difficulty with this, however, is how to cope with the variation of this assemblage in time (seasonal succession) and space (possible contrasted vertical distribution of phytoplankton taxa). Therefore, achieving sufficient resolution with standard microscopic techniques can be time-consuming and costly. Some authors have recommended sampling at one season, in order to reduce sampling effort and the number of samples to be examined (Padisák et al. 2006). By contrast, our application combining assessment of algal groups based on chemotaxonomy (HPLC pigment analysis and CHEMTAX treatment) and identification of dominant taxa by microscopy, allowed processing of numerous samples in a reasonable time frame, while achieving quite adequate temporal and vertical resolution. Obviously, the WFD postulates that phytoplankton diversity should also be covered but, by combining these two techniques, diversity *sensu* Margalef (as the set of core species, responsible for carbon and energy flow in the ecosystem at a particular time, see Pedrós-Alió 2006) is still measurable.

Applying this method to phytoplankton samples from 12 Belgian reservoirs substantially reduced the workload and enabled us to set templates of probability of occurrence of phytoplankton functional groups (discussed below) at the maximal ecological potential (Figs. 7, 8 and 9), based on the reference conditions defined by Reynolds (2005) for natural lakes from other regions. Indeed, the method we developed takes into account taxonomic composition, assesses abundance and biomass of the phytoplankton taxa, and easily detects blooms, thereby meeting the requirements of the WFD. The results obtained with this method were used by the local authorities to access the ecological potential (in a similar way to that described by Carvalho et al. 2005), using lake typologies developed for European lakes (Solheim 2005), with slight adaptations to allow for the obvious physical differences between natural lakes and reservoirs.

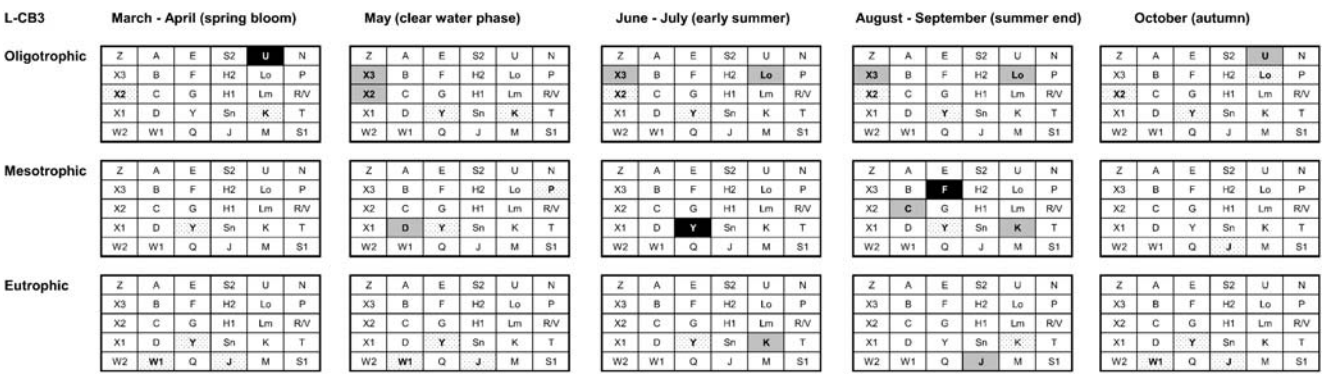

Fig. 9 Template of probability of occurrence of phytoplankton functional groups in heavily modified water bodies type L-CB3 (Central/Baltic type 3) at the maximal ecological potential, following the reference conditions defined by Reynolds (2005) for natural lakes from other regions (see Figs. 2 and 3 for legends)

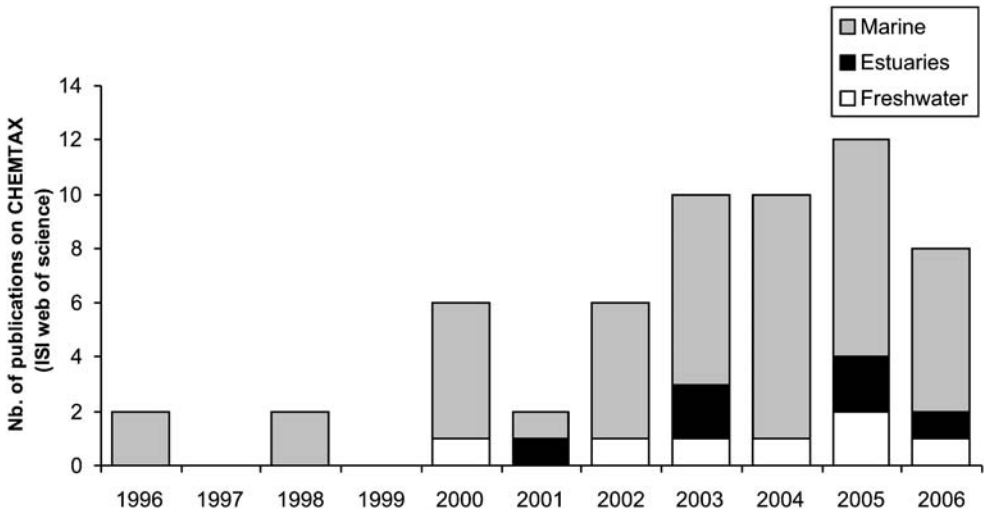

Fig. 10 Number of publications sorted by year and by environment type, containing the word CHEMTAX within article titles, keywords and abstracts (in ISI web science)

In most cases, assigning the observed phytoplankton assemblages to the functional groups of Reynolds et al. (2002) was unequivocal. We determined the functional group based on the dominant taxa identified by microscopy and filled the relative abundance of the functional group grids using the algal group contribution (as a fraction of total chl *a*) obtained from the HPLC-CHEMTAX analysis. Although the same pigment-based class may theoretically span several functional groups, there were few cases were conflicts occurred. In our study, conflicts for assigning pigment-based abundance among functional groups occurred in the following cases: N (*Tabellaria* belonging to diatoms, *Cosmarium* and *Staurodesmus* belonging to chlorophytes), P (*Fragilaria crotonensis* and *Aulacoseira granulata* belonging to diatoms, *Closterium aciculare* and *Staurastrum pingue* belonging to chlorophytes), X2 (*Plagioselmis* belonging to cryptophytes and *Chrysochromulina* belonging to chrysophytes), Lm (*Ceratium* belonging to dinoflagellates, and *Microcystis* belonging to cyanobacteria T1) and W1 (Euglenoids belonging to euglenophytes, *Synura* belonging to chrysophytes and *Gonium* belonging to chlorophytes). When uncertainties occurred in our application, they were easily resolved by a rapid enumeration of the key taxa under the inverted microscope and redistribution of the equivalent chl *a* allocated to the chemotaxonomic group concerned.

The definition of the template grids corresponding to the maximal ecological potential (Figs. 7, 8 and 9) is based largely on expert knowledge. A number of these grids, setting the phytoplankton assemblages in reference conditions for specific European regions, lake types and trophic levels, as well as the whole procedure of the water quality classification and methods of calculating the distance to the reference conditions (ecological quality ratio; EQR) can be found in Carvalho et al. (2005) and Solheim (2005). However, to our knowledge, no template grids of functional groups were available for the Central/Baltic region, and very few examples are available for heavily modified water bodies in any region of Europe. With our dataset we attempted to set these reference template grids, bringing slight changes to the observed values (taking into account both the results from the 2006 survey and historical data), i.e. reducing the proportion of bloom-forming cyanobacteria and the proportion of Y assemblages in lakes situated in the oligotrophic range. We considered that the situation observed in Belgian reservoirs in 2006 did not correspond to the worst status ever observed. At the end of March, some reservoirs were still under ice (quite unusual in this region) and, according to the Royal Belgium Meteorological Institute, August was amongst the coldest and rainiest ever recorded. It is possible that these template grids will find no application in regions other than Belgium; however, as an example, they can be viewed as a comparative tool for other researchers and water policy makers working on the WFD.

Briefly, in the development of monitoring methods in compliance with the WFD, the following question should be addressed: what is more advantageous, few samples with highly detailed taxonomical and quantitative information (achievable by standard, time-consuming, microscopic enumerations), or numerous samples with lower level of detail (given by quasi automatic, cost-effective, chemotaxonomical analysis)? The debate remains open, but in this study we demonstrate that, provided that the chemotaxonomical approach is complemented with dominant taxa identification at a level sufficient for ecological interpretation, adequate assessment of the status of phytoplankton assemblages in lakes can be achieved.

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