Diatoms : an ecoregional indicator of nutrients, organic mater and micropollutants pollution

Frédéric Rimet

UNIVERSITÉ DE GRENOBLE

THÈSE

Pour obtenir le grade de

DOCTEUR DE L'UNIVERSITÉ DE GRENOBLE

Spécialité : Biodiversité, écologie, environnement.

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Présentée par

Frédéric RIMET

Thèse dirigée par **Isabelle DOMAIZON**

préparée au sein du **Laboratoire INRA UMR-CARRTEL**
dans **l'École Doctorale SISEO**

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RESUME

Les diatomées sont des microalgues ubiquistes d'une diversité exceptionnelle. Cela en fait de bons indicateurs de la qualité des écosystèmes aquatiques ; elles sont utilisées dans ce cadre depuis plus de 50 ans. Depuis l'année 2000, la Directive Cadre Européenne sur l'Eau impose leur utilisation pour évaluer la qualité écologique des cours d'eau.

Un cadre typologique doit être utilisé afin de comparer des rivières analogues entre elles, c'est-à-dire des rivières de mêmes régions bioclimatiques, coulant sur les mêmes substrats géologiques et à des altitudes semblables. Différentes classifications écorégionales ont été définies sur la base de ces paramètres. Dans le cadre de cette thèse nous avons montré qu'à une échelle couvrant 4 pays (Espagne, France, Italie, Suisse) et à une échelle régionale (Nord-est de la France), les écorégions et la géologie sont déterminantes pour expliquer les communautés. Les paramètres caractérisant la pollution sont moins importants. Contrairement à certains auteurs, nous n'avons pas observé d'homogénéisation des communautés lorsque le niveau de pollution augmente. D'autre part nous n'avons pas observé de communautés restreintes géographiquement : cela permettrait de rassembler des écorégions distinctes géographiquement mais présentant les mêmes caractéristiques physiques.

Les diatomées présentent une diversité spécifique très importante qui peut être un frein à leur utilisation en routine. Nous avons montré dans un deuxième volet de ce travail, qu'en augmentant la précision de détermination (de la subdivision à l'espèce), les performances d'évaluation de la pollution augmentait mais beaucoup moins que le nombre de taxons. Les performances d'évaluation entre le genre et l'espèce sont d'ailleurs proches, alors qu'il y a dix fois plus d'espèces que de genres. Nous avons montré aussi que des métriques simplificatrices (formes de vie, guildes écologiques) permettaient d'évaluer aussi bien le niveau en nutriments que des indices diatomiques basés sur les espèces. Ces métriques apportent des informations supplémentaires en termes de structure de biofilm qui ne sont pas accessibles aux données en espèce.

Enfin, la pollution des rivières par les micropolluants devient une préoccupation sociétale croissante. Nous avons émis l'hypothèse que les diatomées pouvaient être de bons candidats pour évaluer la pression en herbicides. Afin de tester cette hypothèse, quatre expérimentations de 2 mois ont été réalisées en mésocosmes lotiques. Nous avons ainsi montré que les diatomées vivant entourées de matrices polysaccharidiques épaisses étaient plus résistantes aux pesticides dissous. Au contraire les diatomées présentant une surface cellulaire de contact importante avec l'eau étaient défavorisées. Ce type de métrique pourrait être utilisé *in situ* à plus large échelle.

Nous concluons sur l'intérêt d'intégrer ces métriques à la bioindication par les diatomées. Mais également nous soulignons l'importance de croiser la phylogénie et l'écologie pour mieux comprendre quelles pressions environnementales ont induit des phénomènes d'adaptation chez les diatomées. Ce type d'études contribuera à l'amélioration de la bioindication par les diatomées en eaux douces.

ABSTRACT

Diatoms are extremely diverse ubiquitous microalgae. This makes them good indicators of the quality of aquatic ecosystems, and they have been used for this purpose for the past 50 years. Since 2000, the European Water Framework Directive has required them to be used for assessing the ecological quality of watercourses.

A typological framework has to be devised in order to compare rivers that are comparable, i.e. rivers with the same bioclimatic regions, that flow over the same geological substrate at similar altitudes. Various ecoregional classifications have been defined using these parameters. At a scale covering 4 European countries (Spain, France, Italy, and Switzerland) and at a regional scale (north-east France) we show that ecoregions and geology are determinant in explaining communities, and that pollution-related parameters are less important. Unlike some other authors, we did not observe any homogenization of communities as the level of pollution level increased. Moreover, we did not observe geographically restricted communities, which make it possible to pool data from geographically distinct ecoregions with the same physical characteristics.

Diatoms display a very high degree of species diversity, which can be a problem for their routine use as assessment tools. We showed that when the precision of identification was increased from sub-division to species level, pollution assessment performances also increased, but to a much less marked extent than the number of taxa. Assessment performances at the genus and species levels are similar, whereas there are ten time more species than genera. We also showed that simplifying metrics (life-forms, ecological guilds) can be used to assess nutrient levels as effectively as diatom indices based on species. Furthermore, these metrics provide additional information about biofilm structure that is not available from species-based data.

Finally, micropollutant pollution of rivers is of increasing concern to citizens. We hypothesize that diatoms could be good candidates for assessing herbicide pressure. Four experiments lasting 2 months were conducted in lotic mesocosms. We showed that diatoms surrounded by thick exopolysaccharide matrices were more resistant to dissolved pesticides. On the other hand, diatoms with a high cell surface in contact with the water were disadvantaged. This kind of metric could be used *in situ* at a larger scale.

We conclude that these metrics could be useful for the purposes of diatom bioassessment. However, we also stress the importance of combining phylogeny and ecology to clarify which environmental pressures are forcing diatoms to adapt. Such studies will enhance diatom bioassessment.

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Keywords

Biomonitoring, diatoms, ecoregion, herbicide, life-form, taxonomy,

List of papers

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2. Rimet F., 2009. Benthic diatom assemblages and their correspondence with ecoregional classifications: case study of rivers in north-eastern France. *Hydrobiologia*, 636: 137-151.

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1. Introduction

1. Introduction

a. General framework

Water is an essential resource for human beings. Its uses are crucial for human life and include consumption as drinking water, agriculture, energy generation, and industrial uses. Both the quantity and quality of this fundamental resource warrant special attention. Its management and monitoring is a central concern for Governments, and may affect international relations. As a result, countries have been implementing national water monitoring programs for several decades. Rivers constitute a major aspect of water resources. For instance, in France a dense network of control in rivers throughout the country was set up in the 1980s, and water quality has been periodically assessed using diverse indicators (physical, chemical and biological). The data provided by these indicators have provided the basis for the implementation of restorative actions, where required, such as the construction of waste water treatment plants, financial incitements to reduce agricultural fertilizer inputs etc...

In this context, the European Union sought to federate Member States around a common strategy for water quality policy; this was particularly crucial since many rivers or lakes cross national borders. In 2000, the Member States of the European Community decided to adopt a "Framework for Community action in the field of water policy" (European commission, 2000). This framework for action, known as the Water Framework Directive, set out to prevent or reduce water pollution, promote its sustainable use, improve the health of aquatic ecosystems and mitigate the effects of floods and droughts. One of the main objectives of the Water Framework Directive is to achieve "good ecological status" by 2015. This date is now looming, and delays are already scheduled. However, one of the most important points is that this Directive underlines the importance of assessing the impact of human beings on the aquatic biota, particularly in surface water such as water courses.

b. Bio-assessment in rivers

"Biological assessment is an evaluation of the condition of a waterbody using biological surveys and other direct measurements of the resident biota in surface waters" (Barbour et al., 1999). Assessment of human impact on aquatic biota started more than a century ago with Kolkwitz & Marson (1908) who assessed the impact of organic matter concentration on aquatic vegetation. In particular, among other biological organisms, they proposed a list of micro-algal species that are indicators of the saprobic level for use in assessing the level of organic matter. They had already included a list of diatom species. The main interest of such an approach is that an aquatic biota reflects both the stresses it has encountered over time and the fluctuations of the environment. Methods based on aquatic biota were subsequently developed to assess the pollution of rivers. The concept of bio-indicators emerged. A bio-indicator can be defined as a species or population that,

because of the ecological features of the species that constitute the community, provides an integrated record of the ecological environment - an aquatic ecosystem for instance - and thus provides early detection of biotic and abiotic modifications (adapted from Morin, 2006). It is crucial to use several bio-indicators (e.g. fish, macro-invertebrates, diatoms…) in order to detect the combined effects of various stressors that may have impacts at different levels of ecological complexity. A panel of indicators provides a broad measure of the overall impact on the biota.

Rivers are an important part of water resources, and several bio-indicators are used for their ecological assessment. The Water Framework Directive requires five main bio-indicators to be used for rivers. First, fish populations; they are particularly suitable for warning about interruptions of river connectivity and global pollution. Second, macro-invertebrates are an essential biological element for assessing the diversity of river micro-habitats, and their organic and nutrient pollution. Third, macrophytes are good indicators of river eutrophication and, to a lesser degree, of gross organic pollution. Fourth, Phytoplankton is a bio-indicator used in large lentic rivers and makes it possible to assess the nutrient level. Finally, diatoms indicate the levels of nutrients and organic matter.

c. Diatom biology

Diatoms (phylum of the Bacillariophyta) are eukaryotic, unicellular algae which range in size from 2 µm (e.g. *Minidiscus trioculatus* in Quiroga & Chretiennot-Dinet, 2004) to about 500 µm (e.g. *Coscinodiscus wailesii* Gran et Angst). They belong to the Chromista kingdom, and are sister species of other important algal classes that occur in freshwater ecosystems, such as the Chrysophyceae, Synurophyceae and Eustigmatophyceae. Diatoms are present in a wide range of habitats, ranging from dry habitats (e.g. Bérard et al., 2004), to freshwater and marine habitats.

The particularity of diatom cells is that they are enclosed in a siliceous exo-skeleton also known as the frustule (Figure 1). The frustule is essentially constituted of silicate ($SiO_2.H_2O$) (Round et al., 1990), and consists of two valves (the epivalve and hypovalve) connected by a girdle. The frustule is also ornamented by several important features such as:

- Pores, that allow nutrients to be exchanged between the environment and the cell, and mucilage to be secreted,
- The raphe, which is longitudinal fissure that enables diatoms to move by adhering to substrates after excreting microfibrils through the raphe,
- Spines, which enable connecting cells to form colonies or enable individual cells to be sustained in the plankton.

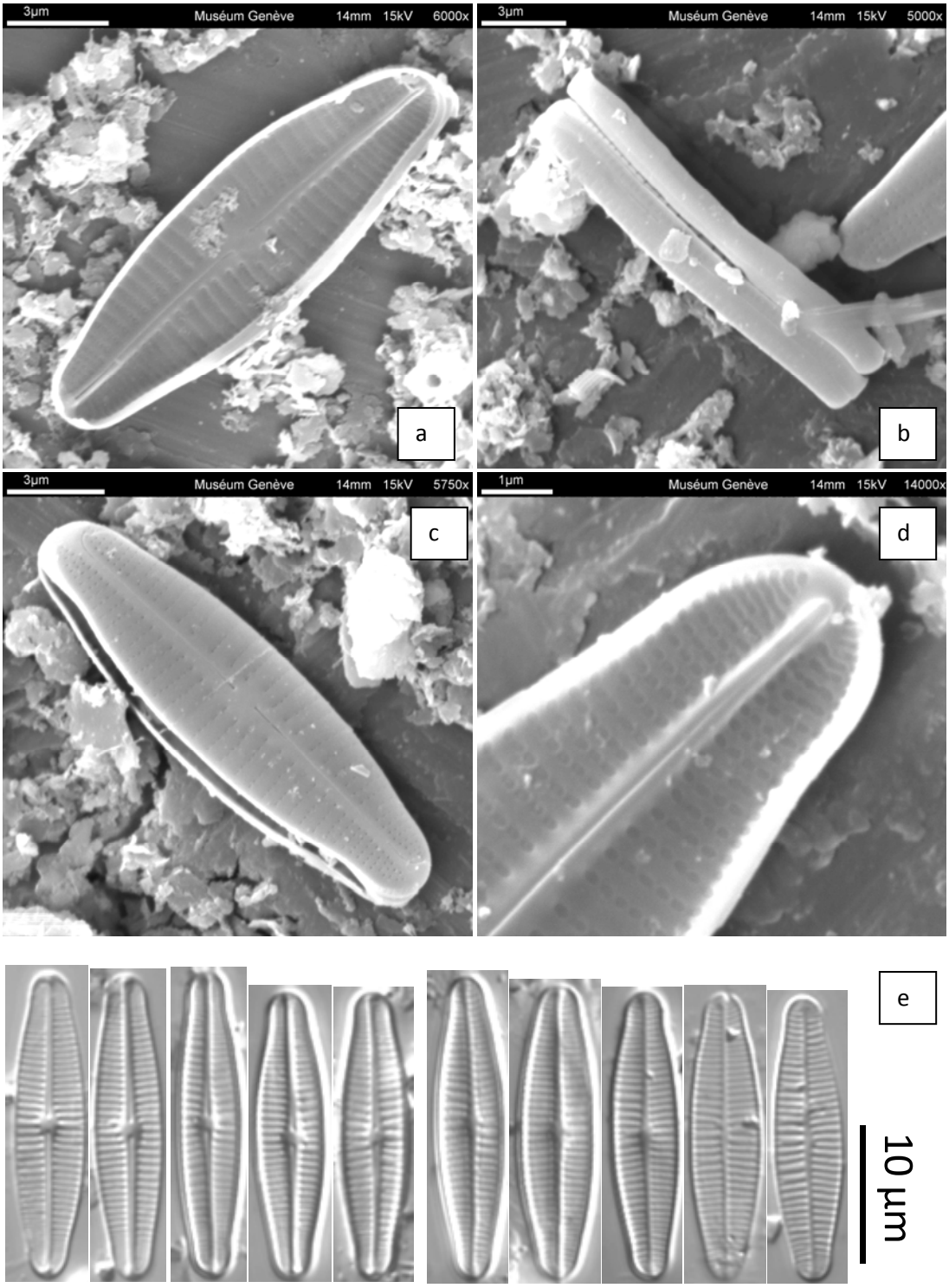

Figure 1: Frustule of *Achnanthidium druartii* Rimet & Couté. (a-d): scanning electron microscopy, (a): valve view, inner side, (b) griddle view, (c): valve view, outer side, (d) detailed view, inner side, (e) light microscopy.

These photosynthetic organisms have one or more chloroplasts, depending on the taxon. The number of chloroplasts is relatively constant within a genus (e.g. 1 chloroplast in *Achnanthidium,* 2

chloroplasts in *Navicula, Nitzschia,* and several in *Diatoma, Melosira*). Chloroplasts consist of chlorophylls a and c, and also contain accessory pigments (fucoxanthin and β-carotene) that give them their characteristic brown color (see the chloroplast in Figure 2, and the biofilm in Figure 4). Cells store energy from photosynthesis in the form of chrysolaminarin and lipids, which form small drops inside the cells (Round et al., 1990). Diatom cells also contain the classical intracellular organites of eukaryotic cells such as the nucleus, mitochondria, and Golgi apparatus.

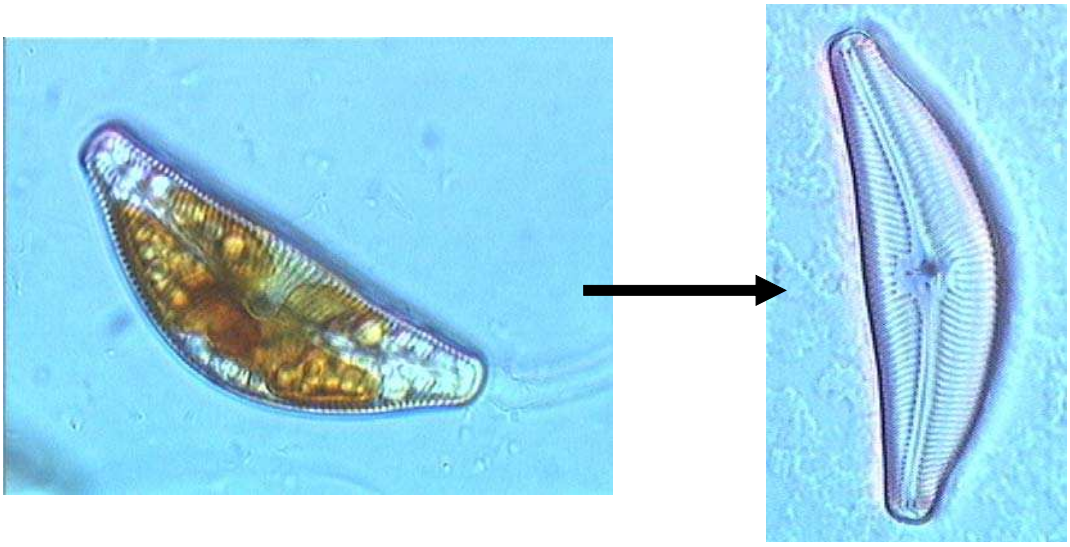

Figure 2: *Cymbella tumida* (from the Thonon Culture Collection, culture TCC519). (left): living material; (right): cleaned frustules after nitric acid treatment.

The diatom life cycle (Figure 3), like that of many other eukaryotic protists, consists of two phases.

The first phase corresponds to asexual reproduction. A simple mitotic division occurs, and each daughter cell keeps one of the parent cell's valves. The new part of the frustule is always constituted inside that of the parent. Because of the rigidity of the cell wall, the new valves formed are slightly smaller than the parental valve. Over time, as the cells go on dividing, a reduction in cell size is observable. Such phenomena can be clearly visible in clonal cultures where sexual reproduction does not occur. In the end, very small cells are formed which can be no longer viable.

Sexual reproduction enables cells to recover their maximal size. During this second phase, gametes are produced. Reproduction is isogamic[1] or heterogamic[2] depending on the taxon. An auxospore is then produced, which goes on to develop and divide by mitosis to produce two cells, each with the maximum size.

[1] Isogamic : the gametes display the same morphology
[2] Heterogamic (=anisogamic) : the gametes display different morphologies

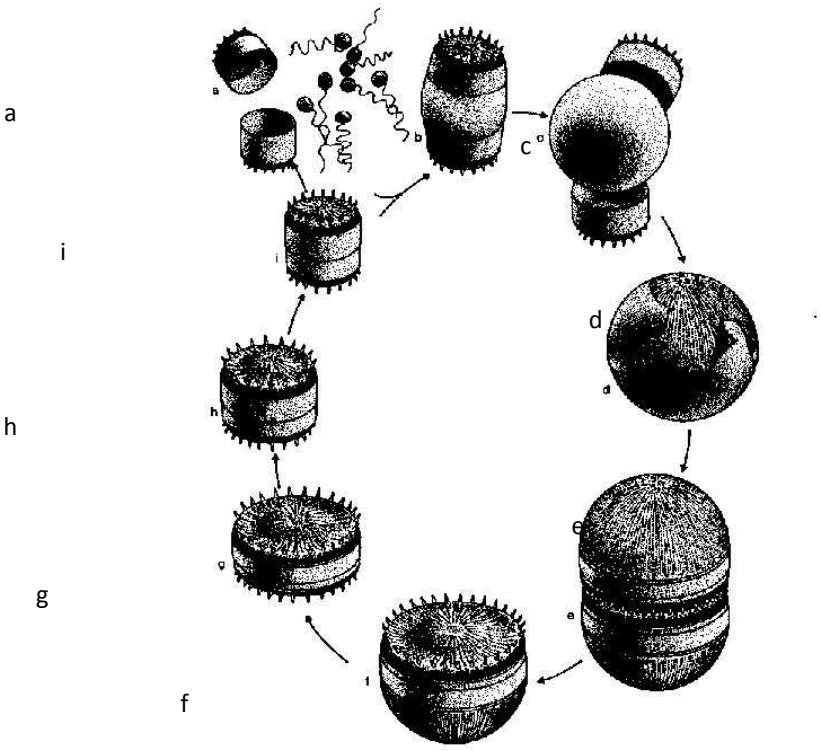

Figure 3: Life cycle of a centric diatom (*Stephanodiscus*), from Round et al. (1990). (a): formation of motile gamete, (b-c): auxospore formation, (d-e): first division, (f): initial cell, (g-i): mitotic division and cell size reduction.

Frustule morphology is an essential feature for both taxonomy and identification. Most taxonomic characteristics are defined on the basis of particular frustule features: symmetry and outline of the frustule, number of raphes, striae density, pore density, pore structure... Some of these characteristics can be observed using light microscopy (e.g. frustule outline, striae density), but others require scanning electron microscopy (e.g. pore density and shape, cf. Figure 1d). To observe these characteristics correctly, the diatom has to be cleaned to remove the protoplast (e.g. chloroplasts, nucleus, lipid drops) (see Figure 2). Hydrogen peroxide or nitric acid are often used for this purpose, and there are European standards to ensure that it is carried out properly (e.g. Afnor, 2003, 2007). Observations can be then carried out using light microscopy (100x immersions lens) or scanning electron microscopy.

d. Diatom diversity: an advantage for bioassessment?

Diatoms constitute an extremely diverse phylum, encompassing about 100,000 taxa (Mann & Droop, 1996). Several hundred new taxa are discovered every year according to the Catalogue of Diatom Names of the California Academy of Sciences (compiled by E. Fourtanier & J.P. Kociolek). Most of these new taxa are described on the basis of frustule morphology and divide species complexes into

numerous species with more restricted ranges of morphological variations. Unfortunately, published studies too rarely provide any additional details about their ecology, but when they do, it often emerges that these new diatom species have narrower ecological spectra than the species complex to which they belong. This is true, for instance, with *Achnanthidium dolomiticum,* which can be pinpointed to water flowing over a particular lithology (carbonate rocks) with low nutrient levels. *A. minutissimum,* the species complex to which *A. dolomiticum* belongs, also shows some preference for low nutrient concentrations, but it has a wider spectrum of lithological preferences (Cantonati & Lange-Bertalot, 2006). Moreover, recent studies based on phylogenetic and mating techniques carried out on species complexes such as *Nitzschia palea* (Trobajo et al., 2009), *Sellaphora pupula* (Evans et al., 2008; Mann et al., 2004), *Navicula cryptocephala* (Pouličková et al., 2010), *N. phylepta* (Vanelslander et al., 2009), *Gomphonema parvulum* (Kermarrec et al., 2012) revealed unexpected cryptic diversity, which is sometimes difficult to relate to particular environmental parameters or geographical distributional patterns. These studies can be expected to increase the estimated total number of diatom taxa known to exist worldwide.

In addition, "amnesic behavior" or incomplete familiarity with the taxonomy literature for established species can also artificially lead to the re-creation of species that have already been described. One example of this is *Cyclotella operculata* f. *minuta,* which was described by Grunow in Van Heurck (1882) and over century later described by Druart & Straub as *C. costei* (Druart & Straub, 1988). Two years later, another conspecific taxon (*C. cyclopuncta*) was introduced (Hakansson & Carter, 1990).

Despite these complications, this diversity makes diatoms excellent bioindicators since both nutrients and organic matter have long been shown to control the relative abundance of species (and even of varieties of species) in rivers (e.g. Patrick, 1961; Lange-Bertalot, 1979). Their ubiquity is also a crucial advantage. Moreover, benthic diatoms constitute a major part of the biomass in temperate rivers. This has led several authors to develop autecological indices based on the ecological preferences of diatom taxa (Butcher, 1947; Fjerdingstad, 1950; Hustedt, 1957; Zelinka & Marvan, 1961). Diatoms are now used worldwide alongside macroinvertebrates, fish, phytoplankton and macrophytes to assess the ecological quality of rivers (e.g. Kelly et al., 1998; Lobo et al., 1995; Chessman et al., 1999 and Coste et al., 1991). Most of these indices are based on the formula of Zelinka & Marvan (1961):

$$\text{Index} = \frac{\sum_{j=1}^{n} aj.sj.vj}{\sum_{j=1}^{n} aj.vj}$$

where a_j = abundance or proportion of valves of species j in sample, s_j = pollution sensitivity ('optimum') of species j, and v_j = indicator value ('tolerance'). This equation is typically a weighted average equation implying a unimodal response curve, even if a large majority of diatom species do not display any such trend, as was demonstrated in USA (Potapova et al., 2004).

For about 14 years, field sampling, diatom preparation and counting have been standardized at the European level (Kelly et al., 1998; Afnor, 2000, 2003, 2004, 2007). This ensures good comparability of the diatom inventories produced during the last decade in the different European countries. Basically, diatoms are scraped from several stones (5 stones for the French standard) taken from lotic zones of rivers (Figure 4).

Figure 4: Sampling diatoms from stones in rivers

Most of the existing biotic indices for diatoms are based on species sensitivity. However, the question of taxonomic resolution is often posed without any clear justifications, assuming that the most precise determination would be the most effective for diatom biomonitoring, even if it has to be applied on large geographical scale by large numbers of people who may not necessarily all have the same identification skills. However, some authors have shown that there was no clear increase in the assessment power when taxonomic resolution was increased for macroinvertebrates (Jones, 2008). Moreover, identification to diatom species level (or the infra-species level) can be challenging, because of the tremendous diversity of these organisms, and because of incessant taxonomical changes. Finally, from a financial point of view, identifying diatoms to species level takes much longer than doing so to genus level, which makes it more expensive. Some authors (e.g. Zampella et al., 2007) think that the large number of diatom species and the difficulty of identifying them limits their use for routine purposes, as numerous identification errors occur at species level. Few studies have attempted to estimate the advantage to reduce the identification precision from species to class or sub-division level for biomonitoring purposes.

e. Diatom life forms and ecological guilds

All diatoms are basically unicellular algae, but they exhibit considerable diversity of life-forms and many of them can form colonies. Taxa may even present several life-forms during their development. This is the case for instance with *Cymbella* species, which can be unicellular and free moving at one

stage, but attached to a peduncle and immobilized at another stage. Life-forms can be classified as follows:

1. If diatoms are **solitary cells**, they may be:

    a. **Unattached**: the diatoms float (e.g. Centric diatoms in plankton) or move freely (e.g. free moving cells of *Nitzschia* or *Navicula*). See *Nitzschia palea* in Figure 5d.

    b. **Attached**: the diatoms can be attached to substrates in several different ways:

        i. **Adnate**: cells are firmly attached by their valve face (e.g. *Cocconeis placentula* var. *euglypta* Figure 5e) or by their girdle view (e.g. *Eunotia*).

        ii. **Mucilage pad**: cells produce mucilage on a pole that sticks to the substrate (e.g. *Diatoma, Ulnaria*)

        iii. **Mucilage stalk**: cells produce a stalk through apical pore fields and this sticks to the substrate. The stalk can be simple (one cell) or can link several cells (see arbuscular colonies). Several genera such as *Gomphonema* and *Achnanthidium* can produce stalks.

2. There is considerable diversity in the types of **colonies,** but for freshwater taxa they can be assigned to the following classes:

    a. **Chain colonies**: centric cells are juxtaposed by their valves. Cells can be linked by spines (*Aulacoseira*) or by granules (*Melosira varians*). In some cases (*Cyclotella, Thalassiosira*) the cells do not touch and are simply held together by threads of polysaccharides.

    b. **Ribbon colonies**: pennate diatoms are juxtaposed by their valves, and are linked by spines (*Fragilaria capucina* var*. vaucheriae* Figure 5a) or adhere by means of mucilage excretions from their whole valve face (*Fragilariopsis,* also observed on *Nitzschia* sp. in cultures, Kermarrec com. pers.).

    c. **Zig-zag colonies**: pennate cells are connected by mucilage at their opposed poles (*Diatoma*).

    d. **Rosette-forming colonies**: pennate cells produce a short stalk at one pole that sticks to a substrate. After several cell divisions, they produce colonies that resemble fans/rosettes.

    e. **Star colonies**: pennate cells are connected by mucilage at their neighboring poles, such as *Asterionella formosa*.

    f. **Arbuscular colonies**: stalks are produced at one pole. The stalks diverge from each cell and form branching colonies (e.g. *Gomphonema* sp. Figure 5c, *Cymbella, Rhoicosphaenia*)

g. **Mucous tubule colonies**: several diatom genera form tubes (e.g. *Encyonema minutum* Figure 5b, *Frustulia, Berkeleya, Parlibellus*), and cells move inside them in file.

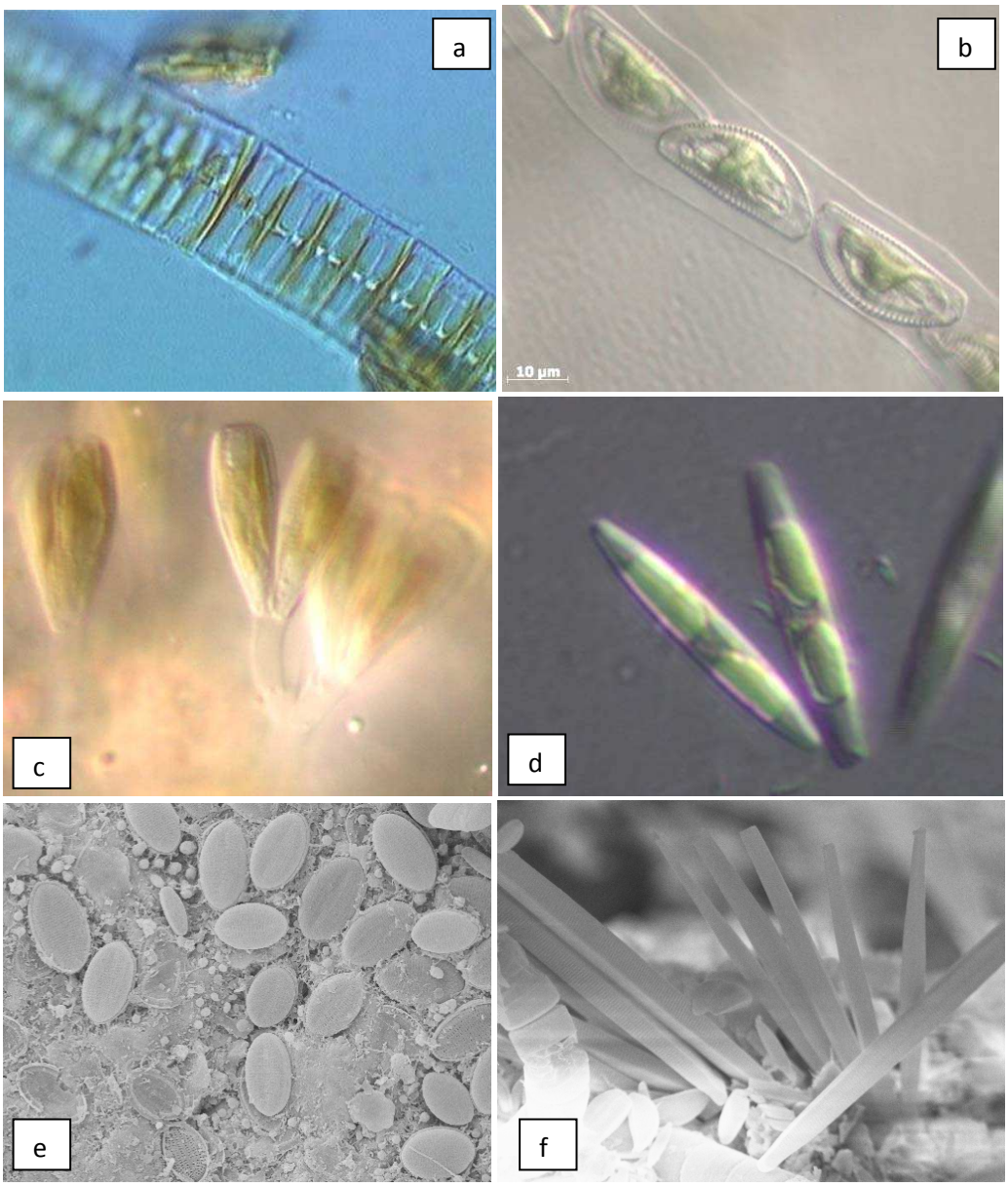

Figure 5: Examples of diatom life-forms. (a) ribbon colony with *Fragilaria capucina* var*. vaucheriae* (from the Thonon Culture Collection, culture TCC372), (b) mucous tubules with *Encyonema minutum* (live sample from the shore of Lake Geneva, 2011), (c) stalks with *Gomphonema* sp. (live sample from the shore of Lake Geneva, 2012), (d) fast-moving diatom, such as *Nitzschia palea* (culture TCC764), (e) prostrated *Cocconeis placentula* var. *euglypta* (dehydrated biofilms from the river Rollingerbaach, Luxembourg, 2003), (f) rosette of *Ulnaria* (dehydrated biofilms from the river Rollingerbaach, Luxembourg, 2003).

As pointed out in Round et al. (1990), the types of colonies and life-forms of diatoms have been subjected to strong selection. Colonies and life-forms are responses to attachment, light and nutrient capture, sinking rate, and habitat selection. Relationships between the abundances of such life-forms

and nutrients have been established in experimental contexts (Hoagland et al., 1982; Pringle, 1990). Diatom cell size is also a response that can be related to the resources available (Finkel et al., 2009). Unfortunately, these different metrics (abundance of cell sizes and life-forms) are seldom used for routine biomonitoring - at least in Europe - even though their use could yield valuable information. The diversity of these life forms can be assigned to larger groups, known as ecological guilds. A guild can be defined as a group of species - equivalent to functional groups in the phytoplankton (e.g. Padisák et al., 2009) - which live in the same environment, but may have adapted differently to abiotic factors. For instance Passy (2007) defined the 'low-profile' guild which encompasses species of short stature including prostrate, adnate, and erect diatoms. This group can withstand physical disturbances, but does not tolerate nutrient enrichment. The 'high-profile' guild comprises large species, or those which tend to form colonies (e.g. tube-forming, filamentous, branched diatoms). This group cannot resist turbulence but benefits from nutrient enrichment. The 'motile' guild consists of fast-moving species (e.g. *Navicula, Nitzschia*) and is adapted to both a turbulent environment and high nutrient concentrations.

f. Geographical distribution of diatom assemblages

The composition of diatom assemblages in rivers is affected by parameters that can be artificially divided into two kinds: those that are affected by anthropogenic activity (e.g. nutrients, organic matter concentration), and those that are not (e.g. geological substrate, climate type, altitude). However, to assess human impact correctly and in order to compare human impact in comparable rivers, natural variability – i.e. parameters that are unaffected by anthropogenic activity - must also be taken into account. River typologies have been defined in order to make it possible to compare rivers that correspond to the same river type. This concept has been taken on board in the Water Framework Directive. Ecoregional classifications have been developed based on abiotic factors such as geology, relief and climate, which are generally acknowledged to determine river functioning at the large scale (Naiman et al., 1992). In France they have been developed by Wasson et al. (2002, 2010) (see Figure 6). An ecoregional classification is also proposed in the Water Framework Directive which includes cross-border ecoregions; these correspond to the ecoregions defined by Illies (1978) (see Figure 6).

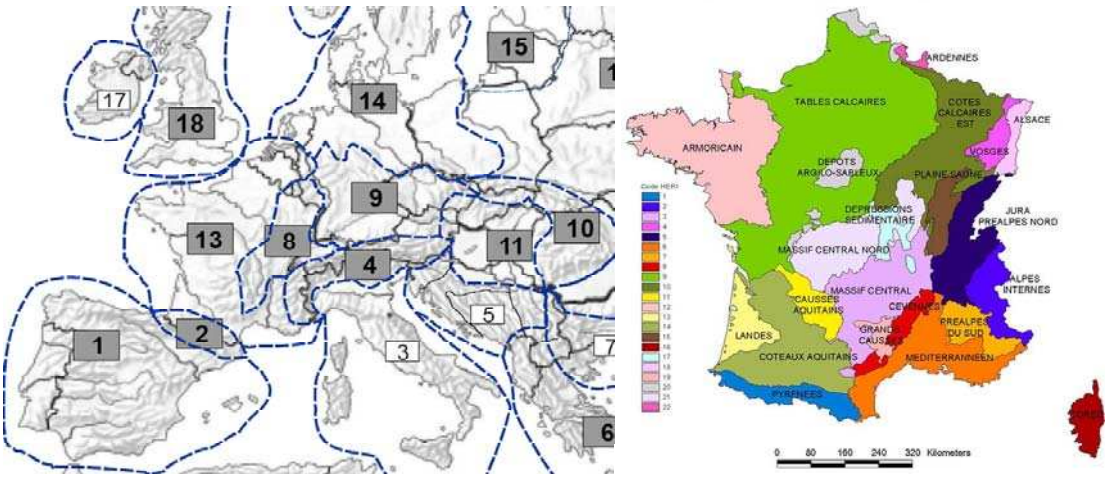

Figure 6: Ecoregional classification in use in Europe. Illies (1978) classification on the left and hydroecoregions (Wasson et al., 2002, 2010) on the right.

However these abiotic classifications were devised without taking into account diatom assemblages or without knowing which parameters could structure them at the large spatial scale. For instance, Illies' ecoregions were defined from river macrofauna. No studies checking the match between ecoregions and diatom assemblages in Europe or in France or indeed the match between diatom assemblages and other parameters such as pollution levels had been conducted before these ecoregions were applied to diatom biomonitoring. Furthermore, several authors pointed out that local factors appear to determine the aquatic biota to a greater extent than large scale factors (Hawkins et al., 2000), and that pollution has homogenized diatom communities in several Spanish river basins (e.g. Leira & Sabater, 2005; Tornes et al., 2007) and American ecoregions (Pan et al., 2000a). These studies support the argument that the species composition of small (especially unicellular) organisms is determined by local factors rather than regional ones, because of their high dispersal abilities (e.g. Finlay, 1996).

g. Diatoms and pesticide contamination

Many human activities are impairing the quality of watercourses. In particular, micropollutants are of increasing concern to both citizens and politicians. This is evident to such an extent that in some regions, such as the Canton of Geneva in Switzerland, the eutrophication of rivers is no longer regarded as a the main problem, and micropollutant contamination is now seen as the primary problem that has to be managed (A. Cordonier from the Service de l'écologie de l'eau in Geneva, com. pers.). About 90% of European rivers are polluted by persistent organic micropollutants, and herbicides are among those most often detected (Loos et al., 2009). This has led governments to launch drives to reduce pesticide use. For instance, the French government plans to reduce pesticide use by 50% over the next 10 years (Ministère de l'Agriculture et de la Pêche, 2008).

The beneficial impact of these actions on the aquatic biota remains to be assessed. So far, bioassessment tools have been developed mainly to assess nutrient and organic matter

concentrations in water. Routine bioindicators of this particular kind of anthropogenic perturbation are only now beginning to be devised for macroinvertebrates and diatoms.

Studying the effects of toxicants on living organisms encompasses a wide variety of methodologies that are known collectively as "ecotoxicology". This scientific discipline includes simple replicable studies, such as single species tests, to define effective concentrations of one or a mixture of toxicant(s) that reduce growth or some other end points (EC50), and also encompasses studies integrating greater ecological complexity by controlling a set of environmental parameters, for instance in mesocosms. Finally, ecotoxicology also encompasses studies that combine high levels of complexity and representativity with *in situ* studies where no/few environmental factors are controlled. Each level of complexity contributes decisive information for understanding the impact of toxicants on the biota. Single-species tests are used to explore mechanistic aspects. In contrast, with *in situ* studies it is often difficult to progress beyond the descriptive stage, because of the diversity of the factors involved and their variability (Boudou & Ribeyre, 1997), but they can be used to validate hypotheses formulated at lower levels of complexity.

Using bioindicators to assess impact of toxicants is challenging and currently still being developed. Several tests have been carried out in Western France (Schafer et al., 2007) using specific metrics to assess the impact of toxicants on macroinvertebrates (Liess & Von der Ohe, 2005; Schafer et al., 2007). The effect of heavy metals on diatom communities has also been investigated (e.g. Gold, 2002; Morin et al., 2007; Peres et al., 1996), and this has revealed a modification of their species composition. Given their mode of action, herbicides can potentially disrupt diatoms, which are photosynthetic organisms. Diatom communities could therefore potentially be used as indicators of herbicide contamination. Nevertheless there have been few studies relating herbicide impact to the composition of diatom communities (e.g. Debenest et al., 2008; Morin et al., 2009a), although this would be of interest given the extent of hydro-ecosystem contamination by herbicides (Loos et al., 2009).

h. Main objectives of the study

Diatoms were first used to monitor rivers about 50 years ago, but their intensive use only started in 2000 in Europe after the introduction of the Water Framework Directive. This has resulted in the generation of a mass of data that has made it possible to address new ecological issues. Moreover, the characteristics of river pollution have changed over recent decades, and this has also opened new fields in diatom bioassessment.

- **Diatoms and ecoregions**

As we have already said, ecoregions were defined on the basis of large scale factors. Diatom assemblages and the parameters that structure them at large spatial scale were relatively little known. Our first objective was therefore to find out whether the existing ecoregions had any relevance to diatom assemblages. We therefore assessed the match between different ecoregional

classifications and diatom assemblages at two different spatial scales, one regional (North Eastern France) and one encompassing several countries (Western European).

Several authors have reported that the composition of diatom assemblages homogenizes over the ecoregions as pollution levels increase (e.g. Leira & Sabater, 2005; Tornes et al., 2007; Pan et al., 2000b). On the other hand, other authors have observed that ecoregions were of prime importance for explaining the diversity of diatom assemblages (Soininen, 2004, 2007; Rimet et al., 2004). Our second objective was therefore to find whether there is a threshold level above which pollution outweighs ecoregions in accounting for diatom assemblages.

- **Taxonomic resolution and alternative metrics in diatom bioassessment**

The second part of this research work concerns the metrics actually used for diatom bioassessment and raises the question of whether they should be updated:

Firstly, diatoms exhibit tremendous diversity at species level. However, this diversity can also restrict their routine use, because of the difficulty of identifying so many species. We therefore wanted to assess the influence of taxonomic resolution on bioassessment with regard to three points: firstly on the description of assemblage composition, secondly on the assessment of environmental parameters (nutrients, organic matter, major-ion content), and thirdly on the correspondence with typological classifications (ecoregions, river size).

Secondly, we tested metrics that offer a potential alternative to the pollution sensitivity of species (which is used in most of the existing diatom bioassessment tools). This second part involved testing the assessment power of several life-forms, size classes and ecological guild abundances over a large area in France and comparing it to existing diatom indices.

- **Diatoms and pesticide contamination**

The final objective of this study was to assess the pressure of pesticides, and herbicides in particular, on river biota. Since diatoms are photosynthetic organisms, the hypothesis is that they should be good indicators of herbicide pollution.

Several studies have already shown that the taxonomic composition of diatom communities in rivers is impacted by herbicides (e.g. Dorigo et al., 2007; Morin et al., 2009b; Guasch et al., 1997, 1998). Nevertheless, diatoms display such wide species diversity that the species compositions identified were different in every study, and the species found to be resistant and sensitive, respectively, were never the same either. No general trend can be deduced from these different studies. We wanted to extend our thinking on metrics by applying it to the herbicides question. Our objective was to reduce diatom species diversity to about ten simple metrics. Moreover, we hypothesized that this should make it easier to develop and test hypotheses concerning herbicide contamination and abundance metrics because the trends should be more general. A lotic mesocosm approach was chosen for this

purpose, because this experimental approach makes it possible to control many environmental factors, making it easier to check hypotheses.

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2. Diatoms and ecoregions

2. Diatoms and ecoregions

a. Preamble and major results

• Introduction

From an anthropocentric point of view, abiotic parameters in rivers can be assigned to two categories: those that are affected (nutrient, organic matter concentrations) and those that are unaffected by human activity (geological substrate, climate, altitude). Both categories have an important structuring effect on diatom assemblages. This has been demonstrated, for instance, for the dominant geology in the river basin (e.g. Tison et al., 2004; Cantonati, 1998), the altitude of the sampling site (Ndiritu et al., 2006; Jüttner et al., 2010), and the distance from the source (Potapova & Charles, 2002b). It has long been known that the concentrations of nutrients and organic matter structure diatom communities (e.g. Butcher, 1947; Fjerdingstad, 1950; Zelinka & Marvan, 1961), and this led scientists to develop tools based on the sensitivity of diatom species to assess the water quality of rivers (see for instance the review of Ector & Rimet, 2005). These tools were usually indices that give a score on a scale. However, for biomonitoring purposes, water managers were soon grouping rivers into homogenous types in order to make it possible to compare river-sites that are in fact comparable: the objective being to eliminate the variability of natural parameters in order to measure only the impact of human activity on aquatic biota. Ecoregions adapted to rivers have been developed for this purpose in Europe and integrated in the Water Framework Directive (European Commission, 2000). Ecoregions have conventionally been defined on the basis of abiotic environmental parameters, such as geology, climate, altitude and river size (e.g. Bailey, 1995).

In Europe, several different methods for constructing ecoregions are proposed in the Water Framework Directive, and these lead to different ecoregional classifications. One classification system A in the Water Framework Directive includes large cross-border ecoregions and is based on the ecoregions defined by Illies (1978). These are biogeographical regions corresponding to the aquatic fauna and are essentially based on macro-invertebrates. System B in the Water Framework Directive proposes a list of the abiotic factors to use, the class boundaries of each parameter are fixed by the author of the ecoregion. This methodology was the one chosen in France, and has led to the definition of 22 hydro-ecoregions (Wasson et al., 2002); these were subsequently reduced to five regions, known as diato-ecoregions, corresponding to the main diatom assemblages present in France (Tison et al., 2005).

The original abiotic classifications - known as hydro-ecoregions - were devised without reference to diatom assemblages and our first aim was to find out whether they reflected diatom assemblages at different spatial scales. We therefore assessed the match between these different ecoregional classifications and diatom assemblages. This assessment was carried out at two different spatial scales: a regional scale (32,700 km²) and a European scale (340,000 km², extending over several countries).

Several authors have observed that local factors explain aquatic biota to a greater extent than large scale factors (Hawkins et al., 2000), and that pollution has homogenized diatom communities in several Spanish river basins (e.g. Leira & Sabater, 2005; Tornes et al., 2007) and American ecoregions (Pan et al., 2000). In contrast, other studies have reported that large scale factors and ecoregions are determinant for diatoms (Soininen, 2004, 2007; Rimet et al., 2004). Our second goal was therefore to find out whether there is a threshold above which factors affected by human activity explain diatom assemblage variability to a greater extent than ecoregions. To address this second point, we tested two areas where diatoms were sampled using the same methodology (European standard). The first area had experienced very little anthropogenic pressure and consisted of high altitude rivers in Spain, France, Italy, and Switzerland. The second area was chosen in one of the most industrialized parts of France, the North-east: this region presents contrasting areas, some of which are highly industrialized, others with intensive agriculture but also including natural areas. We were expecting to find that the match between ecoregions and diatom assemblages would be closer for the high altitude rivers than for the rivers in North-eastern France.

- **Methodology**

The same datasets were used for both purposes. Diatom counts, chemical and physical analyses, and typological information carried out in the framework of routine river quality assessment in Spain, France, Italy, and Switzerland were used to compile a database. Additional data from research studies were also added (Cantonati, 1998; 2001). Diatom samples and counts were carried out in accordance with the European standard (Afnor, 2003).

The first dataset included sampling sites at altitudes of over 800 m. This altitude was chosen because it corresponds to the Water Framework Directive definition of “high-altitude rivers”. The regions covered comprised the Iberian system (Spain), the Pyrenees (Spain and France), the Massif-Central (France), and the Alps (France, Italy, and Switzerland). The second dataset included sampling sites in North-Eastern France, and corresponded to a region presenting highly contrasting zones (ranging from highly industrial to agricultural and natural, from lowlands to low mountains).

- **Results and Discussion**

1. Do ecoregions have any relevance to diatom assemblages?

The first area studied included 261 sampling sites in high altitude rivers sampled between 1993 and 2003. Four different ecoregions, corresponding to system A of the Water Framework Directive, were included: the Iberian region, the Pyrenees, the Alps, and the Western-Highlands (the Massif-Central and the Jura). This classification, initially developed for macroinvertebrates (Illies, 1978), closely matched the diatom assemblages of the area: the assemblages of the different ecoregions were statistically different from each other (MRPP test, $p<0.01\%$). The ecoregions matched the diatom assemblages more closely than other parameters such as altitude, distance from the source, and pollution. System A ecoregions are geographically continuous ecoregions. Nevertheless, we observed that several diatom assemblages were present in separated ecoregions (for instance some

assemblages were present in both the Iberian region and the Western-Highlands, and others in the Alps and the Pyrenees). When diatom assemblages in the Alps were compared to those in the Himalayas, Cantonati et al. (2001) suggested that the prominent role of cosmopolitan taxa in mountain areas could explain the similarities between the assemblages found in these two widely separated mountainous massifs.

The second area included 744 sampling sites in North-Eastern France. It encompassed three major river basins: the Meuse basin - heavily dominated by agriculture, the Moselle basin - mostly dominated by industrial activities, and the Sarre basin - occupied by forest in its upper reaches and by industrial and agricultural activities lower down. Two ecoregional classifications were tested. The first classification was that corresponding to the French hydro-ecoregions (Wasson et al., 2002), the study area encompassed seven of these. The second classification, that of the diato-ecoregions, was much simpler and encompassed just three regions. Both these classifications matched the diatom assemblages, and showed that diatom assemblages were statistically different from one ecoregion to another (MRPP test, $p<0.01\%$). Nevertheless the 7 hydro-ecoregions provided a much better description of the diversity of diatom assemblages than the diato-ecoregions. Similarly to the high altitude mountains study, two hydro-ecoregions, the Vosges and Ardennes displayed similar diatom assemblages. Both areas are mountainous regions characterized by crystalline geologies (schist or granite). They have similar diatom taxa (*Achnanthidium subatomus, Gomphonema rhombicum, Psammothidium subatomoides*).

The results for both study areas show that to make the ecoregional classifications more relevant to diatom assemblages, it might be useful to combine some ecoregions that are geographically separated, but characterized by similar diatom assemblages and environmental descriptors. This approach has already been applied in the USA, where some areas assigned to a single ecoregion are in fact geographically unconnected (Bailey, 1995).

2. Is there a threshold level above which factors affected by human activity explain the variability of diatom assemblages to a greater extent than ecoregions?

In the first study area, corresponding to the high altitude rivers, the pollution level was assessed using the SPI diatom index (Cemagref, 1982). This indicated that in 85% of these rivers water quality was very good, in 11% quality was good, and in 2% it was moderate. No rivers had bad water quality, which meant that the pollution gradient was slight. In the second study area, corresponding to North-eastern France, the pollution level was also assessed using the SPI. This showed that 5.4% of the rivers had very good water quality, 45.4% good quality, 39.6% moderate quality, 9.5% had bad or very bad quality. The pollution gradient was therefore much steeper than in the high altitude rivers.

Several authors did not find any obvious relationship between diatoms and ecoregions, because the most important gradients were the downstream gradient, the mineral content and pH gradients, and the altitudinal and latitudinal gradients (Pan et al., 2000; Potapova & Charles, 2002a). Others observed a homogenization of diatom communities in ecoregions where pollution was increasing (Leira & Sabater, 2005; Tornes et al., 2007).

In the case of high altitude rivers, the ecoregions of System A most closely matched diatom assemblages: the match was closer than those for source distance, altitude, or pollution, and was equivalent to that for geology. This can be explained by the choice of a particular river type which artificially reduced all the other gradients (altitude, pollution, source distance).

In the case of North-eastern France, here too the hydro-ecoregions most closely reflected diatom assemblages. This match was closer than those with river size (assessed by Strahler rank) or pollution (assessed by the SPI). This was also confirmed by a discriminant analysis showing that the most structuring parameters of diatom assemblages were conductivity, pH, and bicarbonate concentration. The variability of these parameters is mainly attributable to the geological substrate, and they are only weakly affected by anthropogenic activity (Agences de l'Eau, 2000). Diatom communities in this area were defined by means of a Twinspan analysis, which showed that the first dichotomy was explained by geological substrate: one community is located on a crystalline substrate, and the other on a sedimentary substrate. Within both of them sub-communities corresponding to polluted and highly polluted areas were present, and these displayed different diatom species compositions. Geology is therefore the primary factor that determines hydro-ecoregions.

Our findings seem to be at odds with those of Pan et al. (2000), Potapova & Charles (2002a), Leira & Sabater (2005) and Tornes et al. (2007). On the contrary, they confirm the results of Soininen (2004, 2007), in Finland and of Rimet et al. (2004) in Luxembourg, who stressed the importance of the ecoregional approach and found that geology, which is a factor that varies at larger spatial scales, had a determining impact on diatom assemblages.

- **Conclusions**

Communities of unicellular organisms such as diatoms have high dispersal capacities. The invasions of rivers by non-native diatom species provide a good demonstration of this ability. New-Zealand lakes provide a good example with *Asterionella formosa* records in sediments: this species was introduced at the time of the European settlement, salmon eggs being the most likely vector (see review of Spaulding et al., 2010). A long-term study (Coste & Ector, 2000) of French rivers has identified the arrival of species such as *Gomphoneis eriense* and *Encyonema triangulum*. Another example, also in French rivers is provided by a period of five years, during which *Achnanthidium druartii* spread from a single river site to 40 river sites some of them several being at hundred kilometers apart (Rimet et al., 2010). Since the statement that “Everything is everywhere: but the environment selects” (Beijerinck, 1913), a fierce debate about diatom endemism reigned until recent years. Some authors argued that diatom endemism should be underestimated (e.g. Mann & Droop, 1996) and others were of the opposite opinion and were favorable to Beijerinck’s law (e.g. Finlay et al., 2002). The spatial structure of the diatom assemblages that we observed was generally congruent with Beijerinck’s law. There were clear correspondences between diatom assemblages and ecoregions. In particular it appeared that if the abiotic factors used to define the ecoregions (altitude, geology, climate) were the same, then the diatom compositions found were comparable. Invasions of new species are probably indicative of recent environmental modifications (e.g. water warming) opening new ecological niches that were rapidly filled by non-native diatom species: the

example of *Achnanthidium druartii* is a good demonstration of this, since within just a few years rivers separated by several hundred kilometers were colonized.

This allowed us to say that in order to improve the effectiveness of ecoregional classifications for diatom assemblages it might be useful to combine some ecoregions that are geographically separated, thus doing away with notions of endemism. These results also reinforce the interest of using an ecoregional approach to developing diatom indices (Grenier et al., 2006; Lavoie et al., 2006).

Despite the findings of Leira & Sabater (2005), Tornes et al. (2007) and Pan et al. (2000), the hypothesis that no relationship between diatoms and ecoregion would be evident for polluted sites was not confirmed in our case. Soininen (2004) also highlights the importance of ecoregions in his study; but he recognized that the location of his polluted sites was biased, and most of them were located within a single ecoregion. In our case, the polluted sites were distributed throughout the study area since diatom communities corresponding to high level of pollutions were found on both crystalline and sedimentary substrates. Nevertheless, land-use differs from one geological substrate to another: agricultural practices, at least, differ depending on the landscape and geology. We can assume that the composition of diatom assemblages would differ as a result of these different practices that result in different kinds of pollution, even when nutrient and organic matter concentrations are equivalent. This would (once again!) highlight the bioindicative power of diatoms, which would offer fine discrimination between different types of anthropogenic perturbations.

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b. Paper 1: Benthic diatoms in western European streams with altitudes above 800 m: Characterisation of the main assemblages and correspondence with ecoregions.

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- **Abstract**

High altitude rivers in European mountains show a large diversity of benthic diatom assemblages. From rivers of the Alps, the Pyrenees, the Massif-Central and the Iberic system, diatoms were studied. The study area spread across four countries, Italy, France, Switzerland and Spain. Since 2000, the European Water Framework Directive (WFD) has required the assessment of stream quality using bioindicators and any deviaton from reference conditions measured. References for each river type and for each bioindicator, such as diatoms, are in the process of being defined.

System A is a typological system proposed by the WFD, in which ecoregions spread over several countries were defined. The first aim of this study was to assess the importance of these ecoregions for diatoms compared to other environmental factors. To reduce the heterogeneity of the diatom assemblages due to the river continuum and, also, pollution, only the rivers higher than 800 meters were selected. These rivers include a majority of sites that are only slightly polluted, or not at all. In total 261 sampling sites were considered from four ecoregions: the Iberic region, the Pyrenees, the Alps and the Western Highlands. The sampling sites were characterised by differences in geology, distance from the source and altitudes. Statistical analysis showed that geographic ecoregions of system A and geology were the most important environmental factors for diatoms. Distance from the source and altitude were less important and pollution was the least important parameter.

The second aim was to describe and to typify the main diatom assemblages of these European mountains. Eight clusters gathered into four main groups were identified. Group I was mainly recorded in the Alps and the Pyrenees; group II had in common its close proximity to the source; group III was often found in the Western Highlands and Iberic region on crystalline geology, and group IV included weakly polluted streams of the Apls and Pyrenees. Some suggestions for the improvement of the ecoregions adapted to benthic diatoms were given in the conclusion.

- **Introduction**

Diatoms are unicellular algae that represent an important part of biodiversity in rivers. Diatom assemblages responses to anthropogenic disturbances have been observed for a long time (e.g. Butcher 1947). Diatoms reproduce and divide rapidly and quickly react to water quality changes (e.g. Round 1991). This prompted water managers to select as one of the tools for assessing a wide range of water quality, alongside macroinvertebrates and fish, benthic diatoms as bioindicators, which investigate environmental quality over longer time period (Stevenson & Pan 1999).

Now, diatom indices are used routinely in different European countries to assess the biological quality of running waters (Prygiel et al. 1999). In 2000, the European Parliament & The Council of the European Union (2000) advised European countries to assess running water quality by using diatoms, as part of the phytobenthos, in addition to phytoplankton, fish, macroinvertebrates and macrophytes. It also calls for applying of the “Ecological Quality Ratio” which uses bioindicators to

evaluate stream quality by assessing the difference between the observed site community to a non-disturbed reference community belonging to the same stream type in the same ecoregion (Ector et al. 2004). Thereby, reference conditions for each stream type had to be defined. Two different typological systems were proposed in the Water Framework Directive –WFD– (European Parliament & The Council of the European Union 2000). The first is system A, in which ecoregions, based on altitude, geology and sizes of the running water catchment area were fixed. This system has the advantage of being easily applicable to all of Europe and the ecoregions proposed in this typological system are large, spread over several European countries.

In the second system B, the requisite factors (altitude, latitude, longitude, geology and size) were used to define the stream types but optional factors can be added to this system typology.

System B is more complex, as each country is currently developing its own typology based more criteria (e.g. Wimmer et al. 2000, Wasson et al. 2001, Munné & Prat 2005). Therefore, comparisons of stream types between different European countries will require complex intercalibration studies.

So far, the ecoregions proposed in system A have never been tested on a large geographical scale, including several countries. A difference in certain catchments characteristics, such as land use, geology and background nutrient fluxes, is expected. For example in Norwegian rivers, macroalgal growth took place after only a small increase of total phosphorus concentrations from 4 to 12 µg.l-1 (Lindstroem 1999) whereas in northern French rivers these concentrations are below the classification range; 500 µg.l-1 is the lowest reference level (Prygiel 1991).

Therefore, the first objective was to assess the importance of the proposed ecoregional system A on diatom assemblages over a large geographical scale including several European countries. Along with this, other environmental factors were also tested; altitude, distance from the source, riverbed geology and pollution level. However, in order to reduce the strong heterogeneity between diatom assemblages usually observed along the river continuum (e.g. Potapova & Charles 2002), a selection of stream types was carried out. In order to reduce the influence of pollution, stream types with low anthropogenic influence were chosen and according to the WFD, the high altitude streams above 800 m were selected because they satisfied these prerequisites.

Benthic diatoms in several regional mountainous areas have already been studied, such as the Pyrenees (e.g. Gomà et al. 2005, Merino et al. 1994), the Alps (e.g. Cantonati 1998, Battegazzore et al. 2004, Ciutti et al. 2005, Rimet et al. 2005, Pipp & Rott 1994, Rott et al. 2003), the Himalayas (e.g. Cantonati et al. 2001, Jüttner & Cox 2000), the Tatras (Kawecka 1980), the Carpathians (Krstic et al.1994) and the Siberians mountains (Potapova 1996). However, a large studies considering biogeography of the main assemblages present in mountains are almost inexistent, except for Cantonati et al. (2001) comparing diatom assemblages of the Alps with the Himalayas, and Kawecka (1980) about diatom assemblages of several Alpine regions of Europe. Furthermore, studies on diatom biogeography including species distribution patterns on a large scale have remained rare (Kociolek & Spaulding 2000) until recently (e.g. Pan et al. 2000, Potapova & Charles 2002, 2003, Tison et al. 2005). The existing assemblage for comparisons are few and far between for European countries and when concerned with high altitude rivers, the number is even more limited. Comparing and typifying assemblages from several European countries was an interesting challenge. For that reason, the second objective was to describe and typify the main diatom assemblages present in the high altitude streams in several western European mountain ranges. Therefore, Diatom samples were gathered from Spain, France, Switzerland and Italy along with their corresponding physical and

chemical data for comparison later on. The main western European mountain ranges were represented: the Alps, the Massif-Central, the Pyrenees and the Iberic system.

- **Methods**

Sampling sites selection

Only sampling sites with an altitude above 800 m and located in Western Europe were chosen and several institutes were contacted for an informal collaboration. Sampling sites with altitudes above 800 m but lacking information on any of the following were rejected: geographical location, epilithic diatom assemblage, geology, altitude or distance from the source.

Diatom sampling, preparation, identification and counts

Sampling was carried out according to European recommendations (Kelly et al. 1998; European Committee for Standardization 2002, 2003). Benthic diatoms were collected from at least five stones from the lotic parts of the sampling sites, avoiding sedimentation zones. Selected stones were those that remain in place under normal hydrological conditions. The upper surface of stones were scratched with a toothbrush. The samples fixed in 4% formaldehyde. From 1993 to 2003, 261 diatom samples were collected under the framework of different European, or national research projects, or national monitoring river networks and respected the European recommendations for sampling (Kelly et al. 1998; European Committee for Standardization 2002, 2003). Twenty sites were located in Switzerland, 67 in Italy, 79 in Spain and 95 in France (Figure 7). A detailed list of the sampling sites is given in Appendix 1.

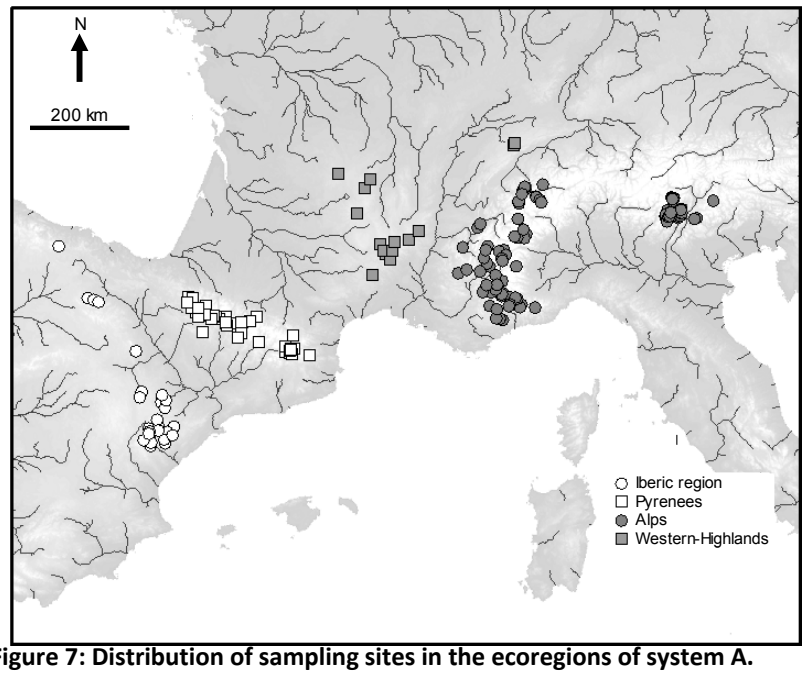

Figure 7: Distribution of sampling sites in the ecoregions of system A.

Following European standard methods (European Committee for Standardization 2002, 2003), the diatom valves were cleaned using 40% hydrogen peroxide to eliminate organic matter and hydrochloric acid to dissolve calcium carbonate (Iserentant et al. 1999). Clean diatom frustules were mounted in Naphrax©. At least 400 valves were counted and identified to species, sub-species or varieties, using an optical microscope with 1000× magnification (Prygiel & Coste 1993; AFNOR 2000). After counting, rare taxa were searched for by scanning the slide at 200× magnification (AFNOR 2000); they were incorporated in the counts as single individuals, whether they were single or double valves. Taxa abundances were expressed in percentage. Diatom floras of Krammer & Lange-Bertalot (1986, 1988, 1991a, b) were used for identifications with other floras such as Diatoms of Europe (Lange-Bertalot 2000-2004), or several volumes of Iconographia Diatomologica (Lange-Bertalot 1995-2004).

Environmental data

For most of the field measurements, multiparametric probes were used to determine water temperature (68% of the samplings), dissolved oxygen (77%), conductivity (81%) and pH (58%). Water samples for some sites were collected and analysed in the laboratory for NO3- (78% of the samples), NO2- (66%), NH4+ (78%), PO43- (69%), according to standard procedures (APHA 1995). All parameters were measured for France and Switzerland, except temperature in Dranse de Bagne River (Switzerland) and dissolved oxygen in La Morge River (Switzerland). For Italy, all the parameters were measured except for pH and dissolved oxygen in the Amola and Avisio rivers; dissolved oxygen and NO2- in the Conca, Cornisello, Larcher, Niscli and Noce Bianco rivers; temperature, dissolved oxygen and NO2- in the Careser and Fersina rivers. For Spain, all parameters measured for the Alp, Duran, Querol and Segre rivers except PO22-. All parameters were measured in the Hijar, Jiloca, Bco Cadajon, Garona, Hijar and Oja rivers, except NO2-. For the Veral and Isuela rivers only pH, temperature, conductivity and dissolved oxygen were available while no physico-chemical data were obtained for the other rivers (Aguas Limpias, Albentosa, Alfambra, Ara, Aragón, Arazas, Aurin, Bco Santa Anna, Bellós, Cabriel, Camarena, Cinca, Cinqueta, Err, Esera, Estarrón, Gállego, Gallo, Gas, Guadalaviar, Guatizalema, Huerva, Isábena, Linares, Mijares, Oropesa, Osía, Pancrudo, Ribera, Turia).
For each sampling site, altitude, distance from the source and ecoregion (according to system A) were determined using 1:20,000 topographical maps. Geology was determined using geological maps and eight classes established: limestone, quaternary sediment, sandstone, mudstone/schist/shale, granite, volcanic, mixed geology, and others geologies.

Data analysis

Before compiling the diatom lists from the different investigators, a taxonomical standardization was carried out using the OMNIDIA software and some slides were checked to ensure a homogenous use of taxa names in the database. Detrended correspondence analysis using the diatom lists showed that the sample distribution on the three first axes were independent from the investigator. This confirmed that taxonomical knowledge was homogenous between investigators even though they sampled different regions.
In order to estimate the pollution level of the sampling sites, the Specific Pollution-sensitivity Index – SPI– (Coste in Cemagref 1982), was calculated with the OMNIDIA software (Lecointe et al. 1993).

Two different group analysis techniques were used to explore the diatom data and determine the diatom assemblages. The first was the K-means partitioning algorithm (MacQueen 1967). The K-means clusters were calculated using the Ginkgo program, a freeware multivariate analysis tool, developed at the University of Barcelona (De Cáceres et al. 2003 a, b). The calculation used the Bray-Curtis distances in determing of the K-means clusters. The second was a hierarchical agglomerative method based on Bray-Curtis distances and Ward's method for group linkage using the PC-Ord software (MacCune & Mefford 1999). The efficiencies of the two methods were calculated with MRPP (Multi-Response Permutation Procedures), and the best method was chosen to carry on the analyses.

After the diatom assemblages were determined for each cluster, an average diatom assemblage was calculated based on all the samples grouped in a cluster. The average diatom assemblages is composed of ten species with the highest percentage. To determine the most indicative taxa characterising each K-means clusters, the Indicator Species Analysis (Dufrêne & Legendre 1997) was performed and given as the underlined species on Table 3. This analysis combines information on the abundance of species within a particular cluster and the faithfulness of its occurrence in a particular cluster. It produces indicator values for each species in each group. Their significance was tested using a Monte Carlo technique, using the PC-Ord software (MacCune & Mefford 1999).

Then, MRPP (Multi-Response Permutation Procedures) was used to assess the importance of environmental factors (expressed as classes: geology, pollution, altitude, distance from the source and ecoregions) on diatom assemblages. This technique was used in ecology by Zimmerman et al. (1985) and more recently and specifically in diatom ecology by Soininen (2004). The classes were defined a priori. The A-statistic is a result given by the MRPP ranging from -1 to +1; it is a descriptor of within-class homogeneity, compared to random expectation:

- if the A-statistic for a class tends towards +1, classes tend to be heterogeneous, therefore they have a meaning for the diatom assemblages,
- if A-statistic for the class tends towards 0, classes are comparable to random expectation, therefore they have no meaning for the diatom assemblages,
- if A-statistic for the class tends towards -1, classes tend to be homogeneous, therefore no meaning for the diatom assemblages.

The number of classes each environmental parameter is divided into has an effect on the analysis, and the results of A-statistics classifications tend to increase when the number of classes increases. Therefore, a comparison of A-statistic classification based on 4 classes with an A-statistic classification based on 8 classes was made.

The four class classification compared 4 ecoregions (the Western Highlands, the Pyrenees, the Iberic region and the Alps), 4 altitudinal classes, 4 distances from the source (0, 21.5, 43, 64.5, 86 km) and 4 pollution classes (SPI – 0, 5, 10 ,15, 20, e.g. AFNOR 2000) with 4 k-means clusters. For the altitudinal as well as the source distance classes, a linear progression of classes intervals (800, 1280, 1760, 2240, 2720 m, and 0, 21.5, 43, 64.5, 86 km, respectively), as well as a logarithmic progression (800, 1086, 1475, 2003, 2720 m and 0, 2, 8.3, 27.5, 86 km were explored. The classification with the highest A-statistic in each case was selected.

In the eight class classification, the following environmental parameters were divided into the 8 classes:

- geologies: limestone, quaternary sediment, sandstone, mudstone/schist/shale, granite, volcanic, others, mixed geology,
- 8 altitudinal classes: either linear progression 800, 1040, 1280, 1520, 1760, 2000, 2240, 2480, 2720 m or logarithmic progression (800, 932, 1086, 1266, 1475, 1719, 2003, 2334, 2720 m,
- source distance classes: either linear progression (0, 10.7, 21.5, 32, 43, 53.7, 64.5, 75.2, 86 km), or logarithmic progression (0, 0.7, 2, 4.3, 8.3, 15.3, 27.5, 48.8, 86 km).
- SPI pollution classes: 0, 2.5, 5, 7.5, 10, 12.5, 15, 17.5, 20

The environmental parameters were then compared with the 8 K-means clusters based on diatom assemblages. Again, the classification with the highest A-statistic was selected to carry on the analyses.

In both comparisons with 4 and 8 classes, A-statistic of K-means clusters was given; classifications based on diatom (K-means) give the highest A-statistic for diatom assemblages. This value was used to give the highest A-statistic value compared to the other classes of environmental parameters (geology, distance from the source, altitude, pollution and ecoregions).

In order to locate sampling sites on a common map, the national coordinates of each country (Lambert II étendu for France, World Geodetic Survey 1984 datum for Italy, Universal Transverse Mercator representation for Spain, Oblique Mercator Projection on Bessel for Switzerland) were transformed using WGS84 (World Geodetic Survey 1984 datum). The maps were drawn using the ArcMap software (ESRI©).

- **Results**

Diatoms identified in the study area

The list of the 498 taxa (species, varieties or sub-species) identified is given in Appendix 1. The most abundant taxa were *Achnanthidium minutissimum* (31% of the diatoms identified), *A. biasolettianum* (11%), *Gomphonema pumilum* (5%), *Fragilaria arcus* (5%) and *Encyonema minutum* (3%). One hundred and sixty-four taxa were identified only once during the study.

Diatom samples locations and environmental characteristics

The sites were located in the four ecoregions of system A (Figure 7): Iberic region (27 samples), Pyrenees (56 samples), Alps (146 samples) and Western Highlands (33 samples). According to the diatom index, SPI, more than 96% of the sampling sites were of good or very good quality (Figure 8) and less than 1% had bad quality. The medians, maximum and minimum values for the other measurements are given on Table 1.

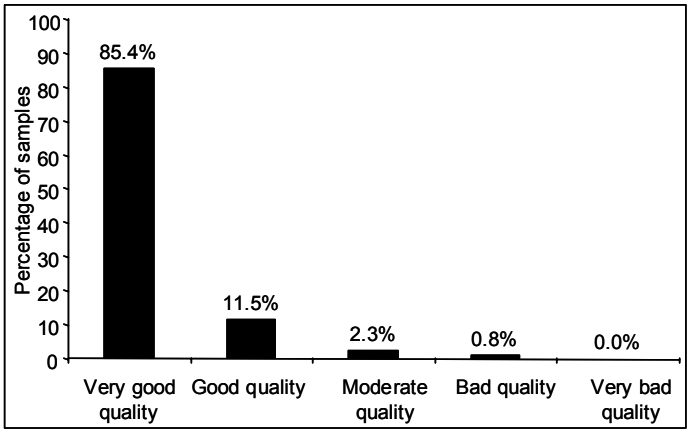

Figure 8: Percentage of samplings in each water quality class using the diatom index SPI (Coste in Cemagref 1982). Water quality classes: 20≤Very good quality<17≤Good quality<13≤Medium quality<9≤Bad quality<5≤Very bad quality<1.

Table 1. Median, maximum and minimum values of the parameters measured in the sampling sites of the studied area. Values are also given for the SPI diatom index (Coste in Cemagref 1982), with: 1≤very bad quality<5≤ bad quality<9≤medium quality<13≤good quality<17≤very good quality≤20.

	Median	Minimum	Maximum	Number of measures
SPI (diatom index)	18.9	6.6	20.0	261
Altitude (m)	1193	800	2720	261
Distance from source (km)	8.0	0.0	86.0	261
Temperature (°C)*	10.0	0.5	24.7	179
pH*	7.8	5.1	8.9	201
Conductivity ($\mu S.cm^{-1}$)*	146.0	2.0	1025.0	213
Dissolved oxygen ($mg.l^{-1}$)*	9.4	3.9	14.2	153
NO_3^- ($mgN.l^{-1}$)*	0.26	0.01	4.36	205
NO_2^- ($mgN.l^{-1}$)*	0.01	0.00	0.18	174
NH_4^+ ($mgN.l^{-1}$)*	0.04	0.00	1.19	205
PO_4^{2-} ($mgP.l^{-1}$)*	0.01	0.00	1.00	182

*: calculated on available data

Ecoregion characteristics are given on Table 2. Site altitudes ranged from 800 to 2720 m (median of 1193 m). Altitudes of the sampling sites in the Alps altitudes were significantly higher (Boniferroni tests $p<0.05$) than in the Iberic region and the Western Highlands, but similar to the Pyrenees. The altitudes of the sampling sites in the Pyrenees, the Iberic System and the Western Highlands had not significantly difference.

For the different sites, the distance from the source ranged from 0 to 86 km (median of 8.0 km). In the Iberic system, it was significantly longer than in the other ecoregions (Boniferroni tests $p<0.05$). Distance from the source of the sampling sites in the Pyrenees, the Alps and the Western Highlands had not significantly difference.

The pH values ranged from 5.1 to 8.9 (median of 7.8) and conductivity from 2 to 1025 μS.cm-1 (median of 146.0 μS.cm-1). These parameters were strongly influenced by geology (e.g. Potapova & Charles 2003, Rimet et al. 2004). The Iberic region was dominated by limestone and had the highest

conductivity of all ecoregions. The Pyrenees, dominated by granite, limestone and sandstone, had the lowest conductivity, while the Alps, dominated by limestone and granite, had an intermediate conductivity in respect to the two former ecoregions. The Western Highlands with the lowest conductivity values was dominated by granite, limestone and volcanic.

Table 2. **Characteristics of the ecoregions. Median values are given for several environmental parameters and for the SPI diatom index (Coste in Cemagref 1982).**

Ecoregion	Iberic region	Pyrenees	Alps	Western highlands
N° of samples	26	56	146	33
SPI	17.1	18.9	19.3	18.1
Altitude (m)	995	1160	1401	953
Distance from source (km)	19.0	12.9	4.0	5.0
Temperature (°C)*	15.0	11.4	6.8	13.6
pH*	7.8	7.8	8.0	7.4
Conductivity ($\mu S.cm^{-1}$)*	306.7	93.3	196.2	37.5
Dissolved oxygen ($mg.l^{-1}$)*	8.4	10.2	9.5	9.1
NO_3^- (mg $NO_3^-.l^{-1}$)*	0.43	0.52	0.23	0.25
NO_2^- (mg $NO_2^-.l^{-1}$)*	not available	0.01	0.01	0.01
NH_4^+ (mg $NH_4^+.l^{-1}$)*	0.10	0.01	0.04	0.03
PO_4^{2-} (mg $PO_4^{2-}.l^{-1}$)*	0.02	0.01	0.01	0.02
Dominant geology	Limestone (74%)	Granite (36%), Limestone (25%), Sandstone (16%)	Limestone (30%) Granite (29%),	Granite (76%), Limestone (15%), Volcanic (6%)

*: calculated on available data

Diatom assemblages

Because of software limitation, only 220 of the most abundant diatom taxa were selected (the selection based on the sum of abundances for each taxa), while the other 282 less abundant taxa were excluded. Based on the 261 diatom lists, ten different K-means analyses and hierarchical analyses were carried out. Between 2 and 15 clusters were defined for both methods and were compared by mean of the A-statistic (Figure 9). The K-means analyses showed the best results and were selected to carry on the analyses.

The K-means analysis defined eight clusters because after eight clusters the A-statistic showed no significant increase in value (Figure 9) and it was also the best compromise in term of the number of clusters characterizing the available data. Table 3 gives the average diatom assemblages for each K-means cluster (average of the most abundant taxa in each cluster). The indicator taxa as defined by an indicator species analysis (Dufrêne & Legendre 1997) are also given for the each clusters (underlined on Table 3).

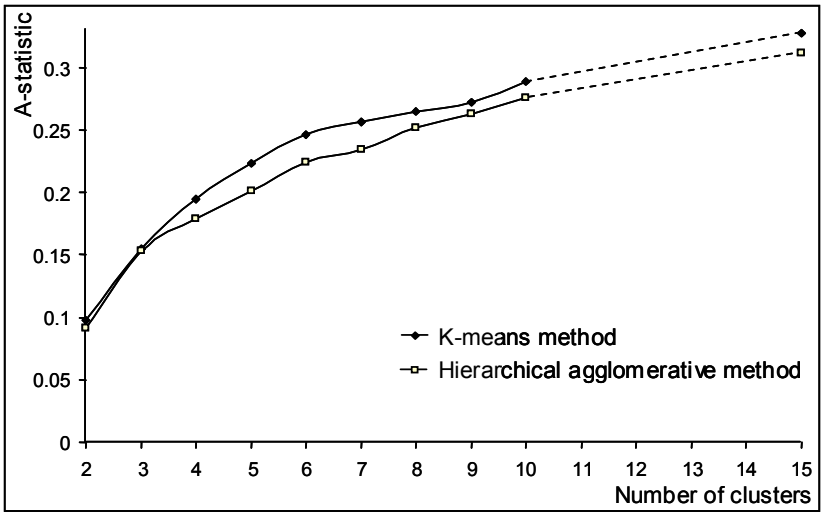

Figure 9: MRPP analysis carried out on two different cluster analysis: K-means method and hierarchical agglomerative method. The A-statistic was calculated from 2 to 15 clusters. A=0 if heterogeneity within clusters equals expectation by chance; A>0 if there is less agreement within clusters than expected by chance, A=1 when all items are different between clusters.

Table 3. **Assemblages of the 8 K-means clusters. These assemblages were established calculating the average abundances of the taxa in each cluster. The 10 most abundant taxa are given with their abundances in bracket. Significant indicator taxa of each cluster, defined with the indicator species analysis, are underlined (Monte-Carlo test, p<0.05).**

Group	K-mean	Taxa names (abundance %), the most indicator taxa are underlined
I	1	Fragilaria arcus (Ehrenb.) Cleve (43.9), Encyonema silesiacum (Bleisch) D.G. Mann (8.9), *Achnanthidium minutissimum* (Kütz.) Czarn. (8.0), Diatoma mesodon (Ehrenb.) Kütz. (7.8), Navicula gregaria Donkin (3.1), *Encyonema minutum* (Hilse) D.G. Mann (2.4), *Diatoma ehrenbergii* Kütz. (2.4), *D. moniliformis* Kütz. (1.8), *Nitzschia palea* (Kütz.) W. Smith (1.8), *Achnanthidium biasolettianum* (Grunow) Round & Bukhtiy. (1.6)
	2	Achnanthidium biasolettianum (49.3), *A. minutissimum* (18.3), Cymbella affinis (4.2), *Gomphonema pumilum* (4.2), G. pumilum var. elegans E. Reichardt & Lange-Bert. (1.9), *Diatoma ehrenbergii* (1.7), *Encyonema minutum* (1.6), *Nitzschia fonticola* (1.5), Gomphonema tergestinum (Grunow) Fricke (1.5), *Encyonopsis microcephala* (1.4)
	3	Gomphonema pumilum (51.4), *Achnanthidium minutissimum* (16.7), *A. biasolettianum* (7.3), *Reimeria sinuata* (3.7), *A. subatomus* (3.3), *Encyonema minutum* (3.2), *Fragilaria arcus* (1.8), *Diatoma ehrenbergii* (1.7), *Gomphonema olivaceum* (Hornem.) Bréb. (1.4), *Cymbella affinis* (1.3)
II	4	Achnanthidium minutissimum (66.1), *A. biasolettianum* (3.2), *Diatoma mesodon* (2.4), *Fragilaria arcus* (1.8), *Encyonema minutum* (1.8), Diadesmis gallica var. perpusilla (Grunow) Lange-Bert. (1.5), *Encyonopsis microcephala* (Grunow) Krammer (1.5), *Gomphonema pumilum* (1.3), *Denticula tenuis* Kütz. (1.3), *Cocconeis placentula* Ehrenb. var. *lineata* (Ehrenb.) Van Heurck (1.1)
III	5	Fragilaria capucina Desm. (10.3), *Amphora pediculus* (Kütz.) Grunow (10.0), Navicula cryptotenella Lange-Bert. (8.3), Fragilaria capucina Desm. var. rumpens (Kütz.) Lange-Bert. (8.3), Fistulifera saprophila (Lange-Bert. & Bonik) Lange-Bert. (5.9), Psammothidium oblongellum (Oestrup) Van de Vijver (4.0), Gomphonema exilissimum (Grunow) Lange-Bert. & E. Reichardt (3.9), *Nitzschia dissipata* (Kütz.) Grunow (3.8), Mayamaea atomus var. permitis (Hust.) Lange-Bert. (3.0), *Eunotia subarcuatoides* Alles Nörpel & Lange-Bert. (2.7)
	6	Cocconeis placentula var. placentula (12.2), Achnanthidium subatomus (9.2), Nitzschia fonticola (6.2), *Diatoma mesodon* (6.0), *Achnanthidium minutissimum* (5.1), *Encyonema silesiacum* (4.8), *Cocconeis placentula* Ehrenb. var. *euglypta* (Ehrenb.) Grunow (4.5), Reimeria sinuata (4.5), *Nitzschia dissipata* (3.6), N. paleacea (Grunow) Grunow (3.4)
IV	7	*Achnanthidium minutissimum* (36.7), *A. biasolettianum* (9.6), Encyonema minutum (6.5), *Fragilaria arcus* (3.9), *Cocconeis placentula* Ehrenb. var. *placentula* (3.9), *Gomphonema pumilum* (Grunow) E. Reichardt & Lange-Bert. (3.2), *Reimeria sinuata* (Gregory) Kociolek & Stoermer (2.9), *Cymbella affinis* Kütz. (2.4), *Achnanthidium subatomus* (Hust.) Lange-Bert. (2.2), *Nitzschia fonticola* Grunow (2.0)
	8	*Achnanthidium minutissimum* (18.6), Gomphonema olivaceum (7.1), *Encyonema minutum* (4.9), *Achnanthidium biasolettianum* (4.8), Encyonopsis microcephala (4.3), *Cocconeis placentula* var. *lineata* (3.1), *Reimeria sinuata* (3.1), *Psammothidium marginulatum* (Grunow) Bukhtiy. & Round (2.3), *Cymbella affinis* (2.3), Diatoma moniliformis (2.3)

The geographical location of the sites for each of the eight clusters is given on Figure 10 and Figure 11. The physical characteristics (geology, distance from the source, altitude, pollution level, ecoregions) for each cluster are listed on Table 4. Considering geographical distribution, distance from the source but also pollution status, some similarities between clusters were identified. Consequently, the 8 clusters were organized into four main groups. Group I predominantly encountered in the Alps and the Pyrenees, Group III was most often recorded in the Western Highlands and on crystalline bedrock, Group IV occurred in all ecoregions. Group II was typically found in close proximity to the source.

Group I, (mostly the Alps and the Pyrenees) is rarely found in the other ecoregions and is composed of clusters 1, 2, & 3. The clusters differed from each other in respect to riverbed geology, distance from the source and pollution status. Cluster 1 includes sites recorded at altitudes ranging from 800 to 1700 m and in river stretches located at a distance from the source between 2 to 32 kms, but had a wide range of geological substrates. *Fragilaria arcus, Encyonema silesiacum* and *Diatoma mesodon* dominated and characterized its average assemblage. Cluster 1 is characterized by lightly polluted sites, with 0.38 mgN.l-1 NO3-, and 0.03 mgP.l-1 PO42- (Table 4). Subdominant taxa such as *Navicula gregaria* (3.1%), *Nitzschia palea* (1.8%) considered as pollution tolerant (Van Dam et al. 1994) were occasionally present in this assemblage.

Cluster 2, as the cluster 1, occurred over a similar range of altitudes (800 to 1400 m) and distances from the source (4 to 32 km). However, it differed from cluster 1 in respect to geology as the substrata was largely dominated by limestone. The average assemblage for this cluster included the following indicator taxa: *Achnanthidium biasolettianum, Cymbella affinis* and *Gomphonema tergestinum*. This cluster sites samples were unpolluted (median values in Table 4: 0.22 mgN.l-1 for NO3-, 0.02 mgP.l-1 for PO42-).

Cluster 3 sample sites were situated on in streams very frequently flowing over granite and limestone and located from the source between 4 to 32 kms . However, they occurred over a wider range of altitudes (from 800 to 2000 m) than clusters 1 and 2. As with Cluster 2, cluster 3 sites were unpolluted (median values in Table 4: 0.16 mgN.l-1 for NO3-, 0.02 mgP.l-1 for PO42-). The average assemblage of cluster 3 was dominated by *Gomphonema pumilum* associated with *Achnanthidium minutissimum* and *A. biasolettianum*.

Group II had only cluster 4 and contained sample sites often found in close proximity to the source (mainly from 0 to 2 km) and at higher altitudes than in Group I (often up to 2695 m). The sites were frequently recorded from the Alps, but also present in the other three ecoregions. The sample sites were unpolluted (median values of Table 4: 0.23 mgN.l-1 for NO3-, 0 mgP.l-1 for PO42-) and had bedrock composed of either limestone or granite. Cluster 4 average assemblage was largely dominated by *Achnanthidium minutissimum* (relative abundance of 66.1%).

Group III contained clusters 5 and 6. The sites were regularly situated on crystalline bedrock thereby explaining the relatively low conductivities. Cluster 5 sites were mainly present in the Western Highlands and the Iberic region and a few were found in the Alps but absent from the Pyrenees. The dominant geological substrate was granite, and the stream had relatively low pH and low conductivities. Typical species of these low conductivity waters included members of the genus *Eunotia* (*E. subarcuatoides, E. bilunaris* (Ehrenb.) Mills, & *E. exigua* (Breb.) Rabenhorst var. *tenella* (Grunow) Nörpel & Alles). The assemblages were present at altitudes lower than 1400 m, and

encountered all along the river continuum (from the beginning of the stream to 64 km from the source). The sample sites were lightly polluted (median values of Table 4: 0.36 mgN.l-1 for NO3-, and 0.02 mgP.l-1 for PO42-), this could explain the presence in low abundances (below 3%) of pollution tolerant taxa such as *Fistulifera saprophila* and *Mayamaea atomus* var. *permitis* (Van Dam et al. 1994).

Cluster 6 occurred mostly in the Western Highlands and to a smaller extent in the Pyrenees and the Alps. Sample sites predominantly occurred below 1400 m on crystalline substrates, rarely on sedimentary geology. The cluster was characterized by waters weakly enriched with nutrient and organic matter (median values of Table 4: 0.81 mgN.l-1 for NO3- and 0.03 mgP.l-1 for PO42-). *Achnanthidium subatomus* was the most important indicator species of this cluster. *Gomphoneis minuta* (Stone) Kociolek & Stoermer occurring at low abundances also characterized this cluster.

Group IV, composed of clusters 7 and 8, was found mostly in the Apls and the Pyrenees. The geological bedrock of cluster 7 was composed of limestone or granite and, to a lower extent, by mudstone, shale and schist. Altitudes ranged mainly from 800 m to 1700 m. The sampling sites rarely had conductivities over 300 µS.cm-1 (median 108 µS.cm-1) and were unpolluted (0.22 mgN.l-1 for NO3-, 0.01 mgP.l-1 for PO42-). The average assemblage for this cluster was dominated by *Achnanthidium minutissimum* (36%). The indicative species were *Gomphonema rhombicum* Schmidt, *G. truncatum* Ehrenb. and *Encyonema minutum*.

Sample sites in cluster 8 covered a large range of altitudes and distances from the source. However, the majority were situated mainly between 4 to 32 km from the source, and rarely above an altitude of 1700 m. They were characterized by light pollution, as the nitrate concentration was rather high (median of Table 4: 0.36 mgN.l-1 for NO3-). The average assemblage was dominated by *Achnanthidium minutissimum* and *Gomphonema olivaceum*, which is the indicator species for the cluster, along with *Encyonopsis microcephala* and *Diatoma monoliformis*.

Assessment of the importance of the different physical parameters for diatom assemblages

The importance of the environmental parameters (the ecoregions, geology, altitude, pollution levels as assessed with the SPI diatom index, and distance from the source) on diatom assemblages was determined with the A-statistic of the MRPP.

The comparison of the linear and logarithmic scales for altitude and source distance using the A-statistic showed that logarithmic classes had higher values than linear classes (altitude with 4 linear classes 0.026, 4 logarithmic classes 0.027, with 8 linear classes 0.030, 8 logarithmic classes 0.035; source distance with 4 linear classes 0.010, 4 logarithmic classes 0.033, with 8 linear classes 0.021, 8 logarithmic classes 0.042). Thus, logarithmic classes were retained.

The A-statistic for the different environmental parameters is given on Figure 12. In the four class comparisons, the ecoregions had the most important influence on the diatom assemblages followed by source distance, altitude and the least was pollution. In the eight class comparisons, geology was the most important parameter followed by distance from the source, then altitude and finally pollution.

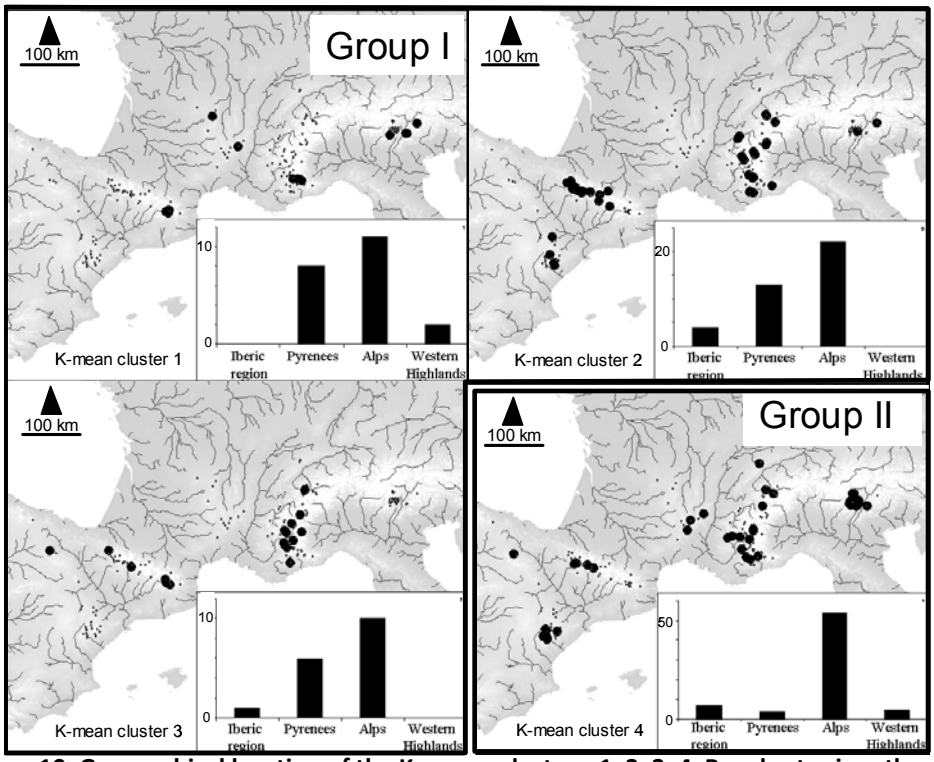

Figure 10: Geographical location of the K-means clusters, 1, 2, 3, 4. Bar charts gives the number of sampling site in each ecoregion for each cluster.

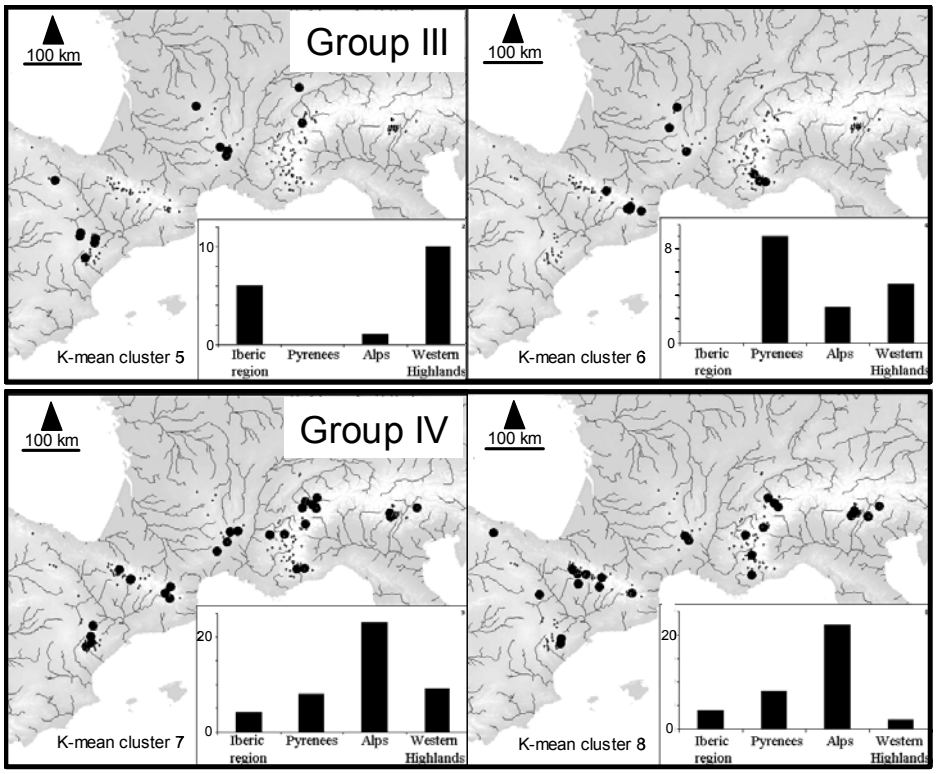

Figure 11: Geographical location of the K-means clusters, 1, 2, 3, 4. Bar charts gives the number of sampling site in each ecoregion for each cluster.

Table 4. Environmental characteristics of the 8 K-means clusters. Median values of some environmental parameters are given. Median values are also given for the SPI diatom index (Coste in Cemagref 1982), with: 1≤very bad quality<5≤bad quality<9≤medium quality<13≤good quality<17≤very good quality≤20. Minimum and maximum values are mentioned in brackets.

Group	K-mean	Ecoregions (% of samples)	Geologies (% of samples) and conductivity (Cond.)	Altitude (m) and distance from source (km)	Pollution level SPI (value /20) Chemicals (mgN or mgP.l-1)
I	1	Alps: 52 Pyrenees: 38 Western H.: 9	Granite: 28 Volcanic: 19 Quaternary sediments: 14 Limestone: 14 Others geologies: 19 Cond.: 79µS.cm-1 (2-291)	Mainly from 820 to 1655m, 1200m (820-1800) 10km (0-30)	Lightly polluted SPI: 19.0 (13.1-20) NO3-: 0.38 (0-1.47) NO2-: 0.01 (0-0.02) NH4+: 0.02 (0-0.08) PO42-: 0.03 (0.00-1.00)
	2	Alps: 56 Pyrenees: 33 Iberic R.: 10	Limestone: 54 Granite: 23 Sandstone: 13 Cond.: 232µS.cm-1 (70-527)	Mainly from 800 to 1400m, 1113m (800-2050) 10km (0-41)	Unpolluted SPI: 19.4 (17.1-20) NO3-: 0.22 (0.06-0.58) NO2-: 0.01 (0-0.01) NH4+: 0.04 (0-0.21) PO42-: 0.02 (0.00-0.11)
	3	Alps: 59 Pyrenees: 35 Iberic R.: 6	Granite: 41 Limestone: 29 Schist, Schale, Mudst.: 17 Cond.: 219µS.cm-1 (25-573)	Mainly from 800 to 2000m, 1250m (848-1800) 10km (1.5-20)	Unpolluted SPI: 19.5 (16.8-20) NO3-: 0.16 (0.01-1.87) NO2-: 0.01 (0-0.18) NH4+: 0.04 (0.01-0.08) PO42-: 0.02 (0-0.03)
II	4	Alps: 77 Iberic R.: 10 Western H: 7 Pyrenees: 6	Limestone: 46 Granite: 30 Others geologies: 17 Cond.: 114µS.cm-1 (6-807)	Present in all altitudes 1379m (782-2694) 1.9km (0-75), frequent near the source (0-2 km).	Unpolluted SPI: 19.5 (17.5-20) NO3-: 0.23 (0.02-1.02) NO2-: 0 (0-0.01) NH4+: 0.02 (0-0.38) PO42-: 0 (0-0.05)
III	5	Western H.: 59 Iberic R.: 35 Alps: 6	Granite: 59 Limestone: 29 Low conductivities Cond.: 32µS.cm-1 (19-1025)	Mainly below 1400m, 885m (795-1250) 5km (1-80)	Lightly polluted SPI: 17.2 (6.6-19.9) NO3-: 0.36 (0.13-4.35) NO2-: 0.01 (0-0.04) NH4+: 0.08 (0.01-0.41) PO42-: 0.02 (0.01-0.33)
	6	Western H.: 53 Pyrenees: 29 Alps: 18	Often on crystalline geologies, Granite: 41 Quaternary sediments: 23 Volcanic: 12 Limestone: 12 Cond.: 132µS.cm-1 (50-353)	Mainly below 1400m 1121m (850-2660) 12km (0-31.2)	Weakly organic and nutrient enriched waters SPI: 16.0 (10.2-19.9) NO3-: 0.81 (0.23-3.14) NO2-: 0.01 (0-0.04) NH4+: 0.08 (0.01-0.46) PO42-: 0.03 (0.02-0.13)
IV	7	All ecoregions Alps: 52 Western H.: 20 Pyrenees: 18 Iberic R.: 9	Granite : 45 Limestone: 32 Schist, Schale, Mudst.: 11 Cond.: 108µS.cm-1 (17-437)	Rare above 1700m 1230m (800-1930) 8.7km (0-34)	Unpolluted SPI: 18.6 (16.6-19.9) NO3-: 0.22 (0-2.28) NO2-: 0.01 (0-0.18) NH4+: 0.04 (0-0.37) PO42-: 0.01 (0-0.23)
	8	All ecoregions Alps: 61 Pyrenees: 22 Iberic R.: 11 Western H.: 5	Limestone: 42 Granite: 28 Cond.: 187µS.cm-1 (9-489)	Rare above 1700m 1172m (800-2720) 11.2km (0-53.7), frequent between 8.5 and 27.8km.	Lightly polluted SPI: 17.6 (9.3-20) NO3-: 0.36 (0.01-1.60) NO2-: 0.01 (0-0.11) NH4+: 0.05 (0.01-1.19) PO42-: 0.01 (0-0.50)

Medians calculated on available data for conductivity, NO_3^-, NO_2^-, NH_4^+ and PO_4^{2-}.

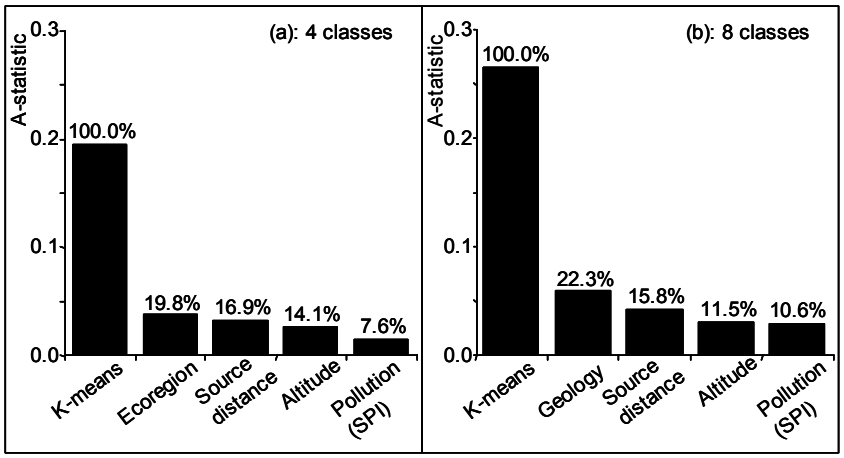

Figure 12: Comparison of the importance of the different environmental parameter for the diatom assemblages assessed with the A-statistic. The A-statistic is given for each parameter classification: (a) comparison of parameters classifications composed of 4 classes, (b) comparison of parameters classifications composed of 8 classes. The A-statistic is expressed as a percentage of the A-statistic of the K-means clusters defined with diatom assemblages. A-statistic of K-means clusters was used to give the classification that had the highest signification for diatom assemblages.

- **Discussion**

Characterization of the main diatom assemblages

Characterization of the diatom assemblages is the first step in assessing the determinant environmental parameters and understanding their distribution in the European mountains. It is also needed in order to define diatom reference assemblages as requested in the Water Framework Directive.

Despite the importance of diatom biogeography in modern diatom research, this subject is infrequently discussed (Kociolek & Spaulding 2000). One of the ideas considered during round tables about diatom biogeography, was cosmopolitanism (Edlund & Jahn 2001). Two of the assemblages characterized in the study area showed a wide distribution and could be considered as cosmopolitan when considering published benthic diatom data of mountain watercourses. For instance, the average assemblage of cluster 1 seems to be worldwide in mountains since *Fragilaria arcus* was frequently observed along with *Diatoma mesodon* in the mountain streams of the Himalaya (Rothfritz et al. 1997), in siliceous springs of the Alps (Cantonati 1998) and in streams of Tatras Mountains in Poland (Kawecka 1980). In the study area this assemblage was observed in lightly polluted waters, as highlighted by the presence of pollution tolerant taxa (*Navicula gregaria*, *Nitzschia palea*). Similarly, in the Spanish Pyrenees' streams, flowing on calcareous and sedimentary substrata, *Fragilaria arcus* was observed in a similiar assemblage accompanied by pollution tolerant taxa such as *Navicula gregaria* and *Fistulifera saprophila* (Gomà et al. 2005). Also in Macedonian streams, *Fragilaria arcus* was quite abundant (9.5%) in waters with BOD of 7.4 mg.l-1 and NO3- concentrations of 3.32 mgN.l-1 (Krstić et al. 1994).

Cluster 6 contained another example of cosmopolitan assemblage that were predominantly found below 1400 m on crystalline substrata and was characterized by *Achnanthidium subatomus*. This

particular species has been observed in relatively high abundance in Himalayan mountain streams of low conductivities (Rothfritz et al. 1997), in small streams of Luxembourg flowing over schist (Rimet et al. 2004), and also in Alpine springs on siliceous substrata (Cantonati 1998). It is therefore possible to consider this species as cosmopolitan. However, cosmopolitan distribution does not prevent taxa from being good indicators of particular geology such as *A. subatomus* always observed on siliceous substrata.

Another example, cluster 2 sites are found in unpolluted streams occurring primarily on limestone. Several of its indicator taxa can be considered as cosmopolitan. *G. tergestinum* occurs in unpolluted rivers of the calcareous Alps (Krammer & Lange-Bertalot 1986, Rimet et al. 2005) or in base-rich waters in the Western Himalayas (Jüttner & Cox 2000). *Cymbella affinis* is also frequent found in unpolluted rivers in the limestone plateau ecoregions of France (Tison et al. 2003) or in Himalayan running waters (Nautiyal et al. 2004). *Achnanthidium biasolettianum*, considered as an alkaliphilous taxon (Cantonati 1998), was also recorded in the "mid-altitude streams and small rivers" type of Austria, with pH of 8 and conductivities of 198 μS.cm-1 (Pipp & Rott 1994).

Achnanthidium minutissimum sensu lato was the most abundant taxon in the studied area. It has the highest abundances (66.1%) in cluster 4. This species is probably one of the most cosmopolitan freshwater diatoms; nevertheless, according to Stevenson & Bahls (in Barbour et al. 1999), it is an attached diatom and often the first species to colonize substrates that have been recently scoured, sometimes leading to the exclusion of all other algae.

Other taxa typical of cluster 4, such as *Diadesmis gallica* var. *perpusilla* and *Diadesmis contenta* (Grunow) Mann, were aerophilous taxa (according to Van Dam et al. 1994). Their presence suggests that the sampled surfaces were either not permanently covered by water or were located at the water/air interface. This kind of assemblage has already been observed in small intermittent streams in the Alps and the Himalayas (Cantonati et al. 2001, Battegazzore et al. 2004).

Similarily, cluster 3 taxa also indicated a particular physical environment. The most common taxa, *Achnanthidium minutissimum*, *Gomphonema pumilum*, were of small size and equipped with a robust mode of attachment in order to resist high current velocities (Rimet et al. 2003). This average assemblage was very uniform and largely dominated by *Gomphonema pumilum*. This average assemblage closely resembles those encountered in Alpine springs on calcareous substrate (Cantonati 1998), or in Carpathian (Kawecka 1980), Alpine (Maier & Rott 1988) or Pyrenean rivers (Merino et al. 1994, Tison et al. 2003, 2004). However, *Gomphonema pumilum* sensu lato as considered in this study is considered to be complex of several taxa (Reichardt 1997) and more detailed studies are needed to understand the ecology of the individual taxon within this complex.

Another taxon belonging to a species complex was *Encyonopsis microcephala*. This species was an indicator species of cluster 8, along with *G. olivaceum* and *Diatoma monoliformis*. *Encyonopsis microcephala* was observed in oligotrophic Alpine lakes (Hustedt 1930) and in unpolluted streams and lakes of the Pyrenees (Besch et al. 1972). According to Krammer (1997), this taxon includes several ecotypes with different ecological requirements: in North Europe it lives in oligotrophic waters, with low electrolyte content, whereas in Central Europe and North America it is frequent in lime-rich waters with middle electrolyte content, as well as in Mediterranean rivers (Gomà et al. 2004).

The three former species complexes (*Achnanthidium minutissimum, Gomphonema pumilum* or *Encyonopsis microcephala*) probably highlight the difficulty in achieving fine taxonomic resolution when confronted with large area studies such as the presented one. Several authors (Kociolek &

Spaulding 2000, Edlund & Jahn 2001) stressed that applying floras and taxonomies beyond their original regional scope could lead to amalgamation (Mann 2000) and therefore to an artificial homogenization of diatom biota. More detailed studies, but also clear identification keys are needed in order to solve this problem.

Rare taxa must not be considered as fuzzy ecological data. Their use as environmental indicators, especially in unpolluted rivers, is valuable (Potapova & Charles 2004). Rare diatoms such as taxa of the *Eunotia* genus are good indicators of low conductivities and low pH waters, and considered as acidobiontic (Krammer & Lange-Bertalot 1991). These taxa characterized cluster 5 sites with riverbeds dominated by granite. Similar assemblages with *Eunotia* species were found in mountain streams flowing over granites in northeastern Siberia by Potapova (1996). In the same way, *Gomphonema rhombicum*, another rare taxon, found in cluster 7 sites that are characterized by conductivities below 300 μS.cm-1 and at altitudes mainly ranging from 800 to 1700 m, was characteristic of low conductivities (about 115 μS.cm-1) and unpolluted rivers of the Belgian Ardennes Mountains flowing over schist substrata and accompanied by *Achnanthidium minutissimum* in relatively high abundances (Leclercq et al. 1996).

Another difficulty encountered was the apparition of exotic species; this implies a regular updating of the floras and documents used for identification. An example was the large size exotic taxon, *Gomphoneis minuta*, recorded in some of studied mountain streams. It occurred frequently in cluster 6 at low abundances (average abundance of 1 %). This exotic species, considered as endemic to New-Zealand and as invasive in France (Coste & Ector 2000) was found for the first time on the Spanish south side of the Pyrenees in high abundance in the Segre River (Gomà et al. 2005). According to Coste & Ector (2000), this species is resistant to relatively high concentrations of organic material.

More recently, *Didymosphenia geminata* (Lyngbye) Schmidt was recorded for the first time in Alpine rivers of Italy in 2004 (Ciutti et al. 2005). This large diatom with worldwide distribution (China, North America, and Turkey) is also known to produce blooms in some rivers of New-Zealand leading to the exclusion of all other kinds of algae. Introduction of new species seems to be a sign of anthropogenic modifications (Edlund & Jahn 2001). This process could become more and more frequent, especially in high altitude mountain where watercourses until recently were far from the main exchange roads.

Mountain diatom assemblages: influence of ecoregions and physical parameters

Seasonal weather effects on diatom assemblage structure in this study were not taken into consideration because sampling was carried out only during the summer. Diatom assemblages are also well known to react to organic pollution and eutrophication of waters, making them particularly good bioindicators for monitoring streams in different parts of the world (Ector et al. 2004). However, in this study, the impact of human activities on diatom assemblages was low because, according to the diatom index SPI, a large majority of the sampling sites were of good or very good biological quality. This was confirmed by the MRPP results with the A-statistic for SPI always the lowest compared to other parameters.

In order to reduce the “river continuum effect”, another well known parameter affecting benthic diatoms, only high altitude streams (over 800m) were selected from the different ecoregions. However, the altitude range of the studied running waters was still large and ranged from 800 to 2720 m. Altitude has already been shown to be an important environmental descriptor indirectly structuring benthic diatom assemblages. Current velocity, light intensity and temperature correlate

with altitude. Temperature directly influences diatom metabolism (e.g. Berges et al. 2002, Medlin & Wilson 1979) and therefore specific composition of the assemblages (Anderson 2000). For instance in the Rhône basin and the Mediterranean region in France, altitude is the most important structuring parameter, before pollution and mineralization level (Rimet et al. 2003).
On the other hand, in the United States (Potapova & Charles 2002), altitude and latitude were related to temperature variation but had minor influence on structuring diatom assemblages compared to pollution and mineral content. The results of our study showed that altitude, even over a relatively large range, had a rather weak effect on the diatom assemblage composition. Other parameters such as snow cover, short vegetation period, also related to altitude in a homogenous latitudinal area, probably affect diatom assemblages but could not be taken into account it this study.
In system A of the WFD, four types of river sizes were defined based on catchment area. Catchment area and distance from the source correlated. On the one hand, these parameters are often main gradients affecting diatom assemblages when a large range of stream types are studied, as shown for instance in a huge scale study in the United States (Potapova & Charles 2002). This inspired authors, such as Descy & Coste (1991) to define hypothetical diatom assemblages occurring along a river continuum in the development of a diatom index. On the other hand, when a homogenous type of stream size was studied, distance from the source should not have significant effects on diatom assemblages, as observed in a study carried out in headwater streams of Luxembourg, Western Europe (Rimet et al. 2004).
In our study, distance from the source varied considerably and ranged from 0 to 86 km and was an important parameter which was more relevant to diatom assemblages than altitude and pollution. In particular, diatom assemblages near the sources, as in cluster 4, were very particular and were dominated by diatoms considered as a pioneer taxa. Species compositions of stretches near the source were very different from those occurring all along the stream, as for instance in cluster 5. Average assemblage of cluster 5 included high abundances of “rosette forming” diatoms (relative abundance of 18% for genus *Fragilaria*). In contrast to algal assemblages in downstream sites, algal assemblages present in upstream stretches are often subject to important physical disturbances, such as fast flooding events reported from prealpine streams (Uehlinger 1991) or dry periods in some Alpine and Himalayan streams (Cantonati et al. 2001). Algal assemblages of upstream sites are usually considered as less mature compared to downstream ones (Margalef 1960).

The most important environmental descriptors for diatom assemblages were geology and the ecoregions. Geology is often observed as the determining factor influencing diatom assemblages at different spatial scales. This was shown at local scale in the headwater streams of Luxembourg, a country divided into two distinct geological regions where geology was the most important parameter, and pollution was a secondary factor (Rimet et al. 2004). At a regional scale in south-eastern France, 4 different assemblages were characterized from unpolluted sites, these diatom assemblages showed a good correspondence with their mountain range origin and the geological substratum (Tison et al. 2004). This was also shown for pristine lakes of Tasmania, where there was a clear correspondence between geological bedrock and benthic diatom assemblages (Vyverman et al. 1996). However, different studies carried out on springs and rivers showed that alkalinity (Cantonati 1998), water conductivity (Sabater & Roca 1990) and ionic composition (Potapova & Charles 2003) were important environmental factors affecting diatom assemblages and species distribution. The

geology in the studied area was complex and very variable, even within a single mountain range. Even though it was the most important physical parameter, but it did not explain all the diatom variability.

The ecoregional classification seemed to reflect differences in diatom assemblages and gave the best A-statistic results, comparable to geology. Correspondence between ecoregions and diatom assemblages is not always evident as shown by Pan et al. (2000) in Mid-Atlantic Highland streams (U.S.) where ecoregional differences between diatom assemblages were rather subtle. In this study (Pan et al. 2000), land use was the most important parameter, and diatom assemblages only corresponded to ecoregions if land use differed between them. Potapova & Charles (2002) also assessed the relationships between diatom assemblages and ecoregions as defined by Omernik (1995); the correlation was not clear, since the most important gradients for diatom assemblages were first a downstream gradient, secondly a mineral content and pH gradient, and thirdly an altitudinal and latitudinal gradient. In our case, the selection of a particular stream type (stream sites above 800 m) reduced the variability of diatom assemblages and the importance of the downstream gradient. The pollution gradient was also reduced since more than 96% of the diatom assemblages indicated a good or very good quality according to the SPI diatom index. Therefore, system A ecoregions reflected differences in diatom assemblages of high altitude streams.

- **Conclusions**

System A ecoregions derived from ecoregions proposed in the Limnofauna Europea of Illies (1978). The map of Illies (1978) shows biogeographical regions of the aquatic fauna, essentially based on macroinvertebrates (Wasson et al. 2001). Even if these ecoregions were not designed primarily for diatoms they can give a first coarse framework for the biogeographical repartition of diatom assemblages. However, some modifications should be carried out to improve the effectiveness of this ecoregional classification. Cantonati et al. (2001), comparing assemblages from the Alps and the Himalayas, pointed out the prominent role of cosmopolitan taxa in mountains. Similarly, in this study several diatom assemblages occurred in different ecoregions, such as cluster 5 was present in the Iberic region and the Western Highlands, or clusters 1, 2 and 3 present in the Pyrenees and the Alps, and others such as clusters 7 and 8 present in all the ecoregions. This also emphasizes the importance of widespread taxa, since similar assemblages were observed in equivalent stream types of geographically separated mountainous areas. Hence, to improve the ecoregional system for diatom assemblages, it might be interesting to regroup some ecoregions, which are geographically separated but characterized by similar diatom assemblages and physical environmental descriptors (e.g. clusters 1, 2, 3 and 5). This approach has already been applied in the United States where some areas belonging to a unique ecoregion are geographically unconnected (Bailey et al. 1995). Our study, focusing on high altitude streams, shows that taking into account ecoregions is necessary for understanding of diatom occurrences. It is therefore also necessary for a better assessment of the stream quality using benthic diatoms. This should also be tested on other stream types to confirm our findings.

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c. Paper 2: Benthic diatom assemblages and their correspondence with ecoregional classifications: case study of rivers in north-eastern France. Rimet F.

Keywords: Bacillariophyceae, biogeography, geology, ionic composition, regional processes, water quality.

- **Abstract**

Diatoms have been routinely used to monitor the ecological status of French rivers. Since 2000, this monitoring has been carried out to comply with the Water Framework Directive. Ecoregions corresponding to the aquatic fauna and flora of rivers were originally defined on the basis of physical descriptors, designated "hydro-ecoregions" in France. They have since been simplified into diato-ecoregions for diatoms. In this study, data sets for benthic diatoms from rivers in Northeastern France were used to identify four main groups of diatom assemblages. The first such assemblage is found in small rivers with crystalline geologies in mountainous massifs with neutral to slightly acidic pH. The second assemblage occurs in small rivers flowing on limestone in lowland regions with an alkaline pH. The third corresponds to the assemblage found in large rivers in limestone lowland regions, and the fourth in highly mineralized rivers with high levels of chloride. Within each of these main assemblages, several communities have been characterized corresponding to different levels of pollution: from pristine to highly polluted. Multivariate analysis showed that the underlying geology was the main factor structuring the diatom assemblages, followed by the pollution level. River size had little impact on diatoms. Statistical tests showed that diato-ecoregions provided little information about diatom assemblages in the studied region, whereas there was a close correlation between diatoms and hydro-ecoregions. Nevertheless, these hydro-ecoregions did not account for the diatom assemblages in the highly mineralized rivers. These ecoregions could be further improved by merging the hydro-ecoregions of the crystalline mountainous massifs, all of which shared the same diatom assemblages.

- **Introduction**

Assemblages of benthic diatoms in rivers are influenced both by environmental descriptors that are not affected by human activities, such as the dominant geology of the river basin (Tison et al., 2004; Cantonati, 1998; Rimet et al., 2007), the altitude of the sampling site (Ndiritu et al., 2006; Rimet et al., 2007), the distance from the source (Potapova and Charles, 2002), and by others that can be affected by human activities, such as the organic load and nutrient concentration of the water (e.g. Van Dam et al., 1994; Kelly and Whitton, 1998; Kovács et al.; 2006). Within this framework, benthic diatoms have been recommended in the last several decades as an appropriate tool for pollution assessment in rivers (e.g. Round, 1991; Coste et al., 1991; Whitton, 1991; Whitton and Kelly, 1995), and have been routinely used throughout France since the late 1990s to indicate pollution levels of watercourses. Several diatom indices, such as the Specific Pollution-sensitivity Index (Cemagref, 1982) and the standardized Biological Diatom Index (Lenoir and Coste, 1996; Afnor, 2000, 2007), are

routinely used for this purpose. The number of sites sampled has increased gradually year by year, especially in response to the strong impetus from the European Water Framework Directive (European Commission, 2000). Since the year 2000, this Directive has required an assessment of the ecological status of rivers using benthic diatoms.

Sampling benthic diatoms is an administrative obligation in order to comply with the requirements of the Water Framework Directive, but it also has the advantage of providing a mass of samples that has made it possible to develop large-scale diatom databases (e.g. Gosselain et al., 2005). These databases can be used to elucidate the ecology and macroecology (Passy, 2007) of benthic diatoms, and to compare the pollution assessments obtained using diatoms in different European countries (Kelly et al., 2008). Moreover, such databases provide a new way to explore the large-scale geographical distribution of diatom assemblages, as has been done for France (Tison et al., 2005b), Finland (Soininen, 2004a), United States (Potapova & Charles, 2002; Pan et al., 2004), and even a combination of several European countries (e.g. Kovács et al., 2006; Rimet et al., 2007). Most of the studies investigating the biogeographical distribution of diatom assemblages demonstrate the importance of taking into account the regional distribution of diatoms, as summarized in the review of Soininen (2007): the dispersal pattern of diatoms is far not ubiquitous.

Since 2000, the Water Framework Directive (European Commission, 2000) has taken on board this variable distribution, which had already been demonstrated for other aquatic organisms, such as macroinvertebrates (Illies, 1978), and now requires the ecological status to be evaluated on the basis of ecoregions. To do this, ecoregions have been defined in various European countries. Ecoregions reflecting the aquatic fauna and flora, are designated as "hydro-ecoregions" in France (Wasson et al., 2002). The methodology for the definition of these “hydro-ecoregions” was based on the classification system B proposed in the Water Framework Directive. This system requires obligatory factors (altitude, geology, geographical coordinates) and optional parameters (morphology, hydrology, climatic parameters). In that framework, a total of 22 hydro-ecoregions of 1^{st} level were defined in mainland France. To take into account more local particularities, 54 hydro-ecoregions of 2^{nd} level were defined from the 1^{st} level hydro-ecoregions using the same methodology. A supplementary work was carried out to test the adequacy of the 1^{st} level hydro-ecoregions to diatom assemblages: based on diatom samplings carried out on the entire national river network, 11 diatom communities in France have been identified using artificial neural network models and were geographically located (Tison et al., 2005a). The 22 hydro-ecoregions have been related to these 11 benthic diatom assemblages, and were simplified to provide five regions (Tison et al., 2005a), known as “diato-ecoregions”. These hydro-ecoregions and diato-ecoregions have already been tested at a national scale, but testing their effectiveness at a regional scale is also of major interest for water managers (Rimet et al., 2006), because this could provide greater detail at a regional scale. This has been done in a particular ecoregion of the United States (Weilhoefer and Pan, 2006), and has led to a critique and subsequent improvements of the ecoregional classification previously in use there. This approach is being considered by the water managers of the Meuse, Moselle and Sarre basins, which are located in North-eastern France.

The study reported here had three objectives. The first was to identify and describe the diatom assemblages in the Meuse, Moselle and Sarre basins. The second objective was to define the environmental parameters that structure diatom assemblages to the greatest extent, and the third was to assess the match between the proposed ecoregions (hydro-ecoregions and diato-ecoregions)

and the diatom assemblages in this region. Several clustering techniques, simple descriptive statistics and multivariate analyses were used for this purpose.

- **Material and methods**

Study area

The sampling area is situated in North-eastern France (Figure 13), and measures approximately 230 km east/west and 250 km north/south. It encompasses three major river basins. First, the Meuse basin, which is largely dominated by agriculture; the River Meuse flows for about 340 km in France, and then continues its course in Belgium. Second, the Moselle basin, which is mostly dominated by industrial activities; the River Moselle flows for 310 km in France; and then continues its course along the border of Luxembourg and Germany. Third, the Sarre basin, is occupied by forestry in its upper part, but industrial and agricultural activities in its lower part. The River Sarre flows for 130 km in France, and then continues its course in Germany.

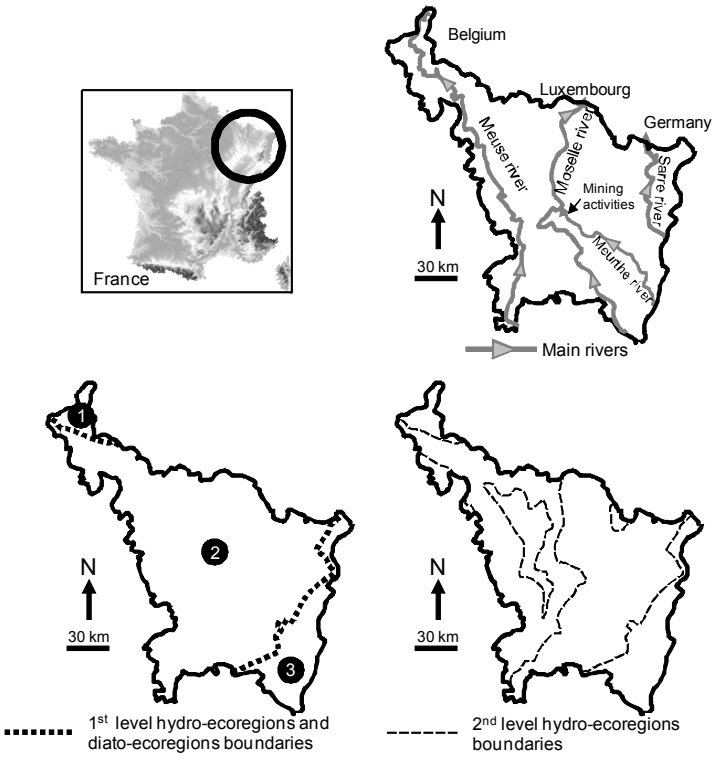

Figure 13: Location of the study area. (a) Main rivers, (b) 1st level hydroecoregions: Ardennes mountains (crystalline geology) (1), Limestone plateaus (2), Vosges mountains (crystalline geology) (3). Diato-ecoregions: region of low conductivities (1 and 3), region of high conductivities (2), (c) 2nd level hydroecoregions.

From 2000 to 2005, diatoms were sampled as part of the biomonitoring program for national river networks (RNB -Réseau National Bassin- and Rref -Réseau de Référence-). The samplings followed the Biological Diatom Index standard (Afnor, 2007), which incorporates the European standard for diatom sampling (Afnor, 2003), and were carried out once a year during the summer period (June - September). Benthic diatoms were collected from at least 5 stones from the lotic parts of the

sampling sites. The upper surface of the stones was scrubbed with a toothbrush. The samples were fixed in 4% formaldehyde. In the laboratory, the diatom valves were cleaned using 40% hydrogen peroxide to eliminate organic matter, and with hydrochloric acid to dissolve calcium carbonate. Clean diatom frustules were mounted in synthetic resin (Naphrax©). At least 400 valves were counted and identified in each sample using a light microscope with a 1000× magnification. Abundances of all observed taxa were expressed as relative counts. The identifications and counts were carried out using Krammer and Lange-Bertalot (1986, 1988, 1991a and b) and Krammer (2000, 2001, 2002).
The counts were carried out by different people, but to ensure a taxonomic homogeneity between them, the counts were systematically checked and, if necessary, identified and counted again by the author of this paper.
Physical and chemical analyses were also carried out at the same sampling sites each month. Water temperature, dissolved oxygen, conductivity and pH were measured in the field. For NO_3^-, NO_2^- NH_4^+, Kjeldahl nitrogen (NKJ, quantification of organic nitrogen), total nitrogen (quantification of organic and mineral nitrogen), total phosphorus, PO_4^{3-}, Na^+, Ca^{2+}, Cl^-, K^+, Mg^{2+}, SO_4^{2-}, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Dissolved Organic Carbon (DOC), suspended matter, carbonates and bicarbonates, water samples were collected and analyzed in laboratory according to standard procedures (APHA, 1995). Since diatom samplings were carried out between June and September (low flow season), an average was calculated for each parameter between June and September in order to have a representative value for the site corresponding to the diatom sampling period. River width was measured in the field. For each sampling site, the Strahler rank was defined according to Strahler (1957). This rank gives a first approximation of the size of the river, and it was used for this purpose in this study. Strahler ranks were calculated using a geographical information system (GIS).

Data analysis
A cluster analysis was used to define groups of samplings with similar diatom assemblages; the Twinspan analysis (Two Way Indicator Species Analysis; Hill, 1979) was computed from the diatom counts (expressed as percentages) with the PcOrd software (McCune and Mefford, 2006). The pseudospecies cut-off levels used were those suggested by the software: 0, 2, 5, 10, 20.
Several MRPPs -Multi Response Permutation Procedures- (Biondini et al., 1985) were computed with the PcOrd software (McCune and Mefford, 2006) to select the best number of Twinspan groups. This analysis calculates an A-statistic, which is a descriptor of within-group homogeneity. This statistic varies between -1 and 1: if the A-statistic approaches 1, the groups are completely different; if the A-statistic approaches 0, the heterogeneity within groups equals what would be expected by chance; if the A-statistic approaches -1 the groups are homogeneous.
A geographical information system (MapInfo©) was used to locate the groups on the studied area. Average diatom assemblages were calculated for each Twinspan group by calculating the average abundance of each taxon in the group. Then, the indicator taxa were defined for each group using the Indicator Species analysis of Dufrêne & Legendre (1997) with the PcOrd software (McCune and Mefford, 2006). This analysis gives an idea of the “consistency” of each taxon for each group. This analysis yields an index calculated on the basis of the abundance and the faithfulness of each taxon in each group. If a taxon has a high Indicator Species analysis index in a group, then the group will be indicated by this taxon. Moreover, this index is tested by mean of a Monte-Carlo test; the indices

presenting a significant p value (<5%) are shown for the corresponding taxa. This analysis calculates the indicator taxa of each group on the basis of its abundance and faithfulness in the group.
In order to determine the most structuring parameters for the 16 diatom assemblages, a forward selection and a discriminant analysis were carried out on 22 environmental parameters (bicarbonates, Ca, chlorides, conductivity, K^+, Mg^{2+}, pH, Na^+, sulphate, NH_4^+, NKJ, dissolved organic carbon, chemical oxygen demand, biological oxygen demand, NO_2^-, O_2, total nitrogen, NO_3^-, PO_4^{2-}, total phosphorus, river width, altitude) and on the 16 groups. The Ginkgo program (De Caceres et al., 2007) was used to compute these analyses. The 22 environmental parameters were standardized before computing the analyses. The standardization was carried out by dividing the difference between the considered value and the average value of the parameter by its standard deviation. Finally the groups were characterized using physical and chemical parameters, and box plot graphs.
In order to comply with the requirements of the WFD, a typological system has been developed in France, leading to the definition of several hydro-ecoregions on the basis of various parameters (altitude, geology, river basin size, river morphology, substrate, climatology; Wasson et al., 2001; Wasson et al., 2002). Several precision levels have been defined in these hydro-ecoregions: France includes 22 1st-level hydro-ecoregions, which are sub-divided into 54 2nd-level hydro-ecoregions. These hydro-ecoregions were then simplified into 5 diato-ecoregions to match the benthic diatoms found in rivers by Tison et al. (2005a and b). The match between diatom assemblages and these various ecoregional classifications were assessed by means of MRPP.
For each sample, based on its diatom composition, the diatom index of water quality, known as the Specific Pollution Sensitivity Index (IPS; Cemagref, 1982), was calculated. This index assesses the water quality according to 5 classes (IPS values: very bad quality < 5 ≤ bad < 9 ≤ intermediate quality < 13 ≤ good < 17 ≤ very good). The rivers in the studied area had Strahler ranks from 1 to 6; rivers with Strahler ranks of 1 and 2 were grouped together, as were rivers of ranks 5 and 6, also according to Chandesris et al. (2006). The water-quality classes and the river-size classes were also compared to the ecoregional classifications by means of MRPP.

- **Results**

Floristic inventories
A total of 744 samplings were carried out from 2000 to 2005, and 567 taxa were identified. For the following statistical analyses and because of software limitations (size of the database which could be used), a selection of the most abundant taxa was carried out: the sum of the abundance of each taxon was calculated over the 744 samplings and the 220 taxa with the highest sum values were selected.

Twinspan analysis and geographical location of the assemblages
The results of the MRPP computed on the Twinspan classification are shown in Figure 14. There is a gradual increase in the A-statistic as the number of groups increases. However, the increase in the A-statistic value was weak above 16 groups, and so a total of 16 final groups was chosen. Figure 15 shows the results of the Twinspan classification Table 5.

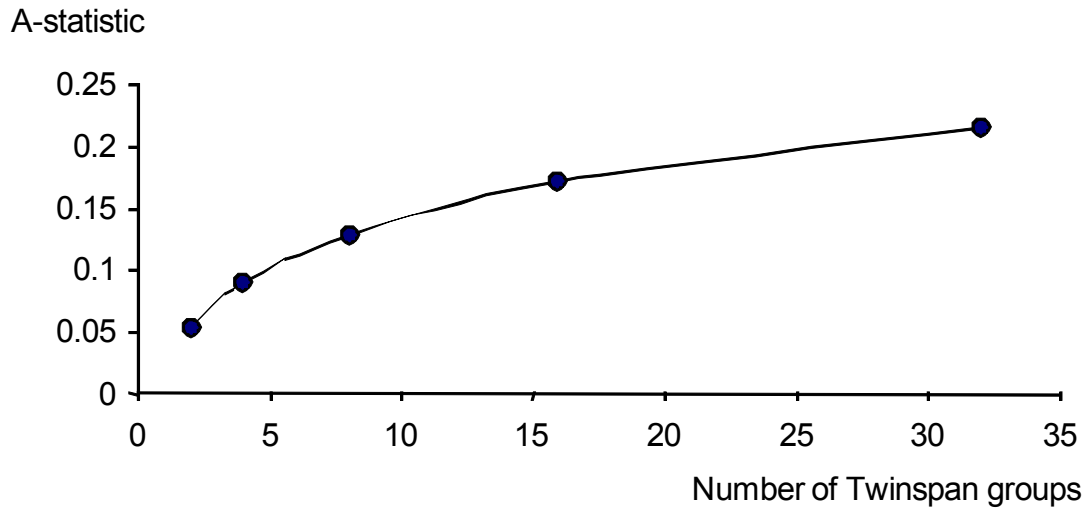

Figure 14: Evolution of the A-statistic value (calculated with MRPP) with the number of Twinspan groups.

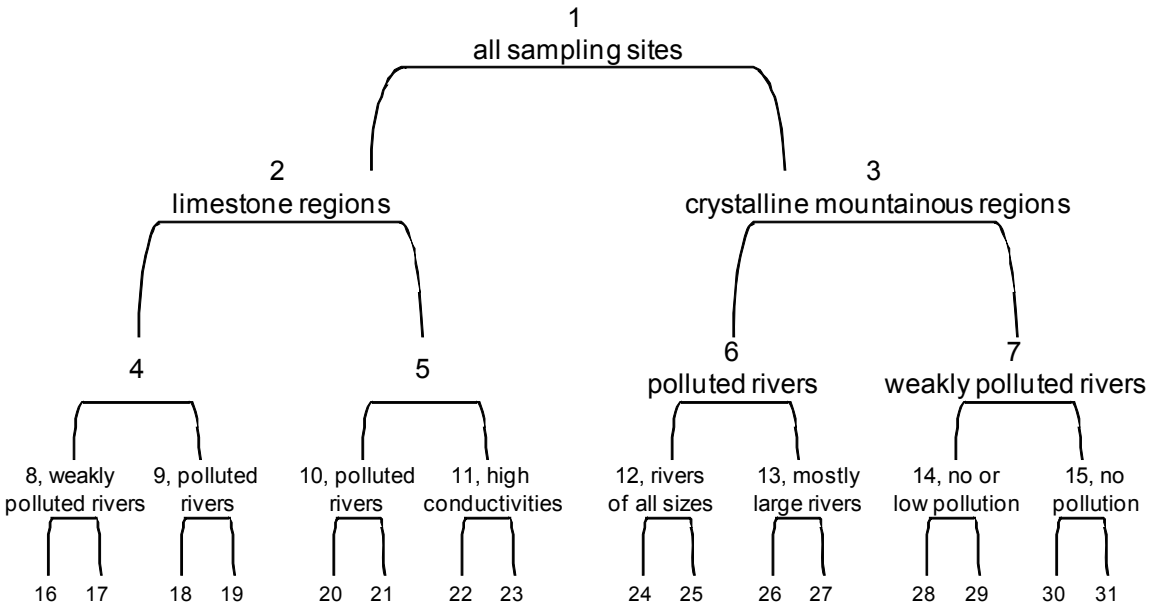

Figure 15: Groups of samples defined on the basis of their diatom composition. The groups were calculated using a Twinspan analysis.

Groups 2 and 3 are plotted on a geographical map in Figure 16. A clear separation between these two groups can be observed: group 2 corresponds to the lowland limestone region, and group 3 to the crystalline and mountainous area. The final 16 groups are plotted on geographical maps in Figure 17, and can be recombined to form the two former groups: group 2 “diatom assemblages of limestone regions”, and group 3 “diatom assemblages of crystalline mountainous regions”.

Table 5: Number of samples and sites constituting the 16 Twinspan final groups.

	Group identification	Number of samples	Number of sites
Limestone regions	16	8	6
	17	63	26
	18	41	27
	19	176	80
	20	77	37
	21	95	41
	22	67	26
	23	65	25
Crystalline mountainous regions	24	26	11
	25	85	29
	26	9	6
	27	19	8
	28	11	4
	29	1	1
	30	9	9
	31	2	2

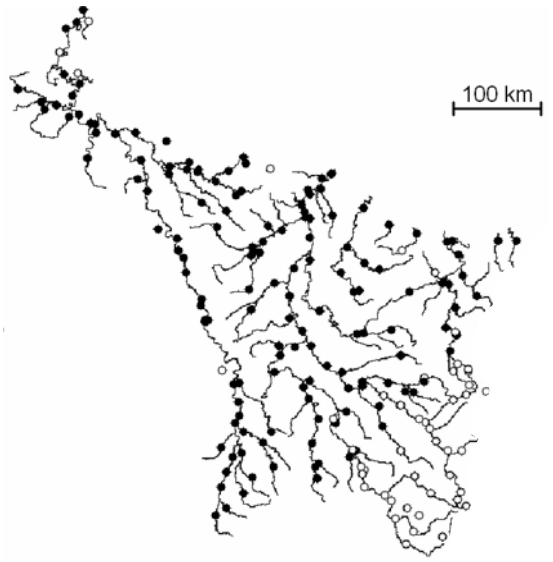

Figure 16: Location of the groups 2 (black spots) and 3 (white spots).

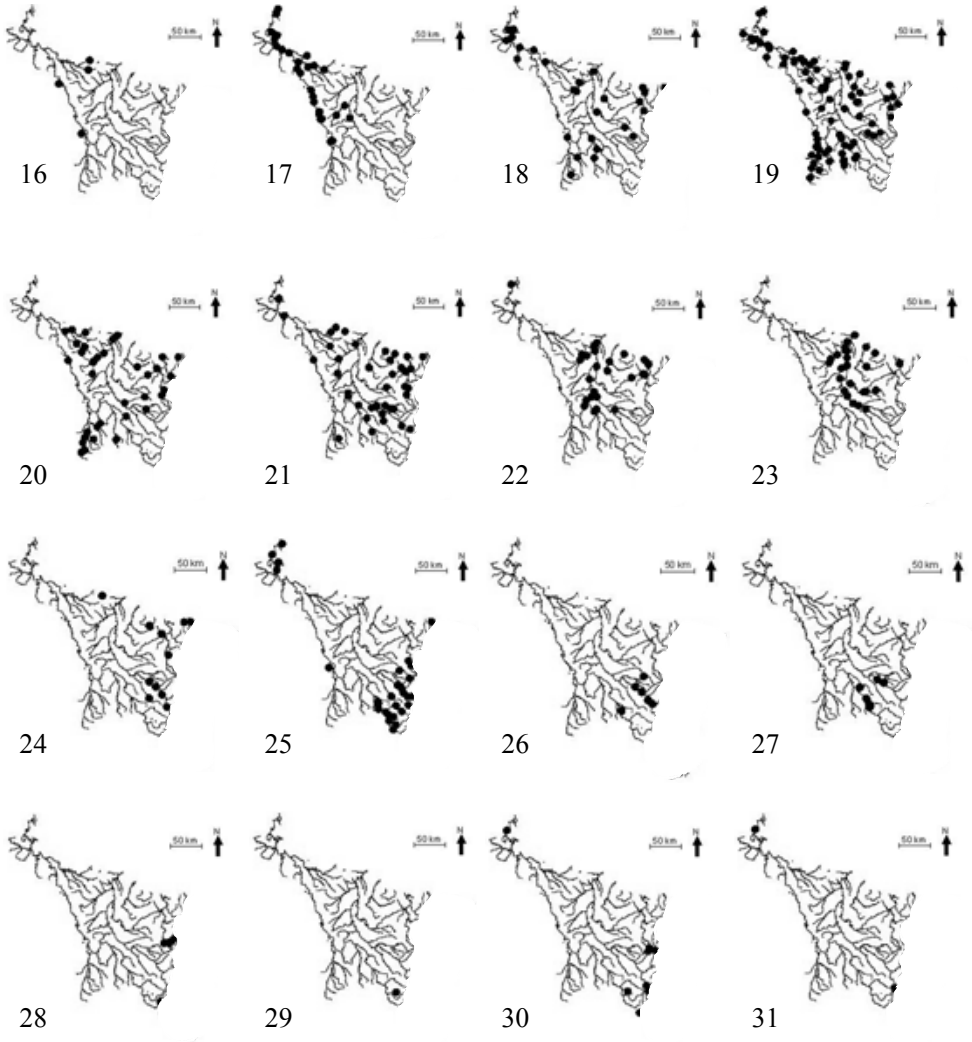

Figure 17: Box-plot of environmental parameters for the 16 diatom assemblages. The horizontal lines across the boxes correspond to the 25% quartile, median and 75% quartile values. The whiskers outside the box show minimum and maximum values.

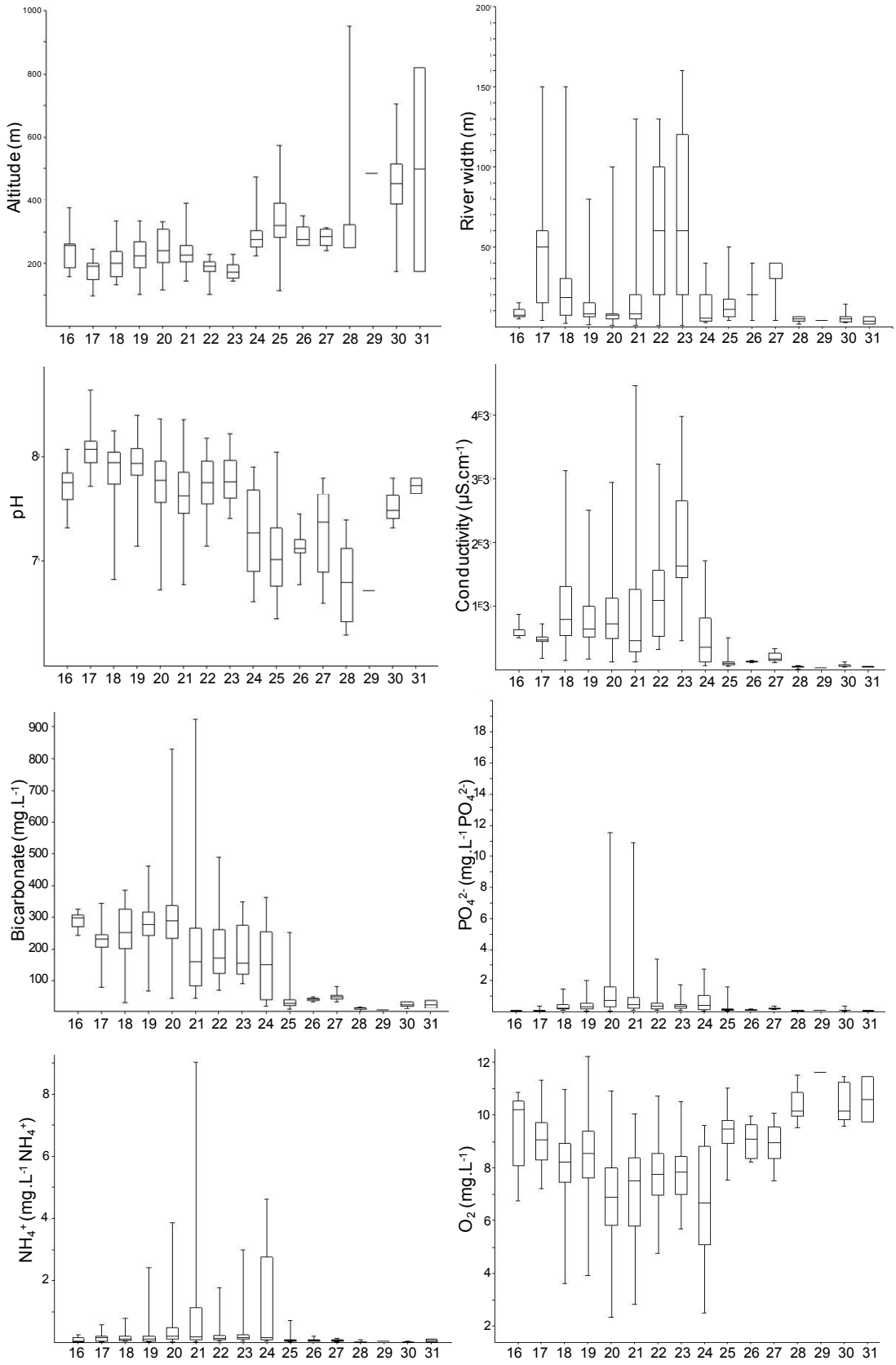

Figure 18: Box-plot of environmental parameters for the 16 diatom assemblages. The horizontal lines across the boxes correspond to the 25% quartile, median and 75% quartile values. The whiskers outside the box show minimum and maximum values.

Diatom assemblages on limestone geology

Diatom assemblages numbers 16 to 23 constitute this set; their average diatom assemblages and indicator taxa are shown in appendix 2.

Only two of them can be considered to be of good and very good ecological quality, respectively. The first (group 16) correspond to small streams (Strahler rank of 3) near the source (median of 12 km), and its most indicator taxa are *Achnanthidium biasolettianum, Denticula tenuis* and *Encyonopsis microcephala*. Several of these rivers are karst resurgences, with low PO_4^{3-} and NH_4^+ concentrations (Figure 18). The second (group 17) correspond to large river sites (Strahler rank of 5) rather far from the source (median distance of 218 km), and most of the sampling sites are located in the Meuse River. *Achnanthidium* cf. *straubianum* and *Cymbella excisa* are its most indicative taxa. Phosphorus concentrations at these sampling sites are relatively low (Figure 18).

Four communities are characteristic of various specific levels of pollution (groups 18 to 21). The communities of groups 18 and 19 correspond to sampling sites with relatively high organic pollution (median DOC of 3.8 mg l^{-1} and 3.5 mg l^{-1} for groups 18 and 19 respectively). The river sizes vary in group 18, whereas they are mostly small to middle sized for group 19 (Strahler rank of 3 and 4). The indicator taxon for group 18 is *Nitzschia sociabilis*. The most indicator taxa of group 19 are *Navicula tripunctata* and *Nitzschia dissipata*.

The communities of groups 20 and 21 are exposed to a higher level of organic pollution (see NH_4^+ and PO_4^{3-} in Figure 18). Indicator taxon of group 20 are *Platessa conspicua* and *Psammothidium lauenburgianum* and *Navicula veneta* is the indicator taxon for group 21.

Finally, diatom communities of groups 22 and 23 correspond to rivers characterized by high electrolyte contents (see conductivity in Figure 18), and in particular, high concentrations of Cl^- (medians of 43 and 371 mg l^{-1} for groups 22 and 23 respectively) and of SO_4^{2-} (medians of 105 and 133 mg l^{-1}). The indicator taxa of these groups are *Cyclotella atomus* (group 22), *Nitzschia frustulum, N. inconspicua, Navicula recens* (group 23). Three kinds of rivers can be identified in these groups. The first kind, which in fact includes most of them, are large rivers (Strahler rank of 5) impacted by chloride discharges from soda extraction plants; the second are small rivers (Strahler rank of 3 and 4) with naturally high electrolyte contents, and the third are rivers downstream of coal mines discharging water with a high SO_4^{2-} content.

Diatom assemblages on crystalline geology

The average diatom assemblages and the indicator taxa of this set of assemblages are shown in appendix 2.

The communities of groups 24 and 25 correspond to organically polluted and lightly polluted rivers respectively (median values for PO_4^{3-} 0.13 and 0.03 mg l^{-1} for groups 24 and 25 respectively). The indicator taxa of these groups are *Eolimna minima, Fistulifera saprophila, Sellaphora seminulum* (group 24) and *Fragilaria capucina* var. *vaucheriae* (group 25).

The communities of groups 26 and 27 are present in transition geologies: these rivers have limestone bedrock, but their conductivities are low (median conductivity 182 µS cm^{-1}), because they are influenced by their catchment areas, which are largely dominated by a crystalline geology. The rivers in group 26 are more polluted than those in group 25 (e.g. PO_4^{3-} concentrations of 0.10 and 0.19 mg l^{-1} for groups 26 and 27, respectively). *Nitzschia fonticola* is the most indicative taxon for group 26 and *Achnanthes subhudsonis* is the most indicative taxon for group 27.

The last four communities (groups 28 to 31) correspond to very small to small rivers with low pollution levels (Strahler rank of 1 to 3). One of them (community 29) is constituted by a single sampling site with a low electrolyte (52 µS.cm^{-1}) content and pH (6.5). Its community is dominated by acidophilic taxa of the genus *Eunotia* (*E. intermedia, E. tenella*).

The communities of groups 28 and 30 correspond to small rivers (Strahler rank of 3), but can be distinguished in terms of pollution level, even if both of them are very lightly polluted. The sampling sites of group 30 are more polluted than those of group 28 (median of PO_4^{3-} 0.05 and 0.1 mg L^{-1} respectively for groups 28 and 30). The most indicator taxa of community 28 are *Psammothidium oblongellum* and *P. subatomoides*. *Achnanthidium subatomus* and *Cocconeis placentula* var. *euglypta* are the most indicator taxon of group 30.

Group 31 corresponds to very small, unpolluted rivers (Strahler ranks 1 and 2). Taxa such as *Fragilaria gracilis*, *Gomphonema micropus* and *Diatoma mesodon* are the most indicative species of this assemblage.

Comparison of the classifications and of the importance of environmental parameters for diatom assemblages

Comparisons of the importance of different classifications (water quality assessed using the IPS diatom index, the diato-ecoregions, the hydro-ecoregions and the river size assessed with the Strahler rank) for diatom assemblages are shown in Figure 19. The hydro-ecoregion classification is the most informative for diatom assemblages; that of water quality is weaker but comparable. River size and diato-ecoregions are much less informative in explaining variability in diatom assemblages.

The results of the forward selection and the discriminant analysis are shown in Figure 20. Fifteen parameters out of 22 were retained by the forward selection to compute the discriminant analysis. The results of the discriminant analysis show clearly that conductivity, pH and carbonates are the most structuring parameters for all 16 diatom assemblages. Parameters related to pollution (NH_4^+, total phosphorus, dissolved oxygen, NKJ) are secondary, as are altitude and river width. Box plot graphs of the most important parameters are shown in Figure 18.

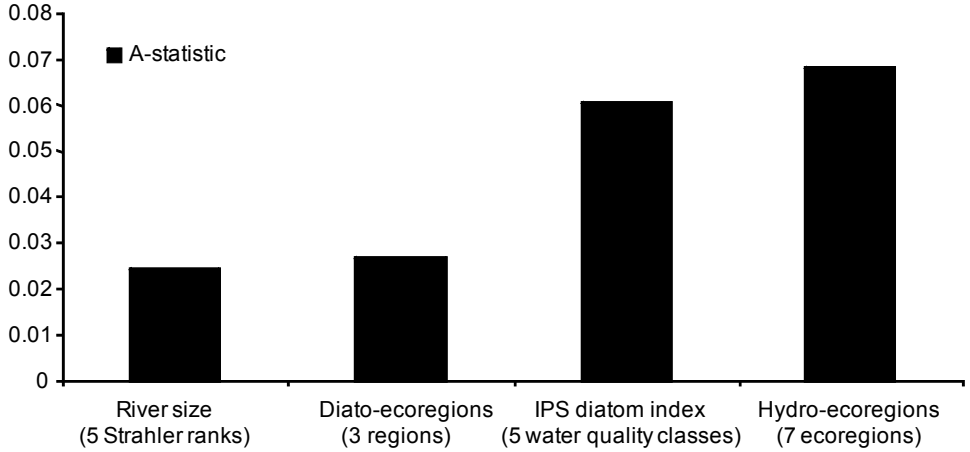

Figure 19: Comparison of the importance of different classifications for the diatom assemblages, by mean of the A-statistic (calculated with MRPP).

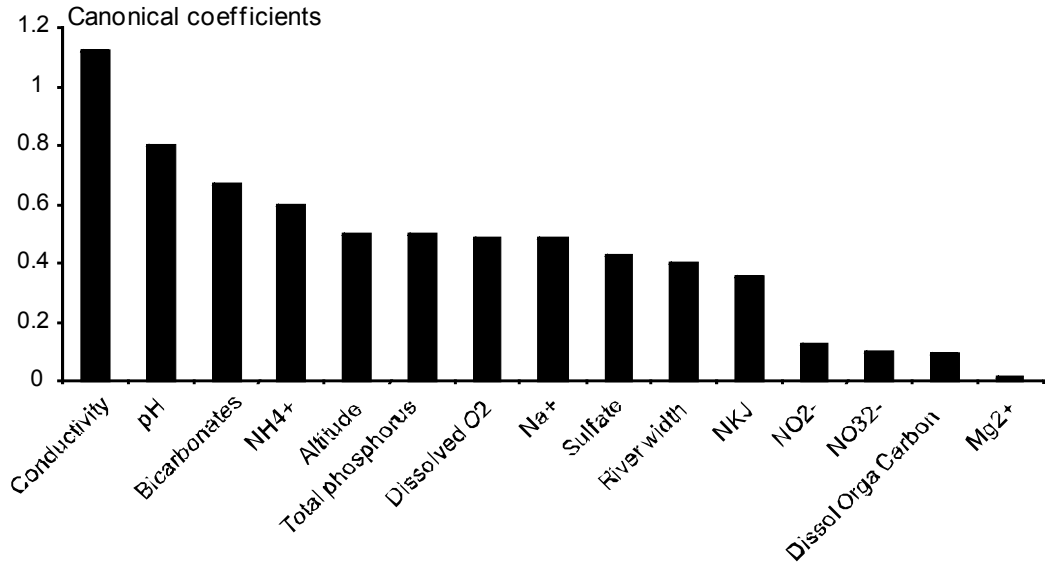

Figure 20: Discriminant analysis carried out on 22 environmental parameters and the 16 final groups. A forward selection was realized to remove 7 redundant parameters. The canonical coefficient of the environmental parameters on the first axis of the discriminant analysis (absolute value) are represented for the 15 lasting parameters.

- **Discussion**

Diatom assemblages of the north-eastern France

Diatom assemblage of the studied river basins (Meuse, Moselle and Sarre basins) can be divided in two main diatom biotypes. The first one corresponds to rivers flowing on limestone regions and the second to rivers flowing on crystalline geology. Inside each type, several particular communities can be described and typified.

Groups 16 to 23 belong to these diatom assemblages of limestone region, some corresponding to low polluted situations (groups 16 and 17). Watercourses of group 16 are mainly small karstic unpolluted rivers and its most indicator taxa are regularly referenced as characteristic of pristine waters as *Achnanthidium biasolettianum* (Rimet et al., 2004) and *Encyonopsis microcephala* (Reichardt, 1997). Group 17 encompassed large rivers relatively weakly polluted and flowing on limestone in regions of low population density; its most indicator taxa *Achnanthidium* cf. *straubianum* and *Cymbella excisa* are considered as indicator of good water quality by diatom indices (e.g. Cemagref, 1982; Lenoir & Coste, 1996).

Four communities (18 to 21) correspond to rivers with various pollution levels and their indicator taxa are characteristic of polluted waters. For instance *Nitzschia sociabilis* for group 18, *Navicula tripunctata, Nitzschia dissipata* for group 19 are considered as mesosaprobic by Van Dam et al. (1994); *Navicula veneta* for group 21 is considered as polysaprobic by Van Dam et al. (1994). Group 20 clusters small polluted rivers and its indicator taxa, *Platessa conspicua* and *Psammothidium lauenburgianum,* were also recorded in the same kind of small polluted streams in Luxembourg (Rimet et al., 2004).

Groups 22 and 23 gather rivers with high electrolyte contents, some of these rivers being naturally salty (salt rock in the river basin), some being impacted by soda industries or coal mines. The

indicator taxa of these groups, *Nitzschia frustulum, N. inconspicua, Navicula recens* are often observed in brackish waters (Witkowski et al., 2000). Several rare taxa observed in these rivers, such as *Entomoneis paludosa*, *Pleurosira laevis, Haslea spicula*, clearly indicate high levels of Cl^-, since they are species found in brackish water or in seawater (Witkowski et al., 2000).

Communities of groups 24 to 31are present in rivers flowing on crystalline geology, or with a hydrographical basin largely dominated by such kind of geology. Some of them correspond to polluted situations as group 24 with indicator taxa (e.g. *Eolimna minima, Sellaphora seminulum*) considered as pollution tolerant according to various diatom indices (Cemagref, 1982; Lenoir and Coste, 1996).
Groups 26 and 27 regroup sampling sites on limestone bedrock, but with a basin dominated by crystalline geology. The water conductivity of these rivers is therefore quite low compared to rivers flowing on limestone geology since the beginning of their course. The most indicator taxa of these groups are *Nitzschia fonticola,* a quite widespread taxon considered as mesosaprobic (Van Dam et al., 1994) and an invasive taxon (Coste & Ector, 2000) *Achnanthes subhudsonis* recorded in base poor rivers in Himalaya (Jüttner et al. 2003).
Communities of groups 28 and 30 gather small rivers characterised by low pollution levels and most of their indicator taxa are referenced in waters of low conductivities, *Psammothidium oblongellum, P. subatomoides, Achnanthidium subatomus* (Rimet et al. 2004), *Cocconeis placentula* var. lineata (Monnier et al. 2007), *Gomphonema rhombicum* (Ector, Iserentant & Ector, 1996).

Structuring importance of environmental descriptors for diatoms assemblages
The geology of the bedrock was an important environmental descriptor in explaining the heterogeneity and geographical distribution of diatom assemblages in the rivers of the studied area. This descriptor has also been shown to be determinant in other rivers: in Luxembourg (Rimet et al., 2004), in France (Tison et al*.,* 2004), in European mountains (Cantonati, 1998; Rimet et al., 2007), and in the rivers of Oregon USA (Weilhoefer and Pan, 2006). Water conductivity, pH, and mineral content such as bicarbonate are directly linked to the composition of the dominant geology in the river basin. They were determinant in our study in explaining diatom communities, as already observed elsewhere (Potapova and Charles, 2003; Soininen, 2004b). In his review covering more than 12 studies of stream diatom assemblages, Soininen (2007) showed that major ion concentration is a primary structuring factor at the scale of a wide geographical range.
Nevertheless, several studies have found geology to be secondary compared to pollution, as is the case in Spanish rivers (e.g. Tornes et al., 2007; Leira and Sabater, 2005), or in mid-Atlantic streams in the US (Pan et al., 1999). The reason given for the relative importance of the effects of geology and pollution on diatoms is that the human impact masks that of geology (Tornes et al., 2007). In our study, human impact appeared to be secondary even though land use pressures are very varied in north-eastern France: some areas are dominated by industrial activities and mining (the north of the region), others are agricultural (the west of the region, especially in the Meuse Basin), and others again are dominated by forestry (the east of the region, mainly in the north of the Vosges hydro-ecoregion). The most important environmental descriptors that represented pollution in the present study were NH_4^+ and total phosphorus. Diatom composition in polluted areas differed depending on the geological substrate of the basin. For instance assemblage 19, present in small to medium size polluted rivers on limestone bedrock, was dominated by *Amphora pediculus*, and had *Navicula*

tripunctata and *Nitzschia dissipata* as indicator taxa. These two indicator taxa were never present at high abundances on crystalline geologies. Diatom assemblage 24, which is also present in small to medium size polluted rivers but on crystalline geology, was dominated by *Eolimna minima* and had *Naviculadicata seminulum, Craticula molestiformis* as indicator taxa. This means that the impact of geology can also be observed in polluted areas, whereas it is usually only under reference conditions that the diatom assemblages are clearly related to geology (Tison et al., 2005b; Rimet et al., 2007). This means that our findings are quite in disagreement with the common idea that pollution homogenizes diatom assemblages (Pan et al., 2000). However, perhaps we need to consider the possibility that geology could determine land use, and that this in turn influences water quality. A detailed study of the agricultural practices should be made, and related to the different diatom assemblages.

Some of the rivers in the studied area have naturally high conductivities. We found that these rivers regularly reached conductivities of 3000 to 4000 µS cm^{-1}, and had chloride concentrations of 500 mg l^{-1} (e.g. River Seille, River Sanon). The taxonomic composition of their diatom assemblages is very different from that found in all the other rivers. These naturally salty rivers (dissolved salt in rivers comes from geological layers containing rock salt) were assigned to diatom assemblage 23, together with rivers impacted by mining activities for sodium exploitation. Mining activities discharge large quantities of $CaCl_2$ and NaCl into these rivers (River Meurthe), artificially increasing conductivity over 2500 µS cm^{-1}, with a chloride concentration over 600 mg l^{-1} and a Na^+ concentration of over 220 mg l^{-1}. Upstream from these mining activities, conductivities are below 500 µS cm^{-1}, the chloride concentration is below 20 mg l^{-1}, and the Na^+ below 12 mg l^{-1}. Indicator taxa of this assemblage are typical of such high conductivities with *Navicula recens* and *Nitzschia frustulum* considered to be indicators of high electrolyte contents by Van Dam et al. (1994) and Krammer and Lange-Bertalot (1986, 1988). Some taxa, such as *Pleurosira laevis, Haslea spicula* and *Entomoneis paludosa*, can be found in these rivers, but more rarely, and are usually considered as brackish and even marine taxa (Witkowski et al., 2000). A second diatom assemblage (n°22) is found in water with high electrolyte contents, but is only present in rivers exposed to artificial saline pollution.
The impact of conductivity on freshwater rivers has already been thoroughly investigated, for instance in Italy (Torrisi and Dell'Uomo, 2006; Torrisi et al., 2008), and a similar diatom flora was found. A diatom index assessing organic pollution and the chloride content of water, has been developed in this context in Italy (Dell'Uomo, 2004) and also in Germany (Ziemann, 1971, 1982, 1999). Mining activities in the studied area were located within a precise, restricted zone, but they impacted on long stretches of river (see Figure 13 for the locations of the mining activities). From the Na^+ extraction facilities on the River Meurthe to the country's boundary with Germany and Luxembourg (more than 100 km downstream), the water of the river is impacted by these high chloride concentrations. The extent of this impact in our study area explains why the structuring effect of Na^+ on diatom assemblages was comparable to that of other environmental descriptors, such as the organic and trophic pollution.

Correspondence between ecoregions and diatom assemblages
Several ecoregional classifications have been proposed to describe the natural variety of diatom assemblages in France. The first classification was the hydro-ecoregional typology, which was constructed on the basis of geology, geomorphology and climate Wasson et al. (2001). A total of 3

main hydro-ecoregions (1st order hydro-ecoregions) were present in the studied area (Vosges, Ardennes, Limestone plateaus) plus seven 2nd level hydro-ecoregions. These seven 2nd level hydro-ecoregions were simplified and adapted to benthic diatoms by Tison et al. (2005a, b). Two diato-ecoregions were present in the studied area (regions of low conductivities and regions of high conductivities). The results clearly show that the 7 hydro-ecoregions describe the diatom assemblage diversity well, even better than the water quality classes given by the IPS diatom index (Cemagref, 1982). These hydro-ecoregions also give a better description of diatom diversity than the river size (Strahler ranks). In other studies, river size is often observed to be the most structuring environmental descriptor for diatom assemblages (e.g. Potapova & Charles, 2002; Pan et al., 2000). This has led some authors to establish diatom biotypologies for bioassessment purposes (Descy and Coste, 1991). This complex descriptor does not have any direct influence on diatoms, but is a proxy for several correlated parameters, such as slope or elevation, which directly influence water turbulence or temperature for instance. These last two parameters do have direct effects on specific diatom composition.

Even though these hydro-ecoregions seem to be appropriate; they do not account for the diatom assemblages of the naturally-salty rivers (e.g. River Seille, River Sanon). In the hydro-ecoregions of Wasson et al. (2001), these particular rivers are classified as the "Limestone Plateaus" hydro-ecoregion, even though they show completely different diatom taxa from these found in the typical diatom assemblages of limestone plateaus. Therefore a particular river type should be defined for these rivers in order to improve the hydro-ecoregion classification of the diatom assemblages in the Meuse, Moselle and Sarre river basins.

A second comment can be made about separating the hydro-ecoregion of "Vosges" from that of "Ardennes". Both these hydro-ecoregions are characterised by crystalline geologies (schist or granite), which confer low conductivities on the water of the rivers. These two mountainous regions display similar diatom assemblages, with indicator taxa characterising low conductivities, such as *Achnanthidium subatomus* (Rimet et al., 2004)*, Gomphonema rhombicum* (Iserentant & Ector, 1996)*,* or slightly acidic pH, such as *Psammothidium subatomoides* (Manoylov, 2007). Therefore, to improve the ecoregional classification for diatom assemblages, these two regional entities could be merged.

The second ecoregional classification tested, with two diato-ecoregions, is a simplification of the 7 hydro-ecoregions. Their accuracy for explaining the diatom assemblage diversity of the studied area was poor, and indeed performed least well of the 4 classifications tested. The diato-ecoregions are probably more suitable for explaining diatom assemblages at larger scale, such as the national scale.

Diatom assemblages in the area studied display considerable diversity. This diversity and the structure of these assemblages were mainly explained by the dominant geology of the catchments area. The structuring effect of geology on diatoms was greater than that of pollution, since diatom assemblages in polluted rivers still reflected the dominant geology of the basin. This finding contrasted with that of other studies showing increasing uniformity of diatom assemblages as the pollution level increases (e.g. Tornes et al., 2007; Leira and Sabater, 2005; Pan et al., 1999). A point to consider in further studies is whether geology influences land use, which in turn could influence the water quality and finally diatom assemblages of polluted areas. In several studies, river size appeared to be a determinant factor controlling the structure if the diatom assemblages (e.g. Descy and Coste, 1991; Molloy, 1992), and the downstream effect is one of the most structuring gradients on river diatoms (Potapova and Charles, 2002). In our study this gradient did not appear to have greater structuring effect than geology or anthropic pressure. In our study, river size was assessed from the riverbed width and the Strahler rank, and detected rather weak impacts. A possible explanation for these disagreements between our findings and the other studies cited is the wide variety of geologies encountered in the area studied (sedimentary and crystalline geologies).
The step, in which the most important structuring parameters and the main diatom assemblages were identified, was necessary to understand the tests of the different ecoregional classifications. The diato-ecoregions tested gave rather poor concordance with the diatom assemblages of the studied area. They only matched the first two groups defined in the Twinspan analysis. The diato-ecoregions are probably more suitable for describing diatom assemblages at the national scale (France) than at a more local scale. The hydro-ecoregions provided good results, and gave a good idea of the distribution of diatom assemblages in the Meuse, Moselle and Sarre catchment basins. Nevertheless they could be improved by merging the hydro-ecoregions of the Ardennes and the Vosges massifs, which have the same geologies and river morphologies, and the same reference diatom assemblages. In contrast, another hydro-ecoregion should be created to take into account the rivers with high conductivities flowing over naturally salted geologies, which have very distinctive diatom floras, which are known and have been thoroughly investigated (Pierre, 1968, 1970, 2001).

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3. Taxonomic resolution and life-forms in diatom biomonitoring

3. Taxonomic resolution and life-forms in diatom biomonitoring

a. Preamble and major results

• Introduction

Diatoms are extremely diverse. In 1996 Mann & Droop (1996) estimated the number of taxa to be around 100,000. Recent studies based on the biological concept of species and on molecular approaches have revealed cryptic diversity within many diatom species complexes, such as *Sellephora pupula* (Evans et al., 2008; Mann et al., 2004), *Nitzschia palea* (Trobajo et al., 2009), *Navicula cryptocephala* (Pouličková et al., 2010), and *N. phylepta* (Vanelslander et al., 2009). More conventional studies based on morphological approaches have revealed tremendous diversity within well known species complexes such as *Achnanthidium minutissimum* (Ector, 2011), and *Amphora copulata* (Levkov, 2009). The number of diatom taxa estimated in the late 1990s now surely needs to be revised upwards.

The diversity and composition of benthic diatom assemblages in rivers are both influenced by the geological context (Tison et al., 2004), latitude (Vyverman et al., 2007), altitude (Ndiritu et al., 2006; Rimet, 2009), river size (Potapova & Charles, 2002) and by factors influenced by human activities, such as organic matter and nutrient concentrations (e.g. Van Dam et al., 1994; Rott et al., 1997; 1998). This diversity and their relationships with pollution make diatoms an excellent bioindicator. Numerous diatom indices (see review of Ector & Rimet, 2005) and calibration models (e.g. Winter & Duthie, 2000; Ponader et al., 2008) have been developed for assessing ecological conditions in rivers. Most of the existing bioassessment tools are based on identifying species or sub-species and on determining their abundance in biofilms, with the exception of a few that are based on genus-level identifications (Rumeau & Coste, 1988; Wu, 1999; Chessman et al., 1999).

Diatom identification requires lengthy training, and the frequent changes in nomenclature can often frighten ecologists (Kociolek & Stoermer, 2001). For instance, 356 new taxa and taxonomic combinations were recorded in 2009 according to the Catalogue of Diatom Names of the California Academy of Sciences (http://research.calcademy.org). The question of what level of taxonomic resolution to choose has long been debated in macroinvertebrate bioassessment, and some authors claim that species determination should be viewed as the gold standard, mainly on the grounds of tradition (Carter & Resh, 2001). Moreover some disadvantages of using a precise taxonomic resolution for river quality assessment have been highlighted (Jones, 2008), and in some cases it does not enhance the assessment. For diatoms, the appropriate level of taxonomic resolution is still debated. Identification difficulties can be seen as limiting their use as routine indicators (Zampella et al., 2007), but it may be possible to reduce the number of species without impairing the assessment (DeNicola, 2000). Even if some taxa with similar ecologies and morphologies are merged, the diatom indices still display a good capacity to assess river pollution (Lenoir & Coste, 1996). Comparison of genus- and species-level resolution has demonstrated the efficacy and robustness of using the genus for bioassessment (Growns, 1999; Wunsam et al., 2002). On the other hand, the importance of using a fine resolution has also been stressed (Ponader & Potapova, 2007), and differing ecologies have been observed between cryptic species within species complexes (Vanelslander et al., 2009).

Diatoms exhibit an interesting diversity of life-forms and cell sizes that merit investigation in relation to water pollution. Using such biological metrics can provide useful information about the structure and architecture of river biofilms. Moreover these metrics are often characteristic of entire genera or families, and can therefore provide another way of drastically reducing the need for taxonomic precision. The relationships between diatom life-forms and nutrient levels are well established in experimental contexts (Hoagland et al., 1982; Pringle, 1990), but have never been tested *in situ* on large areas. Relationships between cell size and the availability of resources have been observed for both phytoplankton and periphyton (Cattaneo et al., 1997; 1998; Wunsam et al., 2002), but failed to provide any interesting data for river quality assessment in some Canadian studies (Lavoie et al., 2010). Finally, Passy (2007) defined ecological guilds of diatoms, the abundance of which is controlled by physical disturbance (water turbulence) and nutrient enrichment. These ecological guilds include diatom species of different genera or families which are able to resist similar environmental perturbations.

Our objectives were to explore alternative ways of assessing river quality to the tools based on classical species pollution-sensitivity. Two main objectives were addressed:

1- We wanted to assess the influence of reducing taxonomic resolution on bioassessment in terms of three goals :
   - First, the reduction of taxonomic resolution should alter the description of the composition of the assemblage.
   - Second, since some metrics that drastically reduce taxonomic information exhibit some relationship with nutrient enrichment (Passy, 2007) we hypothesized that coarser taxonomic resolution may assess environmental parameters, such as nutrient, organic matter and mineral content, just as well as finer resolutions.
   - Third, river size and ecoregion are important factors to be considered in diatom bioassessment (e.g. Pan et al., 2000; Potapova & Charles, 2002; Tison et al., 2005). When taxonomic composition is discussed in research papers, the differences are usually described at species level. We hypothesize that ecoregional classifications can be expected to correlate more closely with a precise taxonomic resolution, and river size with diatom assemblages at a coarse taxonomic level.
2- We wanted to explore the assessment capacities of life-forms, cell sizes and ecological guilds in a large *in situ* area. Two objectives were addressed:
   - First, relationships between diatom metrics and nutrient and organic matter concentrations were explored and compared to data in the literature;
   - Second, the assessment capacities of these metrics were compared to diatom indices for nutrient and organic matter concentrations.

- **Methodology**

The same methodology was used to address the two main objectives. Diatom counts, chemical and physical analyses, and typological information carried out in the framework of routine river quality assessment were entered into a database. Diatom samplings and counts were carried out in accordance with the French standard of the Biological Diatom Index (Afnor, 2000; 2007). The data were then subjected to statistical analyses.

To test the influence of taxonomic resolution, samplings carried out between 2000 and 2008 in the Rhine-Meuse and Rhône-Mediterranean catchments were pooled. 1998 diatom listings with chemical, typological data were entered into a database.

To test the assessment capacities of the other metrics (life-forms, cell-sizes, ecological guilds), samplings carried out in the Rhône-Mediterranean catchment area were pooled. 328 diatom listings with chemical, typological data were compiled.

- **Results and discussion**

Taxonomic resolution had relatively little influence on assemblage structure at the level of species resolution. Similar findings had been obtained for macroinvertebrates (Bowman & Bailey, 1997). We then tested the influence of taxonomic resolution on the assessment capacities of various different parameters (nutrient, organic matter and mineral contents) by comparing the prediction performances of weighted averaging models. We observed an increase in the performance of the model as the taxonomic resolution became finer. Nevertheless, despite the exponential increase in the number of taxa with increasing taxonomic resolution precision, the performance of the model did not followed the same trend. The performance of nutrient models were equivalent at order and species levels, and the models for organic matter and mineral contents displayed similar performances at genus and species resolution levels. Studies based on macroinvertebrates have shown that broader taxonomic resolutions were at least as closely correlated (Warwick, 1988; Doledec et al., 2000) and sometimes more closely correlated (Reynoldson et al., 2001; Feio et al., 2006) to water pollution as finer taxonomic resolutions. Several reasons could explain this finding. First, many diatom taxa are too rare to provide enough information to construct a robust ecological profile and carry out formal analyses (Downes et al., 2000). To avoid this problem, rare species are sometimes eliminated from the models (e.g. Ponader et al., 2008) or from the indices when developed (e.g. Lavoie et al., 2009). Second, the presence of many species and genera does not depend primarily on the nutrient, organic matter or mineral content. For instance, some species and/or genera are aerophilic or have particular requirements for light or turbulence. More environmental descriptors would be necessary to explain the variability of communities at finer taxonomic resolutions. On the other hand, mineral content and class-level resolution gave the best correlation. This can be explained by the Mediophyceae class which includes many halophilous taxa, whereas such taxa are much rarer (Bacillariophyceae) or even absent in the other classes (Coscinodiscophyceae). Third, diatom identification requires lengthy training because of their extreme diversity, and identification errors are frequent at species level. This is even the case for common taxa such as *Gomphonema parvulum* or *Achnanthidium minutissimum,* which include many varieties with intermediate forms. These taxa are regularly the subject of round tables to define common identification criteria (e.g. Ector et al., 2009; Morales, 2002; Kahlert et al., 2009). Identification errors cannot be linked to any environmental parameters. This also calls into question the accuracy of such “shoe-horned” species, especially in the light of molecular studies showing that many “species” in fact consist of cryptic species with different ecologies (Vanelslander et al., 2009; Trobajo et al., 2009), and many species were probably not correctly described in the original papers and taxonomic reappraisals are called for.

Ecoregions are constructed on the basis of abiotic factors (e.g. climate, altitude, geology), but are intended to reflect the biogeography of aquatic assemblages. It has been reported in several regions (Finland in Mykra et al., 2009, France in Tison et al., 2005, Western Europe in Rimet et al., 2007 and USA in Potapova & Charles, 2002) that they do indeed often explain an important part of diatom communities variability. However, in most cases these correspondences have been investigated at species level. We observed that the finer the taxonomic resolution, the more closely the diatom assemblages matched the ecoregion classification. We found that finely resolved taxa (to species or genus level) had more restricted geographical ranges than higher taxonomic groups (e.g. order or family level). Bowman & Bailey (1997) summarized similar observations for benthic macro-invertebrates: "Variation seen in the abundance of individual species resulting from adaptations to a narrow range of natural environmental conditions will not be reflected at higher taxonomic levels […], data noise at the species level due to biogeographic variability may be reduced at higher taxonomic levels."

The correspondence between diatom assemblages and river size (Strahler ranking) showed a different trend. Correspondence increases from subdivision to order resolution, but then from order to species the correspondence remains the same. The Strahler rank is correlated with current velocity and turbulence, and these factors also strongly influence the dominance of diatom life-forms (Lamb & Lome, 1987) and ecological guilds (Passy, 2007). Such metrics are characteristic of high taxonomic levels. For instance, most of the planktic diatom life-forms that are present mainly in large rivers (high Strahler rank) are members of the Mediophyceae class. Similarly, adnate life-forms are mainly found in small rivers, and represented mainly by the Achnanthales order.

Some life-forms and ecological guilds showed clear correlations with nutrient and organic matter concentrations. For instance, the abundance of stalked diatoms increased as organic matter and nutrient concentrations decreased. This is in accordance with the hypothesis of Pringle (1990) that stalked diatoms are less well adapted to incorporating nutrients adsorbed on the substratum, but are better at exploiting nutrients dissolved in the water. Tube-forming diatoms displayed a similar trend to adnate diatoms, and this is consistent with the pollution sensitivities given in the Generic Diatom Index (Rumeau & Coste, 1988). The ecological guilds (Passy, 2007) also displayed close correspondence with nutrient and organic matter concentrations. The motile guild showed an increase with nutrient and organic matter concentrations; a possible reason to explain this is their ability to excrete extracellular enzymes which enable them to use macromolecules adsorbed on substrates or sediments (Pringle, 1990). Conversely, the low-profile guild displayed the opposite trend and is known to colonize bare substrates (Hoagland et al., 1982). However, the abundance of such life-forms and ecological guilds do not depend solely on abiotic factors; grazing pressure and interspecies competition also play major roles.

When comparing the ability to assess the organic matter concentration, the abundance of stalked and tube-forming diatoms performed in a comparable manner to the Biological Diatom Index (Coste et al., 2009). Stalked diatom abundances gave even better results than the Biological Diatom Index for nutrient concentrations assessment. One advantage of using biological traits is that they also provide information about the structure and architecture of biofilms. Another advantage is that this kind of metric could be used to study geographical areas where the diatom taxonomy is unknown, or

where we do not have sufficient data to establish a robust ecological profile using species-level determination, as in the case of the French overseas territories.

- **Conclusions**

We observed that fine taxonomic determination is required for a precise ecoregional bioassessment. This is especially true in the case of the Water Framework Directive which requires the assessment of ecological quality to be organized on the basis of ecoregions.

On the other hand, broad taxonomic resolution appears to be more suitable for the robust bioassessment of nutrient and organic matter concentrations. These conclusions parallel those of several studies carried out of macroinvertebrates (e.g. Hewlett, 2000; Reynoldson et al., 2001; Metzeling et al., 2006) and diatoms (e.g. Growns, 1999; Hill et al., 2001; Wunsam et al., 2002; Raunio & Soininen, 2007).

Moreover, using diatom life-forms and ecological guilds drastically reduces the taxonomic resolution, because these metrics are often characteristic of broad taxonomic resolutions. Such metrics make it possible to obtain valuable information about the architecture and structure of the biofilms, and we observed that the abundance metrics can be used to provide an accurate prediction of nutrient and organic matter concentrations.

These studies provide some indication of how to achieve the best compromise between the taxonomic resolution used, the information the operator wants to obtain, and the time/money available.

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b. Paper 3: Biomonitoring river diatoms: implications of taxonomic resolution.
Rimet F. & Bouchez A.

- **Abstract**

Benthic diatoms are routinely used to assess river pollution. Most of the tools based on these organisms exploit the differences of pollution sensitivity between species; as such, species level identification is required. Accurate determination of diatom species requires rigorous training due to the extreme diversity of the group. Level of taxonomic resolution for biomonitoring is still debated. The objective was to test the influence of taxonomic resolution on diatom bioassessment in an ecoregional framework. We used a database of 1967 diatom samples from biomonitoring programs in two French river basins, that reported three kinds of data for each site: (a) taxa abundance, expressed with 6 separate level of taxonomic resolution: species, genus, family, order, class or subdivision level; (b) physical and chemical characterization; (c) ecoregion and river-size class memberships. Mantel tests showed that the influence of taxonomic resolution on assemblage composition description was weak from species to order level. Mantel tests between chemical parameters and diatom assemblages showed that there was an increase in correlation from subdivision to genera resolution. But species and genus resolutions showed equivalent correlations with chemical parameters. Predictive models using diatom data to reconstruct nutrients, organic matter and mineral content showed an increasing performance from sub-division to species resolution. Nevertheless their performances did not follow the exponential increase of taxa number from sub-division to species: their performances stabilize from order to species resolution when predicting nutrients and are equivalent for genus and species when predicting mineral content and organic matter. Finally, we observed that the more precise the taxonomic resolution, the better the correspondence with ecoregion classification. This can be partly explained by diatom endemism and cosmopolitanism which is mostly observed to species level, rarely to genus level and never above.
For a quick and robust assessment of river pollution coarse identification is sufficient. Hypotheses to explain such results are that: (1) many species are too rare to describe with certainty their ecological requirements; (2) more environmental descriptors are necessary to explain the presence of some species; (3) the dataset is compromised by identification errors, particularly at the species level. On the other hand, a precise ecoregional bioassessment requires a fine taxonomic resolution; this must be stressed for the European Water Framework Directive which requires an assessment in an ecoregion classification.

Keywords: microalgae, taxonomic sufficiency, aquatic pollution, ecoregion, river size.

- **Introduction**

The assemblage composition of benthic diatoms in rivers is influenced by their biogeochemical environment which reflects the catchment geology (Tison *et al.* 2004), altitude (Ndiritu *et al.* 2006; Rimet 2009), stream order (Potapova and Charles 2002) and factors that are subject to human influence, such as organic matter and nutrient concentration (e.g. Van Dam *et al.* 1994; Kovacs *et al.* 2006). Numerous diatom indices (e.g. Coste *et al.* 2009; Dell'Uomo 2004; Lavoie *et al.* 2006; Whitton

and Kelly 1995) and calibration models (e.g. Winter and Duthie 2000, Munn *et al.* 2002, Ponader *et al.* 2008) have been developed, considering species' sensitivities to assess the ecological conditions of rivers.

These models and indices are generally based on species and sub-species levels identifications, with the exception of a few based on genus-level identifications (e.g. Rumeau and Coste 1988; Wu 1999; Chessman *et al.* 1999). Diatom identification requires advanced training and nomenclatural changes challenge consistent identification of taxa (Kociolek 2005). For instance, 356 new taxa and taxonomic combinations were recorded in 2009 according to the Catalogue of Diatom Names of the California Academy of Sciences (http://research.calacademy.org).

Cranston (1990) stated that taxonomic resolution for macroinvertebrate bioassessment in rivers, is often set without explicit justification and selected on subjective criteria, such as sample-processing cost and time. Carter and Resh (2001) criticized that taxonomic resolution is often chosen because of tradition, with species determination being considered the gold standard even if there are some benefits and disadvantages of using precise taxonomic resolution (Jones 2008, Bowman and Bailey 1997). Large number of diatom species and identifications' difficulties can be felt as limits to use them as routine indicators (Zampella *et al.* 2007). DeNicola (2000) observed that the number of species can be lowered; some species could be regrouped because they share the same ecologies and morphologies and this was done for some diatom indices (Lenoir and Coste 1996). Comparisons of bioassessment performances when using a species or a genus resolution were compared (Growns 1999, Hill et al. 2001, Raunio & Soininen 2007, Wunsam et al. 2002) and concluded on robustness and efficacy of genus. Some diatom metrics which drastically reduce taxonomic resolution, such as abundances of life-forms (pedunculate, colonial), *Nitzschia, Achnanthidium minutissimum* or ecological guilds (Passy 2007), show good pollution assessment performances compared to species resolutions methods (Stevenson and Bahls 2002, Berthon et al. 2011). On the other hand, some authors (Kociolek 2005, Patrick & Palavage 1994, Ponader & Potapova 2007) underlined the importance of an accurate species level identification. And most of the diatom bioassessment tools are based on species resolution. This paper concerns the influence of coarser (subdivision) to finer (species) taxonomic resolution on bioassessment. Therefore, we tested three hypotheses.

1- Reducing taxonomic resolution should modify assemblage composition description. The influence of taxonomic resolution on assemblage description was assessed with pairwise Mantel tests ran between different taxonomic resolution datasets.

2- Biotic diatom indices based on species and diatom metrics (ecological guilds of Passy 2007 or stalked diatom abundances) showed similar assessment power for nutrient and organic matter (e.g. Berthon et al. 2011). Since such metrics are based on coarse taxonomic resolution, we hypothesized that coarser taxonomic resolution could assess environmental parameters as nutrient, organic matter and mineral content as well as finer resolutions.

3- Ecoregional classification is important to consider to appreciate diatom assemblage diversity (e.g. Pan *et al.* 2000; Rimet et al. 2007, Tison et al. 2005). When taxonomic composition is discussed in these papers, differences are mostly described at species level. River size is also a major element in determining diatom assemblages (Potapova and Charles 2002). We hypothesized that ecoregional classification should correlate better with a precise taxonomic resolution and that river size should determine diatom assemblage at coarse taxonomic level.

To test these hypotheses, we chose to work on two eastern hydrographic basins in France. This area is large enough to represent a considerable diversity of rivers: from mountainous to lowland rivers;

those subject to a variety of climates (Mediterranean, Alpine and continental); and rivers of a wide range of size (from small streams to rivers with riverbeds wider than 450 m). This should yield results which can be generalized to other rivers in temperate environments.

- **Methods**

Study area

Sampling was carried-out in two hydrographical basins in France (Figure 21). The Rhône-Mediterranean catchment in the south, encompass two major rivers, the Rhône and Saône. The Rhine-Meuse basin in the north, encompass three major rivers, the Moselle, the Meuse and the Rhine. 1002 samples were taken from the Rhône-Mediterranean catchment between 2005 and 2008 and 996 samples from the Rhine-Meuse catchment between 2000 and 2005.

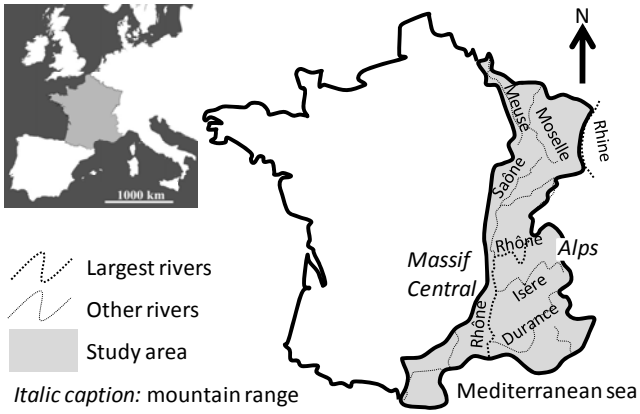

Figure 21: Study area and its major rivers

Diatom sampling and analyses

Diatoms were sampled as part of the biomonitoring program for national river networks. The sampling procedure followed the French standard (Afnor 2007). Diatoms were collected once per year, during the low-flow period. Benthic diatoms were collected from at least five stones from the lotic parts of the sampling sites. The upper surfaces of the stones were scrubbed with a toothbrush to collect the biofilms in which diatoms live. Then the sample was fixed in 4% formaldehyde. In laboratory, the diatom valves were cleaned using 40% H_2O_2 to eliminate organic matter, and HCl to dissolve calcium carbonate. Clean diatom valves were mounted in a resin (Naphrax©). At least 400 valves from each sample were counted and identified using a light microscope (1000× magnification) according to European (European Committee for Standardization 2004) and French (Afnor 2007) standards. The abundances of all observed taxa were expressed as relative counts. Identifications were carried out using Krammer and Lange-Bertalot (1986-1991) to species and sub-species level.

Physical and chemical sample analyses

Physical and chemical analyses were carried-out at the same sampling sites every month. Dissolved oxygen and conductivity were measured in the field. For NO_3^-, NH_4^+, Kjeldahl nitrogen (TKN), PO_4^{3-}, Na^+, Ca^{2+}, Cl^-, K^+, Mg^{2+}, SO_4^{2-}, Biological-Oxygen-Demand (BOD), Chemical-Oxygen-Demand (COD),

water samples were collected and analyzed in the laboratory according to standard procedures (APHA 1995).

Database construction

We combined three kinds of data for each site: (a) taxa abundance, resolved to six different levels of taxonomic resolution: species (including sub-species level), genus, family, order, class and subdivision; (b) physical and chemical characterization; (c) river typology.

(a) For each site, species data were lumped by genus using the updated taxonomy of Omnidia software 5.3 (Lecointe *et al.* 1993). Coarser aggregations (family, order, class and subdivision) were made according to taxonomy in Algaebase (www.algaebase.org update 06/10).

(b) For chemical data, we used the chemical analyses carried out before (maximum interval is one month), and closest in time to each diatom sampling date.

(c) Each sampling site was classified according to the French Typological System used in the Water Framework Directive (European commission 2000): ecoregions (Wasson et al. 2002, Chandesris et al. 2006) have been defined on the basis of geology, climate and relief. Our study area was made up of 17 ecoregions. The Strahler rank (Strahler 1963) calculated by Chandesris et al. (2006) was used to classify sampling sites into homogenous river sizes. Rivers within the area studied held ranks ranging from 1 (very small) to 8 (very large rivers).

Statistical analyses

1. Influence of taxonomic resolution on assemblage composition description

Similarity of assemblages description expressed at the different taxonomic resolutions was assessed with Mantel tests using PcOrd software (McCune and Mefford 2006). Mantel r-value is a measure of similarity which was tested subsequently with Monte-Carlo tests. A high r-value implies similar assemblages. For each taxonomic resolution dataset, Bray-Curtis distances were calculated between the sampling sites. 6 distance matrices were obtained, one for each taxonomic resolution. Then pairwise Mantel tests were ran between these distance matrices.

2. Influence of taxonomic resolution on correlation and prediction of environmental parameters

Mantel tests were carried out to assess the correlations between diatom assemblages described at different taxonomic resolutions and three major environmental gradients, (1) organic matter concentration, (2) nutrient concentration and (3) mineral content. Euclidean distances between sites were calculated for the chemical matrices: organic matter concentration (NH_4^+, TKN, COD, DBO, O_2), nutrient concentration (NO_3^-, PO_4^{2-}) and mineral content (Ca^{2+}, Cl^-, Conductivity, K^+, Mg^{2+}, Na^+, SO_4^{2-}). Bray-Curtis distances between sites were calculated for the diatom matrices (subdivision to species resolution). The correlation coefficient (R) was used to assess the correlation between these matrices.

Weighted-Averaging (WA) models were constructed to predict environmental parameters with diatom assemblages. The influence of taxonomic resolution on performance prediction of the same three major environmental gradients was assessed. To produce a simple metric from the chemical parameters to determine organic matter concentrations of the samples, a PCA (Principal Component Analysis) was calculated with Ginkgo freeware (DeCaceres et al. 2007). The parameters included were NH_4^+, TKN, COD, DBO, O_2. The chemical parameters were standardized (value–mean/standard-deviation) before running the PCA. The first axis showed the highest explained variance and the

samples positions on this axis defined their organic matter concentration. The same methodology was used to define nutrient concentration (NO_3^-, PO_4^{2-}) and mineral content (Ca^{2+}, Cl^-, Conductivity, K^+, Mg^{2+}, Na^+, SO_4^{2-}).
Then, WA models with inverse deshrinking and 1000 bootstrap for validation method were performed with C2 software (Juggins 2010) for each taxonomic resolution (from sub-division to species) and each major environmental gradient (organic matter, nutrients, mineral content). 1498 randomly selected samples were used for calibration and the 500 others for validation. A total of 18 WA models were obtained. Their performances were compared with RMSEP (root mean square error of prediction) and with Pearson correlation coefficients calculated between the 500 predicted and observed values.

3. Influence of taxonomic resolution on relationship between river typology and diatom assemblages
The objective was to assess the correspondence between typological classifications (ecoregions and Strahler rank) and diatom assemblages described at different taxonomic resolutions. To this end, K-means clusters based on Bray-Curtis distances were calculated from the diatom data. For each taxonomic resolution (species, genus, family, order, class, subdivision) nine K-means analyses were computed, each with a different number of groups: 1st analysis with 2 groups, 2nd analysis with 3 groups, 3rd analysis with 4 groups … etc… until the 9th analysis with 10 groups. Then, to assess the influence of taxonomic resolution on typology-biology relationships, we calculated corrected Rand indices (Hubert and Arabie 1985). This index is 0 when there is no coincidence between the typology-biology classifications, and 1 when the correspondence between the classifications is perfect. The results are presented as box-plots; one box-plot for each combination of taxonomic resolution and typological classification is given. Ginkgo freeware was used for these analyses.

- **Results**

1. Influence of taxonomic resolution on assemblage composition description
We identified 931 species. Number of taxa from sub-division to species level resolution follows an exponential trend (Figure 22). Pairwise Mantel tests, calculated among the assemblages described at differing taxonomic resolutions show strong correlations between species, genus, family and order resolution (Figure 23). R-values are even greater between diatom assemblages described at species, genus and family levels. Class and subdivision resolutions are much more weakly correlated to the other taxonomic resolutions. In all cases the correlations are significant between diatom assemblages described at any taxonomic resolution ($p<0.05$), with the exception of those between subdivision/species, and between subdivision/genus.

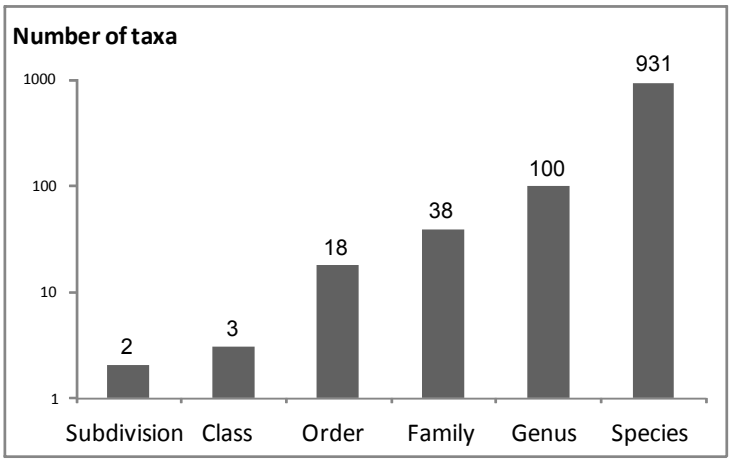

Figure 22: Number of taxa for each taxonomic resolution

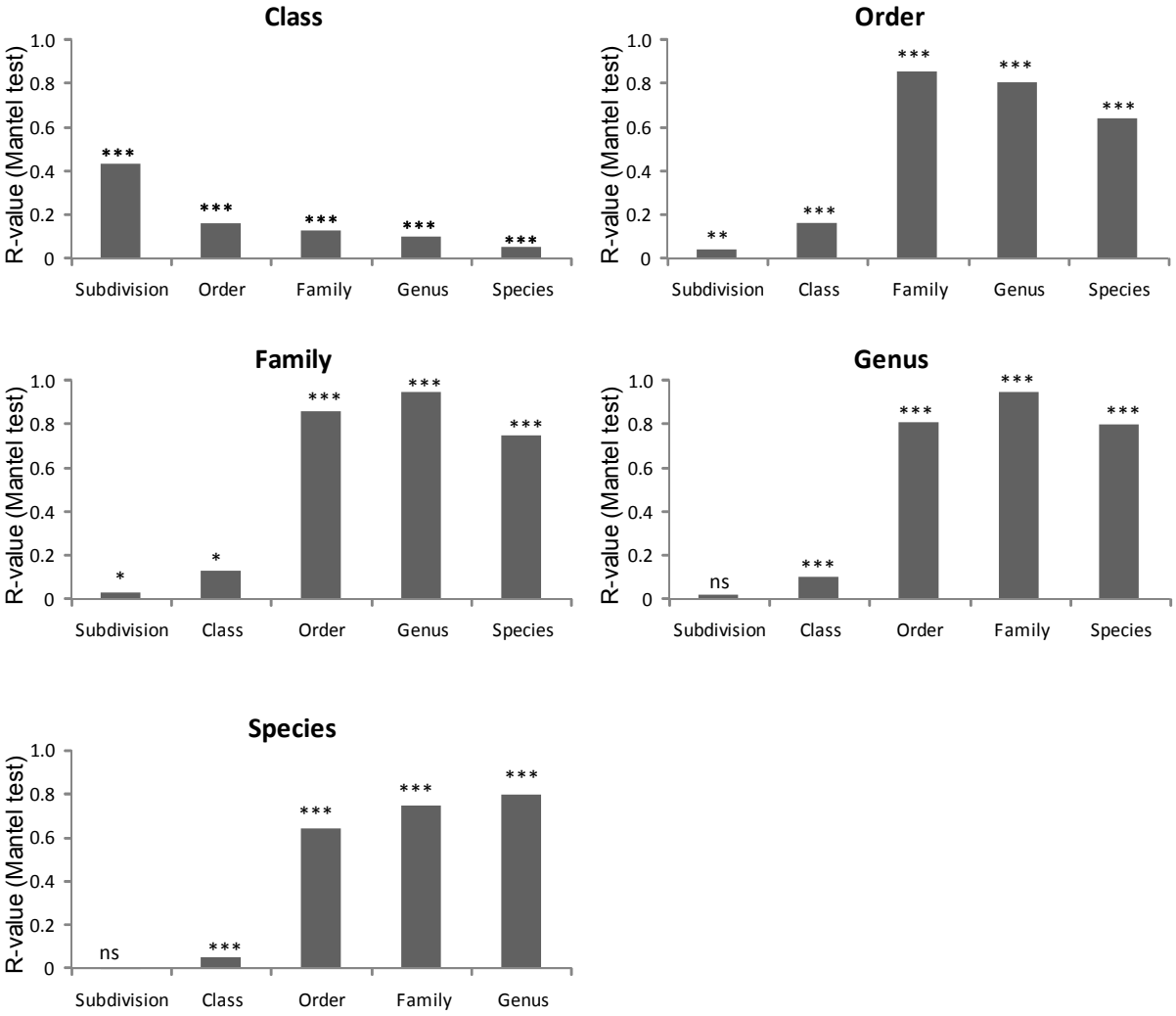

Figure 23: Mantel correlations between diatom assemblages described at 6 different taxonomic resolutions. (*: p<0.001, **: p<0.01, *: p<0.05, ns: p>0.05).**

2. Influence of taxonomic resolution on correlation and prediction of environmental parameters

Correlations between organic matter or nutrients and diatom assemblages increase from sub-division resolution to a maximum value at the genus resolution (Figure 24). Correlations are lower at species resolution. Correlations between diatoms and mineral content show a different trend: the highest correlations are met for class and species resolutions and the lowest for subdivision resolution. Correlations are non-significant from sub-division to order resolution for nutrients, from sub-division to class resolution for organic matter and only for sub-division resolution for mineral content. When summing the correlations of these three parameters, it appears that correlation increases from subdivision to species level, but correlations of genus and species resolutions are comparable.

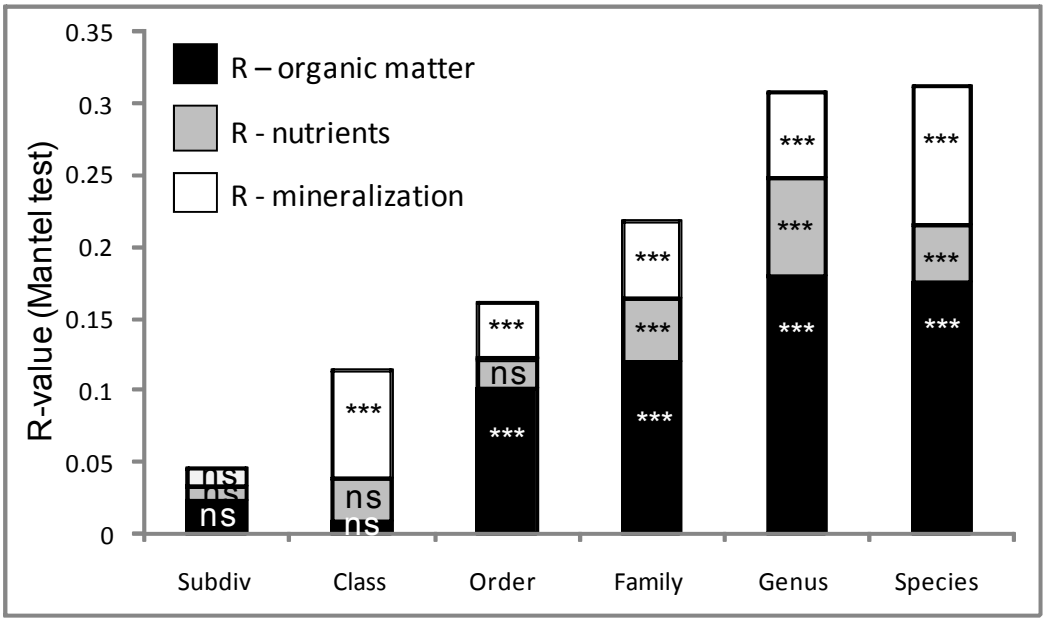

Figure 24: Mantel correlations between diatom assemblages (species to subdivision resolution) and environmental parameters (organic matter, nutrients, mineral content). (*: p<0.001, **: p<0.01, *: p<0.05, ns: p>0.05).**

Predictive models performances increased when taxonomic resolution became more precise (Figure 25). Correlations between observed and predicted values of organic matter and nutrients concentrations were much lower for subdivision and class resolutions than for order to species resolutions. For nutrients, correlations are equivalent from order to species resolution. For organic matter, correlations gradually increase from family to species resolution. Correlations for mineral contents were null for subdivision, and much higher from class to species resolutions. Correlations with mineral content gradually increase from class to species resolution.

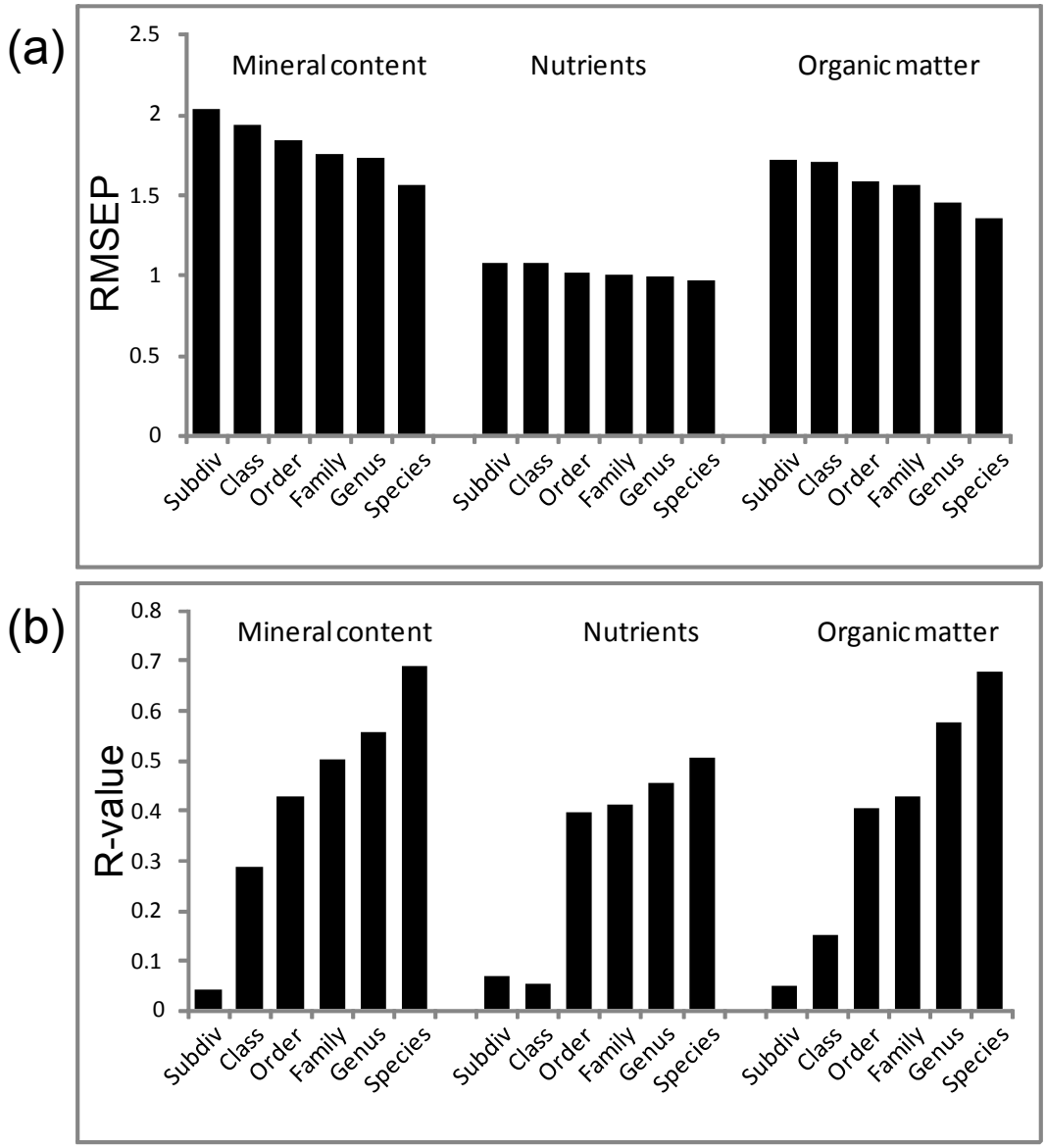

Figure 25: Comparison of WA models performances with (a) RMSEP (Root Mean Square Error of Prediction) and (b) R-value (Pearson correlation coefficient) calculated between predicted and observed values. (*: p<0.001, **: p<0.01, *: p<0.05, ns: p>0.05).**

3. Influence of taxonomic resolution on relationship between river typology and diatom assemblages
There is no correspondence between Strahler rank and diatom described at subdivision resolution, as well as for ecoregion and diatoms at subdivision and class resolutions (Figure 26). For ecoregion, there is a gradual increase of correspondence with diatom assemblages as taxonomic resolution becomes finer. For Strahler rank, the correspondence increases from class to order resolution, then it reaches a plateau for finer resolutions.

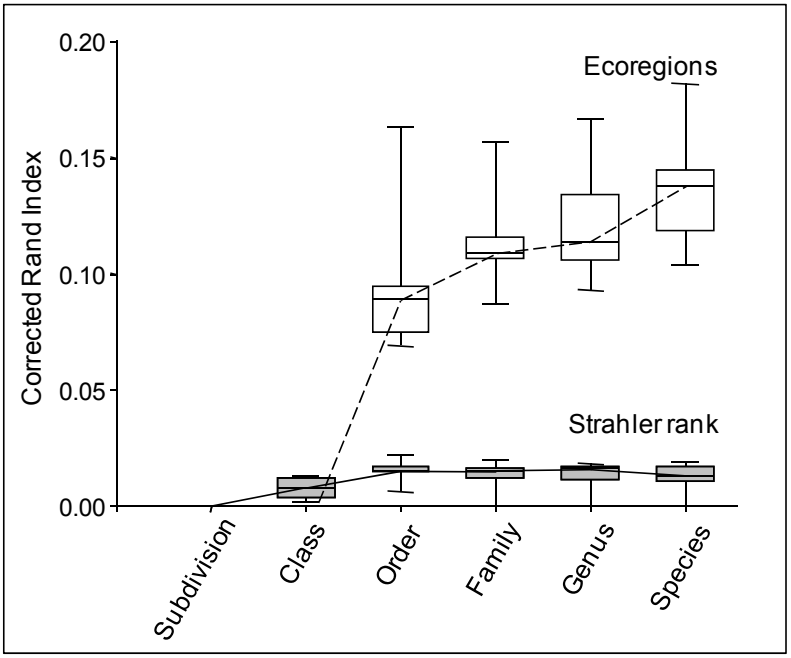

Figure 26: Corrected Rand indexes for each taxa-count at 6 different taxonomic resolutions and the typological classifications (ecoregions and Strahler rank). This index assesses the similarity between diatom assemblages and typological classifications (0: no coincidence, 1: perfect correspondence). Line inside the box is the median, box represent the 25th and 75th percentiles, whiskers the 10th and 90th percentiles.

- **Discussion**

1. Influence of taxonomic resolutions on assemblage composition description
The description of diatom assemblages' structure was very stable from order to species resolution, suggesting that taxonomic resolution had quite little influence on assemblage structure description. These strong correlations suggest that little ecological information is lost when taxonomic resolution decreased from species to order level. Similar findings were obtained for freshwater benthic macroinvertebrates (Bowman and Bailey 1997) when expressed with quantitative data. But this was not the case when the data were expressed qualitatively (Bowman and Bailey 1997; Crawford *et al.* 1992).

2. Influence of taxonomic resolution on correlation and prediction of environmental parameters.

As other predictive models (e.g. Wunsam et al. 2002) or diatom indices (e.g. Chessman et al. 2007, Smucker and Vis 2009) developed elsewhere the models we constructed showed better results with finer taxonomic resolutions than coarser ones. Similarly, correlations between diatom and environmental parameters also increased globally from subdivision to species resolution. Nevertheless, despite the exponential increase of taxa number with taxonomic resolution precision, model performances and correlations did not followed the same trends. Nutrients models performances were equivalent from order to species and both organic matter and mineral content models had similar performances for genus and species resolutions. Studies based on macroinvertebrates, showed that broader taxonomic resolutions were at least as correlated to water pollution as finer taxonomic resolutions in marine (Warwick 1988) and freshwater (Doledec *et al.* 2000) benthic habitats. We even observed stronger correlations with nutrients and organic matter for genus than species resolutions as this was also the case for benthic-invertebrates studies (Reynoldson *et al.* 2001; Feio *et al.* 2006). Several factors could potentially explain this phenomenon; these could be mathematical, ecological, or methodological.

Mathematical considerations

In our study area 11 taxa out of 948 constituted more than 50% of the counted diatoms and 26% were observed in only one sample out of 1967. Similar observations led Downes *et al.* (2000) to state that "the great majority of taxa are usually too rare to provide enough information about their individual abundances for formal analyses". It is indeed difficult to build robust ecological profiles for taxa that present low abundance and low frequency of occurrence. To avoid this problem for the development of assessment methods rare taxa are eliminated, as tested for the Canadian Diatom Index (Lavoie *et al.* 2009): 40% of the rarest taxa (lowest abundance) were removed, and the indication power was still very good. When developing WA models, rare taxa are also removed (e.g. Ponader et al. 2008). This is also done for macroinvertebrates (e.g. Norris and Georges 1993; Cao *et al.* 2001) despite the argument that rare species convey important ecological information both for macroinvertebrates (Jones 2008) and diatom bioassessment (Potapova and Charles 2004).

Ecological considerations

Mathematical considerations do not entirely explain the model performances and the correlations between chemical and diatom data. Nutrients, organic matter and mineral content are only three environmental descriptors among many others, and they are of course not sufficient to completely explain the diatom assemblage structure at fine taxonomic resolution (species and genera). Many others are generally not taken into account in database analyses and are known to be determinant for the presence of some species and/or some genera. For instance, particular species are aerophilic, such as *Nitzschia communis* (Van Dam *et al.* 1994) whose genus is generally considered as strictly aquatic. Other genera are known to be entirely aerophilic, for example *Adlafia, Diadesmis, Frustulia* or *Hantzschia* (Van De Vijver *et al.* 2002). Other parameters such as light intensity (Kawecka 1985; Villeneuve *et al.* 2010), and current velocity (e.g. Song 2007; Cazaubon 1988; Villeneuve *et al.* 2010) are also known to play a crucial role in structuring the diatom assemblage. To explain more variability at fine taxonomic levels, many other parameters would need to be taken into account, which may

also explain why correlations between chemical parameters and genus or species resolution are similar.

Mineral content and class-resolution showed the best correlation and high model performances. An explanation is that Mediophyceae class includes several genera known to be halophilous (*Thalassiosira, Skeletonema, Pleurosira)* (Round *et al.* 1990) and represent an important part of this class (9% of the counted diatoms) compared to the other classes Coscinodiscophyceae which have no halophilous taxa (*Aulacoseira, Melosira, Ellerbeckia*) and Bacillariophyceae which have only a small number of halophilous taxa (less than 1% of the counted diatoms are halophilous species *sensu* Van Dam *et al.* 1994).

Subdivision resolution seems to provide too little data to be significantly correlated to any of the three environmental descriptors (organic matter, nutrients and mineral content). Subdivision level is only divided into two clades, the Bacillariophytina (99% of counted diatoms) and the Coscinodiscophytina. Taxa present in the Coscinodiscophytina are mostly centric diatoms living in filaments. They are typically planktic thus occur only in low flowing sections of rivers (e.g. *Melosira varians*). Habitat preferences may not have been represented by the physical and chemical parameters used in our statistical analyses.

Methodological considerations

Determination of diatom species requires a lot of training due to its extreme diversity. Determination is even difficult for abundant taxa such as *Gomphonema parvulum* (present in more than 51% of the samples of this study) which is regularly the subject of harmonization reports that propose taxonomic agreements to laboratories (Morales 2002, Kahlert *et al.* 2009) and of intercalibration exercises to test technicians skills (reports of Luc Ector in France from 2000 to 2010). This is also the case for the *Achnanthidium minutissimum* species complex (present in more than 85% of the samples of this study) which was the subject of round-tables (Ector *et al.* 2009); this species represents 18 varieties (see Omnidia software v. 5.3, Lecointe *et al.* 1993) which are morphologically difficult to identify and present different ecological profiles according to some authors (Coste et al. 2009). Because of the difficulty in distinguishing many diatom species and varieties, even among those most frequently present in river networks, many taxa can be determined differently from one diatomist to another. Morales *et al.* (2001) underlines the difficulty of correctly determining diatom species using light microscopy, the use of electron microscopy should be employed to avoid these problems. Genetic and inbreeding studies (e.g. Trobajo *et al.* 2009; Evans et al. 2008; Mann et al. 2004) also reveal cryptic diversity which is not taken into account in routine diatom analyses. However, financial limitations make these approaches impossible to apply to thousands of monitoring samples. In his review, Jones (2008) illustrates that as taxonomic resolution increases, information content increases, but taxonomic identifications become less certain. We cannot deny that diatom species identification is often marred by errors. These errors will, at least partially mask assemblage-environment relationships.

3. Influence of taxonomic resolution on relationship between river typology and diatom assemblages

Diatom assemblages and species richnesses show biogeographical structures at regional and global scales (Vyverman *et al.* 2007). Ecoregional classifications have been constructed to reflect the biogeography of assemblages. These classifications have been reported to match diatom assemblages in numerous regions, including Finland (Mykra *et al.* 2009), France (Tison *et al.* 2005),

Western Europe (Rimet *et al.* 2007) and USA (Potapova and Charles 2002). Bedrock and superficial geology strongly influence diatom communities' species compositions (Leland and Porter 2000). But in most cases, these correspondences were studied at the species level. One study (Verleyen *et al.* 2009) assessed the correlation between freshwater diatoms and their biogeographical patterns at the species and genus levels and showed that results were similar for both resolutions. We have shown that, the finer the taxonomic resolution, the better diatom assemblages agree with ecoregion classifications. Many species clearly have ecoregional distributions in European rivers, such as *Achnanthidium bisolettianum,* present in pristine rivers on limestone geology, whereas *Achnanthidium subatomus* is also present in pristine rivers but only those on crystalline geology (Rimet *et al.* 2004). Similarly, *Cocconeis euglypta* is present in rivers on limestone geology while *C. lineata* is found in those on crystalline geology.

Diatom cosmopolitanism, endemism and taxonomic precision may all interact to influence the relationships we observe between diatom assemblage structure and ecoregional variables. Vanormelingen *et al.* (2007) state that numerous species have restricted distribution patterns, whereas at a higher taxonomic level, little evidence is found for endemism as, apart from a few convincing exceptions, diatom genera are cosmopolitan. The review gave only two examples for freshwater genera showing a restricted distribution. The distributions of micro-organisms, like diatoms, are opposed to macro-organisms where entire genera are endemic. Even though our study does not assess diatom endemism, it shows that finely resolved taxa (species, genera) have more restricted geographical ranges than those occurring for higher taxonomic groups (e.g. orders, families). Bowman and Bailey (1997) summarized similar observations for benthic macroinvertebrates: “Variation seen in the abundance of individual species resulting from adaptations to a narrow range of natural environmental conditions will not be reflected at higher taxonomic levels […], data noise at the species level due to biogeographic variability may be reduced at higher taxonomic levels.”

Correspondence between Strahler rank and taxonomic resolution gave a different result. There is an increase correspondence from subdivision to order resolution and then from the order to species resolution the correspondence remains the same. Strahler rank is often correlated with current velocity and water turbulence because small streams are usually more steeper than larger rivers; and current speed strongly influences the dominance of diatom life-forms (e.g. Lamb and Lome 1987) and ecological-guilds (Passy 2007). These metrics cluster diatoms belonging to broader taxonomic resolutions; they often belong to the same classes. For instance, the motile ecological guild is mostly represented by the Naviculales and Bacillariales orders which belong to the Bacillariophyceae class. Another example concerns planktic diatom life-forms; which are mostly members of the Mediophyceae class. A third example concerns the adnate life-forms, particularly represented by the Achnanthales order.

- **Conclusions**

Before starting an ecological survey or developing new bioassessment tools the best compromise between taxonomic resolution, technical drawbacks of mathematical tools to use and time/money to spend, have to be defined. This study provides some answers. For precise ecoregional bioassessment, a fine taxonomic resolution is required. This must be stressed, particularly for the European Water Framework Directive (European commission 2000), which requires assessment of

ecological quality in an ecoregional organization and by means of ecological distances between reference and observed biological assemblages.

On the other hand, several studies require only a quick assessment of ecological quality. Broad taxonomic resolution appears to be well adapted for use in a robust pollution assessment. These findings meet the results of several macroinvertebrate (e.g. Hewlett 2000; Reynoldson *et al.* 2001; Metzeling *et al.* 2006) and diatom (e.g. Growns 1999, Hill et al. 2001, Wunsam et al. 2002, Raunio & Soininen 2007) studies.

Another situation could be in the case of regions with unclear or little-known diatom flora. This is, for instance, the case for tropical regions and French overseas territories, which are subject to the Water Framework requirements and, as such the ecological quality of their rivers must be assessed using diatoms. For such regions, species taxonomy is poorly understood. Coarse taxonomy will therefore, represent the only way forward, at least for the short term.

- **Acknowledgments**

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c. Paper 4: Using diatom life-forms and ecological guilds to assess organic pollution and trophic level in rivers: a case study of rivers in south-eastern France.

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- **Abstract**

The European Union's Water Framework Directive has set a target of achieving good ecological status for all aquatic environments in Europe by 2015. In order to determine the quality of aquatic environments biological indicators such as diatoms are often used. However, biotic diatom indices can be difficult and time consuming to use because of complexity of species determination. We investigated whether the biological traits of diatoms in rivers (life-forms, size classes and ecological guilds) could be used to assess organic pollution and trophic level. We worked on a data set comprising 315 diatom species, determined at 328 river stations of south-east France and a variety of parameters. The abundances of some biological traits differed significantly between the different organic pollution and trophic levels, particularly stalked diatoms, and the motile and low-profile guilds.

Keywords: pollution, freshwater environments, bio-monitoring, algae, biological traits, ecological guilds.

- **Introduction**

Diatoms are microalgae that display exceptional taxonomic diversity; according to Mann and Droop (1996) there are more than 100,000 taxa, and recent studies have highlighted their high degree of cryptic diversity (e.g., Mann et al., 2008, Trobajo et al., 2005, Sabbe et al., 2001, Poulikova et al., 2008). The specific composition of diatom assemblages is affected by, amongst other factors, chemical parameters such as acidification (Mulholland et al., 1986), nutrient concentrations (Kelly, 2003), and organic load (Van Dam et al., 1994). For these reasons, benthic diatoms are routinely used, alongside macrophytes, macroinvertebrates and fish, to assess river quality (Ector et al., 2004). Several biotic indices, such as the Biological Diatom Index (BDI) (Coste, 2009; Afnor, 2007) and the Pollution Sensitivity Index (IPS - Cemagref, 1982) have been developed to assess pollution in rivers. These indices are based on pollution-sensitivity of species, and on their abundance in biofilms. They are now mandatory under the European Water Framework Directive (WFD - European commission, 2000) for the assessment of aquatic environment quality. The WFD is intended to achieve good ecological status for aquatic environments by 2015.

Nevertheless, the use of these indices can reveal some problems. They include numerous species (the BDI uses about 1488 taxa including synonyms, and the IPS about 5300 taxa including synonyms). Some of these taxa are so rare that it is difficult to establish their ecological profile with certainty. Besse-Lotoskaya et al. (2011) have shown that the European indices use different ecological profiles for the same species, indicating that these ecological profiles are difficult to define especially for species presenting low relative abundance in sites and low frequency of occurrence, and therefore are not robust. Another difficulty in attempting to apply diatom indices to different geographical regions is the structuring impact of ecoregion on diatom assemblages. Moreover, from a more technical standpoint, using these indices can be a very lengthy process: identification to species level

is time-consuming and sometimes difficult; several years of training are required before one is able to identify them correctly. Since 2000 and the introduction of the requirements of the WFD, there has also been an increase in the number of samples to be analysed in all European ecoregions (including a number of overseas territories), and so a less time-consuming method could be an attractive alternative.

There are some metrics other than specific pollution-sensitivity that merit investigation in relation to organic pollution and trophic level in waters. The biological traits of diatoms, such as life-forms, size classes of cells, ecological guilds, would yield more robust ecological profiles because they often occur in biofilms and therefore their assignment to a particular pollution level would be statistically more certain. They can also provide useful information about the structure and architecture of biofilms. As most of the biological traits are characteristic of whole genera of diatoms, their identification could be simplified compared to biotic indices.

Three kinds of biological trait were taken into account in this study. The first biological trait is life-forms (e.g. colonial, tube-forming, pioneer, mobile, stalked). Their relationships with nutrient levels have been studied in experimental contexts (Pringle, 1990; Hoagland, 1982). The second trait is size classes. It has already been shown that the size of phytoplankton cells is related both to their abundance (Li, 2002; Irwin et al., 2006), and to the availability of resources (Cattaneo et al., 1997; Wunsam et al., 2002). The third trait is ecological guilds. These can be defined as a group of species, which live in the same environment, but may have adapted differently to abiotic factors. We studied three guilds characterized by Passy (2007). The "low-profile" guild encompasses of species of short stature including for instance prostrate, adnate, and erect diatoms. The "high-profile" guild comprises large species, or those which tend to form colonies (e.g. tube-forming, filamentous, branched diatoms). The "motile" guild consists of fast-moving species (e.g. Navicula, Nitzschia). Passy (2007) showed that "low-profile" species were resistant to physical disturbances, such as those caused by currents, but could not tolerate nutrient enrichment. The "high-profile" and "motile" species showed the opposite tendency (Table 6).

This study has several objectives: (1) to test the response of these biological traits to organic pollution and trophic level in a wide range of rivers, (2) to compare their response to a standardised diatom index (BDI, Afnor, 2007) used to monitor the river network in France. Our study was located in a large river basin, the Rhone-Mediterranean catchment in France, using data from the monitoring network.

Table 6: Ecological guilds' resistance to physical disturbance and nutrient enrichment according to Passy (2007)

	Physical disturbance	Nutrient enrichment
Low-profile	+	-
High-profile	-	+
Motile	-	+

+ resist the perturbation; - do not resist the perturbation

- **Material and Methods**

Study area

Samples were collected in the largest hydrographical basin in France, the Rhone-Mediterranean catchment, comprising 328 stations, located on 212 different rivers in three administrative regions: Rhône-Alpes, Provence-Alpes-Côte d'Azur and Languedoc-Roussillon. Figure 27 shows the localisation of the study area. The area includes two mountainous ranges, the Massif Central on the western side, and the Alps on the eastern side, separated by the Rhône Valley. The River Rhône rises in the Swiss Alps, and flows into the Mediterranean Sea.

The human population density varies considerably within this catchment area. There are high population densities in cities like Lyon (around 1000 inhabitants/km2), and along the Mediterranean coast (around 380 inhabitants.km-2). In contrast, the mountainous regions are less densely populated. Farming activities vary considerably in the different parts of the catchment. In the area between the mountainous regions, in the North and at the top of the Rhône Valley, this activity consists mainly of cereal growing, while further south growing of vines and other fruits dominates. In the mountainous regions, farming mainly involves livestock. Industrial activity is concentrated in the region around Lyon, and in the south of the catchment.

Figure 27: Map of the study area showing its localization and the main watercourses.

Field and laboratory procedures

Diatom samplings were collected in 2007 in accordance with the Biological Diatom Index standard (Afnor, 2007), which incorporates the European standard for diatom sampling (European Committee for Standardisation, 2002). Sampling was carried out during the low flow season, i.e. during the summer in most cases, except for the sampling sites dominated by snow (with low flows during the winter), which were sampled during the winter. Benthic diatoms were collected from at least five stones from the lotic parts of the sampling sites. The upper surfaces of the stones were scrubbed with a toothbrush. The samples were fixed in 4% formaldehyde. In the laboratory, the diatom valves were cleaned using 40% hydrogen peroxide, to eliminate organic matter, and with hydrochloric acid to dissolve calcium carbonate. Clean diatom frustules were mounted in a synthetic resin (Naphrax©). For each sample, at least 400 valves were counted and identified by light microscopy using phase contrast or differential interference contrast with 1000× magnification. The abundances of all the taxa observed were expressed as relative counts. The identifications and counts followed standard methods (Afnor 2007) using the Krammer and Lange-Bertalot floras (1986, 1988, 1991a, 1991b), and other specialised bibliographical data when needed.

Chemical analyses were also carried out at the same sampling sites. Dissolved Oxygen (DO), Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Dissolved Organic Carbon (DOC), NH4+ and NO2- were measured to assess the organic pollution. NO3-, PO43- and total phosphorus were measured to estimate the trophic level. These parameters were analyzed in laboratory according to standard procedures (APHA, 1995). Since the diatom and chemical samplings dates did not coincide, we decided to use the physical and chemical analyses carried out before and closest in date to each diatom sampling.

Calculation of the biological metrics

Biological Diatom Index - We used the method given in Coste (2009) and Afnor (2007) to calculate the Biological Diatom Index (BDI). This is a standard index, and is routinely used to assess the water quality of rivers and streams. It considers the abundance of 1488 species, and the level of pollution sensitivity determined for each of them. The calculated scores range from 1 to 20, and watercourses are assigned to one of five quality classes: very bad if $1 \leq BDI \leq 5$, bad if $5 < BDI \leq 9$, medium if $9 < BDI \leq 13$, good if $13 < BDI \leq 17$, and very good if $17 < BDI \leq 20$. To calculate the scores the Omnidia software v5.3 (Lecointe et al., 1993) was used.

Diatoms and biological traits, life-forms and size classes - Diatom taxa were assigned to various life-forms: mobile, colonial, tube-forming, stalked, and pioneer. The tube-forming life-form is a type of colonial life-form: diatoms live in a protective mucous substance within which they can move freely. The pioneer diatoms are able to colonise bare substrates faster than other species, probably because they are generally small in size and so their exposure to toxic substances is minimal, as is their assimilation (Koshmanesh et al., 1997). Table 7 shows the taxa composition of the different life-forms, one taxon may appear in more than one life-form group. This follows existing published classifications, for instance: Round et al. (1990), Krammer and Lange-Bertalot (1986, 1988, 1991a, 1991b), Germain (1981), and some more specific studies: Hoagland et al. (1982), Katoh (1992), Pringle (1990), Robinson and Rushforth (1987), Allanson (1973).

The biovolumes of the diatom taxa recorded in our study were determined on the basis of the average sizes given in the diatom floras of Krammer and Lange Bertalot (1986, 1988, 1991a, 1991b) and others (Krammer, 2000, 2001, 2002, 2003). We chose basic geometrical forms using their volume formula to approximate diatom species' biovolumes (Hillebrand et al., 1999). They were then assigned to five arbitrarily-defined size classes. The c1 size class includes taxa with biovolumes from 5 to 99 µm3, c2 those from 100 to 299 µm3, c3 those from 300 to 599 µm3, c4 those from 600 to 1499 µm3, and c5 includes all those over 1500 µm3.

Table 7: Taxa assignment to the 6 life-forms

Life forms	Taxa composition
Mobile	*Achnanthes, Achnanthidium, Adlafia, Amphipleura, Amphora, Bacillaria, Brachysira, Caloneis, Cocconeis, Craticula, Cymbella, Cymbopleura, Delicata, Denticula, Diadesmis, Diploneis, Encyonema, Encyonopsis, Eolimna, Epithemia, Eucocconeis, Eunotia, Fallacia, Fistulifera, Frustulia, Geissleria, Gomphoneis, Gomphonema, Gomphosphenia, Gyrosigma, Hippodonta, Karayevia, Kolbesia, Luticola, Mayamaea, Navicula, Nitzschia, Nupela, Planothidium, Platessa, Reimeria, Sellaphora, Simonsenia, Stauroneis, Surirella, Tryblionella.*
Colonial	*Achnanthidium catenatum, Amphipleura, Aulacoseira, Cymbopleura, Delicata, Diadesmis, Diatoma, Encyonema, Eunotia, Fragilaria, Frustulia, Gomphoneis, Melosira, Meridion, Pleurosira, Pseudostaurosira, Staurosira, Staurosirella.*
Tube-forming	*Amphipleura, Cymbopleura, Delicata, Encyonema, Frustulia.*
Stalked	*Achnanthes, Achnanthidium, Cymbella, Diatoma, Encyonopsis, Eucocconeis, Fragilaria, Gomphoneis, Gomphonema, Gomphosphenia, Kolbesia, Planothidium, Platessa, Pseudostaurosira, Reimeria, Rhoicosphenia, Pseudostaurosira, Staurosira, Staurosirella*
Pioneer	*A. minutissimum* and varieties*, A. saprophilum, A. straubianum, Amphora inariensis, A. pediculus*

Ecological guilds - An ecological guild consists of taxa that live in the same kind of environment, but which may have adapted in different ways to survive there (Devito et al., 2004). Passy (2007) defined three ecological guilds. The first, the low-profile guild, consists of species of short stature, including prostrate, adnate, small erect, solitary centrics, and slow-moving species (sensu Passy, 2007). The second, the high-profile guild, consists of species of tall stature, including large erect, filamentous, branched, chain-forming, tube-forming, stalked, and colonial centrics. Finally, the third, the motile guild, consists of fast-moving species (Hudon and Legendre, 1987). Since a large number of the taxa recorded in our study were not referenced in the work of Passy (2007), we extended these guilds by adding all the species we found in our samples. The compositions of these adapted guilds are shown in Table 8. See Appendix 3 for the corresponding references that enable classifying the taxon.

Table 8: Taxa assignment to the three guilds adapted from Passy (2007)

Ecological guilds adapted	Taxa composition
Low-profile	*Achnanthidium, Achnanthes, Amphora, Brachysira, Cymbella, Cyclotella, Cymbopleura, Cocconeis, Cyclostephanos, Delicata, Diploneis, Discostella, Encyonema, Encyonopsis, Eucocconeis, Fragilaria, Karayevia, Kolbesia, Meridion, Nupela, Planothidium, Platessa, Rhoicosphenia, Reimeria, Stephanodiscus*
High-profile	*Aulacoseira, Achnanthidium catenatum, Diadesmis, Diatoma, Eunotia, Fragilaria, Gomphonema, Gomphoneis, Gomphosphenia, Melosira, Pleurosira, Pseudostaurosira, Staurosira, Staurosirella, Tabularia, Ulnaria*
Motile	*Adlafia, Bacillaria, Caloneis, Craticula, Delicata, Denticula, Eolimna, Epithemia, Fallacia, Fistulifera, Geissleria, Gyrosigma, Hippodonta, Luticola, Mayamaea, Navicula, Naviculadicta, Nitzschia, Nupela, Sellaphora, Simonsenia, Stauroneis, Surirella, Tryblionella*

Definition of organic pollution and trophic level classes

To produce a simple metric from the chemical parameters which determine trophic level, a PCA (Principal Component Analysis) was used to classify the samples along the first axis of this analysis (Coste et al. 2009) using PC-ORD 5© software (McCune and Mefford, 2006). The parameters selected for inclusion were those used in France in the Nutrient index of the SEQeau system (Agence de l'Eau 2000): NO3-, PO43- and total phosphorus. These parameters were standardised (value – mean / standard deviation). The first axis of the PCA was strongly correlated with these three chemical parameters. We used the position of the samples on the first axis of the PCA, and clustered them into five classes containing equal numbers of sites to define five classes of increasing trophic level: t1 (66 stations), t2 (66), t3 (66), t4 (66), t5 (64).

The same methodology was used to produce a simple metric for organic pollution. The chemical parameters selected for inclusion in the organic pollution were those used in France in the Organic Matter index of the SEQeau system (Agence de l'Eau 2000): DO, BOD, COD, DOC, NH4+ and NO2-. The first axis of the PCA was strongly correlated with these six chemical parameters. Again we used the position of the samples on the first axis of the PCA, and clustered them into five classes containing equal numbers of sites to define five classes of increasing organic pollution: s1 (66 stations), s2 (66), s3 (66), s4 (66) and s5 (64).

Relationships between organic pollution and trophic level classes, and biological metrics

The range of biological metrics abundances in the five organic pollution and trophic level classes were represented using box-plots graphs.

One-way ANOVA were used to find out whether there were any significant differences between the various biological traits in the organic pollution and trophic level classes (SigmaStat 3.10© software). Since normality tests and equal variance tests failed, Kruskal-Wallis one-way ANOVA tests (i.e. ANOVA on ranks) were used to test whether there were significant differences between the biological traits. The H-value of the Kruskal-Wallis test gives an assessment of the discrimination of the metrics between the trophic classes and between the organic pollution classes (the H-value was comparable for each of the metrics, since the degrees of freedom were identical). The H-value was therefore used to compare the discrimination power of the metrics for the trophic and organic pollution classes. If the H-value was high, the discriminating power was high.

- **Results**

Five trophic level classes were defined on the basis of the first PCA axis (Figure 28-a). Their mean values and standard deviations are shown in Table 9-a. Five organic pollution classes were defined on the basis of the second PCA axis (Figure 28-b). Their mean values and standard deviations are shown in Table 9-b.

Table 9: Mean values and standard deviation of the chemical parameters for each of the trophic level (a) and organic pollution (b) classes (DO: Dissolved Oxygen, BOD: Biological Oxygen Demand, DOC: Dissolved Organic Carbon).

	a: Trophic level classes				
	t1	**t2**	**t3**	**t4**	**t5**
Nitrate*	13.02 ± 9.87	4.74 ± 2.14	2.70 ± 0.79	1.58 ± 0.40	0.81 ± 0.37
Phosphate*	0.76 ± 1.38	0.08 ± 0.06	0.03 ± 0.02	0.01 ± 0.01	0.01 ± 0.00
Total Phosphorus*	0.31 ± 0.47	0.06 ± 0.04	0.03 ± 0.01	0.02 ± 0.00	0.02 ± 0.01

	b: Organic pollution classes				
	s1	**s2**	**s3**	**s4**	**s5**
DO*	7.28 ± 2.00	8.56 ± 0.91	9.20 ± 0.72	10.14 ± 0.77	11.97 ± 1.55
DO (%)	79.32 ± 20.41	91.14 ± 8.05	97.09 ± 6.20	103.07 ± 6.30	108.20 ± 9.61
BOD*	2.05 ± 1.26	1.43 ± 0.69	1.36 ± 0.74	1.16 ± 0.62	1.12 ± 0.55
DOC*	3.33 ± 1.54	2.08 ± 1.16	1.68 ± 0.59	1.47 ± 0.57	1.27 ± 0.65
NH_4^+*	0.81 ± 2.73	0.10 ± 0.12	0.09 ± 0.13	0.08 ± 0.08	0.08 ± 0.06
NO_2^-*	0.35 ± 0.67	0.04 ± 0.06	0.04 ± 0.04	0.04 ± 0.04	0.04 ± 0.08

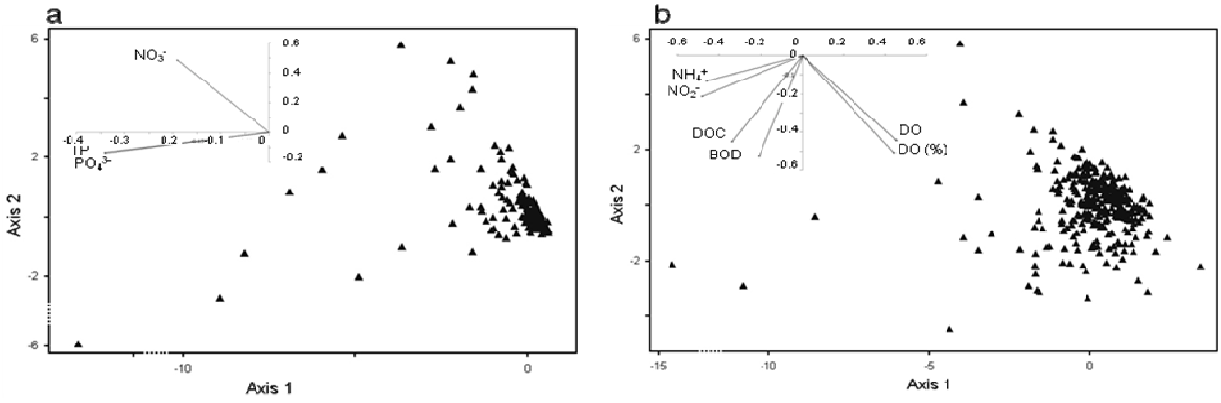

Figure 28: Principal component analysis using (a) the trophic level parameters (Eigen value: axis 1: 71.6%, axis 2: 27.4%) and (b) the organic pollution paramters (Eigen value: axis 1: 44.3%, axis 2: 22.1%).

There are significant differences between the mean values of the BDI in the organic pollution ($p < 0.001$) and trophic level classes ($p < 0.001$) (Table 10 and Figure 29). The watercourses classified in the trophic class t1 corresponded to medium quality watercourses (BDI average in t1 = 12.06). The watercourses classified in the trophic class t2 corresponded to good quality watercourses (BDI

average in t2 = 15.87), and those classified in the trophic classes t3, t4, t5 corresponded to very good quality watercourses (BDI average in t3 = 17.07, t4 = 18.68, t5 = 18.60).
The watercourses classified in the organic pollution class s1 corresponded to medium quality watercourses (BDI average in s1 = 13.83). The watercourses classified in classes s2, s3, s4, s5 corresponded to very good quality watercourses (BDI average in s2 = 16.24, s3 = 16.32, s4 = 17.49, s5 = 18.40).

Table 10: H and p-values of the One-Way Analysis of Variance on Ranks (Kruskall-Wallis test) carried out for the different biological traits and diatom index. Pairwise comparisons of the 5 mean values are also given (Dunn's Method).

	Trophic level			**Organic pollution**		
	H	**p-value**	**Relationships between groups**	**H**	**p-value**	**Relationships between groups**
BDI	137.871	≤ 0.001	t1<t2=t3<t4 ; t3=t5 ; t2<t5 ; t4=t5	53.267	≤ 0.001	s1<s2=s3=s4 ; s1<s2=s3<s5 ; s4=s5
Life-forms						
mobile	5.174	0.27	t1=t2=t3=t4=t5	4.113	0.391	s1=s2=s3=s4=s5
colonial	82.87	≤ 0.001	t1=t2<t4=t5 ; t2=t3<t4=t5 ; t1<t3	26.261	≤ 0.001	s2=s3=s4=s5 ; s1=s2=s3 ; s1<s4=s5
tube forming	96.329	≤ 0.001	t1<t2=t3<t4=t5	40.094	≤ 0.001	s1<s2=s3=s4=s5
stalked	142.528	≤ 0.001	t1<t2<t3<t4=t5	45.838	≤ 0.001	s1=s2=s3<s5 ; s5=s4 ; s2=s3=s4 ; s1<s4
pioneer	19.782	≤ 0.001	t1<t2=t4=t5 ; t2=t3=t4=t5 ; t1=t2	1.208	0.877	s1=s2=s3=s4=s5
Size classes						
c1	1.707	0.789	t1=t2=t3=t4=t5	6.778	0.148	s1=s2=s3=s4=s5
c2	27.078	≤ 0.001	t1=t2<t4=t5 ; t3=t4=t5 ; t1=t2=t3	18.881	≤ 0.001	s1=s2=s3=s4 ; s2=s4=s5 ; s1=s3<s5
c3	33.382	≤ 0.001	t1=t2=t3>t4=t5	2.345	0.673	s1=s2=s3=s4=s5
c4	1.522	0.823	t1=t2=t3=t4=t5	4.797	0.309	s1=s2=s3=s4=s5
c5	14.572	0.006	t1=t2=t3=t5 ; t2=t3=t4=t5 ; t1>t4	36.861	≤ 0.001	s3>s4=s5 ; s1=s2=s4 ; s1=s2=s3 ; s1=s2>s5
Ecological guilds						
high profile	26.768	≤ 0.001	t1=t2=t3 ; t3=t4=t5 ; t1=t2<t4=t5	18.498	≤ 0.001	s1=s3<s5 ; s2=s4=s5 ; s1=s2=s3=s4
low profile	57.633	≤ 0.001	t1<t2<t4=t5 ; t3=t4=t5 ; t1=t2<t4=t5	17.58	≤ 0.001	s1=s2=s3=s4 ; s2=s3=s4=s5 ; s1<s5
motile	123.77	≤ 0.001	t1<t2=t3<t4=t5	32.527	≤ 0.001	s1>s4=s5 : s1=s3 ; s1>s2 ; s2=s3=s4 ; s3>s5 ; s2=s5

There were significant differences in the distribution of the colonial diatoms between the organic pollution and trophic level classes (Table 10 and Figure 30-a and Figure 31-a; $p \leq 0.001$). There were also significant differences in the distribution of the tube-forming diatoms between the five organic pollution classes (Table 10 and Figure 30-b; $p \leq 0.001$) and the five trophic level classes (Table 10 and Figure 31-b; $p \leq 0.001$). Finally, the distribution of the stalked diatoms between the organic pollution (Figure 30-c; $p \leq 0.001$) and trophic level classes (Figure 31-c; $p \leq 0.001$) was significantly different.
As shown in Table 10, size classes c2, c3 and c5 discriminated significantly between the five trophic level classes ($p \leq 0.001$ for c2, c3 and $p < 0.05$ for c5). Classes c1 and c4 ($p > 0.05$) did not show any differences in their abundance between the trophic level classes. Only size classes c2 and c5 could distinguish significantly the organic pollution classes ($p \leq 0.001$) the others did not show any significant differences.
The abundance of the ecological guilds was significant different between the organic pollution classes (Table 10 and Figure 32-a, b, c; $p < 0.001$). There were also significant differences in the abundances of the three ecological guilds between the trophic level classes (Table 10 and Figure 33-a, b, c; $p < 0.001$). The low-profile and high-profile diatoms were more numerous when the trophic level and organic pollution were low. On the contrary, motile diatoms were more numerous when the trophic level and the organic pollution levels were high (Figure 32-c, Figure 33-cFigure 34).

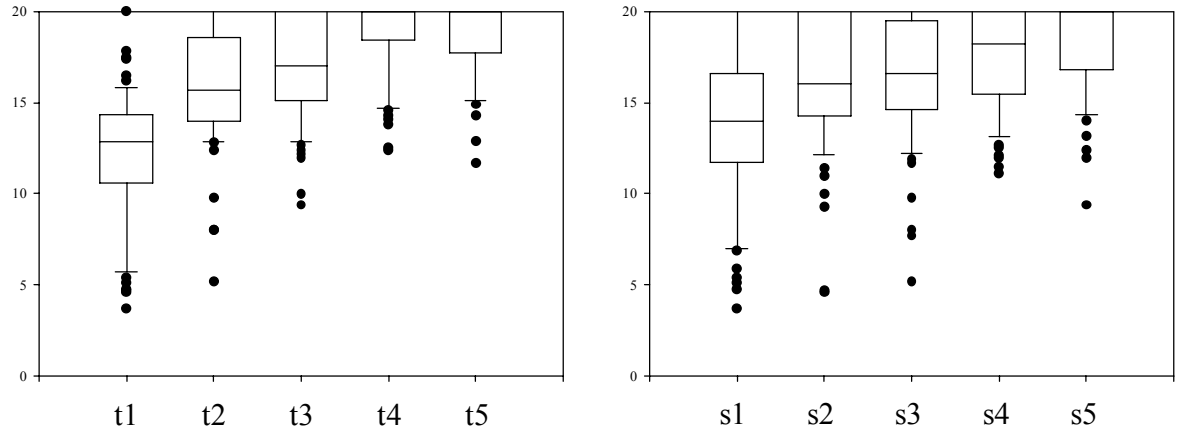

Figure 29: Box plot of the Biological Diatom Index (BDI) value for (a) the trophic level classes (Kruskal Wallis test: p<0.001) and (b) the organic pollution classes (Kruskal Wallis test: p<0.001).

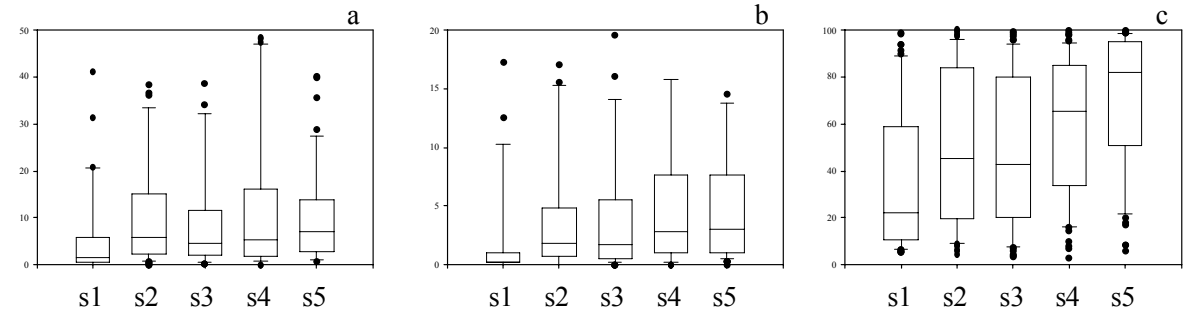

Figure 30: Box-plot of the life-form abundance for the organic pollution classes. (a) colonial diatoms (Kruskal Wallis: p<0.001), (b) tube-forming diatoms (Kruskal Wallis: p<0.001), (c) stalked diatoms (Kruskal Wallis: p<0.001).

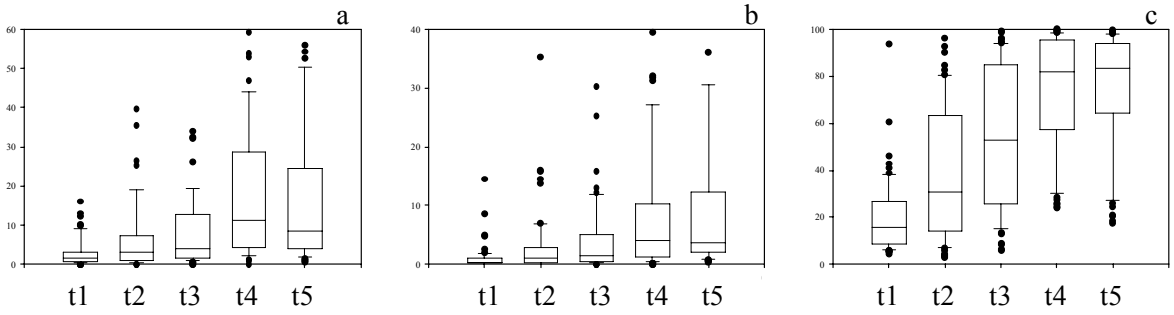

Figure 31: Box-plot of the life-forms abundance for the trophic level classes. (a) colonial diatoms (Kruskal Wallis: p<0.001), (b) tube-forming diatoms (Kruskal Wallis: p<0.001), (c) stalked diatoms (Kruskal Wallis: p<0.001).

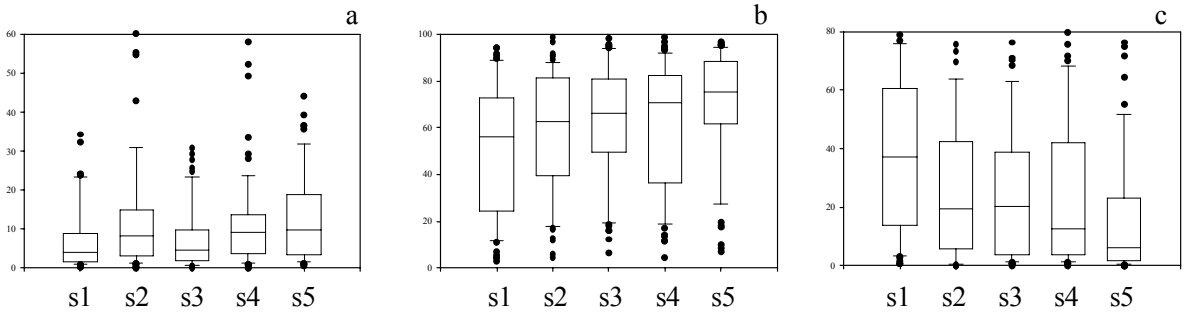

Figure 32: Box-plot of the ecological guild abundance for the organic pollution classes. (a) high-profile diatoms (Kruskal Wallis: p=0.646), (b) low-profile diatoms (Kruskal Wallis: p<0.001), (c) motile diatoms (Kruskal Wallis: p<0.001).

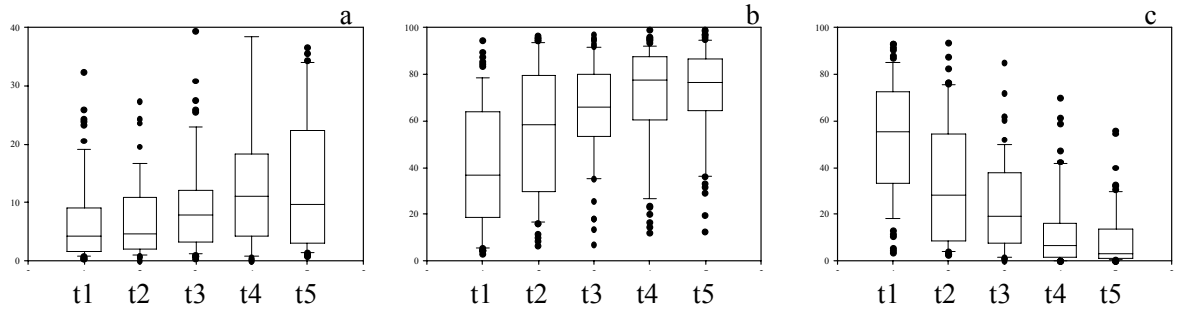

Figure 33: Box-plot of the ecological guild abundance for the trophic level classes. (a) high-profile diatoms (Kruskal Wallis: p<0.001), (b) low-profile diatoms (Kruskal Wallis: p<0.001), (c) motile diatoms (Kruskal Wallis: p<0.001).

For organic pollution the highest H-value (Figure 34-b) was observed for the BDI (H_{BDI} = 53.3). The H-values were also high for stalked diatoms ($H_{stalked}$ = 45.8), tube-forming diatoms ($H_{tube\text{-}forming}$ = 40.1), large sized diatoms (H_{c5} = 36.9) and the motile guild diatoms (H_{motile}= 32.5). For trophic level, the highest H-value (Figure 34-a) was observed for stalked diatoms ($H_{stalked}$ = 142.5) and the second highest was observed for the BDI (H_{BDI} = 137.9). Some metrics other than BDI and stalked diatoms also showed high H-values for trophic level as motile guild diatoms (H_{motile} = 123.8) and tube-forming diatoms ($H_{tube\text{-}forming}$ = 96.3).

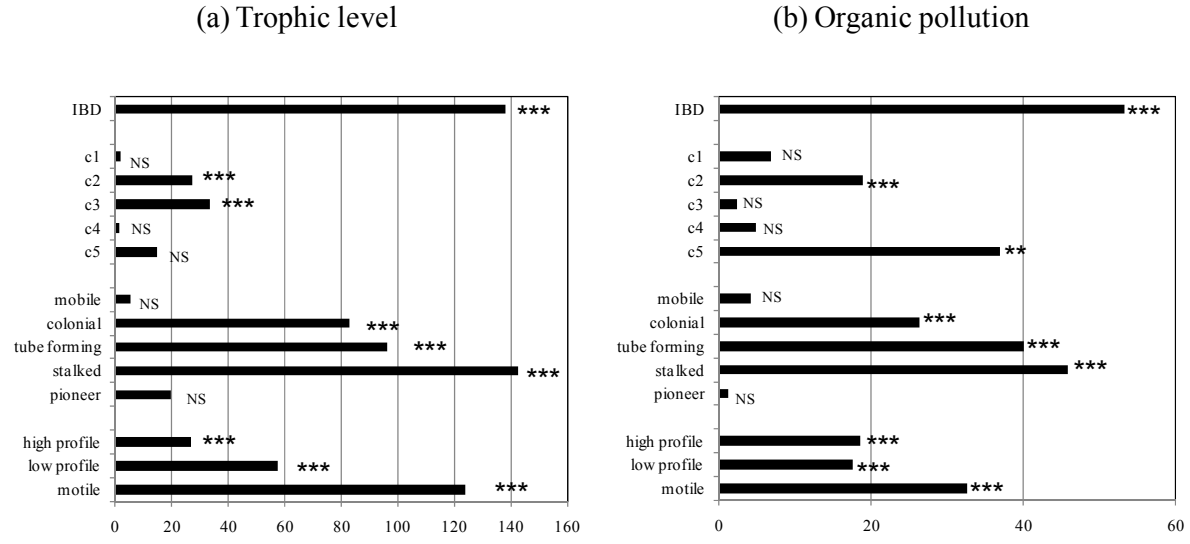

Figure 34: Discrimination power of the different metrics for organic pollution and trophic level as indicated by the H-values of the Kruskal-Wallis tests. The tests were run for all the size-classes, life-forms and ecological guilds tested according to (a) the trophic level and (b) the organic pollution classes. (distribution differences between the classes, N.S.- no significant difference, p > 0.05; ** – significant difference, p ≤ 0.01; * – significant difference, p ≤ 0.001).**

- **Discussion**

The Rhone-Mediterranean catchment encompasses a large variety of river typologies. Some of them are mountainous and pristine and others, particularly in urban sections, are highly polluted. These rivers also display considerable size differences. The geology also varies between different areas. Nevertheless, it appears that even though all these rivers present very different typologies, the distribution of diatom size classes, life-forms and ecological guilds are strongly influenced by trophic level and organic pollution of the water. These relationships were in accordance with our knowledge on ecological preferences.

In our study, the relative abundances of the tube-forming diatoms increased when the organic pollution and trophic level were low. Based on the analysis of a large database including rivers in several French river catchments, Rumeau and Coste (1988) constructed a diatom index based on pollution sensitivities of genera. The pollution sensitivities assigned to tube-forming genera were high ($GDI_{Frustulia}$ = 4.8/5, $GDI_{Encyonema}$ = 4.9/5). Similarly, Leira et al. (2009) investigated diatom diversity in 45 lakes in Ireland. This study showed that at higher taxonomic levels (order and family), diatoms belonging to the Cymbellaceae and Amphipleuraceae were mainly found in oligotrophic and ultra-oligotrophic environments.

Stalked diatoms showed the best discrimination of trophic level and organic pollution of any of the metrics (except the BDI for organic pollution): the relative abundance of stalked diatoms increased when trophic level and organic pollution decreased. In our study area, the most abundant stalk-producing genera were Achnanthidium (63.42% of all the stalked diatom valves in the entire database), and Cymbella (4.50%). Rumeau and Coste (1988), showed that these genera are mostly present in rivers with low nutrient and organic content, and that they are very sensitive to pollution ($GDI_{Cymbella}$ = 4.7 and $GDI_{Achnanthidium}$ = 4.5/5). Leira et al. (2009) found the same at higher taxonomic levels (e.g. Achnanthidiaceae, Achnanthaceae, Gomphonemataceae). Pringle (1990) has advanced the hypothesis that stalked diatoms are less well-adapted at incorporating nutrients adsorbed on the substratum, but better at exploiting the nutrients dissolved in the water. This was also confirmed during experiments where biofilms from nutrient-rich waters were transferred into unpolluted rivers: the abundance of motile diatoms decreased to the advantage of stalked diatoms (Rimet et al., 2009).

It appears that the trend in the relative abundance of pioneer diatoms little depended on the trophic level. Only the most organically polluted rivers had significantly lower abundance of pioneer diatoms than the other classes. Stevenson and Bahls (1999) found a relationship between the abundance of A. minutissimum, considered as a pioneer species, that invade biofilms just after the end of intense chemical disturbance events (e.g. acid mine drainage, toxic pollution). Thus, pioneer diatoms seem to be able to indicate the intensity of particular chemical disturbances (such as mine drainage), but not that of trophic level or organic pollutions.

There are different life-forms which often respond in similar ways to organic pollution and trophic levels. It is therefore possible to reduce the number of forms to a few ecological guilds (Passy, 2007). She) shows that these guilds can be used to distinguish between different sources and levels of disturbance. Our study showed that these guilds can efficiently discriminate between different levels of nutrient and organic pollution. The response observed of the ecological guilds abundances was similar between our study and the work of Passy (2007) who studied two rivers from upstream to downstream (Mesta River in Bulgaria and White Creek River in USA). However, the high diversity of

rivers sampled in our study confirms and enables to generalise the responses observed not solely to an upstream-downstream gradient but to a nutrient and organic matter concentration gradient.

The results observed on the watercourses of the Rhone-Mediterranean catchment have shown that the abundance of motile species increased with organic matter and nutrient concentration, as already observed by Passy (2007). Several reasons could explain the dominance of motile diatoms in resource-rich environments. The first motile diatoms can secrete extracellular enzymes, which enable them to use macromolecules adsorbed on the substrates or sediments (Pringle, 1990). Second motile diatoms are often bigger than low-profile diatoms, which enables them to store more nutrients (Pringle, 1990). Third motile diatoms also have the advantage over sessile species of being able to move rapidly from nutrient-poor microenvironments to ones with higher concentrations of nutrients and organic molecules (Johnson et al., 1997).

Conversely, we observed more low-profile diatoms in the nutrient-poor environments (rivers with very high quality). These taxa are known to be the first to colonise bare substrates, just after the bacteria (Hoagland et al., 1982) and before the stalked diatoms and the filamentous algae. We also found significant relationships between the abundance of high-profile diatoms and the different trophic level classes and organic pollution classes. These diatoms form colonies, which enables them to exploit resources not available to other species that stay closer to the substrate.

The relative abundances of these three guilds in biofilms do not depend solely on abiotic factors. Biotic factors, such as grazing and interspecies competition are also important. Low-profile diatoms, which include the adnate diatoms, have adapted to resist both the severe physical disturbance caused by water turbulence (Passy, 2007; Robinson and Rushford, 1987), and grazing pressure (Luttenton et al., 1986; Katoh, 1992). Nutrient-poor rivers, in which biofilms are rare, show a higher grazing pressure on benthic algae than nutrient-rich rivers, where biofilms are thick and abundant. During transfer experiments in which biofilms were transferred from polluted to unpolluted rivers (Rimet et al. 2009), high-profile diatoms (such as Gomphonema parvulum) disappeared soon after being transferred. Nutrient depletion was probably an important factor for these eutraphentic taxa (Van Dam et al., 1994). Furthermore, these high-profile diatoms were adapted to living in biofilms in which there is significant competition for space (Hoagland et al., 1982). According to Katoh (1992) and Luttenton et al. (1986), vertically positioned diatoms lose their advantage in oligotrophic and oligosaprobic rivers because competition for space is less crucial. Moreover, the high-profile diatoms are subjected to higher grazing pressure than adnate diatoms in unpolluted conditions where scrapers are abundant (Luttenton et al., 1986; Katoh, 1992; Rimet et al. 2009).

Among the different biological traits tested during this study, we showed that the abundance of tube-forming diatoms and stalked diatoms discriminated well between different trophic and organic pollution levels. Passy (2007) showed that the low-profile, high-profile and motile ecological guilds could be used to estimate trophic level. In our study, we showed that the low-profile and motile diatoms could also be used to estimate organic pollution. The Biological Diatom Index showed the greatest ability to discriminate between the organic pollution classes, but abundance of stalked diatom gave the best results for trophic level classes discrimination.

Nevertheless some limits of using diatom traits can be highlighted. First, stalked diatoms did not significantly differentiate the water quality classes showing the lowest trophic levels and the lowest organic pollutions. Another limitation is that such metrics potentially loses some ecological information, such as the influence of geological substrate (Rimet et al., 2004, Rimet 2009) or the impact of shading and temperature (e.g. Kawecka, 1985) which are often observed at species level.

Finally among the traits tested, some did not show any interesting abilities in assessing nutrient and organic pollution. This was particularly the case for size classes even if some trends were observable for small and medium size classes. Similar findings were observed in Lavoie et al. (2010) in Canadian rivers using generalised linear models who concluded that body size should not be used as a proxy for nutrient assessment.

- **Conclusion**

In the framework of studies that aim to assess organic pollution or trophic level, our study demonstrated that some simple biological traits could be used instead or in addition to species-based diatom bioassessment tools. This approach would simplify the taxonomical work because identification to genus level would be sufficient in most cases. Using biological traits would also provide more information about the structure and architecture of biofilms. Another advantage is that this kind of metric could be used to study geographical areas where taxonomy is unknown, or where we do not have sufficient data available to establish a robust ecological profile using species-level determination, as it is the case in French overseas territories.

- **Acknowledgments**

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4. Diatoms and pesticide contamination

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a. Preamble and major results

• Introduction

River quality has deteriorated as a result of many anthropogenic factors, such as increases in the concentrations of organic matter and nutrients, and modifications of river morphology. However, one of the key environmental problems we are facing today is the increasing contamination of freshwater systems by industrial chemical compounds, which are released deliberately into the environment. About 90% of European rivers are polluted by persistent organic micropollutants, and herbicides are some of those most often detected (Loos et al., 2009). Policies are increasingly concerned with this kind of pollution and its effects on the environment. In France, several government-led actions have been initiated since 2009. They require a 50% reduction in pesticide use in agriculture over the next 10 years (Ministère de l'Agriculture et de la Pêche, 2008). These actions have a high societal cost, and so the hypothetical beneficial impact on environmental health has to be quantified. The objective of this study is to assess the remediation of rivers as pesticide pressure declines. Since diatoms are photosynthetic organisms, the hypothesis is that they should be good indicators of herbicide pollution.

The existing bioassessment tools based on diatoms were developed to assess the trophic load (Whitton & Kelly, 1995; Kelly et al., 2007), the saprobic load (Rott et al., 1997; Watanabe et al., 1986), and the overall pollution (Coste et al., 2009). However, recent *in-situ* ecotoxicological studies have provided promising results based on physiological algal activity (e.g. Tlili et al., 2008; Gustavson et al., 2003; Navarro et al., 2002; Guasch et al., 1999) and taxonomical composition (e.g. Dorigo et al., 2007; Morin et al., 2009; Guasch et al., 1997; 1998b), suggesting that diatoms do indeed offer a suitable tool for the bioassessment of pesticide risks.

Benthic diatoms display a wide range of diverse strategies to resist environmental pressures such as grazing and water turbulence, and also to access resources, by nutrient uptake, and light harvesting. This has resulted in a variety of life-forms: benthic, planktic, mobile, colonial, those living in mucous tubules, pedunculate, and pioneering. Diatom species can also be divided into groups or ecological guilds, which have developed various different strategies to resist the same kind of environmental pressures. The interest of using such metrics in an ecotoxicological context is that such metrics are common in biofilms, which implies they are likely to display robust and predictable responses to ecological gradients. This has been clearly demonstrated for gradients of nutrients and organic matter in a wide variety of rivers in France (Berthon et al., 2011). Compared to the pollution affinities of species, only a few species are common and therefore only a few of them display robust and predictable trends; the great majority of taxa are rare, which makes it difficult to assess their pollution sensitivities with certainty.

The objective of this study was to compare a species-based approach to a metric-based approach in assessing the impact of a herbicide on diatom assemblages in a biomonitoring context. The hypothesis was that using diatom life-forms and ecological guilds would make it possible to detect these impacts better than using diatom species data. Several hypotheses about the impact of

pesticides on diatom metrics were also tested. First, the shielding effect of thick exopolysaccharide matrices against dissolved micropollutants is well documented in medical bacteriology (Onbasli & Aslim, 2009) and was tested here: we expected to find an increase in the relative abundance of diatoms living in such thick habitats as herbicide concentration increased. Second, another hypothesis is an increase in small size taxa as herbicide contamination increases (e.g. Wunsam et al., 2002a; Cattaneo et al., 1998; 2004; Morin, 2006). Third, pioneer taxa are often abundant in micropollutant-contaminated rivers (Stevenson & Bahls, 2002), and their relative abundance was expected to increase as herbicide contamination increased. Fourth, taxa that exploit dissolved resources rather than adsorbed resources (e.g. pedunculates) were likely to be more sensitive to dissolved pesticide.

In order to control most of environmental factors known to have an effect on diatom assemblages (e.g. current velocity, nutrients, lights), we used a lotic mesocosm approach. Four outdoor lotic mesocosms experiments were conducted in 63 to 75 day-long experiments from 2006 to 2008. We focused on a herbicide often used in vineyards (diuron), which is used in association with fungicides (azoxystrobin and tebuconazol). The concentrations of these pesticides applied in the mesocosms were similar to those found in natural rivers in vineyard areas.

The sensitivity to pesticides of the different metrics used in the hypotheses was tested and discussed in the context of their use as routine indicators of environmental remediation to be expected after the reduction of agricultural pesticide use.

- **Methodology**

Four lotic mesocosms designed by Volatier (2004) were used. Each channel is 4 m long, 0.4 m wide and 0.35 m deep and made of stainless steel. The channels are isolated from each other and function independently. Water is supplied by pumping from a depth of 36 m in Lake Geneva (720 L/h) four times a day. This provides an adequate supply of nutrients and seeds the channels with natural communities of microorganisms from the lake. Glass slides (7.9 cm^2) were used as artificial substrates to collect the biofilms for further diatom identification and counts.

Contamination concentrations were chosen on the basis of previous studies (Montuelle et al., 2010) carried out in a neighboring river basin in a vine-growing area (Beaujolais, France). Two kinds of contamination were applied to the mesocosms: some were exposed to chronic pollution throughout the experiment and others to acute pollution with high concentrations lasting 4 hours in order to simulate a flooding event. In addition, some channels were contamination-free (controls). The concentrations used for the chronic pollution, depending on the experiment, ranged from 1.55 to 2.82 $\mu g.L^{-1}$ for diuron, from 0.60 to 1.19 $\mu g.L^{-1}$ for azoxystrobin and from 0.39 to 0.59 $\mu g.L^{-1}$ for tebuconazol. For acute pollution they ranged from 11.16 to 13.03 $\mu g.L^{-1}$ for diuron, from 4.70 $\mu g.L^{-1}$ to 6.60 $\mu g.L^{-1}$ for tebuconazol, and was 7.22 $\mu g.L^{-1}$ for azoxystrobin.

Diatom samplings were carried out every week or every two weeks (depending on the experiment) and physical, chemical, and pesticide samples were also taken. The experiments were carried out

from late spring to early summer for periods of 63 to 75 days. Diatom and chemical analyses followed standard French and European methodologies.

Diatoms were identified and counted to species or sub-species level. These data were then transformed into abundances of size-classes, life-forms and ecological guilds according to (Berthon et al., 2011).

- **Results and discussion**

1- Why use diatom metrics instead of taxonomical composition to assess pesticide contamination?

When we look at the overall variability of the diatom assemblages in these four experiments, we can see that they varied considerably from one experiment to another. Some species were abundant in some experiments, but showed very low abundance in others, and some taxa were only detected in some of the experiments. Statistical analyses showed that the diatom assemblages differed significantly from one experiment to another. These differences could be explained by the natural origin of the samples, since diatom communities in Lake Geneva display both seasonal and interannual changes (Rimet et al., 2009). However, these differences could also have purely technical and taxonomical origins. Diatom identification requires advanced training and nomenclature changes undermine the consistent identification of taxa (Kociolek, 2005). Our experimental samples contained several species complexes (e.g. *Fragilaria capucina, Nitzschia palea*) that are difficult to identify (e.g. Trobajo et al., 2009), and which are regularly subjected to intercalibration. Furthermore, the samples from the different experiments were identified by different people, and this probably accounts for some of the variability of the diatom assemblage composition.

If we look at the experiments separately, diatom assemblages changed considerably from the beginning to the end of each experiment. When the mesocosm experiments started the substrates were free of biofilms and the subsequent colonization time was the most structuring parameter of the diatom assemblages in all four experiments. The importance of this parameter is well documented in natural environments such as rivers (e.g. Cazaubon, 1988; Eulin & LeCohu, 1998), and several authors have proposed general models to describe algal successions as colonization progresses (Hoagland et al., 1982).

Despite the importance of interannual and seasonal changes, identification uncertainties and colonization times, pesticides had a significant impact on our diatom assemblages. This has also been observed in other ecotoxicological studies using various types of approach such as microcosms (e.g. Peres et al., 1996), mesocosms (e.g. Schmitt-Jansen & Altenburger, 2005), and *in-situ* studies (e.g. Dorigo et al., 2004; Guasch et al., 1998a; Morin et al., 2009). These authors studied the effects of pesticides using species abundances and identified sensitive and resistant taxa, which differed from one experiment to another. Given the diversity of diatoms, it is difficult to extrapolate from species-based data. These authors did not assess the effect of pesticides on diatom metrics. We observed that impact of pesticides was stronger when we looked at diatom ecological guilds rather than species abundances. Using a metric approach makes it possible to merge several species in a single metric, thus making it easier to compare different studies. Diatom metrics are often characteristic of

entire genera, and so their use reduces the identification difficulties/errors that are often observed at species level. Moreover, trends in diatom metrics versus saproby and trophy are easy to predict (Berthon et al., 2011), and the presence of pesticides can be expected to disturb these trends in natural rivers. The use of diatom metrics therefore provides interesting data for pesticide bioassessment.

2- Which metrics detect pesticide contamination most effectively?

We observed that diatom assemblages in mesocosms are influenced by several environmental parameters. These included pesticide contamination, which had a significant effect on diatom metrics. The main objective of this study was very practical, and was a response to requests from water managers. We postulated four hypotheses, which were detailed in the Introduction, and then tried to validate them by analyzing the results of the mesocosm experiments.

Several studies had shown that small diatom taxa increase in response to herbicide contamination (e.g. Wunsam et al., 2002b; Cattaneo et al., 1998; 2004); Morin, 2006). We explored this phenomenon in our mesocosm experiments by classifying diatoms according to size class. We did indeed find an increase in the smallest size classes and a decrease in the largest size class in the contaminated channels of some experiments, but the trends were lacking repeatability from one experiment to another: only one size class gave the same result on two occasions.

Pioneer taxa are frequently observed in rivers contaminated by micropollutants. For instance, *Achnanthidium minutissimum,* which is considered to be a pioneer in rivers (Sabater, 2000), is often observed downstream from mining activities that discharge high concentrations of heavy metals (e.g. Gold et al., 2003; Ferreira da Silva et al., 2009; Salonen et al., 2006). Stevenson & Bahls (2002) think that pioneer diatoms are able to resist severe “chemical insults”. Nevertheless, the abundance of pioneer diatoms increased in the contaminated channels in only one experiment.

Compared to mobile diatoms, pedunculate diatoms are adapted to exploit dissolved nutrients in water rather than nutrients adsorbed on the substratum (Pringle, 1990). For this reason, we would expect pedunculate diatoms to be more sensitive to dissolved herbicides than motile ones, and therefore to suffer a more marked decrease in abundance. In fact this was only observed in one experiment out of four.

For these three kinds of metric, even though the trends appear to confirm our initial hypotheses, additional mesocosms experiments are required to confirm our observations and validate their interest for really effective pesticide assessment.

Mobile life-forms encompass all the taxa presenting a raphe structure that enables them to move. The motile diatom guild (Passy, 2007) is a selection of fast-moving diatom species, especially the *Nitzschia* s.l. and *Navicula* s.l. genera. Both groups increase when organic matter and nutrient concentrations increase in water (Berthon et al., 2011). Such taxa can secrete extracellular enzymes, which enables them to exploit resources adsorbed on substrates or sediments (Pringle, 1990). Most of the genera that compose the motile guild are characteristic of bad water quality according to the Generic Diatom Index (Rumeau & Coste, 1988), and they usually live in thick biofilms. Our

mesocosms experiments showed an increase abundance of these two metrics in the contaminated mesocosms. This is consistent with the findings of Guasch et al. (1998a) and Dorigo et al. (2004) who observed that species resistant to atrazine belonged to the *Nitzschia* and *Navicula* genera. Similarly in diuron-contaminated rivers, Morin et al. (2009) observed an increase in the abundance of taxa preferring organic matter- and nutrient-rich water. This was also confirmed by Guasch et al. (2003) who used EC50 tests to show that thick biofilms are less sensitive to atrazine. These findings could confirm the shielding effect of thick exopolysaccharide matrices.

Tube-forming life-forms include the diatom taxa that produce tubes and live in colonies. *Encyonema* and *Frustulia* are members of this life-form, and are generally considered to be nutrient-sensitive diatoms (e.g. Rumeau & Coste, 1988). In our mesocosms experiments, their abundance increased in the contaminated channels. One possible hypothesis to explain their resistance to pesticides could be, once again, the shielding effect of their exopolysaccharide tube.

The low-profile guild includes small diatom taxa and adnate diatom taxa attached to the substratum over an entire valve surface. They increased in abundance. In the case of the adnate diatoms this could be because one entire side of the cell is protected from the pesticide by the substratum. In the case of small diatoms, this observation is consistent with the findings of Wunsam et al. (2002b) Cattaneo et al. (1998; 2004) and Morin (2006).

The high-profile guild (Passy, 2007) corresponds to diatoms exposing a large profile to the water current, and which therefore have a large surface area in direct contact with the water. We therefore expected to see a decrease in their abundance. They did indeed show the opposite trend to the motile guild and low-profile guild in the mesocosms. This probably means that they are more exposed to dissolved pesticides than the motile and low-profile diatoms, which could explain their reduced abundance in pesticide-contaminated mesocosms.

- **Conclusions**

The mesocosm experiments allowed us to identify several metrics that could be good candidates for assessing the impact of pesticides on river diatom assemblages at relatively low pollutions levels corresponding to concentrations regularly found in rivers. The metrics found to be good indicators were the mobile and mucous tubule life-forms, and the ecological guilds (Passy, 2007). However, this impact was observed under particular conditions. First, we had chosen to control the variability of several factors known to have an impact on diatom assemblages by performing mesocosm experiments: nutrients, light, and current velocity were the same in all the mesocosms. Second, instead of using the diatom assemblage with species data, we chose to transform them into abundances of size-classes, life-forms, and ecological guilds. This transformed noisy information (species data) into a clearer data set with more robust trends. This simplification also made it possible to test hypotheses that cannot be confirmed using species data. And finally, using these metrics provides more comparable data from different experiments, making it easier to generalize.

The next question that water managers ask is whether it is possible to use such metrics to assess pesticide impact *in situ* on river diatom assemblages. A study was carried out on large datasets including diatom listings, chemical, physical, micropollutant data, and river typology from the Rhine-Meuse and Rhone-Mediterranean basins (Marcel et al., 2011; Bouchez et al., 2010). Early results demonstrated that nutrients and river typology had an overriding impact on diatom metrics (life-forms, ecological guilds, diversity indices). Even if sampling sites of a particular river type with a particular nutrient level were selected, very few correlations could be observed between the metrics that had been identified as being effective in mesocosms and the concentrations of the most commonly detected herbicides (atrazine, diuron, isoproturon). These conflicting findings highlight the need for multi-scale approaches if we are to understand the impact of pesticides on diatom assemblages. *In-situ* surveys on particular rivers sites in agricultural zones with known pesticides inputs (e.g. Montuelle et al., 2010), and simple ecotoxicological tests (EC50) on a large variety of diatom species, in order to range their sensitivity and propose new explanatory hypotheses is now called for.

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b. Paper 5: Use of diatom life-forms and ecological guilds to assess pesticide contamination in rivers: lotic mesocosm approaches.

Rimet F. & Bouchez A.

- **Abstract**

The persistence of pesticides in the environment and their effects are a cause of concern to more and more people, and so in 2009 the French government announced plans to reduce pesticide use in agriculture over the next 10 years. Water managers are to monitor the beneficial impact of this reduction on aquatic environments. It has been suggested that diatoms may be good indicators of pesticides, and more particularly of herbicides, in water. Diatoms have been routinely used to assess organic and nutrient pollution for more than 10 years. The general approach is to develop a diatom-based tool to assess pesticide contamination. Diatom indices are usually based on specific pollution sensitivity. Other metrics, such as life-forms, ecological guilds, or cell size offer other advantages. For instance, the relationships between trends in these metrics and environmental gradients are more robust, and make it easier to establish ecological hypotheses. We have therefore opted for this approach.

To develop such a tool, outdoor, lotic mesocosm experiments lasting about 2 months were conducted from 2006 to 2008. Herbicides (diuron) and fungicides (azoxystrobin, tebuconazole) were tested at environmental concentrations (sum of pesticides concentrations from 1.11 to 3.01 $\mu g.l^{-1}$ for chronic pollutions and from 20.25 to 29.50 $\mu g.l^{-1}$ for short-term acute pollutions). Diatom communities in artificial channels were analyzed by light microscopy using standard European methods. The various parameters structuring diatom communities were assessed, and colonization time appeared to be the most important. However, pesticide contamination was the second most important, and had a more significant impact on the composition of ecological guilds than on species composition. Some metrics did not display any significant trends (benthic/planktic, colonial, pedunculate, pioneer), but others looked promising for use in pesticide contamination assessment: the abundances of motile-guild, low-profile guild and mucous tubule diatoms all increased in contaminated channels, whereas high-profile diatoms showed the opposite trend. Some possible explanations, such as a protective effect of the exopolysaccharide matrix, can be advanced: diatoms living inside a mucous tubule may be shielded from dissolved pesticides, as are motile diatoms, which have a micro-habitat preference for thick matrices which also allows them to withstand higher levels of water contamination. In the same way, high-profile guild diatoms are exposed to dissolved pesticides to a greater extent, and this could explain their lower abundance in contaminated channels.

Keywords: benthic diatoms, biological metrics, fungicide, herbicide, mesocosm, micropollutant.

- **Introduction**

Diatoms constitute the most diverse microalgal class, and include several hundred thousand taxa (Mann and Droop, 1996), they are also frequently dominant in freshwater biofilms in rivers (Blinn et al., 1980). The structuring effect of nutrients and organic matter concentrations on the taxonomical

composition of diatom assemblages has long been observed, and this has led to the development of bioassessment tools centered on this particular algal class. Biotic indices have been developed by numerous authors in Europe since the 1960s (Zelinka and Marvan, 1961; Lange-Bertalot, 1979), especially in the 1980s (e.g. Descy, 1980; Coste in Cemagref, 1982; Leclercq and Maquet, 1987). All these biotic indices are calculated using similar metrics, which are based on species abundances and their specific affinities for organic and/or nutrient concentrations. These biotic indices have been increasingly used in Europe since the European Water Framework Directive (European commission, 2000) has required member states to assess the ecological integrity of their rivers using diatom indices alongside other bioindicators, such as macro-invertebrates, macrophytes and fish.

Beside the problems of pollution caused by increasing nutrient and organic loads, another key issue is pesticide persistence in aquatic environments. Policies are increasingly concerned about this kind of pollution and its effects on the environment. In France, several government-led actions have been initiated since 2009. They require a 50% reduction in pesticide use in agriculture over the next 10 years (Ministère de l'Agriculture et de la Pêche, 2008). The study reported here took place in this framework, since the beneficial impact of these actions on aquatic ecosystems has to be assessed. The idea is to assess the remediation of rivers when pesticide pressure reduces, the key bioindicator used here will be benthic diatoms. Bioassessment tools based on the taxonomical composition of diatoms have been developed to assess the trophic load (Whitton and Kelly, 1995; Kelly et al., 2007), the saprobic load (Rott et al., 1997; Watanabe et al., 1986), and the overall pollution (Prygiel and Coste, 1998; Afnor, 2007). However, recent *in-situ* ecotoxicological studies have provided encouraging results based on physiological algal activity (e.g. Tlili et al., 2008; Gustavson et al., 2003; Navarro et al., 2002; Guasch et al., 1999) and taxonomical composition (e.g. Dorigo et al*.,* 2007; Morin et al*.,* 2009a; Guasch et al., 1997, 1998b) suggesting that diatoms do indeed offer a suitable tool for the bioassessment of pesticide risk.

Benthic diatoms use various different strategies to resist environmental pressures such as grazing, flow disturbance, nutrient resource, and this has resulted in several life-forms: benthic, planktic, mobile, colonial, those living in mucous tubules, pedunculate, and pioneer. Diatoms can also be assigned to ecological guilds, consisting of groups of taxa that live in the same environment, but which may have adapted in different ways to abiotic factors. The ecological guilds used in this study were adapted from Passy (2007). Such diatom metrics provide ways to assess pollution levels other than the usual diatom indices corresponding to specific affinities to nutrient and organic concentrations. The diatom life-forms, cell sizes and ecological guilds found along trophic, saprobic and current velocity gradients can be accurately predicted (e.g. Berthon et al., 2011; Biggs et al., 1998; Passy, 2007). Moreover, the interest of using such metrics is that they are few in number and very common in biofilms, which implies that they are likely to display robust and predictable responses to ecological gradients. Compared to specific pollution affinities of species, only a few species are common and therefore display robust trends, a large majority of taxa are rare, and therefore it is difficult to assess their pollution sensitivities with certainties. Life-forms and ecological guilds can also provide interesting information about the structure and architecture of the biofilm. Another advantage is that such metrics are often characteristic of the genus level, and therefore easier to use for routine monitoring purposes.

The hypothesis of this study is that pesticides disturb the relative abundance of these metrics (ecological guilds, life-forms, cell size). Several hypotheses can be tested, such as the shielding effect against micropollutants of thick exopolysaccharide matrices for diatoms living in these micro-

habitats, as has well been documented in bacteriology (e.g. Onbasli and Aslim, 2009). Another hypothesis that could be tested is the increase of small taxa in response to pesticide contamination (e.g. Cattaneo et al., 1998, 2004; Morin, 2006). The increased abundance of pioneer taxa with pesticide contamination is another hypothesis often advanced (Stevenson and Bahls, 2002), and which could be tested. Another hypothesis to test is that taxa that have adapted to exploit dissolved rather than adsorbed resources (e.g. pedunculate) are likely to be more sensitive to dissolved pesticide.

The experimental framework we used was a lotic mesocosm approach. This has the advantage of controlling most environmental factors in order to focus on the effect of pesticides. Four outdoor mesocosm experiments were conducted in 63 to 75-days experiments from 2006 to 2008 during which the effects of pesticides were tested on benthic diatom metrics. Three water-soluble pesticides were tested, an herbicide (diuron) and two fungicides (azoxystrobin and tebuconazol), at concentrations found in natural rivers.

The potential value of such metrics for assessing pesticide contamination in rivers will be discussed in the context of their use as routine indicators of environmental remediation after the reduction of agricultural pesticide use.

- **Methods**

1. Description of the lotic mesocosms

A set of four artificial outdoor channels designed by Volatier (2004) was used. Figure 35 shows a diagrammatic representation of an artificial channel. Each channel was made of stainless steel (length 4 m, width 0.4 m and depth 0.35 m). A part of the pumping from the lake, originally used for a fish-farm of our institute, was used for our experiment (about 0.72 m^3/h out of 30 m^3/h). This water supply is pumped from a depth of 36 m in Lake Geneva. The semi-open functioning provided a water turnover of 4 times a day. This water turnover made it possible to maintain an adequate supply of nutrients in the channels, and to assume that microorganism seeding occurred that allowed colonization of the artificial substrates placed in each channel. Glass slides (7.9 cm^2) were used as artificial substrates to collect the periphyton for further taxonomical examination and counting. For experiments 1 and 2, 5 glass slides were placed in each channel, for experiments 3 and 4, 6 glass slides were placed in each channel.

Four experiments were carried out from 2006 to 2008, each lasted more than 60 days. The first and second experiments started on 15/MAY/2006, and 11/SEP/2006 (Villeneuve 2008). They were intended to test simultaneously the influence of pesticides and water turbulence on biofilms after 1 month of colonization. The third and fourth experiments started on 2/MAY/2007 and the 9/JUN/2008. These experiments were intended to test the influence of chronic and acute pesticide pollution. The details of each experiment are given in Table 11.

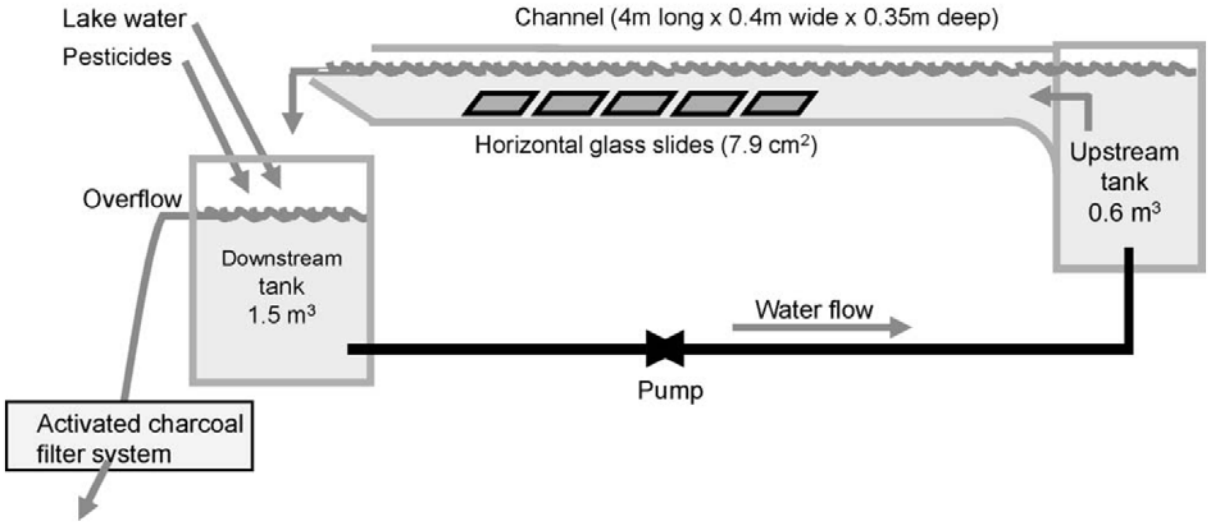

Figure 35: Schematic description of a lotic mesocosm (side view). The experimental platform is equipped with 4 mesocosms with parallel water supplies.

Table 11: Details of the four mesocosm experiments carried out from 2006 to 2008. Average values and standard deviation (between brackets) of pesticide concentrations are given in $\mu g.l^{-1}$ for the pollution period (several days for chronic pollution, and 4 hours for acute pollution).

	Experiment 1	**Experiment 2**	**Experiment 3**	**Experiment 4**
Factor tested	Effect of turbulence/chronic pollution: samplings were carried out simultaneously in laminar and turbulent zones of polluted or unpolluted channels	Effect of turbulence/chronic pollution: samplings were carried out simultaneously in laminar and turbulent zones of polluted or unpolluted channels	Effect of chronic/acute pollutions: samplings were carried out in each channel at the same date.	Effect of chronic/acute pollutions: samplings were carried out in each channel at the same date.
Experiment duration	63 days	67 days	75 days	65 days
Starting date	15/05/2006	11/09/2006	02/05/2007	09/06/2008
Sampling days	14, 28, 35, 49, 63.	15, 30, 37, 52, 67.	12, 27, 40, 47, 54, 75.	15, 30, 38, 44, 51, 65.
Contamination date	Chronic pollution started at day 28	Chronic pollution started at day 30	Chronic pollution started at day 27 and stopped at day 54, acute pollution was applied for 4 hours on day 40.	Chronic pollution started at day 0, acute pollution was applied for 4 hours on days 30, 38 and 44
Current velocity	laminar zones 0-0.2 m/s, turbulent zones 0.2-1.1 m/s	laminar zones 0-0.2 m/s, turbulent zones 0.2-1.1 m/s	0.15 m/s (laminar zones only)	0.15 m/s (laminar zones only)
Channel 1	Uncontaminated, laminar and turbulent zones Diuron: 0.03 (0.06) Azoxystrobin: 0 (0)	Uncontaminated, laminar and turbulent zones Diuron: 0 (0) Azoxystrobin: 0 (0)	Uncontaminated Diuron: 0 (0) Azoxystrobin: 0 (0)	Uncontaminated Diuron: 0 (0.01) Tebuconazol: 0 (0)
Channel 2	Chronic pollution, laminar and turbulent zones Diuron: 1.67 (0.57) Azoxystrobin: 0.69 (0.29)	Chronic pollution, laminar and turbulent zones Diuron: 2.82 (1.99) Azoxystrobin: 1.19 (0.89)	Chronic pollution Diuron: 1.55 (0.60) Azoxystrobin: 0.80 (0.30)	Acute pollution Diuron: 0 (0.01), 11.16 (3.87) averages during acute pollution (3 pulses) Tebuconazol: 0 (0), 4.70 (1.84) averages during acute pollution (3 pulses)
Channel 3	not used	not used	Chronic pollution Diuron: 1.21 (0.46) Azoxystrobin: 0.64 (0.25)	Chronic + acute pollution Diuron: 1.01 (0.82), 12.90 (4.16) averages during acute pollution (3 pulses) Tebuconazol: 0.59 (0.55), 6.60 (2.20) averages during acute pollution (3 pulses)
Channel 4	not used	not used	Chronic + acute pollution Diuron: 1.17 (0.36), 13.03 (1.49) during acute pollution Azoxystrobin: 0.60 (0.17), 7.22 (0.62) during acute pollution	Chronic pollution Diuron: 0.72 (0.31) Tebuconazol: 0.39 (0.33)

2. Choice of pesticides and contamination procedures

This study was carried out in the framework of several research projects about a vineyard area situated in the French Beaujolais region, and more precisely in the Morcille River. This river basin has been studied for several years (2006-2009) and was impacted by high concentrations of Diuron (herbicide), commonly used with a fungicide Azoxystrobin and in the last years with Tebuconazole (Montuelle et al. 2010). These pesticides are commonly used in Europe. Therefore, they were selected for our experiments. The chemical constants of the pesticides tested are provided hereby at the Functional Tools for Pesticide Risk Assessment and Management website (www.eu-footprint.org).

- Diuron: this substituted urea herbicide is used in vineyards. It is the 6th most often detected herbicide in drinkable water in France (Ministère de la Santé et des Solidarité, 2005). It has a Koc of 485 (i.e. it is tightly adsorbed onto soil organic matter, and its adsorption is directly affected by the amount of organic matter present), a kow of 2.7 (low to medium propensity to accumulate in body fat), and low water solubility (35.6 mg/L)
- Azoxystrobin: this strobilurin fungicide is also used in vineyards. Koc=423, Kow= 2.4, low water solubility (6.7 mg/L)
- Tebuconazol: this triazol fungicide is used in vineyards, and also on many vegetable crops. Koc=769, Kow=3.7, low water solubility (36 mg/L).

Pesticide contaminations were applied to several channels and concentrations were maintained by means of peristaltic pumps (Ismatec, IPN8). The overflow from the channels was decontaminated by activated-carbon filtration before being discharged.

3. Diatom sampling and analysis

At each sampling date (Table 11), the biofilm was scraped from one glass slide in each channel and suspended in 20 mL of 0.2 µm-Nucleopore filtered water containing 10% formaldehyde. After sampling, the glass slide was removed for the lotic mesocosm. The sample was then divided into 2 subsamples of equal volumes. One subsample was used for diatom analyses, following the European and French standards for diatom analysis (Afnor, 2004, 2007); this subsample was cleaned using 40% hydrogen peroxide to eliminate organic matter, and with hydrochloric acid to dissolve calcium carbonate. After cleaning, the diatom frustules were mounted in a synthetic resin (Naphrax©). Up to 400 valves were counted and identified in each sample using a light microscope with 1000× magnification (Zeiss AxioImager ©). The abundances of all the taxa observed were expressed as relative counts. The diatom floras of Krammer and Lange-Bertalot (1986, 1988, 1991a, b) and more recent books (e.g. Diatoms of Europe, Iconographia Diatomologica, Bibliotheca Diatomologica floras) and papers (e.g. Diatom Research papers) were used for the identifications.

The second subsample was used for quantitative analysis (results expressed as cells.cm^{-2}) of all algae (diatoms plus all the other algal classes) following the standardized Utermohl technique (Afnor, 2006). These findings are not discussed in this paper.

4. Environmental variables

The pH, temperature, and conductivity of the water were measured daily with a probe both in each channel, and in the incoming water. The chemical composition was measured weekly, including the sampling days, in water samples from each channel and from the incoming water. French standard

operating procedures and protocols were followed for measuring the contents of NH_4^+ (NF T 90-015), NO_3^- (NF EN ISO 13395), NO_2^- (NF EN 26777), SiO_2^- (NF T 90-007), and PO_4^{3-} (NF EN 1189).
Using standardized protocols, the pesticides being tested were analyzed in the water samples by ESI-LC-MS/MS (API 4000, Applied Biosystems) at the Cemagref Water Chemistry Laboratory (Lyon, France).

5. Definition of the diatom biological traits

Three different kinds of biological trait were used; life-forms, size classes and ecological guilds.

Life-forms

Different life-forms were distinguished: benthic, planktic, mobile, colonial, tube-forming, pedunculate and pioneers. The tube-forming life-form is a kind of colonial life-form: the diatoms live in a mucous protective substance within which they move freely. The pioneer diatoms are able to colonize bare substrates faster than other species. Table 12 shows the assignment of taxa to these different life-forms. This was based on existing classifications found in various publications: Krammer and Lange-Bertalot (1986, 1988, 1991a, b), Germain (1981) and more specific studies: Hoagland et al. (1982), Katoh (1992), Pringle (1990), Robinson and Rushforth (1987), and Allanson (1973).

Size classes

The biovolumes of the diatom taxa recorded in the different experiments were determined following average size values given in the diatom floras of Krammer and Lange Bertalot (1986, 1988, 1991a,b) or other books, such as the Diatoms of Europe (e.g. Krammer, 2000, 2001, 2002, 2003). The taxa were then assigned to 5 size classes: class size c1 consisting of taxa with biovolumes of less than 99 μm^3, c2 of those between 100 and 299 μm^3, c3 between 300 and 599 μm^3, c4 between 600 and 1499 μm^3 and c5 greater than 1500 μm^3.

Ecological guild

An ecological guild consists of taxa that all live in the same kind of environment, but which may display different adaptations to living in it (Devito et al., 2004). Passy (2007) defined three ecological guilds. The first, the low-profile guild consists of species of short stature, including prostrate, adnate, erect, solitary centrics, and slow moving species. The second, the high-profile guild, consists of species of tall stature, including erect, filamentous, branched, chain-forming, tube forming, pedunculate, and colonial centrics. And the third, the motile guild, consists of fast-moving species. Since several of the taxa recorded in our study were not referenced in Passy (2007), we adapted these guilds to include all the species and genera we found in our samples. The compositions of these adapted guilds are shown in Table 12.

Table 12: Assignment of taxa to biological metrics (life-forms and ecological guilds).

Biological metrics	Taxa concerned
Benthic	*Achnanthes, Achnanthidium, Adlafia, Amphora, Chamaepinnularia, Cocconeis, Cymbella, Denticula, Diatoma, Encyonema, Encyonopsis, Eolimna, Fallacia, Fistulifera, Fragilaria, Gomphonema, Gyrosigma, Mayamaea, Melosira, Navicula, Nitzschia, Planothidium, Sellaphora, Staurosira, Ulnaria.*
Planktic	*Asterionella, Aulacoseira, Cyclostephanos, Cyclotella, Discostella, Melosira, Puncticulata, Stephanodiscus, Thalassiosira.*
Mobile	*Achnanthes, Achnanthidium, Adlafia, Amphora, Chamaepinnularia, Cocconeis, Cymbella, Denticula, Encyonema, Encyonopsis, Eolimna, Fallacia, Fistulifera, Gomphonema, Gyrosigma, Mayamaea, Navicula, Nitzschia, Planothidium, Sellaphora.*
Colonial	*Asterionella, Aulacoseira, Cymbella, Diatoma, Encyonema, Encyonopsis, Fragilaria, Melosira, Staurosira.*
Mucous tubule	*Encyonema, Encyonopsis.*
Pedunculate	*Achnanthes, Achnanthidium, Diatoma, Fragilaria, Gomphonema, Planothidium.*
Pioneer	*Achnanthidium minutissimum* sensu lato, *Amphora pediculus.*
High profile guild	*Asterionella, Aulacoseira, Diatoma, Fragilaria, Gomphonema, Melosira, Staurosira, Ulnaria.*
Low profile guild	*Achnanthes, Achnanthidium, Amphora, Cocconeis, Cyclostephanos, Cyclotella, Cymbella, Discostella, Encyonema, Encyonopsis, Planothidium, Puncticulata, Stephanodiscus, Thalassiosira.*
Motile guild	*Adlafia, Chamaepinnularia, Craticula, Denticula, Eolimna, Fallacia, Fistulifera, Gyrosigma, Mayamaea, Navicula, Nitzschia, Sellaphora.*

6. Statistical analysis

Prior to assessing the effects of pesticides, the homogeneity of the physical and chemical conditions between the channels was tested for each experiment. One-way ANOVA (experiments 3 and 4) and t-tests (experiments 1 and 2) were carried out on these measures. The same analyses were run to assess the significance of contamination factors.

In order to assess which environmental descriptors best discriminated the diatom assemblages in the artificial channels, MRPPs (Multi Response Permutation Procedure, Biondini et al., 1985) were ran using the Pc-Ord software (McCune and Mefford, 2006). This analysis calculates an A-statistic, which is a descriptor of within-group homogeneity. This statistic varies ranges from -1 and +1: if the A-statistic approaches +1, the groups (here the diatom assemblages) are completely different; if the A-statistic approaches 0, the heterogeneity between groups equals what would be expected by chance; if the A-statistic approaches -1 the groups are homogeneous. The descriptors tested were colonization time (6 different dates for each experiment) and pesticide contamination (presence or absence for each experiment). Other specific factors were also tested: water turbulence for experiments 1 and 2 (turbulent/not turbulent) and acute/chronic pollution for experiments 3 and 4 (experiment 3: no pollution/chronic pollution/acute pollution; experiment 4: no pollution/chronic pollution/acute pollution/chronic+acute pollution)

The MRPP results were computed for the taxonomic composition and the ecological guild composition in order to compare the structuring impact of the environmental descriptor on these different biological metrics. Life-forms were not tested, because the sum of their abundances did not equal 100% (unlike the sums of the taxa and ecological guild composition), which would have interfered with the results. Size classes were not tested, because they did not show any interesting trends.

The second step was to assess which diatom metric best detected the pesticide contamination present in the channel. The discrimination power for pesticide contamination was compared to that for the colonization time. In this framework, two-way ANOVAs were run using pesticide contamination (the presence or absence of contamination) and colonization time (sampling date) as factors. The two-way ANOVAs were computed only on the samplings carried out after pesticide contamination. The p-value of the ANOVA was used to assess the discrimination power. Finally, after these two-way ANOVAs, pairwise comparison tests (Holm-Sidak method) were computed on the diatom metrics to assess the trend of the metrics from the uncontaminated to the contaminated channels. SigmaStat v 3.10 software was used for these analyses.

- **Results**

1. Chemical characteristics of the mesocosms

For the physical and chemical measures, no significant differences were observed between the channels except for pH in experiment 3, and for NH_4^+ in experiment 1. Table 13 shows the average concentrations of main measured parameters.

For pesticides, significant differences were observed between the channels in every experiment. Average concentrations of the pesticides are given in Table 11.

Table 13: Physical and chemical parameters in the channels. Mean values and standard deviations (between brackets) are shown. t-tests (experiments 1 and 2), and one-way ANOVA (experiments 3 and 4) were carried out between the channels for each experiments. Where significant differences were observed, mean values for the different channels (Ch.) are given.

	Exp. 1	**Exp. 2**	**Exp. 3**	**Exp. 4**
pH	8.3 (0.2)	8.0 (0.2)	Ch. 1: 8.1 (0.1) Other ch.: 8.2 (0.1)	8.1 (0.1)
SiO2 (mg/L)	1.12 (0.82)	0.54 (0.48)	0.55 (0.42)	0.85 (0.35)
PO42- (mg/L)	0.005 (0.006)	0.002 (0.001)	0.004 (0.004)	0.003 (0.001)
NO3- (mg/L)	0.26 (0.20)	0.45 (0.10)	0.35 (0.11)	0.50 (0.08)
NO2- (mg/L)	0.003 (0.002)	0.002 (0.001)	0.003 (0.002)	0.002 (0.001)
NH4+ (mg/L)	Ch. 1: 0.008 (0.004) Ch.2: 0.005 (0.003)	0.003 (0.005)	0.009 (0.010)	0.003 (0.003)

2. Results of the diatom analysis

A total of 88 diatom samples were analyzed, and 124 taxa belonging to 34 genera were observed during the 4 experiments. The number of taxa observed during each experiment varied considerably (26 taxa in experiment 1, 46 in experiment 2, 88 in experiment 3, and 46 in experiment 4), and if we consider the dominant taxa that made up 50% of the valves counted during each experiment, we can see that they differed considerably (experiment 1: *Fragilaria capucina* var. *vaucheriae*: 26%, *Diatoma ehrenbergii*: 21%, *Achnanthidium minutissimum*: 26%, experiment 2: *Diatoma vulgaris*: 61%, experiment 3: *Nitzschia fonticola*: 20%, *Diatoma vulgaris* f. *lineare*: 16%, *Fragilaria tenuistriata*: 16%, experiment 4: *Diatoma elongatum*: 28%, *Achnanthidium minutissimum*: 18%, *Nitzschia fonticola*: 11%).

These observations are confirmed by the MRPP tests carried out on the diatom assemblages in all four experiments, which showed that they were highly significantly different from each other (A-statistic: 0.33, p-value<0.01%). The detrended correspondence analysis in Figure 36 illustrates these differences.

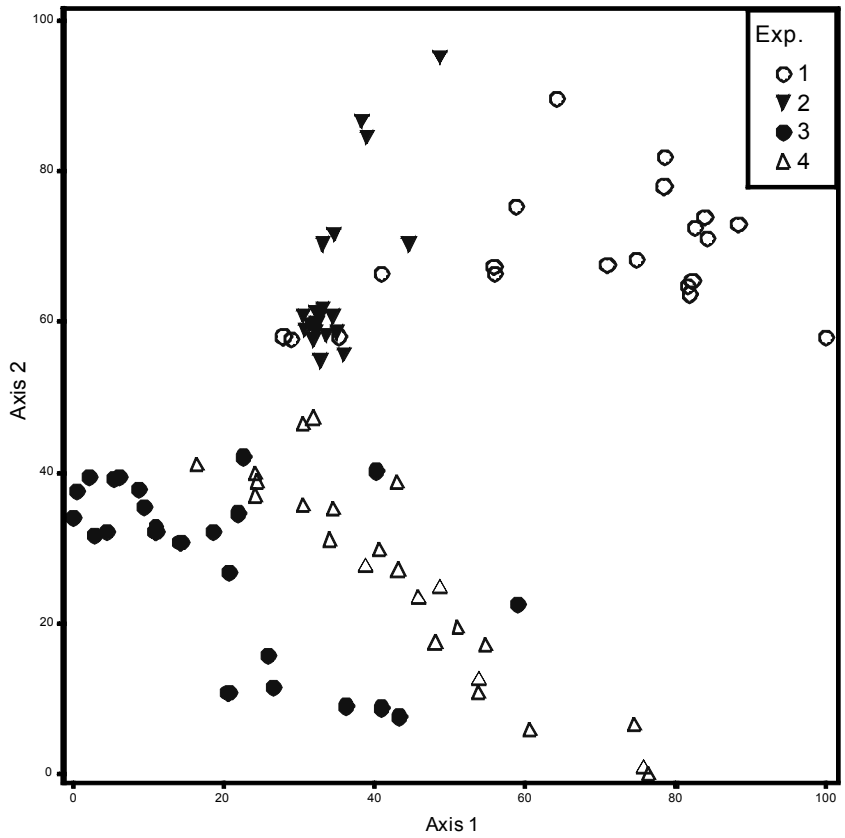

Figure 36: Detrended correspondence analysis carried out on the diatom assemblages of the four experiments. Coefficients of determination for the correlations between ordination distances and distances in the original n-dimensional space: axis 1-R2= 21.9%, axis 2 - R2 = 11.7%.

3. The importance of pesticide contamination in structuring diatoms assemblages

The importance of the various environmental descriptors, biofilm colonization time, pesticide contamination and other specific treatments (water turbulence, acute/chronic pollution) for the structure of the assemblages were assessed using MRPP for the taxonomical and ecological guild composition. Figure 37 and Figure 38 show the results of the MRPP tests for taxonomical and ecological guild composition respectively.

The most important structuring parameter for both taxonomical and ecological guild composition was the colonization time: taxonomical and ecological guild composition both showed significant differences at the different sampling dates.

Pesticide contamination was the second most important structuring parameter for both taxonomical and ecological guild composition. The structuring importance of pesticide contamination was greater for ecological guild composition than for taxonomical composition: the p-values and A statistic showed more significant effects for ecological guild composition than for taxonomical composition.

The other treatments (water turbulence, acute/chronic pollution) did not show any significant structuring effects on taxonomical composition or ecological guild composition.

The impact of colonization time and pesticide contamination on diatom metrics abundance in the channels was assessed by means of two-way ANOVA. Table 14 and Table 15 summarizes the results of these two-way ANOVAs carried out on the diatom metrics abundances (cell size, life-forms, ecological guilds) using colonization time as the first factor, and pesticide contamination

(uncontaminated/contaminated) as the second factor. Overall, we can see that colonization time had an impact on diatom abundance more frequently (25 significant tests out of 45) than pesticide contamination (20 significant tests out of 45).

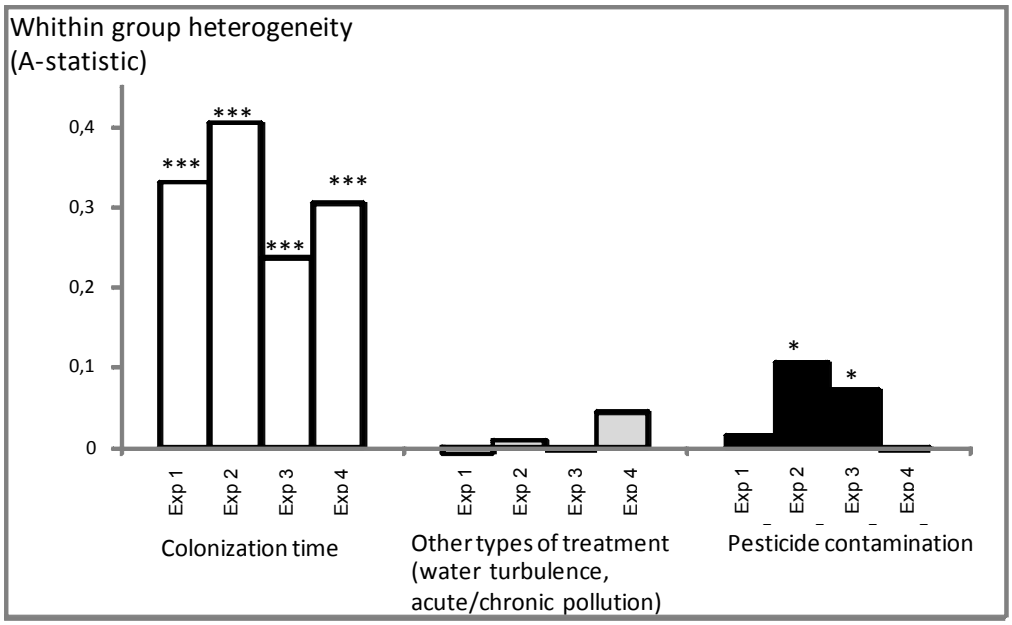

Figure 37: Importance of different descriptors in structuring the taxonomical composition. The A-statistic (calculated by an MRPP) provides an assessment of the assemblage heterogeneity for colonization times (sampling dates), types of treatments (water turbulence, acute/chronic pollution) and pesticide contamination (contaminated/uncontaminated channels, the MRPP tests are calculated from the data obtained during pesticide contamination). This heterogeneity gives an assessment of the structuring effect for each of these 3 descriptors. *: significant difference (p<5%), *: very significant difference (p<0.1%).**

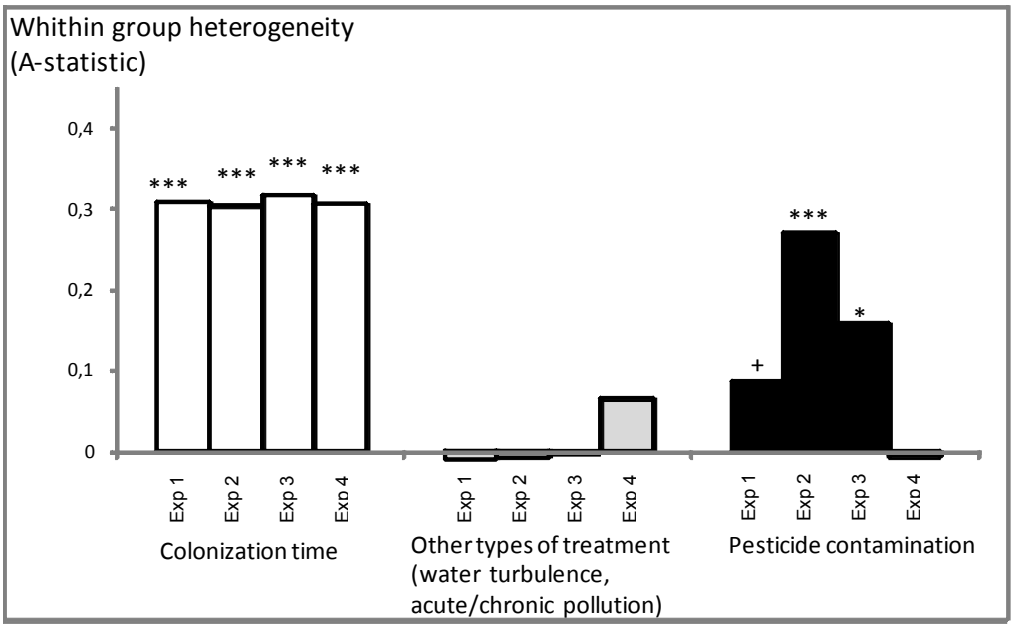

Figure 38: Structuring importance of different descriptors on the ecological guilds. The A-statistic (calculated by mean of a MRPP) gives an assessment of assemblage heterogeneity for colonization times (sampling dates), types of treatments (water turbulence, acute/chronic pollution) and pesticide contamination (contaminated/uncontaminated channels, the MRPP tests are calculated on the data during pesticide contamination). This heterogeneity gives an assessment of the structuring effect of each of these 3 descriptors. +: p-value < 10%, *: p-value < 5%, ***: p-value < 0.1%.

Table 14: Differences in abundance metrics in the unpolluted and polluted channels. Two-way ANOVA (first factor: colonization time, second factor: contamination), and pairwise multiple comparison procedures (Holm-Sidak method) were carried out. ↗: significant (p-value < 5%) abundance increases from unpolluted to polluted channels, ↘: significant (p-value < 5%) abundance decreases from unpolluted to polluted channels, ns: no significant evolution.

Biological metric	Exp.1	Exp.2	Exp.3	Exp.4
Size 1	↗	ns	ns	ns
Size 2	↗	ns	ns	↗
Size 3	↗	ns	↗	ns
Size 4	ns	ns	↗	ns
Size 5	↘	ns	ns	ns
Benthic	ns	ns	ns	ns
Planktic	ns	ns	ns	ns
Mobile	↗	ns	↗	ns
Colonial	ns	ns	↘	ns
Mucous tubule	↗	↗	ns	ns
Pedunculate	ns	ns	↘	ns
Pioneer	↗	ns	ns	ns
High profile guild	↘	ns	↘	ns
Low profile guild	↗	ns	↗	ns
Motile guild	↗	ns	↗	ns

Table 15: Differences in abundance metrics in the colonization times. Two-way ANOVA (first factor: colonization time, second factor: contamination), ns: p-value > 5%, *: p-value < 5%, **: p-value<1%, ***: p-value<0.1%.

Biological metric	Exp.1	Exp.2	Exp.3	Exp.4
Size 1	***	ns	ns	*
Size 2	**	ns	ns	**
Size 3	ns	ns	**	ns
Size 4	ns	ns	ns	ns
Size 5	**	ns	ns	*
Benthic	ns	ns	ns	*
Planktic	ns	ns	ns	**
Mobile	***	ns	*	ns
Colonial	***	ns	*	ns
Mucous tubule	ns	ns	ns	***
Pedunculate	**	ns	*	ns
Pioneer	***	ns	ns	*
High profile guild	**	ns	*	*
Low profile guild	*	ns	ns	*
Motile guild	ns	ns	**	*

4. Difference in the metrics abundances from polluted to unpolluted channels

In order to assess the change in the different diatom metrics from an uncontaminated to a contaminated channel, pairwise comparisons tests (Holm-Sidak method) were carried out after the two-way ANOVA. Table 14 summarizes the differences found.

Benthic and planktic life-forms never showed any abundance differences between uncontaminated and contaminated channels.

Several metrics showed only one significant change over the 4 experiments: taxa presenting sizes below 99 μm^3, taxa between 600-1499 μm^3 and taxa over 1500 μm^3, colonial diatoms, pedunculate diatoms, and pioneer diatoms.

Other metrics showed two significant differences between uncontaminated to contaminated channels: taxa between 100-299 μm^3 and between 300-599 μm^3 in size increased, as did mobile, mucous tubule, low-profile guild, and motile guild taxa. High-profile diatoms decreased from uncontaminated to contaminated channels.

- **Discussion**

1. Why use life-forms or ecological guilds instead of taxonomical composition to assess pesticide contamination?

Many studies investigating the impact of pesticides on phototrophic organisms in biofilms have revealed clear ecophysiological effects. For instance, atrazine contamination has been shown to affect the photosynthetic activity of algae (Bérard et al., 2003; Guasch and Sabater, 1998) which depends on their previous exposure to the toxicant. More to our purpose, diuron exposure reduced the photosynthetic activity of microalgae living in river biofilms, together with a modification of their community structure revealed by DGGE (Pesce et al., 2006; Tlili et al., 2008). The physiological impact of pesticides often differs from one species to another (e.g. for atrazine: Bérard, 1996), and even from one strain to another. This has been clearly shown in the context of the Pollution Induce Community Tolerance –PICT– concept (Blanck et al., 1988). This therefore has direct implications for the taxonomical composition of biofilms.

Ecotoxicological based on diatom composition are usually carried out at the species level (e.g. Guasch et al., 1998b; Morin et al., 2009b; Peres et al., 1996; Dorigo et al., 2004), and various species have been reported to be either tolerant or sensitive to similar pesticides. For instance, the species reported to be atrazine-tolerant by Guasch et al. (1998b) are different from those reported by Dorigo et al. (2004). This depended on the algae present at the beginning of the experiment when the substrate was first seeded. This makes it difficult to identify a general trend when analyzing the experiments as a whole. The same was true for the 4 experiments presented here. Their diatom assemblages were significantly different, and different species dominated. These differences may have been of natural origin, and mainly attributable to the seasonal and interannual changes in the diatom communities of Lake Geneva (Rimet et al., 2009a). But they could also be attributable to taxonomical problems. Diatoms are very diverse, often including cryptic species (Mann, 1999, 2004; Evans et al., 2008), and it is often difficult to identify species with certainty. For instance the *Fragilaria capucina* sensu lato group was abundant in several of our mesocosm experiments. This taxon was determinated into *Fragilaria capucina* in Experiment 2, but divided into *F. capucina* and *F. tenuistriata* in experiments 3 and 4: these taxa are morphologically close, unless some differences in

striae density; they are grouped together in some identification books, such as Krammer and Lange-Bertalot (1991a).

Guasch et al. (1998a) stated that the identification of sensitive species was “not consistent among studies”, and that it was “probably not sufficient to define community tolerance in terms of species composition”. In our opinion, using diatom metrics makes it possible to simplify the signal, and thus make it easier to compare experiments. Trends are easier to identify, because these metrics occur more often than specific taxa. Consequently, trends are more robust. Hypotheses are also easier to establish and can be connected to other fields: for instance observations in medicine showed that bacteria living in biofilms also present higher resistances to antibiotics (e.g. Stewart and Costerton, 2001).

An approach based on diatom metrics is therefore really worth considering for ecotoxicological studies. Trends of diatom metrics versus saproby and trophy are easy to predict (Berthon et al., 2011), and the presence of pesticide can be expected to disturb these trends in natural rivers.

2. Which are the most important structuring factors in mesocosms?

Identification and quantification of the structuring impact of environmental factors on diatom assemblages is a key subject. Variations in physical environment (water turbulence) or in the contamination sequences (acute/chronic) did not show any significant structuring effect on taxonomical composition or ecological guild composition despite frequently reported impacts of minor changes in the environment (see for example Dorigo et al., 2009, Villeneuve et al., 2010).

Seasonality is often observed as playing a key role in explaining diatom assemblage structure in rivers (e.g. Leira and Sabater 2005; Passy 2007). Diatom assemblages showed important heterogeneity between the four experiments, since diatom seeding of mesocosms was coming from the lake and since diatom assemblages of Lake Geneva show important seasonal and to a lesser degree inter-annual changes (Rimet et al. 2009a). These two kinds of changes in the Lake clearly play an overriding role in the overall diatom structuring in the mesocosms and therefore in their response to pesticide pollution. Duong et al. (2008) also underlined the importance of seasonality when studying heavy-metals and diatom composition. In our case two experiments (experiments 2 and 4) out of four showed very minor differences in the biological metrics abundances between polluted and unpolluted channels. For experiment 2 neither differences between polluted and unpolluted channels nor temporal variations were observable in the different metrics abundance. For experiment 4 the significant differences for most of metrics were only along the colonization process of the biofilms.

Many studies have already shown that the taxonomical composition of diatoms changes considerably as the colonization phases of biofilms progress, for instance after a flood that has scoured the biofilms (Cazaubon, 1988), on uncolonized parts of aquatic plants (Ferreira and Seeliger, 1985), or after a new substrate has been immersed in a river (Eulin and LeCohu, 1998). Several authors have also proposed some general models to describe the succession of microalgal species as colonization progresses (Hoagland, 1981; Hoagland et al., 1982). The diatom assemblages observed in our study also displayed major structural modifications as the colonization time line progressed. Indeed, this factor was found to be the most important parameter structuring diatom communities in all 4 mesocosms experiments.

Despite the importance of colonization, diatom species composition has also been shown to be affected by pesticides in several microcosms (e.g. Peres et al., 1996), mesocosms (e.g. Schmitt-Jansen

and Altenburger, 2005), and in *in-situ* studies (e.g. Dorigo et al., 2004; Guasch et al., 1998b, Morin et al., 2009b). However, these authors did not study this effect using metrics other than taxonomical composition or diversity indices. In the four mesocosm experiments presented here, pesticide contamination demonstrated a significant structuring effect on diatoms in most of the experiments, although to a lesser extent than colonization time. Pesticide contamination showed a stronger structuring effect on ecological guilds than on species composition. Another important observation is that even when the concentrations tested were low (around 1.5 µg/L for chronic pollutions, and around 20 µg/L for acute pollution lasting a couple of hours) and comparable to concentrations observed in rivers, their effects were still measurable. Therefore, given the high ecological relevance of such complex experimental systems, we can suppose that these levels of contamination have an impact on diatom life-forms and ecological guilds in natural rivers.

3. Which metrics detect pesticide contamination most effectively?

The main objective of this study was very practical, and is a response to requests from water managers. What water stakeholders want are bioindicator tools that can be used to monitor the recovery of aquatic ecosystems after pesticide application has been reduced. Diatoms seem likely to be good candidates for assessing contamination with pesticides, and in particular with herbicides. These mesocosm experiments can identify which metrics look most promising.

For instance, size classes could be expected to provide interesting metrics. Several authors have already observed a reduction in cell size after heavy-metal contamination (Cattaneo et al., 1998, 2004; Joux-Arab et al., 2000; Morin, 2006). The hypothesis underlying the use of size classes here was that pesticide contamination could select small species. The trends observed in our experiments were rather weak, and would require further experiments, even though it is true that the 4 smallest size classes did indeed increase in the contaminated channels, and the largest sizes decreased.

Several life-forms, such as pedunculate diatoms, were also expected to be affected by pesticide contamination. Such life-form diatoms increase in abundance when nutrient and organic matter concentrations fall in river water (Berthon et al., 2011), and genera that have this life-form are classified as good ecological indicators by the Generic Diatom Index (Rumeau and Coste, 1988). This has also been confirmed in experiments in which biofilms from nutrient-rich water were transferred into unpolluted rivers: the abundance of motile diatoms decreased to the benefit of pedunculate diatoms (Rimet et al., 2009b). According to Pringle (1990), pedunculate diatoms are better at exploiting nutrients dissolved in water than motile diatoms, which tend to utilize nutrients adsorbed on the substratum. We would therefore expect these diatoms would be strongly affected by pesticides dissolved in the water, but this was in fact the case in only one experiment. Once again, further experiments are required to confirm this single significant trend.

Pioneer diatoms were also expected to be good indicators of pesticide contamination. *Achnanthidium minutissimum* is a colonizer that quickly occupies any free space available (Sabater, 2000). For instance it was observed during translocation experiments (Rimet et al., 2005) that when blocks were transferred from a polluted site to a reference site, pollution-resistant taxa disappeared, liberating free space on the substrate where this pioneering colonizer soon developed. Stevenson and Bahls (2002) think that this pioneer diatom is able to resist to severe “chemical insults”. It is frequently observed downstream from mining activities discharging high concentrations of heavy metals, such as Cu (Van Dam and Mertens, 1990; Sabater et al., 2002; Guasch et al., 2004), Cd and Zn (Gold et al., 2003a,b,c; Morin et al., 2007), Pb (Ferreira da Silva et al., 2009; Salonen et al., 2006) into

the water. We observed only one significant trend in our lotic mesocosms, which was consistent with the cited works (i.e. these organisms increased when the water was contaminated), but this needs to be confirmed by further experiments.

In contrast, several metrics showed more promising results. The mobile life-forms and the motile guild are closely-related metrics and showed similar trends. Mobile life-forms encompass all diatoms with a raphe structure that enables them to move, and the motile guild is a selection of fast-moving mobile diatoms, such as *Navicula* sensu lato or *Nitzschia* sensu lato (Passy, 2007). These metrics increase when nutrient and organic matter concentrations increase (Berthon et al., 2011), and the genera composing them are usually classified as pollution tolerant in the Generic Diatom Index (Rumeau and Coste, 1988). Both metrics showed an increasing abundance in the pesticide-contaminated mesocosms, which is consistent with the findings of several ecotoxicological studies. For instance Guasch et al. (1998b) observed that taxa resistant to the herbicide atrazine were also considered to be tolerant to organic pollution, and she cited taxa such as *Navicula menisculus, N. lanceolata, Nitzschia palea*. Similarly in isoproturon- and atrazine-polluted rivers, some pollution-tolerant diatom species, such as *Nitzschia palea*, tend to occur (Dorigo et al., 2004). Morin et al. (2009b) observed an increase of pollution-tolerant taxa in the sense of Lange-Bertalot (1979) on artificial substrates laid in diuron-contaminated rivers. In two of our experiments, these two metrics (motile guild and mobile life-form) also increased with pesticide contamination.

Diatoms living in mucous tubules showed promising results, since their abundance increased in pesticide-contaminated mesocosms. Genera presenting this life-form (*Encyonema, Frustulia*) are generally considered to be pollution sensitive (Rumeau and Coste, 1988). A possible explanation for their resistance here could be that the presence of the tubule shields the cells living inside it from dissolved pesticides. And finally the protective effect of exopolysaccharide (production of thick matrices or mucous tubules) against dissolved biocides in water, which is well documented in bacteriology (e.g. Onbasli and Aslim, 2009), could also be confirmed for diatoms.

The low-profile guild encompasses taxa such as *Achnanthes* sensu lato*, Cocconeis*, *Encyonema*. This guild tends to decrease as nutrient and organic loads increase (Berthon et al., 2011) and its members are classified as good water quality indicators in the Generic Diatom Index (Rumeau and Coste, 1988). They are resistant to turbulence, and are able to use nutrients dissolved in water (Passy, 2007; Pringle, 1990). This could be expected to make them more sensitive to dissolved pesticides; nevertheless their abundance increased in our contaminated mesocosms.

Diatoms of the high-profile guild showed the opposite trend to the motile guild and mucous tubule life-forms, since they showed decreasing abundance in the pesticide-contaminated mesocosms. According to Passy (2007), the taxa composing this guild are not adapted to resist water turbulence or grazing pressure, but have good ability to use dissolved nutrients in water. This is likely to mean that they are more exposed to dissolved pesticides than the motile diatoms, which could explain their reduced abundance in pesticide-contaminated mesocosms.

- **Conclusions**

Using diatom metrics is a way to transform noisy information at the species level into a clearer data set with more robust trends and more easily testable hypotheses. Mesocosm systems offer a way to approach natural complexity by controlling many factors (e.g. turbulence, light, nutrients), and mesocosm experiments analyzed by means of diatom metrics offer possible new tools for assessing pesticide contamination at the low concentrations found *in situ* (around 1.5 $\mu g.l^{-1}$ for chronic pollution, and 20 $\mu g.l^{-1}$ for short-term acute pollution). Several previous studies conducted in mesocosms, microcosms and in natural rivers, by other authors using the classical taxonomical composition (e.g. Guasch et al., 1998b; Morin et al., 2009b; Peres et al., 1996; Dorigo et al., 2004) have given similar results, namely that taxa selected by pesticide pollution tend to belong to the motile guild, but it seemed to be important to confirm these findings using different methods. The first such method is to continue developing mesocosm systems, like the ones presented here, in order to be able to control a maximum of environmental parameters. The idea would be to increase the number of repetitions by increasing the number of experiments in order to confirm (or invalidate) the findings. The second approach would be to work on large datasets supplied by the routine river monitoring networks of the water agencies. These datasets, including diatom listings, classical chemistry, river typology and micropollutant (pesticide) concentrations should allow *in-situ* testing of our findings observed in lotic mesocoms. The third approach would be to monitor pilot river basins in agricultural zones with known pesticide inputs, and in which plans for pesticide reduction are being actively implemented. Diatom monitoring of the rivers analyzed by means of these metrics should provide a way to validate these tools for pesticide assessment.

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5. Conclusions and perspectives

5. Conclusions and perspectives

This thesis had three major objectives; all of them are directly linked to the use of diatoms for bioassessment and how their current use for assessing nutrients and organic matter in an ecoregional framework, and their relatively recent use for assessing the impact of pesticides on diatom assemblages can be improved. The first topic dealt with ecoregions and how they can explain the diversity of diatom assemblages. The second topic looked at questions about the metrics currently used in diatom indices for bio-assessment, and whether better alternatives can be found in terms of taxonomic resolution and the type of metric. The third topic concerned the impact of pesticides on diatom assemblages and involved mesocosm experiments lasting for about 2 months.

From our results and from a detailed analysis of publications in the field of diatoms and river pollution over the last 10 years, we forecast some likely future aspects of diatom bioassessment.

a. Main conclusions

- **Diatoms and ecoregions**

We first assessed the importance of ecoregions in explaining the diversity of diatom assemblages. These microalgae are known to have high dispersal abilities; there are examples of exotic and recent invading species that have rapidly spread over hundreds of kilometers in just a few years (e.g. *Achnanthidium druartii* in Rimet et al., 2010). We observed that their assemblages display strong spatial features, which are closely related to the geology of the river basin. Ecoregional classifications, which are generally based on large-scale factors such as geology, altitude, climate, provide a good explanation of this spatial aspect. The statement of Beijerinck (1913) “Everything is everywhere: but the environment selects” seems to apply in the case of diatoms: ecoregions with similar geological, altitudinal and climatic conditions presented equivalent diatom assemblages even if they were spatially separated from each other. This was the case, for instance, for the two mountainous regions in North-eastern France, the Vosges and the Ardennes, which are in two different ecoregions, but present similar geologies, climates, and altitudes, and therefore similar diatom species compositions. The same was observed for regions with similar geologies and altitudes in the study comparing diatom assemblages in Spain, France, Switzerland and Italy: the same diatom assemblages were found in sites separated by hundreds or thousands kilometers. No taxonomical particularities were restricted to any given area if similar typological features and similar kind of pollutions were also present elsewhere. As stated by Finlay et al. (2002), the concept of diatom endemism should be discarded. Nevertheless, there are some nuances. Studies based on molecular data can show that inside species complexes such as *Nitzschia palea* (Trobajo et al., 2009) or *Gomphonema parvulum* (Kermarrec, 2012) some clades are restricted to particular geographical zones, and also that clades of the same species complexes can be sympatric. For populations of the marine species *Pseudo-nitzschia pungens* a correlation has been observed between geographical and phylogenetic distances, implying that geographical distance can limit dispersal (Casteleyn et al.,

2010). Nevertheless, there are some obvious shortcomings in the data: morphology studies are probably not precise enough to reveal details, and the molecular studies probably did not record enough data to demonstrate that some clades of diatoms really are restricted to particular areas. Moreover, at large spatial scales, historical processes are decisive in explaining diatom biogeography (Vyverman et al., 2007): for instance diatom floras from isolated sub-Antarctic zones include many endemic species (Van De Vijver & Beyens, 1999).

However, we did not observe any taxonomical features restricted to particular geographical zones at the scale of our studies and using our methodology of identifications and counts derived from routine assessments (i.e. light microscopy). We therefore conclude that ecoregion classification for diatom bioassessment can be improved by merging ecoregions that share the same geology, climate and altitude, even if they are geographically separated.

We then looked for a threshold level from which pollution (organic matter and nutrient concentrations) outweighed ecoregions in accounting for the variability of diatom assemblages. Several authors (Leira & Sabater, 2005; Tornes et al., 2007; Pan et al., 2000) have observed that pollution tends to homogenize diatom assemblage composition in different ecoregions, however we did not observe any such homogenization as pollution increased. The first dichotomy found in diatom assemblages in the North-eastern part of France - a highly industrialized area with many severely polluted rivers - was explained by geology and not by the level of river pollution. Diatom communities in rivers characterized by equivalent levels of nutrients and organic matter had differing species compositions. If pollution had homogenized the diatom communities, a dichotomy would have been found instead between diatom communities in highly polluted rivers and those in less polluted rivers. Results similar to ours have also been observed in other study areas, such as Finland (Soininen, 2004) and Luxembourg (Rimet et al., 2004). These results also demonstrate the interest of using an ecoregional framework for diatom bioassessment (e.g. Grenier et al., 2006; Lavoie et al., 2006).

- **Taxonomic resolution and alternative metrics in diatom bioassessment**

Diatom bioassessment in European rivers is primarily carried out using diatom indices. These indices are based on species identification, and the metrics used to calculate the biotic indices are species sensitivities to nutrients (e.g. Kelly & Whitton, 1995; Rott et al., 1998) or global pollution levels including nutrients and organic matter (e.g. Coste et al., 2009), and in some cases conductivity (e.g. EPI-D in Dell'Uomo, 2004 and Torrisi et al., 2010). After using these indices for more than a decade, particularly in the context of the Water Framework Directive, it is now time to step back and think how they could be improved. In this second part, we explored alternative ways of assessing river quality other than the existing tools based on classical species pollution-sensitivity determinations (Ector & Rimet, 2005).

First we tested the assessment performances of diatom bioassessment for nutrients, organic matter and major-ion concentrations when identifications were carried out at coarse or fine levels of taxonomic resolution. These tests were applied to determinations carried out from sub-division to species levels. We observed that assessment performances increased when taxonomic resolution

was finer, but that this increase did not parallel the exponential increase in the number of taxa to be identified: for instance, the number of taxa increased tenfold from genus to species level, whereas performance increased by only 12 to 23% depending on the parameter. The gain in assessment performance does not therefore match the increased number of taxa to be determined, which means that fine resolution does not in fact provide more robust bioassessment. This is something that had already been observed for macro-invertebrates (Jones, 2008).

Second, we tested alternative metrics to diatom taxa composition for assessing river pollution. Using diatom life-forms and ecological guilds is another way to reduce taxonomic resolution drastically. We compared the performance of such metrics to that of existing diatom indices based on species identifications and found that their assessment performances were comparable. The performance of stalked and tube-forming diatom abundances was comparable to that of the Biological Diatom Index (Coste et al., 2009) for assessing the organic matter concentration. Stalked diatom abundances actually gave even better results than the Biological Diatom Index for nutrient concentrations. Ecological guilds also displayed interesting results in terms of assessment power. It was concluded that beyond this assessment power, these metrics also provide valuable information about the architecture and structure of biofilms. Moreover, they can offer a good alternative to species determinations when little is known about the local diatom flora, as in French overseas departments (tropical islands), which are also subject to the Water Framework Directive.

- **Diatoms and pesticide contamination**

The impact of pesticides, and in particular herbicides, on aquatic ecosystems is of increasing concern to western societies. Politicians are investing in actions intended to reduce their impact on the aquatic biota (e.g. reduction of pesticide use in agriculture). Bioassessment methods are needed to assess the benefit of these actions, but so far the existing tools for assessing global pollution (especially organic matter and nutrient concentrations) and not pesticide contamination. Bioassessment tools suitable for this specific type of contamination need to be developed. Given their photosynthetic activity, diatoms should be good candidates for providing early warning of herbicide contamination.

Our last objective was to test the impact of pesticides (herbicides and fungicides) on diatom assemblages. We chose to carry out experiments in outdoor lotic mesocosms in order to control several parameters known to have an important structuring impact on diatoms: current velocity, nutrients and light. The experiments lasted about 2 months and were seeded using algal communities from Lake Geneva.

We observed that the impact of pesticide contamination was greater when diatom data transformed into abundance metrics were considered instead of simply using species abundances. Transforming diatom species abundances into abundance metrics increased the robustness of trends, improved the comparability of experiments and so facilitated generalization. The reason for this was that species composition of diatom communities in the mesocosms differed from one experiment to another because of seasonal and interannual changes in diatom species in the lake. Another reason

was that these experiments were conducted from 2005 to 2008, and different investigators identified some species complexes differently (e.g. *Fragilaria capucina* species complex).

Increased pesticide contamination reduced the high-profile guild. In contrast, the relative abundance of the motile guild and diatoms living in mucous tubules was higher in the contaminated mesocosms. One possible explanation could be that diatoms living in mucous tubules and motile guild diatoms are both surrounded by thick exopolysaccharide matrices, which could shield the diatoms from pesticides. Several authors have reported results that could validate this hypothesis: *Nitzschia* and *Navicula* genera - which belong to the motile guild - were observed to be more resistant to atrazine (Guasch et al., 1998; Dorigo et al., 2004) and EC50 tests have shown that thick biofilms are less sensitive to atrazine (Guasch et al., 2003). The high-profile guild has the opposite strategy, with diatoms exposing a large surface area to direct contact with dissolved pesticides.

Such findings have been validated in ongoing projects using various complexity scales. First, simple diatom cultures for EC50 determinations carried out on a wide range of species gave consistent results (ongoing thesis of F. Larras). Second, the validation of these hypotheses on a large spatial scale also showed that only the high profile guild decreased significantly when atrazine contamination increased (Bouchez et al., 2012). These studies make it possible to offer water-managers diatom metrics that could be good candidates for assessing herbicide contamination.

b. Future prospects

Ecology and environmental pressure are often considered to be key factors in speciation (Wiens, 2004). Many ecologists now think that phylogenetically close species are also ecologically similar. It has been demonstrated that there is a strong correlation between these two aspects in many higher plants and animals (see, for instance, the review of Cavender-Bares et al., 2009). These examples support the hypothesis of phylogenetic niche conservation. This hypothesis postulates that ecological traits are maintained over time among phylogenetically-related species (Wiens et al., 2010).

Several diatoms taxa show that environmental factors can indeed explain speciation or phenotype divergence. For instance, when US lake sediment records were investigated, it was found that some minor environmental changes explained speciation within the *Stephanodiscus niagarae* species complex (Theriot et al., 2006). Other examples can be given of other species complexes which encompass morphologically close taxa with similar ecological preferences: this is the case, for instance, of *Cyclotella comensis* and *C. pseudocomensis,* which both live in the plankton of alpine lakes characterized by carbonate substrates and homogeneous trophic levels (Houk et al., 2010). Similarly, most of the taxa belonging to the *Cymbella excisa* species complex (*C. excisa, C. excisiformis, C. exigua, C. parva*) display the similar ecologies, since they live in oligotrophic water with calcareous geology (Krammer, 2002). Stronger environmental pressures obviously lead to greater divergence. This is shown in the study of Kociolek & Ruck (2004), who related phylogeny and ecology in the Surirellaceae family. They showed that some of the genera grouped in a monophyletic clade were entirely marine; but that others, also grouped in a single monophyletic clade, all lived in freshwater. So in this case ionic content must determine the radical divergence between these clades. These few examples seem to confirm phylogenetic niche conservation. On the other hand,

Vanelslander et al. (2009) found little difference in terms of salt requirements between *Navicula phyllepta* clades living in sympatry, which would seem to imply the opposite phenomenon: i.e. that species displaying similar ecological preferences cannot co-exist if their resource requirements are also too similar, and this therefore leads to sympatric speciation.

Nevertheless, the hypothesis of niche conservation is too rarely addressed with precision even if it would have obvious relevance to conservation biology (Wiens et al., 2010) and also to diatom bioassessment. Indeed, the quality assessment of rivers is based on the establishment of ecological profiles of taxa (e.g., diatoms, macroinvertebrates) in relation to pollution gradients. A good definition of the taxa and taxonomic level to use is crucial to achieve ecological profiles of the taxa and assessment of the environment that are robust, but also ensure that the cost of an analysis is kept as low as possible while preserving good accuracy of the bioindicator, as we demonstrated in the second part of this thesis. Therefore finding out whether there is a phylogenetic signal in the sensitivity to pollution (versus organic matter, nutrients and herbicides) of the most abundant diatoms in European rivers would be crucial for diatom bioassessment.

According to Wiens et al. (2010) the hypothesis of niche conservation has a broader definition than the conservation of the phylogenetic niche, i.e. niche conservation can occur between species, a level at which phylogeny may be irrelevant. In some cases, the environment may select species with similar traits but which may be phylogenetically distant. This is the case when studying diatom life-forms. Some of them are characteristic of genera or of higher taxonomical levels, and often reflect a shared evolutionary history (Julius & Theriot, 2010): “Barriers encountered by taxa requiring adaptations for growth may, in some instances, have provided an advantage to species allowing them to radiate and colonize many similar habitats.” But in other cases “chemical barriers may have represented a greater challenge, and adaptation to nutrient regimes may have provided selective advantage, allowing variations in growth forms to evolve multiple times.” Several cases of morphological convergence can be given, such as pedunculate or monoraphid diatoms (e.g. Bruder & Medlin, 2008; Theriot et al., 2011; Kingston, 2003), which are apparently polyphyletic groups. We demonstrated the interest of using life-forms and ecological guilds to assess the impact of the levels of pesticides, nutrients and organic matter in parts 2 and 3 of the thesis. A more systematic study of their distribution in the phylogenetic trees of the dominant taxa in European rivers is also called for. This would make it possible to achieve a better understanding of the major environmental pressures that have forced diatoms to adapt. If these environmental pressures can be related to anthropogenic pressures, biomonitoring tools could take advantage of these results.

Finally, we demonstrate the importance of ecoregions for explaining the species composition of diatom assemblages. Ecoregions have direct implications for water quality assessment. Here too it is important to adopt a phylogenic perspective. Do differences in terms of ecoregions imply divergences of diatoms at high or low phylogenetic levels? My hypothesis, based on the results of the first and second parts of the thesis, is that ecoregions have only made diatoms diverge recently and therefore can be expected to have had little taxonomical impact.

In a recent bibliographical review about diatoms and river pollution (Rimet, 2012), I included all the papers published in peer-reviewed journals over the past 10 years (1999-2009). A total of 226 papers were selected. Despite the common use of the word "species" (which was the third most commonly-used word in the abstracts of all the papers), taxonomy was very rarely addressed (the word taxonomy appeared in 52nd position). After the claim of Kociolek & Stoermer (2001) that "ecological studies use taxonomy as a means to an end", "taxonomy appears to offer difficult hurdles for ecologists" because of its incessant modifications. The same can be said of phylogeny: papers about diatom bioassessment did not once address phylogenetic concepts. Diatom biomonitoring research is surely lagging behind macroinvertebrate research, where these disciplines have overlapped for a couple of years. These studies (e.g. Carew et al., 2011; Buchwalter et al., 2008) make it possible to define clades composed of species, or genera or mixed taxonomic levels that can be appropriately used as indicators of particular environmental stressors. "Evolutionary considerations can therefore help to guide the development of macro-invertebrate biomonitors and provide insights into processes that produce sensitive and tolerant taxa" (Carew et al., 2011). Diatom bioassessment should take advantage of the more systematic merging of phylogenetic concepts and ecology.

Current methods use optical devices and morphological characteristics to identify diatoms. However, although existing methodologies are fairly robust and standardized, it takes staff several years' experience to develop the necessary skills, and these methods are expensive in terms of manpower. This limits the frequency and intensity of sampling, whilst demand for analyses is steadily increasing. Metagenomics can help in assessing biodiversity in samples. DNA barcoding uses short genetic markers to identify species (Hebert et al., 2003). Metagenomics and DNA barcoding will produce a radical innovation of biomonitoring. Next-generation sequencing revolutionizes these fields, because it enables to sequence large numbers of organisms at low cost. The first tests carried out with diatom samples are promising (e.g. Kermarrec, 2012). Such innovations will have a decisive effect on the technological aspects of diatom biomonitoring, and will probably revolutionize our concepts about diatom ecology.

c. References

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Appendix 1

Appendix 1: List of the sampled sites (high altitude rivers)

Country	River	Station	Sampling date	X-WGS 1960 decimal	Y-WGS 1960 decimal
France	Alagnon	La Veissière	9 August 1999	2.77444	45.10389
France	Alagnon	La Veissière	31 July 2000	2.77444	45.10389
France	Alignon	station ALI 4	8 August 2001	3.82389	44.43139
France	Allier	Chasserades	12 August 1998	3.84000	44.59222
France	Allier	Chasserades	7 August 1999	3.84000	44.59222
France	Allier	Chasserades	29 July 2000	3.84000	44.59222
France	Allier	Langogne	5 September 1996	3.85167	44.75472
France	Allier	Langogne	29 August 1997	3.85167	44.75472
France	Allier	Langogne	12 August 1998	3.85167	44.75472
France	Allier	Langogne	6 August 1999	3.85167	44.75472
France	Allier	Langogne	28 July 2000	3.85167	44.75472
France	Arc	Amont Bonneval	12 September 1997	7.09167	45.38722
France	Arc	Lanslebourg-Mont-Cenis	27 August 2001	6.87556	45.29278
France	Arve	Les Houches	11 September 1997	6.76778	45.90694
France	Averole	Bessans	27 August 2001	7.05806	45.30611
France	Bes	Javie (Esclangon-Peroure)	21 August 2001	6.26722	44.21139
France	Blanche	Seyne les Alpes	21 August 2001	6.32806	44.35333
France	Bléone	Amont Prads	19 September 1997	6.47000	44.23833
France	Ceyssat	Ceyssat	11 August 1999	2.89083	45.75889
France	Ceyssat	Ceyssat	3 August 2000	2.89083	45.75889
France	Challandre	Beuil	2 March 1998	6.96333	44.04944
France	Challandre	Beuil	23 April 1998	6.96333	44.04944
France	Challandre	Beuil	24 August 1998	6.96333	44.04944
France	Chastillon	Isola	4 March 1998	7.13222	44.20472
France	Chastillon	Isola	19 August 1998	7.08500	44.21833
France	Cians	Beuil	2 March 1998	6.98361	44.08333
France	Cians	Beuil	22 April 1998	6.98361	44.08333
France	Clarée	Névache	26 August 2001	6.60528	45.02917
France	Clarée	Val des Prés	2 August 2001	6.67278	44.94806
France	Colagne	Rieutort de Randon	8 August 2001	3.50194	44.65667
France	Diosaz	Servoz	29 August 2001	6.75389	45.94139
France	Diosaz	Servoz	30 August 2001	6.75389	45.94139
France	Dordogne	Mont Dore	31 July 2001	2.80361	45.56972
France	Doron de Termignon	Termignon - Pont du Châtelard	27 August 2001	6.80778	45.30611
France	Doubs	Arçon - Pont N 437	10 September 1997	6.37556	46.94833
France	Doubs	Cluse et Mijoux	5 September 2000	6.35528	46.89278
France	Dourbie	Dourbie	23 July 2001	3.48583	44.09306
France	Drac blanc	Champoleon	22 August 2001	6.24667	44.73167
France	Drac Blanc	Gondouins	19 September 1997	6.24694	44.73194
France	Drac noir	Lagrand	22 August 2001	6.32139	44.68389
France	Durance	Embrun	1 August 2001	6.50389	44.55611
France	Durance	Saint-Martin-de-Queyrières	2 August 2001	6.59167	44.87361
France	Eyrieux	Amont de Saint Agrève	15 September 1997	4.37583	45.04556
France	Giffre	Sixt - Nambride	29 August 2001	6.80806	46.07639
France	Goudeche	Station Diren (GOU3bis)	8 August 2001	3.82389	44.43139
France	Grand Buech	Aspres sur Buech	23 August 2001	5.76722	44.55611
France	Gresse	Saint-Guillaume (Pont Jacquet)	23 August 2001	5.56472	44.93444
France	Guiers Mort	Saint Pierre de Chartreuse - Perquelin	28 August 2001	5.84167	45.33333
France	Guiers Vif	Saint Même	28 August 2001	5.88889	45.40750
France	Guil	Mont-Dauphin	1 August 2001	6.61194	44.83778

France	Guil	Ristolas	2 August 2001	6.97694	44.77917
France	Guisane	Monetier-les-Bains (Le Casset)	26 August 2001	6.48361	44.98833
France	Isère	Seez	11 September 1997	6.81472	45.62389
France	Isère	Val d'Isère	27 August 2001	6.99722	45.45472
France	Issole	Saint-André-les-Alpes (Mourefrey)	21 August 2001	6.49694	43.99528
France	La Vaire	Annot (Pont des Scaffarels)	21 August 2001	6.67944	43.95472
France	Lane	Andon	7 June 2000	6.79444	43.79944
France	Lane	Andon	7 June 2000	6.78611	43.79944
France	Lane	Valderoure	17 April 1996	6.70667	43.79250
France	Lane	Valderoure	20 August 1996	6.70667	43.79250
France	Lane	Valderoure	23 October 1996	6.70667	43.79250
France	Le Lez	Sentein	24 July 2001	0.92556	42.85306
France	Loire	Sainte-Eulalie	28 August 1997	4.18639	44.84167
France	Loire	Sainte-Eulalie	13 August 1998	4.18639	44.84167
France	Loire	Sainte-Eulalie	6 August 1999	4.18639	44.84167
France	Loire	Sainte-Eulalie	28 July 2000	4.18639	44.84167
France	Lot	Chadenet	8 August 2001	3.69528	44.51167
France	Lot	Chadenet	28 July 1994	3.61472	44.56000
France	Loup	Gréolières	7 June 2000	6.87556	43.78556
France	Luye	Gap	22 August 2001	6.09833	44.56972
France	Oriege	700m amont usine EDF	24 July 2001	1.89167	42.69222
France	Oule	Montmorin	23 August 2001	5.57111	44.46139
France	Riou	Saint Etienne	4 March 1998	6.93639	44.21833
France	Romanche	Mizoen (Chambon amont)	26 August 2001	6.17944	45.04278
France	Roya	Tende	6 July 1998	7.61194	44.11028
France	Roya	Tende	15 September 1998	7.61194	44.11028
France	Sanguinière	Entraunes	26 March 2001	6.76750	44.25889
France	Sévéraisse	Villar-Loubière	22 August 2001	6.13889	44.82639
France	Tech	Prats de Mollo la Preste	17 September 1997	2.39083	42.41833
France	Torrent de Parpaillon	Condamine-Châtelard (Sainte-Anne)	21 August 2001	6.69306	44.47500
France	Ubaye	Saint-Pons	1 August 2001	6.57806	44.39389
France	Valat de la Latte	station LATTE 1	8 August 2001	3.82389	44.43139
France	Valat de la Sapine	station SAPI	8 August 2001	3.82389	44.43139
France	Valat des Cloutasses	station CLOU 1	8 August 2001	3.82389	44.43139
France	Var	Entraunes	26 March 2001	6.70639	44.25889
France	Var	Entraunes	26 March 2001	6.73361	44.20472
France	Var	Source	20 September 1997	6.74694	44.23833
France	Vénéon	Vénosc	26 August 2001	6.14583	44.96833
France	Verdon	La Foux d'Allos	19 September 1997	6.56472	44.29944
France	Vésubie	Saint Martin	2 September 1998	7.26750	44.11694
France	Vésubie	Saint Martin	2 September 1998	7.24028	44.06972
France	Vienne	Saint-Setiers	30 August 1996	2.06111	45.70083
France	Vienne	Saint-Setiers	14 August 1998	2.06111	45.70083
France	Vienne	Saint-Setiers	11 September 1999	2.06111	45.70083
France	Vienne	Saint-Setiers	5 September 2000	2.06111	45.70083
Italy	Meledrio	Malghette	8 September 1995	10.81083	46.25917
Italy	Meledrio	Vagliana	9 September 1995	10.88111	46.08639
Italy	Amola	Amola GL	1 July 2000	10.70694	46.21250
Italy	Amola	Amola NGL1	1 July 2000	10.70639	46.21222
Italy	Arnò	M.ga Trivena	4 September 1995	10.61278	46.00056
Italy	Avisio	Campitello1	7 July 1998	11.74083	46.47194
Italy	Avisio	Campitello1	11 April 1999	11.74083	46.47194
Italy	Avisio	Campitello1	10 May 1999	11.74083	46.47194

Italy	Avisio	Campitello1	7 June 1999	11.74083	46.47194
Italy	Avisio	Campitello1	2 August 1998	11.74083	46.47194
Italy	Avisio	Campitello1	7 September 1998	11.74083	46.47194
Italy	Avisio	Campitello1	4 October 1998	11.74083	46.47194
Italy	Avisio	Campitello1	1 November 1998	11.74083	46.47194
Italy	Avisio	Campitello1	30 November 1998	11.74083	46.47194
Italy	Avisio	Campitello1	19 January 1999	11.74083	46.47194
Italy	Avisio	Campitello1	15 February 1999	11.74083	46.47194
Italy	Avisio	Campitello1	15 March 1999	11.74083	46.47194
Italy	Bedù di Pelugo	Val di Borzago	28 May 1996	10.66417	46.10611
Italy	Bedù S.Valentino	Coel di Vigo	28 May 1996	10.63583	46.08556
Italy	Bedù S.Valentino	Miniera	4 September 1995	10.64028	46.08639
Italy	Careser	CR1	2 August 2001	10.71389	46.43667
Italy	Careser	CR1bis	2 August 2001	10.70833	46.43306
Italy	Careser	CR2	2 August 2001	10.70750	46.43139
Italy	Careser	CR3	3 August 2001	10.68139	46.41611
Italy	Chiese	Conca Levade	5 September 1995	10.56500	46.12306
Italy	Chiese	Malga Val di Fumo	5 September 1995	10.55639	46.08278
Italy	Conca	C4	1 August 1997	10.60000	46.10000
Italy	Conca	C7	1 August 1997	10.60000	46.10000
Italy	Conca	C8	1 August 1997	10.60000	46.10000
Italy	Cornisello	V1	1 August 1997	10.68333	46.23333
Italy	Cornisello	V3	1 August 1997	10.68333	46.23333
Italy	Cornisello	V4	1 August 1997	10.68333	46.23333
Italy	Fersina	Palù	19 October 1999	11.36056	46.12861
Italy	Fersina	Palù	28 June 2000	11.36056	46.12861
Italy	Fersina	Palù	20 February 2001	11.36056	46.12861
Italy	Fersina	S. Orsola	19 October 1999	11.30583	46.10528
Italy	Fersina	S. Orsola	28 June 2000	11.30583	46.10528
Italy	Fersina	S. Orsola	20 February 2001	11.30583	46.10528
Italy	Larcher	NB1bis	1 August 2000	10.66000	46.44333
Italy	Larcher	NB2bis	1 August 2000	10.66417	46.43667
Italy	Larcher	NB3bis	1 August 2000	10.67611	46.42667
Italy	Niscli	N0	1 August 1997	10.60000	46.10000
Italy	Niscli	N2	1 August 1997	10.60000	46.10000
Italy	Noce bianco	NB1	1 August 2000	10.65333	46.43583
Italy	Noce bianco	NB2	1 August 2001	10.66667	46.43139
Italy	Noce bianco	NB3	1 August 2000	10.67611	46.42639
Italy	Noce bianco	NB4	1 August 2000	10.67833	46.42417
Italy	Noce bianco	NB5	3 August 2001	10.68056	46.41472
Italy	Rio Ambiez	Senaso	7 September 1995	10.87750	46.11833
Italy	Rio Ceda	M.ga Ceda Bassa	26 August 1993	10.93333	46.13778
Italy	Rio Ceda	Rio Ceda	28 August 1993	10.93778	46.13556
Italy	Rio d'Algone	Nambi	23 August 1993	10.81139	46.13528
Italy	Rio d'Algone	Sacco	23 August 1993	10.82250	46.14639
Italy	Rio d'Algone	Val Genera	23 August 1993	10.81250	46.18861
Italy	Rio S. Maria Flavona	Acqueforti di Pozzol	6 September 1995	10.91917	46.24361
Italy	Rio S. Maria Flavona	Malga Pozzol	6 September 1995	10.92722	46.23472
Italy	Rio Tresenga	Rislà Tovel	6 August 1995	10.94722	46.25750
Italy	Sarca di Brenta	Brenta Alta	3 August 1993	10.85889	46.18500
Italy	Sarca di Genova	Fontanabona	4 June 1996	10.67972	46.16583
Italy	Sarca di Genova	P.te Cambiali	31 August 1993	10.59444	46.19917
Italy	Sarca di Genova	Rifugio Bedole	4 June 1996	10.59583	46.19917

Italy	Sarca di Valagola	Valagola	1 June 1996	10.82944	46.18861
Italy	Sarca di Vallesinella	Sorgente Vallesinella	1 September 1993	10.87278	46.20944
Italy	Sarca Nambrone	Malga Nambrone	3 May 1996	10.75611	46.20833
Italy	Sarca Nambrone	Lago di Cornisello	3 May 1996	10.72583	46.21944
Italy	Torrente Massò	Busa dell'Acqua	29 August 1993	10.93000	46.18167
Italy	Torrente Sporeggio	Fontana Fredda	7 September 1995	10.96889	46.18667
Spain	Aguas Limpias	E. Sarra	25 July 2002	-0.33697	42.79072
Spain	Albentosa	Albentosa	10 August 2003	-0.75917	40.09861
Spain	Alfambra	Aguilar de Alfambra	8 August 2003	-0.76889	40.58139
Spain	Alfambra	Apelluz	8 August 2003	-0.72111	40.49306
Spain	Alfambra	Teruel	9 August 2003	-0.91417	40.39139
Spain	Alp	La Molina	June 1998	1.91253	42.36536
Spain	Alp	La Molina	March 1998	1.91253	42.36536
Spain	Alp	La Molina	September 1998	1.91253	42.36536
Spain	Alp	Malniu	June 1998	1.97783	42.35228
Spain	Alp	Malniu	March 1998	1.97783	42.35228
Spain	Alp	Malniu	September 1998	1.97783	42.35228
Spain	Ara	Broto	12 October 2002	-0.11250	42.59222
Spain	Aragón	Canfranc	17 October 2002	-0.50861	42.72056
Spain	Aragón	Castiello	25 July 2002	-0.54675	42.63325
Spain	Aragón Subordán	Hecho (Selva de Oza)	August 2003	-0.69556	42.85056
Spain	Arazas	Torla (desembocadura)	12 October 2002	-0.09722	42.65444
Spain	Arazas	Torla (pradera Ordesa)	12 October 2002	-0.04611	42.64972
Spain	Aurin	Isín	25 July 2002	-0.40133	42.59936
Spain	Aurín	Isín	August 2003	-0.39889	42.60028
Spain	Bco Cadajon	San Millan de la Cogolla	8 August 2002	-2.87900	42.29625
Spain	Bco Santa Anna	Sort	1 August 2002	1.13897	42.41089
Spain	Bellós	Puértolas (Fuen Blanca	August 2003	0.05972	42.63667
Spain	Cabriel	El Vallecillo	9 August 2003	-0.45194	40.21778
Spain	Camarena	Camarena de la Sierra	9 August 2003	-0.95472	40.16000
Spain	Cinca	Barrosa	October 2002	0.20558	42.69214
Spain	Cinca	Bielsa	August 2003	0.23583	42.61056
Spain	Cinca	Salinas	October 2002	0.22642	42.58594
Spain	Cinqueta	Gistaín	August 2003	0.34556	42.61500
Spain	Cinqueta	Saravillo	August 2003	0.27056	42.56167
Spain	Duran	Bellver	June 1998	1.80708	42.37322
Spain	Duran	Bellver	March 1998	1.80708	42.37322
Spain	Duran	Campllong	June 1998	1.76189	42.46108
Spain	Duran	Campllong	March 1998	1.76189	42.46108
Spain	Duran	Campllong	September 1998	1.76189	42.46108
Spain	Err	Llivia	1 August 2002	2.00028	42.45656
Spain	Esera	Benasque	October 2002	0.61347	42.68517
Spain	Esera	L'Ospital Benasque	August 2003	0.60611	42.68222
Spain	Estarrón	Aisa	13 October 2002	-0.61250	42.68778
Spain	Gállego	Biescas 1	12 October 2002	-0.32208	42.62828
Spain	Gállego	Biescas 2	August 2003	-0.30917	42.63139
Spain	Gallo	Orihuela del Tremedal	9 August 2003	-0.34694	40.54889
Spain	Garona	Valh D'Arau	31 July 2002	0.79500	42.73961
Spain	Gas	Leres	13 October 2002	-0.46444	42.56639
Spain	Guadalaviar	Cella	9 August 2003	-0.68056	40.40333
Spain	Guadalaviar	Torres de Albarracín	9 August 2003	-0.46472	40.42389
Spain	Guadalaviar	Villar del Cobo	9 August 2003	-0.32889	40.39194
Spain	Guatizalema	Nozito	20 October 2002	-0.25933	42.30669

Spain	Hijar	Reinosa-Espinilla	11 August 2002	-4.20644	43.01908
Spain	Huerva	Lagueruela	24 July 2003	-0.80917	41.04083
Spain	Isábena	Obarra	August 2003	0.59778	42.39417
Spain	Isuela	Calcena	October 2002	-1.68503	41.65589
Spain	Jiloca	Calamocha	31 July 2002	-1.30533	40.88328
Spain	Jiloca	Calamocha	24 July 2003	-0.68472	40.97083
Spain	Jiloca	Luco	31 July 2002	-1.29161	40.98950
Spain	Jiloca	Torrijos del campo	August 2003	-0.66639	40.82722
Spain	Linares	Castelvispal	10 August 2003	-0.48639	40.27639
Spain	Mijares	Sarrión	10 August 2003	-0.76528	40.16028
Spain	Mijares	Valbona	10 August 2003	-0.82500	40.22944
Spain	Oja	Ezcaray	8 August 2002	-3.00847	42.30522
Spain	Oropesa	Pradoluengo	8 August 2002	-3.15461	42.30556
Spain	Osía	Jasa	13 October 2002	-0.66417	42.69167
Spain	Pancrudo	Navarrete del Río	24 July 2003	-0.78306	40.91111
Spain	Querol	La Vinyola	June 1998	1.90978	42.45314
Spain	Querol	La Vinyola	March 1998	1.90978	42.45314
Spain	Querol	La Vinyola	September 1998	1.90978	42.45314
Spain	Querol	Talltorta	June 1998	1.89856	42.40036
Spain	Querol	Talltorta	March 1998	1.89856	42.40036
Spain	Querol	Talltorta	September 1998	1.89856	42.40036
Spain	Ribera	Baliera	August 2003	0.64667	42.49250
Spain	Segre	Isovol	June 1998	1.81667	42.37350
Spain	Segre	Isovol	March 1998	1.81667	42.37350
Spain	Segre	Isovol	September 1998	1.81667	42.37350
Spain	Segre	La Granota	June 1998	1.93817	42.42864
Spain	Segre	La Granota	March 1998	1.93817	42.42864
Spain	Segre	La Granota	September 1998	1.93817	42.42864
Spain	Turia	Teruel	9 August 2003	-0.87722	40.34472
Spain	Turia	Teruel (Villaspesa)	9 August 2003	-0.87083	40.30083
Spain	Veral	Ansó	14 October 2002	-0.80278	42.75667
Spain	Veral	Zuriza	October 2002	-0.81950	42.86306
Switzerland	Drance de Bagnes	aval Châble	9 November 1998	7.16667	46.08333
Switzerland	Drance de Bagnes	aval Châble	17 March 1999	7.16667	46.08333
Switzerland	Drance de Bagnes	aval Châble	18 May 1999	7.16667	46.08333
Switzerland	Drance de Bagnes	aval Châble	16 August 1999	7.16667	46.08333
Switzerland	Drance de Bagnes	Bonatchiesse	9 November 1998	7.33333	46.00000
Switzerland	Drance de Bagnes	Bonatchiesse	18 May 1999	7.33333	46.00000
Switzerland	Drance de Bagnes	Bonatchiesse	16 August 1999	7.33333	46.00000
Switzerland	Drance de Bagnes	Champsec	9 November 1998	7.18333	46.06667
Switzerland	Drance de Bagnes	Champsec	17 March 1999	7.18333	46.06667
Switzerland	Drance de Bagnes	Champsec	18 May 1999	7.18333	46.06667
Switzerland	Drance de Bagnes	Champsec	16 August 1999	7.18333	46.06667
Switzerland	Drance de Bagnes	Plamproz	9 November 1998	7.28333	46.03333
Switzerland	Drance de Bagnes	Plamproz	18 May 1999	7.28333	46.03333
Switzerland	La Morge	Malona	21 November 2000	7.30000	46.31667
Switzerland	La Morge	Malona	23 March 2000	7.30000	46.31667
Switzerland	La Vièze	Amont Morgins	9 October 2001	6.85000	46.21667
Switzerland	La Vièze	Amont Morgins	14 February 2001	6.85000	46.21667
Switzerland	La Vièze	Champéry	9 October 2001	6.86667	46.18333
Switzerland	La Vièze	Grand Paradis	9 October 2001	6.81667	46.15000
Switzerland	La Vièze	Grand Paradis	14 February 2001	6.81667	46.15000

Appendix 2

Appendix 2: Average diatom assemblages of the north-eastern french rivers. For each taxon, the average abundance is given (%). The Indicator Species Analysis index is given (Indval), this index indicates that the corresponding taxon is indicator of the group considered; it is significant if p<0,05 (Monte-Carlo test) . The Omndia code has been added because of its wide use among diatom-technicians in Europe.

		Diatom assemblages of limestone regions											
		16 □ Very good water quality, small streams			17 □ Relatively good quality, large rivers			18 □ Polluted rivers of various sizes			19 □ Small to medium sized polluted rivers		
	Group	16	16	16	17	17	17	18	18	18	19	19	19
Omnidia Code	Taxon name	%	indval	p-value	%	indval	p-value	%	indval	p-value	%	indval	p-value
AAMB	Aulacoseira ambigua (Grunow) Simonsen	0.00			1.57	15.8	0.07	1.05			0.01		
AATO	Achnanthes atomus Hustedt	0.00			0.14			0.00			0.21	1.9	0.56
ACHS	Achnanthes sp.	0.00			0.00			0.01			0.01		
ACON	Achnanthes conspicua A.Mayer	0.10			0.27			0.77			0.55		
ACOP	Amphora copulata(Kutzing) Schoeman & Archibald	0.06			0.66			0.82			0.36		
ADBI	Achnanthidium biasolettianum (Grunow) Lange-Bertalot	30.60	82.1	0	0.86			0.00			0.11		
ADCT	Achnanthidium catenatum (Bily & Marvan) Lange-Bertalot	0.00			0.01			0.00			0.01		
ADEU	Achnanthidium eutrophilum (Lange-Bertalot) Lange-Bertalot	0.00			1.99	12.6	0.12	0.03			0.72		
ADKR	Achnanthidium kranzii (Lange-Bertalot) Round & Bukhtiyarova	0.00			0.00			0.00			0.00		
ADLS	Adlafia suchlandtii (Hustedt) Moser Lange-Bertalot & Metzeltin	0.07			0.00			0.00			0.00		
ADMF	Achnanthidium minutissimum (Kutzing) Czarnecki var. affinis (Grunow) Bukht.	0.00			0.04			0.05			0.07		
ADMI	Achnanthidium minutissimum (Kutzing) Czarnecki	40.80	23.1	0.03	16.59			1.20			5.96		
ADMM	Adlafia minuscula var. muralis (Grunow) Lange-Bertalot	0.07			0.00			0.00			0.00		
ADMS	Adlafia minuscula (Grunow) Lange-Bertalot	0.00			0.01			0.01			0.02		
ADSA	Achnanthidium saprophilum (Kobayasi et Mayama) Round & Bukhtiyarova	0.00			0.14			0.13			0.12		
ADSB	Achnanthidium straubianum (Lange-Bertalot)Lange-Bertalot	0.39			4.03	58	0	0.06			0.11		
ADSU	Achnanthidium subatomus (Hustedt) Lange-Bertalot	0.00			0.02			0.01			0.00		
AFOR	Asterionella formosa Hassall	0.00			0.30			0.04			0.00		
AFUG	Achnanthes fugei Carter	0.00			0.03			0.46			0.13		
AINA	Amphora inariensis Krammer	0.00			0.00			0.05	2.6	0.59	0.04		
AMII	Achnanthes minutissima Kutzing var. inconspicua Oestrup	1.91			0.00			0.00			0.00		
AMMO	Amphora montana Krasske	0.00			0.01			0.03			0.04		
AOVA	Amphora ovalis (Kutzing) Kutzing	0.00			0.26			0.34	13.1	0.1	0.13		
APED	Amphora pediculus (Kutzing) Grunow	7.69			31.11	20.4	0	18.59			26.12		
ASHU	Achnanthes subhudsonis Hustedt	0.00			0.00			0.00			0.00		
AUDI	Aulacoseira distans (Ehrenberg) Simonsen	0.00			0.13	2.3	0.61	0.07			0.01		
AUGR	Aulacoseira granulata (Ehrenberg) Simonsen	0.00			0.89	19.3	0.03	0.18			0.00		
AVEN	Amphora veneta Kutzing	0.00			0.02			0.02			0.01		
BPAX	Bacillaria paxillifera (O.F. Muller) Hendey var. paxillifera	0.00			0.00			0.11			0.00		
CAFF	*Cymbella excisa* Kutzing	0.00			1.54	53.6	0	0.01			0.07		
CAGR	Cyclotella atomus var. gracilis Genkal & Kiss	0.00			0.01			0.00			0.03		
CATO	Cyclotella atomus Hustedt	0.00			0.64			0.43			0.35		
CBAC	Caloneis bacillum (Grunow) Cleve	0.13			0.39			0.85	19.3	0.04	0.75		
CDUB	Cyclostephanos dubius (Fricke) Round	0.00			0.44	10.1	0.15	0.31			0.02		
CINV	Cyclostephanos invisitatus (Hohn & Hellerman) Theriot Stoermer & Hakansson	0.00			0.60			0.65			0.07		
CMED	Cyclotella meduanae Germain	0.00			0.01			0.02			0.00		
CMEN	Cyclotella meneghiniana Kutzing	0.00			0.70			3.27			0.81		
CMLF	Craticula molestiformis (Hustedt) Lange-Bertalot	0.00			0.02			0.02			0.04		
COCE	Cyclotella ocellata Pantocsek	0.00			0.04			0.13	7.9	0.11	0.00		
CPED	Cocconeis pediculus Ehrenberg	0.16			0.20			0.23			0.37		
CPLA	Cocconeis placentula Ehrenberg var. placentula	0.06			0.27			0.73			0.60		
CPLE	Cocconeis placentula Ehrenberg var. euglypta (Ehrenberg) Grunow	1.14			1.01			3.65			4.74		
CPLI	Cocconeis placentula Ehrenberg var. lineata (Ehrenberg) Van Heurck	1.20			0.13			0.15			0.29		
CPPL	Cocconeis placentula Ehrenberg var. pseudolineata Geitler	0.07			0.01			0.03			0.00		
CPST	Cyclotella pseudostelligera Hustedt	0.00			2.66			0.96			0.93		
CRAC	Craticula accomoda (Hustedt) Mann	0.00			0.00			0.02			0.02		
CSOL	*Cymatopleura solea* (Brebisson) W.Smith	0.00			0.04			0.09	9.9	0.11	0.02		
CSTE	*Cyclotella stelligera* Cleve et Grunow	0.00			0.02			0.03			0.01		
CTUM	Cymbella tumida (Brebisson) Van Heurck	0.00			0.01			0.02			0.01		
DCOT	Diadesmis contenta (Grunow) Mann	0.00			0.01			0.04			0.01		
DMAR	Diploneis marginestriata Hustedt	0.03			0.20			0.28	13.5	0.07	0.03		
DMES	Diatoma mesodon (Ehrenberg) Kutzing	0.00			0.00			0.00			0.01		
DOBL	Diploneis oblongella (Naegeli) Cleve-Euler	0.06			0.07			0.07	4.2	0.33	0.01		
DOVA	Diploneis ovalis (Hilse) Cleve	0.00			0.02			0.23	2.2	0.4	0.00		
DTEN	Denticula tenuis Kutzing	2.68	35.7	0	0.02			0.00			0.02		
DVUL	Diatoma vulgaris Bory	0.16			0.33			0.37			0.61	10.5	0.21
ECAE	Encyonema caespitosum Kutzing	0.03			0.09	6.2	0.2	0.01			0.01		
EEXI	Eunotia exigua (Brebisson) Rabenhorst	0.00			0.00			0.00			0.00		
EMIN	*Eunotia minor* (Kutzing) Grunow	0.00			0.00			0.00			0.00		
ENCM	Encyonopsis microcephala (Grunow) Krammer	1.29	31.1	0.01	0.71			0.01			0.00		
ENMI	Encyonema minutum (Hilse) D.G. Mann	0.69			0.36			0.11			0.45		
EOCO	Eolimna comperei Ector. Coste et Iserentant	0.00			0.04			0.00			0.01		
EOMI	Eolimna minima (Grunow) Lange-Bertalot	1.13			1.41			1.94			4.31		
EPRO	Encyonema prostratum (Berkeley) Kutzing	0.00			0.02			0.14	11.1	0.13	0.03		
ESBM	Eolimna subminuscula (Manguin) Moser Lange-Bertalot & Metzeltin	0.03			0.06			0.23			2.14		
ESLE	Encyonema silesiacum (Bleisch) D.G. Mann	0.00			0.11			0.01			0.22		
ETEN	Eunotia tenella (Grunow) Hustedt	0.00			0.00			0.00			0.00		
EUIN	Eunotia intermedia (Krasske) Norpel & Lange-Bertalot	0.00			0.00			0.00			0.00		

FARC	*Fragilaria arcus* (Ehrenberg) Cleve	0.00			0.00			0.00			0.00		
FBID	Fragilaria bidens Heiberg	0.00			0.00			0.00			0.00		
FCAP	Fragilaria capucina Desmazieres var. capucina	0.06			0.06			0.01			0.08		
FCRO	Fragilaria crotonensis Kitton	0.00			0.00			0.00			0.00		
FCRP	*Fragilaria capucina* Desmaziere ssp. *rumpens* (Kutzing) Lange-Bertalot	0.03			0.01			0.01			0.06		
FCVA	Fragilaria capucina Desmazieres var. vaucheriae (Kutzing) Lange-Bertalot	0.06			0.10			0.07			0.11		
FGRA	Fragilaria gracilis Ostrup	0.00			0.00			0.00			0.00		
FLEN	Fallacia lenzi (Hustedt) Lange-Bertalot	0.00			0.07			0.24	9.9	0.13	0.05		
FMOC	Fallacia monoculata (Hustedt) Mann	0.00			0.01			0.01			0.01		
FPUL	*Fragilaria pulchella* (Ralfs) Lange-Bertalot	0.00			0.00			0.00			0.00		
FSAP	Fistulifera saprophila (Lange-Bertalot & Bonik) Lange-Bertalot	0.00			0.04			0.00			0.26		
FSBH	Fallacia subhamulata (Grunow) Mann	0.00			0.46			1.22	36.9	0.01	0.51		
FUAC	Fragilaria ulna (Nitzsch) Lange-Bertalot var. acus (Kutzing) Lange-Bertalot	0.00			0.05			0.13	4.4	0.39	0.04		
GACC	Geissleria acceptata (Hustedt) Lange-Bertalot & Metzeltin	0.00			0.02			0.01			0.02		
GANG	Gomphonema angustatum (Kutzing) Rabenhorst	0.00			0.01			0.04	1.4	0.8	0.02		
GEXL	Gomphonema exilissimum (Grunow) Lange-Bertalot & Reichardt	0.00			0.00			0.01			0.00		
GGRA	Gomphonema gracile Ehrenberg	0.00			0.02			0.00			0.02		
GMIC	Gomphonema micropus Kutzing var. micropus	0.00			0.01			0.01			0.01		
GMIN	Gomphonema minutum (Agardh) Agardh f. minutum	0.03			0.24			0.10			0.53		
GNOD	Gyrosigma nodiferum (Grunow) Reimer	0.13			0.39			2.17	56.4	0	0.26		
GOLI	Gomphonema olivaceum (Hornemann) Brebisson var. olivaceum	0.00			0.02			0.08			0.29	15.1	0.08
GOMS	Gomphonema spp.	0.00			0.01			0.02			0.02		
GPAR	*Gomphonema parvulum* (Kutzing) Kutzing	0.03			0.36			0.31			1.10		
GPAS	Gomphonema parvulum var. parvulum f. saprophilum Lange-Bert.&Reichardt	0.00			0.00			0.00			0.01		
GPLI	Gomphosphenia lingulatiformis (Lange-Bertalot & Reichardt) Lange-Bertalot	0.00			0.01			0.05			0.01		
GPRI	Gomphonema pumilum var. rigidum Reichardt & Lange-Bertalot	0.00			0.00			0.01			0.02		
GPUM	Gomphonema pumilum (Grunow) Reichardt & Lange-Bertalot	1.03	12.2	0.09	0.00			0.01			0.09		
GPVL	Gomphonema parvulius Lange-Bertalot & Reichardt	0.00			0.01			0.00			0.01		
GRHB	Gomphonema rhombicum M. Schmidt	0.00			0.00			0.00			0.00		
GTER	Gomphonema tergestinum Fricke	0.00			0.22			0.02			0.14		
GTRU	Gomphonema truncatum Ehrenberg	0.00			0.07			0.04			0.05		
GYAC	Gyrosigma acuminatum (Kutzing)Rabenhorst	0.00			0.04			0.04	3.3	0.47	0.01		
GYAT	Gyrosigma attenuatum (Kutzing) Rabenhorst	0.03			0.17			0.36	25.3	0.02	0.11		
HCAP	Hippodonta capitata (Ehrenberg) Lange-Bertalot Metzeltin & Witkowski	0.00			0.02			0.14	8.1	0.25	0.03		
KCLE	Karayevia clevei (Grunow) Bukhtiyarova	0.00			0.02			0.15			0.01		
KLAT	Karayevia laterostrata (Hustedt) Kingston	0.00			0.00			0.01			0.00		
KPLO	Kolbesia ploenensis (Hustedt) Kingston	0.10			0.12			0.82			0.35		
LGOE	Luticola goeppertiana (Bleisch) Mann	0.00			0.02			0.16			0.03		
LHUN	Lemnicola hungarica (Grunow) Round & Basson	0.00			0.02			0.05			0.02		
LMUT	Luticola mutica (Kutzing) Mann	0.00			0.00			0.02			0.00		
MAAL	Mayamaea atomus var. alcimonica (Reichardt) Reichardt	0.03			0.00			0.00			0.12		
MAAT	Mayamaea atomus (Kutzing) Lange-Bertalot	0.00			0.00			0.07			0.25		
MAPE	Mayamaea atomus var. permitis (Hustedt) Lange-Bertalot	0.13			0.07			0.04			0.70		
MCIR	Meridion circulare (Greville) C.A. Agardh var. circulare	0.00			0.00			0.01			0.04		
MLLC	Mayamaea lacunolaciniata (Lange-Bertalot & Bonik) Lange-Bertalot	0.00			0.00			0.01			0.03		
MVAR	Melosira varians Agardh	0.06			0.14			3.07	20.9	0.08	1.82		
NACI	Nitzschia acicularis (Kutzing) W.M.Smith	0.00			0.03			0.06			0.03		
NACU	Nitzschia acula Hantzsch	0.00			0.04			0.07	6.2	0.17	0.02		
NAGF	Nitzschia angustiforaminata Lange-Bertalot	0.00			0.01			0.06			0.00		
NAMP	Nitzschia amphibia Grunow f. amphibia	0.00			0.24			0.78			0.39		
NANT	Navicula antonii Lange-Bertalot	0.03			0.59			1.80	13.1	0.11	1.62		
NARV	Navicula arvensis Hustedt	0.03			0.00			0.00			0.01		
NASP	Navicula sp.	0.00			0.05			0.01			0.03		
NBRG	Nitzschia bergii Cleve-Euler	0.00			0.08			0.09	5.6	0.28	0.01		
NCIN	*Navicula cincta* (Ehrenberg) Ralfs	0.00			0.03			0.04		0.87	0.02		
NCPL	*Nitzschia capitellata* Hustedt	0.00			0.07			0.19			0.14		
NCPR	Navicula capitatoradiata Germain	0.03			2.37	27.7	0.02	1.30			0.99		
NCRY	Navicula cryptocephala Kutzing	0.03			0.07			0.22			0.13		
NCTE	Navicula cryptotenella Lange-Bertalot	0.47			5.32			7.26			7.27	24.2	0
NCTO	Navicula cryptotenelloides Lange-Bertalot	0.06			0.98	15.6	0.06	0.51			0.58		
NCTV	Navicula caterva Hohn & Hellerman	0.00			0.00			0.00			0.01		
NDIF	Navicula difficillima Hustedt	1.39	10.8	0.09	0.01			0.07			0.00		
NDIS	Nitzschia dissipata (Kutzing) Grunow var. dissipata	1.32			2.03			4.99			5.80	26.8	0.02
NERI	Navicula erifuga Lange-Bertalot	0.00			0.00			0.06			0.01		
NEXI	Navicula exilis Kutzing	0.00			0.00			0.01			0.01		
NFIL	Nitzschia filiformis (W.M.Smith) Van Heurck var. filiformis	0.00			0.00			0.04			0.01		
NFON	*Nitzschia fonticola* Grunow	0.69			2.18			1.15			1.45		
NGER	Navicula germainii Wallace	0.09			0.00			0.42			0.04		
NGRE	Navicula gregaria Donkin	0.19			0.30			2.83			2.31		
NHAN	Nitzschia hantzschiana Rabenhorst	0.00			0.00			0.01			0.00		
NHEU	Nitzschia heufleriana Grunow	0.00			0.04			0.15	16.9	0.07	0.04		
NIAR	Nitzschia archibaldii Lange-Bertalot	0.00			0.03			0.02			0.01		
NIFR	Nitzschia frustulum (Kutzing) Grunow var. frustulum	0.00			0.49			0.41			0.13		
NIGR	Nitzschia gracilis Hantzsch	0.00			0.01			0.10			0.01		
NINC	Nitzschia inconspicua Grunow	0.00			0.70			0.56			0.51		
NING	Navicula ingenua Hustedt	0.00			0.00			0.01			0.00		

NINT	*Nitzschia intermedia* Hantzsch	0.00			0.04			0.07	3.4	0.54	0.02		
NIPF	Nitzschia paleaeformis Hustedt	0.00			0.00			0.02			0.01		
NIPU	Nitzschia pusilla (Kutzing) Grunow	0.00			0.00			0.03			0.04		
NLAN	Navicula lanceolata (Agardh) Ehrenberg	0.00			0.04			1.51			1.12		
NLEV	*Nitzschia levidensis* (W.Smith) Grunow	0.00			0.01			0.15	10.7	0.12	0.06		
NLIN	Nitzschia linearis (Agardh) W.M.Smith var. linearis	0.00			0.04			0.13	8.1	0.21	0.04		
NMEN	Navicula menisculus Schumann var. menisculus	0.06			0.52	8.9	0.22	0.49			0.40		
NMIC	*Nitzschia microcephala* Grunow	0.00			0.00			0.11			0.16		
NNOV	Navicula novaesiberica Lange-Bertalot	0.00			0.00			0.05			0.00		
NPAD	*Nitzschia palea* (Kutzing) W.Smith var. *debilis* (Kutzing) Grunow	0.22	4.2	0.46	0.04			0.01			0.06		
NPAE	*Nitzschia paleacea* (Grunow) Grunow	0.00			0.83			0.11			0.36		
NPAL	Nitzschia palea (Kutzing) W.Smith	0.13			0.58			1.89			1.55		
NRCH	*Navicula reichardtiana* Lange-Bertalot	0.06			0.36			0.34			0.70	16.7	0.06
NRCS	Navicula recens (Lange-Bertalot) Lange-Bertalot	0.00			0.10			0.78			0.06		
NREC	*Nitzschia recta* Hantzsch	0.00			0.12			0.73	31.7	0.01	0.15		
NRFA	Navicula radiosafallax Lange-Bertalot	0.00			0.03			0.04	2.4	0.64	0.04		
NRHY	Navicula rhynchocephala Kutzing	0.00			0.00			0.00			0.01		
NSHR	Navicula schroeteri Meister var. schroeteri	0.00			0.00			0.19	9.7	0.12	0.01		
NSLU	Navicula sublucidula Hustedt	0.06	4.8	0.21	0.04			0.01			0.03		
NSOC	Nitzschia sociabilis Hustedt	0.66			0.86			3.96	40.2	0	0.80		
NSOL	Nitzschia solgensis Cleve-Euler	0.00			0.04			0.00			0.01		
NSSY	Navicula schroeteri Meister var. symmetrica (Patrick) Lange-Bertalot	0.00			0.02			0.81	13.9	0.08	0.04		
NSUA	*Nitzschia subacicularis* Hustedt	0.25			0.05			0.01			0.03		
NTEN	Navicula tenelloides Hustedt	0.00			0.01			0.02			0.02		
NTPT	Navicula tripunctata (O.F.Muller) Bory	0.69			1.66			4.70			5.83	31.4	0.01
NTRO	Nitzschia tropica Hustedt	0.00			0.00			0.00			0.00		
NTRV	Navicula trivialis Lange-Bertalot var. trivialis	0.00			0.02			0.08			0.10		
NVDS	*Sellaphora seminulum* (Grunow) Mann	0.00			0.05			0.02			0.15		
NVEN	Navicula veneta Kutzing	0.00			0.11			0.14			0.09		
NVER	Nitzschia vermicularis (Kutzing) Hantzsch	0.00			0.00			0.13	14.7	0.06	0.02		
NVIR	Navicula viridula (Kutzing) Ehrenberg	0.00			0.02			0.43	11.4	0.1	0.15		
NVRO	Navicula viridula (Kutzing) Ehrenberg var. rostellata (Kutzing) Cleve	0.00			0.00			0.04			0.00		
NZAG	Nitzschia angustatula Lange-Bertalot	0.00			0.20	11.7	0.11	0.17			0.04		
NZLT	Nitzschia linearis (Agardh) W.M. Smith var. tenuis (W.Smith) Grunow	0.00			0.04			0.07	7.6	0.15	0.02		
NZSS	Nitzschia spp.	0.00			0.01			0.00			0.04		
NZSU	Nitzschia supralitorea Lange-Bertalot	0.00			0.00			0.25			0.05		
PBIO	Psammothidium bioretii (Germain) Bukhtiyarova et Round	0.00			0.01			0.00			0.00		
PCLT	Placoneis clementis (Grunow) Cox	0.00			0.00			0.01			0.00		
PDAO	Psammothidium daonense (Lange-Bertalot) Lange-Bertalot	0.00			0.00			0.00			0.00		
PDAU	Planothidium daui (Foged) Lange-Bertalot	0.00			0.00			0.00			0.01		
PGRN	Planothidium granum (Hohn & Hellerman) Lange-Bertalot	0.00			0.00			0.00			0.03		
PHEL	Psammothidium helveticum (Hustedt) Bukhtiyarova et Round	0.00			0.02			0.00			0.01		
PLAU	Psammothidium lauenburgianum (Hustedt) Bukhtiyarova et Round	0.25			0.33			0.12			0.45		
PLFR	Planothidium frequentissimum (Lange-Bertalot) Lange-Bertalot	0.06			0.20			0.31			0.44		
POBG	Psammothidium oblongellum (Oestrup) Van de Vijver	0.00			0.00			0.04			0.01		
PPRO	Parlibellus protracta (Grunow) Witkowski Lange-Bertalot & Metzeltin	0.00			0.00			0.03			0.01		
PRST	Planothidium rostratum (Oestrup) Lange-Bertalot	0.06			0.18			0.04			0.02		
PSAT	Psammothidium subatomoides (Hustedt) Bukhtiyarova et Round	0.00			0.00			0.00			0.00		
PSBR	Pseudostaurosira brevistriata (Grunow) Williams & Round	0.00			0.06			0.06			0.02		
PTDE	Planothidium delicatulum (Kutzing) Round & Bukhtiyarova	0.00			0.00			0.00			0.00		
PTEL	Planothidium ellipticum (Cleve)Round & Bukhtiyarova	0.00			0.01			0.01			0.04		
PTHA	Planothidium hauckianum (Grunow) Round & Bukhtiyarova	0.00			0.00			0.10	4.3	0.29	0.02		
PTLA	Planothidium lanceolatum (Brebisson) Lange-Bertalot	0.19			0.18			0.27			0.44		
RABB	Rhoicosphenia abbreviata (C.Agardh) Lange-Bertalot	0.29			0.38			4.67			3.67		
RSIN	Reimeria sinuata (Gregory) Kociolek & Stoermer	0.13			0.20			0.13			0.26		
RUNI	Reimeria uniseriata Sala, Guerrero & Ferrario	0.00			0.10			0.07			0.06		
SANG	Surirella angusta Kutzing	0.00			0.00			0.09			0.03		
SBKU	Surirella brebissonii var. kuetzingii Krammer et Lange-Bertalot	0.00			0.02			0.35	15.4	0.07	0.14		
SBRE	*Surirella brebissonii* Krammer & Lange-Bertalot	0.00			0.10			0.49			0.51		
SCON	Staurosira construens Ehrenberg	0.00			0.06			0.04			0.03		
SELI	Staurosira elliptica (Schumann) Williams & Round	0.00			0.00			0.00			0.00		
SFSC	Synedra fasciculata Kutzing	0.00			0.00			0.00			0.02		
SHAN	*Stephanodiscus hantzschii* Grunow	0.00			0.36	10.8	0.14	0.35			0.02		
SHTE	Stephanodiscus hantzschii f. tenuis (Hustedt) Hakansson et Stoermer	0.00			0.40	11.6	0.13	0.14			0.03		
SIDE	Simonsenia delognei Lange-Bertalot	0.03			0.43			0.89	16.1	0.09	0.09		
SKPO	Skeletonema potamos (Weber) Hasle	0.00			0.02			0.07			0.00		
SLIN	Surirella linearis Smith	0.00			0.00			0.04			0.01		
SPUP	Sellaphora pupula (Kutzing) Mereschkowksy	0.00			0.09			0.15			0.06		
SRPI	Staurosira pinnata Ehrenberg	0.06			0.51	16.8	0.06	0.22			0.05		
SSVE	Staurosira venter (Ehrenberg) Cleve & Moeller	0.03			0.44	3.7	0.5	0.09			0.07		
TAPI	Tryblionella apiculata Gregory	0.06			0.04			0.63	30.7	0.01	0.21		
TBRA	Thalassiosira bramaputrae (Ehrenberg) Hakansson & Locker	0.00			0.00			0.24			0.00		
THUN	Tryblionella hungarica (Grunow) Mann	0.00			0.00			0.03			0.01		
TPSN	Thalassiosira pseudonana Hasle et Heimdal	0.03			0.20			0.09			0.08		
TWEI	Thalassiosira weissflogii (Grunow) Fryxell & Hasle	0.00			0.02			0.74			0.18		
UULN	Ulnaria ulna (Nitzsch) CompÞre	0.03			0.04			0.18			0.20		

		Diatom assemblages of limestone regions											
		20 □ Organically polluted small rivers			21 - Organically polluted small rivers			22 □ Rivers with high conductivities (caused by human impact)			23 - Rivers with high conductivities (natural and human impact)		
	Group	20	20	20	21	21	21	22	22	22	23	23	23
Omnidia Code	Taxon name	%	indval	p-value	%	indval	p-value	%	indval	p-value	%	indval	p-value
AAMB	Aulacoseira ambigua (Grunow) Simonsen	0.04			0.07			0.20			0.00		
AATO	Achnanthes atomus Hustedt	0.00			0.00			0.09			0.00		
ACHS	Achnanthes sp.	0.00			0.03			0.02			0.00		
ACON	Achnanthes conspicua A.Mayer	7.38	63.9	0	0.10			1.06			0.37		
ACOP	Amphora copulata(Kutzing) Schoeman & Archibald	0.17			0.09			1.28	22.6	0.03	1.11		
ADBI	Achnanthidium biasolettianum (Grunow) Lange-Bertalot	0.02			0.02			0.00			0.00		
ADCT	Achnanthidium catenatum (Bily & Marvan) Lange-Bertalot	0.00			0.01			0.00			0.00		
ADEU	Achnanthidium eutrophilum (Lange-Bertalot) Lange-Bertalot	0.13			0.79			0.91			0.36		
ADKR	Achnanthidium kranzii (Lange-Bertalot) Round & Bukhtiyarova	0.00			0.00			0.00			0.00		
ADLS	Adlafia suchlandtii (Hustedt) Moser Lange-Bertalot & Metzeltin	0.05			0.00			0.00			0.00		
ADMF	Achnanthidium minutissimum (Kutzing) Czarnecki var. affinis (Grunow) Bukht.	0.05			0.03			0.00			0.00		
ADMI	Achnanthidium minutissimum (Kutzing) Czarnecki	1.98			2.72			1.29			0.46		
ADMM	Adlafia minuscula var. muralis (Grunow) Lange-Bertalot	0.00			0.01			0.00			0.00		
ADMS	Adlafia minuscula (Grunow) Lange-Bertalot	0.01			0.03			0.05	1.9	0.79	0.00		
ADSA	Achnanthidium saprophilum (Kobayasi et Mayama) Round & Bukhtiyarova	0.10			0.15			0.08			0.04		
ADSB	Achnanthidium straubianum (Lange-Bertalot)Lange-Bertalot	0.01			0.01			0.00			0.00		
ADSU	Achnanthidium subatomus (Hustedt) Lange-Bertalot	0.00			0.00			0.00			0.00		
AFOR	Asterionella formosa Hassall	0.01			0.01			0.00			0.00		
AFUG	Achnanthes fugei Carter	0.90			0.00			1.85	6.6	0.19	0.00		
AINA	Amphora inariensis Krammer	0.01			0.00			0.02			0.04		
AMII	Achnanthes minutissima Kutzing var. inconspicua Oestrup	0.00			0.00			0.00			0.00		
AMMO	Amphora montana Krasske	0.01			0.04	2.3	0.67	0.03			0.01		
AOVA	Amphora ovalis (Kutzing) Kutzing	0.06			0.04			0.25			0.15		
APED	Amphora pediculus (Kutzing) Grunow	28.89			5.24			19.52			13.78		
ASHU	Achnanthes subhudsonis Hustedt	0.00			0.19			0.13			0.00		
AUDI	Aulacoseira distans (Ehrenberg) Simonsen	0.00			0.05			0.05			0.00		
AUGR	Aulacoseira granulata (Ehrenberg) Simonsen	0.03			0.02			0.05			0.00		
AVEN	Amphora veneta Kutzing	0.09			0.47			0.03			0.04		
BPAX	Bacillaria paxillifera (O.F. Muller) Hendey var. paxillifera	0.13			0.00			0.49			0.61	17.5	0.05
CAFF	*Cymbella excisa* Kutzing	0.00			0.00			0.00			0.00		
CAGR	Cyclotella atomus var. gracilis Genkal & Kiss	0.02			0.02			0.46	19.4	0.04	0.04		
CATO	Cyclotella atomus Hustedt	0.03			0.08			2.92	44.9	0.01	0.50		
CBAC	Caloneis bacillum (Grunow) Cleve	0.43			0.13			0.32			0.44		
CDUB	Cyclostephanos dubius (Fricke) Round	0.03			0.12			0.32			0.20		
CINV	Cyclostephanos invisitatus (Hohn & Hellerman) Theriot Stoermer & Hakansson	0.07			0.13			0.85	17.4	0.07	0.20		
CMED	Cyclotella meduanae Germain	0.00			0.01			0.31	6.4	0.23	0.14		
CMEN	Cyclotella meneghiniana Kutzing	0.86			1.74			4.16	23.6	0.07	2.75		
CMLF	Craticula molestiformis (Hustedt) Lange-Bertalot	0.05			0.10			0.07			0.05		
COCE	Cyclotella ocellata Pantocsek	0.00			0.02			0.01			0.00		
CPED	Cocconeis pediculus Ehrenberg	0.28			0.57	9.4	0.25	0.36			0.10		
CPLA	Cocconeis placentula Ehrenberg var. placentula	0.43			1.16			0.74			0.80		
CPLE	Cocconeis placentula Ehrenberg var. euglypta (Ehrenberg) Grunow	1.84			7.58	15.6	0.09	2.67			1.73		
CPLI	Cocconeis placentula Ehrenberg var. lineata (Ehrenberg) Van Heurck	0.17			0.64			0.44			0.11		
CPPL	Cocconeis placentula Ehrenberg var. pseudolineata Geitler	0.00			0.04			0.02			0.00		
CPST	Cyclotella pseudostelligera Hustedt	0.20			0.63			3.95	20.3	0.08	2.26		
CRAC	Craticula accomoda (Hustedt) Mann	0.01			0.06			0.07			0.00		
CSOL	*Cymatopleura solea* (Brebisson) W.Smith	0.01			0.02			0.02			0.00		
CSTE	*Cyclotella stelligera* Cleve et Grunow	0.00			0.01			0.02			0.00		
CTUM	Cymbella tumida (Brebisson) Van Heurck	0.01			0.04			0.05	2.7	0.52	0.01		
DCOT	Diadesmis contenta (Grunow) Mann	0.04			0.03			0.10	2.8	0.53	0.00		
DMAR	Diploneis marginestriata Hustedt	0.00			0.00			0.02			0.00		
DMES	Diatoma mesodon (Ehrenberg) Kutzing	0.00			0.01			0.03			0.00		
DOBL	Diploneis oblongella (Naegeli) Cleve-Euler	0.02			0.01			0.01			0.01		
DOVA	Diploneis ovalis (Hilse) Cleve	0.00			0.00			0.00			0.00		
DTEN	Denticula tenuis Kutzing	0.00			0.03			0.01			0.02		
DVUL	Diatoma vulgaris Bory	0.04			0.36			0.07			0.02		
ECAE	Encyonema caespitosum Kutzing	0.00			0.01			0.00			0.00		
EEXI	Eunotia exigua (Brebisson) Rabenhorst	0.00			0.01			0.00			0.00		
EMIN	*Eunotia minor* (Kutzing) Grunow	0.00			0.00			0.00			0.00		
ENCM	Encyonopsis microcephala (Grunow) Krammer	0.02			0.00			0.00			0.00		
ENMI	Encyonema minutum (Hilse) D.G. Mann	0.11			0.35			0.10			0.02		
EOCO	Eolimna comperei Ector. Coste et Iserentant	0.00			0.01			0.25	6	0.19	0.00		
EOMI	Eolimna minima (Grunow) Lange-Bertalot	16.78			6.63			6.73			2.67		
EPRO	Encyonema prostratum (Berkeley) Kutzing	0.01			0.01			0.05			0.00		
ESBM	Eolimna subminuscula (Manguin) Moser Lange-Bertalot & Metzeltin	2.17			7.60			1.05			2.90		

ESLE	Encyonema silesiacum (Bleisch) D.G. Mann	0.10			0.11			0.05			0.00		
ETEN	Eunotia tenella (Grunow) Hustedt	0.00			0.00			0.00			0.00		
EUIN	Eunotia intermedia (Krasske) Norpel & Lange-Bertalot	0.00			0.00			0.00			0.00		
FARC	*Fragilaria arcus* (Ehrenberg) Cleve	0.00			0.01			0.02			0.00		
FBID	Fragilaria bidens Heiberg	0.00			0.02			0.01			0.00		
FCAP	Fragilaria capucina Desmazieres var. capucina	0.03			0.04			0.01			0.00		
FCRO	Fragilaria crotonensis Kitton	0.00			0.04			0.00			0.00		
FCRP	*Fragilaria capucina* Desmaziere ssp. *rumpens* (Kutzing) Lange-Bertalot	0.00			0.07			0.02			0.01		
FCVA	Fragilaria capucina Desmazieres var. vaucheriae (Kutzing) Lange-Bertalot	0.06			0.28			0.10			0.01		
FGRA	Fragilaria gracilis Ostrup	0.00			0.00			0.00			0.00		
FLEN	Fallacia lenzi (Hustedt) Lange-Bertalot	0.06			0.02			0.03			0.02		
FMOC	Fallacia monoculata (Hustedt) Mann	0.02			0.11	2.6	0.5	0.00			0.01		
FPUL	*Fragilaria pulchella* (Ralfs) Lange-Bertalot	0.00			0.05			0.00			0.00		
FSAP	Fistulifera saprophila (Lange-Bertalot & Bonik) Lange-Bertalot	0.13			0.48			0.20			0.03		
FSBH	Fallacia subhamulata (Grunow) Mann	0.28			0.11			0.22			0.08		
FUAC	Fragilaria ulna (Nitzsch) Lange-Bertalot var. acus (Kutzing) Lange-Bertalot	0.01			0.02			0.02			0.02		
GACC	Geissleria acceptata (Hustedt) Lange-Bertalot & Metzeltin	0.07			0.07			0.03			0.02		
GANG	Gomphonema angustatum (Kutzing) Rabenhorst	0.00			0.01			0.01			0.00		
GEXL	Gomphonema exilissimum (Grunow) Lange-Bertalot & Reichardt	0.02			0.01			0.00			0.00		
GGRA	Gomphonema gracile Ehrenberg	0.01			0.03			0.03			0.01		
GMIC	Gomphonema micropus Kutzing var. micropus	0.01			0.14			0.00			0.00		
GMIN	Gomphonema minutum (Agardh) Agardh f. minutum	0.25			0.77			0.06			0.03		
GNOD	Gyrosigma nodiferum (Grunow) Reimer	0.06			0.03			0.36			0.08		
GOLI	Gomphonema olivaceum (Hornemann) Brebisson var. olivaceum	0.09			0.15			0.07			0.01		
GOMS	Gomphonema spp.	0.01			0.01			0.02			0.03	1.2	0.93
GPAR	*Gomphonema parvulum* (Kutzing) Kutzing	0.68			4.40	18.5	0.07	0.67			0.60		
GPAS	Gomphonema parvulum var. parvulum f. saprophilum Lange-Bert.&Reichardt	0.00			0.10	1.6	0.51	0.00			0.00		
GPLI	Gomphosphenia lingulatiformis (Lange-Bertalot & Reichardt) Lange-Bertalot	0.05			0.01			0.64	23.2	0.03	0.22		
GPRI	Gomphonema pumilum var. rigidum Reichardt & Lange-Bertalot	0.07	3.7	0.33	0.00			0.00			0.00		
GPUM	Gomphonema pumilum (Grunow) Reichardt & Lange-Bertalot	0.19			0.07			0.00			0.02		
GPVL	Gomphonema parvulius Lange-Bertalot & Reichardt	0.00			0.01			0.00			0.00		
GRHB	Gomphonema rhombicum M. Schmidt	0.00			0.00			0.00			0.00		
GTER	Gomphonema tergestinum Fricke	0.05			0.06			0.05			0.00		
GTRU	Gomphonema truncatum Ehrenberg	0.02			0.01			0.02			0.00		
GYAC	Gyrosigma acuminatum (Kutzing)Rabenhorst	0.01			0.03			0.04			0.04		
GYAT	Gyrosigma attenuatum (Kutzing) Rabenhorst	0.01			0.02			0.03			0.01		
HCAP	Hippodonta capitata (Ehrenberg) Lange-Bertalot Metzeltin & Witkowski	0.05			0.05			0.03			0.02		
KCLE	Karayevia clevei (Grunow) Bukhtiyarova	0.18			0.04			1.11	33.3	0.02	0.10		
KLAT	Karayevia laterostrata (Hustedt) Kingston	0.07	2.3	0.53	0.03			0.00			0.01		
KPLO	Kolbesia ploenensis (Hustedt) Kingston	0.82			0.32			1.24	22.1	0.03	0.47		
LGOE	Luticola goeppertiana (Bleisch) Mann	0.02			0.19			0.70	10.3	0.14	0.03		
LHUN	Lemnicola hungarica (Grunow) Round & Basson	0.14	5.8	0.23	0.03			0.01			0.07		
LMUT	Luticola mutica (Kutzing) Mann	0.01			0.06			0.06	2.8	0.46	0.02		
MAAL	Mayamaea atomus var. alcimonica (Reichardt) Reichardt	0.22	3.8	0.36	0.04			0.00			0.03		
MAAT	Mayamaea atomus (Kutzing) Lange-Bertalot	0.22			0.15			0.14			0.29	7.4	0.33
MAPE	Mayamaea atomus var. permitis (Hustedt) Lange-Bertalot	2.20			2.60			0.47			0.89		
MCIR	Meridion circulare (Greville) C.A. Agardh var. circulare	0.02			0.02			0.00			0.00		
MLLC	Mayamaea lacunolaciniata (Lange-Bertalot & Bonik) Lange-Bertalot	0.00			0.01			0.06			0.00		
MVAR	Melosira varians Agardh	0.75			1.12			1.06			0.46		
NACI	Nitzschia acicularis (Kutzing) W.M.Smith	0.09			0.09			0.06			0.02		
NACU	Nitzschia acula Hantzsch	0.02			0.01			0.01			0.00		
NAGF	Nitzschia angustiforaminata Lange-Bertalot	0.05			0.00			0.08	1.6	0.8	0.05		
NAMP	Nitzschia amphibia Grunow f. amphibia	0.67			2.90			1.49			1.47		
NANT	Navicula antonii Lange-Bertalot	0.73			0.53			1.14			1.48		
NARV	Navicula arvensis Hustedt	0.00			0.07	4	0.32	0.00			0.00		
NASP	Navicula sp.	0.06			0.05			0.03			0.09		
NBRG	Nitzschia bergii Cleve-Euler	0.01			0.03			0.02			0.02		
NCIN	*Navicula cincta* (Ehrenberg) Ralfs	0.01			0.04			0.02			0.04		
NCPL	*Nitzschia capitellata* Hustedt	0.38			0.70			0.08			0.18		
NCPR	Navicula capitatoradiata Germain	0.24			1.02			0.38			0.06		
NCRY	Navicula cryptocephala Kutzing	0.18			0.67			0.07			0.02		
NCTE	Navicula cryptotenella Lange-Bertalot	1.52			2.10			1.67			2.65		
NCTO	Navicula cryptotenelloides Lange-Bertalot	0.21			0.35			0.21			0.12		
NCTV	Navicula caterva Hohn & Hellerman	0.00			0.04			0.08	3.7	0.31	0.00		
NDIF	Navicula difficillima Hustedt	0.00			0.01			0.02			0.00		
NDIS	Nitzschia dissipata (Kutzing) Grunow var. dissipata	0.91			0.66			0.81			1.77		
NERI	Navicula erifuga Lange-Bertalot	0.03			0.01			0.00			0.14	4.4	0.3
NEXI	Navicula exilis Kutzing	0.01			0.03			0.00			0.00		
NFIL	Nitzschia filiformis (W.M.Smith) Van Heurck var. filiformis	0.01			0.01			0.24			0.26	8.8	0.15
NFON	*Nitzschia fonticola* Grunow	0.59			3.29			1.78			0.64		
NGER	Navicula germainii Wallace	0.04			0.44	7.3	0.25	0.11			0.04		
NGRE	Navicula gregaria Donkin	2.26			2.35			0.87			0.88		
NHAN	Nitzschia hantzschiana Rabenhorst	0.01			0.00			0.00			0.00		
NHEU	Nitzschia heufleriana Grunow	0.01			0.01			0.01			0.00		
NIAR	Nitzschia archibaldii Lange-Bertalot	0.01			0.01			0.00			0.00		
NIFR	Nitzschia frustulum (Kutzing) Grunow var. frustulum	0.31			0.28			1.40			12.16	64.7	0

NIGR	Nitzschia gracilis Hantzsch	0.01			0.14			0.37	5.3	0.34	0.04		
NINC	Nitzschia inconspicua Grunow	0.85			3.99			6.85			22.45	33.3	0.01
NING	Navicula ingenua Hustedt	0.04			0.01			0.47	16.7	0.03	0.02		
NINT	*Nitzschia intermedia* Hantzsch	0.07			0.06			0.01			0.00		
NIPF	Nitzschia paleaeformis Hustedt	0.03			0.18			0.02			0.02		
NIPU	Nitzschia pusilla (Kutzing) Grunow	0.04			0.05			0.07			0.02		
NLAN	Navicula lanceolata (Agardh) Ehrenberg	1.04			1.20			0.65			0.42		
NLEV	*Nitzschia levidensis* (W.Smith) Grunow	0.07			0.08			0.10			0.04		
NLIN	Nitzschia linearis (Agardh) W.M.Smith var. linearis	0.05			0.06			0.03			0.00		
NMEN	Navicula menisculus Schumann var. menisculus	0.24			0.24			0.30			0.21		
NMIC	*Nitzschia microcephala* Grunow	0.42			0.03			0.51			0.59	11.9	0.12
NNOV	Navicula novaesiberica Lange-Bertalot	0.00			0.06	4.3	0.28	0.02			0.00		
NPAD	*Nitzschia palea* (Kutzing) W.Smith var. *debilis* (Kutzing) Grunow	0.06			0.15			0.20			0.02		
NPAE	*Nitzschia paleacea* (Grunow) Grunow	0.25			1.51			0.41			0.19		
NPAL	Nitzschia palea (Kutzing) W.Smith	2.06			4.49	16.6	0.12	1.05			1.19		
NRCH	*Navicula reichardtiana* Lange-Bertalot	0.31			0.33			0.19			0.06		
NRCS	Navicula recens (Lange-Bertalot) Lange-Bertalot	0.04			0.47			0.21			1.84	27.6	0.03
NREC	*Nitzschia recta* Hantzsch	0.12			0.11			0.11			0.02		
NRFA	Navicula radiosafallax Lange-Bertalot	0.00			0.02			0.03			0.02		
NRHY	Navicula rhynchocephala Kutzing	0.01			0.05			0.00			0.00		
NSHR	Navicula schroeteri Meister var. schroeteri	0.00			0.02			0.04			0.02		
NSLU	Navicula sublucidula Hustedt	0.00			0.00			0.01			0.00		
NSOC	Nitzschia sociabilis Hustedt	0.47			0.22			1.46			0.59		
NSOL	Nitzschia solgensis Cleve-Euler	0.03			0.02			0.07	4.4	0.33	0.02		
NSSY	Navicula schroeteri Meister var. symmetrica (Patrick) Lange-Bertalot	0.09			0.14			0.09			0.20		
NSUA	*Nitzschia subacicularis* Hustedt	0.01			0.25	5	0.37	0.01			0.01		
NTEN	Navicula tenelloides Hustedt	0.03			0.01			0.02			0.04		
NTPT	Navicula tripunctata (O.F.Muller) Bory	1.43			1.25			1.19			1.08		
NTRO	Nitzschia tropica Hustedt	0.00			0.04			0.02			0.00		
NTRV	Navicula trivialis Lange-Bertalot var. trivialis	0.15	5.3	0.46	0.12			0.02			0.01		
NVDS	*Sellaphora seminulum* (Grunow) Mann	0.80			4.87			0.83			0.19		
NVEN	Navicula veneta Kutzing	0.36			2.70	27.5	0.03	0.25			0.38		
NVER	Nitzschia vermicularis (Kutzing) Hantzsch	0.01			0.03			0.01			0.00		
NVIR	Navicula viridula (Kutzing) Ehrenberg	0.04			0.02			0.07			0.00		
NVRO	Navicula viridula (Kutzing) Ehrenberg var. rostellata (Kutzing) Cleve	0.00			0.08	2.3	0.67	0.11			0.07		
NZAG	Nitzschia angustatula Lange-Bertalot	0.01			0.00			0.10			0.00		
NZLT	Nitzschia linearis (Agardh) W.M. Smith var. tenuis (W.Smith) Grunow	0.03			0.00			0.00			0.00		
NZSS	Nitzschia spp.	0.03			0.07	2.3	0.76	0.05			0.05		
NZSU	Nitzschia supralitorea Lange-Bertalot	0.01			0.08			0.18			0.43	6.9	0.22
PBIO	Psammothidium bioretii (Germain) Bukhtiyarova et Round	0.02			0.03			0.00			0.00		
PCLT	Placoneis clementis (Grunow) Cox	0.00			0.01			0.01			0.00		
PDAO	Psammothidium daonense (Lange-Bertalot) Lange-Bertalot	0.02			0.00			0.00			0.00		
PDAU	Planothidium daui (Foged) Lange-Bertalot	0.03			0.04			0.07			0.00		
PGRN	Planothidium granum (Hohn & Hellerman) Lange-Bertalot	0.11			0.01			0.02			0.00		
PHEL	Psammothidium helveticum (Hustedt) Bukhtiyarova et Round	0.04			0.00			0.01			0.00		
PLAU	Psammothidium lauenburgianum (Hustedt) Bukhtiyarova et Round	2.37	44	0.01	0.20			0.17			0.09		
PLFR	Planothidium frequentissimum (Lange-Bertalot) Lange-Bertalot	1.99			2.52			1.03			0.88		
POBG	Psammothidium oblongellum (Oestrup) Van de Vijver	0.16			0.02			0.03			0.00		
PPRO	Parlibellus protracta (Grunow) Witkowski Lange-Bertalot & Metzeltin	0.00			0.04			0.01			0.00		
PRST	Planothidium rostratum (Oestrup) Lange-Bertalot	0.02			0.16			0.58	9.8	0.15	0.07		
PSAT	Psammothidium subatomoides (Hustedt) Bukhtiyarova et Round	0.09			0.01			0.10			0.00		
PSBR	Pseudostaurosira brevistriata (Grunow) Williams & Round	0.00			0.27			0.12	2.2	0.86	0.01		
PTDE	Planothidium delicatulum (Kutzing) Round & Bukhtiyarova	0.01			0.91	11.8	0.09	0.12			0.36		
PTEL	Planothidium ellipticum (Cleve)Round & Bukhtiyarova	0.08			0.12	3.9	0.49	0.03			0.03		
PTHA	Planothidium hauckianum (Grunow) Round & Bukhtiyarova	0.08			0.02			0.00			0.00		
PTLA	Planothidium lanceolatum (Brebisson) Lange-Bertalot	0.85			0.73			0.25			0.30		
RABB	Rhoicosphenia abbreviata (C.Agardh) Lange-Bertalot	4.24			2.17			4.68			7.73	25	0.03
RSIN	Reimeria sinuata (Gregory) Kociolek & Stoermer	0.48			0.74			0.16			0.08		
RUNI	Reimeria uniseriata Sala, Guerrero & Ferrario	0.07			0.09			0.06			0.12	3.3	0.78
SANG	Surirella angusta Kutzing	0.02			0.06			0.00			0.00		
SBKU	Surirella brebissonii var. kuetzingii Krammer et Lange-Bertalot	0.01			0.10			0.03			0.07		
SBRE	*Surirella brebissonii* Krammer & Lange-Bertalot	0.25			0.61	9.8	0.25	0.25			0.24		
SCON	Staurosira construens Ehrenberg	0.07			0.08			0.07	2.4	0.89	0.03		
SELI	Staurosira elliptica (Schumann) Williams & Round	0.00			0.17			0.42	4.2	0.21	0.00		
SFSC	Synedra fasciculata Kutzing	0.05			0.04			0.20	9.8	0.11	0.12		
SHAN	*Stephanodiscus hantzschii* Grunow	0.05			0.04			0.27			0.08		
SHTE	Stephanodiscus hantzschii f. tenuis (Hustedt) Hakansson et Stoermer	0.03			0.07			0.33			0.04		
SIDE	Simonsenia delognei Lange-Bertalot	0.09			0.52			0.17			0.02		
SKPO	Skeletonema potamos (Weber) Hasle	0.00			0.01			0.76	17.6	0.05	0.11		
SLIN	Surirella linearis Smith	0.00			0.01			0.01			0.07		
SPUP	Sellaphora pupula (Kutzing) Mereschkowksy	0.05			0.38	9.4	0.19	0.07			0.00		
SRPI	Staurosira pinnata Ehrenberg	0.05			0.11			0.06			0.00		
SSVE	Staurosira venter (Ehrenberg) Cleve & Moeller	0.04			0.03			0.04			0.87		
TAPI	Tryblionella apiculata Gregory	0.15			0.13			0.12			0.15		
TBRA	Thalassiosira bramaputrae (Ehrenberg) Hakansson & Locker	0.00			0.02			0.28	15.5	0.07	0.16		
THUN	Tryblionella hungarica (Grunow) Mann	0.04	1.9	0.74	0.02			0.01			0.02		
TPSN	Thalassiosira pseudonana Hasle et Heimdal	0.10			0.10			0.13			0.08		
TWEI	Thalassiosira weissflogii (Grunow) Fryxell & Hasle	0.03			0.08			1.35	19.7	0.04	0.26		

UULN	Ulnaria ulna (Nitzsch) CompÞre	0.05			0.28			0.05			0.08		

		Diatom assemblages of crystalline mountainous regions											
		24 □ Small to medium sized rivers with organic pollution			25 □ Rivers of various sizes with little pollution			26 □ Mostly large rivers moderately polluted, in transition geologies			27 - Mostly large rivers moderately polluted, in transition geologies		
Omnidia Code	Group / Taxon name	24 %	24 indval	24 p-value	25 %	25 indval	25 p-value	26 %	26 indval	26 p-value	27 %	27 indval	27 p-value
AAMB	*Aulacoseira ambigua* (Grunow) Simonsen	0.13			0.19			0.17			0.04		
AATO	*Achnanthes atomus* Hustedt	0.00			0.00			0.00			0.00		
ACHS	*Achnanthes* sp.	0.06	6.6	0.15	0.04			0.00			0.00		
ACON	*Achnanthes conspicua* A.Mayer	0.03			0.11			0.03			0.00		
ACOP	*Amphora copulata* (Kutzing) Schoeman & Archibald	0.00			0.02			0.00			0.00		
ADBI	*Achnanthidium biasolettianum* (Grunow) Lange-Bertalot	0.00			0.72			0.00			0.07		
ADCT	*Achnanthidium catenatum* (Bily & Marvan) Lange-Bertalot	0.02			0.53			0.20			2.31	11	0.06
ADEU	*Achnanthidium eutrophilum* (Lange-Bertalot) Lange-Bertalot	0.00			0.34			0.94			0.31		
ADKR	*Achnanthidium kranzii* (Lange-Bertalot) Round & Bukhtiyarova	0.00			0.00			0.00			0.00		
ADLS	*Adlafia suchlandtii* (Hustedt) Moser Lange-Bertalot & Metzeltin	0.00			0.22			0.28			0.00		
ADMF	*Achnanthidium minutissimum* (Kutzing) Czarnecki var. *affinis* (Grunow) Bukht.	0.02			0.05			0.11	5.9	0.28	0.00		
ADMI	*Achnanthidium minutissimum* (Kutzing) Czarnecki	7.77			16.53			7.07			5.73		
ADMM	*Adlafia minuscula* var. *muralis* (Grunow) Lange-Bertalot	0.00			0.04			0.00			0.00		
ADMS	*Adlafia minuscula* (Grunow) Lange-Bertalot	0.03			0.03			0.00			0.03		
ADSA	*Achnanthidium saprophilum* (Kobayasi et Mayama) Round & Bukhtiyarova	0.00			0.29			0.06			2.50	11	0.14
ADSB	*Achnanthidium straubianum* (Lange-Bertalot)Lange-Bertalot	0.00			0.17			0.00			0.05		
ADSU	*Achnanthidium subatomus* (Hustedt) Lange-Bertalot	0.00			1.09			0.17			0.00		
AFOR	*Asterionella formosa* Hassall	0.00			0.07			0.11	4.7	0.22	0.00		
AFUG	*Achnanthes fugei* Carter	0.00			0.00			0.00			0.00		
AINA	*Amphora inariensis* Krammer	0.00			0.02			0.00			0.00		
AMII	*Achnanthes minutissima* Kutzing var. *inconspicua* Oestrup	0.00			0.00			0.00			0.00		
AMMO	*Amphora montana* Krasske	0.02			0.00			0.00			0.00		
AOVA	*Amphora ovalis* (Kutzing) Kutzing	0.00			0.01			0.00			0.00		
APED	*Amphora pediculus* (Kutzing) Grunow	0.08			0.75			0.08			0.16		
ASHU	*Achnanthes subhudsonis* Hustedt	0.00			5.36			0.00			5.17	40.1	0.01
AUDI	*Aulacoseira distans* (Ehrenberg) Simonsen	0.00			0.04			0.03			0.00		
AUGR	*Aulacoseira granulata* (Ehrenberg) Simonsen	0.00			0.01			0.00			0.00		
AVEN	*Amphora veneta* Kutzing	0.00			0.01			0.08			0.60	23.7	0.01
BPAX	*Bacillaria paxillifera* (O.F. Muller) Hendey var. *paxillifera*	0.00			0.00			0.00			0.00		
CAFF	*Cymbella excisa* Kutzing	0.00			0.02			0.00			0.00		
CAGR	*Cyclotella atomus* var. *gracilis* Genkal & Kiss	0.00			0.00			0.00			0.00		
CATO	*Cyclotella atomus* Hustedt	0.00			0.00			0.00			0.00		
CBAC	*Caloneis bacillum* (Grunow) Cleve	0.00			0.05			0.00			0.03		
CDUB	*Cyclostephanos dubius* (Fricke) Round	0.00			0.02			0.11			0.00		
CINV	*Cyclostephanos invisitatus* (Hohn & Hellerman) Theriot Stoermer & Hakansson	0.03			0.09			0.08			0.01		
CMED	*Cyclotella meduanae* Germain	0.00			0.01			0.00			0.00		
CMEN	*Cyclotella meneghiniana* Kutzing	0.08			0.36			0.06			0.25		
CMLF	*Craticula molestiformis* (Hustedt) Lange-Bertalot	1.74	35.1	0.01	0.05			0.00			0.00		
COCE	*Cyclotella ocellata* Pantocsek	0.00			0.00			0.00			0.00		
CPED	*Cocconeis pediculus* Ehrenberg	0.00			0.02			0.00			0.00		
CPLA	*Cocconeis placentula* Ehrenberg var. *placentula*	0.61			1.44	6.8	0.68	0.11			0.29		
CPLE	*Cocconeis placentula* Ehrenberg var. *euglypta* (Ehrenberg) Grunow	2.32			1.23			2.34			3.45		
CPLI	*Cocconeis placentula* Ehrenberg var. *lineata* (Ehrenberg) Van Heurck	0.80			8.98			1.54			2.38		
CPPL	*Cocconeis placentula* Ehrenberg var. *pseudolineata* Geitler	0.05			0.28			0.03			0.79	14.1	0.07
CPST	*Cyclotella pseudostelligera* Hustedt	0.27			0.46			0.88			0.44		
CRAC	*Craticula accomoda* (Hustedt) Mann	0.08	3.9	0.3	0.00			0.00			0.00		
CSOL	*Cymatopleura solea* (Brebisson) W.Smith	0.02			0.00			0.00			0.00		
CSTE	*Cyclotella stelligera* Cleve et Grunow	0.03			0.10	6.1	0.22	0.03			0.00		
CTUM	*Cymbella tumida* (Brebisson) Van Heurck	0.00			0.01			0.00			0.03		
DCOT	*Diadesmis contenta* (Grunow) Mann	0.05			0.00			0.00			0.00		
DMAR	*Diploneis marginestriata* Hustedt	0.00			0.00			0.00			0.00		
DMES	*Diatoma mesodon* (Ehrenberg) Kutzing	0.05			0.28			0.00			0.00		
DOBL	*Diploneis oblongella* (Naegeli) Cleve-Euler	0.00			0.01			0.00			0.00		
DOVA	*Diploneis ovalis* (Hilse) Cleve	0.00			0.00			0.00			0.00		
DTEN	*Denticula tenuis* Kutzing	0.00			0.02			0.00			0.00		
DVUL	*Diatoma vulgaris* Bory	0.00			0.04			0.00			0.18		
ECAE	*Encyonema caespitosum* Kutzing	0.00			0.11			0.00			0.00		
EEXI	*Eunotia exigua* (Brebisson) Rabenhorst	0.00			0.03			0.00			0.00		
EMIN	*Eunotia minor* (Kutzing) Grunow	0.00			0.08			0.00			0.00		
ENCM	*Encyonopsis microcephala* (Grunow) Krammer	0.02			0.03			0.00			0.00		
ENMI	*Encyonema minutum* (Hilse) D.G. Mann	0.13			3.49			1.60			2.99		
EOCO	*Eolimna comperei* Ector. Coste et Iserentant	0.00			0.00			0.00			0.00		
EOMI	*Eolimna minima* (Grunow) Lange-Bertalot	28.03	27.9	0.02	6.81			5.20			1.27		
EPRO	*Encyonema prostratum* (Berkeley) Kutzing	0.00			0.00			0.00			0.00		
ESBM	*Eolimna subminuscula* (Manguin) Moser Lange-Bertalot & Metzeltin	1.24			0.98			6.07			12.62	32.2	0.01

ESLE	*Encyonema silesiacum* (Bleisch) D.G. Mann	0.53			0.90			0.03			0.78		
ETEN	*Eunotia tenella* (Grunow) Hustedt	0.00			0.00			0.00			0.00		
EUIN	*Eunotia intermedia* (Krasske) Norpel & Lange-Bertalot	0.00			0.00			0.00			0.00		
FARC	*Fragilaria arcus* (Ehrenberg) Cleve	0.00			0.41			0.00			0.08		
FBID	*Fragilaria bidens* Heiberg	0.06			0.44	22.4	0.02	0.00			0.03		
FCAP	*Fragilaria capucina* Desmazieres var. *capucina*	0.11			0.35			0.08			0.11		
FCRO	*Fragilaria crotonensis* Kitton	0.00			0.10			0.31	22.8	0.03	0.00		
FCRP	*Fragilaria capucina* Desmaziere ssp. *rumpens* (Kutzing) Lange-Bertalot	0.22			0.85			0.11			0.04		
FCVA	*Fragilaria capucina* Desmazieres var. *vaucheriae* (Kutzing) Lange-Bertalot	0.45			3.03	28.5	0.02	2.84			0.55		
FGRA	*Fragilaria gracilis* Ostrup	0.00			0.00			0.00			0.00		
FLEN	*Fallacia lenzi* (Hustedt) Lange-Bertalot	0.00			0.00			0.00			0.00		
FMOC	*Fallacia monoculata* (Hustedt) Mann	0.00			0.00			0.00			0.00		
FPUL	*Fragilaria pulchella* (Ralfs) Lange-Bertalot	0.09	2.7	0.41	0.05			0.00			0.01		
FSAP	*Fistulifera saprophila* (Lange-Bertalot & Bonik) Lange-Bertalot	5.31	21.6	0.04	1.23			0.88			4.79		
FSBH	*Fallacia subhamulata* (Grunow) Mann	0.00			0.00			0.00			0.00		
FUAC	*Fragilaria ulna* (Nitzsch) Lange-Bertalot var. *acus* (Kutzing) Lange-Bertalot	0.00			0.01			0.00			0.00		
GACC	*Geissleria acceptata* (Hustedt) Lange-Bertalot & Metzeltin	0.00			0.10			0.08			0.01		
GANG	*Gomphonema angustatum* (Kutzing) Rabenhorst	0.00			0.05			0.00			0.00		
GEXL	*Gomphonema exilissimum* (Grunow) Lange-Bertalot & Reichardt	0.00			0.23			0.20			0.00		
GGRA	*Gomphonema gracile* Ehrenberg	0.00			0.01			0.00			0.04	2.2	0.66
GMIC	*Gomphonema micropus* Kutzing var. *micropus*	0.05			0.10			0.37			0.08		
GMIN	*Gomphonema minutum* (Agardh) Agardh f. *minutum*	0.05			0.20			1.56	21.3	0.03	0.25		
GNOD	*Gyrosigma nodiferum* (Grunow) Reimer	0.00			0.00			0.00			0.00		
GOLI	*Gomphonema olivaceum* (Hornemann) Brebisson var. *olivaceum*	0.02			0.01			0.00			0.00		
GOMS	*Gomphonema* spp.	0.03			0.01			0.00			0.00		
GPAR	*Gomphonema parvulum* (Kutzing) Kutzing	2.66			1.98			2.71			0.75		
GPAS	*Gomphonema parvulum* var. *parvulum* f. *saprophilum* Lange-Bert.&Reichardt	0.00			0.00			0.00			0.03		
GPLI	*Gomphosphenia lingulatiformis* (Lange-Bertalot & Reichardt) Lange-Bertalot	0.00			0.00			0.00			0.00		
GPRI	*Gomphonema pumilum* var. *rigidum* Reichardt & Lange-Bertalot	0.00			0.00			0.00			0.00		
GPUM	*Gomphonema pumilum* (Grunow) Reichardt & Lange-Bertalot	0.03			0.03			0.36			0.00		
GPVL	*Gomphonema parvulius* Lange-Bertalot & Reichardt	0.00			0.09			0.08			0.00		
GRHB	*Gomphonema rhombicum* M. Schmidt	0.00			0.00			0.00			0.00		
GTER	*Gomphonema tergestinum* Fricke	0.00			0.07			0.25	12.9	0.1	0.03		
GTRU	*Gomphonema truncatum* Ehrenberg	0.00			0.02			0.00			0.01		
GYAC	*Gyrosigma acuminatum* (Kutzing)Rabenhorst	0.00			0.01			0.00			0.00		
GYAT	*Gyrosigma attenuatum* (Kutzing) Rabenhorst	0.00			0.00			0.00			0.00		
HCAP	*Hippodonta capitata* (Ehrenberg) Lange-Bertalot Metzeltin & Witkowski	0.00			0.09			0.00			0.03		
KCLE	*Karayevia clevei* (Grunow) Bukhtiyarova	0.00			0.04			0.03			0.01		
KLAT	*Karayevia laterostrata* (Hustedt) Kingston	0.02			0.04			0.03			0.00		
KPLO	*Kolbesia ploenensis* (Hustedt) Kingston	0.00			0.01			0.00			0.01		
LGOE	*Luticola goeppertiana* (Bleisch) Mann	0.00			0.11			0.22			0.04		
LHUN	*Lemnicola hungarica* (Grunow) Round & Basson	0.00			0.01			0.00			0.00		
LMUT	*Luticola mutica* (Kutzing) Mann	0.00			0.01			0.00			0.01		
MAAL	*Mayamaea atomus* var. *alcimonica* (Reichardt) Reichardt	0.00			0.01			0.00			0.00		
MAAT	*Mayamaea atomus* (Kutzing) Lange-Bertalot	0.16			0.07			0.06			0.00		
MAPE	*Mayamaea atomus* var. *permitis* (Hustedt) Lange-Bertalot	16.88	30.7	0.02	2.92			5.52			5.91		
MCIR	*Meridion circulare* (Greville) C.A. Agardh var. *circulare*	0.00			0.06	4.3	0.33	0.00			0.00		
MLLC	*Mayamaea lacunolaciniata* (Lange-Bertalot & Bonik) Lange-Bertalot	0.00			0.05			0.00			0.10	4.2	0.28
MVAR	*Melosira varians* Agardh	0.03			1.70			0.25			0.38		
NACI	*Nitzschia acicularis* (Kutzing) W.M.Smith	0.03			0.07			0.00			0.03		
NACU	*Nitzschia acula* Hantzsch	0.00			0.01			0.00			0.00		
NAGF	*Nitzschia angustiforaminata* Lange-Bertalot	0.02			0.02			0.00			0.00		
NAMP	*Nitzschia amphibia* Grunow f. *amphibia*	0.06			0.05			1.68			3.70	24.4	0.03
NANT	*Navicula antonii* Lange-Bertalot	0.00			0.09			0.00			0.04		
NARV	*Navicula arvensis* Hustedt	0.03			0.01			0.00			0.00		
NASP	*Navicula* sp.	0.00			0.15			0.11			0.03		
NBRG	*Nitzschia bergii* Cleve-Euler	0.00			0.05			0.00			0.00		
NCIN	*Navicula cincta* (Ehrenberg) Ralfs	0.06			0.00			0.00			0.00		
NCPL	*Nitzschia capitellata* Hustedt	0.30			0.25			1.26	22.9	0.03	0.01		
NCPR	*Navicula capitatoradiata* Germain	0.13			0.11			0.00			0.43		
NCRY	*Navicula cryptocephala* Kutzing	0.66			1.66	18.5	0.07	0.50			0.24		
NCTE	*Navicula cryptotenella* Lange-Bertalot	0.44			0.63			0.39			0.15		
NCTO	*Navicula cryptotenelloides* Lange-Bertalot	0.00			0.09			0.31			0.05		
NCTV	*Navicula caterva* Hohn & Hellerman	0.00			0.00			0.00			0.00		
NDIF	*Navicula difficillima* Hustedt	0.00			0.02			0.00			0.01		
NDIS	*Nitzschia dissipata* (Kutzing) Grunow var. dissipata	0.24			0.69			0.31			0.08		
NERI	*Navicula erifuga* Lange-Bertalot	0.00			0.03			0.03			0.00		
NEXI	*Navicula exilis* Kutzing	0.02			0.17			0.14			0.00		
NFIL	*Nitzschia filiformis* (W.M.Smith) Van Heurck var. *filiformis*	0.08			0.01			0.03			0.00		
NFON	*Nitzschia fonticola* Grunow	0.45			2.04			15.40	39.4	0	8.85		
NGER	*Navicula germainii* Wallace	0.02			0.13			0.11			0.03		
NGRE	*Navicula gregaria* Donkin	1.93			4.49			5.04	18	0.06	1.15		
NHAN	*Nitzschia hantzschiana* Rabenhorst	0.00			0.10			0.00			0.01		
NHEU	*Nitzschia heufleriana* Grunow	0.00			0.00			0.00			0.00		
NIAR	*Nitzschia archibaldii* Lange-Bertalot	0.38			0.19			0.00			0.00		
NIFR	*Nitzschia frustulum* (Kutzing) Grunow var. *frustulum*	0.22			0.07			0.42			0.32		

NIGR	*Nitzschia gracilis* Hantzsch	0.05			0.01			0.00			0.00		
NINC	*Nitzschia inconspicua* Grunow	4.58			1.34			10.06			12.28		
NING	*Navicula ingenua* Hustedt	0.00			0.00			0.00			0.00		
NINT	*Nitzschia intermedia* Hantzsch	0.00			0.01			0.03			0.01		
NIPF	*Nitzschia paleaeformis* Hustedt	0.00			0.14			0.00			0.28	10.4	0.09
NIPU	*Nitzschia pusilla* (Kutzing) Grunow	0.06			0.03			0.00			0.04		
NLAN	*Navicula lanceolata* (Agardh) Ehrenberg	0.39			1.88			2.04	17.8	0.08	0.11		
NLEV	*Nitzschia levidensis* (W.Smith) Grunow	0.00			0.05			0.00			0.00		
NLIN	*Nitzschia linearis* (Agardh) W.M.Smith var. *linearis*	0.00			0.07			0.00			0.01		
NMEN	*Navicula menisculus* Schumann var. *menisculus*	0.08			0.05			0.00			0.00		
NMIC	*Nitzschia microcephala* Grunow	0.00			0.01			0.00			0.00		
NNOV	*Navicula novaesiberica* Lange-Bertalot	0.00			0.00			0.00			0.00		
NPAD	*Nitzschia palea* (Kutzing) W.Smith var. *debilis* (Kutzing) Grunow	0.00			0.20			0.03			0.00		
NPAE	*Nitzschia paleacea* (Grunow) Grunow	0.09			3.19			6.76			6.78	29.1	0.02
NPAL	*Nitzschia palea* (Kutzing) W.Smith	4.16			2.11			2.06			1.68		
NRCH	*Navicula reichardtiana* Lange-Bertalot	0.17			0.14			0.03			0.01		
NRCS	*Navicula recens* (Lange-Bertalot) Lange-Bertalot	0.00			0.01			0.06			0.03		
NREC	*Nitzschia recta* Hantzsch	0.02			0.22			0.03			0.00		
NRFA	*Navicula radiosafallax* Lange-Bertalot	0.00			0.01			0.00			0.00		
NRHY	*Navicula rhynchocephala* Kutzing	0.00			0.42			0.11			0.12		
NSHR	*Navicula schroeteri* Meister var. *schroeteri*	0.02			0.01			0.00			0.01		
NSLU	*Navicula sublucidula* Hustedt	0.00			0.00			0.00			0.00		
NSOC	*Nitzschia sociabilis* Hustedt	0.08			0.11			0.08			0.00		
NSOL	*Nitzschia solgensis* Cleve-Euler	0.00			0.00			0.00			0.00		
NSSY	*Navicula schroeteri* Meister var. *symmetrica* (Patrick) Lange-Bertalot	0.00			0.17			0.00			0.00		
NSUA	*Nitzschia subacicularis* Hustedt	0.00			0.06			0.06			0.05		
NTEN	*Navicula tenelloides* Hustedt	0.02			0.02			0.00			0.00		
NTPT	*Navicula tripunctata* (O.F.Muller) Bory	0.20			0.02			0.00			0.00		
NTRO	*Nitzschia tropica* Hustedt	0.00			0.06			0.00			0.73	22.6	0.02
NTRV	*Navicula trivialis* Lange-Bertalot var. *trivialis*	0.05			0.02			0.03			0.00		
NVDS	*Sellaphora seminulum* (Grunow) Mann	7.79	38.4	0.01	0.57			0.64			0.11		
NVEN	*Navicula veneta* Kutzing	0.42			0.03			0.00			0.00		
NVER	*Nitzschia vermicularis* (Kutzing) Hantzsch	0.00			0.00			0.00			0.00		
NVIR	*Navicula viridula* (Kutzing) Ehrenberg	0.00			0.00			0.00			0.00		
NVRO	*Navicula viridula* (Kutzing) Ehrenberg var. *rostellata* (Kutzing) Cleve	0.00			0.03			0.03			0.03		
NZAG	*Nitzschia angustatula* Lange-Bertalot	0.00			0.00			0.00			0.01		
NZLT	*Nitzschia linearis* (Agardh) W.M. Smith var. *tenuis* (W.Smith) Grunow	0.00			0.00			0.00			0.00		
NZSS	*Nitzschia* spp.	0.02			0.04			0.00			0.03		
NZSU	*Nitzschia supralitorea* Lange-Bertalot	0.00			0.02			0.00			0.03		
PBIO	*Psammothidium bioretii* (Germain) Bukhtiyarova et Round	0.00			0.59			0.11			0.08		
PCLT	*Placoneis clementis* (Grunow) Cox	0.00			0.04			0.00			0.00		
PDAO	*Psammothidium daonense* (Lange-Bertalot) Lange-Bertalot	0.02			0.10			0.03			0.00		
PDAU	*Planothidium daui* (Foged) Lange-Bertalot	0.03			0.91	22	0.04	0.03			0.26		
PGRN	*Planothidium granum* (Hohn & Hellerman) Lange-Bertalot	0.00			0.40	9.6	0.1	0.00			0.23		
PHEL	*Psammothidium helveticum* (Hustedt) Bukhtiyarova et Round	0.00			0.10			0.00			0.00		
PLAU	*Psammothidium lauenburgianum* (Hustedt) Bukhtiyarova et Round	0.02			0.05			0.00			0.01		
PLFR	*Planothidium frequentissimum* (Lange-Bertalot) Lange-Bertalot	1.27			2.78	14.7	0.13	0.79			0.89		
POBG	*Psammothidium oblongellum* (Oestrup) Van de Vijver	0.00			0.04			0.00			0.00		
PPRO	*Parlibellus protracta* (Grunow) Witkowski Lange-Bertalot & Metzeltin	0.00			0.10			0.11	7.4	0.15	0.03		
PRST	*Planothidium rostratum* (Oestrup) Lange-Bertalot	0.00			0.05			0.03			0.01		
PSAT	*Psammothidium subatomoides* (Hustedt) Bukhtiyarova et Round	0.31			1.37			0.08			0.13		
PSBR	*Pseudostaurosira brevistriata* (Grunow) Williams & Round	0.00			0.03			0.00			0.01		
PTDE	*Planothidium delicatulum* (Kutzing) Round & Bukhtiyarova	0.00			0.01			0.06			0.00		
PTEL	*Planothidium ellipticum* (Cleve)Round & Bukhtiyarova	0.00			0.03			0.03			0.01		
PTHA	*Planothidium hauckianum* (Grunow) Round & Bukhtiyarova	0.00			0.00			0.00			0.00		
PTLA	*Planothidium lanceolatum* (Brebisson) Lange-Bertalot	0.20			1.40			1.79			0.20		
RABB	*Rhoicosphenia abbreviata* (C.Agardh) Lange-Bertalot	0.95			0.05			0.22			0.07		
RSIN	*Reimeria sinuata* (Gregory) Kociolek & Stoermer	3.35			1.59			5.01	29.8	0.03	4.33		
RUNI	*Reimeria uniseriata* Sala, Guerrero & Ferrario	0.00			0.09			0.03			0.00		
SANG	*Surirella angusta* Kutzing	0.00			0.08			0.14	8.6	0.21	0.00		
SBKU	*Surirella brebissonii* var. *kuetzingii* Krammer et Lange-Bertalot	0.00			0.01			0.00			0.00		
SBRE	*Surirella brebissonii* Krammer & Lange-Bertalot	0.00			0.09			0.08			0.07		
SCON	*Staurosira construens* Ehrenberg	0.02			0.06			0.03			0.03		
SELI	*Staurosira elliptica* (Schumann) Williams & Round	0.00			0.00			0.00			0.00		
SFSC	*Synedra fasciculata* Kutzing	0.00			0.00			0.00			0.00		
SHAN	*Stephanodiscus hantzschii* Grunow	0.00			0.06			0.00			0.00		
SHTE	*Stephanodiscus hantzschii* f. *tenuis* (Hustedt) Hakansson et Stoermer	0.00			0.05			0.06			0.01		
SIDE	*Simonsenia delognei* Lange-Bertalot	0.02			0.03			0.00			0.01		
SKPO	*Skeletonema potamos* (Weber) Hasle	0.00			0.00			0.00			0.00		
SLIN	*Surirella linearis* Smith	0.00			0.03			0.00			0.00		
SPUP	*Sellaphora pupula* (Kutzing) Mereschkowksy	0.02			0.07			0.03			0.01		
SRPI	*Staurosira pinnata* Ehrenberg	0.06			0.08			0.00			0.00		
SSVE	*Staurosira venter* (Ehrenberg) Cleve & Moeller	0.00			0.15			0.00			0.13		
TAPI	*Tryblionella apiculata* Gregory	0.00			0.01			0.00			0.00		
TBRA	*Thalassiosira bramaputrae* (Ehrenberg) Hakansson & Locker	0.00			0.00			0.00			0.00		
THUN	*Tryblionella hungarica* (Grunow) Mann	0.02			0.00			0.00			0.00		
TPSN	*Thalassiosira pseudonana* Hasle et Heimdal	0.00			0.07			0.17	6	0.34	0.17		
TWEI	*Thalassiosira weissflogii* (Grunow) Fryxell & Hasle	0.00			0.00			0.00			0.00		

UULN	*Ulnaria ulna* (Nitzsch) CompÞre	0.09			0.26			0.06			0.05		

		Diatom assemblages of crystalline mountainous regions											
		28 □Small rivers without any pollution, low electrolyte content			29 □Small acidic rivers, with little organic pollution			30 □Small rivers with low conductivities and very low pollution level			31 □Very small rivers without any pollution, low electrolyte content		
	Group	28	28	28	29	29	29	30	30	30	31	31	31
Omnidia Code	Taxon name	%	indval	p-value	%	indval	p-value	%	indval	p-value	%	indval	p-value
AAMB	*Aulacoseira ambigua* (Grunow) Simonsen	0.00			0.00			0.00			0.00		
AATO	*Achnanthes atomus* Hustedt	0.00			0.00			0.00			0.00		
ACHS	*Achnanthes* sp.	0.00			0.00			0.00			0.00		
ACON	*Achnanthes conspicua* A.Mayer	0.12			0.00			0.06			0.00		
ACOP	*Amphora copulata* (Kutzing) Schoeman & Archibald	0.00			0.00			0.06			0.00		
ADBI	*Achnanthidium biasolettianum* (Grunow) Lange-Bertalot	0.17			0.00			0.06			0.00		
ADCT	*Achnanthidium catenatum* (Bily & Marvan) Lange-Bertalot	0.74			0.00			0.61			0.00		
ADEU	*Achnanthidium eutrophilum* (Lange-Bertalot) Lange-Bertalot	0.00			0.00			0.00			0.00		
ADKR	*Achnanthidium kranzii* (Lange-Bertalot) Round & Bukhtiyarova	0.00			0.00			0.03			6.17	49.8	0
ADLS	*Adlafia suchlandtii* (Hustedt) Moser Lange-Bertalot & Metzeltin	0.00			0.00			3.24	46.7	0	0.00		
ADMF	*Achnanthidium minutissimum* (Kutzing) Czarnecki var. *affinis* (Grunow) Bukht.	0.00			0.00			0.00			0.00		
ADMI	*Achnanthidium minutissimum* (Kutzing) Czarnecki	27.07			0.00			24.33			17.20		
ADMM	*Adlafia minuscula* var. *muralis* (Grunow) Lange-Bertalot	0.26			0.00			0.74	29.9	0.01	0.26		
ADMS	*Adlafia minuscula* (Grunow) Lange-Bertalot	0.02			0.00			0.00			0.00		
ADSA	*Achnanthidium saprophilum* (Kobayasi et Mayama) Round & Bukhtiyarova	0.00			0.00			0.00			0.00		
ADSB	*Achnanthidium straubianum* (Lange-Bertalot)Lange-Bertalot	0.00			0.00			0.00			0.00		
ADSU	*Achnanthidium subatomus* (Hustedt) Lange-Bertalot	0.05			0.00			10.61	59.2	0	0.00		
AFOR	*Asterionella formosa* Hassall	0.00			0.00			0.00			0.00		
AFUG	*Achnanthes fugei* Carter	0.00			0.00			0.00			0.00		
AINA	*Amphora inariensis* Krammer	0.00			0.00			0.00			0.00		
AMII	*Achnanthes minutissima* Kutzing var. *inconspicua* Oestrup	0.00			0.00			0.00			18.50	45.3	0
AMMO	*Amphora montana* Krasske	0.00			0.00			0.00			0.00		
AOVA	*Amphora ovalis* (Kutzing) Kutzing	0.05			0.00			0.00			0.00		
APED	*Amphora pediculus* (Kutzing) Grunow	0.52			0.00			0.23			0.00		
ASHU	*Achnanthes subhudsonis* Hustedt	0.00			0.00			0.00			0.00		
AUDI	*Aulacoseira distans* (Ehrenberg) Simonsen	0.00			0.27			0.00			0.00		
AUGR	*Aulacoseira granulata* (Ehrenberg) Simonsen	0.00			0.00			0.00			0.00		
AVEN	*Amphora veneta* Kutzing	0.09			0.00			0.00			0.00		
BPAX	*Bacillaria paxillifera* (O.F. Muller) Hendey var. *paxillifera*	0.00			0.00			0.00			0.00		
CAFF	*Cymbella excisa* Kutzing	0.00			0.00			0.00			0.00		
CAGR	*Cyclotella atomus* var. *gracilis* Genkal & Kiss	0.00			0.00			0.00			0.00		
CATO	*Cyclotella atomus* Hustedt	0.00			0.00			0.00			0.00		
CBAC	*Caloneis bacillum* (Grunow) Cleve	0.02			0.00			0.00			0.00		
CDUB	*Cyclostephanos dubius* (Fricke) Round	0.00			0.00			0.00			0.00		
CINV	*Cyclostephanos invisitatus* (Hohn & Hellerman) Theriot Stoermer & Hakansson	0.00			0.00			0.00			0.00		
CMED	*Cyclotella meduanae* Germain	0.00			0.00			0.00			0.00		
CMEN	*Cyclotella meneghiniana* Kutzing	0.09			0.00			0.00			0.12		
CMLF	*Craticula molestiformis* (Hustedt) Lange-Bertalot	0.05			0.00			0.00			0.00		
COCE	*Cyclotella ocellata* Pantocsek	0.00			0.00			0.00			0.00		
CPED	*Cocconeis pediculus* Ehrenberg	0.00			0.00			0.00			0.00		
CPLA	*Cocconeis placentula* Ehrenberg var. *placentula*	0.54			0.00			0.09			0.12		
CPLE	*Cocconeis placentula* Ehrenberg var. *euglypta* (Ehrenberg) Grunow	0.02			0.00			0.00			0.00		
CPLI	*Cocconeis placentula* Ehrenberg var. *lineata* (Ehrenberg) Van Heurck	0.19			0.00			18.52	51.9	0.01	0.13		
CPPL	*Cocconeis placentula* Ehrenberg var. *pseudolineata* Geitler	0.10			0.00			0.36			0.00		
CPST	*Cyclotella pseudostelligera* Hustedt	0.02			0.00			0.00			0.00		
CRAC	*Craticula accomoda* (Hustedt) Mann	0.00			0.00			0.00			0.00		
CSOL	*Cymatopleura solea* (Brebisson) W.Smith	0.00			0.00			0.00			0.00		
CSTE	*Cyclotella stelligera* Cleve et Grunow	0.00			0.00			0.00			0.00		
CTUM	*Cymbella tumida* (Brebisson) Van Heurck	0.00			0.00			0.00			0.00		
DCOT	*Diadesmis contenta* (Grunow) Mann	0.00			0.00			0.00			0.00		
DMAR	*Diploneis marginestriata* Hustedt	0.00			0.00			0.00			0.00		
DMES	*Diatoma mesodon* (Ehrenberg) Kutzing	2.63			0.00			0.62			4.97	57.7	0
DOBL	*Diploneis oblongella* (Naegeli) Cleve-Euler	0.00			0.00			0.00			0.00		
DOVA	*Diploneis ovalis* (Hilse) Cleve	0.00			0.00			0.00			0.00		
DTEN	*Denticula tenuis* Kutzing	0.02			0.00			0.00			0.00		
DVUL	*Diatoma vulgaris* Bory	0.00			0.00			0.00			0.00		
ECAE	*Encyonema caespitosum* Kutzing	0.00			0.00			0.00			0.00		
EEXI	*Eunotia exigua* (Brebisson) Rabenhorst	1.03	56.4	0	0.82			0.06			0.37		
EMIN	*Eunotia minor* (Kutzing) Grunow	2.17	53.7	0	0.00			0.69			0.00		
ENCM	*Encyonopsis microcephala* (Grunow) Krammer	0.00			0.00			0.00			0.00		
ENMI	*Encyonema minutum* (Hilse) D.G. Mann	0.85			0.00			4.32	16.9	0.08	1.44		
EOCO	*Eolimna comperei* Ector. Coste et Iserentant	0.00			0.00			0.00			0.00		
EOMI	*Eolimna minima* (Grunow) Lange-Bertalot	11.27			0.27			6.12			0.00		
EPRO	*Encyonema prostratum* (Berkeley) Kutzing	0.00			0.00			0.00			0.00		
ESBM	*Eolimna subminuscula* (Manguin) Moser Lange-Bertalot & Metzeltin	0.00			0.00			0.00			0.00		
ESLE	*Encyonema silesiacum* (Bleisch) D.G. Mann	0.10			0.00			1.40			6.28	59.1	0

ETEN	*Eunotia tenella* (Grunow) Hustedt	0.00			13.42			0.11	11.1	0.05	0.00		
EUIN	*Eunotia intermedia* (Krasske) Norpel & Lange-Bertalot	0.00			80.82			0.00			0.00		
FARC	*Fragilaria arcus* (Ehrenberg) Cleve	0.00			0.00			0.17			0.49	20.6	0.03
FBID	*Fragilaria bidens* Heiberg	0.00			0.00			0.00			0.00		
FCAP	*Fragilaria capucina* Desmazieres var. *capucina*	3.05			0.00			0.73			4.29	47.5	0.01
FCRO	*Fragilaria crotonensis* Kitton	0.00			0.00			0.00			0.00		
FCRP	*Fragilaria capucina* Desmaziere ssp. *rumpens* (Kutzing) Lange-Bertalot	0.19			0.00			4.76	41.3	0.01	0.00		
FCVA	*Fragilaria capucina* Desmazieres var. *vaucheriae* (Kutzing) Lange-Bertalot	0.42			0.00			1.67			0.00		
FGRA	*Fragilaria gracilis* Ostrup	0.02			0.00			0.08			25.27	99.5	0
FLEN	*Fallacia lenzi* (Hustedt) Lange-Bertalot	0.00			0.00			0.00			0.00		
FMOC	*Fallacia monoculata* (Hustedt) Mann	0.00			0.00			0.00			0.00		
FPUL	*Fragilaria pulchella* (Ralfs) Lange-Bertalot	0.00			0.00			0.00			0.00		
FSAP	*Fistulifera saprophila* (Lange-Bertalot & Bonik) Lange-Bertalot	0.12			0.00			0.34			0.00		
FSBH	*Fallacia subhamulata* (Grunow) Mann	0.02			0.00			0.00			0.00		
FUAC	*Fragilaria ulna* (Nitzsch) Lange-Bertalot var. *acus* (Kutzing) Lange-Bertalot	0.00			0.00			0.00			0.00		
GACC	*Geissleria acceptata* (Hustedt) Lange-Bertalot & Metzeltin	0.00			0.00			0.08	3.8	0.45	0.00		
GANG	*Gomphonema angustatum* (Kutzing) Rabenhorst	0.00			0.00			0.00			0.00		
GEXL	*Gomphonema exilissimum* (Grunow) Lange-Bertalot & Reichardt	2.14			0.00			0.14			2.62	48.7	0
GGRA	*Gomphonema gracile* Ehrenberg	0.02			0.00			0.00			0.00		
GMIC	*Gomphonema micropus* Kutzing var. *micropus*	0.05			0.00			0.00			5.76	87.7	0
GMIN	*Gomphonema minutum* (Agardh) Agardh f. *minutum*	0.00			0.00			0.00			0.00		
GNOD	*Gyrosigma nodiferum* (Grunow) Reimer	0.00			0.00			0.00			0.00		
GOLI	*Gomphonema olivaceum* (Hornemann) Brebisson var. *olivaceum*	0.00			0.00			0.00			0.00		
GOMS	*Gomphonema* spp.	0.00			0.00			0.00			0.00		
GPAR	*Gomphonema parvulum* (Kutzing) Kutzing	2.52			0.00			1.01			0.49		
GPAS	*Gomphonema parvulum* var. *parvulum* f. *saprophilum* Lange-Bert.&Reichardt	0.00			0.00			0.00			0.00		
GPLI	*Gomphosphenia lingulatiformis* (Lange-Bertalot & Reichardt) Lange-Bertalot	0.00			0.00			0.00			0.00		
GPRI	*Gomphonema pumilum* var. *rigidum* Reichardt & Lange-Bertalot	0.00			0.00			0.00			0.00		
GPUM	*Gomphonema pumilum* (Grunow) Reichardt & Lange-Bertalot	0.00			0.00			1.33			0.00		
GPVL	*Gomphonema parvulius* Lange-Bertalot & Reichardt	2.91	42.4	0	0.00			0.00			0.00		
GRHB	*Gomphonema rhombicum* M. Schmidt	2.89			0.00			2.43	30.4	0.01	0.00		
GTER	*Gomphonema tergestinum* Fricke	0.00			0.00			0.00			0.00		
GTRU	*Gomphonema truncatum* Ehrenberg	0.00			0.00			0.00			0.37	30.2	0.02
GYAC	*Gyrosigma acuminatum* (Kutzing)Rabenhorst	0.00			0.00			0.00			0.00		
GYAT	*Gyrosigma attenuatum* (Kutzing) Rabenhorst	0.05			0.00			0.00			0.00		
HCAP	*Hippodonta capitata* (Ehrenberg) Lange-Bertalot Metzeltin & Witkowski	0.00			0.00			0.00			0.00		
KCLE	*Karayevia clevei* (Grunow) Bukhtiyarova	0.00			0.00			0.00			0.00		
KLAT	*Karayevia laterostrata* (Hustedt) Kingston	0.00			0.00			0.00			0.00		
KPLO	*Kolbesia ploenensis* (Hustedt) Kingston	0.00			0.00			0.00			0.00		
LGOE	*Luticola goeppertiana* (Bleisch) Mann	0.00			0.00			0.00			0.00		
LHUN	*Lemnicola hungarica* (Grunow) Round & Basson	0.00			0.00			0.00			0.00		
LMUT	*Luticola mutica* (Kutzing) Mann	0.00			0.00			0.00			0.00		
MAAL	*Mayamaea atomus* var. *alcimonica* (Reichardt) Reichardt	0.00			0.00			0.00			0.00		
MAAT	*Mayamaea atomus* (Kutzing) Lange-Bertalot	0.05			0.00			0.00			0.00		
MAPE	*Mayamaea atomus* var. *permitis* (Hustedt) Lange-Bertalot	0.33			0.00			1.84			0.79		
MCIR	*Meridion circulare* (Greville) C.A. Agardh var. *circulare*	0.00			0.00			0.00			0.00		
MLLC	*Mayamaea lacunolaciniata* (Lange-Bertalot & Bonik) Lange-Bertalot	0.00			0.00			0.00			0.00		
MVAR	*Melosira varians* Agardh	0.05			0.27			0.20			0.00		
NACI	*Nitzschia acicularis* (Kutzing) W.M.Smith	0.14			0.00			0.00			0.13	8.6	0.19
NACU	*Nitzschia acula* Hantzsch	0.00			0.00			0.00			0.00		
NAGF	*Nitzschia angustiforaminata* Lange-Bertalot	0.00			0.00			0.00			0.00		
NAMP	*Nitzschia amphibia* Grunow f. *amphibia*	0.10			0.00			0.00			0.00		
NANT	*Navicula antonii* Lange-Bertalot	0.00			0.00			0.00			0.00		
NARV	*Navicula arvensis* Hustedt	0.05			0.00			0.00			0.00		
NASP	*Navicula* sp.	0.19	6.5	0.35	0.00			0.00			0.00		
NBRG	*Nitzschia bergii* Cleve-Euler	0.00			0.00			0.00			0.00		
NCIN	*Navicula cincta* (Ehrenberg) Ralfs	0.00			0.00			0.00			0.00		
NCPL	*Nitzschia capitellata* Hustedt	0.12			0.00			0.00			0.00		
NCPR	*Navicula capitatoradiata* Germain	0.00			0.00			0.00			0.00		
NCRY	*Navicula cryptocephala* Kutzing	1.73			0.00			0.45			0.12		
NCTE	*Navicula cryptotenella* Lange-Bertalot	0.17			0.00			0.03			0.00		
NCTO	*Navicula cryptotenelloides* Lange-Bertalot	0.00			0.00			0.00			0.00		
NCTV	*Navicula caterva* Hohn & Hellerman	0.00			0.00			0.00			0.00		
NDIF	*Navicula difficillima* Hustedt	0.02			0.00			0.06			0.00		
NDIS	*Nitzschia dissipata* (Kutzing) Grunow var. dissipata	0.17			0.00			0.87			0.12		
NERI	*Navicula erifuga* Lange-Bertalot	0.02			0.00			0.00			0.00		
NEXI	*Navicula exilis* Kutzing	1.66	33.1	0.01	0.00			0.62			0.52		
NFIL	*Nitzschia filiformis* (W.M.Smith) Van Heurck var. *filiformis*	0.00			0.00			0.00			0.00		
NFON	*Nitzschia fonticola* Grunow	0.61			0.00			0.00			0.00		
NGER	*Navicula germainii* Wallace	0.05			0.00			0.00			0.00		
NGRE	*Navicula gregaria* Donkin	0.40			0.00			2.67			0.38		
NHAN	*Nitzschia hantzschiana* Rabenhorst	0.53	13.2	0.06	0.00			0.06			0.00		
NHEU	*Nitzschia heufleriana* Grunow	0.00			0.00			0.00			0.00		
NIAR	*Nitzschia archibaldii* Lange-Bertalot	1.10			0.00			1.88	23	0.03	0.00		
NIFR	*Nitzschia frustulum* (Kutzing) Grunow var. *frustulum*	0.00			0.00			0.00			0.00		
NIGR	*Nitzschia gracilis* Hantzsch	0.00			0.00			0.00			0.00		

NINC	*Nitzschia inconspicua* Grunow	0.17			0.00			0.00			0.00		
NING	*Navicula ingenua* Hustedt	0.00			0.00			0.00			0.00		
NINT	*Nitzschia intermedia* Hantzsch	0.00			0.00			0.00			0.00		
NIPF	*Nitzschia paleaeformis* Hustedt	0.00			0.00			0.00			0.00		
NIPU	*Nitzschia pusilla* (Kutzing) Grunow	0.00			0.00			0.06	2.9	0.68	0.00		
NLAN	*Navicula lanceolata* (Agardh) Ehrenberg	0.09			0.00			0.83			0.12		
NLEV	*Nitzschia levidensis* (W.Smith) Grunow	0.00			0.00			0.00			0.00		
NLIN	*Nitzschia linearis* (Agardh) W.M.Smith var. *linearis*	0.00			0.00			0.03			0.00		
NMEN	*Navicula menisculus* Schumann var. *menisculus*	0.00			0.00			0.03			0.00		
NMIC	*Nitzschia microcephala* Grunow	0.00			0.00			0.00			0.00		
NNOV	*Navicula novaesiberica* Lange-Bertalot	0.00			0.00			0.00			0.00		
NPAD	*Nitzschia palea* (Kutzing) W.Smith var. *debilis* (Kutzing) Grunow	0.09			0.00			0.23			0.00		
NPAE	*Nitzschia paleacea* (Grunow) Grunow	0.14			0.00			0.20			0.00		
NPAL	*Nitzschia palea* (Kutzing) W.Smith	0.52			0.00			0.69			0.39		
NRCH	*Navicula reichardtiana* Lange-Bertalot	0.05			0.00			0.08			0.00		
NRCS	*Navicula recens* (Lange-Bertalot) Lange-Bertalot	0.00			0.00			0.00			0.00		
NREC	*Nitzschia recta* Hantzsch	0.02			0.00			0.03			0.12		
NRFA	*Navicula radiosafallax* Lange-Bertalot	0.00			0.00			0.00			0.00		
NRHY	*Navicula rhynchocephala* Kutzing	0.56	18	0.04	0.00			0.14			0.00		
NSHR	*Navicula schroeteri* Meister var. *schroeteri*	0.00			0.00			0.00			0.00		
NSLU	*Navicula sublucidula* Hustedt	0.00			0.00			0.00			0.00		
NSOC	*Nitzschia sociabilis* Hustedt	0.07			0.00			0.00			0.00		
NSOL	*Nitzschia solgensis* Cleve-Euler	0.00			0.00			0.00			0.00		
NSSY	*Navicula schroeteri* Meister var. *symmetrica* (Patrick) Lange-Bertalot	0.00			0.00			0.00			0.00		
NSUA	*Nitzschia subacicularis* Hustedt	0.00			0.00			0.00			0.00		
NTEN	*Navicula tenelloides* Hustedt	0.05	1.8	0.82	0.00			0.00			0.00		
NTPT	*Navicula tripunctata* (O.F.Muller) Bory	0.23			0.00			0.06			0.00		
NTRO	*Nitzschia tropica* Hustedt	0.00			0.00			0.00			0.00		
NTRV	*Navicula trivialis* Lange-Bertalot var. *trivialis*	0.00			0.00			0.00			0.00		
NVDS	*Sellaphora seminulum* (Grunow) Mann	0.44			0.00			0.03			0.00		
NVEN	*Navicula veneta* Kutzing	0.00			0.00			0.06			0.00		
NVER	*Nitzschia vermicularis* (Kutzing) Hantzsch	0.00			0.00			0.00			0.00		
NVIR	*Navicula viridula* (Kutzing) Ehrenberg	0.00			0.00			0.00			0.00		
NVRO	*Navicula viridula* (Kutzing) Ehrenberg var. *rostellata* (Kutzing) Cleve	0.00			0.00			0.00			0.00		
NZAG	*Nitzschia angustatula* Lange-Bertalot	0.00			0.00			0.00			0.00		
NZLT	*Nitzschia linearis* (Agardh) W.M. Smith var. *tenuis* (W.Smith) Grunow	0.00			0.00			0.00			0.00		
NZSS	*Nitzschia* spp.	0.00			0.00			0.00			0.00		
NZSU	*Nitzschia supralitorea* Lange-Bertalot	0.00			0.00			0.00			0.00		
PBIO	*Psammothidium bioretii* (Germain) Bukhtiyarova et Round	0.85	22	0.03	0.00			0.14			0.25		
PCLT	*Placoneis clementis* (Grunow) Cox	0.52	24.1	0.03	0.00			0.00			0.00		
PDAO	*Psammothidium daonense* (Lange-Bertalot) Lange-Bertalot	0.63			0.00			0.68	20.2	0.03	0.39		
PDAU	*Planothidium daui* (Foged) Lange-Bertalot	0.17			0.00			0.00			0.00		
PGRN	*Planothidium granum* (Hohn & Hellerman) Lange-Bertalot	0.00			0.00			0.00			0.00		
PHEL	*Psammothidium helveticum* (Hustedt) Bukhtiyarova et Round	2.50	51	0	1.92			0.00			0.00		
PLAU	*Psammothidium lauenburgianum* (Hustedt) Bukhtiyarova et Round	0.00			0.00			0.00			0.00		
PLFR	*Planothidium frequentissimum* (Lange-Bertalot) Lange-Bertalot	2.23			0.55			0.42			0.00		
POBG	*Psammothidium oblongellum* (Oestrup) Van de Vijver	8.33	91.4	0	1.37			0.23			0.26		
PPRO	*Parlibellus protracta* (Grunow) Witkowski Lange-Bertalot & Metzeltin	0.00			0.00			0.00			0.00		
PRST	*Planothidium rostratum* (Oestrup) Lange-Bertalot	0.00			0.00			0.00			0.00		
PSAT	*Psammothidium subatomoides* (Hustedt) Bukhtiyarova et Round	9.07	63.3	0	0.00			0.43			0.12		
PSBR	*Pseudostaurosira brevistriata* (Grunow) Williams & Round	0.00			0.00			0.00			0.00		
PTDE	*Planothidium delicatulum* (Kutzing) Round & Bukhtiyarova	0.00			0.00			0.00			0.00		
PTEL	*Planothidium ellipticum* (Cleve)Round & Bukhtiyarova	0.02			0.00			0.00			0.00		
PTHA	*Planothidium hauckianum* (Grunow) Round & Bukhtiyarova	0.00			0.00			0.00			0.00		
PTLA	*Planothidium lanceolatum* (Brebisson) Lange-Bertalot	2.48	20.9	0.04	0.27			0.85			0.66		
RABB	*Rhoicosphenia abbreviata* (C.Agardh) Lange-Bertalot	0.07			0.00			0.29			0.00		
RSIN	*Reimeria sinuata* (Gregory) Kociolek & Stoermer	0.02			0.00			0.34			0.00		
RUNI	*Reimeria uniseriata* Sala, Guerrero & Ferrario	0.00			0.00			0.00			0.00		
SANG	*Surirella angusta* Kutzing	0.12			0.00			0.00			0.00		
SBKU	*Surirella brebissonii* var. *kuetzingii* Krammer et Lange-Bertalot	0.09			0.00			0.00			0.00		
SBRE	*Surirella brebissonii* Krammer & Lange-Bertalot	0.07			0.00			0.03			0.00		
SCON	*Staurosira construens* Ehrenberg	0.02			0.00			0.00			0.00		
SELI	*Staurosira elliptica* (Schumann) Williams & Round	0.00			0.00			0.00			0.00		
SFSC	*Synedra fasciculata* Kutzing	0.00			0.00			0.00			0.00		
SHAN	*Stephanodiscus hantzschii* Grunow	0.05			0.00			0.00			0.00		
SHTE	*Stephanodiscus hantzschii* f. *tenuis* (Hustedt) Hakansson et Stoermer	0.00			0.00			0.00			0.00		
SIDE	*Simonsenia delognei* Lange-Bertalot	0.00			0.00			0.00			0.00		
SKPO	*Skeletonema potamos* (Weber) Hasle	0.00			0.00			0.00			0.00		
SLIN	*Surirella linearis* Smith	0.21	15.5	0.06	0.00			0.00			0.12		
SPUP	*Sellaphora pupula* (Kutzing) Mereschkowksy	0.00			0.00			0.00			0.00		
SRPI	*Staurosira pinnata* Ehrenberg	0.02			0.00			0.32			0.00		
SSVE	*Staurosira venter* (Ehrenberg) Cleve & Moeller	0.00			0.00			0.00			0.00		
TAPI	*Tryblionella apiculata* Gregory	0.00			0.00			0.00			0.00		
TBRA	*Thalassiosira bramaputrae* (Ehrenberg) Hakansson & Locker	0.00			0.00			0.00			0.00		
THUN	*Tryblionella hungarica* (Grunow) Mann	0.00			0.00			0.00			0.00		
TPSN	*Thalassiosira pseudonana* Hasle et Heimdal	0.00			0.00			0.00			0.00		
TWEI	*Thalassiosira weissflogii* (Grunow) Fryxell & Hasle	0.00			0.00			0.00			0.00		
UULN	*Ulnaria ulna* (Nitzsch) CompÞre	0.00			0.00			0.17			0.64	29.2	0.02

Appendix 3

Appendix 3: References used to assign each taxon to diatom life-forms, ecological guilds and size classes

Genus	reference # 1	reference # 2	reference # 3	reference # 4	reference # 5
Achnanthes	F. E. Round, R. M. Crawford, and D. G. Mann. The diatoms. Biology, morphology of the genera., 1990. 747 pages. + personnal observations on cultures + F. Rimet, L. Ector, H. M. Cauchie, and L. Hoffmann. Changes in diatom-dominated biofilms during simulated improvements in water quality: implications for diatom-based monitoring in rivers. Eur.J.Phycol. 44:567-577, 2009.	K. Katoh. Correlation between cell density and dominant growth form of epilithic diatom assemblages. Diatom Research 7:77-86, 1992.	K. D. Hoagland, S. C. Roemer, and J. R. Rosowski. Colonization and community structure of two periphyton assemblages, with emphasis on the diatoms (Bacillariophyceae). American Journal of Botany 69:188-213, 1982.	F. Rimet, L. Ector, H. M. Cauchie, and L. Hoffmann. Changes in diatom-dominated biofilms during simulated improvements in water quality: implications for diatom-based monitoring in rivers. Eur.J.Phycol. 44:567-577, 2009.	C. M. Pringle. Nutrient spatial heterogeneity: effects on community structure, physiognomy, and diversity of stream algae. Ecology 71:905-920, 1990.
Achnanthidium	F. Straub. Note algologique II. Apparition envahissante de la diatomée Achnanthes catenata Bily & Marvan (Heterokontophyta, Bacillariophyceae) dans le lac de Neuchâtel. Bull.Soc.Neuchât.Sc.Nat. 125:59-66, 2002.	K. Katoh. Correlation between cell density and dominant growth form of epilithic diatom assemblages. Diatom Research 7:77-86, 1992.	K. D. Hoagland, S. C. Roemer, and J. R. Rosowski. Colonization and community structure of two periphyton assemblages, with emphasis on the diatoms (Bacillariophyceae). American Journal of Botany 69:188-213, 1982.	F. Rimet, L. Ector, H. M. Cauchie, and L. Hoffmann. Changes in diatom-dominated biofilms during simulated improvements in water quality: implications for diatom-based monitoring in rivers. Eur.J.Phycol. 44:567-577, 2009.	C. M. Pringle. Nutrient spatial heterogeneity: effects on community structure, physiognomy, and diversity of stream algae. Ecology 71:905-920, 1990.
Actinocyclus	K. Krammer and H. Lange-Bertalot. Bacillariophyceae 3. Teil: Centrales, Fragilariaceae, Eunotiaceae., 1991. 576 pages.				
Adlafia	Bibliotheca Diatomologica 46, 2002, Van de Vijver.				
Amphipleura	H. Germain. Flore des diatomées, eaux douces et saumâtres, Paris:1981. 444 pages.	K. D. Hoagland, S. C. Roemer, and J. R. Rosowski. Colonization and community structure of two periphyton assemblages, with emphasis on the diatoms (Bacillariophyceae). American Journal of Botany 69:188-213, 1982.			

Amphora	K. Krammer and H. Lange-Bertalot. Bacillariophyceae 1. Teil: Naviculaceae. 1986. 876 pages. + F. E. Round, R. M. Crawford, and D. G. Mann. The diatoms. Biology, morphology of the genera., 1990. 747 pages.	F. Rimet, L. Ector, H. M. Cauchie, and L. Hoffmann. Changes in diatom-dominated biofilms during simulated improvements in water quality: implications for diatom-based monitoring in rivers. Eur.J.Phycol. 44:567-577, 2009.			
Aneumastus	F. E. Round, R. M. Crawford, and D. G. Mann. The diatoms. Biology, morphology of the genera., 1990. 747 pages.				
Arachnoidiscus	Van Heurck, H. 1896. A Treatise on the Diatomaceae. Translated by W.E. Baxter. William Wesley & Son, London. 558 pp., 35 pls.				
Asterionella	K. Krammer and H. Lange-Bertalot. Bacillariophyceae 3. Teil: Centrales, Fragilariaceae, Eunotiaceae., 1991. 576 pages.				
Aulacoseira	Iconographia diatomologica, volume 2, 1996	K. Krammer and H. Lange-Bertalot. Bacillariophyceae 3. Teil: Centrales, Fragilariaceae, Eunotiaceae., 1991. 576 pages.	K. D. Hoagland, S. C. Roemer, and J. R. Rosowski. Colonization and community structure of two periphyton assemblages, with emphasis on the diatoms (Bacillariophyceae). American Journal of Botany 69:188-213, 1982.		
Bacillaria	Round, F.E. and Bukhtiyarova, L. 1996. Four new genera based on Achnanthes (Achnanthidium) together with a re-definition of Achnanthidium. Diatom Research 11(2):345-361. - no information on life form of Karayeva, Planothidium and Rossithidium	K. Katoh. Correlation between cell density and dominant growth form of epilithic diatom assemblages. Diatom Research 7:77-86, 1992.			

Berkeleya	F. E. Round, R. M. Crawford, and D. G. Mann. The diatoms. Biology, morphology of the genera., 1990. 747 pages.				
Caloneis	Round, F.E. and Bukhtiyarova, L. 1996. Four new genera based on Achnanthes (Achnanthidium) together with a re-definition of Achnanthidium. Diatom Research 11(2):345-361. - no information on life form of Karayeva, Planothidium and Rossithidium				
Campylodiscus	F. E. Round, R. M. Crawford, and D. G. Mann. The diatoms. Biology, morphology of the genera., 1990. 747 pages.				
Catenula	F. E. Round, R. M. Crawford, and D. G. Mann. The diatoms. Biology, morphology of the genera., 1990. 747 pages.	F. Rimet, L. Ector, H. M. Cauchie, and L. Hoffmann. Changes in diatom-dominated biofilms during simulated improvements in water quality: implications for diatom-based monitoring in rivers. Eur.J.Phycol. 44:567-577, 2009.			
Cavinula	F. E. Round, R. M. Crawford, and D. G. Mann. The diatoms. Biology, morphology of the genera., 1990. 747 pages.				
Chamaepinnularia	Iconographia Diatomologica 2				
Cocconeis	F. E. Round, R. M. Crawford, and D. G. Mann. The diatoms. Biology, morphology of the genera., 1990. 747 pages.	K. Katoh. Correlation between cell density and dominant growth form of epilithic diatom assemblages. Diatom Research 7:77-86, 1992.	M. R. Luttenton, J. B. Vansteenburg, and R. G. Rada. Phycoperiphyton in selected reaches of the Upper Mississippi River: community composition, architecture, and productivity. Hydrobiologia 136:31-46, 1986.	F. Rimet, L. Ector, H. M. Cauchie, and L. Hoffmann. Changes in diatom-dominated biofilms during simulated improvements in water quality: implications for diatom-based monitoring in rivers. Eur.J.Phycol. 44:567-577, 2009.	C. M. Pringle. Nutrient spatial heterogeneity: effects on community structure, physiognomy, and diversity of stream algae. Ecology 71:905-920, 1990.

Craticula	F. E. Round, R. M. Crawford, and D. G. Mann. The diatoms. Biology, morphology of the genera., 1990. 747 pages.				
Cyclostephanos	K. Krammer and H. Lange-Bertalot. Bacillariophyceae 3. Teil: Centrales, Fragilariaceae, Eunotiaceae., 1991. 576 pages.				
Cyclotella	Diatom Research 17, 1, 2002. Hakansson,	K. Katoh. Correlation between cell density and dominant growth form of epilithic diatom assemblages. Diatom Research 7:77-86, 1992.	DRUART, J.C. et STRAUB, F., 1988. - Description de deux nouvelles cyclotelles (Bacillariophyceae) de milieux alcalins et eutrophes : Cyclotella costei nov. sp. et Cyclotella wuethrichiana nov. sp. Schweiz. Z. Hydrol. 50 : 182-188.		
Cymatopleura	F. E. Round, R. M. Crawford, and D. G. Mann. The diatoms. Biology, morphology of the genera., 1990. 747 pages.				
Cymbopleura	Diatoms of Europe 4.				
Delicata	Krammer, K. 2003. Cymbopleura, Delicata, Navicymbula, Gomphocymbellopsis, Afrocymbella. In: H. Lange-Bertalot (ed.), Diatoms of Europe, Diatoms of the European Inland waters and comparable habitats. A.R.G. Gantner Verlag K.G., 4:529 pp.				
Denticula	F. E. Round, R. M. Crawford, and D. G. Mann. The diatoms. Biology, morphology of the genera., 1990. 747 pages.				
Discotella	Houk, V. and Klee, R. 2004. The stelligeroid taxa of the genus Cyclotella (Kützing) Brébisson (Bacillariophyceae) and their transfer into the new genus Discostella gen. nov. Diatom Research 19(2):203-228.				

Ellerbeckia	K. Krammer and H. Lange-Bertalot. Bacillariophyceae 3. Teil: Centrales, Fragilariaceae, Eunotiaceae., 1991. 576 pages.				
Encyonema	Bibliotheca Diatomologica 36	F. E. Round, R. M. Crawford, and D. G. Mann. The diatoms. Biology, morphology of the genera., 1990. 747 pages.			
Encyonopsis	Bibliotheca Diatomologica 37				
Entomoneis	F. E. Round, R. M. Crawford, and D. G. Mann. The diatoms. Biology, morphology of the genera., 1990. 747 pages.				
Eolimna	Bibliotheca Diatomologica 38		M. R. Luttenton, J. B. Vansteenburg, and R. G. Rada. Phycoperiphyton in selected reaches of the Upper Mississippi River: community composition, architecture, and productivity. Hydrobiologia 136:31-46, 1986.	F. Rimet, L. Ector, H. M. Cauchie, and L. Hoffmann. Changes in diatom-dominated biofilms during simulated improvements in water quality: implications for diatom-based monitoring in rivers. Eur.J.Phycol. 44:567-577, 2009.	
Epithemia	F. E. Round, R. M. Crawford, and D. G. Mann. The diatoms. Biology, morphology of the genera., 1990. 747 pages.				
Eucocconeis	K. Krammer and H. Lange-Bertalot. Bacillariophyceae 4. Teil: Achnanthaceae. Kritische Ergänzungen zu Navicula (Lineolatae) und Gomphonema. Gesamtliteraturverzeichnis Teil 14. 1991. 437 pages.				
Eunotia	F. E. Round, R. M. Crawford, and D. G. Mann. The diatoms. Biology, morphology of the genera., 1990. 747 pages.	K. Katoh. Correlation between cell density and dominant growth form of epilithic diatom assemblages. Diatom Research 7:77-86, 1992.			

Fallacia	Round, F.E. and Bukhtiyarova, L. 1996. Four new genera based on Achnanthes (Achnanthidium) together with a re-definition of Achnanthidium. Diatom Research 11(2):345-361. - no information on life form of Karayeva, Planothidium and Rossithidium				
Fistulifera	Diatom of Europe 2			F. Rimet, L. Ector, H. M. Cauchie, and L. Hoffmann. Changes in diatom-dominated biofilms during simulated improvements in water quality: implications for diatom-based monitoring in rivers. Eur.J.Phycol. 44:567-577, 2009.	
Fragilaria	F. E. Round, R. M. Crawford, and D. G. Mann. The diatoms. Biology, morphology of the genera., 1990. 747 pages.	K. Katoh. Correlation between cell density and dominant growth form of epilithic diatom assemblages. Diatom Research 7:77-86, 1992.	K. D. Hoagland, S. C. Roemer, and J. R. Rosowski. Colonization and community structure of two periphyton assemblages, with emphasis on the diatoms (Bacillariophyceae). American Journal of Botany 69:188-213, 1982.	S. C. Roemer, K. D. Hoagland, and J. R. Rosowski. Development of a freshwater periphyton community as influenced by diatom mucilage. Can.J.Bot. 62:1799-1813, 1984.	Iconographia Diatomologica 2. Classé en planctonique aussi vu la forme et taille
Frustulia	H. Germain. Flore des diatomées, eaux douces et saumâtres, Paris:1981. 444 pages.				
Geissleria	Iconographia Diatomologica 2.				
Gomphoneis	F. E. Round, R. M. Crawford, and D. G. Mann. The diatoms. Biology, morphology of the genera., 1990. 747 pages.				
Gomphosphaenia	Nova Hedwigia 60, 1995.				
Gyrosigma	F. E. Round, R. M. Crawford, and D. G. Mann. The diatoms. Biology, morphology of the genera., 1990. 747 pages.				

Hippodonta	Iconographia Diatomologica 4				
Karayevia	Round, F.E. and Bukhtiyarova, L. 1996. Four new genera based on Achnanthes (Achnanthidium) together with a re-definition of Achnanthidium. Diatom Research 11(2):345-361. - no information on life form of Karayeva, Planothidium and Rossithidium				
Kobayasiella	Lange-Bertalot, H. 1999 [ref. 009347]. Kobayasiella nom. nov. ein neuer Gattungsname für Kobayasia Lange-Bertalot 1996. In: Lange-Bertalot, H. (ed.), Iconographia Diatomologica. Annotated Diatom Micrographs. Vol. 6. Phytogeography-Diversity-Taxonomy. Koeltz Scientific Books, Königstein, Germany, 6:pp. 272-275.				
Kolbesia	Round, F.E. and Bukhtiyarova, L. 1996. Four new genera based on Achnanthes (Achnanthidium) together with a re-definition of Achnanthidium. Diatom Research 11(2):345-361.				
Licmophora	F. E. Round, R. M. Crawford, and D. G. Mann. The diatoms. Biology, morphology of the genera., 1990. 747 pages.				
Luticola	Round, F.E. and Bukhtiyarova, L. 1996. Four new genera based on Achnanthes (Achnanthidium) together with a re-definition of Achnanthidium. Diatom Research 11(2):345-361.				
Mastogloia	F. E. Round, R. M. Crawford, and D. G. Mann. The diatoms. Biology, morphology of the genera., 1990. 747 pages.				
Mayamaea	Diatom of Europe 2				

Melosira	F. E. Round, R. M. Crawford, and D. G. Mann. The diatoms. Biology, morphology of the genera., 1990. 747 pages.	K. Katoh. Correlation between cell density and dominant growth form of epilithic diatom assemblages. Diatom Research 7:77-86, 1992.	M. R. Luttenton, J. B. Vansteenburg, and R. G. Rada. Phycoperiphyton in selected reaches of the Upper Mississippi River: community composition, architecture, and productivity. Hydrobiologia 136:31-46, 1986.		
Meridion	Round, F.E. and Bukhtiyarova, L. 1996. Four new genera based on Achnanthes (Achnanthidium) together with a re-definition of Achnanthidium. Diatom Research 11(2):345-361. - no information on life form of Karayeva, Planothidium and Rossithidium				
Microcostatus	Diatom research 13. 1998. Johansen				
Muelleria	F. E. Round, R. M. Crawford, and D. G. Mann. The diatoms. Biology, morphology of the genera., 1990. 747 pages.				
Naviculadicta	Bibliotheca Diatomologica 29				
Neidium	F. E. Round, R. M. Crawford, and D. G. Mann. The diatoms. Biology, morphology of the genera., 1990. 747 pages.				
Nitzschia	Bibliotheca Diatomologica 15. Vue la forme, je classe aussi en planctonique.	K. Katoh. Correlation between cell density and dominant growth form of epilithic diatom assemblages. Diatom Research 7:77-86, 1992.	K. D. Hoagland, S. C. Roemer, and J. R. Rosowski. Colonization and community structure of two periphyton assemblages, with emphasis on the diatoms (Bacillariophyceae). American Journal of Botany 69:188-213, 1982.	F. Rimet, L. Ector, H. M. Cauchie, and L. Hoffmann. Changes in diatom-dominated biofilms during simulated improvements in water quality: implications for diatom-based monitoring in rivers. Eur.J.Phycol. 44:567-577, 2009.	C. M. Pringle. Nutrient spatial heterogeneity: effects on community structure, physiognomy, and diversity of stream algae. Ecology 71:905-920, 1990.
Nupela	Bilbiotheca Diatomlogica 29				
Opephora	F. E. Round, R. M. Crawford, and D. G. Mann. The diatoms. Biology, morphology of the genera., 1990. 747 pages.				

Parlibellus	F. E. Round, R. M. Crawford, and D. G. Mann. The diatoms. Biology, morphology of the genera., 1990. 747 pages.				
Pinnularia	K. Krammer and H. Lange-Bertalot. Bacillariophyceae 1. Teil: Naviculaceae. 1986. 876 pages.	K. Krammer. The genus Pinnularia, Ruggell, Germany:Gantner Verlag, 2000. 703 pages.			
Placoneis	F. E. Round, R. M. Crawford, and D. G. Mann. The diatoms. Biology, morphology of the genera., 1990. 747 pages.				
Planothidium	Round, F.E. and Bukhtiyarova, L. 1996. Four new genera based on Achnanthes (Achnanthidium) together with a re-definition of Achnanthidium. Diatom Research 11(2):345-361. - no information on life form of Karayeva, Planothidium and Rossithidium				
Platessa	Krammer, K. and Lange-Bertalot, H. 2004. Bacillariophyceae 4. Teil: Achnanthaceae, Kritische Erganzungen zu Navicula (Lineolatae), Gomphonema Gesamtliteraturverzeichnis Teil 1-4 [second revised edition]. In: H. Ettl et al., Suesswasserflora von Mitteleuropa. Spektrum Akademischer Verlad Heidelberg, 2(4):468 pp., 93 pls. [first edition was published in 1991]				
Pleurosira	Round, F.E. and Bukhtiyarova, L. 1996. Four new genera based on Achnanthes (Achnanthidium) together with a re-definition of Achnanthidium. Diatom Research 11(2):345-361. - no information on life form of Karayeva, Planothidium and Rossithidium				

Praetriceratium	Sims, P.A. 2001. The genus Praetriceratium gen. nov.: a survey of eupodiscoid genera with a sipho marginalis. Diatom Research 16(2):399-416.				
Psammothidium	Bukhtiyarova, L. and Round, F.E. 1996. Revision of the genus Achnanthes sensu lato. Psammothidium, a new genus based on A. marginulatum. Diatom Research 11(1):1-30.				
Pseudostaurosira	Diatom Research 2, 1987				
Pulchella	K. Krammer. Navicula sensu stricto, 10 Genera Separated from Navicula sensu stricto, Frustulia., Ruggell, Germany:Gantner Verlag, 2001. 526 pages.				
Puncticulata	Diatom Research 17, 1, 2002. Hakansson				
Reimeria	Round, F.E. and Bukhtiyarova, L. 1996. Four new genera based on Achnanthes (Achnanthidium) together with a re-definition of Achnanthidium. Diatom Research 11(2):345-361. - no information on life form of Karayeva, Planothidium and Rossithidium				
Rhoicosphenia	F. E. Round, R. M. Crawford, and D. G. Mann. The diatoms. Biology, morphology of the genera., 1990. 747 pages. + J. Brun. Diatomées des Alpes et du Jura et de la région Suisse et Française des environs de Genève., Amsterdam:A. Asher and Co, 1965. 146 pages.	K. D. Hoagland, S. C. Roemer, and J. R. Rosowski. Colonization and community structure of two periphyton assemblages, with emphasis on the diatoms (Bacillariophyceae). American Journal of Botany 69:188-213, 1982.			
Rhopalodia	F. E. Round, R. M. Crawford, and D. G. Mann. The diatoms. Biology, morphology of the genera., 1990. 747 pages.				

Rossithidium	Round, F.E. and Bukhtiyarova, L. 1996. Four new genera based on Achnanthes (Achnanthidium) together with a re-definition of Achnanthidium. Diatom Research 11(2):345-361. - no information on life form of Karayeva, Planothidium and Rossithidium				
Sellaphora	F. E. Round, R. M. Crawford, and D. G. Mann. The diatoms. Biology, morphology of the genera., 1990. 747 pages.				
Simonsenia	K. Krammer and H. Lange-Bertalot. Bacillariophyceae 2. Teil: Bacillariaceae, Epithemiaceae, Surirellaceae. 1988. 596 pages.				
Skeletonema	Round, F.E. and Bukhtiyarova, L. 1996. Four new genera based on Achnanthes (Achnanthidium) together with a re-definition of Achnanthidium. Diatom Research 11(2):345-361. - no information on life form of Karayeva, Planothidium and Rossithidium				
Stauroforma	Diatom research 11, 1996.				
Stauroneis	F. E. Round, R. M. Crawford, and D. G. Mann. The diatoms. Biology, morphology of the genera., 1990. 747 pages.	Iconographia Diatomologica 13 + Iconographia Diatomologica 46			
Staurosira	F. E. Round, R. M. Crawford, and D. G. Mann. The diatoms. Biology, morphology of the genera., 1990. 747 pages.	K. Krammer and H. Lange-Bertalot. Bacillariophyceae 3. Teil: Centrales, Fragilariaceae, Eunotiaceae., 1991. 576 pages.			
Staurosirella	Diatom Research 21. E. Morales 2006.	F. E. Round, R. M. Crawford, and D. G. Mann. The diatoms. Biology, morphology of the genera., 1990. 747 pages.			

Stenopterobia	F. E. Round, R. M. Crawford, and D. G. Mann. The diatoms. Biology, morphology of the genera., 1990. 747 pages.				
Stephanodiscus	Diatom Research, 17, 1. Hakasson	K. Krammer and H. Lange-Bertalot. Bacillariophyceae 3. Teil: Centrales, Fragilariaceae, Eunotiaceae., 1991. 576 pages.			
Surirella	F. E. Round, R. M. Crawford, and D. G. Mann. The diatoms. Biology, morphology of the genera., 1990. 747 pages.	K. Katoh. Correlation between cell density and dominant growth form of epilithic diatom assemblages. Diatom Research 7:77-86, 1992.	S. C. Roemer, K. D. Hoagland, and J. R. Rosowski. Development of a freshwater periphyton community as influenced by diatom mucilage. Can.J.Bot. 62:1799-1813, 1984.		
Synedra	K. Krammer and H. Lange-Bertalot. Bacillariophyceae 3. Teil: Centrales, Fragilariaceae, Eunotiaceae., 1991. 576 pages.				
Synedrella	Diatom 17, 2001.				
Tabellaria	K. Krammer and H. Lange-Bertalot. Bacillariophyceae 3. Teil: Centrales, Fragilariaceae, Eunotiaceae., 1991. 576 pages.	K. Katoh. Correlation between cell density and dominant growth form of epilithic diatom assemblages. Diatom Research 7:77-86, 1992.			
Tabularia	Round, F.E. and Bukhtiyarova, L. 1996. Four new genera based on Achnanthes (Achnanthidium) together with a re-definition of Achnanthidium. Diatom Research 11(2):345-361. - no information on life form of Karayeva, Planothidium and Rossithidium				
Thalassiosira	K. Krammer and H. Lange-Bertalot. Bacillariophyceae 3. Teil: Centrales, Fragilariaceae, Eunotiaceae., 1991. 576 pages.	Simonsen 1987, Plates 647-648,			

Tryblionella	F. E. Round, R. M. Crawford, and D. G. Mann. The diatoms. Biology, morphology of the genera., 1990. 747 pages.				
Ulnaria	Lange-Bertalot Festchrift, 2001	K. Katoh. Correlation between cell density and dominant growth form of epilithic diatom assemblages. Diatom Research 7:77-86, 1992.	K. D. Hoagland, S. C. Roemer, and J. R. Rosowski. Colonization and community structure of two periphyton assemblages, with emphasis on the diatoms (Bacillariophyceae). American Journal of Botany 69:188-213, 1982.		