Universidade de Aveiro Departamento de Biologia
2018

Andreia Filipa Henriques Mortágua

DNA METABARCODING APPROACH AS A COMPLEMENTARY TECHNIQUE FOR ASSESSMENT OF PORTUGUESE RIVERS USING DIATOMS

ABORDAGEM DE *METABARCODING* DE DNA COMO TÉCNICA COMPLEMENTAR NA AVALIAÇÃO DOS RIOS PORTUGUESES USANDO DIATOMÁCEAS

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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Biologia Aplicada, realizada sob a orientação científica da Doutora Salomé Fernandes Pinheiro de Almeida, professora auxiliar do Departamento de Biologia da Universidade de Aveiro e coorientação científica da Doutora Maria João Feio, investigadora no MARE-UC (*Marine and Environmental Sciences Centre*) da Universidade de Coimbra.

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palavras-chave

Diatomáceas, *metabarcoding* de DNA, DNA ambiental, biomonitorização, rios portugueses, avaliação ecológica da água, sistemas de água doce

resumo

A Directiva Quadro de Água (DQA) é o principal instrumento político de gestão das massas de água na Europa e inclui a avaliação biológica de rios e riachos através das diatomáceas, mediante o cálculo de um índice autoecológico, o *Indice de Polluosensibilité Spécifique* (IPS), adotado oficialmente para Portugal. Este índice requer um alto nível de conhecimento taxonómico para a identificação morfológica dos indivíduos. Avanços na área da genómica, como o *metabarcoding* de DNA combinado com técnicas de sequenciamento de alto rendimento (HTS), oferecem uma alternativa promissora aos métodos clássicos, limitando a exigência de especialização e, portanto, reduzindo o tempo e os custos. O objetivo deste estudo foi testar o potencial do *metabarcoding* de DNA de diatomáceas na avaliação biológica de rios portugueses, comparando as classificações do IPS, obtidas com abordagens morfológica e moleculares. No total, 88 amostras de rios do centro de Portugal foram recolhidas na primavera de 2017, seguindo as normas da DQA. A abordagem morfológica incluiu a identificação taxonómica de pelo menos 400 valvas ao microscópio ótico. A abordagem molecular compreendeu a extração de eDNA seguida de sequenciação (Illumina MiSeq) usando o *barcode* de DNA *rbc*L com 312pb. As sequências foram analisadas com o software Mothur, produzindo Unidades Taxonómicas Operacionais (UTOs) atribuídas à biblioteca de referência R-Syst::diatom. Testou-se também o efeito de um fator de correção (FC) para o biovolume aplicado aos dados moleculares. Os inventários das comunidades de diatomáceas revelaram um total de 306, 125 e 111 espécies identificadas através da morfologia e método molecular sem e com FC, respetivamente. A percentagem total de UTOs atribuídos com sucesso à biblioteca de referência foi de 32%, com uma média de 47,5% por amostra, enquanto a percentagem média de leituras “não classificadas” dos UTOs convertidos em lista de taxa foi de 52,5% e variou entre 2 e 95%, entre todas as amostras. Ao comparar as abundâncias das espécies, os resultados mostraram diferenças estatísticas em relação ao número de espécies dos inventários moleculares e morfológico, embora a aplicação do FC tenha aproximado as duas abordagens. A fonte dessas diferenças pode estar na necessidade de completar as bibliotecas de referência, representando, atualmente, a maior dificuldade na atribuição taxonómica das sequências de eDNA. Em relação aos valores de IPS, os resultados indicaram uma boa correlação entre os métodos morfológico e moleculares, especialmente quando se aplicou o FC. Os diagramas de NMDS e PCO baseados na abundância das espécies revelaram um gradiente de classificações de qualidade em todas as 3 metodologias, apoiando a hipótese de que o *metabarcoding* de DNA pode vir a ser abordagem válida para avaliação da qualidade ecológica. No entanto, ainda há trabalho a ser feito nesta área no sentido de proporcionar uma transição suave entre a abordagem tradicional e a mais recente, sem perder de vista o conhecimento acumulado nas últimas décadas sobre a avaliação da qualidade da água.

keywords

Diatoms, DNA metabarcoding, environmental DNA, biomonitoring, Portuguese rivers, ecological assessment of water, freshwater systems

abstract

The Water Framework Directive (WFD) is the main political instrument for management of the waterbodies in Europe and includes the bioassessment of rivers and streams based on diatoms, through the calculation of an autoecological index, the *Indice de Polluosensibilité Spécifique* (IPS) officially adopted for Portugal. This index requires a high level of taxonomic expertise for morphological identification of individuals. Advances in genomics, such as the DNA metabarcoding combined with high-throughput sequencing (HTS) techniques offer a promising alternative to classical methods, limiting expertise requirement and therefore reducing time and costs. The aim of this study was to test the potential of DNA metabarcoding of diatoms in the bioassessment of Portuguese rivers by comparing the IPS classifications obtained with morphological and molecular approaches. A total of 88 samples from rivers in central Portugal were collected in the spring of 2017 following WFD standards. The morphological approach comprised taxonomic identification of at least 400 valves, under the light microscope. The molecular approach included eDNA extraction followed by DNA sequencing (Illumina MiSeq) using a 312bp *rbc*L DNA barcode. Sequences were analysed with Mothur software, producing Operational Taxonomic Units (OTUs) that were taxonomically assigned to the R-Syst::diatom reference library. It was also tested the effect of a correction factor (CF) for biovolume applied on molecular data. Inventories of diatom communities revealed a total number of 306, 125 and 111 species identified with morphology, molecular method without and with the CF, respectively. The total percentage of successfully assigned OTUs to the reference library was 32%, with an average of 47.5% per sample, while the average percentage of unassigned reads from the converted OTUs to taxa list was 52.5% and varied between 2 and 95%, among all samples. When comparing species' abundances, the results showed statistical differences in the number of species between molecular and morphological inventories although the application of the CF approximated both approaches. The source of these differences may lay on the incompleteness of reference libraries, which currently represents the major difficulty in taxonomic assignment of eDNA sequencings. Regarding IPS values, the results indicated a good correlation between morphological and molecular methods, especially when applying the CF. NMDS and PCO diagrams based on species abundances revealed a gradient of quality classifications in all 3 methodologies. These support the hypothesis that DNA metabarcoding may be a valid approach for ecological quality assessment. Yet, there is still work to be done on this new methodology in order to be able to make a smooth transition between the traditional and this new approach without misspend accumulated knowledge from the last decades on water quality assessment.

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INTRODUCTION

Water is an essential resource for the existence of all living beings. It covers over 70% of the Earth's surface and it is distributed by oceans, lakes, glaciers, rivers, groundwater and as vapour in the atmosphere (Cia.gov, 2017). Particularly freshwater is a valuable and finite resource and humans have been using it over time not only for direct consumption, but also for the development of other means of subsistence such as in agriculture or in industry which led to the evolution of civilizations. However, the exploration of water resources was and still is not always sustainable.

Although it is a very old practice ascending to ancient Mesopotamia, the use of pesticides in agriculture began with elemental sulphur to control insects and rapidly became a routine. Later in the 1940's, the production of synthetic pesticides, which displayed higher selectivity and better control of pests, grew significantly (Unsworth, 2010). However, over-application of agrochemicals (pesticides and fertilizers containing nitrates and phosphates, among others) has shown to contribute to the degradation of soil/water quality and ecosystems. Many studies identified other problems associated with agriculture, namely, the loss of biodiversity due to monocultures, unsustainable water consumption (Horrigan et al., 2002), salinization and erosion of soil (UNEP/WHO, 1996) and eutrophication of freshwater systems (Daniel et al., 1994).

Along with agricultural practices, other means of anthropogenic water contamination have also begun long time ago. During centuries, due to population growth and urbanization, drinking water sources were contaminated with raw sewage leading to the spread of diseases such as cholera, infectious hepatitis or typhoid (Bryan, 1977). In the mid-19th century, the Industrial Revolution introduced new sources of water pollution intensifying the damages on human and environmental health. Since then, chemical wastes, toxic components and hazardous solvents and metals have been disseminated in marine and freshwaters (UNEP/WHO, 1996). Direct discharges of contaminants from mining, smelting or even pharmaceutics also play a significant role in this issue with pollutants leaching from surface to groundwater.

Human activities always had consequences in the environment and ecosystems' balance, however, the impact of these activities dramatically increased in the last few decades due to over-exploitation of resources and wastes generated, as mentioned above. Particularly freshwater resources from which humans use 70 to 80% for economic purposes have become scarce for consumption and its quality decreased (Baroni et al., 2007). The importance of *clean* water gradually became a matter of concern when in 1960 the first environmental movements emerged and symbolic events such as the Earth Day or legislative acts in U.S.A like Clean Water Act (CWA), in 1972 (Congress, 1972), raised awareness on the water pollution issue.

1. Water Monitoring Programs

International organizations and political entities realized the urgency of taking action to protect water resources for meeting human and socio-economic needs and, in 1977, the United Nations Water Conference approved the Mar del Plata Action Plan which stated several goals on water management before the end of the 20th century, namely, the assessment of water resources status based on a national and international standardization of methodologies and instruments for comprehensive analysis, the improvement of new available data on water quantity and quality in order to safeguard its adequate supply and the increment on water use efficiency (Biswas, 2004).

Later, in 1992, the International Conference on Water and the Environment took place in Dublin representing an important marker but this time on freshwater policy (Gleick, 1993). The necessity of developing and implementing a holistic and interdisciplinary approach on a global scale for water protection was reinforced by the international community and set as urgent, following Agenda 21 (Rio de Janeiro, 1992) which proposes a list of activities undertaken by all States from the United Nations including the establishment of "appropriate policy frameworks and national priorities" for water assessment. Following this, other meetings occurred worldwide with environmental sustainability in view: the Convention of Biological Diversity in 1992, Earth Summits in 1995 (New York) and 2002 (Johannesburg) (Hering et al., 2010).

1.1. Biomonitoring in Europe and Portugal

1.1.1. Background

In Europe, an EU Directive based on the Ecological Quality of Surface Waters was drafted to implement several measures, monitoring schemes and water quality standards in the European countries. From here, the European Water Framework Directive (WFD) (Directive 2000/60/EC) was adopted in 2000 sharing some ecological objectives and approaches with the US CWA from 1972.

The WFD became an important political instrument which, by definition, provides economic, social and environmental approaches on sustainable exploitation of water bodies based on the improvement and prevention of its chemical and biological quality at a local, regional, national and European levels (INAG, 2008; Cruz et al., 2009). It requires that European Member States achieve the "good status" of all their water bodies, including estuaries,

coastal waters, rivers and streams, lakes and groundwater (Hering et al., 2018). After its publication, started the identification and characterization of river basins, the establishment of monitoring networks, the execution of the operational programmes of measures until the meeting of environmental objectives in 2015. At the end of that year, the first management cycle ended, however, the objectives were not fully accomplished by the Member States. Due to this default, a second and a third (final deadline) management cycles were established for 2021 and 2027, respectively (Directive 2000/60/EC).

In Portugal, political initiatives concerning water management go back to 1919, when Portuguese government approved the Water Law, which exclusively safeguarded the economic interests on water resources (Costa et al., 2011). Until the 1970's, many attempts to reform water legislation were made, by introducing legal provisions for the preservation of water quality. The first came in the late 1940's, with the creation of a commission to "study and codify measures to avoid pollution of the country's waterways" (Pato, 2007). Since 2000, the legislation concerning to water resources management and protection has been following the norms of the WFD.

1.1.2. Technical Procedures

The assessment of water bodies' ecological quality in lotic systems defined by the WFD strategy is based on biological elements and support physical-chemical and hydromorphological parameters. The biological parameters include organisms such as phytoplankton, phytobenthos, macrophytes, benthic invertebrates and fish fauna and their characterization must contain information of communities' composition and abundance data. The physical-chemical parameters include temperature, pH, dissolved oxygen, conductivity, water flow, transparency, total phosphorus, total nitrogen, Chemical Oxygen Demand (COD), among others (European Commission, 2009).

In order to assess their status, the sampling of biological communities must respond to specific standards and protocols. Table 1 shows a brief description of the major aspects to be considered in the sampling routine established by the Institute of Water (INAG, 2008) for the assessment of biological quality (using phytobenthos) of Portuguese rivers under the WFD norms.

The final ecological quality classification is expressed in 5 quality classes according to the level of alteration of natural conditions (low to high disturbance): High, Good, Moderate, Poor and Bad (INAG, 2009). This approach is based on preliminary establishment of reference conditions for each river type, which should correspond to the absence of human disturbance (including industrial, urban and agricultural influences) (Feio et al., 2014).

Table 1. Methodological norms for the correct sampling and analysis of phytobenthos contemplated in the monitoring program adopted for river systems at a national level.

	Sampling Routine
Time of the year	Spring (recommended)
Location	A section with approximately 50m which include coarse substratum, turbulent flow with current velocity between 10-50 cm/s, no overshadow and similar luminosity
Sampling procedure	Random selection of 5 stones with biofilm and scraped into a tray using a toothbrush and water from the river. In the absence of stones, biofilm is collected from macrophytes
Preservation	The biofilm fixation is made using lugol, stored in 250 mL flasks and kept at 4˚C until analysis
Slides preparation	First, the fixative is removed. Then, cellular organic matter is oxidized, and finally, the definitive slides are mounted for microscopic observation
Identification	Count and identification of at least 400 valves to species level, under light microscope

1.1.3. Biological Element Phytobenthos (Diatoms)

The biological element phytobenthos is usually restricted to benthic diatoms in the majority of the member states of the European Union (INAG, 2008). The diatoms are photoautotrophic microalgae abundant in almost all aquatic systems (Smol and Stoermer, 2010) with life strategies including benthic species that adhere to different substrates (e.g. stones, plants, sand, animals, mud) (Zimmermann et al., 2015). Their structure consists of two valves and siliceous bands forming the frustule (Smol and Stoermer, 2010). They are responsible for 20 to 25% of all organic carbon fixation on the planet representing an important food resource for marine and freshwater organisms (Round et al., 1990). Diatoms are considered good water quality bioindicators due to the easy preservation of the frustules and its sensitivity to several stress factors, which is a consequence of their short generation time (fast response to environmental changes) (Smol and Stoermer, 2010; Keck et al., 2018).

1.1.4. Diatom Indices

Among many algal bioindicator groups, diatoms are the most used due to their sensitivity to contamination, easy sampling, handling and preservation, vast diversity and ubiquity of the species (Martín et al., 2010). The assessment of freshwater resources (rivers and streams) using the entire aquatic community, including diatoms, was firstly approached by Kolkwitz and Marsson (1902, 1908), who published the theoretical foundation of the relations between aquatic organisms and water degradation (organic contamination). Later, several other

ecologists (Butcher, 1947; Patrick, 1949; Zelinka & Marvan, 1961; Lange-Bertalot, 1979) contributed to the development of diatom biotic indices. Many other assessment methods were also developed for other BQEs used in biomonitoring, such as the BQI (Benthic Quality Index), BMWP (Biological Monitoring Working Party Index), FBI (Family Biotic Index) and the IPtI (Indíce Português de Invertebrados) used for benthic macroinvertebrates (Wiederholm, 1980; Armitage et al., 1983; Hilsenhoff, 1988; Ferreira et al., 2008); Damage rating, River Trophic Status Indicator (RTSI), River Macrophyte Nutrient Index (RMNI) used for macrophytes from rivers (Haslam, 1982; Ali et al., 1999, Willby et al., 2012) or, more recently, HeLM (Hellenic Lake Macrophyte) method developed for Greek lakes (Zervas et al., 2018); and the IBI (Index of Biological Integrity) which was adapted for fish, algae, macroinvertebrates and macrophytes, among others (Karr, 1981).

The construction of biological indices comprises years of sampling to collect enough data for evaluation of long-term trends as well as to define the origin of those trends (anthropogenic pressures or natural variation) (Wallace, 1996). They incorporate biological diversity and integrity concepts, providing useful information on the assessment of ecosystems' health (Karr, 1993). The majority of the diatom indices are based on relative abundance combined with a level of sensitivity or tolerance of selected taxa (usually at the species level). Some of those indices are the IPS (*Indice de Polluosensibilité Spécifique*; Cemagref, 1982), the TDI (Trophic Diatom Index; Kelly, 1998), the IBD (*Indice Biologique Diatomées*; Prygiel & Coste, 1998) or the EPI-D (Diatom-Based Eutrophication/Pollution Index; Dell'Uomo et al. 1999). Portugal adopted the IPS method for biomonitoring using diatoms, which addresses specific pressures such as acidification, salinity, eutrophication and organic matter (Almeida et al., 2014). This index is based on the relative abundance of all the taxa present in a set of samples, their indicator values (1 to 3 scale) and their sensitivity values to pollution (1 to 5 scale) (Descy & Coste, 1991). The following Equation (1), describes the IPS, where $IPSV_i$ is indicator value, a_i is the relative abundance of species i in the sample, and $IPSS_i$ is the pollution sensitivity.

$$IPS = \frac{\sum_{i=1}^{n} a_i \times IPSV_i \times IPSS_i}{\sum_{i=1}^{n} a_i \times IPSV_i} \quad (1)$$

In the case of ecological assessment under the WFD strategy, these indices are then converted to Ecological Quality Ratios (EQR). The EQR is calculated by dividing the index value of each sampling site by the median of that index pre-established for the reference sites of the same typology, which results in a value between 0 and 1 (INAG, 2009). According to the watercourse typology, EQR value of 1 represents the reference conditions and values close to 0 indicates bad ecological status (Bund & Solimini, 2006).

2. Morphological *versus* DNA-based Monitoring Tools

To estimate diatom indices, it is required to identify the taxa at species level (as shown in Table 1) and elaborate an accurate taxonomic inventory of the community. The current method used in most countries (morphological approach) requires identifying the taxa by observation of their morphological features. This demand has some limitations/disadvantages, namely:

1) Phenotypic plasticity and genetic variability of organisms (Hebert et al., 2003) leading to misidentifications, nomenclatural divergences and incorrect biodiversity estimation (Belton et al., 2014), although, according to Will and Rubinoff (2004), the bigger problem regarding this topic is the definition of species (or another taxonomic group) which remains in debate among scientists;

2) Cryptic taxa, which is problematic due to the lack of conspicuous differences in external appearance (Pfenninger and Schwenk, 2007). The sympatric coexistence of cryptic species might have considerable consequences not only for bioassessment but also for other areas of study, such as conservation, biogeography or macroecology (Muangmai et al., 2016);

3) Specificity of morphological keys (Hebert et al., 2003): only single "semaphoronts" (*sensu* Hennig, 1965) translated as "an individual during a very small temporal duration of its life" (Havstad et al., 2015) are possible to identify;

4) The personal concept and perception of specific characteristics of a given organism is different between taxonomists which is aggravated by the aspects above mentioned;

5) It is a time-consuming task (Belton et al., 2014; Taberlet et al., 2012; Zimmermann et al., 2014), taking months of work in some cases;

6) It is financially expensive. Ex.: Identification of a small group of known species can cost about $2 per specimen, and when it comes to a larger group of species, identifying a single specimen can cost $50 to $100 (all costs assumed) in North America, according to Hebert and Gregory (2005).

These disadvantages also limit the identification process of diatoms. They are a very diverse group and the morphological features analysed using traditional approach are, among others, the frustule's symmetry [radial (*Centrales*) or bilateral (*Pennates*)], the presence or absence of raphe (in pennate diatoms) and its position, striae pattern, the length and width of

cells and the patterns of pores distributed in a species-specific way (Smol & Stoermer, 2010; De Tommasi, Gielis & Rogato, 2017). It is difficult to identify diatoms beyond the genus level for reasons 1) and 2) and also because there are considerable variations in morphology within a population (Babanazaroya et al., 1996). Besides all the limitations mentioned above, morphological approach also entails expensive equipment (in many cases it is necessary to resort to electron microscopy) for diatom identification.

2.1. The Advent of DNA-Based Approach in Biomonitoring

2.1.1. DNA Barcoding and Metabarcoding (eDNA)

DNA barcoding, as a standardised alternative method for taxonomic identification, was first introduced by Hebert et al. (2003) who demonstrated the reliable assignment of organisms to higher taxonomic categories based on the differences in cytochrome *c* oxidase subunit I (COI) amino-acid. By definition, the DNA barcode is a short sequence of DNA (400-800 bp) easily sequenced in one read (in principle), which unambiguously identifies a given taxon (Kress & Erikson, 2008; Zimmermann et al, 2015). This technique can either be used for assignment of unknown individuals to species, or improvement of new species discovery by using large-scale screening of one or few reference genes (Moritz & Cicero, 2004).

«DNA barcoding is a novel system designed to provide rapid, accurate, and automatable species identifications by using short, standardized gene regions as internal species tags. »

Hebert and Gregory, (2005)

This approach brought solution to the identification of cryptic species (Hebert et al., 2004), but it also can be useful in other fields such as, forensics science to analyse biological samples of crime scenes or in environmental and ecological genomic studies (Li et al., 2014). COI sequence has been used as one of the universal barcodes for animals' sequencing (including macroinvertebrates). For plants, it has been more difficult to find a universal marker due to the lack of correct variation within single loci (Li et al., 2014), however, four plant DNA barcodes have been developed and widely used in systematics: *rbc*L, *matK*, *trnH-psbA* and ITS (Kress, 2017). For protists, Table 2 shows DNA markers used in some studies for identification purposes (Pawlowski et al., 2012, 2016).

Table 2. Alternative protistan DNA barcodes proposed and used for identification.

Gene	Organism	Reference in literature
D1-D2/D2-D3 regions at 5' end of 28S rDNA	Ciliates	Gentekaki and Lynn, 2009
	Haptophytes	Liu et al., 2009
	Acantharians	Decelle et al., 2012
	Diatoms	Rimet et al., 2014
ITS1/ITS2 rDNA	Chlorarachniophytes	Gile et al., 2010
	Dinoflagellates	Litaker et al., 2007; Stern et al., 2012
COI	Euglyphida	Heger et al., 2011
	Dinoflagellates	Stern et al., 2010
	Coccolithophorid haptophytes	Hagino et al., 2011
	Ciliates	Barth et al., 2006
ITS	Diatoms	Evans et al., 2009
***rbc*L**		Kermarrec et al., 2013; Zimmermann et al., 2014
***Cox*1**		Evans et al., 2009; Rimet et al., 2014
4V 18S		Rimet et al., 2014

The number of DNA barcodes for protists' diversity evaluation has greatly increased in the last decade. Reference databases have received up to tenfold more sequences of some eukaryotic supergroups than in the years before 2000 (Pawlowski et al., 2016). The application of high-throughput sequencing (HTS) technologies contributed to the constant emergency of new unidentified eukaryotic sequences which shows that reference databases for protists barcoding still needs more curation compared to the ones for animal or plant species.

Environmental DNA (eDNA) barcoding – metabarcoding – derive from DNA barcoding, however, instead of linking a specimen to a unique sequence, it identifies multiple taxa from mixed samples identifying the community composition of a given environment, using HTS techniques (Zimmermann et al., 2015). Early studies using metabarcoding approach were focused on the discovery of protists' diversity (Pawlowski et al., 2016), resorting to group-specific primers for a given DNA barcode. In the case of diatoms, different DNA barcodes are suitable for different purposes. *Cox*1, ITS and 28S genes are considered suitable for taxonomic

studies, while *rbc*L and 18S genes are recommended for biomonitoring (Pawlowski et al., 2016) (Table 2).

The first works on diatom identification using eDNA barcoding were based on Sanger sequencing of clones (Jahn et al., 2007), however, some years later, Next-Generation Sequencing (NGS) was introduced as a high speed and large-scale approach which revealed interesting results for DNA sequencing (Kermarrec et al., 2014). This HTS technique allowed scientists to improve the DNA metabarcoding method for bioassessment. Calculation of taxa abundances using HTS data derives from the number of DNA sequences (i.e. reads) assigned to each taxon (species) (Vasselon et al., 2018). Each read is clustered into Operational Taxonomic Units (OTU), which will then be assigned to a Linnaean taxon using a reference library (Keck et al., 2018). Afterwards, OTUs list is converted to a taxonomic list and the traditional indices based on the ecological species preferences can be calculated. Figure 1 shows a scheme of the metabarcoding standard steps including eDNA sample processing (1), PCR amplification (2), high-throughput sequencing (3), filtering of the sequence data (4), clustering into OTUs (5) and assignment to morphospecies (6).

2.1.2. Pros and Cons on Using DNA Metabarcoding for Biomonitoring

Species detection using DNA metabarcoding is subjected to some biases, which have been reported in many studies and summarized by Pawlowski et al. (2016):

1. Incompleteness of reference libraries;
2. The choice of DNA barcode (marker);
3. The selection and efficacy of PCR primers.

The incompleteness of reference databases **(1)** is considered the primary reason for high levels of "unassigned" reads/OTUs (i.e. those which have no Linnaean taxon correspondence in the reference library) resulting from HTS data (Vasselon et al., 2017; Rivera et al., 2018; Pawlowski et al., 2016). Presently, in order to calculate biological indices (specially diatom indices) it is necessary to associate autoecological values and other factors to morphospecies. Thus, the more unassigned reads to species in a dataset, the less accurate the calculation of the indices will be for a certain sample site. Visco et al. (2015) reported this gap in the reference database when compared the results of DI-CH calculations based on eDNA and rDNA data from diatoms and verified that only a small fraction (30%) of the sequences matched a species in the library. This represents a problem if we intend to use the metabarcoding approach alone in bioassessment. For example, the COI gene barcode is present in the database of numerous species. A study using benthic macroinvertebrates performed by Emilson et al. (2017) and based

on the comparison between morphological and DNA metabarcoding metrics across streams (i.e. % chironomid, richness, % EPT) benefited from the well-developed COI reference sequence database to support their results on the effectiveness of metabarcoding, which provided higher richness values than morphological approach. According to Valentini et al., (2016), 90% of the fish species have been sequenced (in Western European continental waters). However other markers need to be sequenced to provide also intraspecific and geographic variation data.

Besides diatoms, prokaryotes and eukaryotes in general, require a specific set of eDNA markers **(2)** for each in order to cover the majority of the phyla. The most sequenced eDNA barcode is the 16S, highly represented in reference databases and because of that, it is an obvious choice for prokaryotes. On the other hand, eukaryotes have not yet an ideal marker. Its choice mostly depends on the taxonomic resolution and groups of interest (Drummond et al., 2015). Table 2 shows the DNA markers most applied for the different organisms.

Primer specificity **(3)** or recent divergence are two factors controlling detection limits on genetic identification of taxa. They also reduce species number and alter its composition in molecular inventories (Pawlowski et al., 2018). The PCR is the main step in samples' processing which contributes to these events. Elbrecht et al. (2017), for example, identified primer biases as the primary source of variation, while Vivien et al. (2016) points out the existence of false negatives. PCR reaction generates a number of amplicons which will highly influence the number of sequences attributed to a given taxon resulting in the quantitative ambiguities reported in some metabarcoding studies (Kermarrec et al., 2013; Elbrecht et al., 2017). Sequences of some species are easily amplified than others, which lead to a preferential amplification of those species (that sometimes may be a few) compared to others, contributing to more biases (Pawlowski et al., 2018).

The problematic of abundance biases has tried to be overcome, for example by applying correction factors. Vasselon et al. (2018) tested the efficiency of a correction factor for biovolume on 8 diatom species from pure cultures by comparing the copy number of *rbc*L gene and cells biovolume. Their results showed a reduction of 47% on the differences between morphological and metabarcoding-based water quality indices. Because cells with high biomass can comprise a higher number of copies of the marker compared with small cells (therefore, lower copy number), this correction factor brought both molecular and morphological approaches together.

Because DNA metabarcoding associated with HTS techniques is a relatively recent approach, it still presents obstacles to the correct and accurate identification of species. Optimization and standardization of protocols are necessary. However, it is evident that this method carries great advantages if we intend to apply it in bioassessment. Some of the most denoted benefits are their precision compared with morphological methods, especially for microorganisms, certain life stages (for example, juveniles and pupae) and cryptic taxa (Hering

et al., 2018). The reduction of costs inherent to technical procedures using DNA-based approach compared with morphological one is overly defended, and indeed, NGS technologies have similar costs (in some cases slightly lower, depending on the indicator and number of specimens treated) to traditional method (Stein et al., 2014). In the future, with the advance of technology and competitiveness of the market, the prices will decrease and possibly there will be a burst in the application of DNA metabarcoding in bioassessment. Also, the results of NGS techniques may proportionate easier and faster identifications compared with those under the microscope.

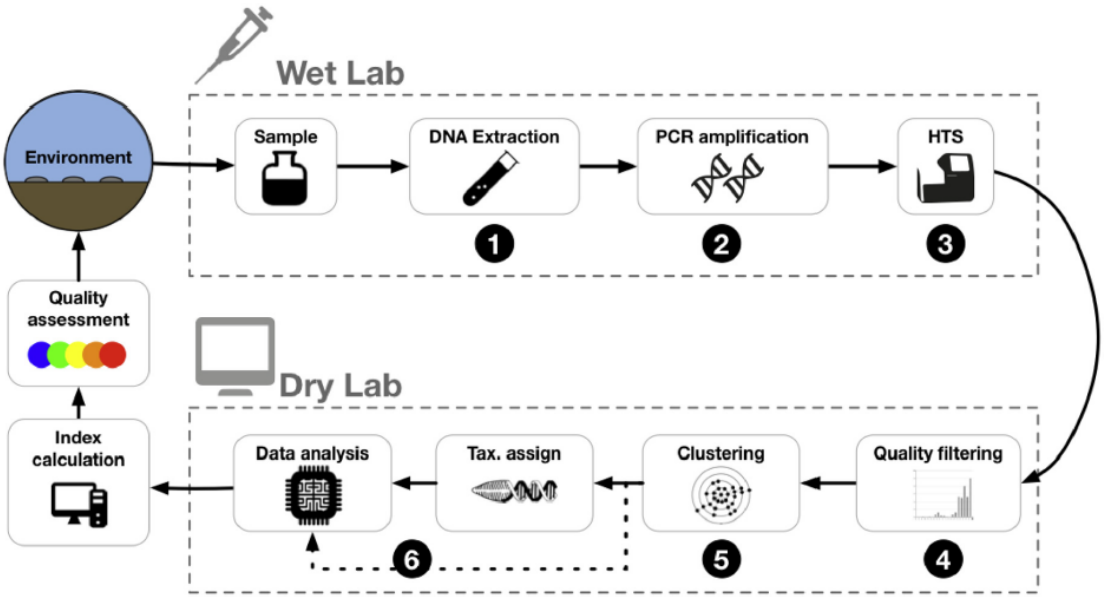

Fig. 1. Scheme of the main steps carried out in DNA metabarcoding procedure for biomonitoring (Pawlowski et al., 2016).

OBJECTIVES & HYPOTHESES

DNA-based methodologies associated with advanced technologies have opened the door to an easier and more resolute approach for water bioassessment. Worldwide, ecologists and taxonomists are studying and debating the efficacy of this new method applied to aquatic ecosystems. For now, in Europe, DNA metabarcoding can be included in biomonitoring under the WFD goals for ecological assessment only for complementing the traditional approaches (Hering et al., 2018). A realistic option would be to replace the process of identification of organisms by molecular procedures for more cost-efficiency and speed of process (Pawlowski et al., 2016) without the need of restructuring the whole framework which would take time and more investigation. However, this is a gradual transition (if it happens) and will take more optimization and standardization of the protocols. For now, and to our knowledge, no studies on this subject have been made in Portuguese rivers.

For those reasons, the major aim of our study is to evaluate the applicability of DNA metabarcoding associated with HTS techniques for biomonitoring on Portuguese rivers based on diatom samples collected from the central region of Portugal, in parallel with the WFD strategy. For that we focused on two main questions:

I) Do indices based on molecular and morphological data provide similar classifications of study sites in Portuguese rivers with different disturbance levels?

II) What is the best approach to obtain realistic abundance data from molecular analyses in Portugal?

To answer these questions, we compared the diatom community structure and ecological quality classifications based on IPS values obtained from the traditional (morphological identification) and more recent approach (eDNA metabarcoding).

And because many authors already pointed out limiting factors on DNA metabarcoding procedures (some of them are explored above), we also considered in this study the application of a correction factor (CF) for cell biovolume already proposed by Vasselon et al. (2018). Their work revealed an evident success on diminishing the discrepancies between molecular and morphological inventories (underestimating the big taxa) by applying an equation to the resulting number of reads from HTS data.

Summarizing, we compared three main aspects between two methodologies (molecular-DNA metabarcoding - and morphological-based approach): abundance, index values and resulting quality classifications, and the response of indices to stressors (given by physical-chemical parameters). Within the molecular approach, we also analysed the effect of a correction factor for biovolume (based on the work of Vasselon et al., 2018) on those three aspects.

MATERIAL & METHODS

Under the WFD norms, Portuguese entities responsible for elaborating the River Basin Management Plans (RBMP) have established 8 hydrographic regions in continental Portugal considered in the water monitoring actions for the 2nd cycle of the management plans in force from 2016 to 2021. These hydrographic regions comprise their main river basins as well as the catchment areas of coastal streams, groundwater and adjacent coastal waters. The river basins which defined the 8 regions are (Agência Portuguesa do Ambiente, 2015):

1. Minho and Lima
2. Cávado, Ave and Leça
3. Douro
4. **Vouga, Mondego and Lis**
5. Tejo and western streams
6. Sado and Mira
7. Guadiana
8. Algarve streams

1. Study Area

The study region is located in central Portugal (Fig. 2) and covers a total area of approximately 11,215 km^2 (Mendes et al., 2014) which comprises three hydrographic basins: **Vouga, Mondego and Lis**. This region belongs to the western part of the Iberian Peninsula, between important paleogeographic and tectonic units and consists of two geomorphological components: the Hesperian Massif and the Western Mesocenozoic Orla (Lisboa et al., 2015; Agência Portuguesa do Ambiente, 2015).

The source of river Vouga lies in Serra da Lapa at 930 m of altitude and runs 148 km until it drains into Ria de Aveiro, a lagoon connected to the Atlantic Ocean. River Vouga basin has a surface area of 3,685 km^2 and is separated from Mondego river basin to the south by Serra do Buçaco (Agência Portuguesa do Ambiente, 2015).

The Mondego catchment region is the second largest basin in national territory covering an area of 6,659 km^2. It lies between Vouga basin (to the East) and Tagus and Lis basins (to the South). The source of Mondego river is in Serra da Estrela at 1,537 m of altitude. It runs approximately 300 km until it flows into the Atlantic Ocean near Figueira da Foz (Agência Portuguesa do Ambiente, 2015).

The Lis basin covers an area of 837 km^2 and has its source in the district of Leiria (Ramos, 2008). It is limited to the north by Mondego's basin, to the south by Alcoa's basin and to the east by Tagus river basin (Vieira et al., 2012). The majority of its area is below 200 m of altitude, with the exception of Estremadura Limestone Massif where it can reach altitudes above 400 m (Agência Portuguesa do Ambiente, 2015).

These three basins include rivers classified into 4 typologies based on their geological and hydrological similarity: mountain (M), littoral (L), medium-large and small northern river types (N1>100 km^2 and N1≤100 km^2, respectively). The definition of each river typology results from the selection of several factors such as geology and size of the drainage area or biological information of diatom communities, invertebrates, macrophytes and ichthyofauna (Agência Portuguesa do Ambiente, 2015).

According to the WFD, surface water bodies can be grouped into 6 categories: rivers, lakes, transitional waters, coastal waters, artificial water body and heavily modified body of water. Vouga, Mondego and Lis basins comprise all of them except lakes. This study is focused on rivers.

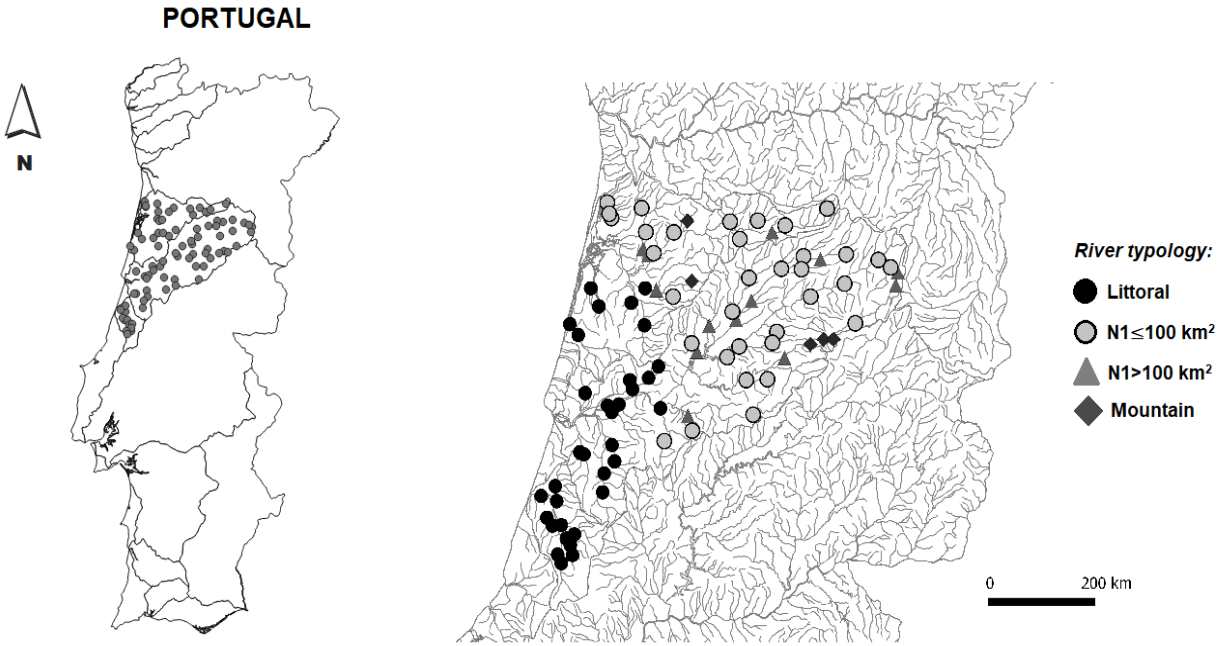

Fig. 2. Localization of the study area in central Portugal. The river sites sampled are indicated with dots (on the left map) and symbols corresponding to different river typologies properly identified on the right map.

2. Sample Collection

In the spring of 2017, a total of 88 river sites (Fig. 3) were selected and sampled following the WFD protocols for collection of phytobenthic organisms in lotic systems (INAG, 2008) and resumed in Table 2 (*Introduction*). For each sampling site, at least 5 randomly selected stones from turbulent flow areas were scraped into a tray using a toothbrush and water from the river. When stones were not the dominant substrate available or were absent, we collected biofilm attached to macrophytes or sediment. In those cases, we obtained samples from more than one substrate when possible:

- Four of the 88 samples were from sand and stone
- Three were from macrophytes and sand
- One was from macrophytes and stone
- Four were from sand only

In the analysis, when there was more than one sample per site, we always preferred the ones extracted from stones, than macrophytes and only in the last case, we used samples from sand. For morphological analysis, the biofilm was stored using formaldehyde in 50 mL glass flasks and kept in a dark and dry environment until treatment. For DNA metabarcoding analysis, 96% ethanol was used for biofilm removal and immediate fixation. About 15 mL of biofilm and 35 mL of ethanol filled 50 mL falcons (Vasselon et al., 2017). These flacks were kept at 4 ˚C until analysis (for approximately 5 months).

Fig. 3. Example of two of the 88 sites sampled showing different features: 09I_06 (a) and 09F_06 (b).

3. Laboratory Procedures

3.1. Microscopic Analysis

After collection of samples from river sites, these were prepared for morphological analysis of diatoms using the light microscope (LM) and according to WFD standards for phytobenthos (INAG, 2008). The first step was oxidation of the biofilm (Fig. 4) adding 5 mL of nitric acid and about 0.25g of potassium dichromate and left to react for 24 hours at room temperature. After that, oxidation by-products were removed with distilled water by centrifugation (5', 2000 rpm). Centrifugation was repeated at least 3 times. The oxidized pellet was resuspended in water and a drop was left to dry on a coverslip. The slides were then mounted using Naphrax® (Fig. 5) and the diatoms identified to the lowest taxonomic rank possible, usually to species level but also to infra-specific levels using Krammer and Lange-Bertalot (1986, 1988, 1991a and 1991b), Krammer (2000, 2001 and 2009) and Prygiel and Coste (2000). Relative abundance of diatoms was determined by enumeration of at least 400 valves per sample under the light microscope (Leitz Biomed 20 EB) using an immersion objective of 100× (numerical aperture: 1.32).

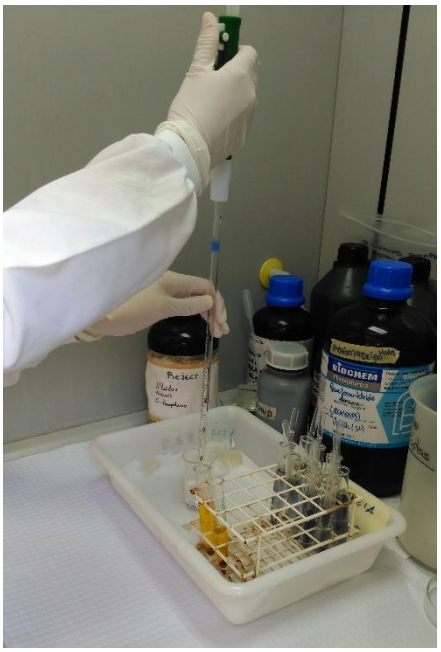

Fig. 4. Oxidation of samples using nitric acid and potassium dichromate.

Fig. 5. Mounting of the permanent slides using Naphrax® for further observation under the light microscope.

3.2. Environmental DNA Metabarcoding

3.2.1. DNA Extraction

For DNA extraction, a volume of 2 mL of each sample was centrifuged for 20 min at 4˚C and 12000 rpm. The supernatant (ethanol) was removed and the DNA contained in the pellet was isolated with commercial kit NucleoSpin® Soil, following the manufacture's recommended protocol (NucleoSpin® Soil User Manual, MACHEREY-NAGEL GmbH & Co. KG, November 2017 / Rev. 07). This protocol includes a first step of diatom cells' mechanical lysis and a second step of enzymatic lysis. First, the sample was resuspended in Lysis Buffer SL1 and 2 (supplemented with the Enhancer SX), and ceramic beads mechanically contributed to its disruption. These reagents along with lysis buffer SL3 precipitated proteins and PCR inhibitors. A centrifugation step followed for 2' and 11000*g*. The tube containing the pellet and reagents was refrigerated at 0-4ºC for 5 minutes, and centrifuged again (1', 11000*g*). The supernatant was removed and added to a first NucleoSpin® Inhibitor Removal Column in order to filter the lysate by centrifugation (Fig.6). A binding buffer was added to the column to remove residual humic substances and other PCR inhibitors. A new volume of the binding buffer and three wash buffers passed through a second Inhibitor Removal Column by successive centrifugation (30'', 11000*g*) and vortex (2'') in the last two steps. This was necessary to remove the diatoms' silica wall. Then, the membrane was dried by centrifugation (2', 11000*g*). Finally, the elution buffer SE was added to the column and left to rest for 1 minute, followed by the last centrifugation (30'', 11000*g*). The eluted DNA was ready to amplify.

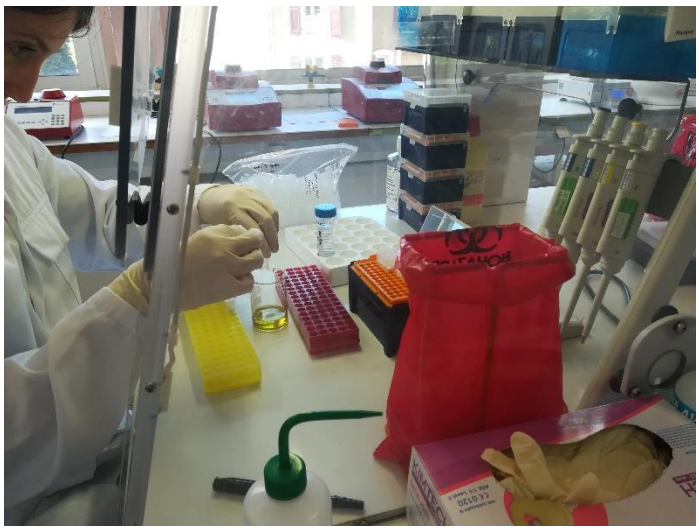

Fig. 6. Removal of the supernatant before centrifugation for filtering of the lysate.

3.2.2. PCR Amplification

For PCR, the ideal amount of DNA in each sample should be 25 ng/µL. In order to quantify the DNA extracted, a NanoDrop™ 1000 Spectrophotometer was used. PCR was

performed by amplification of *rbc*L plastid gene focusing on a 312 bp barcode. *rbc*L was chosen because there is already public information available about it and it is more resolutive than other DNA barcodes (Frigerio et al., 2016). The 312 bp was selected for its suitability in size for NGS sequencing requirements which are limited to <500bp long. The primer pair used to amplify the 312 bp region was Diat_rbcL_708F (forward) and R3 (reverse), with some modifications (Frigerio et al., 2016).

For PCR, three replicates were generated from each sample. Two forward primers and 3 reverse primers of 100 µM each were mixed separately and diluted in molecular biology grade water until a final volume of 150 µL. 15.4 µL of water was mixed with 2.5 µL of 10X Buffer, 2 µL of 2.5 mM of dNTP, 1.25 µL of 10 mg/mL of BSA and the same volume of 10 pmol/ µL of forward and reverse primers, and finally, 0.15 µL of 4U of Takara LA Taq ® polymerase. From this mixture, 24 µL were added to 1 µL of DNA extracted. PCR reactions were performed in PTC-100® Thermal Cycler featuring 33 cycles of 1 min of denaturation at 95ºC, 1 min of annealing at 54ºC and 1 min of extension at 72ºC. At the end of this process, the PCR products from each sample ran in electrophoresis gel (Fig. 7) and the results were analysed in order to verify the success of extraction and the relative amount of DNA available.

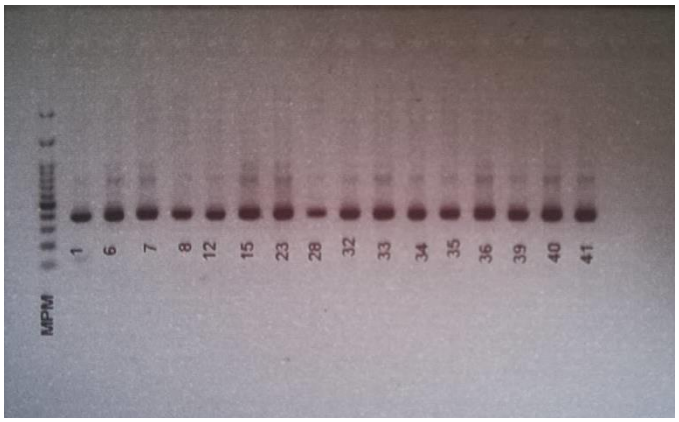

Fig. 7. Example of a DNA run through the electrophoresis gel.

3.2.3. High-Throughput Sequencing

The 3 replicates of DNA per sample resulting from PCR amplification were placed in a 96 well microplate and sent to *Plateforme Génome-Transcriptome* of Bordeaux, France, for sequencing with Illumina MiSeq System.

4. Analysis

4.1. Bioinformatic Analysis

The sequencing platform in Bordeaux, performed the first step of demultiplexing resulting a *fasta* and quality file for each of the total libraries. Paired-end reads were assembled into a contiguous sequence and only reads with an overlap region > 140bp and a maximum of 1 mismatch were kept. Then, only reads with length > 300 bp, Phred quality score > 23 over a moving window of 25 bp, > 1 mismatch in the primer sequence, homopolymer > 8 bp, or with ambiguous base (N) were excluded. All the samples were analysed together using the Mothur software (version 1.39.5, Schloss et al. 2009) following the bioinformatics process described in Vasselon et al. (2017) and outlined in Table 3. Briefly, data was dereplicated in order to work with Individual Sequence Unit (ISU), Chimera were removed using the "chimera.vsearch" command with default parameters, and ISU with 1 read were removed. Selected DNA reads were clustered in OTUs using a distance similarity threshold of 95 % using the Opticlust method with default parameters (Schloss et al., 2009). Finally, all samples were normalized to the same read number (using the smallest read abundance obtained for 1 sample) in order to allow inter-sample comparison. Diatom molecular inventories were obtained using the method described previously in Vasselon et al. (2017) with the R-Syst::diatom library (Rimet et al. 2016, version 17-05-2017, http://www.rsyst.inra.fr/en) for taxonomic assignment of OTUs. Briefly, taxonomy of each DNA read was obtained with the "classify.seqs" command (method=wang, confidence score threshold=60%) and used to determine the consensus taxonomy of OTU with the "classify.otu" command (confidence threshold=80%). From the set of normalised samples, a correction factor was applied to the number of reads in each sample, for each species, based on the biovolume of cells indicated in OMNIDIA version 5.5 (Lecointe et al., 1993) and following the instructions from Vasselon et al. (2018). This was only performed for molecular data.

Table 3. Main data processing steps in Mothur software.

Process	Description of the step	Mothur command	Working file
Trimming	Input (one fastq file per library (1 to n))	-	Pre-treated data (fastq files)
	Checking of file consistancy	fastq.info()	fastq files
	Trimming by sample (quality, length,…)	trims.seqs()	fastq files
	Merging all samples data	merge.file	fasta libraries (1 to n)
	Selecting representative unique reads	unique.seqs()	fasta All
	Performing alignment (*rbc*L barcode)	align.seqs()	"unique reads"/Read number per "unique reads"
		screen.seqs()	
		filter.seqs()	
	Removing chimeras	pre.cluster()	Curated reads alignment
		shimera.uchime()	
		remove.seqs()	
	Affiliating taxonomy to reads	classify.seqs()	Filtered reads
	Removing "non-diatom" reads	remove.lineage()	Filtered reads
Clustering	Clustering reads into OTU	dist.seqs()	"Diatom" reads
		cluster()	
	Removing singleton	split.abund()	OTU list
		remove.seqs()	
	Homogeneizing read number per sample	make.shared()	OTU list
		sub.sample()	
	Affiliating taxonomy to OTU	classify.otu()	Reads taxonomy/OTU list
Analysis	OTU level	-	Final OTU list per sample
	Taxonomic level	-	Final taxonomy list per sample

4.2. Morphological and Molecular IPS

The molecular method was separated in two approaches according to the application or not of a correction factor for biovolume to the matrix of reads' relative abundances per sample. The equation of the correction factor (CF), where *b* is the biovolume of a given species follows:

$$CF = 10^{[0.0703 \times (\log b^{2.4908})]} \quad (2)$$

Annexes I, II and III show morphological, molecular without and with correction factor inventories (based on relative species abundance and DNA reads, respectively) from which the

IPS, *Indice de Polluosensibilité Spécifique* (Cemagref, 1982), was calculated for each river site using OMNIDIA version 5.5 software (Lecointe et al., 1993). Quality classes were obtained based on IPS values and the following boundaries: IPS ≥ 17 - "High", IPS [13-17[- "Good", IPS [9-13[- "Moderate", IPS [5-9[- "Poor" and IPS [1-5[- "Bad".

4.3. Statistical Analysis

The number of families, genera and species were determined for each methodology (morphological, molecular with and without CF) as well as relative abundances of individuals per species and the 5 most abundant species. The numbers of species per method were compared performing pair-wise *T*-tests (univariate - PERMANOVA, Primer 7; Clarke et al., 2014), and boxplots for graphical inspection.

Means and respective standard-deviations of the IPS values for each site and method were determined. The correlation between IPS values from all approaches was determined using the Pearson's coefficient (SigmaStat version 4.0; Systat Software, Inc., San Jose California USA) and visualised using linear regression.

To compare the ecological quality classes attributed to each site, a NMDS (non-metric Multidimensional Scaling) based on Bray-Curtis similarity matrices of abundances was performed for the three methodologies (Primer 7; Clarke et al., 2014).

Principal COordinate analysis (PCO) based on Bray-Curtis similarity was performed based on data from 8 physical-chemical parameters [dissolved oxygen, Habitat Quality Assessment (HQA), conductivity, total nitrogen, total phosphorus, water temperature, Chemical Oxygen Demand (COD) and pH] for quality classes based on species abundances per method. The HQA is derived from the River Habitat Survey (RHS) data and consists of 3 key indicators scored and summed all together: site condition, site context and species habitat index. This parameter indicates the overall habitat diversity deduced by the physical features of the channel or river corridor and it is only comparable between rivers with similar typologies (Raven et al., 1998).

The relative contribution of each parameter to the differences between sites from each class was evaluated using the correlation coefficient. The parameters with correlation coefficients >0.2 between the abiotic factors and ecological quality classes attributed to biological elements on the principal coordinate axes were plotted.

RESULTS

1. Taxonomic Composition and Diversity

Inventories of diatom communities based on morphological identification revealed a total number of 8 families, 71 genera and 306 species. Within 88 samples identified (one per site), the minimum number of species per sample was 7 and the maximum was 64, with an average of 24 species per sample. The most abundant species were *Achnanthidium minutissimum, Karayevia oblongella, Eolimna minima, Cocconeis placentula* and *Cocconeis euglypta*, respectively (Table 4; Fig. 8a, b, c, d; Fig. 9a).

The approach using environmental DNA (eDNA) metabarcoding for all the samples sequenced resulted in a total number of 6,952,790 DNA reads with an average of 63,207 reads per sample. After the quality filtering step, 1,017,833 reads were retained and clustered into 1285 Operational Taxonomic Units - OTUs (95 % similarity threshold) with an average of 63,207 OTUs per sample. At this point and from the 88 samples, the total percentage of successfully assigned OTUs to the reference library was 32%, with an average of 47.5% (Standard-Deviation, SD=0.07%) per sample, while the average percentage of unassigned reads from the converted OTUs to taxa list was 52.5% (SD=0.07%) and varied between 2 and 95%, among all samples. From the 1285 OTUs obtained in the clustering step, 863 corresponded to those unassigned species. As mentioned above, to allow inter-sample comparison, 3 samples characterized with less than 2500 reads were removed, and the remaining samples were rarefied to 2776 reads (lowest read abundance obtained for one sample) for a total of 297,032 reads corresponding to 846 OTUs.

We obtained between 20 and 68 species, per sample, and an average of 39 species in the whole dataset and a total of 125 species using molecular approach after removing low reads and without the correction for biovolume (CF). The most abundant diatom taxa in this case were *Cocconeis sp., Ulnaria ulna, Gomphonema rhombicum, Achnanthidium minutissimum* and *Melosira varians* (Fig. 8a, e, f, g). When applying the correction factor, we obtained between 13 and 58 species with an average of 33 species in the whole dataset and a total of 111 species. The most abundant taxa were *Cocconeis sp., Achnanthidium minutissimum, Achnanthidium sp., Eolimna minima* and *Navicula sp*. (Table 4; Fig. 8a, c; Fig. 9b, c).

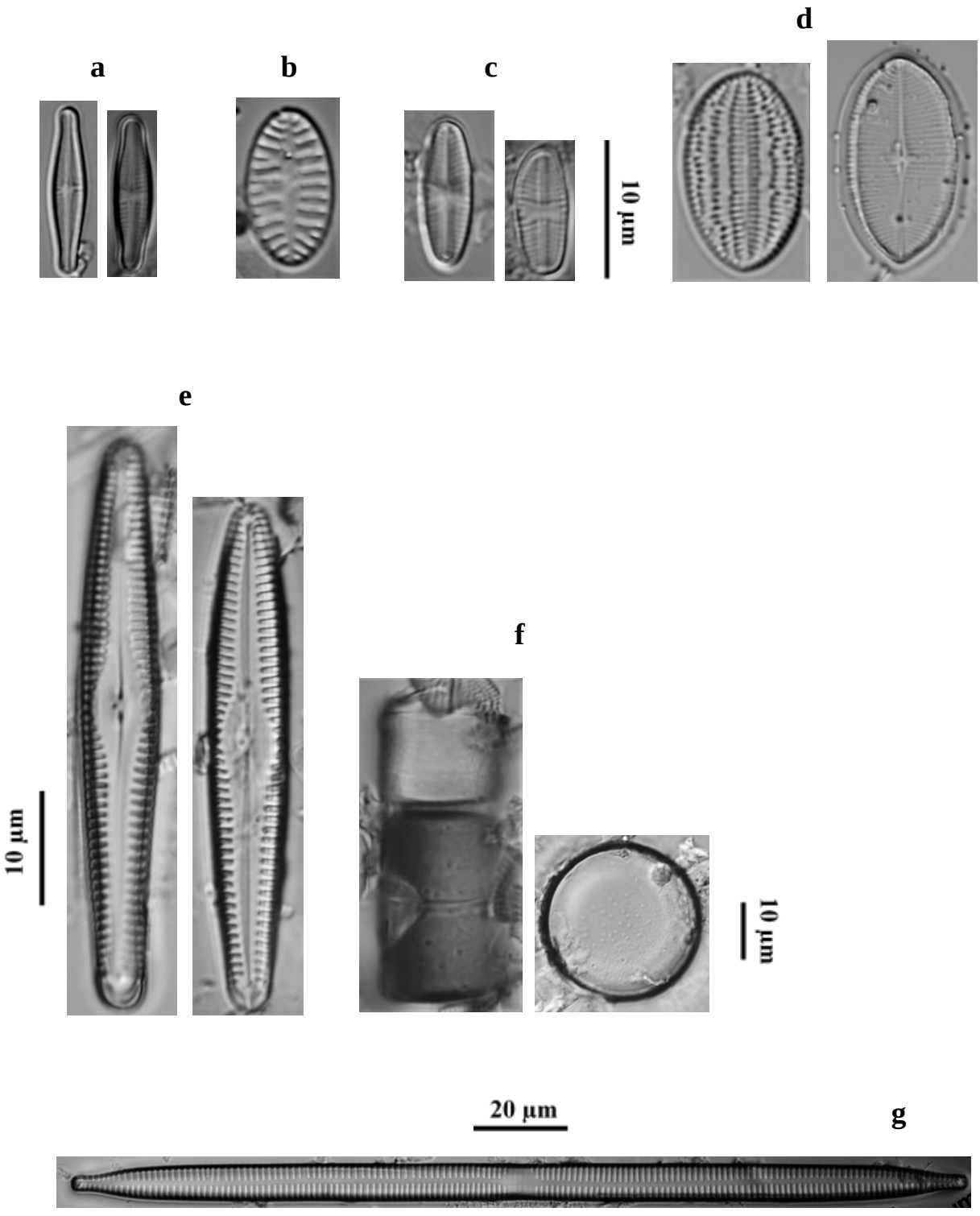

Fig. 8. LM micrographs of *Achnanthidium minutissimum* (a), *Karayevia oblongella* (b), *Eolimna minima* (c), *Cocconeis placentula* (d), *Gomphonema rhombicum* (e), *Ulnaria ulna* (f) and *Melosira varians* (g).

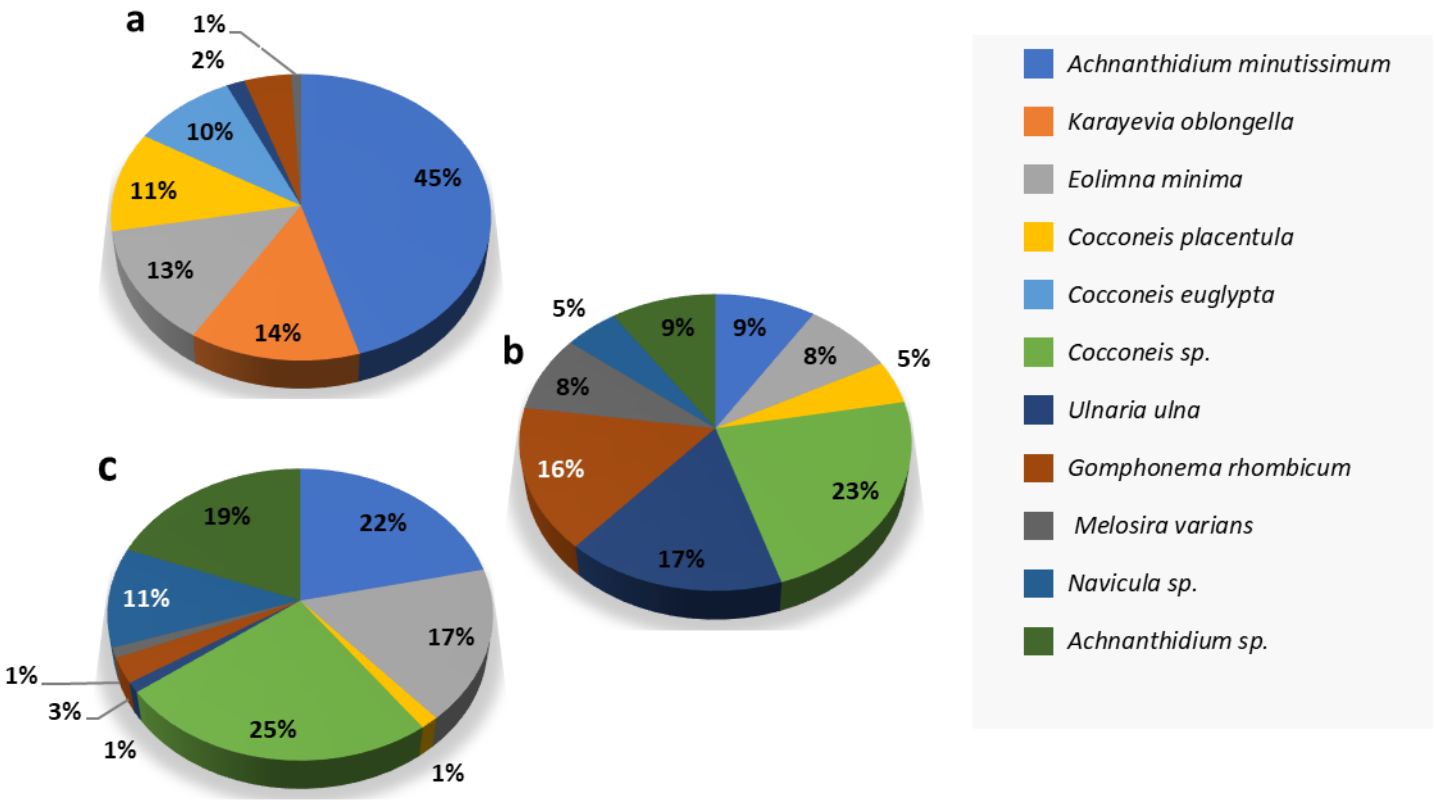

Fig. 9. Dominant species for the morphological (a) and molecular approaches (without biovolume correction – CF (b); and with this correction (c)). The three approaches only share one species within the 5 most abundant: *Achnanthidium minutissimum*.

Table 4. List of the 5 most abundant species per approach.

	Morphology	**Molecular Without CF**	**Molecular With CF**
5 most abundant species	*Achnanthidium minutissimum* (Kützing) Czarnecki	*Cocconeis sp.* Ehrenberg	*Cocconeis sp.* Ehrenberg
	Karayevia oblongella (Østrup) M. Aboal	*Ulnaria ulna* (Nitzsch) Compère	*Achnanthidium minutissimum* (Kützing) Czarnecki
	Eolimna minima (Grunow) Lange-Bertalot	*Gomphonema rhombicum* M. Schmidt	*Achnanthidium sp.* Kützing
	Cocconeis placentula Ehrenberg	*Achnanthidium minutissimum* (Kützing) Czarnecki	*Eolimna minima* (Grunow) Lange-Bertalot
	Cocconeis euglypta Ehrenberg	*Melosira varians* Agardh	*Navicula sp.*Bory

Although the values seem close, PERMANOVA analysis showed significant differences between all methodologies. Morphology *versus* molecular method with and without CF (t=5.76 and t=8.59, $p<0.05$, respectively); Molecular with and without CF (t=3.49 and $p<0.05$) (Fig. 10).

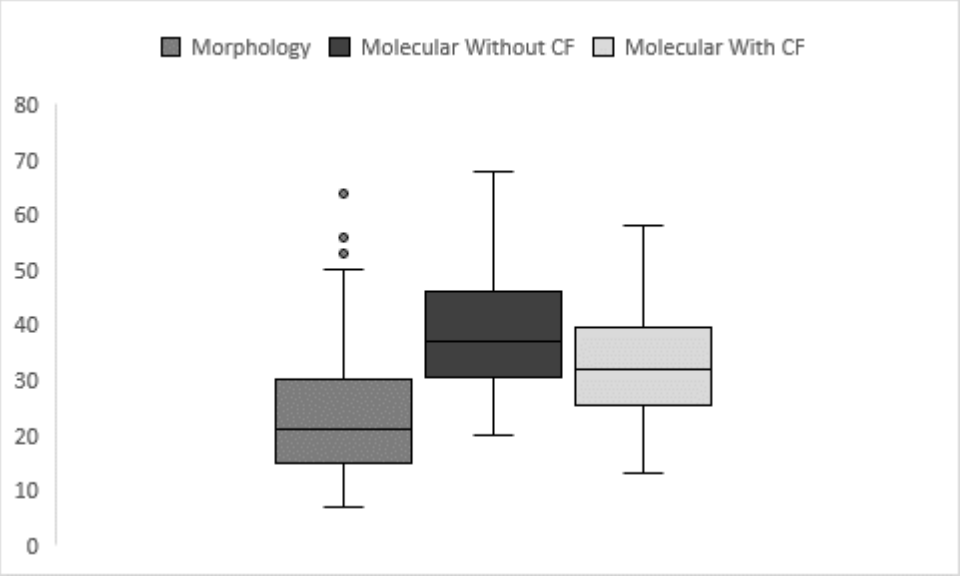

Fig. 10. Boxplot showing the average number of species from all samples (central dash) for the three methodologies. The limits of the boxes indicate the minimum and the maximum of the variances, vertical lines represent the quartiles (the 1st are the lower ones and the 3rd are the upper ones) and the dots are outliers. All methods show significant differences between each other (*T*-test, $p<0.05$).

2. Comparison Between the IPS Values for Morphological and Molecular Approaches

The comparison of IPS values from the morphological and molecular methods was made with the application of a biovolume correction factor (CF) and without the correction factor. The IPS values varied between 6.8 and 19.9, 4.9 and 19.7, 7.5 and 19.9 and the average IPS values were 14.9 (SD=3.16), 14.7 (SD=2.78) and 13.9 (SD=3.4) for the morphological approach, the molecular without CF and the molecular with CF, respectively.

According to Pearson's Correlation performed for the linear regressions shown in Fig. 11, the coefficients R describe a high correlation between the variables for both the molecular method with CF and morphology (R=0.64) (Fig. 11a) and molecular without CF and the morphological approach (R=0.60) (Fig. 11b). As expected, there is a stronger correlation

between both molecular approaches (R=0.88) (Fig. 11c), although the *p*-values are lower than 0.05, which indicates significant differences between all the three combinations of methods.

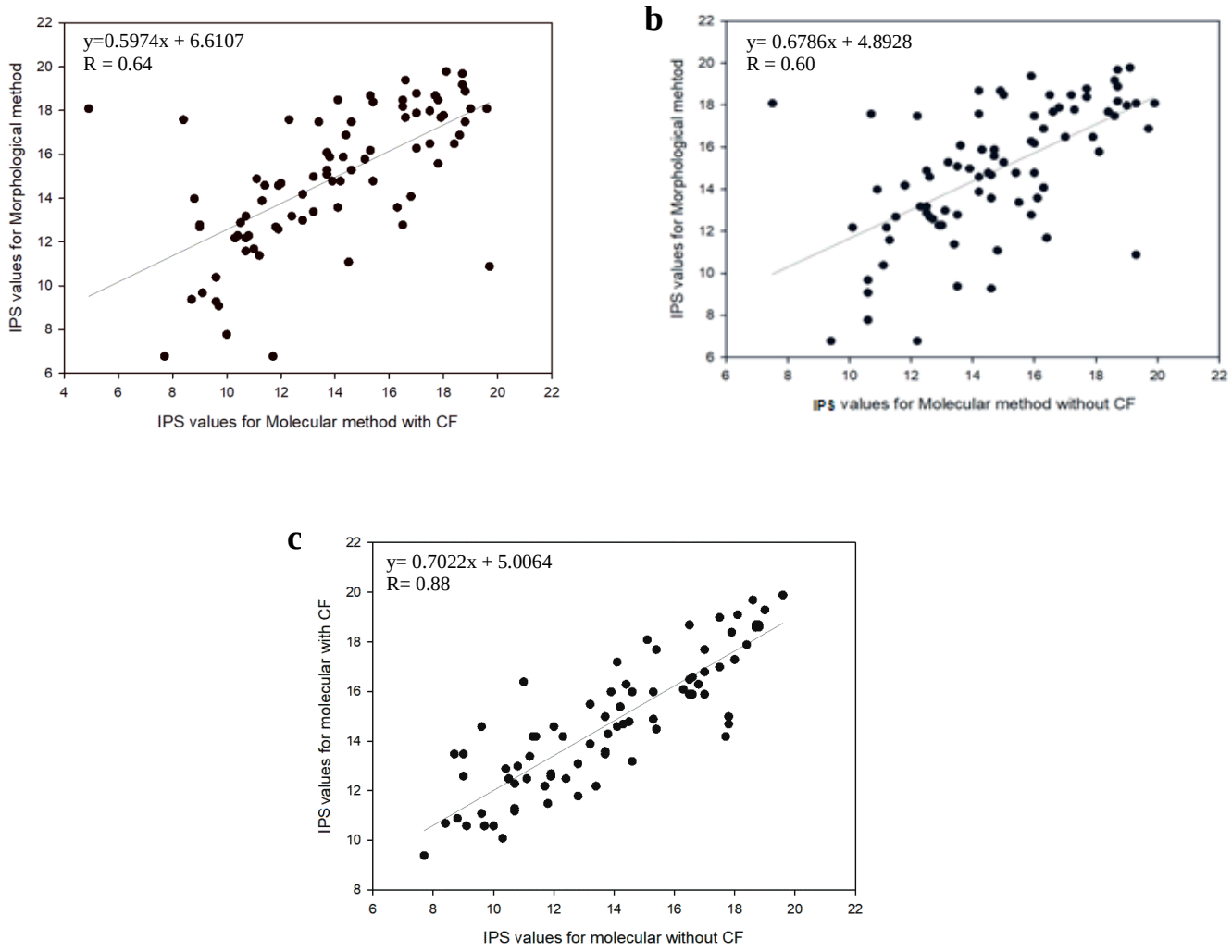

Fig. 11. Linear regression of IPS values from morphological and molecular approaches with CF (a) and without CF methods (b) and both molecular approaches (c). Significant differences between all combinations of methods (*p*-value<0.05).

The great majority of sites were classified in "Good" quality status for all approaches. The molecular method without CF resulted in the highest percentage of samples with class "Good" followed by the morphological approach, as presented in Table 5. The methodology showing the highest percentage of quality classes below "Good" (including 1 sample site with "Bad" status) was the molecular with CF, while the morphological approach resulted in the highest percentage of samples with classifications above "Good".

More than half of the samples had the same quality class from all the methodologies applied. The methodologies sharing more sites with the same quality class were the molecular with and without the correction factor (Table 6). Molecular method with CF is closer to the

morphological approach sharing 57% of samples with the same classification and 6% of samples with 2 or more classes of difference.

Table 5. Percentage of sites by quality class and approach.

	High	Good	Moderate	Poor	Bad
Morphology	32%	42%	22%	4%	0%
Molecular with CF	25%	35%	35%	5%	1%
Molecular without CF	23%	48%	27%	1%	0%

Table 6. Percentage of sites which share the same ecological quality class and have 1 and 2 classes of difference between approach.

		Molecular with CF	Molecular without CF
Share the same class	**Morphology**	57%	56%
	Molecular without CF	69%	-
1 class of difference	**Morphology**	37%	40%
	Molecular without CF	30%	-
≥2 classes of difference	**Morphology**	6%	5%
	Molecular without CF	1%	-

The NMDS (Fig. 12) shows a visible and similar gradient of quality for all approaches, "High" and "Good" classes concentrated on the left side and worst quality classes ("Moderate", "Poor" and "Bad") on the right side of the diagram, for all approaches. Yet, there are some cases where the same site has contrasting classifications. For example, site 14D_53 (in the dark circles) was classified as "Poor" by the molecular method without CF and as "High" by morphology. Another contrasting result occurred for site 09F_06 (in the dotted circles) (Fig. 3b), which was classified as "High" by both molecular methodologies and as "moderate" by the morphological approach.

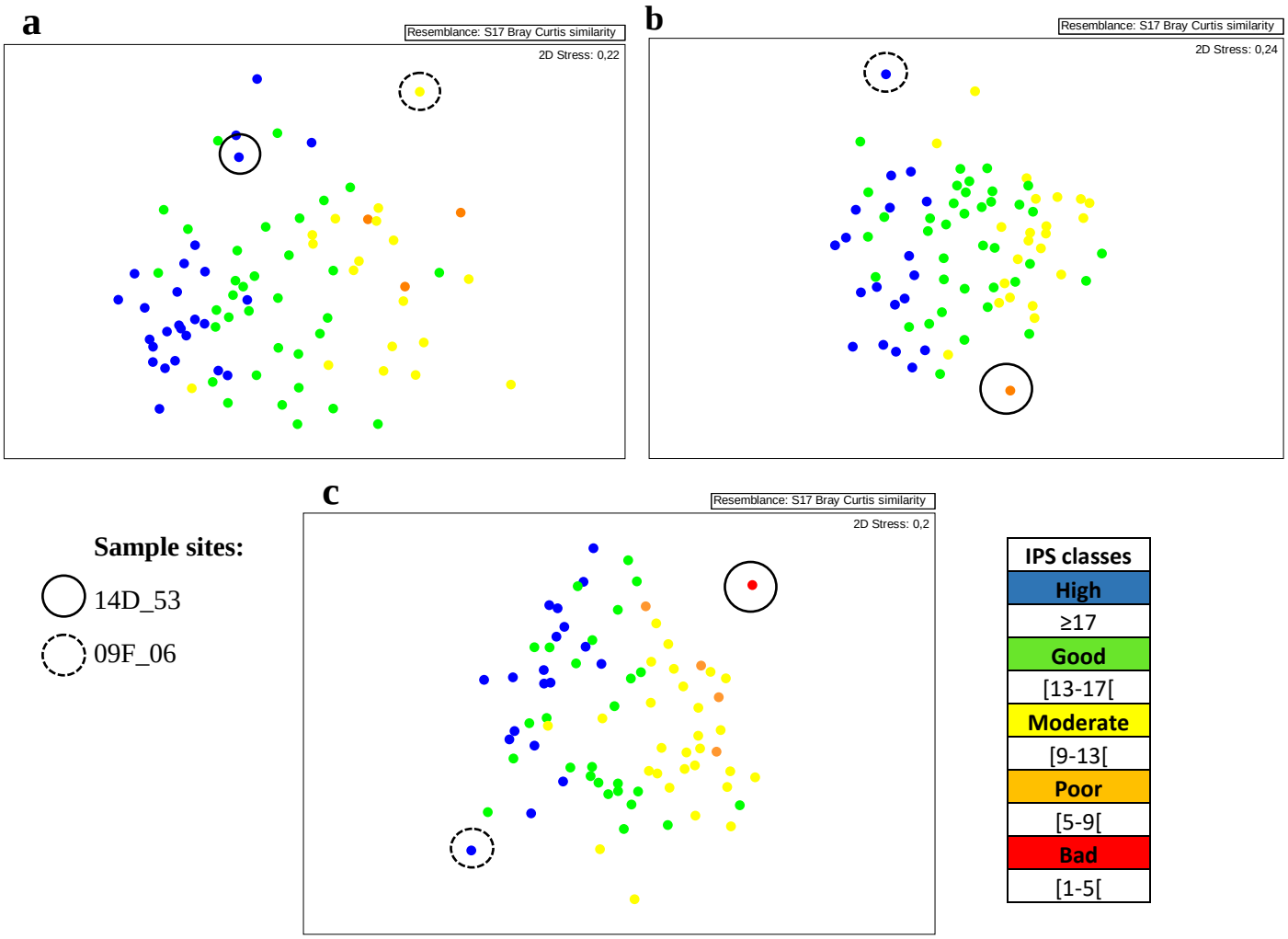

Fig. 12. NMDS plots based on the diatom communities and correspondent ecological quality classes based on IPS values obtained from morphological data (a), molecular data without (b) and with CF (c). The intervals of IPS and respective classes and colours are indicated on the figure, as well as the highlighted sample sites 14D_53 and 09F_06.

The PCO ordination (Fig. 13) shows that 30 to 40% of the variation among methodologies is explained by axes 1 and 2 (31.1 - 41%). The distribution of samples is similar to that obtained with NMDSs (Fig. 12). A higher dissolved O_2 and Habitat Quality Assessment (HQA) scores were associated with better quality classes in the 3 cases. Higher conductivity, nutrients (phosphorus and nitrogen) concentration and Chemical Oxygen Demand (COD) are associated to the worst quality classes of all methods.

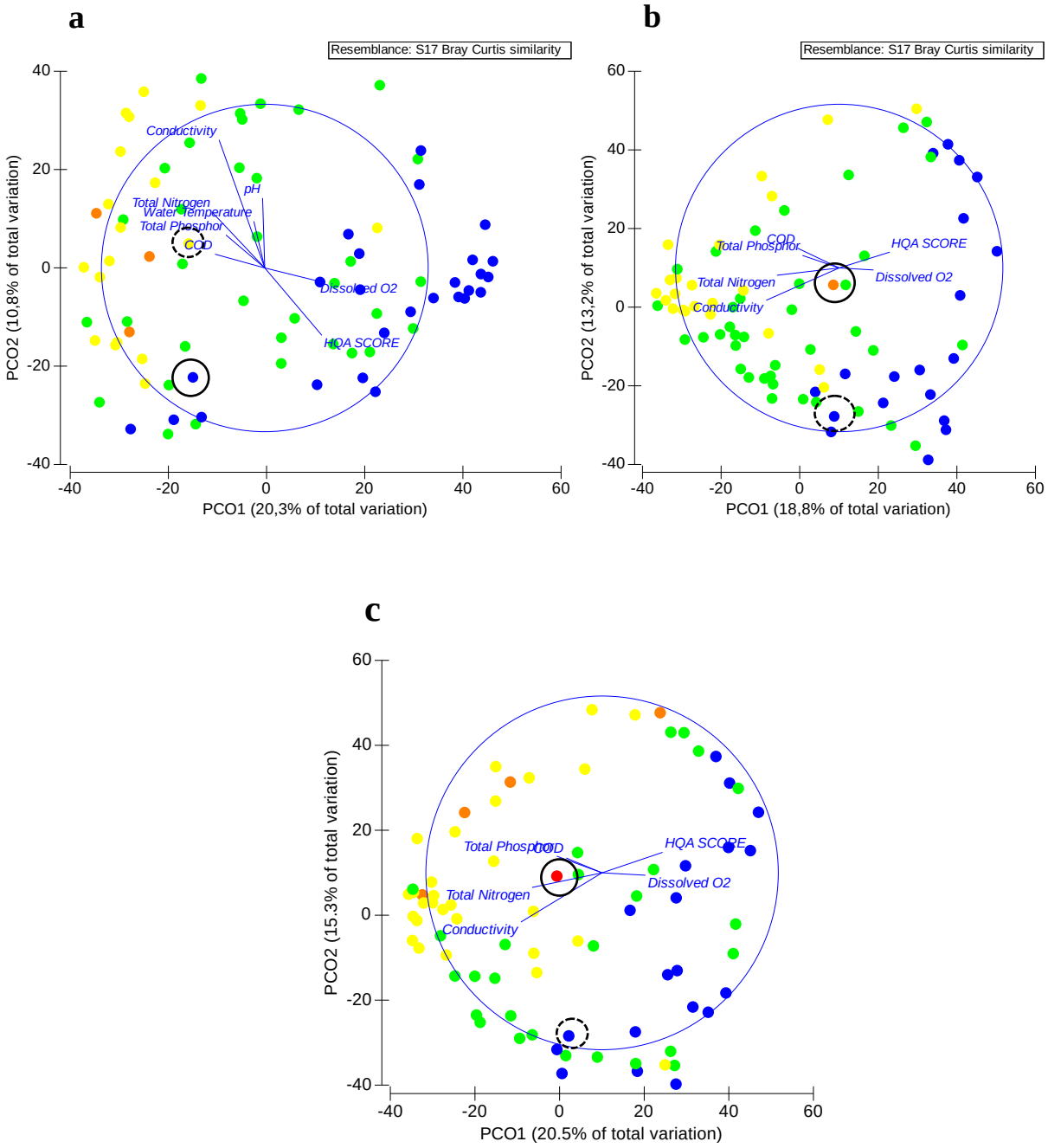

Fig. 13. Principal Coordinates Analysis (PCO) for sites using Bray-Curtis similarity based on diatom communities and corresponding ecological quality classes attributed by IPS index for data obtained with the morphological approach (a), molecular method without (b) and with CF (c). Vectors representing physical-chemical parameters [conductivity, pH, total nitrogen, water temperature, total phosphorus, Chemical Oxygen Demand (COD), dissolved O_2 and Habitat Quality Assessment (HQA)] which present Pearson coefficient > 0.2. The quality classes and respective colours are indicated on Fig. 12, as well as the dark and dotted circles representing 14D_53 and 09F_06 sample sites, respectively.

DISCUSSION

Recent studies demonstrated the effectiveness of DNA-based approaches for water quality assessment using diatom assemblages for different European regions and types of water bodies (Zimmerman et al., 2014; Visco et al., 2015; Vasselon et al., 2017; Rivera et al., 2018). In our study, we compared the eDNA and conventional (based on morphological determination using the light microscope) approaches in terms of community structure and ecological status classifications applied to Portuguese rivers and found similarities but also differences between morphological and molecular approaches.

1. Composition of Diatom Communities

The total number of species identified by both methods varied between 306 with the morphological approach (204, 120 and 534 in Vasselon et al., 2017; Rivera et al., 2018 and Keck et al., 2018, respectively) and 125-111 in DNA metabarcoding (66, 102 and 61 in Vasselon et al., 2017; Rivera et al., 2018 and Keck et al., 2018, respectively). The higher number of species identified in the traditional manner compared with the molecular method reveals a discrepancy between species diversity as was also demonstrated in other studies referred in the former sentence. However, other studies using diatoms (Zimmermann et al., 2014) and fish (Valentini et al., 2016) showed the opposite. Zimmermann et al. (2014) obtained 263 *versus* 102 taxa detected by eDNA and traditional approaches, respectively, while, in the second (Valentini et al., 2016), in 89% of the sites, eDNA-based approach detected a higher or identical number of taxa when compared to the traditional method. The differences between species diversity in the first studies may lay in the high percentage of unassigned reads. In our work, a total of 32% of OTUs were successfully assigned to species level which is congruent with other results (35.7% by Vasselon et al., 2017; 41% by Rivera et al., 2018; 30% by Keck et al., 2018), while 67% of OTUs corresponded to unassigned species.

In our study we also concluded that applying a correction factor (CF) to molecular data approximated it to the morphological results. The correction factor used approximates the two approaches by down weighting the largest taxa which are overestimated with the molecular approach compared to the traditional one which ignores size of diatoms and only regards the number of individuals. Regarding the community structure, this approximation is reflected on the similarity of the dominant taxa as well as their proportion, and consequently the ecological classifications inferred. Other authors obtained similar results when using correction factors for organisms such as bacteria (Angly et al., 2014), oligochaete (Vivien, Lejzerowicz & Pawlowski (2016), arthropods (Krehenwinkel et al., 2017) or fishes (Thomas et al., 2016). Those

corrections reduced bias in reads' abundances increasing correlation between morphological and molecular inventories. Based on their work, Krehenwinkel et al. (2017) suggest that a deeper focus on developing correction factors for groups of larger and more complex taxa may be of great use for comparative studies of invasive species' abundances in different sites. Regarding diatom cells and according to Vasselon et al. (2018), the correction factor based on biovolume differences between individuals brought together the molecular and morphological approaches. These results are built upon the correlation between *rbc*L gene copy number and genome size (linked to biovolume) in diatoms. *rbc*L copy number varies depending on several aspects such as the number of chloroplasts per cell, the number of genomes per chloroplast and/or the number of copies of *rbc*L gene per chloroplast genome (Ersland, Aldrich & Cattolico, 1981; Treusch et al., 2012). In the first case, it is known that there is a great stability in chloroplasts' number within a single genus varying from 1 to approximately 8 chloroplasts per cell between genera. In the second case, unicellular algae show a number of copies of chloroplast genome varying from hundreds to dozens, such as the example of *Olisthodiscus luteus* and *Thalassiosira pseudonana* with 650 and 55 genome copies per cell, respectively, contrarily to higher plants which can display thousands of copies of chloroplast genome per cell. At last, there is only one copy of *rbc*L gene per chloroplast genome (Vasselon et al., 2018). In their study, Vasselon and colleagues assumed that if there is an increase in cells' biovolume, the chloroplasts' biovolume also increases, as well as the genome copies per chloroplast, consequently the DNA quantity and thus, the *rbc*L gene copy number. However, this is not so linear and other factors which were not considered can affect the gene copy number, such as cells' life cycle stage, cells' physiological status, chloroplasts' physiology and DNA integrity (Eberhard, Drapier & Wollman, 2002). Iron limitation, for example, can reduce the number of chloroplasts per cell. Given all those variations and influences, it is important to perform a deeper evaluation of the correction factor for a more realistic and correct metabarcoding quantification.

The variations in the number of species detected by both molecular methodologies (with and without (CF)) were partially due to the removal of species with no biovolume correction factor already established. Therefore, it is important to improve biovolume data as well as other metrics. Also, as mentioned by Weber and Pawlowski (2013), one of the causes of discrepancies in abundances between molecular and morphological approaches may be due to biological biases such as biovolume variation. In this case, although the proximity of abundances with the application of a correction factor for biovolume is significant, this difference still exists. In view of this, some authors (e.g., Zaiko et al. 2015) opted to use only presence-absence molecular data of plankton communities for analysis.

Although 50% of the species recorded in molecular inventories are also present in morphological inventories, only one of the 5 most abundant species is shared by the three

approaches: *Achnanthidium minutissimum*, occurring in 90% of the samples and showing a higher abundance in the morphological method. As it is widely known (Martín et al., 2010; Kermarrec et al., 2014; Rivera et al., 2018; Sun et al., 2018; Keck et al., 2018), *Achnanthidium minutissimum* is a widespread species, identified as one of the most abundant species in morphological inventories for biomonitoring worldwide and also in Portugal (Luís et al., 2009; Almeida and Feio, 2012). *A. minutissimum* has a lower biovolume (76 μm^3, on average) when compared with other species such as *Ulnaria Ulna* and *Melosira varians* (present in both molecular and morphological inventories), with an average of 467 μm^3 and 3267 μm^3 (obtained from OMNIDIA version 5.5), respectively. Based on the correlation between *rbc*L gene copy number and diatom cell biovolume (Vasselon et al., 2018), the correction factor (CF) affected the abundance of the number of reads per sample by underestimating the big cells without interfering significantly with the small cells. This justifies the removal of the two big taxa above mentioned from the list of 5 most abundant species using the molecular method with CF. Additionally to *A. minutissimum*, the presence of *Eolimna minima* in both morphological and molecular with CF approaches of the 5 most abundant species supports the remaining results which shows a positive effect of the correction factor towards the morphological approach. *Eolimna minima* is often found among the most abundant benthic species in freshwaters (Wetzel et al., 2015) and recorded in another study (Keck et al., 2018) as one of the most abundant species in morphological analysis. This naviculoid diatom is similar to *A. minutissimum* in biovolume (88 μm^3) and is also considered a cryptic taxon, suggesting that future changes in nomenclature and insertion of other species in this complex may occur (currently in discussion).

2. Comparison of IPS values and Ecological Status Classifications

Despite the differences, the IPS values obtained from molecular data were well correlated with those obtained with morphological data (R=0.64 and 0.60, with molecular data with and without CF, respectively). More than half of the samples shared the same class either between molecular with CF and morphological approaches (57%), and between molecular method without CF and morphology (56%). A low percentage of the samples showed 2 or more classes of difference between the 3 combinations of methodologies. The morphological approach revealed the most optimistic ecological quality classifications summing up to 74% of the sites with “High” and “Good” status, followed by molecular method without the correction factor (71%) and molecular method with CF (60%).

The NMDS and PCO showed a similar global gradient of quality with the sites with better quality classifications associated to higher dissolved oxygen and higher habitat quality (HQA), independently of the approach used (morphological/molecular). Higher values of the HQA index in streams are also normally associated with sites less affected by anthropogenic

pressure (Raven et al., 1998). Higher nutrient concentration and conductivity is related to sources of pollution/eutrophication (Morrison et al., 2001), supporting the PCO results, which show an association between the worst quality classified sites by the IPS (diatoms) and higher concentrations of phosphorus and nitrogen. In general, the more common species in sites with "moderate" or "poor" quality classifications were *Nitzschia palea* in morphological list and *Eolimna minima* in both inventories. The two species were already reported as tolerant to high nutrient concentration and, organic pollution (Takamura et al., 1990; Thi et al., 2006). Thus, we verified that the molecular approach also responded well to anthropogenic degradation in rivers.

Yet, it is worthwhile to attend to the differences found between methods. In one site the IPSs varied between 18.1 (class "High") with morphological data and 7.5 ("Poor") and 4.9 ("Bad") with molecular data without and with CF, respectively. In this sample, the most abundant species found with the molecular approach was *Sellaphora seminulum*, a tolerant species (sensibility value of 1.5 in the IPS), while the morphological inventory showed high abundance of the sensitive species *Eunotia incisa* (sensibility value of 5 in the IPS). In fact, *Eunotia incisa* was absent from the reference library used and occurred in the inventory as unassigned, which was probably the main reason for the different results. In another sample the opposite situation occurred: the classification of "High" ecological quality (IPS= 19) with molecular data while with morphological data the classification was "Moderate" (IPS= 10.9). This sample showed high abundance of *Achnanthidium minutissimum* (sensibility = 5) in molecular methods, while *Achnanthidium saprophilum* (sensibility = 3) was the most abundant species in the taxonomic list. In this case, there might be a problem of correspondence in molecular databases between species and barcodes or a misidentification under the light microscope in the traditional approach. As it is known, *Achnanthidium* taxa have been clustered into "species complexes" due to difficulties in its identification. The small cell size makes it hard to recognise specific morphological features under the light microscope (Wojtal et al., 2011). Additionally, the constant change of *Achnanthidium* genus concept over time becomes a barrier for taxonomists to name uniformly and correctly these organisms (Ponader et al., 2006). For these reasons, taxa lists based on species' morphology are also subjected to errors and misidentifications which can compromise the accuracy of bioassessment. However, the degradation observed in the river and high conductivity and low dissolved oxygen values support the morphological results, with a higher abundance of *Achnanthidium saprophilum* (more tolerant) than *Achnanthidium minutissimum* (more sensitive). Thus, a meticulous curation of reference libraries is also essential in the process of developing molecular-based methods for ecological assessment of streams.

The effort and time needed were lower for metabarcoding than for the traditional approach. Taxonomic inventories based on the counting of valves per sample took some months and a great taxonomic expertise effort, while the eDNA approach associated with HTS

technique allowed a reduction of the effort and time consumed by standardizing the laboratory protocols and automating the handling of samples (larger spatio-temporal sampling possible), as also verified in other studies (Zimmermann et al., 2014; Vasselon et al., 2017, Hering et al., 2018).

Summarizing, the major problem which molecular ecologists face regarding DNA metabarcoding in biomonitoring is the low accuracy of the taxonomic assignment to HTS data (consequence of the high number of unassigned reads), especially due to incompleteness of reference libraries. Two possible solutions have been proposed to solve this problem (Hering et al. 2018): i) data generation for improvement of barcode reference database by collecting diatom cells from biofilm samples and selecting monoclonal strains for further sequencing (Vasselon et al., 2017), or ii) data generation of ecological preferences of unassigned OTUs. For now, the ideal solution is to continue adding more information to reference libraries since current indices (including IPS) depend on species identification and abundances.

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CONCLUSIONS & FUTURE PERSPECTIVES

Our results confirm previous studies (Visco et al., 2015; Vasselon et al., 2017; Rivera et al., 2017) on the contribution of DNA metabarcoding to the traditional approach for ecological quality assessment of freshwaters using diatoms. Although there is a good correlation between DNA and morphological-based methodologies in terms of ecological classifications, the incompleteness of reference libraries along with the need for standardization and harmonization of laboratory and field procedures, must be improved before replacing the traditional methods by molecular approaches. Currently, the major focus is on completing the reference databases, although a full coverage of all diatom species is not expected in the near future due to the difficulties in isolation and cultivation of some taxa. However, it is possible to explore alternative approaches to overcome this issue, especially because the understanding of species concept continues to evolve (Mann, 2010).

New-generation sequencing (NGS) techniques applied in biomonitoring should become very advantageous due to their promising future lower costs, rapid processing of samples and identification accuracy. The majority of studies have been focusing on measuring biodiversity by determining the species richness of a specific ecological niche. According to this new perspective and the fact that, currently, the end-point of bioassessment is to attribute ecological classifications to the water bodies, the taxonomic correspondence of OTUs to morphospecies could be indeed discarded. Therefore, the direct assignment of HTS data to ecological values already proposed by Keck et al. (2018) for diatom communities can be a way to overcome the problems inherent to taxonomic identification. However, with the target of 2027 in view, it is not feasible to completely replace the current water bioassessment strategies set out in the WFD by a DNA-based approach which would require the establishment of a whole new framework including indices recalculation and adaptation of laboratory protocols. Instead, we recommend the use of molecular methods as a complement to the traditional approach for biomonitoring.

In Portugal, other studies have been made using DNA metabarcoding approach associated with HTS techniques in aquatic ecosystems. One of them, for biomonitoring of estuarine macrobenthic communities (Lobo et al., 2017) and the other, for assessment of crustaceous trophic niches (Siegenthaler et al., 2018). Both agreed on the efficacy and potential of this method when compared to the morphology-based approach. On the other hand, and to our knowledge, the present study is the first one covering the ecological assessment with diatoms of Portuguese river types which constitutes an important basis for further application of metagenomics of microalgae in the country.

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ANNEX I

Table of the species and their relative abundances (%) per sample for morphological approach

	08F_02	08G_02	09F_06	09G_01	09G_03	09G_04	09H_04	09I_06	09J_03	09J_04	09L_02	10E_50	10F_50	10G_02	10H_03	10H_53	10J_52	10K_01	10K_05	10L_02	10M_08	11E_01	11H_04	11H_06	11I_01	11I_09	11J_12	11J_51	11J_02	11K_03	11L_50	11M_01	12E_01	12F_06	12F_07	12F_51	12G_02	12G_50
Achnanthes ploenensis Hustedt var. ***ploenensis*** (=***Kolbesia***)																																						
Achnanthes ricula Hohn & Hellerman																		259		185																		
Achnanthidium atomoides Monnier, Lange-Bertalot & Ector																										200												
Achnanthidium catenatum (Bílý & Marvan) Lange-Bertalot					4421																																	
Achnanthidium exiguum (Grunow) Czarnecki												648																										
Achnanthidium helveticum (Hustedt) Monnier, Lange-Bertalot & Ector														025																	119							
Achnanthidium minutissimum (Kütz.) Czarnecki f. ***anormale***																																						
Achnanthidium minutissimum (Kützing) Czarnecki	936	5450		4198	2033	3888	8188	961	395	1275	4723		167	5760	3022	2824	112	5106	428	1044	048	148		2287	1675		1625	2020	4116	6203	350	7933	100	1981	075	1149	829	3990
Achnanthidium pyrenaicum (Hustedt) Kobayasi																																				073		095
Achnanthidium reimeri (Cambrun) Ponader & Potapova																																166						
Achnanthidium rivulare Potapova & Ponader										4625													6625	4623												4810	
Achnanthidium saprophilum (Kobayashi et Mayama) Round & Bukhtiyarova			8762																																		
Achnanthidium straubianum (Lange-Bertalot) Lange-Bertalot	296				260	636		788																					073					118		196		048
Achnanthidium subhudsonis (Hustdt) H. Kobayasi	345	250		198		196				250	215							165	238						1125		300	673	1162								
Achnantidium subatomoides (Hustedt) Monnier, Lange-Bertalot et Ector				420		929	145			425	453			1961	120	1505		141		418	024				225	1225	1850		024	2193	2200	214						
Adlafia minuscula (Grunow) Lange-Bertalot																				023																		
Adlafia sp. Moser Lange-Bertalot & Metzeltin											024																											
Amphora copulata (Kütz) Schoeman & Archibald																																					048	
Amphora inariensis Krammer	862												048																					165		049		119
Amphora indistincta Levkov																																						
Amphora minutissima W. Smith																																						
Amphora ovalis (Kützing) Kützing													048									025																
Amphora pediculus (Kützing) Grunow	172											100	144						048			074			250				557					377	100	733		689
Aulacoseira distans (Ehr.)Simonsen																																						
Aulacoseira granulata (Ehr.) Simonsen																									050													
Aulacoseira tenella (Nygaard) Simonsen					686																																	
Bacillaria paxillifera (***paxillifer***) O. F. Müller) Hendey var. ***paxillifera***			024									998											150					025				025		1525			
Brachysira neglectissima Lange-Bertalot																																						
Caloneis amphisbaena (Bory) Cleve fo. ***amphisbaena***																																			075			
Caloneis bacillum (Grunow) Cleve																																				073		
Caloneis cf. ***lewisii*** Patrick																																				049		
Caloneis P. T. Cleve																																		047				
Chamaepinnularia evanida (Hustedt) Lange-Bertalot																				023																		
Chamaepinnularia sp. Lange-Bertalot & Krammer			024																																			
Cocconeis euglypta Ehrenberg emend Romero & Jahn	690		024									599	6292						261	023		5605	100			100			048				125	4245	1175	220	047	356
Cocconeis neodiminuta Krammer in Ricard																			148																			
Cocconeis pediculus Ehrenberg																																			350	049		071
Cocconeis placentula Ehrenberg var. ***lineata*** (Ehr.) Van Heurck	172	200	073					3596	4015	125	1050		239	025			494	1247	2613		072	691	575		075	3725	3865	339	047	050			330		024	355	
Cocconeis pseudolineata (Geitler) Lange-Bertalot											095							118	071						100												
Conticribra weissflogii (Grunow) Stachura-Suchoples & Williams																																						
Craticula ambigua (Ehrenberg) Mann																																						
Craticula molestiformis (Hustedt) Lange-Bertalot																				070																024		
Ctenophora pulchella (Ralfs ex Kutz.) Williams et Round										350									024																		
Cyclotella atomus Hustedt												050													150											024		
Cyclotella meneghiniana Kützing		025										125							024			296			050			050	024								
Cymatopleura solea (Brebisson in Breb. & Godey) W. Smith var. ***solea***																																				024		
Cymbella aspera (Ehrenberg) Peragallo																																						
Cymbella excisa Kützing var. ***excisa***																																					024	
Cymbella neolanceolata W. Silva					024																																	
Cymbella perparva Krammer																																					284	
Cymbella tumida (Brebisson) Van Heurck																								024														
Cymbopleura amphicephala Krammer																																						
Denticula tenuis Kützing																																						
Diadesmis contenta (Grunow ex. Van Heurck) Mann																																				024		
Diadesmis perpusilla (Grunow) D. G. Mann in Round & al.																																						
Diatoma mesodon (Ehrenberg) Kützing						073		025		050				049	096	625													354	550	309							
Diploneis marginestriata Hustedt																																						
Diploneis oblongella (Naegeli) Clave-Euler																																		142				
Diploneis oculata (Berbisson in Desmaziéres) Clave																																		047				
Encyonema minutum (Hilse in Rabh.) D. G. Mann in Round Crawford & Mann		025		099						125															125			125		024							
Encyonema neogracile Krammer							072								096																							
Encyonema silesiacum (Bleisch in Rabh.) D.G. Mann									123					686					095	209			400	170	150		150		218							877	
Encyonema ventricosum (Agardh) Grunow in Schmidt & al.					024									025																				307				214
Encyonopsis microcephala (Grunow) Krammer																																						
Encyonopsis minuta Krammer & Reichardt																																		094				048
Eolimna minima (Grunow) Lange-Bertalot	616	375		4099	142	2592	072	567	468		1718	1322	215	270		116	2989	541	1544	3503	2819	198			3675			274	605					118		293		148
Eolimna subminuscula (Manguin) Moser Lange-Bertalot & Metzeltin	245											050																025						024				
Eunotia bilunaris (Ehrenberg) Schaarschmidt		050						123																								648		400			
Eunotia botuliformis Wild, Nörpel-Schempp & Lange-Bertalot							097																														
Eunotia exigua (Brebisson ex Kützing) Rabenhorst																															225							
Eunotia implicata Nörpel-Schempp Alles & Lange-Bertalot in Alles &al.																																						
Eunotia incisa Gregory var. ***incisa***																																						
Eunotia minor (Kützing) Grunow in Van Heureck							242			175				172	144	069					1012																	
Eunotia monodon Ehrenberg var. ***monodon***																						025																
Eunotia mucophila (Lange-Bertalot & Norpel Schempp) Lange-Bertalot															096	069																						
Eunotia paludosa Grunow																																						
Eunotia pectinalis (Dyllwyn) Rabenhorst var. ***pectinalis***																																						
Eunotia pectinalis (Kütz.) Rabenhorst var. undulata f. ***tridon*** Manguin																																723						
Eunotia soleirolii (Kützing) Rabenhorst			049				048					100									072										2544						
Eunotia C. G. Ehrenberg																																						
Eunotia subarcuatoides Alles Nörpel & Lange-Bertalot in Alles et al.							338																															
Eunotia sudetica O. Muller							048																															
Fallacia insociabilis (Krasske) D.G. Mann																																						
Fallacia mitis (Hustedt) D.G. Mann																																			325	024		
Fallacia pygmaea (Kützing) Stickle & Mann ssp. ***Pygmaea*** in Lange-Bertalot & al																																						
Fallacia sp. A. J. Stickle & D. G. Mann																																						
Fallacia subhamulata (Grunow in V. Heurck) D.G. Mann																																				098		024
Fallacia sublucidula (Hustedt) D.G. Mann																																						
Fistulifera saprophila (Lange-Bertalot & Bonik) Lange-Bertalot																	337			302	072				025												
Fragilaria acidoclinata Lange-Bertalot & Hofmann																																						
Fragilaria aff. ***pectinalis*** (O. F. Müller) Grary																													024									
Fragilaria aff. ***perminuta*** (Grunow) Lange-Bertalot																													097									
Fragilaria aff. ***rumpens*** (Kütz.) G.W.F.Carlson											119																				095							
Fragilaria arcus (Ehrenberg) Clave var. ***arcus***																															238							
Fragilaria bidens Heiberg																																						
Fragilaria boreomongolica Kulikovskiy, Lange-Bertalot, Witkoxski & Dorofeyuk																													024									
Fragilaria candidagilae Almeida, C. Delgado, Novais & S. Blanco																																						
Fragilaria capucina Desmazieres var. ***capucina***																															125							

Fragilaria capucina Desmazieres var. *vaucheriae* (Kützing) Lange-Bertalot		2.00		0.49																				6.81				1.75										
Fragilaria cf. *tenera* (W. Smith) Lange-Bertalot f. *anormale*																																						
Fragilaria crotonensis Kitton											2.86														0.50													
Fragilaria gracilis Østrup					1.18			0.25		1.75			0.48	1.23	3.12													1.50										
Fragilaria nevadensis Lineares-Cuesta & Sanchez-Castilho																		0.24																				
Fragilaria pararumpens Lange-Bertalot, Hofmann & Werum in Hofmann & al.																		1.88																				
Fragilaria parva (Grunow in Van Heurck) Tuiji & Williams										2.75																			0.73				1.25				4.27	
Fragilaria pectinalis (O. F. Müller) Grary																																						
Fragilaria perminuta (Grunow) Lange-Bertalot																							2.00														1.90	
Fragilaria capitellata (Grunow) J.B Petersen											0.48								2.38																			
Fragilaria rinoi Almeida & C. Delgado														0.49																								
Fragilaria rumpens (Kütz.) G.W.F.Carlson															1.68										2.00												0.24	
Fragilaria H. C. Lyngbye			0.49													1.16			1.43		1.20													0.24		1.96	0.24	
Frustulia vulgaris (Thwaites) De Toni																							0.25															
Geissleria acceptata (Hust.) Lange-Bertalot & Metzeltin																		0.47																				
Geissleria decussis (Østrup) Lange-Bertalot & Metzeltin																					0.96	2.22													1.25			
Gomphonema acuminatum Ehrenberg var. *acuminatum*					2.60																														1.50			
Gomphonema affine Kützing																									0.75			0.25										
Gomphonema angustatum (Kützing) Rabenhorst																																						
Gomphonema augur Ehrenberg																																						
Gomphonema cf. *pseudoaugur* Lange-Bertalot																																						
Gomphonema clavatum Ehr.																													0.73						1.00			
Gomphonema exilissimum (Grun.) Lange-Bertalot & Reichardt					2.36						0.72																											
Gomphonema gracile Ehrenberg					6.62		1.45												0.48				0.50										0.75		1.25			
Gomphonema ibericum Reichardt																		1.41																				
Gomphonema lagenula Kützing		3.25				1.47		0.74					1.44		0.96						0.24											2.38						
Gomphonema mexicanum Grunow																																						
Gomphonema micropus Kützing var. *micropus*	0.49																												0.48									
Gomphonema minutum (Agardh) Agardh f. *minutum*																																		0.71		0.49		2.61
Gomphonema olivaceum (Hornemann) Brébisson var. *olivaceum*																																						
Gomphonema pala Reichardt																						0.49																
Gomphonema parvulum (Kützing) Kützing var. *parvulum* f. *parvulum*	0.49			0.49	0.24					2.00		0.50	0.24	0.98	1.92						3.37						1.00	1.00	0.24	3.07	5.25	0.95	7.23	1.65	0.50	0.49	0.71	
Gomphonema productum (Grunow) Lange-Bertalot & Reichardt																																						
Gomphonema pseudoaugur Lange-Bertalot																			0.95																			
Gomphonema pumilum (Grunow) Reichardt & Lange-Bertalot	1.72											0.75										0.25																
Gomphonema rhombicum M. Schmidt							2.42	0.99		4.75	1.19				21.34	39.35			0.48													1.43						
Gomphonema rosenstockianum Lange-Bertalot & Reichardt																								0.49														
Gomphonema saprophilum (Lange-Bertalot & Reicardt) Abarca, R. Jahan, Zimmermann & Enke		11.25												0.25																								
Gomphonema C. G. Ehrenberg																3.24		0.47																				
Gomphonema tergestinum (Grunow in Van Heurck) Schmidt in Schmidt & al.																																						
Gomphonema truncatum Ehr.																																			1.50			
Gomphonema uniserhombicum E. Reichardt																												0.25		8.02	5.75							
Gomphosphenia lingulatiformis (Lange-Bertalot & Reichardt) Lange-Bertalot																																						
Gyrosigma attenuatum (Kützing) Rabenhorst																																						
Gyrosigma sciotense (Sullivan et Wormley) Clave																																						0.48
Halamphora montana (krasske) Levkov			0.24																															0.47				
Halamphora veneta (Kützing) Levkov																																						
Hantzschia amphioxys (Ehr.) Grunow in Clave et Grunow 1880																																						
Hippodonta capitata (Ehr.) Lange-Bertalot, Metzeltin & Witkowski			1.46										0.95					0.47	0.48	0.23		0.99							0.24				0.75		2.50	0.49		
Hippodonta hungarica (Grunow) Lange-Bertalot, Metzeltin & Witkowski												0.50																										
Hippodonta lesmonensis (Hustedt) Lange-Bertalot Metzeltin & Witkowski																																						
Hippodonta pseudacceptata (Kobayasi) Lange-Bertalot Metzeltin & Witkowski	8.37																		0.71																			
Karayevia clevei (Grunow) Bukhtiyarova var. *clevei*																			0.48																	0.24		
Karayevia kolbei (Hustdt) Bukhtiyarova																																			4.00			
Karayevia oblongella (Øestrup) M. Aboal	0.74	3.75	1.70			5.13	0.48	22.66	3.69	9.50			0.24	0.25	30.70	1.85	0.45						1.75	5.84		64.50	5.00	3.24	9.93		50.75	2.61	1.25					
Karayevia ploenensis (Hustedt) Bukhtiyarova var. *gessneri* (Hust.) Bukhtiyarova																																		0.71		8.80		
Karayevia suchlandtii (Hustedt) Bukhtiyarova																																						
Lemnicola hungarica (Grunow) Round & Basson												0.50										0.49																
Luticola goeppertiana (Bleisch in Rabenhorst) D.G. Mann in Round Crawford & Mann																																						
Luticola chonii (Hilse) D. G. Mann in Round Crawford & Mann																																						
Luticola mutica (Kützing) D. G. Mann in Round Crawford & Mann																																				0.73		
Mayamaea atomus (Kützuing) Lange-Bertalot var. *atomus*			0.24										0.24	0.25	2.16		1.57		0.48	1.16	0.96																	
Mayamaea permitis (Hustedt) Bruder & Medlin	3.45										0.24		3.35	1.47			27.87		2.38	6.96	8.43	0.49										0.48				0.98	2.37	0.71
Mayameae agrestis (Hustedt) Lange-Bertalot																		0.24			0.48								0.24									
Melosira varians Agardh			0.73						0.25				0.24		0.24		0.22	0.24	0.24				0.25				1.50		1.21				2.00	0.24	2.50		0.71	1.19
Meridion circulare (Greville) C.A. Agardh var. *circulare*																	0.45				1.93																	
Navicula amphiceropsis Lange-Bertalot & Rumrich																																		0.47				
Navicula angusta Grunow							0.24																															
Navicula antonii Lange-Bertalot											0.24		0.96																							0.49	0.47	
Navicula arvensis Hustedt	0.25																																					
Navicula capitatoradiata Germain									0.49																													0.48
Navicula catalanogermanica Lange-Bertalot & Hofmann																																						
Navicula caterva Hohn & Hellerman																																			0.75			
Navicula cf. *cincta* (Ehr.) Ralfs in Pritchard																																						
Navicula cincta (Ehr.) Ralfs in Pritchard																																						
Navicula cryptocephala Kützing		0.50		0.49		1.71		0.74			0.24	0.50	0.48				1.12		0.95	0.23	3.86		0.50			0.25		0.50	0.73		1.00		12.72			1.22		
Navicula cryptotenella Lange-Bertalot										0.50	2.15		1.67					2.59	0.24				0.75		0.75		3.50	0.25	0.24					3.30	1.50	2.44	2.37	23.52
Navicula cryptotenelloides Lange-Bertalot																			0.24															0.24				0.71
Navicula erifuga Lange-Bertalot in Krammer & Lange-Bertalot																																						
Navicula exilis Kützing																																						
Navicula germainii Wallace																																						
Navicula gregaria Donkin	19.21	1.25	1.21									2.74	6.94						7.84		0.72	6.67			1.50	0.25		1.50	2.66				4.49		3.25	7.33	0.95	
Navicula hintzii Lange-Bertalot																																						
Navicula lanceolata (Agardh) Ehrenberg	0.49	1.75				0.24		0.49				2.24							0.95				1.25		0.25	0.50	1.00	4.49	1.69				2.00		0.25	0.98		0.24
Navicula notha Wallace					1.65					0.50				0.74						0.23				4.62														
Navicula phyllepta Kutzing																																						
Navicula pseudoarvensis Hustedt														0.25		0.23																						
Navicula radiosa Kützing																			0.24																			
Navicula radiosafallax Lange-Bertalot																																					0.24	
Navicula reichardtiana Lange-Bertalot var. *reichardtiana*																																		0.24				
Navicula rhynchocephala Kützing																					0.48							0.25					4.74					
Navicula rostellata Kützing												0.50																										
Navicula schroeteri Meister var. *schroeteri*																																						
Navicula simulata Manguin																																						
Navicula J.B.M Bory de St. Vincent															0.24																	0.24				0.24		
Navicula tenelloides Hustedt																																						
Navicula tripunctata (O.F. Müller) Bory												1.00										0.25												3.54	1.25	1.96		3.09
Navicula trivialis Lange-Bertalot																																						
Navicula upsaliensis (Grunow) Peragallo																																				0.49		
Navicula veneta Kützing	0.74	0.25	0.49	0.74								2.24	0.24											0.24				0.50		0.47						0.49		

Navicula vilaplanii (Lange-Bert. & Sabater) Lange-Bertalot & Sabater in Rumrich & al.																																						
Navicula viridula (Kützing) Ehrenberg																																				0.24		
Naviculadicta absoluta (Hustedt) Lange-Bertalot in Lange-Bertalot & Moser																																						
Neidium ampliatum (Ehrenberg) Krammer in Krammer & Lange-Bertalot																															0.50							
Neidium dubium (Ehrenberg) Cleve																																						
Nitzschia acicularis (Kützing) W.M. Smith																						0.74																
Nitzschia acidoclinata Lange-Bertalot													0.24																									
Nitzschia agnita Hustedt																																					0.24	
Nitzschia amphibia Grunow f. *amphibia*	2.22		0.24									17.96					0.45					2.72			0.75								0.25			1.47		
Nitzschia archibaldii Lange-Bertalot														0.74					0.24		0.96															0.49		
Nitzschia capitellata Hustedt in A. Schmidt & al.																																		0.47				1.43
Nitzschia costei Tudesque, Rimet & Ector																																		0.24		0.49	0.24	
Nitzschia disputata Carter														0.49																								
Nitzschia dissipata (Kützing) Grunow ssp. *dissipata*	0.99			0.49		0.24		0.25		0.75		0.25	0.72		0.24	0.93							1.50	2.19	0.50	0.50	0.50	1.50			0.50			0.47	1.25	5.38		1.66
Nitzschia dubia W.M. Smith																																			0.75			
Nitzschia epithemoides Grunow var. *disputata* (Carter) Lange-Bertalot																																0.48						
Nitzschia filiformis (W.M. Smith) Van Heurck var. *filiformis*																																						
Nitzschia fonticola Grunow in Van Heurck																	1.35					0.25							0.48					0.71			2.61	0.71
Nitzschia frequens Hustedt																																						
Nitzschia gracilis Hantzsch																																						
Nitzschia hantzschiana Rabenhorst												0.50									1.45																	
Nitzschia incognita Legler et Krasske																																						
Nitzschia inconspicua Grunow	2.96																				0.48						1.00									0.98		1.19
Nitzschia inconspicua Grunow f. *anormale*																																						
Nitzschia intermedia Hantzsch ex Cleve & Grunow																																						
Nitzschia lacuum Lange-Bertalot																																						0.48
Nitzschia linearis (Agardh) W.M. Smith var. *linearis*																			0.24				0.25												1.25			
Nitzschia linearis (Agardh) W.M. Smith var. *tenuis* (W. Smith) Grunow in Cleve & Grunow																			0.24																	0.49		
Nitzschia media Hantzsch.																			0.71		0.24																0.47	
Nitzschia microcephala Grunow in Cleve & Moller																																						
Nitzschia nana Grunow in Van Heurck					0.47																																	
Nitzschia palea (Kützing) W. Smith var. *debilis* (Kützing) Grunow in Cl. & Grunow		4.00																																				
Nitzschia palea (Kützing) W. Smith var. *palea*			0.49	0.74		1.22						4.74	0.72		0.48		0.67		0.48					2.19				0.50			0.25		6.23		1.25	0.98		
Nitzschia paleacea (Grunow) Grunow in van Heurck												0.75						0.47				0.49																
Nitzschia parvula W. M. Smith																																						
Nitzschia perminuta (Grunow) M.Peragallo					1.89										0.24			0.94	0.48																			
Nitzschia pusilla (Kützing) Grunow emend Lange-Bertalot													0.48																									
Nitzschia recta Hantzsch in Rabenhorst																			0.24																1.00			0.71
Nitzschia rosenstockii Lange-Bertalot																																						
Nitzschia soratensis Morales & Vis																																						0.71
Nitzschia sigma (Kützing) W.M. Smith												0.50																										
Nitzschia sociabilis Hustedt	0.74		0.49																0.24																	2.20		
Nitzschia solgensis Clave-Euler	0.25																																					
Nitzschia A. H. Hassall															0.48																							
Nitzschia subacicularis Hustedt in A. Schmidt et al.			0.49																																			
Nitzschia supralitorea Lange-Bertalot																																					1.66	
Nitzschia umbonata (Ehrenberg) Lange-Bertalot																																						
Nitzschia valdestriata Aleem & Hustedt																																						
Nitzschia vermicularis (Kützing) Hantzsch in Rabenhorst																																						
Nupela lapidosa (Lange-Bertalot) Lange-Bertalot var. *lapidosa*																																						
Parlibellus protractus (Grunow) Witkowski Lange-Bertalot & Metzeltin	0.49											1.50							0.24			0.49														0.73		
Pinnularia lundii Hustedt var. *lundii*			0.49																																			
Pinnularia microstauron (Ehr.) Cleve var. *microstauron*																																						
Pinnularia parvulissima Krammer																																						
Pinnularia C. G. Ehrenberg											0.48																											
Pinnularia subcapitata Gregory var. *elongata* Krammer																0.45			0.24												0.50							
Pinnularia subgibba Krammer var. *subgibba*				0.49																																		
Pinnularia viridis (Nitzsch) Ehrenberg var. *viridis*																												1.25										
Placoneis clementis (Grunow) Cox																																	0.50					
Placoneis clementoides (Hustedt) Cox																																						
Placoneis pseudanglica (Lange-Bertalot) Cox																																						
Placoneis C. Mereschkowsky																																						
Planothidium daui (Foged) Lange-Bertalot			0.73																			0.99							0.24									
Planothidium delicatulum (Kützing) Round & Bukhtiyarova												1.25										0.25					0.50											
Planothidium frequentissimum (Lange-Bertalot) Lange-Bertalot	6.65	1.25		5.19		1.47		3.45	7.39	1.75	1.91	11.97	2.39	0.49	0.48		5.17	1.88	6.41	19.72	3.61	9.63	12.00	0.24	5.50	5.75	7.50	3.49	2.91	0.24			6.73	2.36	23.25	13.94	0.47	
Planothidium granum (Hohn & Hellerman) Lange-Bertalot																																		1.89				
Planothidium hauckianum (Grun.) Round & Bukhtiyarova																																						
Planothidium haynaldii (Schaarschmidt) Lange-Bertalot																				0.46																		
Planothidium lanceolatum (Brébisson ex Kützing) Lange-Bertalot	1.48		0.49			1.96		3.45	2.71		1.19	4.24					2.92	0.24	9.74	7.42	20.24	0.49			3.25	11.50		3.24	2.18		0.50			0.47		0.24		0.48
Planothidium minutissimum (Krasske) Morales	0.74																																				0.47	
Planothidium robustius (Hustedt) Lange-Bertalot																																						
Planothidium rostratum (Østrup) Lange-Bertalot																																						
Platessa conspicua (A.Mayer) Lange-Bertalot							1.69																													0.98		
Platessa hustedtii (Krasske) Lange-Bertalot																																						
Pleurosira laevis (Ehrenberg) Compere f. *laevis* Ehrenberg																																						
Prestauroneis integra (W. Smith) Bruder in Bruder & Medin												0.50																										
Psammothidium sacculum (Carter) Bukhtiyarova et Round																																						
Pseudofallacia monoculata (Hustedt)																																						
Pseudofallacia tenera (Hustedt) Liu Kociolek & Wang																																						
Pseudostaurosira parasitica (W. Smith) Morales																		0.24																				
Reimeria sinuata (Gregory) Kociolek & Stoermer	0.74					1.22			0.25	1.00	0.24						0.45	1.41	0.48	2.32	2.89				1.50	0.25	1.50	1.25								0.24	7.11	0.48
Reimeria uniseriata Sala Guerrero & Ferrario																																				0.98		0.71
Rhoicosphenia abbreviata (C. Agardh) Lange-Bertalot																		0.47	0.48										0.24						4.25	0.49		
Rossithidium linearis (W. Sm.) Round & Bukhtiyarova													2.87																									
Sellaphora pseudopupula (Krasske) Lange-Bertalot																																	0.50					
Sellaphora pupula (Kützing) Mereschkowksy												0.25		0.49								0.49					0.50						2.24			0.24		
Sellaphora seminulum (Grunow) D. G. Mann	3.20	2.25				1.22		5.67	1.97		0.48	2.49	0.72	1.72			17.98	0.24	3.56	3.02	6.02	0.25			5.25				0.48							6.36		
Simonsenia delognei Lange-Bertalot																																		0.47		0.49		
Stauroneis gracilis Ehrenberg																																						
Stauroneis kriegeri Patrick																		0.94	0.24							0.25			0.24									
Stauroneis separanda Lange-Bertalot & Werum																																		0.47				
Stauroneis smithii Grunow																																						
Stauroneis thermicola (Petersen) Lund																																						
Staurosira binodis Lange-Bertalot in Hofmann Werum & Lange-Bertalot																																		0.71				
Staurosira brevistriata (Grunow) Grunow													0.72																									
Staurosira construens Ehrenberg																		8.94																				
Staurosira elliptica (Schumann) Williams & Round																																						
Staurosira martyi (Heribaud) Lange-Bertalot																																				0.98		
Staurosira mutabilis (Wm Smith) Grunow																		0.47																				
Staurosira (C. G. Ehrenberg) D. M. Williams & F. E. Round											3.10																											

	12G_55	13E_50	13F_50	13G_51	14D_51	14D_52	14D_53	14F_51	14F_52	15D_01	15D_03	15E_06	15E_08	15E_10	15E_50	15E_51	16E_02	16E_51	PTRH4A_R_1	PTRH4A_R_10	PTRH4A_R_11	PTRH4A_R_12	PTRH4A_R_13	PTRH4A_R_14	PTRH4A_R_15	PTRH4A_R_16	PTRH4A_R_17	PTRH4A_R_18	PTRH4A_R_2	PTRH4A_R_20	PTRH4A_R_21	PTRH4A_R_22
Achnanthes ploenensis Hustedt var. ***ploenensis*** (=***Kolbesia***)															023																	
Achnanthes ricula Hohn & Hellerman																																
Achnanthidium atomoides Monnier, Lange-Bertalot & Ector																																
Achnanthidium catenatum (Bílý & Marvan) Lange-Bertalot																																
Achnanthidium exiguum (Grunow) Czarnecki						075					142																				268	
Achnanthidium helveticum (Hustedt) Monnier, Lange-Bertalot & Ector																																
Achnanthidium minutissimum (Kütz.) Czarnecki f. ***anormale***																		207														
Achnanthidium minutissimum (Kützing) Czarnecki	6139	074	1022	3163	564	125	620	8057	4793	315		2724	098	876	1408	1476	1214	8575	450	1855	198	4512	1800	072	2947		2100	2847		217		1363
Achnanthidium pyrenaicum (Hustedt) Kobayasi	192											040																				024
Achnanthidium reimeri (Cambrun) Ponader & Potapova																																
Achnanthidium rivulare Potapova & Ponader	384																			5205					266					072		1995
Achnanthidium saprophilum (Kobayashi et Mayama) Round & Bukhtiyarova																																
Achnanthidium straubianum (Lange-Bertalot) Lange-Bertalot												497		049													425					049
Achnanthidium subhudsonis (Hustdt) H. Kobayasi	048																							072	048							
Achnantidium subatomoides (Hustedt) Monnier, Lange-Bertalot et Ector																									411			813				
Adlafia minuscula (Grunow) Lange-Bertalot					098																											
Adlafia sp. Moser Lange-Bertalot & Metzeltin																																
Amphora copulata (Kütz) Schoeman & Archibald																																
Amphora inariensis Krammer														024	704	881	238															
Amphora indistincta Levkov																381	524															
Amphora minutissima W. Smith						150																										
Amphora ovalis (Kützing) Kützing																																
Amphora pediculus (Kützing) Grunow			125	2019				088	1095	048	047	298	024	1217	4718	2357	1190	115			025	1049		096								
Aulacoseira distans (Ehr.)Simonsen																																
Aulacoseira granulata (Ehr.) Simonsen																																
Aulacoseira tenella (Nygaard) Simonsen																																
Bacillaria paxillifera ((***paxillifer***) O. F. Müller) Hendey var. ***paxillifera***		060	025																025					239							
Brachysira neglectissima Lange-Bertalot					025																									024		
Caloneis amphisbaena (Bory) Cleve fo. ***amphisbaena***											024																					
Caloneis bacillum (Grunow) Cleve														073	023	024	095															
Caloneis cf. ***lewisii*** Patrick																																
Caloneis P. T. Cleve						060																		048								
Chamaepinnularia evanida (Hustedt) Lange-Bertalot						025																			024					096		
Chamaepinnularia sp. Lange-Bertalot & Krammer																									314							
Cocconeis euglypta Ehrenberg emend Romero & Jahn	168	2085	274	2506	098			088	1290	581	165		024	1119	399	786	214			048	395	415									217		1606
Cocconeis neodiminuta Krammer in Ricard																																
Cocconeis pediculus Ehrenberg			374	341																												097
Cocconeis placentula Ehrenberg var. ***lineata*** (Ehr.) Van Heurck	600	124					596		170							071						122		694	145	6775	475	2821	673			170
Cocconeis pseudolineata (Geitler) Lange-Bertalot	216																															049
Conticribra weissflogii (Grunow) Stachura-Suchoples & Williams						075																										049
Craticula ambigua (Ehrenberg) Mann																															073	
Craticula molestiformis (Hustedt) Lange-Bertalot						060				073																						
Ctenophora pulchella (Ralfs ex Kutz.) Williams et Round																			025													
Cyclotella atomus Hustedt																			150					024					096	024		
Cyclotella meneghiniana Kützing		025			025						024					048			025					191						024	097	097
Cymatopleura solea (Brebisson in Breb. & Godey) W. Smith var. ***solea***																																
Cymbella aspera (Ehrenberg) Peragallo										024																						
Cymbella excisa Kützing var. ***excisa***												666																				
Cymbella neolanceolata W. Silva																																
Cymbella perparva Krammer																																
Cymbella tumida (Brebisson) Van Heurck																																
Cymbopleura amphicephala Krammer		060																														
Denticula tenuis Kützing																																
Diadesmis contenta (Grunow ex. Van Heurck) Mann					294																									386		
Diadesmis perpusilla (Grunow) D. G. Mann in Round & al.																							680					024				
Diatoma mesodon (Ehrenberg) Kützing																							525			125		287				
Diploneis marginestriata Hustedt																	024															
Diploneis oblongella (Naegeli) Clave-Euler			025																													
Diploneis oculata (Berbisson in Desmaziéres) Clave																024																
Encyonema minutum (Hilse in Rabh.) D. G. Mann in Round Crawford & Mann			1247																100													
Encyonema neogracile Krammer																																
Encyonema silesiacum (Bleisch in Rabh.) D.G. Mann								353																024	145		375					097
Encyonema ventricosum (Agardh) Grunow in Schmidt & al.								419																						024		
Encyonopsis microcephala (Grunow) Krammer									049			239																				
Encyonopsis minuta Krammer & Reichardt																																
Eolimna minima (Grunow) Lange-Bertalot	072				049	060				145		378		876	047	595	333	989						431	1280	125		670	601	048	170	195
Eolimna subminuscula (Manguin) Moser Lange-Bertalot & Metzeltin						060				073				195										096						024	073	
Eunotia bilunaris (Ehrenberg) Schaarscmidt			050																1475				425									
Eunotia botuliformis Wild, Nörpel-Schempp & Lange-Bertalot																																
Eunotia exigua (Brebisson ex Kützing) Rabenhorst																							150									
Eunotia implicata Nörpel-Schempp Alles & Lange-Bertalot in Alles &al.						025															049											
Eunotia incisa Gregory var. ***incisa***							5087				142																					
Eunotia minor (Kützing) Grunow in Van Heureck																							775	048			200	359	1068			
Eunotia monodon Ehrenberg var. ***monodon***																																
Eunotia mucophila (Lange-Bertalot & Norpel Schempp) Lange-Bertalot					074					121																						

Eunotia paludosa Grunow					0.25																											
Eunotia pectinalis (Dyllwyn) Rabenhorst var. *pectinalis*					2.21																											
Eunotia pectinalis (Kütz.) Rabenhorst var. undulata f. *tridon* Manguin																																
Eunotia soleirolii (Kützing) Rabenhorst						2.50				4.84														0.48				1.67	1.44	1.69		
Eunotia C. G. Ehrenberg																																
Eunotia subarcuatoides Alles Nörpel & Lange-Bertalot in Alles et al.																																
Eunotia sudetica O. Muller																																
Fallacia insociabilis (Krasske) D.G. Mann					0.25																											
Fallacia mitis (Hustedt) D.G. Mann					0.74											1.43													0.48			
Fallacia pygmaea (Kützing) Stickle & Mann ssp. *Pygmaea* in Lange-Bertalot & al										0.48						0.48																
Fallacia sp. A. J. Stickle & D. G. Mann																0.71																
Fallacia subhamulata (Grunow in V. Heurck) D.G. Mann																0.48																
Fallacia sublucidula (Hustedt) D.G. Mann																0.71																
Fistulifera saprophila (Lange-Bertalot & Bonik) Lange-Bertalot										0.48			52.68	28.22	0.47		2.38				3.21			0.24	0.48							0.49
Fragilaria acidoclinata Lange-Bertalot & Hofmann					0.49																											
Fragilaria aff. *pectinalis* (O. F. Müller) Grary																																
Fragilaria aff. *perminuta* (Grunow) Lange-Bertalot																								1.20								
Fragilaria aff. *rumpens* (Kütz.) G.W.F.Carlson																				0.48												
Fragilaria arcus (Ehrenberg) Clave var. *arcus*																								0.48	0.48							
Fragilaria bidens Heiberg																			2.00													
Fragilaria boreomongolica Kulikovskiy, Lange-Bertalot, Witkoxski & Dorofeyuk	0.96																0.24															
Fragilaria candidagilae Almeida, C. Delgado, Novais & S. Blanco	1.44																0.48															
Fragilaria capucina Desmazieres var. *capucina*													0.49																			
Fragilaria capucina Desmazieres var. *vaucheriae* (Kützing) Lange-Bertalot																																
Fragilaria cf. *tenera* (W. Smith) Lange-Bertalot f. *anormale*					0.49																											
Fragilaria crotonensis Kitton																																
Fragilaria gracilis Østrup																				2.89			29.50		0.48		7.50	1.91		0.24		10.22
Fragilaria nevadensis Lineares-Cuesta & Sanchez-Castilho																				0.48												
Fragilaria pararumpens Lange-Bertalot, Hofmann & Werum in Hofmann & al.																													0.24			
Fragilaria parva (Grunow in Van Heurck) Tuiji & Williams																																1.70
Fragilaria pectinalis (O. F. Müller) Grary						0.50														0.24												
Fragilaria perminuta (Grunow) Lange-Bertalot																																
Fragilaria capitellata (Grunow) J.B Petersen		0.74																												0.72		1.22
Fragilaria rinoi Almeida & C. Delgado															0.23	1.19																
Fragilaria rumpens (Kütz.) G.W.F.Carlson																																
Fragilaria H. C. Lyngbye										1.45		0.40					0.95															
Frustulia vulgaris (Thwaites) De Toni		0.25	0.25								0.24																		0.48			
Geissleria acceptata (Hust.) Lange-Bertalot & Metzeltin																																
Geissleria decussis (Østrup) Lange-Bertalot & Metzeltin												1.19		0.49																		
Gomphonema acuminatum Ehrenberg var. *acuminatum*																			1.25				1.25									
Gomphonema affine Kützing																																
Gomphonema angustatum (Kützing) Rabenhorst					0.49																											
Gomphonema augur Ehrenberg																			1.25													
Gomphonema cf. *pseudoaugur* Lange-Bertalot					0.49																											
Gomphonema clavatum Ehr.																						3.41			0.48							
Gomphonema exilissimum (Grun.) Lange-Bertalot & Reichardt																							9.75									
Gomphonema gracile Ehrenberg		1.74					1.74		0.24		0.24								3.75													1.95
Gomphonema ibericum Reichardt																																
Gomphonema lagenula Kützing						2.50																		0.96			1.00					2.19
Gomphonema mexicanum Grunow																								0.72								
Gomphonema micropus Kützing var. *micropus*						1.00																							0.48			
Gomphonema minutum (Agardh) Agardh f. *minutum*														0.97	0.94															0.48		
Gomphonema olivaceum (Hornemann) Brébisson var. *olivaceum*								4.86						0.97	1.64		2.38															
Gomphonema pala Reichardt																																
Gomphonema parvulum (Kützing) Kützing var. *parvulum* f. *parvulum*	0.96		0.25		1.47	6.00			0.24	1.45					0.47	0.24			8.00	0.48		0.49	4.25	1.67			0.75	1.20	1.68			
Gomphonema productum (Grunow) Lange-Bertalot & Reichardt																										0.50						
Gomphonema pseudoaugur Lange-Bertalot																																
Gomphonema pumilum (Grunow) Reichardt & Lange-Bertalot	0.96														0.70	3.33	5.95		2.75			26.10		1.44	0.48				0.96	1.45		1.95
Gomphonema rhombicum M. Schmidt	5.52																			23.37					13.04		15.25	12.20				
Gomphonema rosenstockianum Lange-Bertalot & Reichardt																																
Gomphonema saprophilum (Lange-Bertalot & Reicardt) Abarca, R. Jahan, Zimmermann & Enke					0.25	5.75																			0.24						68.37	
Gomphonema C. G. Ehrenberg						4.00																										
Gomphonema tergestinum (Grunow in Van Heurck) Schmidt in Schmidt & al.																																
Gomphonema truncatum Ehr.			2.49																3.00													
Gomphonema uniserhombicum E. Reichardt																										1.75						
Gomphosphenia lingulatiformis (Lange-Bertalot & Reichardt) Lange-Bertalot	0.24																															
Gyrosigma attenuatum (Kützing) Rabenhorst				0.24																												
Gyrosigma sciotense (Sullivan et Wormley) Clave																																
Halamphora montana (krasske) Levkov										0.73																						
Halamphora veneta (Kützing) Levkov					0.25																									0.24		
Hantzschia amphioxys (Ehr.) Grunow in Clave et Grunow 1880																							0.25									
Hippodonta capitata (Ehr.) Lange-Bertalot, Metzeltin & Witkowski					7.60	1.00					5.20								1.00											2.65	0.73	
Hippodonta hungarica (Grunow) Lange-Bertalot, Metzeltin & Witkowski					0.74						1.18																			1.20		
Hippodonta lesmonensis (Hustedt) Lange-Bertalot Metzeltin & Witkowski																														0.48		
Hippodonta pseudacceptata (Kobayasi) Lange-Bertalot Metzeltin & Witkowski		4.22			2.94					1.94	19.39													3.83						7.95		1.46
Karayevia clevei (Grunow) Bukhtiyarova var. *clevei*	0.24																															
Karayevia kolbei (Hustdt) Bukhtiyarova																																
Karayevia oblongella (Østrup) M. Aboal	4.32	2.98	0.75		13.48	5.75	28.54			20.82											22.72		4.50	27.99	3.14	18.50	31.75	6.46	26.20	4.82		0.49
Karayevia ploenensis (Hustedt) Bukhtiyarova var. *gessneri* (Hust.) Bukhtiyarova														0.49		0.71																
Karayevia suchlandtii (Hustedt) Bukhtiyarova																																
Lemnicola hungarica (Grunow) Round & Basson					0.25	0.25																		0.24							0.97	
Luticola goeppertiana (Bleisch in Rabenhorst) D.G. Mann in Round Crawford & Mann												0.20																				
Luticola cohnii (Hilse) D. G. Mann in Round Crawford & Mann					0.49																								0.24			
Luticola mutica (Kützing) D. G. Mann in Round Crawford & Mann					0.49																									0.48		
Mayamaea atomus (Kützuing) Lange-Bertalot var. *atomus*					0.25	0.75				0.48							0.24															2.68
Mayamaea permitis (Hustedt) Bruder & Medlin					2.21	6.00				4.84			0.73	2.68	3.52	0.24	15.95	0.23						2.39						0.72		5.35
Mayameae agrestis (Hustedt) Lange-Bertalot																																
Melosira varians Agardh	0.96		10.97	0.49	1.96					2.91						0.48				0.72				1.91					0.48	4.10		
Meridion circulare (Greville) C.A. Agardh var. *circulare*																		0.46					4.00			0.25	0.50					
Navicula amphiceropsis Lange-Bertalot & Rumrich																																
Navicula angusta Grunow																							0.50					0.24				
Navicula antonii Lange-Bertalot																	0.48													0.24		
Navicula arvensis Hustedt																																
Navicula capitatoradiata Germain							0.74								0.47														3.37			
Navicula catalanogermanica Lange-Bertalot & Hofmann																	0.24															
Navicula caterva Hohn & Hellerman					0.25											0.24	0.24															
Navicula cf. *cincta* (Ehr.) Ralfs in Pritchard						0.25																										

Navicula cincta (Ehr.) Ralfs in Pritchard																0.24																
Navicula cryptocephala Kützing						10.25					0.95								10.75								0.25		1.92	1.45	4.14	1.22
Navicula cryptotenella Lange-Bertalot	1.20		4.74		0.74									5.60	4.69	0.71	5.48								0.48					0.48		
Navicula cryptotenelloides Lange-Bertalot				5.11					4.87							0.24	1.67															
Navicula erifuga Lange-Bertalot in Krammer & Lange-Bertalot		0.50																	2.00													
Navicula exilis Kützing																												0.96				
Navicula germainii Wallace																								0.48						0.96		
Navicula gregaria Donkin		19.11	1.75		7.84	4.00		0.22		9.20	4.26		14.39	4.87	5.63	5.95	3.57		0.25		2.72			17.94	0.48				3.37	11.81		
Navicula hintzii Lange-Bertalot																									0.24							
Navicula lanceolata (Agardh) Ehrenberg		23.82			0.49	0.25	2.48			6.54	0.95		4.39		0.47				1.00		6.67			0.72	0.24				3.37	0.24	0.24	
Navicula notha Wallace																									0.48		3.75		0.48			
Navicula phyllepta Kutzing														0.24	0.23																	
Navicula pseudoarvensis Hustedt																																
Navicula radiosa Kützing																																0.24
Navicula radiosafallax Lange-Bertalot																																
Navicula reichardtiana Lange-Bertalot var. *reichardtiana*		0.50	1.00												0.47	0.71	8.10		12.25													
Navicula rhynchocephala Kützing						0.25																				0.25				0.72		
Navicula rostellata Kützing																														0.24		
Navicula schroeteri Meister var. *schroeteri*																																0.24
Navicula simulata Manguin						0.25																										
Navicula J.B.M Bory de St. Vincent						0.75								0.24			0.48															
Navicula tenelloides Hustedt																																
Navicula tripunctata (O.F. Müller) Bory			16.46	1.22				0.44	4.87	0.48							0.48															
Navicula trivialis Lange-Bertalot																0.24																
Navicula upsaliensis (Grunow) Peragallo																																
Navicula veneta Kützing		4.22			1.96	2.00		0.22		2.18			1.71			0.24	1.19		2.50		0.49	0.24				0.25			0.48	1.20		
Navicula vilaplanii (Lange-Bert. & Sabater) Lange-Bertalot & Sabater in Rumrich & al.						0.50																								0.48		
Navicula viridula (Kützing) Ehrenberg																																
Naviculadicta absoluta (Hustedt) Lange-Bertalot in Lange-Bertalot & Moser		0.25																														
Neidium ampliatum (Ehrenberg) Krammer in Krammer & Lange-Bertalot																																
Neidium dubium (Ehrenberg) Cleve																														0.48		
Nitzschia acicularis (Kützing) W.M. Smith																			1.25											0.24		0.24
Nitzschia acidoclinata Lange-Bertalot																																
Nitzschia agnita Hustedt																																
Nitzschia amphibia Grunow f. *amphibia*		0.25			1.23					0.48		1.19	0.49				0.48					1.71		0.72						0.48		
Nitzschia archibaldii Lange-Bertalot					1.47	0.50				1.69		0.40																				
Nitzschia capitellata Hustedt in A. Schmidt & al.	0.48					0.75																										
Nitzschia costei Tudesque, Rimet & Ector																																
Nitzschia disputata Carter																																
Nitzschia dissipata (Kützing) Grunow ssp. *dissipata*	1.20	0.99	2.24	5.35				1.55	0.24					2.68	0.94	2.62	3.33		9.50					0.48					1.44		0.49	
Nitzschia dubia W.M. Smith			0.25																		0.25									0.24		
Nitzschia epithemoides Grunow var. *disputata* (Carter) Lange-Bertalot																																
Nitzschia filiformis (W.M. Smith) Van Heurck var. *filiformis*																			3.00													
Nitzschia fonticola Grunow in Van Heurck												1.59																				0.97
Nitzschia frequens Hustedt																														0.24		
Nitzschia gracilis Hantzsch						1.25																										
Nitzschia hantzschiana Rabenhorst																																
Nitzschia incognita Legler et Krasske																																0.49
Nitzschia inconspicua Grunow				3.89	1.23			1.10		0.24		0.60		2.43	3.05	3.10	4.52													0.24		
Nitzschia inconspicua Grunow f. *anormale*																	0.24															
Nitzschia intermedia Hantzsch ex Cleve & Grunow											1.42																					
Nitzschia lacuum Lange-Bertalot														0.49																1.93		
Nitzschia linearis (Agardh) W.M. Smith var. *linearis*		0.25	2.00		0.25		0.25			0.24			0.49						0.50							0.25			1.92			
Nitzschia linearis (Agardh) W.M. Smith var. *tenuis* (W. Smith) Grunow in Cleve & Grunow						0.25					0.71																					
Nitzschia media Hantzsch.																														0.24		
Nitzschia microcephala Grunow in Cleve & Moller																	0.24															
Nitzschia nana Grunow in Van Heurck																																
Nitzschia palea (Kützing) W. Smith var. *debilis* (Kützing) Grunow in Cl. & Grunow						2.75																			0.24				0.96			
Nitzschia palea (Kützing) W. Smith var. *palea*			0.25		0.25	2.50	0.50			0.73			0.98	0.73	0.47	0.24			5.25					1.67		0.50				1.93	4.87	
Nitzschia paleacea (Grunow) Grunow in van Heurck		4.71									6.38								3.25													
Nitzschia parvula W. M. Smith					0.49																											
Nitzschia perminuta (Grunow) M.Peragallo																									0.48					0.24		
Nitzschia pusilla (Kützing) Grunow emend Lange-Bertalot																																
Nitzschia recta Hantzsch in Rabenhorst																																
Nitzschia rosenstockii Lange-Bertalot										0.97																						
Nitzschia soratensis Morales & Vis																																
Nitzschia sigma (Kützing) W.M. Smith																																
Nitzschia sociabilis Hustedt																								0.24								
Nitzschia solgensis Clave-Euler																																
Nitzschia A. H. Hassall	0.24				0.74									0.49																		
Nitzschia subacicularis Hustedt in A. Schmidt et al.																														0.48		
Nitzschia supralitorea Lange-Bertalot						1.00																		0.96						2.17		1.46
Nitzschia umbonata (Ehrenberg) Lange-Bertalot																																
Nitzschia valdestriata Aleem & Hustedt					0.25																											
Nitzschia vermicularis (Kützing) Hantzsch in Rabenhorst										0.24						0.24																
Nupela lapidosa (Lange-Bertalot) Lange-Bertalot var. *lapidosa*																										1.00		1.91				
Parlibellus protractus (Grunow) Witkowski Lange-Bertalot & Metzeltin						0.50				5.81	30.97													1.67						9.40		
Pinnularia lundii Hustedt var. *lundii*																																
Pinnularia microstauron (Ehr.) Cleve var. *microstauron*							0.50																									
Pinnularia parvulissima Krammer						2.00																										
Pinnularia C. G. Ehrenberg					0.25																											
Pinnularia subcapitata Gregory var. *elongata* Krammer						0.50																										
Pinnularia subgibba Krammer var. *subgibba*																															0.24	
Pinnularia viridis (Nitzsch) Ehrenberg var. *viridis*																																
Placoneis clementis (Grunow) Cox					2.70					0.24	7.33																			0.48	0.49	
Placoneis clementoides (Hustedt) Cox																														0.48		
Placoneis pseudanglica (Lange-Bertalot) Cox						0.50																										
Placoneis C. Mereschkowsky										0.24																						
Planothidium daui (Foged) Lange-Bertalot																														5.78		
Planothidium delicatulum (Kützing) Round & Bukhtiyarova					0.49					0.48																						
Planothidium frequentissimum (Lange-Bertalot) Lange-Bertalot					19.85	6.50				6.78		0.40		2.43		4.52	0.71		0.50		17.53			4.31	3.86	1.25			5.05	7.47	4.38	0.97
Planothidium granum (Hohn & Hellerman) Lange-Bertalot					0.49		0.50														5.19											
Planothidium hauckianum (Grun.) Round & Bukhtiyarova																					6.91									0.48		
Planothidium haynaldii (Schaarschmidt) Lange-Bertalot																																
Planothidium lanceolatum (Brébisson ex Kützing) Lange-Bertalot	0.72	5.96	2.49		2.45	4.50	0.50		4.38	2.18		0.80	17.32			0.95		0.46	2.25	0.24	26.67	4.39		2.39	7.73	3.25			15.87		2.68	
Planothidium minutissimum (Krasske) Morales										0.73																				0.96		
Planothidium robustius (Hustedt) Lange-Bertalot																																
Planothidium rostratum (Østrup) Lange-Bertalot		0.99							2.43		9.69		0.98	0.73																		
Platessa conspicua (A.Mayer) Lange-Bertalot											2.36																					

Platessa hustedtii (Krasske) Lange-Bertalot													
Pleurosira laevis (Ehrenberg) Compere f. ***laevis*** Ehrenberg													
Prestauroneis integra (W. Smith) Bruder in Bruder & Medin													
Psammothidium sacculum (Carter) Bukhtiyarova et Round													
Pseudofallacia monoculata (Hustedt)													
Pseudofallacia tenera (Hustedt) Liu Kociolek & Wang													
Pseudostaurosira parasitica (W. Smith) Morales													
Reimeria sinuata (Gregory) Kociolek & Stoermer													
Reimeria uniseriata Sala Guerrero & Ferrario													
Rhoicosphenia abbreviata (C. Agardh) Lange-Bertalot													
Rossithidium linearis (W. Sm.) Round & Bukhtiyarova													
Sellaphora pseudopupula (Krasske) Lange-Bertalot													
Sellaphora pupula (Kützing) Mereschkowksy													
Sellaphora seminulum (Grunow) D. G. Mann													
Simonsenia delognei Lange-Bertalot													
Stauroneis gracilis Ehrenberg													
Stauroneis kriegeri Patrick													
Stauroneis separanda Lange-Bertalot & Werum													
Stauroneis smithii Grunow													
Stauroneis thermicola (Petersen) Lund													
Staurosira binodis Lange-Bertalot in Hofmann Werum & Lange-Bertalot													
Staurosira brevistriata (Grunow) Grunow													
Staurosira construens Ehrenberg													
Staurosira elliptica (Schumann) Williams & Round													
Staurosira martyi (Heribaud) Lange-Bertalot													
Staurosira mutabilis (Wm Smith) Grunow													
Staurosira (C. G. Ehrenberg) D. M. Williams & F. E. Round													
Staurosira venter (Ehr.) Cleve & Moeller													
Staurosirella pinnata (Ehr.) Williams & Round													
Surirella angusta Kützing													
Surirella brebissonii Krammer & Lange-Bertalot var. ***brebissonii***													
Surirella brebissonii var. ***keutzingii*** Krammer et Lange-Bertalot													
Surirella linearis W.M.Smith in Schmidt & al.													
Surirella roba Leclercq													
Surirella robusta Ehrenberg													
Tabellaria fenestrata (Lyngbye) Kützing													
Tabellaria flocculosa (Roth) Kützing													
Tabellaria ventricosa Kützing													
Tabularia fasciculata (Agardh) Williamset Round													
Tabularia tabulata (C. A. Agardh) Snoeijs													
Thalassiosira pseudonana Hasle et Heimdal													
Tryblionella calida (Grunow in Cl. & Grun.) D. G. Mann in Round Crawford & Mann													
Tryblionella constricta (Kützing) Poulin in Poulin & al.													
Tryblionella debilis Arnott ex O'Meara													
Tryblionella hungarica (Grunow) D.G. Mann													
Ulnaria biceps (Kützing) Compère													
Ulnaria ulna (Nitzsch.) Compère													

	PTRH4A_R_23	PTRH4A_R_24	PTRH4A_R_25	PTRH4A_R_26	PTRH4A_R_27	PTRH4A_R_28	PTRH4A_R_3	PTRH4A_R_4	PTRH4A_R_5	PTRH4A_R_6	PTRH4A_R_7	PTRH4A_R_9
Achnanthes ploenensis Hustedt var. ***ploenensis*** (=***Kolbesia***)												
Achnanthes ricula Hohn & Hellerman												
Achnanthidium atomoides Monnier, Lange-Bertalot & Ector												
Achnanthidium catenatum (Bílý & Marvan) Lange-Bertalot												
Achnanthidium exiguum (Grunow) Czarnecki												
Achnanthidium helveticum (Hustedt) Monnier, Lange-Bertalot & Ector												
Achnanthidium minutissimum (Kütz.) Czarnecki f. ***anormale***												
Achnanthidium minutissimum (Kützing) Czarnecki												
Achnanthidium pyrenaicum (Hustedt) Kobayasi												
Achnanthidium reimeri (Cambrun) Ponader & Potapova												
Achnanthidium rivulare Potapova & Ponader												
Achnanthidium saprophilum (Kobayashi et Mayama) Round & Bukhtiyarova												
Achnanthidium straubianum (Lange-Bertalot) Lange-Bertalot												
Achnanthidium subhudsonis (Hustdt) H. Kobayasi												
Achnantidium subatomoides (Hustedt) Monnier, Lange-Bertalot et Ector												
Adlafia minuscula (Grunow) Lange-Bertalot												
Adlafia sp. Moser Lange-Bertalot & Metzeltin												
Amphora copulata (Kütz) Schoeman & Archibald												
Amphora inariensis Krammer												
Amphora indistincta Levkov												
Amphora minutissima W. Smith												
Amphora ovalis (Kützing) Kützing												
Amphora pediculus (Kützing) Grunow												
Aulacoseira distans (Ehr.)Simonsen												
Aulacoseira granulata (Ehr.) Simonsen												
Aulacoseira tenella (Nygaard) Simonsen												
Bacillaria paxillifera (|***paxillifer***) O. F. Müller) Hendey var. ***paxillifera***												
Brachysira neglectissima Lange-Bertalot												
Caloneis amphisbaena (Bory) Cleve fo. ***amphisbaena***												
Caloneis bacillum (Grunow) Cleve												
Caloneis cf. ***lewisii*** Patrick												
Caloneis P. T. Cleve												
Chamaepinnularia evanida (Hustedt) Lange-Bertalot												
Chamaepinnularia sp. Lange-Bertalot & Krammer												
Cocconeis euglypta Ehrenberg emend Romero & Jahn												
Cocconeis neodiminuta Krammer in Ricard												
Cocconeis pediculus Ehrenberg												
Cocconeis placentula Ehrenberg var. ***lineata*** (Ehr.) Van Heurck												
Cocconeis pseudolineata (Geitler) Lange-Bertalot												
Conticribra weissflogii (Grunow) Stachura-Suchoples & Williams												
Craticula ambigua (Ehrenberg) Mann												
Craticula molestiformis (Hustedt) Lange-Bertalot												
Ctenophora pulchella (Ralfs ex Kutz.) Williams et Round												
Cyclotella atomus Hustedt												
Cyclotella meneghiniana Kützing												
Cymatopleura solea (Brebisson in Breb. & Godey) W. Smith var. ***solea***												
Cymbella aspera (Ehrenberg) Peragallo												
Cymbella excisa Kützing var. ***excisa***												
Cymbella neolanceolata W. Silva												

Cymbella perparva Krammer												
Cymbella tumida (Brebisson) Van Heurck												
Cymbopleura amphicephala Krammer												
Denticula tenuis Kützing												
Diadesmis contenta (Grunow ex. Van Heurck) Mann												
Diadesmis perpusilla (Grunow) D. G. Mann in Round & al.												
Diatoma mesodon (Ehrenberg) Kützing			049									
Diploneis marginestriata Hustedt												
Diploneis oblongella (Naegeli) Clave-Euler												
Diploneis oculata (Berbisson in Desmaziéres) Clave												
Encyonema minutum (Hilse in Rabh.) D. G. Mann in Round Crawford & Mann		025									047	
Encyonema neogracile Krammer												
Encyonema silesiacum (Bleisch in Rabh.) D.G. Mann			220				171	116			140	
Encyonema ventricosum (Agardh) Grunow in Schmidt & al.					169							
Encyonopsis microcephala (Grunow) Krammer												
Encyonopsis minuta Krammer & Reichardt												
Eolimna minima (Grunow) Lange-Bertalot	300		2088		1304	147	4548	2837	1064	306	4159	
Eolimna subminuscula (Manguin) Moser Lange-Bertalot & Metzeltin											023	
Eunotia bilunaris (Ehrenberg) Schaarscmidt												
Eunotia botuliformis Wild, Nörpel-Schempp & Lange-Bertalot												
Eunotia exigua (Brebisson ex Kützing) Rabenhorst												048
Eunotia implicata Nörpel-Schempp Alles & Lange-Bertalot in Alles &al.												
Eunotia incisa Gregory var. *incisa*												
Eunotia minor (Kützing) Grunow in Van Heureck	950		366		048		049					
Eunotia monodon Ehrenberg var. *monodon*												
Eunotia mucophila (Lange-Bertalot & Norpel Schempp) Lange-Bertalot												
Eunotia paludosa Grunow												
Eunotia pectinalis (Dyllwyn) Rabenhorst var. *pectinalis*												
Eunotia pectinalis (Kütz.) Rabenhorst var. undulata f. *tridon* Manguin												
Eunotia soleirolii (Kützing) Rabenhorst	025		098									
Eunotia C. G. Ehrenberg								070			093	
Eunotia subarcuatoides Alles Nörpel & Lange-Bertalot in Alles et al.												
Eunotia sudetica O. Muller												
Fallacia insociabilis (Krasske) D.G. Mann												
Fallacia mitis (Hustedt) D.G. Mann									025			
Fallacia pygmaea (Kützing) Stickle & Mann ssp. *Pygmaea* in Lange-Bertalot & al												
Fallacia sp. A. J. Stickle & D. G. Mann												
Fallacia subhamulata (Grunow in V. Heurck) D.G. Mann												
Fallacia sublucidula (Hustedt) D.G. Mann												
Fistulifera saprophila (Lange-Bertalot & Bonik) Lange-Bertalot			049		121		098				164	
Fragilaria acidoclinata Lange-Bertalot & Hofmann												
Fragilaria aff. *pectinalis* (O. F. Müller) Grary												
Fragilaria aff. *perminuta* (Grunow) Lange-Bertalot											140	
Fragilaria aff. *rumpens* (Kütz.) G.W.F.Carlson												
Fragilaria arcus (Ehrenberg) Clave var. *arcus*												
Fragilaria bidens Heiberg												
Fragilaria boreomongolica Kulikovskiy, Lange-Bertalot, Witkoxski & Dorofeyuk											023	
Fragilaria candidagilae Almeida, C. Delgado, Novais & S. Blanco												
Fragilaria capucina Desmazieres var. *capucina*												
Fragilaria capucina Desmazieres var. *vaucheriae* (Kützing) Lange-Bertalot									025			
Fragilaria cf. *tenera* (W. Smith) Lange-Bertalot f. *anormale*												
Fragilaria crotonensis Kitton												
Fragilaria gracilis Østrup			561	023								
Fragilaria nevadensis Lineares-Cuesta & Sanchez-Castilho												
Fragilaria pararumpens Lange-Bertalot, Hofmann & Werum in Hofmann & al.												
Fragilaria parva (Grunow in Van Heurck) Tuiji & Williams			098		435		049	140				
Fragilaria pectinalis (O. F. Müller) Grary												
Fragilaria perminuta (Grunow) Lange-Bertalot			073									
Fragilaria capitellata (Grunow) J.B Petersen			244									
Fragilaria rinoi Almeida & C. Delgado												
Fragilaria rumpens (Kütz.) G.W.F.Carlson	175		122									
Fragilaria H. C. Lyngbye							098					
Frustulia vulgaris (Thwaites) De Toni												
Geissleria acceptata (Hust.) Lange-Bertalot & Metzeltin			073		242							
Geissleria decussis (Østrup) Lange-Bertalot & Metzeltin												
Gomphonema acuminatum Ehrenberg var. *acuminatum*												
Gomphonema affine Kützing												
Gomphonema angustatum (Kützing) Rabenhorst												
Gomphonema augur Ehrenberg												
Gomphonema cf. *pseudoaugur* Lange-Bertalot												
Gomphonema clavatum Ehr.												
Gomphonema exilissimum (Grun.) Lange-Bertalot & Reichardt							073	047			140	
Gomphonema gracile Ehrenberg												
Gomphonema ibericum Reichardt												
Gomphonema lagenula Kützing												
Gomphonema mexicanum Grunow												
Gomphonema micropus Kützing var. *micropus*												
Gomphonema minutum (Agardh) Agardh f. *minutum*												
Gomphonema olivaceum (Hornemann) Brébisson var. *olivaceum*												
Gomphonema pala Reichardt												
Gomphonema parvulum (Kützing) Kützing var. *parvulum* f. *parvulum*	225	368						140	124		350	
Gomphonema productum (Grunow) Lange-Bertalot & Reichardt												
Gomphonema pseudoaugur Lange-Bertalot												
Gomphonema pumilum (Grunow) Reichardt & Lange-Bertalot		319			362							
Gomphonema rhombicum M. Schmidt				2974		025		093		088	047	
Gomphonema rosenstockianum Lange-Bertalot & Reichardt					048							
Gomphonema saprophilum (Lange-Bertalot & Reicardt) Abarca, R. Jahan, Zimmermann & Enke												
Gomphonema C. G. Ehrenberg												
Gomphonema tergestinum (Grunow in Van Heurck) Schmidt in Schmidt & al.					048							
Gomphonema truncatum Ehr.												
Gomphonema uniserhombicum E. Reichardt												
Gomphosphenia lingulatiformis (Lange-Bertalot & Reichardt) Lange-Bertalot												
Gyrosigma attenuatum (Kützing) Rabenhorst												
Gyrosigma sciotense (Sullivan et Wormley) Clave												
Halamphora montana (krasske) Levkov												
Halamphora veneta (Kützing) Levkov												048
Hantzschia amphioxys (Ehr.) Grunow in Clave et Grunow 1880												
Hippodonta capitata (Ehr.) Lange-Bertalot, Metzeltin & Witkowski									025			
Hippodonta hungarica (Grunow) Lange-Bertalot, Metzeltin & Witkowski												
Hippodonta lesmonensis (Hustedt) Lange-Bertalot Metzeltin & Witkowski												

Hippodonta pseudacceptata (Kobayasi) Lange-Bertalot Metzeltin & Witkowski						024					
Karayevia clevei (Grunow) Bukhtiyarova var. *clevei*				024							
Karayevia kolbei (Hustdt) Bukhtiyarova		195									
Karayevia oblongella (Østrup) M. Aboal	300		024	351	025	024	116	198	022	1075	8547
Karayevia ploenensis (Hustedt) Bukhtiyarova var. *gessneri* (Hust.) Bukhtiyarova											
Karayevia suchlandtii (Hustedt) Bukhtiyarova							023				
Lemnicola hungarica (Grunow) Round & Basson						196					
Luticola goeppertiana (Bleisch in Rabenhorst) D.G. Mann in Round Crawford & Mann											
Luticola chonii (Hilse) D. G. Mann in Round Crawford & Mann											
Luticola mutica (Kützing) D. G. Mann in Round Crawford & Mann											
Mayamaea atomus (Kützuing) Lange-Bertalot var. *atomus*				048		660	047	644	109	187	
Mayamaea permitis (Hustedt) Bruder & Medlin			293	652		1125	209			397	
Mayameae agrestis (Hustedt) Lange-Bertalot											
Melosira varians Agardh			073								
Meridion circulare (Greville) C.A. Agardh var. *circulare*							047				
Navicula amphiceropsis Lange-Bertalot & Rumrich											
Navicula angusta Grunow											
Navicula antonii Lange-Bertalot			146						022	023	
Navicula arvensis Hustedt											
Navicula capitatoradiata Germain											
Navicula catalanogermanica Lange-Bertalot & Hofmann											
Navicula caterva Hohn & Hellerman											
Navicula cf. *cincta* (Ehr.) Ralfs in Pritchard											
Navicula cincta (Ehr.) Ralfs in Pritchard											
Navicula cryptocephala Kützing				072		024	093	223		140	
Navicula cryptotenella Lange-Bertalot			098	145					066	234	
Navicula cryptotenelloides Lange-Bertalot		098									
Navicula erifuga Lange-Bertalot in Krammer & Lange-Bertalot											
Navicula exilis Kützing			049								
Navicula germainii Wallace											
Navicula gregaria Donkin			049	048		049				187	
Navicula hintzii Lange-Bertalot							023				
Navicula lanceolata (Agardh) Ehrenberg						073		124		164	
Navicula notha Wallace											
Navicula phyllepta Kutzing											
Navicula pseudoarvensis Hustedt											
Navicula radiosa Kützing							023				
Navicula radiosafallax Lange-Bertalot											
Navicula reichardtiana Lange-Bertalot var. *reichardtiana*			244	047		024					
Navicula rhynchocephala Kützing											
Navicula rostellata Kützing											
Navicula schroeteri Meister var. *schroeteri*											
Navicula simulata Manguin											
Navicula J.B.M Bory de St. Vincent											
Navicula tenelloides Hustedt											
Navicula tripunctata (O.F. Müller) Bory											
Navicula trivialis Lange-Bertalot											
Navicula upsaliensis (Grunow) Peragallo											
Navicula veneta Kützing				070							
Navicula vilaplanii (Lange-Bert. & Sabater) Lange-Bertalot & Sabater in Rumrich & al.											
Navicula viridula (Kützing) Ehrenberg											
Naviculadicta absoluta (Hustedt) Lange-Bertalot in Lange-Bertalot & Moser											
Neidium ampliatum (Ehrenberg) Krammer in Krammer & Lange-Bertalot											
Neidium dubium (Ehrenberg) Cleve											
Nitzschia acicularis (Kützing) W.M. Smith											
Nitzschia acidoclinata Lange-Bertalot										304	
Nitzschia agnita Hustedt											
Nitzschia amphibia Grunow f. *amphibia*											
Nitzschia archibaldii Lange-Bertalot											
Nitzschia capitellata Hustedt in A. Schmidt & al.											
Nitzschia costei Tudesque, Rimet & Ector											
Nitzschia disputata Carter											
Nitzschia dissipata (Kützing) Grunow ssp. *dissipata*	275										
Nitzschia dubia W.M. Smith											
Nitzschia epithemoides Grunow var. *disputata* (Carter) Lange-Bertalot											
Nitzschia filiformis (W.M. Smith) Van Heurck var. *filiformis*											
Nitzschia fonticola Grunow in Van Heurck			098	386		171					
Nitzschia frequens Hustedt											
Nitzschia gracilis Hantzsch							047				
Nitzschia hantzschiana Rabenhorst											
Nitzschia incognita Legler et Krasske											
Nitzschia inconspicua Grunow											
Nitzschia inconspicua Grunow f. *anormale*											
Nitzschia intermedia Hantzsch ex Cleve & Grunow											
Nitzschia lacuum Lange-Bertalot											
Nitzschia linearis (Agardh) W.M. Smith var. *linearis*						024					
Nitzschia linearis (Agardh) W.M. Smith var. *tenuis* (W. Smith) Grunow in Cleve & Grunow											
Nitzschia media Hantzsch.						122	047				
Nitzschia microcephala Grunow in Cleve & Moller											
Nitzschia nana Grunow in Van Heurck											
Nitzschia palea (Kützing) W. Smith var. *debilis* (Kützing) Grunow in Cl. & Grunow											
Nitzschia palea (Kützing) W. Smith var. *palea*		098								093	
Nitzschia paleacea (Grunow) Grunow in van Heurck											
Nitzschia parvula W. M. Smith											
Nitzschia perminuta (Grunow) M.Peragallo			073				070				
Nitzschia pusilla (Kützing) Grunow emend Lange-Bertalot											
Nitzschia recta Hantzsch in Rabenhorst										023	
Nitzschia rosenstockii Lange-Bertalot											
Nitzschia soratensis Morales & Vis											
Nitzschia sigma (Kützing) W.M. Smith											
Nitzschia sociabilis Hustedt											
Nitzschia solgensis Clave-Euler											
Nitzschia A. H. Hassall											
Nitzschia subacicularis Hustedt in A. Schmidt et al.						098					
Nitzschia supralitorea Lange-Bertalot											
Nitzschia umbonata (Ehrenberg) Lange-Bertalot											
Nitzschia valdestriata Aleem & Hustedt											
Nitzschia vermicularis (Kützing) Hantzsch in Rabenhorst											
Nupela lapidosa (Lange-Bertalot) Lange-Bertalot var. *lapidosa*											
Parlibellus protractus (Grunow) Witkowski Lange-Bertalot & Metzeltin											

Pinnularia lundii Hustedt var. *lundii*												
Pinnularia microstauron (Ehr.) Cleve var. *microstauron*												
Pinnularia parvulissima Krammer												
Pinnularia C. G. Ehrenberg												
Pinnularia subcapitata Gregory var. *elongata* Krammer								0.70				
Pinnularia subgibba Krammer var. *subgibba*												
Pinnularia viridis (Nitzsch) Ehrenberg var. *viridis*												
Placoneis clementis (Grunow) Cox									0.50			
Placoneis clementoides (Hustedt) Cox												
Placoneis pseudanglica (Lange-Bertalot) Cox												
Placoneis C. Mereschkowsky												
Planothidium daui (Foged) Lange-Bertalot					0.48							
Planothidium delicatulum (Kützing) Round & Bukhtiyarova												
Planothidium frequentissimum (Lange-Bertalot) Lange-Bertalot		5.88	7.07		3.14	0.74	5.13	8.84	3.22	0.88	3.04	1.94
Planothidium granum (Hohn & Hellerman) Lange-Bertalot												
Planothidium hauckianum (Grun.) Round & Bukhtiyarova												
Planothidium haynaldii (Schaarschmidt) Lange-Bertalot												
Planothidium lanceolatum (Brébisson ex Kützing) Lange-Bertalot			12.20	0.70	7.00		10.27	10.00	16.34	0.66	1.87	
Planothidium minutissimum (Krasske) Morales												
Planothidium robustius (Hustedt) Lange-Bertalot												
Planothidium rostratum (Østrup) Lange-Bertalot												
Platessa conspicua (A.Mayer) Lange-Bertalot												
Platessa hustedtii (Krasske) Lange-Bertalot												
Pleurosira laevis (Ehrenberg) Compere f. *laevis* Ehrenberg												
Prestauroneis integra (W. Smith) Bruder in Bruder & Medin												
Psammothidium sacculum (Carter) Bukhtiyarova et Round												
Pseudofallacia monoculata (Hustedt)												
Pseudofallacia tenera (Hustedt) Liu Kociolek & Wang												
Pseudostaurosira parasitica (W. Smith) Morales												
Reimeria sinuata (Gregory) Kociolek & Stoermer			0.24	0.47	2.90	0.25		1.16	2.97		0.93	
Reimeria uniseriata Sala Guerrero & Ferrario												
Rhoicosphenia abbreviata (C. Agardh) Lange-Bertalot		3.43										
Rossithidium linearis (W. Sm.) Round & Bukhtiyarova												
Sellaphora pseudopupula (Krasske) Lange-Bertalot												
Sellaphora pupula (Kützing) Mereschkowksy										0.22		
Sellaphora seminulum (Grunow) D. G. Mann			0.98		0.48		3.67	6.74	2.97		5.14	
Simonsenia delognei Lange-Bertalot												
Stauroneis gracilis Ehrenberg												
Stauroneis kriegeri Patrick							0.73					0.48
Stauroneis separanda Lange-Bertalot & Werum												
Stauroneis smithii Grunow												
Stauroneis thermicola (Petersen) Lund												
Staurosira binodis Lange-Bertalot in Hofmann Werum & Lange-Bertalot												
Staurosira brevistriata (Grunow) Grunow												
Staurosira construens Ehrenberg												
Staurosira elliptica (Schumann) Williams & Round												
Staurosira martyi (Heribaud) Lange-Bertalot												
Staurosira mutabilis (Wm Smith) Grunow												
Staurosira (C. G. Ehrenberg) D. M. Williams & F. E. Round												
Staurosira venter (Ehr.) Cleve & Moeller												
Staurosirella pinnata (Ehr.) Williams & Round												
Surirella angusta Kützing												
Surirella brebissonii Krammer & Lange-Bertalot var. *brebissonii*		0.49										
Surirella brebissonii var. *keutzingii* Krammer et Lange-Bertalot												
Surirella linearis W.M.Smith in Schmidt & al.	1.00			0.23								
Surirella roba Leclercq												
Surirella robusta Ehrenberg												
Tabellaria fenestrata (Lyngbye) Kützing												
Tabellaria flocculosa (Roth) Kützing												
Tabellaria ventricosa Kützing												
Tabularia fasciculata (Agardh) Williamset Round												
Tabularia tabulata (C. A. Agardh) Snoeijs												
Thalassiosira pseudonana Hasle et Heimdal												
Tryblionella calida (Grunow in Cl. & Grun.) D. G. Mann in Round Crawford & Mann												
Tryblionella constricta (Kützing) Poulin in Poulin & al.												
Tryblionella debilis Arnott ex O'Meara												
Tryblionella hungarica (Grunow) D.G. Mann												
Ulnaria biceps (Kützing) Compère			1.46		0.48							
Ulnaria ulna (Nitzsch.) Compère	0.25		0.73	10.77	0.72			0.47				0.24

ANNEX II

Table of the species and corresponding abundance in reads (total number of reads retained: 2500) per sample, in %, for molecular approach without the application of a correction factor (CF)

	08F_02_35C	08G_02	09F_06	09G_01	09G_03	09G_04	09H_04	09I_06	09J_03	09J_04	09L_02	10E_50	10F_50	10G_02	10H_03	10H_53	10J_52	10K_01	10K_05	10M_08	11E_01	11H_04	11H_06	11I_01	11I_09	11I_12	11I_51	11J_02	11K_03	11L_50	11M_01	12E_01	12F_06	12F_07_M	12F_51	12G_02	12G_50	12G_55	13E_50
Achnanthes coarctata																																						004	
Achnanthidium delmontii	122	037	9805	085	974	128	283	226	041	341	325	006	231	959	765	055	011	085	022		065	088	1772	1271	081	015	080	011	024	128	970	015	527	020	187	044	868	966	
Achnanthidium minutissimum	004	095		5248	004	5741	5883	090	141	1758	3282	006	172	1949	965	950		787	018	004	012	887	1772	907		673	678	128	1983	303	245		052		076	982	394	620	
Achnanthidium pyrenaicum																																							
Achnanthidium sp.																																							
Amphora copulata	103		004		004				004	012		095	154	010					007	011	148			092	011			044				026	441	237	850		574	012	
Amphora ovalis												013									012														004				
Amphora pediculus																								008															
Amphora sp.					079																																		
Aulacoseira ambigua										004																				013	016								
Aulacoseira granulata			025									013									037											004		350	008				007
Aulacoseira subarctica																																							
Berkeleya rutilans	011											051																				004	026	026	128		011		004
Caloneis amphisbaena																																	007	007			019	004	
Caloneis fontinalis	192									004	061	083	2678					062	025	004	419	1529	004	015	096	478		011				004	1227	007	008	1243	868	115	245
Caloneis sp.	144	176		556	004	112		6348	8647	885	4730	082	007			004	1191	8055	4958	004	033	040		004	1448	6408	6714	880	1621	1156	004		234		012	423	084	008	004
Cocconeis pediculus	004		007									090	004																								004		022
Cocconeis placentula																																							
Cocconeis sp.																																							
Conticribra weissflogii																																							
Craticula buderi	1536											077												008								018			012				011
Craticula cuspidata												013									004																		
Craticula submuscula										368								004	120					061			011									004		
Craticula sp.	018	176	018	028	012			008				045					1125		301		1356		004			023	011	011				004	015		012		015		026
Ctenophora pulchella								004																															
Cyclotella meneghiniana																																							
Cyclotella sp.																																							
Cymbella excisa	004										004	006											020	004															
Cymbella tumida																																							
Cymbella sp.			004																																				
Diploneis subovalis																																							
Diploneis sp.					004																																		
Discostella stelligera																																							
Discostella sp.				004								006																											
Ellerbeckia sp.													007												004									112		019	211	
Encyonema minutum		015		109	008	060		004	041	286	028	019	007	156			086	019	080	004	012	558	086	084	004	226	037	120	248		327		007			614	1205	044	019
Encyonema prostratum								011																			004						997						004
Encyonema silesiacum																																							
Encyonema sp.																																							
Encyonopsis subminuta	578	146	004	3136	004	2169	005	2226	511	031	199	2068	037	162	124	084	3216	090	1045	506	163	474	086	2688	825	1010	374	204	200	269	012	168	063		331	026	088	004	048
Eolimna minima																																							
Epithemia gibba																																							
Epithemia sorex							021			004		013	007	005	008															004	027	088	004	948				037
Eunotia bilunaris		015			004			080			004	006	004	005	004			008		025	004		224		060	004	004	007			004	077	011					004	104
Eunotia glacialis		033		004	016	074	330	087	065	376	089		004	2448	949	214	018	085	007	5524			004		1387	004	007	011	236	2064	1122	2651	026					015
Eunotia minor										012		082	011	1232	240	004					134		004								051	108						011
Eunotia pectinalis			004				031	294	044	027						013														084	016							
Eunotia sp.													004																					004					
Fallacia pygmaea																																	073						
Fallacia sp.	788	208	004	060		078		004	004		004	013	015	005			465		011	065		117		004	023	031	041	004				022	019		008	018	019		104
Fistulifera saprophila																															004								
Fragilaria arcus	041	238		060	103	033	603	004	007	838	134	006	007	1025	1501	013	127	048	127	029		084	685	122	023	011	100	033	171	252	1941	018	059	007	020	489	057	083	383
Fragilaria capucina var. capucina															028																012								
Fragilaria sp.																							008																
Frustulia erifuga		018		008				004					026		008			004	004				088		004		004					011	004	007	012		004	004
Frustulia sp.													004								073			004														
Frustulia vulgaris																																			088				
Geissleria decussis					004																																		
Geissleria sp.												006																											
Gomphoneis minuta															004							007						037											
Gomphonema affine	1182	015	004	012	231	087	2243	083	041	2052	389	064	084	005	1725	7006	250	082	058	239	016		012	080	096	551	019	015	912	4913	2849	004	104	330	020	430	371	918	041
Gomphonema bourbonense	158	099		101	3176	351	991	109	041	1665	272	141	083	1297	2994	1476	022	055	044	387	033	077	080	077	256	023	074	015	4471	610	2319	361	083	620	191	088	042	068	126
Gomphonema parvulum																																							
Gomphonema rhombicum			004									006	040																				019	040	323		275	044	004
Gomphonema sp.												006																											
Gyrosigma acuminatum															012						004														004				
Halamphora montana																																							
Halamphora veneta																												004											
Hantzschia amphioxys var. major		026												015	032								024		015								007	455			004		
Iconella sp.																																	059	092	347				
Karayevia ploenensis var. gessneri											004	013								014	029												015						
Lemnicola hungarica	689		018	004		004		008				013	092				1321		022	076	016	062	004	019	111	015		007				065	015	007	040		088		189
Mayamaea permitis																																							
Mayamaea sp.																																							
Melosira sp.	895	2000	004	226		058			044	069		1321	1684	025			356	394	512	086	147	2920	369	1799	512	187	433	2000		004		1627	096	3476	383	577	1668	1412	1519
Melosira varians																																							
Navicula capitatoradiata		051		012	028	037				023	012	199	051	010	024		011		044	228		007	086		042		007	007				372	080		084		004		019
Navicula cryptocephala										012	101	068	121					105	018		016	095	008	027		004	156	011	012			015	345	020	259	040	1296	044	
Navicula cryptotenella		022				021	010	008				013		020	012			008	025	022	004			004	008	004				033	094	004	011	167	007	024		004	004	
Navicula cryptotenelloides	1359	205	007						011	004		615	1313	010		004	145		341	047	5485	018		069	115		022	044				700	015	422	1121	007	031	004	609
Navicula gregaria		007																																					
Navicula lanceolata													004																										
Navicula phyllepta																																							
Navicula rostellata		048	011	008	004				022	023	008			010			004		011	033				008	008	011		004		004	270							007
Navicula slesvicensis																																				008			
Navicula tripunctata	527	1218	007			078			104			1199	1053	156			232	019	327	083	130	073		214	233	015	634	153				299	3830	653	1121		1315	048	3124
Navicula sp.		004	018	004					004			090		005	020	004					004				019							011	078	007	092		008	004	037
Navicula veneta		004								004	004	006		005	020	050					004								016	026	023								004
Neidium sp.																																							004
Nitzschia acidoclinata	007		004									588					025				041												007	020	164				
Nitzschia amphibia																																							
Nitzschia capitellata												082																											
Nitzschia cf. bulnheimiana																					004																		
Nitzschia cf. microcephala		015											018						015				004		004							018	089	013	084		023	016	
Nitzschia cf. pusilla																																			004				
Nitzschia communis																																				004			
Nitzschia dissipata	022	044	014	020	004	041	026	008	026	286	082	051	202	313	336	105	018	004	062	043	004	077	220	011	008	004	163	011		051	031	026	063	277	1001	044	382	358	045
Nitzschia dissipata var. media			007	004								442	004									007	060	065										132	012				011
Nitzschia filiformis																																							

	13F_50	13G_51	14D_51	14D_52_M	14D_53	14F_51	14F_52	15D_01	15D_03	15E_06	15E_08	15E_10	15E_50	15E_51	16E_02	16E_51	PTRH4A_R_1	PTRH4A_R_10	PTRH4A_R_11	PTRH4A_R_12	PTRH4A_R_13	PTRH4A_R_14_M	PTRH4A_R_15	PTRH4A_R_16	PTRH4A_R_17	PTRH4A_R_18	PTRH4A_R_2	PTRH4A_R_20	PTRH4A_R_21	PTRH4A_R_22	PTRH4A_R_23	PTRH4A_R_24	PTRH4A_R_25	PTRH4A_R_26	PTRH4A_R_27	PTRH4A_R_28	PTRH4A_R_3	PTRH4A_R_4	PTRH4A_R_5	PTRH4A_R_6	PTRH4A_R_7	PTRH4A_R_9
Achnanthes coarctata																																										
Achnanthidium delmontii																																										
Achnanthidium minutissimum																																										
Achnanthidium pyrenaicum																																										
Achnanthidium sp.																																										
Amphora copulata																																										
Amphora ovalis																																										
Amphora pediculus																																										
Amphora sp.																																										
Aulacoseira ambigua																																										
Aulacoseira granulata																																										
Aulacoseira subarctica																																										
Berkeleya rutilans																																										
Caloneis amphisbaena																																										
Caloneis fontinalis																																										
Caloneis sp.																																										
Cocconeis pediculus																																										
Cocconeis placentula																																										
Cocconeis sp.																																										
Conticribra weissflogii																																										
Craticula buderi																																										
Craticula cuspidata																																										
Craticula subminuscula																																										
Craticula sp.																																										
Ctenophora pulchella																																										
Cyclotella meneghiniana																																										
Cyclotella sp.																																										
Cymbella excisa																																										
Cymbella tumida																																										
Cymbella sp.																																										
Diploneis subovalis																																										
Diploneis sp.																																										
Discostella stelligera																																										
Discostella sp.																																										
Ellerbeckia sp.																																										
Encyonema minutum																																										
Encyonema prostratum																																										
Encyonema silesiacum																																										
Encyonema sp.																																										
Encyonopsis subminuta																																										
Eolimna minima																																										
Epithemia gibba																																										
Epithemia sorex																																										

Eunotia bilunaris				018													627		004	004	163	004							080		014	004										
Eunotia glacialis			031	197	254			124	004										069	004	033	023			040		435				005		004				033	053	018		030	045
Eunotia minor				120	[illegible]		008		011									011	018		852	030	004	089	044	418	1303	004			5739		373		029	111	935	087	1015	008	026	307
Eunotia pectinalis				051	262		008			004							010		015		029	008			007				004		052											
Eunotia sp.					004																033			126	387	650																
Fallacia pygmaea			016					004	052																							004										
Fallacia sp.														004																												
Fistulifera saprophila		012		226		045	028	120	559	586	[illegible]	787	550	020	173	015			058	007		019	007				063	016	015	007		062	043		055		018	015	015	004	081	097
Fragilaria arcus																																										
Fragilaria capucina var. capucina																																										
Fragilaria sp.	022	019	047	004	008	040	028	481	007	175	059	008	100	096	025		105	287	004		754	045	102		113	040	096	024	004	349	588		293	007	146	016	051	024		020	019	170
Frustulia erifuga																					069										005											
Frustulia sp.																																										
Frustulia vulgaris	004	004	010								007						005					015			007		030												004			006
Geissleria decussis		004			030					004		218											004																			
Geissleria sp.																																			029							
Gomphoneis minuta																																										
Gomphonema affine																																										
Gomphonema bourbonense							004		015											088																						
Gomphonema parvulum																																								004		
Gomphonema rhombicum	033	148		055		[illegible]	392	026	266	081	135	518	742	483	[illegible]	015	601	4987		2604	408	015	8260	583	4867	4644	074	004	034	481	005	499	014	1345	510	135	058	387	063	148	085	074
Gomphonema sp.	011	004	052	1411	072	060	065	571	045	574	275	111	013	132	079	022	4067	026	033	363	5962	204	073	085	047	047	089	048	7465	463	469	1409	040		033	095	113	348	331	144	178	335
Gyrosigma acuminatum	040	051					024		030			047	013	016	005		089					015						282				004										
Halamphora montana		004	005																																							
Halamphora veneta																			004																							
Hantzschia amphioxys var. major																					012																					
Iconella sp.							008	017																	007		052	254			005	015										
Karayevia ploenensis var. gessneri		070					044			167		091	071	048																		258										
Lemnicola hungarica				058	008																						007		042								113					
Mayamaea permitis		121	021	368	004	025	024	159	904	047	824	859	200	056	761	040			083	044		015	018				055	024		044		080	029		022		303	048	121		418	449
Mayamaea sp.													004		005						008																					
Melosira sp.																																										
Melosira varians	[illegible]	859	[illegible]	047	008	197	242	[illegible]	[illegible]	811	461	400	313	683	158	202	037	577	3799	172		536	047				1288	2799	027	059	019	080	120	029	102		318	048	015	012	052	341
Navicula capitatoradiata												004																														
Navicula cryptocephala				401	013			026	056	008	015			004	015		324			018		011			007		063	085	144	007							106	034	011	004	115	114
Navicula cryptotenella	007	245				025	303		225	081	004	400	596	068	608	033		011		015			036					004				040	047		022	008	004	034	007	124	030	
Navicula cryptotenelloides	011	043				025			056		004	028	013	048	222	007	010				004		004	004		015	048				014	025	040		026	004	004		029	004	048	006
Navicula gregaria	025	121	[illegible]	689	025	222	016	[illegible]	255		[illegible]	[illegible]	967	643	351		063		127	026		389		004			339	1748	049	004		131			015		015	019			385	460
Navicula lanceolata																																							007		004	
Navicula phyllepta						005						012	017		025																	004										
Navicula rostellata																																										
Navicula slesvicensis								026			004								004			042		004	007		133	089			009						044	034	004		030	023
Navicula tripunctata	011																															004										
Navicula sp.	189	785	063	208	119	111	[illegible]	485	[illegible]	175	[illegible]	546	675	072	741	007			1056	007		328	186	004	058		199	077		004		852	062	004	029		040	140	040	004	1114	204
Navicula veneta	004		084	128			008	223	375	008	139	012	013	040	064	015	058			007		019						016	030												004	
Neidium sp.					224		004	361						004							012					004	018	161										015				
Nitzschia acidoclinata																																										
Nitzschia amphibia		004	005				008		015	008		004			020	055				044		038						016	004			025										
Nitzschia capitellata																												008														
Nitzschia cf. bulnheimiana												004																														
Nitzschia cf. microcephala												004			025																											
Nitzschia cf. pusilla	036	023	010	004		010		013			011	004			015		105										133	149	008			011									004	006
Nitzschia communis											004		004		005																											
Nitzschia dissipata													004		005																											
Nitzschia dissipata var. media	218	121	047	007	004	504	109	047	022	019	004	336	650	607	539	007	1134	007	007	029	016	038	018	011	062	004	055	044			403	138	065		011	004	194	048	018	008	107	261
Nitzschia filiformis								107									983		004									012														
Nitzschia fonticola		008					069			144		016			005	022																040										
Nitzschia inconspicua		630				060					004	182	980	044	054					037		004										004										
Nitzschia lorenziana									007										004									004														
Nitzschia palea	033	054	010	777	004	010	036	249	094	074	154	071	025	016	010		737		004	004	029	053	007		015		103	499	490	011	118	142	018		015		099	063	040	004	104	085
Nitzschia sigmoidea	004						008		004			008	013	048			026											016				011										
Nitzschia supralitorea			021	036				167	026		022	040																217	004			004										
Nitzschia tubicola			016														063											016														
Nitzschia sp.	007	023	[illegible]	062	004		048	064	127	035	022	028	013	068		004	214		018	004	004	023	004		015		410	439	1186	099		349	004		255		851	039	015		107	335
Pinnularia cf. marchica				007																		004		004			004															006
Pinnularia isselana																											007															
Pinnularia stomatophora																					004																					
Pinnularia subcommutata var. nonfasciata																																										006
Pinnularia subgibba			314	2964	008			112			004						016	004			012	026				004	413	008	023				004				058	039	015		007	028
Pinnularia sp.		004	115	084			004	554	030	004							031	004	004			019		018	007	004	299	012	004				007			008	018	048	022		007	028
Pinnularia viridis																																							004			
Placoneis constans																												004										073				
Placoneis sp.				004																								109										174				
Planothidium frequentissimum				004															004								004								007			019		004	004	
Planothidium lanceolatum	029		052	828	080		162	043	472	640	165		017	915	040	180	099	007	3705	198		302	080	306			2421	016		040		033	337	011	900	075	2293	697	1493	016	1488	1102
Planothidium sp.	018	004	073	233	008		210	416	334	206	388	226	067	759	138	018	131	004	784	626		060	022	011			125	149	046	044		400	029		102	008	190	842	066	032	244	182

Pleurosira laevis									6.00			0.04	0.04						0.07																					
Rhoicosphenia abbreviata		0.04					0.04			0.04			0.04	0.60						0.26		0.04			0.04															
Sellaphora pupula			0.10	0.58					0.04											0.04					0.18	1.33	0.23			0.04						0.10				
Sellaphora seminulum		0.04	0.42	1.13	[illegible]		0.16	1.03	3.34		0.59		0.08		0.10		0.10		0.11	0.26					0.89	0.20	0.72	0.07	0.05	0.04	0.14		0.22		3.32	0.82	1.14	0.04	3.67	3.86
Sellaphora sp.		0.04			0.76			0.09				0.08					0.10								0.15					0.04					0.04	0.29				0.17
Skeletonema potamos																																								
Stauroneis phoenicenteron			0.05																						0.04	0.04														
Staurosira brevistriata																																								
Staurosira construens		0.04	0.73		15.71		0.93	0.73	0.60	0.08		0.04								0.04		0.04			0.04	0.20									0.04	0.05	0.04			
Staurosira elliptica					0.21					0.27																														
Staurosira sp.																																								
Stephanodiscus sp.																	0.10																							
Surirella solea									0.04																	0.08														
Surirella sp.	0.04	0.23	0.05	0.18		0.25	0.24	2.83	1.27		1.61	0.12	0.08	1.92	0.10		0.21		0.36						0.41	13.29				1.42										0.11
Tabellaria flocculosa																		0.11			0.53								0.05											
Tabularia tabulata										0.16		0.04					1.15																							
Thalassiosira profunda																																								
Thalassiosira pseudonana									0.04			0.04																												
Tryblionella apiculata	0.04		0.16	0.04							0.04						0.16		0.04						0.26	2.17				0.40										
Tryblionella sp.			2.62			0.05		0.04			0.04	0.08		0.12	0.05				0.04							0.28				0.29										
Ulnaria acus																														0.04										
Ulnaria ulna	[illegible]	0.12	6.08	5.40	0.97	0.50	1.66	0.86	5.96	11.49	0.22	0.24	0.17	2.16	0.20	1.14	0.68	19.63	0.65	3.26	0.73	46.17	0.69	6.32	4.24	3.66	1.63	47.21			76.17	69.36	64.94	0.20	5.99	1.84	0.44	0.28	5.55	11.36
Ulnaria sp.																																								
Urosolenia eriensis																																								

ANNEX III

Table of the species and corresponding abundance in reads (total number of reads retained: 2500) per sample, in %, for molecular approach with the application of a correction factor (CF)

	08F_02_35C	08G_02	09F_06	09G_01	09G_03	09G_04	09H_04	09I_06	09J_03	09J_04	09L_02	10E_50	10F_50	10G_02	10H_03	10H_53	10J_52	10K_01	10K_05	10M_08	11E_01	11H_04	11H_06	11I_01	11I_09	11I_51	11I_12	11J_02	11K_03	11L_50	11M_01	12E_01	12F_06	12F_07_M	12F_51	12G_02	12G_50	12G_55	13E_50	13F_50
Achnanthes coarctata																																					017			
Achnanthidium delmontii	203	120	9765	095	2592	150	429	323	061	794	443	012	684	1947	1865	229	015	054	038		205	332	4022	2167	073	045	024	067	048	446	2733	059	928	087	387	144	1882	4249		895
Achnanthidium minutissimum																																								
Achnanthidium pyrenaicum	006	313		5869	011	6741	8154	129	210	4086	4480	012	510	3955	2353	3977		1203	032	011	089	3358	4022	1547		1033	1054	787	3932	1055	691		091		157	3212	854	2728		081
Achnanthidium sp.																																								
Amphora copulata																																								
Amphora ovalis	176		004		011				006	028		187	467	021					013	035	460			160	028			276				105	796	1073	1796		1272	054		042
Amphora pediculus												000									000														000					
Amphora sp.																								003																
Aulacoseira ambigua																																								
Aulacoseira granulata					054																																		
Aulacoseira subarctica										004																				021	021									
Berkeleya rutilans			001									001									004											001		056	001				001	
Caloneis amphisbaena																																005	014	036	082		008		003	
Caloneis fontinalis	006											080																					001			003	001			
Caloneis sp.																																002	323	004	002	607	281	076	083	055
Cocconeis pediculus	048					001	012	024	1188									014	007	002	197	864	001	004	084		112	010												
Cocconeis placentula	162	389		419	007	1388	4849	0411	0155							012	1116	8293	5888	008	089	102		004	2320	6890	6830	3648	2164	2711	007		277		017	932	123	024	006	064
Cocconeis sp.	002		002									054	003																							003			016	
Conticribra weissflogii																																								
Craticula buderi																																								
Craticula cuspidata	2132											121													015							061			021				021	
Craticula subminuscula												008									004																			
Craticula sp.										153								001	038					019		003									002					
Ctenophora pulchella	005	065	003	005	005			002				013					230		078		628		001			002	005	010				002	004		004		005		009	
Cyclotella meneghiniana								002																																
Cyclotella sp.																																		009		2688				
Cymbella excisa	000										000	000											001	000									000		000					
Cymbella tumida																																								
Cymbella sp.			000																														013	002	001			005		010
Diploneis subovalis																																	004					001		
Diploneis sp.					002																																			
Discostella stelligera				000																																				
Ellerbeckia sp.												007																												
Encyonema minutum													000												000								000		004		001	016		003
Encyonema prostratum		010		026	005	012		001	013	142	008	008	005	068			011	006	030	002	003	452	047	031	002	012	075	159	105		197		376			429	558	041	009	556
Encyonema silesiacum								009																		003												005	
Encyonema sp.																																					009	078	016	102	
Encyonopsis subminuta	904	450	004	3278	010	2380	007	2975	711	068	254	3654	102	306	288	329	4183	128	1726	1480	480	1679	077	4801	1835	538	1477	1177	370	875	031	638	104		689	079				
Eolimna minima																																								
Epithemia sorex							009			002		007	006	003	005															004	021	108	002	1130					023
Eunotia bilunaris		003			001			002			000	001	001	001	001			001		004	001		028		006	000	000	002			001	017	001				001	013
Eunotia glacialis		025		001	010	020	115	028	019	201	028		002	1141	531	206	006	012	003	3966	001		002		757	003	001	015	107	1651	726	2458	011				008	
Eunotia minor										001		002	001	100	023	001					017		000							001	000	008	008					001	
Eunotia pectinalis			000				000	003	000	000			002			000														001	000								
Eunotia sp.																																								
Fallacia pygmaea																																		259		001				
Fallacia sp.	2287	1192	007	117		160		009	010		010	042	075	018			1122		033	353		768		011	095	108	083	089			001	153	057		029	104	072		407	
Fistulifera saprophila																																								
Fragilaria arcus																																							009	
Fragilaria capucina var. capucina	082	368		082	129	018	429	003	005	917	086	006	010	977	1719	025	083	031	105	042		149	731	098	026	072	008	095	159	412	2567	035	049	014	019	751	058	172	405	115
Fragilaria sp.															002																001									
Frustulia erifuga																							002																	
Frustulia sp.		007		001				001					009		002			001	001				023		001	001					005	001	003	003			002	001	005
Frustulia vulgaris													005								105			003										080					
Geissleria decussis																																								
Geissleria sp.																																				003				
Gomphoneis minuta					002																																			
Gomphonema affine												006																												
Gomphonema bourbonense															004							012							081											
Gomphonema parvulum	202	005	000	001	063	010	347	012	006	489	054	012	026	001	430	2997	056	013	010	076	005		003	014	023	003	088	009	185	1749	820	002	019	149	004	144	082	413	009	037
Gomphonema rhombicum	083	103		086	2665	130	473	049	019	1217	117	084	031	830	2303	1948	010	026	024	382	082	092	057	041	192	086	011	028	2796	670	2059	460	019	864	125	091	029	094	090	038
Gomphonema sp.			000									000	005																				001	007	026		024	008	000	018
Gyrosigma acuminatum												008																												
Halamphora montana															001						001														000					
Halamphora veneta																												008												
Hantzschia amphioxys var. major		015												005	014								010		006								005	164			003			
Iconella sp.																																	055	215	377					
Karayevia ploenensis var. gessneri											002	009								016	082												009							
Lemnicola hungarica	1225		020	005		005		011				026	289				1956		041	253	065	250	010	035	280		025	048				235	028	031	088		088		455	
Mayamaea permitis																																								
Mayamaea sp.																																								
Melosira sp.	080	354	000	014		004			004	007		135	268	003			027	032	049	006	025	594	045	165	065	036	016	662		001		353	009	825	043	101	198	334	184	295
Melosira varians																																								
Navicula capitatoradiata		060		005	027	016				020	006	135	054	007	021		005		028	255		010	029		036	004		016				538	019		062		003		015	
Navicula cryptocephala										011	054	048	139					062	012		020	140	007	018		092	002	026	009			023	236	084	208	051	1092	075		082
Navicula cryptotenella		064				021	014	009				021		086	026			010	039	060	011			006	016		005		057	287	010	089	258	026	043		007	015		107
Navicula cryptotenelloides	745	221	002						005	003		388	1274	007		006	066		198	048	5670	023		089	089	011		088				927	009	611	759	008	022	006	451	098
Navicula gregaria		004																																						
Navicula lanceolata													003																											
Navicula phyllepta																																								
Navicula rostellata		029	002	002	002				006	010	002			004			001		004	019					003	003	002		002		002	203						003		
Navicula slesvicensis																																					003			023
Navicula tripunctata	823	3749	007			086			144			2129	2914	297			302	028	539	243	384	258		342	518	903	022	882				1129	5486	2866	2165		2666	196	6589	1978
Navicula sp.		006	009	002					003			088				009					006				022							021	057	014	092		008	009	041	000
Navicula veneta		006								005	003	007		005	026	113					007									017	048	035							004	
Neidium sp.																																						007		
Nitzschia acidoclinata	005		002									477					015				055												006	038	145					
Nitzschia amphibia																																								
Nitzschia capitellata												086																												
Nitzschia cf. bulnheimiana																					012																			
Nitzschia cf. microcephala		080											084						016				006		006							046	099	037	109		031	044		256
Nitzschia cf. pusilla																																				005				
Nitzschia communis																																					002			
Nitzschia dissipata	010	089	004	006	003	013	011	003	010	179	012	026	160	171	220	118	007	002	029	086	003	078	134	015	005	067	002	018		048	024	028	080	328	555	089	223	422	027	655
Nitzschia dissipata var. media			002	001								197	003									006	082	026										137	006				006	
Nitzschia filiformis												021	009								011												055		014		010			
Nitzschia fonticola																																								

	13G_51	14D_51	14D_52_M	14D_53	14F_51	14F_52	15D_01	15D_03	15E_06	15E_08	15E_10	15E_50	15E_51	16E_02	16E_51	PTRH4A_R_1	PTRH4A_R_10	PTRH4A_R_11	PTRH4A_R_12	PTRH4A_R_13	PTRH4A_R_14_M	PTRH4A_R_15	PTRH4A_R_16	PTRH4A_R_17	PTRH4A_R_18	PTRH4A_R_2	PTRH4A_R_20	PTRH4A_R_21
Achnanthes coarctata						006																						
Achnanthidium delmontii					014																							
Achnanthidium minutissimum	878	138		006	7775	3873	192	237	4915	013	784	2181	2074	831	7201	154	502	092	5439	2892		168	042	576	053	011	017	
Achnanthidium pyrenaicum					012																							
Achnanthidium sp.	252				264	912	088	046	1124		220	258	445	128	1953	120	3801		320	686	024	1197	089	1318	092			
Amphora copulata																										000		
Amphora ovalis																												
Amphora pediculus	3103	047			710	1647		263	798		847	1795	4215	2708	052				1220		241			011		221		
Amphora sp.					000						000	000		000													000	
Aulacoseira ambigua																												
Aulacoseira granulata																												
Aulacoseira subarctica																												
Berkeleya rutilans																				024								
Caloneis amphisbaena		363	002				135																				010	
Caloneis fontinalis																												
Caloneis sp.		007	004			052			012		010	015	042	025	005				007							013	042	
Cocconeis pediculus	012				001						005		004	004														
Cocconeis placentula	1275	003			086	052		062		003	378	154	099	586	001		025	015	481		007							005
Cocconeis sp.	509	016		516	019	003	009	013			080	077	115	048			2211		236	070	318	2166	9145	5876	8073	328	227	008
Conticribra weissflogii	003	102	072		004	002		008					006								075						048	
Craticula buderi								004																				
Craticula cuspidata																											042	007
Craticula submuscula			019					016		306	604	005		008		213			012		137						038	
Craticula sp.											002																	
Ctenophora pulchella																					021							
Cyclotella meneghiniana	001	014	002					009					005			005	004				2563		001			002	037	007
Cyclotella sp.																												
Cymbella excisa					084						021			006			189											
Cymbella tumida																000					002							
Cymbella sp.																												
Diploneis subovalis						003	004	004	001				002						001								007	
Diploneis sp.																											022	
Discostella stelligera																												
Ellerbeckia sp.						000														000								
Encyonema minutum																												
Encyonema prostratum	000				000						000	001	001	000														
Encyonema silesiacum	010	010	002	001	105	025					020			023		004	068		002	064	005	128	010	109		002	004	
Encyonema sp.			013	003			015																					
Encyonopsis subminuta					008	022																						
Eolimna minima	076	048	470	131	013	534	454	641	082	408	563	169	166	222	544	096	028	022	216	185	419	227	340	010	080	2907	047	054
Epithemia sorex			016													557		003	002	113	006							066
Eunotia bilunaris		008	084	019			020	000										012	000	005	008			007		089		
Eunotia glacialis			087	369		002		005									010	013		499	043	004	033	080	278	877	004	
Eunotia minor			006	014		000			000							001		002		003	002			001				000
Eunotia pectinalis				000																001			001	008	013			
Eunotia sp.		010					002	014																				
Fallacia pygmaea													006															
Fallacia sp.																												
Fistulifera saprophila	042		1231		108	062	622	1705	1430	4161	2166	1377	059	554	027			320	026		205	065				319	117	080
Fragilaria arcus																												
Fragilaria capucina var. capucina																												
Fragilaria sp.	019	097	005	005	026	017	673	006	115	084	006	068	077	021		161	557	005		903	133	245		161	054	132	048	005
Frustulia erifuga																				005								
Frustulia sp.																												
Frustulia vulgaris	001	005								001						002					011			003		010		
Geissleria decussis	004			019					002		157											008						
Geissleria sp.																												
Gomphoneis minuta																												
Gomphonema affine																												

Gomphonema bourbonense							0.03		0.13											0.89								
Gomphonema parvulum																											
Gomphonema rhombicum	0.32		0.18		2.15	0.61	0.08	0.48	0.12	0.17	0.84	1.09	0.85	2.78	0.02	2.01	21.09		5.42	1.06	0.10	43.28	0.86	15.09	13.77	0.22	0.02	0.11
Gomphonema sp.	0.03	0.73	13.96	0.31	0.26	0.26	5.36	0.25	2.55	1.06	0.55	0.06	0.71	0.46	0.07	41.96	0.84	0.33	2.33	47.95	4.02	1.18	0.48	0.45	0.48	0.82	0.64	71.40
Gyrosigma acuminatum	0.04					0.01		0.02			0.03	0.01	0.01	0.00		0.12					0.04						0.47	
Halamphora montana	0.06	0.16																										
Halamphora veneta																		0.01										
Hantzschia amphioxys var. major																				0.11								
Iconella sp.						0.02	0.09														0.04				0.26	1.85		
Karayevia ploenensis var. gessneri	0.77					0.29			1.23		0.76	0.54	0.48															
Lemnicola hungarica			0.65	0.04																					0.08		0.45	
Mayamaea permitis	2.68	0.98	12.29	0.06	0.37	0.83	5.03	16.90	0.70	10.76	14.48	3.07	1.02	14.95	0.45			2.82	0.95		1.00	0.99			1.73	1.08		
Mayamaea sp.												0.05		0.08						0.18								
Melosira sp.																												
Melosira varians	0.97	2.65	0.08	0.01	0.15	0.16	2.80	1.34	0.61	0.80	0.84	0.24	0.63	0.16	0.12	0.06	1.28	6.49	0.19		1.80	0.13			2.03	6.31	0.04	
Navicula capitatoradiata											0.02																	
Navicula cryptocephala			4.51	0.06			0.27	0.85	0.04	0.06			0.02	0.10		3.80			0.13		0.25			0.08	0.66	1.27	1.57	
Navicula cryptotenella	1.99				0.13	1.48		1.54	0.44	0.02	2.46	3.34	0.45	4.36	0.14		0.18		0.12			0.73				0.07		
Navicula cryptotenelloides	0.79				0.81			0.87		0.04	0.89	0.16	0.72	3.61	0.07	0.80				0.09		0.16	0.05		0.37	1.24		
Navicula gregaria	0.83	3.49	7.08	0.11	1.00	0.07	1.40	1.47		1.00	6.13	4.57	3.61	2.12		0.67		1.32	0.17		7.95		0.02		3.26	24.02	0.49	
Navicula lanceolata																												
Navicula phyllepta					0.02						0.05	0.06		0.12														
Navicula rostellata																												
Navicula slesvicensis							0.14			0.01								0.02			0.48		0.01	0.04	0.73	0.89		
Navicula tripunctata																												
Navicula sp.	15.36	2.59	6.09	1.53	1.48	20.67	13.51	27.79	2.29	21.94	8.09	9.10	1.15	12.80	0.07			31.36	0.14		19.19	8.91	0.06	1.66		5.46	3.00	
Navicula veneta		1.79	1.95			0.05	3.23	3.21	0.05	0.83	0.09	0.09	0.33	0.58	0.08	0.91			0.07		0.57						0.33	0.45	
Neidium sp.				1.66		0.03	5.76						0.04							0.17				0.06	0.29	3.62		
Nitzschia acidoclinata																												
Nitzschia amphibia	0.03	0.10				0.04		0.11	0.05		0.03			0.16	0.25				0.38		1.02					0.29	0.05	
Nitzschia capitellata																										0.10		
Nitzschia cf. bulnheimiana											0.04																	
Nitzschia cf. microcephala											0.06			0.42														
Nitzschia cf. pusilla	0.81	0.29	0.07		0.09		0.24			0.08	0.04			0.17		2.15									2.45	3.93	0.15	
Nitzschia communis										0.02		0.03		0.04														
Nitzschia dissipata												0.02		0.02														
Nitzschia dissipata var. media	0.68	0.66	0.06	0.02	1.86	0.37	0.88	0.11	0.07	0.01	1.43	2.52	2.79	2.67	0.02	9.96	0.08	0.06	0.16	0.11	0.63	0.25	0.05	0.51	0.03	0.44	0.50	
Nitzschia filiformis							0.75									7.53		0.03									0.12	
Nitzschia fonticola	0.07					0.37			0.65		0.11			0.04	0.10													
Nitzschia inconspicua	12.25				0.77					0.04	2.68	13.13	0.70	0.93					0.89		0.22							
Nitzschia lorenziana								0.02										0.02									0.02	
Nitzschia palea	0.44	0.18	9.37	0.02	0.05	0.18	2.85	0.63	0.40	0.73	0.43	0.14	0.11	0.07		9.28		0.04	0.03	0.28	1.27	0.14		0.17	1.17	8.06	5.72	
Nitzschia sigmoidea						0.00		0.00		0.00	0.00	0.00	0.01			0.01										0.01		
Nitzschia supralitorea		0.87	1.08				4.71	0.44		0.25	0.89															8.62	0.11	
Nitzschia tubicola		0.80														0.88										0.29		
Nitzschia sp.	0.19	3.59	0.75	0.02		0.24	0.74	0.86	0.19	0.10	0.17	0.07	0.45		0.02	2.70		0.22	0.03	0.04	0.54	0.07		0.17	4.62	7.08	13.84	
Pinnularia cf. marchica			0.08																		0.08		0.02		0.04			
Pinnularia isselana																									0.04			
Pinnularia stomatophora																				0.01								
Pinnularia subcommutata var. nonfasciata																												
Pinnularia subgibba		0.70	4.69	0.01			0.17			0.00						0.03	0.01			0.02	0.08			0.01	0.61	0.02	0.03	
Pinnularia sp.	0.01	0.74	0.88			0.01	2.40	0.08	0.01							0.15	0.02	0.02			0.17		0.04	0.03	0.02	1.27	0.07	0.02	
Pinnularia viridis																												
Placoneis constans																										0.03		
Placoneis sp.			0.04																							1.49		
Planothidium frequentissimum			0.06															0.07							0.06			
Planothidium lanceolatum		0.77	8.65	0.37		0.68	0.43	2.77	3.00	0.67		0.08	5.22	0.24	0.64	1.08	0.10	39.20	1.34		6.29	1.37	1.65		23.65	0.22		
Planothidium sp.	0.04	1.62	3.66	0.06		1.33	6.21	2.94	1.45	2.39	1.79	0.48	6.51	1.28	0.10	2.14	0.08	12.47	6.38		1.89	0.56	0.09		1.84	3.13		0.69
Pleurosira laevis								0.00			0.00	0.00						0.00										
Rhoicosphenia abbreviata	0.02					0.01			0.02			0.02	0.80						0.15		0.07				0.03			
Sellaphora pupula		0.07	0.28					0.01											0.01						0.03	0.86	0.11	
Sellaphora seminulum	0.08	1.93	3.70	7.58		0.21	3.20	6.12		0.75		0.13		0.19		0.86		0.86	0.54						2.71	0.88	2.29	
Sellaphora sp.	0.08			0.88			0.24				0.12					0.82									0.40			
Skeletonema potamos																												
Stauroneis phoenicenteron		0.00																							0.00	0.00		
Staurosira brevistriata																												
Staurosira construens	0.03	1.14		7.64		0.41	0.77	0.37	0.04		0.12								0.03		0.08				0.04	0.30		
Staurosira elliptica				0.42					0.55																			
Staurosira sp.																												
Surirella sp.	0.09	0.04	0.10		0.06	0.05	1.47	0.89		0.84	0.03	0.02	0.57	0.03		0.12		0.20							0.21	9.71		
Tabellaria flocculosa																	0.15			0.43								
Tabularia tabulata									0.01		0.00					0.13												
Thalassiosira profunda								0.09			0.08																	
Thalassiosira pseudonana																												
Tryblionella apiculata		0.18	0.03							0.01						0.13		0.03							0.20	2.35		
Tryblionella sp.		2.98			0.02		0.03			0.01	0.03		0.05	0.02				0.03								0.31		
Ulnaria acus																												
Ulnaria ulna	0.01	0.93	0.69	0.05	0.02	0.07	0.09	0.86	0.56	0.01	0.01	0.01	0.13	0.01	0.04	0.08	2.82	0.07	0.23	0.07	10.04	0.12		0.67	0.43	0.53	0.17	
Ulnaria sp.																												

	PTRH4A_R_22	PTRH4A_R_23	PTRH4A_R_24	PTRH4A_R_25	PTRH4A_R_26	PTRH4A_R_27	PTRH4A_R_28	PTRH4A_R_3	PTRH4A_R_4	PTRH4A_R_5	PTRH4A_R_6	PTRH4A_R_7	PTRH4A_R_9
Achnanthes coarctata													
Achnanthidium delmontii							0.10						
Achnanthidium minutissimum	33.71	4.38	16.46	1.72	3.94	2.74	0.78	0.07	5.01	1.82	0.53	0.72	0.47
Achnanthidium pyrenaicum													
Achnanthidium sp.	32.67	41.03	2.66	2.57	13.23	8.42	48.33	0.15	1.72	0.25	14.77	0.11	0.09
Amphora copulata				0.00									
Amphora ovalis													
Amphora pediculus		0.22	4.28		0.24			0.23	0.92			0.17	0.19
Amphora sp.											0.00		
Aulacoseira ambigua													
Aulacoseira granulata													
Aulacoseira subarctica													
Berkeleya rutilans													
Caloneis amphisbaena													
Caloneis fontinalis													
Caloneis sp.			0.09										
Cocconeis pediculus	0.04		0.01										
Cocconeis placentula	4.27		15.72	3.46	0.88	0.03		0.42	0.08	0.06	0.01	0.02	
Cocconeis sp.	28.7		0.77	4.82	57.04	23.48	52.55	3.82	31.02	61.98	80.21	17.15	0.06
Conticribra weissflogii	2.65												

Craticula buderi													
Craticula cuspidata													
Craticula subminuscula			0.16										
Craticula sp.													
Ctenophora pulchella													
Cyclotella meneghiniana	0.56		0.04	0.21									
Cyclotella sp.													
Cymbella excisa													
Cymbella tumida			0.00										
Cymbella sp.													
Diploneis subovalis													
Diploneis sp.													
Discostella stelligera													
Ellerbeckia sp.										0.00			
Encyonema minutum													
Encyonema prostratum			0.00										
Encyonema silesiacum	0.15		0.65	3.42	0.05	2.05	0.04	1.79	0.29	0.09	0.20	0.26	0.08
Encyonema sp.										0.04			
Encyonopsis subminuta													
Eolimna minima	1.83	1.09	0.53	1.30	0.43	6.96	1.69	4.28	3.98	1.40	1.80	3.54	5.73
Epithemia sorex		0.09	0.03										
Eunotia bilunaris		0.01		0.02				0.04	0.04	0.02		0.02	0.04
Eunotia glacialis		2.70		6.76		0.86	0.33	4.33	0.81	3.97	0.03	0.09	1.17
Eunotia minor		0.05											
Eunotia pectinalis													
Eunotia sp.			0.01										
Fallacia pygmaea													
Fallacia sp.	0.40		2.81	5.96		5.10		0.64	0.89	0.43	0.10	2.12	2.79
Fistulifera saprophila													
Fragilaria arcus													
Fragilaria capucina var. capucina	5.13	6.22		10.88	0.22	3.68	0.10	0.48	0.18		0.14	0.13	1.33
Fragilaria sp.		0.00											
Frustulia erifuga													
Frustulia sp.										0.01			0.01
Frustulia vulgaris													
Geissleria decussis						0.71							
Geissleria sp.													
Gomphoneis minuta													
Gomphonema affine													
Gomphonema bourbonense											0.03		
Gomphonema parvulum	1.54	0.01	1.33	0.12	8.68	2.80	0.18	0.12	0.61	0.11	0.22	0.13	0.13
Gomphonema rhombicum	4.57	3.34	11.61	0.99		0.56	0.39	0.72	1.70	1.78	0.66	0.84	1.76
Gomphonema sp.			0.00										
Gyrosigma acuminatum													
Halamphora montana													
Halamphora veneta													
Hantzschia amphioxys var. major		0.02	0.07										
Iconella sp.			3.56										
Karayevia ploenensis var. gessneri								0.81					
Lemnicola hungarica	1.47		2.23	2.43		1.25		6.51	0.80	2.20		6.67	7.95
Mayamaea permitis													
Mayamaea sp.													
Melosira sp.	0.10	0.02	0.11	0.51	0.10	0.29		0.84	0.04	0.01	0.01	0.04	0.31
Melosira varians													
Navicula capitatoradiata	0.08							0.77	0.19	0.07	0.02	0.62	0.68
Navicula cryptocephala			0.41	1.44		0.46	0.04	0.03	0.20	0.05	0.70	0.17	
Navicula cryptotenella		0.28	0.59	2.77		1.21	0.05	0.06		0.44	0.05	0.63	0.08
Navicula cryptotenelloides	0.04		1.12			0.26		0.10	0.10			1.89	2.51
Navicula gregaria										0.02		0.01	
Navicula lanceolata			0.02										
Navicula phyllepta													
Navicula rostellata		0.04						0.16	0.10	0.01		0.08	0.07
Navicula slesvicensis			0.02										
Navicula tripunctata	0.11		2.82	4.55	0.22	1.46		0.76	2.03	0.64	0.05	1.56	3.18
Navicula sp.												0.03	
Navicula veneta									0.12				
Neidium sp.													
Nitzschia acidoclinata													
Nitzschia amphibia			0.29										
Nitzschia capitellata													
Nitzschia cf. bulnheimiana													
Nitzschia cf. microcephala													
Nitzschia cf. pusilla			0.18									0.03	0.06
Nitzschia communis													
Nitzschia dissipata													
Nitzschia dissipata var. media		2.44	0.97	1.38		0.16	0.01	1.05	0.20	0.08	0.03	0.43	1.17
Nitzschia filiformis													
Nitzschia fonticola			0.44										
Nitzschia inconspicua			0.09										
Nitzschia lorenziana													
Nitzschia palea	0.13	1.03	1.43	0.55		0.80		0.77	0.37	0.26	0.02	0.60	0.55
Nitzschia sigmoidea			0.00										
Nitzschia supralitorea			0.09										
Nitzschia tubicola													
Nitzschia sp.	1.20		3.52	0.11		5.28		6.60	0.23	0.10		0.62	2.15
Pinnularia cf. marchica													0.03
Pinnularia isselana													
Pinnularia stomatophora													
Pinnularia subcommutata var. nonfasciata													0.01
Pinnularia subgibba				0.01				0.06	0.03	0.01		0.01	0.02
Pinnularia sp.				0.08			0.02	0.05	0.11	0.05		0.02	0.07
Pinnularia viridis										0.00			
Placoneis constans									0.19				
Placoneis sp.									0.88				
Planothidium frequentissimum						0.22			0.17		0.03	0.03	
Planothidium lanceolatum	0.42		0.29	8.86	0.23	16.11	0.33	15.41	3.59	8.47	0.08	7.48	6.11
Planothidium sp.	0.69		5.24	1.15		2.75	0.05	1.92	6.51	0.56	0.23	1.83	1.52
Pleurosira laevis													
Rhoicosphenia abbreviata													
Sellaphora pupula			0.01						0.02				
Sellaphora seminulum	0.24	0.11	0.10	1.19		1.23		7.00	1.33	2.03	0.06	5.73	6.71
Sellaphora sp.			0.09					0.07	0.42				0.27

Skeletonema potamos													
Stauroneis phoenicenteron													
Staurosira brevistriata													
Staurosira construens								0.03	0.03	0.02			
Staurosira elliptica													
Staurosira sp.													
Surirella sp.			0.65										0.03
Tabellaria flocculosa		0.03											
Tabularia tabulata													
Thalassiosira profunda													
Thalassiosira pseudonana													
Tryblionella apiculata			0.27										
Tryblionella sp.			0.20										
Ulnaria acus			0.03										
Ulnaria ulna	5.14			20.93	15.24	12.14	0.01	0.42	0.10	0.03	0.01	0.29	0.66
Ulnaria sp.													