Master's thesis

Assessing past ecological changes in Lake Isojärvi and its suitability as a Finnish lake reference site using sedimentary diatoms

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Greek: διά (dia) = "through" + τέμνειν (temnein) = "to cut", i.e. "cut in half"

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ABSTRACT

Paleolimnology studies the past of inland aquatic ecosystems through the physical, chemical and biological information stored into the sediments of lakes. The usefulness of paleolimnological methods has been acknowledged in defining the reference conditions of watersheds required in the implementation of the European Water Framework Directive (WFD). In this study a diatom record from a sediment core was used to infer the past limnological and trophic status changes in Lake Isojärvi, located in Central Finland, which has been proposed as a reference lake representing oligohumic mid-sized lakes in the Finnish lake typology. Transfer functions were used to reconstruct total phosphorus (TP), total organic carbon (TOC), colour and pH history of the lake. In addition, the physical and chemical data from Meriläinen *et al.* (2006), who studied the same lake and sediment core as in this study, were compared with the diatom record. Some changes were observed in the loss-on-ignition (LOI %), carbon content (C %) and water content (%) of the sediment, which probably reflect catchment erosion caused by logging. The dominant diatom taxa were *Aulacoseira subarctica* (O. Müller) Haworth and *Cyclotella rossii* (Grunow) Håkansson, comprising 25.7–40.5 % and 22.0–32.4 % of the observed taxa. Although minor changes in the diatom assemblages representing the lake's history were observed, the lake is suggested to be a suitable Finnish lake reference site for use in implementation of the WFD.

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TIIVISTELMÄ

Vesiekosysteemien sedimentteihin kerrostunutta fysikaalista, kemiallista ja biologista tietoa voidaan tutkia paleolimnologisin tutkimusmenetelmin. Paleolimnologisten menetelmien käyttökelpoisuus on havaittu mm. Euroopan Unionin Vesipuitedirektiivin (VPD) täytäntöönpanossa tarvittavissa järvien referenssitilaselvityksissä. Tässä tutkimuksessa järvisedimentin piileviä käytetään Keski-Suomessa sijaitsevassa Isojärvessä tapahtuneiden limnologisten ja trofiatason muutosten selvittämisessä. Isojärveä on ehdotettu käytettäväksi VPD:n keskikokoisten ja vähähumuksisten järvien referenssijärvenä. Kokonaisfosforin, orgaanisen hiilen (TOC), värin ja pH:n muutokset rekonstruoitiin käyttämällä valmiita kalibraatioaineistoja. Lisäksi tarkasteltiin samoista sedimenttinäytteistä aiemmin tehtyjen fysikaalisten ja kemiallisten analyysien tuloksia (Meriläinen *et al.* 2006) yhdessä tässä tutkimuksessa kerätyn piileväaineiston kanssa. Muutoksia havaittiin sedimentin hehkutushäviössä (LOI %), hiili- (C %) ja vesipitoisuudessa (%), joiden arvioidaan heijastelevan hakkuiden aiheuttamaa valuma-alueen eroosiota. Hallitsevina piilevälajeina olivat *Aulacoseira subarctica* (O. Müller) Haworth ja *Cyclotella rossii* (Grunow) Håkansson, jotka muodostivat vastaavasti yhteensä 25,7–40,5 % ja 22,0–32,4 % havaituista lajeista. Vaikka järven historiaa kuvastavissa piilevien lajikoostumuksissa havaittiin pieniä muutoksia, ehdotetaan sen olevan sopiva vertailujärvi käytettäväksi vesipuitedirektiivin toimeenpanossa.

Contents

1. INTRODUCTION

Paleolimnology is the multidisciplinary science that studies the ontogeny of lakes and other inland aquatic ecosystems (i.e. their development through time). The record of an ecosystem's development through time is stored into the sediments as physical, chemical and biological information, often called *proxy information*. This proxy information can be sampled, studied and analysed using various paleolimnological techniques (e.g. Frey 1969, Dixit *et al.* 1992, Smol 2002, Cohen 2003, O'Sullivan 2004).

Material generated by processes within the catchment and in the atmosphere of a lake, eventually accumulates its sediments, which is why the sediments can be described as an ecosystem's memory or, merely, its archive (Figure 1). Therefore, paleolimnological analyses can also provide information about both processes in the catchment areas and changes in the atmosphere, in addition to processes within the lake itself (Frey 1969, Smol 2002, Cohen 2003, O'Sullivan 2004).

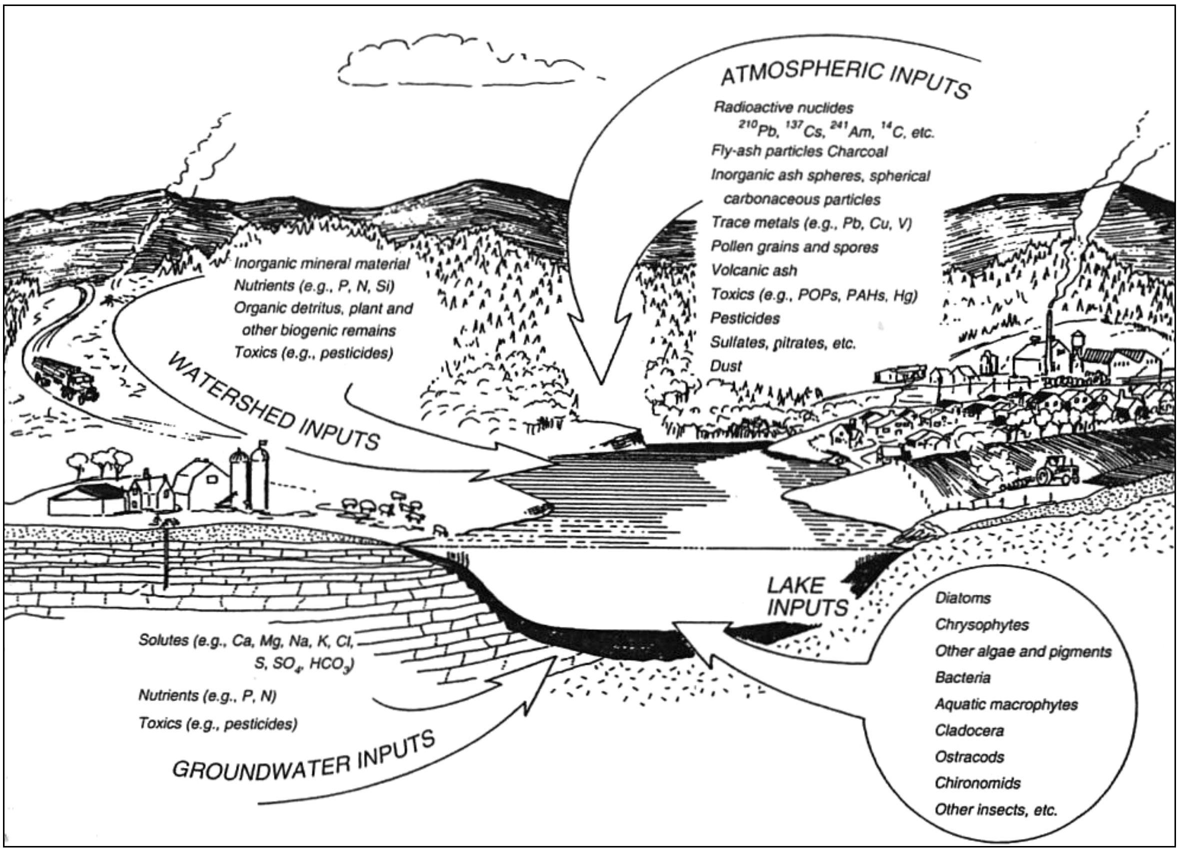

Figure 1. An illustration of different sources of allochthonous and autochthonous material, which accumulate in the bottom sediments of lakes (from Smol 2002).

Paleolimnological methods have proved their usefulness, for example in defining the reference conditions to be used in assessing the ecological status of water bodies. The European Water Framework Directive (hereafter referred to as WFD), which came into force on 22 December 2000, emphasises ecological quality, rather than chemical quality, for assessing the status of a lake, wherein this respect the WFD differs from many earlier water directives and legislation. The hydromorphological and physico-chemical variables are still used, but they are considered as supportive elements or secondary information, and ecological variables as primary elements, when assessing the quality of the watercourses (European Parliament 2000, Bennion *et al.* 2004, Räsänen *et al.* 2004, 2006, 2007, Bennion & Battarbee 2007).

Long-term information about aquatic ecosystems is an acknowledged need for their management and research. However, long-term monitoring data are often missing for various reasons, usually related to the difficulty in implementing long-term monitoring programs (e.g. Battarbee 1999). According to Dr John P. Smol (seminar 23.5.2007, Helsinki, Finland: "Arctic Environments, Lake Mud, and Climate Change: A window on the past and a view to the future"), approximately 90 % of the reviewed environmental assessments were three years or less in duration. In order to detect long-term trends and have statistically defensible results, paleolimnology is usually the only way to get the required data. For instance, even a 50-cm long sediment core from an oligotrophic lake can provide a considerable time span for assessing the trends in a lake more reliably than a short, 3-year study (Dixit *et al.* 1992, Smol 2002).

Diatoms (Class Bacillariophyceae) are a group of microscopic algae, which are considered one of the most valuable group of fossils found from lake sediments and used for paleolimnological investigations. Due to their ability to colonise new habitats quickly, diatoms are sensitive to changes in their environment. Diatoms are also ecologically diverse and they colonise virtually all microhabitats in lakes and rivers. Furthermore, diatoms are nearly always present, which makes them *inter alia* a valuable biological indicator (Hutchinson 1967, Round *et al.* 1990, Dixit *et al.* 1992, Battarbee *et al.* 1999, Wetzel 2001, Smol 2002).

The biology and ecology of diatoms has been studied rather intensively in different habitats, which makes them useful in pollution studies (Round *et al.* 1990, Dixit *et al.* 1992, Stoermer & Smol 1999a, Smol 2002). One of the best known examples of the value of diatoms in assessing the state of the environment has been their use in lake acidification studies (e.g. Dixit *et al.* 1992, Battarbee *et al.* 1999, Smol 2002). The features described above make diatoms ideal ecological indicator species, which can be used to reconstruct lake water pH on the basis of the species composition observed in a sample (Dixit *et al.* 1992). Much cited papers by Birks *et al.* (1990) and Birks (1998) describe the reconstruction as a two step process, where the first step involves modelling of modern diatom abundances responses to pH (*i.e.* regression), and the second step, where the modelled responses are exploited to infer the pH according to the species composition in a sediment sample (*i.e.* calibration).

The aforementioned problem of lack of long-term monitoring data can be overcome with paleolimnological data, which allows the determination of the reference conditions even from already impacted lakes. In contrast to paleolimnological data, there is a problem in comparing the historical and modern monitoring data obtained with conventional methods, which have been changing over time (e.g. changes in the detection limits of laboratory methods). Furthermore, data sets describing the status of a lake in its pre-disturbance condition are very scarce (Battarbee *et al.* 2005, Bennion & Battarbee 2007).

In many areas there are no lakes which can be used as reference lakes *per se* and the only way to obtain information about the pre-impact state is through a paleolimnological analysis, which also allows any baseline date to be selected to represent the pre-impact conditions (*see* e.g. Bennion *et al.* 2004, Weckström *et al.* 2004, Räsänen *et al.* 2006). In the context of the WFD, the reference condition refers to the state of an aquatic ecosystem before human impact and should therefore be defined separately for each study area as it becomes clear that it cannot be the same in every place (European Parliament 2000, Bennion & Battarbee 2007). It is also important to understand that the pre-human state of a lake does not refer to a purely clean natural state, but the state before *intensive* human impact (Räsänen *et al.* 2004, 2006).

This thesis applies paleolimnological methods and especially diatom analysis to infer limnological and trophic status changes from a bottom sediment core from Lake Isojärvi, located in Central Finland. The usefulness of the information from the diatom analyses is discussed from the perspective of the WFD. Several caveats do arise when using paleolimnological methods, and especially diatom analysis, in deciphering the ecological history of aquatic ecosystems, and some of these caveats are also to be discussed in the thesis, but the emphasis is on the applicability of paleolimnology and especially of diatom analysis.

In the case of Lake Isojärvi, the direction and rate of possible changes are the focus of interest. The suitability of Lake Isojärvi as a Finnish lake reference site, as defined in the WFD, is discussed in the end of the thesis.

2. BACKGROUND

2.1. Applications and development of paleolimnology

The paleolimnological approach can be viewed as a set of separate phases, starting in the field and ending with data processing (Figure 2). The collection of samples is the most critical phase in any biological study, and in a paleolimnological approach the sampling technique used becomes emphasized as the bottom sediment is difficult material for sampling. Due to the water content of sediments, it is difficult to get an undisturbed sample, which is the prerequisite for conserving the temporal resolution naturally preserved in the sediments in certain circumstances (Glew *et al.* 2001, Smol 2002).

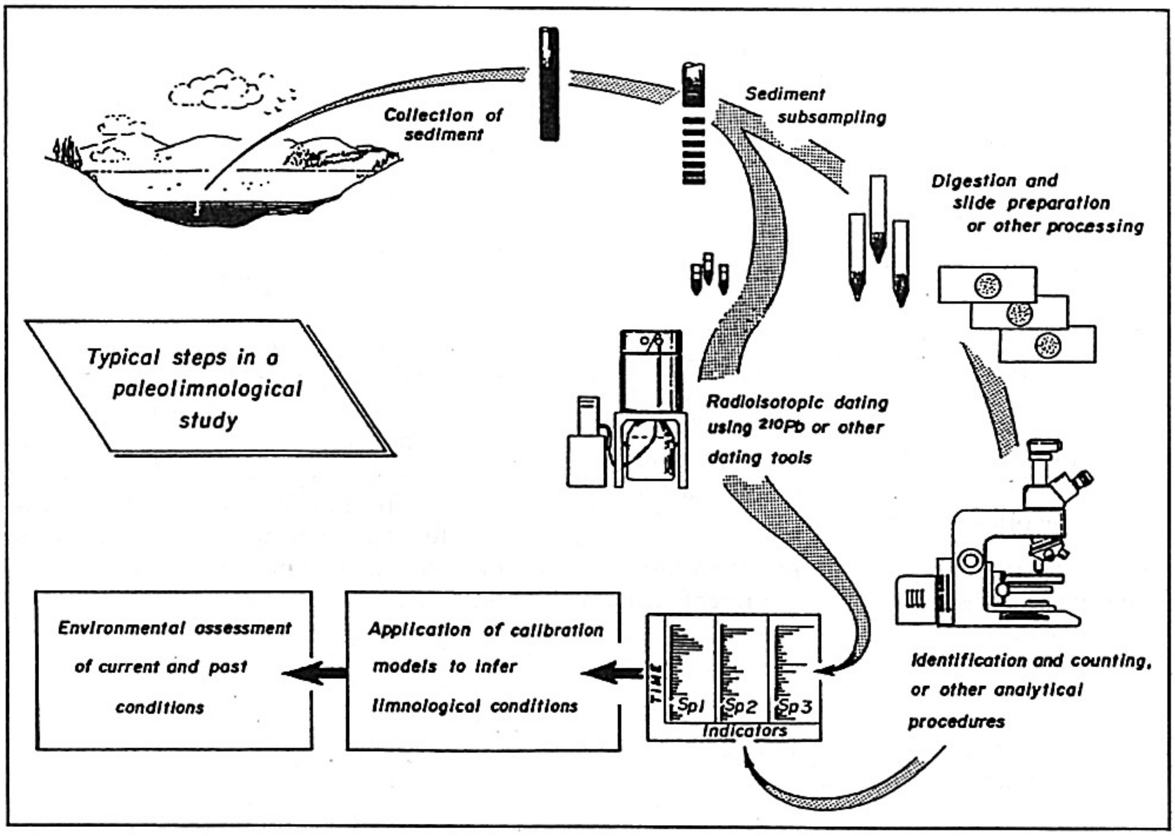

Figure 2. An overview of a typical paleolimnological study (from Smol 2002).

2.1.1. Applications of paleolimnology

Several different applications of paleolimnological methods provide examples from a spectrum of possibilities and applications, such as reconstruction of climatic changes (e.g. Smol *et al.* 2005), human impacts on watercourses like pulp and paper mill pollution (e.g. Meriläinen *et al.* 2001, 2003, Hynynen *et al.* 2004), logging in the catchments of lakes (Räsänen *et al.* 2007), and municipal sewage water (Meriläinen *et al.* 2003). Sarmaja-Korjonen *et al.* (2006) studied the paleolimnological development of Lake Njargajavri, and the effect of climatic and environmental changes on an aquatic ecosystem. Guhrén *et al.* (2007) studied the effects of liming on lakes through diatom records in the sediments, thus providing straightforward information for lake management purposes.

Furthermore, paleolimnological studies provide valuable information for ecosystem monitoring, because systematic monitoring of water courses often has a shorter history than the human impacts on water courses (e.g. Anderson 1995, Simola *et al.* 1996, Battarbee 1999, Anderson 2001, Kauppila *et al.* 2002, Smol 2002). Simola *et al.* (1996) used paleolimnological analyses as an information source in biomonitoring of large lakes and concluded that the methods used can provide detailed information about the past, which is helpful in assessing the current status, as well as future trends. This was also emphasized by Anderson (1995) and Battarbee (1999), who highlighted the role of paleolimnology in predicting the future of a lake through knowledge of the past, and possibly in identifying early warning signs. Anderson (1995) also noted that the information inferred from the sediments enables more efficient modelling of lake dynamics.

Flower *et al.* (1997) and Battarbee (1999) discussed the use of sediments in ecosystem restoration projects, by comparing the diatom assemblages obtained from the sediments with modern sites, thus allowing a more in-depth picture of the desired goal of the restoration and providing valuable information for lake managers and authorities.

Information about the past can be utilised in implementing the WFD, for example in defining a ‘good ecological status’ for a particular lake (e.g. Kauppila *et al.* 2002, Miettinen *et al.* 2005, Leira *et al.* 2006, Räsänen *et al.* 2006, 2007, Guhrén *et al.* 2007). Miettinen *et al.* (2005) used diatom derived transfer functions to assess background and present total phosphorus and water colour in lakes in eastern Finland. At the time of that study, water colour was suggested by Pilke *et al.* (2002) to be one basis for a Finnish lake typology, and thus the information about the past water colour in lakes is an essential starting point when evaluating the ecological status of a lake. The definition of ‘good ecological status’ has produced a straggle of complex connotations, and paleolimnological investigations have provided some answers, which have had an important role in implementing the WFD (Bennion & Battarbee 2007).

Meriläinen *et al.* (2006) suggested using Lake Isojärvi and Lake Saari-Kiekki as reference lakes for WFD, on the grounds of paleolimnological studies. A study by Leira *et al.* (2006) provides another demonstration of the potential use of paleolimnological approach in implementing WFD in Ireland. Bennion *et al.* (2004), Leira *et al.* (2006) and Bennion and Battarbee (2007) cautioned that using the same historical reference state in all lakes is not appropriate when the land use of different regions differs from each other.

Räsänen *et al.* (2006) compared the classification of lakes made on the grounds of the lake chemistry and diatom analysis, and concluded that the inferred status of a lake assessed using diatoms is often better than the chemistry implies. As an example they cited naturally eutrophic lakes, which were already nutrient-rich before intensive human impact, contrary to a common belief about the natural ontogeny of lakes. Battarbee *et al.* (2005) extended this subject by discussing the need to study oligotrophication, which is a rather understudied field, however relating to a very practical question about restoration targets set for human impacted lakes.

Another, rather exotic example by its nature, is a paleolimnological study of Lake Rano Raraku, Easter Island, by Dumont *et al.* (1998), which has shed light on the mystery of what happened on Easter Island, hence providing again a different application of paleolimnology. Dumont *et al.* (1998) studied microfossils of plants and animals from a sediment core obtained from Lake Rano Raraku, located in the quarry where the island’s giant statues were carved. Based on the microfossils and the dated sediment core, Dumont *et al.* (1998) suggested that Amerindians did have a role in the cultural collapse of the island.

2.1.2. Development of paleolimnology

A vast literature about different methods for obtaining information from lake sediments has been published as handbooks by Berglund (1986a), Last & Smol (2001a,b), Smol *et al.* (2001a,b) and Cohen (2003). As a field of science, paleolimnology has developed to be more interdisciplinary from its beginning (O'Sullivan 2004). Both O'Sullivan (2004) and Smol (2002) described the development as rapid and coupled with fast evolution of methods and technology, which have made possible more effective and thorough studies of the sediments.

Anderson (2001) described three areas of progress in paleolimnology, which have had major consequences in the development of modern techniques:

1. Increased concern with quantification of data (e.g. by using weighted averaging (WA) models and constrained ordination).
2. Increased concern about temporal resolution (e.g. the use of annually-laminated sediments and fine-interval sampling).
3. Integrated, multidisciplinary projects, which make it possible to carry out multi-proxy studies.

Although paleolimnology has been, and still is, considered largely a descriptive science because the data obtained are mostly qualitative, it has developed to be a more quantitative and objective science as the methods have been improved (Anderson 1995, 2001, Battarbee 1999). New methods are constantly being implemented and old methods refined (Dixit *et al.* 1992, Smol 2002).

Battarbee (1999) recognized three key developments which have contributed to the increased use of paleolimnological techniques in understanding contemporary lake dynamics, in contrast to more traditional long-term studies. In addition to the aforementioned coring and numerical techniques, accurate methods for dating the younger sediments have made it possible to assess near historical changes.

At the same time, Battarbee *et al.* (2005) acknowledged the need for a closer communication between limnology and paleolimnology, as there is a need to further improve the comparability of observational data and paleolimnological records, and on the other hand, to lengthen the time-scales usually considered in limnological research.

2.2. Diatoms as a source of information

Diatoms (Class Bacillariophyceae) are a group of microscopic algae, which are considered one of the most important lacustrine microfossils that can be used in paleolimnological work (e.g. Dixit *et al.* 1992, Flower *et al.* 1997, Stoermer & Smol 1999a, Cohen 2003, O'Sullivan 2004). As an example of the abundance and commonness of diatoms, according to Werner's (1977a) estimation, diatoms constitute approximately 20 to 25 % of the world net primary production. In addition, according to Anderson's (1990) estimate, already one gram of dry sediment can contain several tens of million diatom frustules.

Diatoms are unicellular and eukaryotic, with a distinctive, ornamented cell wall. The cell walls are composed primarily of resistant opaline silica (SiO_2). As compared to other organic algal cell wall materials, Si uptake and deposition consumes less energy thus making the structure of diatoms energy efficient, and thus could be viewed as an ecological and evolutionary advantage (Werner 1977b, Round *et al.* 1990, Battarbee *et al.* 2001). Diatom cells are pigmented and photosynthetic, although some species can live heterotrophically in the dark, which requires a suitable source of organic carbon (Round *et al.* 1990).

If bicarbonate is not taken into account, silica is often the most abundant acidic substance in inland waters. In some waters, the silica concentration may be even greater than the concentration of bicarbonate. Diatom algae are the main mechanism of loss of silica in lakes and streams, as they assimilate large quantities of silica. If the concentration of silica is decreased under approximately 0.5 mg l^{-1}, many diatoms cannot compete with nonsiliceous algae (Hutchinson 1957, Wetzel 2001).

Diatoms are usually separated into two major groups according to their symmetry: the centric diatoms, which have radial symmetry and the pennate diatoms, which are bilaterally symmetrical. The cells of diatoms consist of two thecae, with one theca a bit larger than the other and working as some kind of lid (

Figure 3). The vegetative cell division involves a successive decrease in population mean cell size, which is a special characteristic of diatoms, also known as the MacDonald-Pfitzer rule (Round *et al.* 1990, Battarbee *et al.* 2001, Wetzel 2001).

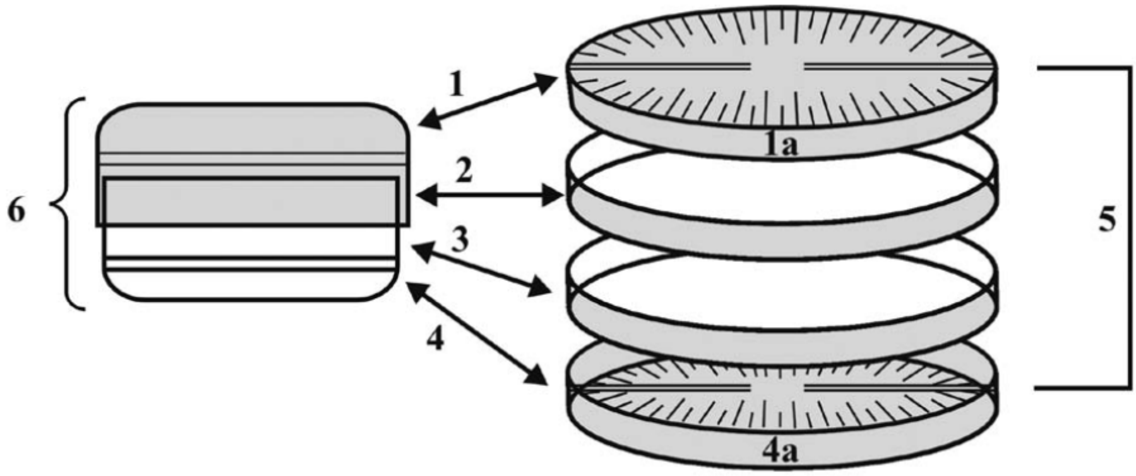

Figure 3. The structure of a diatom frustule. 1 = epivalve, i.e. the lid of the frustule, where the face and the edge (1a) of the lid can be distinguished, 2 = epicingulum, i.e. the middle piece, comprises the epitheca together with the epivalve, 3 = hypocingulum i.e. the middle piece, comprises the hypotheca together with the hypovalve, 4 = hypovalve, i.e. the bottom of the frustule, where the face and the edge (4a) of the bottom can be distinguished, 5 = epitheca and hypotheca together comprise the diatom's frustule, and 6 = the frustule of the diatom, where epicingulum and hypocingulum are overlapping (from Eloranta *et al.* 2007).

The most common mode of multiplication of diatoms is vegetative reproduction. Due to the successive decrease in the cell size during asexual reproduction, the size is restored by auxospore formation. Sexual reproduction takes place periodically when the cells reach a minimum critical size, approximately 30 to 40 % of maximum size. Changes in size and shape during the vegetative and sexual reproduction cause morphological variation (Battarbee 1986, Round *et al.* 1990, Wetzel 2001).

Identification of diatoms is based on their siliceous cell walls (frustules), which are composed of two valves (Figure 3). The number of different recognised species varies between in the order of 10^4 and 10^5, according to author and species concepts. The first classification systems were based on shape, symmetry and ornamentation of the frustules, as seen with light microscopy. As electron microscopy developed, finer details were revealed, which led to refinements in diatom systematics. In recent studies on diatom systematics, more emphasis is put on using living material and on molecular techniques (Dixit *et al.* 1992, Stoermer & Smol 1999a,b, Battarbee *et al.* 2001).

With some exceptions, the siliceous frustules of the diatoms are well preserved in sediments, which is why most fossil taxa can be identified to species level (Flower 1993, Stoermer & Smol 1999a, Battarbee *et al.* 2001). As most sediments contain more than 100 taxa, with a range of different morphological forms, diatom taxonomy is considered rather difficult (Battarbee 1986).

2.2.1. Different diatoms in different environments

Different diatom taxa have their preferred environmental conditions and limits of tolerance to changes. Changes in the environment are seen in the composition of the diatom assemblages, which therefore can be used in inferring the past conditions of a lake (e.g. Birks *et al.* 1990). For example, the main variable affecting the diatom productivity and species change is most often phosphorus, whose fluctuation through time is often reconstructed from diatom records in lake eutrophication studies (Stevenson & Pan 1999, Battarbee *et al.* 2001, Smol 2002). Birks *et al.* (1990) and Dixit *et al.* (1992), *inter alia*, describe diatoms as good ecological indicators for lake water pH, due to which they are often used in lake acidification studies.

An analysis of sedimentary diatoms is relatively robust against the effects of seasonal variation and changing weather conditions, in comparison to single water column samples (Miettinen *et al.* 2005). Hall & Smol (1999) pointed out that the annual ranges of nutrients such as P are greater in more eutrophic lakes, which brings noise into diatom inference models, because of peak populations representing different total phosphorus concentrations during the same year, causing prediction errors to increase.

The distribution of diatoms is controlled by several environmental factors (e.g. Round *et al.* 1990, Battarbee *et al.* 2001, O'Sullivan 2004):

1. *Physical factors*: temperature, light, mixing, turbulence, ice cover, wind;
2. *Chemical factors*: pH, dissolved organic carbon (DOC), salinity, conductivity and nutrient concentration, especially total phosphorus (TP), but also nitrogen (N) and silica (Si) concentration; and,
3. *Biological factors*: grazing, mutualism, and parasitism, which are important, but changes through time are difficult to assess.

Temperature affects the metabolic processes in diatoms, which is why they have been used in inferring the past temperatures. Temperature has an obvious effect on physical factors (e.g. stratification and ice-cover), and on chemical factors (e.g. pH and nutrients) (Battarbee *et al.* 2001). Some authors have found certain diatoms occurring in specific temperatures (see e.g. Bigler *et al.* 2000, Weckström *et al.* 1997), whilst other authors have discussed inferring the past temperatures indirectly through resource-related competition and a temperature-pH relationship (Lotter *et al.* 1999). Strong correlations between diatoms and temperatures have been published especially from extremely cold environments (Cohen 2003). Bigler and Hall (2003) stated that fluctuating lake water pH, nutrient concentrations, and habitat availabilities might influence the diatom communities more than temperature, thus decreasing the accuracy of diatom inferred temperatures. However, it was concluded that if pH seems constant, diatoms can be considered as reliable indicators of temperature in their study sites in northern Sweden.

Light plays a key role in photosynthesis, and therefore its influence can be seen in diatom communities. Turbidity of water is often related to eutrophication or increased allochthonous material (e.g. due to deforestation, agriculture, forest fires, vegetational changes). In many boreal lakes, the light climate is affected by dissolved organic carbon (DOC) due to humic substances. Based on diatom-DOC coupling, it has been possible to assess the optical characteristics of the water column (Battarbee *et al.* 2001, O'Sullivan 2004). In dystrophic lakes and in high-latitude lakes the variation in the diatom communities is more closely explained by the variation in DOC than by pH (Pienitz & Vincent 2000, O'Sullivan 2004).

Diatom communities, especially planktonic diatoms, but also benthic diatoms through resuspension, are affected by turbulence. Mixing of the water column and stratification have an impact on the diatom assemblages according to different adaptations of diatom species. Hence, changes in stratification may be reconstructed from the sediment record (Battarbee *et al.* 2001).

Salinity has been suggested to have the strongest impact from all environmental factors that affect the diatom distribution (Battarbee 1986, van Dam *et al.* 1994, Battarbee *et al.* 2001). Therefore, diatoms can provide information about thalassic (coastal) areas and athalassic (inland salt lake) environments. Changes in salinity can be inferred from diatoms adapted to different salinities (Battarbee 1986, Battarbee *et al.* 2001). As in the case of pH reconstruction, classification of diatoms according to salinity has been replaced by the use of *transfer functions* (Figure 2, Figure 4). Transfer functions can also be used in inferring the past nutrient status (mainly total phosphorus, e.g. Miettinen *et al.* 2005) of a lake (Smol 2002, O'Sullivan 2004). Reconstructions of the changes in nutrient status can be used in studies of lake ontogeny (long-term change) and eutrophication (short-term change) (Battarbee 1986). Miettinen *et al.* (2005) used diatoms and transfer functions in TP and colour reconstructions (DOC). An earlier example of an eutrophication study by Stockner (1972) used simpler methods through calculation of ratios between two diatom orders (*Centrales* and *Pennales*).

Diatom species can be classified according to Hustedt's five pH categories (Table 1), based on studies of diatoms in different habitats (Hustedt 1938-1939). Hustedt's observations about different responses to pH of different species, and consequent classification according to pH, were the earliest of their kind, and hence had an important role in the development of new classifications and statistical methods (Battarbee 1986, Battarbee *et al.* 1999). van Dam *et al.* (1994) use the same categories as Hustedt (1938-1938), but have added a category 'circumneutral' for diatoms occurring at pH-values about 7. van Dam *et al.* (1994) noted that the category 'indifferent' was replaced by later authors, as the 'indifferent', i.e. diatoms insensitive to pH, are extremely rare.

Different indices have been developed on the basis of Hustedt's classification system. An example is Nygaard's indices, where three indices are calculated based on the amount of acid and alkaline taxa (Battarbee *et al.* 1999). For instance, in a much cited study by Meriläinen (1967), Nygaard's indices were used and the results from Finnish lakes were compared with those from Danish lakes, which Nygaard himself had used. Although the indices proved to be a little different between Finnish and Danish lakes, it was concluded that diatom floras can be used to infer the pH of natural water fairly accurately.

Table 1. Hustedt's (1938-1939) five pH categories, which have been widely used in classifying the diatoms. Hustedt has presented categories also for different salinity preferences (Battarbee 1986, Battarbee *et al.* 1999, O'Sullivan 2004).

Category according to pH	**Description**	**Category according to salinity**	**Description**
alkalibiontic	diatoms occurring only at pH > 7	polyhalobous	diatoms that are widely distributed along the salinity gradient
alkaliphilous	diatoms occurring at pH 7 and optimum distribution at pH > 7		
indifferent / circumneutral	diatoms occurring at both sides of pH 7	mesohalobous	diatoms occurring in brackish waters
acidophilous	diatoms occurring at pH 7 and optimum distribution at pH < 7	oligohalobous	diatoms occurring in fresh waters
acidobiontic	diatoms with optimum distribution at pH 5,5 and below	halophobous	diatoms occurring in high salt concentrations

In addition to the common nutrients, phosphorus (P) and nitrogen (N), limiting algal growth, the diatoms can also become limited by silica (Si). P is the most common limiting nutrient for diatom communities. However, in the situation of excess P loading, the Si reserve might become depleted, as the permanent sedimentation of diatoms increases, as it was shown in the Laurentian Great Lakes (Schelske 1999). Si uptake by diatoms can at times be so effective that the dissolved Si can become undetectable in the laboratory water analysis (Round *et al.* 1990).

A variety of different productivity indices have been used, such as the ratio of planktonic to benthic taxa, where the increase in the ratio reflects eutrophication in a shallow lake due to increased concentration of P (O'Sullivan 2004). Other indices that have been used are the *Centrales:Pennales* (C:P) and *Araphinidineae:Centrales* (A:C) ratios, which are based on characteristic species for eutrophic, mesotrophic, and oligotrophic conditions (Meriläinen 1967, Stockner 1972, Battarbee 1986). However, many of the indices have been set aside as they have proved to be too simplistic (Hall & Smol 1999). As the statistical methods and computers have been developed, more detailed analyses of each taxa in a sample has become possible.

2.2.2. Diatom analysis in environmental studies

The use of diatoms as indicators of environmental changes has proven to be a powerful and reliable method (Battarbee 1986). The diatom analyses have been used in reconstruction of the backgrounds for public environmental concerns, such as climate change, eutrophication and surface water acidification. Furthermore, diatom analysis has established a firm position in the assessment and monitoring of running waters (Dixit *et al.* 1992, Battarbee *et al.* 1999, Smol 2002, Eloranta *et al.* 2007).

Diatom analysis of lake sediments can be used in inferring the lake ontogeny, changes in shorelines (fluctuation in water level, e.g. Sarmaja-Korjonen *et al.* 2006), surface water acidification (e.g. Flower & Battarbee 1983), eutrophication (e.g. Stockner

1972, Kauppila *et al.* 2002), and climate change (e.g. Battarbee *et al.* 2001, Smol *et al.* 2005). The sedimentary diatom compositions provide also a reliable way for recognising naturally eutrophic lakes and selection of the reference lakes required by WFD, as noted by Miettinen *et al.* (2005) and Leira *et al.* (2006).

Surface water acidification studies in the 1980s brought diatoms into wider use and, at the same time, started the rapid development of new statistical methods for pH reconstruction, which have been applied also for the other variables (Battarbee *et al.* 1999). For example, a study by Flower and Battarbee (1983) used ^{210}Pb dated sediment cores and diatoms in inferring the past changes in two lakes, which were found out to have become strongly acidified during the past 130 years. Their study also showed differences between lakes with forested and afforested catchments, from which they made an important observation that the acidification process can be partly controlled by catchment characteristics.

In addition to analyzing the diatoms from the whole sediment core, a “top-down” approach has been used successfully in acidification studies, whereby only the bottom and top of the core (preacidification and modern situations respectively) have been analyzed. This top-down approach allows many lakes to be studied in a relatively short time (Battarbee *et al.* 1999, Smol 2002).

In relation to acidification, liming has been used as a lake restoration technique in mitigating its effects on watercourses. Guhrén *et al.* (2007) used diatoms in assessing the effects of liming in 12 lakes in Sweden. The results showed the biological responses to acidification to be complex in different lakes. The importance of knowing the history of a lake and its catchment was emphasised in order to be able to establish sensible restoration goals.

2.2.3. Things to be taken into account with diatoms

The composition of the diatom communities can change within the same lake, depending on the sampling site. Therefore, in order to make more careful estimates of diatoms, a multiple-core approach should be used. In addition, the sediment sample can include diatoms from other sources (catchment soils or upstream habitats), or conversely the sample might not represent all the diatoms in the lake, due to losses through the outflow or from grazing (Battarbee 1986).

Other processes (e.g. slow accumulation rates and bioturbation of the sediments) can weaken the resolution of the reconstruction and thus the selection of the sampling site is of critical importance (Battarbee 1986, Battarbee *et al.* 2001).

Several factors affect the preservation of diatoms in the bottom sediments, which thus have to be taken into account in order to get reliable results. The diatom frustules become easily dissolved in saline and alkaline ($pH > 9.0$) solutions, and the dissolution is further enhanced by high temperatures (Flower 1993, Battarbee *et al.* 2001, Cohen 2003). In general, diatoms are best preserved in acid lakes (with some exceptions), rather than alkaline lakes. Flower (1993) emphasised that dissolution of the frustules is a multivariate problem, thus making the interpretation challenging if the rate of dissolution varies between species.

Benthic marcroinvertebrate activity has an influence on diatom preservation together with the silica content of the cell wall (Battarbee *et al.* 2001). In addition, high pressure in the bottom of deep lakes can break the frustules and thus make their identification impossible. Most of the dissolution of the frustules takes place in the water column, sediment-water interface (SWI), or during the first few decades after deposition (Flower 1993, Battarbee *et al.* 2001, Cohen 2003, O'Sullivan 2004).

Mann and Droop (1996) noted that the existing species-level classification system has been found to be too coarse. Consequently, the old classification system hides some of the potential information, which could be obtained from the diatoms. In some species sympatric phenodemes are reproductively isolated and, hence are considered to be separate species. Bearing this in mind, the total number of diatom species would be at least 2×10^5.

Battarbee *et al.* (2001) discussed recent studies that have been putting more emphasis on living diatoms and on molecular techniques, which again enables more detailed refinements in diatom systematics. Constantly changing taxa is a challenge for a diatom analyst as new genera are created and old genera are refined.

An important note is that diatoms should not be used as the only method in inferring the past of the ecosystems, especially in eutrophication studies, where the complex food-webs are likely to affect the lake. As diatoms are only one of the many algal groups in lakes and represent only one trophic level, other fossil remains should be used as well (Simola *et al.* 1996, Hall & Smol 1999).

For example, Hynynen *et al.* (2004) used chironomids in addition to diatoms in interpreting the changes in Lake Lievestuoreenjärvi. A study by Sarmaja-Korjonen *et al.* (2006) demonstrated the usefulness of using many organisms (Cladocera, chironomids and diatoms), instead of fewer methods. Bigler and Hall (2003) used diatoms in estimating past changes in temperature and were able to justify their results with parallel proxy data. Numerous factors are affecting the diatom communities, which is why multi-proxy approach should be used to get reliable results and information about the complex climate-environment interactions.

2.2.4. Handling the data and development of statistics

Even though Hustedt's classification system (Table 1) is still helpful in assessing the history and development of a lake, it and linear models derived from it have a number of weaknesses and restrictions, which have been taken into account when developing new ways to reconstruct the past. For example, an assumption that a taxon can be classified into an individual class, is problematic. Another invalid assumption is that the species have linear or monotonic relationship to pH (Battarbee *et al.* 1999). Thus, the Hustedt classification system and linear regression models for pH have largely been replaced by transfer functions (Figure 4) (Battarbee 1986, Battarbee *et al.* 1999, O'Sullivan 2004).

In addition to transfer functions, several indices can be used in handling the data from diatom analyses. An example of software designed to analyse the data from diatom analyses is OMNIDIA programme, which is an important and widely used tool in processing the data and producing information, which can be easily presented. All known information about freshwater diatoms has been collected into the programme, which calculates a group of different indices and gives ecological information about the sample, after the identified diatoms and their abundances have been inputted (Lecointe *et al.* 1993, Eloranta *et al.* 2007). Eloranta *et al.* (2007) recommend the use of the OMNIDIA programme for assessing the river water quality by using diatom assemblages.

2.3. Statistical analysis of diatom data

After the quantitative data about the ecological characteristics of diatoms has been obtained, there are various methods available for the statistical analysis of diatom data.

Instead of a linear response, most organisms have a unimodal response to environmental variables (Smol 2002). Birks (1998) stated that the major advances in statistical techniques happened after the numerical unimodal-based techniques for gradient analysis were developed. Reconstruction of an environmental variable (e.g. pH, TP, DOC and TOC) can be done by using weighted averaging (WA) regression and calibration, which assume unimodal relationship between the diatoms and the variable. In WA it is assumed that taxa that have their optima at a certain pH are then also the most abundant at that pH. Taxa that have narrower tolerance can be taken into account by giving them more weight in WA, than for taxa that have wide tolerance (Battarbee *et al.* 1999, Smol 2002). However, some taxa might appear to have linear response to the sampled environmental gradient, if the gradient is not long enough. The selection of the technique can be done objectively by using DCA in determining how long the gradient is, measured in standard deviation (SD) units (Birks 1998, Smol 2002). According to Birks (1998), the linear techniques (e.g. PCA or RDA) are more appropriate if the gradient is < 2 SD units.

Multivariate statistical techniques allow determination of those environmental factors which are mostly affecting the species distribution, and on the other hand the possibility to do eyeballing of otherwise convoluted numerical data. One of the most used statistical approaches in diatom studies is canonical (i.e. direct-gradient) correspondence analysis (CCA), which allows analysis of environmental variables and species data together (e.g. diatom-environment relationships in lake training sets). As an alternative, also redundancy analysis (RDA) can be used, if linear technique is required (Hall & Smol 1999, Smol 2002). PCA and DCA can be used in studying water chemistry and diatom variation independently in a training set (i.e. calibration set), which is then used in constructing the transfer function (Dixit *et al.* 1992, Battarbee *et al.* 1999, Hall & Smol 1999).

Principal component analysis (PCA) is perhaps more widely known statistical method used in ecological studies. Like RDA, PCA is a linear technique. PCA is based on *eigenvalue*-technique. The difference between PCA and RDA is that the PCA is an indirect ordination technique, whereas the RDA is a direct ordination technique, like CCA. The direct ordination technique refers to a technique, which can detect patterns in the species data that can be directly related to measured environmental variables. Indirect techniques (e.g. PCA) can be used for revealing the patterns within multivariate species data (Smol 2002).

The direct ordination techniques (e.g. CCA and RDA) can be compared to multiple regression analysis, but they are multivariate in nature, as the all species in the dataset can be modelled simultaneously with one or more environmental predictors (Battarbee *et al.* 1999, Hall & Smol 1999, Smol 2002).

2.3.1. Transfer functions

Transfer functions have been developed for reconstructing the past of an aquatic system in a quantitative manner, by assuming that the biological remains, e.g. diatoms, reflect the environmental conditions (Jeppesen *et al.* 2001, Smol 2002).

In the case of diatoms, the transfer functions are based on modern observations of diatom distributions in different lakes. These modern data sets are commonly termed *training sets* (Figure 4). Most often surface sediment samples (usually 0.5 or 1 cm thick layer) are used in constructing the training sets. Training sets should cover the environmental gradients under study, with reliable measurements of water chemistry. The diatom taxa observed in the samples are assumed to have a unimodal or Gaussian distribution along the environmental variable, e.g. TP or DOC, the optimum occurring in the conditions where the maximum abundance of the particular taxa is observed (Battarbee *et al.* 1999, Hall & Smol 1999, Jeppesen 2001).

Statistical significance between diatoms and the studied environmental variable can first be tested using Monte Carlo permutation tests. Only after that can a transfer function be constructed. In general, a two-step process, which includes a regression step and a calibration step, is used in generating the transfer function. In practice, the regression step means modelling the relationship between the modern surface sediment diatom assemblages and water chemistry, by using the modern training set. In the regression step, the living optimas and tolerances of each diatom species are taken into account. In the calibration step, the studied environmental variable is inferred from fossil diatom assemblages, by using the function generated during the regression step (Birks *et al.* 1990, Battarbee *et al.* 1999, Hall & Smol 1999).

Due to morphological differences, some diatom species preserve better than others. This can have a significant impact on the accuracy of transfer functions, due to the biases in the composition of the diatom assemblages in the sediment (Flower 1993, Battarbee *et al.* 2001).

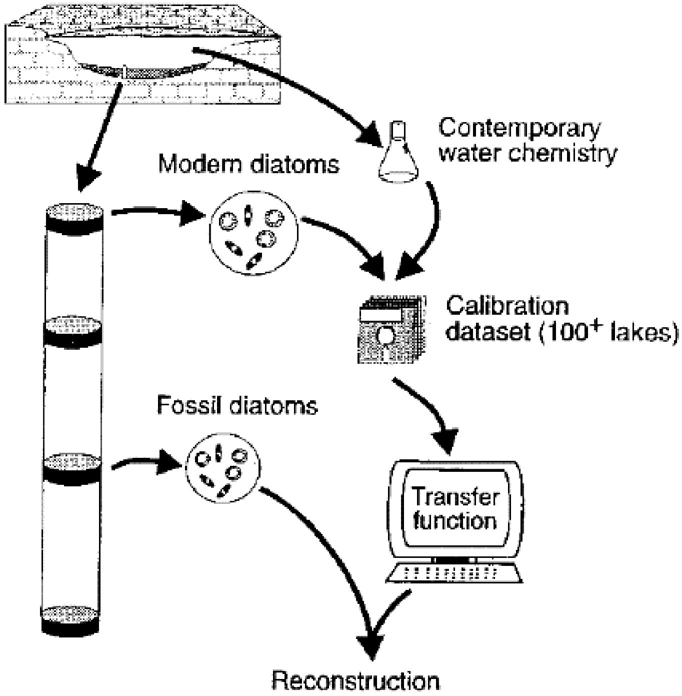

Figure 4. Schematic drawing showing the different steps in constructing a transfer function and finally reconstructing the changes in the measured environmental variables (from Hall & Smol 1999).

2.4. Future perspectives of paleolimnology

Smol (2002) discussed the increasing complexity of environmental problems, as the interlinking factors make it difficult to forecast how individual issues eventually pile-up. According to Smol (2002), paleolimnology can help to answer many of the questions relating to environmental change, as long time series can be used to detect the trends and sudden changes can be dated relatively accurately. Anderson (1995) and Dearing *et al.* (2006) also emphasize the importance of understanding long-term changes, to be able to model the future better by knowing the past. Dearing *et al.* (2006) discussed the role and importance of paleoenvironmental studies in research on human-environment interactions, where the background data is usually missing due to short data series. Issues related to human-environment interactions require long-term perspectives and more studies in degraded human-dominated landscapes and highly-valued ecosystems, hence providing opportunities still unused for the paleolimnologists. Anderson (1995) remarked that although there have been significant improvements in paleolimnology there is still a need to improve paleolimnology towards more quantitative and objective direction.

Diatom taxonomy and nomenclature have received too little attention for the past century, even though these underpin all diatom studies. Resources aimed for this kind of basic research are few, as the science in general has been constrained more and more by directed research programmes (Stoermer & Smol 1999b). Battarbee *et al.* (2001) reflected the constantly changing taxa as a challenge for a diatom analyst, which becomes emphasized when taking into account what Stoermer and Smol (1999b) said about taxonomy and nomenclature related research above.

Stoermer and Smol (1999b) recognized also the need for better knowledge of life cycles and life history processes of diatoms. Consequently, there is also a need for experimental evidence on diatom physiology. As an example, in order to exploit fully the current statistical analyses, more basic data should be used than is available at the present. Stoermer and Smol (1999b) concluded that a lot of potential information through diatom research is still waiting to be utilized.

In conclusion, as Stoermer and Smol (1999b) and Smol (2002) promised a lot of potential information remains to be extracted by going into finer details and analysis techniques. Smol (2002) described the fast development of paleolimnology and concluded that development will undoubtedly continue, but already now a huge amount of data is possible to be collected even from a single sediment sample by using multiproxy techniques. For example, in relation to eutrophication studies and the use of diatoms, Hall and Smol (1999) discussed the importance of also analysing other bioindicators in addition to diatoms from the sediments, as they may reveal essential information about food-webs, which might stay obscure if only diatoms are used.

As the paleolimnological data has had a key role in the implementation of the WFD and in the definition of the reference conditions, there seems to be many opportunities still to become for paleolimnologists (Bennion & Battarbee 2007). Furthermore, Battarbee *et al.* (2005) acknowledge the need to increase the communication between limnology and paleolimnology, which is needed to improve the comparability of the observational data and paleolimnological records.

2.5. Rationale for the study

According to Meriläinen *et al.* (2006), Lake Isojärvi is suitable as a reference lake in implementing the WFD. Although a clear indication of changes supposedly caused by erosion was detected in the sediment profile, it was concluded that this has not caused any significant changes in the ecological state of the lake. The same sediment core slices were analysed for their diatoms in this study as was studied by Meriläinen *et al.* (2006), who analysed the subsamples for their organic content (LOI %), water content, selected chemical elements, and the stable isotope ratios of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$). In addition, the diatoms were analysed from three subsamples by Meriläinen *et al.* (2006).

In comparison to the study by Meriläinen *et al.* (2006), in this study the diatom analysis was done for all the sediment subsamples, in order detect possible changes in the diatom record, which cannot be done reliably by analysing only three subsamples, even though that kind of method is often used due to the laborious nature of diatom analysis (e.g. Smol 2002). Furthermore, the analysis of all subsamples for their diatoms, allows the diatom data to be statistically studied with the chemical data. Hence the underlying rationale was that making a more detailed diatom analysis from all the subsamples would allow an improved evaluation of the preliminary assessment made by Meriläinen *et al.* (2006) as to the suitability of this site as a reference lake.

3. MATERIAL AND METHODS

3.1. Lake Isojärvi

Lake Isojärvi is located in the Province of Western Finland, Central Finland Region, in the municipality of Kuhmoinen (Figure 5). According to the information available from the Finnish Environment Administration's HERTTA-database (the Environmental Information System), the lake is part of the Kymijoki river basin area (lake number 14.263.1.001). North of Lortikanvuori hill Lake Isojärvi drains to Lake Päijänne and south of the hill to Lake Längelmävesi. Lortikanvuori is located on the south side of Lake Isojärvi.

Table 2. Physiographic data for Lake Isojärvi (obtained from HERTTA database). * A.s.l. is the abbreviation for "above the sea level".

Parameter	**Unit**	**Value**
Altitude m a.s.l.*	m	+118.90 (N60)
Lake area	km^2	18.3
Drainage basin	km^2	156.0
Drainage basin to lake area ratio	$km^2\ km^{-2}$	8.5
Maximum lake depth	m	69.7
Mean depth	m	16.4
Shore length	km	117.3
Volume	$10^3\ m^3$	300233

Table 3. Water quality data from Lake Isojärvi (obtained from HERTTA database). All water samples were taken from 1 m depth at site Isojärvi 4, which is a national sampling site for lake water quality monitoring.

Parameter	**Date of the measurements**							
	11.7.2007	*1.9.2005*	*30.6.2005*	*23.2.2005*	*17.2.2004*	*30.5.1990*	*31.3.1977*	*24.1.1964*
TP ($\mu g\ l^{-1}$)	4	4	5	5	4	5	12	-
TN ($\mu g\ l^{-1}$)	310	290	340	430	380	410	600	-
pH	6.9	6.5	7.0	6.4	6.6	6.8	6.7	6.7
O_2 ($mg\ l^{-1}$)	8.5	9.1	8.9	11.6	13.1	11.4	13.1	13.1
O_2 (%)	98	91	91	81	91	102	95	94
Colour ($mg\ Pt\ l^{-1}$)	25	30	35	35	30	30	35	35

According to the Environment Administration's general usability classification, Lake Isojärvi belongs to Class I (HERTTA). According to the Finnish lake typology (Vuori *et al.* 2006) required to implement the WFD, Lake Isojärvi belongs to type *Vh*, i.e. oligohumic lakes, where the water colour is < 30 mg Pt l^{-1} (cf. Table 2, Table 3). In addition, Lake Isojärvi is classified as a medium sized lake (i.e. lakes with surface area 5-40 km^2). Type *Vh* corresponds to the type 4a in an earlier proposal for the Finnish lake typology by Pilke *et al.* (2002). The lake's surface area is 18.3 km^2 and the maximum depth 69.7 m (HERTTA).

The bedrock of the area around the lake consists mainly of granite, quartz diorites, granodiorites, mica schists, and mica gneisses, with occasional spots of amphibolites, gneissic amphibolites, and prophyrites (Vesihallitus 1978, Suominen *et al.* 2002). The catchment of the lake is mostly covered with forests (80.2%) and peatlands (10.2%). Cultivated land comprises only 0.75% of the catchment area (HERTTA). Soil type around Lake Isojärvi is mainly bare rock, with small spots of sand, clay, sedge peat and sphagnum peat (Suominen *et al.* 2002, GEOKARTTA, METSÄHALLITUS). The lake has formed inside a fracture in the earth's crust, i.e. a fault-line in the bedrock (Vesihallitus 1978, METSÄHALLITUS), which is why the lake is relatively deep with a steep morphology (Table 2). The fault-lines in the area of Isojärvi National Park, which formed when the bedrock fractured some 200 million years ago, are in the direction of northwest to southeast.

According to information from METSÄHALLITUS, the first large loggings were carried out around the 1880s in the area of Isojärvi National Park (Figure 5). During the First World War, the increased need for timber and firewood required further extensive felling in the 1920s and 1930s. The last known large forest fire in the area of Isojärvi National Park is documented to have occurred in the 1850s. After Isojärvi National Park was established in 1982, there has been no logging activities in the area of the park, excluding tree felling by Canadian beavers (*Castor canadensis*), which have thrived in the area since the 1990s.

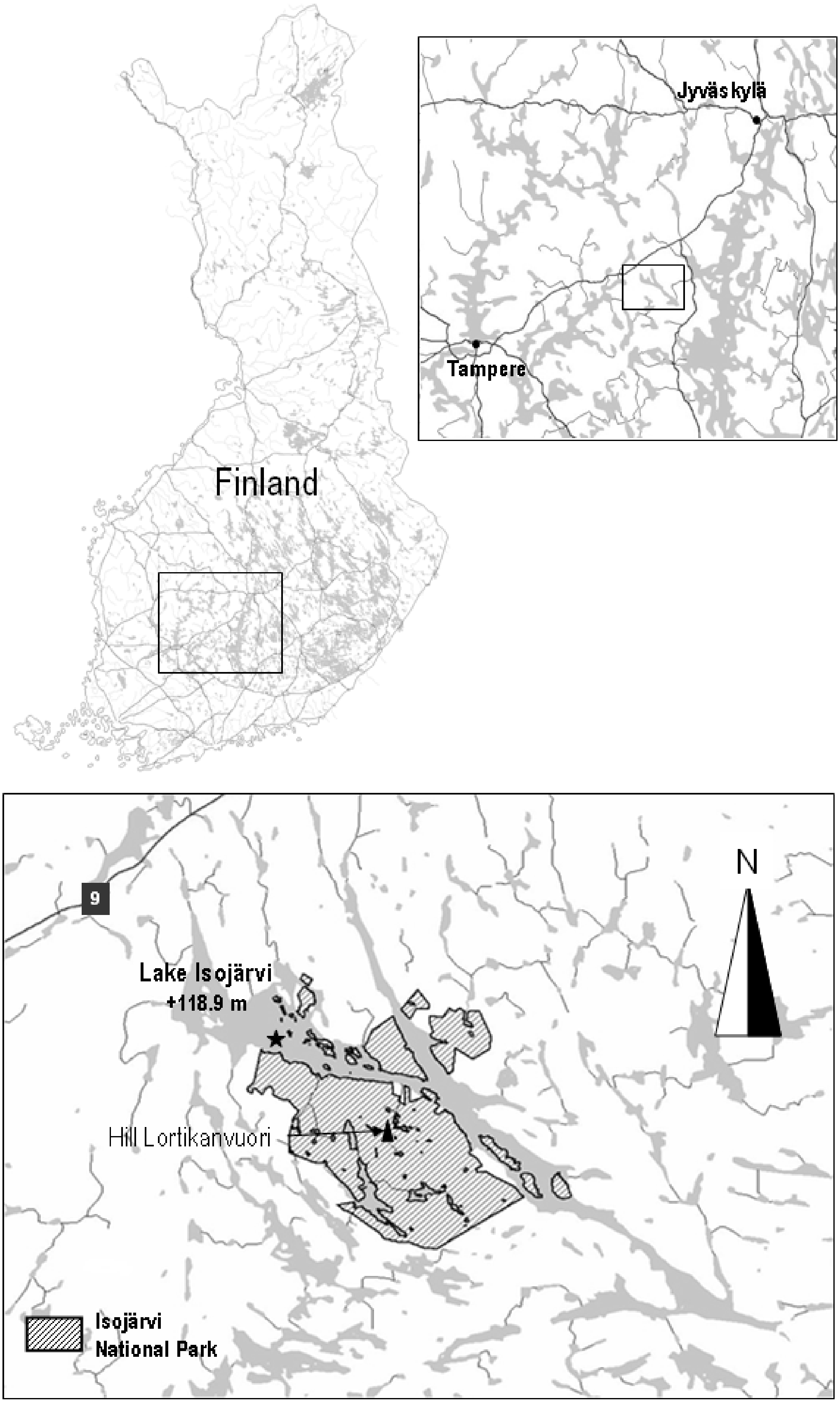

Figure 5. The location of the study site showed with the rectangulars drawn on the map. Lake Isojärvi is partly surrounded by the Isojärvi National Park (diagonal area). The sediment coring site is marked by the star symbol (HERTTA-database). Lake Isojärvi drains to Lake Päijänne north of Hill Lortikanvuori and south of the hill to Lake Längelmävesi (METSÄHALLITUS).

3.1.1. Sediment core characteristics from the previous study

Lake Isojärvi was previously studied by Meriläinen *et al.* (2006), using the same sediment core as that used in this study. Some results from the previous study are summarized below. The carbon content (%) seems to be rather stable (Figure 6), until a marked reduction in carbon concentration at a depth of 10-12 cm, which continues to 4-6 cm. The lowest carbon content is at a depth of 8-10 cm, after which it starts to increase again. The change is visible also in the water content and loss-on-ignition results. The change in the sediment profile indicates that more heavy mineral matter than normal was then being accumulated into the bottom.

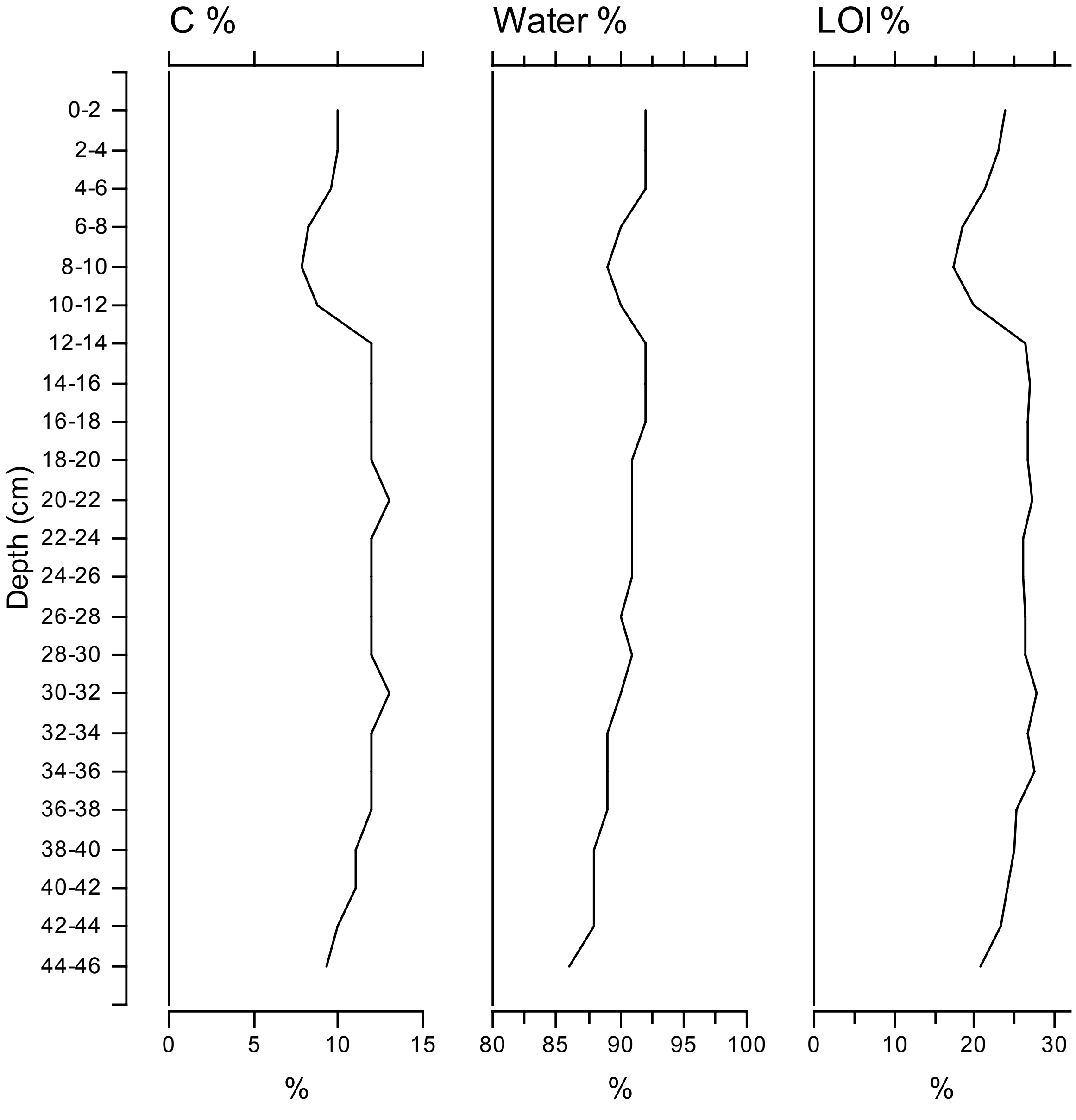

Figure 6. Carbon content (C %), water content (Water %) and loss-on-ignition (LOI %) in the sediment profile of Lake Isojärvi (data from Meriläinen *et al.* 2006).

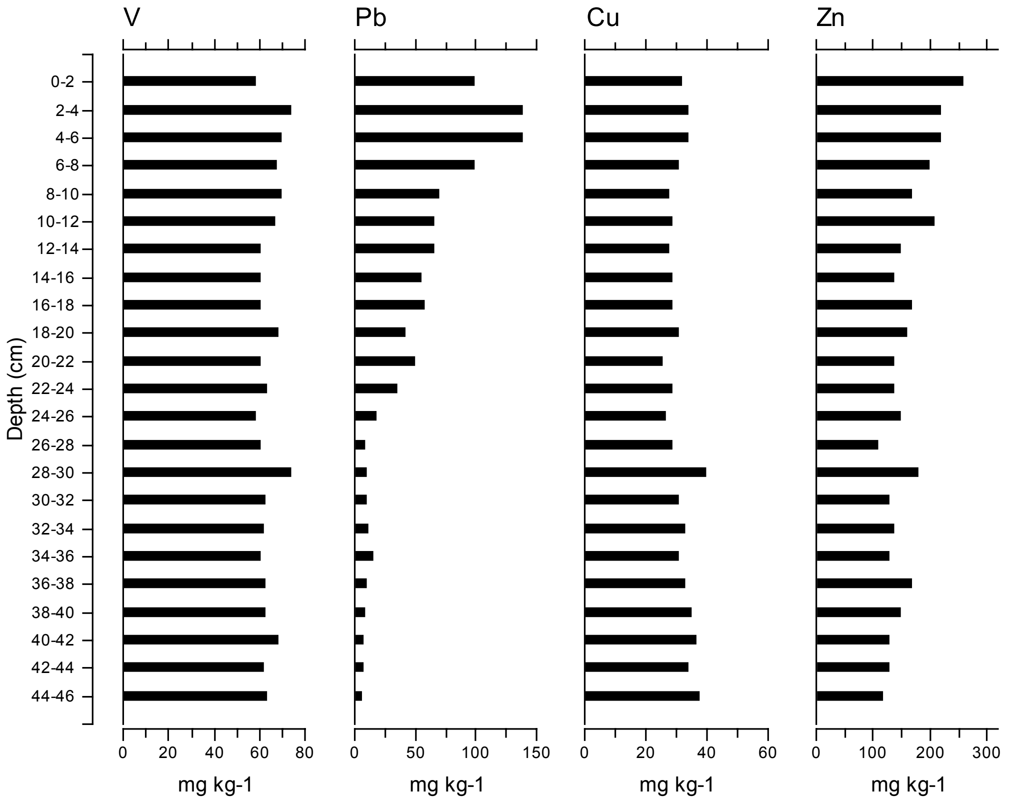

Figure 7. Vanadium (V), lead (Pb), copper (Co) and zinc (Zn) concentrations in the sediment profile of Lake Isojärvi (data from Meriläinen *et al.* 2006).

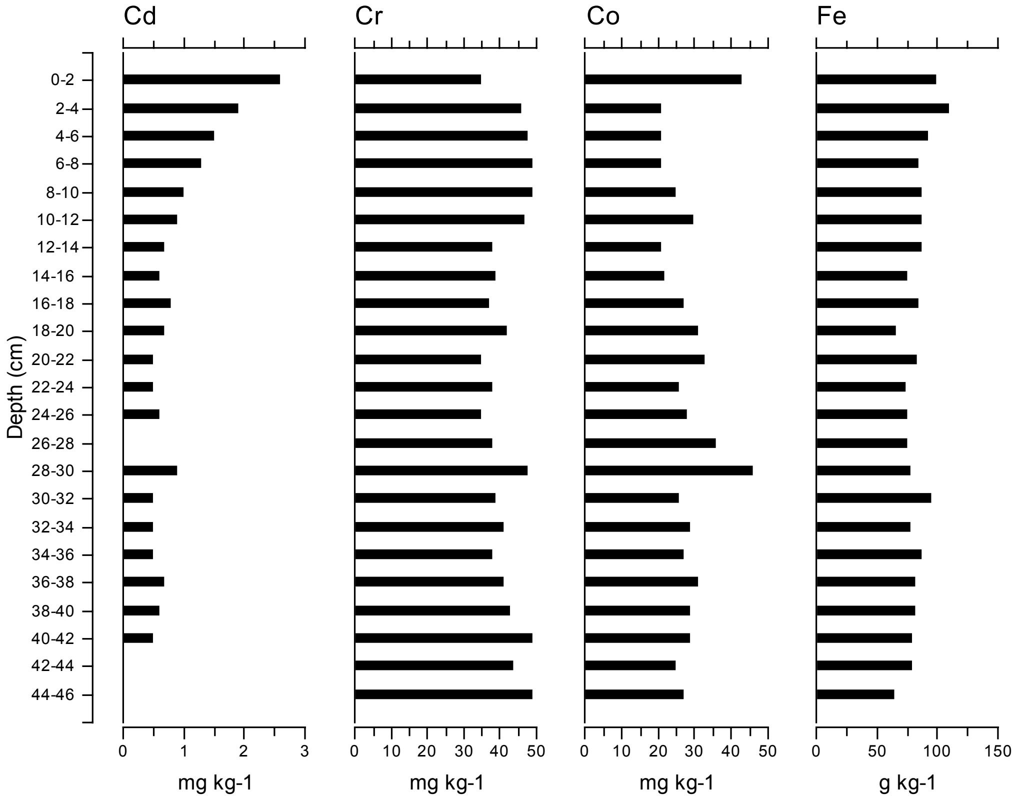

Figure 8. Cadmium (Cd), total chrome (Cr), cobolt (Co) and iron (Fe) concentrations in the sediment profile of Lake Isojärvi (data from Meriläinen *et al.* 2006).

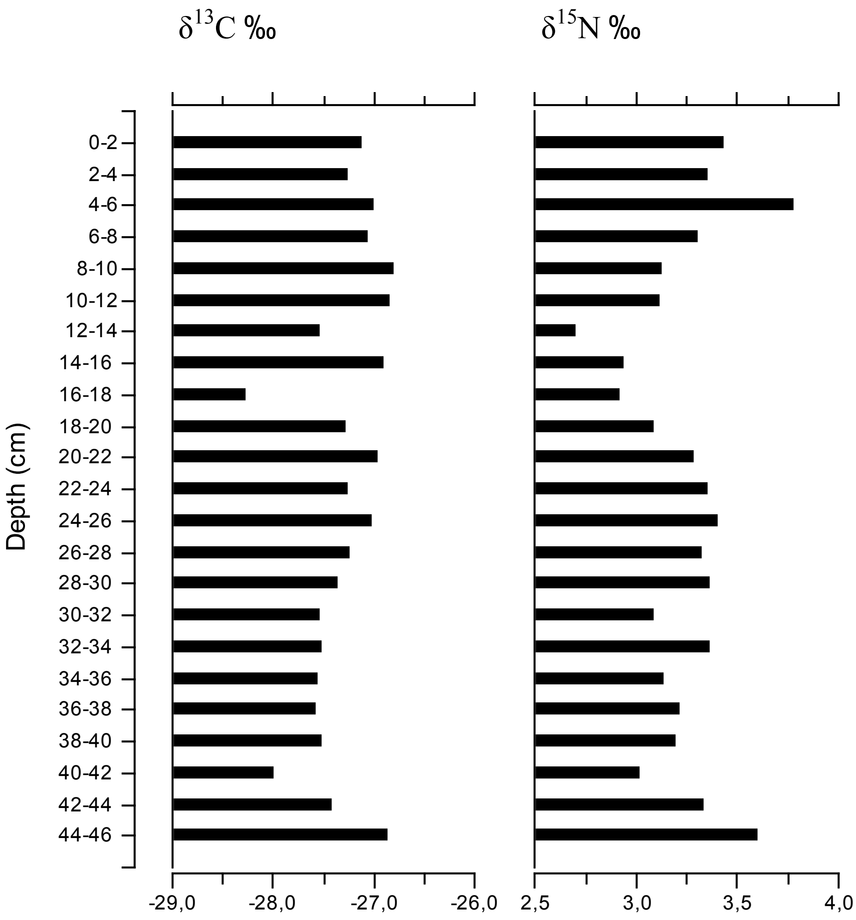

Figure 9. Stable isotope records of carbon ($\delta^{13}C$ ‰) and nitrogen ($\delta^{15}N$ ‰) in the sediment of Lake Isojärvi (data from Meriläinen *et al.* 2006).

The upper layer of the sediment (0-12 cm) is characterised by the elevated Pb, Zn and Cd concentrations (Figure 7 and Figure 8).

According to Meriläinen *et al.* (2006), the variation of $\delta^{15}N$ (Figure 9) in the sediment was 1.1 per mille and it had a strong positive correlation with the content of organic matter and carbon concentration ($r = 0.981$, $p > 0.01$). $\delta^{13}C$ (Figure 9) did not have a significant correlation with any analysed variable. The variation of $\delta^{13}C$ was 1.5 per mille, from -26.8 to -28.3 ‰ (Figure 9).

3.2. Sediment coring

A 46-cm long surface sediment core was collected from a 57 m deep point of the basin with a Kajak type corer on October 2005. The sediment core was sectioned into 23 subsamples of 2 cm intervals. The coordinates of the sampling point are 61° 43’ 21’’ N and 24° 56’ 47’’ E in EUREF-FIN coordinate system, or 6847851 N and 3391599 E in KKJ uniform grid coordinate system (Meriläinen *et al.* 2006).The sampling site is near Isojärvi 4, which is a national sampling site for lake water quality monitoring.

3.3. Diatom analysis

Diatom analysis was performed according to the instructions provided in Battarbee (1986) and Eloranta *et al.* (2007). Instructions regarding the diatom analysis are also provided in standard SFS-EN 13946 (2003).

Subsamples from the consecutive sediment slices were extracted and the organic matter of the subsamples was oxidized with the mixture of 65% HNO_3 and 96-98% H_2SO_4 at 60-80°C. The oxidation of the subsamples continued until the colour of the sediment material was light grey.

After the oxidation procedure, the absence of organic material was confirmed by dropping a few drops of 30% H_2O_2 into the wet digestion suspension. The purpose of H_2O_2 is to remove the organic matter by oxidation, and if any organic matter is left the wet digestion suspension would start to bubble. The oxidized sediment suspensions were washed at least three times with distilled water, until there were no acid anymore in the test tubes. The acid residues in the suspension would react with the resin used in mounting the glass slides and result dimming of the preparate. Between the washes the diatom suspension were let to settle at least 8 h, in order to make sure that no diatoms were lost between the washes.

A drop of the oxidized suspension was dropped on glass slides to dry. The glass slides were mounted with Naphrax® resin, which has a high refractive index. At least 500 frustules were counted from each sample. The counting and identification were done from glass slide preparations at 1000-1250× magnification.

The material was identified according to Mölder and Tynni (1967-1970), Tynni (1975-1980), Houk (2003) and Krammer and Lange-Bertalot (1986-1991) diatom floras. The nomenclature of the diatom follows Krammer and Lange-Bertalot (1986-1991), except for *Aulacoseira distans* var. *humilis* (A. Cleve-Euler) identified according to Houk's (2003) flora.

3.4. Handling of the diatom data

The amounts of different taxa in each subsample were recorded and the total amount of frustules calculated per subsample was calculated using a MS Excel spreadsheet programme. As the number of taxa was relatively low, all taxa were included to the sum. According to Battarbee *et al.* (2002), it is sometimes useful to exclude some groups from the counts, in case some taxa are particularly dominant and hence valuable information might be lost when the variation of less abundant taxa stays unnoticed.

MS Excel spreadsheet software was used to process the data into a suitable format to be able to analyze the data with the other software. OMNIDIA version 4.2 (Lecointe *et al.* 1993) diatom database software was used to calculate the relative proportions of different trophic status and pH level indicator species.

The reconstructions of the lake water pH, total phosphorus and total organic carbon (TOC) concentrations based on the diatom assemblages, counted from the sediment samples, were done by using the European Diatom Database (EDDI) and ERNIE 1.0 software (EDDI, Juggins 2001). The species data was processed into a suitable format by using WinTran Version 1.5 (EDDI).

Lake water pH reconstruction was calculated by using The Surface Waters Acidification Programme (SWAP) calibration set and weighted averaging (WA) calculation method (EDDI). Total organic carbon (TOC) and total phosphorus (TP) reconstructions were calculated by using combined pH and combined TP datasets respectively, (EDDI) and locally-weighted weighted averaging (LWWA) calculation method (Table 4).

According to Juggins (2001), comparisons suggest that the LWWA method is more effective compared to the other available methods in EDDI, e.g. weighted averaging partial least squares (WAPLS), modern analog technique (MAT), and weighted averaging (WA). For pH reconstruction, only the WA method was available (EDDI).

Where the reconstructed values lie close to the mean of the training set values, inverse deshrinking is recommended as it gives an overall lower root mean squared error (RMSE) prediction. Where the reconstructed values lay towards the ends the ends of the gradient sampled by the training set, classical deshrinking is recommended, as it produces more accurate reconstructions at the gradient ends (Juggins 2001).

Table 4. Information about the datasets and numerical methods used in reconstructing the environmental variables from the sedimentary diatoms from Lake Isojärvi.

Reconstructed environmental variable	**pH**	**TP**	**TOC**
Dataset	SWAP	Combined TP dataset	Combined pH dataset
Range of the variable in the dataset, mean value in brackets	4.3-7.3 (5.6)	2-1189 (99) µg l^{-1}	0.1-20 (4.1) mg l^{-1}
Transfer function performance			
RMSE	0.344	0.306	2.13
r-squared	0.82	0.696	0.47
max bias	0.157	0.555	7.15
Number of taxa in the dataset	381	345	536
Numerical method used in the reconstruction	WA (classical deshrinking)	LWWA (classical deshrinking)	LWWA (inversed deshrinking)

In addition, total phosphorus and colour reconstructions were calculated by Juha Miettinen (pers. comm.) also by using a weighted averaging (WA) calibration data set (Miettinen 2003), which consists of 78 lakes in eastern Finland.

A detrended correspondence analysis (DCA) was run with the diatom data in order to choose between unimodal and linear techniques. According to the DCA, the length of the gradient (Axis 1) is 0.769. Birks (1998) recommends using linear techniques when the length of the gradient is less than 2. Principal component analysis (PCA) was chosen to be used for community analysis of the diatom data.

Multivariate analyses (PCA and DCA) of the diatom and other environmental data were performed with PC-ORD for Windows version 4.41 (McCune & Mefford 1999) and with C2 software version 1.5.0 (Juggins 2007). The stratigraphic curves have been processed by using C2 software for ecological and palaeoecological data analysis and visualisation (Juggins 2007) and MS Excel spreadsheet software.

4. RESULTS

4.1. Sedimentary diatoms

Cyclotella rossii (Grunow) Håkansson and *Aulacoseira subarctica* (O. Müller) Haworth were the most abundant taxa observed in the sediment samples of Lake Isojärvi (*see* Appendix 2), respectively comprising 22.0-32.4 % and 25.7-40.5 % from the total amount of counted frustules per sample (Figure 10). Two different forms of *A. subarctica* were observed, the form with short frustules being the most common. The relative abundance of *A. subarctica* with tall (high-celled) frustules increases towards the surface sediment beginning from a depth of 12 cm. A total of 108 diatom taxa were identified in the samples from Lake Isojärvi (Appendix 1).

Other common taxa were *Asterionella formosa* Hassall, *Cyclotella* spp. (*C. radiosa* Grunow and *C. pseudostelligera* Hustedt), *Tabellaria flocculosa* (Roth) Kützing, *Tabellaria flocculosa var. flocculosa* (Roth) Kützing, *Aulacoseira* spp., and *Achnanthes minutissima* Kützing.

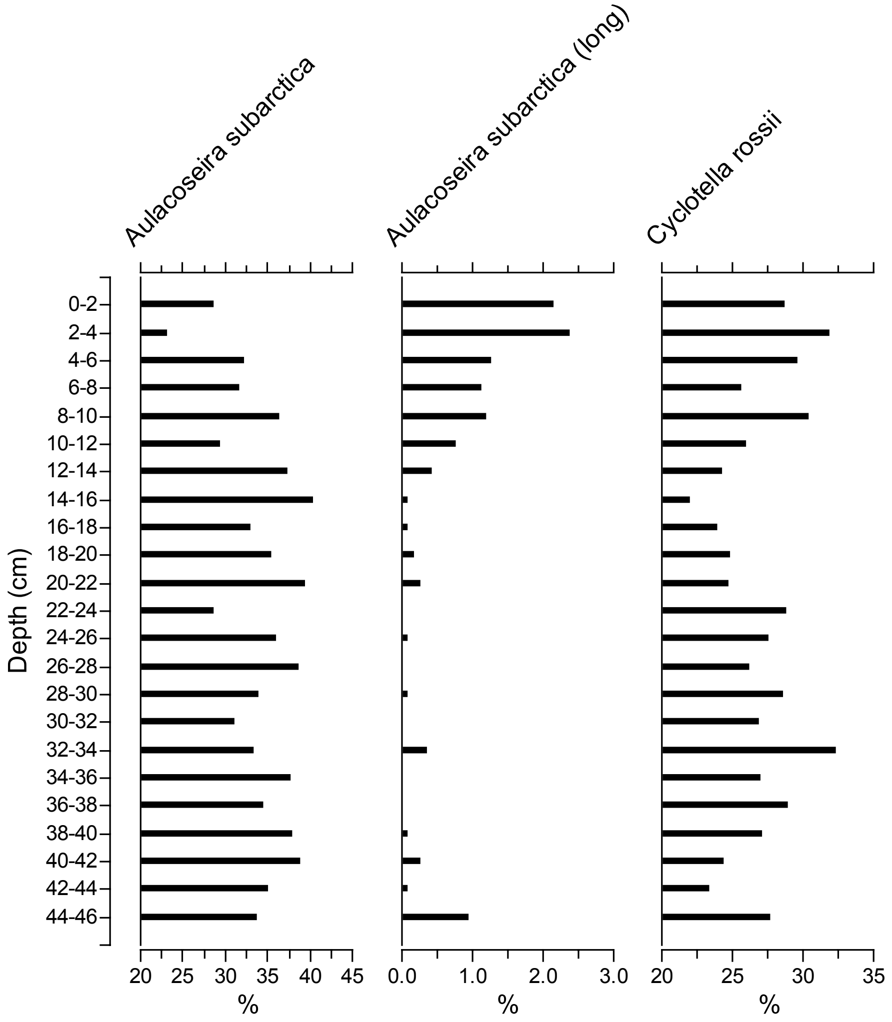

Figure 10. Stratigraphy of the most abundant diatom taxa *Aulacoseira subarctica* (O. Müller) Haworth and *Cyclotella rossii* Håkansson in Lake Isojärvi. Short and tall (high-celled) forms of *Aulacoseira subarctica* were observed in the samples, the first stratigraphy showing both short and long forms and the second showing only the long form.

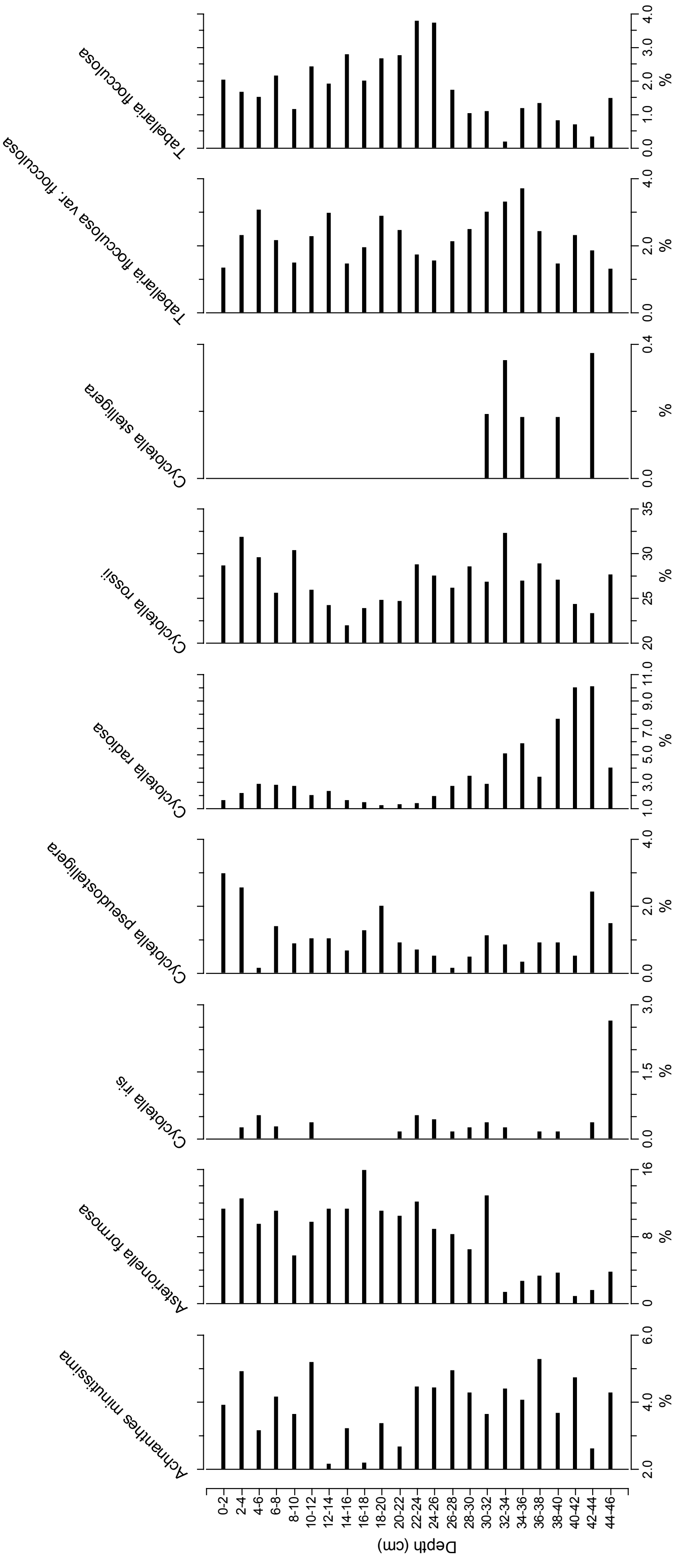

Figure 11. Stratigraphy of some selected relatively common diatom taxa in Lake Isojärvi.

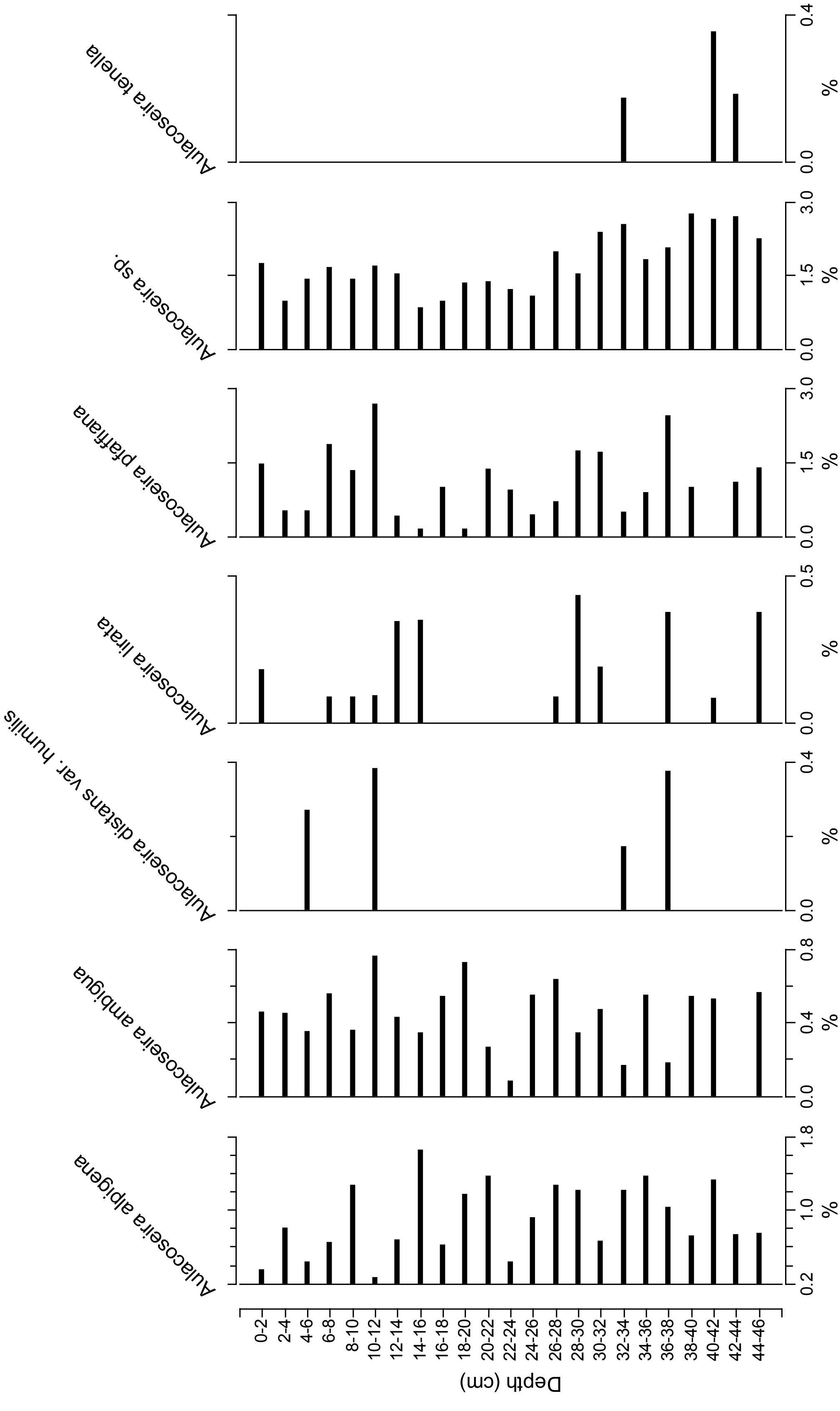

Figure 12. Stratigraphy of *Aulacoseira spp.* in Lake Isojärvi.

4.2. pH, colour, TOC and TP reconstructions

The European Diatom Database (EDDI) transfer functions based on the SWAP dataset, combined TP dataset and combined pH dataset were used for inferring pH, total phosphorus (TP) and total organic carbon (TOC) respectively. According to the functions, TOC has varied between 7.0 and 9.2 mg l^{-1} and pH between 6.2 and 7.2 (Figure 13). For TP, the model gave values, which varied between 1.0 and 1.2 µg l^{-1}. A slight increase in pH is visible from the sample at 32-34 cm on.

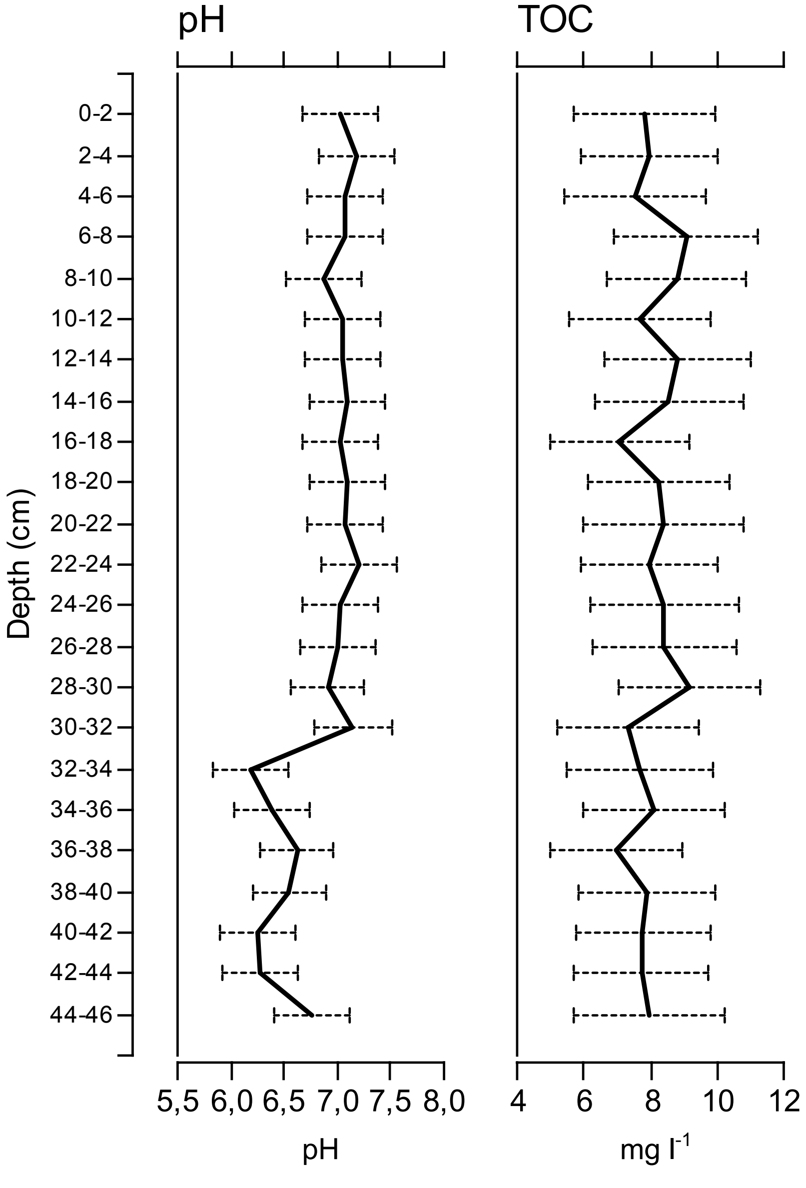

Figure 13. pH and total organic carbon (TOC, mg l^{-1}) concentrations inferred from the diatom record of the sediment core from Lake Isojärvi. Reconstructions are based on the calibration data sets and diatom data sets in the European Diatom Database (EDDI). The dashed lines represent the standard error of the reconstructions.

According to the transfer functions by Miettinen (2003) used for the total phosphorus (TP) concentration and colour reconstructions, phosphorus concentration has been varied between 3.3 and 5.4 µg l^{-1} and colour between 13.8 and 28.2 mg Pt l^{-1} in Lake Isojärvi

(Figure 14). Water colour and TP concentrations seem to have been relatively stable, excluding the period described by the samples from 12 cm to 22 cm, where there is a visible increase in both of the variables.

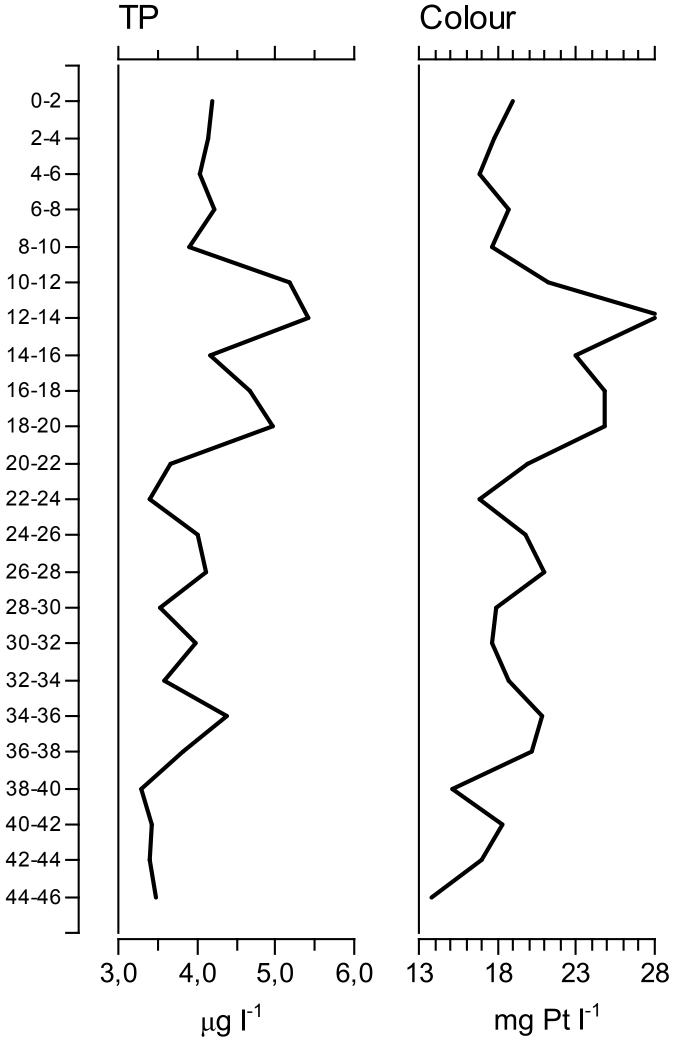

Figure 14. Total phosphorus concentrations (μg l^{-1}) and colour (mg Pt l^{-1}) inferred from the diatom record of the sediment core from Lake Isojärvi. Reconstructions are based on the calibration data sets and diatom data sets of Miettinen (2003).

4.3. Ecological indicator values

According to the trophic status indicator value calculated based on the observed diatom assemblages in the bottom sediment, Lake Isojärvi was oligo-mesotrophic in the beginning of 1700s, which is estimated to be represented by the lowermost sediment sample at the depth of 44-46 cm (Figure 15) and. according to the trophic status indicator by van Dam *et al.* (1994), the lake has remained approximately the same ever since.

The majority of the diatoms were acidophilous or circumneutral (after van Dam *et al.* 1994) in the bottom sediment of Lake Isojärvi, i.e. majority of the diatoms had their optimum for occurrence at pH less than 7 or at pH values approximately 7 (Figure 16). Although there is some variation in the proportions when comparing younger and older sediments, the situation remains substantially the same.

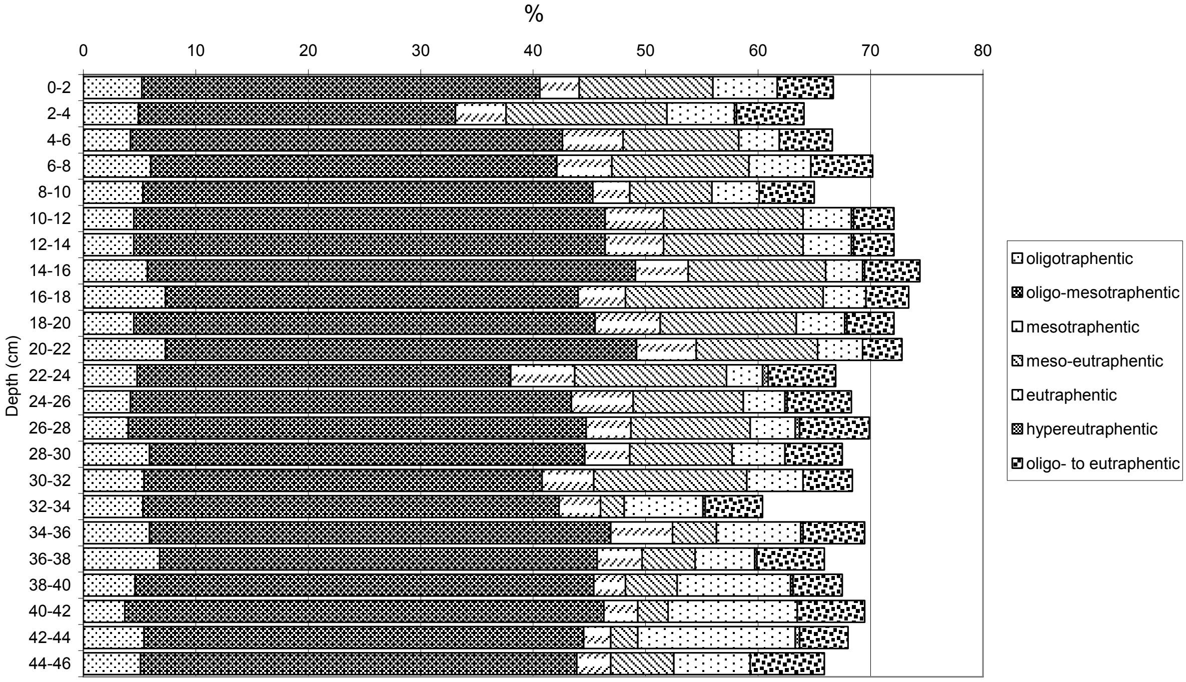

Figure 15. Ecological indicator values for trophic state inferred from the diatom record in the bottom sediment core from Lake Isojärvi, calculated with OMNIDIA software (Lecointe *et al.* 1993, van Dam *et al.* 1994).

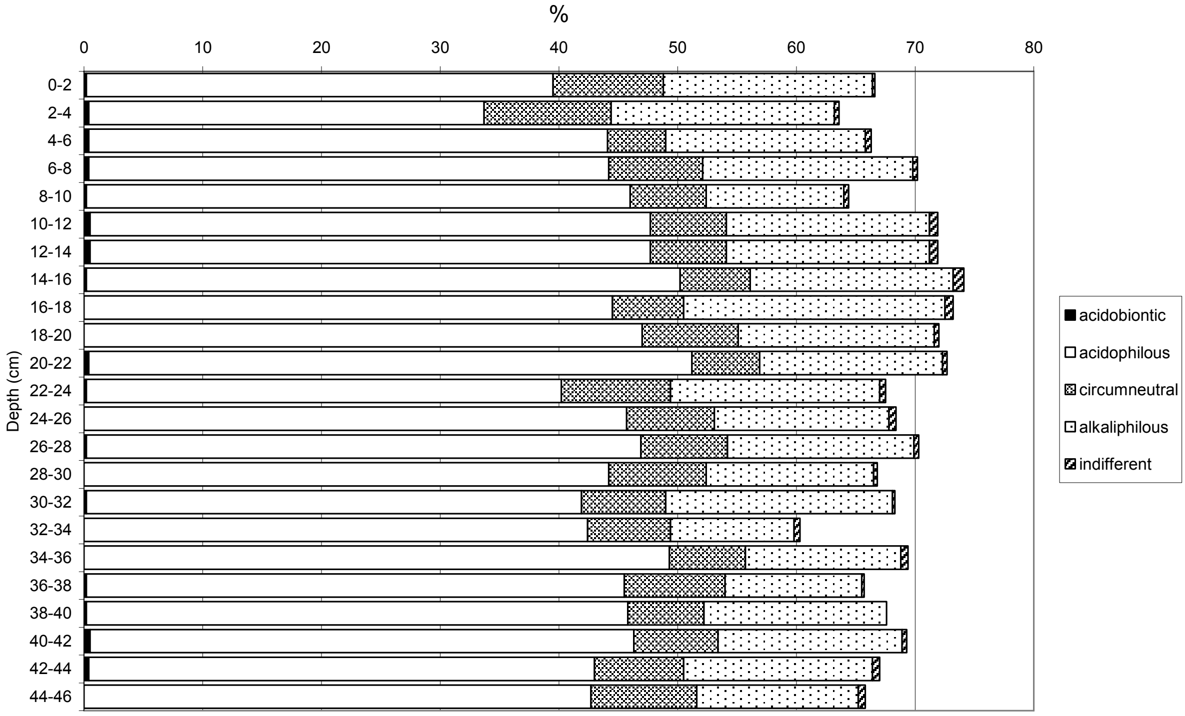

Figure 16. Proportions of the diatom taxa with different pH indicator values in the bottom sediment of Lake Isojärvi, calculated with OMNIDIA software (Lecointe *et al.* 1993). No alkalibiontic taxa were observed and hence it is excluded from the legend (van Dam *et al.* 1994).

4.4. Multivariate analyses

PCA ordination did not distinguish diatom assemblages into clear clusters (Figure 17). However, some differences between the diatom assemblages can be seen when consecutive sediment samples are separated into following clusters: 32-46 cm, 22-32 cm, 12-22 cm, and 0-12 cm.

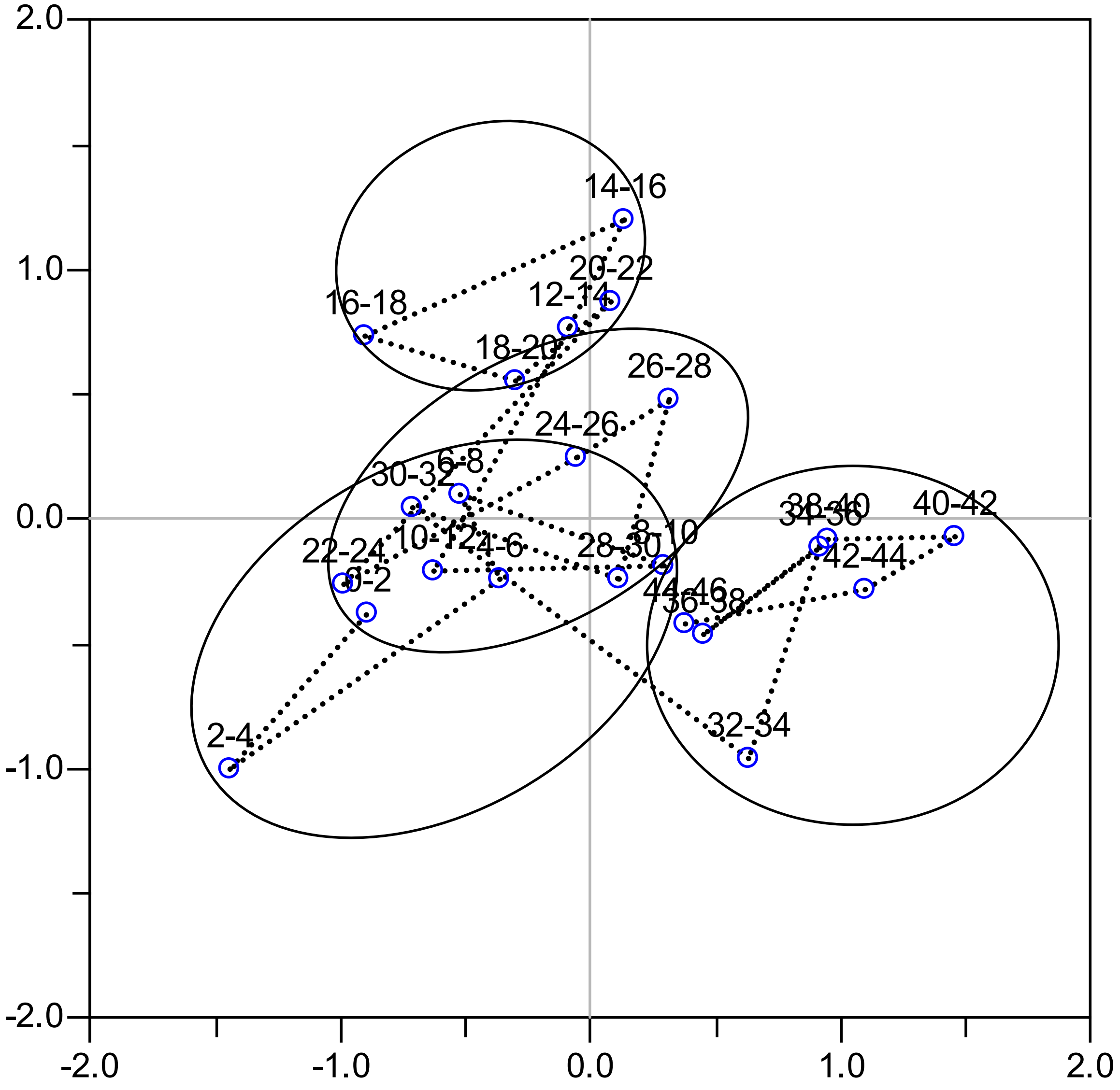

Figure 17. PCA ordination for sedimentary diatoms in Lake Isojärvi (no transformation; eigenvalue for Axis 1 0.52 and for Axis 2 0.29).

5. DISCUSSION

5.1. Sedimentary diatoms

The diversity of different diatom taxa in Lake Isojärvi is relatively low, as only 108 taxa were observed in the samples. One plausible reason for the low amount of diatom taxa observed is inexperience in identifying the diatoms. However, Meriläinen *et al.* (2006) observed 77 diatom taxa in the three samples they studied, which indicates that the 108 taxa observed in this study is not far from the experienced diatom identifier's opinion. On the other hand, 23 samples were studied in this study, compared to the 3 samples studied by Meriläinen *et al.* (2006), due to which the probability for detecting more species is also higher.

Kukkonen (2004) studied Lake Pyhäjärvi, located in Karelia, Eastern Finland, and observed approximately 200 diatom taxa in the studied sediment samples. Lake Pyhäjärvi is an oligotrophic and clear lake, and can thus be compared to Lake Isojärvi. However, Lake Pyhäjärvi has bigger lake area than Lake Isojärvi, which might also contribute to the overall diversity of occurring diatom taxa. *Cyclotella rossii* and *Aulacoseira subarctica* were found to be the dominant species in the samples (Figure 10, Appendix 2). *A. subarctica* is a filamentous, acidophilous and typical pelagic species for oligo-mesotrophic and cold waters (Krammer & Lange-Bertalot 1991, van Dam *et al.* 1994, Gibson *et al.* 2003, Houk 2003). *Cyclotella* spp. are typically planktonic and often referred to as indicator species for oligotrophic conditions, although this is not always the case (Round *et al.* 1990, Wetzel 2001). In fact, *C. pseudostelligera* and *C. radiosa* have both been classified as taxa, which tolerate nutrient enrichment (eutraphentic) by van Dam *et al.* (1994).

Cremer & Wagner (2004) observed that *Cyclotella rossii* is a common to dominant component in the clear, oligotrophic lakes in East Greenland. The description of the environmental conditions where *C. rossii* thrives, is also true for Lake Isojärvi. Approximately 40 % of the observed diatom flora of some sediment samples from Lake Pyhäjärvi, consisted of *C. kuetzingia* and *C. rossii*. Furthermore, also *Aulacoseira subarctica* was observed by Kukkonen (2004) in Lake Pyhäjärvi. Rühland *et al.* (2003) studied ecology and spatial distributions of surface-sediment diatoms from 77 lakes, located in the subarctic Canada, and used DIC, total nitrogen (TN), DOC, depth and SiO_2 as variables. They found that *C. rossii* was less abundant in lakes with higher DIC concentrations and clearly absent in lakes with higher TN concentrations. Bigler *et al.* (2000) constructed a diatom-training set from lakes in northern Sweden, and found *C. rossii*, which is a taxa indicative of more alkaline waters, having its calculatory optimum pH and temperature > 7.26 and 13.0°C, respectively. For *A. subarctica* the calculatory optimum pH and temperature were 6.96 and 12.3°C, respectively. Also data collected by Korhola *et al.* (1999) suggested more alkaline sites showing the dominance of *C. rossii*, and, furthermore, according to their study the high abundance of *Cyclotella* spp. is typical for deeper lakes in Finnish Lapland. Weckström *et al.* (1997) studied diatoms as quantitative indicators of pH and temperature in subarctic Fennoscandian lakes, and found *C. rossii* to prefer cold waters, the weighted-average temperature and pH optima being 10.3°C and 7.20, respectively. According to Gibson *et al.* (2003), *A. subarctica* appears as a response to moderate increases in nutrients, but is disadvantaged by further enrichment.

The other relatively common species observed in the sediment samples of Lake Isojärvi, were *Asterionella formosa*, *Achnanthes minutissima*, and *Tabellaria* spp. (Figure

11). *Asterionella formosa* is a planktonic species, which is alkaliphilous and indicates meso-eutrophic conditions (van Dam *et al.* 1994, Kingston 2003). *Tabellaria flocculosa* is acidophilous and indicates mesotrophic conditions. *Achnanthes minutissima* is circumneutral and thrives in oligo- to eutrophic conditions (van Dam *et al.* 1994). In addition, low number of several different *Eunotia* spp. were observed in the samples. Many of *Eunotia* spp. are indicating acidic conditions (van Dam *et al.* 1994, DeNicola 2000).

The trophic status classes of van Dam *et al.* (1994) have not been defined for all diatoms, due to which less than 100 % of the diatoms can be classified, which can introduce errors into the interpretation of the results. As the results of the diatom analyses can differ between different persons counting and identifying the diatoms, the proportions of different trophic status and pH classes can also differ between different studies. Of the diatoms observed in the samples in this study, the environmental indicator values were not defined for all taxa, which makes the results less reliable.

The proportions of the pH indicators were consistent with Meriläinen *et al.* (2006), indicating the majority of the diatoms in the sediment of Lake Isojärvi to be acidophilous or circumneutral (Figure 16).

The pH, TP, TOC and colour reconstructions made on the basis of the results from the diatom analyses (Figure 13 and Figure 14) show that there has not been any dramatic change in the lake water chemistry. Interestingly, a small change with increasing trends in the reconstructed water colour and TP concentrations is visible at the depth of approximately 12-24 cm in the stratigraphy, based on Miettinen's (2003) models (Figure 14). This change might be due to the past logging activities within the catchment of Lake Isojärvi, during which nutrients from the soil were flushed to the lake with rain and melt water. Eventually, the soil has become nutrient poor and the TP concentrations have started to decrease. Consequently, as the soil has become nutrient poor it has started to erode, which is seen as increased minerogenic matter in the sediment (Figure 6).

The water quality data analysed from Lake Isojärvi (Table 3) in 2005 from the same location as the sedimentary core was taken, show that the pH has varied between 6.4 and 7, water colour between 30 and 35 mg Pt l^{-1}, and TP between 4 and 5 µg l^{-1}. According to the water quality data available from HERTTA-database, water colour in Lake Isojärvi's other sampling sites, has generally been 30 mg Pt l^{-1} and less. The results of the transfer functions are relatively well in line with the water quality analyses in 2005. For the topmost sediment layer (0-2 cm), which represents the time of sampling (2005), the model of Miettinen (2003) gave 4.2 µg l^{-1} and 19 mg Pt l^{-1} for TP and colour respectively. According to the EDDI transfer function, the pH was calculated to be 7.0, based on the diatom assemblage in the topmost subsample.

When comparing the results of this study to the results by Meriläinen *et al.* (2006), the trophic status of Lake Isojärvi was found to be a little different. One plausible reason for this might a different version of the OMNIDIA software, as Meriläinen *et al.* (2006) used an old version of the software in their study and the most recent one was used in this study. Also the proportion of the taxa represented in the OMNIDIA software was higher in the study by Meriläinen *et al.* (2006) as over 80 % of the identified diatoms had been defined in the OMNIDIA. In this study, the proportion of the identified taxa defined in the OMNIDIA software was less than 80 % in all samples. Another reason for the difference is an obvious inexperience in identifying the diatoms. However, the results of both studies are consistent with each other in the sense that no marked trophic status changes were detected (Figure 15).

Other examples of the impacts on lakes of logging are provided by Laird & Cumming (2001), Laird *et al.* (2001), and Räsänen *et al.* (2007). In all of the aforementioned studies the changes of the diatom assemblages in the impacted lakes were small shifts in percent abundance, rather the appearance or disappearance of diatom taxa. They also suggested on the grounds of their studies that diatom assemblages reflect the changes after logging more reasonably than sediment chemistry, which is in line with the results from Lake Isojärvi.

5.2. Remarks on dating of the sediment core and detected changes

Dating of the sediment was not done in this study, even though time is one of the most important dimensions as it gives the time frame for paleolimnological studies. However, it is possible to estimate the age of the sediment using the concentration of Pb in the sediment samples (Figure 7). Meriläinen *et al.* (2006) estimated the age by using a study by Brännvall *et al.* (2001), which showed that a clear and lasting increase in Pb concentrations took place between the 1800s and 1900s. According to Meriläinen *et al.* (2006), another significant increase of Pb concentrations took place in the 1970s, when the use of leaded gasoline was at its highest. On the grounds of Pb accumulation in sediments, Meriläinen *et al.* (2006) concluded that the year 1970 corresponds approximately to a sediment depth of 4-6 cm.

It is commonly assumed that the Industrial Revolution in the 1800s was a clear period for atmospheric Pb pollution. According to Brännvall *et al.* (2001), Pb levels were elevated then, but not as strikingly as after the increase in fossil fuel combustion around the 1950s. Verta *et al.* (1989) observed the start of anthropogenic accumulation of Pb to be evident in all of the studied sediment cores from lakes in southern Finland, whereas in northern Finland, anthropogenic Pb appears during the 1800s. In addition, the Pb concentration profiles were observed to level off or decrease after 1980.

Figure 18. Sales of unleaded and leaded gasoline in Finland between 1970 and 2000 (solid line = sales of leaded gasoline, dotted line = sales of unleaded gasoline) (data from Mr. Jyrki Pohjolainen, Finnish Oil and Gas Federation).

On the grounds of the studies by Verta *et al.* (1989) and Brännvall *et al.* (2001), Meriläinen *et al.* (2006) estimated the year 1900 to be at a depth of approximately 18 cm and the bottom of the core (46 cm) to correspond approximately to the beginning of the 1700s. The sales statistics of gasoline (Finnish Oil and Gas Federation) in Finland support the observations of Meriläinen *et al.* (2006) and Verta *et al.* (1986): the amount of sold leaded gasoline started to decrease in 1988 and after 1995 no leaded gasoline was sold anymore in Finland (Figure 18).

The water content of the sediments in shallow waters tends to be lower, due to the coarser materials, and *vice versa* higher in deeper waters. Due to compaction of the sediment, the water content decreases with depth in a sediment core and the vertical variation can be caused by various factors including the rate of sedimentation and the degrees of compaction and bioturbation (Bengtsson & Enell 1986). Meriläinen *et al.* (2006) assumed the carbon and water content changes in the sediments to have been caused by erosion within the catchment area. Based on the estimated age of the sediment, the changes are estimated to have taken place between the 1930s and 1970s due to logging activities.

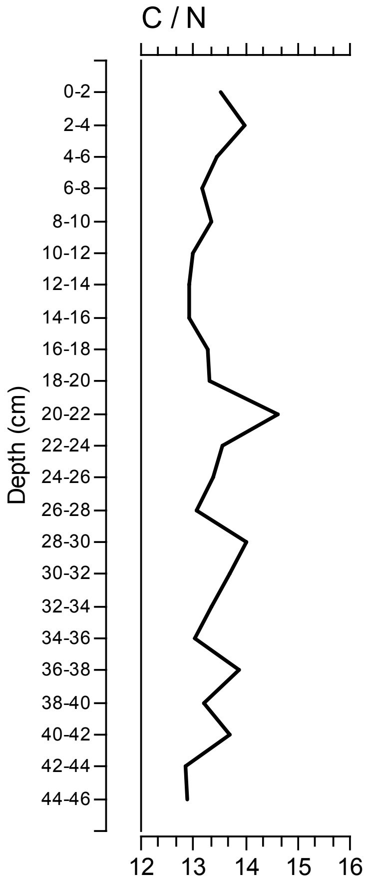

Figure 19. Carbon-nitrogen ratio (C/N ratio) in the sediment profile of Lake Isojärvi (data from Meriläinen *et al.* 2006).

According to the stratigraphy of TP concentrations and water colour (Figure 14), inferred based on Miettinen's (2003) model, a change was observed in the depth of 12-24 cm. According to Meriläinen *et al.* (2006), the year 1900 is approximately in the depth of 18 cm. According to METSÄHALLITUS, the first extensive loggings were carried out around the 1880s, and, in addition, the last extensive forest fire took place in 1850. Thus, assuming the dating is approximately correct, the beginning of extensive logging corresponds approximately to the same time as the change in the TP and water colour stratigraphy is seen (Figure 14). This assumption is further underpinned when looking the stratigraphy of the C/N ratio of the sediment (Figure 19), where the C/N ratio stays somewhat lower and stable after the sample from depth 20-22 cm till the depth of 10-12 cm, compared to the deeper parts of the core. For example, Räsänen *et al.* (2007) observed decreasing C/N ratio (the proportion of allochthonic matter increased) in one of the studied lakes, which they suggested to be a sign of increased erosion caused by the forest harvesting. According to Cohen (2003), the C/N ratio is a useful tool for distinguishing long-term transitions between terrestrial to algal-input dominance in lake sediment organic matter, but should be used carefully as the early diagenetic loss of N sometimes elevates the C/N ratio. In addition, the extensive forest fire might have contributed to the observed change. However, according MacDonald *et al.* 1991, fires of limited spatial extent and intensity are not necessarily detected in the sediment record.

The variation in nitrogen stable isotope ratios have a strong positive correlation between the organic matter content and carbon content of the sediment (Meriläinen *et al.* 2006). Although there are several caveats in making the interpretations on the basis of the changes of δ^{15}N, changes in nitrogen stable isotope ratios are often reflected in sedimentary profiles of plant material from terrestrial versus aquatic sources (Cohen 2003). Due to the erosion, more autochthonous, terrestrial matter has accumulated into the sediment and this might be seen as a correlation between organic matter content and carbon content of the sediment. Naturally this would also be seen in δ^{13}C values, as strong fractionation mechanisms are affecting the isotopic composition of terrestrial plants already during their photosynthesis, which eventually is seen in the isotopic composition of the plant debris entering a lake versus the isotopic composition of autochthonous matter.

The variation in carbon's stable isotope ratios did not have significant correlation between any of the studied variables (Meriläinen *et al.* 2006), which is probably due to the rather complicated fractionation pathways of carbon isotopes in lakes (Cohen 2003). However, it is good to remember that the fractionation pathways of nitrogen isotopes are also complicated (Cohen 2003), and thus also the interpretation of the isotope results can be a complex task.

The TP, TOC, colour and pH reconstructions were made by using different calibration data sets (EDDI and Miettinen 2003). The TOC and pH reconstructions made with the calibration data sets available from the EDDI database are generally in line with the results calculated with the dataset of Miettinen (2003). However, the TP reconstruction made with the EDDI transfer function showed considerably low values (1.0-1.2 µg l^{-1}), which appear to be over the range of the variable in the used dataset (Table 4). According to the study by Meriläinen *et al.* (2006), the TP values were 3, 6 and 5 µg l^{-1}, at the depths of 44-46 cm, 8-10 cm, and 0-2 cm respectively. The TP values calculated based on the diatom calculations in this study cannot be used, as they are under the range of the variable in the EDDI's dataset. Instead, the TP reconstruction made with the transfer function of Miettinen (2003) can be considered a more justified alternative.

There are some obvious sources of error, which should be taken into account when assessing the reconstructions. The most significant error is related to the identification of the diatoms, which is commonly considered a difficult process. The author of this study was unfamiliar with the identification process at the beginning, thus making the misidentification more probable. Also making the diatom preparations was initially an unfamiliar process, thus adding the possibility of errors already in the sample preparations. However, the list of identified diatom taxa from the study by Meriläinen *et al.* (2006) was available during the identification process, which probably decreases the amount of possible misidentifications made during the analysis.

In the previous study by Meriläinen *et al.* (2006) it was concluded that Lake Isojärvi is suitable for use as a reference lake in the WFD. In addition, they detected some changes in the geochemical record of the sediment core, which was assumed to be caused by erosion within the catchment of the lake. This accord well with the historical information about forestry activities within the area. However, Meriläinen *et al.* (2006) concluded that the change, which is clearly visible in the loss-on-ignition, evaporation residue carbon concentration stratigraphy, has not affected the ecological status of the lake. The extended results from this study support the idea that Lake Isojärvi in its natural state and therefore could be used as a reference lake in implementing the WFD.

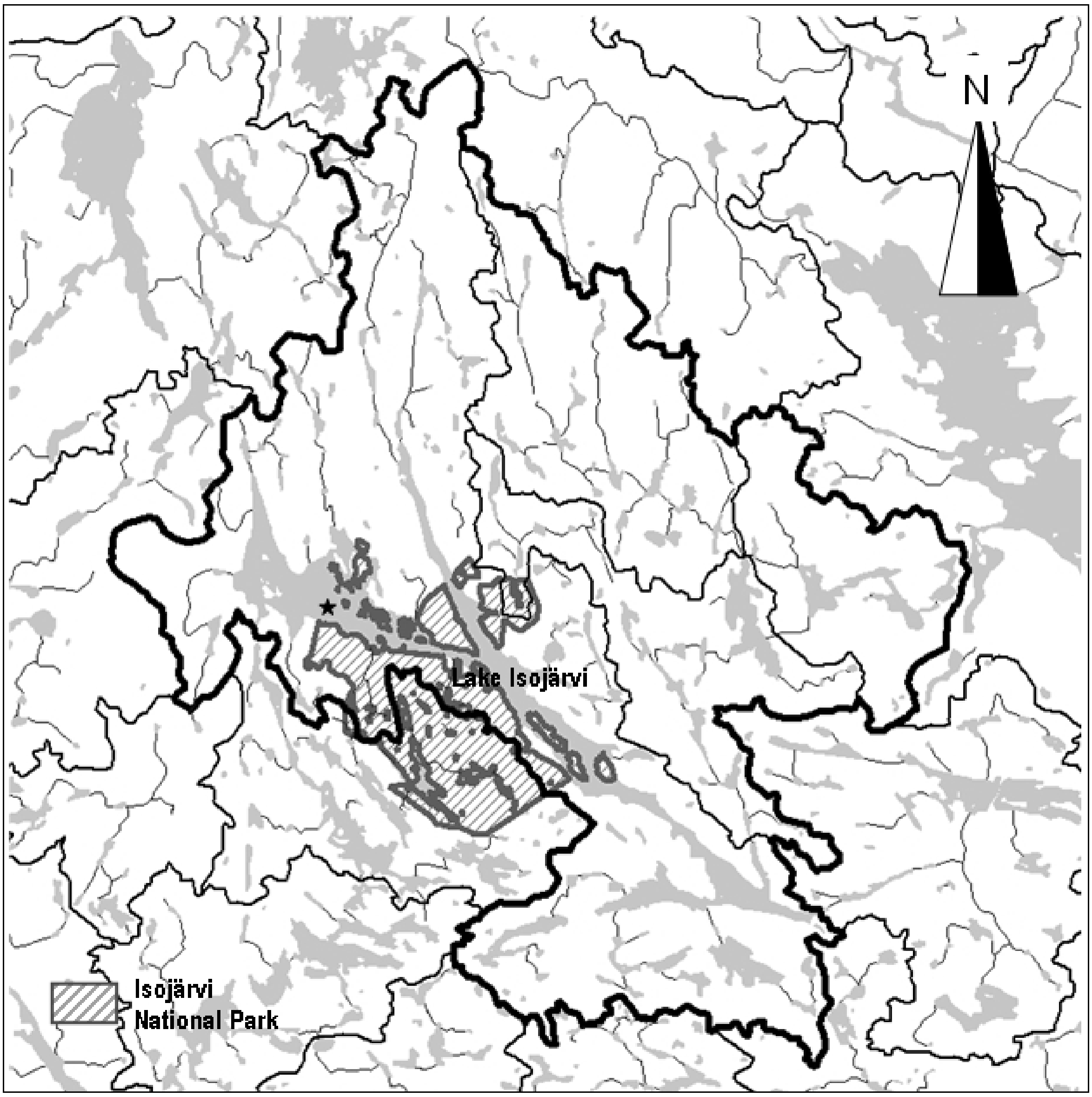

Figure 20. The catchment area of Lake Isojärvi (thin line) is a part of a bigger protected catchment area (thick line). Isojärvi National Park partly covers the catchment area of Lake Isojärvi (diagonal area). Grey areas represent water courses (map from HERTTA database).

Another important thing in assessing the suitability of Lake Isojärvi for use as a reference lake is its location within a protected catchment area (HERTTA-database)

(Figure 20). Furthermore, the lake is partly surrounded by Isojärvi National Park. According to Suominen *et al.* (2002), the forests around Lake Isojärvi have been mainly in forestry use and harvesting has been done by using light selection felling and no extensive logging operations have been taken place. These things together make the area also interesting to be used as a palaeoecological reference area (cf. Berglund 1986b).

At the same time it is noteworthy that, even in a seemingly remote and unaffected lake such as Lake Isojärvi, the signs of long-range transport of airborne pollutants can be clearly visible (Pb, Zn, Cd, and As) (Figure 7 and Figure 8). Brännvall *et al.* 2001 have detected changes in Pb concentrations in the sediment profiles in Sweden, which date back all the way to the time of the Roman Empire, which illustrates how far atmospheric pollution can range.

5.3. Multivariate analyses

Meriläinen *et al.* (2006) ran a PCA for the elements and the stable isotope ratios of carbon and nitrogen. The analysis divided the sediment profile into two clusters: the top 12 cm and the older sediment (12-46 cm). The profile of the surface sediment is characterized by elevated levels of Pb, Cd, Zn, and As.

The PCA run with the diatom data (Figure 17) shows the diatom assemblages analyzed at different depths of the sediment profile to be relatively close to each other and no clear clusters can be observed, although some changes are evident.

5.4. Other studies discussing diatoms, paleolimnology and WFD

Bennion *et al.* (2004) examined the fossil diatom record from 26 Scottish loch basins and evaluated the usability of paleolimnology as a tool for defining reference conditions and ecological status. According to the two-way indicator species analysis (TWINSPAN), four groups, with characteristic diatom assemblages, were identified. The key factors in the data were water depth and productivity. Based on the results of Bennion *et al.* (2004), Lake Isojärvi would well belong to the group of large and mostly deep oligotrophic-mesotrophic lakes. The associated dominant diatom taxa of the group are e.g. *Aulacoseira subarctica* and *Cyclotella* spp. like *C. comensis* and *C. radiosa*. From the studied lochs they found both minimally impacted and enriched lochs, and possible signs of recovery from eutrophication in some lakes. Bennion *et al.* (2004) found it difficult to find minimally impacted lochs from lowland sites and especially shallow systems, to be used as reference sites. This is due to the fact that all sites have been impacted to some degree. It was concluded that paleolimnology can play an important role especially for those lake types, where reference sites cannot be found, by providing site-specific reference conditions.

Leira *et al.* (2006) assessed the ecological status of candidate reference lakes in Ireland. The assessment was carried out by studying a representative selection of 76 oligotrophic and meso-oligotrophic lakes, which have been nominated as candidate refecence lakes by the Irish Environmental Protection Agency. Leira *et al.* (2006) and Bennion *et al.* (2004) both noticed the impact of nutrient enrichment in some the lakes they studied. The results by Leira *et al.* (2006) indicate that the diatom communities especially in large, deep and low alkalinity lakes have been particularly changed and show biologically important deviation from reference conditions. Acidification and nutrient enrichment were noticed to be the most important factors, with a remark that climate change may also be important, as a number of the studied lakes had experienced a rise in various *Cyclotella* taxa compared to the refercence conditions. The rise of *Cyclotella* taxa has been linked to climate change throughout the Canadian Arctic (Karst-Riddoch *et al.*

2005) and also in temperate regions (Wolin & Stoermer 2005). Leira *et al.* (2006) adopted the reference date *c.* 1850 AD used in UK e.g. by Bennion *et al.* (2004), and concluded on the ground of their data, that it might not be appropriate reference baseline in all cases in Ireland, as the history of land use in some areas differ from each other.

The diffculty of finding minimally impacted lakes to be used as a reference site for certain lake types was emphasized in a study by Weckström *et al.* (2004), who studied the coastal waters of Finland, by the Baltic Sea. The coastal waters of Finland by the Baltic Sea have been heavily impacted already before the start of monitoring programs, thus reflecting the same problem identified by Bennion *et al.* (2004). Weckström *et al.* (2004) collected a modern reference dataset of surface sediment samples together with surface-water chemistry data from 45 embayments along the Baltic coast of southern Finland. The constructed diatom-transfer function was applied to a long sediment core from a site, where there is a record of measured water chemistry of approximately 30 years. The accuracy of the diatom-based reconstruction was tested against the available water chemistry data, and a good agreement between the measured and diatom-inferred values was observed. On the basis of their results, Weckström *et al.* (2004) suggested a method, whereby the diatom assemblages in the top 1 cm of a sediment core and the assemblage deeper in the core (representing predisturbance conditions) are compared with each other to establish better targets to be used in restoration programs such as the WFD.

Räsänen *et al.* (2007) studied the impact of logging on six small boreal lakes, located in eastern Finland, and highlighted the relevance of assessing the effects of silvicultural management to a large number of Finnish forest lakes in the implementation of WFD. Their results suggested that logging has not markedly lead to the change of biological elements of the studied lakes and all the studied lakes were found to be close to their background conditions, which is in line with results from Lake Isojärvi, assuming that the detected change in the stratigraphies (Figure 6) is due to the erosion caused by logging. Räsänen *et al.* (2007) concluded that not much would be known about the long-term changes caused by logging in the studied lakes without paleolimnological studies.

A study of naturally and historically eutrophic lakes by Räsänen *et al.* (2006) showed that naturally eutrophic lakes do exist in Finland. The study aimed to identify the pre-impact state of the supposedly naturally eutrophic lakes and their characteristic diatom species. Räsänen *et al.* (2006) found that the diatom assemblages had changed markedly over time in the studied lakes and that there is a wide variation in the diatom assemblage composition of the reference conditions, hence making the unequivocal determination of the lake's restoration target difficult based solely on an assumed natural state diatom flora. Due to this, Räsänen *et al.* (2006) recommend lake-specific careful examination when deciding the needed management actions, as the naturally eutrophic lakes' group appears to be rather heterogenic.

Diatoms *per se* are not included into the biological quality parameters in classification of lakes according to the WFD. Diatoms are used in the assessment of aquatic flora as a part of other phytoplankton and phytobenthos. At the same time the WFD requires that the lakes' natural state or reference conditions should be defined, in order to make the classification possible. In the WFD, paleolimnological studies are mentioned as one possible tool in inferring the past changes and reference status of lakes (European Parliament 2000, Bennion & Battarbee 2007).

Many authors have emphasized the fact that often the only available information about past changes in lakes is obtained using paleolimnological techniques (e.g. Smol 2002, Kukkonen 2003, Bennion *et al.* 2004, Räsänen *et al.* 2004, 2007, Bennion &

Battarbee 2007). For example, Räsänen *et al.* (2004) showed that naturally eutrophic lakes exist in Finland by using paleolimnological study methods. Naturally eutrophic lake type has been suggested e.g. by Pilke *et al.* (2002) to be used in the Finnish lake typology, used in implementation of the WFD. The existence of naturally eutrophic lakes in Finland was previously argued, as it has been difficult or impossible to prove it. In the current lake typology (Vuori *et al.* 2006), naturally eutrophic lakes are included in class *RrRc*, which stands for naturally eutrophic and calcareous lakes.

Räsänen *et al.* (2004, 2006, 2007) and Bennion *et al.* (2004) concluded that paleolimnological diatom analysis and the reconstruction models related to it, are a powerful tool in assessing the reference conditions and the present state of inland waters. Weckström *et al.* (2004) studied the coastal waters by the Baltic Sea, where the problem of finding minimally impacted reference sites is emphasized as most of the coastal waters in Finland are heavily impacted.

5.5. Conclusions

Meriläinen *et al.* (2006) concluded that even though some signs of erosion can be observed in the sediment profile when looking at the carbon content (C %), water content (%) and LOI (%) (Figure 6), the erosion has not caused significant changes in the ecological status of Lake Isojärvi. Based on the results derived from the diatom analyses of the sediment samples, the ecological status of Lake Isojärvi seems to have been relatively stable during the studied history, from approximately the beginning of the 1700s through to 2005, based on the assumptions made from the age of the sediment by Meriläinen *et al.* (2006). Some changes in the diatom assemblages are visible, but the PCA ordination did not distinguished the assemblages into clear clusters (Figure 17). This observation is also in line with the other studies made on the impacts of logging on the diatom fauna of a lake (*see* Laird & Cumming 2001, Laird *et al.* 2001, Räsänen *et al.* 2007).

The pH, TOC, TP and colour reconstructions (Figure 13 and Figure 14), made on the grounds of the diatom assemblages, did not show any significant changes during the lake's history, apart from the slight change in the TP and colour reconstructions based on Miettinen's (2003) models. However, the detected changes can be considered relatively insignificant in the light of the DCA and PCA analyses run on the diatom data.

When inferring past changes, and especially defining reference conditions, the dating of the sediment profile should be done appropriately, for example using ^{210}Pb or ^{137}Cs dating methods. The dating of the sediment core of Lake Isojärvi would be valuable add to the study, as it would give a time-line against which the detected changes could be assessed. As an example, it would be interesting to see how much the sedimentation rate changed, when the assumed catchment's erosion took place. Another issue could be the lack of local, in this case Central Finland, calibration dataset for diatoms. Although the same diatom species can be found all around the globe, the environmental conditions are nevertheless different, due to which valuable information related to local conditions might be lost if a local diatom calibration dataset is not available. On the other hand, the use of other than local diatom datasets emphasizes the cosmopolitan nature of diatoms.

Based on the results of this study and the results by Meriläinen *et al.* (2006), Lake Isojärvi is considered to be suitable for being used as a lake reference site for medium-sized oligohumic lakes (corresponds the type *Vh* according to Vuori *et al.* (2006) in the Finnish lake typology), in the implementation of the European Union Water Framework Directive (WFD). Some minor changes in the ecological status of the lake were detected, which were assumed to be because of the logging in the area of Lake Isojärvi's catchment.

However, according to the diatom analysis, the changes have not significantly affected the biology of the lake. Furthermore, based on the PCA (Figure 17), the ecological status of Lake Isojärvi, inferred from the diatoms, seems to have returned closer to the state it was in the lower parts of the sediment, the depths 12-22 cm representing the time of the biggest changes in the lake. This study has also provided an example of how diatom analysis and other paleolimnological techniques can provide otherwise inaccessible information about the past trophic status and other limnological changes during the history of a lake.

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Appendix 1. Results of the diatom counts

Identified sedimentary diatom taxa in Lake Isojärvi, Finland

Sample depth (cm) Taxa	**0-2**	**2-4**	**4-6**	**6-8**	**8-10**	**10-12**	**12-14**	**14-16**	**16-18**	**18-20**	**20-22**	**22-24**	**24-26**	**26-28**	**28-30**	**30-32**	**32-34**	**34-36**	**36-38**	**38-40**	**40-42**	**42-44**	**44-46**
Achnanthes flexella (Kützing) Brun	1	1	-	-	-	1	-	1	-	-	0,5	-	2	1	-	-	-	-	-	-	-	-	2
Achnanthes kryophila Petersen	1	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	1	1	1	-	-	-	1
Achnanthes laterostrata Hustedt	-	-	-	-	-	1	-	-	-	-	1	-	1	-	-	1,5	1	-	-	-	1	-	-
Achnanthes levanderi Hustedt	-	-	-	-	-	2	1	-	2	-	-	-	-	-	-	-	-	-	1	-	-	-	-
Achnanthes linearis sensu auct. nonnull.	-	0,5	-	1	-	-	-	-	-	1	-	3	1	1	-	-	-	-	1	-	-	-	-
Achnanthes minutissima Kützing	21	27	17,5	22	20	27	12,5	18,5	12	18,5	14,5	25	24	27	24,5	19	25	22	28	20	26,5	14	22,5
Achnanthes oblongella Oestrup	-	-	-	1	-	-	2	-	-	1	-	2	-	-	1	-	1	1	-	-	-	-	-
Achnanthes sp. Bory	-	-	1	-	-	1	-	-	0,5	-	-	-	-	-	-	-	2	1	1	-	2	-	-
Achnanthes subatomoides (Hustedt) Lange-Bertalot & Archibald	-	1	-	1	2	1	1	1	1	1	-	1	-	-	1	-	-	-	1	-	-	-	1
Achnanthes ventralis (Krasske) Lange-Bertalot	-	-	2	1	-	-	-	-	-	1	-	3	-	1	-	3	-	1	1	1	-	-	1
Amphora sp. Ehrenberg ex Kützing	-	1	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Asterionella formosa Hassall	60,5	69	52,5	59	31,5	51	65,5	64,5	86,5	61	57	68	48	45,5	37,5	67	8	15	18	20,5	5,5	9	20,5
Aulacoseira alpigena (Grunow) Krammer	2	4,5	2,5	3,5	7	1,5	4	9,5	3,5	6,5	7,5	2,5	5	7	7	3,5	7	7,5	5,5	4	7,5	4	4
Aulacoseira ambigua (Grunow) Simonsen	2,5	2,5	2	3	2	4	2,5	2	3	4	1,5	0,5	3	3,5	2	2,5	1	3	1	3	3	-	3
Aulacoseira distans var. *humilis* (A. Cleve-Euler)	-	-	1,5	-	-	2	-	-	-	-	-	-	-	-	-	-	1	-	2	-	-	-	-
Aulacoseira lirata (Ehrenberg) Ross	1	-	-	0,5	0,5	0,5	2	2	-	-	-	-	-	0,5	2,5	1	-	-	2	-	0,5	-	2
Aulacoseira pfaffiana (Reinsch) Krammer	8	3	3	10	7,5	14	2,5	1	5,5	1	7,5	5,5	2,5	4	10	9	3	5	13	5,5	-	6	7,5
Aulacoseira sp. Thwaites	9,5	5,5	8	9	8	9	9	5	5,5	7,5	7,5	7	6	11	9	12,5	14,5	10	11	15	15	14,5	12
Aulacoseira subarctica (O. Müller) Haworth	153	127	177,5	167,5	198,5	152,5	214,8	229,5	179,5	193,5	212,5	160	194	211	194	161,5	189,3	203,3	182	205,5	217,5	186,3	177
Aulacoseira subarctica (O. Müller) Haworth long	11,5	13	7	6	6,5	4	2,5	0,5	0,5	1	1,5	-	0,5	-	0,5	-	2	-	-	0,5	1,5	0,5	5
Aulacoseira tenella (Nygaard) Simonsen	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	2	1	-
Brachysira brebissonii f. *brebissonii* R. Ross in B. Hartley	5	-	5	2	1	1	1	4	7,5	1	2,5	2	1	3	2	3	6	5	2	3	2	3	3
Brachysira vitrea (Grunow) R. Ross	12,5	7	11	5	4	4	8,5	9,5	11	11	9	8	9	6,5	8	10	12	10	14	9	15	13	10,5
Brachysira zellensis (Grunow) Round & DG Mann	-	-	-	-	-	-	-	2	0,75	0,5	-	1	-	-	-	1	1	-	1	1	-	-	1
Cyclotella iris Brun et Héribaud	-	1,5	3	1,5	-	2	-	-	-	-	1	3	2,5	1	1,5	2	1,5	-	1	1	-	2	14
Cyclotella pseudostelligera Hustedt	16	14	1	7,5	5	5,5	6	4	7	11	5	4	3	1	3	6	5	2	5	5	3	13	8
Cyclotella radiosa Grunow	9	12	16	15	15	10,5	13,5	9,5	8,25	7,3	7,5	8	10,5	15	20	15	29	32	18	42	56,5	54	21,5
Cyclotella rossii (Grunow) Håkansson	153,5	174	163	135	165,5	134,5	139	125	130	135,5	133,5	161	148,5	143	163	140	183	145,5	153	146,5	136,5	124	145,5
Cyclotella stelligera Cleve & Grunow	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	2	1	-	1	-	2	-
Cymbella cesatii (Rabenhorst) Grunow ex A.W.F. Schmidt	-	-	-	-	1	-	2	1	1	1	-	1	-	-	1	-	-	1	-	-	-	-	-
Cymbella cf. *amphicephala* Nägeli	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Cymbella cuspidata (Kützing)	-	-	-	1	-	-	-	0,5	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-
Cymbella descripta (Hustedt) Krammer & Lange-Bertalot	2	4,5	2	2	2,5	1	0,8	3	0,5	2	1	2	1	1	5	-	-	1	2	1	1,5	6	1
Cymbella gaeumannii Meister	-	2	-	-	1	1	1	-	1	1	1	-	2	-	-	1	-	-	-	1	-	-	-
Cymbella gracilis (Rabenhorst) Cleve	1	-	4	2	1	4	3,5	2	1	3	1	4	-	-	2	4	3	2	2	-	2	3	0,5
Cymbella microcephala Grunow	-	-	-	-	-	-	-	-	1	-	-	-	-	-	1	-	-	-	-	-	-	-	-

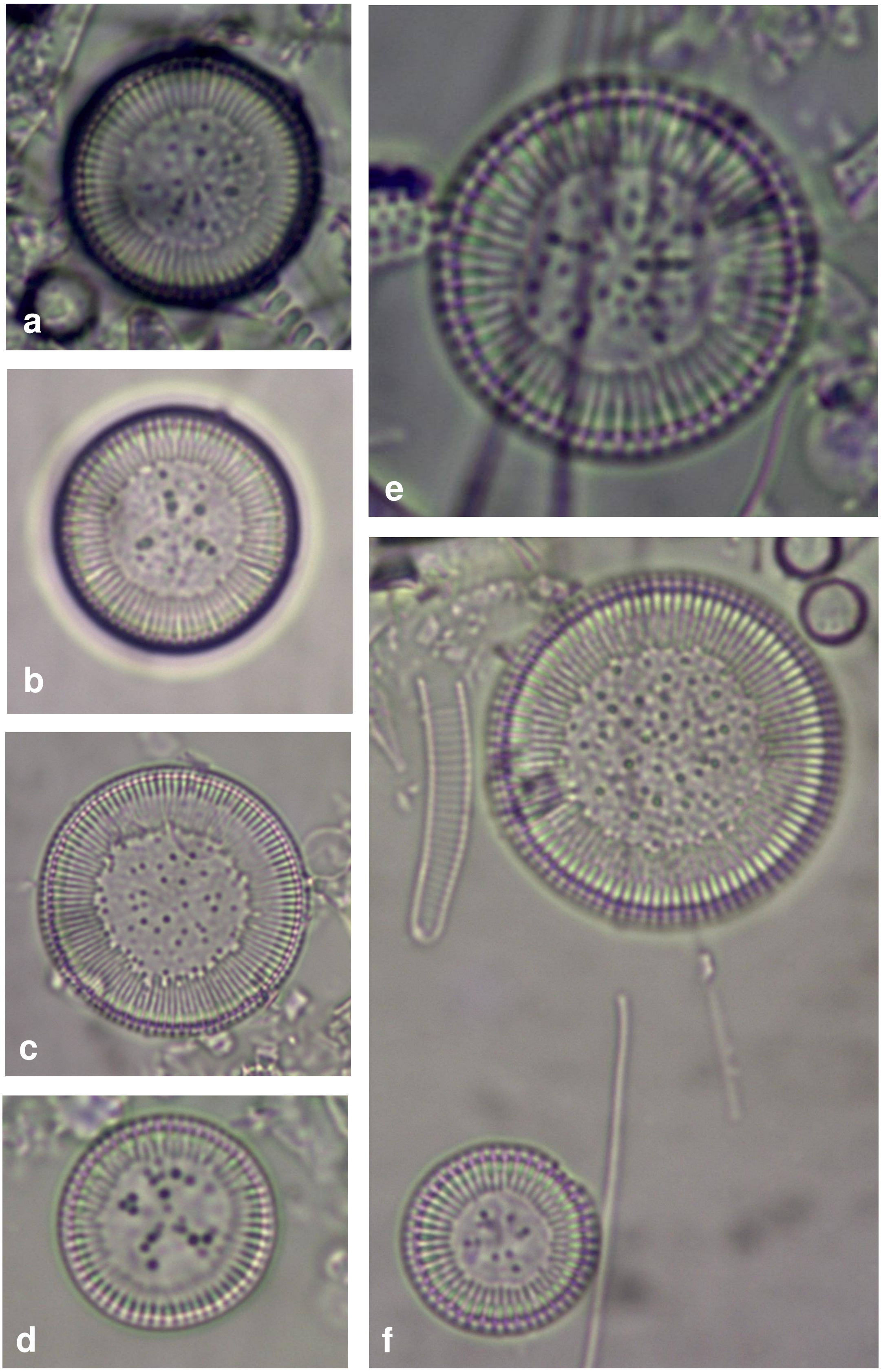
Appendix 1. Results of the diatom counts

Sample depth (cm) Taxa	**0-2**	**2-4**	**4-6**	**6-8**	**8-10**	**10-12**	**12-14**	**14-16**	**16-18**	**18-20**	**20-22**	**22-24**	**24-26**	**26-28**	**28-30**	**30-32**	**32-34**	**34-36**	**36-38**	**38-40**	**40-42**	**42-44**	**44-46**
Cymbella minuta Hilse	1	1	-	-	-	-	-	-	-	-	-	2	-	1	-	-	-	-	-	1	-	-	-
Cymbella sp. C.A. Agardh	-	-	-	-	1	1	0,5	1	-	-	-	-	-	-	-	-	0,5	-	1	-	1	4,5	-
Diploneis sp. Ehrenberg	-	1	-	-	-	-	-	-	1	-	-	-	1	-	-	-	-	1	-	-	-	-	-
Eunotia bilunaris (Ehrenberg) Mills var. *bilunaris*	1	1,5	3	1,5	2	0,5	3,8	4,5	3,5	2	2	3	3	2	2	1	3	2,5	1	-	1,5	3	2,5
Eunotia cf. *hexaglyphis* Ehrenberg	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Eunotia cf. *pectinalis* (Dillwyn) Rabenhorst	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0,5	-	-	0,5	-	1	-	-	-
Eunotia exigua (Brébisson) Rabenhorst	1	1	1	1	0,5	-	1,5	1	-	-	0,5	0,5	-	1	-	1	-	-	1	0,5	2,5	2	-
Eunotia gracilis (Ehrenberg) Rabenhorst	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-
Eunotia incisa Gregory	1,5	1,5	2	1	-	2	1	-	1	2,5	4,5	2	2	1	4	4	4	4	3,5	3	1	3	-
Eunotia meisteri Hustedt	-	1	1	1	-	-	0,5	0,5	4	3	-	-	-	1	-	-	-	-	-	-	-	-	-
Eunotia paludosa Grunow var. *paludosa*	-	-	-	-	-	-	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
Eunotia rhychocephala Hustedt var. *rynchocephala*	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Eunotia serra var. *diadema* (Ehrenberg) Patrick	-	-	-	-	-	1	-	1	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
Eunotia sp. Ehrenberg	0,5	0,5	-	-	-	-	0,3	-	-	-	-	-	1	2	1,5	0,5	4	1	2	-	1	2,5	-
Fragilaria brevistriata Grunow	1	0,5	2	1	1,5	1	0,5	1	1	1	-	0,5	1	1,5	-	1	-	1	-	1	-	-	2,5
Fragilaria cf. capucina var. mesolepta (Rabenhorst) Rabenhorst	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Fragilaria cf. tenera (W. Smith) Lange-Bertalot	6,5	5	7	6	6,5	9	6	4,5	6,8	9,5	2,5	9,5	7,5	3	5	4	-	5	4	2	1	3	6
Fragilaria constricta Ehrenberg	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0,5	-
Fragilaria construens Ehrenberg	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-
Fragilaria construens f. *binodis* (Ehrenberg) Hustedt	-	-	-	2	1	-	2	2	3,5	-	-	1	-	4	4	-	-	-	-	-	-	-	-
Fragilaria construens (Ehrenberg) Grunow f. *construens*	1	3	1	1	3	2	1	3	1	2,5	1	-	-	-	-	-	-	2	3	-	-	1	7
Fragilaria construens f. *venter* (Ehrenberg) Hustedt	-	3	1	1	1	-	1	-	-	-	-	4	4	3,5	4	-	1	-	2	-	1	-	-
Fragilaria crotonensis Kitton	-	-	3	1	-	1	-	-	-	-	-	-	-	-	-	-	1	0,5	-	-	-	-	-
Fragilaria exigua Grunow	4	3,5	2,5	3	2,5	2	3,3	4	2	2	3,8	4,5	2,5	-	1	3	-	1	2	2	-	1	3
Fragilaria fasciculata (Agardh) Lange-Bertalot	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	0,5	-
Fragilaria pinnata Ehrenberg var. *pinnata*	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
Fragilaria sp. Lyngbye	8	7	5,5	6	9	10,5	10,3	10	5	3,5	3,5	5	6	2	3	1	9	2	3,5	5	6	14	2
Fragilaria ulna var. *acus* (Kützing) Lange-Bertalot	-	0,5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fragilaria ulna (Nitzsch) Lange-Bertalot var. *ulna*	1	-	-	-	-	2	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	1
Fragilaria virescens Ralfs	1	-	1	1	-	0,5	1	1	-	-	-	-	-	-	4	-	-	-	1	-	-	1	1
Frustulia rhomboides (Ehrenberg) DeToni	3	2,25	-	5,5	5	3	4	6,3	8,5	2,8	4,8	3	3	4	2	2	5,5	5	3	4,5	7	4,5	3
Gomphonema acuminatum Ehrenberg	-	-	-	-	-	2	0,8	-	2	0,8	2,3	3	1	-	-	1	1	1	-	1	1	1	-
Gomphonema cf. *subtile* Ehrenberg DeToni	-	-	-	-	-	-	-	-	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-
Gomphonema gracile Ehrenberg	1	3	1	1,5	2,5	1	1,5	2	1	1	1	1	1	1	2	2	-	1	1	1	-	1	1
Gomphonema sp. Ehrenberg	-	-	-	-	-	2	1	-	-	1	-	-	-	-	1	-	1	-	1	-	-	1	-
Meridion circulare (Greville) Agardh	1,5	0,5	1	0,5	-	1	-	1	1	0,5	-	0,5	-	0,5	-	-	-	-	0,5	1	1	-	-
Navicula angusta Grunow	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
Navicula arvensis Hustedt	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-

Appendix 1. Results of the diatom counts

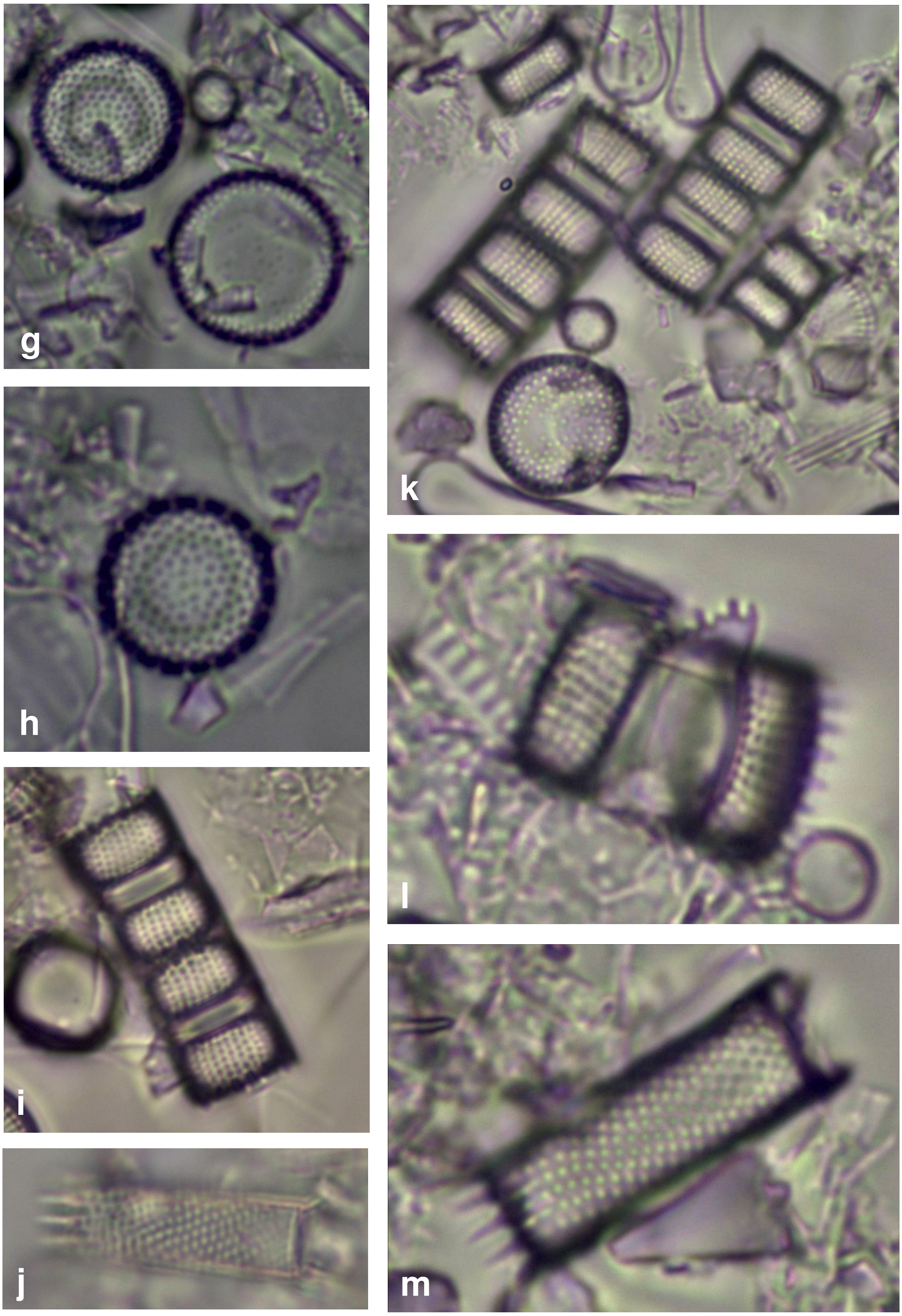
Sample depth (cm)	**0-2**	**2-4**	**4-6**	**6-8**	**8-10**	**10-12**	**12-14**	**14-16**	**16-18**	**18-20**	**20-22**	**22-24**	**24-26**	**26-28**	**28-30**	**30-32**	**32-34**	**34-36**	**36-38**	**38-40**	**40-42**	**42-44**	**44-46**
Taxa																							
Navicula cf. *kriegerii* Krasske	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
Navicula cocconeiformis Gregory ex Greville	1	-	1	-	-	-	1,5	-	-	1,8	2	-	-	-	1	-	-	-	-	-	-	-	-
Navicula cryptocephala Kützing	-	-	-	-	-	1	-	-	-	-	-	-	-	1	1	-	-	-	-	-	1	-	-
Navicula halophila (Grunow) Cleve	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0,5	-	-	-	-	1
Navicula jaernefeltii Hustedt	1	-	1	1	-	1	-	-	-	-	-	-	-	-	1	-	-	-	-	3	-	1	1
Navicula leptostriata Jørgensen	-	-	-	-	-	-	1,5	-	-	-	-	-	-	-	2	-	-	1,5	-	-	-	-	-
Navicula longicephala Hustedt	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
Navicula pupula Kützing	-	1	-	-	1	1	-	-	1	-	-	-	-	1	1	1	-	-	-	1	1	1	1
Navicula radiosa Kützing	1	0,5	1	0,5	1	2,5	1	-	1,5	1	0,8	1,5	1	2	2	2	1	0,5	1	3	4	2	-
Navicula radiosafallax Lange-Bertalot	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
Navicula sp. Bory	-	1	-	-	-	1	-	-	-	-	-	0,5	1	-	-	-	1	-	-	-	1,5	1	0,5
Navicula subtilissima Cleve	-	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Neidium ampliatum (Ehrenberg) Krammer	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-
Neidium sp. Pfitzer	-	-	-	-	-	0,5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nitzschia fonticola Grunow	0,5	1	1	1	1	1	-	-	-	0,5	-	1	-	1	2	1	-	0,5	-	-	2	-	1
Nitzschia palea (Kützing) W. Smith	-	1	-	-	-	-	1	0,5	-	0,5	-	3	1	2	-	-	1	1	1	1	-	2	-
Nitzschia paleacea Grunow	1,5	0,5	-	0,5	1	1	0,25	1	-	0,8	1	2	0,5	-	1	1	2	-	2	2	1	3,5	-
Nitzschia recta Hantzsch	-	-	-	1	1,5	1	-	1	-	-	-	-	-	-	-	1	-	-	-	0,5	-	1	-
Nitzschia sp. Hassal	0,5	1	-	-	-	0,5	-	-	-	0,5	-	-	0,5	1	1	1	1	1	0,5	2,5	2	0,5	-
Pennales spp. *(Cymbella sp.?)*	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Peronia fibula Brébisson	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Pinnularia gibba Ehrenberg	-	1	-	-	-	1	0,75	-	2	-	0,5	1,5	2	-	-	-	1	2	1	-	-	2	4,5
Pinnularia interrupta W. Smith	2	2	-	1	1	-	-	-	1	1,5	-	-	-	-	1	1,5	3,5	-	1	-	1	1	2
Pinnularia microstauron (Ehrenberg) Cleve	-	-	-	-	-	-	-	-	0,5	-	1	-	1	-	-	-	-	-	-	1	-	-	-
Pinnularia sp. Ehrenberg	1	1,25	-	-	1,5	1	-	-	1	-	0,5	2	1	0,5	1	2	1,5	1	1,5	-	1	1	-
Pinnularia viridis (Nitzsch) Ehrenberg	-	-	-	1	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
Stauroneis anceps Ehrenberg	-	1	-	-	-	-	1	-	1	1	-	-	-	-	-	-	-	1	1	-	1	-	-
Stauroneis smithii Grunow	-	-	-	-	-	-	-	-	0,5	-	-	-	-	-	-	-	-	1	-	-	-	-	-
Stenopterobia cf. *curvula* (W. Smith) Krammer	-	1	0,5	1	-	-	0,5	0,5	0,5	-	1,5	-	-	-	-	-	-	-	-	-	-	-	-
Stephanodiscus alpinus Hustedt	2,5	2	2,5	4	6	2,5	-	-	-	-	-	1	-	-	-	3	-	0,5	-	-	1	-	2,5
Surirella angusta Kützing	-	0,5	1	1	-	0,5	0,5	1	1	-	2,5	-	1	1	1	-	-	-	1	1	-	-	-
Surirella minuta Brébisson in Kützing	-	1	-	1	-	-	-	1	-	-	0,5	-	-	1	-	-	1,5	1	1	1	-	1	1,5
Surirella sp. Turpin	-	-	-	-	-	-	-	-	-	-	-	-	0,5	-	-	-	-	-	-	-	-	-	-
Tabellaria fenestrata (Lyngbye) Kützing	-	1	1	-	-	-	1,3	-	-	2,3	-	-	1	-	1	-	-	-	-	-	1	-	-
Tabellaria flocculosa var. *flocculosa* (Roth) Kützing	7,3	12,8	17	11,5	8,3	12	17,1	8,5	10,8	15,9	13,4	9,8	8,5	11,8	14,3	15,8	18,8	20	13	8	13	10	7
Tabellaria flocculosa (Roth) Kützing long	11	9,3	8,5	11,5	6,5	12,8	11,1	16	11	14,6	15	21,3	20,3	9,5	6	5,8	1,3	6,5	7,3	4,5	4	2	8
Tetracyclus glans (Ehrenberg) Mills	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
Total counted	533	546	549	527	544	518	573	568	543	545	538	558	538	544	570	519	565	539	527	540	558	531	525

Appendix 2: Micrographs of *Cyclotella rossii* and *Aulacoseira subarctica* from Lake Isojärvi



Figures a-f. Light micrographs of *Cyclotella rossii* (Grunow) Håkansson (valve views) from Lake Isojärvi. Micrographs are taken with 1000x magnification (figures not in scale). Micrographs are taken by Mika Nieminen.

Appendix 2: Micrographs of *Cyclotella rossii* and *Aulacoseira subarctica* from Lake Isojärvi



Figures g-m. Light micrographs of *Aulacoseira subarctica* (O. Müller) Haworth from Lake Isojärvi. Micrographs are taken with 1000x magnification (figures not in scale). **Figures g,h and k.** The frustules in valve view. **Figures i-m.** The frustules in girdle view. **Figures j and m.** The high-celled form of *A. subarctica*. Micrographs are taken by Mika Nieminen.