Eesti Maaülikool
Estonian University of Life Sciences

HOW PHYTOPLANKTON PIGMENTS REFLECT HISTORICAL AND CONTEMPORARY STATUS OF LARGE SHALLOW LAKES?

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RENÉ FREIBERG

A Thesis
for applying for the degree of Doctor of Philosophy in Hydrobiology

Väitekiri
filosoofiadoktori kraadi taotlemiseks hüdrobioloogia eralal

Tartu 2012

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Opponent: **Prof. Dr. Martin Dokulil**
EX Institute for Limnology
Austrian Academy of Sciences

Supervisors: **Dr. Arvo Tuvikene, Dr. Ilmar Tõnno**
Centre for Limnology,
Institute of Agricultural and Environmental Sciences
Estonian University of Life Sciences

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“...God created the world in six days. On the seventh day, he rested. On the eighth day, he started getting complaints. And it hasn’t stopped since.”

James Scott Bell, *Sins of the Fathers*

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

I **Freiberg, R.**, Nõmm, M., Tõnno, I., Alliksaar, T., Nõges, T. & Kisand, A. (2011) Dynamics of phytoplankton pigments in water and surface sediments of a large shallow lake. *Estonian Journal of Earth Sciences,* **60(2),** 91–101.

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III Leeben, A., Tõnno, I., **Freiberg, R.**, Lepane, V., Bonningues, N., Makarõtševa, N., Heinsalu, A. & Alliksaar, T. (2008) History of anthropogenically mediated eutrophication of Lake Peipsi as revealed by the stratigraphy of fossil pigments and molecular size fractions of pore-water dissolved organic matter. *Hydrobiologia,* **599,** 49–58.

IV Leeben, A., **Freiberg, R.**, Tõnno, I., Kõiv, T., Alliksaar, T. & Heinsalu, A. (2012) A comparison of the palaeolimnology of Peipsi and Võrtsjärv: connected shallow lakes in north-eastern Europe for the twentieth century, especially in relation to eutrophication progression and water-level fluctuations. *Hydrobiologia,* accepted. DOI 10.1007/s10750-012-1209-7

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Manuscript preparation	**RF,** IT**,** TN, TA	IT, **RF**, VL	AL, IT, **RF**, VL, +	AL, **RF**

RF – Réne Freiberg; AL – Aina Leeben; IT– Ilmar Tõnno; TA – Tiiu Alliksaar; AK– Anu Kisand; TK – Toomas Kõiv; TN – Tiina Nõges; MN – Monika Nõmm; VL – Viia Lepane; AH – Atko Heinsalu; + – other authors; * – co-workers.

ABBREVIATIONS

A_{254}	Absorbance at 254 nm
A_{250}/A_{360}	Ratio of absorbances (aromaticity index)
BD-RP	Reductant soluble phosphorus
$CaCO_3$	Calcium carbonate
Cantha	Canthaxanthin
CAR	Sum of α- and β-carotene
CDOM	Chromophoric dissolved organic matter
Chl *a*	Chlorophyll *a*
Chl *b*	Chlorophyll *b*
Chl c_2	Chlorophyll c_2
CRS	Constant rate of supply
CY	Cyanobacteria
Diadino	Diadinoxanthin
Diato	Diatoxanthin
Echi	Echinenone
EU	European Union
FeS	Iron sulphide
Fuco	Fucoxanthin
HCl-RP	Calcium bound phosphorus
HMW	High molecular weight fraction of pore water dissolved organic matter
HPLC	High performance liquid chromatography
HS	Humic substances
IPR	Ion pairing reagent
LMW	Low molecular weight fraction of pore water dissolved organic matter
Lute	Lutein
MMW	Medium molecular weight fraction of pore water dissolved organic matter
M_n	Number average molecular weight of *p*DOM
M_w	Weight average molecular weight of *p*DOM
N	Nitrogen
NAO	North Atlantic Oscillation
NaOH-RP	Metal oxide bound phosphorus
NAO_w	North Atlantic Oscillation winter index
NH_4Cl-RP	Loosely sorbed sedimentary phosphorus
ODS	Octadecyl silane
OM	Organic matter
P	Phosphorus

PCA	Principal Component Analysis
PDA	Photodiode array detector
*p*DOM	Pore-water dissolved organic matter
Pheo *a*	Pheophytin *a*
PhP	Phytoplankton pigment
WLF	Water-level fluctuation
Zea	Zeaxanthin
α-Car	β,ε-carotene (trivial name = α-carotene)
β-Car	β,β-carotene (trivial name = β-carotene)
ΣFR	Sum of sediment phosphorus fractions

1. INTRODUCTION

Phytoplankton is the most important primary producer in the aquatic ecosystems and account for half of all photosynthetic activity on earth, thus creating the base for most aquatic food chains. It also plays key role in the oxygen- carbon dioxide balance of the atmosphere and helping to ameliorate green-house gases, by removing nearly a third of the anthropogenic carbon released to the atmosphere (Sabine and Feely, 2007).

In Europe, the EU has implemented the Water Framework Directive (Directive 2000), which is focused on assessing water quality in all waterbodies spanning from ground water to coastal marine waters. The directive requires that undisturbed or reference conditions should be defined for these aquatic environments. Monitoring records rarely go back more than a few decades and in order to define reference conditions other approaches are needed. Multi-proxy palaeoecological assessments can provide the long-term records of ecological status and natural variability that are needed to interpret changes observed in the environment today and predict future changes.

Reference conditions are most commonly established by regional comparisons, nutrient loading models, and increasingly by palaeolimnological methods (e.g., Jordan et al., 2001; Ramstack et al., 2004). The latter approach, using sediment records to reconstruct limnological change, is in many ways the most robust, and for regionally unique and highly altered systems, the only reliable way to understand predisturbance conditions (Engstrom et al., 2006). Palaeoecological data have many advantages, including good correlations between plankton and fossil abundance, consistent methodology through time, and the ability to obtain equally long time series before and after perturbation (Cottingham et al., 2000)

Algal remains in the sediments can provide historical information at the species level, although such taxonomic resolution is generally limited to groups such as diatoms, which leave siliceous frustules, and green algae, some of which possess cell walls that resist degradation. Phytoplankton pigments (PhPs) that capture and transform solar energy in the water column (Hall et al., 1999) store valuable palaeoclimatic and palaeoenvironmental information if buried in sediments (Fietz et al., 2007; Soma et al., 2007). Although fossil pigments, such as chlorophyll derivatives and carotenoids, do not provide information at the species level, they can be used to make inferences about past algal communities because algal pigments are often taxon-specific (Tsugeki et al., 2010). Carotenoids are useful biomarkers of different classes of phytoplankton, while chlorophyll *a* is not class-specific and is commonly used to estimate the total amount of phytoplankton in aquatic systems (Jeffrey et al., 1997a; Bianchi et al., 2002; Reuss et al., 2005).

Contrary to anaerobic sediments where PhPs may preserve for a long period, the aerobic degradation of PhPs in the water column is usually very rapid and extensive (Leavitt & Carpenter 1990a, 1990b; Leavitt, 1993; Patoine & Leavitt, 2006). The dating of sediments in a shallow, productive system can be highly problematic due to wind-induced resuspension of sediments, diagenetic reactions causing gas generation, bioturbation from benthivorous fish or burrowing insect larvae and detachment of surface sediment from the lake bottom by algal mats (Eilers et al., 2004).

The composition of PhPs preserved in sediments commonly differs from that in the water as the sensibility to decomposition varies among different pigments (Bianchi et al., 2002). Each lake represents a unique environment for deposition and preservation of PhPs, depending on its oxygen and light penetration characteristics, resuspension intensity, digestion through zooplankton grazing and phytoplankton abundance. In anaerobic sediments of deep lakes, where resuspension and bioturbation are negligible, PhPs or their ratios might reflect the history of phytoplankton composition and primary production (Cohen, 2003; Reuss et al., 2005).

In deep lakes the stratification depth is also an important factor for conservation of sediment pigments as their degradation in the water column is rapid (> 90%) due to photo-oxidation, grazing by invertebrates, etc. (Leavitt & Carpenter, 1990a, 1990b; Leavitt, 1993; Descy et al., 1999; Patoine & Leavitt, 2006). In shallow lakes resuspension and aerobic conditions in surface sediments enhance the transformation processes of organic matter, including PhPs, which strongly complicates the interpretation of palaeolimnological information stored in sediments (Leavitt & Carpenter, 1989). However, as most of the World's lakes are shallow (Scheffer, 1998), the evaluation of the possibilities of tracking historical changes in shallow lakes by using fossil sediment records is highly needed.

This dissertation is focused on phytoplankton pigment analysis of two interconnected large shallow lakes Võrtsjärv and Peipsi. The first issue that is covered is how the PhPs in the upper sediment layer of the lake Võrtsjärv follow the annual dynamics of phytoplankton pigments in the water column. Thereafter eutrophication history and palaeolimnological aspects of these two lakes on the 20th century are compared in a multi-proxy studies.

2. REVIEW OF THE LITERATURE

2.1. Phytoplankton pigments in limnological and palaeolimnological studies

2.1.1. Phytoplankton pigment records

Phytoplankton pigments (chlorophylls, carotenoids, phycobiliproteins and their derivatives) have been isolated and identified from aquatic sediments for over 60 years (Fox, 1944; Fox et al., 1944; Vallentyne, 1954). It is now commonly known that such pigments from algae, phototrophic bacteria and higher plants preserve long after all morphological structures have disappeared. In temporal regions the history of lakes can be traced back to the last ice-age and in regions where sediments were undisturbed from the last ice-age processes even an older pigments can be found (Soma et al., 2007). Unmodified algal carotenoids have been recovered from 56,000 year-old marine sediments (Watts & Maxwell, 1977) and 2.8 million years of phytoplankton history in Lake Baikal was reconstructed by Soma et al. in 2001 using residual photosynthetic pigments. Perhydrocarotene, a fully saturated carotenoid derivative, has been isolated from Green River shale deposited in shallow lakes over 50 million years ago (Murphy et al., 1967).

To date more than 700 different naturally occurring carotenoids (Britton et al., 2004) and around 20 chlorophylls have been described, and they are virtually ubiquitous in phototrophic organisms. Many of them have taxonomic specificity that makes them a valuable tool in ecological and palaeoecological studies.

2.1.2. The use and importance of phytoplankton pigments in oceanography and limnology

Pigments act as tracers to elucidate the composition and fate of phytoplankton in the waterbodies and are often associated with important biogeochemical cycles related to, for example, carbon dynamics in the oceans. They are increasingly used in *in situ* and remote-sensing applications, detecting algal biomass and major taxa through changes in light reflecting properties of water.

Some microalgae also contribute significantly to climatic processes, providing nuclei for atmospheric water condensation (Aiken et al., 1992). All microalgae, by their photosynthetic activities, contribute to atmospheric carbon dioxide 'draw-down' (Jeffrey & Mantoura, 1997). Because of the important global role of phytoplankton, monitoring their biomass by measuring ocean colour from space (Sathyendranath et al., 2004; Nair et al., 2008) and increasing the accuracy of *in situ* pigment measurements to determine algal types in the water column (Jeffrey et al., 1997b), have become high priority areas for oceanographic and limnological research.

As at the present time, the marine phytoplankton contribute at least a quarter of the biomass of the world's vegetation, and constitute the base of the food web that supports either directly or indirectly all the animal populations of the open sea, a lot of PhP research has been done in oceanography. Dr S.W. Jeffrey, has been a pioneer in the development of tools and knowledge on pigments in ocean environments. In 1997 a comprehensive book was published *"Phytoplankton Pigments in Oceanography",* edited by Jeffrey, S.W., Mantoura, R.F.C. & Wright, S.W. (UNESCO Press) with a follow up in 2011 *"Phytoplankton Pigments: Characterization, Chemotaxonomy and Applications in Oceanography"*, edited by Roy, S., Llewellyn, C.A., Egeland, S. A. & Johnsen, G., (Cambridge University Press) that summarize well the current knowledge about phytoplankton pigment analysis in the oceans.

Lakes generally display higher production and sedimentation rates than oceans and contain palaeoclimatic and palaeoenvironmental information on the local, regional and continental level. The use of PhPs in palaeolimnology has been reviewed in 2001 by Drs. P.R. Leavitt and D.A. Hodgson in "*Tracking environmental change using lake sediments*", Volume 3, Smol J.P., Birks H.J.B., Last W.M. (eds.). In principle, fossil pigments can be used in any palaeoecological application in which historical changes in lake production or primary producer composition are a key response (e.g., eutrophication, acidification, climate change, food-web interactions and human impacts). For example, past ultraviolet radiation environments in lakes has been successfully restored by using fossil pigments (Leavitt et al., 1997).

Planktic and benthic algal communities are common for shallow lakes. Benthic algae can be substantial primary producers in pelagic and benthic food webs in non-stratified lakes (Zimba, 1995; Bonilla et al., 2005).

However, the development of benthic algae is limited in turbid shallow lakes where the euphotic zone does not penetrate to the sediment surface (Nõges & Laugaste, 1998).

In shallow lakes resuspension and aerobic conditions in surface sediments enhance the transformation processes of organic matter, including PhPs, which strongly complicates the interpretation of palaeolimnological information stored in sediments (Leavitt & Carpenter, 1989). Also dating of sediments from large, shallow, dynamic basin is less than ideal. Chronological uncertainty is greater than in lakes of greater relative depth, and cores from some sites can be unreliable (Engstrom et al., 2006). Despite various methodological problems, some successful palaeolimnological studies of shallow lakes have been published within the last years (Eilers et al., 2004; Engstrom et al., 2006).

2.1.3. Phytoplankton bio-optical sensing and chemotaxonomy

The composition and biomass of algae in marine and freshwaters are traditionally determined by microscopy. During the last two decades, efforts have been made to develop more cost-efficient measures.

Pigment-based *in situ* detection of phytoplankton biomass and different bio-optical groups is most commonly done using a few wavelengths that are greatly impacted by the pigment-specific absorption maxima of the phytoplankton. Most optical underwater *in situ* instruments are well suited for time-series measurements of phytoplankton and particle concentrations, but are limited in the discrimination of different phytoplankton classes, pigment groups and species (Johnsen et al., 2011).

A technique that shows promise in detecting the presence of specific pigment groups and the corresponding *in situ* biomass of various algal groups is artificial light stimulated spectral fluorescence, which examines the light energy transfer by pigments in the wavelength range from 400 to 700 nm. The rise and fall in fluorescence emission reflects the light energy transfer from the photosynthetically active pigments present within the cell. The advantage of this approach is the higher sensitivity when compared to absorption measurements, making it useful even for dilute phytoplankton concentrations. The technique reflects the condition of the light harvesting pigments, the physiological state of the phytoplankton and is insensitive to non-algal particles (e.g. mineral particles from river runoff) and coloured dissolved organic matter, in contrast to light absorption (Johnsen et al., 2011). Currently, *in situ* data from multiple-fixed-wavelength spectral fluorometers, for example Algae Online Analyser®, are able to quantify biomass, species composition and have given comparable data with chromatography derived phytoplankton pigment markers, that are good for first-order approximation of phytoplankton group abundances, but, as is the case for many fluorometry-based instruments, determination of absolute Chl *a* concentrations is less robust (Richardson et al., 2010).

For large-scale monitoring a remote sensing is a fast developing field. Signatures from *in situ* spectral diffuse attenuation coefficients, reflectance, light beam attenuation, absorption, scattering, and Chl *a* fluorescence may be detected from remote sensing using satellites and aeroplane-based spectrometers that detect reflected light from the sea surface. The resolution varies with the platform and with the focus area. Current resolution from satellites ranges from 250 m to 1 km (Ladner et al., 2007), and it is down to tens of centimetres for aircraft ocean colour sensors (e.g. Klonowski *et al.*, 2007).

The most promising and powerful chemotaxonomic method to determine the phytoplankton group composition is to analyse PhPs by High Performance Liquid Chromatography (HPLC). A CHEMTAX program was developed in 1996 for marine environment for calculating algal class abundances from measurements of chlorophyll and carotenoid pigments determined by HPLC. The program uses factor analysis and a steepest descent algorithm to find the best fit to the data based on an initial guess of the pigment ratios for the classes to be determined (Mackey et al., 1996). This method is commonly used for determining algal groups in marine and estuarine areas but is underdeveloped in freshwaters. Chemotaxonomy, used in marine and coastal areas (e.g. Andersen et al., 1996; Schlüter et al., 2000; Havskum et al., 2004), allows determination of even pico-sized algae, for which traditional microscopy methods can be inadequate because of their small size and often low abundance. HPLC has so far been used in only a few chemotaxonomic freshwater studies (e.g. Fietz & Nicklisch, 2004; Descy et al., 2005; Schlüter et al., 2006; Lauridsen et al., 2011), despite the technique being relatively quick compared to microscopy.

Recently some efforts to combine CHEMTAX analysis with satellite-derived distributions of specific phytoplankton pigments to describe the distributions of particular components of the phytoplankton community have been made (Pan et al., 2011).

2.2. Pigment sedimentation process in large shallow lakes

Main autochthonous sources of PhPs of a lake are *in situ* and littoral production. Major pigment fluxes are direct sedimentation of dead cells and through zooplankton grazing loop (Fig. 1.). Vertical fluxes of pigments are used in limnology to monitor phytoplankton abundance, herbivore grazing, ecosystem efficiency, and historical changes in production. However, experimental and mass flux studies indicate that > 90% of pigment is degraded to colourless compounds before permanent burial (Leavitt, 1993). A model of pigment flux has been worked out by Cuddington & Leavitt (1999) to quantify the relative importance of production and degradation as controls of pigment sedimentation. According to this model, pigment deposition increases with production, sinking rate, and phytoplankton depth and declines as lake depth and the

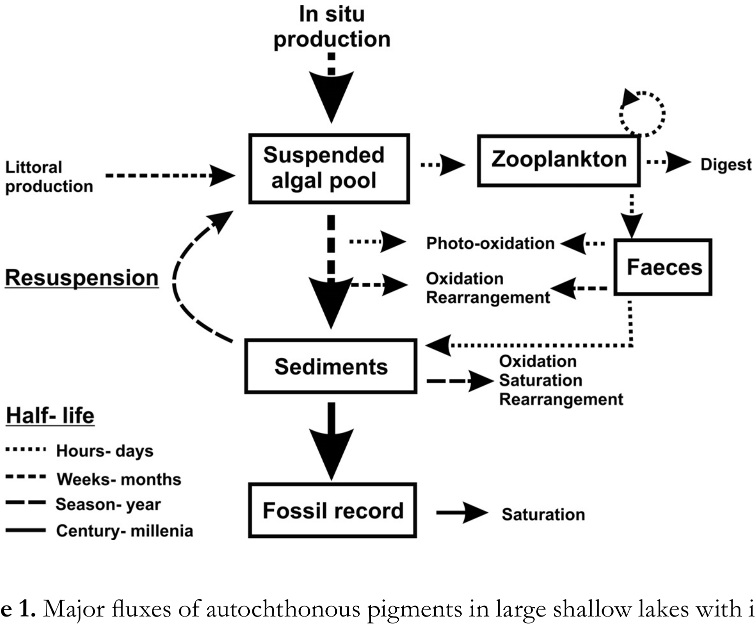

Figure 1. Major fluxes of autochthonous pigments in large shallow lakes with indications of approximate decay coefficients. Most degradation of pigments occurs during deposition through the water column and in the surface sediments. Regular resuspension of the upper layer of the sediment distorts clear historic record in large shallow lakes. Benthic algae and fauna are not included but can have large influence on fossil record in some lakes. Modified from Leavitt (1993).

depth of oxygen penetration increases. Unexpectedly, pigment sedimentation rate was found not to be sensitive to variation in photooxidation rates, even though bleaching accounted for the second greatest amount of pigment loss. Digestion by zooplankton caused the most pigment degradation, but grazing increased pigment deposition when digestive losses were less than those due to oxidation of pigments in ungrazed cells. The model suggests that algal production may be underestimated in sedimentation studies that do not consider variability in water column depth. Further, comparisons with palaeoecological analyses suggest that some inferred increases in production during lake ontogeny may arise from changes in regulation of pigment fluxes rather than from increased algal production (Cuddington & Leavitt, 1999). Similar lake specific PhP flux studies have been conducted on Lake Baikal (Fietz et al., 2005), Lake Mendota (Hurley & Armstrong, 1990) and many others. Vertical fluxes in a large shallow lake are particularly complicated because the upper sediment layer is regularly resuspended.

2.3. Sampling and analysis of phytoplankton pigments with HPLC

Phytoplankton pigment samples from the water column are commonly taken by filtering water thru glass-microfibre filters and following extraction with acetone, methanol, ethanol or mixture of these solvents. Sonication is often used to break up cell walls of microalgae to ensure complete extraction of pigments. Due to the labile nature of pigments certain precautions have to be taken. Keeping the filters in dark, cold (preferably frozen to −20 °C at least) and under inert gas environment is important to prevent pigment degradation.

Sediments for pigment analyses can be collected using any standard palaeoecological technique including gravity, piston and freeze coring, followed by a subsequent sectioning of the cores that determines the maximal resolution of sediment analyses. The same handling and storage precaution procedures should be taken as with filters. Pigments in lyophilized sediment samples are especially vulnerable to degradation and prolonged storage of these samples is not recommended (Reuss et al., 2005). Use of freeze cores has proven excellent for coring surface sediments that often have high water content. Freezing the sediment *in situ* also provides good preservation conditions for pigments. However, the disadvantage of freeze cores is the relatively small sample size obtainable for each level and the limited length of the core.

It is important to understand, that there is no one good HPLC method for pigment analysis. In spite of continuous developments in instruments and columns, analysis of phytoplankton pigments is still a challenge for HPLC techniques. This is because many pigments vary greatly in structure and span a wide range of polarities, while others have similar chemical structures, with differences as small as the position of a double bond (Jeffrey et al., 1997b). It will be particularly problematic for extracts from sediment cores as these often exhibit distributions that are highly complex and display a broad range in polarity (Goericke et al., 1999). Most HPLC methods employ reversed-phase stationary phases, with C_8 to C_{30} chains chemically bonded to silica supports and gradient elution from partially aqueous mobile phases to non-aqueous organic mixtures. Under such conditions, pigments are primarily resolved on the basis of their polarity. In Garrido et al. (2011) 26 different methods are described to analyse pigments with HPLC. To choose the right method can be a difficult task for a researcher and often only empirical experience and modification of methods will lead to successful outcome that is lake and matrix specific and depends from the pigments the analyse is focused on.

3. AIMS OF THE STUDY

In Europe, the EU has implemented the Water Framework Directive requiring that undisturbed or reference conditions should be defined also for large shallow lakes. This dissertation is focused on phytoplankton pigment analysis of two interconnected large shallow lakes Võrtsjärv and Peipsi. Contemporary pigment flux and multi-proxy palaeolimnological studies were conducted to assess the usability of phytoplankton pigments to monitor on-going and historical processes in these lakes.

Specific aims of the studies were:

- To find out to which extent the phytoplankton pigments in the upper sediment layer of the large shallow Lake Võrtsjärv follow the annual phytoplankton dynamics in the water column (**PAPER I**);
- Multi-proxy study was conducted to address how climate-driven and anthropogenic eutrophication interact as stressors to affect Lake Võrtsjärv ecosystem functioning (**PAPER II**);
- Reconstruction of the recent eutrophication history of Lake Peipsi is presented at a high-resolution scale based on fossil pigment records and stratigraphic changes in the molecular size structure of pore-water dissolved organic matter (**PAPER III**);
- A multi-proxy palaeolimnological approach was applied to provide insights into the natural variability and human-mediated trends of two interconnected temperate large shallow lakes, Peipsi and Võrtsjärv, during the 20th century (**PAPER IV**).

The following questions were addressed:

- Does pigment composition in the upper sediment layer follow annual changes in the water column (**PAPER I**)?
- How does water level influence pigment composition in the upper part of the sediment (**PAPER I**)?
- Do fossil pigments reflect anthropogenic and climate-driven changes in lakes Peipsi and Võrtsjärv and how are these results supported by other palaeoindicators and monitoring data **(PAPERS II** & **III**)?
- Are there substantial differences in the eutrophication history of lakes Peipsi and Võrtsjärv (**PAPER IV**)?

4. MATERIALS AND METHODS

4.1. Description of the lakes

Lake Peipsi (surface area 3,555 km^2, mean depth 7.1 m) is the fourth largest lake in Europe. Lake Võrtsjärv is considerably smaller (surface area 270 km^2) and shallower (mean depth 2.8 m) than Peipsi and its catchment (area 3,374 km^2) is a part of the Peipsi catchment (area 47,800 km^2, Fig. 2).

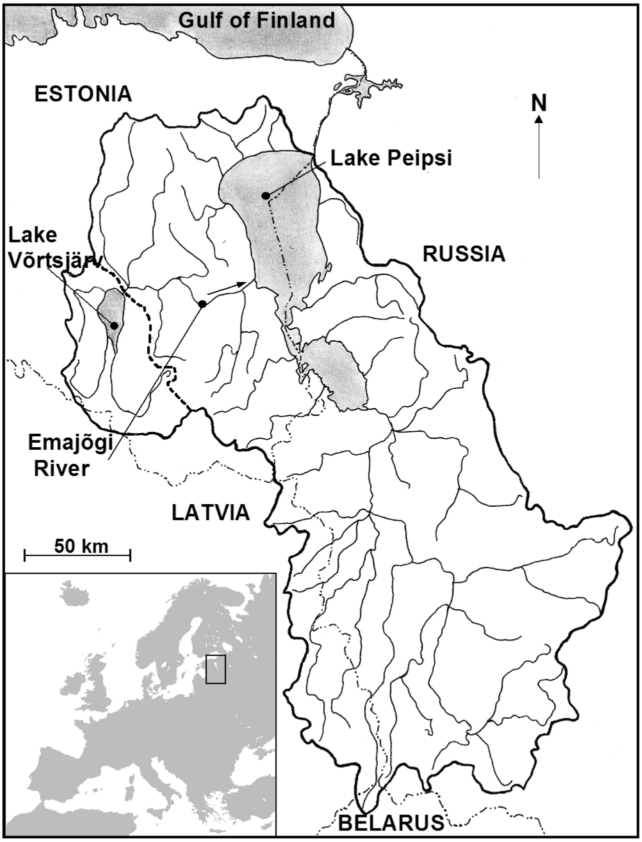

Figure 2. Location of the study area and sampling points.

The two lakes are connected by the Emajõgi River (length 100 km), which drains Võrtsjärv water to Peipsi. The average annual outflow from Võrtsjärv between 1961 and 2000 was ca. 0.74 km^3 (Järvet, 2004). The water level of the lakes fluctuates seasonally: mean annual range is 1.4 m in Võrtsjärv and 1.2 m in Peipsi (Fig. 3).

Due to the shallowness of the lakes their water columns remain thermally unstratified and are relatively rich in oxygen (**PAPER IV**, Table 1), although in wintertime and on hot and calm summer days, when the mixing of the lakes' water columns becomes limited, anoxic conditions may occur near the bottom layers.

According to their hydrochemical characteristics, the waters of lakes Peipsi and Võrtsjärv belong to the Ca-group of the hydrogen carbonate class. The lakes' water has a weak alkaline reaction. In general, the annual average concentration of humic substances in the lakes does not exceed the medium level, although seasonally snowmelt and autumn rains pronouncedly increase the runoff of organic matter from surrounding areas.

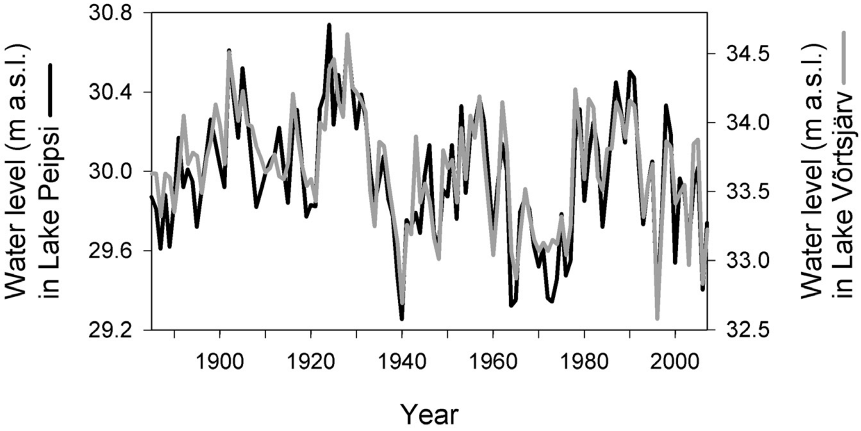

Figure 3. Dynamics in the water level of lakes Peipsi and Võrtsjärv. The data were provided by the Estonian Meteorological and Hydrological Institute. Missing water levels of some years were reconstructed by Jaani (1990, 2001).

The lakes are eutrophic and productive (**PAPER IV**, Table 1). In both lakes diatoms and cyanobacteria prevail in biomass. As Võrtsjärv is richer (approximately twice) in phosphorus, nitrogen and silicon, the biomass of its phytoplankton is higher (more than 2-fold) than in Peipsi. Also, in Võrtsjärv bacteria are more abundant compared to Peipsi. However, the biomass of zooplankton and zoobenthos in Peipsi many times exceeds that of Võrtsjärv.

4.2. Field sampling

In **PAPER I** the sampling site situated in the southern part of Võrtsjärv between the eastern shore and the island of Tondisaar (58.20955° N, 26.0957° E; **PAPER I**, Fig. 2), where the mean water depth is 2.5 m. From the sampling site depth-integrated lake water samples and surface sediment samples were collected weekly in 2007 during two periods– from 15 May to 03 July and from 07 August to 30 October. A Willner-type (Uppsala University) gravity corer (Fig. 4) was used for sediment coring.

Figure 4. A Willner-type gravity corer was used for sample collection in **PAPER I**, photo by A. Kisand.

All samples were placed into an insulated box and immediately transported to the laboratory. Pigment analyses were conducted on sediment layers 0–1cm, 2–3 cm and 4–5 cm.

In **PAPER III** sediment samples were obtained from the middle of the broadest part of the lake Peipsi (58.78722° N, 27.32222° E; water depth 9.2 m) with a freeze corer (Wright, 1980) in winter 2002 and 2006. The 2002 core was subjected to dating and pore-water analyses; pigment analyses were performed on the 2006 core. The two cores were correlated through their loss-on-ignition curves. The same corer was used to take a 120 cm sediment core from the southern part of the Võrtsjärv (58.12833° N, 26.06944° E, water depth 1.40 m; **PAPER II**) in March 2003. *In situ* frozen sediments were carefully cleaned and sliced into continuous 1 cm thick sub-samples.

4.3. Core dating and other palaeoindicators

The cores in **PAPER II– IV** were dated using ^{210}Pb activity measurements. The sediment age was calculated using the model of constant rate of supply (CRS; Appleby & Oldfield, 1978). The ^{210}Pb-based age-scale of the cores was corrected using reference dates of the artificial radionuclides ^{137}Cs and ^{241}Am. The obtained age-scale was then validated by the sediment distribution of microscopically enumerated spheroidal fly-ash particles; the products of high-temperature fossil-fuel combustion, which concentration profile in sediments follow the characteristic features of fuel-burning history of the region (Nõges et al., 2006). Sediment chronology data, methodology, results and reliability of the chronology were published by Heinsalu et al. (2007, 2008).

In multi- proxy studies (**PAPER II–IV**) additional palaeolimnological parameters were analysed from the cores— organic matter, water and calcium carbonate ($CaCO_3$) content, phosphorus (P) fractions, molecular size structure of pore-water dissolved organic matter (*p*DOM) and diatom composition. Details of these methods and results are fully described in corresponding publications.

4.4. Pigment extraction

Pigment samples from the water column in **PAPER I** were collected and extracted as recommended by Mantoura & Llewellyn (1983). Depth-integrated water samples (50−100 mL, depending on water turbidity) were filtered through Whatman GF/C glass microfibre (1.2 μm) filters (precombusted at 400 °C for 4 h) and frozen (−20 °C) until the extraction. Acetone-methanol mixture (80:20 v:v) was added to the frozen GF/C glass-fibre filters in order to extract PhPs, thereafter the filtered samples were sonicated (Branson 1210) for 10 min. All PhP samples were extracted at −20 °C in the dark for 24 h.

Analysis of PhPs from the sediments in **PAPER I–IV** followed the recommendations of Leavitt and Hodgson (2001). The frozen sediment samples were freeze-dried and pigments were extracted with acetone-methanol mixture (80:20 v:v) at −20 °C in the dark for 24 h under a N_2 atmosphere. All pigment extracts were clarified by filtration through a 0.45 μm pore-size filter (Millex LCR, Millipore) before chromatographic analysis.

4.5. Pigment and data analysis

In **PAPER I** reversed-phase high-performance liquid chromatography (HPLC) was applied, using a Shimadzu Prominence (Japan) series system with a photodiode-array (PDA) detector (Fig. 5) to separate the PhPs.

A fluorescence detector with excitation wavelength set at 440 nm and emission at 660 nm was used to confirm pigment identification. The method was adapted from Airs et al. (2001) and slightly modified. As an ion-pairing reagent (IPR) 0.5 M ammonium acetate was added in a volume ratio of 2:3 to each PhP sample before the injection. To avoid chemical decomposition of pigments, the autosampler was cooled down to +5 °C (Reuss & Conley, 2005) and a maximum of 10 samples were loaded at a time. The sample injection volume was 50 μL. Separations were performed in a reversed-phase mode by using two Waters Spherisorb ODS2 3 μm columns (150 mm × 4.6 mm I.D.) in-line with a pre-column (10 mm × 5 mm I.D.) containing the same phase. A binary gradient elution method (Table 1) was used with isocratic holds between 0–2 and 30–43 min.

Figure 5. Shimadzu Prominence series HPLC system with degasser, two high pressure pumps, autosampler, column oven and photodiode-array (PDA) and fluorescence detectors that was used in phytoplankton pigment analysis in **PAPER I**.

Table 1. HPLC elution scheme and solvents used in the separation of phytoplankton pigments in **PAPER I**

	Time, min				
	0	2	30	43	50
Solvent A, %	50	50	100	100	50
Solvent B, %	50	50	0	0	50

Solvent A = 80% methanol : 20% 0.5 M ammonium acetate (pH 7.2) (v:v). Solvent B = 80% methanol : 20% acetone (v:v)

The flow rate remained constant during the elution, 0.8 mL min^{-1}. Absorbance was detected at wavelengths from 350 to 700 nm. The software 'LC solution ver. 1.22' (Shimadzu) was applied to collect and analyse the data. The integration of peak areas was made at each pigment absorbance maximum (Jeffrey et al., 1997b). Commercially available external standards from DHI Water and Environment (Denmark) were used for peak identification and quantification. Standard addition method was used to confirm correct peak identification. Typical chromatograms of this method are shown on Fig. 6.

Different HPLC equipment and slightly different elution scheme was used in **PAPER II** and **III** for sediment PhP analysis, these details are described in respective papers.

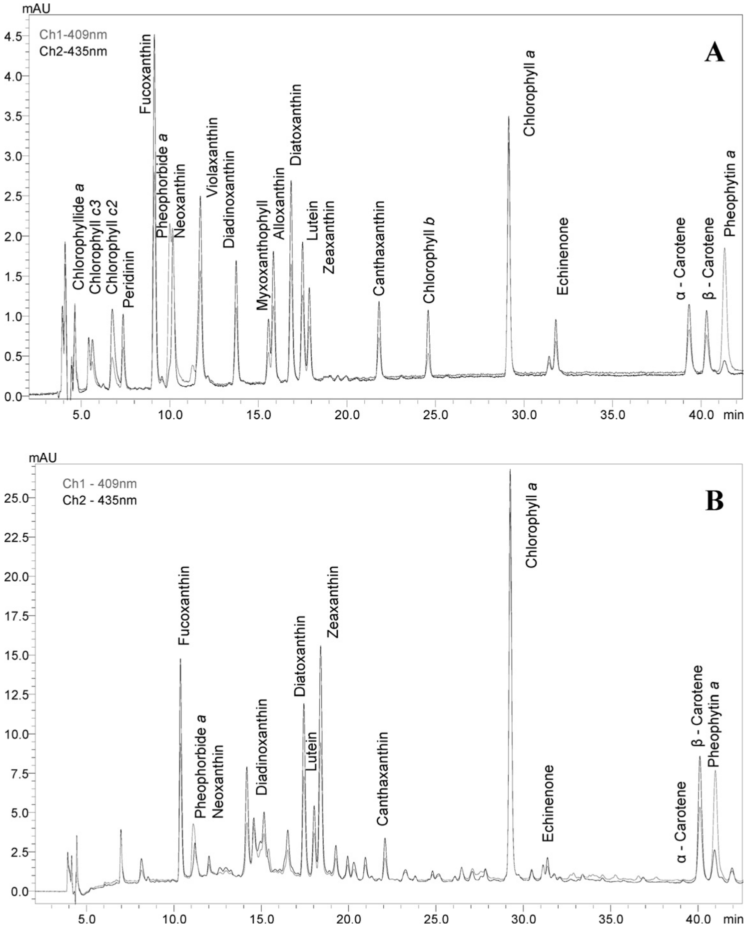

Figure 6. Typical chromatograms at wavelengths 409 nm and 435 nm **A)** Pigment standards and **B)** Pigments in the upper sediment layer of Lake Võrtsjärv in **PAPER I**.

Taking into account that cyanobacteria and diatoms are dominating phytoplankton groups in both of these lakes, only pigments associated with these groups and overall indicators of biomass were taken into analysis (Table 2).

Table 2. List of indicator pigments that were analyzed in **PAPER I–IV** from the water column and from the sediments and their taxonomic affinities, abbreviations and stability values. The relative degree of chemical stability is ranked from most (1) to least (4) stable. Modified from Leavitt & Hodgson (2001).

Pigment	Abbreviation	Stability	Major groups or process
Chlorophylls			
Chlorophyll *a*	Chl *a*	3	All photosynthetic algae, higher plants
Chlorophyll *b*	Chl *b*	2	Green algae, euglenophytes, higher plants
Chlorophyll c_2	Chl c_2	4	Diatoms, dinoflagellates, chrysophytes
Chlorophyll degradation product			
Pheophytin *a*	Pheo *a*	1	Chl *a* derivative (general)
Carotenoids			
β-carotene	β-Car	1	Most algae and plants
α-carotene	α-Car	2	Cryptophytes, prochlorophytes, rhodophytes
Fucoxanthin	Fuco	2	Diatoms, prymnesiophytes, chrysophytes, raphidophytes, several dinoflagellates
Lutein[1]	Lute or L	1	Green algae, euglenophytes, higher plants
Zeaxanthin[1]	Zea or Z	1	Cyanobacteria, prochlorophytes, rhodophytes, chlorophytes
Echinenone	Echi	1	Cyanobacteria
Canthaxanthin	Cantha	1	colonial cyanobacteria
Diatoxanthin[2]	Diato	2	Diatoms, dinoflagellates, chrysophytes
Diadinoxanthin[2]	Diadino	3	Diatoms, dinoflagellates, prymnesiophytes, chrysophytes, raphidophytes, euglenophytes, cryptophytes

[1] Lutein and zeaxanthin were not separated in **PAPER II–IV**, instead the sum of these pigments (Z + L) was used in data analysis

[2] Diatoxanthin may interconvert with diadinoxanthin in lake sediments as was confirmed in **PAPER I**.

Principal Component Analysis (PCA) in **PAPER II–IV** was carried out for ordination and classification of the sub-samples of the sediment core in relation to sediment quality variables. The PCA was applied to the whole data set, although in order to avoid redundancy and perform a more realistic ordination the variables with low percentage of contribution were eliminated. Kaiser's rule, which states that components with eigenvalues under unity should be discarded, was applied to determine the number of components to be retained in the PCA (Kaiser, 1960; Jolliffe, 1986). Sedimentary variables were analysed after centring and standardisation. PCA was performed with the Multivariate Statistical Package (MVSP), release 3.12 (Kovach 1999). For Kruskal-Wallis analyses and Spearman rank order correlations program STATISTICA (version 6.0) was used.

Instrumentally measured data on lake water-levels, annual mean air temperature (T_{air}) and precipitation for town Tartu (~50 km east from Lake Võrtsjärv) were obtained from the Estonian Institute of Hydrology and Meteorology.

Values for the North Atlantic Oscillation (NAO) index for the period 1899- 2001 were available on the website (http://www.cru.uea.ac.uk/cru/data/nao/). For winter NAO index (NAO_w) four months were selected, starting with December of the previous year.

5. RESULTS AND DISCUSSION

5.1. Sedimentation process of phytoplankton pigments in Lake Võrtsjärv

The concentrations of all studied PhPs in the water samples were lower from May to July than from August to October in 2007. However, in the upper sediment layers the contents of Chl *a*, Pheo *a*, Fuco, Diadino and Diato were higher from May to July than from August to October (**PAPER I,** Fig. 3A–G). Zea content in the studied sediment layers remained similar within the investigated periods, while Cantha increased towards autumn (**PAPER I,** Fig. 3F, G). Chl *a*, Fuco and Diadino generally decreased, while Diato increased from the sediment surface (0–1 cm) towards deeper sediment layers. Concentrations of Pheo *a*, Zea and Cantha were rather similar in all investigated sediment layers (**PAPER I,** Fig. 3A–G).

Chlorophyll *a* was the dominant pigment in water and in surface sediment layers within the whole investigated period. The mean concentration of Chl *a* in water was 17 times higher than that of Pheo *a*, while in upper sediment layers the difference was ~ 3 times (Fig. 7A). Chlorophyll *a* concentration in water was inversely correlated with that in

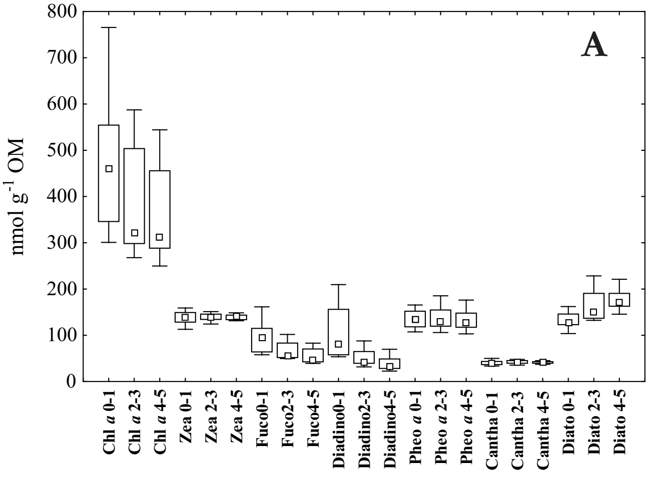

Figure 7. A) Concentrations of phytoplankton pigments in the upper sediment layers of Võrtsjärv in nanomoles per gram organic matter (OM) in 2007. Median, 25th and 75th percentiles as vertical boxes with error bars are presented.

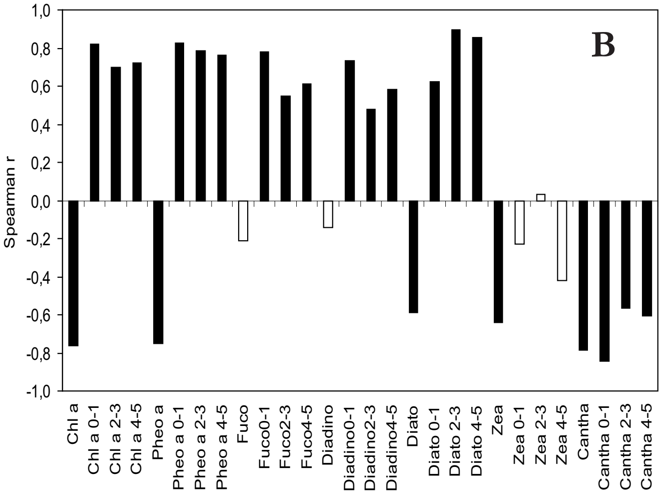

Figure 7. B) Spearman correlation coefficients (black bars significant at $p < 0.05$, white bars nonsignificant) of phytoplankton pigment concentrations and water level in Võrtsjärv in 2007. Chl *a*— chlorophyll *a*; Pheo *a*— pheophytin *a*; Fuco— fucoxanthin; Diadino— diadinoxanthin; Diato— diatoxanthin; Zea— zeaxanthin; Cantha— canthaxanthin. 0–1, 2–3 and 4–5 investigated sediment layers (in cm).

surface sediment layers, and the same was valid also for Pheo *a* and Diato. No correlation was detected between Fuco, Diadino, Zea and Cantha concentrations in water and in upper sediment layers except the positive correlation between Cantha in water and in the 0–1 cm sediment layer. The contents of the same pigment in different sediment layers were generally positively correlated (**PAPER I**, Table 2).

The water level in Võrtsjärv was decreasing during our study period from May to November (**PAPER I,** Fig. 3H). The concentrations of Chl *a*, Pheo *a*, Diato, Zea and Cantha in water were significantly negatively correlated with the water depth, while for Fuco and Diadino this correlation was weak and insignificant. Sediment pigment contents in all studied layers were significantly positively correlated with water depth except for Zea and Cantha. The content of Cantha in sediment was negatively correlated with water depth, while for Zea the correlation was insignificant (Fig. 7B).

The dynamics of PhPs in the upper sediment layers of Võrtsjärv generally did not correspond to their dynamics in the water. Because of the morphology of the lake basin the water column is not stratified, sediments are subjected to mixing by waves and characterized by a high accumulation rate, especially the surface layers (Heinsalu et al., 2008, Fig. 1C). This can be the reason why short-term coupling with water and sediment

PhPs was not detected in that large shallow and turbid lake. In stratified lakes where sediment layers are not disturbed by resuspension, such uncoupling could result from seasonal differences in the phytoplankton sinking rate, which is low during the phytoplankton population growing phase and high in the population decline phase (Sommer, 1984). In large shallow lakes like Võrtsjärv sedimentation is accompanied by intensive resuspension (Scheffer, 1998). Staying longer in the water column, more than 90% of PhPs could be degraded to colourless compounds before permanent burial, whereas the most rapid degradation occurs in dying cells and detritus (Leavitt & Carpenter, 1990a, 1990b; Leavitt, 1993). Resuspension is very strong in Võrtsjärv, and as the sediment trap experiments have shown, the major part (up to 96%) of the downward particle flux (including PhPs) is formed of resuspended material (Nõges et al., 1999). Wind speed and water level fluctuations have strong impact on resuspension intensity in large and shallow lakes, causing much higher resuspension and water turbidity at low water level during windy days. The water level of Võrtsjärv is typically low in winter and high in spring after the snowmelt, and its gradual decrease during summer and early autumn is followed by a small peak in late autumn (Haberman et al., 1998; Järvet, 2004). In 2007 the difference between the minimum and maximum water levels was 1.28 m (**PAPER I**, Fig. 3H). Higher sediment PhP contents (except Zea and Cantha) in spring (**PAPER I,** Fig. 3A–E) could partly originate from the previous autumn. After the formation of the ice cover the phytoplankton could calmly deposit on the bottom of the lake, and quite high PhP amounts were preserved in the sediments due to the low temperature and lack of resuspension under the ice. In spring, when the water level was high (**PAPER I,** Fig. 3H), the impact of resuspension was assumed to be relatively weak and therefore the deposited algal material of the spring phytoplankton maximum remained relatively less disturbed in the sediments. Also, the degradation of PhPs should be slow due to the still low water temperature. Together with the water level decrease towards autumn, the intensity of resuspension assumingly increased, as the earlier sediment trap experiments in Võrtsjärv have shown that the low water periods are characterized by a significantly higher sedimentation of resuspended material and, thus, more intensive resuspension than the high water periods (Nõges et al., 1999).

The degradation of PhPs intensified towards autumn for the higher water temperature and also because due to resuspension the pigments stayed for a relatively longer period in the illuminated and oxygenated water column. We found a strong positive correlation between sediment carotenoids associated with diatoms and the water level of the lake (Fig. 7B). This could indicate that the changes in the water depth are first of all important for settling and resuspension processes of rather heavy cells of diatoms (Stoermer & Smol, 1999). As diatoms in Võrtsjärv have a biomass peak in spring when the water level is high, this correlation could also be caused by periodicity. The coupling of the water level with the sediment pigments (Zea, Cantha) associated with cyanobacteria (CY) proved to be different from the coupling with diatoms (Fig. 7B). The biomass and proportion of CY in phytoplankton of Võrtsjärv generally increase towards the autumn (Nõges et al., 2004). The concentration of the marker pigment of colonial CY (Cantha) both in water and in surface sediments increased markedly towards the autumn (**PAPER I,** Fig. 3G), causing probably negative coupling with the decreasing water level in the lake (Fig.7B). Another CY marker pigment, Zea, associated with their total concentration, did not show any relationship with the water level. As the cells of CY are lighter than those of diatoms, the changes in the sedimentation/resuspension pattern due to the variations in water depth are not as important for CY as they are for diatoms. Due to relatively large cell dimensions, the sinking and floating velocities of colonial CY are enhanced if compared to other non-aggregated CY. *Microcystis* species (one of the dominant colonial CY also in Võrtsjärv) produce large colonies that could actively control their buoyancy and settling rate (Walsby & McAllister, 1987; Nõges & Laugaste, 1998; Roderick et al., 2000; Reynolds, 2006). The positive correlation between Cantha concentration in water and in the sediment top surface layer (0–1 cm) could indicate active migration of colonial CY between water and the sediment surface. The lack of correlation between Cantha concentration in water and in sediment subsurface layers of 2–5 cm (**PAPER I,** Table 2) could also support this hypothesis. Phytobenthos of Võrtsjärv, consisting mainly of CY and diatoms (Pork & Kõvask, 1973), might also confuse the coupling of CY and diatom marker pigment between the water and upper sediment layers. The development of benthic algae in lakes depends on light penetrating to the sediment surface. In the very turbid Võrtsjärv the estimated euphotic zone varied from 1.6 to 3.2 m, remaining lower than the mean (2.8 m) or maximum (6.0 m) water depth (Haberman et al., 1998; Reinart & Nõges, 2004). Monthly phytoplankton monitoring indicates that in the period of low water level resuspended algae from the sediment surface enrich phytoplankton species composition in the water column but do not contribute much to biomass (Nõges et al., 2004). Therefore the development of the benthic algae community in Võrtsjärv is limited.

Decrease in Chl *a*, Fuco and Diadino contents towards the deeper layers of the sediment core might be explained by chemical instability of these PhPs (**PAPER I,** Fig. 3A, C, D), e.g., both Fuco and Diadino contain a 5,6-epoxide group which enhances their quick degradation (Hurley & Armstrong, 1990; Reuss & Conley, 2005). Considerable decrease in unstable PhPs content between the sediment surface and other investigated layers indicates that mainly only the top of the sediment surface is included to resuspension in Võrtsjärv. Consequently, in case of unstable pigments the 'older' PhPs stored in deeper sediments are assumably more degraded than the pigments in the upper sediment layers. In comparison with diatom marker pigments, Zea and Cantha are chemically very stable, whereas Zea could be preserved even in aerobic environment (Hurley & Armstrong, 1990; Bianchi et al., 2000; Leavitt & Hodgson, 2001; Fietz et al., 2005; Reuss et al., 2005; Buchaca & Catalan, 2007, 2008). That could explain why there was no distinct difference between Zea and Cantha concentrations in different sediment layers. As Pheo *a* is the degradation product of Chl *a*, its content was assumed to increase towards the deeper sediment layers. Contrary to unstable Chl *a*, Pheo *a* is very persistent in sediments (Leavitt & Hodgson, 2001). However, Pheo *a* content did not show any distinct downward pattern in sediments and we could not track the degradation of Chl *a* on the basis of Pheo *a* content. Consequently, Pheo *a* and the marker pigments of CY (Cantha and Zea) in the upper sediment layers of Võrtsjärv seem to be rather conservative against degradation and could be used to track the historical changes in total phytoplankton and CY.

The water column irradiance is rather low in the shallow and turbid Võrtsjärv (Nõges & Laugaste, 1998; Reinart & Nõges, 2004). The diatom carotenoid, Diadino, which is active in the xanthophyll cycle could be transformed into Diato at high light to reduce the amount of energy reaching the photosynthetic reaction centres (Louda et al., 2002). Diatoms in Võrtsjärv are probably not under light stress as Diato concentration in the water column was nearly an order of magnitude lower than that of Diadino (**PAPER I,** Fig. 3D, E). On the contrary, in the upper sediment layers the content of Diato was higher than that of Diadino. Moreover, Diato content increased and Diadino content decreased downwards in the sediment core (**PAPER I,** Figs 3D, E; 4B). Although part of the light in Võrtsjärv penetrates to the sediment surface, it is obvious that Diadino transformation to Diato in upper sediment layers could not be caused by excess light and should take place also in darkness. Consequently, transformation of the investigated diatoms marker carotenoids in Võrtsjärv occurs mainly in sediments, not in the water column. Several other studies have found that the xanthophyll cycle is not necessarily linked to protection against excess light (Morales et al., 1990; Torsten et al., 2001; Fietz et al. 2005). Louda et al. (2002) established that Diadino disappeared and transformed to Diato within the first two weeks of dark incubation. The inter-molecular Diadino-Diato transformation mechanism has been described by Patoine & Leavitt (2006).

Previous investigations in Võrtsjärv have shown a rather high sedimentation rate, especially for the upper sediment layers where the compaction of sediments has not occurred yet (Heinsalu et al., 2008). A high sedimentation rate means quick final burial of PhPs, which ensures rather similar preservation conditions within several years. This could explain positive correlations of the contents of the same pigment in different sediment layers (**PAPER I,** Table 2). Quick final burial of chemically stable palaeoindicators favours preservation of the historical information in Võrtsjärv sediments and allows palaeolimnological reconstructions of the lake ecosystem (Heinsalu et al., 2008).

5.2. Lake Võrtsjärv— pigment data comparison with other palaeoindicators

5.2.1. Results of PCA and Spearman rank order correlation analyses

The PCA over the 100 year period was based on all analysed variables except *p*DOM characteristics as these parameters had low percentage of contribution. The first two axes of PCA described 83.4 % of the total variance in the data set. The first factor explained most of the variability (58.2%) and had positive loadings for $CaCO_3$ content, NH_4Cl–RP and sediment pigments. The second factor, accounting for 25.2% of the total variance, was correlated primarily with ΣFR, BD-RP and NaOH-RP.

On the basis of the PCA three time periods were differentiated (Fig. 8): 1900–mid 1950s, mid 1950s–mid 1990s and mid 1990s–early 2000s. According to the Kruskal-Wallis test the investigated variables differed significantly between the first two periods ($CaCO_3$, organic matter, Chl

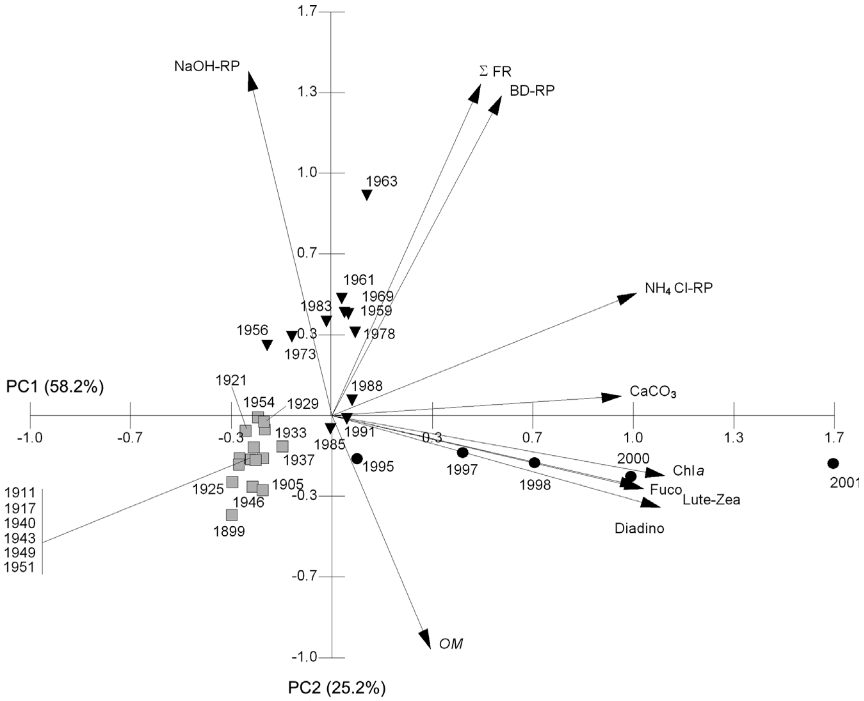

Figure 8. Principal Component Analysis (PCA) biplot showing three time periods differentiated on the basis of selected palaeoparameters (arrows) from the Võrtsjärv dated sediment core over the period from 1900 to 2001. Grey squares: 1900–mid 1950s; black triangles: mid 1950s–mid 1990s; black circles: mid 1990s--early 2000s. Chl *a*— chlorophyll *a*, Fuco— fucoxanthin, Diadino— diadinoxanthin, Lute-Zea— sum of lutein and zeaxanthin, OM— organic matter, $CaCO_3$— carbonate content, ΣFR— sum of sedimentary phosphorus (P) fractions, NaOH-RP— metal oxide bound P, BD-RP— reductant soluble P, NH_4Cl-RP— loosely sorbed P. The first PCA axis (PC1) explains 58.2% and the second 25.2% of the variation in the dataset.

a, ΣFR, NH_4Cl-RP, BD-RP), and between the first and the last period ($CaCO_3$, organic matter, Chl *a*, β-carotene, Z + L, Fuco, Diadino, humic substances (HS), NH_4Cl-RP, BD-RP, NaOH-NRP). Water level changes had strong positive relationship with NAO_w, T_{air} and precipitation in the second half of the 20th century (**PAPER II**, Table 2).

The Spearman rank order correlations of water level and climate parameters with selected palaeodata in different investigated time periods are given in **PAPER II**, Table 3. Palaeopigments (except Pheo *a*) correlated positively with $CaCO_3$ and NH_4Cl–RP and negatively with HS throughout the investigated timespan (Table 3).

Table 3. Spearman rank order correlations between selected palaeoparameters (n= 31) of Võrtsjärv for the period 1900–2001. Significant correlations are in bold ($p < 0.05$).

Palaeopigments	**$CaCO_3$**	**Area HS**	**NH_4Cl–RP**
Chl *a*	**0.747**	**-0.640**	**0.696**
β-Car	**0.590**	**-0.477**	**0.592**
Z + L	**0.745**	**-0.751**	**0.606**
Fuco	**0.577**	**-0.556**	**0.473**
Diadino	**0.664**	**-0.647**	**0.563**

Keys for palaeopigments are in Table 2. $CaCO_3$— carbonate content; Area HS— total peak areas of humic substances in the organic matter dissolved in sediment porewater; NH_4Cl-RP— loosely sorbed sedimentary phosphorus fraction.

5.2.2. Eutrophication history within 20th century

The palaeopigments composition in Võrtsjärv sediments indicates that cyanobacteria and diatoms were the dominant phytoplankton groups in the lake also during the first half of the 20th century (**PAPER II**, Fig. 2), the first period clearly distinguishable on the PCA plot (Fig. 8). In this period no significant increase in phosphorus (P) input from the lake catchment occurred as the content and variability of studied P fractions is rather low (**PAPER II**, Fig. 2). Probably sediment resuspension was the most important P source for Võrtsjärv phytoplankton at that time. The large catchment area ensure naturally higher nutrient inflow to Võrtsjärv while the shallowness of the lake causing strong resuspension level favors nutrient release from sediments to the water. Therefore slightly eutrophic conditions prevailed in the lake already from 1900 to mid1950s. Earlier Heinsalu et al. (2008) also showed that the sedimentary diatom flora since about 1840 indicates of moderately eutrophic shallow-water conditions in Võrtsjärv. The reasons are likely natural– the lake's large catchment area, its shallowness, high resuspension of sediments because of that and thereof sediments as a source of nutrients. The regular oscillation of the water level has a strong impact on Võrtsjärv ecosystem controlling its light climate as well as nutrient cycles, and through that also bioproduction and organic matter (OM) decomposition.

The water level itself is determined mainly by the winter climatic conditions. In Estonia the western airflow from the Atlantic during positive NAO remarkably increases air temperature and the amount of precipitation in winter. Warmer winters cause higher water level in spring and also during the following summer and autumn in Võrtsjärv. With higher water level algal density is rather determined by the light availability than by nutrients resulting in lower phytoplankton biomass. When water level is lower more light is available and nutrient limitation takes over the control of phytoplankton. The shallower lake means higher phytoplankton biomass per unit volume and also per unit area (Nõges et al., 2003; 2010a). As explained in the previous section, the proportion of pigments derived from phytobenthos is negligible in the pigment content accumulated to the Võrtsjärv sediment.

The amount of precipitation showed quite a strong positive relationship with water level in the first half of the 20th century (**PAPER II**, Table 2). Of the fossil pigments only Diadino had significant, but positive correlation with the water level. Also the water level fluctuations and the amount of precipitation significantly correlated with *p*DOM characteristics like HS quantity (expressed as area of HS) and M_w (**PAPER II**, Table 3). Similar positive relationship was obtained by Leeben et al. (**PAPER IV**) suggesting higher transport of land-derived organic particles and substances into the lake during increased water level. According to Toming et al. (2009) water level and yellow substance (constituting up to 90% of dissolved organic matter in boreal region) are in positive correlation in Võrtsjärv. Concentrations of yellow substance have maximum values in spring and generally decrease towards autumn.

The reason why the relationship between the water level and the phytoplankton biomass in the water column (Nõges et al., 2003; 2010b) is not observed for the total phytoplankton biomass (Chl *a* and β-carotene) and the fossil phytoplankton pigments in sediment record can be a result of high resuspension in this shallow lake, what due to wind-induced wave activity constantly mix surface sediment layers and cause decay of settled algae and their pigments. Other arguments can be quite weak correlation between NAO_w and the water level registered for this period (**PAPER II**, Table 2) and the common feature for calculated ^{210}Pb radiometric ages, the relatively high standard error of dates for deeper sediment horizons (see Fig. 1c in Heinsalu et al., 2008), what can complicate temporal comparison of datasets.

Beside water level changes also air temperature fluctuation may influence the entire water column of shallow, non-stratified lakes and therefore these lakes are likely to respond more directly to short-term weather variations (Arvola et al., 2010). Higher water temperature in summer favour cyanobacterial blooms (Blenckner et al., 2010) and therefore the short-term increase of Z + L in the 1930s could be induced by the rise of T_{air} in these years (**PAPER II**, Fig. 2).

The PCA analysis evidences changes in Võrtsjärv ecological conditions since the 1950s (Fig. 8). Increase in palaeopigments (except Pheo *a*), elevated contents of P fractions (except NaOH-NRP and HCl-RP) and $CaCO_3$ as well as decline in HS and M_w are distinctive for the time period from mid 1950s to mid 1990s (**PAPER II**, Fig. 2). Earlier investigations indicate that since 1960s the urbanisation and agricultural activity in Võrtsjärv drainage area accelerated. Long-term monitoring dataset have revealed that higher nutrient loading to the lake enhanced phytoplankton biomass in 1960s demonstrating its maximum values in 1970s (Nõges & Laugaste, 1998; Nõges & Nõges, 2006). Moreover, the analysis of phytoplankton taxonomic indices have shown a continuous deterioration of Võrtsjärv ecological status during the 44-year period of limnological investigations (1964-2007; Nõges et al., 2010a).

Increase in the content of palaeopigments and P fractions from the mid 1950s confirm accelerated nutrient loading to the lake and a rise in bioproduction. The positive correlation of palaeopigments (except Pheo *a*) with NH_4Cl-RP and $CaCO_3$ indicate accelerated eutrophication of the lake (Table 3). NH_4Cl-RP includes phosphates dissolved in sediment porewater and loosely sorbed on sediment particles. Higher concentrations of NH_4Cl-RP encourage diffusion of P from the sediment to the lake water, since diffusion is driven by concentration gradients. If no other processes precipitate phosphates again, diffusion from sediments can continually provide P supply for lake's primary producers.

The increase in $CaCO_3$ content in sediments from the mid 1950s has been considered as indicator of the lake eutrophication as intensive primary production induces rise of water pH which results in accelerated $CaCO_3$ precipitation (Hodell et al., 1998; Dean, 1999). The decline of HS and weight-average molecular weight (M_w) values since 1960s--1970s coincide with phytoplankton maximum values in 1970s as well as with increase of palaeopigments since mid 1950s (**PAPER II,** Fig. 2). The lower HS values and molecular weight of *p*DOM in sediments reflects rather the autochthonous than allochthonous origin of OM (Bergström & Jansson, 2000, Jansson et al., 2000) and hence increased in-lake bioproduction. Negative relationship of HS with palaeopigments is in agreement with this statement. However lower M_w might indicate that microbial mineralization of OM is an important degradation process in Võrtsjärv sediments (Münster & Chróst, 1990).

For the period from mid-1950s to mid-1990s the correlations between all used climate parameters (NAO_w, T_{air}, the amount of precipitation) and the water level were much stronger (**PAPER II,** Table 2). In Võrtsjärv the NAO_w index is positively related with biomass of CY and diatoms as well as with total biomass of phytoplankton in spring (Nõges & Järvalt, 2004, Nõges et al., 2010b). According to our analysis NAO_w, the amount of precipitation and T_{air} had positive correlation with palaeopigments (Z + L, Fuco and Diadino) and negative correlation with P fractions (ΣFR, BD-RP and NaOH-RP) during this period. Accelerated eutrophication and better preservation conditions of palaeopigments during higher water level can be the cause of overall rise in fossil pigments content and their positive relationship with NAO_w index. Higher floods follow usually colder winters because due to the freezing of soils most of the meltwater reaches rivers as surface runoff, carrying large quantities of nutrients to the lake (Nõges & Järvalt, 2004). This could be one possible explanation for negative correlation of NAO_w and T_{air} with ΣFR, BD-RP and NaOH- RP.

Eutrophication problems will enhance because of climate warming as it is expected to lead to increased external nutrient loading. However the effects of climate change on shallow temperate lakes will mimic the effects of human induced eutrophication and to ascertain its magnitude in deterioration process is complicated (Mooij et al., 2007, Nõges et al., 2008). In the case of Võrtsjärv the effects of climate change include change in temperature, but even more hydrological change that is likely to be much more important. Water level oscillations in Võrtsjärv induce changes in the composition of dominant phytoplankton species: in high water period shadow-tolerant species are favoured and vice versa (Nõges et al., 2003, Nõges et al., 2010b). Unfortunately palaeopigments are only bioindicators for phytoplankton groups not species (Leavitt & Hodgson, 2001). In our opinion this is the reason why photosynthetic pigments are poor water level fluctuation indicators in Võrtsjärv.

Together with the collapse of extensive agriculture in early 1990s the external load of nutrients to Võrtsjärv remarkably declined (Järvet, 2004). The weakening of anthropogenic pressure to lake ecosystem is contrarily reflected in the sediment core as investigated P fractions (except NaOH-RP), fossil pigments, sediment OM and $CaCO_3$ content sharply increased since mid 1990s (**PAPER II,** Fig. 2). The most likely explanation would be, that the upper 0-13 cm sediment layers (period from mid 1990s to early 2000s) are yet unconsolidated and therefore they are clearly different from other horizons. Microbial activity and thus degradation processes of settled material in upper sediment layers are much higher than in deeper sediments (Wetzel, 2001). Due to resuspension surface sediment layers are involved in lake's P cycle and a part of settled P is released back to the water column. The peak of Chl *a*/ Pheo *a* ratio in the 1990s reveals that degradation of fossil pigments is in progress (**PAPER II**, Fig. 2). However the sharp increase of palaeopigments coincided with the rise of monthly measured chlorophyll *a* concentration in the water column since the 1990s: from about 20 mg m^{-3} to 50 mg m^{-3} (Fig. 7 in Nõges et al., 2011). According to the monitoring data the biomass of the filamentous cyanobacteria *Limnothrix planctonica* increased since 1990s in Võrtsjärv. This cyanobacteria builds up a considerable standing stock by autumn. High biomass in autumn is characterized by high Chl *a* content stimulating higher primary production (Nõges et al., 2011).

Together with the palaeopigments the T_{air} increased since the 1980s (**PAPER II**, Fig. 2). Generally phytoplankton is the first to benefit from higher water temperatures (Mooij et al., 2007). Thus, the rise of palaeopigments since the mid-1990s could be also favoured by increasing T_{air}

5.3. Lake Peipsi— pigment data comparison with other palaeoindicators

5.3.1. Results of PCA analyses

The PCA over the 100 year period of Peipsi palaeodata was based on phytoplankton pigments, *p*DOM characteristics and diatom remains, as these parameters had high percentage of contribution. The first two axes of PCA described 64.2 % of the total variance in the data set. The first factor explained 43% of the variability and had positive loadings for sediment pigments, planktonic diatoms and organic matter content. The second factor, accounting for 21.2% of the total variance, was correlated primarily with the percentage of low molecular weight *p*DOM and absorbance index. Similar to Võrtsjärv the PCA analysis differentiated three time periods (Fig. 9): 1900– mid 1950s, mid 1950s– mid 1990s and mid 1990s to early 2000s.

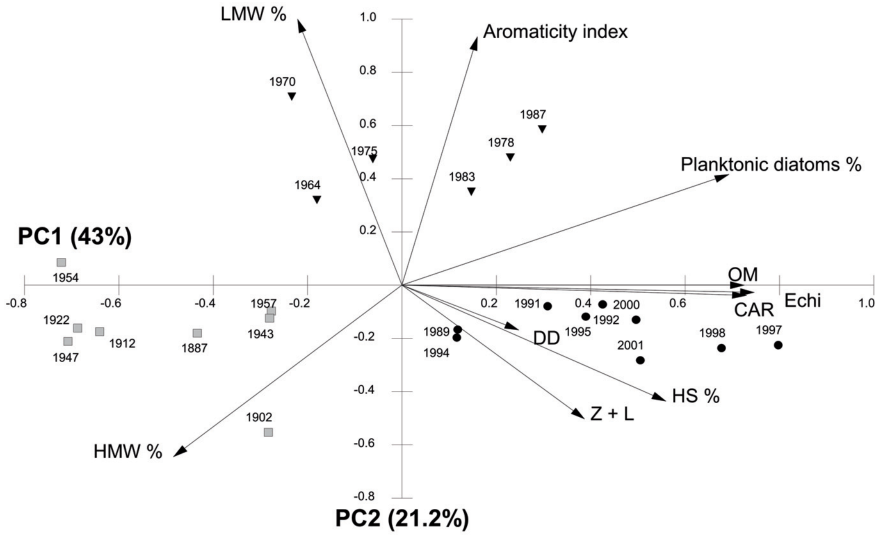

Figure 9. Principal Component Analysis (PCA) biplot showing three time periods differentiated on the basis of selected palaeoparameters (arrows) from the Lake Peipsi dated sediment core over the period 1887– 2001. Grey squares: 1900 to mid 1950s; black triangles: 1960s to 1990s; black circles: 1990 to early 2000s. Aromaticity index— ratio of absorbances A_{250}/A_{360}, HMW % and LMW %— percentage of high and low molecular fraction of pore water dissolved organic matter respectively, HS %— humic substances, OM— organic matter, Echi— echinenone, CAR— sum of α- and β-carotenes, DD— sum of diato- and diadinoxanthin, Z + L— sum of zeaxanthin and lutein. The first PCA axis (PC1) explains 43% and the second 21.2% of the variation in the dataset.

The quantities and profiles of fossil pigments in the sediment record of Peipsi vary greatly. The concentrations of Chl *a* and Chl c_2 in the lower part of the record (50 and 1 nmol g^{-1} OM, respectively) are approximately one quarter of those in the upper part (**PAPER III**, Fig. 1a, c). Their concentrations started to increase in the 1970s. The distribution of Cantha exhibits a reverse pattern, having a slightly higher concentration (around 50 nmol g^{-1} OM) before the 1920s, after which it diminishes somewhat (to 40 nmol g^{-1} OM) and remains stable up to the top of the core (**PAPER III**, Fig. 1f). The concentrations of Echi and Zea vary over the ranges 15–30 and 30–45 nmol g^{-1} OM, respectively (**PAPER III**, Fig. 1d, e). Their lowest concentrations are detected around the mid-20th century. The concentration of Chl *b* varies over the range 5–10 nmol g^{-1} OM (**PAPER III**, Fig. 1g) and its stratigraphy appears similar to those of Echi and Zea. The lowest concentrations of Chl *b* occur in the 1960s and 1970s.

5.3.2. Temporal changes in the quantity and structure of *p*DOM

The concentration of *p*DOM (expressed as carbon) in the sediment varies from 2 to 3 mM (**PAPER III**, Fig. 1k, Table 2). Its highest values are around 1900 and at the end of the 20th century. The weight-average molecular weight (M_w) was less than 7 kDa, varying from 3.5 to 5 kDa (**PAPER III**, Fig. 1l). The number-average molecular weight (M_n) of *p*DOM was less than 4 kDa and mainly in the range 1–3 kDa (**PAPER III**, Fig. 1m). The ratio of M_w to M_n, called polydispersity, has been used as a quantitative measure of the molecular weight distribution of OM in a mixture (Chin et al., 1998). For pure organic substances the ratio is equal to 1; for a mixture of molecules it is greater than 1.

In the sediments of Peipsi, the polydispersity of *p*DOM varies over a very narrow range (**PAPER III**, Fig. 1n), indicating that temporal changes in the molecular weight distribution are small. However, the aromaticity index (ratio of absorbances A250/A360) (Peuravuori & Pihlaja, 1997) estimates of *p*DOM demonstrate slightly elevated values from the 1960s to the late 1980s (Fig. 9; **PAPER III**, Fig. 1o, Table 2), implying that the proportion of substances of smaller size and lower aromaticity has increased in the OM accumulated during this period. Ordinarily, lower aromaticity and molecular weight (hence also size) are characteristic of autochthonous OM, while compounds with higher aromaticity and molecular weight predominate in OM derived from terrestrial sources (McKnight et al., 2001). Stratigraphic changes in the distribution of different molecular weight fractions confirm that the proportion of high molecular weight *p*DOM has decreased somewhat since the 1940s and the proportion of low molecular weight substances has increased (Fig. 9; **PAPER III**, Fig. 1p, Table 2).

5.3.3. Onset and course of eutrophication

Qualitative phytoplankton proxies— the concentrations of Chl *a*, Chl c_2, Echi and Zea— indicate that a shift of the lake ecosystem towards eutrophy started in the 1970s. From that time onwards the concentrations of the pigments are higher than in earlier years, or are gradually increasing. Statistical analysis reveals that changes in the concentration of Chl *a* correlate well with those of Echi and Chl c_2 (**PAPER III**, Table 2), suggesting that the main contributors to the phytoplankton biomass in the lake have been cyanobacteria and diatoms. However, it is difficult to estimate which of these has made the larger contribution. On the one hand, chlorophylls are more photosensitive than carotenoids. On the other, diatom cells sink more rapidly than CY. Moreover, many CY species form surface blooms and even after the bloom collapse, their colonies continue to float on the surface, prolonging their exposure to sunlight. Different sinking rates of CY, and hence different times of exposure to sunlight, could also explain why the concentration of Zea was higher than that of Echi in the sediments of Peipsi, whereas according to the monitoring data, the biomass of filamentous CY in the lake has been higher than that of unicellular *Microcystis* species (Nõges et al., 1996). The findings of Bianchi et al. (2002) suggest that unicellular CY (including picocyanobacteria) may have higher sedimentation rates than the N_2-fixing filamentous CY.

Cantha is the only pigment whose concentration shows no change during the 1970s. As mentioned previously, Cantha has been widely detected also in herbivores. Moreover, when Quiblier- Llobéras et al. (1996) observed the summer planktonic community in an oligo-mesotrophic lake, Cantha was found only in zooplankton, but it was totally absent from phytoplankton. In the case of Peipsi, further investigations should be conducted to determine whether Cantha is a marker pigment of specific CY taxa or whether it is confined to herbivores.

The changes in the pigment ratios in the sediment record imply that the ecosystem changed in the 1960s (**PAPER III**, Fig. 1h–j). The sediment composition data suggest that the sediment deposited since the 1960s clearly differs from the underlying layers: it is richer in OM and less compacted (**PAPER III**, Fig. 1q, r). The preservation index of Chl *a* declines steadily, simultaneously with changes in the pigment ratios, and this also implies that conditions in the water column or/and sediments have changed since the 1960s (**PAPER III**, Fig. 1b).

On the basis of long-term investigations of Peipsi, it is generally considered that the lake trophy started to increase in the 1960s. In the 1970s, the process accelerated and a high level was reached in the 1980s. From the early 1990s until 1996 an improvement in the lake water quality was observed. However, since 1997 there has been a continuous and even accelerating deterioration of the lake status (Nõges et al., 1996; Nõges & Nõges, 2006). Changes in fossil pigments accord well with the monitored course of eutrophication, except for the transient recovery of the lake in the 1990s, which is not clear in the fossil pigment stratigraphy. However, limnological data of phytoplankton biomass from that period suggest that despite a lowered nutrient level in 1988–1994 (Loigu & Leisk, 1996), the average phytoplankton biomass remained high (Laugaste et al., 1996). As shown by the *p*DOM data, changes in the lake OM occurred even before the 1960s. The distributions of molecular weight fractions of *p*DOM indicate a change in the late 1930s to early 1940s (**PAPER III**, Fig. 1p), suggesting a rise in the proportion of autochthonous matter in the OM pool. The increase in the autochthonous contribution to the bulk of OM becomes more and more evident over the following years. According to the *p*DOM characteristics, the autochthonous component in the sediments was highest during the 1970s and 1980s (**PAPER III**, Fig. 1o, p). A subsequent reduction in the proportion of the low molecular weight (LMW) fraction and an increase in the proportion of OM with higher aromaticity in the *p*DOM implies a somewhat decreased contribution of autochthonous matter in the bulk of OM during the 1990s, thus indicating a slight deceleration of eutrophication. Thus, we conclude that even the pre-eutrophication conditions in Peipsi can be assessed by the sedimentary organics.

Comparison of the fossil pigment chromatograms suggests that the pigment composition of phytoplankton has remained relatively unchanged over the entire time span investigated. This finding coincides well with the results of Laugaste et al. (1996). A rather stable, mesotrophic species composition characterizes the sedimentary diatom assemblage for the period 1880–1950 (Heinsalu et al., 2007). The sedimentary profile and preservation index of Chl *a* obtained in the present study indicate a low algal standing crop and high stability of the lake conditions at that time (**PAPER III**, Fig. 1a, b). Besides the low Chl *a* concentrations, the high levels of Echi, Zea and Chl *b* in the sediments dated to the 1900–1920s are noteworthy (**PAPER III**, Fig. 1d, e, g). A slight concurrent increase in the *p*DOC concentration is also discernible (**PAPER III**, Fig. 1k). Constituents of fluorescent *p*DOM were also present in greater quantities in the lower part of the sediment record than in the sediment layers dated to the mid- 20th century (Heinsalu et al., 2007: Fig. 6a, b). This may be explained by the fact that the environmental conditions in the lake during that period were favourable for the preservation of OM and also other pigments, e.g. Cantha (**PAPER III**, Fig. 1f), Diato and β-Car (the last two are not shown). Another possible reason is revealed by comparing the temporal changes of these OM constituents with the changes of water level (**PAPER III**, Fig. 2). Keeping in mind that the dating of the lower part of the core involves a greater error, one could speculate that the runoff of OM from the lake catchment was higher in that period than in previous and subsequent decades. The presence of a greater proportion of large aromatic organic substances supports the hypothesis of intensive terrestrial transport of OM, as they indicate that allochthonous OM predominated in the lake ecosystem before the 1930s.

Unfortunately, the water level observation series is too short to detect any further regularities, especially since the influence of waterlevel fluctuations is severely masked in the upper part of the sediment record by progressive eutrophication of the water body. Therefore, the extent of external loading of OM to the Peipsi ecosystem at the beginning of the 20th century still remains ambiguous.

5.4. Palaeolimnological comparison of lakes Peipsi and Võrtsjärv

5.4.1. Lithostratigraphy and sediment main constituents

The sediments from Võrtsjärv were greenish brown gyttja, with dark layers, probably containing aggregates of iron sulphide (FeS) around the 1950s, 1970s and 1990s (**PAPER IV**, Fig. 3a). The lower part of the core from Peipsi was homogeneous brownish green gyttja, comprising the sediment layers accumulated until the 1930s. Subsequently, black FeS grains appeared and the sediment colour became gradually darker. The Peipsi sediments settled between the mid-1960s and mid-1980s were the darkest. The OM content in the lower part of the records (up to the mid-twentieth century) of both lakes ranged between 24 and 26% (**PAPER IV**, Fig. 3a). Starting from the sediments accumulated around the 1960s its curves for the lakes diverge: while in Peipsi the OM content increased, in Võrtsjärv it decreased. The OM reduction in the latter lake was due to a gradual increase (from 7–8% prior the 1960s to 22% in the 1990s) in carbonate. In the Peipsi sediments the carbonate content was only 1–3% and it remained stable through the whole record.

5.4.2. Records of sub-fossil pigments

The sum of α- and β-carotenes (CAR) in the Võrtsjärv sediments was rather constant around 50 nmol g^{-1} OM up to the mid-twentieth century

(**PAPER IV**, Fig. 3b); thereafter it became very variable (40 and 70 nmol g^{-1} OM). The highest CAR content, 95 nmol g^{-1} OM, was found in sediments dated at 1998. In the Peipsi record, the highest CAR content, of around 90 nmol g^{-1} OM, was observed in the sediments dated to 1900–1905, the mid-1970s–late 1980s and the late 1990s; in the rest of the record the CAR content varied between 71 and 77 nmol g^{-1} OM.

The sum of diatom marker pigments, diadinoxanthin and diatoxanthin (DD) in the Peipsi sediments was quite stable, being between 80 and 100 nmol g^{-1} OM. In the Võrtsjärv sediments, the DD content was ca. half as much— between 40 and 60 nmol g^{-1} OM, which dropped to 32 nmol g^{-1} OM in the midtwentieth century. The average contents of the marker pigments of cyanobacteria (zeaxanthin and lutein (Z + L)) in the sediment records of the two lakes were almost equal— about70–80 nmol g^{-1} OM. In Peipsi, a little higher content of Z + L, of around 90 nmol g^{-1} OM, was detected only in the oldest sediment layers. In Võrtsjärv, these pigments started to increase in the sediments from the mid-1940s. After a short-term drop around the 1950s from 80 to 60 nmol g^{-1} OM the increase continued, reaching 100 nmol g^{-1} OM in the 1980s and over 150 nmol g^{-1} OM in the 1990s. The Echi (pigment in some cyanobacteria) content in the lakes' sediment records was markedly lower than the contents of other subfossil pigments. In Võrtsjärv it fluctuated mainly between 1 and 10 nmol g^{-1} OM. In Peipsi, the Echi content varied from 15 to 30 nmol g^{-1} OM, the highest values were from the early 1980s to the late 1990s.

The DD/CAR ratio in the Peipsi record was constant around 0.025, and the ratio in Võrtsjärv was three times higher, with wider variations. However, in both lakes, clear trends or shifts in its distribution were missing. In contrast, the Echi/CAR ratio exhibited higher values, between 0.2 and 0.3, in the Peipsi sediments and lower values of 0.1 in Võrtsjärv. Like in the case of the profiles of sub-fossil pigments, this ratio varied more in the Võrtsjärv sediments than in Peipsi.

5.4.3. Sub-fossil diatom records

In the diatom assemblage of Võrtsjärv, small-sized *Fragilaria* species (*Fragilaria brevistriata* Grun., *F. construens* (Ehrenb.) Grun. and *F. pinnata* Ehrenb.) dominated (**PAPER IV**, Fig. 3c). Their proportion in the total abundance of diatoms gradually diminished from the 1950s when the relative abundance of *Aulacoseira ambigua*, a eutrophic water diatom, progressively increased, reaching 45% in the uppermost layer.

In Peipsi, small-sized *Fragilaria* species (*F. brevistriata* and *F. heidenii* Østr.) were only half as abundant as in Võrtsjärv. Their relative abundance in the diatom assemblage started to fall in the 1950s like in Võrtsjärv, but the decline in Peipsi was more pronounced. In Peipsi typical large-lake species *Aulacoseira islandica*, *A. granulata* and *A. ambigua* predominated in the diatom assemblage until the 1950s. A decade later the diatom *Stephanodiscus parvus* Stoermer & Håk., characteristic of eutrophic waters, started to increase in Peipsi, making up nearly 30% in the 1990s. Thereafter its importance diminished and *A. islandica* became slightly more abundant. In contrast with Peipsi where in the diatom assemblage planktonic species prevailed, in Võrtsjärv planktonic diatoms contributed only one-third. In both lakes, the proportion of planktonic species started to increase in the 1950s.

5.4.4. DOM in pore water

In the Võrtsjärv sediments, the average absorbances at 254 nm (A_{254}) values were about two-fold higher than in Peipsi (**PAPER IV**, Fig. 3d). In the Peipsi record the A_{254} variations with the depth were minimal: somewhat elevated values occurred only in the 1900s and 1990s. In the Võrtsjärv sediments, where fluctuations in the A_{254} were very large, the highest values were for the early 1970s.

The A_{250}/A_{360} ratio was around 4 in the lower parts of the cores in both lakes. In the Peipsi sediments, this ratio started to vary more widely in the 1950s and attained values as high as 7–8 in the 1980s; however, the ratio fell to the initial level of about 4 in the 1990s.

Weight-average molecular weights (M_w) of porewater DOM in Võrtsjärv were ca. one-half of those in Peipsi (**PAPER IV**, Fig. 3d). In the Peipsi porewater samples M_w of DOM exhibited enormous instability, especially in the sediment accumulated since the mid-1960s. Compounds with medium molecular weights (MMWs) dominated in the pore-water DOM of the sediments in both lakes (**PAPER IV**, Fig. 3d). In the lower part of the Võrtsjärv record the relative abundance of MMW in the total pore-water DOM varied between 55 and 80%, but after the mid-1970s their per cent share decreased in some years to 30%. In Peipsi the proportion of the MMW fraction in the older sediment layers contributed from 70 to 80% to the total pore-water DOM, from the mid-1930s its level slightly increased, reaching values over 95% in some periods.

In the sediment layers of the lakes accumulated in the early twentieth century, compounds with high molecular weight (HMW) comprised about 20% of pore-water DOM. Only in the Peipsi sediments from the late-1930s, did these compounds show a drop in their contribution by 10–15%. At the same time, the proportion of compounds with low molecular weight (LMW), which until the 1940s accounted for 10% in pore-water DOM increased up to 25%. In the 1990s, the proportion of LMW in the Peipsi sediments again fell. In the Võrtsjärv pore-water DOM their proportion varied between 3 and 35%, reaching 56% in the 1990s.

5.4.5. PCA analysis of palaeodata

The PCA analysis of palaeodata (Fig. 10) applying Kaiser's rule revealed five components (plausible factors) for both lakes with eigenvalues > unity. These five factors accounted for 86.7% of the total variance in the Peipsi and 91% in the Võrtsjärv data. The first principal component (PC1) explained 48.3 and 40.7%, in the two lakes, respectively, and the second 14.8 and 18.2%, respectively, of the total variance in the palaeodata. As the PC1 described almost half of the total variance in the data set and its scores plot over the period from the 1970s to the end of the twentieth century contained features attributable to nutrient availability and changes in in-lake production it was assumed that this component could detect the shift in the lakes' trophy.

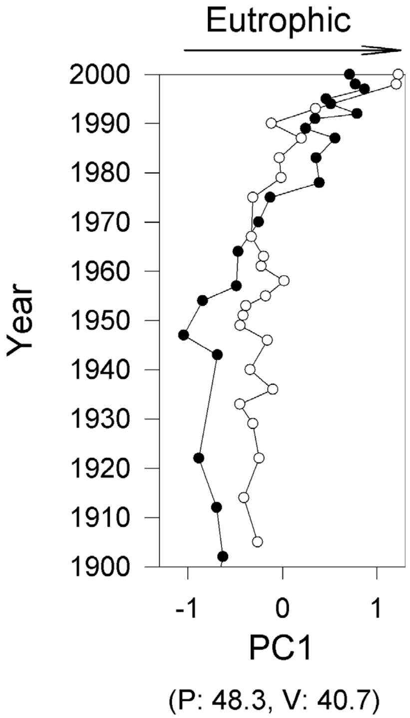

Figure 10. Stratigraphic plots of principal component 1 (PC1) extracted from the palaeoindicators of lakes Peipsi (closed dots) and Võrtsjärv (open dots) by principal component analysis and showing changes in the lakes' trophy over the twentieth century. The percentage of eigenvalues is given in parentheses. **P**— Lake Peipsi, **V**— Lake Võrtsjärv.

5.4.6. Correlations of the proxies of phytoplankton with water-level changes

In both lakes, the relationships between changes in the sub-fossil pigments content and in water level over the 100-year period (1900–2000) were weak (Table 4). Several proxies indicated that in the mid-1950s, the two lake ecosystems started to alter markedly. Therefore, Spearman rank correlation coefficients between the palaeodata and water-level changes were also calculated separately for 1900–1949 and for 1950–2000 (Table 4).

This analysis revealed a strong positive correlation of water level in both lakes with the proportion of planktonic species in the diatom assemblages in the earlier period but not for the period 1950–2000. Before the 1950s the CAR and DD contents showed a strong and the Echi content a moderate positive correlation with the water-level fluctuation (WLF) for Võrtsjärv but a very weak one for Peipsi. In the second half of the twentieth century the relationships were switched: becoming weakly or moderately negative between the contents of sub-fossil pigments and water-level changes for both lake sediments, except for Echi in Peipsi.

Table 4. Spearman rank order correlation between changes in water level and phytoplankton proxies of lakes Peipsi and Võrtsjärv calculated for the period 1900–2000 as well as separately for the pre-eutrophication period (before 1950) and after the enhanced nutrient loading (1950–2000).

Proxy	1900–2000		1900–1949		1950–2000	
	Peipsi	Võrtsjärv	Peipsi	Võrtsjärv	Peipsi	Võrtsjärv
CAR	–0.012	0.043	0.071	**0.724**	–0.002	–0.259
DD	–0.188	0.031	0.129	**0.797**	**–0.401**	–0.276
Echi	**0.309**	0.204	0.050	0.592	0.381	–0.074
Z+L	–0.012	–0.378	0.171	–0.205	–0.095	–0.444
Planktonic diatoms %	0.208	–0.037	0.750	**0.833**	–0.229	–0.211

The palaeodata was detrended using a quintic polynomial. The whole data set was smoothed using the 3 years running average repeated twice— from the beginning and the end of the data. The underlined correlation coefficients are significant at $p < 0.10$, bold at $p < 0.05$. CAR— sum of α- and β-carotenes, DD— sum of diadinoxanthin and diatoxanthin, Echi— echinenone, Z + L— sum of zeaxanthin and lutein

5.4.7. Comparison of the palaeorecords with monitoring data

According to our results the contents of CAR (total biomass), DD (diatoms) and Echi (filamentous CY) were on average 1.4, 1.6 and 3 times, respectively, higher in the Peipsi sediments than in those of Võrtsjärv (**PAPER IV**, Fig. 3b). However, according to monitoring data (1962–2000) the biomass of phytoplankton in Peipsi was 2.5 times and based on Chl *a* concentration, 1.8-fold lower than in Võrtsjärv (Nõges et al., 1996; Haberman et al., 2004, 2008).

The discrepancy between the sub-fossil pigments' content in sediments and the phytoplankton biomass in the water column indicates that the degradation of microalgae and their pigments is more important in Võrtsjärv than in Peipsi. The smaller depth of Võrtsjärv makes it more vulnerable to wind-induced disturbance and mixing. This causes frequent stirring of the settled material, bringing it back to the water column. The algae remain longer in the water, and pigments are exposed again to sunlight and oxic conditions and become available for pelagic microbes.

Our research showed that the sediments of both lakes contained markedly larger amounts of Z + L (*Microcystis* spp. + green algae) than Echi. This also contradicts monitoring data, which demonstrate that in the second half of the twentieth century the biomass of cyanobacteria was formed mainly by filamentous species and the biomass of green algae was very low (Nõges et al., 2003, 2004). The larger proportion of Z + L than Echi in the lakes' sediment, whereas in the water the situation is reverse, may be due to more extensive degradation of filamentous CY during sinking through the water column and in the surface sediment than *Microcystis* spp. (Bianchi et al., 2002). The other explanation is that Echi is minor pigment in CY community compared to Zea (Egeland, 2011).

The high content of sub-fossil pigments in the Peipsi sediments at the beginning of the twentieth century (**PAPER IV**, Fig. 3b) suggests that preservation conditions can alter during various development periods of a single lake. Although according to written historical records intense phytoplankton blooms occurred in Peipsi already at the beginning of the twentieth century, the measured average biomass of predominant phytoplankton groups and the total biomass of phytoplankton were still lower in the first half of the twentieth century than in the 1980s and 1990s (Laugaste et al., 1996).

In addition to more favourable preservation conditions (for example reduced resuspension due to very high water level and absence of a zebra mussel population) compared with other periods, the large content of some pigments in the Peipsi sediments at the beginning of the twentieth century can be also explained by their enhanced allochthonous input originating from macrovegetation growing in the catchment. Some carotenes included in our analysis are also synthesised in higher plants. Although it is commonly believed that land-derived pigments do not survive transport to water bodies (Meyers & Ishiwatari, 1993), when incorporated into the matrix of humic substances these land-derived pigments can become extremely resistant to degradation (Cieslewicz & Gonet, 2004).

Between 1900 and 1930 the water level in the lakes was on average higher (Fig. 3), suggesting a larger run-off of terrestrial material than in subsequent decades. The lower values of the A_{250}/A_{360} ratio and a larger proportion of substances with HMW (**PAPER IV**, Fig. 3d) prior to the 1940s indicate that the share of allochthonous OM in the bulk of OM in the Peipsi sediment was indeed larger than in the latter years. Therefore, an increased rate of input of these pigments from the lake's catchment in the early 1900s cannot be ruled out.

5.5. Signs of eutrophication

5.5.1. Lake Peipsi

The darker colour of sediment layers settled around the 1930s is the first sign of alterations in the lake's ecosystem. We suppose that the change in sediment colour was caused by the occurrence of FeS aggregates. Their appearance in the sediments indicates anoxic decomposition of organic substances. In lake bottom oxygen is rapidly depleted when a large amount of organic material accumulates there and the mixing of the water column is limited. The sediment main constituents and sub-fossil diatoms do not show any changes in the 1930s. As explained in the previous section, the data of sub-fossil pigments in Peipsi over that period should be interpreted with caution. Nevertheless, the appearance of FeS in sediments dated to the 1930s coincides well with the drop in the relative abundance of organic substances with HMW. The reduction in the percentage of HMW suggests that the proportion of allochthonous OM accumulated in the 1930s and 1940s in the sediments started to decrease gradually.

In the subsequent years, the shift in the origin of OM and its quantity become more obvious. The changes in sediment composition in the late 1950s confirm accelerated increment of OM: the sediment is less compacted and the content of OM is higher than in the previous decades. The increase in the relative abundance of planktonic diatoms in the 1960s indicates incipient proliferation of planktonic algae typical for eutrophied water bodies. The elevated A_{250}/A_{360} ratio confirms that in the material settled around the 1960s autochthonous organic substances became predominant. The appearance of the eutrophic diatom *Stephanodiscus parvus* in these years clearly demonstrates increase in nutrient availability.

In the late 1960s the sediment became darker, indicating further acceleration of eutrophication. From the early 1970s, layers consisting of organic substances with very large M_w occur in the Peipsi record.

Earlier, we presumed that such compounds sedimented in the years of massive phytoplankton blooms (**PAPER III**). Also, the CAR and Echi content started to increase in the 1970s, indicating an escalation of phytoplankton blooms, consisting of filamentous CY.

In the sediments settled in the 1990s, some symptoms of the reversal of eutrophication are notable: the sediment contains less FeS aggregates. The relative abundance of planktonic diatoms and *Sthephanodiscus parvus* shows a decrease. Interestingly, also the A_{250}/A_{360} ratio and the proportion of LMW substances show a drop to the pre-eutrophication level. These signs point towards mesotrophic conditions in the lake, which can be explained by the reduced load of nutrients. According to Nõges et al. (2007, Table 2), in the 1990s the nutrient inputs to Peipsi from the Estonian part of its catchment area dropped: 13% for P and of 40% for N compared with the 1980s. However, the Echi content in the Peipsi record remains high suggesting no recovery of the lake between 1990 and 2000.

PCA of palaeodata demonstrates no recovery of the lake's ecosystem in the 1990s either (Fig. 10). This implies that most likely eutrophication was continuing at the turn of the century. Moreover, compared with the 1980s the in-lake P concentration increased 17% by the 1990s (Nõges et al., 2007, Table 2) and CY blooms became more intense (Laugaste et al., 2008). These mass developments of blue-greens in Peipsi during the 1990s were related to the reduction in the N/P ratio (Nõges et al., 2008). The cause of the rise of the P concentration in lake water in spite of the decrease in the external phosphorus load still remains unclear (Nõges et al., 2007).

The results of PCA indicate a short-term recovery of the ecosystem of Peipsi also between the 1940s and 1960s (Fig. 10). This turn towards oligotrophy is not expressed in any individual indicators (**PAPER IV**, Fig. 3). Although statistics and monitoring data about the period are missing, we suppose that this provisional transition of the ecosystem was probably a result of the regression of agriculture and fishing during World War II and the post-war depression.

5.5.2. Lake Võrtsjärv

The first layer with FeS aggregates in the Võrtsjärv sediments appears around the 1960s, when also the carbonate content, total algal biomass and the proportion of planktonic diatoms start to increase (**PAPER IV**, Fig. 3). The source of carbonates in the lake's sediments is not yet determined. One reason of their occurrence can be the proliferation of algae. A massive bloom of microalgae or CY leads to a rapid depletion of free CO_2, increasing the pH of lake water and inducing precipitation of carbonates. However, the decrease in the values of the A_{250}/A_{360} ratio and in the content of Echi does not support this explanation. On the contrary, the decrease in the ratio implies a decrease in the rate of primary production in the lake since the 1960s. Monitoring data of these years demonstrate that in the 1970s the total biomass of phytoplankton as well as the biomass of filamentous CY increased compared with the previous decade (Nõges et al., 2003). However, in the 1980s, although the nutrient loading continued to increase, the biomass of phytoplankton and CY fell back to the level of the 1960s.

The decrease in the A_{250}/A_{360} ratio since the 1960s may have resulted from the increase in the carbonate content in Võrtsjärv sediments. Laboratory experiments by Thimsen & Keil (1998) demonstrated that mineral particles can remove humic substances from water by adsorbing them onto their surface. Substances of small size and LMW would adhere more easily.

In the 1990s, the nutrient load to Võrtsjärv fell even more than to Peipsi: the input of total P decreased ca. 35% and the total N load >50% (Nõges et al., 2007, Table 2) compared with those in the 1980s. However, the palaeolimnological records (**PAPER IV**, Fig. 3) of Võrtsjärv do not show if the lake trophy fell in the 1990s. This corresponds well to limnological monitoring data, which demonstrate that despite the decrease in the external load, the concentration of inlake P and phytoplankton biomass increased in the 1990s (Nõges et al., 2007, Table 2 & 2010a). Nõges & Kisand (1999) argue that these high concentrations of P were due to its release from deep anoxic sediment exposed during heavy storms.

PCA analysis shows that in the sediments of Võrtsjärv the shift towards eutrophy is much less pronounced than in Peipsi (Fig. 10). This can be due to frequent resuspension of sediments in Võrtsjärv, which stirs up and relocates accumulated material at the bottom smoothing differences between the deposits settled in different years.

5.6. Influence of water-level fluctuations

In lakes Võrtsjärv and Peipsi oscillations of water level have a periodicity of ca. 30 years (Nõges & Nõges, 1998; Jaani, 2001). These fluctuations are related to the variations in North Atlantic Oscillation (NAO), which controls the direction and intensity of westerly winds, and with that the transport of heat and moisture to Europe. Increased westerly winds cause mild winters with high precipitation resulting in the rise of water level. When the western airflow is restricted, winters become colder, while summers that are dry and hot cause reduction in water level. Changes in water level considerably affect light conditions and nutrient availability in the water column. During a high water level large amounts of solid and dissolved matter, including nutrients, are transported into lakes. The land-derived substances reduce the quantity and quality of light essential for photosynthetic organisms, at the same time the nutrient availability for primary producers increases. During a low water-level period the input of materials from the catchment is low and light conditions improve. However, in very shallow lakes reduced depth may result in substantial resuspension of sediments, stimulating release of nutrients, but also increasing water turbidity. Limnological measurements since the 1960s have shown that over periods of a high water level the concentration of phytoplankton in the water column of the lakes is commonly lower than over the periods of a low water level (Nõges & Nõges, 1998; Haldna et al., 2008).

Spearman rank correlation analysis of the palaeodata and the water-level series revealed that phytoplankton responded differently to the WLFs during the two study periods in the twentieth century as well as in the two lakes. For entire 100-year period, the content of sub-fossil pigments and abundance of planktonic diatoms exhibited a very weak relationship with the WLF (Table 4). When the periods of pre-eutrophication (up to 1950) and intensive nutrient loads (1950–2000) were analysed separately, in both lakes the relative abundance of planktonic diatoms demonstrated a significant strong positive relationship with the WLF prior to the 1950s, but not in 1950–2000 when the lake gradually eutrophied.

Spearman correlation analysis of sub-fossil pigments and water-level data for Võrtsjärv showed a similar pattern: over years when the external nutrient load was low, the rise of the water level involved an increase in the biomass of dominant algal groups, diatoms (DD) and filamentous CY (Echi), as well as the total algal biomass (CAR). The correlation ceased to exist after the 1950s when the lakes became eutrophic. No such period relation was observed in Peipsi except that the content of DD showed a moderate negative correlation with the WLF after 1950.

The shift in the relationship between the phytoplankton biomass and WLF might be due to the increase of the lakes' internal store of nutrients: before the 1950s the necessary nutrients for algae were brought from the catchment during a high water period, after the 1950s, when lakes started to receive extra amounts of P and N, their 'own' reserves increased making the lakes' ecosystems 'independent' from the delivery from the catchment.

5.7. Recommendations for pigment analysis

Pigment chromatography of sediment samples produces extremely complex chromatograms that are often very difficult to interpret. It is important when undertaking this task that the HPLC system is adequate and correctly optimised to produce the best results. The following suggestions relate to pigment analysis using a reversed-phase method.

Sample preparation

Although not used in our analysis, the use of internal standards is recommended, especially if the HPLC is not using an automated sampling device. Internal standard allows correcting errors in the volume injected and which acts as a reference standard for comparing chromotographic behaviours among runs. Ideal reference standards should be stable compounds with pigment-like chemical characteristics and unique positions on chromatograms, although in complex sedimentary mixtures, this latter condition can be difficult to achieve and synthetic dyes may be used (Leavitt & Hodgson, 2001). More common internal standards are shown in Table 5.

Table 5. Some internal standards used in phytoplankton pigment analysis

Name of the internal standard	Reference
Sudan II	Reuss et al., 2010; Leavitt & Findlay 1994
trans-β-apo-8'-carotenal	Wright et al., 1991
β-apo-8-carotenal	Reuss & Conley, 2005
2-nonadecanone	Huguet et al., 2011
Cu-mesoporphyrin-IX-DME	Louda et al., 2002
Mesoporphyrin-9-dimethyl ester	Hurley & Armstrong, 1990
Vitamin E	Lauridsen et al., 2011

Solvent delivery

A high-quality pulseless pump system capable of delivering at least a binary gradient is required. In some methods, quarternary solvent delivery system is required (Airs et al., 2001). Some form of degassing system, using either helium sparging or more often, vacuum degassing, is essential.

The injector

While it is possible to undertake pigment analysis with a manual injector (**PAPER II-IV**), an autosampler is a valuable addition that substantially increases peak retention time reproducibility. In a typical pigment analysis system, the column never reaches equilibrium with the mobile phase between samples. It is thus very important for each sample to be injected at precisely the same time in the analysis cycle. It is important that the autosampler can be programmed to dilute samples with ion-pairing reagent before the injection. This improves peak resolution and increases the sensitivity of the analysis. The sample must be diluted immediately before injection, otherwise degradation of pigments can be noticed. Another important feature is the ability to refrigerate the samples. Many autosamplers cannot refrigerate below +4°C. Some laboratories use jacketed sample racks that keep samples at about −10°C (Dr. Simon Wright, unpublished).

The column

All modern pigment methods use reverse-phase columns, packed with silica particles (3–5 µm diameter), that are coated with a waxy layer with C_8, C_{18}, or sometimes C_{30} alkyl chains, with monomeric or polymeric coating. Smaller particles produce better column efficiency and better resolution, but higher back-pressures. Despite the high cost of Ultra Performance Liquid Chromatography (UPLC) systems, they are used more frequently and enhance selectivity of pigments that are unresolved with HPLC. To achieve stable retention times a column oven is recommended. Most of them are air ovens although some researchers (Dr. Simon Wright, unpublished) find water baths are more effective.

Column wash

Generally, when using salts in the chromatographic eluent, ammonium acetate or tetrabutylammonium acetate in the case of pigment analysis, it is recommended to wash the salt out from reverse-phase columns before shutting the system down. Mixture of organic solvent with high (50% or more) percentage of water is usually suggested. However, with Waters Sperisorb ODS2 column that was used in **PAPER I–IV**, decrease in retention times could be noticed after washing columns with organic eluent that contained high percentage of water. I found that pure gradient-grade methanol gave much more stable retention times and was the best eluent to wash columns with. In that case the lifetime of a column can be hundreds of samples and more than two years.

Detectors

Although pigment analysis can be carried out with UV detector (**PAPER II–IV**) a photodiode-array (PDA) detector (**PAPER I**) enhances the quality of correct peak identification and increases the sensitivity to low pigment concentrations of field samples. Modern PDA software allows user to record spectra of known standards in the libraries for later comparison with the unknown pigments in the sample. However, absorption library is inaccurate when working with low concentrations of pigments. Fluorescence detection is a valuable addition to photodiode-array detection, due to its sensitivity and selectivity to detect chlorophylls and their derivatives.

Chromatogram integration

Although not often practised, when using PDA detector then integration of peaks at their absorption maximum should be preferred to get lower limit of detection and better selectivity of pigments. The most reliable method to confirm peak identification is standard addition method. This method also helps to avoid matrix effects in quantification but is more time consuming than common calibration with external standards.

6. CONCLUSIONS

As a result of contemporary pigment flux and multi-proxy palaeolimnological studies of lakes Peipsi and Võrtsjärv the following conclusions can be highlighted:

- The short-term dynamics of phytoplankton pigments in the upper sediment layers of Lake Võrtsjärv generally did not correspond to their dynamics in the water. Resuspension in such a large and shallow lake could be the reasons for this phenomenon (**PAPER I)**;

- Higher sediment pigment contents in spring were assumably caused by lower resuspension due to high water level and slow degradation due to low water temperature (**PAPER I**);

- Decrease in chemically unstable phytoplankton pigment contents between the sediment surface and deeper layers indicates that normally only the sediment surface is resuspended in Lake Võrtsjärv (**PAPER I**);

- According to fossil pigments and other palaeoindicators three periods in the evolution of lakes Peipsi and Võrtsjärv can be distinguished on the 20th century: from 1900 to 1950s, from 1950s to 1990s and from 1990s to 2000 (**PAPERS II–IV**);

- Palaeoindicators show that eutrophication of both lakes started to increase rapidly in 1950s. This is also supported by the monitoring data that is available. Although some palaeoindicators peaked in 1970s no significant recovery to more natural state was observed (**PAPERS II–IV**);

- When the lakes became eutrophic, the ‘natural’ pattern where high water level means high phytoplankton biomass, was interrupted. The Lake Võrtsjärv algal records displayed especially well this phenomenon: before the 1950s the content of sub-fossil pigments and the relative abundance of planktonic diatoms had a strongly positive relationship with the water level fluctuations, after the 1950s such correlations vanished (**PAPER IV**);

- Although the conditions of sediment deposition and preservation in sediments of lakes Peipsi and Võrtsjärv differ, a transition in the ecological status experienced by the lakes in the 1950s is clearly evident in the sediment records of the lakes. Some man-made alterations are not distinguishable in the sediment record of Lake Võrtsjärv, apparently due to a stronger influence of sediment resuspension because of the lake's shallowness (**PAPER IV**);

- Rapid pigment degradation can still take place in the upper part of the sediment, distorting somewhat the interpretation of most recent events in the lakes history (**PAPER II**);

- Results do show, that phytoplankton pigments can be used in multi-proxy palaeolimnological studies to track changes in phytoplankton communities and work out reference conditions even in large shallow lakes (**PAPERS II–IV**).

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SUMMARY IN ESTONIAN

Kuidas fütoplanktoni pigmendid peegeldavad suurte madalate järvede ajaloolist ja tänapäevast seisundit?

Sissejuhatus

Fütoplankton on tähtsaim orgaanilise aine esmastootja vee-keskkonnas ning hõlmab umbes pool Maal toimuvast fotosünteesist, pannes aluse enamikele vees olevatele toiduahelatele ning omades olulist rolli atmosfääris olevate kasvuhoonegaaside tasakaaluprotsessides.

Euroopa Liidus jõustunud veepoliitika raamdirektiivi kohaselt tuleb kehtestada veekogude hea seisundi kriteeriumid. Loodusliku ja inimtekkelise eutrofeerumise eristamine on tihti võimalik vaid mitmekülgsete paleoökoloogiliste uuringutega, sest seireandmed ulatuvad enamasti vaid mõnede aastakümnete taha, kui veekogud olid juba tugeva inimmõju all.

Vetikate valgusenergiat püüdvad ning edasikandvad pigmendid on olulised ajaloolise informatsiooni allikad, kuna säilivad settesse mattununa tuhandeid aastaid ning võimaldavad hinnata järvedes toimunud vetikate biomassi ja taksonite vahelisi muutusi. Erinevalt sügavatest järvedest, on pigmentide kasutamine suurte ja madalate järvede paleolimnoloogilistes uuringutes raskendatud ning maailmas seni kajastatud vaid üksikutes töödes, kuna selget ajaloolist settejälge segab vee intensiivse liikumise poolt põhjustatud setete resuspensioon.

Käesoleva töö fookuses on suurtes ja madalates järvedes, Võrtsjärves ja Peipsi järves, toimuvate fütoplanktoni pigmentide voogude ning lähiajaloo uurimine mitmekülgsete paleolimnoloogiliste näitajate kaasabil. Esmalt selgitati Võrtsjärve veesamba ja sette ülemise kihi pigmendivoogude seaduspärasusi ning edasi rekonstrueeriti järvede eutrofeerumise ajalugu 20. sajandil.

Töö peamised eesmärgid olid:

- Selgitada välja, mil määral fütoplanktoni pigmendid järgivad suure ja madala Võrtsjärve sette pinnakihis veesamba pigmentide aastast dünaamikat (**ARTIKKEL I**);

- Teostada multiparameetriline paleolimnoloogiline setteuuring, selgitamaks välja kliima- ja inimmõjude osakaal Võrtsjärve eutrofeerumisprotsessis (**ARTIKKEL II**);
- Taastada Peipsi järve eutrofeerumise lähiajalugu, kasutades selleks fossiilseid pigmente ja sette poorivees lahustunud orgaanilise aine omadusi (**ARTIKKEL III**);
- Kasutades sette paleolimnoloogilisi näitajaid, võrrelda kahe ühenduses oleva suure ja madala järve eutrofeerumise ajalugu 20. sajandil (**ARTIKKEL IV**).

Metoodika

Käesoleva töö läbivaks meetodiks on fütoplanktoni pigmentide mõõtmine kõrgefektiivse vedelikkromatograafia abil. Eestis uudset ning sobivaks kohandatud metoodikat rakendati pigmendivoogude uurimiseks Võrtsjärve veesambas (**ARTIKKEL I**) ja Võrtsjärve ning Peipsi järve settes (**ARTIKLID I–IV**). Lisaks pigmentidele mõõdeti nende järvede ajaloolisest settest ka teisi järvede eutrofeerumist iseloomustavaid parameetreid, nagu sette poorivee lahustunud orgaanilise aine omadused ning juba standardseteks saanud meetoditega hinnati karbonaatide ja fosforisisaldus, ränivetikate ohtrus ja taksonoomiline koosseis.

Töö tulemused võib kokku võtta järgnevalt:

- Fütoplanktoni pigmentide lühiajaline dünaamika Võrtsjärve sette ülemises kihis ei järgi nende muutusi veesambas ning on mõjutatud järve veetasemest;
- Keemiliselt ebastabiilsete pigmentide sisalduste vähenemine sügavamates settekihtides näitab, et tavaliselt on Võrtsjärves resuspensioonist haaratud vaid sette pinnakiht;
- Fütoplanktoni pigmentide ja teiste paleoindikaatorite põhjal võib mõlema järve arengus eristada kolme ajaperioodi: 1900 kuni 1950-ndad, 1950-ndad kuni 1990-ndad ning sajandi viimane kümnend;
- Eutrofeerumine kiirenes mõlemas järves 1950-ndatel, mida toetab ka olemasolev seireandmestik. Kuigi mõned eutrofeerumise

indikaatorid saavutasid oma maksimumi 1970-ndatel ei ole märkimisväärset järvede seisundi paranemist sajandi viimasel kümnendil näha;

- Sajandi keskel toimunud muutused rikkusid järvede eelneva seaduspärasuse, kus kõrge veetase järves tõi kaasa kõrgema fütoplanktoni biomassi;
- Ajaloolised muutused on paremini talletunud Peipsi järve settes, kuna Võrtsjärve madal veetase ning intensiivne vee liikumine segab settearhiivi moodustumist;
- Sette ülemises kihis toimuvad intensiivsed pigmentide lagunemisprotsessid ning seetõttu peab neid tulemusi interpreteerima ettevaatusega;
- Käesolev töö näitab, et fütoplanktoni pigmendid on kasutatavad ka suurte madalate järvede paleolimnoloogilistes uuringutes ning seisundi hindamise kriteeriumite väljatöötamisel.

Töö tähtsus ja vajadused edasisteks uuringuteks

Käesolev töö on autorile teadaolevalt esimene fütoplanktoni pigmentidele fokusseeritud doktoritöö Eestis ning annab esmakordselt väärtuslikku teavet Võrtsjärve fütoplanktoni pigmentide aastase dünaamika ja pigmendivoogude kohta. Samuti on edukalt rekonstrueeritud Võrtsjärve ja Peipsi järve eutrofeerumise ajalugu 20. sajandil, kasutades selleks Eestis uudseid meetodeid— fütoplanktoni pigmentide ja sette poorivee andmeid.

Vaatamata nendele saavutustele on fütoplanktoni pigmendid limnoloogilistes uuringutes nii Eestis kui kogu maailmas alakasutatud ning vastavad kromatograafilised mõõtmise meetodid vajaksid ühtlustamist ja edasist täiustamist. Praegusel hetkel on täiesti kasutamata kemotaksonoomilised võimalused fütoplanktoni pool-automaatseks seiramiseks pigmentide abil, mis võimaldaksid oluliselt tihedamat seiresammu ja vähendaksid ajakulu võrreldes traditsioonilise mikroskoopiaga. Kemotaksonoomiliste meetodite kasutuselevõtt eeldab aga paremat arusaamist vetikate pigmentkoostisest, selle sõltuvust ökoloogilistest ja keskkonnateguritest ning pigmentide lagunemise seaduspärasuste põhjalikumat tundmist.

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I

ORIGINAL PUBLICATIONS

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Dynamics of phytoplankton pigments in water and surface sediments of a large shallow lake

Rene Freiberg[a], Monika Nõmm[a], Ilmar Tõnno[a], Tiiu Alliksaar[b], Tiina Nõges[a] and Anu Kisand[a,b]

[a] Centre for Limnology, Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, 61101 Rannu, Tartumaa, Estonia; rene.freiberg@limnos.ee
[b] Institute of Geology, Tallinn University of Technology, Ehitajate tee 5, 19086 Tallinn, Estonia

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Abstract. Our aim was to find out to which extent fossil phytoplankton pigments in the large shallow and turbid Lake Võrtsjärv carry information on the history of phytoplankton communities. For this purpose we examined how the changes in the pigment composition of surface sediments follow their changes in the water column. Depth-integrated lake water and surface sediment samples were collected weekly in May–October 2007. Considering cyanobacterial and diatom dominance in phytoplankton, we analysed fucoxanthin, diadinoxanthin and diatoxanthin as marker pigments for diatoms, zeaxanthin as a marker pigment for total cyanobacteria and canthaxanthin as a marker pigment for colonial cyanobacteria. Chlorophyll *a* and its derivative pheophytin *a* were applied as indicators for total phytoplankton.

The dynamics of phytoplankton pigments in surface sediments generally did not follow their dynamics in the water column, possibly due to intensive resuspension and a high sedimentation rate in a large and shallow lake. It was noticed that the surface sediment carries information on pigment degradation intensity and on weight and size characteristics of phytoplankton cells, which affect their sinking and floating velocities. Higher pigment contents of sediment in spring were presumably caused by lower resuspension due to high water level and slower degradation in cold water. Pheophytin *a* and the marker pigments of cyanobacteria were found to be persistent against degradation in upper sediment layers, which makes them useful indicators for tracking the historical changes in phytoplankton communities also in a shallow lake. Sharp decrease in chemically unstable pigment contents between the sediment surface and deeper layers indicates that only the uppermost sediment surface is resuspended in Lake Võrtsjärv. The transformation of the diatom marker carotenoid diadinoxanthin to diatoxanthin was found to occur mainly in sediments and not in the water column, and the process is not induced by excess light.

Key words: phytoplankton pigments, large shallow lake, surface sediment layers, resuspension, Lake Võrtsjärv.

INTRODUCTION

Phytoplankton pigments (PhPs) that capture and transform solar energy in the water column (Hall et al. 1999) store valuable palaeoclimatic and palaeoenvironmental information if buried in sediments (Fietz et al. 2007; Soma et al. 2007). Carotenoids are useful biomarkers of different classes of phytoplankton, while chlorophyll *a* (Chl *a*) is not class-specific and is commonly used to estimate the total amount of phytoplankton in aquatic systems (Jeffrey et al. 1997; Bianchi et al. 2002; Reuss et al. 2005).

Planktic and benthic algal communities are common for shallow lakes. Benthic algae can be substantial primary producers in pelagic and benthic food webs in non-stratified lakes (Zimba 1995; Bonilla et al. 2005). However, the development of benthic algae is limited in turbid shallow lakes where the euphotic zone does not penetrate to the sediment surface (Nõges & Laugaste 1998).

Contrary to anaerobic sediments where PhPs may preserve for a long period, the aerobic degradation of PhPs in the water column is usually very rapid and extensive (Leavitt & Carpenter 1990a, 1990b; Leavitt 1993; Patoine & Leavitt 2006). The composition of PhPs preserved in sediments commonly differs from that in the water as the sensibility to decomposition varies among different pigments (Bianchi et al. 2002). Each lake represents a unique environment for deposition and preservation of PhPs, depending on its oxygen and light penetration characteristics, resuspension intensity, digestion through zooplankton grazing and phytoplankton abundance. In anaerobic sediments of deep lakes, where resuspension and bioturbation are negligible, PhPs or their ratios might reflect the history of phytoplankton composition and primary production (Cohen 2003, pp. 257–260; Reuss et al. 2005). In deep lakes the stratification depth is also an important factor for conservation of sediment pigments as their degradation in the water column is rapid (>90%) due to photo-oxidation, grazing by invertebrates, etc. (Leavitt & Carpenter 1990a, 1990b; Leavitt 1993; Descy et al. 1999; Patoine & Leavitt 2006). In shallow lakes resuspension and aerobic conditions in surface sediments enhance the transformation processes of organic matter, including PhPs, which strongly complicates the interpretation of palaeolimnological information stored in sediments (Leavitt & Carpenter 1989). However, as most of the World's lakes are shallow (Scheffer 1998), the evaluation of the possibilities of tracking historical changes in shallow lakes by using fossil sediment records is highly needed. This has become extremely relevant during the last decade in the light of the challenging tasks posed by the EU Water Framework Directive (Directive 2000) requesting the improvement of the ecological status of water bodies considering the type-specific reference conditions. If no historical data or reference water bodies are available, only palaeolimnology and modelling could enable assessment of the historical status of the water body. Despite various methodological problems, some successful palaeolimnological studies of shallow lakes have been published within the last years (Eilers et al. 2004; Engstrom et al. 2006; Leeben et al. 2008). Our aim was to find out to which extent the fossil PhPs in such a large shallow and turbid lake as Võrtsjärv carry the information on the history of phytoplankton communities. For this purpose we examined how the changes in the PhP composition of surface sediments follow their changes in the water column.

STUDY SITE

Lake Võrtsjärv (Fig. 1), the third largest lake (270 km^2) in Eastern Europe, excluding large lakes in Russia, is situated in the Central Estonian depression of preglacial origin. The bedrock is comprised of Middle Devonian deposits, mainly sandstone, and covered with diverse Quaternary sediments. The southern part of the lake bottom is covered with gyttja overlying lake marl. Northwards the mud is gradually replaced by sandy mud and sand. No sediment accumulation occurs in the wind-exposed northern part of the lake and the bottom (Fig. 2) is mainly sandy or stony (Raukas 1995).

Lake Võrtsjärv is very shallow, with a mean depth of 2.8 m (maximum 6 m). Due to bad outflow conditions, water level in the lake fluctuates strongly (annual mean amplitude 1.38 m), depending on the amount of precipitation in the catchment area (3374 km^2). The renewal of the 750 million m^3 water mass takes place on average once a year. Due to shallowness and the large wind-exposed area, L. Võrtsjärv is unstratified and very turbid. The Secchi depth ranges from 0.5 to 1.0 m during the ice-free period. The lake is eutrophic, characterized by mean concentrations of about 2 mg L^{-1} for total nitrogen and about 50 μg L^{-1} for total phosphorus. The dominant phytoplankton groups in the lake are cyanobacteria (CY) and diatoms, forming ~90% of the total phytoplankton biomass (Jaani 1973; Haberman et al. 1998; Nõges et al. 2004).

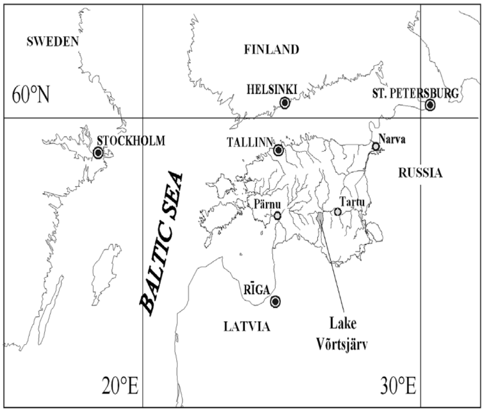

Fig. 1. Location map of L. Võrtsjärv.

MATERIALS AND METHODS

Considering CY and diatom dominance in phytoplankton of L. Võrtsjärv (Nõges et al. 2004), fucoxanthin (Fuco), diadinoxanthin (Diadino) and diatoxanthin (Diato) were analysed as marker pigments for diatoms (Leavitt & Hodgson 2001; Buchaca & Catalan 2007a, 2007b), zeaxanthin (Zea) as a marker pigment for total CY and canthaxanthin (Cantha) as a marker pigment for colonial CY (Leavitt & Hodgson 2001; McGowan et al. 2005; Carreto et al. 2008) to track phytoplankton community changes in water and in upper sediment layers. Diatoxanthin and Diadino were both analysed to track diatoms as in their xanthophyll cycle Diadino could be transformed to Diato at intensive light (Patoine & Leavitt 2006). Chlorophyll *a* and pheophytin *a* (Pheo *a*) were applied as marker pigments for total phytoplankton and for general Chl *a* derivative, respectively (Leavitt & Hodgson 2001). However, one should consider that Chl *a* is also the major pigment in higher plants.

Field sampling

The sampling site is situated in the southern part of L. Võrtsjärv between the eastern shore and the island of

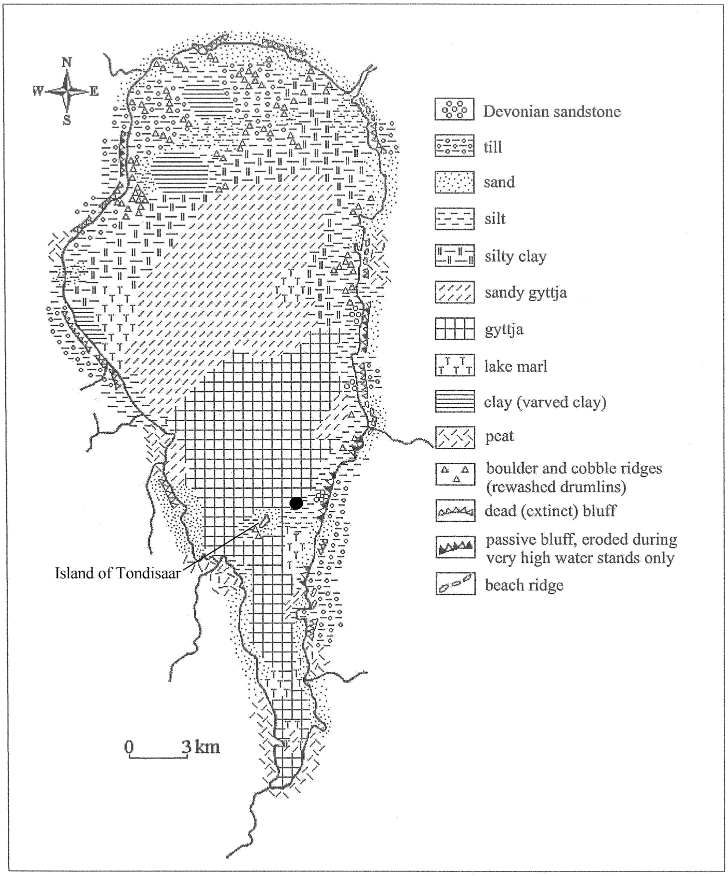

Fig. 2. Bottom sediments in L. Võrtsjärv after R. Pirrus, A. Raukas and E. Tavast (modified from Raukas & Tavast 2002). The solid circle marks the photosynthetic pigments sampling station.

Tondisaar (58°12.573′ N, 026°05.742′ E; Fig. 2), where the mean water depth is 2.5 m. Phytoplankton of L. Võrtsjärv has usually two biomass maxima (Nõges et al. 2004) – in spring (diatoms) and in autumn (CY and diatoms), and the sampling periods of PhPs were selected accordingly. From the sampling site depth-integrated lake water samples and surface sediment samples were collected weekly in 2007 during two periods – from 15 May to 03 July and from 07 August to 30 October. A Willner-type gravity corer was used for sediment coring (Uppsala University). All samples were placed into an insulated box and immediately transported to the laboratory.

Extraction of pigments

Analysis of PhPs followed the recommendations of Leavitt & Hodgson (2001) and Mantoura & Llewellyn (1983). Depth-integrated water samples (50–100 mL, depending on water turbidity) were filtered through Whatman GF/C glass microfibre (1.2 µm) filters (precombusted at 400 °C for 4 h) and frozen (−20 °C) until PhP analyses. From the sediment cores three subsamples from depths of 0–1, 2–3 and 4–5 cm were sectioned. From each homogenized subsample approximately 5 g of wet sediment was weighed and immediately frozen (−20 °C) prior to PhP analyses.

Thereafter frozen sediment samples were freeze-dried. Residual sediment from the subsamples was heated at 550 °C for 4 h for measurements of sediment organic matter content as loss on ignition (Heiri et al. 2001) relevant for PhP calculations. Acetone–methanol mixture (80 : 20 v : v) was added to the frozen GF/C glass-fibre filters and the freeze-dried sediment samples in order to extract PhPs, thereafter the filtered samples were sonicated (Branson 1210) for 10 min. All PhP samples were extracted at −20 °C in the dark for 24 h. Finally the PhP extracts were clarified by filtration through a 0.45 µm pore-size filter (Millex LCR, Millipore).

High-performance liquid chromatography analyses

Reversed-phase high-performance liquid chromatography (RP-HPLC) was applied, using a Shimadzu Prominence (Japan) series system with a photodiode array (PDA) detector to separate the PhPs. The method was adapted from Airs et al. (2001) and slightly modified. As an ion-pairing reagent 0.5 M ammonium acetate was added in a volume ratio of 2 : 3 to each PhP sample. To avoid chemical decomposition of pigments, the autosampler was cooled down to +5 °C (Reuss & Conley 2005) and a maximum of 10 samples were loaded at a time. The sample injection volume was 50 µL.

Separations were performed in a reversed-phase mode by using two Waters Spherisorb ODS2 3 µm columns (150 mm × 4.6 mm I.D.) in-line with a pre-column (10 mm × 5 mm I.D.) containing the same phase. A binary gradient elution method (Table 1) was used with isocratic holds between 0–2 and 30–43 min. The flow rate remained constant during the elution, 0.8 mL min^{-1}. Absorbance was detected at wavelengths from 350 to 700 nm. The software 'LC solution ver. 1.22' (Shimadzu) was applied to collect and analyse the data. The integration of peak areas was made at each pigment absorbance maximum (Jeffrey et al. 1997). Commercially available external standards from DHI (Denmark) were used for peak identification and quantification. Spearman rank order correlation and the program 'Statistica for Windows 6.0' were applied in statistical analyses.

Table 1. Elution scheme and solvents used in the separation of phytoplankton pigments by HPLC

	Time, min				
	0	2	30	43	50
Solvent A, %	50	50	100	100	50
Solvent B, %	50	50	0	0	50

Solvent A = 80% methanol : 20% 0.5 M ammonium acetate (pH 7.2) (v : v).
Solvent B = 80% methanol : 20% acetone (v : v).

RESULTS

Lake sediments at the sampling site were characterized by water-rich (>90%) gyttja, consisting mainly of mineral matter (>60% in dried sediment) and less organic matter and carbonates (about 25% and 11%, respectively).

The concentrations of all studied PhPs in the water samples were lower from May to July than from August to October in 2007. However, in the upper sediment layers the contents of Chl *a*, Pheo *a*, Fuco, Diadino and Diato were higher from May to July than from August to October (Fig. 3A–G). Zea content in the studied sediment layers remained similar within the investigated periods, while Cantha increased towards autumn (Fig. 3F, G). Chl *a*, Fuco and Diadino generally decreased, while Diato increased from the sediment surface (0–1 cm) towards deeper sediment layers. Concentrations of Pheo *a*, Zea and Cantha were rather similar in all investigated sediment layers (Fig. 3A–G).

Chlorophyll *a* was the dominant pigment in water and in surface sediment layers within the whole investigated period. In water Chl *a* was followed by Zea, Fuco, Diadino, Pheo *a*, Cantha and Diato. In upper sediment layers the concentrations of Zea, Diato and Pheo *a* were similar, followed by Fuco and Diadino, while Cantha concentration was the lowest. The mean concentration of Chl *a* in water was 17 times higher than that of Pheo *a*, while in upper sediment layers the difference was ~3 times (Fig. 4A, B).

Chlorophyll *a* concentration in water was inversely correlated with that in surface sediment layers, and the same was valid also for Pheo *a* and Diato. No correlation was detected between Fuco, Diadino, Zea and Cantha concentrations in water and in upper sediment layers except the positive correlation between Cantha in water and in the 0–1 cm sediment layer. The contents of the same pigment in different sediment layers were generally positively correlated (Table 2). The water level in L. Võrtsjärv was decreasing during our study period from May to November (Fig. 3H). The concentrations of Chl *a*, Pheo *a*, Diato, Zea and Cantha in water were significantly negatively correlated with the water depth, while for Fuco and Diadino this correlation was weak and insignificant. Sediment pigment contents in all studied layers were significantly positively correlated with water depth except for Zea and Cantha. The content of Cantha in sediment was negatively correlated with water depth, while for Zea the correlation was insignificant (Fig. 5).

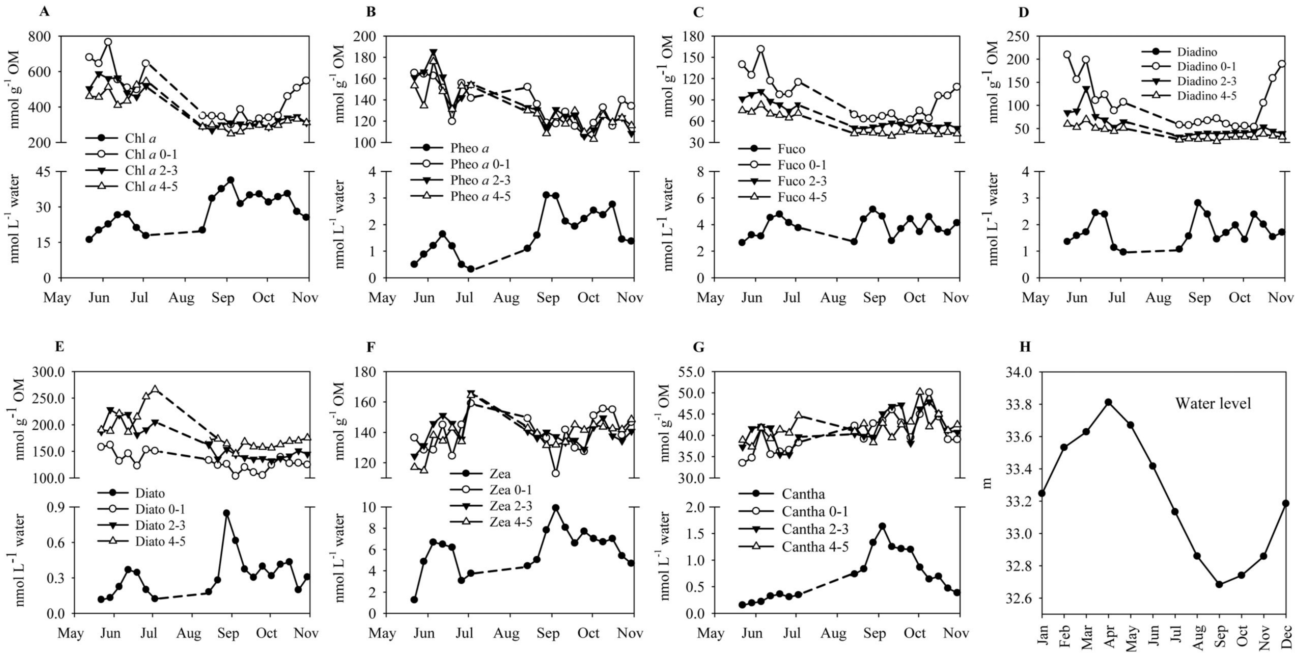

Fig. 3. Dynamics of phytoplankton pigments in L. Võrtsjärv water (nmol L^{-1} water) and in upper sediment layers (nmol g^{-1} OM; OM – organic matter) in 2007 (A–G). The timespan between two analysed periods is marked with dashed lines. (A) Chl *a* – chlorophyll *a*; (B) Pheo *a* – pheophytin *a*; (C) Fuco – fucoxanthin; (D) Diadino – diadinoxanthin; (E) Diato – diatoxanthin; (F) Zea – zeaxanthin; (G) Cantha – canthaxanthin. 0–1, 2–3 and 4–5 – investigated sediment layers (in cm); (H) Water level dynamics (metres above sea level) in L. Võrtsjärv in 2007 (http://www.emhi.ee/).

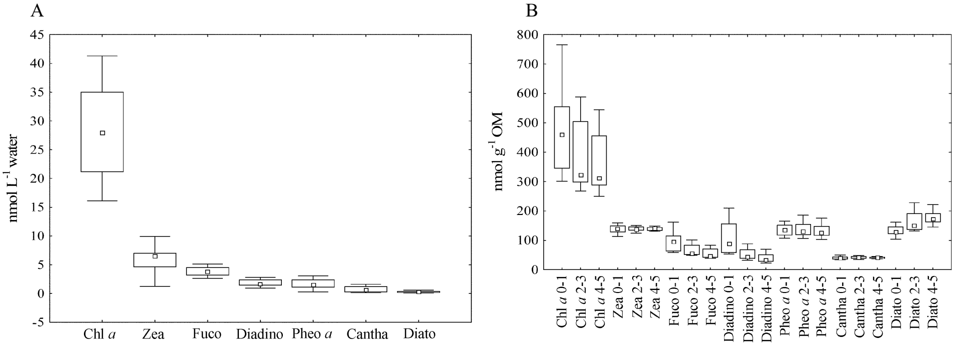

Fig. 4. Concentrations of phytoplankton pigments in L. Võrtsjärv water (A; nmol L^{-1} water) and in upper sediment layers (B; nmol g^{-1} OM; OM – organic matter) in 2007. Median, 25th and 75th percentiles as vertical boxes with error bars are presented. Chl *a* – chlorophyll *a*; Pheo *a* – pheophytin *a*; Fuco – fucoxanthin; Diadino – diadinoxanthin; Diato – diatoxanthin; Zea – zeaxanthin; Cantha – canthaxanthin. 0–1, 2–3 and 4–5 – investigated sediment layers (in cm).

Table 2. Spearman rank order correlations for phytoplankton pigments analysed from L. Võrtsjärv water samples and upper sediment layers in 2007. Significant correlations at $p < 0.05$ are marked in bold. Chl *a* – chlorophyll *a*; Pheo *a* – pheophytin *a*; Fuco – fucoxanthin; Diadino – diadinoxanthin; Diato – diatoxanthin; Zea – zeaxanthin; Cantha – canthaxanthin. Sediment layers 0–1, 2–3 and 4–5 cm

	Chl *a* 0–1	Chl *a* 2–3	Chl *a* 4–5
Chl *a* water	**−0.805**	**−0.540**	**−0.695**
Chl *a* 0–1		**0.823**	**0.826**
Chl *a* 2–3			**0.807**

	Pheo *a* 0–1	Pheo *a* 2–3	Pheo *a* 4–5
Pheo *a* water	**−0.753**	**−0.697**	**−0.721**
Pheo *a* 0–1		**0.754**	**0.735**
Pheo *a* 2–3			**0.942**

	Fuco 0–1	Fuco 2–3	Fuco 4–5
Fuco water	−0.375	−0.274	−0.047
Fuco 0–1		**0.711**	**0.595**
Fuco 2–3			**0.798**

	Diadino 0–1	Diadino 2–3	Diadino 4–5
Diadino water	−0.068	0.052	−0.098
Diadino 0–1		**0.616**	**0.707**
Diadino 2–3			**0.928**

	Diato 0–1	Diato 2–3	Diato 4–5
Diato water	**−0.572**	−0.440	**−0.619**
Diato 0–1		**0.726**	**0.644**
Diato 2–3			**0.800**

	Zea 0–1	Zea 2–3	Zea 4–5
Zea water	−0.179	0.077	−0.214
Zea 0–1		0.446	**0.523**
Zea 2–3			**0.618**

	Cantha 0–1	Cantha 2–3	Cantha 4–5
Cantha water	**0.751**	0.353	0.314
Cantha 0–1		**0.730**	0.367
Cantha 2–3			0.295

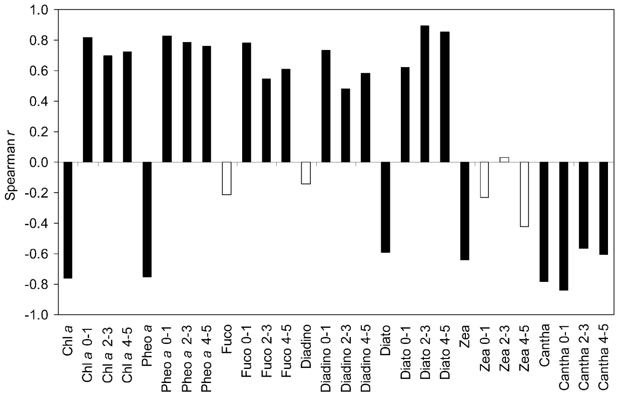

Fig. 5. Spearman correlation coefficients (black bars significant at $p < 0.05$, white bars nonsignificant) of phytoplankton pigment concentrations (marked as in Fig. 4) and water level in L. Võrtsjärv in 2007.

DISCUSSION

The dynamics of PhPs in the upper sediment layers of L. Võrtsjärv generally did not correspond to their dynamics in the water. Because of the morphology of the lake basin the water column is not stratified, sediments are subjected to mixing by waves and characterized by a high accumulation rate, especially the surface layers (Heinsalu et al. 2008, Fig. 1C). This can be the reason why short-term coupling with water and sediment PhPs was not detected in that large shallow and turbid lake. In stratified lakes where sediment layers are not disturbed by resuspension, such uncoupling could result from seasonal differences in the phytoplankton sinking rate, which is low during the phytoplankton population growing phase and high in the population decline phase (Sommer 1984). In large shallow lakes like L. Võrtsjärv sedimentation is accompanied by intensive resuspension (Scheffer 1998). Staying longer in the water column, more than 90% of PhPs could be degraded to colourless compounds before permanent burial, whereas the most rapid degradation occurs in dying cells and detritus (Leavitt & Carpenter 1990a, 1990b; Leavitt 1993). Resuspension is very strong in L. Võrtsjärv, and as the sediment trap experiments have shown, the major part (up to 96%) of the downward particle flux (including PhPs) is formed of resuspended material (Nõges et al. 1999). Wind speed and water level fluctuations have strong impact on resuspension intensity in large and shallow lakes, causing much higher resuspension and water turbidity at low water level during windy days. The water level of L. Võrtsjärv is typically low in winter and high in spring after the snowmelt, and its gradual decrease during summer and early autumn is followed by a small peak in late autumn (Haberman et al. 1998; Järvet 2004). In 2007 the difference between the minimum and maximum water levels was 1.28 m (Fig. 3H). Lake bottom morphometry changes at our sampling site, between the slopes of the shore and the island of Tondisaar (Fig. 2), which might cause varying sedimentation and resuspension conditions (Håkanson & Jansson 1983).

Higher sediment PhP contents (except Zea and Cantha) in spring (Fig. 3A–E) could partly originate from the previous autumn. After the formation of the ice cover the phytoplankton could calmly deposit on the bottom of the lake, and quite high PhP amounts were preserved in the sediments due to the low temperature and lack of resuspension under the ice. In spring, when the water level was high (Fig. 3H), the impact of resuspension was assumed to be relatively weak and therefore the deposited algal material of the spring phytoplankton maximum remained relatively less disturbed in the sediments. Also, the degradation of PhPs should be slow due to the still low water temperature. Together with the water level decrease towards autumn, the intensity of resuspension assumingly increased, as the earlier sediment trap experiments in L. Võrtsjärv have shown that the low water periods are characterized by a significantly higher sedimentation of resuspended material and, thus, more intensive resuspension than the high water periods (Nõges et al. 1999). The degradation of PhPs as well obviously intensified towards autumn owing to higher water temperature and also because due to resuspension the pigments stayed for a relatively longer period in the illuminated and oxygenated water column. We found a strong positive correlation between sediment carotenoids associated with diatoms and the water level of the lake (Fig. 5). This could indicate that the changes in the water depth are first of all important for settling and resuspension processes of rather heavy cells of diatoms (Stoermer & Smol 1999). As diatoms in L. Võrtsjärv have a biomass peak in spring when the water level is high, this correlation could also be caused by periodicity. While biostratigraphic diatom analysis showed great potential of the planktonic/periphytic diatom ratio in the sediment for reconstructing the historical water level changes in L. Võrtsjärv (Heinsalu et al. 2008), the use of diatom marker pigments in sediment for that purpose is questionable. The coupling of the water level with the sediment pigments associated with CY (Zea, Cantha) proved to be different from the coupling with diatoms (Fig. 5). The biomass and proportion of CY in phytoplankton of L. Võrtsjärv generally increase towards the autumn (Nõges et al. 2004). The concentration of the marker pigment of colonial CY (Cantha) both in water and in surface sediments increased markedly towards the autumn (Fig. 3G), causing probably negative coupling with the decreasing water level in the lake (Fig. 5). Another marker pigment for CY, Zea, associated with their total concentration, did not show any relationship with the water level. As the cells of CY are lighter than those of diatoms, the changes in the sedimentation/resuspension pattern due to the variations in water depth are not as important for CY as they are for diatoms. Due to relatively large cell dimensions, the sinking and floating velocities of colonial CY are enhanced if compared to other non-aggregated CY. *Microcystis* species (one of the dominant colonial CY also in L. Võrtsjärv) produce large colonies that could actively control their buoyancy and settling rate (Walsby & McAllister 1987; Nõges & Laugaste 1998; Roderick et al. 2000; Reynolds 2006). The positive correlation between Cantha concentration in water and in the sediment top surface layer (0–1 cm) could indicate active migration of colonial CY between water and the sediment surface. The lack of correlation between Cantha concentration in water and in sediment subsurface layers of 2–5 cm (Table 2) could also support this hypothesis. Phytobenthos of L. Võrtsjärv, consisting mainly of CY and diatoms (Pork & Kõvask 1973), might also confuse the coupling of CY and diatom marker pigment between the water and upper sediment layers. The development of benthic algae in lakes depends on light penetrating to the sediment surface. In the very turbid L. Võrtsjärv the estimated euphotic zone varied from 1.6 to 3.2 m, remaining lower than the mean (2.8 m) or maximum (6.0 m) water depth (Haberman et al. 1998; Reinart & Nõges 2004). Monthly phytoplankton monitoring indicates that in the period of low water level resuspended algae from the sediment surface enrich phytoplankton species composition in the water column but do not contribute much to biomass (Nõges et al. 2004). Therefore the development of the benthic algae community in L. Võrtsjärv is limited. Unfortunately, the HPLC technique does not allow identifying whether PhPs in sediments are from phytoplankton or from phytobenthos (Greisberger & Teubner 2007).

Decrease in Chl *a*, Fuco and Diadino contents towards the deeper layers of the sediment core might be explained by chemical instability of these PhPs (Fig. 3A, C, D), e.g., both Fuco and Diadino contain a 5,6-epoxide group which enhances their quick degradation (Hurley & Armstrong 1990; Reuss & Conley 2005). Considerable decrease in unstable PhPs content between the sediment surface and other investigated layers indicates that mainly only the top of the sediment surface is included to resuspension in L. Võrtsjärv (Fig. 3A, C, D). Consequently, in case of unstable pigments the ‘older’ PhPs stored in deeper sediments are assumably more degraded than the pigments in the upper sediment layers. In comparison with diatom marker pigments, Zea and Cantha are chemically very stable, whereas Zea could be preserved even in aerobic environment (Hurley & Armstrong 1990; Bianchi et al. 2000; Leavitt & Hodgson 2001; Fietz et al. 2005; Reuss et al. 2005; Buchaca & Catalan 2007a, 2007b). That could explain why there was no distinct difference between Zea and Cantha concentrations in different sediment layers. As Pheo *a* is the degradation product of Chl *a*, its content was assumed to increase towards the deeper sediment layers. Contrary to unstable Chl *a*, Pheo *a* is very persistent in sediments (Leavitt & Hodgson 2001). However, Pheo *a* content did not show any distinct downward pattern in sediments and we could not track the degradation of Chl *a* on the basis of Pheo *a* content. Consequently, Pheo *a* and the marker pigments of CY (Cantha and Zea) in the upper sediment layers of L. Võrtsjärv seem to be rather conservative against degradation and could be used to track the historical changes in total phytoplankton and CY.

The water column irradiance is rather low in the shallow and turbid L. Võrtsjärv (Nõges & Laugaste 1998; Reinart & Nõges 2004). The diatom carotenoid Diadino that is active in the xanthophyll cycle could be transformed into Diato at high light to reduce the amount of energy reaching the photosynthetic reaction centres (Louda et al. 2002). Diatoms in L. Võrtsjärv are probably not under light stress as Diato concentration in the water column was nearly an order of magnitude lower than that of Diadino (Fig. 3D, E). On the contrary, in the upper sediment layers the content of Diato was higher than that of Diadino. Moreover, Diato content increased and Diadino content decreased downwards in the sediment core (Figs 3D, E; 4B). Although part of the light in L. Võrtsjärv penetrates to the sediment surface, it is obvious that Diadino transformation to Diato in upper sediment layers could not be caused by excess light and should take place also in darkness. Consequently, transformation of the investigated diatoms marker carotenoids in L. Võrtsjärv occurs mainly in sediments, not in the water column. Several other studies have found that the xanthophyll cycle is not necessarily linked to protection against excess light (Morales et al. 1990; Torsten et al. 2001; Fietz et al. 2005). Louda et al. (2002) established that Diadino disappeared and transformed to Diato within the first two weeks of dark incubation. The inter-molecular Diadino–Diato transformation mechanism has been described by Patoine & Leavitt (2006).

Previous investigations in L. Võrtsjärv have shown a rather high sedimentation rate, especially for the upper sediment layers where the compaction of sediments has not occurred yet (Heinsalu et al. 2008). A high sedimentation rate means quick final burial of PhPs, which ensures rather similar preservation conditions within several years. This could explain positive correlations of the contents of the same pigment in different sediment layers (Table 2). Quick final burial of chemically stable palaeoindicators favours preservation of the historical information in L. Võrtsjärv sediments and allows palaeolimnological reconstructions of the lake ecosystem (Heinsalu et al. 2008).

CONCLUSIONS

- The dynamics of phytoplankton pigments (PhPs) in the upper sediment layers of L. Võrtsjärv generally did not correspond to their dynamics in the water. Sediments mixing by waves and characterized by a high accumulation rate in such a large and shallow lake could be the reasons for this phenomenon.
- Higher sediment PhP contents in spring were assumably caused by lower resuspension due to high water level and slow degradation due to low water temperature.
- Decrease in chemically unstable PhP contents between the sediment surface and deeper layers indicates that only the sediment surface is resuspended in L. Võrtsjärv.
- Pheophytin *a* and the marker pigments of cyanobacteria (Cantha and Zea) in the upper sediment layers of L. Võrtsjärv seem to be rather conservative against degradation and could be used to track the historical changes in total phytoplankton and cyanobacteria.
- Transformation of the diatom carotenoid Diadino to Diato in L. Võrtsjärv occurs mainly in sediments and is not induced by excess light.

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Suure ja madala järve fütoplanktoni pigmentide dünaamika settes ning veesambas

Rene Freiberg, Monika Nõmm, Ilmar Tõnno, Tiiu Alliksaar, Tiina Nõges ja Anu Kisand

On kindlaks tehtud, mil määral annavad settes talletunud fossiilsed fütoplanktoni pigmendid teavet suure ja madala Võrtsjärve ajaloolise fütoplanktoni koosluse kohta. Selle väljaselgitamiseks võrreldi pindmiste settekihtide ja veesamba pigmentide sisaldusi ühenädalase intervalliga perioodil maist juunini ning augustist oktoobrini 2007. aastal. Arvestades sini- ja ränivetikate domineerimist järves, mõõdeti järgmisi pigmente: fukoksantiin, diadinoksantiin ning diatoksantiin kui ränivetikate, zeaksantiin kui sinivetikate ja kantaksantiin kui koloniaalsete sinivetikate marker. Kogu fütoplanktoni biomassi hindamiseks mõõdeti klorofüll *a* ja selle laguprodukti feofütiin *a* sisaldusi.

Uuringu tulemusena selgus, et settes olevate pigmentide sisaldus enamasti ei järginud veesambas toimunud pigmentide sisalduse muutusi. Peamised põhjused selleks on oletatavasti järves toimuv intensiivne resuspensioon ja suur settimiskiirus. Tulemused kinnitavad, et settes olevate pigmentide sisaldus peegeldab nende lagunemise intensiivsust ja vetikarakkude ujuvust. Kevadperioodil täheldati settes suuremat pigmentide sisaldust, mis oli tingitud järve kõrgest veetasemest ja madalast veetemperatuurist. Feofütiin *a* ja sinivetikate markerpigmendid on lagunemisele vastupidavamad ning kasutatavad ajalooliste muutuste kindlakstegemisel ka madalates järvedes. Kergemini lagunevate pigmentide sisalduse järsk vähenemine sügavamates settekihtides kinnitab, et resuspensioon mõjutab Võrtsjärves oluliselt vaid pindmist settekihti. Samuti selgus mõõtmise tulemusena, et ränivetikate markerpigmendi diadinoksantiini muutumine diatoksantiiniks toimub peamiselt settes ja see protsess ei ole põhjustatud liigtugevast päikesevalgusest.

II

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Ecosystem changes in large and shallow Võrtsjärv, a lake in Estonia — evidence from sediment pigments and phosphorus fractions

Ilmar Tõnno[1], Anna-Liisa Kirsi[1], Rene Freiberg[1], Tiiu Alliksaar[2], Viia Lepane[3], Toomas Kõiv[1], Anu Kisand[2] and Atko Heinsalu[2]

[1] *Centre for Limnology, Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Rannu, Tartu County EE-61117, Estonia (corresponding author's e-mail: ilmar.tenno@limnos.ee)*
[2] *Institute of Geology, Tallinn University of Technology, Ehitajate tee 5, EE-19086 Tallinn, Estonia*
[3] *Institute of Chemistry, Tallinn University of Technology, Akadeemia tee 15, EE-12618 Tallinn, Estonia*

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Palaeopigments, organic matter dissolved in sediment porewater (pDOM) and phosphorus fractions were analysed in a sediment core from large shallow Võrtsjärv with the aim to assess whether the trends of climate-driven and anthropogenic changes in lake trophy within the 20th century are reflected in its sediment record. In the first half of the last century, the lake was naturally slightly eutrophic as the accumulation of palaeopigments was low and the load of nutrients stable; investigated variations in palaeoparameters were induced by the regular natural water level fluctuations. Since the mid-1950s eutrophication of Võrtsjärv has accelerated — content of fossil pigments, $CaCO_3$ and nutrients increased, while pDOM revealed high autochthonous matter input. The increase of palaeopigment's concentrations during the last decade may be consistent with regional climate warming. Investigated palaeoindicators and water level changes had a stronger relationship in the second half of the 20th century.

Introduction

Globally, the majority of lakes are classified as shallow waterbodies (Scheffer 1998). Large and shallow lakes are regionally highly important as they offer a wide spectrum of ecosystem services to mankind like sources of drinking water, fishery, recreation sites and waterway. Because of their intensive exploitation large shallow lakes are under multiple pressures via increased nutrient load and toxic pollution, exploitation of bioresources, modification of hydrology and places for waste disposal (Nõges *et al.* 2008). Often the status of these lakes is impaired by exploitation, most commonly by nutrient enrichment causing eutrophication. In very shallow lakes, wind-induced resuspension increase the contact between sediment and water and frequently cause increased concentration of suspended solids in lake water (Ekholm *et al.* 1997, Hamilton and Mitchell 1997). Sondergaard *et al.* (2003) showed that in shallow-lake water sus-

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pended solids and phosphorus can increase by a factor of 5–10 within a few windy days. The need to understand and resolve the problems arising from enhanced trophy has stimulated studies of shallow lakes; both their ecology and management have been receiving increased attention during the last decades (Gulati *et al.* 2007).

Large shallow lakes have large area/mean-depth ratio making them very sensitive to climate change, especially through changes in hydrological regime, causing large fluctuations of water level, change of lake volume and depth. In shallow lakes natural water-level fluctuation can be intra- or interannual, depending on regional climatic conditions (e.g. temperate, semi-arid) as well as on human activities (Coops *et al.* 2003). Fluctuations of water level in shallow lakes may alter the mean water-column irradiance and shift areas of sediment erosion, transportation, and accumulation (Bengtsson *et al.* 1990). Wind-induced resuspension and resulting processes, which frequently happen in large shallow lakes, are even more pronounced during low water-level periods. The dynamics of water level strongly affects lake biota. For instance, high water level during the growing season reduces light availability, while low water-level may damage plants via wave action or ice during winter and desiccation in summer (Coops and Hosper 2002). Water-level fluctuation shifts shallow lakes between clear-water and turbid states, which are independent of nutrient enrichment and top-down effects. Such shifts can enhance species richness and diversity (Blindow 1992, Scheffer *et al.* 1993, Coops *et al.* 2003). As a result of water-level changes, phytoplankton in shallow lakes is exposed to high nutrient availability as well as to permanent mixing and variable light conditions. Climate, both directly and indirectly, affects lake physics (temperature, flushing), chemistry (DOC, pH, nutrients) and biology, therefore, a climate-driven change can potentially obscure or exaggerate the eutrophication process (Carvalho and Kirika 2003, Sondergaard *et al.* 2003). It has been noticed that climate change can significantly alter the functioning of shallow lakes and seasonal patterns in their water quality, but the responses to climate change are not easily predicted (Nõges *et al.* 2008). For instance, increasing water temperatures are likely to increase winter phytoplankton biomass, but will similarly increase spring/autumn populations of grazing zooplankton; the net effect is uncertain (Carvalho and Kirika 2003).

Increasing water temperatures also enhance the potential of cyanobacteria to dominate the phytoplankton community (Elliott *et al.* 2006, Domis *et al.* 2007). Moreover, even moderate climate warming can enhance eutrophication problems as external loading is expected to increase due to shorter freezing time of watershed’s soils and the increased precipitation (Moss *et al.* 2003, Nõges *et al.* 2005, Mooij *et al.* 2007).

Võrtsjärv is one of the largest lakes in eastern Europe by surface area but it is very shallow. About half of its catchment area is used for agriculture and cattle breeding. The modern lake is highly eutrophic and it has experienced anthropogenic pressure since the second half of the 20th century mainly due to accelerated urbanisation and intensive agricultural activity (Haberman *et al.* 1998, Nõges and Järvalt 2004). The lake underwent a rapid eutrophication during the 1970s and 1980s when practices in agriculture and wastewater treatments severely increased nutrient loads (Nõges *et al.* 2010a, Nõges *et al.* 2011). Thereafter, despite a considerable decrease in nutrient loading, no significant decline in nutrient concentrations has been observed (Nõges and Kisand 1999, Nõges *et al.* 2010a). The apparent reason for this is the internal loading, which is affected by the climatically-induced cyclic water-level fluctuations. The long-term mean annual amplitude of water-level change in very shallow Võrtsjärv is 1.4 m and the absolute range is 3.2 m (Nõges *et al.* 2005), having strong influence on the ecosystem. Thus the water-level fluctuations are the leading force controlling light regime as well as nutrient cycles in this lake.

During the last decades a need to examine the water quality of lakes over long time-scales and to determine their current ecological status has become relevant. Sometimes, the palaeolimnological approach through the study of sediment profile is the only way to access past environmental changes (Bennion and Battarbee 2007, Heinsalu and Alliksaar 2009). The mixing of shallow lake surface sediments complicate an interpretation of palaeolimnological information stored in sediments. However, within the last decade successful palaeolimnological investigations have been carried out also in shallow lakes (Eilers *et al.* 2006, Engstrom *et al.* 2006, Leeben *et al.* 2008).

The present study aimed to address how climate and anthropogenic eutrophication interact as stressors to affect the Võrtsjärv ecosystem. This is an important question to answer in terms of lake management. For this purpose, a sediment core representing the last 100 years of the history of the lake was investigated for fossil phytoplankton pigments, sediment porewater dissolved organic matter (pDOM) and phosphorus (P) fractions. Changes in the distribution of these palaeoindicators of lake primary production, origin and sources of organic matter (OM) and eutrophication were compared with the measured climate-change-induced environmental variables, nutrient loadings and phytoplankton indices based on long-term monitoring data.

Study site

Võrtsjärv (Fig. 1) with a surface area of 270 km^2 is a very shallow, unstratified lake with a mean depth of 2.8 m and a maximum depth of 6.0 m. The lake has six main inflows and one outflow to Lake Peipsi via the Suur Emajõgi. Due to the complicated and restricted outflow conditions, water level in the lake fluctuates strongly (annual mean amplitude 1.4 m) depending on the amount of precipitation in the catchment area (3374 km^2). Precipitation, on the other hand, is governed by variations in the North Atlantic Oscillation (NAO) that controls the strength of moisture transport by westerly winds and hence the water-level fluctuations in the lake, that exhibit a periodicity of about 30 years (Nõges and Nõges 1998, Jaani 2001). Because of its large area, shallowness and the predominating westerly winds, sediments of the lake are exposed to wave-induced resuspension making the water permanently highly turbid.

The lake is eutrophic, characterised by mean nutrient concentrations of about 2.0 mg l^{-1} for total N and about 50 μg l^{-1} for total P (P_{tot}). The lake water is weakly alkaline (193 mg HCO_3^- l^{-1}).

Fig. 1. Location of Võrtsjärv. Sediment coring point is marked with a black star.

Diatoms and cyanobacteria dominate the phytoplankton biomass, recently the latter was shown to account for more than two-thirds of the biomass during the summer months (Nõges *et al.* 2010b). Regular limnological investigations of the lake started in the 1960s when the chemical and biological monitoring began; in the 1970s also nutrient loading measurements from four main inflows were initiated.

Material and methods

Sediment sampling and chronology

A freeze corer was used to take a 120 cm sediment core from the southern part of Võrtsjärv (58°09´42´´N, 26°04´10´´E, water depth 1.40 m) in March 2003 (Fig. 1). The *in-situ* frozen sediment core was carefully cleaned and sliced into continuous 1-cm-thick sub-samples and stored at –20 °C in the dark prior to analyses.

For the chronology, the sediment samples were analysed for the activity of ^{210}Pb and ^{226}Ra, and the artificial radionuclides of ^{137}Cs and ^{241}Am by gamma spectrometry using a low background germanium detector (Appleby *et al.* 1986). The ^{210}Pb radiometric ages were calculated applying the constant rate of supply model and corrected by ^{137}Cs and ^{241}Am measurements of the core. The obtained age scale was then validated by the distribution in the sediment of microscopically enumerated spheroidal fly-ash particles — the products of high-temperature fossil-fuel combustion — whose concentration profile in sediments follow the characteristic features of fuel-burning history of the region (Nõges *et al.* 2006). All these dating analyses of the sediment core were undertaken by Heinsalu *et al.* (2008).

Standard methods were used for the determination of the basic sediment composition. The water content was measured by drying the samples to constant weight at 105 °C. Loss-on-ignition (550 °C for 4 h and at 950 °C for 2 h) was measured to evaluate sediment organic matter and carbonate contents (Heiri *et al.* 2001).

Fossil phytoplankton pigments

In Võrtsjärv, ca. 90% of the total phytoplankton biomass is formed by cyanobacteria (mainly filamentous forms) and diatoms (Nõges and Laugaste 1998; Nõges *et al.* 2004). Therefore, carotenoids like fucoxanthin and diadinoxanthin (Fuco and Diadino respectively) were chosen to track changes in sedimentary diatoms (Jeffrey *et al.* 1997, Bianchi *et al.* 2002, McGowan *et al.* 2005, Reuss *et al.* 2005). Lutein (Lute; indicating green algae) and zeaxanthin (Zea; indicating cyanobacteria) were taken together (Lute-Zea) as we failed to separate these pigments properly. According to Võrtsjärv phytoplankton composition where green algae are not the dominant group, Lute-Zea should mostly represent cyanobacteria. As all phytoplankton groups contain β-carotene, although in smaller concentrations, chlorophyll *a* (Chl *a*) together with β-carotene was chosen as a proxy for total phytoplankton biomass, although Chl *a* and β-carotene are also the major pigments in higher plants (Leavitt 1993, Jeffrey *et al.* 1997, Leavitt and Hodgson 2001, Patoine and Leavitt 2006). Pheophytin *a* (Pheo *a*) was used as a general Chl *a* derivative. To evaluate the preservation of palaeopigments the Chl *a*/Pheo *a* ratio was used.

The analysis of sediment pigments followed the recommendations of Leavitt and Hodgson (2001). The frozen sediment samples were freeze-dried and pigments were extracted with an acetone–methanol mixture (80:20 v:v) at –20 °C in the dark for 24 h under N_2. Thereafter, the pigment extracts were clarified by filtration through a 0.45 μm pore-size filter (Millex LCR, Millipore).

The reversed-phase high-pressure liquid chromatography (RP-HPLC) used for pigment separation consisted of a Cecil 1100 series instrument (Cecil Instrument, Cambridge, England) made up of binary pumps fitted with a dynamic gradient mixer (Cecil Instrument) with a system purge and a variable wavelength (200–800 nm) ultraviolet-visible detector (model CE1200, Cecil Instrument) with a 18 μl flow cell. Injection was done using a Rheodyne model 7725 manual valve (Cotati, California, USA) fitted with a 50 μl loop. Prior to the injection, 0.5 M ammonium acetate was added to each sample as an ion-pairing reagent (Wright *et al.* 1991).

Chromatographic separations were performed in the reversed-phase mode using a Waters (Milford, USA) Spherisorb ODS2 3 μm column (150 mm × 4.6 mm I.D.) in line with a pre-column containing the same phase. A binary gradient elution method, adapted from Zapata *et al.* (1987), was used with constant flow rate of 1.5 ml min^{-1}. The mobile phases and the elution program are shown in Table 1. After the analysis, the solvent composition was returned to the initial conditions for 10 min, which allowed the system equilibrium to be restored prior to the next sample injection. Before use, solvents were degassed under vacuum and bubbled with helium during chromatography. All solvents were HPLC gradient grade and chemicals were analytical grade. Analyses were carried out at room temperature (25 °C). The calculation of peak areas was made at 450 nm (Jeffrey *et al.* 1997). Identification and calibration of pigments was performed with commercially available standards from DHI Water and Environment (Denmark). A standard addition method was used to confirm peak identification.

Organic matter dissolved in sediment porewater

The molecular weight distribution of pDOM was evaluated using a high-performance size-exclusion chromatography (HPSEC) system which comprised a Dionex P680 HPLC Pump, Agilent 1200 Series (Agilent Technologies, UK) diode array absorbance detector (DAD) and a Rheodyne injector valve with a 50 μl sample loop. A BioSep-SEC-S 2000 PEEK analytical column (300 × 7.50 mm, Phenomenex, USA) preceded by a suitable guard column (75 × 7.50 mm, Phenomenex, USA) was used for separation. The applied flow rate was 1 ml min^{-1}. The column packing material was silica bonded with hydrophilic diol coating, with particle size of 5 μm and pore size of 145Å. The mobile phase consisted of 0.10 M $NH_4H_2PO_4$–$(NH_4)_2HPO_4$ buffer (pH 6.8). The HPSEC system was calibrated using protein standards (Aqueous SEC 1 Std, Phenomenex, USA).

The frozen sediment samples were thawed at 4 °C, and the porewater was separated by centrifugation at 4500 rpm for 15 min. Samples were filtered through 0.45 μm pore size filters (Millex LCR, Millipore) and analysed in triplicate on the same day. All solutions for the HPLC measurements were prepared using distilled water filtered through a MilliQ water system and degassed. Chromatograms were recorded and processed using an Agilent ChemStation software. Full details of the used method are described by Lepane *et al.* (2004).

Total peak areas of humic substances (HS; molecular size fraction 1.2–2.3 kDa, with shoulder 600–800 Da) were calculated from the chromatograms, representing the total UV-absorbing fraction of pDOM in the porewater sample. Weight-average molecular weight (M_w) of pDOM was calculated using the formula:

$$M_w = \Sigma(h_i M_i)/\Sigma h_i$$

where h_i is the detector output and M_i is the molecular weight, at the *i*th retention time (Mori and Barth 1999).

Sediment phosphorus fractions

Sediment P fractionation was performed in triplicates according to the modified method of Psenner *et al.* (1988). Prior analyses, the frozen sediment subsamples (–20 °C) were melted in darkness at 4 °C during 24 h; 120 mg of melted sediment was extracted in four consecutive steps with 10 ml of the following solution: ammonium chloride (1.0 M NH_4Cl, pH 7), bicarbonate-dithionite (BD; both $NaHCO_3$ and $Na_2S_2O_4$ 0.11 M), sodium hydroxide (0.1 M NaOH) and hydrochloric acid (0.5 M HCl). As the frequently used modification of the scheme of Psenner *et al.* (1988), 0.1 M NaOH was applied instead of the original 1.0 M NaOH. The extracts were centrifuged at 4000 rpm for 10 min. The soluble reactive P (SRP) in each fraction was determined spectrophotometrically with the molybdenum-blue method (Murphy and Riley 1962). This extraction procedure fractionates sedimentary P into loosely sorbed P (NH_4Cl-RP), reductant soluble P (BD-RP), metal-oxide bound P (NaOH-RP) and calcium bound P (HCl-RP). In addition to the obtained reactive P fractions, the amount of NaOH-non-reactive P (NaOH-NRP), representing organic P, was calculated as the difference between P_{tot} in the NaOH extract (NaOH-P_{tot}) and NaOH-RP. NaOH-P_{tot} was measured according to Murphy and Riley (1962) after persulphate digestion.

Statistical analysis of data

A Principal Component Analysis (PCA) was carried out for ordination and classification of the sub-samples of the sediment core in relation to sediment quality variables. PCA was applied to the whole data set, although in order to avoid

Table 1. Analytical gradient program and solvents used for RP-HPLC method.

Time (min)	0.5 M ammonium acetate (pH 7.2) and methanol (2:8 v:v) (%)	Methanol and acetone (8:2 v:v) (%)	Gradient system
0	50	50	Injection
2	50	50	Isocratic hold
15	0	100	Linear
23	0	100	Isocratic hold
25	50	50	Linear

redundancy and perform a more realistic ordination the variables with low percentage of contribution were eliminated. Kaiser's rule — suggesting that components with eigenvalues under 1.0 should be discarded — was applied to determine the number of components to be retained in PCA (Kaiser 1960). Sedimentary variables were analysed after centring and standardisation. PCA was performed with the Multivariate Statistical Package (MVSP) ver. 3.12 (Kovach 1999). Kruskal-Wallis ANOVA was used to check for differences among untreated palaeoparameters among PCA-separated periods. Spearman's rank order correlation (r_S) was used to find dependencies between untreated parameters. For both analyses STATISTICA (ver. 6.0) was used. The data on lake water-levels, annual mean air temperature (T_{air}) and precipitation for the city of Tartu (~50 km east from Võrtsjärv) were obtained from the Estonian Institute of Hydrology and Meteorology. Values of the NAO index for the period 1899–2001 were taken from http://www.cru.uea.ac.uk/cru/data/nao/. For the winter NAO index (NAO_w), four months starting with December of the previous year were selected.

Results

Chronology and lithostratigraphy

The results of the Võrtsjärv core-chronology study are presented in detail in Heinsalu *et al.* (2008). ^{210}Pb measurements of the recent sediments showed that the entire unsupported ^{210}Pb inventory was within the upper 94 cm, indicating an approximate age of 150 years. Peak concentrations of ^{137}Cs and ^{241}Am allowed locating the early 1960s (sediment depth of 35–40 cm) when the atmospheric nuclear tests were carried out. This chronology was confirmed by the sediment distribution of spheroidal fly-ash particle profile, documenting the characteristic changes in the fossil fuel-burning history of the region. According to the chronology, the 70-cm-long sediment core that was under study covered approximately 100 years, the period since about AD 1900.

The uppermost part of the sediment core was poorly compacted (water content was > 90%). The carbonate content started to increase in the mid-1950s and peaked sharply during the 1990s (Fig. 2). The OM content also rose during the past 15 years (Fig. 2).

Pigment stratigraphy

After an increase in the 1930s, the content of Lute-Zea, as well as Fuco decreased in the 1940s (Fig. 2). The content of Chl *a*, β-carotene and Lute-Zea increased since the 1950s while Fuco and Diadino increased since the 1980s. The dynamics of Pheo *a* showed no obvious trend. From the mid-1990s onward, investigated sediment pigments contents distinctly increased. The Chl *a*/Pheo *a* ratio showed an increasing trend since the mid-1990s (Fig. 2).

Changes in pDOM quantity and structure

The dominating UV-absorbing organic fraction in Võrtsjärv sediment porewater was the HS fraction (Lepane *et al.* 2010). The HS fraction content showed no trend until the 1970s, thereafter HS was decreasing (Fig. 2). The temporal distributions of molecular weight of pDOM showed mainly scattered values with no obvious trends (Fig. 2). However, a slight decrease of pDOM molecular weight was observed since the 1970s, thus indicating that the amount of organic compounds of autochthonous origin increased. M_w varied from 1.2 to 2.3 kDa, being close to aquatic fulvic compounds.

Changes in the dynamics of sediment phosphorus fractions

The average content of P fractions sum (ΣFR) was 730 μg g^{-1} DW, ranging from 460 to 1360 μg g^{-1} DW (Fig. 2). The dominating P fraction, BD-RP, accounted for 24%–49% of ΣFR and was strongly positively correlated with ΣFR ($r_S = 0.94, n = 31, p < 0.05$). The most labile P fraction (NH_4Cl-RP) contributed 0.7%–4% to ΣFR. The distinct rise in ΣFR began in the 1950s followed by the decrease in the 1970s on account of BD-RP and NaOH-RP. Another rise in ΣFR occurred in the mid-1990s. Although the con-

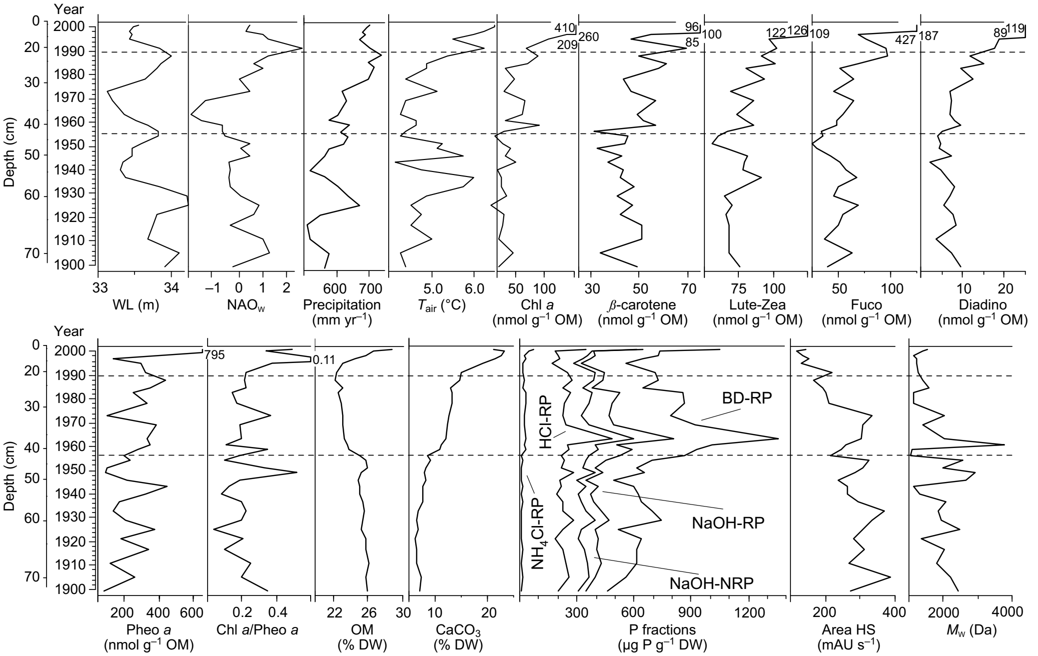

Fig. 2. The dynamics of palaeoparameters in sediments and water level oscillation (WL) in Võrtsjärv together with the fluctuation of annual mean air temperature (T_{air}), North Atlantic Oscillation winter index (NAO$_w$) and amount of precipitation for the period 1900–2001. Chl *a* = chlorophyll *a*; Lute-Zea = lutein and zeaxanthin; Fuco = fucoxanthin; Diadino = diadinoxanthin; Pheo *a* = pheophytin *a*; BD-RP = reductant soluble sedimentary phosphorus (P) fraction; NH$_4$Cl-RP = loosely sorbed P; HCl-RP = calcium bound P; NaOH-NRP = organic P; NaOH-RP = metal oxide bound P; Area HS = total peak areas of humic substances in the organic matter dissolved in sediment porewater (pDOM) (mAU s^{-1} = milli arbitrary units by seconds); M_w = weight-average molecular weight of pDOM; DW = dry weight; OM = organic matter; Da = dalton.

tents of HCl-RP and NaOH-NRP increased in the early 1960s and in the mid-1990s, respectively, their dynamics showed no obvious trend throughout the 20th century (Fig. 2).

Periods distinguished by PCA

The PCA over the 100-year period was based on all analysed variables except pDOM characteristics as the percentage of contribution of these parameters was low. The first two axes of PCA described 83.4% of the total variance in the data set. The first factor explained most of the variability (58.2%) and had positive loadings for CaCO$_3$ content, NH$_4$Cl-RP and sediment pigments. The second factor, accounting for 25.2% of the total variance, was correlated primarily with ΣFR, BD-RP and NaOH-RP. On the basis of the PCA, three periods were distinguished (Fig. 3): 1900–mid-1950s, mid-1950s–mid-1990s and mid-1990s–early 2000s. According to Kruskal-Wallis ANOVA, the investigated variables differed significantly between the first two periods (CaCO$_3$, organic matter, Chl *a*, ΣFR, NH$_4$Cl-RP, BD-RP), and between the first and the last period (CaCO$_3$, organic matter, Chl *a*, β-carotene, Lute-Zea, Fuco, Diadino, HS, NH$_4$Cl-RP, BD-RP, NaOH-NRP).

Water-level changes correlated strongly and positively with NAO$_w$, T_{air} and precipitation in the second half of the 20th century (Table 2). Correlations of water level and climate parameters with selected palaeodata in different investigated periods are given in Table 3. During the investigated period, palaeopigments (except Pheo *a*) correlated positively with CaCO$_3$ and NH$_4$Cl-RP and negatively with HS (Table 4).

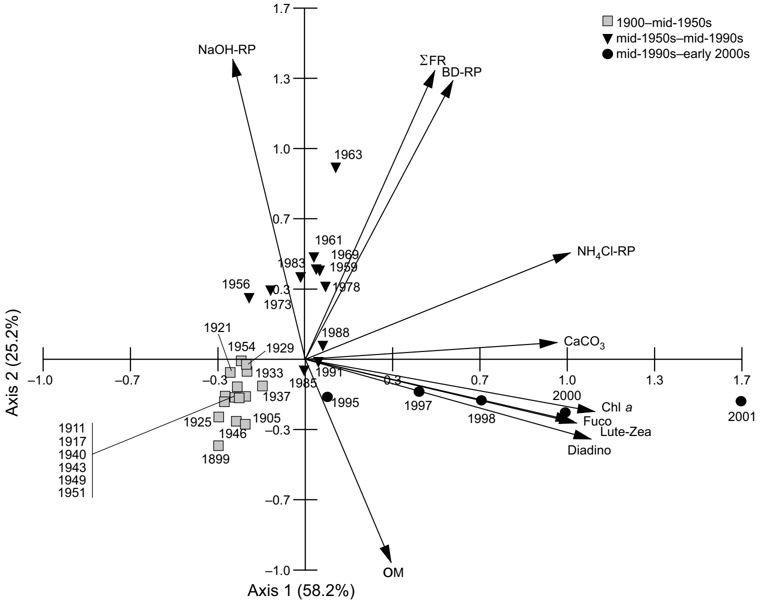

Fig. 3. Principal Component Analysis (PCA) biplot indicating three periods distinguished using selected palaeoparameters (arrows) from the 1900–2001 Võrtsjärv sediment core. For abbreviations *see* Fig. 2. ΣFR = sum of sedimentary phosphorus fractions.

Discussion

The palaeopigment composition in Võrtsjärv sediments indicates that cyanobacteria and diatoms were the dominant phytoplankton groups in the lake during the first half of the 20th century (Fig. 2), the first period clearly distinguishable in the PCA plot (Fig. 3). During this period, no significant increase in P input from the lake catchment occurred as the content and variability of studied P fractions was rather low (Fig. 2). Sediment resuspension was probably the most important P source for Võrtsjärv phytoplankton at that time. The large catchment area ensures naturally higher nutrient inflow to Võrtsjärv while the shallowness of the lake causing strong resuspension favors nutrient release from sediments into water. Therefore, slightly eutrophic conditions prevailed in the lake already from 1900 to the mid-1950s. Earlier Heinsalu *et al.* (2008) also showed that the sedimentary diatom flora since about 1840 indicated moderately eutrophic, shallow-water conditions in Võrtsjärv. The reasons for that were likely natural — the lake's large catchment area, its shallowness resulting in high resuspension of sediments, hence sediments being a source of nutrients.

The regular oscillation of the water level has a strong impact on the Võrtsjärv ecosystem controlling its light climate as well as nutrient cycles, and through that also bioproduction and OM decomposition. The water level itself is determined mainly by the winter climatic conditions. In Estonia, the western airflow from the Atlantic during positive NAO remarkably increases air temperature and the amount of precipitation in winter. Warmer winters cause higher water level in Võrtsjärv in spring and also during the following summer and autumn. With higher water level, algal density is determined by light availability rather than by nutrients which results in lower phytoplankton biomass. When water

Table 2. Spearman rank order correlations of Võrtsjärv water level with climate parameters in different periods in the 20th century. Correlation coefficients set in boldface are significant at $p < 0.05$.

Water level in different periods	Winter NAO_w	Annual mean air temperature (T_{air})	Precipitation
1900 to mid-1950s; $n = 15$	0.352	–0.279	**0.580**
Mid-1950s to mid-1990s; $n = 11$	**0.654**	0.483	**0.636**

level is lower, more light is available and nutrient limitation takes over the control of phytoplankton. The shallower lake means higher phytoplankton biomass per unit volume and also per unit area (Nõges *et al.* 2003, Nõges *et al.* 2010a). In Võrtsjärv, the estimated euphotic-zone depth is 1.6–3.2 m, being generally smaller than the mean (2.8 m) or maximum (6.0 m) water depth (Haberman *et al.* 1998). Thus, euphotic zone penetration to the lake bottom is limited and the benthic algal community development is depressed. Moreover in deeper areas of Võrtsjärv the sediment surface consists of very fine floating material which is not the best substrate for phytobenthos. Phytoplankton investigations in Võrtsjärv indicate that during the low-water-level period, algae from the sediment surface increase the phytoplankton species number in the water column, but do not contribute remarkably to biomass (Nõges *et al.* 2004). Thus the proportion of pigments derived from phytobentos is negligible in the pigment content accumulated in the Võrtsjärv sediment.

In the first half of the 20th century, water level correlated positively with precipitation (Table 2). Of the fossil pigments, only diadinoxanthin correlated significantly and positively with water level. Also the water level fluctuations and the amount of precipitation significantly affected pDOM characteristics like HS quantity (expressed as area of HS) and M_w (Table 3). A positive relationship suggests higher transport of land-derived organic particles and substances into the lake during increased water level. According to Toming *et al.* (2009), in Võrtsjärv water level and yellow substance (constituting up to 90% of DOM in boreal region) correlate positively. Concentration of yellow substance reaches maximum in spring and generally decreases towards autumn. The reason why the relationship between water level and phytoplankton biomass in the water column (Nõges *et al.* 2003, Nõges *et al.* 2010b) was not found for the total phytoplankton biomass (Chl *a* and *β*-carotene) and the fossil phytoplankton pigments in the sediment record can be a result of wind-induced waves constantly mixing surface sediment layers in this shallow lake causing decay of settled algae and their pigments. Other arguments for the lack of this relationship can be

Table 3. Spearman rank order correlations of selected palaeodata from Võrtsjärv dated sediment core with climate parameters and water-level in different time periods within the 20th century. Correlation coefficients set in boldface are significant at $p < 0.05$. Area HS = total peak areas of humic substances in the organic matter dissolved in sediment porewater (pDOM); M_w = weight-average molecular weight of pDOM; ΣFR = sum of sedimentary phosphorus (P) fractions; BD-RP = reductant soluble P; NaOH-RP = metal oxide bound P.

Palaeodata	1900–mid-1950s; $n = 15$				mid-1950s–mid-1990s; $n = 11$			
	NAO$_w$	Annual mean air temperature (T_{air})	Precipitation	Water level	NAO$_w$	Annual mean air temperature (T_{air})	Precipitation	Water level
Chlorophyll *a*	0.137	–0.017	–0.139	–0.196	0.391	0.399	0.384	0.237
β-carotene	–0.078	–0.001	–0.188	0.246	0.300	0.349	0.363	0.363
Lutein and zeaxanthin	–0.237	0.161	–0.353	–0.321	**0.608**	0.526	**0.601**	0.433
Fucoxanthin	0.034	–0.177	–0.15	0.096	**0.629**	0.498	**0.671**	0.405
Diadinoxanthin	0.078	0.095	–0.037	0.478	**0.720**	**0.621**	**0.692**	0.433
Area HS	–0.034	–0.082	**0.559**	**0.646**	**–0.643**	–0.438	**–0.664**	**–0.664**
M_w	0.076	0.087	0.491	0.260	–0.230	0.067	–0.377	–0.377
ΣFR	–0.434	0.310	0.224	–0.032	**–0.888**	**–0.816**	**–0.650**	–0.398
BD-RP	–0.479	0.385	0.127	–0.167	**–0.888**	**–0.869**	**–0.615**	–0.447
NaOH-RP	–0.148	0.143	0.168	0.185	**–0.671**	**–0.703**	–0.489	–0.356

quite weak correlation between NAO_w and water level found for the first half of the 20th century (Table 2), and the relatively high standard error of dates for deeper sediment horizons (*see* fig. 1c in Heinsalu *et al.* 2008), which is the common feature for calculated ^{210}Pb radiometric ages and what can complicate temporal comparison of datasets.

Besides water-level changes, also air-temperature fluctuation may influence the entire water column of shallow, non-stratified lakes and therefore these lakes are likely to respond more directly to short-term weather variations (Arvola *et al.* 2010). Higher water temperature in summer favour cyanobacterial blooms (Blenckner *et al.* 2010) and therefore the short-term increase in Lute-Zea in the 1930s could be induced by the rise of T_{air} in that decade (Fig. 2).

The PCA analysis revealed changes in Võrtsjärv ecological conditions since the 1950s (Fig. 3). Increase in palaeopigments (except Pheo *a*), elevated contents of P fractions (except NaOH-NRP and HCl-RP) and $CaCO_3$ as well as decline in HS and M_w are distinctive for the period from the mid-1950s to the mid-1990s (Fig. 2). Earlier investigations indicated that since the 1960s, urbanisation and agricultural activities in the Võrtsjärv drainage area intensified. A long-term monitoring dataset revealed that due to higher nutrient loading to the lake phytoplankton biomass increased in the 1960s reaching its maximum in the 1970s (Nõges and Laugaste 1998, Nõges and Nõges 2006). Moreover, the analysis of phytoplankton taxonomic indices showed a continuous deterioration of Võrtsjärv ecological status during the 44-year period of limnological investigations (1964–2007; Nõges *et al.* 2010a). Increase in the content of palaeopigments and P fractions from the mid-1950s onwards confirm increased nutrient loading to the lake and a rise in bioproduction. Positive correlations of palaeopigments (except Pheo *a*) with NH_4Cl-RP and $CaCO_3$ indicate accelerated eutrophication of the lake (Table 4). NH_4Cl-RP includes phosphates dissolved in sediment porewater and loosely sorbed on sediment particles. Higher concentration of NH_4Cl-RP facilitates P diffusion from the sediment to the lake water, since diffusion is driven by concentration gradients. If no other processes precipitate phosphates again, diffusion from sediments can constantly provide P supply for lake primary producers. The increase in $CaCO_3$ content in the sediments from the mid-1950s onwards has been considered an indicator of lake eutrophication, as intensive primary production rises water pH which results in accelerated $CaCO_3$ precipitation (Hodell *et al.* 1998, Dean 1999). The decline in the HS and M_w values since the 1960s–1970s coincided with the phytoplankton maximum values in the 1970s, as well as with an increase in palaeopigments since the mid-1950s (Fig. 2). The lower HS values and lower molecular weight of pDOM in sediments indicate autochthonous rather than allochthonous origin of OM (Bergström and Jansson 2000, Jansson *et al.* 2000), and hence increased in-lake bioproduction. Negative relationship of HS with palaeopigments supports the above assumption. However, lower M_w might indicate that microbial mineralization of OM is an important degradation process in the Võrtsjärv sediments (Münster and Chróst 1990).

Table 4. Spearman rank order correlations between selected palaeoparameters ($n = 31$) of Võrtsjärv for the period 1900–2001. All correlation coefficients are significant at $p < 0.05$. Area HS = total peak areas of humic substances in the organic matter dissolved in sediment porewater; NH_4Cl-RP = loosely sorbed sedimentary phosphorus fraction.

Palaeopigments	$CaCO_3$ content	Area HS	NH_4Cl-RP
Chlorophyll *a*	0.747	–0.640	0.696
β-carotene	0.590	–0.477	0.592
Lutein and zeaxanthin	0.745	–0.751	0.606
Fucoxanthin	0.577	–0.556	0.473
Diadinoxanthin	0.664	–0.647	0.563

Between the mid-1950s and the mid-1990s, the correlations between all used climate parameters (NAO_w, T_{air}, the amount of precipitation) and water level were much stronger (Table 2). In Võrtsjärv, the NAO_w index is positively correlated with biomass of cyanobacteria and diatoms as well as with total biomass of phytoplankton in spring (Nõges and Järvalt 2004, Nõges *et al.* 2010b). Our results show that between the mid-1950s and the mid-1990s, NAO_w, precipitation and T_{air} correlated positively with palaeopigments (Lute-Zea, Fuco and Diadino) and negatively with P fractions (ΣFR, BD-RP and NaOH-RP). Accelerated eutrophication and better preservation conditions of palaeopigments during higher water level can be the cause of the overall rise in fossil pigments content and their positive relationship with the NAO_w index.

Higher floods follow usually colder winters because due to frozen soils most of the meltwater reaches rivers as surface runoff, carrying large quantities of nutrients to the lake (Nõges and Järvalt 2004). This could be one possible explanation for negative correlation of NAO_w and T_{air} with ΣFR, BD-RP and NaOH-RP. Eutrophication will increase because of climate warming as it is expected to lead to increased external nutrient loading. However, the effects of climate change on shallow temperate lakes will mimic the effects of human-induced eutrophication and to ascertain its magnitude is complicated (Mooij *et al.* 2007, Nõges *et al.* 2008). In case of Võrtsjärv, the effects of climate change include change in temperature, but even more hydrological changes that are likely to be much more important. Water level oscillations in Võrtsjärv induce changes in the composition of dominant phytoplankton species: during high-water period shadow-tolerant species are favoured and vice versa (Nõges *et al.* 2003, Nõges *et al.* 2010b). Unfortunately, palaeopigments are only bioindicators of phytoplankton groups not species (Leavitt and Hodgson 2001). In our opinion this is the reason why photosynthetic pigments are poor water-level-fluctuation indicators in Võrtsjärv.

Together with the collapse of extensive agriculture in the early 1990s, the external load of nutrients to Võrtsjärv declined remarkably (Järvet 2004). Along with weakening anthropogenic pressure on the lake ecosystem P fractions (except NaOH-RP), fossil pigments, sediment OM and $CaCO_3$ content in the sediment core sharply increased since the mid-1990s (Fig. 2). The most likely explanation would be that the upper 0–13 cm sediment layers (period from mid 1990s to early 2000s) are unconsolidated and therefore clearly differ from other horizons. Microbial activity and thus degradation processes of settled material in upper sediment layers are much more intensive than in deeper sediments (Wetzel 2001). Due to resuspension, surface sediment layers are involved in the lake's P cycle and a part of settled P is released back to the water column. The peak of the Chl *a*/Pheo *a* ratio in the 1990s reveals that degradation of fossil pigments was in progress (Fig. 2). However, the sharp increase in palaeopigments coincided with the rise of monthly chlorophyll *a* concentration in the water column since the 1990s: from about 20 mg m^{-3} to 50 mg m^{-3} (Fig. 7; Nõges *et al.* 2011). According to the monitoring data, the biomass of the filamentous cyanobacteria *Limnothrix planctonica* has increased in Võrtsjärv since the 1990s. This cyanobacteria build up a considerable standing stock by autumn. High biomass in autumn is characterized by a high Chl *a* content (Nõges *et al.* 2011). Together with the palaeopigments the T_{air} increased since the 1980s (Fig. 2). Generally phytoplankton is the first to benefit from higher water temperatures (Mooij *et al.* 2007). Thus, the rise in palaeopigments since the mid-1990s could also resulted from increasing T_{air}.

Conclusions

Our palaeolimnological investigation revealed that distinct changes in the Võrtsjärv ecosystem occurred in the last century. In the first half of the 20th century the lake was naturally slightly eutrophic and the changes in palaeoindices reflect natural variations induced by the regular oscillation of the water level. Accumulation of palaeopigments was low and the load of nutrients was stable. As water level did not correlate strongly with the climate parameters, the relationship between palaeoparameters and water level was also generally weak.

The deterioration of the Võrtsjärv ecosystem from the mid-1950s onwards was caused by increased eutrophication. The increase in $CaCO_3$ content, palaeopigments and nutrients, and low molecular weight of OM in sediments reflect high autochthonous matter input. The situation as compared with that during the pre-eutrophication period — when lake water contained substances with various molecular weights (from in-lake production and catchment-derived) the share of which depended on water-level fluctuations — changed and substances of autochthonous origin with smaller molecular weights started to predominate. Group-specific fossil pigments are poor water-level-fluctuation indicators in Võrtsjärv, as water-level oscillations induce changes in the composition of dominant phytoplankton species.

From the mid-1990s to the early 2000s the increase in palaeopigment concentrations could be caused by risen air and water temperatures. However, degradation of fossil pigments is also in progress in upper sediment layers.

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III

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History of anthropogenically mediated eutrophication of Lake Peipsi as revealed by the stratigraphy of fossil pigments and molecular size fractions of pore-water dissolved organic matter

Aina Leeben · Ilmar Tõnno · Rene Freiberg · Viia Lepane · Nicolas Bonningues · Natalja Makarõtševa · Atko Heinsalu · Tiiu Alliksaar

Abstract We investigated stratigraphic changes in fossil pigments and the molecular structure of the UV-absorbing fraction of pore-water dissolved organic matter in a sedimentary record from Lake Peipsi (Estonia/Russia) temporally covering the 20th century. The aims of the study were to define the onset of eutrophication in the lake and to track its course. An attempt was also made to reconstruct lake conditions before the intensive nutrient loading began. Fossil pigment analysis indicated that the eutrophication of the lake started in the 1960s and accelerated in the 1970s. Sedimentary pigments also indicate a continuing tendency of the lake ecosystem towards eutrophy in the 1980s and 1990s. However, changes in the molecular size structure of pore-water dissolved organic matter indicated that the contribution of autochthonous matter to the organic pool of the lake ecosystem had already started to increase around the end of the 1930s. We conclude that this rise was generated by a coincidence of several anthropogenic and natural factors. The pore-water data also show that a slight relative reduction in the autochthonous organic matter took place in the 1990s. A discordance in the paleodata obtained for the beginning of the 20th century complicates clear conclusions about earlier conditions in the lake. On the one hand, the qualitative characteristics of pore-water dissolved organic matter and the low concentration of chlorophyll *a* indicate that the phytoplankton biomass was low in Lake Peipsi during that period. On the other hand, the concentrations of marker pigments of specific phytoplankton groups are high, comparable with the values in the recent sediments. Possible reasons for the high levels of these pigments in the early 1900s sediments, such as a shift in the preservation conditions of organic substances and their transport from the lake's catchment, are discussed.

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European Large Lakes—Ecosystem changes and their ecological and socioeconomic impacts

A. Leeben (✉)
Marine Systems Institute, Tallinn University of Technology, Akadeemia tee 21, 12618 Tallinn, Estonia
e-mail: aina.leeben@phys.sea.ee

I. Tõnno · R. Freiberg
Centre for Limnology, Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Rannu, Tartu County 61101, Estonia

V. Lepane · N. Bonningues · N. Makarõtševa
Institute of Chemistry, Tallinn University of Technology, Akadeemia tee 15, 12618 Tallinn, Estonia

N. Bonningues
Joseph Fourier University, DSU, 480 Avenue Centrale, 38400 Saint-Martin d'Heres, France

A. Heinsalu · T. Alliksaar
Institute of Geology, Tallinn University of Technology, Ehitajate tee 5, 19086 Tallinn, Estonia

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Introduction

Increased nutrient loading due to intensified agriculture, industry and urbanization has been one of the forcing factors influencing European lakes during recent centuries. In particular, the period since the Second World War has been characterized by the enrichment of water bodies with biogenic substances. However, initial changes and the course of eutrophication in large water ecosystems are often either poorly documented or information on them is entirely missing. In such cases, a paleolimnological approach is often the only way to reconstruct the history of a lake.

Lake Peipsi is the fourth largest lake in Europe by surface area. The catchment consists of fertile soils; there are several municipalities with up to 200,000 inhabitants and numerous industrial establishments. The lake has been subjected to increasing anthropogenic inputs of nutrients from the 1950s onwards, as demonstrated by paleoecological analyses of diatom assemblages (Heinsalu et al., 2007). Monitoring data collected since the early 1960s reveal subsequent nutrient inputs and changes in the lake ecosystem (Nõges, 2001; Pihu & Haberman, 2001).

In this study, we present a reconstruction of the recent eutrophication history of Lake Peipsi at a high-resolution scale based on fossil pigment records and stratigraphic changes in the molecular size structure of pore-water dissolved organic matter (*p*DOM). Two methods were employed in the analyses of pigments and *p*DOM: first, crude samples were analysed spectrophotometrically; thereafter, compounds in the sample were separated and analysed by high-performance liquid chromatography (HPLC). Although measurements of *p*DOM are not yet widely employed in paleoinvestigations, several studies have demonstrated the capacity of these methods to provide insight into the origin and character of organic matter (OM) (Peuravuori & Pihlaja, 1997; Chin et al., 1998; Lepane et al., 2004). Moreover, results from a recent study have proved that the absorbance characteristics of *p*DOM have high potential for tracking the sources of organic carbon and for assessing the paleoproductivity of a lake (Leeben et al., 2005).

Study site

Lake Peipsi is a large (3,555 km^2), shallow (mean depth 7.1 m, max depth 15.3 m), unstratified and eutrophied water body, which consists of two extensive basins (Peipsi proper and Lake Pihkva) joined by a narrow strait (Lämmijärv). The lake receives its water from a catchment area of 47,800 km^2 and has an outflow into the Gulf of Finland. The mean residence time of the water is about 2 years. A characteristic feature of the lake is the fluctuation of the water level (mean annual range 1.2 m). Approximately 40% of the lake's catchment area is covered by coniferous and mixed forests, and approximately another 40% is used for agricultural purposes. The soils of the catchment are very diverse (loamy Cambisols, Luvisols, Podzoluvisols, sandy Podzols, Planosols, Gleysols, Histosols). During wintertime, the lake is covered by ice for an average of 114 days. A comprehensive overview of the catchment and limnology of Lake Peipsi can be found in Nõges (2001) and Pihu & Haberman (2001).

Materials and methods

Sediment coring and dating

Sediment samples were obtained from the middle of the broadest part of the lake (58°47′14″ N, 27°19′20″ E; water depth 9.2 m) with a freeze corer (Wright, 1980) in winter 2002 and 2006. The cores were sectioned into consecutive 1-cm sub-samples. The 2002 core was subjected to dating and pore-water analyses; pigment analyses were performed on the 2006 core. The two cores were correlated through their loss-on-ignition curves.

For dating, the sediment samples were analysed for ^{210}Pb, ^{226}Ra and ^{137}Cs by gamma spectrometry using a well-type coaxial low background intrinsic germanium detector (Appleby et al., 1986). For the ^{210}Pb chronology, the CRS model (Constant Rate of Supply; Appleby et al., 1986) was applied. The methodology, results and reliability of the chronology are presented and discussed in detail in Heinsalu et al. (2007).

Analyses of pore-water dissolved organic matter (*p*DOM)

The frozen sediment samples were thawed at 4°C. The pore water was collected and clarified by centrifugation and filtration (pore size 0.2 μm). Absorption spectra were recorded over the range 200–800 nm with a Cadas 100 scanning spectrophotometer (Dr. Lange) using Milli-Q water as a blank. The ratio of the absorbances at 250 and 365 nm was calculated to evaluate the character and molecular structure of *p*DOM: a high ratio indicates organic substances with a low degree of aromaticity and small molecular size (Peuravuori & Pihlaja, 1997). The absorbance values were converted into carbon concentrations using a linear relationship between the absorbance values of *p*DOM at 250 nm and the concentration of dissolved organic carbon (DOC) in the pore water ($r = 0.87$, $n = 10$). The DOC concentration was determined by the standard method ISO 8245 (1999) using a DC-80 Total Organic Carbon Analyzer (Rosemount-Dohrmann): the organic carbon is converted to CO_2 by ultraviolet-promoted persulphate oxidation, and the CO_2 formed is detected in the infrared part of the spectrum.

High-performance size-exclusion chromatography (HPSEC) of pore-water samples was carried out using an HPLC system with a UV detector (Knauer) coupled to a biocompatible BIOSEP-SEC-S2000 column (7.5 × 300 mm, Phenomenex). A 100-μl sample was injected into the column. Compounds were eluted with 0.02 M phosphate buffer (pH 6.8) at a flow rate of 1 ml min^{-1} and detected by absorbance at 254 nm. The HPLC system was calibrated using protein standards, which were run individually; the corresponding elution times were recorded and plotted against their molecular weights. Full details of the HPSEC equipment and procedures are described in Lepane et al. (2004). The total peak areas were calculated from the chromatograms, representing the total UV-absorbing fraction of the DOM in the pore-water sample. To obtain the percentages of molecular fractions, the chromatograms were divided into three molecular size fractions: 0.01–0.2 kDa (low molecular weight, LMW); 0.2–100 kDa (medium molecular weight, MMW) and 100–2,000 kDa (high molecular weight, HMW). The areas of the respective fractions were calculated and divided by the total peak area. Weight-average and number-average molecular weights of *p*DOM (M_w and M_n, respectively) were determined using the formulae

$$M_w = \Sigma(h_i \cdot M_i)/\Sigma h_i \text{ and } M_n = \Sigma(h_i)/\Sigma(h_i/M_i),$$

where h_i is the detector output and M_i is the molecular weight, both at the ith retention time (Mori & Barth, 1999). Thus, M_n is the weight of an 'average' molecule in the mixture, while M_w is the weight of the molecule to which the 'average' atom belongs (Zhou et al., 2000).

Analyses of fossil pigments

Sediment pigments were extracted and analysed following the recommendations of Leavitt & Hodgson (2001). The frozen sediment samples were freeze-dried and pigments were extracted with an acetone-methanol mixture (80:20 v:v) at −20°C for 24 h under a N_2 atmosphere. Thereafter, the extracts were clarified by filtration through a 0.45 μm pore-size filter (Millex LCR, Millipore). The absorption spectra of the pigment extracts were recorded with a Hitachi U-3010 spectrophotometer over the wavelength range 400–800 nm. The ratio between the absorbances at 410 and 665 nm was calculated from the spectra as an indicator of chlorophyll (Chl) *a* preservation. The degradation products of Chl *a* have an absorbance peak at 410 nm, while the absorbance peak around 665 nm is due to Chl *a* and its derivatives (Rowan, 1989).

The pigments were separated with an HPLC installation consisting of two pumps (CE 1100 Cecil Instruments Ltd, UK), a dynamic mixer (Cecil), an injection valve (Rheodyne 7125) with a 100-μl injection loop and a UV detector (CE1200, Cecil). A Spherisorb ODS2 column (4.6 × 150 mm, 3 μm particle size, Waters) was used for the separation. Prior to the HPLC run, an ion-pairing solution was added to the sample extract. The pigments were eluted using a non-linear binary gradient at a constant flow rate of 1.5 ml min^{-1} (Table 1) and identified by their retention times reported in the literature and

Table 1 Elution scheme and solvents used in the separation of pigments by HPLC

Solvent (%)	Time (min)				
	0	2	15	23	25
A	100	50	0	0	100
B	0	50	100	100	0

Solvent A = 80% methanol : 20% 1 M ammonium acetate (pH 7.2) (v:v)

Solvent B = 80% methanol : 20% acetone (v:v)

standards provided by DHI (Denmark). The chromatograms were quantified by absorbance at 450 nm.

Results

Pigment stratigraphy

According to Laugaste et al. (1996), the assemblage of dominant species in Lake Peipsi has not changed during the last 100 years: the main portion of phytoplankton biomass in the lake is formed by diatoms [*Aulacoseira islandica* (O. Müller) Sim., *Aulacoseira. granulata* (Ehr.) Sim., *Stephanodiscus binderanus* (Kütz.) Krieger], followed by cyanobacteria [*Gloeotrichia echinulata* (J. E. Smith) Richter, *Aphanizomenon*, *Microcystis* and *Anabaena* species]. Third come green algae, but their biomass is considerably smaller than that of the two predominant groups. Unfortunately, because the peaks overlap with those of other pigments, the HPLC method we used failed to separate fucoxanthin (a marker pigment for diatoms) and myxoxanthophyll (a marker pigment for cyanobacteria) properly. Therefore, Chl c_2 (for diatoms) and echinenone (for N_2-fixing filamentous cyanobacteria) were chosen to track changes in the dominant phytoplankton groups in the sediments (Leavitt & Hodgson, 2001; Bianchi et al., 2002). We also present zeaxanthin and canthaxanthin data, because the actual pigment composition of cyanobacteria species occurring in Lake Peipsi is not fully known. Zeaxanthin has been detected in *Microcystis* species (Bianchi et al., 2002; Kupperstein & Boyer, 2005), but it is also a common pigment in vascular plants. Canthaxanthin has been used as a marker pigment for colonial and filamentous cyanobacteria (Lami et al., 2000; Leavitt & Hodgson, 2001); however, sometimes herbivore tissues also contain this xanthophyll (Leavitt, 1993). Chlorophyll *b* was chosen as a proxy for green algae and Chl *a* as a proxy for total phytoplankton. However, one should keep in mind that both these are also major pigments in higher plants.

The quantities and profiles of fossil pigments in the sediment record of Lake Peipsi vary greatly. The concentrations of Chl *a* and Chl c_2 in the lower part of the record (50 and 1 nmol g^{-1} OM, respectively) are approximately one quarter of those in the upper part (Fig. 1a, c; Table 2). Their concentrations started to increase in the 1970s. The distribution of canthaxanthin exhibits a reverse pattern, having a slightly higher concentration (around 50 nmol g^{-1} OM) before the 1920s, after which it diminishes somewhat (to 40 nmol g^{-1} OM) and remains stable up to the top of the core (Fig. 1f). The concentrations of echinenone and zeaxanthin vary over the ranges 15–30 and 30–45 nmol g^{-1} OM, respectively (Fig. 1d, e). Their lowest concentrations are detected around the mid-20th century. The concentration of Chl *b* varies over the range 5–10 nmol g^{-1} OM (Fig. 1g) and its stratigraphy appears similar to those of echinenone and zeaxanthin. The lowest concentrations of Chl *b* occur in the 1960s and 1970s.

Temporal changes in the quantity and structure of *p*DOM

The concentration of *p*DOM (expressed as carbon) in the sediment varies from 2 to 3 mM (Fig. 1k, Table 2). Its highest values are around 1900 and at the end of the 20th century. The weight-average molecular weight was less than 7 kDa, varying from 3.5 to 5 kDa (Fig. 1l). The number-average molecular weight of *p*DOM was less than 4 kDa and mainly in the range 1–3 kDa (Fig. 1m). In two sediment layers, one dated to the late 1960s and the other to the very early 1990s, the pore-water organic substances have very high molecular weights (Fig. 1l, m). We would point out that the values for *p*DOM obtained here are not 'real' concentrations and molecular weights of organic substances in the pore water. Freezing and subsequent thawing of organic material in aqueous solution disintegrates particulate OM and disrupts dissolved organic molecules. Comparison of the molecular characteristics of *p*DOM in fresh and frozen samples showed that the molecular weights are approximately 2–3 times higher in unfrozen samples than in frozen samples (Lepane et al., 2006).

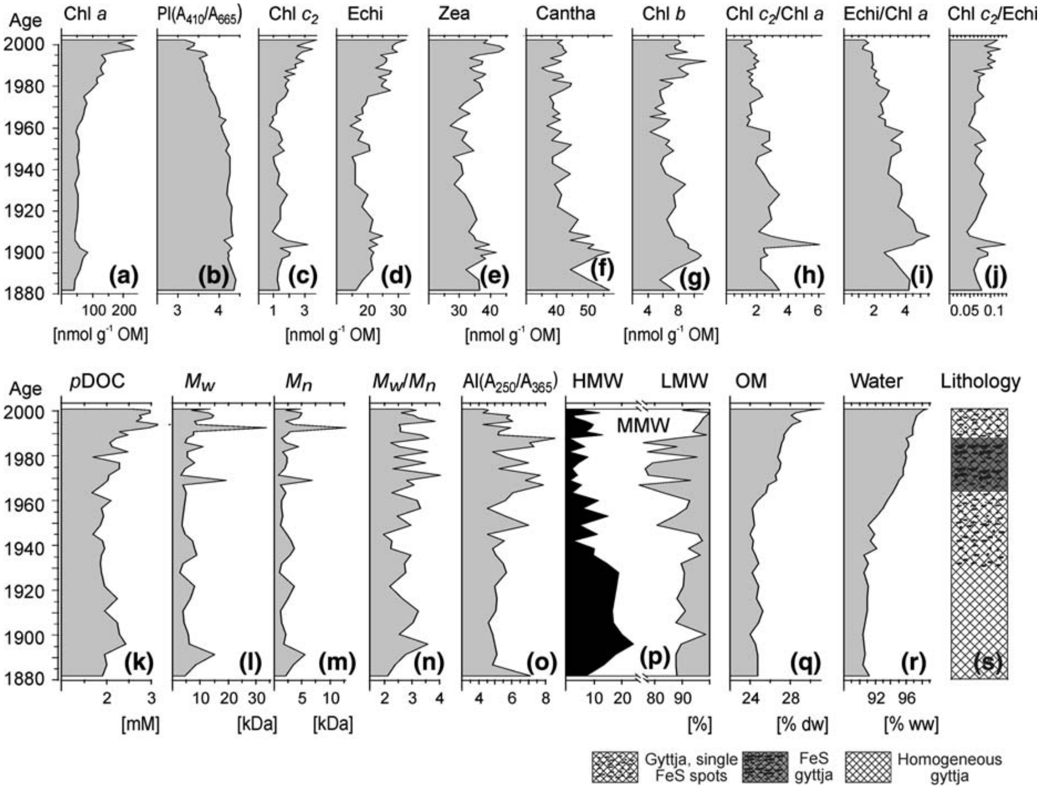

Fig. 1 (*Upper panel*) Age-resolved profiles of the concentrations of selected fossil pigments (**a**, **c**–**g**) and their ratios (**h**–**j**) in the sediments of Lake Peipsi. Preservation index of chlorophyll *a* (PI), calculated as the ratio between absorbances at 410 and 665 nm (A_{410}/A_{665}), is also shown (**b**). (*Lower panel*) Age-related changes in the characteristics of pore-water dissolved organic matter (**k**–**p**). For comparison, the profiles of major sediment constituents (**q**, **r**) and lithology (**s**) (reprinted with permission from Heinsalu et al., 2007) are also shown. Abbreviations: Chl—Chlorophyll; Echi—Echinenone; Zea—Zeaxanthin; Cantha—Canthaxanthin; *p*DOC—Pore-water dissolved organic carbon; M_w and M_n—Weight-average and number-average molecular weight, respectively; AI—Aromaticity index calculated as the ratio between absorbances at 250 and 365 nm (A_{250}/A_{365}); LMW—Low molecular weight; MMW—Medium molecular weight; HMW—High molecular weight; OM—Organic matter; dw and ww—Dry and wet weight of sediments, respectively. For details see Materials and methods

The ratio of M_w to M_n, called polydispersity, has been used as a quantitative measure of the molecular weight distribution of OM in a mixture (Chin et al., 1998). For pure organic substances the ratio is equal to 1; for a mixture of molecules it is greater than 1. In the sediments of Lake Peipsi, the polydispersity of *p*DOM varies over a very narrow range (Fig. 1n), indicating that temporal changes in the molecular weight distribution are small. However, the aromaticity index estimates of *p*DOM demonstrate slightly elevated values from the 1960s to the late 1980s (Fig. 1o, Table 2), implying that the proportion of substances of smaller size and lower aromaticity has increased in the OM accumulated during this period. Ordinarily, lower aromaticity and molecular weight (hence also size) are characteristic of autochthonous OM, while compounds with higher aromaticity and molecular weight predominate in OM derived from terrestrial sources (McKnight et al., 2001). Stratigraphic changes in the distribution of different molecular weight fractions confirm that the proportion of high molecular weight *p*DOM has decreased

Table 2 Spearman rank order correlations for selected characteristics of pore-water dissolved organic matter (*p*DOM) and fossil pigments obtained by different methods (given in parentheses) for the analysed sediments of Lake Peipsi

Pair of characteristics	Spearman	*P*-level
Fossil pigments		
A_{665} (Abs1) and Chlorophyll *a* (HPLC)	0.846	0.000
A_{665} (Abs1) and Chlorophyll *b* (HPLC)	0.247	0.095
A_{665} (Abs1) and Chlorophyll c_2 (HPLC)	0.691	0.000
A_{665} (Abs1) and Echinenone (HPLC)	0.804	0.000
A_{665} (Abs1) and Zeaxanthin (HPLC)	0.484	0.000
A_{665} (Abs1) and Canthaxanthin (HPLC)	−0.257	0.081
*p*DOM		
*p*DOC (PO + Abs2) and Total area (HPSEC)	0.882	0.000
A_{250}/A_{365} (Abs2) and HMW (HPSEC)	−0.545	0.000
A_{250}/A_{365} (Abs2) and MMW (HPSEC)	−0.257	0.109
A_{250}/A_{365} (Abs2) and LMW (HPSEC)	0.274	0.087
M_w/M_n (HPSEC) and HMW (HPSEC)	−0.011	0.947
M_w/M_n (HPSEC) and MMW (HPSEC)	0.440	0.004
M_w/M_n (HPSEC) and LMW (HPSEC)	0.169	0.327

A_{665}—Absorbance of crude pigment extract at 665 nm; *p*DOC—Concentration of pore-water dissolved organic carbon; Total area—Total area of chromatogram; A_{250}/A_{365}—Ratio of absorbances of pore water at 250 nm and 365 nm; HMW, MMW, LMW—Respective areas of the high, medium and low molecular weight fractions in the chromatograms; M_w/M_n—Ratio of weight-average molecular weight and number-average molecular weight; PO—Persulphate oxidation method; Abs1—Absorption spectroscopy of pigment extracts; Abs2—Absorption spectroscopy of pore water; HPLC—High-performance liquid chromatography; HPSEC—High-performance size exclusion chromatography. For details see Materials and methods

somewhat since the 1940s and the proportion of low molecular weight substances has increased (Fig. 1p, Table 2).

Discussion

Onset and course of eutrophication

Qualitative phytoplankton proxies—the concentrations of Chl *a*, Chl c_2, echinenone and zeaxanthin—indicate that a shift of the lake ecosystem towards eutrophy started in the 1970s. From that time onwards the concentrations of the pigments are higher than in earlier years, or are gradually increasing. Statistical analysis reveals that changes in the concentration of Chl *a* correlate well with those of echinenone and Chl c_2 (Table 2), suggesting that the main contributors to the phytoplankton biomass in the lake have been cyanobacteria and diatoms. However, it is difficult to estimate which of these has made the larger contribution. On the one hand, chlorophylls are more photosensitive than carotenoids. On the other, diatom cells sink more rapidly than cyanobacteria. Moreover, many cyanobacteria species form surface blooms and even after the bloom collapse, their colonies continue to float on the surface, prolonging their exposure to sunlight. Different sinking rates of cyanobacteria, and hence different times of exposure to sunlight, could also explain why the concentration of zeaxanthin was higher than that of echinenone in the sediments of Lake Peipsi, whereas according to the monitoring data, the biomass of filamentous cyanobacteria in the lake has been higher than that of unicellular *Microcystis* species (Nõges et al., 1996). The findings of Bianchi et al. (2002) suggest that unicellular cyanobacteria (including picocyanobacteria) may have higher sedimentation rates than the N_2-fixing filamentous cyanobacteria.

Canthaxanthin is the only pigment whose concentration shows no change during the 1970s. As mentioned previously, canthaxanthin has been widely detected also in herbivores. Moreover, when Quiblier-Llobéras et al. (1996) observed the summer planktonic community in an oligo-mesotrophic lake, canthaxanthin was found only in zooplankton, but it was totally absent from phytoplankton. In the case of Lake Peipsi, further investigations should be conducted to determine whether canthaxantin is a marker pigment of specific cyanobacteria taxa or whether it is confined to herbivores.

The changes in the pigment ratios in the sediment record imply that the ecosystem changed in the 1960s (Fig. 1h–j). The sediment composition data suggest that the sediment deposited since the 1960s clearly differs from the underlying layers: it is richer in OM and less compacted (Fig. 1q, r). The preservation index of Chl *a* declines steadily, simultaneously with changes in the pigment ratios, and this also implies that conditions in the water column or/and sediments have changed since the 1960s (Fig. 1b).

On the basis of long-term investigations of Lake Peipsi, it is generally considered that the lake trophy started to increase in the 1960s. In the 1970s, the process accelerated and a high level was reached in the 1980s. From the early 1990s until 1996 an improvement in the lake water quality was observed. However, since 1997 there has been a continuous and even accelerating deterioration of the lake status (Nõges et al., 1996; Nõges & Nõges, 2006). Changes in fossil pigments accord well with the monitored course of eutrophication, except for the transient recovery of the lake in the 1990s, which is not clear in the fossil pigment stratigraphy. However, limnological data of phytoplankton biomass from that period suggest that despite a lowered nutrient level in 1988–1994 (Loigu & Leisk, 1996), the average phytoplankton biomass remained high (Laugaste et al., 1996).

As shown by the *p*DOM data, changes in the lake OM occurred even before the 1960s. The distributions of molecular weight fractions of *p*DOM indicate a change in the late 1930s to early 1940s (Fig. 1p), suggesting a rise in the proportion of autochthonous matter in the OM pool. The increase in the autochthonous contribution to the bulk of OM becomes more and more evident over the following years. According to the *p*DOM characteristics, the autochthonous component in the sediments was highest during the 1970s and 1980s (Fig. 1o, p). A subsequent reduction in the proportion of the LMW fraction and an increase in the proportion of OM with higher aromaticity in the *p*DOM implies a somewhat decreased contribution of autochthonous matter in the bulk of OM during the 1990s, thus indicating a slight deceleration of eutrophication. The stratigraphic changes in the molecular size structure of *p*DOM (Fig. 1o, p) coincide well with the zonal changes in the sediment lithology (Fig. 1s), demonstrating that the dissolved organic fraction in the sedimentary OM has changed in concert with changes in the particulate fraction.

There is no clear explanation for the autochthonous shift in the character of *p*DOM in the late 1930s to early 1940s. A similar qualitative shift in *p*DOM was observed in the measurements of the fluorescence of *p*DOM from the same core in an earlier study (Heinsalu et al., 2007: Figs. 5, 6c, d). We suppose that the cumulative effects of several anthropogenic and natural factors (pre-war increases in agriculture and urban sewage discharges, intensive fishing, establishment of the Zebra mussel *Dreissena polymorpha* Pallas in the lake, numerous extensive forest fires and low water levels) may have triggered these changes in OM.

According to monitoring observations, very large fluctuations in the summer phytoplankton biomass are characteristic of the Peipsi ecosystem: the inter-annual differences can be up to 10-fold (Laugaste et al., 1996). Somewhat unexpectedly, we could detect no prominent changes in the productivity proxies in the sediments. However, the two abrupt peaks of *p*DOM molecular weights (Fig. 1l, m) appear to coincide temporally with the two densest phytoplankton bloom events registered by monitoring observations in Lake Pihkva, in 1972 and 1991 (Laugaste et al., 1996: Fig. 9b), when the biomass of cyanobacteria exceeded the mean annual biomass level by factors of 10 and 22, respectively. However, these bloom events are not detectable in other constituents of the sediments. Moreover, we are unable to surmise whether (1) the blooms extended to Lake Peipsi proper and/or (2) the OM produced in Lake Pihkva could be carried to and settle in Lake Peipsi proper. Therefore, the coincidence may be just accidental.

Conditions prior to the 1930s

The paleolimnological inferences about the nutrient loading response in Peipsi over the past 40 years, based on the stratigraphic changes in fossil pigments and molecular structure of *p*DOM, are consistent with

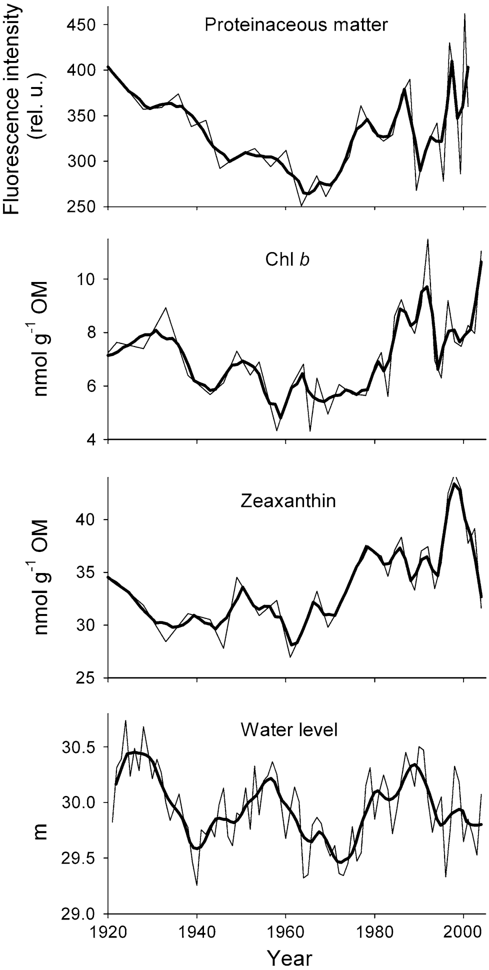

Fig. 2 Comparative curves (fine line—raw data, solid line—smoothed data, Loess curve fitting) of the annual water-level dynamics (elevation, m above sea level) and temporal distribution of proteinaceous matter, zeaxanthin and chlorophyll *b* (Chl *b*) in the settled material of Lake Peipsi. The initial unsmoothed curve of the proteinaceous matter is redrawn with permission from Heinsalu et al. (2007). OM—Organic matter

the monitoring results for the lake. Thus, we conclude that even the pre-eutrophication conditions in Peipsi can be assessed by the sedimentary organics.

Comparison of the fossil pigment chromatograms suggests that the pigment composition of phytoplankton has remained relatively unchanged over the entire time span investigated. This finding coincides well with the results of Laugaste et al. (1996). A rather stable, mesotrophic species composition characterizes the sedimentary diatom assemblage for the period 1880–1950 (Heinsalu et al., 2007). The sedimentary profile and preservation index of Chl *a* obtained in the present study indicate a low algal standing crop and high stability of the lake conditions at that time (Fig. 1a, b).

Besides the low Chl *a* concentrations, the high levels of echinenone, zeaxanthin and Chl *b* in the sediments dated to the 1900–1920s are noteworthy (Fig. 1d, e, g). A slight concurrent increase in the *p*DOC concentration is also discernible (Fig. 1k). Constituents of fluorescent *p*DOM were also present in greater quantities in the lower part of the sediment record than in the sediment layers dated to the mid-20th century (Heinsalu et al., 2007: Fig. 6a, b). This may be explained by the fact that the environmental conditions in the lake during that period were favourable for the preservation of OM and also other pigments, e.g. canthaxanthin (Fig. 1f), diatoxanthin and β-carotene (the last two are not shown). Another possible reason is revealed by comparing the temporal changes of these OM constituents with the changes of water level (Fig. 2). Keeping in mind that the dating of the lower part of the core involves a greater error, one could speculate that the runoff of OM from the lake catchment was higher in that period than in previous and subsequent decades. The presence of a greater proportion of large aromatic organic substances supports the hypothesis of intensive terrestrial transport of OM, as they indicate that allochthonous OM predominated in the lake ecosystem before the 1930s. Unfortunately, the water level observation series is too short to detect any further regularities, especially since the influence of water-level fluctuations is severely masked in the upper part of the sediment record by progressive eutrophication of the water body. Therefore, the extent of external loading of OM to the Lake Peipsi ecosystem at the beginning of the 20th century still remains ambiguous.

Conclusions

The first symptoms of environmental disturbance of Lake Peipsi appeared in the settled matter dated to the late 1930s, where changes in the molecular size composition of *p*DOM indicate an incipient relative increase of autochthonous matter and a corresponding reduction of allochthonous OM. In subsequent decades, this trend becomes more and more obvious. In the fossil pigments, an increase in water column production appears in the 1960s and since then the OM constituents indicate a gradual trend towards eutrophy. The stratigraphic changes in *p*DOM size fractions imply that the nutrient availability for phytoplankton might have declined somewhat in the 1990s. However, these changes are minor and subsequent persistently high concentrations of fossil pigments in the early 2000s do not suggest that the eutrophication level of the lake had been reduced.

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IV

Leeben, A., Freiberg, R., Tõnno, I., Kõiv, T.,
Alliksaar, T. & Heinsalu, A. (2012)

A COMPARISON OF THE PALAEOLIMNOLOGY OF PEIPSI AND VÕRTSJÄRV: CONNECTED SHALLOW LAKES IN NORTH-EASTERN EUROPE FOR THE TWENTIETH CENTURY, ESPECIALLY IN RELATION TO EUTROPHICATION PROGRESSION AND WATER-LEVEL FLUCTUATIONS.

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SHALLOW LAKE ECOSYSTEMS

A comparison of the palaeolimnology of Peipsi and Võrtsjärv: connected shallow lakes in north-eastern Europe for the twentieth century, especially in relation to eutrophication progression and water-level fluctuations

Aina Leeben · Rene Freiberg · Ilmar Tõnno · Toomas Kõiv · Tiiu Alliksaar · Atko Heinsalu

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Abstract We applied a multi-proxy palaeolimnological approach to provide insights into the natural variability and human-mediated trends of two interconnected temperate large shallow lakes, Peipsi and Võrtsjärv, during the twentieth century. The history of the lakes was assessed on the basis of age-related changes in the sediment main constituents (water, organic matter and carbonate), sub-fossil pigments, diatom assemblages and organic matter dissolved in pore water. The temporal changes in the palaeodata indicate an increase of the in-lake biological production in both lakes from about the 1960s, suggesting enhanced nutrient inputs. In subsequent decades, the gradual increase of autochthonous organic matter becomes more obvious, indicating progressive eutrophication of the lakes. Palaeolimnological indicators from the sediment record of Lake Peipsi indicate a slight recession of the lake's eutrophication in the 1990s but not for Lake Võrtsjärv. The results of the study also suggest that after the lakes became eutrophied, the climatically induced water-level fluctuations ceased to be the main driver determining the abundance of phytoplankton. Responses of the lakes to human-induced impacts are better recorded in the sediments of Lake Peipsi than in those of Lake Võrtsjärv, which is shallower of the two and where the wave-induced resuspension of deposits markedly smooths or erases the signals of environmental changes. The results of the investigation expand the knowledge on how large shallow lakes respond to human-mediated and natural perturbations, including those in the lake catchment areas and the capability of the lakes to store the chronology and sequence of these changes.

Keywords Eutrophication · Lake sediments · Multi-proxy study · Water-level changes · Lake Peipsi · Lake Võrtsjärv

Introduction

Many aquatic systems have experienced significant changes in their ecological status over the last 100 years due to increased industrialisation, urbanisation and agriculture. Shallow lakes are especially susceptible to various human impacts such as their

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Guest editors: Zhengwen Liu, Bo-Ping Han & Ramesh D. Gulati / Conservation, management and restoration of shallow lake ecosystems facing multiple stressors

A. Leeben (✉) · R. Freiberg · I. Tõnno · T. Kõiv
Center for Limnology, Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, 61117 Rannu, Tartumaa, Estonia
e-mail: aina.leeben@emu.ee

T. Alliksaar · A. Heinsalu
Institute of Geology, Tallinn University of Technology, Ehitajate tee 5, 19086 Tallinn, Estonia

enrichment with artificial and biogenic substances, drastic changes in their hydrological regime, overfishing and introduction of alien species. However, the information on pristine conditions, initial changes and the subsequent evolution of a lake ecosystem under stressors is often sparse or obtained by research methods that are not comparable. In addition, such information is commonly available only since the second half of the twentieth century, when the first symptoms of perturbation of aquatic environments by agricultural and industrial pollution and exploitation emerged. An important tool that enables to trace the past natural and human-induced developments of water bodies is their palaeolimnological investigation by analysing physical, chemical and biological information preserved in sedimentary profiles (Charles et al., 1994; Smol, 1995; Eilers et al., 2006; Engstrom et al., 2006).

In this article, we present and compare palaeolimnological history, reconstructed for the twentieth century period of two large shallow lakes—Peipsi and Võrtsjärv—in north-eastern Europe. The lakes are interconnected and their hydrological regimes are very similar (Fig. 1). Up to 43% of the catchment area of Lake Võrtsjärv and 40% of the surroundings of Lake Peipsi were used for agricultural purposes during the second half of the twentieth century. Regular monitoring and limnological investigations of the lakes were started in the 1960s. These studies show that from the 1970s to 1990 there was an increased nutrient concentration in the lakes' catchment area from intensive use of fertilisers in the agriculture, and in the case of Lake Peipsi also untreated sewage was discharged directly into the lake. In this period, the load of total nitrogen (TN) and total phosphorus (TP) into Lake Võrtsjärv reached up to 15.7 g TN and 0.7 g TP m^{-2} $year^{-1}$. Lake Peipsi received up to 8.4 g of TN and 0.3 g of TP m^{-2} $year^{-1}$ (Nõges et al., 2010b: fig. 4).

To achieve an integrated insight into common and divergent patterns of the functioning and shifts of the two lakes' ecosystems over the study period, we collated and statistically analysed a number of traditional and novel sediment indicators of palaeolimnological processes and environmental conditions. We used sub-fossil pigments (Leeben et al., 2008) and diatoms (Heinsalu et al., 2007, 2008) to infer changes in the composition and abundance of different taxonomic groups of phytoplankton and to identify the availability of nutrients. Alterations in the intensity of primary production were also assessed from the lithology of the sediments and on the basis of changes in the abundance of basic components of the settled material (Heinsalu et al., 2007, 2008). Molecular size and weight characteristics of the dissolved organic matter (DOM) fraction in pore water were examined to discern allochthonous versus autochthonous matter in sedimentary OM (Leeben et al., 2008; Lepane et al., 2010).

Description of the lakes

Lake Peipsi (surface area 3,555 km^2, mean depth 7.1 m) is the fourth largest lake in Europe (Table 1). Lake Võrtsjärv is considerably smaller (surface area 280 km^2) and shallower (mean depth 2.8 m) than Lake Peipsi and its catchment (area 3,104 km^2) is a

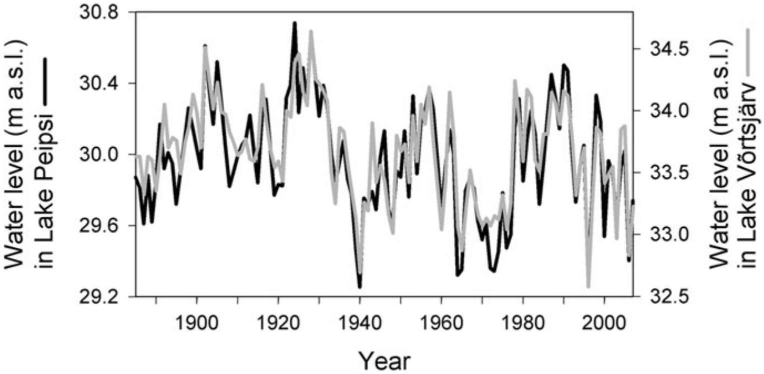


Fig. 1 Dynamics in the water level of lakes Peipsi and Võrtsjärv. The data were provided by the Estonian Meteorological and Hydrological Institute. Missing water levels of some years were reconstructed by Jaani (1990, 2001)

part of the Lake Peipsi catchment (area 47,800 km^2, Fig. 2). The two lakes are connected by the Emajõgi River (length 100 km), which drains Lake Võrtsjärv water to Lake Peipsi. The average annual outflow from Lake Võrtsjärv between 1961 and 2000 was ca. 0.74 km^3 (Järvet, 2004). The water level of the lakes fluctuates seasonally: mean annual range is 1.4 m in Lake Võrtsjärv and 1.2 m in Lake Peipsi.

Both the lakes being quite shallow they do not thermally stratify and are relatively rich in oxygen (10–11 mg l^{-1}, Table 1), although in wintertime and on hot and calm summer days, when the mixing of the lakes' water columns is limited, anoxic conditions may occur near bottom layers.

The lakes are eutrophic and highly productive, with diatoms and cyanobacteria prevailing in phytoplankton biomass (Table 1). Lake Võrtsjärv is about twice richer in phosphorus (mean concentration 52 μg l^{-1}) and nitrogen (mean concentration 1,540 μg l^{-1}) compared with Lake Peipsi. Also, in Lake Võrtsjärv phytoplankton biomass (25.6 g m^{-3}) and abundance of bacteria (6.6×10^6 cells ml^{-1}) are about twice as large as in Lake Peipsi (10.0 g m^{-3} and 3×10^6 cells ml^{-1}, respectively). However, the biomasses of zooplankton and zoobenthos in Lake Peipsi many times exceed those of Lake Võrtsjärv. The more turbid water and unconsolidated sediments in Lake Võrtsjärv (transparency by Secchi disc 1.07 m) seem to adversely affect both planktonic filtrators and bottom inhabitants.

Materials and methods

Sampling and chronology of sediments

Coring was performed on Lake Võrtsjärv in 2002 and on Lake Peipsi in 2002 and 2006. A freeze corer (Wright, 1980) was used to obtain sediment samples, which were sectioned into continuous 1-cm thick slices. The cores were dated using ^{210}Pb activity measurements. The sediment age was calculated using the model of constant rate of supply (CRS; Appleby & Oldfield, 1978). The ^{210}Pb-based age-scale of the cores was corrected using reference dates of the artificial radionuclides ^{137}Cs and ^{241}Am and the distribution of spheroidal fly ash particles (Rose, 1990). Sediment chronology data were published by Heinsalu et al. (2007, 2008).

Laboratory analyses

DOM in sediment pore water

The pore water was separated from the solid fraction by centrifugation at 2,328*g* for 30 min and clarified by filtration. Two methods were used to track quantitative and qualitative changes in DOM in pore water. First, the whole pore-water samples were analysed spectrophotometrically. Absorption spectra from the Lake Peipsi samples were collected over the range from 800 to 200 nm using a Cadas 100 spectrophotometer (Dr. Lange Ltd); the Lake Võrtsjärv samples were scanned from 500 to 200 nm using a V-530 UV–VIS Spectrophotometer (Jasco). Light with a wavelength ranging from 240 to 370 nm is mainly absorbed by humic substances (e.g. Peuravuori & Pihlaja, 1997), which make up 60–90% of the total sedimentary OM (Meyers & Ishiwatari, 1993). Therefore, the absorbance at 254 nm (A_{254}) was used to quantify humic substances. The origin of sedimentary OM was revealed from the ratio of the absorbances at 250 and 360 nm (A_{250}/A_{360}) of pore-water DOM, which according to De Haan & De Boer (1987) and Peuravuori & Pihlaja (1997) is inversely correlated with molecular size and aromaticity. A higher aromaticity is typical of allochthonous OM, while compounds with aliphatic structure dominate in autochthonous OM. Thereafter, the pore-water DOM samples were analysed for molecular size characteristics using a high-performance size-exclusion chromatography (HPSEC) method. Full details of this method and apparatus were described by Leeben et al. (2008) and Lepane et al. (2010). Briefly, the separation of pore-water DOM was achieved on a BIOSEP-SEC-S2000 column (7.5×300 mm with a 7.5×75 mm precolumn, Phenomenex) using phosphate buffer (pH 6.8) as eluent. The eluted solute was detected at 254 nm. The column system was calibrated by recording retention volumes of 4–5 protein standards with different molecular weights. We chose weight-average molecular weight (MW$_w$; Determan, 1968) to characterise the molecular weight of pore-water DOM. Percentage of a certain molecular size fraction was calculated as the area of the respective fraction divided by total peak area.

Sub-fossil pigments

Sedimentary pigments from freeze-dried samples were extracted with an acetone–methanol mixture

Table 1 Main characteristics of the studied lakes (Haberman et al., 2004, 2008)

Characteristic	Lake Peipsi	Lake Võrtsjärv
Sampling site location	58°47′14″N 27°19′20″E	58°07′42″N 26°04′10″E
Surface area (km^2)	3,555	280
Mean depth (m)	7.1	2.8
Average total volume (km^3)	25	0.8
Catchment area (km^2)	47,800	3,104
Water residence time (y)	2	1
Average ice duration (d)	114	130
pH	8.18	8.11
TP (μg l^{-1})	39	52
TN (μg l^{-1})	745	1,540
O_2 (mg l^{-1})	10.30	10.98
Transparency by Secchi disc (m)	1.60	1.07
Abundance of bacteria (cells ml^{-1})	3×10^6	6.6×10^6
Biomass of phytoplankton (g m^{-3})[a]	10.0	25.6
Dominating species		
Cyanobacteria	*Gloeotrichia echinulata* (Smith) P.G. Richt., *Aphanizomenon flos-aquae* Ralfs ex Bornet & Flahault, *Microcystis* spp., *Anabaena* spp.	*Limnothrix planktonica* (Wołosz.) Meffert, *L. redekei* (van Goor) Meffert, *Planktolyngbya limnetica* (Lemm.) Kom.-Legn. & Cronb., *Aphanizomenon skujae* Kom.-Legn. & Cronb.
Diatoms	*Aulacoseira islandica* (O. Müll.) Simonsen, *A. granulata* (Ehrenb.) Simonsen, *A. ambigua* (Grun.) Simonsen, *Stephanodiscus binderanus* (Kütz.) Krieger, *S. neoastraea* Håk. & Hickel	*Aulacoseira* spp. *Cyclotella* spp. *Synedra* spp.
Chlorophyll *a* (mg m^{-3})	18[b]	33
Phytoplankton primary productivity (gC m^2 y^{-1})	200	200
Biomass of zooplankton (g m^{-3})[a]	1.500	0.874
Biomass of zoobenthos (g m^{-2})[a]	12.3	6.6
+*Dreissena polymorpha* (Pallas) (g m^{-2})[a]	235.0	1.8[c]

Coordinates of the sampling sites are also given

Physical, chemical and biological values represent the average over the years of limnological monitoring. Water samples were collected in the surface layer of the lake's pelagic zone during the ice-free period

[a] Wet weight

[b] Nõges et al. (1996)

[c] Timm et al. (1996)

(80:20) at −20°C for 24 h under N_2 atmosphere. Debris from the extracts was removed by membrane filtration (0.45 μm). Pigments were separated using a high-performance liquid chromatography system (model 1100, Cecil Instruments Ltd) fitted with a Spherisorb ODS2 column (4.6 × 150 mm, particle size 3 μm; Waters) and a Rheodyne injection valve (model 7125). Pigments were eluted with two solutions consisting of (1) 80% methanol + 20% 1 M ammonium acetate and (2) 80% methanol + 20% acetone delivered in a nonlinear gradient operational mode at constant flow rate of 1.5 ml min^{-1}. Pigments were detected spectrophotometrically (model CE1200, Cecil Instruments Ltd). The procedures for pigment

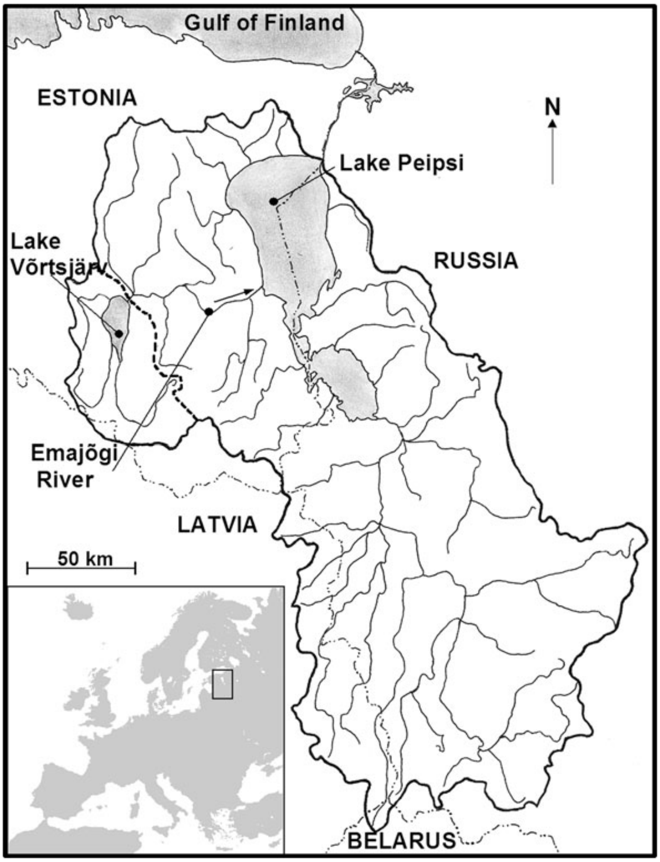


Fig. 2 Location of lakes Võrtsjärv and Peipsi. The *solid line* marks the border of the catchment of the lakes. The *dashed solid line* indicates the border of the catchment of Lake Võrtsjärv

extraction and separation instrumentation were the same as used by Leeben et al. (2008).

Total algal abundance was inferred from the sum of the contents of α-carotene and β-carotene (CAR), which are more stable with respect to photodegradation and chemical alteration than chlorophyll *a*. In the Lake Võrtsjärv sediment samples, zeaxanthin (Z) and lutein (L) could not be separated and therefore for Lake Peipsi the two were also pooled (Z + L). Zeaxanthin is considered as a specific pigment for *Microcystis* species (Bianchi et al., 2002), and lutein has been used to assess palaeochanges in chlorophytes (Leawitt & Hodgson, 2001). In the sediment samples from Lake Peipsi the main marker pigment for diatoms—fucoxanthin—could not be resolved properly by the HPLC used. Instead, we give the quantitative changes in diatom biomass in the lakes as a sum of diatoxanthin and diadinoxanthin (DD). Echinenone (ECHI) was chosen to track changes in filamentous cyanobacteria (Leawitt & Hodgson, 2001).

Sub-fossil diatoms

Pre-weighed sediment samples were treated with 30% H_2O_2 to remove organic debris (Battarbee et al., 2001). For preparing slides, Naphrax® was used as mounting medium. Diatom valves were identified and enumerated under an Axiolab (Zeiss) microscope. For more information on the diatom analysis of sediment samples from the lakes see Heinsalu et al. (2007, 2008).

Sediment constituents

For the sediment samples, the water content (%wet weight) was calculated from weight loss after drying the sediment at 105°C. The contents of OM and carbonate

(%dry weight) were measured as loss on ignition at 550 and 950°C, respectively (Heiri et al., 2001).

Statistical analyses

For detecting patterns and trends in the palaeodata of the lakes, centred principal component analysis (PCA) was carried out using Multivariate Statistical Package (MVSP), release 3.12 (Kovach, 1999). Kaiser's rule, which states that components with eigenvalues under unity should be discarded, was applied to determine the number of components to be retained in the PCA (Kaiser, 1960; Jolliffe, 1986).

To reveal the strength of the relationships between the changes in proxies of algal assemblages and fluctuations in water level Spearman rank correlation coefficients were calculated. Spearman correlation analysis instead of Pearson correlation analysis was chosen because often biological processes are not linearly related to environmental factors (e.g. Kudela et al., 1997; Breton et al., 2006). To eliminate long-term trends, the palaeodata were detrended using a quintic polynomial. Thereafter, the data set was smoothed using the 3 years running average repeated twice—from the beginning and the end of the data.

Results

Lithostratigraphy and sediment main constituents

The sediments from Lake Võrtsjärv were greenish brown gyttja, with dark layers, probably containing aggregates of FeS around the 1950s, 1970s and 1990s (Fig. 3a). The lower part of the core from Lake Peipsi was homogeneous brownish green gyttja, comprising the sediment layers accumulated until the 1930s. Subsequently, black FeS grains appeared and the sediment colour became gradually darker. The Lake Peipsi sediments settled between the mid-1960s and mid-1980s were the darkest.

The OM content in the lower part of the records (up to the mid-twentieth century) of both lakes ranged between 24 and 26% (Fig. 3a). Starting from the sediments accumulated around the 1960s its curves for the lakes diverge: while in Lake Peipsi the OM content increased, in Lake Võrtsjärv it decreased. The OM reduction in the latter lake was due to a gradual increase (from 7–8% prior the 1960s to 22% in the 1990s) in carbonate. In the Lake Peipsi sediments the carbonate content was only 1–3% and it remained stable through the whole record.

Fig. 3 Age-resolved changes in **a** the sediment composition, **b** sub-fossil pigments, **c** sub-fossil diatom assemblages and **d** sediment pore-water dissolved organic matter (DOM) in the sediment records from lakes Peipsi (*closed dots*) and Võrtsjärv (*open dots*). On the upper horizontal axis, the proxy information is given. Complete sub-fossil diatom data over the studied 100 years for Lake Peipsi are presented in Heinsalu et al. (2008), for Lake Võrtsjärv in Heinsalu et al. (2007). *OM* organic matter, *LMW* low molecular weight, *MMW* medium molecular weight, *HMW* high molecular weight. In pore-water DOM samples from Lake Peipsi, HMW = 100–200, MMW = 0.2–100 and LMW = 0.1–0.2 kDa; from Lake Võrtsjärv HMW = 530–1200, MMW = 1.2–2.3 and LMW = <0.15 kDa. Note that part of the horizontal axis in the graph of the molecular weight fractions of Lake Peipsi is omitted ▶

Sediments of both lakes had a large content of water. In Lake Võrtsjärv, the water content during the twentieth century up to the 1990s was around 87% ($\sigma = 1.3$). In the uppermost part of the core (dated at 1992–2002) the water content increased up to 96%. In Lake Peipsi, changes in sediment compaction occurred in the middle of the study period, when the water content, which was 91% until the 1950s, increased gradually to 98% in the 1990s.

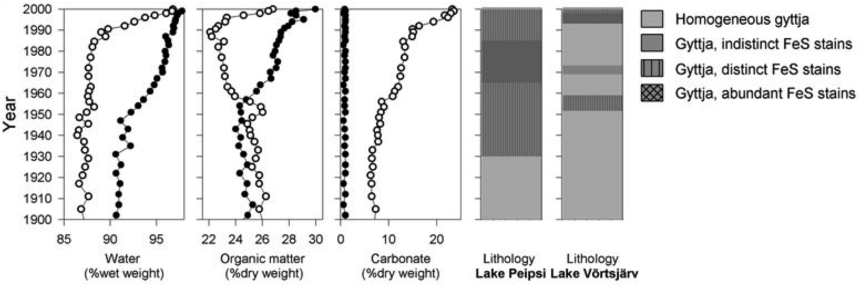
Algal assemblages

Records of sub-fossil pigments

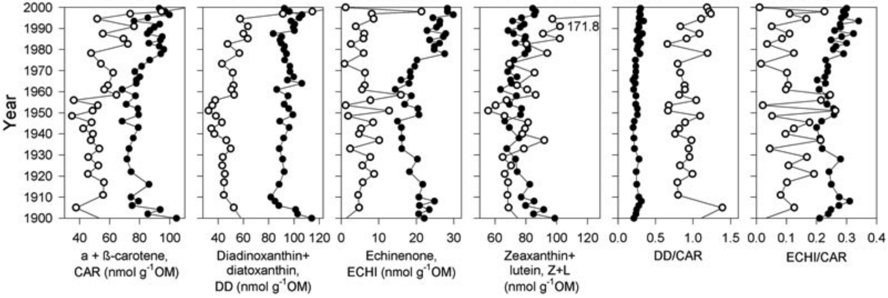
The CAR content in the Lake Võrtsjärv sediments was rather constant around 50 nmol g^{-1} OM up to the mid-twentieth century (Fig. 3b); thereafter it became very variable (40 and 70 nmol g^{-1} OM). The highest CAR content, 95 nmol g^{-1} OM, was found in sediments dated at 1998. In the Lake Peipsi record, the highest CAR content, of around 90 nmol g^{-1} OM, was observed in the sediments dated to 1900–1905, the mid-1970s–late 1980s and the late 1990s; in the rest of the record the CAR content varied between 71 and 77 nmol g^{-1} OM.

The DD content in the Lake Peipsi sediments was quite stable, being between 80 and 100 nmol g^{-1} OM. In the Lake Võrtsjärv sediments, the DD content was ca. half as much—between 40 and 60 nmol g^{-1} OM, which dropped to 32 nmol g^{-1} OM in the mid-twentieth century.

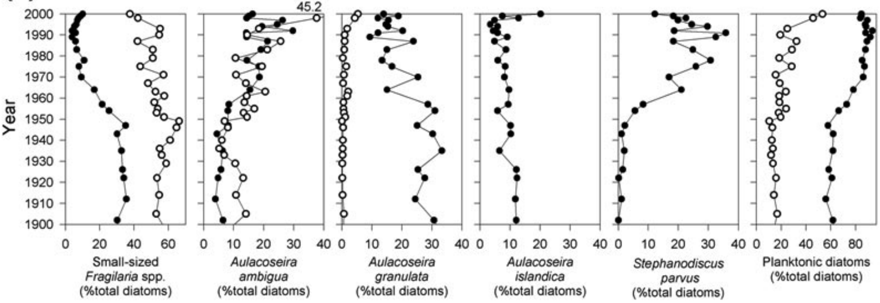
The average contents of Z + L in the sediment records of the two lakes were almost equal—about



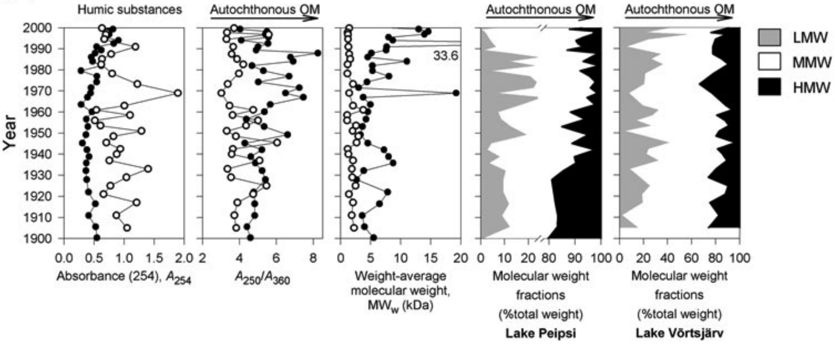
(a) Sediment main constituents and lithology



(b) Sub-fossil pigments



(c) Sub-fossil diatoms



(d) Pore-water DOM

70–80 nmol g^{-1} OM. In Lake Peipsi, a little higher content of Z + L, of around 90 nmol g^{-1} OM, was detected only in the oldest sediment layers. In Lake Võrtsjärv, these pigments started to increase in the sediments from the mid-1940s. After a short-term drop around the 1950s from 80 to 60 nmol g^{-1} OM the increase continued, reaching 100 nmol g^{-1} OM in the 1980s and over 150 nmol g^{-1} OM in the 1990s.

The ECHI content in the lakes' sediment records was markedly lower than the contents of other sub-fossil pigments. In Lake Võrtsjärv it fluctuated mainly between 1 and 10 nmol g^{-1} OM. In Lake Peipsi, the ECHI content varied from 15 to 30 nmol g^{-1} OM, the highest values were from the early 1980s to the late 1990s.

The DD/CAR ratio in the Lake Peipsi record was constant around 0.025, and the ratio in Lake Võrtsjärv was three times higher, with wider variations. However, in both lakes, clear trends or shifts in its distribution were missing. In contrast, the ECHI/CAR ratio exhibited higher values, between 0.2 and 0.3, in the Lake Peipsi sediments and lower values of 0.1 in Lake Võrtsjärv. Like in the case of the profiles of sub-fossil pigments, this ratio varied more in the Lake Võrtsjärv sediments than in Lake Peipsi.

Sub-fossil diatom records

In the diatom assemblage of Lake Võrtsjärv, small-sized *Fragilaria* species (*Fragilaria brevistriata* Grun., *F. construens* (Ehrenb.) Grun. and *F. pinnata* Ehrenb.) dominated (Fig. 3c). Their proportion in the total abundance of diatoms gradually diminished starting from the 1950s when the relative abundance of *Aulacoseira ambigua*, a eutrophic water diatom, progressively increased, reaching 45% in the uppermost layer.

In Lake Peipsi, small-sized *Fragilaria* species (*F. brevistriata* and *F. heidenii* Østr.) were only half as abundant as in Lake Võrtsjärv. Their relative abundance in the diatom assemblage started to fall in the 1950s like in Lake Võrtsjärv, but the decline in Lake Peipsi was more pronounced. In Lake Peipsi typical large-lake species *Aulacoseira islandica*, *A. granulata* and *A. ambigua* predominated in the diatom assemblage until the 1950s. A decade later the diatom *Stephanodiscus parvus* Stoermer & Håk., characteristic of eutrophic waters, started to increase in Lake Peipsi, making up nearly 30% in the 1990s. Thereafter its importance diminished and *A. islandica* became slightly more abundant. In contrast with Lake Peipsi where in the diatom assemblage planktonic species prevailed, in Lake Võrtsjärv planktonic diatoms contributed only one-third. In both lakes, the proportion of planktonic species started to increase in the 1950s.

DOM in pore water

In the Lake Võrtsjärv sediments, the average A_{254} values were about two-fold higher than in Lake Peipsi (Fig. 3d). In the Lake Peipsi record the A_{254} variations with the depth were minimal: somewhat elevated values occurred only in the 1900s and 1990s. In the Lake Võrtsjärv sediments, where fluctuations in the A_{254} were very large, the highest values were for the early 1970s.

The A_{250}/A_{360} ratio was around 4 in the lower parts of the cores in both lakes. In the Lake Peipsi sediments, this ratio started to vary more widely in the 1950s and attained values as high as 7–8 in the 1980s; however, the ratio fell to the initial level of about 4 in the 1990s.

Weight-average molecular weights (MW_w) of pore-water DOM in Lake Võrtsjärv were ca. one-half of those in Lake Peipsi (Fig. 3d). In the Lake Peipsi pore-water samples MW_w of DOM exhibited enormous instability, especially in the sediment accumulated since the mid-1960s. Compounds with medium molecular weights (MMWs) dominated in the pore-water DOM of the sediments in both lakes (Fig. 3d). In the lower part of the Lake Võrtsjärv record the relative abundance of MMW in the total pore-water DOM varied between 55 and 80%, but after the mid-1970s their per cent share decreased in some years to 30%. In Lake Peipsi the proportion of the MMW fraction in the older sediment layers contributed from 70 to 80% to the total pore-water DOM, from the mid-1930s its level slightly increased, reaching values over 95% in some periods.

In the sediment layers of the lakes accumulated in the early twentieth century, compounds with high molecular weight (HMW) comprised about 20% of pore-water DOM. Only in the Lake Peipsi sediments from the late-1930s, did these compounds show a drop in their contribution by 10–15%. At the same time, the proportion of compounds with low molecular weight (LMW), which until the 1940s accounted for 10% in pore-water DOM increased up to 25%. In the 1990s, the proportion of LMW in the Lake Peipsi sediments again fell. In the Lake Võrtsjärv pore-water DOM their proportion varied between 3 and 35%, reaching 56% in the 1990s.

PCA analysis of palaeodata

The PCA analysis of palaeodata (Fig. 4) applying Kaiser's rule revealed five components (plausible factors) for both lakes with eigenvalues ≥ unity. These five factors accounted for 86.7% of the total variance in the Lake Peipsi and 91% in the Lake Võrtsjärv data. The first principal component (PC1) explained 48.3 and 40.7%, in the two lakes, respectively, and the second 14.8 and 18.2%, respectively, of the total variance in the palaeodata. As the PC1 described almost half of the total variance in the data set and its scores plot over the period from the 1970s to the end of the twentieth century contained features attributable to nutrient availability and changes in in-lake production it was assumed that this component could detect the shift in the lakes' trophy.

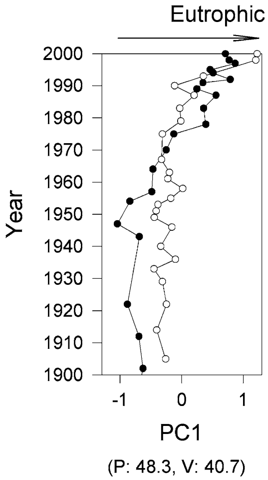


Fig. 4 Stratigraphic plots of principal component 1 (PC1) extracted from the palaeoindicators of lakes Peipsi (*closed dots*) and Võrtsjärv (*open dots*) by principal component analysis and showing changes in the lakes' trophy over the twentieth century. The percentage of eigenvalues is given in parentheses. *P* Lake Peipsi, *V* Lake Võrtsjärv

Correlations of the proxies of phytoplankton with water-level changes

In both lakes, the relationships between changes in the sub-fossil pigments content and in water level over the 100-year period (1900–2000) were weak (Table 2). Several proxies indicated that in the mid-1950s, the two lake ecosystems started to alter markedly. Therefore, Spearman rank correlation coefficients between the palaeodata and water-level changes were also calculated separately for 1900–1949 and for 1950–2000 (Table 2). This analysis revealed a strong positive correlation of water level in both lakes with the proportion of planktonic species in the diatom assemblages in the earlier period but not for the period 1950–2000. Before the 1950s the CAR and DD contents showed a strong and the ECHI content a moderate positive correlation with the water-level fluctuation (WLF) for Lake Võrtsjärv but a very weak one for Lake Peipsi. In the second half of the twentieth

Table 2 Spearman rank order correlation between changes in water level and phytoplankton proxies of lakes Peipsi and Võrtsjärv calculated for the period 1900–2000 as well as separately for the pre-eutrophication period (before 1950) and after the enhanced nutrient loading (1950–2000)

Proxy	1900–2000		1900–1949		1950–2000	
	Peipsi	Võrtsjärv	Peipsi	Võrtsjärv	Peipsi	Võrtsjärv
CAR	−0.012	0.043	0.071	**0.724**	−0.002	−0.259
DD	−0.188	0.031	0.129	**0.797**	**−0.401**	−0.276
ECHI	**0.309**	0.204	0.050	0.592	0.381	−0.074
Z + L	−0.012	−0.378	0.171	−0.205	−0.095	−0.444
Planktonic diatoms%	0.208	−0.037	0.750	**0.833**	−0.229	−0.211

The palaeodata were detrended using a quintic polynomial. The whole data set was smoothed using the 3 years running average repeated twice—from the beginning and the end of the data. The underlined correlation coefficients are significant at $P < 0.10$, bold at $P < 0.05$

CAR sum of α- and β-carotenes, *DD* sum of diadinoxanthin and diatoxanthin, *ECHI* echinenone, *Z* + *L* sum of zeaxanthin and lutein century the relationships were switched: becoming weakly or moderately negative between the contents of sub-fossil pigments and water-level changes for both lake sediments, except for ECHI in Lake Peipsi.

Discussion

Comparison of the palaeorecords with monitoring data

According to our results the contents of CAR (total biomass), DD (diatoms) and ECHI (filamentous cyanobacteria) were on average 1.4, 1.6 and 3 times, respectively, *higher* in the Lake Peipsi sediments than in those of Lake Võrtsjärv (Fig. 3b). However, according to monitoring data (1962–2000) the biomass of phytoplankton in Lake Peipsi was 2.5 times and based on chlorophyll *a* concentration, 1.8-fold *lower* than in Lake Võrtsjärv (Nõges et al., 1996; Haberman et al., 2004, 2008).

The discrepancy between the sub-fossil pigments' content in sediments and the phytoplankton biomass in the water column indicates that the degradation of microalgae and their pigments is more important in Lake Võrtsjärv than in Lake Peipsi. The smaller depth of Lake Võrtsjärv makes it more vulnerable to wind-induced disturbance and mixing. This causes frequent stirring of the settled material, bringing it back to the water column. The algae remain longer in the water, and pigments are exposed again to sunlight and oxic conditions and become available for pelagic microbes.

Our research showed that the sediments of both lakes contained markedly larger amounts of Z + L (*Microcystis* spp. + green algae) than ECHI. This also contradicts monitoring data, which demonstrate that in the second half of the twentieth century the biomass of cyanobacteria was formed mainly by filamentous species and the biomass of green algae was very low (Nõges et al., 2003, 2004). The larger proportion of Z + L than ECHI in the lakes' sediment, whereas in the water the situation is reverse, may be due to more extensive degradation of filamentous cyanobacteria during sinking through the water column and in the surface sediment than *Microcystis* spp. (Bianchi et al., 2002).

The high content of sub-fossil pigments in the Lake Peipsi sediments at the beginning of the twentieth century (Fig. 3b) suggests that preservation conditions can alter during various development periods of a single lake. Although according to written historical records intense phytoplankton blooms occurred in Lake Peipsi already at the beginning of the twentieth century, the measured average biomass of predominant phytoplankton groups and the total biomass of phytoplankton were still lower in the first half of the twentieth century than in the 1980s and 1990s (Laugaste et al., 1996).

In addition to more favourable preservation conditions (for example reduced resuspension due to very high water level and absence of a zebra mussel population) compared with other periods, the large content of some pigments in the Lake Peipsi sediments at the beginning of the twentieth century can be also explained by their enhanced allochthonous input originating from macrovegetation growing in the catchment. Carotenes, lutein and zeaxanthin are also synthesised in higher plants. Although it is commonly believed that land-derived pigments do not survive transport to water bodies (Meyers & Ishiwatari, 1993), when incorporated into the matrix of humic substances these land-derived pigments can become extremely resistant to degrading (Cieslewicz & Gonet, 2004). Between 1900 and 1930 the water level in the lakes was on average higher (Fig. 1), suggesting a larger run-off of terrestrial material than in subsequent decades. The lower values of the A_{250}/A_{360} ratio and a larger proportion of substances with HMW (Fig. 3d) prior to the 1940s indicate that the share of allochthonous OM in the bulk of OM in the Lake Peipsi sediment was indeed larger than in the latter years. Therefore, an increased rate of input of these pigments from the lake's catchment in the early 1900s cannot be ruled out.

Signs of eutrophication

Lake Peipsi

The darker colour of sediment layers settled around the 1930s is the first sign of alterations in the lake's ecosystem. We suppose that the change in sediment colour was caused by the occurrence of FeS aggregates. Their appearance in the sediments indicates anoxic decomposition of organic substances. In lake bottom oxygen is rapidly depleted when a large amount of organic material accumulates there and the mixing of the water column is limited.

The sediment main constituents and sub-fossil diatoms do not show any changes in the 1930s. As explained in the previous section under "Discussion", the data of sub-fossil pigments in Lake Peipsi over that period should be interpreted with caution. Nevertheless, the appearance of FeS in sediments dated to the 1930s coincides well with the drop in the relative abundance of organic substances with HMW. The reduction in the percentage of HMW suggests that the proportion of allochthonous OM accumulated in the 1930s and 1940s in the sediments started to decrease gradually.

In the subsequent years, the shift in the origin of OM and its quantity become more obvious. The changes in sediment composition in the late 1950s confirm accelerated increment of OM: the sediment is less compacted and the content of OM is higher than in the previous decades. The increase in the relative abundance of planktonic diatoms in the 1960s indicates incipient proliferation of planktonic algae typical for eutrophied water bodies. The elevated A_{250}/A_{360} ratio confirms that in the material settled around the 1960s autochthonous organic substances became predominant. The appearance of the eutrophic diatom *Stephanodiscus parvus* in these years clearly demonstrates increase in nutrient availability.

In the late 1960s the sediment became darker, indicating further acceleration of eutrophication. From the early 1970s, layers consisting of organic substances with very large weight-average molecular weight (MW_w) occur in the Lake Peipsi record. Earlier, we presumed that such compounds sedimented in the years of massive phytoplankton blooms (Leeben et al., 2008). Also, the CAR and ECHI content started to increase in the 1970s, indicating an escalation of phytoplankton blooms, consisting of filamentous cyanobacteria.

In the sediments settled in the 1990s, some symptoms of the reversal of eutrophication are notable: the sediment contains less FeS aggregates. The relative abundance of planktonic diatoms and *Sthephanodiscus parvus* shows a decrease. Interestingly, also the A_{250}/A_{360} ratio and the proportion of LMW substances show a drop to the pre-eutrophication level. These decreases point towards mesotrophic conditions returning, which can be explained by the reduced load of nutrients. According to Nõges et al. (2007: table 2), in the 1990s the nutrient inputs to Lake Peipsi from the Estonian part of its catchment area dropped: 13% for P and of 40% for N compared with the 1980s. However, the ECHI content in the Lake Peipsi record remains high suggesting no recovery of the lake between 1990 and 2000.

PCA of palaeodata demonstrates no recovery of the lake's ecosystem in the 1990s either (Fig. 4). This implies that most likely eutrophication was continuing at the turn of the century. Moreover, compared with the 1980s the in-lake P concentration increased 17% by the 1990s (Nõges et al., 2007: table 2) and cyanobacterial blooms became more intense (Laugaste et al., 2008). These mass developments of blue-greens in Lake Peipsi during the 1990s were related to the reduction in the TN/TP ratio (Nõges et al., 2008). The cause of the rise of the TP concentration in lake water in spite of the decrease in the external phosphorus load still remains unclear (Nõges et al., 2007).

The results of PCA indicate a short-term recovery of the ecosystem of Lake Peipsi also between the 1940s and 1960s (Fig. 4). This turn towards oligotrophy is not expressed in any individual indicators (Fig. 3). Although statistics and monitoring data about the period are missing, we suppose that this provisional transition of the ecosystem was probably a result of the regression of agriculture and fishing during World War II and the post-war depression.

Lake Võrtsjärv

The first layer with FeS aggregates in the Lake Võrtsjärv sediments appears around the 1960s, when also the carbonate content, total algal biomass and the proportion of planktonic diatoms start increasing (Fig. 3). The source of carbonates in the lake's sediments is not yet determined. One reason of their occurrence can be the proliferation of algae. A massive bloom of microalgae or cyanobacteria leads to a rapid depletion of free CO_2, increasing the pH of lake water and inducing precipitation of carbonates. However, the decrease in the values of the A_{250}/A_{360} ratio and in the content of ECHI does not support this explanation. On the contrary, the decrease in the ratio implies a decrease in the rate of primary production in the lake since the 1960s. Monitoring data of these years demonstrate that in the 1970s the total biomass of phytoplankton as well as the biomass of filamentous cyanobacteria increased compared with the previous decade (Nõges et al., 2003). However, in the 1980s, although the nutrient loading continued to increase, the biomass of phytoplankton and cyanobacteria fell back to the level of the 1960s.

The decrease in the A_{250}/A_{360} ratio since the 1960s may have resulted from the increase in the carbonate content in Lake Võrtsjärv sediments. Laboratory experiments by Thimsen & Keil (1998) demonstrated that mineral particles can remove humic substances from water by adsorbing them onto their surface. Substances of small size and LMW would adhere more easily.

In the 1990s, the nutrient load to Lake Võrtsjärv fell even more than to Lake Peipsi: the input of TP decreased ca. 35% and the TN load >50% (Nõges et al., 2007: table 2) compared with those in the 1980s. However, the palaeolimnological records (Fig. 3) of Lake Võrtsjärv do not show if the lake trophy fell in the 1990s. This corresponds well to limnological monitoring data, which demonstrate that despite the decrease in the external load, the concentration of in-lake P and phytoplankton biomass increased in the 1990s (Nõges et al., 2007: table 2, 2010a). Nõges & Kisand (1999) argue that these high concentrations of P were due to its release from deep anoxic sediment exposed during heavy storms.

PCA analysis shows that in the sediments of Lake Võrtsjärv the shift towards eutrophy is much less pronounced than in Lake Peipsi (Fig. 4). This can be due to frequent resuspension of sediments in Lake Võrtsjärv, which stirs up and relocates accumulated material at the bottom smoothing differences between the deposits settled in different years.

Influence of water-level fluctuations

In lakes Võrtsjärv and Peipsi oscillations of water level have a periodicity of ca. 30 years (Nõges & Nõges, 1998; Jaani, 2001). These fluctuations are related to the variations in North Atlantic Oscillation (NAO), which controls the direction and intensity of westerly winds, and with that the transport of heat and moisture to Europe. Increased westerly winds cause mild winters with high precipitation resulting in the rise of water level. When the western airflow is restricted, winters become colder, while summers that are dry and hot cause reduction in water level.

Changes in water level considerably affect light conditions and nutrient availability in the water column. During a high water level large amounts of solid and dissolved matter, including nutrients, are transported into lakes. The land-derived substances reduce the quantity and quality of light essential for photosynthetic organisms, at the same time the nutrient availability for primary producers increases. During a low water-level period the input of materials from the catchment is low and light conditions improve. However, in very shallow lakes reduced depth may result in substantial resuspension of sediments, stimulating release of nutrients, but also increasing water turbidity.

Spearman rank correlation analysis of the palaeodata and the water-level series revealed that phytoplankton responded differently to the WLFs during the two study periods in the twentieth century as well as in the two lakes. For entire 100-year period, the content of sub-fossil pigments and abundance of planktonic diatoms exhibited a very weak relationship with the WLF (Table 2). When the periods of pre-eutrophication (up to 1950) and intensive nutrient loads (1950–2000) were analysed separately, in both lakes the relative abundance of planktonic diatoms demonstrated a significant strong positive relationship with the WLF prior to the 1950s, but not in 1950–2000 when the lake gradually eutrophied.

Spearman correlation analysis of sub-fossil pigments and water-level data for Lake Võrtsjärv showed a similar pattern: over years when the external nutrient load was low, the rise of the water level involved an increase in the biomass of dominant algal groups, diatoms (DD) and filamentous cyanobacteria (ECHI), as well as the total algal biomass (CAR). The correlation ceased to exist after the 1950s when the lakes became eutrophic. No such period relation was observed in Lake Peipsi except that the content of DD showed a moderate negative correlation with the WLF after 1950.

The shift in the relationship between the phytoplankton biomass and WLF might be due to the increase of the lakes' internal store of nutrients: before the 1950s the necessary nutrients for algae were brought from the catchment during a high water period, after the 1950s, when lakes started to receive extra amounts of P and N, their 'own' reserves increased making the lakes' ecosystems 'independent' from the delivery from the catchment.

Limnological measurements since the 1960s have shown that over periods of a high water level the concentration of phytoplankton in the water column of the lakes is commonly lower than over the periods of a low water level (Nõges & Nõges, 1998; Milius et al., 2005). The disparity between the monitoring data and the data obtained from sediment analysis can be explained by limitations of the monitoring, which is restricted to data collection from often single and randomly chosen sites, discrete depths, as well as that such data approximate the average biomass in the water column at a certain moment of time. In contrast, sediment data represent the OM produced in the whole water column during a longer period.

Conclusions

Although the conditions of sediment deposition and preservation in sediments of lakes Peipsi and Võrtsjärv differ, somewhat complicating comparison of the natural variability and human-induced impacts in these lakes, a transition in the ecological status experienced by the lakes in the 1960s due to increasing nutrient supplies and subsequent progression of eutrophication of their ecosystems is clearly evident in the sediment records of the lakes. Alterations in the lithology and composition of pore-water OM of the Lake Peipsi sediments suggest that the nutrient loading had started increasing already in the 1930s, but there was a short intermission from 1940 to the early 1950s, when both farming and fishing as well as developmental activities were paralysed due to WWII and the post-war turmoil. Changes in the composition of sub-fossil diatom assemblages, pore-water OM and lithology also show a slight recession in the eutrophication of Lake Peipsi in the 1990s, when the use of agricultural fertilisers in the lake's catchment was drastically decreased. Such man-made alterations are not distinguishable in the sediment record of Lake Võrtsjärv, apparently due to a stronger influence of sediment resuspension because of the lake's shallowness. Our study shows that when the lakes became eutrophic, the 'natural' pattern, high level of water–larger phytoplankton biomass, was interrupted. The Lake Võrtsjärv algal records displayed especially well this phenomenon: before the 1950s the content of sub-fossil pigments and the relative abundance of planktonic diatoms had a strongly positive relationship with the WLFs, after the 1950s such correlations vanished.

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CURRICULUM VITAE

First name: René
Surname: Freiberg
Citizenship: Estonian
Date of Birth: 11.08.1977
Address: Estonian University of Life Sciences,
Institute of Agricultural and Environmental Sciences,
Centre for Limnology,
Rannu Municipality, Tartu County EE61101, Estonia
Telephone: +372 522 9091
E-mail: rene.freiberg@limnos.ee

Education:

2006 – 2012	Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences, Environmental Sciences and applied Biology, Ph.D. studies
1995 – 2000	University of Tartu, Faculty of Biology and Geography, B.Sc. (M.Sc.) studies
1984 – 1995	Tallinn Lilleküla Gymnasium

Additional training

2010	Stuart Jones HPLC Academy, intermediate course (UK) DHI pigment workshop (Denmark)

Employment

Since 2005	Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences, Centre for Limnology, specialist
2002 – 2005	Karula National Park Administration, Life-Nature project "Conservation of Natura 2000 biotopes in Karula National Park", manager
2001 – 2002	Karula National Park Administration, specialist of scientific monitoring

Research interests:

Phytoplankton pigments, aquatic toxicology

Publications

Tõnno, I.; Kirsi, A. -L.; Freiberg, R.; Alliksaar, T.; Lepane, V.; Kõiv, T.; Kisand, A.; Heinsalu, A. (2013). Ecosystem changes in large and shallow Võrtsjärv, a lake in Estonia — evidence from sediment pigments and phosphorus fractions. *Boreal Environment Research*, **18**, [in press]

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ELULOOKIRJELDUS

Eesnimi: René
Perenimi: Freiberg
Sünniaeg: Estonian
Töökoht: 11.08.1977
Aadress: Eesti Maaülikool,
Põllumajandus- ja keskkonnainstituut,
Rannu vald, EE61101 Tartumaa, Eesti
Telefon: +372 522 9091
E-mail: rene.freiberg@limnos.ee

Haridustee

2006 – 2012	Eesti Maaülikool, Põllumajandus- ja keskkonnainstituut, Limnoloogiakeskus, doktoriõpe keskkonnateaduse ja rakendusbioloogia erialal
1995 – 2000	Tartu Ülikool, Bioloogia- geograafia teaduskond, hüdrobioloogia B.Sc. (M.Sc.)
1984 – 1995	Tallinna Lilleküla Gümnaasium

Täiendõpe

2010	Stuart Jones HPLC Academy, kesktaseme kursus (UK) DHI pigmentide seminar (Denmark)

Teenistuskäik

Since 2005	Eesti Maaülikool, Põllumajandus- ja keskkonnainstituut, Limnoloogiakeskus, spetsialist
2002–2005	Karula Rahvuspargi Administratsioon, Life-Nature projekti "Conservation of Natura 2000 biotopes in Karula National Park" koordinaator
2001–2002	Karula Rahvuspargi Administratsioon, seirespetsialist

Uurimissuunad

Fütoplanktoni pigmendid, veetoksikoloogia

Publikatsioonid

Tõnno, I.; Kirsi, A. -L.; Freiberg, R.; Alliksaar, T.; Lepane, V.; Kõiv, T.; Kisand, A.; Heinsalu, A. (2013). Ecosystem changes in large and shallow Võrtsjärv, a lake in Estonia — evidence from sediment pigments and phosphorus fractions. *Boreal Environment Research*, **18**, [trükis]

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VEISTE HERPESVIIRUS 1 NAKKUSE EPIDEMIOLOOGIA, MÕJU KARJA TERVISELE JA TÕRJE EESTI PIIMAVEISTE KARJADES
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