Technische Universität München

Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt

Lehrstuhl für Aquatische Systembiologie

Contribution of the calcifying green alga *Phacotus lenticularis* to lake carbonate sequestration

Sebastian Lenz

Vollständiger Abdruck der von der Fakultät Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt der Technischen Universität München zur Erlangung des akademischen Grades eines

Doktors der Naturwissenschaften

genehmigten Dissertation.

Vorsitzender:	Prof. Dr. Johannes Kollmann
Prüfer der Dissertation:	
	1. Prof. Dr. Jürgen Geist
	2. apl. Prof. Dr. Tanja Gschlößl

Die Dissertation wurde am 21.01.2020 bei der Technischen Universität München eingereicht und durch die Fakultät Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt am 23.04.2020 angenommen.

So remember to look up at the stars and not down at your feet. Try to make sense of what you see and wonder about what makes the universe exist. Be curious.

And however difficult life may seem, there is always something you can do and succeed at.

It matters that you don't just give up.

Professor Stephen Hawking
(† 14 March 2018)

Contents

List of Figures

List of Tables

Preface

The aim of this dissertation is to investigate the role of the calcifying green alga *Phacotus lenticularis* in respect to its contribution to long term carbon (C)-fixation by carbonate sequestration in Bavarian lakes. The thesis is structured in the three following parts.

The introduction presents the state of the art knowledge of C-cycling in alkaline lakes by explaining the most fundamental processes and major sources of C in this freshwater ecosystem. Function and control mechanisms of biogenic calcite precipitation and the outstanding role of *P. lenticularis* are described in the context of a review on the literature about the *Phacotus* calcite shell and reported sedimentary occurrences of *P. lenticularis*. Here, the term **carbonate sequestration** is used in respect to the C that is sequestrated in form of sedimented carbonate at the lake bottom for geologic timescales acting as a C-sink in the way that it reduces the C-emissions from the lake into the atmosphere. Thereafter a brief summary of the objectives and hypotheses of the work is followed by a general materials and methods section that gives an overview on the applied methods.

The main part consists of three core chapters on *Phacotus* carbonate sequestration.

1. Chapter 2 is the basis for all further quantitative calculations on *Phacotus* carbonate sequestration. It describes a new method for the exact determination of the shell mass using the Focussed Ion Beam (FIB) technology and computer based 3D-modelling. It further contains a detailed description of the internal ultrastructure of the *Phacotus* shell as well as a morphometric assessment of *Phacotus* shells from four German hard water lakes.
2. Chapter 3 summarizes the required methodology for a representative monitoring of a *P. lenticularis* population in a lake in the light of its spatiotemporal variation. Cell densities and sedimentation rates of four different lakes are compared and findings about shell dissolution during sedimentation and sample preparation are discussed.
3. Chapter 4 quantitatively determines the *Phacotus* shell content in lake sediments of four lakes to investigate the degree of *Phacotus* shell contribution to long term carbonate sequestration. The *Phacotus* shell content of a gravity core dating back to 1868 is determined and the relevant factors affecting *Phacotus* shell content in sediments are discussed.

The last chapter is the general discussion which is also divided into three parts. In section 5.1, the variance of morphometry and occurrence is discussed as a linking aspect that played a fundamental role in all of the three core chapters. On the basis of scanning electron micrographs, a new hypothesis is presented, which is opposing to the idea of genetically determined *Phacotus* morphotypes as reason for morphometric variance. Further on, the reasons for the spatiotemporal variation of *P. lenticularis* in the water column as

well as in sediment are discussed. Section 5.2 focuses on the relevance of *P. lenticularis* contribution to total carbonate sequestration in lakes. The question if organic carbon also contributes to total *Phacotus* carbon fixation is discussed using an estimation of the C_{org} content of the *P. lenticularis* protoplast. The artificial enhancement of (*P. lenticularis*) carbonate precipitation in lakes is briefly discussed and compared to the potential environmental adaptions that Bavarian lakes might face in the progress of climate change. Finally, the role of *P. lenticularis* in the ecosystem and if *P. lenticularis* is responsible for the sequestration of relevant amounts of carbon in lakes is discussed.

In section 5.3 a universal scheme shows all relevant data on *P. lenticularis* carbonate sequestration for the example of Lake Grosser Ostersee. Finally, it is discussed in which extend the formerly presented methods can be applied for a larger number of lakes. A suitable concept is presented and illustrated on the example of Bavaria.

1 Introduction

1.1 Carbon cycling and carbonate-water system in alkaline lakes

Carbon (C) is the elementary component for all organisms and it is present in several C-pools everywhere on the planet. Currently, the atmosphere, a C-pool holding 0.750 Pg C, gets most attention because of its rising concentration of carbon dioxide (CO_2) causing massive changes of the global climate system and ecosystems (Ciais *et al*, 2013; Einsele, Yan and Hinderer, 2001; Purves, 2011). However, the most important C-pools in terms of quantity are sedimentary deposits including fossil fuels that hold 43,000 Pg C and the oceans where 34 Pg C is stored in form of dissolved carbonate ions. These C-pools are interconnected. Almost the entire C in organisms originates from CO_2 from the atmosphere or bicarbonate ions (HCO_3^-) dissolved in water (Sadava, 2019). Biological processes like photosynthesis of autotroph organisms, as well as physical processes like weathering of rocks and autochthonous carbonate precipitation, drive the transport of atmospheric C into the terrestrial and marine compartments. Marine ecosystems are important hot spots for carbon cycling but also freshwater ecosystems play an appreciable role in regional and global carbon cycles (Cole *et al*, 2007; Einsele *et al*, 2001). The annual C-inflow in global freshwaters is estimated at 2.9 Pg C (Cole *et al*, 2007) and approximately 21 % of the annually entering amount of C are stored as sediment (Tranvik *et al*, 2009). The essential processes of the C-cycle in hard water lakes are depicted in form of arrows in the adopted scheme after (Noges *et al*, 2016).

The most fundamental processes in freshwater ecosystem metabolism are gross primary production (GPP) and respiration. GPP stores solar energy, fixes CO_2 by photosynthesis, and builds up organic C-compounds in form of biomass that finally sediments. The opposed process is the respiration, i.e. mineralization of the organic C-compounds to CO_2 by respiration to generate energy (Finlay, Leavitt, Wissel and Prairie, 2009; Noges *et al*, 2016; Solomon *et al*, 2013). The rates of these processes and their relation to each other is highly dynamic over daily to seasonal timescales (Cremona *et al*, 2014). In the presented study, the investigated lakes are alkaline lakes of the temperate climate zone in southern Bavaria, Germany. These types of lakes generally emit more CO_2 than they would store over a whole year under the assumption that sufficient dissolved inorganic carbon DIC (H_2CO_3, HCO_3^-, CO_3^{2-}) input is provided (Cole *et al*, 2007; Liu, Dreybrodt and Liu, 2011). Therefore, it depends on the observed timescale and on the net ecosystem production (NEP) if the studied lakes act as C-sources or C-sinks (Cole, Pace, Carpenter and Kitchell, 2000).

The major source of carbon of these surface waters is weathering of rocks in their carbonatic catchments. This provides C in form of DIC and dissolved Ca^{2+} ions which are the preconditions for carbonate precipitation. The DIC originates to one half from

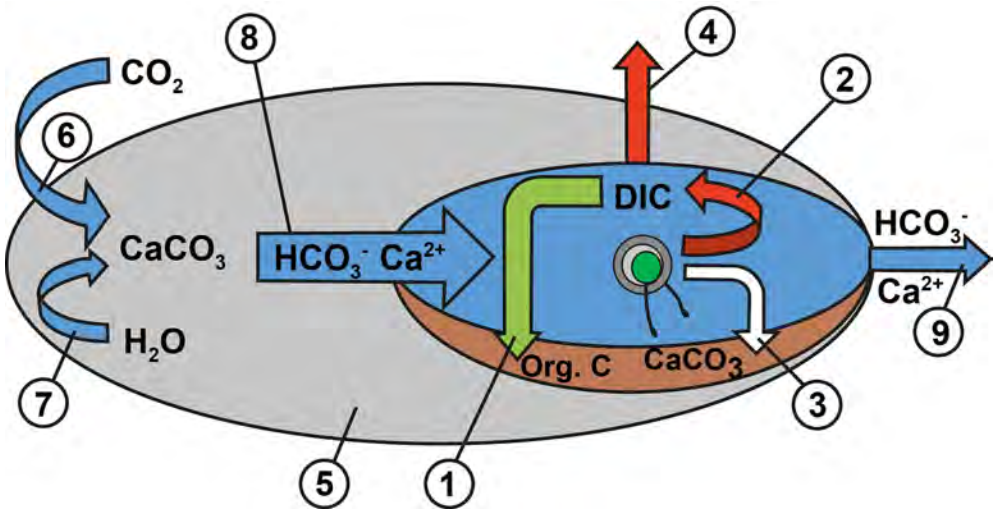

Fig. 1.1: C-related processes in hard water lakes (blue oval): 1 photosynthesis, 2 respiration, 3 physico-chemically and biologically induced calcite precipitation, 4 evasion of CO_2 into the atmosphere, 5 calcareous catchment, 6 inflow from atmosphere and soil, 7 inflow from atmosphere and aquifer, 8 lake inflow from groundwater and surface runoff, 9 outflow; figure adapted und further developed after (Noges *et al*, 2016).

the dissolution of carbonates and to the other half from the atmosphere and soil air (Heathcote and Downing, 2012). In comparison to other gases from the atmosphere, CO_2 has a remarkable solubility in water due to its bipolar character. Therefore, CO_2 solutes 40 times better in water than O_2 under pure atmospheric conditions. This special fact enables the solution of enormous amounts of inorganic carbon in water bodies, despite under natural conditions, the partial pressure of CO_2 in water is significantly lower than of the other atmospheric gases (Schwoerbel and Brendelberger, 2005; Stumm and Morgan, 1996). Once the inorganic carbon is dissolved in water, the lime-carbonic acid balance (eq. 1.1) describes the relation of the different DIC species: Dissolved CO_2 gets hydrated in water and forms carbonic acid (H_2CO_3) which dissociates to free protons and carbonate ions with rising pH of the water.

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^- \rightleftharpoons {}_2H^+ + CO_3^{2-} \tag{1.1}$$

In freshwater lakes with pH values between 6.5 and 8.5 bicarbonate ions dominate the titration alkalinity and offer thereby the necessary DIC for carbonate precipitation. Carbonate saturated or supersaturated conditions arise in the water column when the ionic activity product of Ca^{2+} and carbonate ions (CO_3^{2-}) equals or overpasses the pressure and temperature dependent carbonate equilibrium constant K_c (Stumm and Morgan, 1996). For carbonate crystallization and finally autochthonous calcite precipitation (ACP), the presence or nucleation of crystallisation nuclei are required that form only under critical super saturation and the physicochemical conditions for further crystal growth (Koschel, Proft and Raidt, 1987b). As inhibiting factor, high concentrations of phosphate may delay calcite formation during the early phase of active photosynthesis until the increased primary production finally leads to decreasing phosphate concentrations (Bluszcz, Lücke, Ohlendorf and Zolitschka, 2009). Subsequently caused by phosphate-mediated inhibition,

calcite crystals form rapidly because of the high super saturation and get suspended in the upper water column. There, they interact in various ways with ecosystem processes by limiting the available ions via depletion of Ca^{2+} and HCO_3^- as well as reducing phosphate concentrations (Koschel, 1983; Proft and Stutter, 1993). With a wide range of forms and diameters (Koschel and Raidt, 1988b), the calcite crystals contribute to C-circulation in lakes causing accelerated sedimentation of particulate matter and thereby expediting the carbonate pump (Andersen, Kragh, Martinsen, Kristensen and Sand-Jensen, 2019).

1.2 Biogenic calcite precipitation

During autochthonous calcite precipitation, the dissolved Ca^{2+} and CO_3^{2-} ions from the lake water precipitate according to (eq. 1.2) and form particular suspended carbonate, i.e. calcite ($CaCO_3$).

$$Ca^{2+} + 2HCO_3^- \rightleftharpoons CO_2 + CaCO_3 + H_2O \tag{1.2}$$

The ACP is controlled by a combination of chemical, physical but also biogenic factors where temperature changes seem to play a less important role in directly triggering calcite precipitation than CO_2-assimilation by photosynthesis (Kelts and Hsü, 1978). For this reason, the term biogenic calcite precipitation (BCP) has established. It can be further differentiated according to the site of its origin either in the litoral or in the pelagial (Fig. 1.2). In the pelagial zone, mainly cyanobacterial picoplankton is responsible for the bulk carbonate precipitation (Thompson, Schultze-Lam, Bevertige and Des Marais, 1997) whereas in the litoral zone charaphyceans (Emi, 2001) precipitate most of the calcite.

In alkaline hard-water lakes BCP leads to "whiting" events (Fig. 1.3) as prominent seasonal phenomena that typically happen during the early algal bloom in spring and mid-summer, when intense sunlight in the nutrient enriched surface waters stimulate intense CO_2 assimilation which induces carbonate precipitation (B. Müller, Wang and Wehrli, 2006). This leads to elevated concentrations of suspended particulate calcite ($CaCO_3$) crystals. As a result, up to 5 mg $CaCO_3$ L^{-1} in the topmost water column can be suspended. During whiting events, lakes show a typically turquoise appearance due to the characteristic light scattering of the suspended $CaCO_3$ crystals.

When BCP occurs, for every mole $CaCO_3$ an additional mole of CO_2 is released into the water which is available and used for photosynthesis as long as DIC and Ca^{2+} are abundant (Andersen *et al*, 2019). The carbon inside the sedimented carbonates is finally stored at the lake bottom and stays there sequestered for geologic timescales if conditions are adequate (Einsele *et al*, 2001). For this reason the term **"carbonate sequestration"** is used in respect to the function of BCP acting as a C-sink in the way that it reduces the C-emissions from the lake into the atmosphere (Fig. 1.2).

Phytoplankton is involved in BCP either directly or indirectly. Indirectly involved, though causing the main impact are cyanobacteria (Schneider and Le Campion-Alsumard, 1999). Due to their assimilation activity they were identified as a major influencing factor inducing BCP, since they act in a double way (1) as heterogeneous crystallisation nuclei and (2) by their photosynthetic activity (Dittrich and Obst, 2004; Thompson *et al*, 1997).

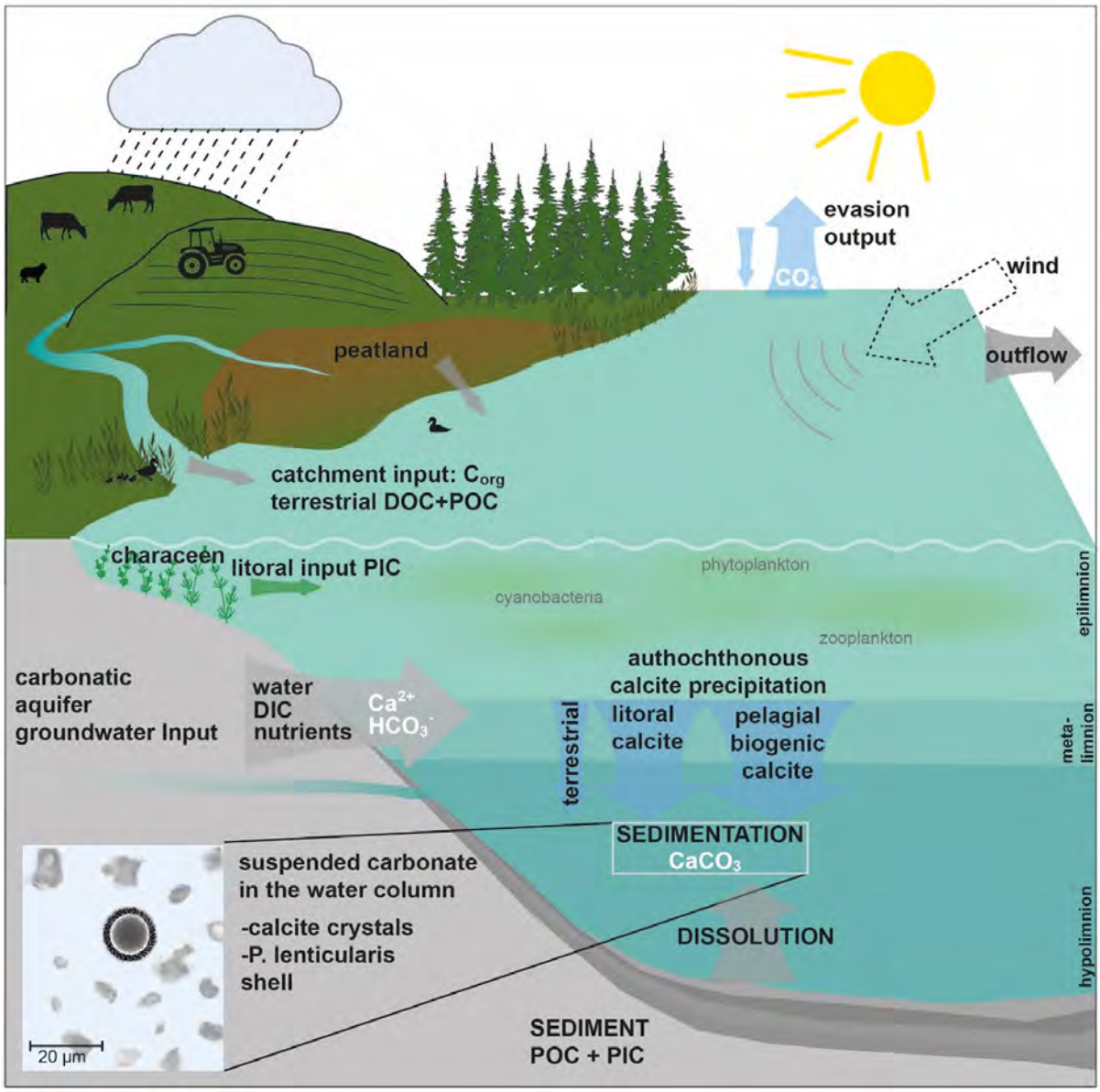

Fig. 1.2: C-sources and related processes for carbonate sequestration: sources for dissolved organic and inorganic carbon (DOC, DIC) are the aquifer, the catchment and the atmosphere. Carbonate precipitation and particulate terrestrial inputs cause sedimentation which potentially is reduced by dissolution. Sediment originates from the sedimentation of particulate organic and inorganic carbon (POC, PIC).

1.3 Calcifying phytoplankton *Phacotus lenticularis*

Calcifying phytoplankton species in lacustrine environments exist very few. Despite the species *Difflugia limnetica* and *Tintinopsis spec.* that incorporate suspended calcite crystals from the water in their external housings (Koschel *et al*, 1987b) the only species that actively precipitates calcite to build its shell by external calcification (Kelts and Hsü, 1978) is the calcifying freshwater alga *Phacotus lenticularis* (Ehrenberg) Diesing 1866 Stein.

Fig. 1.3: Aerial photograph during whiting conditions at lake Grosser Ostersee (LO) while the directly adjacent Lake Fohnsee (LF) appears dark blue (archive LSI); near the lake surface, the suspended calcite crystals in the epilimnion scatter the sunlight in characteristic turquoise.

The taxonomic-systematic classification of *Phacotus lenticularis* is presented in Fig. 1.5.

The unicellular green alga *Phacotus lenticularis* forms a bivalved calcite shell that in addition to BCP contributes to long term C-sequestration at the lake bottom, and thereby reduces the lakes C-emissions into the atmosphere. The calcifying algae is abundant in temperate hard-water lakes worldwide and contributes during mass developments between 10 and 50 % of the total phytoplankton biomass (Schlegel, Koschel and Krienitz, 1998). Therefore, this phytoplankton algae incorporate remarkable amounts of $CaCO_3$ in its

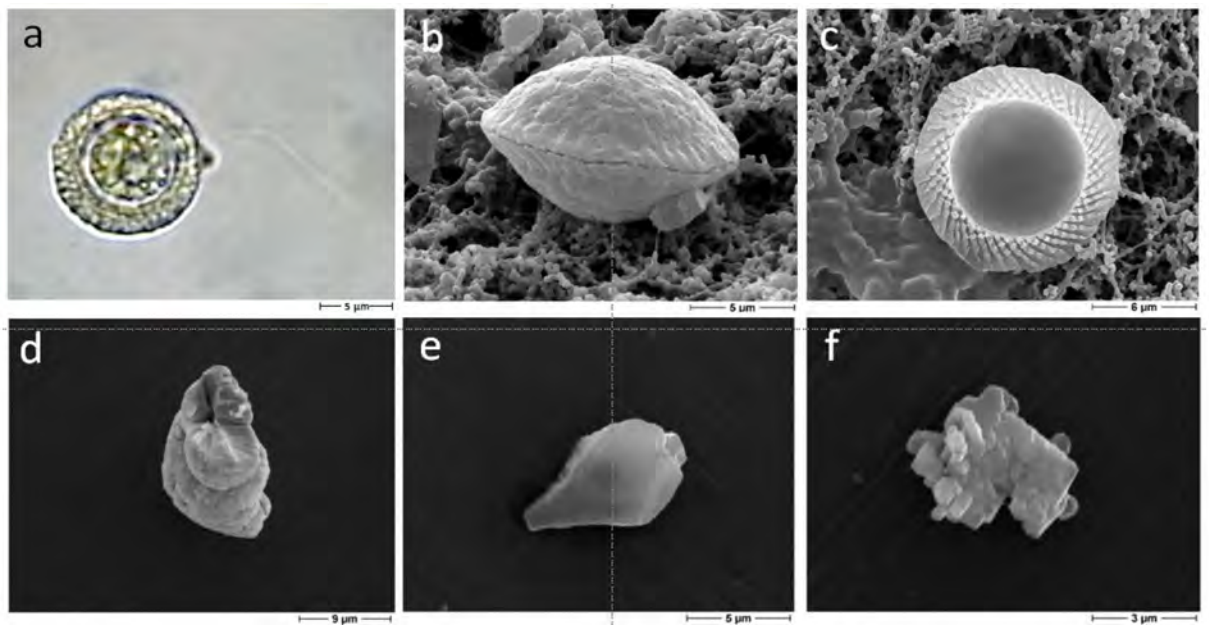

Fig. 1.4: *Phacotus lenticularis* calcite shell (a-c) and autochthonous calcite crystals in diverse habitus as they form simultaneously in the epilimnion due to BCP (d-f).

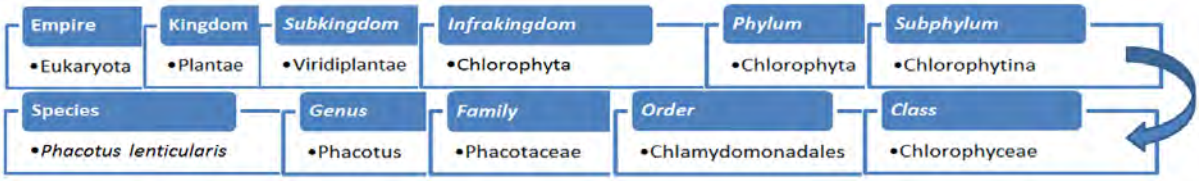

Fig. 1.5: Taxonomy of *Phacotus lenticularis* (Guiry, 2019).

two shells (Giering *et al*, 1990b; Koschel, Proft and Raidt, 1987a; Koschel and Raidt, 1988a; Pocratsky, 1982; Sieminska, 1952; Steinberg and Klee, 1983) that consists of 98 % – 99 % pure $CaCO_3$ (Schlegel *et al*, 1998). During mass developments in the Baltic Lake District of north-eastern Germany, *Phacotus lenticularis* temporarily contributed 100 % to autochthonous calcite precipitation in Lake Ziestsee (Koschel and Raidt, 1988a) and 95 % – 100 % in Lake Tollense and Lake Haussee (Krienitz *et al*, 1993). Consequently, this species brought about high autochthonous $CaCO_3$ inputs in these lakes and played a definite role in limnic carbonate sedimentation as a sediment contributor (Bluszcz *et al*, 2009; Koschel *et al*, 1987a; G. Müller and Oti, 1981). Literature on sedimentary *Phacotus* records goes back to 1902 when Langenheim described over 40 occurrences of *Phacotus* shells in Scandinavia from Kalk Gyttja (pelitic carbonate sediments) originating from places near or under moors. Korde (1966) found *Phacotus* shells in lacustrine sediments from shallow water bodies with high alkalinity that were in the state of progression of their littoral zone and depth reduction. G. Müller and Oti (1981) described a *P. lenticularis* occurrence that formed in the Ries Crater Lake (Germany) during the late Miocene under permanent, sometimes evaporating lake conditions with high ion-concentrations and algal blooms. *Phacotus* shells were even found in sediments that formed under temporarily brackish conditions in shallow eutrophic ponds (Füchtbauer *et al*, 1977).

Consequently, sedimentary *Phacotus* records prove that *Phacotus lenticularis* is capable of forming populations in a wide range of environmental conditions and that its shell is capable of sedimentary preservation. However, until now there have been no quantitative measurements of the total content of *Phacotus* shells in lake sediments.

In order to be able to calculate and evaluate the quantitative contribution to long term C-sequestration of this unique calcifying freshwater organism, the present work shows the first quantitative study of *Phacotus* abundance in limnic sediments. Moreover, precise data on the mass of *Phacotus lenticularis* shells are presented for the first time. This is important because with these data finally a comprehensive evaluation of the impact of *Phacotus* carbonate contribution to total carbonate sequestration in lakes is possible.

1.4 Objectives

The importance of carbonate precipitation by phytoplankton in fresh water lakes has not been sufficiently considered in global C-cycling. As a contribution to that, this work examines the role of the calcifying green alga *Phacotus lenticularis* in regard to its contribution to long term C-fixation by carbonate sequestration in Bavarian lakes.

The objective of this study was to quantitatively determine the amount of C which is bound by *P. lenticularis* in the form of calcite. In order to achieve this goal, the precise determination of the exact mass of a *Phacotus* shell was a prerequisite and the analysis of the spatiotemporal population distribution in the upper water column was necessary. Finally, the determination of the fraction of *Phacotus* shells in lake sediments needed to be investigated in order to evaluate the importance of the calcifying alga for long-term carbonate sequestration.

Against this background, three hypotheses were tested:

1. The calcified shells of *P. lenticularis* are characterised by identical calcium carbonate contents
2. *Phacotus* shells remain preserved in recent lake sediments
3. *P. lenticularis* is responsible for fixation of relevant amounts of carbon in lakes

As a result a better understanding of the role of calcifying phytoplankton *P. lenticularis* in freshwater ecosystems should be achieved.

1.5 Materials and Methods

1.5.1 Study sites and monitoring concept

The project included seven hard water lakes of different trophic state with calcareous catchments in four areas of Bavaria (Fig. 1.6).Lake Großer Ostersee (GOS) in the South of Munich, Lake Igelsbachsee (IGS) and Lake Altmühlsee (ALT) in Franconia, Lake Hopfensee (HOP) and Lake Sulzbergersee (SUL) in the Allgäu, Lake Abtsdorfersee (ABS) in the Berchtesgadener Land and Lake Waginger See (WAG) near Traunstein.

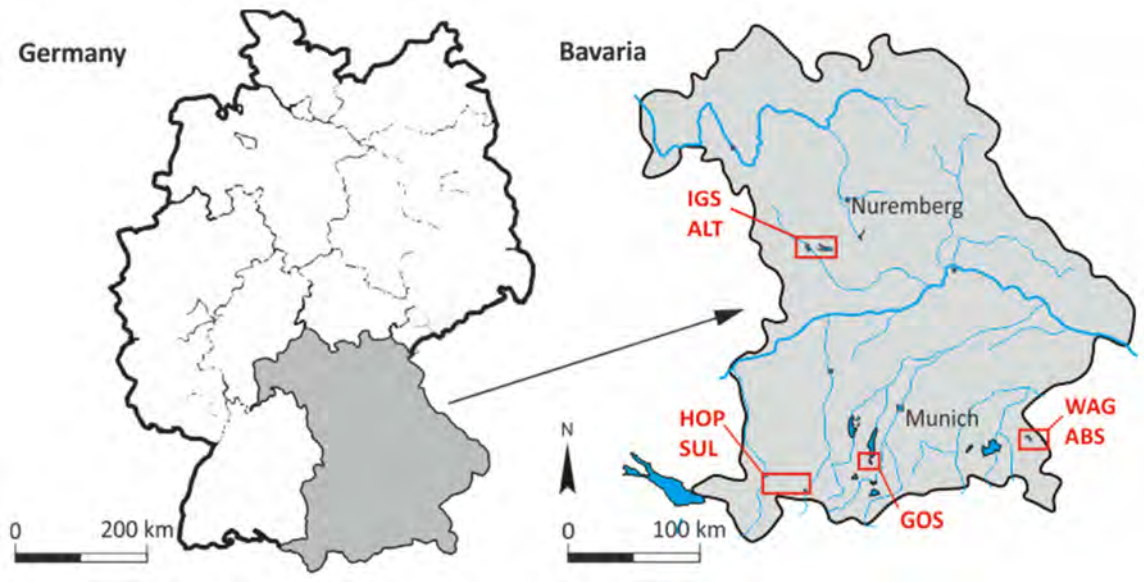

Fig. 1.6: Study area and location of investigated seven hard water lakes in Bavaria, Germany.

Documented *P. lenticularis* occurrences have been reported in each lake for the last ten years (2005-2015) during routine phytoplankton investigations executed by the responsible water management authorities. Thereby all examined and monitored water bodies within the framework of the project were demonstrably suitable habitats for *P. lenticularis*. The lakes HOP, ALT and IGS even hosted *P. lenticularis* mass occurrences with maximum cell densities of $> 500,000$ Individuals per Litre (Ind. L^{-1}). All seven lakes have been analysed for their average *Phacotus* occurrence and the concentration of total suspended carbonate in the upper water column. Detailed investigations on the *Phacotus* shell volume and mass were realized in five of these Lakes (Chapter 2). Furthermore, in four lakes sediment analysis and sediment trap experiments have been realized (Tab. 1.1).

The monitoring concept aimed to assess the *Phacotus* abundance in the epilimnion and the total suspended carbonate concentration of the selected lakes in a time and cost-effective way. Simultaneously, the mean *Phacotus* abundance needed to be detected starting from the beginning of the strongest *Phacotus* growth. Therefore, it was important to detect the beginning of the first growth peak. The onset of growth had to be individually determined for each lake. In order to identify the onset, the water temperatures and pH-values at the lake surface were monitored bi-weekly by water measurements from the beginning of May. The sampling started when the appearance of *P. lenticularis* was to

Table 1.1. Overview of investigated lakes, trophic state and highest reported *P. lenticularis* abundance.

Lake	Trophic state	*Phacotus* (Ind. L^{-1})	Water analysis	Sediment analysis	Sediment traps
GOS	oligotrophic	90,000	✗	✗	✗
IGS	eutrophic	60,000	✗	✗	✗
ALT	hypertrophic	730,000	✗		
HOP	eutrophic	1,490,000	✗	✗	✗
SUL	eutrophic	120,000	✗		
ABS	mesotrophic	180,000	✗	✗	✗
WAG	mesotrophic	200,000	✗		

be expected. According to the findings of Gruenert und Raeder (2014a) the onset of the *P lenticularis* growth is induced when water temperatures at a depth of 0.5 m depth exceed 20° C and rise above pH-values > 7.5. The monitoring was carried out from the beginning of June until the beginning of September 2016. In this period, every lake was sampled at least ten times at a regular frequency. The sampling and measurement of physico-chemical conditions of the water column was conducted at the deepest position of the lake.

In order to investigate the *Phacotus* content in the sedimented material, sediment traps were deployed. A cylindrical sediment trap (CST) (Hydro-Bios, Kiel, Germany), according to Saarso (1995), was exposed at a water depth of 7 m directly below the zone in which *Phacotus* developed. For this purpose, a buoy weight made of concrete was positioned at the deepest point of the lake with a rope attached to a buoy (Fig. 1.9a). The buoy served as a marker for the sampling site, and additionally its rope served as a running line for the CST. In order to exchange the sediment sample bottle the CST was brought to the surface with a second rope (green in Fig. 1.9a). Samples were taken as frequently as feasible at least once a week.

During the vegetative reproduction of *P. lenticularis*, the cells split and their shell halves sink in the water column. Inactive individuals also sink. To trap the sedimented *P. lenticularis* individuals, square sediment traps (SST) at the lake bottom were used to investigate if the shell halves that formed in the water column, arrive and persist above the lake bottom or if they dissolve during sedimentation. These SST were made of plexiglass, had an edge length of 20 × 20 cm and a sliding lid. For the exposition, research divers attached two open SST horizontally on a metal rod. The rod was fixated one metre above the lake bottom on a wooden pole near the buoy weight. The vertical distance x between the CST and the SST varied in the different lakes depending on the water depth. During sampling, the divers brought the closed square traps to the boat. There the contents of the CST were decanted into PE bottles. SST sampling took place at GOS (4×), IGS (3×), HOP (2×) and ABS (5×) at the same time as the CST was emptied.

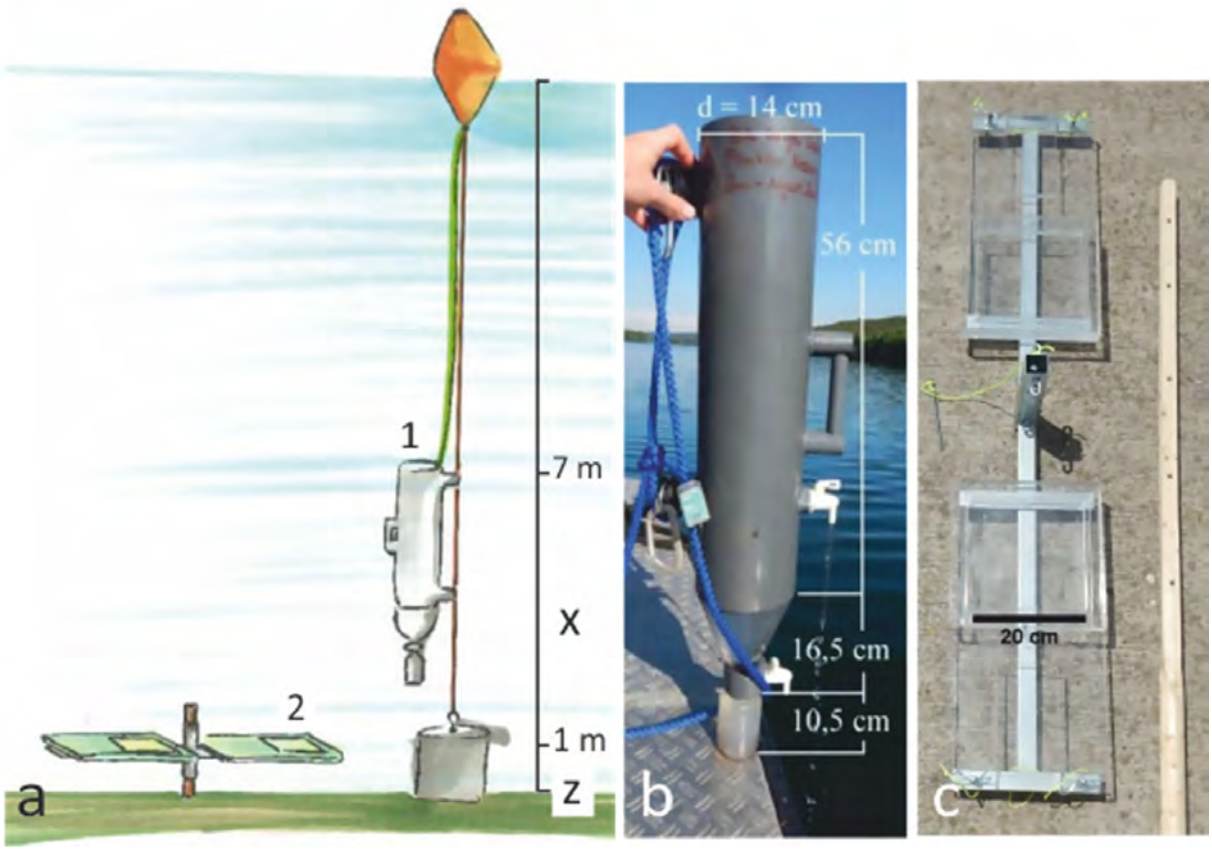

Fig. 1.7: Depth adjustment of sediment traps in a lake, changed according to Chapter 3: cylindrical sediment trap at 7 m depth (1) and square sediment trap 1 m above the lake bottom (2) in the depth z (a); dimensions of cylindrical sediment trap CST (b); dimensions of square sediment trap SST (c).

1.5.2 Sampling for representative monitoring

In an exploratory study, the development of the *Phacotus* population and its vertical distribution in a depth between 0 and 10 m was investigated in Lake Grosser Ostersee with high spatial and temporal resolution, i.e. every metre and in depth and in part daily. For this purpose over 53 days, always 33 water samples were taken with a standard Ruttner water sampler at three different sites (GOS-N, GOS-E and GOS-W). After an initial microscopic analysis of the samples from different points in time in order to determine the emergence of the *Phacotus* population, the 858 plankton samples from 26 selected sampling days were counted completely. At least 300 individual cells were counted per sample to ensure the accurate determination of shell numbers with an inverse microscope (Leitz Labovert, Stuttgart, Germany) at 200–400× magnification according to the Utermöhl (1958). The cell densities in Chapter 3 are average values of 0–10 m depth for 2015.

In 2016 the assessment of *Phacotus* abundance and total suspended carbonate concentration in the water column of the other lakes was undertaken based on the information from the exploratory study with the difference that integrated water samples from depths ranging from 0 - 7 m were taken with a seven-metre-long hose sampler according to the

DIN Technical Committee Water Analysis (2015). A 3 kg weight attached to the open end of the PVC-hose ensured vertical sinking (Fig. 1.8a, b). The top end was closed after the complete hose was let down slowly into the water. To recover the 6 L water sample, the open end was pulled up and emptied into a measuring bucket by lifting the hose from the end (Fig. 1.8c, d). Every first sample was discarded in order to rinse the sampling hose. The second sample was introduced into a canister, which was homogenized by shacking before filling of further sampling bottles from the canister.

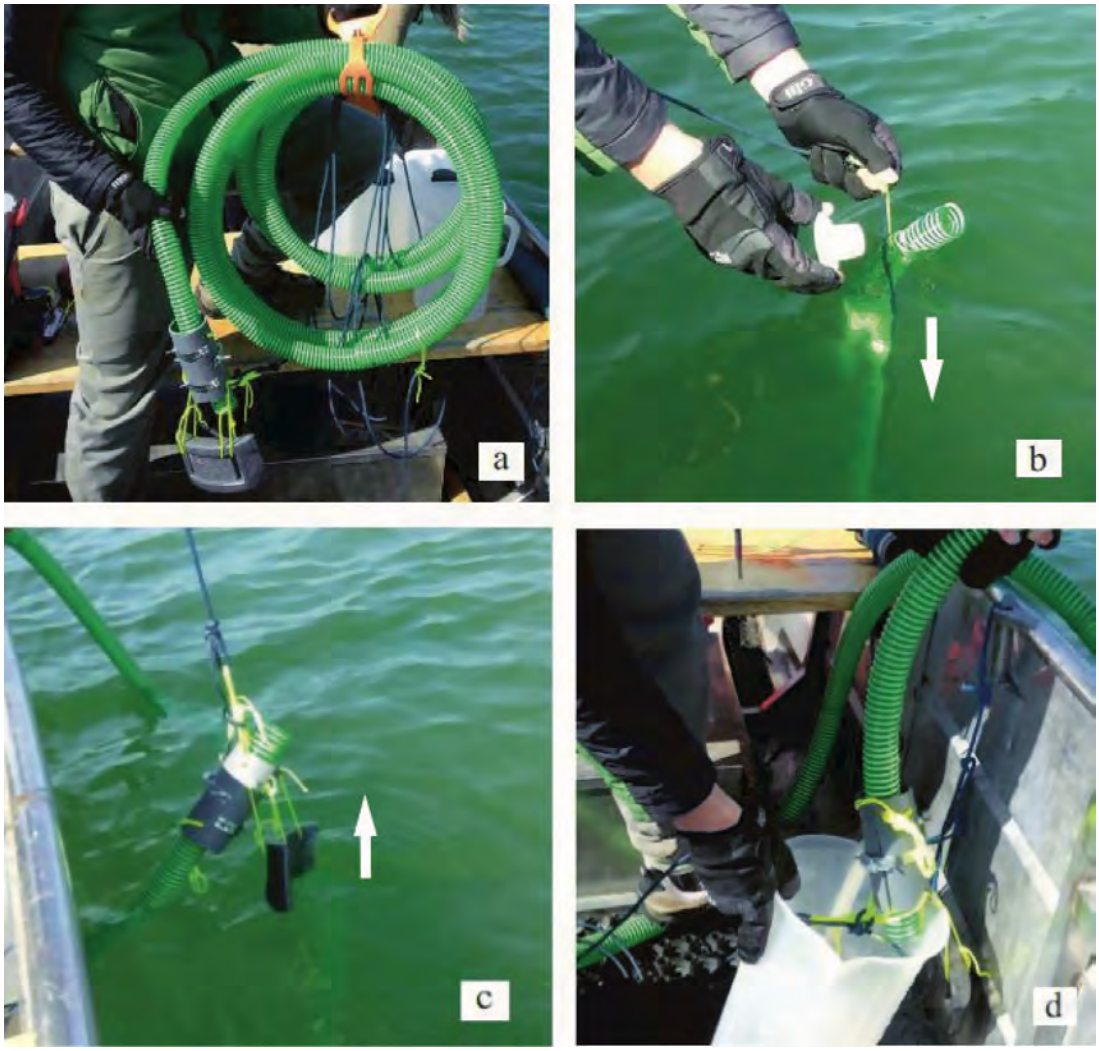

Fig. 1.8: Handling of the sampling hose: open PVC-tube with weight attached to the end (a), closing the top after complete vertical let down of the open hose (b), recovery of the hose by first lifting the down hanging end (c), emptying the hose into a measuring bucket (d).

Finally, two 100 mL brown glass flasks were filled and conserved in 2 % of neutral Lugol's solution for plankton analysis. Right after sampling four water samples (200 mL) were filtrated on 0.45 μm cellulose-nitrate filters (Whatman, Maidstone, UK). Filters were oven dried overnight at 50°C and stored in a desiccator until further analysis. Depth profiles of the physical parameters, water temperature, pH-value and conductivity were measured with a WTW Multi 3430 (Weilheim, Germany) handheld device connected to a 30 m cable with a WTW MPP 930 multiprobe. An integrated pressure sensor determined

the depth of the actual measurement. Additionally, the Secchi depth was determined using a Secchi-visibility disc (Hydrobios, Kiel, Germany).

To investigate the fraction of *Phacotus* shells in the sediment of Lake Grosser Ostersee, a total of six sediment gravity cores were taken with a UWITEC gravity corer (Mondsee, Austria). They were labelled as GOSED for Lake Großer Ostersee Sediment core and the numbers 01 to 06. The sediment cores GOSED01, GOSED02 and GOSED03 were used for the first loose sediment investigations to characterize the carbonate, lacustrine basinal silt and to investigate the heterogeneity of the different sedimentation basins in the Lake Großer Ostersee. All other sediment cores originate from the deepest point (29.7 m) in the Lake Großer Ostersee (WGS 84 lat/lon: 47.79082°, 11.30145°). It was determined that the cores of this site had the least mechanical disturbance and overlay of material. For this reason, these cores were considered most representative for Lake Großer Ostersee. Methods for sample preparation and production of microscopic preparations were optimized using core GOSED04. For sediment suspension a buffered suspension liquid (NH_3+H_2O) with a adjusted pH of ≥ 8.5 was used according to (Bollmann, Brabec, Cortés and Geisen, 1999) so that the *Phacotus* shells did not dissolve during analysis. The *Phacotus* shell concentration of the sediment suspension at which the shells can be clearly identified was determined. The core GOSED06 was finally analysed in detail. The results are presented in Chapter 4.

In addition to the sediment cores, surface sediment samples were taken with a Plexiglas tube from a PVC UWITEC gravity corer in the lakes Großer Ostersee, Lake Abstdorfer, Lake Hopfensee and Lake Igelsbachsee. Research divers removed the topmost 4 cm of basinal sediment next to the square sediment traps. The sediment was completely freeze-dried and carefully homogenised for further analysis analogously to the material from the sediment core as further described below.

1.5.3 Carbonate measurements

To determine the suspended particulate $CaCO_3$ concentration in the water column, the dried material was measured on the membrane filter by infrared (IR) CO_2 analysis. This analysis was performed at the Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB), Stechlin, Germany. A detailed description of the procedure can be found in Chapter 2. The determination of the carbonate fixed in the *Phacotus* shells in the water column was carried out by analysing the fixated plankton samples under an inverted microscope with a polarisation filter (Labovert, Leitz, Stuttgart, Germany) according to the method described by Utermöhl (1958). The content of *Phacotus* carbonate in the water column was calculated by multiplying the *Phacotus* cell density by the lake-specific mass of a shell.

The content of *Phacotus* carbonate, reaching the lake bottom, was determined analogously. For this purpose, the water from the square sediment trap samples in PE bottles was first decanted, and the wet sediment was constricted using membrane filters, dewatered by freeze-drying and carefully homogenized. The organic fraction of this material was then burned for 4 hours at 550°C in a muffle furnace. Then 0.5 mg of this inorganic sediment

material was dissolved in 2 ml of water. In three replicas, 50 μl of this suspension were prepared on cover glasses containing *Phacotus* abundance, determined by using a research microscope with a polarization filter (DM RBE, Leica Mio-systems, Wetzlar, Germany). Moreover, the *Phacotus* carbonate content was calculated as mentioned above.

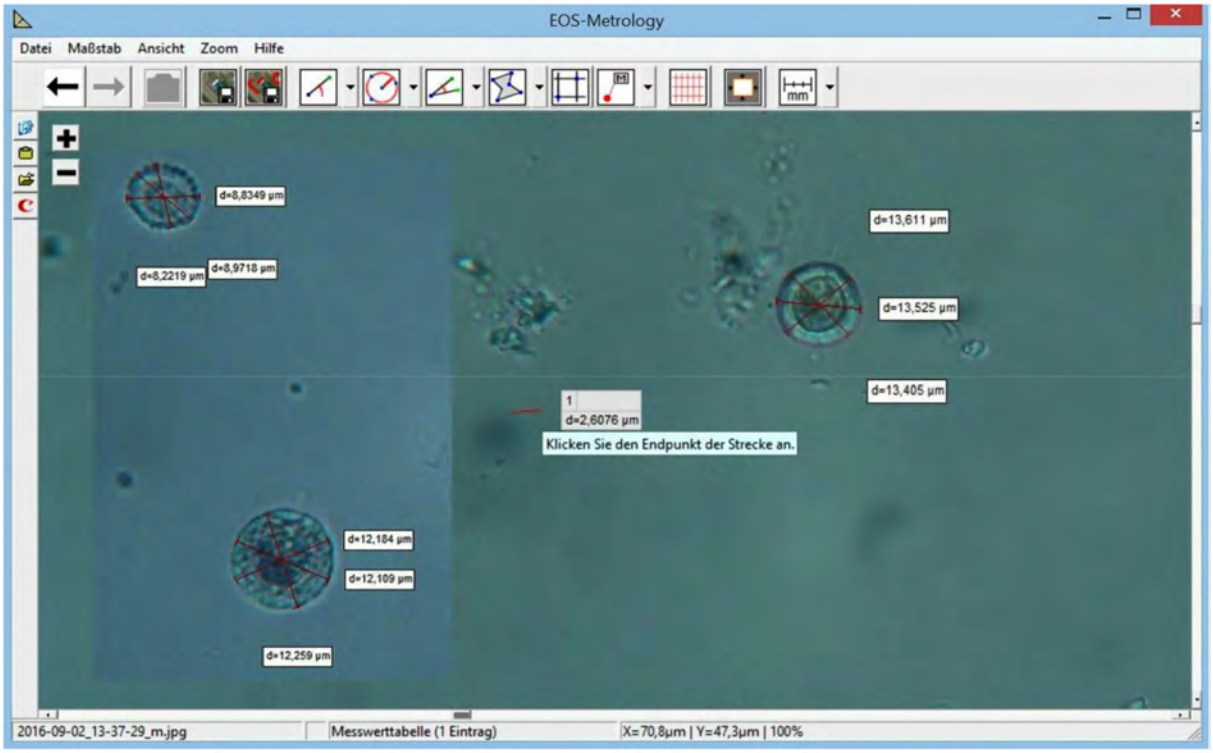

Fig. 1.9: Measurement of the *Phacotus* shells to determine the mean diameter.

The *Phacotus* shells used to determine the exact mass of a *Phacotus* housing originated from Lake Altmuehlsee, Lake Grosser Ostersee, Lake Hopfensee and Lake Igelsbachsee. In the period from June to August 2016 plankton samples were taken from the epilimnion (0–7 m depth). Microscopic plankton analysis was carried out under an inverted, 50 *Phacotus* shells of each lake were photographed at a magnification of 400×. The EOS-Metrology software was used to determine the mean diameter and the standard deviation (SD) from the photos. For this purpose, three measurements of the diameter were carried out on each of the 150 shells per lake (Fig. 1.9).

For a precise volume measurement, detailed investigations of the internal and external morphology of single shells were conducted with a Focused Ion Beam (FIB) technique using a FEI Helios NanoLab G3 UC (Hillsoro, Oregon, USA). The sample material was enriched via membrane filters, prepared on Si-wafers and coated with carbon and platinum for the investigations of thin layer cross-sections. The following investigations were performed at the FIB workstation of the Swiss Federal Laboratories for Materials Science and Technology (EMPA) in Duebendorf, Switzerland. To determine the volume of the shells, 23 individuals were cut vertically in the middle and one shell horizontally, parallel to its flat side. The detailed procedure is illustrated in Chapter 2.

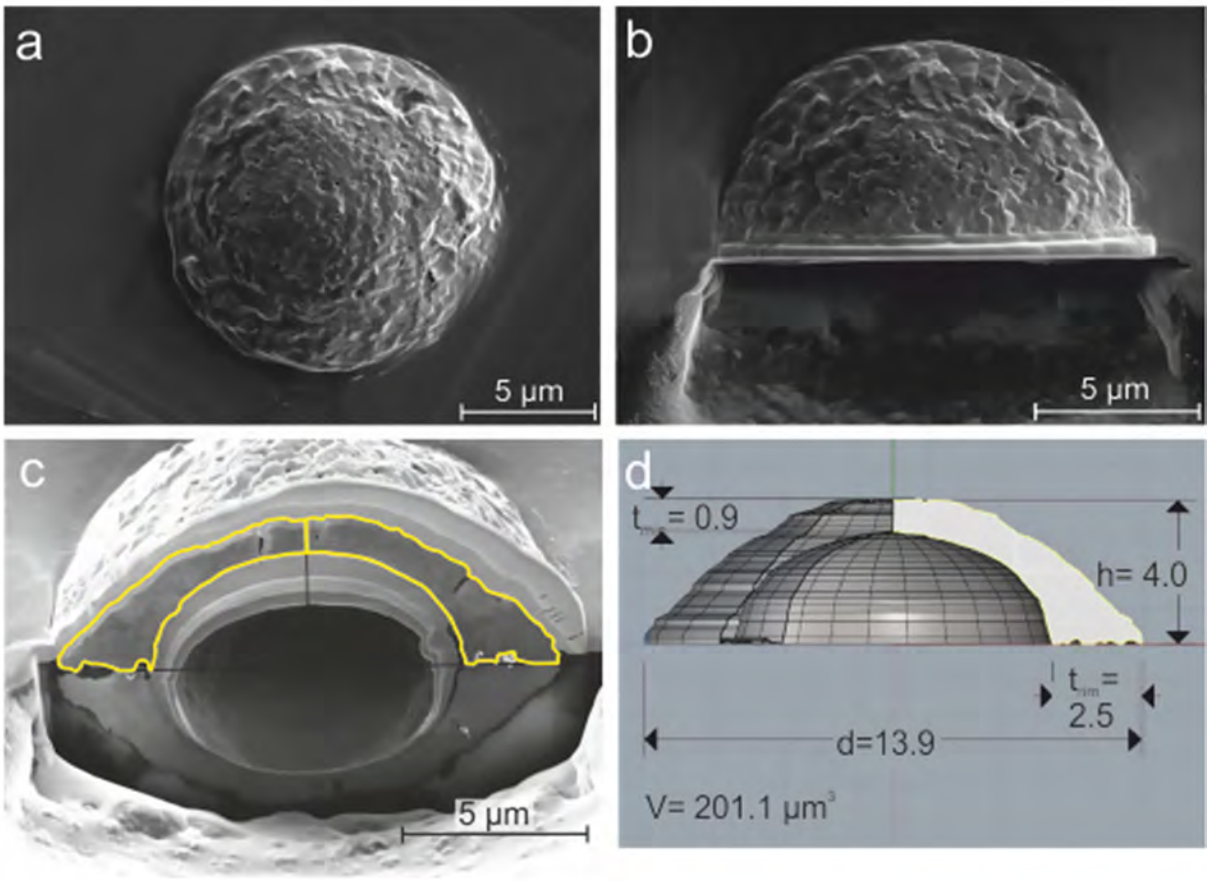

Fig. 1.10: Method for volume determination of a *Phacotus lenticularis* shell, changed according to (Lenz *et al*, 2017): a) scanning electron micrograph of an intact shell with flagella pore at the upper right and several small pores on its surface, plan view prior to FIB milling; b) shell, cut in the middle with narrow Pt-protection layer on cutting edge, plan view; c) shell cross-section with both marked profile lines for creation of d) open 3D-body of rotation with measuring bars in µm for volume determination with Rhinoceros 3D software.

Undamaged individuals that could be positioned favourably for the next step were finally photographed using a scanning electron microscope (Fig. 1.10 a,b). The left and right profiles of each of the cross-sections were drawn (Fig. 1.10c, d). The outcome was 48 profiles of shell halves for cross-section image analysis. For each shell, profiles were converted to surface area and rotated 360° to create a closed-up 3D-body of rotation, from which the volume (μm^3) was determined by using the CAD software Rhinoceros (McNeel, 2013).

Not the entire volume of the *Phacotus* shell consists of carbonate. The biogenic crystallized calcite of the *Phacotus* shell is porous. There are tiny cavities and pores which are not calcified (Fig. 1.11). On a *Phacotus lenticularis* shell from Lake Grosser Ostersee from 4 m depth of the water column sampled in June 2015, the different types of pores and cavities were examined and displayed. At the cut of the contact zone at the shell margin, tabular rhombohedral calcite crystals showed a step-like cut edge where micro pores as well as a pore-canal (black arrow) were exposed in the massive calcite shell (Fig. 1.11a). At a vertical cut that was performed parallel to the shell rim, tabular rhombohedral cal-

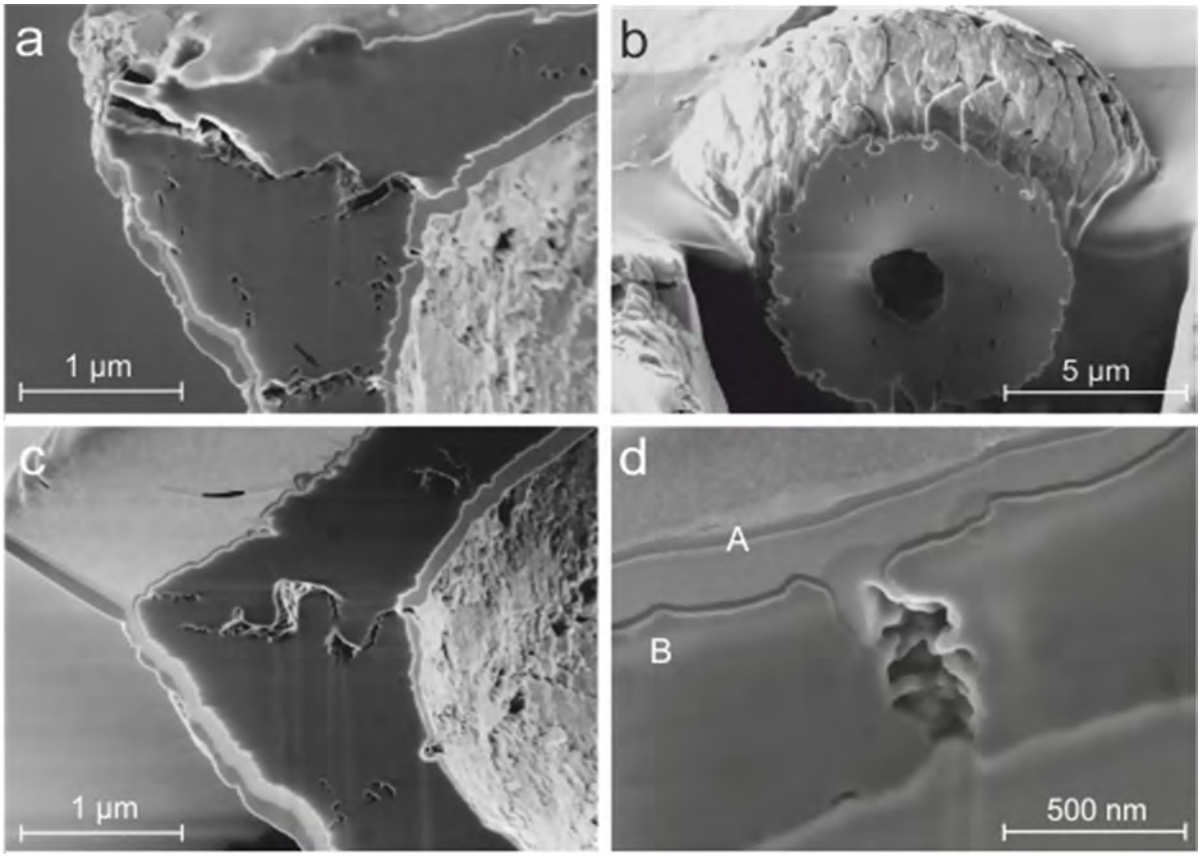

Fig. 1.11: Internal pores and cavities on the shell cross-sections, changed according to (Lenz *et al*, 2017): a) Close-up scanning electron micrograph of the contact zone at the shell margin; b) imaging face in upper shell zone; c) contact zone on the shell rim; d) shell cross-section in the central zone, pore-canal that intersects the complete shell from inside to outside, Pt-protection coating (A) on the dark-grey shell calcite (B).

cite crystals were visible on the outer most two rings of the shell margin. The exposed cut surface revealed very massive inner calcite that is interspersed only with few, small pores that are positioned in a spiral-like way along the rings of calcite crystals (Fig. 1.11b).

At the cut of the contact zone photographed on the other side of the investigated *Phacotus* shell rim, the calcite crystals are interlocked and the cavity between the two shells can be prosecuted deeper inside the shell (Fig. 1.11c). A further type of cavity was investigated at the shell cross section in the central zone of the *Phacotus* shell where pore-canals intersect the complete shell from inside to outside (Fig. 1.11d). Considering these detected cavities in the carbonate, the total porosity was be estimated in order to determine the lime content of a *Phacotus* shell.

For the estimation of total porosity Φ (%), image analysis was conducted on 15 consecutive cross-section micrographs of two *Phacotus* individuals with the image analysis software FiJi ImageJ (Rasband, 2016). The total porosity was determined in terms of percentage of open pore area per unit shell area on the plane shell cut faces. Scale errors, we eliminated by employing different sized analysis windows (0.8×0.8 μm and

2.3×0.7 μm) and repeated the measurement on at least five micrographs in series, of which each represented a 0.04 μm deeper cut face of the investigated shell.

The shell density ρ (ng μm^{-3}) was calculated according to (eq. 1.3), using $\rho_0 = 0.0027$ ng μm^{-3} (pure calcite crystal without pores), and the $CaCO_3$ mass m [ng] of each individual *Phacotus* shell using the determined volume V according to (eq. 1.4).

$$\rho = \rho_0(1 - \Phi) \tag{1.3}$$

$$m = V \cdot \rho \tag{1.4}$$

With the lake-specific mass of the *Phacotus* shells from Lake Grosser Ostersee the amount of fixated carbonate (Chapter 5.3.1) could be estimated using a sum curve (Fig. 1.12). The sum curve was based on sedimentation rates that were calculated over the monitoring period from June to October 2015 ($n = 14$). The basic assumption was that all shells formed in the above water column are deposited in the sediment trap. Only the shells that would actually sediment to the bottom of the lake were taken into account. These measured values were extrapolated for a period of 144 days (June to October). The calculation of the *Phacotus* calcite mass m_{cc} was performed according to (eq. 1.5).

$$m_{CC} = \sum_{144}^{i} \left(S_i \times A_{GOS} \times m_S \right) \tag{1.5}$$

The sedimentation rate used was between 10,000 shells (sh) $m^{-2}d^{-1}$ and 70,000,000 sh $m^{-2}d^{-1}$ with an average value of 15,072,482 sh $m^{-2}d^{-1}$.

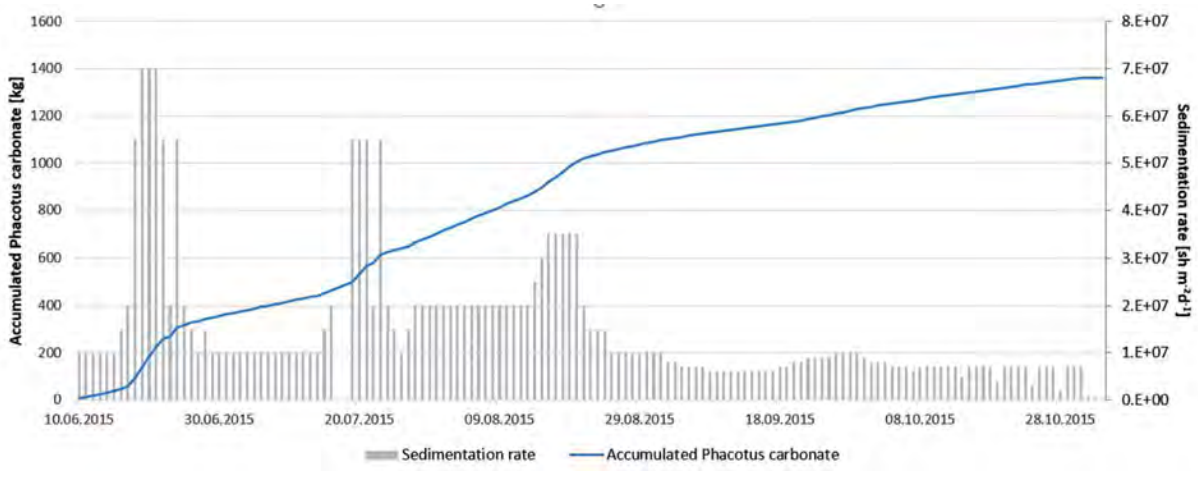

Fig. 1.12: Sum curve of the estimated amount of fixated *Phacotus* carbonate in Lake Grosser Ostersee deduced from sedimentation rates.

For another calculation of the amount of fixated *Phacotus* carbonate in GOS using the TOC-Content of dated sediment core GOSED06, (eq. 1.6) was used.

$$\text{Shell concentration per area lake sediment} \times \text{lake area} \times \text{shell mass} \tag{1.6}$$

1.5.4 Sediment core analysis

The 32 cm long sediment gravity core GOSED06 was divided longitudinally into two equally sized halves for examination. The first half was photographed and described sedimentologically and the second half was divided into exactly 1 cm long sections using a cutting device specially designed for this purpose (Fig. 1.13). The isolated sediment sections were slowly dewatered by freeze-drying and then carefully homogenised. Water

Fig. 1.13: Sediment core division of GOSED06: half of the sediment core was portioned with cutting plates in 1 cm samples.

content and sediment density were determined before and after the drying by weighing. The obtained sediment material was used (1) for the determination of the calcium carbonate content of the sediment, (2) for the determination of the calcium carbonate content of the *Phacotus* shells fraction of the sediment, (3) for ^{210}Pb and ^{137}Cs activity measurements for dating of the sediment layers, and for (4) element analysis to determine the carbon fractions Total Carbon (TC), Total Organic Carbon (TOC) and Total Inorganic Carbon (TIC).

For microscopic analysis, 50 μl of a suspension of the sediment material with a defined sediment concentration were applied to a cover glass with a diameter of 10 mm and embedded in Naphrax. Care was taken to ensure that (1) the pH value of the suspension was alkaline in order to avoid dissolution of calcium carbonate, and (2) the sediment concentration was between 0.25 and a maximum of 0.30 mg L^{-1}, so that microscopic evaluation was not hampered by material overlaps. The preparations were analysed at a suitable magnification (200× or 400×) on a research microscope with polarization filter (DM RBE, Leica Miosystems, Wetzlar, Germany) with regard to the number of *Phacotus* shells. So the resulting slides had a defined amount of sediment on the cover glass, so that the total number of *P. lenticularis* on the entire cover glass N_{shells} (CG) could

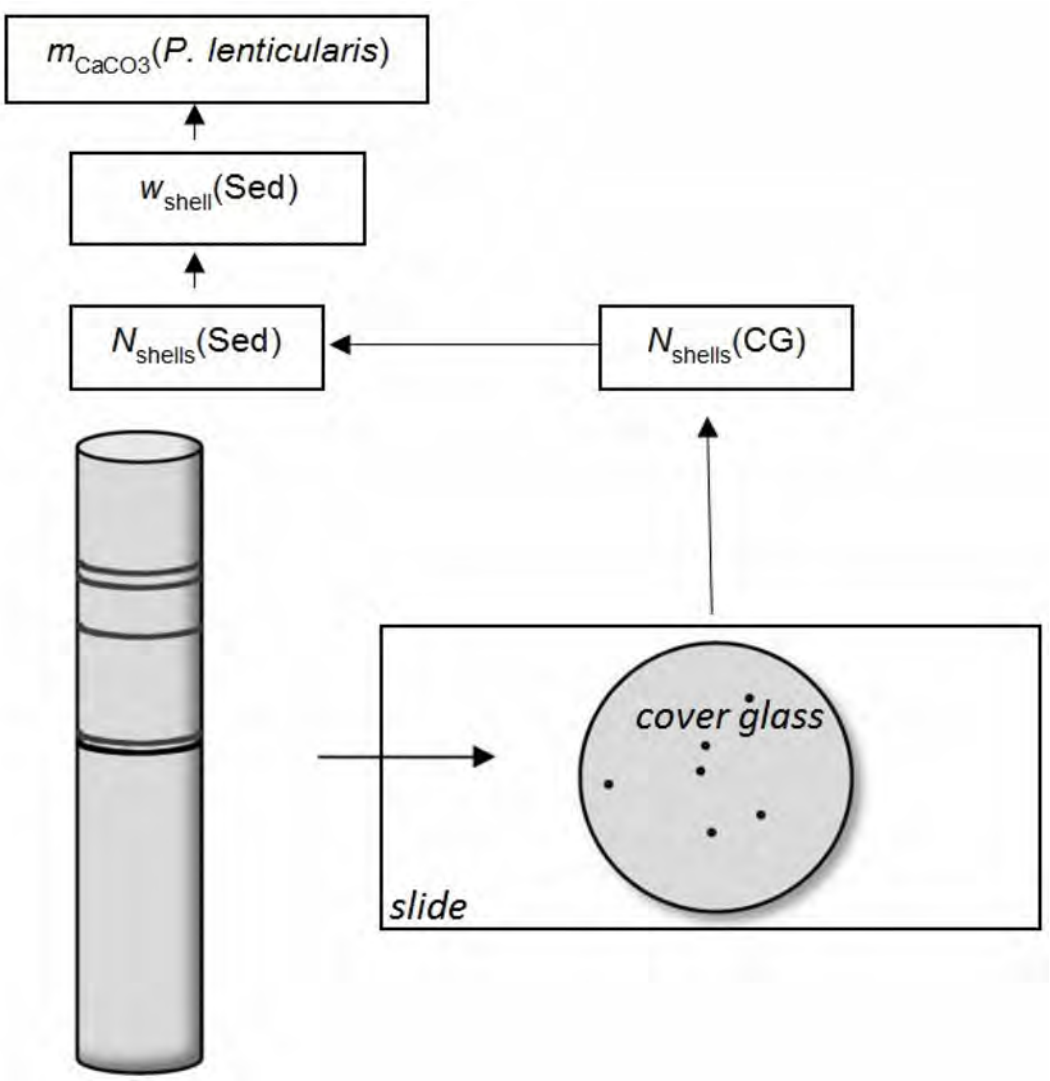

Fig. 1.14: Determination of the contribution of *P. lenticularis* to sedimentary CO_2 fixation from the sediment core via microscopic sediment analysis of cover glases.

be accurately determined. Flowingly, the number of sedimented *Phacotus* shells N_{shells} (Sed) was calculated in relation to the total carbonate sediment mass per cover glass. Consequently the mass fraction of *P. lenticularis* shells w_{shells} (Sed) as well as the fixed $CaCO_3$ mass m_{CaCO_3} (*P. lenticularis*) could be determined (Fig. 1.14). The individual mean *Phacotus* shell mass was used according to Chapter 2.

In the samples, intact *Phacotus* individuals were extremely difficult to distinguish. Therefore, the restriction had to be made to classify all individuals as half shells (individual Loricae). The data provide a conservative approach applying minimum results that guarantee that analysis do not overestimate the actual amount of *Phacotus* shells in the sediment. Dating of the sediment core

The dating of sediment core GOSED06 was conducted employing a Canberra Germanium Well Detector (San Ramon, California, USA) at the Swiss Federal Institute of Aquatic Science and Technology (EAWAG) in Dübendorf, Switzerland, at the department surface waters. A total of 32 samples were tested for ^{210}Pb and ^{137}Cs activity. The ^{210}Pb

and ^{137}Cs dating method was used for 1 cm intervals (Appleby, 2001; McGowan *et al*, 2015). To determine the sedimentation rate by ^{10}Pb, the ^{226}Ra activity was subtracted from its values.

Grain size analysis was realized unsing a Malvern Mastersizer 2000 (Malvern, UK) which uses a laser beam that gets diffracted by the sediment particles. A total of 32 wet unprocessed sediment samples from the sediment core GOSED05 were analyzed. Samples were disaggregated using Na-Pyrophosphate and ultra sound. Then particle size analysis was conducted in a range of 0.02 to 2000 μm. For Capter 4 the obtained information was displayed as the volume weighted mean and the relative content in clay (% of particles between 0.01–2 μm) silt (% of particles between 2–63 μm) and sand (% of particles between 63–2000 μm) were displayed in a graph. The sediment core GOSED05 was obtained at the same location and date as GOSED06. It was also stored and divided in the exact same manner. The comparability was ensured by cross-correlation of the two cores based on the lithological description.

The different carbon fractions were determined as further paleolimnological parameter of the dried sediment material of GOSED06 and the surface sediments from GOS, ALT, IGL and HOP. To quantify the total carbon in the sediment samples, an element analyser Hekatech Euro EA 3000 (Wegberg, Germany) was used. A total of 40 samples were analyzed. The contained total amount of C was calculated in reference to a CHNS standard, with the Callidus analysis (Dublin, UK) software.

The amount of inorganic carbon (TIC, Total Inorganic Carbon) using a titration coulometer UIC CM5015 (Joliet, Illinois, USA). The difference between TC and TIC was used to calculate the fraction of organic carbon (TOC). From the TIC the content of precipitated calcium carbonate or bound carbon dioxide could be calculated according to equation (eq. 1.7).

$$\mathrm{CaCO_3} = \mathrm{TIC} \cdot \frac{\mathrm{M_{CaCO_3}}}{\mathrm{M_C}} = \mathrm{TIC} \cdot 8.33 \tag{1.7}$$

2 | Calcite production by the calcifying green alga *Phacotus lenticularis*

A similar version of this chapter was published: Lenz S, Gruenert U, Geist J, Stiefel M, Lentz M, Raeder U (2017) Calcite production by the calcifying green alga *Phacotus lenticularis*. Journal of Limnology.

2.1 Abstract

The importance of carbonate precipitation by phytoplankton in fresh water lakes has not been sufficiently considered in global carbon cycles and climate change scenarios. The objective of this study was to determine the influence of the calcifying bivalved phytoflagellate *Phacotus lenticularis* (Ehrenberg) Deising 1866 on the total calcite precipitation in five European hard-water lakes. For this purpose, an accurate mass determination of single *Phacotus lenticularis* shells was required. We developed a novel methodological approach to precisely determine the volume and mass of the calcified shells. Focused ion beam (FIB) techniques were employed to investigate internal structural features. Thin layer cross-sections of the shell profiles were reproduced and perforation as well as the crystalline structure of the calcite plates were monitored. 3D-shell models were computed by 360° rotation of the shell cross-sections using a CAD 3D imaging software to calculate precise volumes and estimate realistic masses. In contrast to previous estimates, we determined a 2.8-fold higher shell mass of 0.86 ng $CaCO_3$ (standard deviation $SD = 0.18$) for the highly massive shells at a mean volume per individual of 334.1 μm^3 (SD = 70). An initial shell porosity of less than 5 % was derived from thin layer cross-section images, resulting in a presumed mean shell density of 0.0026 ng μm^{-3}. The shell diameter was significantly influenced by the lake's origin. The shells from each lake displayed substantial variations in diameter and shape. The pores in the shells showed two variations. Wider pore canals penetrated the whole shell wall, whereas small, elongated pores were located along the interspaces between calcite crystals with tabular habit. The approximate average dimensions of these calcite plates were $1.0 \times 1.6 \times 0.2\,\mu m$. The mean lateral wall thickness at the rim and centre of the shell were 1.98 μm ($SD = 0.42$) and 0.79 μm ($SD = 0.17$), respectively. The average carbonate precipitation by *Phacotus lenticularis* in relation to the total epilimnetic suspended calcite precipitation was 6.0 % in the oligotrophic Lake Grosser Ostersee (Bavaria, Germany). During the growing season, *Phacotus lenticularis* contributed up to 21 % of the particulate calcium carbonate in the epilimnion. These findings suggest that *Phacotus lenticularis* should be considered in the assessment of hard-water lake carbon cycling.

2.2 Author contributions

Sebastian Lenz (SL) was part of the team conceiving the study. The development of methods was carried out by him. Carbonate measurements were done at the Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Stechlin, Germany by SL. Processing and interpretation of data were realized by SL. The manuscript was drafted and improved by SL.

2.3 Introduction

The role of inland aquatic systems in C-cycling is poorly understood. In addition to the terrestrial C-input into aquatic systems (Cole *et al*, 2007), there can also be substantial C-fixation by primary producers within aquatic systems. Carbonate precipitation induced by planktonic algae is a process that has been widely investigated in marine environments. However, it also occurs in freshwater ecosystems, but there is less information available about its importance in these ecosystems. The calcifying freshwater flagellate *Phacotus lenticularis* (Ehrenberg) Deising 1866 (Chlorophyceae, Chlamydomonadales, Phacotaceae) is abundant in temperate hard-water lakes worldwide. These unicellular green algae have been reported to incorporate remarkable amounts of $CaCO_3$ in their shells (Giering *et al*, 1990b; Koschel *et al*, 1987a; Koschel and Raidt, 1988a; Pocratsky, 1982; Sieminska, 1952; Sladecek, Cyrus and Borovickova, 1958; Steinberg and Klee, 1983), consisting of 98 % – 99 % pure $CaCO_3$ (Schlegel *et al*, 1998). It is assumed that it temporally contributes up to 100 % of the autochthonous calcite precipitation during mass development (Koschel and Raidt, 1988a). Consequently this species brings about high autochthonous $CaCO_3$ inputs in these lakes and plays a definite role in limnic carbonate sedimentation as a sediment contributor (Koschel *et al*, 1987a; G. Müller and Oti, 1981).

Previous works have described the external and structural features of *Phacotus* shells (often named *lorica*) in great detail. The mineralised external housing has a simple radially symmetric morphology and is formed by rhombohedral calcite crystals that are arranged in several rings around a central rotational axis (Kamptner, 1950; Koschel and Raidt, 1988a). However, several important aspects remain controversial. For instance, it is unknown if the interspaces between the rhombohedral crystals are filled with calcite or remain empty. Also, internal orientation and extensions of the crystals have not been described so far. Moreover, there have been no explicit measurements of their porosity. *Phacotus* shells are likely to vary in morphotype and surface porosity over time and location (Schlegel, Koschel and Krienitz, 2000). Variations in shell diameter between 6–17 μm have been reported (Giering *et al*, 1990b; Koschel and Raidt, 1988a; Pocratsky, 1982; Schlegel *et al*, 1998; Schlegel, Koschel and Krienitz, 2000; Steinberg and Klee, 1983). However, none of these works systematically determined the precise amount of calcite within a shell. Furthermore, there has been no explicit assessment of the individual variations of the general dimensions, nor calculations of the volume of *Phacotus* shells from different lakes.

A single provisional estimation based on conductometry has been reported by Koschel *et al* (1987b), which suggested a shell mass of 0.3 ng $CaCO_3$ per individual shell. This result was derived from a particularly suitable *Phacotus* mass development in which the shells accounted for 100 % of autochthonous calcite. To the best of our knowledge, this value has not yet been verified, and a repetition of the measurement seems difficult because (1) it is exceptionally rare that mass developments account for 100 % of autochthonous calcite precipitation, and (2) in all other cases mass determination of exclusively *Phacotus* shells is generally impeded by co-precipitated calcite crystals.

In our study, we built the basis for a quantitative determination of the potential role of *Phacotus lenticularis* in carbonate precipitation in lakes. Our methodical approach revealed new insights on the internal ultrastructure of the *Phacotus* shell and enabled us to verify and estimate the porosity of the material. We measured the relevant external shell parameters, calculated the precise shell volumes, and estimated the realistic shell masses of 24 individual shells from five different lakes. We investigated if there is a variation of the *Phacotus* diameter in lakes with different trophic states and during the growing season. Finally, we determined the amount of *Phacotus* calcite in relation to the total calcite precipitation in the epilimnetic layer of the oligotrophic Lake Grosser Ostersee (Bavaria, Germany) and addressed the question of whether *Phacotus lenticularis* significantly affects the $CaCO_3$ budget of this lake.

2.4 Methods

2.4.1 Study sites

Phacotus shells for mass determination were derived from five lakes in southern Bavaria, Germany: Lake Abtsdorfer See (ABS), Lake Altmuehlsee (ALT), Lake Grosser Ostersee (GOS), Lake Hopfensee (HOP) and Lake Igelsbachsee (IGS). The lakes differed in surface area, maximum depth, and trophic state (Tab. 2.1), but held significant *Phacotus lenticularis* occurrences with cell densities of more than 1×10^5 individuals per litre (Ind. L^{-1}). Intensive investigations on suspended carbonate and *Phacotus* population dynamics in Lake Grosser Ostersee were conducted. Lake Grosser Ostersee was fed by alkaline groundwater with a catchment area comprising 35 km^2 (Gruenert and Raeder, 2014b).

2.4.2 Sampling

Samples for volumetric shell measurements were taken in June and August 2016 from the epilimnetic layer (0–7 m depth) at the deepest point of each lake. Integrated water samples were taken with a PE sampling tube (7 m length and 4.5 cm diameter) with a 2.5 kg weight at the bottom end. The tube was slowly let down vertically into the water. Its content was filled into a 6 L canister and homogenised by shaking prior to further processing. Samples for the measurements of the *Phacotus* shell abundance and total epilimnetic particulate calcite were taken between 0–5 m depth to ensure comparability with previous works. Sampling was carried out weekly at the deepest point of each lake during the *Phacotus* growing season from June to October 2015. These samples were

Table 2.1. Characteristics of selected hard water lakes and geographic sampling positions in Bavaria, Germany.

	Gr. Ostersee	Abtsdorfersee	Igelsbachsee	Hopfensee	Altmuhlsee
Trophic state	oligotrophic	mesotrophic	mesotrophic	eutrophic	hypertrophic
Circulation type	dimictic	dimictic	polymictic	dimictic	polymictic
Surface area (ha)	118	78	72	185	450
Max. depth (m)	29.7	20.0	11.5	10.4	2.2
Coordinates	47°47′25, 2″	47°54′35, 4″	49°08′45, 3″	47°36′06, 1″	49°08′35, 8″
(WGS 84)	011°18′06, 5″	012°54′22, 4″	010°54′13, 3″	010°40′46, 6″	010°43′34, 4″
Physical, chemical average values of the surface layer (0-7 m) during June till August 2016, SD in brackets					
Water temp. (°C)	17.4 (4.7)	15.5 (5.2)	20.9 (2.7)	17.3 (2.8)	22.1 (1.6)
pH value	8.2 (0.2)	7.9 (0.4)	8.6 (0.5)	8.2 (0.4)	9.0(0.3)
Ca^{2+}conc. (mg L^{-1})	59.2	78.2	37.5	69.3	47.1
TP (μg L^{-1})	> 8.2	> 17.4	> 25*	> 35**	> 200*
Secci depth (m)	3.3 (0.6)	1.1 (0.2)	3.0 (0.6)	1.1 (0.3)	0.5 (0.2)

TP data from Bavarian Environmental Agency *2014, **2013, Ca^{2+} concentration from last sampling in August

taken with a standard Ruttner water sampler and integrated by mixing aliquots inside a measuring cup. Water temperature, pH depth profiles and concentrations of Ca^{2+} in solution were measured according to Gruenert and Raeder (2014a).

2.4.3 Infrared gas analysis for carbonate determination

Particulate $CaCO_3$ concentrations were measured by infrared (IR) CO_2 analysis. We used a revised method with a modified measurement setup (Fig. 2.1) based on the former conductometric CO_2 analysis reported by Proft (1983; 1984). This enabled a more precise determination of the suspended calcite in lake water samples.

Each lake water sample (200 mL) was filtrated through 0.45 μm cellulose-nitrate filters. The filters were oven dried overnight at 50°C and stored in a desiccator before introducing them with tweezers into a reaction vessel containing 10 % HCl solution. A carrier gas was streamed through the reaction vessel and absorbed the emerging CO_2 released by carbonate dissolution. Prior to CO_2 gas analysis with a Saxon Junkalor GmbH (Dessau, Germany) Infralyt 50 gas analyser, the carrier gas was passed through Cu flakes to remove any HCl and dried by passing it through granulated $CaCl_2$. The carrier gas was air from the lab, which was pumped with a constant gas flow of 33 mL s^{-1} through NaOH pellets before use to remove the atmospheric CO_2.

The system was calibrated for a measuring range between 0.005–0.05 [mg C L^{-1}] with an IC-standard solution (containing $NaHCO_3$ and Na_2CO_3). The Infralyt 50 measured the CO_2 content displayed as ppm CO_2 and the LC-Net-Box transformed the analogue signal to a digital signal for processing with the Jasco (Easton, Maryland, USA) Borwin 1.5

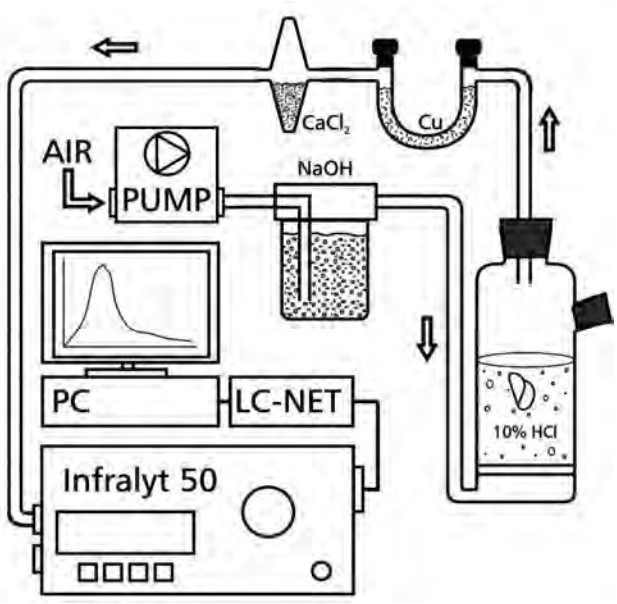

Fig. 2.1: Schematic representation of the carbonate infrared gas analysis measurement setup: 1. membrane air pump; 2. NaOH pellets for carrier gas purification; 3. reaction vessel with 10 % HCl and sample filter; 4. HCl removal by Cu-flakes; 5. gas drying by granulated $CaCl_2$; 6. Saxon Junkalor GmbH Infralyt 50 gas analyser; 7. LC-Net-Box for signal transformation; 8. data acquisition with Borwin 1.5 Software.

software. The output dimension was given as carbon concentration c_C [mg C L^{-1}]. Before each measurement, the background CO_2 ($\leq$ 1 ppm) was set to zero. Measurement time for one sample was 180 s. The 10 % HCl solution in the reaction vessel was renewed after the measurement of 10 samples. The conversion of c_C to c_{CaCO3} concentration was calculated by multiplication of c_C with the ratio of the molecular weights M_{CaCO3} 100.09 g mol^{-1} and M_C 12.01 g mol^{-1} according to Formula (eq. 3.1). After every 20 measurements, a calibration sample was measured to guarantee accurate results. The system was calibrated after every 60 measurements.

$$c_{CaCO_3}\ [mgL^{-1}] = c_C\ [mgL^{-1}] \times \frac{100.09\ gmol^{-1}}{12.01\ gmol^{-1}} \tag{2.1}$$

2.4.4 Plankton analysis

Plankton samples were stored in 100 mL brown glass bottles and preserved with Lugol's solution. Suspended shell concentrations in the water column were determined with an inverted microscope Leitz Labovert at 200–400× according to Utermöhl (1958). We counted single shells as one, and complete *Phacotus* housings as two shells. The cell density was derived by dividing the sum of the shells by two because a complete *Phacotus* housing consists of two shells. At least 300 individuals were counted per sample to ensure accurate determination of the shell numbers. Additionally, the shell diameter was determined by taking two perpendicular measurements. From each lake, 150 individuals (50 from three sampling events at different stages of population growth in June, July, and August 2016) were measured with the EOS-Metrology software. To test for mean shell

diameter differences between lakes and month of sampling, a two-way-analysis-of-variance (ANOVA) was conducted. In case of significance, Tukey-post-hoc-tests were computed. Statistical analyses were carried out using the open source software R 3.2.2 (R Core Team, 2015).

2.4.5 Focused ion beam processing

Thin layer cross-sections of a *Phacotus* calcite shell were cut with a FEI (Hillsboro, Oregon, USA) Helios NanoLab G3 UC FIB. Prior to milling, samples were prepared on Si wafers and coated with 20 nm carbon using a Leica EM ACE600-Coater. To assure clean cut surfaces, the cutting area in the middle of each shell was sealed with a protective layer of Pt (Fig. 2.2b-d) with a thickness of 1 μm, derived from ion induced deposition. The calcite shell was cut and polished using ion currents from 47 nA to 2.5 nA, at an acceleration voltage (AV) of 30 kV. Scanning electron micrographs were obtained at 1.0 kV AV with a viewing angle of 52° to the ion beam. In total, 23 individuals were cut vertically in the middle and one shell horizontally, parallel to its flat side.

2.4.6 Volume and mass determination

A total number of 24 individuals from five lakes were FIB milled to obtain a site-specific view of the shell morphology and structure. On the shell cross-section images, profile lines along the outer margin of the shell were marked. Two profiles were drawn on each shell cross-section (Fig. 2.2d), resulting in 48 profiles of shell halves for cross-section image analysis. On these profiles, the following characteristic parameters (Fig. 2.2e) were measured (precision as relative error): minimal shell thickness t_{min} (11.1 %), rim width t_{Rim} (2.0 %), radius r (0.6 %), diameter d and height h (1.4 %). For each shell, profiles were converted to surface area and rotated 360° to create a closed up 3D-body of rotation from which the volume (μm^3) was determined by using the CAD software Rhinoceros (McNeel, 2013).

For the estimation of total porosity Φ (%), image analysis was conducted on 15 consecutive cross-section micrographs of two *Phacotus* individuals with the image analysis software FiJi ImageJ (Rasband, 2016). The total porosity was determined in terms of percentage of open pore area per unit shell area on the plane shell cut faces. To eliminate scale errors, we analysed with different sized analysis windows ($0.8 \times 0.8\,\mu$m and $2.3 \times 0.7\,\mu$m) and repeated the measurement on at least five micrographs in series, of which each represented a 0.04 μm deeper cut face of the investigated shell. Subsequently, the shell density ρ (ng μm^{-3}) was calculated according to Formula (eq. 2.2) with a density of $\rho_0 = 0.0027$ ng μm^{-3} for pure calcite.

$$\rho = \rho_0(1 - \Phi) \tag{2.2}$$

The mass m of $CaCO_3$ (ng) deposited in each individual *Phacotus* shell was calculated according to Fomula eqrefe23 by multiplying the volume of the 3D-body of rotation with ρ.

$$m = V \cdot \rho \tag{2.3}$$

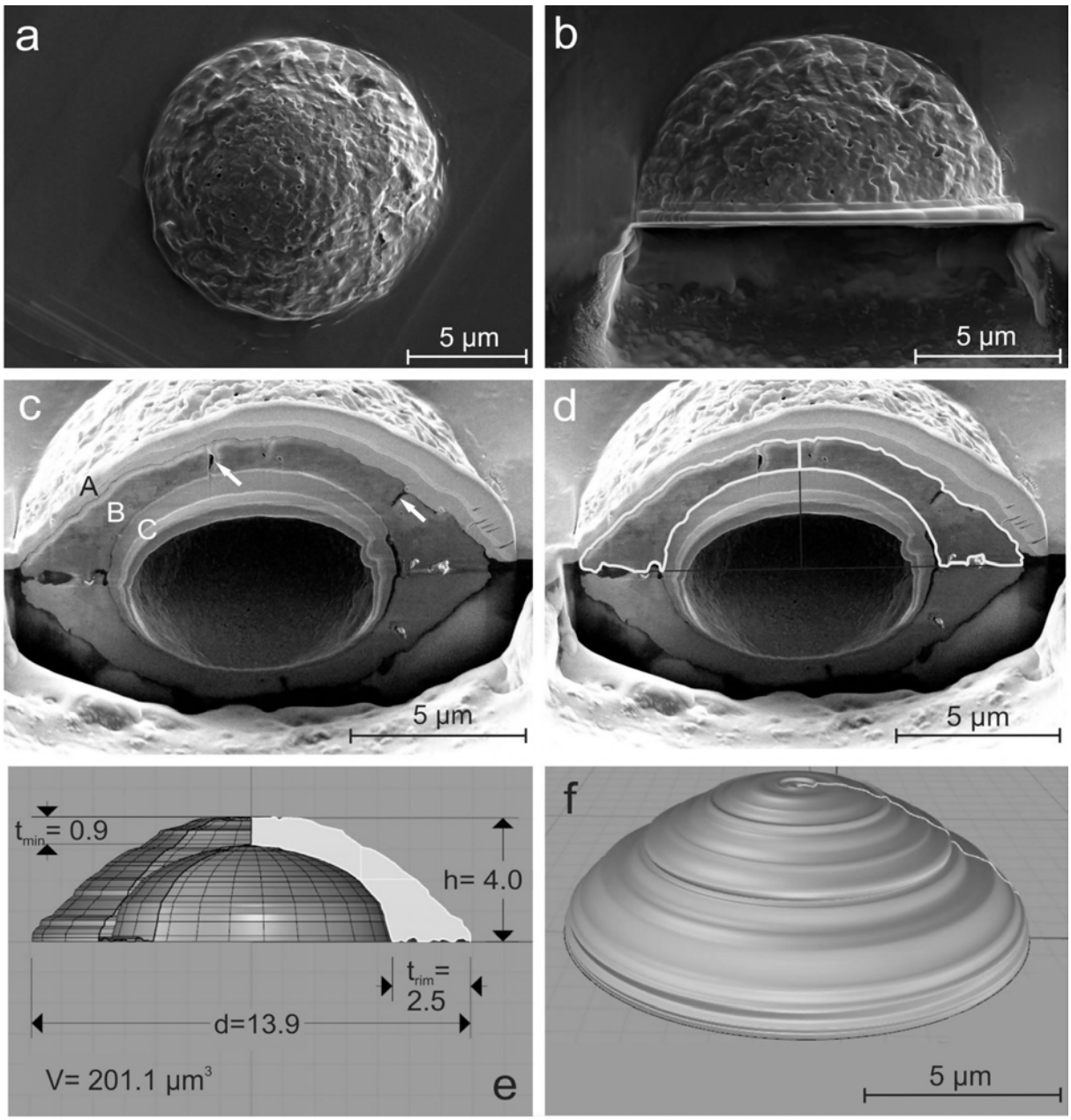

Fig. 2.2: Method for volume determination, scanning electron micrographs of a *Phacotus lenticularis* shell from Lake Grosser Ostersee, 4 m depth, June 2015: a) intact shell with flagella pore at the upper right and several small pores on its surface, plan view prior to FIB milling; b) shell, cut in the middle with narrow Pt-protection layer on cutting edge, plan view; c) imaging face of shell cross-section with two exposed pore canals (white arrows), light grey Pt coating (A) over dark grey shell material (B), layers of cutting debris, accumulated inside the shell during cutting process (C), side view; d) shell cross-section with both marked profile lines for creation of a 3D-body, side view; e) open 3D-body of rotation with profile of the cross-section and measuring bars, dimension are in μm, side view perpendicular to profile; f) closed 3D-body for volume determination with Rhinoceros 3D software.

2.5 Results

2.5.1 Calculation of shell mass

Our measurements of 24 individuals from 5 lakes led to a mean volume of 334.1 μm^3 (standard deviation SD = 70) for a complete *Phacotus* housing with a 13.9 μm diameter (consisting of two shells). Our data showed that shells with the same diameter had a variation in volume up to a factor 1.8. The calculated mean shell mass for a complete specimen (comprising two valves) was 0.86 ng ($SD = 0.18$). The mean shell mass ranged from 1.03 ($SD = 0.11$) ng in Lake Grosser Ostersee to 0.66 ($SD = 0.10$) ng in Lake Igelsbachsee (Tab. 2.2).

Table 2.2. General dimensions, volumes and calculated masses of *Phacotus* shells

	Gr. Ostersee	Abtsdorfersee	Igelsbachsee	Hopfensee	Altmiihlsee
Mass$^+$ (ng)	0.52 (0.06)	0.43 (0.09)	0.33 (0.05)	0.40 (0.06)	0.48 (0.05)
Volume (μm^3)	201.7 (32.6)	167.0 (36.9)	128.4 (21.4)	155.4 (22.8)	186.7 (21.5)
Diameter (μm)	13.6 (0.9)	14.0 (1.2)	12.5 (0.5)	15.0 (1.2)	15.0 (0.4)
Rim width (μm)	2.4 (0.4)	2.1 (0.4)	1.8 (0.2)	1.6 (0.3)	2.0 (0.3)
Thickness (μm)	1.0 (0.1)	0.7 (0.2)	0.8 (0.2)	0.7 (0.1)	0.8 (0.1)
Height (μm)	4.2 (0.3)	4.5 (0.4)	4.2 (0.1)	4.8 (0.6)	4.8 (0.2)

$^+$For a single shell half with 5 % porosity; 48 shell profiles of 24 individuals were measured

2.5.2 Estimation of porosity

Based on image analysis of the shell cut faces of the cross-section micrographs, highly massive calcite shells were identified (data not shown). We decided to calculate the material density according to Formula 2 using total porosity derived from image analysis. The cut faces appeared even and showed only few cut pores. We analysed both a relatively porous and a highly massive appearing shell. The resulting estimated porosities were 2.9 % for the porous and 2.2 % for the massive shell. The smallest analysed pores had a pore-throat size of 50 nm. Nevertheless, close-up scanning electron micrographs also showed mesopores with diameters smaller than 50 nm (Fig. 2.4a). These pores certainly contributed to an increased inner surface area but might not contribute to a significantly higher porosity. However, for further calculations, we assumed a total material porosity of $\Phi = 5\,\%$ to take this fact into account. According to Formula (eq. 2.2), the shell density was $\rho = 0.0026$ ng μm^{-3}.

2.5.3 Variability of general dimensions on shell cross-sections

The shell diameter (d) was significantly influenced by the lake's origin ($p = 0.007$), but the month of sampling had no significant influence ($p = 0.190$). The investigated shells in 2016

had a mean diameter of 13.8 μm for Lake Abtsdorfersee, 13.1 μm for Lake Altmuehlsee, 12.7 μm for Lake Grosser Ostersee, 14.0 μm for Lake Hopfensee, and 12.0 μm for Lake Igelsbachsee (Fig. 2.3). The biggest shell was found in Lake Abtsdorfersee ($d = 21.9\,\mu$m), the smallest shell in Lake Igelsbachsee ($d = 8.3\,\mu$m).

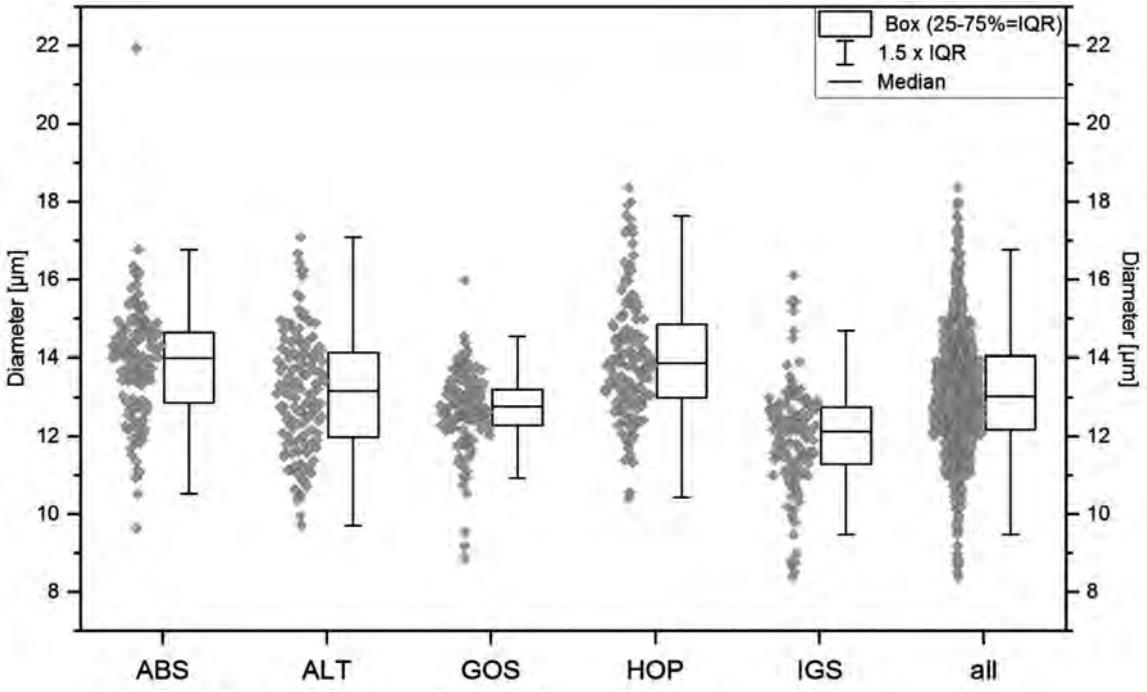

Fig. 2.3: Variation of *Phacotus* shell diameters measured during cell counting 2016: total n was 150 for each lake; 50 from June, July and August each. Data points as grey squares; summary as box including first to third quartile (25–75 % = interquartile range IQR), whiskers describe 1.5× IQR (12.5–87.5 %). The line represents the median. Abbreviations as indicated in methods section.

The shell size parameter height (h) varied significantly between the lakes. The shell thickness (t_{min}) and rim width (t_{Rim}) showed significant variation only for the shells from Lake Grosser Ostersee (Tab. 2.2). The h/d ratios of the *Phacotus* shells from each lake remained constant (0.32; $SD = 0.02$). The characteristic appearance of the shells from different lakes might be better reflected by the ratio of the shell thickness to its diameter. Shells from two lakes showed remarkable characteristics: The shells from Lake Hopfensee had a filigree shell architecture with a low t_{min}/d ratio of 0.045 ($SD = 0.010$), whereas shells from Lake Grosser Ostersee showed a massive architecture with a high t_{min}/d ratio of 0.075 ($SD = 0.011$). In contrast, the t_{Rim}/d ratio was not a good descriptive value because it was dominated by the variation in the shell diameter.

2.5.4 Observations of the shell ultrastructure

The analyses of calcite shell cross-sections from SEM images confirmed that the internal structure consisted of closely stacked tabular calcite crystals. They had approximate dimensions of $1.0 \times 1.6 \times 0.2$ μm. Most of the interspaces were predominately cemented with super-fine-grained calcite lacking a specific crystal structure. Some interspaces were

unfilled and formed cylindrical elongated pores that did not penetrate the entire shell. Most pores were not interlinked and ceased before reaching the outer layer. In the inner most shell areas, small pores were pitched in a circular-spiral arrangement along the smaller lateral edge of the tabular crystals (Fig. 2.2a and Fig. 2.4b). The majority of pores that could be seen at the shell surface were from this type and had an average diameter of 0.11 μm ($SD = 0.03$).

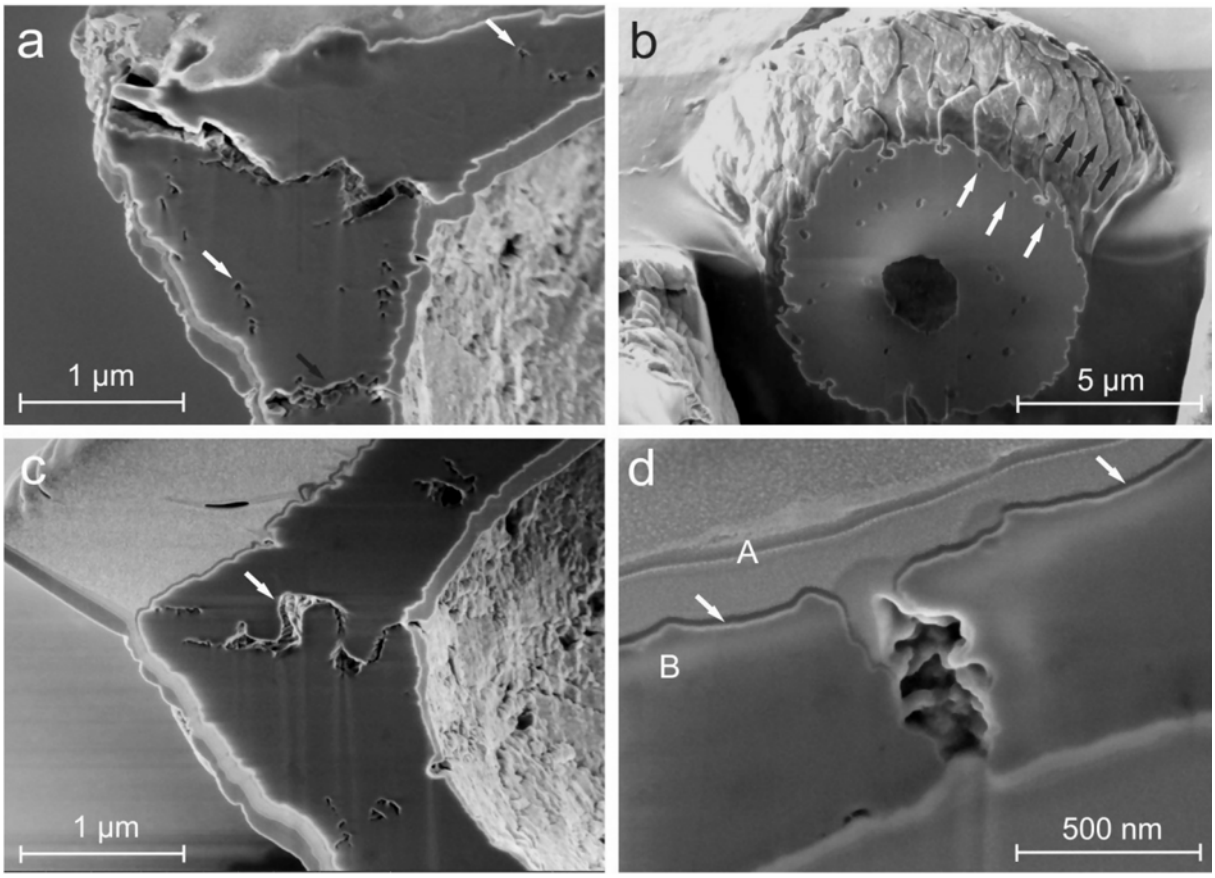

Fig. 2.4: Close-up scanning electron micrographs of internal pores and cavities on the shell cross-sections: a) contact zone at the shell margin, cut tabular rhombohedral calcite crystals show a step-like cut edge, there are micro pores (white arrows) and a pore-canal (black arrow) in the massive calcite shell; b) imaging face in upper shell zone, horizontally cut parallel to contact planes, tabular rhombohedral calcite crystals (black arrows) are visible on the outer most two rings at the shell margin, inner calcite seems to be very massive and interspersed with few, small pores (white arrows), side view; c) contact zone on the shell rim, calcite crystals are interlocked, the cavity between the two shells (white arrow) formed during imaging process, previously filled with an unknown substance volatile in vacuum under an electron beam; d) shell cross-section in the central zone, pore-canal that intersects the complete shell from inside to outside, its cavernous rim appeared during the imaging process and was also filled with the unknown substance, Pt-protection coating (A), dark 20 nm C-sputter-layer (white arrows), actual shell calcite (B).

Another kind of pore, fewer and likely situated in the central shell area, intersected the whole shell (Fig. 2.2c and Fig. 2.4a, d). These pore canals had wider pore-throats

of approximately 0.2 μm and irregular pore walls. According to the behavior during SEM imaging, these pores seemed to be coated or filled with a volatile organic substance that disappeared under the electron beam under vacuum conditions. The space in the contact zone between the two shells (rim interspace) was filled with the same substance and showed tabular crystals almost interlocked with each other (Fig. 2.4c).

2.5.5 *Phacotus* induced calcite precipitation

To quantify the autochthonous calcite precipitation in the epilimnion of Lake Grosser Ostersee, we measured the amount of total calcite in the upper 5 m of the water column. Remarkably high concentrations of total suspended carbonate of over 1.5 mg L^{-1} were reached from mid-July until mid-September, with a maximum of 3.2 mg L^{-1} (Fig. 2.5).

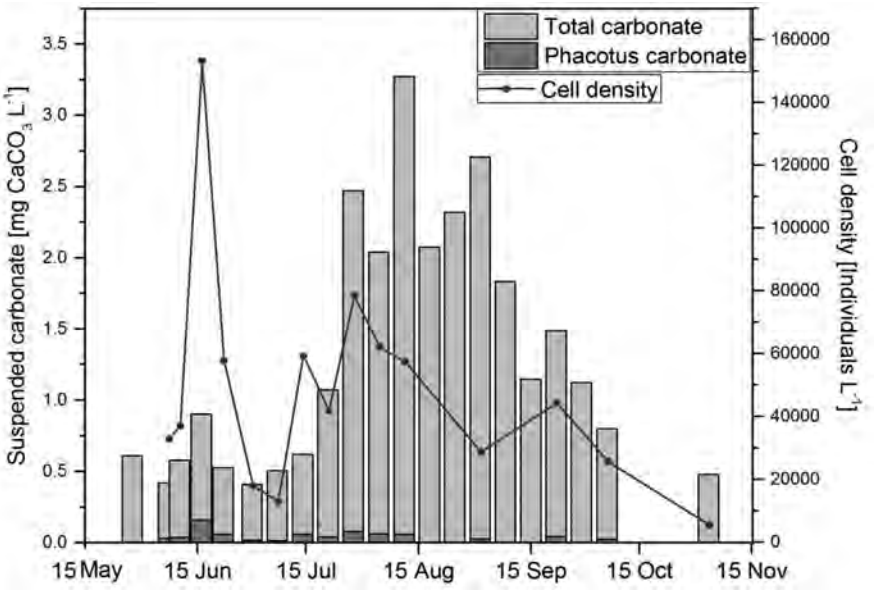

Fig. 2.5: Total particulate carbonate (light bars) and amount of precipitated calcite provided by *Phacotus lenticularis* (dark bars). Total carbonate values were measured by infrared gas-analysis whereas *Phacotus* carbonate values were calculated using an individual's mass of 1.03 ng (two shells). Not all sampling dates have *Phacotus* cell density data (line). All shown data are integrated for 0–5 m of Lake Grosser Ostersee in 2015.

During the peak development of *Phacotus lenticularis*on 16$^{\text{th}}$ June 2015, the maximum cell density reached 477 800 Ind. L^{-1} in 2 m depth. The average value for the epilimnion (0–5 m) was 153 327 Ind. L^{-1}. During this occasion, 21.5 % of precipitated calcite was provided by *Phacotus lenticularis*. This peak was followed by a sudden decrease in cell numbers coinciding with rain- and wind-induced epilimnetic mixing and grazing by zooplankton. Three additional *Phacotus* peaks occurred in early July, in late July, and in mid-September. The average amount of precipitated calcite provided by *Phacotus lenticularis* over the whole monitoring period was 6.0 % in relation to total suspended calcite owing to the high total precipitated calcite concentrations in the epilimnion.

In the epilimnion of Lake Grosser Ostersee the average carbonate concentration per square metre of lake surface was 6.53 g $CaCO_3$ m^{-2} (as average over the whole investigation

period from 7th June – 3rd November 2015 in 0–5 m water depth). At peak concentrations on 11th August 2015, the values reached 16.1 g $CaCO_3$ m^{-2}. The total contribution of *Phacotus lenticularis* (6 %) on the mean value was 0.38 g $CaCO_3$ m^{-2} (0–5 m) and reached 0.80 g $CaCO_3$ m^{-2} during *Phacotus* peak concentrations (21.5 %) on 16th June 2015.

2.6 Discussion

This study provides a novel methodological basis for a quantitative assessment of *Phacotus* precipitated carbonate in relation to the total carbonate precipitation in lakes. We examined the variations of the *Phacotus* shape and determined the resulting weight of its shells, which was the most relevant variable in this investigation. Our investigation revealed that the lake's origin significantly influenced the shell diameters and height. Accordingly, the shell volumes and masses varied strongly between lakes. It seems unlikely that dissolution was the reason for the variation in diameter or mass of the shells because neither the water chemistry (Tab. 2.1), nor the selective electron micrographs of the epilimnetic material (Fig. 2.2 and Fig. 2.3), showed evidence of dissolution of *Phacotus* shells or calcite crystals in any of the lakes. The shells were found to be highly massive with few pores of different types (i.e., porosity $<$ 5 %). This resulted in a shell mass for *Phacotus lenticularis* which was 2.8-fold higher than the current estimation of 0.3 ng $CaCO_3$ per individual reported by Koschel *et al* (1987b).

There might be several reasons that explain why our size-adjusted mass estimation based on 24 individuals from 5 lakes show a higher and more realistic shell mass (0.86 ng $CaCO_3$) compared to current estimates. First, differences in the methodological approaches need to be considered: in contrast to our study, Koschel *et al* (1987b) had the favourable and rare circumstance of lake water samples in which 100 % of the suspended carbonate was derived from *P .lenticularis* cells. Thus, the amount of carbonate from a certain volume of lake water (on a filter) could be measured conductometrically. The individual shell mass was then calculated through division with the microscopically determined cell concentration.

This approach might have resulted in an inferior shell mass value either because of common inaccuracies during microscopic cell counting or, owing to insufficient transfer of shells from the lake water onto the membrane filters. Homogenisation and division are difficult owing to fairly swift sedimentation of the *Phacotus* shells and can lead to depleted *Phacotus* shell concentrations in decanted water samples.

In our opinion, the methodology we used for volume determination can be considered less prone to errors. Using state-of-the-art FIB technology, the accuracy in imaging was high and scaling for the 3D-software had an absolute precision of less than 0.06 μm and the subsequent volume calculation had an extremely low mean error of $1.3 \times 10^{-5}, \mu m^3$ ($SD = 1.2 \times 10^{-5}$). However, our method for calculating the mass is not entirely unproblematic because the proportion of mesoporosity had to be estimated (in our case 2.4 % additional to the measured macroporosity with an average of 2.6 %). This resulted in a presumed total porosity of approximately 5 %. Accordingly, our calculated shell density ($\rho =$

0.0026 ng μm^{-3}, was only slightly lower than the ρ_0 of massive calcite. This value is in accordance with the density of biomineralised calcite of planktonic marine algae (dried coccolith powder) measured with a gas pyknometer (Hariskos, Chairopoulou, Posten, Teipel and Vučak, 2016) and seems plausible in regard to the very massive looking shell cross-sections on the close-up scanning electron micrographs (Fig. 2.4). In accordance with this, values from Koschel *et al* (1987b) do not seem to be realistic: applying their reported shell density of $\rho_s = 0.0006$ ng μm^{-3} to Formula (eq. 2.2), the resulting porosity Φ of 77.8 % seems exorbitantly high.

Secondly, in the different study lakes the natural variation of the general *Phacotus* size also caused large differences in the mean shell masses and could be an explanation for the difference in shell mass results. In previous publications, these natural variations were not subject to special interest. However, they are highly relevant if the quantitative *Phacotus* contribution to the total carbonate precipitation in lakes is being determined. Our measurements on individual cells from five lakes of different trophic states and mixis showed that *Phacotus lenticularis* had a great variability in size and morphology. Also Koschel and Raidt (1988a) identified a strong variation in shell diameter in all of their investigated water bodies. Our investigated shells with diameters in the range of $14.0 \pm 2.4\,\mu m$ are typical. According to Koschel and Raidt (1988a) shell diameters over 14 μm can be considered as large and Giering *et al* (1990b) states that shells with smaller diameters (7–12 μm) "may indicate daughter cells, in which shell development is incomplete".

Our analyses of the internal shell ultrastructure revealed that the small pores from the crystal interspaces were pitched in a circular-spiral arrangement along the smaller lateral edge of the tabular crystals. This means that we can confirm the oval-circular arrangement of the crystals in the shell centre (Fig. 2.4b) and the observations of Kamptner (1950) as well as those by Koschel and Raidt (1988a). They described the rotational symmetry of the crystals at the marginal shell rim and dissymmetrical orientation in the centre of the cell, similar to a "skiodromen model". Predominantly located in the central shell area, we found a second type of pore, the pore canals. In contrast to the descriptions of Giering *et al* (1990b), we could not confirm that all of the pores on the inner shell surface were "openings of the pore canals penetrating the *lorica*". Actually, only a few of them intersected the whole shell from the inside to the outside (Fig. 2.2c and Fig. 2.4a, d). These pore canals were wider than the pores in the crystal interspaces and showed irregular pore walls. Furthermore, we can report that shells of vegetative cells do actually touch each other in the contact zone (Fig. 2.4a) and are sometimes even almost interlocked (Fig. 2.4c). The rim interspace seemed to be filled with a volatile substance, which under an electron beam under vacuum conditions slowly disappears. This substance might represent the mucilaginous sheath (Hepperle and Krienitz, 1996), previously called "gelatinous mother cell wall" (Giering *et al*, 1990b). We observed that the pore channels seem to be coated or filled with this same volatile organic substance. In our opinion, it seems more probable that the exchange of substances during the motile cell state of *Phacotus lenticularis* is carried out through the pore canals rather than through the rim interspace in the contact zone, as suggested by Kamptner (1950).

Finally, we can report a remarkable contribution of *Phacotus lenticularis* to the total suspended calcite precipitation in Lake Grosser Ostersee of 21.5 % at peak cell densities. Krienitz *et al* (1993) reported the mean percentages of *Phacotus* calcite of the total suspended epilimnetic calcite in several hard-water lakes of northern Germany ranged from 5 %–20 % and reached 54 % in exceptional circumstances. Fluctuating *Phacotus* populations and irregularly oscillating total calcite precipitation rates were mentioned as reasons for the wide range of variation. Furthermore, the trophic state of the lake has to be considered as an important influencing factor. Significant *Phacotus* calcite percentages are expected predominantly in eutrophic and highly eutrophic lakes (Schlegel *et al*, 1998).

The total contribution of *Phacotus lenticularis* on the epilimnic mean carbonate concentration per square meter reached up to 21.5 % during peak concentrations and on average 6 % over the whole investigation period (7^{th} June–3^{rd} November 2015). Taking into account that sedimentation is only responsible for about 20 % of total current C-turnover from inland waters (Tranvik *et al*, 2009), and that at least half of the C is precipitated in the form of organic carbon compounds (Noges *et al*, 2016), carbonate precipitation by *Phacotus lenticularis* represents a small but notable part of C-turnover in lakes. In hard-water lakes, it should certainly be considered in the assessment of lake carbon cycling.

2.7 Conclusion

An accurate quantification of the carbonate sequestration of *Phacotus lenticularis* in lakes requires precise estimation of its shell sizes, masses, and the variations of both variables in relation to spatio-temporal variability. The mean diameter from times during highest cell densities should be used as the basis for all calcite mass calculations. During these events, *Phacotus lenticularis* actually provides relevant amounts of epilimnetic suspended calcite. Further investigation is necessary with respect to persistence, and actual amount, of *Phacotus* carbonate stored in recent lacustrine sediments and whether shells tend to be re-dissolved on their way from the epilimnion to the lake bottom.

3 | Representative monitoring of the calcifying alga *Phacotus lenticularis* in lentic ecosystems

A similar version of this chapter was accepted: Lenz S, Geist J, Raeder U (2020): Representative monitoring of the calcifying alga *Phacotus lenticularis* (Chlamydophyceae) in lentic ecosystems. Journal of Limnology.

3.1 Abstract

The biogenic carbonate precipitation by the freshwater alga *Phacotus lenticularis* may play a role in long-term carbon (C) fixation that has not yet been quantified. This is partly due to the absence of a standardised methodology to representatively sample and assess the cell density and sedimentation of *P. lenticularis* in lakes. The objective of the present study was to define an adequate sampling methodology taking into account the spatiotemporal variation of *P. lenticularis* as well as the sedimentation and dissolution of calcite shells. Simultaneous measurements in three different lake sub-basins of Lake Grosser Ostersee, Germany, showed that the spatial cell density of *P. lenticularis* was similar in each lake. At all sites, the vertical *P. lenticularis* cell density maxima corresponded with the slowly downshifting thermocline from depths of 2 to 6 m. During the entire growth period, composite samples from 0 to 7 m included 89 % of the total *P. lenticularis* population. Lake bathymetry, as well as external factors like wind exposure, did not appear to affect the abundance of these calcifying algae. Sediment traps at a depth below the thermocline (at 7 m) and 1 m above the lake bottom recorded sedimentation rates of *P. lenticularis* shell halves (sh) between 1.1×10^6 and 1.1×10^8 sh m^{-2} d^{-1}, while mean cell concentrations in the water column were between 1.1×10^8 and 1.7×10^9 shells per m^2. Sinking velocity ranged between 3 and 4 m d^{-1}. Sediment from traps installed at a depth of 7 m did not reflect mean shell concentrations in the water column above. Dissolution of carbonates reduced the number of shells in sediment traps at the lake bottom and during the storage of samples. A laboratory experiment showed that even distilled water used for dilution during microscopic analysis led to dissolution of *P. lenticularis* shells. In conclusion, combined sampling of *P. lenticularis* from open water and sediment traps close to the lake bottom delivers a most representative assessment of biogenic carbonate precipitation. Due to dissolution effects, high temporal resolution along with appropriate sample preservation are crucial, whereas spatial representativeness was already achieved with low number of sampling sites per lake.

3.2 Author contributions

Sebastian Lenz (SL) was part of the team conceiving the study. The development of methods was carried out by him. The acquisition of data in terms of fieldwork and microscopic analysis was realized by SL. Interpretation of data was realized by SL. The manuscript was drafted and improved by SL.

3.3 Introduction

Carbon fixation by planktonic algae is considered to be an important CO_2 sink in marine environments, and has even led to fertilisation experiments to investigate enhancing algal growth for carbon sequestration (Blain *et al*, 2007; P. J. Morris and Charette, 2013; Smetacek *et al*, 2012). Biogenic carbon sequestration has been less intensely studied in freshwater systems, yet there have also been documented occurrences of algae that form crystalline calcite structures in these environments. Among these algae, *Phacotus lenticularis* has drawn great attention since this species is particularly widespread in calcareous lakes and thus may be one of the most important freshwater algae species involved in carbon sequestration.

Phacotus lenticularis, hereafter referred to as *Phacotus*, is a unicellular, bi-flagellated green alga, belonging to the Chlamydophyceae. It forms regularly-shaped shells (loricae) of crystalline calcite plates that may significantly influence carbonate chemistry and sinking carbon fluxes in lakes (Cărăuş, 2002; John, Whitton and Brook, 2011; Menezes, 2010; Sánchez, J. C., Cobelas, M. Á., Sanjurjo, M. A., 1998; Wehr, J.D., Sheath, R.G., Kociolek P., 2001). *Phacotus* is widespread in the temperate, subtropical and tropical climatic zones of both hemispheres, appearing predominantly as planktonic algae in various morphometric and ecological states in stagnant inland waters (Schlegel *et al*, 1998). In particular, mass developments of *Phacotus* may have a considerable influence on the $CaCO_3$ budget of lakes (Krienitz *et al*, 1993), and the importance of these calcifying algae is demonstrated by the long-term preservation of carbon in the form of remarkable shell deposits in sedimentary records dating back to the Miocene, 120 ka BP (Lagerheim, 1902; G. Müller and Oti, 1981).

The largest body of literature in the context of *Phacotus* has focused on the genesis and properties of its shell. Koschel and Raidt (1988a) studied the morphology of *Phacotus* shells, while Giering *et al* (1990b) and Hepperle and Krienitz (1996; 1997) examined shell calcification patterns. Schlegel, Krienitz and Hepperle (2000) described four morphotypes of *Phacotus* shells, which are suggested to reflect organismic calcification control. The abundance of *Phacotus* and its contribution to suspended autochthonous calcite content in hard-water lakes of northern Germany have been studied (Krienitz *et al*, 1993). Also well understood are the shell growth responses of *Phacotus* to meteorological changes (Gruenert and Raeder, 2014a) and the systematic assessment of natural variations in the general size and particular calcite mass of *Phacotus* shells, in order to quantitatively determine its role in carbonate sedimentation in hard-water ecosystems (Lenz *et al*, 2017).

Despite the intense research into *Phacotus* and its potential importance in biogenic carbon sequestration, a representative sampling method that at the same time considers spatiotemporal resolution as well as sedimentation and dissolution is still lacking. Such a protocol would be highly beneficial in assessing the contribution of this species to calcite precipitation and carbon sequestration in lake ecosystems, as well as for improving the comparability between different studies. Thus, the primary goal of this study was to determine and validate a methodology that facilitates a reproducible and representative assessment of the actual *Phacotus* cell density in a whole lake, considering this organism's spatiotemporal variations. The following hypotheses were tested: (1) a single continuous mixed sample taken from the epilimnion sufficiently describes the *Phacotus* population in a small lake; (2) *Phacotus* shell sedimentation rates at different depths in the hypolimnion mirror the *Phacotus* population in open water; and (3) dissolution of sedimented cells does not play a role under hard-water conditions.

3.4 Material and Methods

3.4.1 Study site

Four Bavarian lakes (Tab. 3.1) with documented *Phacotus* occurrences during the last ten years (2005–2015) were investigated: Lake Grosser Ostersee (GOS), Lake Abtsdorfer See (ABS), Lake Igelsbachsee (IGS) and Lake Hopfensee (HOP) (Fig. 3.1). Lake Igelsbachsee, a Ca-rich artificial reservoir, lies in a basin of late-Triassic quartz sandstones surrounded by Jurassic carbonates. The other lakes are natural hard-water lakes in the pre-alpine basin in Southern Bavaria (Germany). All of the lakes have carbonate-dominated catchments and are fed by alkaline water from the Northern Limestone Alps. They are embedded in an open quaternary aquifer of predominantly fluvioglacial sediments with intercalated basal moraine tills that overlay a tertiary sandstone aquitard (Grimminger, 1982).

Fig. 3.1: Location of the four lakes investigated in Bavaria, Germany.

Table 3.1. Selected hard-water lakes in Bavaria, Germany, and geographic sampling positions at each lake's deepest point.

	Gr. Ostersee	**Abtsdorfersee**	**Igelsbachsee**	**Hopfensee**
Trophic state	Oligotrophic	Mesotrophic	Mesotrophic	Eutrophic
Mixis type	Dimictic	Dimictic	Polymictic	Dimictic
Surface area [ha]	118	78	72	186
Max. depth [m]	29.7	20.0	11.5	10.4
Volume [$10^6 \times m^3$]	14.0	9.4	3.9	8.9
Calculated residence time [d]	247	252	-/-	128
pH value*	8.2 (0.2)	7.9 (0.4)	8.6 (0.5)	8.2 (0.4)
Ca^{2+} conc. [mg L^{-1}]**	69.2	78.2	37.6	69.3

3.4.2 Study concept

Several experiments were performed to obtain a representative *Phacotus* population assessment. In 2015, during an exploratory study at GOS, relevant elements for the representative sampling of *Phacotus* were systematically analysed in two experiments (1, 2). During an intensive sampling campaign during 2016 at GOS, ABS, IGS and HOP, the monitoring methodology consisted of two further advanced experiments (3, 4). Carbonate dissolution was identified as a disruptive factor in IGS, not only in the lake but also after sampling, therefore a dissolution experiment (5) was performed.

1. Water column investigations at three sites with 1-metre resolution and a daily sampling frequency to assess vertical distribution and temporal variation of *Phacotus* in order to determine the optimal sampling depth.
2. Sediment trap experiments at two sites and two different depths to determine the sedimentation velocity as well as differences in the horizontal distribution of *Phacotus*.
3. Water column investigations with mixed samples and a weekly sampling frequency to simultaneously detect *Phacotus* population development in the epilimnion of the four lakes (Fig. 3.2a).
4. Sediment trap experiments in each lake, with cylindrical sediment traps attached to a buoy (Fig. 3.2a, b) and square sediment traps at the lake bottom (Fig. 3.2c).
5. A laboratory experiment showing that the use of distilled water during microscopic examination leads to the dissolution of *Phacotus* shells.

3.4.3 Exploratory study

In 2015, an exploratory study was performed at Lake Grosser Ostersee (Fig. 3.3c), initially with a weekly sampling frequency then a daily sampling frequency during the peak growth period of *Phacotus*. A field study was conducted to reveal spatiotemporal variations in

Phacotus population density and the resulting sedimentation rates. From the lake surface down to a depth of 10 m, each metre was simultaneously sampled using a standard Ruttner water sampler at three sampling sites (GOS-W, GOS-N and GOS-E). Two cylindrical sediment traps (Hydro-Bios, Kiel, Germany), according to Saarso (1995), with an exposure area of 0.015 m^2, were installed at two of these sites (GOS-W and GOS-E) at depths of 16 and 26 m. During two weeks in June 2015, a total of 24 sediment trap samples were collected, with trap exposure times of one to two days. The sedimentation velocity of *Phacotus* shells, v [m d^{-1}], from the epilimnion to the sediment trap, was estimated. This velocity was derived from the time lag between the first increase in *Phacotus* cell density in the water column (0–10 m) and the resulting increase in *Phacotus* shell sedimentation rate at 16 m depth, by dividing the sedimentation distance [m] by the time lag t [d].

3.4.4 Monitoring methodology

The monitoring carried out during 2016 aimed to simultaneously assess *Phacotus* population development in the epilimnion of the four lakes. As a sampling methodology, we used the standard plankton sampling and analysis procedure of the European Water Framework Directive (Mischke and Nixdorf, 2008), with adaptions based to the results of our exploratory study. Special attention was paid to avoid variations in the sampling procedure, as the largest errors in results typically originate from sampling errors in the field (Cairns Jr and Smith, 1993; Kelly, 1998). At the deepest point of each lake, a buoy and two different sediment traps (Fig. 3.2a) were installed. Personnel were trained in sampling techniques and hardware handling prior to beginning the fieldwork. Depth profiles of water temperature and pH values were measured using a WTW MPP 930 multiprobe (Weilheim, Germany), and the Secchi depth was determined using a Hydro-Bios visibility disc.

The beginning of the detailed sampling campaign was dependent on pH values of at least 7.8 and temperatures of more than 20°C at a depth of 0.5 m from the surface. These thresholds determine the expected onset of *Phacotus* growth. Weekly sampling was carried out during the growth period from June to September. To analyse *Phacotus* abundance, continuous mixed water samples from a depth of 0 to 7 m were taken using a 7-metre-long hose sampler (DIN Technical Committee Water analysis, 2015). To facilitate vertical sinking of the hose, a 3 kg weight was attached to the open end (Fig. 3.2a). The entire hose was slowly lowered into the water. After closing the top end of the hose with a plug, the open end was pulled up and its contents were emptied into a 6 L measuring bucket by lifting the hose from the top end. In order to rinse the sampling hose, the first sample was discarded. Brown-glass flasks were previously prepared with neutral pH Lugol's solution so that a 2 % (v/v) concentration would ensure preservation during sample storage, transport and post-sampling. Procedures for plankton analysis of the epilimnion followed the method of (Lenz *et al*, 2017). *Phacotus* shell concentration was determined according to Utermöhl (1958), using an inverted microscope (Leitz Labovert, Stuttgart, Germany) at 200–400× magnification. Samples were analysed immediately as far as possible to avoid carbonate dissolution during storage and at least 300 individual cells were counted per sample to ensure the accurate determination of shell numbers.

Additionally, selected shells from the epilimnion were analysed using a Hitachi S-2300 scanning electron microscope (Tokyo, Japan).

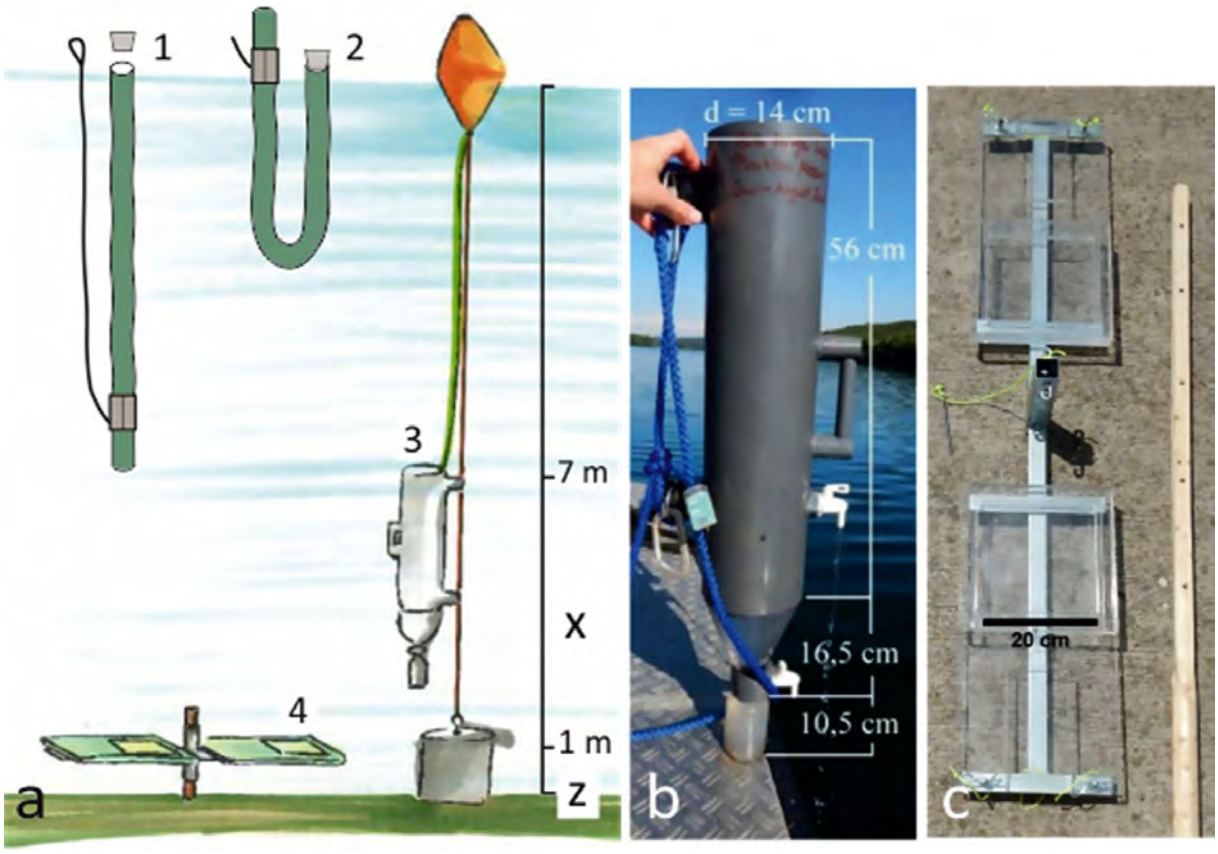

Fig. 3.2: a) Sampling equipment for representative *Phacotus* population monitoring: sampling hose insertion (1) and recovery (2), buoy with hanging cylindrical sediment trap (3) and square-type sediment trap 1 m above the lake bottom (4). b) Dimensions of the sediment traps: cylindrical-type for installation in the water column, c) square-type with a closable opening for installation and recovery on a wooden pole by divers at the lake bottom.

During the detailed sampling campaign in 2016 at GOS, ABS, IGS and HOP, the monitored water column was reduced to the range of 0 to 7 m and the cylindrical sediment traps were set at a depth of 7 m, with exposure times of between 6 and 8 days. Additionally, square sediment traps we constructed ourselves (Fig. 3.2c) were used to investigate the dissolution of particulate carbonate in the hypolimnion. These traps were modified versions of the design of Kozerski and Leuschner (1999), with an exposure area of 0.040 m^2, and were designed to be installed and recovered by divers using a wooden pole, 1 m above the lake bottom. The exposure times varied from 6 to 16 days.

The sediment trap samples were recovered in 200 mL clear PVC bottles, transported at 20°C and stored with a 2 % (v/v) concentration of neutral pH Lugol's solution for no more than two months in a dark room prior to analysis with an inverted microscope at 200–400× magnification, according to Utermöhl (1958). The sediment trap material from the bottom traps was analysed using a technique adapted from the coverslip method described by Koch and Young (2007). After weighing the freeze-dried samples, the organic sediment

content was burned in a muffle furnace (550°C, 4 h). Thereafter, each loss on ignition sample was carefully crumbled in a mortar and 0.5 mg of the homogenised material was added to an Eppendorf tube containing 2 mL buffered suspension liquid comprising NH_3 and H_2O with a pH $\geq$ 8.5, (Bollmann *et al*, 1999). As a calibration standard, 0.5 mg of borosilicate microspheres (Thermo Fisher Scientific #9015, Waltham, Massachusetts, USA) was added and the carbonate aggregates were destroyed in an ultrasonic basin (Bandelin Sonorex TK52, Berlin, Germany) for 60 s (150 W, 35 kHz), according to the method of Bordiga, Bartol and Henderiks (2015). Subsequently, the samples were mixed using a CHS Vortex homogeniser (Prague, Czech Republic) and 50 μL of the suspension was transferred using a pipette to the centre of a circular coverslip, according to the 'drop' method (Bordiga *et al*, 2015; Koch and Young, 2007). The mass of the sediment, m_{cs}, on the coverslip was $50 \pm 1.9\,\mu$g, and was calculated relative to the total amount of microspheres on the coverslip. Three microscope slides were made for each sample. Shell numbers on each coverslip were counted under light microscopy using polarised light at 200× magnification. A complete *Phacotus* housing was counted as two shells (sh). Single shells were counted as one shell.

Phacotus shell sedimentation rates, S [sh m^{-2} d^{-1}], were calculated according to equation 1, with an exposure time, t [d], and an exposure area, A, of 0.015 and 0.04 m^2 for the cylindrical and the square sediment traps, respectively. The total number of shells in a square sediment trap, N_{tr}, was calculated according to equation 2 using the difference between the total sediment dry mass, m_{tr} [g], and the sediment mass on the coverslip, m_{cs} [g], multiplied by the total number of shell halves, N_{cs}, on a coverslip.

$$S = N_{tr}/A \cdot t \tag{3.1}$$

$$N_{tr} = N_{cs} \cdot m_{tr}/m_{cs} \tag{3.2}$$

3.4.5 Dissolution experiment

The effect of bi-distilled water on *Phacotus* shells during storage or sample treatment for microscopic analysis was examined. Representative samples from the field survey were diluted 1:10 with bi-distilled water. The 24 h experiment was performed under an inverted microscope at 40× magnification. Images were acquired using Kappa ImageBase camera software from Kappa Opto-Electronics and Accusoft 2007 (Gleichen, Germany). The temperature (23.7°C ± 0.4°C) and pH values (8.4 ± 0.3) were constantly monitored with a WTW Sentix pH electrode (Weilheim, Germany).

3.5 Results

3.5.1 Spatial variability of *Phacotus* cell density

Over a time span of four months, the mean *Phacotus* cell densities from depths of 0 to 10 m at the three sites, GOS-W, GOS-E and GOS-N, were almost identical (Fig. 3.3a). No significant differences in *Phacotus* density (Fig. 3.3b) were observed, although the different sub-basins varied by depth and wind exposure (Fig. 3.3c). Even the main

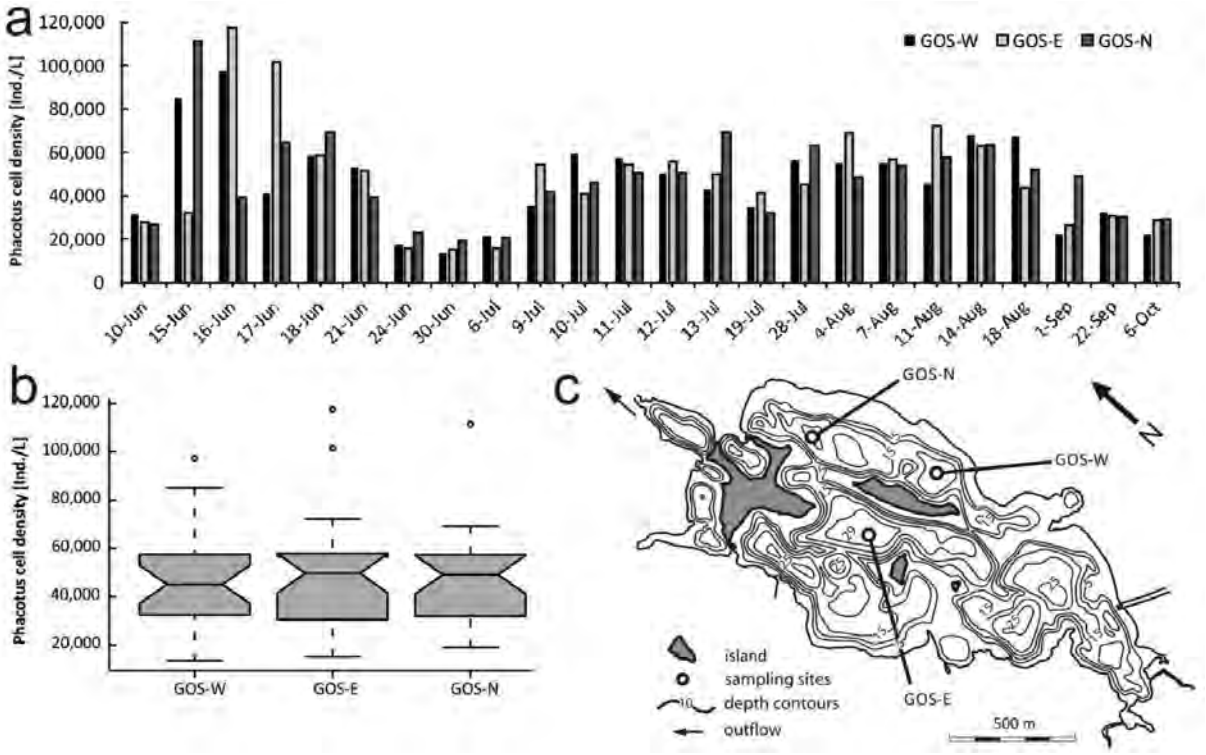

Fig. 3.3: Mean *Phacotus* population density at three different sampling sites in different lake basins from June to October 2015; $n = 24$ synchronized measuring days at depths of 0 to 10 m, a) temporal variation during the growth period (the time axis is not linear), b) boxplots of all data (boxes: 0.25 and 0.75 quartile, whiskers: 1.5× interquartile range, notches, dots: outliers as defined as 3× SD), c) bathymetric map of Lake Grosser Ostersee with the three sampling sites shown.

Phacotus growth period started at almost the same time, with a difference of only two days.

In contrast, the number of cells along the depth gradient showed a strong vertical variation between 0 and 10 m depth. From mid-June, an epilimnion developed and a strong, stable stratification formed, while the highest cell density of 475.000 individuals per litre (Ind. L^{-1}) was measured in 2 m depth during the first growth peak on 16th of June. The thermocline descended continuously and the maximum cell density of *Phacotus* also gradually moved downwards, from 2 to 4 m in June to 5 to 6 m in August. During August, increasing biogenic calcite precipitation led to concentrations of suspended carbonate > 2 mg $CaCO_3$ L^{-1} in the upper water column. During this period average Secchi depth in Lake Grosser Ostersee was reduced to 1.5 m ($SD = 0.5$) in comparison to a total mean of 3.3 m ($SD = 0.6m$). In August, the maximum cell density occurred at a depth of 5 m. The *Phacotus* cell densities were in the range of 4×10^5 Ind. L^{-1} (red), 2×10^5 Ind. L^{-1} (turquoise) and 0 (dark blue) (Fig. 3.4). Below a depth of 7 m, cell densities occasionally reached 5×10^4 Ind. L^{-1}, while the fraction of inactive and dead *Phacotus* individuals from the areas above increasingly adulterated the *Phacotus* shell density to an unknown degree. Over the monitored timeframe, the water column above a depth of 7 m contained 89 % of all *Phacotus* individuals counted.

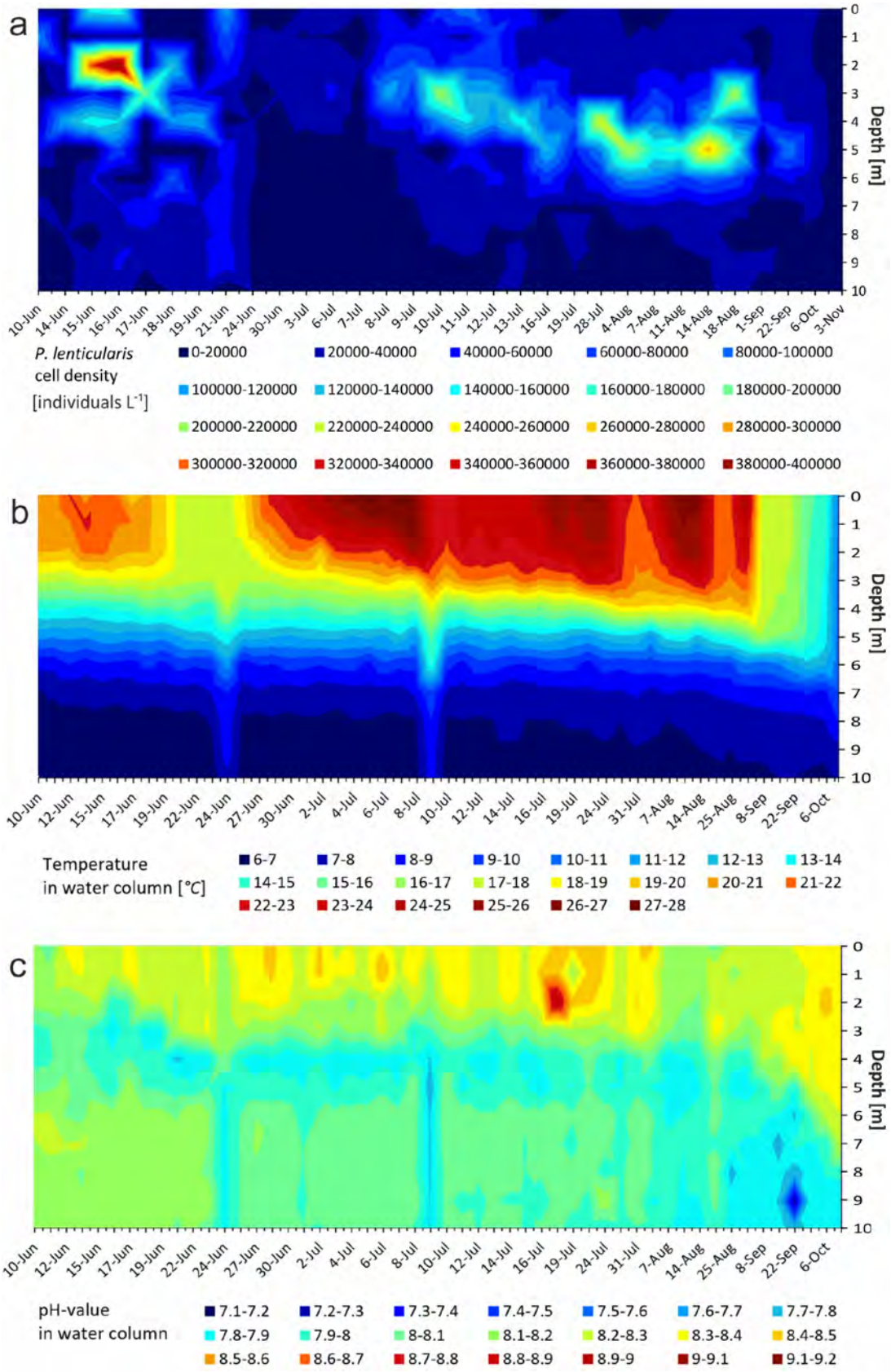

Fig. 3.4: Depth distribution and spatiotemporal variation of a) *Phacotus* cell density, b) temperature, and c) pH-value at site GOS-W between depths of 0 to 10 m during 2015.

3.5.2 *Phacotus* shell sedimentation

Mean sedimentation velocities of 3 to 4 m d^{-1} for *Phacotus* shells were derived from the comparison of peak occurrences of *Phacotus* among water column and the sediment traps placed at different depths in the hypolimnion of Lake Grosser Ostersee.

The first and highest peak in *Phacotus* population growth (Peak 1) in June 2015 led to higher *Phacotus* shell sedimentation rates than the second peak (Peak 2) in July 2015. The two sites GOS-W and GOS-E showed sedimentation rates in a comparable dimension of 1.9×10^7 sh $m^{-2}d^{-1}$ at the same depths (Fig. 3.5b).

In 2016, the sedimentation rates of the cylindrical sediment traps at 7 m depth (dots) were generally lower than those from the bottom sediment traps 1 m above the lake bottom. *Phacotus* abundance and sedimentation rates corresponded in the four lakes investigated. In general, high *Phacotus* cell densities in the water column resulted in high quantities of shells in the sediment traps, but a significant correlation could not be established (Fig. 3.5a).

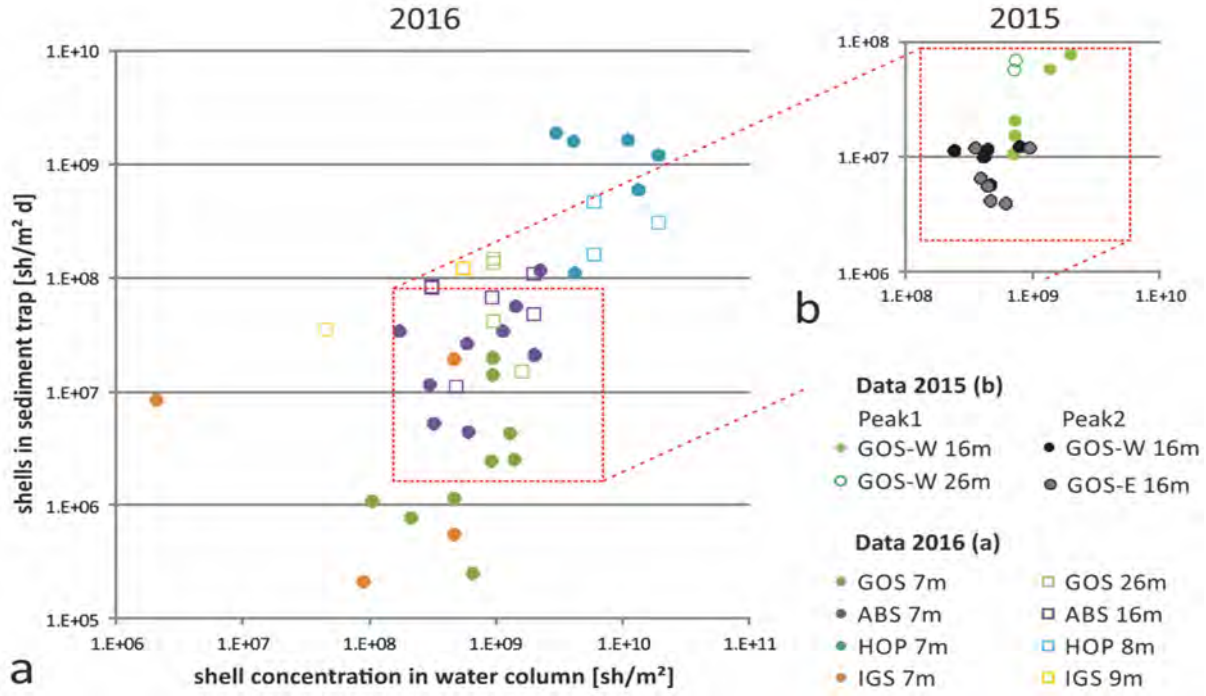

Fig. 3.5: Relationship between suspended epilimnetic *Phacotus* shell concentrations in the upper water column and corresponding Phacotus shell sedimentation rates in sediment traps exposed above (installation depth and place are indicated in the legend). a) Data from 2016 from depths of 0 to 7 m of four Bavarian lakes, b) data from 2015, from depths of 0 to 10 m of Lake Grosser Ostersee. The delay in sedimentation due to sinking was calculated using an estimated shell sedimentation velocity of 3 m d^{-1} for all lakes and all years.

3.5.3 Carbonate dissolution

As indicated by the lowest cell densities and sedimentation rates of all four lakes, carbonate dissolution may have reduced the abundance of *Phacotus* shells in IGS despite of the

alkaline pH in this lake. Observations of *Phacotus* shells from the water column and sediment traps suggest that dissolution took place in the epilimnion as well as at the lake bottom. Even massive calcite crystals showed clear signs of dissolution (Fig. 3.6c, d) at the lake bottom. Microscopic analysis of samples from selected dates showed 28.640 Ind. L^{-} *Phacotus* shells in the epilimnion (0–7 m) shortly after sampling. In addition, following two months of storage, these previously measured cell densities could not be confirmed because the shell abundance was reduced to 60 Ind. L^{-1}.

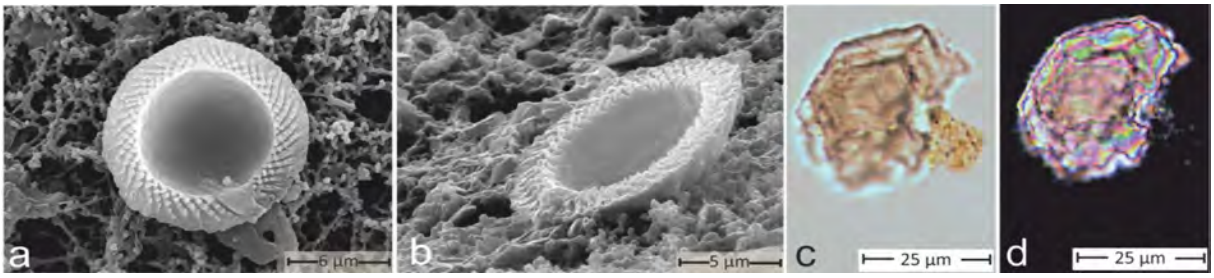

Fig. 3.6: a) Electron micrographs of *Phacotus* shells from the epilimnion in Lake Igelsbachsee: plane view of an open shell, b) diagonal perspective of the shell rim with deep trenches visible on the broad contact plane as evidence of dissolution c) micrograph of a large blocky calcite crystal with dissolution structures indicating carbonate-dissolving conditions at the lake bottom, and d) the same crystal under crossed nicols.

The dissolution of carbonate particles during sample storage and microscopic analysis was also confirmed in the laboratory dilution experiment with bi-distilled water. The process which led to the complete dissolution of a *Phacotus* shell was observed and documented over 24 h inside an Utermöhl chamber (Fig. 3.7). Within the first 10 h, the rim edge of the *Phacotus* shell gradually became thinner and paler in appearance. After 12 h, the regular round outer line across the shell rim disappeared and an obvious loss of material could be observed. The shell rim had clearly been dissolved after 15 h, and the shell diameter was notably reduced from 15 to 11 μm. Eventually, the shell completely disappeared and only the exposed protoplast remained. At this stage, *Phacotus* would not have been recognised as such during microscopic analysis.

3.6 Discussion

In order to determine an adequate sampling methodology for the representative assessment of the calcifying freshwater alga *Phacotus lenticularis*, several aspects of cell density distribution and shell sedimentation were examined. In Lake GOS, the horizontal heterogeneity (1) was negligible while the density changes of *P. lenticularis* throughout time showed a dynamic phenology (2) and the vertical distribution (3) could be representatively assessed by sampling an integrated water sample from the epilimnion. With regard to temporal variation, an approach of assessing cell densities using sediment traps installed beneath the epilimnion (4) revealed that there was little correlation between sedimentation data and cell densities in the water column. One reason was carbonate dissolution during sedimentation (5), which also occurred during microscopic analysis and following

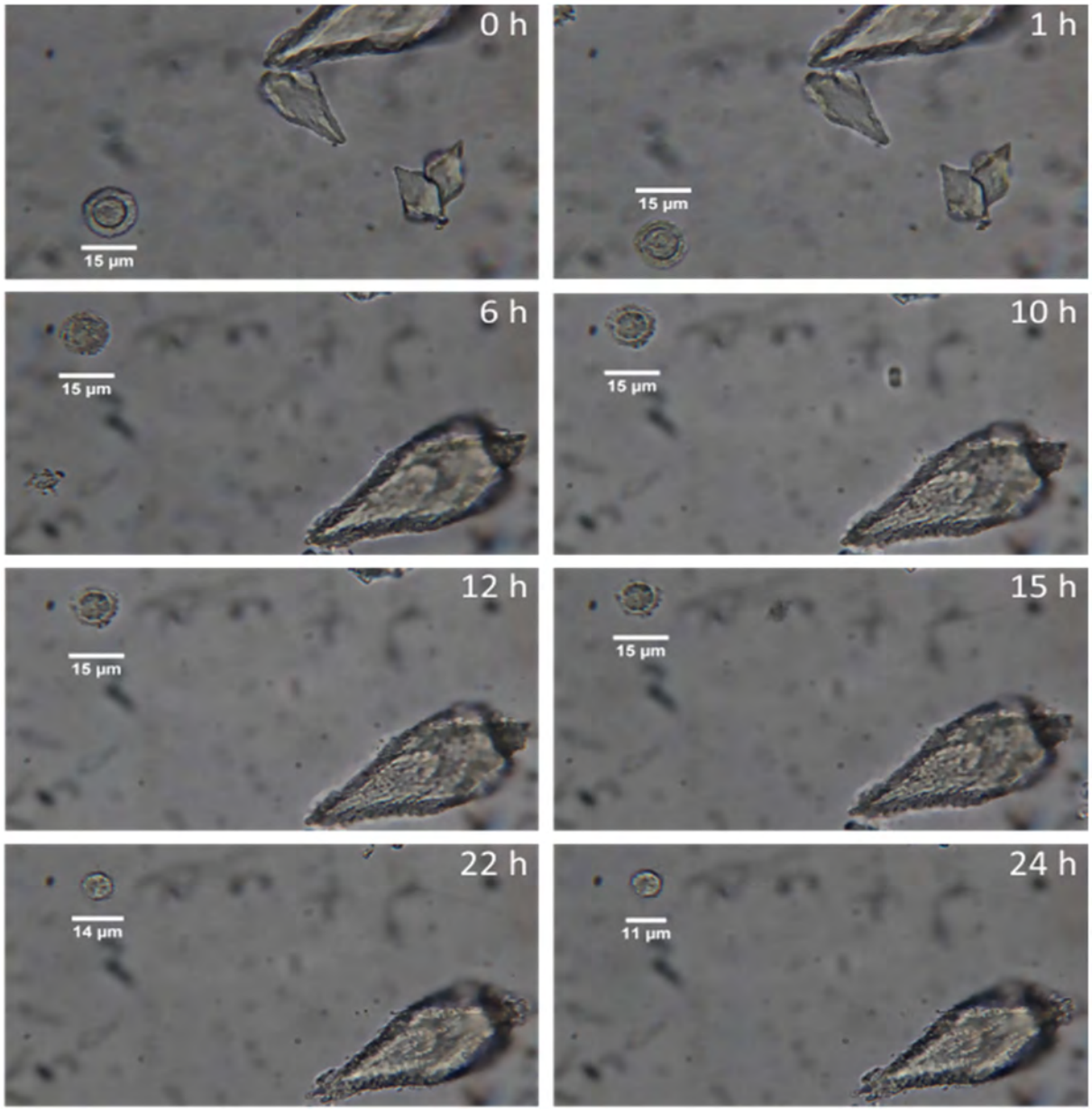

Fig. 3.7: Micrographs from a 24 h experiment to demonstrate carbonate dissolution on the circular shell rim of an individual *Phacotus* cell (left) and a wedge-shaped calcite crystal (right) showing slow dissolution; the experiment was performed under an inverted light microscope at 400× magnification in an Utermöhl chamber (Utermöhl, 1958), diluted 1 : 10 with bi-distilled water.

storage of samples (6). Carbonate dissolution during microscopic analysis was further illustrated by a laboratory experiment (7).

In contradiction to the more general findings of de van de Bogert *et al* (2012), who suggested that single-location estimates can yield errors of more than an order of magnitude when it comes to estimating daily gross primary production and respiration, the horizontal variation (1) in *Phacotus* abundance at the different sites in Lake GOS was negligible. Apparently, the morphological characteristics of the lake basin (bathymetry),

in addition to external factors such as wind exposure, did not control the horizontal distribution of *Phacotus* over a period of more than 10 weeks. It was verified that stable stratification provided conditions that enabled simultaneous *Phacotus* development in the epilimnion at three sites. Therefore, one single sampling site over the deepest point of a lake was sufficient for representative and effective *Phacotus* population monitoring in this lake being smaller 2 km^2. This finding is also in accordance with the standard plankton sampling procedure for the EU Water Framework Directive (Mischke and Nixdorf, 2008).

The density changes of *P. lenticularis* (2) over the vegetation period of five month in Lake Grosser Ostersee resulted in three successive growth peaks. A short and intense first one was followed by two temporally extended population peaks with lower cell numbers. This finding is quite similar to the descriptions of Krienitz *et al* (1993) for Lake Haussee in northern Germany, suggesting that such a temporal dynamics may be characteristic for this species. During the first growth phase in mid-June, mean cell densities extended 100.000 Ind. L^{-1}. Within the following nine days, the population collapsed to the lowest cell number (17.000 Ind. L^{-1}) of the entire study period (Fig. 3.3). This can be explained by at least two factors or their combination: first, increased grazing of filtrating zooplankton that typically arises after the first phytoplankton bloom, and second, several windy days with rainfall leading to the breakdown of the epilimnion. With rising temperatures and the re-establishment of the stratification at the beginning of July, two further growth peaks developed with cell numbers around 30.000 and 65.000 Ind. L^{-1}. The second *Phacotus* growth phase was probably attenuated because it went along with the onset of intensified biogenic calcite precipitation leading to high amounts of suspended carbonate and consequently to a reduction of both light transmission as well as a depletion of dissolved phosphate and dissolved inorganic carbon (DIC) in the upper water column.

The measurement of the vertical spatial distribution (3) of *Phacotus* cell density along with water temperature revealed that the population maxima were always located above the thermocline, which developed from mid-June and descended continously from 2 to 6 m through the summer. This is to be expected since *Phacotus* actively moves near the surface where the light intensity is adequate and favourable for reproduction and photosynthesis (Gruenert, Benda, Müller and Raeder, 2016). Hydrographical parameters such as pH and high temperatures (Fig. 1.14) naturally result in carbonate oversaturation in the epilimnion (Bluszcz *et al*, 2009), which is thought to be a relevant factor for the extracellular calcification of *Phacotus* shells (Hepperle and Krienitz, 1996). This implies that the vertical spatial distribution of *Phacotus* cell density occurs through a combination of active movement of the flagellate and passive accumulation due to the density gradient above the metalimnion, and can explain why 89 % of *Phacotus* cells in the 0 to 10 m water columns were concentrated at depths in the range of 0 to 7 m. Therefore, this supported the hypothesis that an integrated water sample taken over the entire dimension of the epilimnion is sufficient to describe the *Phacotus* population in a small lake.

The sediment trap experiments (4) revealed that *Phacotus* shells contributed a maximum of 15 wt % to the total calcite at the bottom of Lake Hopfensee, where the highest *Phacotus* shell abundance in sediment trap material (Fig. 3.5a) was detected. High cell densities of *Phacotus* in the upper water column resulted in large amounts of shells in the

sediment traps. However, in the majority of the lakes, sedimentation rates were higher at the lake bottom than in the depth to 7 m. Cell densities only revealed weak temporal relationships with the sedimentation rates (Fig. 3.5a). For these reasons the second hypothesis, i.e. that sedimentation rates mirror the *Phacotus* population in the water column, could not be supported. It would be interesting to explore this aspect further by investigating the depletion of phytoplankton by zooplankton inside the sedimentation traps, as suggested by Bloesch and Burns (1980), or whether *Phacotus* individuals living at a depth of 7 m are still fit enough to escape from the sediment traps.

In contrast to the last hypothesis, analysis of plankton from IGS revealed signs of calcite dissolution on the surfaces of *Phacotus* shells despite of the alkaline nature of all lakes. In the plane view, the *Phacotus* shells and their broad shell rim appeared even and compact (Fig. 3.6a), but viewing from an oblique angle revealed pronounced trenches between the edges of the calcite crystals at the shell rims (Fig. 3.6b). This was considered as indicator for carbonate dissolution of *Phacotus* shells already in the water column (5), which was surprising since the conditions in the epilimnion of a hard-water lake, with a Ca^{2+} ionic concentration of 37.6 mg L^{-1}, pH value > 8.6 ($SD = 0.5$) and temperature of 20.9°C ($SD = 2.7$°C) as described by Lenz *et al* (2017), did not indicate carbonate undersaturated conditions. These parameters are within acceptable ranges indicated for the occurrence of *Phacotus* in lakes (Gruenert *et al*, 2016; Schlegel, 2001b; Schlegel, Krienitz and Hepperle, 2000). A *Phacotus* mass occurrence was even documented at IGS in 2006 (Bavarian Environment Agency, 2016), which suggests suitable water conditions for *Phacotus* in this lake. In addition, at the lake bottom, material from the sediment traps showed further features of dissolution on larger carbonate crystals (Fig. 3.6c-d), which also indicated carbonate-diluting conditions in the lower water column – a process that has also been observed in other hard-water lakes (Bluszcz *et al*, 2009; Ohlendorf and Sturm, 2001). The conditions in the hypolimnion of IGS, beyond a depth of 7 m, showed a pH value of 7.64 ($SD = 0.1$) and indicated anaerobic conditions and a marked increase in conductivity towards the bottom of the lake. Mineralisation processes, such as bacterial activity, are factors that can affect calcite sedimentation (Kelts and Hsü, 1978), therefore $CaCO_3$ undersaturated conditions may have developed in IGS. Calcite crystals, as well as *Phacotus* shells, potentially dissolved during sedimentation down the water column, undermining the sediment trap results from IGS. Consequently, in IGS, where dissolution cannot be excluded, sediment traps were not adequate for the representative assessment of the *Phacotus* population in the epilimnion.

Dissolution of carbonates continued in the IGS samples even in the brown-glass flasks (6) during storage. The evidence for this was the absence of *Phacotus* shells in stored samples which had demonstrably contained *Phacotus* shells prior to storage. Incorrect sample treatment can be excluded as a reason for this, since all samples were treated identically and IGS was the only lake which showed a reduction in *Phacotus* shell numbers following storage. The reasons for carbonate dissolution during storage remain unclear, because the pH was > 7.7 in the overhead water of the sampling flasks. To avoid carbonate dissolution during storage it is recommended that Lugol's solution with a pH > 7 is used and that plankton samples are analysed immediately.

A reduction in *Phacotus* shells also occurred during microscopic analysis. A dilution experiment (7) showed that it was not only decreased pH values in sampling bottles but also the use of distilled water during the preparation of samples for microscopic examination that led to dissolution of the calcite shells of *Phacotus* (Fig. 3.7). The experiment demonstrated that, following shell dissolution, no evidence of *Phacotus* remained, apart from the naked protoplast, which could possibly be misinterpreted as a single-celled chlorophyte flagellate such as *Chlamydomonas* sp. To prevent the unnoticed loss of *Phacotus* shells, the use of distilled water for sample preparation is not recommended. Instead, a buffered suspension liquid comprising NH_3 and H_2O with a pH $\geq$ 8.5, after Bollmann *et al* (1999), should be used.

All of the findings concerning dissolution suggest that dense sampling intervals of *Phacotus* as well as appropriate preservation and handling are essential to avoid a bias towards underestimating their abundance and contribution to biogenic carbon sequestration, even in alkaline lakes. This aspect appears equally important as an early start of sampling campaigns to capture the first and strongest population peak of the season.

3.7 Conclusion

In order to assess representative population densities of *Phacotus lenticularis* in a water column throughout the complete growth period, the timely commencement of monitoring is essential to assess what is the first and often largest population peak. For small lakes of < 2 km^2, integrated water samples can be collected down to the metalimnion from one representative sampling site. The present study found that the epilimnetic range of 0 to 7 m provided optimum coverage of *Phacotus* individuals. Due to the rapid sedimentation of *Phacotus* shells, it is essential to homogenise plankton samples before transferring them into previously prepared sampling bottles. Fixation works well with 2 % neutral pH Lugol's solution, which is recommended to be added to a sample at a four-fold concentration (Utermöhl, 1958). Cylindrical sediment traps are recommended to be installed at the bottom of a lake, at a depth of > 10 m. To avoid carbonate dissolution during storage, microscopic analysis should ideally be completed immediately after sampling. As an indicator for carbonate dissolution, the ionic concentration of Ca^{2+} in the overhead water of the sampling flasks can be analysed. During counting under a microscope it is essential to differentiate single shells from intact individuals accounting for two shells. During sample preparation, water with an adjusted pH value of > 8.5 should be used for sample dilution. To track potential carbonate dissolution in the hypolimnion, sediment analysis can reveal whether all shells reaching the lake bottom are stored over geologic timescales or if dissolution in sediment or at the sediment surface reduces the amount of *Phacotus* shells.

4 | *Phacotus lenticularis* content of carbonate sediments and epilimnion in four German hard water lakes

A similar version of this chapter was submitted: Lenz S, Dubois N, Geist J, Raeder U. (2019) *Phacotus lenticularis* content in carbonate sediments and epilimnion in four German hard water lakes (submitted for publication in Journal of Limnology, October 2019).

4.1 Abstract

Autochthonous calcite precipitation is an important process for C-fixation in hard water lakes, which is mainly induced by the photosynthesis of planktonic microorganisms. Among these, the widespread calcifying green alga *Phacotus lenticularis* (Ehrenberg) Diesing contributes to biogenic calcite precipitation in temperate regions. Its role in carbonate precipitation needs to be investigated, because there are no studies dedicated to the quantitative contribution of *Phacotus* shells to long term carbonate sequestration in hard-water lake sediments. In order to fill this gap, the *Phacotus* shell content in the sediments of four German hard water lakes was determined and compared to the fraction of *Phacotus* shells in the total suspended autochthonous calcite of the euphotic zone.

It was found that the *Phacotus* shells contributed at least 10 % to the autochthonous carbonate precipitation in the upper water column in three investigated lakes. During a *Phacotus* mass occurrence with a cell density of 1.8×10^6 Ind L^{-1} in Lake Hopfensee, even 59 % of the 3.6 mg L^{-1} total carbonate concentration consisted of *Phacotus* shells. In contrast to this high amount, the topmost basinal sediment contained a *Phacotus* shell content between 80 and 36,252 individuals per mg dry sediment, representing only 0.02 % to 2.28 % of the total carbonate sediment content. In a gravity core from Lake Grosser Ostersee, dating back $\sim$ 150 years, the *Phacotus* shell content was continuously below 0.24 % whereas the shell diameters remained equal to those of living individuals found in the water column proving that *Phacotus* shells are capable to persist in the sediment after deposition.

A main reason for the large discrepancy between *Phacotus* shell abundance in the euphotic zone and in sediment was found to be the gross authigenic carbonate precipitation, which dilutes the sedimenting *Phacotus* shells that accumulate exclusively during short and intensive population peaks in summer. Additionally, dissolution of the carbonate shells during sedimentation was proven to be a relevant factor in Lake Igelsbachsee by means of reducing the number of *Phacotus* shells reaching the lake bottom. These

facts explain that short-term high *Phacotus* carbonate contents of the total suspended carbonate in the water column do not mirror the contribution of *Phacotus* shells in the sedimentary record.

4.2 Author contributions

Sebastian Lenz (SL) was part of the team conceiving the study. Sediment core sampling, sample preparation and sediment analysis was done by SL at the Swiss Federal Institute of Aquatic Science and Technology, Department Surface Waters EAWAG in Duebendorf. The interpretation of the sediment core data was done by SL. Microscopic analysis was realized by graduate students under supervision of SL. The manuscript was drafted and finalized by SL.

4.3 Introduction

Autochthonous calcite precipitation induced by primary production is a common process in alkaline hard water lakes (Dittrich, Kurz and Wehrli, 2004; B. Müller *et al*, 2006; Stabel, 1986). Similarly, calcite precipitation occurs in surface waters as a result of the warming effects. This phenomenon is also well understood and has been applied in quantitative approaches determining fine sediment deposition Auerswald and Geist (2018). Biological calcite precipitation involves formation of carbonate shells by the planktonic calcifying phytoflagellate *Phacotus lenticularis* (Ehrenberg) Diesing (1866) (Guiry, 2019). *Phacotus lenticularis*, hereinafter abbreviated *Phacotus*, is a widespread alga that lives in temperate, subtropical and tropical regions and occurs worldwide in lime-rich alkaline stagnant inland waters (Schlegel *et al*, 1998).

The epilimnion *Phacotus* substantially contributes to the total suspended epilimnetic calcite precipitation, particularly during mass developments, due to its two lens-like calcite shells. The normal contributions ranges from 5–10 % and can sometimes reach 100 % (Gruenert and Raeder, 2014a; Koschel and Raidt, 1988a; Krienitz *et al*, 1993; Lenz *et al*, 2017). *Phacotus* is also supposed to be a significant source of carbonate in lake sediments as suggested by Kelts and Hsü (1978). Sedimentary records prove that *Phacotus* shells can be preserved and form dominating sediment fractions. The oldest reported occurrences of the calcareous shells were found in an early Miocene (126–138 ka BP) fresh water deposit in Öhningen in southwest Germany and in a middle Miocene (115–126 ka BP) limestone deposit, which had a 'particularly high occurrence' of *Phacotus* shells that it was referred to as '*Phacotus*-Kalk', in Jutland, Denmark (Lagerheim, 1902). In addition, a sediment core from the early Dryas (13–11 ka BP) found in south Argentina contained sediments, in which the calcite fraction almost exclusively consisted of *Phacotus* shells (Haberzettl *et al*, 2007; Jouve *et al*, 2013). G. Müller and Oti (1981) introduced a summary and a detailed description of the documented fossil *Phacotus* occurrences in Eurasia, including a fossil *Phacotus* occurrence in brackish sediments from the Miocene Ries crater (72–116 ka BP) in south Germany. However, all these findings offer only qualitative descriptions of the *Phacotus* records.

This study aimed to quantitatively evaluate the role of *Phacotus* in lacustrine calcite precipitation based on the quantitative assessment of the *Phacotus* shell fraction in the sediment and the water body of four German lakes. The percentage of suspended *Phacotus* shells in the total suspended autochthonous calcite in the epilimnion was determined and compared to the *Phacotus* shell amount in the corresponding sediment deposits. It was hypothesised that *Phacotus* shells significantly contribute to the lacustrine 'carbonate sequestration', because carbon is mineralised as carbonate and sequestered over long geologic timescales.

4.4 Materials and Methods

Study site

Four Bavarian lakes (Tab. 4.1) with documented occurrences of *Phacotus* in the last ten years (2005–2015) were investigated: Lake Grosser Ostersee (GOS), Lake Abtsdorfer See (ABS), Lake Igelsbachsee (IGS), and Lake Hopfensee (HOP) (Fig. 4.1). They are all stratified natural hard water lakes located in the pre-alpine basin in south Bavaria, Germany, except IGS, which is a Ca-rich artificial reservoir lying in a basin of late Triassic quartz sandstones surrounded by Jurassic carbonates. All other lakes have carbonate dominated catchments, are fed with alkaline water from the Northern Limestone Alps, and are a part of an open quaternary aquifer of predominantly fluvioglacial sediments with intercalated basal moraine tills that overlay a Tertiary sandstone aquitard.

Table 4.1. Limnologic characteristics and sampling locations at the deepest point of the examined hard water lakes.

	Gr. Ostersee	**Abtsdorfersee**	**Igelsbachsee**	**Hopfensee**
Trophic state	Oligotrophic	Mesotrophic	Mesotrophic	Eutrophic
Circulation type	Dimictic	Dimictic	Polymictic	Dimictic
Surface area [ha]	118	78	72	186
Max. depth [m]	29.7	20.0	11.5	10.4
Volume [100 m^3]	14.0	9.4	3.9	8.9
Calc. residence time [d]	247	252	-/-	128
Coordinates	$47°47'25,2''$	$47°54'35,4''$	$49°08'45,3''$	$47°36'06,1''$
(WGS 84)	$011°18'06,5''$	$012°54'22,4''$	$010°54'13,3''$	$010°40'46,6''$

Water samples for *Phacotus* abundance and total epilimnetic particulate calcite analysis (Fig. 4.2) were collected on a weekly basis from the surface water (0–7 m depth) at the deepest point of each lake following the method described by Lenz *et al* (2017). Depth profiles of water temperature and pH were measured *in situ* with a WTW MPP 930 mulitprobe. The concentrations of Ca^{2+} in solution were determined following the method described in Gruenert and Raeder (2014a). Particulate $CaCO_3$ concentration was measured using infrared CO_2 analysis on a Saxon Junkalor Infralyt 50 gas analyser (Lenz *et al*,

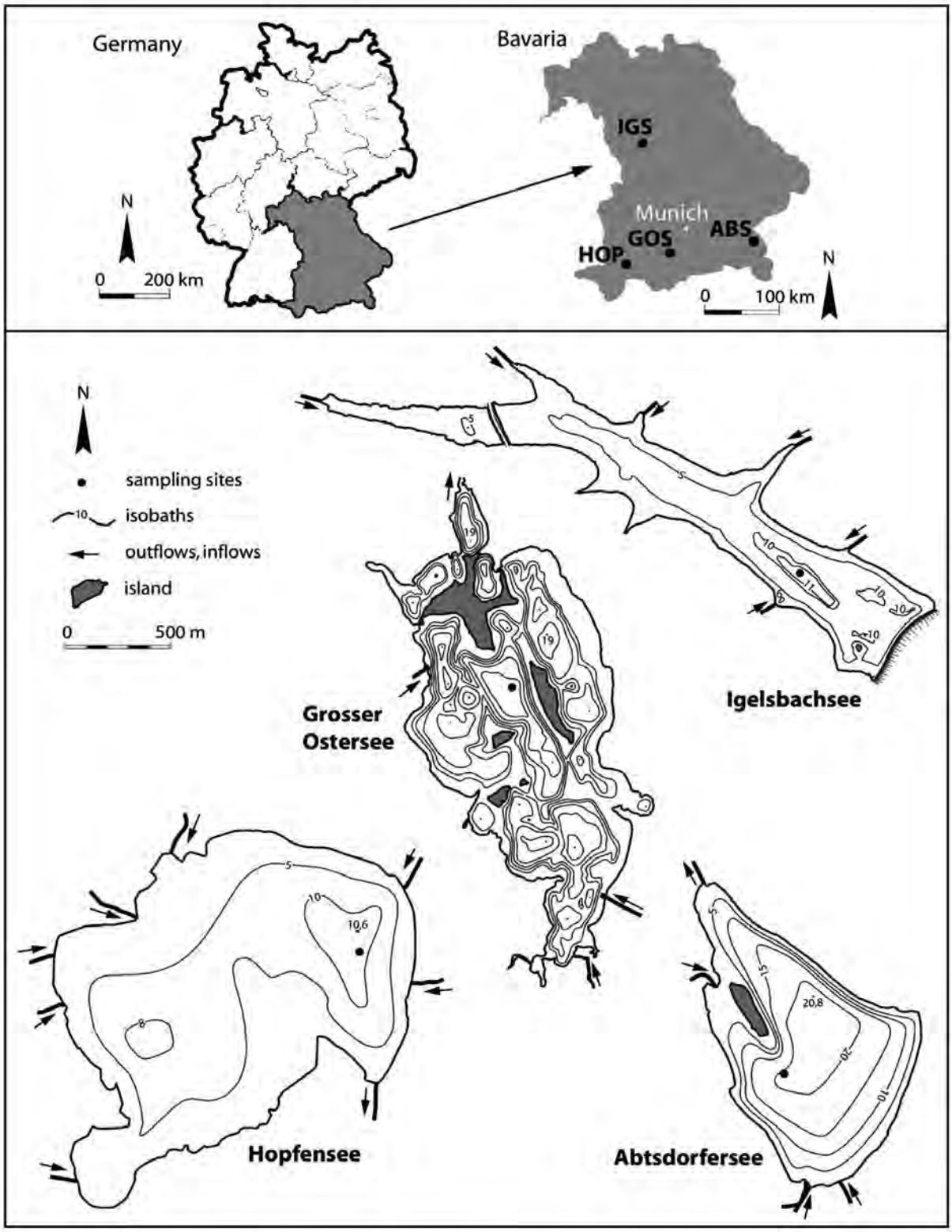

Fig. 4.1: Locations of the study sites in Bavaria, Germany: bathymetry and sampling points at Lake Igelsbachsee (IGS), Lake Grosser Ostersee (GOS), Lake Hopfensee (HOP), and Lake Abtsdorfersee (ABS).

2017), and the plankton abundance was determined with an inverted microscope Leitz Labovert at 200× based on the method mentioned in Utermöhl (1958).

A scientific diver used a tube from a PVC Uwitec gravity corer to collect samples from the surface sediment, i.e. the topmost material from the deepest point of each lake. The upper 4 cm of the composite samples were examined, in order to obtain a representative mean value over several years to diminish the effects of annual variance of *Phacotus* populations and to include carbonate dissolution in the oxic part of the sediment. An aliquot of each sample was suspended in 10 mL buffered suspension liquid (NH_3 + H_2O) with a pH of $\geq$ 8.5 (Bollmann *et al*, 1999). The sediment was mildly dried and carefully homogenised in a mortar. A 0.50-mg homogenised sample mixed with 2 mL of the suspension liquid was transferred into an Eppendorf tube. Next, 0.50 mg of borosilicate microspheres (ThermoFisher Scientific #9015) were used as calibration standard, and the disaggregation of the carbonate aggregates was conducted using a Bandelin Sonorex TK52 (Berlin, Germany) ultrasound bath (one minute, 150 W, 35 kHz) following the method used by Bordiga *et al* (2015). Finally, the sample was mixed using a CHS Vortex homogeniser (Praha, Czech Republic).

A 50-μL sample of the suspension was obtained during the mixing and placed into the centre of a circular coverslip using a laboratory pipette according to the 'drop' method (Bordiga *et al*, 2015; Koch and Young, 2007). This process was replicated five times for each sample. The exact mass of sediment ($m_{sed\_DG\_soll}$) on the coverslips was calculated from the total amount of microspheres on them. Shell number was visually determined for each coverslip using light-microscopy with polarised light at 200× magnification. Intact Phacotus individuals were counted as two shells whereas single shells were counted as half individuals. Rare broken shells were counted as an entire shell, if the fragment was more than half of a shell to avoid overestimation of the *Phacotus* shell abundance. The *Phacotus* shell fraction in the sediment w_{Phacotus} [%] was calculated using the product of shell number and lake specific mean shell mass relative to the total sediment and carbonate mass on the entire coverslip, respectively, according to the method described by Lenz *et al* (2017).

A sediment core from GOS (GOS-06) was selected for further analysis and dating. The core samples were collected using a UWITEC gravity corer (Mondsee, Austria) at the deepest point of the lake in 2017. The core was split lengthwise, lithologically described, and subsequently sub-sampled at 1 cm resolution. One half of the core was used for dating, elemental analysis, and sediment size distribution at Eawag in Dübendorf, Switzerland. The freeze-dried samples were carefully homogenised and dated through ^{137}Cs and ^{210}Pb activity measurements using a Canberra Ge well detector (San Ramon, California) according to the methods described in Appleby (2001) and McGowan *et al* (2015). The sedimentation rate of ^{210}Pb was determined after subtracting the ^{226}Ra activity using a constant flux–constant sedimentation rate model. The elemental analysis of total carbon (TC) and total nitrogen (TN) was conducted using a Hekatech Euro EA 300 elemental analyser (Wegberg, Germany). Total inorganic carbon (TIC) was determined using a UIC Coulometer CM 5015 (Joliet, Illinois). Total organic carbon (TOC) was calculated by subtraction of TIC from TC. The carbonate content ($CaCO_3$) was calculated

from TIC through multiplication by the ratio of the molecular weights of $CaCO_3$ and C (100.09 g mol^{-1} and 12.01 g mol^{-1}, respectively). Total carbon accumulation rate $R_{acc\ TC}$ [g cm^{-2} a^{-1}] was derived from the total carbon fraction TC [wt%] of the sediment mass accumulation rate $R_{acc\ sm}$ [g cm^{-2} a^{-1}] (Formula (eq. 4.1)). $R_{acc\ sm}$ of each sample was individually calculated through the multiplication of sediment density ρ_{sed} [g cm^{-3}] by the sedimentation rate R_{sed} [cm a^{-1}] (Formula (eq. 4.2)).

$$R_{acc\ TC} = R_{acc\ sm} \cdot (TC) \tag{4.1}$$

$$R_{acc\ sm} = \rho_{sed} \cdot R_{sed} \tag{4.2}$$

The particle size analysis was performed on wet unprocessed sediment from the sediment core (GOS-05) that was obtained at the same location and date as GOS-06. It was also stored and divided in the exact same manner. To ensure comparability, both cores were lithologically described and cross-correlated based on their lithological description. Samples were disaggregated using Na-Pyrophosphate and ultra sound, and then particle size analysis was conducted with a Malvern (Malvern, UK) Mastersizer 2000.

The second half of GOS-06 was used to determine the *Phacotus* shell content using the previously described method for surface sediment samples. Additionally, *Phacotus* shell diameter measurements were performed on 136 evenly distributed shells over the complete sediment core using Kappa Opto-Electronics & AccuSoft (Gleichen, Germany) camera software Kappa ImageBase (2007). Micrographs were analysed with FiJi ImageJ image analysis software (Rasband, 2016) using two perpendicular diameter measurements, and the selected shells were examined under scanning electron microscope (SEM) at the Limnological Research Station of TU Munich, Germany, in order to detect potential dissolution features.

4.5 Results

4.5.1 Suspended carbonate in lake water and *Phacotus* fraction

The amount and temporal variation of total suspended carbonate in the lake water and the contributing *Phacotus* fraction measured during the period of the strongest *Phacotus* growth, which took place between June and August 2016, were found to be different in the four investigated lakes (Fig. 4.2). In the eutrophic HOP, the total carbonate concentration reached its highest level at 3.5 mg L^{-1}. In the other three, the values were always below 0.7 mg L^{-1}. Furthermore, in HOP during a *Phacotus* bloom, the cell density was 1.8×10^6 Ind L^{-1}. The *Phacotus* carbonate temporal contribution to the total suspended carbonate in the epilimnion was measured during this period to be 59 %. In ABS, two *Phacotus* growth peaks were observed with a maximum cell density of 183,400 Ind L^{-1} during the first peak when *Phacotus* represented 34 % of the total suspended carbonate. In GOS, the first population peak had a lower maximum cell density of 84,200 *Phacotus* L^{-1} at the end of June, during which the *Phacotus* shells contributed 19 % to the total suspended carbonate in the epilimnion.

In HOP, ABS, and GOS, the pH values and conductivities of the lake water indicated permanent carbonate oversaturation throughout the entire depth of the water column (Tab. 4.2). These conditions did not prevail in IGS, where the actual amount of *Phacotus* could not be accurately recorded, due to the dissolution of *Phacotus* shells caused by the low Ca^{2+} concentrations. This was observed by counting *Phacotus* shells of two identical plankton samples at different times. Cell densities measured immediately after the fieldwork were found to be around 5×10^4 Ind L^{-1}, while *Phacotus* shells disappeared after one month. Therefore, IGS represents a lake with *Phacotus* population in the water column, but with almost no sedimentary *Phacotus* records, because the shells dissolve before being preserved in the sediment.

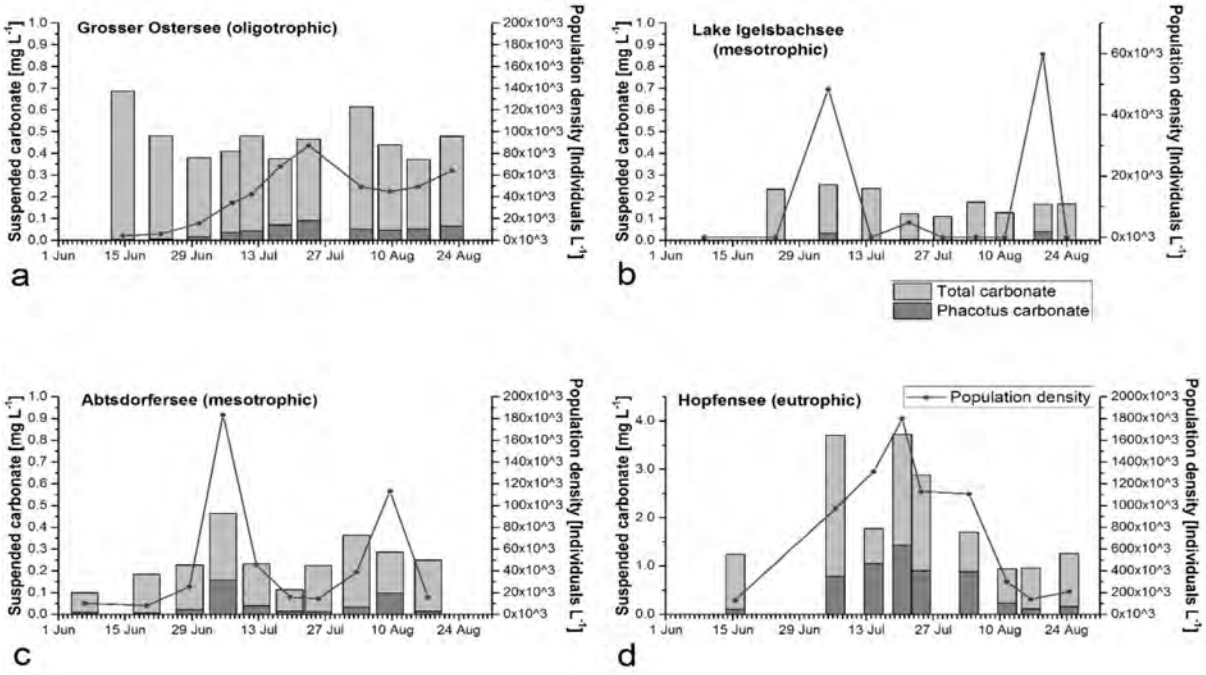

Fig. 4.2: Total suspended epilimnetic carbonate (white bars) and *Phacotus* carbonate fraction (grey bars) with the corresponding *Phacotus* population density (data points) during the growth period in the summer of 2016.

4.5.2 Surface sediment analysis

Surface sediment analysis revealed that the four lakes had significantly different total carbonate contents (Tab. 4.2). This strongly affected the *Phacotus* fraction of the total carbonate sediment with *Phacotus* shell content ranging from 80 (IGS) to 36,252 (HOP) individuals per mg dry sediment [Ind mg^{-1}] in the topmost 4 cm of basinal sediment. This represents total *Phacotus* carbonate content of 0.02 % and 2.28 % in IGS and HOP, respectively. The other two lakes (GOS and ABS) had intermediate *Phacotus* shell contents of 3,255 and 24,401 shells per mg dry sediment, respectively, and the *Phacotus* fraction contributed 0.16 % and 2.45 %, respectively, to the total carbonate in the sediment.

Table 4.2. *Phacotus lenticularis* (P.l.) fraction in surface sediment (0–4 cm) and surface water (0–7 m) with the corresponding epilimnetic physical chemical water conditions (standard deviation indicated between brackets).

	Gr. Ostersee	**Abtsdorfersee**	**Igelsbachsee**	**Hopfensee**
Sediment from deepest point of the lake (0–4 cm):				
P.l. shells [Individuals mg^{-1}]	3,255 (1,074)	24,401 (603)	80 (22)	36,252 (1,539)
P.l. fraction sediment [%]*	0.15 (0.06)	1.05 (0.03)	0.00 (0.00)	1.45 (0.06)
$CaCO_3$ fraction sediment [%]	88.8	42.8	12.5	63.6
P.l. fraction in total carbonate sediment [%]*	0.16 (0.06)	2.45 (0.06)	0.02 (0.01)	2.28 (0.10)
Suspended carbonate from water column (0–7 m): average values of water, June-August 2016				
Mean *P.l.* fraction in suspended carbonate in water [%]*	9.9 (6.1)	13.9 (10.6)	4.3 (7.9)	29.0 (16.9)
Water temp. (°C)	17.4 (4.7)	15.5 (5.2)	20.9 (2.7)	17.3 (2.8)
pH value	8.2 (0.2)	7.9 (0.4)	8.6 (0.5)	8.2 (0.4)
Ca^{2+} conc. (mg L^{-1})	69.2	78.2	37.6	69.3
TP (μg L^{-1})	> 8.2	> 17.4	> 25	> 35
Secci depth (m)	3.3 (0.6)	1.1 (0.2)	3.0 (0.6)	1.1 (0.3)

TP data from Bavarian Environmental Agency, Ca^{2+} concentration from last sampling in August 2016

*the lake specific P.l. shell mass according to Lenz *et al* (2017) was used for mass calculation

4.5.3 Sediment core from Lake Grosser Ostersee

The age model (Fig. 4.3) is based on ^{137}Cs-activity measurements and confirmed by the sedimentation rate derived from the ^{210}Pb-activity measurements. In the topmost section, R_{sed} was determined through the interpolation between the surface (2016), the ^{137}Cs peaks (1963 and 1986), and the first signal of ^{137}Cs activity (1954). The time determination for 1963 was set at the depth of 11.5 cm below the actual ^{137}Cs-activity peak, because the coherent lamination and varve thickness were better fitted with the rest of the sediment core, suggesting that Cs probably has been remobilised in the sediment. In the section from 1954 (13.5 cm) to 1925 (19.5 cm), the age of the samples was determined by counting the biochemical varves downwards. In the non-laminated section above 19.5 cm, the ^{210}Pb a 0.21-cm a^{-1} sedimentation rate was used, resulting in a basal age of $\sim$ 150 a. As sampling was conducted in 1.0 cm steps, every sample represented four to five years.

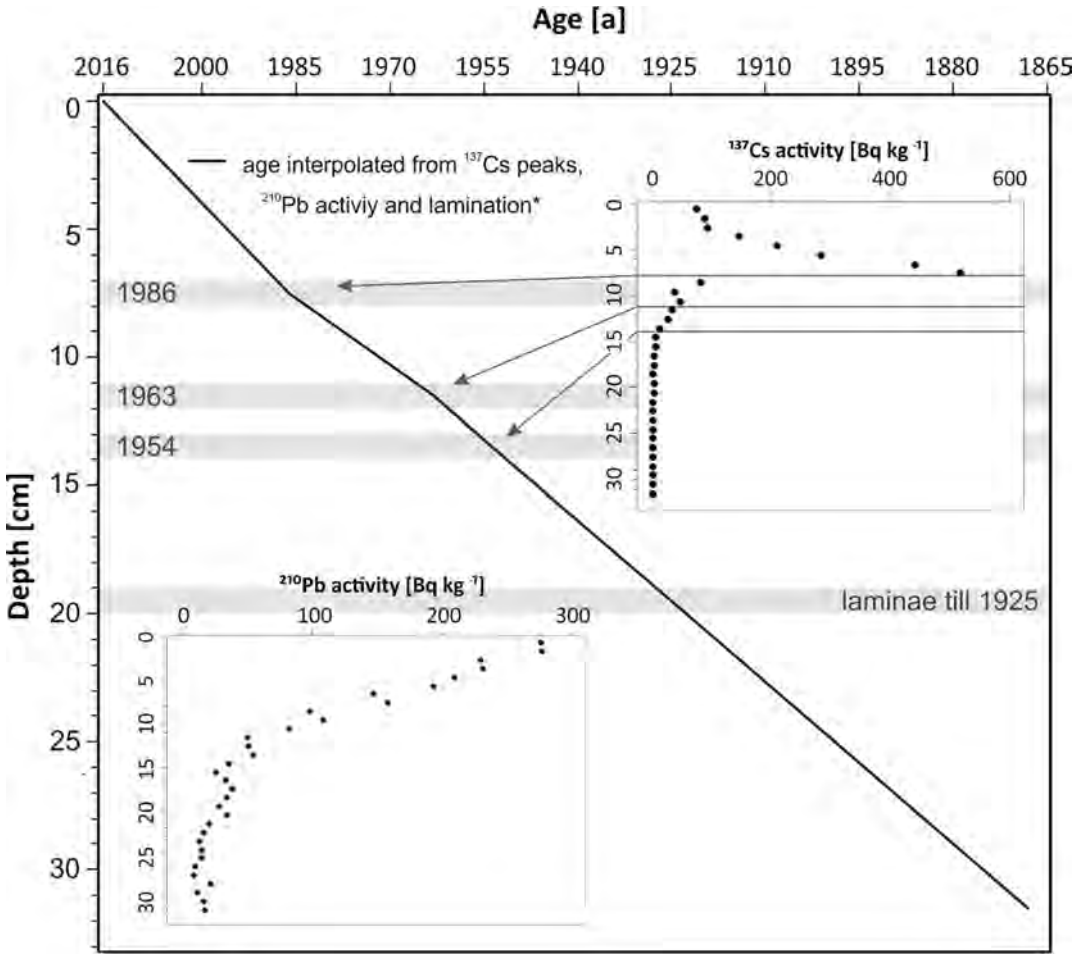

Fig. 4.3: Age-depth model for the GOS-06 sediment core with activity depth profiles for ^{137}Cs and ^{210}Pb.

The core was subdivided into three lithological units: A (0–10 cm), B (10–19 cm), and C (19–31.5 cm) (Fig. 4.4). The sedimentologic characteristics of the three main core units are listed in Tab. 4.3. The sediment of unit C appeared dark grey with few dark streaks and no lamination. At the transition from the unstratified unit C to layered unit B, a steady decline of the mean TOC/TN ratio from the maximum value of 14.6 (20.5 cm) to 9.4 (17.5 cm) indicated a loss in the input of external organic carbon from vascular plants (Fig. 4.5). Unit B was laminated with approx. 40 varve cycles of decreasing thickness. At the transition from unit B (10.5) to unit A (9.5) cm, several proxies changed. Below 10.5 cm, approximately 16 varve cycles showed regular undisturbed sedimentation. From this point upward, the sediment got darker accompanied by a steady rise in the TOC from 1.8 wt% (11.5 cm) to reach its maximum value of 3.1 wt% (9.5 cm). Simultaneously, the $CaCO_3$ content declined to its minimum of 81 wt% (9.5 cm). The well-preserved core section terminated at this point with a dark 2 mm thick autumn-winter layer (10.2–10.0 cm). From 10.2 cm upward, there were four duplets of diffuse and unclear preserved laminations. The grey-beige unit A was found to be sandier. In the section from 10 to 8 cm, the sand fraction increased (+7 wt%) at the expense of the silt fraction. In this section, endobenthic bottom dwellers and plant macro remains were occasionally found

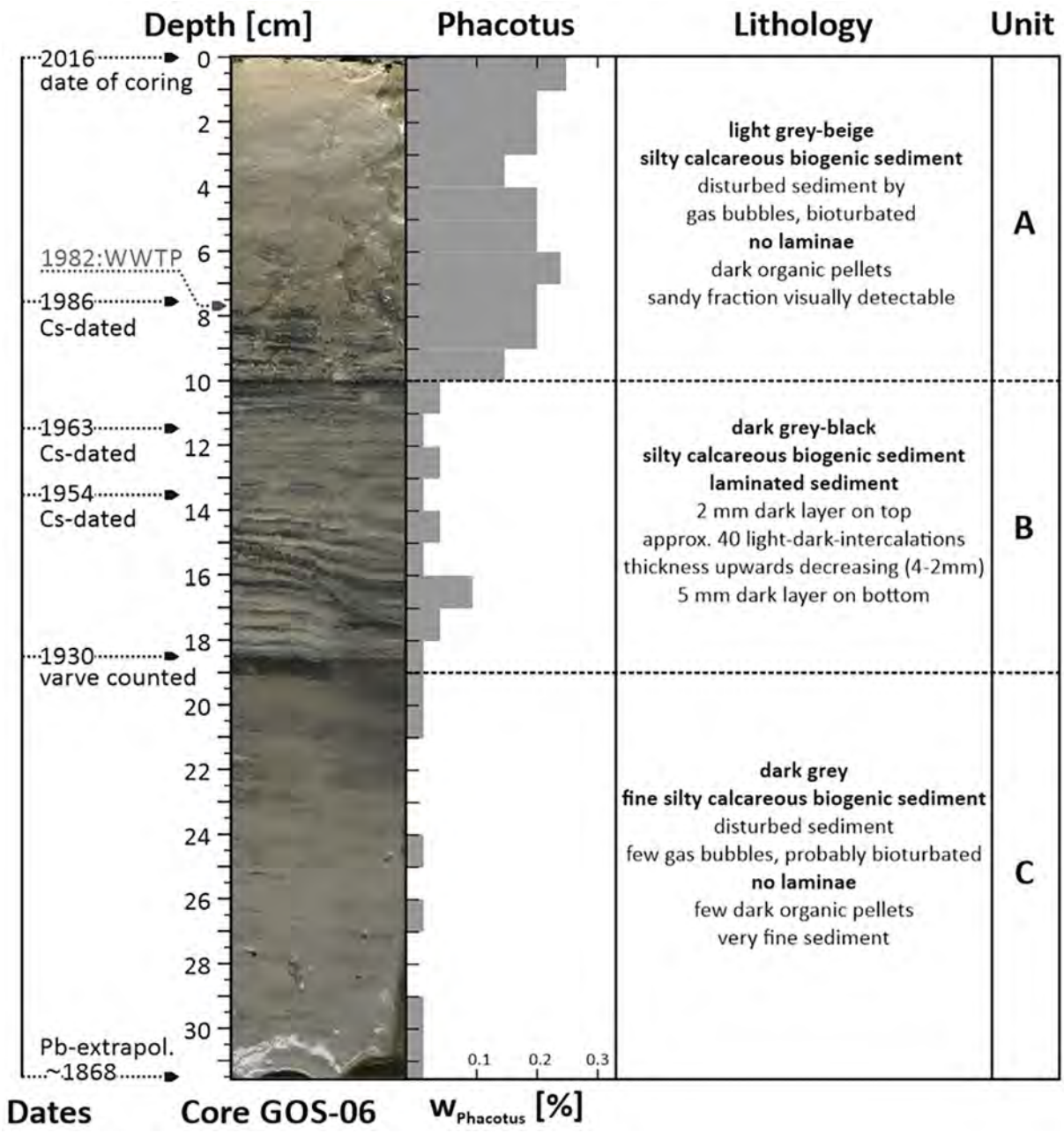

Fig. 4.4: Photograph of the GOS-06 core with lithology and radioisotopic dates from Lake Grosser Ostersee, and the maximum *Phacotus* shell abundance w_{Phacotus} [wt%] is given as weight per cent of dry sediment. (The arrow pointing at 1982 indicates the operation start of a wastewater treatment plant (WWTP)).

in other cores from the same location. Additionally, cavities from gas bubbles were found at 6.0 to 7.5 cm depths.

The GOS-06 sediment core was 31.5 cm long (Fig. 4.4) and showed a continuous record of biogenic silty pelagic sediment with a high carbonate content of more than 87 wt% $CaCO_3$ and a low TOC content of less than 3 wt%. The SEM sediment examination confirmed that the laminations were biochemical non-glacial varves. No allochthonous input was assumed at the deepest point of GOS, because the lake has no natural superficial

inflow and the central basin is surrounded by several smaller basins (Fig. 4.1). This assumption was confirmed by the low mean TOC/TN ratio of 10.2 (standard deviation $SD = 1.8$) (Fig. 4.5).

Table 4.3. Summary of the analysis of the sediment from the GOS-06 core divided into three lithological units: A, B and C.

Unit (depth)	**A (0–10 cm)**	**B (10–19 cm)**	**C (19–31.5 cm)**
year	2016–1970 (46 a)	1970–1927 (43 a)	1927–1868 (59 a)
trophic state of the lake*	Meso-/Oligotrophic	Mesotrophic	Oligotrophic
sand fraction [%]	15.5 ± 2.7	7.6 ± 1.8	6.1 ± 1.2
silt fraction [%]	79.2 ± 2.2	85.3 ± 0.8	86.7 ± 0.8
clay fraction [%]	5.3 ± 2.2	7.1 ± 1.1	7.2 ± 0.6
total organic carbon [%]	2.5 ± 0.3	2.0 ± 0.2	1.8 ± 0.4
carbonate content [%]	87.7 ± 3.3	89.7 ± 1.6	90.0 ± 2.9
Phacotus shells [Individuals mg^{-1}]	3286 ± 903	327 ± 292	34 ± 77

*(Gruenert and Raeder, 2014a; Melzer, 1976)

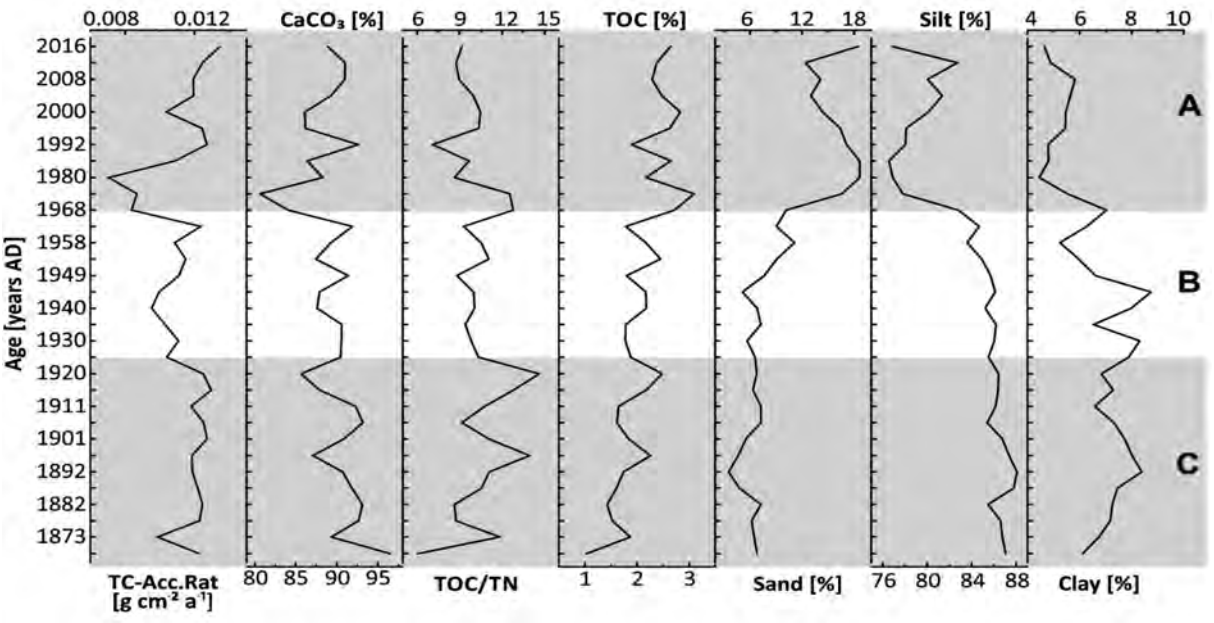

Fig. 4.5: Geochemical and grain size distribution profiles of the GOS-06 core from Lake Grosser Ostersee: all element content [wt-%] is given as weigh per cent of dry sediment, and the Total Carbon Accumulation rate (TC-Acc.Rate) is given in [g C m^{-2} a^{-1}].

w_{Phacotus} was determined relative to the total dry sediment mass, which ranged from 0.00 to 0.24 wt% (Fig. 4.4). The threefold division of the sediment core units was also reflected in terms of the *Phacotus* shell content. The units C and B contained very low amounts of *Phacotus* shells ranging between 34 and 327 Ind mg^{-1} (Tab. 4.3). In contrast,

in unit A the *Phacotus* shell fraction was ten times higher and accounted for 3286 Ind/mg. This represented a w_{Phacotus} of 0.24 wt% of the total dry sediment mass.

Micrographs were used for diameter a w_{Phacotus} of 0.24 wt% measurements during microscopic analysis (Fig. 4.6) and to check the shells for indications of carbonate dissolution. The mean diameter of 136 *Phacotus* shells from evenly distributed core depths was 13.7 μm ($SD = 1.7$). This value was slightly higher than the mean diameter that was determined for living *Phacotus* shells in GOS (13.6 μm, $SD = 0.9$) (Lenz *et al*, 2017).

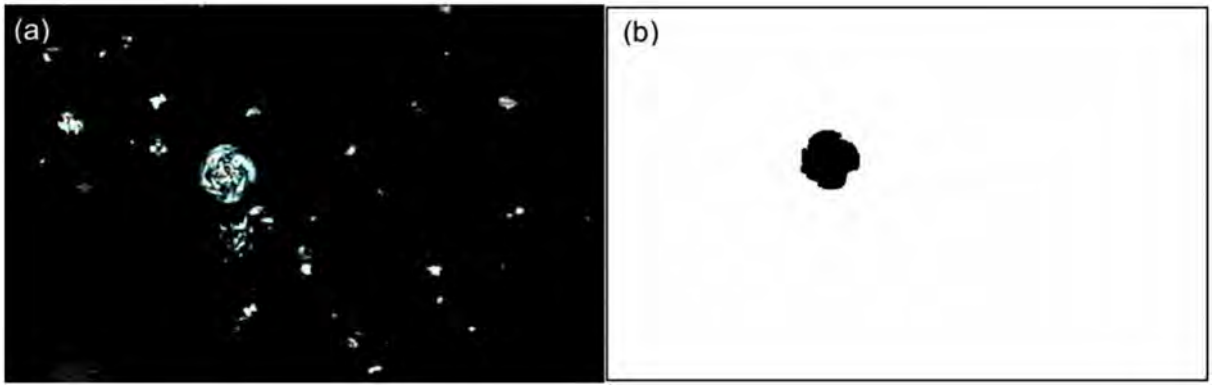

Fig. 4.6: Micrograph of sediment sample from 0.5 cm of the GOS-06 sediment core under crossed nicols (a) and the corresponding picture for diameter measurements after processing with ImageJ Fiji software (b).

4.6 Discussion

The hypothesis that *Phacotus* shells represent a considerable part of carbonate sequestration in lakes was confirmed in part of the samples, in which the calcite shells represented over 10 % of the autochthonous calcite precipitation during the summer season in the investigated hard water lakes.

Several aspects influence the *Phacotus* shell abundance in the water column and in the sediments, including (1) the difference in the specific timeframes of reference between water and sediment samples; (2) the dilution of *Phacotus* shell carbonate by the overall carbonate sedimentation; (3) the dissolution of *Phacotus* shells during sedimentation, which played a subordinate role with the exception of IGS (Fig. 4.2); (4) the reduction of *Phacotus* shell abundance in the lakes sediment, which may be caused by dissolution after deposition, such as in IGS .

For the first aspect (1), Tab. 4.2 shows the large discrepancy between *Phacotus* shell abundance in water and in sediment. This difference lies in the timeframe of reference. For example, water samples from HOP, which represent a single sampling day, contained a *Phacotus* shell fraction of 29 % of the mean suspended calcite, whereas in the sediment samples representing approximately 15 years of carbonate sedimentation, *Phacotus* shells represented only 2.28 % of the total carbonate sediment.

The second aspect (2) indicates that the low *Phacotus* fractions in lake sediments were caused by ‘dilution’. The *Phacotus* populations showed strong growth and high abund-

ances in midsummer (Fig. 4.2), but these periods were generally short (Gruenert and Raeder, 2014a; Koschel *et al*, 1987b; Krienitz *et al*, 1993; Lenz *et al*, 2017). Therefore, the contribution of *Phacotus* shells to the carbonate sediment remains low in comparison to the overall autochthonous biogenic calcite. In spring and late summer, the autochthonous carbonate precipitation induced by diatoms and cyanobacteria blooms (Dittrich *et al*, 2004) is the source of most sedimented carbonate in lakes (Kelts and Hsü, 1978). However, charaphyceans, their crushed stem casts, and oogonia can also be transported as suspended particles from the littoral zone as a result of wind induced wave activity (Emi, 2001), and they can contribute to the carbonate sequestration in the deepest lake sectors. This was especially the case in GOS, where the GOS-06 sediment core contained a low *Phacotus* shell content of not more than 0.24 % which equals an amount of 4593 shells per mg. These low *Phacotus* contents at this location might have resulted from a combination of several factors. The oligotrophic lake with limited primary production showed a high abundance of the picocyanobacterium Synechococcus (Ruber *et al*, 2018), which contributed to intense autochthonous carbonate precipitation (Dittrich *et al*, 2004; Dittrich, Müller, Mavrocordatos and Wehrli, 2003), resulting in an extraordinary high total carbonate content in the sediment ($> 89\,\%$). In addition to the generally moderate *Phacotus* population, the sediment had a very low *Phacotus* content. Nevertheless, *Phacotus* shells were always present over the complete depth of the sediment core. From 1970, the *Phacotus* shell abundance rose to the maximum value of 0.24 % in the topmost sediment layer. The lowest *Phacotus* shell contents were found in the deepest core in unit C, not overpassing 0.02 % which equals an amount of less than 290 shells per mg. From this point upward, the *Phacotus* shell content gradually increased (Fig. 4.4).

Aspect (3) indicates that *Phacotus* shells dissolve in the water column during sedimentation. This aspect was not confirmed and seemed unlikely in the majority of the investigated lakes. For example the groundwater-fed and relatively cold pre-alpine GOS is characterised by constant high alkalinity with permanent carbonate super saturated conditions. In contrast, in IGS, practically no shells were detected in the sediment, although in the epilimnion, numerous *Phacotus* shells had been observed (Fig. 4.2b). Shells from sediment trap experiments at the bottom of IGS (Lenz *et al*, 2019) showed partly dissolved *Phacotus* shells, which was interpreted as an evidence of the carbonate dissolution conditions in the water.

However, aspect (4), which is related to the dissolution of carbonate particles, has to be considered in the sediment. Under oxic conditions, which were indicated by living bottom dwellers found in the bioturbated sediment core sections, carbonates are likely to dissolve, because the decomposition of organic matter provides free protons that lead to the dissolution of carbonate particles (B. Müller *et al*, 2006). For example, based on a sedimentation rate of 0.2 mm a^{-1}, the residence time for sedimented carbonates in the oxic sediment zone of GOS-06 sediment core was found to be approximately two to three years. Therefore, carbonate dissolution was likely to occur in the topmost sediment of GOS. However, the embedded *Phacotus* shells were not alterated. The value of their diameter over the complete depth of the sediment core remained constant at 13.7 μm ($SD = 1.7$), which was also within the same range as the living *Phacotus* shells that

were analysed from the water column (Lenz *et al*, 2017). Furthermore, light microscopic images showed that, in the epilimnion of GOS, small calcite particles ($< 2\,\mu$m) were present in abundance, which were not observed during microscopic examination of the lakes sediment. This result is in accordance with the findings of B. Müller *et al* (2006), which proposed that dissolution in aerated hard water lake sediments preferentially take place at the expense of smaller calcite crystals, due to their elevated weight-specific surface area, instead of reducing the amount of *Phacotus* shells.

4.7 Conclusion

The abundance of *Phacotus* shells in lacustrine sediments is mainly affected by total carbonate sedimentation. Although the dynamic population development of *Phacotus* in the water column temporally provides the *Phacotus* carbonate contents with 59 % of the total suspended carbonate, in the sedimentary record, only low total sediment carbonate contents could be observed. Dissolution can reduce the number of *Phacotus* shells present in the water column before they reach the ground. However, once embedded in the anoxic section of the basinal sediment, shells were found to be preserved in the investigated sediment cores with shell diameters in the same range of recent living *Phacotus* individuals.

Quantitative data are necessary to accurately define the '*Phacotus*-rich' sediments as described by Haberzettl *et al* (2007), Jouve *et al* (2013) and their formation mechanisms. The two most probable mechanisms are supposed to be formation as a result of primary concentration in the water column due to wind action in littoral zones and formation resulting from the secondary concentration caused by the dissolution of smaller carbonate particles around the *Phacotus* shells.Dissertation Sebastian Lenz: Contribution of the calcifying green alga *Phacotus lenticularis* to lake carbonate sequestration.

5 | General Discussion

In order to investigate the role of *P. lenticularis* in hard water lakes in regard to its unique capacity of forming a calcite shell as an outstanding part of limnic phytoplankton, this work addresses the essential aspects for the assessment of its contribution to long-term C-fixation. At first, the lack of precise data on the mass of *Phacotus* shells made it necessary to investigate the natural morphometric variation as a starting point for all further calculations. The newly developed methodology assessing both, volume and mass, represents a new approach on microparticle examination that offered at the same time new insights on the internal microstructure of the calcite shell of *P. lenticularis*. Starting from this basis, the hypotheses presented in the Objectives section of this work (Chapter 1), are discussed across chapters in the light of the aspects variance of morphometry and occurrence, relevance and transferability to other lakes.

5.1 Variance of morphometry and occurrence of *P. lenticularis*

The variability of *P. lenticularis* shells poses a challenge to the investigation of its contribution to carbonate sequestration under various aspects. On the one hand, *Phacotus* shells differ in diameter and morphology, leading to different shell masses (Chapter 2) while on the other hand, the method and frequency for assessing *Phacotus* cell numbers in the water column must be adequate, since the individual density can vary strongly both in time and space (Chapter 3) to obtain a representative mean value. Furthermore, the proportion of shells in the lake sediment is also highly variable (Chapter 4). In all three previous publications, the variance of *P. lenticulais'* morphometry and occurrence is a connecting element that had to be addressed specifically.

5.1.1 Modificatory variability

During the detailed examination of the shells, it was noticed that the diameter and other shell characteristics vary significantly. Whether the reasons are of environmental or genetic origin, is still largely unexplored. The present study has shown that the origin of different lakes significantly affects the diameters of the shells ($p = 0,007$), obviously different conditions in different lakes are responsible for the varying *Phacotus* shells. Additionally, a temporal variability of the shell diameter in the course of population development (Fig. 2.3) was observed. The differences were only moderate but not significant ($p = 0,190$). In the majority of the lakes, the mean value of the shell diameter increased by up to 20 % between June and August (Chapter 2). However, it seems that the reasons for this are not the changing environmental conditions that are the formation of a stable stratification and rising water temperatures with higher carbonate super saturation. Rather at the

beginning of the vegetation season when more adolescent cells with smaller shell diameters (Giering *et al*, 1990b)keep the mean values low in June whereas in August the proportion of larger adult individuals is higher.

The morphometric survey on shell height, thickness and diameter in different lakes (Tab. 2.1) as well as morphological observations (Fig. 5.1) illustrated the lake-specific differences in shell architecture. Only the new method using the Focussed Ion Beam (FIB) technology provided the necessary insights to describe the natural variance of the *Phacotus* shells in such detail. The ratio of shell thickness to shell diameter was found to be a descriptive characteristic value for *Phacotus* shell morphology. For example the architecture of the shells from Lake Hopfensee $t_{\min}/d$ with 0.045 ($SD = 0.010$) is filigree compared to the squat shells from Lake Grosser Ostersee with a $t_{\min}/d$ ratio of 0.075 ($SD = 0.011$). These differences resulted in shell masses ranging from 0.33 ng in Lake Igelsbachsee, over 0.40 ng in Lake Hopfensee to 0.52 ng in lake Grosser Ostersee (Tab. 2.2). Accordingly, the hypothesis that the calcified shells of *P. lenticularis* can be characterized by identical calcium carbonate contents cannot be confirmed without a differentiation between the temporal and the special aspect of variation. While the formerly described variations of *Phacotus* shell diameters over the growth period allow the use of a representative mean value, whereas it seems necessary to investigate and use lake-specific shell masses.

5.1.2 Genetic variability

During the examination of numerous individuals from the different lakes it was obvious that their shape and symmetry differed. A comparison of the surface structure next to the cross sections of the examined shells was possible through the study under the Focussed-Ion-Beam (FIB). Different phenotypes were encountered between the lakes but also within the same lake. The differences could be due to varying margin width, shell thickness and shell surface (Fig. 5.1).

After finding different phenotypes in the same lake, it was suspected that genetic differences in the alleles of individual strains caused variable organismic controlled calcification of the *Phacotus* shell and four morphotypes were described (Schlegel, Koschel and Krienitz, 2000). The investigated shells of the present work can be assigned to three of these morphotypes. However, a corresponding classification does not seem to make sense at this point because the impression was that the different forms and surface structures of the *Phacotus* shells have originated, at least partially, due to different stages of shell development (Hepperle and Krienitz, 1996; Koschel and Raidt, 1988a). It was that young individuals have a particularly uneven surface structure with recognizable individual tabular calcite crystals (Fig. 5.2a). The shells have smaller diameters because the edge is not yet formed in its full width. Only after the complete length of the shell edge has been fromed, the thickness of the shell increases by consecutive external deposition of "micritic" calcite, i. e. the superfine-grained calcite, lacking a specific crystal structure. This only leads to an initially smooth appearing pore-penetrated shell surface (Fig. 5.2b) with increasing maturity of the individual, which subsequently increases in thickness until fewer and fewer pores remain visible. Indeed, only the larger (adult) individuals actually showed flat or smooth surfaces, often without recognizable pores (Fig. 5.2c).

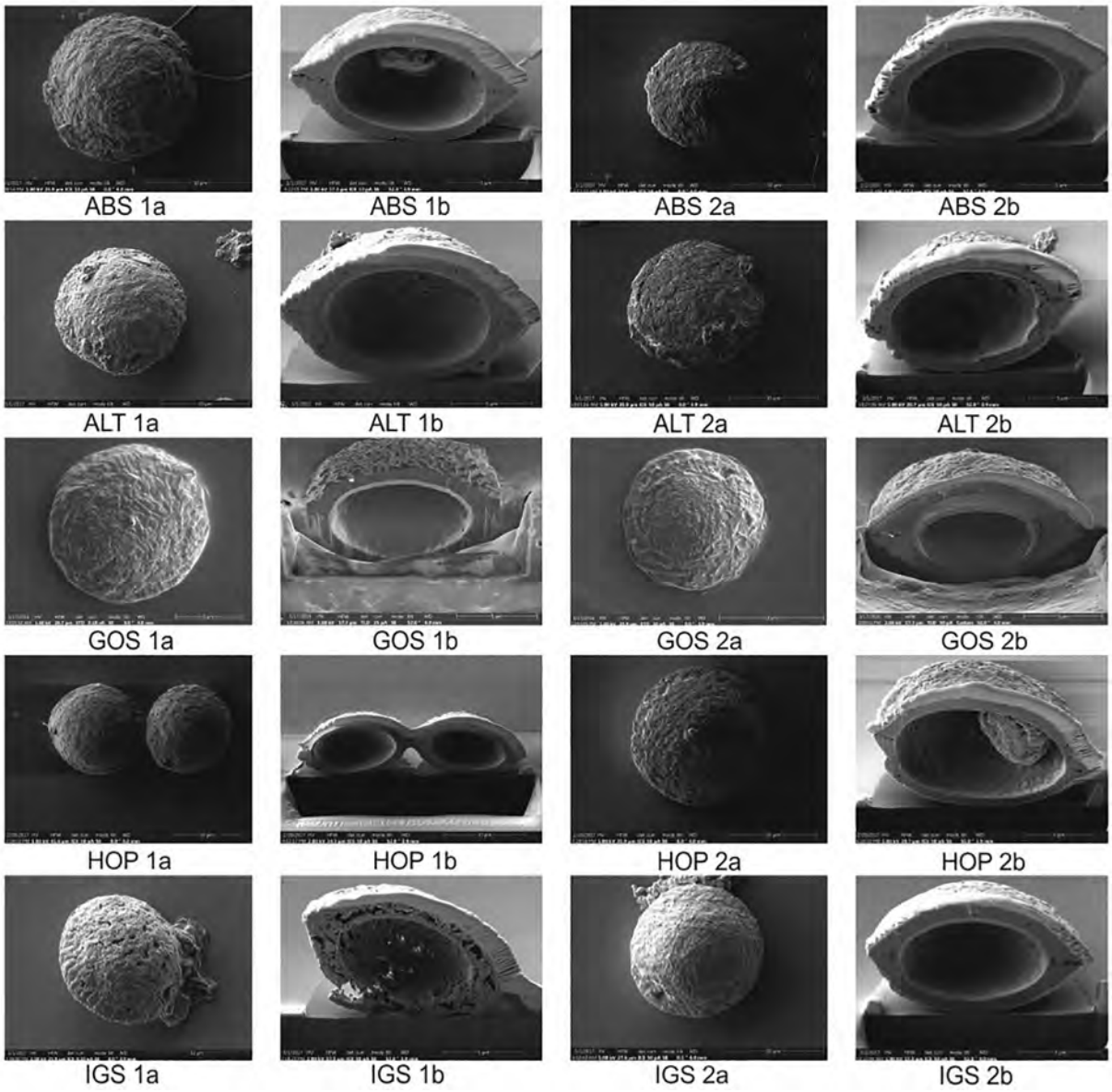

Fig. 5.1: Illustration of varying shell morphometry in the lakes Absdorfersee (ALS), Altmuehlsee (ALT), Grosser Ostersee (GOS), Hopfensee (HOP) and Igelsbachsee (IGS): shell surfaces next to shell cross-sections (REM images left, FIB images right).

However, this impression can only be generalized to a limited extent due to the small number of individuals ($n = 25$) being examined in detail. On the basis of selective laboratory experiments on algae cultures, it would be equally interesting to examine whether different stages of growth (Giering *et al*, 1990b) can at least explain individual morphotypes or whether the assumption of genetic justification of the various phenotypes can be proven by molecular genetics. It is also unclear to what extent environmental conditions impact shell formation, e.g. whether the ratio of shell thickness to shell diameter varies with changes in the algal culture conditions. Adequate parameters to be investigated would be the index of carbonate saturation and calcification inhibitors such as humic acids found in Lake Hopfensee and Lake Abstsdorfersee.

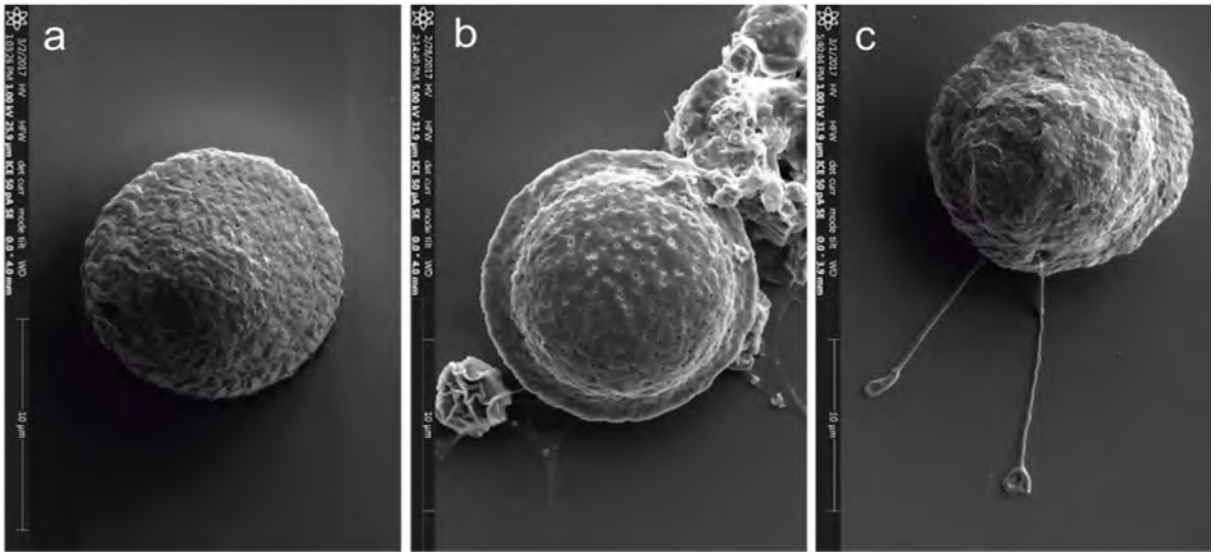

Fig. 5.2: Progress of calcification of *P. lenticularis*: early stage with recognizable tabular calcite crystals (a), advanced stage with numerous pores and smooth surface and further developed shell edge (b), adult stage with two good visible flagella and largely "cemented" surface and less modulated shell edge due to external calcite deposition (c).

5.1.3 Temporal and spatial variance in the sense of the phenology of *Phacotus lenticularis*

P. lenticularis temporal population development is highly dynamic and can be different from lake to lake as well as from year to year. This can be seen both in the correlation of *Phacotus* cell numbers in the comparison of the studied lakes in Bavaria (Chapter 4) and quite clearly in Lake Grosser Ostersee when contrasting the years 2015 and 2016 (Chapter 2 and 3). This was also mentioned in previous studies (Krienitz *et al*, 1993) in lakes of the Mecklenburg Lake District in Northern Germany. Noticeable is that in the hypertrophic Lake Hopfensee and the eutrophic Lake Altmuehlsee the highest individual densities occurred (Chapter 3). Experiments have shown that neither the amount of available N or P has a drastically reducing effect on the growth of *P. lenticularis* (Schlegel *et al*, 1998). Other factors such as $CaCO_3$ super saturation directly influenced by the temperature (T) and pH-value were also relevant parameters for the growth of *Phacotus* in the investigated lakes. It has been shown that T and pH-value are prerequisites for the beginning of exponential growth in given ranges (Gruenert and Raeder, 2014b). To effectively combat these cell count variations, high-frequency monitoring was used to examine the vertical and horizontal distribution of the *Phacotus* population and temporal dynamics at several locations of Lake Grosser Ostersee. The result showed that it is possible to ignore the horizontal heterogeneity of the occurrence of *P. lenticularis* in Lake Grosser Ostersee (Chapter 3). For this reason sampling was limited to one point in each lake for further research, because the other lakes were smaller and were more uniform in bathymetry than Lake Grosser Ostersee.

The spatial (depth) distribution of *P. lenticularis* varies within a lake (Chapter 3) and hydrographic parameters such as temperature and pH-value affect the vertical het-

erogeneity (Gruenert *et al*, 2016). That is why weather-related factors also interfere with the carbonate system: solar radiation and wind movement influence the temperature and pH-value of the water column (Gruenert and Raeder, 2014b), and thus, indirectly impact *P. lenticularis*' vertical distribution. To address this heterogeneity, mixed samples were taken over a depth range of 0-7 m and the mean density of *P. lenticularis* was recorded at the deepest point of the lake. This was the most practical and effective method of determining *Phacotus* contribution to total carbonate precipitation in the investigated lakes.

Only sediment analysis may take into account the temporal variance of the occurrence of *P. lenticularis* and all other factors that reduce the number of shells on the way to the lake bottom and in the sediment. Unlike the "snapshots" during plankton analysis in the water column, the sediment records mirror the long-term occurrence of *P. lenticularis* in a lake. It should be remembered that no clear estimation can be made about absolute cell numbers in the water column neither by the number of shell halves contained in the sediment nor by information provided by sedimentation traps (Chapter 3). Lakes whose sediments do not contain *Phacotus* shells, nevertheless can house a *Phacotus* population. Consequently, the only truly effective approach for evaluating long-term *Phacotus* carbonate sequestration is direct microscopic examination of the sediment to determine the "net fraction" of *Phacotus* shells in the sediment as described in Chapter 4.

5.2 Relevance of *P. lenticularis* contribution to total carbonate sequestration

The investigation of the relevance of *Phacotus* as a calcifying phytoplankton in freshwater ecosystems needs to be regarded in the broader context of C-sequestration where major carbon-fixing processes are the gross primary production (GPP) and respiration (RES). As described in the introduction, the focus of the present work is especially on the biogenic carbonate precipitation (BCP) which can be seen as the essential part of the fixation of inorganically bound C in form of calcite at the lake bottom. This is what is meant by *carbonate sequestration*, in means of a long-term C-sink so as to minimize the CO_2 emissions from the lake into the atmosphere. *Phacotus* actively contributes to that process, and its contribution is discussed below.

5.2.1 Estimation of the carbon bound by *Phacotus lenticularis*

In Chapter 2, the present work includes all the basic information necessary to determine the mass and volume of the *Phacotus* shells and the amount of inorganically fixed carbon (C_{IC}) within the natural variance. On average, one million individuals in *P. lenticularis* contain $\sim$ 1.046 mg $CaCO_3$ ($\sim$ 0.1255 mg C_{IC}). *P. lenticularis* with its very distinctive shell is the only known calcifying green alga, which forms a calcareous shell in fresh water by extracellular calcification (Kelts and Hsü, 1978; Koschel and Raidt, 1988b). The amount in both the BCP and the sediment can be precisely determined by the shell mass and the number of individuals. In this sense the percentage of *Phacotus* shells of the

suspended total carbonate in the water column and the percentage of *Phacotus* shells in lake sediments were demonstrated and discussed in Chapters 3 and 4.

In addition, here *Phacotus*' organic carbon (C_{org}) ratio shall be estimated in order to determining the maximum carbon fixation potential in lake sediments using the example of the displayed shell in Fig. 1.10. The maximum possible cell volume (ellipsoid with a radius of 4.5 μm and a height of 3.1 μm) is 257 μm^3, assuming that the protoplast occupies the entire interior inside of the two shell halves (Giering, Krienitz and Casper, 1992). In this excample a *Phacotus* cell contains up to 51 pg C_{org} (which defines a C_{org} mass of ~ 0.051 mg in 10^6 *Phacotus* individuals), using a "carbon to the cell volume relationship for freshwater alga cells." of 0.2 pg $C_{org}/\mu m^3$ according to (Rocha and Duncan, 1985). Accordingly, the total amount of C (TC) set in 10^6 *Phacotus* cells would be 0.1769 mg TC. Meaning that 29 % are bound as C_{org} in the protoplast besides a ratio of 71 % C_{IC} in the two halves of the shell. This estimate is associated with "measurements of 0.2 mg of fresh biomass L^{-1} in *Phacotus* biomass of a water sample with a population density of about 1 million L^{-1} cells" (Schlegel *et al*, 1998). This 0.2 mg fresh *Phacotus* biomass contains $\sim$ 0.05 mg C_{org} if we use according to Herzog, Golomb *et al* (2004), 1 g algal biomass contains $\sim$ 1.8 g CO_2 ($\sim$ 0.49 g C). The second estimation also results in 10^6 *Phacotus* cells having a TC of $\sim$ 0.17 mg C equivalent to $\sim$ 30 % (0.05 mg) C_{org} in the biomass and 70 % C_{IC} (0.12 mg) in the shell. Summarizing, the ratio of inorganically bound C in the calcite shell to organically bound C in the *Phacotus* protoplast biomass is at most 70 % to 30 % and realistically, within the range of 80 % to 20 % as the protoplast does not fill the inside of the shell's two halves completely.

In order to assess whether the sedimented C_{org} of *Phacotus* is relevant or not, one must ask whether the C_{org} is buried in the sediment across geological time scales or whether it is mineralized to CO_2 and/or CH_4 by microorganisms at the lake bottom. That depends heavily on the sediment surface oxygen exposure time (Sobek *et al*, 2009) and the magnitude and rate of substantial allochthon organic matter catchment supply (Jonsson, Meili, Bergström and Jansson, 2001). In addition, changes in land-cover and associated changes in nutrient loading in the lake catchment as well as atmospheric N-deposition control organic carbon burial in lakes (Anderson, Dietz and Engstrom, 2013). The maximum amount of C_{org} that can be bound by *P. lenticularis*, as just estimated, is relatively low at 20–30 % of a *Phacotus* cell's TC. This circumstance has a reducing effect: sediment trap investigations in Lake Grosser Ostersee in 2015 have shown that an average of 12.3 % ($SD = 4.3$ %) of the shells, arriving at the bottom of the lake, no longer contain a protoplast. The potential proportion of C_{org} caused by *Phacotus* sediment is thereby further reduced. For this reason, in the present work, the organically bound C is considered negligible compared to the inorganically bound C.

Taking the example of the most intensively examined sediment core from Lake Grosser Ostersee, adequate conditions for the decomposition of the moderate amounts of organic matter (Chapter 4) prevail. The residence time in the oxic sediment zone of the oligotrophic lake without surface inflow was approximately two to three years. During this time, loss rates of 25-45 % for C_{org} have been found in the sediment of lakes with oxic hypolimnia (Jonsson and Jansson, 1997; Teranes and Bernasconi, 2000). As a result, based

on Lake Grosser Ostersee, the biogenic silty sediment that originated from the pelagic zone has a low TOC content of less than 3 wt% and a $CaCO_3$ content of 87 wt%. According to this, in the oligotroph hard-water lake, organic carbon fixation in general as well as *P. lenticularis* have no great relevance.

5.2.2 Enhancement of carbonate sequestration by *P. lenticularis* versus natural development of climate and trophic state in lakes

Mass Accumulation Rates (MARs) indicate the amount of C that actually reaches the ground and stays fixated in the bottom sediment. Looking at the high MARs for *P. lenticularis* carbonate from sediment trap experiments during the high growth phase in summer, it seems reasonable to consider if these could artificially be prolonged or intensified to maximize carbon fixation. In Lake Grosser Ostersee the temporal MARs for *P. lenticularis* were between 0.03 and 0.32 g C $m^{-2}a^{-1}$ whereas the average annual inorganic carbon-MAR was 0.009 g $m^{-2}a^{-1}$ for the uppermost sediment core section from 1970-2016. The high discrepancy of an up to 35-fold higher C-accumulation potential of *P. lenticularis* during mass growth periods illustrates the massive potential of *Phacotus* C-fixation. Consequently, recurrently the question arises whether a targeted artificial promotion of the *Phacotus* occurrence in its natural environment is possible. It is important to consider that a lake's trophic condition is the most decisive factor for high *Phacotus* occurrence. In mesotrophic-eutrophic lakes, the highest *P. lenticularis* cell densities have been observed (Schlegel *et al*, 1998), which was also confirmed in this study for Bavarian lakes. In addition, *Phacotus* mass growth occurred in waters with surface temperatures higher than 15°C and high pH-value above 7.5 (Gruenert and Raeder, 2014a) as well as stable stratification conditions. Systematic modification of these parameters specifically for the purpose of facilitating an artificially induced mass occurrence of *P. lenticularis* in order to increase carbonate sequestration in a lake would involve firstly, a massive intrusion on the ecosystem and, secondly, a significant resource and energy expenditure. Considering the unforeseeable consequences for the environment, this is difficult to justify.

In fact, the relevant parameters for *Phacotus* abundance (lake's trophic condition, water temperature and stable stratification) are already changing in Bavarian lakes and rivers. Reasons are reduced nutrient input due to the efforts based on the Water Framework Directive (WFD) and the effects of global climate change. As a result of the WFD the Bavarian government strive constantly to improve the quality of waters to achieve the required ecologically good condition. Therefore, it is to be predicted that the number of eutrophic lakes in Bavaria will decrease over the long term. Consequently, the occurrence of *P. lenticularis* may do as well. Opposing to this, the process of eutrophication caused by climate change is a phenomenon that has be observed in an increasing number of lakes, since it has already been studied and identified in German lakes at the Mecklenburg Lake District (Adrian and Shatwell, 2018) as well as in Denmark. This process could mitigate re-oligotrophication also in Bavaria and is briefly explained below.

In Bavaria, an increase in the overall annual average air temperature trend in southern Germany for the period 1931 to 2015 to an average of +0.8 to +1.2°C/85 years in the summer season was observed (Steinbauer and Komischke, 2017). In temperate dimictic and polymictic lakes in general, this leads to an increase of the temperature difference between surface water and deep water which makes the stratification in the lakes more stable. Thereby the thermocline is no longer broken regularly in summer, but the stratification periods last longer (Hansen, Nedergaard and Skov, 2008). This leads to deeper and more stable thermoclines in lakes, which prevents oxygen transfer into the hypolimnion and increases the risk of oxygen deficiency in the hypolimnion (Jeppesen *et al*, 2013). Higher temperatures increase O_2 consumption and lower solubility of oxygen in the water can lead to P-release from the sediment in the hypolimnion. Therefore, a climate change effect could be eutrophication of lakes induced by changing climatic conditions. Similarly, water surface temperatures warmed in lakes of other regions as well, for example in Denmark by 2°C in the third and fourth quarters of every year between 1989 and 2006. The reason for this was the increase in the continental climate effect, which was responsible for rising lake temperatures due to higher summer air temperatures (Jeppesen *et al*, 2013).

It is impossible to predict which of these two expected future developments –re-oligotrophication due to reduced external nutrient supply or eutrophication due internal fertilisation as consequence of climate change- will play the dominant role in lakes. The interplay of the above evidence, however, could boost future conditions for the mass occurrence of *P. lenticularis*, thereby leading to increased sequestration of carbonate by *P. lenticularis*. Since 2000, the sediment studies in Lake Grosser Ostersee have shown a trend in the green alga *P. lenticularis*, i.e. a steady increase in CO_2 storage, although the former mesotrophic lake has once again become oligotrophic.

5.2.3 Is *Phacotus lenticularis* responsible for fixation of relevant amounts of carbon in lakes?

The answer is yes and no. As highlighted in Chapter 4, the answer depends on the considered spatiotemporal context. Globally viewed, the freshwater system is an important transfer point within the global carbon cycle connecting atmosphere, hydrosphere, geosphere, and biosphere (Kastowski, Hinderer and Vecsei, 2011). Focussing on hard-water lakes in the temperate climate, the superordinate cycle of biogenic calcite precipitation is an important process in which *P. lenticularis* forms such a small proportion over periods of over more than one year, that *P. lenticularis* is not of any relevance. The currently assumed globally relevant process is the increased autochthonous and allochthonous carbon flux, which is related to anthropogenic change compared to long-term mean values (5000-2000 BP) and has resulted in a doubling of carbon fixation in European lakes during anthropocene (Kastowski *et al*, 2011). In the future, the fraction of global carbon buried in lakes will be increasingly important if worldwide trends in anthropogenic eutrophication continue (Heathcote and Downing, 2012). In regional terms, biogenic calcite precipitation is a relevant process in the Bavarian hard water lakes investigated. As already shown in Chapter 5, the *P. lenticularis* fraction represents only a small part of the total calcite precipitation.

On the ecosystem level, regarding one specific lake, *P. lenticularis* is clearly responsible for the fixation of relevant amounts of carbon. Since *P. lenticularis* occupies about 10 % and -in exceptional cases- 50 % of plankton biomass (Schlegel *et al*, 1998) and temporarily up to 100 % of suspended autochthonous calcite in the epilimnion (Koschel *et al*, 1987a), the regional significance for the permanent fixation of C by carbonate sequestration has been clearly demonstrated. Therefore, it is still interesting to place its relevance in the ecological context of lakes. Phacotus plays an outstanding role in limnic phytoplankton due to the mineralization of its calcite shell (findings not yet proven). Autochthonous carbonate precipitation has already been described as a natural control mechanism for eutrophication in freshwater lakes which counteracts eutrophication (Koschel, 1983). Calcium precipitation by *P. lenticularis*, as part of the biogenic carbonate precipitation (BCP) is assumed to have a similar effect by adsorption of N and P on the calcite crystals of its shell (Giering *et al*, 1990b; Krienitz *et al*, 1993; Schlegel *et al*, 1998). Therefore, *P. lenticularis* plays an important role in the lakes, in which it already provides a considerable part in the processes of carbon fixation during mass occurrences. However, this is always limited to short periods of time and cannot be generalized.

Several aspects influencing *P. lenticularis* abundance still remain unclear. For example if *P. lenticularis* benefits during phases of nutrient limitation from the ability to store P and N on its shell helping to overcome scarce periods in a lake or to which degree grazing has influence on the individual numbers of *P. lenticularis* is still unclear and has not been investigated successfully. Concerning its size of 7-20 μm (Lampert and Sommer, 1999), *P. lenticularis* can be ingested by copepods and small cladocera and has already been found in the digestive tract of *Bosmina longirostris* and *Daphnia cucullata*. As no change in the *Phacotus* shells were observed after allowing for intestinal passage of light or using scanning electron microscopy, it was suspected that the *Phacotus* shell might be an effective defence mechanism against zooplankton (Schlegel, 2001b). Based on the finding that the interaction with *P. lenticularis* also rises with the increased feeding pressure of the filtering zooplankton, it was suspected that the *Phacotus* shell offers protection against digestion and is therefore essential to the survival of the flagellate (Schlegel, 2001a).

5.3 Application and transferability of the methods

5.3.1 *Phacotus* carbonate sequestration at Lake Grosser Ostersee

From the beginning of this work, sampling focussed on the assessment of total suspended carbonate concentration on the epi- and metalimnion by water and plankton sampling with the aim of *Phacotus* shell mass determination. Most of the suspended biogenic carbonate in the pelagial at the monitoring sites were found to be autochthonous calcite crystals that crystalized in the upper water body over several month, mainly induced by the assimilation activity of cyanobacteria and phytoplankton (Fig. 5.3). The *Phacotus* contribution to total suspended carbonate sedimentation was intense but of short duration and depended on the dynamic phenology of *Phacotus* population which was influenced by zooplankton (grazing) as well as wind-induced collapse of the epilimnion as described in detail for lake Grosser Ostersee (Chapter 2).

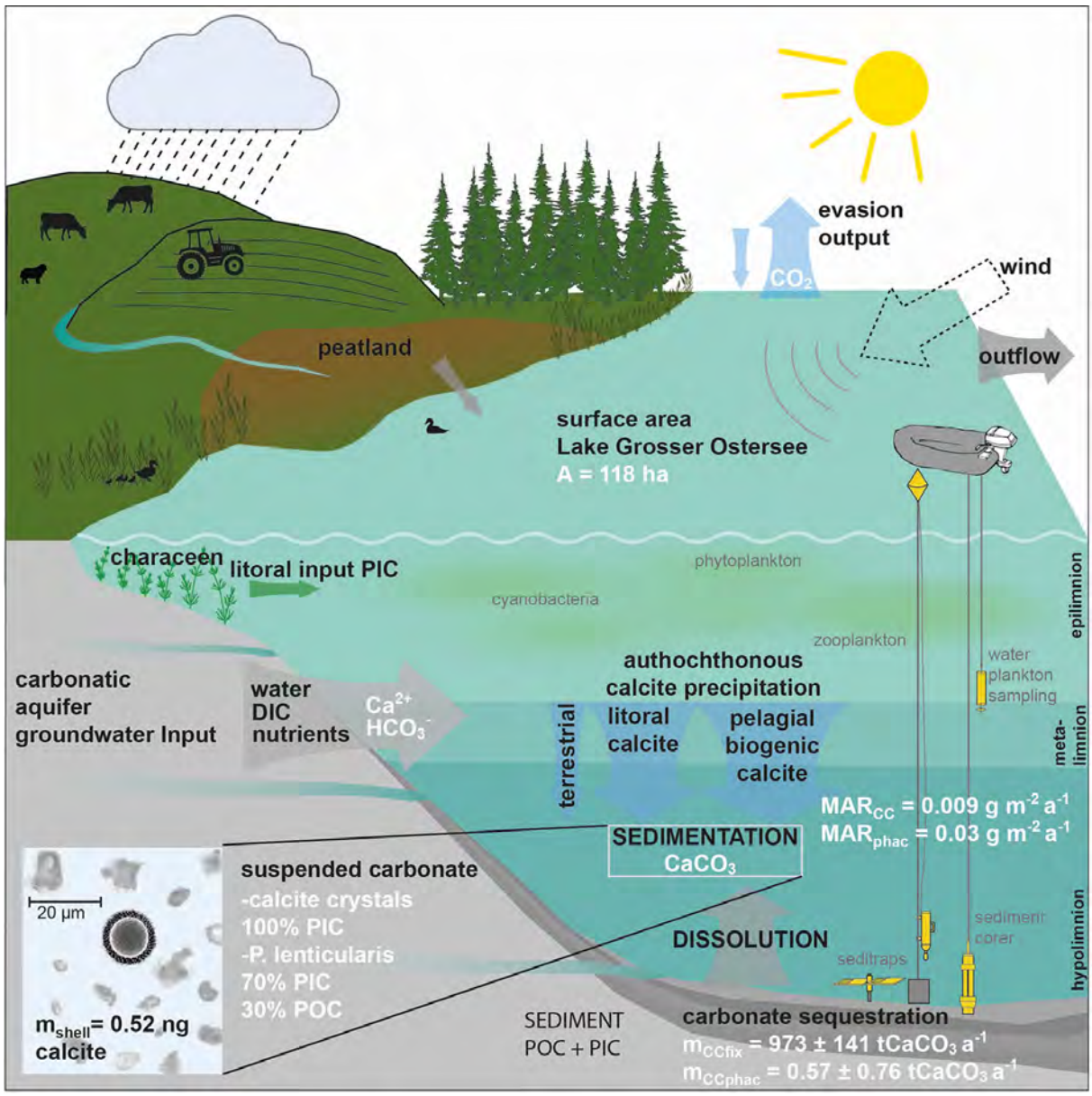

Fig. 5.3: Scheme of carbonate sequestration in in Lake Grosser Ostersee with specific results on the quantitative contribution of *P. lenticularis*. Annual mass of fixated carbonate (m_{CCfix}) and *Phacotus* carbonate (m_{CCphac}) and Mass Accumulation Rates of total carbonate (MAR_{CC}) and *Phacotus* carbonate during highest cell growth in 2015 (MAR_{phac}).

As logical consecutive step, the monitoring concept in the following investigation (Chapter 3) focussed on both: (1) the short-term "carbonate dynamics" of *P. lenticularis* in the upper water column and (2) the hypolimnion where sedimentation traps revealed the surprisingly high mass accumulation rates of *Phacotus* carbonate (MAR_{phac}) during the growth peaks while carbonate dissolution was identified as possible reducing factor in the deeper water column. Finally, the long-term carbonate sequestration at the lake bottom was demonstrated by analysing a dated sediment core (Chapter 4), revealing the comparatively low total carbonate mass accumulation rates (MAR_{CC}) that result at the lake bottom over a whole year. It could be illustrated that the high proportions

of *Phacotus* carbonate on the suspended total carbonate of the water column are not reflected in the sediment. Main reason for that seemed to be dillusion of the *Phacotus* content by the bulk autochthonous carbonate sedimentation which is enhanced by further particulate inorganic carbon input from the litoral (crushed stem casts of charaphyceans) as well as potential terrestrial particulate carbonate input in the case of lakes with superficial inflow. As a result, in the oligotrophic Lake Grosser Ostersee the long-term carbonate sequestration (m_{CCfix}) at the lake bottom resulted to be around 10^3 t $CaCO_3$ a^{-1}, which equals a CO_2e equivalent of 412 t a^{-1} $\pm$60 t a^{-1} CO_2e that potentially would have been released into the atmosphere by evasion. The annual mass of fixated *Phacotus* carbonate (m_{CCphac}) was $0.6 \pm 0,8$ t $CaCO_3$ a^{-1}. This corresponds to the estimates from the sediment trap experiments that resulted in a total carbonate sequestration of 1.4 t $CaCO_3$ for the main growth phase of *P. lenticularis* from June to October 2015 (Fig. 1.12). Because of the low TOC content of the sediment in Lake Grosser Ostersee, for long term C-sequestration mainly particulate inorganic carbon was relevant regarding the TOC content of the sediment of less than 3 wt-%.

5.3.2 Transferability to other lakes

On the basis of our results on the occurrence of *P. lenticularis*, and the resulting permanent carbonate sequestration in the five Bavarian lakes, the question arises whether the findings can also be applied to larger areas. But the methodology described in Chapter 3 is too detailed to assess carbonate sequestration of *P. lenticularis* for an entire region. The relatively low proportion of *P. lenticularis* in the suspended total carbonate in lakes, seems not to justify the high temporal expenditure by plankton analysis for larger areas such as Bavaria. If, however, the focus is reduced to investigating the total carbonate sequestration in the lakes, explicitly excluding the additional accumulation of organic carbon as described by (Dean and Gorham, 1998), an estimate is possible for several lakes at a time by the systematic approach in four steps which is presented in the following.

In the first step, (1) all lakes in the investigated area must be identified in which autochthonous carbonate precipitation can occur in principle. Selection criteria could be water temperatures and pH values or water hardness. Therefore, for example parameters like mean Ca^{2+} concentration in the water column and the trophic status of the lake would be advantageous. The geology of the catchment areas and the resulting water hardness of the groundwater and surface waters would further help for this purpose. Then, reference lakes must be chosen that are representative for several "lake classes" representing low/middle/high carbonate sequestration. The following parameters are needed for their characterization: a) sedimentation rates over one year, b) the TC/TOC/TIC fraction in the uppermost sediment layer, c) the hydrochemical and hydrophysical data from the water column and c) the hydromorphological characteristics of each reference lake. The second step (2) would determine the specific C-accumulation rate for each "lake class". As a third step (3), all lakes in question must be assigned to one of the "lake classes" on the basis of their hydro chemical, physical and morphological characteristics. Thus, a specific C-accumulation rate is assigned to each lake in the entire study area without having to determine it in each individual lake. In the last step (4), a total carbonate precipitation

for each lake would be calculated. Since the carbonate precipitation takes place in the uppermost water column, it seems reasonable to assume that the lake area can be used as a proportional (proxy) value to estimate the total carbonate precipitation. Thereby, an estimate on the total carbonate sequestration for all lakes on the basis of the lake area would be derived.

5.3.3 Application to Bavarian Lakes

Against the background of estimating the potential CO_2 binding capacity of Bavarian lakes by carbonate precipitation, the procedure described was applied throughout Bavaria by means of a database query at the Bavarian Environmental Agency (LfU) office in Wielenbach. First, all lakes were identified which in principle can be considered for autochthonous carbonate precipitation and *P. lenticularis* occurrence. Criteria for the selection were e.g. a pH value of more than 7.5 in the uppermost 2 m of the water column and a temperature of more than 15°C in the summer months May-October. These guideline values were derived from the minimum requirements for the occurrence of *P. lenticularis* (Gruenert and Raeder, 2014a; Schlegel *et al*, 1998).

The result was that 111 of the 212 lakes that are currently listed in Bavaria with a total area of 835.74 km^2, occur for autochthonous carbonate precipitation and the occurrence of *P. lenticularis* (Fig. 5.4). This corresponds to an area of 317.85 km^2 (38 %). Defining three reference lakes and lake classes with C-accumulation rates of 77 t CO_2 km^{-2} a^{-1} (low), 385 t CO_2 km^{-2} a^{-1} (medium) and 538 t CO_2 km^{-2} a^{-1},a potential CO_2 sequestration by carbonate precipitation of approximately 161.883 t CO_2 per year resulted for Bavarian lakes.

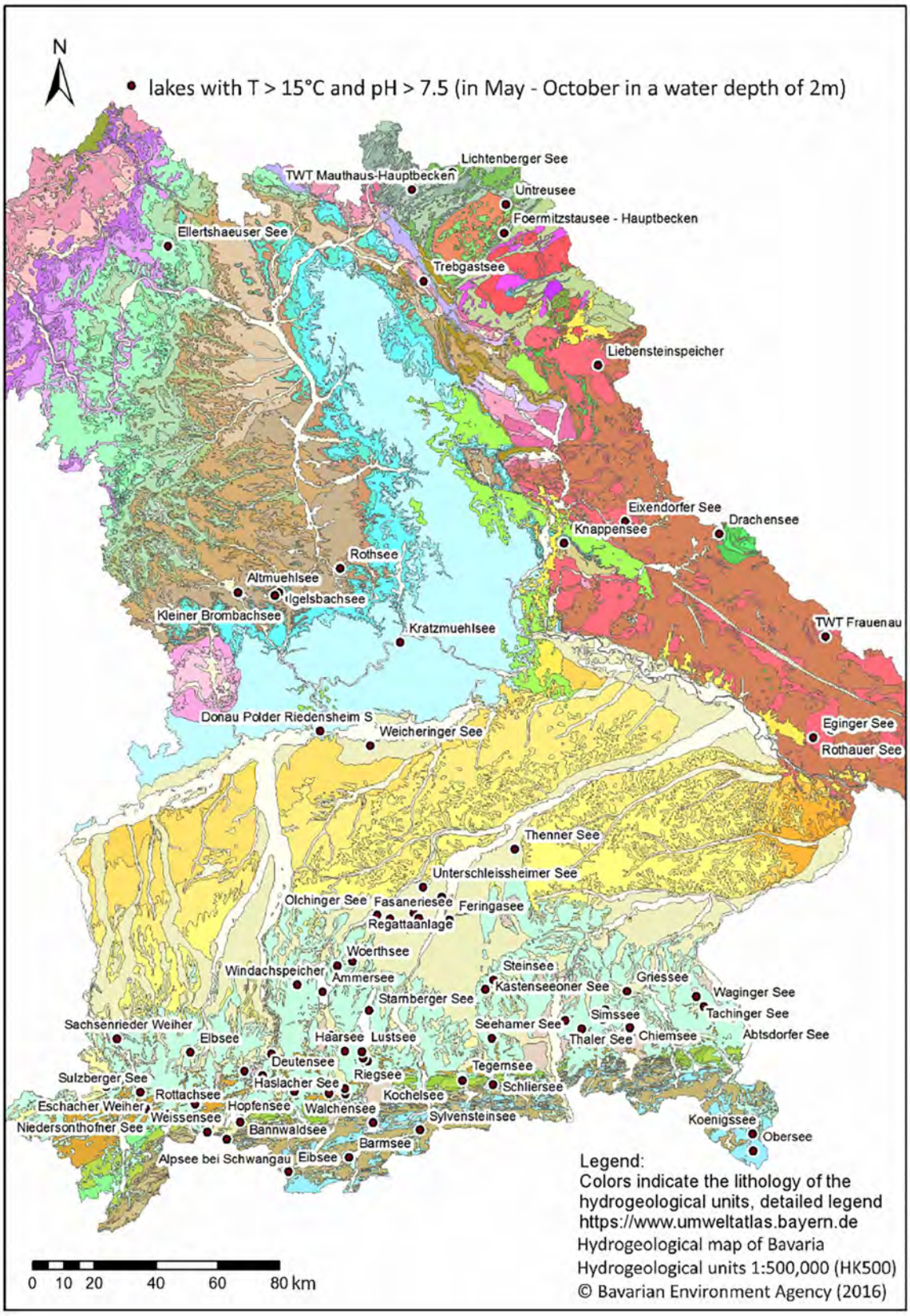

Fig. 5.4: Bavarian lakes that provide accurate conditions for autochthonous carbonate precipitation and the occurrence of *P. lenticularis* according to a database query at the LfU office in Wielenbach: applied criteria: $T > 15$°C and pH > 7.5 in May – October in a water depth of 2 m.

5.4 Author contributions

Sebastian Lenz (SL), Prof. Dr. Jürgen Geist (JG), Dr. Uta Raeder (UR), Dr. Uta Grünert (UG), Michael Stiefel (MS), Maren Lentz (ML), Prof. Dr. Nathalie Dubois (ND)

Chapter 2 Calcite production by the calcifying green alga *Phacotus lenticularis*

This study was primarily designed by SL and UR, with critical revision by JG and UG. The development of methods was carried out by SL in consultation with UG, MS and ML. Data acquisition with the focussed ion beam and selective electron imaging was realized at the Swiss Federal Laboratories for Materials Science and Technology EMPA in Dübendorf by MS and carbonate measurements were done at the Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Stechlin, Germany by SL with the help of ML. Processing and interpretation of data were realized by SL and discussed with UR, UG and JG. The manuscript was drafted by SL and continuously improved thanks to input and revision of JG and UR.

Chapter 3. Representative monitoring of the calcifying alga *Phacotus lenticularis* (Chlamydophyceae) in lentic ecosystems

This study was primarily designed by SL and UR, with fundamental conceptual input of JG. The development of methods was carried out by SL. The acquisition of data in terms of fieldwork and microscopic analysis was realized by SL with the support of the by graduate students Andreas Mayr, Lukas Heinrich and Helena Macke. Interpretation of data was realized by SL and discussed with JG. The manuscript was drafted by SL and continuously improved thanks to revision of JG and UR.

Chapter 4 *Phacotus lenticularis* content of carbonate sediments and epilimnion in four German hard water lakes

This study was primarily designed by SL and UR, in consultation with JG. Sediment core dating and interpretation was done by ND at the Swiss Federal Institute of Aquatic Science and Technology, Department Surface Waters EAWAG in Dübendorf. Further sediment analysis was done by SL at the same institution. The interpretation of the sediment core data was done by SL in consultation of ND. Microscopic analysis was realized by graduate students Simone Rost and Simone Rudolph under supervision of SL. The manuscript was drafted and finalized by SL due to continuous revision of JG and UR.

5.5 Publication List

The following papers were included in this thesis:

Lenz S, Dubois N, Geist J, Raeder U. *Phacotus lenticularis* content in carbonate sediments and epilimnion in four German hard water lakes (Manuscript submitted for publication).

Lenz S, Raeder U, Geist J (2020) Representative monitoring of calcifying alga *Phacotus lenticularis* (Clamydophyceae) in lentic ecosystems. Journal of Limnology.

Lenz S, Gruenert U, Geist J, Stiefel M, Lentz M, Raeder U (2017) Calcite production by the calcifying green alga *Phacotus lenticularis*. Journal of Limnology.

Oral contributions related to the thesis:

Lenz S (2019) Seen als Kohlenstoffsenke – Bedeutung der Planktonalge *Phacotus lenticularis*. Workshop Bergseen im Klimawandel, June 27 2019, Technical University Munich, Iffeldof, Germany.

Lenz S (2017) Vorstellung der kalzifizierenden Süßwasseralge *Phacotus lenticularis* und Erfahrungen aus Kulturversuchen. Kolloquium „Kultivierung kalzifizierender Algen im Labor“, March 29 2017, Karlsruhe Institute of Technology, Karlsruhe, Germany.

Lenz S, Raeder U (2017) Biogene Entkalkung: Die Grünalge *Phacotus lenticularis* und ihr Beitrag zur Karbonatfällung in Seen mit unterschiedlicher Trophie. Jahrestagung der Deutschen Gesellschaft für Limnologie und der deutschsprachigen Sektionen der SIL, September 25-29 2015, Brandenburg University of Technology Cottbus-Senftenberg, Germany.

Lenz S (2016) Seen im Klimawandel – CO_2-Bindungsvermögen der klazifizierenden Planktonalge *Phacotus lenticularis*. Seminar at chair of Hydrology, aquatic ecology and water management, April 04 2016, University of Applied Sciences Weihenstephan-Triesdorf (HSWT), Weidenbach, Germany.

Lenz S (2015) Presentation Thesis: Distribution and CO_2-sequestration of the green alga *Phacotus lenticularis*– Monitoring and adaption to climate change. Institute Seminar at Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB), November 08 2015, Stechlin-Neuglobsow, Germany.

Poster contributions related to the thesis:

Lenz S, Raeder U (2015) Verbreitung und CO_2-Bindungsvermögen der Grünalge *Phacotus lenticularis* in bayerischen Seen – Monitoring und Anpassung an den Klimawandel. Jahrestagung der Deutschen Gesellschaft für Limnologie und der deutschsprachigen Sektionen der SIL, September 21-25 2015, University Duisburg-Essen, Germany.

Acknowledgements

I would like to thank Prof. Dr. Jürgen Geist for his guidance and dedicated efforts that gave me the motivation to constantly improve and advance this work. I would like to express my special gratitude to my advisor and mentor Dr. Uta Raeder for her expert advice and her persistent encouragement as well as continuous support not only in scientific aspects. I highly appreciated the privilege of being able to realize this PhD project at the Limnological Research Station in Iffeldorf as a part of the Limno community.

Furthermore, I would like to thank Prof. Dr. Tanja Gschlößl from the Bavarian State Ministry of the Environment and Consumer Protection for her continuous support of this project as well as Prof. Dr. Peter Jacob who enabled the measurements at the Swiss Federal Laboratories for Materials Science and Technology (EMPA).

My sincere thanks go to my co-doctoral students Dr. Franzi Bauer, Andrea Hofmann, Schteffi Rüegg, Dr. Tine Fritz, Dr. Nico Corti and Simon Baier for sharing joys and sorrows and of course for helping out where ever needed. In the same way, all other colleagues of the Limnological Research Station contributed to the completion of this work. Especially Dr. Markus Hoffmann and Dr. Uta Grünert supported with their expert advice. Further thanks deserve my master students Simone Rost, Mone Rudolph, Andi Mayr, Lukas Heinrich, Helena Macke and bachelor student Claudia Böhme as well as all other student assistants, divers and interns who together have shaped the incomparable Phaco(i)tus Team.

I would like to thank all my friends and family for their direct and indirect support. Especially Franzi and Max for offering their apartment as cohousing space and Thomas, Lilli, Kilian and Markus in their role as pacemaker providing encouragement until the finish line.

Finally, I would like to express my sincere thanks to my spouse Eva for her valuable assistance in the illustration of numerous figures of this work and for her support especially during the last years when she was always ready to take over the care of our daughters. It was her infinite patience and never-ending endurance that made it possible to bring this thesis to a successful completion.

Summary

The unicellular green alga *Phacotus lenticularis* is the only calcifying species of phytoplankton in freshwater and is abundant in temperate hard-water lakes worldwide. It forms a bivalved calcite shell of up to 20 μm diameter that consists of 98 % of pure calcite ($CaCO_3$). Thereby during mass developments in summer, the precipitated calcite contributes to the biogenic induced carbonate precipitation in hard water lakes. The precipitated carbonate sequestrates the carbon (C) effectively and for geologic time scales at the lake bottom. The aim of this dissertation was to investigate the role of the calcifying green alga *P. lenticularis* in respect to its contribution to long term C-sequestration by carbonate precipitation in Bavarian lakes. The work begins with the analysis of the microscopic calcite shell, further investigates the population dynamics and sedimentation rates in order to finally assess the quantitative role of *Phacotus lenticularis* content in lake sediments.

A major outcome was the development of a novel approach on analysing the mass of *P. lenticularis* shells using the Focussed Ion Beam technology. This method was applied and validated in four German hard water lakes and is also applicable to other microparticles. A detailed morphometric assessment of the *Phacotus* shells revealed that shell diameter was significantly influenced by lake origin. The shells from each lake displayed substantial variations in diameter and shape leading to shell masses ranging from 0.33 to 0.52 ng. This enabled a comprehensive and quantitative evaluation of the impact of *Phacotus* carbonate contribution to total carbonate sequestration in lakes.

The work describes a hitherto absent standardized methodology that representatively assesses the spatiotemporal variation of *P. lenticularis* in the water column. Simultaneous measurements in three different lake sub-basins of Lake Grosser Ostersee showed that the spatial cell density was similar. At all sites, the vertical *P. lenticularis* cell density maxima corresponded with the slowly downshifting thermocline. Apparently, the lake bathymetry as well as external factors like wind exposure were no factors that control the abundance of the calcifying alga. Furthermore, cell densities and sedimentation rates were assessed for four lakes and dissolution of carbonates was found to be a disruptive factor that can reduce the shell number in sediment traps at the lake bottom but also during storage of plankton samples.

Concluding, this work demonstrates that the *Phacotus* carbonate content in lake sediments is smaller than in the suspended carbonate of the water column. This is because the contribution to total carbonate precipitation of seasonal appearing *P. lenticularis* with its short-term population peaks is highly dependent on the amount of additional carbonate precipitation and the observed time frame. A sediment core of oligotrophic Lake Grosser Ostersee demonstrated that the *P. lenticularis* contribution resulted to be less than 1 wt-% of the total carbonate content and that dilution by gross authigenic

carbonate precipitation was the main reason. Yet, in the broad context of C-fixation in hard water lakes, the contribution of *P. lenticularis* to limnic calcite precipitation represents a small but notable part of C-turnover that should certainly be considered in the assessment of lake carbon cycling.

Zusammenfassung

Die einzellige Grünalge *Phacotus lenticularis* ist die einzige kalzifizierende Phytoplanktonart im Süßwasser und kommt weltweit in Hartwasserseen der gemäßigten Breitenzone vor. Sie bildet eine zweiteilige Kalzitschale von bis zu 20 μm Durchmesser, die zu 98 % aus reinem Kalzit ($CaCO_3$) besteht. Dadurch trägt das gefällte Kalzit bei Massenentwicklungen im Hochsommer zur biogen induzierten Karbonatfällung in Hartwasserseen bei. Das gefällte Karbonat bindet den Kohlenstoff (C) dauerhaft über geologische Zeiträume am Seegrund. Ziel dieser Dissertation war es, die Rolle von *P. lenticularis* hinsichtlich ihres Beitrags zur langfristigen C-Sequestrierung durch Karbonatfällung in bayerischen Seen zu untersuchen. In der Arbeit werden die Kalzitschalen untersucht sowie Populationsdynamik und Sedimentationsraten bestimmt. Das ermöglicht erstmals die Bestimmung des quantitativen Beitrags der *Phacotus lenticularis*-Schalen am Gesamtkarbonat in Seesedimenten.

Ein wesentliches Ergebnis war die Entwicklung eines neuartigen Ansatzes zur Analyse der Masse von *Phacotus*-Schalen mit der Focussed Ion Beam Technologie. Diese Methode wurde in vier deutschen Hartwasserseen validiert und ist auch auf andere Mikropartikel anwendbar. Eine detaillierte morphometrische Untersuchung der *Phacotus*-Schalen ergab, dass der Schalendurchmesser signifikant durch die Herkunft beeinflusst wurde. Die Schalen aus verschiedenen Seen wiesen erhebliche Unterschiede in Durchmesser und Form auf, was zu Schalenmassen von 0,33 bis 0,52 ng führte. Dies ermöglicht eine quantitative Bewertung des Einflusses des *Phacotus*-Karbonatbeitrags zur Gesamtkarbonatfällung in Seen.

Die Arbeit beschreibt eine standardisierte Methodik zur repräsentativen Untersuchung des räumlich-zeitlichen Auftretens von *P. lenticularis* in der Wassersäule. Gleichzeitige Messungen in drei verschiedenen Seebecken des Großen Ostersees ergaben identische Zelldichten. An allen Standorten gingen die vertikalen *P. lenticularis*-Zelldichtemaxima mit der sich langsam absenkenden Thermokline einher. Offenbar kontrollieren weder die Bathymetrie des Sees noch externe Faktoren wie Windexposition, die Häufigkeit der Kalkalgen. Darüber hinaus wurden in vier Seen die Zelldichten und Sedimentationsraten ermittelt wobei sich die Karbonatrücklösung als Störfaktor herausstellte, der die Schalenzahl sowohl in den Sedimentfallen am Seeboden als auch in den Planktonproben während der Lagerung reduzieren kann.

Zusammenfassend zeigt diese Arbeit, dass der *Phacotus*-Karbonatgehalt in Seesedimenten geringer ist als im suspendierten Karbonat in der Wassersäule, weil *P. lenticularis* saisonal mit kurzfristigen Populationsspitzen auftritt und sein Anteil am Gesamtkarbonat dadurch stark von der Menge der zusätzlichen Karbonatfällung und dem untersuchten Zeitraum abhängt. In einem Sedimentkern des oligotrophen Großen Ostersees konnte gezeigt werden, dass der Beitrag von *P. lenticularis* weniger als 1 Gew.-% des Gesamtkar-

bonatgehaltes betrug und dass dafür die Verdünnung durch die authigene Karbonatfällung der Hauptgrund war. Vor dem Hintergrund der C-Fixierung in Hartwasserseen stellt der Beitrag von *P. lenticularis* zur limnischen Kalzitfällung einen kleinen, aber nennenswerten Teil des C-Umsatzes dar, der durchaus Berücksichtigung finden sollte.

6 | References

Adrian, R. and Shatwell, T. (2018). Seen im Klimawandel: Diagnosen und Prognosen aus der Langzeitforschung. Berlin: Leibniz-Institut für Gewässerökologie und Binnenfischerei (IGB) im Forschungsverbund Berlin e.V. doi: 10.4126/FRL01-006407562

Alefs, J. (1997). Feinstratigrafie und Diatomeensukzession in den Profundalsedimenten des Ammersees und Starnbergersees: Dissertation. Technische Universität München.

Alonso-Zarza, A. M. and Tanner, L. H. (2010). Carbonates in Continental Settings: Facies, Environments, and Processes. Elsevier.

Anas, M. M., Scott, K. A. and Wissel, B. (2015). Carbon budgets of boreal lakes: State of knowledge, challenges, and implications. *Environmental Reviews*, *23*(3), 275–287. doi: 10.1139/er-2014-0074

Andersen, M. R., Kragh, T., Martinsen, K. T., Kristensen, E. and Sand-Jensen, K. (2019). The carbon pump supports high primary production in a shallow lake. *Aquatic Sciences*, *81*(2), 1305. doi: 10.1007/s00027-019-0622-7

Anderson, N. J., D'andrea, W. and Fritz, S. C. (2009). Holocene carbon burial by lakes in SW Greenland. *Global Change Biology*, *15*(11), 2590–2598. doi: 10.1111/j.1365-2486.2009.01942.x

Anderson, N. J., Dietz, R. D. and Engstrom, D. R. (2013). Land-use change, not climate, controls organic carbon burial in lakes. *Proceedings of the Royal Society B: Biological Sciences*, *280*(1769), 20131278. doi: 10.1098/rspb.2013.1278

Appleby, P. G. (2001). Chronostratigraphic Techniques in Recent Sediments. In W. M. Last, J. P. Smol and Birks, H. J. B (Eds.), Tracking environmental change using lake sediments (Vol. 1, pp. 171–203). Dordrecht and Boston: Kluwer Academic Publishers. doi: 10.1007/0-306-47669-X9

Auerswald, K. and Geist, J. (2018). Extent and Causes of Siltation in a Headwater Stream Bed: Catchment Soil Erosion is Less Important than Internal Stream Processes. *Land Degradation & Development*, *29*(3), 737–748. doi: 10.1002/ldr.2779

Balch, W. M., Holligan, P. M. and Kilpatrick, K. A. (1992). Calcification, photosynthesis and growth of the bloom-forming coccolithophore, Emiliania huxleyi. *Continental Shelf Research*, *12*(12), 1353–1374.

Bavarian Environment Agency. (2016). Planktondaten für "Phacotus lenticularis" in den Gewässern Abtsdorfer See, Altmuehlsee, Grosser Ostersee, Hopfensee, Igelsbachsee, Waginger See. Wielenbach. Retrieved from https://www.lfu.bayern.de

Bavarian Regional Statistical Office. (1953). Historisches Gemeindeverzeichnis: Die Einwohnerzahlen der Geimenden Bayerns in der Zeit von 1840–1952 (Vol. Heft 192). Munich: Bayerisches Statistisches Landesamt.

Bayerisches Landesamt für Wasserwirtschaft. (1987). Verzeichnis der Seen in Bayern mit einem Kartenteil: Teil 1 Text u.Teil 2 Karten. München.

Berger, S. A., Diehl, S., Stibor, H., Trommer, G., Ruhenstroth, M., Wild, A., Weigert, A., Jäger, C. G. and Striebel, M. (2007). Water temperature and mixing depth affect timing and

magnitude of events during spring succession of the plankton. *Oecologia*, *150*(4), 643–654. doi: 10.1007/s00442-006-0550-9

Blaauboer, M. C. (1982). The phytoplankton species composition and the seasonal periodicity in Lake Vechten from 1956 to 1979. *Hydrobiologia*, *95*(1), 25–36.

Blain, S., Quéguiner, B., Armand, L., Belviso, S., Bombled, B., Bopp, L., Bowie, A., Brunet, C., Brussaard, C., Carlotti, F., Christaki, U., Corbière, A., Durand, I., Ebersbach, F., Fuda, J.-L., Garcia, N., Gerringa, L., Griffiths, B., Guigue, C., Guillerm, C., Jacquet, S., Jeandel, C., Laan, P., Lefèvre, D., Lo Monaco, C., Malits, A., Mosseri, J., Obernosterer, I., Park, Y.-H., Picheral, M., Pondaven, P., Remenyi, T., Sandroni, V., Sarthou, G., Savoye, N., Scouarnec, L., Souhaut, M., Thuiller, D., Timmermans, K., Trull, T., Uitz, J., van Beek, P., Veldhuis, M., Vincent, D., Viollier, E., Vong, L. and Wagener, T. (2007). Effect of natural iron fertilization on carbon sequestration in the Southern Ocean. *Nature*, *446*, 1070 EP -. doi: 10.1038/nature05700

Bloesch, J. and Burns, N. M. (1980). A critical review of sedimentation trap technique. *Schweizerische Zeitschrift für Hydrologie*, *42*(1), 15–55. doi: 10.1007/BF02502505

Bluszcz, P., Kirilova, E., Lotter, A. F., Ohlendorf, C. and Zolitschka, B. (2008). Global Radiation and Onset of Stratification as Forcing Factors of Seasonal Carbonate and Organic Matter Flux Dynamics in a Hypertrophic Hardwater Lake (Sacrower See, Northeastern Germany). *Aquatic Geochemistry*, *14*(1), 73–98. doi: 10.1007/s10498-008-9026-3

Bluszcz, P., Lücke, A., Ohlendorf, C. and Zolitschka, B. (2009). Seasonal dynamics of stable isotopes and element ratios in authigenic calcites during their precipitation and dissolution, Sacrower See (northeastern Germany). *Journal of Limnology*, *68*(2), 257. doi: 10.4081/jlimnol.2009.257

Bollmann, J., Brabec, B., Cortés, M. Y. and Geisen, M. (1999). Determination of absolute coccolith abundances in deep-sea sediments by spiking with microbeads and spraying (SMS-method). *Marine Micropaleontology*, *38*(1), 29–38. doi: 10.1016/S0377-8398(99)00032-8

Bordiga, M., Bartol, M. and Henderiks, J. (2015). Absolute nannofossil abundance estimates: Quantifying the pros and cons of different techniques. *Revue de Micropaléontologie*, *58*(3), 155–165. doi: 10.1016/j.revmic.2015.05.002

Börtitz, C. D. (2012). Charakterisierung des Phacotus-Vorkommens in Ausgewählten Gewässern der Osterseengruppe: Diplomarbeit. Ernst Moritz Arndt Universität Greifswald.

Braig, E., Mayr, C., Kirchner, G., Hofmann, A., Raeder, U. and Melzer, A. (2013). Fifty years of eutrophication and lake restoration reflected in sedimentary carbon and nitrogen isotopes of a small, hardwater lake (south Germany). *Journal of Limnology*, *72*(2). doi: 10.4081/jlimnol.2013.e21

Brownlee, C. and Taylor, A. R. (2002). Algal calcification and silification. *eLS*.

Bucka, H. (1987a). Ecological aspects of the mass appearance of planktonic algae in dam reservoirs of southern Poland. *Acta Hydrobiologica*, *29*(2), 149–191.

Bucka, H. (1987b). Ecological aspects of the mass appearance of planktonic algae in dam reservoirs of southern Poland. *Acta Hydrobiologica*, *29*(2), 149–191.

Bukovec, K. and Exner, K. (1994). Iffeldorf, Geschichte eines Dorfes. Polling: Esta-Druck.

Cairns Jr, J. and Smith, E. P. (1993). The statistical validity of biomonitoring data. *Lewis publishers, Boca Raton, FL(USA)*.

Campbell, I. D., Campbell, C., Vitt, D. H., Kelker, D., Laird, L. D., Trew, D., Kotak, B., LeClair, D. and Bayley, S. (2000). A first estimate of organic carbon storage in Holocene

lake sediments in Alberta, Canada. *Journal of Paleolimnology*, *24*(4), 395–400. doi: 10.1023/A:1008103605817

Casper, S. J. (2012). Lake Stechlin: A temperate oligotrophic lake. Springer Science & Business Media.

Cermeño, P., Teixeira, I. G., Branco, M., Figueiras, F. G. and Marañón, E. (2014). Sampling the limits of species richness in marine phytoplankton communities. *Journal of Plankton Research*, *36*(4), 1135–1139.

Ciais, P., Sabine, C., Bala, G., Bopp, L., Brovkin, V., Canadell, J., Chhabra, A., DeFries, R., Galloway, J. and Heimann, M. (2013). Carbon and other biogeochemical cycles. Climate change 2013: The physical science basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. *Cambridge University Press Cambridge United Kingdom and New York NY USA*, 465–570.

Cole, J. J., Pace, M. L., Carpenter, S. R. and Kitchell, J. F. (2000). Persistence of net heterotrophy in lakes during nutrient addition and food web manipulations. *Limnology and Oceanography*, *45*(8), 1718–1730.

Cole, J. J., Prairie, Y. T., Caraco, N. F., McDowell, W. H., Tranvik, L. J., Striegl, R. G., Duarte, C. M., Kortelainen, P., Downing, J. A., Middelburg, J. J. and Melack, J. (2007). Plumbing the Global Carbon Cycle: Integrating Inland Waters into the Terrestrial Carbon Budget. *Ecosystems*, *10*(1), 172–185. doi: 10.1007/s10021-006-9013-8

Cremona, F., Kõiv, T., Nõges, P., Pall, P., Rõõm, E.-I., Feldmann, T., Viik, M. and Nõges, T. (2014). Dynamic carbon budget of a large shallow lake assessed by a mass balance approach. *Hydrobiologia*, *731*(1), 109–123. doi: 10.1007/s10750-013-1686-3

Cărăuş, I. (2002). Algae of Romania. *Studii şi Cercetări, Universitatea Bacau, Biologie*, *7*, 1–694.

Daberkow, S. A. (2011). Räumliche Verteilung der Phacotus lenticularis Population im großen Ostersee: Zulassungsarbeit. Technische Universität München.

Dean, W. E. and Gorham, E. (1998). Magnitude and significance of carbon burial in lakes, reservoirs, and peatlands. *Geology*, *26*(6), 535–538.

DIN Technical Committee Water analysis. (2015). Water quality - Guidance on quantitative and qualitative sampling of phytoplankton from inland waters (CEN/TC230 ed.) (No. EN 16698:2015 (E)). Beuth.

Dittrich, M., Kurz, P. and Wehrli, B. (2004). The role of autotrophic picocyanobacteria in calcite precipitation in an oligotrophic lake. *Geomicrobiology Journal*, *21*(1), 45–53. doi: 10.1080/01490450490253455

Dittrich, M., Müller, B., Mavrocordatos, D. and Wehrli, B. (2003). Induced Calcite Precipitation by CyanobacteriumSynechococcus. *Acta Hydrochimica et Hydrobiologica*, *31*(2), 162–169. doi: 10.1002/aheh.200300486

Dittrich, M. and Obst, M. (2004). Are picoplankton responsible for calcite precipitation in lakes? *AMBIO: A Journal of the Human Environment*, *33*(8), 559–564. doi: 10.1579/0044-7447-33.8.559

Dölker, J. (2011). Das Kalkbindevermögen von Phacotus lenticularis und der Einfluss des Nährstoffangebotes auf sein Vorkommen in ausgewählten See der Osterseengruppe: Bachelorarbeit. Technische Universität München.

Dunlap, J. R. and Walne, P. L. (1993). Microarchitecture and mineralization in loricae of phacotacean flagellates. *Acta protozoologica*, *32*(4), 237–244.

Einola, E., Rantakari, M., Kankaala, P., Kortelainen, P., Ojala, A., Pajunen, H., Mäkelä, S. and Arvola, L. (2011). Carbon pools and fluxes in a chain of five boreal lakes: A dry and wet year comparison. *Journal of Geophysical Research*, *116*(G3). doi: 10.1029/2010JG001636

Einsele, G., Yan, J. and Hinderer, M. (2001). Atmospheric carbon burial in modern lake basins and its significance for the global carbon budget. *Global and Planetary Change*, *30*(3-4), 167–195. doi: 10.1016/S0921-8181(01)00105-9

Emi, I. (2001). Application of Stable Isotope Techniques to Inorganic and Biogenic Carbonates. In W. M. Last and J. P. Smol (Eds.), Tracking environmental change using lake sediments (pp. 351–371). Dordrecht and London: Kluwer Academic.

Ettl, H., Gärtner, G., Kadlubowska, J. Z. and Mrozińska, T. (1983-1988). Chlorophyta I: Phytomonadina (Vol. Band 16). Stuttgart: Fischer.

Ferland, M.-E., del Giorgio, P. A., Teodoru, C. R. and Prairie, Y. T. (2012). Long-term C accumulation and total C stocks in boreal lakes in northern Québec. *Global Biogeochemical Cycles*, *26*(4), n/a-n/a. doi: 10.1029/2011GB004241

Finlay, K., Leavitt, P. R., Wissel, B. and Prairie, Y. T. (2009). Regulation of spatial and temporal variability of carbon flux in six hard-water lakes of the northern Great Plains. *Limnology and Oceanography*, *54*(6), 2553.

Forzza, R., Baumgratz, J., Bicudo, C. d. M., Carvalho Junior, A., Costa, A., Costa, D., Hopkins, M., Leitman, P., Lohmann, L., Maia, L. et al. (2010). Catálogo das plantas e fungos do Brasil. v. 1 e 2. *Rio de Janeiro: Andrea Jakobsson Estúdio & Jardim Botânico do Rio de Janeiro*.

Friedlingstein, P., Cox, P., Betts, R., Bopp, L., von Bloh, W., Brovkin, V., Cadule, P., Doney, S., Eby, M., Fung, I., Bala, G., John, J., Jones, C., Joos, F., Kato, T., Kawamiya, M., Knorr, W., Lindsay, K., Matthews, H. D., Raddatz, T., Rayner, P., Reick, C., Roeckner, E., Schnitzler, K.-G., Schnur, R., Strassmann, K., Weaver, A. J., Yoshikawa, C. and Zeng, N. (2006). Climate–Carbon Cycle Feedback Analysis: Results from the C 4 MIP Model Intercomparison. *Journal of Climate*, *19*(14), 3337–3353. doi: 10.1175/JCLI3800.1

Fuchs, A., Selmeczy, G. B., Kasprzak, P., Padisák, J. and Casper, P. (2016). Coincidence of sedimentation peaks with diatom blooms, wind, and calcite precipitation measured in high resolution by a multi-trap. *Hydrobiologia*, *763*(1), 329–344. doi: 10.1007/s10750-015-2388-9

Füchtbauer, H., von der Brelie, G., Dehm, R., Förstner, U., Gall, H., Höfling, R., Hoefs, J., Hollerbach, H., Jankowski, B. and Jung, W. (1977). Tertiary lake sediments of the Ries, research borehole Nördlingen 1973-a summary. *Geologica Bavarica*, *75*, 13–19.

Gälman, V., Rydberg, J., de Luna, S. S., Bindler, R. and Renberg, I. (2008). Carbon and nitrogen loss rates during aging of lake sediment: Changes over 27 years studied in varved lake sediment. *Limnology and Oceanography*, *53*(3), 1076–1082. doi: 10.4319/lo.2008.53.3.1076

Giering, B., Krienitz, L. and Casper, S. J. (1992). Zur Taxonomie von Phacotus lenticularis (Ehrenberg) Stein (Chlamydophyceae, Phacotaceae). *Nova Hedwigia*, *55*(3-4), 367–380.

Giering, B., Krienitz, L., Casper, S. J., Peschke, T. and Raidt, H. (1990a). LM and SEM observations on the asexual reproduction and lorica formation of Phacotus lendneri Chodat (Chlamydophyceae, Phacotaceae). *Archiv für Protistenkunde*, *138*(1), 75–88. doi: 10.1016/S0003-9365(11)80212-5

Giering, B., Krienitz, L., Casper, S. J., Peschke, T. and Raidt, H. (1990b). LM-oder REM-Beobachtungen an Phacotus sphaericus (Wislouch) Giering nov. stat.(Chlamydophyceae, Phacotaceae). *Archiv für Protistenkunde*, *138*(4), 323–332.

Gierlowski-Kordesch, E. H. (2010). Lacustrine Carbonates. In A. M. Alonso-Zarza and L. H. Tanner (Eds.), Carbonates in Continental Settings: Facies, Environments, and Processes (Vol. 61, pp. 1–101). Elsevier. doi: 10.1016/S0070-4571(09)06101-9

Goldman, C. R., Kumagai, M. and Robarts, R. D. (2013). Climatic change and inland waters: Impacts and mitigation for ecosystems and societies. Chichester, West Sussex, UK: Wiley-Blackwell.

Golubić, S., Krumbein, W. and Schneider, J. (1979). The Carbon Cycle. *Studies in environmental science*, *3*, 29–45. doi: 10.1016/S0166-1116(08)71053-7

Grimminger, H. (1982). Verzeichnis der Seen in Bayern mit einem Kartenteil. tl. 1. Text. tl. 2. Karten. Bayerisches Landesamt für Wasserwirtschaft.

Gruenert, U., Benda, J., Müller, C. and Raeder, U. (2016). Vertical segregation of two cell-cycle phases of the calcifying freshwater phytoflagellate Phacotus lenticularis (Chlorophyta). *Journal of Plankton Research*, *38*(1), 94–105. doi: 10.1093/plankt/fbv093

Gruenert, U. and Raeder, U. (2014a). Bedeutung der Planktonalge Phacotus lenticularis für die Speicherung von klimarelevanten Kohlendioxids in bayerischen Seen: Endbericht zum Forschungsvorhaben. Technische Universität München.

Gruenert, U. and Raeder, U. (2014b). Growth responses of the calcite-loricated freshwater phytoflagellate Phacotus lenticularis (Chlorophyta) to the CaCO3 saturation state and meteorological changes. *Journal of Plankton Research*, *36*(3), 630–640. doi: 10.1093/plankt/fbu006

Grunewald, K. and Bastian, O. (2013). Ökosystemdienstleistungen: Konzept, Methoden und Fallbeispiele. Berlin: Springer Spektrum.

Guiry, M. D. (2019). AlgaeBase: World-wide electronic publication. searched on 04 August 2019.. Retrieved from http://www.algaebase.org

Haberzettl, T., Corbella, H., Fey, M., Janssen, S., Lücke, A., Mayr, C., Ohlendorf, C., Schäbitz, F., Schleser, G. H., Wille, M., Wulf, S. and Zolitschka, B. (2007). Lateglacial and Holocene wet—dry cycles in southern Patagonia: Chronology, sedimentology and geochemistry of a lacustrine record from Laguna Potrok Aike, Argentina. *The Holocene*, *17*(3), 297–310. doi: 10.1177/0959683607076437

Haberzettl, T., Fey, M., Lücke, A., Maidana, N., Mayr, C., Ohlendorf, C., Schäbitz, F., Schleser, G. H., Wille, M. and Zolitschka, B. (2005). Climatically induced lake level changes during the last two millennia as reflected in sediments of Laguna Potrok Aike, southern Patagonia (Santa Cruz, Argentina). *Journal of Paleolimnology*, *33*(3), 283–302. doi: 10.1007/s10933-004-5331-z

Hahn, A., Kliem, P., Oehlerich, M., Ohlendorf, C. and Zolitschka, B. (2014). Elemental composition of the Laguna Potrok Aike sediment sequence reveals paleoclimatic changes over the past 51 ka in southern Patagonia, Argentina. *Journal of Paleolimnology*, *52*(4), 349–366. doi: 10.1007/s10933-014-9798-y

Hansen, J. W., Nedergaard, M. and Skov, F. (2008). IGLOO–Indikatorer for globale klimaforandringer i overvågningen, Bilagsrapport. *By-og Landskabsstyrelsen, Miljøministeriet, Denmark*.

Hariskos, I., Chairopoulou, M., Posten, C., Teipel, U. and Vučak, M. (2016). Characterisation of biogenic calcite particles from microalgae-coccoliths as a potential raw material for industrial application. *Ecol. Eng. Environ. Prot*, *7*, 36–41.

Hariskos, I., Chairopoulou, M., Vučak, M., Posten, C. and Teipel, U. (2016). Produktion und Charakterisierung von Mikrostrukturierten Calcitpartikeln aus der Kalkalge Emiliania Huxleyi. *Chemie Ingenieur Technik*. doi: 10.1002/cite.201500128

Heathcote, A. J. and Downing, J. A. (2012). Impacts of Eutrophication on Carbon Burial in Freshwater Lakes in an Intensively Agricultural Landscape. *Ecosystems*, *15*(1), 60–70. doi: 10.1007/s10021-011-9488-9

Hepperle, D. and Krienitz, L. (1996). The extracellular calcification of zoospores of Phacotus lenticularis (Chlorophyta, Chlamydomonadales). *European Journal of Phycology*, *31*(1), 11–21. doi: 10.1080/09670269600651141

Hepperle, D. and Krienitz, L. (1997). Phacotus lenticularis (Chlamydomonales, Phacotaceae) zoospores require external supersaturation of calcium carbonate for calcification in culture 1, 2. *Journal of phycology*, *33*(3), 415–424.

Herzog, H., Golomb, D. et al. (2004). Carbon capture and storage from fossil fuel use. *Encyclopedia of energy*, *1*(6562), 277–287.

Hillaire-Marcel, C. and de Vernal, A. (2007). Proxies in Late Cenozoic Paleoceanography: Chapter Ten Coccolithophores: From Extant Populations to Fossil Assemblages (Vol. 1). Elsevier.

Hutchinson, G. E. (1957). A Treatise on Limnology: I and II (Vol. 1). London: J. Wiley.

Jacques, G. and Luc, B. (2007). Chapter Ten Coccolithophores: From Extant Populations to Fossil Assemblages. In C. Hillaire-Marcel and A. de Vernal (Eds.), Proxies in Late Cenozoic Paleoceanography (Vol. 1, pp. 409–439). Elsevier. doi: 10.1016/S1572-5480(07)01015-9

Jeppesen, E., Kronvang, B., Jørgensen, T. B., Larsen, S. E., Andersen, H. E., Søndergaard, M., Liboriussen, L., Bjerring, R., Johansson, L. S. and Trolle, D. (2013). Recent Climate–Induced Changes in Freshwaters in Denmark. In C. R. Goldman, M. Kumagai and R. D. Robarts (Eds.), Climatic change and inland waters (pp. 155–171). Chichester, West Sussex, UK: Wiley-Blackwell.

John, D. M., Whitton, B. A. and Brook, A. J. (2011). Freshwater algal flora of the British Isles. Cambridge University Press.

Jonsson, A. and Jansson, M. (1997). Sedimentation and mineralisation of organic carbon, nitrogen and phosphorous in a large humic lake, northern Sweden. *Archiv für Hydrobiologie*, *141*, 45–65.

Jonsson, A., Meili, M., Bergström, A.-K. and Jansson, M. (2001). Whole-lake mineralization of allochthonous and autochthonous organic carbon in a large humic lake (örträsket, N. Sweden). *Limnology and Oceanography*, *46*(7), 1691–1700. doi: 10.4319/lo.2001.46.7.1691

Jouve, G., Francus, P., Lamoureux, S., Provencher-Nolet, L., Hahn, A., Haberzettl, T., Fortin, D. and Nuttin, L. (2013). Microsedimentological characterization using image analysis and m-XRF as indicators of sedimentary processes and climate changes during Lateglacial at Laguna Potrok Aike, Santa Cruz, Argentina. *Quaternary Science Reviews*, *71*, 191–204. doi: 10.1016/j.quascirev.2012.06.003

Kamptner, E. (1950). Über den Aufbau des Kalkgehäuses von Phacotus Lendneri CHOD. *Österreichische Botanische Zeitschrift*, *97*(3), 391–402.

Kastowski, M., Hinderer, M. and Vecsei, A. (2011). Long-term carbon burial in European lakes: Analysis and estimate. *Global Biogeochemical Cycles*, *25*(3), n/a-n/a. doi: 10.1029/2010GB003874

Kelly, M. (1998). Quality Assurance for Phytoplankton Data. Durham. Retrieved from www.environmentdata.org

Kelts, K. and Hsü, K. J. (1978). Freshwater Carbonate Sedimentation. In A. Lerman (Ed.), Lakes (pp. 295–323). New York, NY: Springer New York. doi: 10.1007/978-1-4757-1152-3

Kirilova, E. P., Bluszcz, P., Heiri, O., Cremer, H., Ohlendorf, C., Lotter, A. F. and Zolitschka, B. (2008). Seasonal and interannual dynamics of diatom assemblages in Sacrower See (NE Germany): a sediment trap study. *Hydrobiologia*, *614*(1), 159–170. doi: 10.1007/s10750-008-9504-z

Koch, C. and Young, J. R. (2007). A simple weighing and dilution technique for determining absolute abundances of coccoliths from sediment samples. *Journal of Nannoplankton Research*, *29*(1), 67–69.

Koester, J., Brownlee, C. and Taylor, A. R. (2016). Algal Calcification and Silicification. *eLS*.

Korde, N. W. (1966). Algenreste in Seesedimenten: Zur Entwicklungsgeschichte der Seen und umliegenden Landschaften. Stuttgart: Schweizerbatrt'sche.

Kortelainen, P., Pajunen, H., Rantakari, M. and Saarnisto, M. (2004). A large carbon pool and small sink in boreal Holocene lake sediments. *Global Change Biology*, *10*(10), 1648–1653. doi: 10.1111/j.1365-2486.2004.00848.x

Kortelainen, P., Rantakari, M., Pajunen, H., Huttunen, J. T., Mattsson, T., Juutinen, S., Larmola, T., Alm, J., Silvola, J. and Martikainen, P. J. (2013). Carbon evasion/accumulation ratio in boreal lakes is linked to nitrogen. *Global Biogeochemical Cycles*, *27*(2), 363–374. doi: 10.1002/gbc.20036

Koschel, R. (1983). Calcite precipitation as a natural control mechanism of eutrophication. *Arch Hydrobiol*, *98*, 380–408.

Koschel, R., Proft, G. and Raidt, H. (1987a). Autochthonous Calcite Precipitation in Hardwater Lakes of the Mecklenburg Lake District. *Limnologica*, *18*(2), 317–338.

Koschel, R., Proft, G. and Raidt, H. (1987b). Phacotus-Massenentwicklungen - eine Quelle des autochthonen Kalkeintrages in Seen. *Limnologica*, *18*(2), 457–459.

Koschel, R. and Raidt, H. (1988a). Morphologische Merkmale der Phacotus-Hüllen in Hartwasserseen der Mecklenburger Seenplatte. *Limnologica*, *19*(2), 13–25.

Koschel, R. and Raidt, H. (1988b). Morphology of Calcite Crystals in Hardwater Lakes. *Limnologica*, *119*(2), 3–12.

Kozerski, H.-P. and Leuschner, K. (1999). Plate sediment traps for slowly moving waters. *Water Research*, *33*(13), 2913–2922. doi: 10.1016/S0043-1354(98)00510-7

Krienitz, L., Koschel, R., Giering, B., Casper, S. and Hepperle, D. (1993). Phenomenology of organismic calcite precipitation by Phacotus in hardwater lakes and ponds of northeastern Germany. *Internationale Vereinigung für theoretische und angewandte Limnologie: Verhandlungen*, *25*(1), 170–174.

Laas, A., Cremona, F., Meinson, P., Rõõm, E.-I., Nõges, T. and Nõges, P. (2016). Summer depth distribution profiles of dissolved CO2 and O2 in shallow temperate lakes reveal trophic state and lake type specific differences. *The Science of the total environment*, *566-567*, 63–75. doi: 10.1016/j.scitotenv.2016.05.038

Lagerheim, G. (1902). Untersuchungen über Fossile Algen, I, II. *Geologiska Föreningen i Stockholm Förhandlingar*, *24*(7), 475–500. doi: 10.1080/11035890209449975

Lampert, W. and Sommer, U. (1999). Limnoökologie (2., überarb. Aufl. ed.). Stuttgart and New York: Thieme.

Last, W. M. and Smol, J. P. (2001). Tracking environmental change using lake sediments: Physical and Geochemical Methods (Vol. Volume 2). Dordrecht and London: Kluwer Academic.

Last, W. M., Smol, J. P. and Birks, H. J. B. (2001). Tracking environmental change using lake sediments: Basin Analysis, Coring and Chronological Techniques (Vol. Volume. 1). Dordrecht and Boston: Kluwer Academic Publishers.

Le Quéré, C., Moriarty, R., Andrew, R. M., Canadell, J. G., Sitch, S., Korsbakken, J. I., Friedlingstein, P., Peters, G. P., Andres, R. J., Boden, T. A., Houghton, R. A., House, J. I., Keeling, R. F., Tans, P., Arneth, A., Bakker, D. C. E., Barbero, L., Bopp, L., Chang, J., Chevallier, F., Chini, L. P., Ciais, P., Fader, M., Feely, R. A., Gkritzalis, T., Harris, I., Hauck, J., Ilyina, T., Jain, A. K., Kato, E., Kitidis, V., Klein Goldewijk, K., Koven, C., Landschützer, P., Lauvset, S. K., Lefèvre, N., Lenton, A., Lima, I. D., Metzl, N., Millero, F., Munro, D. R., Murata, A., Nabel, J. E. M. S., Nakaoka, S., Nojiri, Y., O'Brien, K., Olsen, A., Ono, T., Pérez, F. F., Pfeil, B., Pierrot, D., Poulter, B., Rehder, G., Rödenbeck, C., Saito, S., Schuster, U., Schwinger, J., Séférian, R., Steinhoff, T., Stocker, B. D., Sutton, A. J., Takahashi, T., Tilbrook, B., van der Laan-Luijkx, I. T., van der Werf, G. R., van Heuven, S., Vandemark, D., Viovy, N., Wiltshire, A., Zaehle, S. and Zeng, N. (2015). Global Carbon Budget 2015. *Earth System Science Data*, *7*(2), 349–396. doi: 10.5194/essd-7-349-2015

Le Quéré, C., Raupach, M. R., Canadell, J. G., Marland et al., G., Le Quéré et al., Corinne, Marland, G., Bopp, L., Ciais, P., Conway, T. J., Doney, S. C., Feely, R. A., Foster, P., Friedlingstein, P., Gurney, K., Houghton, R. A., House, J. I., Huntingford, C., Levy, P. E., Lomas, M. R., Majkut, J., Metzl, N., Ometto, J. P., Peters, G. P., Prentice, I. C., Randerson, J. T., Running, S. W., Sarmiento, J. L., Schuster, U., Sitch, S., Takahashi, T., Viovy, N., van der Werf, Guido R. and Woodward, F. I. (2009). Trends in the sources and sinks of carbon dioxide. *Nature Geoscience*, *2*(12), 831–836. doi: 10.1038/ngeo689

Lenz, S., Dubois, N., Geist, J. and Raeder, U. (2019). *Phacotus lenticularis* content in carbonate sediments and epilimnion in four German hard water lakes (Manuscript submitted for publication).

Lenz, S., Gruenert, U., Geist, J., Stiefel, M., Lentz, M. and Raeder, U. (2017). Calcite production by the calcifying green alga *Phacotus lenticularis*. *Journal of Limnology*. doi: 10.4081/jlimnol.2018.1692

Lenz, S., Raeder, U. and Geist, J. (2020). Representative monitoring of calcifying alga *Phacotus lenticularis* (Clamydophyceae) in lentic ecosystems. *Journal of Limnology*.

Lerman, A. (1978). Lakes. New York, NY: Springer New York. doi: 10.1007/978-1-4757-1152-3

Liu, Z., Dreybrodt, W. and Liu, H. (2011). Atmospheric CO2 sink: Silicate weathering or carbonate weathering? *Applied Geochemistry*, *26*, S292-S294. doi: 10.1016/j.apgeochem.2011.03.085

Mandel, S. J. (2014). Charakterisierung der Vertikalen Verteilung der Kalkbildenden Planktonalge Phacotus lenticularis im Westen und Osten Desgroßen Ostersee: Diplomarbeit. Technische Universität München.

Marcé, R., Obrador, B., Morguí, J.-A., Lluís Riera, J., López, P. and Armengol, J. (2015). Carbonate weathering as a driver of CO2 supersaturation in lakes. *Nature Geoscience*, *8*(2), 107–111. doi: 10.1038/ngeo2341

Martin, J. H., Coale, K. H., Johnson, K. S., Fitzwater, S. E., Gordon, R. M., Tanner, S. J., Hunter, C. N., Elrod, V. A., Nowicki, J. L., Coley, T. L., Barber, R. T., Lindley, S., Watson, A. J., van Scoy, K., Law, C. S., Liddicoat, M. I., Ling, R., Stanton, T., Stockel, J., Collins, C., Anderson, A., Bidigare, R., Ondrusek, M., Latasa, M., Millero, F. J., Lee, K., Yao, W., Zhang, J. Z., Friederich, G., Sakamoto, C., Chavez, F., Buck, K., Kolber, Z., Greene, R., Falkowski, P., Chisholm, S. W., Hoge, F., Swift, R., Yungel, J., Turner, S., Nightingale, P., Hatton, A., Liss, P. and Tindale, N. W. (1994). Testing the iron hypothesis in ecosystems of the equatorial Pacific Ocean. *Nature*, *371*(6493), 123–129. doi: 10.1038/371123a0

McGowan, S., Anderson, N. J., Edwards, M. E., Langdon, P. G., Jones, V. J., Turner, S., van Hardenbroek, M., Whiteford, E. and Wiik, E. (2015). Long-term perspectives on terrestrial and aquatic carbon cycling from palaeolimnology. *Wiley Interdisciplinary Reviews: Water*, n/a. doi: 10.1002/wat2.1130

McNeel, R. (2013). Rhinoceros: CAD software. Barcelona, Spain: Robert McNeel & Associates. Retrieved from http://www.rhino3d.com/

Melzer, A. (1976). Makrophytische Wasserpflanzen als Indikatoren des Gewässerzustandes oberbayerischer Seen: Dargestellt im Rahmen limnologischer Untersuchungen an den Osterseen und den Eggstätt-Hemhofer Seen (Oberbayern) (Vol. 34). Vaduz: Cramer.

Menezes, M. (2010). Chlorophyceae. In R. C. Forzza *et al* (Eds.), Catálogo das plantas e fungos do Brasil. v. 1 e 2.

Mischke, U. and Nixdorf, B. (2008). Bewertung von Seen mittels Phytoplankton zur Umsetzung der EU-Wasserrahmenrichtlinie (Vol. 10; U. Mischke and B. Nixdorf, Eds.). Cottbus: Brandenburgische Techn. Univ., Fak. Umweltwiss. und Verfahrenstechnik.

Morris, J. (op. 2013). Biology - How life works (2nd printing ed.). New York: W.H. Freeman and Company.

Morris, P. J. and Charette, M. A. (2013). A synthesis of upper ocean carbon and dissolved iron budgets for Southern Ocean natural iron fertilisation studies. *Deep Sea Research Part II: Topical Studies in Oceanography*, *90*, 147–157. doi: 10.1016/j.dsr2.2013.02.001

Müller. (2012). Charakterisierung der Vertikalen Verteilung der Kalkbildenden Planktonalge Phacotus lenticularis im großen Ostersee: Diplomarbeit. Technische Universität München.

Müller, B., Wang, Y. and Wehrli, B. (2006). Cycling of calcite in hard water lakes of different trophic states. *Limnology and Oceanography*, *51*(4), 1678–1688. doi: 10.4319/lo.2006.51.4.1678

Müller, G. and Oti, M. (1981). The occurrence of calcified planktonic green algae in freshwater carbonates. *Sedimentology*, *28*(6), 897–902. doi: 10.1111/j.1365-3091.1981.tb01952.x

Myrbo, A. and Shapley, M. D. (2006). Seasonal water-column dynamics of dissolved inorganic carbon stable isotopic compositions (δ13CDIC) in small hardwater lakes in Minnesota and Montana. *Geochimica et Cosmochimica Acta*, *70*(11), 2699–2714. doi: 10.1016/j.gca.2006.02.010

Nixdorf, B., Hoehn, E., Riedmüller, u., Mischke, U., Schönfelder, I. and Bahnwart Mandy. (2008). Anforderungen an Probenahme und Analyse der Phytoplanktonbiozönosen in Seen zur ökologischen Bewertung gemäß der EU-WRRL. In U. Mischke and B. Nixdorf (Eds.), Bewertung von Seen mittels Phytoplankton zur Umsetzung der EU-Wasserrahmenrichtlinie (Vol. 10, pp. 147–184). Cottbus: Brandenburgische Techn. Univ., Fak. Umweltwiss. und Verfahrenstechnik.

Noges, P., Cremona, F., Laas, A., Martma, T., Room, E.-I., Toming, K., Viik, M., Vilbaste, S. and Noges, T. (2016). Role of a productive lake in carbon sequestration within a calcareous catchment. *The Science of the total environment*, *550*, 225–230. doi: 10.1016/j.scitotenv.2016.01.088

Ohlendorf, C. and Sturm, M. (2001). Precipitation and Dissolution of Calcite in a Swiss High Alpine Lake. *Arctic, Antarctic, and Alpine Research*, *33*(4), 410. doi: 10.2307/1552550

Pocratsky, L. A. (1982). Nutritional, chemical and ultrastructural characterization of the lorica and extracellular mucilage of Phacotus lenticularis (Phacotaceae, Volvocales): Dissertation. University Tennessee.

Pollard, R. T., Salter, I., Sanders, R. J., Lucas, M. I., Moore, C. M., Mills, R. A., Statham, P. J., Allen, J. T., Baker, A. R., Bakker, D. C. E., Charette, M. A., Fielding, S., Fones, G. R., French, M., Hickman, A. E., Holland, R. J., Hughes, J. A., Jickells, T. D., Lampitt, R. S., Morris, P. J., Nédélec, F. H., Nielsdóttir, M., Planquette, H., Popova, E. E., Poulton, A. J., Read, J. F., Seeyave, S., Smith, T., Stinchcombe, M., Taylor, S., Thomalla, S., Venables, H. J., Williamson, R. and Zubkov, M. V. (2009). Southern Ocean deep-water carbon export enhanced by natural iron fertilization. *Nature*, *457*(7229), 577–580. doi: 10.1038/nature07716

Porter, k. G. (1973). Selective Grazing and Differential Digestion of Algae by Zooplankton. *Nature*, *244*(5412), 179–180. doi: 10.1038/244179a0

Prairie, Y. T. (2008). Carbocentric limnology: Looking back, looking forward. *Canadian Journal of Fisheries and Aquatic Sciences*, *65*(3), 543–548. doi: 10.1139/F08-011

Proft, G. (1983). Automatische Bestimmung von Kohlendioxid und Carbonat in Wasser nach dem flow-stream-Prinzip. *Acta Hydrochimica et Hydrobiologica*, *11*(2), 235–239. doi: 10.1002/aheh.19830110214

Proft, G. (1984). Die Pelagische Calcitfällung und der Carbonatgehalt von Sedimenten pleistozäner Seen. *Acta Hydrochimica et Hydrobiologica*, *12*(3), 321–326.

Proft, G. and Stutter, E. (1993). Calcite Precipitation in Hard Water Lakes in Calculation and Experiment. *Internationale Revue der gesamten Hydrobiologie und Hydrographie*, *78*(2), 177–199. doi: 10.1002/iroh.19930780203

Purves, W. K. (2011). Biologie (9. Aufl. ed.). Heidelberg: Spektrum Akademischer Verlag.

R Core Team. (2015). R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from https://www.r-project.org/

Raeder, U. (2018). Endbericht Forschungsvorhaben: Bayerns Stillgewässer im Klimawandel - Einfluss und Anpassung: Verbreitung und CO2-Bindungsvermögen der Grünalge Phacotus lenticularis in bayerischen Seen – Monitoring und Anpassung an den Klimawandel.

Rasband, W. S. (2016). ImageJ: public domain Java image processing program. Bethesda, Maryland, USA: National Institutes of Health. Retrieved from https://imagej.nih.gov/ij/

Raymond, P. A., Bauer, J. E. and Cole, J. J. (2000). Atmospheric CO_2 evasion, dissolved inorganic carbon production, and net heterotrophy in the York River estuary. *Limnology and Oceanography*, *45*(8), 1707–1717.

Raymond, P. A., Hartmann, J., Lauerwald, R., Sobek, S., McDonald, C., Hoover, M., Butman, D., Striegl, R., Mayorga, E., Humborg, C., Kortelainen, P., Durr, H., Meybeck, M., Ciais, P. and Guth, P. (2013). Global carbon dioxide emissions from inland waters. *Nature*, *503*(7476), 355–359. doi: 10.1038/nature12760

Reimnitz, E. and Saarso, M. (1990). Studies of sediment transport by ice. *Die Expedition Arktis VII-I, mit FS Polarstern*, 44.

Rocha, O. and Duncan, A. (1985). The relationship between cell carbon and cell volume in freshwater algal species used in zooplanktonic studies. *Journal of Plankton Research*, *7*(2), 279–294.

Ronneberger, D. and Kasprzak, P. (2012). The zooplanktonic population. In S. J. Casper (Ed.), Lake Stechlin (Vol. 58, pp. 243–259). Springer Science & Business Media.

Rõõm, E.-I., Nõges, P., Feldmann, T., Tuvikene, L., Kisand, A., Teearu, H. and Nõges, T. (2014). Years are not brothers: Two-year comparison of greenhouse gas fluxes in large shallow Lake

Võrtsjärv, Estonia. *Journal of Hydrology*, *519*, 1594–1606. doi: 10.1016/j.jhydrol.2014.09.011

Ruber, J., Bauer, F. R., Millard, A. D., Raeder, U., Geist, J. and Zwirglmaier, K. (2016). Synechococcus diversity along a trophic gradient in the Osterseen Lake District, Bavaria. *Microbiology (Reading, England)*, *162*(12), 2053–2063. doi: 10.1099/mic.0.000389

Ruber, J., Geist, J., Hartmann, M., Millard, A., Raeder, U., Zubkov, M. and Zwirglmaier, K. (2018). Spatio-temporal distribution pattern of the picocyanobacterium Synechococcus in lakes of different trophic states: A comparison of flow cytometry and sequencing approaches. *Hydrobiologia*, *811*(1), 77–92. doi: 10.1007/s10750-017-3368-z

Rudolph, S. (2017). Auftreten der kalkbildenden Planktonalge Phacotus lenticularis in bayerischen Seen: Masterarbeit. Technische Universität München.

Saarso, M. (1995). Vertical variability of particulate flux in the Baltic Sea: 3. Symposium Proceedings. Nurmijarvi: NurmiPrint.

Sadava. (2019). Purves Biologie. Springer Berlin Heidelberg.

Sánchez, J. C., Cobelas, M. Á., Sanjurjo, M. A. (1998). Lista florística y bibliográfica de los clorófitos (Chlorophyta) de la Península Ibérica, Islas Baleares e Islas Canarias, No. 14. Asociación Española de Limnología.

Schlegel, I. (2001a). Ökologische und molekularbiologische Charakterisierung von Populationen des kalzithüllentragenden Phytoflagellaten Phacotus (Chlorophyta) (Unpublished doctoral dissertation). Verlag nicht ermittelbar.

Schlegel, I. (2001b). Ökologische und molekularbiologische Charakterisierung von Populationen des kalzithüllentragenden Phytoflagellaten Phatotus (Chlorophyta) (Dissertation). Humbold-Universität, Berlin.

Schlegel, I., Koschel, R. and Krienitz, L. (1998). On the occurrence of Phacotus lenticularis (Chlorophyta) in lakes of different trophic state. *Hydrobiologia*, *369-370*(0), 353–361. doi: 10.1023/A:1017019925927

Schlegel, I., Koschel, R. and Krienitz, L. (2000). Phacotus lenticularis (Chlorophyta) population dynamics in both nature and culture. *Internationale Vereinigung fur Theoretische und Angewandte Limnologie Verhandlungen*, *27*(2), 700–703.

Schlegel, I., Krienitz, L. and Hepperle, D. (2000). Variability of calcification of *Phacotus lenticularis* (Chlorophyta, Chlamydomonadales) in nature and culture. *Phycologia*, *39*(4), 318–322. doi: 10.2216/i0031-8884-39-4-318.1

Schmahl, W. W., Griesshaber, E., Kelm, K., Ball, A., Goetz, A., Xu, D., Kreitmeier, L. and Jordan, G. (2012). Towards systematics of calcite biocrystals: insight from the inside. *Zeitschrift für Kristallographie - Crystalline Materials*, *227*(8), 604–611. doi: 10.1524/zkri.2012.1479

Schneider, J. and Le Campion-Alsumard, T. (1999). Construction and destruction of carbonates by marine and freshwater cyanobacteria. *European Journal of Phycology*, *34*(4), 417–426.

Schröder, H. G., Windolpi, H. and Schneider, J. (1983). Bilanzierung der biogenen Karbonatproduktion eines oligotrophen Sees (Attersee, Salzkammergut—Österreich). *Arch. Hydrobiol*, *97*, 356–372.

Schwenkmeyer, I. (2012). Charakterisierung des Vorkommens der kalkbindenden Planktonalge Phacotus lenticularis an verschiedenen Standorten im Epilimnion des Großen Ostersees: Diplomarbeit. Fachhochschule Weihenstephan, Abteilung Triesdorf.

Schwoerbel, J. (op. 1994). Methoden der Hydrobiologie: Süsswasserbiologie (4., neubearbeitete Aufl. ed., Vol. 979). Stuttgart and Jena: G. Fischer.

Schwoerbel, J. and Brendelberger, H. (2005). Einführung in die Limnologie (9. Aufl. ed.). München and Heidelberg: Elsevier, Spektrum, Akad. Verl.

Sieminska, J. (1952). The plankton of the artificial lake at the Roznow dam. *Academie Polonaise des Sciences et des Lettres*(18), 109 pp.

Sladecek, V., Cyrus, Z. and Borovickova, A. (1958). Hydrobiological investigations of a treatment of beet sugar factory's wastes in an experimental lagoon. *Sbornik vus. skolu chem. techn. paliv a vodu*, 121–150.

Smetacek, V., Klaas, C., Strass, V. H., Assmy, P., Montresor, M., Cisewski, B., Savoye, N., Webb, A., d'Ovidio, F., Arrieta, J. M., Bathmann, U., Bellerby, R., Berg, G. M., Croot, P., Gonzalez, S., Henjes, J., Herndl, G. J., Hoffmann, L. J., Leach, H., Losch, M., Mills, M. M., Neill, C., Peeken, I., Röttgers, R., Sachs, O., Sauter, E., Schmidt, M. M., Schwarz, J., Terbrüggen, A. and Wolf-Gladrow, D. (2012). Deep carbon export from a Southern Ocean iron-fertilized diatom bloom. *Nature*, *487*(7407), 313–319. doi: 10.1038/nature11229

Sobek, S., Durisch-Kaiser, E., Zurbrügg, R., Wongfun, N., Wessels, M., Pasche, N. and Wehrli, B. (2009). Organic carbon burial efficiency in lake sediments controlled by oxygen exposure time and sediment source. *Limnology and Oceanography*, *54*(6), 2243–2254. doi: 10.4319/lo.2009.54.6.2243

Soendergaard, M., Jensen, J. P. and Jeppesen, E. (2005). Seasonal response of nutrients to reduced phosphorus loading in 12 Danish lakes. *Freshwater Biology*, *50*(10), 1605–1615.

Solomon, C. T., Bruesewitz, D. A., Richardson, D. C., Rose, K. C., van de Bogert, M. C., Hanson, P. C., Kratz, T. K., Larget, B., Adrian, R., Babin, B. L., Chiu, C.-Y., Hamilton, D. P., Gaiser, E. E., Hendricks, S., Istvànovics, V., Laas, A., O'Donnell, D. M., Pace, M. L., Ryder, E., Staehr, P. A., Torgersen, T., Vanni, M. J., Weathers, K. C. and Zhu, G. (2013). Ecosystem respiration: Drivers of daily variability and background respiration in lakes around the globe. *Limnology and Oceanography*, *58*(3), 849–866. doi: 10.4319/lo.2013.58.3.0849

Sommer, U. (1994). Planktologie. Berlin, Heidelberg: Springer Berlin Heidelberg.

Sprater, C. V. (2011). Zeitliche Entwicklung der Calcithüllen-tragenden Phacotus lenticularis Population an drei ausgewählten Seen der Osterseengruppe: Zulassungsarbeit. Technische Universität München.

Stabel, H.-H. (1986). Calcite precipitation in Lake Constance: Chemical equilibrium, sedimentation, and nucleation by algae1. *Limnology and Oceanography*, *31*(5), 1081–1094. doi: 10.4319/lo.1986.31.5.1081

Steinbauer, A. and Komischke, H. (2017). Monitoring Bericht 2016: Klimawandel in Süddeutschland: Veränderungen von meteorologischen und hydrologischen Kenngrößen: Klimamonitoring im Rahmen der Kooperation KLIWA. Retrieved from http://www.kliwa.de/publikationen-monitoringberichte.htm

Steinberg, C. and Klee, R. (1983). Röntgenmikroanalyse der Schale einer einzelligen Grünalge: Chemismus einer Phacotus lendneri-Schale. *Mikrokosmos*, *72*(6), 170–173.

Stumm, W. and Morgan, J. J. (1996). Aquatic chemistry: Chemical equilibria and rates in natural waters (3rd ed. ed.). New York: John Wiley & Sons.

Teranes, J. L. and Bernasconi, S. M. (2000). The record of nitrate utilization and productivity limitation provided by δ15N values in lake organic matter—A study of sediment trap and core sediments from Baldeggersee, Switzerland. *Limnology and Oceanography*, *45*(4), 801–813.

Thomas, E. A. (1950). Auffällige biologische Folgen von Sprungschichtneigungen im Zürichsee. *Zeitung für Hydrologie*(12), 1–24.

Thompson, J. B., Schultze-Lam, S., Bevertige, T. J. and Des Marais, D. J. (1997). Whiting events: Biogenic origin due to the photosynthetic activity of cyanobacterial picoplankton. *Limnology and Oceanography*(42), 133–141.

Tranvik, L. J., Downing, J. A., Cotner, J. B., Loiselle, S. A., Striegl, R. G., Ballatore, T. J., Dillon, P., Finlay, K., Fortino, K., Knoll, L. B., Kortelainen, P. L., Kutser, T., Larsen, S., Laurion, I., Leech, D. M., McCallister, S. L., McKnight, D. M., Melack, J. M., Overholt, E., Porter, J. A., Prairie, Y., Renwick, W. H., Roland, F., Sherman, B. S., Schindler, D. W., Sobek, S., Tremblay, A., Vanni, M. J., Verschoor, A. M., von Wachenfeldt, E. and Weyhenmeyer, G. A. (2009). Lakes and reservoirs as regulators of carbon cycling and climate. *Limnology and Oceanography*, *54*(6part2), 2298–2314. doi: 10.4319/lo.2009.54.6part2.2298

Utermöhl, H. (1958). Zur Vervollkommnung der quantitativen Phytoplankton-Methodik (Vol. No. 9). Stuttgart: E. Schweizerbart'sche.

van de Bogert, M. C., Bade, D. L., Carpenter, S. R., Cole, J. J., Pace, M. L., Hanson, P. C. and Langman, O. C. (2012). Spatial heterogeneity strongly affects estimates of ecosystem metabolism in two north temperate lakes. *Limnology and Oceanography*, *57*(6), 1689–1700. doi: 10.4319/lo.2012.57.6.1689

Wachniew, P. and Różański, K. (1997). Carbon budget of a mid-latitude, groundwater-controlled lake: Isotopic evidence for the importance of dissolved inorganic carbon recycling. *Geochimica et Cosmochimica Acta*, *61*(12), 2453–2465. doi: 10.1016/S0016-7037(97)00089-6

Wehr, J.D., Sheath, R.G., Kociolek P. (2001). Freshwater Algae of North America: Ecology and Classification. San Diego: Academic Press.

Weyhenmeyer, G. A., Kosten, S., Wallin, M. B., Tranvik, L. J., Jeppesen, E. and Roland, F. (2015). Significant fraction of CO_2 emissions from boreal lakes derived from hydrologic inorganic carbon inputs. *Nature Geoscience*, *8*(12), 933–936. doi: 10.1038/ngeo2582

Willén, E. (1976). A simplified method of phytoplankton counting. *British Phycological Journal*, *11*(3), 265–278. doi: 10.1080/00071617600650551

Wray, J. L. (1977). Calcareous Algae: Developments in Palaeontology and Stratigraphy, 4. New York: Elsevier Science Ltd.

Wu, P., Gao, C., Chen, F. and Yu, S. (2016). Response of organic carbon burial to trophic level changes in a shallow eutrophic lake in SE China. *Journal of environmental sciences (China)*, *46*, 220–228. doi: 10.1016/j.jes.2016.05.003

Yoon, J.-E., Yoo, K.-C., Macdonald, A. M., Yoon, H. I., Park, K.-T., Yang, E.-J. and Kim, H. C. (2016). Ocean iron fertilization experiments: Past–present–future with introduction to Korean Iron Fertilization Experiment in the Southern Ocean (KIFES) Project. *Biogeosciences Discuss.*, *10*.

Zeh, U. M. (1980). Schwebstoffe im Ammersee - Untersuchungen zur Verteilung, zusammensetzung und Sedimentation suspendierten Materials in einem Voralpensee: Dissertation. Technische Universität München.