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## **Responses in growth, phenology and vertical distribution of the calcite-loricated phytoflagellate *Phacotus lenticularis* (Chlorophyta) to climate related changes of the lake water carbonate chemistry and temperature**

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# Contents

<b>Summary</b>	<b>7</b>
<b>Zusammenfassung</b>	<b>9</b>
<b>1 General Introduction</b>	<b>13</b>
1.1 Atmospheric concentrations of CO <sub>2</sub> . . . . .	13
1.2 Lake carbonate chemistry . . . . .	14
1.3 CaCO <sub>3</sub> saturation state of lakes . . . . .	16
1.4 Calcifying phytoplankton . . . . .	17
1.5 The <i>Phacotus lenticularis</i> cell cycle . . . . .	19
<b>2 Methods</b>	<b>21</b>
2.1 Study sites . . . . .	21
2.1.1 Lake Großer Ostersee . . . . .	22
2.1.2 Lake Eishaussee . . . . .	22
2.1.3 Lake Östlicher Breitenauersee . . . . .	23
2.2 General sampling strategy and analytical methods . . . . .	23
<b>3 Outline of the thesis</b>	<b>27</b>
3.1 The main hypothesis under investigation . . . . .	27
<b>4 List of publications</b>	<b>29</b>
<b>5 General Discussion</b>	<b>31</b>
5.1 Growth responses to CaCO <sub>3</sub> saturation state . . . . .	31
5.2 CaCO <sub>3</sub> saturation state, pH and water temperature . . . . .	33
5.3 Distribution patterns of cell cycle phases . . . . .	35
<b>6 Conclusions and Outlook</b>	<b>39</b>
<b>7 Acknowledgements</b>	<b>43</b>

<b>8</b>	<b>References</b>	<b>45</b>
	<b>Appendix</b>	<b>57</b>
<b>A</b>	<b>Growth responses to CaCO<sub>3</sub> saturation state</b>	<b>57</b>
A.1	Abstract . . . . .	57
A.2	Introduction . . . . .	58
A.3	Methods . . . . .	60
A.3.1	Study site . . . . .	60
A.3.2	Sampling strategy . . . . .	61
A.3.3	Data analysis . . . . .	62
A.4	Results . . . . .	63
A.4.1	Seasonal dynamics in biological and hydrological variables	63
A.4.2	Meteorological conditions . . . . .	64
A.4.3	Onset of exponential growth . . . . .	65
A.4.4	Growth dynamics in relation to hydrological and meteorological variables . . . . .	67
A.5	Discussion . . . . .	71
A.6	Acknowledgements . . . . .	73
A.7	Funding . . . . .	73
A.8	References . . . . .	74
<b>B</b>	<b>Vertical segregation of cell-cycle phases</b>	<b>79</b>
B.1	Abstract . . . . .	79
B.2	Introduction . . . . .	80
B.3	Methods . . . . .	82
B.3.1	Study site . . . . .	82
B.3.2	Sample collection and processing . . . . .	82
B.3.3	Data structure and statistical analysis . . . . .	84
B.4	Results . . . . .	87
B.4.1	Hydrographic conditions and weather . . . . .	87
B.4.2	Vertical distribution and seasonal variability of <i>P. lenticularis</i>	87
B.4.3	Relevance of pH, water temperature and density gradient .	89
B.4.4	Cross-correlation and multi-variate linear regression analysis	91
B.5	Discussion . . . . .	93
B.6	Acknowledgements . . . . .	95
B.7	References . . . . .	95

# List of Figures

1.1	Carbon input into freshwater lakes. . . . .	15
1.2	Calcified lorica and empty shell halves. . . . .	18
1.3	Light microscope image of cell-cycle phases. . . . .	20
2.1	Images of lake Großer Ostersee. . . . .	22
2.2	Images of lake Eishaussee and lake Östlicher Breitenauersee. . . . .	23
2.3	Map of a section of the Osterseen lake district. . . . .	24
A.1	Seasonal variations in parameters. . . . .	64
A.2	Meteorological time series. . . . .	65
A.3	Population growth of <i>Phacotus lenticularis</i> . . . . .	66
A.4	Cross-correlation correlograms. . . . .	69
A.5	Linear regressions. . . . .	70
B.1	SEM images of <i>Phacotus lenticularis</i> cells. . . . .	82
B.2	Map of lake Ostersee showing sampling sites. . . . .	83
B.3	Light microscope images of cell-cycle phased <i>Phacotus</i> cells. . . . .	84
B.4	Depth-time contour plot of seasonal changes in parameters. . . . .	86
B.5	Time series of meteorological parameters. . . . .	88
B.6	Depth-time contour plots of <i>Phacotus</i> cells. . . . .	89
B.7	Tolerance ranges of <i>Phacotus</i> densities against parameters. . . . .	90
B.8	Cross-correlation coefficients. . . . .	91
B.9	Multivariate linear regression analysis. . . . .	92



# List of Tables

A.1	Characteristics of the surface layer of the three lakes. . . . .	63
A.2	Parameters at the onset of <i>Phacotus</i> exponential growth. . . . .	67
A.3	Cross-correlation values ( $r_{xy}$ ) and time lags in lake Ostersee. . . . .	68
A.4	Cross-correlation values ( $r_{xy}$ ) and time lags in three lakes. . . . .	68





# Summary

Increased anthropogenic carbon dioxide emissions enhance carbon inputs into aquatic ecosystems. The consequences are manifold, such as decreased pH and saturation levels with respect to calcium carbonate ( $\text{CaCO}_3$ ) in oceans and increased inflow of dissolved inorganic carbon into freshwater lakes via groundwater due to higher weathering rates and soil microbial respiration. Climate related changes in the carbonate chemistry and inorganic carbon flux of lakes are complex and not yet well understood. The saturation level of  $\text{CaCO}_3$  is mandatory for many aquatic organisms to form calcite shells and skeletons, and precipitation rates tend to decrease when  $\text{CaCO}_3$  saturation levels decline which may as well result in declined growth. This thesis focuses on the abundance, distribution and phenology of the calcifying freshwater green algae *Phacotus lenticularis* in response to climate related changes in lake water carbonate chemistry and water temperature. *Phacotus* shells are composed of tightly attached calcite plates and therefore may significantly contribute to the accretion of  $\text{CaCO}_3$  in lake sediments. Calcifying algae and their calcification process are sensitive to small changes in pH and  $\text{CaCO}_3$  saturation and may act as indicators for climate impacts on lake water carbonate chemistry.

In publication 1 we investigated how meteorological variability in interaction with the  $\text{CaCO}_3$  saturation state influences the timing and intensity of *Phacotus lenticularis* growth. The study revealed that increasing  $\text{CaCO}_3$  supersaturation mediates the onset of exponential growth by *Phacotus* and stimulates seasonal peak development. The algae benefit from increasing  $\text{CaCO}_3$  supersaturation in natural heterogeneous environments, likely because the cells can develop a protective shell of increasingly dense calcite. We found that the relationship between cell density and  $\text{CaCO}_3$  saturation is positive and linear at saturation indices between 3 and 7. Early peak development is possible even at lower water temperatures when  $\text{CaCO}_3$  supersaturation is sufficiently high. Our study provides evidence that wind may indirectly cause a decline in epilimnetic pH followed by

a delayed decline in  $\text{CaCO}_3$  saturation. Solar irradiance positively affected both pH and water temperature.

In publication 2 we explored the vertical distribution of two *Phacotus* cell-cycle phases in the lake water column. *Phacotus* cells, growing in biomass, possess two flagella and a lense-shaped, solid calcite shell. Mitotic (dividing) cells lack flagella, and calcification of daughter cells occurs in the final phase of mitosis. We investigated whether the two cell-cycle phases differ in vertical distribution along gradients that influence calcification, motility and sinking rates. Our results show that growing and mitotic *Phacotus* cells segregated vertically during peak development. Growing cells (GCs) accumulated within the thermocline, while mitotic cells (MCs) aggregated in the epilimnion down to 4 m depth, and seldom below. GCs and MCs differed significantly in their vertical distribution patterns, preference with respect to pH and water temperature, but not to water density. We found that pH and temperature variations in the epilimnion, where mitotic cells aggregate and calcify, explained up to 83% of the variance of growing cell abundance at a time lag of one to three days in a daily sampling campaign. Among the meteorological variables we tested, rainfall and air temperature had a small additional positive effect on GC abundance. The results suggest that *Phacotus* cells migrate upwards prior to loss of motility to layers that reduce sinking but likely enhance calcification rates, which translates into a positive response of growing cell abundance.

# Zusammenfassung

Die Zunahme der anthropogenen CO<sub>2</sub> Emission in der Erdatmosphäre verursacht zunehmend höhere Kohlenstoffeinträge in die Gewässer. In marinen Ökosystemen ist durch den vermehrten Eintrag von Kohlendioxid über die Luft-Wasser-Kontaktzone seit längerem eine 'Versauerung' des Wassers zu beobachten. Die pH Wert Absenkung in den Ozeanen hat eine Abnahme der CaCO<sub>3</sub> Sättigung zur Folge. Dadurch sinkt das Bindevermögen Kalkskelett bildender Lebewesen, mit negativen Auswirkungen für den marinen anorganischen Kohlenstoffkreislauf. Binnengewässer sind als tiefste Punkte in der Landschaft Senken oder Quellen für Kohlenstoff und nehmen damit Einfluss auf den regionalen Kohlenstoffhaushalt. Eine erhöhte Zufuhr von anorganischem Kohlenstoff in Seen durch das Grundwasser entsteht durch wärmebedingte höhere mikrobielle Respirationsraten in der Wurzelzone (Rhizosphäre) und höhere Verwitterungsraten von kalkreichem Sedimentgestein. Wenn Niederschlag auf die Erdoberfläche fällt und in die Rhizosphäre dringt, werden Gase, insbesondere Kohlendioxid, im Wasser gelöst und transportiert. Bei Anwesenheit von gelöstem Kohlendioxid steigt die Löslichkeit von Kalkgestein. Das gelöste Karbonat gelangt dann über das Grundwasser in die Seen. Der See interne Kohlenstoffchemismus ist sehr komplex und über die Photosynthese wiederum stark an regionale Wetterveränderungen gekoppelt. Im Gegensatz zu marinen Systemen sind die Auswirkungen erhöhter Kohlenstoffeinträge und deren Folgen für kalkbildende Lebewesen in Seeökosystemen noch nicht detailliert verstanden.

In dieser Dissertation wurde das Vorkommen, die jahreszeitliche Entwicklung und die vertikale Verbreitung der kalkbildenden Süßwasserualge *Phacotus lenticularis* in Abhängigkeit von chemisch-physikalischen Parametern, die das Kalkbindevermögen beeinflussen, und in Abhängigkeit von lokalen Wetterveränderungen untersucht. Als Untersuchungsgewässer dienten drei alkaline Seen der Osterseenkette. Die zu den Chlamydomonadaceae gehörende Grünalge *P. lenticularis* kann neben dem CO<sub>2</sub> Verbrauch durch Photosynthese dem Gewässer weiteren anorga-

nischen Kohlenstoff entziehen, indem sie an der äußeren Zellwand eine extrazelluläre Kalkfällung durchführt. Die aus zwei uhrglasartigen Hälften zusammengesetzte Zellhülle, die Lorica, besteht aus einem dichten Ring von Kalzitkristallen. Durch die Akkumulation solcher hoher Konzentrationen an  $\text{CaCO}_3$  beeinflussen *Phacotus* Algenblüten erheblich den Kohlenstoffhaushalt von Seeökosystemen. Fossile Kalksedimente, in denen zahlreiche *Phacotus* Loricae nachgewiesen wurden, belegen, dass die Anreicherung der Schalen im Sediment dauerhaft sein kann. Durch seine starke Bindung an die gewässerinterne  $\text{CaCO}_3$  Sättigung ist ein *Phacotus* Vorkommen möglicherweise auch zur Beurteilung der Auswirkungen des Klimawandels auf die zahlreichen Veränderungen im anorganischen Kohlenstoffchemismus in Seen geeignet.

In Veröffentlichung 1 wurde untersucht, ob Veränderungen der  $\text{CaCO}_3$  Sättigung Einfluss auf den Beginn des exponentiellen Wachstums von *P. lenticularis* und auf die Ausbildung von Wachstumsspeaks nimmt. In zwei von drei untersuchten Seen der Osteenkette führte ein zweifacher Anstieg der  $\text{CaCO}_3$  Sättigung zu einem fünffachen Anstieg der Wachstumsrate von *P. lenticularis*. *Phacotus* scheint unter natürlichen heterogenen Bedingungen im Gewässer durch die Ausbildung einer Kalkhülle einen Wachstumsvorteil zu erlangen. In einem weiteren See war ein exponentielles Wachstum auch schon bei niedrigeren Wassertemperaturen um  $13^\circ\text{C}$  möglich, nachdem die  $\text{CaCO}_3$  Sättigung früh in der Vegetationsperiode auf Indexwerte über 5 angestiegen war. In einer Regressionsanalyse konnten wir eine positive lineare Beziehung zwischen dem Sättigungsindex für  $\text{CaCO}_3$  und dem *Phacotus* Wachstum für Indizes zwischen 3 und 7 feststellen. Aus den Analysen zum Einfluss von Wetterparametern auf den Gewässerchemismus ergaben sich erste Hinweise, dass Wind indirekt zu einer Absenkung des pH Werts und mit einer zeitlichen Verzögerung zu einer Absenkung der  $\text{CaCO}_3$  Sättigung führen kann, während die Sonneneinstrahlung sich positiv auf den pH Wert und die Wassertemperatur auswirkt.

In Veröffentlichung 2 wurde die vertikale Verteilung in der photischen Zone von zwei Zellentwicklungsstadien von *P. lenticularis* untersucht, die sich in ihrer Bewegungsfähigkeit und ihrer äußeren Zellbeschaffenheit unterscheiden. *Phacotus* Zellen im Stadium des Zellwachstums, in dem Zellbestandteile ergänzt und aufgebaut werden, verfügen über zwei Flagellen und eine linsenförmige, feste Calcithülle. Zellen im Teilungsstadium (Mitose) haben die Flagellen zurückgebildet, und die Tochterzellen kalzifizieren, eingeschlossen in eine Mutterzelle bereits kurz nach der Teilung. *Phacotus lenticularis* ist ein Vertreter des Frühlings- bzw. des Sommerplanktons in Seen, die eine Temperaturschichtung aufweisen. Durch

diese Schichtung entstehen Tiefenzonen mit unterschiedlichen Durchmischungsverhältnissen und Gradienten physikalisch-chemischer Parameter. Das Ziel dieser Studie war zu untersuchen, ob die beiden Zellstadien unterschiedliche vertikale Verteilungen entlang von Gradienten aufweisen, welche die Beweglichkeit und Kalzifizierung beeinflussen. Die Untersuchungen haben gezeigt, dass wachsende und sich teilende *Phacotus* Zellen in niedrigen Dichten in ihrem Vorkommen einander in einer Tiefenzone zwischen 0 – 8 m Wassertiefe überlagern. In Zeiten hoher Zelldichten konzentrieren sich wachsende und teilende Zellen jedoch in deutlich getrennten Tiefenschichten. Zellen im Mitosestadium fanden sich fast ausschließlich in der durchmischten Zone nahe der Gewässeroberfläche, dem Epilimnion. Eine vom Wind hervorgerufene Durchmischung der oberflächennahen Gewässerschichten verhindert ein schnelles Absinken der nicht beweglichen *Phacotus* Zellen im Mitosestadium. Dadurch verlängert sich die Zeit, die die Alge in der photischen Zone verbringt. Das Epilimnion im Großen Ostersee wies auch die höchsten Wassertemperatur- und pH Werte und damit eine hohe  $\text{CaCO}_3$  Sättigung auf. Dadurch wird das Ausfällen von Kalzitkristallen gefördert. Die Tochterzellen, die am Ende des Mitosestadiums entstehen, finden im Epilimnion die besten Bedingungen zum Ausbilden ihrer Kalzithülle. Wachsende *Phacotus* Zellen in hohen Konzentrationen aggregierten in einer 1 - 3 m breiten Schicht innerhalb des Metalimnions (Sprungschicht). Im nicht durchmischten Metalimnion können Flagellaten ihre bevorzugte Tiefe besser halten. Die tiefenzonale Auftrennung lässt vermuten, dass *Phacotus lenticularis* vor der Zellteilung und dem Verlust seiner Beweglichkeit eine vertikale, aufwärtsgerichtete Wanderung durchführt. Signifikante Unterschiede hinsichtlich der Tiefenverteilung beider Stadien fanden sich in Bezug auf Gradienten des pH Werts und der Wassertemperatur, nicht aber in Bezug auf Gradienten der Wasserdichte. Schwankungen des pH Werts und der Wassertemperatur im Epilimnion konnten bis zu 83 % der Variation in der Zelldichte der häufig auftretenden wachsenden Zellen erklären. Niederschlag und Lufttemperatur, als meteorologische Einflußgrößen, hatten ebenfalls einen positiven Effekt auf die Dichte der wachsenden Zellen.



# 1 | General Introduction

## 1.1 Atmospheric concentrations of CO<sub>2</sub>

The rising atmospheric level of carbon dioxide (CO<sub>2</sub>) is the single most important factor contributing to anthropogenic climate change and warming of the earth's surface. In the known geological past over at least 800 000 years atmospheric CO<sub>2</sub> concentrations were tightly coupled to air temperature changes during ice ages and interglacial periods, as well as variations in ocean CO<sub>2</sub> uptake and release (Sigman et al. 2010, Ziegler et al. 2013). Atmospheric partial pressure of CO<sub>2</sub> (pCO<sub>2</sub>) oscillated between 180 – 280 ppm in the sequence of glacial-interglacial cycle events (Lüthi et al. 2008). Since the beginning of the early industrial era around 1750 CO<sub>2</sub> emissions have increased by more than 30% to around 398 ppm in 2015 (Mauna Loa Observatory). Ongoing industrial and population growth have resulted in even further increases of emission rates of  $\sim 1.9 \text{ ppm yr}^{-1}$  in the past decade compared to an average annual release of  $1.4 \text{ ppm yr}^{-1}$  in the 1960s when continuous direct atmospheric measurements began (NOAA, <http://www.esrl.noaa.gov/gmd/ccgg/carbontracker>, IPCC 2014). Once released to the air, CO<sub>2</sub> persists in the atmosphere for decades to centuries and the climate impacts are global in extend.

One primary effect of increased CO<sub>2</sub> emission is the intensification of the greenhouse effect. Atmospheric green house gases such as CO<sub>2</sub> play a key role in absorbing and re-emitting infrared radiation thereby trapping heat near the earth's surface. Atmospheric CO<sub>2</sub> from human activities contributes the largest single radiative forcing to climate warming and accounts for  $\sim 65\%$  of total forcing due to long-lived gases (NOAA Annual Greenhouse Gas Index, <http://www.esrl.noaa.gov/gmd/aggi/aggi.html>). Anthropogenic climate warming increases the surface temperature of aquatic ecosystems and thereby enhances differences in water density. As a consequence, vertical circulation declines, and

nutrient renewal as well as vertical exchange of gases are reduced. Satellite data records show significant warming trends for over 40 large lakes worldwide (Winder and Schindler 2004, Bleckner et al. 2007, Schneider and Hook 2010, Sharma et al. 2015). In lake Constance, surface water temperature increased by approx.  $0.9^{\circ}\text{C}$  since 1988 compared to the reference time period 1962 – 1987 (KLIWA 2011). Similar ongoing trends of an  $0.3^{\circ}\text{C}$  increase in epilimnetic layer temperature per decade were found in the shallower lakes Müggelsee and Heiligensee (Kirillin 2010). Excess atmospheric  $\text{pCO}_2$  will further significantly increase the influx of inorganic carbon into lake ecosystems. Lakes comprise a geographically distributed network of the lowest points in the surrounding landscape that make them active sites for storage, transport and transformation of considerable amounts of carbon (Finlay et al. 2009). The main sources of carbon inflow into lakes are via exchange between lake surface and atmosphere and by groundwater or surface inflow from the surrounding catchment. The inorganic carbon chemistry of freshwater lakes is a complex system of equilibrium reactions that requires a brief introduction.

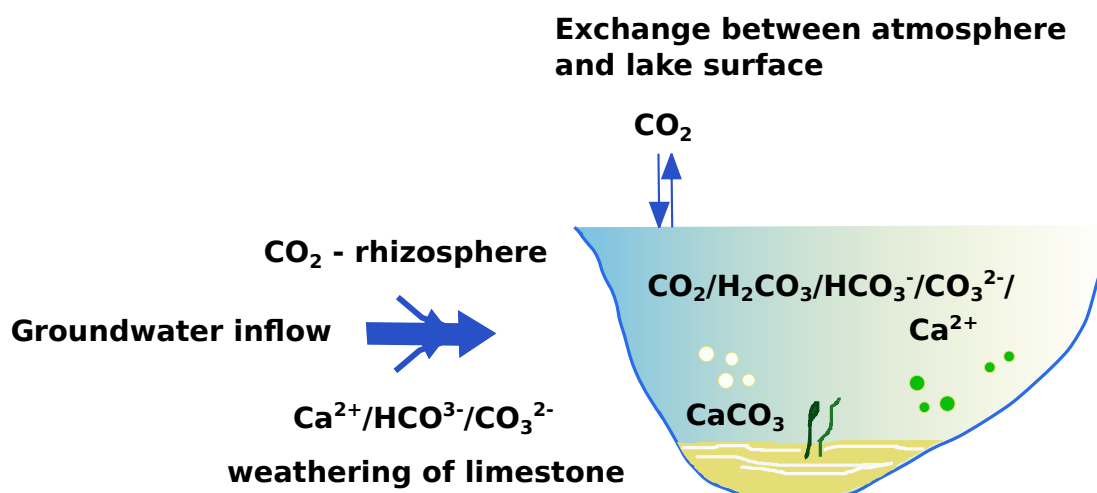
## 1.2 Lake carbonate chemistry

Atmospheric  $\text{CO}_2$  solutes well in water. The solubility is a function of pressure and temperature. Increasing  $\text{CO}_2$  partial pressure increases the solubility of  $\text{CO}_2$ , whereas increasing temperature decreases the solubility of  $\text{CO}_2$  in water. Unlike other gases,  $\text{CO}_2$  reacts with water to form carbonic acid ( $\text{H}_2\text{CO}_3$ , a weak acid that dissociates quickly and releases hydrogen ions  $\text{H}^+$  and bicarbonate ions ( $\text{HCO}_3^-$ ) (Eq. 1.1). The reaction increases hydrogen ion activity and thereby lowers lake water pH. The additional  $\text{H}^+$  ions react with carbonate ions ( $\text{CO}_3^{2-}$ ) and lower their respective concentrations. A reduction in carbonate ions lowers the saturation state of the water with respect to calcite and aragonite, the two common forms of mineralized calcium carbonate. In freshwater systems, bicarbonate and carbonate concentrations establish an equilibrium with dissolved carbon dioxide. The equilibrium reactions act as buffering system and neutralized added acids and bases. The speciation into carbonates allows water to take up more carbon than would be possible due to the solubility of  $\text{CO}_2$  alone.



The ambient concentrations of  $\text{CO}_2$ ,  $\text{H}_2\text{CO}_3$ ,  $\text{HCO}_3^-$  and  $\text{CO}_3^{2-}$  are pH-dependent.  $\text{HCO}_3^-$  is the dominant anion in most hard water lakes of the tem-





**Figure 1.1:** Schematic representation of carbon inputs into freshwater lakes located in a limestone drainage area.

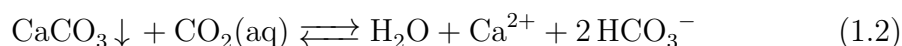
perate regions. Between pH 7 and 9  $\text{HCO}_3^-$  is quantitatively predominant. Below the first dissociation point ( $\text{pK}_1 = 6.3$ )  $\text{CO}_2$  ( $+\text{H}_2\text{CO}_3$ ) is the dominant inorganic carbon species, and carbonate ( $\text{CO}_3^{2-}$ ) dominates above the second dissociation point ( $\text{pK}_2 = 9.1$ ).  $\text{CO}_2$ ,  $\text{H}_2\text{CO}_3$ ,  $\text{HCO}_3^-$  and  $\text{CO}_3^{2-}$  are collectively referred to as dissolved inorganic carbon (DIC). The main origin of lake water bicarbonate and carbonate ions are geological weathering processes. As water flows through the soil of the drainage basin, it becomes enriched with  $\text{CO}_2$  from plant and microbial respiration. The carbonic acid that is formed dissolves carbonate minerals of limestone rocks ( $\text{CaCO}_3$ ) and produces calcium ions ( $\text{Ca}^{2+}$ ),  $\text{HCO}_3^-$  and  $\text{CO}_3^{2-}$  ions that enter the lake via groundwater inflow (Fig. 1.1). Rainfall is acidic, because atmospheric  $\text{CO}_2$  dissolves in the rainwater producing carbonic acid. Acidic rain contributes to the weathering of carbonate minerals. Hardwater lakes that comprise nearly one-third of the volume of all inland waters worldwide, contain high amounts of inorganic carbon (Myrbo and Shapley 2006).

Soil microbial response to warming and seasonal changes in precipitation rates will likely further increase carbon import to aquatic systems (Davidson and Janssens 2006). As carbonates increase in hard water lakes, values of pH also increases from the release of hydroxyl ions ( $\text{OH}^-$ ) generated from the reactions of bicarbonate and carbonate with water. Alkalinity of the lake water increases likewise. Alkalinity is described as the quantity of negative charges that can accept and neutralize  $\text{H}^+$  ions released from the dissociation reaction of carbonic acid. Positive alkalinity of most freshwaters results from conditions where carbonate and bicarbonate ions represent the main buffering components. A typical

alkalinity background of temperate hard water lakes is  $1 - 3 \text{ mmol L}^{-1}$  (Stabel 1986, <http://www.igkb.org/>).

### 1.3 $\text{CaCO}_3$ saturation state of lakes

Lake water carbonate concentrations determine the saturation level of calcium carbonate that is used by many aquatic organisms to form shells and skeletons. Undissociated  $\text{CaCO}_3$  forms an equilibrium with  $\text{Ca}^{2+}$ ,  $\text{HCO}_3^-$ ,  $\text{CO}_3^{2-}$ ,  $\text{CO}_2$  and  $\text{H}_2\text{CO}_3$  in solution. If a portion of  $\text{CO}_2$  required to maintain the equilibrium is lost from the system,  $\text{CaCO}_3$  will precipitate, thereby releasing  $\text{CO}_2$  until the equilibrium is reestablished. When  $\text{CO}_2$  is added to the system, the solution becomes corrosive and  $\text{CaCO}_3$  will dissolve:



The saturation state of  $\text{CaCO}_3$

$$\Omega_{\text{CaCO}_3} = [\text{Ca}^{2+}] \cdot [\text{CO}_3^{2-}] / K_s \quad (1.3)$$

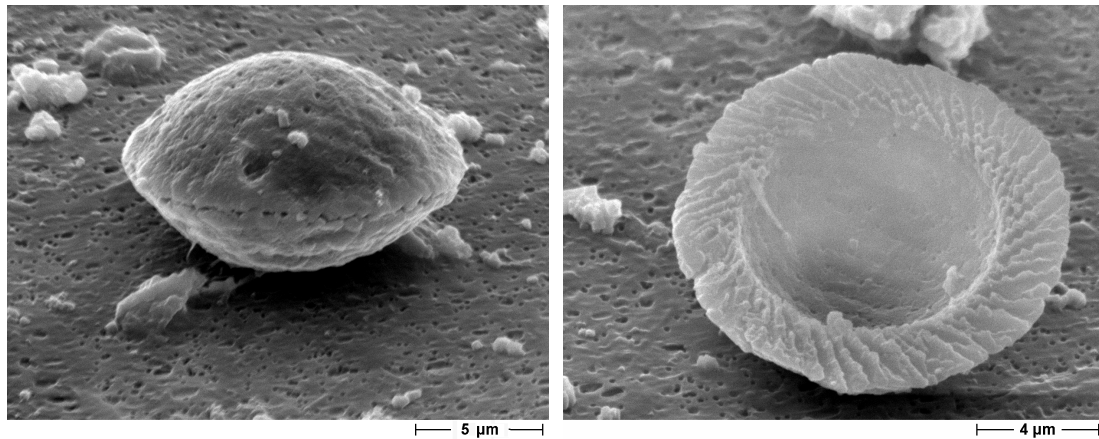
refers to the concentrations or ion activities of the reacting ions  $\text{Ca}^{2+}$  and  $\text{CO}_3^{2-}$  in water in relation to their corresponding concentrations at saturation when the mineral is neither forming or dissolving.

When the ionic activity product of calcium and carbonate in lake water exceeds the solubility product ( $K_s$ ), the system is supersaturated and precipitation of  $\text{CaCO}_3$  occurs (Plummer and Busenberg 1982). Vice versa, when the ionic activity product of calcium and carbonate falls below the solubility product the system is undersaturated and  $\text{CaCO}_3$  will dissolve.  $\Omega$  values above 1 signify supersaturation, whereas  $\Omega$  values below 1 signify undersaturation. Typical mean saturation values for temperate alkaline lakes are  $\Omega = 3 - 20$  (Stabel 1986, Koschel 1997). Most calcite formed in temperate hard water lakes precipitates in the pelagic zone during increased algal blooms in spring and mid-summer when algal cells assimilate large amounts of  $\text{CO}_2$  and  $\text{HCO}_3^-$  for photosynthesis, thereby releasing  $\text{OH}^-$ . The resulting increase in pH further shifts the  $\text{HCO}_3^- / \text{CO}_3^{2-}$  ratio, causing an increase in the chemical activity of  $\text{CO}_3^{2-}$  ions, and the solubility of  $\text{CaCO}_3$  is exceeded (Stabel 1986, Gruber and Samiento 2002). A thermal initiation of  $\text{CaCO}_3$  precipitation can occur as a result of temperature dependent differences in carbon dioxide solubility.  $\text{CO}_2$  is more soluble in colder

than in warmer water. An increase in water temperature will result in a reduction of  $\text{CO}_2$  ( $+\text{H}_2\text{CO}_3$ ) concentrations in solution, and the equilibrium will shift towards the precipitation of calcite. Physically initiated  $\text{CaCO}_3$  precipitation occurs on rare occasions during sudden increases of  $\text{CO}_2$  concentrations in the lake water e.g. via groundwater inflow. This phenomenon can lead to spontaneous emission of  $\text{CO}_2$  to the atmosphere and a temporary lowering of lake water  $\text{CO}_2$  concentrations.

## 1.4 Calcifying phytoplankton

Being small but yet so diverse, phytoplankton cells are among the most important primary producers on the globe. Some algal species stand out, as they are capable of both carbon fixation through photosynthesis and through controlled precipitation of  $\text{CaCO}_3$ , thereby creating elaborate shells of crystalline calcite plates. Marine coccolithophores and foraminifera, a class of amoeboid, heterotrophic protists, form the majority of particulate inorganic carbon (PIC) in the oceans. The calcite fixed in their shells is exported to the ocean floor and acts as long term carbon sink (Riebesell et al. 2000, Beaufort et al. 2011). In freshwater ecosystems, calcite encrustations occur in several prokaryotic and eukaryotic algal taxa both in benthic and pelagic systems (Pentecost 1991, Freydet and Verrecchia 1998, Laval et al. 2000, Riding, 2006). A regular shell of dense calcite plates however is unique to the flagellated green algae of the family Phacotaceae and in particular to its widely distributed member *Phacotus lenticularis* (Fig. 1.2). The dense, regular shell of *Phacotus lenticularis* is composed of two halves each up to  $1.8 \mu\text{m}$  thick (Hepperle and Krienitz 1996). This way precipitation of  $\text{CaCO}_3$  during *Phacotus* bloom events significantly influences the biochemistry of lakes (Koschel 1995). *P. lenticularis* is distributed worldwide and inhabits the euphotic zone of temperate hard water lakes of a broad trophic range (Schlegel et al. 1998, Wehr et al. 2001, Menezes 2010, John et al. 2011, Caraus, 2012). The phytoflagellate requires supersaturation with respect to  $\text{CaCO}_3$  to form a calcite shell and calcification rates tend to decrease in response to lower carbonate ion concentrations (Hepperle and Krienitz 1997). *Phacotus* cells can grow and divide without calcification, but will calcify when the conditions are favourable (Schlegel et al. 2000 a, b). Their calcite shells can be so abundant in alkaline water, that significant amounts of  $\text{CaCO}_3$  are exported to the lake floor, remaining for centuries and millennia (Müller and Oti 1981, Bluszcz et al. 2008, Jouve et al. 2013).



**Figure 1.2:** Side view of a calcified lorica showing dense calcite plates and pores (left), empty shell halve showing ring like arranged calcite crystals (right).

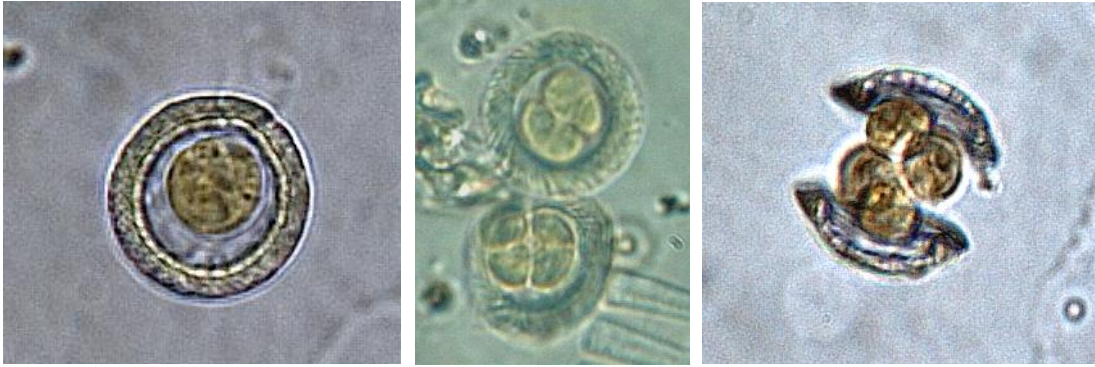
The ability to form a calcified skeleton is very old among marine and freshwater organisms and has evolved several times during the geological past. At present, the oldest records of protists with carbonate impregnated cell walls come from approx. 710 million years old Neoproterozoic rocks (Knoll 2003). A broad simultaneous evolution of mineralized skeletons among organisms appeared during the Cambrian period approx. 540 million years ago, but shell covered protists, such as red and green algae, became abundant much later in the Ordovician period 460 million years ago. Fossil records of *Phacotus* shells can be tracked back to the Miocene approx. 10 million years ago (Lagerheim 1902).

Various hypothesis exist about the advantages and functions of calcite shells. The broad development of animal diversity during the Cambrian period may have increased predation pressure and favoured the evolution of protective shells (Bengston 1994). Other hypothesis propose that environmental changes, such as increased seawater  $\text{Ca}^{2+}$  ion concentrations, that can damage cell content, favoured skeletal biomineralization and storage of calcium (Stanley and Hardy 1998). The periodic structure of many mineralized shells also comprises special optical properties. Here, the size of the plates and their micro-structure are of particular importance. Calcite shells have been found to reflect and scatter light, acting as tiny mirrors in the water (Quintero and Torres 2007). They reduce transmission of light into the cells and can prevent damage to proteins and DNA by elevated UV radiation (Gao et al. 2009).

## 1.5 The *Phacotus lenticularis* cell cycle

A phenomenon of the cell-cycle of many phytoplankton species is that it often forms synchronous populations (Weiler and Karl 1979, Goto and Johnson 1995). The algae grow photoautotrophically during the day, when light is available, and undergo DNA replication and nuclear as well as cell division during the dark. Many green algal cells divide by a complex mechanism called multiple fission. This means that the protoplast divides up into more than two daughter cells. The division number depends on the present growth conditions and is very stable, ranging between 2 – 16 (Bisova and Zachleder 2014). In many unicellular and colonial algal forms reproduction is vegetative, occurring as the cell divides (Van Den Hoek et al. 1995). Sexual reproduction often takes place between population maxima at deteriorating environmental conditions. It includes the formation of gametes (sexual reproductive cells) and the fusion of two gametes to a zygote. Sexual fusion of gametes can also result into development of thick-walled resting cysts (hypnozygotes). Encystment constitutes the overwintering population that will lead to a recurrent bloom in the following growing season (Bravo et al. 2012). It is widely agreed that asexual reproduction is of advantage when stable environmental conditions allow for rapid population growth, whereas sexual reproduction enables maintenance of genetic diversity and thus adaptation to a changing environment and infections (Koester et al. 2007, Krueger-Hadfield et al. 2014). Our current understanding of *Phacotus* life cycle stages and vegetative cell-cycle phases relies mainly on laboratory studies of Giering et al. 1990, Hepperle and Krienitz 1996, Schlegel 2001 and on studies on *Chlamydomonas* cultures (Donnan et al. 1985, Oldenhof et al. 2004, 2006). *P. lenticularis* has been found to mainly divide asexually. The primary alteration of vegetative reproduction is between a prolonged growth phase (G1, nuclear interphase), during which the cell increases in biomass but the nucleus remains intact, and mitosis, during which the nucleus is being replicated and the cell eventually divides (Vaulot, 1995, Donnan et al, 1985) (Fig. 1.3).

*Phacotus* cells growing in biomass possess two flagella and a bivalved calcified lorica. The cells increase in volume until they have achieved all necessary prerequisites to complete the cell cycle. In the division phase, multiple rounds of division occur, each consisting of DNA synthesis, nuclear division (mitosis) and cell division (cytokinesis) (Giering et al. 1990). Prior to entering mitosis, the two flagella are being regressed and the two shell halves separate at their contact zone. Mitosis follows a strict sequence of steps, starting with the breakdown



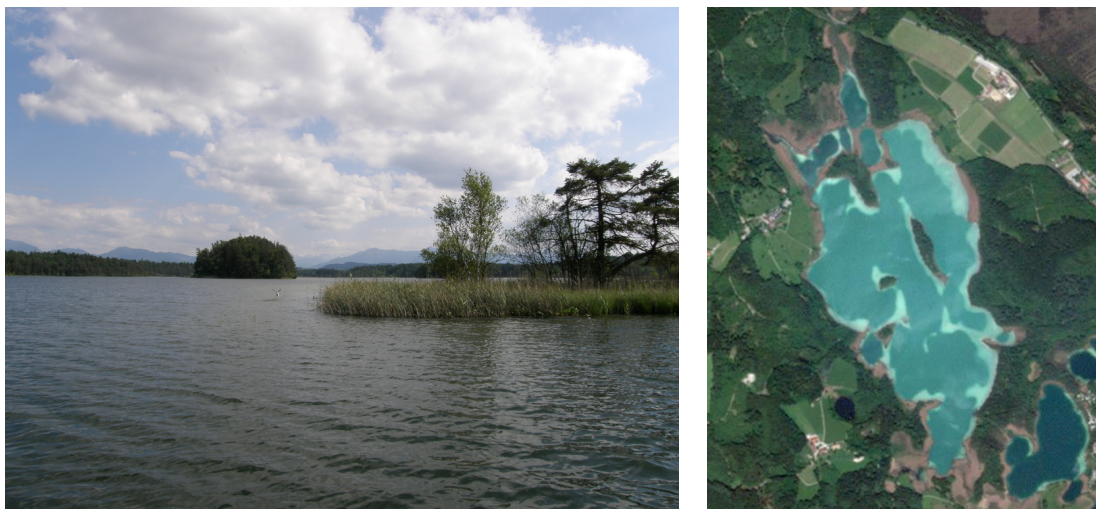
**Figure 1.3:** Light microscope images of a *Phacotus lenticularis* cells from different cell-cycle phases; (A) growing cell - protoplast is small in diameter; (B) mitotic cells - the protoplast has divided by multiple fision; (C) mitotic cell including four daughter cells within the separated mother shell (credits Christine Sprater and Sophie Daberkow).

of the nuclear membrane, the duplicated chromosomes separate and become re-encapsulated in their own nuclei. The remaining cell content divides around the daughter nuclei and the new daughter cells are eventually disconnected. In general, two longitudinal cell divisions lead to the formation of four (but sometimes 2, 8 and 16) naked *P. lenticularis* daughter cells. The daughter cells soon grow new flagella and begin to form new extracellular  $\text{CaCO}_3$ . At the beginning of the calcification process small crystals appear at the outer extracellular layer in constant distances from each other, and eventually a lens-shaped solid lorica is developed. In a final step, the maternal wall ruptures and the daughter cells are liberated into the surrounding water. After separation, the next generation of daughter cells resumes growth during the next period of nuclear interphase. Studies on *Chlamydomonas* cultures showed that the growth phase G1 was dependent on light, whereas after a point designated as transition or commitment point, processes were light independent (Oldenhof et al. 2006). After passing this point, the cell cycle could be completed in the dark, without external light supply. Sexual reproduction exists in the *P. lenticularis* life cycle, but so far was observed only sporadically. Schlegel 2001 documented fusion of gametes, as well as zygote and hypnozygote stages from aged cultures.

## 2 | Methods

### 2.1 Study sites

*Phacotus lenticularis* populations were investigated in three lakes that belong to the Osterseen lake district: lake Großer Ostersee, lake Eishaussee and lake Östlicher Breitenauersee. The Osterseen lake district developed after the last glacial period, the Würm glaciation, when retreating glaciers left behind dead ice fields (Wasserwirtschaft 1987). The extensive ice blocks were surrounded by gravel from melting ice and snow generating marginal terraces (Doppler et al. 2011). After melting, the ice blocks left behind deep basins that were subsequently filled with sediments and water. Nowadays, the Osterseen lake district consists of 19 smaller lakes that are interconnected by natural channels. The lakes are located approx. 50 km south of Munich in the pre-alpine region of Bavaria (47°47'25" N, 11°18'15" E). Weathering of limestone bedrock in the aquifer of the lake districts watershed leads to consistent inflow of calcium and carbonate rich groundwater into the lakes resulting in high alkalinity levels and high buffering capacities. Most lakes of the district exhibit regular groundwater inflow with a small contribution of one tributary. Groundwater influxes and nutrient loadings vary among lakes (Raeder 1990). Originally oligotrophic, the lakes in the southern area of the district were exposed to eutrophication from municipality waste water and agricultural land use. Self purification decreases nutrient levels from lake to lake on a south-north facing direction following the slope of the ground (Melzer 1976). Lakes located in the southern part of the district, such as lake Waschsee, are still classified as eutrophic (Melzer 1976, Raeder 1990, Sandmann 1995, Beck 2005, Keiz 2013), whereas lakes in the northern part, such as lake Lustsee, are classified as oligotrophic.



**Figure 2.1:** Image of lake Großer Ostersee. View over the lake (left) and areal view showing pelagic calcite precipitation (right).

### 2.1.1 Lake Großer Ostersee

Lake Großer Ostersee is a dimictic, alkaline lake encompassing a surface area of 117.63 ha and with a maximum depth of 29.7 m. Lake Großer Ostersee is the largest of the 19 lakes of the Osterseen lake district (Fig. 2.1). The lake basin is divided into several natural sub-basins, whose sediments are dominated by organic matter and calcite-rich marl originating from pelagic  $\text{CaCO}_3$  precipitation. The lakes trophic status is oligotrophic to mesotrophic (Melzer 1976, Raeder 1990, Sandmann 1995, Beck 2005, Keiz 2013). The surrounding landscape is mostly forested with a mixture of coniferous and deciduous trees and a few sites of public access to the lake.

### 2.1.2 Lake Eishaussee

Lake Eishaussee is a smaller, meromictic lake located at the beginning of a side chain of the Osterseen lake district (Fig. 2.2). Surface area of lake Eishaussee is 7.69 ha and maximum depth is 19.1 m. The lake is divided into two natural sub-basins, with one basin being permanently anaerobic. The sheltered location of the lake and its small surface area compared to the depth of this basin prohibit a wind induced mixing of the basins deepest water zones. Hence, in this zones biomineralisation caused a continuous increase in ion concentrations and conductivity. The lakes trophic status is oligotrophic to mesotrophic (Melzer 1976, Raeder 1990, Sandmann 1995, Beck 2005, Keiz 2013).





**Figure 2.2:** Images of lake Eishaussee (left) and lake Östlicher Breitenauersee (right) (credit Sophie Daberkow).

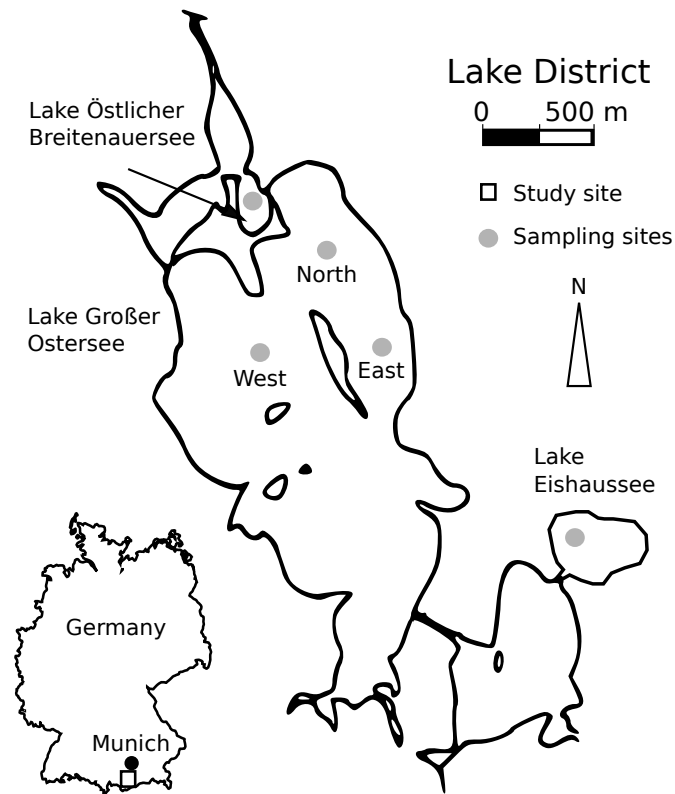
### 2.1.3 Lake Östlicher Breitenauersee

Lake Östlicher Breitenauersee is a dimictic, alkaline lake encompassing a surface area of 2.36 ha and with a maximum depth of 15.6 m (Fig. 2.2). It is thereby the smallest of the three study lakes. Lake Östlicher Breitenauersee is located north of lake Großer Ostersee and receives permanent inflow from the latter via a small natural channel. Both lakes are similar in water chemistry (Melzer 1976, Raeder 1990, Sandmann 1995, Beck 2005, Keiz 2013).

## 2.2 General sampling strategy and analytical methods

The three study lakes were sampled during the *Phacotus* growing season from end of April till end of August in 2011 (Fig. 2.3). In the following years 2012 and 2013 sampling concentrated on lake Großer Ostersee, where the highest *Phacotus* abundances were found in 2011. Always the deepest part of each lake was sampled, and in 2013 two additional sites in the northern and eastern sub-basin of lake Großer Ostersee were included in the sampling campaign. The sampling period 2013 was extended till November.

Physical and chemical sampling included in situ measurements of temperature, pH, conductivity and dissolved oxygen with a multi parameter probe (WTW-Multi 350i) at one metre depth intervals from the surface to the lake bottom. Water samples for laboratory chemical and plankton analysis were retrieved



**Figure 2.3:** Map of a section of the Osterseen lake district with lake Großer Ostersee, lake Östlicher Breitenauersee and lake Eishaussee showing the selected sampling sites (Bavaria, Germany).

as depth-integrated samples between 0 – 5 m depth in lakes Großer Ostersee, Östlicher Breitenauersee and Eishaussee in 2011, and from one metre depth intervals between 0 – 10 m depth in lake Großer Ostersee during the sampling campaigns 2012 and 2013. Sub-samples for quantitative analysis of algal abundance were preserved with Lugol’s solution. Cells were enumerated using the Utermöhl sedimentation method (Utermöhl 1931, 1958). The settling technique uses a sedimentation chamber, into which a 5 – 50 ml sub-sample is placed. Gravity causes the phytoplankton cells to settle on the bottom of the chamber onto an object slide. The settled cells can then be identified and counted using an inverted microscope (Leitz Labovert) at 200 – 400x. In our study, the entire base of the object slide was viewed to ensure accurate determination of *P. lenticularis* abundance. A mean density (cells  $L^{-1}$ ) was calculated from the counts. In 2012 and 2013 cells were additionally examined for morphological criteria that allow to distinguish between two main phases of the vegetative cell cycle of *Phacotus*. Cells in the growing phase of the cell cycle (GC) are flagellated, possess a bi-valved calcite shell and the cellular content often does not fill the entire volume

of the cell. Cells in the mitotic phase (MC) had mostly divided into four daughter cells, that were still enclosed in their maternal cell wall by mid-morning, when samples were taken from the lake. We also enumerated empty calcite-shells that had separated into two shell halves or that had no noticeable cellular structure attached to them.

Additional depth-integrated water samples were collected for further laboratory analysis on the  $\text{CaCO}_3$  saturation state of the lake water in 2011. Quantification of the  $\text{CaCO}_3$  saturation index  $\Omega$  (Berner, 1971) is based on direct measurement of pH, water temperature, alkalinity and concentrations of primary ions. Data on pH and water temperature were obtained from probe measurements. Alkalinity was determined by acidimetric titration following the method described in DIN 38407 (Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung - Summarische Wirkungs- und Stoffkenngrößen (Gruppe H) - Teil 7: Bestimmung der Säure und Basekapazität). In this method alkaline lake water is titrated with an standard acid (0.1 M HCL) to the carbonate endpoint at pH 8.2 and the bicarbonate endpoint at pH 4.3. Changes in pH were measured with a pH metre. Alkalinity was calculated according to DIN 38407. Ion exchange chromatography was used to obtain concentrations of main inorganic ions in the lake water ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{K}^+$ ,  $\text{Na}^+$ ,  $\text{Cl}^-$ ,  $\text{F}^-$ ,  $\text{NO}_3^-$  and  $\text{SO}_4^{2-}$ ). The analysis separates ions and charged molecules based on their affinity to the ion exchanger. In cation exchange chromatography positively charged ions are attracted to a negatively charged solid support (column). Vice versa, in anion exchange chromatography negatively charged ions are attracted to a positively charged solid support. A lake water sample is injected onto the column under conditions where it is strongly retained. A gradient of increasing salt concentrations is then applied to elute the sample components from the column. Ions with weaker ionic interactions will start to elute from the column first, whereas ions with a stronger ionic interaction elute later in the gradient. The ions of interest are then detected by means of conductivity or light absorbance (Dionex, Ion-Exchange Chromatography). The  $\text{CaCO}_3$  saturation index  $\Omega$  was calculated using a freeware input program WinIAP (Sequentix, [http://www.sequentix.de/software\\_winiap.php](http://www.sequentix.de/software_winiap.php)).

Weekly to biweekly measurements on total phosphorus (TP), soluble reactive phosphorus (SRP), nitrate-nitrogen ( $\text{NO}_3^-$  - N) and ammonium-nitrogen ( $\text{NH}_4^+$  - N) provided information of the nutrient status of the lakes. The samples were analysed spectrophotometrically following standard methods (DEV, 1999 for TP and  $\text{NH}_4^+$  - N; Murphy and Riley, 1962 for SRP; Navone, 1964 for  $\text{NO}_3^-$  - N).



## 3 | Outline of the thesis

In this thesis questions related to climate induced changes on the physical and chemical parameters of alkaline lakes, in particular on the  $\text{CaCO}_3$  saturation state, and possible consequences to the calcifying phytoflagellate *Phacotus lenticularis* were addressed. Interest in calcifying phytoplankton increases because of their high relevance to the carbon cycle dynamics and vertical carbon transport in aquatic systems. The study was focussed on the seasonal occurrence and abundance of *P. lenticularis* in response to variations in the  $\text{CaCO}_3$  saturation state, pH, water temperature and meteorological drivers. The study also documents specific vertical distribution patterns of two morphologically distinct main phases of the *Phacotus* cell cycle. The cell cycle remains one of the least studied and less understood aspects of phytoplankton biology. Understanding the requirements and functional roles of individual phases in the life cycle of calcifying phytoplankton still offers interesting and novel insights into their ecology and biology.

### 3.1 The main hypothesis under investigation

We hypothesized that fluctuations in growth of *Phacotus* will to some extent be attributed to variations in  $\text{CaCO}_3$  supersaturation. At elevated  $\text{CaCO}_3$  supersaturation, *Phacotus* cells can develop a shell of increasingly dense calcite. Protective calcite shells are of advantage to the cells in natural heterogeneous environments and likely will allow for higher growth rates. *Phacotus* cells may show a significant sensitive to changes in lake water  $\text{CaCO}_3$  supersaturation.

Variability in epilimnetic  $\text{CaCO}_3$  saturation and pH are governed to a large extent by photosynthetic  $\text{CO}_2$  uptake in the photic zone and will thereby dependent on regional weather conditions. Little is yet known of these complex interactions. We further hypothesized that local meteorological variability: i.e. solar irradi-

ance, rainfall and wind speed, will significantly affect the lake water  $\text{CaCO}_3$  saturation index. With some time delay this interaction will have implications on *Phacotus* growth dynamics (publication 1). The two hypothesis were tested by quantifying seasonal profiles in *Phacotus* abundance,  $\text{CaCO}_3$  saturation, pH, water temperature and nutrients in three lakes of the Osterseen lake district (Bavaria) that differ in morphology, wind exposure and to a small extend in trophic status. Daily measurements, weekly and biweekly measurements covering the *Phacotus* growing season and meteorological measurements, provided by station Rothenfeld (maintained by the Ministry of Bavaria, <http://www.wetter-by.de/>), were used to quantify this potentially short-term interactions.

In a second study, we hypothesized that both the calcified, motile cell-cycle phase (cells growing in biomass - GCs) and the calcite-shell forming, non-motile phase (DNA replication, mitosis, and cell division - MCs) show differentiated distributions along vertical gradients of physicochemical parameters. We investigated GC and MC distribution patterns in relation to pH and water temperature, two parameters that are known to be significant controlling factors setting the boundaries of *Phacotus* reproduction and calcification. Moreover, we hypothesized that water column stratification may differentially affect the vertical distributions of the two cell-cycle phases. Density stratification creates turbulent and nonturbulent layers in the water column that affect sinking rates and motility of phytoplankton cells. Nonmotile cells likely prefer to be high up in the water column to avoid sinking to fast out of the photic zone, while motile cells may seek nonturbulent water zones that enable layer formation. Daily measurements covering a period of exponential growth and decline in *P. lenticularis* were used to investigate temporal responses of GC and MC densities in response to variations in depth specific physicochemical and in meteorological parameters

The two studies are based on current records of physical, chemical and planktonic variables from lakes Großer Ostersee, Eishaussee und Östlicher Breitenauersee. Our data set covers three seasonal cycles and two short-term, daily time series of *Phacotus* exponential growth and decline. The intensive and complex datasets were analysed by correlation and cross-correlation analysis, as well as single and multivariate linear regression analysis. Integrating time lags into the statistical analysis of the daily data subsets allowed to make first conclusions about delayed algal responses to influences of environmental parameter. The present study contributes to the current, relevant research on the relationship between calcifying phytoplankton, water carbonate chemistry and meteorological parameters.

## 4 | List of publications

The present doctoral thesis is based on the following publications:

Gruenert, U., Raeder, U. (2014) Growth responses of the calcite-loricated freshwater phytoflagellate *Phacotus lenticularis* (Chlorophyta) to the  $\text{CaCO}_3$  saturation state and meteorological changes. Featured article in the Journal of Plankton Research, 36 (3), 630 - 640.

Gruenert, U., Benda, J., Müller, C., 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.

The first authors contributions to the publications:

For all publications, the first author devised the hypothesis, experimental concepts and approaches in discussion with Dr. Uta Raeder. The first author conducted the sampling, performed the laboratory analysis and evaluated the data. However, I received much appreciated assistance from dedicated students with the time consuming sampling and laboratory work. The statistical analysis of the complex depth/time data set of the second publication was done in collaboration with Prof. Dr. Jan Benda. All manuscripts were drafted and written by the first author and discussed with the co-authors.





## 5 | General Discussion

Many recent studies confirm, that marine calcifying organisms are among the vulnerable groups in response to alterations of the oceans pH, carbonate system and water temperature caused by climate change (Lombard et al. 2010, Reyes-Nivia et al. 2013, Keul et al. 2013, Freeman and Lovenduski 2015). In this thesis, I explored responses of the calcifying freshwater phytoflagellate *Phacotus lenticularis* to changes in the lakes  $\text{CaCO}_3$  saturation state, pH and water temperature, as well as to changes in meteorological parameters. Combining high frequency sampling and data analysis, that integrates algal response times, I was able to demonstrate a significant sensitivity of *Phacotus lenticularis* cells towards the three lake internal parameters and their meteorological drivers. I was also able to differentiate between varying effects of pH and water temperature on two distinct phases in the vegetative cell cycle. The *Phacotus lenticularis* cell cycle is divided into two characteristic phases and remains a less-studied aspect in *Phacotus* biology. The results of my publications may provide an perspective on future effects of changing lake water carbonate chemistry, pH and temperature on the widely distributed calcifying freshwater algae *Phacotus lenticularis*.

### 5.1 Growth responses of *P. lenticularis* to the $\text{CaCO}_3$ saturation state

In this chapter, I discuss the hypothesis of possible interactions between *Phacotus* population growth and the seasonal dynamics in  $\text{CaCO}_3$  saturation state, pH and water temperature. Although a positive interaction seemed likely, given that  $\text{CaCO}_3$  supersaturation was required in laboratory experiments to cause calcification in cultured *Phacotus* cells (Hepperle and Krienitz 1996), further cultural experiments also showed that non-calcified *Phacotus* cells grew well and at comparable photosynthetic rates under decreased pH and  $\text{CaCO}_3$  saturation con-

ditions (Schlegel et al. 2000b, Schlegel 2001). The question, if changes in  $\text{CaCO}_3$  saturation and pH affect growth in natural *Phacotus* populations still required an answer.

My study confirms the assumption that in a natural heterogeneous environment *P. lenticularis* cells potentially benefit from increasing  $\text{CaCO}_3$  supersaturation and pH, likely because the calcite shell they can develop offers significant protection. Our data showed a clear positive, linear relationship between *Phacotus* population growth and epilimnetic  $\text{CaCO}_3$  saturation indices ( $\Omega$ ) between 3 – 7. In two of three lakes we studied, a twofold increase of the lake waters  $\text{CaCO}_3$  saturation indices resulted into a fivefold increase in *Phacotus* growth rates when seasonal population growth started. Exponential growth was observed at Omega values above 5. A third lake already supported early peak development, likely because  $\text{CaCO}_3$  saturation indices were sufficiently high from early spring on. The coherent picture was complemented when the relationship between pH,  $\text{CaCO}_3$  saturation and *Phacotus* population growth was explored. Seasonal variation in pH was significantly positively correlated with variation in  $\text{CaCO}_3$  saturation at a time lag of two days, and with variation in *Phacotus* abundance at a time lag of three days. The three variables seem to be tightly connected including specific algal response times toward changes in pH and more pronounced to changes in  $\text{CaCO}_3$  supersaturation. However, it is very likely that response times vary among lakes and seasons, according to site specific variations in lake properties. Previous studies confirm our findings, that the saturation equilibrium of  $\text{CaCO}_3$  in lakes is closely related to pH and also to water temperature (Stabel 1986, Stumm and Morgan 1996). Variation in pH follows variation in phytoplankton production and decomposition in the photic zone. In lake Constance, epilimnetic pH and  $\text{CaCO}_3$  saturation indices increased with increasing thermal stratification and phytoplankton growth, while hypolimnetic indices remained low with only little variation (Stabel 1986). Areas of maximum  $\text{CaCO}_3$  supersaturation coincided with high pH values, while there was less similarity in the annual pattern between  $\Omega$  and water temperature. In our study, water temperature affected *Phacotus* net growth significantly and positively during the main part of the growing season in lake Ostersee, but not so obviously in the two additional lakes. However, our data provide some evidence, that a steady increase in water temperature in combination with a distinct rise in pH and  $\text{CaCO}_3$  supersaturation mediated the onset of *Phacotus* exponential growth end of June 2011 in lake Ostersee. A clear correlation between water temperature and  $\text{CaCO}_3$  saturation indices was not found in the time series.

Water temperature is undeniable an important ecological factor in lakes. It controls the seasonally changing physical and chemical structure of the water column and affects the periodicity of phytoplankton populations (Reynolds 1980, Seip and Reynolds 1995, Gerten and Adrian 2000, Weyhenmeyer 2001, Sommer et al. 2012). Water temperature also influences the carbonate system of lakes and lake water pH (Stabel 1986). At saturating solar irradiance, photosynthetic activity of phytoplankton increases with water temperature up to a species-specific optimum (Raven and Geider 1988). Likewise, algal demand of CO<sub>2</sub> increases, withdrawing CO<sub>2</sub> and HCO<sub>3</sub><sup>-</sup> from the system. The removal of CO<sub>2</sub> and HCO<sub>3</sub><sup>-</sup> causes complex shifts in the CaCO<sub>3</sub> saturation state of the water (Otzuki and Wetzel 1972; Stabel 1986). Increasing epilimnetic temperature also contributes to the CaCO<sub>3</sub> equilibrium by decreasing the calcite solubility product (Plummer and Busenberg 1982). On a physiological level, metabolic activity and reproductive development of algal cells depend on water temperature (Thomas 1975, Li and Dickie 1987). Field studies confirmed that *P. lenticularis*, a member of the *Chlamydomonas* family, achieved highest abundances in lakes during warmer periods and water temperature ranges from 16 to 26 °C (Schlegel et al. 1998, Schlegel et al. 2000b). The total range of temperature tolerance for *Chlamydomonas* species in experimental studies ranged from 6 to 35 °C, with accelerated algal growth in a temperature range from 12 to 30 °C (McCombie 1960). Swimming velocity of flagellated *Chlamydomonas* cells increased significantly at increased water temperatures (Majima and Oosawa 1975). In our study, natural *Phacotus* populations grew at elevated growth rates of up to 0.69 d<sup>-1</sup> in an epilimnetic temperature range from 19 to 23 °C (averaged over 0 – 5 m depth). Exponential growth was observed at lower temperatures of ~ 13 °C in lake Eishaussee when CaCO<sub>3</sub> saturation indices rose at levels above  $\Omega > 5$ , but growth rates remained at 20% on average.

## 5.2 Meteorological changes and their effects on the CaCO<sub>3</sub> saturation state, pH and water temperature

It is assumed that lake internal physical and chemical factors are more directly influenced by climatic factors than biological attributes, that are also subject to competition, predation and trophic interactions (George et al. 2000). In the first study I was able to show, that epilimnetic pH levels and with some time delay  $\Omega$

levels in lake Ostersee declined with increasing wind speed. However, pH levels were positively related to changes in solar irradiance. An influence of solar irradiance on the  $\text{CaCO}_3$  saturation state was not clearly detected. Increasing wind speed tends to increase the  $\text{CO}_2$  flux from atmosphere to the lake surface in times of decreased epilimnetic  $\text{CO}_2$  concentrations (Maberly 1996; Finlay et al. 2009). Higher wind speed can displace and redistribute suspended phytoplankton and thus alters their productivity (George and Heaney 1978). Solar irradiance controls lake photosynthetic activity that can decrease dissolved  $\text{CO}_2$  concentrations. My findings suggest that meteorological factors that cause variability in dissolved  $\text{CO}_2$  concentrations in the epilimnetic layer are determined factors of epilimnetic pH and  $\text{CaCO}_3$  saturation state and this way have implications on calcification and growth of *P. lenticularis*. Further studies have shown that pH can be sensitive to changes in precipitation rates and droughts, that alter weathering rates and water balance (Webster et al. 1996).

Starting and end points of the *Phacotus* growing season seem to be strongly linked to meteorological forcing, especially solar irradiance and air temperature. In the sampling year 2013, we were able to show, that *Phacotus*, a typical phytoplankton species of early summer to late summer, can extend its growing season far into autumn along with an extended stratification period. Decreasing water temperature and beginning mixing of the upper strata did not decline *Phacotus* abundances when pH was sufficiently high ( $> 8.1$ ). Circulation of the upper water layer in autumn promotes entrainment of nutrients from the colder hypolimnion. The difference in total phosphorus concentrations between epilimnion and deeper hypolimnion in September 2013 was a twofold and likely resulted in an entrainment of phosphorus during the beginning circulation of the water column. This may have supported peak development as late as mid of September. In October when stratification ceased, the *Phacotus* population eventually declined. Thus, *Phacotus lenticularis* is likely an ideal organism for climate change studies. *Phacotus* population growth and phenology seems to be controlled to a large extent by physical and chemical parameters such as  $\text{CaCO}_3$  saturation state, pH and water temperature, rather than by predation or likely nutrient availability, if a minimum nutrient supply is warranted (Schlegel et al. 2000a, b; Gruenert and Raeder, 2014).

### 5.3 Distribution patterns of two cell-cycle phases of *P. lenticularis* in relation to vertical physicochemical gradients

In this chapter, I discuss the hypothesis of differentiated vertical distributions of *Phacotus* cells from two different cell-cycle phases, motile growing cells (GCs) and non-motile mitotic cells (MCs), across environmental gradients in lake Ostersee. The two cell-cycle phases were expected to chose between turbulent and less turbulent water layers, as well as between layers that differ in pH, water temperature and water density, according to their ability to actively move through the water column and to their sensitivity towards conditions that promote calcification. Data from a daily sampling campaign allowed to make novel conclusions about influences of environmental parameter that might be delayed by only a few days. The two sampling periods 2012 and 2013 allowed us to make reliable conclusions on how patterns in vertical distributions of GCs and MCs vary over the time course of an entire growing season and with changes in abiotic gradients. This, in addition to the depth specific measurements, adds novel insights into the biology of *Phacotus lenticularis*.

Vertical profiles on growing and mitotic cell abundance clearly showed, that some GCs and MCs were always randomly distributed to all depth between 0 – 10 m, and presumably deeper. Population densities of growing *Phacotus* cells were highest in the thermocline at a maximum water density gradient, indicating that non-turbulent conditions, lower pH values and water temperatures are favourite niches for them. High accumulations of GCs followed the seasonal downward shift of the thermocline. In contrast, maximum densities of MCs where noted in the surface mixed layer, when the weather was calm and sunny. The distribution of mitotic cells showed no distinct seasonal pattern. MCs regularly aggregated very near the lake surface at 0 – 2 m depth and sometimes near the upper boundaries of the thermocline at 4 m depth but seldom below. These depth specific aggregations may represent either passive accumulations by turbulences or direct movements of flagellated *Phacotus* cells towards specific points in the water column prior to mitotic cell division (Reynolds 1976).

Vertical distribution patterns of growing and mitotic cells along pH gradients differed significantly. High concentrations of mitotic cells were noted in a pH range between 8.1 – 8.3, whereas high concentrations of growing cells occurred in

a lower pH range between 7.8 – 8.0. Values of pH in lake Ostersee were highest in the surface mixed layer (8.0 – 8.3) and decreased in a downward direction. High values of pH are interrelated to high  $\text{CaCO}_3$  supersaturation (Stabel 1986). An increase in pH shifts the  $\text{HCO}_3^- / \text{CO}_3^{2-}$  ratio, causing an increase in the chemical activity of  $\text{CO}_3^{2-}$  ions, thus causing supersaturation (Plummer and Busenberg 1982). Epilimnetic pH values in lake Ostersee correspond to rather low  $\text{CaCO}_3$  supersaturation of approx.  $\Omega = 5 - 8$  (Gruenert and Raeder 2014, Stabel 1986 for lake Constance). Calcification of *Phacotus* cells in cultures was observed when pH exceeded 8.0 and at much higher  $\text{CaCO}_3$  supersaturation up to  $\Omega = 110$  (but calcification is possible at lower  $\Omega$  values). The results imply that shell formation is favoured in the epilimnetic layer of lake Ostersee. Cross-correlation analysis of the daily data subset 2012 revealed that growing cell densities were positively correlated with pH at water layers where mitotic cells accumulated. Growing cells depend on their formation from dividing cells, and vice versa. High pH in the epilimnetic layer may promote calcification and thus is beneficial for the survival of mitotic *Phacotus* cells. High MC abundance will immediately translate into a positive response of growing *Phacotus* cell abundance.

Significant differences in vertical distributions of GCs and MCs were also found with respect to water temperature. MCs accumulated in warmer surface layers at water temperatures above 20 °C, whereas GCs preferred lower temperature ranges between 12 – 21 °C. The generally higher temperatures within the epilimnion may have a positive influence on generation times. Many studies confirm, that water temperature has a direct effect on the cell cycle. When temperature decreases, all phases of the cell cycle lengthen in duration (Francis and Barlow 1987, Zachleder and Van den Ende 1992). This suggests that the effect of low temperature is due to a general slowing down of biochemical reactions within the cell. Temperature extremes on the opposite, can lead to head shock or completely stop growth and cell division (Kobiyama et al. 2010). However, it is also suggested that the duration of cell-cycle phases is independent of temperature within the optimal physiological tolerance range of the phytoplankton species (Donnan et al. 1985, Vitova et al. 2011). No data exist so far on specific physiological tolerance ranges of the cell division phase of the cell cycle. Epilimnetic temperature changes during summer stratification were moderate and temperature ranges (12 – 25 °C) were generally within the physiological tolerance limits for *Phacotus* found in field studies (9 – 28 °C, Schlegel 2001) and often in the upper half of this range. *Phacotus* can likely progress through the cell cycle under mostly constant favourable temperature conditions in lake Ostersee when weather conditions are

calm and sunny. The upper temperature limit of more than 28 °C was never attained. This likely explains the weak correlations we found in the data sets of all three sampling campaigns between water temperature and seasonal dynamics of *Phacotus* abundance as well as abundance of different cell-cycle phases.

Our data revealed no relationships between vertical distribution patterns of growing and mitotic cells to specific water density gradients. Growing cells accumulated within the thermocline at maximum water density gradients of more than 0.8 g L<sup>-1</sup> m<sup>-1</sup>, but there were growing cells at all water density gradients between 0 – 10 m depth. Mitotic cells tended to aggregate in the surface mixed layer at lower water density gradients of less than 0.4 g L<sup>-1</sup> m<sup>-1</sup>, but higher abundances were also found at higher water density gradients. I could show in my study, that *Phacotus* cells are able to overcome higher water density gradients by swimming upwards from the thermocline into the surface mixed layer. These findings are in line with studies that found flagellates to be able to swim through high water density gradients under strongly stratified conditions (Kamykowski and Zentara 1977, Townsend et al. 2005). Water turbulence in the epilimnetic layer is an important factor to keep phytoplankton in suspension. Phytoplankton cells are normally heavier than water and tend to sink to deeper water zones. In the turbulent surface water of stratified lakes, phytoplankton cells are constantly redistributed (Reynolds 1994; Ptacnik et al. 2003). We repeatedly found non-motile mitotic cells in the turbulent surface water between June and August 2012 and over the entire growing season in 2013, suggesting that these cells prefer the turbulent surface layer to counter the tendency to sink. *Phacotus* cells benefit from this behaviour by maximising the time they remain within the photic zone.

Our data indicate that growing cells migrate upwards prior to cell division and loss of motility. No studies exist so far on migration patterns of *P. lenticularis*, or whether its cell division is phased to diel cycles. Garces et al. 1997 found for many algal species, that DNA synthesis and an associated second growing phase (S, G2 phase) process during the dark, whereas mitosis and cytokinesis (M phase) often occur in the first hours of the day. Diel vertical migration is also known to be an adaptive behaviour, where cells migrate down at night to deeper nutrient-riched water, where they take up nutrient and swim up during daytime to the nutrient depleted surface water where they use the accumulated nutrients for synthesis of biomass gained from solar irradiance (Fauchot et al. 2005, Hall and Pearl 2011). Downward migration during the day can be induced by high light intensities and aims to avoid light-induced cell damage (Eppley et al. 1969). The phasing between migration, cell division, and light cycles is often variable

and modulated by environmental conditions. This offers a wide field of possible new studies on *Phacotus* behaviour and adaptation.

An alternative interpretation of the differences we found in vertical distribution patterns of GCs and MCs could be that varying cell densities at different depth zones could result from active aggregations and growth in optimal regions or from strong losses at non-optimal regions. However, our data do not support differential loss rate as the main factor explaining the observed distributions. In particular the fact that GC densities positively correlate with pH and temperature at depth were we found MC cells, support effects on growth and division, and not on losses. In addition, the loss rate hypothesis is not well supported by the rich literature on flagellate migration (Weiler and Karl 1979, Seo and Fritz 2000, Fauchot et al. 2005, Townsend et al. 2005).



## 6 | Conclusions and Outlook

In summary, this thesis documents that seasonal dynamic patterns of *Phacotus lenticularis* are influenced by the lakes  $\text{CaCO}_3$  saturation state, pH and water temperature. In our study, increasing supersaturation of  $\text{CaCO}_3$  mediated the onset of *P. lenticularis* exponential growth and stimulated seasonal peak development. The  $\text{CaCO}_3$  saturation state explained a high proportion of 60% of the variance in changes in *Phacotus* abundance at  $\Omega$  values between 3 and 7. *Phacotus* abundance showed the highest significance level and shortest response time to changes in the  $\text{CaCO}_3$  saturation state at time lags of one to two days. Responses to changes in pH were significant but included a longer time lag of three days. During pronounced stratification water temperature changes were insufficient to produce a direct significant response of *Phacotus* abundance, but increasing epilimnetic temperatures in June likely stimulated peak development.

The data presented in this thesis have also contributed to a better understanding of spacial and temporal distribution patterns of cell-cycle phased *Phacotus* cells. Vertical distributions of the calcified, motile stage (GC) and the calcite-shell forming, non-motile cell stage (MC) differed significantly during the entire growing season. Peak densities of growing cells accumulated in a 1 – 4 m layer within the thermocline at maximum water density gradients that suppress mixing and enable layer formation. Mitotic cells, on the contrary, aggregated in the surface mixed layer at environmental conditions that minimize sinking rates. The data suggest that non-motile MCs benefit from their presence in the mixed layer by maximizing the time the cells remain in the photic zone. High pH values and thus high  $\text{CaCO}_3$  saturation indices in the epilimnion are beneficial to mitotic cells because they increase calcification rates. In addition warmer water likely shortens the duration of cell division by increasing biochemical reactions time. In the presented data set, vertical distributions of growing and mitotic cells were best differentiated by their preferred pH ranges, but also by their broader water temperature ranges.

The changes in lake internal physicochemical parameters investigated in the first study were shown to be related to meteorological variables. Wind was found to induce an immediate decline in epilimnetic pH followed by a decline in  $\text{CaCO}_3$  saturation about two days later. Solar irradiance positively affected both pH and water temperature. In the second study, rainfall and in addition air temperature had a small positive effect on growing-cell abundance at a time lag of two days. In general, the studies provides evidence that regional-scale climate-induced changes in pH,  $\text{CaCO}_3$  supersaturation and water temperature are important to growth of *P. lenticularis* at small temporal scales. In addition, starting and end points of the *Phacotus* growing season seem to be linked to solar irradiance and air temperature and the duration of thermal stratification.

The so far outlined research findings will contribute to a better understanding of the ecological responses of calcifying phytoplankton and their cell-cycle phases to physicochemical and meteorological parameters in freshwater lakes. Compared to marine calcifying phytoplankton, little is known about freshwater calcifying algae and their phenomenological, behavioural and physiological responses to environmental change. A broad field of research still lies ahead. Further research efforts should be extended to adaptive strategies of *Phacotus lenticularis* to increasing epilimnetic water temperatures, light intensities and broader variations in pH and the  $\text{CaCO}_3$  saturation state. I have shown, that some of these environmental parameters may act differentially on specific cell-cycle phases. Here, the exiting knowledge is still sparse. Whether migration patterns and cell division of *Phacotus* are phased, and what the phasing would be, still needs to be explored. The phasing will be modulated by environmental conditions and can result in fluctuating patterns. If growth and division are indeed phased to a day/night cycle, we still don't know at what time calcification occurs. Calcification rates will vary with variations of the environment that surrounds *Phacotus* cells. How climate variability and its effects on lake internal physicochemical parameters influences calcification rates in *Phacotus* cells is an interesting question. Extend in duration of the *Phacotus* growing season by meteorological variability and a raise in epilimnetic temperature may significantly affect calcification rates. Here we still need to measure how much  $\text{CaCO}_3$  is fixed in a single *Phacotus* shell to be able to calculate the amount of  $\text{CaCO}_3$  an entire *Phacotus* population can precipitate during its growing season. This can be done by accurately measuring diameter and vertical extension of the shell or by measuring the amount of evaporating  $\text{CO}_2$  after an acid was added to the calcite shells. Algal response times are often short-term. If we want to understand how *Phacotus* cells and

their cell-cycle phases interact with environmental conditions, higher frequency sampling is needed and can complement the necessary long term monitoring. An adequate statistical analysis that integrates algal response times will help to illuminate the often complex interactions between *Phacotus* and its environment. The assumption that *Phacotus* cells are not digested by zooplankton so far relies on microscopic observations done by Schlegel (2001). Systematic laboratory experiments are still required to verify the hypothesis that their calcite shell protects *Phacotus* cells from grazing by zooplankton.



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# APPENDIX



# A | Growth responses of the calcite- loricated freshwater phytoflagellate *Phacotus lenticularis* (Chlorophyta) to the CaCO<sub>3</sub> saturation state and meteorological changes

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## A.1 Abstract

The pelagic phytoflagellate *Phacotus lenticularis* creates a shell of highly symmetrical calcite crystals at its outer extracellular layer and thereby constitutes a significant source of calcite in temperate alkaline lakes worldwide. Responses of *P. lenticularis* to a changing lake environment therefore have potential implications for the calcium carbonate flux in lakes. How meteorological variability in interaction with the CaCO<sub>3</sub> saturation state may influence the abundance and phenology of *P. lenticularis* has not been investigated so far. We performed a biweekly to daily sampling campaign on algal abundance, CaCO<sub>3</sub> saturation, pH, water temperature, and nutrient status in three hardwater lakes that varied in lake morphology and wind exposure. Our data provide evidence that increas-

ing supersaturation of  $\text{CaCO}_3$  mediates the onset of *P. lenticularis* exponential growth and stimulates seasonal peak development. However, total cell concentration in the lake water appears to be independent of the  $\text{CaCO}_3$  saturation state and was highest in the most wind exposed lake. *P. lenticularis* abundance and epilimnetic  $\text{CaCO}_3$  supersaturation were significantly positively correlated at a time lag of about one day. Wind caused an immediate decline in epilimnetic pH followed by a decline in  $\text{CaCO}_3$  saturation about two days later. Solar irradiance positively affected both pH and water temperature, but water temperature changes were insufficient to produce a significant response of  $\text{CaCO}_3$  saturation. We conclude that the  $\text{CaCO}_3$  saturation state is a determinant factor driving the timing of *P. lenticularis* peaks in alkaline lakes.

Keywords:  $\text{CaCO}_3$  saturation, meteorological variability, *Phacotus lenticularis*, onset exponential growth, alkaline lakes

## A.2 Introduction

Interest in the interaction between phytoplankton and climate driven changes in the physical and chemical properties of aquatic systems has increased because of the potential consequences of such changes to productivity, resource flow and ecosystem functioning. Special attention is drawn to phytoplankton species that calcify, as these are significant contributors to the carbon cycle dynamics and vertical material flux in marine and limnic systems. Little is known about the physical and chemical conditions that stimulate growth of calcifying phytoplankton in lakes and how climate changes may affect their productivity.

Green algae of the genus *Phacotus* are abundant in temperate hardwater lakes of a wide trophic range, making up to 5 – 36% of the overall phytoplankton biomass during peak development (Sanchez et al. 1998; Wehr et al., 2001; Menezes, 2010; John et al. 2011; Caraus, 2012). The unicellular flagellates form a regular shell of crystalline calcite plates by extracellular precipitation of  $\text{CaCO}_3$  (Diesing, 1866; Bold and Wynne, 1985; Hepperle and Krienitz 1996). In alkaline fresh water external encrustations of calcite have been described for prokaryotic and eukaryotic algae both in pelagic and benthic zones (Pentecost, 1991; Freytet and Verrecchia, 1998; Laval et al., 2000; Riding, 2006). A solid calcite shell of regular morphology analogous to coccolithophorids, is unique to the genus *Phacotus*. *P. lenticularis* constitutes a significant fraction of the phytoplankton in alkaline lakes, affecting the carbonate chemistry of the water through primary

production and fixation of carbon in their shells (Schlegel, 1998; Schlegel et al., 2000a; Schlegel et al., 2000b). *Phacotus* lorica can significantly add to the accretion of new  $\text{CaCO}_3$  in lake sediments (Müller and Oti, 1981; Bluszczyk et al., 2008; Jouve et al., 2013).

Supersaturation with respect to  $\text{Ca}^{2+}$  and  $\text{HCO}_3^-$  in the surface water is a common phenomenon in hard water lakes during periods of stratification. The calcification reaction proceeds according to the calcite solution equilibrium of freshwater (Stumm and Morgan, 1981). When the ionic activity product of calcium and carbonate in lake water exceeds the solubility product, the system is supersaturated and precipitation of  $\text{CaCO}_3$  occurs. The rates of  $\text{CaCO}_3$  nucleation depend on the presence of binding sites in the water, such as organic molecules and pico-plankton (Thompson et al., 1997; Dittrich and Obst, 2004; Dittrich and Sibling, 2010). Increasing epilimnetic temperatures contribute to  $\text{CaCO}_3$  supersaturation by decreasing the calcite solubility product (Plummer and Busenberg, 1982; Langmuir, 1997). Highly dynamic demands of  $\text{CO}_2$  by phytoplankton and exchange of  $\text{CO}_2$  between atmosphere and lake surface govern epilimnetic pH and carbonic ion concentrations, thereby causing complex shifts in the  $\text{CaCO}_3$  saturation state of the water (Otzuki and Wetzel, 1972; Stabel, 1986).

Calcite formation of *Phacotus* cells is characterized by a control of both nucleation and growth of calcite crystals by the cell when  $\text{CaCO}_3$  supersaturation occurs within a physiological tolerance limit (Hepperle and Krienitz, 1996; Hepperle and Krienitz, 1997). If supersaturation is too high, the cells fail to control mineralization, and crystals become randomly directed. Undersaturation cannot be compensated for, and the cells do not calcify. Experimental studies have shown that while extracellular calcification of *P. lenticularis* depended directly on the degree of  $\text{CaCO}_3$  supersaturation in the cultural medium, primary production did not (Schlegel et al., 2000; Schlegel, 2001). Non calcified cultured strains grew with equally high photosynthetic rates in  $\text{CaCO}_3$  undersaturated media at pH values of 4 as they did at pH values up to 9.5. Calcification was observed when pH exceeded 8.0. The extracellular calcite formation in *P. lenticularis* cells may not directly enhance photosynthetic carbon fixation.

It remains to be quantified how  $\text{CaCO}_3$  saturation that stimulates calcite formation and shell development affects *Phacotus* growth and abundance in natural habitats with varying environmental factors. Calcification has long been known to promote resistance to grazing by zooplankton (Littler et al., 1983; Padilla, 1989). It is also known that the periodic structure of calcite crystals that form

the shells reflect or scatter light, thereby reducing the transmission of solar ultraviolet radiation to the cell (Gao et al., 2009; Quintero-Torres, 2006; Gao et al., 2012). The goal of this study was to determine how variability in epilimnetic  $\text{CaCO}_3$  supersaturation influences the seasonal periodicity of *P. lenticularis* in three oligotrophic hard water lakes that differ in morphology, wind exposure and to some extent in water chemistry. *P. lenticularis* is a widely distributed and often dominant species of early-summer phytoplankton communities in alkaline lakes at pH values that allow for controlled calcification. Field and experimental studies have demonstrated that pH values better predict *P. lenticularis* abundance than lake temperature (Schlegel, 1998), but the link to  $\text{CaCO}_3$  saturation is not yet well understood.

One specific objective of this study is to investigate whether  $\text{CaCO}_3$  saturation drives the initiation of exponential growth in natural habitats. We hypothesize that *P. lenticularis* is capable of achieving high growth rates under conditions of increasing  $\text{CaCO}_3$  supersaturation in lakes because the algae can develop a protective shell of increasingly dense calcite. We further hypothesize that variations in seasonal growth may to some extent be attributed to changing  $\text{CaCO}_3$  saturation indices in the lake water. Variability of epilimnetic pH and  $\text{CaCO}_3$  saturation is governed to a large extent by photosynthetic  $\text{CO}_2$  uptake and thereby dependent on regional weather conditions (Stabel, 1986). Phytoplankton responses to such variability are often complex, species specific and short-term. For this reason we used daily measurements to understand the short-term dynamics of meteorological variables, variables of the waters carbonate chemistry and how they drive *P. lenticularis* growth dynamics.

## A.3 Methods

### A.3.1 Study site

Lakes Ostersee, Breitenauersee and Eishaussee are ground water fed, alkaline lakes located in the Osterseen lake district north of the Alpine foreland basin (47°47'25" N, 11°18'15" E, Bavaria, Germany). The basin is underlain by a sedimentary geology. Sediment bedrocks are covered by gravel terraces, morainic soils and wetlands. Previous studies have shown that *P. lenticularis* can be regularly found in the plankton communities of the three lakes, making up to 10% of the algal biomass (Börtitz, 2012). A summary of the physical, chemical and biolog-

ical characteristics of lakes Ostersee, Breitenauersee and Eishaussee is shown in Tab. A.1.

### A.3.2 Sampling strategy

Each lake was sampled for algal abundance weekly to biweekly from 28 April to 29 August 2011. Phytoplankton was sampled daily during the period of *P. lenticularis* exponential growth from 27 June to 15 July 2011 in lake Ostersee, where intense algal growth was observed. Depth-integrated water samples were collected from 0 to 5 metres depth at the deepest point of each lake. The vertical distribution of *P. lenticularis* cells in the water column was investigated and found to be variable, with maximum cell numbers concentrated between 3 – 5 metres depth. A rapid decline of *P. lenticularis* cell numbers was observed below the thermocline, at approx. 5 metres depth. Subsamples for quantitative analysis of algal abundance were preserved with Lugol's solution. Cells were enumerated by inverted microscope using a sedimentation chamber at 200 – 400x (Utermöhl, 1958). The entire chamber, corresponding to a total volume of 25 ml, was viewed to ensure accurate determination of *P. lenticularis* abundance. A mean density (cells L<sup>-1</sup>) in the epilimnetic zone (0 – 5 m) was calculated from the counts. Water temperature, pH and electrical conductivity depth profiles were measured in 1 metre intervals from 0 to 5 metres water depth using a portable multi-electrode meter (WTW-Multi 350i) during the phytoplankton sampling campaign. We computed mean pH, water temperature and conductivity values, averaged over depth, for further analyses. Additional depth-integrated water samples were collected from 0 to 5 metres for further laboratory analysis from 30 May to 8 August 2011 when sufficient *P. lenticularis* cells were present in the water column. Alkalinity was determined by acidimetric titration. Ion exchange chromatography was used to obtain concentrations of inorganic ions in solution (Ca<sup>2+</sup>, Mg<sup>2+</sup>, K<sup>+</sup>, Na<sup>+</sup>, Cl<sup>-</sup>, F<sup>-</sup>, NO<sub>3</sub><sup>-</sup> and SO<sub>4</sub><sup>2-</sup>) (Dionex Dx-120, Weiss, 2004). Weekly measurements on total phosphorus (TP), soluble reactive phosphorus (SRP), nitrate-nitrogen NO<sub>3</sub><sup>-</sup> - N and ammonium-nitrogen NH<sub>4</sub><sup>+</sup> - N) provided information of the nutrient status of the lakes. The samples were analysed spectrophotometrically following standard methods (DEV, 1999 for TP and NH<sub>4</sub><sup>+</sup> - N; Murphy and Riley, 1962 for SRP; Navone, 1964 for NO<sub>3</sub><sup>-</sup>).

The CaCO<sub>3</sub> saturation index  $\Omega$  (Berner, 1971) was calculated using an input program WinIAP (Sequentix, [http://www.sequentix.de/software\\_winiap.php](http://www.sequentix.de/software_winiap.php)) based on direct measurement of pH, temperature, alkalinity and concentrations

of primary ions. Activity coefficients were estimated by means of the extended Debye-Hückel equation, valid for solutions of higher ionic strength and electrical conductivities (mean values Ostersee =  $474 \pm 23.3 \mu\text{S cm}^{-1}$ , Breitenauersee =  $466 \pm 24.7 \mu\text{S cm}^{-1}$ , Eishaussee =  $482 \pm 29.3 \mu\text{S cm}^{-1}$ )(Stumm and Morgan 1996). Meteorological data sets were consulted to gather information on the weather conditions during the sampling campaign. Daily time series of wind speed, global radiation and rain fall recorded at the meteorological station Rothenfeld (maintained by the Ministry of Bavaria, (<http://www.wetter-by.de/>) for the period 13 April – 29 August 2011 were selected and used for further statistical analysis.

### A.3.3 Data analysis

The abundance data were used in a simple exponential model to determine the onset and progress of the exponential growth phase of *P. lenticularis* in the three lakes. From the abundances  $N_i$  measured at times  $t_i$  we calculated the net growth rate  $\lambda_i$  for a time interval  $t_i$  to  $t_{i+1}$  of successive measurements according to

$$\lambda_i = \log_e \frac{N_{i+1}/N_i}{t_{i+1} - t_i} \quad (\text{A.1})$$

The equation describes a constant growth rate for population size  $N(t)$  on continuous time following the ordinary differential equation  $dN/dt = \lambda_i N$  with the solution  $N(t) = c \exp(\lambda_i t)$  that exponentially interpolates the measured data  $N_i = N(t_i)$  and  $N_{i+1} = N(t_{i+1})$ . For sufficiently small values of net growth,  $\lambda_i$  equals  $\mu_i = (N_{i+1} - N_i)/N_i$ . The onset of the exponential growth phase was defined as the first period where  $\lambda_i$  showed positive values over at least three consecutive measurements. An exponential function with a rate constant set to the average of the calculated growth rates  $\lambda_i$  was fitted to this period in order to demonstrate the progress of exponential growth (dashed lines in Fig. A.3). Auto-correlations and cross-correlations between *P. lenticularis* abundance,  $\text{CaCO}_3$  saturation, pH, water temperature, and local meteorological variables were analysed for a period of 19 daily measurements starting at 27 June in lake Ostersee and for biweekly measurements in all three lakes between 6 June and 8 August 2011 (R freeware, CRAN). Cross-correlation analysis provided evidence of linear relations between two discrete series at given time lags (days for lake Ostersee and 3 – 4 day periods for all three lakes). Cross-correlation coefficients were computed for each time lag and tested for significant difference from zero at the 5% level.



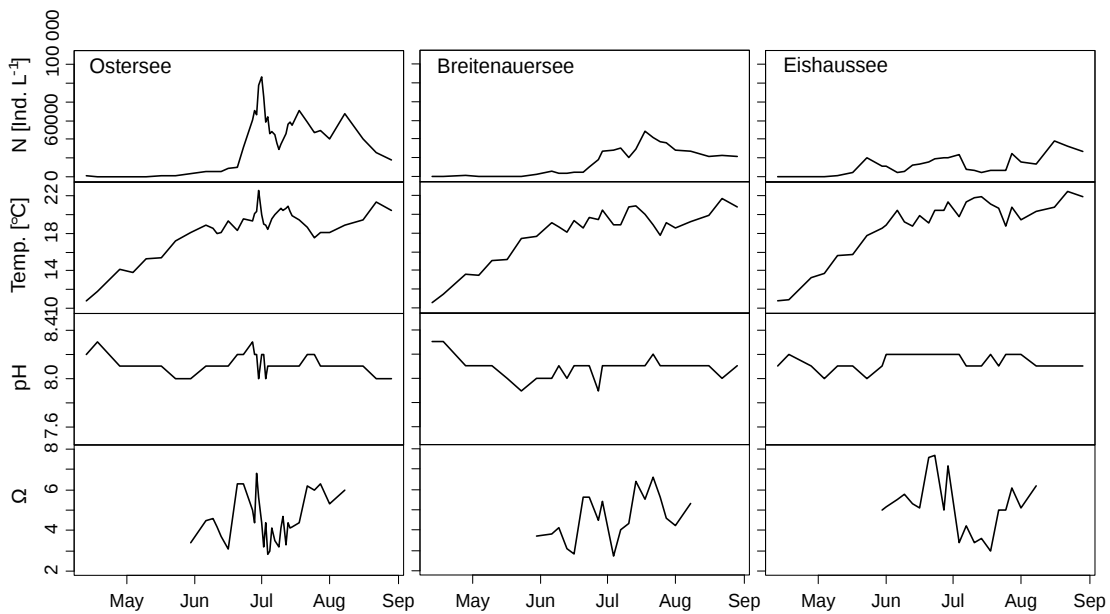
**Table A.1:** Physical, chemical and biological characteristics of the surface layer (0 – 5 m) of the three studied lakes between May and August.

		Ostersee	Breitenauersee	Eishaussee
Circulation type		dimictic	dimictic	meromictic
Surface area	[km <sup>2</sup> ]	1.2	0.03	0.08
Maximum depth	[m]	29.7	15.6	19.1
Water temperature	[°C]	18.7 (± 1.2)	18.9 (± 1.3)	19.8 (± 1.5)
Secchi depth	[m]	3.6 (± 1.1)	3.6 (± 0.9)	5.1 (± 0.8)
pH value		8.1 (± 0.07)	8.0 (± 0.06)	8.2 (± 0.05)
Alkalinity	[mmol L <sup>-1</sup> ]	4.44 (± 0.001)	4.46 (± 0.001)	4.39 (± 0.01)
Ca <sup>2+</sup> concentration	[mmol L <sup>-1</sup> ]	1.5 (± 0.2)	1.4 (± 0.2)	1.4 (± 0.2)
Total phosphorus	[µmol L <sup>-1</sup> ]	0.3 (± 0.1)	0.2 (± 0.1)	0.3 (± 0.1)
Sol. react. phosph.	[µmol L <sup>-1</sup> ]	not detectable	not detectable	not detectable
NO <sub>3</sub> - N	[mmol L <sup>-1</sup> ]	0.09 (± 0.01)	0.08 (± 0.01)	0.04 (± 0.01)
NH <sub>4</sub> <sup>+</sup> - N	[µmol L <sup>-1</sup> ]	2.2 (± 0.006)	1.6 (± 0.007)	8.9 (± 0.005)
Chlorophyll a	[µmol L <sup>-1</sup> ]	0.005 (± 0.003)	0.004 (± 0.002)	0.008 (± 0.003)

## A.4 Results

### A.4.1 Seasonal dynamics in biological and hydrological variables

Seasonal fluctuations in abundance of *P. lenticularis* with two to four peaks during the growing season were observed in all three lakes (Fig. A.1). Maximum peak abundance ranged from 106 300 Ind. L<sup>-1</sup> in lake Ostersee to 48 320 Ind. L<sup>-1</sup> in lake Breitenauersee, and 38 360 Ind. L<sup>-1</sup> in Lake Eishaussee. Timing of the first maxima differed by 6 days between lakes Ostersee and Breitenauersee, whereas in lake Eishaussee an earlier, but small peak developed 32 – 38 days ahead of the other lakes. All lakes were stratified during the entire sampling season. The thermocline varied in depth between 3 and 5 metres. Water temperature in the epilimnetic layer increased through the season from 10.8 – 22.6 °C (average 18,7 ± 1.2 °C) in lake Ostersee, 10.6 – 21.7 °C (average 18.9 ± 1.3 °C) in lake Breitenauersee and 10.8 – 22.5 °C (average 19.8 ± 1.5 °C) in lake Eishaussee. Values for pH varied between 8.0 – 8.3 in lake Ostersee, 7.9 – 8.3 in lake Breitenauersee and 8.0 – 8.3 in lake Eishaussee. There was very little seasonal fluctuation in pH.

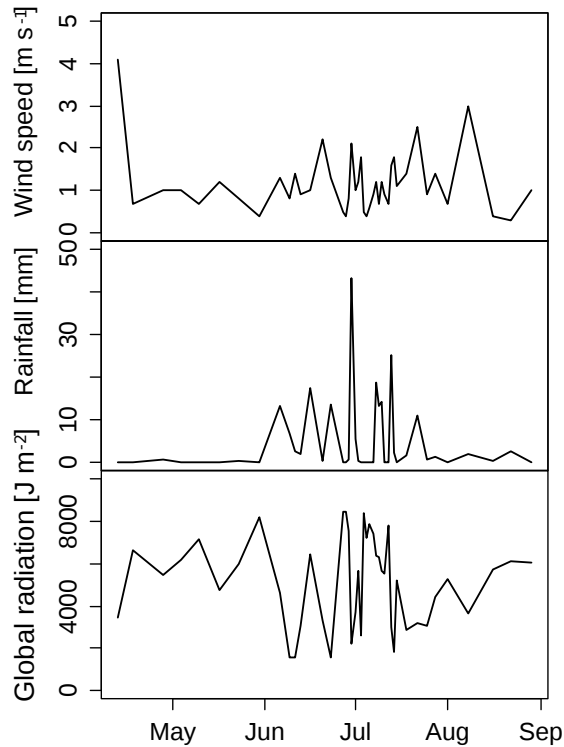


**Figure A.1:** Seasonal variations in cell density, water temperature, pH value and calcite saturation index ( $\Omega$ ) in the stratified upper 5 m water layer during April – August 2011. The values represent midmorning measurements.

The lakes remained supersaturated with respect to  $\text{CaCO}_3$  throughout the sampling season. The  $\text{CaCO}_3$  saturation index  $\Omega$  ranged from 2.8 – 6.8 (average  $4.6 \pm 1.2$ ) in lake Ostersee, 2.7 – 6.6 (average  $4.6 \pm 1.1$ ) in lake Breitenauersee and 3.0 – 7.7 (average  $5.2 \pm 1.4$ ) in lake Eishaussee. The general trophic state of the lakes is oligotrophic (Tab.1). Mean concentrations of TP were  $0.3 \mu\text{mol L}^{-1}$  in lake Ostersee,  $0.2 \mu\text{mol L}^{-1}$  in lake Breitenauersee and  $0.3 \mu\text{mol L}^{-1}$  in lake Eishaussee. Soluble reactive phosphorus was not detectable throughout the sampling period. Concentrations of nitrate-nitrogen (mean values  $42.8 - 92.9 \mu\text{mol L}^{-1}$ , the main source of nitrogen in the lakes, and ammonium-nitrogen (mean values  $1.6 - 8.9 \mu\text{mol L}^{-1}$ ) were combined a 100-fold higher than concentrations in total phosphorus corresponding to classical P limiting conditions for algal growth in the three lakes. Overall, there was little seasonal fluctuation in nutrient concentrations.

#### A.4.2 Meteorological conditions

The late spring season (end of April – early June) started with a period of calm weather, low wind speed, very little rainfall and high light availability (average  $0.8 \text{ m s}^{-1}$ ,  $0.9 \text{ mm}$  and  $5\,974.4 \text{ J m}^{-2}$ , respectively) (Fig. A.2). From 6 June to 22

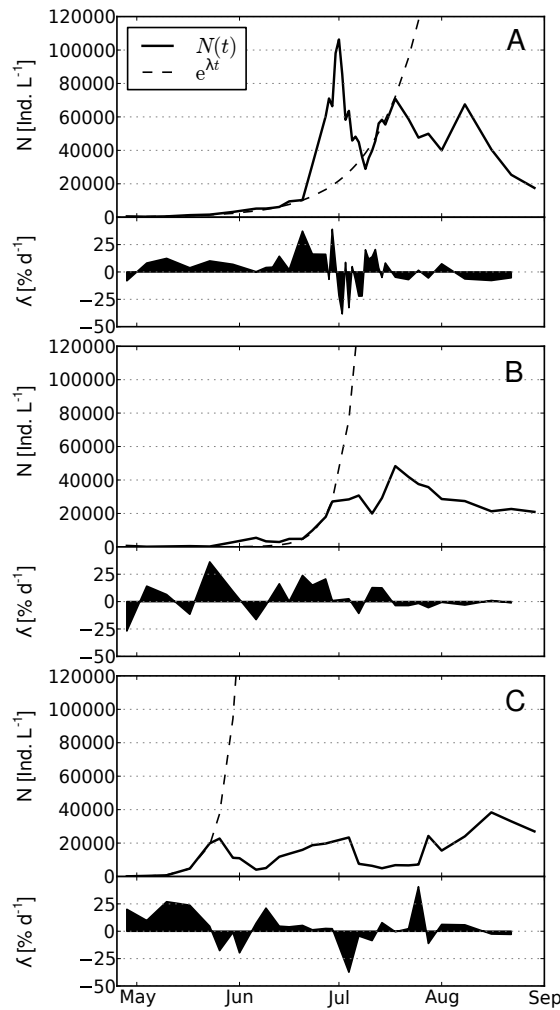


**Figure A.2:** Meteorological time series of wind speed (daily mean), rainfall and global radiation (daily total) during April - August 2011.

July the passage of several atmospheric low pressure systems defined a period of increased rainfall rates (average  $7.4 \text{ mm}$ ) and initiated a heavy rainfall event of  $43.3 \text{ mm}$  on 30 June. Wind speed increased slightly (average  $1.2 \text{ m s}^{-1}$ ) and light availability was variable but remained high (average  $4\,982.4 \text{ J m}^{-2}$ ). Weather conditions during the late-summer season (end July – end August) were again characterized by low rainfall rates, a small decrease in light availability and variable but increasing wind speed (average  $1.9 \text{ mm}$ ,  $4\,317.9 \text{ J m}^{-2}$  and  $1.3 \text{ m s}^{-1}$ , respectively). The growing season of 2011 showed lower rainfall and wind speed rates, but higher solar radiation rates, when compared to average conditions between 1991 – 2011 (Meteorological station Rothenfeld).

### A.4.3 Onset of exponential growth

Exponential growth with a positive growth rate over at least three consecutive measurement days of *P. lenticularis* abundance in lake Ostersee occurred around 4 May (Fig. A.3). Growth rates fluctuated around zero prior to the bloom, whereas



**Figure A.3:** Population growth of *Phacotus lenticularis* in lakes Ostersee (A), Breitenauersee (B) and Eishaussee (C). The temporal evolution of cell numbers (upper panels, solid line) over the measurement period starting on 28 April and net growth rates (lower panels) estimated according to equation 1 are shown. The onset of exponential growth is indicated by exponential functions (upper panels, dashed line) with averaged growth rates taken from the data (lower panels) of  $7\% \text{ d}^{-1}$  for lake Ostersee,  $23\% \text{ d}^{-1}$  for lake Breitenauersee, and  $20\% \text{ d}^{-1}$  for lake Eishaussee.

after the onset of exponential growth, net growth rates averaged  $7\%$  for about 42 days. Around 20 June, the  $\text{CaCO}_3$  saturation index  $\Omega$  showed a twofold increase within 4 days from 3.1 to 6.3 followed by a fivefold increase in *P. lenticularis* net growth from  $7\%$  to  $37\%$  (Fig. A.1 and Fig. A.3). Water temperature had steadily increased from the beginning of the sampling period but dropped slightly from  $19.3$  to  $18.3^\circ\text{C}$  prior to the exponential growth phase. A small increase in pH values from 8.1 to 8.2 was observed around 20 June, that continued to rise to 8.3 when the exponential growth phase was well underway (Fig. A.1, Tab.2).

**Table A.2:** Parameters at the onset of *Phacotus* exponential growth.

	Water temperature [°C]	pH value	Calcite saturation index $\Omega$
Ostersee 20 June	18.3	8.2	6.3
Breitenauersee 20 June	18.5	8.1	5.6
Eishaussee 28 April	13.3	8.1	5.0 (30 May)

*P. lenticularis* growth rates in lake Breitenauersee fluctuated frequently between positive and negative values early in the sampling period (Fig. A.3). Enhanced exponential growth at average net growth rates of 23% per day started at the same day as in lake Ostersee (20 June) following a twofold increase in  $\text{CaCO}_3$  saturation from 2.8 to 5.6. The results suggest that there is a possible area of interaction between saturation index  $\Omega$  values and the onset of *P. lenticularis* exponential growth. Water temperature decreased slightly from 19.3 to 18.5 °C, and a small increase in pH values from 8.0 to 8.1 was observed prior to peak development (Fig. A.1, Tab. A.2). The earliest onset of *P. lenticularis* exponential growth at an average growth rate of 20% was detected in lake Eishaussee around 28 April (Fig. A.3). Water temperature and pH threshold levels for the start of exponential growth were 13.3 °C and 8.1, respectively (Tab. A.2).  $\Omega$  values, that were first recorded on 30 May, averaged 5.0 and were distinctly higher than in lakes Ostersee and Breitenauersee during this time period. As in the other two lakes, abundance of *P. lenticularis* in lake Eishaussee increased steadily until 20 June, when it developed a second peak.  $\Omega$  values remained at levels of around 5 between late May and mid-June, but further increased to 7.6 around 20 June.

#### A.4.4 Growth dynamics in relation to hydrological and meteorological variables

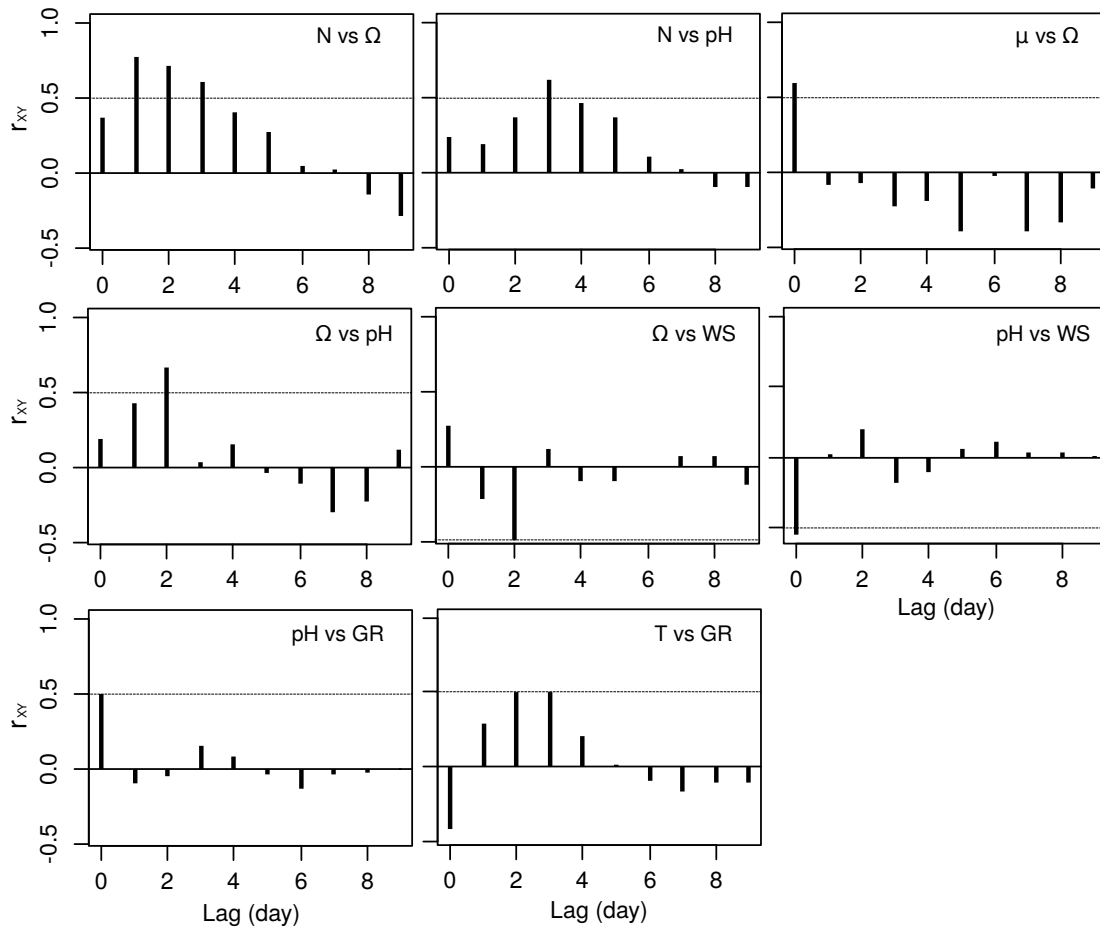
A coherent pattern can be detected in the bivariate cross-correlations among lake Ostersee (27 June – 15 July) time series. The highest significant cross-correlation was observed between abundance of *P. lenticularis* and  $\text{CaCO}_3$  saturation (Tab. A.3, Fig. A.4). An increase in  $\Omega$  values was followed by an increase in net growth at lags of 1 – 3 days (Fig. A.4), while *Phacotus* abundance was likely to decline when  $\text{CaCO}_3$  saturation indices declined. The extended period

**Table A.3:** Significant cross-correlation values ( $r_{xy}$ ) and corresponding time lags between *Phacotus* population size (N), net growth rates ( $\mu$ ), Calcite saturation index ( $\Omega$ ), pH values, wind speed (WS) and global radiation (GB) during 27 June – 15 July 2011 in lake Ostersee. Signal 1 was shifted along the X-axis to determine maximum match between signals.

Signal 1	Signal 2	Time lag [days]	Correlation coefficient ( $r_{xy}$ )
N	$\Omega$	1	0.7745
		2	0.7267
		3	0.6262
N	pH	3	0.5994
$\mu$	$\Omega$	0	0.5946
$\Omega$	pH	2	0.6489
$\Omega$	WS	2	-0.5435
pH	WS	0	-0.5518
pH	GR	0	0.5000
T	GR	2	0.5623
		3	0.5580

**Table A.4:** Significant cross-correlation values ( $r_{xy}$ ) and corresponding time lags between *Phacotus* population size (N), net growth rates ( $\mu$ ), Calcite saturation index ( $\Omega$ ), pH values, wind speed (WS), global radiation (GB) and rainfall (R) during 6 June – 8 August 2011 in lakes Ostersee, Breitenauersee and Eishausee.

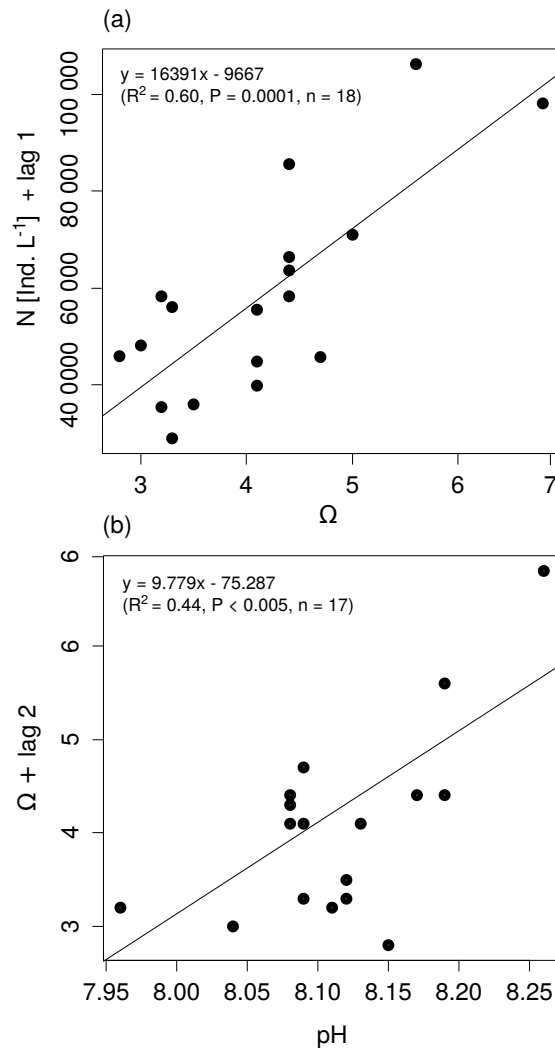
Signal 1	Signal 2	Time lag [days]	Correlation coefficient ( $r_{xy}$ )
Ostersee			
N	T	0	0.5478
T	WS	1	-0.4846
T	GR	1	0.6881
Breitenauersee			
N	$\Omega$	1	0.5941
Eishausee			
N	$\Omega$	1	0.6786
N	pH	0	0.6389
		1	0.7573
$\Omega$	pH	0	0.5071
		1	0.5876
T	R	1	-0.6184



**Figure A.4:** Cross-correlation correlograms ( $r_{XY}$  cross-correlation coefficients) computed from bivariate time series between *Phacotus* population size (N), net growth rates ( $\mu$ ), calcite saturation index ( $\Omega$ ), pH values in lake Ostersee as well as wind speed (WS) and global radiation (GB). Time lags correspond to days. The dashed lines represent 95% confidence limits for the cross-correlation function.  $r_{XY}$  values in Tab. A.3 were computed from this analysis.

of significant cross correlation values over several days probably reflects the fact that the abundance and  $\Omega$  time series are also correlated with their own past and future values. Changes in  $\text{CaCO}_3$  saturation explained 60% of the variability in abundance at lag 1 (Fig. A.5 a). Net growth rates were directly positively correlated with calcite saturation indices. The two results suggest that *P. lenticularis* is sensitive to changing  $\text{CaCO}_3$  supersaturation.

Variations in pH explained 44% of  $\Omega$  changes at lags of 2 days (Fig. A.4 and Fig. A.5). Confirming the relationships between pH,  $\Omega$  and net growth, pH was positively correlated with *Phacotus* abundance at lags of 3 days. The relation



**Figure A.5:** *Phacotus* abundance as a function of changes in the  $\text{CaCO}_3$  saturation index ( $\Omega$ ) (a), and  $\text{CaCO}_3$  saturation as a function of changes in pH (b). Solid lines indicate the best linear fit to the data points.

between  $\Omega$  and water temperature was below the level of significance. However, water temperature affected net growth significantly during the main part of the growing season in lake Ostersee (Tab. A.4). Among the climate variables examined, wind caused a significant decline in pH and  $\Omega$  levels in the epilimnetic layer at time scales of zero and two days, respectively. In addition to the dependence on wind speed, pH was directly positive correlated to global radiation. The analysis also confirmed a positive correlation between global radiation and water temperature with lags of 2 – 3 days. Rainfall was not found to be significantly correlated to any of the tested variables, likely due to the high number of days with no rainfall in the data set. However, it is possible that excess precipitation (43 mm) on 30 June and an inflow of organic acids from the catchment caused



the rapid decline in  $\Omega$  and pH values. The rainfall event may have terminated the development of the first peak in *P. lenticularis* growth. While a significant correlation between abundance and  $\text{CaCO}_3$  saturation was only observed during the early phase of algal growth in lake Ostersee, the extended data set at larger time intervals yielded significant positive correlations between N and  $\Omega$  for the main part of the growing season of *P. lenticularis* in lakes Breitenauersee and Eishaussee (Tab. A.4). A positive correlation between N and pH, as well as between  $\Omega$  and pH was still visible at enhanced time lags for lake Eishaussee.

## A.5 Discussion

We had hypothesized that *P. lenticularis* potentially benefits from increasing  $\text{CaCO}_3$  supersaturation in natural heterogeneous environments, likely because the cells can develop a protective shell of increasingly dense calcite. If so, then the growth potential of *P. lenticularis* during periods of increasing  $\text{CaCO}_3$  supersaturation would exceed the metabolic costs for shell formation.

This study gives first evidence that increasing  $\text{CaCO}_3$  supersaturation mediates the onset of *P. lenticularis* growth and promotes peak development during the growing season. Our data demonstrated that a twofold increase in  $\text{CaCO}_3$  saturation indices was immediately followed by an up to fivefold increase of net growth rates in lakes Ostersee and Breitenauersee. In lake Eishaussee a first peak developed earlier in the sampling season, likely due to the generally higher  $\text{CaCO}_3$  supersaturation in this lake early in the season. Our studies point to thresholds of  $\Omega > 5.0$ ,  $\text{pH} > 8.1$  and water temperature  $> 13.3^\circ\text{C}$  favouring the onset of enhanced exponential growth. The data also suggest that the relationship between cell density and  $\text{CaCO}_3$  saturation is positive and linear at  $\Omega$  values between 3 and 7. Although *Phacotus* is known to be adapted to higher water temperature, we could show for lake Eishaussee that peak development is possible early in the growing season at lower epilimnetic temperatures when  $\text{CaCO}_3$  supersaturation is sufficiently high. Total cell concentration in the water column seems not to be dependent on the degree of  $\text{CaCO}_3$  supersaturation as we found the highest cell concentrations in lake Ostersee, which showed lower  $\text{CaCO}_3$  saturation indices than lake Eishaussee. All studied lakes are oligotrophic and phosphorus is continuously limiting during the stratification period. We found that lake Ostersee is more dynamic in terms of water column stability and temperature change due to its large surface area and wind exposure. Wind forcing may play a significant

role in enhancing the rate of nutrient regeneration and decomposition by sometimes creating internal waves in the stratified layer of lake Ostersee (Moss, 2013). However, detailed studies on nutrient concentration changes would be necessary to verify this hypothesis.

In the studied lakes net growth rates ( $\mu$ ) during the exponential growth phase averaged between  $0.35 \text{ d}^{-1}$  and  $0.69 \text{ d}^{-1}$ . Our calculations underestimate specific growth rates as we did not consider rates of removal due to losses. Still, our values for net growth rates in natural lakes exceeded values measured in mono-culture experiments at light-saturated and nutrient-enhanced conditions where maximum growth rates of  $0.49 \text{ d}^{-1}$  were observed (Schlegel et al., 2000). Mean growth rates of chlorophytes species in culture experiments were found to reach  $0.62 \text{ d}^{-1}$  at  $20^\circ\text{C}$ , comparable to our maximum growth rates (Lürding et al. 2013). Further experimental studies demonstrated that *P. lenticularis* cultures (without calcite-shell development) attained inherently low biomass and P:C ratios compared to other chlorophyte species (Striebel et al., 2009). While laboratory studies suggest that the flagellate is relatively slow growing, our data from three oligotrophic hardwater lakes indicate that *P. lenticularis* is capable of accelerated growth during several short windows of increasing  $\text{CaCO}_3$  supersaturation under conditions of persistent phosphorus deficiency from early-summer until circulation starts in autumn. The exponential growth phase of *P. lenticularis* in lakes Ostersee and Breitenauersee was associated with the clear water phase. Strong grazing pressure is known to cause shifts in phytoplankton composition towards taxa that resist consumption e.g. by calcification (Agrawal, 1998). The low cell P:C ratio may further qualify *P. lenticularis* as P deficient food for grazers. Low phytoplankton biomass supports the high demand of light that was observed in *P. lenticularis* cultures (Striebel et al., 2009). In field studies *P. lenticularis* blooms were often associated with populations of the Cladoceran *Bosmina longirostris* (Schlegel, 2001), but we are unaware of any detailed study of such specific algal defence - grazer - interactions.

Phytoplankton species and communities in lakes have been well-documented over extended time periods providing useful records for extracting weather-related responses (Winder and Schindler, 2004; Adrian et al., 2006; Huber et al., 2008; Thackeray et al., 2008; Binding et al. 2011; Thackeray, 2012; De Senerpont Domis et al., 2013, Lürding et al. 2013). This study provides evidence that regional-scale climate-induced changes in pH and  $\text{CaCO}_3$  supersaturation are important to growth of *P. lenticularis* at small temporal scales. A positive relationship existed between pH and daily solar radiation, while pH was depressed during windy

periods in lake Ostersee, resulting in a lagged response of  $\text{CaCO}_3$  saturation. The response time of  $\Omega$  to changes in epilimnetic pH is likely to be lake-specific and to vary seasonally. The solubility equilibrium of  $\text{CaCO}_3$  is a complex function of factors such as the concentration of dissolved ions, water temperature, primary production, availability and charge of crystal seeds and mixing conditions in the surface water layer (Loewenthal and Marais, 1976; Hodell et al., 1998; Reddy and Hoch, 2012). Solar irradiance controls net lake productivity, while wind activity tends to increase the  $\text{CO}_2$  flux across the air-water interface and to a lesser extent from the hypolimnion (Maberly, 1996; Finlay et al., 2009). Our data suggest that variability in dissolved  $\text{CO}_2$  concentration determines the  $\text{CaCO}_3$  saturation state in the epilimnetic layer during persistent stratification in lake Ostersee. There is some uncertainty over the effects of a future change in wind patterns during summer on *P. lenticularis* growth. Stronger and more frequent exposure to wind may increase the forcing on the lake surface, causing deeper mixing and greater entrainment of cooler water,  $\text{CO}_2$  and nutrients from below. The effects on *P. lenticularis* growth could be oppositional in a low productive alkaline lake. Neither future increase in surface water temperature or ultraviolet radiation are likely to negatively impact this phytoflagellate owing to its ability to build a calcite shell and prevent sedimentation by flagellar motility in warmer and more strongly stratified waters. *P. lenticularis* provides a good model of trends in the lakes carbonate chemistry in response to climate change and how this may affect biomineralization in freshwater systems.

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# B | Vertical segregation of two cell-cycle phases of the calcifying freshwater phytoflagellate *Phacotus lenticularis* (Chlorophyta)

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## B.1 Abstract

Specific responses of cells from different cell-cycle phases to environmental variations strongly impact their ecology and productivity. We quantified responses of both the motile, calcified growing cell phase (G1 phase) and the calcite-shell forming, non-motile mitotic phase (S, G2 and M phase) to changes in depth specific physicochemical parameters and meteorological parameters over two growing seasons. Growing (GCs) and mitotic cells (MCs) segregated vertically during peak development. GCs accumulated within the thermocline, while MCs aggregated in the epilimnion down to 4 m depth, which corresponded to the maximum depth of mixing. GCs and MCs differed in their preference with respect to pH and water temperature, but not to water density. Interestingly, pH and temperature vari-

ations in the epilimnion, where mitotic cells aggregate and calcify, explained up to 83% of the variance of growing cell density at a time lag of one and more days in a daily sampling campaign. Among the meteorological variables we tested, rainfall and air temperature had an additional positive effect on GC density. The results suggest that *Phacotus* cells migrate upwards prior to loss of motility to layers that reduce sinking but likely enhance calcification rates, which translates into a response of growing cell density.

Keywords: cell-cycle, *Phacotus lenticularis*, vertical distribution patterns, pH, temperature, water density gradient, multiple-parameter linear regression model, alkaline lakes

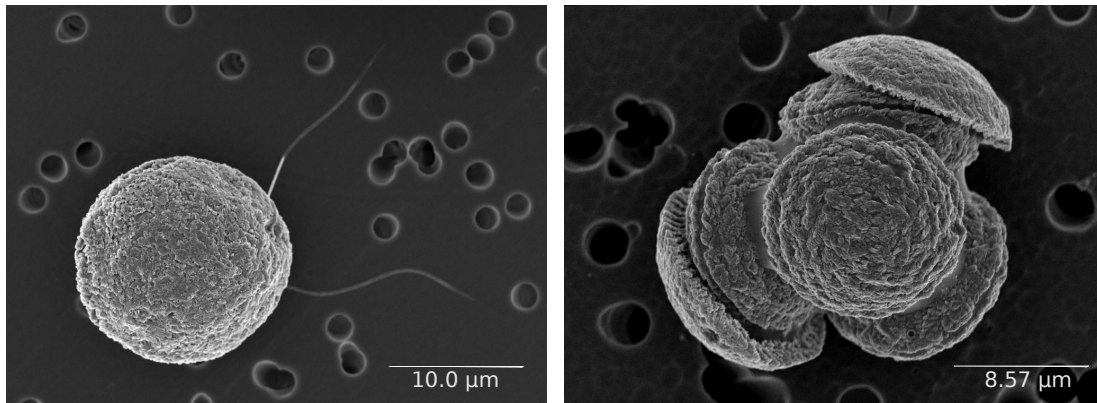
## B.2 Introduction

Phytoplankton species that calcify have been remarkably successful in adapting to a wide range of ocean and freshwater environments, contributing to carbon cycle dynamics and the vertical material flux of these systems (Okada and Honjo, 1973; Brown and Yoder, 1994; Van der Wal et al., 1995; Schlegel et al., 1998; Zondervan et al., 2001; Delille et al., 2005). The manner in which environmental controls affect the vertical distribution and abundance of calcifying phytoplankton and their cell-cycle phases in lakes, is not well explained.

The unicellular green flagellate *Phacotus lenticularis* creates a shell of highly symmetrical calcite crystals at its outer extracellular layer and thereby constitutes a significant source of calcite in temperate alkaline lakes worldwide (Koschel, 1995; Sanchez et al., 1998; Bold and Wynne, 1985; Hepperle and Krienitz, 1996). The vegetative cell cycle of *P. lenticularis* is typical of a large group of algae that can divide by multiple fission into more than two daughter cells (Giering et al., 1990; Zachleder and Van Den Ende, 1992; Bisova and Zachleder, 2014). Schlegel (2001) also documented fusion of gametes, as well as zygote and hypnozygote stages from aged cultures. Vegetative *Phacotus* cells undergo a prolonged G1 phase, during which the cells are motile. They then undergo repeating rounds of alternating S phase, mitosis and cytokinesis to produce mostly 4 – 8 daughter cells. At the onset of mitosis *Phacotus* cells regress flagella and become immobile. Calcification occurs by extracellular precipitation of  $\text{CaCO}_3$  on the cell surface of daughter cells. Little is known about the vertical distribution of cell-cycle phased *Phacotus* cells and yet this question is of significance to the carbonate chemistry in alkaline lakes.

In stratified lakes, vertical gradients and their temporal dynamics determine the environmental variability to which phytoplankton cells and populations are exposed (Klausmeier and Litchman, 2001; Clegg et al., 2004, 2007; Longhi and Beisner, 2010). Previous studies have demonstrated that pH and water temperature are primary controlling factors setting the boundaries of *Phacotus* phenology and growth (Schlegel et al., 1998, Schlegel et al., 2000). Phytoplankton species vary in their physiological tolerance to an upward or downward drift in pH and change in carbon dioxide species and carbon availability (Lane and Burris, 1981; Chen and Durbin, 1994). The direct sensitivity of  $\text{CaCO}_3$  solubility to pH affects the tolerances of calcifying phytoplankton and may be relevant during cell-cycle phases where calcification occurs (Muller et al., 2008; Beaufort et al., 2011; Gruenert and Raeder, 2014). Rates of calcification in *Phacotus* were found to be closely coupled to pH and  $\text{CaCO}_3$  supersaturation (Hepperle and Krienitz, 1996; Hepperle and Krienitz, 1997). In cultures, *P. lenticularis* grew over a broad pH range, but optimum growth was observed at alkaline pH between 8.0 and 9.5 (Schlegel et al., 2000). Water temperature can affect phytoplankton cell-cycle phases in a number of ways. An increase in water temperature increases metabolic activity, rates of photosynthesis and cell division (Jitts et al., 1964; Davison, 1991; Butterwick et al., 2005). Temperature controls the seasonally changing physical and chemical vertical structure of a lake through changes in water density. Increasing water temperature also contributes to  $\text{CaCO}_3$  supersaturation by decreasing the calcite solubility product (Plummer and Busenberg, 1982; Langmuir, 1997). Turbulence influences vertical distribution patterns of motile and non-motile cells. In the turbulent surface water of stratified lakes, gravitating phytoplankton is constantly redistributed (Reynolds, 1994; Ptacnik et al., 2003). Strong density stratification within the thermocline suppresses turbulent mixing and promotes the formation of defined layers by motile phytoplankton species (Etemad-Shahidi and Imberger, 2001; McManus et al., 2003). Non-motile cell-cycle phases require a distribution higher in the water column to counteract losses by sinking.

Our data set covers two seasonal cycles and a daily time series of growing and mitotic cell vertical distribution. This allowed us to detect variations in distribution patterns and growth in response to variations in physicochemical parameters and their meteorological controls on seasonal and short term scales. With this knowledge we better understand the sensitivity of individual cell-cycle phases to the seasonality in lake internal parameters as well as key aspects of weather.



**Figure B.1:** SEM images of a *Phacotus lenticularis* cell in the growing phase (GC) of the vegetative cell-cycle showing the solid arrangement of calcite crystals and two apical flagella (left), and a mitotic cell (MC) after cell division with four calcified daughter cells shortly before liberation (right).

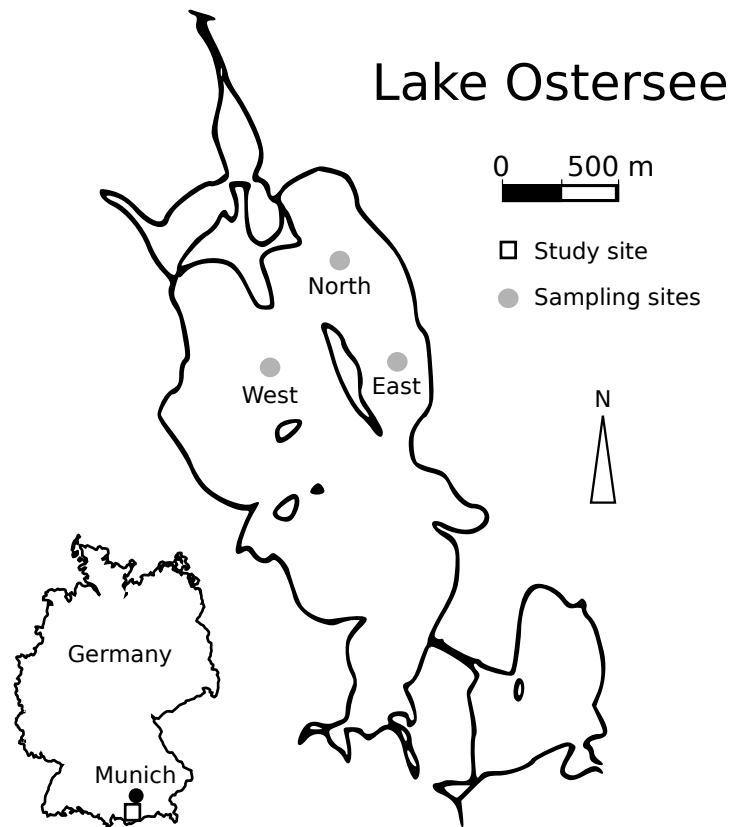
## B.3 Methods

### B.3.1 Study site

Lake Ostersee is a dimictic, alkaline lake located in a lake district north of the Alpine foreland basin (47°47'25" N, 11°18'15" E, Bavaria, Germany). The oligotrophic lake is fed by nutrient poor, carbonate rich ground water and is underlain by a sedimentary geology. Lake surface area is 1.2 km<sup>2</sup>, with a mean depth of 11.90 m and a maximum depth of 29.7 m. The lake floor is divided into several sub-basins. A summary of the physical, chemical, and biological characteristics of lake Ostersee is described in Gruenert and Raeder (2014).

### B.3.2 Sample collection and processing

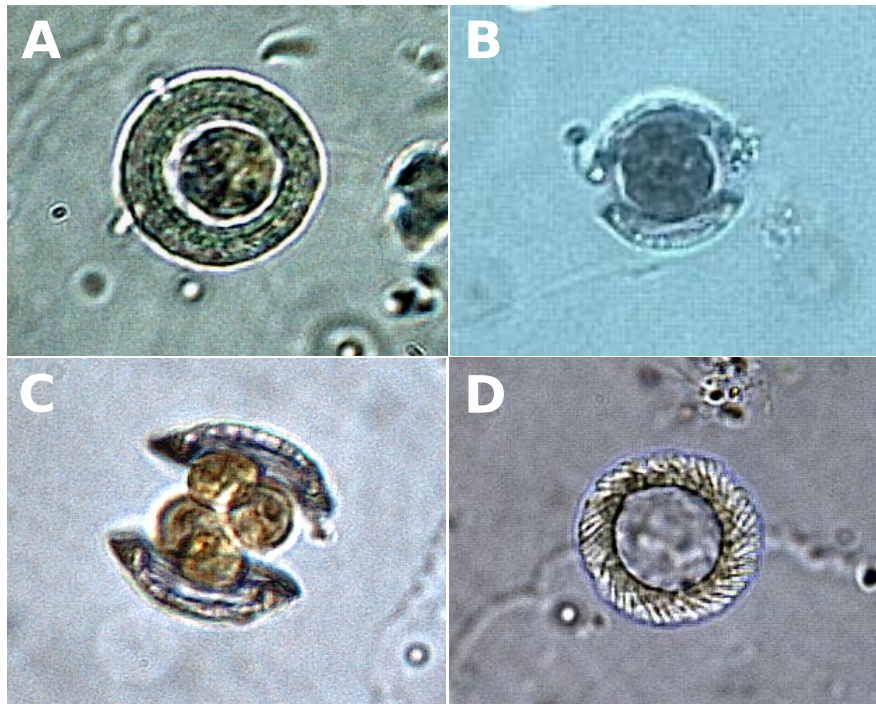
Sampling was carried out at the deepest, western lake sub-basin (Site West) in 2012 and 2013 as well as at two shallower sub-basins in the northern and eastern extensions of the lake (Sites North and East, Fig. B.2) in 2013. The southern lake basin receives irregular water inflow from an artificial channel and a ground water point source and was therefore excluded from the sampling campaign. The three sampling locations span gradients in wind exposure and, to a lesser extent, in nutrient levels. All sites were sampled biweekly from early July to late August 2012, and from early June to late November 2013. A high frequency daily sampling campaign was conducted from 27 July to 17 August 2012 covering a period of *P*.



**Figure B.2:** Map of lake Ostersee showing the selected sampling sites (Bavaria, Germany).

*lenticularis* exponential growth and decline. Plankton samples were collected in a standardized sampling order (West, North, East) and time (between 8 and 10 am) and approx. half an hour time lag between sampling sites.

Vertical profiles of *Phacotus* density, water temperature, pH, and electrical conductivity were collected at 1 m intervals from 0 to 10 m water depth at each sampling site. We chose this depth range because the majority of *Phacotus* cells were observed above 8 m depth. Water transparency was measured with a Secchi disc. Temperature, pH, and conductivity were recorded with portable sensors (WTW Multi 350i). Subsamples for quantitative analysis of algal density in the water column were preserved with Lugol's solution. After homogenisation, 25 ml (sometimes only 10 ml in case of high cell densities) of the sample were placed in a sedimentation chamber. Cells were enumerated by inverted microscope at 200 – 400x (Utermöhl 1958). The entire surface of the chamber was scanned to ensure accurate cell counts (detection limit at 5% significance was 120 Ind. L<sup>-1</sup>; Mazziotti et al. 2013). Cells were examined for morphological criteria characteristic of two stages of the cell-cycle (Fig. B.1) - cells in the growing phase (GC) and cells in the mitotic/post mitotic phase (MC). We use the term mitotic cells to describe cells



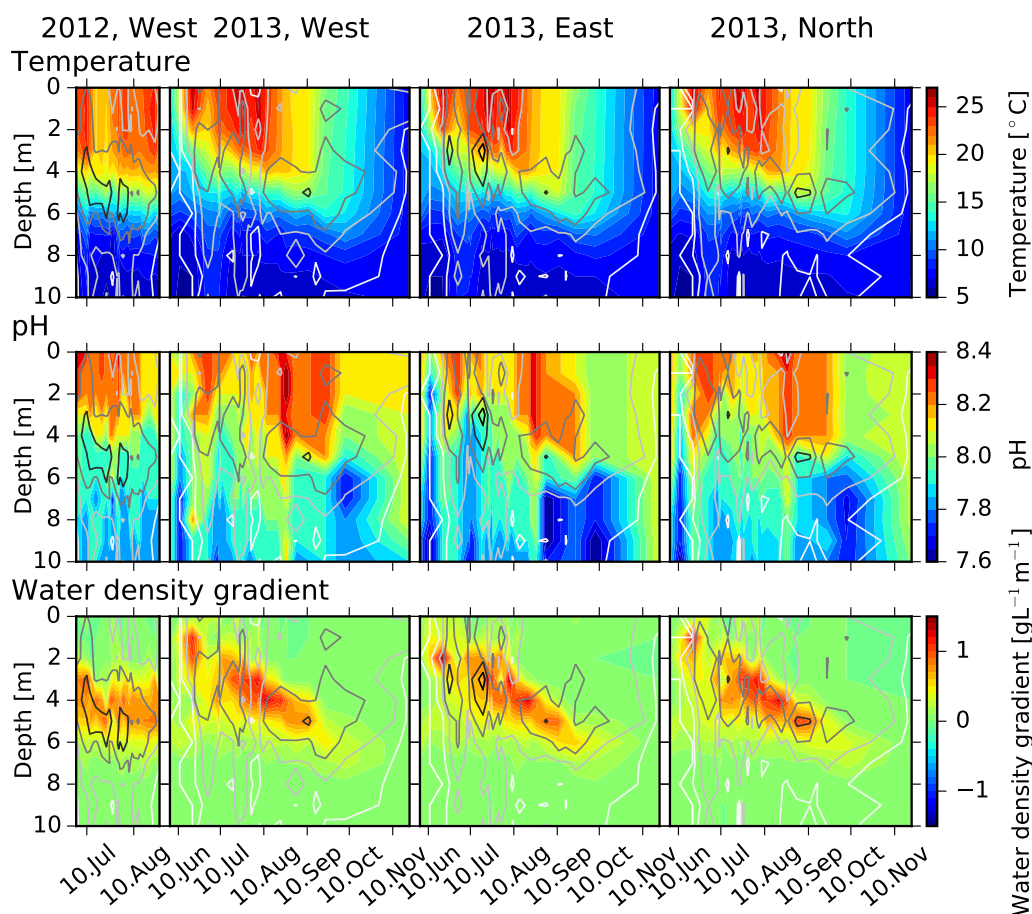
**Figure B.3:** Light microscope images of cell-cycle phased *Phacotus* cells showing a growing cell surrounded by a undivided calcite-shell (A), mitotic cell surrounded by two separate shell halves (B) and four daughter cells enclosed in a separated maternal shell (C) and empty calcite shells (D).

in the DNA replication - division sequence of the cell-cycle. Cells were classified as GC's when the two shell halves were attached to one another, forming a single unit, and the protoplast was either small in diameter or had not divided (Fig. B.3 A). Cells were classified as MC's when the shell had separated along a marginal contact zone or the protoplast had divided and two, four or eight daughter cells were present inside the cell wall (Fig. B.3 B – C). Most mitotic cells we found had already divided and were fully calcified by mid-morning, when the samples were taken. Whether non-calcified cells will be liberated under alkaline conditions is not yet known. We also enumerated empty calcite-shells (CS) that had no noticeable cellular structure attached to them, or calcite shell halves that derived from cell division (Fig. B.3 D). Two shell halves were counted as one shell.

### B.3.3 Data structure and statistical analysis

Water temperature, pH, conductivity, and data of *Phacotus* GC, MC, and CS densities were arrayed on 1 m interval depth - time grids. Water density was calculated for each depth zone using temperature and conductivity data (Imboden und

Wüest, 1995). Density gradients were computed by calculating the difference between two water density measurements separated by 1 m depth. Cross-correlation coefficients were computed between GC, MC, and CS densities, averaged over all depth measurements, and depth specific averages of environmental variables (see below) from periods of highest sampling frequencies (27 July to 16 August, 2012). Cell density and environmental data were interpolated onto daily intervals. To keep the analysis simple we did not resolve cell densities by water depth but averaged them over all depths. Knowing that cells are mostly concentrated at certain depths, the depth-averaged density mainly reflects the density at the cell's preferred depth. The environmental variables were shifted in time by lags ranging from -3 to +3 days relative to the time series of *Phacotus* densities in order to test for delayed algal responses quantified by Pearson's correlation coefficient (white lines in Fig. B.8). The distribution of the null hypothesis of no correlations was generated from the data by 1000 times independently shuffling the data (light grey bands in Fig. B.8 show the central 95% percentile). Leave-one-out cross validations were performed to compute the confidence interval around the correlation coefficient (dark grey bands in Fig. B.8 show the central 95% percentiles). Correlations were considered significant if the two distributions did not overlap by more than 5%, i.e. when the light and dark bands in Fig. B.8 did not overlap. As depth specific averages of water temperature and pH we used (1) total means over 10 m water depth, (2) values at the depth of the respective density maxima, (3) averages over the metalimnion, and (4) epilimnetic averages from the surface down to +1 m above the lower edge of the epilimnion. The latter was defined as the depth where the temperature gradient exceeds  $1\text{ }^{\circ}\text{C m}^{-1}$ . Multivariate linear regression models were applied on the daily time series to test for effects of environmental variables on growing and mitotic cell density. As possible principal independent variables we used water temperature (TW), pH, Secchi depth (S), as a proxy for light transmission, and air temperature (TA), wind speed (W), global irradiance (I), and rain fall (R) as descriptors characterizing meteorological conditions (data obtained from the Ministry of Bavaria at Rothenfeld station, <http://www.wetter-by.de/>). Meteorological data were provided as daily averaged values (air temperature, wind speed) and daily sums (rain fall, solar irradiance). TW and pH were subdivided into 4 different depth specific averages as described above. All independent variables were provided at lags of 0, 1, 2, and 3 days. We generated these many variants of independent variables to better estimate the robustness of the regression results. From each of the principal independent variables exactly one variant and one lag were selected for a single regression. In all regressions we allowed for an additional offset. In a



**Figure B.4:** Depth-time contour plot of seasonal changes in water temperature, pH, and water density gradient. Gray contour lines indicate *Phacotus* GC densities in steps of 1000 (white), 3162, 100 000, 316 227 and 1000 000 (black) cells L<sup>-1</sup>.

first step we regressed each of the 7 principal independent variables individually (4 variants times 4 lags = 16 regressions for each of the TW and pH variables, 4 regressions for each of the S, TA, W, I and R variables). Models with one or more insignificant ( $p > 0.01$ ) independent variables were discarded from further evaluation. For the single linear regression (GCs) only pH contributed significantly to the models. In a next step we tested regression models with two variables by adding in turn each of the remaining predictor variables to the pH variables. In this step only pH and water temperature contributed significantly to the model performance. In the final step we added the remaining predictor variables to pH and water temperature to construct regression models with three variables. All calculations were programmed in Python 2.7 using the numpy, scipy, and matplotlib packages.



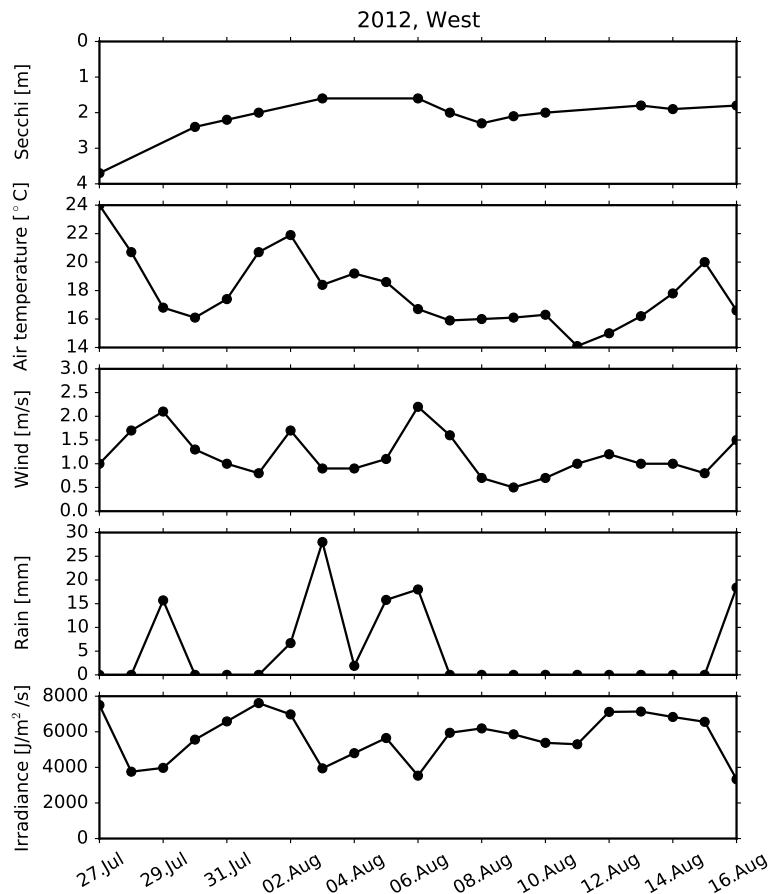
## B.4 Results

### B.4.1 Hydrographic conditions and weather

Environmental variables at the three sampling stations varied in a similar manner during the sampling period in 2013 (Fig. B.4). Water temperatures at each depth and sampling day were highly correlated among pairs of sampling locations (Pearson's correlation coefficient  $r = 1.00$ ,  $p < 10^{-10}$ ,  $n = 297$ ) and ranged over  $5.7 - 27.3^\circ\text{C}$  between 0 and 10 m depth. Values of pH ranged over  $7.4 - 8.4$  and were also correlated between locations ( $r \geq 0.87$ ,  $p < 10^{-10}$ ,  $n = 297$ ). Given that in freshwater lakes water density is mainly a function of temperature and to a small extent of electrical conductivity, we found that water density was significantly negatively correlated with water temperature ( $996.7 - 1000.6 \text{ g L}^{-1}$ ,  $r = -0.99$ ,  $p < 10^{-10}$ ,  $n = 297$ ). Water density gradients were uncorrelated with temperature ( $r < 0.48$ ) and attained highest values  $> 1 \text{ g L}^{-1} \text{ m}^{-1}$  in a temperature range between  $17$  and  $24^\circ\text{C}$  within the thermocline. The thermocline was located at 4 to 6 m depth and had temperature gradients up to  $5^\circ\text{C}$  per metre. Secchi depth decreased over the summer in all stations from approx. 4.5 to 1.5 m. During autumn, light penetration through the water column increased again to values around 6.0 m for Secchi depth. Ranges of water parameter values were very similar between the years 2012 and 2013. Weather conditions during the daily sampling campaign (27 July – 17 August 2012) were characterized by a calm, dry period with the passage of an atmospheric low pressure system during the 2 and 6 August 2012. The average air temperature oscillated between  $14$  and  $24^\circ\text{C}$  (average  $17.8^\circ\text{C}$ ) and solar irradiance was  $5745 \text{ J m}^{-2}$  on average (min:  $3332 \text{ J m}^{-2}$ , max:  $7612 \text{ J m}^{-2}$ ) (Fig. B.5). Meteorological data showed low values for wind speed during most of the sampling campaign except for two days when wind forcing exceeded  $2 \text{ m s}^{-1}$ . Most days exhibited little or no rainfall, but the low pressure system initiated a moderate rainfall event of 28 mm.

### B.4.2 Vertical distribution and seasonal variability of *P. lenticularis*

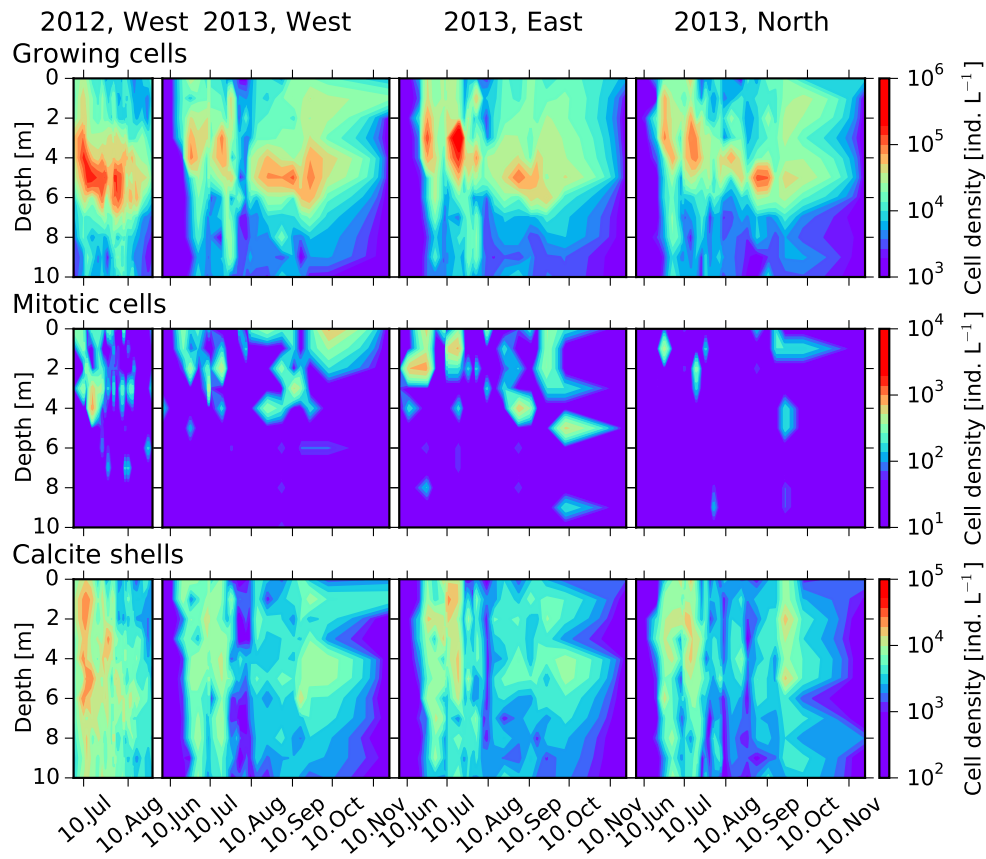
Growing cells were most abundant at about 3 – 4 m depth in June/July and about 5 m depth in August/September, at all stations (Fig. B.6). Maximum GC densities ( $> 70\,000 \text{ Ind. L}^{-1}$ ) occurred in the thermocline, at maximum water



**Figure B.5:** Time series of Secchi depth, air temperature, and wind speed (daily mean), rainfall and global irradiance (daily total) during 27. July – 17. August 2012.

density gradients (Fig. B.4). Layers of maximum GC density varied in thickness between 1 to 3 m over time. Some growing cells were found at all depths to 10 m and presumably deeper. At the end of September, when the thermocline descended, GCs were mixed upwards. Mitotic cells concentrated within the surface mixed layer between 0 to 4 m depth, most of the time clearly above the growing cells. Mean depth of MCs was significantly smaller than mean depth of GCs in 46 out of 66 overall sampling days where MCs were recorded (Welch test with 5% level of significance, Bonferroni corrected by the number of sampling days). MCs were much less abundant than GCs (by more than two orders of magnitude) and showed no distinctive seasonal pattern in vertical distribution. Mitotic cells were uncommon below 6 m depth. Empty calcite shells were found at all depth sampled. CSs were abundant at times of higher growing and mitotic cell densities during June/July and September.

All sampling stations showed three to four peaks in *Phacotus* density. Most cell

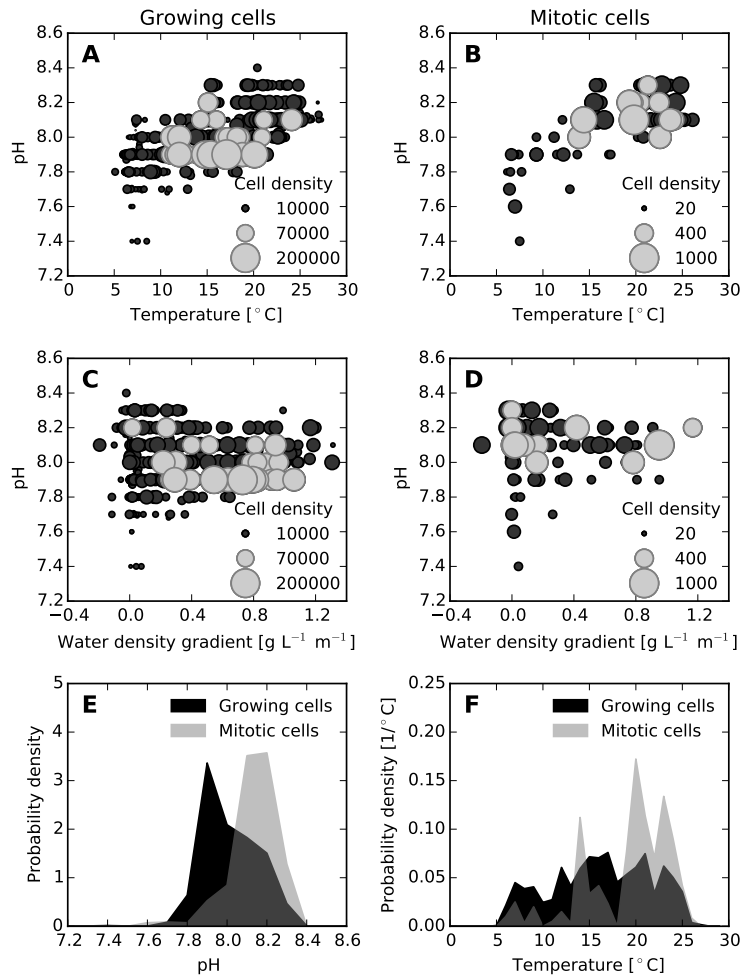


**Figure B.6:** Depth-time contour plots of *Phacotus* growing cell, mitotic cell, and calcite shell distributions at locations 2012 West and 2013 West, East, North.

maxima occurred within the period of pronounced thermal stratification, but the long-living flagellate presented a last peak in September 2013 after an incomplete mixing of the upper 6 m of the water column that likely injected nutrients from the hypolimnion. Hypolimnetic total phosphorus concentrations in lake Ostersee exceeded epilimnetic TP concentrations by twofold by the end of August 2013 ( $TP_{epi} = 9.4 \mu\text{g L}^{-1}$ ,  $TP_{hypo} = 22.8 \mu\text{g L}^{-1}$ ). Water entrainment from deeper water zones into the epilimnetic layer of lake Ostersee could likely have promoted a last *Phacotus* peak.

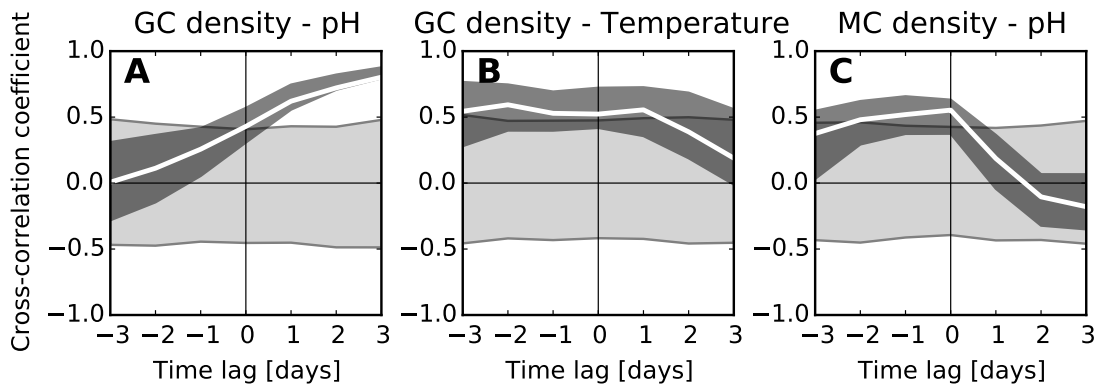
### B.4.3 Relevance of pH, water temperature and density gradient

GC and MC densities from the full data set 2012 and 2013, were plotted against their associated environmental variables (Fig. B.7). The data demonstrate that low densities of GCs and MCs were distributed over a wide range of pH, water



**Figure B.7:** Tolerance ranges of *Phacotus* GC densities (A,C) and MC densities in Ind. L<sup>-1</sup> (B,D) against pH, water temperature, and water density gradients. Circle diameter is proportional to cell density. Grey circles represent highest cell densities (GCs: upper 5%, > 70 000 Ind. L<sup>-1</sup>; less abundant MCs: upper 10%, > 400 Ind. L<sup>-1</sup>). Probability densities of GCs and MCs in dependence on pH and water temperature (E, F, histograms normalized to integral one).

temperature and density gradients, whereas high cell densities aggregated in much narrower pH and temperature ranges. Maximum GC densities (> 70 000 Ind. L<sup>-1</sup>) occurred at a pH range between 7.8 to 8.0 and seldom > 8.0. In contrast, the majority of MCs (> 400 Ind. L<sup>-1</sup>) in lake Ostersee were detected at higher pH from 8.0 to 8.3. The difference between GC and MC distributions along pH gradients was significant (Fig. B.7 E, t-test,  $t=9.5$ ,  $p < 0.001$ ). Water temperature ranges of growing and mitotic cells during peaks were found to partly overlap (GCs: 7 – 25 °C, MCs: 14 – 25 °C). Mitotic cells avoided low temperatures and tended to accumulate near the surface at higher temperatures > 18 °C. The

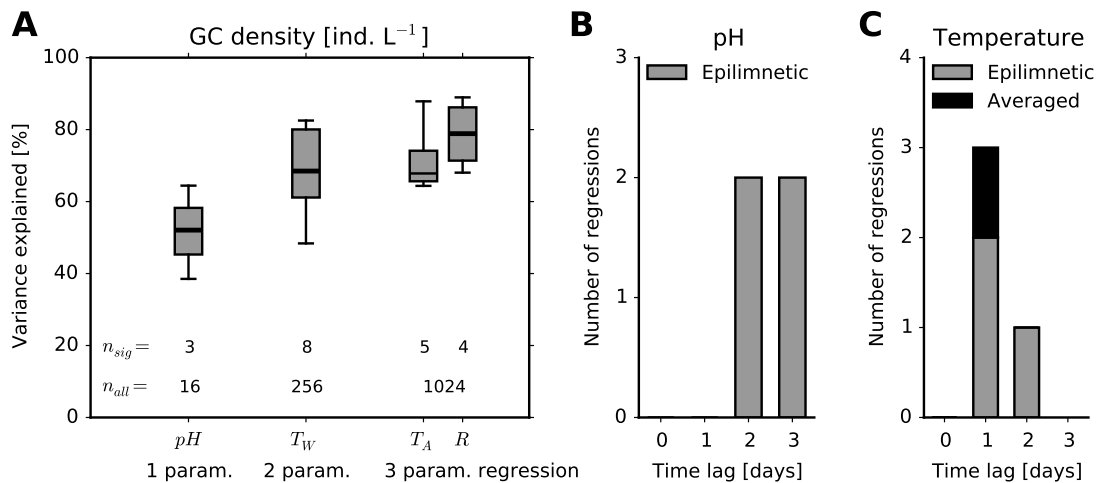


**Figure B.8:** Cross-correlation coefficients computed from the daily time series of 2012 between *Phacotus* GC abundance averaged over the 0 – 10 m water column and epilimnetic pH (A), GC abundance and water temperature (B), and averaged MC abundance and epilimnetic pH (C). The light grey areas are the central 95% of the null hypothesis of no correlation computed from 1000 randomly shuffled data pairs. The dark grey areas mark the 95% confidence intervals computed from a leave-one-out cross validation.

difference between GC and MC distributions along temperature gradients was likewise significant (Fig. B.7 D, t-test,  $t=6.5$ ,  $p < 0.001$ ). We detected no evidence for a preference of either cell-cycle phase to specific water density gradients. Note that the preferences do not necessarily imply a direct causal influence of pH and water temperature on GC and MC vertical distributions.

#### B.4.4 Cross-correlation and multi-variate linear regression analysis

A daily data subset was used to further investigate short-term interactions between densities of cells in the growing and mitotic phase of the cell cycle and lake internal environmental variables from four depth zones as well as meteorological variables. The single physicochemical variable that correlated significantly with depth averaged GC density was epilimnetic pH delayed by at least one day (Fig. B.8). MC density was not significantly correlated with any of the tested variables, likely because of less precise estimates of MC densities resulting from low MC counts in the samples. In line with the correlation analysis, epilimnetic pH was the single significant predictor for GC density in a linear regression analysis (Fig. B.9 A, one parameter regression, upper quartile  $r^2=58\%$ , maximum  $r^2=64\%$ ), and epilimnetic temperature was selected as the sole second significant predictor in addition to pH in the regression analysis (Fig. B.9 A, two parameter regression, upper quartile  $r^2=80\%$ , maximum  $r^2=83\%$ ). The strongest three pa-



**Figure B.9:** Multivariate linear regression analysis of *Phacotus* abundance. A: Box (quartiles) and whisker (minimum and maximum) plot summarizing significant predictors of depth averaged GC abundance from a step-wise multivariate linear regression analysis (TW = water temperature, TA = air temperature, R = rainfall, see methods). B, C: distribution of time lags and depth specific variants of the best (top 50%) two-parameter models with pH and water temperature as independent variables.

parameter regression model included epilimnetic pH, epilimnetic temperature, and rainfall, and explained maximally 89% of the variance in GC density (Fig. B.9 A, three parameter regression). In addition to rainfall, air temperature was the only other significant third parameter selected by the regression analysis, but model predictions were on average not improved. Time lags of up to three days for *Phacotus* response times were required for the regression analysis to generate significant explanatory power of the models. *Phacotus* responses followed pH variations with a delay of 2 – 3 days, whereas responses to water temperature were faster with time lags of 1 – 2 days (Fig. B.9 B, C). The best 50% of all significant two-parameter models used epilimnetic pH and temperature time series, with the exception of one model that uses water temperature averaged over 10 m depth. Interestingly, only mitotic cells concentrated in the epilimnetic layer while growing cells accumulated in the thermocline below MC aggregations (Fig. B.4 and Fig. B.6). However, neither predictor variable we used explained a significant portion of variance in mitotic cell density, likely because MCs were much less abundant than GCs. Considering the vertical distribution patterns of MCs and GCs, our data suggest that epilimnetic pH and temperature may act on mitotic cell density and subsequently influence growing cell density.

## B.5 Discussion

Our study aimed to establish how vertical distribution patterns of cell-cycle phased *P. lenticularis* cells are related to environmental characteristics that promote calcification and minimize sinking rates. Our hypothesis was, that motile growing cells and non-motile mitotic cells may show distinct vertical distributions. The study further sought to better understand and predict seasonal patterns in GC and MC vertical distribution and density. We persistently found maximum concentrations of GCs in the thermocline at maximum water density gradients and lower pH and temperatures. The depth distribution of the thermocline clearly controlled the shape of the vertical profiles of growing cells throughout the stratified period in the survey years 2012 and 2013. Depth distribution of growing *Phacotus* cells was similar to that found for other flagellates and chlamydomonads (Klausmeier and Lichtman, 2001, Clegg et al., 2007). The preference of many chlamydomonads for moderate O<sub>2</sub> and CO<sub>2</sub> concentrations may further account for their frequent occurrence deeper in the water column (Jones, 1988; Tittel et al., 2005; Striebel et al., 2009). The extended sampling campaign in 2013 demonstrated that *Phacotus* is not entirely dependent on strong thermal stratification. Initial mixing of the upper water column in autumn tends to homogenize *Phacotus* vertical profiles, but entrainment of colder, nutrient enriched hypolimnetic water can lead to accelerated growth and peak development of the population late in the season if pH remains above 8.1. Vertical distributions of mitotic cells showed no distinct seasonal pattern. High MC densities were found in the illuminated, mixed surface layer at elevated pH and water temperature. MC distributions were patchy, regularly accumulating very near the lake surface and also near the upper boundaries of the thermocline at 4 m depth but seldom below. Horizontal turbulence and advection in the epilimnion tend to homogenize phytoplankton distribution (Marce et al., 2007; Serra et al., 2007). Aggregations of high mitotic cell concentrations occurred mostly during calm weather conditions and may indicate either passive accumulations or direct movements of flagellated *Phacotus* cells towards specific layers in the water column prior to mitosis and cell division (Reynolds 1976). We repeatedly found maximum accumulations of mitotic cells clearly above layers of growing cells suggesting that *Phacotus* performs an upward migration prior to mitosis. Migrating upwards to turbulent layers will reduce the possibility of sinking out of the photic zone when motility ceases during cell division (Reynolds, 2006). *Phacotus* cells will benefit from this behaviour by extending their presence in the photic zone. We found distributional responses

of growing and mitotic cells to vertical pH and water temperature gradients during peak development. The results refine previous studies that demonstrated an association between *P. lenticularis* density and higher pH and temperature values in lakes (Schlegel et al., 1998). In lake Ostersee, the epilimnion showed the highest pH values, which decreased with depth. An increase in pH shifts the  $\text{HCO}_3^- / \text{CO}_3^{2-}$  equilibrium, causing  $\text{CaCO}_3$  supersaturation (Brunskill, 1969; Stabel, 1986). Consequently, calcification is favoured in the epilimnetic layer of the water column. During our morning sampling campaigns we generally found mother cells that incorporated already calcified daughter cells indicating that DNA replication and cell division took place in the absence of higher light intensities. According to experimental studies of Giering et al., 1990, *Phacotus* cell division and calcification of daughter cells takes approximately 7 hours to complete, whereas growth in biomass can last between 1-3 days. To our knowledge, no studies exist so far on at what time of day calcification occurs or whether cell division of *P. lenticularis* is phased to a diel cycle. However, a vast number of studies have demonstrated that many flagellates migrate vertically and divide on a light-dark cycle (Cullen et al., 1985; Watanabe et al., 1991; Figueroa et al., 1998; Van Dolah and Leightfield, 1999; Fauchot et al., 2005; Townsend et al., 2005).

Our daily data set provides evidence that changes in epilimnetic pH and temperature may be important drivers determining the temporal dynamics of growing cell density in the thermocline. A multivariate linear regression analysis demonstrated that GC density increased as a positive function of pH and temperature in the epilimnetic layer. Only mitotic cells concentrated regularly very near the surface of the epilimnion while growing cells aggregated below in the thermocline. The results suggest that conditions that promote development and liberation of calcified daughter cells, are important factors for growing cell density. The regression analysis also suggests that among the meteorological variables rainfall and air temperature are additional triggers of increased GC density. Previous studies have demonstrated that increased Chla concentrations were related to elevated nutrient loading from the catchment following local rainfall events (Fee et al. 1992, Mallin et al. 1993). Catchment soil composition and intensity of precipitation are likely to play an important role in mediating the impact of rain events on the hydrology and nutrient export to lakes and thus on phytoplankton productivity (Jennings et al. 2009; 2012). Surface and epilimnetic water temperatures can be highly correlated to regional-scale air temperatures and will reflect warming trends in the air (De Stasio et al., 1996; Livingstone and Lotter, 1998).



Given the temperature dependence of a number of biological processes, phytoplankton growth and cell division are generally very sensitive to air temperature changes over a variety of temporal scales (Sorokin and Kraus, 1965; Davison, 1991; Butterwick et al., 2005).

In summary, this survey provides evidence that *Phacotus* cells are able to differentially use spatial heterogeneity in resources during their cell cycle through active motility. Prior to loss of motility in the early cell division phase, motile growing *Phacotus* cells migrate upwards into water layers of increased turbulence, pH and water temperature. Growing cells depend on their formation from dividing cells, and vice versa. Environmental conditions that promote cell division and calcification, and reduce sinking rates are beneficial for non-motile mitotic *Phacotus* cells and hence translate into a response of growing *Phacotus* cell density.

## B.6 Acknowledgements

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Dissertation an der Mathematisch-Naturwissenschaftlichen Fakultät der Humboldt-Universität zu Berlin: 98pp.

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