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Growth responses of the calcite-loricated freshwater phytoflagellate *Phacotus lenticularis* (Chlorophyta) to the CaCO_3 saturation state and meteorological changes

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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_3 saturation state may influence the abundance and phenology of *P. lenticularis* has not been investigated so far. We measured, biweekly to daily, algal abundance, CaCO_3 saturation, pH, water temperature and nutrient status in three hard-water lakes that varied in lake morphology and wind exposure. Our data provide evidence that increasing supersaturation of 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 CaCO_3 saturation state and was the highest in the most wind exposed lake. *Phacotus lenticularis* abundance and epilimnetic CaCO_3 supersaturation were significantly positively correlated at a time lag of about 1 day. Wind caused an immediate decline in epilimnetic pH followed by a decline in CaCO_3 saturation about 2 days later. Solar irradiance positively affected both pH and water temperature, but water temperature changes were insufficient to

produce a significant CaCO₃ saturation response. We conclude that the CaCO₃ saturation state is a determinant factor driving the timing of *P. lenticularis* peaks in alkaline lakes.

KEYWORDS: CaCO₃ saturation; meteorological variability; *Phacotus lenticularis*; onset exponential growth; alkaline lakes

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 for productivity, resource flow and ecosystem functioning. Special attention is drawn to phytoplankton species that calcify, as these are significant contributors to 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 hard-water lakes of a wide trophic range, making up to 5–36% of the overall phytoplankton biomass during peak development (Cambra Sánchez *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 CaCO₃ (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; Freytag 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*. *Phacotus 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 *et al.*, 1998, 2000a,b). *Phacotus* lorica can significantly add to the accretion of new CaCO₃ in lake sediments (Müller and Oti, 1981; Bluszczyk *et al.*, 2009; Jouve *et al.*, 2012).

Supersaturation with respect to Ca²⁺ and HCO₃⁻ 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, 1996). When the ionic activity product of calcium and carbonate in lake water exceeds the solubility product, the system is supersaturated and precipitation of CaCO₃

occurs. The rates of CaCO₃ 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 CaCO₃ supersaturation by decreasing the calcite solubility product (Plummer and Busenberg, 1982; Langmuir, 1997). Highly dynamic demands for CO₂ by phytoplankton and exchange of CO₂ between atmosphere and lake surface govern epilimnetic pH and carbonic ion concentrations, thereby causing complex shifts in the CaCO₃ saturation state of the water (Otsuki 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 CaCO₃ supersaturation occurs within a physiological tolerance limit (Hepperle and Krienitz, 1996, 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 CaCO₃ supersaturation in the cultural medium, primary production did not (Schlegel *et al.*, 2000a,b; Schlegel, 2001). Non-calcified cultured strains grew with equally high photosynthetic rates in CaCO₃ 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 CaCO₃ 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 (Quintero-Torres *et al.*, 2006; Gao *et al.*, 2009, 2012).

The goal of this study was to determine how variability in epilimnetic CaCO₃ supersaturation influences the

Table I: Physical, chemical and biological characteristics of the surface layer (0–5 m) of all studied lakes during May till August

	Ostersee	Breitenauersee	Eishaussee
Circulation type	dimictic	dimictic	meromictic
Surface area (km ²)	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 ⁻¹)	4.44 (±0.01)	4.46 (±0.01)	4.39 (±0.01)
Ca ²⁺ concentration (mmol L ⁻¹)	1.5 (±0.2)	1.4 (±0.2)	1.4 (±0.2)
Total phosphorus (μmol L ⁻¹)	0.3 (±0.1)	0.2 (±0.1)	0.3 (±0.1)
Soluble reactive phosphorus (μmol L ⁻¹)	Not detectable	Not detectable	Not detectable
NO ₃ ⁻ -N (mmol L ⁻¹)	0.09 (±0.01)	0.08 (±0.01)	0.04 (±0.01)
NH ₄ ⁺ -N (μmol L ⁻¹)	2.2 (±0.006)	1.6 (±0.007)	8.9 (±0.005)
Chlorophyll <i>a</i> (μmol L ⁻¹)	0.005 (±0.003)	0.004 (±0.002)	0.008 (±0.003)

seasonal periodicity of *P. lenticularis* in three oligotrophic hard water lakes that differ in morphology, wind exposure and to some extent in water chemistry. *Phacotus 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 *et al.*, 1998), but the link to CaCO₃ saturation is not yet well understood.

One specific objective of this study is to investigate whether CaCO₃ 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 CaCO₃ 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 CaCO₃ saturation indices in the lake water.

Variability of epilimnetic pH and CaCO₃ saturation is governed to a large extent by photosynthetic CO₂ 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 water carbonate chemistry and how they drive *P. lenticularis* growth dynamics.

METHOD

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 biological characteristics of lakes Ostersee, Breitenauersee and Eishaussee is shown in Table I.

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 m 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 m depth. A rapid decline of *P. lenticularis* cell numbers was observed below the thermocline, at ~5 m depth.

Subsamples for quantitative analysis of algal abundance were preserved with Lugol's solution. Cells were enumerated by inverted microscope at ×200–400 (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⁻¹) in the epilimnetic zone (0–5 m) was calculated from the counts.

Water temperature, pH and electrical conductivity depth profiles were measured at 1 m intervals from 0 to

5 m 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 m for further laboratory analysis from 30 May to 08 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 (Ca²⁺, Mg²⁺, K⁺, Na⁺, Cl⁻, F⁻, NO₃⁻ and SO₄²⁻) (Dionex Dx-120). Weekly measurements of total phosphorus (TP), soluble reactive phosphorus (SRP), nitrate-nitrogen (NO₃⁻-N) and ammonium-nitrogen (NH₄⁺-N) provided information on the nutrient status of the lakes. The samples were analysed spectrophotometrically following standard methods (DEV, 2013 for TP and NH₄⁺-N; Murphy and Riley, 1962 for SRP; Navone, 1964 for NO₃⁻-N).

The CaCO₃ saturation index Ω (Berner, 1971) was calculated using an input program WinIAP (Sequentix, 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 rainfall recorded at the meteorological station Rothenfeld (maintained by the Ministry of Bavaria, <http://www.lfl.bayern.de/agm/daten.php?statnr=80>) for the period 13 April–29 August 2011 were selected and used for further statistical analysis.

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 λ_i for a time interval t_i to t_{i+1} of successive measurements according to

$$\lambda_i = \log_e(N_{i+1}/N_i)/(t_{i+1} - t_i)$$

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, λ_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 λ_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 λ_i was fitted to this period in order to demonstrate the progress of exponential growth (dashed lines in Fig. 3).

Auto-correlations and cross-correlations between *P. lenticularis* abundance, CaCO₃ saturation, pH, water temperature and local meteorological variables were analysed for a period of 19 daily measurements starting on 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.

RESULTS

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. 1). Maximum peak abundance ranged from 106 300 Ind. L⁻¹ in lake Ostersee to 48 320 Ind. L⁻¹ in lake Breitenauersee, and 38 360 Ind. L⁻¹ 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 m. Water temperature in the epilimnetic layer increased through the season from 10.8–22.6°C (average $18.7 \pm 1.2^\circ\text{C}$) in lake Ostersee, 10.6–21.7°C (average $18.9 \pm 1.3^\circ\text{C}$) in lake Breitenauersee and 10.8–22.5°C (average $19.8 \pm 1.5^\circ\text{C}$) 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.

The lakes remained supersaturated with respect to CaCO₃ throughout the sampling season. The CaCO₃ saturation index Ω ranged from 2.8–6.8 (average 4.6 ± 1.2) in lake Ostersee, 2.7–6.6 (average 4.6 ± 1.1) in lake

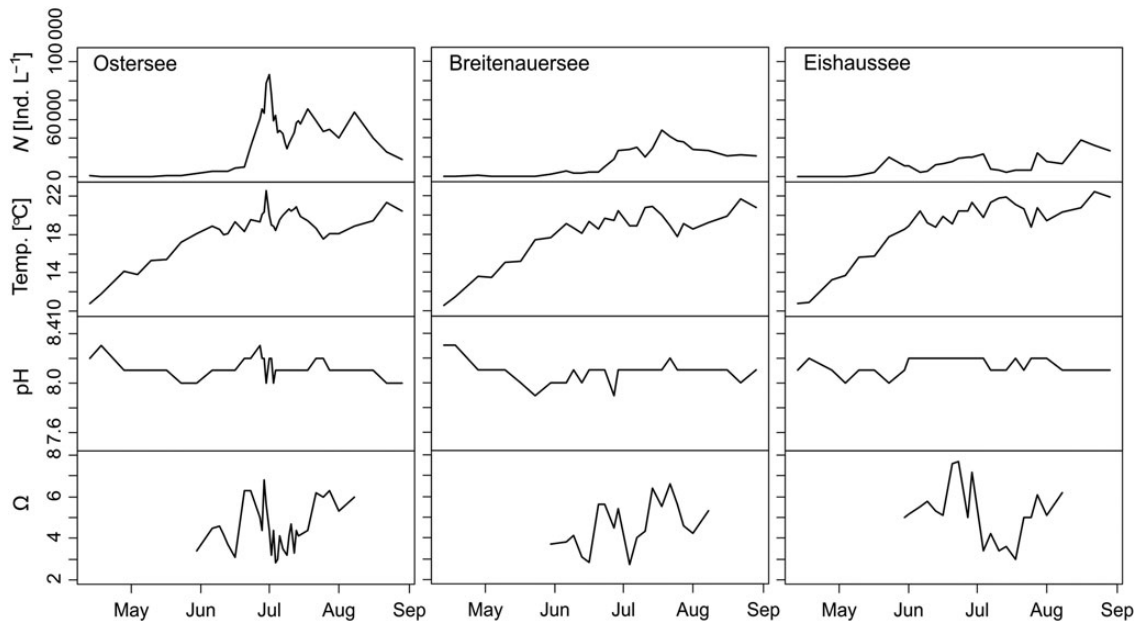


Fig. 1. Seasonal variations in cell density, water temperature, pH value and calcite saturation index (Ω) in the stratified upper 5 m water layer during April–August 2011. The values represent midmorning measurements.

Breitenauersee and 3.0–7.7 (average 5.2 ± 1.4) in lake Eishaussee.

The general trophic state of the lakes is oligotrophic (Table 1). Mean concentrations of TP were $0.3 \mu\text{M L}^{-1}$ in lake Ostersee, $0.2 \mu\text{M L}^{-1}$ in lake Breitenauersee and $0.3 \mu\text{M L}^{-1}$ in lake Eishaussee. SRP was not detectable throughout the sampling period. Concentrations of nitrate-nitrogen (mean values $42.8\text{--}92.9 \mu\text{M L}^{-1}$), the main source of nitrogen in the lakes and ammonium-nitrogen (mean values $1.6\text{--}8.9 \mu\text{M nitrate-N L}^{-1}$) were combined a 100-fold higher than concentrations in TP corresponding to classical P limiting conditions for algal growth in the three lakes. Overall, there was little seasonal fluctuation in nutrient concentrations.

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 m s^{-1} , 0.9 mm and $5\,974.4 \text{ J m}^{-2}$, respectively) (Fig. 2). From 6 June to 22 July, the passage of several atmospheric low pressure systems defined a period of increased rainfall (average 7.4 mm) and initiated a heavy rainfall event of 43.3 mm on 30 June. Wind speed increased slightly (average 1.2 m s^{-1}) and light availability was variable but remained high (average 4982.4 J m^{-2}). Weather conditions during the late-summer season (end July–end August) were again characterized by low rainfall, a small

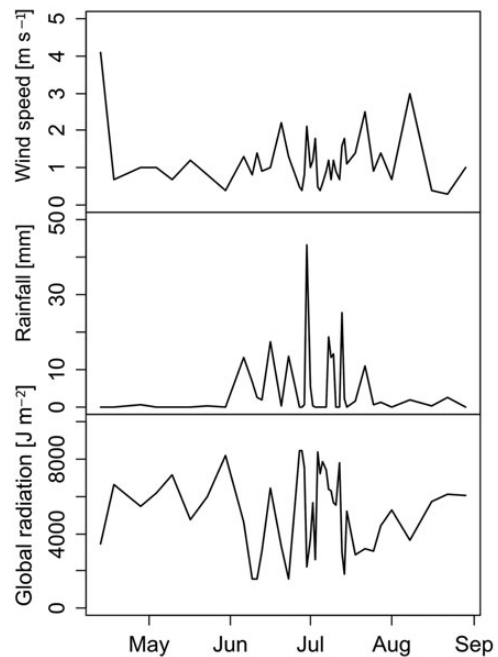


Fig. 2. Meteorological time series of wind speed (daily mean), rainfall and global radiation (daily total) during April–August 2011.

decrease in light availability and variable but increasing wind speed (average 1.9 mm , $4\,317.9 \text{ J m}^{-2}$ and 1.3 m s^{-1} , respectively). The 2011 growing season showed lower rainfall and wind speed rates, but higher solar radiation rates, when compared with average conditions between 1991 and 2011 (Meteorological station Rothenfeld).

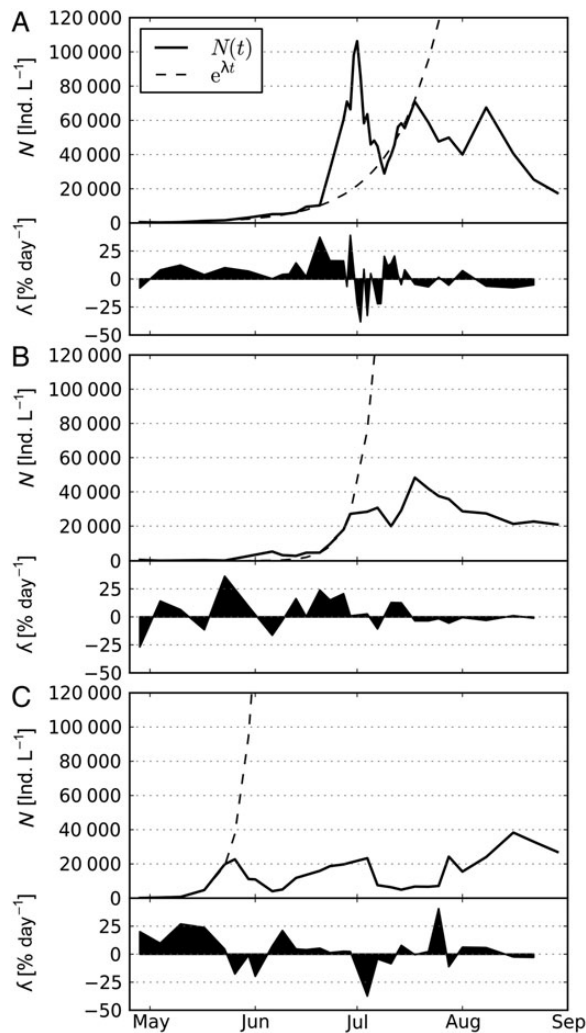


Fig 3. Population growth of *Phacotus lenticularis* in lakes Ostersee (A), Breitenauersee (B) and Eishaussee (C). The temporal evolution of cell numbers (upper panels, solid line) over the measurement period starting on 28 April and net growth rates (lower panels) estimated according to equation (1) 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 $7\% \text{ day}^{-1}$ for lake Ostersee, $23\% \text{ day}^{-1}$ for lake Breitenauersee and $20\% \text{ day}^{-1}$ for lake Eishaussee.

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. 3). Growth rates fluctuated around zero prior to the bloom, whereas after the onset of exponential growth, net growth rates averaged 7% for about 42 days. Around 20 June, the CaCO_3 saturation index Ω showed a two-fold increase within 4 days from 3.1 to 6.3 followed by a five-fold increase in *P. lenticularis* net growth from 7 to 37% (Figs 1 and 3). Water temperature had steadily increased from the beginning of the sampling period but

Table II: Parameters of water temperature, pH and Ω values at the onset of Phacotus exponential growth

	Water temperature ($^{\circ}\text{C}$)	pH value	Calcite saturation index Ω
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)

dropped slightly from 19.3 to 18.3°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. 1, Table II).

Phacotus lenticularis growth rates in lake Breitenauersee fluctuated frequently between positive and negative values early in the sampling period (Fig. 3). Enhanced exponential growth at average net growth rates of 23% per day started on the same day as in lake Ostersee (20 June) following a two-fold increase in CaCO_3 saturation from 2.8 to 5.6. The results suggest that there is a possible area of interaction between saturation index Ω 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. 1, Table II).

The earliest onset of *P. lenticularis* exponential growth at an average growth rate of 20% was detected in lake Eishaussee around 28 April (Fig. 3). Water temperature and pH threshold levels for the start of exponential growth were 13.3°C and 8.1, respectively (Table II). Ω values, which 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. Ω values remained at levels of around 5 between late May and mid-June, but further increased to 7.6 around 20 June.

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 CaCO_3 saturation (Table III, Fig. 4). An increase in Ω values was followed by an increase in net growth at lags of 1–3 days

*Table III: Significant cross-correlation values (r_{XY}) and corresponding time lags between *Phacotus* population size (N), net growth rates (μ), calcite saturation index (Ω), pH values, wind speed (WS) and global radiation (GB)*

Signal 1	Signal 2	Time (d) derived from the r_{XY} lags	Significant cross-correlation values (r_{XY})
N	Ω	1	0.7745
		2	0.7267
		3	0.6262
N	pH	3	0.5994
μ	Ω	0	0.5946
Ω	pH	2	0.6489
Ω	WS	2	-0.5435
pH	WS	0	-0.5518
pH	GR	0	0.5000
T	GR	2	0.5623
		3	0.5580

27 June–15 July 2011 in lake Ostersee. Signal 1 was shifted along the x-axis to determine maximum match between signals.

(Fig. 4), while its abundance was likely to decline when CaCO_3 saturation indices declined. The extended period of significant cross-correlation values over several days probably reflects the fact that the abundance and Ω time series are also correlated with their own past and future values. Changes in CaCO_3 saturation explained 60% of the variability in abundance at lag 1 (Fig. 5A). Net growth rates were directly positively correlated with calcite saturation indices. The two results suggest that *P. lenticularis* is sensitive to changing CaCO_3 supersaturation.

Variations in pH explained 44% of Ω changes at lags of 2 days (Figs 4 and 5). Confirming the relationships between pH, Ω and net growth, pH was positively correlated with abundance at lags of 3 days. The relation between Ω 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 (Table IV).

Among the climate variables examined, wind caused a significant decline in pH and Ω levels in the epilimnetic

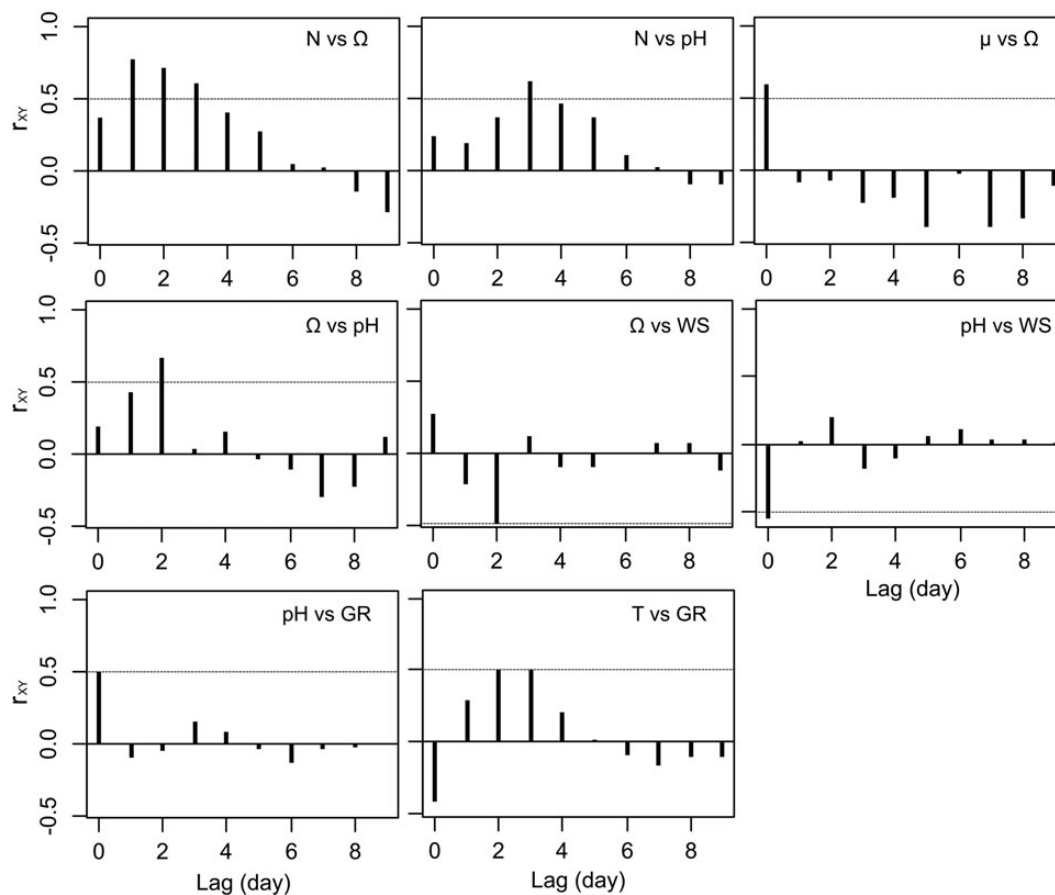


Fig. 4. Cross-correlation correlograms (r_{XY} cross-correlation coefficients) computed from bivariate time series between *Phacotus* population size (N), net growth rates (μ), calcite saturation index (Ω), pH values in lake Ostersee as well as wind speed (WS) and global radiation (GB). Time lags correspond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

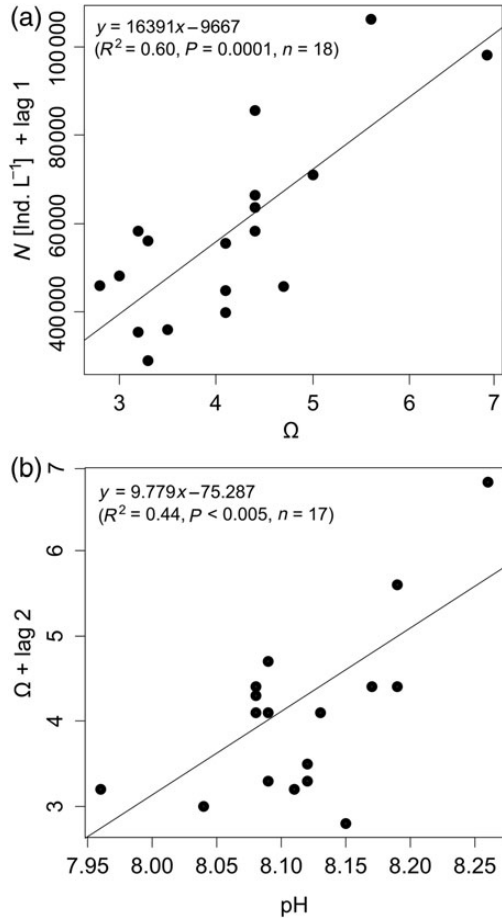


Fig. 5. *Phacotus* abundance as a function of changes in the CaCO_3 saturation index (Ω) (a), and CaCO_3 saturation as a function of changes in pH (b). Solid lines indicate the best linear fit to the data points.

layer at time scales of 0 and 2 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 variables tested, 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 Ω 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 CaCO_3 saturation was only observed during the early phase of algal growth in lake Ostersee, the extended data set at longer time intervals yielded significant positive correlations between N and Ω for the main part of the *P. lenticularis* growing season in lakes Breitenauersee and Eishaussee (Table IV). A positive correlation

Table IV. Significant cross-correlation values (r_{XY}) and corresponding time lags between *Phacotus* population size (N), net growth rates (μ), calcite saturation index (Ω), pH values, wind speed (WS), global radiation (GB) and rainfall (R)

Signal 1	Signal 2	Time (3–4 days) derived from the r_{XY} lags	Significant cross-correlation values (r_{XY})
Ostersee			
N	T	0	0.5478
T	WS	1	−0.4846
T	GR	1	0.6881
Breitenauersee			
N	Ω	1	0.5941
Eishaussee			
N	Ω	1	0.6786
N	pH	0	0.6389
		1	0.7573
Ω	pH	0	0.5071
		1	0.5876
T	R	1	−0.6184

06 June–08 August 2011 in lakes Ostersee, Breitenauersee and Eishaussee.

between N and pH, as well as between Ω and pH was still visible at enhanced time lags for lake Eishaussee.

DISCUSSION

We had hypothesized that *P. lenticularis* potentially benefits from increasing 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 CaCO_3 supersaturation would exceed the metabolic costs for shell formation.

This study gives first evidence that increasing CaCO_3 supersaturation mediates the onset of *P. lenticularis* growth and promotes peak development during the growing season. Our data demonstrate that a two-fold increase in CaCO_3 saturation indices was immediately followed by an up to five-fold 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 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 CaCO_3 saturation is positive and linear at Ω 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 CaCO_3 supersaturation is sufficiently high. Total cell concentration in the water column seems not to be dependent on the degree of CaCO_3 supersaturation as we found the highest cell concentrations in lake Ostersee, which showed lower CaCO_3 saturation indices than lake Eishaussee. All the lakes studied 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 lakes studied, net growth rates (μ) during the exponential growth phase averaged between 0.35 and 0.69 day^{-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 day^{-1} were observed (Schlegel *et al.*, 2000a,b). Mean growth rates of chlorophyte species in culture experiments were found to reach 0.62 day^{-1} at 20°C, comparable with our maximum growth rates (Lürling *et al.*, 2013). Further experimental studies demonstrated that *P. lenticularis* cultures (without calcite-shell development) attained inherently low biomass and P:C ratios compared with other chlorophyte species (Striebel *et al.*, 2009).

While laboratory studies suggest that the flagellate is relatively slow growing, our data from three oligotrophic hard-water lakes indicate that *P. lenticularis* is capable of accelerated growth during several short windows of increasing 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 light demand that was observed in *P. lenticularis* cultures (Striebel *et al.*, 2009). In field studies, *P. lenticularis* blooms are 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; Lürling *et al.*, 2013). This study provides evidence that regional-scale climate-induced changes in pH and CaCO_3 supersaturation are important for 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 CaCO_3 saturation.

The response time of Ω to changes in epilimnetic pH is likely to be lake-specific and to vary seasonally. The solubility equilibrium of 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 (Lowenthal and Marais, 1976; Hodell *et al.*, 1998; Reddy and Hoch, 2012). Solar irradiance controls net lake productivity, while wind activity tends to increase the 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 CO_2 concentration determines the 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, CO_2 and nutrients from below. The effects on *P. lenticularis* growth could be the opposite in a low productivity alkaline lake. Neither future increase in surface water temperature or ultraviolet radiation are likely to negatively impact this flagellate owing to its ability to build a calcite shell and prevent sedimentation by flagellar motility in warmer and more strongly stratified waters. *Phacotus 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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