

## Research



**Cite this article:** Andersen MR, Kragh T, Sand-Jensen K. 2017 Extreme diel dissolved oxygen and carbon cycles in shallow vegetated lakes. *Proc. R. Soc. B* **284**: 20171427. <http://dx.doi.org/10.1098/rsob.2017.1427>

Received: 26 June 2017

Accepted: 7 August 2017

**Subject Category:**

Ecology

**Subject Areas:**

biochemistry, ecology

**Keywords:**

shallow vegetated lakes, charophytes, recurring stratification-mixing, daytime anoxia, diel dissolved oxygen cycles, diel carbon cycles

**Author for correspondence:**

Mikkel R. Andersen

e-mail: [mikkel.andersen@dkit.ie](mailto:mikkel.andersen@dkit.ie)

Electronic supplementary material is available online at <https://dx.doi.org/10.6084/m9.figshare.c.3854731>.

# Extreme diel dissolved oxygen and carbon cycles in shallow vegetated lakes

Mikkel R. Andersen, Theis Kragh and Kaj Sand-Jensen

Freshwater Biological Laboratory, Biological Institute, University of Copenhagen, Universitetsparken 4, 2100 Copenhagen, Denmark

MRA, 0000-0003-2104-2894

A common perception in limnology is that shallow lakes are homogeneously mixed owing to their small water volume. However, this perception is largely gained by downscaling knowledge from large lakes to their smaller counterparts. Here we show that shallow vegetated lakes (less than 0.6 m), in fact, undergo recurring daytime stratification and nocturnal mixing accompanied by extreme chemical variations during summer. Dense submerged vegetation effectively attenuates light and turbulence generating separation between warm surface waters and much colder bottom waters. Photosynthesis in surface waters produces oxygen accumulation and CO<sub>2</sub> depletion, whereas respiration in dark bottom waters causes anoxia and CO<sub>2</sub> accumulation. High daytime pH in surface waters promotes precipitation of CaCO<sub>3</sub> which is re-dissolved in bottom waters. Nocturnal convective mixing re-introduces oxygen into bottom waters for aerobic respiration and regenerated inorganic carbon into surface waters, which supports intense photosynthesis. Our results reconfigure the basic understanding of local environmental gradients in shallow lakes, one of the most abundant freshwater habitats globally.

## 1. Introduction

Small, shallow lakes are extremely abundant everywhere around the world in both natural [1,2] and cultivated landscapes [3,4]. They are vastly more abundant than the larger, deeper lakes [5] that have shaped our view on environmental variability [6,7], evolutionary processes [8], regulation of food webs and carbon dynamics [8,9] for the entire size range of lakes. Small shallow lakes are expected to show extensive seasonal and diel variations in temperature, oxygen and CO<sub>2</sub> because of their small water volume and intimate contact with sediments and water plants [10]. Moreover, small lakes display much greater inter-lake variability of physical, chemical and biological conditions than large lakes [9,11,12]. As a consequence, small lakes support a higher combined species richness of water plants and macroinvertebrates compared to larger lakes or streams within catchments [8,12].

In particular, there is one research area in which the lack of studies has led to skewed perceptions of the ecological characteristics of small, shallow lakes. Small lakes have usually been assumed to be well mixed and exhibit limited chemical variability as a consequence of the shallow water column; however, there is little justification [13,14]. For this reason, small lakes have often been chemically characterized by a single, daytime water sample of surface waters. Nevertheless, recent studies document that stratification-mixing patterns can be very complex in shallow lakes with dense macrophyte stands thus causing extensive attenuation with water depth of both the light flux and the wind-driven turbulence [13,15]. In addition to the high diel variability of temperature, oxygen, CO<sub>2</sub> and pH in small, shallow lakes, the hypothesis is that recurring daily stratification-mixing patterns [15] generate profound vertical variability between surface and bottom waters, and enforce diel variability in surface and bottom waters if the two water layers become isolated from the moderating effects of vertical mixing owing to daytime temperature stratification. Photosynthetic processes alone would then determine the chemical development in surface waters, while respiratory processes alone would determine the chemical development in bottom waters. This could represent a challenge for growth and survival of sessile

**Table 1.** Mean vertical temperature differences in the water column as well as mean oxygen saturation in the surface- and bottom water during day and night at the deepest site in four small vegetated lakes in a 24-h period in early-June on the open alvar of Öland, Sweden. Surface area, maximum water depth and mean charophyte biomass are also shown. Lake 1 is the intensely studied lake.

lake no.	area m <sup>2</sup>	depth m	charophyte biomass	temp diff. (day)	temp diff. (night)	O <sub>2</sub> saturation (day)		O <sub>2</sub> saturation (night)	
			g DW m <sup>-2</sup>	°C	°C	surface %	bottom %	surface %	bottom %
1	600	0.27	796	10.6	-0.5	163.0	0.7	33.6	20.7
2	75	0.37	813	6.0	-0.5	176.1	0.2	51.6	12.4
3	390	0.24	319	9.6	-1.6	142.9	0.1	54.8	8.4
4	2500	0.41	462	9.0	-1.1	172.0	0.7	58.4	20.7

animals, benthic macroalgae and rooted plants which have to withstand the highly variable environmental conditions.

With technical advancements of sensors and loggers which can measure temperature, light, oxygen, pH, conductivity, and water level continuously, it is possible to determine the stratification-mixing patterns along micro-gradients and test how extensive the variability of gases and solutes is in shallow lake habitats. By studying day-to-day dynamics, the four specific objectives were to determine: (i) vertical stratification and mixing patterns, (ii) diel extremes of temperature, oxygen, pH and inorganic carbon in surface and bottom waters, (iii) loss of dissolved inorganic carbon (DIC) by photosynthesis and calcification (i.e.  $\text{Ca}^{2+} + 2\text{HCO}_3^- + \text{H}_2\text{O} \rightarrow \text{CaCO}_3$  (precipitated) +  $\text{CO}_2$  (assimilated) +  $\text{O}_2$ ) in surface waters as well as the replenishment of DIC by respiration and dissolution of  $\text{CaCO}_3$  by the reverse processes in bottom waters [16], and (iv) if the highly dynamic environment challenged the survival of charophytes or handed them a competitive advantage.

These objectives were addressed by measuring environmental conditions at high temporal and vertical resolution in four shallow, oligotrophic lakes with dense covers of characean macroalgae on the open south-Swedish alvar during spring and summer. In addition, the reproducibility of diel variations in temperature and oxygen in surface and bottom waters is reported. By studying diel variations in the vertical distribution of light, oxygen, sulfide, ferrous iron, calcium, total, and individual inorganic carbon species ( $\text{DIC} = \text{CO}_2 + \text{HCO}_3^- + \text{CO}_3^{2-}$ ) their coupling to photosynthesis and respiration were determined. Realizing that  $\text{HCO}_3^-$  is the main determinant of acid neutralizing capacity ( $\text{ANC} = \text{HCO}_3^- + 2\text{CO}_3^{2-} + \text{OH}^- - \text{H}^+$ ) and closely linearly related to conductivity (electronic supplementary material, figure S1), ANC was continuously calculated from conductivity measurements. The determination of pH, ANC and temperature permitted calculation of DIC as well as all three inorganic carbon species. With the determination of  $\text{CO}_3^{2-}$  and calcium, evaluation of the precipitation and dissolution of  $\text{CaCO}_3$  was conducted on a continuous basis. We broaden the perspectives by comparisons with the chemical and hydrological dynamics of larger and deeper lakes with different stratification and mixing patterns.

## 2. Material and methods

The study was conducted in four small, shallow lakes (table 1) located in the sparsely vegetated calcareous grassland around Greby on Öland, Sweden (56.81168° N, 16.6094° E) [10]. The

lakes had variable surface area (75–2500 m<sup>2</sup>), water depth (0.24–0.41 m) and charophyte biomass (319–813 g dry weight (DW) m<sup>-2</sup>; table 1). Water depths were measured in grids across the lakes. Water levels were measured at 10 min intervals by comparing submerged pressure data loggers in each of the investigated lakes (HOBO U-20-001-04, Onset Computers, Bourne, MA, USA) to a similar logger in air. This allowed high-frequency calculations of water depth, surface area and water volume in each lake, while correcting for atmospheric changes in barometric pressure. In the intensely studied lake 1, surface area varied from 630–745 m<sup>2</sup> in late May to 825–859 m<sup>2</sup> in mid-August; maximum depth varied from 0.30 to 0.46 m. According to methods described previously [15], meteorological parameters (incident light, wind speed and temperature) were measured next to the lakes at 2.0 m above the ground. Vertical profiles of light and temperature were measured at the deepest site in the lakes at 5 cm depth intervals at 10 min intervals using temperature-light sensors (HOBO UA-002-64, Onset Computers). Depth profiles of dissolved oxygen (MiniDOT sensors, PME, Vista, Ca, USA) and pH (pH-Temp2000 MadgeTech, Warner, NH, USA) were measured at 7 cm depth intervals every 10 min. Oxygen sensors were placed in anoxic and 100% oxygen saturated water before and after deployment. pH sensors were calibrated in standard buffer solutions at pH 4.0 and pH 7.0 and checked in the same buffer solutions after deployment. Neither the oxygen- nor the pH sensors showed any drift. Conductivity was measured at 10 min intervals (HOBO U24-001, Onset Computers) and corrected to 20°C. Depth profiles of the photosynthetic active radiation (PAR, 400–700 nm) were measured with a small spherical quantum sensor (Waltz, Switzerland).

A vertical steel peg was installed in the centre of lake 1 in order to measure vertical profiles of solutes. Gas-impermeable rubber hoses (inner diameter 4 mm) were mounted at 7 cm intervals along the peg. The rubber hoses led to a platform located 2 m away just above the lake surface, from which water samples were collected with a syringe. The rubber hoses were fitted with valves to prevent reverse flow. During a diel cycle, water samples were collected and analysed by standard methods for DIC, ANC, calcium, ferrous iron, sulfide, ortho-phosphate, nitrate and ammonium [17,18]. Weekly surface samples for measurements of ANC (meq. l<sup>-1</sup>), pH and specific conductivity (Sp. cond.,  $\mu$  Siemens cm<sup>-1</sup>) showed a close linear relationship of conductivity,  $\text{ANC} = 0.008572 \times \text{Sp. cond.} - 0.1286$  ( $R^2 = 0.93$ ,  $p < 0.0001$ ,  $n = 153$ , electronic supplementary material, figure S1). This relationship enabled continuous estimates of DIC and proportions of individual carbon species from measurements of temperature, pH and specific conductivity [19].

The anoxic tolerance of charophytes in darkness was tested in replicate by exposing 5 cm long apical shoots in large closed glass bottles to variable periods (up to 72 h) of anoxia and reduced conditions in bottom waters collected from lake 1 at midday in early June 2016. After anoxic dark exposure, charophyte shoots were

transferred to oxic water, but maintained in darkness for the remaining period (up to 72 h). Following this, their photosynthetic rate was measured in air-saturated lake water in glass bottles (50 ml) exposed to high illumination ( $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), constant temperature ( $20^\circ\text{C}$ ) and stirred conditions for 90 min in an incubator [20]. Glass bottles filled with water served as blanks. Oxygen concentration was measured in the glass bottles by an oxygen optode (Firesting PyroScience, Aachen, Germany).

### 3. Results

#### (a) Recurring daytime stratification and nocturnal mixing

The studied lakes showed profound diel temperature changes and a clear contrast between high surface temperatures and much lower bottom temperatures during daytime (table 1). The water column stratified strongly on four warm days in late May and on all days in June and August (figures 1–3). On cold, windy days in May, the water column remained well mixed (figure 1). Every night, the water column was mixed by vertical convection induced by cooling of surface waters (table 1; electronic supplementary material, table S1). Maximum daytime differences in temperature between surface and bottom waters were  $11.2\text{--}14.4^\circ\text{C}$  in May and  $6.2\text{--}8.9^\circ\text{C}$  in June and August.

The steepest vertical temperature gradient ( $0.3\text{--}1.9^\circ\text{C cm}^{-1}$ ) formed at  $15\text{--}22$  cm depth in lake 1. This exceptionally strong daytime vertical stratification can be explained by the dense vegetation reaching up to  $5\text{--}10$  cm below the water surface. The vegetation effectively attenuates the solar heat flux along with the wind induced turbulent mixing. Thus, in May, 90% of the short-wave radiation was absorbed within the upper  $9\text{--}13$  cm of the charophyte canopy (mean  $10.6$  cm, 95% confidence limits (C.L.)  $\pm 1.3$  cm). The lake water was transparent, and vegetation was responsible for most (98%) of the light attenuation according to comparisons between vertical light profiles measured inside and outside the vegetation. Turbulence generated by wind shear on the lake surface was effectively dissipated by contact with the vegetation, thereby preventing full turbulent mixing, except on cold, windy days. Since the solar heat flux was absorbed within a thin surface layer, it became markedly warmer than the air (electronic supplementary material, figure S2). At night, cooling of surface waters to  $0.1\text{--}4.1^\circ\text{C}$  below bottom temperatures (electronic supplementary material, table S1) produced inverse unstable water density profiles, which drove convective mixing of the shallow water column.

#### (b) Diel dissolved oxygen variations

Alternating daytime stratification and nocturnal mixing along with steep vertical attenuation of photosynthetic irradiance in dense submerged vegetation account for the astonishing vertical and temporal dynamics of oxygen, pH, ANC and inorganic carbon species. Diel dissolved oxygen cycles were similar during warm days in May (figure 1) and all days in August (figure 2). Minimum oxygen concentrations in surface waters occurred at sunrise ( $12\text{--}25\%$  saturation) after a full night with respiration. Maximum oxygen concentrations were attained in the early afternoon ( $205\text{--}235\%$  saturation) after approximately 10 h of photosynthesis. Early in the morning, when photosynthesis and temperature stratification commenced, oxygen concentrations increased rapidly in surface

waters. Meanwhile, in the dark bottom waters, oxygen simultaneously declined by respiration thus turning anoxic around midday and staying so until between 20.00 and 00.00, when convective mixing re-introduced oxygen from surface waters and re-established homogeneous physical and chemical conditions (figures 1–3).

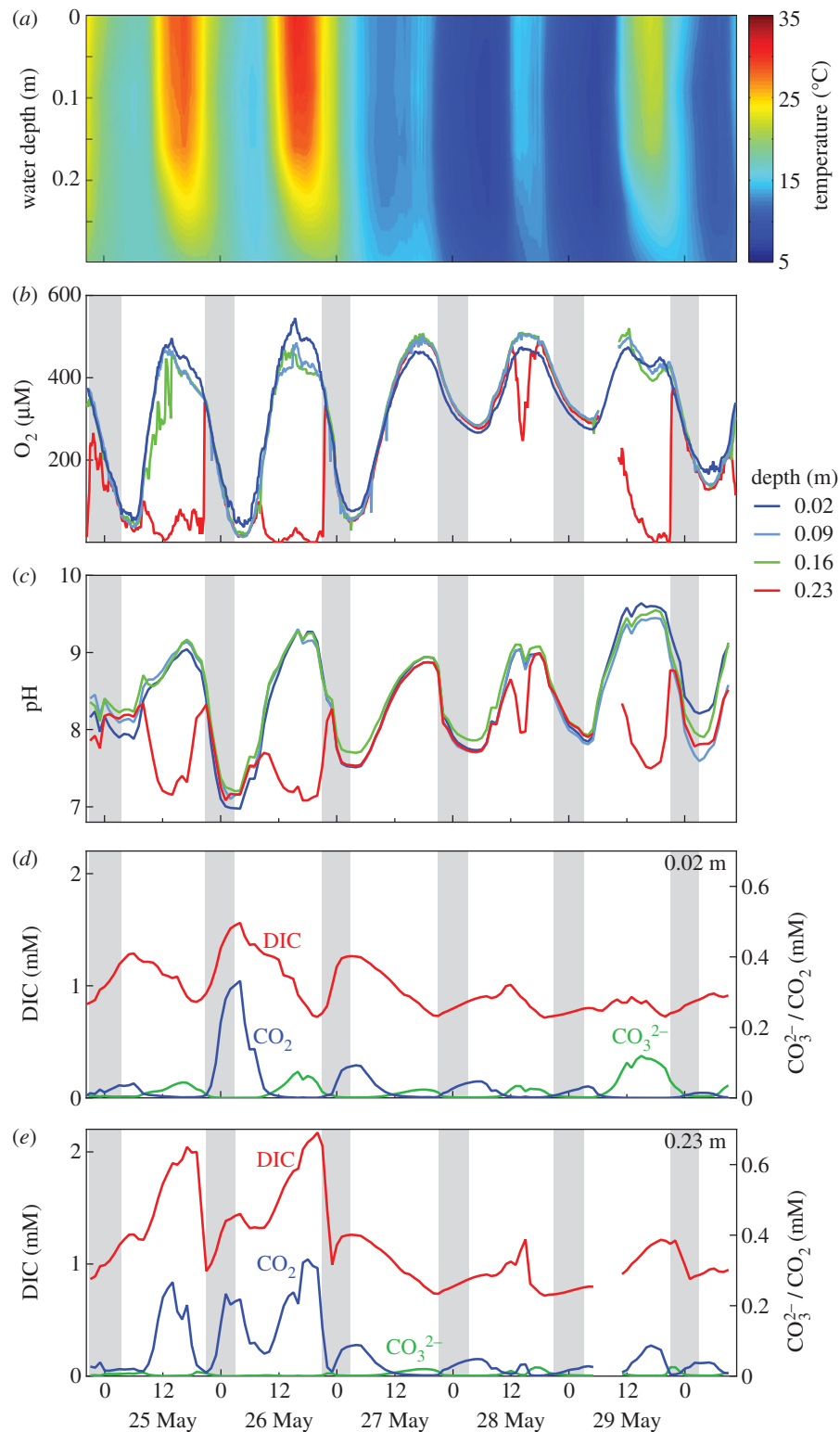
The same remarkable diel temperature and oxygen cycles—with anoxia in bottom waters during daytime stratification and oxic conditions during vertical mixing—were found across the studied lakes (table 1, figure 3). On days in early June 2016, the bottom waters in lakes 1, 2 and 4 all became anoxic from around 10.00 to 20.00, after which nocturnal convective mixing re-established uniform temperatures and oxic conditions until the morning. Lake 3 had almost permanently anoxic bottom waters except for brief periods around 06.00 and 20.00, when equal bottom and surface temperatures suggested particularly efficient vertical mixing of the water.

Anoxic conditions in bottom waters after midday induced accumulation of sulfide and ferrous iron (figure 4). Reduction of sulphate and ferric iron serve as electron acceptors during anaerobic respiration [21]. Sulfide accumulation in bottom waters was small; accumulation of ferrous iron was larger and continued throughout the stratified period, this shows that during anoxia, ferric iron was a more important electron acceptor than sulphate. Sulfide and ferrous iron were re-oxidized when oxygen was reintroduced to the bottom waters by vertical mixing. Ammonium accumulated to a small extent in the bottom waters during temperature stratification, whereas concentrations of nitrate ( $0.009\text{--}0.098 \mu\text{M}$ ) and phosphate ( $0\text{--}0.035 \mu\text{M}$ ) remained close to zero. These low nutrient levels emphasize the oligotrophic nature of the studied lakes.

#### (c) Diel variations of pH and inorganic carbon

Rising pH during continuous photosynthesis in surface waters from morning to afternoon was accompanied by decreasing DIC, ANC and  $\text{CO}_2$  and increasing  $\text{CO}_3^{2-}$  (figures 1 and 2). This time course is the result of coupled  $\text{CO}_2$  assimilation in photosynthesis along with  $\text{CO}_2$  loss by calcification [16] (i.e.  $\text{Ca}^{2+} + 2\text{HCO}_3^- \rightarrow \text{CaCO}_3$  (precipitated) +  $\text{CO}_2$  (assimilated)). Photosynthesis alone without calcification does not reduce ANC or calcium [22] (i.e.  $\text{Ca}^{2+} + 2\text{HCO}_3^- \rightarrow \text{Ca}^{2+} + 2\text{CO}_2$  (assimilated) +  $2\text{OH}^-$ ). The decline of ANC in surface waters could be a result of  $\text{CaCO}_3$  precipitating directly on vegetation surfaces because photosynthesis is coupled to calcification. More likely, it is a result of spontaneous formation and sinking of calcite crystals in the water since high pH converts  $\text{HCO}_3^-$  to  $\text{CO}_3^{2-}$  ( $\text{HCO}_3^- + \text{OH}^- \rightarrow \text{CO}_3^{2-}$ ). The calcite saturation index (the ionic molar product of  $\text{Ca}^{2+}$  and  $\text{CO}_3^{2-}$  relative to the solubility product at ambient temperature) reached high values (around 10) in the surface water in the afternoon on all days in August (figure 2). Consequently, it should promote calcite formation. Daytime ANC loss in surface waters was apparently mainly owing to precipitation and sinking of calcite crystals, which were re-dissolved in bottom waters because of high  $\text{CO}_2$  and low pH. The dissolution of  $\text{CaCO}_3$  resulted in accumulation of DIC, ANC and calcium in bottom waters until nocturnal convection broke the stratification.

Surface water pH was lowest (around 8.0) shortly after sunrise and highest ( $9.4\text{--}9.6$ ) in the afternoon after hours of photosynthesis (figures 1 and 2). This development was accompanied by an almost complete depletion of  $\text{CO}_2$  and



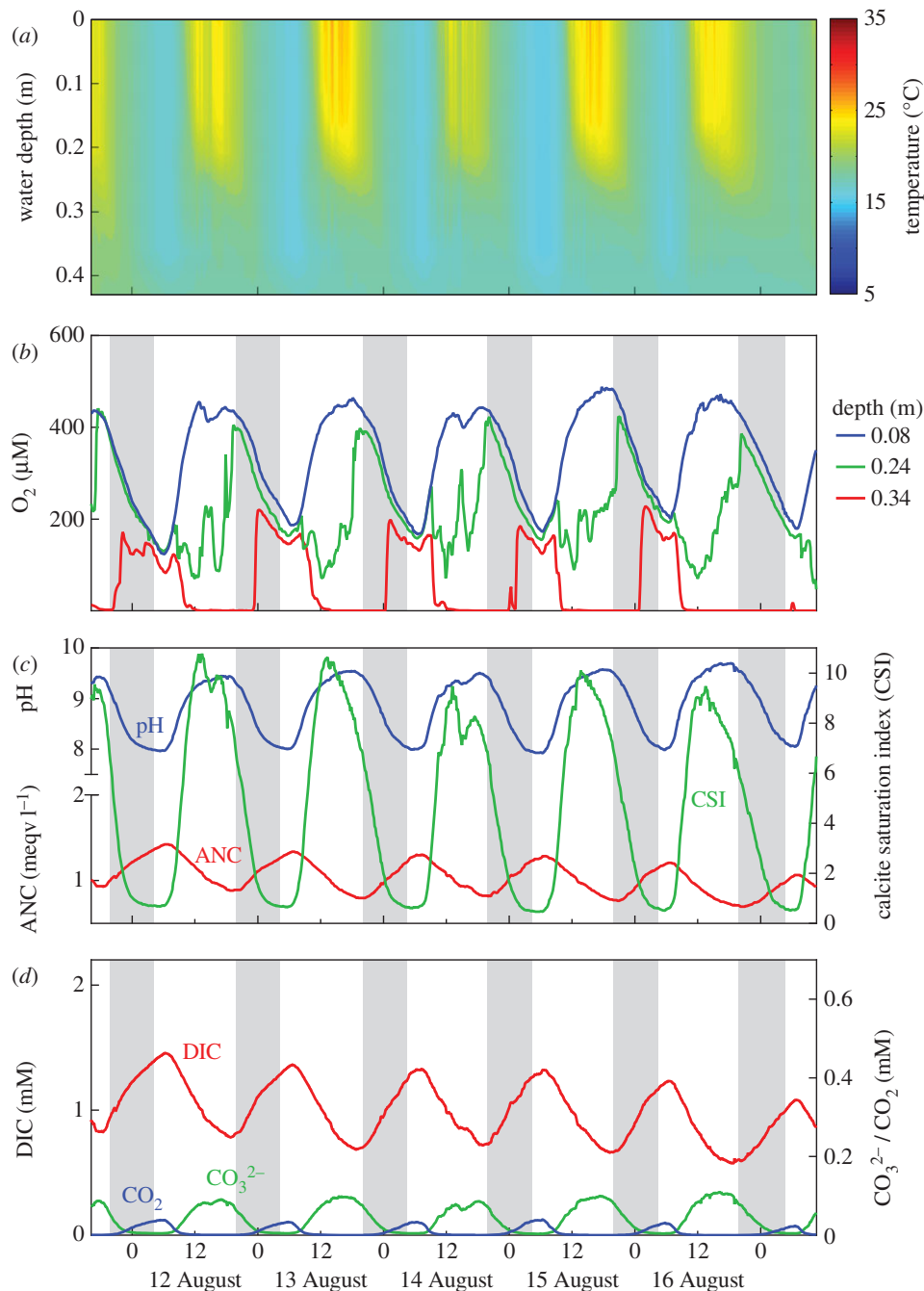
**Figure 1.** Time series of temperature, O<sub>2</sub>, pH, ANC, DIC and individual carbon species with depth in a shallow charophyte lake (lake 1) for a period of 6 days in May. (a) Temperature isopleths calculated from measurements at 5 cm depth intervals. (b,c) Oxygen and pH measured at 0.02 m (dark blue), 0.09 m (light blue), 0.16 m (green) and 0.23 m (red) below water surface. (d) DIC (red), CO<sub>3</sub><sup>2-</sup> (green) and CO<sub>2</sub> (blue) in surface waters (0.02 m). (e) DIC (red), CO<sub>3</sub><sup>2-</sup> (green) and CO<sub>2</sub> (blue) in bottom waters (0.23 m). Background colours show the day/night cycle: white, day; grey, night.

an increase of CO<sub>3</sub><sup>2-</sup>. Daytime ANC loss in surface waters by CaCO<sub>3</sub> precipitation averaged 0.57 meq l<sup>-1</sup> on five consecutive days in August. Most ANC (0.51 meq l<sup>-1</sup>, 91%), calcium and DIC returned to surface waters before sunrise the following day by vertical mixing reflecting that most precipitated CaCO<sub>3</sub> from surface waters was re-dissolved in bottom waters with excess CO<sub>2</sub> and low pH (figure 4). Sediment incubations confirmed the release of two moles DIC for every mole of oxygen consumed owing to

concomitant respiration of organic carbon and CaCO<sub>3</sub> dissolution (organic C + O<sub>2</sub> + CaCO<sub>3</sub> → Ca<sup>2+</sup> + H<sub>2</sub>O + 2HCO<sub>3</sub><sup>-</sup>; electronic supplementary material, table S2).

This interpretation was supported by direct analyses of the vertical distribution of DIC, ANC and calcium during a full diel cycle (figure 4). Daytime ANC and calcium decline in surface waters by calcification was matched by increasing concentrations in bottom waters by CaCO<sub>3</sub> dissolution coupled to respiration. These processes continued throughout





**Figure 2.** Time series of temperature, O<sub>2</sub>, pH, ANC, calcite saturation index, DIC and individual carbon species in a shallow charophyte lake (lake 1) during a period of 6 days in August. (a) Temperature isopleths calculated from measurements at 5 cm depth intervals. (b) Oxygen measured at 0.08 m (dark blue), 0.24 m (green) and 0.34 m (red) below water surface. (c) pH (blue), ANC (red) and CSI (green) in surface waters (0.08 m). (d) DIC (red), CO<sub>3</sub><sup>2-</sup> (green) and CO<sub>2</sub> (blue) in surface waters (0.08 m). Background colours show the day/night cycle: white, day; grey, night.

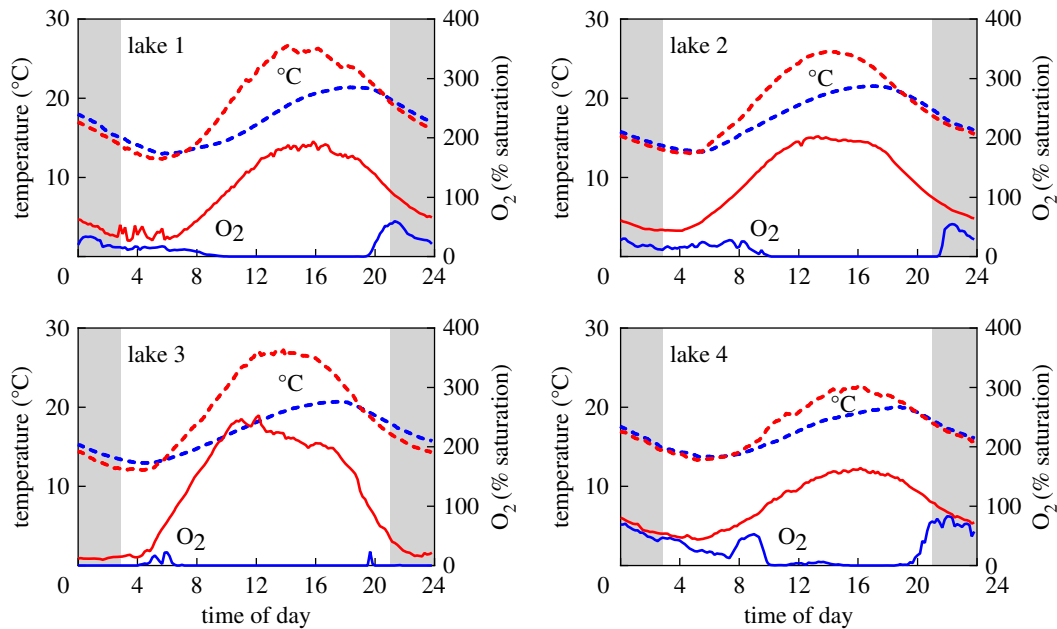
the night and gave rise to increasing concentrations of DIC, ANC and calcium in the water column until photosynthesis picked up at sunrise.

#### (d) Anoxic tolerance of charophytes

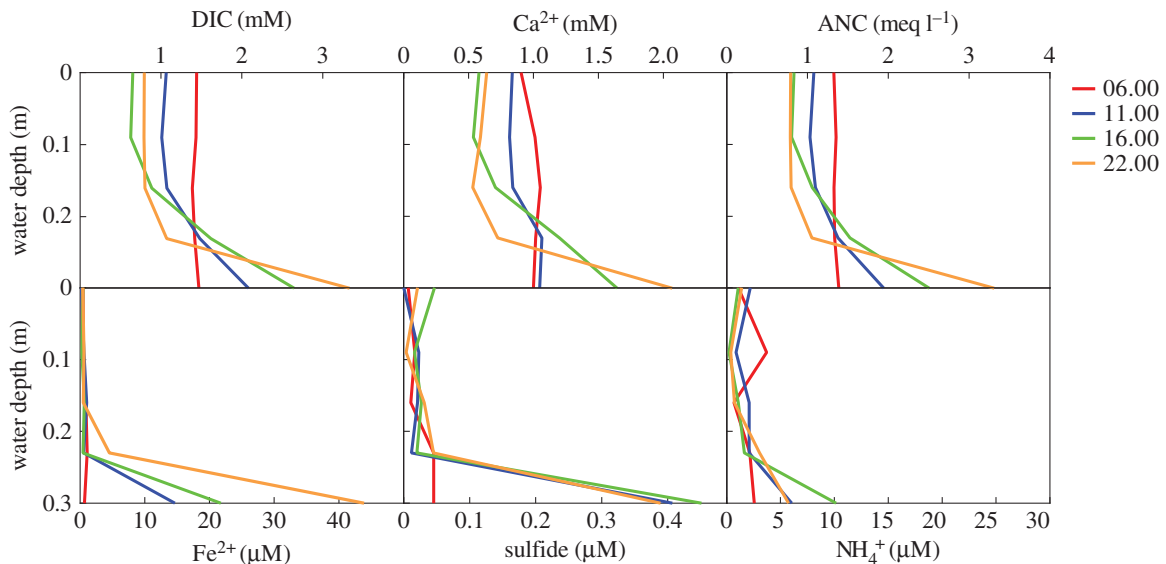
Charophytes were remarkably resilient to the adverse conditions that developed in the bottom waters every day. Tolerance tests showed that charophytes survived 24 h in darkness exposed to anoxic, reduced bottom waters with only modest reductions (16%) of the photosynthetic capacity upon re-exposure to light and aerated surface waters (figure 5). They even survived 72 h of permanent darkness in anoxic, reduced bottom waters, although photosynthetic capacity after this extended anoxic period decreased to one-third of the initial capacity.

## 4. Discussion

This study adds new dimensions to the understanding of environmental conditions and biogeochemistry in shallow vegetated lakes. The results confirm that, during summer, alternating temperature stratification and mixing are recurring daily phenomena in shallow vegetated lakes, because dense vegetation strongly attenuates depth penetration of light, heat flux and wind-driven turbulence during the day. Surface cooling at night induces penetrative convective mixing [15,23]. Hitherto, very shallow lakes in open habitats have often been considered to be permanently mixed, and if stratification and oxygen depletion occurred it was confined to organically polluted lakes or to nocturnal periods during degradation of heavy phytoplankton blooms in hypereutrophic lakes [24,25].



**Figure 3.** Diel cycles of water temperatures (°C, dashed lines) and oxygen saturation (% saturation, solid lines) in the surface waters (red) and bottom waters (blue) in four shallow vegetated lakes. Lakes 2 and 3 were sampled on 6 June 2016; lakes 1 and 4 on 10 June 2015. Grey background shows the night period.

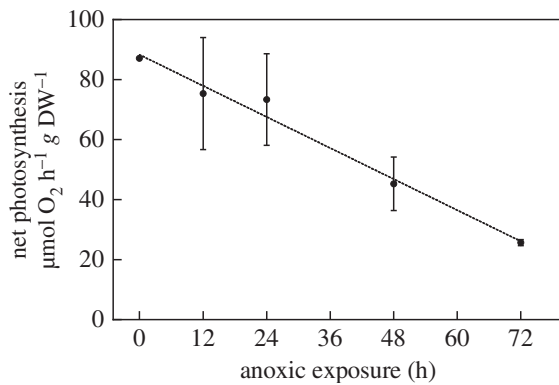


**Figure 4.** Depth profiles of DIC, Ca<sup>2+</sup>, ANC, Fe<sup>2+</sup>,  $\sum\text{H}_2\text{S}$  and NH<sub>4</sub><sup>+</sup> in a shallow charophyte lake (lake 1) during a diel cycle. Measurements were carried out on 26 May at 06.00 (red), 11.00 (blue), 16.00 (green) and 22.00 (orange). The water column was vertically mixed at 06.00, stratified below 0.20 m at 16.00 and below 0.25 m at 22.00.

This study shows that daytime thermal decoupling between photosynthesis in the upper vegetation canopy and respiration in darkness in the lower canopy generated profound chemical gradients. Surface waters underwent oxygen accumulation and DIC depletion, while bottom waters underwent anoxia and DIC accumulation. Nocturnal mixing injected oxygen from surface waters into bottom waters thus re-establishing oxygenic respiration. At the same time, nocturnal mixing supplied regenerated DIC from bottom waters to surface waters for continued photosynthesis on the following day. If, instead of being returned to surface waters, regenerated DIC had been trapped more permanently in stagnant bottom water as in dimictic lakes [26], which show full vertical mixing in spring and autumn and thermal stratification during summer [27], photosynthesis would become severely constrained by DIC depletion since up to half of the DIC pool was lost from the surface waters during a single daytime period.

The contrast of the shallow, vegetation-rich lakes compared to lakes with less vegetation or greater water depth was striking [9,11,27]. Measurements for a full year in nine small, shallow Danish lakes (water depths: 0.3–2.0 m), showed vertical stratification on 67% of the days between early spring and late autumn [28]. On most occasions (94%), full convective mixing occurred during the following night. The likelihood of stratification increased from the shallowest lake (0.3–0.6 m deep) with few macrophytes that rarely stratified, slightly deeper lakes (0.4–1.5 m) with more macrophytes which both stratified and mixed daily, to even deeper lakes (more than 1.5 m) that could remain stratified for several days [28]. The lakes in this study all fit in the second category in accordance with the statistical prediction of Martinsen *et al.* [28].

The recycling of DIC worked as a biological pump, the carbonate pump [29], and was characterized by carbonate precipitation in surface waters, carbonate dissolution in bottom



**Figure 5.** Net photosynthesis of *Chara aspera* after 0, 12, 24, 48 and 72 h of exposure to anoxia in darkness and subsequent exposure to oxic conditions in darkness before photosynthetic measurements after 72 h. Mean photosynthetic rates of replicates and s.d. The dotted line is the linear regression model ( $y = -0.8632x + 88.32$ ,  $p$  value = 0.001,  $R^2 = 0.98$ ).

waters and return of DIC to surface water by mixing. The carbonate pump works on different timescales dictated by the mixing regime and, thus, depends on time of the year, lake morphometry and macrophyte density. In dimictic lakes the carbonate pump operates at timescales of several months such as 9 m deep Williams Lake, Minnesota, USA [27]. Calcifying *Chara* species grew to depths of 5 m and consumed 25–30% of the DIC pool in the surface waters of Williams Lake during summer stratification for 11 consecutive years, while input from tributaries and dissolution of carbonate in sediments and vertical mixing restored the surface water DIC pool from autumn to spring [27]. In comparison, the carbonate pump worked on a diel scale in the charophyte-rich shallow lakes examined here, and it can be absent in permanently mixed lakes [30].

Polymictic lakes undergo mixing more than twice a year [26] and take a position between the daily recurring stratified-mixing lakes and the dimictic lakes. In polymictic lakes, stratification may last for hours [30], days [28,31], weeks [32] or months [32]. Polymictic lakes may therefore share some of the features described here, though being less prominent. For example, the large (270 km<sup>2</sup>) and shallow (6.0 m) Lake Võrtsjärv, Estonia, was usually fully mixed, but occasionally stratified for few hours during summer accompanied by hypoxia and CO<sub>2</sub> build-up in the bottom waters [30]. The charophyte-dominated Lost Pond, Minnesota (1.2 m) stratified for several days accompanied by bottom hypoxia [31]. Finally, prolonged anoxia in the bottom waters of Nakamun Lake, Alberta, Canada, (8.0 m) released sediment phosphorus, which was returned to surface waters by mixing [32]. Thus, polymictic lakes share some characteristics with the lakes in our study as they irregularly experienced oxygen depletion below the thermocline and the subsequent mixing events pumped nutrients and CO<sub>2</sub> from bottom waters to the photic zone. However, since convective mixing was insufficient to fully mix the water column in most polymictic lakes, they relied on wind for mixing and this process takes place on variable time scales depending on wind speed, lake depth and fetch. Productivity in the charophyte-lakes studied here benefit from the stratified and mixed conditions both taking place diurnally and it would be severely constrained in the absence of either process. The stratified conditions create the high CO<sub>2</sub> and low pH conditions in the bottom waters which enhanced dissolution of precipitated carbonates, while

convective mixing returned DIC and other solutes to the surface water every night. This intimate diel coupling between substrate use and release by production and decomposition does not exist in polymictic lakes.

Despite the oligotrophic waters of the study lakes submerged vegetation is capable of building high biomasses [33] through nutrient uptake from bottom waters and sediments [34]. Nutrients released by mineralization from sediments are diluted in a gradually smaller water volume as depth decreases [35]. Shallow lakes also have small water volumes below the thermocline and a large percentage of the sediment is in contact with warm surface water stimulating mineralization and direct nutrient release to plant growth in the photic zone [7]. Overall, internal nutrient recycling can be several-fold higher than external nutrient loading in well-mixed, shallow lakes, while for the same external loading, internal nutrient recycling may be several-fold less than the external loading in deeper dimictic lakes [36]. Consequently, the critical thresholds of external nutrient loading between oligo-, meso- and eutrophic lakes decreases markedly with increasing water depth [35]. Moreover, the sediment nutrient pool can be directly taken up by rhizoids and incorporated in new *Chara* growth. Thus, despite undetectable nutrient concentrations in the water, characteristic of very oligotrophic lakes, the charophytes could attain dense biomasses and high metabolic rates typical of eutrophic lakes [37,38], owing to efficient recycling and sediment exploitation of nutrients in the small study lakes.

The dense vegetation absorbed almost all of the photosynthetic active irradiance and could attain high rates of photosynthesis, particularly before noon, when DIC and CO<sub>2</sub> concentrations were high in surface waters [39]. Depletion of DIC and CO<sub>2</sub> along with accumulation of oxygen in surface waters, as the result of intense photosynthesis from morning to noon, suppressed photosynthesis in the afternoon [39]. On a daily basis, photosynthetic rates relative to water volume in the shallow vegetated lakes were, nonetheless, higher than in nutrient-rich phytoplankton communities in lakes and estuaries [37], because light absorption and photosynthesis were confined to the upper 10 cm of the plant canopy [37,40]. These high volumetric rates of daytime photosynthesis and nocturnal respiration [10,37,39] drive the profound diel and vertical changes of oxygen and inorganic carbon concentrations as well as pH observed in the study lakes. Recent work on whole-lake metabolism has focused on the heterogeneity of metabolic rates within the water column [41,42] and with particular emphasis on the metalimnion [43]. The metalimnion is defined as the layer in the water column where the density gradient is steepest resulting in a highly dynamic physical and chemical environment [7,44]. Thermal decoupling of the surface water (epilimnion) from the bottom water (hypolimnion) means that nutrient, DIC and oxygen gradients build up in the metalimnion. In oligotrophic lakes where the euphotic zone may extend below the metalimnion, the increased access to nutrients means that the metalimnetic phytoplankton communities contribute with a disproportionately large amount of the whole-lake productivity [43]. However, in the oligotrophic and shallow lakes studied here, very little light is available in the metalimnion because the majority of the light is attenuated by the canopy of the charophytes above [15]. Therefore, the metalimnion communities contribute with little to none of whole-lake productivity. This situation is directly analogous to the metalimnion of eutrophic and hypereutrophic lakes in which productivity is also severely constrained by lack of light [41,43].

Organisms have to adapt to the extreme environmental gradients in these shallow lakes. The basal parts of the vegetation below the diel thermocline were exposed to up to 12 h of anoxia and accumulation of potentially toxic sulfide and ferrous iron. Simultaneously, apical tissues experienced wide diel amplitudes of temperature, oxygen and pH. The dominant charophyte vegetation in the lakes, in contrast to rooted plants [45], lack air lacunae for longitudinal oxygen transport from apical to basal tissue located in anoxic waters and sediments [34]. Experiments showed that charophytes possessed efficient anaerobic respiration which generated as much  $\text{CO}_2$  as aerobic respiration [46]. Tolerance tests showed that charophytes survived 24 h in darkness exposed to anoxic, reduced bottom waters with only modest reductions of the photosynthetic capacity upon re-exposure to light and aerated surface waters. They even survived 72 h of permanent darkness in anoxic, reduced bottom waters, although photosynthetic capacity after this extended anoxic period decreased to one-third of the initial capacity. The dominant charophytes were remarkably robust to the adverse conditions that develop below the diel thermocline. The marine seagrass *Zostera marina* also experience anoxic conditions but showed much less resilience to anoxia as it took severe damage after only 1 h and died after 8 h in anoxia at 30°C [47]. By influencing the mixing regime of the lake [15], the dominant *Chara* species contribute to creating adverse conditions in the bottom waters to aquatic plants and their own surprisingly high resilience give them a competitive advance which contribute to their ability to form dense, monospecific stands in these shallow lakes. Highly efficient  $\text{HCO}_3^-$  use and ability to maintain photosynthesis at low DIC levels further contribute to the development of these dense charophyte stands.

The large diel temperature and oxygen amplitudes in the study lakes also present a challenge to animal survival. Motile organisms can move to suitable conditions in the water column [48]. They can escape high temperatures in surface water during the day by moving mere centimetres down the water column, though lower oxygen concentrations there may present a new challenge to meet respiratory needs. Thus, selection is expected to drive adaptations and behaviour of animals towards improved tolerance to high temperature and/or low oxygen concentration. Before sunrise the well mixed water column is hypoxic as oxygen saturation drops to 10–50% which challenge sessile and motile animals alike.

Charophytes and many rooted plants are efficient calcifiers and  $\text{HCO}_3^-$  users [16,49]. Calcification delivers protons and ensures conversion of  $\text{HCO}_3^-$  to free  $\text{CO}_2$  for continued

photosynthesis without increasing pH to inhibiting levels (i.e.  $\text{Ca}^{2+} + 2\text{HCO}_3^- \rightarrow \text{CaCO}_3 + \text{CO}_2$ ). By contrast,  $\text{HCO}_3^-$  use without calcification increases pH ( $\text{HCO}_3^- \rightarrow \text{CO}_2 + \text{OH}^-$ ) which may inhibit further photosynthesis. In the biogeochemical cycling in the shallow lakes, the dissolution of  $\text{CaCO}_3$  in bottom waters and the re-supply of DIC to surface waters during nocturnal mixing can ensure that high photosynthetic rates are maintained day after day. In addition, high photosynthesis can provide the necessary energy to support the high respiratory demands of the dense vegetation [10,37,39].

Our findings of extreme vertical and diel variability of temperature, oxygen, pH and solutes are not confined to shallow lakes with charophytes. Given that the stabilizing effect of the vegetation cover prevents mixing at very shallow depths such diel stratification events are probably also present in flooded marshes and shallow vegetated areas along shores in larger lakes.

## 5. Conclusion

Shallow lakes by far constitute the most common lake type throughout the world [7,50]. The preference for studying large lakes with deeper waters, however, has given us a skewed perception of environmental conditions, species adaptations and ecosystem processes for the natural range of lentic water bodies [6,11]. The results of this study reveal extreme diel variability of temperature, oxygen, pH and DIC along with a novel discovery of anoxia and reduced ions in the bottom waters during daytime stratification. The extreme amplitudes of temperature and oxygen suggest that the evolutionary processes of freshwater species could be particularly important in small, shallow lakes because their high abundance [7,50] and challenging environmental conditions can promote species adaptation to high temperature and oxygen stress [51].

**Data accessibility.** The dataset supporting this article is available from the Dryad Digital Repository: (<http://datadryad.org/resource/doi:10.5061/dryad.c95fk>) [52].

**Authors' contributions.** K.S.-J., M.R.A. and T.K. designed the experiment; M.R.A., K.S.-J. and T.K. performed the fieldwork and data acquisition; K.S.-J., M.R.A. and T.K. analysed the data; M.R.A. and K.S.-J. wrote the paper. All authors gave final approval for publication.

**Competing interests.** We declare we have no competing interests.

**Funding.** This study was funded by a grant from the Carlsberg foundation to K.S.-J.

**Acknowledgements.** The authors thank Mikkel Madsen-Oesterbye for technical assistance, Ole Petersen and Jens Borum for constructive discussions and the anonymous reviewers for their inputs.

## References

1. Anderson N, Stedmon CA. 2007 The effect of evapoconcentration on dissolved organic carbon concentration and quality in lakes of SW Greenland. *Freshw. Biol.* **52**, 280–289. (doi:10.1111/j.1365-2427.2006.01688.x)
2. Emmerton CA, Lesack LF, Marsh P. 2007 Lake abundance, potential water storage, and habitat distribution in the Mackenzie River Delta, western Canadian Arctic. *Water Resour. Res.* **43**, 14485. (doi:10.1029/2006WR005139)
3. Biggs J, Williams P, Whitfield M, Nicolet P, Weatherby A. 2005 15 years of pond assessment in Britain: results and lessons learned from the work of Pond Conservation. *Aquat. Conserv.* **15**, 693–714. (doi:10.1002/aqc.745)
4. Davies B, Biggs J, Williams P, Lee J, Thompson S. 2008 A comparison of the catchment sizes of rivers, streams, ponds, ditches and lakes: implications for protecting aquatic biodiversity in an agricultural landscape. *Hydrobiologia* **597**, 7–17. (doi:10.1007/s10750-007-9227-6)
5. Downing J *et al.* 2006 The global abundance and size distribution of lakes, ponds, and impoundments. *Limnol. Oceanogr.* **51**, 2388–2397. (doi:10.4319/lo.2006.51.5.2388)
6. Hanson PC, Carpenter SR, Cardille JA, Coe MT, Winslow LA. 2007 Small lakes dominate a random sample of regional lake characteristics. *Freshw. Biol.* **52**, 814–822. (doi:10.1111/j.1365-2427.2007.01730.x)
7. Kalf J. 2002 *Limnology: inland water ecosystems*. Upper Saddle River, NJ: Prentice Hall.



8. Biggs J, von Fumetti S, Kelly-Quinn M. 2016 The importance of small waterbodies for biodiversity and ecosystem services: implications for policy makers. *Hydrobiologia* **793**, 3–39. (doi:10.1007/s10750-016-3007-0)
9. Staehr PA, Baastrup-Spohr L, Sand-Jensen K, Stedmon C. 2012 Lake metabolism scales with lake morphometry and catchment conditions. *Aquat. Sci.* **74**, 155–169. (doi:10.1007/s00027-011-0207-6)
10. Christensen J, Sand-Jensen K, Staehr PA. 2013 Fluctuating water levels control water chemistry and metabolism of a charophyte-dominated pond. *Freshw. Biol.* **58**, 1353–1365. (doi:10.1111/fwb.12132)
11. Sand-Jensen K, Staehr PA. 2007 Scaling of pelagic metabolism to size, trophy and forest cover in small Danish lakes. *Ecosystems* **10**, 128–142. (doi:10.1007/s10021-006-9001-z)
12. Williams P, Whitfield M, Biggs J, Bray S, Fox G, Nicolet P, Sear D. 2004 Comparative biodiversity of rivers, streams, ditches and ponds in an agricultural landscape in southern England. *Biol. Conserv.* **115**, 329–341. (doi:10.1016/S0006-3207(03)00153-8)
13. Herb WR, Stefan HG. 2005 Dynamics of vertical mixing in a shallow lake with submersed macrophytes. *Water Resour. Res.* **41**, W02023. (doi:10.1029/2003WR002613)
14. Branco BF, Torgersen T. 2009 Predicting the onset of thermal stratification in shallow inland waterbodies. *Aquat. Sci.* **71**, 65–79. (doi:10.1007/s00027-009-8063-3)
15. Andersen MR, Sand-Jensen K, Iestyn Woolway R, Jones ID. 2016 Profound daily vertical stratification and mixing in a small, shallow, wind-exposed lake with submersed macrophytes. *Aquat. Sci.* **79**, 395–406. (doi:10.1007/s00027-016-0505-0)
16. McConnaughey T. 1991 Calcification in *Chara corallina*: CO<sub>2</sub> hydroxylation generates protons for bicarbonate assimilation. *Limnol. Oceanogr.* **36**, 619–628. (doi:10.4319/lno.1991.36.4.0619)
17. Gran G. 1952 Determination of the equivalence point in potentiometric titrations—part II. *Analyst* **77**, 661–671. (doi:10.1039/an9527700661)
18. University of Copenhagen. 1977 *Limnological methods (in Danish)*. (Ferskvandsbiologisk Laboratorium. Københavns Universitet (Ed.)). København, Denmark: Akademisk Forlag.
19. Lewis E, Wallace D. 1998 *Program developed for CO<sub>2</sub> system calculations*. Oak Ridge, TN: Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, US Department of Energy. ORNL/CDIAC-105.
20. Nielsen ES, Hansen VK. 1961 Influence of surface illumination on plankton photosynthesis in Danish waters (56°N) throughout the year. *Physiol. Plant.* **14**, 595–613. (doi:10.1111/j.1399-3054.1961.tb07917.x)
21. Stumm W, Baccini P. 1978 Man-made chemical perturbation of lakes. In *Lakes* (ed. A Lerman), pp. 91–126. New York, NY: Springer.
22. Madsen TV, Sand-Jensen K. 1991 Photosynthetic carbon assimilation in aquatic macrophytes. *Aquat. Bot.* **41**, 5–40. (doi:10.1016/0304-3770(91)90037-6)
23. Holgerson MA, Zappa CJ, Raymond PA. 2016 Substantial overnight reaeration by convective cooling discovered in pond ecosystems. *Geophys. Res. Lett.* **43**, 8044–8051. (doi:10.1002/2016GL070206)
24. Barica J, Kling H, Gibson J. 1980 Experimental manipulation of algal bloom composition by nitrogen addition. *Can. J. Fish. Aquat. Sci.* **37**, 1175–1183. (doi:10.1139/f80-150)
25. Søndergaard M, Kristensen P, Jeppesen E. 1993 Eight years of internal phosphorus loading and changes in the sediment phosphorus profile of Lake Søbygaard, Denmark. *Hydrobiologia* **253**, 345–356. (doi:10.1007/BF00050760)
26. Lewis Jr WM. 1983 A revised classification of lakes based on mixing. *Can. J. Fish. Aquat. Sci.* **40**, 1779–1787. (doi:10.1139/f83-207)
27. McConnaughey TA, LaBaugh JW, Rosenberry DO, Striegl RG, Reddy MM, Schuster PF, Carter V. 1994 Carbon budget for a groundwater-fed lake: calcification supports summer photosynthesis. *Limnol. Oceanogr.* **39**, 1319–1332. (doi:10.4319/lno.1994.39.6.1319)
28. Martinsen KT, Andersen MR, Sand-Jensen K. In press. Water temperature dynamics and prediction of stratification in small lakes. *Aquat. Sci.*
29. Holligan PM, Robertson JE. 1996 Significance of ocean carbonate budgets for the global carbon cycle. *Glob. Change Biol.* **2**, 85–95. (doi:10.1111/j.1365-2486.1996.tb00053.x)
30. Laas A, Cremona F, Meinson P, Rööm E-I, Nöges T, Nöges P. 2016 Summer depth distribution profiles of dissolved CO<sub>2</sub> and O<sub>2</sub> in shallow temperate lakes reveal trophic state and lake type specific differences. *Sci. Total Environ.* **566**, 63–75. (doi:10.1016/j.scitotenv.2016.05.038)
31. Cole J, Fisher SG. 1978 Annual metabolism of a temporary pond ecosystem. *Am. Midl. Nat.* **100**, 15–22. (doi:10.2307/2424773)
32. Orihel DM, Schindler DW, Ballard NC, Graham MD, O'Connell DW, Wilson LR, Vinebrooke RD. 2015 The 'nutrient pump': iron-poor sediments fuel low nitrogen-to-phosphorus ratios and cyanobacterial blooms in polymictic lakes. *Limnol. Oceanogr.* **60**, 856–871. (doi:10.1002/lno.10076)
33. Geertz-Hansen O, Montes C, Duarte CM, Sand-Jensen K, Marbà N, Grillas P. 2011 Ecosystem metabolism in a temporary Mediterranean marsh (Doñana National Park, SW Spain). *Biogeosciences* **8**, 963–971. (doi:10.5194/bg-8-963-2011)
34. Beilby MJ, Casanova MT. 2014 *The physiology of characean cells*. Berlin, Germany: Springer-Verlag.
35. Vollenweider RA. 1968 *Water management research*. Paris, France: OECD. DAYCSU68.27. Mimeo.
36. Fee E. 1979 A relation between lake morphometry and primary productivity and its use in interpreting whole-lake eutrophication experiments. *Limnol. Oceanogr.* **24**, 401–416. (doi:10.4319/lno.1979.24.3.0401)
37. Martinsen KT, Andersen MR, Kragh T, Sand-Jensen K. In press. High rates and close diel coupling of primary production and ecosystem respiration in small, oligotrophic charophyte-rich lakes. *Aquat. Sci.*
38. Hoellein TJ, Bruesewitz DA, Richardson DC. 2013 Revisiting Odum (1956): a synthesis of aquatic ecosystem metabolism. *Limnol. Oceanogr.* **58**, 2089–2100. (doi:10.4319/lno.2013.58.6.2089)
39. Kragh T, Andersen MR, Sand-Jensen K. In press. Distinct diurnal patterns of ecosystem metabolism in a shallow lake: afternoon depression of photosynthesis and nocturnal decline of respiration. *Oecologia*.
40. Krause-Jensen D, Sand-Jensen K. 1998 Light attenuation and photosynthesis of aquatic plant communities. *Limnol. Oceanogr.* **43**, 396–407. (doi:10.4319/lno.1998.43.3.0396)
41. Obrador B, Staehr PA, Christensen JP. 2014 Vertical patterns of metabolism in three contrasting stratified lakes. *Limnol. Oceanogr.* **59**, 1228–1240. (doi:10.4319/lno.2014.59.4.1228)
42. Staehr PA, Christensen J, Batt RD, Read JS. 2012 Ecosystem metabolism in a stratified lake. *Limnol. Oceanogr.* **57**, 1317–1330. (doi:10.4319/lno.2012.57.5.1317)
43. Giling DP *et al.* 2017 Delving deeper: metabolic processes in the metalimnion of stratified lakes. *Limnol. Oceanogr.* **62**, 1288–1306. (doi:10.1002/lno.10504)
44. Wetzel RG. 2001 *Limnology: lake and river ecosystems*. San Diego, CA: Academic Press.
45. Armstrong W. 1980 Aeration in higher plants. *Adv. Bot. Res.* **7**, 225–332. (doi:10.1016/S0065-2296(08)60089-0)
46. Thieme K. 2016 *Metabolism of Chara aspera from a small shallow pond*. Copenhagen, Denmark: University of Copenhagen.
47. Pulido C, Borum J. 2010 Eelgrass (*Zostera marina*) tolerance to anoxia. *J. Exp. Mar. Biol. Ecol.* **385**, 8–13. (doi:10.1016/j.jembe.2010.01.014)
48. Vad CF, Horvath Z, Kiss KT, Toth B, Pentek AL, Acs E. 2013 Vertical distribution of zooplankton in a shallow peatland pond: the limiting role of dissolved oxygen. *Ann. Limnol. Int. J. Limnol.* **49**, 275–285. (doi:10.1051/limn/2013060)
49. Lucas WJ. 1985 *Inorganic carbon uptake by aquatic photosynthetic organisms*, pp. 229–254. Rockville, MD: American Society of Plant Physiology Rockville.
50. Wetzel RG. 1992 Gradient-dominated ecosystems: sources and regulatory functions of dissolved organic matter in freshwater ecosystems. In *Dissolved organic matter in lacustrine ecosystems: energy source and system regulator* (eds K Salonen, T Kairesalo, R Jones), pp. 181–198. Dordrecht, The Netherlands: Springer.
51. Verberk WC, Bilton DT. 2013 Respiratory control in aquatic insects dictates their vulnerability to global warming. *Biol. Lett.* **9**, 20130473. (doi:10.1098/rsbl.2013.0473)
52. Andersen MR, Kragh T, Sand-Jensen K. 2017 Data from: extreme diel dissolved oxygen and carbon cycles in shallow vegetated lakes. Dryad Digital Repository. (<http://dx.doi.org/10.5061/dryad.c95fk>)