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PHYSIOLOGICAL ECOLOGY - ORIGINAL RESEARCH

Complementary ecophysiological strategies combine to facilitate survival in the hostile conditions of a deep chlorophyll maximum

Mark R. Clegg · Ursula Gaedke · Bertram Boehrer · Elly Spijkerman

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Abstract In the deep, cooler layers of clear, nutrient-poor, stratified water bodies, phytoplankton often accumulate to form a thin band or "deep chlorophyll maximum" (DCM) of ecological importance. Under such conditions, these photosynthetic microorganisms may be close to their physiological compensation points and to the boundaries of their ecological tolerance. To grow and survive any resulting energy limitation, DCM species are thought to exhibit highly specialised or flexible acclimation strategies. In this study, we investigated several of the adaptable ecophysiological strategies potentially employed by one such species, *Chlamydomonas acidophila*: a motile, unicellular, phytoplanktonic flagellate that often dominates the DCM in stratified, acidic lakes. Physiological and behavioural responses were measured in laboratory experiments and were subsequently related to field observations. Results showed moderate light compensation points for photosynthesis and growth at 22°C, relatively low maintenance costs, a behavioural preference for low to moderate light, and a decreased compensation point for photosynthesis at 8°C. Even though this flagellated alga exhibited a physiologically mediated diel vertical migration in the field, migrating upwards slightly during the day, the ambient light reaching the DCM was below compensation points, and so calculations of daily net photosynthetic gain showed that survival by purely autotrophic means was not possible. Results suggested that strategies such as low-light acclimation, small-scale directed movements towards light, a capacity for mixotrophic growth, acclimation to low temperature, in situ exposure to low O_2, high CO_2 and high P concentrations, and an avoidance of predation, could combine to help overcome this energetic dilemma and explain the occurrence of the DCM. Therefore, corroborating the deceptive ecophysiological complexity of this and similar organisms, only a suite of complementary strategies can facilitate the survival of *C. acidophila* in this DCM.

Keywords DCM · Photosynthesis · Growth · Behaviour · Phytoplankton

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M. R. Clegg (✉) · U. Gaedke · E. Spijkerman
Institute of Biochemistry and Biology, University of Potsdam, Am Neuen Palais 10, 14469 Potsdam, Germany
e-mail: clegg@uni-potsdam.de

B. Boehrer
Department of Lake Research, Helmholtz Centre for Environmental Research-UFZ, Brückstrasse 3a, 39114 Magdeburg, Germany

Introduction

Throughout a diverse range of freshwater, brackish and marine ecosystems, photosynthetic planktonic microorganisms, or phytoplankton, frequently accumulate in thin strata or layers (e.g. Cullen 1982; Klausmeier and Litchman 2001), at depths as low as 120 m (Kirk 1994). Such distinctive accumulations are called "deep chlorophyll maxima" (DCMs) and are one of the most remarkable and consistent features of these aquatic environments. They are often most prominent and of greatest ecological importance in nutrient-poor, oligotrophic, physically and chemically stratified water bodies, such as clear water lakes or the open ocean (e.g. Cullen 1982; Saros et al. 2005), contributing substantially to primary productivity (Moll et al. 1984; Wollmann et al. 2000), influencing the structure, dynamics and efficiency of the aquatic food web (Fennel

and Boss 2003; Tittel et al. 2003) and shaping the global biogeochemical cycling of resources (Huisman et al. 2006).

DCMs may be comprised of a single- or multi-species assemblage of phytoplankton that is typically on the boundaries of light and nutrient compensation points, as light decreases exponentially towards the base of the euphotic zone and nutrient concentrations generally begin to increase with depth (e.g. Coon et al. 1987). When organisms exist at these limits of their ecological tolerance, it can open up the potential for a diversity of flexible, adaptable strategies to promote survival. These strategies are widely utilised in both aquatic and terrestrial ecosystems and range from the biomolecular adaptations of microbes (Pakchung et al. 2006) to the timing of foraging in reindeer (Loe et al. 2007). Adaptable strategies can also be employed by phytoplankton, and may help overcome the potential environmental constraints encountered within a DCM. Indeed, even though DCMs can be influenced by many physical, chemical and biological factors (e.g. Longhurst 1976; Cullen 1982; Steinbuck et al. 2009), it is often believed that the complexity associated with explaining their formation, magnitude and species composition may be due to the numerous adaptable strategies and ecophysiological influences that contribute to growth and aggregation within this layer.

Although the broader physical, chemical and biological influences on DCMs have been extensively studied and reviewed (Cullen 1982; Reynolds 2006), the complex contribution of these ecophysiological strategies is still debated and is less well understood. Adaptations have been investigated in isolation, and some examples of integrated studies do exist, in which various biotic and abiotic factors have been incorporated into elegant models predicting vertical distribution (e.g. Moll et al. 1984; Kamykowski and Yamazaki 1997; Klausmeier and Litchman 2001; Yoshiyama et al. 2009). However, further empirical investigation of the strategies employed by the component phytoplankton would help expand existing knowledge of DCM ecology. Such research is becoming increasingly important, as global change theory suggests that the duration and prominence of stratification may increase with time (Fang and Stefan 1999; Huisman et al. 2006), and is of additional benefit for the study of harmful species that comprise many DCMs (e.g. Smayda 1997; Cullen and MacIntyre 1998). In this study, we therefore present a rare, detailed, integrated laboratory and field examination of a number of these adaptable strategies, their ecophysiological importance, and their potential combined influence on a DCM species.

A DCM dominated by the unicellular green flagellated alga *Chlamydomonas acidophila* regularly forms and persists throughout summer (Tittel et al. 2003) in the stratified, acidic Lake 111 (Lusatia, Germany). Despite only being situated at a depth of approximately 7 m, the light reaching this DCM at midday is less than 1% of subsurface irradiance (i.e. <20 and usually ~ 8 µmol photons m^{-2} s^{-1}). In situ measurements have shown that this quantity is relatively stable due to the strong attenuation primarily caused by high concentrations of dissolved iron, which also leads to a long-wavelength (600–750 nm), red-shifted, spectral environment (Gerloff-Elias et al. 2005a).

The magnitude and composition of a DCM can be influenced by the quantity and quality of ambient light. For example, DCMs in oligotrophic lakes and oceans are often dominated by cyanobacteria and cryptophytes (e.g. Padisak et al. 1997) and DCMs comprising green algal chlorophytes are less frequently observed (Coon et al. 1987; Gaevsky et al. 2002). This has largely been attributed to the poorer ability of green algae to grow under light-limiting conditions (Richardson et al. 1983). To a lesser extent, the predominantly green light (λ of 500–600 nm) encountered in many DCMs may also encourage species containing the accessory pigment phycoerythrin (e.g. cyanobacteria and cryptophytes), whereas red light at depth may favour a pigment stoichiometry that is better suited to harvest longer wavelengths (Gerloff-Elias et al. 2005a). We therefore aimed to investigate the minimal light requirements of *C. acidophila*; focussing on the potential photosynthetic and respiratory abilities of these cells, their possible acclimation to the cooler, low-light, spectral conditions at depth in Lake 111, and their potential contribution to the formation of this DCM.

In addition to photosynthetic strategies, many algal flagellates are also able to use dissolved organic carbon (DOC) as an alternative source of energy, with the exploitation of increased concentrations in deeper waters often assisting growth in light-limited conditions (e.g. Laybourn-Parry 2002). However, in contrast to many chlamydomonads, the hetero- or mixo-trophic growth of *C. acidophila* is restricted to the osmotrophic use of glucose (Cassin 1974) and some basic photolytic products of recalcitrant DOC (Tittel et al. 2009). Although only low concentrations of glucose have been detected in the DCM of Lake 111, and low light might prevent substantial photolysis, slow rates of mixotrophic growth have been observed (Tittel et al. 2003), and so the potential role of mixotrophy was also considered in this study.

The survival and growth of phytoplankton within a DCM may also be influenced by active changes in a cell's vertical position. Altered buoyancy, depth regulation and, particularly, diel vertical migration (e.g. Walsby 1977; Sommer and Gliwicz 1986; Cullen and MacIntyre 1998) can be employed to exploit opposing gradients of essential resources and may improve the light and nutrient climate of the organism (Jones 1993; Klausmeier and Litchman 2001). Given limited turbulent mixing (as found in the wind-protected, stratified Lake 111; Karakas et al. 2003),

photosynthetic flagellates such as *C. acidophila* are generally able to move in a controlled manner, actively and rapidly responding to changes in their environment. The most well studied and potentially the most important of these responses is the behavioural response to light (e.g. Feinleib and Curry 1971; Diehn et al. 1977; Clegg et al. 2004a). This "photoresponse" helps to direct vertical migration and offers a mechanism by which to locate and maintain position in preferred or optimal conditions (Heaney and Eppley 1981; Smayda 1997), often resulting in the formation of discrete layers within the water column (e.g. Gervais 1997; Clegg et al. 2003; Beckmann and Hense 2004). Therefore, due to its potential importance in this low-light environment, for the first time we also investigated and quantified the behavioural response of *C. acidophila* to light, and in situ chlorophyll *a* monitoring was used to determine the possible role of vertical migration in Lake 111.

Finally, the potential interaction of photosynthesis, respiration, growth, behavioural response and migration was explored, to assess the nature and extent of any combined influence in the DCM. Only an integrated study of the possible driving mechanisms can deliver a complete ecophysiological picture, and so through combined laboratory analyses and measurement of vertical distribution and migration in the field, we aimed to determine the potential significance of these adaptable strategies, their implications for survival, and their contribution to the occurrence and vertical position of this DCM.

Materials and methods

Study site

Lake 111 is a small (10 ha), meromictic lake, with a maximum depth of 10 m, located in an abandoned mining area of Lusatia, eastern Germany (51°29′N; 13°38′E). It has a geogenically derived pH of 2.7 and contains high concentrations of dissolved iron and sulfate. During summer, a stronger, stable thermal and chemical stratification develops (Karakas et al. 2003), during which concentrations of soluble reactive phosphorus, total dissolved inorganic carbon (DIC) and dissolved organic carbon (DOC) are low in the, upper, mixolimnion and increase with depth across an, intermediate, chemocline to peak in the, deep, monimolimnion (Tittel et al. 2003; Spijkerman et al. 2007). As the carbon equilibrium is dependent on pH, more than 99% of the DIC available for photosynthesis in this acidic water is in the form of CO_2. Species richness and biomass are generally low (Wollmann et al. 2000). The lake's truncated food web comprises bacteria, two phytoplanktonic flagellates (the chrysophyte *Ochromonas* sp. and the chlorophyte *Chlamydomonas acidophila*), two rotifers (*Cephalodella hoodi* and *Elosa worallii*) and one heliozoan (*Actinophrys sol*). Over 90% of the biomass of the DCM that forms at a depth of approximately 7 m is composed of *C. acidophila*, which allows focus on an almost monospecies layer. The low biodiversity and relatively few consumers also facilitate the investigation of ecophysiological strategies.

Culture conditions and growth rates

An axenic clone of *Chlamydomonas acidophila* Negoro (CCAP strain 11/137) isolated from Lake 111 (Bissinger et al. 2000; but distributed worldwide in acidic habitats) was grown semi-continuously in Erlenmeyer flasks at 22 ± 2°C (and later at 8°C) in Woods Hole medium, adapted and adjusted to a pH of 2.7 (Gerloff-Elias et al. 2005b). Cells were grown in a 16:8 h light:dark regime at six "culture" irradiances, ranging from 11 to 390 µmol photons m^{-2} s^{-1}, verified using a 4π quantum sensor (US-SQS, Walz, Germany). The in situ light quality of Lake 111 was simulated using neon lamps (TLD 58 W/930, Philips) that provided spectral wavelengths above 440 nm. All cultures were mixed regularly to prevent wall growth, and a total volume of 250 ml in each 500 ml flask ensured a large surface area for gas exchange. Cultures were diluted daily by adjusting optical density to 0.05 (measured at 750 nm in a 1 cm cuvette; using a Shimadzu UV mini-1240 spectrophotometer, Japan), daily growth rates (μ) were calculated, assuming exponential growth, and cell densities were determined with an automatic cell counter (CASY 1, Schärfe, Germany).

Chlorophyll concentration and photosynthesis

Culture material was concentrated to an optical density of 0.15–0.2 (at 750 nm) and dark adapted for 30 min (to standardise physiological base levels). Aliquots were subsequently taken for the measurement of chlorophyll *a* (Chl *a*) concentration, photosynthesis and cell density. To determine Chl *a* concentration, cells were collected by centrifugation (12,000×*g*, 5 min) and extracted in 90% acetone using a Mini Beadbeater 1 (Biospec Products Inc., The Netherlands). Absorption was measured by spectrophotometer (Shimadzu UV2401PC, Japan) at 750, 664 and 647 nm, and concentrations were calculated using the equations in Jeffrey and Humphrey (1975).

Oxygen evolution was measured at the cultivation temperature (22 or 8°C) over a range of eleven actinic light intensities (0–1,500 µmol photons m^{-2} s^{-1}) using a light pipette system (PLD 2, Topgallant LLC, USA). Rates of oxygen evolution, indicating photosynthesis (*P*), were corrected for Chl *a* and fitted to the equation

$$P = P_{\max}(1 - e^{-\alpha E/P\max}) + E\beta + R_{\mathrm{d}}, \quad (1)$$

where E is the photon irradiance, $P_{\max}$ is the maximum gross oxygen production, R_{d} is the respiration rate in the dark, α is the initial slope of the photosynthesis–irradiance (P–E) curve, and β is the slope at high irradiances that describes photoinhibition.

The threshold irradiance at which net photosynthesis equals zero ($E_{\mathrm{c,p}}$) was calculated by dividing R_{d} by α. Apparent photosynthesis (P_{app}) was defined as the P at culture irradiance E calculated from Eq. 1. Light compensation points for apparent photosynthesis and growth ($E_{\mathrm{c,app}}$ and $E_{\mathrm{c,g}}$) were derived from the points at which linear regressions, calculated from measurements of P_{app} and μ at the three lowest culture irradiances, intercepted the x axis.

Behavioural response

Live cells cultured at seven irradiances, spanning 10–180 µmol photons m^{-2} s^{-1} and from within the range used for physiological measurements, were exposed to a gradient of photon irradiance in a laboratory preference chamber. The chamber was comparable with that verified for ecological applicability in meso-scale trials in Clegg et al. (2003) and consisted of a glass microscope slide modified to form an enclosed cavity (55 mm long, 20 mm wide and 0.33 mm deep). The chamber was secured on the stage of an inverted microscope (Axiovert 25, Zeiss, Germany) and a gradient was produced across its length using a series of neutral density filters (Lee Filters, USA) fixed in position 30 mm above the chamber, beneath a 20 W halogen light source (Osram, Germany). This created six gradually increasing light zones with average irradiances of 10, 25, 50, 100, 180 and 230 µmol photons m^{-2} s^{-1}, quantified using a 2π sensor (LI-190, Li-Cor Products, USA). Small fans adjacent to the chamber prevented the build up of radiant heat, and thermocouples, attached to a digital thermometer (935, Testo, UK) and fixed in each end, verified that internal temperatures remained constant over time at 22 ± 0.5°C.

Culture suspension was injected into the chamber and population response and individual cell behaviour were recorded in separate experiments, following methods described in Clegg et al. (2003). Population response, defined as the difference between the initial and final cell distribution, was measured five times for each culture suspension. Differences in distribution were assessed using a log linear model (McCullagh and Nelder 1983) constructed within a GenStat (Lawes Agricultural Trust, UK) program, and the proportion of cells displaying a preference for each light zone was tested in a three-model nested F test (Hurley 1996). Finally, additional Tukey tests of pairwise differences distinguished the specific zones in which significant accumulations or decreases in cell concentration occurred.

Measurements of individual cells determined the factors driving population response, and characterised any behaviour as phototactic, ortho- or klino-photokinetic (Feinleib and Curry 1971). In four replicate experiments at each culture irradiance, the swimming tracks of 25 randomly selected cells in each light zone were recorded and measured. Cell swimming directions were expressed relative to chamber orientation, and were analysed using orientation analysis software (Oriana, Kovach Computing, UK). Watson's F test and Rayleigh's test of uniformity assessed if significant variation in swimming direction occurred across the chamber and if a preferred, phototactic, swimming direction existed. Swimming speeds were calculated and analysed statistically using one-way analysis of variance (ANOVA) and Tukey tests, to determine the ortho-photokinetic response. Klino-photokinetic response was tested by additional measurements of the frequency with which cells turned and stopped. Final measurements of the percentage of cells turning within each transition region, between the six light zones, identified potential photophobic reactions (after Diehn et al. 1977).

In vivo Chl *a* fluorescence monitoring

From May to August 2001, a multi-parameter probe (Ocean Seven 316), with an integrated Seapoint chlorophyll fluorometer (Idronaut, Italy), measured selected physical, chemical and biological parameters in Lake 111 at 10 cm depth intervals. Data were collected daily at 0:00, 6:00, 12:00 and 18:00 h, and the vertical migration of the DCM was determined by tracking the maximum point of the in vivo Chl *a* fluorescence profile. After verification by microscopic enumeration (to overcome interpretive fluorometric concerns: Cullen 1982), and due to the high sampling resolution and monospecies nature of the DCM, this point could be used as a reliable approximation of the vertical accumulation of *C. acidophila*. A typical midday fluorescence profile of Lake 111, illustrating the distinctive peak of the DCM, is shown in Fig. 1. The accompanying depth profiles are typical of the sampling period and show a steep thermal stratification, with the predominant algal species (*C. acidophila*) accumulating in oxic conditions at irradiances less than 1% of subsurface values. The vertical attenuation of light was independently verified using a 4π sensor (SQL, Li-Cor Products, USA), and remains largely constant throughout the summer. The quantity of light reaching *C. acidophila* in the DCM was calculated using an average attenuation coefficient ($k_{\mathrm{d(PAR)}}$) of 0.69 m^{-1} and global irradiances recorded at a nearby weather station, and was corrected

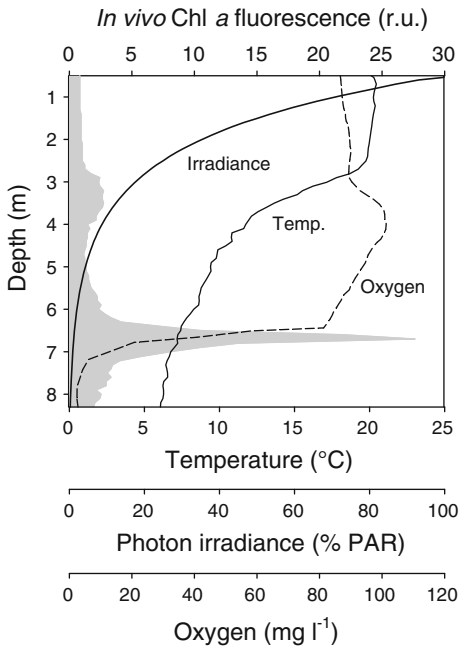

Fig. 1 Depth profiles showing the vertical distribution of chlorophyll *a* fluorescence (relative units, *shaded curve*), photon irradiance (percent of photosynthetically active radiation, % PAR, *thick line*), temperature (*thin line*) and oxygen (temperature-corrected percentage, *dashed line*) measured in Lake 111 at 12:00 h on a typical day (20 July 2001) using a multiparameter probe

for reflective loss at the water's surface (following methods in Kirk 1994).

Photosynthesis in the DCM

Finally, *C. acidophila* was grown in the laboratory under conditions simulating those found in the DCM of Lake 111. Cells were grown in Woods Hole medium at pH 2.7, at 8°C, and with 8 (±1) µmol photons m^{-2} s^{-1} of orange light, provided using a filter that only transmits wavelengths above 520 nm (filter no. 105, Lee Filters, UK), to emulate the spectral quality at depth in Lake 111. To determine the potential effects of the increase of CO_2 and DOC concentrations with depth (Tittel et al. 2003), cultures were either enriched with carbon (by aerating with 4.5% CO_2 and adding glucose to give 1.4 mmol DIC l^{-1} and 60 mmol DOC l^{-1}, respectively), or they received no additional carbon (beyond a headspace-derived equilibrium level of 0.075 mmol DIC l^{-1}). As the acclimation of organisms at low temperatures occurs slowly, incubations lasted three weeks, with fresh medium added weekly. Photosynthetic rates were determined as above, and the P_{app} of *C. acidophila* at the average DCM depth, and over the course of a day, was calculated using Eq. 1, measured photosynthesis parameters, and average hourly photon irradiances.

Results

Photosynthesis, respiration, and growth

Selected physiological characteristics of *C. acidophila* grown at different photon irradiances at 22°C and at DCM conditions (low irradiance, higher spectral wavelengths and 8°C) are summarised in Table 1. At 22°C, the maximum photosynthetic capacity (P_{max}) and dark respiration rate (R_d) increased with increasing culture irradiance and the ratio (R_d:P_{max}) remained similar. From a nadir at 44 µmol photons m^{-2} s^{-1}, the light compensation point for photosynthesis ($E_{c,p}$) derived from *P–E* curves increased with increasing and decreasing culture irradiances and ranged from 12 to 25 µmol photons m^{-2} s^{-1}. Both the P_{max} and R_d of algae grown at DCM conditions were comparable with those grown at similarly low culture irradiances at 22°C (*t* test, $df = 14$, $P = 0.27$ and $P = 0.68$), suggesting no significant temperature- or spectral-dependent reduction. There was also no significant effect of CO_2 aeration and glucose on the P_{max} and R_d of cultures grown at 8°C (*t* test, $df = 14$, $P = 0.15$ and 0.15, respectively). However, there was a significantly lower $E_{c,p}$ at 8°C (both with and without additional carbon) than at the lowest culture irradiance at 22°C (Mann–Whitney *U*, $df = 14$, $P < 0.01$), with $E_{c,p}$ values at 8°C comparable with (and not above) the culture irradiance, implying that purely autotrophic growth is just possible at the lower temperature.

Further analysis of photosynthesis and growth rates recorded over the range of culture irradiances (Fig. 2) allowed the calculation of light compensation points for apparent photosynthesis and growth at 22°C, giving an $E_{c,app}$ of 14 ± 1 and an $E_{c,g}$ of 19 ± 3 µmol photons m^{-2} s^{-1}, respectively (all values: mean ± SE). This implies that a minimum irradiance of 14 µmol photons m^{-2} s^{-1} is needed to maintain a population of *C. acidophila* at 22°C, and 19 µmol photons m^{-2} s^{-1} will allow population growth. Additional comparison of the rates of growth and dark respiration (Fig. 3) showed that growth was positively correlated with R_d over the range of culture irradiances, and the minimum dark respiration (R_0) was −15 ± 4 mmol O_2 g Chl a^{-1} h^{-1} (mean ± SE).

Behavioural response

Population response curves (Fig. 4a–g) showed that cells taken from all seven culture irradiances displayed a significant change from an initial, homogeneous distribution (modified log-linear model *F* test, $P < 0.01$). At culture irradiances of 10 to 70 µmol photons m^{-2} s^{-1}, there was a significant, preferential accumulation of cells at moderately low irradiances of 50 µmol photons m^{-2} s^{-1} (Tukey test, $P < 0.05$), and an avoidance of very low and high light.

Table 1 The oxygen evolution of *C. acidophila* at different culture irradiances (μmol photons m^{-2} s^{-1}), temperatures (*T*, °C), at DCM light ($\lambda > 520$ nm), and with 4.5% CO_2 aeration (+CO_2) and the addition of glucose (+Gluc)

Irradiance	*T*	Treatment	P_{max}	R_d	R_d:P_{max}	$E_{c,p}$
390 ± 29	22	–	285 ± 20	−48 ± 8	17 ± 3	25 ± 4
199 ± 5	22	–	280 ± 8	−40 ± 1	14 ± 0	19 ± 1
87 ± 4	22	–	180 ± 9	−24 ± 3	13 ± 2	15 ± 2
44 ± 2	22	–	123 ± 6	−16 ± 2	13 ± 2	12 ± 2
22 ± 1	22	–	128 ± 27	−21 ± 2	18 ± 3	16 ± 3
11 ± 1	22	–	104 ± 34	−15 ± 1	17 ± 5	22 ± 8
8 ± 1	8	$\lambda > 520$ nm	78 ± 13	−14 ± 4	17 ± 3	8 ± 2
8 ± 1	8	$\lambda > 520$ nm +CO_2 +Gluc	107 ± 14	−20 ± 3	17 ± 2	10 ± 1

Data presented include maximal oxygen evolution at saturating irradiance (P_{max}, mmol O_2 g Chl a^{-1} h^{-1}), dark respiration rate (R_d, mmol O_2 g Chl a^{-1} h^{-1}), dark respiration as a proportion of P_{max} (R_d:P_{max} in %), and the light compensation point for photosynthesis based on *P–E* curves ($E_{c,p}$, μmol photons m^{-2} s^{-1}). Means ± SE are given

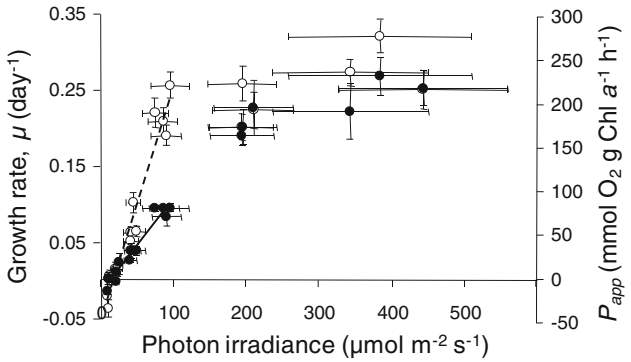

Fig. 2 The growth rates, μ (day^{-1}, *empty circles*), and apparent net photosynthesis, P_{app} (mmol O_2 g Chl a^{-1} h^{-1}, *filled circles*), of *C. acidophila* recorded over a range of culture photon irradiances at 22°C (means ± SE of three independent experiments). Compensation points for growth, $E_{c,g}$ (*dashed line*), and apparent photosynthesis, $E_{c,app}$ (*solid line*), were derived from the intercept of linear regressions of low-irradiance measurements with the *x*-axis

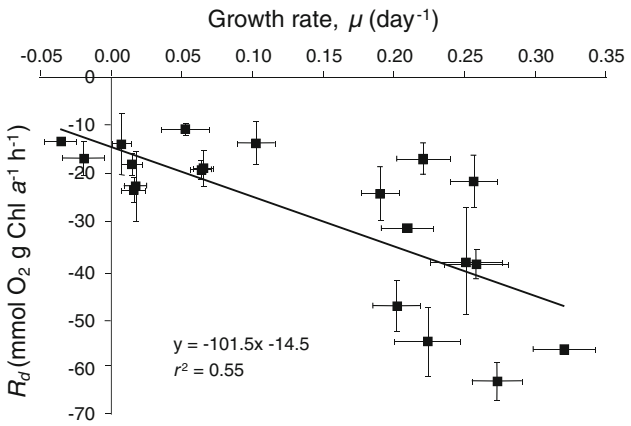

Fig. 3 A comparison of the growth rates, μ (day^{-1}) and dark respiration, R_d (mmol O_2 g Chl a^{-1} h^{-1}) in *C. acidophila* (means ± SE of three independent experiments). The linear trend shows a reduced major axis regression, with the *y* intercept representing the minimum dark respiration (R_0)

However, at culture irradiances of 100 and 140 μmol photons m^{-2} s^{-1}, cells displayed a significantly wider preference (modified *F* test, $P < 0.05$) for 50–100 μmol photons m^{-2} s^{-1} (Tukey test, $P < 0.05$). This acclimation was most apparent at the highest behavioural culture irradiance, 180 μmol photons m^{-2} s^{-1}, with cells displaying a broad preference for 50–180 μmol photons m^{-2} s^{-1} (Tukey test, $P < 0.05$). Although there was an adaptation of preference with increasing culture irradiance (modified *F* test, $P < 0.05$), the correlation is imperfect, with preferences generally skewed towards 50 μmol photons m^{-2} s^{-1}.

Measurement of the swimming behaviour of individual cells re-enforced population response results and identified the nature of the photoresponse. The mean swimming directions (Table 2) of *C. acidophila* cells cultured at 10 μmol photons m^{-2} s^{-1}, for example, showed a clear directionality of response, with cells orientated towards the 50 μmol photons m^{-2} s^{-1} zone, within which, directionality was significantly reduced (Rayleigh's test of uniformity, $P > 0.05$). In low-irradiance zones, cells displayed a positive photo-orientation towards higher irradiance, whereas in high-irradiance zones there was a significant switch to negative photo-orientation (Watson's *F* test, $P < 0.001$), as cells swam away from high light towards lower irradiances. Similar results were found for the other six culture irradiances, with cells swimming towards the widening preferences observed in population response experiments, indicating the existence of a flexible, tactic photoresponse in *C. acidophila*.

Simultaneous measurement of cell swimming speed showed no significant variation (from a mean of 87 ± 20 μm s^{-1}) across the light gradient, or with increasing culture irradiance (two-way ANOVA, $P > 0.2$), discounting a possible ortho-photokinetic influence. Similarly, no significant variation in turning or stopping frequency (two-way ANOVA, $P > 0.2$) suggested no klino-photokinetic

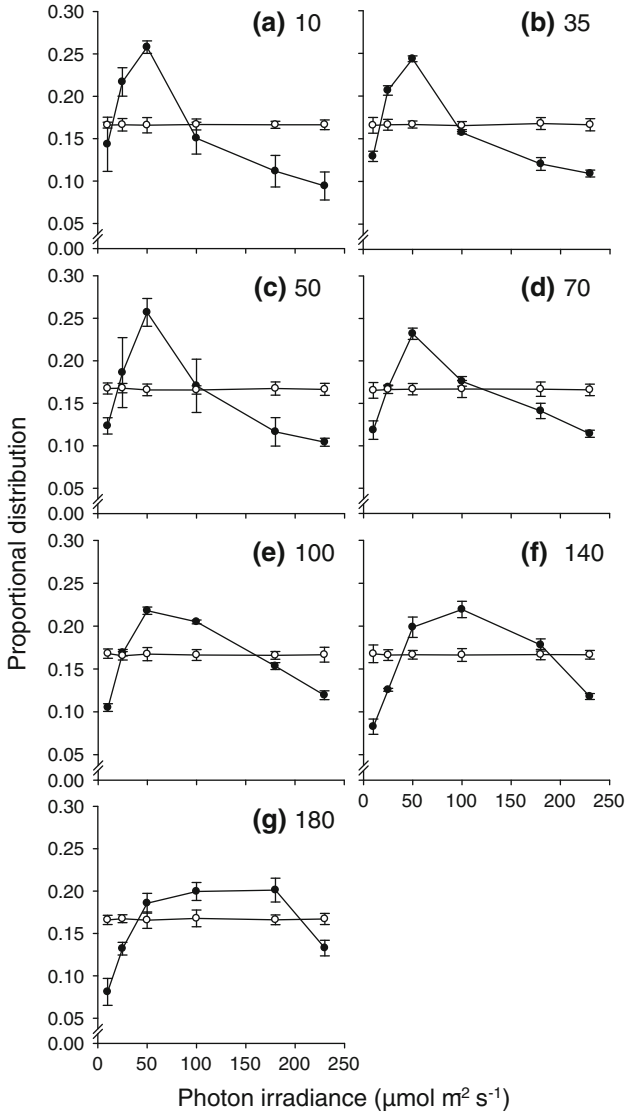

Fig. 4 Population response curves showing the distribution of *C. acidophila* taken from seven different culture irradiances: **a** 10 **b** 35 **c** 50 **d** 70 **e** 100 **f** 140 **g** 180 µmol photons m^{-2} s^{-1}, and exposed for a duration of 35 min to the gradient of photon irradiance (10 to 230 µmol m^{-2} s^{-1}) within the preference chamber. The initial (*empty circles*) and final (*filled circles*) distributions of cells are presented as a proportion of 1 (with maximum likelihood estimates ±2SE)

influence. However, final kinetic measurements did show that phobic response might affect behaviour, as significant increases (from a mean of 33%, up to 60%) in the percentage of cells turning at the boundaries of zones of preference (ANOVA, $P < 0.005$; Tukey test, $P < 0.05$) facilitated accumulation within these zones.

Migration

In addition to laboratory analyses of behaviour, in vivo fluorescence was used to determine variations in the average population depth of *C. acidophila* in Lake 111 over time, compared with changes in the irradiance reaching these algal maxima (Fig. 5). Results showed that the population of *C. acidophila* frequently exhibited small, diel, vertical migrations that were synchronised and correlated with the changing light. For example, the mean value of the difference in depth of the Chl *a* fluorescence maximum from 0:00 to 6:00 h was significantly higher than zero (*t* test $P < 0.001$, $n = 74$), suggesting an upward migration of cells in the early morning. Conversely, the mean difference in depth from 18:00 to 0:00 h was lower than zero (*t* test $P < 0.001$, $n = 74$), indicating downwards migration in the evening.

There was also a positive linear correlation (Fig. 6) between the extent of upwards migration (i.e. the difference in depth between 0:00 and 12:00 h) and the depth of the population at 0:00 h ($P < 0.001$, $n = 73$). This indicated that cells typically migrated over a larger distance when they were deeper in the water column at 0:00 h and less when shallower. For example, no upward migration occurred on 35% of the sample days, when the DCM was already relatively shallow. When upward migration did occur, a significant positive linear relationship was observed between the quantity of light at dawn (an average of the irradiances at 5:00 and 6:00 h) and the extent of upward migration ($P < 0.01$, $n = 41$). On average, the amplitude of this upward migration was only 0.3 m, and the population density maximum never migrated to a depth shallower than 6.4 m (Fig. 5).

Daily photosynthesis and the energetic impact of mixotrophy and migration

Calculation of the light reaching in vivo Chl *a* fluorescence maxima indicated that *C. acidophila* was found at a depth with an average irradiance of 8 (±1) µmol photons m^{-2} s^{-1} at 12:00 h: a level close to the $E_{c,p}$ at 8°C (Table 1), but lower than compensation points at 22°C and behavioural preferences. Moreover, the daily average irradiance was only 4.6 µmol photons m^{-2} s^{-1} (assuming a light:dark regime of 16:8 h and an average population depth taken from all in vivo maxima). Consequently, estimations of the apparent photosynthetic rates in the DCM showed that *C. acidophila* was only able to produce O_2 for a small proportion of the day (Fig. 7). However, when cultures were acclimated to DCM conditions with higher concentrations of CO_2 and glucose, the calculated amount and duration of O_2 production increased (Fig. 7). Similarly, on days when migrations occurred, the amount and duration of photosynthesis also increased significantly (*t* test $P < 0.001$). However, as illustrated in Fig. 7, calculations indicated that this migration would not result in sufficient photosynthesis to outweigh respiratory losses (as R_d:P_{app} at 4.6 µmol photons m^{-2} s^{-1} was −1.9). The minimum daily average irradiance needed to

Table 2 Mean swimming directions and orientation of *C. acidophila* cells, cultured at seven different irradiances, in each of the six zones of the preference chamber (10, 25, 50, 100, 180 and 230 μmol photons m^{-2} s^{-1})

Culture irradiance (μmol photons m^{-2} s^{-1})	Preference chamber zone (μmol photons m^{-2} s^{-1})						Cell preference (μmol photons m^{-2} s^{-1})
	10	25	50	100	180	230	
10	101 (23)+	92 (23)+	22 (46)	273 (30)−	291 (28)−	271 (15)−	50
35	97 (25)+	84 (34)+	85 (65)	239 (31)−	265 (25)−	267 (19)−	50
50	80 (24)+	86 (29)+	357 (132)	245 (20)−	279 (21)−	260 (22)−	50
70	88 (18)+	92 (31)+	305 (82)	292 (29)−	288 (35)−	263 (16)−	50
100	74 (23)+	74 (29)+	81 (89)	331 (218)	281 (24)−	284 (19)−	50–100
140	92 (12)+	76 (35)+	97 (65)	336 (120)	261 (19)−	256 (19)−	50–100
180	88 (17)+	94 (24)+	66 (200)	336 (60)	274 (87)	249 (17)−	50–180

Swimming directions are expressed relative to chamber orientation (±95% confidence intervals), with a 90° swimming direction indicating movement towards higher light and a 270° movement towards lower light. Where a significant, unimodal directionality is suggested by Rayleigh's test of uniformity, positive photo-orientation (+) towards higher irradiances and negative photo-orientation (−) away from higher irradiances are indicated, and individual cell preferences are quantified

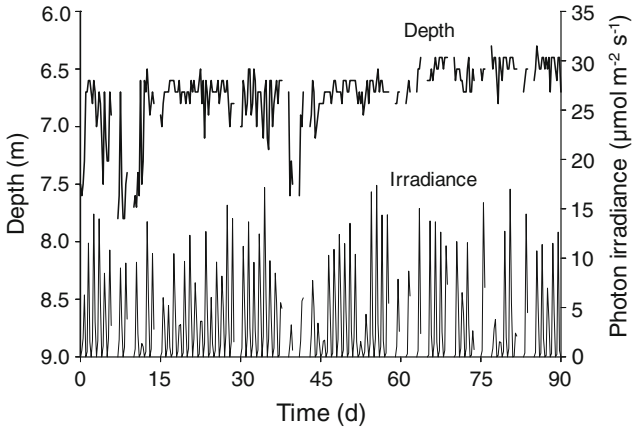

Fig. 5 A time series showing the vertical position of in vivo Chl *a* fluorescence maxima in Lake 111, measured at 0:00, 6:00, 12:00 and 18:00 h for 74 days during stratification from May to August 2001 (depth, *thick line*), and the corresponding underwater photon irradiance (PAR, μmol m^{-2} s^{-1}) at these depths (irradiance, *thin line*). The depths of the in vivo Chl *a* fluorescence maxima shown here represent the vertical position of the *C. acidophila* DCM. Short interruptions in the collection of data were due to periods of technical maintenance

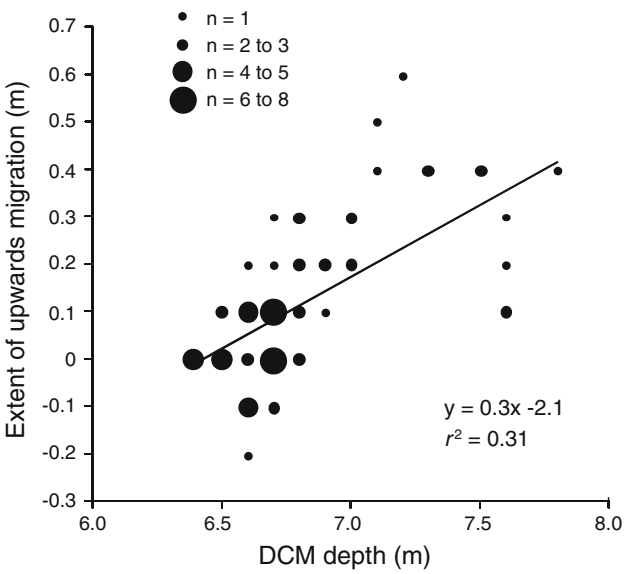

Fig. 6 The impact of DCM depth at midnight (0:00 h) on the extent of upwards migration of *C. acidophila* on the following day ($n = 73$; derived from 74 sampling days, with one outlier omitted). The extent of upwards migration was derived from depth differences between 0:00 and 12:00 h, with negative values signifying downward movement. Data point frequency (n) is indicated by the size of *filled circles*

achieve an R_d:P_{app} of −1, required for photosynthesis to exceed respiration, would be 24 μmol photons m^{-2} s^{-1} (at high CO_2 and glucose); considerably more light than reached *C. acidophila* in the DCM of Lake 111.

Discussion

Physiological acclimation and its influence within the DCM

Species accumulating in DCMs are regularly subjected to extremely low light, which may increase maintenance costs and divert energetic resources from growth, protein synthesis and cell replication. Ideally, it would therefore be beneficial if DCM species were able to reduce metabolic maintenance costs and/or acclimate their photosynthetic apparatus.

One possible mechanism for decreasing metabolic costs is through the reduction of respiratory losses (e.g. Geider and Osborne 1989). The proportion of dark respiration to maximum photosynthesis (R_d:P_{max}) measured here was 13–18% (Table 1). Although lower than in some motile green algae (Striebel et al. 2009), this is not exceptionally

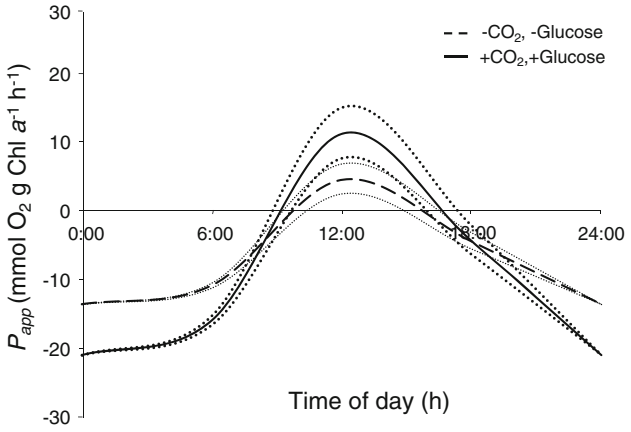

Fig. 7 Estimated diel variation in the P_{app} (mmol O_2 g Chl a^{-1} h^{-1}) of *C. acidophila* in the DCM of Lake 111; calculated using Eq. 1, average hourly irradiances, and photosynthesis and respiration data from cultures acclimated to DCM conditions either without (*dashed line*) or with (*solid line*) 4.5% CO_2 aeration and glucose. In each case, the *dashed* or *solid line* represents the P_{app} realised at the average DCM depth of the *C. acidophila* population, and *dotted lines* represent the potential maximum and minimum P_{app} resulting from migration

low and falls well within the range of 5–22% observed in other Chlorophyceae (Geider and Osborne 1989). Another commonly used indicator of metabolic costs is minimum dark respiration (R_0), which is often determined by calculating the intercept of the linear relationship between R_d and growth. Using this method, and assuming a photosynthetic quotient of 0.714 mol CO_2 mol^{-1} O_2 (for nitrate media: Quigg and Beardall 2003) and a largely stable Chl *a*: C ratio (0.023 g Chl *a* g C^{-1}: own measurements), the R_0 of −15 mmol O_2 g Chl a^{-1} h^{-1} (extrapolated from Fig. 3) corresponds to a value of 0.07 d^{-1} for *C. acidophila*. This value is lower than the basal respiration rate of 0.29 d^{-1} displayed by another chlamydomonad (Striebel et al. 2009; calculated assuming a 16 h light period), but is well within the range (0.01–0.20 d^{-1}) of green algae listed in Geider and Osborne (1989). The R_0 value therefore suggests that *C. acidophila* exhibits relatively average (although perhaps slightly low) respiratory maintenance costs compared to other phytoplankton that, alone, are not fully conducive to survival in the DCM. This potential energetic problem might be partially overcome, however, if *C. acidophila* were also able to increase, adapt or acclimate its photosynthetic abilities.

Photosynthesis depends on the interception and absorption of photons, and both aquatic and terrestrial organisms have developed multifarious solutions for the difficulties caused by low light. Problematically, however, green algae are generally thought to have higher light compensation points than other algal phyla (Richardson et al. 1983) and, in addition to spectral considerations, this may be one reason why they are often less abundant in deeper waters. For example, Richardson et al. (1983) reported $E_{c,g}$ values for several green microalgae of around 20 µmol photons m^{-2} s^{-1} (under continuous illumination), although lower values have been recorded elsewhere (Rhee and Gotham 1981; Striebel et al. 2009). In this study, we calculated an $E_{c,app}$ of 14 and an $E_{c,g}$ of 19 µmol photons m^{-2} s^{-1} for *C. acidophila* at 22°C, and an $E_{c,p}$ of 8–10 µmol photons m^{-2} s^{-1} at 8°C. These values are comparable with the $E_{c,g}$ of 20 µmol photons m^{-2} s^{-1} determined by Tittel et al. (2005) at 20°C, using a different medium, and the $E_{c,app}$ at 22°C is also within the range of other motile green algae (Striebel et al. 2009). Even though these values do indicate some propensity for low-light acclimation, the scale of any photophysiological adjustment again appears rather limited, compared with other DCM species that can grow at as low as 2 (e.g. *Planktothrix rubescens*: Bright and Walsby 2000) or even 1 µmol photons m^{-2} s^{-1} (e.g. *Phaeodactylum tricornutum*: Geider et al. 1985).

In contrast to the energetic burden of low light, low temperatures (as encountered in this and many DCMs) can often decrease metabolic activities, lower nutrient demands, and assist survival (e.g. Richardson et al. 1983). Interestingly, *C. acidophila*'s $E_{c,p}$ did decrease with temperature, however, its P_{max} and R_d were unaffected, so only differences in photosynthetic efficiency (or spectral influences) could have resulted in these lower values at 8°C. Importantly, as the relative respiration rates did not differ with temperature (R_d:P_{max} was constant), *C. acidophila* can compensate respiration at a lower photon irradiance at 8°C, which may influence in situ growth and partially assist survival in the DCM. A schematic summary of potential physiological influences is presented in Fig. 8a. In general, however, *C. acidophila* remains less well acclimated to low light and temperature than some species, and results imply that other ecophysiological mechanisms might also contribute to its survival.

Behavioural influences within the DCM

The controlled behavioural response of *C. acidophila* (summarised in Fig. 8b), for example, may supplement acclimation by providing a flexible spatial and temporal strategy by which to avoid the harshest of environmental conditions. For the first time (although postulated in the field: Doi et al. 2003), laboratory experiments discovered and characterised a light-dependent behavioural response in *C. acidophila* (Fig. 4; Table 2), showing a controlled phototactic orientation towards a preference of around 50 µmol photons m^{-2} s^{-1}, maintained by photophobic reactions. This was comparable with the preference for 35 µmol photons m^{-2} s^{-1} displayed by a member of the same genus, *C. moewusii* (Clegg et al. 2003). It was,

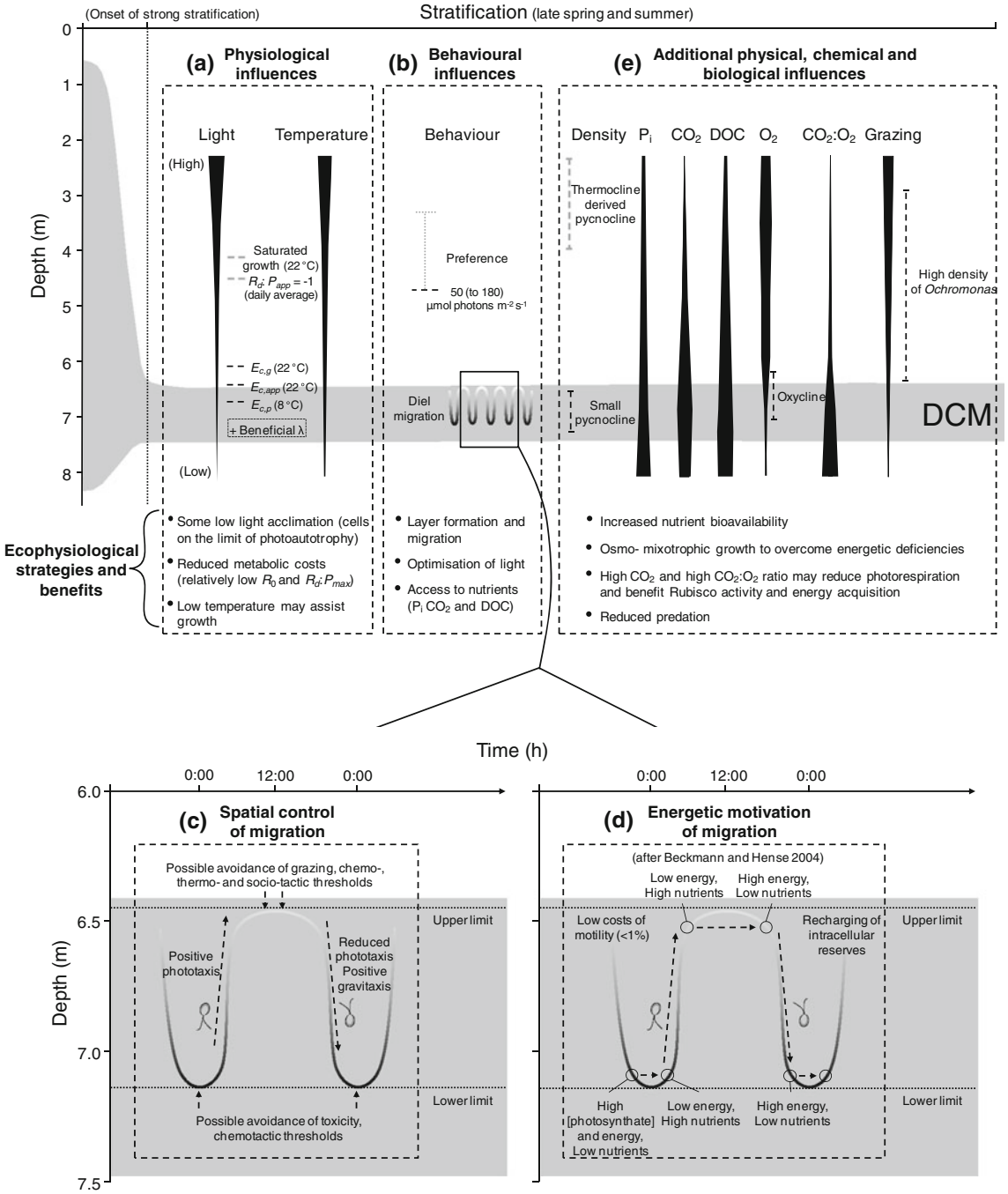

Fig. 8 A schematic diagram of the major factors that are potentially responsible for the formation, position, and persistence of the *C. acidophila* DCM in Lake 111, illustrating: **a** the major physiological influences; **b** the major behavioural influences, including the potential **c** spatial control and **d** energetic motivation of diel vertical migration within this layer; and **e** the additional physical, chemical and biological influences. In each case, the resulting ecophysiological consequences and benefits of adaptable strategies are also indicated

however, slightly higher than physiological compensation points; although, when behavioural acclimation or plasticity is also considered (preferences spanned 50–180 µmol photons m^{-2} s^{-1}), it was comparable with irradiances saturating growth (~80 µmol photons m^{-2} s^{-1}; Fig. 2). In addition to this possible physiological link, it is likely that photoresponse also helps control the relatively regular, small-amplitude, diel vertical migrations observed in Lake 111 (Fig. 5).

Diel vertical migration in phytoplankton can exceed several metres (Kamykowski 1981; Sommer and Gliwicz 1986) or, as in this study, can be in the range of centimetres

(Arvola et al. 1992; Gervais 1997). The diel synchronization of light and distribution in Lake 111 suggests that photoresponse may influence migration and the position of this DCM, as part of an overriding circadian rhythm. At dawn, positive phototaxis may trigger an ascent of cells towards higher light, photophobic reactions and negative phototaxis away from high light could then help maintain cells deep in the water column, and, at dusk, the superseding importance of gravitaxis (Kessler et al. 1992) could initiate a downward migration (Fig. 8c).

The vertical position of aggregated cells and their separation into discrete layers may be influenced by quantifiable behavioural preferences (e.g. Clegg et al. 2007) or the end limits of vertical migrations (e.g. Klausmeier and Litchman 2001). However, neither photoresponse nor migration fully explains the position of this DCM. For example, if *C. acidophila* were to respond solely to preferences for irradiance, it would accumulate at a depth of about 4.7 m (Fig. 8b). Moreover, when the average swimming speed of 87 μm s^{-1} is related to the low-amplitude migration of 0.3 m, it is clear that *C. acidophila* does not fully exploit its capacity for migration (a trend also found in cryptomonads: Gervais 1997). Of course, additional factors, such as secondary tactic responses to chemical gradients (e.g. Clegg et al. 2004b), gradients of temperature (Kamykowski 1981), grazing pressure (Tittel et al. 2003), nutrient limitation (Beckmann and Hense 2004), or even an avoidance of toxicity (Gervais et al. 2003), might also influence vertical position and potentially curtail this migration (Fig. 8c).

Importantly, however, and in addition to these spatial considerations, behavioural preferences and migration also have the potential to interact energetically with physiological responses, and only when laboratory measurements are considered in the context of in vivo field observations is it possible to assess this potential combined influence on survival.

An integrated analysis of physiology and behaviour

Integrated results showed that rates of photosynthetic O_2 production realised during the day cannot generally compensate for respiratory losses during the night (Fig. 7), and *C. acidophila* therefore cannot grow purely photoautotrophically in the DCM of Lake 111. In fact, photoautotrophy alone is insufficient to provide a net excess of energy below a depth of about 4.5 m (with its average light of 24 μmol photons m^{-2} s^{-1}, needed to achieve an R_d:P_{app} of −1).

Laboratory analyses also suggested that *C. acidophila* should actively position itself between light compensation and saturation points for growth (Fig. 8a, b). However, field observations showed that cells aggregated at an average daily irradiance below compensation points. The energetic problems associated with this may be partially resolved over time by small migrations (e.g. Fig. 7), which can even be triggered by, or synchronised with, physiological or nutritional status (Kamykowski and Yamazaki 1997; Clegg et al. 2003), whereby cells might only migrate when they are running out of energy (Heaney and Eppley 1981; Beckmann and Hense 2004), recharging intracellular reserves via a temporal and vertical decoupling of photosynthesis and nutrient assimilation. Interestingly, this physiological mediation may occur in Lake 111 (Fig. 8d); where *C. acidophila* migrated upwards significantly more when deeper, in more detrimental light conditions (Fig. 6).

Even though such migrations confer possible advantages by optimising diurnal light utilisation and increasing nocturnal access to nutrients (Jones 1993), they are not without physiological expense. However, the consistent flagellar propulsion of *C. acidophila* appeared unconstrained by proportional costs for motility of 0.1–1% (calculated using the metabolic cost model: Crawford 1992), even at low light (Raven and Richardson 1984), and was therefore unlikely to restrict migration energetically or limit the position of this DCM.

Although helpful, the small-scale migrations observed in Lake 111 do not compensate fully for any photoautotrophically derived energetic deficiency (Fig. 7), and so this may advocate the potential importance of alternative energetic strategies in this species and of additional factors within, and, as controls upon the position of, the DCM (Fig. 8e).

Additional influences in the DCM

In addition to the constraints of low light, the growth of *C. acidophila* in Lake 111 is believed to be limited or co-limited by either inorganic phosphorus (P_i: Spijkerman et al. 2007) and/or carbon (CO_2, DOC: Tittel et al. 2005). Similarly, the formation of thin vertical strata has been linked to the bioavailability of mineral nutrients and the position of nutriclines (e.g. Cullen 1982). However, although P_i concentrations generally increase with depth in Lake 111 (Fig. 8e), the gradient is probably insufficiently steep to provide a satisfactory explanation for the biomass in this DCM, or its precise vertical position. Clear gradients of CO_2 and DOC also occur (Fig. 8e), but as concentrations are already significantly increased at a depth of 5 m (Tittel et al. 2003), the aggregation below 6.4 m may not be dictated by carbon limitation. Nevertheless, *C. acidophila* cultures aerated with CO_2 did display enhanced photosynthesis (Table 1; Fig. 7), and so the increased carbon deeper in Lake 111 might assist in situ growth.

Additionally, slow rates of growth ($\mu = 0.05$ d^{-1}) have previously been observed in laboratory experiments conducted at low light (7 μmol photons m^{-2} s^{-1}) with filtered DCM water (Tittel et al. 2003), even though the use of

DOC by *C. acidophila* is restricted (Tittel et al. 2009). Hypothetically, the nutritional versatility afforded by a switch to heterotrophy in darker conditions may help survival by supplementing carbon fixation and energy budgets (as in Antarctic cryptophytes; Laybourn-Parry 2002). Results from this study (Fig. 7) suggest that a periodic, mixotrophic acquisition of energy (via combined auto- and osmotrophy) may help balance metabolic rates, thereby contributing to the persistence of *C. acidophila* in this DCM.

The depth of the DCM also coincides with a slight increase in water density (0.15 kg m^{-3}: Karakas et al. 2003). This small pycnocline (situated below the main thermocline-induced increase: Fig. 8e) is unlikely to restrict behavioural movement, but may help cells to maintain their position and prevent some passive sinking (Steinbuck et al. 2009). Perhaps more importantly, the initial occurrence of the DCM during April, and its depth, also corresponds with a decrease in O_2 concentration and the start of a steep oxycline (Figs. 1, 8e).

In some eutrophic waters, green algal DCMs can occur close to the oxycline (Gaevsky et al. 2002; Gervais et al. 2003), as lower oxygen (and temperatures) may help decrease losses via zooplankton grazing (e.g. Arvola et al. 1992; Kessler and Lampert 2004). Top-down control by herbivory, however, is rather limited in Lake 111, due to the lack of crustacean zooplankton (e.g. Weithoff 2004). In fact, "intra-guild" predation by the phago-mixotrophic *Ochromonas* sp. may be the most significant loss to grazing (Tittel et al. 2003). Indeed, its abundance at shallower depths (Fig. 8e) may help explain the low density of *C. acidophila* in surface waters, and may influence the upper boundary of this DCM (cf. depth-differential grazing: Longhurst 1976). Active migration away from such predation (potentially mediated by kairomones: Latta et al. 2009) may also contribute to the vertical position and discrete nature of this layer (Fennel and Boss 2003).

It is also possible that the more anoxic conditions close to the oxycline might benefit *C. acidophila* physiologically. Many chlamydomonads are often found in oxygen-deficient habitats, and can even show a direct behavioural affinity for low concentrations of O_2 and for high CO_2 (Clegg et al. 2004b). Changes in the CO_2:O_2 ratio associated with an oxycline (e.g. Fig. 8e) may also reduce photorespiration and benefit Rubisco activity. The energetic advantages of increased CO_2 have been described (Table 1; Fig. 7); however, O_2 concentrations in these experiments approached 100% saturation. Consequently, a slight reduction in O_2 at depth may increase carbon fixation rates, improve P_i bioavailability, and present increased photoautotrophic possibilities. This may assist survival, and so its potential effect on the formation of the DCM in Lake 111 should also not be overlooked.

Synthesis and concluding remarks

Theoretically, species in extreme environments or at the boundaries of their ecological limits can be (or become) more specialised, in which case they may be prolific competitors, although more vulnerable to environmental change, or they may be (or become) more adaptable generalists, in which case they may be less competitive and yet more able to deal with a fluctuating, often ephemeral environment. In many cases, an ecophysiological balancing act of specialisation, adaptable strategies, overlapping mechanisms and broad, flexible tolerances may be required to enable a species to survive and thrive under seemingly inhospitable ambient environmental conditions that would prove fatal to most organisms.

The metabolic, photosynthetic, behavioural and migratory characteristics of *C. acidophila* do not fully explain its accumulation in a distinct DCM below 1% of subsurface light. Although moderate low-light acclimation, small-scale directed movements towards light, mixotrophic nutrition, and advantageous low temperatures in the DCM do help support growth, laboratory measurements indicated that the low photon irradiances reaching the DCM were insufficient for a net positive energetic balance of photosynthesis and nocturnal respiratory losses. The small-scale, flexible migration observed may be spatially and energetically important, and appears to be physiologically mediated, but is regulated by parameters other than light alone. Photoautotrophy in the DCM may also be influenced by the changing chemical conditions associated with the oxycline, which include an increase in the bioavailability of nutrients, and may delineate the interface of physically and chemically beneficial environments and the position of an ecological niche in which light and nutrient requirements can be balanced most advantageously. Equally, intra-guild predatory control by *Ochromonas* might also influence the final vertical position of the DCM.

Illustrated schematically in Fig. 8a–e, we therefore suggest that the DCM in Lake 111, and its vertical position, is maintained by a complex combination and reciprocal interaction of growth and maintenance strategies, behavioural movements and trophic characteristics, with additional influences potentially exerted by predation pressure, P_i, CO_2, and O_2 concentrations. Corroborating the deceptive ecophysiological complexity of *C. acidophila*, it is most likely that only a suite of endogenous physiological, behavioural and nutritional strategies (analogous to those typically displayed by adaptable, *K*-, rather than opportunistic, *r*- type, strategists) can combine to surmount the detrimental environmental conditions, optimise fitness, and facilitate survival in the DCM of this stratified system.

This study emphasises not only the potential importance of adaptable ecophysiological strategies for the survival

and occurrence of organisms in deep chlorophyll maxima but also their potential contribution to the formation and maintenance of these significant components of the aquatic environment. These findings help improve our understanding of the complex ecophysiological strategies invoked within a DCM, and can be applied to a wide range of freshwater and marine ecosystems. Even at the microbial level, this investigation therefore highlights the importance of an integrative approach to the study of the ecology of organisms, and particularly to those that comprise deep chlorophyll maxima.

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