Comparison of the depth where *Planktothrix rubescens* stratifies and the depth where the daily insolation supports its neutral buoyancy

Anthony E. Walsby, Gabrielle Ng, Catherine Dunn and Phillip A. Davis

School of Biological Sciences, University of Bristol, Bristol BS8 1UG, United Kingdom

Author for correspondence:
Anthony E. Walsby
Tel: +44 (0)117 9287490
Email: a.e.walsby@bristol.ac.uk

Received: *26 August 2003*
Accepted: *28 November 2003*

doi: 10.1111/j.1469-8137.2004.01020.x

Summary

- Comparisons were made of the depths where the cyanobacterium *Planktothrix rubescens* stratified in lakes and the depths where the daily insolation supported neutral buoyancy of the organism.
- The percentage of filaments floating and sinking were measured in cultures grown under light : dark cycles at different irradiances. Q_n, the daily insolation for neutral buoyancy, was determined as that at which 50% of the filaments were floating, averaged over the 24-h period.
- For *P. rubescens* 9316 from Lake Zürich, Q_n was 0.28 mol m^{-2}; during the summer period of stratification in Lake Zürich, the neutral buoyancy depth (z_n) at which this insolation occurred varied between 5 and 14 m, depending on cloud cover and light attenuation. The weekly mean depth of the *Planktothrix* population maximum (z_p) was usually within 1 m of the mean z_n. For *P. rubescens* 9972 from Blelham Tarn, Q_n was 0.51 mol m^{-2}; during the stratified period in Blelham Tarn, z_n varied between 2.3 and 6.2 m, also similar to z_p; the shallower stratification depth is explained by the steeper light attenuation.
- The depth at which *Planktothrix* stratifies in lakes is therefore explained by buoyancy regulation in relation to the irradiance. Metalimnetic stratification occurs only when z_n exceeds the mixed depth, z_m; when $z_m > z_n$, the organism becomes entrained in the surface mixed layer.

Key words: cyanobacteria, *Planktothrix rubescens*, buoyancy regulation, stratification, Blelham Tarn, Lake Zürich.

Introduction

In stratified lakes, cyanobacteria often form a population maximum in the metalimnion (Thomas & Märki, 1949; Lund, 1959; Zimmermann, 1969; Klemer, 1976; Konopka, 1989). At the depths where they stratify there is usually a steep temperature gradient and a low irradiance, close to the compensation points for photosynthesis (Micheletti *et al.*, 1998) and growth (Walsby & Schanz, 2002). The temperature gradient provides the stable density gradient required to resist vertical mixing that would otherwise disperse the population. It is thought to be the gradient of irradiance, however, that the cyanobacteria use to regulate their position in the metalimnion (reviews by Reynolds, 1987; Oliver, 1994; Walsby, 1994; Oliver & Ganf, 2000).

The role of light in stratification was first suggested by observations that cyanobacteria with gas vesicles floated in cultures kept at low irradiance but sank at high irradiance (Walsby, 1969; Meffert, 1971). The buoyancy increase at low irradiance results from a relative increase in gas-vesicle volume and decrease in the denser cell components, especially carbohydrate, which is respired or converted to less-dense protein. At high irradiance, buoyancy is lost because of the relative decrease in gas vesicle volume and increase in carbohydrate and other dense components (Oliver & Walsby, 1984; Thomas & Walsby, 1985; Utkilen *et al.*, 1985). Details of the response, and its variation with nutrient conditions in different species,

Dedicated to the memory of John Gallon (1944–2003), Editor of *New Phytologist*

are reviewed by Konopka (1984), Reynolds (1987), Oliver (1994) and Walsby (1994).

Buoyancy change in response to irradiance has been observed in many cyanobacteria including species of *Anabaena* (Dinsdale & Walsby, 1972; Walsby *et al.*, 1989), *Aphanizomenon* (Konopka *et al.*, 1987b, Porat *et al.*, 2001), *Dactylococcopsis* (Walsby *et al.*, 1983b), *Microcystis* (Konopka *et al.*, 1987a) and *Planktothrix* (*Oscillatoria*) (Walsby *et al.*, 1983a). In their natural habitats many of these organisms show vertical migrations that might be explained by buoyancy regulation but those best known for forming stable metalimnetic populations are species of *Planktothrix*.

Evidence for the role of buoyancy change by cyanobacteria stratifying in lakes has come from experiments in which samples from the metalimnion were suspended in transparent containers at different depths: when samples of *Planktothrix* (*Oscillatoria*) *agardhii* from the population maximum at a depth of 5 m in Deming Lake, Minnesota, were incubated nearer the water surface the percentage of filaments that were floating decreased but at greater depths the percentage that were floating increased (Walsby & Klemer, 1974). The buoyancy of *P. rubescens* (a red coloured *O. agardhii*) in Lake Gjersjøen, Norway similarly increased with depth; at all depths buoyancy increased during the night and decreased during the day, suggesting control was by light. Filaments suspended in darkened containers became buoyant at all depths, proving that the buoyancy loss in uncovered containers was in response to light (Walsby *et al.*, 1983a). Kromkamp & Walsby (1990) made quantitative measurements of the light-dependent rate of density change by *P. rubescens* isolated from Lake Gjersjøen and used the measurements in a computer model to show that the stratification and vertical migration by filaments in the lake could be explained by irradiance-driven buoyancy regulation.

While these studies have suggested a plausible mechanism for stratification by metalimnetic cyanobacteria, the evidence for it is incomplete. The expectation is that, at equilibrium, the depth of the population maximum (z_p) should be similar to the depth at which filaments become neutrally buoyant (z_n) in response to the ambient irradiance. In the few observations made, however, there are inconsistencies. In Lake Gjersjøen, the depth of the *Planktothrix* population maximum was at 6 m whereas the filaments were neutrally buoyant at a depth closer to 4 m (Walsby *et al.*, 1983a). In Lake Zürich, about 80% of the *Planktothrix* filaments were buoyant at the 11-m depth of the population maximum, and they remained buoyant after incubation for 7 h in the light at that depth (Walsby *et al.*, 2001). In each case it was suggested that the population was migrating up in response to previous decreases in irradiance caused by shortening day length, greater cloud cover or increased light attenuation in the overlying epilimnion. In fact, discrepancies between z_p and z_n are to be expected because it takes time for a filament to adjust its buoyancy in response to the changed irradiance and then to move depth. With constantly changing irradiance, the system will never be at equilibrium. Another possible cause of the difference is that the buoyancy state at a given irradiance may vary with the availability of different nutrients (Walsby, 1994); at low irradiances, the increase in gas vesicle production and buoyancy may depend on the availability of combined nitrogen (Klemer, 1978; Klemer *et al.*, 1982; Spencer & King, 1985) and phosphate (Konopka *et al.*, 1987a, b).

Although it may be difficult to account for the finer details of movement involved in vertical stratification, it should be possible to account for the major changes in population depth in different lakes by making broad comparisons between z_p and z_n over longer periods. As a test of this, we have determined the buoyancy state expressed as the percentage of filaments floating (% F) of a Lake Zürich strain of *P. rubescens* grown on light : dark cycles giving different daily insolations (Q, the integral of irradiance received over the 24-h period; Kirk, 1994) to find the insolation (Q_n) that supports neutral buoyancy, taken as 50% filaments floating, averaged over the day. From continuous records of irradiance and vertical light attenuation in Lake Zürich (Walsby & Schanz, 2002) we then determined the depth at which Q_n occurred each day, which we used as a measure of z_n, the 'the neutral buoyancy depth' at which 50% of the *Planktothrix* population would be buoyant, averaged over the daily cycle. This was compared with z_p, the depth of the population maximum, to determine which of the movements of the population over the annual cycle can be explained by buoyancy regulation. We have also performed a similar investigation with *P. rubescens* isolated from Blelham Tarn, a much smaller lake in which the light is more steeply attenuated and the population maximum occurs at a shallower depth (Davis *et al.*, 2003b).

Methods

Cultures of *Planktothrix*

The strains of *P. rubescens* used were from the Bristol Collection: *Pla* 9316 (CCAP 1459/41) isolated from Lake Zürich (Walsby *et al.*, 1998); *Pla* B9972 (CCAP 1460/19) from Blelham Tarn (Davis *et al.*, 2003a). Stock cultures and experimental cultures were grown at 20°C on 12 : 12 h light–dark cycles. For determination of buoyancy change, the cultures were grown in 250-mL Erlenmeyer flasks in glass tanks illuminated from below with fluorescent tubes, using the apparatus and culture media described by Bright & Walsby (2000). Measurements of photon irradiance (photon flux density) were made with a Macam Quantum Sensor SD101Q Cos, sensitive to wavelengths of 400–700 nm. The photon irradiance was measured at the illuminated bottom of the flask (E_f) and just above the surface of the culture (E_b). All of the irrandiance values in the Results section are mean photon irradiances (E_m) calculated from the equation of Van Liere & Walsby (1982): $E_m = (E_f - E_b)/\ln(E_f/E_b)$. Different photon irradiances were obtained by interposing neutral density filters between the lamps and the flasks. Cultures were left for 3 d to acclimate to new irradiances before making buoyancy measurements.

Measurements of buoyancy change

Samples from the cultures were rapidly drawn up into a 50-µl Terumo microsyringe, which sheared long filaments without affecting their buoyancy, and slowly discharged into 0.5 ml of culture medium. This 10-fold diluted suspension was placed on counting chambers, formed from 38 × 76 mm glass slides with a central well (measuring 13 × 30 mm and 1.2 mm deep) under a coverslip. The sample was left for 15 min to allow buoyant filaments to float up to the coverslip and others to sink on to the slide. (A filament sinking 1 m d^{-1} sinks 1.2 mm in 1.7 min.) The floating and sinking filaments were then enumerated by systematically scanning transects across the 13-mm width of the counting chamber with a Leitz Orthoplan microscope using a 10× objective, first at the focal plane at the coverslip and then at the slide (Walsby & Booker, 1980). The total number of transects scanned per sample (using two or three slides) was between 10 and 24, depending on the filament concentration; the mean number of filaments counted per sample was 400 and the mean number per transect was 30. The microscope measurements were made without delay to minimize postsampling changes. It was impractical to continue this manual counting for more than a 12-h period: measurements were first made during successive light phases at different irradiances; the culture was then phase-shifted by 12 h and after 3–5 d acclimation to the new light–dark cycle, measurements were made of buoyancy change during the 12-h dark period that followed exposure to light of the same irradiances.

Calculation of Q_z, z_n and z_m in lakes

Calculations of Q_z, z_n and z_m in Lake Zürich and Blelham Tarn were made from information on the photon irradiance, temperature and *Planktothrix* populations at different times and depths in the two lakes, stored in the Excel spreadsheet DATA-SET.XLS described by Walsby (2001). Data are arranged in five tables (T1–T5), with a separate column for each day. T1 contains date and growth coefficients. Table T2 contains the *Planktothrix* filament biovolume concentration at each depth (N_i, in cm^3 m^{-3}) where the depth at the ith point is $z_i = i\Delta z$ and Δz is the depth interval. T3 contains data on the ratio of irradiance at each depth (E_i) relative to that at the water surface (E_0). T4 contains data on the water temperature (Θ_i, in °C) at each depth. T5 lists the photon irradiance (in µmol m^{-2} s^{-1}) immediately under the water surface at each time of day, $E_j = j\Delta t$, where Δt is the time interval in the table. The data-set for Lake Zürich (in which Δz = 1.0 m and Δt = 1 h) was that used by Walsby & Schanz (2002) for August 1998 to September 1999. The data set for Blelham Tarn was that of Davis *et al.* (2003) for August 1999 to October 2000 (Δt = 1 h, Δz = 0.5 m). The actual sampling depths varied with the vertical distribution of the organisms and the season of the year: when Lake Zürich was stratified, the depth of the *Planktothrix* maximum was located with a horizontal beam transmissometer and samples for determination of filament concentration were then taken at that depth and at intervals of 1 m above and below. When the lakes were mixed, the measurements were made at larger intervals and the values at intermediate depths were calculated by linear interpolation (Walsby, 1997).

The neutral buoyancy depth, z_n, was found by the following procedure: (i) the daily photon insolation at the surface, Q_0 (in mol m^{-2}) was calculated as the numerical integral of the photon irradiance measurements over each 24-h period in T5; (ii) the daily insolation at each depth, Q_z, was calculated as the product of Q_0 and E_i/E_0 from T3; (iii) the sampling depths immediately above (z_i) and below (z_{i+1}) where $Q_z = Q_n$ were located and the insolation at these depths (Q_i and Q_{i+1}) was determined; (iv) the depth z_n, where the daily insolation was equal to Q_n, was calculated by logarithmic interpolation (because irradiance decreases exponentially with depth):

$$z_n = z_i + \Delta z[\ln(Q_i/q) - \ln(Q_n/q)]/[\ln(Q_i/q) - \ln(Q_{i+1}/q)]$$

where Δz is the sampling depth interval and q = 1 mol m^{-2} (Walsby, 1997). For sensitivity analysis, the same calculation was also performed for $0.67Q_n$ and $1.5Q_n$.

The values for the mixed depth, z_m, were those calculated by Walsby & Schanz (2002) for Lake Zürich and by Davis *et al.* (2003) for Blelham Tarn. These values were calculated for each day as the depth where the Wedderburn number (ratio of forces of buoyancy and mixing) was equal to 1.0 for Lake Zürich and to 0.4–0.7 (depending on the wind direction relative to the lake topography) for Blelham Tarn; it was determined from data on the vertical temperature gradient and the wind speed and direction, using equations of Spigel & Imberger (1987) and procedures described by Walsby & Schanz (2002).

Results

Preamble on buoyancy changes in relation to irradiance

In a *Planktothrix* culture on a light–dark cycle, if all filaments behaved identically, all would be buoyant at the end of the dark period, would lose buoyancy at the same time in the light period and regain buoyancy at the same time in the dark period. In a higher irradiance buoyancy loss would occur earlier in the light period and buoyancy would be regained later in the dark period; above a certain threshold irradiance, the filaments would not regain buoyancy by the end of the dark period. Conversely, below a certain low irradiance threshold the filaments would remain buoyant throughout the light period. At a certain intermediate irradiance the filaments would be buoyant for half the day and would sink for the other half. Such filaments could be considered to be neutrally buoyant on average over time if they sank down and floated up equal distances over the daily cycle. In practice, not all

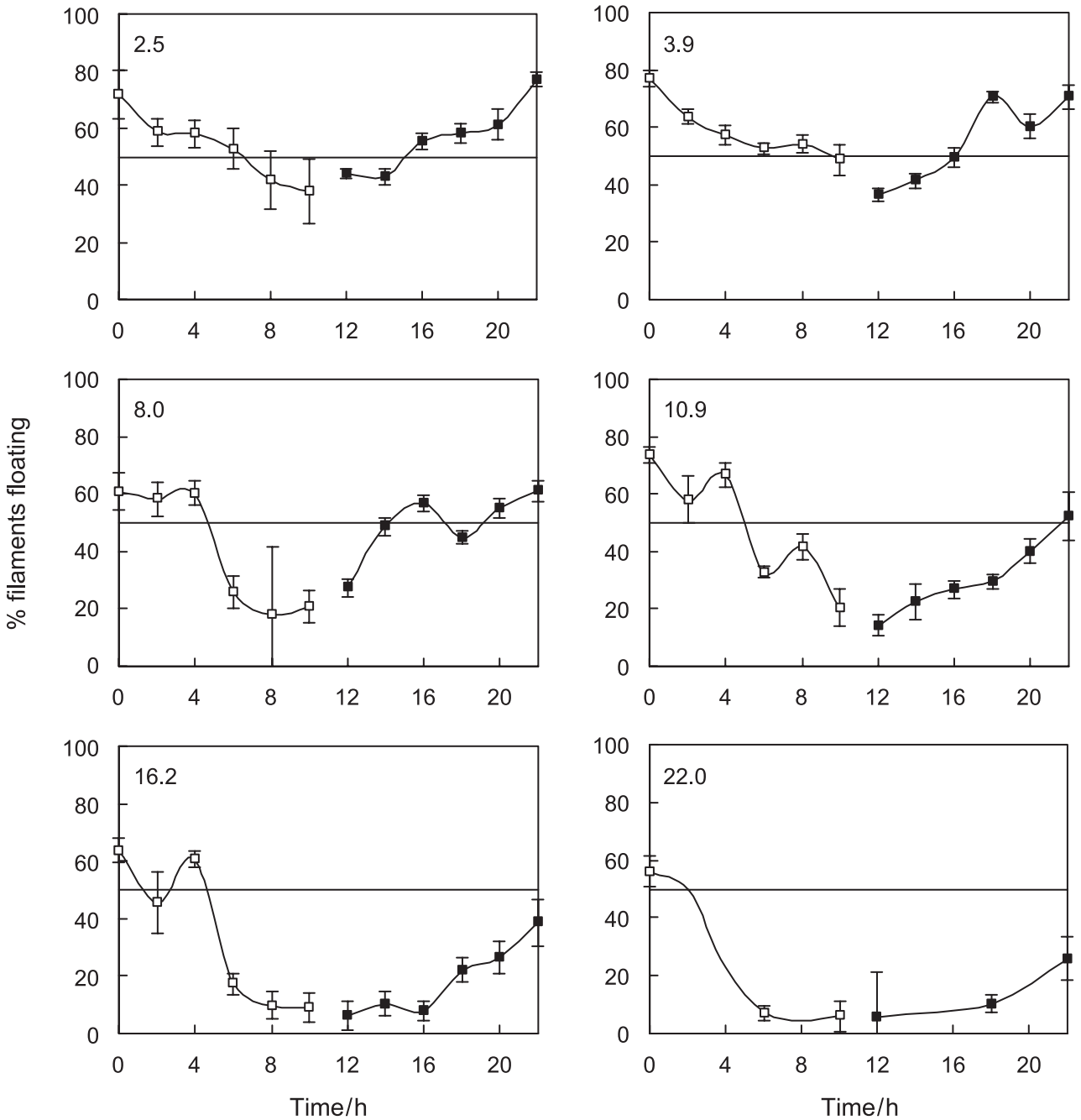

Fig. 1 Changes in the percentage of filaments floating (% *F*) in cultures of *P. rubescens* strain 9316, first during the light phase (open squares) and then the dark phase (closed squares) of cultures maintained for 12 h each day at the different photon irradiances (μmol m^{-2} s^{-1}) indicated at the top left of each panel. The horizontal line indicates neutral buoyancy (50% floating).

filaments in a culture behaved identically and the filaments did not lose or gain buoyancy simultaneously (Fig. 1).

Buoyancy changes of *P. rubescens* strain 9316 from Lake Zürich

Previous investigations with *P. rubescens* strain 9316 from Lake Zürich showed growth at irradiances as low as 2 μmol m^{-2} s^{-1} in the light phase of a 12 : 12 h light–dark cycle (Bright & Walsby, 2000). Most of the filaments were buoyant throughout the daily cycle under these conditions; at irradiances exceeding 25 μmol m^{-2} s^{-1}, which supported the maximum growth rate (Bright & Walsby, 2000), most filaments sank. Intermediate irradiances were investigated to find the conditions giving neutral buoyancy.

Cultures were acclimated for 3–5 d at one of six selected irradiances during the 12-h light phase; buoyancy changes

were then monitored over the daily cycle. In the first experiment, the buoyancy state (% F) was determined at intervals of 2 h over a single cycle. The results are shown in Fig. 1. At each irradiance % F decreased during the light phase, reaching a minimum at the end of the 12-h light period. The decrease was not even: at 8.0 μmol m^{-2} s^{-1}, for example, the largest decrease occurred between 4 and 6 h after the start of the light period. During the dark period the % F increased, reaching its highest value at the end of the period, and the rate of change was generally more gradual than the rate of buoyancy loss in the light. For each treatment the mean % F measured at 2-h intervals over the 24-h cycle was calculated: it was > 50% for cultures at or below 3.9 μmol m^{-2} s^{-1} and < 50% for cultures at or above 8.0 μmol m^{-2} s^{-1}, which indicates that the photon irradiance in the light phase supporting neutral buoyancy (50% floating) occurs between 3.9 and 8.0 μmol m^{-2} s^{-1}.

It is expected that the diel cycle of buoyancy change would eventually become stable in a culture maintained under a uniform light–dark cycle and that the buoyancy would be the same at 0 and 24 h, the end of the dark period. This was true for those cultures at the lower irradiances, but for those at the higher irradiances the % F was lower after 24 than 0 h, suggesting that they had not achieved a steady-state cycle.

In the second set of experiments, measurements of buoyancy state were made over three daily cycles, but only at the beginning and end of the light and dark phases (Fig. 2). The average % F remained fairly constant during the 3 d, indicating that the cultures were acclimated to the conditions used. The mean of the 12 measurements made on each culture (two in the light phase, two in the dark phase on each of 3 d) was calculated. From these results it is inferred that the irradiance in the light phase supporting neutral buoyancy (50% filaments floating over the daily cycle) occurs between 3.9 and 10.9 μmol m^{-2} s^{-1}.

The results from the two experiments were combined in a graph of mean % F at each irradiance (Fig. 3). The photon irradiance giving a mean of 50% filaments floating was determined from the plot of a fourth-order polynomial equation ($y = 0.0005614x^4 - 0.01518x^3 + 0.01080x^2 - 0.7974x + 57.91$, between the limits $2.5 < x < 22$) where y is the % F and x is (E_m/μmol m^{-2} s^{-1}): from this, the irradiance giving neutral buoyancy is 6.51 μmol m^{-2} s^{-1}. Multiplying this value by the length of the 12-h light period, 43 200 s, gives the daily photon insolation supporting neutral buoyancy, Q_n = 0.281 mol m^{-2}. The precise value of Q_n is sensitive to the method of interpolation; the consequences of using smaller and larger values in

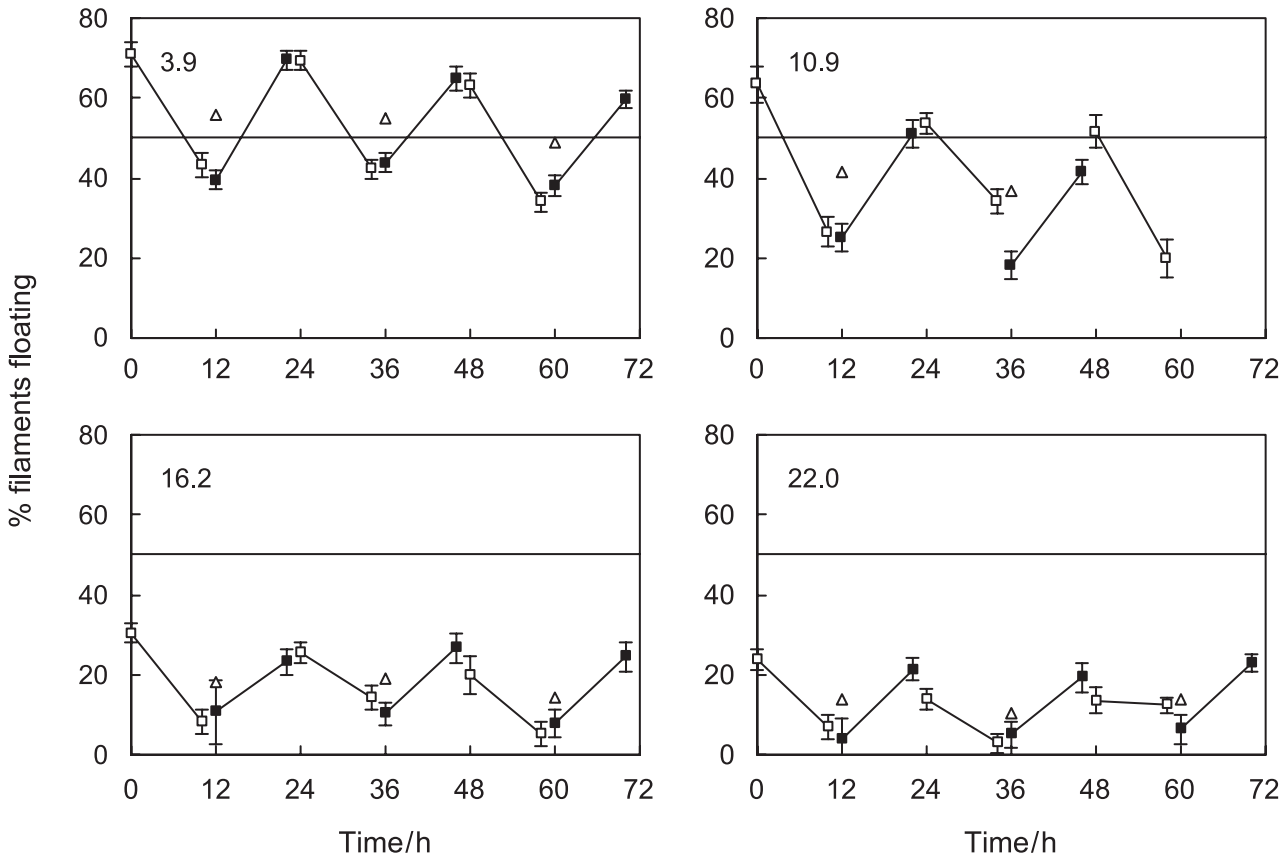

Fig. 2 Change in the percentage of filaments floating (% F) at different times of day over three light–dark cycles, with the different photon irradiances (μmol m^{-2} s^{-1}) indicated at the top left of each panel. Open symbols, during 12-h light phase; closed symbols, 12-h dark phase. Triangles indicate mean of four values over each day.

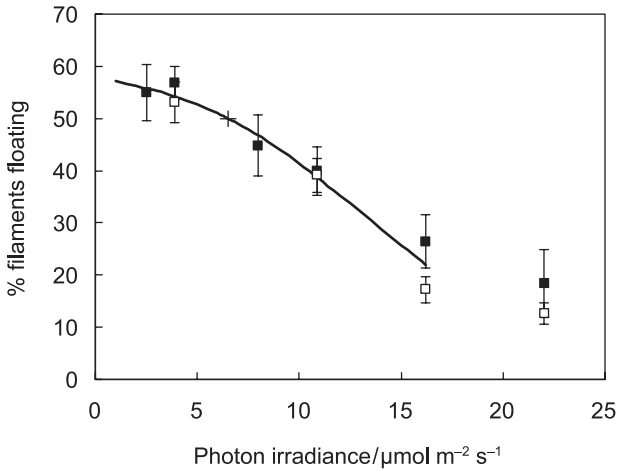

Fig. 3 Mean percentage of filaments floating (% *F*) over 24 h of cultures of *P. rubescens* strain 9316 kept at different photon irradiances during the 12-h light periods, from data in Fig. 1 (closed symbols) and Fig. 2 (open symbols). The irradiance giving neutral buoyancy is indicated by a cross.

calculating neutral buoyancy depths are therefore included in the analyses made below. (The polynomial expression is purely a device for the interpolation described and is not suitable for predicting the buoyancy state at other irradiances.)

The neutral buoyancy depth in Lake Zürich

Using the value of Q_n = 0.281 mol m^{-2}, the neutral buoyancy depth, z_n, was calculated for each day in two periods of thermal stratification in Lake Zürich. The z_n values were compared with the depth of the *Planktothrix* population maximum, z_p, for periods when this exceeded the mixed depth, z_m, i.e. when the population was not entrained by mixing (Fig. 4).

(a) 17 August–23 September 1998 *Planktothrix rubescens* was initially confined to the metalimnion. On 17 August the population maximum, z_p, was at a depth of 13 m but by 26 August it had moved up to 10 m and remained there until 9 September, after which the mixed depth exceeded 10 m and entrainment of the population began. Over the period of stratification there was a similarity between z_p and z_n: on 17 August z_n was also at 13 m and then gradually decreased, because of increased cloud cover and the decreasing insolation with shortening days (the change is steepest at the equinox). The changes in z_n would therefore explain the observed decrease in z_p: the calculated values of z_n were less than 13 m on each of the subsequent 9 d before z_p reached 10 m, and were equal to or less than the interpolated z_p values on seven of the days. Over the next 15 d the calculated value of z_n showed little change, and remained within 1 m of the z_p at 10 m (Fig. 4). It is noted here that the recorded value of z_p is based on a sampling interval of 1 m, and that the depth of the maximum

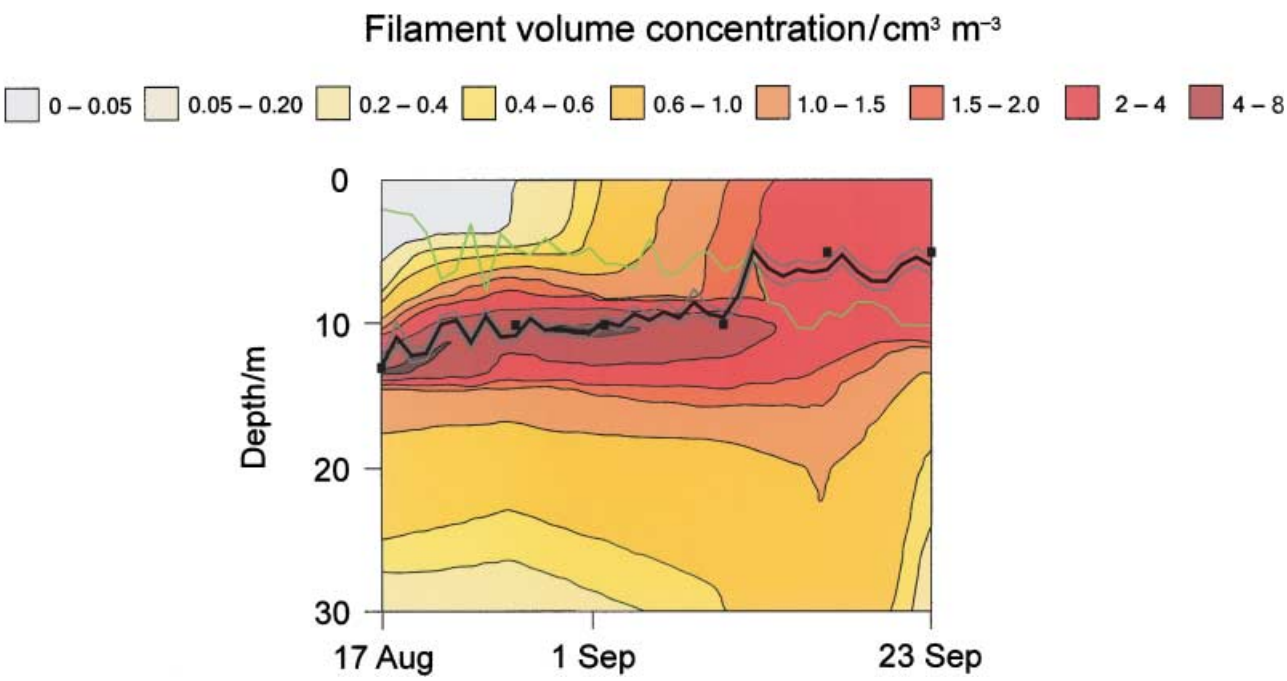

Fig. 4 Changes in the vertical distribution of *P. rubescens* (isopleths), the depth of mixing (z_m, green line) and the neutral buoyancy depth (z_n, black line), calculated as the depth at which the insolation was equal to the neutral buoyancy insolation (Q_n); faint grey lines above and below are calculated from 1.5Q_n and 0.67Q_n, respectively; black squares indicate the depth of the measured population maximum (z_p) on the sampling dates. Lake Zürich, 17 August–23 September 1998.

may have fluctuated by up to 1 m, because of seiche movements (Walsby *et al.*, 2001). For hydrodynamic reasons, filaments adjusting their buoyancy do not move by more than 1 m d^{-1} (Kromkamp & Walsby, 1990) and it is likely that filaments will take at least 1 wk to float up as much as 3 m.

During the next 9 d (10–18 September), there were nine cloudy days with a mean insolation of only 14 mol m^{-2} (Walsby & Schanz, 2002): this affected the vertical distribution in two ways. The first was that there was cooling of the surface water (by 4°C in the top 10 m; by 1.6°C in the top 20 m), so that with a less-steep density gradient, wind mixing caused the mixed depth, z_m, to exceed 10 m, and much of the metalimnetic *Planktothrix* population became entrained into the epilimnion. The second consequence of the decrease in Q_0 was the sharp decrease in z_n, to a minimum of 5.2 m on 11 September, allowing the *Planktothrix* filaments to float up into the influence of the deepening surface mixed layer (Fig. 4). Together, these changes can explain the observed decrease in z_p; the *Planktothrix* concentration was fairly uniform in the top 10 m but peaked marginally at 5 m (see Fig. 1 of Walsby & Schanz, 2002). From this time onwards the *Planktothrix* population became mixed progressively deeper and for this period further comparisons of z_n and z_p are meaningless.

The comparisons between z_p and z_n made above are based on values of z_n calculated from a single estimate of Q_n = 0.281 mol m^{-2} (Fig. 3). Similar sets of analyses were based on values of Q_n that are 50% higher or lower than this estimate; the results are indicated by the ancillary loci plotted above and below z_n in Fig. 4 (and subsequent figures). Because of the exponential change in irradiance with depth, these large changes in Q_n translate to rather small differences in the calculated value of z_n, with a maximum difference of 0.91 m and a minimum of 0.36 m. The general solution is that when Q_n is increased by the proportion x, the change in the calculated value of z_n is given by the equation:

$$\Delta z_n = \ln[1/(1 + x)]/K_d \qquad \text{Eqn 1}$$

For example, when $x = 0.5$ and $K_d = 0.4$ m^{-1}, z_n decreases by 1 m.

(b) 1 April–14 September 1999 Thermal stratification started in early April after the winter period in which the *Planktothrix* population was uniformly mixed through the top 60–100 m, and even part to the lake bottom at 136 m. In late April and early May a substantial part of the *Planktothrix* population collected at the metalimnion: the maximum there can be explained by movement from other depths, where the concentration decreased (Walsby & Schanz, 2002). Because of the weak thermal gradients, z_m varied between 5 and 12 m over this period and z_p was not well defined (the sampling was at 5-m intervals). Nevertheless, the calculated z_n values (Fig. 5) track the broad changes in z_p (and especially so later in this period when the mixed depth became confined to the shallower depths above z_p and z_n). This supports the idea that the initial formation of the metalimnetic maximum in early May is caused by buoyancy regulation of *P. rubescens* in response to the irradiance. The total *Planktothrix* biomass, integrated through the top 60 m of the water column, did not change much during this period but in early June the population collapsed, perhaps through parasitism or grazing, and only 1% survived. The summer population developed from this remnant, which survived mainly in a band between 15 and 30 m.

The *Planktothrix* population increased steadily throughout the summer and most of it was confined to a 3-m thick layer with z_p varying between 10 and 13 m. For much of this period z_p followed the general trend in z_n, which fluctuated daily with the changing cloud cover (Fig. 5). The only days on which there was a divergence of more than 2 m between z_n and z_p was on 9 and 19 July; apart from this, peaks and troughs in z_p followed six of the seven peaks and troughs in z_n shown in Fig. 5.

Buoyancy changes of *P. rubescens* strain B9972 from Blelham Tarn

Measurements of buoyancy change were made in the course of experiments conducted primarily to determine the growth rate of *P. rubescens* strain B9972 isolated from Blelham Tarn (Davis & Walsby, 2002). Cultures were grown at 16°C in 12 : 12 h light–dark cycles over a range of photon irradiances in the light phase and the % F was determined in the middle of the light phase (between 5 and 7 h after the start of the light period). The results for different irradiances are shown in Fig. 6. The line of fit is the plot of a third-order polynomial equation ($y = -0.0189x^3 + 0.1936x^2 - 2.9188x + 88.207$, between the limits $0 < x < 17$), from which the irradiance supporting 50% floating was calculated, 11.7 µmol m^{-2} s^{-1}. Multiplying this value by the length of the light period, 43 200 s, gives the corresponding daily photon insolation, 0.507 mol m^{-2}. This experiment differs from those conducted with *P. rubescens* 9316, but reference to the data in Fig. 1 shows that the % F half-way through the light period is similar to the mean value over the 24 h, and this value is therefore taken as an estimate of Q_n for this strain, which can be used to calculate the neutral buoyancy depth in Blelham Tarn.

The neutral buoyancy depth in Blelham Tarn

Davis *et al.* (2003) recorded the vertical distribution of *P. rubescens* in Blelham Tarn from August 1999 to October 2000. This lake is much smaller, shallower and less stable than Lake Zürich and although the population of *P. rubescens* stratified in the metalimnion in the summers of both years, it did so at shallower depths, 2–6 m (Fig. 7). The top of this shallow layer was more susceptible to penetration by wind-driven mixing and consequently some filaments were always present

New Phytologist DWR-752

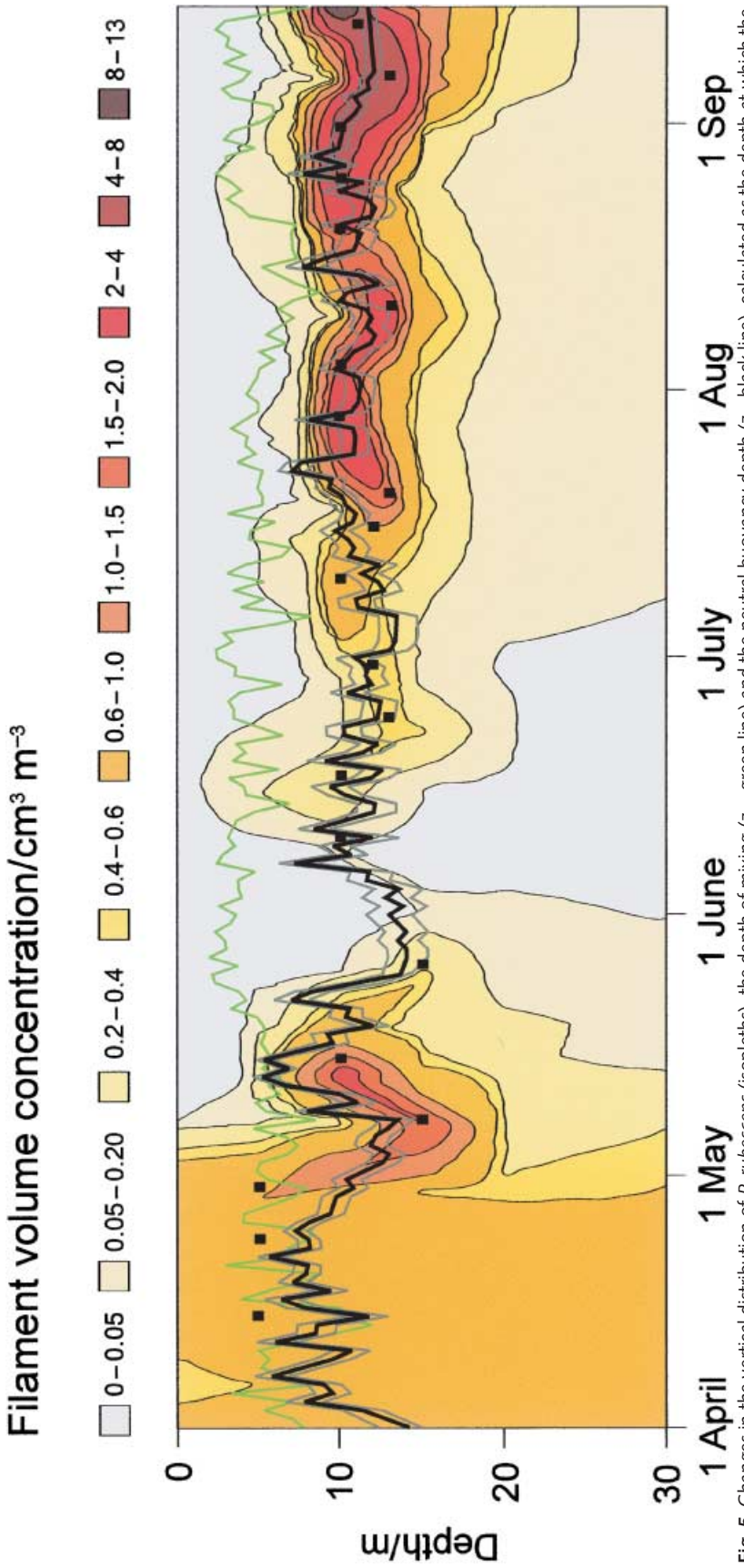

Fig. 5 Changes in the vertical distribution of *P. rubescens* (isopleths), the depth of mixing (z_m, green line) and the neutral buoyancy depth (z_n, black line), calculated as the depth at which the insolation was equal to the neutral buoyancy insolation (Q_n); faint grey lines above and below are calculated from $1.5Q_n$ and $0.67Q_n$, respectively; black squares indicate the depth of the measured population maximum (z_p) on the sampling dates. Lake Zürich, 29 March–15 September 1999.

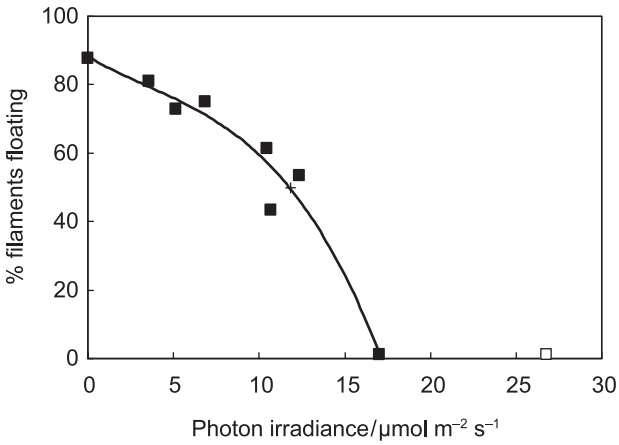

Fig. 6 Percentage of filaments floating (% *F*) in the middle of the light phase in cultures of *P. rubescens* strain B9972 kept at different photon irradiances during the 12-h light period of a light–dark cycle. The irradiance supporting 50% buoyancy is indicated by a cross.

in the epilimnion. Nevertheless, the population maximum occurred in the metalimnion during late spring and summer and the values z_p and z_n could be compared over this period (Fig. 7).

The stratification of *P. rubescens* coincided with the onset of thermal stratification in mid-April 2000 (Davis *et al.*, 2003b); during the first month z_n varied between 3.0 and 4.9 m, within the broad depth band occupied by the organism. During the month of May the population became more sharply stratified at a depth of about 5 m; the mean value of z_n increased to 5.2 m, corresponding closely to the depth of the population maximum. In June the upward movement of the stratified population followed the decrease in z_n after about 1 wk: the mean value was 3.7 m for the month of June and 3.0 m for the first 2 wk of July, when the population reached its minimum depth (Fig. 7). The similarity between z_p and z_n held until the population disappeared in early September, possibly because of parasitism by chytrids and grazing (Davis *et al.*, 2003b).

General discussion

Insolation for neutral buoyancy in other cyanobacteria

Although light-driven buoyancy regulation has been demonstrated in many other cyanobacteria, there have been few direct measurements of the irradiance that results in neutral buoyancy. Another strain of *P. rubescens* from Lake Gjersjøen, Norway, grown under continuous light, showed neutral buoyancy at about 5 μmol m^{-2} s^{-1}, equivalent to a Q_n of 0.43 mol m^{-2} (Walsby *et al.*, 1983a), intermediate between the values for the two strains investigated here. The values of Q_n might be different for cultures grown on light–dark cycles because cyanobacteria use light less efficiently under continuous illumination (Foy *et al.*, 1976). Walsby & Booker (1980) showed that in cultures of *Anabaena flos-aquae* grown in continuous light, neutral buoyancy occurred at 13 μmol m^{-2} s^{-1}, equivalent to a Q_n of 1.12 mol m^{-2}. An explanation for this higher value is that the photosynthetic production of carbohydrate, on which buoyancy loss depends (Oliver, 1994), will be less efficient at very low irradiances in *Anabaena*, which lacks phycoerythrin and has a lower light-affinity coefficient (but a higher E_k value) than *Planktothrix* (Van Liere & Walsby, 1982). A consequence of the higher Q_n in *Anabaena* is that z_n is more likely to occur above the stable thermocline region. Lund (1959) found that during the summer in Esthwaite Water, *Anabaena circinalis* was largely restricted to the epilimnion while *Planktothrix* (*Oscillatoria*) *agardhii* var. *isothrix* was stratified in the metalimnion.

Although irradiance is the main controlling factor of buoyancy, other factors, such as temperature and nutrient availability, must also affect Q_n, because they also affect the photosynthetic accumulation of carbohydrate and the synthesis of gas vesicles, the major determinants of buoyant density (Oliver & Walsby, 1984). It has been shown that enrichment with both combined nitrogen (Klemer, 1978) and phosphate (Konopka *et al.*, 1987a, 1987b) stimulated the increase in gas-vesicle content and buoyancy at low irradiances. There has been no direct investigation of the effect of nutrient concentration on Q_n: the expected trend would be for Q_n to increase at high nutrient concentration and to decrease with nutrient limitation. Our measurements of Q_n were made in cultures containing nutrients at concentrations higher than occur in these lakes; it is therefore unlikely that Q_n would be any higher in the lake.

Similarity between z_n and z_p

The general agreement between z_n and z_p for *P. rubescens* in Lake Zürich and Blelham Tarn supports the theory that buoyancy regulation in response to irradiance explains how the organism stratifies in the metalimnion of a lake. The value of z_n calculated from the measured Q_n predicts the depth to which the organism will migrate, explains the major changes in z_p over the season of stratification, and the gross differences between the depths of the *Planktothrix* layer in the two lakes.

Concerning possible effects of temperature, the metalimnetic *Planktothrix* population often straddles layers of different temperatures and the temperature also varies at the population peak: in Lake Zürich it was at 9°C in early July and 17°C in mid September, 1995. It seems unlikely, therefore, that temperature provides an essential cue for the stratification depth, although it will modulate the response to irradiance. Concerning possible effects of nutrients, neither nitrate nor soluble phosphate was limiting during the main growth period in Lake Zürich (Walsby & Schanz, 2002). Similarly in Blelham Tarn, the elements most likely to limit phytoplankton biomass were not exhausted from the water column (Davis *et al.*, 2003b).

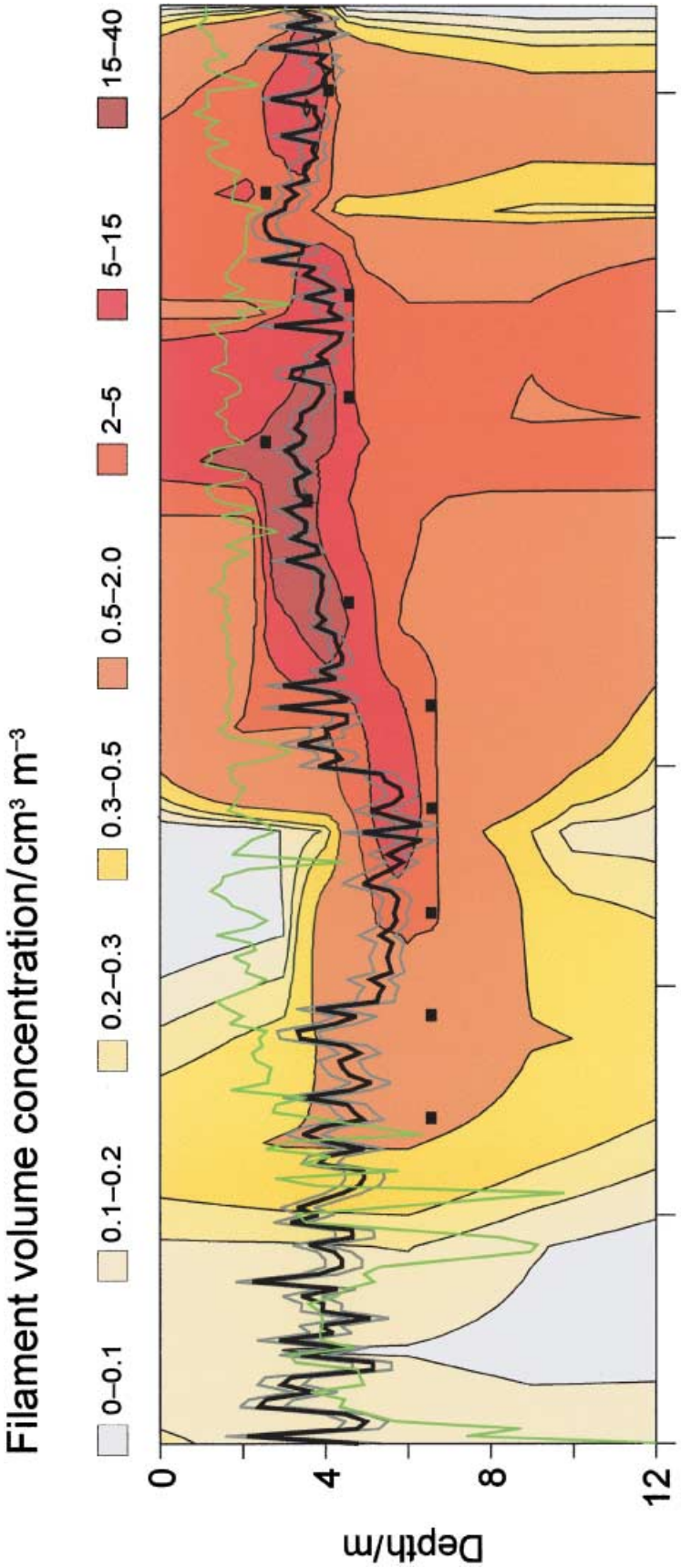

Fig. 7 Changes in the vertical distribution of *P. rubescens* (isopleths), the depth of mixing (z_m, green line) and the neutral buoyancy depth (z_n, black line), calculated as the depth at which the insolation was equal to the neutral buoyancy insolation (Q_n); faint grey lines above and below are calculated from $1.5Q_n$ and $0.67Q_n$, respectively; black squares indicate the depth of the measured population maximum (z_p) on the sampling dates. Blelham Tarn, April–September 2000.

Difference between the two lakes

The calculation of z_n provides a quantitative explanation for the differences in depth of stratification of the *Planktothrix* populations in the two lakes. During the main period of stratification, 1 June–1 September, the mean depth of the *P. rubescens* maximum in Blelham Tarn is z_p = 3.6 m, which compares with the mean calculated value, z_n = 3.1 m. The corresponding mean values in Lake Zürich are much greater, z_p = 11.2 m and z_n = 11.0 m. A small part of the difference is caused by the higher Q_n value of the Blelham Tarn strain: if this is substituted in the calculation for Lake Zürich, the mean of z_n is decreased from 11.0 to 9.5 m. The residual threefold difference must be caused by the underwater light field. Since there is only a small difference between the mean daily insolation at the water surface over this period (29.0 mol m^{-2} in Blelham Tarn and 31.4 mol m^{-2} in Lake Zürich), the remaining difference in z_n must be caused by the greater light attenuation in the more eutrophic Blelham Tarn: over the same period, the mean K_d between depths of 2 and 6 m was 1.2 m^{-1} in Blelham Tarn, compared with only 0.42 m^{-1} in Lake Zürich. The higher K_d in the epilimnion of Blelham Tarn is partly caused by the higher concentration of phytoplankton supported by the more eutrophic conditions and the *Planktothrix* population itself contributes to the K_d there. This in turn affects the stratification depth, as can be demonstrated by the following calculation.

Measurements made on populations in different lakes indicate that the relationship between *Planktothrix* biovolume concentration (N) and light attenuation is:

$$K_d = 0.1(N/\text{cm}^3\ \text{m}^{-3})\text{m}^{-1} \qquad \text{Eqn 2}$$

(Walsby *et al.*, 2001; Davis *et al.*, 2003b). During late May in Blelham Tarn, part of the *Planktothrix* population became entrained in the epilimnion (Fig. 7), where the concentration increased by 2 cm^3 m^{-3}: this would have raised the K_d by 0.2 m^{-1}. If K_d had been uniform through the surface layer, then the depth of z_n would have been:

$$z_n = \ln(Q_0/Q_n)/K_d \qquad \text{Eqn 3}$$

An increase in K_d from 0.68 to 0.88 m^{-1} would have had the effect of decreasing z_n from 6.0 to 4.6 m. The decrease in z_n caused by the observed entrainment of part of the population could therefore have caused the stratified layer to move up. At the end of June, as more of the population became entrained in the surface mixed layer, K_d was increased further and the underlying stratified population moved up even nearer the surface. The same effect is seen in Lake Zürich during September (Fig. 5) where there is a marked decrease in z_n once the population has become entrained in the surface layer.

Another consequence of self-shading is a local steepening of the light gradient within the stratified population: for example, in Blelham Tarn on 4 July 2000 the *P. rubescens* population reached a maximum of 32 cm^3 m^{-3} at a depth of 3.5 m and at that depth K_d exceeded the background attenuation at other depths by 3.2 m^{-1} (Davis *et al.*, 2003b). For a *Planktothrix* filament regulating its buoyancy according to the irradiance, this will affect the depths at which buoyancy is lost and gained.

Conditions required for stratification in the metalimnion

Metalimnetic stratification of cyanobacteria does not occur in eutrophic lakes because the light attenuation by the high concentration of phytoplankton in the epilimnion reduces the neutral buoyancy depth to less than the mixed depth. A formal quantitative explanation is provided by calculations based on equations 2 and 3. An extreme example is provided by Lake Rotongaio, New Zealand. The very high concentration of phosphorus (total P, 266 mg m^{-3}; dissolved reactive P, 147 mg m^{-3}; Vincent, 1989) supported the development in the surface mixed layer of an *Anabaena* population exceeding 40 cm^3 m^{-3}, which would have generated a K_d exceeding 4 m^{-1}, similar to that observed (Walsby *et al.*, 1989). Adopting values for Q_0 = 30 mol m^{-2} and Q_n = 1.12 mol m^{-2} (as for *A. flos-aquae*), then the predicted value of z_n would have been 0.82 m (Walsby *et al.*, 1989 calculated an equilibrium depth of 0.88 m using the computer model of buoyancy regulation of Kromkamp & Walsby, 1990). This depth was exceeded by z_m, because of convectional mixing of the cooled surface layer at night, and the organism was therefore dispersed through the epilimnion (Walsby *et al.*, 1989).

Klemer (1978) found that in Perspex columns suspended in Deming Lake, Minnesota, filaments of *P.* (*Oscillatoria*) *agardhii* var. *isothrix* moved up into the epilimnion when the columns were shaded and also when the enclosed water was enriched with combined N (supplied as NH_4Cl). Enrichment may affect the vertical distribution in two ways, through its direct effect on buoyancy in this organism (Klemer, 1978) and by the indirect effect of increased growth causing a rise in light attenuation.

Planktothrix rubescens formed a metalimnetic population each summer for many years in Lake Zürich, but it disappeared for a few years in the late 1960s when the lake became eutrophic (Schanz & Thomas, 1981). This disappearance may also be explained by the increase in light attenuation by phytoplankton in the surface layers that caused the *Planktothrix* filaments to rise into the epilimnion where they were outcompeted by other organisms.

Depth of stratification and the optimal depth for growth

The occurrence of *P. rubescens* in deep layers of lakes suggested to earlier investigators that it was both a cold stenotherm (Ruttner, 1937) and a shade-requiring organism (Findenegg,

1943), which would not tolerate the higher temperatures and irradiances in the epilimnion. Investigations in culture, however, showed not only that it grows at temperatures of 25°C and photon irradiances up to 200 µmol m^{-2} s^{-1} (equivalent to a Q of 8.6 mol m^{-2}) but it grows fastest under these conditions (Bright & Walsby, 2000; Davis & Walsby, 2002). The conditions where it stratifies, temperatures of 8–18°C and Q_n of 0.5 mol m^{-2}, are therefore not optimal for its growth. Why has this behaviour evolved?

Consider first the fate of *Planktothrix* filaments with a higher Q_n that attempt to stratify where the irradiance and temperature are optimal: because these conditions occur well above the thermocline region, the filaments will be entrained in the surface mixed layer (as do the *Anabaena* spp. considered above). These filaments, with a maximum growth rate of $\mu_m <$ 0.35 d^{-1} (Davis & Walsby, 2002), would be outcompeted by *A. flos-aquae*, with μ_m = 0.8 d^{-1} (Van Liere & Walsby, 1982) and by many diatoms, cryptophytes and chlorophytes with growth rates exceeding 0.8 d^{-1} (Reynolds, 1984). *Planktothrix* filaments that lose buoyancy when Q exceeds 0.5 mol m^{-2}, however, will stratify in the metalimnion where the irradiance is too low for many of the other phytoplankton; some diatoms could grow under these conditions but they lack buoyancy and would sink through the steep density gradient in the metalimnion. The development by *P. rubescens* of a low Q_n resulting in stratification at depths providing suboptimal conditions free of competitors therefore constitutes an evolutionarily stable strategy. Moreover organisms that stratify in the metalimnion are less susceptible to removal by flushing than phytoplankton in the epilimnion, particularly in small lakes such as Blelham Tarn, which have a short retention time (Davis *et al.*, 2003b).

Although stratification depends on z_n coinciding with the thermocline region, this coincidence is not uncommon. The establishment of a thermocline also depends on the gradient of radiation and the lowest depth of the thermocline region, where the irradiance is 1–2% of the surface irradiance, may initially be similar to z_n. Because of wind-induced mixing, however, the depth of the seasonal thermocline will be further extended (Spigel & Imberger, 1987), broadening the density gradient below that required for cyanobacterial stratification. The relationship between the depth of the thermocline and the gradient of irradiance will vary in different lakes and may result in the selection of *Planktothrix* variants that have different values of Q_n, and hence of z_n.

In both of the lakes studied, *P. rubescens* is capable of growth when it becomes entrained in the epilimnion and if there are few competitors present when this happens, the population may continue to increase (Reynolds *et al.*, 1984; Walsby & Schanz, 2002; Davis *et al.*, 2003b).

Acknowledgements

We are grateful to Professor F. Schanz for data on Lake Zürich and Professor C. S. Reynolds and the Centre for Ecology and Hydrology (CEH) of NERC for data on Blelham Tarn. We thank Annette Richer for maintaining the cultures and Tim Colburn for drawing the contour plots. This work was supported by a grant from the UK Natural Environment Research Council to A.E.W. P.A.D. was supported with a NERC CASE award by the CEH.

References

Bright DI, Walsby AE. 2000. The daily integral of growth by *Planktothrix rubescens* calculated from growth rate in culture and irradiance in Lake Zürich. *New Phytologist* **146**: 301–316.

Davis PA, Walsby AE. 2002. Comparison of measured growth rates with those calculated from rates of photosynthesis in *Planktothrix* spp. isolated from Blelham Tarn, English Lake District. *New Phytologist* **156**: 225–239.

Davis PA, Beard SJ, Walsby AE. 2003a. Variation in filament width and gas vesicles of red and green isolates of *Planktothrix* spp. *Archiv für Hydrobiologie* (Suppl.). *Algological Studies* **108**: 15–30.

Davis PA, Dent M, Parker J, Reynolds CS, Walsby AE. 2003b. The annual cycle of growth rate and biomass change in *Planktothrix* spp. in Blelham Tarn, English Lake District. *Freshwater Biology* **48**: 852–867.

Dinsdale MT, Walsby AE. 1972. The interrelations of cell turgor pressure, gas-vacuolation, and buoyancy in a blue-green alga. *Journal of Experimental Botany* **23**: 561–570.

Foy RH, Gibson CE, Smith RV. 1976. The influence of daylength, light intensity and temperature on the growth rates of planktonic blue-green algae. *British Phycological Journal* **11**: 151–163.

Findenegg I. 1943. Untersuchungen über die Ökologie und die Produktionsverhältnisse des Planktons im Kärtner Seengebiet. *Internationale Revue der Gesamten Hydrobiologie* **43**: 368–429.

Kirk JTO. 1994. *Light and photosynthesis in aquatic ecosystems, 2nd edn.* Cambridge, UK: Cambridge University Press.

Klemer AR. 1976. The vertical distribution of *Oscillatoria agardhii* var. *isothrix*. *Archiv für Hydrobiologie* **78**: 343–362.

Klemer AR. 1978. Nitrogen limitation of growth and gas vacuolation in *Oscillatoria rubescens*. *Mitteilungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie* **20**: 2293–2297.

Klemer AR, Feuillade J, Feuillade M. 1982. Cyanobacterial blooms: carbon and nitrogen limitation have opposite effects on the buoyancy of *Oscillatoria*. *Science* **215**: 1629–1631.

Konopka A. 1984. Effect of light–nutrient interactions on buoyancy regulation by planktonic cyanobacteria. In: Klug MJ, Reddy CA, eds. *Current perspectives in microbial ecology*. Washington, DC, USA: American Society for Microbiology, 41–48.

Konopka A. 1989. Metalimnetic cyanobacteria in hard-water lakes: buoyancy regulation and physiological state. *Limnology and Oceanography* **34**: 1174–1184.

Konopka A, Kromkamp J, Mur LR. 1987a. Buoyancy regulation in phosphate-limited cultures of *Microcystis aeruginosa*. *FEMS Microbiology Ecology* **45**: 135–142.

Konopka A, Kromkamp J, Mur LR. 1987b. Regulation of gas vesicle content and buoyancy in light- or phosphate-limited cultures of *Aphanizomenon flos-aquae* (Cyanophyta). *Journal of Phycology* **23**: 70–78.

Kromkamp J, Walsby AE. 1990. A computer model of buoyancy and vertical migration in cyanobacteria. *Journal of Plankton Research* **12**: 161–183.

Lund JWG. 1959. Buoyancy in relation to the ecology of the fresh water phytoplankton. *British Phycology Bulletin* **1**: 1–17.

Meffert M-E. 1971. Cultivation and growth of two *Oscillatoria* species. *Mitteilungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie* **19**: 189–205.

Micheletti S, Schanz F, Walsby AE. 1998. The daily integral of photosynthesis by *Planktothrix rubescens* during summer stratification and autumnal mixing in Lake Zürich. *New Phytologist* **138**: 233–249.

Oliver RL. 1994. Floating and sinking in gas-vacuolate cyanobacteria. *Journal of Phycology* **30**: 161–173.

Oliver RL, Ganf GG. 2000. Freshwater blooms. In: Whitton BA, Potts M, eds. *The ecology of cyanobacteria.* Dordrecht, The Netherlands: Kluwer Academic, 149–194.

Oliver RL, Walsby AE. 1984. Direct evidence for the role of light-mediated gas vesicle collapse in the buoyancy regulation of *Anabaena flos-aquae* (cyanobacteria). *Limnology and Oceanography* **29**: 879–886.

Porat R, Teltsch B, Perelman A, Dubinsky Z. 2001. Diel buoyancy changes by the cyanobacterium *Aphanizomenon ovalisporum* from a shallow reservoir. *Journal of Plankton Research* **23**: 753–763.

Reynolds CS. 1984. *The ecology of freshwater phytoplankton*. Cambridge, UK: Cambridge University Press, 384 pp.

Reynolds CS. 1987. Cyanobacterial water-blooms. *Advances in Botanical Research* **13**: 67–143.

Reynolds CS, Wiseman SW, Clarke MJO. 1984. Growth- and loss-rate responses of phytoplankton to intermittent artificial mixing and their potential application to the control of planktonic algal biomass. *Journal of Applied Ecology* **21**: 11–39.

Ruttner F. 1937. Limnologische Studien an einigen Seen der Ostalpen. *Archiv für Hydrobiologie* **32**: 167–193.

Schanz F, Thomas EA. 1981. Reversal of eutrophication in Lake Zürich. *WHO Water Quality Bulletin* **6**: 108–112.

Spencer CN, King DL. 1985. Interactions between light, NH_4^+, and CO_2 in buoyancy regulation of *Anabaena flos-aquae* (Cyanophyceae). *Journal of Phycology* **21**: 194–199.

Spigel RH, Imberger J. 1987. Mixing processes relevant to phytoplankton dynamics in lakes. *New Zealand Journal of Marine and Freshwater Research* **21**: 361–377.

Thomas EA, Märki E. 1949. Der heutige Zustand des Zürichsees. *Mitteilungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie* **10**: 476–488.

Thomas RH, Walsby AE. 1985. Buoyancy regulation in a strain of *Microcystis. Journal of General Microbiology* **131**: 799–809.

Utkilen HC, Oliver RL, Walsby AE. 1985. Buoyancy regulation in a red *Oscillatoria* unable to collapse gas vacuoles by turgor pressure. *Archiv für Hydrobiologie* **102**: 319–329.

Van Liere L, Walsby AE. 1982. Interactions of cyanobacteria with light. In: Carr NG, Whitton BA, eds. *The biology of the cyanobacteria.* Oxford, UK: Blackwell Scientific Publications, 9–45.

Vincent WF. 1989. Cyanobacterial growth and dominance in two eutrophic lakes: review and synthesis. *Archiv für Hydrobiologie; Ergebnisse der Limnologie* **32**: 239–254.

Walsby AE. 1969. The permeability of blue-green algal gas-vacuole membranes to gas. *Proceedings of the Royal Society of London B* **173**: 235–255.

Walsby AE. 1994. Gas vesicles. *Microbiological Reviews* **58**: 94–144.

Walsby AE. 1997. Numerical integration of phytoplankton photosynthesis through depth and time in a water column. *New Phytologist* **136**: 189–209.

Walsby AE. 2001. Determining the photosynthetic productivity of a stratified phytoplankton population. *Aquatic Sciences* **63**: 18–43.

Walsby AE, Booker MJ. 1980. Changes in buoyancy of a planktonic blue-green alga in response to light intensity. *British Phycology Journal* **15**: 311–319.

Walsby AE, Klemer AR. 1974. The role of gas vacuoles in the microstratification of a population of *Oscillatoria agardhii* var. *isothrix* in Deming Lake, Minnesota. *Archiv für Hydrobiologie* **74**: 375–392.

Walsby AE, Schanz F. 2002. Light-dependent growth rate determines changes in the population of *Planktothrix rubescens* over the annual cycle in Lake Zürich, Switzerland. *New Phytologist* **154**: 671–687.

Walsby AE, Utkilen HC, Johnsen IJ. 1983a. Buoyancy changes of a red coloured *Oscillatoria agardhii* in Lake Gjersjøen, Norway. *Archiv für Hydrobiologie* **97**: 18–38.

Walsby AE, Van Rijn J, Cohen Y. 1983b. The biology of a new gas-vacuolate cyanobacterium, *Dactylococcopsis salina* sp. nov., in Solar Lake. *Proceedings of the Royal Society of London B* **217**: 417–447.

Walsby AE, Reynolds CS, Oliver RL, Kromkamp J. 1989. The role of gas vacuoles and carbohydrate content in the buoyancy and vertical distribution of *Anabaena minutissima* in Lake Rotongaio, New Zealand. *Archiv für Hydrobiologie; Ergebnisse der Limnologie* **32**: 1–25.

Walsby AE, Avery A, Schanz F. 1998. The critical pressures of gas vesicles in *Planktothrix rubescens* in relation to the depth of winter mixing in Lake Zürich, Switzerland. *Journal of Plankton Research* **20**: 1357–1375.

Walsby AE, Dubinsky Z, Kromkamp JC, Lehmann C, Schanz F. 2001. The effects of diel changes in photosynthetic coefficients and depth of *Planktothrix rubescens* on the daily integral of photosynthesis in Lake Zürich. *Aquatic Sciences* **63**: 326–349.

Zimmermann U. 1969. Ökologische und physiologische Untersuchungen an der planktischen Blaualge *Oscillatoria rubescens* D.C. unter besonderer Berücksichtigung von Licht und Temperatur. *Schweizerische Zeitschrift für Hydrologie* **31**: 1–58.