

Behavioral response as a predictor of seasonal depth distribution and vertical niche separation in freshwater phytoplanktonic flagellates

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Abstract

The distribution of phytoplanktonic flagellates in aquatic ecosystems has been widely attributed to a number of driving factors. In this study, we evaluated the influence of behavior on the daytime, seasonal depth distribution and vertical niche separation of five phylogenetically contrasting species of freshwater flagellates. A model predicting distribution was formulated using the dominant behavioral preferences for light, oxygen, and carbon dioxide, previously quantified in laboratory experiments, and was subsequently applied to the physical and chemical conditions measured in a small, strongly stratifying, hypertrophic lake. This model predicted the daytime depth distributions of natural populations of flagellates well, with an average areal fit of above 56% for all species; above 74% for *Ceratium furcoides*, *Chlamydomonas* sp., and *Dinobryon sertularia*; and of up to 93% during stratification. Regression analyses showed no significant variation from a 1:1 relationship between the predicted and observed average depths of species in the water column. The model also predicted the constriction into discrete vertical niches upon stratification and delineated the progression from *Plagioselmis nannoplanctica* in surface waters, through *C. furcoides* and *D. sertularia*, to *Chlamydomonas* sp. and *Euglena gracilis* deeper in the water column. Changes in observed distributions could not be directly correlated with other members of the plankton community or explained by the segregation of nutrients. The model's wider, transferable applicability suggested that behavioral response to environmental gradients may predict many patterns of distribution, particularly during stratification. In addition to known physiological and biochemical influences, this investigation emphasized the importance of behavioral response in the functional ecology of phytoplanktonic flagellates.

Phytoplanktonic flagellates are a diverse group of unicellular or colonial eukaryotic microorganisms characterized by controlled motility combined with an ability to photosynthesize. Commonly occurring in both freshwater and marine habitats, they form an important functional

component of the phytoplankton community (Salonen et al. 1984; Stone et al. 1993) and are frequently the principal contributor to harmful algal blooms and red tides (Smayda 1997). The spatial and temporal distributions of flagellates in aquatic ecosystems have been attributed to a large number of driving factors such as physiological response (e.g., Harris et al. 1979), competitive interactions (Riegman et al. 1996), succession (Gasol et al. 1992), the changing distribution of resources (Klausmeier and Litchman 2001), differential predation (Elser and Carpenter 1988), and regimes of turbulent mixing propagated by wind or wave action (Kamykowski 1995). In much the same way, cell motility is also widely considered to be an important influence on distribution; however, to date and due to its complexity, the full ecological significance of active behavioral response still remains uncertain (Jones and Ilmavirta 1988; Kamykowski et al. 1998; Cullen and MacIntyre 1998).

During periods of thermal stratification in freshwater lakes, when flagellates are often most abundant and when the water column typically exhibits an increased heterogeneity brought about by the development of strong vertical gradients of essential resources, active motility may allow

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flagellates to select and maintain a favorable position within the water column. Indeed, many studies have shown that under these conditions species frequently employ complex behavioral strategies, such as depth regulation (Cullen and MacIntyre 1998), layer formation (Gervais 1998), and strong diel vertical migrations (Salonen et al. 1984), which, in combination with physiological and nutritional flexibility (Smayda 1997), could help to alleviate any spatial limitation or segregation of resources and might contribute to the common vertical niche separation of species in a water column (Sommer 1982). Such strategies may confer significant physiological advantages by maximizing growth and survival (Raven and Richardson 1984; Smayda 1997) and are widely assumed to be important controls of distribution. However, despite the many field observations of vertical distribution and migration, the underlying mechanisms by which these behavioral strategies are controlled have not yet been fully established (Jones 1993; Kamykowski and Yamazaki 1997; Cullen and MacIntyre 1998).

Extensive investigations have, nevertheless, found that the movements of phytoplanktonic flagellates are often biochemically mediated (Miller and Diehn 1978; Iseki et al. 2002) and are usually driven by sensory responses to environmental stimuli such as light (Kessler et al. 1992), gravity (Häder and Lebert 2001), temperature (Kamykowski 1981), chemical gradients (Lee et al. 1999; Clegg et al. 2004a), and even salinity (Erga et al. 2003). Moreover, recent observations indicate that this behavior may be deceptively sophisticated (Kamykowski and Yamazaki 1997; Bearon et al. 2004; Clegg et al. 2004b). For example, in an investigation of the behavioral response of five phylogenetically contrasting species of flagellates, significant preferences for light (Clegg et al. 2003a), temperature (Clegg et al. 2003b), and chemical gradients (Clegg et al. 2004a) were described. Subsequently, when cells were exposed to multiple conflicting gradients, these preferences for different environmental factors could be arranged into a hierarchy of dominant response (Clegg et al. 2004b). Until now, however, the direct ecological influence of many of these sensory-mediated responses remains untested.

This investigation therefore takes this hierarchy of behavioral preferences, determined in laboratory conditions (Clegg et al. 2004b), and applies it to field data collected from a small hypertrophic, freshwater lake to assess the impact of behavioral response on the daytime distribution of phytoplanktonic flagellates. This “daytime” distribution of cells is equivalent to the upper vertical extent of the diel migratory cycle. It is the most commonly sampled distribution and, in predominantly photosynthetic species, is arguably the time during which the acquisition of energy should be greatest. A model was formulated that predicts this distribution by applying quantified preferences to the physical and chemical gradients that develop over the course of a year. Although several models have previously conceptualized aspects of flagellate behavior (e.g., the biophysical swimming trajectory model of Kamykowski et al. 1998; the adaptive biochemical composition model of Yamazaki and Kamykowski 2000; the cell motility model of Bearon et al. 2004; and the marine migration model of

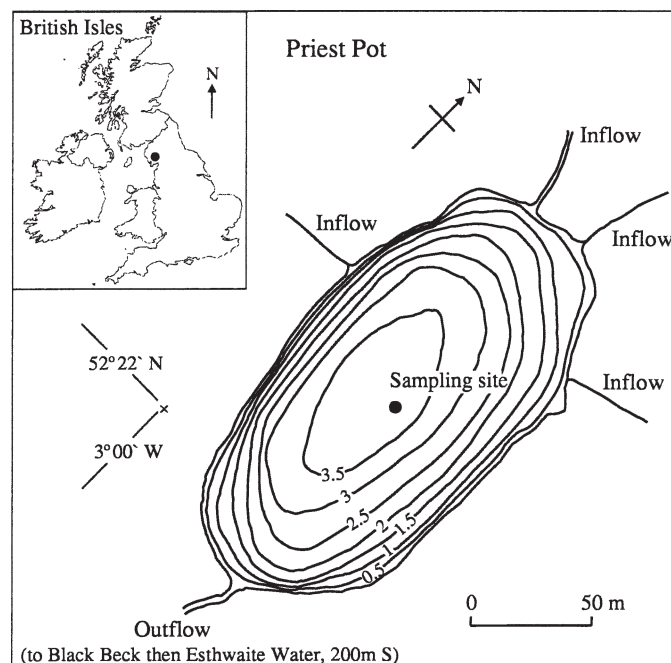


Fig. 1. Bathymetric map of Priest Pot (adapted from Finlay and Maberly 2000) with contours showing the depth in meters. The location of the lake within the British Isles and the position of the sampling site are also shown.

Beckmann and Hense 2004), this is the first example of an expansive model that predicts flagellate distribution based on a suite of quantified behavioral preferences.

Predictions are then compared with the distribution of natural populations of flagellates to assess the extent to which active behavioral preferences may control variations in spatial and temporal distribution. After evaluating the contribution of behavioral response to the delineation of discrete strata of different species of flagellates commonly encountered in many aquatic ecosystems (Sommer 1982; Reynolds 1992), the influence of other potential controls of distribution are considered, the interpretive value of the model is assessed, and its ecological implications are discussed.

Methods

Study site and collection of field data—Field data for testing the behavioral model were collected from Priest Pot (Fig. 1), a small hypertrophic lake with a high microbial diversity, situated 200 m to the north of Esthwaite Water in the English Lake District at 52°22'N, 3°00'W. The lake is ellipsoid in shape and has a surface area of 0.01 km², an average maximum depth of 3.5 m, and is encircled by trees, fen, and encroaching beds of emergent and submerged macrophytes. The resulting shelter from wind-induced turbulence, combined with a high biological productivity comprising an extensive photosynthetic component, typically leads to intense summer stratification (Finlay and Maberly 2000).

In situ samples were collected from a site close to the center of the lake (and with a depth of about 3.5 m), at 50-

cm depth intervals prior to stratification, and at 30-cm intervals from stratification onward, fortnightly from March to November 2000 at approximately 11:00 h. Sampling was completed within 2 h, to coincide with the theoretical upper limit of vertical migration (e.g., Jones 1988). Samples were collected in order of increasing disturbance, to minimize disruption of the water column. First, dissolved oxygen and temperature were recorded using a model 57 integrated meter (Yellow Spring Instruments). Subsequently, the attenuation of downwelling light was measured with a 2π , cosine-corrected, photosynthetically active radiation sensor (Macam Photometrics) and was adjusted for changes in surface irradiance, measured simultaneously with a Q102 Radiometer (Macam Photometrics). Depth profiles of underwater light were calculated later using these attenuation coefficients and the average surface photon irradiance for the 3 h preceding sampling (recorded every 10 min on an automatic monitoring station at the adjacent Esthwaite Water and corrected for 7% surface reflection). Finally, algal samples were collected using a pneumatic, close-interval syringe sampler (Heaney 1974) and immediately fixed, while a weighted tube and peristaltic pump were used to extract 1 liter of water from corresponding depths. This water was kept cool and dark, sealed in airtight bottles, and used for chemical and chlorophyll analyses. Except for sulfide samples, which were fixed in the field, the processing and analysis of samples was performed immediately on return to the laboratory.

Sample analysis and phytoplankton enumeration—Sulfide (H_2S , HS^- , and S^{2+}) concentration was measured spectrophotometrically (Mackereth et al. 1989) after acidification and addition of ammonium ferric sulfide in the field. Nitrate was reduced to nitrite by hydrazine and following the diazotization of sulfanilamide; both concentrations were differentiated spectrophotometrically (Mackereth et al. 1989). Ammonia concentrations were determined colorimetrically using the phenol hypochlorite method (Mackereth et al. 1989); soluble reactive phosphorus (SRP) was quantified after reduction of a phosphomolybdate complex (Stephens 1963); and total phosphorus was measured after persulphate digestion of samples (Eisenreich et al. 1975). The pH was measured using a combination electrode (Hanna Instruments) and was subsequently corrected to lake temperature, while alkalinity was measured by Gran titration (Mackereth et al. 1989). Total inorganic carbon concentration was measured by passing the carbon released from acidified samples through an infrared gas analyzer, with CO_2 concentrations then calculated using the carbon speciation approach of Mackereth et al. (1989) and equations in Maberly (1996).

Chlorophyll and bacterial chlorophyll concentrations were determined after a measured volume of water from each depth was filtered through glass-fiber GF/C filter paper (Whatman International). Two replicates were boiled in 100% methanol and extracted in 90% acetone to determine algal chlorophyll *a* content and to differentiate bacterial chlorophyll *a*, *c*, and *d* (Caraco and Puccoon 1986). This latter differentiation provided a measure of one

of the most substantial bacterial assemblages in Priest Pot (Finlay and Maberly 2000). Separate, 12-mL algal samples taken from each sampling interval were immediately fixed with Lugol's iodine solution and left for 14 d in settling cylinders. After removal of the upper 8 mL, algae (and protozoa) in the remaining 4 mL were enumerated microscopically at $\times 100$ and $\times 400$ magnifications using the Lund chamber technique (Lund 1959). Where possible, at least 100 cells, 100 colonies, or for less abundant species, 100 fields were counted to give a statistical measure of counting accuracy ($\pm 20\%$ for 95% confidence limits: Lund et al. 1958).

Construction of a predictive model—The model was created using the behavioral preferences of five phylogenetically contrasting species of freshwater phytoplanktonic flagellates, determined in previous laboratory studies. These test species from the Culture Collection of Algae and Protozoa (CCAP) had been isolated from a variety of freshwater sources (thereby not restricting the applicability of results to Priest Pot) and included the mixotrophic dinophyte, *Ceratium hirundinella* fo. *furcoides* Schröder CCAP Strain 1110/4 (taxonomically recognized in Heaney et al. 1988 and henceforth referred to as *C. furcoides* Levander Langhans); a phototrophic chlorophyte, *Chlamydomonas moewusii* Gerloff CCAP 11/5B; a mixotrophic chrysophyte, *Dinobryon sertularia* Ehrenberg CCAP 917/2; a mixotrophic euglenophyte, *Euglena gracilis* Klebs CCAP 1224/5Z; and a phototrophic cryptophyte, *Plagioselmis nannoplanctica* Skuja Novarino (formerly *Rhodomonas minuta* Skuja or *Rhodomonas lacustris* var. *nannoplanctica* Javornicky) CCAP 995/3. Since behavioral measurements made with these isolates were comparable with those from different sources (e.g., with 12 other *Chlamydomonas* spp. and strains of *C. moewusii* from several freshwater, brackish, and marine sources: Clegg unpubl. data), they were deemed representative of their species. Similarly, the stability of their behavior over time (>3 yr) and with changing culture conditions (e.g., light, Clegg et al. 2003a; or phosphate, Clegg et al. 2004a) also suggested that results from these cultured isolates had a broad ecological relevance.

Cells of each species cultured in chemostats were exposed to representative physical and chemical gradients in preference chamber experiments. The behavioral responses of populations and individual cells were measured by cell-track analysis, and preferences for light (Clegg et al. 2003a), temperature (Clegg et al. 2003b), phosphate, oxygen, carbon dioxide, and pH (Clegg et al. 2004a) were quantified. Subsequently, the comparative strength of these preferences was determined in experiments testing the response of flagellates to multiple gradients, and dominant preferences were ranked in order of importance (Clegg et al. 2004b). Using this hierarchy, quantitative limits for the model were derived from the three most important preferences for light, oxygen, and carbon dioxide (Table 1), and these were applied to the changing physical and chemical gradients in Priest Pot. For each species, this predicted distribution was then compared with the observed distribution of the natural population.

Table 1. Quantitative limits and sources of the three dominant behavioral preferences used to construct a model predicting spatial distribution. P_1 to P_3 signifies the order of importance of preferences for photon irradiance (I), oxygen (O_2), and carbon dioxide (CO_2) derived from a hierarchy of dominance established in previous laboratory studies (Clegg et al. 2004b).

Species	Order of dominance	Factor	Limit of preference	Source of preference limits
<i>Ceratium furcoides</i>	P_1	Irradiance	60–120 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$	I + O_2 (Clegg et al. 2004b)
	P_2	Oxygen	150–200 mmol m^{-3}	I + O_2 (Clegg et al. 2004b)
	P_3	Carbon dioxide	>70 mmol m^{-3}	CO_2 threshold (Clegg et al. 2004a)
<i>Chlamydomonas</i> sp.	P_1	Carbon dioxide	>80 mmol m^{-3}	O_2 + CO_2 (Clegg et al. 2004b)
	P_2	Oxygen	>150 mmol m^{-3}	O_2 threshold (Clegg et al. 2004a)
	P_3	Irradiance	4–40 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$	I + O_2 (Clegg et al. 2004b)
<i>Dinobryon sertularia</i>	P_1	Irradiance	4–120 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$	I + CO_2 (Clegg et al. 2004b)
	P_2	Carbon dioxide	>93 mmol m^{-3}	CO_2 threshold (Clegg et al. 2004a)
	P_3	Oxygen	>70 mmol m^{-3}	O_2 + CO_2 (Clegg et al. 2004b)
<i>Euglena gracilis</i>	P_1	Carbon dioxide	>75 mmol m^{-3}	CO_2 threshold (Clegg et al. 2004a)
	P_2	Irradiance	4–20 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$	I preference (Clegg et al. 2003a)
	P_3	Oxygen	<120 mmol m^{-3}	O_2 threshold (Clegg et al. 2004a)
<i>Plagioselmis nanoplanctica</i>	P_1	Irradiance	>120 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$	I preference (Clegg et al. 2003a)
	P_2	Oxygen	>150 mmol m^{-3}	O_2 + CO_2 (Clegg et al. 2004b)
	P_3	Carbon dioxide	>60 mmol m^{-3}	CO_2 threshold (Clegg et al. 2004a)

Where possible, most preference limits have been derived from the behavioral responses of cells to combinations of gradients (e.g., light and O_2 , light and CO_2 , CO_2 and O_2 ; Clegg et al. 2004b), since these are considered more ecologically realistic. In these cases, limits were extrapolated from measurements of population response that represent the range of light, O_2 , or CO_2 within which cells preferentially accumulate. When the arrangement of gradients was not representative of natural conditions (e.g., when high light corresponded with low concentrations of oxygen), quantitative limits were derived either from distinct bands of preference for single gradients (e.g., the preference of *E. gracilis* for 4–20 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) that are driven by phototactic or chemotactic responses and phobic reactions, which help position and maintain cells within preferred conditions, or from thresholds, above which cells accumulate (e.g., >70 mmol m^{-3} CO_2 in *C. furcoides*). These limits also took into account any flexibility in behavioral responses previously associated with light acclimation (exemplified by the preference of *C. furcoides* for a light range of 60 to 120 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$; Clegg et al. 2003a) or nutrient depletion (Clegg et al. 2004a) to help broaden the model's ecophysiological applicability.

For each species, these three quantitative limits were taken one by one and applied to depth profiles of irradiance, O_2 , and CO_2 from each sample date to produce bands of predicted distribution for each preference. Then, for each sample date, the final predicted depth range of distribution (Z) was defined as the vertical range within which all three preferences or the two dominant preferences overlapped (Fig. 2a). Although largely parametric, this model of prediction can be described by a series of equations.

The predicted depth range of distribution Z is based on the overlap of a series of predicted depth coordinates z_1 , z_2 , and z_3 derived from the upper (u) and lower (l) vertical

limits of primary (P_1), secondary (P_2), and tertiary (P_3) behavioral preferences:

$$z_1 = P_1u, P_1l; \quad z_2 = P_2u, P_2l; \quad z_3 = P_3u, P_3l \quad (1)$$

This means that Z at time t_1 can normally be expressed as a coordinate specifying the upper and lower limits of a vertical range delineated by the deepest upper limit (u_{\max}) and the shallowest lower limit (l_{\min}) of behavioral preferences:

$$Z(t_1) = > P_{(1...3)}u_{\max}, < P_{(1...3)}l_{\min} \quad (2)$$

with a series of these values $Z(t_{1...n})$ giving a temporal prediction of distribution. In effect, this superposes behavioral preferences onto depth–time contour plots of seasonal variations in physical and chemical conditions. In the event that these bands of preference failed to overlap, which occurred only rarely in this study, four additional rules were applied to weight Z for the dominance of preferences (these are described graphically and mathematically in Fig. 2b–e).

Analysis of model fit and ecological relevance—Regression analyses and parallel t -tests assessed the amount of significant variation from a 1:1 relationship between predicted mean depths (planimetrically derived as the mid-point of Zt) and observed average modal depths (at which cell abundance was greatest, and equivalent to the weighted centroids of Herman 1983), and gave a statistical measure of model fit. In addition, using the trapezoidal rule (that approximates the spatial area under a curve), measurements of the total area under the depth profile produced by the natural population on each sampling date were compared with the area of the depth profile that fell inside the predicted vertical limits, to give an indication of the percentage of the natural population for which the distribution is correctly predicted. Subsequently, for each

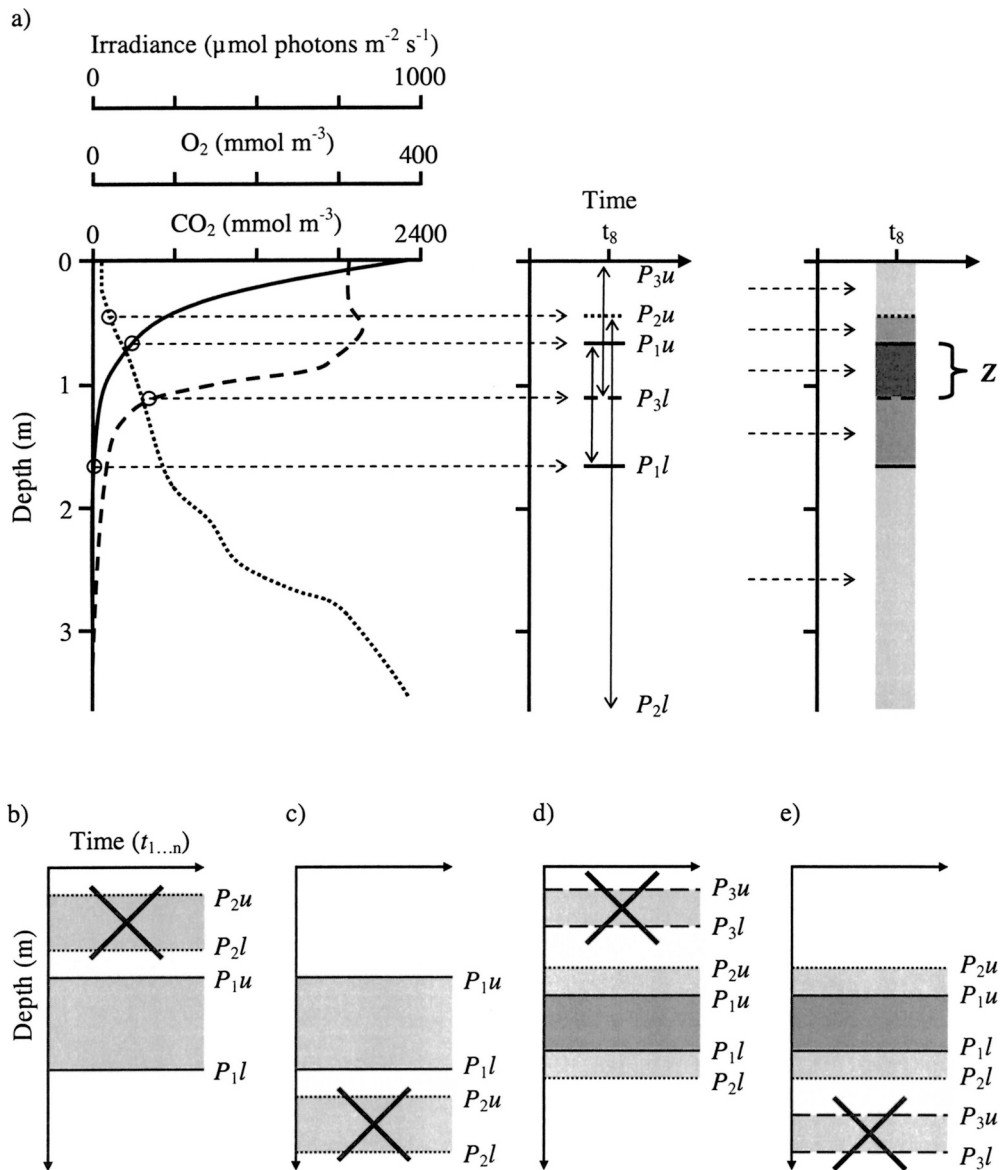


Fig. 2. (a) An example extrapolation of the predicted depth range of distribution (Z) for *D. sertularia* on one sample date (t_8 : 20 July 2000) in Priest Pot. Preference limits were applied to depth profiles of irradiance (solid line), O_2 (dashed line), and CO_2 (dotted line); bands of predicted distribution for each preference were produced; and the overlap of three preferences or the two dominant preferences were used to derive Z . Light gray, mid gray, and black shading indicates the overlap of 1, 2, and 3 bands of preference, respectively. (b–e) The four additional rules applied to weight Z for the dominance of behavioral preferences: (b) if the depth of P_{2u} or $P_{2l} < P_{1u}$, then disregard either P_{2u} or P_{2l} ; (c) if the depth of P_{2u} or $P_{2l} > P_{1l}$, then disregard either P_{2u} or P_{2l} ; (d) if the depth of P_{3u} and $P_{3l} < P_{1u}$ or P_{2u} , then disregard P_3 ; and (e) if the depth of P_{3u} and $P_{3l} > P_{1l}$ or P_{2l} , then disregard P_3 .

species, the average percentage of correct prediction and percentages of correct prediction within and outside periods of stratification were calculated and compared.

In a separate analysis, the degree of overlap of test species in the natural population and in the model's prediction was also assessed to determine the influence of behavioral response on the potential vertical segregation of flagellates in Priest Pot.

Finally, since the influence of behavior cannot be considered in isolation, the distribution of the natural

population and any vertical segregation was also related to potential influences such as the segregation and limitation of nutrients (derived from a comparison of molar inorganic N to SRP ratios with Redfield ratios) and to the distribution of other members of the plankton community such as algae, phototrophic bacteria, and protozoa (derived from chlorophyll and microscopic analyses). After appropriate tests for normality, nonparametric Spearman rank correlation analyses were used to compare the observed vertical distributions of each factor (expressed as an

average modal depth) with the observed modal depth of each species over the 13 sampling dates (r_{depth}). Additional analyses of any relationship between variations in depth from one sampling date to the next (r_{var}) helped to assess the potential for a causal link between these factors and the distribution of test species, while background analyses of any correlations of concentration at each depth at times $t_{1...n}$ (r_{time}) assessed the potential distorting influence of seasonal changes on distribution.

Results

Physical, chemical, and biological data—Strong physical stratification developed in Priest Pot during 2000. Although average irradiance in surface waters increased over the summer (Fig. 3a), often exceeding $1000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, light penetration through the water column decreased as a result of increasing vertical attenuation. During summer, this resulted in a 1% euphotic depth of between 1 and 2 m. Temperature depth profiles (Fig. 3b) showed that thermal stratification eventually stabilized during May to produce a steep thermocline of 7°C over 1.5 m, which persisted until the end of September.

Prior to thermal stratification, concentrations of SRP (Fig. 3c) were moderate. During stratification, however, release of phosphate from the sediment resulted in concentrations exceeding 100 mg P m^{-3} in the hypolimnion and the development of a steep phosphate gradient. Accordingly, only during a single period in late summer at depths of between 0.5 and 2.5 m did concentrations decrease to levels likely to limit algal growth ($<3 \text{ mg P m}^{-3}$). Nitrate and nitrite concentrations (Fig. 3d) were generally low and decreased to a minimum of 6 mg N m^{-3} (close to the detection limit) in the hypolimnion during stratification.

Concentrations of dissolved oxygen (Fig. 3e) were high prior to stratification and temporarily achieved supersaturation in early May (140% : 490 mmol m^{-3} at 9°C). However, upon stratification, the sediment and hypolimnion rapidly became anoxic and a strong oxycline developed and persisted until the autumn overturn. The position of this oxycline remained stable at a depth of 1.5 m, with concentrations typically decreasing from 300 to $0 \text{ mmol O}_2 \text{ m}^{-3}$ over as little as 0.7 m. Anoxic conditions in the hypolimnion also caused high concentrations of sulfide to develop during the summer (Fig. 3f), with peak concentrations of 45 mmol m^{-3} observed in July and late August.

During periods of turbulent mixing, concentrations of CO_2 (Fig. 3g) were close to equilibrium with air. However, during stratification, levels in the epilimnion sporadically became depleted, coinciding with increased algal productivity, whereas in the hypolimnion, concentrations increased considerably due to high rates of respiration. At times, the resulting CO_2 gradient ranged from undetectable to $>3000 \text{ mmol m}^{-3}$. The alkalinity of Priest Pot (not presented) also increased with depth, from 0.6 to 1.5 equivalents m^{-3} , whereas pH (Fig. 3h) decreased from 7.3 to 6.0 with increasing depth.

Phytoplankton in Priest Pot showed a clear seasonal periodicity. The proportion of flagellate species increased during stratification to account for 69% of the phytoplankton (in terms of the number of species). In addition, five flagellate taxa of an identical species (or genus in the case of *Chlamydomonas* sp.) to those tested in the laboratory experiments were observed and exhibited vertical distributions that varied considerably with time and between species.

Predicted versus observed distribution—Application of the model to field data showed that the final predicted depth range of distribution (Z) varied considerably with time and species. Predictions suggested that *C. furcoides* (Fig. 4a), *D. sertularia* (Fig. 4c), and *P. nannoplanctica* (Fig. 4e) would be positioned higher in the water column than *Chlamydomonas* sp. (Fig. 4b) and *E. gracilis* (Fig. 4d) and that all species should be more narrowly distributed during stratification.

Comparisons of predicted and observed distributions (Fig. 5a–e) showed that in all five species there was a good correlation of the model's prediction and the observed distribution of flagellates in Priest Pot. For *C. furcoides* (Fig. 5a), the model slightly overestimated the small upward movement of the population before stratification but accurately predicted the position (at around 0.5 m) and subsequent constriction of the population during stratification, and the small-scale changes in depth over summer. For *Chlamydomonas* sp. (Fig. 5b), the predicted distribution directly matched observed distributions throughout the year. The model also predicted the narrowing distribution of the sporadic population of *D. sertularia* (Fig. 5c) and its vertical position after stratification.

Perhaps the species for which the model fit least well was *E. gracilis* (Fig. 5d). The behavioral model did not predict the general downward movement with the onset of stratification. However, the constriction upon stratification was predicted, and, although the depth of the relatively small population at 1.5 to 2 m during summer was slightly underestimated, all the major peaks of population density fell within anticipated spatial limits. For the final species, *P. nannoplanctica* (Fig. 5e), the model predicted not only surface accumulations and fluctuations in position during the summer months, but also the vertical constriction with the onset of stratification and dispersal before the autumn overturn.

The accuracy of the model was quantified, and the spatial and temporal fit of predicted and observed data was calculated (Table 2). Regression analysis showed that in all five species there was no significant variation of predicted and observed average depths from a 1:1 relationship. In addition, areal measurements of the percentage of the natural population for which distribution was correctly predicted were generally high. For all species, the average areal fit of the model was $>56\%$ and was $>74\%$ for *C. furcoides*, *Chlamydomonas* sp., and *D. sertularia*. Further analysis also suggested that the fit of the model during stratification was exceptionally good, predicting 87%, 91%, and 93% of the distribution of populations of *C. furcoides*, *Chlamydomonas* sp., and *D. sertularia*, respectively.

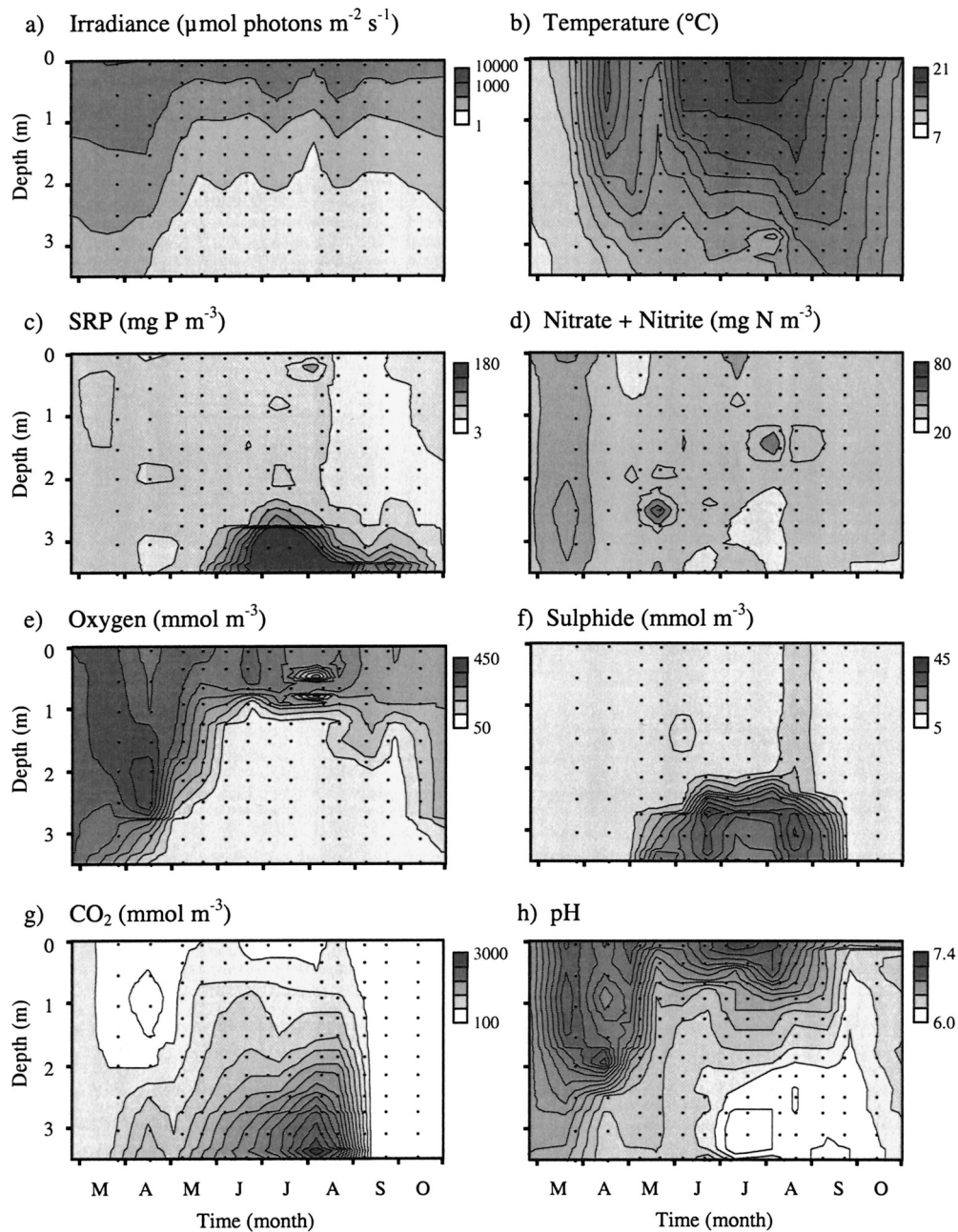


Fig. 3. Depth-time contour plots depicting the seasonal and vertical variation of selected physical and chemical properties (a-h) in Priest Pot in 2000. Dots indicate spatial and temporal sampling intervals.

Niche separation—The comparison of predicted and observed data (Fig. 5a-e) also highlighted the potential spatial separation of the five species of flagellates into discrete vertical niches. This is more apparent if only the average modal and mean depths of each species are compared (Fig. 6). In both the natural population (Fig. 6a) and the model (Fig. 6b), there was a general downward progression from *P. nannoplanctica* in surface waters, through *C. furcoides* and *D. sertularia*, to *Chlamydomonas* sp. and *E. gracilis* deeper in the water column. This progression was more distinct during stratification, and,

although less variable than in the natural population, the relative position of each species was well predicted by the behavioral model.

The formation of discrete layers or vertical niches by these motile species of flagellates also contrasts with the distribution of nonmotile species found in Priest Pot. For example, the distribution of a neutrally buoyant alga, *Chlorella* sp. (Fig. 5f), and other nonmotile species tended to be more homogeneous with a greater abundance in the epilimnion during summer and a wider vertical band of distribution than was typically exhibited by the flagellate species (Fig. 5a-e).

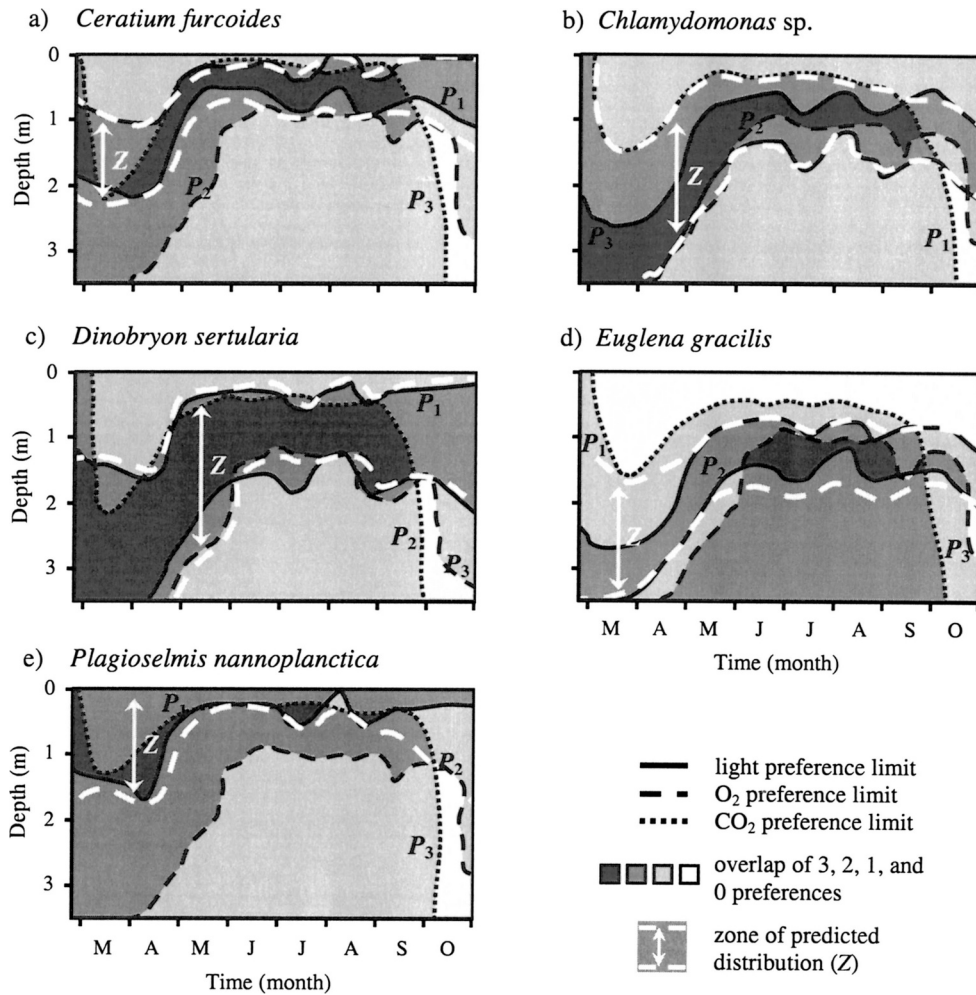


Fig. 4. Application of the quantitative limits of dominant laboratory behavioral preferences to the seasonal and vertical variation of light, oxygen, and carbon dioxide in Priest Pot. For each species (a–e), the overlap of two or more primary (P_1), secondary (P_2), and tertiary (P_3) behavioral preferences forms the basis of the final predicted depth range of distribution Z ($t_{1...n}$), shown by white, dashed lines.

Additional influences on flagellate distribution—Finally, comparison of the distribution of the five test species with additional factors (Fig. 7a–g) placed any spatial and temporal variations within the context of the broader plankton community found in Priest Pot and helped to assess the importance of the behavioral model. Although it is often difficult to separate correlations within the plankton community from their potential regulation by physical or chemical factors, comparison of vertical variations in the observed average modal depths of the five test species with variations in the modal depths of additional factors (not presented graphically) found no suggestion of clear causal relationships, even when distributions appeared related. For example, although the distribution of the test species and algal chlorophyll (Fig. 7a) were similar, with both abundant in the epilimnion during stratification, more detailed statistical comparison showed no correlation of modal depth ($-0.518 < r_{\text{depth}} < 0.518$, $df = 11$, $p > 0.1$) and no synchronization of

changes in vertical position ($-0.559 < r_{\text{var}} < 0.559$, $df = 9$, $p > 0.1$). Similarly, the spatial separation of all species from bacterial chlorophyll (Fig. 7b) could not be directly correlated with changes in the vertical distribution of test species ($r_{\text{depth}} p > 0.05$; $r_{\text{var}} p > 0.1$).

Statistical analyses also suggested no clear relationship with the distribution of cyanobacteria (Fig. 7c, $r_{\text{depth}} p > 0.1$, $r_{\text{var}} p > 0.1$) or diatoms (Fig. 7d, $r_{\text{depth}} p > 0.1$, $r_{\text{var}} p > 0.05$), and while there was a similarity with the distribution of photosynthetic flagellates (Fig. 7e) and a positive correlation of depth in two species (*C. furcoides* and *D. sertularia*, $r_{\text{depth}} > 0.702$, $df = 11$, $p < 0.05$), there was no “causal” synchronization of changes in vertical position ($-0.543 < r_{\text{var}} < 0.543$, $df = 9$, $p > 0.1$). Although there also appeared to be a separation of *C. furcoides*, *D. sertularia*, and *P. nannoplantica* from the increased population of ciliate and heterotrophic flagellate protozoa at the oxycline during stratification (Fig. 7f), statistical analyses again suggested no significant correlation ($r_{\text{depth}} p > 0.1$) or synchronization

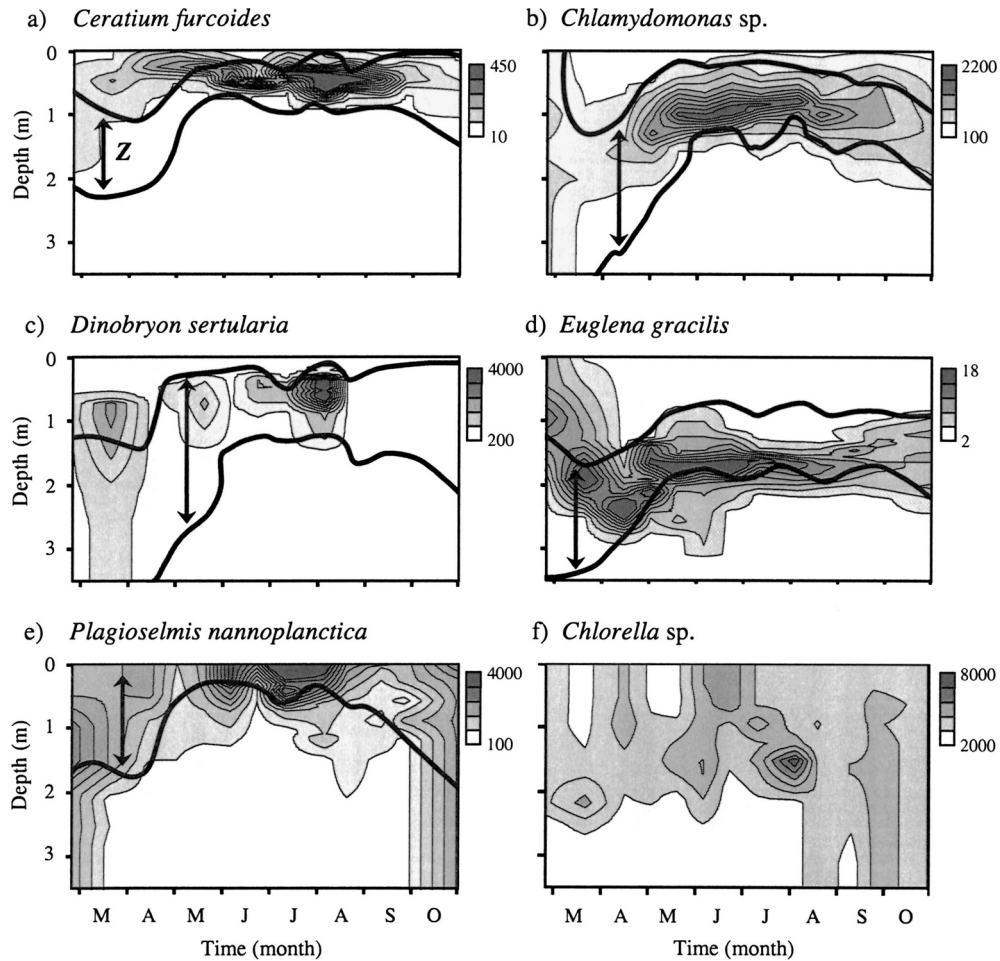


Fig. 5. A comparison of observed field data and the predicted seasonal vertical distribution of phytoplanktonic flagellates (a–e) in Priest Pot in 2000. Final predicted depth range of distribution, Z , derived from laboratory tests of behavioral response, is superposed onto depth–time contour plots of natural populations of flagellates. The abundance of natural populations is shown in cells mL^{-1} ; and Z , taken from Fig. 4, is now delineated by thick, black lines. A depth–time profile of *Chlorella* sp. is also presented (f), to illustrate the comparatively homogeneous distribution of nonmotile species in Priest Pot.

($r_{\text{var}} p > 0.1$) of depth distributions. Similarly, no correlation was found with the distribution of zooplankton (data not presented, $r_{\text{depth}} p > 0.1$, $r_{\text{var}} p > 0.1$). Background analyses of correlations at each depth and time (r_{time}) also stressed the

influence of seasonal change on distribution, since many of the correlations caused by mixing-derived homogeneity became insignificant during stratification, upon the development of vertical heterogeneity.

Table 2. Statistical and quantitative analysis of model fit. Regression analyses and associated t -tests show the variation of predicted and observed average depths for each species from a 1:1 relationship. Areal measurements indicate the percentage of the natural population for which the distribution was correctly predicted.

Species	Variation of predicted and observed average depths from a 1:1 relationship p (multiple t -tests, $n = 13$)	Areal fit (% \pm SD) of the observed population within the predicted vertical limits		
		Average (Mar–Nov)	During stratification	During mixing
<i>Ceratium furcoides</i>	>0.113	74.5 \pm 24.8	86.7 \pm 11.3	63.9 \pm 35.0
<i>Chlamydomonas</i> sp.	>0.301	82.5 \pm 13.6	91.3 \pm 7.7	75.0 \pm 14.9
<i>Dinobryon sertularia</i>	>0.183	87.1 \pm 12.5	92.9 \pm 6.3	82.2 \pm 16.7
<i>Euglena gracilis</i>	>0.062	58.6 \pm 23.4	67.1 \pm 12.5	51.3 \pm 27.7
<i>Plagioselmis nannoplanctica</i>	>0.072	56.6 \pm 21.3	61.1 \pm 15.7	52.7 \pm 23.1

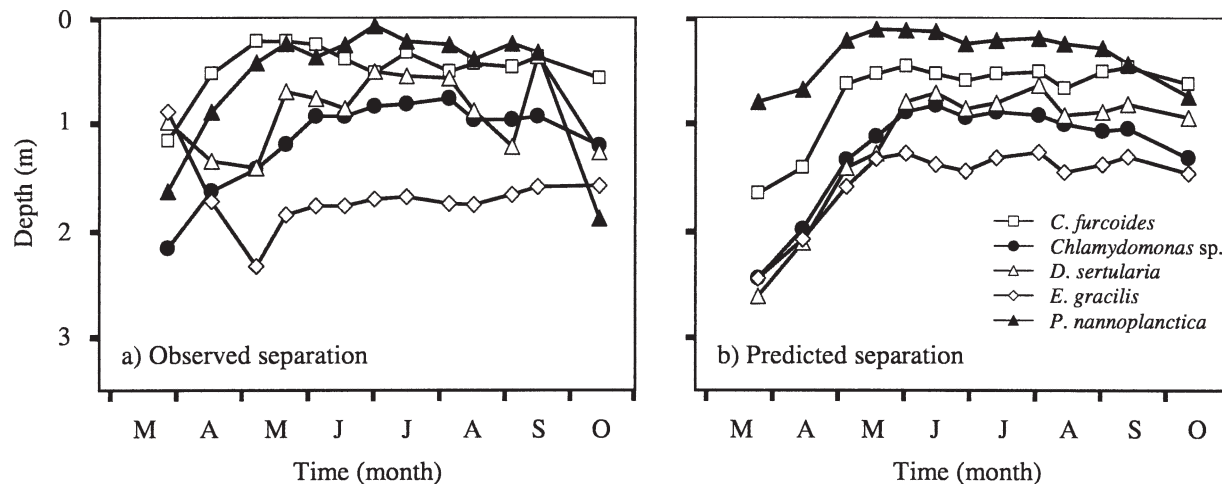


Fig. 6. Vertical niche separation of the five test species of flagellates in Priest Pot and the separation predicted by the behavioral model. Data plotted show (a) the observed population maxima based on the average modal depth and (b) the mean predicted vertical distribution over the sampling period.

For most of the year, comparison of molar inorganic N to SRP ratios with the Redfield ratio of 16N:1P suggested that the lake showed a potential for nitrogen limitation (Fig. 7g). However, only briefly was this realized when concentrations fell below detection limits in the epilimnion early in the spring and close to the oxycline later in the summer. The most severe nutrient limitation occurred when phosphorus became depleted at depths of between 0.5 m and 2 m during late summer, producing a spatial segregation of resources (light from dissolved inorganic nutrients). Statistical comparison again suggested no relationship between the average modal depth of nutrient limitation and flagellate distribution ($r_{\text{depth}} p > 0.1$). Although there was some suggestion that *D. sertularia* moved deeper to overcome this limitation, there was no direct correlation between changes in the vertical position of the test species (during the day) and the development of nutrient depletion in Priest Pot ($r_{\text{var}} p > 0.1$).

This absence of any clear spatial or temporal correlation of study species with the distribution of other members of the plankton community, or with periods of nutrient depletion, therefore highlighted the likely importance of behavioral response as a control of the distribution and vertical separation of flagellates within Priest Pot.

Discussion

Spatial and temporal variations in flagellate distribution—The seasonal distribution of phytoplankton in Priest Pot is typical of that found in many small lakes (Moss 1967; Reynolds 1976). With the onset of stratification, there was an increase in species diversity and a shift in composition toward flagellated species, reflecting an increased number of available niches (Reynolds 1992) and a propensity for flagellates to exploit opposing gradients of natural resources (Beckmann and Hense 2004). The development of steep physical and chemical gradients during summer was the result of reciprocal interactions among organisms

and their environment, and, while gradients were often extreme, they were not exceptional and are analogous to those found in many lakes (e.g., Salonen et al. 1984; Gervais 1998).

The heterogeneous distribution of flagellates in Priest Pot (Fig. 5a–e) was also comparable with that previously encountered elsewhere. Where species persisted, the onset of stratification, development of chemoclines, and increased light attenuation in the upper epilimnion all appeared to coincide with changes in vertical position. Such shifts in position are often difficult to interpret since they may be susceptible to distortion by strategies of competitive growth (Huisman et al. 1999; Klausmeier and Litchman 2001) and must always be considered within the broader context of diel migration (Flynn and Fasham 2002) and physiological or nutritional versatility (Cullen and MacIntyre 1998; Yamazaki and Kamykowski 2000). Nevertheless, comparison here with thresholds derived from laboratory experiments suggested that quantified behavioral preferences may be an important control of the vertical position of flagellates. Since the behavioral model predicted the vertical distribution of cells during the daytime phase of the diel migratory cycle, when different taxa often exhibit different vertical positioning in the water column, it is largely independent of the substantial upward and downward movements of cells associated with diel migration (e.g., Jones 1988). Furthermore, the relative importance of behavior over some aspects of physiological response (such as the adaptation of photosynthesis or pigment suites to different depths) can also be inferred by contrasting the narrow bands of concentrated distribution formed by flagellates (cf. Reynolds 1992) and their vertical separation (Fig. 5a–e) with the more homogeneous distribution of nonmotile photosynthetic species (Fig. 5f, Fig. 7a). Indeed, application of the model to field data suggested that, in addition to established factors such as the physiological versatility or nutritional capability of cells (e.g., Smayda 1997; Tittel et al. 2003), preferences may even help to prevent competition and promote the

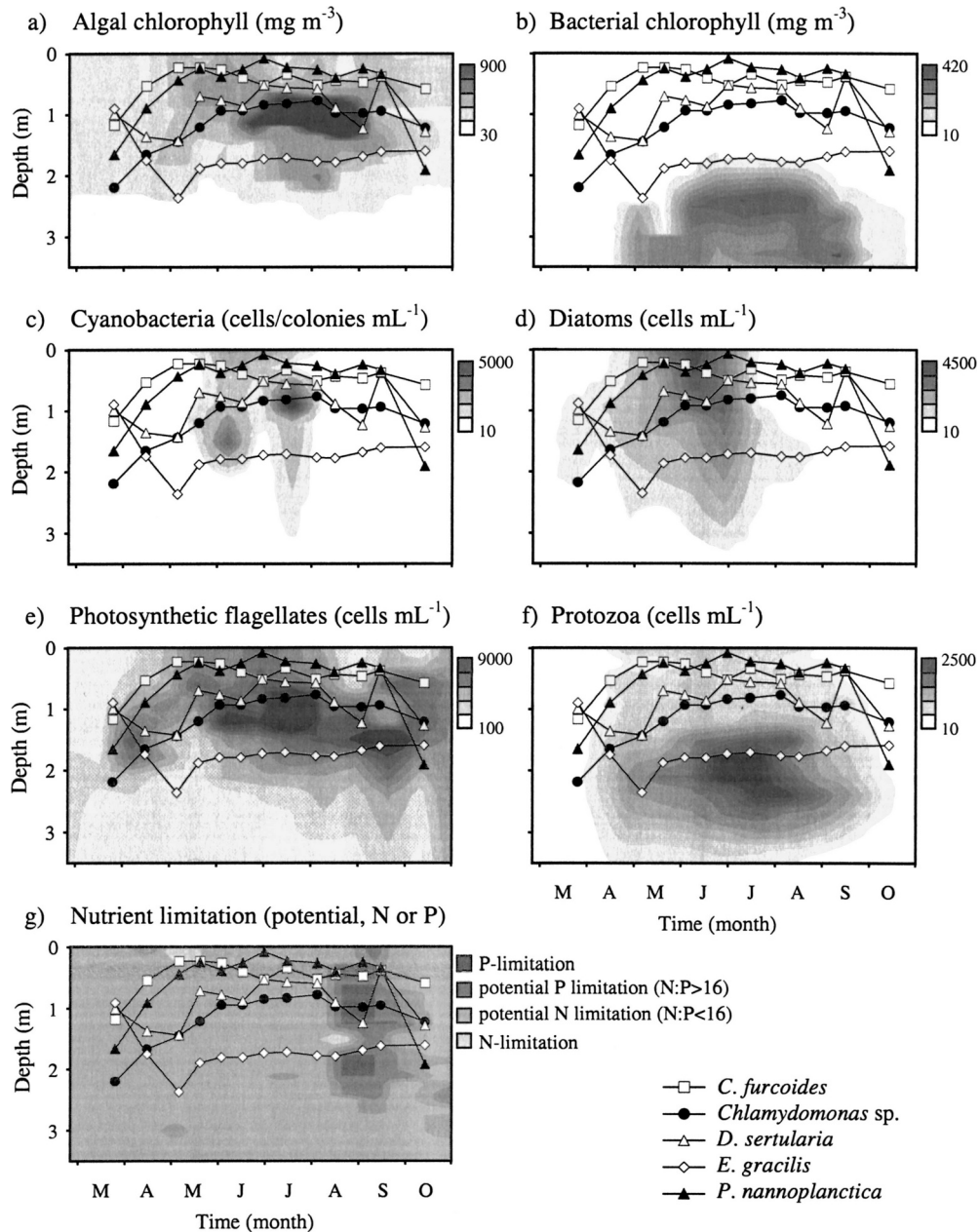


Fig. 7. Comparison of the spatial and temporal distribution of the five test species with additional factors of potential influence in Priest Pot. Observed average modal depth distributions (taken from Fig. 6a) are compared with depth–time contour plots depicting (a–f) the seasonal vertical distribution of planktonic groups and (g) variations in potential and actual nutrient limitation defined by Redfield ratios or when N and P concentrations fall below detection limits, respectively.

diversity of flagellates by assisting and maintaining a vertical niche separation.

The additional absence of any direct spatial or temporal correlation of flagellate distribution with other members of the plankton community, or with periods of nutrient depletion (Fig. 7a–g), also reinforced the importance of behavioral responses to environmental gradients as a potential controlling influence on vertical distribution.

Assessment of model fit and interpretative value—A more detailed assessment of model fit suggested that behavioral preferences for light, oxygen, and carbon dioxide combine to influence the daytime distribution of flagellates in Priest Pot (Fig. 5a–e). For example, the vertical distribution of *C. furcoides* appeared to be regulated by sensory preferences for moderate to high irradiance, high O_2 concentrations, and a weaker preference for moderate levels of CO_2 . This

positioned cells within oxic waters, in the center of the epilimnion. Similarly, the vertical positioning of *Chlamydomonas* sp. near the oxycline, the broad epilimnetic distribution of *D. sertularia*, the hypolimnetic distribution of *E. gracilis*, and the accumulation of *P. nannoplanctica* in surface waters all appeared to be controlled by characteristic behavioral preferences for different quantities of light, O₂, and CO₂ (Table 1).

The good general correlation of predicted and observed distributions was confirmed by regression analyses and an areal fit above 56%, which increased to over 90% during stratification (Table 2). The discrete vertical niches of the five test species (Fig. 6); their downward progression; the separation of *P. nannoplanctica* and *E. gracilis*; and the overlap of *C. furcoides*, *Chlamydomonas* sp., and *D. sertularia* were also well predicted by the model, particularly during stratification. This improved fit during stratification is most likely caused by the ability of flagellates to control their vertical position more precisely under less turbulent conditions (dependent on the relationship between swimming speeds and the velocity of the water field; Kessler 1986). Indeed, regression analysis suggested that model accuracy was inversely related to average wind speed (data not shown, $p < 0.05$, $r^2 = 0.68$, $n = 13$) and, consequently, the degree of vertical and horizontal mixing (regulated by eddy diffusivity).

Of course, the aim of any ecological model is to simulate natural occurrences and their underlying principles mathematically, in an attempt to improve the understanding of designated processes. The model presented here generally supported the use of behavioral responses derived in the laboratory as predictors of distributions in the field. However, there were some discrepancies that might reduce its applicability under certain environmental conditions and some limitations that must also be considered. For example, the underestimation of the depth of *E. gracilis* during stratification (Fig. 5d) may be explained by an affinity for sulfidic conditions (Camacho et al. 2001) or by an increased heterotrophic growth in the high concentrations of nutrients and phototrophic bacteria (e.g., *Thiopedia rosea*) found lower in the water column (Fig. 7b). Indeed, species-specific biochemical, physiological, and nutritional characteristics may affect the interpretative value of the model. For example, variations in the metabolic status of cells (Beckmann and Hense 2004), their photosynthetic (chromatic or bioenergetic) adaptation to light (e.g., Tandeau de Marsac 1977; Cullen and Lewis 1988), and their nutrient uptake abilities (Lieberman et al. 1994) can all influence vertical position. In many cases behavioral preferences and physiological optima may even be linked (e.g., light and growth, Clegg et al. 2003a), complicating prediction further. However, since the model presented here does not attempt to predict the abundance of cells, only their vertical distribution, some of these effects will be lessened.

The mixotrophic tendencies of flagellates might also contribute to changes in vertical position as cells seek to overcome the spatial segregation of nutrients by exploiting alternative, substitutable resources (e.g., Bird and Kalf 1986; Caron et al. 1993). This seemed unlikely in Priest Pot

since there was no direct correlation of changes in flagellate distribution with changes in either nutrient limitation or bacterial abundance (r_{depth} , $r_{\text{var}} p > 0.1$). It may be that observed temporal strategies of diel migration and nutrient retrieval (data not presented), or the ecologically robust K -selected tendencies common in some flagellates (e.g., improved nutrient storage capabilities in *C. furcoides*), were able to overcome this brief period of resource limitation. Further investigations are certainly required to help improve our understanding of the ecophysiological relationship between the quantitative behavioral preferences used in this model, nutrient limitation, and the physiological status and characteristics of cells.

In a similar way, more severe grazing or increased mixing in more turbulent waters might also affect the predictive power of the model. Although evidence suggested no direct correlation with the distribution of protozoa or the relatively low numbers of zooplankton in Priest Pot (Fig. 7f; r_{depth} , $r_{\text{var}} p > 0.1$), it is not possible to discount completely the potential effect of grazers on flagellate abundance, if not distribution. Likewise, although for most of the year swimming speeds in excess of $50 \mu\text{m s}^{-1}$ (Clegg et al. 2003a) could overcome the sheer stresses and velocity fields induced by low wind speeds across the sheltered surface of Priest Pot, the effect of behavior in more turbulent waters might only be expressed if mixing subsides for a sufficient period of time (as in Croome and Tyler 1988).

Although it is not possible to rule out altogether the underlying influence of these factors, the general importance of behavioral response as a control of distribution and the robustness of the behavioral model are supported by comparison with distributions of phylogenetically similar species reported in other studies. For example, the preferences of *C. furcoides* for moderate light and O₂ concentrations could contribute to its characteristic avoidance of high irradiance and anoxic conditions (e.g., Harris et al. 1979; Frempong 1984). The preference of *Chlamydomonas* sp. for low light and moderate O₂ and CO₂ could help explain the frequent occurrence of chlamydomonads deeper in the water column (e.g., Jones 1988; Tittel et al. 2003). The affinity of *D. sertularia* for high CO₂ also offers an alternative explanation to bacterial abundance for the metalimnetic distribution reported by Bird and Kalf (1986), indirectly supporting the argument that photosynthesis may be more important in this genus than the phagotrophic uptake of bacteria (e.g., Caron et al. 1993). The preferences of *E. gracilis* for low light, low O₂, and high CO₂ predicted the deep distributions observed by Häder (1987) and Lindholm (1992) equally well and corroborated the benthic tendencies of many euglenoids (e.g., Kingston 2002; Spilmont et al. 2005). Similarly, preferences for high light and O₂ in *P. nannoplanctica* might also contribute to the distribution reported by Sommer (1985) and account for its frequent abundance (and that of rhodomonads; Teubner et al. 2003) in the surface waters of many clear lakes (Lepisto and Holopainen 2003).

Such comparisons can be justified, in principle, due to the stability of the behavioral preferences on which the model is based and the use of isolates taken from a variety

of freshwater sources. They also suggest that the model may be applicable in a range of lakes and, importantly, since the model also held during the brief time of nutrient depletion in Priest Pot, in lakes of varying trophic state or with clear regimes of nutrient depletion.

The findings presented here should also be considered in a broader behavioral and ecological context. For example, this behavioral model provides a mechanistic explanation of one of the behavioral strategies (depth regulation) that flagellates employ and focuses on the daytime position of populations of flagellates. It does not predict the diel migration of species (that was also observed in Priest Pot) or any small vertical movement associated with changes in the nutrient or physiological status of individual cells. However, it does suggest, as an alternative to the metabolism-influenced thresholds of Kamykowski and Yamazaki (1997), that behavioral preferences are an important, stable control of the upper vertical limit of diel migration. The good general fit of the model also suggests that they should be considered as part of the battery of flexible behavioral, nutritional, and physiological strategies (e.g., phosphorus retrieval by diel migration, switching to heterotrophic nutrition) that flagellates employ to help promote survival or alleviate problems brought about by the segregation of resources in a pelagic ecosystem.

The ecological significance of behavioral response—As the first example of an expansive model that predicts flagellate distribution based on a suite of behavioral responses, our model supplements and expands existing models conceptualizing flagellate behavior. While confirming the underlying importance of photoresponse as a control of motility and distribution (Bearon et al. 2004; Hedger et al. 2004), it shows that response to chemical factors also influence the distributions of some species (such as *Chlamydomonas* sp. and *E. gracilis*). On a broader level, it highlights the potential ecological significance of behavioral response per se as a control of distribution, suggesting that its influence might be even stronger than already widely acknowledged (e.g. Smayda 1997; Kamykowski and Yamazaki 1997), and that quantified preferences should assume increased importance in future models.

For example, integration of the comparatively simple hierarchical approach demonstrated here could strengthen existing models in which internal, physiological (Beckmann and Hense 2004) and external, environmental factors (Liu et al. 2001) are shown to influence the distribution of phytoplankton and the formation of subsurface chlorophyll maxima. It might prove helpful in motility-based models (Liu et al. 2001; Bearon et al. 2004) investigating the formation of harmful algal blooms (e.g., of *Gonyostomum semen* and red-tide-forming dinophytes). Similarly, future iterations and an incorporation in Lagrangian and Eulerian models of bloom formation might also help improve our understanding of the link between behavioral preferences and migration (Beckmann and Hense 2004), cell metabolism (Kamykowski and Yamazaki 1997), water dynamics (Nagai et al. 2003), or diel circadian influences (Janowitz and Kamykoswki 1991), and may have theoretical,

practical, and economic value (Cullen and MacIntyre 1998).

The model presented here suggests that behavioral response can be used to help explain the seasonal depth distribution and vertical niche separation of phytoplanktonic flagellates, particularly in a stratified water column. It also supports the view that behavioral response may be ecologically influential, conferring functional and ecophysiological advantages over nonmotile species (Jones 1993; Broekhuizen 1999). It showed that the ability of cells to locate favorable microenvironments can influence population distribution and might therefore have physiological implications. For example, a correlation between behavioral and physiological optima in *P. nannoplanctica* (Riegman et al. 1996; Clegg et al. 2003a) positions cells in surface waters and may optimize primary production (Kamykowski and Yamazaki 1997). In the same way, the formation of metalimnetic peaks by *Chlamydomonas* sp. and *E. gracilis* might facilitate simultaneous access to light and organic or inorganic nutrients. Accordingly, behavioral response might influence community structure, food web interactions, and even geographical distribution, depending on the strength and flexibility of this link with physiology.

Through the application of laboratory derived quantitative limits to field data, this study therefore suggested that, in combination with a number of interacting physiological and ecological factors, active behavioral response and its diversity may be important controls of the temporal coexistence and the spatial, vertical niche separation of flagellates in a lake ecosystem.

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