Diel migration of phytoplankton and spectral light field in the Ría de Vigo (NW Spain)

Abstract
Diel migration of *Mesodinium rubrum*, *Eutreptiella* sp., *Scrippsiella trochoidea*, *Dinophysis acuminata* and *Ceratium furca* throughout a 24 h cycle is described for a stable, well-stratified estuary (Ría de Vigo, NW Spain). Daily changes in light quantity and in spectral light ratios i.e. red:far-red, blue:red, green:red and blue:green have been analysed. The spectral light ratios changed at twilight and around noon at various depths. Some of the downward migrations were well predicted by Stokes’ law, while other migrations were faster and deeper than calculated. The coincidence of these movements with abrupt changes in red:far-red, green:red and blue:green light ratios is discussed. Some species are able to migrate through the pycnocline, whereas others do not seem to be able to do so. Several species are present in maximum numbers at depth at night, while others display upward migration independent of light, suggesting the existence of endogenous rhythms. Upward migration at dusk began with dispersal of populations, with renewed aggregation at the sea surface coincident with an increase in the red:far-red ratio at 6 m and the green:red ratio at 6 and 10 m. Based on direct evidence for the control of flagellar mobility by light quality reported by other authors from laboratory studies, it is suggested that, together with other cues, spectral light ratios of different light qualities modulate vertical phytoplanktonic migration.

Introduction
Phytoplanktonic organisms have been found to adjust their position in the water column by means of vertical migrations (Forward 1976; Levandowsky and Kaneta 1987; Häder 1988). The motile organisms respond to gravity (Taylor 1987), to chemical (MacNab 1985) and thermal (Poff 1985) gradients, to the magnetic field of the Earth (Frankel 1984) and also to light (Forward 1975, 1976). Light is the main energy source for photosynthetic organisms, but the different wavelengths could play an important role as “informational factors” regarding the environmental conditions (Rüdiger and López-Figueroa 1992; Smith 1994). All these stimuli may act together, but the present study focusses on light-quality changes related to the vertical migration of the most abundant motile phytoplanktonic species in an estuary (Ría of Vigo; study site: 42°15′N; 8°45′W).

Phytoplanktonic organisms have developed at least three different responses to light as an “informational factor”: phototaxis, photokinesis and photoinhibition of migration (Forward 1975). The position of phytoplankton throughout the day is the result of a combination of positive and negative phototactic and photophobic responses. The dynamics of such interactions plus the gravitational effects of sinking determine the position of phytoplankton in the water column (Nultsch and Häder 1988). Irradiance and its changes were early recognised by Aschoff (1960) as providing active stimulus for phytoplanktonic cell movements. It is also well known that specific light qualities, mainly the ultraviolet/blue (UV/B) and green (G) spectral wavelengths modulate the degree of phototactic orientation under laboratory conditions (see Häder 1988).

Although the action of B, G and red/far-red (R/FR) photoreceptors in controlling flagellar movements has been demonstrated in the laboratory, the role of these photoreceptors in the natural environment, as well as daily light-quality changes and their influence and ecological significance have rarely been investigated (Levandowsky and Kaneta 1987; Rüdiger and López-Figueroa 1992).

The aim of the present study was to determine the coincidence of various changes in light quality with changes in the position of motile microplankton in the
water column. The vertical positions of the autotrophic ciliate *Mesodinium rubrum*, the euglenoid flagellate *Eutreptiella* sp. and of the dinoflagellates *Scrippsiella trochoidea*, *Dinophysis acuminata* and *Ceratium furca* were followed in the Ría de Vigo, with simultaneous records of the spectral light field in the water column. Migration in these species has previously been reported (Villarino et al. 1995), but the relationship between light and migration has not been evaluated. The light-quality changes are expressed as ratios between specific wavebands: red to far-red (R:FR), blue to red (B:R), green to red (G:R) and blue to green (B:G). These ratios were selected because of their previously demonstrated involvement in the perception mechanism controlling several physiological responses in higher plants (Smith 1994) and algae (Lipps 1973; Faust et al. 1982; Rüdiger and López-Figueroa 1992), including flagellar motility connected with vertical movements through the water column.

### Materials and methods

Light-field changes and distributions of motile microplankton were recorded over a 24 h cycle at a fixed station in the stable stratified estuarine system of Ría de Vigo (42°15′N; 8°45′W), which has a permanent well-defined pycnocline at ~3 to 4 m depth (Fig. 1). The station was sampled from 10:50 hrs Greenwich Mean Time (GMT) on 19 September to 11:00 hrs GMT on 20 September 1991 at ~2 h intervals.

#### Light measurements

The underwater spectra (350 to 800 nm) were measured during two cloudless, consecutive, summer days with a LiCor spectroradiometer Model Li-1800 UW at 11:00, 14:00, 15:00, 16:30, 17:30 and 18:30 hrs and at 06:30, 7:30, 8:30, 10:00, 11:00 hrs GMT, respectively. The photon-fluence rate, expressed in μmol m⁻² s⁻¹, was measured between 350 and 800 nm at 1 nm intervals at depths of 1, 2, 3, 4, 6, 8, 10, 12 and 16 m. Quantum integration (QI) was determined for total radiation (QI, \( \lambda = 350–800 \text{ nm} \)) and for several wavelength ranges: blue (B, \( \lambda = 410 \text{ to } 460 \text{ nm} \)), green (G, \( \lambda = 500 \text{ to } 550 \text{ nm} \)), yellow (Y, \( \lambda = 562 \text{ to } 612 \text{ nm} \)), red (R, \( \lambda = 655 \text{ to } 685 \text{ nm} \)) and far-red (FR, \( \lambda = 705 \text{ to } 735 \text{ nm} \)).

### Results

#### Underwater light ratios

General light conditions (defined in a previous study; Figueroa et al. 1994) revealed a maximum photon fluence at ~12.00 hrs GMT (local noon = 12:35 hrs GMT), with an attenuation coefficient, \( K_{dQI} \), ranging from 0.18 to 0.39 m⁻¹ (Fig. 2). Green (G) and yellow (Y) radiation both displayed a pattern of attenuation similar to that of total radiation (QI), with \( K_{dG} \) and \( K_{dY} \) ranging from 0.15 to 0.35 m⁻¹. Blue (B) light showed greater attenuation (\( K_{dB} \) near 0.6 m⁻¹) at dusk and dawn, remaining constant during the rest of the day (\( K_{dB} 0.35 \text{ and } 0.4 \text{ m}^{-1} \)). Red (R) light was more attenuated still, with values of \( K_{dR} 0.5 \text{ m}^{-1} \) and occasionally as high as 0.7 m⁻¹. The penetration of far-red (FR) radiation was very low and just reached 6 m depth, so \( K_{dF} \) was one order of magnitude greater than the attenuation coefficient for other radiations (Fig. 2). Changes in the light-quality field expressed as percentage total radiation (QI, \( \lambda = 350 \text{ to } 800 \text{ nm} \)) revealed that in the Ría de Vigo light was mainly comprised of green (G) and yellow (Y) wavelengths (Fig. 3). The relative importance of G and Y in relation to total radiation increased with increasing depth. B as a percentage of total radiation decreased with increasing depth from dawn to noon. Blue and red (B, R) bands displayed a complementary distribution: at noon 9% B was minimum and 9% R was maximum. The percentage of green light (G) decreased slightly from dawn to noon in contrast to Y light. The percentage of FR was very low, and values increased from 8:00 hrs to a maximum at noon (Fig. 3).

The ratios between different light qualities varied as a function of depth and time of day (Figs. 4 and 5). The R:FR ratio increased with increasing depth. High
variations in the G:R ratio were recorded, with constant values at the surface (~12), increasing with increasing depth to values of >100 as a result of the high penetration of green light. At 4 m depth, the G:R ratio displayed three maxima: at 08:00, 14:00 and 18:00 hrs GMT. Both R:FR and G:R ratios displayed greatest variations at the same time (during the morning). The B:R ratio displayed only two maxima – at dusk and at dawn. All these patterns were more pronounced at greater depths (Fig. 4). The only ratio that decreased with increasing depth was B:G (Fig. 5), which was maximum at the surface at dawn.

Microplankton distribution

Fig. 6 shows the daily distribution of the whole motile phytoplankton. The five species examined during this study were the most abundant among the motile
phytoplankton with the exception of cryptomonads (Figuerola et al. 1994). A general pattern emerged which reflected daily migration of the phytoplankton community (Fig. 6). Aggregation occurred at 2 to 4 m depth during the night, and maximum cell concentrations were recorded at the surface around noon. Chlorophyll a fluorescence and biovolume distribution maxima were roughly coincident (Fig. 6). Cells were usually found in the surface layers above 5 to 6 m depth. They dispersed during the night and aggregated during the day. Only 25% of cells were found deeper than 6 m.

Each species had its own migration pattern. The ciliate Mesodinium rubrum was highly active; it was able to move across the pycnocline during the night and was present in maximum concentrations at the surface during the day (Fig. 7a). Euglenophyta of the genus Eutreptiella aggregated at the surface in maximum numbers at noon and migrated through the pycnocline at night (Fig. 7 b). Small numbers of Scrippsiella trochoidea migrated through the pycnocline around dawn (Fig. 7c); between 11:00 and 15:00 hrs GMT this species migrated into the water column with no cells present in surface samples; from 15:00 to 17:00 hrs it was present in maximum numbers at intermediate (2 to 3 m) depths.

Dinophysis acuminata (Fig. 7d) followed a pattern similar to that of Eutreptiella sp, but was unable to cross the pycnocline during the night; this species remained mainly above the density gradient (Fig. 7d). Ceratium furca cells were located below the pycnocline at dusk (Fig. 7e); throughout the day they were present throughout the water column, but with a tendency to aggregate near the surface and to form a subsurface maximum from noon to 17:00 hrs GMT.

Thus, Mesodinium rubrum, Eutreptiella sp. and Ceratium furca were able to cross the pycnocline. During the night, cells dispersed and were probably present in maximum densities below 10 m. No relationship between migratory behaviour and taxonomic groups or size was found.

In general, all species investigated displayed alternative phases of aggregation and dispersal, with dispersal occurring at night. Whereas Dinophysis acuminata and Ceratium furca dispersed during the first half of the night, the other three species dispersed during the second half (Fig. 7).

**Discussion**

It does not seem not very probable that plankton motility is a random process. The causes of displacement and the resulting alternating phases of aggregation and dispersal have been related to internal rhythmicity (Häder 1988). These responses seem to be regulated by environmental variables. In addition, passive sinking by sedimentation due to the inhibition of flagellar movement by high irrater-
diances of visible light (Niell 1989) or specific UV-B ra-
diation (Häder 1988; Ekelund 1990) has been reported.

Light has received preferential attention among the
environmental variables held to be responsible for up-
ward and downward migrations (Taylor 1987). How-
ever, the relationship of plankton movements with light
quality in addition to light quantity has been considered
in only a very few studies (see Kirk 1983; Taylor 1987;
Häder 1988), despite the fact that parts of certain
wavebands have been proposed as environmental signals
through which several physiological responses are reg-
ulated (López-Figueroa 1992; Smith 1994). Thus, the
relationship of plankton migration to daily changes in
specific light-quality ratios is still a quite unknown as-
pect of plankton ecology.

The values of $K_d$ in Fig. 2 enable the interpretation of
plankton movements: cells displayed successive bouts of
aggregation and dispersion, and high values of $K_{d,Q}$,
$K_{d,G}$ and $K_{d,Y}$ and low values of $K_{d,R}$ and $K_{d,FR}$ from
12:00 to 15:00 hrs were evident during the accumulation
of cells at the surface (Figs. 6 and 7). Vertical displace-
ments in the early morning and in the evening imply cell
dispersion. Re-aggregation of cells at depth at dusk were
accompanied by increased $K_d$ values. The amount of
activity necessary to produce downward movements
were estimated by comparison of the expected speed at
which cells sink according to Stokes’ law calculated be-
tween two di/
cerent successive depths (Fig. 7). Fig. 8
shows the estimated speeds of sinking and the upward
speed of swimming for each species. Sinking was calcu-
lated assuming a di/
cerence in density between cells and
water of 0.02 g cm$^{-3}$, which seems reasonably realistic
(Taylor 1987). With this assumption, the cells descended
faster than estimated by Stokes’ law; this supports the
concept of active swimming by spherical cells such as
Scrippsiella trochoidea and Dinophysis acuminata. The
shape coefficients were near unity, indicating that the
displacements of these cells agree quite well with those
predicted by Stokes’ law. These species do not move
across the pycnocline and are present in highest densities
at a maximum depth of 6 m. The other three species
exhibited faster downward movements than calculated

Fig. 5 Green to red ($G:R$) and blue to green ($B:G$) waveband ratios at
surface (0 m) and at different depths during daily cycle in Ría de Vigo

Fig. 6 Distribution of biovolume of total motile phytoplankton
($\mu$m$^3$x10$^3$ ml$^{-1}$) in water column during daily cycle in Ría de Vigo
[Dashed line position of chlorophyll fluorescence maximum (5 to 6
relative units); BPL base of pycnocline; arrows show direction of cell
migration; D dispersed cells; black bar on top abscissa night]
by Stokes’ law; they cross the pycnocline and are found down to a depth of 10 m. Upward migration is linearly well related ($r^2 = 0.86$) to cell size (Fig. 8a). However, downward speed increases exponentially with increasing cell size (Fig. 8b). Thus, the relationship between upwards and downwards speed was not linear, suggesting two different strategies of displacement: *Eutreptiella* sp., *S*. *trochoidea* and *Dinophysis acuminata* move faster upwards than downwards, whereas *Mesodinium rubrum* and particularly *Ceratium furca* move faster downwards. Lombard and Capon (1971) reported similar differences for *Peridinium gregarium*.

Under conditions of high irradiance, the maximum density of Dinophyceae displaced from the surface to deeper levels between 11:00 and 14:00 hrs GMT. This noon-time pattern of distribution may be a direct effect of the high irradiances (300 to 500 µmol m$^{-2}$ s$^{-1}$) at this time of day on flagellar motility. In fact, these irradiances are in the range used by Levandowsky and Kaneta (1987) to cause immobilisation of cells under laboratory conditions, for instance in *Gonyaulax polyedra* (250 µmol m$^{-2}$ s$^{-1}$) and *Gyrodinium* sp. (319 µmol m$^{-2}$ s$^{-1}$). In the neighbouring Ría de Pontevedra, high irradiances were suggested to have the same photoinhibitory effect on growth, photosynthesis and movement of *Gyrodinium cf. aureolum* in surface waters (Jiménez et al. 1992).

In the early morning and before dusk, the majority of cells were at the surface (Table 1). Stokes’ law allowed us to calculate a speed of passive sinking of 2.4 m h$^{-1}$ for *Ceratium furca*, 0.9 m h$^{-1}$ for *Scrippsiella trochoidea* and 1.6 m h$^{-1}$ for *Mesodinium rubrum*. At the end of the light period, the cells moved down faster than predicted, suggesting the existence of vigorous downward swimming (Table 1). This migration occurred shortly before dusk at the same time as a slight decrease in R:FR and G:R ratios and an increase in the B:G ratio was recorded. Under laboratory conditions, Häder (1987) observed that cells of *Euglena gracilis* at high fluence rates move away from a bright surface (negative phototaxis). In weak light they move upwards in the water column towards the light (positive phototaxis); the antagonism of the two responses causes cell aggregation in a band with a fluence rate of 132 µmol m$^{-2}$ s$^{-1}$, (very close to the 60 to 120 µmol m$^{-2}$ s$^{-1}$ at which the freshwater dinoflagellate *Ceratium hirundinella* aggregates: Gálvez et al. 1988). Two dinoflagellates, *Peridinium faeorense* and *Amphidium carterae*, show a
Table 1  General pattern of plankton movement in five phytoplankton species and changes in light-quality ratios in Ría de Vigo during three different periods: between noon and dusk, during the night, and from dawn to noon. Light-quality ratios are considered high (H) or low (L). (N no change during period indicated) Bottom of table shows those values considered high and low for the individual ratios. R:FR: H = 20 to 80, L = 1 to 10; B:R: H = 3 to 6, L = 0.8 to 1.5; G:R: H = 20 to 100, L = 3 to 10; B:G: H = 0.5 to 0.3, L = 0.1

<table>
<thead>
<tr>
<th>Period of time</th>
<th>Migration (direction)</th>
<th>Light-quality ratios</th>
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<td></td>
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<td>R:FR</td>
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<td>Mesodinium rubrum</td>
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<td>noon to dusk</td>
<td>down</td>
<td>L to H</td>
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<td>night</td>
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<td>dawn to noon</td>
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<td>Eutreptiella sp.</td>
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<td>Scrippsiella trochoidea</td>
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<td>Dinophysis acuminata</td>
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<td>dawn to noon</td>
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<td>Ceratium furca</td>
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<td>noon to dusk</td>
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<td>dawn to noon</td>
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<td>H to L</td>
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pronounced positive phototaxis at low and intermediate fluence rates and a diaphotaxis (perpendicular to the light beam) at high fluence rates (Häder and Häder 1989; Eggersdorafer and Häder 1991). As a result of its orientation mechanisms, P. faeorens was found near the surface in the late morning hours and late afternoon and was more randomly distributed (dispersed) the rest of the time. The behaviour described for P. faeorens under laboratory conditions (Eggersdorafer and Häder 1991) and its resultant pattern of distribution were similar to those found for M. rubrum and C. furca in the present study.

At dawn, upward migration takes place in most species (Table 1). This could be triggered by factors other than downward displacement (Niell 1989). Downward sinking and upward swimming seem to be linearly correlated for cells with a biovolume ranging from 8275 µm³ (Scrippsiella trochoidea) to 24 600 µm³ (Dinophysis acuminata) (Fig. 8b). For the larger Mesodinium rubrum and Ceratium furca, downward speed is faster than upward speed, suggesting different controls for downward and upward movements. Upward migration occurred in all species at dawn, when irradiance is very low (5 µmol m⁻² s⁻¹ at the surface and 1 µmol m⁻² s⁻¹ at 6 m depth). Some singular changes in light quality occur at dawn: B:R and G:R ratios increase at 10 m, whereas at 6 m B:R remains constant. R:FR and G:R ratios are also maximal in the morning, and there is no FR radiation in the early morning at 10 m. The G:R ratio displays more notable changes at the time of upward migration from 10 m, whereas R:FR and G:R ratios change at the beginning of upward migration from 6 m. M. rubrum and S. trochoidea show clear responses during the same periods in which changes in the light ratios occur. C. furca migrates upwards to surface waters during the night, Eutreptiella sp. before dawn, and M. rubrum just at dawn. Cell dispersion is more marked in C. furca than in the other two species. An endogenous rhythm may operate in Eutreptiella sp. and C. furca, since their upward migration begins during the hours of darkness (Table 1), apparently independent of the changes in light ratios. The five species considered in this study showed the same migration pattern at dawn, when a drastic increase in the G:R ratio occurs.

There is no empirical evidence to support a cause–effect relationship between plankton migration and changes in light-quality ratios, especially since other light-quality changes could be responsible or have an interactive effect. Nevertheless, despite the paucity of experimental work, several parallels are worthy of further experimental investigation. The coincidence of certain plankton movements with changes in light-quality ratios (summarised in Table 1) should be tested under laboratory conditions. The involvement of some photoreceptors, for example, blue-light photoreceptors, as flavoproteins (Galland and Senger 1988 a, b) and pterins (Ghetti et al. 1985; Galland et al. 1990), G-light photoreceptors (Foster and Smyth 1980; Uhl and Hegemann 1990) and phytochrome (Song et al. 1979) has been demonstrated in the control of flagellar movements, although the actual role of these photoreceptors in aquatic environments is unknown.
The present study indicates that light-quality ratios may act as complex switches controlling phytoplankton migration, since these ratios change dramatically at twilight and around noon and can be detected by photoreceptors involved in controlling flagellar movement in some phytoplankton. The only laboratory evidence for light-quality ratios affecting flagellar movements was reported by Forward and Davenport (1968) and Forward (1973); the inhibition of flagellar movement in Gyrodinium dorsum is regulated by B light and is affected by the environmental R:FR ratio. Song et al. (1979) suggested the involvement of a B-light photoreceptor and phytochrome in this alga. On the other hand, recent biochemical analysis has revealed the presence of at least four different chromoproteins, independent of the photosynthetic apparatus, which were separated from a membrane fraction. These chromoproteins absorb at 380, 638, 667 and 710 nm, and control phototaxis in Peridinium gatunense (Haupt and Häder 1994). The interaction of two photoreceptor systems has also been reported to control flagellar movement in P. gregarium (Lombard and Capon 1971), in which B light stimulates swimming away from but not swimming towards light, whereas a yellow \((\lambda = 579 \text{ nm})\) photoreceptor induces moderate positive phototaxis.

The present study report on the coincidences of daily movements and changes in light-quality ratios and suggests the possible control of plankton movements by light changes. If this hypothesis is correct, a mechanism of light detection by specific photoreceptors must exist. This speculation is supported by theoretical knowledge of the perception mechanisms of informational light in plant cells that are presumed to be general by several authors (Chambers and Spence 1984; López-Figueroa 1992; Smith 1994). The influence of daily changes in different light-quality ratios on plankton movements cannot be disregarded, and further experimental and field research is required.

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