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Blue light regulates the rhythm of diurnal vertical migration in the raphidophyte red-tide alga *Chattonella antiqua*

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We examined the effects of photoperiod, wavelength and light fluence rate on diurnal vertical migration (DVM) cycle in a coastal raphidophyte, *Chattonella antiqua*. We first observed the DVM under different combinations of light–dark (LD) cycles and light spectra. Under continuous white, UV-A or blue light, DVM followed the LD cycle established during the white light pre-conditioning, for one cycle, and then became arrhythmic. Under red light, however, the DVM rhythms under the different LD regimes continued approximately as during pre-conditioning. When *C. antiqua* cultured under continuous red light was exposed to a 2-h pulse of blue light at the beginning or end of artificial 'night', the DVM was delayed or advanced, respectively. The fluence rate–response curve indicates a blue-light threshold of 10^{-2} μmol photons m^{-2} s^{-1} for the DVM phase shift. The equal-quantum action spectra for phase shift peaked in the UV-A/blue region (360–480 nm), which is the part of the light spectrum most transmitted in its natural habitat. We show that *C. antiqua* can sense the weak blue component of

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sunlight throughout its depth range, allowing it to cue its DVM to the day–night cycle regardless of weather and transparency.

KEYWORDS: flagellate; gravitaxis; harmful algal blooms; phototaxis; wavelength

INTRODUCTION

Light is a source of both energy and information for the many biota, especially plants. The spatial, temporal and spectral variability of light experienced by aquatic plants represented by phytoplankton differs significantly from that experienced by terrestrial ones, due to the selective attenuation of solar irradiance in the aquatic medium (Ragni and Ribera d'Alcalà, 2004). In the ocean, the full visible spectrum of white light is found only at the ocean's surface, since the sun's electromagnetic energy is selectively absorbed and filtered as it penetrates the upper layers. Infrared and red light is absorbed and filtered by water itself with green, blue and blue-green light penetrating the furthest (Jeffrey, 1984). However, in other seas, and especially coastal waters, blue light fluctuates more than the light of other wavelengths, and phytoplankton dynamics follow the attenuation of blue light (Shikata *et al.*, 2009).

Generally speaking, blue light may thus be a stronger candidate for exploitation as an environmental cue by marine plankton (Jeffrey 1984; Losi and Gärtner, 2008). Shikata *et al.* (Shikata *et al.*, 2009, 2011b) found that blue light is the most effective for promoting germination of resting stage cells and growth of vegetative cells in coastal microalgae such as diatoms. Furthermore, some blue-light receptors occur in aquatic microalgae (Iseki *et al.*, 2002; Kasahara *et al.*, 2002; Ishikawa *et al.*, 2009), although how these work in the actual environment has not yet been demonstrated.

It has been well known for many years that motility in flagellate algae is controlled by light. Phototaxis in microflagellates is a representative example, and blue light is the most effective out of other components of visible light for phototaxis in many flagellates (e.g. Matsunaga *et al.*, 1998; Horiguchi *et al.*, 1999; Iseki *et al.*, 2002). Furthermore, based on the modeling of spectral ratios of different light wavelengths together with measured abundances of major phytoplankton species, it has also been suggested that spectral ratios of light may act as complex switches controlling phytoplankton DVM (Figueroa *et al.*, 1998).

DVM is a biological phenomenon observed in some flagellate algae including some raphidophytes and dinoflagellates. They start to swim up to the surface before dawn and down to deeper layers at dusk (Yamochi and Abe, 1984; Olsson and Granéli, 1991; Koizumi *et al.*, 1996; Park *et al.*, 2001). This DVM enables flagellates to optimize photosynthesis regardless of the weather and underwater transparency (Ault, 2000), to acquire nutrients over a wide depth range (Watanabe *et al.*, 1991) and to avoid predation by zooplankton which swim to the surface at night and return to deeper layers in the daytime (Lampert, 1989). DVM thus aids in competition with other microalgae such as diatoms, which do not have this ability (Watanabe *et al.*, 1995; Smayda, 1997; Kamykowski *et al.*, 1998; Salonen and Rosenberg, 2000). Hence DVM is one of the most important physiological adaptations for survival and growth of flagellate algae.

It has been suggested that the motility related to DVM is driven by physiological controls such as phototaxis or geotaxis, and timed by an endogenous clock (Kohata and Watanabe, 1986; Wada *et al.*, 1986; Roenneberg *et al.*, 1989; Eggersdorfer and Häder, 1991; Kamykowski *et al.*, 1999; Lebert and Häder, 1999), while being influenced by temperature (Heaney and Eppley, 1980), salinity (Erga *et al.*, 2003; Bearon *et al.*, 2006; Jephson and Carlsson, 2009), light (Kingston, 1999; Richter *et al.*, 2007) and nutrient concentrations (Heaney and Eppley, 1980). Although there have already been many studies of DVM, the difficulty of detailed and close observations has hindered progress in studies on the endogenous mechanisms of DVM in relation to environmental factors.

We have developed a system for automatically observing DVM and measuring its periodicity. Using this system, we have observed how light spectra and light–dark (LD) cycles 'key in' a circadian rhythm to determine the timing of DVM. In the present study, we used this system with a raphidophyte, *Chattonella antiqua* (Hada) Ono. This species causes massive fish kills around Japan, estimated to cost >100 million US dollars in summer during the last few years, occurring also in Korea, the Netherlands, USA and Brazil (Hallegraeff and Hara, 2003; Imai and Yamaguchi, 2012).

METHOD

Culture

We isolated the clonal strain of *C. antiqua* (strain number: 8820) used in this study from the Yatsushiro

Sea, Japan in 20 August 2008. The strain was not axenic. Cultures have been maintained in 175-mL Nunc flasks containing 150 mL of modified SWM-3 medium (Shikata *et al.*, 2011a) with a salinity of 30 at 25°C under 150 μmol photons m^{-2} s^{-1} of white fluorescent irradiation on a 14:10 h LD cycle since it was isolated. Fluence rate in the incubator was measured with a Quantum Scalar Laboratory Irradiance Sensor (QSL-2101, Biospherical Instruments, Inc., San Diego, CA, USA).

Monitoring of DVM rhythms

DVM rhythms were monitored using both analog and digital systems (Figs 1 and 2). These two systems were similar in that near-infrared light was scattered by the flagellate cells and captured with a charge-coupled device (CCD) video camera. It is known that near-infrared light generally has no effect on most biological processes. The first system used an analog camera and the second used a digital camera. These systems were operated in a temperature-controlled (25°C) dark room.

The analog system consisted of several components (Fig. 1). A rectangular acrylic chamber (basal aspect: 1-cm wide × 5-cm long) containing 20 mL of cell suspension (i.e. depth, 4 cm) was held in place with a clamp. The cell suspension was illuminated by a photography light source (CS-15, Asanuma & Co. Ltd, Tokyo, Japan) with an LX-903 optical filter transparent only to light with wavelengths >800 nm (Mitsubishi Layon Co. Ltd, Tokyo, Japan). Light scattered by the cells was captured by a color CCD camera (WAT-2215, Watec Co. Ltd, Yamagata, Japan) from which the infrared cut-off filter had been removed and the LX-903 optical filter was mounted in front of the camera lens. The images were recorded by a time-lapse video recorder (AG-6730, Panasonic, Tokyo, Japan) every hour for 2 days.

The software *Image J* (http://rsbweb.nih.gov/ij/) was used for digitizing, processing and analysis of analog images. First, each color image was split into red, green and blue components. Vertical profiles of gray values for an area of cell suspension were extracted from the images produced by the blue channel, which had the highest contrast. Because *Chattonella* cells did not remain in the middle layer under any of the conditions used in the present study (Fig. 3), the time–course variation of the ratio of the average gray value in the surface layer (0–0.5 cm) to that in the bottom layer (3.5–4.0 cm) (hereafter described as 'surface accumulation ratio') was used to represent the DVM rhythm.

We developed the following digital system to simplify image processing such as capturing images and to observe multiple samples simultaneously. The digital system is illustrated in Fig. 2. A monochromatic digital CCD camera (CFW, Scion Corporation, Frederick, MD, USA) sequentially photographed up to 36 samples (12 groups of 3 samples) held in a synchronous motor-driven wheel which made one complete revolution every 5 min. The infrared cut-off filter in the CCD camera had been previously removed and the LX-903 optical filter was mounted in front of the camera lens. The samples were illuminated by a projection lens-combined light-emitting diode (LED; peak wavelength, 850 nm; Pi Photonics, Inc., Shizuoka, Japan) when in position to be photographed, and the camera captured the light scattered by the cells. Each sample was time-lapse photographed every 5 min for 13 h 35 min to obtain raw data for determining the DVM rhythm. The motion of the wheel and the timing of photography were collectively controlled, via RS-232c and IEEE1394 interfaces, respectively, by a 'BeanShell script' file running on Micromanager software (http://valelab.ucsf.edu/~MM/MMwiki/index.php/Main_Page).

Processing and analysis of digital images were conducted as described below. First, the vertical profiles of the gray values were extracted from the areas of cell suspension in the captured images using *Image J*. The surface accumulation ratios were then calculated as the ratio of an average of gray values in the surface layer (0–0.375 cm) to that in the bottom layer (2.625–3.000 cm). The time–course variation of the surface accumulation ratios was used to represent the DVM rhythm; a 3-point moving average was used for smoothing. The amplitudes of DVM rhythms in different samples from a set of video-frame observations were normalized to compare phases among different samples. The method was as follows: the minimum value of accumulation ratio in an observation of a sample was uniformly subtracted from all values of the accumulation ratios in the observation to adjust the minimum value among different samples in a set of observations, and then each value was uniformly divided by the maximum accumulation ratio to adjust the maximum value among different samples in a set of observations. Furthermore, a regression line of the normalized surface accumulation ratios between 0.25 and 0.75 was drawn by least-square method using Excel. Finally, the degree (time) of phase shift was determined at a reference phase 0.50 in the regression line by a comparison with the control.

Effects of LD cycle on DVM

Chattonella antiqua at ~2500 cells mL^{-1} were precultured under 150 μmol photons m^{-2} s^{-1} of white fluorescent irradiation with a 14:10 h LD cycle (light period, 0600–2000 h local time). Cultures were then

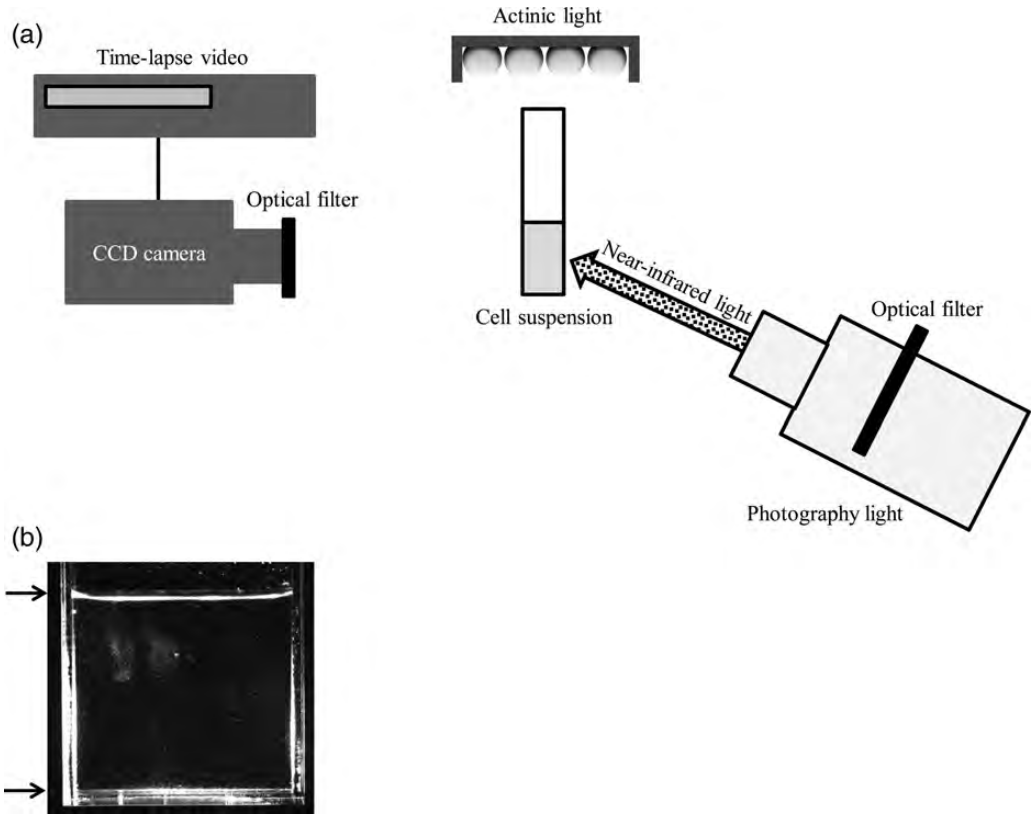

Fig. 1. (**a**) Schematic of the analog system for monitoring DVM. In this system, near-infrared light from a photographic light source with an optical filter that transmits only wavelengths >800 nm was scattered by cells in suspension, captured by a color analog CCD camera without any infrared cut-off filter and recorded by time-lapse video. A matching optical filter was also attached in front of the camera lens. (**b**) An example of the images captured by this system. In this image, the arrows show that cells are near the top and the bottom.

incubated under 50 μmol photons m^{-2} s^{-1} of white light from a fluorescent lamp (FL20SW, Toshiba Lighting and Technology Corporation, Kanagawa, Japan), or under 16.5 μmol photons m^{-2} s^{-1} of UV-A LED (Holorer-it, Pi Photonics, Inc.; peak wavelength: 365 nm), blue LED (Sanyo MIL-C1000T + MILU200, Sanyo Electric Co. Ltd, Tokyo, Japan; peak wavelength: 430 nm) or red LED (Sanyo MIL-C1000T + MILU200, Sanyo Electric Co. Ltd; peak wavelength: 660 nm). Four light regimes were used—14:10 h LD (the same as in pre-conditioning culture); 14:10 h LD with the light period shifted to 1600–0600 h; continuous light (LL) and DD (DD). DVM was monitored using the analog system. Each new light regime was imposed on the *C. antiqua* cultures at 1400 local time, and DVM monitoring began at 2000 h.

Blue-light irradiation: effects of timing and fluence rate

Chattonella antiqua was pre-cultured under 150 μmol photons m^{-2} s^{-1} of white fluorescent irradiation on a 14:10 h LD cycle (light period, 1900–0900 h). Three milliliters of the pre-culture (cell density: ~2500 cells mL^{-1}) was poured into small acrylic chambers (three chambers per treatment) and incubated under 16.5 μmol photons m^{-2} s^{-1} of continuous red LED light (described above) for 22–34 h to stabilize the cells and supply energy for survival and swimming. The irradiation experiment described above had already shown that this quality and fluence rate of light did not cause a phase shift in DVM. With the red light as the background irradiation, the cells were then exposed to a 2-h pulse of blue LED light ('Actinic light' in Fig. 1) (peak wavelength, 480 nm; Sanyo MIL-C1000T + MILU200, Sanyo Electric Co. Ltd) at 1 μmol photons m^{-2} s^{-1}. Seven sequential 2-h periods were used, from the 2 h just before the subjective nighttime on Day 1 (0700–0900 h) to the 2 h following the end of the subjective nighttime on Day 1 (1900–2100 h). These periods were chosen because light-induced phase shifts of circadian rhythm generally occur during the subjective nighttime (Pittendrigh, 1993). On Day 2, DVM was monitored using the digital system for 10 h 35 min starting at 0930 h.

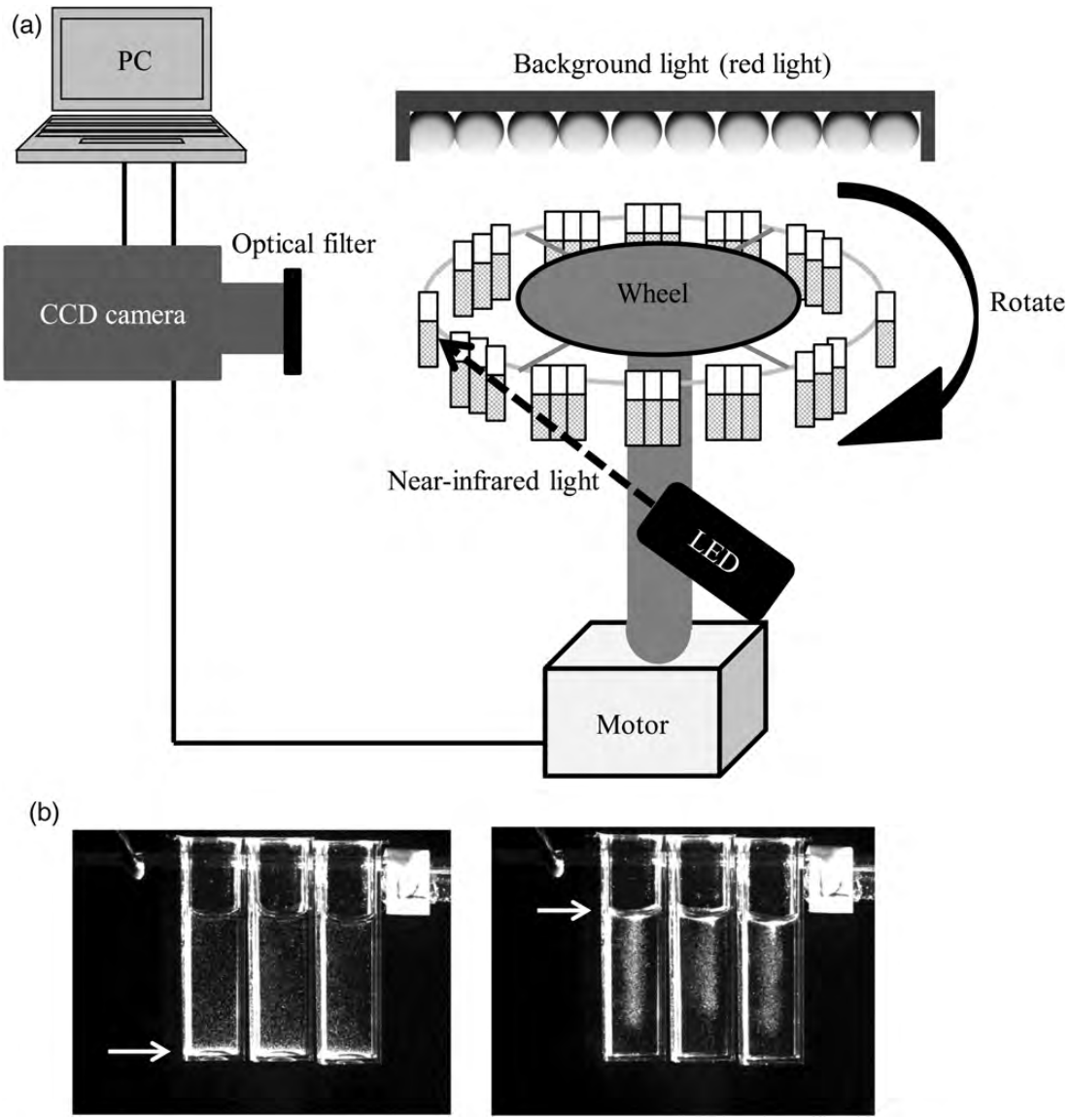

Fig. 2. (**a**) Schematic of the digital system for monitoring DVM. In this system, near-infrared light from an LED scattered by a cell suspension was captured by a digital CCD camera and recorded by PC. An optical filter that transmits only wavelengths >800 nm was attached in front of the camera lens. Up to 36 rectangular chambers (1 × 1 × 5 cm) containing 3 mL of cell suspension were fixed in a turn table that was rotated by an electric motor. The red LED provided continuous background light during the monitoring. (**b**) Two examples of images captured by this system. The left and the right images show accumulations of cells at the bottom and surface, respectively.

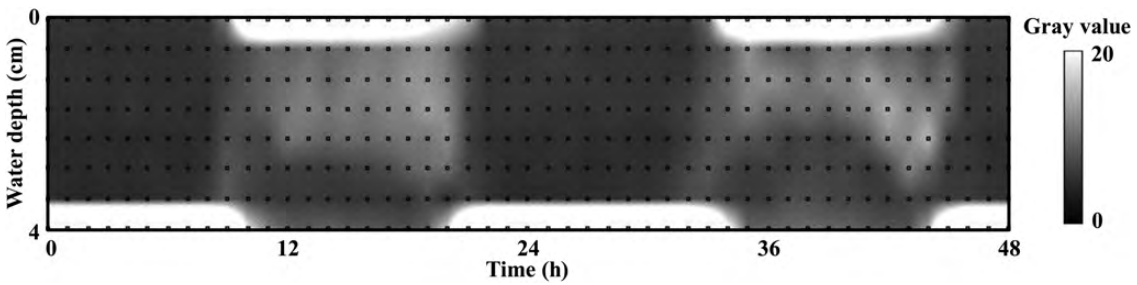

Fig. 3. An example of time–course variations of gray value in a series of photographing. A gray value is represented with color tone (black–white).

The effects of blue-light fluence rate on DVM were observed using pulsed blue light (peak wavelength: 480 nm) from the Okazaki Large Spectrograph (described in the following section) at different fluence rates from 0.003 to 0.3 μmol photons m^{-2} s^{-1}. Three milliliters of the pre-culture (cell density: ~2500 cells mL^{-1}) was poured into small acrylic chambers (six chambers per treatment) and incubated under continuous red LED light. Then, the cultures were exposed to the blue light from 0900 to 1100 on Day 1, and DVM was monitored using the digital system for 13 h 35 min from 0930 h. The time of phase shift was determined as described previously.

Action spectroscopy

Chattonella antiqua was pre-cultured under 150 μmol photons m^{-2} s^{-1} of white fluorescent irradiation on a 14:10 h LD cycle (light, 1900−0900 h). Three milliliters of the pre-culture (cell density: ~2500 cells mL^{-1}) was poured into small acrylic chambers (six chambers per treatment) and incubated under 16.5 μmol photons m^{-2} s^{-1} of continuous red LED light in a small acrylic chamber for 24 h. Then, with this red light as the background irradiation, the cell suspensions were incubated under eight wavelengths between 280 and 560 nm at a fluence rate of 0.03 μmol photons m^{-2} s^{-1} from the Okazaki Large Spectrograph (described in the paragraph that follows). Following 2 h of irradiation by monochromatic light during the first half of the subjective nighttime, samples were incubated under the background light for 22 h and then monitored for DVM rhythm and phase-shift using the digital system.

Monochromatic light for action spectroscopy was provided by an Okazaki Large Spectrograph with a 30-kW xenon arc-lamp (Ushio Electric Co., Tokyo, Japan) at the National Institute for Basic Biology, Okazaki, Japan (Watanabe *et al.*, 1982). Fluence rates were measured with a silicone photodiode-based photon meter (QTM-101, Monotech, Inc., Saitama, Japan) and adjusted for each light fluence rate using neutral-density filters (Fujitok Co., Tokyo, Japan). Each sample was placed in a threshold sample box (Watanabe *et al.* 1982).

Data treatment

For measurements using the digital system, mean values and standard errors were calculated from six independent replicates. Moreover, the data from the six-replicated experimental manipulations were tested for homogeneity of variances using Levene's test. Significance of differences among phase shifts in different treatments was determined using one-way analysis of variance; multiple comparisons were made using Tukey's honestly significant difference (HSD) test. All data that did not show homogeneous variances were log-transformed, and a Levene's test was performed once again. A significance level of $P < 0.05$ was used in all tests. All analyses were performed using SPSS for Windows (SPSS, Chicago, IL, USA).

RESULTS

Effects of LD cycle on DVM

DVM was observed under different LD cycles for 2 days. In DD, most *C. antiqua* cells ascended from the bottom to the surface on Day 1 and remained at the surface thereafter (Fig. 4 "White"). Under white light with an LD cycle, most cells migrated from the bottom to the surface 1−3 h before the start of the light period and then from the surface to the bottom 1−3 h before the dark period (bold line and dashed line in Fig. 4 "White"). Under continuous white light, as in DD, cells migrated to the surface on Day 1 but thereafter most cells stayed at the surface (fine line in Fig. 4 "White").

When the cells were incubated under monochromatic light (UV-A, blue or red) with the same LD regime as in pre-culture, DVM was synchronous with the LD cycle, as was the case under white light. Under UV-A irradiation with an LD regime different from that in pre-culture, most cells accumulated near the surface on Day 1 at the nominal 'light-on' time of the pre-culture LD regime, but thereafter the accumulations of cells at the bottom or at the surface were synchronized with the change from light to dark and dark to light, respectively (Fig. 4 "UV-A"). Similarly, under blue light with different LD regimes, most cells on Day 1 accumulated near the surface just before the time of the nominal light-on time of the pre-culture LD regime, but thereafter most cells accumulated at the bottom or at the surface 0−3 h before the start of the dark or light period, respectively (Fig. 4 "Blue"). Under continuous UV-A or blue light, *C. antiqua* cells ascended from the bottom toward the surface on Day 1, but thereafter DVM became arrhythmic (Fig. 4 "UV-A" and "Blue"). In contrast, under continuous red light, DVM was kept synchronized with pre-culture LD under all LD regimes (Fig. 4 "Red"). In all experiments, whenever a clear DVM rhythm was observed, the period of one complete cycle was taken to be the duration between the time when a minimum accumulation ratio was observed and the time when it was observed again. This period was 22−25 h.

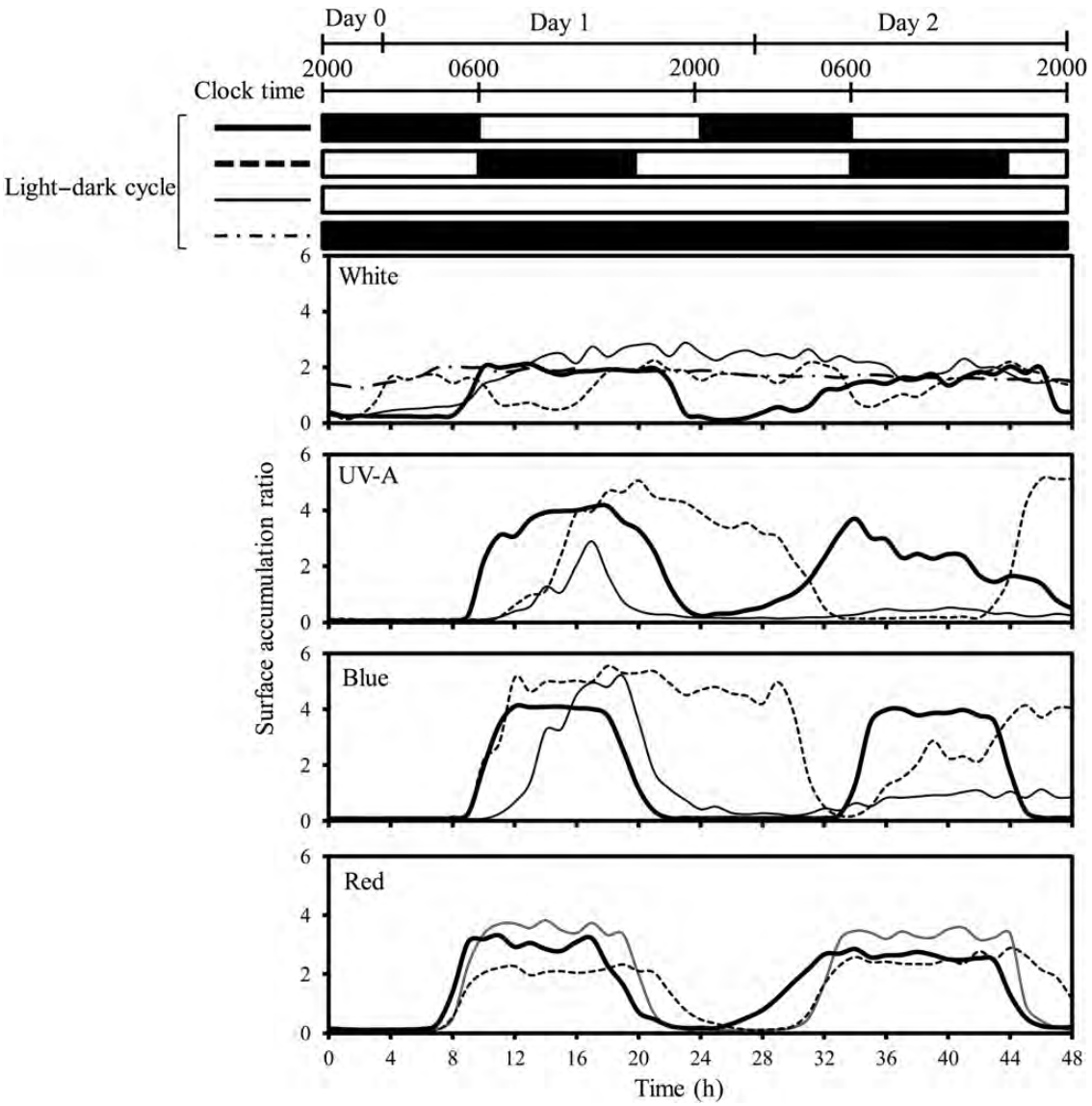

Fig. 4. Patterns of DVM in *C. antiqua* under different light–dark cycles and wavelengths of light. White and black bars represent the periods of light and dark, respectively and the line graphs represent the DVM patterns under white, UV-A (peak wavelength: 365 nm), blue (peak wavelength: 430 nm) and red light (peak wavelength: 660 nm), respectively. Surface accumulation ratio means the ratio of the average gray value, which is synonymous with the ratio of the cell density in the surface layer (0–0.5 cm) to that in the bottom layer (3.5–4.0 cm).

Effects of timing and fluence rate of irradiation on DVM

Exposure to blue light just before or just after the subjective nighttime period did not induce a noticeable phase shift in the DVM rhythm (irradiations a and g in Fig. 5). In contrast, when exposed to blue light during the first or second half of the subjective nighttime period, cells started to ascend 100–150 min later (irradiations b and c in Fig. 5) or 100–200 min earlier (irradiations e and f in Fig. 5), respectively, than without irradiation. In addition, the DVM rhythm disappeared following irradiation by blue light in the middle of the nighttime period (irradiation d in Fig. 5).

We compared the DVM phase under different intensities of blue light irradiation during the subjective nighttime period. Compared with DVM with no blue-light irradiation, the DVM phase was delayed in a fluence rate-dependent manner within a fluence range from 0 to 0.03 μmol photons m^{-2} s^{-1} (Fig. 6). According to the multiple comparisons by Tukey's HSD test, the phase delays in intensities from 0.03 to 0.3 μmol photons m^{-2} s^{-1} were significant in comparison with no blue-light

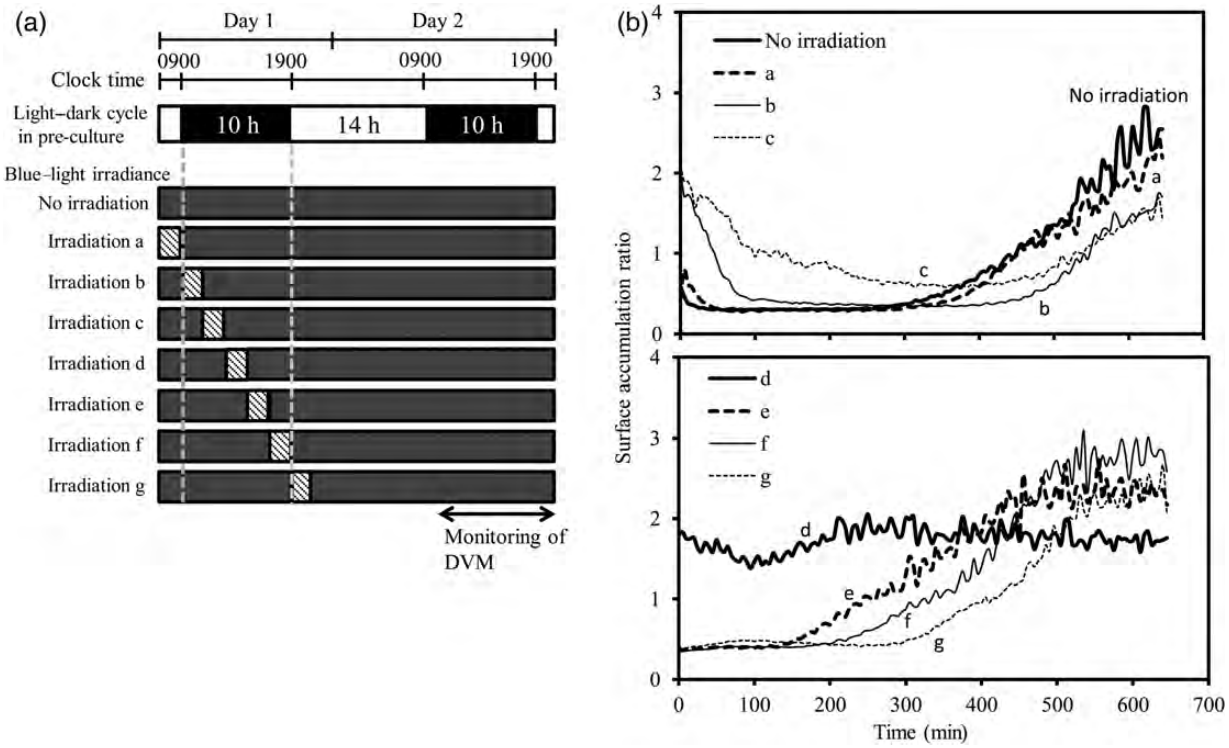

Fig. 5. Effects of the timing of a 2-h pulse of blue-light (peak wavelength: 480 nm) irradiation on the DVM phase shift in *C. antiqua*. (**a**) Schedule of irradiation and period of DVM monitoring. The gray and diagonal areas indicate the periods of red light only and those with red light and blue light added, respectively. (**b**) Time–courses of surface accumulation ratios under each regime of blue-light irradiation. Each line represents the mean of triplicate experiments.

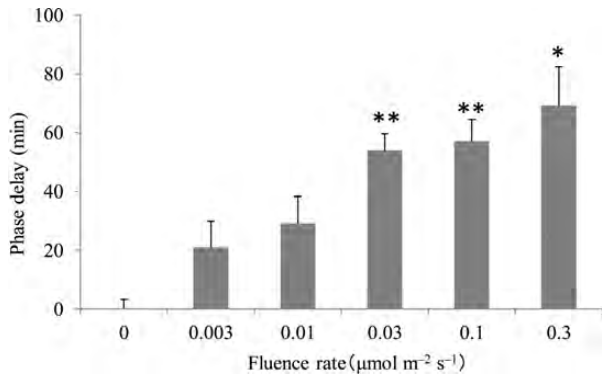

Fig. 6. Relationship between the DVM phase delay and the fluence rate of blue pulsed light. *C. antiqua* cultures were exposed to a 2-h pulse of blue light (peak wavelength: 480 nm) from the Okazaki Large Spectrograph during the first half of a subjective nighttime period (irradiation b in Fig. 5). Each point represents the mean (±SE; $n = 6$). An single asterisk and double asterisks indicate that the phase shift was significantly longer than with no irradiation, or that the phase shift was significantly longer than with no irradiation and also with 0.003 μmol photons m^{-2} s^{-1}, respectively ($P < 0.05$).

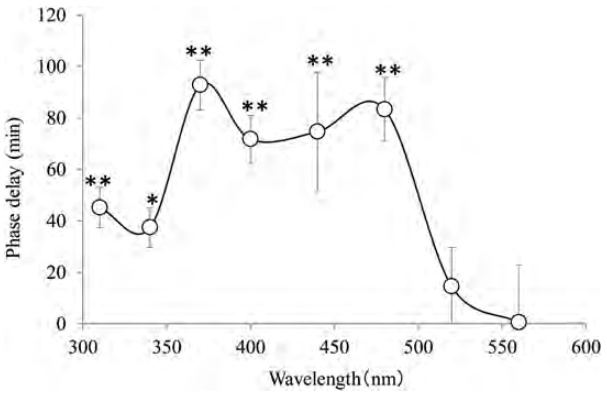

Fig. 7. Equal-quantum action-spectra for the phase delay of DVM. Cultures of *C. antiqua* were exposed to monochromatic light from the Okazaki Large Spectrograph for 2 h during the first half of a subjective nighttime period (irradiation b in Fig. 5). Each point represents the mean (±SE; $n = 6$). An single asterisk and double asterisks indicates that the phase shift were significantly longer than no irradiation and that the phase shift were significantly longer than no irradiation and 560 nm, respectively ($P < 0.01$).

irradiation, and there were also significant differences between 0.03 and 0.1 μmol photons m^{-2} s^{-1} and 0.003 μmol photons m^{-2} s^{-1} ($P < 0.05$).

Action spectra

We obtained equal-quantum action spectra for the phase-shift in the DVM rhythm by using the Okazaki

large spectrograph. The most notable phase shifts were observed under wavelengths from 370 to 480 nm at 0.03 μmol photons m^{-2} s^{-1} (Fig. 7). According to the multiple comparisons by Tukey's HSD test, compared with no blue-light irradiation, the DVM phase shift under wavelengths from 310 to 480 nm was significant ($P < 0.01$). Moreover, the phase shifts under wavelengths from 310 to 480 nm except for 340 nm were also significantly longer than for 560 nm. These action spectra, with a UV-A peak at 370 nm and a blue peak at 440 or 480 nm, are indicative of a flavin-based blue-light photoreceptor (Watanabe, 2004; Hegemann, 2008) such as phototropin (Kasahara *et al.*, 2002), aureochrome (Takahashi *et al.*, 2007; Ishikawa *et al.*, 2009) and photoactivated adenylyl cyclase (Iseki *et al.*, 2002).

DISCUSSION

Organisms in general react quickly to some external environmental changes, but ignore other changes and try to maintain homeostasis. Such homeostasis is often controlled by endogenous clocks. In microalgae, many physiological phenomena are controlled by an endogenous clock, for example metabolic reactions such as nitrogen fixation of cyanobacteria (Grobbelaar *et al.*, 1986; Kondo *et al.*, 1994; Ishiura *et al.*, 1998), phototaxis of green algae (Kondo *et al.*, 1991), cell cycle in euglenids (Edmunds *et al.*, 1982) and bioluminescence in dinoflagellates (Hastings, 1989).

For some flagellate algae such as dinoflagellates, it has been suggested or presumed that DVM is a circadian rhythm controlled by an endogenous clock (Eppley *et al.*, 1968; Weiler and Karl, 1979; Kohata and Watanabe, 1986; Roenneberg *et al.*, 1989). An important condition for the cycle being controlled by an endogenous clock is autonomy. As observed in some other flagellate algae (e.g. Kohata and Watanabe, 1986), *C. antiqua* starts to ascend toward the surface just before the transition from dark to light and to descend toward the bottom just before the transition back to dark and the overall period of the cycle is always ~24 h. Moreover, our observations showed that under constant temperature and LL, the cycle continued for 1–2 days at least (Fig. 4). However, under continuous white light, UV-A or blue light, as well as in DD, DVM became arrhythmic within 1 day (Fig. 4). We emphasize that under continuous red light and under discontinuous red light of any rhythm, the DVM continues with the rhythm in the original culture. In the dark, however, the DVM is lost after one cycle (perhaps due to lack of energy from photosynthesis, see below).

Generally, it has been found that physiological rhythms that are cued to an environmental cycle continue fully only once after the environmental cycle changes to constant conditions, and then the rhythms disappear or gradually decrease and become arrhythmic within a few cycle periods (Lees, 1973; Rence and Loher, 1975; Abe *et al.*, 1997; Levy *et al.*, 2007). Especially in LL, the shift to arrhythmic cycles is well known, but the mechanism remains unknown (Ascoff, 1979; Abe *et al.*, 1997). Although the present study has still not explained why the DVM rhythm disappeared under some sources of LL, the phenomenon may cause the DVM of cells to become arrhythmic when exposed to blue or UV-A light in the middle of the subjective nighttime period (Fig. 5). In contrast, the cessation of DVM in DD could be due to an energy shortage, because the cycle persisted in continuous red light, which is generally effective for photosynthesis, and which does not result in a phase shift of DVM (Fig. 4). These observations suggest that DVM in *C. antiqua* is circadian, controlled by an endogenous clock cued through a receptor sensitive to blue light but not to red light.

Light is the most effective *Zeitgeber* (timing cue) to reset an endogenous clock, and a light-activated cue may also apply to the DVM patterns observed in the present study. Of the wavelengths of visible light, blue light is the most effective for resetting the DVM rhythm in *C. antiqua* and is also the most transmissive underwater (Kirk, 1994). We found the threshold fluence rate of blue light for the DVM phase-shift in *C. antiqua* to be ~10^{-2} μmol m^{-2} s^{-1} (Fig. 6). Considering that only blue light reaches the bottom of the euphotic zone, this is the spectral range of available sunlight that, at this fluence rate, can reach depths of 10–40 m over the summer in a coastal area where *C. antiqua* often blooms according to the recalculated result of field data obtained in our previous study (Shikata *et al.*, 2010). These depths are greater than the maximum depth of 7.5 m to which *C. antiqua* has been found to descend by swimming (Watanabe *et al.*, 1995). All of these observations strongly suggest that in its natural habitat *C. antiqua* can nearly always sense sunlight after going to deeper layers to maintain its DVM rhythm regardless of weather and transparency.

In the red-tide dinoflagellate *Lingulodinium polyedrum* (F. Stein) J.D. Dodge, both blue and red light produce phase shifts in the circadian rhythm (Roenneberg and Deng, 1997). In contrast, the present study indicates that the phase shift in *C. antiqua* occurs only under light from the UV-A and blue wavelengths, and that a flavin-based blue-light photoreceptor (Watanabe, 2004) likely plays a role in the phase shift (Fig. 7). Generally, cryptochrome is well known as a blue-light receptor that plays a role in the adjustment of endogenous clocks in plants and insects (Lin and Todo, 2005; Chaves *et al.*, 2011),

but it has not yet been found in any raphidophyte, including *C. antiqua*. In contrast, a homolog of aureochrome, which has been identified as a blue-light receptor for morphogenetic response in the genus *Vaucheria* (Xanthophyceae) (Takahashi *et al.*, 2007), is found in *C. antiqua*, but its physiological functions remain unknown (Ishikawa *et al.*, 2009). Aureochrome, which consists of a flavin-binding LOV ('light, oxygen or voltage') domain and a b-ZIP ('basic leucine zipper') domain, acts as a blue-light-dependent transcriptional factor (Takahashi *et al.*, 2007). As a negative feedback loop in transcription is thought to be a basis of circadian clock oscillation, aureochrome is a strong candidate for the photoreceptor for DVM adjustment in *C. antiqua*. On the basis of the phenomena observed in the present study, we therefore expect that photoreceptor and clock genes will be identified in *C. antiqua* in the near future, clarifying the molecular mechanism(s) and, therefore, the ecophysiology and evolution of flagellate algae.

Lastly our findings show that pulses of weak blue light and UV-A at precisely controlled times can enhance or disrupt DVM cycles. Our findings may allow, in the future, time-controlled pulses of weak underwater illumination to form the basis for harmful algae mitigation in aquaculture areas subject to harmful blooms of *Chattonella* and perhaps other flagellates.

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