

Diurnal variability of *Synechococcus* abundance in Sagami Bay, Japan

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Abstract *Synechococcus*, the most dominant picophytoplankton in coastal regions exhibits diurnal variations in the open ocean. The aim of this study was to assess its short term population dynamics and cell cycle phases through DNA analysis in a coastal region (Sagami Bay, Japan) by sampling at fixed intervals vertically across the euphotic zone. Day to day variations in physical processes such as mixing, induced by wind, and advective events played an important role in structuring *Synechococcus* population. Compared to sub-surface population, surface cells entered S-phase earlier in the day and were arrested and accumulated in the S-phase for a longer time suggesting an inhibitory effect by higher irradiance. Increase of G₂-phase cells during the dark suggests progression of cells through S-phase in absence of light. Although length of G₂-phase was not known, subsequent increase in G₁-phase cells in the early morning and late evening suggests occurrence of two cohorts of cells which divided in the dark phase or late evening of the cycle. This study showed that the population responds differently across the euphotic zone depending on the physical structure of the water column and its cell cycle phases are controlled by the vertical irradiance availability.

Keywords Picophytoplankton · *Synechococcus* · Cell cycle · Sagami Bay · Diurnal variation

Introduction

In the marine environment, picoplankton ($< 3 \mu\text{m}$; Chen et al., 2011) constitutes an important component of the microbial community. Picoplankton includes autotrophic picophytoplankton (*Prochlorococcus*, *Synechococcus* and picoeukaryotes) as well as heterotrophic bacteria. *Synechococcus* and picoeukaryotes are abundant in the coastal waters, whereas *Prochlorococcus* is dominant in the open ocean (Zubkov et al., 2000). A lot of information has been generated with respect to the distribution and abundance of picophytoplankton on seasonal and spatial scales across the globe (Massana, 2011). Some of the studies include closer monitoring of picophytoplankton at frequencies of days to hours (Vaulot & Marie, 1999; Jacquet et al., 2002). With such close monitoring it is possible to identify and study characteristic phenomena such as synchronized division (Vaulot & Marie, 1999) and also delineate various environmental factors influencing populations on short time scales, all of which will give a better understanding of the population dynamics. Most of these observations are reported from the open ocean where the waters are stable with the comparatively lesser influence of physical forcing. Unlike in the open ocean, the coastal regions are dynamic where the hydrography is continuously changing. In such regions, high frequency observations have revealed large variations in the picophytoplankton populations (Vaulot & Marie, 1999; Jacquet et al., 2002).

There are two ways in which the short term variations in picophytoplankton can be studied, 1) based on the interpretation of the diurnal abundance data obtained through flow cytometric analysis (Jacquet et al., 2002; Tsai et al., 2005) and 2) through the quantification of a deoxyribonucleic acid (DNA) staining dye wherein cells with a single copy of DNA can be distinguished from those in which DNA is replicating, and the proportion of the cells that prepare for division each day can be determined by sampling at regular intervals through the day (Vaulot et al., 1995; Zubkov et al., 2000). Through this method the percentage of cells in the different phases of the cell cycle at any given time can be estimated (Jacquet et al., 1998; Vaulot & Marie, 1999). The latter method when applied for *in situ* studies can provide reliable information on cell division of a particular group of picophytoplankton. Prokaryotic picophytoplankton cell cycle displays two gaps in DNA synthesis, G_1 and G_2 (containing one and two genome copies, respectively) separated by discrete DNA synthesis phase, S. Phytoplankton cell cycles are tightly coupled to the light: dark cycles in cultures (Nelson & Brand 1979; Chisholm 1981) and natural populations (Zubkov et al., 2000). *In situ* observations have shown that the process of cell cycle gets arrested in the S phase under high irradiance (Jacquet et al., 1998) and rapidly passes

from G₁ to G₂ phase under the supply of phosphorus (Vaulot et al., 1996;). In culture, lower nutrient concentrations arrested the cells in the G₁ phase, whereas lower light intensity/dark conditions arrested the cells in the G₁ and G₂ phases (Armbrust et al., 1989; Vaulot et al., 1990; Vaulot & Partensky, 1992).

Studies on short term variations in picophytoplankton cell abundance are mostly limited to open ocean regions, focusing on the surface populations which are more accessible (Jacquet et al., 1998; Tsai et al., 2005) with only a few on the sub-surface populations (Andre et al., 1999; Vaulot & Marie, 1999). Vertical *in situ* DNA cell cycle analysis has been done for *Prochlorococcus* (Liu et al., 1997; Vaulot & Marie, 1999) and for *Synechococcus* (Llabres et al., 2011) to better understand the population dynamics of these cyanobacteria in the open ocean. There are no such reports on the population variations of *Synechococcus* from the coastal regions. Since the coastal regions are dynamic, variations in cell abundance are controlled by environmental factors such as light and nutrients and biological factors such as cell division along with physical processes such as vertical mixing and advection (Jacquet et al., 2002). Inclusion of all these factors can facilitate the delineation of factors controlling the dynamics of picophytoplankton. The vertical distribution of picophytoplankton is influenced by light (Liu et al., 1997; 1998) and sampling across the water column will help us understand the influence of this factor on the diurnal progression of the cell cycle. For the first time we made an attempt to understand the population dynamics of the most dominant picophytoplankton, *Synechococcus* in the coastal regions. The main aim of this study was to examine the short term variability of *Synechococcus* abundance and cell cycle phases. This was done by sampling at fixed intervals at different depths in the water column in order to investigate whether the surface *Synechococcus* populations behave differently from that at the sub-surface and assess the influence of physical factors such as vertical mixing, advection and light.

In view of this, a coastal embayment located at the southeastern part of Honshu, Japan, opening southward to the North Pacific was identified as the site to study the short term variations in the *Synechococcus* cell abundance and cell cycle phases. This bay is constantly under the influence of physical forcing and has typical characteristics of a temperate bay and its hydrography is primarily related to the fluctuations of the Kuroshio Current axis and to waters originating from the coastal areas (Hogetsu & Taga, 1977). The seasonal cycle of biological production in this bay is influenced by the interactions between the water masses of different characteristics. It is seasonally characterized by a diatom spring bloom, high chlorophyll *a* in summer stratification and low chlorophyll *a* in late fall and winter (Kanda et al., 2003). In the Sagami Bay *Synechococcus* is the dominant picophytoplankton

during the warm period with an annual picophytoplankton contribution to the photosynthetic biomass of $32 \pm 4\%$ (Mitbavkar et al., 2009). Information on the short term variations in *Synechococcus* cell abundance and cell cycle phases will be vital for understanding their population dynamics in coastal regions which are constantly under the influence of physical forcing.

Materials and Methods

Sampling

Sampling was carried out at station S3 ($35^{\circ} 00'N$; $139^{\circ} 35'E$) in Sagami Bay (Fig. 1) on board the RV *Tansei Maru* from the Japan Agency for Marine-Earth Science and Technology. Samples were collected for two consecutive days with about two to four hour's temporal resolution from 04:00 to 20:00 h in June (18-19) 2004 from seven depths (0, 5, 10, 15, 25, 40, 60 m). Hydrographic data (water temperature, salinity) and water samples were collected with the help of Teflon-coated Niskin bottles attached to a CTD (Falmouth Scientific) rosette system. The vertical photosynthetically active radiation (PAR) profiles were measured with a natural fluorescence sensor (PNF 2300, Biospherical Instruments). For flow cytometric analysis, samples were preserved in paraformaldehyde (0.2% final concentration), transferred to liquid nitrogen and then stored at $-80^{\circ}C$, until further analysis.

Chlorophyll a

Water samples (200-300 ml) were filtered under gentle aspiration (< 200 mm Hg) through glass fibre filters (Whatmann GF/F). Chlorophyll *a* (chl *a*) was extracted immediately in *N, N*-dimethyl formamide (Suzuki & Ishimaru, 1990) and the samples were preserved at $-20^{\circ}C$ until analysis in the laboratory. Chl *a* concentration was measured using a Turner Design Model 10-AU fluorometer calibrated with commercial chl *a* (Wako Pure Chemical Industries) (Parsons et al., 1984).

Flow cytometric analysis

A Beckman coulter (Epics XL) flow cytometer equipped with 15-mW laser was used for picophytoplankton analysis. Forward light scatter, right angle light scatter, red fluorescence from chlorophyll (> 650 nm, FL4) and orange fluorescence from phycoerythrin (564-606 nm, FL2) were measured after excitation by a 488 nm laser beam. *Synechococcus* group could be discriminated and enumerated based on the orange fluorescence (Fig. 2). Fluorescent beads (2 μm "Fluoresbrite",

polysciences) were used as internal standards and for calibration of the above parameters. Flow cytometric data were collected and saved as listmode files.

Cell cycle analysis

For the *Synechococcus* cell cycle analysis, stored samples were thawed and incubated for 30 min at 37°C in the presence of 0.1 g L⁻¹ of a mixture of RNase A and B (Sigma R-4875 and R-5750) (1:1 [wt/wt]). Subsequently, the samples were stained with SYBR Green-I (Molecular Probes Inc.; final concentration, 10⁻⁴) for at least 10 min (Marie et al., 1997). Samples were run either for 10 min or until 20,000 *Synechococcus* cells were measured. The acquisition was performed at a medium rate (25-30 µl min⁻¹) that allowed good discrimination between the different cell cycle phases (Jacquet et al., 1998).

Data analysis

Linear regression analyses were performed in order to assess the relationship between chlorophyll, *Synechococcus* abundance and salinity. The relation between *Synechococcus* cell cycle phases and PAR was also subjected to linear regression. Cell cycle analyses were performed using MultiCYCLE software (P.S. Rabinovitch, Phoenix Flow Systems, San Diego, CA). DNA histograms obtained from the cell cycle analysis exhibited a peak, which corresponds to the cells in the G₁ phase containing one genome copy. The subsequent increase in the DNA fluorescence marks the beginning of chromosome replication representing the cells in the S phase. This is followed by the cells in the G₂ phase, which have completed DNA replication and contain two genome copies but not yet divided (Fig. 2).

Resultss

Hydrography

Temperature and salinity ranged from 15 to 23.5°C and 33 to 34.5, respectively (Fig. 3c-f). The depth at which the temperature change from the surface temperature is 0.5°C is defined as the Mixed Layer Depth (MLD). On June 18, temperature did not exhibit significant diurnal variations within the MLD. Low saline water mass (33.1 to 33.6) was observed from 04:00 h to 08:00 h up to 7-8 m which increased to 14 m at 10:00 h and shallowed to 6 m at 12:00 h. From 14:00 h to 16:00 h, the mixed layer deepened to around 18-22 m and the resulting water mass was characterized by higher salinity. Subsequently, the MLD reduced to 15 m. On June 19, lower water temperature was observed up to 12:00 h which

subsequently increased up to 20:00 h. Low saline water mass (33.1 to 33.6) was observed from 04:00 h to 10:00 h wherein the MLD reduced from 11 m to 4 m. Thereafter, the mixed layer deepened steadily (13 to 26 m) from 12:00 h to 20:00 h with high saline water mass. Temperature and salinity were higher as compared to that on June 18. The presence of low saline waters in the morning was due to the influx of water from coastal regions during low tide whereas the subsequent high salinity was due to the receding coastal waters and entry of offshore waters during high tide. Similar to June 18, on this day the wind speed increased from 5 m s^{-1} (04:00 h) to 12 m s^{-1} (14:00 h) (Sarma et al., 2006). PAR values ranged up to 2139 and $1239 \mu\text{E m}^{-2} \text{ s}^{-1}$ at the water surface on June 18 and 19, respectively. The euphotic zone depth (depth where PAR reaches 1% of surface radiation) varied between 25 m and 40 m.

Chl *a* concentration ranged up to 3.4 mg m^{-3} (Fig. 3g-h) and were influenced by the tidal variations on the two sampling days. On June 18, chl *a* concentrations were high from 04:00 h to 12:00 h up to 10 m depth during low tide. After 12:00 h, lower chl *a* concentrations were observed in the upper 5 m strata. During this period high saline offshore waters advected into the bay due to the high tide. Subsurface chl maxima was observed below 5 m. On June 19, chl *a* concentrations exhibited an opposite trend to that observed on June 18. On this day, the chl *a* concentrations were lower during the low tide and subsequently increased during the high tide. Chl *a* concentrations did not exhibit any significant relationship with salinity on June 18, but showed a positive relationship on June 19 ($P \leq 0.02$, $r = 0.47$).

Vertical diurnal variation in Synechococcus distribution

Synechococcus cell abundance ranged from 1 to $90 \times 10^3 \text{ cells ml}^{-1}$. On June 18, abundance was high in the upper 5 m at 04:00 h and 20:00 h. At 10 m and 15 m, abundance peaked between 06:00 h to 12:00 h and 16:00 h to 20:00 h. From 12:00 h to 14:00 h cell numbers dropped in the 5 m to 15 m strata. At 25 m, abundance peaks were observed between 04:00 h to 06:00 h, 10:00 h, 14:00 h to 16:00 h and at 20:00 h. The last peak corresponded with high sub-surface chl *a* maxima. On June 19, higher abundance was observed at 04:00 h, 08:00 h to 10:00 h and 16:00 h to 20:00 h in the 0 m to 5 m strata (Fig. 4). In the 10 m to 15 m strata, cell abundance increased at 06:00 h and between 10:00 h to 12:00 h. At 25 m, abundance peaked at 06:00 h and 10:00 h. *Synechococcus* abundance maxima was below 5 m up to 12:00 h and subsequently reduced during high tide. On June 18, similar to chl, *Synechococcus* abundance did not exhibit a significant relationship with salinity. However, on June 19, a negative

relationship was observed between *Synechococcus* abundance and salinity ($P \leq 0.001$, $r = 0.6$). No significant relationship was observed between chl and *Synechococcus*.

On June 18, a higher percentage of *Synechococcus* population was in the G₁ phase from 04:00 h to 08:00 h up to 25 m depth except at 5 m where it was observed up to 06:00 h (Fig. 5 and Fig. 6). In the 0 m to 15 m strata, the percentage of cells entering the S phase started progressing from 04:00 h. At 0 m, this increase continued up to 12:00 h and remained steady up to 18:00 h (14 h duration) whereas at 10 m it was observed up to 14:00 h (10 h duration). Thereafter a decline was observed after 18:00 h and 14:00 h at 0 m and 10 m, respectively. At 5 m it increased up to 08:00 h, after which fluctuations were observed. At 25 m the entry of cells into the S phase started increasing prominently after 06:00 h and continued up to 14:00 h. On June 19, the percentage of cells entering the S phase was comparatively lower and the vertical changes in cell abundance and cell cycle phases were less pronounced than that on June 18. A higher percentage of the *Synechococcus* population was in the G₁ phase from 04:00 to 06:00 h from the surface to a depth of 25 m except at 15 m where it was observed up to 08:00 h (Fig. 6). In the 0 m to 10 m strata, the percentage of cells entering the S phase progressed from 04:00 h and continued up to 20:00 h. At 15 m and 25 m, comparatively lower percentage of cells entered the S phase with a prominent increase after 06:00 h. At 15 m it continued up to 12:00 h, whereas at 25 m a sharp decline was observed at 10:00 h followed by an increase up to 14:00 h and thereafter remained steady.

On both the sampling days, almost 80% of the population was in the S phase by 16:00 h to 18:00 h in the 0 m to 25 m strata. However, the subsequent decline in the percentage of S phase cells at 0 m was comparatively lower than that observed in the sub-surface. On June 18, an increase in the percentage of cells in the G₂ phase was observed at 20:00 h (0 m, 5 m, 15 m and 25 m) and at 18:00 h (10 m) corresponding to a decline in the percentage of cells in the S phase. On June 19, at 18:00 h increase in the percentage of cells in the G₂ phase was observed at 5 m and 15 m. On both days, peak in G₂ cells was also observed on certain occasions during the day. On June 18, at 25 m, an increase in the percentage of cells in the G₂ phase occurred at 06:00 h and on June 19 a comparatively higher percentage was observed between 08:00 h to 10:00 h at 10 m and 25 m. These peaks were followed by an increase in percentage of G₁ cells. Although the decline in S phase cells was not followed always by an increase in G₂ phase cells, a corresponding increase in the percentage of cells in the G₁ phase was prominent.

Discussion

In the Sagami Bay, *Synechococcus* population exhibited diurnal variations in cell abundance. Day to day variations in physical processes such as mixing, induced by wind, and advective events played an important role in structuring the *Synechococcus* population as well as chl biomass. On June 18, the water movements such as tidal variability did not impose any significant variation in the chl concentrations and *Synechococcus* abundance. However, on June 19, the positive relationship of chl with salinity above the MLD suggests influx of chl from the offshore waters during high tide. Also, the negative relation of *Synechococcus* abundance with salinity suggests dilution of its cell abundance by the offshore seawater harboring a lower *Synechococcus* abundance. *Synechococcus* is known to be dominant in the coastal waters as compared to the offshore regions (Mitbavkar et al., 2009). The strong influence of offshore waters was also reflected in the comparatively higher salinity on June 19. Since chl, which is a proxy for phytoplankton biomass was influenced positively and *Synechococcus* abundance was influenced negatively by the influx of offshore waters, no significant relationship was observed between the two suggesting that the bulk chl biomass was mostly contributed by the $> 3 \mu\text{m}$ phytoplankton groups.

In such a dynamic environment, an effort was made to assess the cell cycle of *Synechococcus*. In comparison to methods such as dilution experiments that involve experimental manipulations of the samples for studying phytoplankton growth and grazing mortality rates, cell cycling and cell division are the phenomena more amenable to analysis because they can be very precisely visualized using cell DNA staining (Vaulot & Marie, 1999). However, the cell numbers of the particular group to be analyzed should be sufficiently high ($10,000 \text{ cells ml}^{-1}$). In the Sagami Bay, *Synechococcus* is the dominant picophytoplankton in summer with a high cell abundance (Mitbavkar et al., 2009). Hence, in the present study, cell cycle analyses could be carried out for *Synechococcus*.

In natural populations of *Synechococcus*, light is an important parameter for synchronization of cell cycles, growth and cell division (Liu et al., 1998; Vaulot & Marie, 1999). The importance of irradiance for the entry of G_1 cells into the S phase was shown by Armbrust et al. (1989). Liu et al. (1997) suggested that the amount of light is critical for the *Synechococcus* cells to start DNA synthesis and cell division (Chisholm et al., 1986). In the natural environment, the depth of the euphotic zone is dependent on the sun's position, atmospheric and water characteristics throughout the day. In the present study, the euphotic zone extended up to 25 m to 40 m throughout the sampling period. Since the euphotic depth

progressively increased from morning to noon, vertical differences were observed in the timing of entry of the cells from the G₁ to the S phase wherein G₁ cells in the deeper layers started entering the S phase later in the day. The comparatively higher cell abundance and also the percentage of cells entering the S phase on June 18 than on 19 could be due to the higher PAR intensity observed on the former day. Also, the increasing percentage of cells in the S phase with the passage of time at 0 m shows that higher light intensity at the surface does not inhibit the cells in the G₁ phase from entering the S phase. In the surface Mediterranean waters on sunny day's cells entered the S phases earlier, and the maximum fraction of cells in S phase was higher than on cloudy days (Jacquet et al., 1998).

Studies have shown that cell cycle progression through G₁ requires light whereas the S interval transit is light independent (Armbrust et al., 1989). However, the accumulation of cells in the S phase for a comparatively longer period at 0 m indicates that the cells were arrested at this stage and were not allowed to progress to the G₂ phase. This shows that in *Synechococcus* the DNA replication gets initiated and later blocked. The delay in entry of S to G₂ phase in *Synechococcus* for surface populations could be due to photoinhibition and high ultraviolet (UV) radiation, preventing the DNA replication and causing serious damage to the cell machinery which can be non-recoverable resulting in the loss of cell population as suggested by earlier workers (Vaulot et al., 1995; Jacquet et al., 1998; Partensky et al., 1999; Vaulot & Marie, 1999). The occurrence of cell abundance maxima below 10 m could be an indication of the harmful effects of UV radiation at the surface. Hence a progressive decrease in the length of the S phase with an increase in depth was observed. Other factors such as the spectral composition of underwater light and nutrient concentration are also reported to influence the vertical distribution of picophytoplankton. Hauschild et al. (1991) suggested that light quality through the effect on growth rate may be an important factor controlling the distribution and abundance of various pigment types of *Synechococcus* with green light favoring phycoerythrin and red light favoring phycocyanin containing strains. Brunet et al. (2007) reported high pigment diversity of picophytoplankton in the deep chlorophyll maxima and a vertical segregation of their groups and species indicating an influence of quantity and quality of light and nutrient concentration (Vaulot & Marie, 1999) within the water column.

Although the decline in percentage of S phase cells was not always reflected in the percentage increase in G₂ phase cells, a corresponding increase in the percentage of cells in G₁ phase suggests the occurrence of cell division. On June 19, although the trends on the percentage of cells in G₁ and S phases were similar to that obtained on June 18 until 25 m, the percentage increase in G₂ cells was not observed as on

June 18 at 20:00 h. This mismatch suggests that we could have missed the actual peaks in G₂ phase due to the sampling resolution and such observations have to be carried out several times so as to obtain a good statistical representation.

Increase in the percentage of cells in the G₂ phase during the dark suggests that cells can progress through S in the dark (Armbrust et al., 1989). Llabres et al. (2011) reported cell division during the late afternoon or night. In our study, the frequency of sampling was two hours, and sampling was not done between 20:00 h and 04:00 h. However, we could detect the beginning of G₂ phase at 18:00 h to 20:00 h. Although we do not have any information on the length of the G₂ phase, the subsequent increase in the percentage of cells in the G₁ phase in the early morning (04:00 to 06:00 h) and late evening (20:00 h) suggests that cell division occurred in the dark phase and late evening of the cycle.

Some percentage of cells were in the G₂ phase during the daylight hours which could have divided later in the day. On June 19, the highest percentage of G₂ cells that subsequently reflected in an increase in G₁ cells suggests that cell division occurred during the day on certain occasions. On one such occasion at 10:00 h at 10 m, when the MLD had shallowed below 10 m, the S to G₂ to G₁ transformation happened within a short span of time. The combination of environmental conditions such as suitable light, nutrients and stable water conditions could have triggered such a behavior. In the surface Mediterranean waters, addition of phosphorus induced a shortening of the G₁ phase resulting in cells entering and leaving the S and G₂ phases earlier (Vaulot et al., 1996). Otherwise, due to mixing, the cells are subjected to varying environmental conditions such as light and nutrient conditions which delays the cell cycle progression and completion. This could be one of the reasons for the high picophytoplankton abundance in stratified water conditions (Bouman et al., 2011). Hence the cells within the mixed layer probably complete cell cycle during the night, according to the endogenous clock (Johnson et al., 1996) that is driven by the favorable external forcing such as light and nutrients during the day, thus leading to a diel variability in cell cycle progression pattern. This observation was possible due to the vertical sampling and simultaneous monitoring of the hydrography. Although our study agreed with the earlier observations that within the MLD the population response is not significantly different, but below MLD the behavior differed and was controlled by a combination of factors. Jacquet et al. (2002) through diel surface sampling, concluded that the growth rate of cyanobacteria seems poorly affected in the Alboran Sea of western Mediterranean despite strong hydrological gradients compared to more stable systems such as those found in the tropical ocean. This clearly points out the fact that growth rate regulation is

related to other key parameters such as light and nutrient levels. Other studies in the Mediterranean Sea showed that the growth rate of picophytoplankton could vary over a two-fold range in response to cloud cover or nutrient availability (Jacquet et al., 1998; Vaultot & Partensky, 1992). In the equatorial Pacific, growth processes occurred in parallel at the top and the bottom of the mixed layer inducing uniform profiles for cell abundance and scatter (Vaultot & Marie, 1999). In short term investigations related to population dynamics in coastal waters, water samples collected at a fixed station may not represent the same population, physiologically and genetically due to the ever changing hydrography. This aspect is further complicated if only surface samples are taken into consideration. Diel changes in MLD may also influence diel patterns in cell abundance at a given depth, particularly if there exists a strong vertical gradient in abundance (Binder & DuRand, 2002; Andre et al., 1999). Our study in which samples were collected vertically through the euphotic zone, the MLD did not undergo significant variations. As a result, the diel trend of cellular abundance and cell cycle was not very much distinct. Although advection resulted in lowering of cell abundance on June 19, the cell cycle progression pattern was similar between the two sampling days, with a small difference in the percentage of cell cycle phases. However, results obtained from the dynamic coastal regions should be assessed with care taking into consideration the prevailing short term hydrographic conditions so as to decipher the biological, physical and chemical influences on population dynamics.

With respect to the number of cell divisions per day, observations from the Arabian Sea have reported dual maxima of S and G₂ fractions of cells, which implied more than one division per day. It was hypothesized that a large portion of the cells may have accomplished cell cycles over two cell division peaks, i.e., one cohort of cells entered S phase in the morning and finished cell division around sunset and another cohort started DNA replication in the evening and completed division in the following morning (Liu et al., 1998). This observation in the Arabian Sea and the Pacific Ocean in the present study suggests that ultradian growth mode may be a common phenomenon observed for *Synechococcus* or it could be a case of asynchronous growth or dephased growth of mixed species or strains. The generation time of *Synechococcus* can be as rapid as 5 to 6 hours and can be controlled easily by changing the light fluence or temperature wherein with increase in light intensity from 25 to 50 $\mu\text{E m}^{-2} \text{s}^{-1}$, the average cell cycle duration shortened from 18 to 10 h (Yang et al., 2010). The relation of the cell cycle and circadian rhythm is well studied for *Synechococcus* wherein it has been demonstrated that cell division cycling does not interfere with its circadian clock which maintains a 24 h period regardless of

cell division rate or cell density. Cyanobacteria that divide twice or more per day display circadian rhythms of bioluminescence and levels of mRNA (Kondo et al., 1997). Mori et al. (1996) found that cell division in *Synechococcus* is prohibited for several hours in the circadian cycle, but the cell length continues to increase, thereby leading to an exponential increase in the total cell mass in the batch culture.

The lowered output rate at the surface after the entry of S to G₂ and from G₂ to G₁ in the Sagami Bay suggests cell loss. A clear pattern of diel variation in the cell mortality of *Synechococcus* was observed in the open Mediterranean Sea, where percentage mortality increased during the day and decreased as the night progressed with minimum occurring in the early morning when the maximum number of cells in the G₁ phase was observed (Llabres et al., 2011). They also observed higher variability in percentage dead cells along the diel cycle for the surface populations than for the deeper ones, indicative of higher cell death dynamics at the surface. In addition, to high light and UV radiation, picocyanobacterial cells can get damaged by several environmental factors such as viral infection (Brussard et al., 1999), nutrient limitation (Mann & Chisholm, 2000) and grazing. Suttle (1994) estimated that viral lysis is responsible for the loss of an average of 3% of *Synechococcus* cells. In the Sagami Bay, the nutrient concentrations are high in winter and lower in the summer due to convective mixing and stratification, respectively (Mitbavkar et al., 2009). Since stratified conditions favor picophytoplankton, their abundance is higher in summer (Mitbavkar et al., 2009) when the present study was conducted. This suggests that nutrients may not be an important factor regulating the cell division of picophytoplankton in this region. Grazing by nanoflagellates, which are important grazers of picophytoplankton, is an important factor that needs to be taken into consideration in future studies related to diurnal variability (Dolan & Simek, 1999). Automortality in unicellular phytoplankton is a common fate in the marine environment and may be of substantial importance in reducing the population size, next to grazing and sedimentation (Veldhuis et al., 2001).

Summary

Strong diurnal variations were observed in the *Synechococcus* abundance. Day to day variations in physical processes such as mixing, induced by wind, and advective events played an important role in structuring the *Synechococcus* population as well as chl biomass. Through cell cycle analysis of *Synechococcus* it was observed that the entry of cells into different cell cycle phases is probably

controlled by the solar radiation wherein higher intensity delayed the cell division process in the cells present in surface waters by arresting the cells in the S phase as compared to that at lower depths. Hence there was a progressive decrease in the length of the S phase with an increase in depth. This affected their population size, resulting in a comparatively lower abundance at the surface and maxima in the sub-surface. Other factors such as the vertical spectral quality and quantity of light and nutrient concentrations can also influence the cell cycle progression within the water column. These vertical differences will in turn influence the availability of food for the higher organisms. Observations of such differences through sub-surface sampling are of prime importance, which gives an entirely different picture of the population dynamics from that at the surface. Such information, along with a knowledge of the physical processes helps in improving the understanding of the variability in population cell numbers on short time scales in dynamic areas.

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Legend to figures

Fig. 1. Location of the study area.

Fig. 2. Flow cytometric analysis of *Synechococcus* in the Sagami Bay (15 m, 20:00 h, June 18 2004). (a) Unstained sample. *Synechococcus* identified based on the phycoerythrin orange fluorescence *vs.* red chlorophyll fluorescence. (b-c) Samples stained with the DNA dye SYBR Green-I. (b) DNA SYBR Green-I fluorescence *vs.* Red chlorophyll fluorescence (both expressed in arbitrary units on four-decade log scales). (c) DNA fluorescence histogram (linear scale) representing the *Synechococcus* cell cycle analysis.

Fig. 3. Diurnal and vertical profiles of photosynthetically active radiation (PAR) (a, b), temperature (c, d), salinity (e, f) and chlorophyll *a* (g, h) (black line indicates the mixed layer depth).

Fig. 4. Diurnal and vertical profiles of *Synechococcus* cell abundance on June 18 and 19, 2004. (a-b) Contour plots from 0 m to 60 m and (c-g) line plots from 0 m to 25 m. Grey shades indicate dusk.

Fig. 5. Contour plots of *Synechococcus* cells (%) in the S, G₂ and G₁ phases of the cell cycle from 0 m to 40 m. Grey shades indicate dusk.

Fig. 6. Line plots of *Synechococcus* cells (%) in the S, G₂ and G₁ phases of the cell cycle from 0 m to 25 m (a-e). Grey shades indicate dusk.

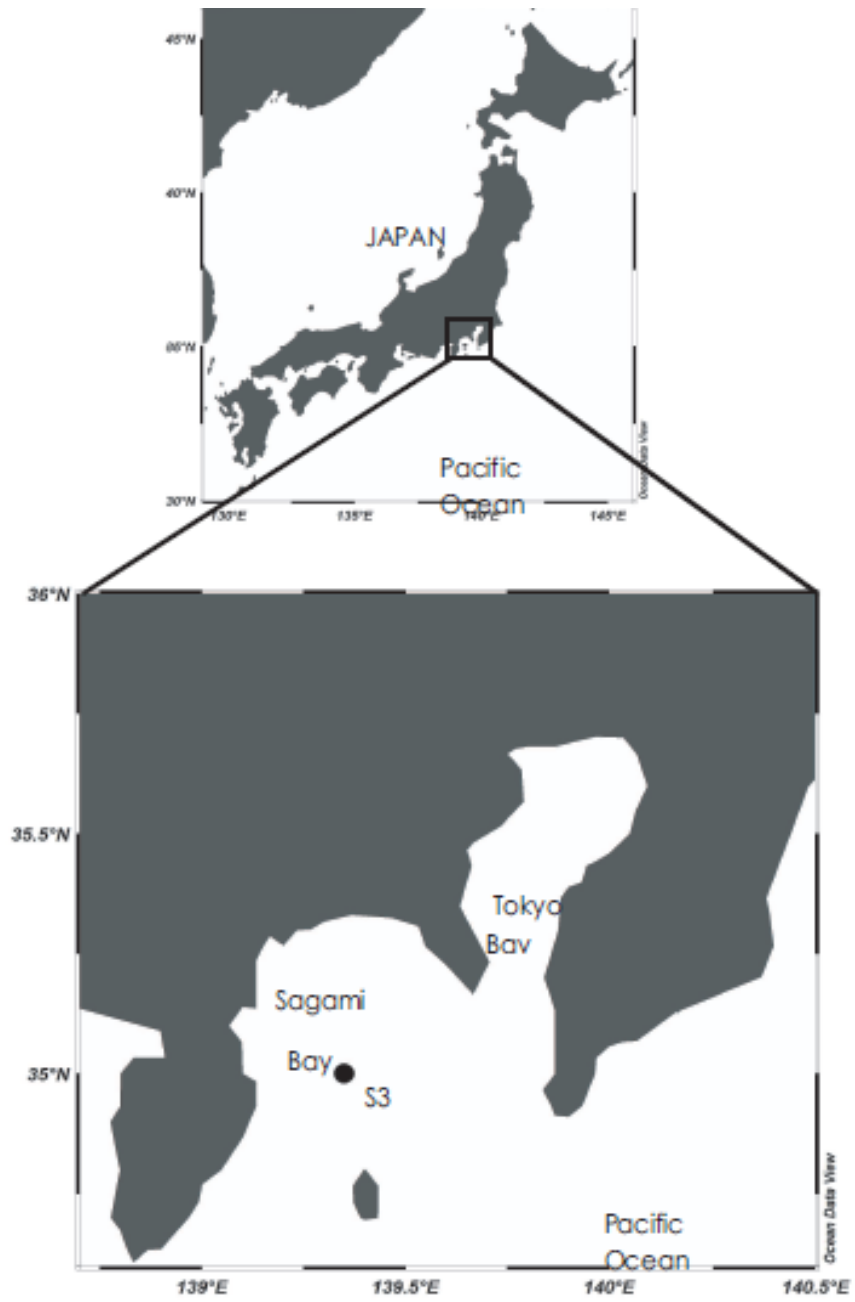


Fig. 1

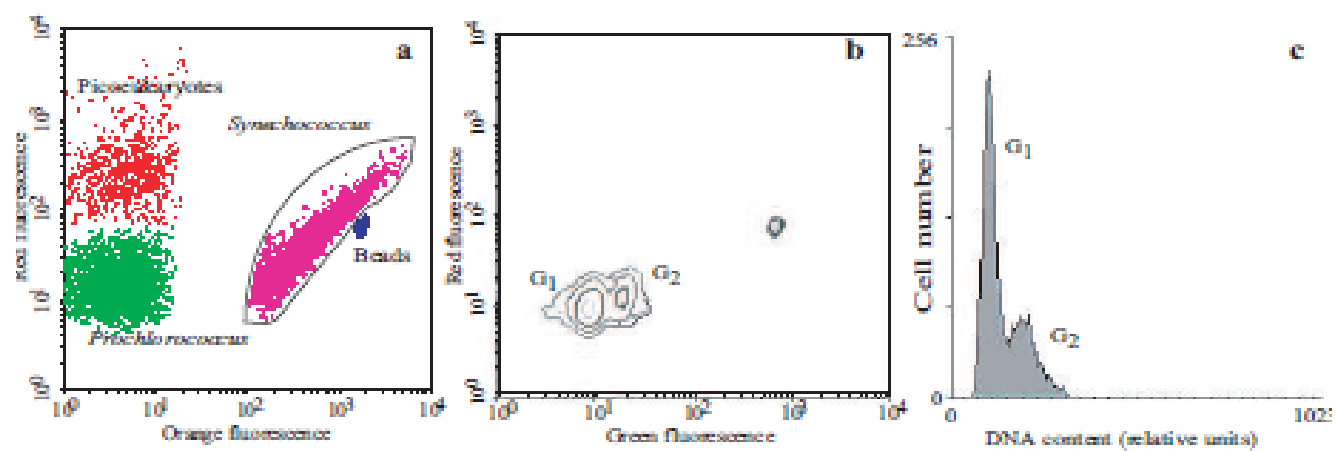


Fig. 2

Figure 3 (a) June 18, 2004 (b) June 19, 2004

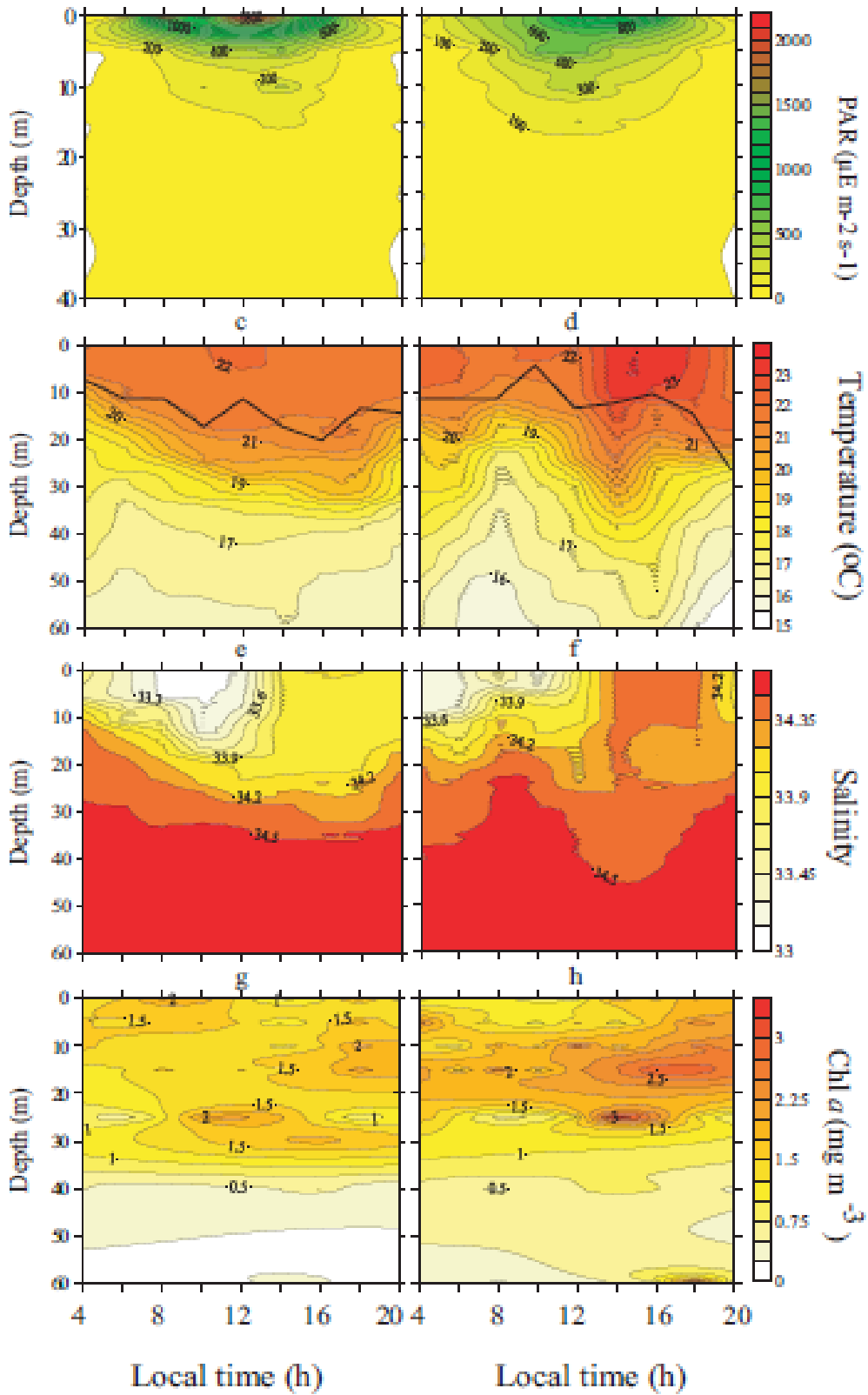


Fig. 3

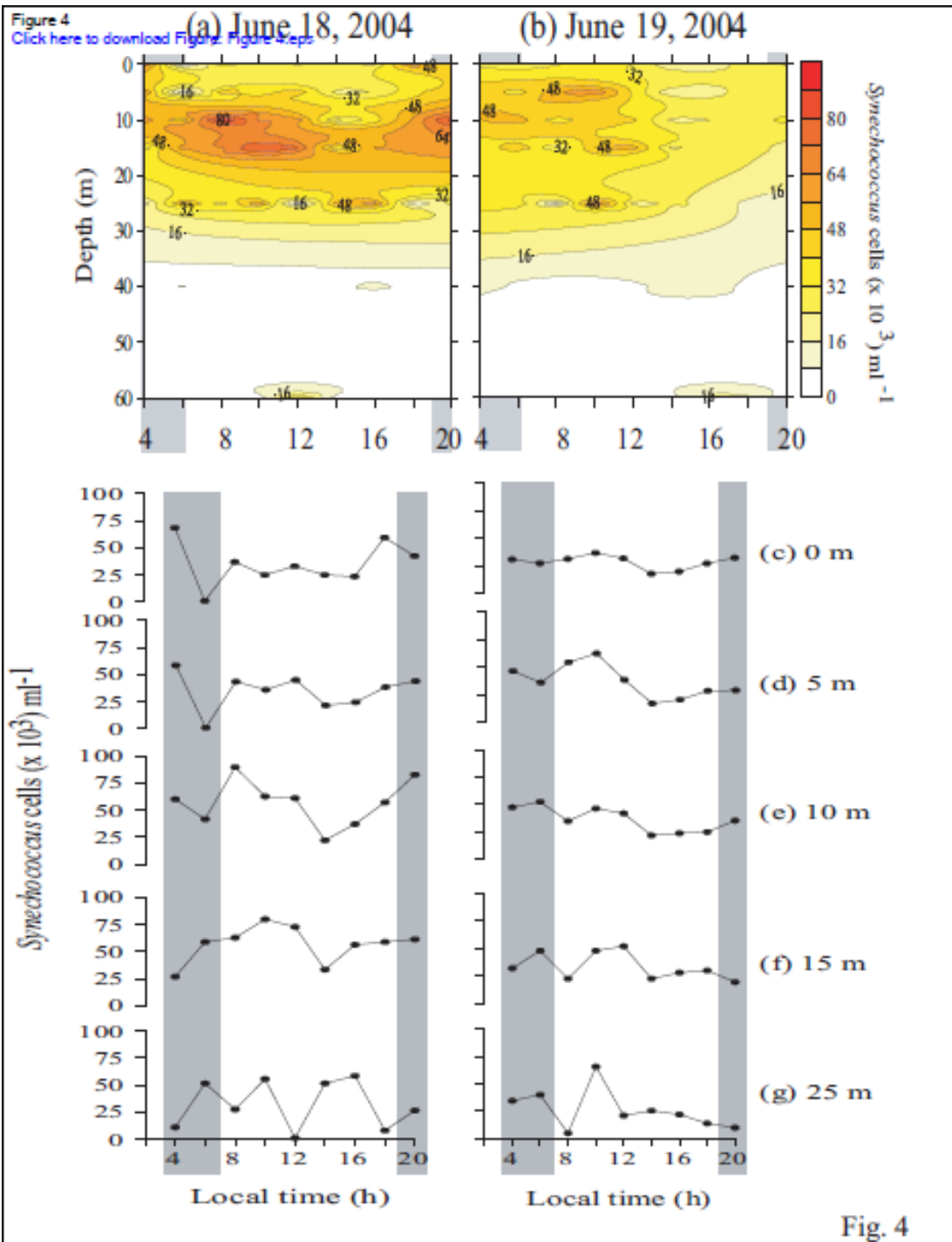


Fig. 4

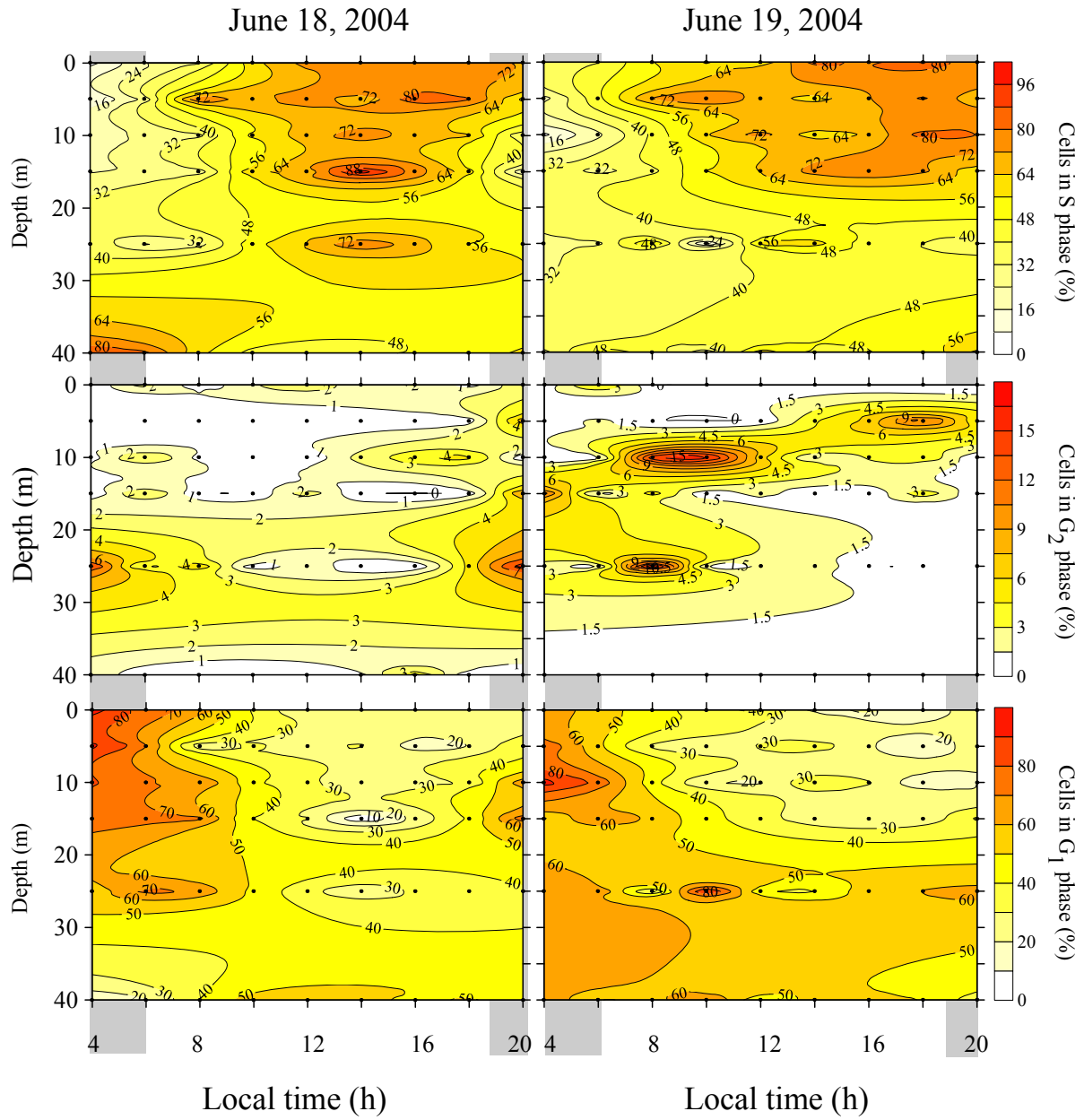


Fig. 5

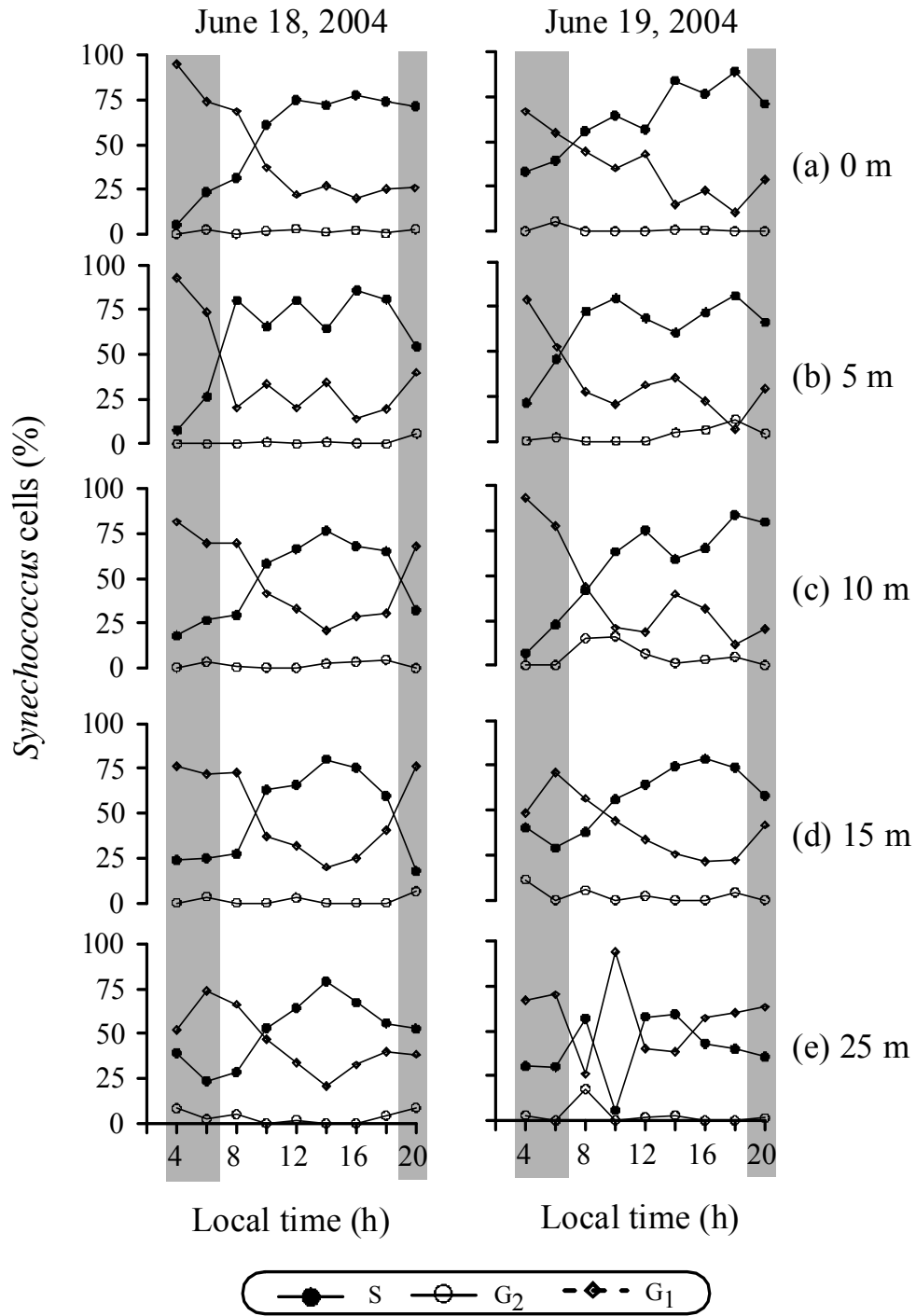


Fig. 6