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Key Points:

- Picophytoplanktonic quasi-antiphase diel cycles in abundance and cell size/biomass are likely a general feature of the oligotrophic ocean
- Grazing pressure on *Prochlorococcus* and *Synechococcus* is as high during the day as during the night

Supporting Information:

Supporting Information may be found in the online version of this article.

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Quasi-Antiphase Diel Patterns of Abundance and Cell Size/Biomass of Picophytoplankton in the Oligotrophic Ocean

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Abstract Picophytoplankton are the smallest, most abundant photosynthetic organisms in the ocean. Knowledge of the diel variability of these tiny microbes has important implications for the structure of microbial food webs and key biogeochemical processes. However, insight into the mechanisms that underlie picophytoplanktonic diel dynamics is limited. By combining a field survey with a published dataset, we found that cell numbers and cell sizes/biomasses of picophytoplankton were tightly synchronized to the day-night cycle, but they were in a quasi-antiphase relationship to each other. This pattern is a confirmation and extension of previous studies. Mortality rates showed that *Prochlorococcus* and *Synechococcus* were subject to considerable grazing pressure throughout the day and night. The quasi-antiphase diel cycles in abundance and cell size/biomass are likely determined by the light-dependent diel behavior of cell growth and division and continuous losses to grazing. This work significantly improves our understanding of autotrophic picoplankton in the oligotrophic ocean.

Plain Language Summary Picophytoplankton are tiny, single-celled photosynthetic organisms that contribute to almost all primary production in the vast euphotic zones of the oligotrophic ocean. Understanding their roles in that environment is critical but challenging, mainly because of their minuscule size and the complexity of microbial processes and interactions. Time-series observations based on flow cytometry, a powerful technique that provides information about the numbers and sizes of picophytoplankton cells, have elucidated many ecological and biogeochemical processes associated with picophytoplankton, but some questions remain. A field survey in the northern South China Sea combined with a published dataset revealed that picophytoplankton cell size and biomass tended to decrease (increase) during the night (day) when cell numbers were increasing (decreasing). Such quasi-antiphase cycles are likely a general feature of near-steady-state oligotrophic ecosystems and reflect the cycles of carbon fixation, energy storage, and cell growth during the daytime and cell division and energy depletion during the night. Mortality rates estimated via modified dilution experiments showed that *Prochlorococcus* and *Synechococcus* were subject to considerable grazing pressure throughout the day and night. This work significantly improves our understanding of these microorganisms and may have implications for the carbon cycle in oligotrophic marine ecosystems.

1. Introduction

The oligotrophic ocean accounts for approximately half of Earth's surface and is the habitat of the greatest number of phototrophs in the world. In that nutrient-deficient environment, the food web is dominated by the microbial loop, and picophytoplankton (*Prochlorococcus*, *Synechococcus*, and picoeukaryotes) account for most of the photosynthetic biomass and primary production (Fenchel, 2008). Picophytoplankton cells are quite small (<2 μm in diameter), but they are numerically the dominant photosynthetic organisms in the ocean and play pivotal roles in shaping planktonic community structure and regulating the carbon cycle in oligotrophic marine ecosystems. With an expansion of oligotrophic regions caused by climate change, these smallest of marine phytoplankton will increase in both numerical abundance and biomass in the future ocean (Flombaum et al., 2013, 2020).

Food webs in the oligotrophic ocean are highly dynamic. Diel cycles of cell division, cell numbers, and cell size of picophytoplankton are significant and well documented in tropical and subtropical near-surface oceanic waters (Binder & DuRand, 2002; Vaulot et al., 1995; Vaulot & Marie, 1999). An increase of cell numbers at night is driven primarily by concurrent cell division, and the decline of cell numbers during the day reflects an imbalance between gains from cell division and losses to grazing and viral lysis. However, it seems paradoxical that the decline of cell abundance is synchronized with photosynthetic production. Recent research performed in the North Pacific Subtropical Gyre (NPSG) has shown that picophytoplankton biomass exhibits marked diel oscillations—a diurnal increase and nocturnal decrease—that are synchronized with the concentrations of particulate organic carbon inferred from optical measurements (Boysen et al., 2021; Henderikx-Freitas et al., 2020). Although the diel periodicity of photosynthesis and cell division undoubtedly contribute to this cycle, mortality associated with grazing and viral infection are equally important determinants of picophytoplankton abundance (Binder & DuRand, 2002). Knowledge of the diel pattern of cell numbers and biomass of picophytoplankton combined with estimates of their growth rates as well as losses to grazing and viral lysis allow consideration of both bottom-up and top-down control.

In this study, we conducted time-series observations and parallel incubation experiments in the northern South China Sea (SCS), which is a typical oligotrophic marginal sea (Wong et al., 2007). We combined flow cytometric (FCM) analysis with an empirical laboratory calibration for cell size determination to assess the diel patterns of three picophytoplankton groups and found that there was a quasi-antiphase relationship between cell numbers and cell size/biomass of *Prochlorococcus* and *Synechococcus*. We complemented this study with a compiled dataset to ascertain the prevalence of these highly synchronized, quasi-antiphase diel cycles of cell numbers and cell size/biomass of picophytoplankton. Furthermore, the incubation experiment results and a simple model facilitated understanding of the mechanisms responsible for the quasi-antiphase diel patterns.

2. Materials and Methods

2.1. Sampling and Environmental Variables

The MARCO summer cruise (KK1904), a survey of the northern SCS, was conducted from 17 June to 04 July 2019 on board the R/V Tan Kah Kee (Figure S1 in Supporting Information S1). Three time-series stations, two on the slope (M4, K11) and one in the basin (SEATS), were occupied during the cruise. We conducted time-series surveys at K11, while Lagrangian observations following drifting sediment traps were carried out at SEATS and M4. During 24-hr or 48-hr sampling periods, surface water samples (~5 m) in triplicate were collected for FCM analysis every ~1.5 hr using a CTD rosette sampler or a plexiglass water sampler. On each observation day, seawater samples were collected twice, at approximately 06:00 and 18:00 (local time), for modified dilution experiments.

2.2. Flow Cytometric (FCM) Analysis and Population-specific Carbon Biomass Calculation

Cell numbers and light scatter of three picophytoplankton populations (*Prochlorococcus*, *Synechococcus*, and picoeukaryotes) were determined using a BC CytoFLEX flow cytometer following procedures described previously (Marie et al., 1999). In accord with the commonly used flow cytometer–specific calibrations (DuRand et al., 2001; Jacquet et al., 2001; Worden et al., 2004), forward light scatter (FSC) was converted to cell size (expressed as equivalent spherical diameter, ESD) using an empirical relationship ($\log ESD = 0.2504 \log FSC + 0.1351$, Figure S2 in Supporting Information S1) between FSC measured by this instrument and cell size determined by epifluorescence microscopy (Zeiss Imager.A2) for different exponentially growing phytoplankton cultures (eight marine picophytoplankton cultures at three different times of day). Additional details regarding FCM analysis and the empirical size-FSC calibration are described in the Supporting Information S1. The resulting cell size was used to calculate biovolume assuming spherical shape. Cell biovolume was then converted to carbon with a conversion factor of 280 fg C μm^{-3}, which was derived from an equatorial Pacific *Prochlorococcus* strain (Heldal et al., 2003). Carbon biomass of each group was estimated by multiplying per-cell carbon by cell numbers.

2.3. Growth and Loss Rate Estimates

To identify the biological factors responsible for the diel variations of picophytoplankton, modified dilution experiments were performed following the protocol of Kimmance and Brussaard (2010) in parallel to the time series. At about sunrise or sunset, natural surface seawater, gently passed through a 20-μm nylon net filter to remove microzooplankton, was combined with grazer-free filtrate (<0.1 μm) or virus-and-grazer-free filtrate (<30 kDa) in proportions of 27%, 55%, 82%, and 100%. All mixtures in quadruplicate were incubated for 9 hr in an on-deck Plexiglas incubator, which was screened with neutral density filters (LEE 298) to simulate the photosynthetically active radiation (PAR) intensity at ∼5 m. Incubation temperature was controlled by continuously flowing surface seawater. FCM samples were taken at the beginning and end of the incubation as described above. Mortality and intrinsic growth rates were calculated from linear regressions of apparent growth rate versus dilution factor (for more details see Text S2 in Supporting Information S1). The low abundance of picoeukaryotes made the rate estimates unreliable, and the calculated grazing rates were generally negative. In the following analysis, we therefore concentrated on *Prochlorococcus* and *Synechococcus*.

For a qualitative comparison, we calculated diurnal/nocturnal net growth rates from time-series observations and incubation experiments. The former rates were estimated from the changes between the two time points closest to sunrise and sunset, that is, $ln(P_2/P_1)/(t_2 - t_1)$, where P is the corresponding abundance or biomass, and t represents time. The latter rates were determined from the difference between the intrinsic growth rates and the loss rates due to grazing and viral lysis. Sometimes the loss rates were negative, especially those associated with virus-induced mortality rates. The differences were therefore calculated in two different ways: the negative loss rates were included in the calculations, and the negative loss rates were set to 0. All the calculations were based on both abundance and biomass.

2.4. Joint Analysis With SeaFlow Dataset

To demonstrate the general relevance of the results of the local time-series study, we extended a published dataset (SeaFlow data v1.3, Ribalet et al., 2020) that consisted of high-resolution, underway FCM observations in surface waters of the North Pacific and South Atlantic. The data set included cell sizes and biomasses of picophytoplankton populations estimated using methodologies similar to ours. After data cleansing, data from 38 cruises (including our SCS cruise) under oligotrophic conditions remained for the subsequent analysis (Figure S3, Table S2 in Supporting Information S1). Details of data cleansing and other data processing procedures are presented in the Supporting Information S1.

In order to make a direct comparison of the data from different geographical regions and dates with differing daylight hours, we adopted a normalized time scale in which sunrise and sunset were fixed at 06:00 and 18:00, respectively. We standardized the measured values by dividing by the mesor (midline-estimating statistic of rhythm, a rhythm-adjusted mean calculated by the cosinor method) value in a 24-hr rolling window. Because the time intervals between the data points in the datasets were not all the same and because many data gaps existed, the mesor could provide a better estimate of central tendency than the arithmetic mean (Refinetti, 2016). Normalization resulted in a mean value of 1. In addition, the normalization led to the elimination of long-range trends, which could influence the assessment of 24-hr periodicity (Leise, 2017). After identification and replacement of outliers and rolling smoothing, the final data were binned to half-hour intervals. To further explore diel variations of picophytoplankton, the diel periodicity analyses were conducted using the cosinor method by fitting a cosine curve with a 24-hr period (Refinetti, 2016). A day with no more than 20 (41.7% of 48 half-hour intervals) missing values was considered a valid day. Altogether, 200 valid days from 250 days were included in the analysis. Because the reliability of the periodicity analyses and the accuracy of phase estimation based on a single-cycle's data were low (Leise, 2017), especially when there were many missing values, we conducted cosinor analyses using a rolling window of 3 days. Diel periodicity was statistically validated with $R^2 \geq 0.36$, and the corresponding clocktimes of the acrophases were analyzed using circular boxplots (Buttarazzi et al., 2018).

3. Results

3.1. Diel Variations of the Picophytoplankton Community in the Northern SCS

Survey results at three stations along the continental slope (K11 and M4) and in the basin (SEATS) indicated that environmental conditions in the northern SCS during the summer were relatively stable. Fluctuations of temperature and salinity were small, and PAR was more-or-less constant (Figure S1, Table S1 in Supporting Information S1). The surface Chl *a* concentrations at the three stations were quite low (0.11–0.13 μg L^{-1}), and biological variables at these three stations were strikingly similar (Table S1 in Supporting Information S1). Picophytoplankton community composition was dominated by *Prochlorococcus* (average abundance reached 156–170 × 10^3 cells mL^{-1}); the cell numbers of *Synechococcus* and picoeukaryotes were 2–3 orders of magnitude lower, respectively. These characteristics indicated that the three stations could be considered typical of oligotrophic environments.

During 24-hr or 48-hr time series, the picophytoplankton in surface waters exhibited clear diel periodicity in cell numbers, cell size, and biomass (Figure 1a). Compared to *Prochlorococcus*, the oscillations were noisier for the cell numbers and biomasses of *Synechococcus* and picoeukaryotes, the abundances of which were relatively low. Generally, the cell numbers of the three picophytoplankton groups increased at night and decreased during the day, whereas cell sizes increased during the day and decreased at night. Analysis by the cosinor method revealed that the average daily percent increases (from trough to peak) in cell numbers of *Prochlorococcus*, *Synechococcus*, and picoeukaryotes were 27.7%, 25.9%, and 30.9%, respectively. The corresponding increases of cell sizes were 18.0%, 14.8%, and 7.5%, respectively. The biomasses of *Prochlorococcus* and *Synechococcus* displayed a pattern similar to those of cell sizes but almost antiphase with those of cell numbers; the biomasses and cell sizes increased during the day and decreased at night. The percent increases of the biomasses of *Prochlorococcus* and *Synechococcus* over the diel cycle were 29.2% and 34.6%, respectively. However, no diel periodicity was detected for the biomass of picoeukaryotes, and there were no diel patterns in the biomasses of heterotrophic bacteria and viruses (Figure S4 in Supporting Information S1).

3.2. Daytime Versus Night-Time Rate Estimates From FCM Abundances or Biomasses

For *Prochlorococcus* and *Synechococcus*, diel patterns of intrinsic growth rates based on cell numbers and biomass were completely opposite (Figure 1b, Table S3 in Supporting Information S1). The estimated intrinsic growth rate based on the rate of change of cell numbers was significantly lower during the day than at night, whereas the biomass-based intrinsic growth rate was obviously higher during the day than at night. Furthermore, net growth rates based on cell numbers were negative during the photoperiod and positive at night, whereas biomass-based estimates were just the opposite, no matter whether the calculation was based on incubation experiments or time-series data (Figure 1c). In contrast, nanoflagellate grazing rates derived from FCM abundances were not significantly different between the day and night, whereas biomass-based grazing rates were significantly higher during the daytime than at night (Figure 1b). We cannot speculate about the reason for these differences, and the later discussion will be based on the commonly used abundance parameters. Both abundance- and biomass-based virus-induced mortality rates were lower than nanoflagellate grazing rates, and most of them were even negative (Figure 1b). It is unclear whether the negative viral mortality is biologically meaningful (i.e., viral effects are stimulatory) or just a methodological artifact (Pasulka et al., 2015).

3.3. The Prevalence of the Diel Patterns of Picophytoplankton Community

As expected, the results of the joint analysis were consistent with the local time series. When all the normalized data were simply aggregated, all three picophytoplankton populations showed pronounced diel patterns, especially in terms of cell size and biomass (Figure 2a). Abundance data displayed great variability, and their diel patterns were less dramatic, even slightly different from those of the local study, that is, the abundances of *Prochlorococcus* and picoeukaryotes rose in the early afternoon. This pattern suggested that the diel variation in abundance was less significant, or that the rhythmic pattern was less consistent.

Further analysis at the daily level confirmed this suggestion (Figure 2b, Figures S8–S11 in Supporting Information S1). Cosinor analysis showed that diel patterns of cell numbers of *Prochlorococcus*, *Synechococcus*, and picoeukaryotes were detectable in 66.0% ($n = 132$), 39.5% ($n = 79$), and 53.5% ($n = 107$), respectively, during the 200 valid days. For all three populations, cell numbers exhibited a diel peak mainly between midnight and sunrise,

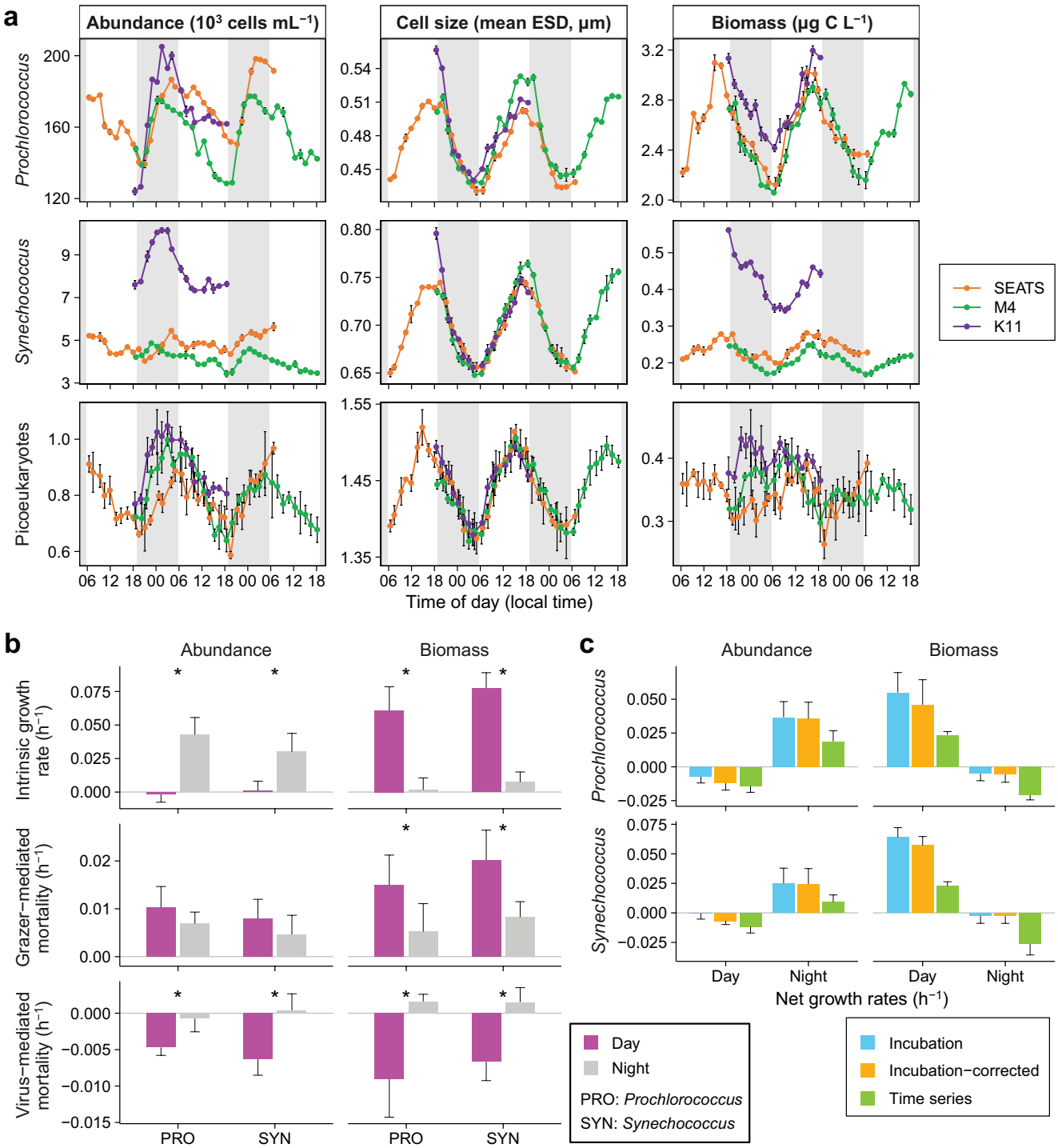

Figure 1. The field study in the northern South China Sea. (a) Time series of picophytoplankton (*Prochlorococcus*, *Synechococcus*, and picoeukaryotes) cell numbers, cell size, and biomass in surface waters at three stations during the MARCO cruise (June–July 2019). Night-time periods are shaded gray. (b) Incubation-based day versus night comparisons of picophytoplankton intrinsic growth, virus- and nanoflagellate-mediated mortality based on cell numbers and biomass. Significant differences between day and night are indicated by asterisks ($p < 0.05$, Student's *t*-test). (c) Day versus night comparisons of picophytoplankton abundance- and biomass-based net growth rates estimated from incubation experiments and time series of in situ changes. Negative incubation-based loss rates are included or corrected to 0. Error bars denote standard deviations ($n = 3$ in [a]; $n = 5$ in [b, c]) and are smaller than the data points when not apparent.

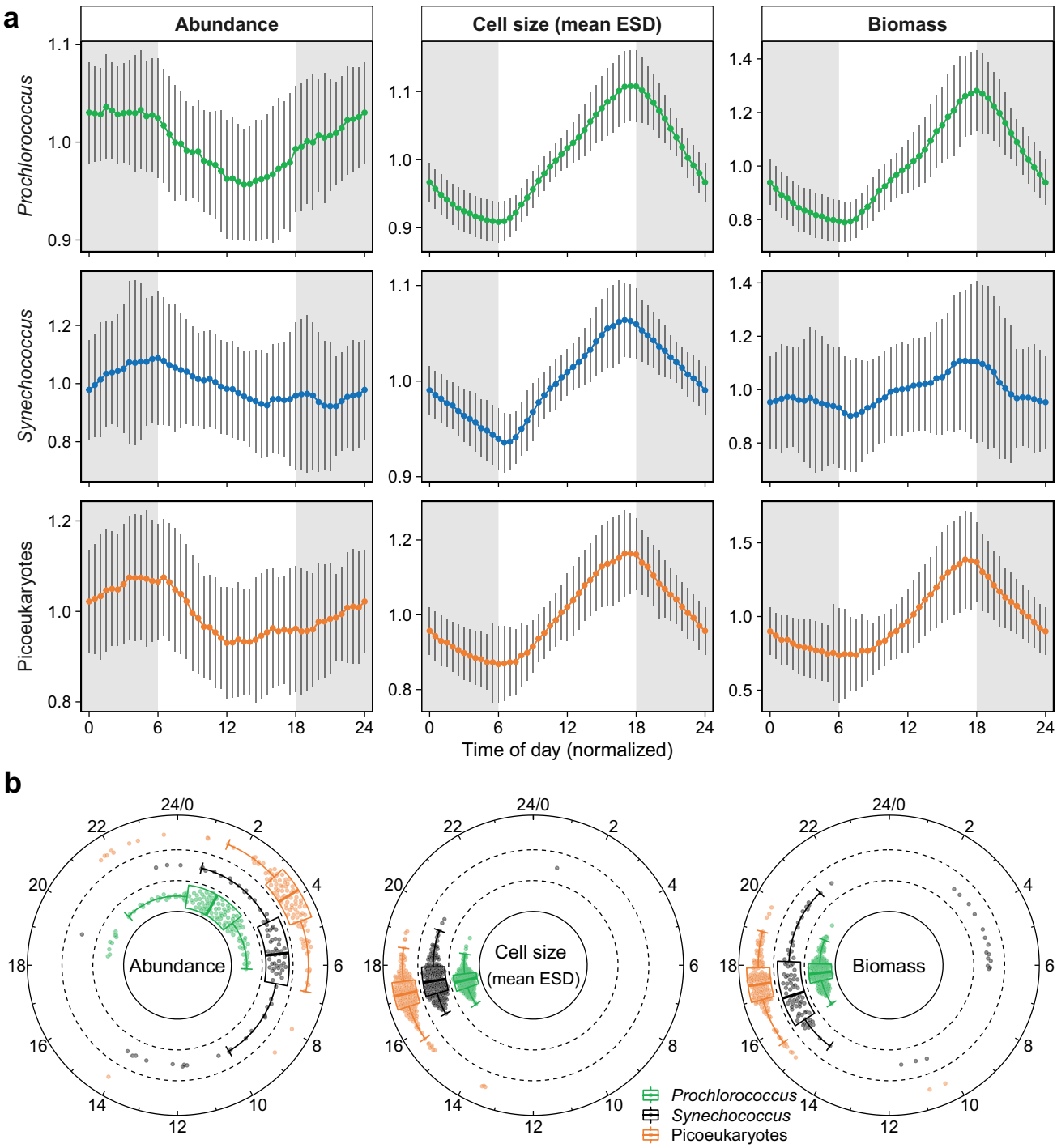

Figure 2. Joint analysis of the South China Sea observations and SeaFlow dataset. (a) Average half-hourly values of cell numbers, cell sizes, and biomasses of three picophytoplankton groups across all 38 cruises. Note that because the values have been normalized, they are unitless and fluctuate about 1. Error bars denote standard deviations (n ranges from 181 to 218). (b) Circular boxplots overlaid with beeswarm plots showing the distribution of the acrophases (peak times) of the diel periodicity in cell numbers, cell sizes, and biomasses of three picophytoplankton groups. Each dot represents a valid day with a statistically significant 24-hr periodicity. From left to right, n = 132, 200, and 195 for *Prochlorococcus*; n = 79, 190, and 97 for *Synechococcus*; and n = 107, 188, and 169 for picoeukaryotes. The boxes contain the central 50% of the data surrounding the circular median; whiskers correspond to 1.5 times the interquartile range.

in which *Prochlorococcus* (01:59 [00:32–03:30], median and interquartile range) peaked earliest, followed by picoeukaryotes (03:52 [03:10–04:41]) and then *Synechococcus* (05:35 [04:18–06:42]). In the case of cell size, all three picophytoplankton populations almost always exhibited a clear diel variability (200 [100%], 190 [95.0%], and 188 [94.0%] for *Prochlorococcus*, *Synechococcus*, and picoeukaryotes, respectively). Diel oscillations of cell size were highly synchronized and phased almost identically for all three populations (*Prochlorococcus*, 17:11 [16:45–17:32]; *Synechococcus*, 17:24 [16:55–17:56]; and picoeukaryotes, 17:10 [16:47–17:32]) during nearly all valid days. Of all 200 valid days, 195 (97.5%), 97 (48.5%), and 169 (84.5%) were characterized by diel patterns of the biomasses of *Prochlorococcus*, *Synechococcus*, and picoeukaryotes, respectively. The corresponding acrophases occurred around sunset (*Prochlorococcus*, 17:35 [17:08–18:04]; *Synechococcus*, 16:51 [15:49–18:08]; and picoeukaryotes, 17:27 [16:59–17:56]) at times similar to the peaks of cell size.

4. Discussion

For obvious reasons, the physiological processes of phytoplankton are closely correlated with the light-dark cycle. In this study, we found marked differences in picophytoplanktonic diel cycles between cell numbers and cell size/biomass in the northern SCS (Figure 1a). The differences were consistent with the net growth rates estimated during the day/night with our modified dilution experiments (Figure 1c). The cycles of abundance and cell size in our study were similar to those observed in the equatorial Pacific, although phased slightly differently (Vaulot & Marie, 1999). In the case of *Prochlorococcus* and *Synechococcus*, the cycles of biomass were consistent with the observations in studies from the NPSG, in which biomass has exhibited marked diel oscillations and coherent synchronization with bulk optical properties (Boysen et al., 2021; Henderikx-Freitas et al., 2020). We did not find a similar diel pattern in picoeukaryotic biomass, possibly because of their low abundance and the fact that the upper threshold (2 μm) that we used may have excluded some cells that became larger with growth (Figure S12 in Supporting Information S1). Direct measurement of group-specific picophytoplankton biomass under field conditions has not been possible, although such measurements are simple in laboratory cultures. Field studies normally make use of some indirect method, such as photosynthetic pigments (Mackey et al., 1996) and FCM-derived estimates (Boysen et al., 2021; Ribalet et al., 2019). Estimates of carbon per cell based on FCM analyses are confounded by uncertainties associated with the conversions of forward scatter to size, size to biovolume (assuming spherical shape), and biovolume to carbon. However, we found that the daily percent increases in biovolume based on the FCM-derived sizes of picophytoplankton were similar in the field and laboratory (Figure S12 in Supporting Information S1). Furthermore, when four different empirical functions (Ribalet et al., 2019; Worden et al., 2004) were used to convert cell biovolume to carbon, the cellular quotas of carbon differed, but the broad trends of biomass were unchanged (Figure S13 in Supporting Information S1). We therefore felt that the patterns in the diel oscillations of picophytoplanktonic biomass were robust.

The findings of the local investigation were further confirmed and generalized in the joint analysis (Figure 2). Of the three picophytoplankton groups, cycles with a period of 24 hr were more evident for *Prochlorococcus* than for *Synechococcus* and picoeukaryotes, which were present in relatively low abundance. The evidence of a diel pattern was strongest for cell size, weakest for cell numbers, and intermediate for biomass, which depended on the product of cell numbers and cell size. It should be noted that only a few underway observations were conducted in the same water mass, and the obtained percentages of significant diel variation were definitely lower than that obtained using Lagrangian observations (similar to stations SEATS and M4). In addition, the acrophase distribution was more concentrated for cell size and biomass than for abundance. These differences reflected the fact that cell size was regulated by a combination of the cell division cycle and the 24-hr photosynthesis/respiration cycle (Binder & DuRand, 2002), whereas cell numbers were regulated by a complex combination of biotic and abiotic factors. Moreover, the daily cycles of biomass were clearly driven largely by changes in cell size and less by the dynamics of abundance. Cell size is controlled mainly by the light-dark cycle, whereas grazing and physical processes have little effect on cell size, but they do affect cell numbers. Mixing of different water masses due to horizontal advection or vertical mixing could add or remove cells and dramatically perturb the diel patterns of cell numbers (André et al., 1999). Tsai et al. (2009) have reported that strong winds and heavy rains during the passage of a typhoon seriously disturbed the diel pattern of *Synechococcus* abundance but had little effect on the cell division cycle.

Furthermore, the joint analysis revealed that the almost antiphase cycles between abundance and cell size/biomass were evident in most cases (Figure 2b). In fact, the underlying mechanism of such quasi-antiphase relationships is easy to understand. In the absence of any physical disturbance, picophytoplankton diel dynamics

are driven primarily by three processes: cell division, cell growth, and loss processes. For a photosynthetic organism, biomass in terms of carbon increases only during the day, decreases at night, and peaks at the end of the photoperiod. Although the timing varies among these three groups and between different times and locations, cell division generally occurs at night and/or in the late afternoon, whether in culture or in the field (Binder & DuRand, 2002; Jacquet et al., 2001). In addition, cell division during steady state growth occurs when the biomass per cell has doubled, and cellular biomass can increase only during the photoperiod. Carbon fixed by photosynthesis is therefore used mainly for cell growth during the daytime. Cell size tends to decrease at night because of cell division, respiration, and exudation. Thus, like biomass, cell size exhibits significant diel periodicity and peaks near sunset. In contrast, diel variations of abundance are determined by cell division and loss processes (e.g., grazing, viral lysis). The fact that these two processes can occur simultaneously and affect abundance in opposite directions confounds deconvolution of diel abundance cycles (Binder & DuRand, 2002) and explains, to some extent, the relatively heterogeneous behavior of those cycles (Figure 2b). However, decreases in cell numbers can result only from loss processes, whereas increases can result only from cell division. The cell numbers increase when the division rate exceeds the loss rate and peak when the former declines and/or the latter increases until they are equal. Figure 2b shows that peak times of abundance occurred mainly between midnight and sunrise. This pattern was consistent with the results of most previous studies (Binder & DuRand, 2002, and references therein), with the exception of Ribalet et al. (2015). The acrophases of *Prochlorococcus* abundance during the two cruises (CN11ID and TN271) studied by Ribalet et al. (2015) are the outliers in Figure 2b. During those cruises, cell numbers and cell sizes of *Prochlorococcus* both increased during the day and decreased at night (Figures S9 and S10 in Supporting Information S1). This pattern differed from the pattern we observed in most cases and may have been an exception to the more common diel cycle of *Prochlorococcus* cell numbers. Returning to Figure 2b, considerable proportion of acrophases of *Prochlorococcus* abundance are distributed around midnight, and this is in accordance with the daily increase in *Prochlorococcus* abundance in the early afternoon in Figure 2a. These results, however, were inconsistent with the general conclusion that cell division in *Prochlorococcus* generally occurs at night and/or in the late afternoon, and might be attributed to an underestimation of *Prochlorococcus* abundance in the early afternoon (because of very low midday fluorescence) (Binder & DuRand, 2002).

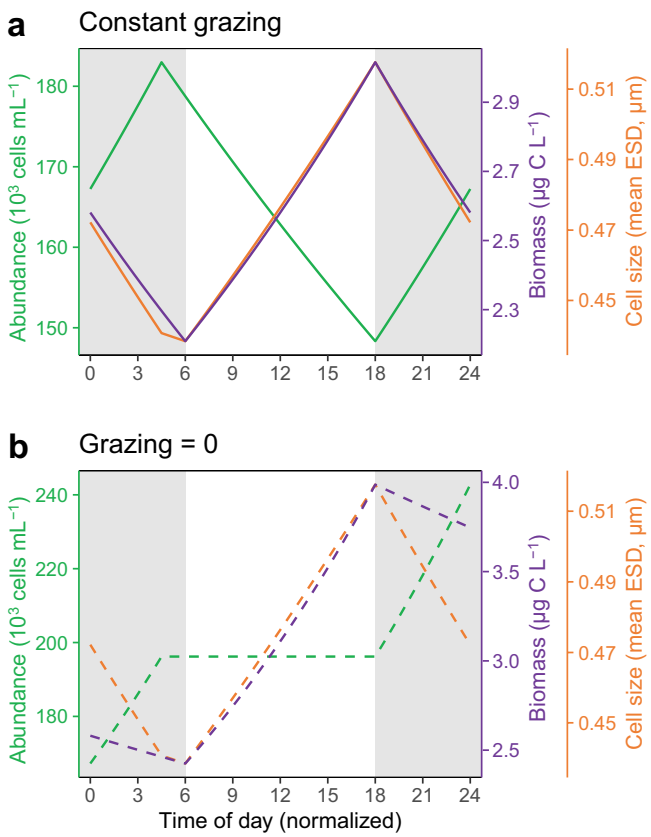

Figure 3. A simple model simulating the daily patterns of cell numbers, cell size, and biomass of *Prochlorococcus* when the grazing rate is constant (a) or equal to 0 (b). All vital rates are assumed to be constant, with net primary production during the day, respiration throughout the night, grazing throughout 24 hr, and cell division beginning at sunset and continuing through the first 10.5 hr of darkness (the average peak time was ~04:30 for *Prochlorococcus* in the South China Sea [SCS] study). The model was optimized using the SCS *Prochlorococcus* data (Figure 1a) and adjusted so that the numbers were almost identical after 24 hr and at the beginning, that is, the system was in steady state.

The idea that grazing generally accounts for the majority of phytoplankton mortality in the ocean (Calbet & Landry, 2004) is consistent with the observations in Figure 1b. However, the diel pattern of protistan grazing on picophytoplankton has not been well defined, and some contradictory findings have been reported (Connell et al., 2020; Fowler et al., 2020). Some reports (Connell et al., 2020; Ribalet et al., 2015; Tsai et al., 2005) have indicated that protistan grazing occurs primarily at night. The discovery in our study of comparable grazing loss during the daytime and night (Figure 1b) was similar to the results of some other studies (Dolan & Šimek, 1999; Fowler et al., 2020; Ng & Liu, 2016). This grazing coincided with a diurnal decrease in cell numbers (Figure 1a). As mentioned above, the *Prochlorococcus* abundance cycles observed by Ribalet et al. (2015) may have been exceptions to the general pattern, and if so, the diel patterns of *Prochlorococcus* mortality would have been exceptions as well. Tsai et al. (2005) have calculated diurnal/nocturnal growth rates of *Synechococcus* based on cell abundance and then multiplied those growth rates by in situ biomasses (derived from a constant carbon conversion factor) to obtain biomass-based production rates and grazing rates. We feel that their calculations were biased by the fact that they did not consider diel patterns of cell size and biomass. Furthermore, the pattern of grazing can also be inferred from laboratory incubations, in which clear diel variations of picophytoplankton abundance have been observed when cells were grown under light-dark cycles. The growth curves

in conventional batch cultures follow a step-like pattern with an ascending period during the night and a stable period during the day (e.g., Jacquet et al., 2001; Zinser et al., 2009; Waldbauer et al., 2012), whereas temporal periodicity of abundance similar to those of natural populations can be observed only under continuous culture conditions (Mori et al., 1996; Claustre et al., 2002). The key features that distinguish continuous culture systems from batch cultures are continuous removal of culture and continuous addition of fresh medium in the former. The implication is that loss processes under realistic field conditions may be continuous and may even occur at a more-or-less constant rate.

Based on the aforementioned results and discussion, we developed a simple mathematical model to simulate the behavior of cell numbers, cell size, and biomass of picophytoplankton over a period of 24 hr (Figure 3a, for more details see Text S4 in Supporting Information S1). In this simulation, there was clearly an almost antiphase relationship between cell numbers and cell size/biomass. If the grazing rate is adjusted to 0 (with other parameters unchanged), the diel patterns would be very similar to that of laboratory batch cultures (Figure 3b). Although this model is very simplistic, it can explain the diel patterns of picophytoplankton in the field (Figure 1a) and in culture and supports the rationale of the above explanation.

5. Conclusions

By combining a field survey with a published dataset, this study confirmed and extended previous results of diel patterns of autotrophic picoplankton in the oligotrophic ocean. We suggest that the quasi-antiphase diel cycles in abundance and cell size/biomass of picophytoplankton are likely a general feature of near-steady-state oligotrophic ecosystems. Light is recognized as a key driver in the dynamics of microbial food webs. The quasi-antiphase diel patterns of cell numbers and cell size/biomass of these tiny phototrophs reflect the cycles of carbon fixation, energy storage, and cell growth during the daytime and cell division and energy depletion during the night. The loss processes through grazing seem to occur throughout the day and night, but many details remain to be discovered.

Data Availability Statement

Data and source code for this study are available on Zenodo (https://doi.org/10.5281/zenodo.5835992).

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