# FINE-SCALE SPATIAL AND TEMPORAL HETEROGENEITY OF PHYTOPLANKTON IN THE INNER TOKYO BAY, JAPAN

### Khin Khin Gyi<sup>1</sup>

## Abstract

The spatial and temporal variations of phytoplankton were governed by the physicochemical properties of the water column such as water stratification, vertical mixing, temperature and salinity gradients, and nutrient supply. In this study, the salinity gradient was pronounced in the upper layer, and a strong stratification developed in the water column which in turn influenced large variability in the vertical distributions and cell concentrations of phytoplankton. In terms of cell concentration, nine species such as *Lauderia annulata, Skeletonema* sp., *Thalassiosira* spp., *Chaetoceros* sp., *Thalassionema frauenfeldii, T. nitzschioides, Pseudo-nitzschia* sp., and *Heterosigma akashiwo* dominated the phytoplankton community, and the percentage of their abundance comprised 89.5% of the total phytoplankton concentration. Among these species, the cells of *Pseudo-nitzschia* sp. and *H. akashiwo* increased at night, approximately 4 to 8 times higher than in the daytime, due to the changes in the water mass. At the most depth range, all species had higher cell concentrations in the upper layers above the pycnocline where Chl-*a* showed the maximum. However, a significant decrease in cell concentration was noted below the pycnocline where the hypoxic water mass was observed.

Keywords hypoxic, phytoplankton, pycnocline, salinity, stratification

# Introduction

The phytoplankton community in the vertical water column is highly heterogeneous and related to the small-scale physical hydrodynamic gradients such as water mass stability (Mellard et al., 2011), mixing (Maznah et al., 2016), and light availability (Tilzer and Goldman, 1978). Abiotic water conditions varied naturally at different timescales in a day (Gast et al., 2014), related to the periodic oscillations of the tidal currents, which consecutively influence short-term changes in the phytoplankton community (Blauw et al., 2012; Khin Khin Gyi, 2020).

The inner region of Tokyo Bay is a eutrophic semi-closed coastal embayment due to urban runoff, river discharge, and industrial wastes from metropolitan areas (Furukawa and Okada, 2006). As a result of freshwater discharge from rivers in Tokyo Bay, a sharp horizontal and vertical salinity gradient was prominent. Moreover, the formation of anoxic or hypoxic water mass during stratification and the vertical distribution of nutrients may result in great fluctuations of phytoplankton abundance, composition, and the vertical patchiness of algal blooming in the inner Tokyo Bay. Therefore, to understand the controlling factors of the phytoplankton community, many previous studies from various aspects of the ecological approaches had already been conducted in Tokyo Bay (e.g., Han, 1988; Han and Furuya, 2000; Koibuchi and Isobe, 2007; Nakane et al., 2008; Bouman et al., 2010). However, their works mainly focused on seasonal and monthly variations, and a few studies have achieved a sampling frequency of nearly weekly intervals. It is remaining to explore how the phytoplankton fluctuate at a short timescale of the day. Here in this paper, a fine-scale vertical structure of phytoplankton was observed at 1 m depth intervals, dealing with the environmental properties of the water column between day and night hours. The objectives of the present study were (1) to clarify the fine-scale spatiotemporal heterogeneity of phytoplankton distributions in species-level, and (2) to observe the species-specific distribution patterns within the communities during different hours of the study period.

<sup>&</sup>lt;sup>1</sup> Department of Marine Science, Sittway University

# **Materials and Methods**

#### Sampling site and sample collection

Phytoplankton samples were collected using a vortex submersible pump to sample simultaneously throughout the vertical water column from 0 to 20 m at every 1 m interval at a fixed station of 23 m deep in the inner Tokyo Bay (35°30'30" N, 139°50'00" E), Japan (Fig. 1). Day and night samplings were conducted at the same point of the above-mentioned fixed station from a training and research vessel "Hiyodori" of Tokyo University of Marine Science and Technology at a local time 10:00 and 20:45 (JST) on 21 September 2017. A total of 42 samples were collected from 21 distinct vertical water layers within 0-20 m depth to understand the fine-scale distribution of phytoplankton.

### Physical and chemical analyses

Hydrographic profiles of water temperature, salinity, sigma-t, and dissolved oxygen were observed using a CTD (AAQ-PRO 2, JFE Advantech, Tokyo, Japan) equipped with sensors for in vivo chlorophyll-*a* fluorescence (Chl-*a*) and turbidity. For inorganic nutrient analyses (NO<sub>3</sub>+NO<sub>2</sub>, PO<sub>4</sub>, SiO<sub>4</sub>, and NH<sub>4</sub>), 15 mL of water sampled at each depth was filtered through a 0.20  $\mu$ m pore size syringe filter (DISMIC-25CS, Advantec, Tokyo, Japan) on the boat deck and frozen at -20°C until analysis. Nutrient concentrations were determined using an automated nutrient analyzer, QuAAtro (SEAL Analytical, Hampshire, UK) in the laboratory.

#### **Phytoplankton analysis**

Water samples of 500 mL were taken for phytoplankton analysis during each CTD cast by attaching a pump intake hose with a CTD and immediately fixed with formalin (final concentration 1%). For the abundance, phytoplankton samples were analyzed with a FlowCAM (Fluid Imaging Technologies, Inc., ME, USA) in triplicate under the autoimage mode.



Figure 1 Sampling site in the inner Tokyo Bay, Japan.

## **Results and Discussion**

#### Hydrological conditions

In the daytime (10:00), the upper layer (top 6 m) was marked with a strong pycnocline, being recognized by the drastic changes in the water salinity (27.95-30.96). At the same time, the fluctuations in the water temperature (22.83-23.13°C) and dissolved oxygen concentration (4.54-5.85 mg L<sup>-1</sup>) were noted, especially in the lower limit of the pycnocline (4-6 m). The concentration of Chl-*a* showed a maximum value (> 5 µg L<sup>-1</sup>) in the upper layer (3-4 m) but decreased sharply in the lower limit of the pycnocline, then gradually decreased to the minimum value (1.5 µg L<sup>-1</sup>) in the 14-20 m depth. Below the pycnocline, a well-mixed water layer was observed at 7-11 m, but a weakly stratified layer (3 m thick) existed underneath, which separated the intermediate (7-11 m) and the bottom mixed layer depth (14-20 m). Simultaneously, the bottom water became hypoxic ( $\leq 2.5$  mg L<sup>-1</sup>) at 14-20 m depth (Fig. 2. a).

At night-time (20:45), a multi-stepped structure in the water column was eroded, while the pycnocline deepened to 5-10 m with the vertical salinity gradients (28.47-32.45) which separated the surface and the bottom layers. The maximum values of water temperature (23.45°C), dissolved oxygen (8.96 mg L<sup>-1</sup>), and Chl-*a* concentration (11.77 µg L<sup>-1</sup>) were observed in the surface layer coincided with the upper limit of the pycnocline but decreased rapidly in the pycnocline till its lower limit, then gradually decreasing towards the bottom. Below the pycnocline, the water mass became nearly hypoxic ( $\leq 2.5$  mg L<sup>-1</sup>), and the thickness increased to 10 m. Based on the profiles, salinity variations resemble density variations, indicating that salinity is more important than water temperature in determining the density field in our observation area. Turbidity and dissolved oxygen concentration showed a negative trend in both observation periods (Fig. 2. b).

# **Dissolved inorganic nutrients**

The vertical distribution of inorganic nutrient concentrations during the day and night times (Figs. 3. a, b) showed that nitrate and nitrite, phosphate, silicate, and ammonium concentrations varied between 8.1 and 39.2  $\mu$ M L<sup>-1</sup>, between 1.1 and 3.5  $\mu$ M L<sup>-1</sup>, between 22 and 56.8  $\mu$ M L<sup>-1</sup>, and between 1.6 and 14.7  $\mu$ M L<sup>-1</sup>, respectively during the study period. The concentration of each inorganic nutrient showed significant peaks in the surface layer above the pycnocline but decreased below it.



**Figure 2** Day and night vertical distributions of water temperature (°C), salinity, sigma-*t*, Chl-*a* ( $\mu$ g L<sup>-1</sup>), dissolved oxygen (mg L<sup>-1</sup>), and turbidity (mg L<sup>-1</sup>).

#### Spatial and temporal distributions of total phytoplankton

The vertical distribution of the total phytoplankton community showed the same pattern between day and night (Figs. 4. a, b). The phytoplankton concentration was higher in the surface layer where Chl-*a* showed the maximum but decreased sharply in the pycnocline till its lower limit, then little variation was found below the pycnocline towards the bottom. During the observation, the pycnocline was deepening at night which increases the surface layer of phytoplankton abundance. Moreover, the phytoplankton concentration and Chl-*a* values increased double at night due to a significant increase in the cell concentrations of *Pseudo-nitzschia* sp. and the Raphidophycean flagellate, *Heterosigma akashiwo* occurred in the upper 0-5 m and 5-8 m depths, respectively.



**Figure 3** Day and night vertical distributions of nitrate and nitrite ( $\mu$ M L<sup>-1</sup>), phosphate ( $\mu$ M L<sup>-1</sup>), silicate ( $\mu$ M L<sup>-1</sup>), and ammonium concentrations ( $\mu$ M L<sup>-1</sup>).

## Phytoplankton assemblage composition

During the study period, a total of 26 phytoplankton taxa, including 11 diatoms (Bacillariophyceae), 13 dinoflagellates (Dinophyceae), and 1 species of Raphidophyceae and Dictyochophyceae were identified. In the phytoplankton community, diatoms were the most abundant group, accounting on average for 77% of the total cell concentration of phytoplankton. Besides, dinoflagellates comprised only 8% of the community, and other phytoplankton groups (such as Raphidophyceae, Dictyochophyceae, and the unidentified phytoplankton), altogether accounted for 15% of the total cell concentration of phytoplankton (Table 1).

In terms of percentage composition, nine phytoplankton species such as *Pseudo-nitzschia* sp. (4,411 cells mL<sup>-1</sup>, 20.6%), *Heterosigma akashiwo* (3,026 cells mL<sup>-1</sup>, 14.2%), *Thalassiosira* sp. 2 (3,013 cells mL<sup>-1</sup>, 14.1%), *Thalassionema frauenfeldii* (2,226 cells mL<sup>-1</sup>, 10.4%), *T. nitzschioides* (2,158 cells mL<sup>-1</sup>, 10.1%), *Skeletonema* sp. (1,316 cells mL<sup>-1</sup>, 6.2%), *Lauderia annulata* (1,235 cells mL<sup>-1</sup>, 5.8%), *Thalassiosira* sp. 1 (882 cells mL<sup>-1</sup>, 4.1%) and *Chaetoceros* sp. (855 cells mL<sup>-1</sup>, 4.0%), were noted as the dominant species and the percentage of their abundance comprised 89.5% of the total cell concentration of phytoplankton (Table 1).



Figure 4 Day and night vertical distributions of total phytoplankton and Chl-a concentration.

Table 1 A list of phytoplankton in	Tokyo	Bay	with	total	abundance	(cells	mL <sup>-1</sup> )	and
percentage composition (%).								

Phytoplankton	Total abundance					
	cells mL <sup>-1</sup>	%				
Bacillariophyceae						
Lauderia annulata	1,235	5.8				
Skeletonema sp.	1,316	6.2				
Thalassiosira sp. 1	882	4.1				
Thalassiosira sp. 2	3,013	14.1				
Coscinodiscus sp.	55	0.3				
Chaetoceros sp.	855	4.0				
Thalassionema frauenfeldii	2,226	10.4				
Thalassionema nitzschioides	2,158	10.1				
Meuniera membranacea	54	0.3				
Pleurosigma sp.	162	0.8				
Pseudo-nitzschia sp.	4,410	20.6				
Dinophyceae						
Prorocentrum minimum	272	1.3				
P. micans	108	0.5				
Dinophysis sp.	135	0.6				
<i>Gyrodinium</i> sp.	109	0.5				
Ceratium furca	502	2.3				
C. fusus	68	0.3				
C. tripos	14	0.1				
Gymnodinium mikimotoi	14	0.1				
Oxyphysis oxytoxoides	54	0.3				
Pyrophacus sp.	27	0.1				
Protoperidinium oblongum	41	0.2				
P. quinquecorne	95	0.4				
Scrippsiella trochoidea	366	1.7				
Raphidophyceae						
Heterosigma akashiwo	3,027	14.2				
Dictyochophyceae						
Dictyocha speculum	41	0.2				
Unidentified phytoplankton	136	0.6				

#### Species-specific distributions of dominant phytoplankton

Fig. 5 showed species-specific patterns in the vertical distributions of nine dominant phytoplankton species during day-and-night times. In general, the samples of these dominant phytoplankton species showed higher cell concentrations during the night than day. Among these species, the cell concentrations of *Pseudo-nitzschia* sp. and *H. akashiwo* increased at night, approximately 4 to 8 times higher than in the daytime. At the most depth range, all species had higher cell concentrations in the upper layer (0-8 m) coinciding with the Chl-a maximum. Below it, their abundance significantly decreased and was quite steady at the greater depths, suggesting these species reveals distinct depth habitats. However, the variability does not markedly affect the pattern of diel vertical migration because most dominant phytoplankton species showed almost similar patterns in the vertical distributions between day and night. Out of these, some species such as Thalassionema frauenfeldii, T. nitzschioides, and Skeletonema sp. exhibited distinct vertical distribution patterns between day and night. In the daytime, Thalassionema frauenfeldii had a fairly even vertical distribution in the water column, but they concentrated in the upper layer (0-5 m) during the night. T. nitzschioides were found throughout the water column during the day; however, they were mainly distributed in the upper layer (0-5 m) during the night and were not observed below 10 m depth. Skeletonema sp. distributed almost at all sampling depths during the day, but they concentrated in the upper 0-5 m during the night and did not occur below 6 m depth.

## Discussion

The spatial and temporal variations of the phytoplankton community in the coastal waters are governed by the physicochemical properties of the water column such as water stratification, vertical mixing, temperature and salinity gradients, and nutrient supply (Pennock, 1985). In the present study, the salinity gradient was pronounced at the upper layer (10 m) due to the influence of freshwater discharge from rivers (Horie et al., 2010). Dealing with this characteristic of water properties, a strong stratification developed in the water column, which in turn influenced the vertical distribution pattern of phytoplankton. Sprintall and Cronin (2001) reported water mass stability or formation of pycnocline which may act as a barrier in the water column, where the sedimentation of particles may be prevented by its marked density gradients. Therefore, in the present study, the vertical distribution of phytoplankton was significantly different above and below the pycnocline (see Figs. 4. a, b).



**Figure 5** Species-specific vertical distribution of dominant phytoplankton (cells mL<sup>-1</sup>) during day and night.

During the study period, diatoms were found to be the most dominant group in the phytoplankton community, accounting for 77% of the total cell concentration of phytoplankton. This result was consistent with the previous finding by Nakane et al. (2008) who reported the diatoms encompassed a major component (84.4%) in the phytoplankton community in inner Tokyo Bay. It was suggested that diatoms can grow rapidly in eutrophic conditions (Tada et al., 2009; Guo et al., 2014) because Tokyo Bay (especially the inner section) is one of the most eutrophic embayments in Japan (Furukawa, 2015). In terms of cell concentration, *Lauderia annulata, Skeletonema* sp., *Thalassiosira* sp. 1 and 2, *Chaetoceros* sp., *Thalassionema frauenfeldii, T. nitzschioides, Pseudo-nitzschia* sp. and *Heterosigma akashiwo* were noted as the dominant species in the community. All these species had higher cell concentrations in the upper layer (10 m) but significantly decreased below 10 m depth (Fig. 5). These distinct depth habitats may deal with the presence of pycnocline which can restrict the phytoplankton distribution. It was further considered that the nutrient concentration was higher in the upper layer due to river discharge which could give favorable conditions for their abundance.

*T. frauenfeldii*, *T. nitzschioides*, and *Skeletonema* sp. showed distinct patterns in their vertical distributions between day and night. They were found throughout the water column during the day but were not observed below 10 m depth during the night with exception of *T. frauenfeldii*, which was detected below 10 m depth at night (but with low concentration) (see Fig. 5). This may due to the increase of the bottom hypoxic water layer (10 m thick) at night, which can limit the distribution of phytoplankton (Rabalais *et al.*, 2010). Moreover, a significant increase in the cell concentrations of *Pseudo-nitzschia* sp. and *H. akashiwo* were observed in the upper layer at night. This may probably be related to the changes in the water mass.

# Conclusion

The fine-scale spatial and temporal distributions of phytoplankton were observed in the inner Tokyo Bay. The vertical profiles of the most dominant phytoplankton species did not show day and night differences in their distribution patterns; however, each species occupied well-defined depth habitats. Large variability in the vertical distribution and concentration of phytoplankton was resulting from the presence of pycnocline, which acts as a barrier in the water column, and the nutrient loads from freshwater discharge from rivers in the upper layers that might increase the cell concentration of phytoplankton. On the other hand, the water mass tends to become hypoxic below the pycnocline which might limit the distribution of phytoplankton. Therefore, it was noted that changes in the vertical distribution of phytoplankton were strongly influenced by the strength and thickness of the pycnocline and the associated hydrodynamic properties.

# Acknowledgments

The author's sincere thanks are extended to Dr. Khin Maung Zaw, Rector of Sittway University, and Dr. Khin Thet Kyaw, Pro-Rector of Sittway University, for their permission to submit this research paper. I wish to express my sincere thanks to Dr. Mya Kyawt Wai, Professor (Head), of the Department of Marine Science, Sittway University for her kind suggestions in preparing the manuscript. Special thanks are deeply indebted to the academic supervisor Professor Dr. Yuji Tanaka and co-supervisor Dr. Takuo Omura for their kind supervision and supportive help throughout the research. Many thanks are also to the Laboratory of Aquatic Science Consultant Co., Ltd. which provided the use of a FlowCAM and lab facilities, the captains, and crew members of "Hiyodori" and students of Plankton Lab who helped during field sampling. This research was partially supported by the Monbukagakusho Scholarship for Khin Khin Gyi and also by JSPS KAKEN-HI Grant Numbers JP18H02263 for Yuji Tanaka.

#### References

- Blauw, A. N., E. Benincà, R. W. P. M. Laane, N. Greenwood and J. Huisman (2012): Dancing with the tides: fluctuations of coastal phytoplankton orchestrated by different oscillatory modes of the tidal cycle. *PLoS ONE*, 7, e49319-e49319.
- Bouman, H. A., T. Nakane, K. Oka, K. Nakata and K. Kurita (2010): Environmental controls on phytoplankton production in coastal ecosystems: a case study from Tokyo Bay. *Estuar. Coast. Shelf Sci.*, 87, 63-72.
- Furukawa, K. (2015): Eutrophication in Tokyo Bay. In Yanagi, T. (Ed.), Eutrophication and oligotrophication in Japanese estuaries: the present status and future tasks. Springer Science +Business Media Dordrecht, pp. 5-38.
- Furukawa, K. and T. Okada (2006): Tokyo Bay: its environmental status- past, present, and future. *In* Wolanski, E. (Ed.), *The environment in Asia Pacific harbours*. Springer, Dordrecht, pp. 15-34.
- Gast, L., A. N. Moura, M. C. P. Vilar, M. K. Cordeiro-Araújo and M. C. Bittencourt-Oliveira (2014): Vertical and temporal variation in phytoplankton assemblages correlated with environmental conditions in the Mundaú reservoir, semi-arid north-eastern Brazil. *Braz. J. Biol.*, 74, 93-102.
- Guo, S., Y. Feng, L. Wang, M. Dai, Z. Liu, Y. Bai and J. Sun (2014): Seasonal variation in the plankton community of a continental-shelf sea: The East China Sea. *Mar. Ecol. Prog. Ser.*, 516, 103-126.
- Han, M.-S. (1988): Studies on the population dynamics and photosynthesis of phytoplankton in Tokyo Bay. Ph.D. thesis. The University of Tokyo. 172 pp.
- Han, M.-S. and K. Furuya (2000): Size and species-specific primary productivity and community structure of phytoplankton in Tokyo Bay. J. Plankton Res., 22, 1221-1235.
- Horie, T., K. Furukawa, T. Okada (2010): Spatial distribution of hypoxic water mass based on a monitoring campaign of bay environment at Tokyo Bay, Japan. Ann. J. Civ. Eng. Ocean, 32, 1-10.
- Khin Khin Gyi (2020): Short-term changes in the vertical distribution of phytoplankton communities in response to tidal fluctuations in Tateyama Bay, Japan. *Mawlamyine University Research Journal*, 12(2): 324-334.

- Koibuchi, Y. and M. Isobe (2007): Phytoplankton bloom mechanism in an area affected by eutrophication: Tokyo Bay in spring 1999. *Coast. Eng. J.*, 49, 461-479.
- Maznah, W. O. W., S. Rahmah, C. C. Lim, W. P. Lee, K. Fatema and M. M. Isa (2016): Effects of tidal events on the composition and distribution of phytoplankton in Merbok river estuary Kedah, Malaysia. *Trop. Ecol.*, 57, 213-229.
- Mellard, J. P., K. Yoshiyama, E. Litchman and C. A. Klausmeier (2011): The vertical distribution of phytoplankton in stratified water columns. *J. Theor. Biol.*, 269, 16-30.
- Nakane, T., K. Nakaka, H. Bouman and T. Platt (2008): Environmental control of short-term variation in the plankton community of inner Tokyo Bay, Japan. *Estuar. Coast. Shelf Sci.*, 78, 796-810.
- Pennock, J. R. (1985): Chlorophyll distributions in the Delaware Estuary: regulation by light-limitation. *Estuar. Coast. Shelf Sci.*, 21, 711-725.
- Rabalais, N. N., R. J. Díaz, L. A. Levin, R. E. Turner, D. Gilbert and J. Zhang (2010): Dynamics and distribution of natural and human-caused hypoxia, *Biogeosciences*, 7, 585-619.
- Sprintall, J. and M. F. Cronin (2001): Upper Ocean vertical structure. *In* Steele, J. H., S. A. Thorpe and K. K. Turekian (Eds.), *Encyclopedia of ocean science*. San Diego: Academic Press, 6, pp. 3120-3129.
- Tada, K., M. Suksomjit, K. Ichimi, Y. Funaki, S. Montani, M. Yamada and P. J. Harrison (2009): Diatoms grow faster using ammonium in rapidly flushed eutrophic Dokai Bay, Japan. J. Oceanogr., 65, 885-891.
- Tilzer, M. M. and C. R. Goldman (1978): Importance of mixing, thermal stratification and light adaptation for phytoplankton in Lake Tahoe (California, Nevada). *Ecology*, 59, 810-821.