



Seasonal variation in alkaline phosphatase activity in the central part of Tokyo Bay, 2013

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Abstract

In surface water of the eutrophic Tokyo Bay, the dissolved inorganic phosphorus (DIP) concentration has decreased in recent decades. DIP is generally utilized by phytoplankton; however, under low DIP conditions, phytoplankton utilize dissolved organic phosphorus using alkaline phosphatase, together with bacteria. This study investigated seasonal variation in alkaline phosphatase activity (APA) in the central part of Tokyo Bay during 2013 to understand the P regime in recent years. Water samples were collected monthly from surface (0 m) and bottom (20–25 m) layers at two stations and were filtered through 0.2, 0.7, and 190 μm -pore-sized filters to examine APA in different fractions, approximately corresponding to dissolved components ($<0.2 \mu\text{m}$), bacteria (0.2–0.7 μm), and phytoplankton (0.7–190 μm). APA was assayed by a fluorometric method with the addition of 4-methylumbelliferyl-phosphate. Potential maximum APA in total fraction ranged from the undetectable level to 2117 $\text{nmol L}^{-1} \text{h}^{-1}$. The APA in the phytoplankton fraction showed a large contribution to that in the total fraction ($69 \pm 37\%$), followed by that in bacterial ($17 \pm 32\%$) and dissolved ($14 \pm 28\%$) fractions. The total APA was generally higher in the surface layer than in the bottom layer and peaked at the surface in July. Past data on the total APA in the central part of Tokyo Bay also showed peaks at the surface during summer. However, the total APA at the surface in July 2013 was 1–2 orders of magnitude higher than that in July 1973, suggesting that phytoplankton DIP stress has considerably emerged in recent years.

Keywords Alkaline phosphatase · Phosphorus · Phytoplankton · Seasonal variation · Tokyo Bay

1 Introduction

Phosphorus (P) is one of the vital elements for life. In marine environments, the dissolved P pool consists of both inorganic and organic forms. Generally, autotrophic phytoplankton take up dissolved inorganic P (DIP), which mostly consists of orthophosphate. However, under DIP-depleted conditions, dissolved organic phosphorus (DOP) can be utilized by phytoplankton, together with heterotrophic bacteria, using alkaline phosphatase (AP) (Hoppe 2003; Kuenzler and Perras 1965). AP is an enzyme that is located on the microbial cell surface and catalyzes the hydrolysis

of organic phosphate monoester. With the fluorometric or spectrophotometric method using an artificial phosphate monoester substrate, potential maximum AP activity (APA) has been measured in various marine environments, such as coastal regions (e.g. Hashimoto et al. 1985; Huang and Hong 1999; Ivančić et al. 2010; Kwon et al. 2011; Labry et al. 2005; Taga and Kobori 1978; Yamaguchi et al. 2004; Yoshimura and Kudo 2011), oligotrophic open ocean (e.g. Duhamel et al. 2011; Mather et al. 2008; Perry 1972; Sato et al. 2013; Suzumura et al. 2012; Yamaguchi et al. 2019), and upwelling regions (Sebastián et al. 2004a). These data indicate that the volumetric APA is significantly enhanced in DIP-depleted conditions ($<0.03 \mu\text{mol L}^{-1}$) as seen in the oligotrophic open ocean (reviewed by Mahaffey et al. 2014). In DIP-replete coastal and upwelling regions, significant APA is generally not observed despite abundant microbial biomass. However, even in these regions, there are several lines of evidence indicating that seasonal or intermittent DIP

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depletion enhances the APA (Ivančić et al. 2010; Labry et al. 2005; Sebastián et al. 2004a; Yoshimura and Kudo 2011).

Tokyo Bay is characterized as a semi-enclosed system and eutrophic area. After a Japanese water pollution control law was enacted in 1970, nutrient loading in the bay has been controlled and its concentration through the water column of the bay has decreased gradually (Ando et al. 2021; Kubo et al. 2019). In 1970s and early 1980s, DIP concentrations in surface water of the central part of Tokyo Bay were frequently greater than $1 \mu\text{mol L}^{-1}$ (Miyata and Hattori 1986; Taga and Kobori 1978). However, after 2000, DIP concentrations below the detection limit ($0.05 \mu\text{mol L}^{-1}$) of standard analytical method were occasionally observed in the surface water during spring and summer periods (Kubo et al. 2019). Molar ratios of nitrogen to P in dissolved inorganic nutrients in the central part of the bay were mostly greater than the Redfield ratio of 16 for the period from the 1980s to recent years (Kubo et al. 2019; Miyata and Hattori 1986). These results imply that phytoplankton DIP stress is severe in recent Tokyo Bay. However, primary productivity in the central part of the bay has not been severely limited by ambient nutrients, even after 1997 (Bouman et al. 2010; Hamana et al. submitted to this special section). For the period from 2006 to 2015, surface DOP concentrations in the central part of the bay had significantly decreased, whereas the surface DON concentrations did not show such a trend (Kubo et al. 2019), suggesting that the selective utilization of DOP by phytoplankton has sustained their productivity in recent years.

In this study, we investigated seasonal variation in APA and associated environmental variables in the central part of Tokyo Bay during 2013. Monthly, we conducted fluorometric APA assay with the single addition of excess 4-methylumbelliferyl phosphate (MUF-P) as an artificial substrate (Hoppe 1983; Sebastián and Niell 2004) to obtain time-series data on the potential maximum APA (hereafter referred to as “APA”). Furthermore, in summer and winter, we conducted the APA assay with the addition of multiple different MUF-P concentrations to obtain kinetic parameters of K_m (half-saturation constant), V_{max} (maximum hydrolysis rate), and K_m/V_{max} (potential turnover time), as sensitive indicators of DIP stress (Labry et al. 2005; Sato et al. 2013). The APA in ambient coastal waters includes that of phytoplankton, bacteria, and dissolved components, and the proportion of phytoplankton APA to total APA tends to be low in DIP-replete eutrophic conditions (Ivančić et al. 2010; Labry et al. 2005; Yoshimura and Kudo 2011). Given the low DIP concentrations in surface water of the recent Tokyo Bay (Kubo et al. 2019), the proportion of phytoplankton APA might be larger at present than in the past. Thus, we also examined the APA in three size fractions approximately corresponding to phytoplankton, bacteria, and dissolved components. The obtained results in the present study were

compared to past P regimes of Tokyo Bay (Hashimoto et al. 1985; Taga and Kobori 1978), and the importance of APA in the recent P regime was evaluated.

2 Materials and methods

2.1 Time-series observation

Field observations were conducted monthly from January to December 2013 on the cruises of the training vessel *Seiyō-Maru* of the Tokyo University of Marine Science and Technology. Seawater samples were collected from the surface (0 m) and bottom (20–25 m) layers at Stations F3 and F6 (Fig. 1) in the central part of Tokyo Bay, using 8 L Niskin bottles mounted on a conductivity-temperature-depth (CTD) rosette system (Falmouth Scientific Inc.). Station F3 is closer to the mouth of the Tamagawa River and more influenced by fresh water than Station F6. Both stations generally possess a seasonal water column structure with summer stratification and winter mixing as described in Nakano et al. (submitted to this special section). The bottom layers at both stations were located at the depth of ~5 m above from the seafloor. According to data on photosynthetically active radiation (PAR) measured by a Biospherical sensor, the bottom layer was regarded as the aphotic zone (< 1% PAR relative to surface PAR) throughout the seasons. Vertical profiles of temperature, salinity, and density (σ_t) were obtained from CTD observations. Using the density profile, mixed layer depth (MLD) was determined as the depth where the σ_t was 0.2 kg m^{-3} greater than that at 2 m depth.

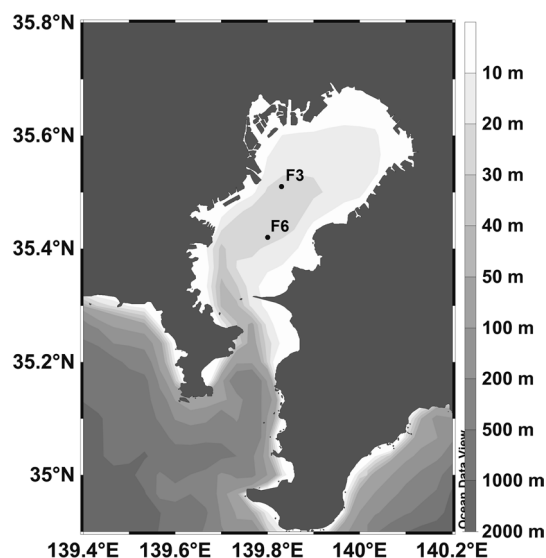


Fig. 1 The two sampling stations in the central part of Tokyo Bay. Background contour shows seafloor bathymetry

2.2 Chlorophyll *a* analysis

Seawater samples for chlorophyll *a* (Chl *a*) analysis were filtered through 25 mm-diameter Whatman GF/F filters (0.7 μm pore size). Pigments on the filters were extracted in *N,N*-dimethylformamide (Suzuki and Ishimaru 1990) and were kept at $-20\text{ }^{\circ}\text{C}$ until analysis ashore. Chl *a* concentrations in the pigment extracts were measured with a Turner Designs 10-AU fluorometer (Strickland and Parsons 1972).

2.3 Determinations of dissolved P pools

Seawater samples for DIP and total dissolved P (TDP) analyses were filtered through cellulose acetate membrane filters (0.2 μm -pore size, DISMIC-25CS, ADVANTEC). The filtrates for DIP and TDP analyses were frozen at $-20\text{ }^{\circ}\text{C}$ until analysis ashore. Concentrations of DIP were determined with a QuAatro-Marine 5 channel autoanalyzer (BLTEC) based on molybdenum blue spectrophotometry with a detection limit ($3\times$ standard deviation of blank) of $0.05\text{ }\mu\text{mol L}^{-1}$ (Kubo et al. 2019). Concentrations of TDP were determined with a QuAatro TN-TP autoanalyzer (BLTEC) based on persulfate oxidation and molybdenum blue spectrophotometry with a detection limit ($3\times$ standard deviation of blank) of $0.04\text{ }\mu\text{mol L}^{-1}$ (Yasui-Tamura et al. 2020). DOP concentrations were calculated by subtracting concentrations of DIP from those of TDP.

2.4 APA assay

Seawater samples for APA assays were filtered through 190 μm -pore-sized nylon meshes, 0.7 μm -pore-sized GF/F filters, and 0.2 μm -pore-sized DISMIC-25CS filters to obtain three size-fractionated filtrates. APA was measured by a fluorometric method with a single addition of $200\text{ }\mu\text{mol L}^{-1}$ MUF-P (Sigma) to the filtrates. The stock solution of MUF-P at 20 mmol L^{-1} in 2-methoxyethanol (Wako) was stored at $-20\text{ }^{\circ}\text{C}$ and diluted with autoclaved-filtered seawater that was prepared by autoclaving ($120\text{ }^{\circ}\text{C}$ for 30 min) surface water at the mouth of Tokyo Bay (35.053°N , 139.740°E in October 2012) and passing it through an ultrafiltration membrane with a nominal molecular weight cut-off of 5000 Da (Vivaflow 200, Vivascience). The autoclaved-filtered seawater was also used for an experimental control. The filtrates with MUF-P addition were incubated for 9 h in the dark at ambient temperature within $\pm 1.5\text{ }^{\circ}\text{C}$. At 0, 3, 6, and 9 h, 4-methylumbelliferone (MUF), as formed through the hydrolysis of MUF-P (Hoppe 1983), was measured using a Turner Designs TD-700 fluorometer with excitation at 300–400 nm and emission at 410–600 nm. The concentrations of MUF were calculated from a standard curve of MUF (Sigma). The coefficient of variation of MUF signals at $200\text{ }\mu\text{mol L}^{-1}$ level was 3%

($n=6$). The APA was determined from the positive slope of the regression line ($r^2 \geq 0.845$, $p < 0.05$) of the MUF concentrations against incubation times. Because the regression lines with $r^2 < 0.845$ included weak negative and insignificant ($p > 0.05$) slopes, these were regarded as $0\text{ nmol L}^{-1}\text{ h}^{-1}$ in this study. The APA in the $< 190\text{ }\mu\text{m}$ fraction was referred to as total APA, whereas the APA in the $< 0.2\text{ }\mu\text{m}$ fraction was regarded as dissolved APA. As the APA in the $< 0.7\text{ }\mu\text{m}$ fraction approximately corresponds to that in bacteria plus dissolved components, we estimated bacterial APA by subtracting the APA in the $< 0.2\text{ }\mu\text{m}$ fraction from that in the $< 0.7\text{ }\mu\text{m}$ fraction. Phytoplankton APA was estimated by subtracting the APA in the $< 0.7\text{ }\mu\text{m}$ fraction from total APA. By dividing the phytoplankton APA based on the Chl *a* concentration, phytoplankton-specific APA was estimated.

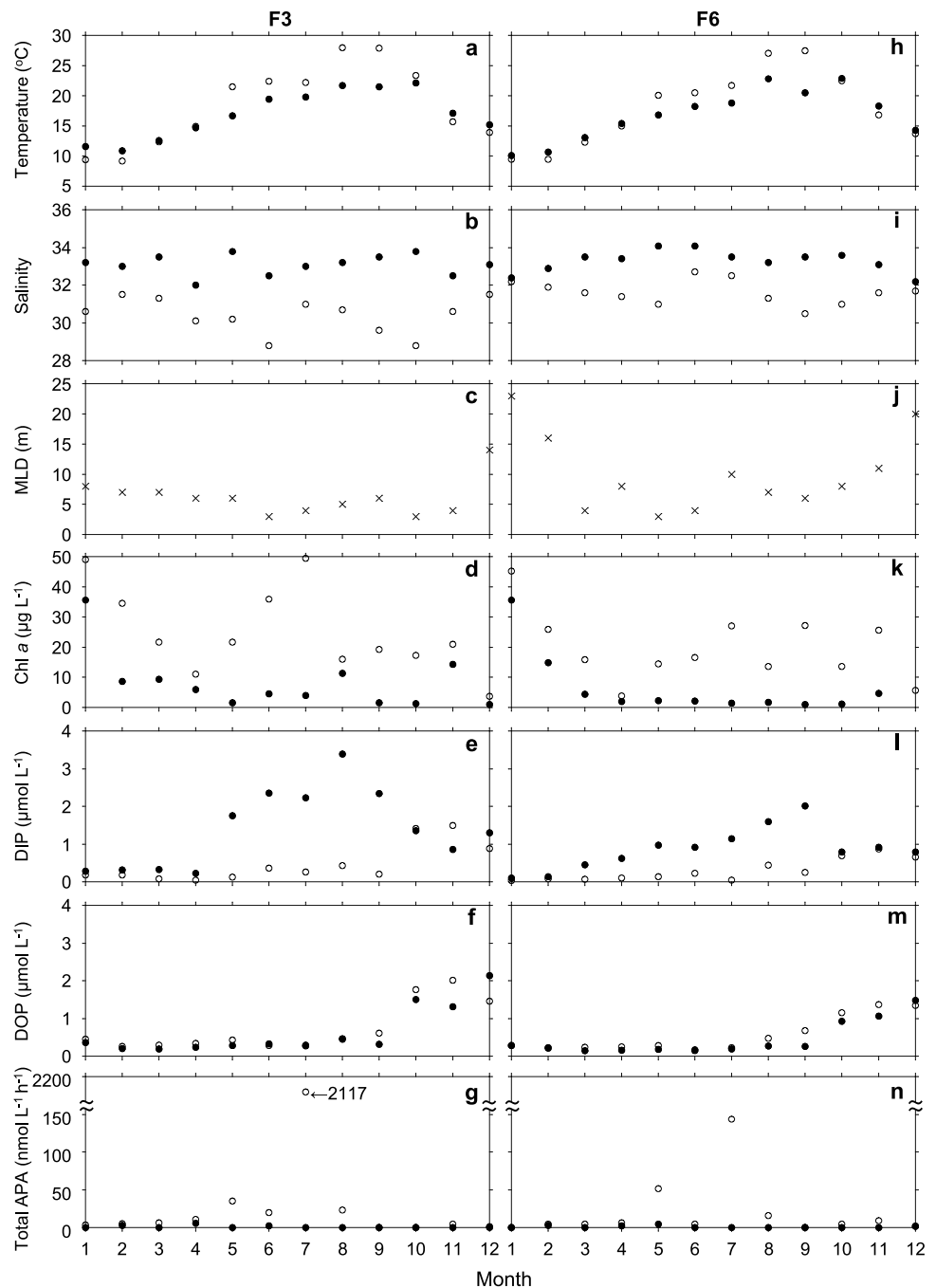
In addition to the APA, kinetic parameters of V_{max} and K_{m} were determined based on the addition of multiple different MUF-P concentrations (0.02, 0.2, 1, 2, 20, 100, and $200\text{ }\mu\text{mol L}^{-1}$) to seawater in the $< 190\text{ }\mu\text{m}$ (total) fraction in May, June, July, November, and December. Each APA with multiple MUF-P additions was estimated in the same manner as that with a single MUF-P addition, mentioned previously herein. Using the multiple APA data, the V_{max} and K_{m} were calculated using the Michaelis–Menten equation and Lineweaver–Burk plot (Suzumura et al. 2012) and were accepted when the plots showed regression lines with $r^2 \geq 0.932$ ($p < 0.05$). Potential turnover time was also calculated by dividing K_{m} by V_{max} (Labry et al. 2005; Sato et al. 2013).

3 Results

3.1 Temperature, salinity, MLD, and Chl *a*

At two stations, temperature varied from 9.2 to $28.0\text{ }^{\circ}\text{C}$ in the surface and bottom layers throughout the study period (Fig. 2a, h). Temperature in the surface layer was maintained at more than $20\text{ }^{\circ}\text{C}$ from May to October, and it was consistently higher than that in the bottom layer, except at Station F6 in October. For the periods from January to April, November, and December, a similar temperature was observed in the two layers. Salinity varied from 28.8 to 34.1 at two stations and was lower in the surface layer than in the bottom layer (Fig. 2b, i). Low salinity below 30 was occasionally observed at the surface at Station F3. A clear seasonal pattern in salinity was not observed. MLD ranged from 3 to 23 m (Fig. 2c, j). A well-mixed water column with $> 10\text{ m}$ MLD was observed in December at Station F3 and in January, February, November, and December at Station F6. Chl *a* concentrations ranged from 0.96 to $49.45\text{ }\mu\text{g L}^{-1}$ (Fig. 2d, k). The Chl *a* peaks ($\sim 49.5\text{ }\mu\text{g L}^{-1}$) were observed at the surface at both stations in January and July.

Fig. 2 Seasonal variations in temperature (**a, h**), salinity (**b, i**), MLD (**c, j**), Chl *a* (**d, k**), DIP (**e, l**), DOP (**f, m**), and total APA (**g, n**) at Stations F3 (*left panels*) and F6 (*right panels*) during 2013. *Blank circles* and *solid circles* indicate data from the surface and bottom layers, respectively. *Crosses* indicate MLD data



The Chl *a* concentrations in the bottom layer were consistently lower than those in the surface layer. Exceptionally high Chl *a* concentrations in the bottom layer ($> 30 \mu\text{g L}^{-1}$) were observed at both stations in January.

3.2 Dissolved P pools

DIP concentrations ranged from < 0.05 to $3.39 \mu\text{mol L}^{-1}$ (Fig. 2e, l). Surface DIP concentrations were consistently lower than $0.50 \mu\text{mol L}^{-1}$ from January to September, and concentrations below the detection limit ($0.05 \mu\text{mol L}^{-1}$)

were observed at Station F3 in April and at Station F6 in January and July. For the period between October and December, surface DIP concentrations were moderate (0.66 – $1.50 \mu\text{mol L}^{-1}$). DIP concentrations in the bottom layer were higher than those in the surface layers, especially from May to September. DOP concentrations varied from 0.10 to $2.14 \mu\text{mol L}^{-1}$ (Fig. 2f, m). Unlike DIP, DOP was not depleted to the detection limit level ($0.04 \mu\text{mol L}^{-1}$). At both stations, DOP concentrations in the surface and bottom layers showed a similar seasonal pattern and

were consistently low between January and September, which increased from October to December.

3.3 APA

APA in the total fraction showed a large variation of $\sim 2117 \text{ nmol L}^{-1} \text{ h}^{-1}$ (Fig. 2g, n). A paired *t* test showed that the total APA at Station F3 was not significantly different from that at Station F6 ($p > 0.05$). The total APA in the surface layer were higher between May and August ($> 16 \text{ nmol L}^{-1} \text{ h}^{-1}$, except in June at Station F6) than in other months ($< 11 \text{ nmol L}^{-1} \text{ h}^{-1}$), whereas that in the bottom layer was consistently lower than $6 \text{ nmol L}^{-1} \text{ h}^{-1}$. The highest peaks of surface total APA were observed at both stations in July ($2117 \text{ nmol L}^{-1} \text{ h}^{-1}$ at Station F3 and $144 \text{ nmol L}^{-1} \text{ h}^{-1}$ at Station F6). APA values in phytoplankton, bacteria, and dissolved fractions were ~ 2005 , ~ 94 , and $\sim 18 \text{ nmol L}^{-1} \text{ h}^{-1}$, respectively (Fig. 3a–d). At the surface, the APA in each fraction tended to be high at both stations between May and August. Throughout the study period, the phytoplankton APA showed a large contribution to the total APA ($69 \pm 37\%$, mean \pm standard deviation), followed by the bacterial APA ($17 \pm 32\%$) and the dissolved APA ($14 \pm 28\%$).

At the peaks of the total APA at the surface at Stations F3 and F6 in July, the phytoplankton APA accounted for 95 and 81%, respectively. Phytoplankton-specific APA ranged from 0 to $40.5 \text{ nmol } \mu\text{g}^{-1} \text{ h}^{-1}$ (Fig. 4a, b). Seasonal pattern of phytoplankton-specific APA was similar to that of the phytoplankton APA, with the highest peaks at the surface in July. Although most phytoplankton-specific APA was lower than $1 \text{ nmol } \mu\text{g}^{-1} \text{ h}^{-1}$, higher values ($> 1 \text{ nmol } \mu\text{g}^{-1} \text{ h}^{-1}$) were frequently observed in the surface and bottom layers between April and July.

3.4 Kinetic parameters

Kinetic parameters could be determined for nine and four samples in the surface and bottom layers, respectively (Table 1). The K_m ranged from 0.35 to $30.0 \mu\text{mol L}^{-1}$. Most of the K_m values were lower than $6 \mu\text{mol L}^{-1}$, except at the surface at Stations F3 and F6 in November ($> 10 \mu\text{mol L}^{-1}$). V_{\max} varied from 1 to $172 \text{ nmol L}^{-1} \text{ h}^{-1}$. Whereas the V_{\max} values were often lower than $10 \text{ nmol L}^{-1} \text{ h}^{-1}$, high V_{\max} values ($\geq 28 \text{ nmol L}^{-1} \text{ h}^{-1}$) were observed at the surface from May to July, except at Station F6 in June. Potential turnover times varied from 14 to 3773 h. Short turnover

Fig. 3 Seasonal variations in APA in phytoplankton, bacterial, and dissolved fractions in the surface (a, c) and bottom (b, d) layers at Stations F3 (left panels) and F6 (right panels) during 2013

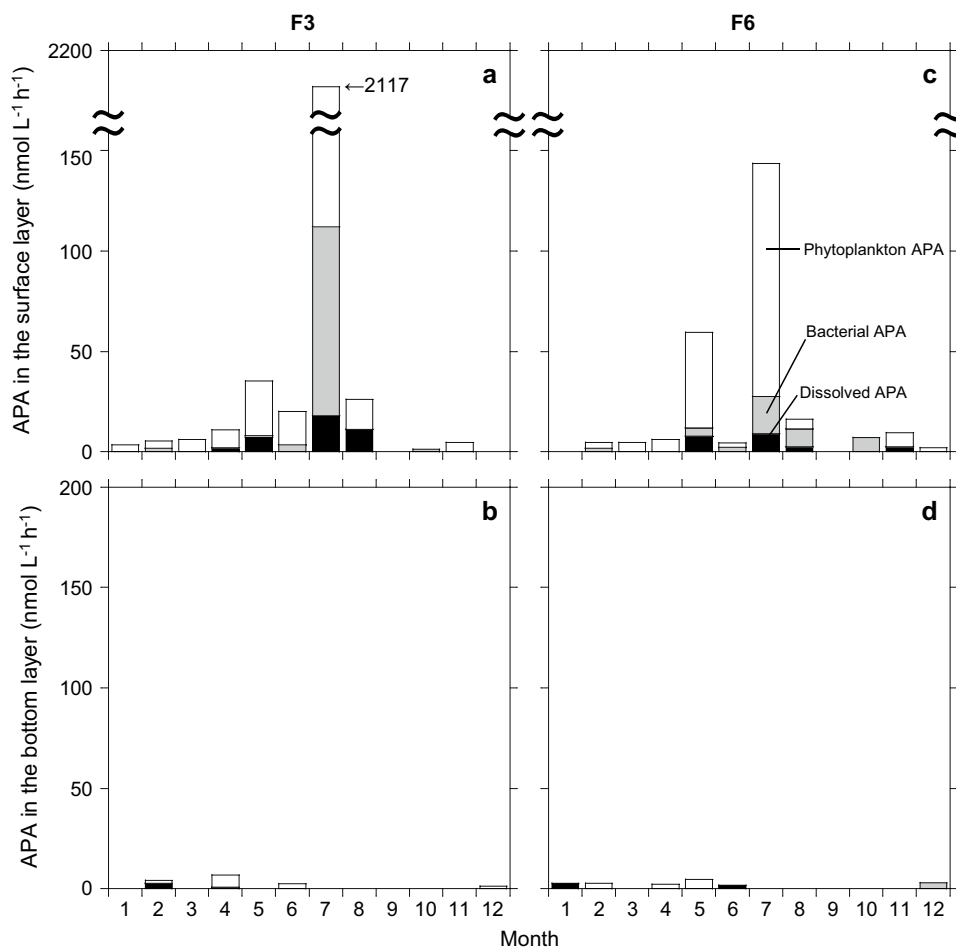


Fig. 4 Seasonal variation in phytoplankton-specific APA in the surface (*blank circles*) and bottom (*solid circles*) layers at Stations F3 (**a**) and F6 (**b**) during 2013

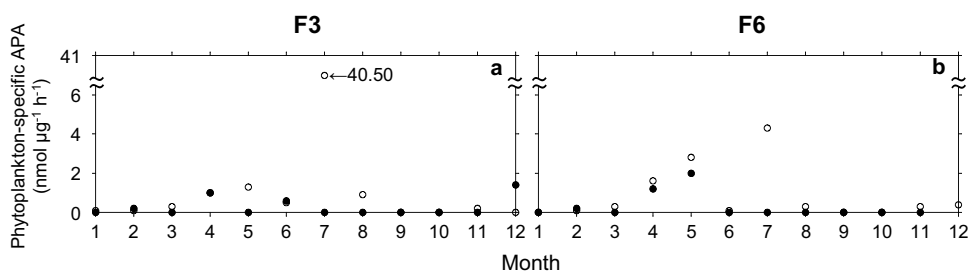


Table 1 Kinetic parameters in the surface and bottom layers at two stations in May, June, July, November, and December 2013

Layer	Month	Station	Temperature (°C)	DIP ($\mu\text{mol L}^{-1}$)	K_m ($\mu\text{mol L}^{-1}$)	V_{max} ($\text{nmol L}^{-1} \text{h}^{-1}$)	Potential turnover time (h)
Surface	May	F3	21.5	0.13	0.75	52	14
	May	F6	20.1	0.14	nd	nd	nd
	June	F3	22.4	0.37	1.55	28	55
	June	F6	20.5	0.23	5.31	5	1054
	July	F3	22.2	0.26	3.95	172	23
	July	F6	21.7	0.05	2.21	95	23
	November	F3	15.7	1.50	30.0	8	3773
	November	F6	16.8	0.87	11.7	5	2344
	December	F3	13.9	0.88	4.20	2	1852
	December	F6	13.7	0.66	3.03	2	1527
Bottom	May	F3	16.7	1.75	1.03	3	331
	May	F6	16.8	0.97	nd	nd	nd
	June	F3	19.4	2.35	nd	nd	nd
	June	F6	18.2	0.92	nd	nd	nd
	July	F3	19.8	2.23	nd	nd	nd
	July	F6	18.8	1.14	nd	nd	nd
	November	F3	17.1	0.86	0.35	3	118
	November	F6	18.3	0.92	nd	nd	nd
	December	F3	15.2	1.30	0.78	1	532
	December	F6	14.3	0.79	1.89	2	952

nd not determined

times with <1 day (24 h) were observed in the surface layers at Station F3 in May and both stations in July.

4 Discussion

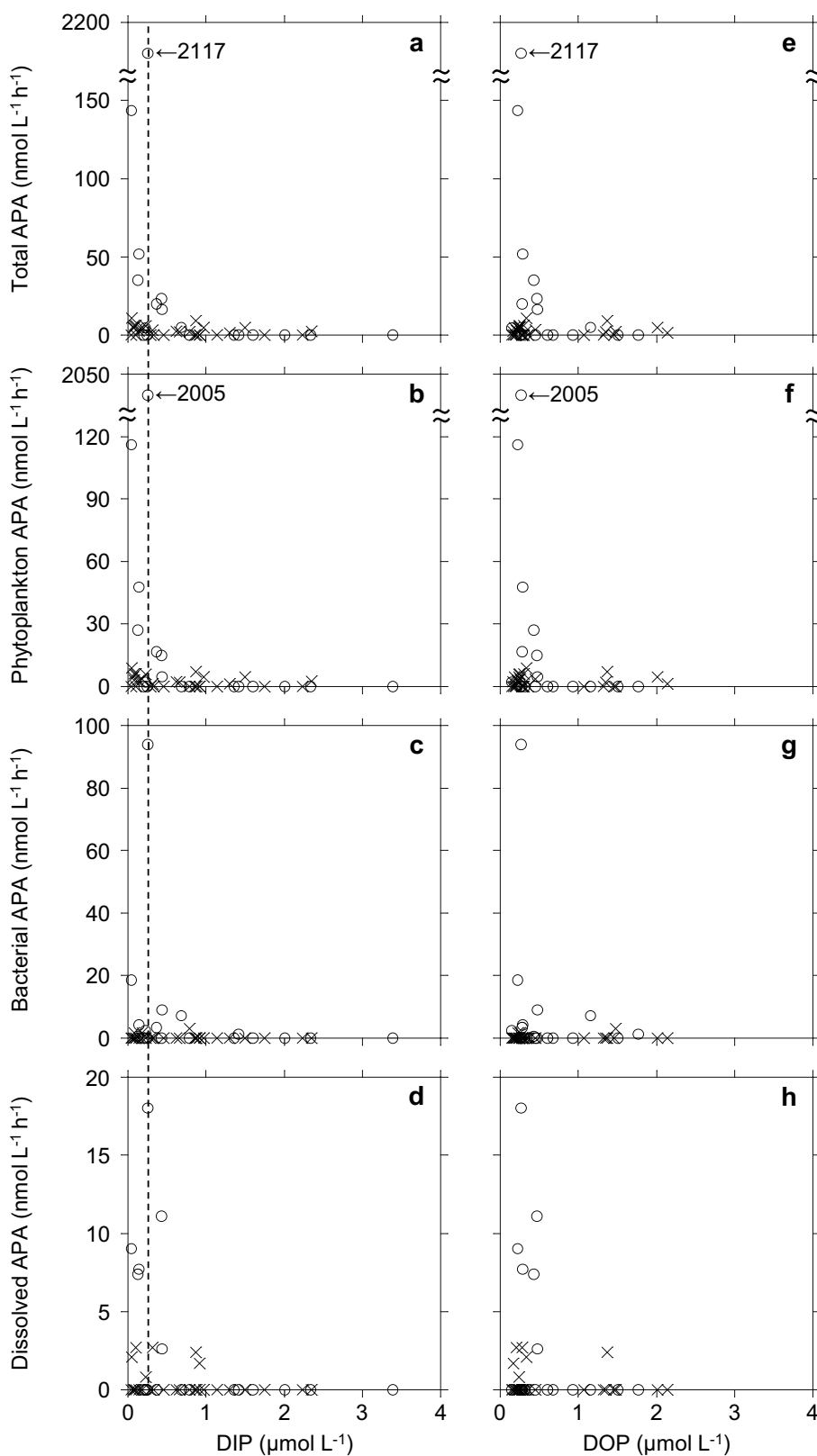
4.1 Seasonal variation in APA related to environmental variables

In the central part of Tokyo Bay during 2013, the total APA showed a large seasonal variation ranging from 0 to $2117 \text{ nmol L}^{-1} \text{ h}^{-1}$ (Fig. 2g, n). The highest values of the total APA at the surface at Stations F3 ($2117 \text{ nmol L}^{-1} \text{ h}^{-1}$) and F6 ($144 \text{ nmol L}^{-1} \text{ h}^{-1}$) in July were comparable to those in the Southeast Bay of Biscay ($\sim 400 \text{ nmol L}^{-1} \text{ h}^{-1}$, Labry et al. 2005), northern Adriatic Sea ($\sim 4640 \text{ nmol L}^{-1} \text{ h}^{-1}$,

Ivančić et al. 2010), and Funka Bay ($\sim 84 \text{ nmol L}^{-1} \text{ h}^{-1}$, Yoshimura and Kudo 2011), which were determined by a fluorometric method with excess MUF-P addition. These high values were all detected under low DIP conditions ($<0.3 \mu\text{mol L}^{-1}$), suggesting that DIP is an important factor controlling the total APA in coastal regions as demonstrated in oligotrophic open ocean (Mahaffey et al. 2014).

A scatter plot of total APA versus DIP concentrations in the present study showed that the total APA was enhanced at less than $0.3 \mu\text{mol L}^{-1}$ of DIP (Fig. 5a). This trend was also seen in the plots of phytoplankton APA (Fig. 5b), mainly owing to a large contribution of phytoplankton APA to total APA ($69 \pm 37\%$, Fig. 3). Furthermore, a similar trend against the DIP concentration was observed in the plots of bacterial and dissolved APA (Fig. 5b–d). These results indicate that along with phytoplankton, bacteria were stressed by low

Fig. 5 Scatter plots of APA in total (**a, e**), phytoplankton (**b, f**), bacterial (**c, g**), and dissolved (**d, h**) fractions against DIP (*left panels*) and DOP (*right panels*) in the present study. *Blank circles* and *crosses* indicate data with temperature of $>20\text{ }^{\circ}\text{C}$ and $<20\text{ }^{\circ}\text{C}$, respectively. A *dashed vertical line* in *left panels* indicates $0.3\text{ }\mu\text{mol L}^{-1}$ of DIP concentration



DIP in the study area, and the increase in dissolved APA was likely ascribed to active free AP derived from the DIP-stressed microbes, as the dissolved free AP is stable and

remains active for several days (Chrost et al. 1986; Li et al. 1998).

Although the ambient DIP concentration is an important factor controlling the APA, temperature may also influence the APA, because unlike salinity and MLD, temperature showed a similar seasonal pattern with the APA (Fig. 2). We observed low total APA ($< 11 \text{ nmol L}^{-1} \text{ h}^{-1}$, Fig. 2g, n) even under low DIP conditions ($< 0.5 \text{ } \mu\text{mol L}^{-1}$, Fig. 2e, l) at the surface from January to April. For this period, surface temperature was consistently lower than $20 \text{ }^\circ\text{C}$ (Fig. 5) with a decrease to $9.2 \text{ }^\circ\text{C}$ (Fig. 2a, h). Seasonal difference in surface temperature in the study area was approximately $20 \text{ }^\circ\text{C}$. According to AP Q_{10} values of 1.5–2.0 (Huber and Kidby 1984 and references therein), the total APA in winter would be 3–4 times lower than that in summer based on the temperature effect itself. This is reasonable when considering the total APA at the surface from January to April ($5 \pm 3 \text{ nmol L}^{-1} \text{ h}^{-1}$) and from May to August, except July ($25 \pm 16 \text{ nmol L}^{-1} \text{ h}^{-1}$). Exceptionally high values of the total APA at the surface in July ($144\text{--}2117 \text{ nmol L}^{-1} \text{ h}^{-1}$) were probably driven by a factor other than temperature. According to Ueno et al. (submitted to this special section), whereas *Skeletonema* spp., *Pseudo-nitzschia* spp., and *Thalassiosira* spp. were cosmopolitan species at Station F3 between 2003 and 2017, *Cylindrotheca closterium* was exceptionally dominant in the phytoplankton community at the surface in July 2013. Although the dominance of *C. closterium* was unclear at Station F6, the exceptionally high total APA might reflect their high AP expression.

In addition, DOP is essential for APA because of an AP substrate. All scatter plots of the APA in different fractions against DOP concentrations showed that the APA was high under low DOP conditions ($< 0.5 \text{ } \mu\text{mol L}^{-1}$), suggesting that active DOP hydrolysis maintained a low concentration of ambient DOP (Fig. 5e–h). During the cool period with an ambient temperature of $< 20 \text{ }^\circ\text{C}$, high APA was not observed even under low DOP conditions ($< 0.5 \text{ } \mu\text{mol L}^{-1}$, Fig. 5e–h). This relationship might be explained by less active DOP hydrolysis along with low DOP production owing to low temperature.

The APA is controlled by not only DIP stress and temperature but also microbial biomass. Surface Chl *a* concentrations in the central part of Tokyo Bay ($\sim 50 \text{ } \mu\text{g L}^{-1}$, Fig. 2d, k) are relatively high compared to those of low-DIP coastal regions ($\sim 5 \text{ } \mu\text{g L}^{-1}$, Labry et al. 2005; Yoshimura and Kudo 2011). At the surface at Stations F3 and F6 in July, Chl *a* concentrations were 49.5 and $27.1 \text{ } \mu\text{g L}^{-1}$, respectively (Fig. 2d, k) and likely contributed to the highest values of the total APA (Fig. 2g, n). However, surface phytoplankton-specific APA (Fig. 4a, b) showed a seasonal pattern similar to that of surface phytoplankton APA (Fig. 3a, c). Furthermore, surface phytoplankton-specific APA at Stations F3 ($40.5 \text{ nmol } \mu\text{g}^{-1} \text{ h}^{-1}$) and F6 ($4.3 \text{ nmol } \mu\text{g}^{-1} \text{ h}^{-1}$) in July

were comparable to Chl *a*-normalized APA in the low-DIP coastal regions ($5\text{--}300 \text{ nmol } \mu\text{g}^{-1} \text{ h}^{-1}$, Labry et al. 2005; Yoshimura and Kudo 2011) as well as in the oligotrophic open oceans (subtropical Atlantic: $29 \pm 2 \text{ nmol } \mu\text{g}^{-1} \text{ h}^{-1}$; subtropical Pacific: $4 \pm 1 \text{ nmol } \mu\text{g}^{-1} \text{ h}^{-1}$, Mahaffey et al. 2014). These results imply that the highest total APA at the surface in July was not solely attributed to biomass accumulation of the DIP-unstressed population. Most microbial cells at the surface in July would be severely stressed by low DIP and hence actively utilize ambient DOP, as seen in the low-DIP coastal regions and oligotrophic open ocean.

4.2 Evaluation of kinetic parameters

In contrast to that of the surface samples, most samples from the bottom layer did not fit the Michaelis–Menten curve, and hence, their K_m and V_{max} values were not determined (Table 1). This was due to unstable, low fluorescent signals of MUF in the incubated samples with different MUF-P concentrations. As phytoplankton biomass (Chl *a*) was lower in the bottom layer than in the surface layer (Fig. 2d, k), kinetic parameters in the bottom layer seem to be derived from a more complex community (phytoplankton and bacteria) than those in the phytoplankton-dominant surface layer. The unstable MUF signals in the bottom layer might be ascribed to different characteristics of AP in the complex community.

The range of K_m values in the present study ($0.35\text{--}30.0 \text{ } \mu\text{mol L}^{-1}$, Table 1) was larger than that in the coastal regions ($0.43\text{--}9.72 \text{ } \mu\text{mol L}^{-1}$, Labry et al. 2005; Ivančić et al. 2010) and DIP-depleted open ocean ($0.07\text{--}0.30 \text{ } \mu\text{mol L}^{-1}$, Sato et al. 2013; Suzumura et al. 2012). If excluding the K_m values at the surface in November ($> 11 \text{ } \mu\text{mol L}^{-1}$), the range ($0.35\text{--}5.31 \text{ } \mu\text{mol L}^{-1}$) is comparable to that in the coastal regions. The K_m values in the DIP-depleted open ocean were lower than those in this study and the coastal regions, suggesting that the DIP concentration is an important factor controlling the K_m . A scatter plot of K_m versus DIP concentrations in this study showed a significant positive relationship when excluding data from the bottom layer ($r^2 = 0.720$, $p < 0.05$, Fig. 6). This indicates that the surface microbial community has a high affinity for DOP under low DIP conditions. The K_m values in the bottom layer were relatively low ($0.35\text{--}1.89 \text{ } \mu\text{mol L}^{-1}$), even under high DIP conditions ($0.79\text{--}1.75 \text{ } \mu\text{mol L}^{-1}$). Labry et al. (2005) reported that K_m values were lower in the bacterial fraction ($2.09\text{--}3.19 \text{ } \mu\text{mol L}^{-1}$) than in the phytoplankton fraction ($9.00\text{--}13.2 \text{ } \mu\text{mol L}^{-1}$). Thus, the low K_m values in the bottom layer in this study likely reflect bacterial values, and those under high DIP conditions might be explained by bacterial requirements for carbon, rather than P, in organic matter (Hoppe and Ullrich 1999).

V_{max} values were aligned with the total APA, as derived from the single addition of excess MUF-P, except for data

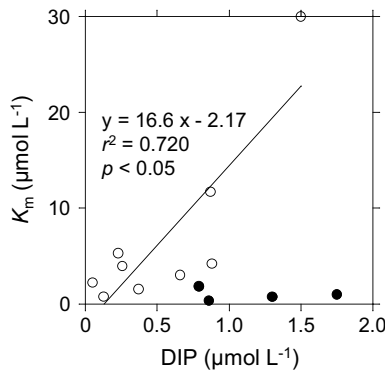


Fig. 6 A scatter plot of K_m versus DIP in the present study. *Blank circles* and *solid circles* indicate data from the surface and bottom layers, respectively. A linear regression line was drawn based on data from the surface layer (*blank circles*)

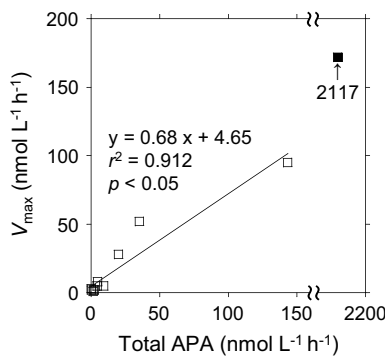


Fig. 7 A scatter plot of V_{max} versus total APA in the present study. A *solid square* indicates data from the surface layer at Station F3 in July. A linear regression line was drawn based on data (*blank squares*), except for those of the surface at Station F3 in July

at the surface at Station F3 in July ($r^2 = 0.912$, $p < 0.05$, Fig. 7). At the surface at Station F3 in July, the total APA was 12 times higher than the V_{max} . Such differences were also observed in previous kinetic studies (Sebastián et al. 2004b; Suzumura et al. 2012), and they suggested the coexistence of different microbes with high and low-affinity systems depending on a range of substrate concentrations in kinetic experiments. At the surface at Station F3 in July, the Chl *a* concentration ($49.5 \mu\text{g L}^{-1}$, Fig. 2d) was at a red tide level in Tokyo Bay ($> 30 \mu\text{g L}^{-1}$, Han et al. 1992), and *C. closterium* ($2985 \text{ cells mL}^{-1}$) was dominant, followed by the cosmopolitan *Pseudo-nitzschia* spp. (86 cells mL^{-1}) (Ueno et al. submitted to this special section). Probably, red tide species (*C. Closterium*) and background species (including *Pseudo-nitzschia* spp.) coexisted there, and they might have different affinities for DOP.

Short potential turnover times with < 1 day (14–23 h) were observed under warm ($> 20^\circ\text{C}$) and low DIP ($< 0.3 \mu\text{mol L}^{-1}$) conditions at the surface in May and July (Table 1). The turnover times were comparable to those in the DIP-depleted summer coastal region (11–16 h, Labry et al. 2005) and oligotrophic open ocean (5–13 h, Sato et al. 2013), indicating that considerable DIP stress recently occurs seasonally at the surface in Tokyo Bay. In contrast, quite long potential turnover times (532–3773 h) were observed under cool ($< 17^\circ\text{C}$) and high DIP ($> 0.6 \mu\text{mol L}^{-1}$) conditions in the surface layer in November and in both layers in December. Similar results were also observed in the DIP-replete winter coastal region (3021–4585 h, Labry et al. 2005).

4.3 P regime in Tokyo Bay: present and past

We compared total APA, DIP, and Chl *a* in the central part of Tokyo Bay in the summer of 1973, 1983, and 2013 (Table 2). The total APA in each year was determined by different fluorometric methods using 3-*o*-methylfluorescein

Table 2 Summer P regimes in the central part of Tokyo Bay in 1973, 1983, and 2013

Date	Layer	Station	DIP ($\mu\text{mol L}^{-1}$)	Chl <i>a</i> ($\mu\text{g L}^{-1}$)	Total APA ($\text{nmol L}^{-1} \text{h}^{-1}$)	Chl <i>a</i> -normalized total APA ($\text{nmol } \mu\text{g}^{-1} \text{h}^{-1}$)	References
July 1973	Surface	T3 (near F3)	4.0	200	10	0.05	Taga and Kobori (1978)
		T4 (near F6)	3.0	300	14	0.05	
	Bottom	T3 (near F3)	0.5	20	3	0.15	
		T4 (near F6)	0.7	20	5	0.25	
June 1983	Surface	5 (near F3)	< 1	nda	300	nda	Hashimoto et al. (1985)
		8 (near F6)	< 1	nda	300	nda	
July 2013	Surface	F3	0.26	49.5	2117	42.8	This study
		F6	0.05	27.1	144	5.32	
	Bottom	F3	2.23	3.96	0	0.00	
		F6	1.14	1.46	0	0.00	

nda no data available

phosphate (Taga and Kobori 1978) and MUF-P (Hashimoto et al. 1985; this study). However, the total APA at the surface in July was 1–2 orders of magnitude higher in 2013 than in 1973, and such a large difference could not be explained by only the methodological differences (Pettersson and Jansson 1978). Since surface DIP concentrations in July drastically decreased from 1973 (3–4 $\mu\text{mol L}^{-1}$) to 2013 ($<0.3 \mu\text{mol L}^{-1}$), the higher total APA in 2013 could be induced by a drastic change in the DIP condition. According to Taga and Kobori (1978), a summer peak in the total APA was observed in the 1970s, similar to that in the present study. Therefore, unlike seasonal temperature, the DIP concentration was more influential on the total APA in 1973 and 2013. Surface Chl *a* concentrations in July were one order of magnitude higher in 1973 than in 2013. However, Chl *a*-normalized total APA was 1–3 orders of magnitude higher in 2013 than in 1973. This means that the total APA was driven by microbial DIP stress rather than biomass accumulation. Although we did not collect Chl *a* data in 1983, relatively high total APA and low DIP was recorded in 1983 (300 $\text{nmol L}^{-1} \text{h}^{-1}$ and $<1 \mu\text{mol L}^{-1}$, respectively, Hashimoto et al. 1985), suggesting that the 1980s were a turning point for the P regime in Tokyo Bay after the water pollution control law was enacted.

5 Conclusion

We revealed seasonal variation in APA and associated environmental factors in the central part of Tokyo Bay during 2013. High values of total APA were detected in the summer surface layer. The total APA was mainly driven by the phytoplankton APA, whereas the bacterial and dissolved APA had relatively minor contributions throughout the seasons. The low concentration of ambient DIP was a major factor inducing the recent summer peak in the total APA, although the high temperature and high Chl *a* concentration partly contributed to this. Along with a drastic decrease in the surface DIP concentration in the bay during recent decades, P sources for summer phytoplankton might have been shifted from DIP to DOP. As inferred from kinetic parameters, a degree of DIP stress in the summer surface water of the central part of Tokyo Bay is currently comparable to that in the DIP-depleted coastal regions and oligotrophic open ocean. If the ambient DIP concentration in the bay continues to decrease, the DIP stress on phytoplankton might be severe in summer and could extend to other seasons, which would possibly affect annual primary productivity in the bay, although this also depends on DOP availability. To monitor the future P regime in Tokyo Bay, time-series observations of APA and associated parameters using research and training vessels would essentially be required.

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Author contributions FH and JK designed the study. SS performed APA assay and P measurements. NH collected Chl *a* data. FH and SS wrote the manuscript. All authors approved the final manuscript.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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