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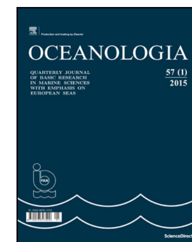

SHORT COMMUNICATION

Spatiotemporal variation of alkaline phosphatase activity in coastal waters off Trivandrum

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Summary Phosphatase is an extracellular enzyme which releases inorganic phosphate (Pi) from dissolved organic phosphate and indirectly organic carbon as nutrients for aquatic communities. Here, we have examined spatiotemporal variation in total alkaline phosphatase activity (APA) over a short period off Trivandrum, SW India. Sampling was at 50 m water depth at 5, 15, 25 and 45 m for 5 consecutive days at 6 h intervals during post-monsoon season. Total APA and phosphatase producing bacteria (PPB) were estimated along with pertinent environmental parameters. APA increased with depth up to 3.98 μM P h^{-1} at 45 m. Increase in pigment concentration with depth is responsible for an increase in APA and Pi uptake. There is a marginal increase in APA towards 18—24 h suggesting feeding activities of secondary producers. On the whole, chlorophyll and phaeophytin were responsible for nearly 45 and 55% variation in APA ($p < 0.01$, $p < 0.001$, $n = 16$), respectively. Total bacterial count (TBC) was responsible for 32% ($p < 0.05$, $n = 16$) and total viable direct counts-aerobic (TVCa) for 24% ($p < 0.05$, $n = 16$) APA variation. About 38% ($p < 0.01$, $n = 20$) variation of APA was linked to chlorophyll at noon and 22% ($p < 0.001$, $n = 20$) to PPB at dawn. Thus, it is possible that bacteria and chlorophyll/phytoplankton could be responsible for variation in APA, with the latter contribution greater than the former at noon. Such studies would help to profile the fertility of coastal waters in terms of bioavailable Pi. Laboratory experiments are underway to help us discern the extent of light-dependent contribution of chlorophyll/phytoplankton to APA and light independent participation of bacteria to the process.
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The availability of organic carbon and inorganic nutrients in water limits the biological productivity of aquatic environments. Of the essential elements involved in the biogeochemical cycle of the marine environment, phosphorus and nitrogen are most often considered as limiting factors. Dissolved and dead organic matter unusable by other organisms is subjected to microbial decomposition. Hence, the 'microbial loop' is important for recycling of phosphate and nitrogen in both water and sediment (Azam et al., 1983, 1995). The extensive number of microbial ectoenzyme activities detected in the water column has been noticed. Also the importance of heterotrophic bacteria and phytoplankton in biochemical/chemical processing and biogeochemical cycling in the ocean has been reported (Benitez-Nelson, 2000; Dyhrman et al., 2006). Hydrolytic enzymes include alkaline phosphatase, chitinase, lipase, protease, aminopeptidase, glucosidase, and others. These enzymes convert organic matter to more labile form and thus influence the growth dynamics of different microbes and phytoplankton (Azam et al., 1995; Chrost, 1991; Martinez et al., 1996). Phytoplankton and bacteria can satisfy their phosphorus (P) requirements by producing hydrolytic enzymes such as alkaline phosphatase (AP) to access Pi from organic P. Pi gets limited in coastal areas with high nitrogen availability or in oceans where there is high rates of nitrogen fixation (Ammerman et al., 2003; Labry et al., 2005; Vidal et al., 2003; Yucel, 2018).

Alkaline phosphatases have a broad range of organic substrate specificities, and therefore hydrolyze a wide variety of organic phosphomonoesters (Ammerman, 1991). These enzymes are attached to cell surface or, are freely dissolved in the water column resulting from the cell lysis or excretion (Hoppe, 2003; Jansson et al., 1988; Li et al., 1998). Upon enzyme hydrolysis, phosphomonoesters release inorganic P (Pi) into the water, along with their organic moiety, thereby increasing Pi availability for planktonic, as well as benthic organisms in shallow marine systems (Dyhrman and Ruttenberg, 2006; Koch et al., 2009). APA has been identified and associated with major groups of algae, cyanobacteria (Dyhrman and Ruttenberg, 2006; Martinez et al., 1996) and heterotrophic bacteria (De Souza et al., 2000) and also studied in seawater (Koch et al., 2009; Mamatha et al., 2012) and sediment (Taga and Kobori, 1978). Generally, it is reported that APA gets expressed when P is limiting and suppressed when P level is replete. It is presumed that APA promotes organic P mineralization and recycling within P-limited systems (Ammerman et al., 2003; Koch et al., 2009; Labry et al., 2005; Mamatha et al., 2015; Sebastian et al., 2012; Vidal et al., 2003).

The concentration of P in coastal waters could be increased by either land runoff or by upwelling process. In the rainy season (June—September), strong south-west monsoonal winds cause coastal upwelling in the Arabian Sea, which is one of the most productive areas in the world (Naqvi et al., 2010; Prell et al., 1991). However, upwelling weakens in the southernmost part of the west coast of India by October, and as a result, the high concentrations of nutrients available at the surface water get exhausted due to autotrophic production (Peetersa et al., 2002). During the post-monsoon season, the weaker north-east monsoon winds result in deeply stratified water and deeper euphotic zone (Peetersa et al., 2002; Pillai et al., 2000). Based on this information, we have examined the spatiotemporal variation in APA along with other environmental parameters over a short period off Trivandrum to appreciate the extent of changes in fertility as shown by the variation in APA. Previously, Mamatha et al. (2015) have noticed that low APA could be indicative of P sufficiency in coastal waters and higher activity suggestive of deficiency in off-shore waters off Trivandrum during upwelling season. In this communication on spatiotemporal variation during post upwelling, there is an increase in APA with depth due to increase in pigment and also Pi utilization without any temporal changes. The paper discusses these remarkable aspects.

The study station was off Trivandrum coast, south-west of India (8°26.04′N, 76°30′E) in the eastern Arabian Sea. Seawater samples were collected onboard FORV "Sagar Sampada" (cruise No. 282) during a post-monsoon season (November 2010) at lat. 08°27.742′N and long. 76°46.709′E at the maximum water depth of 50 m. The sampling was done at 4 depths i.e. 5, 15, 25 and 45 m bss (below sea surface) at 6 hourly intervals for 5 days. The sampling hours were 0 (24), 6, 12 and 18 where 6 and 18 h were designated as dawn and dusk and 12 and 24 h as noon and midnight, respectively. The data with time intervals 6, 12, 18 and 24 h for 5 days were averaged to discern the spatiotemporal variation (supplementary Table S1). Each data point is derived from triplicates.

Samples for dissolved oxygen (DO) were collected in 125 mL stoppered glass bottles avoiding air bubbles and were immediately fixed with Winkler's reagents. Fixed samples were stored in the dark until analysis (Strickland and Parsons, 1965). Sub-samples were taken for analysis of various parameters like total APA, chlorophyll *a*, phaeopigment, phosphate and other nutrients. Seawater sub-samples for the total bacterial count (TBC) were collected in sterile containers immediately after retrieval and fixed with buffered formalin. Seawater sub-samples for other microbiological analyses were similarly collected and stored at 4°C till analysis.

Salinity and temperature were derived from the Sea Bird CTD connected to the rosette Niskin sampler that operated vertically all the time. The pH was checked onboard using pH meter.

Winkler's titrimetric method (Strickland and Parsons, 1965) was used for estimating the dissolved oxygen (DO) concentration onboard using a Dosimeter (Metrohm 785 DMP Titrino). Nutrients were also analyzed on board by SKALAR auto analyzer as described by Wurl (2009).

Chlorophyll *a* (Chl*a*) and phaeophytin (phaeo) concentrations were determined fluorometrically (Turner Designs, USA) by filtering 1 L water samples from each depth with GF/F filters and extracting with 10 mL of 90% acetone in the dark for 24 h under refrigeration. The extract was measured following UNESCO (1994) protocol. The readings were measured before and after acidification using Turner Design (10 AU) fluorometer and then both Chl*a* and phaeo concentrations were calculated.

Total bacterial abundance (TBC) was counted using epifluorescence microscopy (Olympus Fluorescent Microscope Model BX61) by acridine orange direct count (AODC) method (Hobbie et al., 1977) and expressed as numbers per litre (nos L^{-1}). Total direct viable counts (TVC), were estimated as outlined by Kogure et al. (1980) using a mixture of piromedic, pipemedic, nalidixic acid and yeast extract.

Table 1 Range and average of physico-chemical and biological parameters in whole water column (5 days, 4-time interval, 4 depths; n = 80).

Parameter	Range	Mean
Temperature [°C]	27.2—28.7	28 ± 0.57
Salinity	33.07—35.46	34.9 ± 0.57
pH	8.19—8.35	8.31 ± 0.04
DO [ml L^{-1}]	1.19—4.68	3.47 ± 0.64
NO_2^-N [μM]	ndl—0.795	0.16 ± 0.21
NO_3^-N [μM]	0.681—20.026	5.32 ± 4.68
PO_4^-P [μM]	0.093—2.37	0.27 ± 0.29
Chl*a* [μg L^{-1}]	0.71—2.90	1.39 ± 0.5
Phaeo [μg L^{-1}]	0.15—1.29	0.5 ± 0.3
TBC [cells L^{-1}]	2.48×10^8—1.54×10^{10}	$5 \times 10^9 \pm 2.85 \times 10^9$
TVC [cells L^{-1}]	6.37×10^8—8.16×10^9	$3.3 \times 10^9 \pm 2 \times 10^9$
PPB-MPN [cells L^{-1}]	ndl—1.5×10^6	$0.27 \times 10^6 \pm 0.46 \times 10^6$
APA [μM P h^{-1}]	ndl—13.79	2.34 ± 2.71

DO — dissolved oxygen; Chl*a* — chlorophyll *a*; Phaeo — pheaophytin TBC — total bacterial count; TVCa — total viable bacteria; PPB — phosphatase producing bacteria; APA — alkaline phosphatase activity; ndl — non detectable level.

The end point dilution method (MPN — Most Probable Number) is based on a series of dilutions prepared from a sample, where selected liquid media are inoculated with each dilution using 3 parallels. After the incubation period, estimation of the microbial count was done, using statistical tables based on the number of positive wells (showing colour formation i.e. colourless to yellow colour). Three tube method was suitably adopted for microtiter plate method. Phosphatase producing bacteria (PPB) were enumerated by most probable numbers (PPB-MPN) by microtitre plate method using para-nitrophenol phosphate (pNPP) as substrate (1 mM) (Fig. 1s). The hydrolysis of phosphate was indicated by the release of colored para-nitrophenol (pNP) from colourless para-nitrophenol phosphate (pNPP) in proportion to the Pi released. The samples were incubated in the dark at room temperature (28 ± 2°C) for scoring positive and negative growth/reactions. Scoring/calculations were done using McCrady's table (Rodina, 1972). It is assumed that the color development is attributed only to bacteria and free enzymes in the diluted inoculum.

The alkaline phosphatase activity (APA, EC 3.1.3.1) was estimated using the method of Taga and Kobori (1978) and Mamatha et al. (2015). Measurements were run against a control containing an equivalent volume of the seawater samples previously autoclaved. The results represent the average of triplicates corrected for controls. Experimental values were calculated against a standard (para-nitrophenol, pNP) curve prepared to cover concentrations from 10 nM to 1000 nM.

Microbiological data were log(x+1) transformed for statistical analysis. Inter-relationships between biological and environmental parameters were examined using Statistica ver. 6.0 and analysis tool pack in Microsoft Excel. Analysis of variance (ANOVA) was carried out to find out significant spatiotemporal variations in pertinent parameters.

In the present study, there is little variation in salinity with the minimum of 33.07 and maximum of 35.46. The increase of salinity from the surface to bottom is evident but it is not statistically significant. Variation in temperature and pH were also minimal as shown in Table 1. The dissolved oxygen in the water column ranged between 1.19 ml L^{-1} in 45 m depth to 4.42 ml L^{-1} in 5 m layer (Table 1). There was significant variation in vertical distribution with depth and time in temperature and DO ($p < 0.05$). Higher concentration of nitrate (20.02 μM) was measured at 45 m while nitrite (1.55 μM) was measured at surface waters (Table 1). Nitrate and nitrite showed significant variation with depth but not with time. The average concentration of phosphate over the study period was 0.268 ± 0.246 μM (n = 80) and it ranged from 0.08—2.37 μM. Phosphate concentration was higher at 25 m depth (2.37 μM) than at the 5 m depth (0.08 μM). However, the spatial and temporal variation was not statistically significant.

The Chl*a* and bacterial numbers can effectively represent algal and bacterial biomass. Chl*a* concentration varied from 0.71—2.90 μg L^{-1} with an average 1.39 ± 0.50 μg L^{-1} (Table 1). Surprisingly, it was high at 45 m depth (2.9 μg L^{-1}) at all time intervals except noon when it is uniform throughout (Fig. 1a). Like Chl*a*, phaeopigment also follows the same trend in distribution (Fig. 1b).

TBC ranged from 2.4×10^8 to 1.54×10^{10} cells L^{-1} (av. $5 \times 10^9 \pm 3 \times 10^9$ cells L^{-1}) with the maximum at the 45 m, waters showing one order higher than the other depths (Table 1). TVCa was one order less than the TBC and was marginally higher at 45 m where it varied from 6.37×10^8—8.16×10^9 cells L^{-1} (av. $3.7 \times 10^9 \pm 2 \times 10^9$ cells L^{-1}). PPB-MPN ranged from ndl—1.5×10^6 cells L^{-1} (av. $0.27 \times 10^6 \pm 0.46 \times 10^6$ cells L^{-1}). Though there are large standard deviations in the average values of the bacterial parameters, ANOVA showed that there was no significant variation in distribution with depth and time (Fig. 2).

Total APA of the whole water column ranged from ndl to 13.79 μM P h^{-1} (n = 80) (Table 1). It increased with depth to a maximum of 3.98 μM P h^{-1} (n = 16, Fig. 3) at 45 m ($p < 0.05$) corresponding to increase in Chl*a* and phaeo concentration. There is not much variation with time, however, marginal increase towards 18 to 24 h was discernible. Two way ANOVA showed that depth variation is stronger ($p < 0.05$) than the temporal variation in APA.

On the whole, APA is positively related to depth (r = 0.63, $p < 0.05$, n = 80), and negatively with DO (r = −0.594, $p < 0.05$, n = 16). Nitrate influenced 41% (r = 0.637, $p < 0.01$)

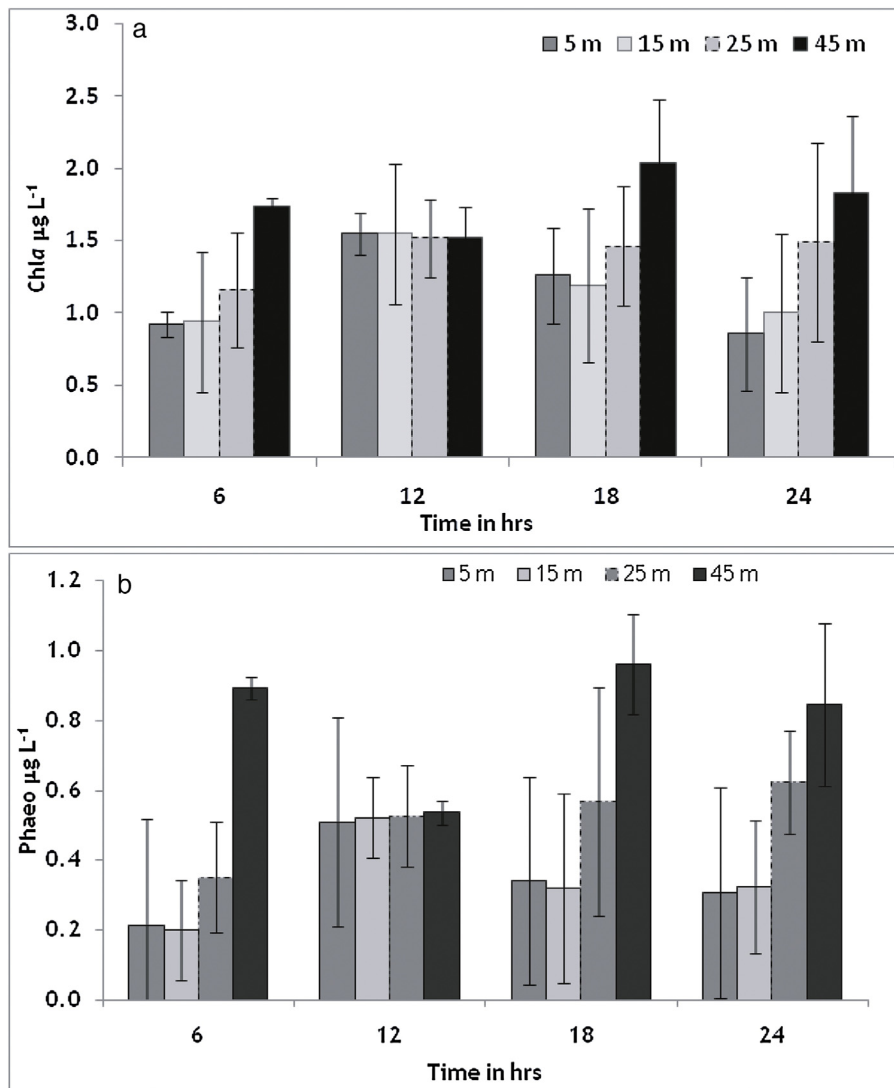

Figure 1 Spatio-temporal variations in (a) chlorophyll and (b) phaeophytin off Trivandrum.

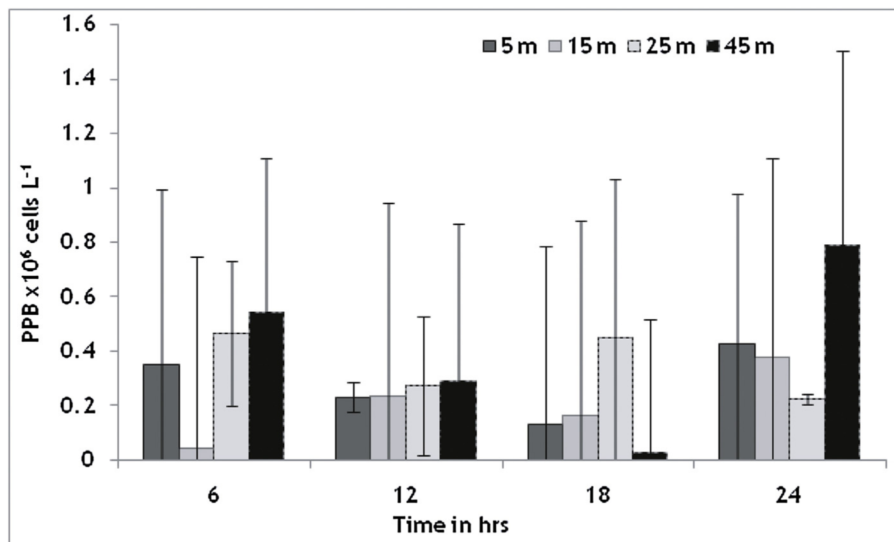

Figure 2 Spatio-temporal variations in phosphatase producing bacteria (PPB-MPN) off Trivandrum.

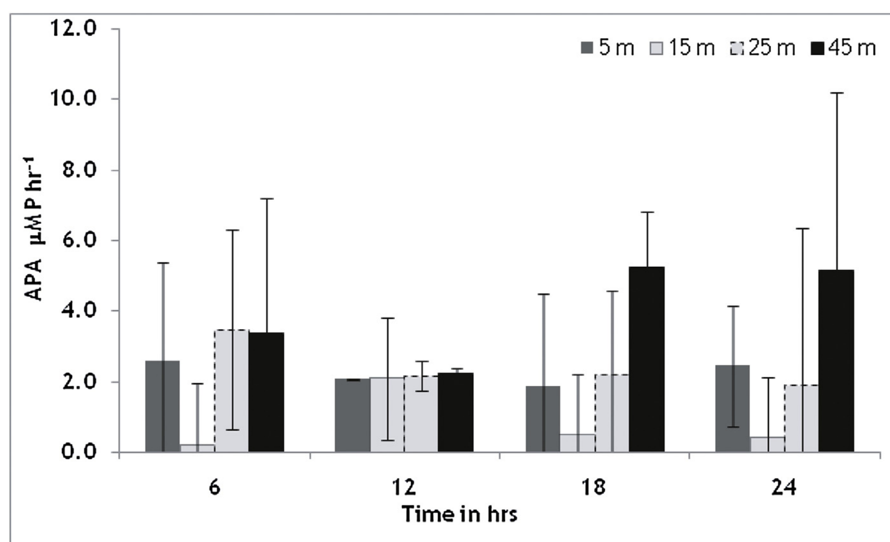

Figure 3 Spatio-temporal variations in alkaline phosphatase activity (APA) off Trivandrum.

Table 2 Relationships of alkaline phosphatase activity (APA) with environmental parameters.

	Whole water (n = 16)	6 h (n = 20)	12 h (n = 20)	18 h (n = 20)	24 h (n = 20)
Depth	0.616 [b]	—	0.631 [b]	—	0.468 [c]
DO	−0.547 [c]	—	−0.608 [b]	—	—
NO_3-N	0.637 [b]	—	—	0.625 [b]	—
Chl*a*	0.670 [b]	—	0.619 [b]	—	—
Phaeo	0.740 [c]	—	0.638 [b]	—	0.491 [c]
TBC	0.561 [a]	—	—	—	—
TVC	0.491 [a]	—	—	—	—
PPB-MPN	—	0.465 [c]	—	—	—

Superscripts denote different significant levels; [a]$p < 0.05$; [b]$p < 0.01$ and [c]$p < 0.001$. DO — dissolved oxygen; Temp. — temperature; Chl — chlorophyll *a*; Phaeo — pheaophytin; TBC — total bacterial count; TVC — total viable count; PPB — phosphatase producing bacteria; APA — alkaline phosphatase activity.

variation in APA. Chl*a* and phaeo were responsible for 45 and 55% variation in APA, respectively ($p < 0.05$, $p < 0.001$, n = 16) (Table 2). Bacterial fractions examined contributed to the changes in APA with the TBC being responsible for nearly 32% ($p < 0.05$, n = 16) and TVCa for 24% ($p < 0.05$, n = 16). Time dependent study showed that 38% ($p < 0.01$, n = 20) variation of APA at noon was linked to Chl*a* and 22% ($p < 0.001$, n = 20) variation in APA to PPB-MPN at dawn (Table 2).

The spatial variation (depth) is discernible in these waters (Nyadjro et al., 2012). The variation with temperature and DO confirms that there is stratification in these shallow coastal waters during this season but restricted to physico-chemical parameters. The higher concentration of nitrate at the bottom suggests that the source could be from sediment. Chl*a* and phaeo were also high at 45 m all the time, suggesting that the settling of the pigments was high throughout the observation. The Chl*a* maxima were observed at noon at all the depths. This peaking could be a reflection of the penetration of PAR (photosynthetically active radiation) up to 45 m depth. However, the depth of the euphotic zone could vary in different waters. Qasim (1982) reported that euphotic zone in the southern Arabian Sea was 60 m and in the northern part it was 40 m. Pillai et al. (2000) also found that the average depth of the euphotic zone in the west coast is 60 m during post-monsoon season. Lack of significant variation in the different bacterial parameters with depth and time suggests that this could be due to their stronger association with phytoplankton (Riemann et al., 1999; Rooney-Varga et al., 2004). A rise in bacterial population at the bottom may be a consequence of nocturnal feeding and excretion by benthic organisms. Nandakumar and Damodaran (1998) found that there is a diurnal variation in the feeding intensity of speckled shrimps at Kochi. They feed more in the night than day. This benthic activity may enrich the bottom area with essential nutrients subsequently allowing bacteria including PPB to proliferate rapidly. Thus there is less heterogeneity in the microbial distribution in the coastal waters, suggesting that the representative sampling can be at any depth.

In the present study, APA peaks were observed from dusk to dawn (1/4 observations — dusk, 1/4 — midnight and 2/4 — dawn) (Fig. 3). These APA peaks could be linked to prolonged grazing by zooplankton. Their sloppy feeding generally leads

to release of phytoplankton exudates (Fouilland et al., 2014; Lignell, 1990; Yucel, 2018). Besides, higher APA at 45 m layer could also be a reflection of higher phosphatase producing bacterial population from sediments below (Ayyakkannu and Chandramohan, 1970, 1971; Barik et al., 2001). Increased expression of APA with depth is noticeable in spite of the same level of Pi. Hence, it is inferred that uptake rate could also be high with depth. On the other hand, Kobori and Taga (1979) found that APA was high at the surface layer and decreased with increasing depth at Tokyo Bay. This difference could be because the water is shallower in the present study than Tokyo Bay. Experimentally, Ivancić et al. (2010) and Lin et al. (2011, 2012) have shown that bacterial contribution to APA could also be as important as phytoplankton. This inference could be particularly true for coastal waters.

The relation of APA with DO and nitrate are indirectly related to depth. APA relates with both pigments and bacterial parameters. The variation of Chl*a* and phaeo with nitrate could be partly due to their requirement of this nutrient and also because nitrate and Chl*a* co-vary with depth. The relationships between APA and biological parameters are discernible in scatter plots (Fig. 2s(a—d)). Thus, it is possible that both bacteria ($r = 0.561$, $p < 0.05$) and Chl*a* ($r = 0.670$, $p < 0.01$) could be responsible for variation in APA, with Chl*a* ($r = 0.619$, $p < 0.01$) contribution being prominent at noon. Kwon et al. (2011) in the northern part of Gamak Bay, Korea and Hino (1988) in Lake Barato, Japan also found a strong linear relation between APA and Chl*a*. Thus, it could be inferred that major part of APA might have been induced by phytoplankton during the daytime. Chrost and Overbeck (1987) also observed that phytoplankton were major APA producers in the photic zone of the lake plusee of East Holstain (North Germany). During summer or winter, phytoplankton APA constituted, on an average, 49% of the total APA in the plusee water but bacteria were the dominant APA producers in winter (41.3—44.9%). Increased phaeo is indicative of degradation of Chl*a*. When Chl*a* degrades, dissolved phosphate gets released into the environment and may indirectly supply the Pi requirement to phytoplankton and microbial communities. Dissolved AP can also be liberated into the environment through the lysis of dead phytoplankton cells (Chrost, 1991). Taga and Kobori (1978) found a positive relationship between phosphatase and Pi in the eutrophic seawater samples in Tokyo Bay. In our study, the phosphate concentration was similar over time and depth (ca 0.2 μM). This lack of variation could be attributed to the balance between uptake and release of this nutrient by the water column community. Induction of APA by phytoplankton may be ecologically significant, allowing dominance by these organisms under phosphate limiting conditions (Kwon et al., 2011; Ly et al., 2014). Thus, the synchronization between the APA-Chl*a* and APA-TBC ($n = 16$) during the whole time series suggests that both bacteria and phytoplankton contribute to total APA.

We, therefore, conclude that there is a positive synchronization of APA with biotic variables like Chl*a*, phaeo and bacterial abundance (Fig. 2s(a—d)). Phytoplankton contribution to variation in total APA is more pronounced at noon, while bacteria contributes during dawn ($r = 0.465$, $p < 0.001$). Like phytoplankton, bacteria also have a major role in continuously making this important nutrient, phosphate, bioavailable to the surrounding waters, especially in the sub euphotic layers. Continuing the observation for the longer time will strengthen our inference about the extent of contribution by primary producers and bacteria to APA in the coastal system. Such studies will help us to understand the changes in productivity of coastal waters in space and time.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at https://doi.org/10.1016/j.oceano.2018.06.004.

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