

Seasonal variations in phytoplankton composition and transparent exopolymer particles in a eutrophicated coastal environment

Neelam Ramaiah*, Ken Furuya

Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo, Tokyo 113-8657, Japan

ABSTRACT: Annual variations in phytoplankton composition and concentrations of transparent exopolymer particles (TEP) were studied in Tokyo Bay, one of the most eutrophicated coastal environments in central Japan. This study aimed at evaluating the influence of anthropogenic input on phytoplankton composition, bloom formations and their subsequent impact on TEP concentrations. Phytoplankton composition was dominated by diatoms, particularly *Skeletonema costatum*, during most of the year. However, raphidophytes represented by *Heterosigma akashiwo* became abundant from late spring to summer. TEP concentrations varied between 14 and 1774 μg Xanthan equiv. l^{-1} , with higher values in the inner bay ($169 \pm 182 \mu\text{g}$ Xanthan equiv. l^{-1} , which in terms of carbon is equivalent to $118 \pm 127 \mu\text{g}$ C l^{-1}) than the bay mouth ($112 \pm 70 \mu\text{g}$ Xanthan equiv. l^{-1} , $\equiv 78 \pm 49 \mu\text{g}$ C l^{-1}). TEP concentrations followed an empirical relationship with chlorophyll *a*. In addition, peak TEP coincided with the peak chlorophyll *a* and raphidophyte abundance. Multivariate analysis between TEP and the different groups of phytoplankton indicated a predominant influence of diatoms and raphidophytes. TEP-carbon percent of chlorophyll-carbon tended to be higher during winter, which coincided with low bacterial counts and implied low or no utilization of TEP and hence an accumulation during such periods. Laboratory experiments were conducted with cultures of *S. costatum* and *H. akashiwo* to test the species-specific variation, if any, in total organic carbon (TOC) production and TEP formation. The consistently higher chlorophyll equivalent TEP production in *H. akashiwo* confirmed our hypothesis that, in addition to well-known contributions by diatoms, groups like the raphidophytes contribute substantially to TEP as well as organic matter production in eutrophicated coastal regions. TOC and TOC equivalent TEP production revealed that, in addition to the biomass and composition, the growth stage(s) of the phytoplankton is an important factor controlling the C-rich TEP concentrations in aquatic ecosystems subjected to anthropogenic perturbances.

KEY WORDS: TEP · Phytoplankton blooms · *Heterosigma* · Eutrophic Tokyo Bay

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INTRODUCTION

Phytoplankton blooms in marine ecosystems account for a major portion of annual primary production, and are responsible for the availability of organic matter and the control of energy flow and food web dynamics (Li et al. 1993, Lignell et al. 1993, Brussaard et al. 1996, Turley et al. 2000). Senescence and crash of blooms lead to aggregate formation (Riebesell 1991a,b, Kiør-

boe et al. 1994, Tiselius & Kuylenstierna 1996). An important component of these aggregates are the transparent exopolymer particles (TEP) formed largely from the dissolved organic matter released by phytoplankton (Alldredge et al. 1993, Mari 1999, Passow 2000). Substantial formation of TEP from macroalgal exudates in coastal waters subjected to aquaculture activities was recently reported (Ramaiah et al. 2001).

Rapid industrialization and human settlement along the coasts lead to voluminous disposal of anthropogenic waste into nearby aquatic areas and pose a major threat to the ecosystem, resulting in alteration

*Present address: SAD 35, NIO Quarter, Dona Paula, Goa 403 004, India. Email: ramaiah@darya.nio.org

of the coastal plankton dynamics (Ramaiah et al. 1995, Pedersen & Borum 1996). The fact that the TEP formation is rapid and their concentrations elevated during phytoplankton blooms has been recently proved by a variety of investigators (Hong et al. 1997, Mari et al. 2001, Ramaiah et al. 2001). We thus hypothesized that the unusually large phytoplankton biomass during red tides, which are frequent occurrences in eutrophicated environments, might, among other biogeochemical episodes, lead to concomitantly high TEP concentrations. Several recent studies have shown the varied biogeochemical roles of TEP in marine ecosystems (Passow & Alldredge 1994, 1995a, Schuster & Herndl 1995, Mari & Kiørboe 1996, Alldredge 1998, Engel 2000, Liu & Buskey 2000, Kiørboe & Jackson 2001, Mari et al. 2001, Turner 2002). However, despite important implications of these particles in several ecological processes and the fact that coastal biology largely governs the organic flux on the shelf, no information exists on the quantification of TEP in relation to phytoplankton composition and red-tide occurrences in eutrophic coastal regions. The present study was conducted to understand the influences the frequently occurring red tides exert on the concentrations of TEP. The site selected for this study was the eutrophicated Tokyo Bay. This bay, surrounded by several major cities of central Japan, is connected to the Pacific Ocean by a narrow channel.

On an annual average, inflowing rivers discharge about $300 \text{ m}^3 \text{ s}^{-1}$ of fresh water and nutrient supplies of 300 t N and 20 t P daily (Matsukawa & Sasaki 1990). Continuous influx of anthropogenic waste has led to phytoplankton community changes, enhanced biomass and frequent occurrences of red tides (Marumo & Murano 1973, Han et al. 1992, Nomura & Yoshida 1997, Nomura 1998). Laboratory experiments were conducted for a comparative estimate of the total organic matter production and resultant TEP formation by *Skeletonema costatum* and *Heterosigma akashiwo*, the 2 major bloom-forming phytoplankters and contributors to organic matter production in this bay.

MATERIALS AND METHODS

Sampling. Sampling was carried out on board the RV 'Seiyou Maru' of the Tokyo University of Fisheries from December 1997 to November 1998. Samples were collected monthly from 6 stations in Tokyo Bay, Japan (Fig. 1); 4 located in the inner bay (Stns F1, F3, F4, F7) and 2 in the bay mouth (Stns 02 and 06). Depths at these stations are shown in Fig. 1. Water samples were obtained from the surface using a clean bucket and from 5 and 10 m depths (10 and 20 m at Stn 06, considering the deeper light penetration depth at this station) using a Van Dorn sampler. Parameters studied were phytoplankton biomass and composition (based on HPLC pigment analyses), TEP concentrations, bacterial counts, TEP sizes, and TEP attached bacterial counts. Stormy weather prevented sampling at Stn 06 in the bay mouth in April and July 1998.

Hydrographic parameters. Temperature and salinity values were obtained from CTD data. Water samples for nutrient estimations were frozen immediately after collection and stored at -20°C until analysis. Nitrate and phosphate concentrations were measured following the standard procedures in Parsons et al. (1984).

Pigments and phytoplankton composition. The Mantoura & Llewellyn (1983) protocol modified by Furuya et al. (1998) was followed for the HPLC pigment analysis. Briefly, 0.25 to 1.7 l of sample was filtered through a 47 mm Whatman GF/F filter at $\sim 100 \text{ mm Hg}$. The filter paper was then quickly frozen and stored at -80°C until analysis. Concentrations of diagnostic marker pigments, viz., chlorophyll *a* (chl *a*), chlorophyll *b* (chl *b*), peridinin (Perid), fucoxanthin (Fuco), 19'-hexanoyloxyfucoxanthin (Hex-fuco), prasinoxanthin (Pras), violaxanthin (Viola), alloxanthin (Allo) and zeaxan-

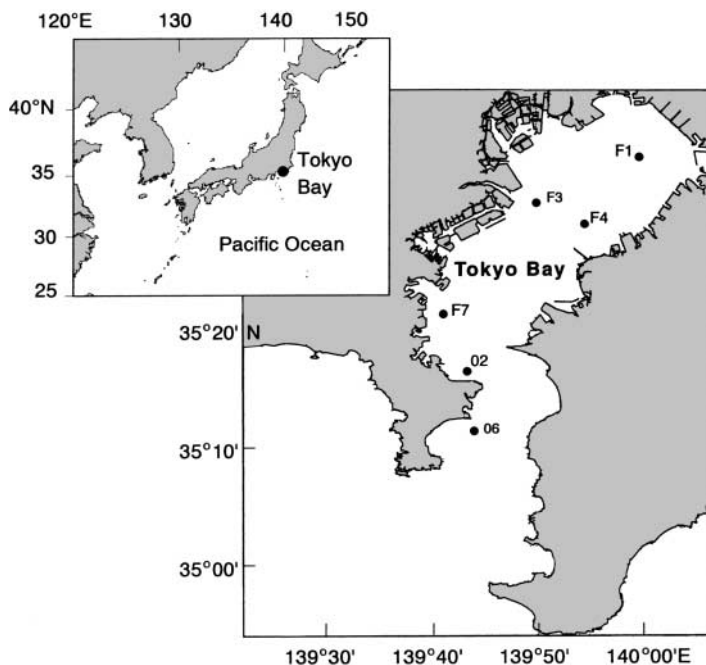


Fig. 1. Location of sampling stations in Tokyo Bay, Japan. Average depths at Stns F1, F3, F4, F7, 02 and 06 respectively were 12, 23, 19, 42, 41 and 213 m

thin (Zea) were estimated from the respective peaks calibrated against pure standards (Water Quality Institute). For estimating phytoplankton composition, concentrations of diagnostic marker pigments obtained by HPLC were subjected to ChemTax—a matrix factorization program running under Matlab (Mackey et al. 1996, 1997). Based on the composition of major pigments, taxonomic groups considered were the diatoms, dinoflagellates, prasinophytes, haptophytes, chlorophytes, cryptophytes and raphidophytes. Initial ratios for groups other than the raphidophytes used in the ChemTax calculation were derived after minor modifications following Mackey et al. (1996) and Furuya et al. (2002). As raphidophytes in the Tokyo Bay are almost totally represented by *Heterosigma akashiwo* (Han et al. 1992, Nomura & Yoshida 1997, Nomura 1998), pigment ratio for this group was obtained by exposing cultures of *H. akashiwo* to light intensities ranging from 75 to 700 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Viola and Fuco were used as marker pigments for raphidophytes (Jeffrey et al. 1997, Mostaert et al. 1998) with respective ratios of 0.12 and 0.69 normalized to chl *a*. The taxonomic groups, marker pigments and initial ratios used for ChemTax analysis are presented in Table 1. Phytoplankton carbon was estimated from the chlorophyll concentrations using an *F* ratio (C:chl *a*) of 25 (Strickland 1965).

TEP concentrations. The method of Passow & Alldredge (1995b) was followed for the quantification of TEP concentrations. A 50 ml sample in 2 replicates was filtered onto 0.4 μm Nuclepore filter paper. Particles retained on the filter were stained with Alcian Blue (8GX, Sigma) solution, the filters were soaked in 80% sulfuric acid for 3 h and absorbance read at 787 nm in a spectrophotometer (Hitachi, U-2000). TEP concentration was expressed in terms of Xanthan equivalents (μg Xanthan equiv. l^{-1}). These values were converted to TEP-carbon (TEP-C) from the relationship $y = 0.75x + 110$ (Engel & Passow 2001). However, we excluded the 110 intercept to avoid the C other than TEP-C (Passow pers. comm.) and hence TEP-C was calculated from the relationship $y = 0.75x$.

TEP sizes. Formalin-preserved samples from various months and stations were randomly selected, filtered and stained with Alcian Blue, and slides were then prepared for microscopy. Stained filters were mounted on glass slides in immersion oil. TEP were enumerated under a light microscope at 200 \times magnification. The sizes at maximum length and width were noted following the method described by Passow & Alldredge (1994). Between 35 and 200 particles varying from 1 to <100 μm were measured for size determination.

Total bacterial counts. For enumeration of bacterial numbers, seawater samples fixed in 4% glutaraldehyde were filtered onto 0.2 μm blackened Nuclepore filters and stained with DAPI (4',6-diamidino-2-phenylindole dihydrochloride, added to obtain a final concentration of 0.5 μg DAPI ml^{-1} sample). Blue fluorescing bacterial cells were enumerated under an epifluorescence microscope (Nikon) equipped with a UV excitation filter following Porter & Feig (1980).

TEP attached bacterial counts. The double staining method of Passow & Alldredge (1994) was followed for estimating the number of bacteria attached to TEP. Samples were filtered under low constant vacuum (<100 mm Hg) through 0.2 μm Nuclepore filters and double stained with DAPI and Alcian Blue. After rinsing with Milli-Q water to remove excess stain, filters were mounted on glass slides in immersion oil. TEP size and number of attached bacteria from the same field were counted with an epifluorescence microscope by switching alternately between the visible and UV light.

Quantification of total organic carbon production by *Skeletonema costatum* and *Heterosigma akashiwo*. Time course experiments were conducted to compare the growth phase associated variations in the production of total organic carbon (TOC) and TEP by these 2 bloom forming species. Cultures of *S. costatum* (N 323) and *H. akashiwo* (N4) originally obtained from the National Institute for Environmental Studies (NIES) culture collection (Tsukuba, Japan) were used for the experiments. Exponential stage cultures (500 μl) were inoculated into experimental flasks.

Table 1. Initial pigment ratio matrix used for the ChemTax analysis. Refer to the text for abbreviations and names of the marker pigments used

Group	Perid	Fuco	Hex-fuco	Pras	Viola	Allo	Zea	Chl <i>b</i>	Chl <i>a</i>
Diatoms	0.00	0.47	0.00	0.00	0.00	0.00	0.00	0.00	1.00
Dinoflagellates	1.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
Raphidophytes	0.00	0.69	0.00	0.00	0.12	0.00	0.00	0.00	1.00
Haptophytes	0.00	0.00	1.71	0.00	0.00	0.00	0.00	0.00	1.00
Cryptophytes	0.00	0.00	0.00	0.00	0.00	0.23	0.00	0.00	1.00
Chlorophytes	0.00	0.00	0.00	0.00	0.06	0.00	0.01	0.46	1.00
Prasinophytes	0.00	0.00	0.00	0.72	0.06	0.00	0.32	0.00	1.00

These cultures (in 4 l of f/5 medium) were incubated at 20°C and $700 \mu\text{mol m}^{-2} \text{s}^{-1}$. Subsamples were drawn daily, 24 h after inoculation, to measure chl *a* and TEP and to estimate the photosynthetic rates. Experiments were designed taking into account various physiological and ecological factors that would lead to overestimation of organic matter production (Williams 1990). We followed a modification of the small volume incubation procedure of Lewis & Smith (1983). $\text{NaH}^{14}\text{CO}_3$ (specific activity of the undiluted stock was 54 mCi mmol^{-1} , Amersham) was added at ca. $0.91 \mu\text{Ci ml}^{-1}$ of the sample and gently mixed. Five ml aliquots were then dispensed into small vials, sealed with parafilm, transferred to a photosynthetron and incubated for 2 h under 10 different light intensities varying between 0 and $2500 \mu\text{mol m}^{-2} \text{s}^{-1}$. Initial (time-zero, T_0) readings

were obtained by adding Aquasol II to 3 vials containing the sample aliquots immediately prior to incubation. At the end of incubation, the samples were acid fumed for 24 h. Aquasol II was then added, the vials were capped and the contents were mixed thoroughly and activity measured in a liquid scintillation analyzer (Packard Tri-cab 1500). Daily photosynthetic rates were normalized to chl *a*. Maximum TOC production was calculated by fitting the hyperbolic tangent function of Jasby & Platt (1976) to the *P-E* (photosynthesis-irradiance) curve.

Statistical analysis. To evaluate the influence of different groups of phytoplankton on TEP, data were subjected to statistical analysis using the Statview J.4.02 program. For comparison, we divided the data into inner bay (Stns F1, F3, F4, F7) and bay mouth (Stns 02 and 06). Statistical computation was based largely on the regression models described in Sokal & Rohlf (1995), with TEP concentrations as the *y*-axis and phytoplankton classes as the *x*-axis variables. Correlation coefficients were obtained between the chl *a* of different classes and TEP. Classes showing significant correlation coefficients were then subjected to multivariate regression analysis to study the influence of phytoplankton blooms and composition on TEP in the inner bay and the bay mouth. Phytoplankton classes exhibiting insignificant relationships were grouped together as 'others'.

RESULTS

Hydrographic parameters

We considered December to February as winter, March to May as spring, June as the monsoon month, July to August as summer and September to November as autumn. The temperature and salinity profiles (Fig. 2) indicated that the water column was well mixed at all the stations during the winter and mid-spring period (December to April). Temperature stratification later became pronounced at all the inner bay stations, with maximum difference between the surface and 10 m observed in July. Salinity profiles suggested a rather homogenous water column until April, with salinity in the inner bay region and at Stn 02 fluctuating between 30 and 33.

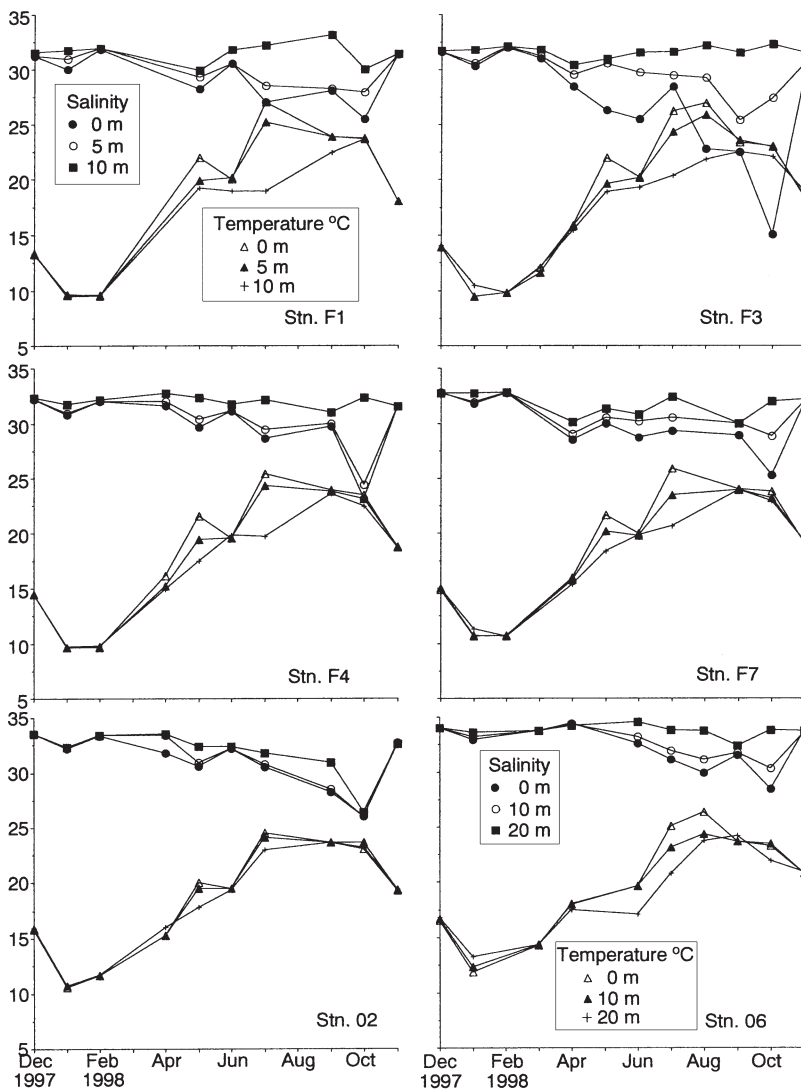


Fig. 2. Variations in temperature and salinity in Tokyo Bay from December 1997 to November 1998. Note differences in sampling depths for Stn 06

A marked stratification was observed at all stations from May onwards, with the lowest surface salinity in October. Maximum salinity gradients between the surface and subsurface depths were recorded at Stns F1 and F3.

Nitrate concentrations were high at the inner bay stations, with a maximum at Stn F3 (Fig. 3). Values at Stns F1, F4 and F7 ranged between 0.2 and 40 μM , while at Stn F3 the maximum was 63.8 μM at the surface in October 1998. Concentrations at Stns 02 and 06 varied between 0.3 and 27.3 μM . Except for a few times, particularly at Stn F3, vertical profiles did not indicate significant variations with regard to depth.

Phosphate concentrations varied between 0.03 and 3.2 μM at the inner bay stations. Concentrations at 10 m were generally higher (Fig. 4). Variations at the outer bay stations were from 0.7 to 1.6 μM .

Chlorophyll a and phytoplankton composition

Chl a concentration was highest in the inner bay and decreased towards the bay mouth (Figs. 5 & 6). Chl a generally varied from <5 to 30 $\mu\text{g l}^{-1}$, except for an increase in concentration at the inner bay stations during April-May. A maximum chl a concentration of 81.2 $\mu\text{g l}^{-1}$ was recorded at Stn F3 in May. On a seasonal scale, the low winter concentrations increased during spring and summer. Concentrations were generally higher during summer at 10 m.

Diatoms were dominant at all the stations, contributing 0.05 to 32.44 $\mu\text{g l}^{-1}$ chl a, followed by the raphidophytes that were prominent during late spring and summer (April to September) at Stns F1, F3 and F4 (Fig. 5). Raphidophytes contributed 0.001 to 38.98 $\mu\text{g l}^{-1}$ chl a, with a maximum recorded at Stn F3 in May 1998, which accounted for about 48% of the total chl a concentration. A congregation of raphidophytes at 10 m was invariably higher at Stn F1, contributing substantially to total chl a. Dinoflagellates were the next in abundance; they increased in biomass from mid-spring (April), with peak contributions of up to 5 $\mu\text{g l}^{-1}$ chl a in either June or July at all the stations. In the bay mouth stations, diatoms were the most abundant during the study period (Fig. 6). Other groups that were frequently observed but not contributing significantly to the chl a were the chlorophytes, prasinophytes and haptophytes. Prasinophytes at most

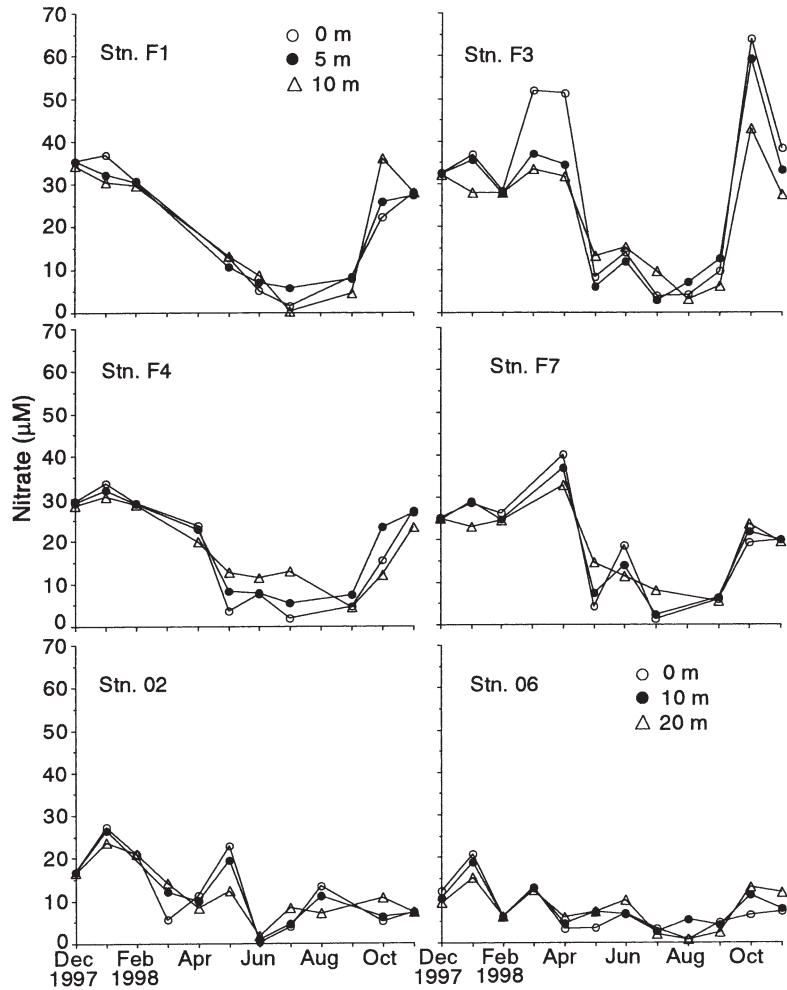


Fig. 3. Variations in nitrate concentrations (μM) in Tokyo Bay from December 1997 to November 1998. Note differences in sampling depths for Stn 06

stations were mainly observed from May to October and remained almost absent during winter. Cryptophytes occurred on a few occasions.

TEP concentration

As shown in Fig. 7, TEP varied from 14 to 1774 $\mu\text{g Xanthan equiv. l}^{-1}$, with higher values inside the bay (avg. 169 $\mu\text{g Xanthan equiv. l}^{-1}$) than towards the bay mouth (avg. 112 $\mu\text{g Xanthan equiv. l}^{-1}$). A general trend observed was of higher concentrations at the surface that decreased at the subsurface. Seasonally, TEP concentrations were the highest during late spring (May) at all stations and persisted well into early autumn (September). An unusually high concentration of 1774 $\mu\text{g Xanthan equiv. l}^{-1}$ was recorded at Stn F3 in May. TEP was consistently high at this station and decreased towards the mouth of the bay.

Contribution of phytoplankton taxa to TEP variations

Table 2 presents the correlation coefficients between the different taxa and TEP observed in the inner bay and bay mouth. In the inner bay, TEP exhibited a highly significant correlation ($p < 0.001$) with diatoms, raphidophytes, chlorophytes and cryptophytes. At the bay mouth, a similarly significant association was observed between TEP and diatoms, raphidophytes and prasinophytes.

Multivariate regression analysis revealed a general influence of diatoms and raphidophytes on TEP concentrations. The following relationships were obtained:

Inner bay:

$$\text{TEP} = 72.65 + 4.21_{\text{diatoms}} + 26.54_{\text{raphidophytes}} + 19.92_{\text{chlorophytes}} + 27.68_{\text{cryptophytes}} + 1.54_{\text{others}}$$

where others = dinoflagellates + prasinophytes + haptophytes.

Bay mouth:

$$\text{TEP} = 91.41 + 1.51_{\text{diatoms}} + 36.3_{\text{raphidophytes}} + 179.14_{\text{prasinophytes}} - 8.67_{\text{others}}$$

where others = dinoflagellates + haptophytes + chlorophytes + cryptophytes.

Total and TEP attached bacterial counts

Total numbers of bacteria were generally higher inside the bay and decreased towards the bay mouth. The numbers varied between 0.5 and 112.8×10^8 cells l^{-1} at Stns F1, F3, F4, F7, O2 and O6 (Fig. 8). Seasonal variations were concomitant with chl *a* and TEP concentrations, with low counts in winter and a maximum during the summer at most stations. Following the peak TEP concentrations, bacterial counts increased during late spring, with surface and subsurface bacterial densities being substantially high during summer. Bacterial numbers were higher in the inner bay (avg. $28.2 \pm 4.84 \times 10^8$ cells l^{-1}) and decreased towards the bay mouth (avg. $21.7 \pm 7.27 \times 10^8$ cells l^{-1}).

Correlation coefficients calculated to evaluate the association between TEP concentration and total number of bacteria yielded a significant relationship (Fig. 9a). The relationship was not altered either with or without inclusion of the anomalous TEP concentration of $1774 \mu\text{g Xanthan equiv. } l^{-1}$ recorded at Stn F3 in May 1998. A significant relationship ($r^2 = 0.146$, $n = 174$, $p < 0.01$) was obtained by including the anomalous TEP concentration.

TEP-C calculated following Engel & Passow (2001) ranged from $30.3 \mu\text{g C } l^{-1}$ at the surface at Stn F4 during February to as high as $1214 \mu\text{g C } l^{-1}$ at Stn F3 during May 1998, which corresponded with the highest chlorophyll concentration. In general, the TEP-C was low during winter and increased during spring, corresponding with chlorophyll concentrations. In the bay mouth, the TEP-C ranged from $9.8 \mu\text{g C } l^{-1}$ in February at the surface at Stn O6 to $249.2 \mu\text{g C } l^{-1}$ in May at the surface at Stn O2. With respect to percent of chlorophyll carbon (chl-C), TEP-C accounted for as low as 6% at Stn F4 at the surface during February to as high as 298.9% at Stn F7 at 5 m depth during January (Fig. 10) in the inner bay. TEP-C accounted from about 1% at 20 m at Stn O6 during July to as high as 298.9% of chl-C during January, May, June and October at 10 m depth at Stn O6.

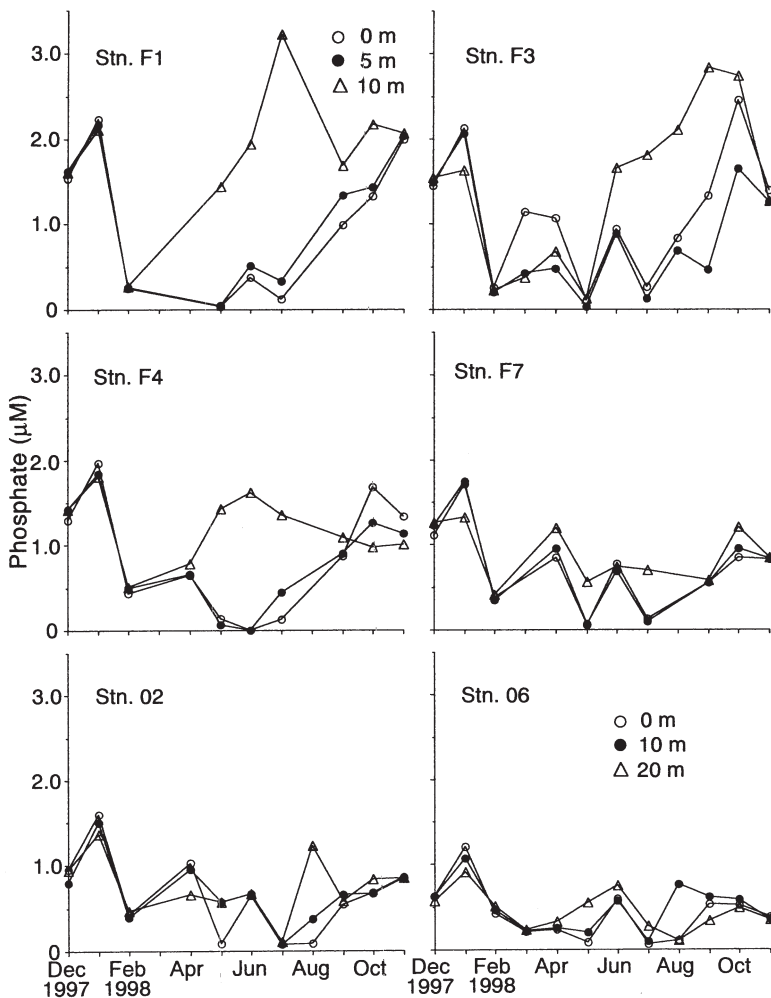


Fig. 4. Variations in phosphate concentrations (μM) in Tokyo Bay from December 1997 to November 1998. Note difference in sampling depths for Stn O6

Production of TOC and TEP by *Skeletonema costatum* and *Heterosigma akashiwo*

Growth as well as organic carbon production differed between the 2 species (Fig. 11a). As reflected from chl *a* concentrations, growth was much faster in *Skeletonema costatum*, which increased rapidly and reached a peak on Day 4. After this, the culture

entered stationary phase, as observed from the drop in chl *a*. In *Heterosigma akashiwo* however, chl *a* increased steadily up to Day 5 and leveled off thereafter. Although TEP production relative to chl *a* was higher during the early exponential phase in the 2 cultures, it decreased through the stationary and senescent phases in *H. akashiwo* (Fig. 11b). Despite a similar trend of higher TEP production relative to chl *a* during

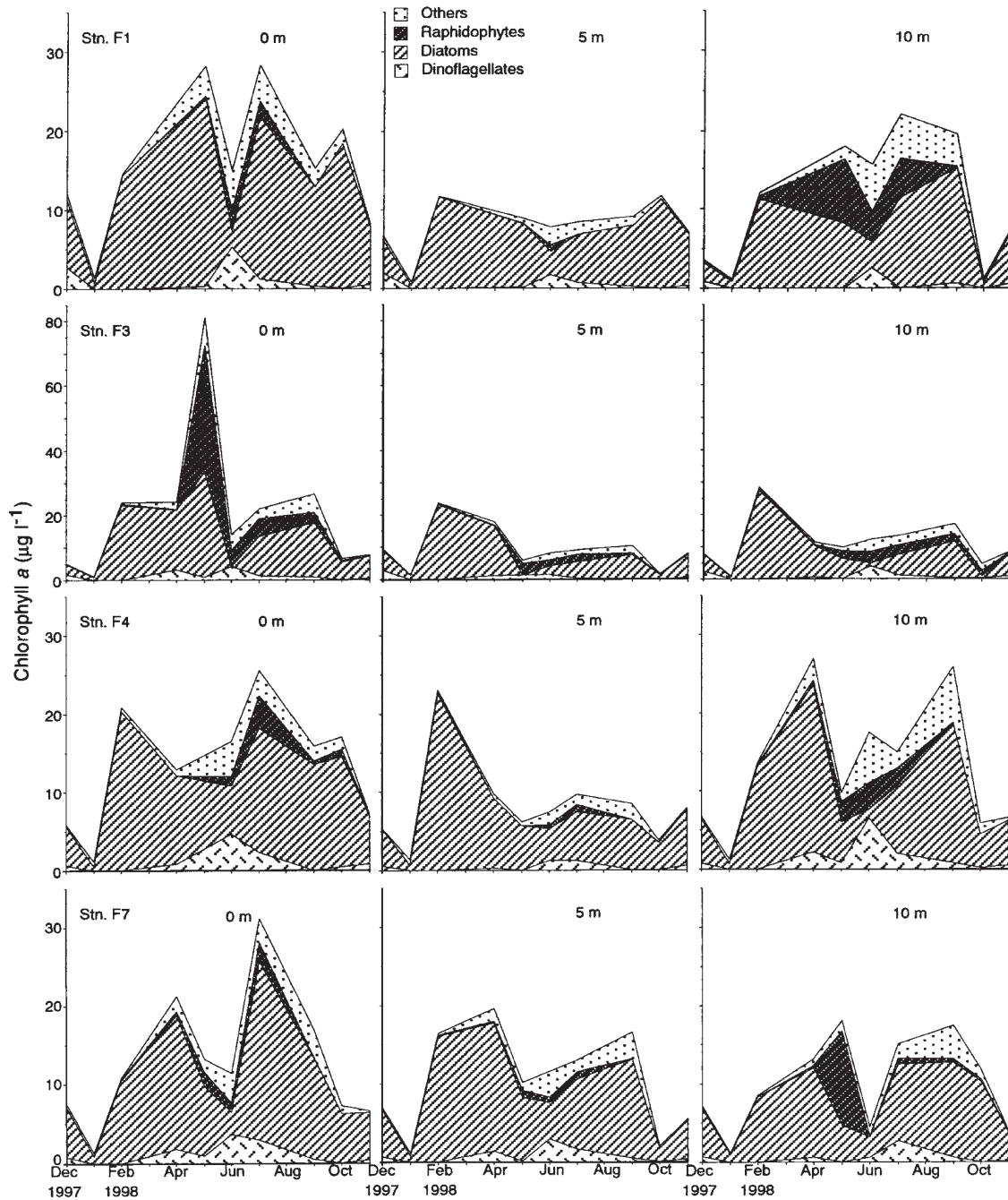


Fig. 5. Variations in chlorophyll *a* and phytoplankton composition at the inner bay stations from December 1997 to November 1998. Note the different y-axis scale for Stn F3

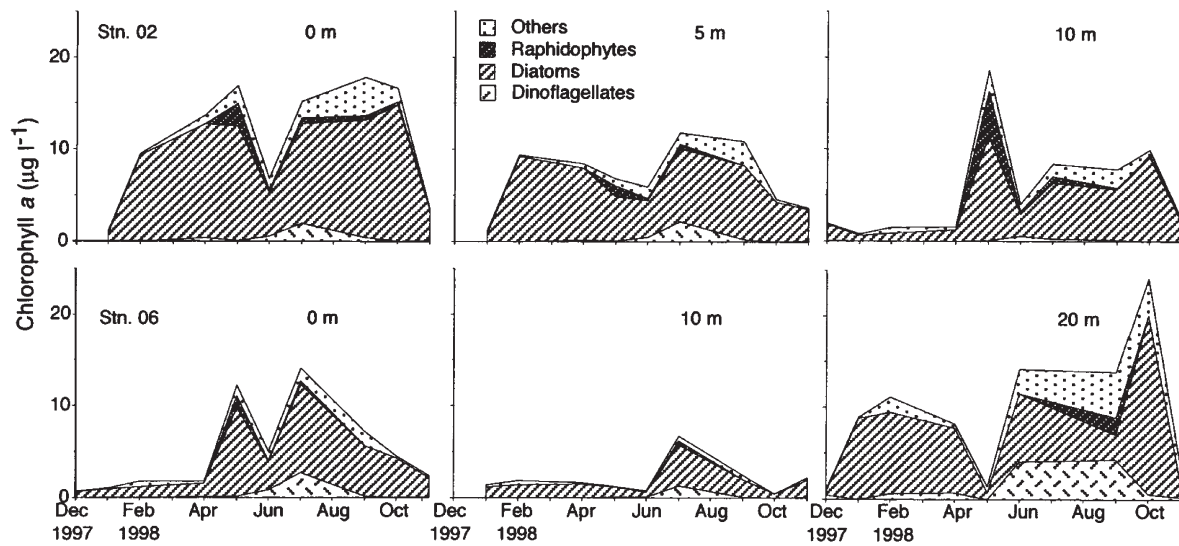


Fig. 6. Variations in chlorophyll *a* and phytoplankton composition at the bay mouth stations from December 1997 to November 1998. Note the different sampling depths for Stn 06

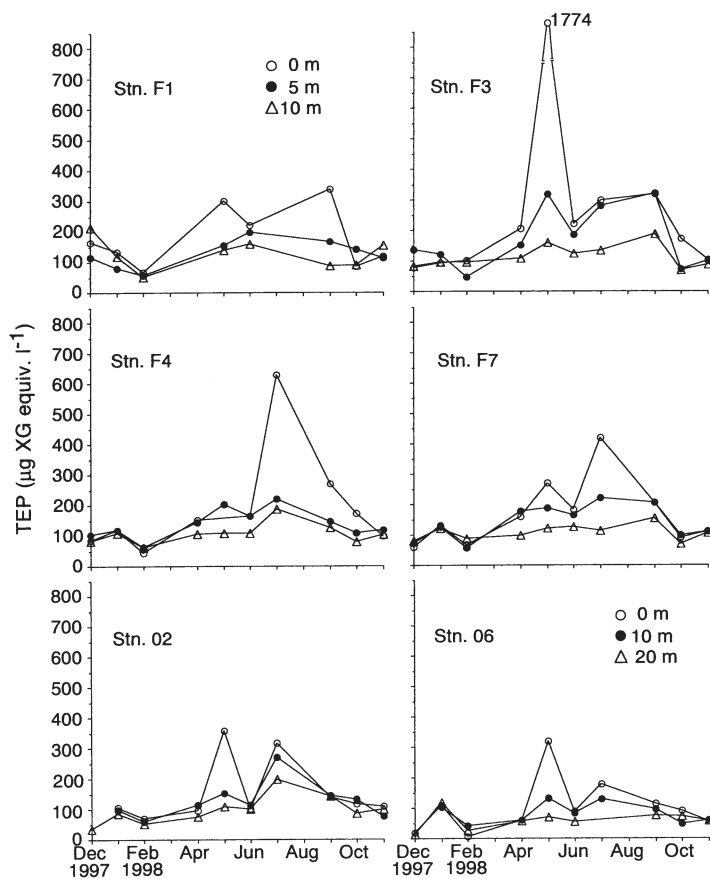


Fig. 7. Variations in transparent exopolymer particle (TEP) concentrations in Tokyo Bay from December 1997 to November 1998. Note differences in sampling depths for Stn 06

the early exponential phase in both the species, the values were consistently higher in *H. akashiwo* during all the phases, with a steady decrease in TEP production relative to chl *a* with growth. In *S. costatum* however, TEP production showed an increase when the culture entered senescence (Fig. 11b). TOC production in *S. costatum* followed a pattern similar to that of the growth rate, which increased exponentially and reached a peak on Day 3 after which there was a steep decline. In *H. akashiwo* however, TOC production was higher during the early exponential stage, decreased on Day 2 and varied little thereafter (Fig. 11c). TEP in relation to TOC production was higher in *H. akashiwo* during the early exponential phase and increased steadily with growth. In *S. costatum* however, TEP relative to TOC unusually increased during the

Table 2. Regression relationships between transparent exopolymer particle (TEP) concentration and biomass (chl *a*) of the phytoplankton groups observed in Tokyo Bay, Japan, during this study. Significant at *** $p \leq 0.001$, ** $p \leq 0.01$ and * $p \leq 0.05$

Group	Correlation coefficient (r^2)	
	Inner bay (n = 116)	Bay mouth (n = 56)
Diatoms	0.117***	0.134***
Raphidophytes	0.572***	0.139***
Dinoflagellates	0.017	0.016
Prasinophytes	0.049*	0.178***
Haptophytes	0.005	0.016
Chlorophytes	0.231***	0.013
Cryptophytes	0.392***	0.057**

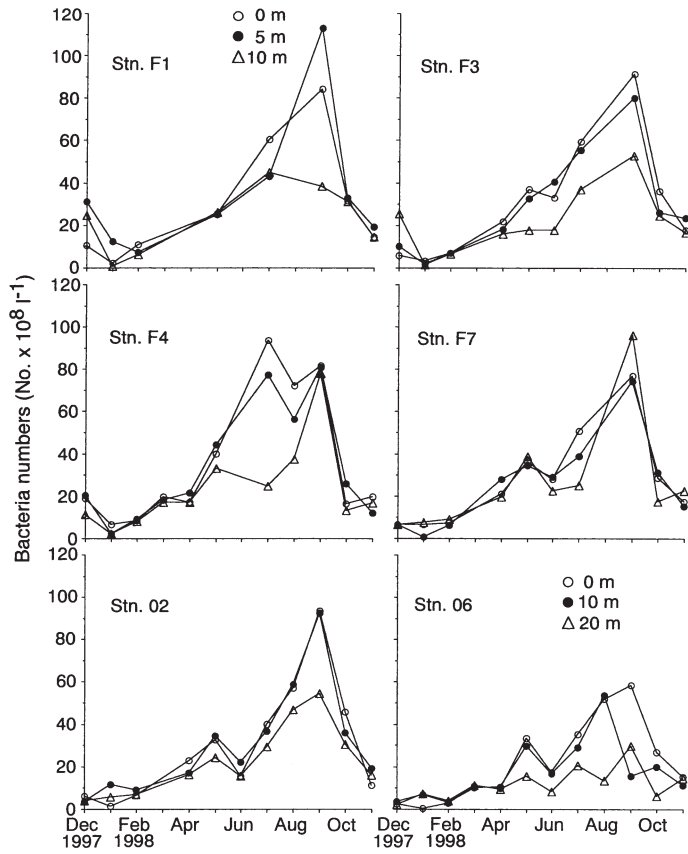


Fig. 8. Variations in bacterial numbers in Tokyo Bay from December 1997 to November 1998. Note differences in sampling depths for Stn 06

stationary phase (Fig. 11d). TEP-C as percent of chl-C was calculated using a C:chl *a* factor of 18 for *H. akashiwo* following Kohata & Watanabe (1987), and of 25 for *S. costatum* as per Strickland (1965). Similar to the trends of the TEP:chl *a* ratio, the TEP-C contribution was higher in *H. akashiwo* compared to that of *S. costatum*. TEP-C was about 360% (or 3.6 times) more than that of chl-C on Days 1 and 2, and decreased to about 98% on Day 6. In the case of *S. costatum*, the percent of TEP-C was 106 on Day 1, decreased to 29 on Day 3 and increased to 52% on Day 5.

DISCUSSION

The perennial supply of excessive nitrates and phosphates, which increased enormously between the 1960s and 1990s (Nomura 1995), has led to extensive eutrophication in Tokyo Bay, Japan. Ogawa & Ogura (1990) reported N:P ratios as high as 380 in the inner parts of the bay. N:P ratios during the present study were nearly always higher than 16 at all the stations in

the inner bay, indicating nitrogen-enriched conditions (prominent at Stns F1 and F3) with respective maxima of 415 and 191 recorded during May. Overall, N:P ratios were higher at the surface at Stn F1, while at Stn F3, the subsurface had higher ratios. As reviewed by Han & Furuya (2000), N and P are never limiting factors for phytoplankton growth in Tokyo Bay. Nutrients are added into this system mostly through the regular anthropogenic inputs, by regeneration from bottom sediments (Matsukawa & Sasaki 1990, Matsumura et al. 2001) and through advection by wind-induced water mixing (Miyata & Hattori 1986) albeit in small quantities.

As can be expected in eutrophic coastal regions, significant negative correlation obtained between nitrate and chl *a* ($r^2 = -0.529$, $n = 170$, $p \leq 0.001$) indicated

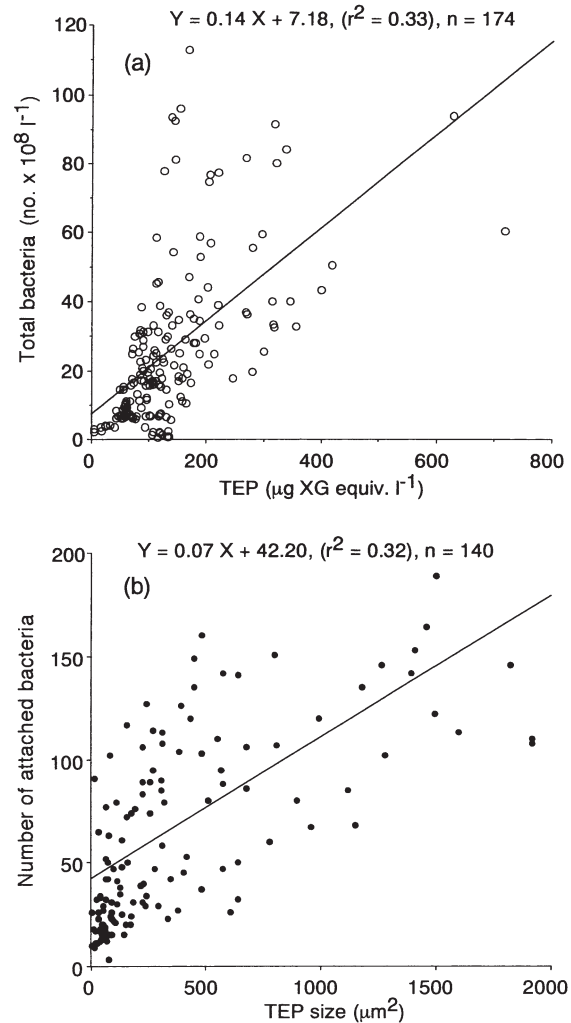


Fig. 9. Relationships between (a) TEP concentration and total number of bacteria, and (b) TEP size and number of bacteria attached to TEP from samples collected in Tokyo Bay from December 1997 to November 1998

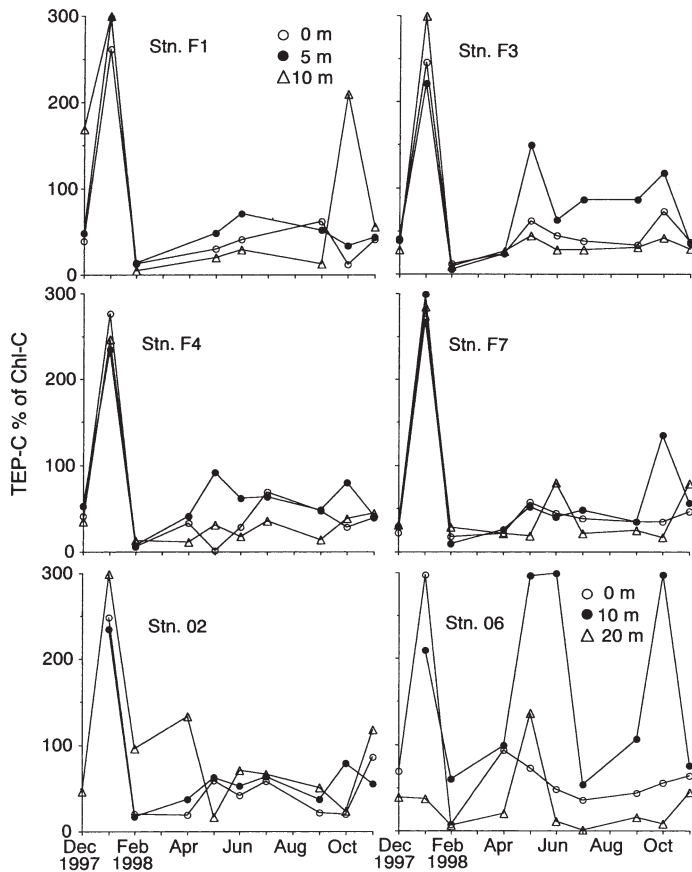


Fig. 10. Variations in TEP-C as percent of chlorophyll-C in Tokyo Bay during December 1997 to November 1998. Note differences in sampling depths for Stn 06

apparent utilization of nutrients resulting in the abundance of phytoplankton in the bay. Unlike the coastal regions of the north Adriatic (Schuster & Herndl 1995) and Kattegat (Mari & Burd 1998), intensity of eutrophication is far higher in Tokyo Bay, which is obvious from the high phytoplankton biomass (avg. $12 \mu\text{g l}^{-1}$ chl *a*) and frequent red tide events dominated by the diatom *Skeletonema costatum* and the raphidophyte *Heterosigma akashiwo* (Han et al. 1992, Nomura 1998). In most cases reported so far from coastal regions (Passow & Alldredge 1994, Ramaiah et al. 1995, Schuster & Herndl 1995), diatoms were the main contributors to the total phytoplankton biomass and subsequently to total chl *a*. In Tokyo Bay however, although 7 phytoplankton classes were observed during this study, and diatoms were dominant, seasonal occurrence

and bloom abundance of raphidophytes were marked. The abundance of the 2 dominant groups, viz., diatoms and raphidophytes, almost covaried with nutrients. Thus, changes in structure and abundance of phytoplankton community in eutrophic coastal waters are direct and linear responses to nutrient dynamics. Use of ChemTax analysis in our study enabled us to quantify the respective chl *a* concentrations of different groups in a phytoplankton community. This revealed that raphidophytes significantly contributed to the phytoplankton biomass. The importance of red tide organisms other than diatoms in coastal ecosystems modified due to anthropogenic input thus cannot be overlooked. Although Nomura & Yoshida (1997) reported microscopic-analysis-based phytoplankton composition from a location close to Stn F3 of this study, phytoplankton composition for the whole bay was not available prior to our study. Based on our HPLC pigment analysis, in addition to reporting the occurrence, we could evaluate the chlorophyll contribution of chrysophytes and cryptophytes, which are fragile and lost during preservation, and hence not available for identification by microscopic analyses. These groups were, however, not abundant throughout the year, and whenever they were present, the TEP also had higher concentrations. Thus, as also revealed by the multivariate analysis, these groups appear to be important as sources of TEP. From this observation, it is possible to suggest that in addition to the major role played by the diatoms, other little-known groups also have a significant influence on TEP formation.

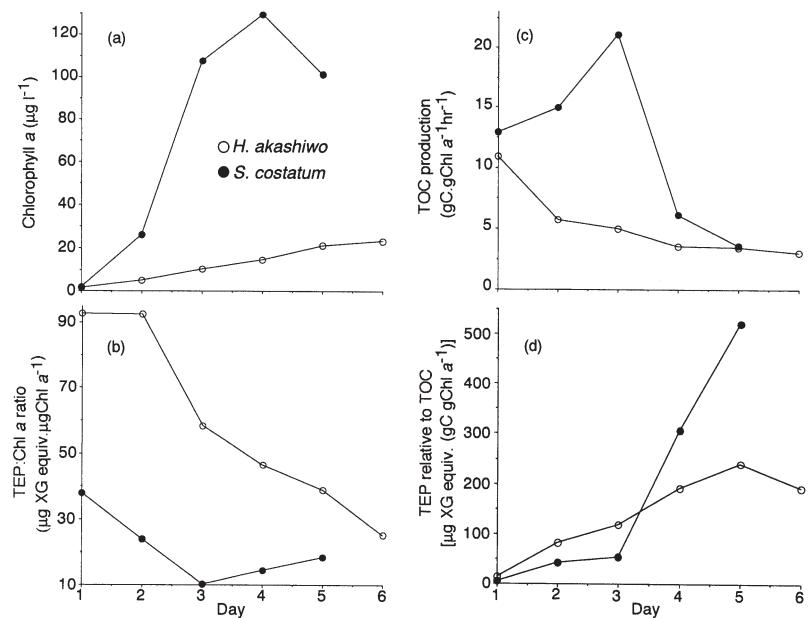


Fig. 11. Time course variations in (a) chlorophyll *a*, (b) TEP:chl *a* ratio, (c) total organic carbon (TOC), and (d) TEP relative to TOC in culture experiments with *Heterosigma akashiwo* and *Skeletonema costatum*

The high species-specific productivity of *Skeletonema costatum* and *Heterosigma akashiwo* (Han et al. 1992) and the substantially high primary productivity (Yamaguchi et al. 1991) in the bay led us to expect a concomitantly enhanced organic matter production during the bloom periods. TEP, formed from the dissolved organic matter largely released by phytoplankton (Allredge et al. 1993), exhibited a significant relationship with chl *a* in Tokyo Bay ($r^2 = 0.42$; $p < 0.001$). TEP peaks coincided with, or followed, the chl *a* highs observed during late spring and summer. As TEP is rich in both C and N, formation of TEP would ultimately lead to higher concentrations of inanimate organic particles that are important for marine snow formation (Turner 2002), various heterotrophic processes, and vertical flux (Passow et al. 2001), thereby influencing the biogeochemistry. Considering the higher than Redfield C:N ratio of TEP (Mari et al. 2001), the quantitative data on TEP and phytoplankton dynamics studied together in the eutrophicated Tokyo Bay indicate that particulate carbon formed during the periodic blooms is substantial and pivotal in the biogeochemistry of enclosed, anthropogenically perturbed environments.

In contrast to Santa Barbara Channel (Passow & Allredge 1995b), where small diatoms predominated, in Tokyo Bay diatoms and raphidophytes are most abundant. The TEP-chl *a* relationship is more or less similar for both regions (Fig. 12). However, TEP concentrations were significantly higher in Tokyo Bay, suggesting that TEP formation is primarily chl *a* dependent. A major difference in the Tokyo Bay scenario, however, is the anthropogenic input of nutrients that are responsible for phytoplankton blooms and higher chl *a* concentrations. In the Ross Sea (Hong et al. 1997) and Indian Ocean (Kumar et al. 1998), peak TEP abundance was associated with a *Phaeocystis* bloom, while in Monterey Bay (Passow & Allredge 1995b) it was associated with a floccing *Chaetoceros* bloom and upwelling. Variations in TEP concentrations in these regions thus confirm that composition of the existing phytoplankton population appears to be a major factor influencing TEP formation and concentrations. From our experiment it is also clear that TOC production as well as the TEP:chl *a* ratio were higher in *Heterosigma akashiwo* during the initial phases of growth. As there were no discrete TEP seen at the time of inoculation (the size of inoculum being 0.5 ml into 4 l of medium), we believe that the formation of TEP is rapid in the early stages of growth in *H. akashiwo*. The C:chl *a* ratio of 18 reported by Kohata & Watanabe (1987) for *H. akashiwo* which is much lower than for diatoms (Strickland 1965), suggests that *H. akashiwo* exudes a large part of the organic carbon as extracellular release. From the gradual increase in chl *a* concentra-

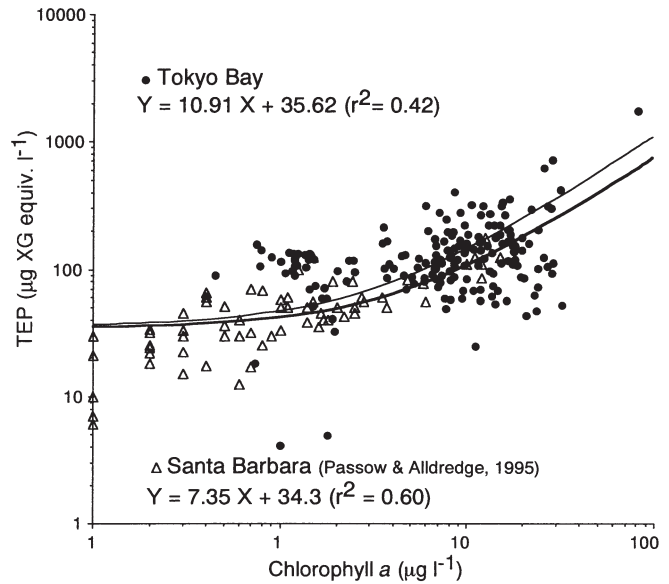


Fig. 12. Comparison of the relationship between TEP and chlorophyll *a* observed in Tokyo Bay during the present study and that reported by Passow & Allredge (1995) from Santa Barbara Channel

tions (Fig. 11a) and decrease in TEP:chl *a* ratio (Fig. 11b), it is apparent from our experiment that the exudates from this raphidophyte are higher during the early stages of growth. This is also confirmed by the TOC production rates (Fig. 11c). The role of *H. akashiwo* in contributing to C-rich TEP in eutrophic coastal environments was thus clearly elucidated in this study. The TEP-chl *a* relationship in Tokyo Bay suggested a dependence on chl *a* as well as phytoplankton composition, the latter being affected by the anthropogenic inputs into the bay.

Corzo et al. (2000) have recently examined the influence of N-nutrient sufficiency and limitation on TEP formation in laboratory cultures of the diatom *Chaetoceros calcitrans*. The net rate of TEP production was higher by 1 order of magnitude in N-limited conditions. Myklestad (1977) described the enhancement of extracellular polysaccharide production by the diatom at high N:P ratios. However, in coastal regions like the Tokyo Bay where nutrients are not a limiting factor, one ought to consider other relevant factors that control the organic matter production and TEP variations. The laboratory experiments conducted during this study were useful in delineating the influence of species-specific variation and growth stage of the phytoplankton. Williams (1990) reported that extracellular release rate was proportional to biomass during the logarithmic growth phase of the diatoms, and maximum exudation coincided with the stationary growth phase. This was also observed in our experiment with

Skeletonema costatum. In *Heterosigma akashiwo* however, TOC as well as the production of TEP relative to TOC were high during the exponential phase and decreased in the stationary phase.

Fogg (1983), Alldredge et al. (1993 & 1998), Mari (1999), Engel & Schartau (1999) and Corzo et al. (2000) have ascertained the major role of diatoms in extracellular organic matter and TEP production. Detailed analyses of one full annual cycle and statistical treatment of our field data revealed the influence of not only diatoms, but also of raphidophytes and, to some extent, chlorophytes, cryptophytes and prasinophytes in TEP formation. It is evident from this study that in eutrophicated coastal environments phytoplankton classes other than diatoms contribute significantly to chl *a* and TEP.

In accordance with the observation by Han & Furuya (2000), and in our experiment also, despite low biomass, the dissolved organic matter released by *H. akashiwo* was at least 3 times higher than that for *Skeletonema costatum*. In all likelihood, the bloom forming species are thus of greater importance in the production of C-rich TEP and the associated biogeochemical processes in coastal regions. During field sampling however, we observed that raphidophyte abundance was not always associated with high TEP and vice versa. The results of our experiment imply the importance of growth stage(s) and not biomass alone, in TEP formation, and help in understanding the non-commensurably lower TEP during periods of considerable phytoplankton biomass as was observed in Tokyo Bay. Although adequate for studying the annual variations, the once a month sampling may have missed out on the important active growth phases, thus leading to a visible anomaly in the covariation of TEP and chl *a*. As is well illustrated by Mari & Burd (1998), TEP appears to be closely related to pelagic primary production rather than phytoplankton biomass alone.

Considering the importance of TEP-bacterial interaction, free and TEP-attached bacterial numbers were also estimated during this study. Total bacteria were abundant in the Tokyo Bay when compared with other marine regions (Passow & Alldredge 1994, Kumar et al. 1998, Ramaiah et al. 2000), with higher counts at the inner bay stations. Higher counts at most stations observed during September coincided with lower values of TEP. It is well known that bacterioplankton often constitutes a bulk of carbon biomass in the marine environments and utilizes a large fraction of the sinking particulate organic matter (Smith et al. 1995, Azam & Long 2001). Bacterial colonization of TEP and microaggregates (Passow & Alldredge 1994, Mari & Kiørboe 1996, Brachvogel et al. 2001, Knoll et al. 2001) sufficiently proves the utilization of these particles as a substrate and possible source of nutrition. The demonstration of dissolution of aggregates by colonizing bacteria and utilization/dependence on the organic solute plumes from such aggregates (Kiørboe & Jackson 2001) is a step further in understanding the role these aggregates play in the biogeochemical processes. In addition, the review by Mari et al. (2001) clearly reveals the important role of TEP in the degradation and recycling of DOC. The significant correlations we observed between TEP and total bacteria and between TEP size and TEP-attached bacteria imply the dependence of bacteria on particulate organics in such eutrophicated coastal environs. Further, from our study, it is evident that the TEP-C sometimes far exceeded the chl-C. This was especially so during winter when both chl-C and bacterial counts were low. As also shown by Passow & Alldredge (1994) and Knoll et al. (2001), TEP are of importance to bacterial growth and abundance in Tokyo Bay as well. Low temperatures during winter and late autumn do not permit normal metabolism of bacteria, leading to an accumulation of TEP-C. To substantiate this observation, we

show, as an example, the pattern of bacterial number versus TEP-C as a percent of chl-C in Fig. 13.

In conclusion, the dynamics of TEP in coastal marine environments subjected to a variety of anthropogenic perturbances is quite complex and influenced by various biological, chemical and environmental factors. Sampling of 1 complete annual cycle and relevant laboratory experiments carried out in this study have helped in confirming the significance of phytoplankton groups other than diatoms in contributing to both chl *a* and TEP production in the eutrophicated Tokyo Bay and similar coastal environments. Further, the re-

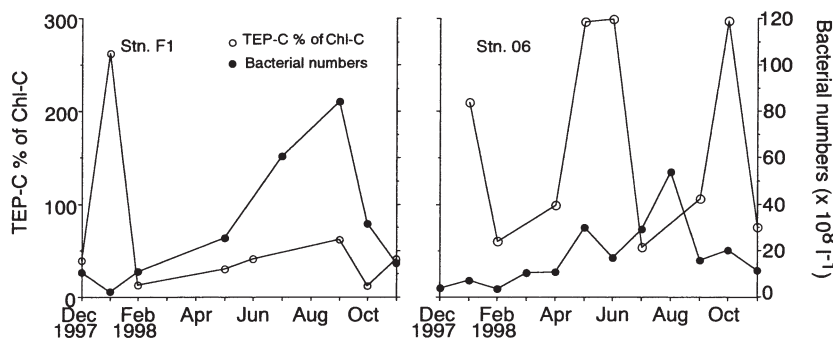


Fig. 13. Variations in TEP-C percent of chl-C and bacterial numbers at the surface at Stns F1 and 06 showing higher percentages of TEP-C during winter. As can be seen from Figs. 8 & 10, higher TEP-C percent during the periods with low bacterial counts suggest under-utilization (and accumulation) of TEP-C

sults of this study suggest that in addition to the biomass and composition, growth stage(s) of the bloom-forming organisms is an important criterion for organic matter production and TEP formation.

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