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Seasonal variations in microbial abundance and transparent exopolymer particle concentration in the sea surface microlayer of temperate coastal waters

Youta Sugai[1,*], Kenji Tsuchiya[1,2], Shinji Shimode[3], Tatsuki Toda[1]

[1]Laboratory of Restoration Ecology, Graduate School of Engineering, Soka University, 1-236 Tangi-cho, Hachioji, Tokyo 192-8577, Japan

[2]National Institute for Environmental Studies, 16-2 Onogawa, Tsukuba, Ibaraki 305-8506, Japan

[3]Graduate School of Environment and Information Science, Yokohama National University, 79-7 Tokiwadai, Hodogaya-Ku, Yokohama, Kanagawa 240-8501, Japan

ABSTRACT: The sea surface microlayer (SML) is defined as the less than 1 mm uppermost layer of the ocean water column, and it plays critical roles in global biogeochemical cycles and climate change. In the SML, organic matter and microorganisms are usually enriched compared to the subsurface water (SSW). To describe seasonality in the enrichment of microorganisms and transparent exopolymer particles (TEPs; gelatinous particles of acidic polysaccharides) in the SML of temperate coastal waters, annual monthly variations in microbial abundance and TEP concentration in the SML and SSW (0.5 m depth) were investigated in Sagami Bay, Japan, from September 2013 to September 2015. The abundance of microorganisms, such as bacteria and heterotrophic nanoflagellates, and TEP concentrations were significantly higher in the SML than in the SSW during the study period. No significant relationship between the enrichment of TEPs and microorganisms in the SML indicated that the enrichment of microorganisms in the SML is not enhanced by that of TEPs. By collecting monthly samples over a period of 2 consecutive years, seasonality in the behavior of microorganisms and TEPs in the SML was observed. Specifically, the enrichment of both microorganisms and TEPs in the SML was particularly high in spring (April 2014 and May 2015). The present study indicates that the formation of the unique SML of temperate coastal waters during spring phytoplankton blooms is possibly due to particular accumulation of TEPs.

KEY WORDS: Sea surface microlayer · SML · Microorganism · Transparent exopolymer particle · TEP · Seasonality · Temperate coastal water · Phytoplankton bloom

INTRODUCTION

The sea surface microlayer (SML) is defined as the less than 1 mm uppermost layer of the ocean water column (Liss & Duce 1997), located at the boundary interface between the atmosphere and the ocean. Thus, the SML plays critical roles in global biogeochemical cycles and climate change through the regulation of the air–sea exchange of atmospheric trace gases and aerosol particles (Cunliffe et al. 2013). The SML forms a physically and chemically distinct environment compared to the subsurface water (SSW) due to its proximity to the atmosphere, which makes the SML an extreme marine environment (Zhang et al. 1998, 2003). For example, the SML is subject to a high magnitude of salinity change and is exposed to high levels of ultraviolet and visible solar radiation.

Even under such a harsh environment, microorganisms exist in the SML. Conrad & Seiler (1988), Frost (1999), and Upstill-Goddard et al. (2003) indicated that microbial activity in the SML significantly changes the air–sea gas transfer velocity of atmospheric trace gases such as methane, carbon monoxide, and nitrous oxide. Furthermore, functional

*Corresponding author: youtasugai@soka.gr.jp

gene analysis detected methane-oxidizing and carbon monoxide-dehydrogenating bacteria in the SML (Cunliffe et al. 2008). Thus, microbial communities in the SML are actively involved in the biogeochemical cycling of both the atmosphere and the ocean (Cunliffe et al. 2011).

Previous studies reported significantly higher microbial abundance in the SML than in the SSW of various ocean areas (e.g. Obernosterer et al. 2005, 2008, Joux et al. 2006, Nakajima et al. 2013) (Table 1). The enrichment of microorganisms in the SML results from higher microbial production in the SML relative to the SSW and/or the upward transport of microorganisms from the SSW to the SML through physical processes such as the adsorption of microbial cells on rising bubbles and buoyant particles (Liss & Duce 1997). In tropical coral reef waters, particularly high enrichment of microorganisms in the SML was observed, probably because gel-like coral mucus colonized by microorganisms ascended in the water column and accumulated in the SML (Nakajima et al. 2013).

Previous studies reported significant enrichment of transparent exopolymer particles (TEPs) in the SML (Wurl & Holmes 2008, Wurl et al. 2009), and thus the SML is referred to as a gelatinous biofilm (Cunliffe & Murrell 2009). TEPs are one of the most ubiquitous gelatinous particles in the ocean and operationally defined as particles of acidic polysaccharides stainable with alcian blue solution (Alldredge et al. 1993). In the water column, TEPs are mainly formed abiotically by the spontaneous coagulation of dissolved precursors released by phytoplankton (Myklestad 1995, Chin et al. 1998, Zhou et al. 1998, Passow 2000). TEPs readily form aggregates with solid particles including microbial cells through passive adsorption to the sticky surface of the TEPs (Alldredge et al. 1993, Passow & Alldredge 1994, Mari & Kiørboe 1996, Verdugo et al. 2004). Azetsu-Scott & Passow (2004) experimentally demonstrated that TEP-based aggregates containing pico- and micro-sized particles ascend in the water column because of the low density of the TEPs. These studies suggest that microbial abundance in

Table 1. Comparative summary of the mean enrichment factors (the ratio of values in the surface microlayer to those in the subsurface water) of transparent exopolymer particle (TEP) concentration and microbial abundance in various studies. Chl *a*: chlorophyll *a* concentration; ANF: autotrophic nanoflagellate abundance; bacteria: bacterial abundance; HNF: heterotrophic nanoflagellate abundance

Study sites	TEP	Chl *a*	ANF	Bacteria	HNF	References
North Atlantic Ocean (Block Island Sound–Azores)		1.2				Sieburth et al. (1976)
Palo Alto Salt Marsh, USA				1.7		Harvey & Young (1980)
Sequim Bay, USA		1.8				Hardy & Apts (1984)
East Coast of Isla Cedros, Mexico		1.0		1.4		Carlucci et al. (1986)
Sequim Bay, USA		0.5				Hardy & Apts (1989)
Stony Brook Harbor, USA				1.7		Kuznetsova & Lee (2001)
Coast of NW Mediterranean Sea, France and Spain		1.8		1.1	5.4	Agogué et al. (2004)
North Atlantic Ocean (Woods Hole–Sargasso Sea)				1.4		Kuznetsova et al. (2004)
Coast of NW Mediterranean Sea, France and Spain		2.0				Momzikoff et al. (2004)
Bay of Banyuls-sur-Mer, France				1.2	2.7	Obernosterer et al. (2005)
Bay of Banyuls-sur-Mer, France		1.3	3.7	1.1	3.7	Joux et al. (2006)
South Pacific Ocean (West of Marquise Islands)		1.2	1.2	1.0	1.8	Obernosterer et al. (2008)
Western Mediterranean Sea (Algerian basin)				1.1		Reinthaler et al. (2008)
Singapore Strait, Singapore	1.3					Wurl & Holmes (2008)
Santa Barbara Channel, USA	1.7					Wurl et al. (2009)
Coast of Southern Baltic Sea, Germany				2.1		Stolle et al. (2010)
North Pacific, offshore Hawaii, and Arctic Ocean (non-slick)	2.0					Wurl et al. (2011)
North Pacific, offshore Hawaii, and Arctic Ocean (slick)	10.5					Wurl et al. (2011)
West coast of Bidong Island, Malaysia		7.1	18.8	6.0	22.6	Nakajima et al. (2013)
Central Arctic Ocean (open sea)	1.1			1.0		Galgani et al. (2016)
North Pacific, South China Sea, and Baltic Sea (non-slick)	1.6			1.2		Wurl et al. (2016)
North Pacific, South China Sea, and Baltic Sea (slick)	12.5			2.8		Wurl et al. (2016)
Sagami Bay, Japan[a]	1.4	1.5	2.9	1.6	4.0	Present study
Sagami Bay, Japan[b]	6.1	7.4	40.8	7.8	37.0	Present study
Sagami Bay, Japan[c]	3.0	3.9	4.6	6.9	4.7	Present study

[a]During the study period except in April 2014 and May 2015
[b]Only in April 2014
[c]Only in May 2015

the SML increases due to the upward transport of the aggregates of TEPs and microorganisms in the water column, and microbial abundance in the SML may increase more because of the high activity of TEP-attached microorganisms (Simon et al. 2002). Thus, the enrichment of microorganisms in the SML may be enhanced by that of TEPs. Recent studies observed the biofilm-like properties of the SML with excessive accumulation of both TEPs and microorganisms in natural slicks (visible wave-damped areas at the sea surface caused by the accumulation of surface-active organic matter) (Wurl et al. 2016).

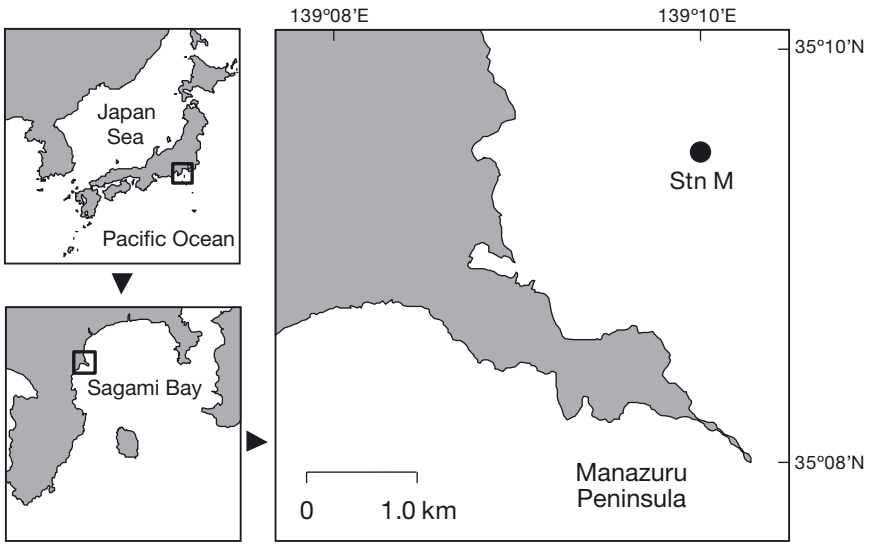

Fig. 1. Location of sampling station (Stn M) in Sagami Bay, Japan

In the water column, the peaks of TEP concentration are generally associated with phytoplankton blooms (Passow & Alldredge 1994, Mari & Kiørboe 1996, Mari & Burd 1998, Passow et al. 2001). Thus, enrichment of TEPs and microorganisms in the SML of temperate coastal waters may vary temporally due to seasonality in TEP concentration in the water column, and high enrichment in the SML may be observed during phytoplankton blooms. Previous studies have conducted opportunistic samplings of the SML, but time-series observation at the same sampling station is required to understand the complex and dynamic temporal interactions in the SML (Cunliffe et al. 2013). Therefore, the present study aimed to describe seasonal variations in the enrichment of TEPs and microorganisms in the SML of temperate coastal waters.

MATERIALS AND METHODS

Study area and samplings

This study was conducted at Station M (Stn M, 120 m depth) in the temperate coastal waters of Sagami Bay, Japan (Fig. 1); details are described in Sugai et al. (2016). Surveys were carried out monthly from September 2013 to September 2015 on the R/V 'Tachibana' of the Manazuru Marine Center for Environmental Research and Education, Yokohama National University. Surface seawater for water temperature and salinity measurement was collected using a bucket. Water temperature was immediately measured with a mercury thermometer, and salinity was measured in a laboratory within 1 h with an inductive salinometer (inductively coupled salinometer model 601 Mk IV; Yeo-Kal Electronics).

SML samples were collected from the bow of the research vessel (12 m long) on the leeward side, using a mesh screen sampler to collect microorganisms within a wide range of sizes and obtain a large number of samples over a relatively short period (Garrett 1965, Agogué et al. 2004, Momzikoff et al. 2004). Nylon mesh (mesh size: 1.25 mm; nylon diameter: 0.43 mm) was stretched over a 60 × 80 cm plastic frame. The screen was lowered vertically through the ocean surface 1.5 to 2 m away from the research vessel to minimize the disruption of the natural SML near the hull, raised horizontally through the ocean surface, and tilted to drain the SML sample into 2 l polypropylene bottles. Approximately the first 100 ml of seawater draining from the screen was discarded to prevent the inclusion of seawater adhering to the frame (Obernosterer et al. 2005). To collect a 2 l SML sample, about 20 successive dips were conducted within 1.5 h. The thickness of the collected SML was calculated to be 380 ± 9 µm following Cunliffe & Wurl (2014). SSW samples were collected at 0.5 m depth using a horizontal Niskin bottle. Seawater samples except for the analysis of TEPs were pre-filtered through 180 µm nylon mesh to remove large plankton and debris.

Wind velocity data were obtained from the Japan Meteorological Agency (www.jma.go.jp/) at the Odawara Office (35° 16′ 36″ N, 139° 09′ 18″ E), located less than 15 km from our sampling station.

Analytical methods

The analyses of inorganic nutrients, dissolved organic carbon (DOC), chlorophyll *a* (chl *a*) concentration, bacterial abundance, and the abundance of autotrophic and heterotrophic nanoflagellates (ANFs and HNFs) are described in detail in Sugai et al. (2016). Briefly, inorganic nutrient samples were filtered through 0.45 µm pore size filters. Nitrate (NO_3) and phosphate (PO_4) concentrations were measured according to Parsons et al. (1984). DOC samples were filtered through 0.22 µm pore size filters. DOC concentration was measured by a high temperature Pt catalyst oxidation method following Ogawa et al. (2003). The detection limit of DOC concentration was 0.001 mgC l^{-1}. Chl *a* samples were filtered onto Whatman GF/F filters (GE Healthcare Life Sciences). Chl *a* pigment was extracted with *N*,*N*-dimethylformanide (Suzuki & Ishimaru 1990), and chl *a* concentration was determined fluorometrically as described by Welschmeyer (1994). Bacterial abundance samples were fixed with buffered and pre-filtered formaldehyde (2% final concentration). The fixed samples were stained according to Shibata et al. (2006) and filtered onto 0.2 µm pore size filters. Bacterial cells were counted using an epifluorescence microscope (Axioskop 2 plus; Zeiss) at 1000× magnification. ANF and HNF abundance samples were fixed with glutaraldehyde (1% final concentration). The fixed samples were filtered onto 0.8 µm pore size filters and stained following Sherr et al. (1993). ANF and HNF cells were counted using the epifluorescence microscope.

Triplicate subsamples of 50 ml for the analysis of chromophoric dissolved organic matter (CDOM) were filtered through 0.22 µm pore size filters (Durapore PVDF; Millipore) under dark conditions. Spectral absorbance was measured using a single-beam UV-visible spectrophotometer (UV-2450; Shimadzu) with 10 cm pathlength quartz cells referenced against Milli-Q water. Spectral scans were carried out from 200 to 800 nm, and the baseline for each spectrum was corrected for absorbance at 750 nm (Blough & Del Vecchio 2002). The absorption coefficient of CDOM at wavelength λ ($a_{CDOM}[\lambda]$) was determined, as described by:

$$a_{CDOM}(\lambda) = 2.303\, D(\lambda)\, l^{-1}$$

where $D(\lambda)$ is the absorbance at wavelength λ and l is the quartz cell pathlength (m).

TEP concentration was measured colorimetrically by a dye-binding assay (Passow & Alldredge 1995, Engel 2009). Triplicate subsamples of 40 to 100 ml were filtered onto 0.4 µm pore size Nuclepore filters (Track-Etched Membrane Black; GE Healthcare Life Sciences) under low, constant vacuum (<20 kPa). The filters were then stained with 500 µl of 0.02% (w/v) alcian blue solution adjusted to a pH of 2.5. After being rinsed twice with 1 ml of Milli-Q water, the filters were stored at −20°C until analysis. The filters were soaked for 3 h in 6 ml of 80% (v/v) sulfuric acid to dissolve dye prior to the spectrophotometrical measurement of the absorbance of the sulfuric acid solution at 787 nm. TEP concentration was expressed in mg of an acidic polysaccharide, Xanthan Gum (Sigma), equivalent per liter (mg $X_{eq.}$ l^{-1}). The detection limit of TEP concentration was 0.04 mg $X_{eq.}$ l^{-1}.

Triplicate subsamples of 100 to 400 ml for the analysis of particulate organic carbon (POC) were filtered onto pre-combusted (450°C; 4 h) Whatman GF/F filters. The filters were then treated with HCl fumes for 2 h to remove inorganic carbon, dried at 60°C for 12 h, and stored in a desiccator until analysis. POC concentration was determined using an organic elemental analyzer (Flash 2000; Thermo Scientific).

Data analysis

Enrichment in the SML was expressed as the enrichment factors (EFs), defined as the ratio of values in the SML to those in the SSW (Sieburth et al. 1976, Harvey & Young 1980). Wilcoxon signed-rank test was applied to the monthly medians of each parameter between the SML and SSW. Correlation analysis was performed using Pearson's correlation coefficient. A probability of $p < 0.05$ was considered significant in all statistical analyses.

RESULTS

Physical and meteorological parameters

Surface water temperature gradually decreased from 26.1°C in September 2013 to 13.0°C in March 2014 and increased to 24.8°C in August 2014 (Fig. 2a). Afterward, surface water temperature showed similar variation from August 2014 to September 2015. Surface salinity of >32.0 was observed during the study period except in October 2013 (30.6) and July 2015 (30.4) (Fig. 2b). Mean wind velocity during sampling was <4.0 m s^{-1} throughout the study period except in December 2014 (5.4 m s^{-1}) and March 2015 (7.3 m s^{-1}) (Fig. 2c).

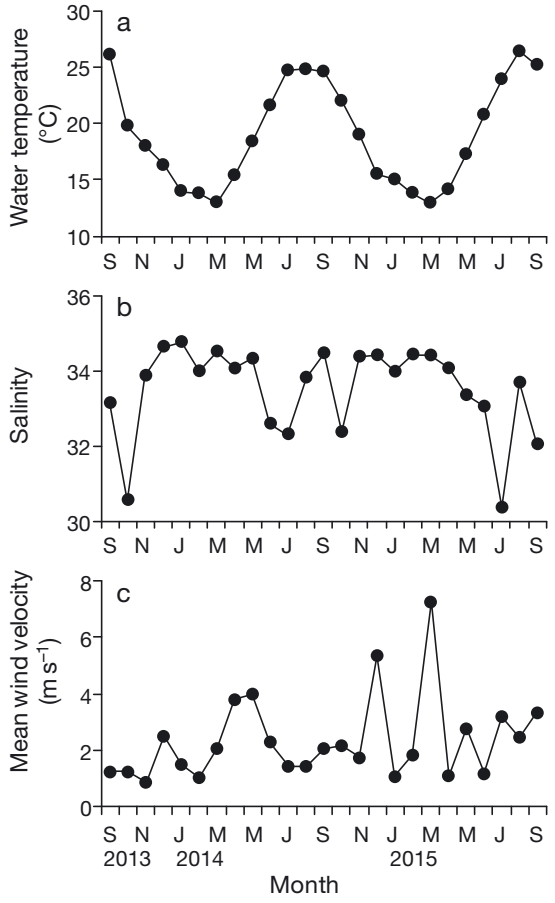

Fig. 2. Monthly variations of (a) surface water temperature, (b) surface salinity, and (c) mean wind velocity during the sampling period

Chemical and biological parameters in SML and SSW

NO_3 concentration ranged from under the detection limit to 14.4 µM in the SML and from under the detection limit to 13.2 µM in the SSW (Fig. 3a). During the study period, NO_3 concentration in the SML (median: 3.04 µM) was slightly but significantly higher than in the SSW (median: 2.27 µM) (Wilcoxon signed-rank test, $p < 0.001$). PO_4 concentration fluctuated between 0.02 and 0.74 µM in the SML and between 0.02 and 0.69 µM in the SSW (Fig. 3b). Significantly higher PO_4 concentrations were observed in the SML (median: 0.19 µM) than in the SSW (median: 0.14 µM) (Wilcoxon signed-rank test, $p < 0.01$).

DOC concentrations were relatively high in April 2014 (6.60 mgC l^{-1}), May 2014 (3.36 mgC l^{-1}), and May 2015 (6.29 mgC l^{-1}) in the SML and in June 2014 (1.67 mgC l^{-1}) and May 2015 (2.18 mgC l^{-1}) in the SSW (Fig. 3c). Significantly higher DOC concentrations were observed in the SML (median: 1.20 mgC l^{-1}) than in the SSW (median: 1.11 mgC l^{-1}) (Wilcoxon signed-rank test, $p < 0.001$). $a_{CDOM}(320)$ showed relatively high values in April 2014 (2.04 m^{-1}) and May 2015 (2.67 m^{-1}) in the SML and in May 2015 (1.50 m^{-1}) in the SSW (Fig. 3d). $a_{CDOM}(320)$ in the SML (median: 0.66 m^{-1}) was significantly higher compared to the SSW (median: 0.56 m^{-1}) (Wilcoxon signed-rank test, $p < 0.001$).

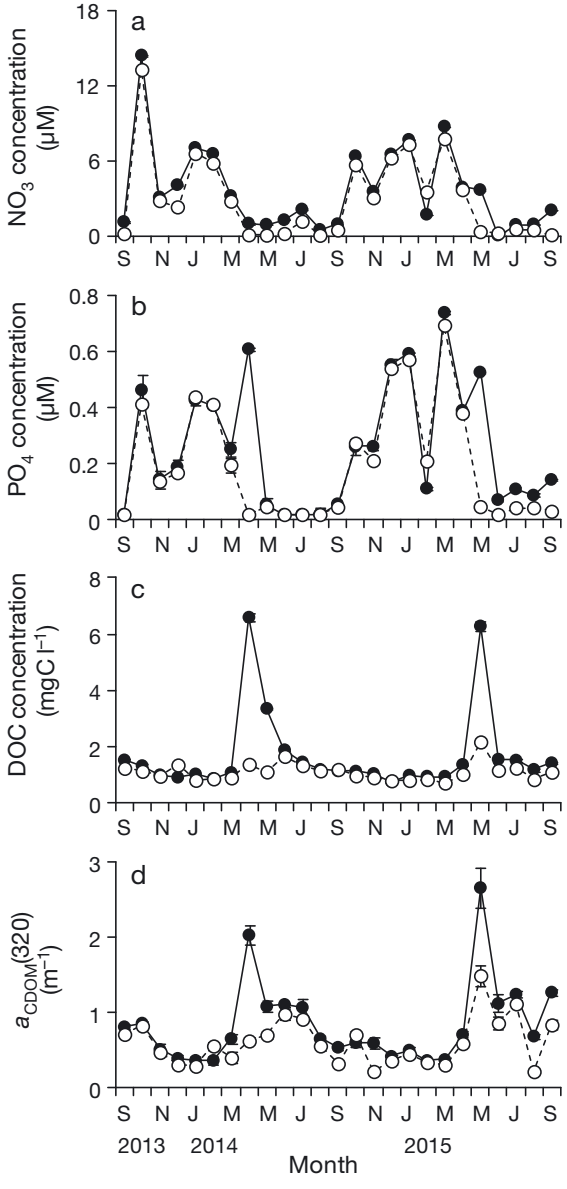

Fig. 3. Monthly variations (mean ± SD) of (a) nitrate (NO_3) concentrations, (b) phosphate (PO_4) concentrations, (c) dissolved organic carbon (DOC) concentrations, and (d) the absorption coefficients of chromophoric dissolved organic matter at 320 nm ($a_{CDOM}[320]$) in the surface microlayer (●) and subsurface water (○)

TEP concentrations were relatively high in April 2014 and May 2015 in the SML (5.44 and 2.11 mg $X_{eq.}$ l^{-1}, respectively) and SSW (0.89 and 0.71 mg $X_{eq.}$ l^{-1}, respectively) (Fig. 4a). Significantly higher TEP concentrations were observed in the SML (median: 0.32 mg $X_{eq.}$ l^{-1}) than in the SSW (median: 0.26 mg $X_{eq.}$ l^{-1}) (Wilcoxon signed-rank test, $p < 0.001$). POC concentrations showed relatively high values in April 2014 (5.17 mgC l^{-1}), May 2015 (1.98 mgC l^{-1}), June 2015 (1.60 mgC l^{-1}), and September 2015 (1.11 mgC l^{-1}) in the SML (Fig. 4b). POC concentration in the SML (median: 0.50 mgC l^{-1}) was significantly higher than in the SSW (median: 0.27 mgC l^{-1}) (Wilcoxon signed-rank test, $p < 0.001$).

Chl *a* concentration was relatively high in April 2014 (129 µg l^{-1}), May 2015 (31.4 µg l^{-1}), and June 2015 (25.9 µg l^{-1}) in the SML and in September 2013 (8.82 µg l^{-1}), March 2014 (9.34 µg l^{-1}), April 2014 (17.5 µg l^{-1}), February 2015 (11.8 µg l^{-1}), May 2015 (7.99 µg l^{-1}), and September 2015 (10.5 µg l^{-1}) in the SSW (Fig. 4c). Significantly higher chl *a* concentration was observed in the SML (median: 3.67 µg l^{-1}) than in the SSW (median: 3.25 µg l^{-1}) (Wilcoxon signed-rank test, $p < 0.05$). ANF abundance showed relatively high values in April 2014 (46.3×10^6 cells l^{-1}), May 2015 (25.5×10^6 cells l^{-1}), June 2015 (17.4×10^6 cells l^{-1}), and September 2015 (21.0×10^6 cells l^{-1}) in the SML and in July 2014 (5.48×10^6 cells l^{-1}), May 2015 (5.48×10^6 cells l^{-1}), and September 2015 (8.51×10^6 cells l^{-1}) in the SSW (Fig. 4d). ANF abundance in the SML (median: 4.33×10^6 cells l^{-1}) was significantly higher than in the SSW (median: 1.35×10^6 cells l^{-1}) (Wilcoxon signed-rank test, $p < 0.001$).

Bacterial abundance was relatively high in April 2014 (16.1×10^9 cells l^{-1}), July 2014 (6.56×10^9 cells l^{-1}), May 2015 (10.4×10^9 cells l^{-1}), and September 2015 (4.97×10^9 cells l^{-1}) in the SML and in July 2014 (5.41×10^9 cells l^{-1}) in the SSW (Fig. 4e). Significantly higher bacterial abundance was observed in the SML (median: 2.52×10^9 cells l^{-1}) than in the SSW (median: 1.33×10^9 cells l^{-1}) (Wilcoxon signed-rank test, $p < 0.001$). HNF abundance showed relatively high values in April 2014 (69.9×10^6 cells l^{-1}), May 2015 (24.0×10^6 cells l^{-1}), and September 2015 (21.6×10^6 cells l^{-1}) in the SML and in July 2014 ($4.73 \times$

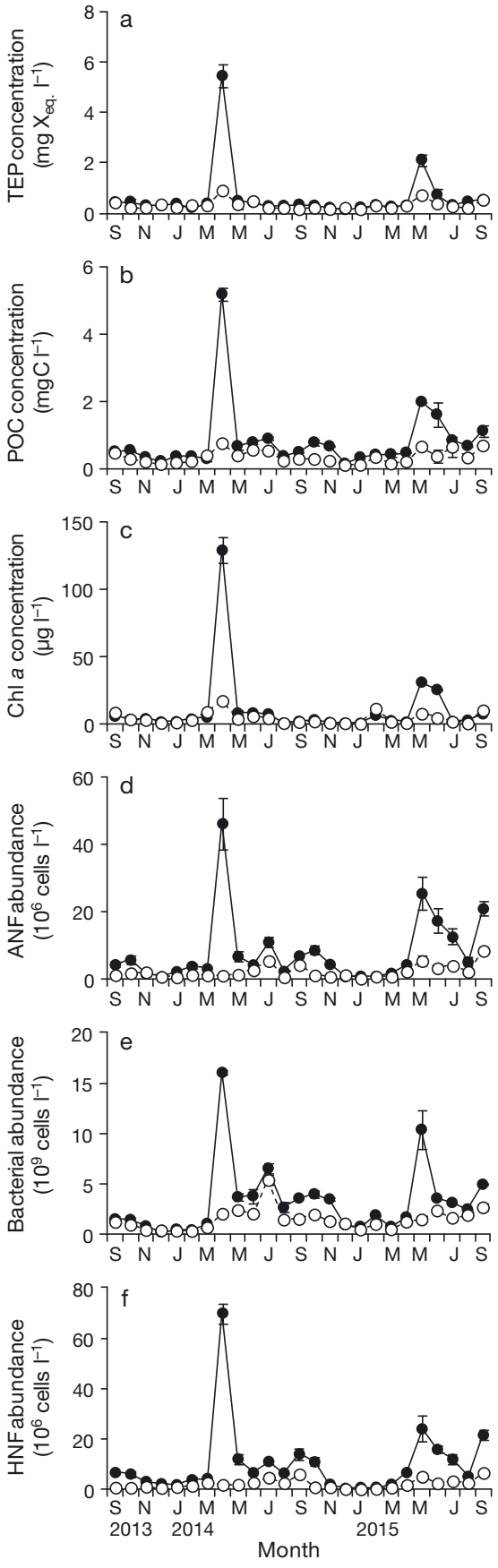

Fig. 4. Monthly variations (mean ± SD) of (a) transparent exopolymer particle (TEP) concentrations, (b) particulate organic carbon (POC) concentrations, (c) chlorophyll *a* (chl *a*) concentrations, (d) autotrophic nanoflagellate (ANF) abundance, (e) bacterial abundance, and (f) heterotrophic nanoflagellate (HNF) abundance in the surface microlayer (●) and subsurface water (○)

10^6 cells l^{-1}), September 2014 (6.05 × 10^6 cells l^{-1}), May 2015 (5.10 × 10^6 cells l^{-1}), and September 2015 (6.62 × 10^6 cells l^{-1}) in the SSW (Fig. 4f). HNF abundance in the SML (median: 6.50 × 10^6 cells l^{-1}) was significantly higher than in the SSW (median: 1.83 × 10^6 cells l^{-1}) (Wilcoxon signed-rank test, $p < 0.001$).

Relationships between parameters

Significant positive correlations were observed between each chemical and biological parameter in the SML and SSW during the study period except in April 2014 and May 2015 (Table 2). In April 2014, parameters except NO_3 concentration showed higher values in the SML than in the SSW. In May 2015, all parameters in the SML were higher compared to the SSW. Salinity did not show a significant relationship with TEP concentrations in the SML or SSW during the study period.

No significant relationship was observed between wind velocity and the EFs of TEP concentration or microbial abundance during the study period except in April 2014 and May 2015. During that period, the EF of TEP concentrations were significantly positively correlated with that of chl *a* concentration ($r = 0.469$, $p < 0.05$, $n = 23$; Fig. 5a). However, the EF of TEP concentrations did not show significant relationships with those of ANF abundance ($p = 0.19$; Fig. 5b), bacterial abundance ($p = 0.14$; Fig. 5c), or HNF abundance ($p = 0.41$; Fig. 5d).

Table 2. Pearson's correlation coefficients (r) of each chemical and biological parameter between the sea surface microlayer (SML) and subsurface water (SSW) during the study period except in April 2014 and May 2015. n: number of data points; NO_3: nitrate; PO_4: phosphate; DOC: dissolved organic carbon; $a_{CDOM}(320)$: absorption coefficient of chromophoric dissolved organic matter at 320 nm; TEP: transparent exopolymer particle; POC: particulate organic carbon; chl *a*: chlorophyll *a*; ANF: autotrophic nanoflagellate; HNF: heterotrophic nanoflagellate

Parameter	r	n	p
NO_3 concentration	0.979	23	<0.001
PO_4 concentration	0.982	23	<0.001
DOC concentration	0.443	23	<0.05
$a_{CDOM}(320)$	0.846	23	<0.001
TEP concentration	0.556	23	<0.01
POC concentration	0.634	23	<0.01
Chl *a* concentration	0.439	23	<0.05
ANF abundance	0.837	23	<0.001
Bacterial abundance	0.919	23	<0.001
HNF abundance	0.799	23	<0.001

DISCUSSION

Enrichment in SML except in April 2014 and May 2015

The mean EFs of TEP concentration and the abundance of each microbial community during the study period except in April 2014 and May 2015 were within the ranges reported by previous studies except Wurl et al. (2011, 2016), who collected slick samples and Nakajima et al. (2013), who conducted their study in tropical coral reef waters (Table 1). During the study period, significant positive correlations were observed between each parameter in the SML and SSW (Table 2). The relationships show that the parameters in the SML varied seasonally similar to those in the SSW. Furthermore, wind velocity did not show a significant relationship with the EFs of TEP concentration and microbial abundance during the study period, although previous studies have indicated the removal of particles from the SML due to deep mixing and bubble bursting under strong wind conditions (e.g. Obernosterer et al. 2008, Wurl et al. 2009).

Wetz et al. (2009) reported a significant positive correlation between salinity and TEP concentration in a river-dominated estuary, suggesting the role of cation availability in TEP formation. However, salinity showed no significant relationship with TEP concentrations in the SML or SSW during our study period. Generally, high TEP concentrations were associated with a phytoplankton bloom in the ocean water column, and the relationship between chl *a* and TEP concentrations can be fitted to TEP = $a(\text{chl } a)^b$ (Passow 2002). In the present study, the following regression formulae were obtained between the parameters: TEP = $231(\text{chl } a)^{0.292}$ ($r^2 = 0.531$, $p < 0.001$ $n = 23$) in the SML during the study period except in April 2014 and May 2015, and TEP = $188(\text{chl } a)^{0.356}$ ($r^2 = 0.544$, $p < 0.001$, $n = 25$) in the SSW (Fig. 6). However, the value *a* in the SML (231) was similar to that in the SSW (188). This indicates that TEPs in the SML were mainly derived from the spontaneous coagulation of TEP precursors released by phytoplankton in the SML, since the value *a* in the SML should have been much higher if TEP concentration increased through other processes, such as the upward transport of TEPs from the SSW.

No significant relationship was observed between the EFs of TEP concentration and ANF abundance, bacterial abundance, or HNF abundance during the study period except in April 2014 and May 2015 (Fig. 5b–d). Although the EF of TEP concentration was significantly positively correlated with that of

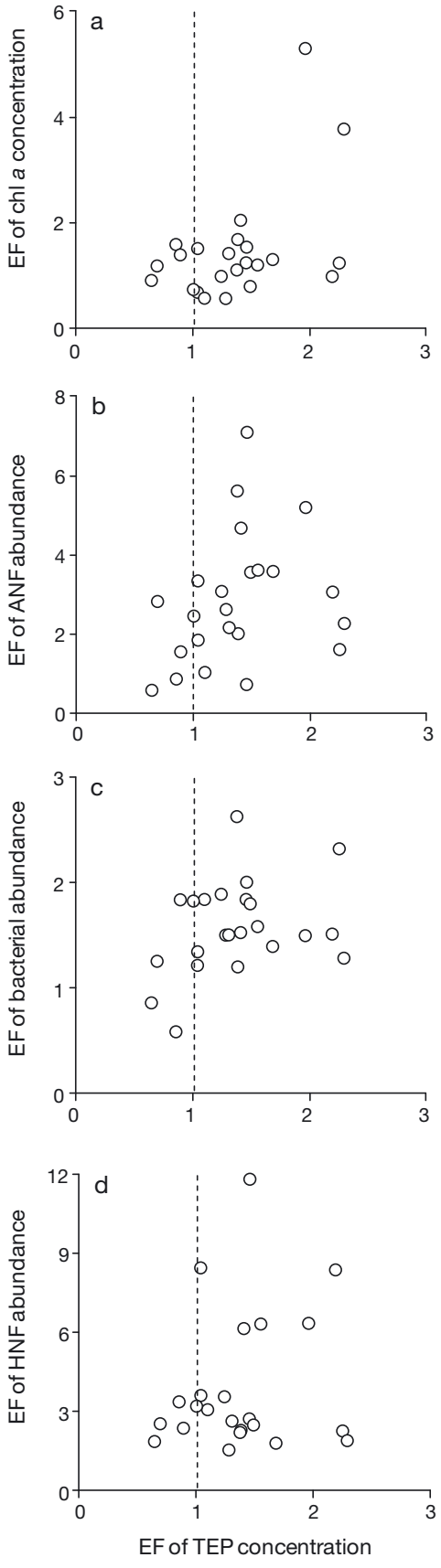

Fig. 5. Relationships between the enrichment factors (EFs) of transparent exopolymer particle (TEP) concentration and (a) chlorophyll *a* (chl *a*) concentration, (b) autotrophic nanoflagellate (ANF) abundance, (c) bacterial abundance, and (d) heterotrophic nanoflagellate (HNF) abundance during the study period except in April 2014 and May 2015. Dashed lines indicate that the EF of TEP is 1 (TEP concentration in the sea surface microlayer [SML] equals that in the subsurface water [SSW])

chl *a* concentration (Fig. 5a), this relationship likely resulted from the extracellular release of TEP precursors by phytoplankton (Myklestad 1995, Chin et al. 1998, Zhou et al. 1998, Passow 2000) because of the significant relationship between TEP and chl *a* concentrations in the SML (Fig. 6). Therefore, the present study indicates that the enrichment of microorganisms in the SML is not enhanced by that of TEPs in temperate coastal waters. Significant enrichment of microorganisms in the SML may have been caused by a combination of several processes, such as higher microbial production in the SML due to significantly enriched inorganic nutrients and dissolved organic materials (Fig. 3), the adsorption of microorganisms on rising bubbles, and high activity of TEP-attached microorganisms (Simon et al. 2002).

Enrichment in SML in April 2014 and May 2015

TEPs and microorganisms were particularly enriched in the SML in April 2014 and May 2015, and the EFs of the parameters except ANF and HNF abundance in May 2015 were much higher than those reported in previous studies except Wurl et al.

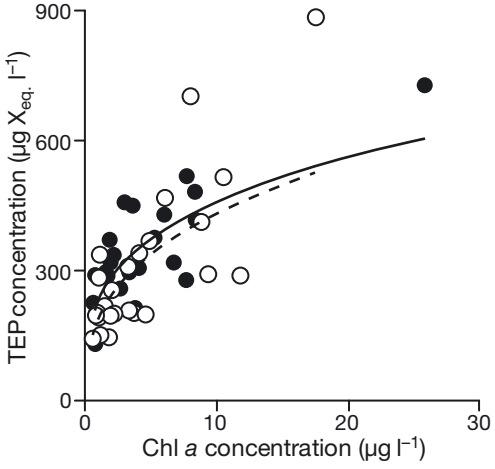

Fig. 6. Relationships between chlorophyll *a* (chl *a*) and transparent exopolymer particle (TEP) concentrations in the surface microlayer (●, solid line) and subsurface water (○, dashed line)

(2011, 2016) and Nakajima et al. (2013) (Table 1). These results indicate the formation of a unique SML in temperate coastal waters during spring phytoplankton blooms.

Since TEP concentrations in the SML showed higher values (5.44 and 2.11 mg $X_{eq.}$ l^{-1}, respectively) than in the SSW (0.89 and 0.71 mg $X_{eq.}$ l^{-1}, respectively) in April 2014 and May 2015 (Fig. 4a), enrichment of TEPs in the SML during this period was probably due to TEP production in the SML rather than the upward transport of TEPs from the SSW. Interestingly, NO_3 and PO_4 concentrations high enough to relieve the nutrient limitation of phytoplankton were detected in the SML in April 2014 and May 2015 despite considerably lower values in the SSW. These results suggest that TEPs and microorganisms showed enrichment in the SML in April 2014 and May 2015 due to phytoplankton blooms in the SML induced by the supply of inorganic nutrients. Furthermore, particularly high EFs of microbial abundance in April 2014 and May 2015 were possibly because the SML was not disrupted by wind-induced wave mixing under the moderate wind conditions (3.8 and 2.8 m s^{-1}, respectively) due to the enhanced viscosity of the SML caused by accumulation of TEPs. The TEP-matrix may have provided a microhabitat favorable for the growth of microorganisms in the extreme environment of the SML, as indicated by Wurl et al. (2016). Otherwise, Wurl et al. (2011) found a high EF of TEP concentration (2.8) under strong wind conditions (7.8 m s^{-1}) only when the concentration of TEP precursors in the SSW was high. In April 2014 and May 2015, moderately high wind velocity and DOC concentration in the SSW (1.38 and 2.18 mgC l^{-1}, respectively) were observed, and wave-induced bubbles rising through the SSW may have promoted TEP formation as suggested by Mopper et al. (1995) and Zhou et al. (1998).

The EF of bacterial abundance in May 2015 (6.9) is comparable to that in April 2014 (7.8), whereas the EFs of ANF and HNF abundance were much lower in May 2015 (4.6 and 4.7, respectively) than in April 2014 (40.8 and 37.0, respectively) (Table 1). A possible reason for this difference is sampling timing; ANF and HNF abundance may have increased several days after sampling occurred.

Enrichment of each microbial community in the SML

The EFs of each microbial community were largely different (Table 1). During the study period except in May 2015, the EFs of ANF and HNF abundance were higher than those of chl *a* concentration and bacterial abundance, as reported by previous studies (e.g. Agogué et al. 2004, Obernosterer et al. 2005, Joux et al. 2006, Nakajima et al. 2013). This may be because ANFs and HNFs had adapted to the SML environment due to their higher resistance to intense UV radiation (Buma et al. 2001, Wängberg et al. 2008). Furthermore, Agogué et al. (2004) reported the depletion of ciliates in the SML (EF = 0.63). Lower abundance of their predators in the SML than in the SSW is another potential reason, although ciliate abundance was not measured in this study. On the other hand, a lower EF of chl *a* concentration may have resulted from a smaller amount of chl *a* per cell in the SML (Richardson et al. 1983, Geider 1987) and/or a negative effect on phytoplankton growth in the SML (Daumas et al. 1976, Hardy & Apts 1984) under more intense light conditions. Joux et al. (2006) and Nakajima et al. (2013) pointed out higher grazing pressure of HNFs on bacteria in the SML than in the SSW. In the present study, the relative abundance of bacteria compared to HNFs in the SML (median: 281) was significantly lower than in the SSW (median: 735) (Wilcoxon signed-rank test, $p < 0.001$), which indicates that the lower EF of bacterial abundance was caused by strong top-down control on bacteria by HNFs. These results show different microbial food web structure in the SML and SSW.

CONCLUSIONS

This study reported annual monthly variations in TEP concentration and microbial abundance in the SML and SSW of Sagami Bay, Japan for 2 consecutive years, to describe the seasonal variations in the enrichment of TEPs and microorganisms in the SML of temperate coastal waters. Relationships between the EFs of TEP concentrations and microbial abundance indicate that the enrichment of microorganisms in the SML is not enhanced by that of TEPs. Particularly high EFs of TEP concentration and microbial abundance in spring indicate the formation of a unique SML of temperate coastal waters during spring phytoplankton blooms. Future studies should investigate bacterial activity and community structure in the SML to better understand the role of microorganisms in the SML in the air–sea exchange of atmospheric trace gases and aerosol particles.

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