



Dynamics and Origin of Transparent Exopolymer Particles in the Oyashio Region of the Western Subarctic Pacific during the Spring Diatom Bloom

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The seasonal biological drawdown of the partial pressure of CO₂ ($p\text{CO}_2$) in the surface waters of the Oyashio region of the western subarctic Pacific is one of the greatest among the world's oceans. This is attributable to spring diatom blooms. Transparent exopolymer particles (TEPs) are known to affect efficiency of the biological carbon pump, and higher TEP levels are frequently associated with massive diatom blooms. However, TEP dynamics in the Oyashio region remain unclear. We investigated the TEP distribution from three cruises during the spring diatom bloom periods in 2010 and 2011. TEP concentrations varied from < 15 to $196 \pm 71 \mu\text{g}$ xanthan gum equiv. L^{-1} above 300 m and generally declined with depth. Vertical TEP concentrations were significantly related not only to chlorophyll *a* concentrations but also to bacterial abundance. Average TEP concentrations within the mixed layer (> 30 m) were significantly higher during the bloom ($155 \pm 12 \mu\text{g}$ xanthan gum equiv. L^{-1}) than in the post-bloom phase ($90 \pm 32 \mu\text{g}$ xanthan gum equiv. L^{-1}). In contrast, bacteria abundance within the mixed layer changed little during the bloom to post-bloom phases. These results suggest that the abundance of phytoplankton greatly contributed to dynamics of the TEP distribution. To evaluate the ability of the phytoplankton to produce TEP, an axenic strain of the diatom *Thalassiosira nordenskioldii*, which is a representative species of Oyashio blooms, was examined within a batch culture system. Cell abundance-normalized TEP and dissolved organic carbon (DOC) production rates changed simultaneously with growth of the strain. Although these production rates were significantly higher in the stationary phase than in the exponential growth period, values of the TEP/DOC ratio changed little throughout incubation. These findings suggest that TEP production in the Oyashio region may be enhanced by an increase in DOC production from spring diatoms.

Keywords: transparent exopolymer particles, primary production, dissolved organic carbon, diatom bloom, *Thalassiosira nordenskioldii*, subarctic Pacific

INTRODUCTION

Transparent exopolymer particles (TEPs) are defined as $> 0.4\text{-}\mu\text{m}$ transparent particles that consist of acidic polysaccharides, and are stainable with the dye Alcian blue (Alldredge et al., 1993). These particles are very sticky, so that they can facilitate the aggregation of particles in seawater (e.g., Passow, 2002a; Wurl et al., 2011; Mari et al., 2017). Generally, the presence of TEP may increase the efficiency of the biological carbon pump, because TEP-attached particles sink readily (e.g., Passow, 2002a; Wurl et al., 2011). In contrast, TEPs can decrease the sinking rates of aggregates, because the density of TEPs is lower than that of seawater (Azetsu-Scott and Passow, 2004; Mari et al., 2017). TEPs also can be consumed by heterotrophic organisms, such as bacteria, zooplankton, and fish (e.g., Grossart et al., 1998, 2006; Prieto et al., 2001), indicating that the particles are important in marine food webs. It is known that many marine organisms, including phytoplankton and bacteria, can generate extracellular polysaccharides such as TEPs (Passow, 2002a). However, TEPs and their precursors in seawater can be utilized as organic substrates by heterotrophic organisms (Passow, 2002a). TEP levels in the world's oceans are highly variable ($0\text{--}14,800\ \mu\text{g}$ xanthan gum equivalent L^{-1} ; e.g., Hong et al., 1997; Radić et al., 2005). Higher TEP levels are sometimes associated with blooms of phytoplankton, such as diatoms (e.g., Passow et al., 1995), *Phaeocystis* spp. (e.g., Hong et al., 1997), dinoflagellates (e.g., Berman and Viner-Mozzini, 2001), cryptophytes (e.g., Passow et al., 1995), and cyanobacteria (e.g., Grossart and Simon, 1997). In particular, diatoms are known to excrete large amounts of polysaccharides, which can form TEPs during their actively growing and/or senescent phases (Mari and Burd, 1998; Passow, 2002b). Using batch cultures, Passow (2002b) reported that TEP production varied widely among phytoplankton species. Fukao et al. (2010) showed that TEP productivity of the diatom *Coscinodiscus granii* was greater in the exponential growth phase than in stationary and decline periods, whereas those of diatoms *Eucampia zodiacus*, *Rhizosolenia setigera*, and *Skeletonema* sp. were greater in the stationary growth and decline phases than in the exponential growth period. To our knowledge, the relationship between the physiology of diatoms and TEP production remains poorly understood.

In the Oyashio region of the Northwest Pacific, massive diatom blooms occur during spring (e.g., Kasai et al., 1998; Saito et al., 2002; Suzuki et al., 2011). These blooms are generally triggered by water-column stratification during the transition from winter to spring (Kasai et al., 1997). The chlorophyll (Chl) *a* concentration and primary production integrated within the water column can reach $500\ \text{mg Chl } a\ \text{m}^{-2}$ (Kasai et al., 1998) and $3,200\ \text{mg C m}^{-2}\ \text{d}^{-1}$ (Isada et al., 2010), respectively. The enormous spring blooms cause an increase in meso- and macro-zooplankton biomass and fishery production in the Northwest Pacific (Sakurai, 2007; Ikeda et al., 2008). Takahashi et al. (2002) reported that the Northwest Pacific including the Oyashio is one of the regions where the seasonal drawdown effect of $p\text{CO}_2$ by marine organisms is maximized. This is mainly caused by spring diatom blooms (Midorikawa et al., 2003; Ayers and Lozier, 2012). Honda (2003) reported that among the world's oceans,

efficiency of the biological carbon pump was relatively high in the Northwest Pacific. Hence, it can be inferred that the relatively high flux of settling particles in the Northwest Pacific is caused by vigorous primary production of diatoms that aggregate and sink. However, TEP dynamics in the Oyashio region during the spring diatom blooms are still poorly understood, even though they are pivotal in biogeochemical and ecological processes.

The main aims of this study were to understand TEP dynamics in the Oyashio region during the spring diatom bloom periods of 2010 and 2011 and to evaluate the relationship between TEP and other parameters. Those parameters include particulate organic carbon (POC) and dissolved organic carbon (DOC) concentrations, POC and DOC production, and phytoplankton and bacterial abundance. In addition, to understand TEP productivity in greater detail, we examined the characteristics of TEP production of an axenic strain of the Oyashio diatom *Thalassiosira nordenskioeldii*, which is a representative species of spring blooms (Chiba et al., 2004; Suzuki et al., 2011). This was grown in a batch culture system. The importance of TEP production by this strain was assessed.

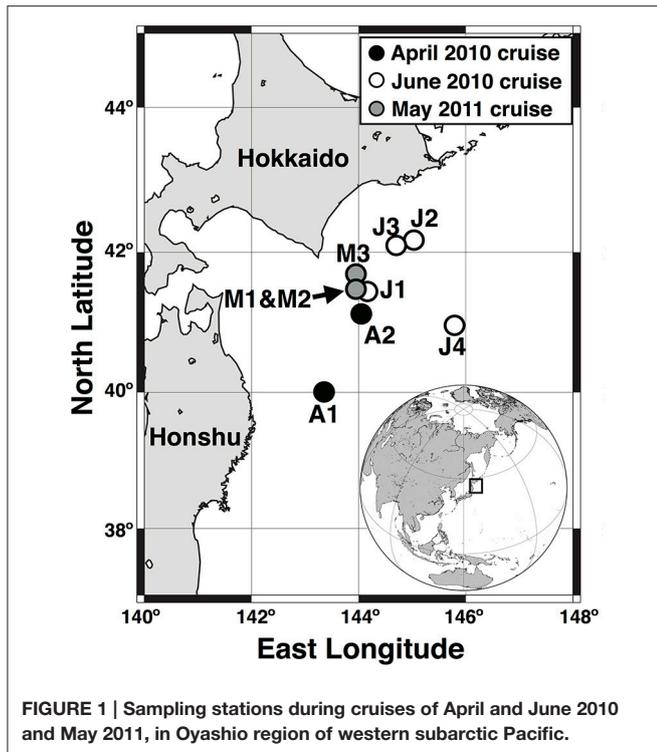
MATERIALS AND METHODS

Research Cruises

Three field campaigns were conducted, 13–23 April 2010, 7–16 June 2010, and 5–13 May 2011 (Figure 1). The FR/V *Wakataka-Maru* (Fisheries Research Agency of Japan) and R/V *Tansei-Maru* (JAMSTEC/AORI, University of Tokyo) were used for two cruises in 2010 and a single cruise in 2011, respectively. In April, June, and May, two (A1 and A2), four (J1, J2, J3, and J4) and three (M1, M2, and M3) sampling stations were visited, respectively. In all cruises, incident photosynthetic available radiation (PAR) above the sea surface was continuously measured on deck with a PAR sensor (ML-020P, EKO Instruments Co., Ltd., Japan) every 10 min on average, and values were recorded with a data logger. Seawater sampling at all stations was accomplished using a CTD-CMS equipped with acid-clean Niskin bottles. The samples were corrected from 5, 10, 20, 40, 60, 80, 100, 200, and 300 m depths in April 2010, and from the those depths plus 30, 50, and 150 m layers in June 2010 and May 2011. Nutrients (nitrite plus nitrate, hereafter referred to as nitrate, phosphate, and silicate) were determined with a BRAN + LUEBBE auto-analyzer following manufacturer protocol. Basal depth of the mixed layer was defined as a threshold value of temperature or density from a near-surface value at 10-m depth ($\Delta T = 0.2^\circ\text{C}$ or $\Delta\sigma_\theta = 0.03\ \text{kg m}^{-3}$; Montégut et al., 2004).

Phytoplankton Pigments and CHEMTAX Processing

Phytoplankton pigments were analyzed using high-performance liquid chromatography (HPLC). Water samples (500 mL) collected from 5 to 300 m depths were filtered on 25-mm Whatman GF/F filters under a gentle vacuum ($< 0.013\ \text{MPa}$). The filter samples were folded, blotted with filter paper, and stored at -80°C . Phytoplankton pigments were extracted with sonication in *N,N*-dimethylformamide (DMF) according to the protocol of Suzuki et al. (2005). HPLC pigment was analyzed according to



the method of Van Heukelem and Thomas (2001), except that the flow rate was 1.2 mL min^{-1} . Details of the analysis method are in Suzuki et al. (2014).

To estimate the contribution of each algal group in the two layers (5–20 and 30–50 m depths) to total Chl *a* biomass, the pigment data were interpreted by factorization using the CHEMTAX program (Mackey et al., 1996). The initial and final pigment ratios were calculated following the method of Latasa (2007) (Supplementary Table 1). Initial ratios were based on Suzuki et al. (2002, 2005, 2009), who estimated the community composition of phytoplankton in the northwestern Pacific. Prymnesiophytes, pelagophytes, and prasinophytes in our CHEMTAX analysis are synonymous with type 3 haptophytes, type 2 chrysophytes, and type 3 prasinophytes according to Mackey et al. (1996), respectively. Chl *a* biomass of each algal group was calculated by multiplying *in situ* Chl *a* concentrations by the CHEMTAX outputs.

To confirm the development and decline in Chl *a* concentration from spring to summer, we also examined monthly satellite images of that concentration as observed by a MODIS/Aqua satellite sensor. MODIS/Aqua monthly Chl *a* data (4-km resolution, OC3M algorithm) for the period February–July 2010 and 2011 were obtained from the Distributed Active Archive Center (DAAC)/Goddard Space Flight Center (GSFC), NASA (<http://oceancolor.gsfc.nasa.gov/cgi/l3>).

Identification and Cell Abundance of Phytoplankton

Water samples (500 mL) were taken at 5-m depth to count diatoms and coccolithophores, and these samples were fixed with buffered formalin (pH 7.8, 1% final concentration). An aliquot

(3–11 mL) of the sample was filtered onto a Nuclepore membrane (1- μm pore size) filter set in a glass funnel (3-mm in diameter at the base) in a vacuum (0.013–0.026 MPa). The filter membrane was rinsed with Milli-Q water to remove salts and immediately dried for 3 h in an oven at 60°C . To identify and count the algal cells, the entire area of the membrane ($\sim 7 \text{ mm}^2$) was examined by a scanning electron microscope (SEM, VE-8800, Keyence Corp., Japan) at magnification approximately $2,000\times$. Phytoplankton species were identified according to Fukuyo et al. (1990); Tomas et al. (1997), Young et al. (2003), Scott and Marchant (2005), and Round et al. (2007). In our study, the dominant diatom species were defined such that they contributed $> 25\%$ to total diatom abundance.

Abundance of Heterotrophic Bacteria

Duplicate water samples (each 2 mL) from 5 to 300 m depths were preserved with paraformaldehyde (0.2% final concentration) and stored at -80°C until analysis on land. An EPICS flow cytometer (XL ADC system, Beckman Coulter, Inc., USA) equipped with a 15-mW air-cooled argon laser exciting at 488 nm and a standard filter setup were used for enumeration of heterotrophic bacteria. Prior to analysis, samples were thawed and drawn through a 35- μm , nylon-mesh-capped Falcon cell strainer (Becton–Dickinson) to remove larger cells. Cells were stained with the nucleic acid stain SYBR Gold solution (Invitrogen). The stock SYBR Gold stain (10^4 -fold concentrations in the commercial solution) was diluted to 10-fold concentrations with Milli-Q water. Twenty-five microliters of the 10-fold SYBR Gold stain was added to the 225- μL bacteria sub-sample and incubated in the dark at room temperature (25°C) for 30 min prior to analysis. The incubated samples were mixed with 250 μL of Flow-Count fluorophores (Beckman Coulter) and then analyzed. Details of the flow cytometric analysis are given in Suzuki et al. (2005).

Particulate Organic Carbon (POC) Concentration

The POC concentration was determined only for the 5-m depth. The 300-mL water sample was filtered onto pre-combusted Whatman GF/F filters (25-mm in diameter, 450°C for 5 h) under a gentle vacuum ($< 0.013 \text{ MPa}$) and stored at -20°C until analysis. On land, the samples were thawed at room temperature, exposed to HCl fumes to remove inorganic carbon, and completely dried in a vacuum desiccator for $> 24 \text{ h}$. The POC concentrations on the filters were determined with an online elemental analyzer (FlashEA1112, Thermo Fisher Scientific, Inc., USA).

Dissolved Organic Carbon (DOC) Concentration

Samples of DOC were collected from 12 depths between 5 and 300 m. Inline filter holders (PP-47, Advantec MFS, Inc., USA) and tubes (Tygon R-3603, United States Plastic Corp., USA) were pre-cleaned by soaking in 1 M HCl and then rinsed with Milli-Q water. Before sampling, pre-combusted Whatman GF/F filters (47 mm diameter) were set in the inline filter holders, and the tubes were mounted. The water samples were taken by connecting the tube with the spigot of the Niskin bottle. At the start of sampling, filtrates were well drained to pre-wash the tubing, filter holder and filter.

Afterward, triplicate samples were collected in pre-combusted 24-mL screw vials with acid-cleaned PTFE septum caps. The samples were immediately stored at -20°C until analysis on land. The frozen samples were thawed and well mixed. DOC concentrations were determined with a total organic carbon analyzer (TOC-V CSH, Shimadzu Corp., Japan). The accuracy and variance of the measured DOC concentrations were checked by analyzing deep seawater reference material provided from the consensus reference material (CRM) program (Prof. Dennis Hansell, University of Miami).

Daily POC and DOC Production

Daily POC and DOC production were estimated using a simulated *in situ* incubation technique at 5-m depth, except at Station M3 during the May 2011 field campaign. The samples were dispensed into three 300-mL, acid-cleaned polycarbonate bottles (two light and one dark) and inoculated with a solution of $\text{NaH}^{13}\text{CO}_3$ (99 atom% ^{13}C), equivalent to $\sim 10\%$ of total inorganic carbon (TIC) in the seawater. All bottles were incubated for ~ 24 h. The incubated POC samples were filtered onto pre-combusted Whatman GF/F filters (25 mm in diameter, 450°C for 5 h) with a gentle vacuum (< 0.013 MPa), and the filtrate was collected in pre-combusted, 500-mL glass bottles (450°C for 5 h). The filter and water samples were stored at -20°C until analysis.

The filter samples of POC production were thawed at room temperature, exposed to HCl fumes to remove inorganic carbon, and then completely dried in a vacuum desiccator for > 24 h. POC concentrations on the filters and ^{13}C abundance were determined using a mass spectrometer (DELTA V, Thermo Fisher Scientific, Inc., USA) with online elemental analyzer (FlashEA1112, Thermo Fisher Scientific). Daily primary production was calculated according to Equation (1) (Hama et al., 1983):

$$\text{POC production} = \frac{(a_{is} - a_{ns})}{(a_{ic} - a_{ns})} \times \frac{[C_p]}{t} \times f, \quad (1)$$

where a_{is} is the ^{13}C atom% in an incubated sample, a_{ns} is the ^{13}C atom% in a natural (i.e., non-incubated) sample, a_{ic} is the ^{13}C atom% in inorganic carbon, $[C_p]$ is the concentration of POC in the incubated sample, t is the incubation duration (day), and f is the discrimination factor of ^{13}C (i.e., 1.025). In our study, TIC concentrations were determined with a total alkalinity analyzer (ATT-05, Kimoto Electric Co., Ltd., Japan).

DOC production was estimated according to Hama and Yanagi (2001) with a few modifications. The frozen samples were thawed at room temperature (25°C) and were desalinated using an electro dialyzer (Micro Acilyzer S3, ASTOM Corp., Japan) equipped with AC-220-550 cartridge (CMX-SB/AMX-SB, ASTOM Corp., Japan). Conductivity of the water samples decreased from ~ 53 to 3.0 mS cm^{-1} within 2–3 h. Before and after desalination, the DOC concentrations were examined. The recovery percentage of those concentrations ranged from 62 to 96%, whereas the conductivity decreased to 6% of the initial value (Supplementary Table 2). The desalinated seawater samples were concentrated to ~ 5 mL with a rotary evaporator at 45°C using

a pre-combusted 500-mL eggplant-shaped flask. HCl was added to the concentrated 5-mL samples to reduce the pH to 2. The low-pH concentrates were purged with N_2 gas for ~ 9 min to remove dissolved inorganic ^{13}C . Thereafter, the samples were further concentrated to ~ 0.5 – 1 mL with a rotary evaporator at 45°C using a pre-combusted 10-mL pear-shaped flask. The ^{13}C atom% of DOC absorbed onto the Whatman GF/F filters combusted in a muffle furnace (450°C , 5 h) were determined using a mass spectrometer (DELTA V, Thermo Fisher Scientific) with an online elemental analyzer (FlashEA1112, Thermo Fisher Scientific). Daily DOC production was calculated according to Equation (2) (Hama and Yanagi, 2001):

$$\text{DOC production} = \frac{(a_{is} - a_{ns})}{(a_{ic} - a_{ns})} \times \frac{[C_d]}{t}, \quad (2)$$

where $[C_d]$ is the DOC concentration (mg m^{-3}).

Furthermore, we estimated the ratio (PER) of DOC production/total production by phytoplankton according to

$$\text{PER (\%)} = \frac{\text{DOC production}}{(\text{POC} + \text{DOC production})} \times 100, \quad (3)$$

Transparent Exopolymer Particle (TEP) Analysis

Water samples of TEP were taken from depths 5–300 m. Triplicate water samples of 250 mL were filtered using Whatman 0.4- μm Nuclepore filters, applying a gentle vacuum (< 0.013 MPa). TEPs captured by the filter were stained with Alcian blue solution (Passow and Alldredge, 1995). The stained filters were immediately rinsed with Milli-Q water and then stored at -20°C until analysis on land.

For the standard TEP curve, the method of Claquin et al. (2008) was modified. As reported by Claquin et al. (2008), the standard curve described in Passow and Alldredge (1995) is applicable only for low concentrations of TEP (i.e., calibration standard weight of xanthan gum ranging between 0 and 40 μg). One milligram of xanthan gum was put into a 15-mL centrifugal tube and added to 10 mL of the Alcian blue stain. The tube was shaken well for ~ 1 h to combine the xanthan gum and Alcian blue. Thereafter, it was centrifuged at $3,200 \times g$ for 20 min, and the supernatant liquid was carefully removed using micro pipets. Ethanol (99.5%) was added to the tube, which was then centrifuged, and the supernatant liquid was removed via the ethanol precipitation method. The ethanol precipitation was repeated at least five times until the solution became transparent. Finally, as much of the ethanol in the tube as possible was removed. To dry the blue-stained xanthan gum, N_2 gas was gently sprayed into the tube. Subsequently, the xanthan gum was completely dried in a desiccator under vacuum for more than 24 h. The blue-stained xanthan gum was dissolved with 6 mL of 80% H_2SO_4 using a volumetric pipette (stock solution of 1 mg xanthan gum 6-mL^{-1}). The stock solution was diluted with 80% H_2SO_4 , to make a dilution series with five concentrations (10, 50, 100, 150, and 200 μg xanthan gum 6-mL^{-1}). Absorption at 787 nm was measured with a Shimadzu spectrophotometer (MPS-2400) in 1-cm cuvettes, with

reference to Milli-Q water. Four slopes of the TEP standard curve were compared with those of Passow and Alldredge (1995) and Claquin et al. (2008) (Supplementary Table 3). Also we conducted a comparison between the standard curves made with this centrifugation and conventional filtration (Passow and Alldredge, 1995) methods. The comparison revealed that the slope (136) of the centrifugation method was lower than that (207) of the conventional method (Supplementary Figure 1). Hence, all TEP concentrations in this study were corrected by multiplying a factor of 1.52. Passow and Alldredge reported that average absorption of the filter blank with 0.4- μm polycarbonate filters was between 0.07 and 0.09. In our study, the filter blank (average \pm standard deviation) was 0.081 ± 0.003 ($n = 4$).

The natural filter samples were transferred into 20-mL vials. Six milliliters of 80% H_2SO_4 was added to the vials, and the filters were soaked for 2 h. The vials were gently agitated five times over this period. Absorption at 787 nm was measured with the spectrophotometer in 1-cm cuvettes against Milli-Q water as reference. The TEP levels (μg xanthan gum equivalent L^{-1}) were determined according to Passow and Alldredge (1995).

TEP Production by the Oyashio Diatom *Thalassiosira nordenskiöldii*

A single cell of *T. nordenskiöldii* Cleve was isolated from Oyashio waters during the May 2011 field campaign. The strain was sterilized in f/2 medium (Guillard and Ryther, 1962; Guillard, 1975) with penicillin and streptomycin (25 U mL^{-1} of penicillin and 25 $\mu\text{g mL}^{-1}$ of streptomycin, 17-603E, Lonza Group Ltd., Switzerland) following the methods of Sugimoto et al. (2007). For the culture experiment, salinity of the medium was adjusted to 33.0, which is a typical value of surface Oyashio waters in spring (e.g., Hattori-Saito et al., 2010). Initial nutrient levels in the medium were also modified from f/2 medium to mimic Oyashio water: 20 μM NaNO_3 , 35 μM $\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$, 2.0 μM $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, 600 nM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 600 μM $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$, 46.7 nM $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 3.9 nM $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 2.2 nM $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 2.0 nM $\text{CuCO}_3 \cdot 5\text{H}_2\text{O}$, 1.3 nM $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, 15 nM Thiamine $\cdot \text{HCl}$, 100 pM Biotin, 19 pM Cyanocobalamin. The axenic strain was inoculated in the medium and grown for ~ 30 generations in an incubator (MIR-554, Sanyo Electric Co., Ltd., Japan) at 5°C , under $100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ from white fluorescent lamps (FL20SS \cdot BRN/18, Toshiba) with a light-dark cycle of 12 h. At the start of this experiment, the well-acclimated axenic strain was inoculated in Oyashio-simulated medium (17 L) in two acid-cleaned 20-L culture vessels (P/N 2600-0012, Thermo Fisher Scientific) and cultured for 40 days.

Nutrient, Cell Abundance, and Chl *a* Measurements in the Culture Experiment

Nutrients were sampled every other day. Duplicate water samples per culture vessel were dispensed into acrylic tubes with screw caps, and the samples were immediately stored at -20°C . The frozen samples were thawed at room temperature and well mixed. The nutrient concentrations (nitrate, phosphate, and silicate) were determined with an auto-analyzer (QuAatro 2-HR, BLTEC Corp., Japan) as described above. Sampling of cell abundance was done every other day. Ten to fifty milliliters

of the water samples were transferred into 15 or 50-mL centrifuge tubes. During days 0–14, *T. nordenskiöldii* was filtered using a 25-mm Whatman Nuclepore membrane filter of 1.0- μm pore size and was immediately resuspended in the non-concentrated water samples. One point five milliliters of the concentrated sample (days 0–14) or non-concentrated sample (days 16–40) was transferred to a micro-slide glass chamber of thickness 1.1 mm (S109502, Matsunami Glass Ind., Ltd., Japan), and covered with a cover glass. To estimate cell abundance, duplicate samples were observed per culture vessel using an epifluorescence microscope (BZ-9000, Keyence Corp., Japan) equipped with a GFP optical filter (OP-66835 BZ filter GFP, Keyence Corp., Japan). The Chl *a*-derived fluorescence in *T. nordenskiöldii* was automatically photographed with 28 photos and $4\times$ magnification, and the fluorescence was summed using image analysis software (Keyence Corp., Japan). Therefore, cell abundance was estimated by dividing the observed volume (mL) by the total value of fluorescence. The specific daily growth rate (μ , d^{-1}) during the culture experiment was estimated by the method of Guillard (1973):

$$\mu = \frac{(\ln N_t - \ln N_0)}{t_t - t_0}, \quad (4)$$

where N_0 is initial cell abundance (cells mL^{-1}), N_t the abundance after t days, t_0 the initial time (d), and t_t the time after t days.

Chl *a* concentrations were determined in the mid-exponential phase. Triplicate samples per vessel (each 200 mL) were filtered onto Whatman GF/F filters with a gentle vacuum (< 0.013 MPa). The filters were folded, blotted with filter paper, and stored at -80°C . The pigment analysis is described in Section Phytoplankton Pigments and CHEMTAX Processing.

TEP Measurement in the Culture Experiment

TEP was sampled once every 4 days between days 0 and 36. Triplicate water samples (1–20 mL) per culture vessel were filtered using Whatman 0.4- μm Nuclepore membrane filters in a gentle vacuum (< 0.013 MPa). Post-treatment used the same method described in Section Transparent Exopolymer Particle (TEP) Analysis.

DOC Determination in the Culture Experiment

DOC was sampled once every 4 days between days 0 and 36. A filter funnel (25-mm in diameter, PN 4203, PALL Corp., USA) was pre-cleaned by soaking in 1 M HCl, and then rinsed with Milli-Q water. Pre-combusted Whatman GF/F filters were placed in the filter funnel, which was equipped with a suction vessel. The water sample was filtrated in a gentle vacuum (< 0.013 MPa). At the start of sampling, a few milliliters of the filtrates were drained to prewash the GF/F filter and filter funnel. Then, duplicate samples were collected in pre-combusted 24-mL screw vials with acid-cleaned PTFE septum caps. The samples were immediately stored at -20°C until analysis. The analysis method was described in Section Dissolved Organic Carbon (DOC) Concentration.

Daily TEP and DOC Productivities

Based on TEP or DOC concentration in the culture vessels, cellular TEP productivity [pg xanthan gum equiv. (cell)⁻¹ d⁻¹] and DOC productivity [pg C (cell)⁻¹ d⁻¹] averaged during the exponential growth and stationary phases (initial and final) using two data points of those phases were estimated via

$$\text{Cellular TEP or DOC productivity} = \frac{(C_t - C_0)}{(t_t - t_0) \times (N_t - N_0)}, \tag{5}$$

where C₀ is TEP concentration (pg xanthan gum equiv. mL⁻¹) or DOC concentration (pg C mL⁻¹) at the initial time of the exponential growth phase or stationary phase, C_t is concentration of the TEP or DOC at the final time after *t* days, t₀ is the initial time (d) of the exponential growth phase or stationary phase, t_t is the final time after *t* days, N₀ is initial cell abundance (cells mL⁻¹) of the exponential growth or stationary phase, and N_t is cell abundance at the final time after *t* days. Carbon-based TEP productivity was estimated by multiplying TEP productivity by a factor of 0.75 (Engel and Passow, 2001).

RESULTS

TEP Observations during the Oyashio Spring Diatom Bloom Hydrography

Seawater temperatures at 5 m increased from April through June 2010 (Table 1). By contrast, salinity only changed slightly throughout the cruises (32.8–33.3). Relatively high nutrient levels were found in April 2010, which declined in June. The surface mixed layer was above 30 m at stations A2, M1, and M2, whereas it was shallower than 10 m at the other stations.

Chlorophyll *a* Concentrations and Community Composition as Estimated from the CHEMTAX Program

Chl *a* concentrations generally decreased from April through June 2010 and from 5 to 300 m depths (Figures 2A–C). Satellite surface Chl *a* images also indicated that decreases in the algal pigment concentration from April to June in 2010 and 2011 (Supplementary Figure 2). The highest Chl *a* concentration at 5-m depth was observed at station A1 in April 2010 (8.8 μg L⁻¹), whereas the lowest was at station J2 in June 2010 (0.4 μg L⁻¹) (Table 1). Chl *a* within the mixed layer significantly decreased from April to June (Kruskal-Wallis ANOVA, *p* < 0.01, Steel-Dwass test, April vs. May: *p* < 0.01; April vs. June: *p* < 0.01; May vs. June: *p* < 0.01).

The final ratio matrix in the 5–20 m layer estimated with the CHEMTAX program (Supplementary Table 1B) was within the range of Mackey et al. (1996), except for the alloxanthin:Chl *a* ratio (0.25), which was slightly larger than that of the maximum (0.23) of Mackey et al. The final ratio matrix in the 30–50 m layer (Supplementary Table 1D) was within the range of Mackey et al. The contributions of each phytoplankton group to Chl *a* biomass from 5 to 50 m depths are shown in Figure 3. Diatoms dominated in both April 2010 (average ± standard deviation: 94

TABLE 1 | Hydrographic conditions and phytoplankton productivity at 5 m depth during Oyashio spring diatom blooms in April and June 2010 and May 2011.

Cruise	Station	Temperature (°C)		Salinity (‰)	Nitrate (μM)	Silicate (μM)	Phosphate (μM)	Chl <i>a</i> (μg L ⁻¹)	Diatom abundance (×10 ³ cells L ⁻¹)	Heterotrophic bacteria abundance (×10 ⁵ cells mL ⁻¹)	TEP (μg xanthan gum equivalent L ⁻¹ d ⁻¹)	POC (μg C L ⁻¹)	DOC (μg C L ⁻¹)	POC production (mg C m ⁻³ d ⁻¹)	DOC production (mg C m ⁻³ d ⁻¹)	PER (%)
		5 m	100 m													
April, 2010	A1	3.1	3.1	32.9	8	20	0.9	8.8	382	4.2 ± 0.3	167 ± 33	503	704 ± 18	290 ± 17	1.6 ± 0.04	0.5
April, 2010	A2	4.5	3.9	33.3	11	24	1.0	4.2	288	5.4 ± 0.4	166 ± 40	404	688 ± 14	100 ± 1	4.2 ± 0.1	4.1
June, 2010	J1	7.0	1.3	32.9	8	8	1.0	0.6	11	3.5 ± 0.1	79 ± 8	143	736 ± 12	25 ± 1	1.9 ± 0.02	7.1
June, 2010	J2	7.0	0.6	32.9	8	8	1.0	0.4	4	2.2 ± 0.1	52 ± 23	137	715 ± 8	28 ± 0.3	1.7 ± 0.4	5.8
June, 2010	J3	9.0	1.3	32.8	4	9	0.8	0.7	1	4.1 ± 0.3	108 ± 18	139	753 ± 10	43 ± 2	2.6 ± 0.2	5.7
June, 2010	J4	7.8	0.8	32.9	8	13	1.0	0.8	3	3.6 ± 0.1	105 ± 17	154	749 ± 17	36 ± 1	1.3 ± 0.02	3.5
May, 2011	M1	5.0	1.7	33.1	13	11	1.1	1.0	23	2.2 ± 0.03	50 ± 9	163	685 ± 14	43 ± 0.4	2.3 ± 0.9	5.1
May, 2011	M2	4.3	2.9	33.0	9	6	0.7	2.0	247	5.4 ± 0.02	184 ± 52	213	704 ± 10	100 ± 5	4.2 ± 0.4	4.0
May, 2011	M3	4.4	2.0	32.9	7	2	0.6	1.7	320	N.D.	132 ± 73	235	728 ± 8	N.D.	N.D.	N.D.

N.D., No data.

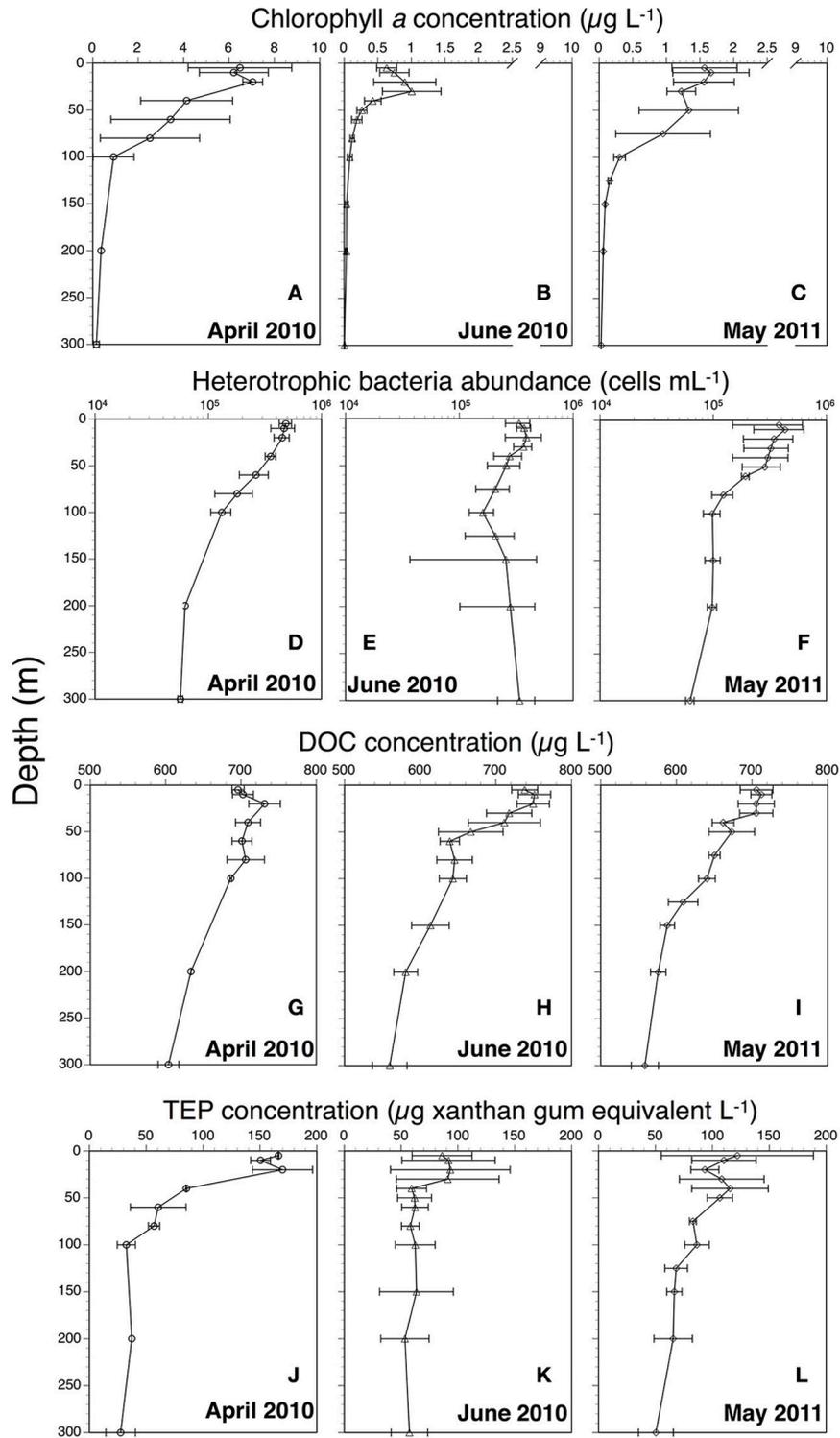


FIGURE 2 | Vertical profiles of chlorophyll *a* (A: April 2010; B: June 2010; C: May 2011), heterotrophic bacteria abundance (D: April 2010; E: June 2010; F: May 2011), dissolved organic carbon (DOC) (G: April 2010; H: June 2010; I: May 2011), and transparent exopolymer particles (TEP) (J: April 2010; K: June 2010; L: May 2011). All profiles show average values. Error bars represent range for April 2010 ($n = 2$) and standard deviation for June 2010 ($n = 4$) and May 2011 ($n = 3$).

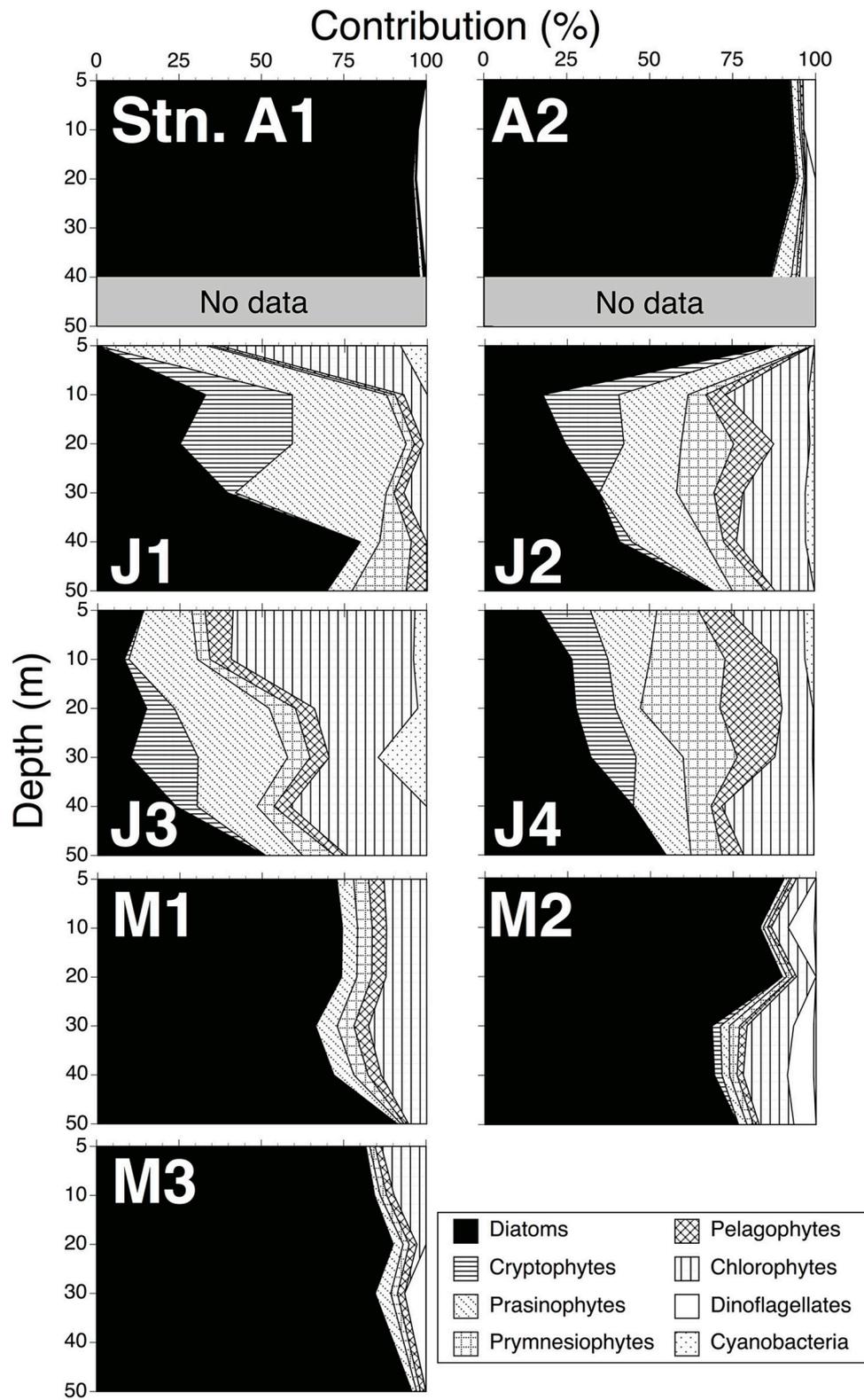


FIGURE 3 | Contributions of each phytoplankton group to chlorophyll a biomass within 0–50 m depths, estimated by CHEMTAX.

$\pm 4\%$) and May 2011 ($80 \pm 9\%$). By contrast, the contributions in June 2010 ($35 \pm 23\%$) were relatively small. The contributions of the other phytoplankton groups, such as cryptophytes ($20 \pm 17\%$), prasinophytes ($19 \pm 10\%$) and prymnesiophytes ($9 \pm 6\%$), were larger than those in April 2010 (cryptophytes: $1 \pm 2\%$; prasinophytes: $2 \pm 2\%$; prymnesiophytes: $0.4 \pm 0.5\%$) and May 2011 (cryptophytes: $9 \pm 5\%$; prasinophytes: $3 \pm 2\%$; prymnesiophytes: $3 \pm 1\%$).

Cell Abundance and Composition of Diatoms and Coccolithophores

Cell abundances of diatoms at 5-m depth were relatively high in April 2010 (335×10^3 cells L^{-1} on average) and low in June 2010 ($5 \pm 4 \times 10^3$ cells L^{-1}) (Table 1). Diatom abundance in May 2011 was highly variable ($150 \pm 115 \times 10^3$ cells L^{-1}). Using SEM, we identified 34 centric diatom species, 13 pennate diatom species, and 3 coccolithophore species, including a holococcolith species (Table 2). The dominant diatom species in April 2010 were *Thalassiosira nordenskiöldii* and *Chaetoceros* spp. In June 2010, *Fragilariopsis pseudonana* and *Neodenticula seminae* were predominant in the diatom community. In May 2011, *Ch. atlanticus* and *Chaetoceros* spp. dominated the diatom assemblages. Chl *a* concentrations were significantly correlated with the cell abundance of centric diatoms (Spearman's rank correlation $\rho = 0.90$, $p < 0.005$, $n = 9$), but only weakly correlated with that of pennate diatoms ($\rho = 0.45$, $p = 0.17$, $n = 9$). The cell abundance of coccolithophores was relatively low throughout the surveys ($0\text{--}3.6 \times 10^3$ cells L^{-1}).

Heterotrophic Bacterial Abundance Estimated by Flow Cytometry

Bacterial abundances in April 2010 and May 2011 were higher in the surface waters than at depth (Figures 2D,F, Table 1). However, in June 2011, little difference was observed from 5 to 300 m (Figure 2E, Table 1). Bacterial abundance within the mixed layer changed only slightly between April 2010 ($4.8 \pm 0.9 \times 10^5$ cells mL^{-1}), June 2010 ($3.5 \pm 0.9 \times 10^5$ cells mL^{-1}), and May 2011 ($3.8 \pm 1.7 \times 10^5$ cells mL^{-1}) (Kruskal-Wallis ANOVA, $p = 0.15$). Bacteria abundances from 5 to 300 m depths were significantly correlated with those of Chl *a* concentrations (Spearman's rank correlation, $\rho = 0.65$, $p < 0.001$, $n = 94$). Significant relationships between these were also observed on each cruise (Spearman's rank correlation, April: $\rho = 0.75$, $p < 0.001$, $n = 16$; June: $\rho = 0.49$, $p < 0.005$, $n = 45$; May: $\rho = 0.94$, $p < 0.001$, $n = 33$). Furthermore, there was a significant relationship between bacterial abundance and Chl *a* within the mixed layer (Spearman's rank correlation, $\rho = 0.57$, $p < 0.01$, $n = 22$). Values of bacteria abundance/Chl *a* concentration within the mixed layer increased significantly over time, e.g., April 2010 [85 ± 40 cells (pg Chl *a*) $^{-1}$], May 2011 [242 ± 68 cells (pg Chl *a*) $^{-1}$] and June 2010 [524 ± 70 cells (pg Chl *a*) $^{-1}$] (Kruskal-Wallis ANOVA, $p < 0.01$, Steel-Dwass test, April vs. May: $p < 0.01$; April vs. June: $p < 0.01$; May vs. June: $p < 0.01$).

POC Concentration

The POC concentration at 5-m depth decreased from April through June 2010 (Table 1; April 2010: $454 \mu\text{g } L^{-1}$ on average;

June 2010: $143 \pm 8 \mu\text{g } L^{-1}$), and that of May 2011 was $203 \pm 37 \mu\text{g } L^{-1}$. The ratio of POC/Chl *a* increased from April through June 2010 (April: 77 on average; June: 237 ± 58), and that in May 2011 was 134 ± 25 .

DOC Concentration

DOC concentrations generally decreased from 5 to 300 m depths (Table 1, Figures 2G-I). The highest concentration ($\sim 768 \mu\text{g } L^{-1}$) was found at 10 and 20 m depths at station J2 and at 10 m at station J3. The lowest concentration ($\sim 540 \mu\text{g } L^{-1}$) was observed at 300 m depth at stations M2, J3, and J4. The DOC concentration within the mixed layer in June 2010 ($744 \pm 24 \mu\text{g } L^{-1}$) was significantly higher than that in April 2010 ($696 \pm 12 \mu\text{g } L^{-1}$) and May 2011 ($708 \pm 12 \mu\text{g } L^{-1}$) (Kruskal-Wallis ANOVA, $p < 0.01$, Steel-Dwass test, April vs. May: $p = 0.99$; April vs. June: $p < 0.05$; May vs. June: $p < 0.01$).

POC and DOC Production

POC production at 5 m depth was between $25 \text{ mg C m}^{-3} \text{ d}^{-1}$ at the J1 station and $290 \text{ mg C m}^{-3} \text{ d}^{-1}$ at the A1 station (Table 1). Average productivity in April and June 2010 and May 2011 decreased from April to June ($195 \pm 95 \text{ mg C m}^{-3} \text{ d}^{-1}$ in April 2010, $72 \pm 29 \text{ mg C m}^{-3} \text{ d}^{-1}$ in May 2011, and $33 \pm 8 \text{ mg C m}^{-3} \text{ d}^{-1}$ in June 2010). The average DOC production at 5 m depth in April and June 2010 and May 2011 was $2.9 \pm 1.3 \text{ mg C m}^{-3} \text{ d}^{-1}$, $1.9 \pm 0.5 \text{ mg C m}^{-3} \text{ d}^{-1}$, and $3.2 \pm 1.0 \text{ mg C m}^{-3} \text{ d}^{-1}$, respectively (Table 1). PER ratios were estimated. The average ratios increased slightly from April to June ($2.3 \pm 1.8\%$ in April 2010, $4.6 \pm 0.6\%$ in May 2011, and $5.5 \pm 1.5\%$ in June 2010) (Table 1).

TEP Concentration

Minimum and maximum TEP concentrations in our surveys were observed at station A1, at 300 m ($< 15 \mu\text{g}$ xanthan gum equiv. L^{-1}) and 20 m ($196 \pm 71 \mu\text{g}$ xanthan gum equiv. L^{-1}) respectively (Figures 2K-L, Table 1). TEP concentrations from 5 to 300 m depths were significantly correlated with those of Chl *a* concentrations throughout the observation period (Spearman's rank correlation, $\rho = 0.65$, $p < 0.001$, $n = 98$) and on each cruise (Spearman's rank correlation, April: $\rho = 0.85$, $p < 0.001$, $n = 17$; June: $\rho = 0.52$, $p < 0.001$, $n = 47$; May: $\rho = 0.78$, $p < 0.001$, $n = 34$). The TEP concentrations were also significantly correlated with bacteria abundance throughout the observation period (Spearman's rank correlation, $\rho = 0.65$, $p < 0.001$, $n = 98$). Additionally, there were significant relationships between the two on each cruise (Spearman's rank correlation, April: $\rho = 0.86$, $p < 0.001$, $n = 17$; June: $\rho = 0.36$, $p < 0.05$, $n = 48$; May: $\rho = 0.58$, $p < 0.001$, $n = 33$).

Average TEP concentrations within the mixed layer in April and June 2010 and May 2011 were 155 ± 12 , 90 ± 32 , and $112 \pm 38 \mu\text{g}$ xanthan gum equiv. L^{-1} , respectively. TEP levels within the mixed layer in April 2010 were significantly higher than those in June 2010, whereas those in May 2011 were not significantly different from those in April and June 2010 (Kruskal-Wallis ANOVA, $p < 0.01$, Steel-Dwass test, April 2010 vs. June 2010: $p < 0.01$, April 2010 vs. May 2011: $p = 0.07$; May vs. June: $p = 0.48$). TEP concentrations within the mixed layer in April and

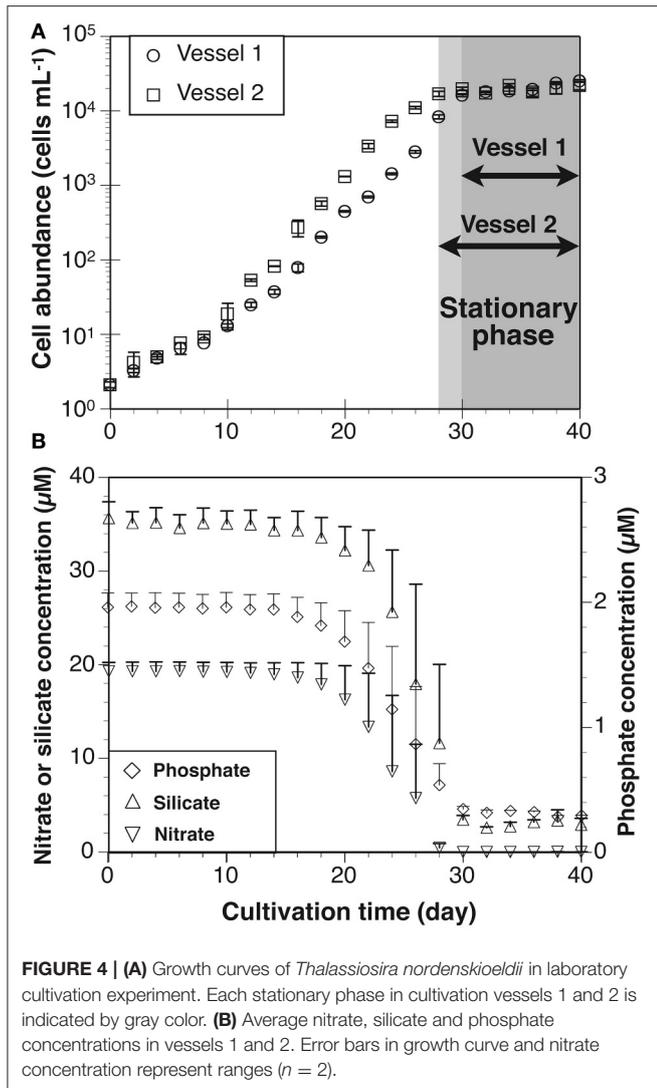
TABLE 2 | List of phytoplankton species identified in Oyashio diatom bloom surveys.

April 2010	June 2010	May 2011
Centric diatoms	Centric diatoms	Centric diatoms
<i>Actinocyclus curvatulus</i>	<i>Asteromphalus hyalinus</i>	<i>Actinocyclus</i> spp.
<i>Actinocyclus</i> spp.	<i>Chaetoceros atlanticus</i>	<i>Asteromphalus hyalinus</i>
<i>Asteromphalus hyalinus</i>	<i>Ch. concavicornis</i>	<i>Chaetoceros atlanticus</i>
<i>Chaetoceros atlanticus</i>	<i>Ch. neglectus</i>	<i>Ch. concavicornis</i>
<i>Ch. concavicornis</i>	<i>Corethron criophilum</i>	<i>Ch. radicans</i>
<i>Ch. decipience</i>	<i>Co. inerne</i>	<i>Chaetoceros</i> spp.
<i>Ch. debilis</i>	<i>Coscinodiscus</i> spp.	<i>Rhizosolenia</i> spp.
<i>Ch. diadema</i>	<i>Rhizosolenia</i> spp.	<i>Thalassiosira eccentrica</i>
<i>Ch. neglectus</i>	<i>Thalassiosira lineata</i>	<i>T. lineata</i>
<i>Ch. pseudocurvisetus</i>	<i>T. nordenskiöldii</i>	<i>T. nordenskiöldii</i>
<i>Ch. radicans</i>	<i>T. oestrupii</i>	<i>T. pacifica</i>
<i>Chaetoceros</i> spp.	<i>T. pacifica</i>	<i>T. trifulta</i>
<i>Corethron criophilum</i>	<i>Thalassiosira</i> spp.	<i>Thalassiosira</i> spp.
<i>Co. inerne</i>	Pennate diatoms	Pennate diatoms
<i>Odontella aurita</i>	<i>Fragilariopsis atlantica</i>	<i>Fragilariopsis atlantica</i>
<i>Rhizosolenia</i> spp.	<i>F. cylindriciformis</i>	<i>F. oceanica</i>
<i>Stephanopyxis turris</i>	<i>F. cylindrus</i>	<i>F. pseudonana</i>
<i>Thalassiosira allenii</i>	<i>F. oceanica</i>	<i>Fragilariopsis</i> spp.
<i>T. angulata</i>	<i>F. pseudonana</i>	<i>Neodenticula seminae</i>
<i>T. eccentrica</i>	<i>Fragilariopsis</i> spp.	<i>Thalassionema nitzschioides</i>
<i>T. hyalina</i>	<i>Neodenticula seminae</i>	Coccolithophores
<i>T. lineata</i>	<i>Thalassionema nitzschioides</i>	<i>Emiliana huxleyi</i>
<i>T. lineoides</i>	<i>Thalassiothrix longissima</i>	
<i>T. mala</i>	Coccolithophores	
<i>T. nordenskiöldii</i>	<i>Coccolithus pelagicus</i> ssp. <i>Pelagicus</i>	
<i>T. oceanica</i>	<i>Emiliana huxleyi</i>	
<i>T. pacifica</i>	<i>Coccolithus pelagicus</i> ssp. <i>Pelagicus</i> HOL*	
<i>T. leptopus</i>		
<i>T. trifulta</i>		
<i>Thalassiosira</i> spp.		
Pennate diatoms		
<i>Fragilariopsis atlantica</i>		
<i>F. cylindrus</i>		
<i>Navicula directa</i>		
<i>Navicula</i> spp.		
<i>Neodenticula seminae</i>		
<i>Nitzschia</i> spp.		
<i>Pseudonitzschia</i> spp.		
<i>Thalassionema nitzschioides</i>		
Coccolithophores		
<i>Coccolithus pelagicus</i> ssp. <i>Pelagicus</i>		

Genus and species names are arranged alphabetically, not systematically. Dominant species in April, May and June are shown in bold. *HOL, holococcolith.

June 2010 and May 2011 were also significantly higher than those below the mixed layer in April ($64 \pm 49 \mu\text{g}$ xanthan gum equiv. L^{-1}) and June 2010 ($67 \pm 27 \mu\text{g}$ xanthan gum equiv. L^{-1}), and May 2011 ($81 \pm 23 \mu\text{g}$ xanthan gum equiv. L^{-1}) (Wilcoxon rank-sum test, April: $p < 0.01$, $n = 17$; May: $p < 0.05$, $n = 34$; June: $p < 0.05$, $n = 48$). TEP values were significantly correlated not only with Chl *a* concentrations but also diatom-derived Chl *a* biomass (Spearman's rank correlation, TEP vs. Chl *a*: $\rho = 0.70$,

$p < 0.001$, $n = 23$; TEP vs. diatom-derived Chl *a*: $\rho = 0.66$, $p < 0.001$, $n = 23$). However, no significant relationship was found between TEP levels and Chl *a* biomass of other phytoplankton groups (Spearman's rank correlation, TEP vs. cryptophytes-Chl *a*: $\rho = -0.22$, $p = 0.31$, $n = 23$; TEP vs. prasinophyte-derived Chl *a*: $\rho = -0.27$, $p = 0.22$, $n = 23$; TEP vs. prymnesiophyte-derived Chl *a*: $\rho = -0.28$, $p = 0.19$, $n = 23$). The TEP/Chl *a* ratio increased significantly from April to June (26 ± 10 in April 2010,



76 ± 24 in May 2011, and 128 ± 31 in June 2010) (Kruskal-Wallis ANOVA, $p < 0.01$, Steel-Dwass test, April vs. May: $p < 0.01$; April vs. June: $p < 0.01$; May vs. June: $p < 0.05$). In contrast, values of TEP concentration/bacteria abundance changed little from April and June [$0.33 \pm 0.06 \mu\text{g xanthan gum} (\times 10^6 \text{ bacterial cells})^{-1}$ in April 2010, $0.34 \pm 0.16 \mu\text{g xanthan gum} (\times 10^6 \text{ bacterial cells})^{-1}$ in May 2011, and $0.25 \pm 0.07 \mu\text{g xanthan gum} (\times 10^6 \text{ bacterial cells})^{-1}$ in June 2010] (Kruskal-Wallis ANOVA, $p < 0.01$, Steel-Dwass test, April vs. May: $p < 0.01$; April vs. June: $p < 0.01$; May vs. June: $p < 0.05$).

Laboratory Experiments

Growth Curves, Nutrient Levels, and Chl *a* Concentrations

Cell abundances in vessels 1 and 2 increased from 2 to $\sim 20,000$ cells mL^{-1} (Figure 4A). As estimated from the specific growth rate, the exponential growth phase was between days 0 and 30 in Vessel 1 and days 0 and 28 in Vessel 2. The stationary phase was observed between days 30 and 40 in Vessel 1 and days 28 and 40 in Vessel 2. The average specific growth rate in both

vessels during the exponential growth and stationary phases was $0.31 \pm 0.14 \text{ d}^{-1}$ and $0.03 \pm 0.07 \text{ d}^{-1}$, respectively (Table 3). The average nitrate, silicate and phosphate levels in both vessels are shown in Figure 4B. During the exponential growth phase, these levels decreased with time. In the stationary phase, nitrate depleted ($< 0.1 \mu\text{M}$), whereas silicate and phosphate concentrations maintained values of $3.1 \pm 0.7 \mu\text{M}$ and $0.3 \pm 0.03 \mu\text{M}$. Chlorophyll *a* concentrations in Vessel 1 (day 20) and 2 (day 18) in the mid-exponential growth phase were $6.1 \pm 0.1 \mu\text{g L}^{-1}$ and $7.1 \pm 0.1 \mu\text{g L}^{-1}$, respectively. Ratios calculated from the Chl *a* concentrations and cell abundance sampled at the same time were 14 pg cell^{-1} in Vessel 1 and 13 pg cell^{-1} in Vessel 2 (average $13 \pm 1 \text{ pg cell}^{-1}$).

TEP and DOC Concentrations

The initial (day 0) TEP concentration in Vessel 1 was undetectable, so this concentration was assumed to be $0 \mu\text{g xanthan gum equiv. L}^{-1}$. The average TEP concentration in both vessels increased with time (Figure 5). The concentrations ranged from $12 \pm 12 \mu\text{g xanthan gum equiv. L}^{-1}$ to $1,488 \pm 93 \mu\text{g xanthan gum equiv. L}^{-1}$. Average DOC concentration also generally increased with time (Figure 5). The concentrations were between $1,148 \pm 62 \mu\text{g C L}^{-1}$ and $1,647 \pm 10 \mu\text{g C L}^{-1}$. TEP concentrations in the vessels increased with DOC concentrations ($\rho = 0.91$, $p < 0.001$, $n = 21$).

Cellular TEP and DOC Productivities

Cellular TEP production rates during the exponential and stationary phases were $2.7 \pm 0.5 \text{ pg xanthan gum equiv. [cell]}^{-1} \text{ d}^{-1}$ and $8.1 \pm 0.9 \text{ pg xanthan gum equiv. [cell]}^{-1} \text{ d}^{-1}$, respectively (Table 3). Cellular DOC productivity was $1.0 \pm 0.2 \text{ pg C [cell]}^{-1} \text{ d}^{-1}$ and $4.0 \pm 2.2 \text{ pg C [cell]}^{-1} \text{ d}^{-1}$ during the exponential and stationary phases, respectively. The cellular TEP and DOC productivities during the exponential growth phase were also converted to a Chl *a*-normalized TEP production rate ($0.21 \mu\text{g xanthan gum} [\mu\text{g Chl } a]^{-1} \text{ d}^{-1}$) and DOC production rate [$0.07 \mu\text{g C} (\mu\text{g Chl } a)^{-1} \text{ d}^{-1}$], using the factor of Chl *a* concentration per cell. The cellular TEP and DOC productivities were 2.9 and 4.0-fold greater in the stationary than exponential growth phase, respectively. Ratios of carbon-based TEP/DOC productivities were 2.2 ± 0.02 in the exponential growth phase and 2.0 ± 0.9 in the stationary phase.

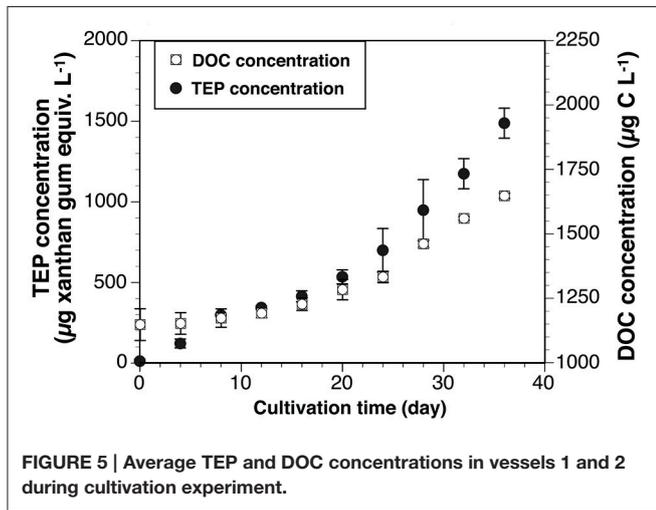
DISCUSSION

Factors Controlling TEP Levels in Oyashio Region

According to Kawai (1972), the Oyashio was defined as the water mass where temperatures at 100 m were $< 5^\circ\text{C}$. Therefore, all stations in this study were in the Oyashio (Table 1). However, hydrographic conditions were variable throughout the observation period (Table 1). In the Oyashio region, it is known that spring diatom blooms occur during April and May, and decline toward summer every year (e.g., Saito et al., 2002). Indeed, decreases in Chl *a* concentration were observed from April to June in both 2010 and 2011 as estimated from the satellite images (Supplementary Figure 2).

TABLE 3 | Summary of results in exponential growth and stationary phases, obtained from laboratory cultivation experiments.

Parameters	Exponential growth phase		Stationary phase	
	Vessel 1	Vessel 2	Vessel 1	Vessel 2
Term (day)	0–30	0–28	30–36	28–40
Specific growth rate ± S.D. (d^{-1})	0.30 ± 0.12	0.32 ± 0.16	0.03 ± 0.02	0.02 ± 0.09
Cellular TEP productivity ($\mu\text{g xanthan gum equiv. [cell]}^{-1} d^{-1}$)	3.2	2.3	7.1	9.0
Cellular DOC productivity ($\mu\text{g C [cell]}^{-1} d^{-1}$)	1.1	0.8	1.8	6.2
Ratio of cellular carbon-based TEP/DOC productivities	2.2	2.1	3.0	1.1

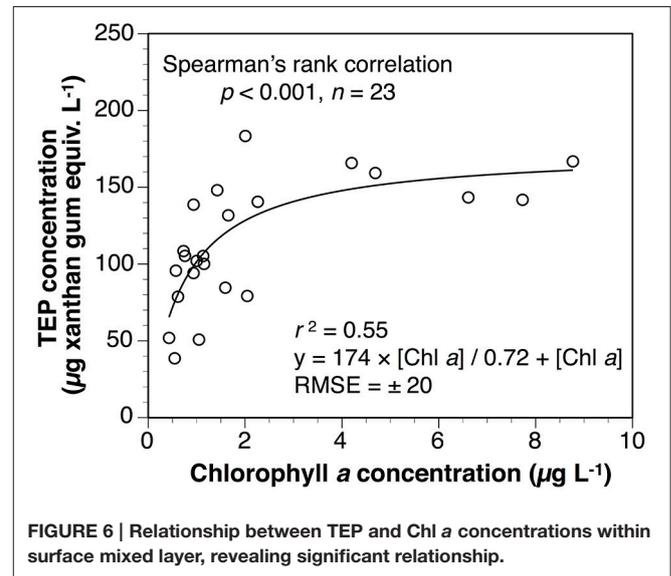
**FIGURE 5 | Average TEP and DOC concentrations in vessels 1 and 2 during cultivation experiment.**

TEP concentrations from 5 to 300 m depths were significantly correlated with those of Chl *a* concentrations and with bacteria abundances. Similarly, the TEP concentrations were higher in the mixed layer than in the layer below. These results indicate that TEP was derived from these microorganisms. The Chl *a* concentrations within the mixed layer decreased significantly from April to June 2010, whereas bacterial cell abundances changed little in that period. The difference in temporal variation between the Chl *a* concentrations and bacteria abundances within the mixed layer suggest that the main TEP producer was phytoplankton. We used the following Michaelis-Menten-type equation for estimating TEP levels within the surface mixed layer, using Chl *a* concentration during the spring Oyashio diatom blooms (Figure 6 and Equation 6).

$$[\text{TEP}]_{\text{SML}} = \frac{174 \times [\text{Chl } a]}{0.72 + [\text{Chl } a]}, \quad (6)$$

where $[\text{TEP}]_{\text{SML}}$ is TEP concentration within the surface mixed layer ($\mu\text{g xanthan gum equiv. L}^{-1}$) and $[\text{Chl } a]$ is Chl *a* concentration within the mixed layer ($\mu\text{g L}^{-1}$). The root mean square error (RMSE) of this curve was estimated at $\pm 20 \mu\text{g xanthan gum equiv. L}^{-1}$. These findings also suggest that TEP within the surface mixed layer was mainly from phytoplankton.

Assuming that TEP was largely produced by phytoplankton, data of each phytoplankton group to Chl *a* biomass estimated from the CHEMTAX program were useful to discover which

**FIGURE 6 | Relationship between TEP and Chl *a* concentrations within surface mixed layer, revealing significant relationship.**

group contributed predominately to TEP concentrations. These data showed that diatom-derived Chl *a* concentrations were dominant in April 2010 ($94 \pm 4\%$) and May 2011 ($80 \pm 9\%$) (Figure 3). This suggests that the diatoms contributed to the relatively high TEP concentration in our study. In addition, data of phytoplankton species composition by SEM showed that *T. nordenskiöldii* and *Chaetoceros* spp. dominated phytoplankton assemblages in April 2010, and *C. atlanticus* and *Chaetoceros* spp. were dominant in May 2011 (Table 2). The relatively high TEP levels may have been caused by these diatom species. Compared to April 2010 and May 2011, the dominant phytoplankton groups in June 2010 were diatoms ($35 \pm 23\%$), cryptophytes ($20 \pm 17\%$), prasinophytes ($19 \pm 10\%$), and prymnesiophytes ($9 \pm 6\%$) (Figure 3). It has been reported that cryptophytes and prymnesiophytes contribute to high TEP concentrations (Passow et al., 1995; Hong et al., 1997). These phytoplankton groups may have also affected the TEP concentration in June 2010.

Data on TEPs are very limited for the Northwest Pacific. Ramaiah et al. (2005) investigated changes in TEP levels in the Western Subarctic Gyre (WSG) of the North Pacific during an *in situ* iron fertilization experiment. According to that work, the centric diatom *Ch. debilis* bloomed in the WSG after iron enrichment, and TEP levels (~ 50 – $190 \mu\text{g xanthan gum equiv. L}^{-1}$) in an iron-enriched patch increased with Chl *a*

concentration ($\sim 1\text{--}19 \mu\text{g L}^{-1}$) at 5 and 10 m depths. TEP levels found by Ramaiah et al. were similar to those observed in our study. However, Passow (2002a) reported that TEP concentration during diatom blooms can reach $> 1,000 \mu\text{g}$ xanthan gum equiv. L^{-1} in various regions (Engel, 2000; Prieto et al., 2002). Environmental factors such as temperature, nutrient concentrations, and UV intensity may modulate the TEP production rate of phytoplankton (Mari et al., 2017). An increase of water temperature can raise the TEP production rate (Claquin et al., 2008; Fukao et al., 2012). Seawater temperatures in April 2010 and those in Ramaiah et al. (2005) were relatively low, at $3.1\text{--}4.5$ and $7.5\text{--}9.5^\circ\text{C}$, respectively. The depletion of nutrients can also increase the TEP concentration because of enhanced release of extracellular polysaccharides (Myklestad, 1995). It has been reported that the ratio of silicate and nitrate concentrations during diatom blooms is relatively constant (approximately 1:1; Brzezinski, 1985; Kudo et al., 2000), and the half-saturation constants of uptake kinetics were $\sim 3 \mu\text{M}$ in silicate and $\sim 1 \mu\text{M}$ in nitrate (e.g., Kanda et al., 1985; Nelson and Tréguer, 1992). In April 2010, nutrient concentrations were abundant ($> 20 \mu\text{M}$ in silicate and $> 8 \mu\text{M}$ in nitrate). These results suggest that the lower temperatures and higher nutrients in April 2010 did not increase the TEP levels in seawater much. Recently, Annane et al. (2015) reported lower TEP levels ($< 200 \mu\text{g}$ xanthan gum equiv. L^{-1}) during the centric diatom bloom ($> 20 \mu\text{g L}^{-1}$ in terms of Chl *a* level, mainly consisting of *T. nordenskiöldii*) in the lower St. Lawrence Estuary of Canada. The temperature and nitrate concentrations in Annane et al. were similar to those in our study. Therefore, TEP productivity can vary with diatom species and can be relatively low during Oyashio spring diatom blooms when *T. nordenskiöldii* becomes dominant (Chiba et al., 2004; Suzuki et al., 2011).

Heterotrophic organisms such as eubacteria can also be important in production (e.g., Passow, 2002b; Sugimoto et al., 2007), decomposition (e.g., Passow et al., 2001), and transformation (e.g., Gärdes et al., 2011; Yamada et al., 2013) processes of TEP. Although the present study suggests that the main TEP producer was likely phytoplankton as described above, bacteria abundance was significantly correlated with not only TEP concentration but also Chl *a* concentration. This indicates close interaction between these microorganisms and TEP. Values of bacteria abundance/Chl *a* concentration within the mixed layer increased significantly from April 2010 to June 2010, suggesting that relative abundance of bacteria to phytoplankton was low in April 2010. In contrast, TEP levels within the mixed layer were significantly higher in April 2010 than in June 2010. It has been suggested that TEP produced by phytoplankton may serve as protection against hydrolases of attached bacteria (Azam and Smith, 1991; Smith et al., 1995). Hence, the relatively lower Chl *a* normalized TEP concentrations in April 2010 may have partially contributed to the relative abundance of bacteria to phytoplankton. The lower TEP concentrations may also mean that bacteria had colonized and degraded TEPs. Indeed, the TEPs and their precursors produced by diatoms can be utilized by bacteria. Recently, Cisternas-Novoa et al. (2015) stated that bacterial abundance affected TEP production by *T. weissflogii*. However, interactions between TEPs and bacteria in nature

are complex because of combinations of various environmental factors, as reviewed by Passow (2002b).

Relationship between TEP Production and Phytoplankton Physiology

The TEP levels appeared to decrease from April to June, together with the decline of diatom blooms in the Oyashio region. In contrast, the TEP/Chl *a* ratio increased significantly from April to June. If the main source of TEP is phytoplankton assemblages, the increase in ratios of TEP/Chl *a* concentrations from April to June may show that the ability to produce TEP was weaker in diatoms than in other phytoplankton groups, such as cryptophytes, prasinophytes and prymnesiophytes. In our laboratory experimentation, the TEP production rate of the axenic strain of *T. nordenskiöldii* was estimated at $0.21 \mu\text{g}$ xanthan gum equiv. $[\mu\text{g Chl } a]^{-1} \text{d}^{-1}$ during the exponential growth phase. Hong et al. (1997) showed that Chl *a*-normalized TEP production rates of *Phaeocystis antarctica* were $> 500 \mu\text{g}$ xanthan gum equiv. $[\mu\text{g Chl } a]^{-1} \text{h}^{-1}$ at photon flux density $\sim 100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. These findings suggest that *T. nordenskiöldii* was a weaker TEP producer than *P. antarctica*. In this study, although the sampling from each culture vessel was performed very carefully on a clean bench to avoid any contamination, perhaps bacteria could be slightly contaminated during incubation, and that might underestimate the TEP productivity by *T. nordenskiöldii* due to its utilization by bacteria. On the other hand, TEP decomposition by bacteria could increase the refractory form of gel particles, because it has been reported that microbial processes can alter the molecular structure of dissolved organic matter (Ogawa et al., 2001). This would contribute to increase in the TEP/Chl *a* ratio between the bloom and post-bloom phases.

Although diatom species composition possibly affects TEP productivity as mentioned above, the incubation experiment using *T. nordenskiöldii* revealed that cellular TEP productivity during the stationary phase was 2.9-fold greater than that during the exponential growth phase (Table 3). Changes in physiological state from the exponential to stationary growth phases in this batch culture experiment were likely caused by nitrate depletion (Figure 4B). Therefore, we speculate that nitrate availability probably influences TEP levels during spring diatom blooms in the Oyashio region.

TEP precursors have been considered to be dissolved acid polysaccharides (Thornton et al., 2007; Wurl et al., 2011), which may mainly be from DOC excreted by phytoplankton (Passow, 2002a). Hence, we examined the relationship between TEP levels and DOC production at 5 m depth, but this was not significant (Spearman's rank correlation, $p = 0.42$, $n = 8$). In addition, the relationship was evaluated using the axenic strain of *T. nordenskiöldii* (Chiba et al., 2004; Suzuki et al., 2011). Productivities of cellular TEP and DOC generally varied simultaneously with growth of the diatom strain. These results suggest that TEP production was linked to DOC in the experiment. The *in situ* DOC production was clearly lower than POC production (Table 1) and was comparable to those ($< 0.5\text{--}1.2 \text{ mg C m}^{-3} \text{d}^{-1}$) in the Mediterranean Sea between June and

July (Lopez-Sandval et al., 2011). However, the PER values were smaller than those (30–41%) in the Mediterranean. In general, relatively low PER values can be found in eutrophic waters (e.g., Hama and Yanagi, 2001; Marañón et al., 2004) and relatively high values were observed in oligotrophic regions (e.g., Alonso-Sáez et al., 2008; Lopez-Sandval et al., 2011). The relatively low PER possibly weaken the relationship between TEP levels and DOC production.

Interestingly, however, the averaged ratio (2.2 ± 0.02) of carbon-based TEP/DOC rates for *T. nordenskiöldii* in the exponential growth phase was nearly the same as the stationary growth phases (2.0 ± 0.9) (Table 3). If all TEP were formed from DOC released by *T. nordenskiöldii*, the carbon-based TEP/DOC production ratio would be < 1 . Our laboratory experiment also found that TEP was rich at the cell surface of *T. nordenskiöldii* (Supplemental Figure 3). This indicates that some of the DOC excreted by *T. nordenskiöldii* were trapped on the cell surfaces. Therefore, the carbon-based TEP/DOC production rate > 1 suggests that the TEP was partly formed at cell surfaces. Moreover, the similar carbon-based TEP/DOC production ratios in the exponential and stationary growth phases imply that the formation rate of TEP to DOC was not significantly different between the two phases. The results of our field observations and laboratory experiments imply that high primary production by diatoms leads to relatively strong DOC production, and that subsequently, the excretion of acid polysaccharides (a type of DOC) can generate higher TEP levels in seawater. This was even though the activity of heterotrophic organisms possibly masked the relationship between TEP concentrations and DOC productivity, together with decay of the diatom bloom. Thus, TEP production in the Oyashio region may be enhanced with the increase in DOC production from spring diatoms.

CONCLUSIONS

This study revealed TEP dynamics and DOC productivity in the Oyashio region of the Northwest Pacific during spring diatom blooms. Our results showed that TEP levels within the mixed layer were correlated with diatom-derived Chl *a* biomass,

suggesting that diatoms were the main TEP producers. However, TEP levels were not correlated with DOC productivity in the Oyashio region. In contrast, we found that the average TEP and DOC productivities of *T. nordenskiöldii* varied simultaneously with growth of the strain and that the carbon-based TEP/DOC ratio changed little between the growth phases. These results suggest that TEP productivity in the Oyashio region increases with DOC productivity during spring diatom periods and that TEP removal (e.g., through consumption by heterotrophic organisms) must be considered in evaluating the relationship between TEP levels and DOC productivity. Further laboratory experiments using isolated diatom and bacteria strains should be made to clarify these issues.

AUTHOR CONTRIBUTIONS

YN and KS designed this research. All authors performed the field observations. The laboratory cultivation experiment was executed by YN. YN analyzed the samples and wrote the paper with KS and YY.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <http://journal.frontiersin.org/article/10.3389/fmars.2017.00079/full#supplementary-material>

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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