Vol. 212: 79–88, 2001 | MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser | Published March 8

Temporal variations in transparent exopolymer particles (TEP) associated with a diatom spring bloom in a subarctic ria in Japan

Neelam Ramaiah*, Takashi Yoshikawa, Ken Furuya

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

ABSTRACT: Temporal variations in transparent exopolymer particles (TEP) were studied during a spring bloom (January to April 1998) in a subarctic ria on the northeast coast of Japan. A diatom-dominated bloom developed from mid-February. Two major peaks were recorded in the first and last week of March, during which chlorophyll concentrations reached the maximum of 12 µg l^{-1}. The TEP concentration increased sharply after the first peak, the pre-bloom average of 901 rising to 1442 µg xanthan equiv. l^{-1}. The maximum TEP value of 2321 µg xanthan equiv. l^{-1} recorded at the surface coincided with the second bloom peak. After this peak, TEP concentration continued to be relatively high. The number of particles fluctuated between 1 and 3.4×10^5 ml^{-1} (size = 4 to 520 µm) with an increase in small-sized particles following the bloom decline. TEP concentrations in this bay were much higher (avg. 1344 µg xanthan equiv. l^{-1}) than reported elsewhere (avg. 147 to 308 µg xanthan equiv. l^{-1}). Although TEP increased considerably following the bloom, it was interesting to note the high pre-bloom concentrations despite low chlorophyll concentrations. This implied that a source other than phytoplankton was responsible for the release of exudates leading to TEP formation. Laboratory experiments confirmed our assumption that the significantly high background TEP was due to the additional formation of these particles from the extracellular exudates released by the macroalga *Undaria pinnatifida*, cultivated commercially in this bay. Thus, besides providing information from the subarctic coastal waters of Japan for the first time, our study also confirms the role of macroalgal exudates as an important additional source for the formation of TEP.

KEY WORDS: TEP · Spring bloom · Phytoplankton · Macroalgal culture · Subarctic ria

Resale or republication not permitted without written consent of the publisher

INTRODUCTION

Phytoplankton spring blooms, which account for a large portion of annual primary production and accumulation of organic matter, are important in determining the flow of energy in marine ecosystems (Li et al. 1993, Lignell et al. 1993, Brussaard et al. 1996). Senescence of blooms often leads to the formation of marine aggregates (Riebesell 1991a,b, Kiørboe et al. 1994, Tiselius & Kuylenstierna 1996). Transparent exopolymer particles (TEP), a class of sticky polysaccharides (Engel 2000) formed abiotically from dissolved and colloidal organic matter (Alldredge et al. 1993, Passow & Alldredge 1994, Mari & Kiørboe 1996, Passow 2000), are an important component of these aggregates. The rapid turnover rate of these exopolymers enhances the importance of TEP in the transformation of dissolved organic matter to colloidal organic matter (Mari 1999). These particles are abundant in coastal areas (Passow & Alldredge 1995a, Schuster & Herndl 1995, Mari & Burd 1998) and in the open ocean (Hong et al. 1997, Kumar et al. 1998). TEP play an important ecological role for the flux of organic matter, directly through aggregate formation and sedimentation (Passow et al. 1994, Alldredge et al. 1995), and indirectly, as an

*E-mail: neelam@aujaghi.fs.a.u-tokyo.ac.jp

© Inter-Research 2001

essential component of aggregates providing substrate and nutrition for microheterotrophs (Passow & Alldredge 1994, Schuster & Herndl 1995, Mari & Kiørboe 1996). This has been clearly documented in the recent surge of research papers on several biogeochemical aspects of TEP (Alldredge 1998, Mari 1999, Engel 2000, Passow 2000). For a better understanding of TEP dynamics, it is important to determine spatio-temporal variations in TEP in varied ecosystems to identify the different sources responsible for TEP formation, and to investigate their utilization by microheterotrophs.

The present study estimated variations in the TEP concentration associated with a spring bloom in the coastal waters of Japan, and examined the possibility of a TEP source other than phytoplankton, viz. macroalgae.

MATERIALS AND METHODS

Study site. Otsuchi Bay (39° 20′ N, 141° 56′ E) is a subarctic ria (a long narrow wedge-shaped inlet, uniformly widening and deepening towards the sea) opening into the northwest Pacific Ocean (Fig. 1). In addition to the commercial-scale cultivation of bivalves and seaweed, annual phytoplankton spring blooms add to the organic matter production in this bay (Yoshikawa et al. unpubl. data). Of the macroalgae cultivated here — *Undaria pinnatifida* forma *distans* and *Laminaria* spp. (locally called 'Wakame' and 'Kombu' respectively) — *U. pinnatifida* is commercially more important, with 1000 tons in wet weight harvested during March and April every year. The long, narrow topography of this bay, associated with wind stress along the bay axis (arising from a prevailing west to northwest wind during winter and spring) results in an intense water-mass exchange between the bay and coastal waters (Shikama 1980). As a result, surface water from inside the bay is transported seaward, whereas dense, more saline, nutrient-enriched subsurface coastal waters enter the bay along the bottom. Furuya et al. (1993) studied the effect of these physical factors on nutrient concentrations, spring bloom formation and phytoplankton productivity, and Kawamiya et al. (1996) confirmed Furuya et al.'s findings using a simulation model coupling the physical and biological parameters.

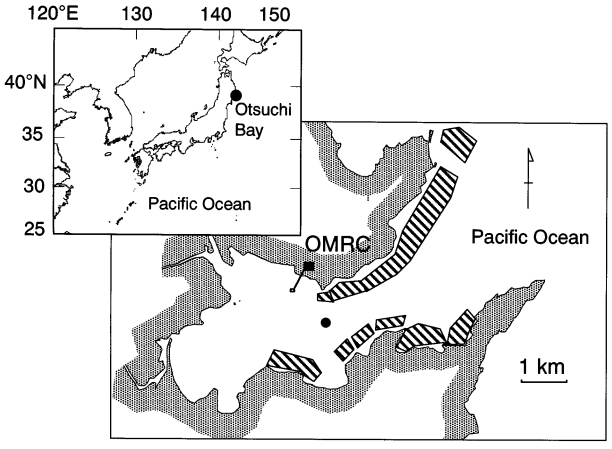

Fig. 1. Location of sampling station in Otsuchi Bay (●). Hatched areas: locations of the rafts used for commercial cultivation of the macroalga *Undaria pinnatifida* forma *distans*. OMRC: Otsuchi Marine Research Center

Sampling was carried out 2 to 3 times a week between January 19 and April 27, 1998 from a station located centrally in the Otsuchi Bay (Fig. 1). Temperature and salinity profiles were obtained from CTD casts (Alec Electric, Kobe, Japan) while water samples were collected with a Van Dorn sampler from the surface, and from 7.5 and 15 m. Post-sampling filtration and part of the analyses were conducted at the Otsuchi Marine Research Center (OMRC), a field station of the University of Tokyo.

TEP. TEP concentrations were estimated following the method of Passow & Alldredge (1995a). A 50 ml sample was filtered onto 0.4 µm Nuclepore filter paper. Particles retained on the filters were stained with Alcian Blue (8GX, Sigma) solution, the filters were soaked in 80% sulfuric acid for 3 h, and absorbance was read at 787 nm in a spectrophotometer (Hitachi, U-2000). A soaking time of 3 h was found to be ideal; extended soaking did not increase absorbance. TEP concentration was expressed in terms of xanthan gum equivalents (µg xanthan equiv. l^{-1}).

TEP sizes and numbers. Formalin-preserved samples from 7.5 m representing different phases of the spring bloom were filtered and stained as above with Alcian Blue. Stained filters were mounted on glass slides in immersion oil. Enumeration and size measurements of TEP were done under the light microscope following the method described by Passow & Alldredge (1995b). More than 350 particles were counted for the enumeration of TEP particles. Size measurements were made on 35 particles chosen at random from different fields, to obtain a general estimate of the size variations of TEP during the bloom.

Phytoplankton pigments and composition. The protocol described by Furuya et al. (1998) was followed for pigment analysis by HPLC. Briefly, 4 to 8 l of water were filtered through GF/F filters; the filters were quickly frozen in liquid nitrogen and stored at –80°C until analysis. Concentrations of the diagnostic marker pigments, viz. chlorophyll *b*, zeaxanthin, fucoxanthin, 19′-hexanoyloxyfucoxanthin, 19′-butanoyloxyfucoxanthin, peridinin, violaxanthin and alloxanthin were estimated from the respective peaks calibrated against

pure standards (Water Quality Institute, Horsholm, Denmark). The data obtained on the concentrations of diagnostic marker pigments was interpreted using CHEMTAX, a matrix factorization program running under Matlab (Mackey et al. 1996, 1997). Diatoms, dinoflagellates, cryptophytes, haptophytes, chrysophytes, chlorophytes and cyanobacteria were the 7 taxonomic groups considered in the present analysis. The initial ratio for each marker pigment used in the CHEMTAX calculation was derived after minor modifications following Mackey et al. (1996) and Furuya et al. (in press). Formalin-preserved samples representing different phases of the bloom were observed under the microscope to determine the diatom species composition. For frequent monitoring of the bloom development, chlorophyll *a* (chl *a*) was estimated fluorometrically (Turner Designs, Sunnyvale, California, USA) after extraction in N, N-dimethylformamide (Suzuki & Ishimaru 1990).

Bacterial counts. Bacterial counts were obtained between February 27 and April 27, 1998. Water samples fixed in 4% glutaraldehyde were filtered onto 0.2 µm blackened Nuclepore filters (prepared by flotation for 12 h in a clear solution of Sudan Black plus acetic acid) and stained with DAPI (4′ 6-diamidino-2-phenylindole; added to obtain a final concentration of 0.5 µg DAPI ml^{-1} sample). Utmost care was taken to prevent bacterial contamination of the filters during staining. In addition, filter blanks were determined and the values deducted to avoid overestimation of total bacterial density. Blue fluorescing bacterial cells were enumerated under an epifluorescence microscope (Nikon, Tokyo, Japan) equipped with an UV excitation filter (Porter & Feig 1980). At least 400 bacteria were counted per sample to enable a statistically significant enumeration of bacterial counts.

Nutrients. Samples were frozen immediately and stored at −20°C until analysis. Concentrations of dissolved inorganic nitrate, ammonium and phosphate were determined using an Auto Analyser II (Technicon, New York, USA).

Quantification of TEP formed from macroalgal exudates. Experiments were conducted to estimate the formation of TEP from the extracellular exudates released by the macroalga *Undaria pinnatifida* forma *distans*. Intact, healthy fronds of *U. pinnatifida* belonging to different age groups were obtained from the culture rafts of the Otsuchi Marine Research Center. After rinsing gently in 0.2 µm filtered seawater (FSW) to remove the epiphytes, these fronds were incubated in 3.5 l of FSW and held for 4 d under different light intensities (110 and 1100 µmol m^{-2} s^{-1}) and temperature conditions (4, 8 to 10 and 20°C). FSW without algae was used as a control. About 47 to 83 g of juvenile or mature fronds were used. The bottles were gently stirred from time to time, taking care to prevent any mechanical damage to the alga. Sub-samples were drawn daily to monitor changes in TEP concentration and bacterial numbers. TEP analyses were made in triplicate. The fronds were dried after termination of the experiment to obtain the dry weight of the alga. Wet weights of the alga were converted to carbon equivalents using a conversion factor of 26.6% (Yoshikawa et al. unpubl. data). TEP formation rates were calculated per gram carbon of the macroalga.

To estimate the differences in TEP concentrations between the regular study location and culture rafts of the macroalga, water samples were collected on 3 consecutive days from both locations during December 1999. Samples obtained from the surface and from 5 m depth were filtered in triplicate for TEP quantification.

RESULTS

Phytoplankton bloom and nutrients

Intermittent freshwater input associated with river discharge led to a weak stratification, with the top 2 to 3 m of water being less saline than the rest of the water column (Fig. 2). A salinity of 28 to 29 recorded at the surface coincided with the freshwater influx. Below this layer, the salinity remained almost constant at around 32 to 33. The water column between 3 and 40 m was well mixed during February and March, with temperatures around 6°C increasing to 9°C towards the end of April.

Two bloom peaks developed during the calm-weather period. Frequent wind-driven water exchange that occurred prior to the first peak prevented the accumulation of phytoplankton biomass. The first diatom bloom was initiated between 5 and 7.5 m depth in late February and spread throughout the water column reaching its first peak (chl *a* = 10 µg l^{-1}) during early March (Fig. 3). As indicated by an abrupt drop in chl *a* concentration, this peak was disrupted during mid-March by prolonged wind stress and wind driven currents (H. Otobe pers. comm.) that resulted in intense water exchange between the bay and coastal waters. A second peak developed during late March, which was more prominent. Chl *a* reached 12 µg l^{-1}, and maintained this level for 1 wk. It began decreasing in early April, indicating the decline of the spring bloom.

Pre-bloom high nitrate concentrations of 4–10 µM decreased to 0.6–6 µM during the first phytoplankton bloom (Fig. 3). Between the 2 bloom peaks, water-mass exchange and inflow of coastal water appeared to replenish the water column nitrate, which increased to >6 µM. Nitrate concentrations decreased to <0.1 µM after the second bloom peak about mid-April. From

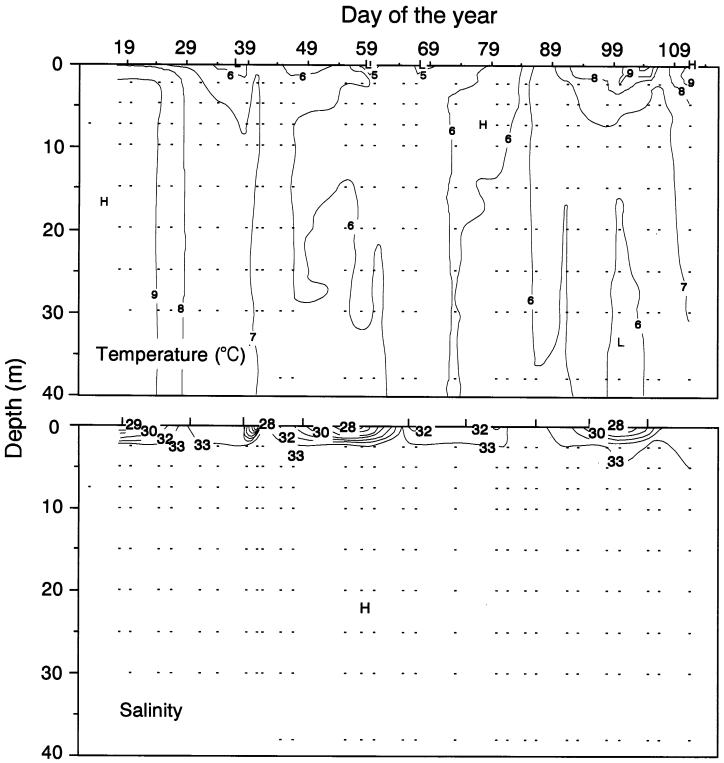

Fig. 2. Temperature and salinity profiles in Otsuchi Bay during the study period

mid-March until the end of the study period, the ammonium concentration in the upper 30 m was generally <0.5 µM; however, below 30 m, values ranged between 1 and 1.5 µM. Phosphate varied between 0.2 and 0.9 µM and declined following the bloom build-up. The N/P ratio generally varied between 0.4 and 14.9, increasing to 20.4 on a few occasions. The decline of the second bloom peak was apparently caused by nutrient depletion.

Phytoplankton composition

Fucoxanthin and 19′-hexanoyloxyfucoxanthin were the most abundant diagnostic pigments during the peak bloom period. Peridinin and zeaxanthin concentrations increased following the decline in diatom abundance towards the end of April. As can be seen from the phytoplankton composition determined by CHEMTAX analysis (Fig. 4), diatoms were the most dominant group throughout the spring bloom period. Prior to and after the diatom abundance, cryptophytes, haptophytes, chrysophytes and chlorophytes together constituted around 40 to 60% of the total phytoplankton. Of the diatoms, chain-forming *Chaetoceros debilis*, *C. socialis*, *C. curvisetus* and *Thalassiosira* spp. were the most common. *Stephanopyxis turris*, *Pseudo-nitzschia pungens*, *Asterionellopsis glacialis*, *Thalassionema nitzschoides*, *Coscinodiscus* spp. and *Guinardia flaccida* were also recorded.

TEP

Associated with the first chl *a* peak, TEP concentration increased sharply from a pre-bloom average of 901 to 1442 µg xanthan equiv. l^{-1} (Fig. 5). Prior to

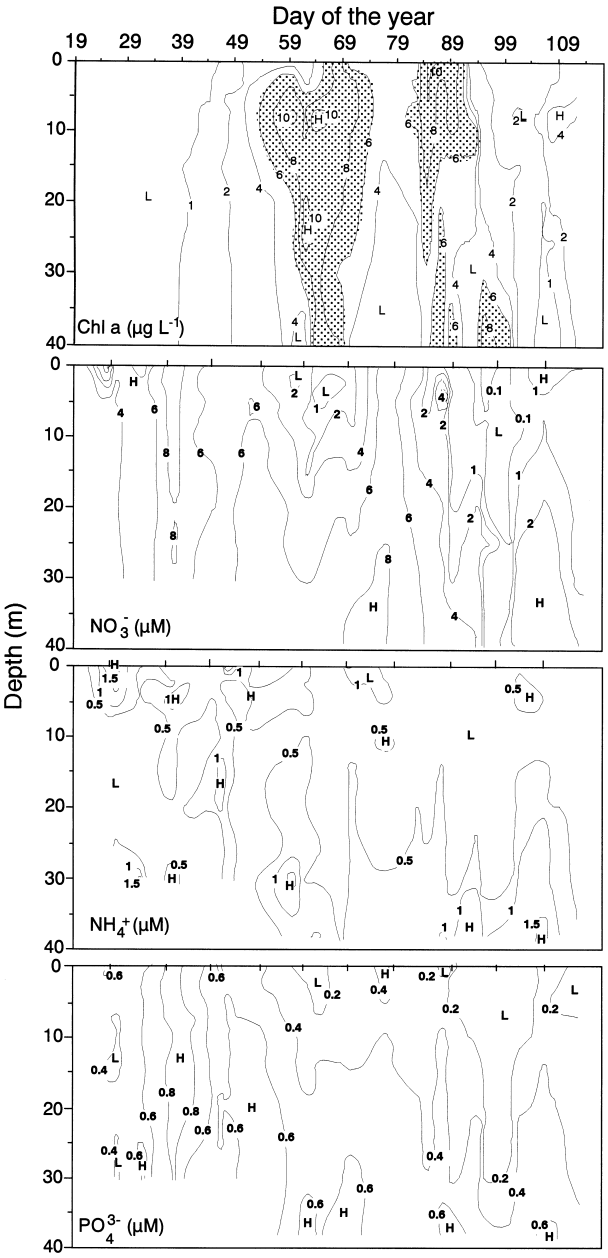

Fig. 3. Vertical profiles of (nitrate, ammonium and phosphate) and nutrients chlorophyll *a* during the 1998 spring bloom in Otsuchi Bay. Shaded areas: peak bloom period. H: high; L: low

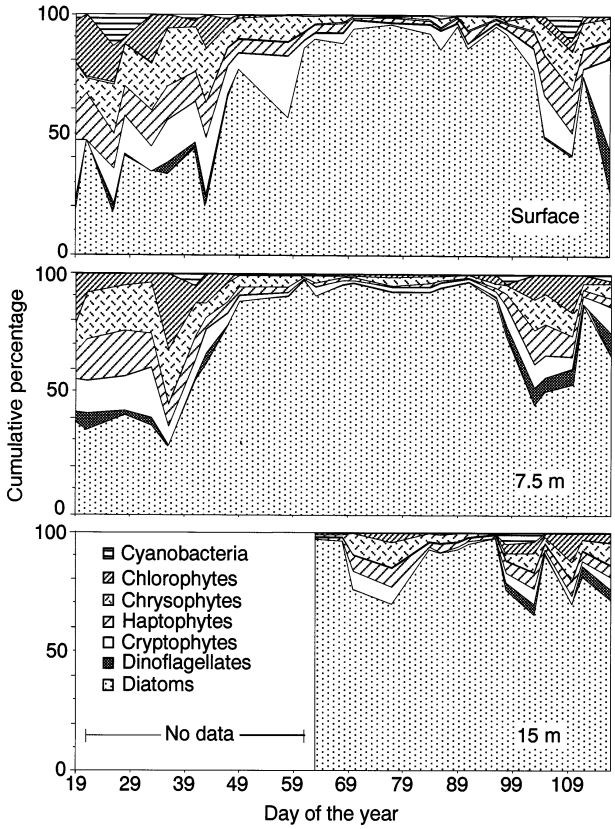

Fig. 4. Percentage contribution of the various classes of phytoplankton during the spring bloom period. Data set on the concentrations of diagnostic marker pigments derived from HPLC pigment analysis was subjected to the CHEMTAX program to obtain the phytoplankton class composition

this peak, TEP at the surface increased steadily from 136 to 1162 µg xanthan equiv. l^{-1} and dropped to 623 before rising to 1500 µg xanthan equiv. l^{-1}. The pre-bloom concentrations at 7.5 and 15 m were fairly constant, varying between 628 and 1209 µg xanthan equiv. l^{-1}. After February, TEP exhibited an almost uniform distribution in the water column. A maximum concentration of 2321 µg xanthan equiv. l^{-1} was recorded at the surface after the second bloom peak. TEP remained at high concentrations during the post-bloom period. Interesting was the increase in TEP concentrations during the pre-bloom period despite a low phytoplankton biomass at this time

TEP occurred in significant numbers at 7.5 m depth during the entire study period. Abundance increased steadily from the pre-bloom (avg. $1.7 \pm 0.4 \times 10^5$ ml^{-1}) through the bloom (avg. $2.82 \pm 0.3 \times 10^5$ ml^{-1}) to the post-bloom (avg. $3.1 \pm 0.2 \times 10^5$ ml^{-1}) periods. The size of the particles varied between 4 and 520 µm, with a maximum average size during the first peak of the bloom of 80 ± 7 µm and average sizes of 64 ± 16 and 61 ± 7 µm during the pre- and post-bloom periods respectively. However, TEP abundance was maximum (3.4×10^5 ml^{-1}) after the second bloom peak, coinciding with an increase in small-sized particles.

Bacterial abundance

Bacterial numbers fluctuated randomly prior to and during the bloom period, varying between 3.1 and

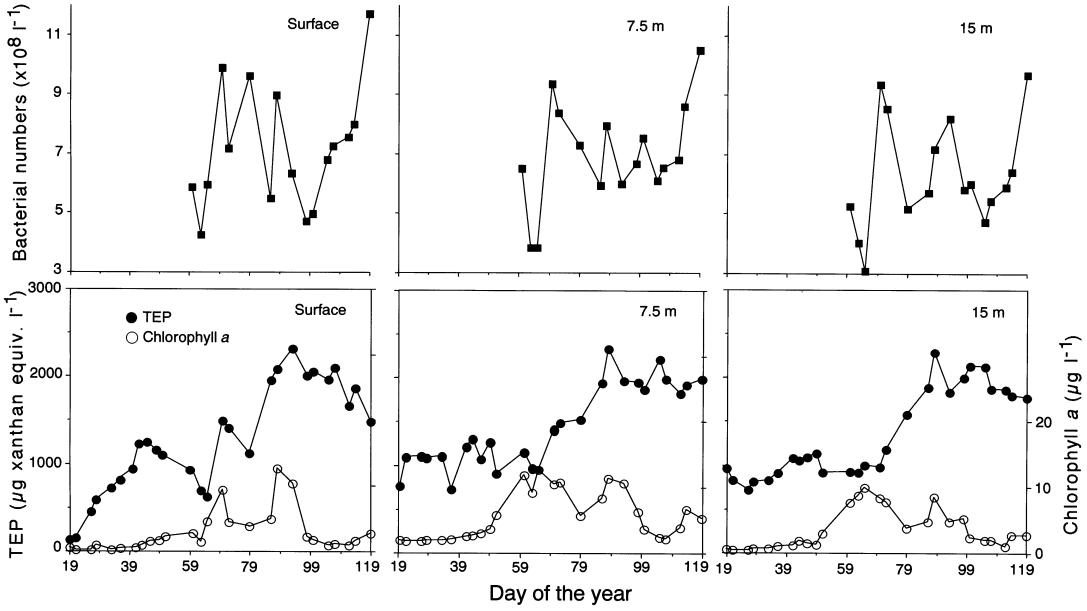

Fig. 5. Temporal changes in TEP, chlorophyll *a* concentrations and bacterial numbers in Otsuchi Bay at the surface and 7.5 and 15 m depths

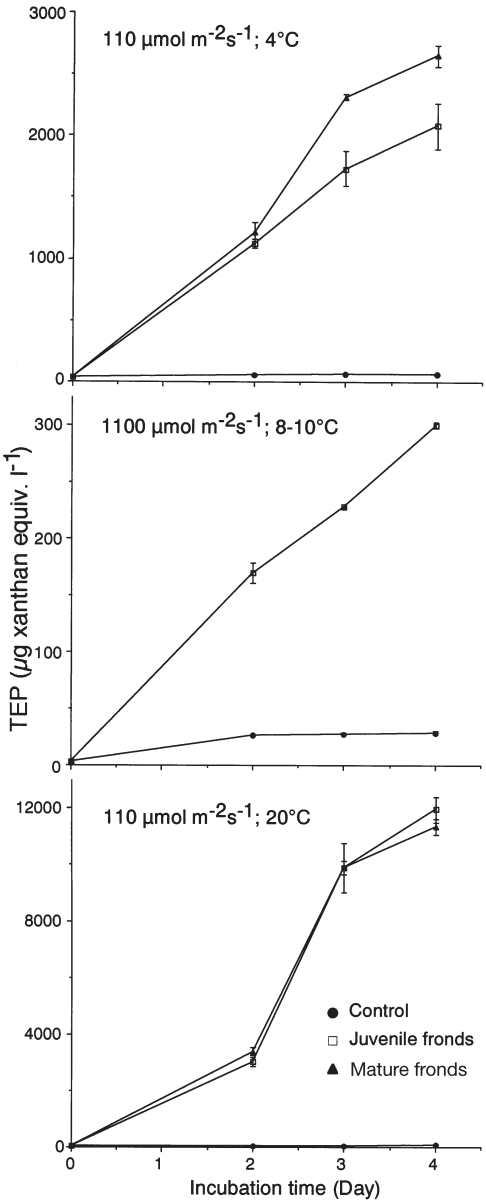

Fig. 6. Time-course variations in the formation of TEP from extracellular exudates of the macroalgae *Undaria pinnatifida* forma *distans* incubated under different light intensities and temperature conditions. Data are averages ±SE for analyses of 3 replicates

9.9×10^8 l^{-1} (Fig. 5). A steady increase was observed following the decline of the spring bloom, reaching a maximum density of 1.2×10^9 l^{-1}. Following a trend similar to that of TEP concentrations, bacterial numbers were relatively higher at the surface (avg. 7.3 ± 2.05×10^8 l^{-1}) than at 7.5 m (avg. 6.9 ± 1.73×10^8 l^{-1}) or 15 m (avg. 6.3 ± 1.82×10^8 l^{-1}). However, no definite association was observed between TEP concentration and bacterial counts at any given depth.

Formation of TEP from exudates of *Undaria pinnatifida* forma *distans*

In the bottles with juvenile fronds of *Undaria pinnatifida* exposed to light intensity of ca. 1100 µmol m^{-2} s^{-1} at 8 to 10°C, TEP increased from 2.4 to 301 µg xanthan equiv. l^{-1} during the 4 d period, yielding a TEP formation rate of 3.3 ± 0.7 mg xanthan equiv. d^{-1} g^{-1} C. In the set with juvenile and mature fronds exposed to 110 µmol m^{-2} s^{-1} and 4°C, TEP concentration increased from 37.5 to 2081.4 and 37.5 to 2651.2 µg xanthan equiv. l^{-1} (Fig. 6). Formation rates of 1.8 ± 0.5 and 1.9 ± 1.1 mg xanthan equiv. d^{-1} g^{-1} C were recorded for the juvenile and mature groups. A higher temperature (20°C) at the same light intensity (110 µmol m^{-2} s^{-1}) tremendously enhanced exudation, leading to increases in TEP concentrations from 37.5 to 11965 µg and from 37.5 to 11351 µg xanthan equiv. l^{-1} by the juvenile and mature algae respectively. Formation rates were 34.4 (±16.4) and 25.8 (±17.4) mg xanthan equiv. d^{-1} g^{-1} C respectively.

Bacterial counts in the control bottles for the 4, 8 to 10, and 20°C sets were in the range of 0.96 to 1.48 (±0.26 to 1.41 SD), 1.3 to 1.77 (± 0.34 to 0.4) and 1.45 to 1.98 (±1.45 to 1.98) × 10^8 l^{-1} respectively during the 4 d period. In the set with juvenile alga at 8 to 10°C, the counts increased from 2.81 to 7.57 (±0.42 to 1.17) × 10^8 l^{-1}. Numbers were higher in the bottle at 4°C, with juvenile alga increasing from 4 to 10.55 (±1.53 to 2.3) × 10^8 l^{-1} with relatively lower counts in the mature alga bottle varying between 1.84 and 6.21 (±1.1 to 1.85) × 10^8 l^{-1}. Maximum bacterial growth was recorded in the 20° set. The increase in density was almost of similar magnitude, from 1.6 to 26.22 (±0.2 to 0.51) and 1.5 to 25.41 (±0.1 to 0.35) × 10^8 l^{-1} for the juvenile and mature alga respectively.

TEP recorded during the December 1999 sampling at the regular study station and near the macroalga culture rafts indicated a clear difference, with higher values close to the culture rafts. TEP concentration at the regular station was 59 ± 2.3 and 64 ± 1 µg xanthan equiv. l^{-1} at the surface and 5 m respectively, while near the culture rafts the respective concentrations were 97 ± 1.5 and 104 ± 3.1 µg xanthan equiv. l^{-1}.

DISCUSSION

An intense water-mass exchange between the bay and coastal waters caused by wind-driven currents exerted a strong influence on phytoplankton growth and spring bloom formation in Otsuchi Bay. Characteristic circulation pattern and influx of coastal waters into this bay constantly replenished the water-column nutrient concentration. A lower than 16 Redfield (N/P)

ratio implied generally phosphate-rich conditions, except on few occasions when nitrate incursions along the bottom associated with an influx of coastal waters increased the ratio to 20.

Formation and stability of the bloom were nutrient- and weather-dependent. The bloom developed from mid February onwards with 2 peaks during the first and last week of March coinciding with calm weather conditions. Chl *a* concentrations that were generally <1 µg l^{-1} during the pre-bloom period (Iizumi et al. 1990) increased to 12 µg l^{-1}. The spring bloom was characteristically dominated by diatoms, with *Chaetoceros* spp. and *Thalassiosira* spp. being the most abundant.

TEP increased simultaneously with the bloom peak and continued to remain relatively high during the post-bloom period. According to Kepkay et al. (1993), increased concentrations of dissolved and colloidal organic matter during or shortly after a bloom and the process of coagulation operating at the height of a bloom result in aggregate formation and the downward flux of organic matter. Elevated concentrations of TEP during the bloom's decline should thus be due to the continued coagulation of dissolved organic matter. The importance of phytoplankton in organic matter production and TEP formation is thus clearly evidenced by the significant rise in TEP associated with bloom build-up. The TEP concentrations recorded in the present study were comparable to the high values observed by Hong et al. (1997) during a *Phaeocystis* sp.-dominated bloom in the Ross Sea, and were higher than concentrations reported elsewhere (Table 1). Noteworthy was the high TEP concentration despite low chl *a* in the pre-bloom period. Anthropogenic waste does not appear to cause an increase in TEP, since TEP concentrations in eutrophicated Tokyo Bay are much lower, although Tokyo Bay regularly receives various kinds of anthropogenic wastes (N.R. & K.F. unpubl. data). This implies that the contributions by autotrophs other than phytoplankton are responsible for the release of the polysaccharide-containing extracellular exudates that are a pre-requisite for TEP formation (Passow 2000). In regard to the special situation in Otsuchi Bay, in addition to phytoplankton, the naturally occurring and commercially cultivated macroalga *Undaria pinnatifida* forma *distans* provides an important additional source of organic matter production. Biomass of this macroalga is estimated to be similar in magnitude for both naturally occurring and cultivated populations (H. Iizumi pers. comm.). Nearly 30 to 45% of the net production of macroalgae either exuded or leached as dissolved organic matter is reported to be rich in soluble proteins, carbohydrates and lipids (Sieburth 1969, Valiela et al. 1985, Buchsbaum et al. 1991). In view of the fact that macroalgal exudates contain the pecursors of TEP, we hypothesized that the generally high background TEP are due to the additional formation from macroalgal exudates.

Our experiment with *Undaria pinnatifida* provided confirmatory evidence of its contribution to TEP concentrations. According to Valiela et al. (1985), maximum DOM is released by macroalgae during the post-senescent phase. However, our experiments showed that TEP formation rates were higher for juvenile fronds than for mature alga. This explains the high pre-bloom TEP concentrations and the generally high background level in the Otsuchi Bay, where cultivation of

Table 1. Variations in TEP concentrations and numbers reported from different geographic locations. Mean values are given in parentheses wherever possible. –: no data

TEP size (µm)	TEP numbers (no. ml^{-1})	TEP concentration (µg xanthan equiv. l^{-1})	Location	Source
Field data				
–	–	0 – 2800 (308)	Ross Sea	Hong et al. (1997)
–	–	14 – 252 (147)	Santa Barbara Channel	Passow & Alldredge (1995a)
–	–	46 – 310 (271)	Monteray Bay	Passow & Alldredge (1995a)
–	–	100 – 255 ((190)	Norwegian Fjord	Passow & Alldredge (1995a)
–	$0.3 – 6 \times 10^4$	–	Kattegat (spring bloom)	Mari & Kiørboe (1996)
–	$0.5 – 3.8 \times 10^5$	–	Kattegat (annual variation)	Mari & Burd (1998)
7 – 97	$0.1 – 7.8 \times 10^3$	70 – 500	Indian Ocean	Kumar et al. (1998)
–	$0.6 – 2.4 \times 10^3$	–	Adriatic Sea	Schuster & Herndl (1995)
4 – 520	$1.0 – 3.4 \times 10^5$	24 – 2321 (1344)	Otsuchi Bay	Present study
Laboratory studies				
–	–	2000 – 12000	*Phaeocystis* sp. culture	Hong et al. (1997)
–	–	210 – 3630	Mesocosm	Passow & Alldredge (1995a)
–	$5 – 1.5 \times 10^2$	20 – 380	*Chaetoceros* sp. culture	Passow & Alldredge (1995b)
–	$1 – 13 \times 10^3$	–	*Chaetoceros* sp. culture	Schuster & Herndl (1995)
23 – 114	$2.5 – 8.6 \times 10^3$	8 – 1862 (775)	*Skeletonema costatum* culture	(N.R. & K.F. unpubl. data)

U. pinnatifida begins in December. Juvenile plants are grown in the bay during the pre-bloom period, with a standing crop of 57 to 226 (avg. 129.4 ± 72.8) tons wet weight and 2.1 to 8.3 (4.6 ± 2.7) tons C (Yoshikawa et al. unpubl. data). At a gross estimate, with a TEP formation rate of 3.3 mg xanthan equiv. d^{-1} g^{-1} C, approximately 15.1 (± 8.9) kg xanthan equiv. TEP d^{-1} can be expected in the bay from macroalgal contribution alone. The effect of elevated temperatures on extracellular release by *U. pinnatifida* was clearly evidenced by our experiment. Young and mature fronds incubated at 20°C respectively yielded a 19- and 14-fold increase in TEP formation rate compared to that observed at 4°C. As the macroalga increases in size, light penetration of the water column is reduced. The experiment conducted at a low light intensity of 110 µmol m^{-2} s^{-1} simulated the effect of reduced light conditions on extracellular release. Based on the results of our experiment, an increase in TEP production associated with a simultaneous increase in biomass and temperature may thus be expected during the spring, which, however, is the harvesting period of *U. pinnatifida*. It can be further suggested that the input from macroalga during the active growth period plays a significant role in augmenting the DOM concentration in the water column. Although we did not quantify DOC during our experiments, from the increase in TEP concentrations it is likely that the amount of DOM contributed by the macroalga is quite substantial. Also, the C:N:P ratio may prove even more indicative of TEP production.

High TEP concentrations recorded near the macroalgae culture rafts during December 1999, long before the phytoplankton bloom, further confirm the role of macroalgae in TEP formation. Hence, the higher availability of TEP near the macroalgae beds compared to our regular station is a sure indication of the important role of *Undaria pinnatifida* in TEP formation.

Although TEP increased sharply following the first bloom peak, no further increase was observed subsequent to the second peak. Randomly fluctuating bacterial numbers increased towards the end of April. The higher availability of TEP after the phytoplankton bloom may explain the post-bloom increase in bacterial density. Wiebe & Smith (1977) estimated that algal exudates contribute nearly 50% of the bacterioplankton energy. Bacteria are known to readily colonize TEP, to solubilize larger particles through elaboration of extracellular enzymes, and finally to utilize the resulting DOM (Smith et al. 1992, Passow & Alldredge 1994, Schuster et al. 1998). Mari & Kiørboe (1996) found TEP to be positively related to bacterial concentration, both being dependent on the pool of dissolved and colloidal organic matter. An increase in small-sized particles associated with a rise in bacterial density leads us to presume that colonization and solubilization of TEP at several points by bacteria break up the particles into smaller-sized ones. Their polysaccharide nature and the amino acids (Decho 1990, Schuster et al. 1998) and metals (Niven et al. 1997) adsorbed on their surfaces make these particles ideal sites for bacterial colonization. A statistically significant correlation obtained between chl *a* and TEP (r^2 = 0.83) and TEP and bacteria (r^2 = 0.98) in our culture experiment with *Skeletonema costatum* (N.R. & K.F. unpubl. data) confirms that TEP production is a function of phytoplankton growth and the dependence of bacterial growth on exudates released by phytoplankton. Recently, Aluwihare & Repeta (1999) demonstrated that TEP include a wide and yet not exactly defined variety of acid polysaccharides, such as aceyl heteropolysaccharides, that may be recalcitrant to bacterial solubilization through ectoenzyme hydrolysis. However, it is quite unlikely that the higher concentration of TEP during the pre-bloom period was due to non-utilization by bacteria. In our experiment with the macroalga, increases in bacterial numbers at all temperatures with concomitant increases in TEP concentrations clearly indicated active bacterial growth, confirming that TEP produced from macroalgal exudates are indeed utilizable and support bacterial growth.

In conclusion, our field study revealed the indispensable role of phytoplankton in TEP formation and a laboratory experiment confirmed the pre-bloom high levels of TEP in Otsuchi Bay to be due to the extracellular exudates copiously released by *Undaria pinnatifida*. According to the statistics provided by the Japan Ministry of Agriculture, Forestry and Fisheries, of the 70 054 tons of cultured *U. pinnatifida* obtained from coastal waters of Japan in 1997, nearly 66% was harvested from the northeast coast alone. Similar to Otsuchi Bay, macroalgae occurring naturally and those cultivated commercially in several coastal rias of Japan may increase TEP concentrations, resulting in the transport of TEP-rich bay waters into the coastal region. The fact that the TEP concentration remained consistently high despite a drop in chl *a* between the 2 peaks during mid-March indicates the influx of TEP-rich coastal waters into the bay. The effect of human activities in modifying organic matter dynamics in the coastal areas of Japan is thus clearly evident. As reviewed by Campbell & Burridge (1998), *U. pinnatifida*, originally native to the temperate coasts of Japan, Korea and China, has extensively invaded several coastal inlets of the north and south Pacific. The spread of this macroalga may lead to conditions similar to those we observed in Otsuchi Bay.

Acknowledgements. The field sampling and laboratory experiments were carried out smoothly thanks to the kind assistance of H. Otobe, I. Takeuchi and all members of the

Otsuchi Marine Research Center (OMRC). We thank M. Hayashi for co-operation with HPLC, H. Ogawa for nutrient analysis and K. Nanba for preliminary suggestions concerning the DAPI method. Critical evaluations of and valuable suggestions about the earlier versions of our manuscript by 3 anonymous reviewers were extremely helpful. This work was supported by Grant-in-Aid (09660191 and 11660181) from the Ministry of Education, Science, Sports and Culture (Monbusho) to K.F. N.R. is grateful to the Monbusho for a Fellowship.

LITERATURE CITED

Alldredge AL (1998) The carbon, nitrogen and mass content of marine snow as a function of aggregate size. Deep-Sea Res 45:529–541

Alldredge AL, Passow U, Logan BE (1993) The abundance and significance of a class of large, transparent organic particles in the ocean. Deep-Sea Res 40:1131–1140

Alldredge AL, Gotschalk C, Passow U, Riebesell U (1995) Mass aggregation of diatom blooms: insights from a mesocosm study. Deep-Sea Res 42:9–27

Aluwihare LI, Repeta DJ (1999) A comparison of the chemical characteristics of oceanic DOM and extracellular DOM produced by marine algae. Mar Ecol Prog Ser 186: 105–117

Brussaard CPD, Gast GJ, van Duyl FL, Riegman R (1996) Impact of phytoplankton bloom magnitude on a pelagic microbial food web. Mar Ecol Prog Ser 144:211–221

Buchsbaum RI, Valiela I, Swain T, Dzierzeski M, Allen S (1991) Available and refractory nitrogen in detritus of coastal vascular plants and macroalgae. Mar Ecol Prog Ser 72:131–143

Campbell SJ, Burridge TR (1998) Occurrence of *Undaria pinnatifida* (Phaeophyta: Laminariales) in Port Phillip Bay, Victoria, Australia. Mar Freshw Res 49:379–381

Decho AW (1990) Microbial exopolymers secretions in ocean environments: their role(s) in food webs and marine processes. Oceanogr Mar Biol Annu Rev 28:73–153

Engel A (2000) The role of transparent exopolymer particles (TEP) in the increase in apparent particle stickiness (α) during the decline of a diatom bloom. J Plankton Res 22: 485–497

Furuya K, Takahashi K, Iizumi H (1993) Wind-dependent formation of phytoplankton spring bloom in Otsuchi Bay, a ria in Sanriku, Japan. J Oceanogr 49:459–475

Furuya K, Hayashi M, Yabushita Y (1998) HPLC determination of phytoplankton pigments using N,N-dimethylformamide. J Oceanogr 54:199–203

Furuya, K, Hayashi M, Yabushita Y, Ishikawa A (in press) Phytoplankton dynamics in the East China Sea in spring and summer as revealed by HPLC-derived pigment signatures. Deep-Sea Res Part II: Top Stud Oceanogr

Hong Y, Smith WO Jr, White A (1997) Studies on transparent exopolymer particles (TEP) produced in the Ross Sea (Antarctica) and by *Phaeocystis antarctica* (Prymnesiophyceae). J Phycol 33:368–376

Iizumi H, Furuya K, Takeuchi I, Kutsuwada K, Tada A, Kawaguchi K (1990) Dynamics of nutrients and chlorophyll *a* at Otsuchi Bay: analyses of the data of monthly observation. Rep Otsuchi Mar Res Cent Jpn 16:63–65

Kawamiya M, Kishi MJ, Kawser Ahmed MD, Sugimoto T (1996) Causes and consequences of spring phytoplankton blooms in Otsuchi Bay, Japan. Deep-Sea Res 16: 1683–1698

Kepkay PE, Niven SHE, Milligan TG (1993) Low molecular weight and colloidal DOC production during a phytoplankton bloom. Mar Ecol Prog Ser 100:233–244

Kiørboe T, Lundsgaard C, Olsen M, Hansen JLS (1994) Aggregation and sedimentation processes during a spring phytoplankton bloom: a field experiment to test coagulation theory. J Mar Res 52:297–323

Kumar MD, Sarma VVSS, Ramaiah N, Gauns M, Desouza SN (1998) Biogeochemical significance of transparent exopolymer particles in the Indian Ocean. Geophys Res Lett 25:81–84

Li WKW, Dickie PM, Harrison WG, Irwin BD (1993) Biomass and production of bacteria and phytoplankton during the spring bloom in the western North Atlantic Ocean. Deep-Sea Res 40:307–327

Lignell R, Heiskanen AS, Kuosa H, Gundersen K, Kuuppo-Leinikki P, Pajuniemi R, Uitto A (1993) Fate of a phytoplankton spring bloom, sedimentation and carbon flow in the plankton food web in the northern Baltic. Mar Ecol Prog Ser 94:239–252

Mackey MD, Mackey DJ, Higgins HW, Wright SW (1996) CHEMTAX—a program for estimating class abundances from chemical markers: application to HPLC measurements of phytoplankton. Mar Ecol Prog Ser 144:265–283

Mackey MD, Mackey DJ, Higgins HW, Wright SW (1997) CHEMTAX user's manual: a program for estimating class abundance from chemical markers—application to HPLC measurements of phytoplankton pigments. Rep Mar Lab CSIRO Aust 229:41

Mari X (1999) Carbon content and C:N ratio of transparent exopolymeric particles (TEP) produced by bubbling exudates of diatoms. Mar Ecol Prog Ser 183:59–71

Mari X, Burd A (1998) Seasonal size spectra of transparent exopolymeric particles (TEP) in a coastal sea and comparison with those predicted using coagulation theory. Mar Ecol Prog Ser 163:63–76

Mari X, Kiørboe T (1996) Abundance, size distribution and bacterial colonization of transparent exopolymer particles (TEP) during spring in the Kattegat. J Plankton Res 18: 969–986

Niven SHE, Kepkay PE, Bugden JBC (1997) The role of TEP in ^{234}Th scavenging during a coastal diatom bloom. Radioprotection-Colloques 32:213–218

Passow U (2000) Formation of transparent exopolymer particles, TEP, from dissolved precursor material. Mar Ecol Prog Ser 192:1–11

Passow U, Alldredge AL (1994) Distribution, size and bacterial colonization of transparent exopolymer particles (TEP) in the ocean. Mar Ecol Prog Ser 113:185–198

Passow U, Alldredge AL (1995a) A quantitative method to determine the concentration of transparent exopolymer particles (TEP). Limnol Oceanogr 40:1326–1335

Passow U, Alldredge AL (1995b) Aggregation of a diatom bloom in a mesocosm: the role of transparent exopolymer particles (TEP). Deep-Sea Res 42:99–109

Passow U, Alldredge AL, Logan BE (1994) The role of particulate carbohydrate exudates in the flocculation of diatom blooms. Deep-Sea Res 41:335–357

Porter GK, Feig YS (1980) The use of DAPI for identifying and counting aquatic microflora. Limnol Oceanogr 25:943–948

Riebesell U (1991a) Particle aggregation during a diatom bloom. I. Physical aspects. Mar Ecol Prog Ser 69:273–280

Riebesell U (1991b) Particle aggregation during a diatom bloom. II. Biological aspects. Mar Ecol Prog Ser 69: 281–291

Sieburth J McN (1969) Studies on algal substances in the sea. III. The production of extracellular organic matter by littoral marine algae. J Exp Mar Biol Ecol 3:290–309

Schuster S, Herndl GJ (1995) Formation and significance of transparent exopolymer particles in the northern Adriatic Sea. Mar Ecol Prog Ser 124:227–236

Schuster S, Arrieta JM, Herndl GJ (1998) Adsorption of dissolved free amino acids on colloidal DOM enhances colloidal DOM utilization but reduces amino acid uptake by orders of magnitude in marine bacterioplankton. Mar Ecol Prog Ser 166:99–108

Shikama N (1980) Current measurements in Otsuchi Bay. Bull Coast Oceanogr 18:1–8 (in Japanese)

Smith DC, Simon M, Alldredge AL, Azam F (1992) Intense hydrolytic enzyme activity on marine aggregates and implications for rapid particle dissolution. Nature 359: 139–142

Suzuki R, Ishimaru T (1990) An improved method for the determination of phytoplankton chlorophyll using N,N-dimethylformamide. J Oceanogr Soc Jpn 46:190–194

Tiselius P, Kuylenstierna M (1996) Growth and decline of a diatom spring bloom: phytoplankton species composition, formation of marine snow and the role of heterotrophic dinoflagellates. J Plankton Res 18:133–155

Valiela I, Teal JM, Allan SD, van Etten R, Goehungel D, Volkman S (1985) Decomposition in salt marsh ecosystems: the phases and major factors affecting disappearance of above-ground organic matter. J Exp Mar Biol Ecol 89:29–54

Wiebe WJ, Smith DF (1977) Direct measurement of dissolved organic carbon released by phytoplankton and incorporation by microheterotrophs. Mar Biol 42:213–223

Editorial responsibility: Otto Kinne (Editor), Oldendorf/Luhe, Germany

Submitted: October 27, 1999; Accepted: August 23, 2000
Proofs received from author(s): February 12, 2001