

Submicrometer particles in northwest Pacific coastal environments: Abundance, size distribution, and biological origins

Abstract—Submicrometer particles (SMP) are suggested to be a critical component for organic matter transitions in seawater, but little is known about variations and controls of SMP in coastal systems. We examined vertical and horizontal distributions of SMP (0.4–1 μm in equivalent spherical diameter) as measured by a resistive pulse particle counter) and biological variables (chlorophyll *a* concentration, abundance of bacteria, and heterotrophic nanoflagellates) in northwest Pacific coastal environments. The abundance and total volume of SMP in the upper 200 m varied in the range of 5×10^4 – 3×10^7 particles ml^{-1} and 4×10^3 – $3 \times 10^6 \mu\text{m}^3 \text{ml}^{-1}$, respectively. Over a large trophic gradient (Chl *a*, 0.02–4 $\mu\text{g liter}^{-1}$), the total volume of SMP was strongly positively correlated with Chl *a* concentration ($r = 0.90$, $P < 0.0001$, $n = 47$) and with other microbial variables ($r = 0.84$ – 0.90), consistent with a hypothesis that SMP dynamics are closely related to microbial food-web processes. Notably, size distribution of SMP in upper waters often exhibited a distinctive peak at a size range of 0.6–0.7 μm , which was most pronounced in productive nearshore waters and became less evident with depth and with distance from the shore. A sonication experiment revealed that the 0.6–0.7- μm particles are primarily nonliving. We hypothesize that SMP, particularly the 0.6–0.7- μm component, are directly produced by biological processes. Our data suggest that SMP are a highly reactive and abundant component of detrital colloids and play important roles in material cycles within coastal systems.

The discovery of many organic submicrometer particles (SMP, 0.4–1 μm in equivalent spherical diameter) in seawater (Koike et al. 1990) has substantially stimulated the interest of oceanographers in the role of SMP and other organic colloids in biogeochemical cycles and food-web processes (reviewed by Nagata and Koike 1995; Nagata and Kirchman 1997). Colloidal organic carbon including SMP and nanometer-size particles (5–200 nm; Wells and Goldberg 1991, 1994) accounts for a large fraction (10–50%) of dissolved organic carbon in seawater (Benner et al. 1992; Guo et al. 1994), providing a critical component for organic matter transitions between soluble and particulate pools (Allredge et al. 1993; Kepkay 1994). Furthermore, SMP provide large solid–water interfaces, which may affect turnover and transport of radionuclides and organic compounds that readily adsorb to surfaces (Moran and Buesseler 1992; Nagata and Kirchman 1996). Thus, clarifying dynamics and distribution pattern of SMP and colloids is fundamentally important to understanding the cycling of matter in the oceans.

Data on SMP distributions are quite limited. In the subarctic Pacific Ocean, Koike et al. (1990) used a resistive pulse particle counter (Elzone 80XY) to find high abundance of SMP (10^7 particles ml^{-1}) in the upper layer, which decreased rapidly with depth, covarying well with Chl *a* concentrations. From the results of physical treatments (soni-

cation, ultracentrifugation, and filtration) of seawater, Koike et al. suggested that SMP are mostly nonliving, fragile, and flexible particles, possibly with a very high water content. In northwest Atlantic shelf water, Longhurst et al. (1992) confirmed high abundance of SMP, but did not examine relationships between SMP abundance and environmental variables. Very little is known about SMP distributions in productive environments (Chl *a* concn of $>1 \mu\text{g liter}^{-1}$) where complex interactions between SMP and microorganisms may occur (Sieracki and Viles 1992). Recent research has suggested that several biological source (protist egestion: Koike et al. 1990; Nagata 1997; viral infection: Shibata et al. 1997) and sink (grazing: Tranvik et al. 1993; enzymatic hydrolysis: Nagata and Kirchman 1996) processes may be involved in the production and degradation of SMP in seawater. The effect of these processes on in situ dynamics of SMP is not known. To understand variations and controls of SMP in marine systems, we examined distributions of SMP in the northwest Pacific. Our purpose was to investigate spatial variabilities in abundance and size distribution of SMP in relation to biological variables over a large trophic gradient in coastal environments.

Water samples were collected at four sampling stations along a nearshore–offshore transect from the Sagami Bay to off the Izu islands, Japan, on the RV *Tansei Maru* cruise (KT-96-9) during 3–9 June 1996. At the time of our survey, the Kuroshio Current was flowing ~ 50 km off the Bohso Peninsula (Japan Maritime Agency). Our sampling stations were located in the middle of the Kuroshio (Sta. A, 34°36'N, 140°27'E, water depth of 3,100 m), off the Kuroshio (Sta. B, 32°28'N, 140°32'E, 2,300 m), and between the Kuroshio and the shore (Sta. C, 34°40'N, 139°53'E, 2,500 m). Sta. D (35°00'N, 139°21'E, 1,500 m) was located at the center of Sagami Bay. Additional samples were collected at the center of Otsuchi Bay (39°20'N, 141°57'E, 43 m), a semienclosed bay in the northeast coast, Japan, on 17 and 20 May 1996. Water was collected with a 10-liter Niskin CTD rosette sampler (Neil Brown Co.) (RV *Tansei Maru* Cruise) or with a 10-liter Van Dorn sampler (Otsuchi Bay). Samples for determinations of abundance of SMP and bacteria were fixed with formaldehyde (final concn 2%), whereas those for flagellates were fixed with glutaraldehyde (final concn 1%). These samples were stored at 4°C in the dark for the later analysis. To avoid artificial aggregation or breakage of SMP (Koike et al. 1990), care was taken to minimize agitation and bubbling of water during sample handling.

Size distribution and abundance of SMP were determined with a resistive pulse particle counter (Elzone 80XY, Particle Data) equipped with a 12- μm orifice tube, which has the capability of counting particles with a spherical diameter of 0.43–2.53 μm . The instrument was calibrated according to Kogure and Koike (1987). Samples were prefiltered through

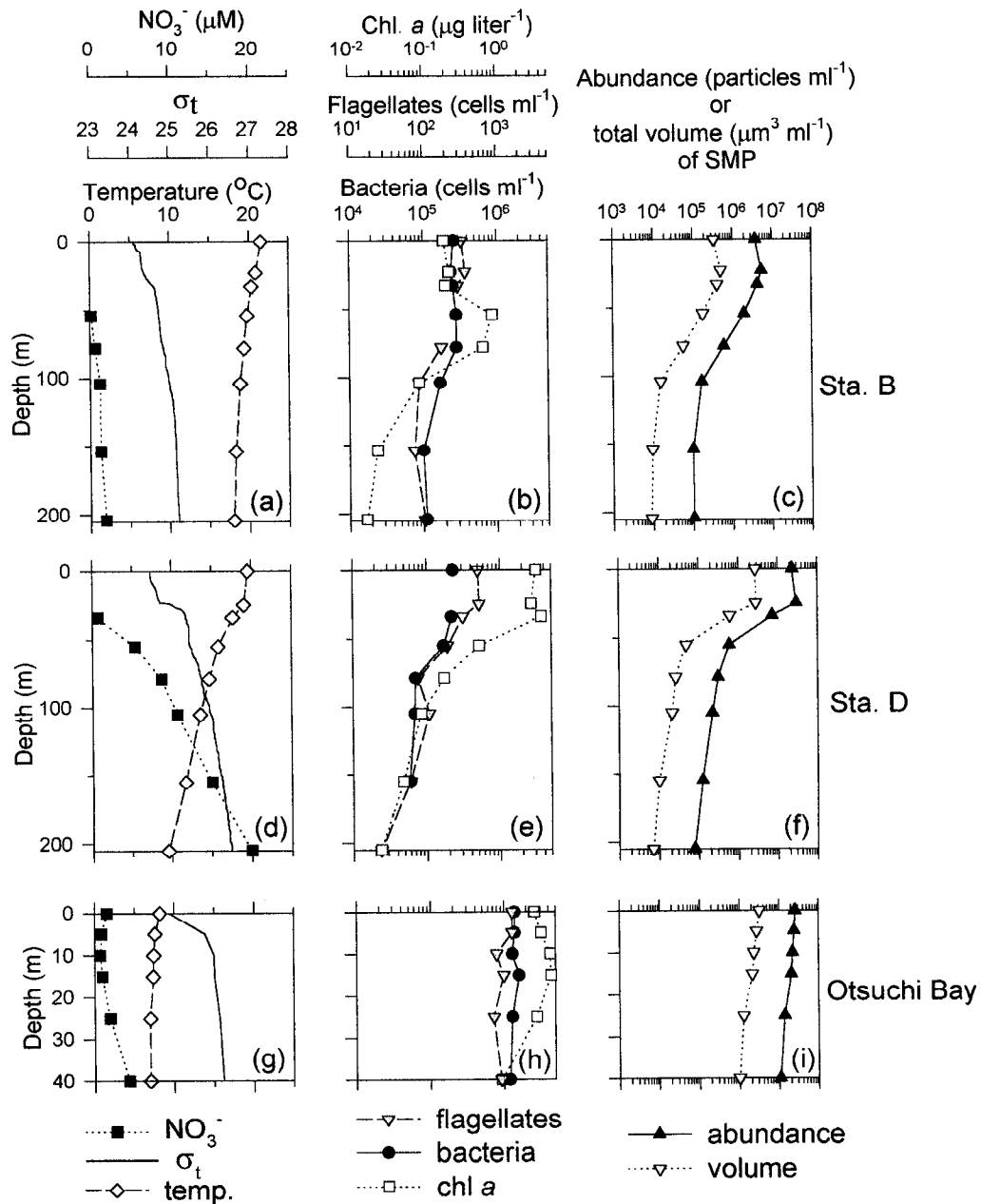


Fig. 1. Depth profiles of σ_t , temperature, nitrate concentration, Chl *a* concentration, bacterial abundance, abundance of heterotrophic nanoflagellates, and the abundance and total volume of SMP at Sta. B (a-c), Sta. D (d-f), and the station in Otsuchi Bay (g-i).

a 10- μm net to avoid clogging of the orifice. Here, we present data of SMP in the size range between 0.43 and 1 μm . Note that this analytical window (0.43–1 μm) is narrower than that of early study in the subarctic Pacific (0.38–1 μm ; Koike et al. 1990); hence, the abundance of SMP that we report here is conservative. The detection limit of SMP was 10^2 particles ml^{-1} . Estimates of SMP abundance are the averages of replicated measurements ($n = 3\text{--}4$; c.v. $<10\%$).

Bacteria were counted by the epifluorescence microscopy after staining with 4',6-diamidino-2-phenylindole (DAPI; Porter and Feig 1980). Heterotrophic nanoflagellates were counted by the epifluorescence microscopy after double

staining with fluorescein isothiocyanate and DAPI (Sherr and Sherr 1983). Nitrate concentrations were determined colorimetrically (Autoanalyzer AA-2, Technicon; Armstrong et al. 1967). Chl *a* concentrations were determined fluorometrically (Fluorometer 10-AU, Turner Designs) after extraction with *N,N*-dimethylformamide (Suzuki and Ishimaru 1990).

We examined effects of sonication on abundance and size distribution of SMP and abundance of bacteria by using water collected from a 10-m depth in Otsuchi Bay on 14 May 1996. The sample was gravity filtered through glass-fiber filters (GF/C, Whatman) to eliminate larger particles that could be destroyed by sonication to produce SMP. The fil-

trate was contained in polystyrene tubes (10-ml capacity) and mildly sonicated (Ultrasonic cleaner B-220, Branson Cleaning Equip). SMP and bacterial abundance were determined (*see above*) over time (0, 0.5, and 1 h).

To examine relationships among SMP and biological variables, we used all the data from the survey on the *Tansei Maru* cruise and that in Otsuchi Bay. The data were log transformed to stabilize the variance that tended to increase with the increase of estimates and to meet the homoscedasticity assumption for regression analysis (Table 1). The data were fit to the model, $\log_{10} Y = a + b \times \log_{10} X$, using ordinary least-squares regression. Because both X and Y variables were measured with error, model II slopes were also calculated (Ricker 1973).

Depth profiles of submicrometer particles—Fig. 1a–f shows depth profiles (0–200 m) at representative stations (Sta. B and D). At all the stations, the water column was stratified with high temperatures (19.5–22.0°C) in the upper 30–40 m and depletion of nitrate (<0.1 μM) in the upper 40–50 m (Fig. 1a,d), except that nitrate concentration was high (1 μM) at 10-m depth at Sta. C (data not shown). Chl a concentrations in the upper 200 m varied greatly (0.02–4 $\mu\text{g liter}^{-1}$) with a general tendency of decrease with distance from the shore. At two stations off (Sta. B) and in the middle of (Sta. A) the Kuroshio, subsurface Chl a maxima (Chl a concn 0.5–0.8 $\mu\text{g liter}^{-1}$) were developed at depths of 50–70 m (Fig. 1b). The abundance of bacteria in the upper 30–100 m varied between 2 and 4 $\times 10^5$ cells ml^{-1} , which decreased to 0.5–1 $\times 10^5$ cells ml^{-1} at depths below 100 m (Fig. 1b,e). The general pattern of depth profiles of heterotrophic nanoflagellates was similar to that of bacteria—the abundance was high (2–5 $\times 10^3$ cells ml^{-1}) in the upper 50 m and low (10–40 cells ml^{-1}) at deeper depths (Fig. 1b,e).

Depth profiles of SMP varied little among four stations with high abundance (0.5–3 $\times 10^7$ particles ml^{-1}) in the upper 20 m, declining sharply with depth. Both number and total volume of SMP at depths of 100–200 m were about two orders of magnitude lower than those in the surface layer (Fig. 1c,f). Over depth, abundance and total volume of SMP generally covaried with Chl a concentrations at Sta. C and D (Fig. 1e,f). At Sta. A and B, depth profiles of SMP did not correspond to Chl a profiles that exhibited subsurface maxima (Fig. 1b,c). Consistent with the general tendency of horizontal distributions of Chl a and flagellate abundance, abundance and total volume of SMP in the upper layer (0–20 m) at nearshore stations (Sta. D, C; 1.5–2.9 $\times 10^7$ particles ml^{-1} , 1.4–2.8 $\times 10^6 \mu\text{m}^3 \text{ml}^{-1}$) were greater than those at offshore stations (Sta. A, B; 0.5–1.4 $\times 10^7$ particles ml^{-1} , 0.5–1.2 $\times 10^6 \mu\text{m}^3 \text{ml}^{-1}$).

At Otsuchi station (40-m depth), the water column was stratified with differences of temperature and salinity between the surface (temp., 8.2°C; salinity, 32.0‰) and deeper (5–40 m; 7.0–7.6°C, 33.0–33.5‰) layer(s) on 17 May (Fig. 1g). Chl a varied in the range of 0.9–4.4 $\mu\text{g liter}^{-1}$ with higher values at 10 and 15 m (Fig. 1h). Abundances of bacteria and heterotrophic nanoflagellates were relatively invariant over depth with ranges of 1.2–1.6 $\times 10^6$ and 0.7–1.3 $\times 10^3$ cells ml^{-1} , respectively (Fig. 1h). Abundance and total volume of SMP were highest at the surface (2.6 $\times 10^7$

Table 1. Regressions fitted to the model, $\log_{10} Y = a + b \times \log_{10} X$. (SMP No., abundance of SMP; SMP vol, total volume of SMP; Bac, bacterial abundance; Hfl, abundance of heterotrophic nanoflagellates; Chl, Chl a concn. P -values for two-tailed t -tests of the null hypothesis: $b = 0$, were <0.0001 for all the regressions. Model II slopes are also shown.)

Y	X	a	b	n	r^2	Model II slope
SMP No.	Chl	6.71	1.12	47	0.803	1.25
SMP vol	Chl	5.68	1.15	47	0.802	1.28
SMP No.	Bac	-2.06	1.54	45	0.688	1.86
SMP vol	Bac	-3.36	1.59	45	0.699	1.90
SMP No.	Hfl	2.07	1.79	44	0.795	2.01
SMP vol	Hfl	0.912	1.84	44	0.807	2.05
Bac	Chl	5.64	0.544	45	0.645	0.677

particles ml^{-1} , 3.2 $\times 10^6 \mu\text{m}^3 \text{ml}^{-1}$), and gradually decreased down to 40 m (1.1 $\times 10^7$ particles ml^{-1} , 1.0 $\times 10^6 \mu\text{m}^3 \text{ml}^{-1}$) (Fig. 1i). On 20 May, depth profiles of measured variables were generally similar to those obtained on 17 May (data not shown).

Abundance and volume of SMP increased with Chl a (Table 1, Fig. 2a)—the regressions explained >80% of the variance in the total number ($r^2 = 0.80$) and volume ($r^2 = 0.80$) of SMP. Abundance and volume of SMP were also significantly ($P < 0.0001$) related to bacterial ($r^2 = 0.688$ and 0.699 for SMP abundance and volume) and flagellate abundance ($r^2 = 0.795$ and 0.807 for SMP abundance and volume) (Table 1; Fig. 2b,c). The slope of the SMP abundance vs. Chl a regression (1.12) was significantly (ANCOVA, $P < 0.001$) larger than the slope of the bacteria vs. Chl a regression (0.544) (Table 1). A similar trend was observed with model II slope estimates (Table 1). Consequently, the SMP:bacteria abundance ratios (0.8–100) increased with the increase of Chl a concentrations (Pearson's $r = 0.302$; $P = 0.04$, $n = 45$; Fig. 2d), indicating that the contribution of bacteria to SMP was small in productive waters.

Size distribution of SMP in the surface waters from Otsuchi Bay and at Sta. D (Sagami Bay) had a pronounced peak at $\sim 0.6 \mu\text{m}$ (Fig. 3c,d). In these stations, the peak gradually became less pronounced with depth; at deeper depths, abundance of SMP simply decreased with increasing particle size. The successive change in size distribution pattern of SMP also occurred in surface waters along the nearshore-offshore gradient; the 0.6- μm peak observed at a nearshore station (Sta. D; Fig. 3c) gradually became less pronounced over the distance from the shore (Fig. 3a,b).

The unimodal size distribution of SMP in upper waters is even clearer when data are plotted in terms of particle volume (Fig. 3e–h)—the peak is formed by 0.6–0.7- μm particles. The peak became less distinctive with depth as well as with distance from the shore, a trend similar to that just described for size distribution in abundance. Relative contribution of the 0.6–0.7- μm particles to total SMP volume varied in the range of 0.17–0.39. This proportion was positively correlated with Chl a (Pearson's $r = 0.543$; $P < 0.0001$, $n = 47$).

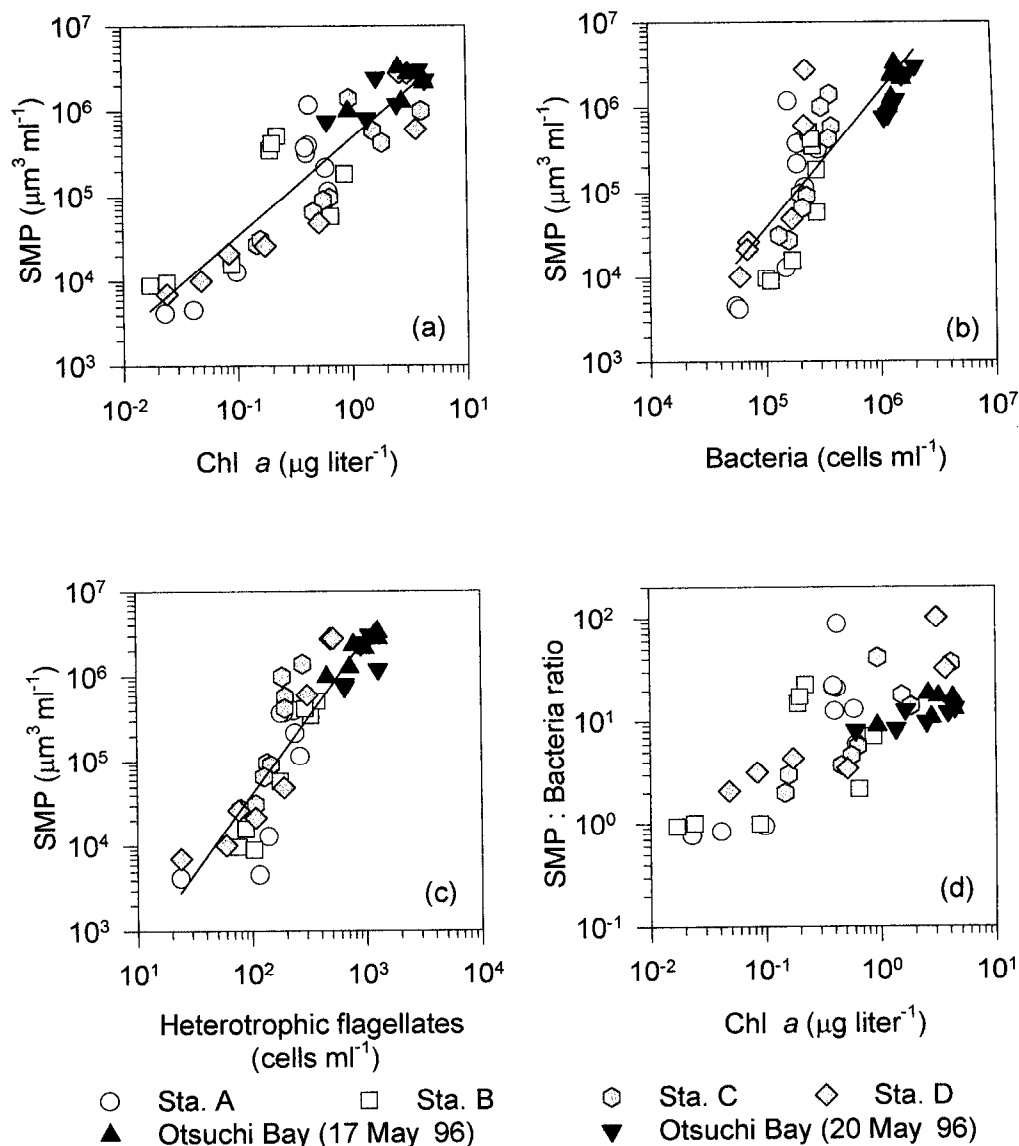


Fig. 2. Relationships between the total volume of SMP and Chl *a* concentration (a), bacterial abundance (b), or the abundance of heterotrophic nanoflagellates (c). Regression parameters are given in Table 1. The relationship between Chl *a* concentration and the ratio of total SMP abundance to bacterial abundance is also presented (d).

Origin of submicrometer particles—SMP are destroyed by mild sonication which does not affect abundance of bacteria, the major living particles in this size (Koike et al. 1990). By using this difference in physical strength between SMP and bacteria, we examined to what extent bacteria contribute to the 0.6–0.7- μm particles in productive waters. An Otsuchi Bay water sample was sonicated, and SMP and bacterial abundance were determined over time. Sonication caused 75% reduction in SMP abundance after 1 h of sonication, but did not reduce bacterial abundance (Fig. 4). The sonication also caused changes in size distribution of SMP—the 0.6–0.7 μm peak disappeared after the sonication (Fig. 5a,b). Although we did not determine size distribution of bacteria, the results of the sonication experiment clearly

demonstrate that SMP and their 0.6–0.7 μm component were primarily nonliving.

Our data show that not only the abundance but also the size distribution of SMP vary successively over a wide trophic gradient in coastal systems. Size distribution of oceanic particles including colloids is largely influenced by physical particle interactions such as aggregation of small particles and break up of large aggregates (McCave 1984; Johnson and Kepkay 1992; Jackson 1995). In fact, size spectra of oceanic particles in a wide size range (10^{-2} – $10^2 \mu\text{m}$) generally fit a power function model (McCave 1983; Wells and Goldberg 1994). However, the formation of a sharp peak (size range of 0.6–0.7 μm) that we found for SMP in productive waters cannot be explained by the power function

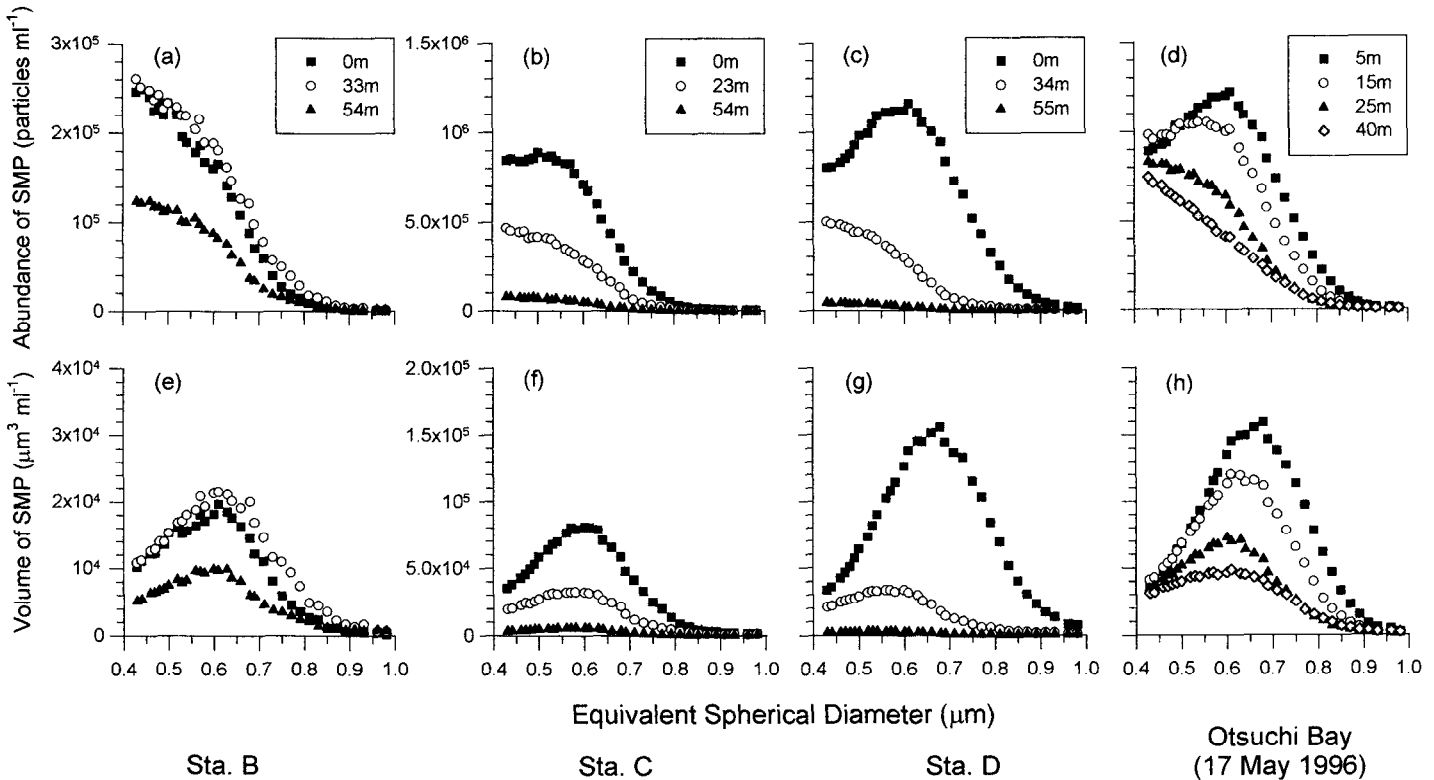


Fig. 3. Size distributions of SMP in waters at Sta. B, C, and D and at the station in Otsuchi Bay. Distributions are plotted in terms of abundance (a–d) and volume (e–h). Data from selected depths are presented to emphasize changes in distribution pattern over the depth. The size distribution at Sta. A is not shown, but it was similar to that at Sta. B. Note that the scales of y-axes for the abundance and volume of SMP at Sta. B (a, e) are different from those for other stations (Sta. C, D, and Otsuchi Bay).

model alone because this model predicts that the number of particles simply increase with decrease of particle size. Alternatively, we hypothesize that the 0.6–0.7- μm particles are directly produced by microorganisms. Previous studies have demonstrated that SMP can be produced along with egestion

by flagellates grazing on bacteria (Koike et al. 1990; Nagata 1997) and by viral infection of bacteria (Shibata et al. 1997). Some of the SMP may be bacterial “ghosts” (Zweifel and Hagstrom 1995), but recent evidence indicates that they could be simply dormant cells (Choi et al. 1996; Karner and Fuhrman 1997). Release of exopolymers, autolysis of microbial cells, and disintegration of large aggregates may also contribute to the formation of SMP in seawater (reviewed by Nagata and Kirchman 1997). A recent study in our laboratory revealed that the size distribution of SMP produced by flagellates grazing on bacteria was remarkably similar to that we observed in productive surface waters (H. Fukuda unpubl.), suggesting that egestion by flagellates could be an important mechanism by which the 0.6–0.7- μm particles are produced.

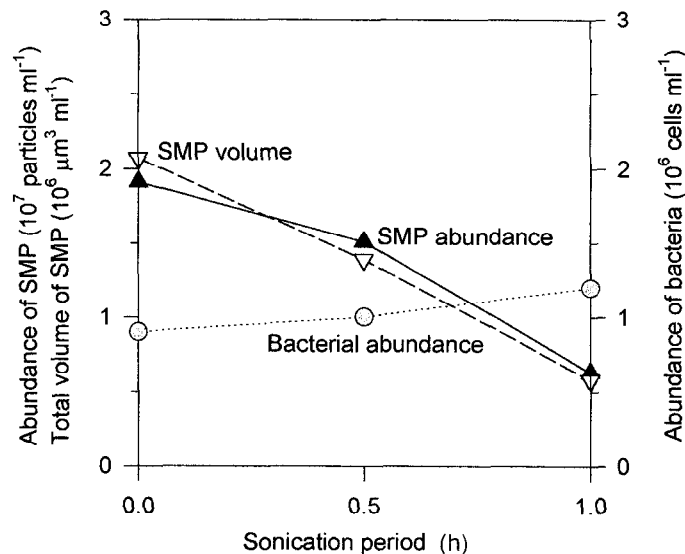


Fig. 4. Effects of sonication on SMP (abundance and volume) and bacteria (abundance).

Recent studies have found various detrital particles with different size and properties in marine environments. These particles include transparent exopolymers stained by Alcian Blue (Alldredge et al. 1993), proteinoous particles stained by Coomassie Brilliant Blue (Long and Azam 1996), DAPI-positive yellow particles (Mostajir et al. 1995), and dimly fluorescent particles stained by Acridine Orange (Sieracki and Viles 1992). Nanometer-size (5–200 nm) detrital colloids have been also counted by electron microscopy (Wells and Goldberg 1991, 1994). Comparison of abundance and size of those detrital particles and SMP (Fig. 6) emphasizes the significance of SMP. We suggest that SMP play a critical

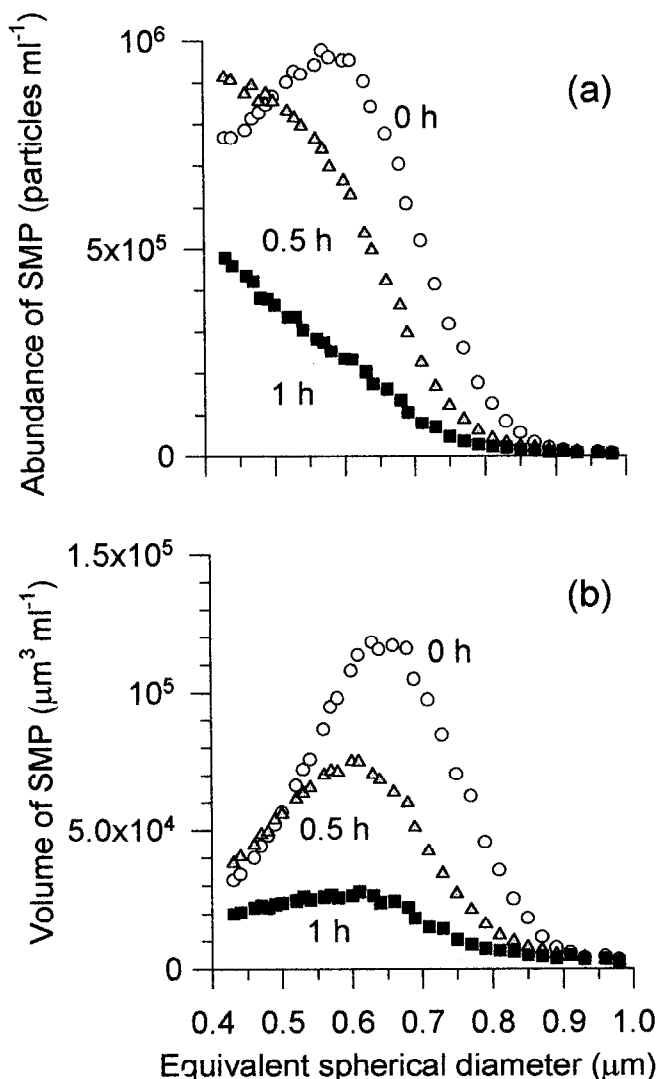


Fig. 5. Effects of sonication on the size distribution of SMP in terms of abundance (a) and volume (b).

role in marine detrital dynamics. Although the size range of SMP examined in this study is quite limited (0.4–1 μm), the total volumes of SMP that we determined in the upper layer ($0.5\text{--}3.2 \times 10^6 \mu\text{m}^3 \text{ml}^{-1}$) are equivalent to or even exceed those of total large particles within a much broader size range (1–100 μm, $0.1\text{--}1.0 \times 10^6 \mu\text{m}^3 \text{ml}^{-1}$; Sheldon et al. 1972) and those of smaller colloids (5–200 nm, $0.02\text{--}0.7 \times 10^6 \mu\text{m}^3 \text{ml}^{-1}$; Wells and Goldberg 1994) in other coastal environments.

Our data suggest that production of SMP is coupled with biological processes. Data in support of this hypothesis include a strong positive correlation between SMP abundance and Chl *a* (Fig. 2a) and sharply peaked size distributions of SMP, particularly in productive waters (Fig. 3c,d). These results are contrasted with previous data on smaller colloidal particles. Wells and Goldberg (1994) found that nanometer-size colloids (5–200 nm) are only marginally correlated with Chl *a* concentrations, and that their size distribution tends to be consistent with the power-law distribution even in pro-

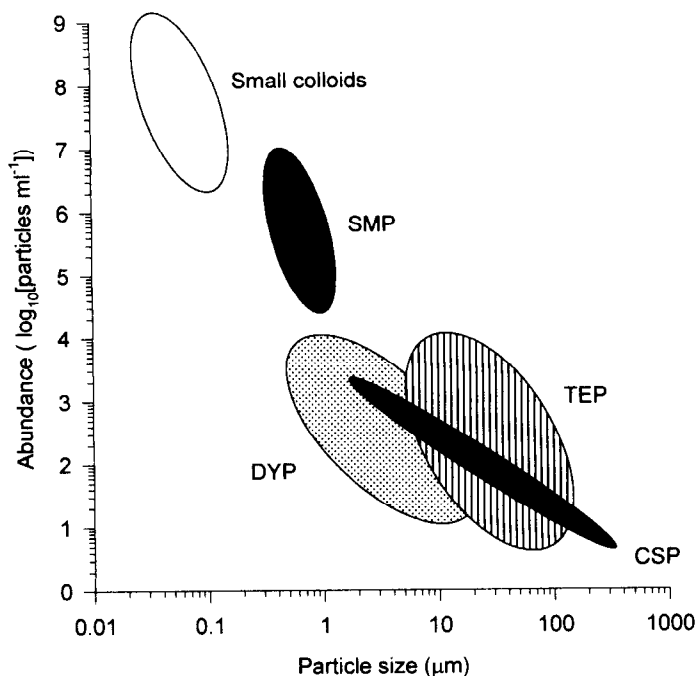


Fig. 6. Approximate ranges of the abundance and size of various marine detrital particles including small colloidal particles examined by electron microscopy (Wells and Goldberg 1991), SMP (this study), DAPI-positive yellow particles (DYP, Mostajir et al. 1995), transparent exopolymers stained by Alcian Blue (TEP, Allredge et al. 1993), and proteineous particles stained by Coomassie Brilliant Blue (CSP, Long and Azam 1996).

ductive upper waters, suggesting that the dynamics of smaller colloids are predominantly controlled by physical aggregation processes. Based on the above comparisons, we hypothesize that SMP in coastal water represent a freshly produced and reactive component among colloidal detrital particles. To test this hypothesis, future studies should clarify turnover rates of SMP and biological mechanisms by which SMP dynamics are regulated.

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