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CONTRIBUTION OF BACTERIAL PRODUCTION TO SINKING CARBON FLUX IN A JAPANESE COASTAL AREA: A MARINE MESOCOSM STUDY

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Abstract

To understand carbon dynamics within coastal and estuarine waters, we examined the activity of sinking particulate organic carbon (POC). Dissolved inorganic ^{13}C ($NaH^{13}CO_3$) was enriched at the surface layer of the mesocosm (marine enclosure; 5 m in diameter and 18 m in depth), and their vertical profiles of POC and $PO^{13}C$ flux below 5 m were measured. Sinking $PO^{13}C$ flux decreased with increasing depth suggesting that POC degrades while sinking. On the other hand, sinking POC flux increased with increasing depth suggesting that POC is produced while sinking. Photosynthetic production below 5 m within mesocosm was negligible, indicating a significant contribution of bacterial production to the total sinking flux. In reality, phytoplankton carbon that was estimated according to the Chlorophyll a concentration within the sinking particles could explain only about 30 % of the sinking POC. The total bacterial production within the water column, calculated assuming a rate of 3.0-5.0 μg C l^{-1} h^{-1} (Koshikawa *et al.* 1999), was of the same order of the sinking POC. Our results suggest that high rates of decomposition and production, resulted in the high turnover rate of sinking particles.

KEYWORDS: *sinking particles, marine mesocosm, bacterial production, microbial loop, ^{13}C tracer*

1. Introduction

Characterization of sinking particle flux in the ocean is essential to understand global carbon dynamics. The activity of sinking particle flux in coastal and estuarine waters should be investigated, in particular, because production is higher in these areas than in open ocean.

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In coastal and estuarine waters, a high proportion (40-70%) of particulate organic carbon (POC) sinks during phytoplankton blooms (e.g. Peinert *et al.* 1982; Laws *et al.* 1988; Lignell *et al.* 1993; Ziemann *et al.* 1993). However, these are net proportions, indicating the end results of physical and biochemical transformation during sinking. The actual quantity of sinking particles both decreases (through decomposition, grazing, etc.) and increases (through photosynthetic and bacterial pathways). These phenomena are not always clear and what amount of sinking particles produced through bacterial pathways (microbial loop; Azam *et al.* 1983) remains unknown.

We examined this issue using a large-scale *in situ* mesocosm (marine enclosure). The mesocosm was about 18 m deep, and stratification was created below the surface mixing layer (0-5 m) by a circulation system (Watanabe *et al.* 1995). Thus, the mixing regime in the water column was similar to that of coastal waters (Watanabe *et al.* 1995) and therefore as a suitable model with which to examine sinking particles.

Watanabe *et al.* (1995) explained the strength of the mesocosm in phytoplankton dynamics and examined mechanism of *Chattonella antiqua* red tide outbreak. We used the same system in present study to examine carbon cycling within the mesocosm.

In an experiment in 1992, we enriched ^{13}C-dissolved inorganic carbon (DIC) at the surface of the mesocosm and monitored the activity of sinking particulate organic carbon (POC) and the $PO^{13}C$ flux. Together with supplemental data obtained in 1994, we discuss here, the increase of sinking particles produced through a bacterial pathway.

2. Materials and Methods

2.1 The N.I.E.S. Mesocosm and Experiment Design

The mesocosm was placed in the Seto Inland Sea, Japan, in summer 1992. The mesocosm consists of a cylindrical water column (Figure 1; 5 m in diameter and 18 m deep) separated from the surrounding water by ethylenevinilacetate reinforced with polyester grids. It is extremely strong, flexible and translucent with no elution from the surface. The novelty of this mesocosm is the vertical circulation system within the surface layer that creates stable stratification around 5 m, which is similar to the surrounding water during summer and suspends non-motile phytoplankton such as diatoms. Details of the structure of the mesocosm have been reported by Watanabe *et al.* (1995).

Immediately after enclosing the water column on 30 July (day 0), the vertical circulation system was installed within the surface layer. Nutrients (1500 g $NaNO_3$, 180 g $NaH_2PO_4 \cdot 2H_2O$, 450g $Na_2SiO_3 \cdot 9H_20$) were enriched from 0 to 10 m in the mesocosm to promote clear species succession (Watanabe *et al.* 1995). On the next day (day 1), 115 g $NaH^{13}CO_3$ was added to the upper mixing layer (0 to 5 m).

2.2 Measurements

(1) Physical Environment

Vertical profiles (0, 1, 2.5, 5, 7.5, 10, 12.5 and 15 m) of seawater temperature, salinity, dissolved

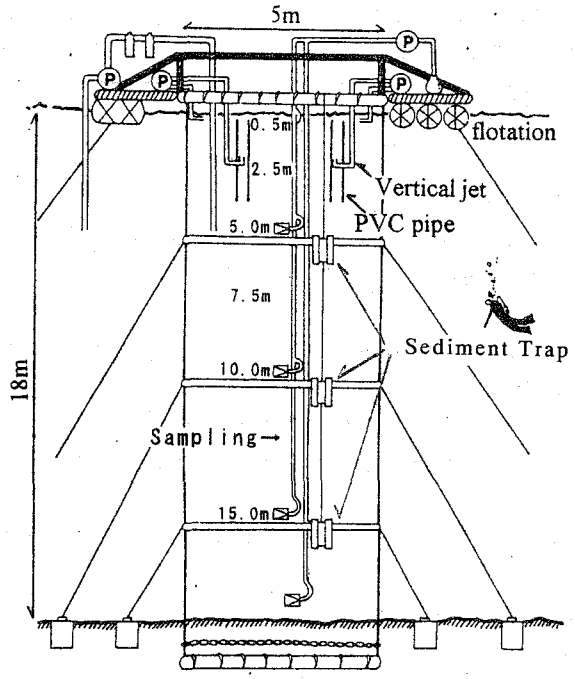

Figure 1. Schematic view of mesocosm. P indicates pumps.(after Watanabe *et al.* 1995)

oxygen (DO) and pH were measured daily at 09:00 with a Surveyor II (Hydrolab Co.). Secchi depth was also measured at 09:00 every day.

(2) Chemical and Biological Environment

Seawater samples were collected at fixed depths (0, 2.5, 5, 7.5, 10 and 15 m) at 09:00. After passing through a precombusted Whatman GF/F filter, NO_2^-+NO_3^-, NH_4^+, PO_4^{3-} and $Si(OH)_4$ were determined in the filtrate (Technicon Autoanalyzer).

Phytoplankton species composition, number and cell volume were determined following the method used in Watanabe *et al.* (1995). Seawater (5 liters) was fixed with 6-10 % Formalin after collection and left for 3 d in a still condition. The original 5 liter sample was concentrated to 1 liter after sedimentation, fixed with glutaraldehyde, and stored in a dark cool place. Live samples were also investigated by light microscopy at a field laboratory to observe nonthecate flagellates. Phytoplankton carbon was calculated using the equation of Strathman (1967) as shown below.

$$\log C = -0.422 + 0.758 \log V \text{ (for diatoms)} \quad \text{eq. (1)}$$

$$\log C = -0.460 + 0.866 \log V \text{ (for other phytoplankton)} \quad \text{eq. (2)}$$

where C is carbon content (pg cell $^{-1}$) and V (μm^3) is volume of phytoplankton species.

The composition of >100-μm zooplankton species was determined in the 0 and 10 m seawater samples.

(3) Sinking Flux and Isotopic Analyses

From 31 July (day 1), two sediment traps (diameter, 10 cm length, 50 cm. Since lateral currents

were insignificant within the mesocosm, we used an open-top collector; Watanabe *et al.* 1995) were placed at 5, 10 and 15 m. The traps were removed and emptied at 08:30 every day from day 2 before water sampling, so the sediment collection period was 1 d. On days 5, 9 and 10, typhoons passed close to the study site and we could not remove and reset the traps. Hence, no data were obtained for days 5, 6, 9, 10 and 11.

Samples were passed in duplicate through precombusted (450 ºC for 4 h) Whatman GF/F filters (thus, the sinking particles were larger than the mean pore size of GF/F filters (0.7 μm)). Chlorophyll a was determined in one filter (Kohata *et al.* 1991). ^{13}C and POC were measured in the other. The filters were stored at -20 ºC until analysis.

Based on the sealed glass-tube combustion and cryogenic purification described by Minagawa *et al.* (1984), all carbon in each sample was converted into CO_2. The percentage of ^{13}C atoms (atom %) in the CO_2 was determined by isotope-ratio mass spectrometry (Finnigan Mat 252/B). The volume of the CO_2 was determined by a vacuum gauge (Edwards capacitance manometer), then the POC in each sample was determined.

Excess $PO^{13}C$ ($PO^{13}C_{ex}$) was calculated as follows (Koshikawa *et al.* 1996):

$$PO^{13}C_{ex} (\mu g\ ^{13}C\ l^{-1}) = (a_s - a_n) \times POC \qquad \text{eq. (3)}$$

where a_s and a_n are the ^{13}C atom % in labeled and natural samples, respectively. $PO^{13}C_{ex}$ and POC were converted into flux form to obtain sinking POC (g C m^{-2} d^{-1}) and sinking $PO^{13}C_{ex}$ flux (mg ^{13}C m^{-2} d^{-1}).

2.3 Supplemental Data Obtained in 1994

(1) Vertical Profile of Photosynthetic Production Ability

Seawater was collected from depths of 0, 2.5, 5 and 10 m inside the mesocosm and transferred into 500-ml light and dark polycarbonate bottles. About 5.0 mg $NaH^{13}CO_3$ (ISOTEC, Inc., USA) was added to the bottles which were then sealed. Each bottle was suspended for 2 h (11:00 to 13:00) at the depth from which bottled sample had been collected. The samples were then filtered through precombusted Whatman GF/F filters. The POC and ^{13}C abundance were determined using a system comprised of an elemental analyzer (Fisons EA1108) and an isotope-ratio mass spectrometer (Finnigan Mat 252/B). Photosynthetic production rates were calculated based on Hama *et al.* (1983). Experiments were repeated three times (Run 1 to 3).

(2) Size of Produced POC

Seawater was collected from 10 m and transferred into 4.5-l transparent polycarbonate bottles. After adding [^{13}C]glucose (ISOTEC, Inc., USA; 22.5 mg per bottle), bottles were suspended for 4 h (09:00 to 13:00) at 10 m. The samples were size-fractionated by sequential filtration using plankton nets (100 and 20 μm), GF/F (mean pore size: 0.7 μm) and Whatman Anopore filters (mean pore size: 0.2 μm) (Koshikawa *et al.,* 1999). Measurements were repeated 6 times.

3. Results

3.1 Environmental Variables

(1) Physical Environment

Stable stratification beneath the surface mixed layer (0 to 5 m), similar to that shown by Watanabe *et al.* (1995), was developed by the circulation system (see also Figure 2). Secchi depth varied from 2.5 to 5.0 m, averaging 3.3 m during the experimental period.

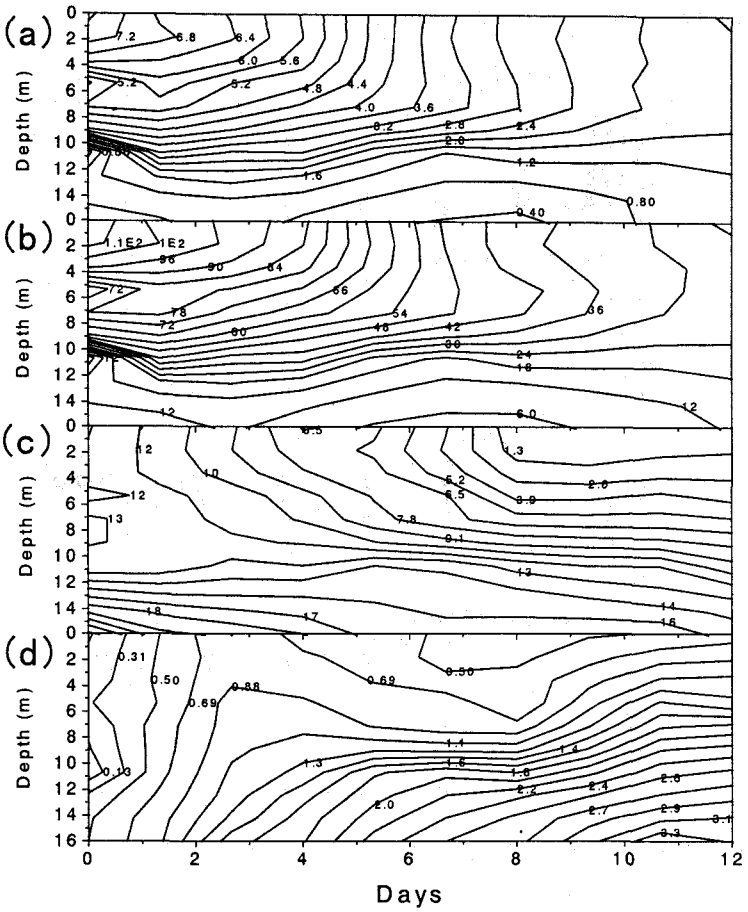

Figure 2. Distribution of nutrients (μM): (a) PO_4^{3-}, (b) NO_2^-+NO_3^-, (c) $Si(OH)_4$, (d) NH_4^+

(2) Chemical Environment

Nutrients decreased quickly throughout the surface area, but apart from silicate, were not depleted (Figure 2). At the end of the experiments, 27.8 μM NO_2^-+NO_3^-, 1.8 μM PO_4^{3-}, and 0.7 μM $Si(OH)_4$ remained in the surface area. In the lower layer of the mesocosm, nutrients remained abundant at the

end of experiment: >20.0 μM NO_2^-+NO_3^-, >1.0 μM PO_4^{3-}, and >10.0 μM $Si(OH)_4$. The distribution of the nutrients suggests that stratification formed below 5 m because of the circulation within the surface layer (Figure 2).

(3) Biological Environment

At the beginning of the experiment, centric diatoms were dominant (Figure 3). As they decreased, levels of dinoflagellates and other flagellates increased. Table I indicates the major species during the experimental period.

Copepods *(Paracalanus parvus, Oithona davisae, O. similis, Corrycaeus spp. and Microsetella norvegica)* dominated the zooplankton community.

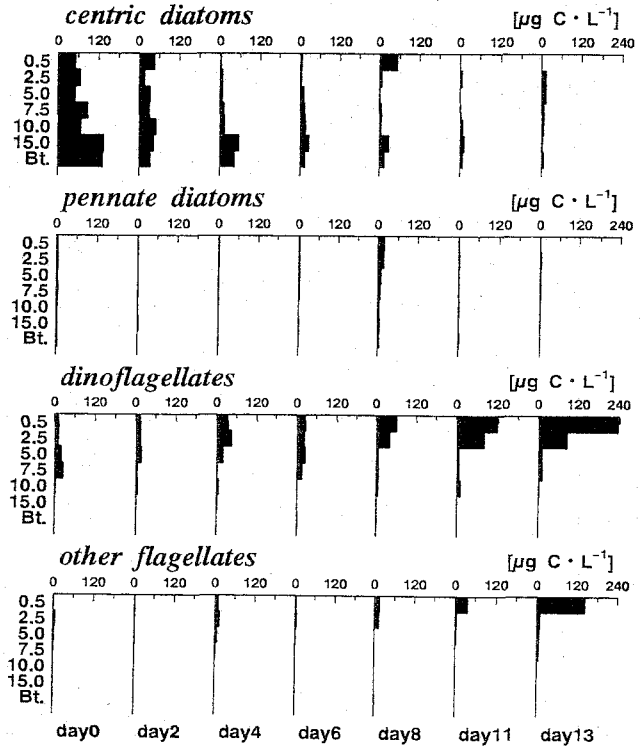

Figure 3. Variation in dominant phytoplankton species in 1992 study.Bt. indicates depth just above bottom sediment

3.2 Sinking POC and $PO^{13}C$ Flux

During most of the experimental period, the sinking $PO^{13}C_{ex}$ flux decreased with depth (Figure 4 a). The cumulative fluxes at 10 and 15 m were 76 and 49%, respectively, of that at 5 m (Figure 4 b), suggesting that 24 and 51% of the sinking POC flux at 5 m were lost by grazing, decomposition, etc. between 5 to 10 m and between 5 to 15 m, respectively. However, while a decrease in the POC flux was suggested by the sinking $PO^{13}C_{ex}$ flux observation, the sinking POC flux did not decrease with depth during most of the experimental period (Figure 5 a). The cumulative flux at 10 and 15 m were

Table Ⅰ. Dominant phytoplankton species during the experiment in 1992 study

	dominant species	volume μm^3	Equivalent spherical diameter μm
centric diatoms	*Alacnoidiscus* sp.	2,123,000	79.7
	Chaetoceros lauderi	6,100	11.3
	Chaetoceros lorenzianus	18,800	16.5
	Coscinodiscus sp.	2,826,000	87.9
	Dactyliosolen mediterraneous	2,400	8.3
	Ditylum brightwellii	45,000	22.1
	Rhizosolenia styliformis	1,213,000	66.2
	Skeletonema costatum	2,300	8.2
	Stephanopyxis palmeriana	7,000	25.6
pennate diatoms	*Bacillaria paradoxa*	1,200	6.6
	Nitzschia closterium (C. clost.)	150	3.3
	Nitzschia delicatissima	110	3.0
	Nitzschia longissima	2,200	17.4
	Nitzschia pungens v. atlantica	850	5.9
dinoflagellates	*Ceratium fusus*	9,500	13.1
	Ceratium kofoidii	4,500	10.2
	Ceratium macroceros	38,900	21.0
	Dinophysis fortii	42,000	21.6
	Dinophysis ovum	29,600	19.2
	Dinophysis rotundata	18,500	16.4
	Gymnodinium sanguineum	9,500	13.1
	Gyrodinium sp.	12,500	14.4
	Prorocentrum micans	6,800	11.8
	Prorocentrum triestinum	580	5.2
	Protoperidinium bipes	390	4.5
	Protoperidinium claudicans	57,800	24.0
	Protoperidinium fatulipes	369,000	44.5
	Protoperidinium granii	38,100	45.0
	Scrippsiella trochoidea	11,500	14.0
other flagellates	*Chattonella* sp.	35,300	20.3
	Eutreptiella sp.	5,100	10.7

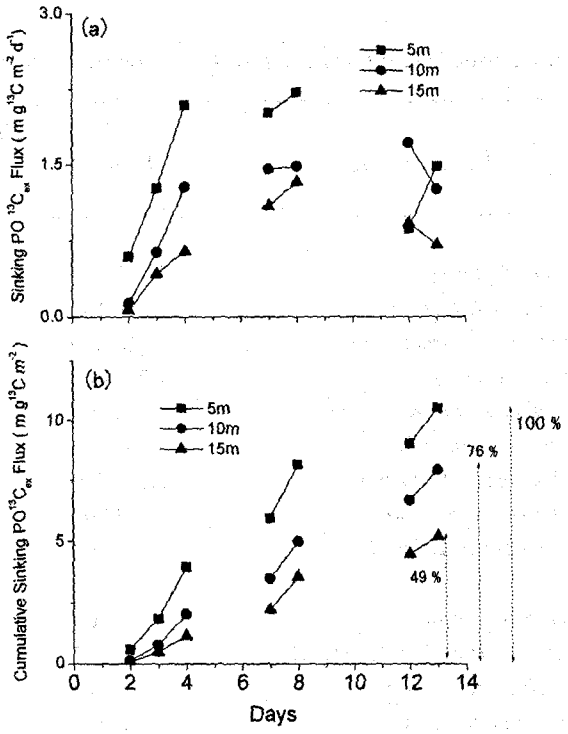

Figure 4. (a) Daily sinking $PO^{13}C_{ex}$ flux and (b) cumulative sinking $PO^{13}C_{ex}$ flux at 5, 10 and 15 m

94 and 112%, respectively, of that at 5 m (Figure 5 b). These findings suggest that POC equivalent to 18% (24 - 6) and 63% (51 + 12) of the sinking POC flux at 5 m was produced while sinking between 5 to 10 m and between 5 to 15 m, respectively, on average during the experimental period. The average sinking POC flux at 5 m was 1.0 g C m^{-2} d^{-1} (Figure 5 b). Thus, daily averages of 0.18 and 0.63 grams of POC per square meter were produced between 5 and 10 m, and between 5 and 15 m, respectively.

3.3 Supplemental Data Obtained in 1994

(1) Vertical Profile of Photosynthetic Ability

Photosynthetic ability decreased quickly with depth (Table II) because of light depletion. The amount of photosynthesis at 10 m was about 0.2 % of that at 0m.

(2) Size of Produced $PO^{13}C$

The particles produced through the bacterial pathway were mostly bigger than the mean pore size of GF/F (Fig. 6). This indicates that the ratio of $PO^{13}C_{ex}$ in the fraction of the Whatman Anopore filter (mean pore size: 0.2 μm) - GF/F was always less than 10% (mean: 5.9%).

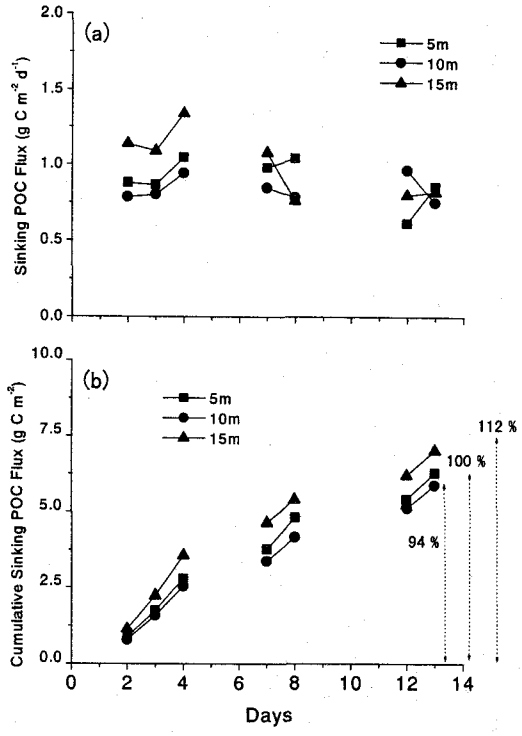

Figure 5. (a) Daily sinking POC flux and (b) cumulative sinking POC flux at 5, 10 and 15 m

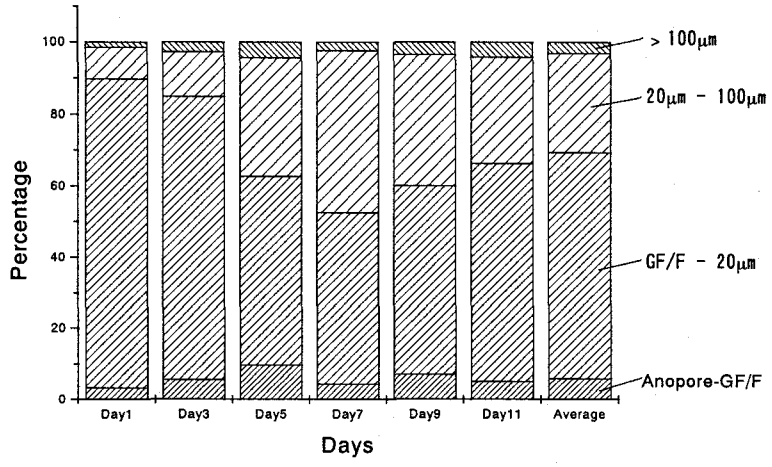

Figure 6. Proportion of $PO^{13}C_{ex}$, in 4 size fractions, that was originally given as [^{13}C] glucose

Table Ⅱ Vertical profile of photosynthetic production ability (μg C l^{-1} h^{-1}).

	Photosynthetic production (μg C l^{-1} h^{-1})			
	Run 1 (Aug.2 1994)	Run 2 (Aug. 6 1994)	Run 3 (Aug 10 1994)	Average
Depth				
0.0 m	24.73	61.79	26.41	37.6
2.5 m	17.06	31.18	16.07	21.4
5.0 m	3.46	6.96	3.15	4.5
10.0 m	0.52	1.03	0.40	0.65

4. Discussion

(1) Negligible Photosynthesis Below 5m: Implication of Significance of Bacterial Production

Photosynthetic and bacterial pathways both produce particles in the aquatic environment. In our mesocosm, photosynthetic production below 5 m was negligible because of light depletion.

Table Ⅱ shows the vertical profiles of primary production within the mesocosm measured on 3 occasions (runs 1 to 3) using ^{13}C technique in 1994. The species emerging in the 3 runs (Table Ⅲ) were similar to those of the same experiment in 1992 (Table Ⅰ). Also, the transparency in runs 1 to 3 (Table Ⅲ) was similar to the average value (3.3 m) in the present study. Moreover, the overall results of the three runs themselves were similar (Table Ⅱ). Thus, the vertical profiles shown in Table Ⅱ were reproducible, including 1992 study. These results show that the bacterial pathway dominates the production of particles at depths below 5 m, especially between 10 and 15 m.

The particles produced through the bacterial pathway should be larger than the mean pore size of Whatman GF/F filters (0.7 μm), which constitutes our operational definition of sinking particles in this study. The results shown in Figure 6 indicates that the ratio of $PO^{13}C_{ex}$ in the fraction of the Whatman Anopore filter (mean pore size: 0.2 μm) - GF/F was always less than 10% (mean: 5.9%). These results indicate that most of the particles produced through the bacterial pathway are larger than the 0.7-μm mean pore size of Whatman GF/F filters.

(2) Quantitative Analyses

Disregarding the photosynthetic pathway, the ratio of particle production through the bacterial pathway was 0.18 and 0.63 g C m^{-2} d^{-1}, as shown above, corresponding to 1.5 and 2.6 μg C l^{-1} h^{-1} between 5 and 10, and between 5 and 15 m, respectively. However these values are minimal estimates. We did not include bacterial production using $DO^{13}C$ below 5 m. Labile $DO^{13}C$ might be found below 5 m may be due to a portion of labile $DO^{13}C$ produced above 5 m diffusing through the stratification and some of the sinking $PO^{13}C$ undergoing decomposition while sinking between 5 and 15 m. In reality, Koshikawa *et al.* (1999) indicated that bacterial production within the surface of mesocosm was about 3 - 5 μg C l^{-1} h^{-1} in 1994 study.

The amount of cumulative sinking POC at 5, 10 and 15 m was 6.3, 5.9 and 7.0 g m^{-2} , whereas cumulative sinking Chlorophyll a flux at 5, 10 and 15 m was 51, 61 and 69 mg C m^{-2} (Fig.7). Assuming a ratio of C to Chlorophyll a equal 30, POC attributed to phytoplankton is 1.5, 1.8 and 2.1 g C m^{-2} at 5, 10 and 15 m , respectively. These values were about 30 % of the sinking POC flux. Thus, phytoplankton carbon would not be a major component of the sinking flux.

On the other hand, using the value for bacterial production defined by Koshikawa *et al.* (1999), 3 - 5 μg C l^{-1} h^{-1} corresponds to 4.3 – 7.2 and 8.6- 14.4 g C m^{-2} between 5 – 10 and between 5 – 15 m, respectively. These values not only represent sinking POC but the sum of sinking and suspended POC. Thus, these values exceed the cumulative sinking POC. However, these results are consistent with the high contribution of the bacterial production to sinking POC.

Table Ⅲ Major phytoplankton species and Secchi depth in each incubation (Runs 1 to 3) in 1994

	Run 1 (Aug.2 1994)	Run 2 (Aug.6 1994)	Run 3 (Aug.10 1994)
centric diatoms	*Coscinodiscus* sp.	*Chaetoceros curvicetus*	*Coscinodiscus* sp.
	Ditylum brightwellii	*Chaetoceros socialis*	*Rhizosolenia fragilissima*
		Rhizosolenia calcar-avis	*Skeletonema costatum*
		Rhizosolenia fragilissima	*Stephanopyxis palmeriana*
		Skeletonema costatum	*Thalassiosira* sp.
		Thalassiosira sp.	
pennate diatoms		*Navicula membranacea*	*Bacillaria paradoxa*
		Nitzschia closterium (C. clost.)	*Entomoneis hyalina*
		Nitzschia pungens v. atlantica	*Nitzschia pungens v. atlantica*
			Thalassiothrix flauenferdii
dinoflagellates	*Ceratium fusus*	*Ceratium kofoidii*	*Ceratium fusus*
	Ceratium kofoidii	*Gonyaulax* sp.	*Ceratium kofoidii*
	Ceratium tripos	*Gymnodinium mikimotoi*	*Gymnodinium sanguineum*
	Gymnodinium mikimotoi	*Gyrodinium* sp.	*Gyrodinium* sp.
	Protoperidinium cerasus	*Prorocentrum triestinum*	*Prorocentrum micans*
	Protoperidinium claudicans	*Protoperidinium cerasus*	*Scrippsiella trochoidea*
	Scrippsiella trochoidea	*Scrippsiella trochoidea*	
other flagellates		*Chattonella* sp.	*Chattonella* sp.
Secchi depth (m)	3.8	3.0	3.5

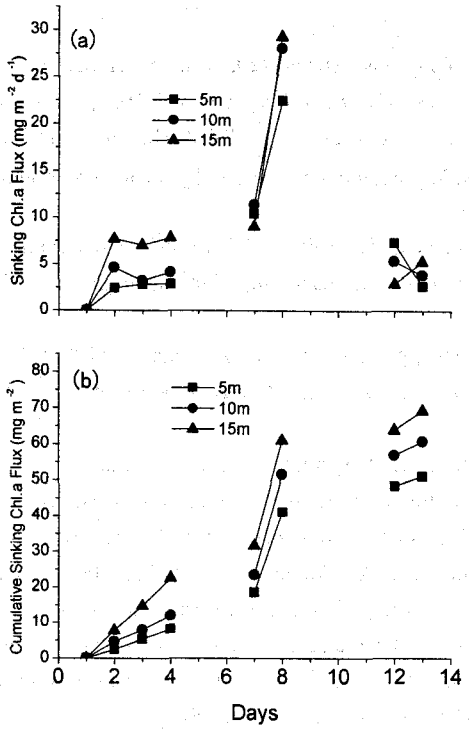

Figure 7. (a) Daily sinking Chlorophyll a flux and (b) cumulative sinking Chlorophyll a flux at 5, 10 and 15m

(3) Another Possibility

Resuspension of bottom sediment could increase the amount of POC with increasing depth. However the water column within the mesocosm was highly stratified below 5m (Figure 3), therefore the effects of this were excluded.

5. Conclusions

The results of the present study indicate that the turnover rate of sinking particles is high in that they are quickly lost and produced, and that production through the bacterial pathway significantly contributes to the numbers of particles sinking below the euphotic zone.

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