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Significance of Estuarine Mixing in Distribution of Bacterial Abundance and Production in the Estuarine System of the Mankyung River and Dongjin River, Korea

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만경강 및 동진강 하구의 박테리아 개체수와 생산량 분포에 있어서의 하구 혼합(estuarine mixing)의 중요성

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Bacterial abundance, production, and environmental parameters were investigated three times to study distribution of bacterial variables and to examine how estuarine mixing would influence the distribution of bacterial variables in the euphotic zone of the estuarine system of the Mankyung river and Dongjin river during a period of October, 1990-August, 1991. Although a limited number of investigations were made, bacterial abundance and production showed large variations from 0.4 to 5.8×10^9 l^{-1} and from 0.1 to 22.2 μg C l^{-1} d^{-1}, respectively. The wide ranges of bacterial variables indicated very dynamic changes in conditions of bacterial growth in the estuary. Interestingly, bacterial abundance substantially increased with depth in most stations of shallow depth, but bacterial production remarkably decreased with depth. We propose that the observed distribution of bacterial abundance and production would be explained by estuarine mixing in the estuary. Analyses of mixing diagrams showed that estuarine mixing would mix conservatively bacteria and bacterial production. Further, estuarine mixing often seemed to cause an increase in bacterial abundance and reduction of bacterial production presumably due to resuspension of sediment. This suggests that roles of estuarine mixing would be significant in the distribution of bacterial abundance and production, and thus in biogeochemical cycles in the estuary.

박테리아의 개체수와 생산량의 분포를, 그리고 하구 혼합(estuarine mixing)이 이들 분포에 미치는 영향을 조사하기 위해, 1990년 10월부터 1991년 8월까지 만경강 및 동진강 하구의 유광대에서 박테리아의 개체수, 생산량, 수온 및 염분도를 3번 조사하였다. 제한된 조사에도 불구하고, 조사기간에 나타난 박테리아의 개체수와 생산량은 넓은 범위의 값을 보였다; 각각 0.4-5.8×10^9 l^{-1}와 0.1-22.2 μg C l^{-1} d^{-1}이었다. 이는 박테리아의 성장이 하구에서 매우 유동적임을 나타냈다. 흥미롭게도, 박테리아의 개체수는 대부분의 수심이 얕은 정점의 표면 아래에서 증가하였으나, 박테리아의 생산량은 뚜렷이 감소하였다. 이러한 현상은 하구 혼합에 의해서 설명될 수 있을 것으로 판단되며, mixing diagrams의 분석은 하구 혼합이 박테리아 개체수와 생산량을 conservative하게 혼합하기도 하나, 박테리아 개체수를 증가시키며, 생산량을 감소시키는 작용을 하였음을 보여 주었다. 이것은 하구 혼합의 역활이 박테리아의 개체수와 생산량의 분포에, 따라서 하구에서의 생지화학적 순환에 있어서 중요함을 제시하였다.

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INTRODUCTION

Estuary is a unique environment and different in many aspects from the coastal and open oceanic environments (Day et al., 1989). Typically, estuary is physically subject to riverine inputs and periodic tidal cycles. Further, in a shallow, well mixed estuary, a turbidity maximum zone is formed (Lee and Kim, 1987; Painchaud and Therriault, 1989). Thus, various microbial activities and distribution of microorganisms along gradients of salinity and turbidity have been reported (Kirchman et al., 1989; Griffith et al., 1990; Painchaud and Therriault, 1989). Bacterial abundance (Kirchman et al., 1989; Painchaud and Therriault, 1989) and production (Griffth et al., 1990), linked to salinity, decreased in a seaward direction in estuaries. Also, effects of periodic tidal cycles in the estuary on bacterial activities have been reported: In the estuary of Naktong river, the main environmental factor affecting uptake of the ^{14}C-glucose was salinity (Ahn et al., 1991). Especially, the effect of salinity on the heterotrophic activity was more pronounced in the upper region of the estuary than at the mouth. Thus, physical forces in the estuary seem to predominantly determine the distribution of bacterial abundance, and activity and production. However, bacteria are reported to respond to destratification due to high spring tides in the York river estuary, Virginia, (Ducklow, 1982) with steady increases in bacterial abundance but delayed increases in bacterial production. In spite of the ability shown in the long-term responses in bacteria to destratification, tidal mixing which seems to cause continually changing microenvironments to bacteria makes bacteria often fail to adapt to the changing environments (Chin-Leo and Kirchman 1990). In the Rhône river plume, microbial loop relationships have been reported to be disrupted because of input of allochtonous carbon and because of rapid changes in growth conditions caused by mixing (Kirchman et al., 1989).

Although distribution of bacterial abundance, activity and production has been related to salinity or turbidity in estuaries, effects of physical factors

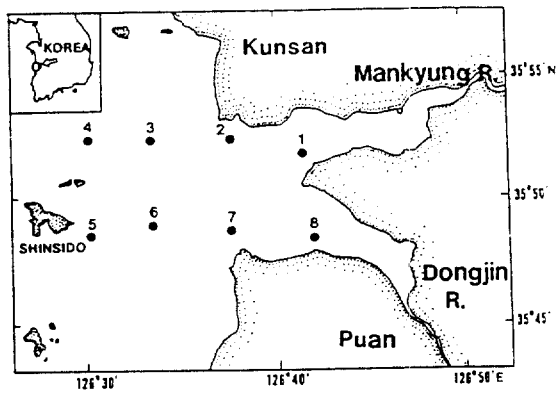

Fig. 1. A map showing sampling stations during a study period of October, 1990 - August, 1991.

(i.e. estuarine or tidal mixing) on distribution of bacterial abundance and production in the estuary have not been systematically assessed. We have studied (1) how bacterial abundance and production varied spatially and temporally, and distributed along a gradient of salinity, and (2) whether or not the distribution of bacterial abundance and production in the estuary could be explained by the mixing in the estuary. We intensively collected seawater samples in the euphotic zone for measurements of bacterial abundance and production, along with measurements of salinity and seawater temperature. Here, we report that estuarine mixing in the shallow estuary would conservatively mix bacteria and bacterial production. Furthermore, estuarine mixing might often increase bacterial abundance and cause reduction of bacterial production, presumably due to releases of sediment-associated bacteria and inhibitory substances on bacterial growth upon resuspension of sediments by the mixing, respectively.

MATERIALS AND METHODS

Sampling Area and Sample Collection

The estuarine system of the Mankyung river and Donjin river is located at the midwest coast of Korea (Fig. 1). The study area is a well-mixed, shallow (usu. <10 m depth) estuary (Shim et al., 1991) subject to a semidiurnal tidal cycle. Stations

1 (1 m depth) and 8 (ca 5 m depth) are located close to the mouth of the Mankyung river and Dongjin river, respectively. We started sampling always in the morning around 9-10 o'clock at station (Stn) 1 or Stn 8, steamed to seaward Stn 4 (ca 5 m depth) and Stn 5 (ca 20 m depth), then returned to the estuary in the afternoon around 2-3 o'clock local time. Whenever we visited the study area, it was 1-3 days before or after the neap tide. Sampling was done in October, 1990, and in April and August, 1991. Seawater was collected by a Van-Dorn sampler (5 *l*) in the euphotic zone.

Measurements of Bacterial Variables and Environmental Parameters

Bacterial abundance was measured by epifluorescence microscopy technique after staining cells with acridine orange (Hobbie et al., 1977). Samples for bacterial abundance were fixed with 3% of 0.22 μm filtered, buffered formalin. Bacterial production was measured basically by the method of Fuhrman and Azam (1982). Briefly, [^{3}H]thymidine (Amersham, sp. act. =25 Ci mmol^{-1}) was added to 5 m*l* seawater to give a final concentration of 10 nM. The treated samples were incubated in the dark at *in situ* temperature for about 1 hour. After incubation, the samples were extracted for the radiolabeled DNA using the method of Robarts et al. (1986). Briefly, thymidine incorporation was stopped by adding NaOH and putting samples in ice. Within 10 hours, trichloroacetic acid (TCA) was added to the samples, then filtered through 0.22 μm Millipore filters. The filters were then washed with phenol:chloroform (50% wt/vol) solution and 80% ethanol (vol/vol). This procedure was more convenient for use in field than extraction with hot-TCA. The incorporated radioactivity was converted to cells produced by using a conversion factor of 1.1×10^{18} cells per mole of thymidine incorporated into DNA (Riemann et al., 1987). To estimate bacterial biomass and production, 20 fg C cell^{-1} (Lee and Fuhrman, 1987) was used. Salinity and water temperature were measured with a T-S Bridge (Hydrobios type MC5).

RESULTS

Hydrography

Most of vertical profiles of salinity in the estuary showed vertically homogeneous distributions (top-to-bottom salinity difference <1‰, Ducklow, 1983) of salinity in the water columns, indicating that water columns were homogeneously mixed at both seaward and estuarine stations (Fig. 2). Only Stn 2 in October, Stns 2 and 8 in April and Stn 8 in August showed increase in salinity with depth (>1‰ difference) in the water columns. Salinity in the estuary ranged from 23.5 to 30.5‰ (October, 1990), 16.6-31.1‰ (April, 1991), and 26.0-30.6‰ (August, 1991). Salinity was always the highest at the seaward Stn 5 and the lowest at the estuarine Stn 1 or 8; at the seaward Stn 5, salinity was ca 30‰ with the smallest variation (29.9-31.1‰), but at the estuarine stations it varied from 16.6 to 29.8‰. Stations located between the estuarine and the seaward stations showed intermediate values of salinity (22.4-30.5‰). Apparently, top-to-bottom difference of salinity was greater at estuarine stations than seaward stations (Fig. 2) during the investigation period.

Vertical profiles of seawater temperature as well as those of salinity showed almost homogeneous distribution in the estuary (Fig. 2), indicating that mixing in the estuary would make distribution of seawater temperature and salinity homogeneous in the water columns. At seaward stations (Stns 4 and 5 in April and August) and at estuarine stations (Stns 7 and 8 in August, Fig. 2) weak thermal stratification was found. The differences of seawater temperature among stations during the study period were small (<6℃). In October, seawater temperature among stations ranged from 16.5 to 17.6 ℃, in April, 6.5 to 12.2℃, and in August, 25.5 to 28.4℃. Thus, temporal variation of seawater temperature was demonstrated in the estuary. In contrast to salinity, top-to-bottom difference of seawater temperature in the euphotic zone was much greater at seaward stations than at estuarine stations (Fig. 2) during the investigation period.

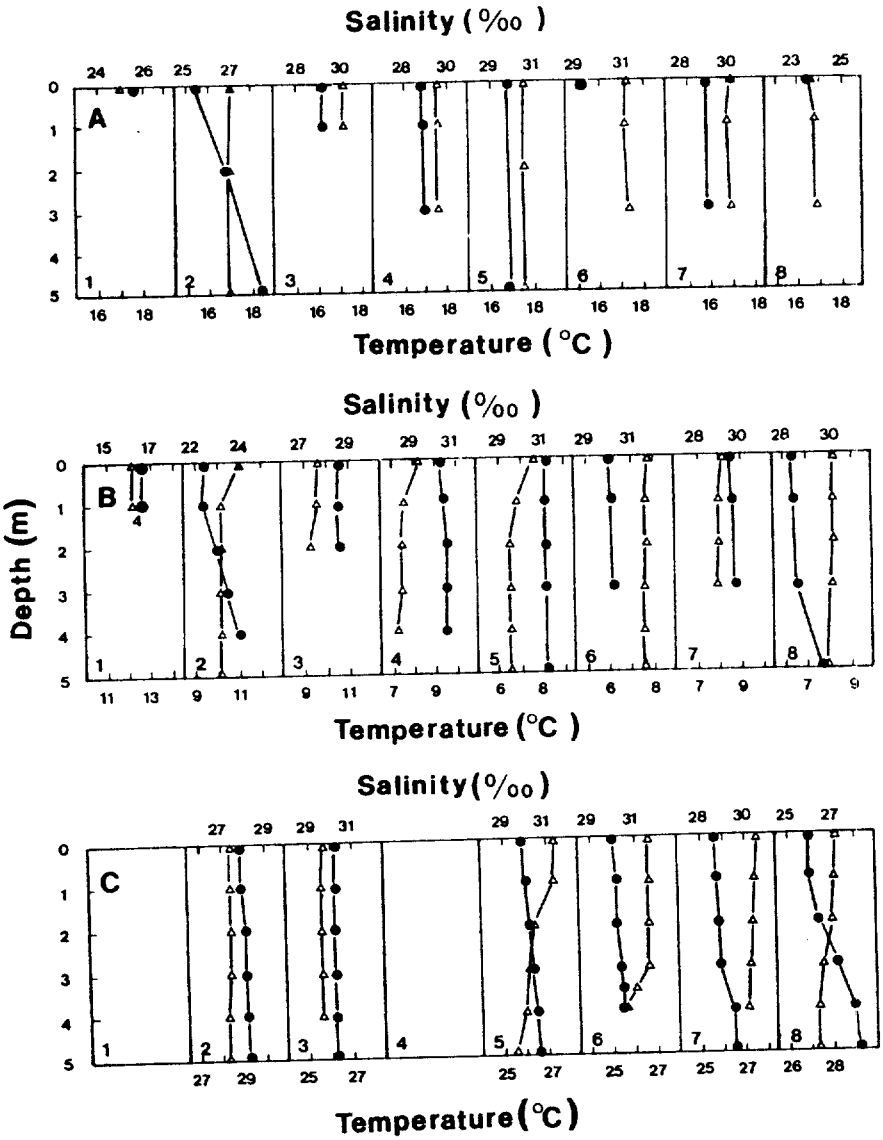

Fig. 2. Vertical profiles of salinity and seawater temperature during October, 1990 (A), April, 1991 (B), and August, 1991 (C). Closed circles represent salinity and open triangles seawater temperature. During August, 1991, stations 1 and 4 were not visited. Numbers in the panels represent station numbers.

Distribution of Bacterial Abundance and Production

Distribution of bacterial abundance and production did not show any distinct trends along a gradient of salinity in the estuary (Fig. 3). Bacterial abundance showed a large variation (14.5 fold) in the euphotic zone during the study period: The highest bacterial abundance (5.8×10^9 l^{-1}) during the study was found at the subsurface at Stn 6 in April, 1991 and the lowest abundance (0.4×10^9 l^{-1}) at the subsurface at the seaward Stn 5 in October, 1990 (Fig. 4). At the seaward Stns 4 and 5, bacterial abundance was the lowest in October ($0.4\text{-}0.9\times10^9$ l^{-1}), but increased during April ($1.1\text{-}2.0\times10^9$ l^{-1}) and August (ca 2×10^9 l^{-1}). At the estuarine Stns 1 and 8, bacterial abundance was also low in October ($0.7\text{-}1.6\times10^9$ l^{-1}), but increased during April ($1.6\text{-}2.8\times10^9$ l^{-1}) and August (1.4-2.3

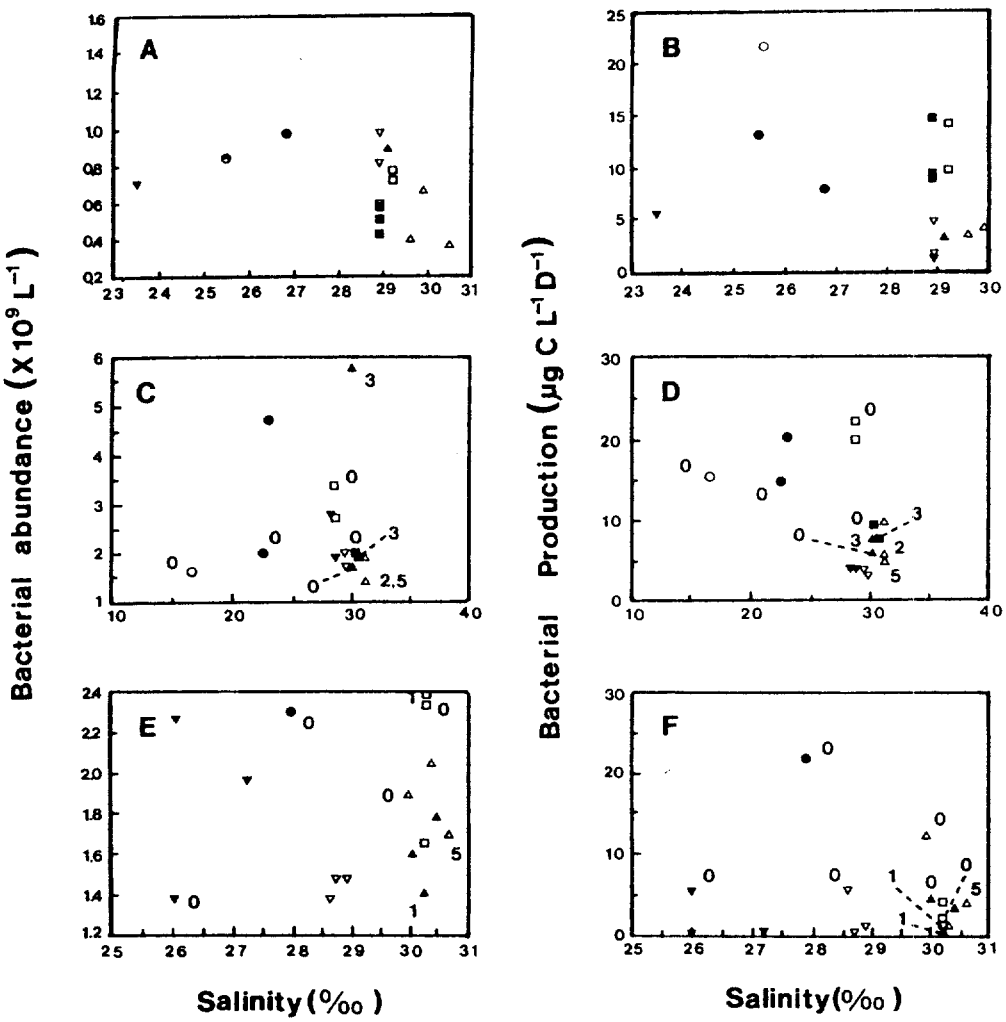

Fig. 3. Diagrams showing bacterial abundance vs salinity and bacterial production vs salinity in the estuary during October, 1990 (A & B), April, 1991 (C & D), and August, 1991 (E & F). Open circles represent station (Stn) 1, closed circles Stn 2, open squares Stn 3, closed squares Stn 4, open triangles Stn 5, closed triangles Stn 6, open inverted triangles Stn 7, and closed inverted triangles Stn 8. Numbers marked beside symbols represent sampling depth for the stations in which analyses of mixing were made in Table 1.

$\times 10^9$ l^{-1}). Bacterial abundance in the middle region of the estuary was low in October (0.4-1.0$\times$ 10^9 l^{-1}), but increased in April (1.1-5.8$\times 10^9$ l^{-1}) and August (1.4-3.5$\times 10^9$ l^{-1}). Depth-variation of bacterial abundance within the water columns in the whole estuary was less than 2 fold. However, interestingly, in most stations of shallow depth (5 m depth), bacterial abundance tended to substantially increase with depth; Stns 2 and 8 always showed such trends during the investigations. Further, Stn 7 in October, 1990 and Stn 6 in April and August, 1991 showed such trends (Fig. 4).

Bacterial production ranged from 0.1 to 22.2 μg C l^{-1} d^{-1}, showing also a large variation (up to 222 fold) in the euphotic zone of the estuary. The highest bacterial production (22.2 μg C l^{-1} d^{-1}) was found at the surface at Stn 3 in April and August, 1991 and the lowest production (0.1 μg C l^{-1} d^{-1}) was found at the subsurface (1 or 2 m) at the estuarine Stns 7 and 8 in August, 1991.

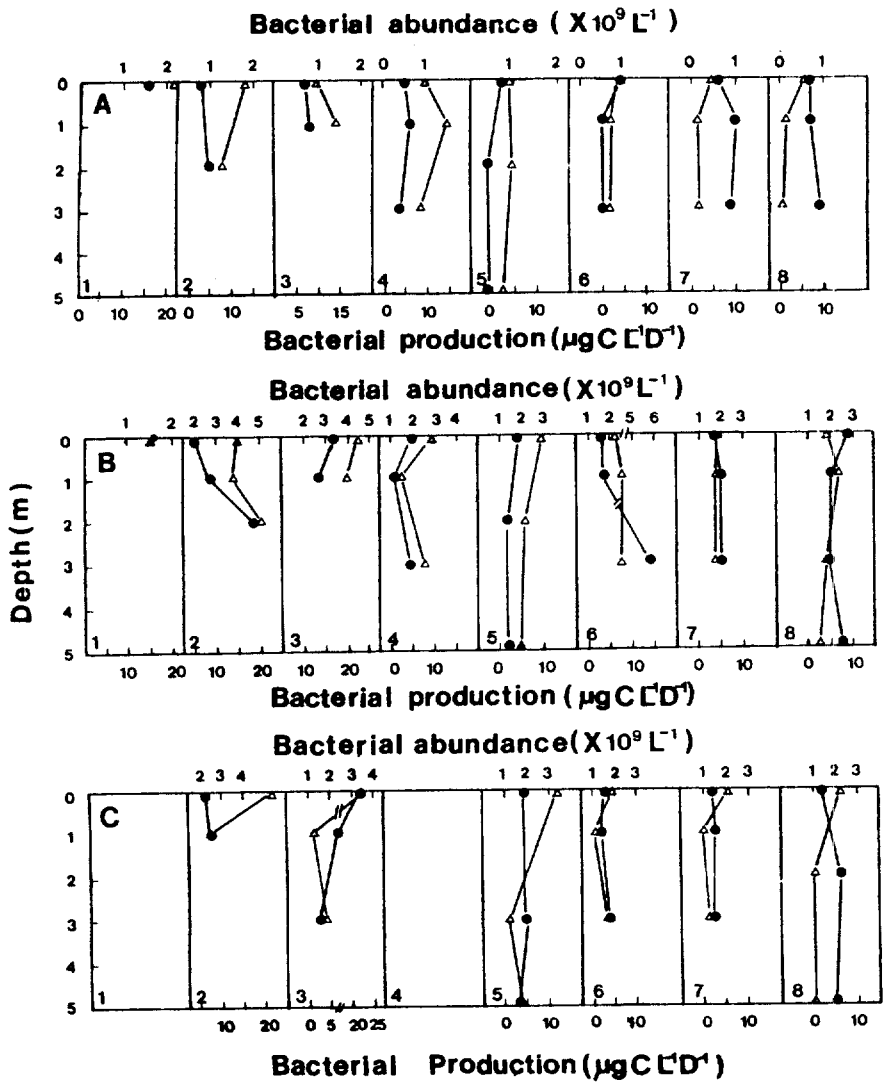

Fig. 4. Vertical profiles of bacterial abundance and production in the euphotic zone during October, 1990 (A), April, 1991 (B), and August, 1991 (C). Closed circles represent bacterial abundance and open triangles bacterial production. During August 1991, stations 1 and 4 were not visited. Numbers in the panels represent station numbers.

At the seaward Stns 4 and 5, bacterial production did not show distinctive temporal variation; it ranged from 2.5 to 14.7 μg C l^{-1} d^{-1} in October, 2.6 to 9.8 μg C l^{-1} d^{-1} in April, and 1.2 to 12.0 μg C l^{-1} d^{-1} in August. At the estuarine Stn 8, bacterial production did not show any distinctive temporal variations either. Values of bacterial production were always much higher at Stns 1 and 2 than at Stns 7 and 8, suggesting that more sources of organic matter for bacterial growth were available at Stns 1 and 2 than at Stns 7 and 8. Depth-variation of bacterial production within the euphotic zone showed >2 fold variation in about half of the stations investigated. In most stations of shallow depth (Stns 2, 3, 7, and 8), bacterial production tended to markedly decrease with depth (Fig. 4).

DISCUSSION

The distribution of bacterial abundance and

production over the investigation period showed wide ranges of variation despite of limited investigations: The range of bacterial abundance found during the study (0.4-5.8$\times 10^9$ l^{-1}) was typical for the estuarine, nearshore and coastal environments (Ducklow, 1982; Ducklow and Kirchman, 1983; Kirchman et al., 1989; Cho and Azam, 1990; Griffith et al., 1990). For example, bacterial abundance in estuarine and nearshore waters of Georgia ranged from 2 to 6$\times 10^9$ l^{-1} (Griffth et al., 1990), and that in coastal Southern California Bight ranged from 0.2 to 10.0$\times 10^9$ l^{-1} (Cho and Azam, 1990). Also, the range of bacterial production found in the estuary during the study (0.1-22.2 μg C l^{-1} d^{-1}) was typical for the estuarine, nearshore and coastal waters (Fuhrman and Azam, 1980, 1982; Ducklow, 1982; Ducklow and Kirchman, 1983; Cole et al., 1988; Kirchman et al., 1989; Griffith et al., 1990). For example, bacterial production in estuarine and nearshore waters of Georgia ranged from ca 10 to 75 μg C l^{-1} d^{-1} (Griffth et al., 1990), and that in coastal Southern California Bight ranged from 0.1 to 15 μg C l^{-1} d^{-1}. (Fuhrman and Azam, 1980, 1982; Cho and Azam, 1988). The above comparisons indicate that the temporal and the spatial variations of bacterial variables were large in the estuarine system of the Mankyung river and Dongjin river, and that conditions of bacterial growth were dynamically changed in the shallow euphotic zone (1-5 m depth) of the estuary.

The most intriguing observation from this investigation was the major trends that bacterial abundance substantially increased with depth but bacterial production remarkably decreased with depth in most shallow stations, despite of almost homogeneous conditions of water column indicated by vertical distributions of salinity and seawater temperature. The magnitudes of the top-to-bottom differences of salinity and seawater temperature in the water column were small in the estuary (Fig. 2), even though salinity always increased from the estuarine stations to the seaward stations and seawater temperature decreased towards the seaward stations. Thus, mixing seemed to be active in the estuary. The studied area has large tidal range (ca 3-6 m) and is subject to a semidiurnal tidal cycle. Therefore, tidal mixing must contribute to the observed homogeneous hydrographic conditions in the estuary. Since we do not have any data or literature which shows the presence of tidal mixing in the estuary, we use a term of 'estuarine mixing' to describe the observed hydrographic conditions.

Estuarine mixing is expected to mix water bodies and therefore constituents of the water bodies (including bacterially utilizable organic matter). Thus, the mixing in the estuary is expected to mix bacteria and bacterial production, too. In addition, tidal mixing in shallow estuary has been known to have another important role of resuspending sediment (Rhoads et al., 1975). Sediment-associated organic matter, metabolites from sediment community activities, and bacteria will be released into the water column upon resuspension of sediment by the mixing in the estuary. Bacterial abundance in sediment near the study area has been reported to be much higher than that in the water column (Lee and Lee, 1991). The consequences of the mixing in the shallow estuary will be an increased bacterial abundance and an increased concentration of organic matter in the water column near the bottom. Thus, the process of resuspension has been thought to allow greater bacterial abundance (Wainright, 1990) and bacterial production (Rhoads et al., 1975) in the water column. In the estuarine system of Mankyung river and Dongjin river bacterial abundance increased with depth below the surface in most of shallow stations as expected. However, bacterial production decreased remarkably with depth below the surface in most of shallow stations.

We think these observations could be simply explained by the mechanism of sediment resuspension associated with estuarine mixing. Wainright (1990) demonstrated in the laboratory that sediment resuspension was detectable at shear velocities of 0.95 to 1.35 cm s^{-1} in sediments ranging from fine to coarse sands. The flux of bacteria via sediment resuspension was estimated to double typical water column concentration of bacteria in the field within a few hours. Microscopic observations of our samples showed that more sediment

particles were found in bottom samples than in surface samples in the estuary (not shown). Thus, increased bacterial abundance seemed to be due to sediment resuspension. Since much lower values of bacterial production were observed below the surface, the observed increases in bacterial abundance could not be due to growth of bacteria of the water column. The reduced bacterial growth at the bottom of the euphotic zone, which is close to the bottom of the water column in shallow stations, could be due to some inhibitory substances released from sediment upon resuspension. For example, hydrogen sulfide, recently reported to inhibit bacterial growth in the sea (Hoppe et al., 1990), would be released from sediment due to anaerobic bacterial metabolism in subsurface sediments and inhibit bacterial growth of the water column close to the bottom.

We further tested the above proposal that estuarine mixing would increase bacterial abundance and cause reduction of bacterial production in shallow estuary by analysing mixing diagrams (Figs. 3 and 5). Analysis of mixing diagrams can only be applicable to the stations which can be explained by mixing between end-member sources. It must be noted that the estuarine mixing were always complex (i.e. at least 3 end-member sources were present in the estuary-the Mankyung river, Dongjin river, and sea). We limited our analysis to April and August when mixing seemed to be rather simple. Table 1 lists results of the above analyses applied to the corresponding stations indicated in Figure 5. For example, in April, water body at the surface of Stn 2 could be regarded as a result of conservative mixing between two water bodies of Stn 1 and Stn 3 (Fig. 5b): Diagrams of bacterial abundance vs salinity for Stns 1, 2, and 3 (Fig. 3c) showed that a slight decrease in bacterial abundance was found compared with the predicted value of bacterial abundance on the basis of the conservative mixing (i.e. total bacterial abundance was unchanged). Considering counting error (ca<7%, unpublished data) of bacterial abundance, it could be regarded that bacterial abundance was conservatively mixed. Diagram of bacterial production vs salinity (Fig. 3d) showed that

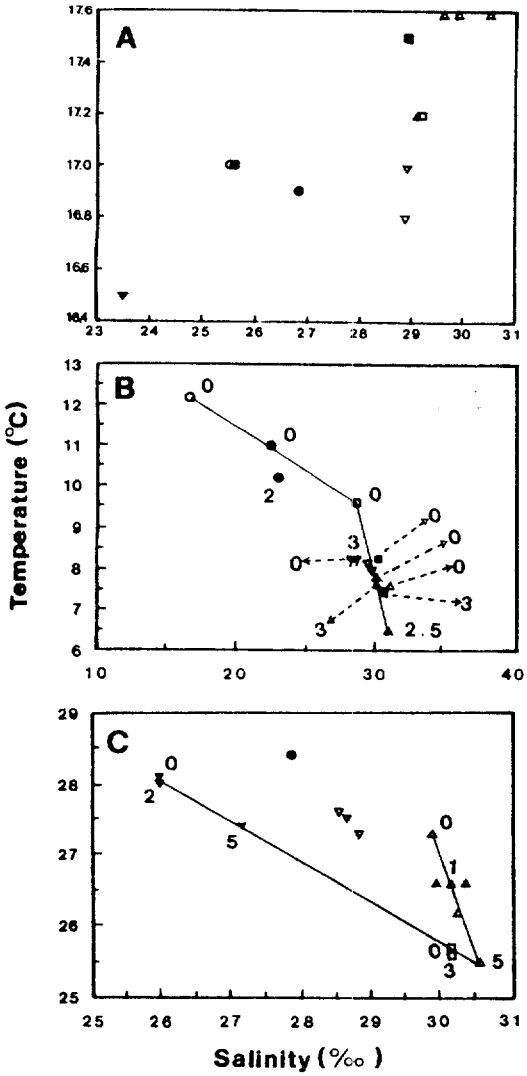

Fig. 5. Mixing diagrams (seawater temperature vs salinity) in the estuary during October, 1990 (A), April, 1991 (B), and August, 1991 (C). Symbols are the same as in Figure 3. Numbers marked beside symbols represent sampling depth.

bacterial production at 0 m depth at Stn 2 substantially decreased as a result of mixing of two water bodies, indicating that estuarine mixing caused reduction of bacterial production. The results listed in Table 1 can be summarized as follows: Estuarine mixing would mix conservatively or increase bacterial abundance, and mix conservatively or cause reduction of bacterial production. Thus, analyses of mixing diagrams are consistent with

Table 1. Summary of analyses of estuarine mixing. It is indicated whether estuarine mixing in the estuarine system of the Mankyung river and Dongjin river resulted in conservative mixing of bacterial abundance (AODC) and production (BSP), or increased or caused reduction of AODC and BSP.

Stn	Depth (m)	Date	Estuarine mixing acted as: Increase AODC	Increase BSP	Reduction AODC	Reduction BSP	Conservative mixing AODC	Conservative mixing BSP
2	0	4, 1991				+	+	
4	0-3	4, 1991					+	+
6	0	4, 1991				+	+	
6	3	4, 1991	+					+
3	0	8, 1991	+			+		
3	1	8, 1991	+			+		
6	1	8, 1991			+	+		

the above hypothesis that the mixing in the estuary would often increase bacterial abundance, but decrease bacterial production of the water column near sediment. Apparently, the roles of estuarine mixing in distribution of bacterial abundance and production would depend on shear stress. Thus, estuarine mixing, causing sediment resuspension, would have great influence on biogeochemical cycles mediated by bacteria in the estuary. Further, estuarine mixing would explain the observed large variation of bacterial production in the estuary.

In summary, estuarine mixing in the estuary would mix conservatively bacteria and bacterial production. However, estuarine mixing especially in the shallow estuary would cause resuspension of sediment and subsequent releases of bacteria, organic matter, and toxic metabolite(s) into water column. Thereby, estuarine mixing would increase bacterial abundance and cause reduction of bacterial production. The significant roles of mixing in the estuary should be considered to understand the roles of bacteria in biogeochemical cycles in the estuary.

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