Abundance and Production of Bacterioplankton along a Transect of Ise Bay, Japan

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Abundance and production of bacterioplankton were investigated along with chlorophyll-a (chl-a) concentration over a transect through Ise Bay, Japan. The transect was designed to cross the thermohaline front of the bay, which assumes a distinct form in winter. Average bacterioplankton abundance was $6.4 \times 10^9$ cells l$^{-1}$ in winter and $8.3 \times 10^9$ cells l$^{-1}$ in summer. Average bacterioplankton production was estimated to be $13.8 \mu g C l^{-1} day^{-1}$ in winter and $35.6 \mu g C l^{-1} day^{-1}$ in summer. Chl-a maxima were observed in the inner area and the front area, while the maximum bacterioplankton abundance and production were found at the inner to innermost area of the bay. Thus, the bacterioplankton abundance or production in Ise Bay was not necessarily coupled with the front-associated chl-a maximum.

Keywords: Bacterioplankton, distribution, abundance, production, Ise Bay.

1. Introduction

Bacteria are recognized as a major component of microbial food webs (e.g., Sherr and Sherr, 1987; Pomeroy and Wiebe, 1993) and as a major pathway in biogeochemical cycling of carbon (see, for example, Cho and Azam, 1990; Turley and Mackie, 1994). Of the aquatic bacterial abundance and productivity, the major fraction is dominated by free-living, planktonic bacteria, or bacterioplankton (Cho and Azam, 1990; Turley and Mackie, 1994). Planktonic bacteria also have considerable heterotrophic versatility (Egli, 1995). Thus bacterioplankton may affect the whole pelagic food web structure through microbial loops (e.g., Rheinheimer, 1991; Valiela, 1995).

The contribution of microbial loops to marine production is closely associated with the ratio of new production to regenerated production. Heinänen et al. (1995) expected that decreased bacterial contribution accompanies the increased new production in strongly mixed waters (fronts); however, they observed unchanged bacterial contribution. Cho et al. (1994) also reported the uncoupling of bacteria and phytoplankton productions at a tidal front. Along the same lines as these, the present communication reports the distribution of bacterioplankton abundance and production as regards phytoplankton abundance associated with the thermohaline front of Ise Bay, Japan (Yanagi et al., 1997). The abundance and production of bacterioplankton showed a distinct decrease toward the outer bay, but there was little correspondence to the physical structure and the associated phytoplankton maxima. Thus, uncoupling of phyto- and bacterioplankton is discussed.

2. Materials and Methods

2.1 Sample collection

Field sampling was conducted at 10 sites along a semi-longitudinal transect of Ise Bay (Fig. 1). Ise Bay, with a surface area of 1600 km$^2$ and a volume of 30 km$^3$ (average depth 18 m), is one of the most polluted bays in Japan (Sekiguchi, 1976). Water samples were collected with the Rosette Multi Sampler mounted on a CTD (Neil Brown Mark III) at known depths during the Tanseimaru cruise KT-95-2 (February 1995; winter) and KT-96-12 (July 1996; summer). Water temperature and salinity were measured with the CTD; a distinct thermohaline front was observed between the sites 7 and 8 in Fig. 1 (Yanagi et al., 1997).

Chlorophyll-a (chl-a) concentration data were collected using a Turner Design fluorometer (chl-a data from Yanagi, personal communication).

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2.2 Bacterioplankton abundance and production

The abundance of bacterioplankton (cells l$^{-1}$) was determined by direct counting and epifluorescence microscopy. Bacterial samples were fixed with formalin (final 5%) and refrigerated on board. Planktonic bacteria in a known volume of a sample were collected on a Nuclepore filter (pore size 0.2 µm) pre-blackened with Sudan Black. The collected
cells were stained with 0.01% acridine orange (AO; Hobbie et al., 1977), and the AO-stained cells were counted with an epifluorescence microscope (Olympus BH-2).

The production of bacterioplankton (µg C l⁻¹ day⁻¹) was estimated by multiplying the abundance (cells l⁻¹) by the specific growth rate (h⁻¹) assuming a conversion factor based on: an approximate average cell volume of 0.1 µm³; a specific gravity of 1.1; and a carbon content of 22% of the wet weight (Bratbak and Dundas, 1984; Simon and Azam, 1989; Moriarty, 1990). The specific growth rate was determined only for the surface water samples, and thus bacterioplankton production was accordingly estimated only for the surface water samples.

The specific growth rate of bacterioplankton was determined by a dilution-incubation technique. Sample water was first passed through a 5 µm filter to remove bacterivores such as heterotrophic nanoflagellates. The 5 µm filtrate was diluted 10 times in the same water, filtered through a 0.2 µm filter. In the 0.2 µm filtrate, bacterial cells were present at a density of less than 10³ cells l⁻¹, and the diluting water was thought to be virtually bacteria-free. Specific growth rates (µ, h⁻¹) of the bacterioplankton were calculated from the increase of bacterial numbers during incubation over 12 h at room temperature. The linearity of the regression line for time vs. logarithm of bacterial cell numbers was always significant, with regression coefficients higher than 0.9 for sample numbers of at least 3 (Naganuma and Miura, 1997).

3. Results and Discussion

3.1 Distribution of bacterioplankton abundance

There were distinct horizontal gradients in bacterioplankton abundance and production along the Ise Bay transect, both in winter and summer (Figs. 2 and 3). Higher abundance (e.g., >10⁹ cells l⁻¹) and production (e.g., >40–50 µg C l⁻¹ d⁻¹) were observed at the innermost sites 1 and 2, while lower values were seen at the outermost sites, except for low production at site 2 in February but high production at sites 3 and 5 in July. Average abundance was 6.4 [± SD 5.2] × 10⁸ cells l⁻¹ in winter and 8.3 [± SD 5.2] × 10⁸ cells l⁻¹ in summer.

The horizontal gradient in bacterial abundance is thought to correspond to eutrophication gradient, as previously shown in Shimoda Bay (Naganuma and Seki, 1993). Eutrophication parameters such as nitrate and phosphate concentrations were not determined in this study, however, the geography and hydrography of Ise Bay suggest that the bay

![Fig. 1. Sites of sample collection along a transect through Ise Bay, Japan. The thermohaline front in February, 1995, was located between the sites 7 and 8 (Yanagi et al., 1997).](image1)

![Fig. 2. Horizontal and vertical distribution of planktonic bacterial abundance along the Ise Bay transect in winter (February 1995) and summer (July 1996).](image2)
receives considerable amounts of polluting substances through major river discharge (e.g., Ota, 1975).

Adding to the horizontal gradients, there was a distinct vertical zonation (i.e., stratification) in bacterial abundance in summer, although there was some vertical stratification observed in February (Fig. 2). Thermal stratification is better developed in summer, and the bacterial gradient might be formed accordingly. By contrast, fluctuations of the typically strong southeast-ward winter wind caused advection of the water masses in and out of the bay (Yanagi et al., 1997), to which the weak stratification of bacterioplankton abundance has been attributed.

3.2 Bacterioplankton growth and production

The bacterial growth rate, on which production estimation was based, did not show well-defined gradients (data not shown). The growth rate in winter varied within a low, narrow range from 0.035 h⁻¹ (site 6) to 0.058 h⁻¹ (sites 9 and 10) with an average of 0.046 [± SD 0.008] h⁻¹. The growth rate in winter varied within a slightly higher and wider range from 0.059 h⁻¹ (sites 2 and 10) to 0.103 h⁻¹ (site 5) with an average of 0.076 [± SD 0.013] h⁻¹. The small site-to-site variation in the bacterial growth rate has also been observed in other coastal waters such as Shimoda Bay (Naganuma and Seki, 1993) and the Seto Inland Sea (Naganuma and Miura, 1997). This was possibly because the growth rate was profoundly affected by the water temperature, which varied little among the sites. Another possible reason may be that the bacterioplankton communities had adapted to the local dissolved organic matter (DOM) to maintain their growth at a certain rate. Ise Bay receives riverine DOM (Ota, 1975), some of which is terrigenous and is rapidly remineralized by microorganisms (Opsahl and Benner, 1997). Thus, the bacterioplankton growth rate in Ise Bay may be affected by the availability of the riverine DOM, as well as phytoplankton-derived DOM.

The estimated production of bacterioplankton was within the range from 8.3 (site 10) to 41.5 µg C l⁻¹ d⁻¹ (site 1) in winter; and from 12.5 (site 10) to 70 µg C l⁻¹ d⁻¹ (site 2) in summer (Fig. 3). Overall average production was 13.8 [± SD 10.1] µg C l⁻¹ d⁻¹ in winter and 35.6 [± SD 18.3] µg C l⁻¹ d⁻¹ in summer. This production is within the known range from eutrophic Shimoda Bay (36–140 µg C l⁻¹ d⁻¹; Naganuma and Seki, 1993) to the Seto Inland Sea without red tides (2.4–17.2 µg C l⁻¹ d⁻¹; Naganuma and Miura, 1997) and to physical fronts (about 4–12 µg C l⁻¹ d⁻¹; Cho et al., 1994; Heinänen et al., 1995).

It is unlikely that the primary production in Ise Bay at the times of observation exceeded 800 µg C l⁻¹ d⁻¹ in winter and 300 µg C l⁻¹ d⁻¹ in summer, assuming the following figures for estimation: the assimilation index of 10 µg C [µg Chl-a]⁻¹ h⁻¹; daylight of 10 h in winter and 14 h in summer;

![Fig. 3. Horizontal gradient of phytoplankton abundance (left), bacterioplankton abundance (middle) and bacterioplankton production rate (right) in the surface waters along the Ise Bay transect in February 1995 (upper) and July 1996 (lower). Average values are shown.](image-url)
and the overall average chl-a concentration of 8.2 [± SD 6.9] µg l⁻¹ in winter and 2.2 [± SD 3.2] µg l⁻¹ in summer. The average of surface chl-a concentration was 7.8 [± SD 5.3] µg l⁻¹ in summer and 3.3 [± SD 2.9] µg l⁻¹ in winter. Therefore, the bacterioplankton production in Ise Bay corresponds to 2–12% (or more) of the estimated primary production, which is compared with values of 4–16% estimated for physical fronts, where bacterial and primary productions are uncoupled (Cho et al., 1994; Heinänen et al., 1995).

3.3 Phytoplankton-bacterioplankton correlation

The overall correlation between chl-a concentration [CA; µg l⁻¹] and bacterioplankton abundance [BA; ×10⁸ cells l⁻¹] was significant for the winter data set, and less significant for the summer data set, expressed as follows:

Winter \[ \text{BA} = 0.35 \text{[CA]} + 1.51 \ (r = 0.61, n = 58, p < 0.001) \]

Summer \[ \text{BA} = 0.34 \text{[CA]} + 4.75 \ (r = 0.29, n = 62, p < 0.05) \]

where \( r \) is the correlation coefficient, \( n \) is the sample number, and \( p \) is the statistical probability. This was only a general trend using the overall data from euphotic and deeper zones collectively. This expression tells us little about the site-to-site variation and associated correlation. The site-to-site variation of phyto- and bacterioplankton variables is affected by incident sunlight and river discharge, and seems to be best shown for the surface waters. When focusing on the surface waters, a more significant correlation was found for the summer data set:

Winter \[ \text{BA} = 0.25 \text{[CA]} + 4.40 \ (r = 0.26, n = 10, p < 0.5) \]

Summer \[ \text{BA} = 1.30 \text{[CA]} + 4.08 \ (r = 0.71, n = 10, p < 0.01) \]

Thus, the correlation between phyto- and bacterioplankton variables was examined using the data sets from surface waters.

Phytoplankton abundance, expressed as chl-a concentration, showed maxima in the areas of sites 2–3 and 5–6 (Fig. 3). The maximum at sites 2–3 was located just outward of a major river discharge, and corresponded to the input of riverine inorganic nutrients. On the other hand, the maximum at sites 5–6 is thought to correspond to the occurrence of a thermohaline front, which was a well-mixed type formed mainly by strong wind in winter (Yanagi et al., 1997). In contrast, the summer front was rather a weakly-mixed type, and the associated chl-a maximum was accordingly less well developed.

Bacterioplankton abundance and production showed a tendency to decrease toward the outer bay (Fig. 3). Based on the pattern of phyto- and bacterioplankton distribution, the areas of Ise Bay areas were characterized as:

- Innermost area (site 1), low [CA] and high [BA];
- Inner area (sites 2–3), high [CA] and mid (winter) to high (summer) [BA];
- Front area (sites 5–6), high [CA] high and mid [BA]; and
- Outer area (sites 8–10), low [CA] and low [BA].

Discrepancy, or uncoupling, between phyto- and bacterioplankton abundances was observed in the innermost area and the frontal area. The uncoupling in the innermost area, particularly in winter, was probably due to the greater dependence of bacterioplankton abundance on riverine DOM. It is likely that phytoplankton production in the major river discharge is slowed down by high fluctuating salinity, and the riverine DOM becomes more important to bacterioplankton than phytoplankton-derived DOM.

The uncoupling in the frontal area has been reported in previous studies (Cho et al., 1994; Heinänen et al., 1995). The abundance-to-abundance or abundance-to-production type analysis is applicable to stable or large scale systems (e.g., Cho and Azam, 1990), but is not necessarily applicable to fluctuating, small scale systems (Pomeroy and Deibel, 1986). The uncoupling at the Ise Bay front is thought to be due to the fact that phytoplanton-derived DOM was not available in winter when photosynthetic DOM excretion is slowed (Larsson and Hagström, 1982; Fogg, 1983). Another explanation is that frontal mixing facilitates the sinking of phytoplankton (Cho et al., 1994) and thus uncouples the photosynthetic DOM production from secondary production by surface water bacteria.

The frontal uncoupling of phyto- and bacterioplankton may be explained differently. Bacterial production in mid-latitude estuaries and coastal waters is lowered by low temperature (Pomeroy and Deibel, 1986; Shiah and Ducklow, 1994). In contrast, a high temperature in summer increases bacterial production, but bacterivory is also activated and cancels the increase of bacterial production. The coupling of phyto- and bacterioplankton was thus obscured, though it was potentially possible. This suggests that the phytoplankton to bacterioplankton carbon flow cannot always be understood in straightforward terms, and the same may be true for the subsequent food web.

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