Journal of Oceanography, Vol. 54, pp. 285 to 295. 1998

Standing Stocks and Production Rates of Phytoplankton and Abundance of Bacteria in the Seto Inland Sea, Japan

KUNINAO TADA[1], KOJI MONAKA[1], MASAKAZU MORISHITA[1] and TOSHIYA HASHIMOTO[2]

[1]*Faculty of Agriculture, Kagawa University, Miki, Kagawa 761-0795, Japan*
[2]*Faculty of Applied Biological Science, Hiroshima University, Higashi-Hiroshima 739-8528, Japan*

(Received 9 August 1997; in revised form 31 March 1998; accepted 31 March 1998)

Standing stocks and production rates of phytoplankton and abundance of bacteria were investigated at 39 stations in the Seto Inland Sea, Japan during four cruises in October 1993, January, April and June 1994. Primary productivity was measured by the ^{13}C tracer method. Photosynthetic rate varied from 0.41 to 32.1 μgC/l/h with an average value of 4.67 μgC/l/h. Annual primary production was estimated to be 218 gC/m^2/year. Annual primary production in this study was 1.8 times as high as the values which were previously reported at same area. The reason for the disagreement between our primary production value and previous values is not thought to be due to the difference of methods used for measuring primary production or the different Chl.*a* concentrations but to the method of estimating the primary production in the euphotic zone from the *in vitro* measurements. The distribution of bacterial cells in surface seawater was examined during the same cruises. Bacterial cell density ranged from 0.32 to 3.4 $\times$ 10^6 cells/ml. The density was relatively high in the eutrophic regions of Hiroshima Bay and Osaka Bay. In addition, a high density of bacteria was also observed in an area within Suo Nada where Chl.*a* was relatively low. The disparity between Chl.*a* and bacterial density in Suo Nada suggests that bacterial abundance can be controlled by the availability of substrates other than phytoplankton exudate.

Keywords:
· Primary production,
· Seto Inland Sea,
· ^{13}C tracer method,
· bacteria.

1. Introduction

Primary production by phytoplankton supports the organisms of higher trophic levels in most marine ecosystems. The ^{14}C method has long been applied for the determination of primary production in natural waters, since the method was first introduced by Steeman-Nielsen (1952). However, measurements of primary production by the ^{14}C method have seldom been made in the past few decades in Japan because the use of ^{14}C in the natural environment has been restricted. Recently the ^{13}C method for the measurement of primary production was adopted (Hama *et al.*, 1983), but there have been few measurements of primary productivity in the coastal waters of Japan using this method.

The role of bacteria as transformers of dissolved organic matter mainly released by phytoplankton into particulate organic matter has been recognized, although heterotrophic bacteria in seawater are usually thought to be major decomposers of organic matter (Cole *et al.*, 1988). There have been remarkable developments in the studies on aquatic microbial ecology since the introduction of the improved techniques such as epifluorescence microscopy (Hobbie *et al.*, 1977). Production by heterotrophic bacteria is an important link between detritus, dissolved organic matter and organisms of higher trophic levels, i.e., the microbial food chain (Azam *et al.*, 1983).

The Seto Inland Sea, Japan, is a eutrophic region and red tides often occur during summer. The history of water pollution in this semi-enclosed sea is reviewed in Yanagi and Okaichi (1997). Several studies have been carried out on the association between red tides and eutrophication in this sea (e.g., Okaichi, 1983; Okaichi, 1997). Earlier, in the Seto Inland Sea, Endo (1970) investigated the primary production by the ^{14}C method and reported an annual primary production of 0.33 gC/m^2/day (120 gC/m^2/year) for this area. This value has been widely cited as a typical value for that region. However, the measurement was made more than 25 years ago and it has been thought that this value may be an underestimate for several reasons. For example Nixon (1988) synthesized published data from many areas of the world and suggested that fishery yield increases with primary production in aquatic systems. But the values of primary production reported by Endo (1980) and Uye *et al.* (1987) and the total annual fish catch in this sea did not conform well with the pattern exhibited by other coastal waters. In particular, using Nixon's (1988) relationship and those primary production estimates, fish catch was underpredicted.

This suggested that either fish catch was overestimated or primary production was underestimated. Uncertainty about the level of the primary production is a serious problem in studying the transfer of organic matter to fish production and the biogeochemical cycles in the Seto Inland Sea.

Because phytoplankton and bacteria are key organisms in coastal ecosystems, it is very important to determine the biomass of phytoplankton and bacteria. Furthermore, an accurate understanding of primary production is very important for the development of plans for the sustainable development of fishery resources in the Seto Inland Sea. Evaluation of the role of bacteria is also very important in studies of pelagic carbon flow in the Seto Inland Sea. Therefore, it is important to accumulate available data of biomass and its productivity of phytoplankton and bacteria in the coastal sea. However, only a few studies have been undertaken to measure the densities of bacteria and phytoplankton simultaneously in coastal waters (e.g., Naganuma and Miura, 1997; Naganuma, 1997). In this study, standing stocks and primary production rates of phytoplankton were investigated in the Seto Inland Sea in 1993 and 1994. Moreover, the biomass of heterotrophic bacteria in surface seawater was estimated on the same cruises.

2. Materials and Methods

Oceanographic observations were made and samples were collected at 39 stations (21 main stations (M) and 18 small stations (S)) throughout the Seto Inland Sea (Fig. 1) by *T.R.V. Toyoshio-Maru* of Hiroshima University during four seasons: Cruise I:12–22 October 1993, Cruise II: 8–21 January, Cruise III: 12–22 April and Cruise IV: 20–30 June 1994.

2.1 Oceanographic observation and processing of water samples

At all 39 stations, vertical profiles of temperature, salinity and Chl.*a* (with a Sea Tech fluorometer) were measured with a Sea Bird CTD. Surface seawater samples were collected with a clean plastic bucket at all stations. In addition, water samples for measurement of nutrient concentrations and primary production rates were collected with Van Dorn bottles from several depths at 21 main stations. Water samples for Chl.*a* were immediately filtered through a precombusted Whatman GF/F filter (450°C, 2 h) and preserved in N,N-dimethylformamide until analysis at –20°C (Suzuki and Ishimaru, 1990). Chl.*a* concentrations were determined by Lorenzen's (1967) spectrophotometric method (Parsons *et al.*, 1984). *In vivo* fluorescence values from CTD casts were calibrated with extracted Chl.*a* values determined by the spectrophotometric method. Water temperature, salinity and nutrient concentration data have been reported in Hashimoto *et al.* (1996).

2.2 Estimation of phytoplankton production

Primary production was measured by the ^{13}C tracer method (Hama *et al.*, 1983). Incubation of water samples was done on deck, using a tank that was equipped with artificial light. This method is relatively insensitive to weather conditions and does not require the vessel to be stopped during the measurement. The measurements of photosynthetic rate were conducted at 10 to 12 stations during every cruise (Table 1).

Water samples were taken from five depths corresponding to 100, 57, 42, 27, and 17% photon fluxes just above the sea surface, using clean plastic buckets and a 10-

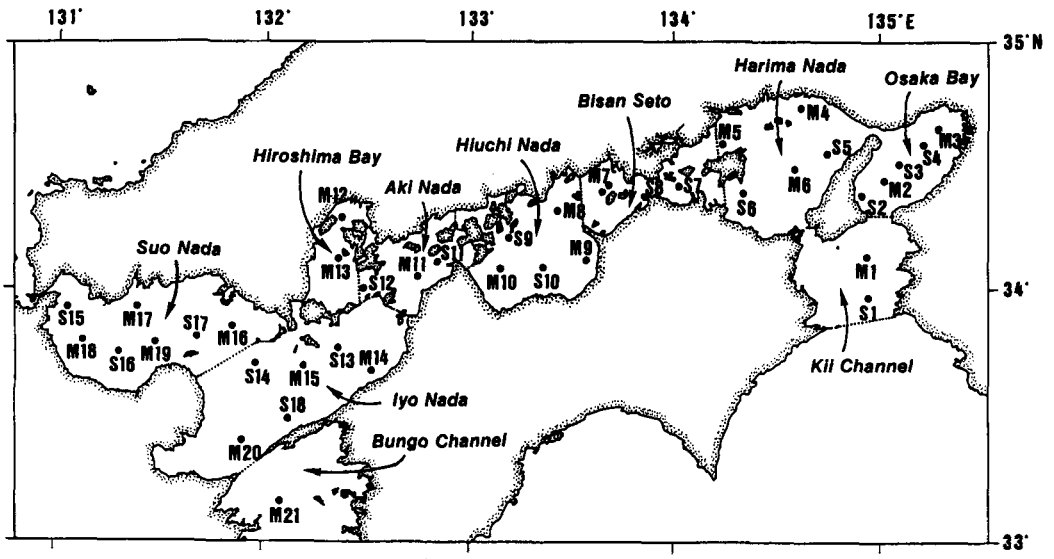

Fig. 1. Location of sampling stations in the Seto Inland Sea, Japan.

Table 1. Sampling stations where primary production was measured in each cruise.

October 1993	M1, M2, M4, M7, M11, M12, M15, M18, M19, M20, M21
January 1994	M1, M2, M4, M8, M11, M12, M16, M17, M19, M21
April 1994	M1, M2, M4, M7, M8, M11, M12, M15, M18, M19, M20, M21
June 1994	M1, M2, M4, M8, M11, M12, S13, M15, M18, M19, M20, M21

l Van Dorn sampler. The depths corresponding to these light levels were estimated from the transparency measured by Secchi disk, assuming that the thickness of the euphotic layer was 2.8 times as deep as the transparency (Hashimoto and Tada, 1997). The seawater samples for production rate analyses were immediately filtered through a 300 μm mesh screen to remove large zooplankton and transferred into 1-l polycarbonate bottles. After the addition of $NaH^{13}CO_3$ (about 10% of total inorganic carbon in the ambient water), the samples were incubated for about 2–4 hours in a tank. The incubations were conducted at in situ temperature with running surface seawater under the corresponding light intensity, which was regulated by density filters. The light intensity inside the tank was about 220 μE m^{-2} sec. All the samples were filtered through precombusted 47 mm Whatman GF/F filter (450°C for 4 h), after incubation. The filters were frozen and stored at –20°C until isotope analysis. After the filter papers were treated with HCl fumes to remove inorganic carbon, the ^{13}C to ^{12}C isotope ratios in the samples were analysed by an infrared spectrophotometer (JASCO $^{13}CO_2$ Analyzer EX-130S), using the method of Satoh *et al.* (1985). Photosynthetic rate was calculated according to Hama *et al.* (1983). A trapezoidal integration was applied to calculate primary production throughout the euphotic zone. For estimating daily primary production, we assumed a day length of 12 h.

2.3 Cell density of bacteria in surface seawater

Surface seawater samples were collected with a clean plastic bucket at 39 stations all over the Seto Inland Sea. The bacterial cells in surface seawater samples (0 m depth) were stained with 4'6'-diamidino-2-phenylindole (DAPI) and filtered on Nuclepore black filters (pore size: 0.2 μm), according to the procedure of Kogure (1990, which is a modification of the method of Hobbie *et al.*, 1977) and counted using an epifluorescence microscope (Nikon Y-2F-E). The coefficient of variation for all procedures was 2.2% (n = 5).

3. Results

3.1 Physicochemical conditions

The detailed physicochemical conditions during the four cruises have been reported previously (Hashimoto *et al.*, 1996; Yamamoto *et al.*, 1997). Surface water temperature was lowest in January (11–14°C) and highest in October (21–23°C). Surface salinity varied from 28.45 to 33.74 psu and was high (>ca. 33 psu) in Kii Channel and Bungo Channel and low (<30 psu) in Osaka Bay and Hiroshima Bay. Furthermore, in October 1993, salinity was unusually low (1.0 to 1.5 psu lower than other periods) over the entire Seto Inland Sea, which was due to heavy rainfall during June to September in 1993. Dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphorus (DIP) concentrations in the surface water varied from 0.04 to 18.7 μg-at N/l and from 0.03 to 1.11 μg-at P/l, respectively, and were relatively high in Osaka Bay, Kii Channel and Bisan Seto. Moreover, DIN

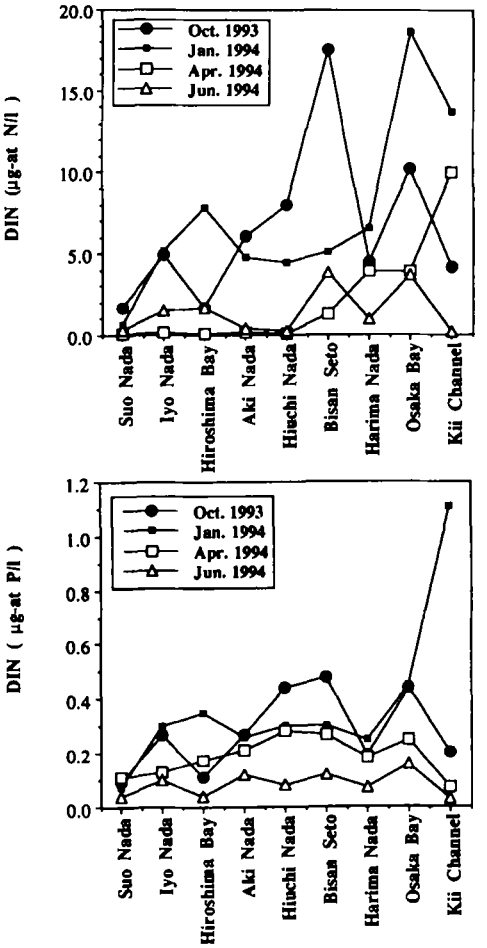

Fig. 2. Average concentrations of dissolved inorganic nitrogen (DIN) and phosphorus (DIP) in surface seawater at each area in the four cruises.

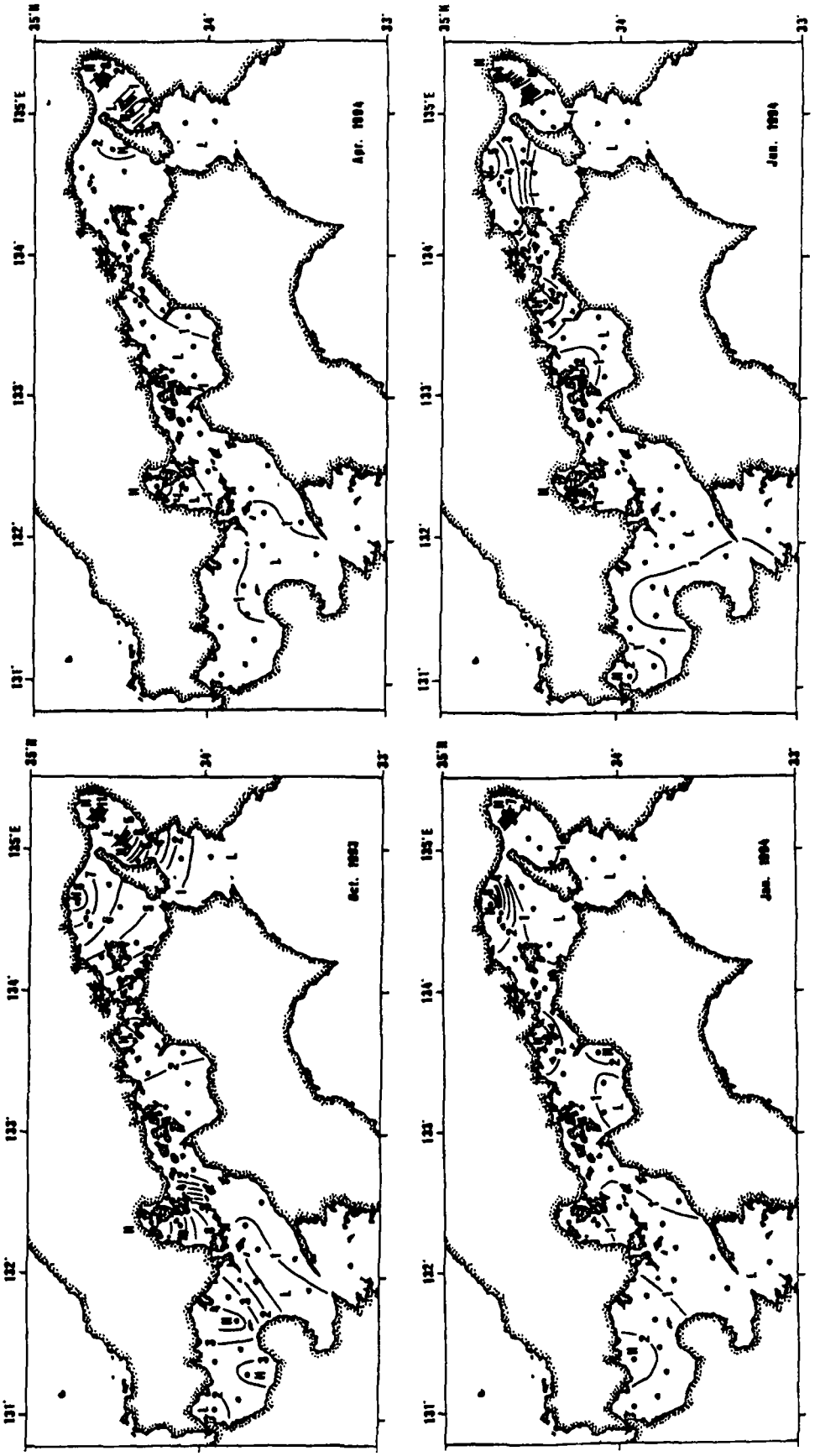

Fig. 3. Horizontal distribution of chlorophyll *a* (*μg/l*) in surface water (0 m) in the Seto Inland Sea.

and DIP were relatively high in January (regional average: 7.1 μg-at N/l and 0.32 μg-at P/l) and October (4.8 μg-at N/l and 0.26 μg-at P/l) but low in April (1.4 μg-at N/l and 0.15 μg-at P/l) and June (2.1 μg-at N/l and 0.13 μg-at P/l) (Fig. 2).

3.2 *Chlorophyll a*

Geographical variations in Chl.*a* concentrations in the surface seawater (0 m) are shown for each cruise in Fig. 3. Low Chl.*a* concentrations were seen in Bungo Channel and Kii Channel (about 1 μg/l), whereas high Chl.*a* concentrations (>4 μg/l) were often seen in Osaka Bay, Hiroshima Bay, and the northern part of Harima-Nada. Particularly high concentrations of Chl.*a* were always seen in the inner part of Osaka Bay, and red tide outbreaks of *Heterosigma* sp. were observed in June 1994, when the Chl.*a* concentration was higher than 100 μg/l.

Chl.*a* standing stocks in the surface layer (0–10 m) at each area in the Seto Inland Sea are shown in Fig. 4. In general, Chl.*a* content was relatively high in October and June and low in January. The overall average of Chl.*a* standing stock in the surface layer was 25.7 mg/m^2. Standing stocks of Chl.*a* in the surface layer were remarkably higher in the eutrophic region than in other areas (Osaka Bay; 51.5 mg/m^2, Hiroshima Bay; 47.4 mg/m^2, Harima-Nada; 36.9 mg/m^2).

Chl.*a* standing stocks in the euphotic layer at each area in the Seto Inland Sea are shown in Fig. 5. The thickness of the euphotic layer ranged from 0 to 4.2–58.8 m depth. There was a tendency for high Chl.*a* concentrations in October and low ones in January, like that of the surface layer. The overall average of Chl.*a* standing stocks in the euphotic

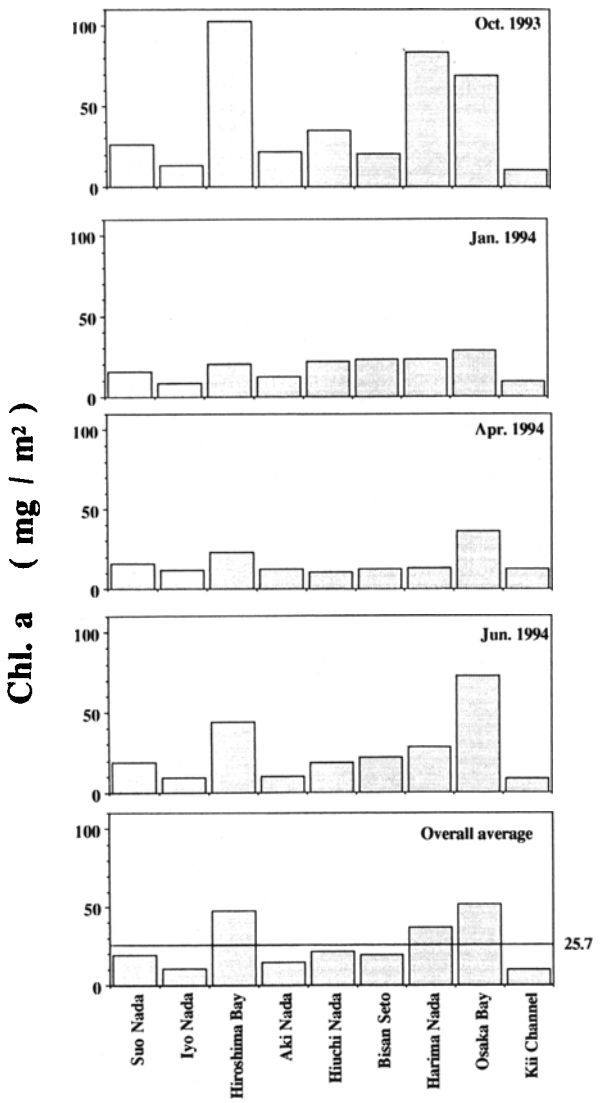

Fig. 4. Standing stocks of chlorophyll *a* in surface layer (0 to 10 m deep) in the Seto Inland Sea.

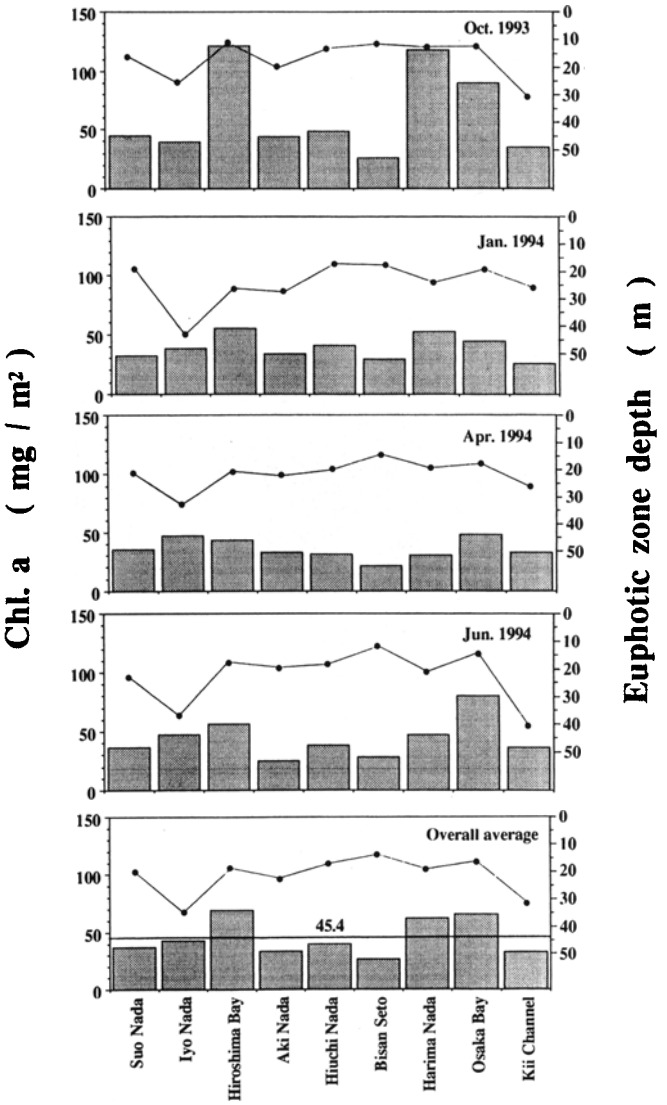

Fig. 5. Standing stocks of chlorophyll *a* (bars) in euphotic layer (0 to 4.2–58.8 m deep) and the euphotic zone depth (dots and lines) in the Seto Inland Sea.

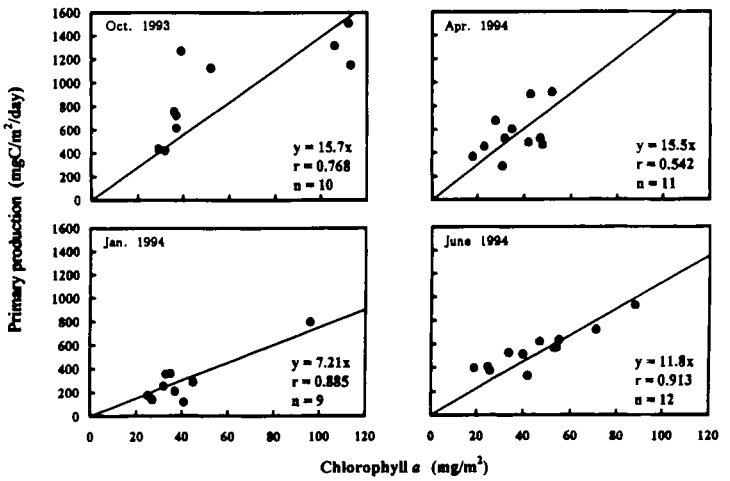

Fig. 6. Correlation between chlorophyll *a* standing stocks and primary production in euphotic layer in the Seto Inland Sea.

layer was 45.4 mg/m^2 and its thickness was 21.6 m. Overall averages of Chl.*a* standing stocks were highest in the Osaka Bay (65.4 mg/m^2), Hiroshima Bay (69.1 mg/m^2) and Harima-Nada (61.8 mg/m^2). Also, the thickness of the euphotic layers was shallower in Osaka Bay (16.0 m) and Hiroshima Bay (18.9 m). However, standing stocks of Chl.*a* in the euphotic layer in these areas were not remarkably higher than in other areas.

3.3 Primary production

The photosynthetic rate at each depth varied from 0.41 to 32.1 μgC/l/h and the overall mean photosynthetic rate was 4.67 μgC/l/h. Photosynthetic rate was measured at 10 to 12 stations of the total 39 stations (Table 1). The depth-integrated primary production rate varied from 118 to 1550 mgC/m^2/day. For stations where photosynthetic rate was not measured, it was calculated from the regression equation shown in Fig. 6 and Chl.*a* data from those stations. Mean ratios of primary production to Chl.*a* standing stock, the Chl.*a* specific productivity, were 15.7, 7.21, 15.5 and 11.8 mgC/mgChl.*a*/day and mean primary production rates in each cruise were 968, 294, 565 and 538 mgC/m^2/day in October, January, April and June, respectively. Integrated annual primary production was 0.596 gC/m^2/day (218 gC/m^2/year). Primary production in each area for every season is summarized in Fig. 7. Primary production was generally high in October and lowest in January. The production was high in Hiroshima Bay, Harima Nada and Osaka Bay and low in Suo Nada, Aki Nada and Bisan Seto. This is similar to the distribution of Chl.*a* content in the euphotic layer.

3.4 Bacteria

Geographical variation in bacterial density in the surface seawater (0 m) is shown for each cruise in Fig. 8. The density of bacteria ranged from 0.32 to 3.4 × 10^6 cells/ml,

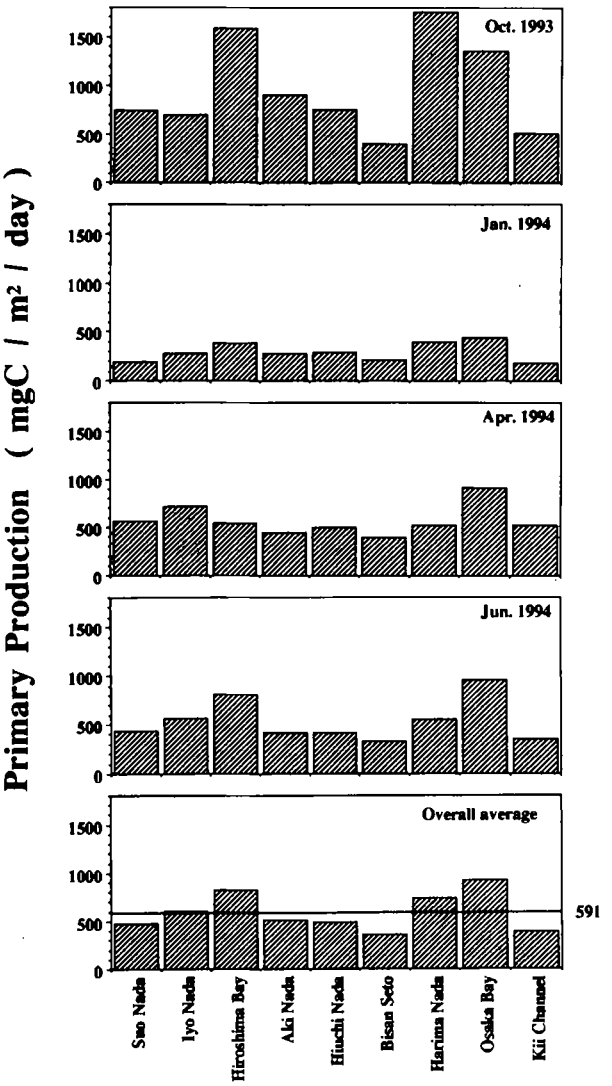

Fig. 7. Primary production in the Seto Inland Sea.

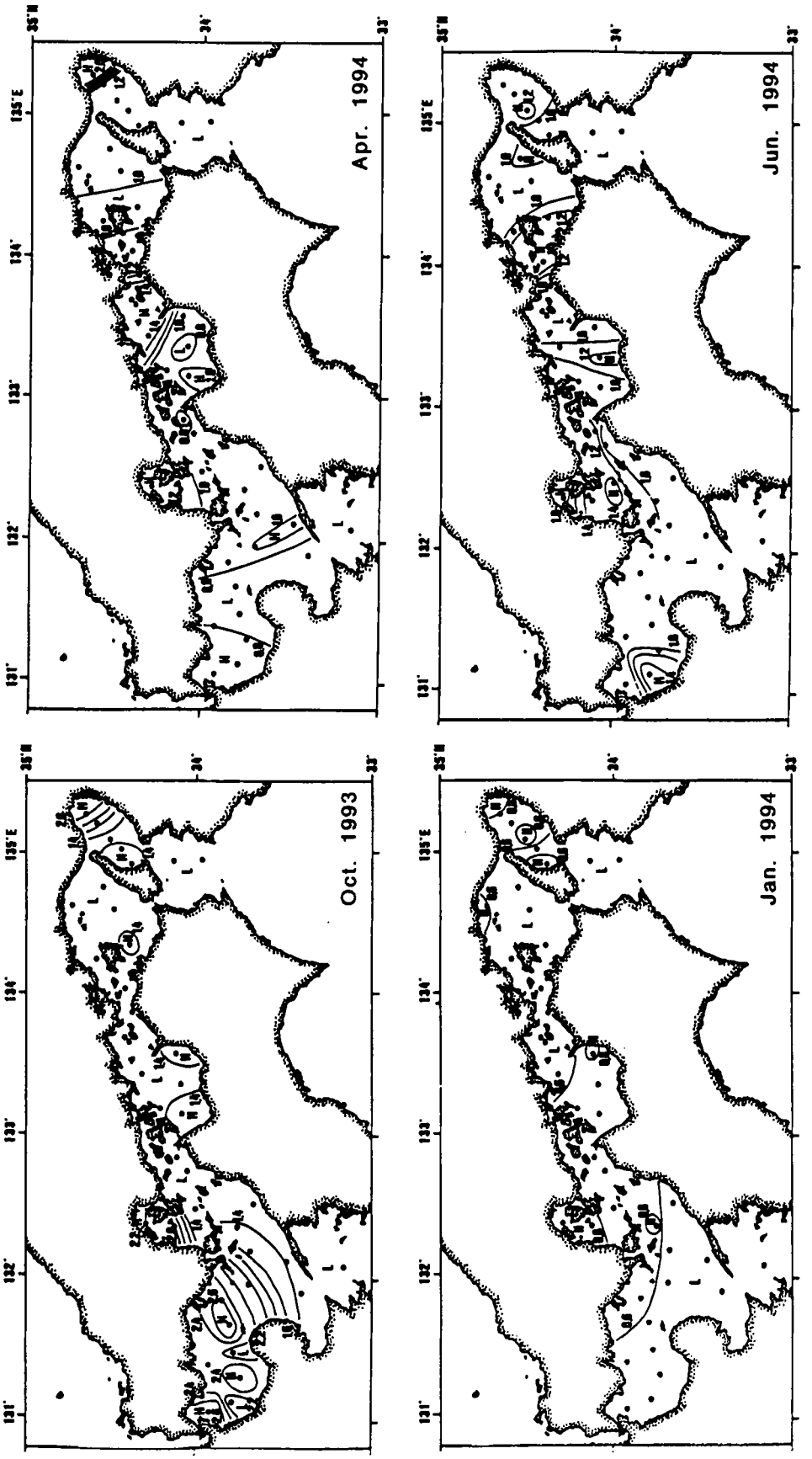

Fig. 8. Horizontal distribution of bacterial density ($\times 10^6$ cells/ml) in surface seawater in the Seto Inland Sea.

with much higher densities in October (1.7×10^6 cells/ml) than in other months (0.55×10^6, 0.99×10^6 and 0.76×10^6 cells/ml in January, April and June, respectively). The overall average for the whole Seto Inland Sea was 1.0×10^6 cells/ml. In general, the annual average density of bacteria was relatively high in Hiroshima Bay, Osaka Bay and Suo Nada.

4. Discussion

4.1 Phytoplankton standing stock

There was a tendency for Chl.*a* to be high in October and the low in January. Precipitation was higher than normal years during June to September in 1993 and hence the salinity of surface seawater during this period was lower (Yamamoto *et al.*, 1997). As shown in Fig. 2, DIN and DIP concentrations were relatively high in October. The high level of nutrients in October was probably due to a large nutrient input from the unusually high precipitation. This large nutrient input may have caused the phytoplankton biomass to increase.

In the eutrophic region, the standing stock of Chl.*a* in euphotic layer was not remarkably higher, because the euphotic layer was relatively shallow. However, the Chl.*a* content in surface layer (0–10 m) was much higher than in other areas. The higher Chl.*a* content causes rapid attenuation of solar radiation and therefore decreases the euphotic zone depth. Thus eutrophication may give rise to the shallowing euphotic zone and, as a result, the total primary production does not increase in spite of the high Chl.*a* concentration.

4.2 Primary production

The photosynthetic rate measured in this study (0.41 to 32.1 μgC/l/h, mean = 4.67 μgC/l/h) compares with previously measured rates for the Seto Inland Sea of 0.96 to 17.1 μgC/l/h by ^{14}C method (Endo 1970, Uye *et al.*, 1987), 0 to 21 μgC/l/h at Hiuch Nada from June to September by the ^{13}C method (Handa *et al.*, 1984), and 0 to 3.6 μgC/l/h at Suo Nada in May and July by the ^{13}C method (Yamaguchi and Anraku, 1984). Our measured values, expressed on a per liter basis, are similar to the values reported by Endo (1970), Uye *et al.* (1987) and Handa *et al.* (1984). Photosynthetic rates reported by Yamaguchi and Anraku (1984) at Suo Nada were lower, although values measured at Suo Nada in the present study (0.05 to 8.60 μgC/l/h, mean value of 3.14 μgC/l/h) were lower compared to other areas within the Seto Inland Sea. Low production rates were probably due to low standing stocks of Chl.*a* at Suo Nada (Fig. 5).

The photosynthetic assimilation ratio determined in this study varied from 0.03 to 9.75 μgC/μgChl.*a*/h (mean of 2.32 μgC/μgChl.*a*/h) in the euphotic zone and was similar to values reported in other studies. Yamaguchi and Anraku (1984) reported assimilation ratios from 1.0 to 2.3 μgC/

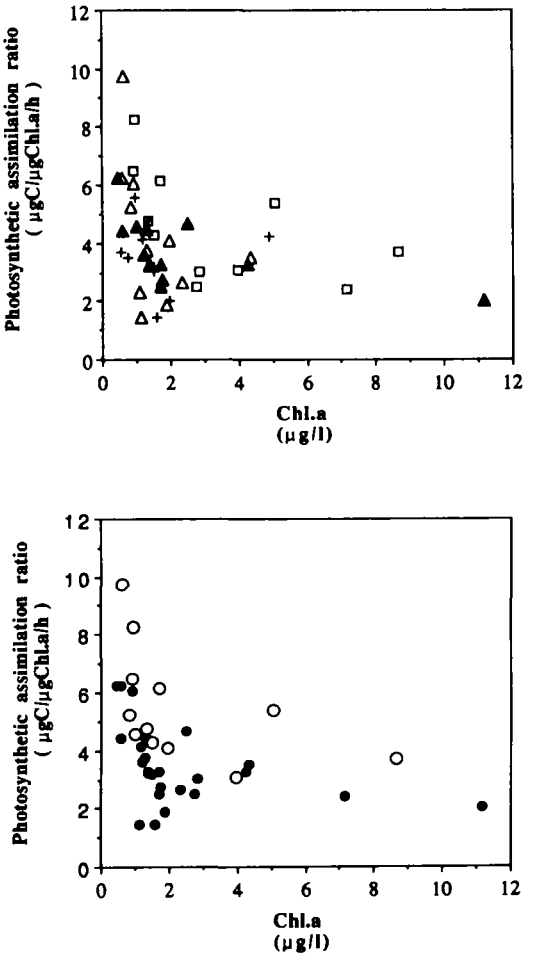

Fig. 9. Relationships between chlorophyll *a* concentration and photosynthetic assimilation ratio in surface waters during four cruises. (upper): distinguished by four seasons. (□); October, (+); January, (△); April, (▲); June. (lower): distinguished by the difference of nutrient concentrations. (●); DIN < 1.0 μg-at N/l, DIP < 0.1 μg-at P/l, (○); others

μgChl.*a*/h in May and from 4.8 to 11.0 μgC/μgChl.*a*/h in July at Suo Nada. Harrison and Platt (1980) reported assimilation ratios ranging from 2.0 to 13.1 for a 22-month period in a shallow marine basin. Photosynthetic assimilation ratios were relatively low in waters with higher Chl.*a* concentrations (>2 μg/l) (Fig. 9) and showed large variation when Chl.*a* concentrations were low (<2 μg/l). The photosynthetic assimilation ratios were lower in lower nutrient waters (DIN < 1.0 μg-at N/l, DIP < 0.1 μg-at P/l) and the values from January were also lower than those in other waters. This indicates that low nutrient concentrations, low water temperatures, or a combination of these factors, may have caused the low assimilation ratio. Phytoplankton species composition and physiological condition may also have contributed to the variation in assimilation ratio. Our mean depth-integrated photosynthetic assimilation ratios (15.7,

7.21, 15.5 and 11.8 mgC/mgChl.*a*/day in October, January, April and June, respectively) were similar to previous measurements made by the ^{14}C method in the same area (13.1, 3.2, 15.8 and 14.5 mgC/mgChl.*a*/day, respectively (Uye *et al.*, 1987)). Recently, Hama *et al.* (1977) determined the depth-integrated Chl.*a*, photosynthetic rate and photosynthetic assimilation ratio in the East China Sea (22 to 37 mg/m^2, 280 to 750 gC/m^2/day and 13 to 23 mgC/mgChl.*a*/day, respectively). It is interesting to note that although the depth-integrated assimilation ratio determined in this study for the semi-enclosed Seto Inland Sea is comparable to that of the East China Sea (13 to 23 mgC/mgChl.*a*/day), the thickness of euphotic zone was about three times deeper, and Chl.*a* concentrations were about one-tenth lower in the East China Sea.

It has been reported previously that annual primary production in the Seto Inland Sea is 120 to 122 gC/m^2/year (Endo, 1970; Uye *et al.*, 1987). Annual primary production in this study was 1.8 times as high as the values which were previously reported, although photosynthetic rate and depth integrated photosynthetic assimilation ratio in our study were similar to those of previous studies. We believe that the reason for the disagreement was not related to the different method for measurement of primary production or the different Chl.*a* concentration. Hama *et al.* (1983) reported that photosynthetic rates determined by the ^{13}C method showed a remarkable agreement with those determined by the ^{14}C method. In fact, however, we believe that the method used to estimate primary production based on the euphotic zone depth contributed to the discrepancy. In this study we calculated the primary production in the euphotic zone by a trapezoidal integration, while Endo (1970) and Uye *et al.* (1987) used the equation of Steemann-Nielsen (1952), assuming that the depth of 1% surface irradiance was twice the Secchi depth. However we determined that the thickness of euphotic layer was about 2.8 times the Secchi depth (Hashimoto and Tada, 1997).

Using the average value of the production from our study, 218 gC/m^2/year, the annual production of the Seto Inland Sea is estimated at 4,080,000 tons of carbon.

4.3 Bacteria

Our overall range of 0.32 to 3.4 $\times$ 10^6 cells/ml was in good agreement with the range of 0.4 to 2.2 $\times$ 10^6 cells/ml for the whole Seto Inland Sea (Naganuma and Miura, 1997) and of eutrophic coastal waters in general (Sanders *et al.*, 1992). Previously reported that bacterial cell densities in the Seto Inland Sea ranged from 0.9 to 4.9 $\times$ 10^6 cells/ml in Hiroshima Bay (Iwamoto *et al.*, 1993, 1994; Imai and Yamaguchi, 1996), 0.45 to 3.0 $\times$ 10^6 cells/ml in Suo Nada (Imai, 1984) and 0.40 to 5.1 $\times$ 10^6 cells/ml in Osaka Bay (Imai and Yamaguchi, 1997). In the present study, bacterial cell density was somewhat lower (0.76 to 2.2 $\times$ 10^6 cells/ml) in Hiroshima Bay and approximately the same (0.29 to 3.3 $\times$ 10^6 cells/ml) in Suo Nada and Osaka Bay as was found in those previous studies. The discrepancy in Hiroshima Bay is probably due to the fact that Iwamoto *et al.* (1993, 1994) and Imai and Yamaguchi (1996) conducted their studies at a pier of the Nansei National Fisheries Research Institute, where the water was very shallow (about 8 m) and the waters were more eutrophic. The range of bacterial densities obtained in this study was also in good agreement with the result of Nakamura *et al.* (1994) who reported that the bacterial cell density in the surface layer ranged from 1.1 to 2.3 $\times$ 10^6 cells/ml around the Ie-shima Islands in the Seto Inland Sea during summer.

The seasonal and horizontal patterns of bacterial density were similar to the distribution of Chl.*a* concentration in surface seawater except at the Suo Nada stations (Fig. 3). A correlation between Chl.*a* concentration and bacterial density of surface seawater is shown in Fig. 10. Bacterial cell density seemed to correlate with Chl.*a* concentration except at the Suo Nada stations (the relationship was not statistically significant). This result is not inconsistent with previous studies by Cole *et al.* (1988) and Bird and Kalff (1984). Recently, Naganuma and Miura (1997) reported that there was a clear correlation between phytoplankton (Chl.*a*) and bacterial abundances in the Seto Inland Sea. It has been thought that water column bacteria utilize DOM (dissolved organic matter) as an energy source, and this is mainly of phytoplankton origin. However the release of DOC by healthy phytoplankton is a small percentage of the photosynthetic rate (5–10%, Mague *et al.*, 1980), making it difficult to explain the high rate of bacterial production in the ocean (10–50% of primary production, Larsson and Hagstoröm 1982; Azam *et al.*, 1983; Cole *et al.*, 1988). Interestingly, in the Seto Inland sea, Imai and Yamaguchi (1996, 1997) and Naganuma and Miura (1997) reported that bacterial production may exceed primary production. Furthermore, Uye *et al.* (1996) suggested that, taking the amount of carbon required to support bacterial production into consideration, the remaining primary production appears to be insufficient to meet the requirements by microzooplankton and net-zooplankton.

Nagata and Kirchman (1992) suggest that DOM release by protozoa is a potentially important process in aquatic ecosystems. On the other hand, it was recently suggested that coastal primary production may be supported by microbial regeneration of nutrients from terrigenous DOM (Opsahl and Benner, 1997). Furthermore, Naganuma and Miura (1997) reported that bacterial production was not correlated with abundance, although bacterial abundance had a good correlation with phytoplankton abundance in the Seto Inland Sea. They suggested that this correlation is still maintained when the DOM is heavily supplemented with allochthonous organic matter. In our data, in addition to high density of bacteria in eutrophic areas like Hiroshima Bay and Osaka Bay, it is interesting to note that a high density of

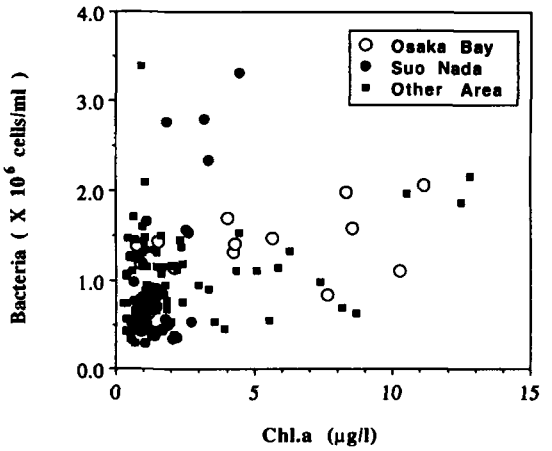

Fig. 10. Correlation between chlorophyll *a* concentration and bacterial density of surface seawater in Osaka Bay, Suo Nada and other areas in the Seto Inland Sea.

bacteria also occurred in Suo Nada where Chl.*a* concentration was relatively low. When correlations between Chl.*a* concentration and bacterial density were made for Osaka Bay and Suo Nada stations separately (Fig. 10), the correlation was clearly different between the two areas. This indicates that the role of the microbial loop in Suo Nada may be more important than in other parts of the Seto Inland Sea. The higher densities of bacteria per unit chlorophyll in Suo Nada indicate that bacterial abundance may depend on the availability of substrates other than phytoplankton exudate. A different trophic phasing dynamics (coupling of the different trophic levels), as reported by Parsons (1988), may also be another explanation. Furthermore, it was reported that the transfer efficiency from primary production to tertiary production was extremely low in Suo Nada (Uye and Hashimoto, 1997). More environmental and ecological research is needed in this area.

In this study, we have investigated the density of bacteria for surface waters only and did not measure bacterial productivity. Bacterial biomass and production throughout the water column should be determined. Further research is needed to assess the role of bacteria in the microbial food chain and to determine the dynamics of organic matter in coastal waters.

Acknowledgements

The authors thank Drs. O. Matsuda, S. Uye, T. Yamamoto and H. Hashimoto, Hiroshima University, for their discussion and critical comments. We also thank Drs. Sam C. Wainright, Rutgers, The State University of New Jersey and S. Montani, Kagawa University for their critical comments and encouragement during preparation of the manuscript. We are much obliged to anonymous reviewers for their useful comments on the manuscript. We greatly appreciate Dr. A. Shiomoto, National Research Institute of Far Seas Fisheries, for his advice on the ^{13}C analysis and for his kindly permitting our use of the infrared absorption analyzer. We are grateful to the captain and crew of the *T.R.V. Toyoshio-Maru* for their kind assistance during the field observation and the collection of seawater samples. Finally, the authors thank Drs. T. Okaichi and T. Yanagi for giving us the opportunity to do this research. This work was supported by "Interdisciplinary Study on the Sustainable Production of Valuable Fishes and Preservation of Environment in Seto Inland Sea, Japan" under the sponsorship of the Nippon Life Insurance Foundation.

References

Azam, F., T. Fenchel, J. G. Field, J. S. Gray, L. A. Meyer-Reil and F. Thingstad (1983): The ecological role of water-column microbes in the sea. *Mar. Ecol. Prog. Ser.*, **10**, 257–263.

Bird, D. F. and J. Kalff (1984): Empirical relationships between bacterial abundance and chlorophyll concentration in fresh and marine waters. *Can. J. Fish. Aquat. Sci.*, **41**, 1015–1023.

Cole, J. J., S. Findlay and M. L. Pace (1988): Bacterial production in fresh and saltwater ecosystems: a cross-system overview. *Mar. Ecol. Prog. Ser.*, **43**, 1–10.

Endo, T. (1970): On primary production in the Seto Inland Sea. *J. Fac. Fish. Anim. Husb. Hiroshima Univ.*, **9**, 177–221 (in Japanese).

Hama, T., T. Miyazaki, Y. Ogawa, T. Iwakuma, M. Takahashi, A. Otsuki and S. Ichimura (1983): Measurement of photosynthetic production of a marine phytoplankton population using a stable ^{13}C isotope. *Mar. Biol.*, **73**, 31–36.

Hama, T., K. H. Shin and N. Handa (1997): Apatial variability in the primary productivity in the East China Sea and its adjast waters. *J. Oceanogr.*, **53**, 41–51.

Handa, N., H. Matsueda, K. Matsunaga and T. Manabe (1984): The vertical fluxes and decomposition of organic matter in Hiuchi Nada. Report for Special Research Project on Environmental Science supported by Grants in Aid for Scientific Research Ministry of Education, Culture, Sports and Science, Japan (B210-R01–3), p. 63–75 (in Japanese).

Harrison, W. G. and T. Platt (1980): Variations in assimilation number of coastal marine phytoplankton: Effects of environmental co-variates. *J. Plankton Res.*, **2**, 249–260.

Hashimoto, T. and K. Tada (1997): Optical properties of sea water in Hiroshima Bay, Japan. *Umi no Kenkyu*, **6**, 151–155 (in Japanese).

Hashimoto, T., T. Yamamoto, K. Tada, O. Matsuda, T. Nagasue, M. Tada, A. Go and K. Nakaguchi (1996): Seasonal investigation of water quality characteristics of the Seto Inland Sea. *J. Fac. Appl. Biol. Sci. Hiroshima Univ.*, **35**, 243–273 (in Japanese).

Hobbie, J. E., R. J. Daley and S. Jasper (1977): Use of Nuclepore filters for counting bacteria by fluorescence microscopy. *Appl. Environ. Microbiol.*, **33**, 1225–1228.

Imai, I. (1984): Size distribution and biomass of bacteria in Suo Nada, western Seto Inland Sea. *Bull. Nansei Reg. Fish. Res. Lab.*, **17**, 183–196 (in Japanese).

Imai, I. and M. Yamaguchi (1996): Seasonal fluctuations in

abundance and production of bacteria, and quantitative relation with heterotrophic microflagellates in Northern Hiroshima Bay, Seto Inland Sea. *Bull. Nansei Natl. Fish. Res. Inst.*, **29**, 75–86 (in Japanese).

Imai, I. and M. Yamaguchi (1997): Abundance and productivity of bacteria in Osaka Bay, eastern Seto Inland Sea, Japan. *Bull. Nansei Natl. Fish. Res. Inst.*, **30**, 173–181 (in Japanese).

Iwamoto, N., I. Imai and S. Uye (1993): Numerical fluctuation of bacteria and heterotrophic microflagellates in Hiroshima Bay of the Inland Sea of Japan in summer 1990. *Bull. Plankton Soc. Japan*, **40**, 55–66 (in Japanese).

Iwamoto, N., I. Imai and S. Uye (1994): Seasonal fluctuation in abundance of bacteria, heterotrophic nanoflagellates, autotrophic nanoflagellates and nanodiatoms in Hiroshima Bay, the Inland Sea of Japan. *Bull. Plankton Soc. Japan*, **41**, 31–42.

Kogure, K. (1990): *A Manual of Observations for Coastal Environment II.: Water Quality and Microorganisms*. The Oceanographic Society of Japan, Koseisya-Koseikaku, Tokyo, 386 pp. (in Japanese).

Larsson, U. and A. Hagstoröm (1982): Fractionated phytoplankton primary production, exudate release and bacterial production in a Baltic eutrophication gradient. *Mar. Biol.*, **67**, 57–70.

Lorenzen, C. J. (1967): Determination of chlorophyll and pheopigments: spectrophotometric equations. *Limnol. Oceanogr.*, **12**, 343–346.

Mague, T., E. Friberg, D. J. Hughes and I. Morris (1980): Extracellular release of carbon by marine phytoplankton: a physiological approach. *Limnol. Oceanogr.*, **25**, 262–279.

Naganuma, T. (1997): Abundance and production of bacterioplankton along a transect of Ise Bay, Japan. *J. Oceanogr.*, **53**, 579–583.

Naganuma, T. and S. Miura (1997): Abundance, production and viability of bacterioplankton in the Seto Inland Sea, Japan. *J. Oceanogr.*, **53**, 435–442.

Nagata, T. and D. L. Kirchman (1992): Release of dissolved organic matter by heterotrophic protozoa: Implications for microbial food webs. *Arch. Hydrobiol., Beih. Ergeb. Limnol.*, **35**, 99–109.

Nakamura, Y., K. Fukami, S. Sasaki and J. Hiromi (1994): Population dynamics of bacteria and heterotrophic nanoflagellates following the summer diatom bloom in the Seto Inland Sea. *Bull. Plankton Soc. Japan*, **41**, 1–8.

Nixon, S. W. (1988): Physical energy inputs and the comparative ecology of lake and marine ecosystems. *Limnol. Oceanogr.*, **33**, 1005–1025.

Okaichi, T. (1983): Marine environmental studies on outbreaks of red tide in neritic waters. *J. Oceanogr. Soc. Japan*, **39**, 267–278 (in Japanese).

Okaichi, T. (1997): Red tides in the Seto Inland Sea. p. 251–304. In *Sustainable Development in the Seto Inland Sea, Japan*, ed. by T. Okaichi and T. Yanagi, Terra Scientific Publishing Company, Tokyo.

Opsahl, S. and R. Benner (1997): Distribution and cycling of terrigenous dissolved organic matter in the ocean. *Nature*, **386**, 480–482.

Parsons, T. R. (1988): Trophodynamic phasing in theoretical, experimental and natural pelagic ecosystems. *J. Oceanogr. Soc. Japan*, **44**, 94–101.

Parsons, T. R., Y. Maita and C. M. Lalli (1984): *A Manual of Chemical and Biological Methods for Seawater Analysis*. Pergamon Press, Oxford, 173 pp.

Sanders, R. W., D. A. Caron and U.-G. Berninger (1992): Relationship between bacteria and heterotrophic nanoplankton in marine and fresh waters: an interecosystem composition. *Mar. Ecol. Prog. Ser.*, **86**, 1–14.

Satoh, H., Y. Yamaguchi, N. Kokubu and Y. Aruga (1985): Application of infrared absorption spectrometry for measuring the photosynthetic production of phytoplankton by the stable ^{13}C isotope method. *La mer*, **23**, 171–176.

Steeman-Nielsen, E. (1952): The use of radio-active carbon (^{14}C) for measuring organic production in the sea. *J. Cons. Int. Explor. Mer.*, **18**, 117–140.

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

Uye, S. and H. Hashimoto (1997): Trophic structure and transfer efficiency in the pelagic ecosystem. p. 41–46. In *Sustainable Development in the Seto Inland Sea, Japan*, ed. by T. Okaichi and T. Yanagi, Terra Scientific Publishing Company, Tokyo.

Uye, S., H. Kuwata and T. Endo (1987): Standing stocks and production rates of phytoplankton and planktonic copepods in the Inland Sea of Japan. *J. Oceanogr. Soc. Japan*, **42**, 421–434.

Uye, S., N. Nagano and H. Tamaki (1996): Geographical and seasonal variations in abundance, biomass and estimated production rates of microzooplankton in the Inland Sea of Japan. *J. Oceanogr.*, **52**, 689–703.

Yamaguchi, M. and M. Anraku (1984): Primary productivity in Suo-Nada, Western Seto Inland Sea. *Bull. Nansei Reg. Fish. Res. Lab.*, **17**, 135–149 (in Japanese).

Yamamoto, T., O. Matsuda, T. Hashimoto and K. Tada (1997): Environmental conditions to support biological production. p. 19–27. In *Sustainable Development in the Seto Inland Sea, Japan*, ed. by T. Okaichi and T. Yanagi, Terra Scientific Publishing Company, Tokyo.

Yanagi, T. and T. Okaichi (1997): Seto Inland Sea-Historical background. p. 9–14. In *Sustainable Development in the Seto Inland Sea, Japan*, ed. by T. Okaichi and T. Yanagi, Terra Scientific Publishing Company, Tokyo.