Effects of nutrient supplies on the growth rates of planktonic bacteria in a bay of the Uwa Sea, Japan

Daisuke Ichinotsuka[1], Toshiya Katano[2, 3], Hidetaka Takeoka[2], Shin-ichi Nakano*[1, 4]

[1] LAFWEDY, Ehime University, Tarumi 3-5-7, Matsuyama 790-8566, Ehime, Japan

[2] Center for Marine Environmental Studies, Ehime University, Bunkyo-cho 3, Matsuyama, 790-8577, Japan

[3] Ariake Sea Research Project, Saga University, 1 Honjo-cho, Saga, 840-8502, Japan

[4] Center for Ecological Research, Kyoto University, Hirano-cho 2 chome, 509-3, Otsu, Shiga 520-2113, Japan

Running head: Bacterial growth rate in a bay

*Corresponding author: e-mail, nakano@ecology.kyoto-u.ac.jp

ABSTRACT

In the oligo- to meso-trophic waters of Uchiumi Bay, Japan, there is an intermittent physical event which supplies nutrients to the sea: bottom intrusion of deep, cool, nutrient-rich water that slides through just above the continental shelf. To clarify effects of nutrient supply by this bottom intrusion on bacterial growth, we followed seasonal changes in bacterial growth rates and conducted nutrient amendment experiments with single or mixed-nutrient additions of nitrogen and phosphorus. In addition, we examined the importance of organic matter supply for bacterial growth rates using glucose. Bacterioplankton bulk growth rates ranged between 0.37 and 1.86 d^{-1}, with relatively high growth rates during the period of strong thermal stratification. Neither positive nor negative effects on bacterial growth rates were detected by amendments of nutrients or glucose. A significant positive relationship ($p < 0.001$) between cell density and production of bacteria was found, though the regression slope of bacterial cell density on bacterial production was relatively low (0.34). Our results suggested that bacterial growth rates in Uchiumi Bay are nearly saturated at ambient levels of nutrients and/or organic matter, and that bacterial abundance is subject to a relatively weak bottom-up control.

Key words: bacterial growth rate, nutrient limitation, bottom-up control

INTRODUCTION

Planktonic heterotrophic bacteria constitute a large portion of the planktonic biomass in both marine and freshwater ecosystems (Cho & Azam 1990, Geller et al. 1991, Kawabata & Nakanishi 1996, Simon et al. 1992). Thus, bacterial utilization of inorganic and organic matter and, resultant production of bacterial biomass may account for a substantial part of material cycling in an aquatic system. Bacterial production depends on bacterial abundance, cell size and growth rate. It has been considered that abundance and growth rates of bacteria in natural aquatic environments are susceptible to subtle changes in environmental conditions. Studies on the seasonal dynamics of bacterial abundance frequently show a relative constancy of cell density over time (Azam et al. 1983, Pace 1988). In contrast, bacterial growth rates vary depending on environmental variables: temperature, nutrient and energy supplies as bottom up control, and grazing and viral lysis as top down control (Church et al. 2000, Sala et al. 2002). Bacterial growth rates are therefore a useful measure in elucidating environmental factor(s) which may cause changes in bacterial production. There is a significant

correlation between bacterial growth rates and phytoplankton biomass (Cole et al. 1988, White et al. 1991). Thus, it is well accepted that bacterial growth rates are closely related to the productivity of an aquatic ecosystem.

Owing to their bulk in nucleic acids and proteins, the nitrogen and phosphorus requirements of bacteria are large. This necessitates bacterial cells to maintain low intracellular C:N:P ratios (Goldman et al. 1991, Tezuka 1990). The amount of available nutrients can therefore constrain bacterial growth rates (Caron et al. 2000, Hoch & Bronk 2007, Rivkin & Anderson 1997). Evidences have been accumulated that bacteria in the Atlantic Ocean, the Mediterranean Sea, Baltic Sea are limited by inorganic nutrients, mainly phosphorus (Caron et al. 2000, Pinhassi et al. 2006, Rivkin and Anderson 1997). Also, nutrient limitation of bacterial growth in lakes has been intensively studied (Elser et al. 1995, Morris and Lewis 1992, Jansson et al. 1996). However, such information is relatively limited for marine coastal waters especially in Western Pacific Ocean.

The Uwa Sea is located along southwestern coastal area of Shikoku Island, Japan. The trophic state of the sea has been considered meso- to oligo-trophic (Tomaru et al. 2002). Within this system, there is an intermittent physical event which supplies nutrients to the sea: the bottom intrusion of deep, cool, nutrient-rich Pacific water which

slides just above the continental shelf (Kaneda et al. 2002 a & b). Large amounts of nitrate and phosphate supplied by bottom intrusion are the major nutrient source for phytoplankton primary production in the sea (Koizumi 1991, Koizumi & Kohno 1994, Koizumi et al. 1997, Takeoka et al. 2000). Since inorganic nitrogen (Rivkin and Anderson 1997) and/or phosphorus (Caron et al. 2000, Pinhassi et al. 2006, Rivkin and Anderson 1997) supplies stimulate bacterial growth in marine environments, bottom intrusion may also serve as an important supply of nutrients for bacteria in the Uwa Sea. The importance of nutrients supplied through bottom intrusion on bacterial growth rates remains to be resolved.

In the present study, to elucidate the effects of nutrient supply by the bottom intrusion on bacterial growth rates, we followed seasonal changes in bacterial growth rates and conducted nutrient amendment experiments in a bay of the Uwa Sea. To identify which nutrient is important for accelerating bacterial growth rates, we made single or mixed-nutrient addition experiments. We also examined the importance of organic matter supply for bacterial growth rates.

MATERIALS AND METHODS

Monthly monitoring on physicochemical and biological parameters

There are two major physical events in the Uwa Sea: *kyucho* and bottom intrusion both of which occur mainly in summer. The former is an intrusion of surface, warm, oligotrophic water from the south of the Bungo Channel to the west coast of Shikoku Island (Takeoka et al. 1993, Takeoka & Yoshimura 1988). Bottom intrusion consists of deep, cold, nutrient-rich water that flows just over the continental shelf (Kaneda et al. 2002 a & b, Takeoka et al. 2000), serving as the major nutrient input to the coastal areas (Koizumi 1991, Koizumi & Kohno 1994, Koizumi et al. 1997, Takeoka et al. 2000). While the effect of *kyucho* on phytoplankton growth is negligible, diatom blooms are observed to be promoted by nutrient inputs by bottom intrusion (Koizumi & Kohno 1994, Koizumi et al. 1997). Thus, the trophic status of the Uwa Sea is temporarily changed when a bottom intrusion happens, and the dominant biological matter cycling may be changed by a shift of the dominant food linkages from a microbial food web to a herbivorous food web due to the occurrence of bottom intrusion (Nakano et al. 2004).

The present study was conducted at the station Ub (Fig. 1, 33° 2` N, 132° 28` E; water depth, ca 53 m) in Uchiumi Bay located in Iegushi, Uchiumi Village, Ehime Prefecture, Japan (Nakano et al. 2004), which opens to the Bungo Channel within the

Uwa Sea (Fig. 1). The Channel is strongly affected by an oligotrophic Kuroshio front (Takeoka et al. 2000). There are no rivers or streams flowing into the Bay so there is negligible effect on water quality by nutrient loading of inflows. The sampling station, Ub was located in the center of a pearl oyster culture farm. Water samples were collected monthly using a 6 L Van-Dorn water sampler from 0, 2, 5, 10, 15, 20, 30, 40 and 50 m depths from June 2002 to June 2003. Vertical distribution of water temperature was determined with a Chlorotech profiler (Arec Electronics Co., ACL-208-DK). To monitor the occurrence of bottom intrusion, the water temperature was measured every 30 min with a thermistor chain at 2 and 60 m depths at the station Ut (Fig. 1).

A 100 ml portion of the water sample was filtered through a 0.2 μm Nuclepore filter, and the filtrate was used for measuring the concentration of dissolved inorganic nitrogen (DIN = NO_3^- + NO_2^- + NH_4^+) and soluble reactive phosphorus (SRP) using an AutoAnalyzer 3 (BRAN-LUEBBE co. Ltd.).

Another 100 ml portion of the water sample was filtered through a GF/F filter (Whatman) precombusted at 450 ºC for 2 hours, and the filtrate was used to measure the concentration of dissolved organic carbon (DOC) using a total carbon analyzer (TOC-V, Shimadzu Co. Ltd.). DOC measurements were conducted between February 2003 and

August 2003.

To determine chlorophyll *a* concentration, a 100 ml water sample was filtered through a 0.2 μm Nuclepore filter to retain seston. Each filter retaining seston was taken into a glass test tube, and 6.5 ml of N, N-dimethylformamide (DMF) was added to extract chlorophyll *a*. The amount of chlorophyll *a* thus extracted was determined using the fluorometric method (Moran & Porath 1980).

For enumeration of heterotrophic bacteria, a water sample (100 ml) was fixed with glutaraldehyde at a final concentration of 1%. Bacteria were stained with 4'6 – diamidino – 2 - phenylindole (DAPI) and filtered on black polycarbonate filters (0.2 μm pore size, Porter & Feig 1980). At least 300 bacterial cells, in most cases > 400 cells on the filter were counted using an epifluorecence microscope under UV excitation.

Nutrient amendment experiment

We used the dilution experiment for determining nutrient limitation on bacterial growth rates. The method proposed by Kirchman et al. (1982) and used for studies on nutrient limitation on bacterial growth in various environments (Felip et al. 1996, Middelboe et al. 2002, Morris & Lewis Jr 1992, Rivkin & Anderson 1997) was followed. Water sample collected from 10 m depth, where high abundances of phytoplankton and

bacteria have been previously observed (Ichinotsuka et al. 2006, Katano et al. 2004, Katano et al. 2005) was used. The sample was subjected to size-fractionations as follows: 0.8 μm Nuclepore filter for removing comsumers of bacteria such as protists under negative pressure at 0.05 MPa, and 0.2 μm Gelman culture capsule filter for removing all planktonic organisms using gravity. We discarded the first one liter of the filtrate of Gelman culture capsule filter to minimize the contamination of dissolved organic matter released from the filter. We diluted the < 0.8μm filtrate into the < 0.2 μm filtrate at a dilution level of 0.1, and 200 ml portions of the diluted water sample were poured into twenty 250 - ml polycarbonate bottles. In our microscopic observation, cell size of heterotrophic nanoflagellates was mostly 4 - 5 μm. Thus, our manipulation removed most protist cells from the seawater. Moreover, the removal of grazers and the dilution of the sample resulted in a reduction in the amount of grazing on bacteria. NH_4Cl (NH_4) as inorganic nitrogen, NaH_2PO_4 (PO_4) as inorganic phosphorus and glucose as organic matter were added to each of four bottles to attain final concentrations of 15 μmol N l^{-1}, 1 μmol P l^{-1} and 30 μmol C l^{-1}, respectively. Mixed supplements were also made. Thus, our experiment was carried out in four replications. In that NH_4 and PO_4 were added to four bottles. The remaining four bottles served as the control. The 20 bottles thus prepared were incubated at 10 m depth in the near shore

waters of Uchiumi Bay for 24 hours. A recent study on bacterial production in a marine environment has shown that, although the incubation duration of one day was enough to detect changes in bacterial production by the leucine uptake method, changes in bacterial abundance could not be discerned during the period (Pinhassi et al. 2006). However, we also conducted the experiments using 48 hours incubation to determine bacterial growth rate between June 2002 and February 2003, and found that the bacterial growth rates derived from 24 hours incubation were not significantly different from those of 48 hours incubation (data not shown). So, we decided to use bacterial growth rates determined in experiments of 24 hours incubation. At time zero and at the end of the incubation, subsamples for the enumeration of bacteria were taken and fixed as mentioned above. The growth rate, μ, was calculated from the equation, $\mu = \ln (N_f / N_0) / t$, where N_0 and N_f are cell densities at time zero and after 1 day of incubation, respectively, and t is the incubation period. One-way ANOVA was used to test for difference among treatments: subsequent multiple comparisons were performed using Tukey's test with a statistical significance level of $p < 0.05$.

Estimation of bacterial production

Bacterial production, BP (μg C l^{-1} d^{-1}), was calculated as follows:

$$BP = N\,(e^{\mu c}-1) \times V \times F$$

where N, bacterial counts (cells ml^{-1}); μc, mean bacterial growth rate in the control bottles; V, the bacterial cell volume of 0.146 μm^3 (Nagata & Watanabe 1990); and F, the C/V ratio of 120 fg C μm^{-3} (Nagata & Watanabe 1990).

RESULTS

From June to October 2002, we detected 11 times of occurrence of bottom intrusion (Fig. 2). Among our sampling dates, 02 July, 2 August, and 9 October 2002 were during the occurrence of bottom intrusion (Fig. 2, Table 1). Our CTD monitoring data revealed that bottom intrusion also occurred on 18 June 2003 (data not shown). From late August to mid September, water temperature at 2 m depth increased from 23.8˚C to 27.6˚C, indicating the occurrence of *kyucho*, which was intrusion of warm, oligotrophic surface water from the south of the Bungo Channel to the west coast of Shikoku Island (Takeoka et al. 1993, Takeoka & Yoshimura 1988). In Uchiumi Bay, thermal stratification usually develops between May and September (Ichinotsuka et al. 2006, Katano et al. 2004, Katano et al. 2005). This was also the case during the sampling period for this study, though thermal stratification in May 2003 was still minor (Fig. 3).

Thus, we can divide the study period into stratified (from May to September) and well mixed (from October to March) periods.

Seasonally changing patterns were found in concentrations of DIN and DIP (Fig. 3b, c). High concentrations of these nutrients were detected in 20-50 m depth in July, August and October when bottom intrusion occurred (Figs. 2 and 3b, c). In October 2002, nutrient concentrations throughout the water column were high: 2.44 to 6.67 μmol N l^{-1} (Fig. 3a), and 0.27 to 0.40 μmol P l^{-1} (Fig. 3b). Such high concentrations of DIN and DIP continued during mixing period from October to March. In September 2002, concentrations of these nutrients were low throughout the water column, due to the intrusion of *kyucho* (Fig. 2).

A high concentration of DOC was detected in February 2003 in 0-15 m depth (176.7 μmol C l^{-1}) and it decreased to 50.4 μmol C l^{-1} in April (Fig. 3d). The information about DOC concentration in the present study is limited. However, we continued to determine DOC concentration in 2003, 2004 and 2005 and observed no seasonal change in the vertical profile of DOC concentrations (unpublished observations).

Chlorophyll *a* concentrations ranged between 0.17 and 1.77μg l^{-1} during the investigation (Fig. 3e). The concentrations from August to December 2002 did not show

a clear vertical distribution, and cyclic changes between high and low concentrations were found from month to month (Fig.3e).

Bacterial cell counts ranged between 0.4 $\times 10^6$ cells ml^{-1} and 2.7 $\times 10^6$ cells ml^{-1} (Fig. 3f). Higher bacterial numbers were detected throughout the water column from June to September 2002 and from April to June 2003 (Fig. 3f). The period of high bacterial density roughly corresponded to the stratified period (Fig. 3a, f), otherwise the difference in bacterial density between 0-15m and 20-50m depth was not clear.

Bacterial growth rates (control treatment) at the 10 m depth in Uchiumi Bay ranged between 0.37 and 1.86 d^{-1} (Fig. 4) and showed a clear seasonal change with relatively high growth rates during the stratified period (Figs. 3a and 4). In most cases, nutrients or glucose addition did not have an effect on bacterial growth rates, and a statistically significant stimulation of bacterial growth rates was only detected in the experiment of phosphate addition in January 2003 (Table 1).

There was no significant correlation between bacterial growth rates and each environmental variable (water temperature and concentrations of DIN, DIP, and chlorophyll *a*) examined in the present study. Seasonally changing pattern of bacterial production was almost identical to that of bacterial growth rates (data not shown). We tried Billen's approach (Billen et al. 1990) to examine the relationship between cell

density and production of bacteria, and found a significant logarithmic relationship between them (Fig. 5, $r^2 = 0.78$, $p < 0.001$, $n = 13$, slope = 0.34).

DISCUSSION

Bacterial cell density is suggested to increase with the increase in bacterial production, implying that bacterial biomass at steady state is a direct function of substrate supply (Billen et al. 1990, Pace & Cole 1994). Previous studies have used Billen et al. (1990) to examine bottom-up control of bacterial biomass from the regression slope of bacterial biomass on bacterial production (Dufour & Torreton 1996, Pace & Cole 1994). Low slopes found in open ocean (e.g. 0.28) (Dufour & Torreton 1996) suggest that bottom-up control on bacterial biomass is weak, while high slopes found in productive waters (e.g. 0.7) (Billen et al. 1990) signify substantial bottom-up control on bacterial biomass. In the present study, the regression slope of bacterial cell density on bacterial production was found as 0.34, suggesting bottom-up control on bacterial abundance in Uchiumi Bay is weak relative to that of other waters (Billen et al. 1990, Pace & Cole 1994). In our previous study (Ichinotsuka et al. 2006), turnover rates of bacteria due to grazing by nanoflagellates and ciliates (1 to 16 % d^{-1}) were seasonally

high, indicating seasonal importance of top-down control on bacterial abundance. This may be reason why we detected weak bottom-up control on bacterial abundance in the bay.

Most bacterial production rates in previous studies have been determined by the ^{3}H-thymidine or ^{14}C-leucine incorporation methods (Choi et al. 2001, Lee et al. 2001, Riemann et al. 2002, Vaque et al. 2001). We used a bottle incubation method because radioactive tracers are restricted from use in natural environments in Japan. Since bacterial growth rates are within the range of bacterial growth rates of previous studies (Table 2), bacterial growth rates determined in Uchiumi bay are comparable to those in other environments.

Previous studies have demonstrated nutrient (Caron et al. 2000, Hoch & Bronk 2007, Pomeroy & Diebel 1986, Sala et al. 2002) and organic matter (Carlson et al. 2002, Caron et al. 2000, Flaten et al. 2003, Sala et al. 2002) limitation on growth of marine bacteria. Caron et al. (2000) reported that the supplement of glucose to a water sample from the eutrophic waters of Georges Bank stimulated bacterial growth. Whereas, bacterial growth in water samples from oligotrophic waters of the Sargasso Sea was enhanced by a supplement of inorganic nitrogen and phosphorus. Bacterial growth rates of the present study in almost all cases were not stimulated by nutrient or glucose

addition (Table 1), though the water in the present study is oligo- to mesotrophic. Hence, our results suggest that bacterial growth rates of Uchiumi Bay is nearly saturated at ambient levels of nutrients and/or organic matter.

The bacterial-growth saturation by inorganic nutrients and/or organic matter (Fig. 4) seems to contradict the bottom up control of bacteria abundance (Fig. 5). One explanation for this is inappropriate use of carbon source in the experiment. The use of glucose as supply of organic matter to marine bacteria needs some caution. Recent studies have demonstrated that not all marine bacteria use glucose (Alonso & Pernthaler 2006, Alonso-Saez & Gasol 2007, Malmstrom et al. 2005). In Uchiumi Bay, one of the dominant bacterial phylogenetic group throughout the year is *Cytophaga-Flavobacterium* (unpublished observations), and it has been reported that the bacteria included in the group show active uptake of glucose at high concentrations (Alonso & Pernthaler 2006). Since glucose was added at a high concentration (30 μmol C l^{-1}) in our nutrient amendment experiment, stimulation of bacterial growth rates would be expected. However, almost no response was found in bacterial growth rates (Table 1). Other organic substances, such as amino acids (Schweitzer & Simon 1995), might be stimulative for bacterial growth in Uchiumi Bay, though we did not examine them in the present study.

Our monthly monitoring data from 2001 to 2005 at the station showed significant positive relationship between bacterial cell density and chl. a concentration ($p < 0.05$, $n = 48$, data not shown). This supports that bacterial abundance highly depends on the primary production. Thus organic matters derived from exudates of phytoplankton possibly control bacterial growth rates.

The other explanation for the contradiction is temperature. In the present study, bacterial cell densities and bacterial growth rates tend to be high during stratification period (Figs. 3 and 4), although the relationships were not significant as mentioned above. Our monthly monitoring data showed similar trend; Bacterial cell density was high during the warm water season, although significant relationship was not detected (data not shown). Therefore, the other possible factor to control bacterial abundance is water temperature.

Cross system analyses using data from various marine and freshwater ecosystems showed that bacterial abundance had a positive correlation with chlorophyll *a* concentration (Bird & Kalff 1984, Cole et al. 1988, Sommaruga & Robarts 1997). These findings suggest that bacterial abundance was mainly supported by DOC derived from phytoplankton. However, seasonal changes in vertical bacterial density of the present study (Fig. 4B) were different from those of chlorophyll *a* concentration (Fig. 4A) and

DOC concentrations, and we did not find any significant relationship between bacterial growth rates and chlorophyll *a* concentrations. These results suggest that DOC supply by phytoplankton was of minor importance for bacterial density in Uchiumi Bay.

Our previous study indicated that phytoplankton in Uchiumi Bay was subjected to systemic and/or growth rate limitations by N, P or Si (Hashimoto & Nakano 2003). Hence, we expected bacterial growth rates would have been stimulated by the addition of nutrients or organic matter. However, this was not the case (Table 1). In Uchiumi Bay, differences in the response to nutrient supply between large and small phytoplankton have been reported (Nakano et al. 2004). Major nutrient supply to the Bay is primarily due to bottom intrusion (Takeoka et al. 2000), and the high abundance of larger phytoplankton such as diatoms is detected when nutrients are supplied by bottom intrusion (Nakano et al. 2004). In contrast, the abundance of small phytoplankton such as picophytoplankton is high during warmer water temperature, independent of nutrient supply, due to the occurrence of bottom intrusion (Nakano et al. 2004). The responses of heterotrophic bacteria to nutrient supply or temperature changes have been observed to be similar to that of picophytoplankton (Nakano et al. 2004). It is likely that bacterial growth rates in Uchiumi Bay is nearly saturated at ambient levels of nutrients and/or organic matter, and that bacterial abundance is subject to relatively weak bottom-up

control. Although we did not find any significant relationship between temperature and bacterial abundance or the growth rates, bacterial abundance seasonally high during the thermally stratified period (Fig. 3 and our unpublished data). Thus, temperature possibly regulates them. Although we did not evaluate other organic matter for the experiment than glucose, probably other organic matter is a factor to regulate the bacterial abundance. Effect of temperature and other organic substarete should be further investigated to understand the bacterial dynamics in the sea.

Acknowledgements

We thank K. Hyodo, T. Hirose and the staff of Ainan Institute of Oceanic and Fishery Center, and the students of Ehime University, for their help in field monitoring. Thanks are also due to Prof. Suzuki and other members of the Aquatic Biology and Ecology Laboratory of CMES, Ehime University, for their advice, discussions and encouragement throughout the study. We thank Dr. Todd W. Miller for correction of our English and constructive comments on the manuscript. The present study was partly supported by the Grant-in-Aid for Scientific Research No. 16201004, JSPS, and the Research Fund of coastal environment in Uchiumi Bay, Ainan Town, Ehime Prefecture.

References

Alonso C, Pernthaler J (2006) Concentration-dependent patterns of leucine incorporation by coastal picoplankton. Appl Environ Microbiol 72: 2141-2147

Alonso-Saez L, Gasol JM (2007) Seasonal variations in the contributions of different bacterial groups to the uptake of low-molecular-weight compounds in Northwestern Mediterranean coastal waters. Appl Environ Microbiol 73: 3528-3535

Azam F, Fenchel T, Field JG, Gray JS, Meyer-Reil LA, Thingstad F (1983) The ecological role of water-column microbes in the sea. Mar Ecol Prog Ser 10: 257-263

Billen G, Servais P, Becquevort S (1990) Dynamics of bacterioplankton in oligotrophic and eutrophic aquatic environments: bottom-up or top-down control? Hydrobiologia 207: 37-42

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

Carlson CA, Giovannoni SJ, Hansell DA, Goldberg SJ, Parsons R, Otero MP, Vergin K, Wheeler BR (2002) Effect of nutrient amendments on bacterioplankton production, community structure, and DOC utilization in the northwestern Sargasso Sea. Aquat

Microb Ecol 30:19-36

Caron DA, Lim EL, Sanders RW, Dennet MR, Berninger UG (2000) Responses of bacterioplankton and phytoplankton to organic carbon and inorganic nutrient additions in contrasting oceanic ecosystems. Aquat Microb Ecol 22:175-184

Center for Marine Environmental Studies, Ehime University, 2003. http://ccserv.adm.ehime-u.ac.jp/~cmes/database/kako-suion.htm

Cho BC, Azam F (1990) Biogeochemical significance of bacterial biomass in the ocean's euphotic zone. Mar Ecol Prog Ser 63:253-259

Choi KH, Dobbs FC, Cowen RK (2001) Short-term temporal and spatial dynamics of bacterioplankton near Barbados in the Caribbean Sea. Aquat Microb Ecol 25:43–53

Church MJ, Hutchins DA, Ducklow HW (2000) Limitation of Bacterial Growth by Dissolved Organic Matter and Iron in the Southern Ocean. Appl Environ Microbiol 66:455-466

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

Dufour PH, Torreton JP (1996) Bottom-up and top-down control of bacterioplankton from eutrophic to oligotrophic sites in the tropical northeastern Atlantic Ocean. Deep-Sea Res I 43: 1305-1320

Elser J, Stabler L, Hassett R (1995) Nutrient limitation of bacterial growth and rates of bacterivory in lakes and oceans: a comparative study. Aquat Microb Ecol 09:105-110

Felip M, Pace ML, Cole JJ (1996) Regulation of planktonic bacterial growth rates: the effects of temperature and resources. Microb Ecol 31: 15-28

Flaten GAF, Castberg T, Tanaka T, Thingstad TF (2003) Interpretation of nutrient-enrichment bioassays by looking at sub-populations in a marine bacterial community. Aquat Microb Ecol 33:11-18

Geller W, Berberovic R, Gaedke U, Muller H, Pauli HR, Tilzer MM, Weisse T (1991) Relationship among the components of autotrophic and heterotrophic plankton during the seasonal cycle 1987 in Lake Constance. Verh Internat Verein Limnol 24: 831-836

Goldman JC, Dennett MR (1991) Ammonium regeneration and carbon utilization by marine bacteria grown on mixed substrates. Mar Biol 109:369-378

Hamels I, Muylaert K, Casteleyn G, Vyverman W (2001) Uncoupling of bacterial production and flagellate grazing in aquatic sediments: a case study from an intertidal flat. Aquat Microb Ecol 25:31–42

Hashimoto T, Nakano S (2003) Effect of nutrient limitation on abundance and growth of

phytoplankton in a Japanese pearl farm. Mar Ecol Prog Ser 29:43-50

Hoch MP, Bronk DA (2007) Bacterioplankton nutrient metabolism in the Eastern Tropical North Pacific. J Exp Mar Biol Ecol 349:390-404

Ichinotuska D, Ueno H, Nakano S (2006) The relative importance of nanoflagellates and ciliates as consumers of bacteria in a coastal sea area (Japan), where the oligotrichous *Strombidium* spp. and *Strobilidium* spp. dominate. Aquat Microb Ecol 42:139-147

Jansson M, Blomqvist P, Jonsson A, Bergstroem A-K (1996) Nutrient limitation of bacterioplankton, autotrophic and mixotrophic phytoplankton, and heterotrophic nanoflagellates in Lake Oertraesket. Limnol Oceanogr 41:1552-1559

Kaneda A, Takeoka H, Koizumi Y (2002a) Periodic occurrence of diurnal signal of ADCP backscatter strength in Uchiumi Bay, Japan. Est Coast Shelf Sci 55:323-330

Kaneda A, Takeoka H, Nagata E, Koizumi Y (2002b) Periodic intrusion of cold water from the Pacific Ocean into the bottom layer of the Bungo Channel, Japan. J Oceanogr 58:547-556

Katano T, Hirose M, Nakano S (2004) Discrimination between two phycoerythrin-pigment types of *Synechococcus* and their seasonal succession in the Uwa Sea. Microb Environ 19:7-12

Katano T, Kaneda A, Takeoka H, Nakano S (2005) Seasonal changes in abundance and composition of picophytoplankton in relation to occurrence of *kyucho* and bottom intrusion in Uchiumi Bay, Japan. Mar Ecol Prog Ser 298:59-67

Kawabata K, Nakanishi M (1996) Food web structure and biodiversity in lake ecosystems. In T. Abe S. Levin and M. Higashi (eds.), Biodiversity: An ecological perspective. Pp. 202-213. Springer, New York.

Kirchman DL Ducklow DH, Mitchell R (1982) Estimates of bacterial growth from changes in uptake rates and biomass. Appl Environ Microbiol 44: 1296-1307

Koizumi Y (1991) A process of water exchange in Shitaba Bay during the phenomenon of *kyucho*. Bull Coasat Oceanogr 29:82-90

Koizumi Y, Kohno Y (1994) An influence of the *kyucho* on a mechanism of diatom growth in Sitaba Bay in summer. Engankaiyou Kenkyu Note 32:81-89

Koizumi Y, Nishikawa S, Yakushiji F, Uchida T (1997) Germination of resting stage cells and growth of vegetative cells in diatoms caused by *kyucho* events. Suisankaiyou Kenkyuu 61:275-287 (in Japanese with English abstract)

Lee CW, Kudo I, Yanada M, Maita Y (2001) Bacterial abundance and production and heterotrophic nanoflagellate abundance in subarctic coastal waters (Western North Pacific Ocean). Aquat Microb Ecol 23:263–271

Malmstrom RR, Cottrell MT, Elifantz J, Kirchman DL (2005) Biomass Production and Assimilation of Dissolved Organic Matter by SAR11 Bacteria in the Northwest Atlantic Ocean. Appl Environ Microbiol 71: 2979-2986

Middelboe M, Nielsen TG, Bjornsen PK (2002) Viral and bacterial production in the North Water: *in situ* measurements, batch-culture experiments and characterization and distribution of a virus-host system. Deep-Sea Res II 49: 5063-5079

Moran R, Porath D (1980) Chlorophyll determination in intact tissues using *N*,*N*-dimethylformamide. Plant Physiol 65:478-479

Morris DP, Lewis Jr WM (1992) Nutrient limitation of bacterioplankton growth in Lake Dillon, Colorado. Limnol Oceanogr 37: 1179-1192

Nagata T, Watanabe Y (1990) Carbon- and nitrogen-to-volume ratios of bacterioplankton grown under different nutritional conditions. Appl Environ Microbiol 56: 1303-1309

Nakano S, Tomaru Y, Katano T, Kaneda A, Makino W, Nishibe Y, Hirose M, Onji M, Kitamura SI, Takeoka H (2004) The dynamics of microbial and herbivorous food webs in a coastal sea with special reference to intermittent nutrient supply from bottom intrusion. Aquat Ecol 38:485-493

Pace ML (1988) Bacterial mortality and the fate of bacterial production. Hydrobiologia

159:41-49

Pace ML, Cole JJ (1994) Comparative and experimental approaches to top-down and bottom up regulation of bacteria. Microb Ecol 28: 181-193

Pinhassi J, Gomez-Consarnau L, Alonso-Saez L, Sala MM, Vidal M, Pedros-Alio C, Gasol. JM (2006) Seasonal changes in bacterioplankton nutrient limitation and their effects on bacterial community composition in the NW Mediterranean Sea. Aquat Microb Ecol 44: 241-252

Pomeroy LR, Deibel D (1986) Temperature regulation of bacterial activity during the spring bloom in Newfoundland coastal waters. Science 233:359-361

Porter KG, Feig YS (1980) The use of DAPI for identifying and counting aquatic microflora. Limnol Oceanogr 25:943-479

Riemann L, Steward GF, Azam F (2002) Dynamics of bacterial community composition and activity during a mesocosm diatom bloom. Appl Environ Microbiol 66:578-587

Rivkin RB, Anderson MR (1997) Inorganic nutrient limitation of oceanic bacterioplankton. Limnol Oceanogr 42:730-740

Sala MM, Peters F, Gasol JM, Pedrós-Alió C, Marrasé C, Vaqué D (2002) Seasonal and spatial variations in the nutrient limitation of bacterioplankton growth in the northwestern Mediterranean. Aquat Microb Ecol 27:47-56

Schweitzer and Simon (1995) Growth limitation of planktonic bacteria in a large mesotrophic lake. Microb Ecol 30:89-104

Sherr EB, Sherr BF, Cowles TJ (2001) Mesoscale variability in bacterial activity in the Northeast Pacific Ocean off Oregon, USA. Aquat Microb Ecol 25:21-30

Simon M, Cho BC, Azam F (1992) Significance of bacterial biomass in lakes and the ocean: comparison to phytoplankton biomass and biogeochemical implications. Mar Ecol Prog Ser 86:103-110

Sommaruga R, Robarts RD (1997) The significance of autotrophic and heterotrophic picoplankton in hypertrophic ecosystems. FEMS Microbiol Ecol 24:187-200

Takeoka H, Akiyama H, Kikuchi T (1993) The *kyucho* in the Bungo Channel, Japan–Periodic intrusion of oceanic warm water. J Oceanogr 49:369-382

Takeoka H, Koizumi Y, Kaneda A (2000) Year-to-year variation of a *kyucho* and a bottom intrusion in the Bungo Channel, Japan. In Yanagi T (eds) Interactions between Estuaries, Coastal Seas and Shelf Seas. Terra Scientific Publishing company, Tokyo, Japan p 197-215.

Takeoka H, Yoshimura T (1988) The *kyucho* in Uwajima Bay. J Oceanogr 44:6-16

Tezuka Y (1990) Bacterial regeneration of ammonium and phosphate as affected by the carbon:nitrogen:phosphorus ratio of organic substrates. Microb Ecol 19: 227-238

Tomaru Y, Udaka N, Kawabata Z, Nakano S (2002) Seasonal change of seston size distribution and phytoplankton composition in bivalve pearl oyster *Pinctada fucata martensii* culture farm. Hydrobiologia 481:181-185

Vaqué D, Casamayor EO, Gasol JM (2001) Dynamics of whole community bacterial production and grazing losses in seawater incubations as related to the changes in the proportions of bacteria with different DNA content. Aquat Microb Ecol 25:163–177

White PA, Kalff J, Rasussen JB, Gasol JM (1991) The effect of temperature and algal biomass on bacterial production and specific growth rate in freshwater and marine habitats. Microb Ecol 21:99-118

Figure Captions

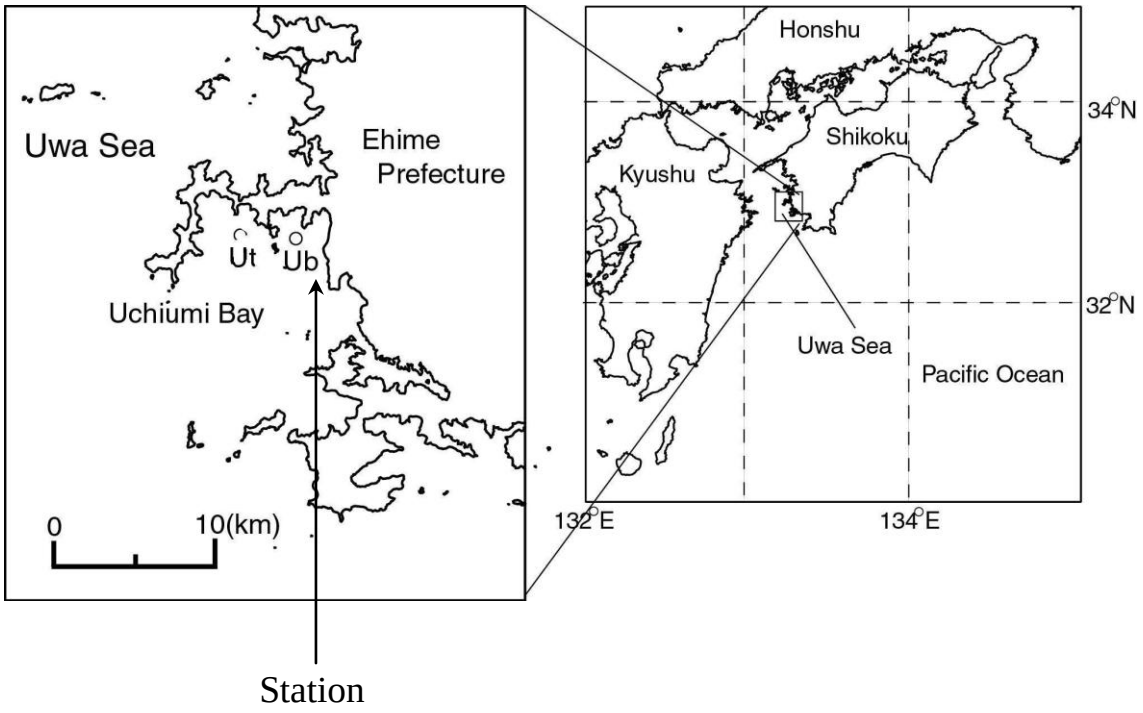
Fig.1. Locations of the sampling station (Stn. Ub) and temperature monitoring station (Stn. Ut) in Uchiumi Bay.

Fig.2. Changes in water temperature at 2- and 60 m depths at Stn. Ut. Arrows with characters indicate the occurrence of bottom intrusion.

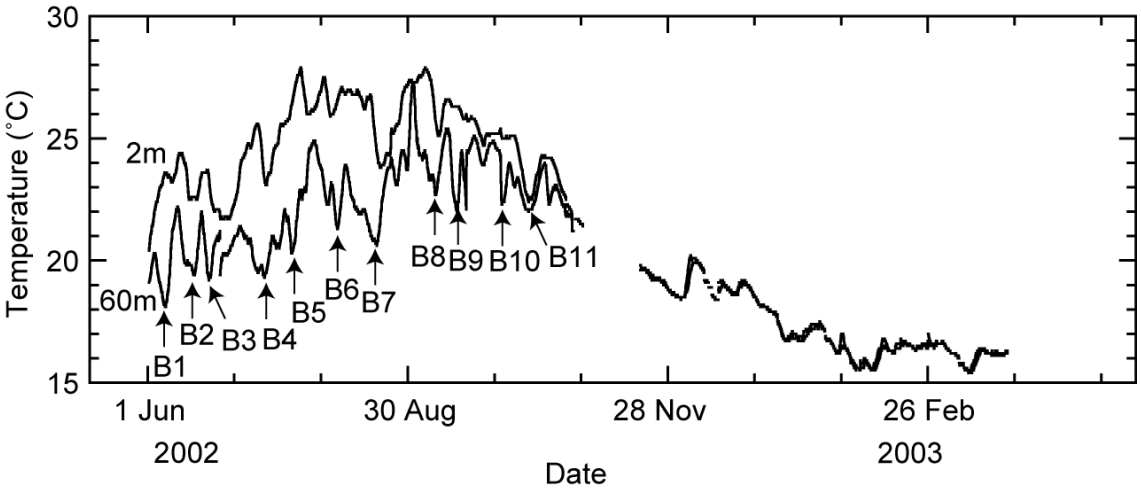
Fig.3. Seasonal changes in water temperature (panel a), dissolved inorganic nitrogen concentrations (DIN, panel b), dissolved inorganic phosphorus concentrations (DIP, panel c), dissolved organic carbon concentrations (DOC, panel d), chlorophyll *a* concentrations (chl. *a*, panel e), and bacterial cell densities (panel f),. White and black triangles in panel a show data from 0- and 50 m depths, respectively, and white circles and white squares in panels b, c, d, e, f, and g show mean values of samples collected from 0 – 15 m depth and 20 - 50 m depth, respectively.

Fig.4. Growth rates of bacteria obtained in the nutrient amendment experiments. Bars and error bars indicate mean values and standard deviations for four replications, respectively. Asterisk (*) on the phosphate treatment in January shows significant difference ($p < 0.05$, Tukey's test after one-way ANOVA) among the treatment in January.

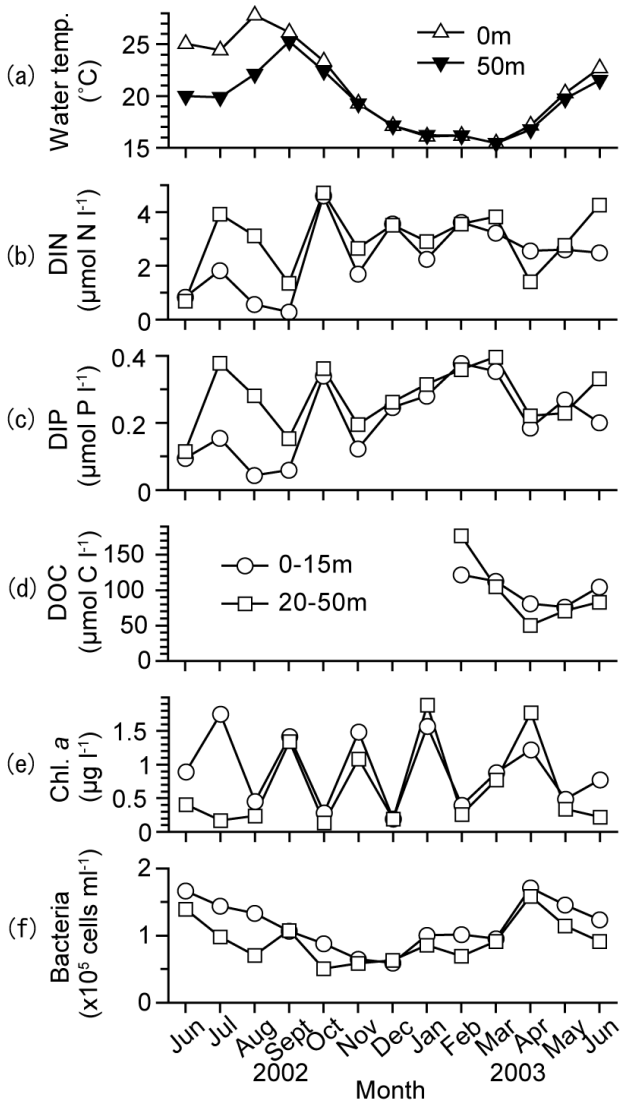
Fig.5. Logarithmic relationship between cell density and production of bacteria from June 2002 to June 2003 in Uchiumi Bay. It should be noted that the bacterial production was calculated from the growth rate and the cell density.



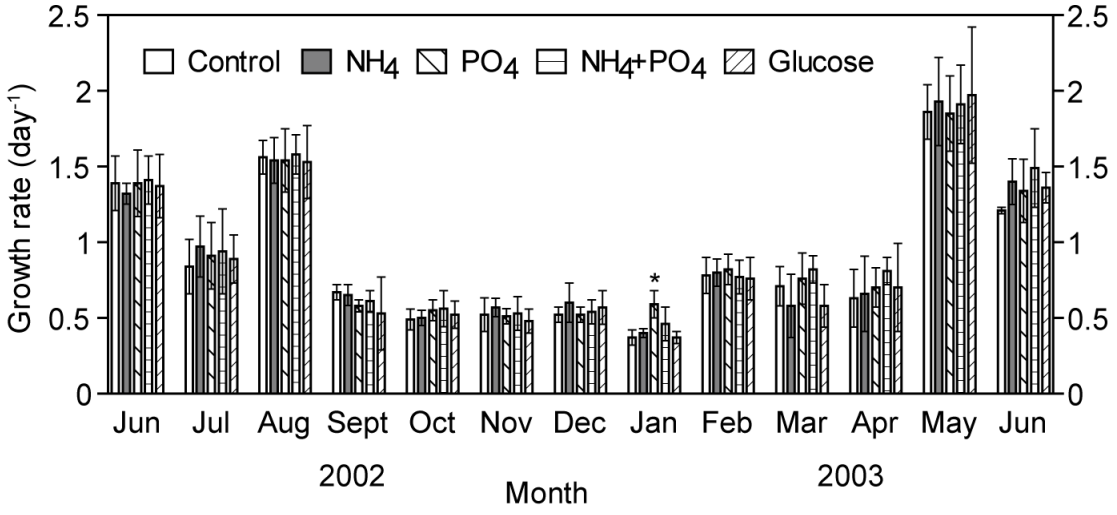
Ichinotsuka et al. Fig. 1



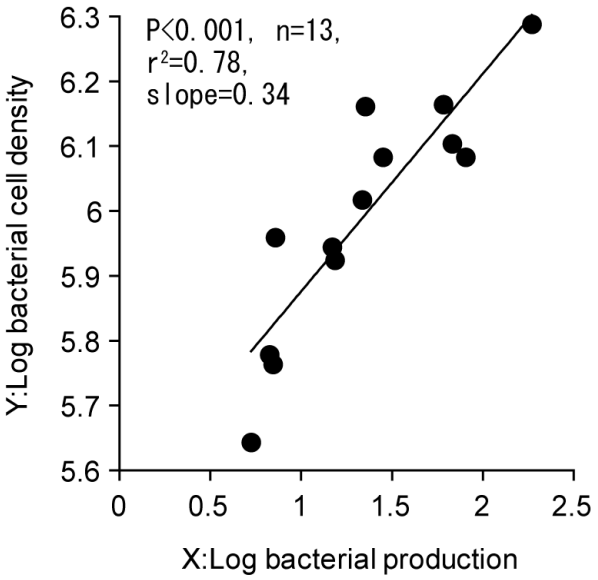
Ichinotsuka et al. Fig. 2



Ichinotsuka et al. Fig. 3



Ichinotsuka et al. Fig. 4



Ichinotsuka et al. Fig. 5

Table 1 Sampling dates and the days after the bottom intrusion.

Date	Days after the bottom intrusion	Event number*[2]
13 Jun 02	-	
12 Jul 02	1	B4
2 Aug 02	0	B6
11 Sep 02	-	
9 Oct 02	0	B11
8 Nov 02	ND*[1]	
10 Dec 02	-	
17 Jan 03	-	
13 Feb 03	-	
12 Mar 03	-	
16 Apr 03	ND*[1]	
20 May 03	ND*[1]	
18 Jun 03	0	-*[3]

*[1]ND, not determined

*[2] cf. Fig. 1

*[3] Identified with other unpublished data

1

Table 2 Growth rates of bacteria in the literature and the present study.

Location	Growth rate (d^{-1})	Source
Catalano Balearic Basin, Mediterranean Sea	0.19 - 1.54	Sala et al. (2002)
Funka Bay	0.02 - 2.57	Lee et al. (2001)
NW Mediterranean Sea	0.01 - 0,35	Vaque et al. (2001)
Oregon Coast	0.01 - 0.31	Sherr et al. (2001)
Schelde estuary	0.1 - 1.2	Hamels et al. (2001)
Scripps Pier	0.3 - 2.2	Riemann et al. (2002)
Southern Ocean	0.02 - 0.78	Church et al. (2000)
West of Barbados, Caribbean Sea	0.16 - 1.46	Choi et al. (2001)
Uchiumi Bay	0.37 - 1.86	This study

2