

Different interrelationships among phytoplankton, bacterial and environmental variables in dumping and reference areas in the East Sea

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ABSTRACT: Approximately several hundred million tons of waste, composed mostly of dredged materials and sewage sludge, are disposed annually in the world's ocean; millions of tons of waste are dumped yearly into the East Sea, Korea. To assess the impacts of such dumping on bacteria and phytoplankton in the pelagic ecosystem, and to understand the mechanisms behind any observed changes, environmental, bacterial, and phytoplankton variables in dumping and reference areas in the East Sea were measured 10 times from January 1996 to August 1998. The effects of waste on bacteria and phytoplankton were also tested *in vitro* by directly adding various types of waste to seawater. Physicochemical characteristics such as temperature, salinity, and concentrations of ammonium, nitrate, and phosphate were generally similar between dumping and reference areas; similar values were observed in bacterial biomass and chlorophyll *a* concentrations between the 2 areas. However, higher or lower bacterial production (BP) and lower primary production (PP) were frequently observed in dumping areas compared to reference sites. Microbial ecological relationships (e.g. sea surface temperature vs. BP/PP ratio, and nitrate concentration vs. PP) were significant at the reference sites but not in the dumping areas. Comparisons of values and relationships of the environmental, phytoplankton, and bacterial variables between dumping and reference sites suggested that lower BP and PP in dumping areas might be due to the disposal of inhibitory substances. This inference seemed to be supported by the waste-addition experiments, in which thymidine incorporation rates were inhibited at concentrations >1% of human and animal wastes and acid waste, and aminopeptidase activity was inhibited at concentrations >1% of human and animal wastes and >5% of acid waste. Thus, continual disposal of waste might impose inhibitory effects on activities of phytoplankton and bacteria in surface waters of the dumping area, but also stimulatory effects on bacteria in more diluted waters. Dumping of organic waste in the ocean has the potential to affect functions of the marine ecosystem at the bottom level of microbial food webs.

KEY WORDS: Ocean dumping · Organic waste · Bacterial production · Primary production · Aminopeptidase · β -glucosidase · East Sea

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INTRODUCTION

Oceans have been utilized as disposal sites of various anthropogenic waste (Kennish 1998), which continued without any international regulations until the early 1970s. Thereafter, the London Convention came

into force and since 1975 has existed as the framework to control all sources of marine pollution and minimize pollution of the sea through the regulation of dumping of waste materials. Nevertheless, a huge amount of waste (hundreds of millions of tons per year), composed mostly of dredged materials, sewage sludge,

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and low level radioactive waste, has been disposed of in the world's ocean (Kennish 1998, IMO 2002). In Korea, the dumping of non-toxic water-soluble or organic waste has been permitted in assigned areas since 1988; thereafter the amount of waste disposed of at 3 dumping sites in the Yellow Sea and East Sea has increased steadily from 552 kt in 1988 to 5188 kt in 1996 (Ministry of Environment 1998).

Disposed wastes can release considerable quantities of nutrients, pollutants, and other substances into marine environments, and consequently may affect the chemical and biological environment of the dumping area. The dumping of organic wastes into the ocean provides new organic matter and nutrients to microorganisms living in the pelagic environment. The allochthonous organic matter might have a different composition to organic matter derived from phytoplankton (Rieley et al. 1997), and a bulk C/N ratio that was higher than the Redfield ratio was reported in sewage sludge by Li et al. (2001). In addition, chemical contaminants (e.g. halogenated hydrocarbon compounds, polycyclic aromatic hydrocarbon, and heavy metal) and other substances derived from hazardous wastes pose a potential danger to marine communities (Kennish 1998). Thus, it would be expected that the growth of bacteria and phytoplankton might be affected.

To date, most studies on the impacts of ocean dumping were carried out on sediments of dumping sites, and mainly examined the distribution of toxic chemical compounds, benthic organisms (Kennish 1998, Friedman et al. 2000, Stronkhorst et al. 2003), and sewage contamination-indicator bacteria (Hill et al. 1993, 1996). For years, benthic infauna has been an important element in monitoring the biological effects of pollutants, because they are sensitive to habitat disturbance related to waste disposal. However, the impacts of waste dumping on pelagic microorganisms have not been investigated extensively (Peele et al. 1981, Cavari & Colwell 1988, Himelbloom & Stevens 1994), even though surface water is the first environment exposed to the wastes and is important in the production of organic matter and transfer of materials through food webs.

In assessing the effect of waste dumping on pelagic microbes, it would be most straightforward to make direct comparisons of measured quantitative and qualitative microbial parameters between dumping and reference areas. However, identical reference environments that have the same natural conditions and biological compositions and activities as dumping sites are rarely found in the ocean. In reality, natural variations of marine microbial variables in space (Choi et al. 2001) would complicate the detection of waste-induced changes between 2 sites. Alternatively, the

effects of dumping materials on phytoplankton and bacterial activities can be directly measured in a mesocosm. The nature of confinement of the mesocosm may not adequately represent processes occurring *in situ*, such as continual dilution of the dumped material with seawater due to turbulent mixing, diffusion, and convection. Nonetheless, important insights into the effects of waste on pelagic microbes may be gained through mesocosm experiments.

As marine microbial ecology progresses, the responses of phytoplankton and bacteria to nutrients, organic matter, and diverse pollutants (Hudak & Fuhrman 1988, Kirchman 1990, Riemann & Lindgaard-Jørgensen 1990, Gustavson et al. 1999, Choi et al. 2002) have been reported from various marine environments. Several empirical relationships among environmental, phytoplankton, and bacterial variables have also been described (Cole et al. 1988, Falkowski & Woodhead 1992, Shiah & Ducklow 1994a,b, Kirchman & Rich 1997, Cho et al. 2001, Hoppe et al. 2002). Thus, if allochthonous organic matter or pollutants were to have certain effects on bacteria and/or phytoplankton, we could detect changes in their responses and interrelationships. For example, ecologically relevant relationships like temperature versus bacterial production are broken in coastal areas affected by thermal effluents (Choi et al. 2002). We analyzed microbial variables in reference and dumping sites, and compared relationships between the 2 types of sites, in order to gain insights into the effects of the dumping of organic waste into the ocean on marine microorganisms. Further, we examined the responses of bacteria and phytoplankton to various dilutions of dumping materials.

MATERIALS AND METHODS

Study area and sample collection. There are 2 waste disposal sites in the East Sea (Fig. 1): 'Byung' (ca. 3500 km² and 1500 m deep; 'B'), and 'Jeong' (ca. 1800 km² and 150 m deep; 'J'). This region is characterized by the strong north or northeastward flow of the Tsushima Warm Current that varies in strength with season (strongest in October and weakest in January) (Chang et al. 2004). The seasonal thermocline is found between 50 and 150 m depth (Ministry of Environment 1998). By the Asian winter monsoon, northwesterly winds prevail in the region with strong seasonal and synoptic band variations (Nam et al. 2005). In general, the direction of mean current and local winds is uniform over the study area. The amount of waste disposed at the 2 dumping sites exponentially increased from 5 kt in 1988 to 2.4 Mt in 1994, and then the rate slowed till 1998. From 1998, the amount steadily

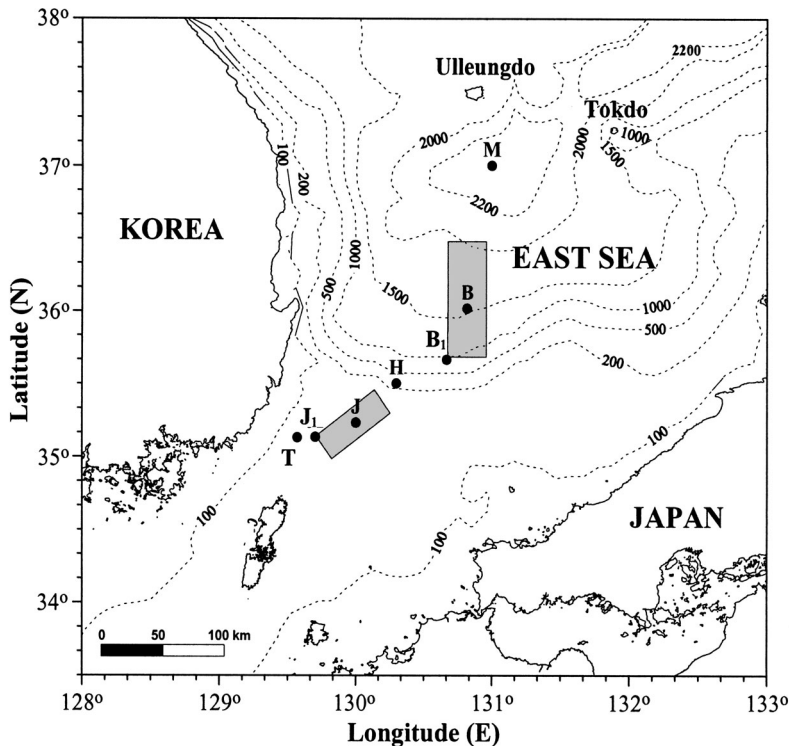


Fig. 1. Study area and sampling stations in the East Sea. Shaded areas indicate dumping areas (B: Byung, J: Jeong). Depth contour lines shown in meters

increased to 6 Mt in 2002 (Fig. 2). During the study period 1996 to 1998, the annual amount of waste disposed into the Jeong area was on average 1410 kt, and the waste was composed mostly of human and animal wastes (ca. 92%), waste water (7%), and sludge from waste water (1%). In the Byung area, an annual average of 2151 kt was dumped, comprising sludge from waste water (54%), acid waste (11%), human and animal wastes and waste water (9%), sewage sludge (8%), and inorganic sludge (7%). During the study period, ocean dumping was conducted by ca. 30 vessels of 200 to 1600 t, and their annual navigation frequency was ca. 3000 times (Ministry of Environment 1998). During 10 cruises in the East Sea (Fig. 1), seawater samples from reference and dumping sites were collected with 10 l Niskin bottles on a CTD-rosette sampler. Stn H and Stn M were chosen as reference stations for Byung (Stn B and Stn B₁) and Jeong (Stn J and Stn J₁), respectively. Each sample was analyzed for bacterial abundance and production, enzyme activity, primary production, and chemical composition.

Bacterial abundance and production. Detailed descriptions of the methods used to determine bacterial abundance and production can be found in Cho et al. (2001) and Choi et al. (2003). Briefly, bacteria, stained with DAPI (4',6-diamidino-2-phenyl-indole;

final concentration $1 \mu\text{g ml}^{-1}$) and collected on $0.2 \mu\text{m}$ polycarbonate black filters, were counted under UV excitation using an epifluorescence microscope (Porter & Feig 1980). Biovolumes of bacteria were determined using microphotographs and projecting slides onto a paper screen. Projected slides of fluorescent beads of known diameter (0.4 and $1.0 \mu\text{m}$, Polysciences) were used for calibration (Moran et al. 1991). Bacterial production was measured using the [methyl- ^3H] thymidine incorporation method (Ducklow et al. 1992). Triplicates of 10 ml samples were put in sterile polypropylene tubes, and [methyl- ^3H] thymidine (specific activity of 80 to 84 Ci mmol^{-1}) was added to the tubes at 10 nM (final concentration). The tubes were incubated at *in situ* water temperature in the dark for ca. 1 to 2 h depending on activity. Extraction and radioassay procedures were performed in accordance with Choi et al. (2003). The incorporated radioactivity was converted to cell number produced using a conversion factor of 2.65×10^{18} cells mol^{-1} thymidine incorporated (Ducklow et al. 1992). Bacterial carbon biomass (BOC) was calculated using biovolumes in accordance with Simon & Azam (1989).

Primary production. Primary productivity (PP) was measured using ^{14}C -bicarbonate in accordance with Parsons et al. (1984). Water samples for production measurements were collected at 3 to 6 depths within the euphotic zone. The euphotic depth was determined by multiplying the Secchi depth by 2.7. Detailed descriptions of the method used to determine PP can be found in Cho et al. (2001). Since the incubation of

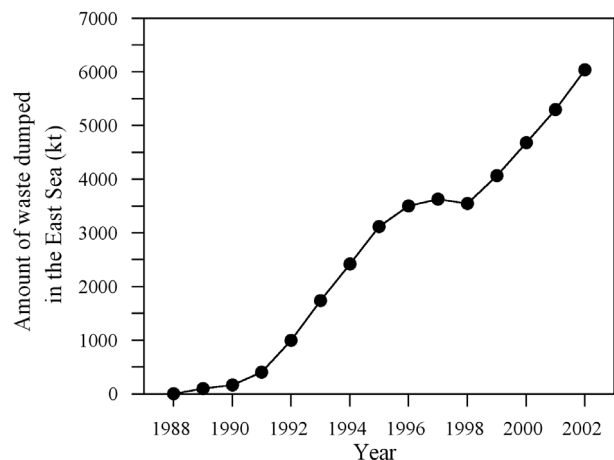


Fig. 2. Yearly variations in amount of waste dumped into the East Sea. Data from Ministry of Environment, Republic of Korea

samples was conducted in an on-deck incubator circulated with surface seawater, PP was corrected for differences between *in situ* and incubation temperatures (for detailed description see Cho et al. 2001). When corrected for temperature, PP decreased by $9 \pm 5\%$ (mean ± 1 SD) in May and July 1996 and July 1997. However, in the other investigations the decreases were $<2\%$. Daily PP was calculated as hourly productivity value \times (daily incident irradiance/incident irradiance during incubation).

Aminopeptidase and β -glucosidase activities. To estimate activities of aminopeptidase and β -glucosidase, we used fluorogenic substrate analogs 4-methylumbelliferyl- β -D-glucopyranoside (MUF-Glc) and L-leucine-4-methyl-coumarinyl amide (Leu-MCA), respectively (Hoppe 1983, Martinez & Azam 1993). Fluorescence of samples supplied with each substrate analog was measured before and after incubation in the dark at *in situ* temperature. The final concentrations of added substrates were 200 μ M for Leu-MCA, and 250 μ M for MUF-Glc. At these substrate concentrations, enzyme activity was nearly saturated (Song et al. 1999). We used 4-methylumbelliferone as a standard for MUF-Glc, and 7-amino-4-methylcoumarin as a standard for Leu-MCA. Fluorescence was measured at 365 nm excitation and 460 nm emission wavelengths using a TKO 100 fluorometer (Hoefer Scientific Instruments).

Waste-addition experiments. To assess the effects of waste on bacterial activities in seawater, dilution experiments of human and animal wastes and acid waste were carried out with surface water from Stn T close to Stn J₁ (Fig. 1) in August 1998. In this experiment, each waste sample was initially diluted with seawater 20- and 100-fold. Then, 100 ml of 100-fold diluted samples were subsequently diluted to 2000-fold by adding seawater at a flow rate of ca. 4 ml min⁻¹ for ca. 8 h. Using a similar procedure, 20 000- and 100 000-fold diluted samples were prepared for ca. 5 h. In each diluted sample and the control (seawater used for the dilution), bacterial abundance and production and aminopeptidase activity were measured. The concentration of [³H]thymidine added for measurement of bacterial production in both control and diluted samples was increased to 50 nM (final concentration) to reduce the isotope dilution.

Environmental variables and statistical analyses.

Water temperature and salinity were measured with a CTD system (SBE-911) mounted on a rosette sampler. For measurements of nutrients, seawater samples were filtered through Whatman GF/F filters, frozen at -20°C , and brought to the laboratory. Ammonium, nitrate and phosphate concentrations were determined using the method of Parsons et al. (1984). Concentrations of chlorophyll *a* (chl *a*) were measured spectrophotometrically in accordance with Parsons et al.

Table 1. Summary of environmental, bacterial and phytoplankton variables in reference (Stns M and H) and dumping (Stns J₁, B and B₁) sites. BCB: bacterial carbon biomass; Chl *a*: chlorophyll *a*; AMPase: aminopeptidase activities; β -GLCase: β -glucosidase activities; BP: bacterial production; PP: primary production. All data except euphotic depth were integrated within the euphotic zone

	Euphotic depth (m)	NH ₄ (mmol m ⁻²)	NO ₃ (mmol m ⁻²)	PO ₄ (mmol m ⁻²)	Chl <i>a</i> (mg m ⁻²)	BCB (mg C m ⁻²)	AMPase (μ mol m ⁻² h ⁻¹)	β -GLCase (μ mol m ⁻² h ⁻¹)	BP (mg C m ⁻² d ⁻¹)	PP (mg C m ⁻² d ⁻¹)
Reference										
Range	16–81	0.5–22.0	1.9–242.0	0.5–20.8	1.3–30.2	178.7–975.4	211.2–870.7	29.9–50.3	10.9–185.4	58.1–2247.5
Mean \pm 1 SD	38 \pm 18	13.6 \pm 8.3	91.3 \pm 82.7	9.3 \pm 7.5	12.8 \pm 8.5	535.5 \pm 242.9	420.2 \pm 194.4	40.0 \pm 8.7	67.8 \pm 62.3	939.1 \pm 746.9
n	9	13	13	13	13	13	9	5	12	8
Dumping										
Range	16–70	3.5–96.9	0.5–267.4	0.2–24.8	1.1–26.8	149.0–1389.5	98.8–1095.2	22.7–84.0	4.2–261.7	96.5–560.4
Mean \pm 1 SD	37 \pm 12	15.7 \pm 20.2	84.3 \pm 78.9	7.9 \pm 7.5	12.7 \pm 8.2	602.1 \pm 357.3	361.7 \pm 270.0	54.1 \pm 21.7	69.3 \pm 67.4	254.6 \pm 123.3
n	20	20	20	20	20	20	14	10	18	10

(1984). Statistical analyses including regression analysis, *t*-test, ANOVA and ANCOVA were conducted using SPSS for Windows (version 8.0, SPSS 1997).

RESULTS

Euphotic depth, water temperature and salinity

The euphotic depth was deepest at Stns B and M (70 and 81 m, respectively) in September 1997, and shallowest at Stns J and H (16 m) in August 1998 (Table 1). In most cases, the euphotic depth was similar for both the dumping and reference stations (*t*-test, $p > 0.05$; data not shown). Water temperature in the euphotic zone varied seasonally, and ranged from 10.3 to 27.6°C (Fig. 3a). Stratification in the euphotic zone was observed from late spring to early autumn, and the depth of the mixed layer ranged from 8 to 58 m in this study (data not shown). Differences in mean water temperature in the mixed layer were <1°C in most investigations, except that between Stns B and M in January 1996 (Fig. 3a). Mean water temperature was generally similar for both the reference and dumping stations; however, temperature deviations in the lower euphotic zone tended to be somewhat larger than in the mixed layer (Fig. 3b). In the mixed and lower euphotic zones, mean salinities and sigma-*t* were similar between dumping and reference stations in most investigations, with the exception of salinity data from Byung in August 1998 (Fig. 3c–f). Thus, physical characteristics including euphotic depth, temperature, and sigma-*t* seemed to be generally similar between dumping and reference areas during the study period.

Nutrients

Depth-integrated ammonium concentrations throughout the euphotic zone were generally similar between dumping and reference areas except for three 6- to 9-fold higher values measured at Stn J in March 1997 and April 1998, and at Stn B in July 1996 (Table 1). Euphotic depth-integrated nitrate concentrations showed wide variations over the study period,

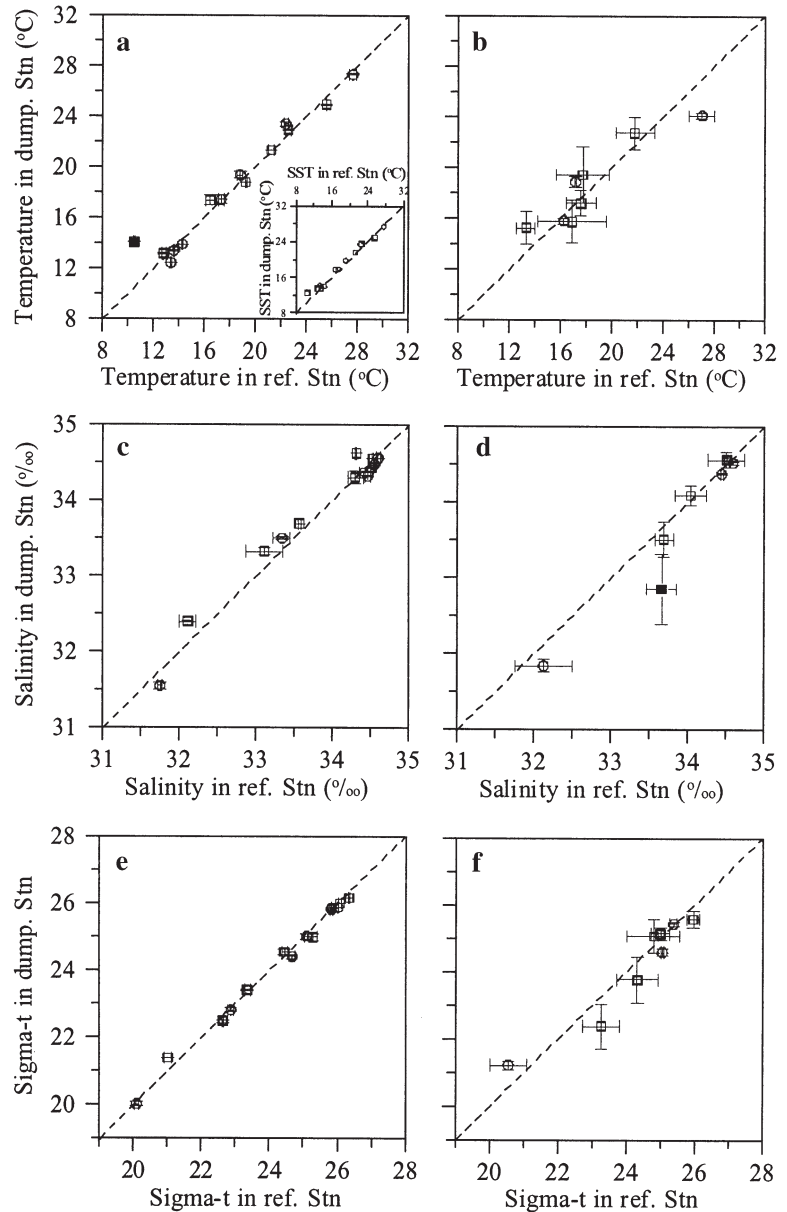


Fig. 3. Comparisons of temperature, sea surface temperature (SST), salinity, and sigma-*t* measured in the upper mixed layer of the euphotic zone (a,c,e) and lower layer of the euphotic zone (b,d,f) between dumping and reference stations at Byung (\square) and Jeong (\circ). Symbols, error bars and dashed line represent mean, ± 1 SD, and 1:1 line, respectively. (a,d) Closed squares represent data from stations at Byung in January 1996 and August 1998, respectively

and in most cases were generally similar between the dumping and reference areas (Table 1). Occasionally, nitrate concentrations that were 2-fold higher (Stn J in May 1998, Stn B in July 1996) or 2-fold lower (Stn B₁ in September 1997 and August 1998) than those at corresponding reference stations were observed at the dumping stations (data not shown). In the euphotic zone, depth-integrated phosphate concentrations were generally similar between the dumping and reference

areas (Table 1), but lower phosphate concentrations at dumping stations relative to reference stations were observed at Stn B₁ in September 1997 and Stns J and B in August 1998.

Chl *a* and primary production

Depth-integrated chl *a* over the euphotic zone in dumping areas ranged from 1.1 to 26.8 mg m⁻², which was generally comparable to the ranges (1.3 to 30.2 mg m⁻²) measured in reference areas (Table 1). PP in reference areas ranged from 58.1 to 2247.5 mg C m⁻² d⁻¹, whereas PP in dumping areas showed a relatively restricted range between 96.5 and 560.4 mg C m⁻² d⁻¹ (Table 1). At Stn B in January and May 1996 and in May 1998, PP was ca. 4-fold lower than that at Stn M. Also, PP was 4-fold lower at Stn J than at Stn H in March 1997 when high ammonium concentration was observed.

Bacterial carbon biomass and bacterial production

The euphotic depth-integrated BCB showed relatively small seasonal variations over the study area, and was generally similar between dumping and reference areas (Table 1). However, depth-integrated BCB was ca. 3-fold lower at Stn J₁ in September 1997 compared to Stn H, although depth-integrated chl *a* was 2.5-fold higher at Stn J₁ (data not shown). The euphotic depth-integrated bacterial production (BP) showed a relatively large seasonal variation (ca. 50 times), and generally similar ranges were observed between dumping and reference stations (Table 1). However, BP at dumping stations was usually either >2-fold higher or >2-fold lower than at reference stations (7 out of 14 cases).

β-glucosidase and aminopeptidase activity

In general, the euphotic depth-integrated β-glucosidase activity in the dumping areas tended to be similar to or higher than those in the reference areas (Table 1). At Stn B in May 1996 and Stns J₁ and B in September 1997, the depth-integrated β-glucosidase activities ranged from 67.6 to 84.0 μmol m⁻² h⁻¹, and were ca. 2-fold higher than those at each corresponding reference station. With the exception of a high value at Stn J in August 1998 (1095.2 μmol m⁻² h⁻¹, 2.5-fold higher than at reference station), the euphotic depth-integrated aminopeptidase activity tended to be similar between dumping and reference stations (Table 1).

Interrelationships among environmental, phytoplankton and bacterial variables

BP and PP showed significant positive and negative correlations with sea surface temperature (SST), respectively, in the reference areas ($p < 0.05$, Fig. 4a,b). Thus, the BP/PP ratio showed a strong positive correlation with SST ($p = 0.004$, Fig. 4c) at the reference stations. However, none of these relationships were significant in dumping areas (Fig. 4). These results seemed to be due to lower values of PP (at Stn B in May 1996 and 1998), lower values of BP (at Stns J and J₁ in July 1997, and Stns J₁ and B in September 1997) or higher values of BP (at Stn B in May 1996 and 1998) compared to

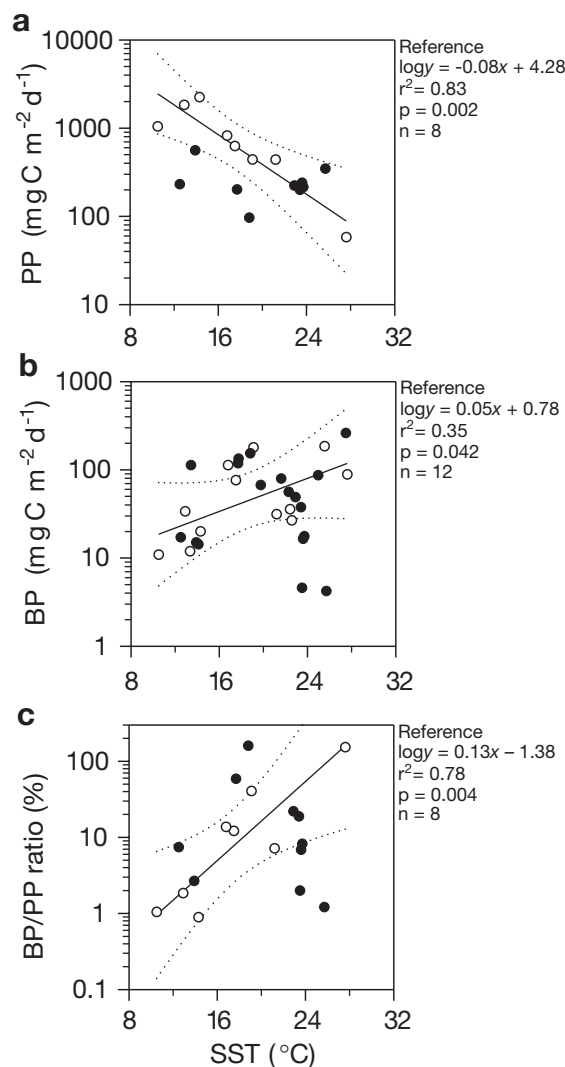


Fig. 4. Scatter plots of depth-integrated (a) primary production (PP) and (b) bacterial production (BP) over the euphotic zone versus SST, and (c) BP/PP ratio versus SST in dumping (●) and reference (○) areas. Solid and dotted lines represent regression line and 99% confidence interval of data from reference sites, respectively

those in corresponding reference areas (Fig. 4). Remarkably, more than half of the PP data from dumping stations were outside the confidence interval of the relationships between PP and euphotic depth-integrated nitrate, and between PP and euphotic depth-integrated chl *a* in the reference areas (Fig. 5a,b). However, depth-integrated chl *a* was significantly correlated with depth-integrated nitrate in both dumping and reference areas (Fig. 5c), and the relationships in both areas were statistically identical (ANCOVA, $p = 0.001$). During the study period, BP did not significantly correlate

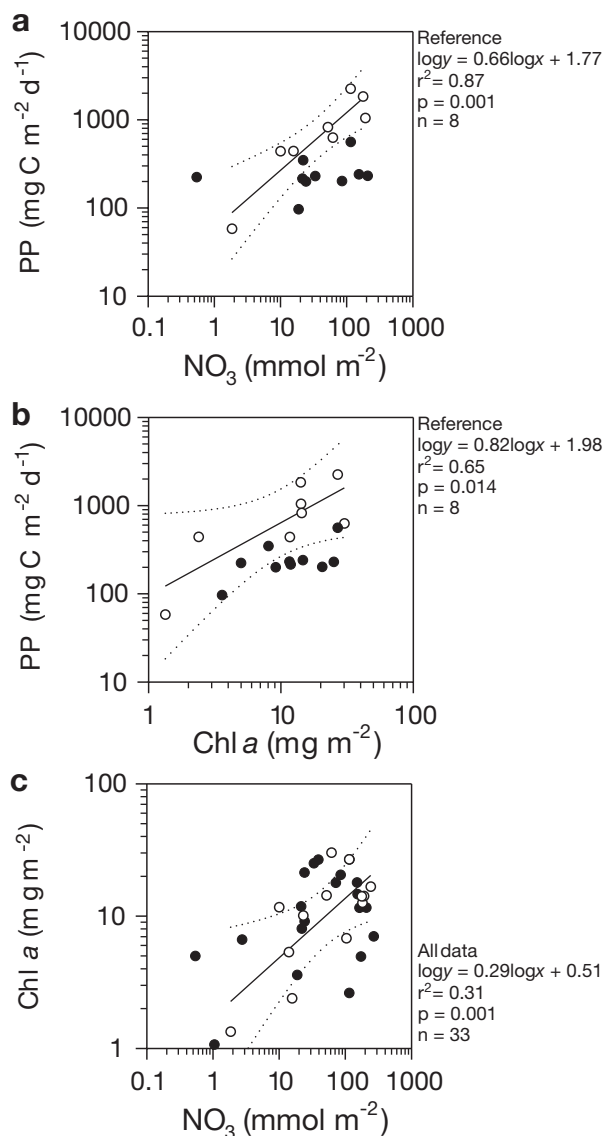


Fig. 5. Relationships between (a) PP and nitrate concentrations (NO_3), (b) PP and chl *a*, and (c) chl *a* and NO_3 in dumping (●) and reference (○) areas. All data were integrated over the euphotic zone. Solid and dotted lines (a,b) represent regression line and 99% confidence interval of data from reference sites, respectively. In (c), relationships in dumping and reference areas were statistically identical; thus, regression line and 99% confidence interval from all data were represented

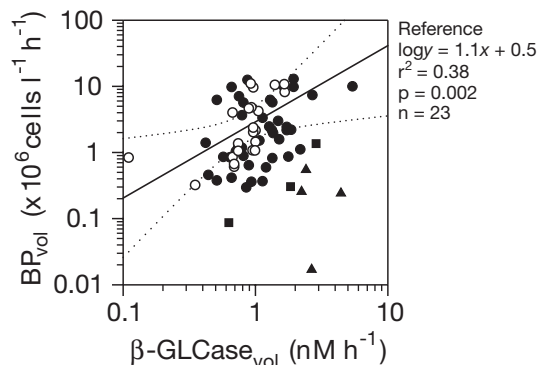


Fig. 6. Scatter plots of bacterial production per unit volume (BP_{vol}) versus β -glucosidase activity per unit volume ($\beta\text{-GLCase}_{\text{vol}}$) in the euphotic zone of dumping (●) and reference (○) areas. Solid and dotted lines represent regression line and 99% confidence interval of data from reference sites, respectively. ■, ▲: data from Stns J₁ and B in September 1997, respectively

with PP in either dumping or reference areas (data not shown). Volumetric BP in reference areas was significantly correlated with β -glucosidase activity ($r^2 = 0.38$, $p = 0.002$, Fig. 6). β -glucosidase activity was also significantly correlated with aminopeptidase activity in the reference areas ($r^2 = 0.24$, $p = 0.03$, data not shown). However, in the dumping areas, these relationships were not statistically significant ($p > 0.05$, Fig. 6).

Waste-addition experiment

Bacterial abundances in stock solutions of acid waste and human and animal wastes were 0.3×10^6 and 0.13×10^6 cells ml^{-1} , respectively. Despite substantial bacterial abundances in stock solutions, BP was not detectable in stock solutions of the wastes. When wastes were added to seawater at 5%, bacterial abundances tended to be slightly decreased (Fig. 7a). At higher dilutions, bacterial abundances were similar among treatments (ANOVA, $p > 0.05$). However, thymidine incorporation rates and aminopeptidase activity were negatively affected at a waste concentration of 5% (Fig. 7b,c). Both human and animal wastes and acid waste exhibited inhibitory effects on thymidine incorporation rates at concentrations of 5 and 1% (ANOVA, $p < 0.05$); however, at greater dilutions, no inhibitory effects were observed (ANOVA, $p > 0.05$, Fig. 7b). In contrast, thymidine incorporation rates were slightly stimulated at the concentration of 0.005% human and animal wastes. Further, concentrations of 1% acid waste were observed to have a stimulating effect on aminopeptidase activities (Fig. 7c). At higher dilutions of acid waste and human and animal wastes, no significant differences in aminopeptidase activities were observed among treatments and control.

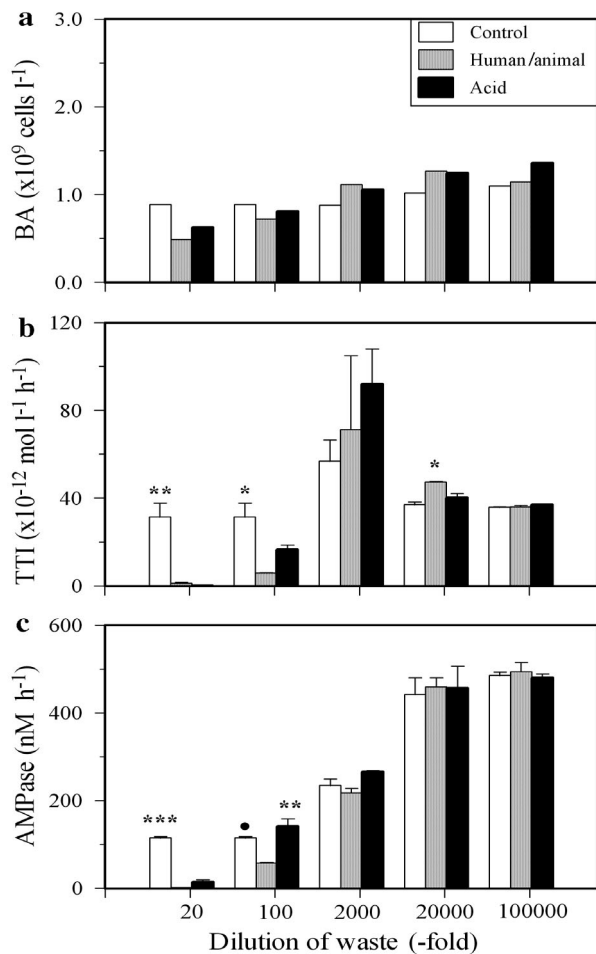


Fig. 7. Changes of (a) bacterial abundance (BA), (b) ³H-thymidine incorporation rates (TTI), and (c) aminopeptidase activities (AMPase) at various concentrations of human and animal wastes and acid waste. Error bars represent 1 SD. BA were counted in singles. *: significant differences between marked and other treatments (ANOVA, **p* < 0.05, ***p* < 0.01, ****p* < 0.001) at the indicated dilution of wastes; ●: significant differences between control and human and animal wastes treatment (**p* < 0.05)

DISCUSSION

A noteworthy observation made during this study was that microbial ecologically relevant relationships established in reference areas were mostly disturbed in dumping areas. The most pronounced observation was that BP/PP ratios did not correlate with SST in the dumping areas.

Recently, it has been reported that BP/PP ratios in the sea can be well explained by SST (Cho et al. 2001, Hoppe et al. 2002). Likewise, SST explained major variations in BP/PP ratios observed in reference areas during this study but, interestingly, not in the dumping areas. In dumping areas, only 4 out of 10 data were within the 99% confidence interval of the relationship.

This pronounced bias may have resulted from differences in physical and chemical characteristics between the 2 types of area, or from the effects of organic waste in the dumping areas, or a combination of both. Since temperature and turbulent mixing seem to be the underlying physical forces regulating variations in BP/PP ratios in the sea (Cho et al. 2001), physicochemical conditions including temperature, salinity, and sigma-*t* could be important factors influencing this difference between reference and dumping areas. However, during the study period, physical parameters including temperature, salinity, and sigma-*t* were similar between reference and dumping areas (Fig. 3). Thus, differences in observed relationships were more likely due to the effects of organic waste dumped in the dumping areas (see below)

Outliers of the 99% confidence interval of the relationship between SST and BP/PP ratio (Fig. 4c) were the data representing lower PP, lower BP, or higher BP than those expected from SST (Fig. 4a,b). As PP can be restricted by the availability of light and nutrients in the ocean (Lalli & Parsons 1997), low PP could be due to limitations caused by these factors. However, comparison of the solar radiation data measured on each investigation day showed that lower PP in the dumping stations did not always seem to relate with limitation of light intensity (data not shown). In addition, nutrient concentrations in the dumping stations with lower PP values were mostly similar to those in corresponding reference stations (Table 1). Further, ammonium concentration was unusually high at Stn J in March 1997, and at this time PP was 4 times lower than at the corresponding reference station. In the dumping areas, PP also did not show a tendency to be limited by nitrate concentration, which seemed to be a major regulating factor of PP in the reference areas (Fig. 5a). In actual fact, PP in the dumping stations was lower than that expected from nitrate concentration (Fig. 5a) and did not increase with increases in chl *a* (Fig. 5b), indicating that phytoplankton efficiency (PE) (defined as the ratio of depth-integrated PP to depth-integrated chl *a*) (Conan et al. 1999) sometimes decreased in the dumping areas. In fact, PE in the dumping stations (mean ± SD = 1.0 ± 0.5) was significantly lower than in reference areas (mean ± SD = 3.4 ± 2.2; *t*-test, *p* = 0.02, data not shown). Thus, it appears that low values for PP occasionally observed in the dumping stations did not result from limitation of nutrient or light intensity, but was probably due to inhibiting effects caused by waste disposal in the area.

The higher or lower BP values frequently observed at the dumping stations (Fig. 4b) require further explanation. In marine environments, temperature and substrates are regarded as important factors that regulate BP (Shiah & Ducklow 1994a, Pomeroy & Wiebe 2001).

Similarly, BP in reference areas showed a positive correlation with SST (Fig. 4b). However, although seawater temperature did not differ significantly between dumping and reference stations in this study (Fig. 3a,b), BP did not correlate significantly with SST in dumping areas (Fig. 4b), suggesting that BP in dumping stations might have been somehow affected. As allochthonous organic matter has been disposed at dumping areas, higher bacterial activities can be expected at these sites (Peele et al. 1981, Cavari & Colwell 1988). As expected, increases in BP were occasionally observed at dumping stations in this study. Further, the relationship between volumetric BP and β -glucosidase activities (Fig. 6) indicates that a high concentration of polymeric substrates for β -glucosidase might have been dumped at Stns J₁ and B in September 1997, where lower BP was observed. This indicates that lower BP in dumping areas might not have resulted from a lack of substrate. Since organic wastes from sewage, dredging, industrial factories etc. tend to contain various inhibitory substances (Kennish 1998), the observed low BP may have been due to inhibitory effects of the organic waste dumped into the areas.

The inhibition of bacterial activities by organic waste was directly confirmed by dilution experiments of wastes with seawater. With up to 100-fold dilution of wastes, BP was significantly inhibited (Fig. 7b). Similarly, phytoplankton growth showed similar responses to various types of waste (Han 2000). Han (2000) reported that phytoplankton growth was inhibited at various concentrations of wastes (up to 625-fold dilution of waste water from a leather factory, 1250-fold dilution of acid waste, and 10 000-fold dilution of alkali waste). However, in this study, slight stimulation of BP was also observed at a 20 000-fold dilution of human and animal wastes (Fig. 7a), and phytoplankton growth also increased at higher dilutions (Han 2000). Therefore, responses of bacteria and phytoplankton to wastes dumped into seawater could be dependent on the constituents of wastes—such as nutrients, organic matter, and small quantities of toxic compounds—and the dilution factor of the wastes (i.e. time elapsed after dumping).

There are few pre-existing studies on impacts of waste dumping on pelagic microorganisms available for comparison with our research (Peele et al. 1981, Cavari & Colwell 1988). At domestic-sewage outfall and chemically polluted sites in coastal waters of the eastern Mediterranean Sea, bacterial abundance and amino acid uptake was 4- to 50-fold higher than those at the control site (Cavari & Colwell 1988). In addition, at a Puerto Rico dump site of pharmaceutical waste in the Atlantic Ocean, the cell-specific uptake of amino acids increased near the dump site, but bacterial abundance was relatively stable (Peele et al. 1981). In con-

trast, 2- to 7-fold decreases as well as 2- to 3-fold increases in BP were occasionally observed at dumping sites of the East Sea during our study period; however, bacterial biomasses were relatively similar between dumping and reference stations. As the volume of waste dumped was much higher in the East Sea compared to the eastern Mediterranean Sea and Puerto Rico dump sites, and because different types of waste were disposed of, different effects of wastes on pelagic bacteria most likely result from differences in the amount and (probably) composition of the wastes.

Finally, we noted that our sampling was not conducted at the time of dumping of wastes. Unfortunately, time intervals between dumping of wastes and water sampling were not made available to this study. It will be necessary to investigate the nature of wastes (e.g. particle size distributions, soluble constituents, chemical compositions), waste distribution, and residence time of wastes in the water column for accurate diagnosis of environmental health of the dumping areas. Nonetheless, as the amount of waste disposed of into the East Sea has been increasing continuously ever since this study was initiated, disturbances on the microbial food webs of the East Sea ecosystem may have become greater. Thus, it would be wise to take measures to reduce the impacts of waste dumping. As termination of sewage disposal into the sea might result in a relatively rapid recovery from the effects of ocean dumping (Cavari & Colwell 1988), introducing a resting period between dumps may be one way to reduce adverse effects of ocean dumping on the pelagic ecosystem of dumping sites.

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LITERATURE CITED

- Cavari B, Colwell RR (1988) Effect of pollution on the bacterial community structure in the coastal waters of the Eastern Mediterranean Sea. *Prog Oceanogr* 21:147–157
- Chang KI, Teaque WJ, Lyu SJ, Perkins HT and 6 others (2004) Circulation and currents in the southwestern East/Japan Sea: overview and review. *Prog Oceanogr* 61:105–156
- Cho BC, Park MG, Shim JH, Choi DH (2001) Sea-surface temperature and *f*-ratio explain large variability in the ratio of bacterial production to primary production in the Yellow Sea. *Mar Ecol Prog Ser* 216:31–41
- Choi DH, Park JS, Hwang CY, Huh SH, Cho BC (2002) Effects of thermal effluents from a power station on bacteria and heterotrophic nanoflagellates in coastal waters. *Mar Ecol Prog Ser* 229:1–10
- Choi DH, Hwang CY, Cho BC (2003) Comparison of virus- and bacterivory-induced bacterial mortality in the eutrophic Masan Bay, Korea. *Aquat Microb Ecol* 30:117–125

- 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
- 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
- Conan P, Turley C, Stutt E, Pujo-Pay M, Van Wambeke F (1999) Relationship between phytoplankton efficiency and the proportion of bacterial production to primary production in the Mediterranean Sea. *Aquat Microb Ecol* 17: 131–144
- Ducklow HW, Kirchman DL, Quinby HL (1992) Bacterioplankton cell growth and macromolecular synthesis in seawater cultures during the North Atlantic spring phytoplankton bloom, May, 1989. *Microb Ecol* 24:125–144
- Falkowski PG, Woodhead AD (1992) Primary production and biogeochemical cycles in the sea. Plenum Press, New York
- Friedman GM, Mukhopadhyay PK, Moch A, Ahmed M (2000) Waters and organic-rich waste near dumping grounds in the New York Bight. *Int J Coal Geol* 43:325–355
- Gustavson K, Petersen S, Pedersen B, Stuer-Lauridsen F, Pedersen S, Wängberg SÅ (1999) Pollution-induced community tolerance (PICT) in coastal phytoplankton communities exposure to copper. *Hydrobiologia* 416:125–138
- Han TP (2000) Effects of oceanic waste dumping on marine environments and on primary productivity. MSc thesis, Gwangju University, Gwangju
- Hill RT, Knight IT, Anikis MS, Colwell RR (1993) Benthic distribution of sewage sludge indicated by *Clostridium perfringens* at a deep-ocean dump site. *Appl Environ Microbiol* 59:47–51
- Hill RT, Straube WL, Palmisano AC, Gibson SL, Colwell RR (1996) Distribution of sewage indicated by *Clostridium perfringens* at a deep-water disposal site after cessation of sewage disposal. *Appl Environ Microbiol* 62:1741–1746
- Himmelbloom BH, Stevens BG (1994) Microbial analysis of a fish waste dump site in Alaska. *Bioresour Technol* 47: 229–233
- Hoppe HG (1983) Significance of exoenzymatic activities in the ecology of brackish water: measurements by means of methylumbelliferyl-substrates. *Mar Ecol Prog Ser* 11: 299–308
- Hoppe HG, Gocke K, Koppe R, Begler C (2002) Bacterial growth and primary production along a north-south transect of the Atlantic Ocean. *Nature* 416:168–171
- Hudak JP, Fuhrman JA (1988) Effects of four organic pollutants on the growth of natural marine bacterioplankton populations. *Mar Ecol Prog Ser* 47:185–194
- IMO (International Maritime Organisation) (2002) Convention on the prevention of marine pollution by dumping of wastes and other matter, 1972 (London Convention 1972). Final report on permits issued in 1998. LC.2/Circ.423, International Maritime Organisation, London
- Kennish MJ (1998) Pollution impacts on marine biotic communities. In: Kennish MJ, Lutz PL (eds) Marine science series, Vol 14. CRC Press, Boca Raton, FL
- Kirchman DL (1990) Limitation of bacterial growth by dissolved organic matter in the subarctic Pacific. *Mar Ecol Prog Ser* 62:47–54
- Kirchman DL, Rich JH (1997) Regulation of bacterial growth rates by dissolved organic carbon and temperature in the Equatorial Pacific Ocean. *Microb Ecol* 33:11–20
- Lalli CM, Parsons TR (1997) Biological oceanography: an introduction, 2nd edn. Butterworth-Heinemann, Oxford
- Li GX, Zhang FS, Sun Y, Wong JWC, Fang M (2001) Chemical evaluation of sewage sludge composting as a mature indicator for composting process. *Water Air Soil Pollut* 132: 333–345
- Martinez J, Azam F (1993) Periplasmic aminopeptidase and alkaline phosphatase activities in a marine bacterium: implications for substrate processing in the sea. *Mar Ecol Prog Ser* 92:89–97
- Ministry of Environment (1998) Marine environmental monitoring and assessment technology: development of monitoring technology for the wastes disposal sea areas. KORDI Report, Korea Ocean Research and Development Institute, Ansan
- Moran MA, Wicks RJ, Hodson RE (1991) Export of dissolved organic matter from a mangrove swamp ecosystem: evidence from natural fluorescence, dissolved lignin phenols, and bacterial secondary production. *Mar Ecol Prog Ser* 76: 175–184
- Nam S, Kim YH, Park KA, Kim K (2005) Spatio-temporal variability in sea surface wind stress near and off the east coast of Korea. *Acta Oceanol Sin* 24:107–114
- Parsons T, Maita Y, Lalli CM (1984) A manual of chemical and biological methods for water analysis. Pergamon Press, Oxford
- Peele ER, Singleton FL, Deming JW, Cavari B, Colwell RR (1981) Effects of pharmaceutical wastes on microbial populations in surface waters at the Puerto Rico dump site in the Atlantic Ocean. *Appl Environ Microbiol* 41:873–879
- Pomeroy LR, Wiebe WJ (2001) Temperature and substrates as interactive limiting factors for marine heterotrophic bacteria. *Aquat Microb Ecol* 23:187–204
- Porter KG, Feig YS (1980) The use of DAPI for identifying and counting aquatic microflora. *Limnol Oceanogr* 25:943–948
- Rieley G, VanDover CL, Eglinton G (1997) Fatty acids as sensitive tracers of sewage sludge carbon in a deep-sea ecosystem. *Environ Sci Technol* 31:1018–1023
- Riemann B, Lindgaard-Jørgensen P (1990) Effects of toxic substances on natural bacterial assemblages determined by means of [³H]thymidine incorporation. *Appl Environ Microbiol* 56:75–80
- Shiah FK, Ducklow HW (1994a) Temperature and substrate regulation of bacterial abundance, production and specific growth rate in Chesapeake Bay, USA. *Mar Ecol Prog Ser* 103:297–308
- Shiah FK, Ducklow HW (1994b) Temperature regulation of heterotrophic bacterioplankton abundance, production and specific growth rate in Chesapeake Bay, USA. *Limnol Oceanogr* 39:1243–1258
- Simon M, Azam F (1989) Protein contents and protein synthesis rates of planktonic marine bacteria. *Mar Ecol Prog Ser* 51:201–213
- Song KD, Choi DH, Lee Y, Hong GH, Chung CS, Kim SH, Cho BC (1999) Responses of bacterial production and enzyme activities to ocean-dumping of organic wastes in the euphotic zone of the East Sea, Korea. *J Korean Soc Oceanogr* 4:275–288
- Stronkhorst J, Ariese F, van Hattum B, Postma JF and 6 others (2003) Environmental impact and recovery at two dumping sites for dredged material in the North Sea. *Environ Pollut* 124:17–31