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# Temporal changes in chlorophyll a concentrations and bacterial, viral, and heterotrophic nanoflagellate abundances in the coastal zone of Sagami Bay, Japan: implications of top-down and bottom-up effects

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**Abstract** — Spatio-temporal distribution patterns of bacterial abundance (BA) and production (BP), heterotrophic nanoflagellate abundance (HNFA), viral abundance (VA), and chlorophyll a concentrations (Chl a) were examined by annual monthly sampling of water from the surface to 100 m depth in a coastal area of Sagami Bay, Japan to investigate the changes of top-down and bottom-up effects on bacterial abundance. During the colder period (November to March), BA and BP were not significantly correlated through the water column, indicating that top-down effects controlled bacterial abundance. In contrast, BA was significantly positively correlated with BP during the warmer period (April to October). The ratio of the estimated potential grazing rate of nanoflagellates to BP during the colder period tended to be greater than that during the warmer period. The change of BA/HNFA suggested that HNF populations did not immediately reduce BA as much as might have been expected during the warmer period. The low values of BA/HNFA during the warmer period also suggested that predation effects were more important in regulating the population dynamics of HNF relative to bottom-up control by the bacterial food supply.

**Key word:** Bacteria, bottom-up, chlorophyll a, HNF, top-down, virus

## Introduction

Bacteria, protozoa, and viruses comprise large fractions of the biomass within planktonic ecosystems (Gasol et al. 1997), and microbial activity often dominates ecosystem functions (del Giorgio et al. 1997), such as the transfer of carbon through the planktonic food web (e.g., Azam et al. 1983). Among microbes, bacterial communities are of central importance in the study of microbial food webs, because bacterial metabolic processes play an important role in transforming dissolved organic matter (DOM), which is generally only available to bacteria, into forms available to higher trophic organisms, particularly the bacterial grazers, heterotrophic nanoflagellates (HNF; McManus and Fuhrman 1988, Sherr and Sherr 1994).

Heterotrophic bacterial communities primarily depend on phytoplankton-derived DOM, which is a common bacterial resource (Shiah and Ducklow 1994, Simon et al. 1998); however, variation in phytoplankton communities (biomass and production) does not always coincide with variation in bacterial abundance (BA) or rates of bacterial production (BP; Cho et al. 1994, Naganuma 1997). This absence of a relationship between phytoplankton and bacterial communities may involve grazing effects on bacteria. However, bacterial and HNF abundances may not be strongly coupled across systems, and consequently, HNF are not always important grazers of bacteria (Gasol and Vaque 1993).

Such resource-supply and grazing effects on bacterial communities are often referred to as bottom-up and top-

down processes, respectively, and the regulatory mechanisms of these factors have been investigated in a variety of ecosystems (McQueen et al. 1986). The importance of resource supply to BA and BP is supported by positive correlations between phytoplankton and bacterial parameters (Bird and Kalff 1984, Cole et al. 1988, Ducklow 1999). In contrast, previous studies have reported that protozoan grazing (Rassoulzadegan and Sheldon 1986, Sherr et al. 1992) and viral lysis (Proctor and Fuhrman 1992, Suttle 1994) are also related to BA in various aquatic environments. In addition, other research has demonstrated that physical processes in the water column (e.g., vertical mixing) that largely control trophic conditions determine the distribution and production of bacteria and their quantitative role in microbial food web processes (Cushing 1989, Cho et al. 2001). These differences among studies suggest that mechanisms controlling bacterial communities are system- dependent. To understand the factors affecting bacterial communities in a given environment, it is important to characterize both the trophic interactions and water column structure in space and time (Sanders et al. 1992, Dufour and Torreton 1996).

Our goal was to examine the temporal changes in microbial food web trophic relationships mediated through top-down and bottom-up regulation of heterotrophic bacteria in the temperate coastal waters of Sagami Bay, Japan. Specifically, we aimed to reveal the mechanisms structuring microbial communities in this system. To this end, we evaluated the relative effects of top-down versus bottom-up regulation of bacterial communities by comparing variation in bacterial abundance (BA) and production (BP) to changes in: (i) concentrations of chlorophyll a (Chl a), (ii) abundance of HNF (HNFA), and (iii) viral abundance (VA).

## Materials and Methods

### Study site and sample collection

Seawater sampling was conducted monthly (mid-month) using 6-L Niskin bottles at depths of 0, 10, 20, 30, 40, 60, and 100 m between March 2004 and February 2005 at a fixed sampling station (St. M; Fig. 1), located 2 km off of the Manazuru Peninsula in Sagami Bay, Japan (35°9′ N, 139°10′ E). Polypropylene tubes (50 ml) were rinsed with seawater three times and then filled. Samples for bacterial and viral analyses were fixed with 2% formalin, and HNF samples were fixed with 1% glutaraldehyde (final concentration) immediately after collection. Water samples for the BP analysis were not fixed. Fixed microbial samples were stored in the dark at 4°C and slide prepared within 24 h and counted within 72 h. At the same time as sample collection, water temperature was measured using a mercury thermometer.

### Measurement of biological parameters

Bacteria and viruses were enumerated using SYBR Green I direct-count methods (Noble and Fuhrman 1998). Each water sample (3 ml) was filtered onto 0.0–2 $\mu$m pore size Anodisc filters. Samples were counted under epifluorescent microscopy (Zeiss Axiophot; filter set: Blue #F 22, Carl Zeiss, Inc.). At least 400 each of bacteria and viruses were counted per filter. HNF-preserved water samples (50 to 100 ml) were stained with DAPI and FITC and filtered at low pressure through a 0.8-$\mu$m pore size Nucleopore filter (Sherr and Sherr 1983). HNF cells were enumerated under epifluorescent microscopy (filter set: UV #F 06 and Blue #F 22). One hundred microscopic fields were examined per sample, and at least 50 HNF were counted per filter.

Rates of BP were estimated from $^{3}$H-thymidine incorporation rates, following Fuhrman and Azam (1982). Freshly collected samples (20 ml) from each depth were spiked with 20 nM $^{3}$H-thymidine (Amersham). Samples were incubated for 1 h in the dark at the ambient water temperature of the collection depth (±2°C). After incubation, samples (20 ml) were filtered through 0.22-$\mu$m membrane filters (GSWP, Millipore) and rinsed three times each with ice-cold 5% trichloroacetic acid (TCA) and ice-cold 80% ethyl alcohol. Samples were radioactively-assayed for TdR incorporation using a liquid scintillation counter (TRI-CARB 460CD, Packard Instrument Co.). Duplicate samples and one TCA-

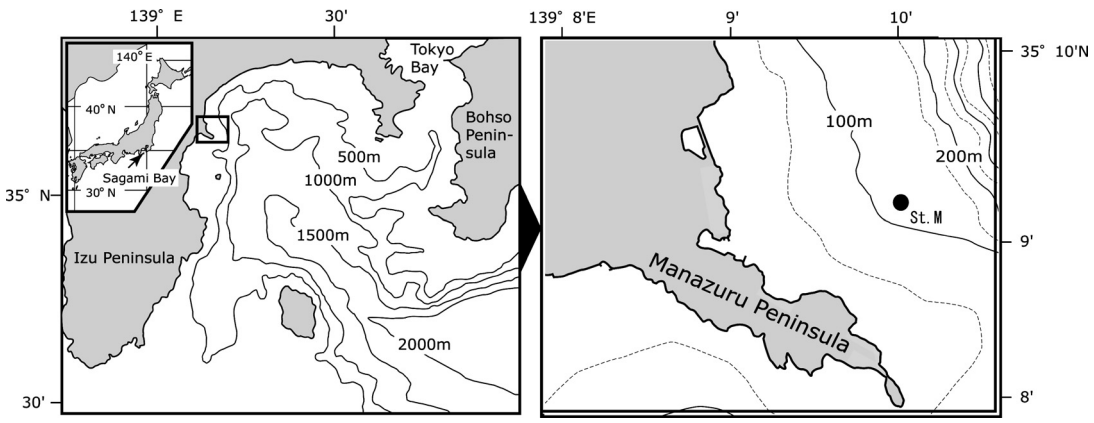


**Fig. 1.** Location of the sampling station (St. M) in Sagami Bay, Japan.

killed control were prepared for each sampling depth. Abiotic absorption rates of TdR to particulate matter were corrected by subtracting the incorporation rate of TdR in killed controls from those in experimental cultures. The radio-isotope (RI) experiment was conducted at the RI center of Yokohama National University, Japan. TdR incorporation rates were converted to bacterial cell production using a theoretical conversion factor of $3.9\times10^{17}$ cells $mol^{-1}$ (Kirchman 2000).

Chlorophyll a concentrations (Chl *a*) in the samples were determined using a fluorometer (10-AU; Turner Design), following the methods of Suzuki and Ishimaru (1990).

### Statistical analysis

Simple correlation (Pearson product moment) analyses were performed to examine BA and BP regulation by top-down and bottom-up processes on the basis of relationships between bacterial parameters and other variables (HNFA, VA, water temperature, and Chl *a*). The normality of data was tested using Kolmogorov-Smirnov tests. Because log-transformation shortens the Kolmogorov-Smirnov distance, log-transformed variables were used in calculations of Pearson correlation coefficients. Means were compared using *t*-tests, and a significance level of $P<0.05$ was used in all statistical analyses.

For correlational analyses of biological parameters, data from different depths and months were combined, because 1 month is too long an interval for analysis of temporal variation of microbial organisms, the turnover of which occurs every few days to months in the upper ocean layers (Bode et al. 2001, Nagata et al. 2001, Teira et al. 2003). To facilitate discussion of the mechanisms regulating BA and BP, data were also grouped into a colder (November to March) and warmer period (April to October) based on the physical condition of the water column, which can affect the metabolism and population dynamics of planktonic organisms. The warmer period included the summer season of stratification, whereas the colder period included the winter periods of mixing. Furthermore, the water column was divided into shallow (0 to 30 m) and deep (40 to 100 m) layers, because seasonal variation in BA and VA differed between the two layers.

## Results

### Hydrography

Throughout the year and at different depths, water temperatures varied from 12.3 to 28°C, (Fig. 2). The highest temperature was recorded in August or September, and the lowest was observed in March. From November to March, the difference in water temperature between 0 and 100 m depth was<3°C, indicating that the water column was vertically well-mixed during this period. Thereafter, the water column became gradually stratified, and the difference in water temperature between 0 and 100 m depth increased to>3°C. In October and November, temperatures were lower at 0 m than at 10, 20, and 30 m.

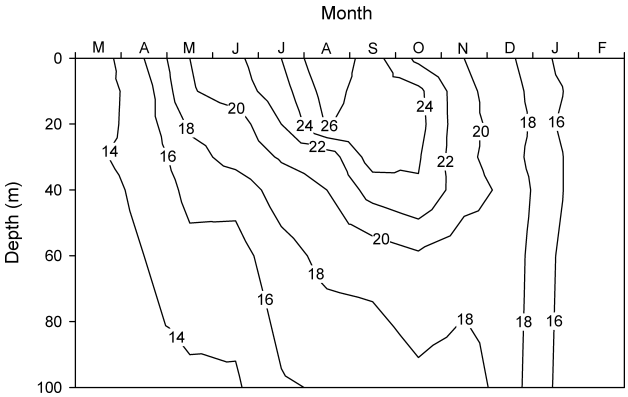


**Fig. 2.** Annual changes in water temperature at St. M from the surface to 100 m depth.

Across seasons and depths, salinity ranged from 28.5 to 35.1 PSU. Surface salinity was higher (ca. 35 PSU) during April to May and lower at the beginning of October and November. Low surface temperatures and salinities in October and November were due to heavy typhoon rainfalls.

### Spatio-temporal changes in biological parameters

Large peaks in Chl *a* occurred in April–May at all depths during spring phytoplankton blooms and in September from 10 to 40 m depth (Fig.3). Subsequent to the April–May peaks, Chl *a* decreased gradually until October (except for the September peaks at depths of 10 to 40 m; Fig. 3). Lower values of Chl *a* occurred at 60 and 100 m depth from August to November (Fig. 3). In October sampling, Chl *a* concentrations at the surface (0 m) were exceptionally high. Fujiki et al. (2004). were observed 5 times Chl *a* peaks from April to July at the depth of 0 to 5 m in this area. They concluded that the increase of Chl *a* concentrations was due to freshwater discharge. Kanda et al. (2003) discussed that exception intense of phytoplankton during summer period may have associated with abnormal hydrographic condition of Sagami Bay.

BP varied from 0.02 to $3.79\times10^8$ cells $l^{-1}d^{-1}$, and higher values were observed at shallow depths (<40 m) during the warmer period. Peaks in BP coincided with the Chl *a* maxima at 30–100 m depth in April, and lower BP values were observed at depths of 60 and 100 m from August to November (Fig. 3). Fluctuations in BP paralleled those of Chl *a* from October to February (i.e., the colder period) in nearly the entire water column (Fig. 3). Moreover, BP was significantly correlated with Chl *a*, except at shallow depths (0–30 m) during the colder period (Table 1).

BA ranged from 0.31 to $5.06\times10^9$ cells $l^{-1}$ throughout

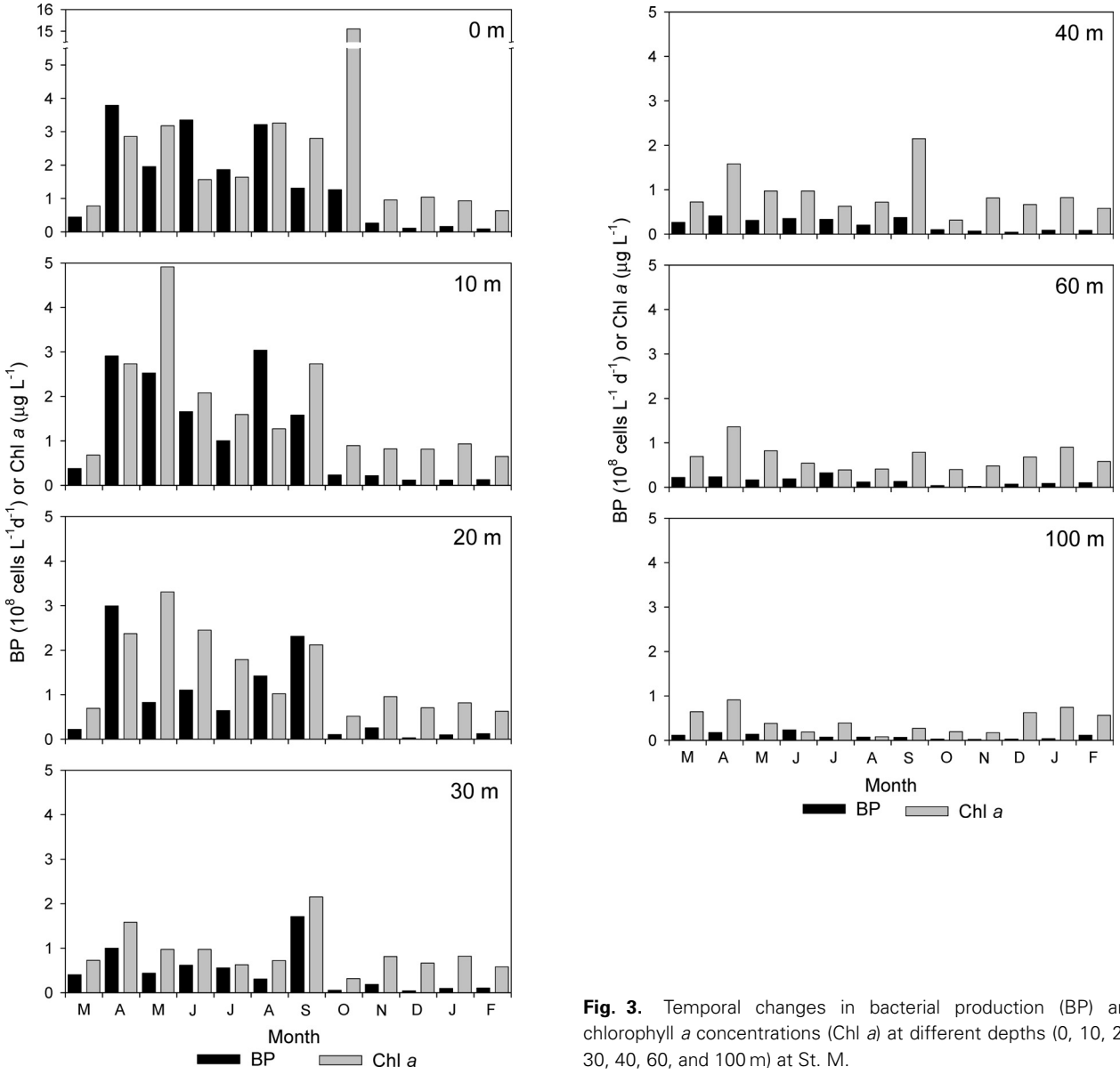


**Fig. 3.** Temporal changes in bacterial production (BP) and chlorophyll *a* concentrations (Chl *a*) at different depths (0, 10, 20, 30, 40, 60, and 100 m) at St. M.

the water column, with higher abundances at depths<40 m than at deeper depths during the warmer period. In May, the maximum value of BA at shallow depths (0 to 30 m) coincided with the maximum Chl *a* (Figs. 3 and 4). BA tended to be lower during the colder period than during the warmer period at depths<40 m. BA increased gradually after April and peaked in July (10 to 30 m depth) and August (0 m). Subsequently, BA leveled off and decreased slightly in August and September, followed by steep declines in October. In contrast, BA at deeper depths (40, 60, and 100 m) was less temporally variable, and after October when the water column turned over, BA gradually increased and peaked in January.

Although VA was one order of magnitude higher than BA, temporal changes in VA were strongly positively correlated with those in BA (Table 1, Fig. 4). Across seasons and depths, VA ranged from 0.38 to $5.64\times10^{10}$ viruses $l^{-1}$ (Fig. 4) and tended to be higher during the warmer period at depths< 40 m. After April, VA markedly increased and peaked in July (0 to 30 m depth). In August, values of VA at depths<40 m remained high, and subsequently, VA decreased rapidly until October. In contrast, temporal fluctuations in VA at depths of 60 and 100 m (with peaks in January) were inconsistent, and annual patterns in VA at these depths were inversely related to those at shallow depths (0 to 30 m).

HNFA was three orders of magnitude lower than BA and varied from 0.10 to $2.31\times10^6$ cells $l^{-1}$ (Fig. 4). HNFA exhibited relatively high values (mean=$0.65\times10^6$ cells $l^{-1}$) from November to February and relatively low values (mean= $0.39\times10^6$ cells $l^{-1}$) from June to October. Temporal changes in HNFA were small, and bimodal peaks occurred in April–May and December–January. The highest peaks were observed at depths between 0 and 30 m in May (Fig. 4). During the colder period, fluctuations in HNFA were similar to patterns in BA, and the two variables were significantly corre-

**Table 1.** Summary of correlation coefficients among log-transformed bacterial production (Log BP), log-transformed bacterial abundance (Log BA), log-transformed viral abundance (Log VA), log-transformed abundance of heterotrophic nanoflagellates (Log HNFA), log-transformed chlorophyll *a* concentrations (Log Chl *a*) and water temperature (Temp) during colder (November to March) and warmer (April to October) periods. Then water column was divided into shallow (0 to 30 m) and deep (40 to 100 m)layers.

| Period | Layer | | Log BA | Log VA | Log HNFA | Log Chl *a* | Temp |
|---|---|---|---|---|---|---|---|
| Colder | Shallow (n=20) | Log BP | −0.466** | −0.397 | −0.584** | 0.232 | −0.301 |
| | | Log BA | | 0.917* | 0.795** | 0.141 | 0.618** |
| | | Log VA | | | 0.665** | 0.086 | 0.532** |
| | | Log HNFA | | | | 0.338 | 0.766** |
| | | Log Chl *a* | | | | | 0.488** |
| | Deep (n=15) | Log BP | 0.106 | −0.229 | −0.472 | 0.685** | −0.682** |
| | | Log BA | | 0.898* | 0.527** | 0.635 | 0.169 |
| | | Log VA | | | 0.41** | 0.398 | 0.413 |
| | | Log HNFA | | | | 0.114 | 0.826* |
| | | Log Chl *a* | | | | | 0.169 |
| Warmer | Shallow (n=28) | Log BP | 0.432* | 0.369 | 0.281 | 0.690** | −0.123 |
| | | Log BA | | 0.665* | 0.458* | 0.410* | 0.216 |
| | | Log VA | | | 0.089 | 0.149 | 0.323 |
| | | Log HNFA | | | | 0.270 | −0.244 |
| | | Log Chl *a* | | | | | −0.167 |
| | Deep (n=15) | Log BP | 0.685** | 0.544* | 0.716** | 0.736 | −0.112 |
| | | Log BA | | 0.701** | 0.446* | 0.654** | 0.060 |
| | | Log VA | | | 0.299 | 0.496* | 0.352 |
| | | Log HNFA | | | | 0.775** | −0.283 |
| | | Log Chl *a* | | | | | 0.073 |

*: $p<0.05$. **: $p<0.01$

lated (Table 1, Fig. 4). In contrast, values of HNFA were low during the warmer period, whereas BA and BP were high at shallow depths (Table 1, Fig. 4).

## Discussion

We monitored the spatio-temporal changes in bacterial variables (BA and BP) and related parameters (water temperature, Chl *a*, VA, and HNFA) in Sagami Bay, Japan. The relationships among these variables can be examined within the framework of top-down and bottom-up regulation of microbial community structure in coastal waters (Šolić et al. 2001, Hyun et al. 2003, Tanaka and Rassoulzadegan 2004). For example, effects of resource supply, water temperature, predation, and viral lysis on the spatio-temporal variation in the abundance and production of bacterial communities have been investigated throughout the world's oceans (Albert et al. 2001, Lee et al. 2001). The importance of resource supply in regulating bacteria (across seasons and geography) has been examined using correlation and/or regression analysis in several aquatic systems (Jonas and Tuttle 1990, Nagata et al. 2001, Vazquez-Dominguez et al. 2005). Positive correlations between BA and Chl *a* often reflect a dependency of bacteria on organic matter fixed by phytoplankton (Cole et al. 1988). Furthermore, many studies have reported that BA and/or BP are positively correlated with primary production and/or Chl *a* concentrations in various aquatic systems (Bird and Kalff 1984, Nagata 1984, Carlson et al. 1996, Kirchman and Rich 1997, Ducklow 1999).

We observed a general significant positive correlation between BP and Chl *a* (with the exception of at shallow depths during the colder period), suggesting that BP was controlled by the amount of substrate supplied by phytoplankton. However, a strong correlation between BP and Chl *a* could also be caused by a common external factor affecting both bacteria and phytoplankton, such as water temperature (Shiah and Ducklow 1994). However, the correlation analysis indicated that resource supply was more important than temperature in regulating bacteria in this study area (Table 1).

BA and BP were significantly positively correlated during the warmer period, suggesting that bottom-up effects on the abundance of bacterial communities were more important than top-down effects (Fig. 5 and Table 1). Similar couplings of BA and BP were reported by Krstulović et al. (1997), further supporting the hypothesized resource regulation of bacteria (i.e., bottom-up control). It is also possible that a quanti-

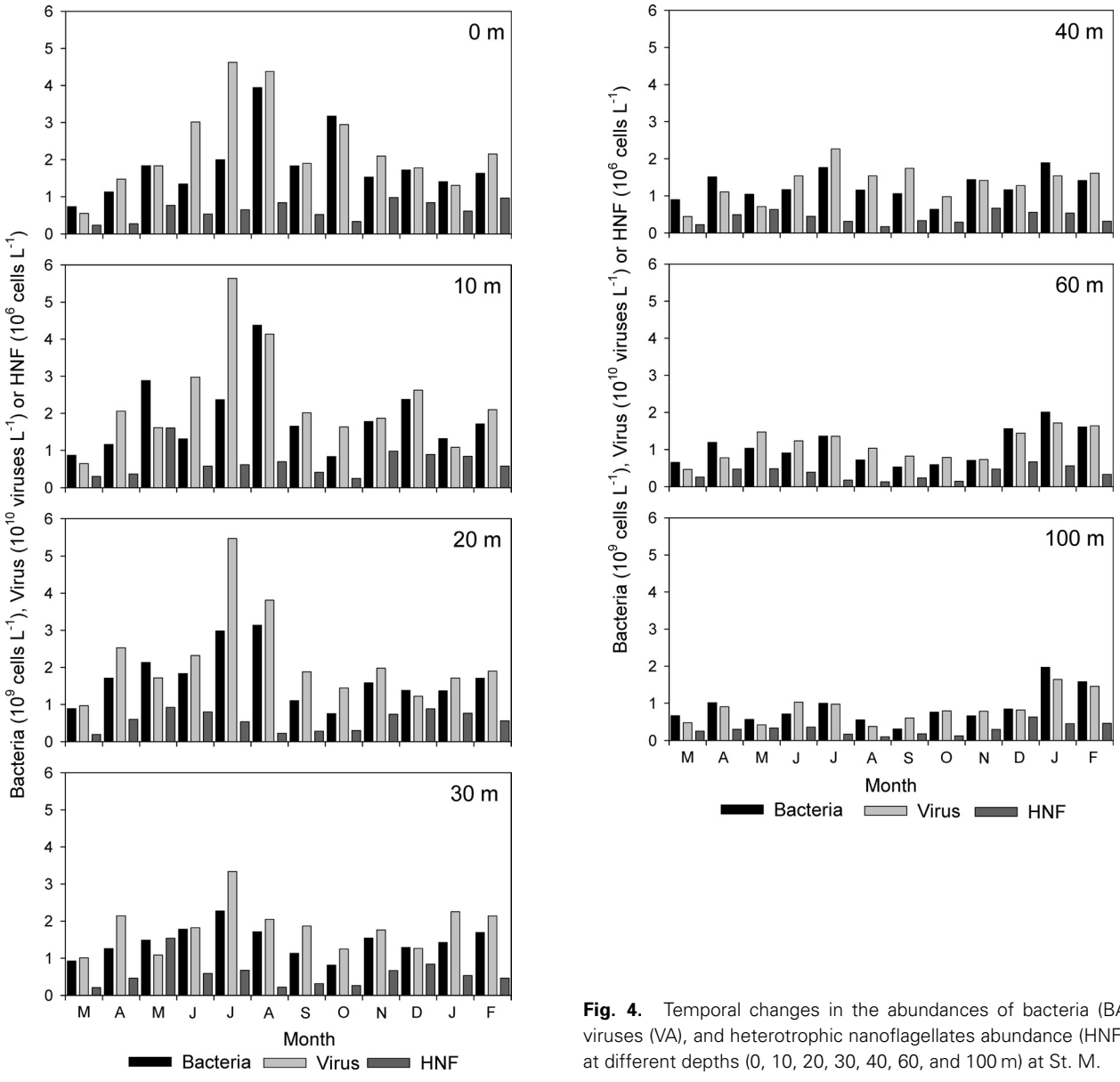


**Fig. 4.** Temporal changes in the abundances of bacteria (BA), viruses (VA), and heterotrophic nanoflagellates abundance (HNFA) at different depths (0, 10, 20, 30, 40, 60, and 100 m) at St. M.

tatively- and qualitatively-rich resource supply (i.e., high Chl *a*, rapid algal growth, and the existence of phytoplankton consumers) coupled with high water temperatures accounted for the sustained high productivity of bacteria in the stratified shallow depths during the warmer period. In contrast, BA and BP were not significantly correlated during the colder period, implies that bacterial production are temperature dependent and/or top-down control of BA dominated or both (Fig. 5 and Table 1). Strong bottom-up control of BA only during summer months has also been observed in Chesapeake Bay, USA (Shiah and Ducklow 1994).

The association of low BA, high HNFA and the strong positive relationship between BA and HNFA during the colder period supported the dominance of top-down regulation of bacterial abundance by HNF grazing (Fig. 4 and Table 1). To qualitatively address the importance of grazing by HNF, the potential grazing rate (PGR; cells $ml^{-1}h^{-1)}$ was estimated using the following equation (Vaque et al. 1994),

$$LogPGR = 3.2 + 0.99\,LogHNFA + 0.28\,T + 0..55\,LogBA \qquad (1)$$

where HNFA is the abundance of heterotrophic nanoflagellates (cells $ml^{-1}$), $T$ is water temperature (°C), and BA is bacterial abundance (cells $ml^{-1}$). The ratio of PGR to BP tended to be higher during the colder period than during the warmer period (Fig. 6), and the uncoupling of *BA* and BP during the colder period also supports this trend. In the central Adriatic Sea, Solić et al. (2001) suggested that an inconsistent relationship between BA and BP indicates that the substrate supply is saturated, and mortality factors such as bacterivory and viral lysis strongly affect bacterial communities. During the colder period in Sagami Bay, top-down effects imposed by HNF were potentially stronger than bottom-up effects in regulating bacterial communities. During seasons in which PGR

largely exceeded BP, HNF would actively consume other resources, such as suspended organic matter and phytoplankton (Sherr and Sherr 1994).

The ratios of BA to HNFA (BA/HNFA) have been used to examine the relative effects of bottom-up and top-down factors on BA and HNFA (e.g., Krstulović et al. 1997, Gasol and Vaqué 1993 ). BA/HNFA increased from the colder (2700±1000, mean±SD, n=35) to the warmer period (3900±2500, n=49) (Fig. 7A). The observed increase in BA/HNFA

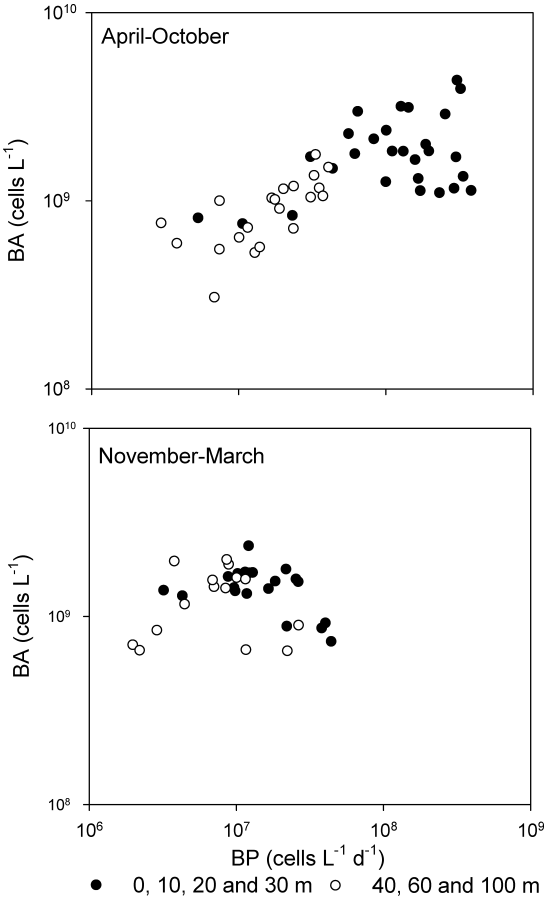


**Fig. 5.** Relationships between bacterial production (BP) and bacterial abundance (BA) during the warmer period (April to October; upper panel) and the colder period (November to March) at St. M. Closed and open circles indicate data obtained from the shallower (0, 10, 20, and 30 m) and deeper layers (40, 60, and 100 m), respectively.

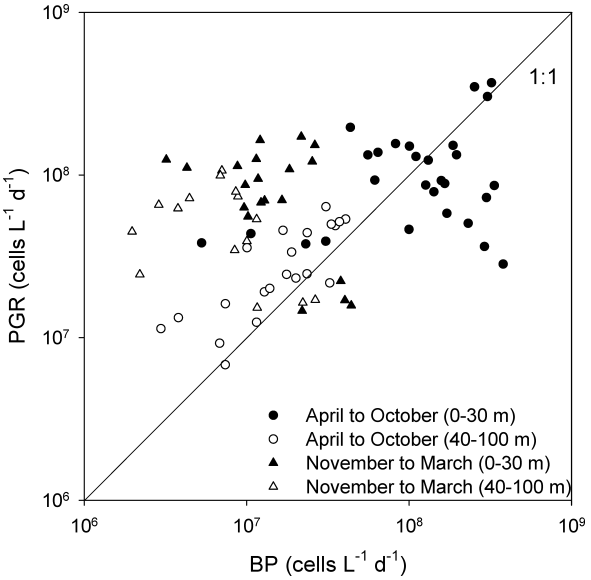


**Fig. 6.** Relationships between bacterial production (BP) and potential grazing rate (PGR) of the assemblage of heterotrophic nanoflagellates at Stn. M. PGR (cells $ml^{-1} h^{-1}$) was estimated using the equation of Vaqué et al. (1994); see text for details. Circles and triangles indicate data obtained during the warmer and colder periods, respectively. The water column was divided into a shallow (0 to 30 m, open symbols) and deep layer (40 to 100 m, closed symbols).

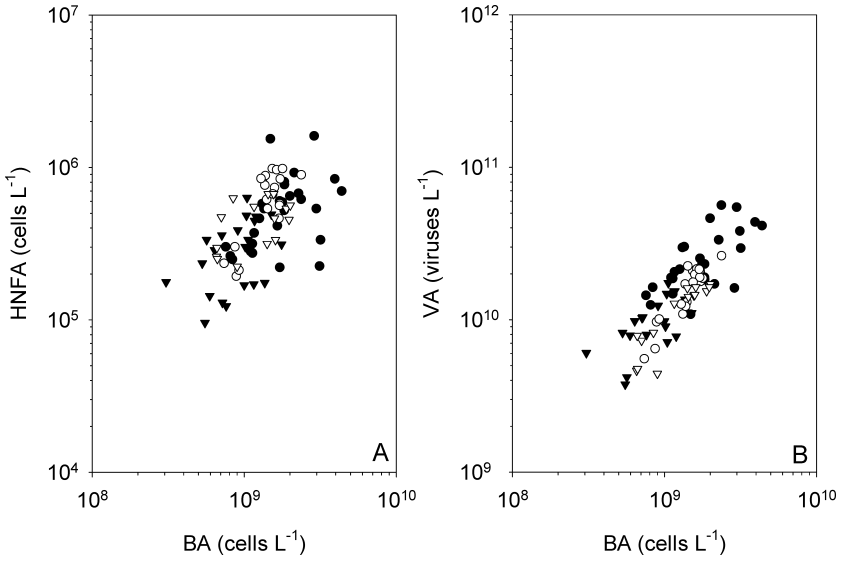


**Fig. 7.** Relationships between bacterial abundance (BA) and (A) abundance of heterotrophic nanoflagellates (HNFA), and (B) viral abundance (VA) at St. M. Circles and triangles indicate data obtained during the warmer and colder periods, respectively. The water column was divided into a shallow (0 to 30 m, open symbols) and deep layer (40 to 100 m, closed symbols).

(i.e., relative effect of bottom-up and top-down factors) from the colder to the warmer period suggested that HNFA did not immediately reduce BA as much as expected during the warmer period. This trend is consistent with the lower PGR/BP during the warmer period. The low levels of HNFA throughout the water column in the summer may have been due to top-down predatory effects on HNF (Verity 1991, Weisse 1991, Jurgens et al. 1996, Šimek et al. 1997). Dinoflagellates, such as *Ceratium furca* is abundant in Sagami Bay in the summer (Baek et al. 2006). Analysis of food vacuole contents revealed that *C. furca* rapidly ingested flagellates, ciliates found during the summer in the natural water samples of the Chesapeake Bay (Smalley et al. 1999). Smalley and Coats (2002) also reported that *C. furca* acting as mixotrophs in the Chesapeake Bay. Safi et al. (2002) reported a similar trend in Manukau Harbour, New Zealand, in that HNF populations were heavily grazed by microzooplankton on May and July and minimally grazed in October. Previous studies on ideal food chain models and laboratory culture experiments demonstrated that strong top-down control on HNF by predators can lead to the dominance of bottom-up effects on BA (Kaunzinger and Morin 1998). The observed bottom-up effect on BA during the warmer period may indicate a strong cascading top-down effect on HNF, although the impacts of viral lysis and grazing by ciliates are not clear in this study.

Spatio-temporal variation in VA paralleled fluctuation in BA. Steward et al. (1992) reported that the rate of viral production increased with increasing bacterial density in coastal and oceanic environments. Seasonal variation in VA and BA and strong positive correlations between the two variables have been reported in a variety of aquatic systems and reflect the trophic dependence of these microbes on one another (Li 1998, Wommack and Colwell 2000). Although previous studies have reported that maximum VA occurs when bacteria and phytoplankton are more abundant, the general conclusion from previous correlative studies in various environments is that Chl *a* is likely a better predictor of VA than BA (Cottrell and Suttle 1995, Maranger and Bird 1995, Wommack and Colwell 2000). However, we observed strong correlations between VA and BA and relatively weak correlations between VA and Chl a throughout the study period (Table 1), which may point to the dominance of bacteriophages in viral populations in this study area. In contrast to BA/HNFA, BA/VA during the warmer period ($0.0850 \pm 0.0326$, $n=49$) was lower than that during the colder period ($0.103 \pm 0.026$, $n=35$). Although it is difficult to evaluate the regulatory role of viral infections in BA using only correlation analyses, the high abundance of viruses and strong correlation between viruses and bacteria together with the decrease of BA/VA during the warmer period indicate that the importance of viral lysis as a source of bacterial mortality may increase during this period.

In summary, the correlational analyses suggested that the relative importance of nutrient supply (bottom-up factors) and grazing pressure (top-down factors) to the regulation of BA fluctuated temporally. During the winter-spring period, top-down regulation of bacterial abundance (likely) by HNF grazing dominated, whereas during the summer-fall period, bottom-up regulation (with high bacterial production sustained by abundant photosynthetic products and high temperatures) exceeded overall top-down effects of grazing and viral lysis. The reduction of top-down effects during the summer-fall period may have been caused by the decline in HNF grazing rather than viral lysis. Further studies are required to test this hypothesis and to reveal the mechanisms structuring microbial communities.

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## References

Albert, C., Michael, R. L. and Scott, N. 2001. Bacterial-flagellate interactions in the microbial food web of the oligotrophic subtropical North Pacific. Aquat. Micro.Ecol. 32: 283–292.

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

Beak, S. H., Shimode, S. and Kikuchi, T. 2006. Reproductive ecology of dominant dinoflagellate, *Certium furca*, in the coastal area of Sagami Bay. Coast. Mar. Sci. 30: 344–352.

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

Bode, A., Barqueros, S., Varela, M., Braun, J. G. and de Armas, D. 2001. Pelagic bacteria and phytoplankton in ocean waters near the Canary Islands in summer. Mar. Ecol. Prog. Ser. 29:1–17.

Carlson, C. A., Ducklow, H. W. and Sleeter, T. D. 1996. Stocks and dynamics of bacterioplankton in the northwestern Sargasso Sea. Deep-Sea. Res. 43: 491–516.

Cho, B. C., Choi, J.-K., Chung, C.-S. and Hong, G. H. 1994. Uncoupling of bacteria and phytoplankton during a spring diatom bloom in the mouth of the Yellow Sea. Mar. Ecol. Prog. Ser. 115: 181–190.

Cho, B. C., Park, M. G., Shim, J. H. and Choi, D. H. 2001. Sea-surface temperature and f-ratio explain large variability in the bacterial production to primary production in the yellow sea. Mar. Ecol. Prog. Ser. 216: 31–41.

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

Cottrel, M. T. and Suttle, C. A. 1995. Dynamics of lytic viruses:the effect of Micromonas pusilla virus on natural populations if the photosynthetic picoflagellate *Micromonas pusilla*. Limnol. Oceanogr. 40: 730–739.

Cushing, D. H. 1989. A difference in structure between ecosystems in strongly stratified waters and in those that are only weakly stratified. J. Plankton Res. 11: 1–13.

del Giorgio, P. A., Cole, J. J. and Cimbleris, A. 1997. Respiration rates in bacteria exceed phytoplankton production in unproductive aquatic systems. Nature. 385: 148–151.

Ducklow H. W. 1999. The bacterial content of the oceanic euphotic zone. FEMS Microb. Ecol. 30: 1–10.

Dufour, P. H. and Torreton, J. -P. 1996. Bottom-up and top-down control of bacterioplankton from eutrophic to oligotrophic sites in the tropical northeastern Atlantic Ocean. Deep-Sea Res. 43: 1305–1320.

Fujiki, T., Toda, T., Kikuchi, T., Aono, H. And Taguchi, S. 2004. Phosphorus limitation of primary productivity during the spring-summer blooms in Sagami Bay, Japan. Mar Ecol Prog Ser. 283: 29–38.

Fuhrman, J. A. and Azam, F. 1982. Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: evaluation and fields results. Mar. Biol. 66: 109–120.

Gasol, J. M., del Giorgio, P. A. and Duarte, C. M. 1997. Biomass distribution in marine planktonic communities. Limnol. Oceanogr. 42: 1353–1363.

Gasol, J. M. and Vaqué, D. 1993. Lack of coupling between heterotrophic nanoflagellates and bacteria: A common phenomenon across aquatic systems? Limnol. Oceanogr. 38: 657–665.

Hyun, J.-Ho. and Kim, K.-Ho. 2003. Bacterial abundance and production during the unique spring phytoplnakton bloom in the central Yellow Sea. Mar. Ecol. Prog. Ser. 252: 77–88.

Jonas, R. B. and Tuttle, J. H. 1990. Bacterioplankton and Organic carbon Dynamics in the Lower Mesohaline Chesapeake Bay. Appl. Environ. Microbiol 56: 747–757.

Jürgens, K., Wickham, S. A., Rothhaupt, K. O. and Santer, B. 1996. Feeding rates of macroandmicrozooplankton on heterotrophic nanoflagellates. Limnol. Oceanogr. 41: 1833–1839.

Kanda, J., Fujiwara, S., Kitazato H. and Okada , Y., 2003. Seasonal and annual variation in the primary production regime in the central part of Sagami Bay. Prog. In Oceanography 57:17–29.

Kaunzinger, C. M. K. and Morin, P. J. 1998. Productivity controls food-chain properties in microbial communities. Nature 395: 495–497.

Kirchman, D. L. 2000. Microbial ecology of the oceans published by A John Willey & Sons, Inc. pp. 103–105.

Krichman, D. L. and Rich, J. H. 1997. Regulation of bacterial growth rates by dissolved organic carbon and temperature in the equatorial Pacific Ocean. Microb. Ecol. 33: 11–20.

Krstulović, N., Šolić, M. and Marasović, I. 1997. Relationship between bacteria, phytoplankton and heterotrophic nanoflagellates along the trophic gradient. Helgolander Meeresunter. 51: 433–443.

Lee, C. W., Kudo, I., Yanada, M. and 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.

Li, W. K. W. 1998. Annual average abundance of heterotrophic bacteria and Synecbococcus in surface ocean waters. Limnol. Oceanogr. 43: 1746–1753.

Maranger, R. and Bird, D. F. 1995. Viral abundances in aquatic systems: a comparison between marine and fresh waters. Mar. Ecol. Prog. Ser. 121: 217–226.

McManus, G. B. and Fuhrman, J. A. 1988. Control of marine bacteriplankton populations: measurement and significance of grazing. Hydrobiol. 159: 51–62.

McQueen, D. J., Post, D. J. and Mills, R. J. 1986. Trophic relationships in freshwater pelagic ecosystems. Can. J. Fish. Aquat. Sci. 43: 1517–1581.

Naganuma, T. 1997. Abundance and Production of Bacterioplankton along a Transect of Ise Bay, Japan. J. Oceanogr. 53: 579–583.

Nagata, T. 1984. Bacterioplanlton in Lake Biwa: annual fluctuations of bacterial numbers and their possible relation with some environmental variables. Jpn. J. Limnol. 45: 126–133.

Nagata, T., Fukuda, R., Fukuda, H. And Koike, I. 2001. basin-Scale Geographic Patterns of Bacterioplankton Biomass and Production in the Subarctic Pacific, July–September 1997. J. Oceanogr. 57: 301–313.

Noble, R. T. and Fuhrman, J. A. 1998. Use of SYBR Green I for rapid epifluorescence counts of marine viruses and bacteria Aquat. Microb. Ecol. 14: 113–118.

Proctor, L. M. and Fuhrman, J. A. 1992. Viral morality of marine bacteria and cyanobacteria. Nature. 343: 60–62.

Rassoulzadegan, F. and Sheldon, R. W. 1986. Predator–prey interaction of nanozooplankton and bacteria in an oligotrophic marine environment. Limnol. Oceanogr. 31: 1010–1021.

Safi, A. K., William, N. V. and Julie, A. H. 2002. Growth and grazing within the microbial food web of a large coastal embayment. Aquat. Microb. Ecol. 29: 39–50.

Sanders, R. W., Caron, D. A. and Berninger, U. G. 1992. Relationships between bacteria and heterotrophic nanoplankton in marine and fresh waters: an inter-ecosystem comparison. Mar. Ecol. Prog. Ser. 86: 1–14.

Sherr, B. F. and Sherr, E. B. 1983. Enumeration of heterotrophic micro-protozoa by epifluorescent microscopy. Estuar. Coast. Shelf Sci. 16: 1–7.

Sherr, B. F., Sherr, E. B. and McDaniel, J. 1992. Effect of protistan grazing the frequency of dividing cells in bacterioplankton assemblages. Appl. Environ. Microbiol. 58: 2381–2385.

Sherr, E. B. and Sherr, B. F. 1994. Bacterivory and herbivory: Key roles of phagotrophic protistis in pelagic food webs. Microb. Ecol. 28: 223–235.

Shiah, F. K. and Ducklow, H. W. 1994. Temperature regulation of heterotrophic bacterioplankton abundance, production and specific growth rate in Chesapeake Bay. Limnol. Ocenogr. 39: 1243–1258.

Šimek, K., Hartman, J., Pernthaler, D., Springmann, J. V. and Paenner, R. 1997. Community structure, picoplankton grazing and zooplankton control of heterotrophic nanofalgellates in a eutrophic reservoir during the summer phytoplankton maximum. Aquat. Microb. Ecol. 12: 49–63.

Simon, M., Tilzer, M. M. and Muller, H. 1998. bacteriplankton dynamics in a larger mesotrophic lake: I. Abundance, production

and growth control. Arch Hydrobiol. 143: 385–407.

Smalley, G. W. and Coats, D. W. 2002. Ecology of the red tide dinoflagellate *Ceratium furca*: distribution, mixotrophy, and grazing impact on ciliate populations of Chesapeake Bay. J. Eukaryot Microbiol. 49: 64–74

Smalley, G. W., Coats, D. W. and Adam, E. J. 1999. A new method using fluorescent microspheres to determine grazing on ciliates by the mixotrophic dinoflagellate *Ceratium furca*. Aqua Microbi Ecol. 17: 167–179.

Šolić, M., Krstulović, N. and Šestanović, S. 2001. The roles of predation, substrate supply and temperature in controlling bacterial abundance: interaction between spatial and seasonal scale. Acta Adriat. 42: 35–48.

Steward, G. Wikner, J., Cochlan, W. P., Smith, D. C., and Azam, F. 1992. Estimation of virus production in the sea: II. Field results. Mar. Microb. Food Webs 6: 79–90.

Suttle, C.A. 1994. The significance of viruses to mortality in aquatic microbial community. Microb. Ecol. 28: 237–243.

Suzuki, R. and Ishimaru, T. 1990. An improved method for the determination of phytoplankton Chlorophyll using N, N-Dimethylfolmamide. J. Oceanogr. Soc. Jpn 46: 190–194.

Tanaka, T. and Rassoulzadegan, F. 2004. Vertical and Seasonal variations of bacterial abundance and production in the mesopelagic layer of the NW Mediterranean Sea: bottom-up and top-down controls. Deep-Sea Res. 51: 531–544.

Teira, E., Pazó, M. J., Auevedo, M., Fuentes, M. V., Niell, F. X. and Fernádez, E. 2003. Rates of dissolved organic carbon production and bacterial activity in the eastern North Atlantic Subtropical Gyre during summer. Mar. Ecol. Prog. Ser. 249: 53–67.

Vaqué, D., Gasol, J. M. and Marrase, C. 1994. Grazing rates on bacteria: the significance of methodology and ecological factors Mar. Ecol. Prog. Ser. 109: 263–274.

Vázquez-Dominguez, E., Gasol, J. M., Agusti, S., Duarte, C. M. and Vaqué, D. 2005. Growth and grazing losses of prokaryotes in the central Atlantic Ocean. J. Plankton Res. 27: 1055–1066.

Verity, P. G. 1991. Feeding in planktonic protozoans:evidence for non-random acquisition of prey. Mar. Microb. Food Webs. 5: 69–76.

Weissse, T. 1991. The annual cycle of heterotrophic fresh water nanofalgellates: role of bottom-up versus to-down control J. Plankton. Res. 13: 167–185.

Wommack, K. E. and Colwell, R. R. 2000. Virioplankton: Viruses in Aquatic Ecosystems. Microb. Mole.Biol. Revie. March pp. 69–114.