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Seasonal and daily changes in bacterivory in a coastal plankton community

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ABSTRACT: Fluorescently labeled bacteria (FLB) were used to examine the long-term changes (monthly for 8 mo) and short-term changes (daily for 10 d) of bacterial grazing in a natural plankton community. Two preliminary experiments also were performed to evaluate the effect of sample volume, incubation time, and light regime on the results. Experimental results indicate a 'bottle effect' for the 3 smallest sample volumes employed (100, 200, 500 ml); lower grazing rates were always observed during 24 h incubations than during 48 h incubations. The light regime (continuous darkness, continuous light, 12L:12D) did not affect grazing rates during a 48 h experimental period. Bacterivory in the seasonal study in Vineyard Sound, Massachusetts (USA) was highly responsive to water temperature and was the primary factor determining grazing pressure over the winter-summer period. Samples incubated at 20°C during winter consistently exhibited higher rates of consumption of bacteria than a triplicate set of samples incubated at ambient water temperature or 5°C. Daily fluctuations in the rates of bacterivory during a 10 d period in May were significant, but were ca 1/10 the magnitude of the seasonal fluctuation. Maximal rates of bacterivory removed up to 60% of the bacterial assemblage daily.

INTRODUCTION

The contribution of bacteria to the total biomass of most aquatic communities is highly significant (Ferguson & Rubblee 1976, Watson et al. 1977, Holligan et al. 1984, Fuhrman et al. 1989). Because of this, quantifying the rates of consumption of these microorganisms has been perceived as essential for modeling energy and nutrient flow in these ecosystems (Azam et al. 1983, Cole et al. 1988). Numerous studies have been conducted to investigate the rates of ingestion of bacterivorous organisms in a wide variety of planktonic environments. Most of these studies, however, have examined the feeding rates of the bacterivore community at a particular time of year, and have stressed the spatial differences in bacterivore activity rather than temporal fluctuations in this activity. Rarely have studies been conducted to examine the seasonal changes in bacterivory (Pace et al. 1990). This information is important, however, particularly in light of the controlling role that temperature plays in the activity of natural bacterial assemblages (Pomeroy & Wiebe 1988). Moreover, the magnitude of the short-term (day-to-day) changes, relative to the seasonal fluctuations in grazing pressure, is poorly known.

The processes affecting bacterivory at these 2 temporal scales, seasonal and daily, may be fundamentally different. Seasonal changes in microbial activity in a temperate environment would be expected to be strongly affected by physical parameters, most notably temperature, which could directly affect the metabolic rates of microorganisms or result in changes in the species composition and density of the bacteria and bacterivore assemblages. Short-term changes in grazing activity, in contrast, might be less affected by physical parameters, but may be responsive to biological features of the community (i.e. rapid oscillations in the abundances of bacteria and their consumers due to predator-prey relationships within the plankton). Transient storm events also may significantly affect microbial processes on short time scales. The magnitudes of these daily and seasonal fluctuations in bacterivore grazing pressure in a particular environment are virtually unknown.

This study was designed to analyze and compare short-term and long-term fluctuations of bacterivory

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in a coastal plankton community. Experiments were conducted with seawater from Vineyard Sound, Massachusetts (USA), to establish the magnitude of the seasonal variation in bacterivore activity, and to compare this variation with the daily fluctuations in the grazing pressure occurring during a warm-water period. For these studies we employed fluorescently labeled bacteria (FLB) to estimate bacterivory. Most studies of bacterial grazing to date employing FLB have been directed at evaluating the clearance rates of specific microorganisms in plankton assemblages (Sherr et al. 1987, Sherr et al. 1989a, b, Gonzalez et al. 1990). These studies were based on an accurate determination of the uptake of labeled bacteria by consumers. Such investigations have provided detailed and valuable information on the species composition of the bacterivore assemblage and the grazing rates of specific groups but, because the entire grazer community can rarely be examined, the results of these studies have been difficult to extrapolate to estimates of total bacterivory in the water sample.

We have employed a method to evaluate total bacterivore grazing activity which relies on the disappearance of FLB from a water sample over an extended incubation period (24 to 48 h). Based on the rate of disappearance of FLB and the density of natural bacteria, bacterial grazing was quantified monthly for an 8 mo period, and daily for a 10 d period during late spring. Two experiments were conducted prior to the grazing study to examine the implications of sample volume and experimental conditions (light regime and length of incubation time) on the results.

METHODS

Sample collection and processing. All samples employed in this study were collected from Vineyard Sound at the Woods Hole Oceanographic Institution Shore Laboratory facility, Woods Hole, Massachusetts (USA). Samples were collected from 0 to 1.0 m depth. All samples employed for cell counts in this study were preserved with 10% glutaraldehyde (final concentration of 1%) prepared in Vineyard Sound Seawater and filtered through 0.22 µm pore size Millipore filters. All bacterial grazing rates were obtained by measuring the rate of disappearance of fluorescently labeled bacteria (FLB) in seawater samples. FLB were prepared from late stationary phase cultures of the bacterium *Pseudomonas halodurans* according to the procedure of Sherr et al. (1987). These starved cells were small in size (cell volumes ≈ 0.1 to 0.3 μm^3; cell lengths ≈ 0.5 to 1.2 µm) relative to bacteria from the exponential growth stage, and more closely resembled the size of natural bacterioplankton in this system. Labeled cells were added to natural seawater samples at concentrations that were 20 to 50% of the abundance of natural bacteria.

FLB and total bacterial abundances were determined by epifluorescence microscopy (Leitz Laborlux) on 1 ml subsamples stained with 4',6-diamidino-2-phenylindole (DAPI) stain and filtered onto black Nuclepore (0.2 µm pore size) filters. Twenty fields were examined from 4 diametral transects on each slide. The number of bacteria counted in each slide varied from 400 to 600. Nanoflagellates were also enumerated by epifluorescence microscopy on 5 to 15 ml DAPI-stained subsamples (Porter & Feig 1980) filtered onto black (0.8 µm pore size) Nuclepore filters. At least 100 flagellates were counted in each data point examining 20 to 30 fields of 3 slides.

We calculated the grazing and net growth rates (g and a respectively) and the number of bacteria grazed as follows:

$$F_t = F_0\, e^{-gt}$$
$$N_t = N_0\, e^{at}$$

where F_t and F_0 = number of FLB at time t and time 0; N_t and N_0 = number of natural bacteria at time t and time 0. Then,

$$g = -1/t\ (\ln[F_t/F_0])$$
$$a = 1/t\ (\ln[N_t/N_0])$$

Assuming that FLB and natural bacteria are removed at the same rate, the number G of natural bacteria grazed during time t can be calculated as follows:

$$G = -g/a\ (N_t - N_0),$$

and the total number TG of bacteria grazed during time t is:

$$TG = F_0 - F_t + G.$$

Several extensive laboratory experiments were carried out with natural samples prior to the long-term and short-term bacterivory experiments in order to establish the most appropriate conditions for conducting these grazing studies. The preliminary experiments examined the effects of the volume of the incubated sample (to examine the 'bottle effect'), the length of the incubation period and the light regime on the rates of bacterivory in Vineyard Sound seawater.

Incubation volume. Seawater was collected from the surface waters of Vineyard Sound on August 10, 1989. Six different volumes (100 ml, 200 ml, 500 ml, 1 l, 2 l, 20 l) of seawater were incubated at 19 °C in continuous light (fluorescent light at 230 to 260 µEinsteins $m^{-2}\ s^{-1}$). All incubation vessels were composed of clear polycarbonate. The 100 and 200 ml volumes were incubated in 250 ml flasks, the 500 ml, 1 l and 2 l aliquots were incubated in 2.5 l flasks, and the 20 l volume was

incubated in a 21 l carboy. Surface:volume ratios were 0.95, 0.81, 0.55, 0.45, 0.39 and 0.17 for the 100 ml, 200 ml, 500 ml, 1 l, 2 l and 20 l volumes, respectively. Quadruplicate containers of each volume were incubated and examined. The caps were kept loose and the containers were not stirred during the incubations. Samples were removed initially and every 6 h over a 36 h period for the enumeration of FLB.

Incubation time and light regime. The effect of the light regime and the sampling time on the rates of bacterivory were examined using 20 l aliquots of Vineyard Sound seawater incubated in clear polycarbonate carboys. Seawater was collected on October 11, 1989, and incubated at 19°C. Aliquots were incubated in continuous darkness, continuous light (fluorescent light at 230 to 260 μEinsteins m^{-2} s^{-1}), or in a 12h/12h light/dark cycle. A set of 1 l aliquots also was incubated in the light/dark cycle. Duplicate aliquots were incubated for each treatment. Samples were taken from each container initially, and after 12, 24, 36 and 48 h of incubation to examine the effect of incubation time on the rates of bacterivory.

Long-term (seasonal) and short-term (day-to-day) grazing measurements. Based on the results of the preliminary experiments described above, an experimental procedure was designed to examine the long-term and short-term fluctuations in bacterivory in Vineyard Sound. Seasonal changes in the rate of bacterivory were determined from samples collected monthly for 8 mo beginning in December 1989. Short-term changes were determined from samples collected daily for a 10 d period beginning May 16, 1990.

All samples were 1 l volumes incubated in 2 l clear polycarbonate flasks. In all experiments conducted, we collected one sample from the field which was divided into subsamples. Samples were incubated at 2 temperatures, ambient (or 5°C, whichever was higher) and 20°C. Winter samples were not incubated below 5°C because of the difficulties with maintaining an ice-free incubation system. The light regime for all experimental periods was a 12h/12h light/dark cycle (fluorescent light at 230 to 260 μEinsteins m^{-2} s^{-1}). Triplicate samples were incubated at each temperature. FLB concentrations were determined from samples taken immediately following the addition of FLB to the samples, and from samples taken after 48 h of incubation.

Control experiments were conducted to examine the possibility of losses of FLB that were unrelated to grazing. These experiments consisted of incubating 3 volumes of 1 l of autoclaved sea water at 5 and 20°C. The light intensity and regime were identical as in the long term and short term grazing measurements. The quantity of FLB introduced in each control volume was the same as that introduced into the experimental containers. We determined the FLB concentrations from samples taken immediately following the addition of FLB, and from samples taken after 48 h. No significant differences between FLB concentrations at $t = 0$ and at $t = 48$ h were observed.

Chlorophyll concentrations for these samples were determined from subsamples filtered onto 0.22 μm Millipore GS filters, extracted in 90% acetone overnight at 5°C, and read on a Turner Fluorometer Model 111.

RESULTS

Investigations of the experimental design

The volume of sample incubated had a measurable effect on the disappearance of fluorescently labeled bacteria in our study. The most apparent effect of incubation volume was the effect on replicability. There was considerably more variability in the rates of bacterivory observed among the 4 replicate flasks for each of the 3 smallest volumes examined than for the largest volumes. In addition, the average rates of bacterivory (total bacteria consumed per unit volume per unit time), calculated from the rate of disappearance of FLB and the abundance of the natural bacterial assemblage, were lower for the 3 smaller volumes (100, 200, 500 ml) relative to the rates measured in the 3 larger volumes (Fig. 1). These differences were significant (1-way ANOVA, $p < 0.05$), however, only between the 500 ml volume and the 3 largest volumes (1, 2 and 20 l). Moreover, the abundance of natural bacteria did not change significantly in vessels with sample volumes ≥ 500 ml, but increased significantly over a 36 h period in the 100 and 200 ml vessels (increase of 1.5× and 2.7×, respectively).

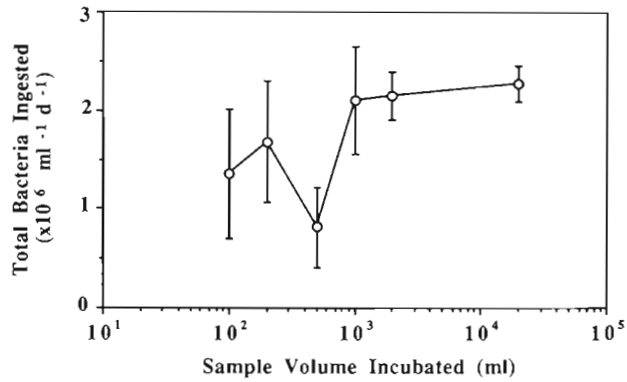

Fig. 1. Effect of sample volume on rates of bacterivory in Vineyard Sound seawater. Experiment conducted August 10, 1989. Error bars: ± 1 SE of 4 replicate flasks at each volume

The surface:volume ratio of the incubation vessels

varied with the different incubation volumes in our experiment (see 'Methods'). We cannot, therefore, conclusively establish whether the effect on grazing rates was due to sample size itself, the surface:volume ratio, or a combination of both factors. We considered that the incubation in a 20 l vessel was the best approximation (if not the ideal) because, due to the large volume, the plankton assemblages were better represented and the surface:volume ratio was the lowest. Because grazing rates in the 20 l and the 1 l volumes were not significantly different (see below), we chose the 1 l volumes for conducting our experiments thereafter because of the convenience of the smaller volume.

The effect of incubation time on the grazing measurements was examined in order to determine the shortest period of time over which significant changes in FLB density (and, thereafter, grazing rates) could be measured. As a conservative measure we calculated grazing rates only when the average rate of the replicates was higher than twice the standard deviation. The time period required to obtain this condition was dependent upon the experimental conditions (most notably temperature), but a minimum period of 24 h was generally required even during warm water periods. Decreases in FLB concentration after 12 h of incubation were, in general, not significant.

The length of the incubation period affected the rate of bacterivory determined by the disappearance of FLB (Fig. 2). Grazing rates averaged for the duplicate incubation vessels in each treatment were always lower for the 0 to 24 h period than for the 0 to 48 h period, but these differences were significant ($p < 0.05$) only for the 20 l aliquots in continuous darkness. Because we found much lower rates of bacterivory in Vineyard Sound during winter, 48 h incubations were employed in all subsequent experiments to maintain consistency.

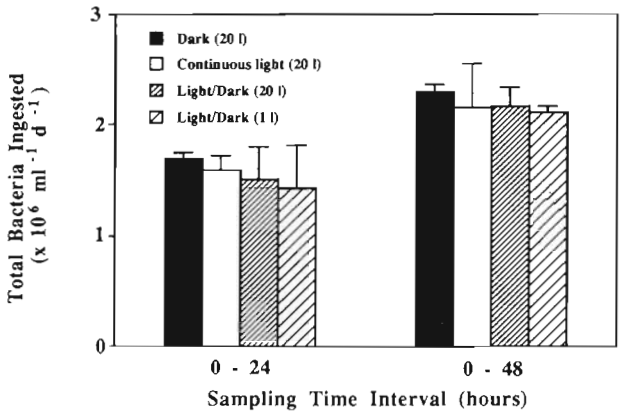

Fig. 2. Effect of incubation period (0 to 24 h, or 0 to 48 h) and light regime (continuous light, 12 h/12 h light/dark cycle, or continuous darkness) on rates of bacterivory in Vineyard Sound seawater. Experiment conducted October 11, 1989. Error bars: ± 1 SE of duplicate flasks

For a particular incubation period (either 24 or 48 h), the light regime had virtually no effect on the rate of bacterivory (Fig. 2). Samples incubated in continuous light, continuous dark or in a 12 h/12 h light/dark cycle showed no significant differences in the rate of disappearance of FLB for a given incubation period. There were no significant differences in the grazing rates between 1 and 20 l incubation volumes in this experiment (Fig. 2).

Grazing studies

Monthly grazing measurements were conducted in Vineyard Sound water over a time period that spanned the annual temperature range (≈ 1 to 20 °C; Fig. 3A). Water temperature remained cold (< 5 °C) for the first 4 monthly samples, and then rose steadily for the next 4 mo. A large diatom bloom occurred during the study and was apparent as a single large peak in chlorophyll *a* concentration in the February sample. Bacteria and heterotrophic nanoplankton (HNAN) also increased in abundance during the spring, and a large peak in the abundance of both assemblages also occurred during the February phytoplankton bloom (Fig. 3B).

Rates of bacterial removal (bacteria consumed ml^{-1} d^{-1}) during the seasonal study (Fig. 3C) ranged from ca 4×10^4 to 2×10^6 bacteria ml^{-1} d^{-1}, and were correlated with water temperature. The correlation coefficient between temperature and the rate of bacterial removal in incubations at ambient temperatures was 0.90, n = 8. Triplicate incubations, at 5 °C (or ambient, see 'Methods') and 20 °C, were carried out on 7 of the 8 sampling dates (water temperature in July was ≈ 20 °C). Grazing rates for the samples at 5 °C or ambient were significantly lower than the corresponding samples at 20 °C in 5 of the 7 comparisons ($p < 0.05$). These latter 5 samples included all the samples for which the in-situ temperature was less than 14 °C. The largest difference between samples incubated at 5 and 20 °C occurred for the December sample (≈ a factor of 20×). The rates of bacterial removal observed in the samples incubated at ambient temperatures or (5 °C, see 'Methods') varied by a factor of ca 25× over the course of the study (filled squares in Fig. 3C). Lowest values occurred in the winter (with the exception of February during the phytoplankton bloom) and highest values occurred in the summer. The samples collected over the seasonal study period but incubated at 20 °C, by contrast, varied by only a factor of ca 3×, and showed little or no relationship to the season.

Rates of bacterial removal were used to estimate the percentage of the natural bacterial assemblage that could be removed by grazing each day (Fig. 3D). Values ranged from 3 % up to 41 % for the samples incubated at 5 °C or ambient temperature and increased

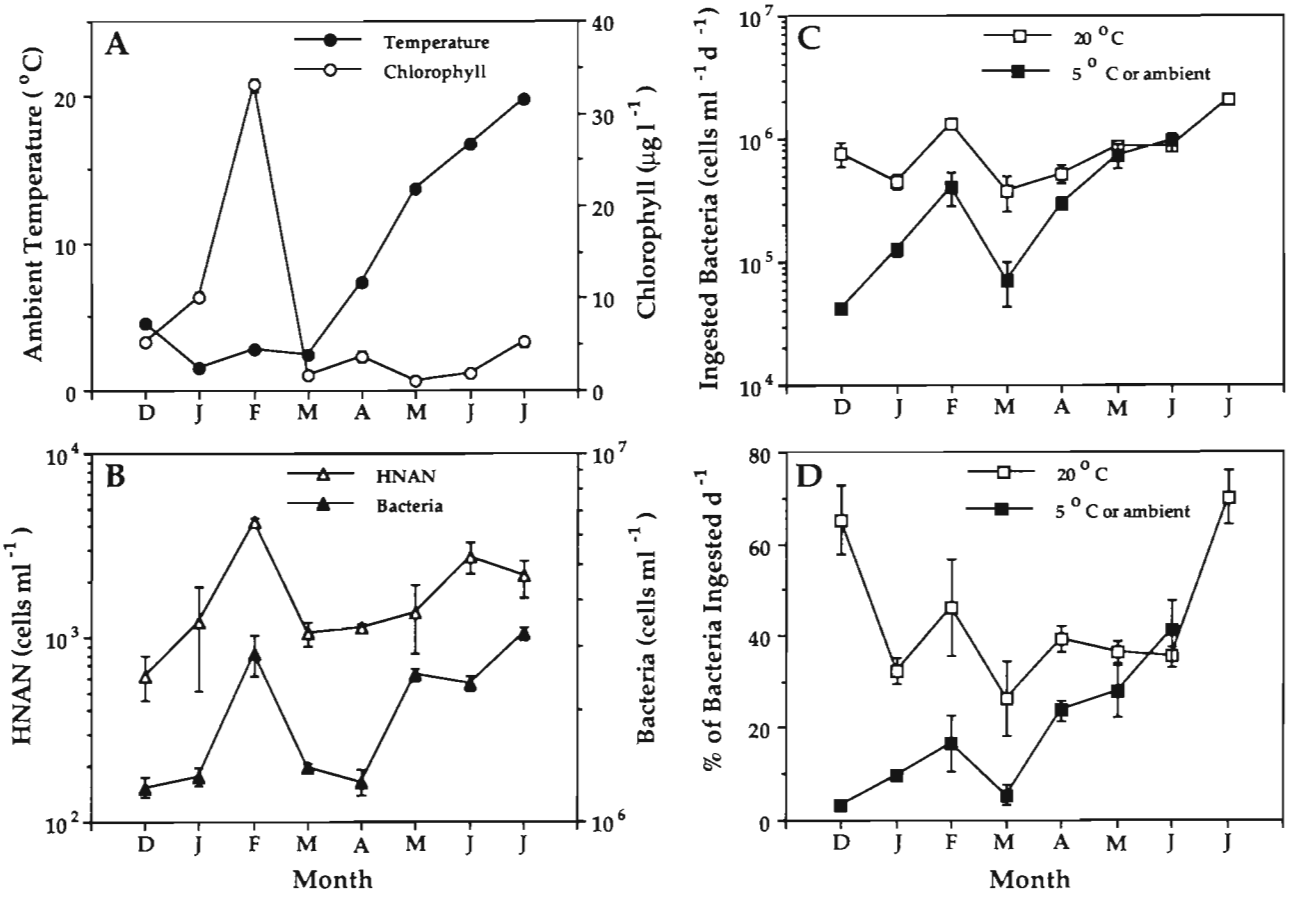

Fig. 3. Seasonal changes in the ambient water temperature and chlorophyll concentration (A), bacterial and heterotrophic nanoplankton (HNAN) density (B), total number of bacteria ingested ml^{-1} d^{-1} in 2 experimental temperature regimes (C), and calculated percentage of the natural bacterial population grazed d^{-1} in 2 experimental temperature regimes (D). Samples used for the measurement of rates of bacterivory (C and D) were incubated at 5°C or ambient water temperature, whichever was higher
Error bars: ± 1 SE of triplicate flasks

steadily from winter to summer. The values for the samples incubated at 20°C ranged from 26% up to 65% and showed no relationship to season. Percentages of the bacterial assemblage removed per day during the winter-spring period were always significantly greater for the samples incubated at 20°C compared to the corresponding samples incubated at 5°C or ambient temperature. The implication of this result is that the potential for the bacterivore assemblage to impact significantly the bacterial community was present in the winter samples but its activity was restrained by the water temperature. As the water temperature increased to 13 to 15°C this effect was no longer apparent.

The effect of incubation temperature on the rate of bacterial growth in the samples was qualitatively similar to its effect on the rate of bacterivory (Fig. 4). Bacterial growth rates were estimated from changes in the bacterial densities during the incubations corrected for losses due to the removal of bacteria by grazers

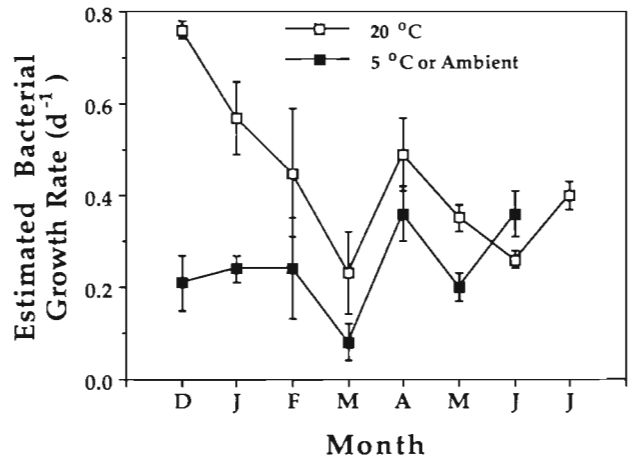

Fig. 4. Estimated bacterial growth rates during the seasonal study. Growth rates were calculated from changes in bacterial abundances between the beginning and end of each incubation, and the number of bacteria consumed during each incubation (determined by FLB disappearance)

during the incubations (estimated from FLB disappearance). The growth rates of the bacterial assemblages in all of the monthly experiments performed December to May were greater in the samples incubated at 20°C than in identical samples incubated at ambient temperature or 5°C. The magnitude of this difference was greatest for the winter samples.

The temperature variation during the 10 d grazing study in May was less than 2°C for the entire period (Fig. 5A). A small peak in chlorophyll *a* concentration occurred on the 6th day of sampling following a storm but the magnitude of this peak was quite small compared to the seasonal range in chlorophyll concentration (Fig. 3A). Bacterial and heterotrophic nanoplankton abundances fluctuated significantly over the 10 d period (Fig. 5B). The largest change in these abundances coincided with the storm event and may have been partly a consequence of resuspension. Bacterial abundances were higher after the chlorophyll peaked, and we observed coinciding with that (although we did not quantify it) a proliferation of lamelibranchia larvae which may have impacted the bacterivore populations.

The grazing activities of the bacterivores during the 10 d study at ≈ 13 to 15°C removed ≈ 5×10^5 bacteria ml^{-1} d^{-1} on average (Fig. 5C). The daily rate, however, varied by a factor of ca 3 over the 10 d period. A similar degree of variability was observed in the percentage of the bacterial assemblage removed by grazing each day (Fig. 5D).

DISCUSSION

Experimental design

Virtually all grazing-rate methods in common use today require the containment and incubation of water samples for some period of time. In practice, this constraint has resulted in a compromise between the

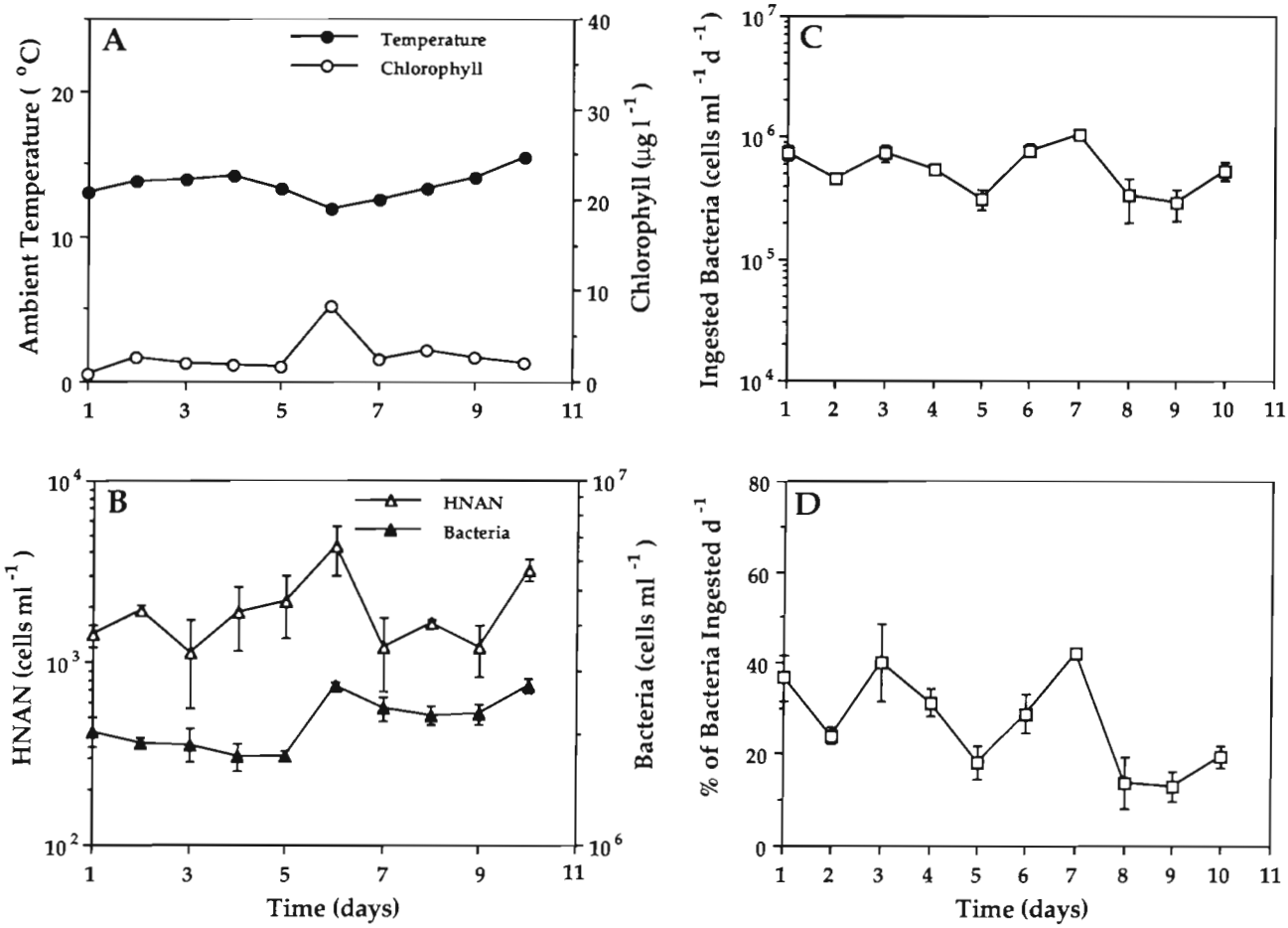

Fig. 5. Daily changes in ambient water temperature and chlorophyll concentration (A), bacterial and heterotrophic nanoplankton (HNAN) density (B), total number of bacteria ingested ml^{-1} d^{-1} (C), and calculated percentage of the natural bacterial population grazed d^{-1} (D). Error bars: ± 1 SE of triplicate flasks

desired accuracy of the measurement on the one hand, and the potential for altering the community's activity on the other hand. In the absence of 'ideal' conditions, it is imperative to try to establish the extent to which a particular experimental protocol might introduce artifacts into these measurements.

It is clear from the results of our study that sample size (i.e. the volume of water incubated) and length of the incubation period may be important considerations in the design of microbial grazing studies. This result is not a new finding. Only rarely, however, have the effects of these potential artifacts been quantitatively examined. Based on the results of our experiment, we conclude that the rates of bacterivory in small (< 1 l) sample volumes may underestimate grazing rates (Fig. 1).

Probably the most important finding of the 'bottle effect' experiment was the demonstration of a large variability among replicate vessels for small incubation volumes (< 1 l). The cause for this result is unknown but may relate to features of the microbial community that were not addressed by the small volumes (i.e. patchiness, aggregates, presence or absence of some rare organisms, etc.). The poor replicability of the microbial processes in the 3 smallest volumes is an important consideration for experimental design because this large variability makes it more difficult to obtain significant differences when examining the effects of different experimental treatments.

Increasing the incubation period from 24 to 48 h had only a small effect on the grazing rates measured in this study (Fig. 2). Changes in the abundance of bacteria may have contributed to this result. Although the effect of increasing incubation time was not significant in a light/dark regime, it is still possible that the use of a 48 h incubation period for the seasonal and short-term grazing measurements in the present study may have slightly overestimated the actual rates of bacterivory.

The size of the FLB also could have affected the observed grazing rates. Our FLB were more uniform in size than the natural bacterial assemblages and equivalent to the larger bacteria in most samples. The addition of FLB at a significant fraction of the abundance of natural bacteria also could have increased grazing rates somewhat. Although these considerations would not have affected our comparisons between treatments they may have affected the absolute rates of bacterial removal calculated in this study. Nonetheless, the rates observed in this study ($\approx 4 \times 10^4$ to 2×10^6 bacteria consumed ml^{-1} d^{-1}) were well within the range of published rates for total community grazing (Fenchel 1982b, Landry et al. 1984, Wright et al. 1987, Pace 1988, Bloem & Bar-Gilissen 1989, Sherr et al. 1989a, Weisse 1989, Wikner et al. 1990).

Other studies have obtained information that indicates that microbial grazing rates may exhibit diel periodicity, although the time of maximal grazing pressure seems to be questionable (Waterbury et al. 1986, Wikner et al. 1990). We examined in this study the grazing rates over 24 and 48 h periods, hence we could not elucidate any diel periodicity. Nevertheless it is interesting that the daily rates of bacterivory were not strongly affected by the imposed light regime (Fig 2).

Bacterivory in Vineyard Sound

Temperature dramatically affected the activity of the bacterivore assemblage of Vineyard Sound during this study. Day-to-day fluctuations in the grazing rate were observed during the short-term study in May, but these fluctuations were not of the magnitude experienced during the seasonal study (the coefficient of variation, CV, for ingested bacteria per day was 116 % for the monthly data and 43 % for the daily data).

The important effect of temperature was clearly evident by comparing the results of the monthly samples incubated at ambient water temperature (or 5 °C) and 20 °C (Figs. 3C and D). The temporal variability of the amount of bacteria removed per day was reduced when the incubation temperature was higher (CV for the samples incubated at ambient temperature was 116 %, and for the incubations at 20 °C it was 62 %). The grazer assemblage present during the winter months had consistently higher rates of consumption at the higher incubation temperature. The grazing rates observed in the samples incubated at 20 °C during winter were comparable to the rates observed in summer samples. Moreover, we observed a significant positive correlation between bacterial removal and temperature for the monthly samples incubated at ambient water temperature. We infer from these results that the bacterivores present during winter possessed the potential to significantly impact the bacterial assemblage but their activities were depressed by low temperature.

Based on the estimated percentage of the bacterial assemblage removed each day, and considering that bacterial growth replaced losses due to grazing, the turnover times for the bacterial community in winter may be as long as 3 wk, while turnover times in summer may be on the order of 2 d. These estimates are in good agreement with a recent summary of 30 studies in which it was concluded that the average turnover time for planktonic bacterial assemblages was 4.5 d (Pace 1990).

Bacterial growth also was depressed by the water temperature during winter (Fig. 4). The incubation of winter samples at 20 °C resulted in bacterial growth rates that were more than 3× the rates at 5 °C. It is possible that the activity of the bacterivore community

was, in part, a response to this seasonal depression in bacterial productivity. There is evidence that bacterivores require a minimal bacterial abundance to benefit from feeding (Fenchel 1980), although the threshold at which this occurs has been a point of discussion (Sherr & Sherr 1987).

It is possible that some of the effect of temperature that we observed in this study was the result of an indirect effect mediated by other components of the microbial food web. For example, phytoplankton growth could have been significantly affected by increasing the water temperature which in turn may have affected the rate of substrate supplied to the bacterial community during the incubations. In addition, it is probable that species composition changed somewhat during the 48 h incubations and this compositional shift might explain some of the differences between samples incubated at ambient or elevated temperature. A direct effect of temperature on bacterial and bacterivore metabolism, however, seems to be the most plausible explanation for our results. The evidence for direct effects of temperature on both bacterial and bacterivore activities is increasing (Caron et al. 1986, Pomeroy et al. 1991). In any event, the overall consequence is that winter assemblages of bacteria and their consumers are capable of rapidly ($\leq$ 48 h) responding to an elevation in the water temperature.

Finally, it should be noted that the effect of temperature on bacterial growth and grazing was probably underestimated in this study. Winter samples were incubated at temperatures no lower than 5 °C because of limitations of the incubators, even though in-situ water temperatures were lower than 5 °C from January through March. Incubation at in-situ winter temperatures might have resulted in lower bacterial growth rates and lower bacterivore grazing rates than were observed for these latter samples in our study.

In summary, our results showed that (1) of the variables associated with the experimental protocol of the grazing rate experiments, the use of sample sizes less than 1 l had the greatest effect on the rate estimate (particularly on the variability between replicates); (2) seasonal fluctuations in the rate of bacterivory were substantially greater than the day-to-day fluctuations. The range of the day-to-day fluctuations in the grazing rates were comparable to the range of the seasonal fluctuations only when the effect of temperature was eliminated (e.g. by incubating all samples at 20 °C).

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