Rate of Bacterial Mortality in Aquatic Environments

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A method is proposed which provides a minimum estimate of the rate of bacterial mortality in growing natural populations of planktonic bacteria. This estimate is given by the rate of decrease of radioactivity from the DNA of a [³H]thymidine-labeled natural assemblage of bacteria after all added thymidine has been exhausted from the medium. Results obtained from river water, estuarine water, and seawater show overall bacterial mortality rates in the range 0.010 to 0.030 h⁻¹, in good agreement with the range of growth rates measured in the same environments. Use of selective filtration through Nuclepore filters (pore size, 2 µm) allowed us to determine the contribution of microzooplankton grazing to overall bacterial mortality. Grazing rates estimated by this method ranged from 0 to 0.02 h⁻¹.

Heterotrophic bacteria have now been recognized as important components in the function of aquatic ecosystems, often utilizing a significant part of the organic matter formed by primary producers (1, 4, 5, 14, 23, 32). Although the value of carbon conversion efficiency (or growth yield) of bacteria in natural environments is still the subject of much controversy in the literature (estimates ranging from 40 to 80% [26] to 7 to 23% [21, 24, 25]), it is nevertheless clear that bacterial biomass production does represent an important flux in the carbon cycle of aquatic systems.

The fate of this biomass is presently not very well known. Much attention has recently been paid to the investigation of the role of microzooplankton as possible bacterial grazers. A method for measuring grazing rates on natural populations of bacteria labeled with [³H]thymidine was proposed by Hollibaugh et al. (17). With this method, King et al. (19) showed that net zooplankton mucous filter feeders like larvaceans have minimal influence on the population dynamics of bacteria. Fenchel (6) showed that ciliates are generally incapable of growing efficiently by grazing on free bacteria at the densities normally encountered in open waters. On the other hand, nanoplanktonic heterotrophic flagellates were shown to be capable of actively grazing on bacteria at the natural densities in most aquatic environments (0.5×10^6 to 5×10^6 bacteria per ml) (7-10). Calculations based on laboratory feeding rates and direct counts of microflagellates suggest that these organisms could exploit up to 60% of bacterial biomass production in some coastal marine environments (10, 21).

Besides grazing, bacterial biomass can undergo different fates, including lysis, either spontaneous or induced by viral or bacterial predators (3). No method is available for estimating the quantitative importance of these processes.

In this paper, we propose a method for measuring the overall rate of bacterial mortality in natural environments.

By mortality, we refer to the process of loss of functional and morphological integrity of bacteria, including lysis and destruction of the genetic material. This definition is not equivalent to loss of viability in the sense used by Postgate (27). In the classic work of Postgate and co-workers (28, 29), viability is estimated as the ratio between colony counts and

total bacterial counts: a bacterium is defined as dead if it does not multiply in a supposedly optimal medium, even if it maintains its morphology and some of its biological functions. For this reason, the loss of viability does not represent the flux of mortality (i.e., the flux of detritus production from bacterial biomass), which is important from a biogeochemical point of view.

Moreover, as pointed out by Gray (13), the slide culture technique developed by Postgate and Hunter (29) "is inappropriate for investigations on mixed cultures of natural origin since a single recovery medium does not allow all microbial cells to develop and, even if it did, differential growth rates would lead to difficulties in counting colonies, and the presence of motile cells would make colony counts inaccurate."

In addition, the derivation of bacterial death from viability measurements can only be used for conditions under which growth is stopped: "cryptic growth" is presented as an artifact that experimental design has to eliminate or reduce as much as possible (27). On the contrary, the measurement of bacterial mortality, which is needed from an ecological point of view, must be workable under conditions of net bacterial growth and not only under conditions of starvation.

Only a few workers were concerned with the problem of measuring bacterial mortality under growth conditions. By measuring the release of radioactive compounds from pure cultures of *Escherichia coli* prelabeled in their protein or nucleic acids content, Koch (20) showed that cell death followed by lysis is less than 1% per h under growth conditions but is enhanced to 5% per h by experimental stresses such as chilling or centrifugation.

The method we propose here consists of following the disappearance of radioactive tracer from the DNA of [³H]thymidine-labeled natural assemblages of bacteria. It is applicable to any natural samples, whatever the growth stage of the bacteria may be, and involves no experimental stresses besides confinement of the sample in a bottle.

MATERIALS AND METHODS

The experiments reported here were performed on freshly collected samples from a variety of natural waters, including fresh water (Meuse river at Heer-Agimont or Huy, Belgium),

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FIG. 1. Time course study of [^3H]thymidine incorporation into the cold TCA-insoluble fraction of a water sample from the Scheldt estuary (Hemiksen, 17/2/1983). [^3H]thymidine (250 kdpm/ml) was added at time zero. The arrow indicates the time of a second, identical addition of [^3H]thymidine to a subsample.

brackish water (Scheldt estuary, Hemiksem or Doel Belgium), or coastal seawater (Belgian coastal zone).

From a practical point of view, the procedures we used were derived from that described by Furhman and Azam (11, 12). To a 100-ml natural water sample, 20 μCi of [$\text{methyl-}^3\text{H}$]thymidine (50 Ci/mmol) or [$\text{methyl-1',2'-}^3\text{H}$]thymidine (110 Ci/mmol) (Amersham Corp.) is added. The sample is incubated in the dark at 20°C. Subsamples (5 ml) are collected every 10 to 20 h for several days to determine the radioactivity associated with the following fractions.

The cold trichloroacetic acid (TCA)-insoluble fraction is determined by pouring a 5-ml subsample into 5 ml of ice-cold 10% TCA and filtering it after 10 min through a Sartorius membrane (pore size, 0.2 μm). This fraction is supposed to comprise nucleic acids and proteins. The hot TCA-insoluble fraction is obtained after 1 h of digestion of the sample in 10% TCA at 95 to 100°C. This treatment is supposed to hydrolyze nucleic acids but not proteins (22).

It has been assumed here that labeling of RNA from [$\text{methyl-}^3\text{H}$]thymidine is not significant with respect to DNA labeling. This assumption is made by most authors working with thymidine incorporation (11, 12), in view of the fact that there is no thymine residue in RNA. It was demonstrated for marine bacteria (17) by treatment with 0.5 N NaOH at 60°C for 30 min. With the same treatment, we verified that [^3H]thymidine incorporation into RNA is insignificant in water from the Scheldt estuary. Note, however, that Karl (18) and Rieman et al. (30) found that [^3H]thymidine incorporation into RNA can account for a significant part (in some cases up to 80%) of total incorporation into the cold TCA-insoluble fraction in some freshwater bacterial communities. In these cases, a more specific method of DNA separation should be developed.

The total particulate fraction results from direct filtration of the sample through a 0.2- μm -pore-size membrane. The

filtrate fraction comprises both unincorporated thymidine remaining in solution and the excreted products of its catabolism (mainly $^3\text{H}_2\text{O}$). The latter can be separated from the former by evaporating the sample to dryness in an oven at 80°C. Control experiments run with [^3H]thymidine in sterile water showed that this treatment does not cause significant ^3H exchange between [^3H]thymidine and water.

The radioactivity was determined by liquid scintillation with a Packard Tri-Carb scintillation counter. Unless stated, incubation of the samples was done at 20°C in the dark. Enumeration of bacteria was carried on by epifluorescence microscopy after acridine orange staining; the standard procedure of Hobbie et al. (16) was used.

RESULTS

Thymidine metabolism during standard [^3H]thymidine incorporation experiments. Figure 1 shows the results of a typical time course study of [^3H]thymidine incorporation into the cold TCA-insoluble fraction by a natural water sample. During the first 2 h, thymidine incorporation was linear with time. After about 3 h, a plateau was reached. If a new addition of tritiated thymidine was made at that stage, incorporation resumed at about the same rate as after the first addition. This suggests that the plateau corresponds to the exhaustion of the thymidine added to the medium, although the radioactivity incorporated into the cold TCA-insoluble fraction at the plateau represents only 20% of the radioactivity added as thymidine (10 to 30% in other similar experiments).

Figure 2 shows the time course of total thymidine incorporation, incorporation into the cold TCA-insoluble fraction, thymidine remaining in the filtrate, and total radioac-

FIG. 2. Time course of [^3H]thymidine incorporation into the cold TCA-insoluble fraction (●) and in the total particulate fraction (▲); disappearance of radioactivity from the filtrate fraction (■) and of radioactive thymidine in the filtrate (○) sample from Meuse river (Heer Agimont, 28/9/1983).

tivity in the filtrate. These results show very clearly that (i) thymidine was entirely exhausted from the medium after about 4 h in this experiment (10 to 20 h in others); (ii) incorporated thymidine was mostly present in the cold TCA-insoluble fraction; and (iii) 80% of the tracer was converted into volatile compounds (probably $^3\text{H}_2\text{O}$) which are liberated in the external medium instead of being incorporated.

The difference between the radioactivity incorporated into the cold and the hot TCA-insoluble fractions varies a lot with the origin of the samples. In seawater, incorporation into the hot TCA-insoluble fraction generally represents no more than 10 to 20% of the incorporation into the cold TCA-insoluble fraction. In fresh water, it often amounts to 40 to 50% of the incorporation into the cold TCA-insoluble fraction. Similar observations were reported by Riemann et al. (30).

Long-term decrease of radioactivity in the DNA. When the radioactivity in the cold TCA-insoluble fraction was followed over several days after exhaustion of the labeled thymidine from the medium, a gradual decrease was observed. In most cases, this decrease obeys first-order kinetics (straight line in semi-log plot) and is therefore characterized in the following discussion by a first-order constant k (hour^{-1}). In a few instances, a biphasic logarithmic decrease was observed, with a lower rate after about 70 h. In these cases, only the first phase was considered here. The rate of decrease is independent of the concentration of radioactive thymidine added (Fig. 3).

In several experiments, we verified that the same rate of

FIG. 3. Long-term decrease of radioactivity in the cold TCA-insoluble fraction of a sample from the Scheldt estuary (Hemiksen, 13/2/83) after ^3H thymidine incorporation at three different concentrations (0.32 nmol/liter [Δ]; 3.2 nmol/liter [$\bullet$]; and 32 nmol/liter [$\blacksquare$]).

FIG. 4. Long-term decrease of radioactivity in the cold TCA-insoluble fraction ($\bullet$), the hot TCA-insoluble fraction ($\circ$), and the DNA fraction ($\blacksquare$), taken as the difference between cold and hot TCA-insoluble fractions (sample from Hemiksen, 6/4/1983).

experimental decrease is observed for the hot and cold TCA-insoluble fractions (an example is given in Fig. 4). The rate of decrease of the radioactivity from the cold TCA-insoluble fraction can therefore be considered as reflecting the rate of radioactivity decrease from bacterial DNA.

As DNA is basically a conservative molecule, undergoing little turnover within the cell (if we except the processes of DNA repair which are probably quantitatively insignificant), the decrease of radioactivity from the DNA must be attributed to the disappearance of the cells themselves and therefore constitutes a minimal estimate of bacterial mortality.

The validity of this estimate rests on the condition that hydrolysis products of labeled DNA from dead cells are not significantly reincorporated by living cells during the experiments. In view of the fact that most ^3H thymidine is rapidly converted into $^3\text{H}_2\text{O}$ (see above), such reincorporation into DNA is unlikely. In fact, we observed that a large addition of unlabeled thymidine (30 mg/liter) after radioactive thymidine has been exhausted does not affect the rate of radioactivity decrease from the cold TCA-insoluble material, suggesting that reincorporation of regenerated ^3H thymidine is insignificant.

Another condition for the validity of our estimate of bacterial mortality is that the rate of hydrolysis of labeled DNA from dead cells is higher than the rate at which the cells are dying. Otherwise, the method could only measure this rate of DNA hydrolysis and would underestimate mortality. In other words, the rate-limiting step has to be cell mortality, and hence hydrolyzable DNA production, instead of DNA hydrolysis itself. The rate of DNA hydrolysis has been measured in river water after addition of $[2-^{14}\text{C}]$ thymidine-labeled DNA (from *E. coli*; Amersham) (Fig. 5). It is at least twice as high (0.046 h^{-1}) as the rate of radioactivity decrease in the cold TCA-insoluble fraction of a ^3H thymidine-labeled bacterial population from the same sample (0.023 h^{-1}). Note that the very small positive difference between the rate of radioactivity decrease in the cold TCA-insoluble fraction and the total particulate fraction observed in most experiments (Fig. 2 and 5) also indicates that only very small amounts of dissolved labeled DNA from dead cells accumulate, as would be the case if DNA hydrolysis were slower than cell lysis.

FIG. 5. Long-term decrease of radioactivity in the cold TCA-insoluble (●) and the total particulate (○) fractions of a sample of river water (Huy, 4/6/84) after ^3H thymidine incorporation. ■, Decrease of ^{14}C -labeled DNA added to a sample of the same water.

FIG. 6. Long-term decrease of radioactivity in the cold TCA-insoluble fraction of five subsamples of river water (Huy, 12/8/1984) after ^3H -thymidine incorporation. Symbols: ●, untreated control; □, autoclaved subsamples; ■, autoclaved subsample reinoculated with 50% untreated water; △, sonicated subsample; ▲, sonicated subsample reinoculated with 50% untreated water.

To further investigate the factors controlling DNA degradation after cell death, the following experiment was performed. A sample of water from the Meuse river was labeled with ^3H thymidine for 20 h. Two subsamples were autoclaved for 10 min. One of them was kept sterile, and the other was reinoculated with 50% unlabeled water from the same station. Two other subsamples were sonicated for 10 min. Again, one of them underwent no further treatment and the other was reinoculated with fresh water. The decrease of radioactivity in the cold fraction was then followed for 4 days in the four subsamples and in an untreated control. The results are shown in Fig. 6. In the autoclaved and the sonicated nonreinoculated subsamples, the rate of decrease of DNA was very low, although there is no doubt that the treatment undergone by these samples resulted in the death of most, if not all, of the bacteria. In these subsamples, the difference in radioactivity between the cold TCA-insoluble and the total particulate fractions was up to 50% that of the cold TCA-insoluble fraction. This indicates that a large part of the DNA was liberated outside the cellular structures in these samples. In the reinoculated subsamples, on the other hand, the rate of decrease of radioactivity in the DNA fraction was much higher, even than in the control sample (at least after a 24-h lag). This indicates that DNA degradation after bacterial death is a microbiological process. The validity of our method for measuring bacterial mortality thus depends on the presence of an active population of living microorganisms able to hydrolyze the DNA liberated by dead cells. Our method is therefore suitable for measuring natural mortality of bacterial populations not submitted to extreme stress that would kill most microorganisms. Under other conditions, as for instance in the extreme cases of the latter experiment or in the case of the addition of toxic substances or antibiotics, the method only provides an underestimate of bacterial death rate.

Effect of temperature. The effect of temperature on the rate of mortality has been measured by following the decrease of radioactivity from the cold TCA-insoluble fraction of several subsamples from identical ^3H thymidine-labeled microbial populations incubated at different temperatures. Two typical results are shown in Fig. 7. As seen in the figure, the rate of mortality increases with increasing temperature.

Effect of the growth stage. To check the effect of growth stage and organic matter starvation on the mortality rate, two experiments were performed. In the first, Casamino Acids (Difco Laboratories) were added to five subsamples

FIG. 7. Effect of incubation temperature on the rate of bacterial mortality in river water (Heer Agimont, 25/5/1983) (○) and estuarine water (Hemiksem, 3/1/83) (●).

from a sample of water from the Scheldt estuary to obtain a range of final concentrations between 1 and 100 mg/liter. Tritiated thymidine was immediately added, and the decrease of radioactivity in the cold TCA-insoluble fraction, after the plateau was reached, was followed for 4 days. Although the bacterial growth was obviously very different in the five subsamples and in a control with no addition, no differences in the rate of mortality ($k = 0.0115 \text{ h}^{-1}$) were observed.

In another experiment, organic-rich water from the upper part of the Scheldt estuary was filtered through a $0.2\text{-}\mu\text{m}$ -pore-size membrane and reinoculated with 1% unfiltered water from the same site. After acridine orange staining, the growth of bacteria was followed by epifluorescence microscopy for 40 days (Fig. 8). Rapid growth occurred during the first 5 days and was followed by a rapid decrease in the number of bacteria, which ultimately stabilized around 1.5×10^6 bacteria ml^{-1} for the next 20 days. Measurement of mortality were performed in the initial phase and in the late starvation phase of the culture. Decay rates of 0.014 and 0.0098 h^{-1} were observed, respectively, showing that mortality is by no means lower in actively growing than in starved natural populations of bacteria. The same experiment was repeated with river water and yielded similar results.

Effect of microplankton grazing. A sample of river water which had reached its plateau of [^3H]thymidine incorporation was filtered through a $2\text{-}\mu\text{m}$ -pore-size Nuclepore membrane to remove eucaryotic organisms. This sample was divided into four subsamples to which was added, respectively, 0, 4, 10, and 20% of a protozoan enrichment obtained by the procedure described by Fenchel (7). Epifluorescence microscopic observation after acridine orange staining of the protozoan enrichment revealed the presence of about 3.3×10^4 microflagellates (diameter, $5 \mu\text{m}$) and 5.5×10^3 ciliates (diameter, $20 \mu\text{m}$) per ml. On the other hand, direct acridine orange counts of the subsamples after 35 h of incubation varied between 1×10^6 and 3×10^6 bacteria ml^{-1} .

The decrease of radioactivity from the cold TCA-insoluble fraction of each subsample was followed for 3 days. The results are given in Fig. 9 and show an increased mortality rate with increasing additions of protozoa.

As an approach to the evaluation of the grazing rate on bacteria in natural samples, we developed the following procedure. A freshly collected water sample was incubated for 10 to 20 h with [^3H]thymidine and then divided into two subsamples, one of which was filtered through a $2\text{-}\mu\text{m}$ -pore-size Nuclepore membrane for retaining microflagellates. The

FIG. 8. Bacterial density in a sample of estuarine water (Hemiksen, 6/10/1983) filtered through a $2\text{-}\mu\text{m}$ -pore-size filter and reinoculated with 1% untreated water. The arrows indicate the time of two mortality rate determinations which yielded, respectively, the values 0.014 and 0.0098 h^{-1} .

FIG. 9. Long-term decrease of radioactivity in the cold TCA-insoluble fraction of a sample of river water (Huy, 15/5/1984), after [^3H]thymidine incorporation, filtration through a $2\text{-}\mu\text{m}$ -pore-size Nuclepore filter, and addition of 0% (●), 4% (○), 10% (■), and 20% (□) of a protozoan enrichment culture.

decrease of radioactivity from the cold TCA-insoluble fraction was followed in both subsamples for 3 to 5 days. A typical result obtained in river water is shown in Fig. 10.

The $2\text{-}\mu\text{m}$ filtration retained a part of the radioactivity (especially in river water samples, for which this part can be as high as 30%). Invariably, however, the mortality rate observed in the filtered subsample was lower than in the unfiltered control. The difference between these two rates is considered to be the consequence of microzooplankton grazing, whereas the rate found in the filtered sample represents a "residual" mortality rate unattributable to grazing. Systematic application of this procedure to samples from the Meuse river during a whole-year cycle showed a residual mortality rate in the range 0.002 to 0.012 h^{-1} , whereas the grazing contribution to total mortality varied between 0.005 and 0.021 h^{-1} . Similar figures were obtained in the Belgian coastal waters (0.010 to 0.020 h^{-1} for residual mortality and 0 to 0.010 h^{-1} for grazing mortality).

Comparison with growth rates. When a steady state is

FIG. 10. Long-term decrease of radioactivity in the cold TCA-insoluble fraction of a sample of river water (Huy, 15/5/1984) after [^3H]thymidine incorporation, filtered (○) or unfiltered (●) through a $2\text{-}\mu\text{m}$ -pore-size Nuclepore membrane at the time indicated by the arrow.

TABLE 1. Simultaneous measurements of bacterial growth rates and first-order mortality constants in seawater and freshwater environments

Environment	Date (day/ mo/yr)	Mortality constant (h ⁻¹)	Growth rate (h ⁻¹)
Belgian coastal zone	10/4/1984	0.015	0.005
	17/4/1984	0.010	0.014
	24/4/1984	0.019	0.021
	2/5/1984	0.017	0.012
	14/6/1984	0.033	0.012
Meuse river (Heer Agimont)	20/3/1984	0.017	0.021
	30/8/1984	0.014	0.030
	27/9/1984	0.011	0.022
	9/10/1984	0.021	0.041
Meuse river (Tihange)	20/3/1984	0.017	0.026
	15/5/1984	0.031	0.030
	7/8/1984	0.021	0.021
	18/9/1984	0.022	0.015
	16/10/1984	0.021	0.022

maintained in a bacterial population, the mortality rate should exactly balance the growth rate. If it is lower or higher, the population increases or decreases, respectively. In the environments we studied, we never observed extremely rapid change in bacterial populations. A typical relative rate of variation of bacterial numbers is 0.005 h⁻¹.

Mortality rates should therefore be close to the rates of growth of the bacterial populations in the same environments. On a few occasions, we measured bacterial mortality and growth rate for the same samples. Growth rate was calculated as the ratio between cell production rate and cell number. Cell production was determined from the rate of [³H]thymidine incorporation into the cold TCA-insoluble fraction by using the conversion factors established by Fuhrman and Azam (12) for seawater samples (A. Fontigny, unpublished data). For Meuse water samples, calibration of [³H]thymidine incorporation was established on the basis of observed growth in reinoculated filtered water samples (P. Servais, unpublished results). Table 1 shows the data obtained for simultaneous measurements of mortality and growth rate. Although both estimates rest on the use of [³H]thymidine labeling, they are completely independent of each other. Their close similarity is therefore a very good check of the validity of our method.

DISCUSSION

The method we propose here allows determination of the overall rate of bacterial mortality in the natural aquatic environment.

Strictly speaking, the method only measures the degradation rate of DNA liberated by dead cells and therefore only provides an underestimate of the rate of bacterial death. However, in natural conditions, i.e., in the absence of severe and sudden stresses and in the presence of an active microbial population, no accumulation of liberated DNA occurs, and the rate of DNA degradation thus reflects bacterial mortality rates.

A limitation of the method is that preincubation with [³H]thymidine has to be long enough to deplete the tracer from the medium. Preincubations of 5 to 20 h are needed in most cases. Moreover, the decrease of tracer from the DNA fraction must be followed for an additional period of 25 to 50 h to obtain an accurate determination of the mortality rate. It

could be feared that changes in bacterial physiology as well as in types and numbers of bacteriophages may become significant during such long incubation periods. However, the time course of DNA disappearance observed in our experiments with natural water samples shows no significant changes in the rate of mortality for more than 70 h.

The range of values recorded for samples from the Meuse River, the Scheldt estuary, and the Belgian coastal waters were, respectively, 0.0011 to 0.031, 0.012 to 0.016, and 0.010 to 0.033 h⁻¹. This compares well with the range of growth rates (0.005 to 0.04 h⁻¹) recorded for planktonic bacteria from the same environments.

By using size fractionation for excluding protozoan grazers, we were also able to distinguish the contribution of grazing to total mortality. Grazing rates ranged from 0.005 to 0.021 h⁻¹ in samples from the Meuse river and from 0 to 0.010 h⁻¹ in the Belgian coastal waters. These figures are consistent with the few available quantitative data on grazing by microflagellates on bacteria in natural waters. The range of microflagellate densities in estuarine and coastal marine waters is 0.3×10^3 to 4×10^3 cells ml⁻¹ (10, 31). The maximum clearance rates, determined by Fenchel (8) for six species of flagellates, are 2×10^{-6} to 8×10^{-5} ml h⁻¹ cell⁻¹. Combination of these two estimates yields a relative grazing rate on free bacteria of 0.0006 to 0.320 h⁻¹. More accurate calculations presented by Fenchel (10) for Limfjorden, Denmark, give grazing rates in the range 0.005 to 0.030 h⁻¹.

The mechanisms responsible for the residual mortality rate, i.e., the mortality recorded after exclusion of grazers by filtration through a 2-μm-pore-size filter, is unclear. Predation by bacteria or by protozoa passing through 2-μm-pore-size filters, virus-induced lysis, or spontaneous autolysis are possible processes the relative importance of which is not known. Our data show that the mortality rate is lower at lower temperature and is not enhanced by starvation. This is probably easier to explain if some form of predation is the dominant process in residual mortality.

The observation that the kinetics of mortality is usually first order requires a few comments. Hinshelwood (15) showed theoretically that an exponential law of bacterial mortality implies that the death of the cells is governed by a conjunction of independent chance events at the cellular level. This is probably the case for "spontaneous" mortality under stress conditions. First-order kinetics is less obvious for mortality caused by grazing. Flagellates grazing on bacteria are known to obey saturation kinetics similar to the Michaelis-Menten equation (8). However, the half-saturation constants for grazing reported for most heterotrophic flagellates are in the range 1.3×10^6 to 38×10^6 bacteria ml⁻¹. At natural bacterial densities in aquatic environments (0.5×10^6 to 5×10^6 bacteria ml⁻¹), the grazing rates are therefore nearly proportional to bacterial concentrations and can be considered as first order provided the biomass of grazers does not vary too much during the observation period.

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