The ecological importance of algal phagotrophy to lake plankton communities

by

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Abstract

The aim of this thesis is to incorporate the smallest organisms, in particular the heterotrophic bacteria and their predators, more fully into current descriptions of aquatic community structure and dynamics. A strong, positive empirical relationship was found between bacterial abundance and chlorophyll concentration in freshwater and marine systems. Common members of the photosynthetic phytoplankton (all chrysophyceans) were shown to be major, even dominant, grazers of these bacteria. This phagotrophic capability is quantitatively important to the bacterioplankton, to the mixotrophs themselves, and in some cases, to the structure of the limnological community as a whole. Grazing by mixo- and heterotrophic protozoans is concentrated on the largest bacterial cells that also have the greatest growth rates. Relatively lower removal rates of the tiniest cells, with low growth rates, is proposed to explain their numerical dominance in lakes.

Résumé

Le but de cette thèse est d'incorporer de manière plus satisfaisante les plus petits organismes, notamment les bactéries hétérotrophes et leurs prédateurs, dans les descriptions de la structure et de la dynamique des communautés aquatiques. Une relation positive étroite entre l'abondance bactérienne et la concentration en chlorophylle est décrite pour des systèmes marins et dulcicoles. Les brouteurs les plus importants de ces bactéries peuvent être des phytoplanctontes qui photosynthetisent également (uniquement des Chrysophytes). La capacité qu'ont ces organismes de se nourrir par phagotrophie a des implications quantitatives pour le bactérioplancton, les mixotrophes eux-mêmes, et pour la structure de la communauté planctonique tout entière. Le broutage des bactéries par les protozoaires mixotrophes et hétérotrophes se concentre sur les bactéries les plus grosses qui sont également celles qui croissent le plus rapidement. Il est suggéré que le faible taux de broutage sur les plus petites bactéries explique leur dominance dans les lacs.

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Preface

As required by the Faculty of Graduate Studies and Research of McGill University, the following statements are made.

"The Candidate has the option, subject to the approval of the Department, of including as part of the thesis the text of an original paper, or papers, suitable for submission to learned journals for publication. In this case the thesis must still conform to all other requirements explained in Guidelines Concerning Thesis Publication, (available at the Thesis Office). Additional material (experimental and design data as well as descriptions of equipment) must be provided in sufficient detail to allow a clear and precise judgement to be made of the importance and originality of the research reported. Abstract, full introduction and conclusion must be included, and where more than one manuscript appears, connecting texts and common abstracts, introduction and conclusions are required. A mere collection of manuscripts is not acceptable; nor can reprints of published papers be accepted."

"While the inclusion of manuscripts co-authored by the Candidate and others is not prohibited by McGill, the Candidate is warned to make an explicit statement on who contributed to such work and to what extent, and Supervisors and others will have to bear witness to the accuracy of such claims before the Oral Committee. It should also be noted that the task of the External Examiner is made much more difficult in such cases, and it is in the

Candidate's interest to make authorship responsibilities perfectly clear."

This thesis has been prepared in the format of five separate papers, suitable for submission to learned journals, as permitted by faculty regulations. All 5 chapters are co-authored by Dr. Jaap Kalff, my thesis supervisor. Therefore the first person plural is used throughout. The "first growth study" referred to in Chapter 5 formed part of a joint project between Dr. David J. Currie, Dr. J. Kalff, and myself. All aspects of that study described in the Chapter were performed by me, except where explicitly attributed to Dr. Currie as unpublished data. The role of Dr. Currie in that study was in the discussion and interest he showed in my bacterial counts, which did not conform with expectations based on his measurements of inorganic phosphorus uptake rates within the bacterioplankton. The decision was made to perform simultaneous measurements on a single water sample in order to compare our results. The results of that joint study have not yet been published; the description of the study given in Chapter 5 represents only my own work. Chapter 1 was published in Canadian Journal of Fisheries and Aquatic Sciences 41: 1015-1023. Chapter 2 was published in Science 231: 493-495. Chapter 3 was published in Limnology and Oceanography 32: 277-284. Chapter 4 has been accepted for publication in Limnology and Oceanography, with minor revision. Chapter 5 will be submitted to Applied and Environmental Microbiology.

The Faculty of Graduate Studies and Research also requires the Candidate to indicate what elements in the thesis represent original

contributions to knowledge. These are: 1) the construction of a predictive relationship, with the appropriate statistical details, between bacterial abundance as measured using modern reliable techniques and chlorophyll concentration, spanning a wide range of trophic conditions in both marine and freshwater sites, 2) these relationships are indistinguishable in lakes and marine sites, 3) a major grazer of bacteria in some lakes is Dinobryon, a member of the photosynthetic plankton, 4) fluorescent latex beads of bacterial size are phagocytosed by Dinobryon at roughly the rate of natural bacteria, 5) Dinobryon can depend more heavily on phagocytosis for sustenance than on photosynthesis, under low light conditions in Nature, 6) members of many other chrysomonad genera are also demonstrated to be mixotrophic, 7) Dinobryon's grazing rate is cued more to temperature than to light availability, 8) Dinobryon and other protozoan grazers in general ingest the tiniest bacteria at a lesser rate than they do the larger ones, 9) Dinobryon's assimilation rate is similar to that for purely heterotrophic organisms, 10) deep chrysophyte peaks may be subsisting, even if temporarily, on phagocytosis rather than photosynthesis, 11) the tiny bacterial cells that are most abundant can be the slowest growing, 12) the fastest growing cells can be responsible for the majority of thymidine isotope dilution during in situ growth rate determinations, and 13) current practices for deriving bacterial growth rate from thymidine incorporation data may give strongly biased results.

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My warmest thanks are for my wife Alison, whose love and

encouragement have meant so much to me during the course of the investigations described herein.

This thesis is dedicated to my father, Francis Henry Bird.

General Introduction

Feeding relationships are of central importance to the structure and dynamics of aquatic communities. Because of this, they provide a powerful organizing force for the description of lake community characteristics, and much of biological limnology is taken up with their analysis and prediction. In light of the recent and growing recognition of the disproportionate influence that smaller size classes of organisms can have on lake metabolism, it is not surprising that microbial feeding relationships are of intense current interest to aquatic ecologists. In particular, the extent of movement of stored bacterial nutrients and energy into higher levels of consumers is "one of the most significant unanswered questions" about aquatic food webs (Pomeroy 1983, 1986).

There has always been limnological interest in bacterial activity though in the past, investigators have been hampered by a lack of trustworthy techniques. Ruttner (1963, p. 157) acknowledged that the contemporary picture of the occurrence and activity of bacteria was "extremely fragmentary". At the time most investigators avoided determination of the total number of bacteria, preferring instead to demonstrate the existence of specialized subpopulations by monitoring changes occurring in nutrient broths inoculated with lake water. A review of bacterial enumeration techniques showed that different methods gave results that varied over 4 orders of magnitude (Jannasch and Jones 1959). A major advance of the past decade has been the development of a technique that allows accurate total counts of bacteria to be made (Hobbie et al. 1977). We can now reliably estimate

the number of bacteria in a particular aquatic system (Bowden 1977), and we can predict from other variables of lakes and marine environments roughly how many bacteria to expect to find there (as I will show in Chapter 1). We still don't know, however, how many are active, inactive, alive or dead, what they subsist on, or what controls their numbers.

The development of methods for measuring bacterial activity has also stimulated interest in bacterial ecology. Strickland and Parsons (1962) introduced the use of radiolabelled organic substrates to assess in situ heterotrophy. Though the information this method provided was largely qualitative, it focussed attention on the importance of bacteria to aquatic metabolism. Early studies on community respiration had sought largely to differentiate the efforts of net zooplankton from the rest of the plankton. Pomeroy (1974) wrote an influential essay that helped change the focus of oceanographers from the diatom-copepod food link to smaller organisms and their rapid metabolism. Interest has now switched almost completely among planktonic oceanographers to the tiniest organisms, following studies showing that, after all, the crustacean zooplankton are responsible for less than 10% of respiration in situ, whereas bacteria are contributing at least 50% in some environments (Williams 1981). Usually 10 to 20%, and up to 75%, of primary production is funneled directly into bacteria through extracellular organic carbon release from the phytoplankton (Larsson and Hagstrom 1979; Blaauboer et al 1982; Cole et al. 1982; Wolter 1982; Lancelot 1983). Most bacterial production is by the tiny free-living cells, subsisting on dissolved organic matter, and not by bacteria attached to particles (Azam and Hodson 1977; Azam et al. 1983). Bacteria are no longer regarded as simple decomposers, but also as a

potentially major food resource, returning lost fixed carbon to higher consumers (Sieburth 1984; Porter et al. 1985).

Though production rates can now be measured reasonably well (it is believed) (Fuhrman and Azam 1980; Karl 1981), there is still little known about what controls production or numbers in situ. Bacterial concentrations are remarkably constant from day to day, given their short life spans, and are constant over the year as well (Hobbie and Wright 1979). For instance, bacteria in MacPherson Bay, Lake Memphremagog, in 1984 varied only 3-fold over the annual cycle whereas phytoplankton biomass varies at least 10-fold (Watson 1979). This constancy of numbers led some researchers to claim that bacteria were being maintained at about a million cells per ml by ciliate grazers that could only subsist in the water column at bacterial densities above that threshold amount, but that were efficient at reducing the bacterial population once densities were high enough to support their growth (Fenchel 1980). This position did not take into consideration evidence produced by East European workers that showed that bacterial numbers did vary considerably in relation to the trophic status of the water body (Godlewska-Lipowa 1976, 1979; Straskrabova 1968). My own work confirmed this, and brought the relationship up to date by using only epifluorescence counts of bacteria. Using values from the literature for both freshwater and marine habitats I found that there was a strong positive relationship between bacterial abundance and chlorophyll concentration, and that this relationship was indistinguishable in fresh and salt water.

Because bacterial numbers are effectively constant on a day-to-day basis, the growth of bacterial populations must be balanced somehow

by cell loss. Conceivable loss agents are sedimentation, lysis, and predation, either by bacteriophage or by zooplankton. The sedimentation rate of an inanimate object the size of a bacterium is only millimeters per day, and it is well established that the majority of bacterial cells in the water column of most environments are free living, not attached to particles, so sedimentation losses are likely to be negligible. Bacteria in nature are too sparse to support important levels of bacteriophage. The third alternative loss factor that has been suggested, autolysis of cells, is difficult to test, and even somewhat illogical, so that it should be disregarded until simple mechanisms have been excluded. The simplest explanation for the loss of bacterial production is through grazing by zooplankton.

Those who believe that zooplankton can control the standing stock of bacteria are mostly marine workers and attribute the major part of grazing to protozooplankton. Though belief in the efficacy of the protozooplanktonic grazers is almost endemic among microbial ecologists, hard evidence is exceedingly rare and mostly inferential (Sherr and Sherr 1984; Sieburth 1984). For some the claim is an explicit assumption based on the necessity of input-output equilibrium (Fuhrman and Azam 1980; Friebele et al. 1978). Others hold with Fenchel (1980), based on laboratory studies with ciliates and microflagellates, that bacterial feeders maintain bacteria at a "threshold" concentration (Porter 1984; Linley et al. 1983). The most convincing arguments for zooplanktonic removal of bacterial production are based on counts of heterotrophic microflagellates in marine waters (Sieburth and Davis 1982; Andersen and Fenchel 1986) and filtration-dilution manipulations (Wright and Coffin 1984; Landry et al. 1984). Wright and Coffin (1984) did not count protozoans, but claimed to have shown that grazers

passing a 3 μm filter could control estuarine bacterial abundance. Andersen and Fenchel (1986) inferred from the succession of bacterial abundance peaks by flagellate peaks in seawater cultures that these protozoans control bacterial populations in situ. Not all who have looked for microflagellates have found them, however. When Parsons et al. (1981) added glucose to marine mesocosms, the bacteria increased dramatically in number and this production was passed to zooplankton with no change in microflagellate population size. Sherr and Sherr (1987) used feeding experiments with fluorescently labelled cultured bacteria to suggest that ciliate grazers alone could account for 100% of bacterial removal in a Georgia estuary.

The broad goal of my thesis was to incorporate the tiniest members of the plankton more fully into current quantitative descriptions of aquatic communities. The final form it took represented a major redirection of interest that resulted from a surprising and intriguing discovery: that major grazers of bacteria in the lakes I studied were members of the photosynthetic plankton.

This discovery came as a consequence of efforts to determine zooplanktonic grazing rates in situ. Attempts by other workers to quantify grazing on bacteria through the use of radiolabelled prey had failed because of the impossibility of separating predators from prey by filtration (Hollibaugh et al. 1980). Therefore I developed a method using bacteria-sized fluorescent latex particles that could be employed for in situ rate determinations. Examination of the first samples collected using this technique revealed undeniable uptake of particles by chlorophyll-bearing members of the class Chrysophyceae.

The first chapter of this thesis, then, tests the general

hypothesis that bacterial abundance in lakes and sea can be predicted from phytoplankton abundance measured as chlorophyll concentration. It was published in the Canadian Journal of Fisheries and Aquatic Sciences in 1984. The second chapter outlines the discovery that common species of algae are quantitatively important grazers on lake bacterioplankton. Included is a comparison of clearance rate of the Dinobryon population to the rates of other bacterial feeders, as well as electron micrographic sections demonstrating the presence of ingested bacteria within algal cells. The colonial habit of Dinobryon was exploited to perform bead-bacteria uptake comparisons. The paper was published in Science (1986).

Chapter 3 provides a more detailed investigation of mixotrophy in Dinobryon. We compared photosynthetic carbon fixation to phagotrophic particle ingestion by a metalimnetic population of the alga in a Laurentian lake. We also made a comparison of day-night grazing rates, and examined the effects of light, temperature and particle size on clearance rate. This paper was published in Limnology and Oceanography in 1987.

Whereas Chapter 3 shows that phagotrophy is potentially important to mixotrophic algae themselves, Chapter 4 shows that it can also be important to the phytoplankton as a whole. This study was done in Lac Gilbert, a small mesotrophic lake with unusually strong vertical stratification of the plankton community. Our estimates of phagotrophy in a dense layer of chrysomonads living at the 7 m depth in the lake showed that secondary production by algae was apparently 4 times greater than community photosynthesis at that depth. Laboratory experiments with the algae collected from this layer confirmed an earlier assumption that assimilation efficiency by Dinobryon was as

great as that of strictly heterotrophic algae. This paper has been accepted for publication in Limnology and Oceanography.

Chapter 5 expands on a suggestion made in Chapter 3, that size selective grazing by microflagellates could have a major impact on the size-abundance and size-activity spectra of the bacterioplankton. This chapter will be submitted to Applied and Environmental Microbiology.

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Chapter 1

Empirical relationships between bacterial abundance and chlorophyll concentration in fresh and marine waters

Abstract

A strong, positive empirical relationship was found between bacterial abundance and chlorophyll concentration in fresh and marine waters. Freshwater and marine linear regression equations are statistically indistinguishable. The overall equation is log AODC = 5.867 + 0.776 log chl *a*, r^2=0.88, where AODC (acridine orange direct count) is number of bacteria per ml and chl *a* is µg chlorophyll *a* per l. It is apparent that planktonic bacteria and algae are tightly linked in lakes and the sea. The slope of the regression line, however, shows that bacterial numbers do not increase as rapidly as algal biomass with an increase in nutrient concentration. We suggest that this disproportionately smaller increase in bacterial numbers need not signify a smaller role for bacteria in lake metabolism with increasing nutrient availability, if bacterial productivity per unit bacterial biomass increases as total bacterial biomass increases between systems.

Introduction

Many authors have suggested that bacterial abundance increases with the trophic state of freshwater and marine systems (Azam et al. 1983; Faust and Correll 1976; Ferguson and Palumbo 1979; Fuhrman et al. 1980; Godlewska-Lipowa, 1976, 1979; Hobbie and Wright 1979; Jones 1972, 1977; Kuznetsov 1970; Rao et al. 1979; Saunders 1980; Silvey and Roach 1964; Straskrabova 1968). Straskrabova (1968) and Kuznetsov (1970) produced tables linking bacterial direct counts to trophic classification. In a study of a large number of lakes and reservoirs in Poland, Godlewska-Lipowa (1976) showed that ranking lakes by bacterial abundance also ranks them by degree of eutrophication and organic matter pollution. A similar multi-lake study by Spencer (1978) uncovered a weak but significant positive correlation between bacterial numbers and both total nitrogen (R^2 = 0.31) and total phosphorus (R^2 = 0.16) in New Zealand lakes. Aizaki et al. (1981), and more recently Linley et al. (1983), first put the relationship into an explicitly predictive form, in fresh and marine systems respectively. Our objective was to quantify and test empirically the hypothesized relationship between bacterial abundance and trophic condition, measured as the concentration of chlorophyll a in surface waters.

Aizaki et al. (1981) found a strong statistical relationship between bacterial number and chlorophyll a in Japanese lakes. We attempted to extend that result in two ways. First, we tested the general applicability of their findings to other lakes and to the sea, using both literature values and observations collected in eleven Quebec lakes spanning a wide trophic range. Second, we considered the mathematical form of the resulting equations in the light of current

ideas about the role of bacteria in aquatic systems. The work was undertaken to provide a firm basis for the prediction of bacterial abundance but would also allow us to draw inferences about algal-bacterial interactions.

Methods

Literature values

Records of simultaneous observations of bacterial abundance and chlorophyll concentration were collected from the literature (Bunch 1979; Chapra and Dobson 1981; Coveney 1982; Daley et al. 1981; Field et al. 1980; Fuhrman et al. 1980; Hodson et al. 1981; Kilham 1981; Kogure et al. 1980; Krempin and Sullivan 1981; MacIsaac et al. 1980; Meyer-Reil et al. 1979; Rao et al. 1979; Rao et al. 1981; Riemann et al. 1982; Schleyer 1981; Vaatanen 1980; Valdes and Albright 1981. The data used in all analyses are available from the Depository of Unpublished Data, CISTI, National Research Council of Canada, Ottawa, Ontario K1A 0S2). We restricted the study to those papers that used the acridine orange-epifluorescence direct count (AODC) method of Francisco et al. (1973) as modified by Hobbie et al. (1977), a method that is now in general use for bacterial enumeration. The AODC method has the needed contrast, not attainable even with the transmitted light-acridine orange technique, to visualize the very numerous small bacteria (0.1-0.6 μm) and to distinguish them from detritus (Daley 1979). Counts using electron microscopy have confirmed that all bacteria trapped on the 0.2 μm filter are counted using AODC (Bowden 1977; Larsson et al. 1978). Loss of the small bacteria that pass through this filter is accepted because these bacteria contribute little to total bacterial biomass, and

because the low flow rate of finer filters makes them unsuitable for routine use (Zimmermann 1977).

Bacterial observations taken at a depth of 1 m were used when possible. This was the most common depth for bacterial sampling, making up 62% of the data used, and selection of these samples created a more homogeneous data set. However, since the use of 1-m samples exclusively would have reduced the data to only 23 points, we accepted AODC data collected elsewhere in the euphotic zone together with chlorophyll a measurements there. Some data represent single samples of both bacteria and chlorophyll at discrete depths (39%), some represent the summer average at a discrete depth (21%), some represent the summer average of epilimnetic integrated samples (39%),and one is an annual average. Single sample observations are more likely to be in error than many-sample averages; we looked for the effect of this error on our analysis by using a weighting method, described in the results section. We accepted the results of any spectrophotometric or fluorometric analyses of chlorophyll a concentration. The specific routine used for chlorophyll measurement was outlined for 54% of the literature observations. Of these, 15% of the marine and 50% of the freshwater estimates were corrected for phaeopigment interference. However, we could find no systematic differences between corrected and uncorrected observations in any subsequent analyses.

We performed a simple linear regression analysis on the data using both SAS (Helwig and Council 1979) and BMDP9R (Dixon 1981), depending on the statistical features desired. The data were transformed to logarithms to more closely satisfy the assumptions of ordinary least squares (Draper and Smith 1981). Regression coefficients were tested for significance following Edwards (1967); confidence limits

for coefficients were calculated according to Draper and Smith (1981). An independent bacteria-chlorophyll data set (27 points) collected from the literature following the original analysis, was used to test the model's predictive capabilities. Nonparametric testing of these results followed Conover (1971). Marine observations from this same data set were used to test the predictive model of Linley et al. (1983).

When appropriate we compensated for two possible types of error in using regression estimates. First, the simple antilog of the value predicted by a log-transformed regression equation underestimates the expected value of the untransformed variable (Land 1972). To correct for this bias, the antilog of the prediction must be multiplied by a correction factor calculated as antilog(2.651 X RMS), where RMS is the residual mean square of the regression (Neyman and Scott 1960). We calculated correction factors for all predictive equations presented. Unbiased confidence limits for the mean and for individual predictions were calculated using Cox's direct method (Land, 1972). Second, when there is error in the independent variable, the ordinary least squares slope estimate can underestimate the slope that would be obtained if error were absent. In the usual case in ecology where the magnitude of independent variable error is unknown, there is no certain method of determining this underlying ideal or "functional" slope (Sprent and Dolby 1980). We derived the Model II, geometric mean slopes recommended by Ricker (1973) in an attempt to compensate for this bias when we were seeking functional relationships.

Quebec lakes

Eleven Quebec lakes were sampled for bacteria during July-August

1982 at 1 m with a Van Dorn bottle. The bacteria were preserved in buffered formalin and counted according to Hobbie et al. (1977). We stained 2 mL samples with 0.2 mL of 0.1% acridine orange, and filtered the samples through dyed (Irgalan black) 0.2 μm pore-size Nuclepore filters. The filters were mounted in oil and examined at 1000X with a Leitz Orthoplan microscope equipped with Ploem Pack 2, an HBO 100 W lamp, Cube G, 3 mm excitation filter BG 12, mirror 510, and barrier filter 515. We counted 10 to 50 randomly selected fields from all parts of the filter so that confidence limits for the mean count were always less than 10% of the sample mean.

Chlorophyll values for the lakes (M. Pace, now Department of Oceanography, University of Hawaii, Honolulu, unpublished data) are the means of monthly samples taken throughout the summer, analyzed according to Strickland and Parsons (1972), and corrected for phaeopigment content with the equations of Lorenzen (1967). Samples were taken with a tube at one station over the deepest part of the lake. Depending on the depth of the lake, samples were integrated over depths of between 0-8 and 0-10 m. Because of the importance of phosphorus in determining different aspects of lake productivity, total phosphorus samples were collected on the same dates and at the same depths as the chlorophyll samples, and analyzed according to Johnson (1971).

Results

We found a highly significant relationship between AODC data from both the freshwater and marine literatures and the associated chlorophyll *a* concentrations (F-test: $P<0.0001$) (Fig. 1) (Table 1). The overall relationship using data from both sources is:

$$\log \text{AODC} = 5.855 + 0.844 \log \text{chl } \underline{a}, \qquad (1)$$

where AODC is acridine orange direct count (number of bacteria mL^{-1}) and chl _a_ is chlorophyll _a_ concentration ($\mu g\ L^{-1}$). There was a weak but significant negative correlation between the absolute value of the residual error term associated with a particular point and the number of separate observations represented by the point (t-test: $P < 0.05$). We reanalyzed the data, weighting each data point by the number of separate bacteria-chlorophyll samples it represented. This procedure reduced the effect of single sample observations on the coefficient estimates and emphasized multi-sample observations. The weighted least squares analysis lowered the slope:

$$\log \text{AODC} = 5.877 + 0.783 \log \text{chla}. \qquad (2)$$

The major reason for the change in the equation was revealed by scrutiny of the individual residuals using Cook's distance measure, D_i (Cook and Weisberg 1982). The numerical value of Cook's distance associated with a particular observation is a simple and objective index of the influence of that observation on the overall regression estimates (Cook 1977). The value of D_i associated with an individual observation reflects the amount of change that would occur in the regression equation coefficients were that observation to be removed from the analysis. A high D_i associated with Lake Elmenteita, Kenya (AODC: Kilham 1981; chlorophyll: Kalff 1983) showed that the point was having a strong influence on the equation. Cook's D_i for Elmenteita was 1.22; a value greater than 1.00 in this case indicates a highly influential point (Cook and Weisberg 1982, p. 118). We recalculated the coefficients

excluding this point; the revised equation is:

$$\log AODC = 5.867 + 0.776 \log chla. \qquad (3)$$

This corresponds very closely to the weighted least squares regression and is probably a more accurate representation of the relationship between bacterial abundance and chlorophyll concentration than the original equation using all data (equation 1). By excluding Lake Elmenteita from the analysis, its influence was removed, though the useful range of the equation was reduced at the same time. The range of chlorophyll a concentrations over which this equation (equation 3) is now useful is from 0.05 to 120 $\mu g\ L^{-1}$.

We split the data into components for hypothesis testing. First, the slope of the equation using just freshwater data is not significantly different from the one obtained by Aizaki et al. using data from Japanese lakes (Fig. 1, Table 1) although the probability was borderline (t-test: $0.05<P<0.1$). However, the intercept difference is highly significant (t-test: $P<0.0001$). Next, the marine data (Fig. 1) were analyzed separately (Table 1). Neither the slope nor intercept was significantly different from the freshwater literature model (intercept: t-test; $0.2<P<0.4$; slope: t-test; $0.5<P<0.6$), whereas again the intercept was significantly higher than the Aizaki model (intercept: t-test; $P<0.0001$; slope: t-test, $0.3<P<0.4$). When we tested the freshwater literature equation against the Quebec lakes equation (Table 1, Table 2, Fig. 2), we found that again the slopes were not statistically distinguishable (t-test; $0.2<P<0.4$) but that the intercepts were different (t-test; $P<0.001$).

Figure 1

Scatter diagram of the points obtained from the literature used to derive regressions of acridine orange direct counts of bacterial abundance and chlorophyll a concentration. Solid lines represent separate freshwater and marine regressions we derived; broken line from Aizaki et al. (1981). Closed circles are freshwater data; open circle is Lake Elmenteita, Kenya (Kilham 1981; Kalff 1983); stars are marine data. Equations are given in Table 1.

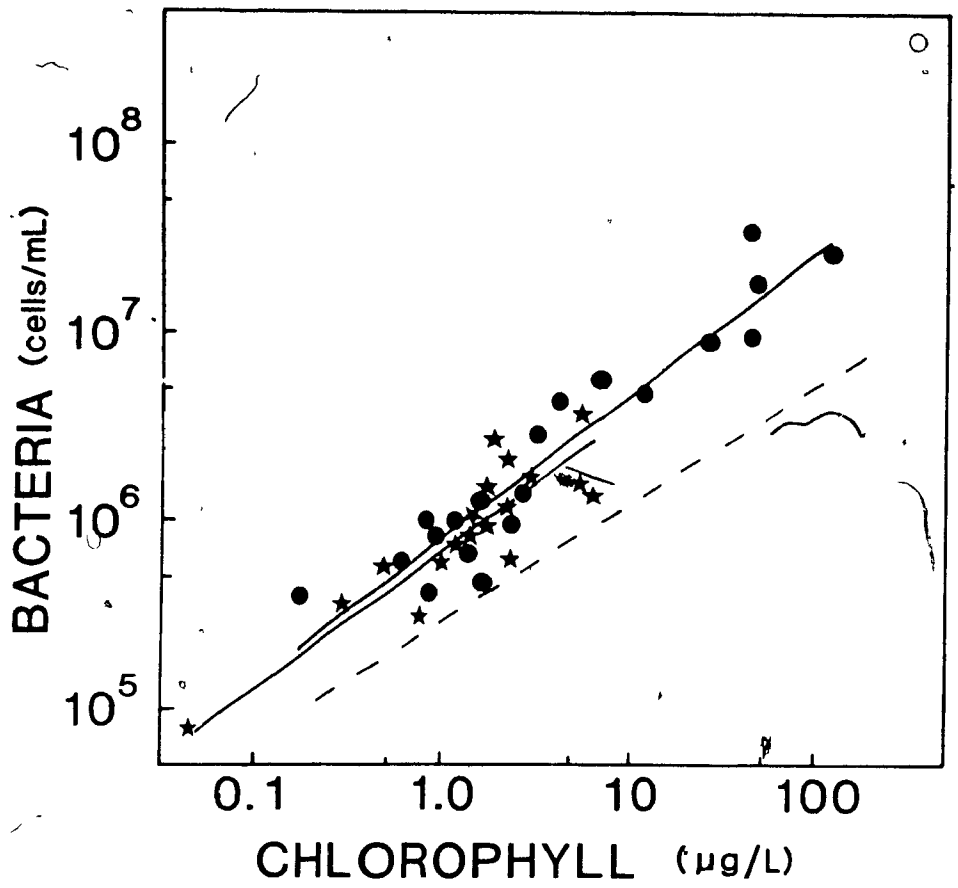

BACTERIA (cells/mL)
10^8
10^7
10^6
10^5
0.1
1.0
10
100
CHLOROPHYLL (µg/L)

Table 1. Regression equations derived in this paper, with descriptive statistics. Variables are AODC (acridine orange direct count, number of bacteria mL^{-1}), chl*a* (chlorophyll *a* concentration, $\mu g.L^{-1}$), BB (bacterial biomass, $\mu g\ C.L^{-1}$), and TP (total phosphorus concentration, $\mu g.L^{-1}$). All variables were log-transformed. Confidence limits around an individual prediction can be calculated using mean X, ΣX^2, information on the residual mean square contained in the correction factor (see text), and standard confidence limits formulae. Predictions of the equations, retransformed to the arithmetic scale, must be multiplied by the correction factor given in order to correct for a bias inherent in the use of log-transformed equations. The model II (geometric mean) slope is provided as an estimate of the functional or "true" numerical relationship between variables when there is error in the independent variable X.

TABLE 1.

Source	Y,X	N	intercept (confidence limits)	slope (confidence limits)	r^2	Mean X	ΣX^2	Correction factor	Model II slope
Overall Literature (including Lake Elmenteita)	AODC, chl*a*	40	5.85 (±0.080)	0.844 (±0.093)	0.90****	0.430	29.5755	1.132	0.89
Overall Literature (excluding Lake Elmenteita)	AODC, chl*a*	39	5.867 (±0.072)	0.776 (±0.094)	0.88****	0.375	22.998	1.105	0.83
Freshwater Literature	AODC, chl*a*	20	5.911 (±0.120)	0.763 (±0.125)	0.90****	0.591	18.393	1.114	0.80
Marine Literature	AODC, chl*a*	19	5.835 (±0.096)	0.736 (±0.194)	0.79****	0.148	4.6047	1.099	0.83

. . . continued

Table 1 (continued)

Source	Y,X	N	intercept (confidence limits)	slope (confidence limits)	r^2	Mean X	ΣX^2	Correction factor	Model II slope
Aizaki et al., 1981	AODC, chla	23	5.445 (±0.094)	0.630 (±0.088)	0.92****	0.748	26.074	1.066	0.66
Québec Lakes (this study)	AODC, chla	13	6.277 (±0.190)	0.569 (±0.270)	0.66***	0.611	6.6624	1.087	0.70
Es and Meyer-Reil, 1982	BB, AODC	10	-3.80 (±1.77)	0.79 (±0.30)	0.82***	5.878	356.3	1.599	0.87
Québec Lakes	AODC, TP	12	5.953 (±0.229)	0.663 (±0.206)	0.83****	1.052	14.782	1.036	0.73
Québec Lakes	chla, TP	12	-0.486 (±0.272)	1.043 (±0.245)	0.90****	1.052	14.782	1.049	1.098

**** $p < .0001$

*** $p < .001$

Figure 2

Bacterial count (AODC) and chlorophyll concentration in 11 Quebec lakes. Solid line is the line of best fit to these data; dotted lines are 95% confidence limits for predictions of the freshwater literature equation (Fig. 1).

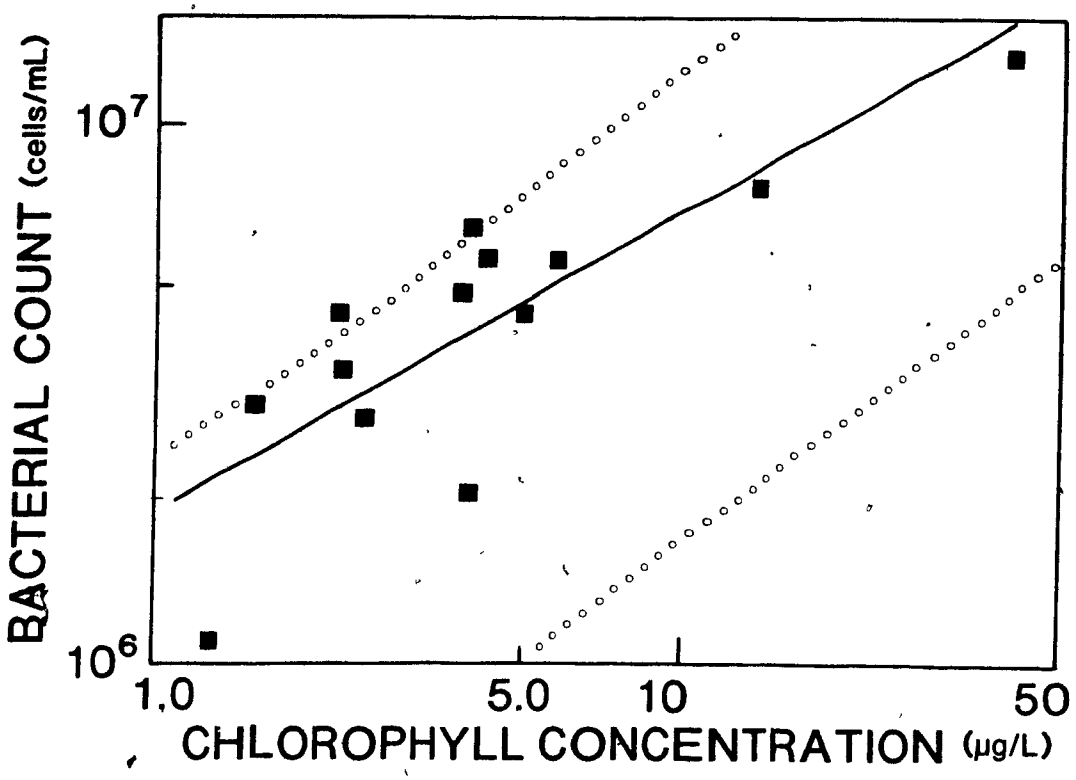

BACTERIAL COUNT (cells/mL)
10^7
10^6
1.0
5.0
10
50
CHLOROPHYLL CONCENTRATION (µg/L)

TABLE 2 Bacterial abundance, (millions of cells $\cdot mL^{-1}$), chlorophyll concentration ($\mu g.L^{-1}$), and total phosphorus concentration ($\mu g.L^{-1}$) in 11 Quebec lakes. These values were used in the Quebec Lakes regressions of Table 1.

Lake	AODC	chla*	TP*
Waterloo	13.4	34.98	59.70
Brome	6.50	3.95	14.64
Argent	4.47	2.22	10.70
Brompton	3.56	1.97	5.96
Bowker	1.10	1.30	3.19
Orford	3.00	1.53	4.77
Massawippi	5.72	4.20	12.71
Magog	7.75	13.55	47.39
Lovering	2.85	2.50	7.04
Maryjo	2.08	4.0	-
Memphremagog			
North basin	3.80	4.92	9.10
Quinn Bay	4.90	4.57	10.05
South basin	5.86	5.64	12.79

* M. Pace unpubl.

We used a dummy intercept variable to get an estimate of the average difference between observations from the literature and from Aizaki et al. (1981) (Draper and Smith 1981). This variable has a value of one for all Aizaki data points, and zero for the others that serve as reference points. When used in a multiple regression, a dummy variable has the effect of moving the center of mass of the group of observations to lie on the reference regression line. The coefficient of the dummy is then the average difference in the dependent variable values between groups. The resulting equation is:

$$\log AODC = 5.953 + 0.692 \log chla - 0.554\ D1 \qquad (4)$$

where chla is chlorophyll a concentration ($\mu g\ L^{-1}$), AODC is number of bacteria $\cdot\ mL^{-1}$, and D1 is the dummy variable. The dummy variable coefficient reveals that on average the Aizaki et al. bacterial counts are about 60% lower than those from the literature at comparable chlorophyll concentrations. The difference seems to stem from differences in methodology (see discussion).

The statistical relationships between bacteria and total phosphorus concentration, and between chlorophyll and total phosphorus concentration (Table 1) were determined using the Quebec data (Table 2) in order to approach algal-bacterial interactions from a different perspective. Bacteria-phosphorus relations have become of interest recently following indications that bacteria might play an unexpectedly large, even dominant, role in orthophosphate uptake in situ (Faust and Correll 1976; Harrison et al. 1977; Krempin et al. 1981; Lean and White

1983). The slope of the bacteria-phosphorus regression was significantly less than one (t-test;$P<0.01$) while the slope of the chlorophyll-phosphorus regression was not different from one. This means that though the algal biomass rises at the same rate as total phosphorus concentration, the bacteria make up a smaller proportion of the total phosphorus standing stock at eutrophic than at oligotrophic sites.

Bacterial and algal data collected from the literature since the original analysis was done were used to test the model (equation 3). Two observations out of 27 fell outside the prediction confidence intervals (Table 3, Fig. 3), which is not an unexpected number (X^2-test: $P > 0.4$).

Particular results are of some interest. First, the bacterial concentrations observed at elevated chlorophyll levels in estuarine waters (Hoppe 1983) were below the predicted values 9 times out of 10. It is likely that the sampling period for these observations (winter-spring bloom) is responsible for this discrepancy because chlorophyll levels were then elevated above their mean levels. However, we have no longterm bacteria-chlorophyll observations at productive marine sites to corroborate this, and therefore cannot be sure that the model does not overestimate bacterial abundance in such cases. Second, the overestimate of bacterioplankton numbers in eutrophic Lake Norrviken (Bell et al. 1983) can be considered a failure of the model in this case (t-test: $P<0.001$). Bell et al. noted the low number of bacteria in the lake and suggested that an allelopathic interaction with blue-green algae in late summer might be responsible. Other blue-green dominated

Figure 3

Comparison of literature regression line (equation 3) with observations collected from the literature (Table 3) subsequent to the original analysis. Broken lines are 95% confidence limits for an individual prediction. a) Observations from inland waters. b) Observations from marine and estuarine waters. Lower curved line corresponds to the predictive equation of Linley et al. (1983).

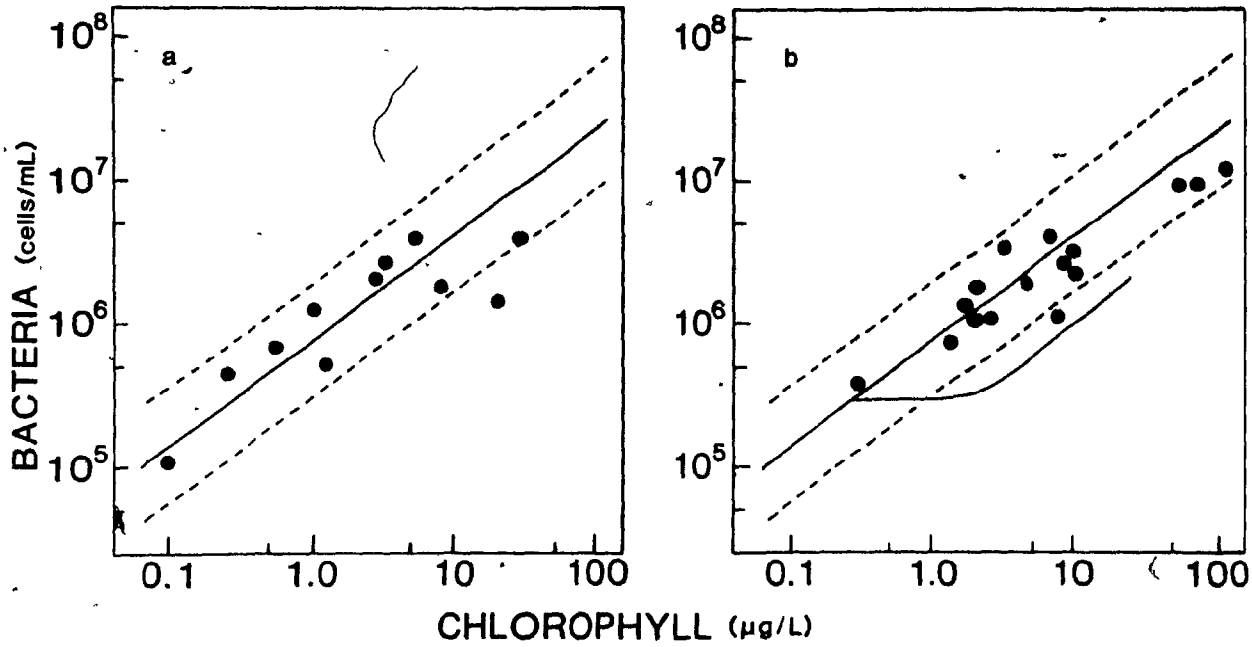

a
b
10^8
10^7
10^6
10^5
BACTERIA (cells/mL)
0.1
1.0
10
100
CHLOROPHYLL (µg/L)

Table 3: Observed total bacterial abundance at marine, estuarine and inland sites from the published literature, with predicted abundance from equation 3 and 95% confidence limits. Abundances are given as millions of cells mL^{-1}; N refers to the total number of separate observations pooled to get average value.

Location	Observed bacterial abundance	Predicted abundance	Lower and Upper 95% confidence limits	N	Source
Marine and Estuarine					
Kiel fjord					
(Haten)	2.60	4.16	1.49 - 9.48	1	Hoppe 1983
(Friedrichsort)	2.48	4.44	1.59 - 10.1	1	Hoppe 1983
(Laboe)	1.11	4.09	1.47 - 9.31	1	Hoppe 1983
(Feuerschiff)	1.05	1.66	0.60 - 3.75	1	Hoppe 1983
(Kieler Bucht Mitte)	1.03	1.60	0.58 - 3.63	1	Hoppe 1983
Schlei fjord					
(Gr. Breite)	14.09	31.0	10.5 - 75.1	1	Hoppe 1983
(Missunde)	9.81	23.6	8.08 - 56.5	1	Hoppe 1983
(Kappeln)	9.11	17.8	6.16 - 42.1	1	Hoppe 1983
(Schleimünde)	2.46	1.34	0.48 - 3.03	1	Hoppe 1983
(Boknis Eck)	1.78	2.43	0.88 - 5.51	1	Hoppe 1983
Tvarminne archipelago					
(Storfjärd)	2.0a	3.29a	1.31 - 8.29	14	Vaatanen 1982

. . . continued

Table 3 continued ...

(Maskskär)	1.7[a]	1.86[a]	0.74 - 4.65	14	Vaatanen 1982
Cape Hatt, N.W.T.	0.386	0.369	0.13 - 0.85	108	Bunch et al. 1981
Kiel Fjord	3.09	4.80	1.72 - 10.9	13	Bolter et al. 1977
Kiel Bight	1.50	1.32	0.48 - 2.99	12	Bolter et al. 1977
St. Croix, Virgin Islands	0.745	1.05	0.38 - 2.39	10	Lasker et al. 1983
Freshwater					
Merrill Lake	0.5	0.32	0.11 - 0.74	1	Wissmar et al. 1982
McBride Lake	0.6	1.00	0.36 - 2.25	1	Wissmar et al. 1982
June Lake	0.1	0.14	0.05 - 0.32	1	Wissmar et al. 1982
Blue Lake	0.8	0.55	0.20 - 1.25	1	Wissmar et al. 1982
Lake Norrviken	1.55	8.54	3.02 - 19.8	4	Bell et al. 1983
Pyramid Lake	2.93	2.05	0.74 - 4.65	17	Galat et al. 1981; Hamilton-Galat and Galat 1983
Lake Gardsjon	1.31	0.84	0.30 - 1.92	16	Olsson 1983; Johansson 1983
Lake Vechten	1.89	4.78	1.71 - 10.9	16	Blaauboer et al 1982
Eastern Lake Erie	2.00	1.82	0.72 - 4.49	6	Lean et al. 1983
Central Lake Erie	3.93	2.73	1.07 - 6.76	6	Lean et al. 1983
Lake Tystrup	4.10	10.50	3.69 - 24.49	17	Riemann 1983

[a] Geometric means

lakes are not similarly affected (Coveney 1982; Hamilton-Galat and Galat 1983; Kilham 1981; Riemann et al. 1982) so that special circumstances must be involved. The model performed well for the other observations in Table 3. Observations were evenly distributed above and below the prediction line (binomial test: $P>0.20$) and there was no apparent bias at low or high bacterial concentrations (2 X 2 contingency table: $P>0.60$). The mean ratio of predicted abundance to observed abundance was not significantly different from 1.0 for either freshwater (ratio=0.96: t-test; $P > 0.50$) or marine data (ratio=1.17: t-test; $P>0.20$).

We used marine observations from the independent data set (Table 3) to test the predictions of a model developed by Linley et al. (1983). Their equation predicts AODC from chlorophyll a concentration, based on data the authors collected at 7 upwelling and coastal marine stations. The 13 observations from the independent set that fell within the range of their equation (0.10-26.22 µg chlorophyll a L^{-1}) all had more bacteria than predicted by their equation (Fig. 3), and 11 of the 13 were outside that model's 95% prediction confidence limits (χ^2-test: $P=0.0$).

Discussion

Bacteria and phytoplankton are apparently quantitatively tightly linked both in lakes and the sea. Nevertheless, some considerable scatter remains to be explained and the confidence limits for an individual prediction are wide (Fig. 3). Reasons for this scatter probably include temporal changes in the relationship between chlorophyll a and algal biomass (Granberg and Harjula 1982), differences in bacterial cell volume, within-lake plankton dynamics, variation in

allochthonous carbon loading, and systematic errors resulting both from differences in microscope configuration (Daley 1979) and from between-operator variability. The systematically lower counts by Aizaki et al. (1981) are at least partially attributable to their use of 0.4 µm, undyed Nuclepore filters (M. Aizaki, pers. comm.) rather than the 0.2 µm, dyed filters recommended (Hobbie et al. 1977) and used by all others in this data set. Cole et al. (1982) noted that one quarter to one half of the bacteria trapped on a 0.2 µm filter passed through a 0.4 µm filter. It appears that on average, three times more bacteria would have been counted by Aizaki et al. had they used the finer filters. The Japanese lakes were excluded from our overall equation since any systematic changes in bacterial diameter along a trophic gradient, allowing greater or lesser proportions of bacteria through the larger pores, would bias the slope estimate. Why our own counts should be significantly higher than literature values is unclear, except that we were exceedingly concerned not to miss even the smallest bacteria that are visible only as minute blurs of light, and we may have counted too many questionable particles in our zeal. Alternatively, our midsummer bacterial observations may overestimate growing season means. The lower and higher intercepts of the Japanese and the Quebec lakes, respectively, are probably due to differences in the bacterial count and not to differences in chlorophyll data, since regressions of chlorophyll on total phosphorus for both cases were indistinguishable from each other, and from established relations (Schindler 1978; Vollenweider and Kerekes 1980).

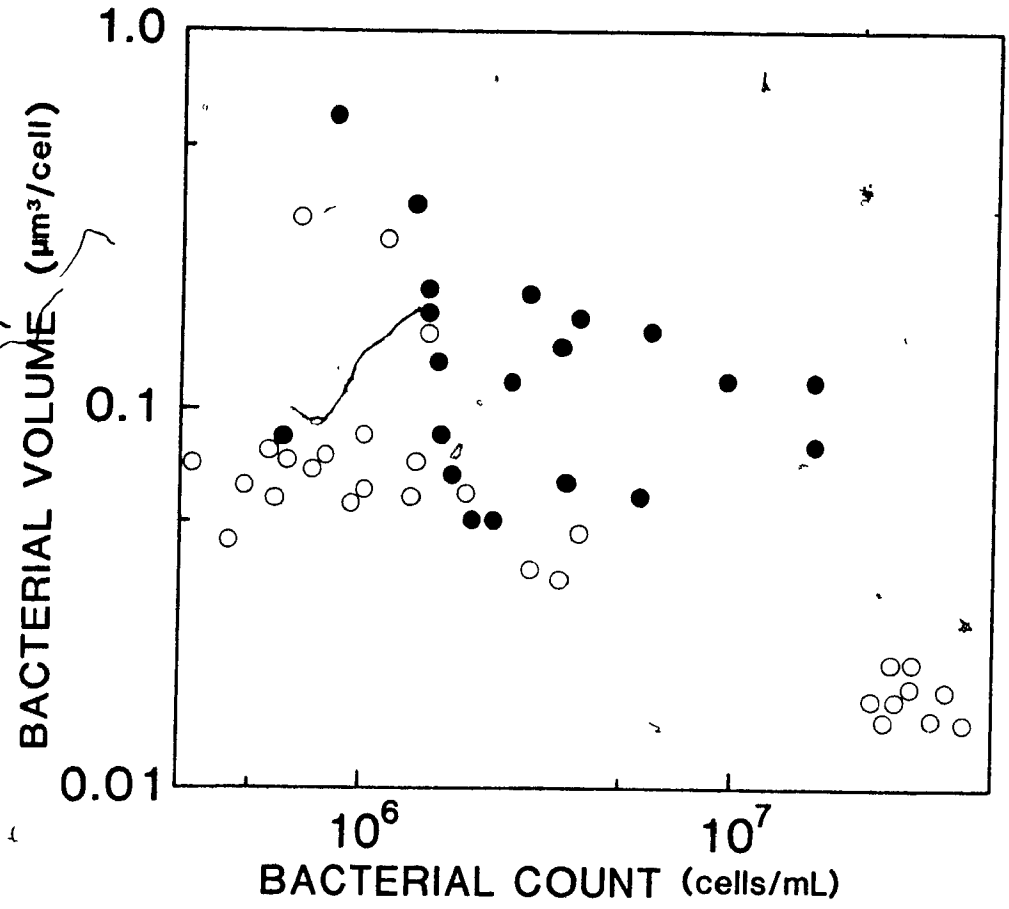
Lake Elmenteita could not be justifiably removed from the data set on the basis of prior information. However, its remoteness from the other points in the sample space accorded it considerable influence in

the analysis. Elmenteita's chlorophyll level on the date sampled, measured within 3 weeks of the bacterial sampling, was 367 $\mu g\ L^{-1}$, while the next highest level in our data set was another Kenyan lake at 120 $\mu g\ L^{-1}$. We decided that until adequate coverage is obtained of lakes with chlorophyll concentration greater than 120 $\mu g\ L^{-1}$, estimation and prediction beyond that point is premature.

The slope of the overall regression equation, at about 0.78 (model I) or 0.83 (model II), means that the increase in the number of bacteria per unit volume does not keep pace with the increase in chlorophyll a as lake trophy changes. This is not peculiar to the bacteria but has also been observed for the crustacean zooplankton (McCauley and Kalff 1981) and for fish (reanalysis of equations in Hanson and Leggett (1982), and Jones and Hoyer (1982) on volumetric yield basis). If bacteria are dependent on primary production for reduced carbon, then there is no obvious reason why this disproportionately lower increase should occur. It was suggested by Pedros-Alio and Brock (1982) that bacterial cell size is positively correlated with trophy, so that bacterial biomass would increase more rapidly than number. Though this would help to explain the discrepancy, we found no evidence for such a relationship (Fig. 4). Indeed, based on the scanning electron microscope cell-size estimates there seems to be a trend toward smaller bacteria in eutrophic waters (t-test on slope: $P<0.05$) though its statistical significance depends on the cluster of Plon lakes (Krambeck et al. 1981) in the lower right corner of figure 4, and should probably be confirmed at other eutrophic sites before being accepted. Care was taken during this analysis to distinguish biomass estimates based on epifluorescence microscopy from those based on scanning electron microscopy, which have been shown to be significantly

Figure 4

Relationship between bacterial cell volume and bacterial abundance. Solid circles are observations made using acridine orange - epifluorescence, open circles are observations made using scanning electron microscopy.


1.0
0.1
0.01
BACTERIAL VOLUME (μm³/cell)
10⁶
10⁷
BACTERIAL COUNT (cells/mL)

different using identical bacterial samples (Fuhrman, 1981). There was no significant relationship between cell size and abundance when only epifluorescence estimates were used. Furthermore, an analysis of data collected from the marine literature by Es and Meyer-Reil (1982) showed the relationship between biomass and abundance to be indistinguishable from linear over a 1000-fold range in bacterial abundance (Table 1). Therefore it is unlikely that the use of abundance rather than biomass as dependent variable has biased the conclusion that chlorophyll concentration and bacterial standing stock do not increase in parallel along a trophic gradient.

Alternatively, it is possible that bacterial abundance is not a proportionate measure of biological activity. It may be that bacterial productivity per unit bacterial biomass increases at a faster rate with increasing trophy than primary productivity per unit chlorophyll. This would mean that bacterial productivity would be approximately linearly related to primary productivity, in inter-lake comparisons, and that the decline in bacterial numbers relative to chlorophyll concentration is a necessary consequence. Unfortunately, there is still considerable uncertainty in measurements of bacterial productivity. There are at least nine methods in current use (Newell and Christian 1981) and some of these give widely different results (Cole et al. 1982). It is, therefore, not yet possible to test this hypothesis. However, it appears that the relationship between daily primary productivity and chlorophyll a concentration is roughly linear between lakes (Smith 1979). If bacterial productivity is linearly related to algal productivity, it must follow that bacterial productivity increases disproportionately faster than bacterial density, given our algal-bacterial equations. This might occur if bacteria were more

heavily grazed in eutrophic than in oligotrophic systems (cf. Gliwicz 1969;Beaver and Crisman 1982) or subject to higher sedimentation losses (Pedros-Alio and Brock 1982;Cole et al. 1982), or if fewer bacteria were dormant and, if so, for shorter periods in eutrophic systems (cf. Morita 1982).

The form of the empirical relationship between bacterial abundance and total phosphorus concentration in Quebec lakes (Table 1) is of considerable interest because bacterial orthophosphate uptake has recently been shown to be important to the cycling of phosphorus in lakes (Currie and Kalff 1984, Lean and White 1983), estuaries (Faust and Correll 1976) and marine coastal waters (Harrison et al. 1977; Krempin et al. 1981). It is known experimentally (Faust and Correll 1976;Mayfield and Inniss 1978;Rhee 1972) and might have been predicted from body size considerations (Smith and Kalff 1982) that bacteria have a decided advantage over the algae in orthophosphate uptake. Yet the epilimnetic bacteria of eutrophic waters appear to contain a smaller proportion of the total phosphorus (TP) present in the system than do the bacteria of oligotrophic waters. This follows from the curvilinearity of the relationship between AODC and TP for the Quebec lakes (Table 1: model I slope=0.66; model II slope=0.73). The slope of the regression line linking chlorophyll concentration to total phosphorus is greater than that linking AODC to TP, suggesting that as total phosphorus increases, an increasing proportion moves into the algae relative to that of the bacteria. If the bacteria are not subject to disproportionately higher loss rates and if their specific production does not increase with trophic state, then they must be limited by some factor that does not increase at the same rate as phosphorus availability with trophy.

A regression equation linking bacterial counts to chlorophyll concentration in several marine locations has recently been published by Linley et al. (1983). Their model, based on data from the English Channel and an upwelling region east of South Africa, yields predictions of bacterial abundance that are on average 3.0 times lower than observed abundances at the independent sites compatible with our model (Fig. 3). The Linley et al. model and our literature model are both internally consistent yet they yield mutually incompatible predictions. If the differences are not methodological, they point to (more interesting) biological differences between the sites sampled by Linley et al. and the sites described by our literature-based equation. In this case the distinguishing attributes are not known.

Lakes can be grouped into trophic categories according to bacterial abundance using a chlorophyll a based classification (Forsberg and Ryding 1980) and the regression equation (Table 1). Oligotrophic waters should contain less than 1.7 million bacteria $\cdot mL^{-1}$, mesotrophic 1.7 - 6.5 million $\cdot mL^{-1}$, and eutrophic waters more than 6.5 million $\cdot mL^{-1}$. A comparison of these ranges with literature values based on older direct count methods is difficult because the trophic classification criteria used there were often not defined (e.g., Kuznetsov 1970, p. 122; Sorokin 1972). However, by converting Romanenko's (1973) Latvian lakes primary production rates to chlorophyll a concentrations (Smith 1979) an approximate comparison can be made. This reveals that acridine orange-epifluorescence direct counts are 2-7 times higher than Romanenko's transmitted light counts in oligotrophic and mesotrophic lakes, and up to 20 times higher in eutrophic lakes. Unfortunately there does not seem to be any simple conversion method that would make the older bacterial values comparable.

The purpose of the study was to demonstrate that aquatic bacterial abundance is predictable, albeit with some uncertainty for the present. It was intriguing to discover that bacteria relate to algal biomass in much the same way in the sea and in freshwater. The surprising fact that bacterial biomass does not increase at the same rate as that of the phytoplankton is currently unexplained, though hypotheses consistent with recent literature results can be advanced for test. Our ability to predict bacterial abundance from chlorophyll data need not be an end in itself, but provides an opportunity to pose more specific questions that should help to reduce the scatter about the line of best fit, and to gain insight into the role that bacteria play in aquatic environments.

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Chapter 2

Bacterial grazing by planktonic algae

Abstract

It was found that six common species of lake algae ingest bacteria. The ingestion rates measured were of the same magnitude as those recorded for marine microflagellates totally dependent on external sources of carbon. A large biomass of Dinobryon species removed more bacteria from the water column of a lake than crustaceans, rotifers, and ciliates combined.

The view that phytoplankton receive all their energy through photosynthesis was first placed in doubt when it was shown that some algae supplement their carbon supply by taking up dissolved organic carbon (1). The phytoplankton could no longer, therefore, be viewed as a strictly autotrophic community even though this uptake is normally modest and provides only a small fraction of the carbon acquired by photosynthesis (1). We now provide evidence that at least some natural phytoplankton are phagotrophic and apparently obtain a substantial fraction of their energy and nutrients by ingesting bacteria at rates very similar to those measured for nonphotosynthetic microflagellates.

The work was carried out in Lac Cromwell, Quebec, on 7 to 8 July 1984. Tracer quantities of bacteria-sized fluorescent latex beads (0.6 um in diameter) (Polysciences) were released into the plankton caught in a Haney in situ grazing chamber (2) at a depth of 3 m. After 1, 4, 7, 10, 13, or 17 minutes, the chamber was retrieved and the plankton were preserved and stained (3). Sample aliquots were filtered onto 10 um pore-size Nuclepore filters for epifluorescence counting of beads ingested by natural grazers. We confirmed that bead uptake rate was representative of bacterial uptake by performing experiments in which algae were exposed to mixtures of beads and tritium-labelled natural bacteria (4, 5).

Four species of the common planktonic alga Dinobryon were major consumers of bacteria in Lac Cromwell (D. sertularia, D. sociale v. americanum, D. cylindricum (Fig. 1a), and D. bavaricum). Other members of the Chrysophyceae, Uroglena americana (Fig. 1b) and U. conradii, also ingested particles. The "grazing" algal community was found to be most concentrated in a thin layer within the thermocline; there

Figure 1

Electron micrographs showing bacterial cells inside chrysophycean algae from Lac Cromwell, Quebec (scale bar 1 μm). A. A Dinobryon cylindricum cell has 2 food vacuoles containing bacteria (b). Chloroplast (c) is also indicated. Fibre-containing vesicles (lv) are for secretion of lorica (l) (7). B. Thin section of Uroglena americana showing 2 large food vacuoles.

a

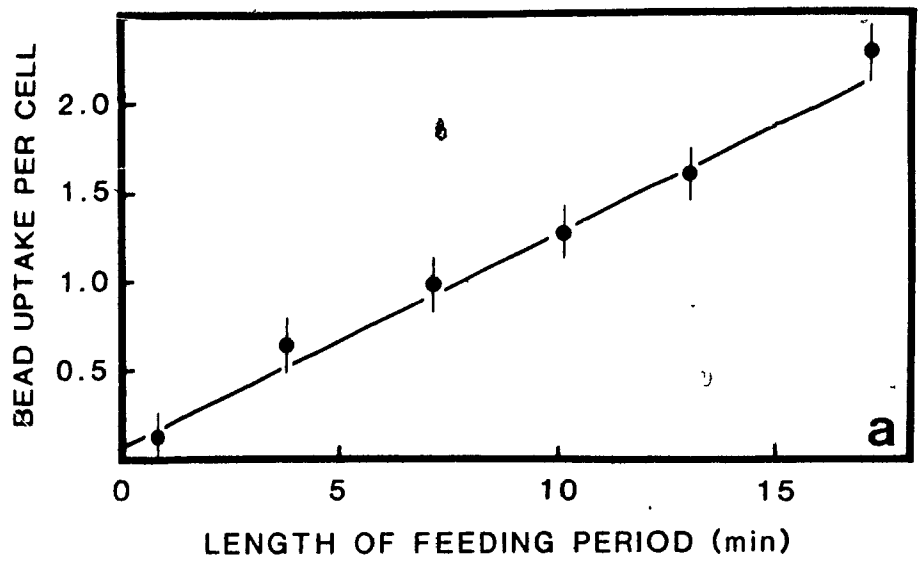
b

Dinobryon ingested 0.125 beads per minute per cell (95% C.L. ± 0.016) meaning that an average of three bacteria were consumed by each algal cell every 5 minutes (Fig. 2). This is equivalent to an individual Dinobryon cell removing bacteria from a volume equal to 1,500,000 times its cell volume, and ingesting almost 30% of its weight in bacteria per day. Uroglena's ingestion rate (0.022 beads per minute per cell (95% C.L. ± 0.007)) was very much lower than Dinobryon's both per cell and per unit biomass.

Although microscopists have noted bacterial ingestion by Dinobryon previously (6,7), the present study also demonstrates that phytoplanktonic phagotrophy is quantitatively important in nature. The grazing rates are of the same magnitude as those measured for marine microflagellates that lack photosynthetic pigments and are therefore totally dependent on external sources of carbon (8). Comparisons of carbon gain under the low light conditions in the metalimnion show that Dinobryon obtained at least 50 percent of its total carbon by bacterivory and thus at most 50 percent by photosynthesis (9). Furthermore, in Lake Memphremagog, Dinobryon can remove more bacteria from the water column than the crustacean, rotifer, and ciliate communities combined (Table 1) (10). Such bacterivory may be both a major factor in bacterial loss and a major source of carbon for some algae growing under low-light conditions in nature.

Figure 2

Time course of bead uptake by Dinobryon species in the metalimnetic algal biomass peak, Lac Cromwell (3 m depth, 12 °C). Each point represents the mean bead count, with 95% confidence limits, for 300 to 600 cells. Uptake rate was determined from the slope of the relationship between bead content per cell (y) and incubation time in minutes (x): $y = 0.064 + 0.125x$, $r^2 = 0.99$. B. Comparison of bead and natural bacteria uptake by Dinobryon. Experiments were performed on 28 November 1984, when a Dinobryon population nearly free of extraneous bacterivores was found beneath the lake ice. Each point represents a separate bead-bacteria feeding experiment (5). Bacteria and beads were offered in the ratio 17.3 ±1.2 to one (n=2); observed uptake was 22.85 ± 2.5 to one (n=11) so that bacteria were ingested 1.32 ± 0.11 times as readily as beads. These results are expressed in the figure as the ratio of the number of beads found inside Dinobryon cells to the number expected to be there were there no discrimination.


BEAD UPTAKE PER CELL
2.0
1.5
1.0
0.5
0
5
10
15
LENGTH OF FEEDING PERIOD (min)
a

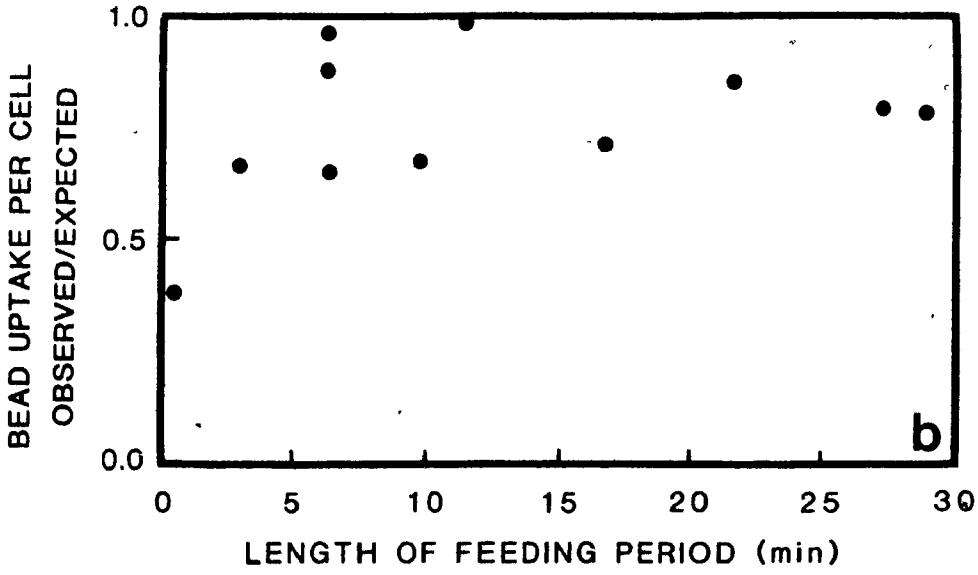

BEAD UPTAKE PER CELL
OBSERVED/EXPECTED
1.0
0.5
0.0
0
5
10
15
20
25
30
LENGTH OF FEEDING PERIOD (min)
b

Table 1. Results of feeding experiments in Quinn Bay, Lake Memphremagog on 18 June 1983. Bacterial abundance was 5 million cells per milliliter. Clearance rate is the volume of water from which bacteria were removed per day. Results comparable to these can be derived from many lakes, since Dinobryon is a common dominant alga (11). Dinobryon abundance in Eastern U.S. lakes averages 142,000 cells per liter when present and 633,000 cells per liter when it is dominant (12).

Organism	Mean abundance per liter	Individual mean clearance rate (milliliters per day)	Group mean clearance rate (95% confidence limits) (milliliters per day per liter)
Crustaceans	19.4	0.22	4.3 ± 2.1
Rotifers	238	0.014	3.3 ± 1.3
Ciliates	15000	0.0010	15.6 ± 11
Dinobryon	479000	0.00014	69.1 ± 19.8

Notes

1. G.W. Saunders, Limnol. Oceanogr. 17, 704 (1972); H.-G. Hoppe, Mar. Biol. 36, 291 (1976); W.F. Vincent and C.R. Goldman, Limnol. Oceanogr. 25, 89 (1980); B.K. Ellis and J.A. Stanford, Limnol. Oceanogr. 25, 89 (1982).

2. J. Haney, Limnol. Oceanogr. 16, 971 (1971).

3. 120 milliliter subsamples were fixed with a solution of 2 parts saturated mercuric chloride to 1 part 95% ethanol (final fixative concentration 3%), then stained with bromophenol blue (M.L. Pace and J.D. Orcutt, Limnol. Oceanogr. 26, 822 (1981)). The stain allows living and nonliving particles to be distinguished with epifluorescence illumination (with excitation under green light, organisms glow red).

4. Natural bacteria were labelled by the procedure of J.T. Hollibaugh, J.A. Fuhrman, and F. Azam, Limnol. Oceanogr. 25, 172 (1980).

5. Iodine-fixed samples were filtered through 105 um Nitex screen and trapped organisms were rinsed with jets of filtered lake water. This removed most unincorporated beads, bacteria, and extraneous bacterivores such as microflagellates and ciliates. The trapped plankton was then washed onto 10 um Nuclepore filters and mounted in 65% glycerin for counting. Unincorporated beads always formed less than 5% of the total. After bead enumeration, organisms were washed into scintillation vials and digested with Protosol (New England Nuclear) at 55 ^{o}C for 12 hours before tritium counting (see Appendix 4 for further information on tritium counting). Bacteria to bead ratio confidence limits followed W.G. Cochran, Sampling Techniques (Wiley & Sons, Toronto, 1977), p. 156; confidence limits for observed to expected ratio by Monte Carlo

method (S.T. Buckland, Biometrics 40, 811 (1984)).

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7. D.E. Wujek, Cytologia 34, 71 (1969).

8. T. Fenchel, Mar. Ecol. Prog. Ser. 9, 35 (1982).

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10. Experiments were done at dawn (4-5 a.m., 18 June 1983) in Quinn Bay, Lake Memphremagog, Quebec-Vermont. Haney grazing experiments were conducted at 3 and 5 m in triplicate using 0.6 um fluorescent beads at 10 and 5% of in situ bacterial concentration. Crustaceans were narcotized with 0.1% nicotine, preserved with formalin and cleared with sodium hypochlorite. All other organisms were preserved with mercuric chloride. Crustaceans were enumerated from 4 75 μm net tows (epilimnion 0-5 m). At least 400 animals were counted per tow. At least 50 rotifers and 50 protozoans were enumerated from each of three integrated whole-water samples drawn with a 2.5 cm i.d. Nalgene tube. Bead uptake was determined for 80 crustaceans, 80 rotifers, 50 ciliates, and 196 Dinobryon cells. Abundant genera were Mesocyclops, Daphnia, and Bosmina (Crustacea), Conochilus and Keratella (Rotifera), and Halteria (Ciliata). The Dinobryon population was largely D. bavaricum with some D. sociale.

11. W.D. Taylor et al., U.S. EPA, National Eutrophication Survey Working Paper No. 710 (1979).

12. S.C. Hern et al., U.S. EPA, National Eutrophication Survey Working Paper No. 707 (1978).

13. We thank G. Nurnberg for translation from German, M. Neuwirth for

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Chapter 3.

Algal phagotrophy: Regulating factors and importance relative to photosynthesis in *Dinobryon* (Chrysophyceae)

Abstract

Simultaneous measurements of inorganic carbon fixation and phagotrophic particle uptake by Dinobryon in a metalimnetic algal peak showed that this alga depended more strongly on ingested bacteria for nutrition than on photosynthesis. Measurements of the grazing rate at different depths in Lac Gilbert, Quebec, showed that the particle ingestion rate depends on water temperature rather than light availability. Phagocytosis of bacteria proceeded at a similar rate both day and night in most lakes where Dinobryon was found. Since other chrysomonad genera (Chrysophaerella, Uroglena, Catenochrysis, Ochromonas, Chromulina, and Chrysococcus) were also found to ingest particles, it will not be possible to estimate grazing on bacteria by counting nonpigmented cells and ignoring those containing chlorophyll. In oligotrophic Lac Bowker, 30% of the phytoplankton cells were actively ingesting small particles, and it was these phytoplankton and not the less numerous zooplankton that were responsible for most bacterial grazing in the lake. Phagotrophy by algae appears to be an important but unexpected pathway for energy flow in lakes.

The flagellated Chrysophyceae, or chrysomonads, bear affinities to both plants and animals and have been claimed for study by zoologists as well as by botanists (Allen 1969). Coexistence of auto- and heterotrophy in the chrysomonads, and the widespread tendency to complete loss of chlorophyll, has fostered the suggestion that these algae may model the progenitors of the Metazoa (Hutner and Provasoli 1951). The ecological diversity and versatility of the group is perhaps most strongly expressed in the genus Ochromonas, which is capable of phagotrophy, heterotrophy, photoheterotrophy and photoautotrophy (Pringsheim 1955; Aaronson and Baker 1959). However, Ochromonas is one of only a few chrysomonads to have been studied extensively in the laboratory and the nutritional capabilities of other chrysomonads are virtually unknown, especially in their natural setting.

Pascher (1943) observed under the microscope that the colonial chrysophycean alga Dinobryon could capture and digest small particles, and thus concluded that at least some part of this alga's nutrition had to be heterotrophic. This unusual phagotrophic ability was not studied further until the recent discovery that several Dinobryon spp. are not only phagotrophic in nature, but can be major grazers of bacteria in lakes (Bird and Kalff 1986). Here we present further work on four different aspects of the capabilities of this alga: first, the relative importance of bacterivory and photosynthetic carbon fixation to Dinobryon's nutrition; second, a comparison of day and night grazing rates; third, the relationship of grazing rate to light and temperature in situ; and fourth, the dependence of grazing rate on particle size.

We would like to acknowledge the technical assistance of L. Glennie, L. Pope and Y. Feig. C. Duarte and R. Krohn commented on early drafts and P. Chambers helped with the measurement of light availability.

Methods

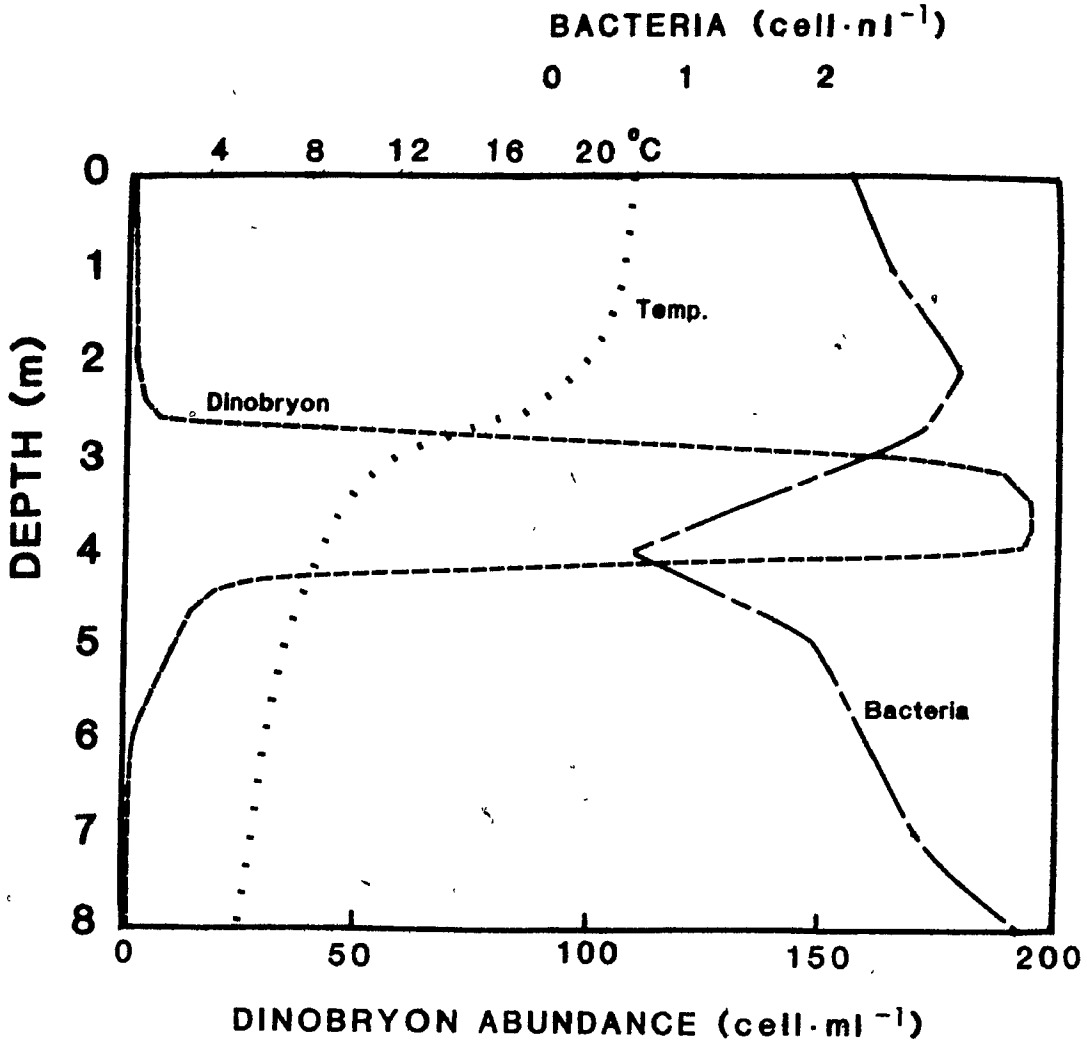
Work on the relative importance of grazing and photosynthesis was done in Lac Cromwell, Quebec, on 7-8 July, 1984. Cromwell is a small (8.7 ha), shallow (max. depth 9 m), dystrophic lake (color 50 Pt units), and is protected from wind-mixing by surrounding hills so that a sharply defined, shallow epilimnion develops (Fig. 1). We withdrew water from the top of the algal biomass peak at 3 m (Fig. 1) for studies of photosynthetic carbon uptake. Three 125 ml Pyrex reagent bottles of this water were incubated with 4 µCi $NaH^{14}CO_3$ at 0, 1, 3, and 5 m in the lake, corresponding to photosynthetically active radiation (PAR) levels of 920, 200, 15 and 1 µEinst m^{-2} s^{-1}. Three dark bottle blanks were incubated at the surface. Samples were fixed after 3 h with Lugol's iodine solution and refrigerated until they could be sorted, within 10 days.

In the laboratory, the fixed samples were filtered through 105 um Nitex screens, rinsed with 10 µm filtered lake water and the trapped plankton were washed into a shallow zooplankton counting tray. Individual Dinobryon colonies were removed from the rest of the plankton with a 10 ul automatic pipet, and collected on 25 mm 10 µm pore-size Nuclepore filters. Filters were mounted in 65% glycerol, cells were counted, washed into a scintillation vial with 3.0 ml of water and suspended in a gel with 10 ml Aquasol II (New England Nuclear) for proper counting geometry. Each incubation bottle yielded 3,000 to 6,000 individual cells of Dinobryon.

Integrated pyrheliometer readings recorded at Lake Memphremagog

Figure 1

Depth profile of temperature, Dinobryon abundance, and bacterial abundance in Lac Cromwell, Quebec on 7-8 June 1984.


BACTERIA (cell·ml⁻¹)
0
1
2
4
8
12
16
20 °C
0
1
2
3
4
5
6
7
8
DEPTH (m)
Temp.
Dinobryon
Bacteria
0
50
100
150
200
DINOBRYON ABUNDANCE (cell·ml⁻¹)

(180 km SE) were used to convert incubation period carbon fixation to total daily photosynthesis. We assumed that 50% of total light was PAR, and that 10% of surface PAR was lost due to reflection (Strickland 1958). Then PAR at a particular depth could be calculated using the Lake Cromwell attenuation coefficient of 1.33 m^{-1}.

Bacterial uptake rate at 3 m was estimated as reported previously (Bird and Kalff 1986). Briefly, bacterial-sized fluorescent microspheres (0.6 um diameter) were released into the water in an in situ grazing chamber (Haney 1971) and counted inside Dinobryon cells after ingestion. Bead number was 27% of the bacterial abundance of 2.04 million cells per ml, which was determined by epifluorescence counts of DAPI-stained bacteria using the method of Porter and Feig (1980) with modifications due to Coleman (1980) (final stain concentration 0.5 $\mu g.ml^{-1}$ in McIlvaine's buffer).

Phagotrophic carbon uptake was calculated using the measured cell volume of 0.09 μm^3 (60 randomly chosen cells), and a bacterial volume to carbon content conversion factor of 220 fg C μm^{-3} (Bratbak and Dundas 1984) so that each bacterium was estimated to contain 19.8 fg carbon. We assumed that assimilation efficiency was 50%. Fenchel (1982) found that net bacterial assimilation by heterotrophic microflagellates and Ochromonas was 60%. Therefore our assumption is likely to underestimate rather than overestimate assimilation rate.

Daytime and nighttime grazing rates were compared in August 1984 in 3 lakes in the Eastern Townships, Quebec: mesotrophic Lake Memphremagog (total phosphorous 15 $\mu g.liter^{-1}$), oligotrophic Lake Orford (TP 5 $\mu g.liter^{-1}$), and oligotrophic Lake Bowker (TP 4 $\mu g.liter^{-1}$). Similar

comparisons were made in Lake Magog (TP 32 $\mu g.liter^{-1}$) in August 1985 and Lac Croche (TP 8 $\mu g.liter^{-1}$) in May 1986. Grazing rates were determined by releasing tracer quantities of fluorescent beads into the plankton trapped in a 10 l Haney in situ grazing chamber (Haney 1971). After 5-10 minutes feeding, the chamber was retrieved and the plankton preserved with a mixture of 1 part $HgCl_2$ to 2 parts EtOH and stained with bromophenol blue (Pace and Orcutt 1981) (1984 samples) or preserved with 0.1% Lugol's iodine, destained with thiosulfate, and stained with DAPI (Pomroy 1984) (1985-86 samples). Preserved samples were mounted on 10 um Nuclepore filters in immersion oil for counting of phagocytosed beads. Coefficients of variation in bead uptake per cell ranged from 56% to 231%; we counted bead content of between 114 and 1322 cells per filter (mean 478), so that 95% confidence limits were always within ± 16% of the mean.

An abundance of Dinobryon colonies throughout the entire euphotic zone of Lac Gilbert, Quebec (45°12'N 72°17'W), surface area 8 ha, Secchi depth 4.2 m, max depth 16 m) provided an opportunity to compare the broad effects of light and temperature on grazing rate. Light was measured as PAR using a KAHLSICO underwater irradiometer with a selenium photocell (425-665 nm), corrected for above-surface changes with a KAHLSICO ambient photocell. Haney-type grazing experiments similar to those described above were conducted on July 10, 1985, at each meter depth down to the 0.5% light level at 10 m. Experiments were conducted on the afternoon of a sunny, calm day, so that mixing was minimal and colonies found at each depth must have been subject to ambient light conditions for an extended period.

Finally, we examined the size-selective characteristics of Dinobryon's particle grazing by offering a mixture of fluorescent beads of

different diameters to grazers in Haney grazing chamber experiments. This study was done in Lac Gilbert on 30 July 1985. Beads were offered in the ratio 21.89 small (0.28 μm) beads to 3.94 medium (0.57 μm) beads to 1 large (0.99 μm) bead. Beads of all sizes were counted inside 60 cells of Dinobryon (largely D. sociale Ehrenberg in this lake). We tested for uptake discrimination on the basis of size of particle, using the chi-squared selectivity index developed by Pearre (1982). In this case, the test had the power to detect particle-size preference on the order of ± 20%.

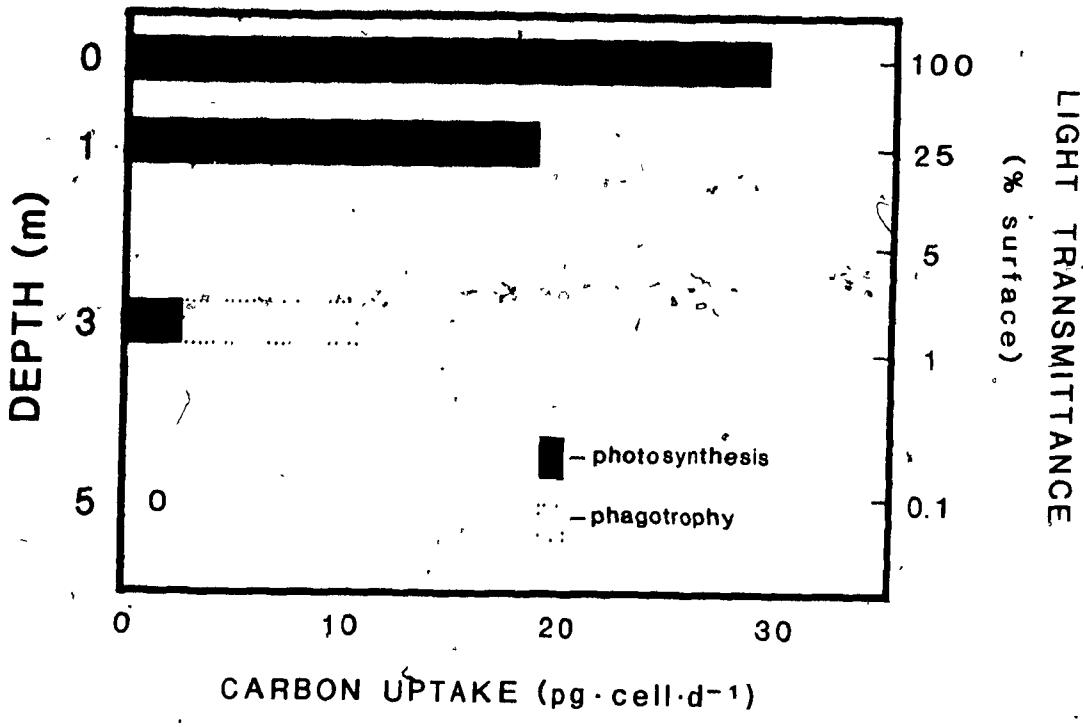
Results

Photosynthesis by Dinobryon cells in Lac Cromwell was greatest at the surface and was 8.3% of this surface value at 3 m where the cells were concentrated (Fig. 2). No net photosynthesis occurred at the 5 m depth. Light use was most efficient at 3 m. Bead uptake rate at the 3 m depth was 0.125 beads per cell per minute. Since the bead concentration was 27% of the bacterial abundance of 2.04 million cells per ml, and natural bacteria were ingested preferentially over beads by a factor of 1.3 to 1 (Bird and Kalff 1986), we estimate that Dinobryon cells consumed on average about 3 bacteria every 5 minutes. Daily assimilation of carbon from ingested particles was therefore at least 8.6 pg C $cell^{-1}$ d^{-1} (Fig. 2).

At face value, photosynthesis at 3 m provided only 28% of the carbon taken up through bacterial ingestion at the same depth. However, the measured photosynthetic rate at all depths is subject to two known sources of error. First, since Dinobryon is phagotrophic, an unknown part of its labelled carbon content is derived from ingestion of labelled picophytoplankton and bacteria. This would cause an overestimation of

Figure 2

Depth profile of photosynthesis and irradiance in Lac Cromwell on 7 June 1984. The open bar at 3 m represents estimated carbon gain through bacterial ingestion and assimilation at the depth of the metalimnetic Dinobryon population maximum.


DEPTH (m)
0
1
3
5
0
100
25
5
1
0.1
LIGHT TRANSMITTANCE
(% surface)
– photosynthesis
– phagotrophy
0
10
20
30
CARBON UPTAKE (pg · cell · d⁻¹)

photosynthesis. For instance, if the specific activity of bacterial and picoplanktonic carbon was 50% of that of the phytoplankton, then photosynthesis by Dinobryon would be overestimated by 90%. We will make the conservative assumption that all Dinobryon's labelled carbon is derived directly from photosynthesis. A second and more important source of error derives from the need to preserve the phytoplankton. Use of Lugol's solution causes radioactive label to leak into the medium at a species-specific rate, leading to an underestimation of photosynthesis (Silver and Davoll 1978; Paerl 1984). Observed leakage losses for other species range from 10% (Lehmusluoto and Niemi 1977) to 60% (Paerl 1984). If 60% of the ^{14}C label was lost due to leakage, than the corrected photosynthetic carbon fixation at 3 m would still only be 70% of carbon assimilation via phagocytosis. Phagocytosis would certainly predominate at 4 m where light was 4 times weaker than at 3 m though the Dinobryon population was equally large. These results show clearly that this alga was not merely supplementing photosynthesis by phagotrophic feeding, but was dependent largely on captured prey for subsistence.

The daytime grazing rates observed in Lac Cromwell fell among values for other sites at similar temperature (Fig. 3). Rates in the other lakes ranged from an estimated 1.2 bacteria per cell per h under the ice in Memphremagog (0°C, 4 Feb. 1984) to a high of 95 bacteria per h in eutrophic Lake Magog (21.9°C, 11 Aug. 1985). These rates either did not change from day to night (lakes Memphremagog, Orford, Magog and Croche) or actually increased at night (Bowker) (Fig. 4). This led us to enquire further into the relationship between grazing, light, and temperature. The results of the experiments conducted in Lac Gilbert in July 1985 show that grazing

Figure 3

Bacterial ingestion rate by Dinobryon species in our study lakes. Lac Gilbert values are from different depths within the lake on one occasion, 10 July 1985. One very high value (95 bacteria·h^{-1}, Lake Magog, 21.9 ^{o}C, August 1985) was not included in this figure.

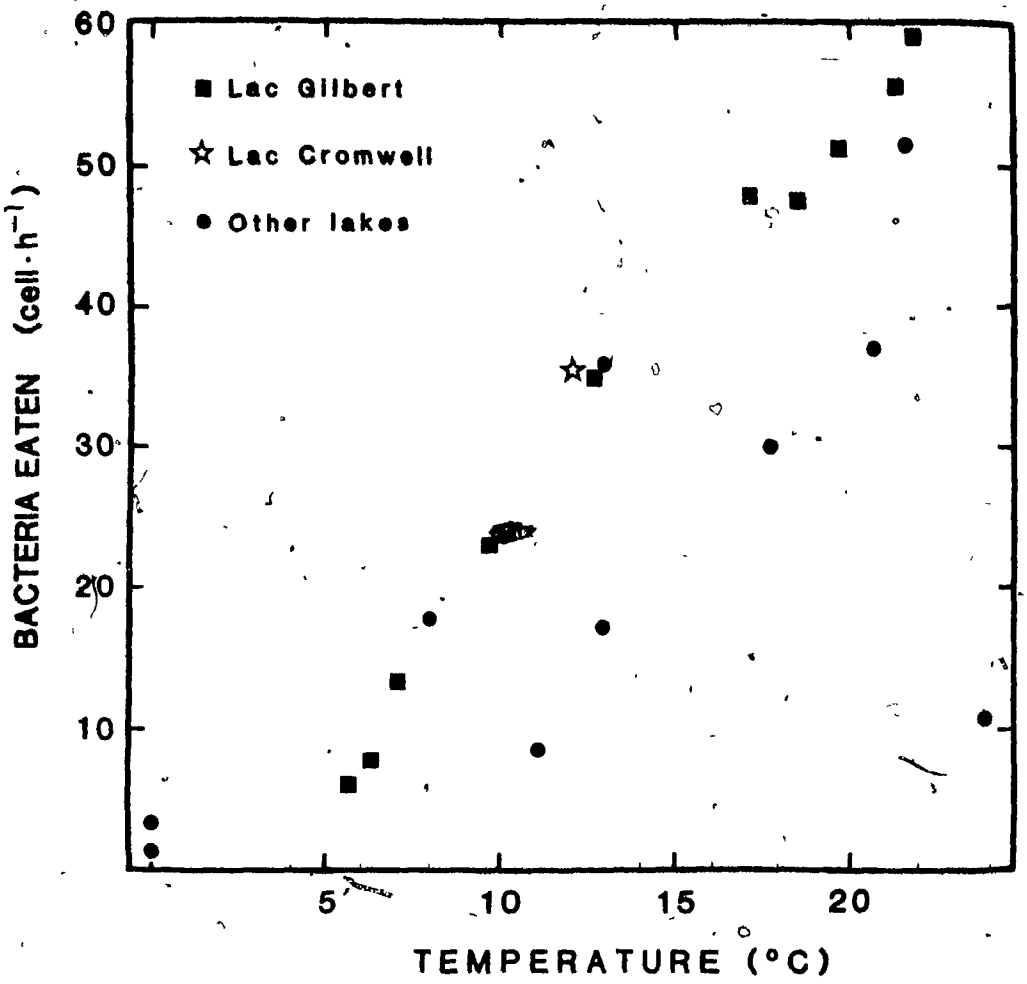
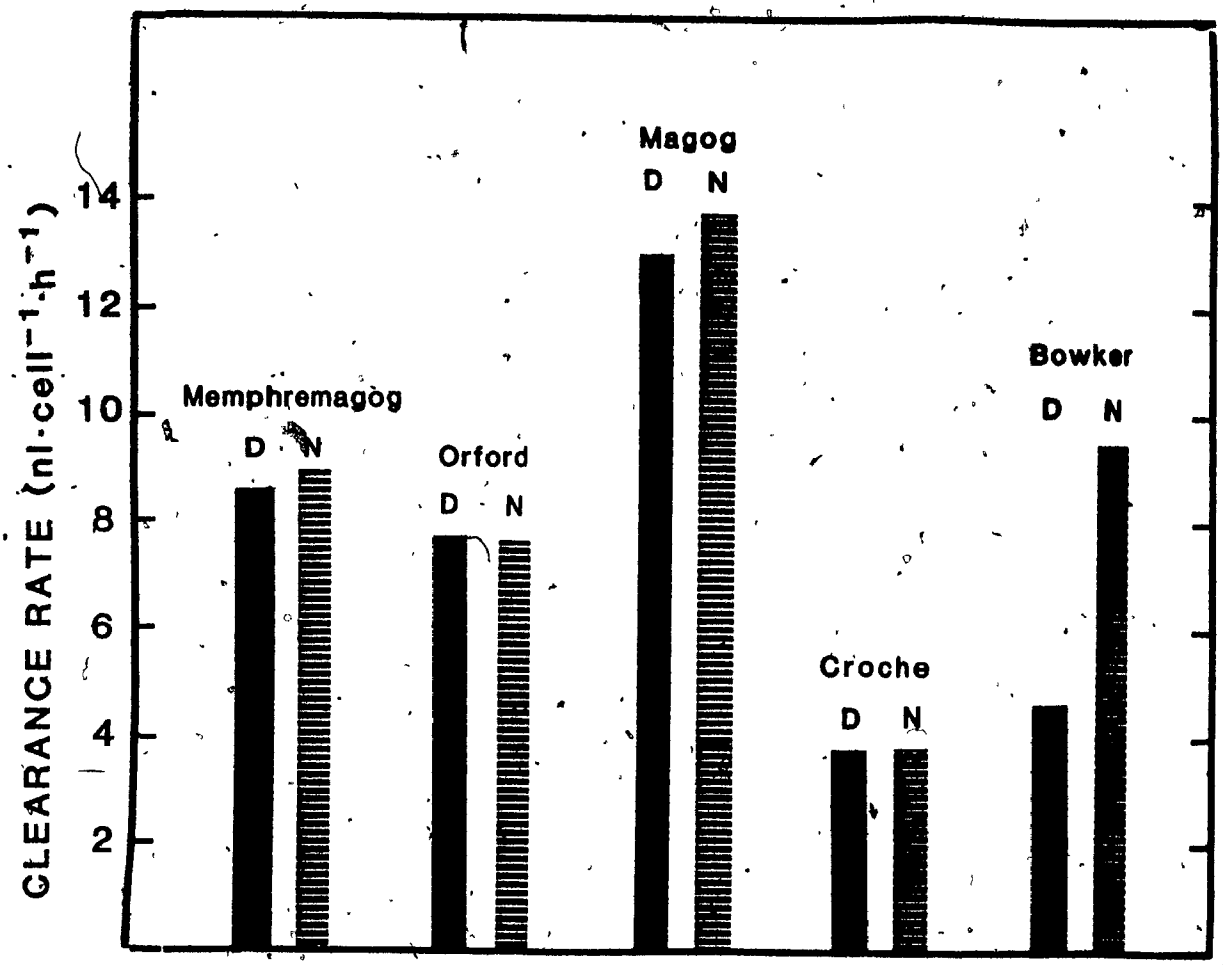

■ Lac Gilbert
☆ Lac Cromwell
● Other lakes
BACTERIA EATEN (cell·h⁻¹)
60
50
40
30
20
10
5
10
15
20
TEMPERATURE (°C)

Figure 4

Diurnal comparison of Diñobryon's clearance rate within Eastern Township and Laurentian lakes, Quebec.


CLEARANCE RATE (nl·cell⁻¹·h⁻¹)
14
12
10
8
6
4
2
Memphremagog
D N
Orford
D N
Magog
D N
Croche
D N
Bowker
D N

rate was closely linked to temperature in this lake and only marginally affected by light (Fig. 5). The partial correlation (r) between bacterial consumption rate and temperature with the effect of light excluded was 0.98 ($p < 0.0001$) whereas the correlation between bacterial consumption and light, excluding the effect of temperature, was -0.29 ($p > 0.40$). This indication of the importance of temperature, rather than light, is consistent with the observed constancy of grazing from day to night in lakes Memphremagog and Orford, and a similar day-night constancy of bacterial ingestion by Ochromonas in the laboratory (Aaronson 1980). The nighttime increase in grazing in Lake Bowker is unexplained.

Finally, the size-selection expetiments show that Dinobryon is almost incapable of ingesting the very tiny 0.28 μm particles (Table 1). Beads of this size correspond to the smallest, though often most numerous, of the freshwater bacteria. The probability of finding such a low number of these tiny beads inside cells by chance is negligibly small. The two larger bead sizes, corresponding in volume to the largest bacteria (0.6 μm diameter) and the cyanobacteria (1 μm diameter), were not discriminated between.

Discussion

The discovery that a common algal genus derives large amounts of carbon from phagocytosis adds a further complication to studies of food web dynamics in lakes and perhaps the sea as well. These studies had already been complicated by the discovery that some algae supplement photosynthetic carbon fixation by taking up dissolved organic carbon at in situ concentrations (briefly reviewed by Harris 1978). With some algae deriving large amounts of their carbon and nutrients from ingested bacteria, trophic dynamics become even less straightforward (Porter et al. 1985).

Figure 5

Depth profile of temperature (T), irradiance (I), Dinobryon abundance (D) and clearance rate (C) in Lac Gilbert, Quebec, on 10 July 1985.

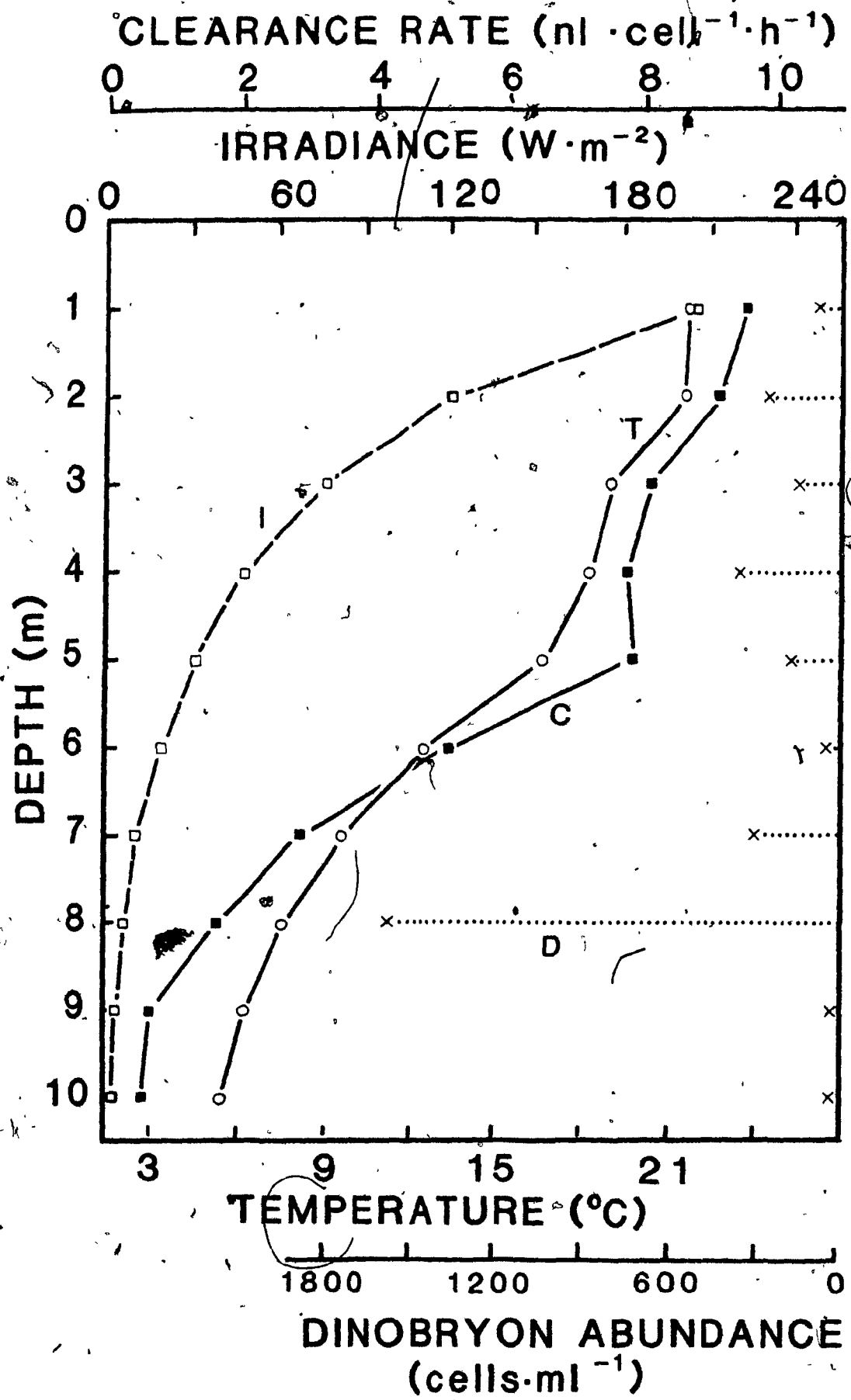

CLEARANCE RATE (nl·cell^{-1}·h^{-1})
0
2
4
6
8
10
IRRADIANCE (W·m^{-2})
0
60
120
180
240
DEPTH (m)
0
1
2
3
4
5
6
7
8
9
10
I
T
C
D
3
9
15
21
TEMPERATURE (°C)
1800
1200
600
0
DINOBRYON ABUNDANCE
(cells·ml^{-1})

Table 1. Results of the size-selection experiment conducted in Lac Gilbert on 30 July 1985. For clarity, all bead abundances have been transformed to abundance relative to the number of large, 0.99 µm beads. A chi-square test provided the probability that the other bead sizes are ingested at the same rate as the largest beads. 95% confidence limits are in brackets.

Bead diameter (µm)	Bead concentration in the water column relative to 0.99 µm beads	Beads ingested by Dinobryon relative to 0.99 µm beads	Probability of no selectivity, relative to 0.99 µm beads
0.99	1.00 (±0.16)	1.00 (±0.26)	
0.57 (±0.01)	3.94 (±0.60)	4.02 (±0.90)	0.97
0.28 (±0.02)	21.89 (±2.57)	0.29 (±0.13)	1×10^{-50}

A second complication is that, until it is established which algae are phagotrophic and which are not, it will not be possible to estimate grazing on bacteria by counting nonpigmented cells and ignoring those containing chlorophyll. Anecdotal reports of phagotrophy implicate the prymnesiophyte Chrysochromulina (marine species only: Manton 1972; Pienaar and Norris 1979), the coccolithophorid Coccolithus pelagicus (Parke and Adams 1960), the xanthophyte Chlorochromonas (Gavaudin 1931), and the chrysophytes Phaeaster (Belcher and Swale 1971), Chrysamoeba (Hibberd 1971), and Pedinella (Swale 1969). Steinberg (1980) showed that the unicellular chrysophyte Spumella ingests small diatoms. Bacteria have been seen in food vacuoles of the photosynthetic dinoflagellate Ceratium hirundinella Muller (Dodge and Crawford 1970). Porter (unpublished, Porter et al. 1985) found experimental evidence for bacterial uptake by Cryptomonas ovata Ehrenberg. Heterotrophy by Ochromonas has been well investigated and was recently reviewed (Aaronson 1980) and Fenchel (1982) noted that it ingested up to 190 bacteria (Pseudomonas) per hour in culture.

Our own investigations have shown that many if not most chrysomonad algae are actively phagotrophic in nature. Wujek's (1976) report of phagotrophy by field-collected Uroglena was verified by Kimura and Ishida (1985) and by us using fluorescent beads and electron microscopy (Bird and Kalff 1986). Kimura and Ishida (1985) reported that Uroglena does not grow in culture in the absence of bacterial prey. We have noted bead uptake by a variety of other chrysomonad species as well: Catenochrysis hispida Phillips, Chrysosphaerella longispina Lauterborn, the epiphytic Dinobryon eurystoma (Stokes) Lemmermann, the microflagellates Ochromonas miniscula Conrad, Chromulina elegans Doflein, and Chrysococcus cystophorus, and one

unidentified colonial species (similar to Chrysostephanosphaera). Indeed, it now seems unusual to come across a chrysomonad genus that does not ingest particles. The genera Synura, Mallomonas, and freshwater Chrysochromulina appear to be three of these exceptions. The importance of phagotrophic feeding by algae is underscored by the recent discovery that 30% of the phytoplankton cells in oligotrophic Lake Bowker were supplementing photosynthetic gains by ingesting bacteria, so that together, the phytoplankton were removing more bacteria from the water column than were the less numerous zooplankton (Bird and Kalff unpublished).

The inability of Dinobryon to capture the smallest bacteria is probably of little importance to Dinobryon itself. By capturing only larger cells, this alga misses 60% of the bacteria by number in Lake Memphremagog, but consumes 85% of picoplanktonic biomass from the water it clears. This selective ability cannot be explained on the basis of a simple "direct interception" model of particle capture, whereby clearance rate would be proportional to the square of the radius of the food particle (Fenchel 1982). Such a model would predict uptake ratios of 1 to 4.1 to 12.8 for the smallest to largest beads, whereas observed ratios were 1 to 76 to 76. The difference is not an effect of cell placement within a lorica on water flow over the cell, since we have noted a similar preference for large beads by the free-living chrysomonads Ochromonas and Chromulina (unpublished data). Indeed, among the bacterivores, only the choanoflagellates and certain species of the ciliate Vorticella take bacteria in proportion to their abundance by size, an ability that is associated with a low clearance rate (cf. Fenchel 1982). Further work on this topic is needed since the existence of such discrepant feeding on

bacteria of different sizes could strongly influence the observed size-abundance and size-activity relationships within bacterial communities.

Though the present study of chrysomonad capabilities further complicates algal nutrition, it also helps to explain apparently odd results from the limnological literature. There has always been some puzzlement over the presence of healthy, growing phytoplankton below the euphotic zone as determined by ^{14}C production (Schindler and Holmgren 1971). In humic lakes and in oligotrophic lakes where the euphotic zone extends into the hypolimnion, highly concentrated populations of chrysophycean cells often accumulate (Fee et al. 1977; Ilmavirta 1983). For example, a dense peak of algae developed in the hypolimnion of Lake 302N of the Experimental Lakes Area (ELA), Ontario, in the summer of 1974 (Fee 1976). The biomass peak was 100 times more concentrated than the phytoplankton biomass in the epilimnion and Dinobryon sertularia Ehrenberg made up 99% of the population in the peak. While this was an extreme case, resulting from experimental fertilization of the hypolimnion with sucrose and inorganic nutrients, deep peaks of Dinobryon, Uroglena, Synura or Chrysosphaerella can occur in all lakes where the euphotic zone extends into the hypolimnion. Fee (1978) thought these peaks to be the result of a summer-long slight excess of inorganic carbon fixation over respiration. His experimental attempt to demonstrate this showed instead that respiration always exceeded ^{14}C production. Unfortunately, these experiments were confounded by the presence of respiring bacteria (Fee 1978). It is nevertheless probable that deep chlorophyll layers composed of algae with phagotrophic abilities are subsisting largely by bacterial

ingestion rather than by photosynthesis alone. Our analysis of other results from the literature supports this idea. The photosynthetic rates of hypolimnetic algae from Lake 266SW (ELA) were measured by track autoradiography (Denoyelles et al. 1980; Knoechel and Denoyelles 1980). Whereas photosynthetic carbon fixation was sufficient to explain observed population growth for Synedra and Ankistrodesmus, photosynthesis could explain at best 40% of the change in population size for the dominant alga Dinobryon sertularia living at the 1% light level during the study period. Our finding would suggest that the fraction not accounted for was attributable to gains through bacterial ingestion.

It is clear from the above that bacterial grazing is important to some members of the phytoplankton community. It is also evident that this source of predatory losses can be important to the bacteria, although the relative importance of algal phagotrophy to the overall flow of energy and materials in lakes remains to be determined. The chrysomonads are most abundant in oligotrophic waters (Kalff and Watson in press) and it is there that the impact of algal phagotrophy can be expected to be the greatest.

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Chapter 4

Phagotrophic subsistence of a metalimnetic phytoplankton peak

Abstract

Evidence is provided for the importance of phagocytosis to mixotrophic chrysophycean algae and to the phytoplankton community as a whole. First, we confirmed the major assumption of past work that the constituents of ingested particles are incorporated efficiently into algal tissue. Cells of the phagotrophic chrysomonad Dinobryon sertularia collected from Lac Gilbert, Quebec, incorporated carbon from radio-labelled bacterial prey with 54% efficiency over a 5 h period. When removed from labelled bacteria, the loss of previously incorporated label from Dinobryon was undetectable over a 4 h period. We conclude that this alga's quantitative utilization of prey tissue is indistinguishable from that of purely heterotrophic organisms. Second, simultaneous measurements were made of phagotrophic feeding and photosynthesis within a highly concentrated layer of chrysomonads (Dinobryon and Ochromonas) in the metalimnion of Lac Gilbert on 3-4 July, 1986. Chrysomonad abundance ranged from 300 cells per ml at 6 m, to 9000 cells per ml at 7 m, and back to less than 100 cells per ml at 9m. At the 7 m peak, phagotrophy accounted for 79% of algal community carbon assimilation on this overcast day.

It is customary to ascribe the development and maintenance of algal biomass in lakes to photosynthetic carbon fixation. In most cases, this is a realistic approximation. For instance, although many algae require externally produced fixed carbon in the form of vitamins, such "heterotrophy" represents a deficiency of on the order of a millionth of a percent of complete autotrophic capacity (Provasoli and Pintner 1953). Studies that have looked for more substantial algal organotrophy have generally concluded that this source of production is of negligible importance compared to photoautotrophy (e.g. Allen 1969). Therefore, it was surprising to discover that algae of some common freshwater genera (Dinobryon, Uroglena, Chrysosphaerella, Ochromonas) are voracious particle feeders that may depend more on consumed prey for growth than on photosynthesis, at least under low light conditions (Bird and Kalff 1986, 1987).

Phagotrophic capability appears to be more than a simple facultative supplement in some cases at least. Though Dinobryon can be maintained axenically (Lehman 1976), other pigmented chrysomonads are obligate mixotrophs. Kimura and Ishida (1986) showed that U. americana would only grow in the presence of bacterial prey. Uroglena apparently cannot synthesize phospholipids and must rely on phagotrophy to supply them (Ishida and Kimura 1986). Estep et al. (1986) showed that photosynthetic species of Ochromonas and Chrysameba that are common in the plankton of the open ocean are also unable to grow well in axenic culture and are bacterivorous at certain stages of their life cycles. Nevertheless, particulate prey alone was insufficient to support growth in these Uroglena, Ochromonas, and Chrysameba species, as light was also required. The literature on algal mixotrophy was recently reviewed by

Sanders and Porter (1987).

We report here on two additional aspects of bacterial grazing by phagotrophic algae in nature. First, we attempted to measure the proportion of the bacterial biomass being ingested by Dinobryon that was assimilated. Second, we examined the validity of an earlier claim, that deep algal peaks composed largely of phagotrophic algae might be maintaining themselves primarily through phagotrophy rather than through photosynthesis (Bird and Kalff 1987).

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Methods

Work was done either in situ, or using lake water samples collected from Lac Gilbert, Quebec, a small oligo-mesotrophic lake (8 ha, 5 µg/l chlorophyll a, 14.6 m max. depth, elevation 180 m) located in the Eastern Townships of Quebec (45°12'N 72°17'W).

Assimilation study. A study of the assimilation efficiency of the lake's most important phagotrophic alga, Dinobryon (in this case, largely D. sertularia), was conducted on 1 August 1986. Labelled bacteria were prepared using a modification of the procedure of Hollibaugh et al. (1980). Two hundred and fifty ml raw lakewater were passed through a 3 µm pore-size Nuclepore filter in a Millipore Sterifil apparatus 7 days prior to the experiment. Twenty µCi of a ^{14}C-labelled amino acid mixture (ICN #10147, 1.89 mCi/mg) were added to the filtrate that was kept at 4 °C until needed. Twenty four hours before the

experiment, 5.7 μCi of ^{14}C-glucose were added to it. Concentration, washing, and depletion of soluble label pools were carried out as described in Hollibaugh et al. (1980) except that sufficient unlabelled amino acids (Sigma #AA-S-18) were added to reach a final concentration of 2.5 μM for all amino acids.

Dinobryon cells were collected from the population maximum at 7 m. Just prior to the experiment, the sample was filtered through 200 um Nitex screening to remove crustaceans, and the Dinobryon colonies were concentrated on a 35 μm screen and resuspended in 50 ml of 0.22 μm pore-size filter-sterilized lake water. The labelled bacteria were added, mixed in by inversion, and a 3 ml zero-time blank was withdrawn immediately. The blank and all subsequent 3 ml samples were collected on a 35 μm screen, washed with jets of filtered lakewater to remove extraneous bacterivores and unincorporated bacteria, washed into a Millipore tower and collected on 10 μm Nuclepore filters to be mounted in oil for enumeration. Prior to this, all 3 ml subsamples except the zero-time blank were examined with a dissecting microscope and rotifers and nauplii were removed with a 10 μl automatic pipet. Samples were then collected at 17 min and at roughly 40 min intervals for 3 h. At that time, half of the remaining labelled culture was collected and washed on a 35 μm screen and resuspended in filter-sterilized water. Both the labelled and unlabelled cultures were then sampled as usual. The washed culture samples allowed us to estimate the rate of incorporated label loss.

We counted Dinobryon cells on the filters, in 55 to 165 fields at 500X with an epifluorescence microscope (rhodamine filter set). Fields were chosen randomly using an HP-11C programmable calculator. Since

these cells are colonial and therefore contagiously distributed, an average 1550 cells were counted per filter so that coefficient of variation for the total number of cells on a filter was 10 to 15%. Filters were transferred into a scintillation vial by washing the slide with 0.6 to 0.8 ml xylene. The cover slip, to which cells adhered, was broken up and also added to the vial. Cells were digested with 1 ml Protosol (New England Nuclear) for 3 h at 50 °C. Radioactivity was assayed in 10 ml Econofluor.

Confidence limits for the radioactivity (DPM) taken up per cell on a filter were determined by a combination of Monte Carlo and bootstrap techniques (Efron and Gong 1983). First, 200 random normal deviates were generated with mean equal to the observed total DPM on a filter, and standard deviation appropriate for a Poisson variable. Next, 200 bootstrap samples of cell counts per filter were generated by randomly sampling, with replacement, the observed population of cell counts per microscope field and converting these to cell counts per filter. The resulting 200 pairs of DPM and cell count estimates yielded 200 simulated DPM per cell ratios, which were then sorted, and the average of the fifth and sixth lowest, and of the fifth and sixth highest, were taken to represent 95% confidence limits. The final assimilation efficiency was calculated as the incorporation rate (0.9 to 4.9 h) divided by uptake rate (0 to 17 min); confidence limits for these rates were also bootstrap percentiles.

Comparison Study. A study comparing total community photosynthesis to total algal phagotrophy was done on 3-4 July 1986. Two clear and 2 dark 125 ml Pyrex reagent bottles were filled with water at each meter depth between 0 and 10 m and incubated in situ for 5 h with 8 μCi $NaH^{14}CO_3$. Samples were returned to the lab in a cooler filled with

icewater, and the phytoplankton were collected on 47 um diameter, 1.0 um pore-size Nuclepore filters. These filters were dissolved in Protosol and counted in Econofluor. Samples of the filtrate were taken to assay total ^{14}C added, as well as to determine labelled dissolved and <1.0 um particulate carbon content following acidification and bubbling (Schindler et al. 1972). Integrated pyrheliometer readings recorded at Lake Memphremagog (10 km to the south) were used to convert incubation period carbon fixation to total daily photosynthesis. Available carbon at each depth was estimated from Gran alkalinity titrations (Kramer 1982) and pH measurement.

Bacterial uptake rate by algae was estimated at each meter depth using bacterial-sized fluorescent microspheres, as described earlier (Bird and Kalff 1987). Bead concentration was 1.5 to 7.7% of bacterial abundance, and the in situ grazing chamber incubation time was 10 minutes. Sufficient chrysomonads were counted to produce coefficients of variation for mean cell abundance and mean bead content per cell of 5%. Phagotrophic carbon uptake was calculated using picoplanktonic cell volumes measured from Dapi-stained samples. We used a carbon content conversion factor of 106 fg C um^{-3}, derived using a natural mixture of bacteria and picophytoplankton (Nagata 1986), and our measure of assimilation efficiency described above. Note that Nagata's conversion factor is much lower than the one used in Chapter 3.

Light was measured as PAR using a Kahlsico underwater irradiometer with a selenium photocell (425-665 nm), corrected for above-surface changes with an ambient photocell. Optical density (= $-\log_{10}$ transparency) was measured with a Philipp Schenk in situ transparency meter calibrated to 0.0 optical density in air, and oxygen concentration

was measured with an in situ cell. We counted rotifers in 40 ml subsamples so that C.V. ranged from 40% when rotifers were rarest to 10% when they were abundant. Heterotrophic microflagellates were counted in transects at 1250X on Dapi-primulin stained samples (Caron 1983); C.V. was 10%. A rough estimate of crustacean zooplankton abundance was obtained by counting all animals in 250 ml samples (C.V. 6 to 20%). Phaeopigment-corrected chlorophyll a estimates were made using boiling 90% ethanol extraction (Sartory and Grobbelaar 1984). Since diatoms were rare, we used biovolume as an estimate of algal biomass. Cells were enumerated and sized in transects across 0.2 μm pore size (2 ml subsample) and 10 μm pore size (40 ml subsample) Nuclepore filters. Phytoplankton were stained with primulin before counting.

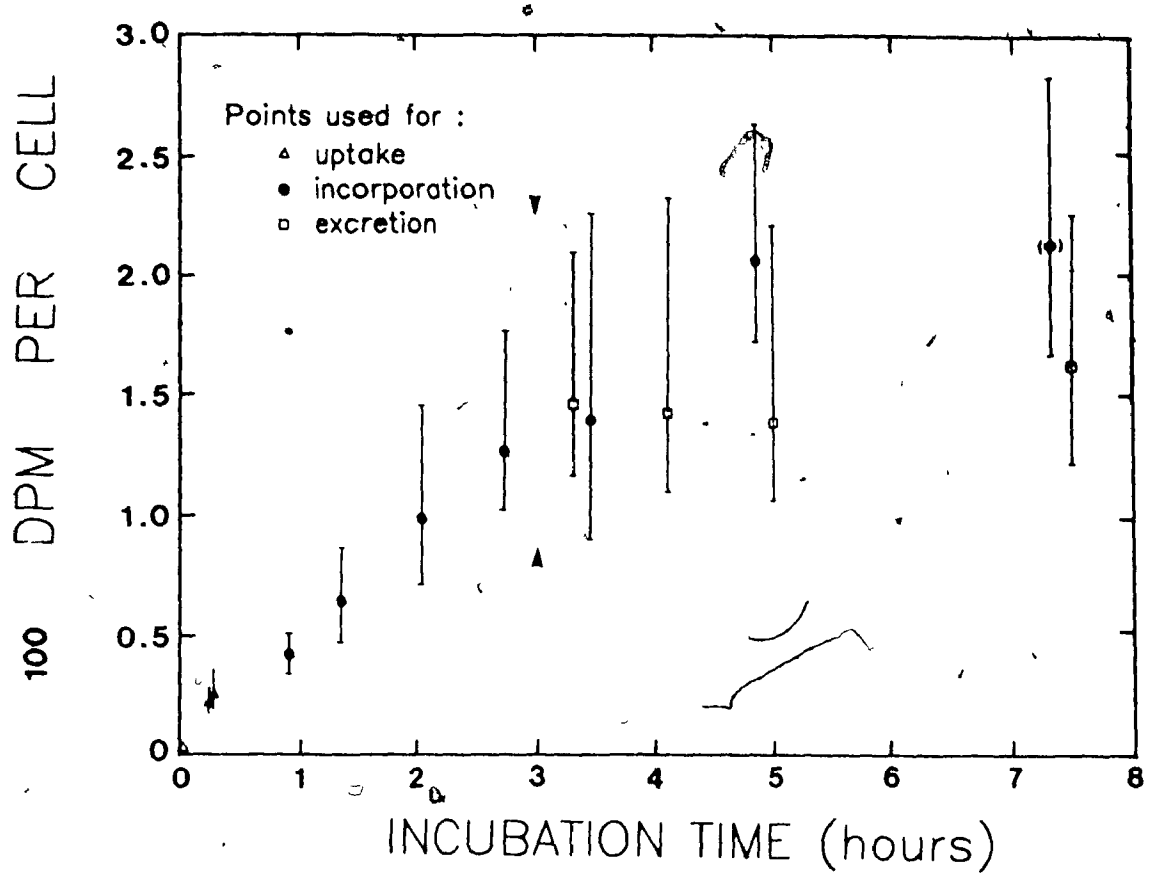
Results

Dinobryon incorporated ^{14}C from labelled bacteria with 54% efficiency (Fig. 1). This is not a robust conclusion since the 95% confidence limits encompass the range 40 to 79%. However, it is more likely that the "true" value was underestimated than overestimated, because the observations used to calculate uptake rate (at 15 min) had positive residual error from the incorporation regression line. The true value should, therefore, lie closer to the 60% figure derived by Fenchel (1982) for Ochromonas, a close relative of Dinobryon. Nevertheless, we used our value for the comparison study estimations. When the algae were removed from the labelled prey, they did not lose the previously incorporated label at a detectable rate. The loss rate was indistinguishable from zero over 4 h ($p > 0.50$). Given the statistical error level of our data, we would have detected loss rates greater than 7% per hour.

The sky was overcast on 3 July, the day of the photosynthesis

Figure 1

Time course of uptake of radioactive label from bacteria by Dinobryon. Excretion of label does not take place for at least 20 min, therefore we used the ratio of incorporation rate (0.9 to 4.9 h) to uptake rate (0 to 17 min) as an estimate of assimilation rate. Arrows at 3 h represent division of culture into labelled and washed - open circles are from washed culture used to estimate loss rate of incorporated label.


100 DPM PER CELL
3.0
2.5
2.0
1.5
1.0
0.5
0
Points used for :
uptake
incorporation
excretion
0
1
2
3
4
5
6
7
8
INCUBATION TIME (hours)

measurements. Total daily insolation was only 40% of the average for the preceding and succeeding 10 day periods, and 22.5% of the seasonal maximum. Profiles of light, temperature, oxygen concentration, optical density, algal biovolume and chlorophyll concentration in Lac Gilbert are shown in Fig. 2. The upper water column was well mixed on this date; the metalimnion extended from 5 to 9 m. Oxygen in the epilimnion was equilibrated with the surface saturation value. There was a slight (17%) oxygen deficit at 7 m, below which oxygen declined to 0.3 $mg \cdot l^{-1}$ at 9 m and to <0.1 $mg \cdot l^{-1}$ at 10 m. There were two sharp peaks in optical density at about 7 m followed by a broad maximum starting at 9.6 m. There was also a pronounced deep chlorophyll peak at 7 m, where the chlorophyll concentration was 5 times higher than in the epilimnion. Chlorophyll samples were taken with a 60 cm Van Dorn sampler, so that profile values represent an average rather than the maximum concentration. There was a second chlorophyll peak, that we will not consider here, in the low oxygen layer at 10 m.

Photosynthesis was greatest at 1 m and was 17% of this value at the 1.4% light level at 7 m (Fig. 3). There was a chrysomonad maximum at 7 m (5862 +- 550 Dinobryon and 3277 +- 346 Ochromonas per ml) that was responsible for the largest peak in optical density. There were no Dinobryon cells at the meter above, 1238 +- 102 cells per ml a meter below, and none at 9 m. The remainder of phagotrophic chrysomonads were all Ochromonas sp. (3-5 μm diameter), with the rare exception of a few Chrysameba seen at 5 m. Though Ochromonas was present at all depths, including the anoxic zone at 11m, it comprised less than 1% of total phytoplankton biomass away from the peak, at depths 0 to 5 m and 9 to 11 m. An abundance of colonial and unicellular cyanobacteria of size 0.6 to

Figure 2

a. Profiles of light, temperature, oxygen concentration (closed squares) and oxygen saturation values (open squares) in Lac Gilbert, Quebec, 3 July 1986. b. Profiles of algal biovolume, optical density and chlorophyll concentration. Phagotrophic chrysomonads made up 1.5% of total algal biovolume at 6 m, 58% at 7 m, 29% at 8 m, and less than 1% at 9 m.

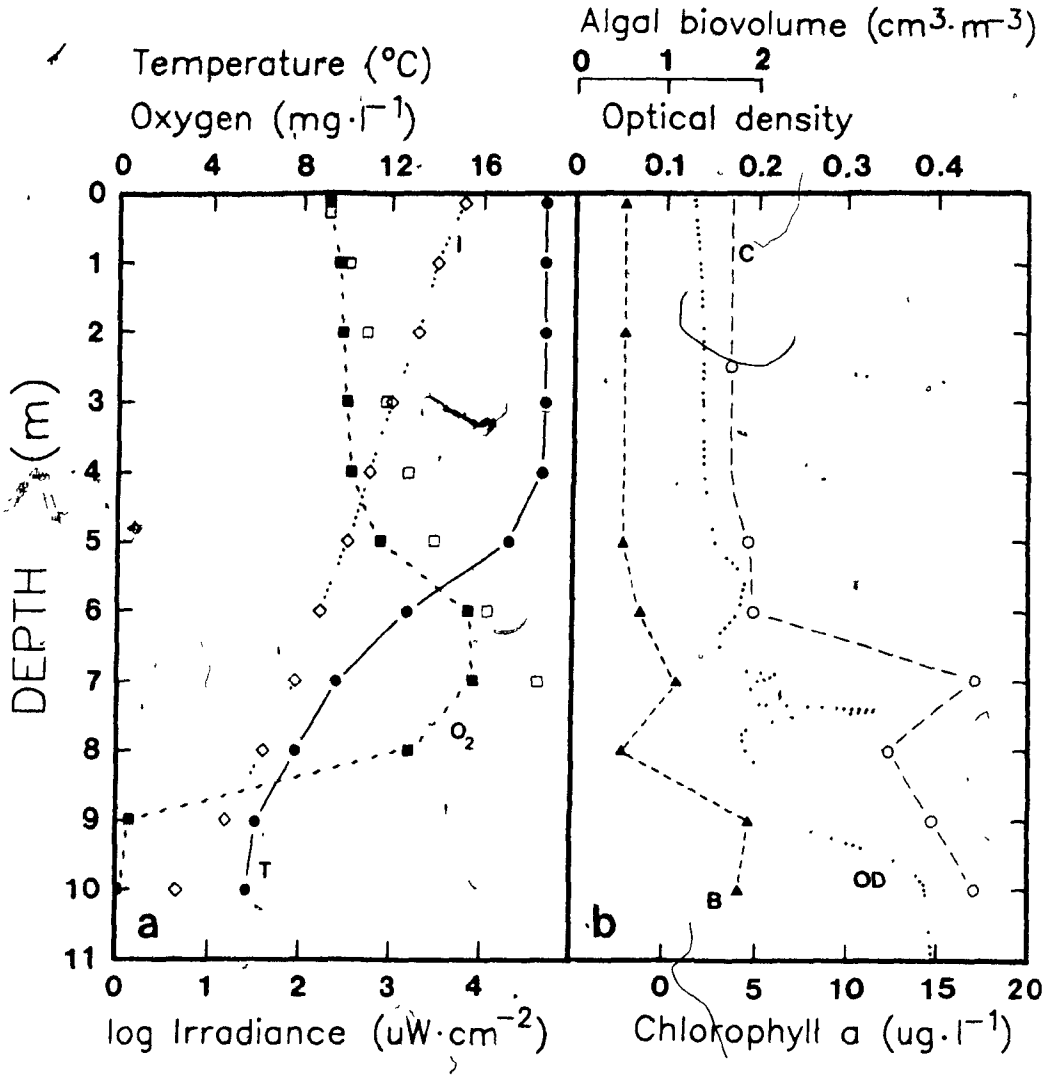
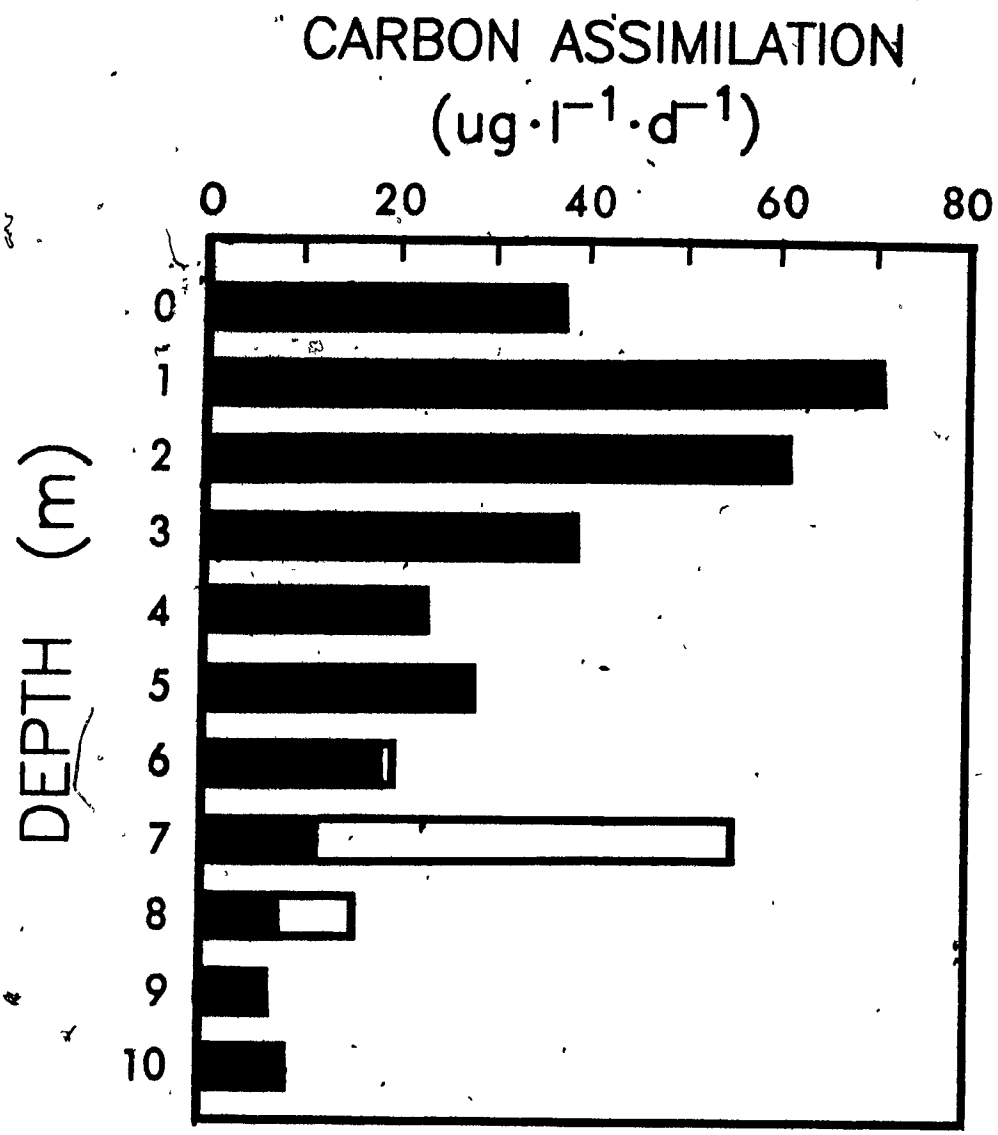

Algal biovolume (cm3·m-3)
0 1 2
Temperature (°C)
Oxygen (mg·l-1)
Optical density
0 4 8 12 16
0 0.1 0.2 0.3 0.4
DEPTH (m)
0 1 2 3 4 5 6 7 8 9 10 11
C
I
O2
T
B
OD
a
b
0 1 2 3 4
log Irradiance (uW·cm-2)
0 5 10 15 20
Chlorophyll a (ug·l-1)

Figure 3

Carbon assimilation rate via photosynthesis (closed bars) and algal phagotrophy (open bars) in Lac Gilbert, Quebec, 3-4 July 1986.


CARBON ASSIMILATION
(ug·l⁻¹·d⁻¹)
0
20
40
60
80
DEPTH (m)
0
1
2
3
4
5
6
7
8
9
10

2 um made up most of the remainder of the metalimnetic (and epilimnetic) algal community.

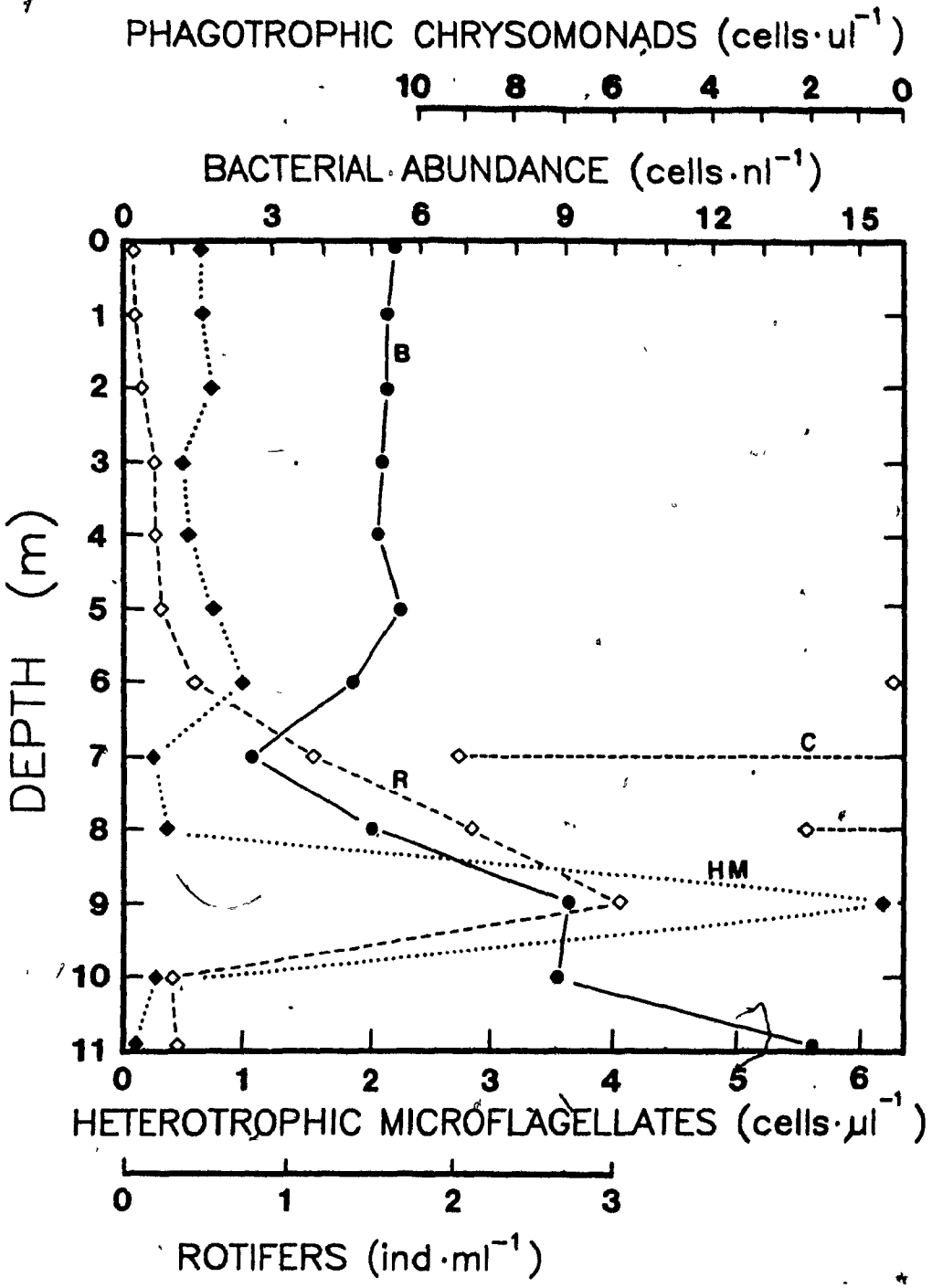
Although some phagotrophic production was evident at all depths, it was negligible outside the biomass peak (Fig. 3). Within the 7 m maximum, however, particle feeding by chrysomonads accounted for 44 +- 9.4 ug C assimilated$\cdot l^{-1} \cdot d^{-1}$, about 79% of the total, whereas primary production was only 12 +- 1 ug C$\cdot l^{-1} \cdot d^{-1}$. Dinobryon's clearance rate was 2.9 nl$\cdot$cell$^{-1} \cdot h^{-1}$ which is identical to the figure we measured for this alga in this lake a year earlier at this temperature and depth (Bird and Kalff 1987). Although Ochromonas is smaller, it was a more voracious feeder, clearing about 4.8 nl$\cdot$cell$^{-1} \cdot h^{-1}$. Particle feeding accounted for 4.3% of algal carbon assimilation at 6 m, 51% at 8 m, and less than 2% at 9 m, compared to the 79% at the 7 m peak.

Discussion

Secondary production based on particle feeding by Dinobryon and Ochromonas contributed more than three quarters of the algal carbon fixation in the 7 m chlorophyll peak of Lac Gilbert on 3 July 1986. These findings provide evidence for our suggestion (Bird and Kalff 1987) that such might well be the case in lakes with deep layers dominated by algal phagotrophs. Such blurring of the roles of 'primary' and 'secondary' producer by these organisms makes a mockery of our neat trophic schemes in this case. Indeed, the quantitative indistinguishability of bacterial feeding by chrysomonad and by protozoan flagellates (Bird and Kalff 1986) complemented by the present demonstration of a high chrysomonad assimilation efficiency (cf. also Fenchel 1982) that was coupled with a decline in heterotrophic microflagellate numbers at 7 m (Fig. 4), makes it look as if algae have outcompeted the zooplankton for prey. The chrysomonad algae provide a

Figure 4

Profiles of abundance of bacteria, phagotrophic chrysomonads, rotifers, and heterotrophic microflagellates in Lac Gilbert. Intense grazing by the zooplankton is concentrated at 9 m, just above the anoxic zone, where picoplanktonic biomass is high. Not shown are the crustacean zooplankton; these were evenly distributed with depth at 50 to 100 individuals per l, with the exception of a single abundance peak of 250 copepods per l (Leptodiaptomus siciloides) that were ingesting picoplankton at 8 m. There was a distinct minimum in bacterial abundance at the level of the chrysomonad peak at 7 m. Bacterial cell volume was 0.05 +- 0.014 μm^3 (0-5 m) and 0.12 +- 0.02 μm^3 (6-11 m).


PHAGOTROPHIC CHRYSOMONADS (cells·ul^{-1})
10 8 6 4 2 0
BACTERIAL ABUNDANCE (cells·nl^{-1})
0 3 6 9 12 15
DEPTH (m)
0 1 2 3 4 5 6 7 8 9 10 11
B
C
R
HM
0 1 2 3 4 5 6
HETEROTROPHIC MICROFLAGELLATES (cells·µl^{-1})
0 1 2 3
ROTIFERS (ind·ml^{-1})

solid counterexample to the dictum that a jack-of-all-trades will be master of none, and will be ecologically excluded (MacArthur and Connell 1966, p. 97-98).

It is evident, however, that the dominance of heterotrophy over primary production in this layer could not be sustained for long, because 75% of the prey organisms were photosynthetic picophytoplankton. We suspect that the the proportion of carbon assimilated via phagotrophy is inversely related to light availability, for which 3 July 1986 represents the low light, heterotrophic extreme. Fee (1976) showed that the photosynthetic response of deep chrysophyte peak algae to increased light was a linear function of the logarithm of illumination at low light levels, with a negative intercept. If also applicable to Lac Gilbert, this means that on sunny days, when light at 7 m would be more than 4 times stronger, photosynthesis at 7 mwould be dominant over secondary production, but with the proportions changing daily and seasonally.

How important is chrysomonad mixotrophy to the lake community? It is a small percentage of total algal production in this lake, especially when volume-weighted by depth strata. It is undoubtedly the primary loss factor for the bacteria in the peak, evidenced by the sharp falloff in bacterial abundance at 7 m (Fig. 4) (see also the similar minimum in Lac Cromwell (Bird and Kalff 1987)). Bacterial volume per cell increased markedly in the colder metalimnetic water (data not shown) so that essentially all bacteria were in the vulnerable size range for chrysomonad feeding (Bird and Kalff 1987). The 7 m chrysomonad population was clearing the water column of 66 to 92% of vulnerable particles daily, necessitating a prey growth rate that might be

difficult to sustain in these relatively cool (9.5 °C) waters.

The concentration of the chrysomonads into a thin layer in Lac Gilbert resulted in intense but localized effects. However, such effects need not be localized. For example, Lake Biwa, Japan, is plagued with nuisance blooms of chrysomonads throughout the water column that are many times denser than those in the peak in Gilbert (Ohno et al. 1983). A 1982 spring bloom of chrysomonads produced 69 µg chlorophyll a per l, contained 68000 cells of Uroglena per ml, and turned the water of the lake red. Similar freshwater "red tides" have occurred every spring in the lake since 1977. Using our measured clearance rates for Uroglena (Bird and Kalff 1986 and unpublished data), it is possible to estimate that during the 1982 bloom mentioned, the algae were probably clearing the water column twice daily. Phagotrophy may have been important in the development of these blooms, sustaining production of those algae that were mixed below the narrow euphotic zone. Lastly, Sanders and Porter (1987) documented the overwhelming importance of chrysophytes in the removal of bacteria during a spring bloom of Dinobryon in Lake Oglethorpe, Georgia. The relative impact of the chrysophytes was greatest at the surface, where they were responsible for more than half of all bacterial grazing. Algal grazers consumed on average about a third of all bacteria ingested at different depths throughout the euphotic zone.

How does this study fit in the context of what is known of deep chlorophyll peak formation? Though many authors have reported the presence of metalimnetic and hypolimnetic maxima of chlorophyll and algal biomass, few general patterns have emerged that might allow us to predict their occurrence. The reason may be, as Cullen (1982) suggested

in a review of deep chlorophyll maxima in the ocean, that deep peaks can arise under a variety of quite different circumstances. Cullen's review included maxima that form as a result of enhanced primary production in the presence of nutrients regenerated from aphotic waters, those that represent an increase in chlorophyll per unit biomass as an adaptation to lower light levels but reflect little or no biomass increase, transient peaks wherein sedimentation rate has been slowed on contact with higher nutrient levels or denser water, and peaks that represent behavioral aggregation by motile algae. Peaks in freshwater, as well, can occur as a result of in situ growth (Fee 1978; Abbott et al. 1984), as sedimentation relics of epilimnetic populations (Fee 1978), and through aggregation (Meffert and Overbeck 1985; Pick et al. 1984). The chrysophyte peak in Gilbert may encompass most of these categories: that is, it may depend on upwelling nutrients, in situ growth, is undoubtedly a manifestation of "behavioral" aggregation, and chlorophyll per unit biomass was two times higher at 7 m than at 2 m. We cannot discount the possibility that Dinobryon settled to the metalimnion from the epilimnion, though the marked concentration at 7.3 m did not depend on any physical discontinuity that we could measure. All these models describing the formation of deep water phytoplankton peaks assume that the biomass observed was a direct product of photosynthetic carbon fixation. However, the finding that the metalimnetic peak in Lac Gilbert generated biomass through the ingestion of bacteria and picophytoplankton adds a new dimension to our knowledge of the formation and maintenance of deep water algal layers.

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Chapter 5

Protozoan grazing and the size-activity structure of limnetic bacterial communities

Abstract

We measured bacterial growth rates by labelled thymidine incorporation, grazing loss rates with fluorescent latex particles, and bacterial cell size and abundance within narrow size classes of freshwater bacterioplankton. Contrary to the predictions of standard allometric relationships, the smallest bacteria showed the lowest incorporation rate per cell and per unit DNA content. Cells trapped by 1 um filters grew most quickly, and were responsible for 100% of detected thymidine biosynthesis. Grazing studies in 6 lakes showed that the large bacterial cells were probably subject to 2 to 40 times more intense grazing pressure from the flagellated protozoa and mixotrophic algae, than were the tiniest cells. We suggest that reduced grazing pressure on the tiniest bacteria allows them to dominate numerically, despite their slower growth.

The development of the epifluorescence technique for enumerating aquatic bacteria (Hobbie et al. 1977; Zimmermann 1977) allowed the subsequent conclusion that the bacteria were not only unexpectedly abundant, but also far smaller than is typical for bacteria in culture. Epifluorescence and scanning electron microscope counts showed that most native bacteria were unexpectedly small, at the limit of resolution of the light microscope and ranging down to the size of large viruses. Understandably, there was some immediate skepticism regarding the meaningfulness of the new counts. Wangersky (1977) and Stevenson (1978) argued that most native aquatic bacteria were in a state of dormancy brought on by starvation. This dormancy, it was suggested, would explain the discrepancy between plate and direct counts (e.g. Jannasch and Jones 1959). Stevenson's argument was based in part on a study by Novitsky and Morita (1976) who found that when a bacterium they cultivated from Antarctic waters (mean volume 0.75 μm^3) was placed in mineral medium, it divided several times without growth, producing great numbers of tiny, inactive but viable cells (mean volume 0.07 μm^3). Since these tiny "resting" cells were similar in size to native bacteria, it was contended that the free-living bacterioplankton might be similarly dormant. In contrast, bacteria living in rich microzones, attached to particles, were suggested to be responsible for the majority of bacterial heterotrophic activity in situ.

Concern over the relative activity of free-living versus particle-attached bacteria has spawned a large number of studies addressing the question (e.g. Schleyer 1981; Kirchman and Mitchell 1982; Pedros-Alio and Brock 1983; Li 1984; Kato 1984; Lovell and Konopka 1985; Jeffrey and Paul 1986). However, as pointed out by Simon

(1985) general conclusions cannot yet be drawn from these studies since the results are not consistent. Some have found no difference in activity between free and attached bacteria (Schleyer 1981), some have found marked differences (Palumbo et al. 1984), whereas others have shown that the activity can be variably partitioned within one environment over the course of the seasons (Cammen and Walker 1982; Simon 1985; Lovell and Konopka 1985).

Stronger support for the dormancy hypothesis, albeit in an altered form, has come from microautoradiography and other direct microscopic methods for assessing viability. Though these methods have demonstrated that many free-living bacteria are undeniably active (Maki and Remsen 1981; Tabor and Neihof 1982; Marcussen et al. 1984), a residual fraction of free bacteria always appears to be dormant, moribund or dead. A difficulty with most claims of differential activity within the bacteria, however, is how to reconcile them with current information that seems to show that bacteria are being preyed upon at a high rate (Andersen and Fenchel 1985; Sherr et al. 1987). In the face of such heavy losses, how could dormant bacteria persist? The purpose of this study was to look at size-activity relationships within the bacterioplankton on a finer scale than has been done in the past, by measuring growth rates, abundances and cell sizes within finely divided size classes. An attempt was also made to determine probable predation loss rates within these classes.

Methods

First growth study

The first study examined the size distribution of bacterial abundance, biomass and thymidine incorporation in Quinn Bay of

mesotrophic Lake Memphremagog, Quebec-Vermont. An integrated water sample taken on 21 September 1984 over the depth of the epilimnion using rinsed Tygon tubing (2.5 cm I.D.) was used for all experiments. Sample processing began within an hour of collection.

Thymidine uptake into macromolecules was measured using a modification of the protocol of Fuhrman and Azam (1982). Methyl-^{3}H-thymidine (69 Ci·mmol^{-1}) was added to 100 ml of whole lakewater to a final concentration of 20 nM. Three ml aliquots of this water were filtered onto 0.2, 0.4, and 1.0 um filters at geometrically spaced intervals over 3.5 hours. The filters were added to 8 ml of ice-cold 5% trichloroacetic acid (TCA) in polypropylene test-tubes held in an ice-bath; extraction was for 15 to 30 minutes. The extractant was collected on a 0.2 um Nuclepore filter and rinsed 3 times with 4 ml TCA that had been used to rinse the original filter. The original and the rinse filters were digested together for 24 h, with 1 ml Protosol (New England Nuclear) in a scintillation vial. After adjusting the pH with 50 ul glacial acetic acid to discourage chemiluminescence, the samples were mixed with 10 ml Econofluor (NEN) and counted. Efficiency was monitored by external standard ratio and checked with internal ^{3}H-water standards. An identical procedure was used to prepare blanks from a formalin-killed sample. Incorporation rate was linear over time in all fractions. Therefore, we used the slope of the regression of total incorporation against time to estimate incorporation rate and its confidence limits.

Bacterial abundance and biomass were determined within size fractions collected on filters of different pore size at the same gentle pressure used for the thymidine fractionation (5 cm Hg). Abundance was estimated from direct epifluorescence counts after DAPI

staining (Porter and Feig 1980; Coleman 1980). Four to six hundred bacteria were counted on each of three replicate filters of each pore size. At the same time, the dimensions of approximately 40 randomly selected cells were measured using an ocular micrometer scale, to determine the mean size of bacteria retained by each filter type. In order to minimize glare and maximize resolution, the field stop from the mercury burner was closed almost completely during measurement. Cell dimensions were estimated to the nearest 0.1 um in the presence of fluorescent microspheres of known dimension (0.3, 0.6, and 1 um diam.). The proportion of attached bacteria in the whole lakewater was determined by counting all attached bacteria in 4 transects across a 3 um pore-size filter. A total of 3,648 attached bacteria were counted, so the proportion of attached bacteria could be estimated with a coefficient of variation of 5%. The abundance or activity of cells in a given size interval was found by difference. Throughout the paper, unless otherwise noted, we use the terms biomass and biovolume interchangeably. The equation of Shuter et al. (1983) was used to estimate DNA content of cells of a given size:

$$\ln \text{volume } (\mu m^3) = 20.4 + 3.52 \ln [\text{DNA}] \text{ (pg)},$$

$$r^2 = 0.72, \quad CF = 1.05,$$

where CF is the correction factor necessary to correct transformation bias (reverse prediction of DNA from volume) (cf. Bird and Prairie 1985).

Second growth study

A second growth study was conducted on 10 May 1987 in eutrophic Lake Waterloo (chlorophyll concentration 14.3 $ug \cdot l^{-1}$ on this date, max depth 6 m). The first part consisted of an isotope dilution experiment

to determine the degree of participation of added label in macromolecule synthesis in different size classes of bacteria. Tritium-labelled thymidine (Thd) was added to 65 ml whole lakewater in 65 ml BOD bottles, to a final concentration of 4 nM. Different samples received additions of cold Thd to levels of 0, 6, 19, and 30 nM. A fifth sample was fixed with 0.1 ml Lugol's iodine immediately before isotope addition. Samples were incubated at in situ temperature (15.2 ^{o}C) in a lakewater-filled cooler for 1 h, before fixation with Lugol's solution. Two additional bottles were incubated with 2 nM labelled thymidine, to be used for macromolecule partitioning. Fixed samples were refrigerated until they could be filtered. Duplicate or triplicate 3 ml subsamples were collected on Nuclepore filters of pore sizes 0.2, 0.4, 0.6, 1.0 and 5.0 um. The procedure for extraction of TCA-insoluble macromolecules was similar to that in the first growth experiment. At least 5,000 disintegration events were counted in all samples, including blanks, to avoid the bias problems associated with timed counts. Incorporation rate without added cold Thd was calculated as the reciprocal of the Y-intercept of a weighted regression (see Appendix 1).

We distinguished DNA from other labelled macromolecules enzymatically, rather than by the standard ultracentrifugation (e.g. Moriarty and Pollard 1982; Findlay et al. 1984). Quadruplicate 3 ml subsamples of labelled and control bacteria were collected on either 0.2 or 1.0 um Nuclepore filters (i.e., 4 sets of 4 replicates, or 16 filters in all) and incubated (1 h, 0 ^{o}C) in 0.5 ml ice-cold PBS (phosphate-buffered saline: 0.14 M NaCl, 0.01 M sodium phosphate, pH 7.8) containing 27% sucrose, 10 mM EDTA, and 50 ul of a 0.1% solution of lysozyme (24,000 units; Sigma). We added 1.95 ml prewarmed

PBS:sucrose:EDTA plus 10 mg sodium dodecyl sulfate (SDS) in PBS (10 min, 37 °C), then added 50 ul of 1% proteinase K (Boehringer Mannheim) (Ebeling et al. 1974) and incubated for 4-5 more hours at the same temperature. Macromolecules were then precipitated and collected on 0.45 um pore-size Millipore filters with 8 ml ice-cold 5% trichloroacetic acid (TCA). Two replicates from each set of 4 were assayed for total incorporated tritium following dissolution of the filters with an appropriate solvent (Protosol for Nuclepore, methyl cellosolve for Millipore). The remaining two replicates were incubated (37 °C, 4 h) in 1 ml 40 mM Tris-HCl (pH 7.9) with 10 mM NaCl, 6 mM $MgCl_2$, and 5,000 units Type I DNAase (Boehringer Mannheim). Residual macromolecules were precipitated with TCA and collected. The difference between pairs of replicates in each set was considered to represent incorporation of Thd into DNA.

Another set of 16 subsamples (0.2 vs. 1.0 um, treatment vs. preserved blank) was collected, in a manner analogous to the above, on 8 filters that were washed in ice-cold 5% TCA and 8 that were held in 2.5 ml 5% TCA at 100 °C for 1 h before cooling and precipitation. The difference between pairs in this case was taken to represent DNA plus residual, nonproteinaceous macromolecules (cf. Moriarty (1984) on improbability of labelling RNA with ^{3}H-Thd).

As a check on the veracity of the isotope dilution technique, we did four additional 1h, 65 ml incubations with the following characteristics. The first received 4 nM ^{3}H-Thd plus 2.9 nM fluorodeoxyuridine (FdU); the second, 4 nM ^{3}H-Thd plus 17.2 nM FdU; the third, 40 nM ^{3}H-Thd plus 17.2 nM FdU; the fourth, 40 nM ^{3}H-Thd alone. All 4 bottles received 11.4 nM uracil. FdU has been used to block endogenous synthesis of Thd in leukemic cells because it binds

irreversibly to thymidylate synthetase (Ellwart and Dormer 1985). The uracil was added to circumvent interference with RNA synthesis that sometimes accompanies FdU action (Heidelberger 1965). These samples were filtered only through 0.2 μm filters and were not otherwise size-fractionated.

We did a filtration-dilution experiment (Kirchman et al. 1982) on 10 May to determine a factor to convert ^{3}H-Thd incorporation rate to growth rate. Twenty-five ml whole lakewater were added to 225 ml water that had been filtered through 0.6 um filters, then 0.2 μm pore size Nuclepore filters at 5 cm Hg. A sample of the diluent was preserved to check for bacterial contamination (cf. Li and Dickie 1985). We waited 3 h before taking the first samples (henceforth "0 h") in order to reduce the effect of any lag period on growth rate calculations. At the 3, 9 and 20 h marks of the incubation, bacterial samples for enumeration, and 50 to 70 ml samples for Thd incorporation (4 nM ^{3}H-Thd, 1 h), were collected. Macromolecule collection and bacterial enumeration were as above. No correction was made to attached bacterial counts for obscured bacteria, since essentially all particles were transparent or translucent, so that attached and embedded bacteria could be seen by focussing through the particles.

Grazing experiments

Size-selective grazing experiments were conducted in 5 lakes in southern Quebec in July-August 1985, following the protocol outlined earlier (Bird and Kalff 1987). Beads of three sizes (0.27, 0.57, and 1.0 μm) were fed to grazers trapped in a 2 litre Haney in situ grazing chamber. Feeding was stopped after 10 minutes by the addition of 1:1000 v/v Lugol's iodine solution to a 250 ml subsample of the

trapped plankton.

Bacteria were enumerated on 0.2 μm filters after destaining with 0.4 mM thiosulfate (Pomroy 1984); beads of all sizes were counted on the same filters. Flagellated grazers were counted using a 4 ml sample collected on 1 um filters. These samples were stained either with DAPI to identify cells with ingested bacteria (0.5 $\mu g \cdot ml^{-1}$), primulin to visualize cell morphology and flagellar conformation (250 $\mu g \cdot ml^{-1}$) (Caron 1983), or both DAPI and bromophenol blue to visualize both inclusions and flagellation (10 $\mu g \cdot ml^{-1}$) (Bird and Kalff 1986). Rotifers and large ciliates were located by scanning the entire filter at a magnification of 125X, and counting ingested beads at 1250X. Nanoplanktonic grazers were counted in transects across the filter at 1250X. We also counted the number of microflagellates attached to particles by locating particles at 125X and examining them at 1250X. Ciliate abundances could only be determined reliably at 1250X. We usually counted the bead contents of 30 ciliates and 60 microflagellates (both heterotrophic and mixotrophic).

We also conducted in situ grazing experiments on 10 May in Waterloo, similar to those described above. The duplicate incubations lasted for 15 minutes each. One subsample of each incubation was preserved with Lugol's iodine.

Results

First growth experiment

The water temperature in the epilimnion of Quinn Bay on 21 September 1984 was 17.4 °C and Secchi disk depth was 5.1 m. The abundance and mean size of the bacteria present on that date are listed in Table 1. Most bacteria (63%) were in the 0.2 to 0.4 μm size

interval. The greatest bacterial biomass (49%) was found in the 0.4 to 1.0 um interval. Only a minor fraction (1%) of the bacteria were attached to particles. However, particle-attached bacteria made up a fifth of the number trapped on the 1 um filter. "Attached" bacteria included those contained in protozoan grazers, and some of the larger, heavily-colonized "particles" were clearly ciliates that had exploded on contact with formalin. Attached bacteria were not larger than free-living cells, except that none of the tiny (0.2 um) "minibacteria" could be seen on particles.

Uptake of thymidine into macromolecules was linear in all size fractions over the course of the incubation (Fig. 1). There was measurable uptake in all size classes, so that there was no dormant size fraction. Thymidine incorporation rate was roughly equal in all size classes (2 - 4 $fmol \cdot ml^{-1} \cdot h^{-1}$) (Fig. 2). However, because most cells were in the smallest size interval, the thymidine incorporation rate of the smallest cells was less than 10% of the rate of the largest cells, on a per-cell basis (Fig. 2). This would on the face of it point to a strong disparity in growth rate within the bacterioplankton. There are, however, several alternatives to consider before tendering such a conclusion. One alternative is that cells of different sizes represented different cell cycle stages of the same organisms, wherein only the largest cells carried out DNA synthesis and division. If such were the case, we would expect to see a similar uptake rate of organic growth substrates per unit biomass by all cells. Size fractionation of uptake of amino acids and glucose showed this alternative to be insufficient to explain the observed disparity. Cells trapped by the 1 um filter took up roughly twice as much of these substrates per unit biomass as did cells in the smaller

Table 1. The mean bacterial size retained on filters of various pore sizes. Also, the estimated size of bacteria in given size ranges, their equivalent spherical diameter, and predicted DNA content.

pore size	abundance	size	interval	size	diameter	predicted DNA content
μm	$10^6 \cdot ml^{-1}$	μm^3	μm	μm^3	μm	fg
0.2	5.01	0.038 ±.028	0.2 - 0.4	0.019	0.33	1.03
0.4	1.85	0.072 ±.034	0.4 - 1.0	0.060	0.49	1.43
1.0	2.76	0.140 ±.056	1.0	0.140	0.64	1.82

Figure 1

Time course of labelled thymidine incorporation in Quinn Bay, Lake Memphremagog, 21 September 1984. Each line represents incorporation into organisms trapped by Nuclepore filters of the stated size.

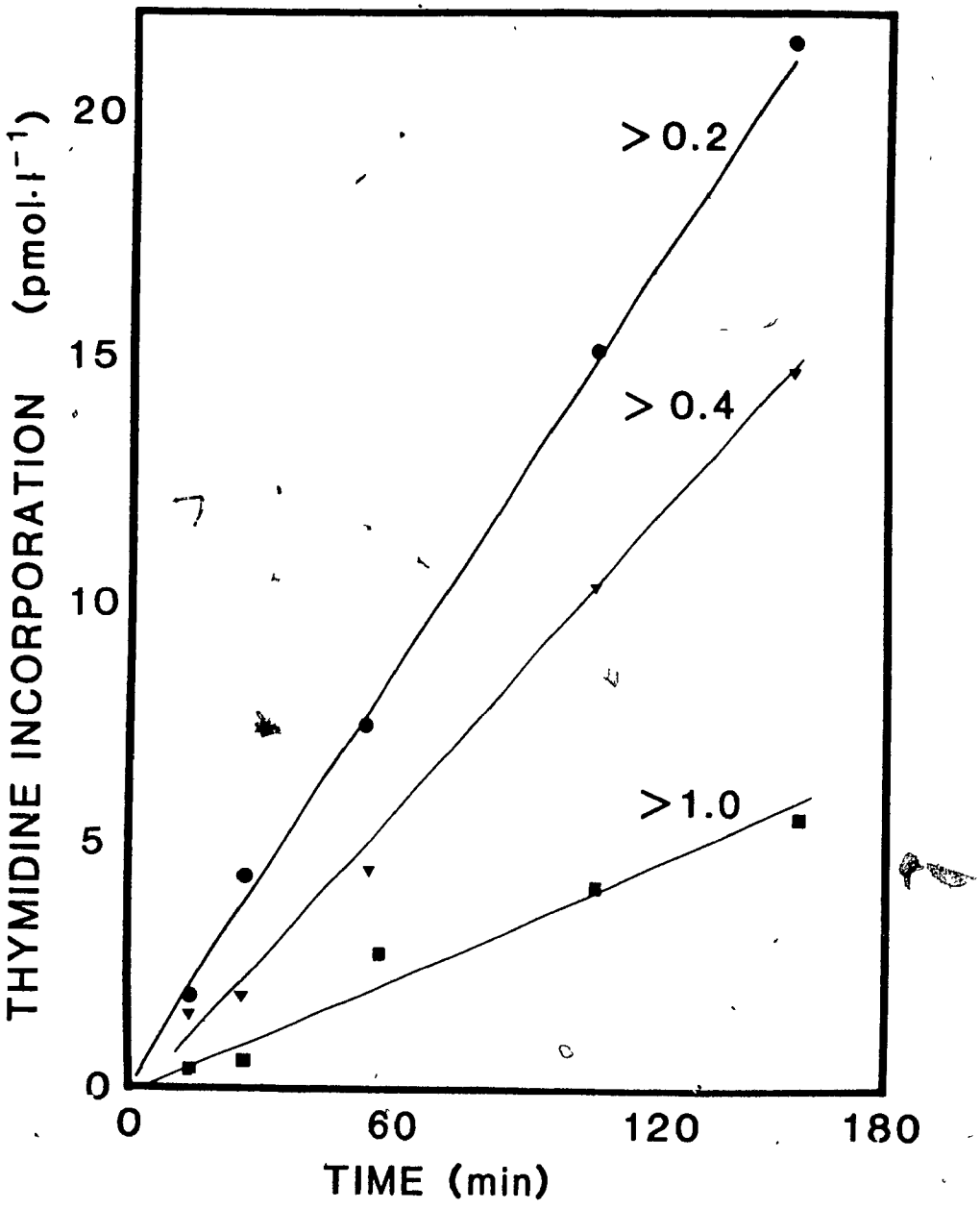
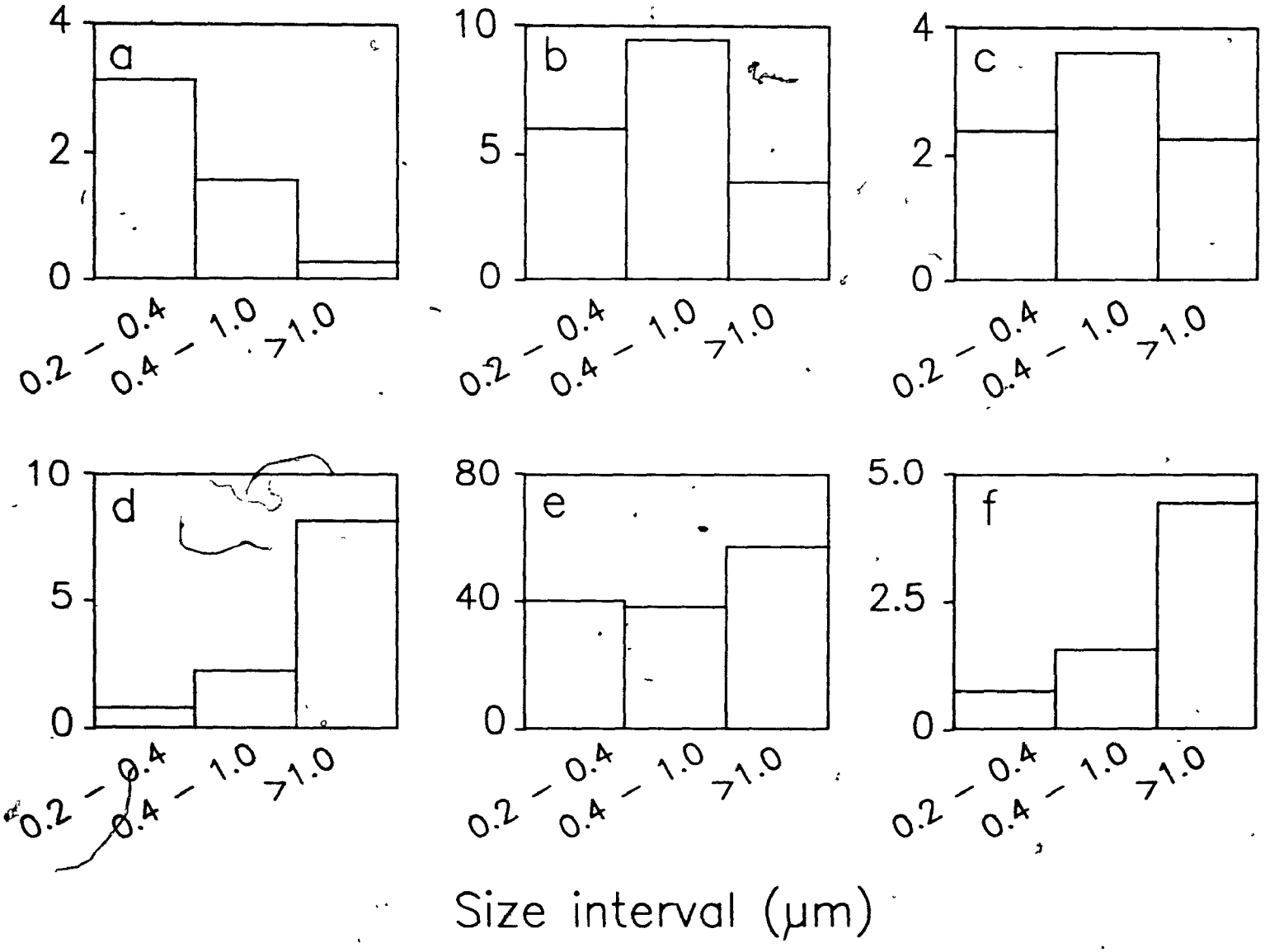

THYMIDINE INCORPORATION (pmol·l⁻¹)
20
15
10
5
0
0
60
120
180
TIME (min)
> 0.2
> 0.4
>1.0

Figure 2.

Abundance, biomass, and thymidine incorporation activity within 3 size classes of plankton in Quinn Bay, 21 September 1984. a: bacterial abundance ($10^6 \cdot ml^{-1}$); b: bacterial biovolume (10^4 $\mu m^3 \cdot ml^{-1}$); c: thymidine incorporation rate ($pmol \cdot l^{-1} \cdot h^{-1}$); d: cell-specific thymidine incorporation rate ($fmol \cdot (10^6$ $cells^{-1})$); e: volume-specific incorporation rate ($pmol \cdot mm^3$); f: genome-specific incorporation rate ($\mu mol \cdot g$ DNA^{-1}).


a
b
c
d
e
f
4
2
0
10
5
0
4
2
0
10
5
0
80
40
0
5.0
2.5
0
0.2 – 0.4
0.4 – 1.0
>1.0
Size interval (μm)

cell size categories (D.J. Currie, U. of Ottawa, unpublished data). Additionally, average volume of cells in the smallest class was less than 10% of those in the largest. Normally growing bacterial cells usually grow to only slightly more than double their volume before dividing (Ammerman et al. 1984).

Secondly, the disparity in thymidine incorporation rate might have stemmed from differences in DNA content with size. It is known that the average quantity of genetic material per bacterial cell varies as the 0.2 power of cell volume among bacterial species (Shuter et al. 1983). This difference is apparent in the examination of any DAPI-stained preparation of bacterioplankton, wherein differences in total light output from individual cells can be large. The use of a single conversion factor to transform thymidine incorporation rate into growth rate would therefore be misleading in this case. When we prorated incorporation rate per cell to cell DNA content estimated on the basis of mean cell volume, using the equation of Shuter et al., the gap between the growth rates of large and small cells narrowed but still did not close (Fig. 2F). Large cells trapped on the 1 μm filter were apparently growing more than six times as fast as the tiniest bacteria.

Finally, there are two alternative explanations that could not be checked with the data of the first growth study (Quinn Bay). First, Riemann et al. (1984) suggested that there was a weak indication in their data that endogenously synthesized thymidine was diluting the specific activity of the thymidine pool most strongly in the slowly growing bacteria (contrary to a suggestion by Moriarty and Pollard (1982)). If an unequal dilution effect was occurring unrecognized,

then straightforward interpretation of the Thd-incorporation results would be misleading. Second, since we did not extract DNA from the macromolecule pool labelled with tritium, we can not be sure that DNA did not form a larger percentage of the labelled pool in the smaller bacteria. Both of these possibilities, if correct, would tend to bias the presumptive growth rate represented in Fig. 2 in favor of the largest cells. These possibilities were examined in the second growth study.

Second growth experiment

The early spring diatom bloom in Lake Waterloo on 10 May 1987, the date of the second growth study, was in decline. Though colonial cyanobacteria had not yet appeared in significant numbers, many filaments of the dominant alga Melosira had been colonized by bacteria, and heavily-colonized detrital aggregates, often with Melosira at the core, were abundant. Once again, most bacteria (45%) fell into the smallest size class and the greatest biomass was trapped by the larger filters, 51% by the 1 um filter and 19% by the 5 um filter. The attached bacteria in Waterloo were larger than free cells. Attached bacteria (75% of the cells on the 5 um filter) made up 4.4% of the population but 14% of bacterioplankton biomass.

Thymidine incorporation rate in the different size classes, uncorrected for isotope dilution, varied roughly two-fold, from 7.4 to 17.3 $pmol \cdot l^{-1} \cdot h^{-1}$, the greatest amount being due to those cells in the 0.4 to 0.6 um filter size class (Table 2). Once again, therefore, no bacteria were dormant as a class. Calculated per cell, the tiniest cells were incorporating Thd at one-half to three-quarters the rate of the largest cells.

The isotope dilution experiments (Fig. 3) show clearly that most

isotope dilution was occurring among the largest, attached bacteria, and progressively less as filter size decreased and tinier bacteria were included. The degree of participation of added labelled Thd in macromolecule synthesis for the smallest (> 0.2 μm) to largest (> 5 μm) size class bacteria was 76, 68, 61, 52, and 42%, repectively. By subtracting uncorrected Thd incorporation rate from estimates corrected for isotope dilution, we could calculate the absolute amount of Thd diluting the labelled pool. This calculation (Table 2) showed that roughly 60% of all isotope dilution was due to bacteria in the greater than 5 um size class, the remaining 40% was due to those in the 1 to 5 μm class, and no detectable isotope dilution occurred in bacteria passing through the 1 μm filter.

The occurrence of isotope dilution in the large, fast growing cells exacerbated the disparity in cell-specific growth rate (Table 2). Thymidine incorporation per cell in the 0.2 to 0.4 μm interval was only 10% of the rate in cells in the greater than 5 μm interval. Cells in intermediate classes incorporated the DNA precursor at roughly 30% of this maximum. Adjustments for probable differences in DNA content closed the gap only slightly (Table 2). An adjustment should also be made for proportion of label occurring in DNA as distinct from that in RNA and protein in the different sizes of bacteria. Though more label did appear in DNA in bacteria passing the 1 μm filter (70% DNA, 18% protein, 12% residual) than in those trapped by the filter (52% DNA, 39% protein, 8% residual), once again this effect was insufficient to account for the apparent growth rate discrepancy between different-sized bacteria in Lake Waterloo.

Addition of 40 nM ^{3}H-Thd successfully repressed endogenous synthesis of Thd (Table 3). Incorporation of labelled Thd at this

Figure 3.

Isotope dilution curves (weighted regression) for 5 size classes of bacteria in Lake Waterloo, 10 May 1987. Labelled thymidine was present at 4 nM; X-intercepts (from which degree of participation was calculated) are, from smallest pore-size to largest, -5.2, -5.9, -6.5, -7.7, and -9.4 nM.

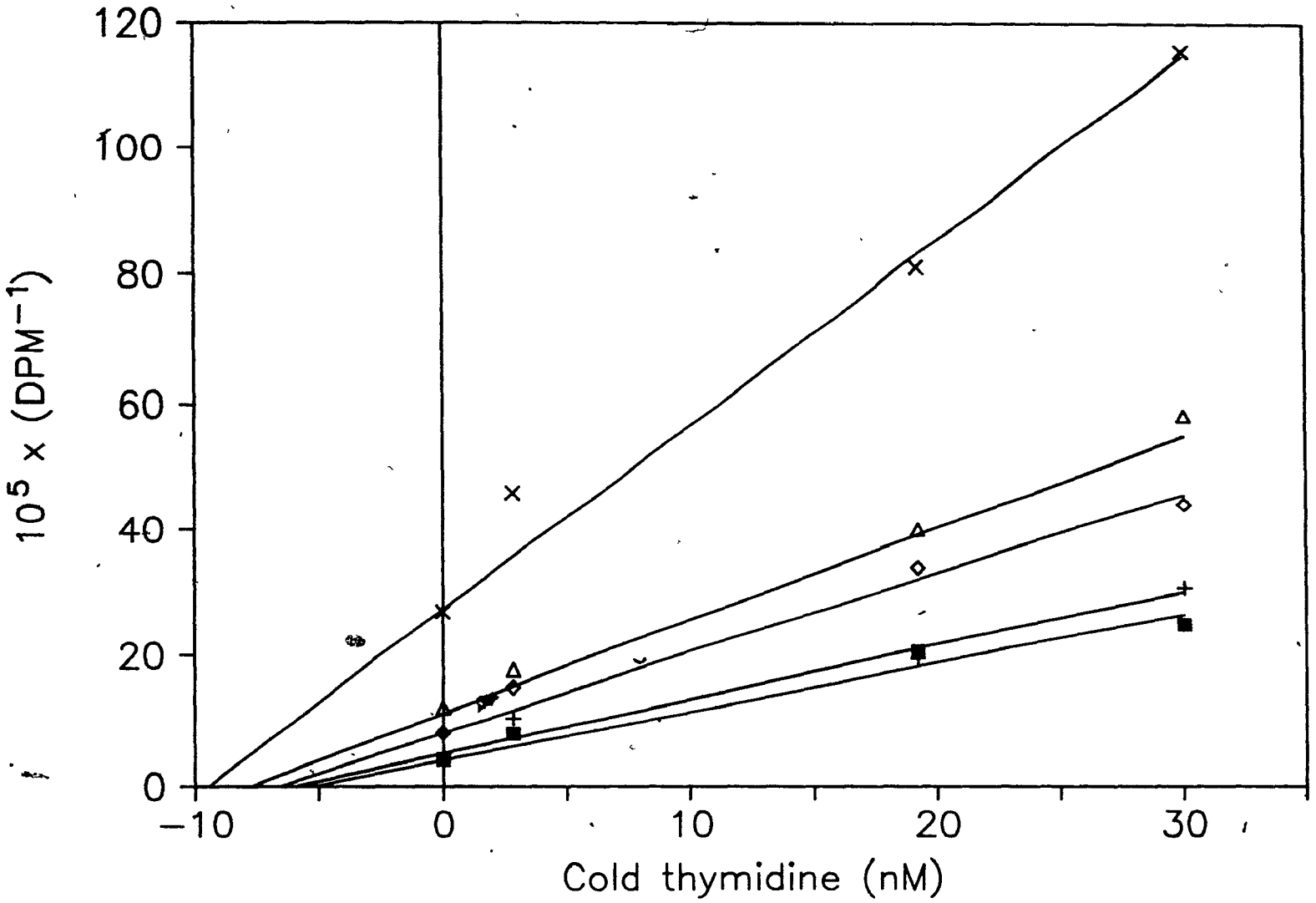

120
100
80
60
40
20
0
10^5 x (DPM^{-1})
−10
0
10
20
30
Cold thymidine (nM)

Table 2a. Abundance, cell volume, estimated DNA content and total bacterial biovolume in 5 filtration size classes, Lake Waterloo, spring 1987.

interval	abundance	size	predicted DNA content	biovolume
µm	$10^6 \cdot ml^{-1}$	μm^3	fg	$\mu m^3 \cdot ml^{-1}$
0.2 - 0.4	2.14	0.010	0.87	20600
0.4 - 0.6	1.21	0.022	1.07	26300
0.6 - 1.0	0.335	0.059	1.42	19800
1.0 - 5.0	0.871	0.076	1.53	66600
> 5.0	0.284	0.110	1.70	31300

Table 2b. Thymidine incorporation rate in Lake Waterloo, spring 1987, partitioned according to Nuclepore filter size-interval.

interval	Thymidine incorporation rate				
	uncorrected	dilution corrected	corrected per cell	corrected per cell vol.	corrected per fg DNA
µm	$fmol \cdot ml^{-1} \cdot h^{-1}$	$fmol \cdot ml^{-1} \cdot h^{-1}$	$fmol \cdot h^{-1}$	$fmol \cdot \mu m^{-3} \cdot h^{-1}$	$fmol \cdot h^{-1}$
0.2 - 0.4	11.2	11.2 (7.4)	5.2E-6	5.1E-4	6
0.4 - 0.6	17.2	17.2 (21.1)	1.4E-5	6.5E-4	13
0.6 - 1.0	7.4	7.4 (6.3)	2.2E-5	3.7E-4	15
1.0 - 5.0	11.4	18.7 (18.7)	2.2E-5	2.8E-4	14
> 5.0	7.9	16.7 (18.7)	5.9E-5	5.4E-4	35

Table 3. Results of pilot study on use of fluorodeoxyuridine to force bacteria to use exogenously supplied thymidine. Expected rates and confidence limits are from the isotope dilution study.

[^{3}H]thymidine concentration (nM)	Fluorodeoxyuridine concentration (nM)	Thymidine uptake rate (fmol·ml^{-1}·h^{-1})	Expected rate (fmol·ml^{-1}·h^{-1})
4	2.9	30	55 (38 - 99)
4	17.2	44	55 (38 - 99)
40	17.2	59	72 (50 - 130)
40	-	67	72 (50 - 130)

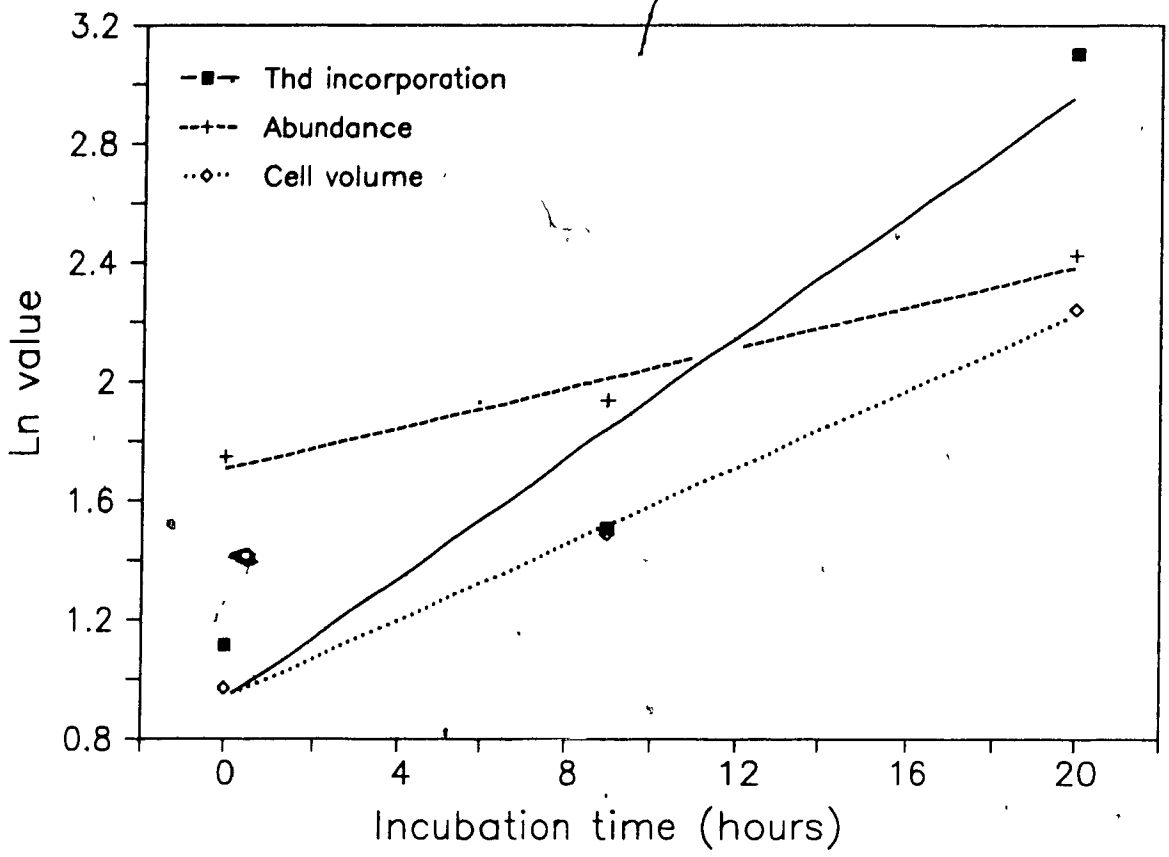
concentration was not different from what would have been predicted on the basis of the dilution assay. However, fluorodeoxyuridine did not act as expected. First, it did not block synthesis of Thd, since an increase in ^{3}H-Thd concentration resulted in increased incorporation in the presence of FdU. Second, FdU was apparently toxic to the cells, since added FdU at the lowest level significantly reduced Thd incorporation. Though this first, unreplicated test is not powerful, we conclude that the use of FdU is probably not appropriate for lake plankton community studies.

How fast were the bacteria growing? The results of the time course study to determine an extrapolation factor from Thd incorporation to growth rate are shown in Fig. 4. Growth rate ($\mu + s$, using the notation of Kirchman et al. 1982) calculated on the basis of Thd incorporation rate was $0.101 \cdot h^{-1}$. The increase in cell numbers (μ) over the same period was $0.034 \cdot h^{-1}$. There was a marked increase in average cell size over the 20 h of the study, going from 0.026 μm^3 at 0 h to 0.044 μm^3 at 9 h, to 0.094 μm^3 at 20 h. The rate of increase of cell-specific volume (s) was $0.064 \cdot h^{-1}$. The sum of cell-specific and population growth rates, equivalent to the rate of increase of total bacterial biomass, was $0.098 \cdot h^{-1}$. Since this rate is almost identical to the rate of increase of Thd incorporation, it appears that the latter rate was coupled to total production of bacterial biomass and not just to production of new cells. Therefore the appropriate extrapolation factor is not the standard one, calculated without regard to biomass accretion (2.28×10^{19} cell$\cdot$mol^{-1}). Rather, the proper factor converts thymidine incorporation to biomass growth rate (5.92×10^{17} $\mu m^3 \cdot$mol^{-1}).

Because we measured growth rate in separate size classes of the

Figure 4.

Dilution assay results for estimation of growth rate extrapolation factors. All values were transformed to $\log_e$. Thymidine incorporation rate expressed as $fmol \cdot ml^{-1} \cdot h^{-1}$, abundance as 10^5 $cell \cdot ml^{-1}$, and cell volume as $100x(\mu m^3 \cdot cell^{-1})$.


Thd incorporation
Abundance
Cell volume
Ln value
3.2
2.8
2.4
2
1.6
1.2
0.8
0
4
8
12
16
20
Incubation time (hours)

bacteria, we can, however, carry the analysis further to test an assumption important to these incubation studies: that the process of dilution and incubation does not alter the pattern of growth of the bacteria, it merely reduces predation losses. Calculations shown in Table 4 show that this assumption is not tenable in this case, based on the effect on the growth rate estimates of the application of different conversion factors to the observed Thd incorporation pattern. First, if a constant Thd-to-biomass extrapolation factor is used in each size class, then the overall μ is $0.24 \cdot d^{-1}$ and s is zero. These are not the relative importances of the population and specific growth rates observed in the dilution study, on which the conversion factor was based. Alternatively, when a constant Thd-to-abundance factor is used, then the relative importance of cell-specific growth increases, but does not surpass the population growth rate. Therefore neither of these approaches to growth rate estimation is compatible with observed size-incorporation rate data. Furthermore, we know that the proportion of Thd that was incorporated into DNA rather than other macromolecules was least in the larger size classes, and that DNA content per cell was probably greatest in the largest bacteria, so that adjustments must be made that would decrease the volume growth rate even further. On the basis of these observations, we tentatively conclude that a large portion of the cell-specific growth-rate (s) occurred in response to filtration, dilution, or confinement, and as a result, the Thd-to-biomass conversion factor overestimates in situ growth rate.

Our calculations show that the population growth rate found in the growth experiment ($0.034 \cdot h^{-1}$) would have been expected if an extrapolation factor of 2×10^{18} $cell \cdot mol^{-1}$ had been used (3×10^{18}

cell•mol^{-1} thymidine incorporated into DNA alone). This factor is similar to the standard one advocated in the literature (2 x 10^{18} cell•mol^{-1}) (Bell et al 1983; Moriarty 1986). The population growth rate would then be accompanied by a cell-specific volume growth rate of 0.015•h^{-1}. The corresponding hourly population growth rates per class (Table 4) show a 6-fold increase from the smallest to the largest cells. If this best estimate is correct, the population of bacterioplankton in Lake Waterloo was turning over slightly more than twice daily (2.2 times), while producing new bacterial biomass equal to 3.2 times bacterial standing stock.

Grazing studies

There were 3940 +/- 520 phagotrophic flagellates per ml in Waterloo on the date of the production experiment (10 May). Average clearance rate was 0.16 ul•cell^{-1}•d^{-1} on 0.6 um beads and 0.18 ul•cell^{-1}•d^{-1} on 1 um beads. Clearance rate on 0.27 um particles was not sufficiently large to be quantifiable with precision, but was no greater than 10% of the rate on the larger beads, and probably was less. Measured average clearance rate (0.6 um beads) for particular flagellate types ranged from zero for bodonids, to 0.04 ul•cell^{-1}•d^{-1} for unidentified organisms 2 um in diameter, to 0.29 ul•cell^{-1}•d^{-1} for Ochromonas. The microflagellate community was clearing 67% (+/- 22%) of the water column of large particles per day.

The rotifer population was not yet abundant in the lake (about 200•l^{-1}) and consisted of genera that are not adept at bacteria-feeding (Keratella, Kellicottia, Trichocerca). While individual ciliates had high clearance rates (e.g. Vorticella, 6.7 ul•ind^{-1}•d^{-1} (0.6 um beads), equivalent to 200,000 body volumes d^{-1}; unidentified

Table 4. Application of different extrapolation factors to thymidine incorporation data, Lake Waterloo, spring 1987. The first is the Thd-to-biomass factor derived from dilution assay, the second is the standard Thd-to-abundance factor from the literature, the third is the standard factor, corrected for estimated DNA content per cell.

Factor =	5.92×10^{17} $\mu m^3 \cdot mol^{-1}$		2×10^{18} $cell \cdot mol^{-1}$		2×10^{18} $cell \cdot mol^{-1}$ adjusted		
interval (μm)	biovolume (μm^3)	abundance ($10^4 \cdot ml^{-1}$)	biovolume (μm^3)	abundance ($10^4 \cdot ml^{-1}$)	biovolume (μm^3)	abundance ($10^4 \cdot ml^{-1}$)	μ (h^{-1})
0.2 - 0.4	6620	64.2	230	2.2	380	3.7	0.017
0.4 - 0.6	10200	47.1	750	3.4	1040	4.8	0.039
0.6 - 1.0	4360	7.4	870	1.5	870	1.5	0.043
1.0 - 5.0	11000	14.4	2900	3.7	2700	3.5	0.039
> 5.0	9800	8.9	3700	3.3	3200	2.9	0.098
μ (h^{-1})	0.26		0.029		0.033		
s (h^{-1})	-0.03		0.021		0.015		

Table 5. Clearance rate of monitored bacterial grazers in Eastern Township lakes, on fluorescent microspheres of three sizes.

	Clearance rate ($\mu l \cdot cell^{-1} \cdot d^{-1}$)			Abundance (ml^{-1})
Bead diameter (um)	0.27	0.57	1.0	
Lake Gilbert (30/7/85)				
Keratella	0.07	1.3	81	
choanoflagellates	0.04	0	0	280
all flagellates	0.025	0.13	0.05	1180
Lake Trousser (30/7/85)				
Halteria	0.02	6.8	13	
all ciliates	0.09	2.7	4.9	13
choanoflagellates	0.34	0	0	
all flagellates	0.01	0.15	0.36	850
Lake Brome (27/7/85)				
all ciliates	0.004	3.1	5.4	6
all flagellates	0.04	0.19	0.1	510
Lake Stukely (9/9/85)				
heterotrophic flagellates	0.007	0.16	0.24	370
Ochromonas	0.007	0.34	0.54	270
Dinobryon	0.014	0.29	0.14	25
epiphytes on *Dinobryon*	1.0	0.2	0.08	4
all flagellates	0.013	0.24	0.36	670
Lake Bowker (9/9/85)				
heterotrophic microflagellates	0.02	0.14	0.54	200
Dinobryon	0.017	0.59	0.29	130
other chrysomonads	0.009	0.31	0.87	240
all flagellates	0.016	0.32	0.62	580

small ciliate (335 μm^3), 7.5 $\mu l \cdot ind^{-1} \cdot d^{-1}$ (1 μm beads), 20 million volumes d^{-1}),

Microflagellate clearance rates in the other five lakes studied were also greatest on the two largest sized particles (Table 5). Only free choanoflagellates were consistently found to be consuming 0.2 μm particles at a rate closely proportional to abundance. Other cells, particularly the mixotrophic chrysomonads, concentrated heavily on larger particles. These large-particle feeders were always numerically dominant, particularly in the richer lakes. In those lakes where sufficient ciliates were examined to make counts reliable, a more exaggerated disparity in feeding rates on particles of different sizes was found (Table 5). On top of this, rotifers cannot graze effectively on 0.2 μm particles (with the notable exception of Conochilus), and the crustaceans show a similar grazing preference for the largest bacteria (not shown).

Discussion

All other factors equal, smaller organisms have the potential for greater mass-specific metabolic and growth rates, and shorter generation times than do larger one (Fenchel 1974; Banse 1982; Fenchel and Finlay 1983; Peters 1984). Since the bacterial size- growth relationships we inferred and observed in Lakes Memphremagog and Waterloo do not follow this pattern, however, we must conclude that other factors are not equal. The smallest cells were less active in both metabolism and growth than the largest cells, contrary to the predictions of allometric relationships, and unless this is unique to free-living bacteria, then it provides further circumstantial evidence for metabolic dormancy of some fraction of the bacterioplankton.

No size fraction was dormant as a class, however. A decline in growth rate with a decreasing average size could have been due to a uniform decrease in specific growth rate with decreasing average size, or an increased proportion of completely inactive cells in the smaller classes. Whichever effect is responsible, these results do not support an earlier claim that the smallest bacterial cells are most active per cell and per unit biomass (Fuhrman 1981). The size data and activity data supporting the earlier claim were preliminary only (collected on separate dates in different locations by different methods; cf. Fuhrman 1981). We therefore tentatively suggest that the contrary is the general rule, that the smallest aquatic bacteria are less metabolically active than larger cells.

There is evidence of another sort in the literature that makes this suggestion more credible. Fenchel and Finlay (1983) developed the argument that the ability to reduce mass-specific respiratory rate in response to starvation is more pronounced in smaller than in larger cells among the Protozoa. This capability was suggested to be a necessary adaptation to the rigors of the very high specific rates associated with small size, that would mean rapid starvation and death upon removal from nutrients if no moderating mechanism were available. Laboratory data provided by the authors can be used to make this argument more quantitative. Recalculation of the data presented in Fenchel and Finlay, Fig. 4, yields the equation:

$$\text{Log}_{10} \text{ resp. rate } (\text{nl } O_2 \cdot \text{cell}^{-1} \cdot \text{h}^{-1}) = -3.9 + 0.67 \log \text{size}_{max} + 2.91 \text{ size}_{diff} - 0.31 (\log \text{size}_{max} * \text{size}_{diff}),$$

$$F = 663, \; R^2 = 0.97.$$

where $size_{max}$ is maximum cell volume (μm^3) and $size_{diff}$ is the difference between observed $\log_{10}$ cell volume and maximum $\log_{10}$ cell volume. All coefficients were highly significant ($p < 0.001$) and the model can be interpreted as follows. Since the positive coefficient of the $size_{max}$ term is significantly less than unity, respiration rate per cell does not increase at the same rate as cell volume among species. The positive coefficient of the $size_{diff}$ term means that smaller cells (i.e. more starved, in the context of the experiments) had lowered respiration rates. The negative coefficient of the interaction term means that the starvation effect grew more pronounced as maximum cell size of a species of protozoan decreased, strongly diverging from the standard allometric trend once maximum cell size fell below about 100,000 μm^3. On the basis of this equation, we can calculate that an organism of the dimensions of Escherichia coli is expected to be able to alter its growth and respiratory rate over an 800-fold range in response to altered nutrient conditions. Indeed, this is close to the reduction in respiration rate observed in the study by Novitsky and Morita (cf. review by Morita 1982). In light of this theoretical potential, the fact that the average growth rate of different sized cells in the present study had only a 2 to 10-fold range, a minor fraction of the model-based range, suggests that nutrient starvation was perhaps a contributing but not dominant factor determining the bacterial size - activity spectrum. That is, the cells in the smallest size class were one-tenth the volume of the largest cells, but were not one eight-hundredth as active, such as would be expected if the tiny cells were merely starved versions of the largest, according to the model.

So far we have treated the abundance measures as if they could be determined without uncertainty. An alternative that cannot be discounted is that at least some of the smallest DAPI-staining particles were not bacteria at all, but large viruses (cf. also Sieracki et al. 1985). If this were the case, it would explain the apparently low specific metabolic activity of particles in the smallest fraction. It might also explain our finding (unpublished) that 50% of DAPI-staining particles (> 0.1 μm) in Lake Memphremagog, July 1985, passed through a 0.2 μm filter. These particles, when isolated, did not increase in abundance during a 48 hour incubation at in situ temperature.

It was interesting to discover that comparatively few large bacteria were responsible for all of the thymidine dilution. If we adjust the dilution attributable to bacteria on the 5 μm filter for the effect of free-living cells trapped there, by assuming that the free cells have the same characterisics as those in the 1 to 5 μm size class, then the 4 to 5% of the bacterioplankton attached to particles appeared to be associated with 51% of the isotope dilution. This positive relation between dilution and size (and hence growth rate) is in agreement with the contention of Moriarty and Pollard (1982) and contrary to that of Riemann et al. (1984). One might speculate from the observed pattern that large cells with the greatest growth rate have the stored resources and machinery to synthesize a portion of their own thymidine. Small cells, on the other hand, with surface-to-volume characteristics favoring uptake but possessing no stored energy, depend heavily on exogenously supplied substrates for growth. On the other hand, isotope dilution in the largest cells may have been due simply to the inadequacy of external supply to meet the demands of

the fastest growing cells (Moriarty 1984).

The smallest cells grew most slowly in both lakes, but were several times more abundant than larger cells. If cells do not change size classes during their development, then loss rates must be disproportionately high for the largest cells. The size-selective feeding observed in these lakes and the other lakes in which grazing was measured is consistent with this. Feeding rates were measured using latex particles rather than bacterial cells, but the similarity of the rates we measured to those in the literature, measured using bacteria (Fenchel 1986), lends our estimates credibility. Furthermore, if anything, the grazing rates we measured are unexpectedly high, being as they are average rates for a heterogeneous assemblage of protozoa that feed by different mechanisms, including the bodonids that apparently specialize almost exclusively on attached bacteria (Caron 1987), as well as protozoans that seem to feed on bacteria trapped by extracellular mucus. Bodonids have never been found to contain beads in any lake we have studied (nor have cells with conformation of Pleuromonas), and we try not to include beads trapped on the outside of protozoans in our counts to avoid counting randomly adsorbed beads as ingested ones. As a result, our measured clearance rates are probably somewhat lower than true rates.

The disparity in feeding rates on particles of different size may allow a partial reconciliation of differential activity, elevated abundances of the most slowly growing cells, and high clearance rates by the entire grazing community. The contention that grazers are implicated in the observed size-activity structure is not unique to this paper. Several authors have alluded to the possible influence of

grazing on bacterial size distribution (Bird and Kalff 1987; Ammerman et al. 1984; Hagstrom 1984; Kjelleberg 1984; Wright and Coffin 1983). The concept is a natural extension of the known influence of algal size on vulnerability to grazing (Gliwicz 1967). In both cases, the less heavily preyed upon fraction displays a lower specific metabolic rate.

Cell-specific clearance rates by microflagellates were roughly similar in all lakes examined, ranging only two-fold among lakes. Other studies have shown that flagellate specific clearance varies only 25% over the course of the growing season in both Waterloo and Memphremagog (unpublished data). This suggests that the microflagellates are food-limited, clearing as much water as is physically possible, independently of bacterial and picoplanktonic abundances. Variability in community clearance rate among lakes at the same temperature would thereby depend solely on microflagellate abundances. The marked tendency of zooplankton to concentrate grazing on the largest particles is suggested to result in increased overall quantity of food consumed, given the inevitable reduction in clearance rate associated with lower capture-size limits (cf. Fenchel 1982, 1986). For example, though bacteria were 7 times more abundant than picophytoplankton in Lake Bowker on the date of the grazing study (Table 5), the average bacterial cell was only 6% of the volume of a picophytoplanktonic cell. Therefore the picophytoplankton represented about 75% of the picoplanktonic biomass, and the loss of available biomass to protozoan grazers with higher clearance rates on larger cells, but with a concomitant inability to consume the smallest bacteria, would be minor indeed.

Food limitation is consistent with the importance of mixotrophs

in oligotrophic lakes. Though Ochromonas was present in all lakes examined in this study, its relative and absolute importance was greatest in oligotrophic Lake Bowker, where chrysomonad mixotrophs made up 65% of the phagotrophic microflagellate community. Following this logic, the majority of grazers in the oligotrophic ocean should also be mixotrophs but this remains to be determined (Estep et al. 1986).

Finally, it is important that future experimental designs take into account the strong size-dependence of growth and grazing rates within the picoplankton. The present results verify and underscore the importance of the suggestion of Fenchel (1982b) that large variations in clearance rate between protozoans can be explained as a consequence of variable capabilities of capturing particles of different sizes. A consequence of this variability is that attempts to demonstrate a protozoan feeding preference for bacteria over latex particles are of reduced value in the absence of evidence for a close correspondence in size between the two food items (e.g. Bird and Kalff 1986; Sherr et al. 1987). The apparent increase in growth rate with cell size means that a simple Thd-to-abundance extrapolation factor will underestimate the biomass production rate. In Lake Waterloo, the biomass growth rate was 50% greater than the population growth rate.

In conclusion, the central results of this analysis of bacterial growth and loss rates are the following. First of all, the tiny bacterial cells that dominate the bacterial community numerically are apparently the slowest growing, both per cell and per unit biomass. This contradicts standard allometric relationships. The result provides circumstantial evidence for the existence of reduced

metabolism as a response to unfavorable conditions by some members of natural bacterial communities. Secondly, current practices for deriving bacterial growth rates based on the incorporation of labelled precursors into DNA may give biased, or at least inaccurate, results. Pleomorphic growth by bacteria inflates the incorporation rate, thereby inflating the growth rate extrapolation factor. Heteroscedasticity in isotope dilution data compromises the accuracy of "degree of participation" calculations (Appendix 1). Finally, it is likely that the dominance of smaller bacterial cells in lakes is a result of their inaccessibility to grazers, because growth rate among the bacteria in limnetic communities increases with size.

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Conclusions

The objectives of this thesis were,

1) to build a predictive model of bacterial abundance in lakes, estuaries, and oceans, that could be used to narrow the range of probable planktonic interactions and to generate further hypotheses relating to microbial activity in those systems,

2) to quantify the impact of predators, in particular the mixotrophic phytoplankton, on bacteria in situ,

3) to determine the importance of bacterial prey to the mixotrophic flagellates, relative to photosynthetic rates in situ,

4) from a larger perspective, to reconcile the conflicting pressures of bacterial population growth and size-dependent grazing as a way of explaining the numerical dominance of the slowest-growing fraction, those passing through 0.4 μm Nuclepore filters.

I conclude by summarizing the more important contributions to microbial ecology.

In Chapter 1, a strong, positive relationship was found between bacterial abundance and chlorophyll concentration in fresh and marine waters. The relationship to total phosphorus concentration, another indicator of nutrient availability, was stronger than to chlorophyll concentration in Quebec lakes. There was no evidence to support the claim that bacteria increase in size in richer lakes. The suggestion was made (on the basis of data not in the thesis) that production per unit bacterial biomass might increase along a trophic gradient, so that the disproportionately lower increase in abundance need not

entail reduced bacterial community metabolism in eutrophic aquatic systems.

Chapter 2 reported that common members of the phytoplankton could be major grazers of bacteria in lakes. In particular, species of the genus Dinobryon consumed bacteria at a rate similar to that of obligate heterotrophs, and consumed more bacteria from mesotrophic Lake Memphremagog on the date studied than did the crustacean, rotifer, and ciliate communities combined.

The focus shifted in Chapter 3 from the impact on the bacteria, to the importance of phagotrophy to the phytoplankton themselves. It was shown that under the low light conditions in which it was most abundant, Dinobryon could rely more on phagotrophy than photosynthesis for subsistence. The total number of chrysomonad species observed to ingest particles was increased to 13. Particle ingestion rate depended on temperature and not on light, so that day and night grazing rates were usually indistinguishable. Dinobryon consumed the larger picoplankton many times faster than it did the tiniest bacterial cells. In an oligotrophic lake, the phytoplankton (Ochromonas, Dinobryon) were responsible for more bacterial grazing than were the zooplankton.

Work in Chapter 4 complemented the preceding chapters by demonstrating that Dinobryon is an efficient bacterivore that incorporated 40 to 80% of ingested bacterial carbon. The community forming a large peak in phytoplankton biomass in the metalimnion of a small mesotrophic lake derived more fixed carbon from mixotrophic particle ingestion than from photosynthesis. It was suggested that

particle-feeding by Uroglena might be a factor in allowing this alga to form toxic blooms in Japanese lakes, and that phagotrophic supplementation of photosynthesis by deep-living algae is responsible for the maintenance of some deep phytoplankton peaks.

Finally, an in depth look at the relationship between size-fractionated growth rate, bacterial abundance, and protozoan grazing showed that the smallest bacteria were growing most slowly, contrary both to earlier claims in the literature and to standard allometric relationships. The largest cells grew most quickly and were responsible for the majority of thymidine isotope dilution. These large bacteria, along with the picophytoplankton, were subject to 2 to 40 times greater grazing pressure by unicellular grazers than were the small cells. It was shown that assumptions behind current procedures for calculating growth rates from thymidine incorporation data are not supportable. First, pleomorphic changes in bacteria in response to filtration, dilution, or confinement bias the calculated thymidine-to-growth extrapolation factor. Second, the accepted method for calculating the degree of participation of labelled thymidine in DNA synthesis is statistically incorrect, so that misleading conclusions may have been drawn in the past on the basis of improper methods.

Appendix 1

An investigation into the statistics of isotope dilution experiments

While analyzing the results of the isotope dilution procedure described in the second growth study of Chapter 5, we became doubtful of the appropriateness of using ordinary least squares to find x and y intercepts (Moriarty 1986; Findlay et al. 1984; Bell 1986). The standard procedure starts with the assumption that labelled thymidine will be incorporated into DNA in proportion to its abundance relative to unlabelled sources. That is (using the notation of Findlay et al. (1984)):

$$C_{obs}/C_{max} = L/(L + P + A) \quad (1)$$

where C_{obs} is observed radioactivity incorporated in a sample, C_{max} is the quantity of radioactivity that would be incorporated if labelled thymidine were the only source available to the bacteria, L is the constant concentration of labelled thymidine added, P is the unknown pool (exogenous and endogenous) of thymidine naturally available to the bacteria, and A is the amount of "cold", unlabelled thymidine added in graded amounts to a series of samples. This equation is inverted and rearranged to yield a mathematically more tractable form:

$$1/C_{obs} = (L + P)/(L \cdot C_{max}) + (1/(L \cdot C_{max})) \cdot A \quad (2)$$

In this form, the equation is analysed by linear regression. The X-intercept yields the total thymidine concentration that is incorporated into DNA (L + P). The degree of participation of labelled thymidine can be calculated as L/(L + P). The Y-intercept will be the most efficient estimator of radioactivity incorporated in the absence of added cold thymidine. Together, the X- and Y-intercepts provide the estimate of total thymidine incorporation during the incubation period.

Once statistical analysis begins of a relationship derived

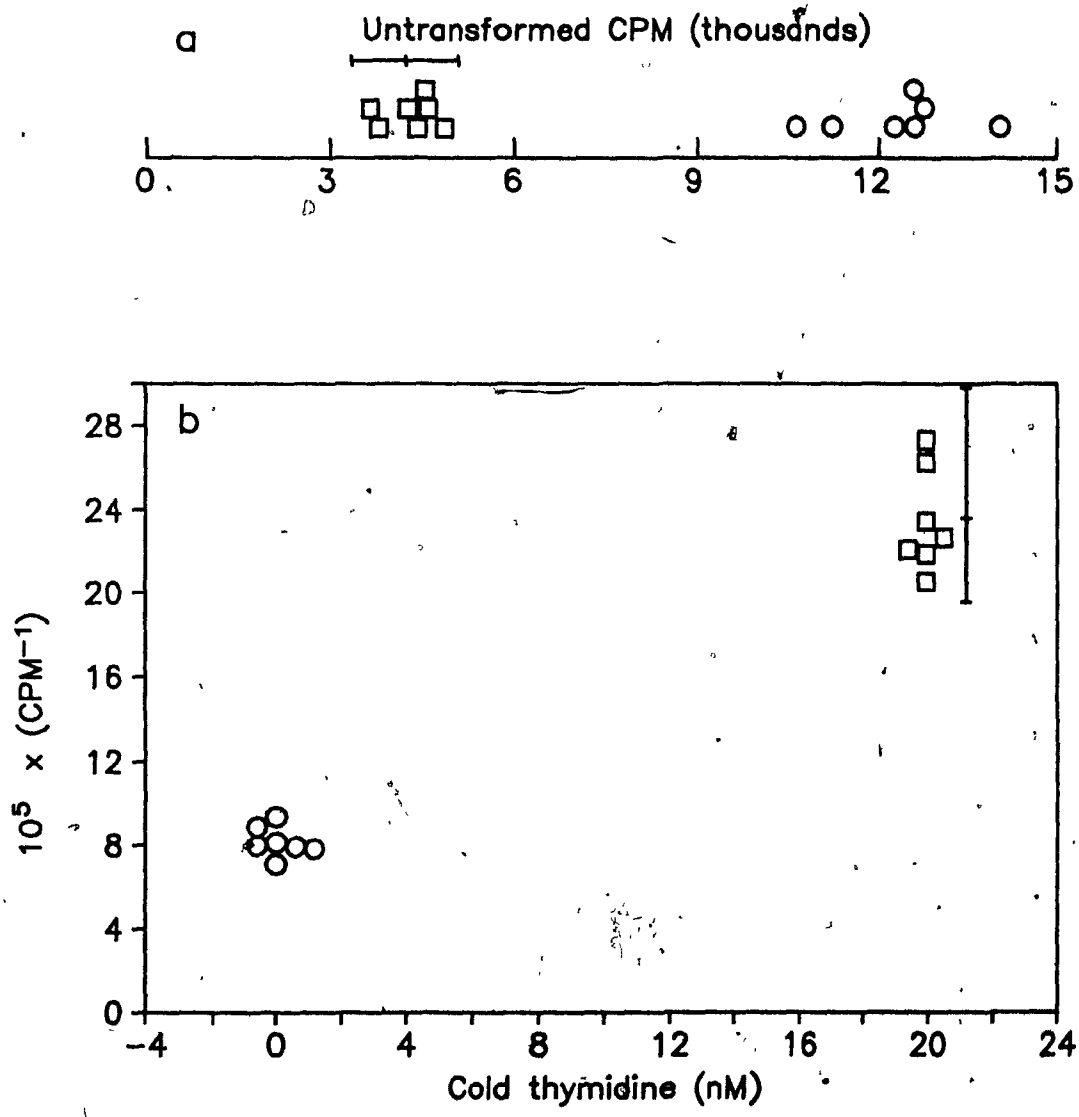
mathematically, however, the procedural rules change, and the assumptions of the statistical tool used must be met in order that trustworthy results be obtained. In particular it appeared likely to us that simple dilution with cold thymidine would not alter the relative variability in incorporation rate among samples, but only change the mean rate. Accordingly, we determined the error structure of label uptake using a new sample collected from Lake Waterloo on 26 May 1987. This sample was divided into 20 ml aliquots in clean glass scintillation vials. Seven vials received 4 nM ^{3}H-Thd, seven vials received 4 nM ^{3}H-Thd plus 20 nM cold Thd, and three vials were Lugol's solution-preserved blanks. Incubations lasted 1 h and were stopped with Lugol's solution, then collected on 0.2 µm Nuclepore filters for TCA extraction as described in Chapter 5.

The results of this study showed that ordinary least squares regression was not appropriate for the analysis of isotope-dilution experiments. The coefficient of variation (CV) of isotope incorporation in the undiluted samples (9%) was almost identical to the CV for the diluted samples (10%) (Fig. 1a). This means that the CVs for the reciprocals at each level are also nearly identical (9 and 10.5%) (Fig. 1b), and therefore that the errors around the mean at each point are not homogeneous as is necessary for maximum precision in determining the X and Y intercepts (Draper and Smith 1981, p. 110). The appropriate model to use in this case is weighted least squares, weighting each point by the reciprocal of its variance. We did this in analysing the experiment described in Chapter 5 by assuming the coefficient of variation at each point was 10%.

A closer consideration of standard isotope dilution methodology

Figure 1.

Isotope dilution results show that ordinary least squares should not be used to find line of best fit. a: Variance of untransformed counts increases with the mean; b: Variance of count reciprocals also increases with the mean. The bar illustrates the effect of reciprocal transformation on symmetrically distributed values. Points are shifted perpendicularly to avoid superimposition.


a
Untransformed CPM (thousands)
0
3
6
9
12
15
b
28
24
20
16
12
8
4
0
10^5 x (CPM^{-1})
−4
0
4
8
12
16
20
24
Cold thymidine (nM)

reveals that there are several statistical problems associated with it, some more serious than others, some more easily remedied than others. The problem of heteroscedasticity is most serious, but also most easily remedied. It requires that effort be made to determine the variability of thymidine incorporation measurements in the system of interest (see Draper and Smith 1981, pp. 35 & 38, on proper replication). Most statistics packages (e.g. SAS, BMD, SPSS) will perform the required weighted least squares calculations if estimates of the variance at each point are available. Note that one should construct or estimate a relationship between variance and X-, not Y-, values, to avoid under- or overestimation of the variance due to residual error in Y. Coefficients of variation in the literature range from 8% (Bell 1986) to 40 to 60% (Meyer et al. 1987). Unless a weighted analysis is done, literature claims related to nonlinearity of isotope dilution plots should be greeted with skepticism, in light of the likelihood of greatly increased residual errors at greater dilution levels. Such claims must be backed by proper tests of lack of fit to a linear model, cast in a framework of weighted least squares, and having sufficient true replication at each dilution level. Note that the nonlinear least squares approach advocated by Kirchman et al. (1986) is as subject to the problems associated with heteroscedasticity as is the standard one.

The weighted least squares procedure does not, unfortunately, remove all statistical problems with this technique. First, the residuals around the regression line will not in general be normally distributed, tending instead to be positively skewed (see Fig. 1). This means that confidence limit statements will be inaccurate. This problem is relatively minor given the more serious concerns about the

biological validity of the technique (cf. Moriarty 1986). Nevertheless, the problem should be kept in mind when constructing confidence statements - these are best made nonparametrically (cf. Efron and Gong 1983). Second, use of the simple reciprocal of the Y-intercept will probably underestimate the true mean value of the thymidine incorporation rate without added cold thymidine. This is because the mean of the reciprocal of a positive random variable (incorporated radioactivity, in this case) is always greater than the reciprocal of its mean (Chiang 1966). In the standard case where the residual errors are normally distributed, the underestimation could be compensated for by applying the following equation (Miller 1984):

$$\text{Corrected DPM} = (1/y_0)\{1 + s^2/y_0\}$$

where y_0 is the Y-intercept of the regression equation and s^2 is the residual mean square. Because the error distribution around the Y-intercept is positively skewed, however, this correction is likely to overcompensate, so that the desired value lies somewhere between the simple reciprocal and the corrected value. This problem was not of concern in the study described in Chapter 5 because the residual error at the intercept of the weighted equations was so small that corrected and uncorrected estimates differed by only 1%. In cases where such a felicitous lack of error does not hold, we recommend the application of Miller's equation, since the transformation bias is likely to be the more important of the two conflicting influences.

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Appendix 2

Data used in the construction of empirical equations of Chapter 1

Table 1. Data used in regression equations in Bird and Kalff (1984). Chla is chlorophyll concentration in surface waters, in $\mu g \cdot L^{-1}$. AODC is acridine orange direct count of bacteria in millions of cells$\cdot mL^{-1}$. N refers to the number of separate observations pooled to get average value.

Site	Chla	AODC	N	Reference
Freshwater				
Erie, east	3.3	2.9	5	Chapra & Dobson 1981; Rao et al. 1981
Erie central	4.5	4.3	10	Chapra & Dobson 1981; Rao et al. 1981
Huron	1.2	1.0	5	Chapra & Dobson 1981; Rao et al. 1981
Ontario	7.04	5.76	24	Rao et al. 1979
Bonilla	1.04	0.67	12	Maclsaac et al. 1980
Bowser	0.17	0.41	2	Maclsaac et al. 1980
Great Central	0.94	0.85	12	Maclsaac et al. 1980
Kitlope	0.59	0.60	12	Maclsaac et al. 1980
Long	2.36	0.98	12	Maclsaac et al. 1980
Love	0.85	0.98	12	Maclsaac et al. 1980
Meziadin	1.68	0.49	12	Maclsaac et al. 1980
Woss	0.87	0.42	12	Maclsaac et al. 1980
Mossø	49.22	13.36	5	Riemann et al. 1982
Bysjön	45.2	9.53	6	Coveney 1982

Table 1 (Cont'd)

Site	Chla	AODC	N	Reference
Haljasjön	27.6	9.4	1	Coveney 1982
Elmenteita	367	360	1	Kalff 1983; Kilham 1981
Sonachi	44	36	1	Kalff 1983; Kilham 1981
Bogoria	121	35	1	Kalff 1983; Kilman 1981
Navaisha	12	3.7	1	Kalff 1983; Kilham 1981
Kootenay 77	2.75	1.3	7	Daley et al. 1981
Kootenay 78	1.7	1.2	7	Daley et al. 1981
Marine and estuarine				
South Davis Strait	0.296	0.322	74	Bunch 1979
Natal, S.A.	2.2	2.13	25	Schleyer 1981
San Pedro Channel 79	5.5	1.6	6	Krempin et al. 1981
San Pedro Channel 78	1.5	1.1	3	Krempin et al. 1981
S.E. Baltic Sea	1.9	2.75	1	Meyer-Reil et al. 1979
Fraser Plume to Georgia Straight				Valdés & Albright 1981
S 0%.	1.9	0.59	1	Valdés & Albright 1981
7%.	1.6	1.43	1	Valdés & Albright 1981
15%.	1.4	0.78	1	Valdés & Albright 1981

Table 1 (Cont'd)

Site	Chla	AODC	N	Reference
19%	1.4	0.77	1	Valdés & Albright 1981
25%	6.5	1.33	1	Valdés & Albright 1981
Nearshore South Africa				Field et al. 1980
Stn A	1.00	0.61	23	Field et al. 1980
B	1.46	0.81	23	Field et al. 1980
C	1.94	1.11	23	Field et al. 1980
D	2.07	1.6	23	Field et al. 1980
Coastal Finland	2.6	1.6	71	Vaatanen 1980
Japan	5.76	3.75	8	Kogare et al. 1980
S. California Bight	0.743	0.305	31	Fuhrman et al. 1980
S. California Bight	0.478	0.568	58	Fuhrman et al. 1980
McMurdo West 10, Antarctica	0.052	0.061	1	Hodson et al. 1981

Table 2. Data used in cell size - cell abundance regression. Volume per cell is measured in μm^3. Methods used were AO-acridine orange-epifluorescence and SEM-scanning electron microscopy. AODC refers to bacterial abundance (millions of cells $\cdot mL^{-1}$).

FW source	Volume $cell^{-1}$	Method	AODC	Reference
Mirror Lake	0.088	AO	1.63	Jordan & Likens 1980
Mendota	0.159	SEM	1.5	Pedros-Alio & Brock 1982
Bysjon	0.160	AO	6	Coveney et al. 1977
Plon Lakes				Krambeck et al. 1981
	0.019	SEM	31	Krambeck et al. 1981
	0.015	SEM	26	Krambeck et al. 1981
	0.022	SEM	26	Krambeck et al. 1981
	0.017	SEM	24	Krambeck et al. 1981
	0.018	SEM	29	Krambeck et al. 1981
	0.022	SEM	28	Krambeck et al. 1981
	0.018	SEM	38	Krambeck et al. 1981
	0.015	SEM	26	Krambeck et al. 1981
	0.015	SEM	34	Krambeck et al. 1981
	0.015	SEM	43	Krambeck et al. 1981
Paajarvi	0.126	AO	1.38	Salonen 1977

Table 2 (Cont'd)

FW source	Volume · cell^{-1}	Method	AODC	Reference
Norrviken	0.179	AO	1.55	Bell et al. 1983
Ice House Pond	0.063	AO	2	Kirchman et al. 1982
Gardsjon	0.342	AO	1.31	ANdersson 1983
Botjarn	0.203	AO	3.1	Johannson 1983
Vitalampa	0.186	AO	3.7	Johannson 1983
Fraser Estuary	0.080	SEM	0.59	Valdes & Albright 1981
	0.072	SEM	1.4	Valdes & Albright 1981
	0.076	SEM	0.78	Valdes & Albright 1981
	0.071	SEM	0.77	Valdes & Albright 1981
	0.061	SEM	1.33	Valdes & Albright 1981
Kiel area	0.06	SEM	1.9	Zimmermann 1977
Upwelling, S. Africa	0.597	AO	0.83	Linley & Field 1982
Reef, S. Africa	0.0516	AO or SEM	2.02	Schleyer 1981
Sargasso Sea	0.279	SEM	1.15	Watson et al. 1977
California Bight	0.046	SEM	0.44	Fuhrman and Azam 1980
	0.132	AO	1.6	
CEE	0.322	AO	0.66	
Antarctic	0.12	AO	2.5	

Table 2 (Cont'd)

FW source	Volume cell^{-1}	Method	AODC	Reference
	0.072	SEM	0.62	
	0.075	SEM	0.30	
	0.058	SEM	0.96	
Scripps Pier	0.038	SEM	2.9	
Offshore Georgia	0.12	AO	9.56	Newell & Christian 1981
Duplin	0.12	AO	16.1	Newell & Christian 1981
Skidaway	0.08	AO	16.7	Newell & Christian 1981
Scripps Pier	0.14	AO	3.26	Fuhrman 1981
	0.036	SEM	3.26	Fuhrman 1981
California Bight	0.081	AO	0.576	Fuhrman 1981
	0.058	SEM	0.576	Fuhrman 1981
Baltic	0.06	SEM	0.5	Hagstrom et al. 1979
Newport estuary	0.047		3.86	Bowden 1977
York estuary	0.082	AO	6.3	Ducklow 1982
Stn. 1 kiel	0.067	AO	3.7	Meyer-Reil 1977
Stn. 5	0.071	AO	1.7	Meyer-Reil 1977

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Appendix 3

Data used to construct figures

Chapter 2, Figure 2a.

Incubation time (min)	bead content per cell
0.9	0.13
3.9	0.65
7.6	0.95
10.1	1.25
13.1	1.64
17.1	2.28

Chapter 2, Figure 2b.

Incubation time (min)	DPM	beads
0.5	226.5	1787
3.0	70	953
6.3	246	4438
6.3	246	3305
6.3	55	1078
9.7	91	1270
11.5	62	1265
16.7	927	13501
21.5	1444	25370
27.2	1384	22479
28.8	1820	29397
control 1	14403	315200
control 2	6189	118200

There were 365 bacteria per DPM.

Chapter 3, Figure 1.

Depth(m)	Temperature (°C)	Dinobryon (cell/ml)	Bacteria (cell/nl)
0	21.8	<1	2.2
1	21.5	<1	2.5
2	19.3	<1	3.0
3	12.1	176	2.0
4	8.7	184	0.6
5	7.0	10	2.0
6	6.1	<1	2.3
7	5.6		2.7
8	5.4		3.4

Chapter 3, Figure 2.

Depth (m)	Carbon uptake (pg/cell/day) [phagotrophic]
0	29.4
1	18.6
3	2.45 [8.6]
5	0

Chapter 3, Figure 3.

Lake	Temperature °C	Ingestion rate (cell/hour)
Memphremagog	0	3.3
Memphremagog	0	1.2
Orford	20.6	37
Bowker	21.5	51.6
Magog	21.9	95.4
Croche	11	8.5
Orford	12.9	17.0
Memphremagog	8	17.6
Memphremagog	17.7	30
Cromwell	12.1	36.1
Bowker	23.9	10.8

(see also Figure 5 data)

Chapter 3, Figure 4.

Lake	Daytime clearance	Night clearance (nl/cell/h)
Memphremagog	8.64	8.89
Orford	7.72	7.72
Bowker	4.62	9.46
Croche	3.80	3.82
Magog	12.96	13.75

Chapter 3, Figure 5.

Depth (m)	Irradiance	Temperature	Clearance	Abundance
0	310	21.9		
1	201	21.85	9.5	72.5
2	120	21.35	8.6	250
3	75	19.75	7.9	145
4	45	18.2	7.4	330
5	30	16.8	7.4	165
6	18	12.6	4.8	40
7	10.5	9.7	2.7	300
8	5.7	7.65	1.6	1575
9	3.2	6.3	0.67	37
10	1.5	5.65	0.53	10

Chapter 4, Figure 1.

Time (h)	cells on filter	DPM ((100 cells)$^{-1}$)	used for
0	37126	0.025	uptake
0.283	48750	0.250	uptake
0.283	35329	0.216	uptake
0.9	34019	0.423	incorp
1.367	30774	0.638	incorp
2.033	18414	0.980	incorp
2.75	27260	1.260	incorp
3.317	14337	1.447	loss
3.483	23600	1.382	incorp
4.1	20219	1.421	loss
4.867	25890	2.006	incorp
5.0	16050	1.410	loss
7.317	28767	2.132	not used
7.483	12624	1.620	loss

Chapter 4, Figure 2.

Depth (m)	Light (uW/cm^2)	Temp °C	O_2 (ppm)	biomass (ppm)	chl a (ppb)
0	6300	18.7	9.1	0.5	
1	3200	18.7	9.5		
2	1900	18.7	9.7	0.53	2.5
3	1050	18.7	9.9		
4	550	18.6	10.1		
5	320	17.2	11.5	0.52	4.55
6	160	12.7	15.4	0.78	4.8
7	90	9.5	15.6	1.12	16.9
8	40	7.8	12.8	0.52	12.25
9	15	6.1	0.7	1.94	14.7
10	7	5.7	0.1	1.80	16.8

Chapter 4, Figure 3.

Depth (m)	Photosynthesis (ppb/d)	Phagotrophy (ppb/day)
0	35.5	
1	69.8	
2	61.0	
3	38.7	
4	23.0	
5	28.4	
6	19.0	0.85
7	12.0	44
8	7.6	7.8
9	7.5	
10	9.8	

Chapter 4, Figure 4

Depth (m)	Chrysomonads	Bacteria	Microflagellates	Rotifers	Photopico
0		5.5	0.620	0.03	
1		5.4	0.643	0.05	
2		5.35	0.700	0.13	0.28
3		5.2	0.506	0.20	
4		5.15	0.540	0.22	0.23
5		5.64	0.737	0.25	0.42
6	330	4.65	0.965	0.44	0.59
7	9139	2.55	0.280	1.16	[illegible]
8	1977	5.02	0.389	1.80	0.51
9	100	10.16	6.202	3.28	1.43
10		9.97	0.751	0.32	1.56
11		13.43	0.427	0.35	1.65

Chapter 5, Figure 1.

Time (min)	Thymidine incorporation (pmol/l)
>0.2 um filters	
16.3	1.81
27.1	4.54
54.9	7.22
105.2	14.36
148.3	20.42
>0.4 um filters	
16.5	1.43
27.3	1.74
55.0	4.26
105.4	9.70
148.4	13.90
>1.0 um filters	
16.6	0.44
27.5	0.48
55.2	2.63
105.6	3.93
148.6	5.26

Chapter 5, Figure 2

Size interval:	0.2-0.4	0.4-1.0	>1
abundance	3.16	1.57	0.28
biovolume	5.93	9.46	3.88
incorporation	2.35	3.61	2.25
cell-specific	0.74	2.30	8.14
volume-specific	39.6	38.2	57.9
DNA-specific	0.72	1.61	4.46

Chapter 5, Figure 3.

Tritium disintegrations per minute

Cold thymidine added (nM):	0	2.83	19.22	30.04
Filter pore size (um)				
0.2	24777	12389	4853	4010
0.4	21071	9514	5119	3241
0.6	12112	6599	2946	2271
1.0	8218	5615	2506	1721
5.0	3702	2174	1234	867

Chapter 5, Figure 4.

Time (h)	Thd incorporation	Abundance	Cell volume
0	3.05	5.74	2.64
9	4.52	6.94	4.43
20	22.23	11.28	9.41

Appendix 4

Assaying tritium by liquid scintillation counting

Tritium is used in biological work primarily because it provides chemicals with very high specific activity. However, the relatively weak emissions produced by tritium decay make this isotope the most difficult to count of those commonly used in limnology. My procedures for tritium counting were based on Kobayashi and Harris (1978). In particular, the following account is taken from Notes 1, 3, 16, 17, 19, and 21.

Filter dissolution

Two things must be maximized for reliable counting of tritium: isotope recovery and counting efficiency. Recovery is most important, and requires that a homogeneous sample be prepared, to minimize self-absorption and maintain proper 3-D scintillation detection. This is achieved by dissolving the filters and hydrolyzing the labelled organic molecules.

i. Nuclepore filters

Polycarbonate filters can be dissolved in 1 ml Protosol (NEN). Be sure to use a vial with a plastic-lined lid. Heat the vial at 55 °C in a water bath for 10 minutes (macromolecules) or overnight (large-bodied crustaceans). Cool to room temperature and add 50 ul of glacial acetic acid. The acid counteracts the basicity of the Protosol to restore a low pH to the fluor, thereby discouraging chemiluminescence. Add 10-13 ml Econofluor, shake well and count. Monitor for chemiluminescence on machines that do not subtract these counts automatically. Sometimes the acidification step is not a sufficient preventative, and samples must be heated overnight at 55 °C to speed up the slow natural subsidence of chemiluminescent events.

ii. Millipore filters

Membrane filters cannot be dissolved with Protosol - the sample will turn dark yellow and be strongly quenched. The most accurate results are obtained by adding 0.5 ml of 0.5 N HCl to the filter in a 20 ml vial. Cap the vial (cap must be plastic), and place it in a boiling water bath for 15-20 minutes (do not submerse cap!). Cool and add 1 ml ethyl acetate, which will dissolve the filter in 5-15 minutes. Add 10 ml Aquasol (Universol) and shake vigorously before counting.

A faster alternative which yields only slightly lower recoveries is to add 1 ml of 2-methoxy- or 2-ethoxy-ethanol-1 to the filter in a 20 ml vial. The filter dissolves in 15 minutes. Add 10 ml Aquasol and count.

Quenching must be determined for each sample. This is most easily and accurately done using the the external standard channels ratio. This is in turn calibrated using a series of variably quenched tritiated water standards. Be sure to use a relationship developed with the vial-sample-fluor combination that was employed for the experimental samples.

References

Kobayashi, Y. and W.G. Harris. 1978. LSC applications notes #1-30. New England Nuclear.