PREFACE

In keeping with the newly accepted regulations for thesis style which have been authorized by the Graduate Training Committee of the Department of Biology at McGill University, the main body of this thesis has been written in a form suitable for publication. Use is made of the style recommended by the Style Manual for Biological Journals and, with minor changes, the manuscript will be submitted to a suitable journal.

BACTERIAL DYNAMICS IN TWO HIGH ARCTIC LAKES

by

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ABSTRACT

The heterotrophic planktonic bacteria in two high arctic lakes were studied by direct microscope count and the enzymatic uptake of ^{14}C labelled glucose and acetate. Bacterial uptake of glucose generally conformed to Michaelis-Menten kinetics, but uptake of acetate was irregular and uninterpretable. Bacterial numbers and activity in oligotrophic Char Lake ranged from .1 to 2 x 10^{8} bacteria/liter and a maximum uptake velocity (Vmax) of 1 to 8 x 10^{-3} µg glucose $l^{-1}hr^{-1}$. Nearby Meretta Lake received waste water from the Department of Transport Base at Resolute and this eutrophication was reflected in higher bacterial numbers of 5 to 80 x 10^{8}/liter and Vmax of 1 x 10^{-2} to 7.5 x 10^{-1} µg glucose $l^{-1}hr^{-1}$. The regression equation showed that 10^{8} bacteria in Char Lake had a Vmax of 3.4 x 10^{-3} µg glucose $l^{-1}hr^{-1}$. Bacterial cycles could not be related to phytoplankton cycles in either lake. Uptake at only 0.25 µg/liter of added glucose was demonstrated and a very low K_t was suggested. Comparison of kinetic data from several lakes suggests a relationship between the bacterial uptake rate of glucose and phytoplankton production.

INTRODUCTION

Although the bacteria of lakes and oceans have been studied since the turn of the century and their importance in the general turnover and transport of materials well established, the ecology of natural populations has until recently received little attention (see Henrici, 1937; Taylor, 1940; Kuznetsov, 1959).

Aquatic bacteria were first enumerated by cultural methods, but it was soon realized by some workers (Cholodny, in Kuznetsov and Romanenko, 1964; Henrici, 1937) that such techniques did not quantify the 'true' water bacteria. Russian workers (Cholodny, Kuznetsov and Karsinkin, and Razumov, in Kuznetsov and Romanenko, 1964) proposed a total bacterial count, which involved the direct microscope examination of stained slide or filtered material. However, a direct microscope count of the filtered organisms, or for that matter, the isolation of bacteria on organically enriched media, do not provide satisfactory estimates of the heterotrophic potential of the natural aquatic bacteria (Vaccaro and Jannasch, 1966).

The fairly recent availability of radioactively labelled compounds now makes it possible to measure the bacterial turnover of organic substrates in the aquatic habitat. Parsons and Strickland (1962), using this technique with marine plankton, found that the velocity of uptake of ^{14}C labelled organics rapidly approached a maximum uptake velocity with increasing substrate concentration,

and that such uptake could be analyzed by Michaelis-Menten enzyme kinetics. Subsequently, Wright and Hobbie (1965a) reported two distinct uptake mechanisms operating simultaneously in natural populations. Uptake at substrate concentrations less than 500 µg/liter glucose or acetate followed Michaelis-Menten enzyme kinetics and was attributed to the bacteria. Uptake at higher concentrations followed simple diffusion kinetics and was found to be associated with algae. They suggested that bacterial uptake keeps substrate concentrations low and thus effectively eliminates heterotrophy by most algae in nature (Wright and Hobbie, 1965b).

The purpose of the present study was to determine the dynamics of the open water bacteria in two high arctic lakes both by direct microscope count and the uptake of labelled organic substrates, and to investigate a possible relationship between the bacterial and phytoplankton cycles. The work was done from January to December, 1970 at Char and Meretta Lakes (74°42'N, 94°57'W) on Cornwallis Island, N.W.T., Canada. The study was part of Char Lake Project, a Canadian contribution to the International Biological Program.

Char Lake (area 52.6 ha, maximum depth 27.5 m, mean depth 10.2 m) lies in a dolomitic limestone basin. Physicochemical characteristics such as high light transmission, low concentrations of most plant nutrients, high dissolved oxygen levels, and extremely low rates of phytoplankton production characterize the lake as very oligotrophic. The lake, with a maximum ice cover of about 2.3 m, is usually ice free only from mid-August to mid-September and has a maximum temperature of less than 5 C (Rigler, 1971). Nearby Meretta Lake (area 26.2 ha,

maximum depth 12 m, mean depth 3 m) lies below the Department of Transport Base at Resolute, receives sewage and other waste water inflow from the base and has some eutrophic features.

METHODS

Water samples were taken with a PVC van Dorn bottle. Sampling stations were established at 2, 7.5, 15, and 25 m and at 2, 5 and 7.5 m in Char and Meretta Lakes, respectively. Glucose uptake rates were determined at weekly intervals from early June to mid-September, 1970 and biweekly for the remainder of the year, while bacterial numbers and acetate uptake were investigated only during the June-September period. All samples were processed within one hour after collection.

Bacterial numbers were determined by the direct count method of Razumov (Kuznetsov and Romanenko, 1964). Ten milliliters of water from Char Lake and one milliliter of water from Meretta Lake were filtered under vacuum through Ø,450nm membrane filters. The filters were placed on filter paper soaked with erythrosin B stain and stained for three to four hours. The filters were then partially destained on filter paper soaked with sterile filtered water before being dried, mounted in immersion oil and placed under a cover slip. Twenty fields per filter, in a cross from edge to edge, were counted at 1250 X with a light microscope. The amount of clumping, interference by detrital particles, and the confusion of detritus with cocciform bacteria determine the accuracy of a direct microscope count (Jannasch and Jones, 1959). None of these seriously affected the enumeration

in the two lakes, but poor colour contrast of bacteria against filter background likely resulted in spuriously low counts of some filters (Appendix 1).

Uptake of labelled organic substrates was determined following Hobbie and Wright (1968). From the original Michaelis-Menten equation of Parsons and Strickland (1962) for uptake at a given substrate concentration, Wright and Hobbie (1965a) derived the equation

$$C\mu t / c = \frac{(K_t + S_N)}{V} + \frac{A}{V}$$

which describes the uptake kinetics of a natural population when the natural substrate concentration is unknown. C = cpm from 1 μCi of ^{14}C; c = cpm of the filtered organisms; μ = number of microcuries added to the sample; t = length of incubation time, in hours; S_N = the natural substrate concentration, in μg/liter; A = the added substrate concentration in μg/liter; K_t (a constant similar to the original Michaelis-Menten constant) is the substrate concentration in μg/liter when the uptake velocity is half the maximum uptake velocity; and V = the maximum uptake velocity, in μg liter^{-1}hr^{-1}.

Radioactive glucose and acetate of high specific activity were used (Glucose-U-^{14}C 1.7 x 10^3μCi/mg, Amersham/Searle; Sodium acetate-U-^{14}C, 667 μCi/mg, Amersham/Searle). Fifty millimeters of lake water were added to a series of five or six acid-washed 125 ml reagent bottles. Labelled substrate was then added by micro-syringe to give a low concentration range of 0.25 or 0.50 to 2.0 or 3.0 μg/liter of

glucose, and 1.0 to 6.0 or 8.0 µg/liter of sodium acetate. One control per series was fixed with lugol iodine immediately after the addition of the isotope. The Char and Meretta samples were incubated in the dark between 1 and 2 C for 6 to 8 hours and 2 to 3 hours respectively, after the addition of glucose, and for 18 to 24 hours and 2 to 3 hours respectively, after the addition of acetate. Uptake was ended by adding lugol iodine. The samples were filtered under vacuum through Ø,450nm membrane filters, the filters dried, glued to planchets and their activity determined with a Geiger-Müller counter. Control counts were subtracted from experimental counts, and the data were plotted by eye and the method of least squares in the form of a modified Lineweaver-Burk plot as S/v (Cµt/c) against S (A). The inverse of the slope gives Vmax, the y-intercept S/v or T, the turnover time of the substrate by the natural population, and the x-intercept ($K_t + S_N$), a rough estimate of the maximum natural substrate concentration. For derivation of equations and fuller explanation of terms see Wright and Hobbie (1966). Hobbie and Wright believe Vmax to be a measure of the heterotrophic potential or number of bacteria in a lake, and T a measure of the physiological activity of the bacterial population.

RESULTS

The planktonic bacteria of Char and Meretta Lakes are predominantly single-celled 1 - 2 x ,5^{00}nm rods. Larger rods up to 5^{000}nm with some chains to 50^{000}nm were occasionally encountered in Meretta Lake.

Bacterial numbers in Char Lake, which varied between 0.1 and 2 x 10^8/liter, showed no vertical stratification except for high counts at the deep stations in early June. There was a trend towards increasing numbers at all depths during the summer, with minima in June and maxima in August and September (Table 1), (Appendix 2). Bacterial populations in Meretta Lake were 10 to 100 times greater than in Char Lake. Except for a sharp peak in numbers during the spring melt (June 29-July 25) when the count reached as high as 80 x 10^8/liter, the population was between 0.5 and 2 x 10^9/liter (Table 2), (Appendix 3).

The uptake pattern of glucose in Char and Meretta Lakes generally conformed to Michaelis-Menten kinetics, with substrate saturation evident at 3 µg/liter of added glucose. With only five or six data points, a fit by least squares loses significance with only a single point appreciably off the line, and thus only 14% of the plots were significant at $P < .1$. However, the pattern of uptake was sufficiently clear in 68% of the plots to allow an eyefit, and all kinetic data for glucose are from eyefitted plots, (Appendix 4). As variation in the slope of the line is exaggerated at the y-intercept, T is often difficult to determine accurately (Riggs, 1963), and therefore small changes in T in Char and Meretta Lakes need not be real. By the nature of the plot there is inevitable correlation between ($K_t + S_N$) and Vmax (Riggs, 1963). Thus changes in ($K_t + S_N$) may be largely generated by the cycle of Vmax, and therefore cannot be interpreted as an independent cycle.

In Char Lake maximum glucose uptake velocities ranged between 1 and 8 x 10^{-3} µg $l^{-1}hr^{-1}$ (Figure 1), (Appendix 5). Glucose uptake at

Table 1. Monthly range and mean of bacterial numbers (10^7/liter) per depth in Char Lake, summer 1970.

	Bacterial Numbers (10^7/liter)			
Depth (m)	June	July	August	September
2	2.4-6.3 $\bar{x}$ 4.3 (N6)	5.7-14.6 $\bar{x}$ 8.6 (N7)	8.6-18.0 $\bar{x}$ 14.0 (N7)	15.4-22.7 $\bar{x}$ 19.6 (N3)
7.5	1.8-6.8 $\bar{x}$ 4.0 (N5)	5.2-11.3 $\bar{x}$ 7.4 (N4)	8.0-14.1 $\bar{x}$ 10.3 (N3)	16.2-18.5 $\bar{x}$ 17.3 (N2)
15	1.0-8.7 $\bar{x}$ 5.6 (N6)	5.3-9.0 $\bar{x}$ 7.6 (N6)	8.4-12.7 $\bar{x}$ 10.9 (N5)	13.2-14.6 $\bar{x}$ 13.9 (N2)
25	4.0-12.9 $\bar{x}$ 9.1 (N5)	6.9-10.0 $\bar{x}$ 9.0 (N3)	10.8-15.2 $\bar{x}$ 13.0 (N2)	14.4-16.2 $\bar{x}$ 15.3 (N2)

Table 2. Range and mean of bacterial numbers (10^8/liter) per depth in the premelt, melt and postmelt periods in Meretta Lake, summer 1970.

Depth (m)	Bacterial Number (10^8/liter) Premelt (June 6-June 13)	Melt (June 29-July 25)	Postmelt (Aug. 1-Sept. 18)
2	2.9-3.6	10.2-82.7	6.2-11.2
	$\bar{x}$ 3.3 (N2)	$\bar{x}$ 38.5 (N5)	$\bar{x}$ 8.9 (N5)
5	2.7-4.6	8.5-73.7	6.9-73.7
	$\bar{x}$ 3.7 (N2)	$\bar{x}$ 29.2 (N5)	$\bar{x}$ 10.5 (N5)
7.5	2.1-4.4	15.0-79.0	6.8-10.1
	$\bar{x}$ 3.3 (N2)	$\bar{x}$ 36.4 (N5)	$\bar{x}$ 8.5 (N5)

the 25M station was usually higher than at the other depths. The Vmax at 2 and 7.5 m was relatively high from late February to early April, minimal in May and June, and increased to maximum values in September and October. The Vmax at 15 and 25 m did not peak early in the year, was relatively high during the early spring decline at 2 and 7.5 m, but was also maximal from September to early November (Figure 1).

In Meretta Lake maximum uptake rates of glucose were 10 to 100 times greater than in Char Lake, ranging from 1×10^{-2} to 7.5×10^{-1} $\mu g\ l^{-1} hr^{-1}$. Maximum uptake rates in the water column were vertically uniform, such that the average Vmax of the three depths is representative of events at any one depth (Figure 2), (Appendix 6). Vmax was low prior to early June, peaked sharply in the spring melt from mid-June to late July, declined in August, recovered in September, and returned to low winter values in October.

The bacterial turnover time of glucose in Char Lake ranged from 43 to 1700 hours and was inversely related to Vmax (Appendix 5). Turnover times ranged between 200 and 600 hours at all depths from February to April, were greater than 1000 hours during the June bacterial minimum and less than 100 hours in the deep stations during the September-October peak. The glucose turnover time in Meretta Lake was considerably faster than in Char Lake, and was also inversely related to Vmax (Figure 2), (Appendix 6). The Meretta turnover time at all depths was 50 to over 100 hours from February to June, as low as 5 to 10 hours in the bacterial peak in July and more than 50 hours after October. No seasonal or vertical pattern in the maximum natural

Figure 1. The maximum velocity of uptake of glucose versus time of year and depth in Char Lake. The units of Vmax are 10^{-3} μg glucose $liter^{-1}$ hr^{-1}.

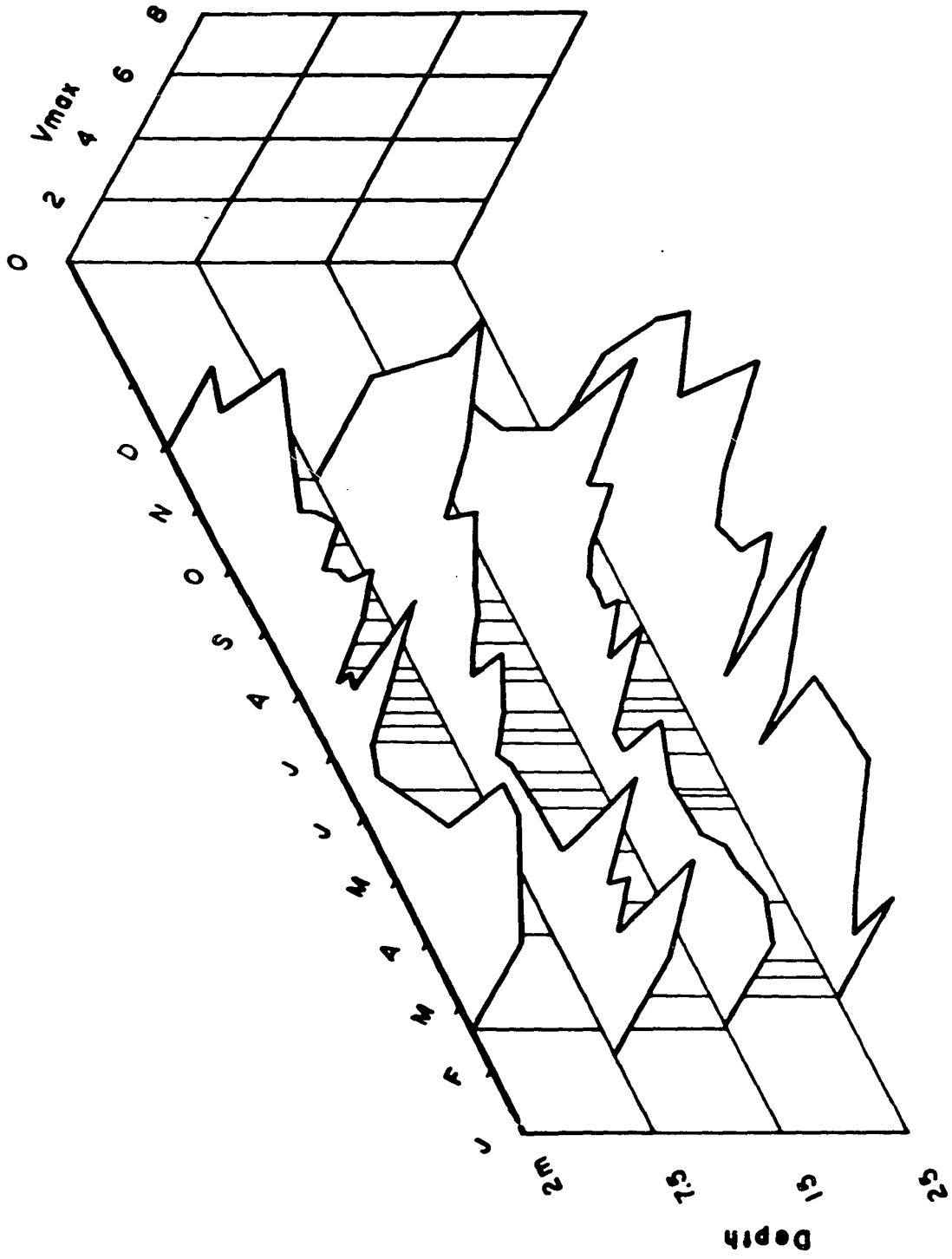
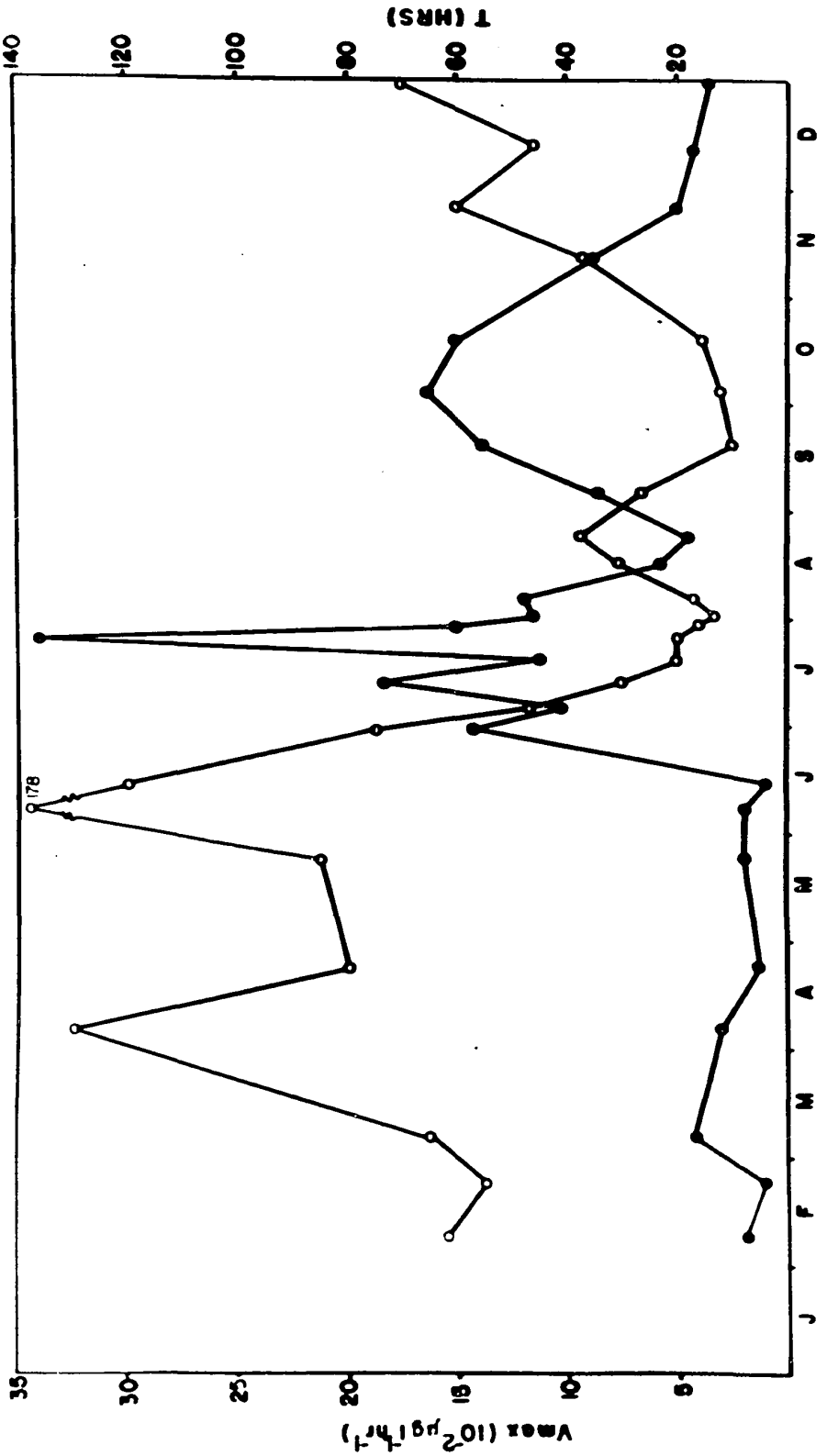

Vmax
0
2
4
6
8
D
N
O
S
A
J
J
M
A
M
F
J
2m
7.5
5
2.5
Depth

Figure 2. Average maximum velocity of uptake, Vmax (—●—) and turnover time, T (—o—) of glucose in Meretta Lake, 1970.


T (HRS)
140
120
100
80
60
40
20
178
J
F
M
A
M
J
J
A
S
O
N
D
35
30
25
20
15
10
5
Vmax (10⁻²µg l⁻¹hr⁻¹)

glucose concentration ($K_t + S_N$) could be discerned in either lake, but estimated Char Lake values ranged from less than 0.5 to approximately 5.0 µg/liter, and in Meretta Lake from less than 0.5 to approximately 25 µg/liter (Appendices 5 and 6).

Bacterial uptake of acetate was not interpretable by Michaelis-Menten kinetics in either lake (Appendices 7, 8 and 9). Uptake was irregular or approached a diffusion (0° order) uptake pattern and Vmax, T and ($K_t + S_N$) values could not be calculated. In the absence of suitable data the velocity of uptake of acetate at 4 µg/liter of added substrate was made to serve as a relative measure of acetate activity in the two lakes. Acetate uptake in Char Lake ranged from 0.4 to 6.0 x 10^{-3} µg $l^{-1}hr^{-1}$, while uptake of 0.58 to 30 x 10^{-2} µg $l^{-1}hr^{-1}$ in Meretta Lake was, as for glucose, 10 to 100 times greater than in Char Lake. In both lakes the pattern of acetate uptake between May and September approximated that for glucose (Appendices 10 and 11).

DISCUSSION

Total bacterial counts from a number of east European lakes, which the authors considered oligotrophic, were generally less than 5 x 10^8 bacteria/liter, while the number of bacteria in eutrophic lakes was greater than 2 x 10^9/liter (Straškraba and Straškrabová, 1969). The population in Char Lake of 0.1 to 2 x 10^8 bacteria per liter would, according to the above scheme, denote that lake as extremely oligotrophic, with one of the lowest total counts reported

from a freshwater system, but similar to some marine populations (Jannasch and Jones, 1959). Bacterial numbers of 0.2 to 8 x 10^9/ liter, on the same basis, would alone characterize Meretta Lake as meso- to eutrophic, and this larger bacterial population is consistent with primary production work on the two lakes showing annual production to be also about ten times higher in Meretta than in Char Lake (Kalff, et al., in prep.). Thus, the number of planktonic bacteria appear to be a direct function of the degree of trophy of lakes.

Bacteria commonly possess permease systems (Cohen and Monod, 1957; Kepes, 1963) which are inducible and which allow the active transport of a number of organic compounds. Such compounds as dissolved sugars, amino acids and fatty acids have been found in 10^{-8} to 10^{-7} M concentrations in nature (Shapiro, 1957; Degens, et al., 1964; Vallentyne and Whittaker, 1966; Siegel and Degens, 1966) and a measure of their in situ uptake by bacteria may provide a good index of the heterotrophic potential of natural waters (Vaccaro and Jannasch, 1966).

Bacterial uptake of glucose and acetate has been measured by enzyme kinetics in aquatic environments of widely different trophic nature (Wright and Hobbie, 1965a; Vaccaro and Jannasch, 1966; 1967; Hobbie and Wright, 1968; Vaccaro, et al., 1968; Allen, 1969; Wetzel, 1969; Hamilton and Preslan, 1970). The kinetic data appear to be related to the trophy of the habitat as indicated by phytoplankton primary production (Figure 3) and suggests the possible utility of this approach in assessing the trophic status of lakes. Thus the

Figure 3. Maximum uptake velocity of glucose versus primary production in several lakes. Turnover time (hrs) in brackets.

1. Char Lake (Kalff et al., in prep.)
2. Lapland lakes (Hobbie and Wright, 1968; Rodhe, 1969)
3. Torne Träsk (Rodhe et al., 1966; Rodhe, 1958)
4. Nedre Laksjön (Rodhe et al., 1966; Rodhe, 1958)
5. Meretta Lake (Kalff et al., in prep.)
6. Crooked Lake (Wetzel, 1968; 1966)
7. Lake Erken (Hobbie and Wright, 1968; Rodhe, 1969)
8. Little Crooked Lake (Wetzel, 1968; 1966)
9. Lake Lötsjön (Allen, 1969; Rodhe, 1969).

maximum uptake rates in Char and Meretta Lakes appear to categorize these lakes as oligotrophic and mesotrophic, respectively.

The glucose turnover time similarily appears to be related to the trophy of the system (Figure 3). As stressed by Hobbie (1967) a measure of the turnover of organic compounds is much more important than the absolute concentration for a study in aquatic ecology. Turnover rates of 500 hours or more, occurring commonly in Char Lake, indicate virtually no bacterial activity, but a T of 10 hours or less (Hobbie, 1967), reached in Meretta Lake during the summer, indicates that a considerable amount of substrate is being transformed.

Hobbie (1967) and Allen (1969) reported estimations of natural substrate concentrations ($K_t + S_N$) to be less than 6 and 10 µg/liter in Lakes Erken and Lötsjön respectively, and to vary little throughout the year. The absence of a yearly cycle suggested to them that the processes of supply and removal were in balance. Recent support for this view was provided by Wright (1970) who reported that the Vmax for glycollate and the rate of production of excreted organic carbon in a pond were within the same order of magnitude. The low ($K_t + S_N$) in Char Lake of less than 3 µg/liter is in accord with the low nutrient content of the lake (Rigler, 1971) yet, except at time of maximum glucose uptake, ($K_t + S_N$) in Meretta Lake is no greater. Thus ($K_t + S_N$) does not appear to be closely related to trophic status.

Since the natural substrate concentration (S_N) is unknown, the actual velocity of uptake cannot be determined and the rate must be measured over a series of concentrations to give a maximum velocity of uptake. The Vmax thus obtained is an estimate of the enzymatic uptake

by the heterotrophic community, but gives no information on the uptake constants of individual species. As Michaelis-Menten kinetics were derived from uniform single reactions systems, it is unknown whether it is reasonable to expect a metabolically heterogeneous population to exhibit a similar enzymatic response to added organic substrates. Nevertheless, natural bacterial populations usually exhibit linear uptake, therefore either the difference between uptake constants is too small to be resolved or the uptake response is that of a single dominant species (Vaccaro and Jannasch, 1967). Given that the uptake constants measured are a composite of many constants, their validity is still dependent on a number of assumptions. In the first place there must be no appreciable reproduction or death of bacteria in the bottles, no significant removal of substrate, and constant uptake over time. These conditions are approximated if the incubation time is kept short, preferably less than 5% of the turnover time (Wright and Hobbie, 1965a, b; Allen, 1969). The second assumption is that there is no immediate respiration of the newly acquired substrates. Recent research, however, has shown that the amount of radioactive carbon retained in the cells is an inaccurate measure of the carbon assimilated, as a significant percentage of the original substrate added is respired as $^{14}CO_2$ during the course of the experiment (Hamilton and Austin, 1967; Hobbie and Crawford, 1969; Williams, 1970; Williams and Gray, 1970). Bacterial respiration of glucose was determined after Hobbie and Crawford (1969) three times in the present study and indicated that 38 to 71% ($\bar{x}$ = 51%) and 8 to 50% ($\bar{x}$ = 22%) of

the glucose assimilated was respired in 8 hours in Char Lake and 2 hours in Meretta Lake, respectively, indicating that the presently reported Vmax values are underestimated and T overestimated (Appendix 12). The third assumption is that there is no appreciable uptake of the substrate by the phytoplankton. Although Wright and Hobbie (1965b) suggested that algae were responsible for less than 10% of the uptake at the low substrate concentrations commonly found in lakes, more recent work (Shrift, 1966; North and Stephens, 1967; Hellebust, 1970) has shown that some phytoplankton possess permease systems similar to those of bacteria and are capable of active transport of dissolved organic matter present in very low concentrations. It is unknown to what extent the uptake measured in Char and Meretta Lakes is attributable to the phytoplankton.

Vaccaro (1969) was able to analyze the uptake of certain organic substrates in the Atlantic and Pacific Oceans by enzyme kinetics only after preconditioning the bacteria with a small amount of substrate. He attributed this response to both a population increase and a reduction in the number of species and hence a more uniform metabolism, or to the induction of a temporarily inactive uptake system. Similar preconditioning experiments after Vaccaro in the present study, in which water samples were enriched by 5 and 500 µg/liter of unlabelled sodium acetate for up to 48 hours, still failed to yield populations whose acetate uptake could be kinetically analyzed (Appendix 13). In other experiments uptake of both glucose and acetate was measured after only two hours at substrate concentrations of 0.25 and 1.0 µg/liter,

respectively. It thus appears as if at least a fraction of the populations in the two lakes can immediately respond to a very small increase in their nutrient supply without the necessity of prior enzyme induction.

In many lakes periods of relatively high phytoplankton biomass are followed by a rise in the heterotrophic bacterial population utilizing the organic matter produced by the algae (Henrici, 1937; Kuznetsov, 1959; Overbeck, 1967; 1968; Schmidt, 1969). Other workers have noted a positive relationship between Vmax and phytoplankton abundance (Hobbie and Wright, 1968; Wright, 1970; Seki and Hardon, 1970; Hamilton and Preslan, 1970).

In Char Lake where the phytoplankton biomass was both extremely low (less than 200 x 10^6 μ^3/liter) and varied only about threefold over the year (Kalff et al., in prep.) the existence of such relationships could not be ascertained. In more eutrophic Meretta Lake, such a correlation was not evident during the summer either, likely because bacterial numbers and activity were substantially influenced by the organic matter inflow from the Department of Transport Base. The single large bacterial bloom coincided with rather than followed a large burst of phytoplankton activity, suggesting that both groups of organisms derived much of their nutrition from the large slug of sewage which flowed into the lake at this time. The absence of any marked increase in bacterial numbers in Char Lake after the onset of the spring melt-off and the subsequent inflow of water from the watershed indicates little terrestrial contamination (Fred et al., 1924; Taylor, 1940; Boyd and Boyd, 1963). Highest bacterial numbers and Vmax values

(Table 1; Figure 1) were found after a large moat had developed around the lake and during the open water period, suggesting that the bacteria might be responding to the return of nutrients from the mud-water interface as a result of increased water circulation (Ruttner, 1963) or that this circulation swept benthic bacteria into a planktonic environment.

Although the number of heterotrophic bacteria on nutrient agar has been related to glucose uptake (Allen, 1969; Gorden, 1969; Seki and Hardon, 1970; Seki and Kennedy, 1970), total bacterial numbers and Vmax appear not to have been related in any previous study. In the present study a significant correlation ($P < .01$) was found between total bacterial numbers and Vmax in Char Lake and from the regression equation (Figure 4) 10^8 bacteria in Char Lake had a Vmax of 3.4×10^{-3} $\mu g\ l^{-1} hr^{-1}$. This correlation shows that despite large scale changes in the physical environment ranging from a solid ice cover, through a period of inflow to a period of open water, the average physiological activity per bacterium did not change. Bacterial numbers were not determined during the winter, but the estimation of such numbers from the winter Vmax values suggest there was no appreciable decline during the polar night. The absence of an interpretable relationship between bacterial numbers and activity in Meretta Lake suggests much variation in the activity per cell between periods of high and low inflow of organic matter. Such variation may result from an irregular wash-in of dead and resting bacteria from the inflow stream or from changes in the species composition and metabolic character of the bacterial population.

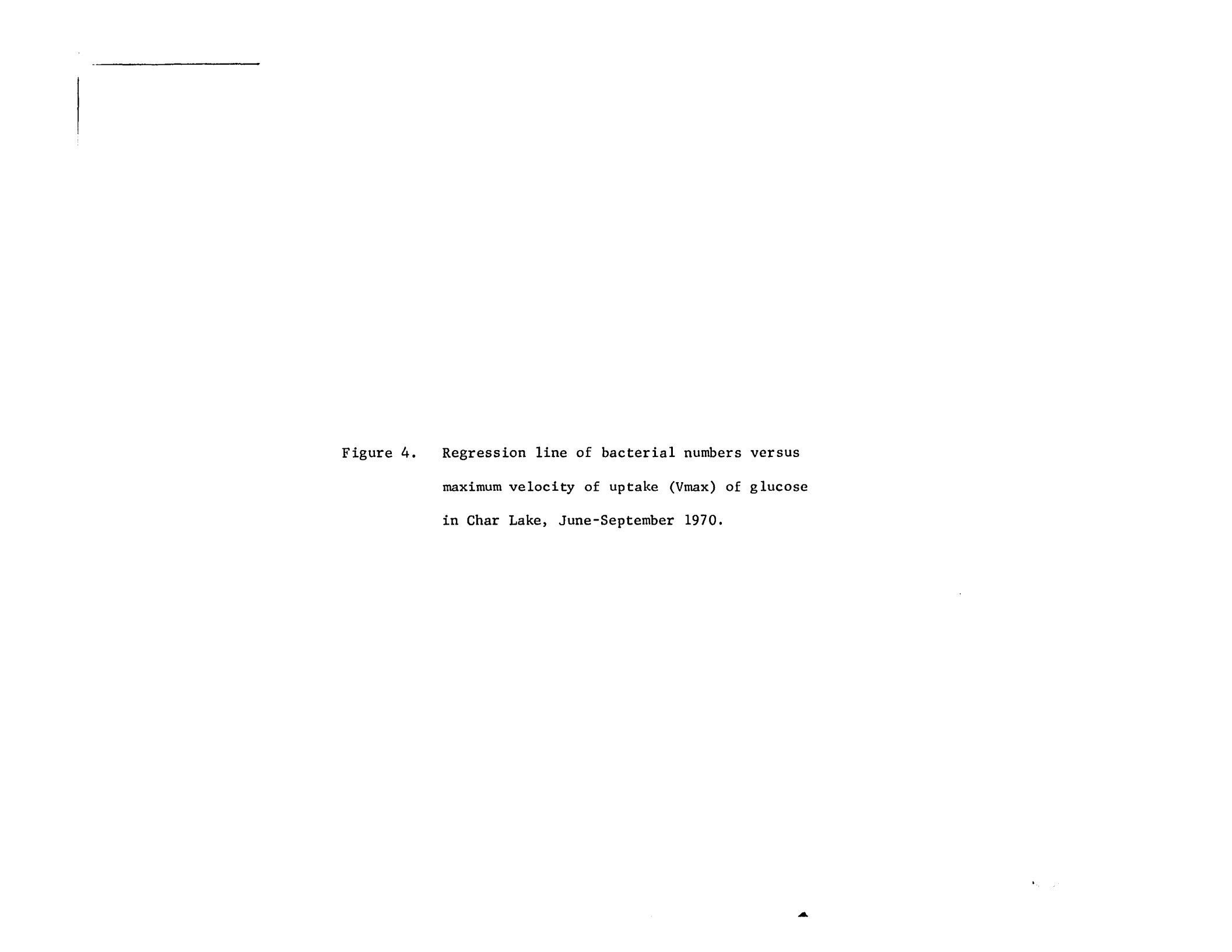
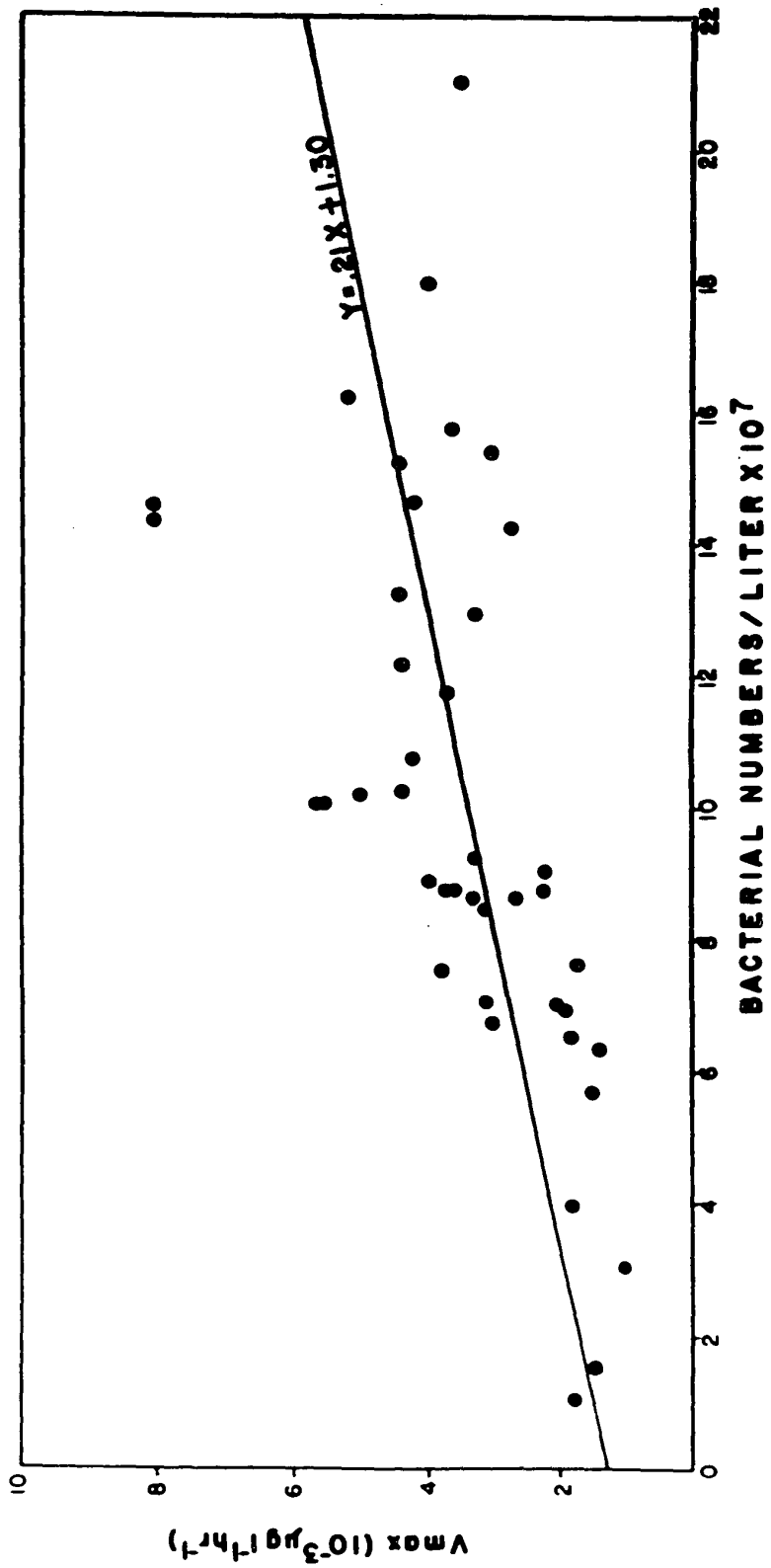


Figure 4. Regression line of bacterial numbers versus maximum velocity of uptake (Vmax) of glucose in Char Lake, June-September 1970.


Y=.21X+1.30
BACTERIAL NUMBERS/LITER X10^7
Vmax (10^-3 µg l^-1 hr^-1)
0
2
4
6
8
10
12
14
16
18
20
22

The Michaelis-Menten constant (K_t) of naturally occurring bacteria has been determined in chemostats (Jannasch, 1967, 1968; Hamilton, et al., 1966) and by bioassay (Hobbie and Wright, 1965; Vaccaro and Jannasch, 1966) and lies in the order of 10^{-6} to 10^{-9} M. Such K_t's have been used as a measure of an organism's affinity to a particular substrate, such that a low K_t value expresses adaptation to low substrate concentrations (Jannasch, 1967, 1968). A crude estimate of the K_t's of the bacterial populations in Char and Meretta Lakes can be obtained from ($K_t + S_N$) data, if S_N is taken as zero when ($K_t + S_N$) is minimal, and yields an approximate K_t of 0.4 µg/liter (5.5×10^{-9} M) in both lakes. By using glucose of high specific activity, uptake was measured at only 0.25 µg/liter (3.3×10^{-9} M) and this, in conjunction with the estimated K_t, indicates that these arctic bacterial populations are able to utilize extremely low substrate concentrations.

Although work by Hamilton et al. (1966) shows that some bacteria are more adept than others at glucose uptake at low temperatures, and Morita and Burton (1970) reported greater glycine uptake at 2.5 C than at 10 C in Alaskan waters, low temperatures do, in general, reduce growth by bacterial communities (Lamanna and Malette, 1965). Within low temperature environments, those with a higher nutrient regime, such as Meretta Lake during the summer, permit more bacterial growth and faster turnover than in low nutrient environments such as Char Lake. Yet it would seem reasonable that turnover rates more closely approximating those found in polluted Lötsjön (Allen, 1969) might well

be encountered in Meretta Lake under a warmer temperature regime.

If the dynamics of the bacteria in unpolluted Char Lake are typical of other polar lakes, then such lakes would be characterized by low bacterial numbers, small numerical fluctuations during the year and very slow turnover times of the available organic matter.

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REFERENCES

Allen, H.L. 1969. Chemo-organotrophic utilization of dissolved organic compounds by planktic algae and bacteria in a pond. Int. Revue ges Hydrobiol. 54: 1-33.

Boyd, W.L. and J.W. Boyd. 1963. A bacteriological study of an arctic coastal lake. Ecology 44: 705-710.

Cohen, G.N. and J. Monod. 1957. Bacterial permeases. Bact. Rev. 21: 169-194.

Degends, E.T., J.H. Reuter and K.N.F. Shaw. 1964. Biochemical compounds in offshore California sediments and seawaters. Geochim. Cosmochim. Acta 27: 45-65.

Fred, E.B., F.C. Wilson and A. Davenport. 1924. Distribution and significance of bacteria in Lake Mendota. Ecology 5: 322-339.

Gorden, R.W. 1969. Ecology of heterotrophic aerobic bacteria of plaza lakes. (Abstr.) Aquatic Biology. Vol. 1, No. 10.

Hamilton, R.D. and K.E. Austin. 1967. Assay of relative heterotrophic potential in the sea: the use of specifically labelled glucose. Can. J. Microbiol. 13: 1165-1173.

----------, K.M. Morgan and J.D.H. Strickland. 1966. The glucose uptake kinetics of some marine bacteria. Can. J. Microbiol. 12: 995-1003.

----------, and J.E. Preslan. 1970. Observations on heterotrophic activity in the Eastern Tropical Pacific. Limnol. Oceanogr. 15: 395-401.

Hellebust, J.A. 1970. The uptake and utilization of organic substances by marine phytoplankters, p. 225-256. In: D.W. Wood (ed.) Symposium on organic matter in natural waters. University of Alaska. 625 pp.

---------- and R.R.L. Guillard. 1967. Uptake specificity for organic substances by the marine diatom Melosira nummuloides. J. Phycol. 3: 395-401.

Henrici, A.T. 1937. Studies of freshwater bacteria. IV. Seasonal fluctuations of lake bacteria in relation to plankton production. J. Bact. 35: 129-139.

Hobbie, J.E. 1967. Glucose and acetate in freshwater: concentration and turnover rates. In: H.L. Golterman and R.S. Clymo (eds.) Chemical environment in the aquatic habitat. North-Holland. 322 pp.

---------- and C.C. Crawford. 1969. Respiration corrections for bacterial uptake of dissolved organic compounds in natural waters. Limnol. Oceanogr. 14: 528-532.

---------- and R.T. Wright. 1965. Bioassay with bacteria uptake kinetics: glucose in freshwater. Limnol. Oceanogr. 10: 471-474.

---------- and ---------. 1968. A new method for the study of bacteria in lakes: description and results. Mitt. Intern. Verein. Limnol. 14: 64-71.

Jannasch, H.W. 1967. Growth of marine bacteria at limiting concentrations of organic carbon in seawater. Limnol. Oceanogr. 12: 264-271.

Jannasch, H.W. 1968. Growth characteristics of heterotrophic bacteria in sea water. J. Bacter. 95: 722-723.

---------- and G.E. Jones. 1959. Bacterial populations in sea water as determined by different methods of enumeration. Limnol. Oceanogr. 4: 128-139.

Kepes, A. 1963. Permeases: identification and mechanism, p. 38-48. In: N.E. Gibbons (ed.) Recent progress in microbiology. 8th Intern. Congr. for Micro., Montreal. 1962. University of Toronto Press.

Kuznetsov, S.I. 1959. Die Rolle der Mikroorganismen in Stroffkreislauf der seen. Deutscher Verlag der Wissenschaften. Berlin. 301 pp.

---------- and V.I. Romanenko. 1964. Microbiological investigation of inland reservoirs. U.S. Dept. of Comm. Joint Publication Research. 22, 802. Washington, D.C. 169 pp.

Lamanna, C. and M.F. Mallette. 1965. Basic Bacteriology: Its biological and chemical background. 3rd ed. Williams and Wilkins. 1001 pp.

Morita, R.Y. and S.D. Burton. 1970. Occurrence, possible significance and metabolism of obligate psychrophiles in marine waters, p. 275-286. In: D.W. Wood (ed.) Symposium on organic matter in natural waters. University of Alaska. 625 pp.

North, B.B. and B.C. Stephens. 1967. Uptake and assimilation of amino acids by Platymonas. Biol. Bull. 133: 391-400.

Overbeck, J. 1967. Zur Bakteriologie des suβwassersees - ergebnisse und probleme. Sonderdruck aus GWF "Das Gas - und wasserfach" 108: 1258-1260.

Overbeck, J. 1968. Prinzipielles zur vorkommen der bakterien im see. Mitt. Internat. Verein. Limnol. 14: 134-144.

Parsons, T.R. and J.D.H. Strickland. 1962. On the production of particulate organic carbon by heterotrophic processes in sea water. Deep-Sea Res. 8: 211-222.

Riggs, D.S. 1963. The mathematical approach to physiological problems. The M.I.T. Press. 445 pp.

Rigler, F.H. 1971. The Char Lake Project: A study of energy flow in a high arctic lake. Proceedings, IBP-UNESCO Symposium on Productivity Problems of Freshwaters. Pol. Acad. Sci. (in press).

Rodhe, W. 1958. Primarproduktion und Seetypen. Verh. Internat. Verein. Limnol. 13: 121-141.

--------- 1969. Crystallization of eutrophication concepts in Northern Europe, p. 50-64. In: Symp. on Eutrophication: causes, consequences, correctives. Academy of Sciences. 661 pp.

---------, J.E. Hobbie and R.T. Wright. 1966. Phototrophy and heterotrophy in high mountain lakes. Mitt. Internat. Verein. Limnol. 16: 302-313.

Ruttner, F. 1963. Fundamentals of limnology. 3rd. ed. University of Toronto Press. 295 pp.

Schmidt, G.W. 1969. Vertical distribution of bacteria and algae in a tropical lake. Int. Revue Ges Hydrobiol. 54: 791-797.

Seki, H. and M. Hardon. 1970. Microbial studies relevant to a lobster introduction in Fatty Basin, B.C. J of Oceanographical Society of Japan, 26: 38-51.

Seki, H. and O.B. Kennedy. 1970. Marine bacteria and other heterotrophs as food for zooplankton in the Strait of Georgia during the winter. J. Fish Res. Bd. 26: 3165-3173.

Shrift, A. 1966. Methionine transport in Clorella vulgaris. Plant Physiol. 41: 405-410.

Siegel, A. and E.T. Degens. 1966. Concentration of dissolved amino acids from saline waters by ligand-exchange chromatography. Science 151: 1098-1101.

Straškraba, M. and V. Straškrabová. 1969. Eastern European Lakes, p. 65-97. In: Symp. on Eutrophication: causes, consequences, correctives. National Academy of Science. 661 pp.

Taylor, C.B. 1940. Bacteriology of freshwater. I. Distribution of bacteria in English lakes. J. Hyg. 40: 616-640.

Vaccaro, R.F. 1969. The response of natural microbial populations in seawater to organic enrichment. Limnol. Oceanogr. 14: 726-735.

----------, S.E. Hicks, H.W. Jannasch and F.G. Carey. 1968. The occurrence and role of glucose in sea water. Limnol. Oceanogr. 13: 356-360.

---------- and H.W. Jannasch. 1966. Studies on heterotrophic activity in seawater based on glucose assimilation. Limnol. Oceanogr. 11: 596-607.

---------- and ---------- 1967. Variations in uptake kinetics for glucose by natural populations in sea water. Limnol. Oceanogr. 12: 540-542.

Vallentyne, J.R. and J.R. Whittaker. 1966. On the presence of free sugars in filtered lake water. Science 124: 1026-1027.

Wetzel, R.G. 1966. Productivity and nutrient relationships in marl lakes of northern Indiana. Verh. Internat. Verein. Limnol. 16: 321-332.

---------- 1968. Dissolved organic matter and phytoplankton production in marl lakes. Mitt. Internat. Verein. Limnol. 14: 261-270.

---------- 1969. Dissolved organic compounds and their utilization in two marl lakes. Hidrólogiai Közlöny 47: 298-303.

Williams, P.J. Le B. 1970. Heterotrophic utilization of dissolved organic compounds in the sea I. Size distribution of population and relationship between respiration and incorporation of growth substrates. J. Mar. Biol. Ass. U.K. 50: 859-870.

---------- and R.W. Gray. 1970. The effect of abrupt increase in substrate concentration upon heterotrophic response in sea water. (Abstr.) 33rd Annual Meeting of Limnol. Oceanogr.

Wright, R.T. 1970. Glycollic acid uptake by planktonic bacteria, p. 521-536. In: D.W. Wood (ed.), Symposium on organic matter in natural waters. University of Alaska. 625 pp.

---------- and J.E. Hobbie. 1965a. The uptake of organic solutes in lake water. Limnol. Oceanogr. 10: 22-28.

---------- and ----------. 1965b. The uptake of organic solutes by planktonic bacteria and algae. Ocean Science and Ocean Engineering Trans. Limnol. Oceanogr. Mar. Technol. Soc. 1: 116-127.

Wright, R.T. and J.E. Hobbie. 1966. The use of glucose and acetate by bacteria and algae in aquatic ecosystems. Ecology 47: 447-464.

APPENDIX 1

Sampling for variability in bacterial numbers in Char Lake, August 24-26, 1970.

Bacterial numbers were sampled with a PVC van Dorn bottle and the water samples transported in one liter polyethylene bottles, from which subsamples were taken for enumeration. The accuracy of the sampling procedure was determined only one time in Char Lake during the summer. Three van Dorn samples were taken at 2, 5, 7.5 and 25 m, and from each sample three 10 ml subsamples were taken directly by bulb-pipette and filtered through ,450nm filters. The contents of the third van Dorn bottle from each depth were placed in three polyethylene bottles, from each of which was taken three 10 ml subsamples. All filters were treated as stated in the Methods section.

Standard deviations of the triplicate counts were low (generally $S\bar{x} < 3.0$), and indicate that neither uneven distribution of bacteria in the samples nor variability in the filter counts was a serious problem in the enumeration. The direct count method of Razumov (Kuznetsov and Romanenko, 1964) is statistically reliable only if clumping of bacteria and interference from detritus is minimal. Jannasch and Jones (1959) state that erythrosin stain often proved superior to methylene blue for differentiating individual cells.

Depth (m)	Number of Bacteria (10^7/liter)					
	van Dorn sample 1	van Dorn sample 2	van Dorn sample 3	Polyethylene bottle 1	Polyethylene bottle 2	Polyethylene bottle 3
	10.8	12.5	9.0	11.2	18.5	19.2
2	16.5	17.3	11.1	10.9	11.3	13.1
	9.9	10.2	13.6	16.8	12.5	11.8
$\bar{x}$	12.4	13.4	11.2	13.0	14.2	14.7
$S\bar{x}$	2.1	2.1	1.9	1.9	2.2	2.3
	9.5	10.1	14.7	13.4	12.5	16.0
7.5	19.8	12.2	23.3	10.9	12.9	16.0
	15.1	19.0	24.5	11.8	8.5	10.3
$\bar{x}$	14.8	13.8	20.8	12.0	11.3	14.1
$S\bar{x}$	3.0	2.7	3.9	0.7	1.4	1.9
	20.0	21.7	13.9	18.9	17.4	18.7
15	23.9	13.0	17.2	13.8	9.1	15.0
	16.2	18.6	15.9	10.2	17.5	11.5
$\bar{x}$	20.1	17.8	15.7	14.3	14.7	15.1
$S\bar{x}$	2.2	2.5	1.0	2.5	2.8	2.1
	26.4	14.3	20.4	14.0	21.0	15.1
25	16.1	15.0	12.0	12.0	15.8	18.0
	14.7	19.9	18.7	16.3	12.4	16.2
$\bar{x}$	19.1	16.4	17.0	14.2	16.4	16.4
$S\bar{x}$	3.7	1.8	2.5	1.2	2.5	0.8

APPENDIX 2

Number of bacteria in Char Lake by depth, summer 1970.

Date	Number of Bacteria (10^7/liter) 2 m	7.5 m	15 m	25 m
June 5	3.01	1.84	8.73	10.23
7	4.88	-	8.71	-
10	4.62	5.29	6.12	6.07
11	2.39	2.17	1.50	3.95
18	6.32	3.77	1.05	12.93
25	4.62	6.79	7.57	12.13
June $\bar{x}$	4.31	3.97	5.61	9.06
July 2	10.18	5.18	5.28	10.02
9	6.51	-	8.85	-
14	6.99	-	6.68	-
16	5.68	7.40	9.04	6.90
22	7.54	-	-	-
23	8.57	11.30	8.76	-
30	14.64	5.84	7.07	9.96
July $\bar{x}$	8.57	7.43	7.61	8.96
August 6	15.76	8.04	9.21	10.76
10	15.02	-	-	-
13	8.57	8.67	8.38	-
21	11.00	-	-	-
22	14.24	-	11.72	-
27	17.98	14.10	12.20	15.20
30	15.45	-	12.74	-
August $\bar{x}$	14.00	10.27	10.85	12.98
Sept. 5	20.78	18.47	13.21	16.23
6	22.65	-	-	-
16	15.42	16.20	14.58	14.36
Sept. $\bar{x}$	19.62	17.34	13.90	15.30

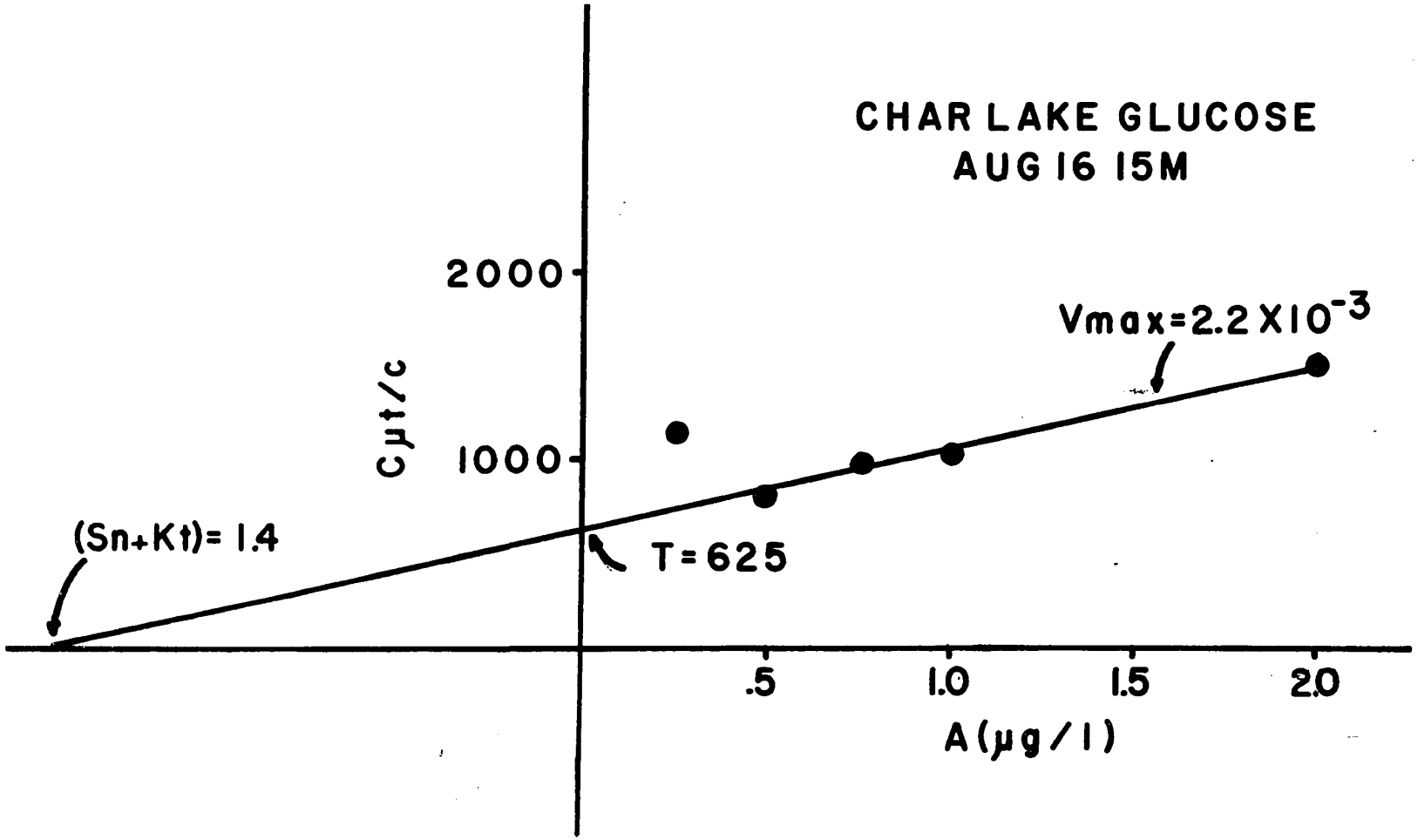
APPENDIX 3

Number of bacteria in Meretta Lake by depth, summer 1970.

	Number of Bacteria (10^8/liter)		
Date	2 m	5 m	7.5 m
June 6	2.89	2.70	2.11
13	3.62	4.62	4.41
Premelt $\bar{x}$	3.26	3.66	3.26
June 29	35.00	30.20	38.80
July 4	10.20	8.51	15.00
11	82.70	73.70	79.01
18	30.01	21.06	25.02
25	34.73	12.63	24.41
Melt $\bar{x}$	38.52	29.20	36.44
August 1	9.16	8.68	7.58
15	6.23	6.92	6.84
23	9.32	8.40	8.84
Sept. 6	8.72	17.43	9.31
18	11.20	10.90	10.10
Postmelt $\bar{x}$	8.93	10.47	8.53

APPENDIX 4

Typical pattern of glucose uptake in Char Lake. Plot is by eyefit and is not significant by least squares at $P < .1$.


CHAR LAKE GLUCOSE
AUG 16 15M
2000
1000
Cμt/c
Vmax=2.2X10⁻³
(Sn+Kt)= 1.4
T=625
.5
1.0
1.5
2.0
A(μg/l)

APPENDIX 5

Kinetic data from glucose uptake experiments in Char Lake, 1970.

Date	Depth (m)	Eyefitted Data			Least Squares Data*		
		Vmax (10^{-3} µg $l^{-1}hr^{-1}$)	T (hrs)	($K_t + S_N$) (µg l l)	Vmax (10^{-3} µg $l^{-1}hr^{-1}$)	T (hrs)	($K_t + S_N$) (µg l l)
Feb. 7	2	-	-	-**			
	7.5	1.74	450	0.7			
	15	-	-	-			
	25	-	-	-			
21	2	3.08	450	1.4			
	7.5	5.97	300	1.8			
	15	2.86	300	0.9			
	25	-	-	-			
March 4	2	-	-	-			
	7.5	2.11	450	1.0			
	15	3.76	575	2.2			
	25	3.33	200	0.5			
19	2	-	-	-			
	7.5	3.64	600	2.2			
	15	-	-	-			
	25	1.78	250	0.4			
24	2	4.76	575	2.7			
	7.5	2.67	300	0.8			
	15	3.08	600	2.0			
	25	2.351	450	0.9			
April 5	2	4.70	650	2.0			
	7.5	5.76	250	1.0			
	15	2.89	367	1.0			
	25	-	-	-			

Appendix 5 - continued

Date	Depth (m)	Eyefitted Data			Least Squares Data		
		Vmax (10^{-3} µg l^{-1} hr^{-1})	T (hrs)	($K_t + S_N$) (µg l l)	Vmax (10^{-3} µg l^{-1} hr^{-1})	T (hrs)	($K_t + S_N$) (µg l l)
April 23	2	2.30	375	0.9			
	7.5	1.80	200	0.35			
	15	2.35	250	0.5			
	25	4.76	380	1.8			
May 17	2	-	-	-			
	7.5	1.82	1660	3.0			
	15	2.11	250	0.3			
June 5	2	1.05	1700	1.8			
	7.5	1.63	875	1.4			
	15	3.63	450	1.6			
	25	4.40	150	0.7			
11	2	-	-	-			
	7.5	-	-	-			
	15	1.48	525	0.7			
	25	1.80	900	1.6			
18	2	1.40	1120	2.0			
	7.5	1.29	1275	1.7			
	15	1.87	900	1.5			
	25	3.30	680	2.0			
25	2	-	-	-			
	7.5	2.10	800	2.0			
	15	1.67	900	1.5			
	25	4.40	700	3.0			
July 2	2	5.00	370	1.9			
	7.5	-	-	-			
	15	-	-	-			
	25	5.70	350	1.8			

Appendix 5 - continued

Date	Depth (m)	Eyefitted Data			Least Squares Data		
		Vmax (10^{-3} µg $l^{-1}hr^{-1}$)	T (hrs)	($K_t + S_N$) (µg l l)	Vmax (10^{-3} µg $l^{-1}hr^{-1}$)	T (hrs)	($K_t + S_N$) (µg l l)
July 9	2	1.82	850	1.5			
	7.5	3.30	350	1.3			
	15	4.00	500	2.0	3.67	480	1.8
	25	7.30	350	2.3			
16	2	1.50	700	0.8			
	7.5	1.86	575	1.1			
	15	2.20	625	1.4			
	25	1.90	350	0.6	1.68	259	0.4
23	2	2.67	1350	1.0			
	7.5	2.10	300	0.6	1.92	286	0.6
	15	3.75	475	1.8	3.83	475	1.8
	25	4.21	300	1.4	4.50	284	1.4
30	2	4.20	475	2.0			
	7.5	3.10	320	1.0	3.05	310	1.0
	15	3.10	525	2.0	3.20	500	2.2
	25	5.70	325	1.9	3.94	209	0.8
Aug. 6	2	3.65	620	1.8			
	7.5	3.50	400	2.0			
	15	3.30	450	2.0	3.20	420	1.9
	25	4.20	350	1.5			
13	2	3.30	850	2.6			
	7.5	3.95	350	1.5	4.18	333	1.4
	15	3.10	700	3.2			
	25	Not done					

Appendix 5 - continued

Date	Depth (m)	Eyefitted Data			Least Squares Data		
		Vmax (10^{-3} µg $l^{-1}hr^{-1}$)	T (hrs)	($K_t + S_N$) (µg l l)	Vmax (10^{-3} µg $l^{-1}hr^{-1}$)	T (hrs)	($K_t + S_N$) (µg l l)
Aug. 27	2	4.0	375	1.0			
	7.5	4.3	250	2.0	6.44	334	2.2
	15	5.4	200	1.1	5.16	219	1.1
	25	4.0	150	0.7	4.23	151	0.6
Sept. 5	2	3.50	300	0.8	3.40	290	0.8
	7.5	3.70	375	1.8			
	15	4.40	320	1.5	4.30	320	1.5
	25	5.20	250	1.0	5.00	245	1.0
16	2	3.06	180	0.6	3.33	180	0.6
	7.5	5.50	250	1.25			
	15	8.10	325	2.60	8.02	301	2.4
	25	8.00	325	2.60	8.00	320	2.6
Oct. 5	2	-	-	-			
	7.5	8.00	300	3.0			
	15	4.24	43	0.38	4.24	43	0.38
	25	-	-	-			
15	2	-	-	-			
	7.5	6.21	180	1.50			
	15	-	-	-			
	25	4.75	77	.4	4.81	76	0.37
30	2	4.40	600	2.50			
	7.5	3.76	320	1.75			
	15	2.61	100	0.35			
	25	6.58	207	1.4			

Appendix 5 - continued

Date	Depth (m)	Eyefitted Data			Least Squares Data		
		Vmax (10^{-3}µg $l^{-1}hr^{-1}$)	T (hrs)	($K_t + S_N$) (µg l l)	Vmax (10^{-3}µg $l^{-1}hr^{-1}$)	T (hrs)	($K_t + S_N$) (µg l l)
Nov. 13	2	2.14	1350	3.0			
	7.5	-	-	-			
	15	-	-	-			
	25	5.45	350	1.9			
30	2	2.50	700	1.75			
	7.5	-	-	-			
	15	-	-	-			
	25	3.00	550	1.1			
Dec. 14	2	-	-	-			
	7.5	-	-	-			
	15	1.46	800	0.8			
	25	Not done					

* Least squares data from plots significant at P $\leq$ .1.

** Uptake pattern not interpretable by eyefit.

APPENDIX 6

Kinetic data from glucose uptake experiments in Meretta Lake, 1970

Date	Depth (m)	Eyefitted Data			Least Squares Data*		
		Vmax (10^{-2}µg $l^{-1}hr^{-1}$)	T (hrs)	($K_t + S_N$) (µg l l)	Vmax (10^{-2}µg $l^{-1}hr^{-1}$)	T (hrs)	($K_t + S_N$) (µg l l)
Feb. 8	2	2.56	115	3.0			
	5	1.74	40	0.6	1.70	42	0.6
	7.5	1.90	30	0.5			
20	2	-	-	-**			
	5	1.14	55	0.7			
	7.5	-	-	-			
March 5	2	-	-	-			
	5	2.86	65	1.9			
	7.5	5.71	65	3.7			
20	2	-	-	-			
	5	-	-	-			
	7.5	-	-	-			
April 6	2	3.07	130	4.0			
	5	Not done					
	7.5	-	-	-			
24	2	-	-	-			
	5	Not done					
	7.5	1.39	80	0.4			
May 24	2	-	-	-			
	5	1.30	90	0.4			
	7.5	2.67	80	1.1			
June 6	2	1.90	285	5.3			
	5	2.96	160	5.8			
	7.5	1.30	90	1.1			

Appendix 6 - continued

Date	Depth (m)	Eyefitted Data			Least Squares Data		
		Vmax (10^{-2} µg l^{-1} hr^{-1})	T (hrs)	($K_t + S_N$) (µg l l)	Vmax (10^{-2} µg l^{-1} hr^{-1})	T (hrs)	($K_t + S_N$) (µg l l)
June 13	2	1.00	119	4.7			
	5	-	-	-			
	7.5	1.18	120	1.4			
29	2	10.5	80	12.5			
	5	18.2	70	1.5			
	7.5	-	-	-			
July 4	2	10.00	60	6.0			
	5	11.80	35	4.0			
	7.5	9.02	45	0.8			
11	2	28.6	48	14.0			
	5	15.4	29	4.5			
	7.5	11.0	15	1.7	10.9	16	1.8
18	2	-	-	-			
	5	10.0	26	2.6			
	7.5	12.5	14	1.8			
25	2	75.00	31	23.3			
	5	15.05	17	2.5			
	7.5	12.60	11	1.3			
28	2	9.68	37	3.6			
	5	16.70	7	1.2	14.80	4	0.5
	7.5	18.80	4	0.9	18.60	4	0.9
Aug. 1	2	8.85	20	1.8			
	5	15.00	15	2.2			
	7.5	10.70	5	0.5			

Appendix 6 - continued

Date	Depth (m)	Eyefitted Data			Least Squares Data		
		Vmax (10^{-2} µg l^{-1} hr^{-1})	T (hrs)	($K_t + S_N$) (µg l l)	Vmax (10^{-2} µg l^{-1} hr^{-1})	T (hrs)	($K_t + S_N$) (µg l l)
Aug. 5	2	-	-	-			
	5	9.05	19	1.8	10.90	20	2.1
	7.5	15.00	15	1.3	16.70	10	1.6
15	2	7.23	27	2.0			
	5	5.00	30	1.0	6.00	27	1.6
	7.5	5.00	35	0.7	4.42	30	0.6
23	2	-	-	-			
	5	4.29	45	3.0	2.78	48	1.4
	7.5	4.92	30	0.4			
Sept. 6	2	9.91	31	1.8			
	5	7.20	34	2.0			
	7.5	8.79	15	1.0			
18	2	16.0	13	2.0			
	5	7.14	7	0.5			
	7.5	18.60	9	2.6			
Oct. 3	2	-	-	-			
	5	16.70	12	3.3			
	7.5	-	-	-			
17	2	-	-	-			
	5	15.50	15	2.5			
	7.5	-	-	-			
31	2	-	-	-			
	5	Not done					
	7.5	Not done					

Appendix 6 - continued

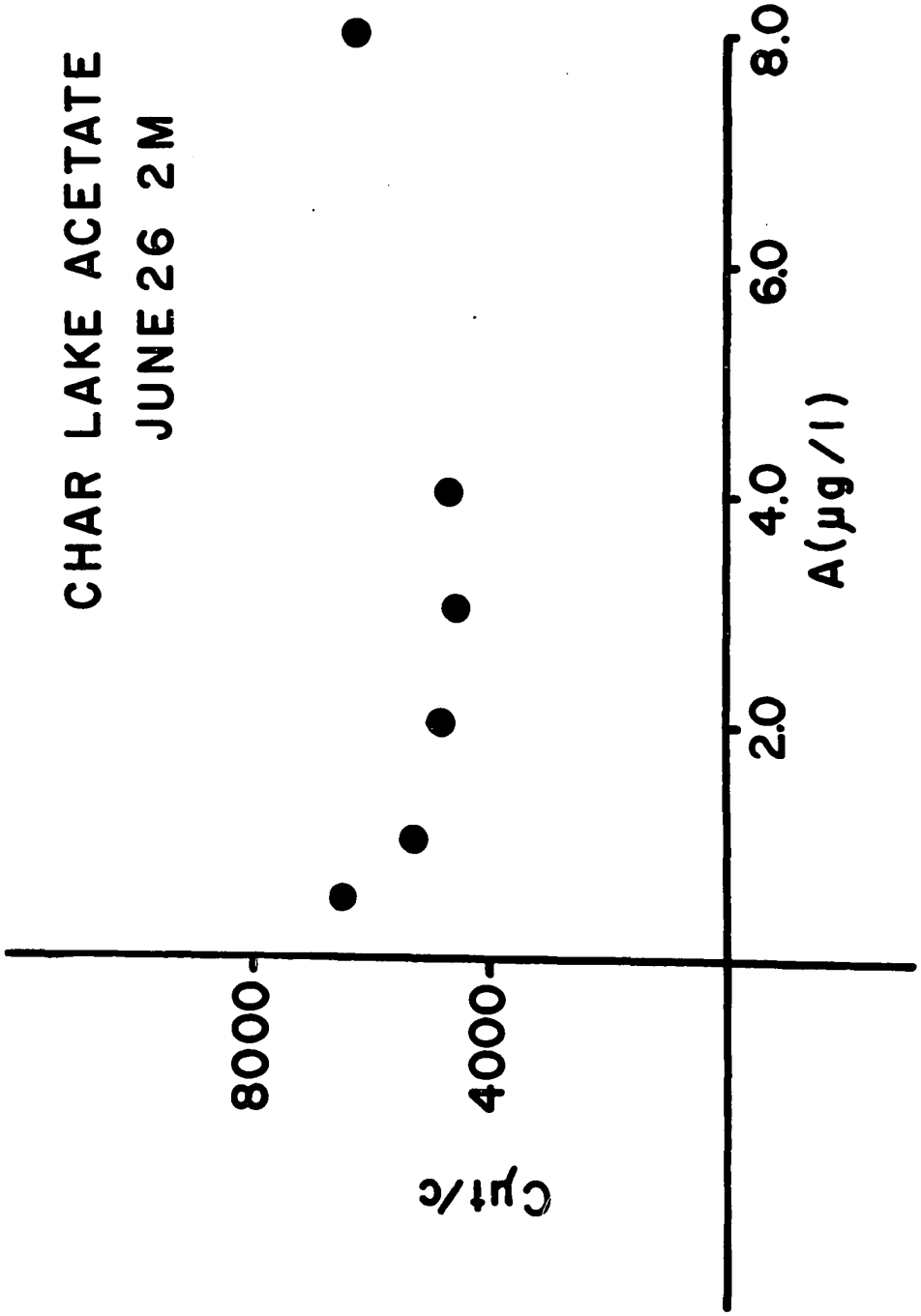
Date	Depth (m)	Eyefitted Data			Least Squares Data		
		Vmax (10^{-2}µg $l^{-1}hr^{-1}$)	T (hrs)	($K_t + S_N$) (µg l l)	Vmax (10^{-2}µg $l^{-1}hr^{-1}$)	T (hrs)	($K_t + S_N$) (µg l l)
Nov. 12	2	-	-	-			
	5	-	-	-			
	7.5	8.82	37	3.3			
26	2	-	-	-			
	5	-	-	-			
	7.5	5.0	60	3.0			
Dec. 11	2	1.81	60	1.3			
	5	-	-	-			
	7.5	6.67	31	1.1			
31	2	2.22	90	1.2			
	5	-	-	-			
	7.5	5.0	50	2.5			

* Least squares data from plots significant at $P < .1$.

** Uptake pattern not interpretable by eyefit.

APPENDIX 7

Typical pattern of acetate uptake in Char Lake. Plot is not interpretable by Michaelis-Menten kinetics.


CHAR LAKE ACETATE
JUNE 26 2M
8000
4000
Cμt/c
2.0
4.0
6.0
8.0
A(μg/l)

APPENDIX 8

Cut/c data (x 10^4) from acetate uptake experiments in Char Lake, summer 1970.

Cμt/c (x 10^4)
A (μg acetate/liter)

Date	Depth (m)	0.5	1.0	1.5	2.0	3.0	4.0	6.0	8.0
May 12	2	.15	.16		.16		.06		.18
	7.5	.05	.08		.06		.13		.09
	15	.05	.10		.06		.07		.11
	25	.14	.08		.14		.20		.22
June 10	2	.24	.60	1.20	1.40	.26	.77		
	7.5	.68	.32	.61	.55	.53	.52		
	15	.48	.29	.36	.48	.45	.54		
	25	.42	.29	.21	.38	.34	.41		
June 17	2	.06	.23	.36	.50	.81	.69		
	7.5	.17	.41	.23	.23	.81	1.02		
	15	.23	.20	.64	.45	.79	.98		
	25	.74	.61	.58	.68	.68	.74		
June 26	2	.65	.53		.48	.45	.46		.62
	7.5	1.22	.84		.79	.77	.76		.85
	15	.54	.51		.62	.52	.60		.81
	25	.59	.76		.48	.53	.58		.71
July 8	2	.22	.20		.27	.24	.28		.23
	7.5	.24	.16		.21	.18	.26		.20
	15	.27	.24		.25	.28	.10		.22
	25	.19	.17		.19	.19	.12		.19
July 28	2		.88		1.11	1.31	.76	.94	1.42
	7.5		.37		.54	.56	.60	.64	.43
	15		.61		.41	1.12	.62	.80	.76
	25		.60		.72	.66	.66	.82	.88

Appendix 8 - continued

$C\mu t/c$ ($\times 10^4$)

A (μg acetate/liter)

Date	Depth (m)	0.5	1.0	1.5	2.0	3.0	4.0	6.0	8.0
August 3	2		-		.27	.31	.78	-	.44
	7.5		.40		.36	.32	.34	.44	.57
	15		.31		.48	.34	.48	.46	.46
	25		.37		.31	.34	.52	.41	.37
August 11	2		.74		.47	1.3	.37	.84	1.6
	7.5		.39		.35	.74	.48	1.3	1.1
	15		.84		.63	.81	.78	.74	.97
	25	Not done							
August 26	2		.96		1.0		.66	.94	1.1
	7.5		1.24		.76		1.1	1.0	1.0
	15		1.9		.72		.85	1.2	2.0
	25		.50		.62		.82	.64	.64
August 31	2		.34		.59		.43	.39	.48
	15		.71		1.25		.94	.80	.97
Sept. 12	2		.12		.07		.21	.15	.38
	7.5		.19		.19		.32	.25	.28
	15		.21		.22		.33	.27	.49
	25		.20		.34		.27	.27	.48

APPENDIX 9

Cμt/c data (x 10^3) from acetate uptake experiments in Meretta Lake, summer 1970.

Date	Depth (m)	Cμt/c (x 10^3) A (μg acetate/liter) 0.5	1.0	2.0	3.0	4.0	6.0	8.0
May 12	2	.27	.13	.15		.25		.44
	5	.39	.15	-		.23		.74
	7.5	.10	.07	.09		.11		.19
June 6	2	1.02	.87	.87		.75		.90
	5	1.2	.93	.76		.58		.76
	7.5	.80	.82	.74		.42		.43
June 13	2	.80	.49	.57		.47		.60
	5	.50	.72	.64		.52		.55
	7.5	.50	.31	.34		.38		.38
June 27	2	.21	.11	.14		.10		.19
	5	.17	.07	.07		.09		.18
	7.5	.29	.20	.08		.12		.14
July 4	2	.03	.06	.07		.07		.14
	5	.11	.18	.09		.09		.10
	7.5	.19	.16	.16		.12		.18
July 12	2	.03	.03	.02	.02	.02		.02
	5	.07	.10	.05	.05	.03		.04
	7.5	.03	.01	.03	.02	.01		.01
July 15	2	.10	.04	.05	.05	.08		.05
	5	.10	.11	.04	.05	.03		.03
	7.5	.07	.04	.03	.03	.02		.04
July 25	2		.28	.21	.21	.21	.28	
	5		.43	.28	.26	.30	.33	
	7.5		.23	.19	.14	.16	.19	
August 1	2		.16	.11	.09	.12	.18	
	5		.21	.12	.12	.11	.17	
	7.5		.20	.10	.11	.10	.16	

Appendix 9 - continued

Date	Depth (m)	Cμt/c (x 10^3) A (μg acetate/liter) 0.5	1.0	2.0	3.0	4.0	6.0	8.0
August 5	2		.36	.32	.26	.43	.32	
	5		.48	.36	.41	.38	.34	
	7.5		.42	.35	.20	.17	.16	
August 15	2		.24	.28	.23	.17	.20	
	5		.57	.39	.51	.42	.39	
	7.5		.18	.46	.18	.15	.26	
August 23	2		.16	.19	.31	.15	.21	
	5		.17	.22	.16	.16	.19	
	7.5		.25	.23	.22	.21	.28	
Sept. 6	2		.27	.23	.19	.14	.18	
	5		.18	.24	.17	.17	.30	
	7.5		.19	.19	.10	.11	.19	
Sept. 18	2		.14	.14		.13	.23	
	5		.09	.12		.17	.13	
	7.5		.16	.05		.08	.12	

APPENDIX 10

Char Lake acetate uptake by depth, summer 1970.

Velocity of uptake at 4 µg/liter of added substrate.

Date	(10^{-3} µg l^{-1} hr^{-1})			
	2 m	7.5 m	15 m	25 m
May 12	2.50	3.20	5.97	1.93
June 10	.51	.80	.74	.97
June 17	.61	.58	.70	.57
June 26	.89	.55	.69	.69
July 8	1.05	.87	.93	1.00
July 28	.56	.82	.67	.75
August 3	1.58	1.17	.84	.76
August 11	1.13	.82	.51	-
August 26	.51	1.32	.54	1.73
August 31	1.03	-	.49	-
Sept. 12	1.68	1.29	1.30	1.57

APPENDIX 11

Meretta acetate uptake by depth, summer 1970.

Velocity of uptake at 4 µg/liter of added substrate.

Date	(10^{-2} µg l^{-1} hr^{-1}) 2.0 m	5.0 m	7.5 m
May 12	1.63	1.74	3.60
June 6	.58	.70	1.20
June 13	1.20	1.10	1.30
June 27	4.00	4.70	3.89
July 4	6.00	4.15	3.17
July 12	23.10	13.10	29.90
July 15	5.45	13.78	11.75
August 1	3.39	3.96	3.82
August 5	1.53	1.04	2.13
August 15	2.30	1.01	2.73
August 23	2.72	2.70	2.01
Sept. 6	3.10	2.65	3.70
Sept. 18	3.22	2.52	5.00

APPENDIX 12

Bacterial respiration of glucose in Char and Meretta Lakes, 1970

Bacterial respiration of glucose was determined after Hobbie and Crawford (1969), using phenethylamine as a $^{14}CO_2$ trap. Twenty-five milliliters of water were placed into a series of five 50 ml erlenmeyer flasks. Glucose -U- ^{14}C was added to Char and Meretta Lake samples in a concentration range of 1 to 4 µg/liter and 0.5 to 4 µg/liter, respectively. One control per series was fixed with lugol iodine. The flasks were sealed with rubber serum stoppers to which metal cups containing chromatographic paper were attached, and incubated at 1-2 C for 8 hours (Char Lake) and 2 hours (Meretta Lake). Working through the septum, 1 ml of 2N H_2SO_4 was then injected into the sample and 1 ml of phenethylamine added to the chromatographic paper. The samples were magnetically stirred for one hour, after which the chromatographic paper was placed in vials for shipping and the water samples filtered through ,450nm membrane filters. At a later date the chromatographic paper was transferred to scintillation vials containing 10 ml of a mixture of 1-4 dioxane, naphalene and PPO, and the counts obtained adjusted to the same efficiency as those from the filters. Crawford (pers. comm.) states that it is safe to transport the chromatographic paper in sealed vials without scintillation cocktail.

Results showed that 38 to 71% ($\bar{x}$ = 51%) of the glucose assimilated at each concentration was respired in 8 hours in Char Lake, and 8 to 50% ($\bar{x}$ = 22%) in 2 hours in Meretta Lake. The limited data suggest that the

turnover time of glucose in Char Lake is seriously overestimated and Vmax underestimated if the respiration of glucose is ignored, however, the pattern is not sufficiently clear to determine the amount of error. The lower percentage loss of activity as $^{14}CO_2$ in Meretta Lake may be simply a result of the shorter incubation time.

Bacterial respiration of glucose in Char Lake, 1970

I. Char Lake, August 7, 1970; 8 hour experiment.

Depth (m)	A (µg glucose/l)	Filter Count (cpm)	Scintillation Count (cpm)	Total Count (cpm)	Scintillation Count as % of Total Count
2	1	121	257	378	68
	2	225	312	537	58
	3	392	447	839	53
	4	392	693	1085	64
	2 + lugol	120	291	411	71
15	1	339	257	596	43
	2	442	720	1162	62
	3	589	538	1127	48
	4	791	-	-	-
	2 + lugol	136	136	272	50

Bacterial respiration of glucose in Char Lake, 1970 - continued

II. Char Lake, August 10, 1970; 8 hour experiment.

Depth (m)	A (µg glucose/l)	Filter Count (cpm)	Scintillation Count (cpm)	Total Count (cpm)	Scintillation Count as % of Total Count
2	1	331	311	642	48
	2	679	631	1310	52
	3	804	788	1592	49
	4	524	782	1306	60
	2 + lugol	137	210	347	61
15	1	433	272	705	38
	2	839	611	1450	42
	3	809	755	1564	48
	4	528	1000	1528	65
	2 + lugol	125	125	250	50
					$\bar{x}$ 51%

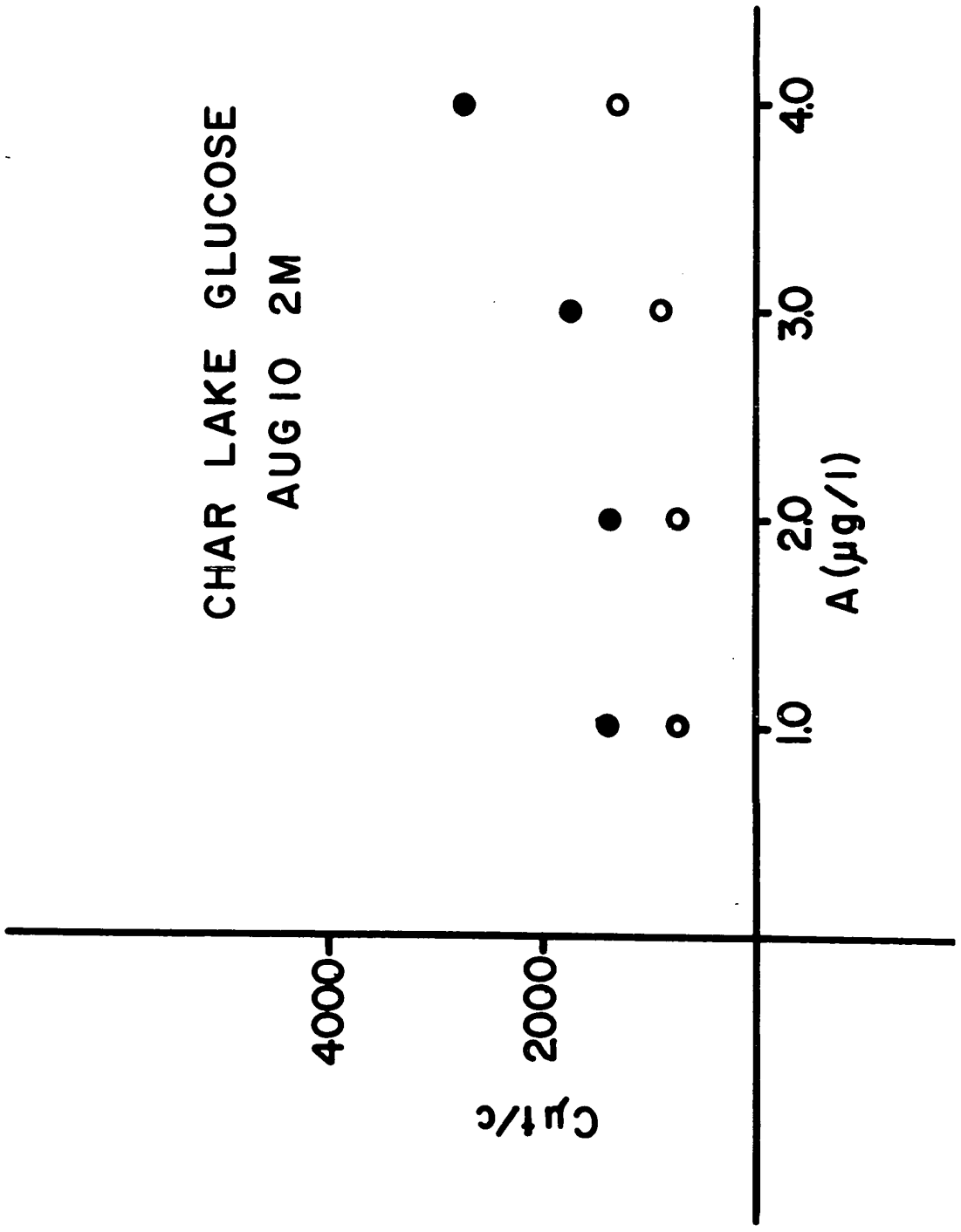
Bacterial respiration of glucose in Meretta Lake, 1970.

Meretta Lake, August 8; 2 hour experiment.

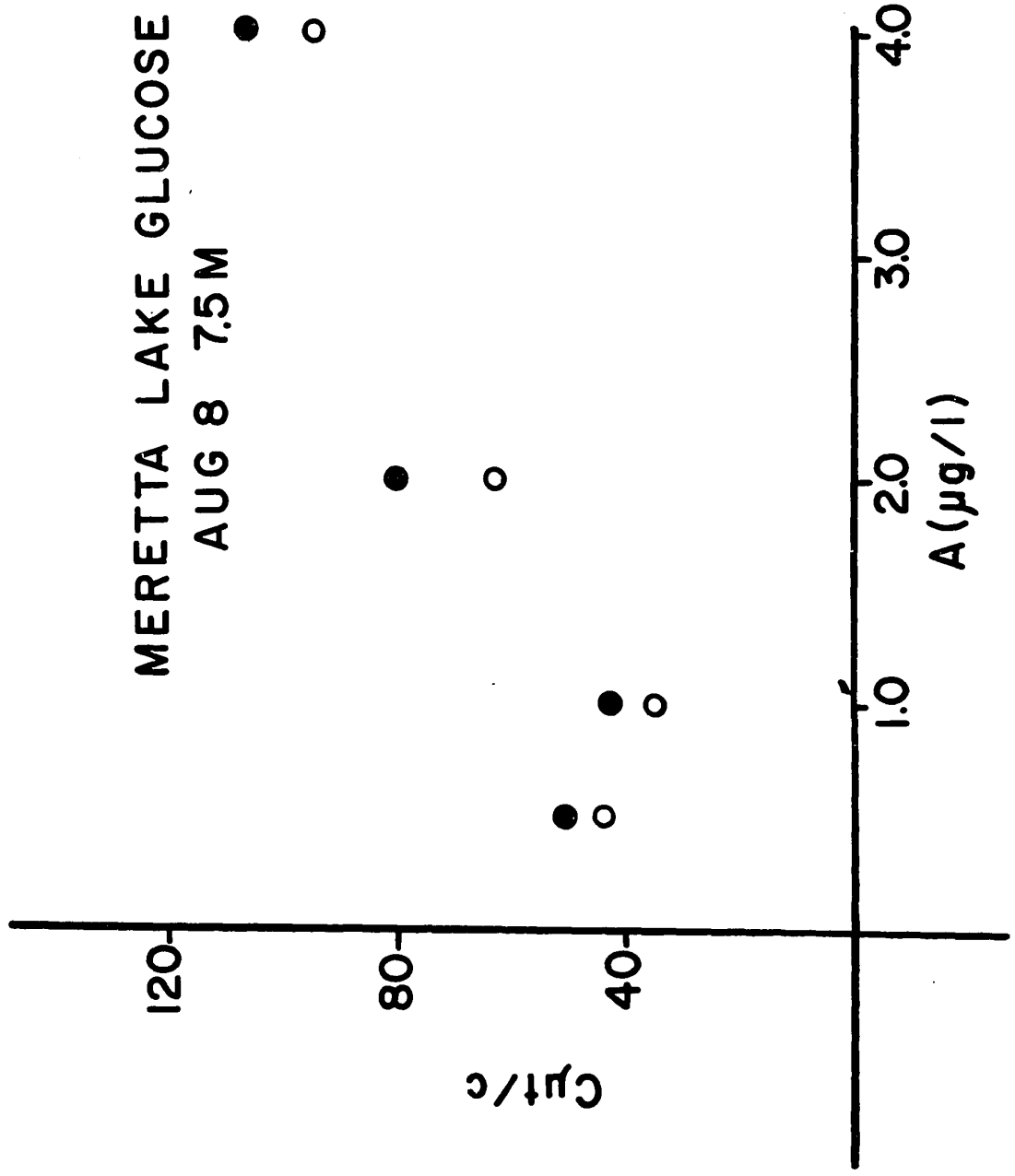
Depth (m)	A (µg glucose/l)	Filter Count (cpm)	Scintillation Count (cpm)	Total Count (cpm)	Scintillation Count as % of Total Count
2	0.5	958	130	1088	12
	1.0	1401	165	1566	10
	2.0	2717	978	3695	26
	4.0	4717	427	5144	8
	1.0 + lugol	72	73	145	50
5	0.5	1701	497	2198	23
	1.0	1087	880	1961	45
	2.0	3425	247	3672	7
	4.0	3650	900	4550	20
	1.0 + lugol	77	49	126	39
7.5	0.5	1182	150	1332	11
	1.0	2778	555	3333	17
	2.0	2924	778	3702	21
	4.0	4425	523	4948	11
	1.0 + lugol	86	47	133	35

$\bar{x}$ 22%

Bacterial respiration of glucose in Char Lake, August 10, 1970. (●) isotope remaining in the bacteria (net uptake); (o) net uptake plus the amount of isotope respired.


CHAR LAKE GLUCOSE
AUG 10 2M
Cμt/c
4000
2000
1.0
2.0
3.0
4.0
A (μg/l)

Bacterial respiration of glucose in Meretta Lake, August 8, 1970. (●) isotope remaining in the bacteria (net uptake); (o) net uptake plus the amount of isotope respired.


MERETTA LAKE GLUCOSE
AUG 8 7.5 M
Cμt/c
120
80
40
1.0
2.0
3.0
4.0
A(μg/l)

APPENDIX 13

Acetate preconditioning experiments in Char Lake, 1970.

Two preconditioning experiments with acetate were done in Char Lake after Vaccaro (1969). Water samples from 2 m were placed in three 1 liter polyethylene bottles and unlabelled sodium acetate added to give concentrations of 5 µg/liter and 500 µg/liter. No acetate was added to the third bottle, from which a zero hour control was determined. The bottles were kept cold (0-5 C) and magnetically stirred. Twenty-four and forty-eight hours after the addition of the unlabelled acetate, 250 ml of water from each bottle were filtered through 47 mm ,200nm membrane filters; the filters were repeatedly washed with filtered lake water and the bacteria resuspended in 250 ml of cell-free lake water. These samples were placed in five 125 ml reagent bottles and acetate uptake experiments were run.

Storage of Char Lake water in the presence and absence of unlabelled acetate failed to change the uptake pattern to one consistent with Michaelis-Menten kinetics. The low uptake of ^{14}C acetate in samples from the August 16-19 500 µg/liter bottle may have resulted from contamination of the sample with unlabelled acetate. More thorough washing in the second experiment appears to have largely eliminated this problem. The bacterial populations in both lakes are responsive to acetate, yet the uptake pattern is uninterpretable. The pattern obtained may result (despite preconditioning) from a varied response of a number of bacteria species, each dominant at a particular concentration. The significance of uptake by phytoplankton (especially µ-algae) is unknown.

I. August 16-19, 1970

	A (µg acetate/l)	Cµt/c (x 10^4) Time after addition of unlabelled acetate 0 hours	24 hours	48 hours
Control	1.0	.50	.41	.69
	2.0	.43	.27	.19
	4.0	1.10	.29	.31
	8.0	.79	.34	.21
5 µg/l Acetate	1.0		.57	.43
	2.0		.59	.40
	4.0		.38	1.21
	8.0		.49	.48
500 µg/l Acetate	1.0		.41	2.31
	2.0		1.24	.78
	4.0		.93	.99
	8.0		.76	2.05

II. September 10-13, 1970

	A (µg acetate/1)	Cµt/c (x 10^4) Time after addition of unlabelled acetate 0 hours	24 hours	48 hours
Control	1.0	.17	.13	.18
	2.0	.17	.31	.23
	4.0	.23	.17	.20
	8.0	.16	.10	.08
5 µg/1 Acetate	1.0		.17	.21
	2.0		.24	.08
	4.0		.26	.24
	8.0		.31	.27
500 µg/1 Acetate	1.0		.32	.23
	2.0		.19	.29
	4.0		.47	.27
	8.0		.46	.19