THE BIOMASS AND ACTIVITY OF BACTERIA IN THE SEDIMENTS OF MARION LAKE, BRITISH COLUMBIA.

by

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A THESIS SUBMITTED IN PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

in the department
of
Zoology

We accept this thesis as conforming to the
required standard

THE UNIVERSITY OF BRITISH COLUMBIA
August, 1974

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ABSTRACT: THE BIOMASS AND ACTIVITY OF BACTERIA IN THE SEDIMENTS OF MARION LAKE, BRITISH COLUMBIA.

Two biomass indicators (direct counts and ATP analysis) and two activity estimators (glucose uptake and dehydrogenase activity) were used to study the bacteria at 1 m water depth in Marion Lake sediments. Direct count-biomass estimates for bacteria averaged 0.61 gC/m^2, were high in summer, declined rapidly in fall, then increased during the winter. Microorganisms less than approximately 30 μm diameter had a mean biomass of 1.28 gC/m^2 as measured by ATP analysis. Seasonal variations in this figure paralleled changes in the algal population, although algal contributions to the microbial biomass were less than 50 per cent. ATP analysis was also used to estimate the biomass of the sediment community, excluding animals greater than approximately 5 mm in length. The mean community biomass was 4.69 gC/m^2.

Comparison of ATP data with enumeration data obtained by others, suggests that ATP is a good biomass indicator, except when cellular ATP levels are changed in reaction to biotic or abiotic environmental factors. It is proposed that, in situations such as intense grazing or rapid increases or decreases in temperature, ATP measurements reflect not only biomass but also activity. At such times ATP-biomass data may indicate biomass potential, or the capacity of the population to maintain its biomass under abnormally high rates of loss.

Dehydrogenase activity, or respiratory potential, of the sediment bacteria was assayed using triphenyl tetrazolium

chloride. The estimate for annual rate of carbon loss as CO_2 from the bacterial population (19.3 g C/m^2.year), was almost identical to previously reported data obtained by measuring oxygen consumption in normal and antibiotic-treated sediment cores.

Uptake of ^{14}C-glucose was determined in mixed, diluted sediments. The maximum uptake rate (9.6 g C/m^2.year), natural uptake rate (5.3 g C/m^2.year) and the natural turnover time (0.31 h) were similar to previous data for these sediments. This suggests that, at least in terms of function, the bacterial population is quite stable from year to year.

Biomass and activity of the Marion Lake sediment bacteria were found to be in phase in late spring through early fall, but activity remained low in winter despite increases in the microbial biomass, and increased much more rapidly than biomass in early spring. The dynamics of the bacterial population are discussed in terms of these relationships.

The size of the algal and bacterial populations and organic matter reservoirs, and the flux rates for carbon between these compartments are summarized.

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ACKNOWLEDGMENT

This research was conducted as part of the Canadian IBP Marion Lake Project, located at the University of British Columbia, and funded by the National Research Council (NRC) of Canada. I would like to acknowledge financial support from NRC and from British Columbia Packers Limited and Fisheries Association of British Columbia.

All the members of the Marion Lake team were helpful and encouraging throughout the project. In particular, I would like to thank Drs. I. E. Efford, P. Kleiber, K. Hall and B. K. Burnison for their ideas and discussions.

I would like to acknowledge the constructive comments and criticism of the members of my research committee, including Drs. P. Larkin, H. Blackburn, K. Hall and I. E. Efford. Dr Blackburn was very generous, allowing me to monopolize his research facilities.

Finally I would like to thank Corinne Perry, for being the way she is.

INTRODUCTION

1. Objectives and background.

Many biological characteristics of Marion Lake, British Columbia, have been studied in detail in an effort to describe the rate and control of energy flow between the major compartments of the system (Hall and Hyatt, 1974). The studies since 1963 were supported by the International Biological Program as part of its commitment to assess productivity in diverse environments. This emphasis on ecological energy flow stems historically from the qualitative description of food webs by Forbes in 1887 (quoted in Odum, 1968) and the energy flow by trophic level concept introduced by Lindeman (1942). The quantitative approach to aquatic ecosystem description using the Lindeman concept has been used in Silver Springs, Florida (Odum, 1957), a temperate cold spring (Teal, 1957) and a salt marsh (Teal, 1962).

Marion Lake is situated 50 km east of Vancouver, British Columbia, at an elevation of 300m. The climate is typically coastal being relatively mild and wet with an annual precipitation of 240 cm. The lake is small (13 hectares), shallow (mean depth 2.4 m) and subject to short turnover time (as short as three days during periods of high water inflow). Planktonic production is extremely low due, directly or indirectly, to the high flush rate (Efford 1967, 1969). Recent work has therefore focused on determining compartment size and carbon or energy fluxes between the compartments of the benthic environment.

Algal biomass in the Marion Lake sediments is approximately 0.043 gC/m^2 (Efford, unpublished). Bacterial numbers were estimated by Fraker, using plate counts, to vary from 5×10^4 cells/ml in winter to 2×10^6 cells/ml in summer (unpublished). Using microscopic counting methods, Ramey (1972) and Burnison (unpublished) estimated numbers of bacteria at the sediment surface to be 10^8 cells/ml and 10^{10} cells/g respectively. Both these workers found a decrease with depth below 2 cm. Hall and Hyatt (1974), using Burnison's data and an average cell volume of $0.36\mu m^3$ derived a fresh weight of 3.9 mg/g sediment. This is equivalent to approximately 0.39 gC/m^2, or about nine times the algal biomass.

Net dissolved and particulate allochthonous inputs to the lake are 28 gC/m^2/year and 36 gC/m^2/year respectively (Geen, unpublished; Odum, unpublished). Autochthonous inputs include primary production by phytoplankton (8 gC/m^2/year - Efford, 1967), epibenthic algae (40.4 - 44.2 gC/m^2/year - Hargrave, 1969; Greundling, 1971).

Carbon losses as carbon dioxide from the benthic community total 57 gC/m^2/year (Hargrave, 1969). Estimates of bacterial respiration were obtained by Hargrave by comparing oxygen uptake in normal versus antibiotic-treated cores but this approach has been criticized (Cameron, 1973; Yetka and Wiebe, 1974). An estimate of 4.2 - 24 gC/m^2/year for bacterial respiration was obtained by Kleiber (1972) using the data of Hall *et al.* (1972).

Within the sediment ecosystem, transfers from the chemical environment to microorganisms in the form of dissolved and particulate material have been studied by Hall et al. (1972) and Hall et al. (1973), using radioactive chemicals and leaf material. Kleiber (1972) reported the flux from algae to the chemical environment was 1.5 - 8.8 $gC/m^2/year$. Using the data of Hall et al. (1972) he further estimated the carbon flux from the environment to bacteria to be 21 - 120 $gC/m^2/year$. The importance of the benthic bacterial population is evident from these data. Total net inputs to the lake are about 132 $gC/m^2/year$ and the use of organic material by bacteria (21 - 120 $gC/m^2/year$) is equivalent to 16 - 91% of this net input.

The purpose of this study was to assess biomass and activity of bacteria in Marion Lake sediments. As described above some measurements have been obtained but the bacterial biomass was not measured during the course of the heterotrophic uptake studies (Hall et al., 1972), and bacteria have not been quantified seasonally except by plate count methods.

No approach to either biomass or activity measurement is accepted as a "standard method". Many papers report information on various parameters of microbial populations but methodology is diverse. A few studies comparing multiple approaches simultaneously have been reported (Witkamp, 1973, in soils; Hobbie et al., 1972, in the ocean). Work comparing two methods is more abundant and is referred to in the discussion.

During this study, two biomass estimators (adenosine-

5′-triphosphate (ATP) and direct microscopic counts) and two activity estimators (dehydrogenase activity and ^{14}C-glucose utilization) were employed. The changes, within and between these methods, seasonally, are presented.

2. Bacterial Biomass

Bacterial counts

Plate count methods have long been used to estimate the numbers of microorganisms in water, soils and sediments. Many reports on various experiments ranging from response to fertilization to vertical distribution of bacteria in soils still use this approach. There is no doubt that the technique is useful for looking at specific physiological groups of microorganisms but the use of the aerobic, heterotrophic plate count as an indicator of total viable bacterial numbers is invalid (Schmidt, 1973).

Microscopic counts of bacteria with conversion of the numbers to biomass using an average cell volume have a number of inherent difficulties. Besides being time consuming, it is difficult to differentiate between detrital particles, dead bacteria and viable bacteria. In sediments particularly, many particles are in the size range of bacteria and many of these adsorb chemicals used to stain cells. The irregular shape of many bacteria makes accurate estimation of cell volume difficult. The technique was used in this study to give an upper limit for the bacterial biomass.

Direct counting methods have been employed in diverse environments including water (Sorokin, 1970; Hobbie et al., 1972), soil (Babiuk and Paul, 1970; Trolldenier, 1973), beach-sands (Khiyama and Makemson, 1973) and sediments (Olah, 1972). Fluorescent stains are helpful in making small bacterial

cells on particles more visible. Quantitive work using fluorescent stains such as acridine orange (Trolldenier, 1973) and fluorescein isothiocyanate (FITC) (Babiuk and Paul, 1970) has been reported. One problem involved in the method is to obtain a sample uniformly dispersed on a microscope slide so that random sampling yields results with minimal variance. In previous research, samples have been spread in thin layers on slides and allowed to dry (Trolldenier, 1973, Babiuk and Paul, 1970) or thin layers of agar containing soil samples were formed (Jones and Mollison, 1948). Unfortunately materials prepared in this manner may have a variable vertical distribution so repeated focusing of the objective lens is required because of its short depth of field. In addition the objective of uniform distribution is often not achieved.

An ideal way to ensure random dispersion of samples would be by filtration through bacteria-retaining membranes. Previous use of this technique has been limited to light microscopy probably because of interference in fluorescent work from the oil used to clear the filters. A method, devised by B. Kent Burnison (unpublished), which employs filtration of samples, clearing in acetone vapors and FITC staining has been used in this study. This technique allows cells to be uniformly distributed with negligible background fluorescence and the cells are bound in the surface layer of the cleared membrane filter.

ATP Analysis

ATP is present in all living cells (Mahler and Cordes, 1966) but is not associated with dead cells (Holm-Hansen and Booth, 1966; Lee et al., 1971a; Patterson et al., 1970; this study). Presence of ATP is therefore indicative of life. Knowledge of numerical relations between ATP and organic carbon associated with living organisms (biomass carbon) permit extrapolation of ATP concentrations to biomass carbon. This relationship has been studied in bacteria (Ausmus, 1973; Ernst, 1970; Hamilton and Holm-Hansen, 1967), algae (Berland et al., 1972; Ausmus, 1973) actinomycetes (Ausmus, 1973), fungi (Ausmus, 1973) and nematodes (Ernst, 1970). Considerable work has been done determining ATP content per bacterial cell (for example Chapelle and Levin, 1968) but information on numbers is less relevant to ecosystem studies than is information on biomass. The ATP to biomass carbon relation varies between groups of organisms, between species and within species at different times of the life cycle. Despite these difficulties a weight relation of carbon to ATP of 250 seems to be meaningful.

The ATP assay is based on the finding of McElroy (1947), that luminescent reactions in firefly tail extracts require ATP. Since then, light production has been shown to be proportional to ATP if other reactants are in excess (Strehler, 1965):

$$\text{ATP} + \text{luciferin} \xrightarrow[\text{luciferase}]{Mg^{++}} \text{adenyl-luciferin} + PP_i$$

$$\text{adenyl-luciferin} \xrightarrow{O_2} \text{adenyl-oxyluciferin} + H_2O + \text{light}$$

ATP has been measured in fresh water (Rudd and Hamilton, 1973; Holm-Hansen and Paerl, 1972), oceans (Hobbie et al., 1972; Holm-Hansen and Paerl, 1972), sewage (Patterson et al., 1970; Brezonik and Patterson, 1971), soils (Ausmus, 1973; Conklin and MacGregor, 1972; MacLeod et al., 1969), marine sediments (Ernst, 1970) and lake sediments (Lee et al., 1971 a and b). Only two previous studies on temporal changes of biomass as measured by ATP have been reported, that being the work of Rudd and Hamilton (1973) and Holm-Hansen and Paerl (1972) on lake water columns.

Application of the method to estimation of bacterial biomass is difficult. Measurements in water masses are often preceded by filtration through nets (60 - 150 μm mesh) to remove large zooplankton (Holm-Hansen and Paerl, 1972), but analyses still include bacteria, algae, protozoa and many other organisms. These authors further attempted to measure bacterial biomass by subtracting algal biomass, determined by direct counting, from total biomass of organisms less than 60 μm measured by ATP analysis. Even in this size range there is the possibility of protozoa biomass being included. Another problem is the uncertainty of the condition of the algae. Direct counts often include dead cells, so overestimation of algal biomass and therefore underestimation of bacterial mass could result. Rudd and Hamilton (1973) followed changes in biomass of various size groups by differential filtration of water samples but no estimate of bacterial biomass was

obtained because of the presence of small algae less than 10 μm, particularly during summer. Size fractionation, while not simple nor precise in aquatic samples, is even more difficult in sediments. No previous attempts have been made to categorize biomass of different size groups by ATP analysis in soils or sediments. In this study, an estimate of the microbial biomass ($<$ approximately 30 μm) was obtained by physical removal of larger size classes.

3. Activity of Microorganisms

It is difficult to assess what microorganisms are doing *in situ*. It is not possible to "see" what they are eating, assimilating and excreting and as a result chemical and radiotracer techniques are used.

Respiration and dehydrogenase (oxidoreductase) activity

The traditional method for determinimg microbial activity involves measuring respiration by changes in oxygen and/or carbon dioxide concentrations (Hargrave, 1969; Beyers *et al.*, 1963). The oxygen method was used rigourously in Marion Lake for one year but the validity of carbon flow information from this data depends on the accuracy of the assumption of a respiratory quotient (RQ)of 0.85 (Hargrave, 1969). Unfortunately, RQ values for sediments range from 0.27 - 0.96 (Teal and Kanwisher, 1961; Pamatmat, 1968) and for epilithophyton the value of 1.1 $\pm$ 0.9 has been reported (Schindler *et al.*, 1973).

The measurement of inorganic carbon is difficult in lakes that do not exhibit relatively high alkalinity, unless sophisticated equipment is available at the site (Schindler, 1973).

One conclusion from the above discussion is that reports of carbon exchange based on gas analysis are often relative rather than absolute. Another way to estimate respiration is by measuring dehydrogenase activity. In such assays triphenyl tetrazolium chloride (TTC) is used to intercept electrons flowing through the electron transport system ,

and competes with oxygen as electron acceptor. This assay is a relative activity estimator since it measures respiratory potential rather than respiration, but it is a convenient method for comparing activity of samples given different treatments or obtained at different times of the year.

Dehydrogenase activity has been used as an activity index in water (Olah, 1972), sewage (Lenhard, 1968), soil (Casida et al., 1964; Lenhard, 1956) and sediments (Olah, 1972; Pamatmat and Bhagwat, 1973; Edwards and Rolley, 1965).

The experimental approach suggests there should be a correlation between respiration as measured by oxygen uptake or carbon dioxide evolution and respiratory potential as measured by dehydrogenase. Such correlation has been demonstrated in some soil studies (Skujins, 1973; Casida et al., 1964) but not others (Howard, 1972). Stevenson (1959) found oxygen uptake was related to dehydrogenase activity in 24 soils ($r = 0.837$) but in 43 soils which were amended with decomposing plant matter correlation was reduced ($r = 0.511$). Howard (1972) reported that formazan production was always less than expected from oxygen consumption data. This may be due to a negative response by microorganisms to the presence of TTC since the insoluble reduced formazan is deposited within the cells. Furthermore,the substitution of TTC in place of oxygen as electron acceptor reduces the amount of ATP (energy) obtained by the cell per mole of substrate reduced.

Pamatmat and Bhagwat (1973) reported correlation between heat production and dehydrogenase activity in Lake Washington sediments but oxygen uptake by sediment cores consistently

underestimated heat production, presumably because oxygen uptake did not measure anaerobic activity while the dehydrogenase assay did. Edwards and Rolley (1965) also found no correlation between oxygen consumption and dehydrogenase activity in sediments. That both anaerobic and aerobic bacterial activity could be assayed by TTC reduction was demonstrated by Olah (1972). He followed ATP levels and dehydrogenase activity in aerated and anaerobic incubation flasks enriched with powdered Phragmites. During aerobic incubation changes in dehydrogenase seemed to parallel changes in ATP, but anaerobic conditions yielded higher ATP concentration and lower dehydrogenase activity than was found in the aerated culture.

Sediments are a complex environment, and even anaerobic sediments may contain a community with populations other than bacteria (Fenchel, 1969). Does the dehydrogenase measurement assay the activity of these other organisms? Pamatmat and Bhagwat (1973) noted the presence of chironomid larvae in some of their samples but did not know whether or not their activity was included in dehydrogenase measurements. Packard (1970) and Curl and Sandberg (1961) homogenized animal tissues to assay dehydrogenase activity. Packard noted the necessity to disrupt not only the outer walls of zooplankton but also the mitochondria before the assay could be performed. Bacteria however can reduce TTC during normal growth (Eidus et al., 1959). On the basis of present knowledge it seems probable that the dehydrogenase assay measures activity of prokaryotic organisms only, unless

samples are homogenized.

The enzyme dehydrogenase was assayed during this study to determine aerobic and anaerobic, prokaryotic activity at different times of the year.

Uptake of radiotracers

The utilization of radioactive compounds by microorganisms is another approach to the estimation of microbial activity. The most common experimental design for utilization measurements is to test uptake at different concentrations of the radioactive substrate. These data are treated by one or more of the available linear transformations (Hall et al., 1972) of the Michaelis - Menten enzyme kinetics equation. Information may be obtained on the turnover time of the substrate (T), the maximum rate of substrate uptake (V_m), and a transport constant plus natural substrate concentration ($K_t + S_n$). If S_n can be determined independently the actual rate of uptake (U_n) may be calculated.

The original low substrate concentration application of Michaelis - Menten kinetics analysis to study active uptake by heterogeneous bacterial populations was by Wright and Hobbie (1965). The work of Parsons and Strickland (1962) was at substrate concentrations high enough that diffusion into algae probably occurred. Wright and Hobbie (1966) assumed respiratory losses of carbon as CO_2 would be negligible but many studies (Hall et al., 1972; Burnison and Morita, 1973; Crawford et al., 1974) have disproved this. Most experimenters

now use modified techniques which allow measurement of gross uptake, that is, the sum of assimilated or particulate uptake and respiratory losses.

There is, unfortunately, no theoretical justification for application of Michaelis - Menten kinetics to heterogeneous populations. The analytical technique, originally the Langmuir isotherm of Michaelis - Menten equation, was developed to describe relatively simple reactions of gases or enzymes. In fact, the equation has been found inapplicable in a number of studies in natural systems (Vaccaro and Jannasch, 1967; Hall et al., 1972; Kleiber, 1972; Crawford et al., 1974).

The use of it to analyze multispecies reactions with variable values of V_m and K_t was tested with a computer model by Williams (1973). He reported that $(K_t + S_n)$ and T are sensitive to deviations from the expected relation between uptake rate and substrate concentration, particularly at low concetrations, but V_m showed little change. Since natural substrate concentrations are often very low (1-50 μg/l), erroneous estimates for T would in turn effect U_n calculations.

Burnison and Morita (1973) tested the occurrence of competitive inhibition for amino acid uptake in Klamath Lake waters. Even at low substrate concentrations, competition was evident in some cases. This is in contrast to the report of Crawford et al. (1974), that competition between amino acids in estuarine waters seemed to be of little consequence. Burnison and Morita found that V_m was not effected by competitive inhibition, while T and $(K_t + S_n)$ were both in-

creased.

From the discussions of Williams (1973) and Burnison and Morita (1973), it is apparent that V_m is the most useful parameter for comparing heterotrophic uptake potential in different water masses because it is less sensitive than T and $(K_t + S_n)$, and therefore U_n, to changes in inhibitory interractions between substrates and to non-kinetic responses of uptake velocity to substrate concentrations. V_m is a "potential" uptake estimate though, and U_n and T_n (the actual rate of uptake and turnover) are the values required for estimates of carbon flux in an ecosystem. The actual rates for glucose have been used in this study as an index of microbial activity.

4. Introduction to the appendices

A number of diverse topics have been relegated to the appendices in an attempt to make the methods and results sections as concise as possible. It is hoped that comprehension of the work does not require reference to the appendices.

Appendices I - III are details of methods used to extract ATP in three different experimental situations.

Appendix IV includes preparation of enzymes used to measure ATP concentrations, and an example of a standard curve obtained for the assay.

Appendix V describes the approach and results of experiments designed to estimate the efficiency of the three ATP extraction procedures.

Appendix VI describes the approach and results of experiments designed to study the relation between biomass carbon and ATP.

Appendix VII outlines the effect of incubation time and oxygen concentration on the assay for dehydrogenase activity.

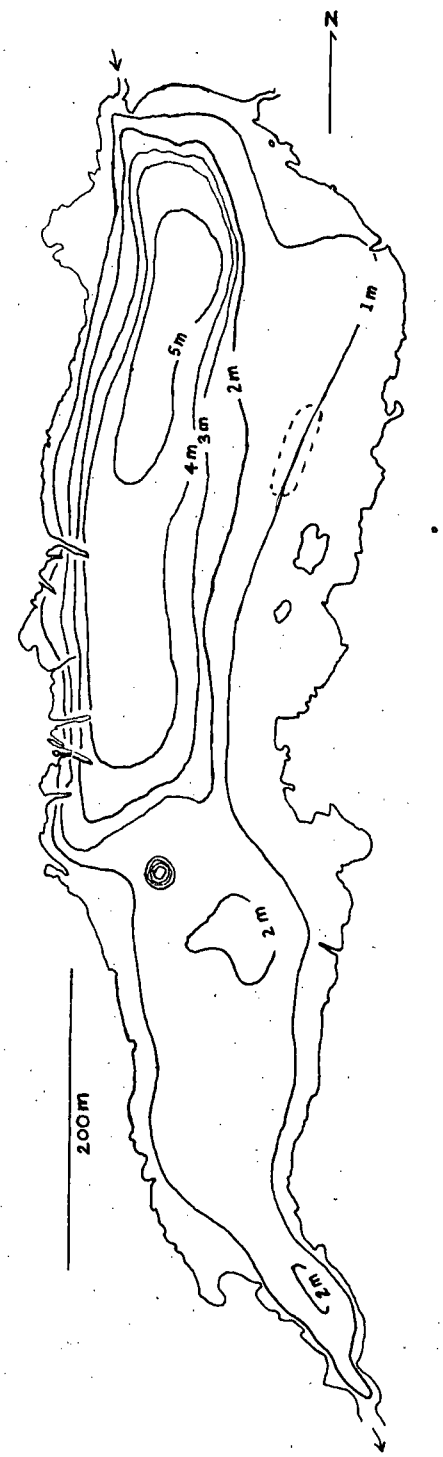
Appendix VIII gives the mathematical analysis used to calculate V_m, T, $(K_t + S_n)$, T_n and U_n in heterotrophic uptake studies.

MATERIALS AND METHODS

Sampling

Undisturbed sediment samples (Hargrave, 1969) were obtained along a 1 m transect at approximately monthly intervals (Figure 1). From each of four samples, three subsamples were taken with glass corers (12.5 cm long, 5.0 cm diameter). The cores were sectioned at the site and the upper 2 cm of sediment were transferred to a sterile jar. The mixed sample was kept on ice during transport, then stored at 2-3°. All analyses requiring fresh sediment (ATP, total organic matter, dehydrogenase activity and radiotracer experiments) were carried out within 2 - 3 days. Within 2 - 3 hours of sampling, interstitial water for glucose analysis was extracted and stored at -20° and sediment subsamples were lyophilized for later use (direct bacterial counts, ATP and carbohydrate analyses).

Figure 1. Marion Lake - morphometry and sampling area (- - - -).


N
5m
4m
3m
2m
1m
2m
2m
200m

Methods

Chemical analyses

Duplicate fresh samples for total organic matter were filtered (10 psi) through previously combusted (550°/1h) glass-fibre filters (Reeve-Angel 934 AH - 2.4 cm dia.). The samples were dried (80°/24h), weighed, combusted (550°/3h) then reweighed. Carbohydrate concentrations in the lyophilized sediments were measured colorimetrically with the phenol-sulphuric acid method (Gerchakov et al., 1972). The assay was standardized with β-D-glucose. Organic carbon content of the sediments was analyzed using a carbon analyzer (Beckman model 915).

Methane production was measured by analysis of the atmosphere above sealed sediment cores (obtained April 21, 1974), incubated at either 4° or 20° for 3 days, using gas chromatography.

To obtain interstitial water approximately 100 ml of mixed sediment were gravity-filtered through Whatman no. 1 filter paper at 4°. The filtrate was filtered through a Millipore GS membrane filter, pore size 0.22μm using a vacuum of 7 psi. The water was stored at -20° in clean test tubes.

Glucose concentrations in the interstitial water were determined enzymatically by the method of Hicks and Carey (1968). A fluorometer (Turner no. 110), equipped with a green phosphorescent lamp (GE-F4T5-G), and a constant temperature door (25°), was used to measure unknown glucose concentrations relative to standard solutions of β-D-glucose. Filters used were, on the excitation side, a Wratten 58, a 1% neutral density and a polaroid lens , on the emission

side, a Wratten 23A. Blanks were identical to standard or interstitial water samples except glucose-6-phosphate dehydrogenase was omitted from the reaction mixture. The detection limit for the assay is about 1μg glucose/l.

Direct counts of bacteria

Bacteria were counted in samples prepared from lyophilized sediments using a method developed by B. Kent Burnison (unpublished). All solutions were filter-sterilized. To 2 ml of distilled water plus 0.5 ml of 0.5 N KOH in a tissue homogenizer tube, was added 10mg of lyophilized sediment. The dry weight was determined by drying parallel samples (100°/24h). The sample was homogenized for 2 min at which time very few, or no, large particles remained. The mixture was transferred to a scintillation vial using 4 ml of distilled water and sonicated for 15 sec at 50-60 cps with an intensity of 50 (Bronwill Scientific Biosonik II). The sample was then adjusted to 100 ml in a volumetric flask with distilled water, and mixed. From this suspension 1.00 ml was transferred to a filtration apparatus and adjusted to 10 ml with water. The sample was filtered (10 psi) through a membrane filter (0.22μm) previously boiled in 0.1% sodium pyrophosphate for 2 min and rinsed with bacteria-free water. The filter was dried at 60°. One-half the area of a slide was coated with transparent glue. The dried filter was placed on this film, then cleared in acetone fumes, air dried and sealed with Permount.

The cells were stained with a solution of FITC using the buffer system suggested by Babiuk and Paul (1970). Cells

were stained for 30 min with a solution of 1.3 ml of 0.5 M sodium carbonate buffer (pH 9.6), 6.0 ml of 0.01 M potassium phosphate buffer (pH 7.2), 5.7 ml of 0.8% saline and 5.3 mg of FITC. The slides were washed in 0.5M sodium carbonate buffer (pH 9.6) for 20 min, and in 1% sodium pyrophosphate for 2 min.

Duplicate slides were prepared for each sample and cells in 15 ocular grid fields per slide counted under oil immersion using a Reichert microscope equipped with a HBO 200 mercury vapour lamp.

ATP Analysis

Determination of unknown ATP concentrations requires extraction of the compound and assay of its abundance in the extract. A correction factor for extraction efficiency must be determined. The methods summarized below are presented in detail in appendices I - VI.

(a) Total ATP extraction from fresh sediments

The method used was that of Lee et al. (1971a). Three replicate 3 ml samples of mixed sediment (150-200 mg dry wt.) were extracted for each monthly sample. The main features are extraction of ATP with ice-cold 0.6 N H_2SO_4 and removal of interfering cations with cation exchange resin (for details see appendix I).

(b) Total ATP extraction from lyophlized sediments

In an attempt to check the fresh sediment ATP values obtained each month, lyophilized sediments which had been stored for up to 1 year at -20°, were extracted on one occasion and assayed using single enzyme and standard ATP

preparations.

Bromosuccinimide extraction was found to be the most efficient of eight procedures tested (Appendix II) and was used for subsequent experiments (for details see appendix II).

(c) Microbial ATP extraction from fresh sediments

Sediment samples were cleaned of large organisms (< approximately 30μm) using micropipettes in an attempt to measure microbial ATP. The separation method and subsequent ATP extraction is described in appendix III.

(d) ATP assay

The method used was the luciferin-luciferase bioluminescence assay. Preparation of the enzymes in appropriate buffer solutions, addition of standard or unknown ATP samples and measurement of light production are described in appendix IV.

(e) Calculation of biomass carbon concentration

The equation used for conversion of raw assay data to biomass carbon per gram dry weight sediment was:

$$\text{ATP assayed} \times \frac{1}{\text{sample dry weight}} \times \text{dilution factor} \times \frac{1}{\text{extraction efficiency}} \times \frac{\text{biomass carbon}}{\text{ATP}} \times 10^{-3}$$

The assayed ATP had units ng/ml; dry wt. was in grams; the dilution factor was 5 for experiments on lyophilized sediments and microbial ATP and (50 x extract volume (ml) x $\frac{1}{2}$) for total ATP in fresh sediments; extraction efficiency was .125, .106 and .205 for total fresh, total lyophilized and microbial fresh ATP extractions respectively (Appendix V); C/ATP was 250 (Appendix VI); the factor 10^{-3} converted the

data from ng to μg.

Dehydrogenase activity

Dehydrogenase activity was measured using a method modified from that of Sorokin and Kadota (1972). The reagent consisted of a solution of 0.1 M tris buffer, adjusted to pH 7.5 with 2N HCl, to which was added 0.9 M 2, 3, 5-triphenyltetrazolium chloride (TTC). This reagent was stored at 2° in the dark.

To perform the assay, 5 ml of fresh mixed sediment was pipetted into a 125 ml erlenmeyer flask. Control samples were routinely steam-killed then treated as normal samples. Blanks of this type were equivalent to adding formalin, or to leaving TTC out of the reagent. To each sample 10 ml of the TTC reagent was added. Rubber stoppers with inlet and outlet tubes were fitted and the samples were bubbled with nitrogen for 10 min to remove oxygen. The flasks were sealed, wrapped in aluminum foil and incubated 4 h and 24 h at 30° with shaking at 100 RPM. Four replicates and one control were used for each incubation period.

Extraction of the water-insoluble formazan was with an acetone-methanol solution (9/1, V/V). Twenty-five ml was added to sample flasks, shaken for 1 h, then filtered through cheesecloth under subdued light conditions. The filtrate was adjusted to 50 ml with extractant poured through the sediment particles. Absorption was measured at 540 nm against an acetone-methanol blank.

A standard curve was obtained by dissolving 2, 3, 5-

triphenyl formazan in extractant solution.

The effects of incubation time and oxygen on dehydrogenase activity measurements are described in appendix VII.

Heterotrophic uptake of ^{14}C-glucose

All experiments were performed using fresh mixed sediments, diluted with autoclaved lake water (final dilution 50:1). Standard solutions of ^{14}C-glucose (U), 215 mc/mM (New England Nuclear), were stored frozen in filter-sterilized distilled water. These solutions were adjusted to 0.1 μc/ml and 1.0 μg glucose/ml prior to use. Two live and one control (formalin-killed) samples were equilibrated for 1 h at lake temperature after dilution. The radiotracer was then added at four solute concentrations (10, 40, 100, 200 μg/l), and incubated under subdued light conditions for 40 or 60 min with shaking at 100 RPM. The incubation flasks, ^{14}C-CO_2 trapping system and filtration of the particulate fraction were described in detail by Kleiber (1972).

Bray's scintillation solution (Bray, 1960) was used for sample radioactivity measurements. Quench curves were prepared with ^{14}C-toluene (417,000 dpm/ml, New England Nuclear) and chloroform in Bray's solution for the liquid scintillation counters used (Nuclear Chicago Mark I and Isocap 300), by the external standard ratio (ESR) method.

Efficiency of ^{14}C-CO_2 counting was determined by comparing the sample ESR to the quench curve. This value was further corrected for ^{14}C-CO_2 trapping efficiency (mean of 84% in 4 samples using ^{14}C-Na H CO_3).

The efficiency of counting particulate material sus-

suspended with Aer - O - Sil (Degussa Chemicals) in Bray's solution was calculated from an efficiency versus weight-of-sediment curve. The curve was obtained by incubating sediment with ^{14}C - glucose overnight. Aliquots of different volumes were filtered through tared membrane filters (0.22 μm pore size). The samples were dried (100°/24 h) and dry wt. determined. One-half of the samples were combusted at 900° in a tube furnace (Lindberg Hevi-Duty model 55035). The evolved ^{14}C-CO_2 was collected in 8 ml of ethanolamine and ethylene glycol monomethyl ether (1/7, V/V) and counted in 10 ml of toluene fluor (0.5% PPO, 0.03% POPOP). The method has been described by Burnison and Perez (1974). Suitable quench curves were prepared for the toluene scintillation solution. The remainder of the samples were counted in the usual way, that is dissolution of the filters in Bray's solution and suspension with Aer - O - Sil.

Comparison of the dpm per g dry wt. obtained by combusting samples, to cpm per g dry wt. by suspending samples, yielded an efficiency-weight curve. Sediment counting efficiency ranged from 54.2 - 58.7%.

The methods used to calculate V_m, T_t, U_n and T_n are described in appendix VIII.

Statistical methods

Seasonal data obtained from ATP and dehydrogenase experiments and bacterial counts were analyzed for differences between the means by analysis of variance (anova). Logarithmic transformations were required for dehydrogenase data and for the total fresh ATP data to correct hetero-

geneous variance within the data. Specific means were then compared at the 1% probability level using the new multiple range test of Duncan (1955).

Heterotrophic uptake data were not statistically analyzed due to the complexity of obtaining information on the errors involved. Kleiber (1972) has proposed a method to handle this problem.

RESULTS AND DISCUSSION

1. Physical and chemical data

The sediments in Marion Lake are a deep flocculent ooze except near the springs and the inlet. The sample area was free of macrophytic growth. Macroscopically the sediments contain some large invertebrates, algal colonies and mats, chironomid tubes and leaf and twig fragments. The sediment particles as seen microscopically are a complex conglomeration of mineral and organic matter with a size range of 20 - 400μm although most are between 70 - 200μm (Figure 2). The particles shown were prepared by critical point drying and coated twice with gold, then examined using a Cambridge Stereoscan scanning electron microscope.

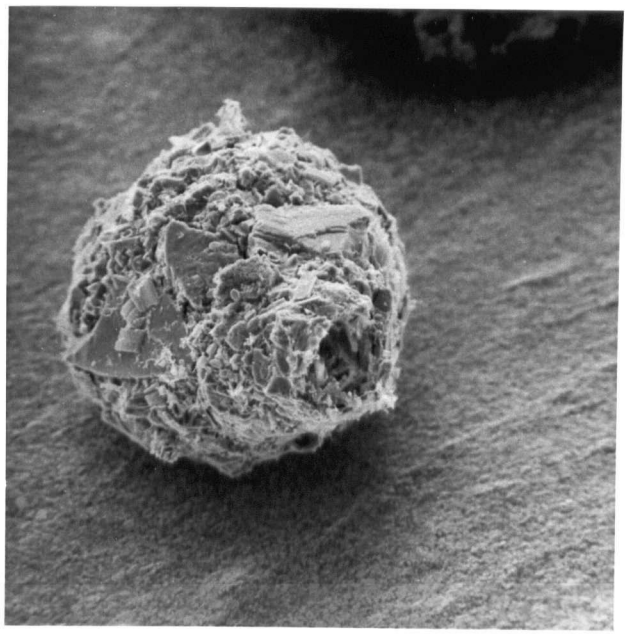
Temperature during the study was typical of Marion Lake (Efford, 1967), although the warming trend usually evident in May was delayed in 1974 (Figure 3a).

Changes in total organic matter in the sediments were not dramatic, fluctuating around a mean of 414 mg/ g dry wt. except in September (Figure 3b). The maximum deviation within samples was 3.6% of the mean. The September peak may be due to algal or invertebrate growth within the lake, or allochthonous inputs although the latter do not peak until late fall (Odum, unpublished). Organic carbon (dissolved plus particulate) was about 20% of the sediment dry weight.

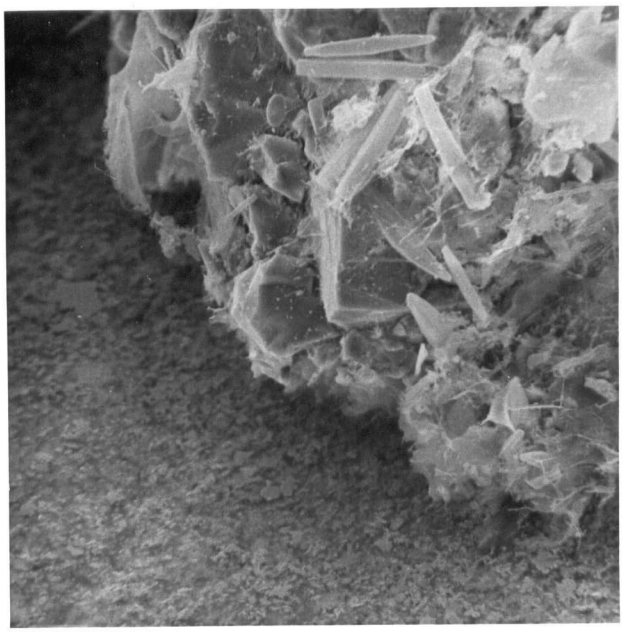
Glucose concentrations in the interstitial water were

high in December and January (about 50µg/l) and very low in April and May (5-20µg/l) (Figure 3c). The low spring concentrations may reflect heterotrophic activity of bacteria, a possibility which is assessed in section 4. Total carbohydrates in the sediments fluctuated irregularly with an annual range of 135-184 mg/g dry wt. or 32.6-44.5% of the total organic matter. These carbohydrate concentrations are approximately twice as high as those previously found by Hall and Doel (1972) and may reflect variations in methodology.

Figure 2. Scanning electron micrographs of Marion Lake sediment particles.

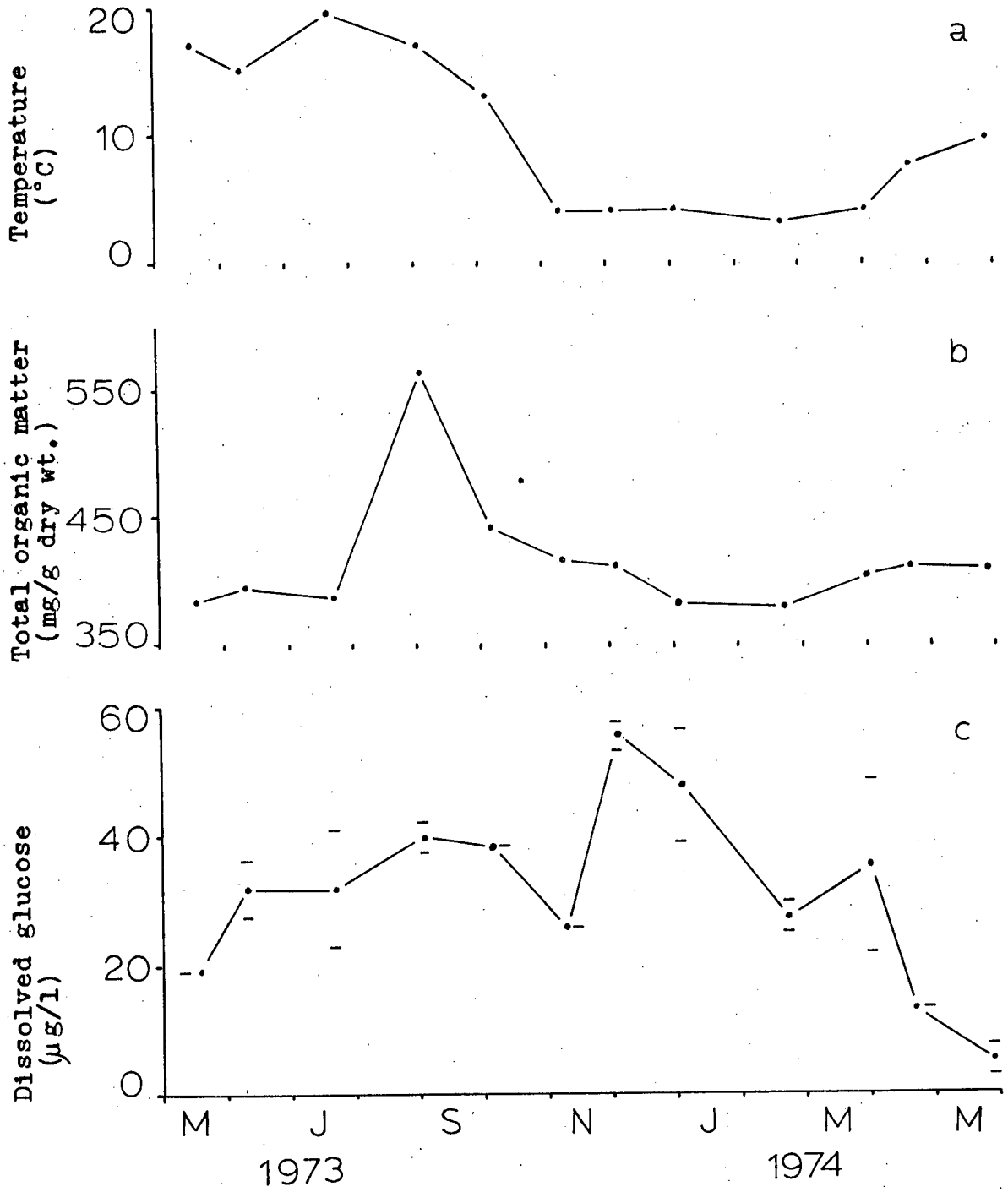


100μm



10μm

Figure 3. Seasonal changes in temperature (a), total organic matter (b) and dissolved glucose (c) in the sediment (bars indicate standard deviation of each sample).


a
Temperature (°C)
20
10
0
b
Total organic matter (mg/g dry wt.)
550
450
350
c
Dissolved glucose (µg/l)
60
40
20
0
M
J
S
N
J
M
M
1973
1974

2. Biomass estimates

(a) Total sediment biomass estimated by ATP analysis

Recovery of cellular ATP added to sediments was low in this study (12.5% for the fresh sediment extraction method - Appendix V) compared to other work that has been reported. Lee et al. (1971a) obtained recoveries of ATP added as bacteria of 24-85% for nine different sediments. Ernst (1970) reported recoveries of cellular ATP and pure ATP added to marine sediments of 60-97% and 61-104% respectively. Low recovery efficiency in Marion Lake sediments may be due to high adsorptive capacity, however, pure ATP added to these sediments was recovered at 52-63%. This indicates the poor recovery is related to extraction from the cells in addition to subsequent recovery from the extraction mixture.

Cell carbon to ATP ratios vary considerably between species (Table I). Although ATP per cell also varies a great deal within the life cycle of a single species (Ausmus, 1973; Lee et al., 1971b), the ATP to carbon ratio may remain relatively constant unless energy sources are completely exhausted (Harrison and Maitra, 1969; Holms et al., 1972). The conversion of ATP concentrations to cell or biomass carbon by a factor of 250 (Hamilton and Holm-Hansen, 1967) was supported by experiments during this study (Appendix VI). It is recognized that biomass carbon estimates thus obtained are subject to error but it is the best estimate available.

The total biomass carbon at 1 m water depth in Marion

Table I. Carbon to ATP ratios from various sources.

references	organism	growth stage	C/ATP ratio range (mean)
Ausmus (1973)	bacteria-5spp.	exponential	357-833(500)
	fungi-8 spp.	"	179-313(233)
	actinomycetes-6 spp.	"	179-238(217)
	algae-6spp.	"	104-278(143)
Berland *et al.* (1972)	algae-7spp.	"	180-592(366)
Hamilton and Holm-Hansen (1967)	*Vibrio spp.*	stationary[1]	1220
		exponential	153
	bacteria-7spp.	exponential and stationary	91-333(250)
Holms *et al.* (1972)	*Escherichia coli*	variety of growth rates	485-808
Appendix VI	bacteria-3spp.	stationary	112-439(281)
	Streptomyces spp.	"	376
	Anacystis nidulans	"	218

[1] The authors assumed carbon content per cell was the same in stationary and exponential phase.

Lake sediments, as measured by ATP analysis, is presented in Figure 4b. This graph summarizes data obtained by extraction of fresh sediment samples. Two features of these data are the peaks in May each year and the homogenity of the total biomass throughout the remainder of the year. A posteriori comparisons among means by the new multiple range test (NMRT) (Table II) indicate that biomass in May, 1973 was significantly higher ($P < .01$) than on any other sampling date. Biomass estimates in June, 1973 and May, 1974 were not significantly different (joined by a line), but are significantly higher than samples in July to April. All other samples were not significantly different from each other (joined by a line).

Analysis of total ATP in lyophilized sediments confirmed the peaks in May each year but introduced much more variability between other samples (Figure 4a, Table II). The increased variability compared to fresh sediment extraction and the high biomass in May, 1974 and December, 1973 may result from the fact that the sample size was only 20 mg dry wt. for the lyophilized samples compared to 150-200 mg dry wt. for the fresh samples. The biomass estimate is generally higher using lyophilized sediments compared to fresh sediments (Figures 4a, b), but this may be due to an inaccurate estimation of extraction efficiency for lyophilized sediments. The efficiency of 10.6% was based on only one experimental organism, compared to fresh extraction efficiency which was measured using six organisms (Appendix V). The

Figure 4. Seasonal changes of total biomass carbon in lyophilized (a) and fresh (b) sediments and of microbial biomass carbon (c).

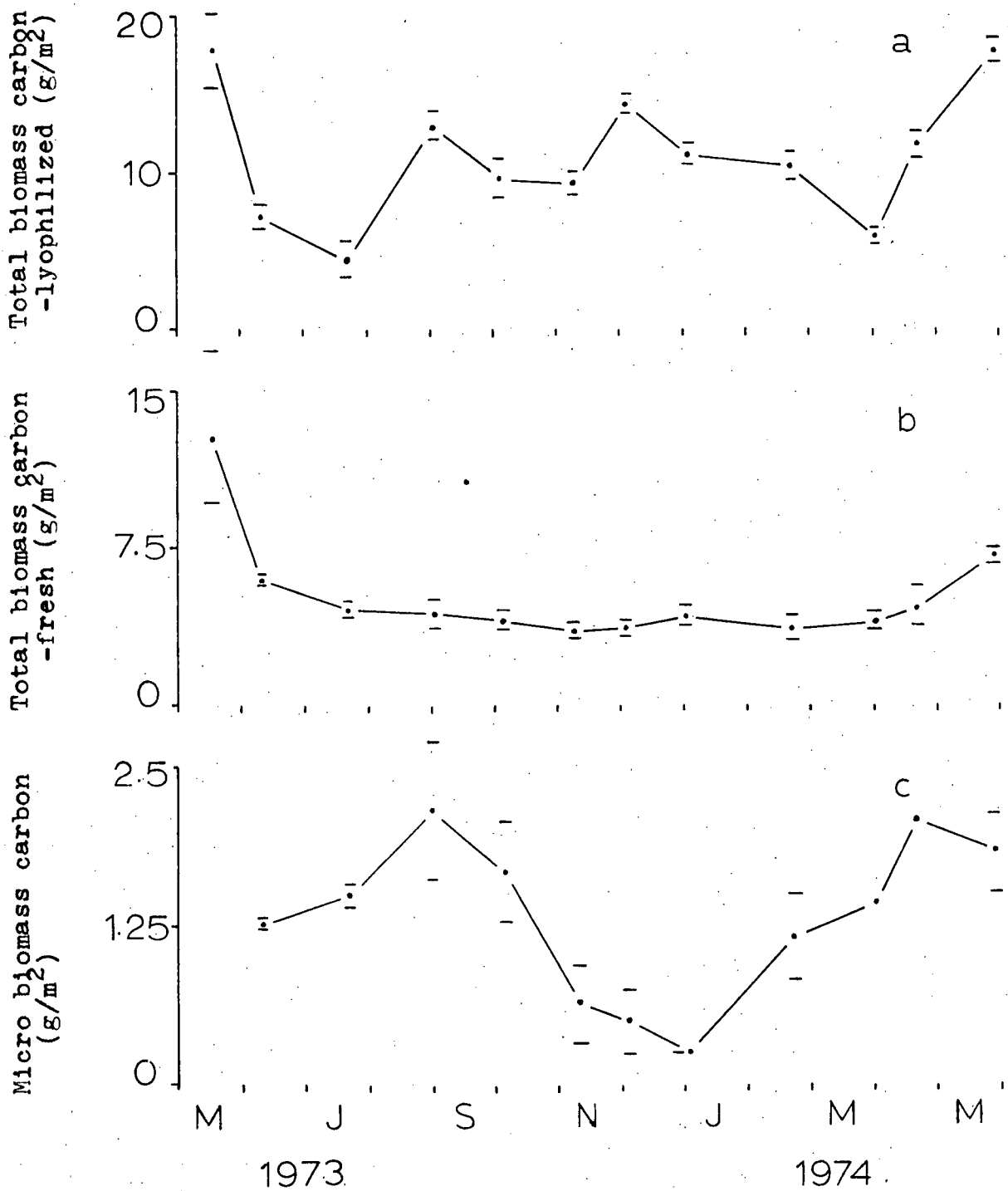

Total biomass carbon -lyophilized (g/m²)
20
10
0
a
Total biomass carbon -fresh (g/m²)
15
7.5
0
b
Micro biomass carbon (g/m²)
2.5
1.25
0
c
M
J
S
N
J
M
M
1973
1974

Table II. Statistical analyses of biomass data.

1. Total fresh sediment ATP analysis

Analysis of variance (Anova)

source	df	ss	ms	F
among samples	11	.6635	.0603	7.5044**
within samples	23	.1849	.0080	
total	34	.8484		

$F_{.01(10, 23)} = 3.21$ ** significant at $P<0.01$

New multiple range test (NMRT) - Samples are ranked by increasing mean values. Lines join samples not significantly different at the 1% level.

Nov Feb Dec Oct Apr 1 Sep Jan Jul Apr 21 June May 74 May 73

2. Total lyophilized sediment ATP analysis

Anova

source	df	ss	ms	F
among	11	78.6379	7.1489	11.6124**
within	13	8.0032	.6156	
total	24	86.6411		

$F_{.01(10, 13)} = 4.10$

NMRT

Jul Apr 1 June Nov Oct Feb Jan Apr 21 Sep Dec May 73 May 74

(continued)

3. Microbial ATP analysis

Anova

source	df	ss	ms	F
among	8	3.8935	.4867	4.5400*
within	9	0.9648	.1072	
total	17	4.8583		

$F_{.01(8, 9)} = 5.47$

$F_{.025(8, 9)} = 4.10$

* significant at $P<0.025$

NMRT

Jan Dec Nov Feb June Jul Oct May 74 Sep

Two samples in April, 1974 were omitted from the statistical analyses because only single values were obtained.

4. Microscopic bacterial counts

Anova

source	df	ss	ms	F
among samples	11	44.498	4.045	20.733**
among slides	12	2.341	.195	.567NS
within slides	336	115.576	.344	
total	359	162.416		

$F_{.01(6, 12)} = 4.8$

$F_{.01(12, \infty)} = 2.2$

$F_{.05(12, \infty)} = 1.8$

NS not significant at $P<.05$

NMRT

Oct Jan Nov Apr 1 May 74 Feb May 73 Apr 21 Sep June Dec Jul

results suggest that quick freezing of samples at the site until lyophilization and ATP analysis could be performed, would be a good method for storing samples.

Concentrations of ATP in sediments have been reported by other workers (Table III). Ernst (1970) converted his data to biomass carbon using a C/ATP ratio of 50/1. To compare his data and that of Lee et al. (1971a) and Karl and LaRock (1974) to data obtained during this study, a C/ATP ratio of 250 was assumed. The main features of the estimated biomass concentrations are the low values in marine sediments and the high values in Marion Lake. Concentrations of biomass carbon of 100μg/g are equivalent to about 2×10^{10} bacteria/g (Appendix VI) which is higher than plate count methods indicate for many sediments (eg. Bianchi, 1973) but much lower than direct counts indicate (eg. Antipchuk, 1972). Any sediment with 100μg biomass would contain virtually no organisms other than bacteria. This would be unusual even in anaerobic environments (Fenchel, 1969). Low values in marine sediments may indicate either very small living populations or that the C/ATP ratio is incorrect, although even a 10 fold increase in the ratio would not have too great an effect. It is hard to evaluate the data in Table III since no information was reported on the nature of the sediment communities, but Marion Lake sediment biomass has been independently estimated by counting organisms of various species (Efford, unpublished). This offers a unique opportunity to compare ATP-biomass carbon estimates to enumeration-biomass carbon estimates. The mean biomass of microorganisms and invertebrates in

Table III. Sediment ATP-biomass carbon data.

reference	location	reported data	biomass carbon (μg/g dry wt.)[1]
Ernst (1970)	6 sites North Sea (28 - 345m)	6.6 - 33.4 μg biomass carbon/ml	165 - 835
Lee *et al.* (1971a)	9 Wisconsin lakes	0.34 - 9.5 μg ATP/g	82 - 2,375
Karl and LaRock (1974)	beach sand	145 - 228 ng ATP/g	36 - 57
	Atlantic ocean (4000 m)	9 - 10.3 ng ATP/g	2 - 3
This study	Marion Lake (seasonal)		2,810 - 12,600

[1] Assuming C/ATP is 250 and, for Ernst's (1970) data, that 1 ml = 200 mg dry wt.

Marion Lake sediments by enumeration is approximately 4-6g C/m^2. ATP-measured biomass carbon in fresh samples ranges from 3.5 - 12.8 g C/m^2 with an integrated mean of 4.7 g C/m^2. Discrepancies may be due to inclusion in the enumeration data of large invertebrates (eg. Sialis) which were removed prior to ATP extraction, variations in the benthos from year to year (not all organisms were enumerated during the same 12 month period), variations in the depth of sediment sampled (eg. in this study the top two cm) and variations in different parts of the lake (not all counts were obtained at 1 m water depth or along the same transect as were ATP data). Despite all these inconsistencies, the data suggest that ATP analysis does provide a quick approximation of sediment biomass in Marion Lake and that the data presented in Table III are approximately correct.

(b) Microbial biomass in the sediments estimated by ATP analysis

Biomass of microorganisms less than approximately 30 μm diameter was quantified by removing larger organisms from sediment samples and measuring residual ATP. This method is subject to all the assumptions concerning efficiency of extraction and biomass carbon to ATP ratios discussed in the previous section.

Seasonal changes in microbial biomass, including bacteria, fungi, actinomycetes, some algae and a few protozoa, do not parallel changes in total biomass. Peaks occurred in late summer and early spring with a nine-fold

difference between maximum and minimum concentrations compared to an approximately four-fold variation in total biomass (Figures 4b, c). Microbial biomass ranged from 0.25 - 2.16 g C/m^2 (January - September). Statistical analysis of the data support the conclusion that warm months have a higher microbial biomass than winter months (Table II). Data for April 1 and 21, 1974 were excluded from the analyses because replicate samples were lost. Temperatures in July, October, September, 1973 and May, 1974 were higher than 9°. Mean microbial biomass is significantly higher in these months than in January, December and November when the temperature was 4°. June biomass data are also higher than in winter months but not at the 1% confidence level.

Seasonal changes in ATP associated with plankton in the 0.22 - 250 μm size range were reported by Rudd and Hamilton (1973). Their data should represent biomass more equivalent to total ATP-biomass than microbial ATP-biomass in this study considering the size range, but in fact resembles the latter (Figure 4c). This reflects the stability of total benthic compared to planktonic communities (Cameron, 1973).

Biomass of microorganisms may be calculated for sediment at 1 m water depth using enumeration data. The annual mean biomass of bacteria is 0.61 g C/m^2 (next section), of protozoa is 0.0013 g C/m^2 (Kool and Stachurska, unpublished) and of algae is approximately 0.23 g C/m^2 (calculated from Greundling, 1971). Actinomycetes are included in the bacterial counts. Fungi have been quantified

only by plate count methods and their numbers are low compared to total bacterial counts (1.8 - 8.2 x 10^7 fungal propagules/m^2 - Chang, unpublished; compared to 10^{13} bacteria/m^2). Dick (1971) suggested many fungi were present as spores of allochthonous origin. Very few healthy fungal filaments were seen during this study except on decaying fish in the lake or on sediments in nutrient-enriched microcosms. A rough estimation of fungal biomass may be obtained by assuming a weight of 5 x 10^{-12}g C per propagule (Shields et al., 1973, reported a mean diameter of 2.5μm for fungi in soil; an average hyphal of spore length of 10μm was assumed here to allow the calculation to be made). Cultivable, fungal biomass is then 9-41 x 10^{-5} g C/m^2. The sum of the enumeration-microbial biomass is 0.84 g C/m^2. The biomass of protozoa and fungi is negligible in this system. The annual mean ATP-measured microbial biomass is 1.28 g C/m^2, indicating the C/ATP ratio may be inaccurate or that the enumeration data underestimate the biomass. The discrepancy is even greater than this indicates because a fraction (large filaments, colonies and diatoms) of the algae were removed prior to ATP analysis.

(c) Bacterial biomass in the sediments estimated by microscopic counts

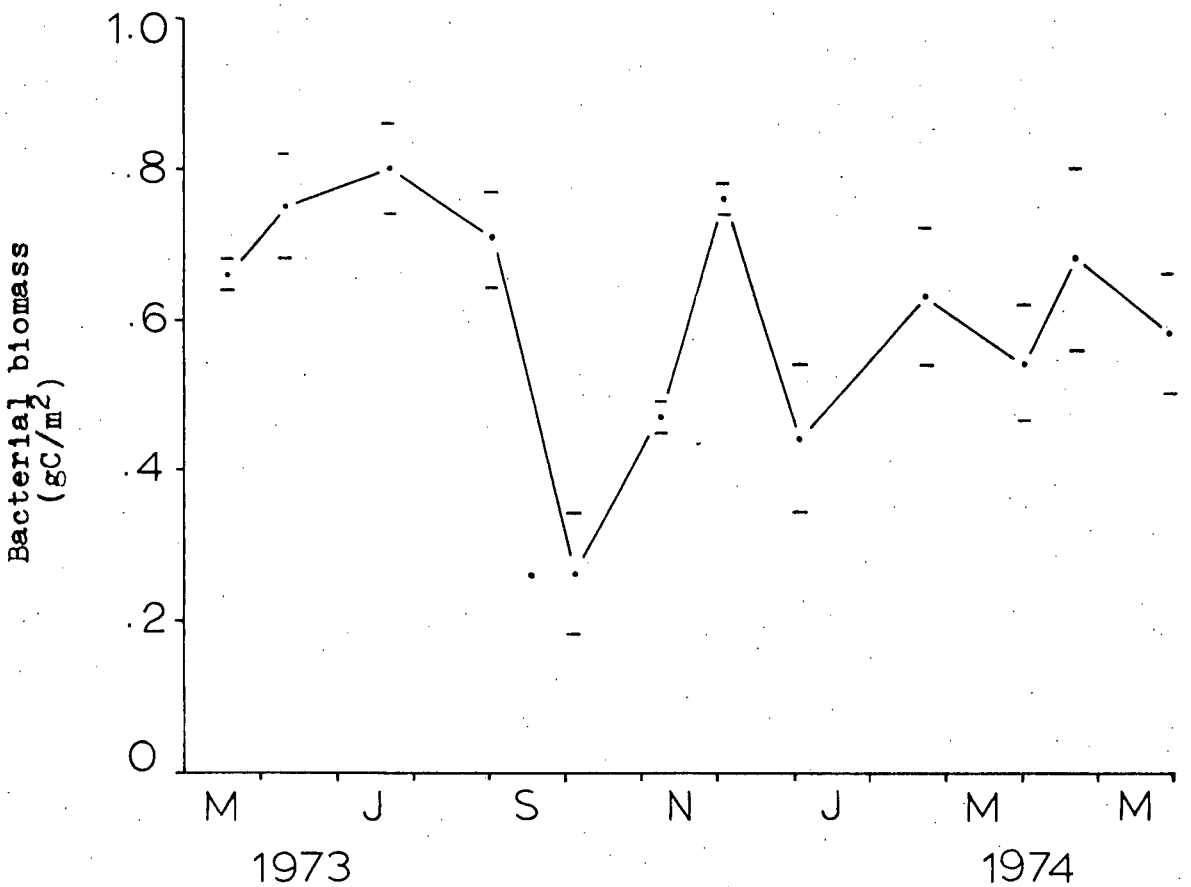
Bacterial biomass was estimated by counting cells in known dilutions of sediment and converting the numbers to biomass assuming that cells have a density of 1g/cc, are 80% water and that 50% of the dry wt. is carbon (Shields et al., 1973). Another requirement for this calculation is the average size per cell, but this is difficult to measure in

sediment systems.

Some microbiologists have measured bacterial cells in soil. These data were converted here to give an average of 1.9×10^{-14}g dry wt./cell (Bae et al., 1972), 5.7×10^{-14} g/cell (Babiuk and Paul, 1970) and 1.6×10^{-14}g/cell (Zvaginsev, 1973). Mean sizes for sediment bacteria measured by Antipchuk (1972) are 3.7×10^{-13}g/rod and 5.8×10^{-14} g/coccus. Burnison (unpublished) suggested a mean volume of $0.36 \mu m^3$ for Marion Lake sediment bacteria which corresponds to 7.2×10^{-14}g/cell. During this study very large spirochaetes (50 - 100μm length) were seen a few times, but never while counting bacteria. Cells up to 5.6×10^{-12}g ($4 \times 3 \mu$m) were more common while at the other extreme cells weighing 2.0×10^{-14}g ($0.8 \times 0.4 \mu$m) were noted. Most bacteria were 3.9×10^{-14}g - 2.4×10^{-13}g (1 - 1.5 x .5 - 1μm). Not enough cells were measured to determine an accurate mean, but 10^{-13}g dry wt./cell, or 5×10^{-14} g C/cell was chosen as a representative value.

Bacterial biomass peaked in July and was at a minimum in October (Figure 5). July biomass data are significantly higher than only those in October, January, November and early April (Table II). With the exception of the high biomass in December the seasonal trend was a maximum in early to mid-summer, a rapid decline in early fall then a slow recovery during the winter. Plate counts were similar in pattern except that the crash occurred in August in 1969 (Fraker, unpublished).

Figure 5. Seasonal changes of bacterial biomass carbon.

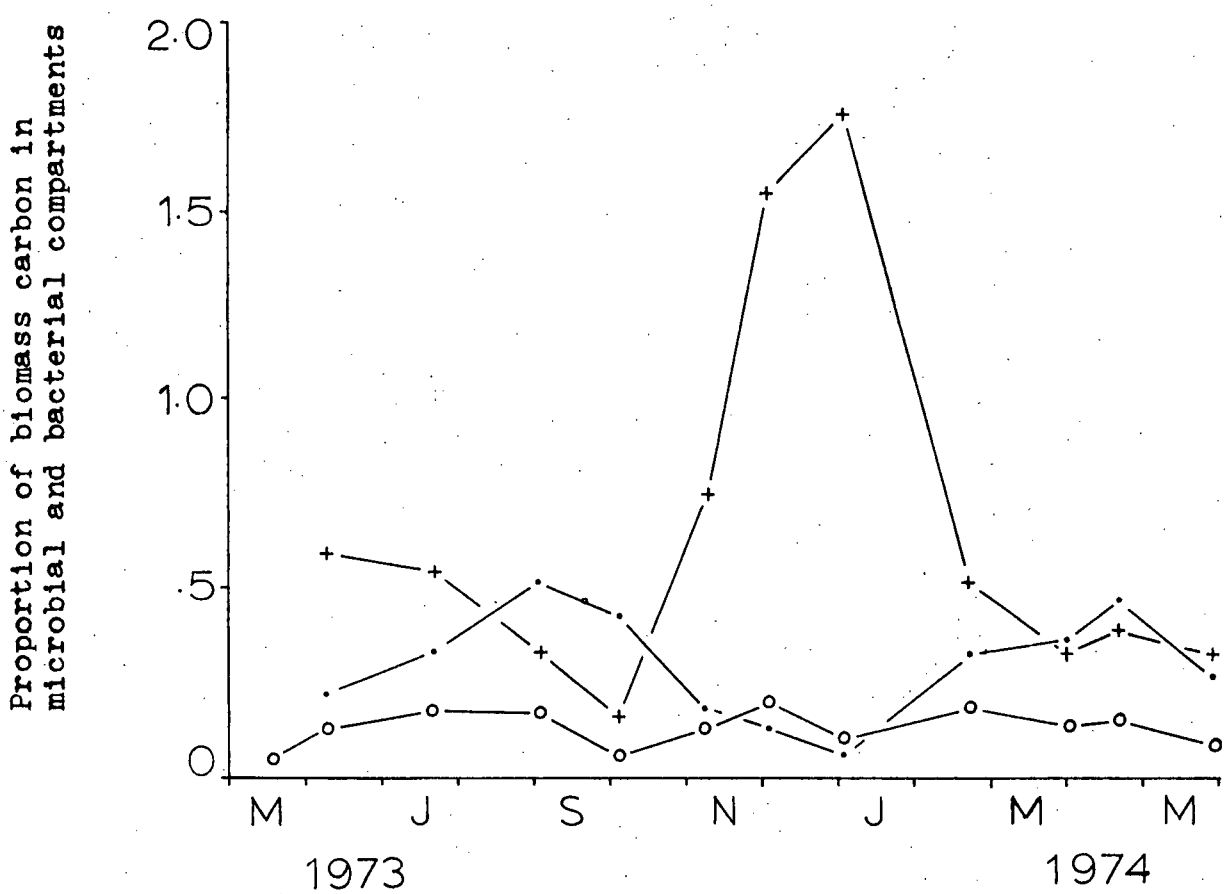

1.0
.8
.6
.4
.2
0
Bacterial biomass
(gC/m²)
M
J
S
N
J
M
M
1973
1974

Most published estimates for bacterial biomass in sediments are low because they were based on plate counts. Data are usually reported as numbers per g so the conversion factor of 5×10^{-14} g C/cell was applied to information reported by the authors cited below. Zobell (1963) reviewed the literature and found bacterial biomass estimated from 5×10^{-7} µg C - 50 µg C/ g sediments. Khiyama and Makemson (1973) used light microscopy and found 1 - 10 µg C/g in beach sands. Surface sediments in fish ponds were found by Antipchuk (1972) to contain approximately 1000 - 2000 µg C/g.His biomass estimates for one sample at each of six locations in three seasons tend to peak in spring or summer then decline in fall, a pattern not unlike that in Marion Lake. The biomass of Marion Lake sediment bacteria ranges from 340 - 650 µg C/g (January - July). These estimates are much higher than reported concentrations prior to the last decade, but that they are not overestimates is suggested by the microbial ATP data, and the similarity with previous direct counts in Marion Lake sediments (Ramey, 1972; Burnison, unpublished).

(d) Relations between biomass estimates

The various biomass data may be compared by examinimg proportional changes in each fraction relative to the others (Figure 6). Bacterial biomass fluctuates between 5 -20% of the total biomass, reflecting changes in the bacterial concentration except when total biomass estimates were high in May. The microbial fraction of the community

Figure 6. Seaonal changes in the proportion of sediment biomass carbon in bacterial compared to total biomass (o——o); bacterial compared to microbial biomass (+——+); microbial compared to total biomass (·——·).


Proportion of biomass carbon in microbial and bacterial compartments
2·0
1·5
1·0
·5
0
M
J
S
N
J
M
M
1973
1974

is high in the summer and low in midwinter, increasing during late winter to another peak in early spring. This pattern resembles that for epibenthic algae at 1 m (Greundling, 1971). The proportion of bacteria in the microbial biomass decreases during the summer, rises sharply in the late fall, dropping again in late winter. This fits a model in which the bacterial contribution is diluted by seasonal increases in the algal biomass.

In December and January, bacteria were more than 150% of the ATP-microbial biomass. This suggests bacterial counts overestimated the bacterial population or that the microbial population was at a physiological low, probably with a C/ATP ratio greater than 250. Fast temperature declines to 0° were shown by Cole et al. (1967) to cause a sharp rise in C/ATP ratios in E. coli. If this applies to natural microbial communities living at 4° for several months, then low ATP concentrations, and therefore underestimates of the community biomass may be expected. The subsequent increase in microbial ATP in February may reflect not only the increase in algal biomass (Greundling, 1971), but also acclimatization of the bacteria. Such a possibility is suggested by the data of Cole et al. (1967).

Comparison of the ATP-total biomass data (Figure 4b) to seasonal variation of the enumeration data (Efford, unpublished) reveals another discrepancy. ATP-biomass peaks in May (12 g C/m^2) and is almost constant for the remainder of the year (4 g C/m^2). Enumeration data are low in spring (about 4 g C/m^2) but constant for the remainder of the year (about 6 g C/m^2). ATP analysis using a C/ATP of ratio of 250

appears to be hypersensitive in May. Cole et al. (1967) reported periods of over- and under-production of ATP in E. coli. The C/ATP ratio decreased during high growth but then increased despite continuing high growth rates. If this is generally true (Holms et al., 1971, reported conflicting data), and considering that prior to May the benthos has been restricted by low temperatures, and that in May respiration in Marion Lake sediments increases exponentially (Hargrave, 1969), then over-production of ATP might occur. Using a C/ATP ratio of 250 would overestimate the community biomass in such circumstances.

One conclusion from this discussion is that ATP is an adequate indicator for biomass at "average" physiological conditions, but under periods of stress, ATP data may be confounded by representing both biomass and activity. Published data for natural systems support this. Holm-Hansen and Paerl (1972) found high primary production, high ATP-biomass and low algal biomass in surface waters of Lake Tahoe, but at 80 m, recorded low production, low ATP-biomass and high algal biomass. Rudd and Hamilton (1973) reported a similar phenomenon in Lake 227 of the Experimental Lakes Area, Ontario. ATP may then be thought of, not as measuring biomass at any instant, but measuring the potential biomass through time. An example is the bacteria in Marion Lake sediments. In January, microbial ATP is low (Figure 4c) and bacterial biomass is proportionally high (Figures 5,6). At any instant in time more bacterial carbon is available

to grazers than ATP data indicate. However, if bacteria are grazed at this time, biomass replacement may be slow due to temperature (or other factor) limitations. Such replacement occurs within a few days at warm temperatures (Fenchel, 1970). In September, ATP-microbial biomass in the sediment is ten-fold higher but enumeration-microbial biomass is only two-fold higher than in January. The suggestion is that in September there is not twice as much potential microbial biomass available for consumption, but up to ten times as much. Stated another way, this means that in September there should be 5 times more biomass available if the system is stressed than is indicated by direct count data.

This prediction could be tested by increasing grazing pressure on the microbial population to find their maximum growth rate. Hargrave (1970) did this type of experiment using a range of densities of Hyalella azteca. Assuming that his measurement of bacterial respiration using antibiotics approximates bacterial growth or production rates, then his data may be applied to the problem. Maximum stimulated bacterial respiration was about 13 times that in fresh, unaltered cores. Considering the inconsistencies in temperature, time, etc. between the experiment and the model it appears that ATP is in fact a measure of both biomass and activity, or simply biomass potential. Under average conditions a C/ATP ratio of 250 represents biomass. Under stress conditions such as intense grazing, cold temperatures or rapid temperature increases the cellular ATP balance is changed and, by definition, ATP becomes an activity indicator.

Interpretation of ATP-biomass data may therefore be difficult. High values may reflect large, inactive populations or small, productive populations. In studies concerned with trophic dynamics, however, more concise information may not be required.

3. Activity estimates

(a) Dehydrogenase activity measurements

Dehydrogenase activity in the sediments is higher in summer than any other time of the year (Figure 7, Table IV). Assays incubated for 24 or 4 hours usually showed similar directional response to changes in sediment respiration, but the absolute rate of response was greater using the short incubation time. This was expected (Appendix VII). The range of data for dehydrogenase activity (.0655-.6825 mg formazan/g.h) for the two incubation times over the 12 months is similar to the range reported by Pamatmat and Bhagwat (1973) for different locations in Lake Washington (.13-.42 mg formazan/g.h, converting their absorption readings to formazan equivalents), although their methodology was quite different.

The dehydrogenase data probably reflects aerobic and anaerobic potential activity of prokaryotes in the sediments since the samples were not homogenized. The suggestive evidence for this was presented in the Introduction. This observation may explain the lack of correlation between oxygen uptake and dehydrogenase activity in some natural samples. Howard (1972) reported soil dehydrogenase activity was always less than that predicted by oxygen uptake data. Pamatwat and Bhagwat (1973) reported that sediment oxygen uptake was less than that predicted by dehydrogenase activity. It is suggested here that, in aerobic soils, respiration due to eukaryotes is significant, but not measured by the dehydrogenase assay. Anderson and Domsch (1973) reported that

Figure 7. Seasonal changes of dehydrogenase activity in the sediments (4h incubation o — o; 24 h incubation . —— .).

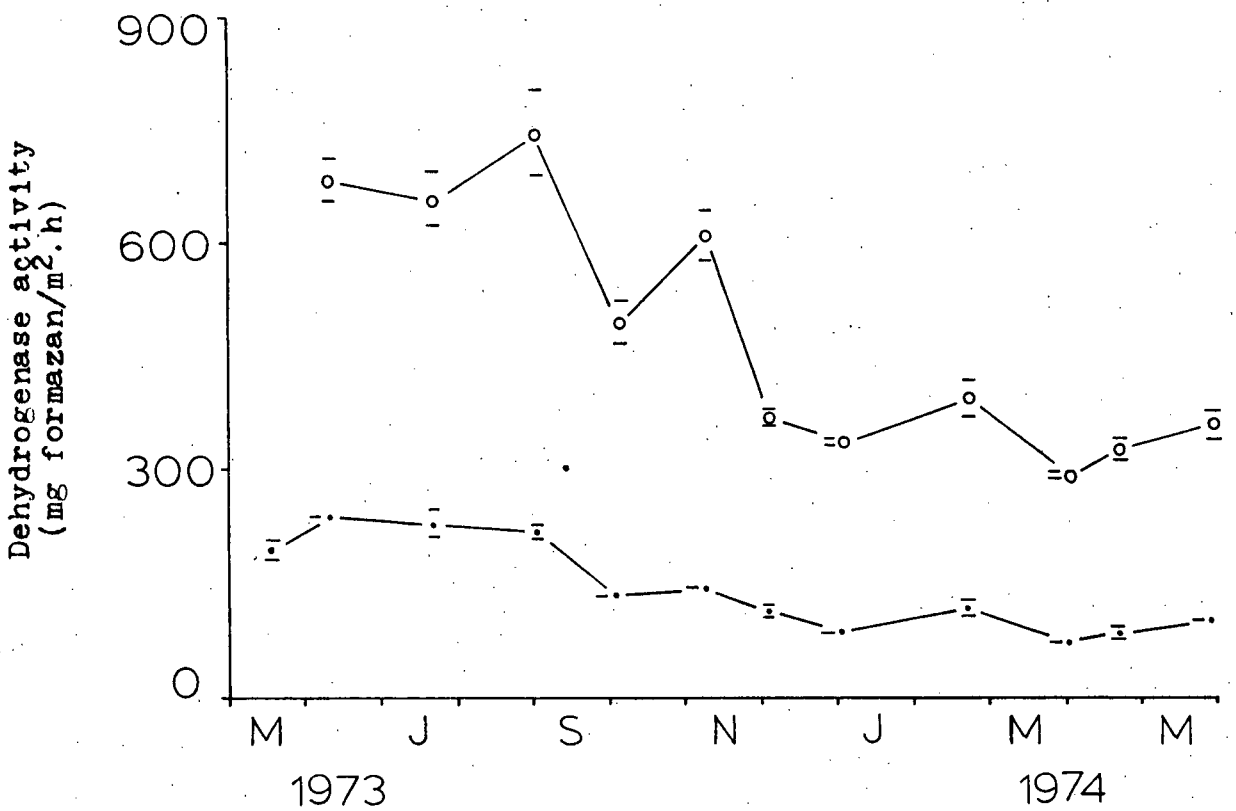

Dehydrogenase activity
(mg formazan/m².h)
900
600
300
0
M
J
S
N
J
M
M
1973
1974

Table IV. Statistical analysis of dehydrogenase activity data.

1. Dehydrogenase activity measured with 4 hour incubation

Anova

source	df	ss	ms	F
among	10	.7396	.0740	42.6292**
within	27	.0468	.0017	
total	37	.7865		

$F_{.01(10, 25)} = 3.13$

NMRT

Apr 1 Apr 21 Jan May 74 Dec Feb Oct Nov Jul June Sep

2. Dehydrogenase activity measured with 24 hour incubation

Anova

source	df	ss	ms	F
among	11	1.2581	.1144	63.3919**
within	29	.0523	.0018	
total	40	1.3104		

$F_{.01(10, 25)} = 3.13$

NMRT

Apr 1 Apr 21 Jan May 74 Dec Feb Oct Nov May 73 Sep Jul June

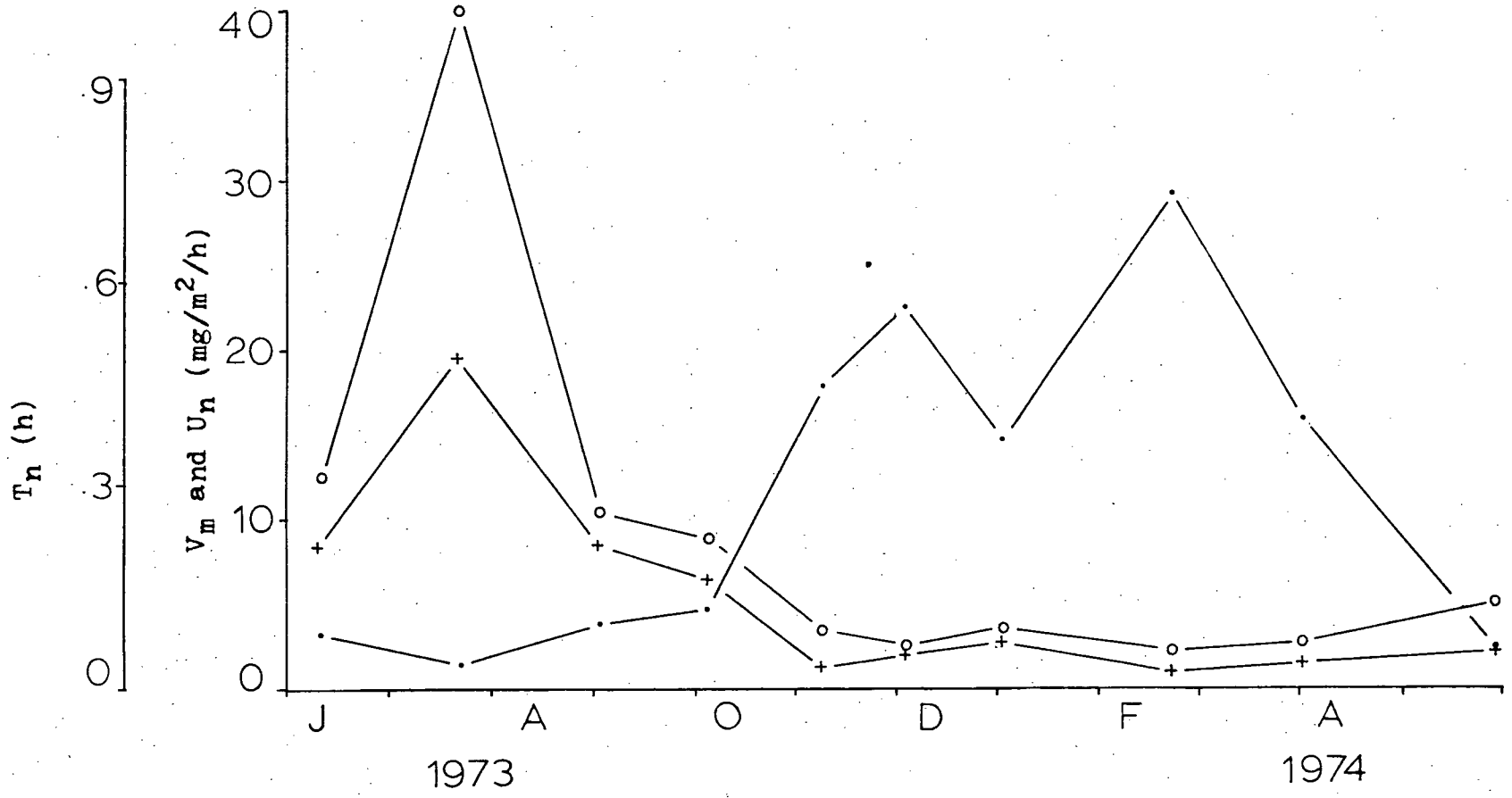
bacteria were responsible for only 22% of total soil oxygen consumption in their studies. In sediments, many anaerobic processes occur, particularly in reduced environments, which are not measured by oxygen uptake but do react with TTC (Pamatmat and Bhagwat, 1973; Olah, 1972).

Hargrave (1969) estimated bacterial oxygen consumption in Marion Lake sediments by subtracting oxygen uptake in antibiotic-treated cores from that in normal cores. Although the usefulness of antibiotics to selectively eliminate specific populations in natural environments is dubious (Cameron, 1973; Yetka and Wiebe, 1974), comparison of his results with dehydrogenase activity measurements is useful. Hargrave found bacterial respiration decreased gradually between September and December then increased gradually until May. When the sediments reached approximately 10°, bacterial activity increased rapidly with increasing temperature. This basic pattern is repeated for the dehydrogenase data (Figure 7), although the November sample was inexplicably high in activity and maximum activity was recorded in September (4h incubation) or June (24h incubation) which were not the dates of maximum temperature (19.5° in July).

(b) Heterotrophic uptake of ^{14}C-glucose

Gross uptake (respired plus assimilated uptake) of ^{14}C-glucose in mixed, diluted sediments followed a dramatic seasonal pattern (Figure 8). Both potential uptake rate (V_m) and natural uptake rate (U_n) were maximum in July when the sediments reached 19.5°. Uptake during winter was consistently

Figure 8. Seasonal changes in the sediments of maximum (V_m) and actual (U_n) rates of glucose uptake, and the actual turnover time (T_n).(V_m o—o ; U_n + — + ; T_n · —— ·).


T_n (h)
V_m and U_n (mg/m^2/h)
0
3
6
9
0
10
20
30
40
J
A
O
D
F
A
1973
1974

low (<4 mg glucose/m^2.h) but started to increase as the temperature climbed above 4° (9.5° in May,1974). The April 21 data were discarded because they did not fit Michaelis-Menten kinetics. Natural turnover time (T_n) displayed an inverse relationship to uptake rate (Figure 8). The time required for the bacteria to completely use glucose equivalent to the *in situ* concentration (S_n) increased as uptake rate declined.

Hall *et al.* (1972) reported similar values for V_m (2.6 - 38.0 μg glucose/g.h) and T_n (.061 - .400h) as were found here (V_m 2.2 - 39.3 μg glucose/g.h, T_n .033 - .727h). The pattern of seasonal variation was almost identical, indicating that the bacterial population, with respect to glucose metabolism, does not change radically from year to year (1971 - 72 compared to 1973 -74).

The natural uptake rate of glucose (U_n) was estimated by Kleiber (1972) to be about 1.8 - 10.6 μg glucose/g.h following a seasonal trend similar to that of V_m. During the present study U_n ranged from 0.6 - 15.9 μg glucose/g.h. Wood (1970) found heterotrophic uptake was maximal in the spring and low in winter in estuarine sediments, but had no data for June and July.

Some previously reported data for parameters of heterotrophic uptake of glucose in natural environments are summarized in Table V. Wood (1970) did not determine S_n so T_n may be low. Harrison *et al.* (1971) used mixed but not diluted sediments, so T_n could be determined directly by extrapolation to zero added substrate (Appendix VIII). Uptake in

Table V. Maximum uptake rates, and natural turnover times and uptake rates for glucose in sediments and water.

reference	location	T_n (h)	V_m ($\mu g/g.h$)	V_m ($\mu g/l.h$)	U_n ($\mu g/g.h$)	U_n ($\mu g/l.h$)
Hobbie et al. (1972)	ocean water (10 - 200 m)			.0005 - .006		
Azam and Holm-Hansen (1973)	ocean water (10 - 200 m)	10 - 128 days				
Crawford et al. (1974)	estuarine water	7.2		25.6		
Harrison et al. (1971)	Klamath Lake sediments	2.25	2.4	170		
Wood (1970)	estuarine sediments	.06	299			
Hall et al. (1970) Kleiber (1972)	Marion Lake -mixed sediments	.26	9.4	627	3.9	260
	-undisturbed sediments	.89-420				
This study	Marion Lake -mixed sediments	.31	9.6	535	5.3	295

undisturbed cores of Marion Lake sediment occurred with a longer T_n than was found in mixed sediment experiments (Hall et al., 1972; Kleiber, 1972). The faster uptake in mixed samples is probably due to increased availability of dissolved organics to the heterotrophic organisms. In open water compared to sediments, T_n is much longer and V_m is much lower. This reflects differences in the population density of heterotrophs in the two environments (eg. Marion Lake waters in March, 1973 contained an equivalent of 74 μg dry wt. of bacteria per 1 while the top two cm of the sediment contained approximately 60,000 μg dry wt. of bacteria/1), and emphasizes the importance of the benthos in nutrient cycling.

(c) Relations between activity estimators

Different activity assays need not, necessarily, measure the same parameters of microbial populations due to their heterogeneous biochemical abilities. Glucose is not a substrate for all bacteria. The ability to reduce TTC to formazan in the dehydrogenase assay may be restricted; degrees of the ability certainly exist (Eidus et al., 1959). There is usually, however, good correlation between the various methods. Skujins (1973) reported a high degree of correlation between soil oxygen consumption, proteolytic ability, nitrification potential and dehydrogenase activity. Hobbie et al. (1972) found a reasonable degree of relation between estimates of marine plankton respiration using oxygen uptake, dehydrogenase activity and ATP.

All the activity estimators applied to Marion Lake sediments indicate bacteria respond to increased temperatures

in May, reaching a peak sometime in late May to early September, then gradually slow down as the temperature drops. Bacterial activity remains low all winter. Heterotrophic uptake of glucose (Hall et al., 1972; Figure 8), glycine and acetate (Hall et al., 1972), and oxygen consumption (Hargrave, 1969) demonstrate sharper seasonal maxima than dehydrogenase activity (Figure 7). This may be due to the fact that dehydrogenase is a measure of potential, although V_m, the potential uptake rate of glucose, has a very sharp peak in midsummer.

Radiotracer uptake studies, particularly those in which U_n and T_n are determined, are perhaps the best way to estimate energy flow from particular carbon sources into bacterial populations. But assumptions often made, for example, that glucose uptake is representative of uptake of all other dissolved carbohydrates, may be invalid. The solution would be to have a uniformly labelled pool of all the natural substrates in their in situ concentration, but this is not practical considering present technology. Dehydrogenase activity may be more indicative of total microbial production than either heterotrophic uptake or oxygen consumption as suggested by Pamatmat and Bhagwat (1973). Their data correlating heat production to dehydrogenase activity in sediments is promising because heat production, which is very difficult to measure in natural communities, may be the ultimate tool for measuring total microbial activity (Brock, 1967).

The relation between heterotrophic uptake, oxygen uptake and dehydrogenase activity in Marion Lake sediments may be examined quantitatively. Hargrave (1969) estimated bacterial respiration to be 19.5 g C/m^2.year which indicates total uptake of 97.5 g C/m^2.year assuming assimilation efficiency of 80% (Hall et al., 1972). Kleiber (1972) estimated carbon flux into the bacteria at 22 - 120 g C/m^2. year with respiratory losses of 4 - 24 g C/m^2.year. Using a regression for dehydrogenase activity on heat production (Patmatmat and Bhagwat, 1973), and assuming approximately 100 Kcal heat are produced per mole of carbon oxidized (Giese, 1968), the potential bacterial respiration is 19.3 g C/m^2.year using the 4 hour incubation assay data. This is equivalent to gross uptake of 96.5 g C/m^2.year. This agreement between potential and actual respiratory data is unexpected, but, despite possible errors in the various assumptions, it increases confidence in the carbon flow estimates.

4. Biomass and activity of Marion Lake bacteria

(a) Qualitative seasonal description

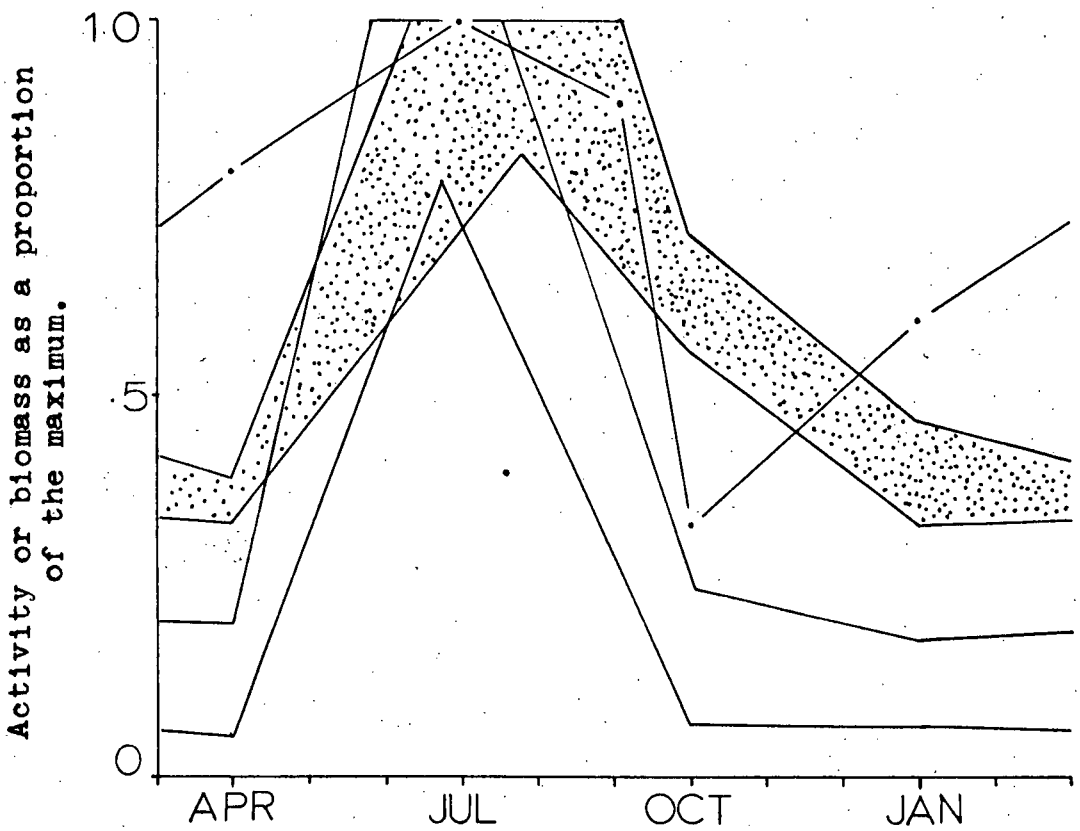
There is evidence that biomass of bacteria in natural environments does not always correlate with activity measurements. Hobbie et al. (1972) reported that high heterotrophic uptake of radiotracers in ocean water was associated, not necessarily with high numbers of bacteria, but a high number of motile bacteria. Skujins (1973) showed there was no correlation between dehydrogenase activity and numbers of cultivable bacteria in soil. Wood (1970) however, found correlation for heterotrophic uptake of acetate and glucose against direct counts of bacteria in sediments. Uptake of six of nine organic acids tested was shown to be correlated with numbers of cultivable bacteria with the ability to utilize each of the six acids as a source of carbon (Robinson et al., 1973). Their estimatesof the population size of each biochemical group are, however, questionable. Considering the number of colonies formed per ml of sample, and the methods they used, most of the sole carbon source plates contained fewer than 15 colonies. The normally accepted limits for plate counts are 20 - 200 colonies per plate, with numbers less than 20 considered statistically unreliable (Parkinson et al., 1971). An important contribution to understanding natural populations of bacteria was made by Stanley and Staley (1974). They demonstrated, applying the uptake approach used in this study and autoradiography, that heterotrophic assimilation of ^{3}H-acetate in an aeration lagoon was due to bacteria, that the uptake per cell increased

linearly over time, and that within the population there were differences in net uptake rate of at least ten-fold by different species. This work suggests that uptake rate may not be proportional to numbers of bacteria because of the heterogeneity between bacterial metabolic abilities.

This discussion is inconclusive. There are reports of correlation and reports of unrelatedness between biomass and activity of bacteria. A simple explanation is that biomass and activity are sometimes out of phase even in one particular environment. Holm-Hansen and Paerl (1972) found maximum heterotrophic uptake of acetate lagged behind maximum ATP-biomass of bacteria in Lake Tahoe. In Marion Lake, sediment bacterial biomass and activity appear to be in phase in late spring, summer and early fall (Figure 9). During the remainder of the year activity either remains constant while biomass increases, or increases very rapidly compared to biomass. The shaded area in Figure 9 indicates the range of relative activity at different times of the year, calculated using the data of Hargrave (1969) on bacterial respiration and uptake of glucose, acetate and glycine reported by Hall *et al.* (1972). Similar bounds for potential respiration are indicated. Relative biomass in different seasons was calculated from direct count data.

These shifts between activity and biomass are intimately related to physical and chemical fluctuations, and resultant changes in biological factors such as primary production and grazing. Considering the relations found (Figure 9), a

Figure 9. Diagrammatic representation of the seasonal relations between activity and biomass of the sediment bacteria as proportions of their maxima. (bacterial biomass ·——· ; range for heterotrophic uptake of glucose, glycine and acetate and for oxygen consumption ▭ ; range for dehydrogenase assay ▨)


Activity or biomass as a proportion of the maximum.
1.0
.5
0
APR
JUL
OCT
JAN

qualitative, hypothetical description of the bacterial population dynamics is possible.

During summer, there are high numbers of bacteria, probably experiencing relatively high grazing pressure, but at a production level high enough to maintain their numbers. In fall, this reproductive capacity declines sharply, despite fresh allochthonous inputs to the lake, due to decreasing temperature and day length and all their biological ramifications. Bacterial biomass drops simultaneously probably as a result of grazing pressure. The bacterial biomass then increases gradually during the winter and early spring during which period growth rate, although not at its maximum, appears to be greater than removal due to grazing. When the temperature climbs above 4° bacteria appear to respond quickly. This is suggested by the sharp decline in glucose concentrations in the interstitial water in April and May (Figure 3c). The total population is probably still at a low activity level however, due to a limited supply of readily-available carbon sources. Detrital material breakdown into molecules of a size which bacteria can transport across their membranes is probably the rate-limiting step at this time. The consequence of this is the low activity measurements obtained in April and sometimes in early May(eg. Figure 8). With still higher temperatures, primary production by epipelic algae increases rapidly (Hargrave, 1969; Greundling, 1971). Carbon and other nutrients become available to bacteria as algal exudates (Kleiber, 1972).

Complex molecules in the detrital material are decomposed more rapidly at higher temperatures. The bacterial population should, theoretically, explode at this time of year. And it probably does. The population is very "active" in terms of all the activity indicators applied to the system. Biomass, however, does not respond as dramatically, presumably due to increased outputs to higher trophic levels.

This descriptive model could be examined with two bits of information. Grazing effects on microbial populations have been studied (eg. Hargrave, 1970; Fenchel, 1970), but data on seasonal variation in this factor are lacking. The other requirement is information on bacterial growth rates at different times of the year under natural conditions. Attempts to obtain such data in this study failed due to heterogeneity between subsamples of the sediments. If the activity estimators already applied to the sediments do reflect bacterial growth rate (as suggested by the work of Stanley and Staley, 1974), and if grazing pressure is relatively high in May to September, and low in winter, then confidence in the above description of bacterial population dynamics would be increased.

(b) Quantitative description

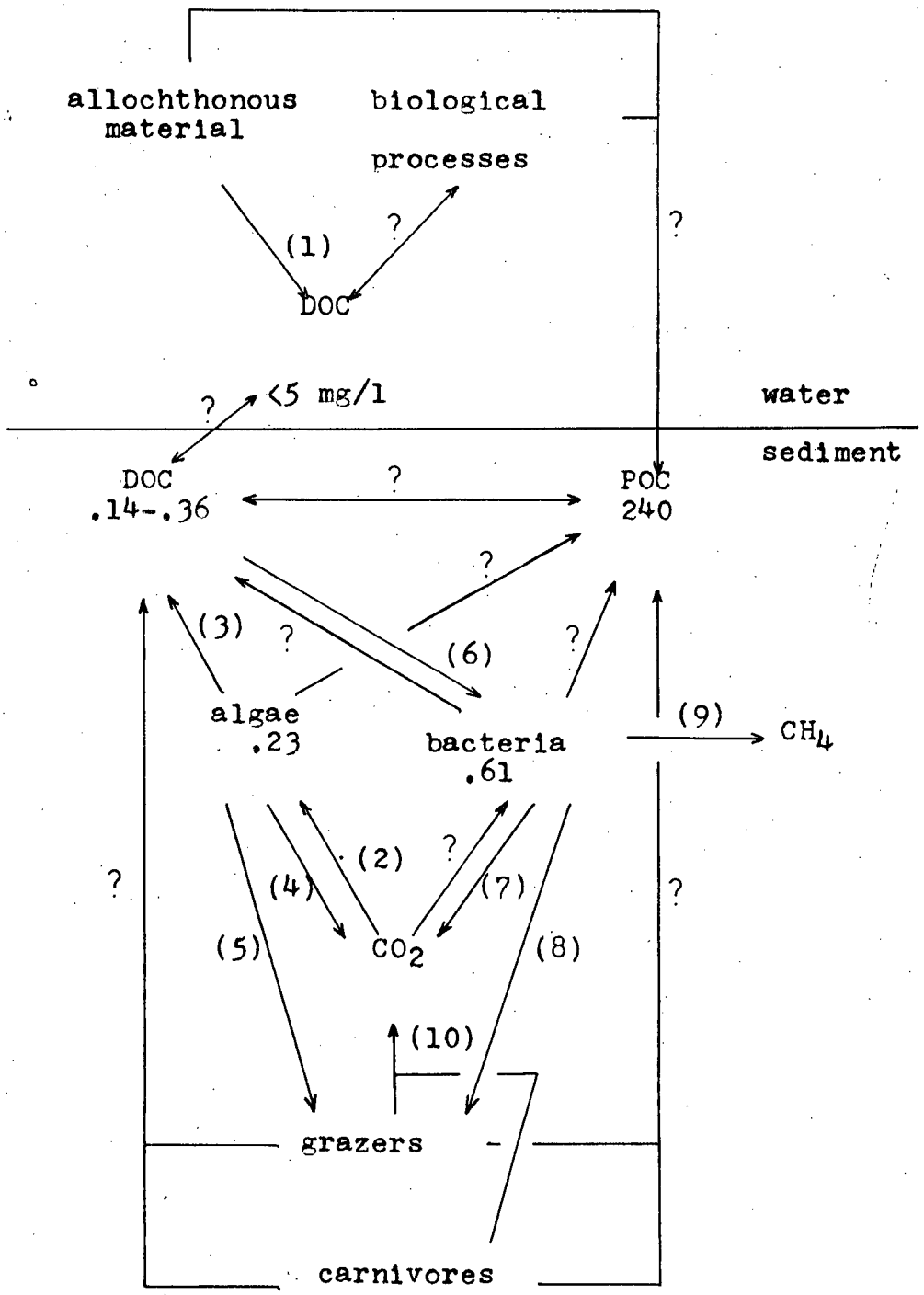
The annual mean inputs and outputs of the bacterial population (integrated mean size of .61 g C/m^2 based on direct counts) are summarized in Figure 10. The range for gross uptake and respiratory losses of organic carbon are from Kleiber (1972). The values in brackets indicate the estimates from oxygen uptake(Hargrave, 1969) and respiratory potential. The loss of carbon as CH_4 was estimated from experiments with sediment cores, and appears to be a minor component of the system.

Epibenthic algal biomass was calculated from Greundling (1971) and gross productivity from his data and that of Hargrave (1969). Excretion of algal photosynthate to the dissolved organic carbon (DOC) pool (pool size calculated from Hall and Hyatt, 1974) was determined by Kleiber (1972). Algal respiration was calculated from Hargrave's (1969) estimate of respiration due to bacteria and algae and corrected for bacterial respiration.

Losses to grazing organisms were calculated by difference between inputs and outputs to the algal and bacterial populations. Recycling of material to the dissolved and particulate organic carbon (POC) pools from higher trophic levels is unknown but may be significant (eg. Hargrave, 1970).

Hall et al. (1973) estimated the contribution of organic carbon to the DOC of the water column from allochthonous leaf material was 1.1 - 3.1 g C/m^2.year. Net DOC inputs from the inlet are 28 gC/m^2.year(Geen,unpublished).Other

Figure 10. Structure and function of the Marion Lake sediment ecosystem (all compartment sizes are expressed in gC/m^2 and all fluxes are in gC/m^2.year, unless otherwise specified - see text for explanation and sources).



(1) 29.1-31.1
(2) 42.3
(3) 1.5-8.8
(4) 6.9-26.7(11.5)
(5) 22.0-29.3
(6) 21.0-120(97.0)
(7) 4.2-24.0(19.4)
(8) 76.6
(9) < 0.5
(10) 28

inputs to this pool come directly or indirectly from phytoplankton and macrophytes and the communities they support. Exchange between water column DOC and sediment DOC has not been quantified, but may be small since diffusion processes would be expected to operate in the opposite direction.

The major unknown component in the system is the rate of decomposition of natural sediment POC (including living and dead organic matter, but primarily the latter) to utilizable DOC. This occurs as a result of chemical and biological processes, but quantitative assessment of its importance will require further development of technology.

SUMMARY AND CONCLUSIONS

The prime functional roles of bacteria are conversion of dissolved and particulate organic matter into bacterial tissues which are then available to higher trophic levels, and mineralization of dissolved and particulate organic nutrients, making them available to photosynthetic organisms. The latter process has not been studied in Marion Lake. The major sources of organic matter for the heterotrophic population appear to be allochthonous inputs from the watershed and epibenthic algal production. Much of the latter is consumed by higher trophic levels and is not, therefore, directly available to bacteria.

During this study, it was demonstrated that ATP analysis offers rapid estimation of both total and microbial sediment biomass. Bacterial biomass was estimated by direct counts, and was the largest component of the microbial ($<$ approximately 30 μm) standing crop. Changes in the proportion of bacterial and microbial biomass in the total community biomass were attributed to changes within the bacterial and microbial populations. Analysis of the discrepancies between ATP- and enumeration-biomass data indicate that ATP is not only a biomass measurement, but, in stress situations, is confounded by reflecting activity. In such situations, ATP may reflect biomass potential, or the ability of a population to maintain its size if ex-

posed to unnaturally high losses, for example, to higher trophic levels.

Previous estimates of bacterial activity in Marion Lake sediments were reviewed and compared to heterotrophic uptake and respiratory potential measurments obtained during this study. Close correlation between heterotrophic uptake of glucose in 1971-72 and 1973-74 indicated that the bacterial population is comparable, at least in function, from year to year.

The seasonal response of bacterial biomass was interpreted with respect to several activity measurements, assuming these measurements reflected growth rate, and that grazing intensity was relatively high in months when temperatures were above approximately 10°. The quantitative role of bacteria in the carbon budget of the sediments was described using acquired data and that of several previous students of the Marion Lake benthos.

The contribution of allochthonous compared to autochthonous inputs is unclear, although estimates of their contributions to the dissolved organic matter pools are available. It was noted that all the estimated total uptake of organic carbon by bacteria need not come directly from either of these primary carbon sources, since there is an unknown quantity from recycling within the community. Temperature, which limits the availability of detritus and dissolved organic matter to bacteria and, particularly in the May to September period, grazing, appear to be the key

factors controlling bacterial production.

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APPENDICES

Appendix 1. Method for extraction of total ATP from fresh sediments (based on Lee *et al.*, 1971a).

Extraction

Add 3 g wet wt. (150-200mg dry wt.) sediment to a chilled centrifuge tube. Add 5 ml. of ice-cold 0.6NH_2SO_4 to tube, mix intermittently for 5 min keeping sample cold then store on ice for 20 min. Centrifuge (5000g/5min) and transfer the supernatant to a 10 ml graduated cylinder. Record the volume (approximately 7 ml). Transfer the pellet to a tared weighing pan for dry wt. determination.

Purification

Transfer 2 ml of extract to a test tube keeping it ice-cold. Add 0.4 mls settled volume in a wide mouth pipette of cation exchange resin (Amberlite IR-20, converted to Na - form by washing with 1N NaOH and distilled water). Mix the contents for 3 min. Strain the sample through glass wool using 3 - 1 ml aliquots of distilled water to wash the tube. Repeat the resin treatment, this time using 2 - 1 ml washes. Adjust the pH to 7.8 with NaOH (.6N, .06N, .006N). The pH changes very rapidly above pH 3. Adjust the volume of the extract to 10 ml with cold Tris buffer (.02 M, pH 7.8). Assay the ATP immediately or store at -20°. Prior to assay it was found that 1:5 dilution of the extract in Tris buffer

gave optimum results, but this ratio varies with sediment type. The purification step increased recovery of ATP ten-fold.

Appendix II. Method for extraction of total ATP from lyophilized sediments.

Add 3 ml of ice-cold bromosuccinimide extraction reagent (.01 M n-bromosuccinimide, .01 M EDTA, .02 M Na_2HAsO_4 ,pH 7.4) to 20 mg of lyophilized sediment in a centrifuge tube. Keep on ice for 25 min with occasional mixing, then centrifuge (5000g/ 5 min). Add 2 ml of ice-cold water to 1 ml of the supernatant, and adjust pH to 7.8 with 1N HCl. Adjust final volume to 5 ml with Tris buffer (.02M, pH 7.8) and assay immediately or store at -20°. No dilution is necessary prior to assay.

Several reagents were tested before n-bromosuccinimide was chosen for this procedure (Table A 1). H_2SO_4 and acidic DMSO with EDTA added are almost as efficient as this reagent.

The amount of sediment extracted (20 mg) can be accurately weighed, but does not contain high enough concentrations of cations to interfere with the assay as suggested by the fact that cation exchange treatment of H_2SO_4 extracts had little effect, and the recovery per unit weight of sediment was reduced when a sediment weight of 100 mg was used.

Table A1. Methods tested for extraction of ATP from lyophilized sediments (sample size 20 mg lyophilized wt. of sediment).

reagent	reference	EDTA(.01 M)	ATP[1] (μg/g oven dry wt)
Tris buffer (.02 M, pH 7.8)	Holm-Hansen and Booth (1966)	-	0.55
		+	0.56
Na HCO_3(.1M)	Bancroft et al. (1974)	-	3.75
$HClO_4$(.6N)	Lee et al. (1971a)	-	1.49
		+	ND [2]
DMSO (neutral -90% in .05 M Tris buffer)	Lee et al. (1971a)	-	6.05
		+	3.40
DMSO(acidic -90% in 0.1 N H_2SO_4)	Lee et al. (1971a)	-	5.34
		+	10.24
Bromosuccinimide (see text)	MacLeod et al. (1969)	+	10.70
H_3PO_4 (.6N)	B. K. Burnison(unpublished)	-	5.05
		+	6.05
H_2SO_4 (.6N)	Lee et al. (1971a)	-	7.54
		+	1.13
+ cation exchange treatment[3]		-	8.22

[1] Values not corrected for extraction efficiency

[2] ND = not detectable

[3] this was the only sample receiving cation exchange treatment

Appendix III. Method for extraction of microbial ATP from fresh sediments.

Dilute 1 ml of fresh sediment with 6 ml filter- sterilized lake water. Aliquots of this suspension are dried for dry wt. determination. Place 0.35 ml in a spot dish and add 0.5 ml lake water. Examine using 16x and 25x magnification of a binocular microscope with a black sample stage. Remove all visible animals and algae with micropipettes controlled via rubber tubing by the mouth. Tease large particles and algal clumps apart. This process takes 20 - 40 min per sample.

Transfer the residue to a centrifuge tube in an ice bath, adjusting the volume to 1 ml with water. Add 1 ml ice-cold 0.6 N H_2SO_4 and mix. After 10 min, centrifuge (5000g/5min), adjust supernatant to pH 7.8 with Na OH (.6, .06, .006N) and add Tris buffer (.02M, pH 7.8) to 5 ml. Store the extract at -20° or assay immediately. Recovery is not improved by dilution of the sample.

Variance between replicates tends to be large, but the sample size is limited by the time required to run the extraction.

Appendix IV. Method for ATP assay.

Preparation of standard solutions

Prepare standard solutions of ATP (1mg/ml, Sigma Chemical Co.) and store in 0.2 ml aliquots at -20°. These are stable for at least 7 months. For the assay prepare dilutions in Tris buffer (.5, 1.0, 2.0, 5.0, 10.0 ng/ml). These are stable for several hours if kept ice-cold.

Preparation of luciferin -luciferase enzyme system

Homogenize 200 mg of lyophilized enzyme (Sigma Chemical Co., FLE -50) in 5 ml fresh, cold .1 M Na_2HAsO_4, .04 M $MgSO_4$, .03 M mercaptoethanol (pH 7.4) in a chilled tissue homogenizing tube. Rinse the homogenizer with 5 ml of the buffer. Store the extracts overnight at 2°, then centrifuge (5000g/10 min). Add 1000 ml fresh .01 M Na_2HAsO_4, .004 M $MgSO_4$, .03 M mercaptoethanol, .5% bovine serum albumin (Sigma A 4503) (pH7.4). Equilibrate at 2° for 1 h prior to use. The enzyme shows little deterioration of response even 6 h after initial use if kept on ice.

This preparation method, devised by Hammerstedt (1973), was not used during the early stages of this study. It is superior to other methods in which activity decreased over time so samples measured at different times were not directly comparable.

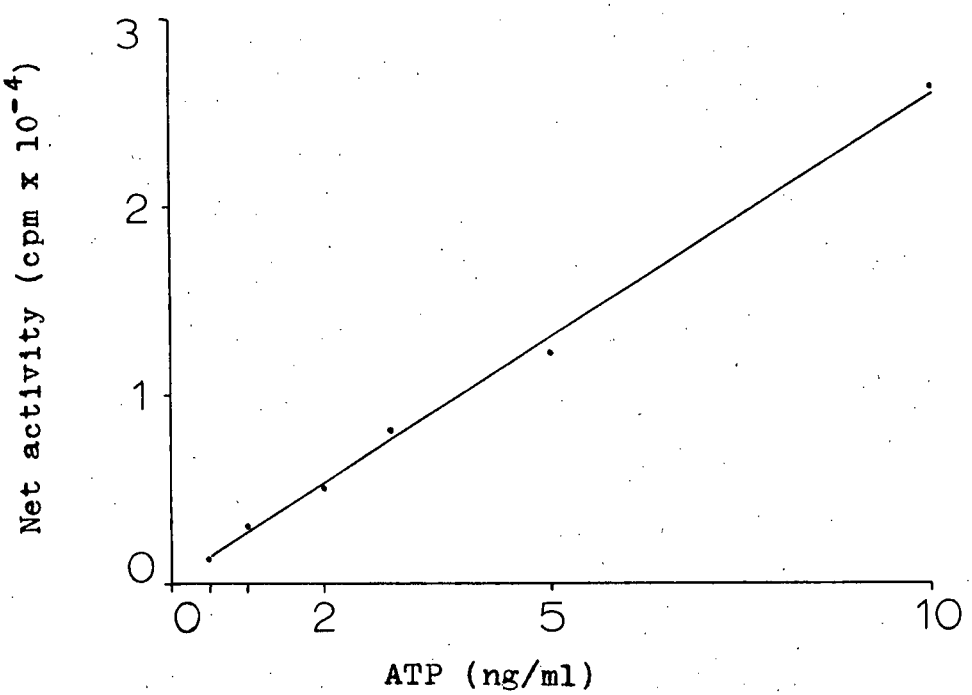
Assay procedure

Set up the counting apparatus as required. In this study a liquid scintillation counter was used (Nuclear Chicago Mark I or Unilux II) at 4°. Windows were wide open (00 - 99) with attenuation at ^3H settings (A 200). Coincidence circuitry was eliminated.

Add 1 ml of .04 M glycylglycine, .003 M $MgSO_4$ (pH 7.4) to a scintillation vial (washed in acid and distilled water). Then add 1 ml of the equilibrated enzyme mixture. Lower the vial into the counting chamber and wait 5 sec to reduce phosphorescence. Count the background for 1 min. Five seconds after completion of the background count, add 1 ml of unknown or standard ATP sample (automatic pipettes giving quick delivery are advantageous), and lower the vial into the counting chamber. Twenty seconds after completion of the background count, initiate a 1 minute gross count. Subtract background to calculate net activity.

Plot net cpm versus ATP concentration and read unknown concentrations from this curve. The relationship is linear over a wide concentration range (Figure A 1).

Figure A I. Typical standard curve for ATP assay (May 18, 1974).


Net activity (cpm x 10^{-4})
3
2
1
0
0
2
5
10
ATP (ng/ml)

Appendix V. Recovery of ATP added to sediments.

The efficiencies of the extraction procedures were determined in a series of experiments in which pure cultures of organisms were added to sediment samples. Measurement of ATP in the pure cultures (using a method analogous to that in Appendix III), in the sediment with added organisms and in control samples permits solution of the following relationship:

$$\text{efficiency} = \frac{\text{ATP(sediment + organisms) - ATP sediment}}{\text{ATP organisms}}$$

The results using a number of species for the three extraction methods are shown in table A II. The isolates from Marion Lake were the most abundant organisms obtained on brain heart infusion agar plates in two plate count series. Isolates 73-1 and 74-1 are small Gram negative rods and 74-2 is a large Gram negative rod.

Table AII. Efficiency of the various ATP extraction methods.

extraction method	organism	efficiency		
H_2SO_4 "total" (fresh sediment)	Isolate 73-1	.091		
	73-1	.129		
	74-1	.052	.128	
	74-2	.209		.125
	Bacillus subtilis	.161		
	Oscillatoria spp.	.102	.116	
	Anacystis nidulans	.129		
H_2SO_4 "micro" (fresh sediment)	Isolate 73-1	.248		
	Bacillus subtilis	.161	.205	
Bromosuccinimide (lyophilized sediment)	Bacillus subtilis	.106		

Appendix VI. Relation between ATP concentration and biomass carbon.

The ratio of organic carbon associated with living organisms to ATP is variable, but an average of 250:1 has been suggested (Hamilton and Holm-Hansen, 1967). This ratio was determined for a number of organisms in this study (Table A III).

Bacteria were grown in nutrient broth + 0.5% yeast extract, the actinomycete in a medium reported by Rodina (1972, p. 373), and the blue-green bacterium inBG-11 (Stanier et al., 1971). The bacteria were in early stationary phase when harvested while the blue-green culture was approximately three weeks old and appeared healthy. Cells were concentrated by centrifugation (3000g/5min) and resuspended after one wash with water.

ATP was extracted from 1 ml of the suspension using the method described for microbial ATP in fresh sediments (Appendix III). Organic carbon was measured in a carbon analyzer (Beckman Model 915). Viable counts were determined on agar plates of the same medium as the cultures were grown in.

The mean C/ATP ratio of 287 (Table A III) was interpreted as a confirmation of the popular value of 250.

Table AIII. The relationship between ATP, biomass carbon and cell numbers.

organism	ATP/cell (μg x 10^9)	biomass carbon/ATP	
isolate 74-1	2.35	112	
74-2	4.62	292	
Bacillus subtilis	14.90	439	287
Streptomyces spp.	–	376	
Anacystis nidulans	7.33	218	

Appendix VII. The effect of incubation time and oxygen on dehydrogenase activity measurements.

Incubation time

The incubation time-activity measurement response is shown in figure A 2. This experiment shows that activity, measured as formazan production, is rapid during the first few hours, then drops to a lower rate. The results suggest incubation times less than 6 h would be most sensitive to differences in respiratory potential in natural samples.

In response to previous studies (Patterson et al., 1970; Sorokin and Kadota, 1972) 24 h incubation was used. Four hour samples were run simultaneously to test sensitivity changes. The seasonal data (presented in Results) indicate activity measurements at the two incubation periods are closely correlated. Sensitivity was greatest using the shorter incubation time - the maximum difference was 456 mg formazan produced/m^2.h (Sep. 1973 - Apr. 1974) for 4 h incubation compared to 168 (June, 1973 - Apr. 1, 1974) for 24 h incubation.

Oxygen

Prior to incubation, sample flasks were flushed with nitrogen for 10 minutes. The rationale for this is that oxygen and TTC compete for electrons in the electron transport system and variable oxygen concentrations in samples would reduce the comparability of TTC-measured dehydrogenase activity in different samples. The minimization of this competition not only makes activity measurements more consistent, but also higher (Table A IV). The data from different depths in the sediments further support this point. The

Figure A 2. Effect of incubation time on dehydrogenase activity measurements.

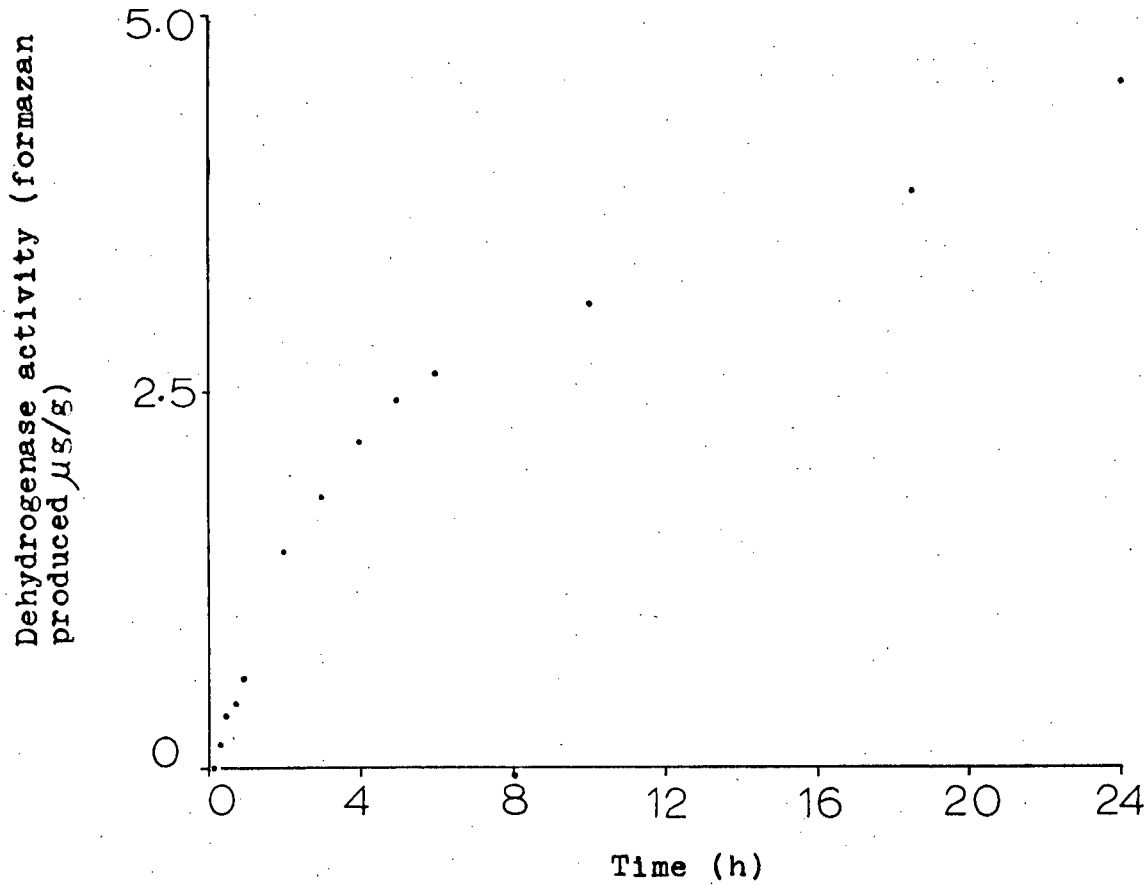

Dehydrogenase activity (formazan produced µg/g)
5.0
2.5
0
0
4
8
12
16
20
24
Time (h)

Table AIV. The effect of aerobic versus anaerobic conditions on dehydrogenase activity assayed at different depths in the sediment (March 14, 1973).

sediment horizon (cm)	incubation condition	formazan production (μg/g dry wt./h)
0 - 1	aerobic	20
	anaerobic	103
1 - 2	aerobic	15
	anaerobic	108
2 - 3	aerobic	44
	anaerobic	88

aerobic value from 2-3 cm depth is probably high relative to the anaerobic value because of the normal absence of oxygen in this layer. This is in agreement with the findings of Lenhard (1968) that removal of oxygen from anaerobic sludge had little effect on dehydrogenase activity.

Appendix VIII. Calculations of heterotrophic uptake parameters.

Theory

Many descriptions of the method for calculating uptake parameters in water samples have been published since 1965. The following is an outline of a procedure developed by Kleiber (1972) for diluted sediment samples.

Turnover time, T, is related to substrate concentration, S, and uptake rate, V, by the formula:

$$T = \frac{S}{V} \qquad E1$$

If the natural turnover time and substrate concentration, T_n and S_n, are known then the real rate of uptake, U_n, may be calculated:

$$U_n = \frac{S_n}{T_n} \qquad E2$$

S_n can be measured independently of uptake experiments. The experimental design of the experiments does not permit direct estimation of T_n because S in the incubation flasks is not S_n. It is instead the added radioactive substrate concentration (S_a), plus the quantity naturally in the sediments corrected for the dilution factor, d, such that:

$$S = S_a + \frac{S_n}{d} \qquad E3$$

T is a function of S, so determination of T_n must be performed at S_n. In non-kinetic experiments, for instance using ^{14}C-bicarbonate to measure primary production in water samples, this is not a problem if S_n is not greatly changed by the added substrate. The sediment studies require dilution

of the samples, however. External substrate is then added at incremental levels which hopefully encompass the natural, undiluted substrate level.

In each experiment, there is an estimated turnover time, T_e, for each S_a. This is calculated by:

$$T_e = \frac{R_a}{R_u} t \qquad E4$$

where R_a is radioactivity (dpm) added to the sample, R_u is the gross uptake of radioactivity and t is the incubation time.

These values of T_e can be measured without information about S (ie. S_a or S_n), but are not independent of S. The variety of linear transformations (Hall et al., 1972) of the hyperbolic Michaelis-Menten function assume that T_e is a linear function of S. The transformation used in this study has the form:

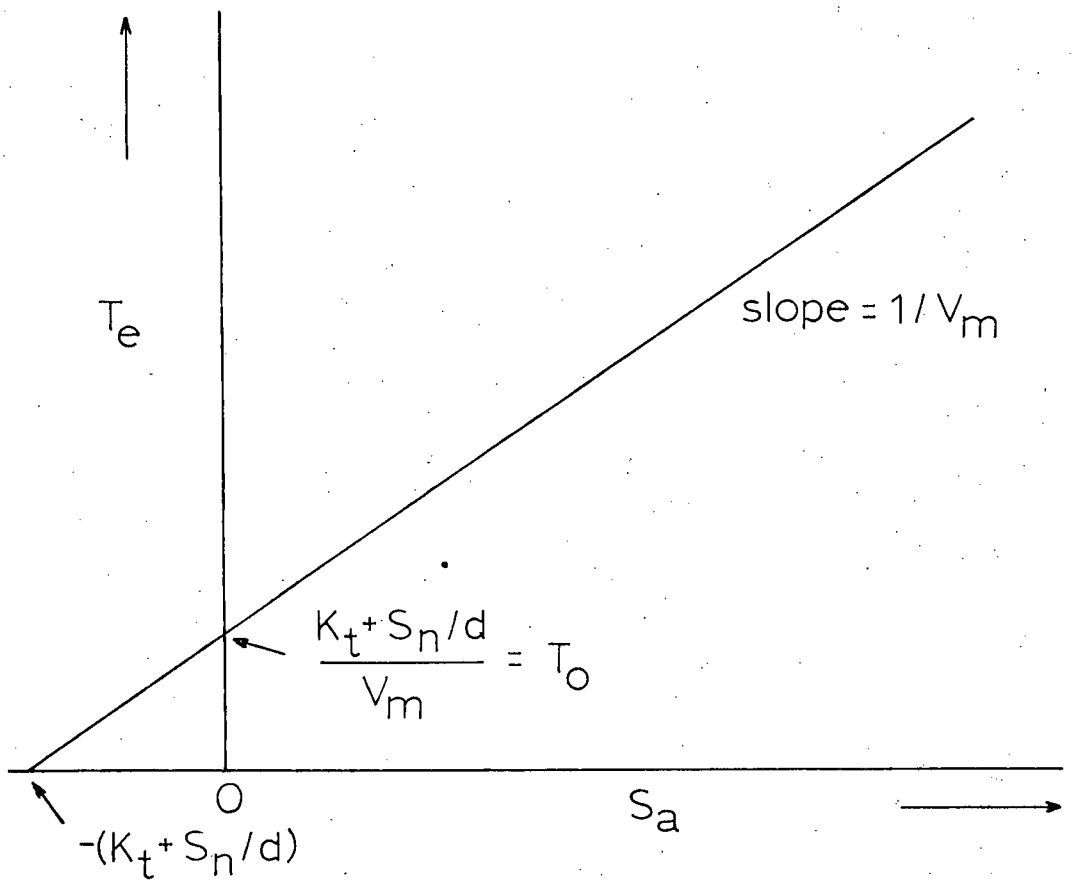
$$T_e = \frac{K_t + S_n}{V_m} + \frac{1}{V_m} S_a \qquad E5$$

where K_t is the transport constant and V_m is the maximum uptake rate. By plotting T_e versus S_a (modified Lineweaver-Burke plot) estimates of V_m, $(K_t + \frac{S_n}{d})$ and T_o, the turnover time for $S_a = 0$ or $S = \frac{S_n}{d}$ (E3) may be obtained (Figure A3).

The work of Williams (1973) and Burnison and Morita (1973) discussed in the Introduction is applicable here. Estimates of V_m and $(K_t + \frac{S_n}{d})$ are both dependent for their accuracy on the validity of the assumption of linearity. T_o is an empirically derived quantity and independent of this assumption.

Determination of T_n, the turnover time at S_n, is

Figure A 3. Graphic representation illustrating the modified Lineweaver-Burk plot.


T_e
slope = $1/V_m$
$\frac{K_t + S_n/d}{V_m} = T_o$
0
S_a
$-(K_t + S_n/d)$

accomplished by determining the value of S_a which corresponds to S_n. This value is:

$$S_a' = S_n - \frac{S_n}{d} \qquad E6$$

From the T_e versus S_a plot, T_e', the turnover time corresponding to S_a', may be determined. Division of this quantity by d accounts for dilution of sediment bacteria in the experiment so:

$$T_n = \frac{T_e'}{d} \qquad E7$$

T_n calculated in this manner, and the independent value of S_n are plugged into E2 to give U_n.

Data processing

The experimental data consisted of the dry weight of sediment per flask, the incubation time, the added ^{14}C-glucose (Ra = cpm /μg), the respired and particulate radioactivity (cpm) for two sample flasks and one control at each of four values of S_a. Interstitial glucose concentrations were determined independently but on the same mixed batch of sediment.

Gross uptake (R_u) was calculated as the sum of the average, net, living, respired and particulate uptake, converted to dpm. T_e (h) was calculated for each level of S_a (μg/l) and a linear regression equation determined (E5). By substituting $S_a' = S_n - S_n/50$, where 50 was the dilution factor, into the regression equation, T_e' was obtained. T_n was calculated by E7 and U_n by E2. The regression coefficient was $1/V_m$.