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ABSTRACT

CHEMO-ORGANOTROPHIC UTILIZATION OF DISSOLVED ORGANIC COMPOUNDS BY PLANKTIC ALGAE AND BACTERIA IN A POND

by Harold LeRoy Allen

A substrate dilution technique, using high specific-activity ^{14}C labeled organic compounds and based on Michaelis-Menten enzyme kinetics, was employed to monitor in situ bacterial metabolism of glucose and acetate over an annual period in Lake Löttsjön, a small pond in southern Sweden. Mathematical and graphical analyses of population responses to added labeled substrate permitted an estimation of (1) natural substrate concentrations ($\mu\text{g l}^{-1}$), (2) the maximum velocity of substrate removal ($\mu\text{g l}^{-1}\text{hr}^{-1}$), and (3) the in situ regeneration time for the substrate (hours). Algal utilization of dissolved organic compounds was determined by bioassay of diffusion uptake kinetics over a portion of the annual period. Concentrations of glucose and acetate were apparently reduced to sufficiently low levels by bacterial activity that appreciable in situ algal heterotrophy could not occur. As correlative measures to the study of annual cycles of organic solutes in

freshwater, the following environmental and biological parameters were routinely monitored: temperature, total algal volume, dissolved organic carbon, particulate organic carbon, and bacterial biomass.

Selected references on heterotrophy of phytoplankton were reviewed in an effort to draw some valid conclusions on the ability of algae to utilize directly dissolved organic compounds.

Seasonal and vertical variations in the distribution and in situ rates of utilization of glucose and acetate by planktic algal and bacterial populations were studied for one year. Natural substrate concentrations of glucose varied little during the annual period and generally remained below $10 \mu\text{g l}^{-1}$. A yearly maximum of $24 \mu\text{g l}^{-1}$ occurred in late spring; concentrations most frequently detected during the ice-free period were $2\text{-}5 \mu\text{g l}^{-1}$. Acetate concentrations were highest ($350 \mu\text{g l}^{-1}$) during March and April under ice cover above the sediment. Winter values ranged from $5\text{-}30 \mu\text{g l}^{-1}$ while summer concentrations in the upper strata (0.5 and 1.5 m) approached $50 \mu\text{g l}^{-1}$ with markedly decreased concentrations available near the sediment (2.5 m). On one instance acetate depletion was detected in the sample.

Rates of bacterial utilization, measured as uptake velo-

cities, were highest for both substrates immediately above the sediments in early June. Maximum rates of uptake of acetate were 10-13 times greater than those of glucose. Annual observed ranges of uptake velocity of acetate were between 5-190 $\mu\text{g l}^{-1}\text{hr}^{-1}$; those for glucose were between 1-15 $\mu\text{g l}^{-1}\text{hr}^{-1}$. Seasonal and vertical profiles of bacterial activity correlate well with changes observed in water temperature. During summer months, changes in maximum velocities of substrate uptake are directly proportional to fluctuations in bacterial biomass.

Turnover times for the regeneration of glucose were not as rapid as those of acetate. During the ice-free season, from late April through mid-November, turnover times for both substrates were generally below 5 hours with intense cycling during mid-summer as low as 0.4-1.0 hours. Winter values decreased to as much as 300 hours at low ambient temperatures. Rates of substrate utilization, approximations of substrate concentration and turnover times are much higher in Lake Löttsjön than those few comparative data presently available from mildly eutrophic and non-polluted waters.

Algal populations metabolized glucose at a faster rate than acetate for most of the ice-free period. Rates of diffusion of these substrates were greater (expressed as K_d , diffusion constant, 10^{-3} to 10^{-2} hours^{-1}) than similar values previously

reported in the literature. Fluctuations in diffusion rates correlated directly with changes in phytoplankton volume.

Seasonal and vertical distribution of dissolved and particulate organic carbon were closely related to changes in temperature. Concentrations of dissolved organic matter were 5-30 mg C l⁻¹ and higher than previous determinations from other fresh waters. Maximum annual concentrations were closely related to high algal production and accumulation. The distribution of particulate carbon was more uniform than the dissolved fraction and exhibited a close relationship to changes observed in phytoplankton volume. Winter concentrations were within a range of 0.5-3.0 mg C l⁻¹, while annual maxima (9-10 mg C l⁻¹) occurred in July and October.

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I. Literature Review

A. Introduction

The utilization of dissolved organic matter by freshwater phytoplankton is well documented and has received much attention in recent years (Myers, 1951; Fogg, 1953; Saunders, 1957; Krauss, 1958; Pringsheim, 1959; Danforth, 1962; Hutner and Provasoli, 1964; among others). Mechanisms available to algal and bacterial populations for the removal of organic solutes from lakewater have also attracted much investigation (Wright and Hobbie, 1965a, 1965b, 1966; Hobbie and Wright, 1965a, 1965b; Wetzel, 1967a, 1967b; Allen, 1967, in preparation); further, most studies include some speculation as to the in situ ecological significance of such findings.

One of the basic problems in efforts to evaluate the role of dissolved organic compounds in natural water and attempts to discern the processes by which algal and bacterial communities may utilize them is that the literature suffers from a lack of uniformity in the terminology used. Comparisons of data and interpretations from different sources are difficult since little or no distinction has been made between growth

on organic substrates and alternative modes of metabolism, e.g. autotrophic growth. For example, little differentiation, if any, has been made between organic growth substances (whether they be accessory factors, growth promoting substances or vitamins), which are required by the vast majority of planktic microorganisms in extremely low concentrations, and growth on organic compounds which are utilized as sole carbon and energy sources. Further, the terminology describing the various means by which a phytoplankter may utilize organic solutes, under laboratory conditions or in situ, is not clearly indicated.

Saunders (1957) points out that algal and protozoan forms have evolved to such an extent that a scale of metabolic pathways exists, from complete photoautotrophy (obligate) through auxotrophy (requiring only accessory growth factors, co-factors, vitamins and related dissolved organic compounds), to complete heterotrophy (obligate) and sole dependence upon organic compounds in total darkness.

Inasmuch as the present study is primarily concerned with the utilization of dissolved organic compounds, a much simplified definition of the phototrophic assimilation of carbon, or photosynthesis, is used. Photosynthetic metabolism is considered as the conversion of dissolved carbon dioxide,

in the presence of sufficient quanta of light energy, into fixed cell carbon. In the absence of sufficient light intensities, this form of metabolism ceases to function. Further, the algae are fully capable of synthesizing all essential metabolites required for normal growth and reproduction from inorganic sources.

B. Auxotrophy

Auxotrophy is perhaps best considered the capacity for growth from an exogenous source of one or more accessory growth factors, essential micro-metabolites, co-factors, and vitamins or vitamin complexes, among others. Such growth promoting substances would commonly include the B vitamin complex, vitamin B₁₂, thiamine, biotin and compounds of a similar structural nature and function. Pertinent literature concerned with auxotrophic growth, the utilization of these micro-nutrients, ecological implications, and suspected physiology of their action are reviewed by Hutchinson (1941), Lucas (1947), Provasoli and Pintner (1953), Saunders (1957), Provasoli (1958a, 1958b), Lewin (1959), Vishniac and Riley (1959) and Droop (1957, 1962). Further, auxotrophic growth implies the organic growth promoting substance is not in

any way utilized for a carbon or an energy source. Concentrations required for optimal growth by most phytoplankters are usually within the milli-microgram range.

Many limnologists and oceanographers have attempted for some time the correlation of phytoplankton succession and changes in organic nutrient supply. However, until more precision is obtained in measuring the distributional properties and annual cycles of many of these nutrients and factors, few meaningful generalizations of the function and ecological importance of these compounds in the ecosystem can be made.

C. Mixotrophy and Photoassimilation

Mixotrophy may be defined as the ability of an organism to concentrate and utilize an organic carbon source from the surrounding medium and simultaneously conduct photosynthesis in the light. Mixotrophy differs from auxotrophy in that the organic compound that is taken into the cell is used as a carbon and energy source in addition to the carbon dioxide assimilated. Hence, the term "supplementary substrate" should be applied to the organic compound associated with mixotrophic uptake. If, in the absence of exogenous carbon dioxide, the cell is capable of removing the organic substrate from the

growth medium at rates greater than those demonstrated in total darkness, the term "mixotrophic photoassimilation" should be applied. For purposes here, this capability will be denoted by "photoassimilation".

Probably the separation of the two processes, photoassimilation and true heterotrophy, does not occur in nature, or at least our present methodology has not been able to show this to be the case. It is quite possible that cells are able to exist during long winter months, under extreme environmental conditions (i.e. thick snow and ice cover), by utilizing both of these processes simultaneously. Actually, photoassimilation may be taking place in situ where heterotrophic growth is suspected, since the lowest levels of light intensity and light quality that will support growth are poorly known. Further, short bursts of light penetrating thick ice and snow cover or to great depths in a lake or an ocean, may be sufficient to stimulate these processes and thus aid in the survival of a species which would otherwise disappear from the system.

Although numerous laboratory studies of mixotrophic growth and photoassimilation have been reported in the literature, little attempt has been made to assemble and interpret some of the findings. Studies of algal mixotrophic growth and photoassimilation have been limited mostly to the utilization of

glucose and acetate at low light intensities (Bristol-Roach, 1928; Pringsheim and Wiessner, 1960; Oaks, 1962a, 1962b; Eppley, et al., 1963; Cheung, et al., 1964; Wiessner and Gaffron, 1964a, 1964b; Tanner, et al., 1965; Griffiths, 1965b; Hoare and Moore, 1965; Wiessner, 1965; Rodriguez-Lopez, 1966; Cheung and Gibbs, 1966; Merrett and Goulding, 1967; and others).

D. Heterotrophy

To avoid confusion in this presentation, the term heterotrophy will be restricted to mean the ability to grow and reproduce entirely in the dark, on one or more organic compounds. The organic compound(s) must therefore serve as the carbon donor and as the energy source.

Fogg (1953) has presented a nomenclature based on different metabolic pathways utilized by algae. The term applied to growth which is dependent upon oxidation or fermentation of exogenous organic substrates, chemo-organotrophy, could equally well be substituted for heterotrophy, the only objection being that there are perhaps other mechanisms available than just oxidation and fermentation, and thus this term may be somewhat limited in its interpretation.

It should be clearly stated that vitamins and essential

metabolites (i.e. all growth promoting substances, accessory factors, co-factors, etc.) do not serve as organic substrates and their utilization in laboratory growth studies should not be taken to constitute algal heterotrophy. Subsequently, studies encountered in the literature that stress the need and utilization of exogenous metabolites, and entitled "heterotrophic" studies of some type, these have either been ignored or placed in the section on auxotrophic growth. Also, because of the vast amount of literature on the subject of algal heterotrophy, it has been necessary to select those papers considered most relevant to the questions we are asking. Further, discussion has been limited to laboratory studies of heterotrophic growth and, where possible, the ecological implications that may be derived from such studies are pointed out. Most of the studies cited concern common freshwater species although studies involving certain marine genera are included because these genera are also prevalent in freshwater.

1. Chlorella (Chlorococcales, Chlorophyta)

Neish (1951) has reported that heterotrophic growth of Chlorella vulgaris (Columbia strain) can be supported on glucose, galactose, fructose, cellobiose, lactose, and four

beta-glucosides. However, Chlorella vulgaris is not known to frequently occur in natural freshwaters, and for this reason a thorough discussion of the heterotrophic capabilities of this species under laboratory growth conditions is not warranted. Other work pertinent to the heterotrophic growth of Chlorella vulgaris has been accomplished by Merrett and Syrett (1960), Griffiths, et al. (1960), Syrett, et al. (1964), Griffiths (1963, 1965a, 1965b), and Karlander and Krauss (1966).

Samejima and Myers (1958) tested Chlorella pyrenoidosa (Emerson strain) and C. ellipsoidea for growth in the dark and at low light intensities on several hexoses, sugar phosphates, and organic acids. During the first 30-70 hours of growth in total darkness limited assimilation of xylose, arabinose, fructose, and maltose occurred. A longer incubation time on the same substrates failed to result in increased growth. Both chlorellas demonstrated good heterotrophic growth on glucose and galactose, but other hexoses were not utilized. It is of some interest to note that C. ellipsoidea was capable of utilizing glycolate, but that C. pyrenoidosa was not, as Tolbert and Zill (1956) have shown C. pyrenoidosa (Emerson strain) is capable of reabsorbing exogenous glycolic acid from the growth medium, which had previously been excreted from the cells during photosynthesis.

Carbon assimilation by C. pyrenoidosa under heterotrophic conditions was shown by Samejima and Myers (1958) to be 45% for glucose, 37% for galactose and 26% for acetate (when utilizing nitrate as the sole nitrogen source), as compared with carbon fixation (100%) under normal light intensities. With ammonia as the sole nitrogen source, and using glucose as the organic substrate, the maximum cell-carbon assimilation under heterotrophic conditions was 58% of that found in the light.

Samejima and Myers (1958) were unable to demonstrate growth in the dark by either Chlorella on any of the sugar phosphates tested. Because fatty acids are toxic in an undissociated form, their use as potential substrates for heterotrophic growth studies is severely limited. Acetic acid caused a 50% inhibition of glucose respiration at pH 4.5, and the sodium salt was toxic at pH 6.7 in concentrations greater than 1% (w/v). Acetate (1% w/v) was found to support growth of both chlorellas, although formate, propionate, butyrate, lactate, succinate, alpha-ketoglutarate, pyruvate, malate, glycolate, fumarate, tartrate, malonate, and glutamate would not support growth. Further, the presence of low light intensities did not increase the assimilation of any of the sugars which were tested.

At low light intensities growth rates supported by photosynthesis and glucose assimilation were found to be additive (Samejima and Myers, 1958), but in total darkness carbon assimilation on glucose could not be increased by the addition of equal concentrations of galactose or acetate, although the latter substrates are utilized independently under heterotrophic conditions. This suggests, in part, that this particular strain of C. pyrenoidosa is capable of supplementing heterotrophic growth on glucose with photosynthetic metabolism. Unfortunately, Samejima and Myers did not indicate the light intensities used in their experimental procedure, which would be important from an ecological point of view. Wright (1964) has shown that light penetration through a thick layer of snow and ice may be reduced to as little as 0.2% of total incident radiation, in which case heterotrophy of organic compounds and photosynthesis might be operative simultaneously in certain populations.

Direct comparisons of laboratory findings with Chlorella, and to some extent with Scenedesmus, to in situ conditions where these two taxa occur are difficult to make. Different isolated strains of these species have been shown to possess quite different metabolic requirements (Bristol-Roach, 1927; Algeus, 1946; Myers, 1947; Neish, 1951; Fogg, 1953; and Killam and

Myers, 1956). From these and other studies it would appear the Emerson strain of C. pyrenoidosa is more limited in its ability to utilize directly organic compounds in the dark than other strains of Chlorella which have been isolated. Samejima and Myers (1958) have suggested that the inability to demonstrate heterotrophic growth of C. pyrenoidosa on certain of the substrates tested, is due primarily to lack of permeability of the cellular membrane to these compounds. Further, it seems probable that numerous generations of Chlorella, and other similar laboratory organisms which are kept continuously in sterile culture, may eventually develop mutants which have different metabolic requirements. It appears that only limited interpretations can be made from laboratory findings involving Chlorella species (cf. Lewin, 1950).

Fogg and Belcher (1961) also investigated the heterotrophic growth characteristics of Chlorella pyrenoidosa, which was isolated from a Lappland lake in northern Sweden and maintained in axenic culture. To test for heterotrophic growth, cultures were started on media solidified with agar and containing the following organic substrates at 0.01M concentrations: acetate, fumarate, succinate, citrate, ethanol, glycerol, xylose, arabinose, glucose, fructose, galactose, mannose, lactose, sucrose, maltose, cellobiose, mannitol, and

starch. Liquid media, enriched with 0.5% carbon dioxide, consisting of the following substrates at 0.01M concentrations were also tested: acetate, glucose, glutamate plus glucose, and glutamate plus acetate (all work done at 25C). None of the above mentioned media supported the growth of C. pyrenoidosa in the dark. Neither could growth be demonstrated at 3C, 15C, or at 20C. However, normal photosynthetic growth occurred, following a lag phase, when cultures were placed in the light.

Although heterotrophic growth on glucose could not be shown (Fogg and Belcher, 1961), glucose (0.01M) did stimulate photosynthetic growth between 1600 and 15,000 meter-candles at temperatures between 15 and 30C. As growth was rapidly stimulated by exogenous glucose, these authors suggested the mechanism for utilization was direct rather than through conversion to carbon dioxide and normal photosynthetic pathways. Here we have an instance of a dual pathway involving photo-assimilation. In a comparison of three different strains of C. pyrenoidosa, similar responses were shown for photosynthetic growth, yet none of the strains were capable of growth in the dark. In conclusion they found C. pyrenoidosa was obligately phototrophic and suggested their C. pyrenoidosa was similar to C. vulgaris (Pearsall and Bengry, 1940; Killam and Myers, 1956), in which cell division is photo-dependent. Rodhe (1955)

first asked whether this species of Chlorella which occurs in large numbers at great depths in certain Lapland lakes throughout the dark winter months, is capable of utilizing dissolved organic matter as a means of survival. This question is still unanswered.

Some work has been accomplished in determining the labeling-patterns which follow the heterotrophic uptake of uniformly labeled (^{14}C) glucose by Chlorella pyrenoidosa (Griffiths, 1965b). Over a 15 minute period, glucose degradation occurred and the label could be traced into sugar mono- and diphosphates, and from these compounds into phosphoglyceric acid and alanine. After a longer incubation period, the label was detected mostly in aspartate and glutamate. Griffiths further demonstrated in labeling experiments, that TCA (tricarboxylic acid) cycle intermediates generally serve as poor substrates for heterotrophic growth, and that hexoses and related compounds are more readily utilized. The mechanisms controlling physiological responses were indicated, but no attempt was made to interpret the findings from an ecological point of view.

2. Other Chlorococcales

Parker, et al. (1961) has investigated the heterotrophic abilities of some of the Chlorococcaceae. All species tested of the genera Bracteacoccus, Spongiochloris, Dictyochloris and some species of Neochloris and Spongiococcum are facultative heterotrophs which readily live on glucose, and in some instances on acetate. On the other hand, the genus Chlorococcum is obligately photoautotrophic. The organic compounds, glucose, glucose-1-phosphate, sodium acetate, galactose, arabinose, and glycerol were used to test for heterotrophic growth, under conditions of total darkness on 1.5% algal slants. Interestingly enough, Parker, et al. (1961) was able to show a striking correlation between heterotrophic growth on glucose and possession of 1) a discoid, net-like or sponge-like chloroplast, and 2) naked zoospores, i.e. lacking a rigid cell wall. The genus Chlorococcum, in which all 16 species are obligate photoautotrophs, possesses a cup-shaped chloroplast and thick-walled zoospores. Parker, et al. (1961) further suggested morphological criteria might be used to determine facultative heterotrophy in members of the Xanthophyceae (see Belcher and Miller, 1960).

Prototheca is considered to be a colorless counterpart of the microalga, Chlorella, and has the specific metabolic requirement of an exogenous organic carbon source (Barker, 1935;

Anderson, 1945). Growth of Prototheca zopfii under heterotrophic conditions was marked on glucose, fructose, galactose, and mannose (Barker, 1935). No growth was supported on any of the disaccharides tested (lactose, maltose, sucrose, arabinose and xylose) or on mannitol. Dihydroxyacetone and glyceraldehyde did support heterotrophic growth, as well as straight-chain fatty acids from acetic (C_2) to decanoic (C_{10}), and even-numbered acids from C_{10} to C_{16} . Branched-chain fatty acids (isobutyric, isocaproic, alpha-crotonic and oleic) also served as substrates. Of the alcohols tested only ethyl, n-propyl, n-butyl and n-amyl supported continuous growth. Samejima and Myers (1958) were unable to demonstrate growth of Chlorella pyrenoidosa or C. ellipsoidea on fatty acids or alcohols, indicative of much more restricted metabolic requirements.

Barker (1935) was unable to show growth in the dark by Prototheca on any of the Krebs-cycle intermediates tested: lactic, pyruvic, citric, succinic, fumaric, malic, acetoacetic, glyoxylic, or glycolic acids. Also, no growth could be detected on glycine or asparagine. Subsequently, Anderson (1945) tested the heterotrophic growth of Prototheca at pH 4.5-5.0 on the substrates as Barker had, and demonstrated successful growth. As pointed out by Anderson, Erickson, et al.

(1955), and Samejima and Myers (1958), the undissociated acid ion may be impermeable to the cellular membrane in near neutral media, but can often support growth under more acid conditions. A single, yet valid criticism of the above cited laboratory studies is that it is highly unlikely that either Chlorella or Prototheca would occur in natural waters possessing an acid pH.

Scenedesmus sp. (strain D₃) was shown by Samejima and Myers (1958) to be capable of heterotrophic growth on glucose, although slow and continued growth could also be sustained on 1% (w/v) concentrations of galactose, mannose, fructose and three disaccharides, maltose, sucrose, and lactose. Further tests indicated the D₃ strain of Scenedesmus was unable to utilize mannitol or glycerol.

Taylor (1960a, 1960b) found Scenedesmus quadricauda capable of growth in the dark on glucose or mannose. Further, organic substrates which did not stimulate respiration (galactose, fructose, and sorbose) were only able to penetrate the cellular membrane after a long incubation period, indicative of passive uptake or diffusion processes. The uptake of glucose could be inhibited by the addition of low concentrations of mannose, and by other compounds which possess structural similarities to glucose. By adding low

concentrations of extracellular ATP and inorganic phosphate, simultaneously with low concentrations of glucose, Taylor suggested kinetics of glucose assimilation could be followed within the cell. The mechanism for concentration he concluded was active transport, although this explanation has recently been questioned by Wright and Hobbie (1966) in view of more recent experimental data.

3. Tribonema and other Xanthophyceae

Belcher and Fogg (1958) have shown that Tribonema aequale is capable of heterotrophic growth on glucose at a rate nearly identical to that obtained through photosynthetic metabolism under normal light intensities (approximately 10,000 lux). The only substrate on which this alga exhibited continued growth was sucrose, although heterotrophy could only be demonstrated after a 21 day period of adaptation. Of the acids tested, only acetic and citric were capable of sustaining growth. Also, the following organic substrates were tested, none of which were able to support growth under dark conditions: fructose, mannose, galactose, rhamnose, arabinose, xylose, lactose, cellobiose, ethanol, glycerol, fumarate, and succinate.

Tribonema minus, another of the common Xanthophyceae which is often found in the littoral regions of lakes and ponds, was shown by Belcher and Miller (1960) to have certain heterotrophic abilities. However, only glucose would support growth of the numerous hexoses, fatty acids, and metabolic intermediates which were tested.

Chemo-organotrophic growth habits of several xanthophytes have been investigated by Casselton (1966). Viability and effective growth were monitored in the dark on 1% (w/v) glucose, galactose, mannose, fructose, maltose, acetate, citrate, succinate, lactate, mannitol, ribose, xylose, and sucrose. Pleurochloris commutata was incapable of growth on any of the substrates tested; Chloridella neglecta grew well on glucose and slightly on galactose. Botrydiopsis arhiza demonstrated effective growth only on glucose; Chlorocloster solani was able to grow on all hexoses provided, yet best growth was sustained on glucose, galactose, and fructose. Visheria stellata could not utilize any of the substrates tested. Bullimilleriopsis petersenian grew on glucose, but another closely related species, B. filiformis, was unable to grow on any of the substrates. Lastly, Heterococcus caespitosus was only able to grow on glucose.

Monodus subterraneus was shown by Belmont and Miller

(1965) to be capable of growth on glutamine between pH 4 and 7.

4. Diatoms (the Bacillariophyceae)

Lewin (1953) tested the heterotrophic growth of 42 cultures of bacteria-free freshwater and soil pennate diatoms. Only 13 species were capable of heterotrophic growth: 7 varieties of Navicula pelliculosa, 5 species of Navicula spp. and 1 species of Nitzschia sp. These species were only capable of growth in the dark on glucose. One isolate of Navicula pelliculosa was tested for heterotrophic growth on 60 different organic substrates, including the usual hexoses, Krebs-cycle intermediates, phosphorylated sugars, fatty acids, alcohols, and amino acids. Only glucose was able to sustain continued growth at concentrations of 0.0073-3.7% (w/v), and the best growth was generally detected at 1-2%. One might critically question the determination of successful heterotrophic growth (based on pigment development as measured in a Klett colorimeter with a No. 66 ruby glass filter), since as in photosynthetic algae, chlorophyll production does not always or necessarily parallel growth rates, metabolic state and physiological conditions. As pigment development might be photo-dependent, and living cells

might be mixed with dead, a more appropriate estimation of heterotrophic growth might be obtained from direct counts (cf. Utermohl, 1958), in combination with the analysis of chlorophyll degradation products (Moss, 1967a, 1967b; Eaton and Moss, 1966).

Lewin (1953) further found this species of Navicula was capable of utilizing glycerol and fructose in a carbon dioxide-free medium, but only in the light; glucose was utilized in the light and in the dark without carbon dioxide.

Lewin and Lewin (1960) tested 44 cultures of marine diatoms for heterotrophic growth on glucose, lactate, and acetate. Sixteen cultures were only capable of growth on glucose; 8 could utilize glucose or lactate; 1, glucose or acetate; 1, glucose, lactate or acetate; and 2, only lactate. Although galactose was only tested in several instances, no positive growth could be detected on this substrate.

A later study was undertaken by Lewin (1963) to determine the heterotrophic ability of 39 species of axenic marine diatoms. Tests were performed in the light and in the dark on media containing 0.5% (w/v) glucose or 0.2% lactate. Half of the 24 species of littoral pennate diatoms which were tested were able to grow heterotrophically, including species of Navicula, Amphora and Nitzschia. Only one centric

diatom, Cyclotella sp., was capable of heterotrophic growth. It is noteworthy that Lewin was unable to show sustained heterotrophic growth of any of Guillard's (1963) centric marine species, with the exception of the Cyclotella sp. Further, some of the laboratory studies conducted by Lewin (1963) seem somewhat questionable since 0.1% (w/v) tryptone was added to the glucose media prior to testing for heterotrophic growth. Presumably, heterotrophic growth could still be demonstrated on glucose alone, but sustained higher growth rates could be obtained if low concentrations of tryptone were supplied.

Lewin (1963) also compared growth rates, or doubling-times, for diatom species capable of growth on glucose and lactate. For Cyclotella, autotrophic growth (3000 lux; medium: seawater plus vitamins and minerals; 20C) allowed division to occur every 18 hours; heterotrophic growth on glucose in total darkness permitted a division to occur every 30 hours. For Nitzschia closterium, division occurred under autotrophic conditions every 12 hours; under heterotrophic conditions division occurred every 78 hours on lactate. For Nitzschia marginata, growth in the light allowed division to occur every 12 hours; growth under heterotrophic conditions on glucose also showed a doubling-time of 12 hours.

Several of Lewin's (1963) conclusions seem quite important, from an ecological point of view, since his predicted pattern of heterotrophic utilization could be demonstrated with axenic laboratory cultures. More of the littoral pennate diatoms, which are found in situ in areas which are assumably rich in organic materials, were capable of utilizing organic substrates in the dark. Since centric diatoms generally inhabit neritic or pelagic waters, where there are relatively low concentrations of organic compounds available, they should be incapable of heterotrophic growth comparable to the diatoms from the littoral region. Lewin (1963) has pointed out that the diatoms which require low concentrations of tryptone, in addition to glucose and lactate, are probably not very successful at in situ heterotrophic survival; yet large populations of diatoms are found in the deeper and trophogenic zones of marine environments (cf. Wood, 1956, 1959; Kimball, et al., 1963).

Guillard (1963) tested 15 clones of centric marine diatoms, 1 pennate diatom, 2 estuarine flagellates, 1 marine green flagellate, and 3 coccolithophorides for heterotrophic growth on 100 μ M of nitrate, nitrite, ammonia, urea, uric acid, glycine, glutamate, and glutamine. Melosira and Coscinodiscus were able to grow well on glutamate and

nitrate; yet only poor growth could be detected on alpha-amino-acids. For the majority of the centric diatoms, glutamine appeared to be the best growth substrate. Also, urea and uric acid were consistently better substrates for heterotrophic growth of estuarine and neritic clones, but not for the true marine and oceanic species. Although very few data were presented, preliminary results suggest that uric acid may be utilized directly by certain of the centric diatoms and the uptake of uric acid could be significant in natural environments.

Several pertinent conclusions were presented by Guillard (1963): 1) Cosinodiscus, Asteromphalus, and Melosira are probably heterotrophic; all species were capable of extended growth on glutamine and glutamate, and utilized these substrates as readily as inorganic nitrogen; 2) clones which were capable of efficient growth on uric acid generally came from polluted and estuarine waters; and 3) the ability to grow and reproduce on uric acid could generally be correlated with the dominant planktic group. It is important to note the ability of Melosira to utilize organic nitrogen substrates, as this genus is prevalent in numerous fresh waters and frequently is a dominant form of the epipelagic algal community.

Sloan and Strickland (1966) investigated the heterotrophic

ability of four marine phytoplankters, Coccolithus, Skeletonema, Thalassiosira, and Cyclotella, the latter represented in fresh waters as well. Experimental conditions consisted of growing these species on glucose, acetate, and glutamate at low concentrations (equivalent to 0.25 mg organic carbon l^{-1}) in total darkness, and in the light. Cyclotella cryptica had previously been shown by Lewin and Lewin (1960) to be a facultative heterotroph. Although similar photosynthetic rates were demonstrated by all of the phytoplankters, with the exception of Cyclotella none of the other algae tested were capable of extended growth on any of the organic compounds provided. Carbon dioxide uptake in the dark was rapid in all forms, but only slight in Cyclotella. In the presence of low light intensities, even after a period of adaptation, assimilation rates for the organic substrates were not equivalent to those estimated for respiration.

One exception to the negative evidence for heterotrophic growth of the marine algae tested was the ability of Thalassiosira to grow and reproduce on glutamate. Further, uptake of glutamate was not suppressed by low light intensities. Sloan and Strickland (1966) suggest, because of the low concentrations of organic matter available in the sea (cf. Duursma, 1960), that appreciable in situ uptake of organic

compounds probably does not exist. One might repeat their experiments at yet lower concentrations (10 μg up to 2.0 mg glucose or acetate l^{-1}), in keeping with naturally detected concentrations of organic solutes.

5. Acetate Flagellates

The acetate flagellates are a heterogeneous group consisting of members of the Volvocales (Chlorophyta), Cryptophyta, Euglenophyta, Chrysophyta, and Pyrrophyta. Their phylogenetic relationships, biochemistry, and physiology have been extensively investigated by Hutner and Provasoli (1951, 1955) and Lwoff (1951). The acetate flagellates, or the so-called "phytoflagellates", share in common 1) the inability to utilize sugars and phosphorylated sugar intermediates, and 2) the ability to utilize directly certain Krebs-cycle intermediates, especially acetate, fatty acids, alcohols, and related compounds. Several species of acetate flagellates have received much attention in an effort to determine their specific metabolic requirements.

Danforth (1962) has presented a summary of substrates utilized by Chilomonas paramecium (Cryptophyta, non-photo-synthetic) and Polytomella caeca, a volvocalian chlorophyte,

which appears to be incapable of photosynthesis. Both species are considered to be typical "phytoflagellates", and neither species is capable of sustained heterotrophic growth on certain of the common hexoses, sugar derivatives or phosphorylated intermediates. Krebs-cycle intermediates, pyruvic, alpha-ketoglutaric, succinic, fumaric, malic, oxaloacetic acids, and lactic acid are readily utilized by Chilomonas; however, Polytomella is somewhat more restricted in that only pyruvic and succinic acids are utilized as substrates. It would appear that the tricarboxylic acids, citric, cis-aconitic and isocitric, are not utilized to any large extent. It seems reasonable to suggest that the inability to utilize these ionized and highly hydrophilic acids reflects suspected impermeability of the cell (cf. Anderson, 1945; Erickson, et al., 1955; Samejima and Myers, 1958).

Chilomonas is reported (Danforth, 1962) to be capable of growth on even-numbered, straight-chain fatty acids, from C_1 to C_8 . Polytomella is capable of utilizing odd and even-numbered fatty acids up to C_5 . Heterotrophic uptake of acids by both species is pH dependent at all concentrations, much as Chlorella (cf. Samejima and Myers, 1958); further, Chilomonas was unable to grow on any of the 20 amino-acids tested.

physiological requirements, metabolic patterns utilized, and biochemical characterization of Euglena (mostly E. gracilis) has received much attention (Cramer and Myers, 1952; Baker, et al., 1955; Danforth and Wilson, 1957; Wilson and Danforth, 1958; and Danforth, 1953, 1961). The species of Euglena which have been tested appear to be highly variable in their specific physiological and nutritional characteristics, and thus, generalized statements of heterotrophic ability are difficult. For example, certain of the strains (cf. Cramer and Myers, 1952) are able to utilize glucose, certain Krebs-cycle intermediates, amino-acids and related compounds (E. gracilis var. bacillaris); while another strain (E. gracilis, Vischer strain) is only capable of utilizing acetate and butyrate. Evidently, the metabolic requirements of the Vischer strain are more similar to those of the typical acetate flagellates (Cramer and Myers, 1952; Danforth, 1962), since the sugars, Krebs-cycle acids and amino-acids which were tested were only able to support growth under heterotrophic conditions in a limited number of instances. On the other hand, the "bacillaris" strain utilized almost every substrate tested and demonstrated little or no specificity. Further, growth by both species on most substrates showed a strong correlation between concentrations which supported growth and pH of the medium

(Cramer and Myers, 1952).

Danforth (1962) has pointed out that certain strains of Euglena are capable of greater rates of glucose uptake when concentrations of carbon dioxide are increased. Cramer and Myers (1952) found that not only was glucose uptake pH dependent, but that rates of uptake were related to concentrations tested. In effect, it was possible to show that uptake of glucose, measured as the growth response of the population, was directly proportional to the concentration up to 1% (w/v). For almost all other substrates tested, on all strains thus far reported, there is no correlation between growth rates and concentrations. Further, Cramer and Myers (1952) demonstrated that their strain of Euglena could easily switch from photosynthetic metabolism to complete heterotrophic growth on glucose in total darkness. Although a lag phase in adaptation to heterotrophic growth was encountered, the mere fact that a species which frequently inhabits freshwater ponds and shallow lakes, and can switch from one mode of metabolic growth to another with facility, is probably of ecological significance. Species of euglenophytes and diatoms which appear to be capable of autotrophic and heterotrophic growth may well be supplementing one pathway with the other in nature. Further research should be undertaken to

determine if species with these dual properties, which grow on glucose or acetate in the dark, are capable of accelerated growth rates in the presence of minimal light intensities.

Also, from a taxonomic point of view, it might be useful to group the Euglenophyta and other of the so-called "phytoflagellates" on the basis of heterotrophic ability and substrates utilized. This would be no more artificial than their present taxonomic treatment has afforded (cf. Prescott, 1962; Firtsch, 1935), although somewhat more difficult to use. The "glucose block", or the inability of many of the acetate flagellates to utilize carbohydrates (Hutner and Provasoli, 1951; Danforth, 1962) is thought to be due to the absence of a hexokinase, and thus free hexoses are not converted to phosphorylated derivatives. This metabolic characteristic might be used to place certain of the "phytoflagellates" into artificial classification schemes. Although much literature exists on the "glucose block", simple impermeability of the cell membranes to sugars of certain taxa may explain the failure to demonstrate direct utilization.

The genus Chlamydomonas is represented by species which are obligate phototrophs, although certain species are capable of heterotrophic growth and possess metabolic patterns similar

to those of the acetate flagellates (Lewin, 1950; Lewin, 1954; Wetherell, 1958; Danforth, 1962; and others). Chlamydomonas Moewusii has been shown capable of utilizing acetate, pyruvate, and succinate although cell multiplication does not occur on these substrates (Lewin, 1950). This suggests that not all substrates which can stimulate respiration are suitable energy sources, although the substrates permeate the cellular membrane. Eppley, et al. (1963) were able to show Chlamydomonas munda is capable of heterotrophic growth on acetate in total darkness, as well as under conditions of low light intensity. A planktic Chlamydomonas sp. isolated from natural lake water is capable of continued growth under autotrophic or heterotrophic conditions on glucose (Wright and Hobbie, 1966). Good growth could be maintained on concentrations of 10 mg glucose l^{-1} .

Wright (1964) has shown that two species of Cryptomonas are capable of heterotrophic growth in the laboratory on acetate, but not on glucose. Growth was tested on different concentrations of acetate (0.1, 1.0 g l^{-1}) and glucose (0.135, 1.35 g l^{-1}) at 3-5°C under the following conditions of 1) total darkness, 2) approximately 65 lux, and 3) at nearly 1400 lux, normal culture intensities for many of the Cryptomonas species. Over a period of 30 days, two of the

three cryptomonads tested showed effective growth in total darkness on 0.1 g acetate l^{-1} . None of the species was able to grow on glucose in total darkness. At low light intensities (ca. 65 lux) optical density measurements of growth were significantly increased over those in total darkness. Further, the percentage of transmitted surface light available just beneath the ice, from which the isolates were obtained, was in the order of 0.2%. Low light intensities such as these further strengthen the possibility that photosynthesis, or at least photoassimilation, is being combined with heterotrophic uptake of organic compounds during the winter months under ice cover.

6. Dinoflagellates (Dinophyceae, Pyrrhophyta)

Heterotrophic abilities of several species of photosynthetic dinoflagellates, Gyrodinium, Peridinium, Gymnodinium, Amphidinium, and one example of Exuviaella were assayed by Provasoli and McLaughlin (1963). Growth conditions were maintained at 15-20C, ca. 2150-4300 lux light intensity, and media containing 10 $\mu g\%$ of various vitamins, factors, co-factors and vitamin analogues. Almost all genera studied required certain of the vitamin complexes. Growth on amino-acid

substrates did not produce greater cell numbers than did nitrate substrates. Gyrodinium, the exception, was able to produce 2-3 times more cells when additional carbon sources were provided. Peptones and various hydrolysates, which were added to defined media did not increase the growth of any of the species tested. The authors sought to show that complete mineralization of materials is not necessarily a requirement for them to be "metabolically" consumed. Further, similar nutritional requirements were exhibited by the various taxa; all were able to utilize some organic nitrogen and phosphorus compounds and all required a minimum of two vitamins for optimal growth. In algal succession, particularly in pond and shallow water environments where many of the dinoflagellates can play a significant role, such limited heterotrophy of organic compounds may be of ecological significance.

In studies of phytoplanktic utilization of dissolved organic matter in lake water, Wright and Hobbie (1966) found Gymnodinium inversum is capable of cultured growth in total darkness on extremely high concentrations of 100 mg acetate l^{-1} .

7. Blue-greens (Cyanophyta)

Some of the nutritional requirements of several genera of marine blue-green algae, Agmenellum, Lyngbya and Tolypothrix, have been studied by van Baalen (1962). Both Lyngbya and Tolypothrix are widespread in many fresh waters, and often a significant portion of the epipelagic community. Tolypothrix was found to be capable of slow growth, whereas Lyngbya was capable of continuous and rapid growth on 1% (w/v) glucose. Van Baalen concluded that heterotrophy was not an important mode of metabolism among the marine cyanophytes.

Cheung, et al. (1964) found the uptake of glucose by Tolypothrix tenuis in total darkness was equivalent to uptake at the 1% light level. Labeling studies with glucose indicated glucose is metabolized via the pentose-phosphate shunt in total darkness. Uptake at the light compensation point (assumed by this investigator to be approximately 1% of total incident light) was probably effected through the Embden-Myherhoff-Parnas pathway. Later work (Cheung and Gibbs, 1966) confirmed that these two metabolic systems were operative.

Of the following organic substrates which were provided at concentrations of 0.1M: glucose, sucrose, maltose, glycine, and glutamine, heterotrophy of Chlorogloea Fritschii was shown to be limited primarily to the utilization of sucrose (Fay,

1965). In the presence of nitrate, good growth could be supported on maltose, and slight growth occurred on the other substrates. In the absence of nitrogen, growth occurred on all substrates tested with the exception of glucose and, in one instance, mannitol. Even at concentrations of less than 0.1M, sucrose allowed ammonia, nitrite and nitrate to be fixed at greater rates than on glucose.

8. Summary and Conclusions

1. Most attempts to demonstrate growth in total darkness on organic substrates have either failed entirely, or have proven inconclusively that in situ algal heterotrophy occurs. Several possible reasons for this might be offered. In laboratory cultures abnormally high concentrations of organic compounds have been used to test for growth in the dark (usually concentrations of 100-1000 mg l⁻¹), and are probably too high to even induce uptake. It is not unreasonable to suggest that inhibitory or even toxic effects would preclude the detection of a heterotrophic response at such high concentrations. In certain instances where Chlorella has shown a heterotrophic response, the cells accumulate polysaccharides and starch reserves to where they are

considered metabolic "giants". Cells which accumulate such storage products are incapable of normal cell division.

The importance of replicating in situ conditions in metabolic and physiological studies that are conducted in the laboratory should be stressed. Concentrations of organic solutes in natural waters (Birge and Juday, 1934; Vallentyne, 1957; Menzel, 1964; Wangersky, 1965; Wright and Hobbie, 1966; Wetzel, 1967a, 1967b; Allen, 1967, in preparation; and others), range from 0.2-50 mg l⁻¹, and generally less than 10 mg l⁻¹ is available in fresh waters. As only a small fraction of this amount is probably available for metabolic use, perhaps less than 1 mg l⁻¹, it seems reasonable to test for heterotrophic growth in this range of concentrations. Further, the compounds most likely to serve as substrates would be hexoses, acetate, lactate, short-chain amino-acids, representative high energy intermediates of glycolysis, and other small molecules which might be able to enter the cell readily without incurring a permeability problem.

One of the most important considerations in demonstrating heterotrophic uptake would be to use compounds which are readily permeable at near in situ pH values. The difficulty encountered in growing cells on certain organic acids may only reflect their unavailability in nature.

2. Although only a comparatively few algae have been tested for heterotrophic abilities, this property seems to be limited to several distinct genera in 1) the Chlorococcales and Volvocales, including Chlorella, Scenedesmus, Chlamydomonas, and Prototheca, 2) certain of the Xanthophyceae, but mainly Tribonema, 3) several of the Cyanophyta, namely Lyngbya and Tolypothrix, 4) many of the Euglenophyta, including Euglena and Chilomonas, 5) Cryptophyta, including Cryptomonas, 6) several of the Bacillariophyceae, including Navicula, Nitzschia, and Cyclotella, and 7) several of the Pyrrophyta, inclusive of Gymnodinium, Peridinium, Gyrodinium, and Amphidinium.

3. It seems likely that we should find numerous heterotrophic genera among the pond and littoral species, and many of the following groups probably have rather universal heterotrophic tendencies 1) Euglenophyta, 2) Bacillariophyceae (mostly the pennate species), 3) Cryptophyta, 4) Volvocales and Chlorococcales; also related filamentous greens, 5) certain of the blue-greens, including Oscillatoria, Anabaena, Lyngbya, and Tolypothrix, and 6) certain of the dinoflagellates, including Gymnodinium, Ceratium, Glenoidinium, Gyrodinium, and Peridinium.

II. Research

A. Introduction

Much investigation has recently focused on the intricate relationships which exist between dissolved organic matter in freshwater and marine environments and the natural populations of algae and bacteria which are capable of producing, transforming and utilizing it (Parsons and Strickland, 1962; Hobbie and Wright, 1965a, 1965b; Wright and Hobbie, 1965a, 1965b, 1966; Hamilton, et al., 1966; Vaccaro and Jannasch, 1966; Wetzel, 1967a, 1967b; Allen, 1967, in preparation; and others). For the most part, the emphasis has been 1) to determine mechanisms of utilization which are available to in situ micro-organisms and 2) to determine annual cycles of dissolved organic matter as related to algal and bacterial periodicity. Primary interest has been placed on actual rates of utilization by in situ populations, annual fluctuations of the qualitative and quantitative aspects of organic compounds in aquatic ecosystems, and on learning basic principles of aquatic metabolism.

Bacterial heterotrophs in freshwater and marine environments are thought to be the most important constituents of the plankton which are capable of direct utilization of dissolved organic compounds (Fred, et al., 1924; Henrici, 1937; Henrici and McCoy, 1938; Taylor, 1940; Waksman, 1941; Zobell, 1946; Potter and Baker, 1956, 1961; Potter, 1964). Some indication that algal communities may be capable of direct utilization of organic solutes in nature has been indicated by Rodhe (1955, 1962), McAllister, et al. (1961), Wood (1956, 1959, 1963), Wright (1964), and Rodhe, et al. (1966).

Dissolved organic matter present in natural fresh and marine waters is generally within the range $0.1-50 \text{ mg l}^{-1}$ (Birge and Juday, 1934; Dazko, 1939, 1951, 1955; Plunkett and Rakestraw, 1955; Vallentyne, 1957; Skoptinsev, 1959; Duursma, 1960; Hood, 1963; Menzel, 1964; and others). Most frequently, concentrations of organic solutes in seawater are consistently lower ($0.1-5.0 \text{ mg l}^{-1}$) than concentrations found in lakes, ponds and estuarine environments ($5.0-15.0 \text{ mg l}^{-1}$). Few compounds have been identified in aquatic environments (cf. Provasoli, 1963); however, some effort has been exerted in identifying the presence of certain compounds in natural environments, e.g. free-sugars

(Vallentyne and Whittaker, 1956); amino-acids (Tatsumoto, et al., 1961); organic acids (Shapiro, 1957; Koyama and Thompson, 1959); carbohydrates (Lewis and Rakestraw, 1955); organic catalysts and free enzymes (Kreps, 1934); and numerous other compounds.

The amount of organic matter available for metabolic consumption in natural water is estimated to be but a small fraction of the total concentration detected through various assay techniques (cf. Keys, et al., 1935; Waksman, 1941; Fogg, 1959; Kusnetzow, 1959; Provasoli, 1963). Wright and Hobbie (1966) have stressed that in evaluating the importance of dissolved organic matter in freshwater and marine ecosystems, we should attempt to determine actual rates of supply and turnover of compounds, rather than concentrating our efforts on the descriptive analyses of determining the quality and quantity of organic compounds present.

B. Dissolved Organic Matter in Freshwater and Marine Ecosystems

Prior to the application of labeled organic compounds to the study of production and utilization of organic matter in natural aquatic systems, techniques were limited to the

estimation of bacterial numbers and physiological responses of these microorganisms to various media and growth conditions. Further, crude analyses of dissolved organic carbon and related bioassay techniques were time-consuming and indicated nothing of the nature of the compounds which were present. By means of enumerating directly on plates or membrane filters, and by dilution-counting techniques, it is possible to estimate the number of bacterial organisms present in a known volume of water (Collins, 1963). Whether total numbers can be correlated with concentrations of dissolved organic matter, and thus be used as an index of concentrations present, it not well established. Enrichment and physiological studies suggest that such correlations must be made with caution, as pH, temperature, inorganic and organic nutrients, vitamins, and numerous other parameters have a pronounced effect on the magnitude of in situ growth which cannot be demonstrated under laboratory conditions (cf. Potter, 1964). As Wright and Hobbie (1966) point out, total numbers of bacteria and the ability to grow selectively on certain organic substrates yield little concerning in situ processes involved in the utilization and regeneration of this material.

Goldman, et al. (1967), Saunders (1958), and Rodhe (1962), among others, have applied radioactive substrates to lake

water samples and have found that labeled organic compounds are readily utilized by natural populations under in situ conditions over short periods of time.

A new radiochemical method for estimating the "relative heterotrophic potential" of microorganisms in seawater was developed in an attempt to compare autotrophic and heterotrophic processes in marine environments (Parsons and Strickland, 1962). The uptake of ^{14}C -labeled glucose and acetate by natural marine heterotrophic bacteria could be kinetically analyzed by Michaelis-Menten enzyme kinetics or the Langmuir adsorption isotherm (cf. also Hinshelwood, 1946; Johnson, et al., 1954; and Fruton and Simmonds, 1958, for a description of the kinetic aspects of enzyme chemistry which are associated with bacterial permeation and uptake processes). By adding $250 \mu\text{g C l}^{-1}$ (glucose or acetate) to an untreated seawater sample, they reasoned the $0\text{--}20 \mu\text{g C l}^{-1}$ already in the sample as dissolved organic compounds could be ignored, and the uptake detected would be primarily that of the added material. In their method, a series of concentrations of labeled and unlabeled substrate was added to natural samples and uptake was measured in the dark over a specific time interval (≤ 4 hours) at near in situ temperatures.

From the uptake kinetics, it was possible to calculate $(K + S)$ which represents the sum of a constant similar to the Michaelis constant (K) and the in situ concentration of the organic solute (S). Assuming that (K) is quantitatively much smaller than (S), this technique has made it possible to 1) estimate rates of removal of organic compounds by natural heterotrophic populations or communities and compare this to photosynthetic assimilation, 2) to compare these uptake parameters in various aquatic ecosystems as an estimate of heterotrophic activity, and 3) to investigate annual cycles of organic compounds and to assess the role of their in situ production.

Wright and Hobbie (1965a) used ^{14}C -labeled glucose and acetate to measure the uptake of these solutes by freshwater planktic algae and bacteria. In natural samples they found glucose and acetate uptake also followed Michaelis-Menten kinetics, and that it was possible to calculate the maximum theoretical velocity of uptake, V . After further investigation, it was necessary to modify the earlier methods to give more precise information about the uptake kinetics and to distinguish between, and measure separately, uptake by planktic bacteria and uptake by the algae. In analyzing their kinetic data, they used the Lineweaver and Burk modification (cf. Fruton and

Simmonds, 1958:251 ff) of Michaelis-Menten enzyme kinetics.

Two distinct uptake mechanisms, which were simultaneously operating in natural populations, were detected (Wright and Hobbie (1965a). Uptake at low substrate concentrations (0-ca. 500 μg glucose or acetate l^{-1}) followed Michaelis-Menten enzyme kinetics, and was attributed to the bacteria. Uptake detected at higher concentrations (0.5-4.0 mg l^{-1}) complied with simple diffusion kinetics and was found to be associated with the algae. By controlling the amount of substrate added to a natural lakewater sample, the two systems of uptake, active transport by the bacteria and passive diffusion by the algae, can be separated fairly well and the various kinetic parameters associated with each system calculated.

Experiments conducted with axenic algal and bacterial cultures, separately and combined, have confirmed that these two distinct processes probably exist in nature (Wright and Hobbie, 1966), and offer an explanation for mechanisms available to planktic populations in directly competing for organic compounds in lake water (Hobbie and Wright, 1965b). A discussion of active transport, and permease systems, which typify bacterial uptake effected through enzymatic processes is given by Cohen and Monod (1957) and Kepes (1963). Diffusion processes, as utilized by the planktic algae and typical of

uptake due to a simple concentration gradient, is discussed at length by Jennings (1963).

Kinetics derived from the measurement of bacterial metabolism (Wright and Hobbie, 1965a, 1966) permit an estimation of 1) natural substrate concentrations of organic compounds ($\mu\text{g l}^{-1}$), 2) the maximum theoretical velocity of substrate removal ($\mu\text{g l}^{-1}\text{hr}^{-1}$), and 3) the turnover time of substrate regeneration (hours). Kinetic parameters obtained from the measurement of algal diffusion are limited to 1) a constant describing the maximum rate of diffusion of an organic solute (hr^{-1}) and 2) a measure of the turnover time of substrate regeneration due to diffusion mechanisms (hours).

C. Description of Study Area

Lake Löttsjön is located approximately 5 km northwest of Stockholm on the eastern Baltic coast of Sweden (1.2 km NNW of the train station in Sundbyberg; latitude $59^{\circ} 23' \text{ N}$ and longitude $17^{\circ} 58' \text{ E}$). Although age analysis by carbon- ^{14}C dating of core samples has not been conducted on sediments removed from the lake, some indication that the lake was connected to a larger inland waterway was shown by the

discovery of a "dug-out" ("ekstock") canoe on the eastern side of the lake during dredging procedures in 1960-1962. The estimated construction date of the boat was 700-800 B.P. Further evidence of age can be seen in several maps of the lake, produced in 1939, where sediment analysis indicated 14-17 m of sediment in the basin. However, because of recent extensive landscaping surrounding the lake, presently the focal point of the city park in Sundbyberg, a direct evaluation of the geological formation of the lake basin is difficult. There is little question that deglaciation and rising and falling ocean levels have created many of the "tectonic" basins (Hutchinson, 1957), which are characteristic of this region of the Baltic coast bordering on Scandinavia. According to the classification scheme presented by Hutchinson (1957), Lake Löt sjön would be classed as a Type 1 lake.

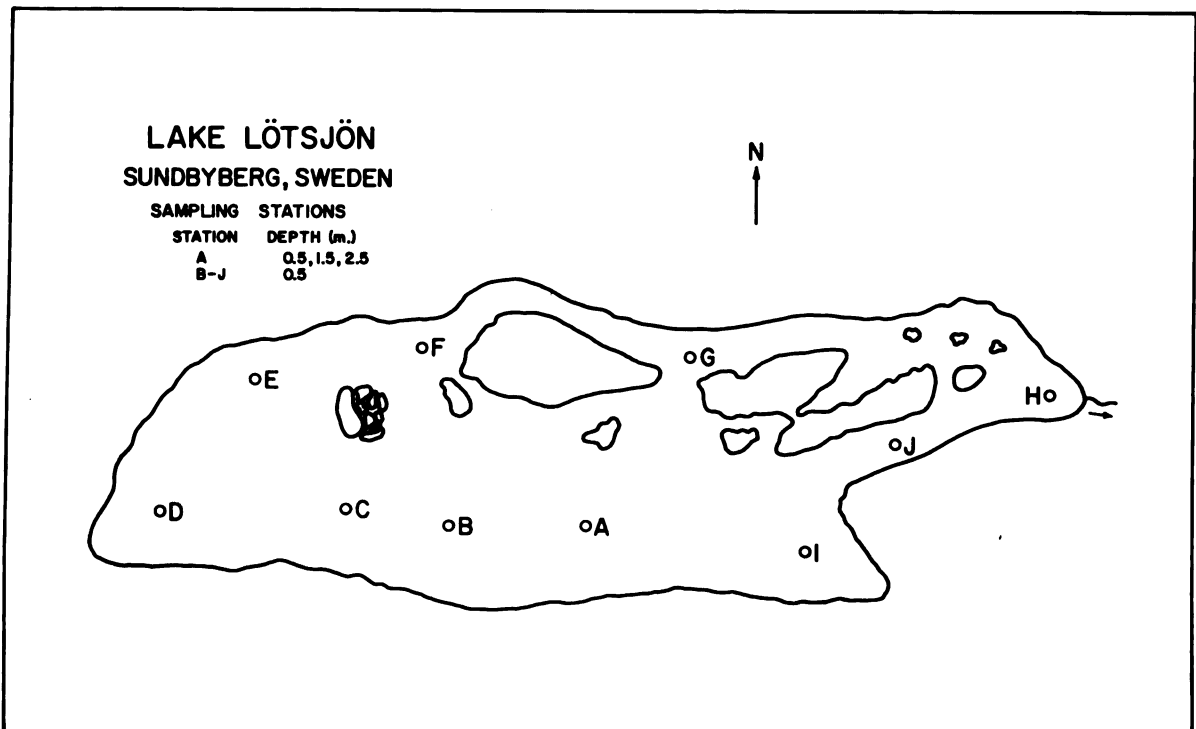
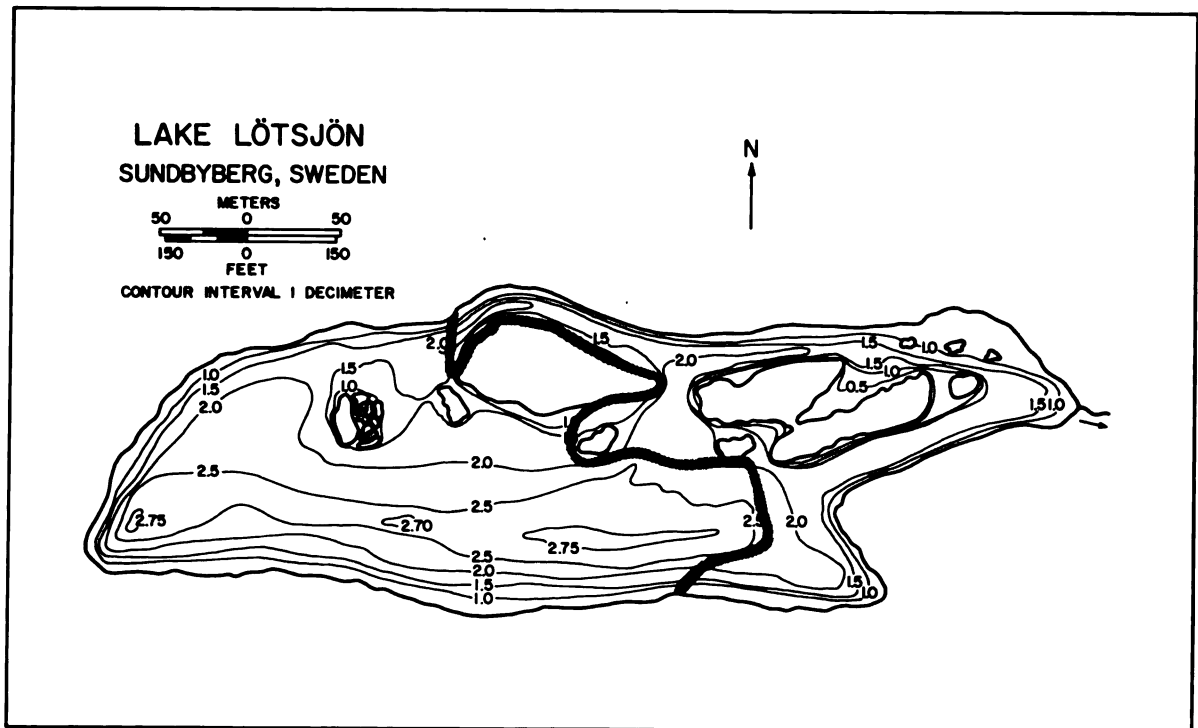
In the early 1940's, Lake Löt sjön was acquired by the City of Sundbyberg, with the intention of eventually converting the area into a park, inclusive of artificial islands, large stands of aquatic plants and colonies of birds. Between 1952-1954 sewage inlets into the basin were examined and sealed, a road was constructed around the lake, and a major effort went into relocating the drainage system which flows

from Lake Löttsjön into Lake Råstasjön. In 1955 a regulatory dam was constructed at the outlet, and the drainage system was made deeper by removing undesirable water plants. Between 1958-1960 ground water culverts were constructed to feed the lake, draining a watershed of approximately 1.5-2.0 km². The two large artificial islands (Fig. 1) were constructed in 1960 from wooden poles and bundles of Phragmites reeds. From 1960-1962 the eastern portion of the lake was dredged to a mean depth of 1.5-2.0 m (the area to the right of the shaded line in Figure 1), enlarging the surface area by 30-40% (lake perimeter was increased from 920 m to 2200 m; surface area was increased from ca. 30,000 m² to over 57,000 m²). During the last three years more artificial islands have been added, in addition to various park facilities surrounding the lake.

The present basin of Lake Löttsjön lies along a ENE-WSW axis, at an elevation of 4.0 m above sea level. During July and August, 1965, the lake was sounded on predetermined transverse and diagonal transects. The morphometric map of the lake was constructed and morphometric parameters were calculated by planimetry (Welch, 1948): volume: 102,190 m³; total surface area excluding islands: 57,110 m²; total surface area of islands: 9370 m²; maximum length: 544 m;

Figure 1. Morphometric map of Lake Löttsjön, Sundbyberg, Sweden. The lake was surveyed by the author and C. Allen on 30 July-2 August 1965. Shaded-line reveals old shoreline prior to dredging in 1960-1962.

Figure 2. Sampling stations in Lake Löttsjön, Sundbyberg, Sweden. Station A (0.5, 1.5 and 2.5 m) represents the collection site of the vertical profile used in the annual cycle study. Samples collected from other stations at 0.5 m only.



maximum width: 184 m; maximum effective length: 484 m;
maximum effective width: 175 m; maximum depth: 2.78 m;
mean depth: 1.79 m; and mean depth-maximum depth relation-
ship: 0.72.

Hydrological data, concerned with surface evaporation, direct precipitation, average outflow, etc., are not given here, although such information is readily available from the Sveriges Meteorologiska och Hydrologiska Institut, Fridhemsgatan 9, Stockholm 12. Surface evaporation often exceeds water input by direct precipitation and ground-water, especially between the months of June and September. When this condition occurs, vast areas of the littoral vegetation, emergent hydrophytes and attached algae become desiccated and rapid decomposition follows. This closed condition contributes to the concentration of organic and inorganic nutrients.

Total ionic concentration (meq l^{-1}) and ionic composition (milliequivalents %) were routinely determined throughout the summer period, June through September, 1965 (Table 1), as a portion of a research program initiated by the Sundbyberg Park Commission to characterize the water chemistry of lakes Löttsjön and Råstasjön. Comparisons are made of mean determinations of cations and anions from lakes Löttsjön

Table 1. Total ionic concentration (meq l^{-1}) and ionic composition ($\text{meq } \%$) for Lake Lötsjön with interconnecting ponds and stream to L. Råstasjön, lakes Norrviken and Ösbysjön. Comparative data are given for 25 lakes in the Uppland district (Sweden) and for river and lake waters of the world. As a baseline measure, "Standard Composition" of fresh waters is included.

	Total Ions		% Total Ions						
	Cations	Anions	Cations				Anions		
			Ca	Mg	Na	K	HCO ₃	SO ₄	Cl
a. Löttsjön ¹	6.253	6.257	54.3	16.6	26.1	3.0	60.8	13.4	25.8
Pond 1	6.278	6.243	58.0	16.8	22.2	3.0	60.1	26.8	19.7
Pond 2	6.532	6.535	58.1	17.1	22.0	2.8	57.9	18.5	23.6
Stream	7.125	7.129	60.9	16.8	19.5	2.7	55.6	16.8	27.1
Råstasjön	5.684	5.727	49.2	18.5	29.1	3.3	52.1	21.1	26.8
b. Ösbysjön ²	3.892	3.882	54.4	17.1	24.1	4.4	64.8	15.3	19.9
Norrviken	3.921	3.792	56.9	18.1	16.0	9.0	55.5	32.6	11.9
Uppland(1-21) ³	1.952	1.998	67.3	16.9	13.6	2.2	74.3	16.2	9.5
Uppland(22-25) ⁴	4.879	4.879	52.0	13.5	22.2	2.3	65.3	17.3	16.9
c. River waters									
of the world ⁵	1.425	1.441	52.6	24.0	19.2	4.2	67.9	16.5	15.6
Standard									
Composition ³	-	-	63.5	17.4	15.7	3.4	73.9	16.0	10.1

(1) Mean values for 6 subsurface (0.5 m) samples from 5 August 1965. Only one sampling station was used in each pond, stream and inflow to L. Råstasjön. (2) Ahl, 1963. (3) Rodhe, 1949. (4) Rodhe, 1958. (5) Livingstone, 1963.

and Råstasjön, the two small ponds and stream connecting them, two other lakes on the eastern Swedish coast, lakes Ösbysjön and Norrviken, two series of lakes investigated in north central Sweden, river waters of the world, and "Standard Composition" of natural fresh waters. Although total concentrations of cations and anions are high in Lake Lötsjön for an inland body of water, only sodium and chloride ions are in greater concentration than values given as mean "Standard Composition". Calcium, magnesium, potassium, bicarbonate, and sulfate ions are below concentrations of "Standard Composition".

Selected physical and chemical parameters were determined during the summer period, June through September, for the whole lake (mean values from stations A-J; Fig. 2). Throughout the entire summer, there was little evidence of stratification of any of the parameters which were monitored.

Hydrogen ion concentration (pH) was generally 7.80-8.05 in the surface waters. Specific conductance (10^6 ohms^{-1} @ 20C) was generally 550-575; dissolved oxygen as determined by the modified Winkler technique normally was within the range 7-11 mg l^{-1} , equivalent to approximately 70-125% saturation at ambient temperatures. Alkalinity (A') was consistently 3.4-3.9 meq l^{-1} , with bicarbonate concentrations

of 200-240 mg HCO_3^- l^{-1} , carbon dioxide concentrations of 2-5 mg CO_2 l^{-1} , and carbonate concentrations of 0.8-1.5 mg CO_3 l^{-1} . Ammonia concentrations were variable, but usually within 100-500 $\mu\text{g NH}_4\text{-N}$ l^{-1} ; nitrite concentrations were generally 5.0-13.0 $\mu\text{g NO}_2\text{-N}$ l^{-1} ; whereas concentrations of nitrate were 3-22 $\mu\text{g NO}_3\text{-N}$ l^{-1} . Total nitrogen (Kjeldahl-N, nitrite and nitrate) ranged from 0.75-1.30 mg l^{-1} . Soluble phosphate concentrations were 0.19-35.0 $\mu\text{g PO}_4\text{-P}$ l^{-1} , while total phosphorus was 175-700 μg l^{-1} . Total iron was 375-1800 μg l^{-1} ; particulate oxidizable organic carbon was within the range 3.6-6.7 mg C l^{-1} . Temperature was monitored on an annual basis and will be discussed in some detail. A more complete summary of water chemistry and physical properties of Lake Löttsjön, in which methods of analysis are indicated, has been published elsewhere (Allen, 1967).

Minor mention should be made of the littoral vegetation surrounding Lake Löttsjön. On the west side of the lake, large stands of Nuphar luteum are found, growing adjacent to stands of Typha angustifolia and T. latifolia. Also, the south periphery of the lake is dominated by N. luteum, with scattered stands of T. latifolia, T. angustifolia, Phragmites communis, Lysimachia vulgaris, and Carex gracilis. The southeastern tip of the lake has prominent stands of

Sparganium ramosum, Carex gracilis, and T. angustifolia.

The region near the outlet is dominated with Carex gracilis.

Further, almost all of the islands are interconnected with N. luteum. Dominant among the attached algal flora were two species of Cladophora.

Inasmuch as seasonal periodicity of total numbers and species of phytoplankton was monitored over an annual period, and will be discussed later, only a brief statement concerning composition will be offered here. The various algal groups, in order of dominance during the summer at the surface (0.5 m) were: Chlorophyta (Scenedesmus, Ankistrodesmus, Chlamydomonas), Cryptophyta (Cryptomonas), Euglenophyta (Euglena, Trachelomonas), Chrysophyta (Phacae, Mallomonas), and Bacillariophyceae (Cyclotella, Synedra). At the middle depth (1.5 m), dominance was maintained by the Chlorophyta and Chrysophyta, with equal subdominance by Euglenophyta, Bacillariophyceae, and Cryptophyta. Immediately above the sediments, at 2.5 m, dominance was shown by the Chlorophyta, and secondarily by the Bacillariophyceae, Chrysophyta, and the Euglenophyta. The Cyanophyta were never found to contribute significantly to the plankton biomass, the only exception being the presence of Anabaena sp. at 0.5 and 1.5 m in June. The number of planktic algal species present was

generally 25-30, with a large percentage of these less than 10-15 μ in diameter.

Dominance in the zooplankton was generally shown by the Rotatoria (Keratella spp., Polyarthra spp., Trichocera sp., Asplanchna sp., Synchaeta sp., Filinia sp., and Kellicottia sp.). Secondary dominance was maintained by several copepods, Diaptomus sp. and Cyclops sp., and two species of Bosmina spp., members of the Cladocera. Generally, a large number of ciliates were present.

There were several reasons for selecting Lake Löttsjön for this study. Foremost, Wright and Hobbie (1965a, 1966) conducted most of their studies on a mildly eutrophic lake, Lake Erken, and some comparative studies from other waters were thought to be necessary. Lake Löttsjön offered a high concentration of organic matter, and a rapidly growing algal and bacterial biomass, as shown through preliminary studies. Further, a rapid turnover and concentration of organic and inorganic nutrients were suspected in this lake, which would offer ideal conditions for testing the newly developed techniques in a near polluted aquatic environment.

D. Methods

1. Kinetic Aspects: Theory and Measurements

Steeman Nielsen (1952) presented an equation for the uptake of inorganic ^{14}C -labeled material by natural populations in the dark over short periods of time. Parsons and Strickland (1962) used ^{14}C -labeled glucose, acetate, and carbon dioxide to measure uptake by natural heterotrophic marine populations, and modified the equation presented by Steeman Nielsen to give a formula which assessed the velocity of kinetic uptake from such measurements:

$$v \text{ (mg C m}^{-3} \text{ hr}^{-1}\text{)} = \frac{c \cdot f \cdot (S_n + A)}{C_{pt}} \quad (1)$$

where v is the rate of uptake in $\text{mg m}^{-3} \text{ hr}^{-1}$; c , the radioactivity of the filtered organisms (cpm); f , a factor to correct for possible isotopic discrimination (1.05; the same as that used in ^{14}C measurements of photosynthesis); S_n , the in situ concentration ($\mu\text{g l}^{-1}$) of the organic substrate; A , the concentration ($\mu\text{g l}^{-1}$) of added substrate (labeled and unlabeled); C , the cpm from 1 μC of ^{14}C -labeled substrate, which should be corrected for geometry and self-absorption in the counting assembly used; μ , is the quantity of ^{14}C added to the sample; and t , the incubation time in hours. This

equation is suitable provided 1) isotopic discrimination is minimal, 2) ^{14}C excretion is insignificant, 3) ^{14}C is actually being incorporated as cell-carbon subsequent to uptake, and 4) natural substrate concentrations (S_n) are much smaller than (A). Vaccaro and Jannasch (1966) have recommended that (S_n) be determined by independent analysis or bioassay procedure, if in situ concentrations are suspected to be substantial.

Parsons and Strickland (1962) found the uptake (v) of ^{14}C compounds by marine populations was non-linear over a limited range of substrate concentrations. However, by applying the Michaelis-Menten equation, in a modified form as the Lineweaver-Burk plot, it is possible to analyze the uptake kinetics more completely. The original Michaelis-Menten equation is given as:

$$v = \frac{V(S)}{K_m + S} \quad (2)$$

where $\underline{v}$ is the velocity of uptake established at a substrate concentration $\underline{S}$; $\underline{V}$, the maximum velocity attained when all uptake sites are saturated with substrate; $\underline{K_m}$, the Michaelis-Menten constant and is defined as the substrate concentration when the velocity $\underline{v}$ is exactly one-half of the maximum velocity,

V.

By inverting equation (2), and multiplying through by (S), the Lineweaver-Burk modification (which converts the hyperbolic saturation curve of equation (2) into its linear form) is obtained:

$$\frac{S_n}{v} = \frac{K_t}{V} + \frac{S_n}{V} \quad (3)$$

or

$$\frac{S_n + A}{v} = \frac{K_t + S_n}{V} + \frac{A}{V} \quad (S_n = S_n + A)$$

where v is the rate of uptake at a specific substrate concentration; V, the theoretical maximum velocity of uptake at saturation, and K_t, a constant similar to the Michaelis-Menten constant (see explanation in Fruton and Simmonds, 1958).

By rearranging equation (1) to:

$$\frac{(S_n + A)}{v} = \frac{C_{ut}}{c} \quad (4)$$

and by substituting into equation (3), we may obtain:

$$\frac{C_{ut}}{c} = \frac{(K_t + S_n)}{V} + \frac{A}{V} \quad (5)$$

which describes uptake kinetics of natural bacterial populations (Wright and Hobbie, 1965a, 1966).

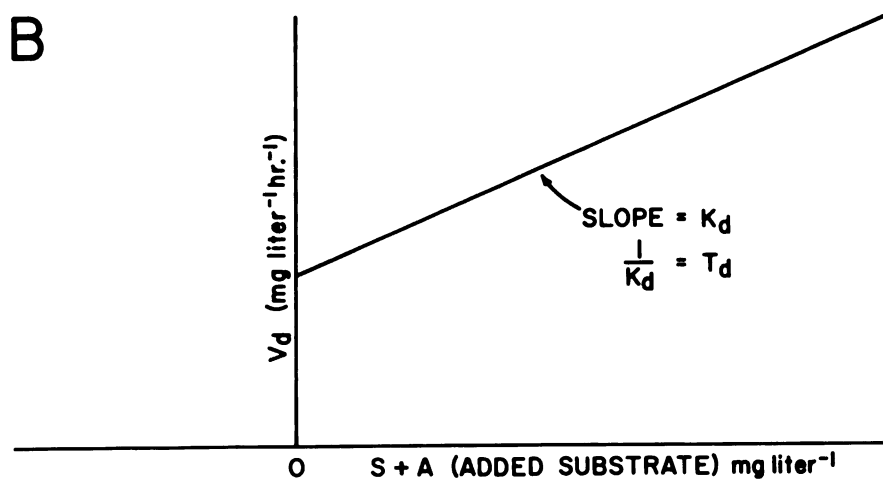
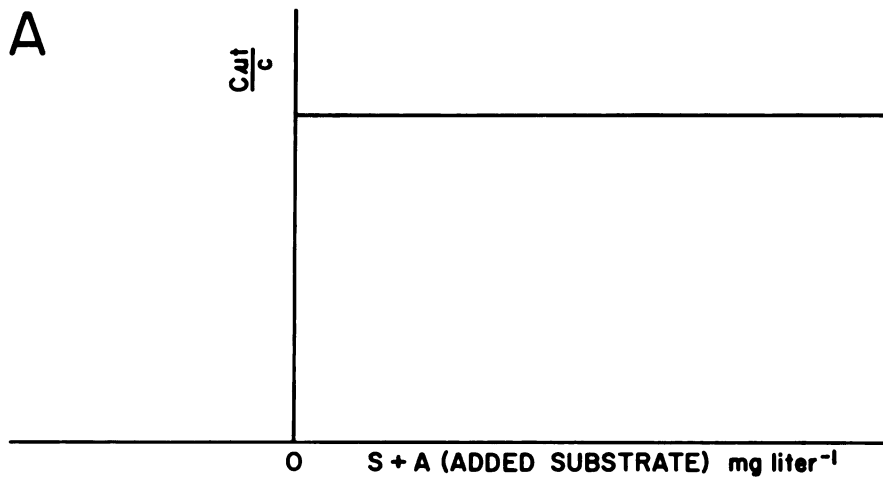
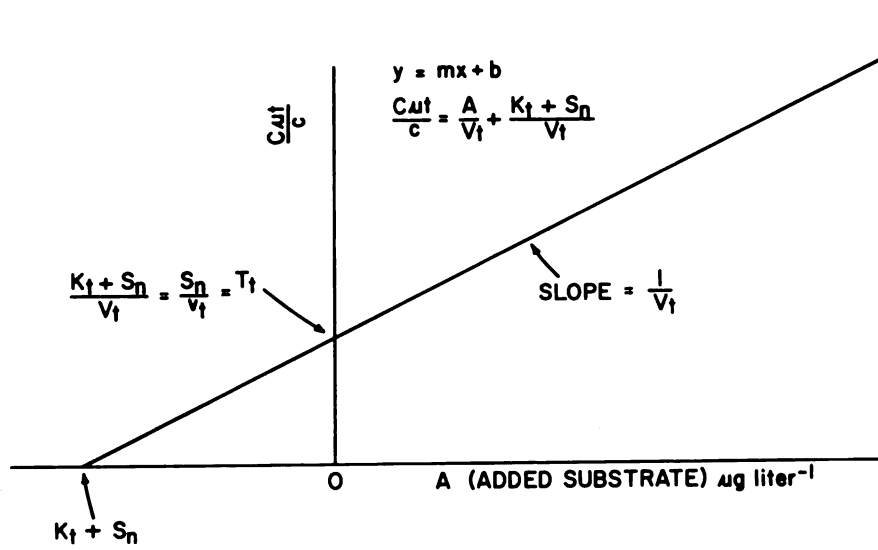
By plotting C_{ut}/c against A (Fig. 3), the intercept on the negative abscissa is equal to $(K_t + S_n)$; the reciprocal of the slope is V , the maximum rate of uptake. The ordinate intercept is equivalent to the turnover time, T_t , which represents the time required for complete removal of the natural substrate. The sum $(K_t + S_n)$ approximates the in situ substrate concentration, S_n , if K_t is very small. Quantitative estimations of K_t were shown by Hobbie and Wright (1965a) in studies of freshwater microbial bioassays to be approximately $5 \mu\text{g l}^{-1}$ for glucose.

Basically, the same assumptions apply to the Wright and Hobbie techniques which were indicated for the Parsons and Strickland procedures. In addition, however, these new procedures require 1) that a constant rate of removal and regeneration of the organic solute are taking place in situ, which implies steady-state conditions, 2) that the ^{14}C organic substrate which is being taken up is not immediately respired by the organisms, and 3) that the organic substrates

Figure 3. Graphical analysis of bacterial uptake at low substrate concentrations following Michaelis-Menten enzyme kinetics. Plot of C_{pt}/c against increasing substrate concentrations (S), illustrating derivation of (1) natural substrate concentration ($K_t + S_n$) as $\mu\text{g l}^{-1}$ (2) maximum velocity of bacterial uptake (V) as $\mu\text{g l}^{-1}\text{hr}^{-1}$ and (3) turnover time for substrate regeneration (T_t) as hours.

Figure 4a. Graphical analysis of algal uptake at high substrate concentrations following kinetics of simple diffusion. Plot of C_{pt}/c against increasing substrate concentrations (S + A), indicating constancy in slope of response line.

Figure 4b. Graphical analysis of algal uptake at high substrate concentrations following kinetics of simple diffusion. Plot of uptake velocity due to diffusion (V_d) against increasing substrate concentrations (S + A), illustrating derivation of diffusion constant (K_d) as hours^{-1} and turnover time of substrate regeneration (T_d) as hours.



added to the natural samples be of a very high specific activity.

Uptake of ^{14}C -labeled organic compounds by natural plankton populations at high substrate concentrations (greater than 0.5 mg l^{-1}), does not exhibit rate limitation or substrate saturation of uptake sites, and likewise does not follow Michaelis-Menten kinetics. Wright and Hobbie (1965a) were able to show uptake velocity continually increases as substrate concentration is increased. The slope of the response line was shown to be a constant, K_d , derived from diffusion kinetics (Figs. 4a and 4b). Wright and Hobbie (1966) have used this constant to estimate diffusion uptake by natural plankton algae. If S_n is known, the rate of uptake due to diffusion (V_d) can be estimated:

$$V_d = (K_d) (S_n) \quad (6)$$

Also, the turnover time due to diffusion, T_d , may be calculated as:

$$T_d = \frac{S_n}{V_d} = \frac{1}{K_d} \quad (7)$$

or it may be obtained as the reciprocal of the slope of V_d plotted against S_n . Concentrations of substrate used in calculating these parameters correspond to those concentrations of labeled and unlabeled substrate which were added to incubated samples. Further comparisons of removal by natural bacterial and algal organisms can be made by comparing the ratio of the two uptake mechanisms as:

$$\frac{T_t}{T_d} = \frac{V_d}{V} \quad (8)$$

Moreover, these measurements, and their ratios may be used for comparisons of different natural fresh and marine waters even though natural substrate concentrations, composition, and rates of uptake are not known.

Assumptions made in monitoring algal diffusion are 1) that materials are incorporated into cell-carbon as they diffuse into the cell, and 2) that insignificant excretion of ^{14}C -labeled material, or respiration, is taking place during the brief incubation periods employed.

a. Measurement of Bacterial Utilization

Lake water samples were collected using a Ruttner sampler from a depth of 0.5, 1.5 and 2.5 m (maximum depth 2.8 m) at station A and transported to the laboratory in the dark at slightly below in situ temperatures. Samples from each depth were promptly (≤ 1 hour) dispensed in 25 ml (summer) or 50 ml (winter) aliquots into each of five 100 ml glass-stoppered bottles. Serially increased amounts of uniformly labeled glucose or acetate (usually 25, 50, 100 and 200 μl or 50, 100, 200 and 400 μl ; equivalent to 1-60 $\mu\text{g l}^{-1}$) were added with micropipettes to four bottles (labeled organic substrates were supplied by Radiochemical Centre, Amsham, England; glucose- ^{14}C and acetate- ^{14}C were diluted 1 $\mu\text{C ml}^{-1}$ and sealed in sterile ampoules according to procedures given in Strickland and Parsons, 1960; specific activity: glucose, 50-150 mc mM^{-1} ; sodium acetate, 20-40 mc mM^{-1}). Blanks were prepared by adding 25 or 50 μl of ^{14}C -labeled substrate, and immediately fixing the sample with I_2KI to inhibit further metabolic activity. In some measurements, an entire series of blanks was prepared for each series of incubated samples. The samples were then incubated in the dark at near in situ temperatures (within 1C) for 0.5 to 3.0 hours. During the summer months large natural populations allowed shorter incubation periods; conversely, during winter months the

incubation period was lengthened to 3.0 hours. Incubation times were found to be inversely related to ambient water temperatures and the density of natural populations. It is absolutely necessary, however, that the incubation time be less than the turnover time, T_t , for the solute being measured.

After incubation, the samples were immediately fixed with iodine-acetic acid (Lugol's) solution and filtered in a multiple filtration unit onto Göttingen membrane filters (pore size $< 0.45\mu$), dried, desiccated, and the radioactivity measured in a Tracer Lab proportional counter of known efficiency. Loss of radioactivity from the cellular pool by fixation with Lugol's solution, rather than by direct and immediate filtration, was checked and estimated to be less than 10% for glucose and acetate.

Activity from the counted filters, after subtraction of blank values, was then analyzed both graphically and mathematically to determine V , $K_t + S_n$, and T_t , as described above. Several examples of the Lineweaver-Burk plot showing the maximum, minimum and normal deviation from a true linear response for the kinetic uptake of glucose and acetate are given in Appendix 1. It should be stressed that these parameters may only be determined through a population response by aquatic bacteria to the addition of labeled

organic substrates, and not through a response of the algal fraction. Serious error may be interjected into these measurements 1) if some aquatic bacteria present in the sample lack permease-active transport mechanisms for the substrate added, 2) if uptake is purely an induced phenomenon, which is doubtful because of the low concentrations added, or 3) if small planktic " μ -algae" have similar active transport abilities. For a critical partial evaluation of this technique, see Vaccaro and Jannasch (1966) and Hamilton, et al. (1966).

b. Measurement of Algal Utilization

The measurement of algal diffusion is identical to that given by Wright and Hobbie (1966). A 250 ml lake water sample is placed in a 1 liter automatic pipette, and 0.5 mg l^{-1} of unlabeled glucose or acetate is added. Concentrations of unlabeled substrate are likewise added to three 100 ml glass-stoppered bottles, such that when 50 ml aliquots of lake water are pipetted into each of 5 bottles, the final concentration of substrate will be 0.5, 1.0, 1.5, 2.0 and 0.5 (for blank) $\text{mg substrate l}^{-1}$. Prior to pipetting the 50 ml aliquots into each bottle, $1-2 \mu\text{C}$ ($1 \mu\text{C ml}^{-1}$) of ^{14}C -glucose or acetate was added to the 250 ml sample in

the automatic pipette, thoroughly mixed and decanted. The blank was immediately fixed (I_2KI), and the samples incubated in the dark for a specific length of time (0.25-6 hours) and then treated identically to the above samples for measuring bacterial activity. Similarly, activity data from counted filters were treated graphically and mathematically to determine kinetic parameters of algal diffusion.

Because of the lengthy preparations necessary to conduct measurements of bacterial and algal substrate removal, measurements of algal diffusion were only made at the surface depth (0.5 m) at station A, on a monthly basis from mid-March through mid-December, 1965. Bacterial metabolism was assayed at 0.5, 1.5 and 2.5 m at station A from March through December, 1965, at two week intervals. During the period of study, the following parameters were monitored in all samples where bacterial and algal activity were measured: temperature, total algal volume; total particulate organic carbon; total dissolved organic carbon; and from mid-June through July, 1965, bacterial enumeration.

Ambient water temperature ($^{\circ}C$) was measured at the time of collection by a thermometer attached to the inner surface of the Ruttner sampler.

2. Phytoplankton

Phytoplanktic periodicity was followed throughout the annual study. Preserved samples (Lugol's solution) were sedimented in 5 or 10 ml counting chambers, depending upon the suspected density, and counted directly with an inverted Zeiss microscope (Utermöhl, 1958); by using appropriate conversion factors (cf. Nauwerck, 1963), annual changes in the phytoplankton volume ($10^8 \mu^3$) were calculated.

3. Bacterial Enumeration

Samples for bacterial enumeration were rapidly transported to the laboratory in total darkness at below in situ thermal conditions. In most measurements the membrane filter technique was applied to determine total numbers (Millipore Manual ADM 40, 1965). Duplicate known volumes, or diluted suspensions thereof, were filtered (ca. 0.5 atm) onto grided Millipore type HA filters (Millipore Filter Corp., Bedford, Mass.), which in turn were placed on absorbant pads saturated with 2.0 ml of common nutrient medium (M-TGE) and incubated at 35C for 24 hours. The filter was then removed and stained with methylene blue to increase

the contrast of the colonies. The filters were then rendered transparent with immersion oil (R.I.= 1.51), and mounted. A suitable number of fields were counted, after which statistics were applied to determine total bacterial numbers present in the original sample.

4. Particulate (Oxidizable) Organic Carbon

Particulate (oxidizable) organic carbon was measured according to a method presented by Strickland and Parsons (1960) with several minor exceptions. The modified method essentially consists of 1) filtering a known volume of lake water (200-500 ml for Lake L8tsjön) through a pre-combusted (500C for 1 hour) glass-fiber ultrafilter (type 984H; Reeve Angel Co., Clifton, N. J.), 2) "wet ashing" the concentrated material with a mixture of potassium dichromate and concentrated sulphuric acid, and 3) spectrophotometric determination of the decrease in extinction of the yellow dichromate upon reduction by organic matter present on the filter. Carbon values are indicated in terms of glucose carbon, where each sample batch of the oxidant mixture was calibrated against known glucose standards. Some evidence was obtained during the annual study, that the oxidizing

power of the mixture is unstable over long periods of time, and subsequently, periodic calibration was necessary. Replicated measurements were not attempted, although duplicate blanks were prepared with each sample batch.

5. Dissolved Organic Carbon

Concentrations of dissolved organic carbon were measured according to a method presented by Menzel and Vaccaro (1964), slightly modified for freshwater studies. Dissolved organic matter is here defined as that fraction of a lake water sample which will pass through a 984H glass-fiber ultra-filter. Thus, a small portion of the sample filtered to determine particulate organic carbon may be used to measure dissolved organic carbon.

The method consists of 1) filtering a lake water sample as described above and pipeting a known volume (in this study 1.0 ml was found to be sufficient) into a glass ampoule, 2) add suitable quantities of potassium persulphate and phosphoric acid, 3) purge the sample with nitrogen gas to remove carbon dioxide present and seal the ampoule, 4) oxidize the dissolved organic material to carbon dioxide at 130C, 5) measure the quantity of CO₂ produced by infrared

absorption (Beckman model IR 215), and 6) determine the correct concentrations of dissolved organic carbon contained in the sample from a calibration curve prepared from CO₂ standards. Samples were generally measured in triplicate.

6. Statistical Analysis

Computer analysis was used to correlate the many parameters for each point in time and space. The data were punched onto cards using the IBM Key punch (model 026), and the Bastat (BS) routine for the IBM CBC3600 Computer was used as the precoded computer format. All assumptions of parametric statistics were demonstrated in the data subjected for analysis. The Bastat routine automatically printed out 1) basic statistics of sums, means, sums of squares, standard deviations, etc., 2) all possible correlations which may exist between any two parameters, and 3) the levels of significance for parameters which are shown to be correlated. The Least Squares (LS) multiple regression routine was also used but without any marked success, as the particular matrix of parameters and observations indicated an infinite number of correlations were possible. In order to obtain meaningful multiple correlations, it

would be necessary to code a special format statement which would recall only those correlations which would be of biological importance.

It was necessary to partition the annual cycle data into the following three segments: 1) data collected from 13 March to 7 June, 1965, 2) data from 15 June through 26 July, 1965, and 3) data from 5 August through 10 December, 1965, to correspond with increasing water temperatures in the spring, minor fluctuations exhibited during the summer, and declining temperatures associated with fall and early winter. To have compared all data collected, from March through December, 1965, would fail to point out certain biological phenomena dependent upon seasonal changes in thermal conditions. There were 7 observations during the spring, 6 observations during the summer period, and 12 observations during the fall and early winter from each depth (0.5, 1.5 and 2.5 m) at station A.

Correlations were conducted between the following parameters, at each individual depth for the time periods indicated: temperature ($^{\circ}\text{C}$); total algal volume ($10^8 \mu^3$); particulate organic carbon (mg C l^{-1}); dissolved organic carbon (mg C l^{-1}); maximum uptake velocity for acetate (V ; $\mu\text{g l}^{-1}\text{hr}^{-1}$); maximum uptake velocity for glucose

(V ; $\mu\text{g l}^{-1}\text{hr}^{-1}$); acetate substrate approximations ($K_t + S_n$; $\mu\text{g l}^{-1}$); glucose substrate approximations ($K_t + S_n$; $\mu\text{g l}^{-1}$); acetate turnover time (T_t ; hours); and glucose turnover time (T_t ; hours). All correlations possible between any two parameters indicating a correlation coefficient of 0.5 (negative or positive) through computer analysis are indicated in Appendix 2. Only levels of significance of 0.05 or less were accepted. Correlations which may indicate certain biological phenomena occurring during the annual cycle are discussed in the text and are also marked by an asterisk (*) in the appendix. No correlations were made with the kinetic parameters of algal diffusion (K_d and T_d), as these were measured on a monthly basis.

E. Results and Discussion

The analysis of bacterial utilization of glucose and acetate at low substrate concentrations nearly always exhibited a linear population response to increasing concentrations between 1-60 $\mu\text{g l}^{-1}$. Substrate concentrations above this quantity (1-4 mg l^{-1}) consistently demonstrated rate limitation in a manner similar to the results of Wright and Hobbie (1965a, 1965b, 1966; Hobbie and Wright,

1965b). Wright and Hobbie (1966; Rodhe, et al., 1966) presented evidence that the bacterial flora of freshwater communities is effective in keeping concentrations of glucose and acetate sufficiently low that appreciable in situ algal heterotrophy cannot occur. At existing substrate concentrations found in Lake Löt sjön (cf. Figs. 5 and 9), it is apparent that natural uptake and effective utilization of these two organic compounds are attributable to the planktic bacterial populations.

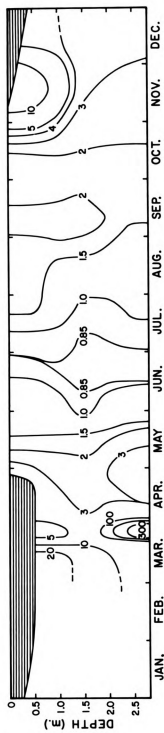
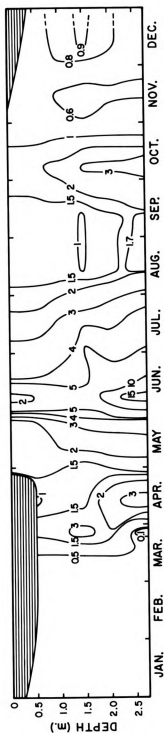
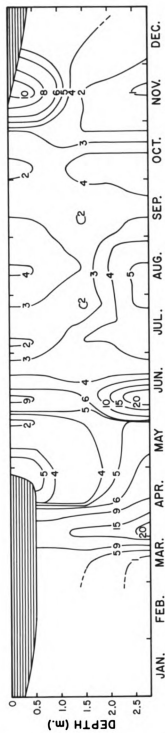
1. Annual Cycle of Glucose: Production and Utilization

Natural substrate approximations of glucose ($K_t + S_n$) vary little throughout the annual period, with concentrations remaining generally below $10 \mu\text{g l}^{-1}$ (Fig. 5). During March and April, 1965, under 0.5 m ice cover, concentrations of $20 \mu\text{g l}^{-1}$ were detected immediately beneath the ice and at 1.5 m. From mid-April to mid-November, the ice-free portion of the year, concentrations were generally low, between $2\text{-}5 \mu\text{g l}^{-1}$, with an observed annual maximum of $24 \mu\text{g l}^{-1}$ at 2.5 m during the early part of June. Little change is noted through the fall months, although $10 \mu\text{g l}^{-1}$ was found at the surface almost immediately after ice-formation in November. Concentrations of glucose at 0.5 m in the summer

Figure 5. Seasonal distribution of natural substrate concentrations of glucose, $K_t + S_n \mu\text{g l}^{-1}$. Lake Löttsjön, Sundbyberg, Sweden, 1965. Sampling frequency: 10-14 days; depths sampled: 0.5, 1.5 and 2.5 m at station A.

Figure 6. Seasonal distribution of heterotrophic capacity as maximum bacterial uptake velocity of glucose, $V \mu\text{g l}^{-1}\text{hr}^{-1}$. Lake Löttsjön, Sundbyberg, Sweden, 1965. Sampling intervals and depths same as Figure 5.

Figure 7. Seasonal distribution of substrate regeneration time of glucose, T_t in hours. Lake Löttsjön, Sundbyberg, Sweden, 1965. Sampling intervals and depths same as Figure 5.



were strongly correlated with concentrations of particulate organic matter (level of significance: 0.02), and may reflect bacterial activity and liberation of soluble organic compounds into the water.

There appeared to be a lower limit of glucose production in Lake Lötsjön, as always a minimum of $2 \mu\text{g l}^{-1}$ was found present in the water column. This suggests the presence of a stable and persistent system for the regeneration of glucose back into the water. In view of some recent work on the physiology of marine bacteria, and associated glucose uptake kinetics (Vaccaro and Jannasch, 1966), one might question the constancy of finding a minimum of $2 \mu\text{g glucose l}^{-1}$ during the annual cycle. K_t values for glucose were shown by these workers to be near $3 \mu\text{g C l}^{-1}$; thus, the continually detected minimal value may represent the "natural" K_t for aquatic bacterial populations. According to Hamilton, et al. (1966), a K_t as low as $2 \mu\text{g l}^{-1}$ or less would indicate a bacterial population especially well adapted to removing and utilizing extremely low levels of substrate. However, as there exists no other single study of complete annual cycles of dissolved organic compounds in lake water as was determined here, the present findings remain in question.

The heterotrophic growth capacity (V) for glucose by

bacterial populations, as indicated by uptake velocities, reaches maximum values ($10-15 \mu\text{g l}^{-1}\text{hr}^{-1}$) immediately above the sediments during late spring following ice loss (Fig. 6). During the summer months uptake rates are decreased markedly in the entire water column to $1-3 \mu\text{g l}^{-1}\text{hr}^{-1}$, and remained within this range well into December under ice cover. During the fall months with decreasing thermal conditions (cf. Fig. 15), ambient water temperatures and uptake velocities for glucose are well correlated at the surface (level of significance: 0.04).

Turnover times (T_t) for glucose were lowest during March (300 hours) at 2.5 m under ice cover, and presumably reflect the presence of a small bacterial population (Fig. 7); although on the sampling date, a turnover time of 5 hours was noted just beneath the ice. As bacterial enumeration was not undertaken at this time, it cannot be said with certainty whether the population was much smaller at the lower depth, or merely unable to kinetically remove glucose (actually $20 \mu\text{g l}^{-1}$ was present above the sediment; see Fig. 5). According to Vaccaro and Jannasch (1966), K_t values are less subject to variability at lower temperatures (6-10C), due to increased physiological control of enzyme systems. Thus, very poor efficiency is shown through

turnover times of 300 hours (12.5 days) if, indeed, large viable populations are there. Turnover times during late spring, summer, and fall were consistently below 2.5 hours, and between June and August decreases to less than 1.0 hour were not uncommon. This indicated a very rapid turnover and regeneration of organic substrates. Regeneration times from mildly eutrophic and other fresh waters have been shown to be very much slower than those from Lake Löttsjön (Wright and Hobbie, 1966; Wetzel, 1967a).

Turnover times for glucose correlated with fluctuations in temperature at 0.5 and 1.5 m (significance levels: 0.01 and 0.04, respectively) during the summer months. With decreasing water temperatures in the fall and early winter, there was a direct corresponding increase in turnover time at all depths in the pond (significance levels: 0.005, 0.005 and 0.005, respectively). Presumably the turnover time is inversely related to water temperature, as increased bacterial biomass is directly associated with thermal increases, and turnover time is inversely proportional to biomass and cell number (cf. Wright and Hobbie, 1966). If this latter relationship is valid, then estimates of bacterial standing crop, or biomass, are possible from measures of the turnover time, much in the same way as

heterotrophic potential or capacity (V) is directly related to bacterial biomass (Fig. 8a, b and c). During June and July, 1965, bacterial enumeration was determined in samples from all depths at station A, in which uptake kinetics were monitored. For both organic substrates, glucose and acetate, total bacterial count is well correlated with rates of direct utilization at all depths, thus confirming a portion of the studies previously conducted by Wright and Hobbie (1966) with pure microbial cultures.

2. Annual Cycle of Acetate: Production and Utilization

The seasonal distribution of acetate concentrations and parameters of kinetic utilization in Lake Löttsjön bare little resemblance to those of glucose (Fig. 9). Concentrations ($K_t + S_n$) of acetate were generally much higher than those of glucose, and reached $350 \mu\text{g l}^{-1}$ in March and April under ice cover immediately above the sediments. Normal winter values were within a range $5\text{-}30 \mu\text{g l}^{-1}$; summer values in the upper strata reached $50 \mu\text{g l}^{-1}$, with concentrations much reduced to $5\text{-}10 \mu\text{g l}^{-1}$ at the lower depths (1.5 and 2.5 m). The minimum observed concentration was near $5 \mu\text{g l}^{-1}$, and again may represent the "natural" K_t due to uptake by

Figure 8. Relationship of total bacterial biomass (total count $\times 10^5 \text{ ml}^{-1}$) to maximum velocities of substrate uptake (V , $\mu\text{g l}^{-1} \text{ hr}^{-1}$) for glucose and acetate at (A) 0.5 m (B) 1.5 m and (C) 2.5 m at Station A. Lake Löttsjön, Sundbyberg, Sweden, June-July, 1965.

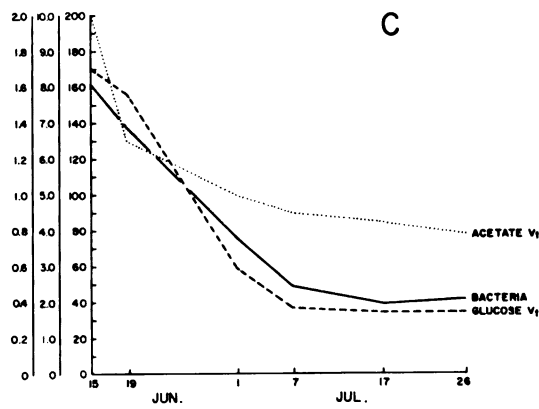
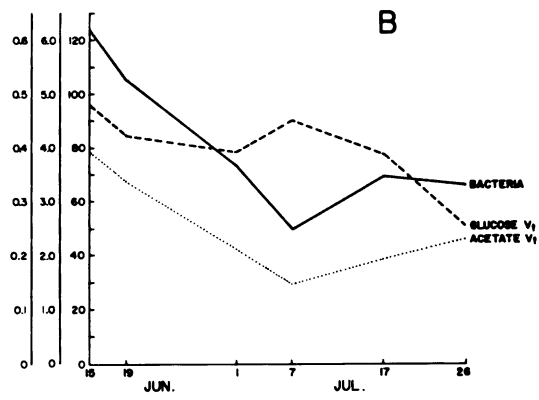
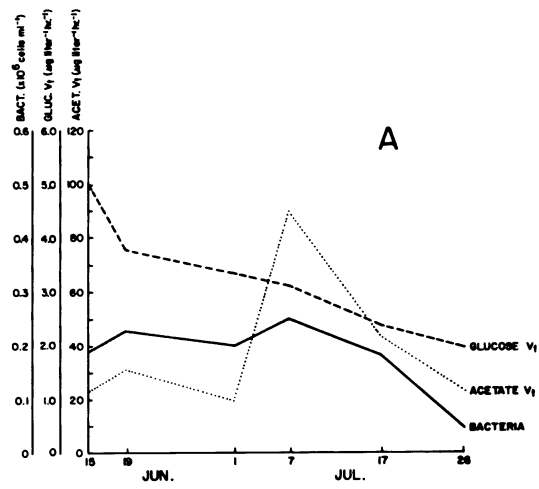
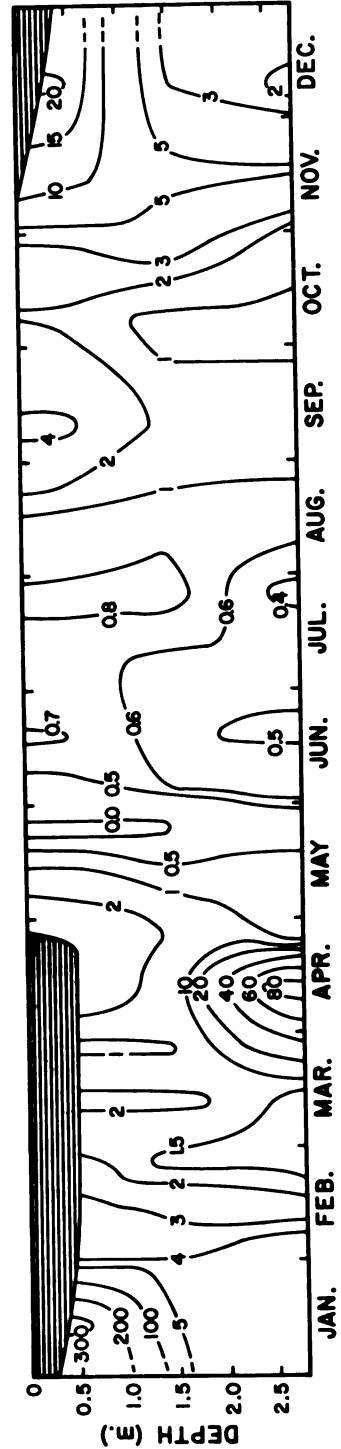
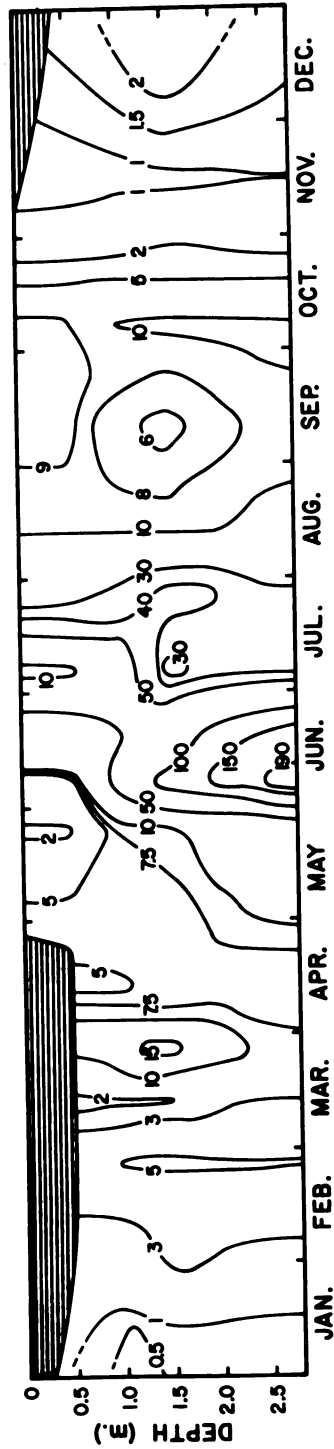
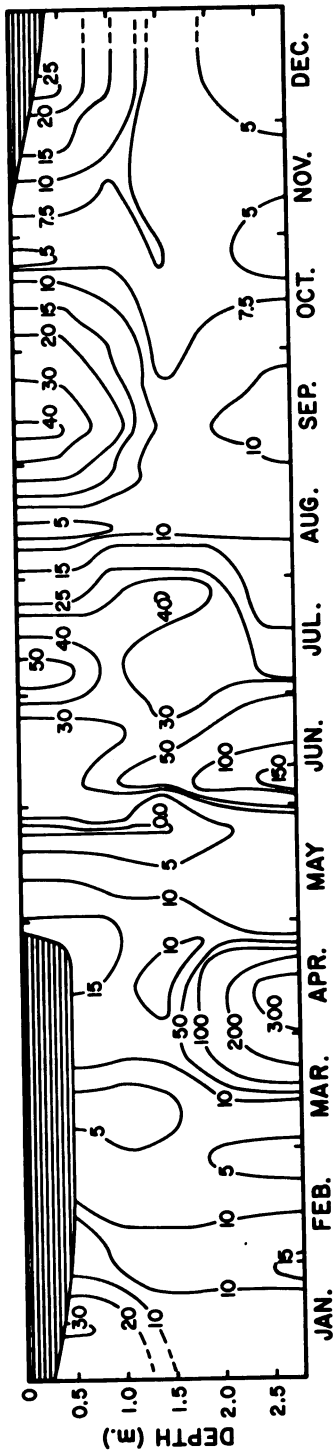


Figure 9. Seasonal distribution of natural substrate concentrations of acetate, $K_t + S_n \mu\text{g l}^{-1}$. Lake Löttsjön, Sundbyberg, Sweden, 1965. Sampling intervals and depths same as Figure 5.

Figure 10. Seasonal distribution of heterotrophic capacity as maximum bacterial uptake velocity of acetate, $V \mu\text{g l}^{-1}\text{hr}^{-1}$. Lake Löttsjön, Sundbyberg, Sweden, 1965. Sampling intervals and depths same as Figure 5.

Figure 11. Seasonal distribution of substrate regeneration time of acetate, T_t in hours. Lake Löttsjön, Sundbyberg, Sweden, 1965. Sampling intervals and depths same as Figure 5.



heterogeneous bacterial populations. The only exception to this minimum was detected near the end of May when acetate was entirely depleted from 0.5 and 1.5 m (0 ug l^{-1} indicated). There is a possibility that the response is below the sensitivity of the technique, although this is unlikely. Good uptake kinetics were shown in these two laboratory measurements, yet the $(K_t + S_n)$ values were positive in both instances i.e., the intercept of the linear response was to the right of the abscissa, instead of to the left. Reasonable explanations for this depletion are presently unknown, although certainly the organisms in the sample possessed uptake mechanisms for acetate (low standard deviation of the linear uptake response). Responses similar to the one described have not been previously detected by other investigators working both in freshwater and marine environments. No relationships could be demonstrated between concentrations of acetate present and other parameters monitored, e.g. temperature, phytoplankton volume, dissolved and particulate organic carbon content, etc.

Whereas the uptake of glucose was uniform and constant throughout most of the ice-free period, acetate uptake (V) increased beneath the ice in March and April, decreased as

ice loss occurred, and increased to an observed annual maximum in June ($190 \mu\text{g l}^{-1}\text{hr}^{-1}$ removed) just above the sediments (Fig. 10). Maximum rates of substrate removal for acetate are nearly 20 times those for glucose. From mid-June this heterotrophic potential (V) decreased rapidly to a removal rate of $6 \mu\text{g l}^{-1}\text{hr}^{-1}$ in September, increased slightly in October to $10 \mu\text{g l}^{-1}\text{hr}^{-1}$, and then decreased again to $1-5 \mu\text{g l}^{-1}\text{hr}^{-1}$ in the winter under ice cover.

Rates of acetate uptake at 2.5 m were well correlated with vernal increases in water temperature (significance level: 0.04). During the fall and early winter, rates of utilization at all depths were closely associated with decreasing water temperatures (0.005, 0.01 and 0.005, respective levels of significance). Inasmuch as temperature regulates metabolic rates and various growth parameters, declining temperatures in the fall would be expected to be a major factor controlling the maximum number of bacteria present.

The general pattern for the regeneration of acetate (T_t) is much the same as that found for glucose (Figs. 11 and 7) with several notable exceptions. During a large portion of the ice-free vegetative period, the turnover time for

acetate was much more rapid than that for glucose, frequently as low as 0.5 hours. Typical winter values appeared to be within the range 1-10 hours (similar for glucose), while the interval from early May through August showed turnover times of 1 hour or less. On one occasion in May, the turnover time at the upper two depths was indicated to be 0 hours. Theoretically, this implies the bacterial populations were utilizing acetate as readily as it was being produced, possibly creating brief periods of actual deficiency in the water column. Since the acetate concentration ($K_t + S_n$) was $0 \mu\text{g l}^{-1}$ on this sampling date, the uptake detected must have been due entirely to the labeled substrate that was added to the sample.

The low turnover times found for both glucose (300 hours in March and April; Fig. 7) and acetate (300 hours in January; Fig. 11) appear to be due to the presence of very small bacterial populations. It could also be that the bacteria were largely absent, and that the uptake detected was primarily due to small phytoplankters (less than 10μ) with uptake mechanisms similar to those possessed by the bacteria (cf. Wright and Hobbie, 1966).

Turnover times for acetate were correlated with temperature at all three depths sampled, during summer

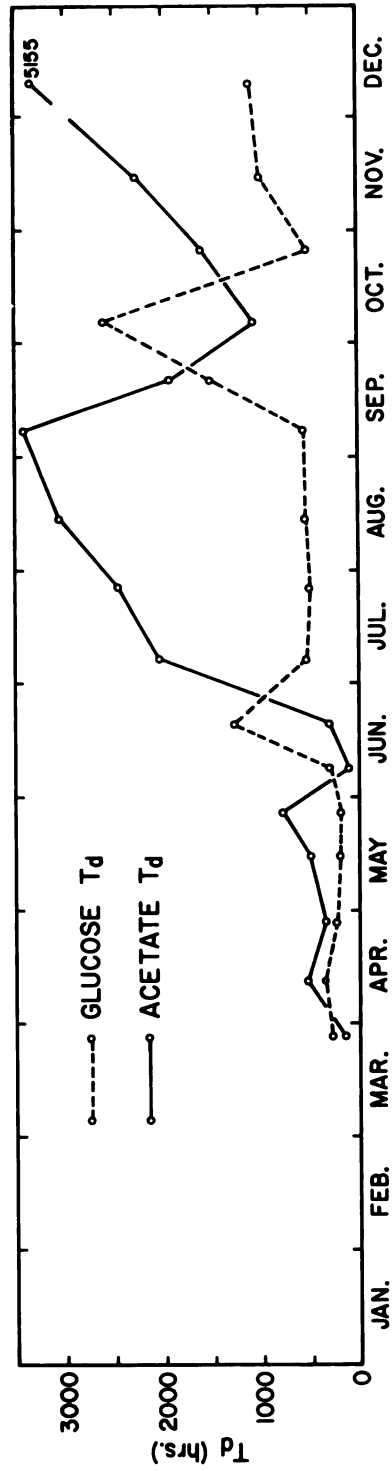
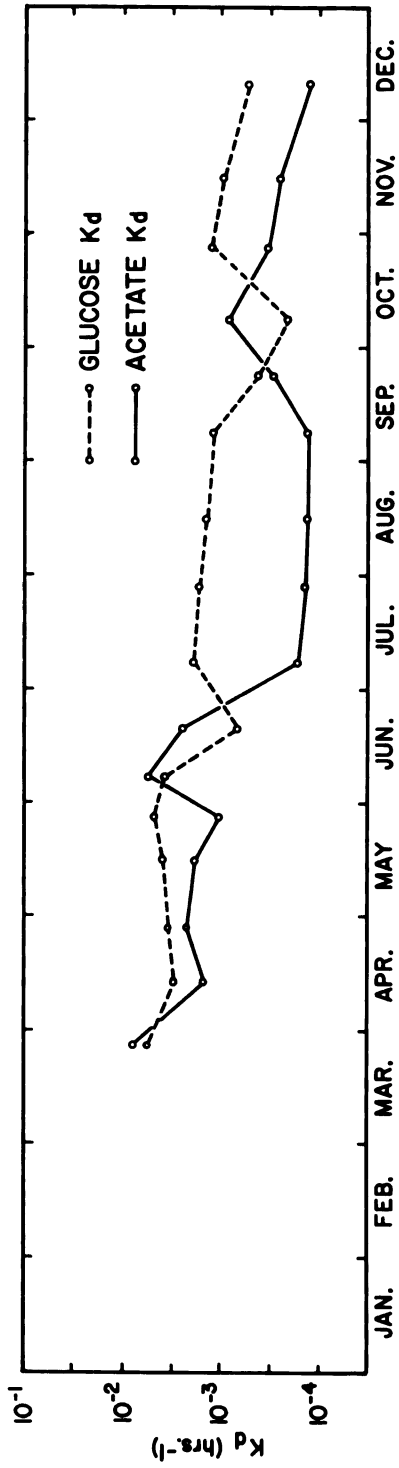
fluctuations of thermal conditions (0.05, 0.01 and 0.06, respective levels of significance). During fall and early winter under ice cover, the relationship of turnover time, and thus biomass, to temperature is highly correlated at all depths (0.005, level of significance at each depth). It is interesting to note that the turnover time for acetate is also correlated with concentrations of particulate organic carbon in many instances, which is difficult to explain unless the bacterial biomass represents a constant fraction of the particulate material and is continuously regenerating acetate through decomposition. Other metabolic interrelationships may exist, however, of which we are ignorant at the present time. In several instances both acetate and glucose turnover times were found to be correlated with concentrations of total dissolved organic carbon.

3. Algal Utilization of Dissolved Organic Compounds

Algal diffusion of glucose occurred at a faster rate than that of acetate throughout the year except on three separate occasions (Figs. 12 and 13). This may indicate that the various phytoplankters had a slightly increased

Figure 12. Algal diffusion of glucose and acetate at 0.5 m (station A) in Lake Löttsjön, Sundbyberg, Sweden, 1965. Diffusion rates (K_d) expressed as hours^{-1} . - - - - - = glucose; ——— = acetate.

Figure 13. Turnover time of substrate regeneration due to algal diffusion (T_d ; expressed in hours) at 0.5 m in Lake Löttsjön, Sundbyberg, Sweden, 1965. - - - - - = glucose; ——— = acetate.



permeability for the glucose molecule in comparison to the acetate ion. Also, species composition within the phytoplankton may, in part, determine those substrates most preferred for uptake. Another possibility is that since the bacterial uptake was shown to be higher for acetate, glucose may be an alternative or supplemental substrate available to the algae without direct bacterial competition for it. This latter possibility seems somewhat dubious as concentrations of either substrate in Lake Löttsjön are present in quantities far below those required for effective algal heterotrophy to occur as discussed above. It should be remembered, however, that glucose and acetate represent only two of the many organic solutes present in lake water. Measurements would have to be made in a variety of natural waters, using a large array of labeled organic substrates through an annual period, to assess actual metabolic pathways utilizing these substrates.

Diffusion of organic compounds was greatest under ice cover, decreasing slightly as the ice cover retreated and then increasing to a second subtle peak. From mid-June through December, diffusion of both substrates gradually decreased; during the summer, diffusion of glucose occurred

more rapidly than acetate, and except for a minor inversion of diffusion rates in October, this same relationship was still evident in the fall and early winter.

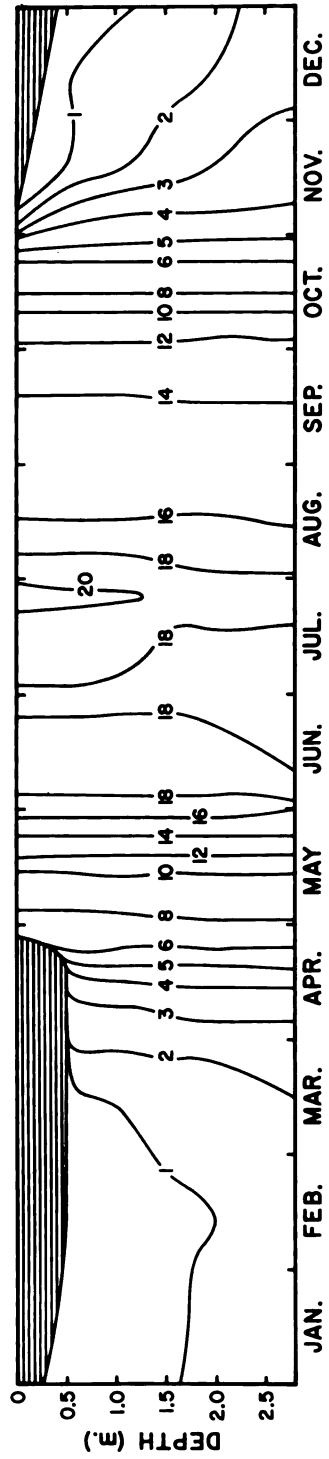
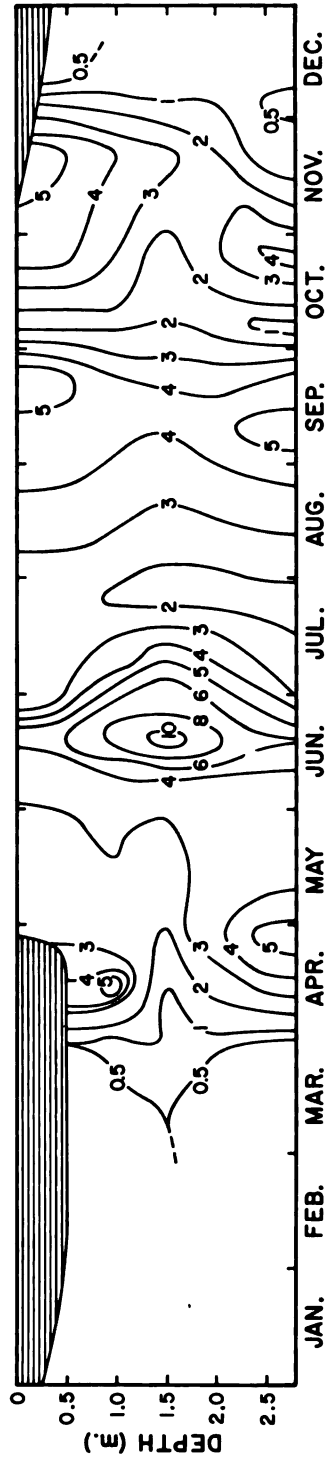
Rates of utilization (K_d) were much greater in Lake Löttsjön (10^{-3} to 10^{-2} hours $^{-1}$) than in other waters thus far tested (approximately 10^{-5} to 10^{-4} hours $^{-1}$ in Wright and Hobbie, 1966; Rodhe, et al., 1966), but too few data have been presented in either of the studies mentioned to draw broad and general conclusions. The rising and falling K_d values for the substrates tested without well defined maxima and minima occurring during the year follow, to some extent, changes observed in phytoplankton volume (Fig. 14). This is very similar to the limited results reported by Wright and Hobbie (1966).

Turnover times (T_d) due to algal diffusion for glucose and acetate remained between 150 and 500 hours for most of the ice-free period, while acetate diffusion decreases sharply after mid-June to over 300 hours, only once dropping in the fall to near 1000 hours (Fig. 13). In the work presented by Wright and Hobbie (1966), turnover times due to diffusion (for a sample from 11 m in Lake Erken, Sweden) are more rapid for glucose than acetate during the winter months, much in the same manner as those limited data obtained

Figure 14. Seasonal distribution of total phytoplankton volume $\times 10^8 \text{ u}^3$.

Lake Löttsjön, Sundbyberg, Sweden, 1965. Sampling intervals and depths same as Figure 5.

Figure 15. Seasonal distribution of water temperature ($^{\circ}\text{C}$). Lake Löttsjön, Sundbyberg, Sweden, 1965. Thermal conditions represent vertical series (0.5, 1.5 and 2.5 m) monitored at station A. Sampling frequency: 10-14 days.



in this study. Wright and Hobbie (1966) found turnover times for glucose in November and December of 1280 and 1800 hours (Lake Löttsjön: 1000 and 1475 hours), and for acetate in November and December, 3300 and 3400 hours (Lake Löttsjön: 2100 and 5155 hours).

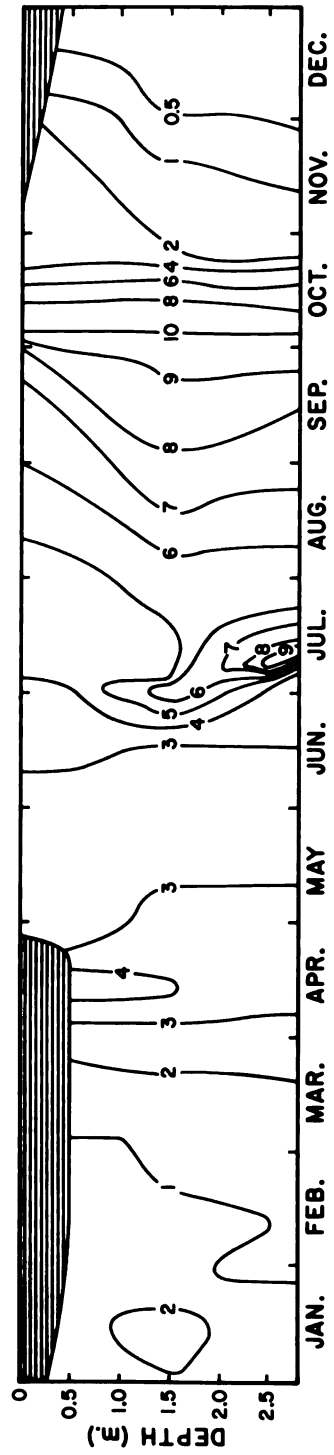
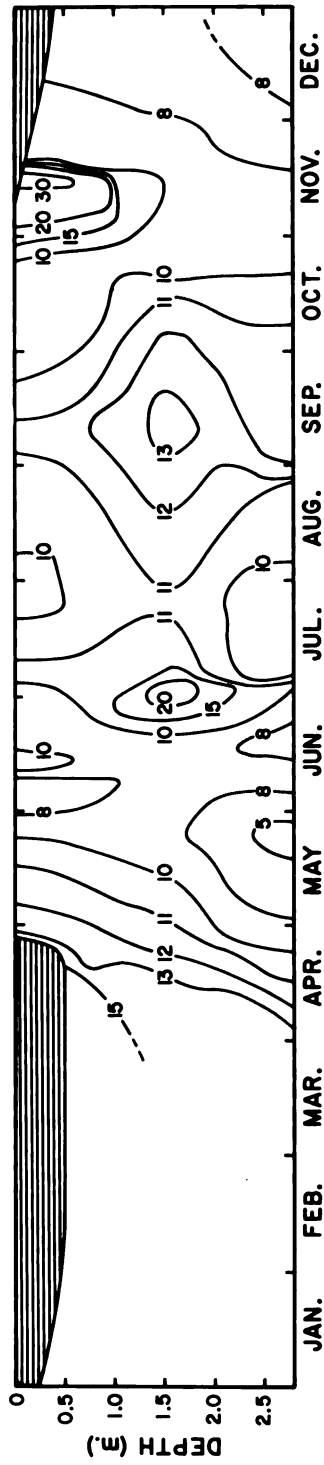
4. Annual Cycles of Dissolved and Particulate Organic Carbon

Concentrations of dissolved organic carbon at all three depths followed one another closely with two maxima noted during the year, one of 24.8 mg C l^{-1} during the summer at 1.5 m, and 30.6 mg C l^{-1} detected during early November with the formation of ice cover at 0.5 m (Fig. 16). These concentrations may be the direct result of metabolic processes, i.e. exogenous materials produced as extra-cellular excretions, etc., but the mechanisms involved have not been identified.

In the spring at the surface (0.5 m), increases in temperature (Fig. 15) were directly associated with decreases in dissolved carbon (0.005, significance level). Whether this material was being directly utilized by planktic algae and bacteria is not known. In the middle strata (1.5 m), spring and fall concentrations of organic carbon

Figure 16. Seasonal distribution of total dissolved organic carbon, mg C l^{-1} . Lake Löttsjön, Sundbyberg, Sweden, 1965. Sampling intervals and depths same as Figure 5.

Figure 17. Seasonal distribution of total particulate (oxidizable) organic carbon, mg C l^{-1} . Lake Löttsjön, Sundbyberg, Sweden, 1965. Sampling intervals and depths same as Figure 5.



were well correlated with temperature changes (0.005, significance level at each depth); during summer temperature fluctuations, the carbon content likewise fluctuated. As the water cooled in late fall, increases in carbon availability were apparent. At 2.5 m, just above the sediment, spring and fall concentrations were also associated with changes in temperature (0.002 and 0.005, respective levels of significance). This correlation is perhaps suggestive of metabolically controlled processes which were temperature dependent, i.e. decomposition, excretion, etc. No doubt allochthonous materials contribute to this fraction of organic matter through spring runoff, wind-blown additions, etc., in addition to the materials produced within the pond itself.

Interesting correlations between changes in phytoplankton volume and dissolved organic carbon were indicated through statistical analysis. At the middle strata (1.5 m), decreases in algal volume were paralleled by markedly decreased concentrations of dissolved organic carbon during the spring (0.005, significance level). During the fall at the same depth, increases in dissolved organics were associated with production and accumulation of planktic algae (0.03). At the lower depth (2.5 m) both parameters were closely

associated with one another as ice formation proceeded to take place (0.01). Although there was no direct evidence for the production of dissolved organic matter by the phytoplankton present, it is known that freshwater and marine species are capable of excreting large quantities of simple organic compounds (Fogg, 1962; Leferve, 1964; Fogg and Watt, 1965; Hellebust, 1965; Forsberg and Taube, 1967; and others), and this may be reflective of a true in situ metabolic pattern. Further, it is noteworthy that in all instances in which large concentrations of dissolved organic matter were detected (greater than 15 mg C l^{-1}), these were found to be immediately following periods of high algal biomass.

From these few preliminary data it would appear that there is a distinct cycling of dissolved organic carbon in this pond, as is probably the case in most natural fresh waters. As indicated earlier from the literature, there probably is a minimum concentration of dissolved material which is fixed and relatively unavailable, while a certain quantity of dissolved compounds are continuously utilized and recycled back into the water. In this manner, a balance is maintained between the processes which utilize dissolved compounds and those which replenish the supply. In Lake

Lötsjön, the minimum value appears to be 8-10 mg dissolved C l⁻¹, although concentrations are generally higher than this. From what little comparative information there is available, the pond-like nature of this lake is reflected in its high concentrations of dissolved organic matter. Lake Erken (Sweden) in which Wright and Hobbie conducted their studies, concentrations were seldom greater than 10 mg C l⁻¹ and usually less than 5-8 mg C l⁻¹.

Particulate organic (oxidizable) carbon was monitored by oxidation (wet ashing) with phosphoric acid and sulphuric acid-dichromate throughout the annual period (Fig. 17). Undoubtedly, there are many forms of organic matter in lake water which are not oxidizable by this technique and thus excluded. From a metabolic standpoint, however, such compounds are probably relatively unimportant and are thought to constitute a minor portion of the total organic matter present. Hence, their omission probably does not quantitatively lend much error to the analysis. Included by this method would be all phytoplankton, bacteria, organic detritus, large organic aggregates, allochthonous materials, and any other suspended matter which could be collected by the filtration procedure.

Winter values of particulate carbon in Lake Lötsjön

were within $1\text{--}2 \text{ mg C l}^{-1}$ and considerably higher than winter values reported from Lake Erken ($0.2\text{--}0.6 \text{ mg C l}^{-1}$; Wright and Hobbie, 1966). An increase was observed immediately beneath the ice in mid-April, which correlated well with the spring phytoplankton maximum (Fig. 14). A slight decrease in particulate carbon occurred with ice loss in late spring, followed by a steady increase to 9 mg C l^{-1} just above the sediment in mid-July. This maximum followed closely the large phytoplankton accumulation at 1.5 m in June. Slight decreases in particulate material were found in August, when $6\text{--}8 \text{ mg C l}^{-1}$ was present, but the annual maximum was detected in October, representing over 10 mg particulate C l^{-1} . A large portion of this material could be attributed to detritus and extraneous matter coming into the lake during mid and late fall. In October this suspended matter began to sediment out.

The particulate organic carbon content was uniformly distributed from the surface to the sediment, and probably reflects the complete circulation of a shallow lake of this type. The dissolved carbon fraction was less uniformly distributed, and was perhaps influenced by diurnal cycles of excretion and uptake of organic compounds by phytoplankton during photosynthesis. Deeper lakes, such as Lake Erken,

have revealed interesting stratification profiles of particulate organic carbon (Dr. J. E. Hobbie, personal communication), which follow closely changes observed in phytoplankton density.

Numerous correlations between particulate organic matter and other parameters determined throughout the annual cycle were evident through numerical analysis. At all depths during the fall and early winter, concentrations of particulate carbon were closely associated with decreasing water temperatures (0.01, 0.005 and 0.005, respective levels of significance). Such correlations are in good agreement with those limited data previously published (Wright and Hobbie, 1966). Phytoplankton volume and particulate organic matter were correlated at 2.5 m during late spring (0.05, significance level) and seem to indicate, through direct counting and identification, a shift of the populations of Cryptophyta, Chrysophyta and Euglenophyta towards the sediment with increasing light intensities after ice loss.

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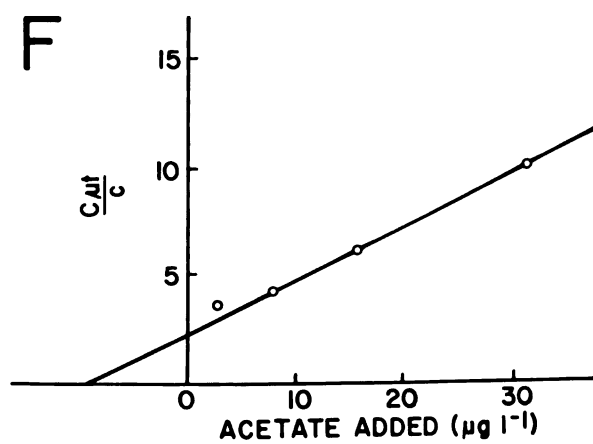
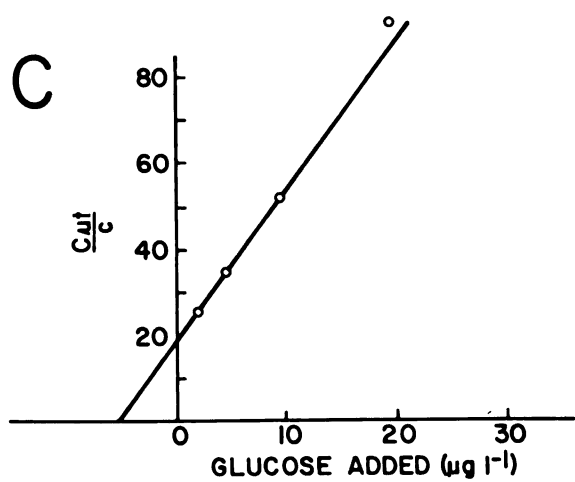
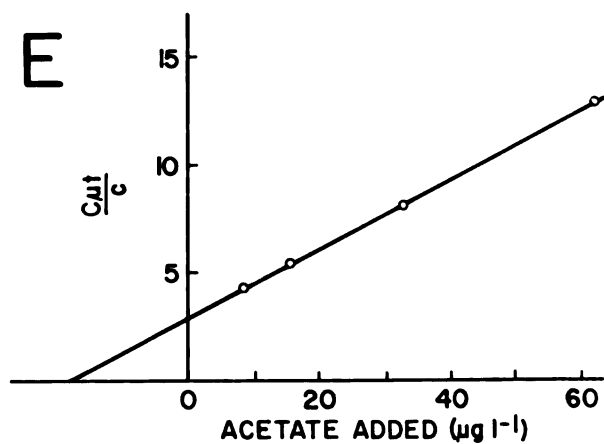
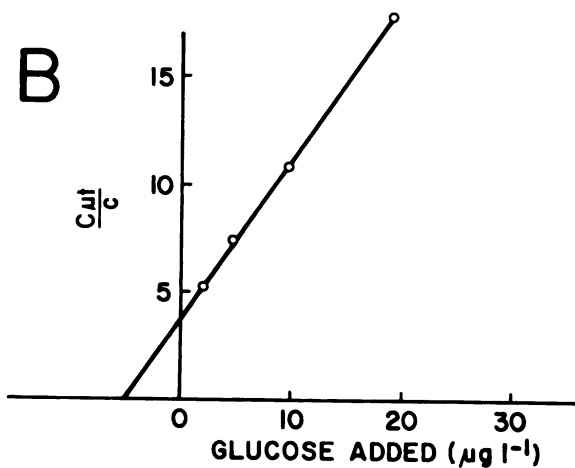
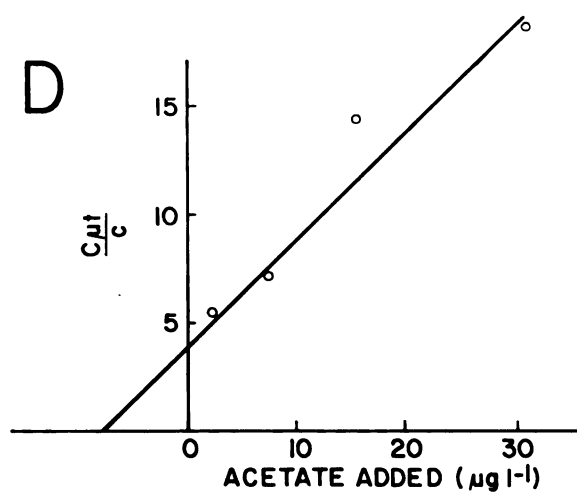
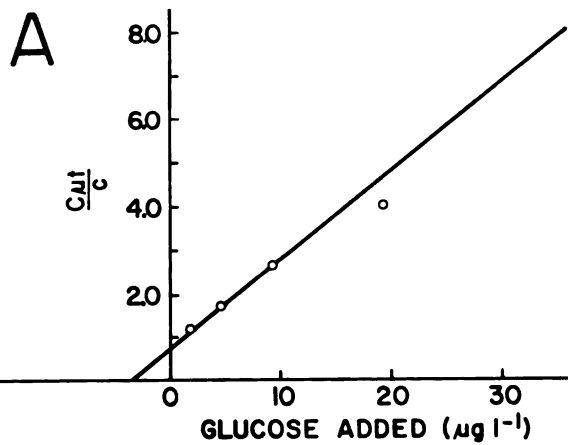
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APPENDIX 1

Several examples of the Lineweaver-Burk plot showing the maximum, minimum, and normal deviation from a linear response for the kinetic uptake of glucose (A-C) and acetate (D-F) are given. See text for an explanation of the graphs.

Plot	Depth (m.)	Date	Indicated (K + S) ($\mu\text{g l}^{-1}$)	Standard Deviation ($\mu\text{g l}^{-1}$)
A	1.5	19 June 1965	3	1.5 Maximum
B	0.5	28 April 1965	6	1.0 Minimum
C	1.5	13 March 1965	6	1.0 Normal
D	0.5	30 January 1965	8	3.0 Maximum
E	0.5	28 April 1965	18	1.0 Minimum
F	2.5	13 February 1965	10	2.0 Normal



APPENDIX 2

All correlations possible between any two parameters indicating a correlation coefficient of 0.5 (positive or negative) or greater through computer analysis are given. The first line immediately beneath the abbreviations of the parameters is the correlation coefficient. The second line represents levels of significance. Only levels of significance of 0.05 or less were accepted, and those marked with an asterisk (*) are considered correlations of biological significance which are discussed in the text.

Appendix Code

Temp	- Temperature
Alg Vol	- Algal Volume
Part OC	- Particulate Organic Carbon
Diss OC	- Dissolved Organic Carbon
AC Vt	- Maximum velocity of kinetic uptake of acetate
GL Vt	- Maximum velocity of kinetic uptake of glucose
AC K+S	- Approximations of <u>in situ</u> acetate concentrations
GL K+S	- Approximations of <u>in situ</u> glucose concentrations
AC Tt	- <u>In situ</u> regeneration time of acetate
GL Tt	- <u>In situ</u> regeneration time of glucose

Appendix 2(A) 0.5 m 13 March-7 June 1965

Temp	Alg Vol	Diss OC*	AC K+S	AC Tt	GL Tt
	0.636	-0.996	-0.521	-0.623	0.596
	0.13	0.005	0.23	0.14	0.16
Alg Vol	Part OC	Diss OC	GL Tt		
	0.696	-0.628	-0.736		
	0.08	0.14	0.06		
Part OC	GL Tt				
	-0.729				
	0.06				
Diss OC	AC K+S	AC Tt	GL Tt		
	0.518	0.609	0.588		
	0.23	0.14	0.16		
AC Vt	GL K+S				
	-0.507				
	0.24				
AC K+S	GL K+S	AC Tt			
	0.573	0.834			
	0.18	0.02			
GL K+S	AC Tt	GL Tt			
	0.705	0.620			
	0.08	0.15			

Appendix 2(B) 0.5 m 15 June-26 July 1965

Temp	GL Vt	GL Tt*	AC Tt*
	0.546	0.916	0.804
	0.26	0.01	0.05
Alg Vol	Part OC	AC K+S	GL K+S
	-0.791	-0.606	-0.694
	0.06	0.20	0.13
Part OC	AC Vt	AC K+S	GL K+S*
	0.749	0.785	0.897
	0.09	0.06	0.02
Diss OC	AC Vt	GL Tt	
	-0.743	0.511	
	0.09	0.30	
AC Vt	GL K+S		
	-0.602		
	0.21		
GL Vt	AC Tt	GL Tt	
	0.749	0.565	
	0.09	0.24	
AC K+S	GL K+S	AC Tt	
	-0.842	0.537	
	0.04	0.27	
AC Tt	GL Tt		
	0.921		
	0.01		

Appendix 2(C) 0.5 m 5 August-10 December 1965

Temp	Part OC*	AC Vt*	GL Vt*	AC Tt*	GL Tt*
	0,717	0.897	-0.591	-0.791	-0.792
	0.01	0.005	0.04	0.005	0.005
Part OC	AC Vt	AC Tt	GL Tt		
	0.616	-0.712	-0.677		
	0.03	0.01	0.02		
Diss OC	GL Tt				
	0.688				
	0.01				
AC Vt	AC Tt	GL Tt			
	-0.654	-0.690			
	0.02	0.01			
GL Vt	GL Tt				
	0.510				
	0.10				
AC Tt	GL Tt				
	0.642				
	0.02				

Appendix 2(D) 1.5 m 13 March-7 June 1965

Temp	Alg Vol	Diss OC*	AC Vt	AC Tt	GL Tt
	0.985	-0.956	0.719	-0.623	-0.609
	0.005	0.005	0.07	0.14	0.15
Alg Vol	Diss OC*	AC Vt	AC Tt	GL Tt	
	-0.981	0.676	-0.585	-0.657	
	0.005	0.10	0.17	0.11	
Part OC	GL K+S	GL Tt			
	-0.748	-0.744			
	0.05	0.06			
Diss OC	AC Vt	AC Tt	GL Tt		
	-0.521	0.620	0.680		
	0.23	0.15	0.10		
AC Vt	AC K+S				
	0.670				
	0.10				
GL Vt	GL K+S	AC Tt			
	0.526	-0.531			
	0.22	0.22			
AC Tt	GL Tt				
	0.699				
	0.49				

Appendix 2(E) 1.5 m 15 June-26 July 1965

Temp	Alg Vol	GL K+S*	AC Tt*	GL Tt*
	-0.575	-0.823	0.899	0.823
	0.23	0.04	0.01	0.04
Alg Vol	AC Vt	GL K+S	AC Tt	GL Tt
	0.589	-0.520	-0.873	-0.922
	0.22	0.29	0.02	0.01
Part OC	Diss OC	AC Vt	GL Vt	
	0.847	-0.689	0.546	
	0.03	0.13	0.26	
Diss OC	GL Vt			
	0.586			
	0.22			
AC Vt	AC K+S			
	-0.640			
	0.17			
GL K+S	AC Tt	GL Tt		
	-0.761	-0.715		
	0.08	0.11		
AC Tt	GL Tt			
	0.985			
	0.005			

Appendix 2(F) 1.5 m 5 August-10 December 1965

Temp	Part OC*	Diss OC*	AC Vt*	AC Tt*	GL Tt*
	0.822	0.862	0.743	-0.868	-0.870
	0.005	0.005	0.01	0.005	0.005
Alg Vol	Diss OC				
	0.631				
	0.03				
Part OC	Diss OC	Ac Vt	AC Tt	GL Tt	
	0.899	0.506	-0.864	-0.756	
	0.005	0.10	0.005	0.005	
Diss OC	AC Tt	GL Tt			
	-0.723	-0.649			
	0.01	0.02			
AC Vt	AC K+S	AC Tt	GL Tt		
	-0.690	-0.673	-0.800		
	0.01	0.02	0.005		
AC Tt	GL Tt				
	0.841				
	0.005				

Appendix 2(G) 2.5 m 13 March-7June 1965

Temp	Alg Vol	Diss OC*	AC Vt*	AC K+S		
	0.553	-0.818	0.778	0.590		
	0.20	0.02	0.04	0.16		
Alg Vol	Part OC*	Diss OC	GL Vt	GL K+S	AC K+S	GL Tt
	0.748	-0.659	0.606	0.851	0.679	-0.606
	0.05	0.11	0.15	0.02	0.10	0.15
Part OC	GL K+S					
	0.791					
	0.03					
Diss OC	GL Vt	AC K+S	GL K+S			
	-0.616	-0.751	-0.525			
	0.15	0.05	0.23			
GL Vt	GL K+S	GL Tt				
	0.856	-0.829				
	0.01	0.02				
AC K+S	AC Tt	GL Tt				
	-0.789	-0.592				
	0.03	0.16				
GL K+S	GL Tt					
	-0.689					
	0.09					

Appendix 2(H) 2.5 m 15 June-26 July 1965

Temp	Alg Vol	AC K+S	AC Tt*	GL Tt
	-0.529	0.666	-0.798	0.732
	0.28	0.15	0.06	0.10
Alg Vol	Part OC	AC Vt	GL K+S	GL Tt
	-0.649	0.915	-0.699	-0.898
	0.16	0.01	0.13	0.01
Part OC	AC Vt			
	-0.742			
	0.09			
Diss OC	AC Vt			
	-0.511			
	0.30			
AC Vt	GL K+S	GL Tt		
	-0.767	-0.659		
	0.08	0.16		
GL Vt	GL K+S	GL Tt		
	0.631	0.539		
	0.18	0.27		
AC K+S	GL K+S	AC Tt		
	0.598	-0.760		
	0.21	0.08		
GL K+S	AC Tt			
	-0.766			
	0.08			

Appendix 2(I) 2.5 m 5 August-10 December 1965

Temp	Alg Vol	Part OC*	Diss OC*	AC Vt*	GL K+S	AC Tt*	GL Tt*
	0.555	0.817	0.804	0.896	0.824	-0.767	-0.857
	0.06	0.005	0.005	0.005	0.005	0.005	0.005
Alg Vol	Diss OC*	GL Tt					
	0.741	-0.503					
	0.01	0.10					
Part OC	Diss OC	AC Vt	GL K+S	AC Tt	GL Tt		
	0.880	0.706	0.796	-0.742	-0.712		
	0.005	0.01	0.005	0.01	0.01		
Diss OC	AC Vt	GL K+S	AC Tt	GL Tt			
	0.574	0.607	-0.700	-0.704			
	0.05	0.04	0.01	0.01			
AC Vt	GL K+S	AC Tt	GL Tt				
	0.851	-0.733	-0.754				
	0.005	0.01	0.005				
GL K+S	AC Tt	GL Tt					
	-0.879	-0.622					
	0.005	0.03					
AC Tt	GL Tt						
	0.515						
	0.09						

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