PRIMARY PRODUCTIVITY, CHEMO-ORGANOTROPHY, AND NUTRITIONAL INTERACTIONS OF EPIPHYTIC ALGAE AND BACTERIA ON MACROPHYTES IN THE LITTORAL OF A LAKE

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ABSTRACT

PRIMARY PRODUCTIVITY, CHEMO-ORGANOTROPHY, AND NUTRITIONAL INTERACTIONS OF EPIPHYTIC ALGAE AND BACTERIA ON MACROPHYTES IN THE LITTORAL OF A LAKE

By

Harold LeRoy Allen

Assessment of epiphytic algal and bacterial *in situ* community metabolism, and physiological-nutritional interrelationships of macrophyte-epiphyte systems, were investigated in the littoral zone of a small temperate lake from April 1968 through May 1969. Annual primary productivity, chemo-organotrophic utilization of dissolved organic compounds, and field and laboratory studies of macrophyte-epiphyte interactions were monitored by carbon-14 techniques. Qualitative and quantitative photosynthetic pigment composition, and a brief taxonomic examination of the sessile complex, accompanied measurement of field parameters.

Productivity measurements of epiphytic algae on artificial substrata colonized in emergent (*Scirpus acutus* Muhl.) and submergent (*Najas flexilis* L. and *Chara* spp.) macrophytic vegetation sites were compared over an annual period with pigment (chlorophyll *a* and total plant carotenoids) estimates of biomass. The results indicate biomass

estimates are not indicative of photosynthetic activity, except during periods of intense productivity. The annual mean productivity of epiphytic algae was higher per unit surface area of the submerged portions of emergent plants (336 mg C m^{-2} day^{-1}) than on that of submergent forms (258 mg C m^{-2} day^{-1}); annual mean productivity per unit area of the littoral zone, for all of the macrophytic surface area colonized, was 195 and 1807 mg C m^{-2} day^{-1} in each of the respective sites. The results indicate that algal epiphytes on submerged vascular vegetation may be the dominant primary producers in shallow-water ecosystems, and may easily exceed the phytoplankton. Spatial and temporal rates of epiphytic productivity are discussed in relation to pigment composition and algal distribution, organic utilization, structural integrity of the matrix, and taxonomic composition of the epiphytic community. Deposition of ^{14}C-monocarbonates during *in situ* measurements of productivity represented 38.5 to 71.7% of the total intracellular fixed carbon. Acidification of ^{14}C-productivity samples by rinsing with dilute hydrochloric acid (0.001 *N*) removed 24% of previously incorporated carbon and is not recommended as a routine procedure.

Potential physiological interactions in macrophyte-epiphyte systems were investigated by bioassay procedure. Inorganic iron added at less than 10 µg l^{-1}, and at 100 µg l^{-1} in combination with organic compounds of

chelatory or complexatory ability, stimulated photosynthesis of epiphytic algae. Results of bioassay experiments with vitamins, trace metals, and inorganic phosphorus are discussed.

Chlorophyll a (corrected for pheophytin degradation products) and total plant carotenoid levels are among the highest standing crops reported in the literature (annual maximum of chlorophyll a: 7.3 g m^{-2}; plant carotenoids: 40.7 SPU m^{-2}). Maximum concentrations were found during winter under ice cover.

Epiphytic bacterial chemo-organotrophy with glucose and acetate substrates, measured at ecologically reasonable concentrations (11 to 160 µg l^{-1}), was evaluated through Michaelis-Menten enzyme kinetic analysis. First-order active transport kinetics dominated throughout the annual period with uptake of acetate (submergent substrata annual mean rate: 893 µg l^{-1} hr^{-1} dm^{-2}; emergent: 106 µg l^{-1} hr^{-1} dm^{-2}) being greater than that of glucose (submergent: 586 µg l^{-1} hr^{-1} dm^{-2}; emergent: 54 µg l^{-1} hr^{-1} dm^{-2}). Sources of dissolved organic matter at the epiphytic surface are discussed in relation to high rates of utilization and to *in situ* metabolism of attached primary producers.

Scirpus acutus was photosynthetically labelled *in situ* with natural concentrations of $^{14}CO_2$. Uptake of labelled materials of macrophytic origin by the epiphytic complex was determined. Extracellular release of ^{14}C-dissolved organic matter with respect to depth in the littoral water

column was followed in three plants over a 5 hour period. The nature of extracellular release, in comparison with $^{14}CO_2$ fixed by the hydrophyte and incorporation by the epiphytic complex, suggests functional interactions that may be prevalent in other macrophyte-epiphyte systems.

Najas flexilis, germinated and grown under axenic conditions in defined medium, was photosynthetically labelled and placed into Plexiglas chambers partitioned into compartments by organic matter-free membrane filters. Uptake of extracellularly released, labelled dissolved organic materials by cultured algal and bacterial epiphytes, separately and mixed in simulated natural communities, was followed under variously controlled conditions. Comparisons of uptake of known concentrations of glucose and acetate at 5, 11-12 and 21-23C by cultured epiphytes permitted evaluation of both laboratory macrophyte-epiphyte systems and field studies. Nutritional and physiological interactions of simulated communities of algae and bacteria are discussed.

Functional aspects of macrophyte-epiphyte metabolism in littoral ecology and lake trophic dynamics are described in the form of a model. Major metabolic and nutritional interactions in the littoral zone of a representative freshwater ecosystem are discussed. Macrophyte-epiphyte metabolism is stressed as a source of dissolved organic materials and extracellular metabolites, potentially capable of regulatory effects on autotrophic productivity

in the pelagial environment and, as a dynamic system, nutritionally and physiologically interacting to sustain high levels of primary productivity and chemo-organotrophy.

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I. INTRODUCTION

A substantial portion of temperate North America, inclusive of the Great Lakes region, is represented with a large number of lakes, ponds, marshes, and similar aquatic habitats of shallow to moderate depth. A large majority of these aquatic ecosystems characteristically possess well-defined littoral zones, with extensive development of submerged and emergent vascular hydrophytic vegetation.

The communities of shallow habitats are commonly thought to contribute significantly to the total energy fixation, utilization, and transformation. Little investigative effort, however, has been directed to comprehensive studies of littoral metabolism and the functional interactions and dynamics of these communities (see discussion by Wetzel, 1964). Frequently, a quantitatively significant portion of the total biomass of primary producers and decomposers constitutes the sessile community (herein referred to as the periphytic or attached algal and bacterial flora) and strongly suggests biomass that is often quantitatively dominant over their pelagial counterpart. Thus, the littoral community may be capable of exerting a strong influence on observed annual cycles and dynamics of pelagic phyto- and zooplankton through direct and indirect

interactions. Such influences, based on the function of dissolved organic compounds and metabolites in natural waters (see discussion of Wetzel, 1968), may have pronounced effects in regulation of eutrophicational ontogeny within many of these fresh waters.

Wetzel and Allen (1970) have suggested interactive mechanisms, functioning in littoral and pelagial environments, which are potentially responsible for regulation of various aspects of trophic dynamics. Detailed theoretical and experimental evidence continues to reaffirm the close physiological interrelationships and nutritional interdependencies existent between the epiphytic, epibenthic, and pelagial floras.

Exceedingly little information exists with regard to the quantitative contribution of epiphytes (hereafter considered the sessile microflora colonizing only vascular macrophytic vegetation) to the total primary producers pool within an aquatic ecosystem. There is, however, a voluminous literature generally applicable to the periphytic community, with stress being placed on the descriptive aspects of spatial and temporal population dynamics and community structure (Wetzel, 1964), but little consideration of nutrition, energetics, or physiological metabolism is evident.

Few investigators have sought to elucidate the interactions existing between the host macrophytic vegetation and the attached algal and bacterial flora in

freshwater environments. Linskens (1963) has determined the direction of transport of inorganic phosphorus between several marine macro-algal species and certain of their dominant epiphytic algae and noted specifically in which cells (epiphytes) and morphological structures (host macro-forms) the translocated isotope became localized. Other physiologically related studies on marine algae and their attached algal flora include Funk (1954), v. d. Ende and Linskens (1962), v. d. Ende and v. Oorschot (1963), and Beth and Linskens (1964), but similar studies on freshwater systems are lacking.

Studies contributing to our present understanding of the distribution of algal (or bacterial) epiphytes and causal mechanisms influencing attachment and subsequent growth are exiguous in the literature. Annual periodicity of the attached algal flora, especially with reference to changes in environmental and water-quality parameters (light, thermal conditions, inorganic chemistry, wave activity, etc.) has received cursory attention (see representative studies by Willer, 1923; Godward, 1934; Knudson, 1957; Cannon, et al., 1961; Kowalczewski, 1965; and others). Many of the studies in this area have not dealt with the epiphytic vegetation in its natural heterogeneity, but have isolated representative taxa from natural substrata to evaluate their individual growth and metabolic requirements under controlled laboratory conditions. Such studies would be of greater value in the interpretation of observed

in situ changes in population densities, species compositional shifts, and community metabolism, if experimental conditions simulated natural conditions.

Some studies (Godward, 1934; Prowse, 1959) have demonstrated a distinct specificity of certain epiphytic algae for select macrophytic hosts. If their observations are representative and extrapolatable to the dominant taxa and vascular hydrophytes commonly colonizing littoral areas, then a host of potential interactive mechanisms will require eventual investigation. Certain of these mechanisms are most likely to function and be detectable at very subtle levels and probably involve both commonly shared and more specific unidirectional metabolic interactions. Jørgensen (1957) suggested that the epiphytic diatom, *Tabellaria flocculosa*, is capable of utilizing silica directly from the stems of *Phragmites*, especially since Si is easily dissolved and decreases synchronously within the hydrophyte during periods of peak population density of the diatom. His circumstantial evidence is further supported by spring and fall epiphytic maxima which do not coincide with the maximum development of planktonic diatom communities. Such proposed nutritional supplementation by the macrophyte may be beneficial in the development of seasonal growth patterns distinctly different from those of the epibenthic or planktonic communities, and further decrease the probability of direct competition for specific nutritional elements or essential metabolites.

The epibenthic habitat (epilithic, epipelic, and epipsammic communities) has been little studied functionally. Studies are beginning to emerge where estimates of their integrated ecological importance and various aspects of their productivity and overall metabolism are considered. Much of the exhaustive literature prior to 1963 has been reviewed by Wetzel, 1964; similarly, much literature specific to epipelic and epilithic habitats has been included by Round, 1964. Other lotic and lenitic studies of particular interest, primarily since 1963, include Grøntved, 1960, 1962; Eichelberger, 1963; Sládeček and Sládečková, 1963, 1964; Wetzel, 1963, 1965a, 1969c; Pieczyńska, 1965; McIntire and Phinney, 1965; Kevern and Ball, 1965; Kevern, et al., 1966; King and Ball, 1966; Szczepanski and Szczepanskà, 1966; Cushing, 1967; Moss and Round, 1967; Backhaus, 1967, 1968; Grzenda, et al., 1968; Peters, et al., 1968; Steele and Baird, 1968; Moss, 1968, 1969; Pamatmat, 1968; and Hargrave, 1969). As in previous studies of epiphytic algal metabolism, there have been no investigations which emphasize synthesis or integration of community parameters that would, in turn, allow a direct estimate of their function or contribution to the lake as a whole.

For at least 40 years the majority of these studies were concerned with assessment of biomass (dry weight or organic matter content), and species composition, and distributional changes. Recently, emphasis has shifted to

in situ measurements of primary productivity. Variations of the carbon-14 technique have been employed in a few cases to measure photosynthetic rates of attached communities under natural conditions.

Unfortunately, much of the work accomplished with artificial substrata is of little relevance in determining the importance of in situ metabolism by attached communities. Where slides (or other substrata) have been suspended vertically in reservoirs and deeper waters, and subsequently allowed to undergo colonization, communities which are atypical in a planktonic environment may have developed. Attachment during prolonged stratification may reveal periphytic communities which are representative of natural populations and distributions of organisms in the plankton (especially applicable to the bacterial microflora). The real value in employing artificial substrata is to obtain attached community development where the natural substrata do not rapidly lend themselves to reproducible sampling procedures in littoral areas, and where attached communities are normally found, in littoral and shallow water zones.

Sampling of the natural heterogeneous community complex presents many obvious problems and depends upon the question being asked and precision required. Undoubtedly such problems are responsible in part for the lack of supporting information on littoral ecology that exists for the pelagial environment. The littoral is frequently

deleted from even the most thorough and comprehensive investigations. Further, of those studies which have attempted to look at the ecological role of attached communities from their overall systems-importance, few have achieved the necessary partitioning of the littoral environment into some of its basic structural components, i.e. into macrophytic vegetation, periphyton, etc., in order to make more direct and valid comparisons with the open water communities (Wetzel, 1964; Hargrave, 1969).

The present studies were designed to elucidate the relative importance of primary productivity and chemo-organotrophy (with selected supporting descriptive parameters) of epiphytic algae on submerged and emergent aquatic vegetation within a small lake to lake metabolism in general. Certain of the nutritional and metabolic interactions of host vascular hydrophytes and their attached heterogeneous algae and bacteria were studied. Potential direct and indirect regulatory effects of the collective littoral community on the pelagial environment and subsequent control over aspects of lake trophic ontogeny were considered. Estimates of *in situ* primary productivity and chemo-organotrophic utilization of dissolved organic compounds, as employed in this study were based on modifications of ^{14}C-methodology. Metabolism of extracellularly released organic materials by axenic cultures of a freshwater macrophyte (*Najas flexilis* L.), and subsequent partitioning of uptake by cultured algal and bacterial

epiphytes in specially constructed chambers, also employed ^{14}C tracer techniques. A technique designed for the *in situ* labelling (with $^{14}CO_2$) of an emergent hydrophyte (*Scirpus acutus* Muhl.), with potential applicability to other littoral metabolic studies, was developed and used under field conditions.

Resultant data, from both field and concomitant laboratory studies of epiphytic metabolism, strongly suggest that this community may easily be a dominant primary producer in many of our typical freshwater ecosystems, and through effective interaction in the cycling of dissolved organic compounds function at subtle levels to accelerate or retard eutrophicational processes and lake ontogeny. From the magnitude of measured annual rates of *in situ* photosynthesis, it is becoming increasingly obvious that we can no longer justify neglect of this part of the aquatic ecosystem, especially when evaluating trophic dynamics at the inter- and intrasystems level.

II. METHODS

A. Lawrence Lake, Michigan

Lawrence Lake (85° 21' W, 42° 27' N) is located in the southwestern corner of lower Michigan (Barry County) which, and in consociation with a smaller lake and surrounding marsh area, forms a basin confluent with the southern outwash apron of the Kalamazoo morainic system (Rich, 1970). The surrounding terrain is characterized geologically by the presence of glacial till and undulating plains. The origin of the lake, as evidenced by the steepness of the basin (Wetzel, et al., in preparation), probably followed melting of a buried terminal ice block. The cultural history of Lawrence Lake, indicating post-settlement influences to the lake system proper, and surrounding watershed, are documented in considerable detail by Rich (1970).

Lawrence Lake lies along a NE-SW axis at an elevation of 275.3 m above sealevel, and receives visible drainage from two very small inlets (Figure 1). The single outflow eventually terminates at Augusta Creek. There is some evidence that vernal springs may also contribute to the lake. The watershed is approximately 10 times the surface area of the lake and consists primarily of fallow fields.

Figure 1.--Morphometric map of Lawrence Lake, Barry County, Michigan, showing Stations I (dominant macrophyte: Scirpus acutus) and II (dominant macrophytes: Najas flexilis and Chara spp.). This map was constructed with the aid of sonar (200kc sec-1, Model F-850-A, Furuno Electric Co., Ltd., Japan) measurements along predetermined transects; at depths of less than 2m direct measures were employed (cf. Wetzel, et al., in preparation, for further details).

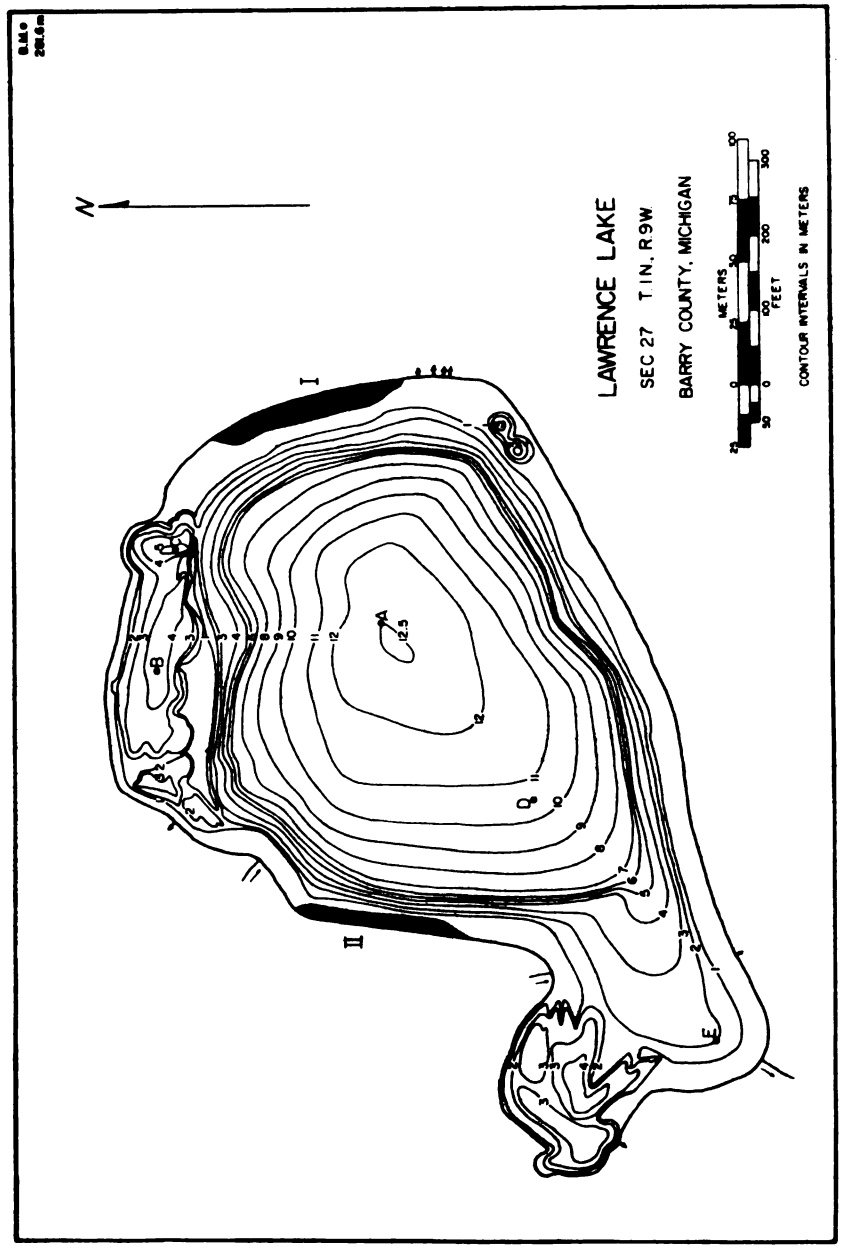

LAWRENCE LAKE
SEC 27 T.1N. R.9W
BARRY COUNTY, MICHIGAN
METERS
FEET
CONTOUR INTERVALS IN METERS
N
I
II
A
B
C
D
E

Figure 1.

Selected morphometric parameters, calculated from the bathymetric map (Figure 1), are total surface area: 4.96 hectares; total volume: 292,349 m^3; maximum depth: 12.6 m; mean depth: 5.89 m; shore development: 1.29, and relative depth: 5.01%. Lawrence Lake is calcareous and contains extensive deposits of marl, nearly pure calcium carbonate. Over a number of years (Rich, 1970) marl was excavated from the basin and has accounted for an addition of 5,250 m^2 to the surface area (10.6%), and 16,445 m^3 (5.6%) to the volume (Wetzel, et al., in preparation).

The subaquatic vegetation in Lawrence Lake occupies a significant percentage of the littoral surface area, and extends to a depth of 6 to 7 m. Dominant macrophytic vegetation includes representative submergent genera: Chara (8-10 species), Najas flexilis, Scirpus subterminalis, Myriophyllum sp., and Ceratophyllum sp.; floating-leaf genera: Potamogeton (5-7 spp.), Nuphar (2 spp.), and Nymphaea (2 spp.); and emergent genera: Scirpus (3 spp.), Sagittaria (3 spp.), and Potentilla fructicosa. An intensive limnological survey of numerous physical, chemical, and biological parameters has been conducted in Lawrence Lake to evaluate the autotrophic dynamics in a marl environment (Wetzel, et al., in preparation).

There are a number of reasons for the selection of Lawrence Lake, as a representative system, for the evaluation of epiphytic metabolism and subsequent comparisons to the pelagial environment. The morphometry of the basin

lends itself to such a study because of the well developed littoral zone of both submerged and emergent macrovegetation, and a well developed epiphytic flora. Moreover, nutrient interactions existing in marl environments, which perpetuate low rates of primary productivity by pelagic phytoplankton (Schelske, 1962; Wetzel, 1956b, 1966, 1967, 1968; Wetzel, *et al.*, in preparation), offer ideal conditions for determining potentially functional interrelationships between the macrophyte-epiphyte system and the open water. Many of these interactions (operational within the pelagial regions) have been alluded to, or are described, in the previous papers by Wetzel.

Two sites were selected for the study of various aspects of *in situ* epiphytic metabolism (*cf.* Figure 1), and include the attached flora on the dominant emergent hydrophyte, *Scirpus acutus* Muhl. (Station I), and the submerged *Najas flexilis* L. and *Chara* spp. (Station II). Station I is located on a marl bench, while Station II, on the western shore of the lake, is characterized by a highly flocculent organic sediment. Choice of these particular sites permits an internal comparison between emergent (submergent portions of the emergent plant) and submergent epiphytic growth and metabolic dynamics.

B. *In situ* assessment of epiphytic community metabolism

Studies were designed initially to determine the suitability of various sampling procedures for measurement of reproducibility of community responses with natural, heterogeneous, epiphytic complexes. Initial studies were also necessary to determine anticipated ranges of rates of primary productivity, chemo-organotrophic velocities, quantitative and qualitative pigment composition under *in situ* conditions. One of the goals of this study has been to attempt to demonstrate the constancy in metabolic responses under natural conditions, even though wide taxonomic differences exist within these communities.

Replicate samples of standing-crops of epiphytes from natural macrophytic surface areas were obtained by removal of uniform surface areas (0.785 cm^2) with a cork-borer. Attached community samples were carefully removed from the macrophytic discs with small pads of precombusted (1 hour at 525C) glassfiber ultrafilters (type 984H; Reeve Angel Co., Clifton, N. J.) and analyzed for chlorophyll *a* content by standard techniques (Strickland and Parsons, 1965). From variances in pigment concentration of the attached community, it became evident that macrophytic tissue was being removed along with the epiphyton (confirmed through microscopic examination of resuspended epiphyte samples). Similar initial tests were performed with colonized,

identical surface areas of various stem sections, leaf portions, and internodal segments with the same effects.

Tests to demonstrate ranges of metabolic parameters were conducted on replicated samples obtained as described above. Methodology employed for measuring carbon-14 uptake (e.g. primary productivity, and chemo-organotrophy) is discussed in detail below. Rates of photosynthesis and uptake of organic compounds were measured at different heights on Scirpus, Potamogeton, Nuphar, and Nymphaea to determine parameter variance and ranges of rates of metabolism. Similarly, measurements of these parameters were replicated at depth (Scirpus, Nymphaea, and Nuphar substrata) and within the same macrophytic stand (Scirpus, Chara).

From preliminary analysis of these data, it was decided to employ artificial substrata to overcome sampling problems associated with contamination by host macrophytic tissue. Artificial substrata consisted of 1" x 3" Plexiglas^{R} slides, wrapped with non-toxic, black polyethylene tape, with 3 standard surface areas (1.6 cm^2 each) exposed for colonization after precleaning the slide surfaces. Two such slides, when placed back-to-back, have an exposed surface area of 9.6 cm^2, and were considered one sample (Figure 2). Artificial substrata were affixed to Plexiglas rods (3/16" diameter) such that when the rods were positioned in the Najas and Chara areas of the littoral (Station II), colonizable surface areas would be exposed

at distances of 5 and 10 cm above the sediments. Substrata placed on the marl bench among the Scirpus acutus stand (Station I) were exposed at 5, 15, and 25 cm above the sediments. Vertical and horizontal placement of substrata permitted adequate characterization of spatial and temporal changes in the colonized epiphyton and was closely representative of vertical distribution existing in the natural vegetative stands. Rods with artificial substrata were placed at the intersection of 1 m^2 quadrats in a randomized-block design at both Stations. All substrata were identical and precleaned prior to placement in situ and allowed to start colonization at the same time, on both sides of the lake. All substrata were colonized for the same period of time prior to initial measurements and sampling was dependent upon random-number selection. After a suitable period of colonization (6 weeks), comparisons of parameters (pigments, primary productivity, and chemo-organotrophy) on natural and artificial substrata were undertaken.

1. Preliminary studies

Measurements of rates of photosynthesis, chemo-organotrophy, and pigment composition were made at biweekly (on two occasions, triweekly) intervals over the annual period of 13 April 1968 through 30 May 1969. Sampling intervals were selected, after considerable prior testing, in relation to the accumulatory development of the attached community. Rapid oscillations in rates of primary

productivity, for example, were not observed when measurements were made at 2-3 day intervals, as would be characteristic for some plankton populations.

Random-numbered substrata were selected on sampling occasions such that the routine parameters could be estimated on one sample (6 replicate, colonized surfaces) from each of the vertical positions within each of the two stations. Colonization of all substrata at each specific depth was assumed to be identical. Systematic treatment of individual colonized areas for each sample (= two slides) is outlined in Figure 2.

2. Measurement of annual cycle parameters

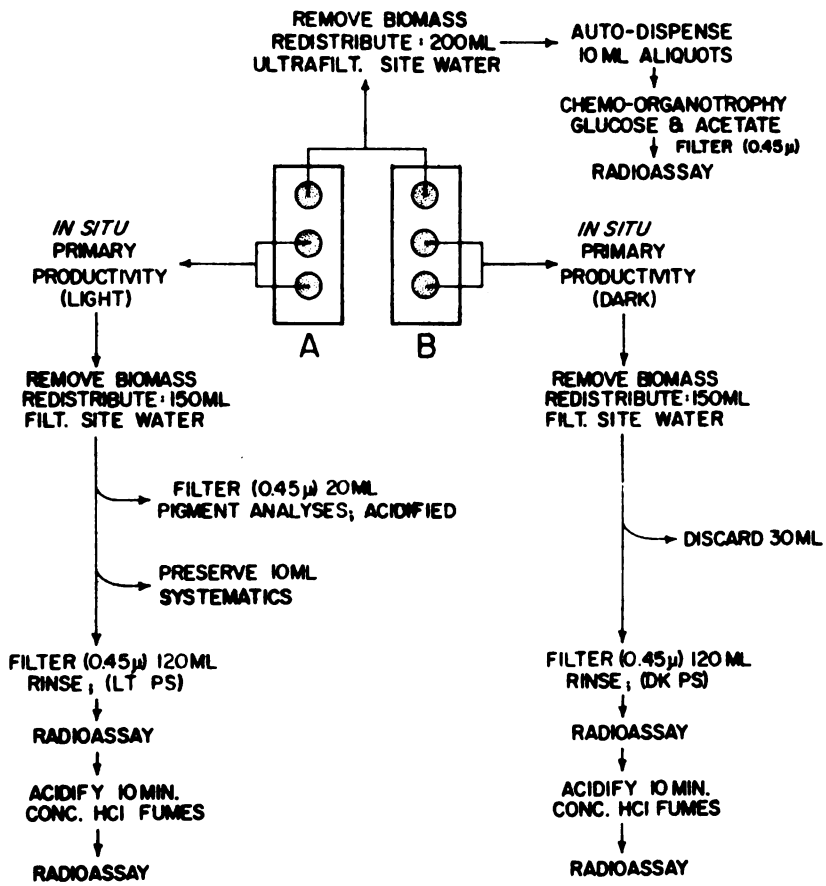
a) Primary productivity

Rates of primary productivity by algal organisms within the epiphytic complex was measured by slight modification of the carbon-14 technique, originally introduced by Steeman-Nielsen (1951, 1952). Various applications of ^{14}C methodology have been used for assessment of natural rates of photosynthesis in both freshwaters (see Goldman, 1963, among others) and marine environments (Strickland, 1960), and more recently within periphytic communities (Wetzel, 1964).

The colonized biomass from both of the upper surface areas of the two slides (Figure 2) was gently removed in the field, with precombusted glassfiber ultrafilter pads held with forceps. The pads were placed into 5 to 10 ml

Figure 2.--Systematic treatment of samples for determination of routinely monitored community metabolic parameters of primary productivity, chemo-organotrophy, and pigment composition within the epiphytic complex (see text).

Figure 2.


REMOVE BIOMASS
REDISTRIBUTE : 200ML
ULTRAFILT. SITE WATER
AUTO-DISPENSE
10 ML ALIQUOTS
CHEMO-ORGANOTROPHY
GLUCOSE & ACETATE
FILTER (0.45μ)
RADIOASSAY
IN SITU
PRIMARY
PRODUCTIVITY
(LIGHT)
IN SITU
PRIMARY
PRODUCTIVITY
(DARK)
A
B
REMOVE BIOMASS
REDISTRIBUTE: 150ML
FILT. SITE WATER
REMOVE BIOMASS
REDISTRIBUTE: 150ML
FILT. SITE WATER
FILTER (0.45μ) 20ML
PIGMENT ANALYSES; ACIDIFIED
DISCARD 30ML
PRESERVE 10ML
SYSTEMATICS
FILTER (0.45μ) 120ML
RINSE; (LT PS)
FILTER (0.45μ) 120 ML
RINSE; (DK PS)
RADIOASSAY
RADIOASSAY
ACIDIFY 10 MIN.
CONC. HCl FUMES
ACIDIFY 10 MIN.
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RADIOASSAY

aliquots of ultrafiltered lakewater from the growing site and returned to the laboratory for chemo-organotrophic measurements. The remaining two slides were placed into wide-mouth (27 mm), ground-glass stoppered, clear and opaque Pyrex^{R} bottles (125 ml). A 0.50 ml solution of tracer ^{14}C, as predominantly $Na(HCO_3)_2$, was injected into each bottle. The isotope was of known radioassay through previous gas phase combustion (see Wetzel, 1964; Goldman, 1968; concentration of absolute activity determined by Dr. R. G. Wetzel: 4.77 $\mu Ci\ ml^{-1}$). The samples were then resuspended at the same depth and position as original colonization took place. After a 3 to 4 hour incubation period, samples were retrieved from the field in a light-free box, and immediately transported to the laboratory. The biomass was removed with precombusted filter pads (cf. Figure 2; light photosynthesis sample), redistributed into 150 ml ultrafiltered lakewater from the growth site, and aliquots filtered (Millipore^{R}, type HA; porosity: 0.45 $\pm$ 0.02μ) for light bottle photosynthesis (120 ml) and pigment composition (20 ml). The final 10 ml aliquot was fixed with 2 drops of Lugol's acetic acid solution for subsequent microscopical examination. The epiphyton of the remaining slide (dark photosynthesis sample) was similarly redistributed into 150 ml of filtered lakewater; a 30 ml portion was discarded and the remainder filtered for radioassay. Filtered epiphyton were dried under desiccation, and the radioactivity determined with a minimum of 2000 counts

(occasionally 1000 counts for dark bottle photosynthesis samples during winter) on a gas-flow Geiger-Muller counter (Nuclear Chicago, Model 6010, with micromil window: D-47). Decontamination procedures (Wetzel, 1965c) were adherred to, and all filtered algal samples were radioassayed prior to and following brief (10 min.) exposure to fumes of concentrated hydrochloric acid. In calcareous aquatic environments precipitation of mono-carbonates proceeds at especially rapid rates, and leads to significant errors where neglected.

Calculation of autotrophic assimilation followed standardized procedures, as used for phytoplankton (see for example, the technique and detailed procedure outlined by Wetzel, 1964). An allowance of 6% is made for potential isotopic discrimination effects. A diurnal factor for surface light received during the in situ incubation was determined by planimetric measurement, the period of incubation, from a recorded diel pyrheliogram (Belfort pyrheliometer; Belfort Inst. Co., Baltimore, Md.). Total available inorganic carbon was calculated from sample temperature, chemical titration of alkalinity, electrometric determination of pH, and appropriate conversion factors (Saunders, et al., 1962). Volumes used for redistribution of organisms (with dilution factors) were equated with the original surface areas colonized. Data are expressed both on a per m^2 of artificial substratum basis and a m^2 of littoral zone

from estimates of the total macrophytic surface area per m^2 of the littoral zone.

b) Chemo-organotrophy

Samples for estimation of chemo-organotrophic utilization of organic compounds were returned to the laboratory within 20 to 30 minutes after collection. Similar to the photosynthetic measurements, the attached biomass was suspended into ultrafiltered lakewater (200 ml; see Figure 2) from the growth site and rapidly auto-pipetted in 10 ml aliquots into screw-capped, Pyrex test-tubes (15 ml).

Procedures for measurements of bacterial utilization of organic substrates follows the identical methodology of Allen (1969), and need be only briefly reiterated here. Serially increasing amounts of uniformly labelled (see discussion in Hamilton and Austin, 1967) glucose or acetate-^{14}C (usually 25, 50, 100, and 200 µl for glucose, equivalent to 11 to 86 µg l^{-1} at a specific activity of 1.044 µCi ml^{-1}; and 10, 20, 40, and 80 µl for acetate, equivalent to 21 to 160 µg l^{-1} at a specific activity of 0.981 µCi ml^{-1}) were added with micropipettes to four of the screw-capped tubes. Adsorption and non-metabolic blanks were prepared by addition of 10 or 25 µl of ^{14}C substrate, and immediately fixing the sample with Lugol's I_2KI. Blanks were incubated along with regular samples in total darkness at near <u>in situ</u> temperatures (± 1C during the vegetative season; during the winter: ± 0.5C) for a period not exceeding 1.5 hours on an

oscillating table (movement only strong enough to disrupt any temporary stratification of the isotope). The low concentrations of added organic substrate used and the short incubation periods should be emphasized. Both glucose and acetate have been detected in freshwater and marine environments at exceedingly low concentrations by both enzymatic or direct assay technique (Wright and Hobbie, 1965a, 1965b, 1966; Hobbie and Wright, 1965a, 1965b; Vaccaro and Jannasch, 1966, 1967; Allen, 1967, 1968a, 1969; Wetzel, 1967; Vaccaro, et al., 1968; among others). As stressed by Allen (1967) and others, estimations of in situ organic metabolism should be made at or near existing natural concentrations. Whether dominance by bacterial transport or algal diffusion kinetics is observed, is of little importance, if added quantities of organic substrates are close to those found in nature. Uptake demonstrated to occur at higher concentrations (e.g. > 500 $\mu g\ l^{-1}$) has little relevance in interpretation of naturally observed bacterial-decomposer and chemo-organotrophic activity, in that detectable responses may be induced or the result of site-inhibition phenomena. Short incubation periods are necessary to avoid possible recycling of metabolites through extracellular release (or as accumulated by-products) and subsequent re-utilization, in accordance with previously established rates of bacterial turnover of organic substrates (see discussion in Allen, 1969). For a more detailed discussion

of the suitability, and applicability, of these measurements based on Michaelis-Menten enzyme kinetic analyses and active transport capabilities, to the study of freshwater microbial metabolism, the reader is referred to the original work of Wright and Hobbie (1965a, 1965b, 1966).

Glucose and acetate were routinely employed because of their representative involvement in more generalized microbial metabolic schemes. On several occasions, other hexose sugars (fructose, galactose), amino-acids (glycine, serine, alanine), and Krebs-Cycle intermediates (succinate, glycolate) were tested. As with the photosynthetic measurements, maximum rates of utilization of organic substrates by the microflora (µg substrate removed l^{-1} hr^{-1}) were equated to surface areas of natural or artificial substrata. In strict interpretation, the new parameter of uptake would become: µg of substrate removed per liter per hour by the microflora colonizing 1 dm^2 of plant or artificial substrata. Presentation of chemoorganotrophic data on a dm^2 as opposed to per m^2 basis emphasizes the dynamics of utilization from small, colonized surface areas where the bacterial component is very heterogeneously distributed in the epiphytic community.

c) Pigment composition

Small aliquots (20 ml; Figure 2) of resuspended epiphytic organisms were concentrated onto Millipore HA membrane filters under low vacuum (< 0.5 atm), placed

between absorbant pads, and immediately frozen under desiccation until extraction and analysis could be performed (1 to 2 weeks). Extraction was accomplished by cell homogenization (1 minute; compressed-air driven Teflon-glass homogenizer) in reagent grade, aqueous 90% basic acetone. After centrifugation, standard spectrophotometric (Hitachi-Perkin Elmer, Model UV-VIS 139, or Beckman DK-2A, ratio-recording spectrophotometer; 10 cm light path) techniques were applied (Strickland and Parsons, 1965). Computation of concentrations of chlorophyll a and total plant carotenoids followed equations given by Strickland and Parsons. Subsequent to acidification of samples (1 drop of 1 N HCl) in spectrophotometer cuvettes, absorption peaks were redetermined to permit estimations of the concentration of pheo-degradation pigment products. Calculations of concentrations of chlorophyll a corrected for the presence of pheophytin products (see discussion by Moss, 1967a, 1967b) followed equations of Lorenzen (1967). Although absorption data were determined for chlorophylls a, b, and c, quantitative evaluation of b and c was not attempted in this study. As with primary productivity field data, chlorophyll a, and total plant carotenoids are expressed as mg or g, and millispecific pigment units (MSPU) or MSPU $\cdot 10^3$, per m^2 of artificial substrata or littoral zone.

d) Taxonomic treatment

Samples of epiphytic communities (10 ml; Figure 2) were routinely preserved from each depth, at each of the two stations, throughout the annual period. Evaluation of species composition and distribution was limited to a cursory examination by the settling-chamber technique (Utermöhl, 1958) and an inverted microscope (Wild, Model M-40). Dominance among the attached flora was usually determined, however, through the use of orthodox prepared slides and a compound light microscope. Both, living and preserved samples of intact, epiphytic community structure were examined with a Zeiss Photomicroscope, after embedding thin-sections of natural macrophytic cross-sections and surfaces in vaspar (1:1 mixture of vaseline and paraffin wax). Photographic documentation has revealed distinctly stratified patterns within natural communities of epiphytes.

3. Determination of naturally colonized surface areas of macrophytes

Quantitative and qualitative data of rates of photosynthesis, chemo-organotrophy, and pigment composition from artificial substrata were expressed in two ways: 1) as a rate or concentration per dm^2 or m^2 of colonized surface area at a specific depth in either of the two stations or 2) by employing factors for the macrophytic surface area present per dm^2 or m^2 of littoral zone, proportionally corrected for the macrophyte biomass present, per unit

area (dm^2 or m^2) of the littoral zone. Expression of these data on the basis of total macrophytic surface areas per unit area of littoral permits a direct comparison of monitored values with identical parameters in the pelagial.

Several procedures were used to obtain estimates of macrophytic surface area supported per m^2 of the littoral zone. Station II is dominated by Najas flexilis and several species of Chara. Owing to the high heterogeneity in natural distribution, representative plants were excised at the sediment-water interface by removal of replicated cores (diameter: 14.9 cm^2) along predetermined transects parallel and perpendicular to the shoreline. The macrophytic biomass was carefully rinsed in a floating sieve, blotted to remove excess water, and gently placed in a tared, shallow aluminum pan containing a known quantity (by weight) of surfactant (Teepol 610^R, Shell Chem. Corp., N. Y.; and a 50% dilution of Tween 80^R, E. H. Sargent & Co.; both were independently tested). Plants, after 10 to 15 seconds immersion, were drained for several seconds and removed. Loss in weight of the surfactant is equivalent to the area covered as determined by previous calibration with various objects of known surface area (technique closely followed that of Harrod and Hall, 1962). Differences in the weight of surfactants, used for conversion to surface area equivalents, were not corrected for evaporative losses during in situ measurements. Transport of the

surfactant to and from the field in air-tight, screw-capped containers minimized such losses.

Surface areas of individual Scirpus acutus plants (Station I) were determined by computation, using the formula for a subtended cone, with data of the leaf (stem) diameter at various heights above the sediment and the vertical distance to the surface. The mean number of Scirpus plants per m^2 was determined in transects perpendicular and parallel to the shoreline, and multiplied by the mean colonizable surface area per plant for a measurement of the total colonizable area exposed per m^2 of the littoral zone.

Estimates of macrophytic surface area were determined during spring and fall; interpolated surface areas for sampling intervals throughout the annual period were applied to monitored parameters only to gain a better understanding of the magnitude of natural biomass change. The surface area conversions represent only approximate values. Computation of all annual cycle data for Scirpus substrata includes correction in surface area per m^2 of littoral zone, for fluctuation in surface water level. Data from the uppermost sample (25 cm above the sediments) are used for calculation of rates or concentrations for the interval from 30 cm to the surface. Sample data from 5, 15, and 25 cm above the sediments (Station I) were used for calculation of parameters in isopleth figures (0-10 cm; 10-20 cm; and 20-30 cm); at Station II, data from 5 and 10 cm

samples are used to express the vertical intervals (0-10 cm; 10-20 cm). Mean height of the submergent plant story by transect measurement was 20 cm.

Although expression of data in isopleth form may be criticized for potential over-resolution, such presentation is of definite value in visualizing dynamic changes which would be obscured, or lost entirely, in individual line-drawings and histograms. Annual cycle data are graphically presented in several forms: 1) as individual parameters at depth versus time, 2) as isopleths, integrative of several depth intervals against time, and 3) as integrated line-drawings of the summation of a parameter with time.

Estimates of the contribution of epiphytic metabolism to the total lake were obtained by multiplication of annual mean rates (planimetrically obtained) by the surface areas (means for vegetative season) of the littoral zone from 0 to 60 cm depth in which species of Chara, Najas flexilis, and Scirpus acutus are found. These latter estimates were determined by planimetry of species vegetational maps of Lawrence Lake, from 0 to 60 cm depth, for the above species.

C. Field experimental studies

Several experimental approaches were applied in an attempt to elucidate some of the physical, chemical, and biological interactions occurring within the epiphytic community complex under natural conditions and to aid in

interpretation of observed annual fluctuations in assessed metabolic parameters.

1. Precipitation of ^{14}C-monocarbonates and decontamination procedure

Measurements of rates of photosynthetic activity in aquatic environments employing inorganic carbon-14 have been demonstrated to be erroneously high in certain instances, owing to extracellular precipitation of ^{14}C monocarbonates (e.g. Paasche, 1963 cited by Wetzel, 1965c). Various procedures have been used for removal of deposited carbonates, including immediate rinsing of filtered algae with dilute acid (Strickland, 1960; Goldman, 1963), and by brief exposure to fumes of concentrated HCl (Steeman-Nielsen, 1952; Wetzel, 1965c). McAllister (1961) and Paasche (1963) have demonstrated that even 1 to 2 minute exposure to fumes of concentrated hydrochloric acid is sufficient to remove the contaminant activity. A 10 minute exposure has been suggested by Wetzel (1965c) as a standard procedure in radioassay studies of photosynthetic activity. Such decontamination procedures are necessary in studies involving the epiphytic complex. Deposition of ^{14}C as monocarbonates by epiphytic algae and bacteria is rapid, even during 3 to 4 hour *in situ* incubation periods. This precipitation was especially evident in the dark-bottle samples for photosynthesis during winter periods under ice cover and poor illumination.

Epiphytic samples were removed from natural Scirpus acutus substrata (15 cm above the sediments; 14 March 1969) after in situ photosynthetic labelling and redistributed into 150 ml of ultrafiltered lakewater from the growth site. Thirty duplicate aliquots (5 ml each) were filtered onto Millipore HA filters. Triplicate samples were dried, desiccated, and exposed to fumes of concentrated HCl for varying periods of time. Identical triplicate samples were also rinsed with 5 ml aliquots of various concentrations of dilute acid. In both treatments $^{14}CO_2$ evolved was precipitated with barium hydroxide, concentrated onto membrane filters, and radioassayed in a G.-M. proportional counter. Loss of intracellularly fixed carbon from the algae by treatment with small aliquots of HCl solution was estimated by conversion of the removed dissolved organic material to $^{14}CO_2$ by persulfate oxidation (Menzel and Vaccaro, 1964). Radioassay of the evolved carbon dioxide was accomplished with a vibrating-reed ionization-chamber electrometer system. Data obtained from all samples analyzed by a conventional Geiger-Muller end-window, gas-flow system were similarly converted to absolute radioactivity by combustion of replicate control samples in gas-phase. A series of filtered samples was also treated by direct immersion in 0.10 N hydrochloric acid for 30 seconds.

2. Application of bioassays to the detection of physiological-nutritional interactions

The *in situ* carbon-14 bioassay of limiting factors for stimulatory or inhibitory response of actively photosynthesizing algal organisms has been applied in both freshwater and marine environments (for example, see Ryther and Guillard, 1959; Menzel and Ryther, 1961; Goldman and Mason, 1962; Goldman, 1960a, 1960b, 1962, 1963, 1964, 1965, 1967; Goldman and Wetzel, 1963; Wetzel, 1964, 1965b; Goldman and Carter, 1965; and others). Although this technique is most frequently applied to planktonic algal populations for detection of nutrient limiting factors to primary productivity, its sensitivity and rapidity is suitable for a general assay of nutritional and metabolic interactions between host macrophytes and their epiphytic community complexes. Detection of the level of bacterial or algal stimulation or inhibition or causal mechanisms involved in perpetuating the response, was not the parameter sought here. Rather the composite community response was desired.

Responses to added micro-nutrients by autotrophic organisms within the epiphytic complex might reveal the direction and magnitude of movement of certain nutritionally important micro-nutrients and metabolites. The likelihood is that materials are translocated between the macrophyte and its epiphytes.

Epiphytic samples were removed from natural *Scirpus* substrata (15 cm above the sediments; 8 September 1968)

and redistributed into ultrafiltered lakewater (2000 ml) collected from the growth site. Identical 100 ml aliquots were dispensed into ground-glass stoppered, transparent Pyrex bottles (125 ml) and labelled. The bottles were incubated under controlled laboratory conditions (ca. 1000 lux; 25C; on a 3 rpm rotating table). Prior to incubation, micro-additions of the following were made: (1) inorganic iron ($FeCl_3$) separately and in combination with low concentrations of artificial and natural complexing agents, (2) inorganic phosphorus (K_2HPO_4), (3) vitamin mixture of B_{12}, biotin, and thiamine hydrochloride, and (4) trace metal mixture of $CuSO_4$, $ZnSO_4$, $CoCl_2$, $MnCl_2$, Na_2MoO_4, and H_3BO_3. Algae were concentrated onto Millipore HA filters and treated as described for photosynthetic samples. Activity of prefiltered controls were subtracted from those of the experimental, to eliminate possible error by inorganic precipitation of ^{14}C where micro-additions of iron were used (Goldman and Mason, 1962).

D. In situ macrophyte-epiphyte interactions

Although extracellular release of dissolved organic compounds has been demonstrated with axenic laboratory cultures of Najas flexilis (Wetzel, 1969a, 1969b), there have been no confirmatory studies which would support the occurrence of a similar release under completely natural conditions in the field (Sieburth, 1969; Sieburth and

Jensen, 1969; and Khailov and Burlakova, 1969, have documented in situ exudation by marine macroalgae). The purpose of this experiment was to demonstrate the presence of extracellular dissolved organic matter (DOM) production by an emergent hydrophyte, Scirpus acutus, and chemo-organotrophic utilization by epiphytic organisms of these materials arising from the plant.

Small, light (< 4 g) chambers of transparent Handi-Wrap^{R} (Dow Chemical Co., Midland, Mich.) with reinforced polystyrene supports (8 x 8 x 16 cm; approximately 1 liter volume) were placed over the emergent tips of three Scirpus acutus plants (Station I, Lawrence Lake). A small ampoule was affixed to the innerside of each chamber, containing $Ba^{14}CO_3$ of known radioassay (52.0 mCi mM^{-1}, 261 μCi mg^{-1}; 84% isotopic abundance in the carbon atom). The exact amount of barium carbonate-^{14}C was determined with gravimetric precision (1.89579 mg $Ba^{14}CO_3$ per ampoule; Cahn Electrobalance) such that when 0.20 ml of 1 N HCl was injected through the chamber wall, $^{14}CO_2$ would be evolved at near atmospheric concentrations (0.035% v/v) similar to the technique employed by Dahlman and Kucera (1968) in translocation studies of grassland vegetation. Diffusion of radioisotopes in liquid (glucose and acetate-^{14}C) and gaseous ($^{14}CO_2$) phase through the membrane could not be demonstrated.

Three types of experimental treatment were designed. On one hydrophyte the epiphytic complex was removed by

gentle friction of glassfiber filter pads, after which a transparent Plexiglas tube (inside diameter: 4.5 cm) was carefully inserted into the sediments surrounding the plant. The top of the Plexiglas tube remained above the water surface and effectively isolated the immediate environment of the Scirpus plant. A second tube was positioned around arother Scirpus plant with the epiphytic community intact. A third plant, without a tube enclosure and with its natural attached flora undisturbed, served as a control.

Upon evolution of the $^{14}CO_2$ in the field water samples adjacent to the plants and samples of the attached communities were systematically collected at 0, 15, 30, 60 minutes, and 1 to 5 hours.

At the termination of the experiment, the Scirpus plants were sectioned at 2 cm intervals from the emergent tip into the rhizome tissue to determine internal labelling patterns. All samples, except those of the attached complex, were assayed for the presence of radioactivity by liquid scintillation techniques (employing a Beckman LS-150 ambient temperature Scintillation counter). Epiphytic samples were assayed after concentration, by solid-counting procedures.

Dissolved organic matter samples were collected in the field by a simple suction-tube device, filtered, and pipetted (1 ml duplicate aliquots) directly into glass vials containing 10 ml of scintillation fluid (8.05 g

Fluoralloy^{R} mix (Beckman Instruments, Inc., Calif.) dissolved in 1 liter of toluene; 2-aminoethanol, a carbon dioxide absorbant, was added at a ratio of 1:10 parts toluene in certain samples, but Triton-X^{R} (Packard Inst. Co., Ill.) at 6:7 (v/v) of toluene plus Fluoralloy mix was primarily used; the latter solvent:scintillator mixture is especially recommended where the sample to be assayed contains considerable water). Carbon dioxide-^{14}C contained within the water, and within the plant tissue at the termination of the experiment, was determined by immediate acidification (4 drops of 1 N HCl) of the counting fluid to a pH of less than 4.0, and trapping the evolved $^{14}CO_2$ in an NCS^{R} (0.6 N solubilizer solution in toluene; Amersham/Searle, Ill.)-based scintillator fluid; NCS functions as a quantitative carbon dioxide absorbant (Zimmerman, 1967). Activity remaining in purged samples was present as dissolved organic compounds. Digestion of Scirpus plant tissue was accomplished with NCS (1.4 ml added to 8.6 ml of toluene-Fluoralloy mixture) at 4C for 48 hours in total darkness. Similar digestion of plant material was effected with Bio-Solv solubilizer (Beckman; Formula BBS-3) at the same concentrations.

Severe color-quenching from chlorophylls resulted from digested macrophytic tissue and required standardization of the instrument with a color-quenching series prior to assay of plant samples. Standardization with liquid, organic isotopes (in the above toluene-based

scintillator fluids) of known radioassay was necessary prior to each assay. Additional standards were prepared by addition of organic (glucose and acetate-^{14}C) isotopes to various concentrations of extracted pigments from unlabelled Scirpus plants. All samples assayed by liquid-scintillation techniques were counted to a preset error of $\pm$ 2.0% (equivalent to 10^5 disintegrations per minute).

E. Axenic macrophyte-epiphyte interaction studies

Studies of epiphytic algal and bacterial metabolism, with isolated and purified cultures under controlled laboratory conditions, have not previously been reported. Interactions of epiphytic organisms and their host macrophytes under natural field conditions are also exiguous in the current literature. For correlative purposes and evaluation of *in situ* monitored parameters and observable growth patterns, some knowledge of the interactive mechanisms and interrelations of bacterial, algal, and macrophytic metabolism under fixed conditions is desirable.

For purposes of conducting experiments with direct interpretative value to field conditions, isolation and purification of several species of algae and bacteria from natural macrophytes was initiated. Resuspended (into ultrafiltered lakewater) epiphytic samples from *Scirpus acutus*, *Najas flexilis*, and *Chara* sp. were repeatedly streaked onto plates of solidified (0.5% agar) Lawrence

Lake water from the growth site. Small aliquots were also introduced into 1) Rodhe's VIII medium (Rodhe, 1948), 2) solidified and liquid WC medium (Dr. R. R. L. Guillard, personal communication), 3) solidified and liquid medium II of Forsberg (1965, as modified by Wetzel and McGregor, 1968), and 4) artificial lakewater with an ionic composition and total ionic content similar to that of the parent lakewater of Lawrence Lake (Table 1). The latter medium did not support growth of algal organisms beyond three routine transfers. Repeated streaking on plates and slants and serial dilution transfers in liquid media involved one treatment with dilute concentrations of antibiotics (penicillin "G"-sodium, and streptomycin sulfate) at 0, 10, 50, 100, and 400 $\mu g\ l^{-1}$ of each. Procedures and concentrations of each bactericidal agent are similar to those used for algal organisms by Droop (1967). Cultures of epiphytic bacteria included Pseudomonas sp., and Caulobacter sp. (see Allen, 1968b for details of isolation of two similar species of freshwater Caulobacter). The isolated epiphytic algae were Gomphonema sp., Cyclotella sp. (cultures of Cyclotella nana were later obtained from Dr. R. R. L. Guillard, Woods Hole Oceanogr. Inst., which were morphologically identical to the epiphytic Cyclotella), and Chlorella sp. Occasional sterility tests with a broad spectra of media (Wetzel and McGregor, 1968) demonstrated axenic conditions throughout the period of laboratory experimentation.

TABLE 1.--Composition of artificial lakewater patterned after that of Lawrence Lake, Barry County, Michigan.

Constituents[1]	Concentration (mg l^{-1})
Ca	80.96
Mg	17.50
Na	7.50
K	3.01
HCO_3	116.68
SO_4	69.23
Cl	10.04
Fe	0.020
PO_4	0.010
NO_3	0.300
Chelator[2]	40.0
Buffer[3]	1000.0

[1]Vitamins and trace metal mix are identical to those recommended in WC medium (see text).

[2]NTA (Nitrilotriacetic acid); Eastman Organic Chemicals, Rochester, N. Y.; No. 5417.

[3]TRIS-(hydroxymethyl)-aminoethane; ultrapure; supplied by Mann Research Laboratories, N. Y.

Upon isolation, minimal nutritional requirements were established for the algae and bacteria. Constant growth conditions were employed for the cultured organisms at 16 to 20C with 800 to 1000 lux intensities. Growth curves were established for the bacteria in liquid media (WC and medium II) with glucose or acetate added. Growth was recorded as optical density of the culture at 650 nm. Algal growth was also monitored in liquid media using similar methods, but was recorded as optical density at 750 nm. Cultures, grown for rate of population increase studies, were incubated in 100 ml of media in side-arm flasks. Cell numbers were determined in a Petroff-Hausser Bacterial Counter with a standard compound light microscope at 1000x magnification and oil immersion optics. Prior to experimental treatment, all cultures were grown until log phase of growth population densities were obtained (optical density monitored).

Najas flexilis, a common taxon of submerged macrophytic species inhabiting freshwater littoral zones, was grown and maintained under axenic conditions from surface-sterilized seeds, until seedlings reached 2 to 3 cm in length (medium II; 750 lux; 20C; see Wetzel and McGregor, 1968, for details of growth conditions). Initially, rates of photosynthetic incorporation of inorganic carbon (^{14}C) by Najas were determined in essentially the same manner as Wetzel and McGregor (1968) with the exception of the radioassay procedure of labelled plant biomass. A

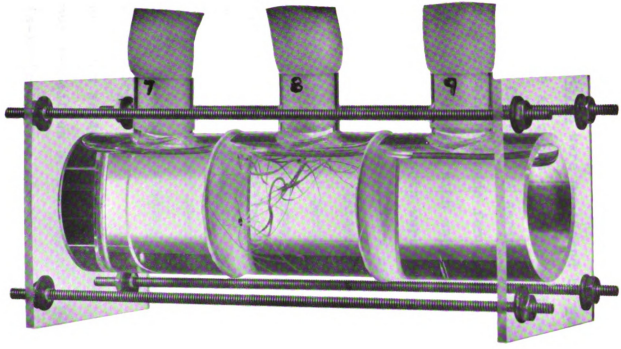
homogenization technique and planchette-plating and solid-counting procedure was used (O'brien and Wardlaw, 1961). Extracellular release of ^{14}C-labelled organic compounds by Najas, subsequent to photosynthetic labelling, was determined by combustion of DOM to $^{14}CO_2$ and radioassay in gas-phase.

Studies on the transfer of labelled dissolved organic compounds and $^{14}CO_2$ from axenic Najas into cultured epiphytic algae and bacteria were accomplished with specially constructed chambers (Figure 3). The chambers were fabricated from highly transparent Plexiglas tubing (inside diameter: 3.75 cm; length of each section: 5.0 cm) in three sections, where each section was separated from the adjacent one by pre-eluted (with 60 ml of 0.1 N HCl, following recommended procedures of Parker, 1967) Millipore GS (porosity: 0.22 $\pm$ 0.02 μ) membrane filters. Tests were made to determine the time interval required for small concentrations of ^{14}C-labelled organic compounds to move and equilibrate from the center chamber section into the two outer sections under exact conditions of normal transfer experiments. Rapid movement of labelled organic compounds resulted in equilibrium activities within 3 to 11 minutes. Adsorption of the isotopes onto filter surfaces was found to be negligible over several hours.

Najas flexilis plants (5 to 6 per flask) were pulse-labelled with inorganic carbon in 200 ml of medium II for a period of 4 hours and carefully transferred to the central

Figure 3.--Experimental Plexiglas chamber used for partitioning uptake kinetics of extracellular products from macrophytes by algal and bacterial epiphytes.

Figure 3.



section of the experimental chamber. After brief acclimation, aliquots of algae or bacteria at known population densities in log phase of growth were introduced into the adjoining chamber sections containing 50 ml of medium. Uptake by the epiphytes of materials extracellularly released by the Najas were monitored by periodically removing small duplicate aliquots of these cultures and filtering onto GS and HA filters. A serial dilution of the extracellular materials was made for application of Michaelis-Menten enzymatic assays with separate cultures of epiphytes. Similar culture experiments were performed on macrophytes, algae, and bacteria present in a single 250 ml flask to gain further insight into interaction phenomena.

Studies were further performed to determine the rates of chemo-organotrophic utilization of glucose and acetate-^{14}C (at the same concentrations and incubation intervals as routinely used in the field) at 5, 11 to 12, and 21 to 23C, by cultured algae and bacteria, to aid in evaluation of previously described studies with partitioned chambers in the laboratory.

Data from separate and mixed cultures are expressed as maximum velocities of utilization (bacterial V_{max} of Michaelis-Menten enzyme kinetics) and rates of diffusion (algal K_d; cf. discussion in Allen, 1969). Resultant data on utilization of organic compounds are further expressed per unit optical density (cell numbers in log phase of

growth) to allow direct comparisons of rates under different experimental conditions.

III. RESULTS AND DISCUSSION

A. In situ methodological studies

A brief survey of rates of primary productivity of epiphytic algae removed from identical surface areas of several natural macrophytic substrata revealed similar rates among communities on different hydrophytic species (Table 2). In that measurements were conducted during two consecutive days at approximately the same time of day, diel photosynthetic patterns for autotrophic metabolism are largely negated. Differences shown are likely to be representative for the season, depth, and specific macrophyte sampled. Although species composition was observed to be different on each of the supporting macrophytes and between sample replicates, rates of inorganic carbon fixation were similar. It is noteworthy that rates of carbon fixation by epiphytes on the stem of Potamogeton sp. were lower than rates from other macrophytes tested.

Replicate measurements of rates of carbon fixation by epiphytic algae were made on 11 samples from Scirpus acutus to determine variance associated with epiphytic communities from the same macrophytic species within the same vegetational stand. At a depth of 15 cm above the sediments a mean rate of photosynthetic productivity of

TABLE 2.--Rates of primary productivity by heterogeneous epiphytic algal communities on several natural macrophytic substrata. Lawrence Lake, Barry County, Michigan; 19-20 September 1967.

Plant and Depth Above Sediments	Productivity (mg C m^{-2} day^{-1})[a]	
	Mean	Range
Scirpus acutus Muhl.		
10 cm	821	± 75
20 cm	1300	± 264
Potamogeton sp.		
50 cm	403	± 49
75 cm	465	± 31
Nuphar sp.		
25 cm	1515	± 110
60 cm	1297	± 218
Nymphaea sp.		
25 cm	1136	± 230
40 cm	809	± 137

[a]Rates (N = 2) expressed as net mg C assimilated per m^2 of macrophytic surface area at depth indicated; samples acidified to remove radiocontaminated carbonates.

1162 (range = ± 409) mg C was assimilated per m^2 of plant substrata per day by attached samples on spatially separated hydrophytes. Plants selected for epiphyte removal were randomly chosen from within an area of approximately 80 m^2 on the marl bench in Lawrence Lake (Figure 1).

Variance estimates of rates of photosynthesis by epiphytes from 15 cm above the sediments on the same emergent hydrophyte were 892 (range = ± 116) (3 replicates of 0.785 cm^2 each; 17 September 1967), 1264 (range = ± 201) and 990 (range = ± 83) mg C m^{-2} of macrophytic substrata day^{-1} for three spatially separated plants. These few results suggest that rates of photosynthesis are relatively uniform despite variation in species composition for epiphytes on the same plant within a homogeneous stand at a specific height in the littoral water column. Additional support was demonstrated for similar rates from replicated measurements on the same plant equidistant above the sediments. Uniformity of epiphytic response appears to be more closely related, at least for Scirpus acutus substrata, to constancy in light and thermal conditions at a particular vertical stratum than to a high degree of overall homogeneity in species composition of attached communities.

Photosynthetic rates were measured for epiphytes on both young and second year Scirpus acutus plants (Station I; 17 September 1967). The mean response for communities attached to over-wintered substrata dominated by diatoms was 1042 (range = ± 381) mg C m^{-2} of plant substrata day^{-1}

(for 5 replicates of two samples each). Epiphytes on plants produced during the same vegetative season had photosynthetic rates of 697 (range = ± 103) mg C m^{-2} of plant substrata day^{-1} (same number and type of replicates) which reflects a distinctly different community structure. Ranges of rates reported here only allow an intrasystem evaluation of the sampling procedure employed for the ^{14}C method for measuring primary productivity rates of algal epiphytes. No attempt was made to place these photosynthetic rates on a littoral zone basis, as variance in natural macrophytic surface area per m^2 of the littoral zone would obscure the differences observed per unit surface on individual plants.

Owing to problems in quantitative removal of the epiphyton from natural plant surfaces, artificial substrata were employed for studying the annual cycle of *in situ* epiphytic metabolism. Prior to initiation of routine monitoring the Plexiglas substrata were allowed to undergo colonization for six weeks, before comparative productivity of epiphytes on natural and artificial substrata was measured. These measurements were conducted on *Scirpus* substrata (natural and artificial at 15 cm above the sediments) at Station I. Rates of photosynthesis measured on 12 replicates of equal surface area (1.6 cm^2) from young *Scirpus* plants were 135 (range = ± 51) mg C m^{-2} of plant substrata day^{-1}; the mean rate for equivalent areas on artificial substrata was 82 (range = ± 26) mg C m^{-2} day^{-1} (12 April

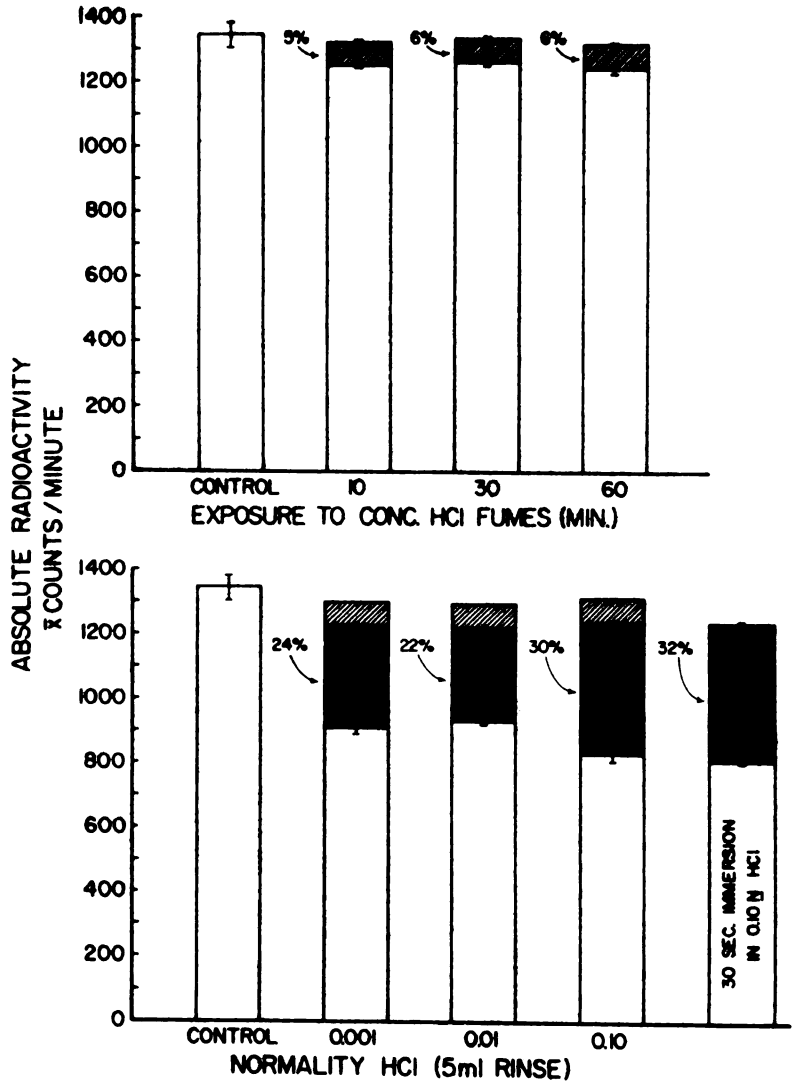
1968). Community structure in the attached algae on both substrata was poorly developed with colonization of only 6 weeks subsequent to ice retreat. Comparisons of over-wintered Scirpus plants with artificial substrata were not undertaken during initial studies. The following spring (19 April 1969) an independent comparison of photosynthetic rates (^{14}C method) of epiphytes from over-wintered surfaces of Scirpus (271 (range = $\pm$ 59) mg C m^{-2} of plant day^{-1}; N = 3; 15 cm above the sediments) showed favorable agreement with similarly placed artificial substrata (162 (range = $\pm$ 48) mg C m^{-2} substrata day^{-1}; N = 3).

Resultant data from initial studies have shown the ^{14}C technique to be adequately suited to the measurement of primary productivity of algal epiphytes. Incubation periods were limited to a 3 to 4 hour interval during mid-day. Rates were additive where carbon fixation was measured from (1) 0900 to 1200, (2) 1200 to 1500, and from 0900 to 1500. A larger portion of the inorganic carbon was fixed during the 0900 to 1200 interval than during the afternoon period (27%).

A major source of inherent error in applying the ^{14}C method of epiphytic complexes, especially true in calcareous environments, is the rate at which deposition of ^{14}C-labelled monocarbonates occurs during in situ incubation. Data from photosynthetically labelled epiphytic samples subjected to two procedures of decontamination are summarized in Figure 4. Filtered samples exposed to fumes of concentrated

Figure 4.--Mean percentage loss of ^{14}C activity (as precipitated monocarbonates) from filtered epiphytic algae upon exposure to fumes of concentrated HCl for varying periods of time (upper), and loss of activity (as intracellular fixed carbon) by rinsing filters with various concentrations of dilute acid (lower). Natural epiphytic algae removed from Scirpus acutus following in situ photosynthetic ^{14}C fixation in Lawrence Lake (Station I), Michigan, 14 March 1969.

Figure 4.


ABSOLUTE RADIOACTIVITY
X COUNTS / MINUTE
1400
1200
1000
800
600
400
200
0
5%
6%
6%
CONTROL
10
30
60
EXPOSURE TO CONC. HCl FUMES (MIN.)
24%
22%
30%
32%
CONTROL
0.001
0.01
0.10
NORMALITY HCl (5ml RINSE)
30 SEC. IMMERSION IN 0.10 N HCl

acid over 10 to 30 minute intervals lost from 5 to 6% of their absolute activity. Identical epiphytic algal samples treated by rinsing with small aliquots of dilute acid released intracellular fixed carbon, equivalent to 22 to 30% of their total absolute activity in addition to $^{14}CO_2$ evolved from the dissolution of monocarbonates. A single set of triplicate samples immersed in 0.1 N HCl released 32% of the fixed organic carbon as dissolved organic compounds, a loss similar to that of rinsed samples. It cannot be over-emphasized that 0.001 N HCl, although very dilute, effectively removes incorporated carbon from ^{14}C-labelled algae upon rinsing. From these findings it was concluded that all photosynthetic samples (both light and dark bottles following in situ incubation) should be decontaminated by standard 10-minute exposure to fumes of concentrated hydrochloric acid prior to radioassay (Wetzel, 1965c). The quantitative significance of precipitated monocarbonates over the annual period is discussed below.

To evaluate the contribution of epiphytic metabolism on a lake basis, it was necessary to recompute data obtained from known surface areas of substrata at depth on the basis of total macrophytic surface areas present per square meter of the littoral zone in each of the two sites. For Scirpus acutus during the vegetative season (spring to fall) the range for each 10 cm segment of the stem above the sediments was 0.07728 to 0.1595 m^2 of colonizable surface area per m^2 of the littoral zone. The total

available surface from the sediments to a height of 30 cm in the water column was 0.2318 to 0.4785 m^2 of plant surface m^{-2} of the littoral zone for the vegetative stand. Factors were determined to correct all annual cycle data at each depth (5, 15, and 25 cm above the sediments), on each sampling occasion, on the basis of a square meter of littoral zone for each 10 cm interval on the plant, by random counting estimates of changes in the quantity of Scirpus plants per m^2 of the littoral zone over the growing season. Initially, in the spring (1968) a mean of 51.3 plants per m^2 of the littoral was found. During the fall (1968) 64.8 occurred per m^2 and in the spring of 1969, 56.3. The assumption of these conversions is that single sample measurements (from 2 x 1.6 cm^2 surface area) are representative of the natural vertical heterogeneity in epiphytic metabolism and thus permit extrapolation of data from each of these points (5, 15 and 25 cm vertical distance) to each of the three 10 cm intervals.

Adsorbed surfactant procedures for obtaining surface areas of Najas flexilis and Chara spp. depended upon core samples of natural macrophytes along predetermined transects both parallel and perpendicular to the shore line at Station II. A high variance in availability of Najas and Chara surfaces at station II was associated with in situ heterogeneity in distribution of the submerged plants (range = 9.0 to 214.9 cm^2 of macrophytic surface area per 14.90 cm^2 of littoral zone; spring, 1969). An annual

range of 6.15 to 10.63 m^2 plant surface/m^2 littoral zone was determined for the vegetative season of 1968. Since the mean vertical height of the submerged plant story was 19.8 cm, the actual macrophytic surface areas per m^2 of littoral zone were divided by 2 to provide factors to correct point measurements of metabolic parameters at 5 and 10 cm intervals to a vertical height of 0 to 10 cm and 10 to 20 cm, respectively. Factors were extrapolated for all sampling intervals to proportionally correct point measurements to an aerial littoral zone basis. Approximately 23 to 27 times more surface area exists on submerged macrophytic species per m^2 of the littoral zone than on the emergent Scirpus acutus. Considering the morphological similarities in submerged and emergent freshwater vegetation, these conversions are likely to be suitable to other shallow aquatic habitats where these taxa occur, provided distributional patterns are shown to be quantitatively and qualitatively similar and the length of the growing season is congruous.

B. Epiphytic algal and bacterial community metabolism

1. Productivity of epiphytic algal primary producers

The current literature on annual cycles of primary productivity in lakes and marine environments contains no single study on epiphytic algae. As discussed in the introductory section, there have been several extensive

studies on primary productivity within the epibenthos, with considerable work having been accomplished with periphytic communities, and to some extent with epipelic communities. The importance of epiphytic carbon fixation and annual contribution to the total primary productivity of an aquatic ecosystem is frequently thought to be insignificant. Observations of large standing crops of attached algal biomass, however, suggest that macrophytes may well support quantitatively dominant primary producers in many of our shallow and more productive natural systems.

Rates of photosynthesis by algae attached to artificial substrata, representative of epiphytes of emergent and submerged vegetation, were monitored in Lawrence Lake for one year (Figures 5 and 6). Data are expressed in each of these figures on the basis of a square meter of macrophytic surface area, permitting a direct comparison of photosynthetic activity between attached communities in two spatially separated macrophytic stands. Rates of photosynthesis on substrata in a stand of Scirpus acutus located on a wind-swept calcareous bench were markedly different over the annual period at each of the three depths monitored, even though separated vertically only by 10 cm intervals. A significant feature is that rates near the surface showed considerable oscillation, with a progressively more stable and persistent response close to the sediment. Wave and mechanical activity throughout the ice-free season was likely responsible for this

Figure 5.--*In situ* primary productivity (mg C m^{-2} macrophytic surface area day^{-1}) by attached algae at 5, 15, and 25 cm above the sediments at the *Scirpus* site (Station I), Lawrence Lake, Michigan. Data were collected from artificial substrata. The area between the upper and lower lines for each depth represents quantitative ^{14}C precipitation as inorganic carbonates by attached microflora during measurements of photosynthesis.

Figure 5.

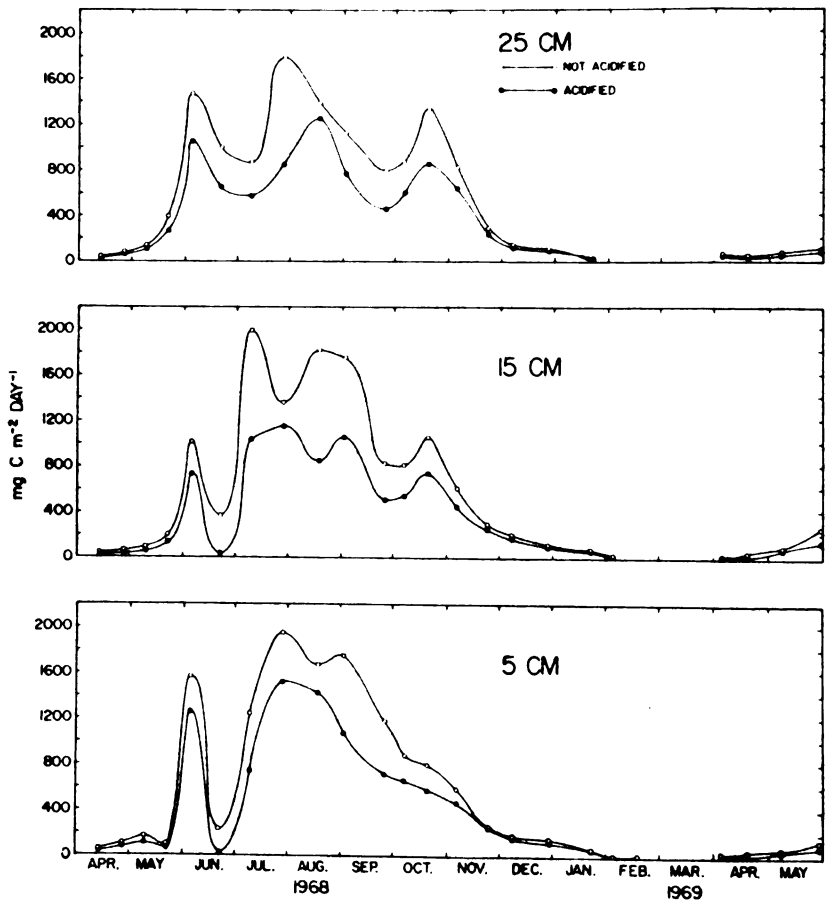

25 CM
NOT ACIDIFIED
ACIDIFIED
15 CM
5 CM
mg C m⁻² DAY⁻¹
0
400
800
1200
1600
2000
APR. MAY JUN. JUL. AUG. SEP. OCT. NOV. DEC. JAN. FEB. MAR. APR. MAY
1968
1969

Figure 6.--*In situ* primary productivity (mg C m^{-2} macrophytic surface area day^{-1}) by attached algae at 5 and 10 cm above the sediments at the *Najas*-*Chara* site (Station II), Lawrence Lake, Michigan. Data were collected from artificial substrata.

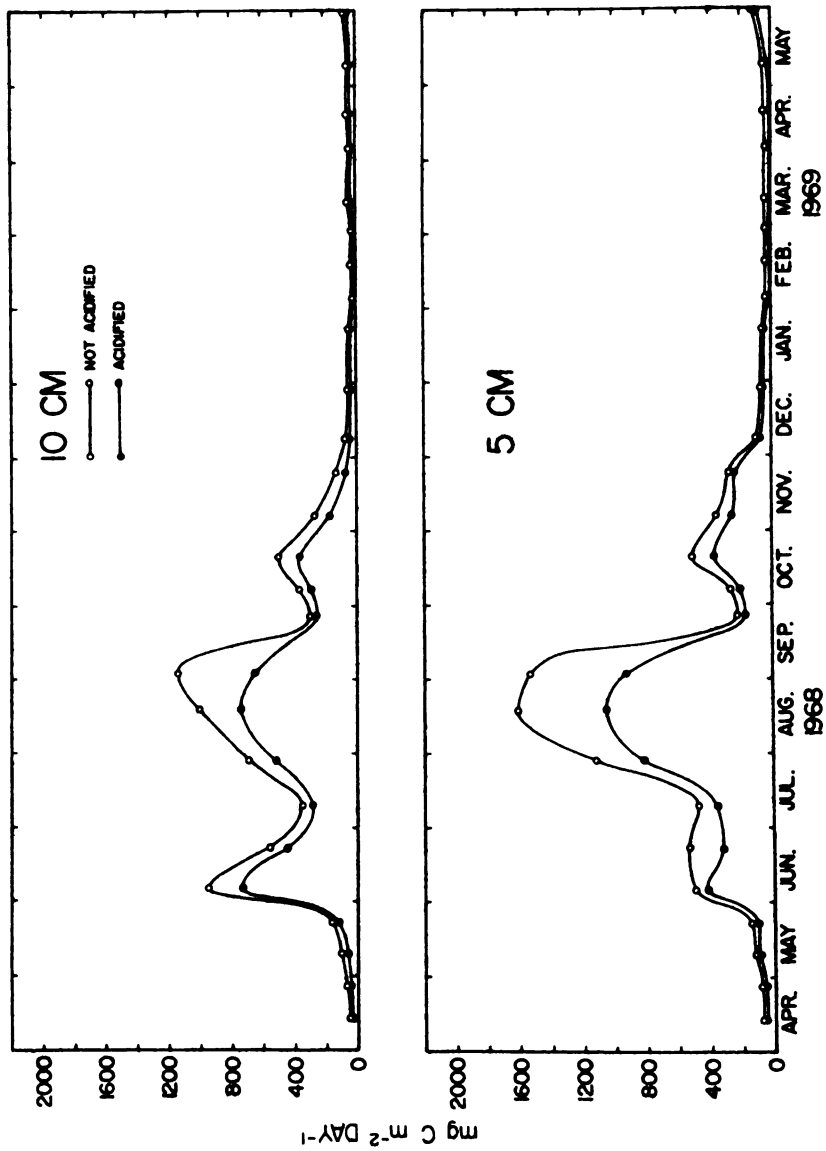

10 CM
NOT ACIDIFIED
ACIDIFIED
5 CM
mg C m-2 DAY-1
2000
1600
1200
800
400
0
APR. MAY JUN. JUL. AUG. SEP. OCT. NOV. DEC. JAN. FEB. MAR. APR. MAY
1968
1969

Figure 6.

stratified pattern. Community cohesiveness and the ability to maintain structural integrity (probably related to the matrix of deposited monocarbonates and biotic components), in a position essentially perpendicular to the macrophytic substrata, probably would be decreased by such physical perturbations. Factors contributing to metabolic fluctuations near the surface may be related to surface dilution effects of nutrients through direct precipitation and run-off, oscillatory movements of the upper portion of the plant, and diel patterns of high light intensity and thermal heating at the surface.

Rates of photosynthesis at the Scirpus site at all depths exhibited annual patterns similar to the general growth patterns of the macrophytes themselves. Photosynthesis at all depths after six weeks colonization increased steadily until the first annual maximum was reached in early June. Growth then decreased to 22 mg C m^{-2} of plant substrata day^{-1} 5 cm above the sediments and coincided with a period of intense precipitation and possible nutrient dilution effects. The annual maxima for all depths occurred during July and August and rapidly decreased to winter levels in mid-November.

A trimodal photosynthetic response was evident in the Najas-Chara site (Figure 6). Although rates from both depths were similar temporally, maximum annual primary productivity occurred near the sediments. The similarity

of annual cycles from both depths probably reflects the small 5 cm vertical distance between the colonized samples.

A comparison of annual mean productivity from equivalent surface areas (Table 3) shows epiphytes on substrata of the Scirpus site were fixing 22.8% more carbon per unit time than attached algae from the submergent site. Productivity maxima, corrected for precipitation of ^{14}C-labelled monocarbonates, at the Scirpus site ranged from 1154 to 1517 mg C assimilated m^{-2} of plant substrata day^{-1} for all depths; samples from the Najas-Chara site ranged from 742 to 1055 mg C m^{-2} day^{-1} for both depths.

Mean annual contamination by deposition of ^{14}C carbonates amounted to 38.5 to 71.7% of the actual carbon fixed for Scirpus substrata. Over the annual period 40.3 to 45.7% of the determinations of mean annual productivity were due to errors of ^{14}C deposition as inorganic carbon within the submerged site. These high percentages stress the necessity for decontamination procedures and indicate the potential error if neglected.

Rates of deposition of carbonates were commonly found to be highest during periods of maximum photosynthesis but were not directly proportional to carbon fixation rates. As much as 53% of the radioactivity was present in precipitated form during the vegetative season in both experimental sites but average proportions varied from 10 to 30%. Deposition of ^{14}C during incubation was greatest during winter conditions under ice cover in dark

TABLE 3.--A comparison of mean annual productivity rates for epiphytes on artificial substrata at the Scirpus (Station I) and Najas-Chara site (Station II), Lawrence Lake. Carbon fixation data are expressed on the basis of equivalent square meters of macrophytic surface.

Depth above Sediments	$\bar{x}$ Annual Productivity (mg C m^{-2} of substrata day^{-1})
Scirpus Site	
25 cm	330.1
15 cm	297.7
5 cm	281.9
Najas-Chara Site	
10 cm	241.3
5 cm	278.8

photosynthesis samples (where precipitation was likely due largely to bacterial activity, see Kusnezow, 1966). Loss of activity through acidification predominated in dark photosynthesis bottles and supports findings reported by Wetzel (1965c).

High standing crops of attached organisms remained on the artificial (cf. discussion on pigments) and natural substrata during winter periods. Rates of net carbon fixation, however, decreased to almost indetectable levels. During the winter season several dark bottles from photosynthesis measurements possessed more ^{14}C activity than did their complementing light bottles, prior to acidification treatment ("net" photosynthesis = 0.01 to 0.09 mg C m^2 of plant substrata day^{-1}). This finding is commonly observed in phytoplankton and has significant ecological implications. Two alternatives are available to the attached algal flora for maintenance of high standing crops under conditions seemingly adverse to photosynthesis. Either the cells are supporting themselves energetically through utilization of metabolic storage products at low respiratory rates, or they are supplementing photosynthesis with chemo-organotrophy by direct uptake of macrophytically, bacterially, or allochthonously derived organic compounds. It is ecologically feasible that both alternatives are functional to some extent under natural conditions.

Visualization of the more dynamic changes in photosynthetic activity based on rates of carbon fixation per square meter of macrophytic surface area at depth, is enhanced by presentation of data in isopleth form for the two macrophyte sites (Figures 7 and 8). Although single data measurements are not decipherable, overall patterns of annual change become accentuated and lend these data to a better interpretation of the dynamics involved.

Epiphytic productivity in the Scirpus site remained less than 50 mg C m^{-2} plant substrata day^{-1} throughout the first week in May and was followed by simultaneous increases near the surface and the sediments. Light and temperature would appear to be critical factors during initial colonization and may lead to "light" and "shade" adaptation during this period. A strong stratification occurred during the latter half of July and August (surface maximum = 1251 mg C m^{-2} plant substrata day^{-1}; near sediment maximum = 1517 mg C m^{-2} plant substrata day^{-1}). Similar pronounced shifts occurred in pigment development (cf. Figures 15 and 16) and correlate with the establishment of stratified diatom communities (Gomphonema sp. and Eunotia sp.) which persisted to some extent into winter months. By early fall a gradual decrease in photosynthesis was found and by the end of December low winter rates prevailed. At Station I natural and artificial substrata were frozen to within 4 cm of the sediments throughout

Figure 7.--Isopleths of *in situ* primary productivity (mg C m^{-2} macrophytic surface area day^{-1}) by attached algae at 5 to 25 cm above the sediments at the *Scirpus* site (Station I), Lawrence Lake, Michigan. Data were corrected for radiocontamination as ^{14}C inorganic carbonates precipitated during measurements of photosynthesis. (Shaded area = ice cover).

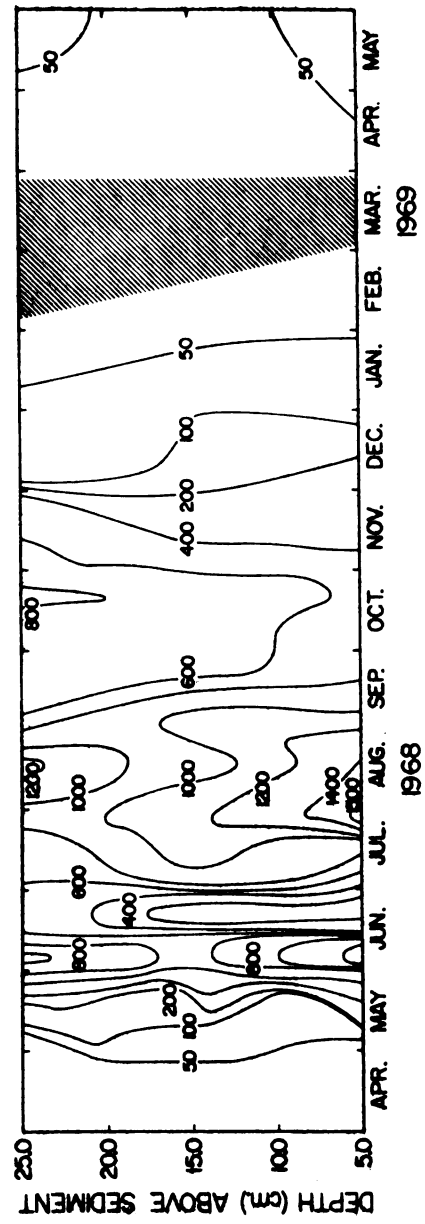

DEPTH (cm) ABOVE SEDIMENT
250
200
150
100
50
APR.
MAY
JUN.
JUL.
AUG.
SEP.
OCT.
NOV.
DEC.
JAN.
FEB.
MAR.
APR.
MAY
1968
1969
50
100
200
400
600
800
1000
1200
1400

Figure 7.

Figure 8.--Isopleths of *in situ* primary productivity (mg C m^{-2} macrophytic surface area day^{-1}) by attached algae at 5 to 10 cm above the sediments at the *Najas*-*Chara* site (Station II), Lawrence Lake, Michigan. Data were corrected for radiocontamination as ^{14}C inorganic carbonates precipitated during measurements of photosynthesis.

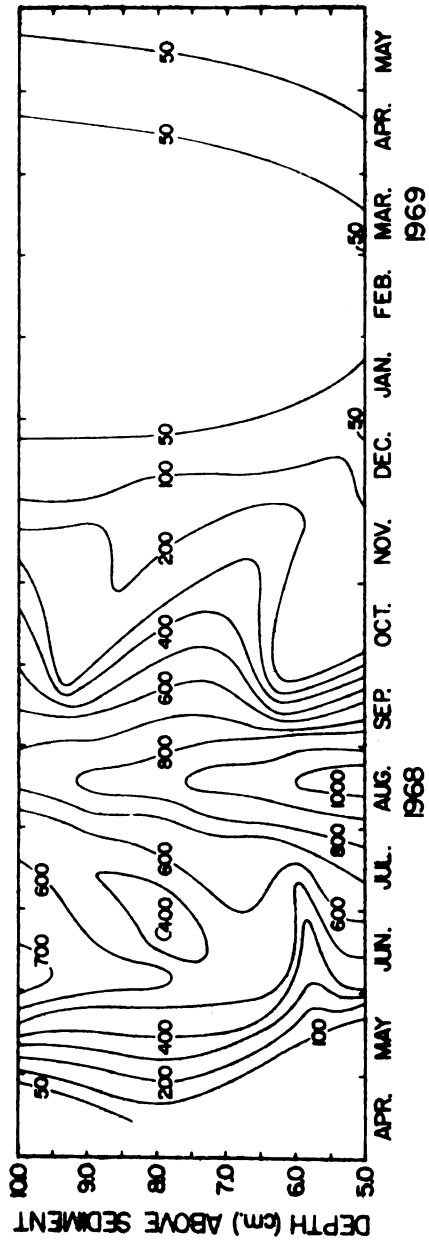

DEPTH (cm.) ABOVE SEDIMENT
10.0
9.0
8.0
7.0
6.0
5.0
APR. MAY JUN. JUL. AUG. SEP. OCT. NOV. DEC. JAN. FEB. MAR. APR. MAY
1968
1969

Figure 8.

most of the month of March. Subsequent to ice-retreat on 27 March 1969, rates increased in nearly identical patterns to those of spring, 1968.

Rates of photosynthesis of algae in the Najas-Chara (Figure 8) showed a much stronger initial intensification during the spring months through May (500 mg C m^{-2} plant substrata day^{-1}) than occurred on the Scirpus substrata. However, stratification in early summer was weak, possibly in response to changes in day length and photoperiod. Contrary to the pronounced stratification observed in mid-summer at Station I, maximum rates of slightly over 1000 mg C m^{-2} plant substrata day^{-1} were observed at 5 cm above the sediments. It is likely that a 5 cm interval was not sufficient to detect a strong vertical difference, if it existed to any significant extent, in the attached algal metabolic rates. Pigment distribution, discussed below, was strongly correlated with rate increases towards the sediment in mid-year samples (see Figures 17 and 18). A gradual loss of vertical stratification in rates of productivity occurred from September through November. By mid-December winter values of 50 mg C m^{-2} plant substrata day^{-1} or less were found in all samples. Station II had a significant ice cover (19 cm), but did not freeze to the sediments. All samples between 22 January and 13 March, 1969, with one exception, had photosynthetic rates of less than 50 mg C m^{-2} plant substrata day^{-1}, and confirm the independence of pigment concentration (e.g. biomass)

and intensities of photosynthesis. It may be deduced from data discussed thus far that monitoring productivity rates on the basis of changes in pigments for epiphytic communities may lead to erroneous conclusions (Wetzel, 1964).

In situ epiphytic primary productivity, expressed per square meter of the littoral zone for the two colonization areas (Figures 9 and 10), immediately demonstrates that the attached algal flora on emergent hydrophytic vegetation contributed only slightly to the total littoral primary production on an annual basis. Much higher epiphytic productivity per area of littoral zone is shown by the communities on submergent vegetation, as was anticipated from the ratio of surface areas per m^2 of benthic area for the two types of vegetation. Rates of ^{14}C fixation at Station I for the three depths monitored (Figure 9) reveal annual maxima of 196 to 232 mg C m^{-2} of littoral zone day^{-1} with winter minima of 0.3 to 5 mg C m^{-2} day^{-1}. For epiphytic algae on submergent simulated substrata (Figure 10) annual maxima also occur during the middle of the vegetative period (July and August) with 3.84 to 5.46 g of carbon fixed per m^2 of the littoral zone day^{-1}. Minima observed from December through April were 57 to 248 mg C m^{-2} of the littoral zone day^{-1}. It is noteworthy that winter rates for carbon fixation under ice cover by algae supported on simulated submergent macrophytic substrata are equivalent to summer maxima observed for sessile forms on simulated emergent vegetation.

Figure 9.--*In situ* primary productivity (mg C m^{-2} of littoral zone day^{-1}) by attached algae at 25 cm (A), 15 cm (B), and 5 cm (C) above the sediments at the *Scirpus acutus* site (Station I), Lawrence Lake, Michigan. Data were collected from artificial substrata. The area between the upper and lower lines for each depth represents productivity error the result of ^{14}C precipitation as inorganic carbonates by attached microflora during measurements of photosynthesis.

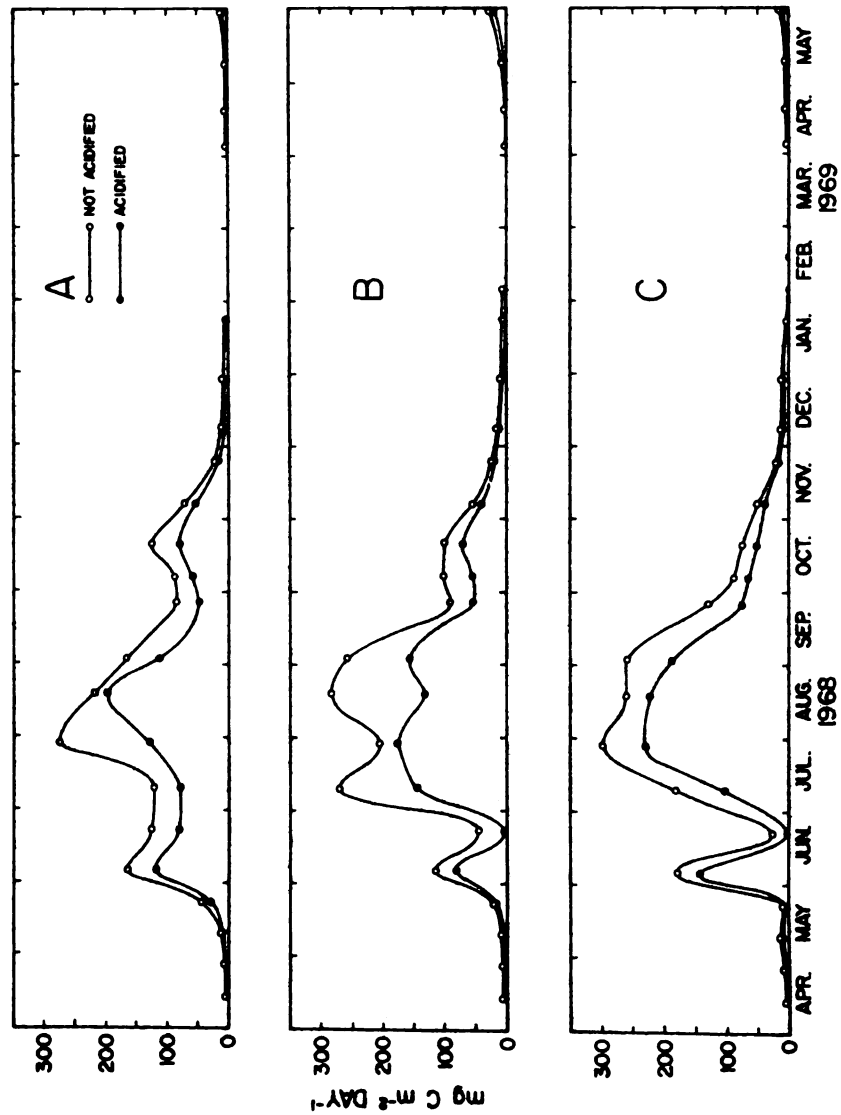
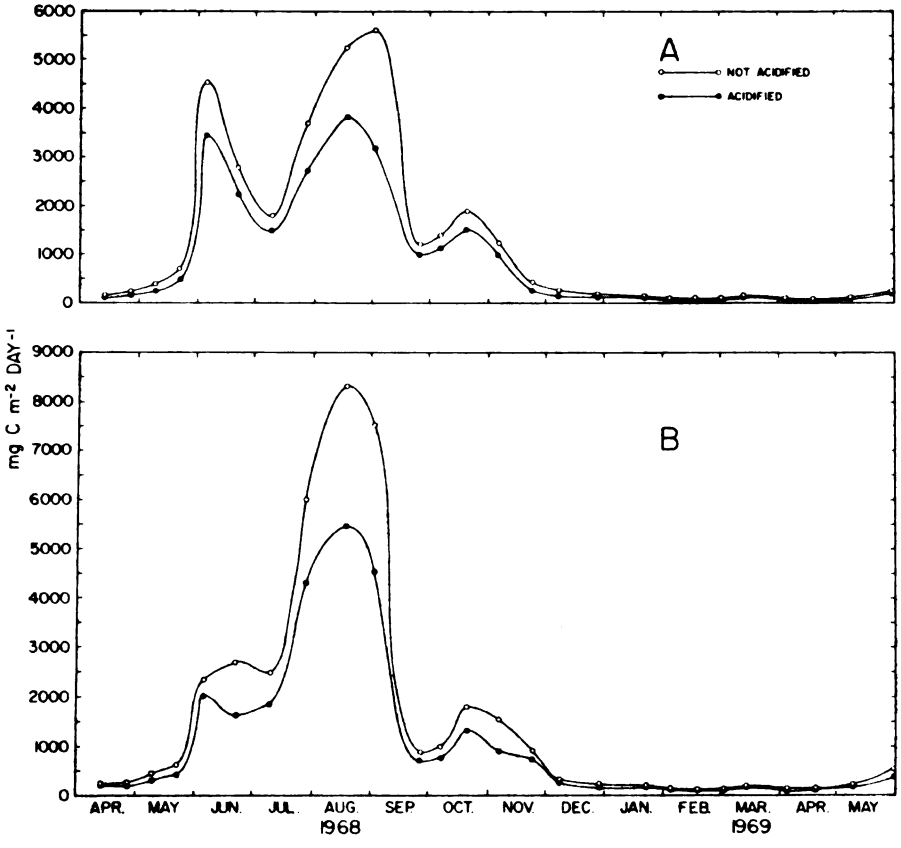

A
NOT ACIDIFIED
ACIDIFIED
B
C
mg C m⁻³ DAY⁻¹
0
100
200
300
APR.
MAY
JUN.
JUL.
AUG.
1968
SEP.
OCT.
NOV.
DEC.
JAN.
FEB.
MAR.
1969
APR.
MAY

Figure 9.

Figure 10.--*In situ* primary productivity (mg C m^{-2} of littoral zone day^{-1}) by attached algae at 10 cm (A) and 5 cm (B) above the sediments at the *Najas*-*Chara* site (Station II), Lawrence Lake, Michigan. Data were collected from artificial substrata. The area between the upper and lower lines for each depth represents productivity error the result of ^{14}C precipitation as inorganic carbonates by attached microflora during measurements of photosynthesis.

Figure 10.


A
NOT ACIDIFIED
ACIDIFIED
B
mg C m⁻² DAY⁻¹
0
1000
2000
3000
4000
5000
6000
7000
8000
9000
APR.
MAY
JUN.
JUL.
AUG.
SEP.
OCT.
NOV.
DEC.
JAN.
FEB.
MAR.
APR.
MAY
1968
1969

Isopleths of rates of photosynthetic intensity for the two types of substrata simulated are presented in Figures 11 and 12. Extrapolated macrophytic substrata surface area per unit area of the littoral zone alter only slightly the patterns of these rates (compare Figures 11 and 12 to Figures 7 and 8). Annual maxima (acidified samples) for both substrata occurred during the late fall period adjacent to the sediments. Winter rates for the Najas-Chara substrata were 100 to 200 times those on Scirpus substrata. In general, similar stratificational patterns were found at both stations. An increase occurred near the surface and sediments during spring and was followed by an annual maximum associated with the sediments and rapid erosion from September into winter conditions under ice cover. The general pattern suggests a stringent regulatory effect by environmental and physical conditions common to both areas.

Productivity rates for attached algae from both vegetative sites were integrated (Figure 13) by summation of rates per vertical increment in the littoral water column. The total productivity per square meter of the littoral zone for the Scirpus substrata include rates extrapolated from the uppermost point measurements (i.e. data from 25 cm above the sediments which were expanded to the 20 to 30 cm interval) to the surface area from 30 cm above the sediments to the air-water interface. Data

Figure 11.--Isopleths of *in situ* primary productivity (mg C m^{-2} of littoral zone day^{-1}) by attached algae from the sediment-water interface to 30 cm above the sediments at the *Scirpus acutus* site (Station I), Lawrence Lake, Michigan. Data were collected from artificial substrata and are corrected for radio-contamination as ^{14}C inorganic carbonates precipitated during measurements of photosynthesis. (Shaded area = ice cover).

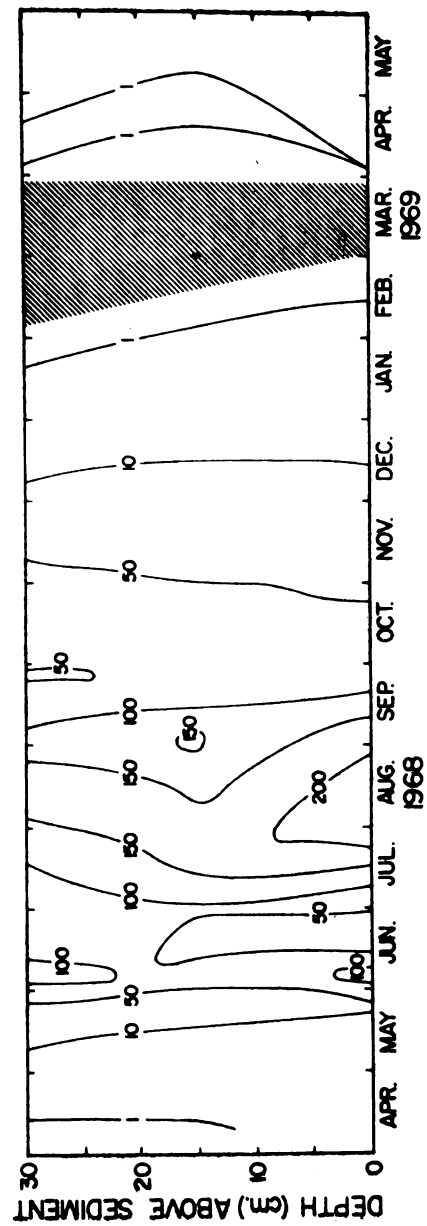

DEPTH (cm.) ABOVE SEDIMENT
30
20
10
0
APR.
MAY
JUN.
JUL.
AUG.
1968
SEP.
OCT.
NOV.
DEC.
JAN.
FEB.
MAR.
1969
APR.
MAY
10
50
100
150
200
250
300

Figure 11.

Figure 12.--Isopleths of *in situ* primary productivity (g C m^{-2} of littoral zone day^{-1}) by attached algae from the sediment-water interface to 20 cm above the sediments at the *Najas*-*Chara* site (Station II), Lawrence Lake, Michigan. Data were collected from artificial substrata and are corrected for radio-contamination as ^{14}C inorganic carbonates precipitated during measurements of photosynthesis.

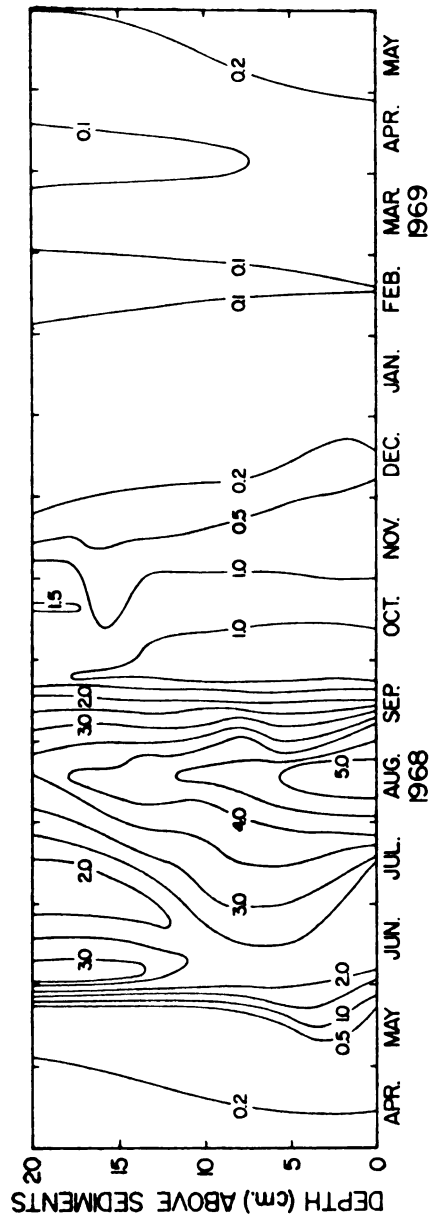

DEPTH (cm.) ABOVE SEDIMENTS
0
5
10
15
20
APR.
MAY
JUN.
JUL.
AUG.
SEP.
OCT.
NOV.
DEC.
JAN.
FEB.
MAR
APR.
MAY
1968
1969
0.2
0.1
0.1
0.1
0.2
0.5
1.0
1.5
1.0
2.0
3.0
5.0
4.0
3.0
2.0
3.0
2.0
1.0
0.5

Figure 12.

Figure 13.--*In situ* integrated primary productivity (g C m^{-2} of littoral zone day^{-1}) by attached algae at the *Scirpus acutus* site (A; Station I), and at the *Najas flexilis* and *Chara* spp. site (B; Station II), Lawrence Lake, Michigan. Data were collected from artificial substrata. The area between the upper and lower lines for each vegetative site represents productivity errors the result of ^{14}C precipitation as inorganic carbonates by attached microflora during measurements of photosynthesis.

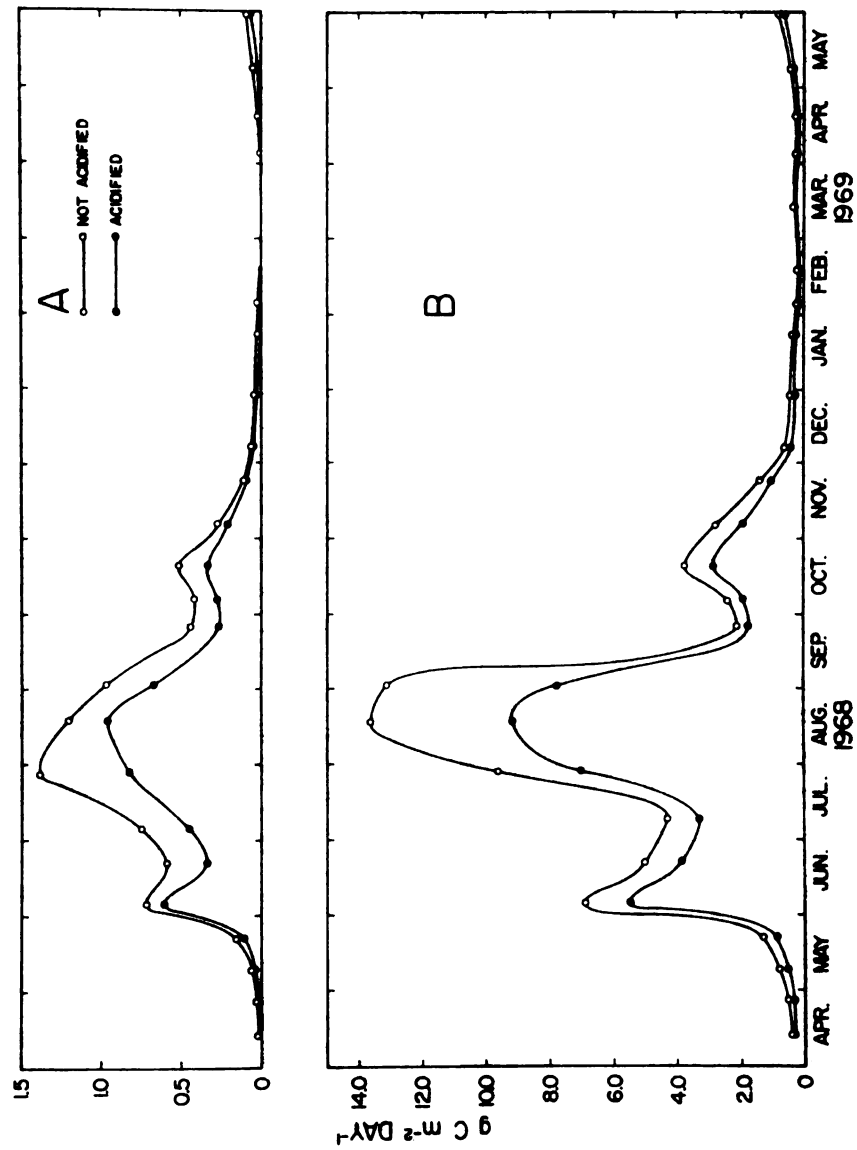

A
NOT ACIDIFIED
ACIDIFIED
1.5
1.0
0.5
0
B
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0
g C m⁻² DAY⁻¹
APR. MAY JUN. JUL. AUG. SEP. OCT. NOV. DEC. JAN. FEB. MAR. APR. MAY
1968
1969

Figure 13.

from all measurements at the Scirpus site were corrected for fluctuations in water level during the ice-free season.

In summary, annual maximum rates of productivity for the attached algal microflora for the entire water column above 1 m^2 of benthic area on the calcareous bench was 0.962 g C assimilated m^{-2} day^{-1}. A minimum rate of 0.506 mg C m^{-2} day^{-1} was observed under ice cover in February for samples. An order of magnitude difference was found for colonized algal species in the zone dominated by submergent macrophytes. A maximum of 9.299 g of carbon m^{-2} day^{-1} was fixed during August while winter values reached a low of 155 mg C m^{-2} day^{-1}. The annual maximum rates noted probably reflect near maximum rates sustained by this system in relation to available light. It is notable that variations in annual patterns at individual depths give markedly similar annual patterns. Each exhibits a sharp increase in photosynthesis by attached communities during the first week in June, followed by a decrease, and the annual observed maximum in mid-August. A third peak, the smallest of the year, occurred in both sites during the latter half of October. Subsequent rates decreased proportionately from October into December with falling water temperatures to persistent winter ranges. By early spring, 1969, intensified rates were observed and agree closely with the previous spring pulse, subsequent to initial colonization.

Mean annual primary productivity rates for epiphytic algae per m^2 of the littoral zone are summarized with respect to vertical increments monitored (Table 4). Surface area effects are readily apparent in the order of magnitude difference between the two mean annual rates for both of the vegetative sites. By summation of individual increments and extrapolation to the maximum height of each of the macrophytic stands, planimetrically determined values for the entire littoral water column above 1 square meter for the two representative sites were estimated (Table 5). Although rates of algal carbon fixation for Scirpus substrata were low, they are probably collectively significant in a system such as Lawrence Lake where suppressed pelagic photosynthetic activity is in effect (cf. earlier discussion on planktonic photosynthesis in relation to chemical-physical and nutritional interactions in a marl lake). Westlake (1963, p. 404) in a review of plant productivity, has stated that "freshwater benthic and epiphytic algae usually account for only a small part of the production of communities where they are present." Without question, an annual mean rate of productivity of 1.807 grams of carbon assimilated per m^2 per day represents one of the dominant producers within this system.

To further emphasize the importance of total epiphytic primary productivity in Lawrence Lake, and to place these data into proper perspective, comparison is made with annual production rates in various aquatic ecosystems

TABLE 4.--Mean annual primary productivity by attached algae on artificial substrata at the *Scirpus* site (Station I) and *Najas*-*Chara* site (Station II), Lawrence Lake, per m^2 of the littoral zone.

Depth above Sediments	$\bar{x}$ Annual Productivity (mg C m^{-2} of littoral zone day^{-1})
Scirpus Site	
20-30 cm	37.2
10-20 cm	37.2
0-10 cm	43.3
Najas-*Chara* Site	
10-20 cm	840.7
0-10 cm	966.5

TABLE 5. Mean annual primary productivity for attached algal organisms on artificial substrata at the *Scirpus* (Station I) and *Najas*-*Chara* site (Station II), Lawrence Lake, integrated for the entire littoral water column.

Site	$\bar{x}$ Annual Productivity (mg C m^{-2} of littoral zone day^{-1})
Scirpus acutus Muhl.	196
Najas flexilis and *Chara* spp.	1807

(Table 6). Such a comparison must be viewed as only approximate as techniques of assessment, conversion factors, and units of expression differ considerably among the investigations cited. Annual mean planktonic algal productivity of Lawrence Lake (1968) was 73.6 g C m^{-2} $year^{-1}$. It is apparent from this current study that epiphytic algae represent the dominant form of primary producer in a shallow-water ecosystem. In aquatic systems where the littoral zone is well colonized by macrophytes, particularly submerged species, attached algal production is likely to exceed that occurring in the pelagial water column. An exception might be polluted pond systems and shallow water habitats of advanced eutrophic conditions. From these comparative data it can be seen that the epiphytic algal community ranks among the highest for both fresh water and marine environments and is likely to be more productive than most epibenthic habitats, especially if the submerged vascular flora is extensively developed.

Mean annual productivity rates of attached algae per square meter of littoral zone per day were used to estimate the approximate magnitude of epiphytic carbon fixation for the entire lake, within restricted depth ranges. Planimetric analyses of vegetational maps of Scirpus acutus, Najas flexilis, and Chara spp. from the shoreline to a depth of 60 cm in Lawrence Lake were used to calculate the total surface area colonized by these

TABLE 6.--A comparison of mean annual production by algal epiphytes in Lawrence Lake, Michigan, to yearly community production values from various aquatic ecosystems.

Community Type	Production ($g\ C\ m^{-2}\ yr^{-1}$)	Method of Measurement	Reference
Lawrence Lake			
Epiphytic algae			
Emergent substrata	71	^{14}C	Present study
Submergent substrata	660		Present study
Phytoplankton	74	^{14}C	Wetzel, et al. (in prep.)
Marion Lake			
Epibenthic algae	40	Oxygen	Hargrave (1969)
Phytoplankton	8	^{14}C	Efford (1967)[1]
Macrophytes	18	^{14}C	Davis (MS 1968)[1]
Borax Lake			
Periphyton	267	^{14}C	Wetzel (1964)
Phytoplankton	91		
Macrophytes	28		
Phytoplankton (Freshwater)	80		Westlake (1963)[2]
Phytoplankton (Marine)	80		
Phytoplankton (Coastal)	120		
Phytoplankton (polluted lake)	240		
Submerged macrophytes	240		
Epibenthic algae (Salt marsh)	200	Oxygen	Pomeroy (1959)[1]
Epibenthic algae (Sandflat)	143-226	Oxygen	Pamatmat (1968)[1]
Intertidal marine sediments	115-178	^{14}C	Grøntved (1962)[1]
Marine epipsammnon	4-9	^{14}C	Steel and Baird (1968)

[1]Cited by Hargrave (1969).

[2]Data from Westlake (1963) were calculated from organic productivity values assuming 40% carbon content.

species (emergent: 1987 m^2; submergent: 2634 m^2). Multiplication of the mean annual epiphytic productivity per m^2 of the littoral water column by these aerial estimates permits the calculation of the mean annual epiphytic production per lake (littoral zone: 0 to 60 cm depth only) per day (Table 7). Although these data represent a minor portion of the total littoral zone and do not include all taxa of hydrophytic vegetation supporting epiphytic algal floras, they easily exceed the total mean annual productivity (as kg C $lake^{-1}$ day^{-1}) of macrophytes in Borax Lake, California (Wetzel, 1964, p. 30).

2. Physiological interactions through bioassay

Potential regulation of epiphytic primary productivity by availability of trace micronutrients and external metabolites has been investigated to a limited extent. Two hypotheses were considered: (1) that the high sustained rates of productivity observed are due to the movement of trace materials and micronutrients outward from the macrophyte during the vegetative season, i.e. a unidirectional flow into the attached communities, and (2) that materials released by the macrophytes during normal photosynthesis as dissolved organic compounds may functionally serve as complexing agents for required metabolites which are being supplied from the littoral water column (an example might be Fe).

TABLE 7.--Total mean annual production by attached algae on artificial substrata at the Scirpus site and Najas-Chara site, Lawrence Lake, Michigan.

Substratum Type	$\bar{x}$ Annual Production (kg C littoral zone^{-1} day^{-1})[a]	$\bar{x}$ Annual Production (kg C littoral zone^{-1} year^{-1})
Scirpus acutus Muhl.	0.389	142
Najas flexilis and Chara spp.	4.760	1737

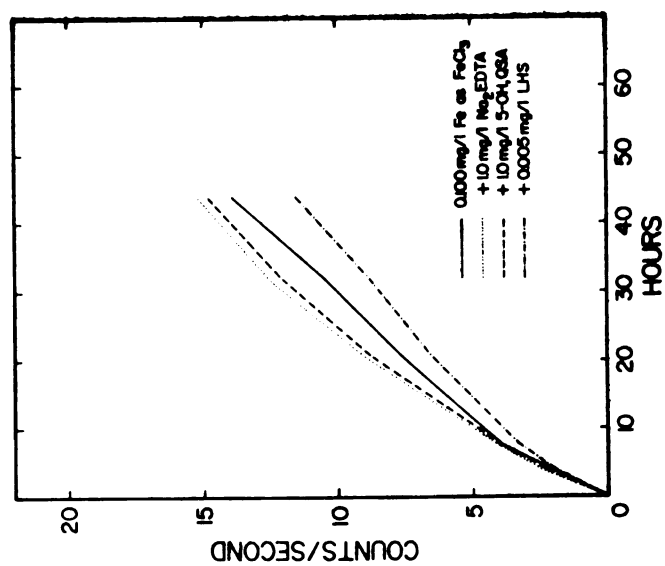
[a]From the shoreline to a depth of 60 cm where the above named emergent and submerged hydrophytic taxa occur.

Quantitatively small and ecologically relevant concentrations of added vitamins, trade metals, and inorganic phosphate failed to stimulate increased rates of carbon fixation by natural epiphytes from Scirpus acutus above controls. Presumably, concentrations of these materials from (1) bacterial metabolic activity, (2) extracellular macrophytic release, (3) entrappment in the muco-organo-carbonate complex of the macrophytic substrata, and (4) availability from the littoral free water area were sufficient that limiting effects to the community response were not evident at the time (8-10 September 1968) of analysis.

Addition of inorganic iron and organic compounds stimulated rates of photosynthesis (Figure 14). Concentrations of inorganic iron in the epilimnnetic waters of the pelagial of Lawrence Lake are frequently in the range of < 1 to 5 μg l^{-1}, and are often below the sensitivity of standard techniques (Wetzel, et al., in preparation). Increased productivity by epiphytes in response to added Fe below 10 μg l^{-1} suggests a previous limitation. Iron, added at 10 to 100 μg l^{-1}, was inhibitory.

Stimulatory effects upon provision of sodium-EDTA and quinolinesulfonic acid, an artificial chelator, at concentrations of 1 mg l^{-1}, together with 100 μg l^{-1} of $FeCl_3$ (inhibitory at this level when added independent of the chelator) has several important ecological implications: (1) macrophytically released dissolved organic matter may

Figure 14.--Effect of iron and chelating agents (ethylenediamine-tetraacetic acid, disodium salt; 8-hydroxy-5-quinoline sulfonic acid; and extracted limnohumic substances) on growth of natural epiphytic algae removed from Scirpus acutus in Lawrence Lake (Station I), Michigan, 8 to 10 September 1968.


COUNTS/SECOND
HOURS
0.000 mg/l Fe as FeCl3
+ 1.0 mg/l Na2EDTA
+ 1.0 mg/l 5-OH,QSA
+ 0.005 mg/l LHS

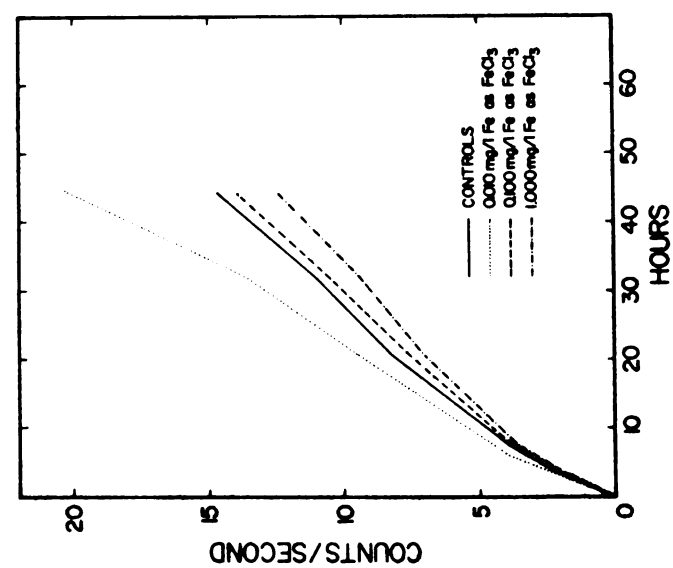

COUNTS/SECOND
HOURS
CONTROLS
0.000 mg/l Fe as FeCl3
0.100 mg/l Fe as FeCl3
1.000 mg/l Fe as FeCl3

Figure 14.

be largely labile and serve only as a carbon and energy source to the attached microflora or largely escape the sessile community, and (2) that organic compounds of allochthonous or autochthonous origin in the littoral water column do not serve as efficient and satisfactory chelators. Limnohumic substances, known to be closely associated with inorganic iron in freshwater ecosystems (see for example Shapiro, 1957), was strongly inhibitory to the epiphytic complex even at 5 μg l^{-1} levels. It should be emphasized that photosynthetic responses were evident in experimental samples within 1 to 2 hours of initiation, and were not reliant upon long term responses in which recycling of micronutrients or respiratory $^{14}CO_2$ may have occurred.

3. Pigment composition and distribution in epiphytic standing crops

Pigment composition (chlorophyll a and plant carotenoids) of the epiphytic complexes have been summarized in Figures 15, 16, 17, and 18. All estimates for chlorophyll a is isopleth figures have been corrected for the presence of pheo-degradation products and theoretically show annual changes in the active chlorophyll pigments. Further, collection of samples at the same time of day has reduced errors due to possible diel changes in pigment content.

Vertical stratification patterns are evident for both chlorophyll a and total plant carotenoids for

Figure 15.--Isopleths of corrected chlorophyll a concentrations (mg m^{-2} of littoral zone) of attached algae at the Scirpus site (Station I), Lawrence Lake, Michigan.

Figure 16.--Isopleths of plant carotenoid concentrations (milli-specified plant pigment units m^{-2} of littoral zone) of attached algae at the Scirpus site (Station I), Lawrence Lake, Michigan.

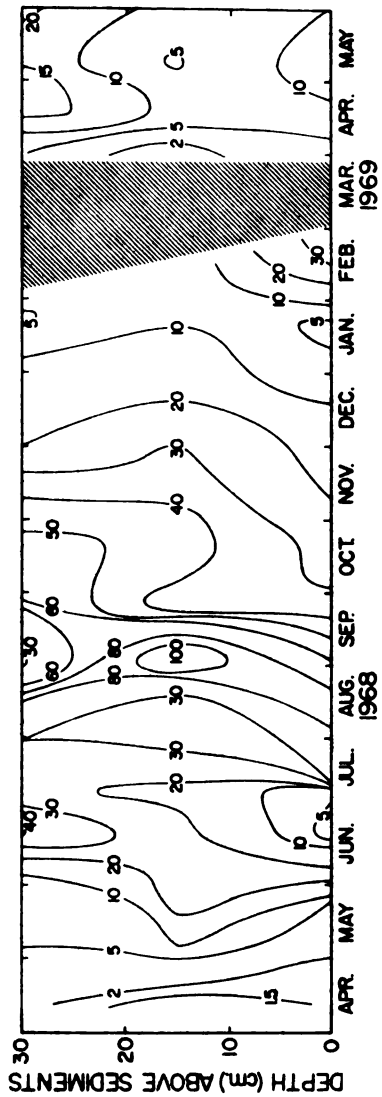


Figure 15.

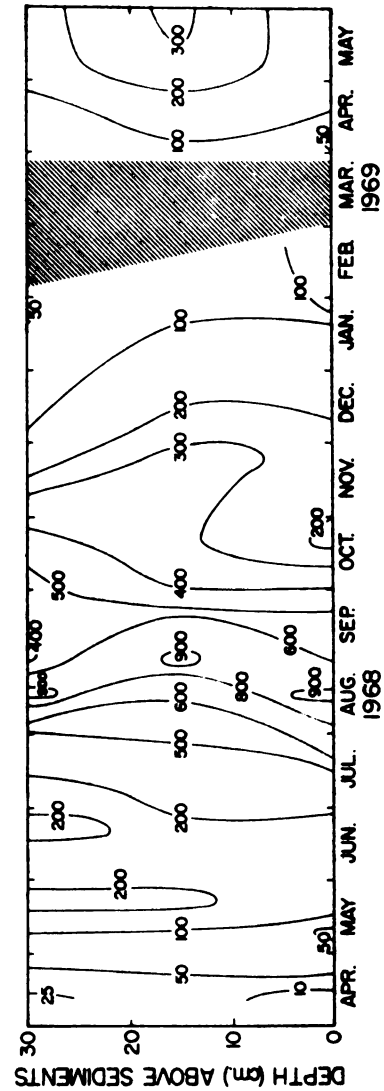


Figure 16.

Figure 17.--Isopleths of corrected chlorophyll *a* concentrations ($g\ m^{-2}$ of littoral zone) of attached algae at the *Najas*-*Chara* site (Station II), Lawrence Lake, Michigan.

Figure 18.--Isopleths of plant carotenoid concentrations (milli-specified plant pigment units $\cdot\ 10^3\ m^{-2}$ of littoral zone) of attached algae at the *Najas*-*Chara* site (Station II), Lawrence Lake, Michigan.

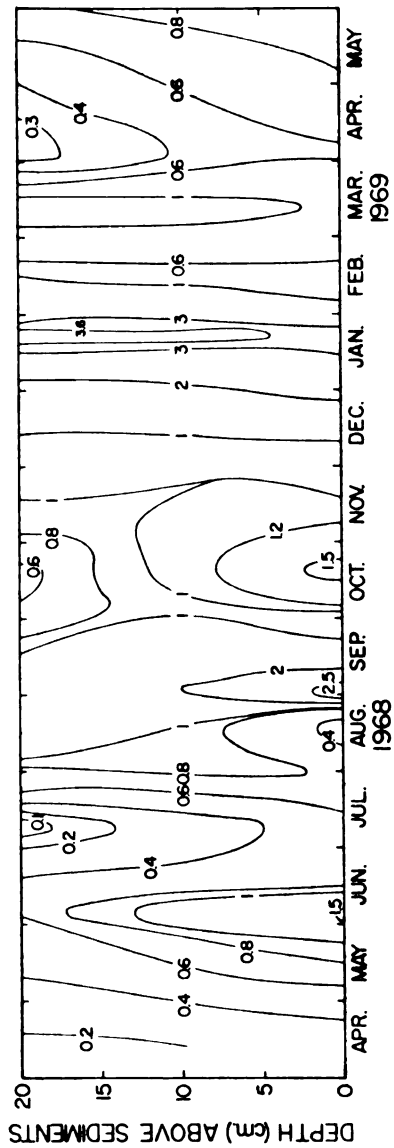


Figure 17.

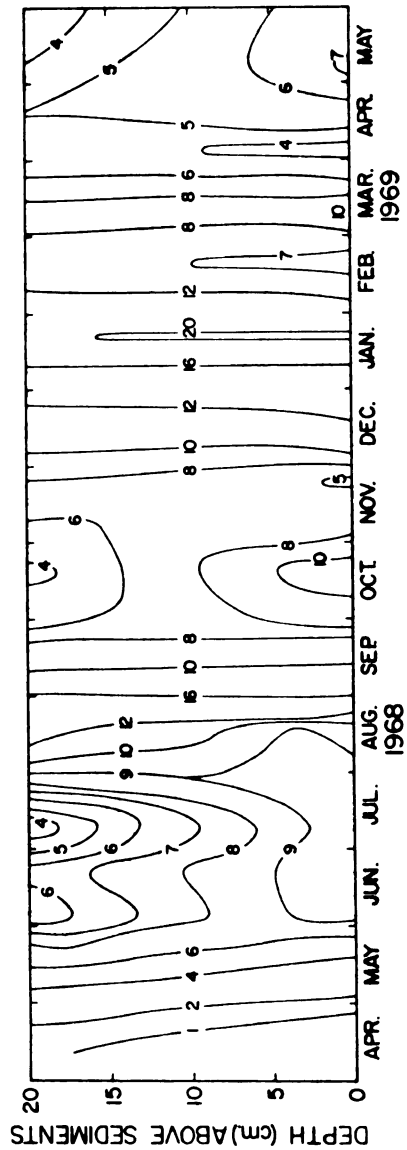


Figure 18.

epiphytes colonizing both the emergent and submerged type of simulated substrata. Initial studies with epiphytes from natural substrata showed considerable uniformity in vertical position of pigment concentration on the same macrophytic species, but significant disagreement among macrophytic species and especially between emergent and submergent forms. Standing crops of both pigments occurred in identical concentrations per unit surface area of the macrophytes in the initial studies. This was especially true for artificial substrata colonized on opposing littoral zones, following six weeks incubation. Differences in summary figures are primarily related to surface area effects and differences in colonizable macrophytic area per m^2 of the littoral zone.

Patterns of annual change in pigment concentration at both stations showed a number of spatial and temporal similarities in their development. Chlorophyll *a* increased rapidly at both Stations (Figure 15 and 17) during initial phases of colonization through the vernal months. A paucity of diatoms were present by early June (*Synedra* sp., *Eunotia* sp., and some *Tabellaria* sp.), with concomitant development of several cyanophytes. Bacterial compactions were also present during this phase of colonization. Similarities in community development at both stations up to this period were marked. A spring peak in chlorophyll *a* adjacent to the surface and the sediments on *Scirpus* substrata correlated with an increase in *Fragilaria* and

Tabellaria respectively at the upper and lower depths. Early summer developmental activity of diatoms in the submerged stands consisted of Fragilaria, Tabellaria, and Cymbella. No pronounced vertical stratification was seen on emergent simulative substrata. By mid-summer at both sites vertical stratification was lost. Community structure from July through early fall was dominated on both substrata by Gomphonema sp., primarily perpendicular to the macrophytic substrata on secreted mucilaginous stalks. A secondary community structure of epiphytes adherent to the Gomphonema developed, primarily consisting of Eunotia and small forms of Cymbella, Fragilaria, and Synedra.

Dominant forms possessing hold-fasts or growing prostrate during much of the vegetative season were: Oedogonium, Bulbochaete, Zygnema, Chaetophora, Navicula, Cyclotella, Synedra, and Chlorella. Many other taxa, including common planktonic and littoral forms, were occasionally identified, but their quantitative contributions were minor. Considerable numbers of epiphytic stalked Caulobacter and Hyphomicrobium were observed attached to the secreted stalks of diatoms. Frequency of attachment of stalked bacteria and the presence of fungi correlated strongly with algal epiphytes undergoing decomposition. Compactions of blue-green algae (Gloeotrichia) and mucilaginous communities of bacterial organisms were occasionally the dominant understory of the epiphyton. Deposition of calcium carbonate crystals and chlorotic diatoms interwoven

in a mucilaginous matrix believed to be cell wall residues of initial colonization by adsorbed bacteria, effectively cover the plant surface beneath the stratified and epiphytic climax community.

Annual maxima of both pigments at the two growth sites are temporally separated (Figures 15, 16, 17, and 18). Accumulations of both pigments from simulated substrata in the submerged zone (Station II) were very high under ice cover (greater than 3.6 g chlorophyll _a_ and 20 SPU m^{-2} of the littoral zone). On the opposite side of the lake (emergent substrata) samples were frozen over much of the January to April interval. Yet pigment concentrations were increasing just prior to freezing of all but 4 cm of the water column.

Annual maximum and minimum pigment concentrations for both sides of the lake were weakly correlated with periods of intense primary productivity. For Scirpus substrata, maximum rates of carbon fixation in the spring and fall coincide to within one week with peak pigment concentrations. No similar relationship was found from midsummer through autumnal overturn. Following ice retreat in the spring (1969), pigments rapidly developed a vertical stratification. Photosynthesis, on the other hand, remained at low pre-ice cover winter rates. For epiphytic algae on substrata in the submergent stand, very little proportionality and direct correspondence between rates of carbon fixation and standing crops of chlorophyll _a_ or

plant carotenoids was found to occur. The maxima noted during the annual study (greater than 3.6 g a m^{-2}, and greater than 20 SPU m^{-2}), coincided with the lowest observed rate of photosynthesis (0.1 g m^{-2} of littoral zone day^{-1}) for this site.

Periods of intense precipitation of ^{14}C carbonates during photosynthesis measurements by epiphytes on artificial Scirpus substrata (see Figure 9) temporally show some visual agreement with periods of maximum pigment development of chlorophyll a. Such a relationship may reflect deposition by algal rather than bacterial microorganisms. Maximum intensities of monocarbonate formation in epiphyte samples from the submergent simulated site bear no correlation with changes observed in pigment concentrations. Certainly, from a physical point of view, the structural integrity of the sessile communities would be enhanced by the deposited crystalline understory which resists decomposition and allows increased standing crops.

Patterns of integrated concentrations of chlorophyll a and plant carotenoids for the entire littoral water column (Figures 19 and 20) over an annual basis accentuate the differences between the two types of substrata. Maximum annual concentrations of chlorophyll a for algal epiphytes at Station I (Scirpus substrata) were 0.195 and 0.383 g m^{-2} at the spring and early fall peaks. Annual maxima for submergent substrata (Station II) were 4.24

Figure 19.--Integrated chlorophyll a concentrations (g m^{-2} of littoral zone) of attached algae at (A) the Scirpus acutus site (Station I), and (B) the Najas flexilis and Chara spp. site (Station II), Lawrence Lake, Michigan. Data were collected from artificial substrata and are corrected for the presence of pheophytin degradation products.

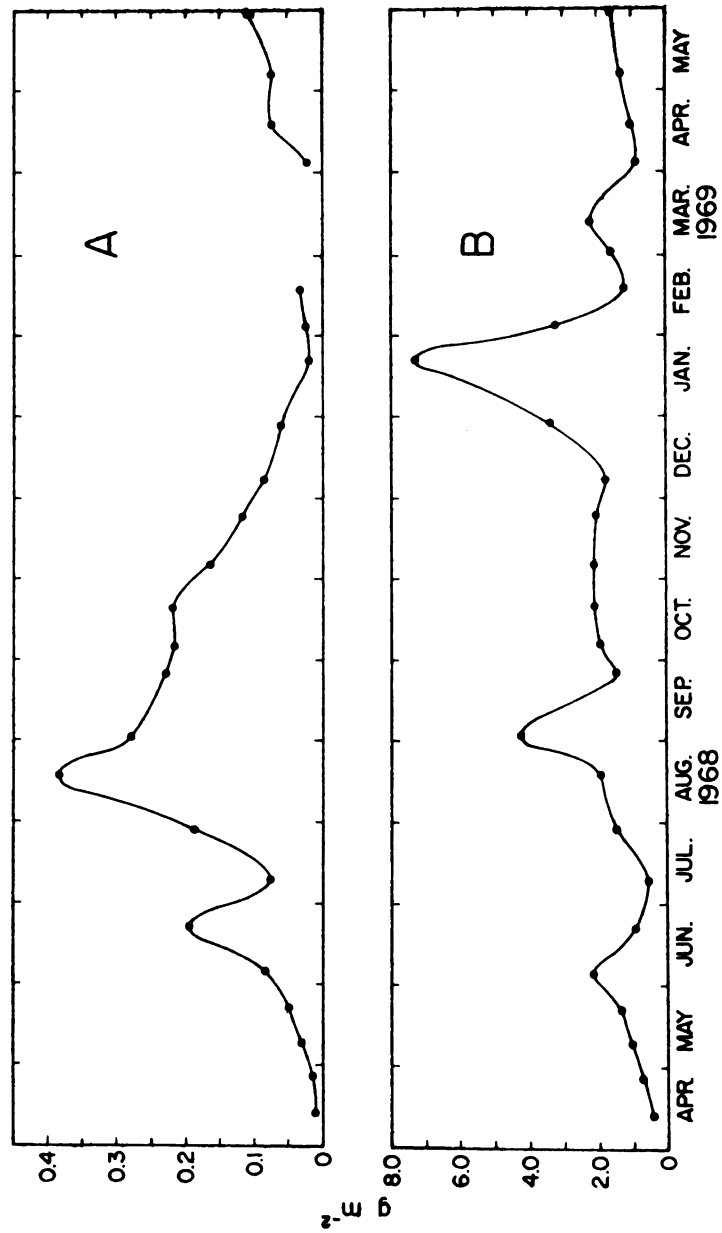

A
B
0.4
0.3
0.2
0.1
0
8.0
6.0
4.0
2.0
0
APR MAY JUN. JUL. AUG. SEP. OCT. NOV. DEC. JAN. FEB. MAR. APR. MAY
1968
1969

Figure 19.

Figure 20.--Integrated plant carotenoid concentrations (milli-specified plant pigment units · 10^3 m^{-2} of littoral zone) of attached algae at (A) the Scirpus acutus site (Station I), and (B) the Najas flexilis and Chara spp. site (Station II), Lawrence Lake, Michigan. Data were collected from artificial substrata.

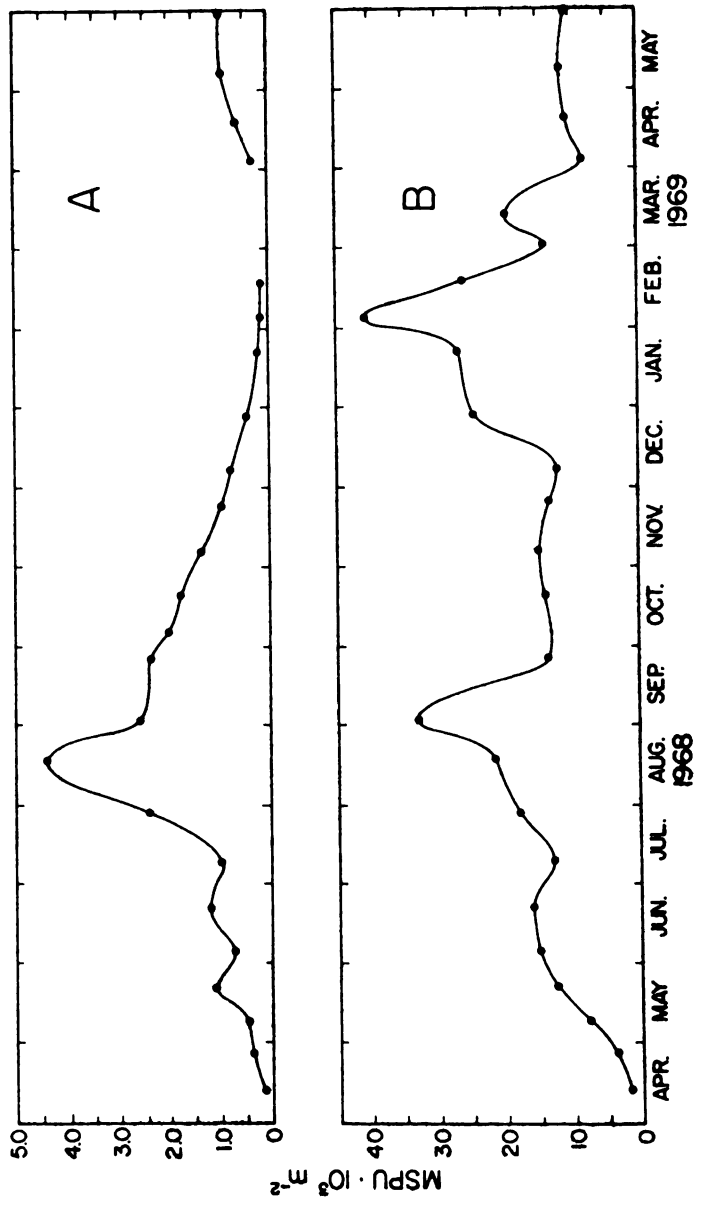

A
B
MSPU · 10³ m⁻²
5.0
4.0
3.0
2.0
1.0
0
40
30
20
10
0
APR. MAY JUN. JUL. AUG. SEP. OCT. NOV DEC. JAN. FEB. MAR. APR. MAY
1968
1969

Figure 20.

and 7.31 g m^{-2} in early September and mid-January, respectively.

The range of chlorophyll a for epiphytes on simulated emergent vegetation (9 to 383 mg m^{-2}) compares favorably with data reviewed by Moss (1968). The annual range of chlorophyll a on submergent simulated substrata (Station II) of 405 to 7312 mg m^{-2} is considerably higher than other epiphytic standing crops (Moss, 1968; Odum, 1957; Odum, et al., 1958), and quantitatively greater per unit area than other data summarized for benthic algal (Moss, 1968) and partitioned freshwater and marine communities (Odum, et al., 1958).

Plant carotenoids on an integrated basis (Figure 20) showed an order of magnitude difference between forms colonizing typified submergent and emergent substrata. Several maxima were found at Station II, similar to those of chlorophyll a, and reached 40.7 SPU m^{-2} during January under ice cover. A second maximum of 33.27 SPU m^{-2} occurred on 2 September. A single maximum at Station I of 4.48 SPU m^{-2} was observed in early August.

In spring of 1969, concentrations of plant carotenoids and chlorophyll a were 100% higher per unit area than values observed in spring of 1968. Colonization was probably incomplete at the first and second sampling periods of 1968.

Annual changes in the ratio of pheopigments (pheophytin + pheophorbide) to chlorophyll a have not been

quantitatively evaluated. From the absorption shifts during spectrophotometric analyses of epiphytic extracts prior to and after acidification, an indication of temporal change in this ratio was determined. Quantities of pheopigments increased slightly during spring colonization on both types of substrata. By early summer approximately 30% of total a pigments was present in degraded form. During fall and early winter oscillations in the percentage were evident, the magnitude and periodicity of which have not been determined. During winter months under ice cover a gradual increase in pigment degradation products was found.

4. Chemo-organotrophy of dissolved organic compounds

Our current understanding of the dynamics of primary producer-decomposer interrelationships in natural freshwater and marine environments is limited. Much of the reason for this is that approaches to in situ microbial metabolism have usually been relegated to static measurements of total viable plate count, membrane filter count, and more recently to fluorescent dye studies of viable cell numbers. Many of the more classical and accepted procedures of studying bacterial activity have relied on various culture techniques and laboratory growth studies. All too frequently, results of controlled environmental studies where organisms are carried through numerous

generations on solidified and liquid media, have little applicability or interpretative value to in situ observations.

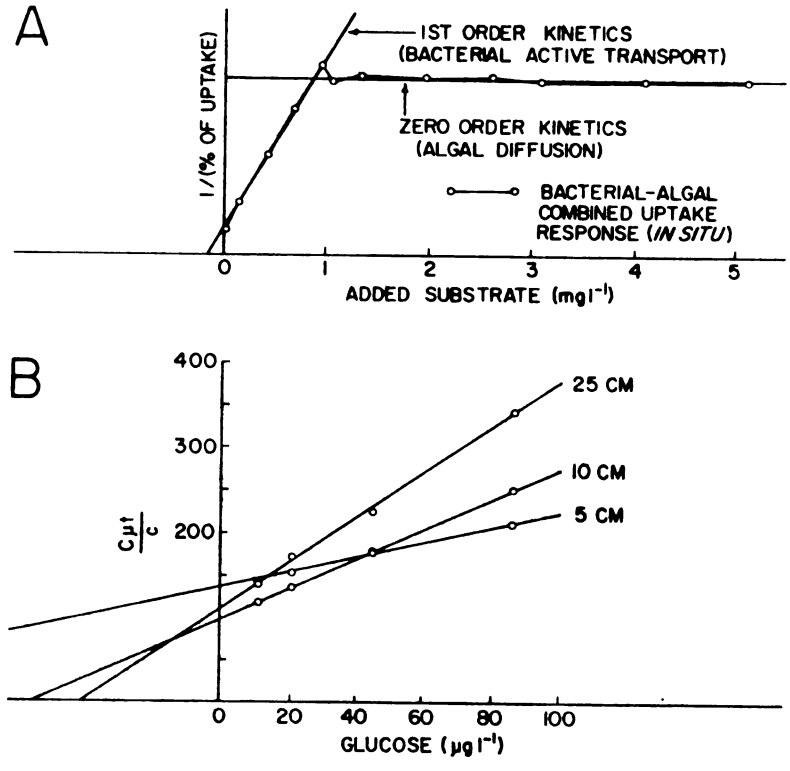
With the advent of labelled organic compounds and advancement in tracer technology, in situ assessment of microbial activity has expanded considerably. Procedures have been developed to measure rates of microbial utilization of organic compounds under natural conditions (Wright and Hobbie, 1965a, 1965b, 1966; Hobbie and Wright, 1965a, 1965b; and others). These procedures are especially pertinent in that they allow measurements to be made on heterogeneous populations. The separation of algal from bacterial utilization of naturally occurring organic substrates can be made through evaluation of enzyme kinetic responses. Planktonic bacterial utilization of organic compounds at low concentrations has been used as an estimate of chemo-organotrophic activity in both freshwater and marine ecosystems to evaluate mechanisms of utilization in relation to annual cycles of dissolved organic matter (for example Allen, 1967, 1969; Wetzel, 1967, 1968; and others). In the epiphytic complex with a large surface area available for bacterial and algal colonization, a significant portion of the soluble organic carbon "pool" in the littoral zones of shallow water ecosystems may be utilized by the attached microflora. High rates of epiphytic removal of the more labile organic compounds, whether of allochthonous or autochthonous origin, if

suitable as carbon or energy sources, may be responsible for the majority of carbon "turnover" in such systems. These mechanisms may indirectly exert sufficient nutritional interactions and restrictions via respired CO_2 or actual depletion of certain compounds to effectively regulate autotrophic metabolism. Within this frame of reference, the epiphytic bacterial complex may assume a position of importance in relation to the rate of eutrophication of a lake or be regulatory by creating specific interactions within individual trophic levels.

Diffusion of organic compounds into algae has been shown to occur under <u>in situ</u> conditions when external concentration gradients reach upwards of 0.3 to 0.6 mg l^{-1} (see Wright and Hobbie, 1965a, 1966; Allen, 1969). At low, near natural concentrations (seldom exceeding 300 µg l^{-1}) bacterial uptake kinetics predominate. From the magnitude of their velocities of uptake, bacteria are effective in removal of potential substrates before accumulated concentrations would permit algal chemo-organotrophy to occur. A graphical presentation (Figure 21, Part A) of uptake response kinetics with increasing concentrations of the added organic compound easily distinguishes between zero-order diffusion (algal) and first order kinetics (bacterial), the latter representing active transport. There were indications during initial studies of resolution of several first- and zero-order responses, when concentrations of glucose from 0 to 5 mg l^{-1} were added. These

Figure 21.--Kinetics of chemo-organotrophic utilization of dissolved organic compounds by freshwater microflora, illustrating (A) differentiation of bacterial and algal uptake in response to increasing substrate concentrations, and (B) graphical representation of uptake kinetics of dilute concentrations of glucose-^{14}C by attached bacteria from 5, 15, and 25 cm above the sediments in Scirpus acutus site (Station I), Lawrence Lake, Michigan, 28 July 1968 (Cμt/c = 1/% of uptake; see text).

Figure 21.


A
1/(% OF UPTAKE)
1ST ORDER KINETICS
(BACTERIAL ACTIVE TRANSPORT)
ZERO ORDER KINETICS
(ALGAL DIFFUSION)
BACTERIAL-ALGAL
COMBINED UPTAKE
RESPONSE (IN SITU)
0
1
2
3
4
5
ADDED SUBSTRATE (mgl⁻¹)
B
400
300
200
Cμt/c
25 CM
10 CM
5 CM
0
20
40
60
80
100
GLUCOSE (μgl⁻¹)

responses likely reflect epiphytic bacterial and algal enzyme and diffusion systems that are highly specific for only certain concentration ranges of the solute. On addition of near natural concentrations of glucose and acetate, epiphytic microbial populations nearly always responded with first order kinetics. A typical kinetic response to the addition of glucose-^{14}C to suspended epiphytic communities from substrata in the Scirpus acutus site on 28 July 1968 is graphically represented in Figure 21, Part B. Velocity of organic uptake in routine measurements usually increased with depth and lowest velocities were observed near the surface or upper portion of the macrophytes. Although duplicate measurements were seldom made after the initial studies, some indication of measurement variance and reliability was obtained by calculation of standard deviations and correlation coefficients for each of the linear responses to the addition of labelled organic isotopes (standard deviation range = 1 to 10%; r = 0.950 or greater for over 90% of the field measurements).

Loss of respiratory $^{14}CO_2$ during incubation with organic isotopes was estimated by precipitation as barium carbonate and liquid scintillation procedures. In late fall and early spring respiratory losses were equivalent to 25 to 40% of the carbon remaining intracellularly (cf. discussion by Hobbie and Crawford, 1969). Annual cycle data were not corrected for such losses.

Experiments were designed initially to assess ranges of rates of utilization of glucose and acetate (0 to 100 µg l^{-1}) from several equal surface areas removed from natural macrophytic substrata. Summarized results (Table 8) indicate minor differences in rates between epiphytes on various types of aquatic vegetation. The small differences in rates may reflect variations in actual bacterial biomass or species within the adherent complexes. It is noteworthy that (1) rates of organic utilization for the same substrate are quantitatively very close to one another, and (2) that acetate is utilized much more rapidly than glucose (the ratio of acetate to glucose uptake = 1.5 to 3.2: 1). The increased uptake of acetate over glucose has been observed for planktonic bacterial populations in a near polluted pond, also by ^{14}C methods (Allen, 1969). In other freshwater studies on utilization of dissolved organic compounds, their in situ concentrations, and turnover times (Hobbie, 1967; Wetzel, 1967), this preference is not markedly shown.

Assessment of rates of chemo-organotrophy of glucose was made on triplicate surface areas (each 0.785 cm^2) from the same plant at depth (Scirpus acutus; 15 cm above the sediments; 14 September 1967; V_{max} range: 11.7 to 19 µg l^{-1} hr^{-1} dm^{-2}) and from six plants spatially separated within the same macrophytic stand (Scirpus acutus; V_{max} range: 8 to 21 µg l^{-1} hr^{-1} dm^{-2}). Statistical differences could not be demonstrated between uptake responses on

TABLE 8.--Rates of chemo-organotrophic utilization of glucose and acetate by heterogeneous epiphytic communities on several natural macrophytic substrata, Lawrence Lake, Michigan, 21-23 September 1967.

Plant	Velocity of Utilization ($\mu g\ l^{-1}\ hr^{-1}$ per dm^2)[a]	
	Glucose	Acetate
Scirpus acutus Muhl.		
10 cm[b]	11.3	37.9
20 cm	12.9	25.6
Chara sp.		
5 cm	9.0	17.6
15 cm	8.7	16.4
Nuphar sp.		
25 cm	13.4	21.2
60 cm	17.8	30.9
Najas flexilis L.		
5 cm	7.3	14.5
15 cm	6.2	10.5

[a]Uptake rates expressed as velocity of removal of organic compounds by epiphytic bacteria from 1 dm^2 of macrophytic surface area at depth indicated. Standard deviations of response lines were within the range of 1 to 10%.

[b]Depth above the sediments for stem, petiole, and internodal substrata collection.

artificial substrata and on the natural substrata subsequent to six weeks in situ colonization of the Plexiglas substrata (only glucose and acetate tested; ranges and standard deviations overlapped considerably). There appeared to be a more uniform response in microbial activity with regard to spatial distribution of samples, but this may be an artifact of the small surface to which uptake velocities were extrapolated (1 dm^2). Expressing organic uptake data per dm^2 attempts to reduce the error involved as species heterogeneity and area covered probably have a very high variance. Secondly, the high rates per small area emphasize dynamics of in situ removal of organic compounds.

Chemo-organotrophy of several organic compounds was tested in addition to glucose and acetate (Table 9). Utilization kinetics of glycolate, glycine, and serine showed unusually high variance and poor linearity at low substrate levels. It may be concluded from these limited data, and from other studies (see Allen, 1969, for example), that glucose and acetate are two of the most suitable organic substrates with which to monitor annual chemo-organotrophic metabolism. Certainly testing of annual cycles with a large array of organic compounds would be necessary to offer any statement of their quantitative importance to one another or to the total soluble organic carbon pool. Were in situ concentrations of fructose, galactose, glycolate, succinate, and the amino acids very

TABLE 9.--Comparison of chemo-organotrophic utilization of dissolved organic compounds by epiphytic communities removed from the emergent hydrophyte, Scirpus acutus, Lawrence Lake, Michigan; 24 September 1968.[a]

Organic Compound	Velocity of Utilization (V_{max}; $\mu g\ l^{-1}\ hr^{-1}$ per dm^2)[b]
Glucose	12.5 ± 1.3
Fructose	1.5 ± 0.25
Galactose	2.0 ± 0.59
Acetate	31.0 ± 3.5
Glycolate	4.5 ± 3.6[c]
Succinate	3.9 ± 1.8
Glycine	9.7 ± 3.0[c]
Alanine	7.7 ± 1.6
Serine	2.0 ± 1.03[c]

[a]Natural epiphytic samples removed from 15 cm above the sediments; resuspended in 400 ml of ultrafiltered lakewater from the site prior to measurements.

[b]Concentration range for added substrates: 0-160 $\mu g\ l^{-1}$; each mean velocity is based on duplicate measurements; ± = range.

[c]Linearity of response kinetics poor.

low in comparison with those of glucose and acetate, the ecological importance of their utilization would assume much larger proportions.

Two further tests were conducted to establish the effect of experimental conditions upon the use of Michaelis-Menten enzyme kinetics for routine field studies of microbial populations. Replicate epiphytic samples from Scirpus acutus substrata, incubated in the dark in the presence of glucose-^{14}C, were subjected to (1) a slow oscillatory movement (Eberbach shaker; approximately 60 oscillations per minute), (2) a fast oscillatory movement of approximately 180 oscillations per minute, and (3) no movement. Rates of uptake under the respective conditions were 26, 29, and 18 $\mu g\ l^{-1}\ hr^{-1}\ dm^{-2}$. The increase is probably due to dispersion of aggregated bacteria and provision of more bacterial cell wall surface area for isotopic uptake. Annual cycle samples were subjected to shaking (wrist-action Burrel shaker), primarily to destratify the isotope and to provide constant dispersion of nutrients naturally occurring in the sample.

Variations in incubation periods of 1 to 4 hours showed rate of change of velocity decreased considerably during the 3rd and 4th hours, suggesting high bacterial mortality or a severe "bottle effect", although other explanations are possible. Incubation of 1 to 1.5 hours was routinely employed throughout the annual period.

On two occasions rates of organic utilization observed on substrata near the sediments (5 cm above the sediments in the Scirpus acutus site) were nearly identical for both substrates to rates from similar surface areas of epibenthic (upper 1 mm) samples. There is a possibility that microbial floras are migratory between the lower plant portions and the sediments but it is more likely that species composition within epiphytic and epibenthic habitats is similar.

Annual cycles of glucose (Figures 22 and 24) and acetate uptake (Figures 23 and 25) by epiphytic communities showed similar patterns of utilization. Highest rates of uptake of both substrates occurred in communities near the sediments. Acetate was preferentially utilized over glucose and had two annual peaks of utilization in both sites (July and September, although temporal agreement is not exact), ranging from 90 to 130 $\mu g\ l^{-1}\ hr^{-1}\ dm^{-2}$ for substrata in Station I to 40 to 70 $\mu g\ l^{-1}\ hr^{-1}\ dm^{-2}$ in Station II. Minimal rates of acetate utilization (5 to 20 $\mu g\ l^{-1}\ hr^{-1}\ dm^{-2}$) generally occurred from October through the period of ice deposition with increases near the bottom under ice cover at both sites. This latter phenomenon, pronounced near the sediments prior to freezing of substrata in mid-February at the Scirpus site, appeared in the Najas and Chara site just prior to loss of ice. Neither of these pulses coincided with increases in photosynthetic rates for the same periods. Development of both chlorophyll a,

Figure 22.--Chemo-organotrophic utilization of glucose (V_{max}; µg glucose removed l^{-1} hr^{-1}) by attached bacteria from 1 dm^2 of colonized surface area at 5, 15, and 25 cm above the sediments at the Scirpus site (Station I), Lawrence Lake, Michigan. Data were collected from artificial substrata.

Figure 22.

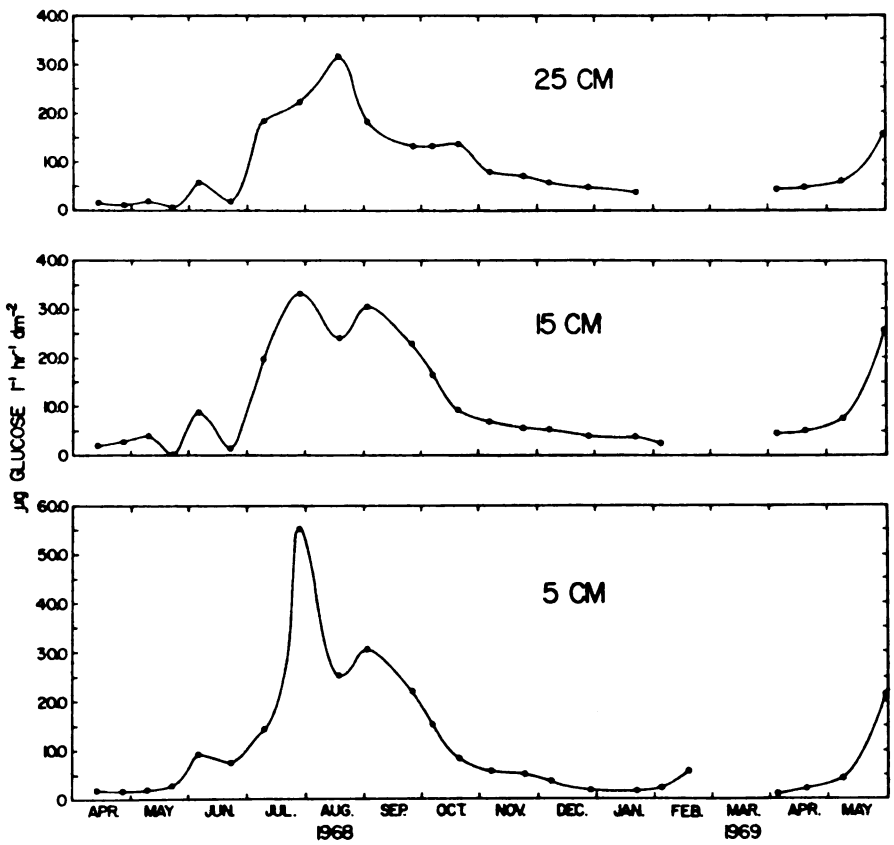
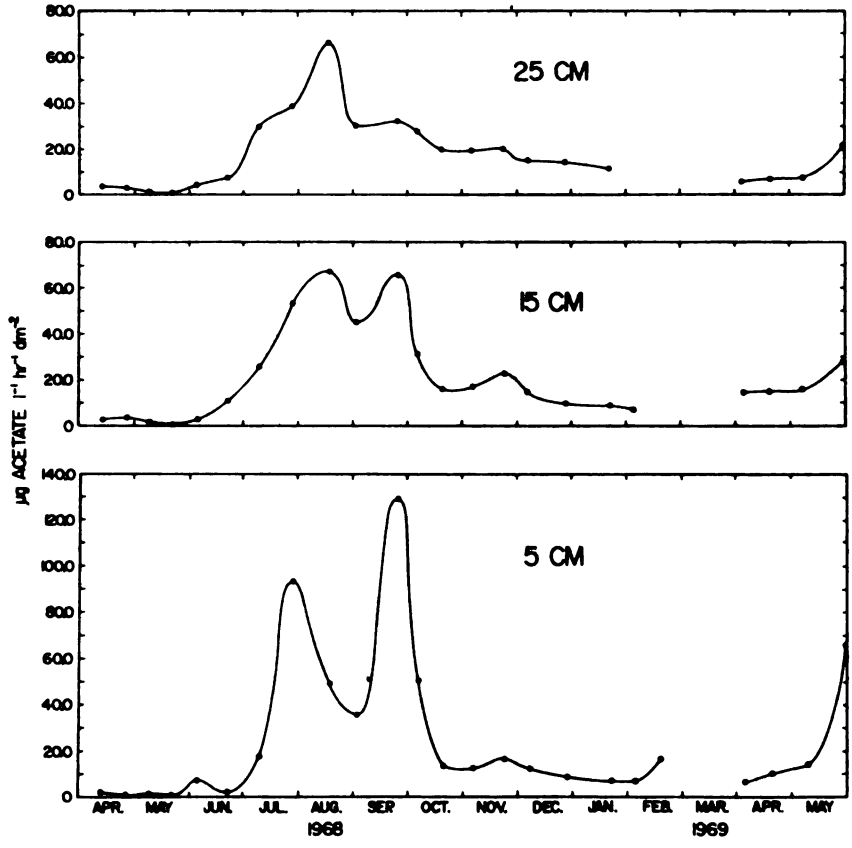

25 CM
15 CM
5 CM
µg GLUCOSE l⁻¹ hr⁻¹ dm⁻²
APR. MAY JUN. JUL. AUG. SEP. OCT. NOV. DEC. JAN. FEB. MAR. APR. MAY
1968
1969

Figure 23.--Chemo-organotrophic utilization of acetate (V_{max}; µg acetate removed l^{-1} hr^{-1}) by attached bacteria from 1 dm^2 of colonized surface area at 5, 15, and 25 cm above the sediments at the Scirpus site (Station I), Lawrence Lake, Michigan. Data were collected from artificial substrata.

Figure 23.


25 CM
15 CM
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μg ACETATE l⁻¹ hr⁻¹ dm⁻²
APR. MAY JUN. JUL. AUG. SEP OCT. NOV. DEC. JAN. FEB. MAR. APR. MAY
1968
1969

Figure 24.--Chemo-organotrophic utilization of glucose (V_{max}; µg glucose removed l^{-1} hr^{-1}) by attached bacteria from 1 dm^2 of colonized surface area at 5 and 10 cm above the sediments at the Najas-Chara site (Station II), Lawrence Lake, Michigan. Data were collected from artificial substrata.

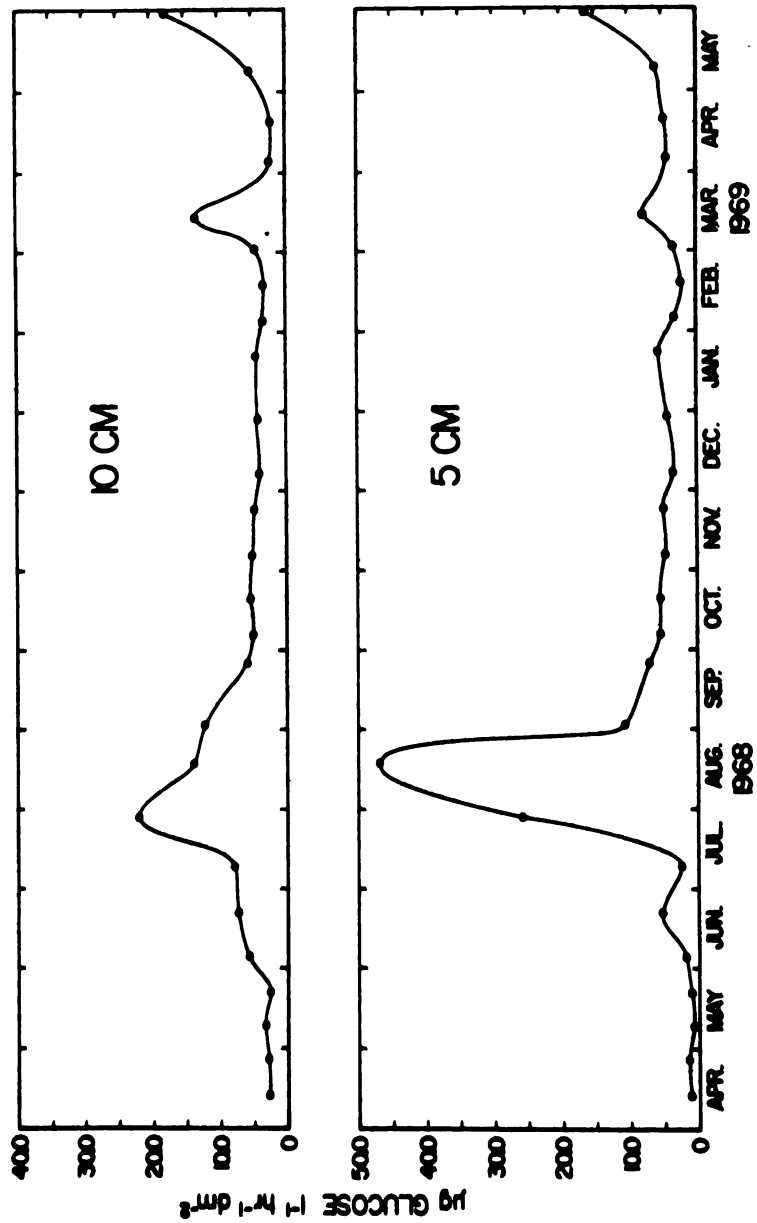
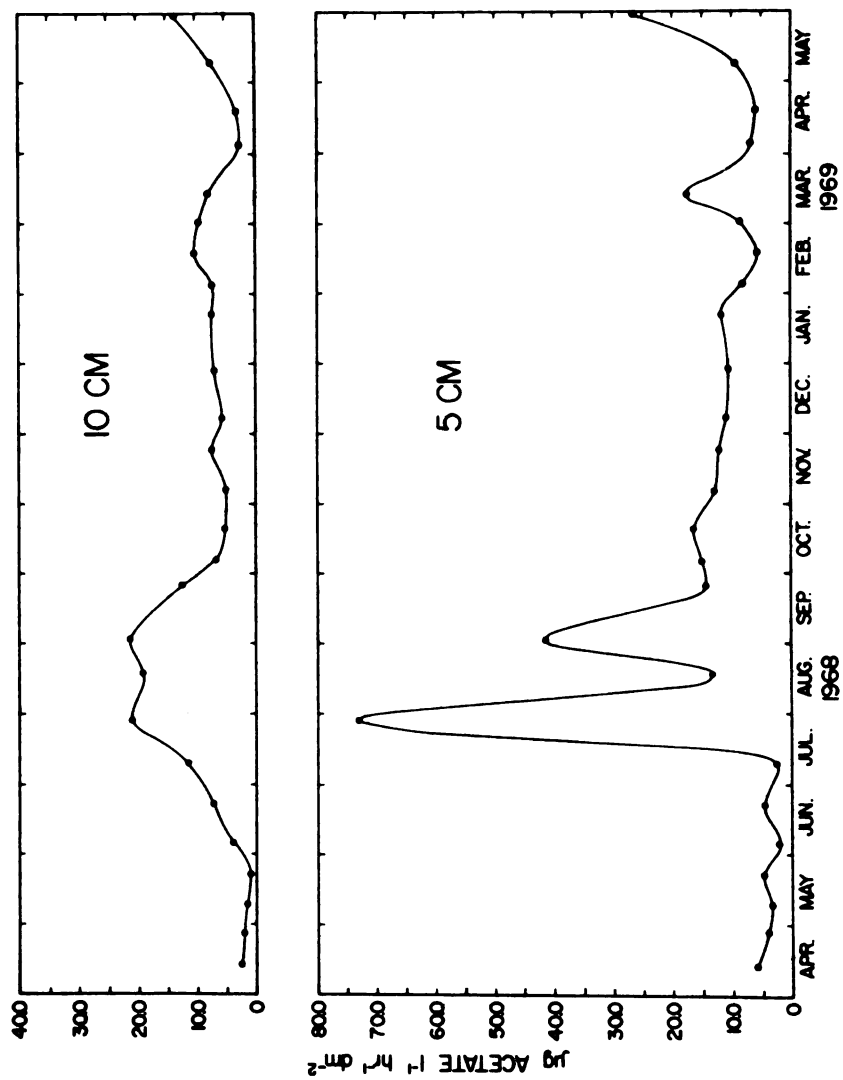

10 CM
5 CM
µg GLUCOSE l⁻¹ hr⁻¹ dm⁻²
0
100
200
300
400
500
APR. MAY JUN. JUL. AUG. SEP. OCT. NOV. DEC. JAN. FEB. MAR. APR. MAY
1968
1969

Figure 24.

Figure 25.--Chemo-organotrophic utilization of acetate (V_{max}; µg acetate removed l^{-1} hr^{-1}) by attached bacteria from 1 dm^2 of colonized surface area at 5 and 10 cm above the sediments at the Najas-Chara site (Station II), Lawrence Lake, Michigan. Data were collected from artificial substrata.


10 CM
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µg ACETATE l⁻¹ hr⁻¹ dm⁻²
APR. MAY JUN. JUL. AUG. SEP. OCT. NOV. DEC. JAN. FEB. MAR. APR. MAY
1968
1969

Figure 25.

and total plant carotenoids correlated strongly with the increase in chemo-organotrophy at the Scirpus site.

Rate changes in spatial and temporal utilization of glucose were similar to those seen for acetate. Periodicity of epiphytic utilization of glucose on the marl bench area was nearly identical to acetate near the surface but was reduced by 50%. Bimodal responses for glucose uptake were found at 5 and 15 cm above the sediments and agreed with acetate, but differed again by 50%. The uppermost substratum at the Scirpus site revealed a peak which occurred between those seen at the lower levels. A strong bimodal response was not evident for glucose uptake at the Najas-Chara site. At the submerged site an annual maximum spanning July and August was found. Rate increases observed for acetate during ice cover were similar for glucose. Glucose annual maxima (greater than 30 to 55 $\mu g\ l^{-1}\ hr^{-1}\ dm^{-2}$ for Station I; greater than 20 to 45 $\mu g\ l^{-1}\ hr^{-1}\ dm^{-2}$ for Station II) were considerably lower than those of acetate; minima values were in the range of 5 to 10 $\mu g\ l^{-1}\ hr^{-1}\ dm^{-2}$ for glucose uptake.

Several summarizing remarks with respect to chemo-organotrophic utilization per unit of macrophytic surface area are pertinent here. Annual patterns showed gradual and continuous change without rapid oscillation (several short-term studies during initial investigations confirm this), contrary to changes seen in annual pelagic patterns of eutrophic waters (see Allen, 1969, for example). Peak

activities of utilization of the organic compounds seldom demonstrated strong agreement, spatially or temporally, although increases towards the sediment were commonly present. This relationship suggests separate sources of *in situ* organic compounds, physiological differences in bacterial species, or, indeed, a community composition distinctly different at each of the sampled strata. Rates of uptake for both substrates during the vegetative season showed extremely close temporal correspondence with rates of primary productivity and may indicate interdependencies in nutritional metabolism via algal excretion, bacterial respiration, etc. Rates per unit area of substratum were higher on substrata colonized on the calcareous bench than in submerged vegetation. Annual rates determined in this study are among the highest presently known, and reflect high rates of metabolism in this portion of the epiphytic system.

Isopleths of epiphytic utilization of glucose and acetate per square meter of the littoral zone for the *Scirpus acutus* site (Figure 26 and 27) and the *Najas-Chara* site show dynamic changes over the annual period (Figures 28 and 29). The differences in available macrophytic surface area per m^2 of the littoral zone now obscures differences seen previously on a unit growth substrata basis. Potential removal of organic carbon exceeds 2000 to 3000 $\mu g\ l^{-1}\ hr^{-1}\ dm^{-2}$ by attached microflora colonizing

Figure 26.--Isopleths of chemo-organotrophic utilization of glucose (V_{max}; µg glucose removed l^{-1} hr^{-1}) by attached bacteria from 1 dm^2 of littoral zone at the Scirpus site (Station I), Lawrence Lake, Michigan. Data were collected from artificial substrata.

Figure 27.--Isopleths of chemo-organotrophic utilization of acetate (V_{max}; µg acetate removed l^{-1} hr^{-1}) by attached bacteria from 1 dm^2 of littoral zone at the Scirpus site (Station I), Lawrence Lake, Michigan. Data were collected from artificial substrata.

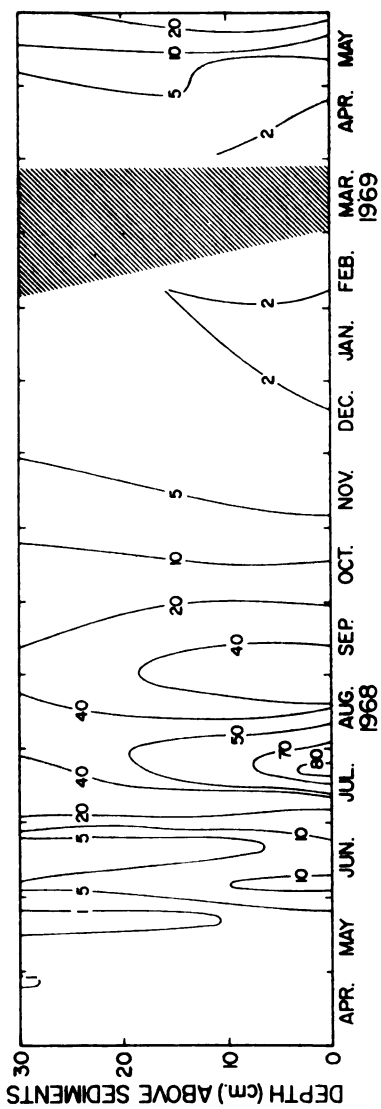


Figure 26.

Figure 27.

Figure 28.--Isopleths of chemo-organotrophic utilization of glucose (V_{max}; µg glucose removed l^{-1} hr^{-1}) by attached bacteria from 1 dm^2 of littoral zone at the Najas-Chara site (Station II), Lawrence Lake, Michigan. Data were collected from artificial substrata.

Figure 29.--Isopleths of chemo-organotrophic utilization of acetate (V_{max}; µg acetate removed l^{-1} hr^{-1}) by attached bacteria from 1 dm^2 of littoral zone at the Najas-Chara site (Station II), Lawrence Lake, Michigan. Data were collected from artificial substrata.

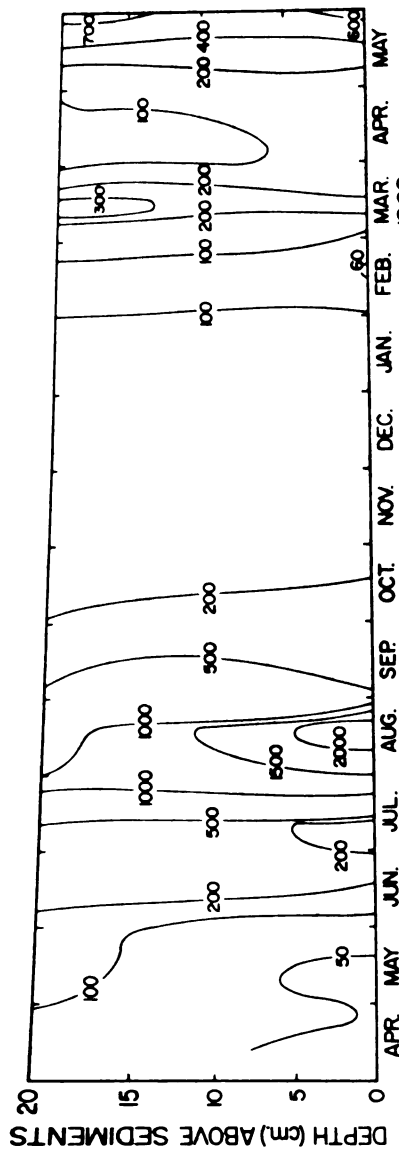


Figure 28.

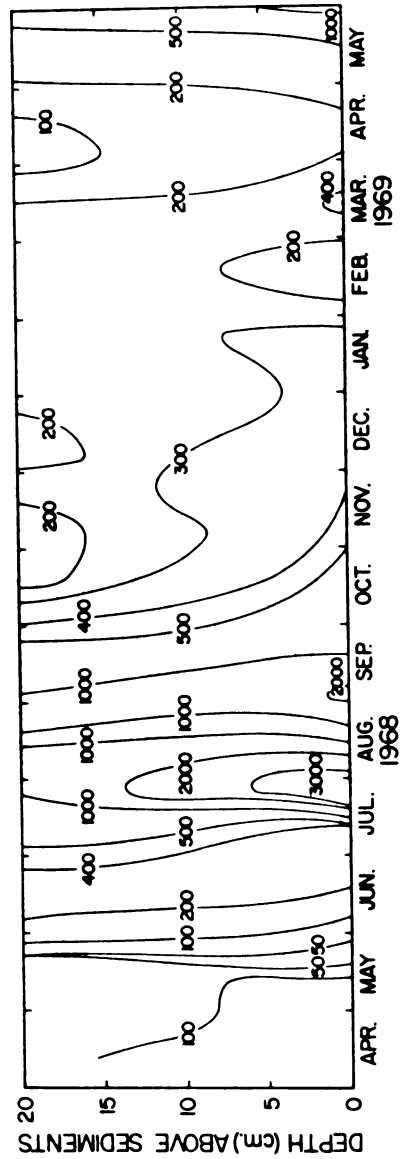


Figure 29.

1 dm^2 of the littoral zone (for both strata = 4978 µg l^{-1} hr^{-1} dm^{-2}). Assuming the water column above a littoral surface area of 1 dm^2 contains an estimated 2 to 4 liters of water, each liter containing 2 to 10 mg dissolved organic carbon, epiphytic bacteria (together with epibenthic forms) are probably responsible for removal of much of the labile and refractory organic compounds which occur there. It is impossible to assume such a large portion of the labile soluble carbon pool exists as glucose and acetate, but high substrate approximations (as $K_t + S_n$ values through graphical analyses of the enzyme kinetics; 100 to 300 µg l^{-1} of each) may be indicative of extremely rapid regenerative mechanisms followed by efficient removal. Wave and mechanical activity in the littoral area probably contributes to replacement of littoral water and an increased organic nutrient availability against "dead water spaces" at the epiphyte-macrophyte surface. During normal thermal stratification, with cycling probably limited to the epilimnetic waters, and normal stagnant water of macrophyte beds, the size of the littoral and pelagial dissolved organic matter pools may be strongly affected by epiphytic metabolism.

Little evidence for significant vertical stratification was found prior to or following the vegetative and ice-free season. Freezing of all samples for a minimum of twenty days during the winter at the Scirpus site was probably partially responsible for the low rates seen

subsequent to ice retreat, as populations may have been severely damaged with respect to enzymatic activity. It seems apparent that incomplete colonization had occurred by the conclusion of the first spring, i.e. rates in May of 1969 were already equivalent to those observed during July of 1968.

Annual glucose and acetate utilization rates were integrated for the total macrophytic surface area present for colonization per dm^2 of the littoral zone, for each of the respective sites (Figures 30 and 31). Scant, non-reticulate emergent substrata (50 to 60 Scirpus acutus plants m^{-2} of the littoral zone) for colonization on the marl bench explains the disparity in annual rates. Maximum summer rates of removal per unit of littoral zone for Scirpus site substrata did not equal winter rates for submergent site substrata. In essence, if these data are representative, the only significant epiphytic removal of dissolved organic compounds occurs in littoral zones within submerged macrophytic vegetation, and is due only to provision of considerable biomass on an expanded surface area. Integrated data accentuate the bimodal response of maximum acetate uptake and the single peaks for glucose. Secondary peaks for Station II epiphytic metabolism occur during the winter months and are probably of species-specific significance. Annual ranges for chemo-organotrophic uptake of glucose are 3.7 to 244 $\mu g\ l^{-1}\ hr^{-1}\ dm^{-2}$ for the Scirpus site and 119.8 to 3155.0 $\mu g\ l^{-1}\ hr^{-1}\ dm^{-2}$ for the submergent

Figure 30.--Integrated chemo-organotrophic utilization of glucose (V_{max}; µg glucose removed l^{-1} hr^{-1}) by attached bacteria from 1 dm^2 of littoral zone at (A) the Scirpus acutus site (Station I), and (B) the Najas flexilis and Chara spp. site (Station II), Lawrence Lake, Michigan. Data were collected from artificial substrata.

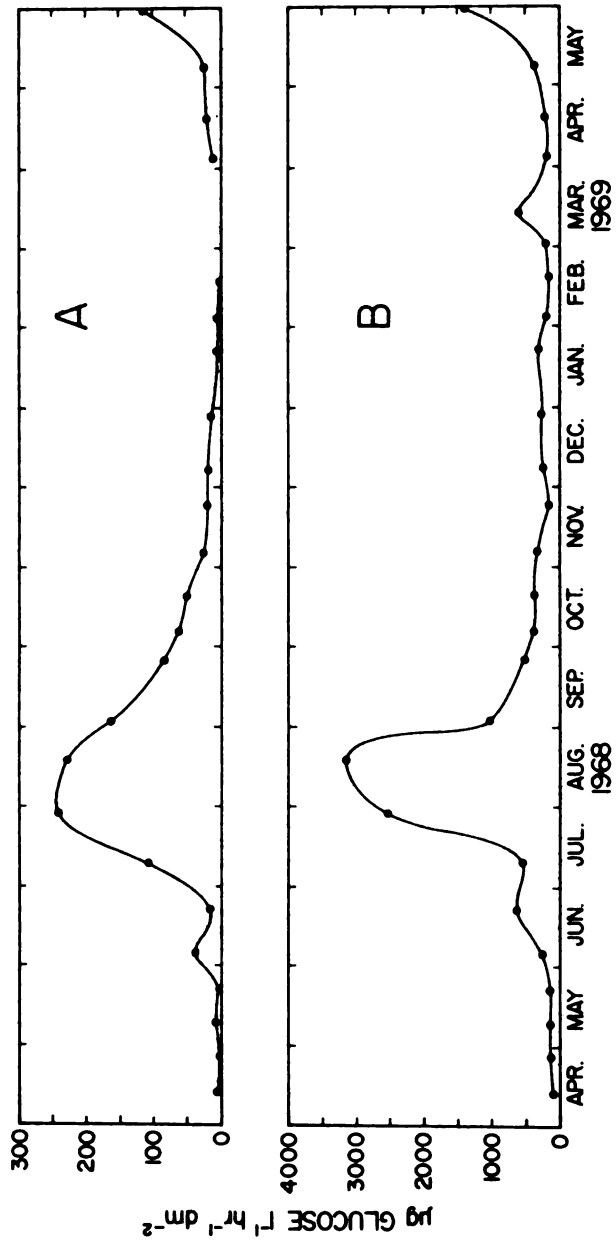
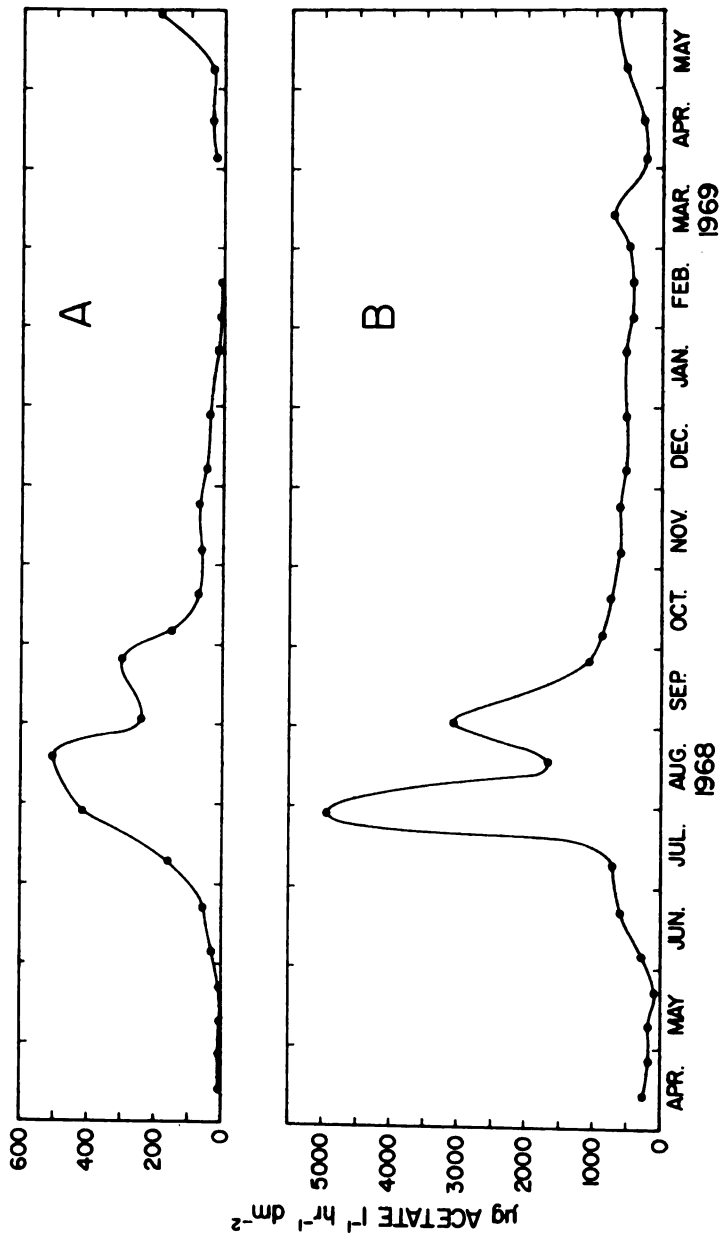

A
B
300
200
100
0
4000
3000
2000
1000
0
mg GLUCOSE l^{-1} hr^{-1} dm^{-2}
APR. MAY JUN. JUL. AUG. SEP. OCT. NOV. DEC. JAN. FEB. MAR. APR. MAY
1968
1969

Figure 30.

Figure 31.--Integrated chemo-organotrophic utilization of acetate (V_{max}; µg acetate removed l^{-1} hr^{-1}) by attached bacteria from 1 dm^2 of littoral zone at (A) the Scirpus acutus site (Station I), and (B) the Najas flexilis and Chara spp. site (Station II), Lawrence Lake, Michigan. Data were collected from artificial substrata.


A
B
600
400
200
0
5000
4000
3000
2000
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µg ACETATE l⁻¹ hr⁻¹ dm⁻²
APR. MAY JUN. JUL. AUG. SEP. OCT. NOV DEC. JAN. FEB. MAR. APR. MAY
1968
1969

Figure 31.

site. Annual acetate utilization ranges for 1 dm^2 of the water column are 10.4 to 502.2 µg l^{-1} hr^{-1}, and 71.2 to 4977.8 µg l^{-1} hr^{-1} at these respective sites. These latter values suggest a paramount effect upon prevailing dissolved organic matter in shallow water littoral plant beds, regardless of its origin.

Annual mean values of chemo-organotrophic utilization of glucose and acetate were determined by planimetric measurement of the respective integrated curves (Table 10). If these mean values are summed together for both sites and extrapolated to a square meter basis over a 24 hour day, and converted to the equivalent percentage of carbon in glucose and acetate, a total of 610 mg of glucose and 958 mg of acetate are potentially used per m^2 per day in the littoral zone by epiphytic communities. Assumptions are that these rates persist over a 24-hour period (which is likely to be an overestimate), and that a continuous regeneration of these substrates occurs. A comparison of these data to net mean annual primary productivity by algal epiphytes (1807.2 + 195.7 = 2002.8 mg C assimilated m^{-2} of the littoral zone day^{-1}) indicates 78% of the carbon produced on a mean daily basis could be removed if it were directly converted to free glucose and acetate. If autolysis of attached algal forms and excretion by them accounted for 5 to 10% of the dissolved organic matter at the plant surface, this would not be enough to sustain the rates of chemo-organotrophy observed, and further suggests the use

TABLE 10.--Mean annual rates of chemo-organotrophic utilization of dissolved organic compounds by epiphytic communities, Lawrence Lake, Michigan. Data were collected from artificial substrata.

Vegetation Site	$\bar{x}$ Annual Velocity of Utilization ($\mu g\ l^{-1}\ hr^{-1}$ per dm^2)[a]	
	Glucose	Acetate
Scirpus acutus Muhl.	54	106
Najas flexilis and *Chara* spp.	586	893

[a]Based on total macrophytic surface area present per unit area of littoral zone.

of organic solutes from other sources, e.g. macrophytic release and surrounding littoral water column. If the assumption that natural chemo-organotrophic uptake is limited to the 6 to 8 hours of diel darkness is made, the annual rates assume even greater importance in littoral metabolism.

C. In situ ^{14}C-labelling of macrophyte-epiphyte systems

Extracellular release of dissolved organic compounds has been established and reconfirmed for axenic laboratory grown Najas flexilis (Wetzel, 1969a, 1969b; Allen, 1970). Similar measurements have not been attempted to demonstrate the occurrence of extracellular organic release under natural conditions in the field. Further, no studies have been conducted to establish if natural epiphytic communities can utilize such products. Circumstantial evidence derived from laboratory cultures presents a very strong case for this, but field evidence for this interactive system is a necessary prerequisite to application of laboratory data to in situ observations.

To document the release of extracellular materials under natural conditions the emergent hydrophyte, Scirpus acutus, was pulse-labelled above the water surface with evolved $^{14}CO_2$, of high specific activity, at close to ambient concentrations (0.035% v/v). Liberation of dissolved organic materials was followed with respect to

depth and time to show patterns of release for plants with and without their epiphytic communities (Table 11). The difference in activities of dissolved organic matter escaping from the plant surface can be attributed to efficiency of removal through epiphytic metabolism. A third plant, serving as a control, was also labelled with carbon dioxide, but without a Plexiglas tube surrounding the plant and restricting its external aquatic milieu (Table 12). Vertical distribution of dissolved organic matter release is not a prominent feature for either of the plants surrounded by Plexiglas tubes. Loss of exchange ability and water renewal may have had adverse effects upon the hydrophytes themselves and disrupted any effects upon the epiphytic communities. Removal of the epiphytes showed little effect upon dissolved carbon loss from the macrophytes during the first 2 hours of incubation. Activities increased where epiphytes were present following this period and after 5 hours *in situ* incubation values were approximately twice those where epiphytes were removed. These data suggest interactive mechanisms may exist between the epiphytic community and the host vascular hydrophyte, and further may indicate that sessile communities are fundamentally important in inducing the physiological release by the macrophyte. A second possibility is that the response is due to physiological damage sustained by the plant during physical removal of its epiphytic vegetation. A similar increase in extracellular release is seen in the

TABLE 11.--Release of dissolved organic matter (^{14}C-labelled) by the emergent hydrophyte, Scirpus acutus Muhl., with and without a natural epiphytic community, simultaneous to in situ photosynthesis in the presence of $^{14}CO_2$. Station I, Lawrence Lake, Michigan; 30 June 1969.

System	Dissolved Organic Matter (cpm)[a] ml^{-1} of lakewater at depth						
	Time Course Sampling Intervals						
	0-3 min	11-16	28-34	59-66	2hr	3hr	5hr
Scirpus acutus Muhl. with epiphytes[b]							
0 cm[c]	381	318	349	371	332	308	316
10 cm	626	339	270	352	360	309	332
20 cm	427	319	313	317	361	350	374
30 cm	340	377	255	259	498	487	515
Scirpus acutus Muhl. without epiphytes							
0 cm	320	328	349	333	408	538	870
10 cm	344	450	289	301	457	515	765
20 cm	330	350	337	251	484	499	812
30 cm	419	318	349	325	479	635	914

[a]Counts per minute radioactivity.

[b]Scirpus acutus plants with and without epiphytes were enclosed in Plexiglas tubes.

[c]Depth below the water surface samples were collected.

TABLE 12.--Release of dissolved organic matter (^{14}C-labelled) by the emergent hydrophyte, *Scirpus acutus* Muhl., simultaneous to *in situ* photosynthesis in the presence of $^{14}CO_2$).[a] (Station I, Lawrence Lake, Michigan; 30 June 1969.

System	Dissolved Organic Matter (cpm ml^{-1} of lakewater at depth)						
	Time Course of Sampling Intervals						
	0-3 min.	11-16	28-34	59-66	2hr	3hr	5hr
Scirpus acutus Muhl. with epiphytes							
0 cm[b]	355	679	272	354	326	512	583
10 cm	608	407	277	293	369	619	421
20 cm	938	400	465	599	505	835	761
30 cm	1346	843	824	814	736	765	887

[a]Serves as a control to those plants surrounded by Plexiglas tubes.

[b]Depth below the water surface samples were collected.

control plant after 1 hour incubation, especially towards the sediments. Whether the release is light dependent and a true stratification exists in nature is not known. These experiments were conducted at mid-day from 0950 to 1450, and results may be correlated with day length and photoperiod, i.e. response of increase in early afternoon may be related to diel photosynthesis patterns of the host plant.

In the above dissolved organic matter samples $^{14}CO_2$ was purged from the scintillation fluid, to yield only carbon-14 in the dissolved organic phase. On several occasions this sparged $^{14}CO_2$ was trapped in an NCS solubilizing agent (see methods), or in a diamino-ethanol based fluor mixture for quantification. Estimates of 12 to 29% as ^{14}C of the total amount released as dissolved organic compounds (for 6 samples only), indicates epiphytic autotrophs may be obtaining a certain amount of their inorganic carbon from the supporting plant.

Non-quantitative samples of attached communities were removed by gentle friction on the plant surface, followed by suction into a glass vial containing solubilizer and fluors, at time intervals of dissolved organic matter collection only to determine if they were labelled and if a transfer had occurred. Scintillation counting procedures confirmed that ^{14}C was being utilized by the epiphytes; the label was detectable within a three-minute period.

Following termination of the field experiments, all three plants were removed from their growth site. Epiphytes from known surface areas (0.785 cm^2) were removed at 6 cm intervals from the portion of the Scirpus plant normally underwater (on 2 plants), resuspended into fresh lakewater from the site, filtered and radioassayed. All three plants were sectioned at 2 cm intervals and 1 mm thick discs of stem tissue were dissolved in NCS and Triton-X, plus fluors to establish localization of the isotope following photosynthetic labelling.

Vertical distribution of carbon incorporation by the epiphytic complex shows little quantitative change (Table 13), although too few data were obtained to make any definitive statements. It is apparent, however, that under natural conditions carbon transfers do occur within this particular epiphyte-macrophyte system. Such data may suggest that a host of physiological interactions are probably functional within epiphyte-macrophyte systems elsewhere.

Attempts were made to determine patterns of carbon incorporation by Scirpus via liquid scintillation techniques, but errors may have been introduced into the data due to pigment quenching. Precautions were taken to minimize these effects. Internal translocation of the ^{14}C label (Table 14) showed little similarity between the three Scirpus plants after 5 hours incubation. Activity with

TABLE 13.--Intracellular fixed carbon-14 in the epiphytic community of Scirpus acutus Muhl., following in situ photosynthetic ^{14}C-labelling of the emergent vascular hydrophyte. (5 hour incubation; Station I, Lawrence Lake, Michigan; 30 June 1969).

Emergent Substrata and Depth Sampled	Epiphytic Activity (cpm per 0.785 cm^2 area)[a]
Scirpus acutus Muhl.[b]	
0 cm = surface	669
6 cm	326
12 cm	464
18 cm	469
24 cm	302
30 cm	291
36 cm	311
42 cm	655
46 cm = sediments	
Scirpus acutus Muhl.[c]	
0 cm = surface	463
6 cm	821
12 cm	325
18 cm	342
24 cm	329
30 cm	221
36 cm	275
42 cm	339
48 cm	271
51 cm = sediments	

[a]Samples processed by gas-flow counting techniques and were treated by acidification for removal of monocarbonate contaminant activity.

[b]Submerged portion incubated with a Plexiglas tube enclosure.

[c]Considered "control" plant; no experimental treatment.

TABLE 14.--Intracellular fixed carbon-14 in Scirpus acutus Muhl., following in situ photosynthetic labelling with $^{14}CO_2$. (5 hour incubation; Station I, Lawrence Lake, Michigan; 30 June 1969).*

Depth Below Water Surface	Activity Incorporated (cpm per mg dry weight)		
	Scirpus No. 1	Scirpus No. 2	Scirpus No. 3
0 cm = surface	4052	1870	1132
4 cm	6608	1431	1562
8 cm	4730	1542	3901
12 cm	2199	1307	2842
16 cm	-	1986	3652
20 cm	4688	2146	2143
24 cm	6772	6342	3144
28 cm	3465	2879	1555
32 cm	1760	3161	2008
36 cm	2297	4107	7681
40 cm	4633	5517	7239

*Measurements of incorporated activity were made at 2 cm intervals from the emergent tip into the rhizoid-horizontal stem; Scirpus No. 1 = epiphytes present, Plexiglas enclosure; Scirpus No. 2 = epiphytes absent, Plexiglas enclosure; Scirpus No. 3 = control plant, epiphytes present, no surrounding enclosure.

depth expressed as counts per minute per mg dry weight was obtained by extrapolation of dry weights from 1 mm thick discs adjacent to those sampled on the plant for radioassay. (Activities above the water surface approached 100,000 cpm per equivalent dry weights, in the vicinity of the $^{14}CO_2$ chambers).

Considerable dissolved organic matter was present at distances of 1 to 3 meters from the plant after 2 hours *in situ* incubation (depth: 20 cm below the surface; 1 m distance: 381; 2 m: 31, and 3 m: 18 cpm ml^{-1}). When the amount of water above a square meter of the littoral zone is taken into consideration, these activities assume greater importance, and indicate that products of macrophyte-epiphyte metabolism are detectable at some distance from their origin. Such documentation furthers the possibility of macrophytic-epiphyte interactions in littoral metabolism, particularly with epibenthic communities in close association.

D. Nutritional and physiological interactions of axenic *Najas flexilis* and cultured epiphytes

Prior to assessment of mixed community interactions between isolated algal and bacterial epiphytes, and the freshwater vascular angiospern, *Najas flexilis* studies were undertaken to establish rates of uptake of glucose and acetate-^{14}C by the epiphytes. Initially, however,

some familiarity with growth kinetics and minimum nutritional requirements of cultured epiphytes was necessary.

Procedures for determination of optical density and equivalent cell numbers have been described in detail in the methods section. The period over which log phase of growth kinetics could be observed was established by spectrophotometric readings prior to each measurement of organic utilization in media II and WC (for media, see Wetzel and McGregor, 1968; and Dr. R. R. L. Guillard, personal communication, respectively). For bacterial species (Caulobacter and Pseudomonas) log phase was observed within the optical density (O.D.) range of 0.01 to 0.150, extending over a 1 to 3 day period, followed subsequently by a population plateau (O.D. = 0.240 to 0.400 maximum). Algal species (Gomphonema, Cyclotella, and Chlorella) generally reached maximum rates of cell increase within 4 days of inoculation and persisted in log growth for 2 to 5 days and occasionally longer. Bacteria cultured in medium II required the presence of a vitamin mix (same as used for WC), trace metal mix (as for WC), ammonium source (NH_4Cl), and glucose (1 mg l^{-1}). The presence of glucose at 1 to 100 mg l^{-1} had no effect upon enzyme kinetic responses when determined by ^{14}C uptake, i.e. apparently there was no adaptive response to higher external concentrations. Epiphytic algal cultures required vitamins and trace metals but no additional carbon or

energy sources. Further, they were not enriched with CO_2; pH of all experimental media was 7.5 to 8.2.

All species of epiphytic organisms were grown into log phase, washed twice, concentrated, and placed into 100 ml of fresh medium. Near in situ concentrations of high specific-activity, uniformly labelled-^{14}C glucose and acetate were added by micropipettes to cultures of individual and mixed algal and bacterial epiphytes. Concentrations were extended to 5 mg l^{-1} where algal species were used. Uptake measurements monitored by Michaelis-Menten and diffusion kinetic graphical and mathematical analyses, at temperature ranges simulating winter (5C), vernal and autumnal (11 to 12C), and summer (21 to 23C) conditions in shallow-water littoral areas are summarized in Tables 15 and 16. Numbers of organisms extrapolated from optical density measurements did not change appreciably during the course of incubation and were usually within the range of 10^4 to 10^5 ml^{-1} for bacteria and algae. While cell concentrations are likely to be higher than in many freshwaters, they were lower than numbers commonly employed in microbiological and algological studies (frequently 10^6 to 10^9 ml^{-1}).

Rates of organic uptake for individual algal and bacterial cultures showed a strong general correlation with temperature. In several instances low temperatures seemed to inactivate enzymatic and diffusion mechanisms.

TABLE 15.--Comparison of rates of chemo-organotrophic utilization of glucose and acetate by individual axenic cultures of algal and bacterial epiphytes under different thermal conditions. (G = glucose; A = acetate).

Cultures	Active Transport Kinetics (V_{max}; $\mu g\ l^{-1}\ hr^{-1}$)						Diffusion Kinetics (K_d; hr^{-1})					
	5C		11-12C		21-23C		5C		11-12C		21-23C	
	G	A	G	A	G	A	G	A	G	A	G	A
Caulobacter	5.9	2.4	3.7	1.2	1.1	4.8						
Pseudomonas	0.1*	0.2*	5.4	3.1*	6.8	7.1						
Gomphonema							.012*	-	.306	.589	.425	.607
Cyclotella					4.3**		.036	.178*	.410	.624	.870	.821
Chlorella							.072*	-	.172*	.304	.210	.510

*Indicates population response exhibited poor linearity.

**Response of Cyclotella at low substrate concentrations suggests first order kinetics, in addition to the horizontal slope shown at higher concentrations.

TABLE 16.--Comparison of rates of chemo-organotrophic utilization of glucose and acetate by mixed axenic cultures of algal and bacterial epiphytes under different thermal conditions. (G = glucose; A = acetate).

Cultures	Active Transport Kinetics (V_{max}; $\mu g\ l^{-1}\ hr^{-1}$)						Diffusion Kinetics (K_d; hr^{-1})					
	5C		11-12C		21-23C		5C		11-12C		21-23C	
	G	A	G	A	G	A	G	A	G	A	G	A
Caulobacter + Gomphonema	6.9	-	2.8	3.4	2.5	6.4	.034*	-	.597	.735	.839	.591
Caulobacter + Chlorella	3.8*	-	1.0*	0.9*	2.5	2.4	.016*	-	.108*	.296	.530	.722
Pseudomonas + Chlorella	0.1*	-	5.1	2.9	8.4	8.7	.031*	-	.380	.665	.431	.507
Pseudomonas + Gomphonema	0.4*	-	6.1	2.8	7.4	8.4	.063	-	.517	1.065	.960	1.40
Pseudomonas + Gomphonema + Chlorella	0.4*	0.9*	5.5	6.4	4.5	9.2	.104	.100	.830	1.057	1.960	2.001
Caulobacter + Chlorella + Gomphonema	0.9	1.6	4.8	5.5	3.2	1.6	.162	.106	.710	2.165	.990	2.360

*Indicates population response exhibited poor linearity.

Rates of glucose uptake of Caulobacter increased at lower temperatures, indicative of highly efficient and specialized enzyme systems under cold conditions. Pseudomonas showed an increase in rates of uptake for both substrates at 11 to 12C, and at 21 to 23C. A common response by algal organisms to substrate concentrations greater than 100 µg l^{-1} for all species except Cyclotella was an increase in rate of diffusion of both substrates. Rates of increase of both algal and bacterial cultures were not proportional to increases in temperature and likely reflect optimal enzymatic activity. Uptake by Cyclotella is of noteworthy importance in that first order kinetics were expressed at concentrations generally dominated by bacterial uptake. This observation may be indicative of direct competition with bacteria for organic substrates. Similar results were not observed with the remaining cultures.

Uptake responses with mixed populations of algae and bacteria in medium II showed definite metabolic interactions (Table 16). Bacterial responses definitive at the lower concentration ranges as in monoculture experiments showed little change in rates of organic uptake in the presence of algae although, as noted, linear responses for glucose at 5C were characteristically poor. Algae, on the other hand, with very few exceptions showed increased accumulation of radioactivity over monoculture conditions. Additional uptake may have been due to uptake of $^{14}CO_2$ previously respired by the bacteria. Increases in

diffusion rate of the algae were greatest for diatom-bacteria and bacteria-diatom-chlorophyte combinations. Nutritional interactions such as these may, in part, be responsible for high rates of organic uptake in natural epiphytes and further be indicative of CO_2 cycling within the attached community. Increases in the cycling speed within the solute phase and recovery of respiratory losses as carbon dioxide by autotrophs and metabolic forms capable of using inorganic carbon probably represents an ecological advantage to the epiphytic complex in comparison with spatially separated communities, e.g. pelagial.

Although measurements employing Michaelis-Menten enzyme kinetics and diffusion mechanisms permit the derivation of turnover times of the organic substrate being tested, such parameters have little relevance in that regenerative rates (as T_t and T_d; see Allen, 1969) cannot be linearily added in heterogeneous communities to explain effects of one taxon on another. Velocity and rate measurements are far more applicable to the interpretation of results where fixed or known population numbers are used.

Extracellular production of dissolved organic materials by Najas flexilis in response to variously modified inorganic nutrient conditions has been previously established (Wetzel, 1969a, 1969b). For purposes of this study, autotrophic assimilation of ^{14}C-labelled inorganic carbon and subsequent release of ^{14}C-labelled organic compounds by axenic cultures of Najas flexilis were

redetermined (Figures 32 and 33). Techniques of sterilization of seed surfaces, procedures for germination and general nutritional responses of this angiosperm have been presented elsewhere (Wetzel and McGregor, 1968). Constant labelling of macrophytic tissue occurred subsequent to 3 hours incubation under constant laboratory conditions (750 lux; 20C; medium II). The assumption was made that rates of excretion would be maximal only after the entire metabolic pool became labelled to a constant specific activity. To substantiate the pattern of extracellular release in medium II, plants (5 or 6 plants per flask; in triplicate) were pulse-labelled with inorganic carbon-14 for four hours, followed by placement into label-free fresh media, after which changes in absolute activity in the dissolved organic carbon phase were followed (Figure 33). Over a 3.45 hour incubation interval, under conditions identical to those used for autotrophic assimilation, a mean excretion rate of 7% of the total intracellular fixed carbon was released.

Utilization of macrophytic extracellular products by cultured epiphytes was determined in partitioned chambers of special design (see Figure 3). Following 4 hours of photosynthesis on ^{14}C-labelled carbon, the plants (5 or 6 per flask) were introduced into the center chamber section of each Plexiglas container. After brief acclimitization by the macrophytes, aliquots of algal and bacterial cultures in log phase of growth were added under aseptic

Figure 32.--Rates of photosynthetic incorporation of carbon-14 (μCi g^{-1} dry weight $hour^{-1}$) by cultured axenic Najas flexilis L.

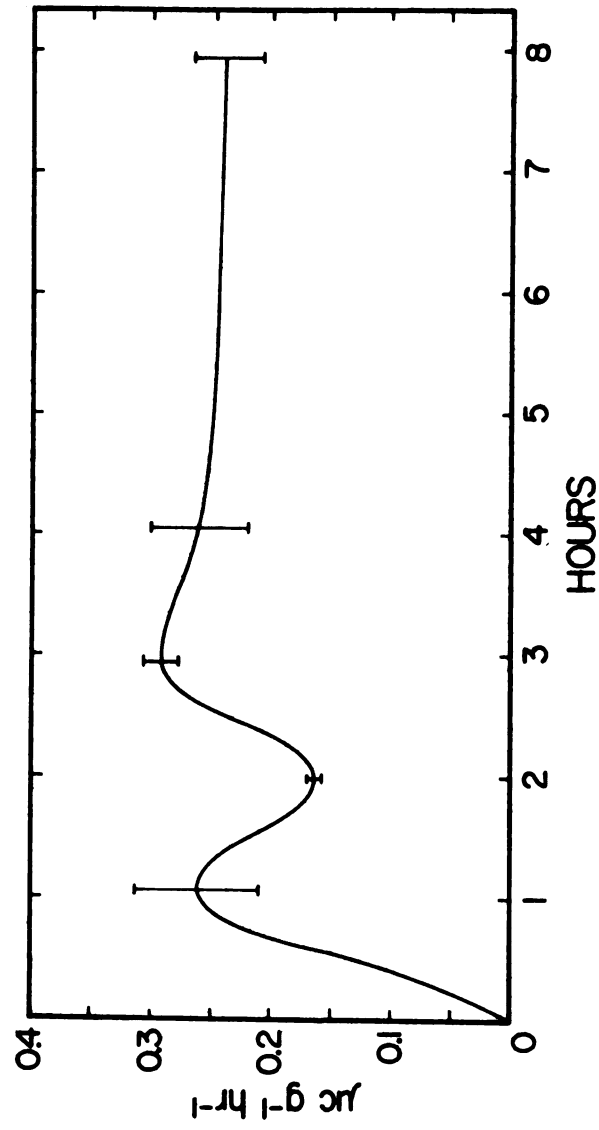


Figure 32.

Figure 33.--Percentage of mean excretion of dissolved organic compounds to the mean rates of photosynthetic carbon fixation by axenic Najas flexilis L.

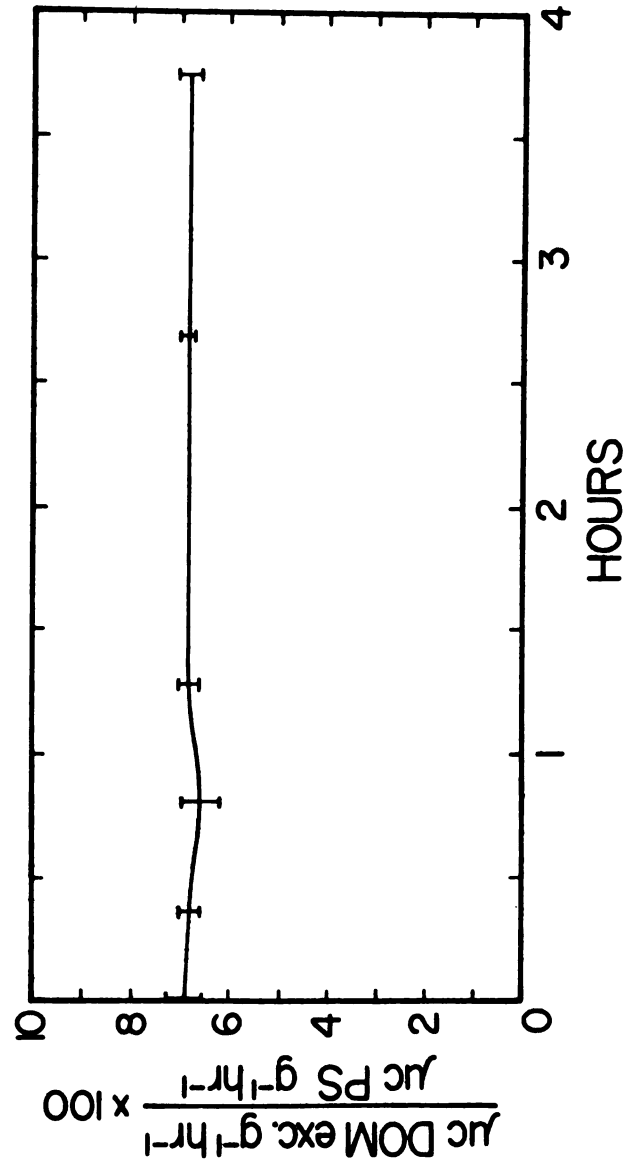


Figure 33.

conditions to the two outer chamber sections. Aliquots of the algal and bacterial cultures were removed with time, dried on planchetes or filters, and radioassayed.

A summary of intracellular uptake by cultured epiphytes (Table 17), under light and dark conditions at 20C confirms that a significant transfer occurs from the macrophytes into the adherent taxa during brief incubation periods. Data expressed were obtained by equating optical density measurements in aliquots removed from the chambers, immediately after inoculation, to dry weights (105C) of equal aliquots of organisms on tared filters using a Cahn Electrobalance. Activities from dried, filtered or plated, organisms were used to compute specific activities on a dry weight basis. Such data presentation readily permits a comparison of epiphytic utilization of macrophytic extracellular organic products to the total amount released (ca. 7% of the macrophyte photosynthate). Based on the maximum epiphytic removal rates (Table 17; 0.72 to 11.45 $\mu Ci \cdot 10^{-3}$ mg dry weight^{-1}), 0.00413 to 0.0545% of the total extracellular carbon can be removed if 17.4 to 21.0 $\mu Ci\ mg^{-1}\ hr^{-1}$ is produced by Najas flexilis under these conditions. Patterns of uptake of extracellular macrophytic products for algae and bacteria were similar, with increased uptake occurring initially, followed by a decrease and gradual plateau. Rates of uptake in total darkness are higher than in the light but these data fail to discriminate if this is due to (1) increased

TABLE 17.--Uptake of ^{14}C-labelled extracellular products of axenic Najas flexilis by cultured algal and bacterial epiphytes. (20C; medium II; see text for experimental procedure). (L = light; D = dark incubation).

Cultures		Specific Activity (μCi 10^{-3} mg dry weight^{-1})						
		Time Course (min.)						
		0	15	30	60	120	180	240
Caulobacter	L	11.50	8.70	8.14	6.50	6.57	3.88	4.12
Pseudomonas	L	9.22	6.96	7.01	5.99	6.12	4.36	5.15
	D	11.45	7.35	8.70	8.61	7.44	3.56	2.18
Gomphonema	L	1.94	2.31	3.12	0.87	1.42	1.40	0.96
	D	2.47	3.33	2.80	1.95	1.86	1.47	1.03
Chlorella	L	2.47	1.75	1.33	1.01	0.98	0.93	0.72
Cyclotella	L	3.25	1.95	1.56	2.07	1.42	1.36	1.21

extracellular release by Najas, (2) increased uptake efficiency by epiphytes, or use of different pathways, or more probably, (3) a combination of these. Rates of bacteria were approximately 10 times those of the algae during the first 15 minutes and gradually decreased to rates only twice those of the algae.

A comparison of mixed algal and bacterial uptake was made by removal of culture aliquots of algae and bacteria, growing simultaneously in the presence of Najas flexilis in Pyrex flasks (Table 18). Optical density measurements were equated for mixed cultures to their dry weights to gain an acceptable expression of their specific activities per unit of time. Certain of these responses are difficult to interpret. Only in mixed cultures of one alga and one bacterium were uptake activities greater than those of individual cultures. Mixed communities of a single bacterium, a chlorophyte, and a diatom, showed poor uptake efficiencies with time. Such results suggest interspecific interactions where competition for specific external metabolites or organic solutes may have existed or where accumulation of extracellular products caused toxicity effects. The brief time interval of sampling in these studies needs to be stressed. There exists the inherent problem of recycling of $^{14}CO_2$ and external metabolites, confounding interpretation, if experiments under fixed conditions are extended over lengthy periods of time.

TABLE 18.--Uptake of ^{14}C-labelled extracellular products of axenic Najas flexilis by mixed cultures of algal and bacterial epiphytes. (20C; medium II).

Mixed Cultures	Specific Activity ($\mu Ci \cdot 10^{-3}$ mg dry weight^{-1})						
	Time Course (min.)						
	0	15	30	60	120	180	240
Caulobacter + Gomphonema	18.3	15.6	19.1	13.7	13.5	13.0	12.8
Caulobacter + Chlorella	8.4	5.9	8.3	2.8	2.7	3.0	-
Pseudomonas + Gomphonema	14.6	15.3	22.1	20.6	18.7	18.4	-
Caulobacter + Gomphonema + Chlorella	6.7	8.3	8.3	8.9	7.7	7.7	-
Pseudomonas + Gomphonema + Chlorella	4.5	4.7	9.7	8.8	5.6	6.0	-

Two additional experiments were performed to elucidate existing nutritional interactions between the cultured epiphytes and Najas flexilis. Najas plants were photosynthetically labelled, placed into fresh media, and allowed to produce extracellular products for 2 hours in the light. The plants were carefully removed and aliquots of medium were dried on planchetts to obtain control values of extracellular activity. Bacteria were then inoculated into this medium and permitted to grow there for 2 hours before they were concentrated and removed (membrane filter). Algal cultures were then allowed to grow on this and media to which no bacteria had been added. A significant increase in algal activity, in both light and dark incubation conditions, following bacterial metabolism (Table 19), suggests materials (either $^{14}CO_2$ or ^{14}C-labelled by-products) released by the Najas were not wholly suitable as carbon and energy substrates without prior microbial degradation. Similar pathways are certainly functional in the natural environment. Aliquots of ^{14}C-extracellular products of Najas were diluted in series (1:0.5, 1:2, 1:4, 1:8, and 1:16) with fresh unlabelled medium II and used for estimation of uptake response kinetics by cultured algae and bacteria. No diffusion or zero-order kinetics could be demonstrated with any validity and may have been related to the low proportions of dissolved organic carbon released or possibly to its unsuitability as a C or energy source. Velocities of substrate uptake did follow enzyme kinetics

TABLE 19.--Utilization of extracellular products of axenic Najas flexilis by cultured algal epiphytes subsequent to microbial metabolism of the released material. See text for explanation. (20C; medium II; L = light; D = dark).

Cultures	Incubation	Specific Activity of Algae ($\mu Ci \cdot 10^{-3}$ mg dry weight^{-1})	
		Without Bacteria	With Bacteria*
Gomphonema	L	1.36	1.89
	D	1.92	2.45
Cyclotella	L	2.45	2.93
Chlorella	L	1.91	-

*Medium II control before bacteria (Caulobacther + Pseudomonas) were added: 0.00236 $\mu Ci\ ml^{-1}$; medium II control after two hours of microbial activity in the light, with bacteria removed: 0.00205 $\mu Ci\ ml^{-1}$.

with a definite first order linear response (r = 0.994; significant at the 0.05% level). Maximum uptake rate for Pseudomonas was 0.023 µg l^{-1} hr^{-1}, and is two orders of magnitude lower than respective rates of glucose and acetate at approximately the same temperatures (20C; see Table 15) and optical densities.

A final laboratory experiment to demonstrate the percentage of $^{14}CO_2$ release by Najas flexilis in pure culture was undertaken. Through precipitation as barium carbonate-^{14}C release of extracellular $^{14}CO_2$ was 15 to 19% of the total fixed carbon at 20C (incubation = 4 hours). Data herein discussed for the various laboratory studies were not corrected for these losses, or for respiratory losses in individual and mixed uptake experiments.

E. Functional aspects of macrophyte-epiphytic metabolism in littoral ecology and lake trophic dynamics

The magnitude of extracellular release of dissolved organic matter (DOM) under natural conditions in the lake, as well as the subsequent rapid incorporation into the epiphytic complex and loss to the surrounding littoral zone, suggests (1) the littoral vegetation and attached periphytic growth are capable of significant contributions to the total DOM "pool", and (2) that considerable utilization and transformation of the macrophytically produced DOM is likely to occur prior to its availability in the littoral and pelagial areas.

Many of the potential ramifications of macrophytically released DOM on profiles, annual patterns, and rates of primary productivity of autotrophic microorganisms in the pelagial environment, have been derived through (1) bioassay responses of pelagial plankton communities to varying inorganic and organic nutrient conditions, and (2) through further knowledge of succinct physical-chemical-nutritional interactions existing and operating in specific freshwater habitats, e.g. in marl lakes (see relevant discussion in Wetzel and Allen, 1970). The relationships of dissolved organic matter, whether allochthonously or autochthonously produced, to sustained and persistent rates of autotrophic metabolism, through direct and indirect interactions intra-primary producer trophic level, are becoming more defined (Wetzel, 1968). Indeed, the presence or absence of an abundance of organic materials functioning as physiological chelators, for example, by provision of inorganic iron to autotrophic plankton metabolism, may be responsible for significant differences in daily rates of openwater photosynthesis. Similar interactions, if persistent and characteristic for the system, may very well lead to advanced or retarded eutrophicational development, regardless of lake morphometry, basin characteristics, depth and surrounding geological features, although the latter certainly play important roles in this process.

A point to be considered in this discussion is that functional interactions of organic matter on pelagial

dynamics would likely be pronounced and accentuated in a system where annual mean primary productivity is low and where the ratio of the littoral to the pelagial zone is high. Prolifically developed submerged and emergent vegetation, with its epiphytic flora in close nutritional association, is probably capable of imposing severe demands on dissolved organic materials at the micro- and macro-levels. It may ultimately deprive plankton communities of the more labile carbon and energy rich compounds, and trace organic micronutrients. Certainly the intensity of rates of primary productivity and chemo-organotrophy reported in this study (among the highest in the literature) for epiphytic algae and bacteria, must have an impact in a system where plankton photosynthesis is suppressed. Even in aquatic ecosystems where significant amounts of allochthonous and autochthonous dissolved and particulate organic matter are available to pelagial metabolism, and are reflected in increased carbon fixation rates, the epiphytic complex may represent the dominant producer and may still be capable of indirectly affecting the population dynamics and community metabolic patterns of the phytoplankton (see relevant discussion by Khailov and Gorbenko, 1967).

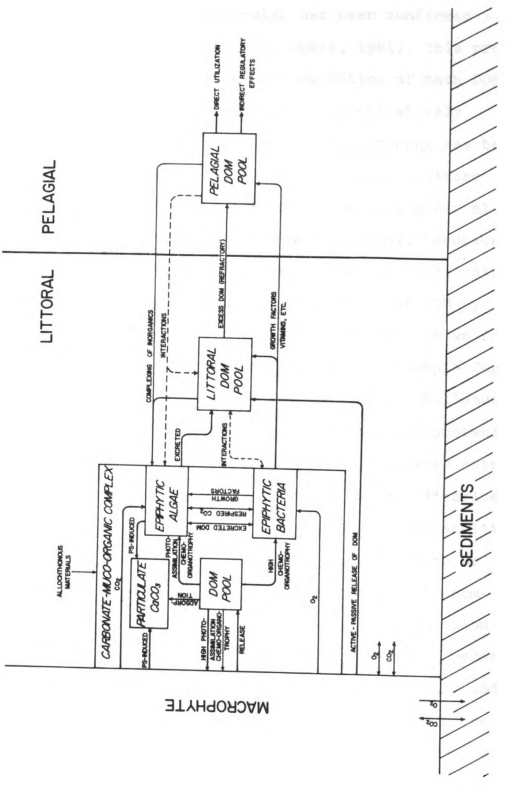
Quantification of various epiphyte metabolic parameters, coupled with descriptive observations of communities, under laboratory and field conditions have permitted

a more thorough understanding of epiphytic dynamics and host macrophytic interactions (Figure 34).

During initial colonization littoral bacteria adhere and probably form a mono-cellular layer on the new vegetative substratum interwoven with time by deposition of calcium carbonate by the macrophyte and attaching algal forms. Such a matrix formation may enhance the integrity of community structure and is probably fundamental in the adherence of large standing crops of organisms. Deposition of particulate monocarbonates is especially prevalent in a calcareous environment such as Lawrence Lake. As colonization intensifies (late spring and early summer for natural substrata) a dissolved organic carbon "pool" is probably established within the matrix of deposited carbonates. Sources contributing to this pool would probably include (1) extracellular release from the macrophyte, (2) active excretion by attached algal and bacterial flora, (3) decomposition products following autolysis of epiphytes, and (4) allochthonously and autochthonously derived particulate and dissolved carbon present in the littoral zone. High rates of chemo-organotrophy and primary productivity are probably sustained to a large extent by provision of trace elements, P, N, biotics, growth factors, etc., as well as labile and more refractile compounds from within the pool. The bioassay conducted, indicating stimulation of epiphytic primary productivity by addition of chelators and inorganic iron, may reflect deficiencies in the qualitative

Figure 34.--Diagrammatic representation of metabolic and nutritional interactions existing in the littoral zone of a representative calcareous aquatic ecosystem. (See text; PS = photosynthesis; DOM = dissolved organic matter).

Figure 34.


LITTORAL
PELAGIAL
MACROPHYTE
ALLOCHTHONOUS MATERIALS
CARBONATE-MUD-ORGANIC COMPLEX
PARTICULATE CaCO3
EPIPHYTIC ALGAE
EPIPHYTIC BACTERA
DOM POOL
LITTORAL DOM POOL
PELAGAL DOM POOL
PS-REDUCED
ASSIM. PHOTO-OXIDATION
ASSIM. CHEMO-OXIDATION
ADSORPTION
DESORPTION
HIGH PHOTO-ASSIMILATION FROM CHEMO-TROPHY
RELEASE
EXCRETED
EXCRETED DOM
REMINERALIZATION
GROWTH FACTORS
INTERACTIONS
EXCESS DOM INTERACTION
GROWTH FACTORS VITAMINS, ETC.
DIRECT UTILIZATION
INDIRECT REGULATORY EFFECTS
ACTIVE - PASSIVE RELEASE OF DOM
SEDIMENTS

composition of this pool or competition by attached bacteria for such organic substrates. In that adsorption of organic compounds on carbonates has been confirmed (Wetzel and Allen, 1970; see also Chave, 1965), this process may contribute to removal and retention of much DOM of epibenthic and eulittoral origin. Quantitatively insignificant, but detectable, chemo-organotrophy has been established for Najas flexilis grown in axenic culture under controlled conditions (Dr. R. G. Wetzel, personal communication), and may represent a nutritional feedback mechanism from the epiphytic flora to the host substratum.

Although not yet documented, excretion of DOM by epiphytic algae is likely to occur (as has been shown for marine and freshwater phytoplankton, cf. for example Fogg, 1962, 1966; Lefèvre, 1964; Fogg and Watt, 1965; Hellebust, 1965; Forsberg and Taube, 1967; and others), and probably contributes together with bacterial and macrophytic extracellular products to the presence of a littoral dissolved organic matter pool. In that specific organic metabolites required for epiphytic metabolism are likely not to be continuously regenerated under natural conditions, compounds of littoral or even pelagial origin may function as feedback mechanisms through the supply of these necessary materials. Without prior knowledge the quality and quantity composition of each of the DOM pools are suspected to be different, in that communities contributing to each

of these pools maintain distinct species composition, which in turn upon autolysis or in situ excretion probably release a specific array of compounds. As an example, intense epiphytic microbial activity may supply certain of the much needed water-soluble vitamins (B-complex) to littoral producers.

The effect of release of CO_2 by the macrophyte, algae and bacteria has not been investigated, but intricate mechanisms of combined respiratory cycles may lead to provision of considerable localized CO_2 accumulations for epiphytic algae and epibenthic communities for early morning photosynthetic requirements. Certainly the release of oxygen by the macrophyte regulates to some extent rates of decomposition processes within the muco-organo-carbonate complex by reducing the intensity to which anaerobic metabolism may proceed. Compactions of sulfur bacteria were observed microscopically on natural Scirpus substrata.

Other nutritional advantages to epiphytes over pelagial communities can be speculated upon briefly. Autotrophic metabolism can probably more easily and readily be shifted on the attached surface to chemo-organotrophic supplementation during periods of poor light and adverse inorganic nutrient conditions. There is the possibility that exosmotic release by macrophytes and maintenance of a surface adsorbed dissolved organic carbon source (the distinction is not herein made between the actual dissolved phase and particulate-dissolved, in various stages

of decomposition; functionally, they may be the same) provides organic compounds easily capable of serving in more than one functional capacity, as a chelator, complexing agent, or as a carbon or reductive substrate.

In summary, the potential importance of the littoral zone as a functional unit within a lake has been emphasized from the standpoint of (1) a source of dissolved organic matter and (2) as a dynamic macrophyte-epiphyte system, nutritionally and physiologically interacting to sustain high levels of primary productivity and chemo-organotrophy. A detailed discussion of the epiphyte-macrophyte interactions alluded to in this study, coupled with functional aspects of epibenthic and pelagial metabolism, has been presented by Wetzel and Allen (1970). Such proposed causal mechanisms and nutritional interrelationships as suggested here (for macrophyte-epiphyte interacting systems), while derived from studies in a typical marl lake, are thought to be generally applicable to representative aquatic ecosystems of shallow to moderate depth.

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